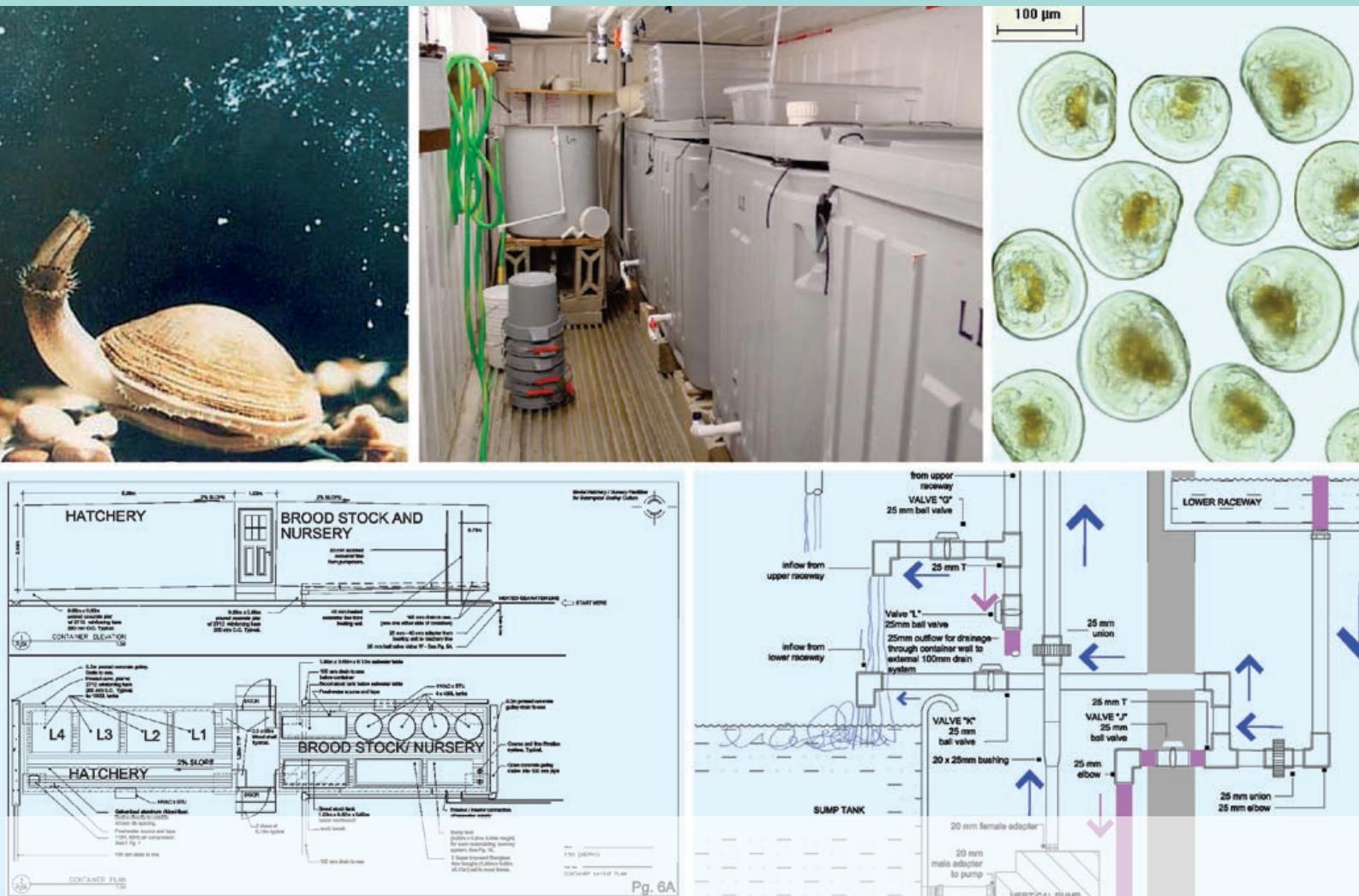


# Installation and operation of a modular bivalve hatchery



**Cover photographs and drawings:**

*Clockwise from top left:* A spawning female Manila clam (courtesy of Brian Edwards); interior of a small bivalve hatchery; photomicrograph of 6-day old sand scallop (*Euvola ziczac*) larvae; technical layout plan of a modular bivalve hatchery; technical detail of a semi-recirculating nursery raceway system (drawings of Souhaila Sarkis).

# Installation and operation of a modular bivalve hatchery

FAO  
FISHERIES  
TECHNICAL  
PAPER

492

Prepared by

**Samia Sarkis**  
FAO Consultant  
Bermuda

and

Compiled and edited by

**Alessandro Lovatelli**  
Inland Water Resources and Aquaculture Service  
FAO Fisheries Department  
Rome, Italy

The mention or omission of specific companies, their products or brand names does not imply any endorsement or judgement by the Food and Agriculture Organization of the United Nations.

The designations employed and the presentation of material in this information product do not imply the expression of any opinion whatsoever on the part of the Food and Agriculture Organization of the United Nations concerning the legal or development status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

ISBN 978-92-5-105595-3

All rights reserved. Reproduction and dissemination of material in this information product for educational or other non-commercial purposes are authorized without any prior written permission from the copyright holders provided the source is fully acknowledged. Reproduction of material in this information product for resale or other commercial purposes is prohibited without written permission of the copyright holders. Application of such permission should be addressed to:

Chief  
Electronic Publishing Policy and Support Branch  
Information Division  
FAO  
Viale delle Terme di Caracalla, 00153 Rome, Italy  
or by e-mail to:  
[copyright@fao.org](mailto:copyright@fao.org)

© FAO 2007

# Preparation of this document

This manual is part of the publication programme of the Fisheries Department Inland Water Resources and Aquaculture Service of the Food and Agriculture Organization of the United Nations (FAO) and complements the FAO Fisheries Technical Paper No. 471 entitled “Hatchery culture of bivalves: a practical manual” published in 2004.

This manual was written for those interested in establishing an aquaculture operation, with minimal experience in this activity, limited technical support and restricted access to information. It stems directly from one of the author’s experience and differs from other manuals by its precision and amount of detail, going one step further in providing a practical template in the building of a hatchery which can be directly copied or modified if needed. Because it focuses on developing aquaculture in resource-limited areas, the manual has been written with the assumption that the user has little, if any, information sources. For this reason, the manual stands as an entity, providing not only the technicalities of setting up and operating a hatchery, but also makes some of the scientific background, deemed useful to the aquaculturist, readily accessible.

The interest in producing this technical manual was generated by the efficiency of the modular hatchery developed and tested over the course of 4 years at the Bermuda Biological Station for Research Inc. (BBSR) for tropical scallop culture. This facility was designed around the facts that little space was available for the building of a hatchery, the budget was small, and proof techniques needed to be developed, prior to a substantial investment. This resulted in a cost efficient, compact, portable hatchery housed in insulated containers. It is a concept, which can be adapted to any region with, as its only requirement, access to “clean” seawater. This modular hatchery may be easily expanded or modified for other bivalve species.

This manual is therefore a technical guide enabling the replication of such a modular hatchery, and also includes detailed protocols pertaining to all aspects of scallop culture, as developed in Bermuda. Protocols reported here were initially written for unskilled personnel, providing a complete and basic outline of the procedures required. However, these protocols have also proved useful throughout years of operation, as they provide an easy routine to follow for all, including more experienced personnel, preventing any careless mistakes easily made throughout the course of a chaotic hatchery day!

The manual is divided into chapters, each of them focusing on a rearing phase and providing: a) Technical drawings with descriptive text; b) Scientific background on biology and culture aspects; and c) Operational and culture protocols. The manual concludes with an economic summary of the set-up of such a modular hatchery and of the labour requirements based on operation in Bermuda. Although the costs given are specific to Bermuda, a detailed equipment list is provided as an appendix, presented as a template which can be used for the calculation of region-specific costs. Other appendixes provide details on required calculations, techniques, equipment list and templates to be copied for routine/maintenance checks.

Last but not least, all those involved in the development of this manual have to be gratefully acknowledged for their dedication, hard work, long hours and enthusiasm. Dr Neil Bourne was the first to provide us with the confidence to pursue our ideal. We also thank him for his constructive criticisms of this manual. Those involved in the aquaculture technique developments and construction of the hatchery from its infancy,

were: Doerte Horsfield, Paul Farrington, Mike Helm, Andrew Cogswell and Claudia Hohn. Data for the economic summary was compiled by Charles King.

Technical drawings were prepared by Souhaila Sarkis, registered Landscape Architect. She is gratefully thanked for her thorough and accurate work. The amount of detail given is a first in the description of an aquaculture facility. For printing purposes the original drawings have been reduced and are not to scale. Scaled drawings are available as PDF files in the enclosed CD-ROM at the back of the manual allowing the reader to print any of these for ease of use and consultation (original paper size format: 11"x17"). The CD-ROM also contains PDF files of all chapters.

A final thank you to Dr Sandra Shumway and Dr LeRoy Creswell, for putting in the time to ensure that this manual is scientifically sound and clear in its presentation. Preparation of the manual has been under the overall coordination of Alessandro Lovatelli, Fishery Resources Officer (Aquaculture), FAO.

The graphic layout of the manual was prepared by J.L. Castilla Civit.

Photos are courtesy of Mike Helm, Souhaila Sarkis and Samia Sarkis, unless otherwise noted.

# Abstract

Limiting factors such as minimal capital investment, lack of technical support or expertise, and available physical space, may put severe restrictions on setting up a hatchery. Not all investors have the means or the will to take the risk to support a large commercial aquaculture operation without substantial proof of its production capacity. For these reasons, the set-up of an inexpensive modular hatchery may be a simpler option to the start-up of a large commercial operation, or maybe sufficient to the needs of a smaller operation.

This manual is intended to stand on its own, as a guide for installation and operation of a bivalve hatchery. Based on years of experience in a resource-limited region, the need for optimal space usage coupled with a restricted budget, has resulted in a modular “portable” hatchery/nursery complex housed in insulated fiberglass containers. With its only requirement being access to “clean” seawater, this model may be easily adaptable to any region. Although the described facility is compact, it is by no means an experimental laboratory, but a hatchery geared towards production. Its functionality has been repeatedly tested over a four-year period, focusing on culture of subtropical/tropical scallops. The developed procedure is suitable for commercial production, the scale of which is dependent on the tankage capacity. In other words, the modular hatchery described here may be expanded by the addition of identical modules, increasing the number of tanks available and hence production.

The described hatchery comprises basic culture facilities for the rearing of bivalve species such as a dedicated seawater system providing a continuous supply of filtered seawater, a temperature control system for seawater, larval rearing tanks for closed or flow-through systems, and flexible usage stacked raceways for spat rearing. The detailed to scale drawings provide a clear guide intended for ease of replication of the facility. An accompanied written text provides further description of the physical facility. Nonetheless, it is not the intent of this guide to dwell into engineering details, but simply to describe a system that works.

This guide also considers the operation of the hatchery, and for this reason contains simple stepwise protocols. These protocols include both maintenance of the hatchery, such as the cleaning of raceways during spat rearing, and culture procedure, as spawning induction.

The modular hatchery is designed for flexibility and may be used for a range of bivalve species and some gastropods. However, protocols given for culture techniques are based on rearing procedures of subtropical/tropical scallop species developed in the pilot hatchery. For additional support, at times necessary in more isolated regions, concise scientific information is provided on various biological aspects of bivalve reproduction and growth.

The manual is divided into chapters for each stage of rearing: broodstock conditioning, algal culture, hatchery, nursery, growout and economic considerations. Every chapter is an entity, and the first five include both the physical requirements and culture considerations and procedures for the relevant rearing stage. The final chapter on economic considerations provides an insight into the labour involved for each stage of production, along with a list of equipment and supplies, which may be used as a template for a new installation.

**Keywords:** modular hatchery, installation, operation, culture, bivalves, technical drawings, rearing protocols.

**Sarkis, S.; Lovatelli, A.** (comp./ed.)  
Installation and operation of a modular bivalve hatchery.  
*FAO Fisheries Technical Paper*. No. 492. Rome, FAO. 2007. 173p.  
Contains a CD-ROM.

# Contents

Preparation of this document .....	iii
Abstract .....	v
List of figures .....	xii
List of tables .....	xiii
List of protocols .....	xiv
List of technical drawings .....	xv
List of appendixes .....	xvii
Glossary .....	xviii
Abbreviations, acronyms and conversions .....	xxi

## Chapter 1 – Scallop broodstock: facilities, reproduction and spawning

<b>1.1 CULTURE FACILITIES .....</b>	<b>1</b>
1.1.1 Master layout plan .....	2
1.1.2 Seawater supply .....	4
1.1.2.1 Primary seawater supply .....	4
1.1.2.2 Secondary seawater supply – link to main intake line .....	6
1.1.2.3 Secondary seawater supply – link to secondary intake line .....	6
1.1.2.4 Main seawater supply to hatchery complex .....	8
1.1.3 Heating unit .....	8
1.1.4 Hatchery/broodstock/nursery complex .....	13
1.1.4.1 Container layout plan .....	13
1.1.4.2 Container plan diagram .....	13
1.1.4.3 Hatchery and nursery ceiling plan .....	16
1.1.4.4 Broodstock: tank and seawater supply .....	18
<b>1.2 SCIENTIFIC BACKGROUND – NATURAL HABITAT AND REPRODUCTIVE CYCLE ..</b>	<b>20</b>
1.2.1 Habitat .....	20
1.2.2 Reproductive cycle .....	22
1.2.2.1 The sand scallop, <i>Euvola (Pecten) ziczac</i> .....	22
1.2.2.2 The calico scallop, <i>Argopecten gibbus</i> .....	22
1.2.3 Life cycle .....	24
<b>1.3 TECHNIQUES – BROODSTOCK .....</b>	<b>25</b>
1.3.1 Gonadal and muscle indices .....	25
PROTOCOL-1 – Determining gonadal and muscle indices .....	25
1.3.2 Maintenace and conditioning of broodstock .....	27
1.3.2.1 The sand scallop, <i>Euvola (Pecten) ziczac</i> .....	28
PROTOCOL-2 – Collecting and holding of sand scallop broodstock ..	28
1.3.2.2 The calico scallop, <i>Argopecten gibbus</i> .....	28
PROTOCOL-3 – Conditioning of calico scallop broodstock .....	29
1.3.3 Spawning induction of scallops .....	30
PROTOCOL-4 – Spawning induction .....	33

## Chapter 2 – Algal cultures: facilities and techniques

<b>2.1 ALGAL CULTURE FACILITIES .....</b>	<b>37</b>
2.1.1 Algal culture container .....	38

---

2.1.1.1 <i>Elevation and connection to outside</i> .....	38
2.1.1.2 <i>Floor plan</i> .....	38
2.1.1.3 <i>Ceiling plan</i> .....	41
2.1.1.4 <i>Details of air supply and 100 l culture vessels</i> .....	41
2.1.2 Chamber unit for master cultures .....	43
<b>2.2 SCIENTIFIC BACKGROUND – LIVE ALGAE AS FOOD</b> .....	43
2.2.1 Algal growth and composition .....	45
2.2.1.1 <i>Selecting algal species</i> .....	45
2.2.1.2 <i>Requirements for algal cultures</i> .....	47
<b>2.3 TECHNIQUES – GROWING ALGAE</b> .....	48
2.3.1 Master cultures .....	48
PROTOCOL-5 – Preparation of culture flasks (125 ml – 500 ml) .....	49
PROTOCOL-6 – Inoculation of 125 ml master cultures .....	50
2.3.2 500 ml batch cultures .....	52
2.3.3 4 litres batch cultures .....	52
PROTOCOL-7 – Inoculation of 500 ml flasks .....	53
PROTOCOL-8 – Inoculation of 4 litres flasks .....	54
2.3.4 100 litres cultures: semi-continuous method .....	55
PROTOCOL-9 – Inoculation and semi-continuous culture of 100 litres vessels .....	56
2.3.5 Monitoring of algal cultures .....	57
PROTOCOL-10 – Estimating cell density using a haemocytometer cell .....	57
2.3.6 Alternate feed for spat .....	58

### **Chapter 3 – Hatchery: facilities and techniques for larval culture**

<b>3.1 HATCHERY FACILITIES</b> .....	59
3.1.1 Larval tanks .....	61
<b>3.2 SCIENTIFIC BACKGROUND – EMBRYONIC AND LARVAL DEVELOPMENT</b> .....	62
3.2.1 Embryonic development to D-larvae .....	62
3.2.1.1 <i>Fertilized eggs: characteristics and developmental requirements</i> ...	64
3.2.2 Larval development .....	66
3.2.2.1 <i>Veliger larvae</i> .....	66
3.2.2.2 <i>Pediveliger larvae</i> .....	66
<b>3.3 SCIENTIFIC BACKGROUND – FACTORS INFLUENCING LARVAL REARING</b> .....	68
3.3.1 Temperature .....	68
3.3.2 Density .....	69
3.3.3 Salinity .....	69
3.3.4 Food ration .....	70
3.3.4.1 <i>Effect of food ration on calico scallop larvae</i> .....	71
3.3.5 Culture systems: flow-through vs. static .....	72
<b>3.4 TECHNIQUES – STANDARD PROTOCOL FOR REARING CALICO AND ZIGZAG SCALLOP LARVAE</b> .....	74
3.4.1 Larval rearing procedure .....	76
3.4.1.1 <i>Water change</i> .....	76
PROTOCOL-11 – Take-down of larval tanks: larval collection and re-distribution .....	77
3.4.1.2 <i>Standard rearing density</i> .....	78
3.4.1.3 <i>Standard food ration</i> .....	79

3.4.1.4 Counting larvae and determining survival rate and shell growth ...	79
3.4.1.5 Setting of larvae .....	80

## Chapter 4 – Nursery: facilities and culture of post-larvae

<b>4.1 NURSERY FACILITIES .....</b>	<b>83</b>
4.1.1 Semi-recirculating raceway system (indoor) .....	84
4.1.1.1 Details of sump tank .....	86
4.1.2 Outdoor raceway .....	86
4.1.2.1 Seawater supply to outdoor raceway .....	89
4.1.2.2 Sieve layout .....	92
4.1.2.3 Outdoor raceway elevations and algal supply .....	92
4.1.3 Circular tanks .....	95
<b>4.2 SCIENTIFIC BACKGROUND – SETTLEMENT AND METAMORPHOSIS .....</b>	<b>97</b>
4.2.1 Factors affecting settlement and metamorphosis .....	99
<b>4.3 SCIENTIFIC BACKGROUND – POST-LARVAL DEVELOPMENT .....</b>	<b>100</b>
<b>4.4 TECHNIQUES – SETTING SYSTEMS AND PROTOCOLS .....</b>	<b>101</b>
4.4.1 Calico and zigzag scallop settlement .....	101
4.4.1.1 Rapid transfer approach .....	102
PROTOCOL-12 – Set of mature larvae in 450 litres tanks – rapid transfer approach .....	104
4.4.1.2 Setting density for raceway system .....	105
4.4.1.3 Raceway set .....	105
PROTOCOL-13 – Setting mature larvae in raceway – maintenance and care .....	106
<b>4.5 TECHNIQUES – POST-LARVAL REARING REQUIREMENTS .....</b>	<b>107</b>
4.5.1 Food ration for spat .....	107
4.5.1.1 Standard food ration protocol for calico and zigzag scallops .....	108
4.5.2 Strategy for efficient use of space in rearing spat .....	108
4.5.2.1 Characteristics of outdoor raceway .....	109
4.5.2.2 Density effect on spat growth .....	110
4.5.3 Raceway weekly maintenance .....	110
PROTOCOL-14 – Rearing spat in outdoor raceway .....	111
4.5.3.1 Maintaining a critical biomass .....	112
PROTOCOL-15 – Weighing and counting of spat for thinning and grading .....	113
4.5.4 Shell growth of calico and zigzag scallop spat .....	114

## Chapter 5 – Growout of juveniles: transfer from nursery to field

<b>5.1 HOLDING AND GROWOUT FACILITIES .....</b>	<b>117</b>
5.1.1 Exterior holding tanks .....	117
5.1.2 Longlines .....	119
5.1.3 Bottom cages .....	120
<b>5.2 TECHNIQUES – TRANSFER OF SPAT FROM NURSERY TO FIELD .....</b>	<b>121</b>
5.2.1 Transfer of 1.5 mm spat from 450 litres tank set .....	121
PROTOCOL-16 – Transfer and retrieval of spat on cultch to field .....	122
5.2.2 Transfer of 2–4 mm spat from raceway to longlines .....	123
5.2.3 Transfer of spat >4 mm .....	123

---

<b>5.3 TECHNIQUES – GROWOUT OF JUVENILES .....</b>	<b>124</b>
5.3.1 Calico scallop growout .....	124
5.3.2 Zigzag scallop growout .....	125
<b>5.4 TECHNIQUES – TRANSPORT OF JUVENILES .....</b>	<b>126</b>
PROTOCOL-17 – Procedure for long transport periods of juvenile scallops .....	127
 <b>Chapter 6 – Economic considerations: costs of set-up and labour requirements</b>	
<b>6.1 SET-UP COSTS OF A MODULAR HATCHERY .....</b>	<b>129</b>
<b>6.2 OPERATIONAL LABOUR REQUIREMENT .....</b>	<b>130</b>
<b>6.3 FINAL PRODUCT .....</b>	<b>131</b>
 <b>Appendices .....</b>	<b>133</b>
 <b>Literature cited .....</b>	<b>167</b>

# List of figures

<b>Figure 1.1:</b> Photograph of a live <i>E. ziczac</i> , the sand scallop or zigzag scallop .....	21
<b>Figure 1.2:</b> Map indicating the distribution of the calico scallop, <i>A. gibbus</i> .....	21
<b>Figure 1.3:</b> Generalized diagram of a pectinid (taken from Bourne, Hodgson and Whyte, 1989) alongside an open calico scallop specimen showing major organs ..	22
<b>Figure 1.4:</b> Calico scallop, <i>A. gibbus</i> , showing gonads with both mature ovaries (bright orange) and sperm (white) .....	23
<b>Figure 1.5:</b> Gonadal indices and muscle indices for calico scallop, <i>A. gibbus</i> in Bermuda waters .....	23
<b>Figure 1.6:</b> Reproductive patterns in cultured <i>A. gibbus</i> from Bermuda .....	23
<b>Figure 1.7:</b> Generalized life history of a pectinid (taken from Bourne, Hodgson and Whyte, 1989) .....	24
<b>Figure 1.8:</b> Spawning attempts with calico scallops, <i>A. gibbus</i> , collected from the growout sites .....	31
<b>Figure 1.9:</b> Isolating sand scallops, <i>E. ziczac</i> , once gamete release is initiated .....	31
<b>Figure 1.10:</b> Sequence of events following fertilization of <i>E. ziczac</i> eggs .....	32
<b>Figure 1.11:</b> Warm water bath set-up for spawning induction of scallops .....	34
<b>Figure 1.12:</b> Measuring eggs or larvae on a Sedgewick-Rafter cell .....	35
<b>Figure 2.1:</b> Theoretical growth curve of typical algal culture showing lag, exponential and stationary phase (taken from Bourne, Hodgson and Whyte, 1989) .....	45
<b>Figure 3.1:</b> Developmental changes of sand scallop larvae to metamorphosis .....	62
<b>Figure 3.2:</b> One-day old <i>E. ziczac</i> veligers showing extended velum .....	63
<b>Figure 3.3:</b> Straight-hinge or D-larvae stage of <i>E. ziczac</i> .....	64
<b>Figure 3.4:</b> Day-6 sand scallop larvae showing initial development of umbones (Um) compared to straight-hinge characteristic (SH) of D-larvae .....	67
<b>Figure 3.5:</b> Day-11 pediveligers of <i>E. ziczac</i> showing eyespot and a well-developed foot in and out of the shell .....	67
<b>Figure 3.6:</b> Conical tank modified to a flow-through system for larval rearing .....	73
<b>Figure 3.7:</b> Results of shell growth (length) for calico scallop larvae reared in Bermuda. Two curves show maximal and minimal range obtained over 4 years of operation .....	75
<b>Figure 3.8:</b> Shell growth (length) for zigzag scallop larvae reared in Bermuda. Two growth curves show maximal and minimal length obtained over 4 years of operation ....	75
<b>Figure 4.1:</b> Development of sand scallop, <i>E. ziczac</i> , following settlement, showing dissoconch in Day-8 scallops, byssal notch formation and pigmentation in Day-23 scallops and similarity to adults in 2 months old scallops .....	101
<b>Figure 4.2:</b> Cultch made of 3 mm black polyethylene mesh filling 450 litres tanks used for set .....	102
<b>Figure 4.3:</b> Evaluation of calico scallop, <i>A. gibbus</i> , set in 450 litres tanks .....	103
<b>Figure 4.4:</b> Mature larvae set on meshed sieve suspended as downwelling system in raceway .....	106
<b>Figure 4.5:</b> Weighing spat on a Sartorius balance ( $\pm 0.01$ gram). ....	113
<b>Figure 4.6:</b> Shell height for calico scallop, <i>A. gibbus</i> , spat reared in raceway system .....	115
<b>Figure 4.7:</b> Shell growth (height) of the zigzag scallop, <i>E. ziczac</i> , following settlement .....	115

<b>Figure 4.8:</b> Survival rate of calico scallop, <i>A. gibbus</i> , post-larvae following settlement. Survival rate is calculated from number of larvae set .....	115
<b>Figure 4.9:</b> Wet weight of calico scallop, <i>A. gibbus</i> , spat (gram per spat) as determined during the nursery stage .....	116
<b>Figure 5.1:</b> Sub-surface longline system used in Bermuda .....	119
<b>Figure 5.2:</b> Schematic diagram of bottom cages made of rebar and plastic mesh used for protection of sand scallop ( <i>Euvola ziczac</i> ) juveniles and adults. Fouling on meshed lid shown close-up, necessitating retrieval of cages from field. ....	120
<b>Figure 5.3:</b> Transfer of one month old calico scallop spat in green collector bags for transfer to growout trays .....	121
<b>Figure 5.4:</b> Spat pouches held in seawater prior to transfer to the field .....	121
<b>Figure 5.5:</b> Securing of pouches into trays for growout in the field on longlines .....	122
<b>Figure 5.6:</b> Transferring 2–4 mm spat into fly-screen pouches used as inserts for growout trays .....	123
<b>Figure 5.7:</b> Spat reared in raceway until 4–9 mm shell height and ready for direct transfer into pearl nets for growout. Pearl nets shown suspended from experimental system .....	124
<b>Figure 5.8:</b> Shell growth of calico scallop, <i>A. gibbus</i> , juveniles reared on longlines in Bermuda .....	125
<b>Figure 5.9:</b> Adult zigzag scallops, <i>E. ziczac</i> , showing natural recessive behaviour in the sand .....	125
<b>Figure 5.10:</b> Comparative growth between zigzag scallops cultured directly on the sandy bottom and suspended in pearl nets .....	126
<b>Figure 6.1:</b> Zigzag and calico scallops (adults and juveniles) ready for market and sold fresh to restaurants in Bermuda .....	131

# List of tables

<b>Table 2.1:</b>	Commonly used species of micro algae in bivalve hatcheries .....	47
<b>Table 3.1:</b>	Yields of Day-2 larvae obtained for several hatchery seasons following controlled fertilization of <i>E. ziczac</i> and <i>A. gibbus</i> in the hatchery. Ranges shown indicate yields obtained for all larval tanks in one spawning. Single numbers indicate mean yield for one spawning .....	65
<b>Table 3.2:</b>	Daily growth rate for both zigzag and calico scallop larvae reared in Bermuda. Results are shown for two larval batches, one illustrating minimal shell growth and the other illustrating maximal shell growth .....	75
<b>Table 3.3:</b>	Relationship between mesh size and larval size retained on it as well as mesh sizes used specifically for the sand scallop and the calico scallop throughout larval development .....	77
<b>Table 3.4:</b>	Larval densities in rearing tanks throughout larval life during a typical hatchery cycle .....	79
<b>Table 3.5:</b>	Food ration and composition used in rearing of calico and zigzag scallop larvae ..	79
<b>Table 4.1:</b>	Standard food ration for rearing of calico and zigzag spat .....	108
<b>Table 4.2:</b>	Relationship between density tested and number of spat per sieve .....	110
<b>Table 4.3:</b>	Aperture size, measured diagonally, for each mesh used in grading of spat .....	112
<b>Table 4.4:</b>	Procedure for maintenance of biomass in raceway system and transfer to outdoor raceway. Sieve size for indoor ( $532 \text{ cm}^2$ ) with 150 or 120 $\mu\text{m}$ mesh. Sieve size for outdoor ( $696 \text{ cm}^2$ ) with 1.2 mm (green) mesh .....	112
<b>Table 4.5:</b>	Ambient temperature recorded in raceway systems in Bermuda during post-larval growth. Range represents monthly change .....	114
<b>Table 6.1:</b>	Summary of the set-up costs for the hatchery/nursery complex. Set-up costs do not include shipping, PVC pipe/connections, construction materials and electrical components. An estimated figure is given separately for PVC parts based on expenses made in Bermuda .....	130
<b>Table 6.2:</b>	Operational costs as a percentage of time for full time aquaculture activities from spawning to growout .....	131

# List of protocols

PROTOCOL-1 – Determining gonadal and muscle indices .....	25
PROTOCOL-2 – Collecting and holding of sand scallop broodstock .....	28
PROTOCOL-3 – Conditioning of calico scallop broodstock .....	29
PROTOCOL-4 – Spawning induction .....	33
PROTOCOL-5 – Preparation of culture flasks (125 ml – 500 ml) .....	49
PROTOCOL-6 – Inoculation of 125 ml master cultures .....	50
PROTOCOL-7 – Inoculation of 500 ml flasks .....	53
PROTOCOL-8 – Inoculation of 4 litres flasks .....	54
PROTOCOL-9 – Inoculation and semi-continuous culture of 100 litres vessels .....	56
PROTOCOL-10 – Estimating cell density using a haemocytometer cell .....	57
PROTOCOL-11 – Take-down of larval tanks: larval collection and re-distribution .....	77
PROTOCOL-12 – Set of mature larvae in 450 litres tanks – rapid transfer approach .....	104
PROTOCOL-13 – Setting mature larvae in raceway – maintenance and care .....	106
PROTOCOL-14 – Rearing spat in outdoor raceway .....	111
PROTOCOL-15 – Weighing and counting of spat for thinning and grading .....	113
PROTOCOL-16 – Transfer and retrieval of spat on cultch to field .....	122
PROTOCOL-17 – Procedure for long transport periods of juvenile scallops .....	127

# List of technical drawings

<b>Technical drawing, Pg. 1*</b> :	Master layout plan .....	3
<b>Technical drawing, Pg. 2:</b>	Primary seawater supply .....	5
<b>Technical drawing, Pg. 3:</b>	Secondary seawater supply .....	7
<b>Technical drawing, Pg. 4:</b>	Primary seawater supply to hatchery complex .....	9
<b>Technical drawing, Pg. 5A:</b>	Seawater heating unit .....	11
<b>Technical drawing, Pg. 5B:</b>	Seawater heating unit: Photographs .....	12
<b>Technical drawing, Pg. 6A:</b>	Container layout plan .....	14
<b>Technical drawing, Pg. 6B:</b>	Hatchery and nursery: Photographs .....	15
<b>Technical drawing, Pg. 7:</b>	Hatchery and nursery: Ceiling plan .....	17
<b>Technical drawing, Pg. 8:</b>	Broodstock: Tank and seawater supply .....	19
<b>Technical drawing, Pg. 9:</b>	Algal culture unit: Elevation and connection to outside .....	39
<b>Technical drawing, Pg. 10:</b>	Algal culture unit: Floor plan .....	40
<b>Technical drawing, Pg. 11:</b>	Algal culture unit: Ceiling plan .....	42
<b>Technical drawing, Pg. 12:</b>	Algal culture unit: Details and photos .....	44
<b>Technical drawing, Pg. 13:</b>	Hatchery: Larval tank section and detail .....	60
<b>Technical drawing, Pg. 14:</b>	Nursery: Semi-recirculating raceway system section .....	85
<b>Technical drawing, Pg. 15A:</b>	Nursery: Sump tank detail .....	87
<b>Technical drawing, Pg. 15B:</b>	Nursery: Semi-recirculating system photographs .....	88
<b>Technical drawing, Pg. 16A:</b>	Outdoor raceway: Plan view and photos .....	90
<b>Technical drawing, Pg. 16B:</b>	Outdoor raceway: Details and photographs .....	91
<b>Technical drawing, Pg. 17:</b>	Outdoor raceway and algal supply elevations .....	93
<b>Technical drawing, Pg. 18:</b>	Outdoor raceway: Algal and seawater supply photographs .....	94
<b>Technical drawing, Pg. 19A:</b>	Circular tanks: Plan and elevation .....	96
<b>Technical drawing, Pg. 19B:</b>	Circular tanks: Open and closed system photographs .....	98
<b>Technical drawing, Pg. 20:</b>	Exterior holding tanks: Plan and elevation .....	118

\* "Pg" refers to the number of the technical drawing.

## Technical drawing notes and pipe conversion

### Please note:

- 1) All valves, pipes and other fittings are of PVC (Schedule 40) unless otherwise specified in drawings.
- 2) The subtropical scallop culture model for hatchery and nursery facilities represents an existing complex in Bermuda, built in accordance with the Bermuda environmental regulations. Installation in other countries is subject to local federal and regional environmental regulations and building code.

---

3) All drawings are metric. Please refer to chart below for imperial conversions.

**Nominal NPT pipe size to nominal  
metric size conversion chart**

Nominal pipe size (Inches/U.S.)	Nominal pipe size (Metric)
1/8"	6 mm
3/16"	7 mm
1/4"	8 mm
3/8"	10 mm
1/2"	15 mm
5/8"	18 mm
3/4"	20 mm
1"	25 mm
1-1/4"	32 mm
1-1/2"	40 mm
2"	50 mm
2-1/2"	65 mm
3"	80 mm
3-1/2"	90 mm
4"	100 mm
4-1/2"	115 mm
5"	125 mm
6"	150 mm

# List of appendixes

<b>Appendix 1:</b> Description of oocyte developmental stages .....	134
<b>Appendix 2:</b> Sample data sheet for gonadal and muscle indices .....	135
<b>Appendix 3:</b> Broodstock check sheet .....	136
<b>Appendix 4:</b> Heating unit set-up and take-down .....	137
<b>Appendix 5:</b> Maintenance and cleaning of seawater system .....	139
<b>Appendix 6:</b> Pump room log .....	140
<b>Appendix 7:</b> Cleaning hatchery after spawning .....	141
<b>Appendix 8:</b> Details of materials .....	142
<b>Appendix 9:</b> Preparation of culture media .....	143
<b>Appendix 10:</b> Chemical sterilization procedure .....	146
<b>Appendix 11:</b> Set-up and take-down of seawater supply in algae container .....	147
<b>Appendix 12:</b> Bactopeptone test .....	149
<b>Appendix 13:</b> Algal culture check list .....	150
<b>Appendix 14:</b> Haemocytometer cell diagram .....	151
<b>Appendix 15:</b> Larval check sheet .....	152
<b>Appendix 16:</b> Determination of dry weight and ash-free dry weight .....	153
<b>Appendix 17:</b> Sieve construction for larval and post-larval collection .....	154
<b>Appendix 18:</b> Raceway check list .....	155
<b>Appendix 19:</b> Cleaning of raceway .....	156
<b>Appendix 20:</b> Counting grid for spat .....	157
<b>Appendix 21:</b> Preparation and ration for dry algae .....	158
<b>Appendix 22:</b> List of equipment: template for costing out set-up of modular hatchery .....	159
<b>Appendix 23:</b> List of selected suppliers .....	166

# Glossary

<b>Adductor muscle</b>	large muscle near centre of scallop that pulls the two valves together
<b>Algae</b>	aquatic plants that reproduce by cell division or spores
<b>Anoxia</b>	deficiency or absence of oxygen in the blood and tissues
<b>Anterior</b>	front or head
<b>Banjo filters</b>	in hatchery terminology, a ring meshed on both sides affixed to the outflow of a tank preventing larval loss through drain
<b>Blastula</b>	a hollow ball of cells, one of the early stages of embryonic development
<b>Bivalve</b>	mollusc of the Class Pelecypoda, having a shell of two valves that are joined by a hinge
<b>Byssus</b>	thread-like filaments used by bivalves to attach themselves to a substrate
<b>Cilia</b>	hair-like structures whose rhythmic beat induces a water current in bivalves
<b>Cleavage</b>	the series of mitotic divisions, usually occurring with no increase in cytoplasmic mass that first transforms the single-celled zygote into a multicellular blastula
<b>Ctenidia</b>	leaf-like appendages that function in respiration and filtration of food from water (used interchangeably with the term gills)
<b>Cultch</b>	material used to collect bivalve spat
<b>Detritus</b>	fragmented or decomposing organic material from plant and animal remains
<b>Diatom</b>	a single-celled algae of the Class Bacillariophyceae; cells are enclosed in a siliceous shell called a frustule, cells can form chains
<b>D-larva</b>	the early veliger larval stage of bivalves, also known as straight-hinge larva
<b>Dribble spawners</b>	in this case used for scallops which do not spawn completely, but partially over a period of time
<b>Dorsal</b>	the back or part of an organism away from the ground
<b>Downwelling</b>	in hatchery terminology, a growing system in which the flow of water enters at the top of a spat holding container (compare with upwelling)
<b>Ectometabolites</b>	a product of metabolism
<b>Embryo</b>	organism in early stages of development; in bivalves, prior to larval stage
<b>Epiphytes</b>	animals or plants living on the surface of the seabed or other substratum
<b>Eye spot</b>	simple organ that develops near centre of mature larvae of some bivalves and is sensitive to light

---

<b>Fecundity</b>	the potential reproductive capacity of an organism or population expressed in the number of eggs (or offsprings) produced during each reproductive cycle
<b>Fertilization</b>	union of egg and sperm
<b>Flagellate</b>	group of single-celled algae characterized by having a locomotory organ called a flagellum
<b>Follicle</b>	small sac-like structure in the ovary, a group of cells surrounding the oocyte and probably concerned with its nutrition
<b>Gamete</b>	mature, haploid, functional sex cell capable of uniting with the alternate sex cell to form a zygote
<b>Gametogenesis</b>	process by which eggs and sperm are produced
<b>Gastrula</b>	the embryonic stage of development consisting of two layers of cells enclosing a sac-like central cavity with a pore at one end
<b>Gill</b>	a leaf-like appendage that functions in respiration and filtration of food from water (see ctendia)
<b>Gonadal Index</b>	in this case the relationship of gonad weight to shell weight, reflecting gonad growth or depletion
<b>Gonads</b>	the primary sexual organ: testis producing sperm or ovary producing eggs
<b>Growout</b>	the process of growing seed to market size
<b>Hermaphrodite</b>	having both male and female reproductive organs in the same individual (animal)
<b>Hypoxia</b>	insufficient levels of oxygen in blood or tissue (short of anoxia)
<b>Inoculum</b>	culture of an organism (alga, rotifer), which is used as a starting point for another culture
<b>Larva</b>	a stage of bivalves from the embryo to metamorphosis
<b>Mantle</b>	the soft fold enclosing the body of a bivalve which secretes the shell
<b>Meiotic division</b>	process in which normal number of chromosomes ( $2n$ ) is reduced to the haploid ( $n$ ) number
<b>Metamorphosis</b>	in bivalves, the period of transformation from the larval to the juvenile stage
<b>Microalgae</b>	small cell-size algae, either single celled or chain forming diatoms, cultured as foods for larvae and spat in a hatchery
<b>Muscle Index</b>	in this case, the relationship of muscle weight to shell weight, reflecting muscle growth or depletion
<b>Oocyte</b>	Cell, which develops into an ovum
<b>Ovary</b>	the sex organ which produces the egg or eggs in a female organism
<b>Pediveliger</b>	Larval stage of molluscs that still has the swimming ciliated organ (velum) and sensitive foot needed for settlement and attachment
<b>pH</b>	a measure of acidity
<b>Plankton</b>	floating or weakly swimming aquatic organisms, can be phytoplankton (plants) or zooplankton (animals)

---

<b>Polar body</b>	minute cells released during meiotic division of the egg after the sperm has penetrated the egg; contains excess chromosomal material to produce a haploid egg
<b>Posterior</b>	the rear, away from the head
<b>Primary oogonia</b>	arising from primordial germ cells during the initial (premeiotic) stage of oocyte development, and differentiates into an oocyte in the ovary
<b>Prodissococonch</b>	Bivalved shell formed by larva prior to metamorphosis. It may be possible to distinguish an earlier, smaller prodissococonch-I from a later, larger prodissococonch-II that encloses the entire animal
<b>Pseudofaeces</b>	false faeces, waste material not taken into the digestive tract
<b>Seed</b>	a young scallop with no specific definition to size
<b>Settlement</b>	behavioural process when mature bivalve larvae seek a suitable substrate for attachment
<b>Shell height</b>	in scallops, the straight line distance measured perpendicularly from the umbo to the ventral margin of the shell
<b>Shell length</b>	in scallops, the straight line distance from the anterior to the posterior margins of the shell
<b>Spat</b>	a newly settled or attached bivalve (also termed post larval or juvenile in bivalves)
<b>Spatfall</b>	the settling or attachment of young bivalve molluscs, which have completed their larval stages
<b>Spawning</b>	release of ova, fertilized or to be fertilized
<b>Statocyst</b>	formed by invagination of the epithelium in bivalve larvae potentially providing the ability to detect gravity
<b>Straight-hinge larva</b>	early part of larval stage, sometimes termed D-stage
<b>Testis</b>	male reproductive organ
<b>Trochophore</b>	the first free-swimming planktonic stage of a mollusc larvae or bivalve embryo
<b>Umbo</b>	beak-like projections at the dorsal part of the shell; it is the oldest part of a bivalve shell (also called the umbone)
<b>Upwelling</b>	in hatchery terminology, a growing system in which a flow of water is induced through the base of a spat holding container (compare with downwelling)
<b>Veliger larva</b>	the larval stage of most molluscs, characterized by the presence of a velum
<b>Velum</b>	ciliated locomotory organ of the larva
<b>Ventral</b>	pertaining to the under or lower side of an animal
<b>Vitellogenesis</b>	formation of the yolk of an egg
<b>Zygote</b>	diploid cell resulting from union of male and female gametes

# Abbreviations, acronyms and conversions

<b>AFDW</b>	Ash-Free Dry Weight
<b>ANOVA</b>	Analysis Of Variance
<b>BBSR</b>	Bermuda Biological Station for Research Inc.
<b>C</b>	Control
<b>CCMP</b>	Centre of Culture for Marine Phytoplankton
<b>CI</b>	Condition Index
<b>CO<sub>2</sub></b>	Carbon Dioxide
<b>DOPA</b>	Dihydroxyphenylalanine
<b>DW</b>	Dry Weight
<b>EDTA</b>	Ethylene Diamine Tetraacetic Acid
<b>Fisher's PSLD</b>	Fisher's Protected Least Significant Difference
<b>FT</b>	Flow-Through
<b>GF/C</b>	Glass Fibre with particle retention of 1.2µm
<b>GI</b>	Gonadal Index
<b>HCL</b>	Hydrochloric Acid
<b>ID</b>	Inner Diameter
<b>int-ext</b>	Interior-Exterior Connection
<b>LNSW</b>	Low Nutrient Seawater
<b>MI</b>	Muscle Index
<b>NPT</b>	National Pipe Thread
<b>MNPT</b>	Male National Pipe Thread
<b>No</b>	Number
<b>OD</b>	Outer Diameter
<b>PLSD</b>	Protected Least Significant Difference
<b>pers.obs.</b>	Personal Observation
<b>PPT</b>	Parts Per Thousand
<b>PUFA</b>	Polyunsaturated Fatty Acid
<b>PVC</b>	Polyvinyl Chloride
<b>Q-water</b>	De-ionised Water
<b>SA</b>	Surface Area
<b>SCUBA</b>	Self-Contained Underwater Breathing Apparatus
<b>SD</b>	Standard Deviation
<b>TFS</b>	Typical Filtration System
<b>USD</b>	United States Dollar
<b>UV</b>	Ultra-Violet

Not all of the following abbreviations have been used in this manual. However, they are provided as reference when reading other documents.

<	less than
>	greater than
n.a.	not analysed or not available (also written as N/A)
µm	micron
mm	millimetre
cm	centimetre
m	metre
km	kilometre

---

<b>inch</b>	inch
<b>ft</b>	foot
<b>yd</b>	yard
<b>mi</b>	mile
<b>ft<sup>2</sup></b>	square foot
<b>yd<sup>2</sup></b>	square yard
<b>mi<sup>2</sup></b>	square mile
<b>m<sup>2</sup></b>	square metre
<b>ha</b>	hectare
<b>km<sup>2</sup></b>	square kilometre
<b>cc</b>	cubic centimetre (= ml)
<b>m<sup>3</sup></b>	cubic metre
<b>ft<sup>3</sup></b>	cubic foot
<b>yd<sup>3</sup></b>	cubic yard
<b>µl</b>	microlitre
<b>ml</b>	millilitre (= cc)
<b>l</b>	litre
<b>µg</b>	microgram
<b>mg</b>	milligram (milligramme)
<b>g</b>	gram (gramme)
<b>kg</b>	kilogram (kilogramme)
<b>mt</b>	metric tonne (1 000 kg) (also written as tonne)
<b>oz</b>	ounce
<b>lb</b>	pound
<b>cwt</b>	hundredweight [value differs in UK ('Imperial') and US units - see weight conversions]
<b>t</b>	ton [value differs in UK ('Imperial') and US units - see weight conversions]
<b>psi</b>	pounds per square inch
<b>psu</b>	practical salinity units
<b>gpm</b>	('Imperial' = UK) gallons per minute
<b>mgd</b>	million ('Imperial' = UK) gallons per day
<b>cfm</b>	cubic feet per minute
<b>ppt</b>	parts per thousand (also written as %)
<b>ppm</b>	parts per million
<b>ppb</b>	parts per billion (thousand million)
<b>min</b>	minute
<b>hr</b>	hour
<b>kWhr</b>	kilowatt-hour

---

## Conversions

This section of the annex should be used in conjunction with the abbreviations section. Please note that the words gallon and tonne have different values depending on whether the source of the text you are reading is 'British' or 'American' in origin.

### Length:

<b>1 µm</b>	0.001 mm = 0.000001 m
<b>1 mm</b>	0.001 m = 1 000 µm = 0.0394 inch
<b>1 cm</b>	0.01 m = 10 mm = 0.394 inch
<b>1 m</b>	1 000 000 µm = 1 000 mm = 100 cm = 0.001 km = 39.4 inch = 3.28 ft = 1.093 yd
<b>1 km</b>	1 000 m = 1 093 yd = 0.621 mi
<b>1 inch</b>	25.38 mm = 2.54 cm

---

<b>1 ft</b>	12 inch = 0.305 m
<b>1 yd</b>	3 ft = 0.914 m
<b>1 mi</b>	1 760 yd = 1.609 km

**Weight:**

<b>1 µg</b>	0.001 mg = 0.000001 g
<b>1 mg</b>	0.001 g = 1 000 µg
<b>1 g</b>	1 000 000 µg = 1 000 mg = 0.001 kg = 0.0353 oz
<b>1 kg</b>	1 000 g = 2.205 lb
<b>1 mt</b>	1 000 kg = 1 000 000 g = 0.9842 UK t = 1.102 US t
<b>1 oz</b>	28.349 g
<b>1 lb</b>	16 oz = 453.59 g
<b>1 UK cwt</b>	112 lb = 50.80 kg
<b>1 US cwt</b>	100 lb = 45.36 kg
<b>1 UK t</b>	20 UK cwt = 2 240 lb
<b>1 US t</b>	20 US cwt = 2 000 lb
<b>1 UK t</b>	1.016 mt = 1.12 US t

**Volume:**

<b>1 µl</b>	0.001 ml = 0.000001 l
<b>1 ml</b>	0.001 l = 1 000 µl = 1 cc
<b>1 L</b>	1 000 000 µl = 1 000 ml = 0.220 UK gallon = 0.264 US gallon
<b>1 m<sup>3</sup></b>	1 000 l = 35.315 ft <sup>3</sup> = 1.308 yd <sup>3</sup> = 219.97 UK gallons = 264.16 US gallons
<b>1 ft<sup>3</sup></b>	0.02832 m <sup>3</sup> = 6.229 UK gallons = 28.316 l
<b>1 UK gallon</b>	4.546 l = 1.2009 US gallons
<b>1 US gallon</b>	3.785 l = 0.833 UK gallon
<b>1 MGD</b>	694.44 GPM = 3.157 m <sup>3</sup> /min = 3 157 l/min

**Concentration – dissolving solids in liquids:**

<b>1 %</b>	1 g in 100 ml
<b>1 ppt</b>	1 g in 1 000 ml = 1 g in 1 l = 1 g/l = 0.1%
<b>1 ppm</b>	1 g in 1 000 000 ml = 1 g in 1 000 L = 1 mg/l = 1 µg/g
<b>1 ppb</b>	1 g in 1 000 000 000 ml = 1 g in 1 000 000 l = 0.001 ppm = 0.001 mg/l

**Concentration – dilution of liquids in liquids:**

<b>1 %</b>	1 ml in 100 ml
<b>1 ppt</b>	1 ml in 1 000 ml = 1 ml in 1 l = 1 ml/l = 0.1%
<b>1 ppm</b>	1 ml in 1 000 000 ml = 1 ml in 1 000 l = 1 µl/l
<b>1 ppb</b>	1 ml in 1 000 000 000 ml = 1 ml in 1 000 000 l = 0.001 ppm = 0.001 ml/l

**Area:**

<b>1 m<sup>2</sup></b>	10.764 ft <sup>2</sup> = 1.196 yd <sup>2</sup>
<b>1 ha</b>	10 000 m <sup>2</sup> = 100 ares = 2.471 acres
<b>1 km<sup>2</sup></b>	100 ha = 0.386 mi <sup>2</sup>
<b>1 ft<sup>2</sup></b>	0.0929 m <sup>2</sup>
<b>1 yd<sup>2</sup></b>	9 ft <sup>2</sup> = 0.836 m <sup>2</sup>
<b>1 acre</b>	4 840 yd <sup>2</sup> = 0.405 ha
<b>1 mi<sup>2</sup></b>	640 acres = 2.59 km <sup>2</sup>

**Temperature:**

<b>°F</b>	$(9 \div 5 \times ^\circ\text{C}) + 32$
<b>°C</b>	$(^\circ\text{F} - 32) \times 5 \div 9$

**Pressure:**

<b>1 psi</b>	70.307 g/cm <sup>2</sup>
--------------	--------------------------

## Scientific units

Scientists have a different way of writing some of the units described in this glossary. They use what is called the Système International (SI). The units are referred to as SI units. For example: 1 ppt, which can be written as 1 g/l (see concentration above) is written as 1 g l<sup>-1</sup> in scientific journals, 1 g/kg as 1 g kg<sup>-1</sup>, 12 mg/kg as 12 mg kg<sup>-1</sup>, and 95 µg/kg as 95 µg kg<sup>-1</sup>. A stocking density of 11 kg/m<sup>3</sup> would be written as 11 kg m<sup>-3</sup>. This SI system is not normally used in daily hatchery records, however for the purpose of standardization, it is used throughout this publication.

## Chapter 1

# Scallop broodstock: facilities, reproduction and spawning

<b>1.1 CULTURE FACILITIES .....</b>	<b>1</b>
1.1.1 Master layout plan .....	2
1.1.2 Seawater supply .....	4
1.1.2.1 Primary seawater supply .....	4
1.1.2.2 Secondary seawater supply – link to main intake line .....	6
1.1.2.3 Secondary seawater supply – link to secondary intake line .....	6
1.1.2.4 Main seawater supply to hatchery complex .....	8
1.1.3 Heating unit .....	8
1.1.4 Hatchery/broodstock/nursery complex .....	13
1.1.4.1 Container layout plan .....	13
1.1.4.2 Container plan diagram .....	13
1.1.4.3 Hatchery and nursery ceiling plan .....	16
1.1.4.4 Broodstock: tank and seawater supply .....	18
<b>1.2 SCIENTIFIC BACKGROUND – NATURAL HABITAT AND REPRODUCTIVE CYCLE ..</b>	<b>20</b>
1.2.1 Habitat .....	20
1.2.2 Reproductive cycle .....	22
1.2.2.1 The sand scallop, <i>Euvola (Pecten) ziczac</i> .....	22
1.2.2.2 The calico scallop, <i>Argopecten gibbus</i> .....	22
1.2.3 Life cycle .....	24
<b>1.3 TECHNIQUES – BROODSTOCK .....</b>	<b>25</b>
1.3.1 Gonadal and muscle indices .....	25
PROTOCOL-1 – Determining gonadal and muscle indices .....	25
1.3.2 Maintenance and conditioning of broodstock .....	27
1.3.2.1 The sand scallop, <i>Euvola (Pecten) ziczac</i> .....	28
PROTOCOL-2 – Collecting and holding of sand scallop broodstock ..	28
1.3.2.2 The calico scallop, <i>Argopecten gibbus</i> .....	28
PROTOCOL-3 – Conditioning of calico scallop broodstock ..	29
1.3.3 Spawning induction of scallops .....	30
PROTOCOL-4 – Spawning induction .....	33

## 1.1 CULTURE FACILITIES

The model hatchery custom-built for rearing of subtropical and tropical scallop species at the Bermuda Biological Station for Research Inc. (BBSR), is shown in great detail in the technical drawings (labelled Pg 1-20). This was done in order to provide a clear understanding of the requirements for such a culture operation, and facilitate building of a similar facility; thus, details of equipment utilized, pipe diameter, valves, etc. is provided. Although this facility was built for rearing scallops, it is a flexible design

making it easily adaptable to the culture of other bivalves, and even to that of certain gastropod species. It is not the intent of this guide to dwell into the engineering of such a hatchery, but to provide enough information to allow replication of this modular facility and to facilitate its costing. There are publications available which provide a wealth of information on various engineering aspects such as materials selection, seawater flow control, heating and cooling, etc.; a recommended book for answers to such questions is that of Huguenin and Colt (2002).

In the described modular hatchery, all piping materials used were made of rigid polyvinyl chloride (PVC) schedule 40, unless otherwise specified. It is one of the most common materials used in seawater culturing systems; it is biologically acceptable, as it is resistant to seawater, has a smooth surface minimizing the burrowing or fouling by marine organisms in the seawater, and does not leach any toxic compound. Schedule 40 piping has thinner walls and is sufficient for a seawater system, such as the one described here. PVC can be joined by solvent or flanging. It has been found in Bermuda that it can be prone to cracking overtime, especially when exposed to sunlight and associated higher temperatures. PVC piping is readily available, easy to work with and relatively affordable. Care must be taken when costing out PVC materials, as fittings can be significant to the overall expenses.

The modular hatchery was built in Bermuda according to environmental laws of the country. Because of its size and its strict use for filter-feeding organisms (bivalves), effluent water was low in pollutants, and discharge was not considered an issue. However, any replication of this facility in another country must be adjusted to the regulations of the country. Please note that throughout the text, the terms “excess outflow” and “effluent” are used interchangeably.

The facility was designed to be flexible in its use, and of easy maintenance. For this reason, unions are used throughout the facility before and after valves or equipment to facilitate routine cleaning and replacement of parts when required. Cleanliness of the seawater supply is primordial in rearing of the early life stages, and procedures used for maintaining various parts of the seawater system are provided as appendixes or protocols throughout this guide.

Culture facilities of the described hatchery comprise:

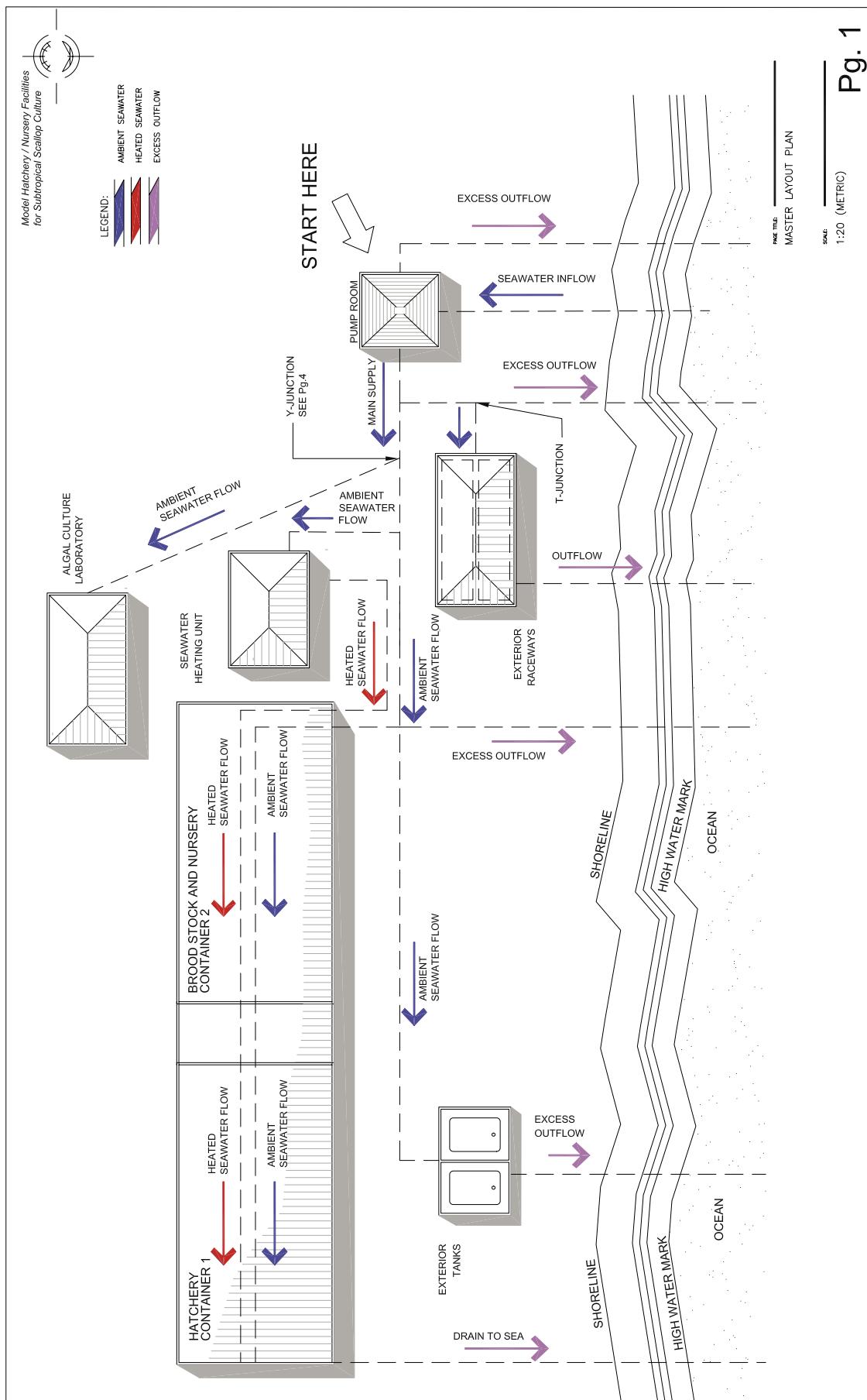
1. A dedicated seawater system providing a continuous supply of filtered seawater.
2. A temperature control system for seawater in support of broodstock conditioning and requirements for larval and juvenile rearing.
3. 1 000 litres insulated, polyethylene tanks for standard larval rearing in closed systems.
4. Flexible usage 450 litres round tanks, and stacked raceways for setting pediveligers and growing spat. Raceways may be connected either as, a single open-flow, a semi-recirculating system, two independent troughs, or holding tanks for a series of small, independent aquaria.

### **1.1.1 Master layout plan**

Refer to Technical Drawing – page 1. The entire facility is comprised of a hatchery/nursery/broodstock complex housed in two fiberglass insulated air-conditioned containers, a separate container for algal cultures, a heating unit housed in a wooden shed providing heated seawater to the hatchery/nursery facility, and an exterior tank system providing additional space for maintenance of broodstock and spat. The pump unit located on the shoreline pumps in raw seawater and supplies the entire facility.

## Technical drawing, Pg. 1

### Master layout plan



General seawater flow is shown, including main ambient supply, heated seawater supply, and outflow. For ease of understanding, these are colour coded blue for ambient seawater, red for heated and purple for outflow. This colour code remains the same for all the technical drawings. Raw seawater is pumped from a 5 m depth and supplied via a main line to the hatchery complex; this was the maximum depth from which it could be pumped at this location, and is not a guideline for optimal pumping. Seawater is diverted from the main line to various pathways through T-junctions and regulated by one-way ball valves. Branching off from the main line, are supply lines to the exterior raceways, the heating unit, a Y-junction to the algal culture container, to the hatchery complex, and finally to the exterior holding tanks. A number of pathways are provided to allow excess water to flow out of the system back to sea; the first is found at the pump room. (Note: Throughout most of the operation, seawater supply exceeds the demand and excess water pumped in is sent to drain). Other outflow pathways are provided for the exterior raceway system, the hatchery complex, and the exterior tanks. These pathways also enable the regulation of water flow to various tanks, by altering the volume of water retained within the supply lines.

All tank systems receive ambient seawater from the pump room. The hatchery complex also receives temperature-controlled seawater from the heating unit via a second pipeline.

### **1.1.2 Seawater supply**

The seawater system includes a primary pumping system with a main intake line (see technical drawing – page 2), a secondary pump linked to the main intake line (see technical drawing – page 3), and a secondary pumping system with a second smaller intake line linked to the secondary pump (see technical drawing – page 3). The pump house is housed within a 4 m<sup>2</sup> stone building with ventilation and a cement floor.

#### **1.1.2.1 Primary seawater supply**

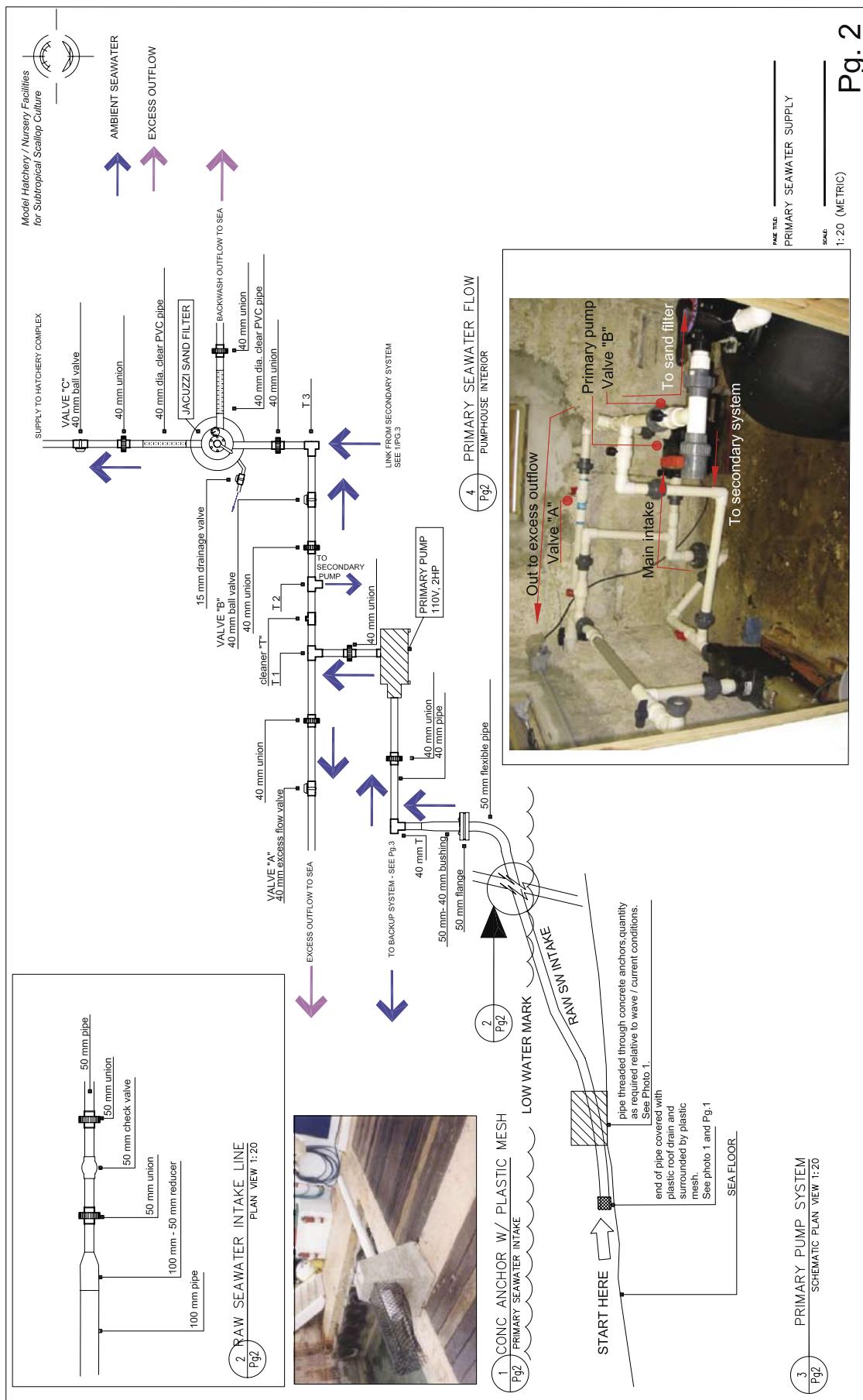
Refer to Technical Drawing – page 2. The main seawater intake is located at approximately 5 m depth. A 100 mm pipe is maintained in place by a 1 m high concrete anchor (1 m<sup>2</sup> base, tapering to 0.1 m<sup>2</sup> at top) (see technical drawing photo – 1/Pg2). The seawater intake is therefore approximately 1 m off the sea bottom; in areas where depth is greater, it is recommended to install the intake higher above the sea surface to avoid additional re-suspended sediments. A plastic roof drain is screwed into the end of the intake to prevent large objects and animals from being sucked in. In order to further ensure that the seawater pipe remains unclogged, a screen, made of 25 mm mesh, is tied with plastic cable around the intake. This mesh is changed once a year, prior to the beginning of the hatchery season.

The 100 mm intake line is weighted above the sea bottom with concrete blocks bridging the pipe. It is made up of 6.5 m pipe lengths glued together. Flange connections located every 30 m allow for dismantling of the pipe in case of blockages. Diagram 2/Pg2 (see technical drawing – page 2) shows details of the intake at the low tide mark, prior to connection to the pump in the pump house. The 100 mm pipe is reduced to a 50 mm diameter pipe, where a 50 mm clear check valve is installed to stop any loss of water in the pump in the event of a power blackout. Unions on either side of the check valve, allow for routine cleaning or changing of the valve. A flexible 50 mm pipe leads to a 50 mm flange for connection to the pump.

As seen in Diagram 3/Pg2 (see technical drawing – page 2), in the pump house, a 50 mm to 40 mm bushing reduces the diameter of the intake, to fit a 40 mm union connecting to the main pump. Seawater is pumped continuously at a flow of about 200 litres per minute from the primary centrifugal pump (Sweetwater PS-6 2 hp). Pumped water

## Technical drawing, Pg. 2

### Primary seawater supply



is passed through the first T-junction (T1) diverting the water either through the effluent (excess outflow) pipe to the sea, or towards the sand filter (Jacuzzi Sand Filter JF4) for removal of coarse material. A cleaner T fitting (T connection with threaded cap) provides the ability to clean the entire pipe system on a routine basis, by adding chlorine. A second T-junction (T2) links this main intake system to the secondary pump. Direction of flow is regulated by one-way ball valves (Valve B to sand filter, Valve A to excess flow). Unions installed before valves facilitate replacement and maintenance of system. Prior to the sand filter, a third T-junction (T3) links the secondary pump to the sand filter. Photo 4/Pg2 illustrates the system described.

The sand filter is set up for daily backwash; Appendix 6 provides detailed backwash procedure conducted every morning prior to any other routine operations, along with a sample check sheet. A 40 mm clear pipe aids in monitoring the clarity of the water supplied to the hatchery complex. Seawater supply from the sand filter to the hatchery complex is monitored by a one-way ball valve (Valve C) (see technical drawing – 2/Pg3).

The entire seawater pumping system, including the sub-surface pipeline is cleaned once a year, at the end of the hatchery/nursery season, in order to control marine biofouling. Some sites may have higher biofouling rates and may require more frequent cleaning. Huguenin and Colt (2002) outline various methods for controlling biofouling. The procedure used at the Bermuda hatchery is outlined in Appendix 5. At this time, complete drainage of the sand filter is done, and usually new sand is purchased for the following season. Cleaning of all parts, including the pipeline is done in Bermuda using commercially available chlorine (or bleach). The use of “pigs” is being considered; these are contractible bullet-shaped plastic parts, which are launched through a clean-out and pushed though the piping under pressure (<100 psi). These “pigs” clean the pipes by friction.

#### **1.1.2.2 Secondary seawater supply – link to main intake line**

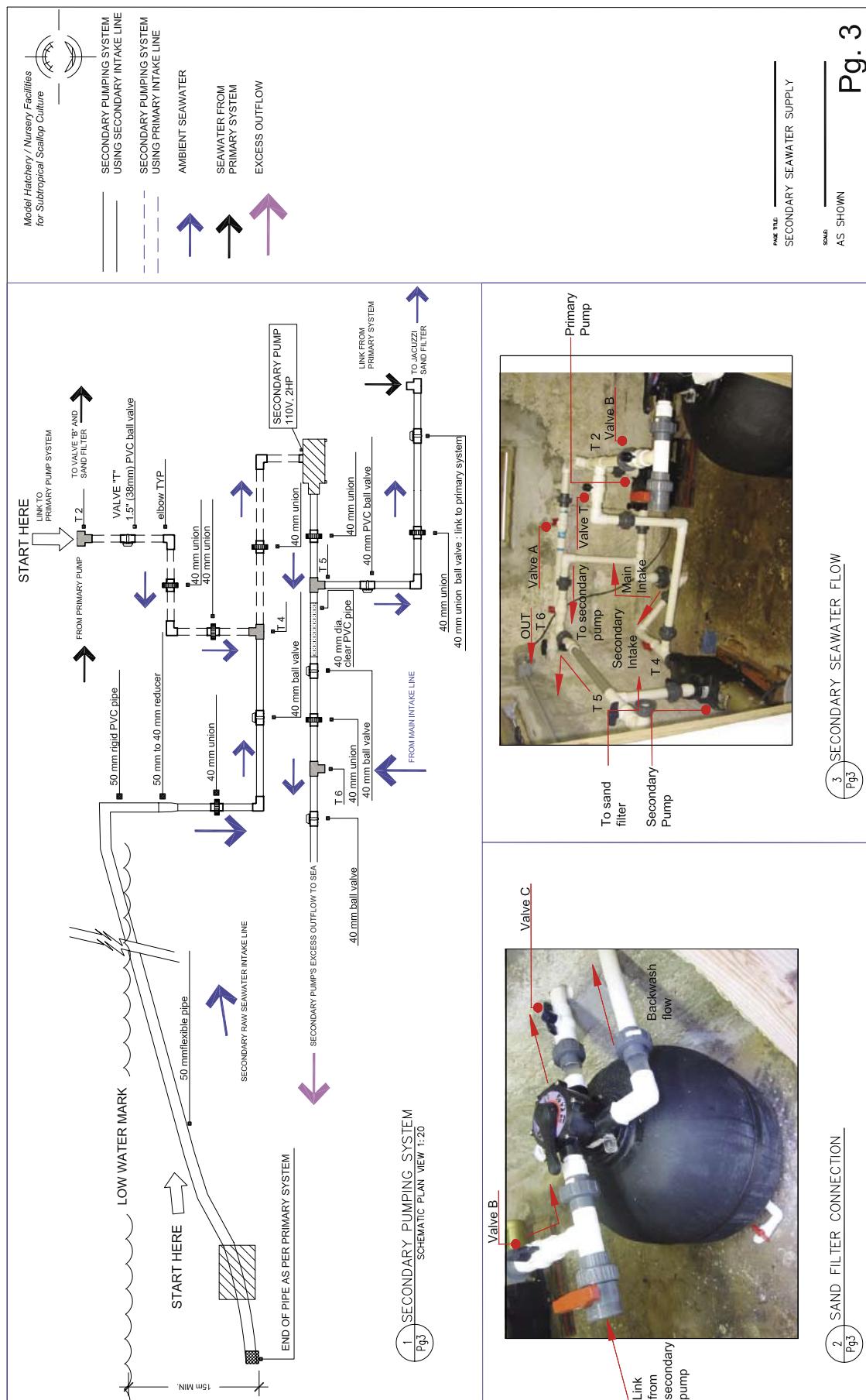
Refer to Technical Drawing – page 3. In the event of a primary pump failure, the secondary pump may be utilized for supplying seawater to the hatchery complex by pumping water from the main intake line. As seen in Diagram 1/Pg3, this can be done by diverting water from T2 toward the secondary pump (Hayward self-priming centrifugal pump 1.5 hp), by opening Valve C and closing Valve B going through junction T4 (T4 allows the inflow of water to come from the main intake line or from the secondary line, see paragraph below). After passing through the secondary pump, water is passed through junction T5, and links either to the sand filter for supply to the hatchery complex, or to the excess outflow pipe. As a further option, junction T6 also provides a more direct route from the main intake line to the secondary pump, bypassing the primary pump. The pumped water then flows back towards the sand filter via T4. Photo 3/Pg3 illustrates the seawater flow for the secondary system.

#### **1.1.2.3 Secondary seawater supply – link to secondary intake line**

Refer to Technical Drawing – page 3. A 50 mm line pumping water from a 1.5 m depth is installed as a secondary line; again this was the maximum depth of this location and is not a guide for optimal pumping depth. Ideally, the secondary line should parallel the main intake line. This was not possible in this case; hence a smaller line was set up in the event of clogging or other emergency rendering the use of the main intake line difficult. In practicality, during four years of operation, this line was used only to facilitate priming of the main pump; nonetheless, it is advised to have a back-up system.

This secondary intake line is connected to the secondary pump through a 40 mm pipe (Diagram 1/Pg3 and Photo 3/Pg3). At T4, water is directed towards the secondary

## Technical drawing, Pg. 3 **Secondary seawater supply**



pump, flows through junction T5 and passed through the sand filter for supply. Any excess water is returned to sea through the excess outflow pipe connected at T5.

#### **1.1.2.4 Main seawater supply to hatchery complex**

Refer to Technical Drawing – page 4. The plan view on Diagram 1/Pg4 shows the main supply line to the entire facility via a 50 mm pipe, starting at the pump house and fixed alongside a land path leading to the complex. The supply line is made up of 6.5 m lengths segments connected using 50 mm straight, 45 °, or flexible rubber couplings, depending on the terrain. Drain valves are at low points in the supply line to ensure complete drainage of the line during cleaning. A clean-out Y-junction is installed at the beginning of the line after the sand filter, for chlorination purposes (see Appendix 5). All seawater passing through the main supply line is coarsely filtered through the sand filter, and is at ambient temperature. Any further filtration or temperature control of the seawater is conducted at specific points in the system when needed.

For ease of reading, water lines are colour coded – ambient supply lines in blue, and effluent (or excess outflow) in purple. At junction T7 sand filtered seawater is diverted to the outdoor raceway. This secondary line is also used for draining the entire line; for this reason, a one-way ball valve is installed immediately after T7, followed by a T-junction providing direct flow to the exterior raceway. One-way ball valves on either side of the T-junction control the flow rate. Excess seawater is sent to sea through the excess outflow pipe line.

Returning to the main supply line, the second main junction is a Y-junction providing seawater to the algal container. In this case, the algal container is located uphill of the hatchery complex. This provides a future option to gravity feed algae to the broodstock and spat. This option is not shown here. Seawater supply to the algal container is regulated by Valve Y. The Y-junction reduces the 50 mm supply line to a 40 mm line and connects to a typical filtration system installed on the exterior wall of the algal container. *Note: Details of the filtration system are provided in 1/Pg5A (see technical drawing – page 5A).* Algal supply line is drained by gravity through a reduced 25 mm pipe, and controlled by Valve Z. As this drain is used only following cleaning of the algal supply pipe, only chlorinated seawater or fresh water seep into the soil.

After the Y-connection, junction T8 on the main intake line, supplies coarsely filtered seawater to the heating unit for the temperature control necessary for larval rearing.

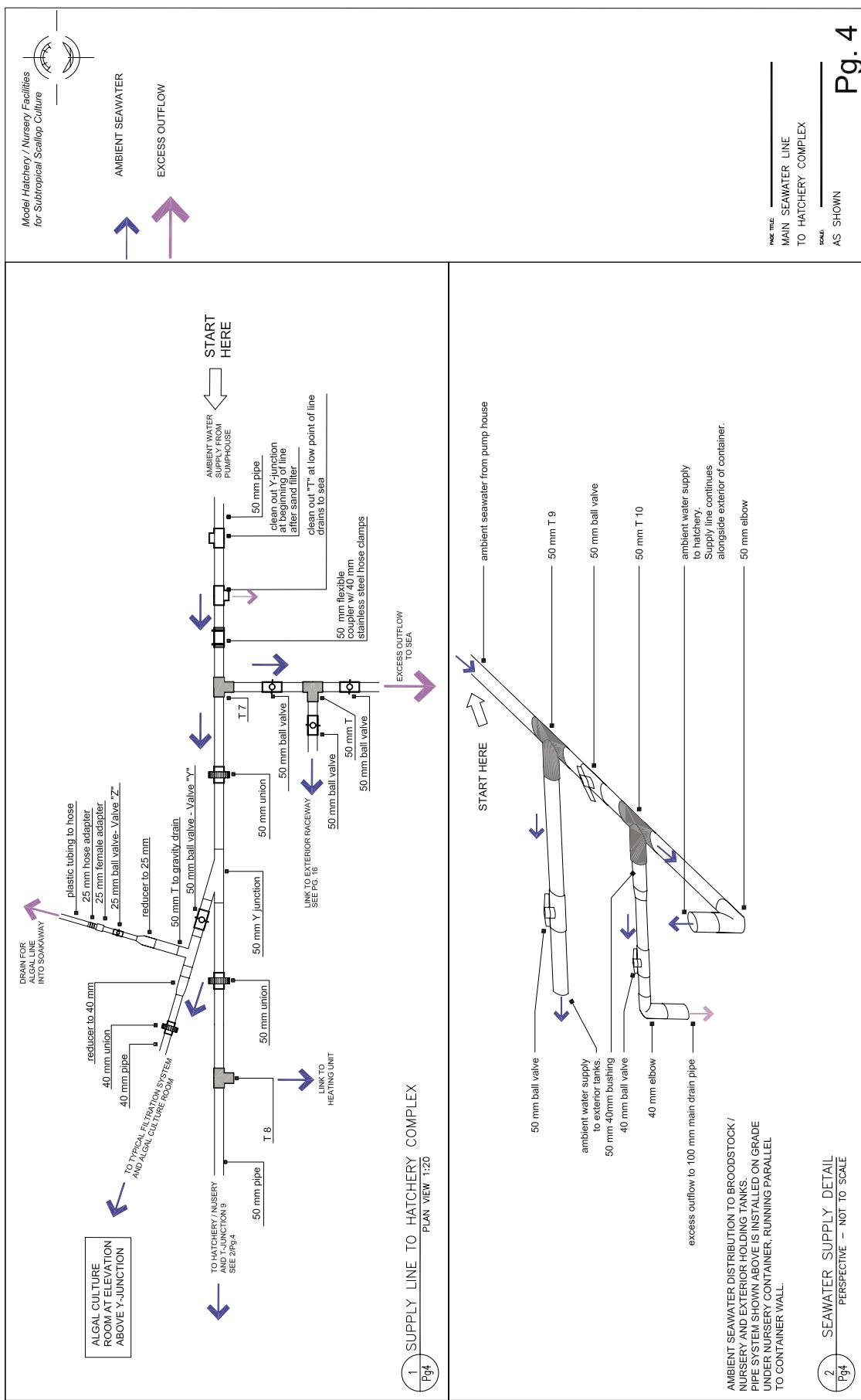
The distribution of seawater (both ambient and heated) to the hatchery complex and to the exterior tanks is shown in greater detail in the second diagram below (see technical drawing – 2/Pg 4). These junctions are located under the hatchery complex containers. Junction T9 provides ambient seawater to the exterior tanks; the flow of which is regulated by 50 mm one-way ball valves. Junction T10 is the last junction supplying ambient seawater to the hatchery complex, by a 50 mm pipe running alongside the wall of the container, and entering the complex at the top of the container, parallel to the ceiling. Flow to the hatchery is regulated inside the hatchery (see technical drawing Diagram – 1/Pg 8). The excess seawater is directed back to sea and regulated by 40 mm valve connected to T10.

#### **1.1.3 Heating unit**

Refer to Technical Drawings – pages 5A and 5B. Description of the heating unit is provided in technical drawing – page 5A; for ease of understanding, labelled photographs are given on technical drawing – page 5B. This heating unit is housed separately from the hatchery complex; mainly due to lack of space in the hatchery. A wooden shed of 2x2.6 m is used for this purpose (see Photo 1/Pg5B).

## Technical drawing, Pg. 4

### Primary seawater supply to hatchery complex



This unit consists of a square 1 000 litres tank, used for semi-recirculation of 1 µm filtered seawater; where, a continuous inflow of ambient seawater is heated and maintained at a set temperature through partial recirculation. Heated seawater is supplied to the hatchery on demand and is mainly required during water changes for larval rearing.

Ambient seawater, coarsely filtered by the sand filter, flows from the pump house through the 50 mm supply line (see technical drawing Diagram – 1/Pg5A). The line is reduced to 40 mm for connection to the filtration system, which is typical of that used throughout various points of the facility. A Hayward swimming pool filter (C250) removes coarser particles (to 25 µm). A 15 mm valve is fitted to the filter housing for cleaning and drainage after use. On the outflow side of the C250 filter, a reducer to 20 mm connected to two cartridge filters, 10 µm and 1 µm filters in that order. Unions before and after the coarse filter allow for replacement of parts and/or cleaning of the system when required. The inflow of 1 µm filtered seawater to the heating tank is done through a 20 mm pipe (Refer to Photo 3/Pg5B for illustration).

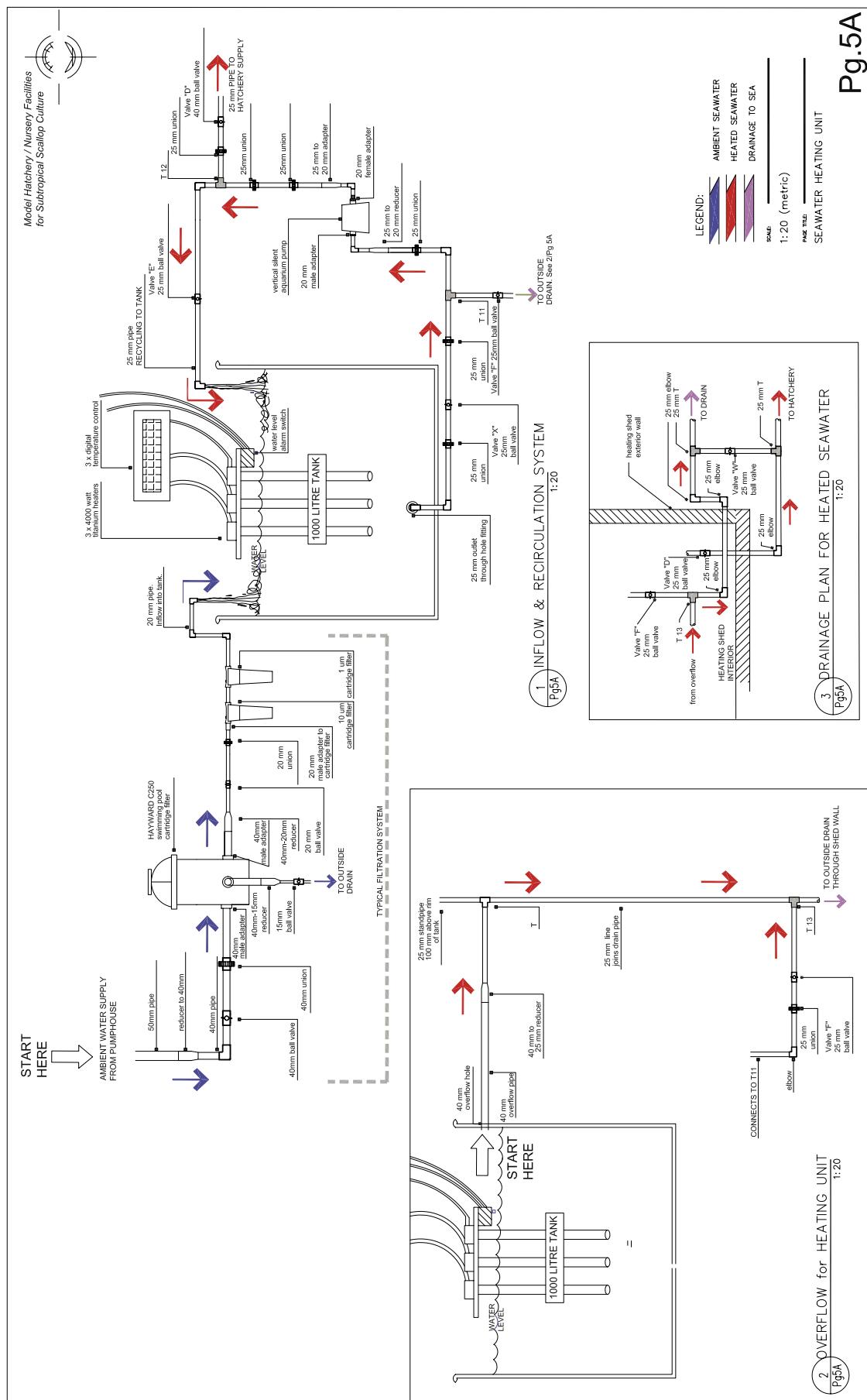
Within a 1 000 litres insulated BONAR tank are three titanium heaters (4 000 watts each) suspended so that maximum water level in the tank remains below the heads of the heaters. Each heater is connected to a digital temperature controller (one-phase), with an automatic function for turning the heater off when the desired temperature is reached. A water level alarm switch regulates the onset of the delivery pump; such that the switch closes contact and turns off the pump, when the water level drops (see Photo 2/Pg5B). This prevents the heaters from burning and the pump from running dry. The outflow is located at the base of the tank, and water flows through a 25 mm pipe; a 25 mm ball valve (Valve X) outside of the outlet controls the outflow of heated seawater. For delivery of heated seawater, Valve X is opened, distributing seawater through junction T11 prior to the pump. T11 allows for delivery of seawater to the pump when heated seawater is required or to outside drain via Valve F, when system is no longer in use. For heated seawater supply, flow is directed via T11 to a vertical delivery pump (Quiet One Centrifugal Pump), fitted with 20 mm adapters. (You may require yearly replacement of the pump and for this reason unions are fitted before and after the pump for ease of replacement). From the pump, the line is increased to 25 mm, and is passed through junction T12; this allows for partial recycling of the water to the heating tank (via Valve E), and for supply to the hatchery (via Valve D). In this way, water temperature is maintained constant through partial recirculation of heated water, and a continuous supply of heated seawater is available for spawning and larval rearing. Proper adjustment of valves D and E is critical to maintaining the balance between inflow of water-through the main supply and recycled pipe – and outflow to hatchery (refer to Photo 4/Pg5B).

A side view diagram of the tank indicates details of the overflow pipe (see technical drawing – 2/Pg5A). This is an additional security for preventing overflow of water over the sides of the tank, should the balance between inflow and outflow be disrupted. A 40 mm hole is drilled into the tank and fitted with a 40 mm pipe cemented into the tank, and reduced to 25 mm. A 100 mm standpipe is added to the top using a T-junction. The drain pipe from the overflow by-passes the heated outflow seawater pipe, and connects to a general drain pipe via T13.

Appendix 4 indicates the procedures used for setting up the heating unit, achieving the proper balance between inflow and outflow, and cleaning the system after use. Diagram 3/Pg5A (see technical drawing – page 5A) shows the heated seawater supply to the hatchery as it comes out of the heating shed, as well as the pathway for draining of the heated seawater system after use. The heating supply to hatchery is provided from

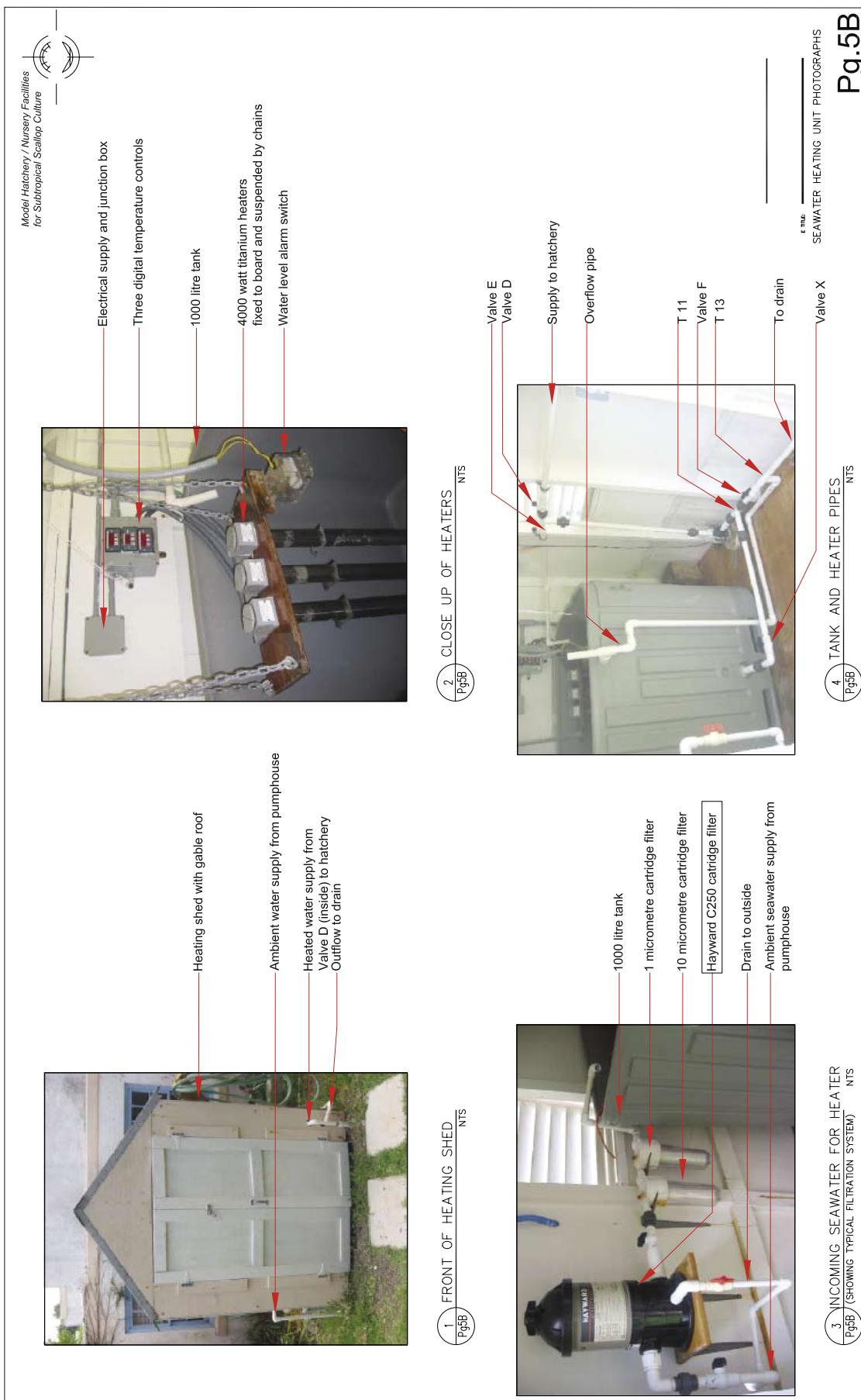
# Technical drawing, Pg. 5A

## Seawater heating unit



## Technical drawing, Pg. 5B

### Seawater heating unit: Photographs



Valve D; in-line to the hatchery is a T-junction allowing for drainage of hatchery heated seawater line after use; this is regulated by a one-way ball Valve W, which opens into a main draining pipe. This main draining pipe also collects water from the overflow of the heating tank, and from the bottom outlet (regulated by Valves X and F) when emptying of the heating tank is needed. Pipes for supply to hatchery and drain are buried in the ground.

This heating system allows for an 8 °C heating differential. It takes approximately 2 hours to fill the heating tank, and obtain the desired temperature. This design allows for the continuous supply of heated water during spawning procedures for maintenance of broodstock and gamete solutions at desired temperature, and supplies 4 000 litres of heated seawater for larval rearing.

#### **1.1.4 Hatchery/broodstock/nursery complex**

Refer to Technical Drawings – page 6A, 6B and 7.

##### **1.1.4.1 Container layout plan**

Refer to Technical Drawing – page 6A. Two reefer containers (6.5x2.5 m) are linked together to create the hatchery complex, comprising facilities for broodstock holding and conditioning, larval rearing and post-larval rearing (see technical drawing – 1/Pg6A and 2/Pg6A). Each container is installed on a concrete slab poured for each corner of the container. Containers are installed on a 2 percent slope, decreasing from the centre to the end, allowing for ease of drainage, namely during water exchanges to larval tanks; this also facilitates spraying clean the floor with a jet of freshwater to remove any debris and maintain cleanliness. Any water or debris washes under the container door into the side open gulleys installed on the outside of the containers. The floors of the containers are ribbed aluminium facilitating routine maintenance. The internal walls of the container are of glass-fibre, allowing for climate control and enabling the drilling of holes for seawater pipe entry. A small hallway is created between the two containers, closed in by doors on either side; such that the complex could be entered from either side. Large container doors are left on the end of each container facilitating movement of tanks or large equipment in and out of the containers. The total surface area of the complex is 33 m<sup>2</sup>.

The container elevation diagram (see technical drawing – 1/Pg6A) also shows the two seawater supply lines, ambient and heated, running alongside the exterior wall of the containers, and entering the complex by an elbow through the wall parallel to the ceiling. The heated seawater line connects to the heating system drain via a T-junction for routine cleaning of the hatchery heated seawater line after use; drainage is controlled by Valve W as described on technical drawing – 3/Pg5.

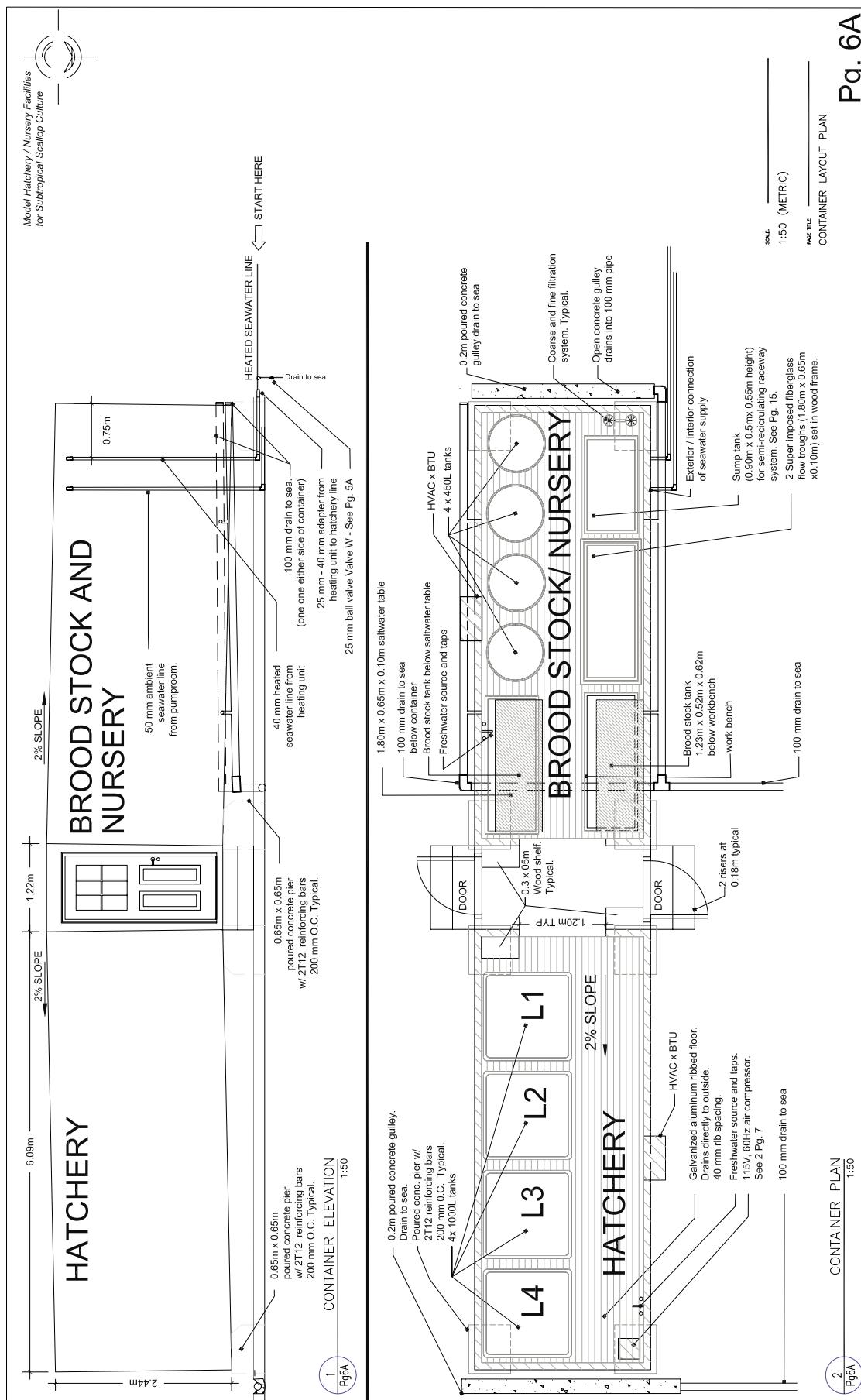
##### **1.1.4.2 Container plan diagram**

Refer to Technical Drawings – pages 6A and 6B. The overall plan of the container complex is shown separately (see technical drawing – 2/Pg6A). Photos are given on Page 6B for clarity. The left container consists of the hatchery facility with four 1 000 litres insulated BONAR tanks (see technical drawing – 2/Pg6B). Shelving is installed in the right hand corner of the container for storage of sieves used during water change. Other equipment used for hatchery purposes, such as trays, hoses and brushes are hung on the side of the container wall on hooks. One air conditioning unit in the hatchery maintains adequate climate control. Further shelving space, located in the hallway, stores spat sieves and other small equipment.

The right container houses the broodstock tanks, saltwater table, bench space, and nursery tanks and raceways (see technical drawing – 1/Pg6B). The seawater supply

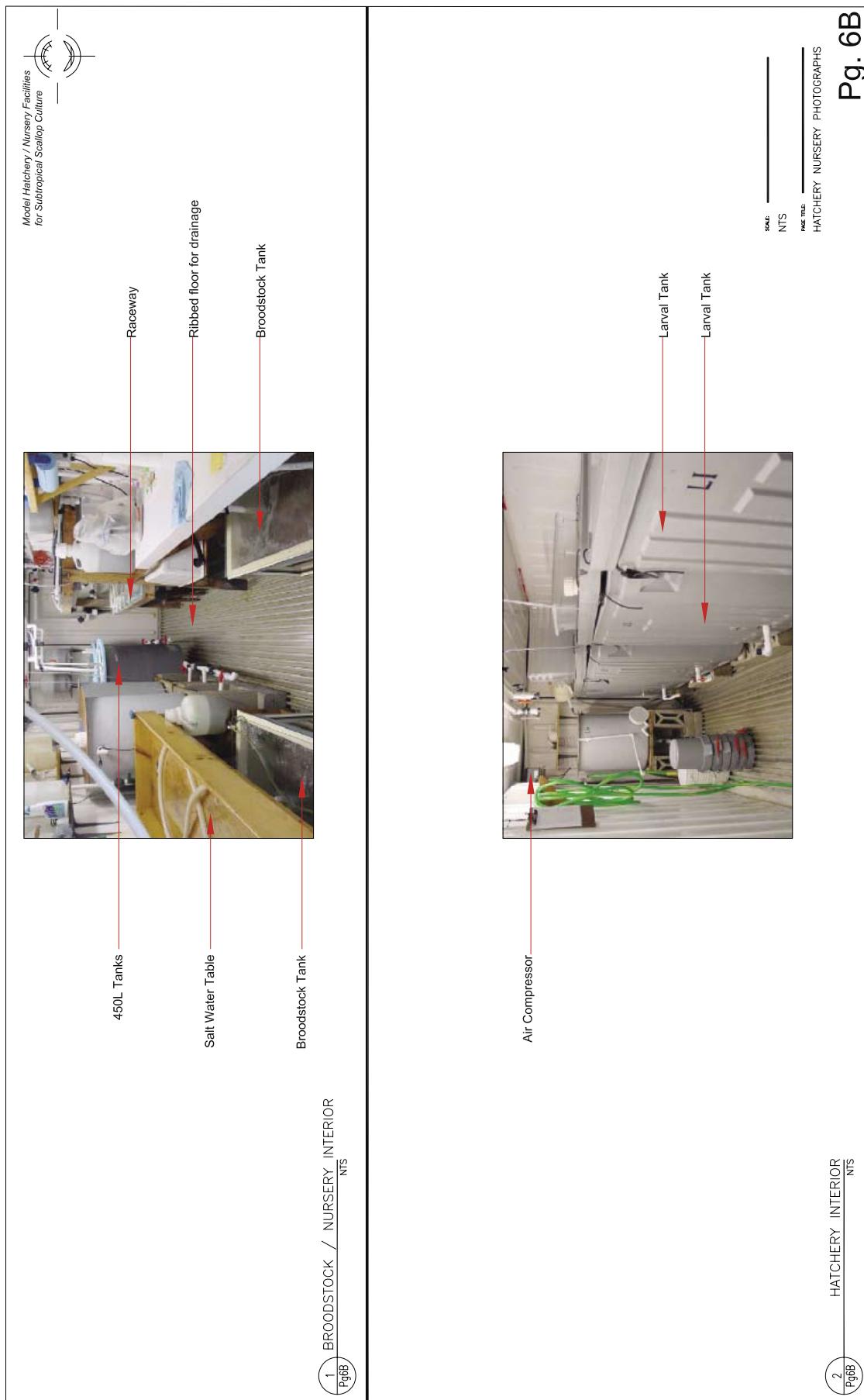
## Technical drawing, Pg. 6A

### Container layout plan



## Technical drawing, Pg. 6B

### Hatchery & nursery: Photographs



lines enter the far right corner of the container and are affixed to the ceiling by plastic clips. The installation of the plumbing on the ceiling is fundamental, as not only are the pipes out of the way but safety is ensured within the facility. Both lines run down the middle of the whole complex, supplying all tanks. A typical filtration system is shown at the right hand corner of the broodstock/nursery container for filtration of ambient seawater to 1 µm, available to all tanks except broodstock tanks. A 220 litres sump tank, used in conjunction with the raceways, is located next to the filtration system (see technical drawing – Page 14 for detail). Two 200x60x15 cm deep flow-troughs are mounted on a sidewall, one above the other, acting as raceways. This system is extremely versatile; each raceway can be used independently as an open, closed, or semi-recirculating system. This allows for setting of larvae on sieves, conducting experiments in a series of independent aquaria, or using both raceways as one larger system with a total capacity of 510 litres, in, for example, the early nursery culture of spat.

Two broodstock tanks are located on the exterior of the container, one below the bench area, and the other below the saltwater table. Finally, space is available for 450 litres nursery tanks, used for the setting of larvae, and rearing prior to the transfer at sea. A second air conditioning unit is located on the side of the broodstock/nursery container to maintain climate control. The air compressor is located at the far left on a shelf, supplying air to all tanks.

Two concrete gullies are located on the exterior of the complex to deliver waste seawater from the hatchery/nursery complex to drain pipes. Main drain pipes are constructed of 100 mm perforated pipe to receive excess seawater from various sources. Two drainage pipes run alongside the broodstock/nursery container receiving outflow from the broodstock tanks, saltwater table, the round nursery tanks, and raceway system.

#### **1.1.4.3 Hatchery and nursery ceiling plan**

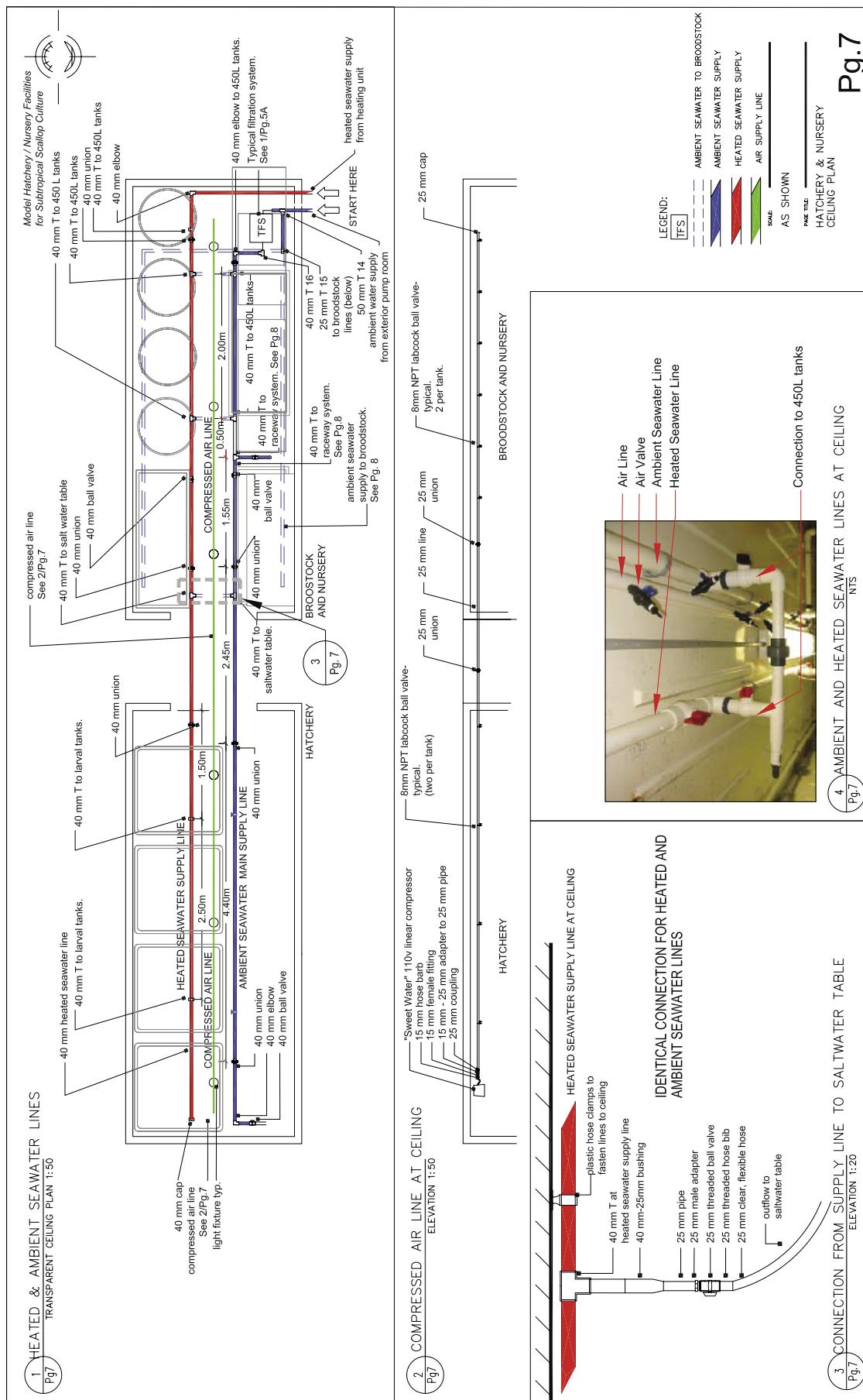
Refer to Technical Drawing – Page 7. The ceiling plan for both the ambient and heated seawater lines in the complex, and for the air supply are shown. The ambient filtered seawater line is coded blue; the heated seawater line is coded in red, and the airline in green. The raw seawater supply to the broodstock is dotted in blue, and bypasses the filtration system. In the hatchery, valves controlling seawater supply are also colour coded with respect to the temperature of the water – red for heated seawater and blue for ambient. This colour coding ensures clarity for all users, and minimizes any technical error which may be detrimental to larval and post-larval cultures.

#### Heated and ambient seawater supply

Refer to Technical Drawing – 1/Pg7. The ambient seawater supply comes directly from the pump room, having passed through a sand filter before entering the hatchery/nursery complex. A 50 mm pipe is affixed on the exterior of the container, and passes through the wall of the container, parallel to the heated seawater line. Inside the container, a 50 mm T (T14) diverts the water into: a) A typical filtration system (TFS), supplying filtered seawater to all hatchery/nursery tanks and saltwater table; and b) Broodstock tank 1, Broodstock tank 2 via junction T15. *Note: Details for this main diversion junction (T14) are given in technical drawing – 1/Page 8.*

After passing through the typical filtration system the 1 µm filtered ambient seawater is distributed to all tanks via junction T16. The main (1 µm filtered) seawater line is affixed to the ceiling in the middle of the container parallel to the heated seawater line (see technical drawing Photo – 4/Pg7). T-junctions are fitted in-line for diverting filtered seawater to the remaining separate tank units; such that in the first container (nursery/broodstock area), there are three T-junctions supplying ambient (and heated)

## Technical drawing, Pg. 7



seawater to the circular 450 litres tanks, one T-junction supplying ambient seawater to the raceways, and one T-junction supplying ambient seawater to the saltwater table. One-way ball valves regulate the flow to various areas. *Detail of the flow to the saltwater table is similar to that given for the heated seawater supply in technical drawing – 3/Pg7. Details of other junctions are given when appropriate in respective sections of the manual.* In the second container, specific to larval rearing, there is less need for ambient seawater; hence there is only one ball valve at the end of the water line for supply of ambient seawater, if needed.

#### Heated seawater line

The heated seawater line supply coming directly from the heating shed runs parallel to the filtered ambient seawater line. T-junctions are similarly fitted in line to supply heated seawater to the separate tanks. In the first nursery/broodstock container, three T-junctions supply heated seawater to the 450 litres tanks, and one to the saltwater table. *Connection to the saltwater table is described in technical drawing – 3/Pg7 and is installed parallel to that of ambient seawater. Similarly, details of connections to other tanks are provided in later sections of the manual.* In the second hatchery container, two T-junctions supply heated seawater to 4 larval tanks (one junction for two tanks). The heated seawater line is capped at the end with a 40 mm cap.

#### Air supply

Refer to Technical Drawing – 2/Pg7. The line for compressed air runs the length of the complex, affixed to the ceiling and parallel to the ambient and heated seawater lines, as shown in the ceiling plan. The location of a small compressor on a shelf at one end of the complex is shown on the diagram. Air is supplied through a 25 mm pipe to larval, broodstock, experimental and raceway tanks. Outlets are placed frequently, and airflow is controlled by labcock ball valves, to which a male barb is fitted for connection to an appropriate length of Tygon tubing (7 mm ID) to the tanks.

#### Details of seawater supply to saltwater table

Refer to Technical Drawing – 3/Pg7. Once diverted through the 40 mm T-junction at the main line, reduction of the pipe to 25mm is done with a 40 mm to 25 mm bushings. A 25 mm male adapter fits into a 25 mm threaded ball valve, which in turn is fitted on the outflow end with a 25 mm threaded hose barb. A 25 mm clear flexible hose supplies water to the saltwater table when needed.

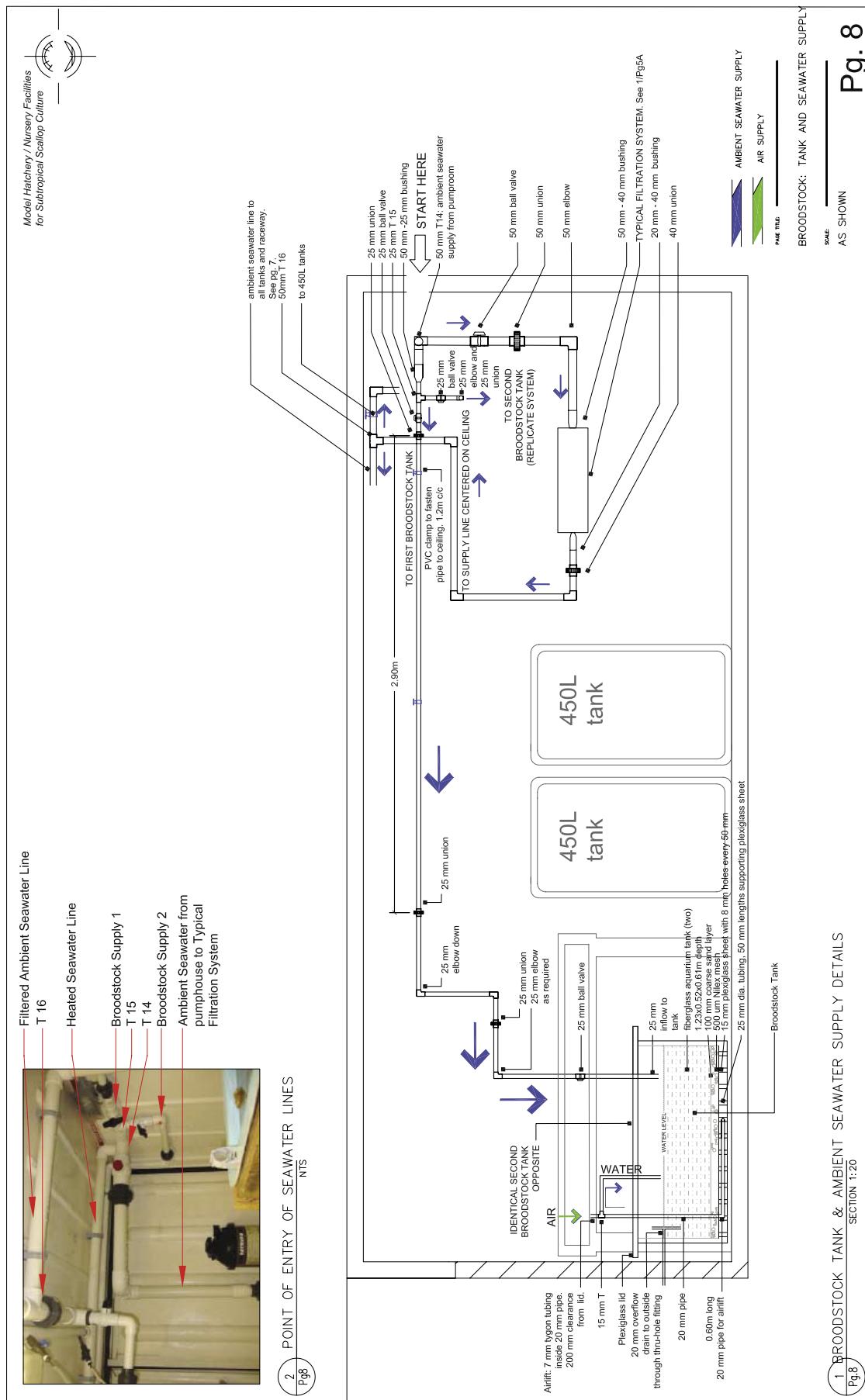
#### **1.1.4.4 Broodstock: tank and seawater supply**

##### Broodstock seawater supply details

Refer to Technical Drawing – page 8. Sand filtered ambient seawater line entering the complex from the exterior, first passes through junction T14, located at the point of entry of the seawater pipe into the container (Diagram 1/Pg8). Water flow is directed to the filtration system and/or to a reduced 25 mm junction T15 for supply to broodstock tanks. The point of entry of both ambient and heated seawater lines is illustrated and labelled on the technical drawing photo – 2/Pg8.

For fine filtration of the ambient seawater, flow coming from T14 is regulated by a 50 mm one-way ball valve to a coarse Hayward filter (25 µm). The 50 mm pipeline is reduced 40 mm prior to the filter for proper fittings. Unions after the valve and on either side of the filter allow for replacement of parts and cleaning of system. A 10 µm and 1 µm cartridge filter housings are installed in-line following the coarse filter as detailed in the typical filtration system (see technical drawing – 1/Pg5A). From the 1 µm filter, the seawater is distributed to the raceways, tanks, and saltwater table, by a 40 mm line connected to the centre of the container ceiling using plastic clamps. Water

## Technical drawing, Pg. 8



is then distributed to all tanks, via junction T16 along the supply line located on the ceiling of the complex.

For supply to the broodstock tanks, ambient seawater diverted through T14 flows through T15. This directs the coarsely filtered seawater (from sand filter in pump house) to Broodstock tank 1 and Broodstock tank 2. The flow for these tanks is regulated by a 25 mm ball valve for each tank. Unions (25 mm) located prior to elbows facilitate the necessary dismantling of pipes for cleaning. A second 25 mm ball valve located prior to the inflow into the tank allows for refinement of flow rate.

#### Broodstock tank

Refer to Technical Drawing – page 8. The two broodstock tanks are identical, 380 litres in volume (120x50x60 cm) and made of fiberglass with a clear viewing window in the front. A Plexiglas lid prevented any debris falling from above. A sub-sand filtration system is installed as follows: 25 mm diameter supporters are cut in 50 mm length and spaced out on the bottom of the tank; these supported a Plexiglas sheet drilled with 8 mm holes every 50 mm. On top of the Plexiglas sheet, is a 500 µm Nitex mesh covering the entire surface. Finally a layer of sand, previously washed and passed through a 1 mm screen, covers the bottom of the tank. Each broodstock tank is set for a semi-recirculation system, thus maximizing the residence time of added food, and allowing for some control in seawater temperature. Ambient coarsely filtered seawater (via the sand filter in the pump house) is supplied by a 25 mm pipe, and inflow is regulated by a 25 mm one-way PVC ball valve at the end of the line. Seawater flows in at the top of the tank. The semi-recirculation system is driven by an air lift, consisting of a 20 mm pipe placed on the bottom of the tank, and connected to a vertical pipe via an elbow. The airline (7 mm Tygon tubing) is inserted into the pipe. A T-junction at the top of the pipe allows for recirculated water to flow back into the tank. Outflow is achieved at the surface of the water, via a thru-hull fitting and hose discharging through the container wall and into the main drain pipes outside.

At certain times, during broodstock conditioning, temperature of seawater needs to be adjusted. Seawater within each tank can be cooled using a 1/5 HP chiller unit installed on a nearby shelf. The chiller is matched in size with the capacity of the tanks to allow for cooling through a 5 °C differential from ambient seawater temperature. For heating the ambient seawater, 250 Watts Ebo-Jaeger aquarium heaters were immersed in the tank; with a flow of 40 litres.h<sup>-1</sup>, two 250 Watt heaters are sufficient to heat the water 5 °C above that of ambient.

Feeding of broodstock is done using one 20-litre carboy for each tank. Carboys are purchased with an existing spigot, located at the bottom of the carboy. This spigot is fitted with a stopcock valve and 7 mm tubing for a finer adjustment of algal flow to the tank. A fast drip-feed is usually adequate to supply algae from one carboy over 24 hours.

## **1.2 SCIENTIFIC BACKGROUND – NATURAL HABITAT AND REPRODUCTIVE CYCLE**

### **1.2.1 Habitat**

The scallop, *Euvola (Pecten) ziczac* (L.), also known as the sand scallop, zigzag scallop or Bermuda scallop, is a sub-tropical and tropical species (Figure 1.1). It is similar to other pectinids in that the right (lower) valve is very convex, whereas the left (upper) valve is usually flat but has been seen to slightly convex or concave in some cases. It has been fished recreationally and/or commercially in Brazil (Pezzuto and Borzone, 1997)

and along the Caribbean coast of Venezuela and Columbia (Velez and Lodeiros, 1990), and has also been seen off Florida (USA) as a by-catch in the calico scallop fishery. Its northernmost distribution is Bermuda (Lodeiros *et al.* 1989).

In Bermuda, the sand scallop inhabits protected inshore waters, lying on grassy, sandy bottoms, ranging in depth from 2–10 m (Sterrer, 1986). In its natural state, this scallop is recessed in the sand with the rim of its outer left valve showing. It will swim when disturbed, but does not cover great distances. It has also been observed to bury completely 5–10 cm into the sand when faced with unfavourable conditions. Maximum shell height recorded in Bermuda is 130 mm (Sterrer, 1986). Its life span is thought to approximate 5 years. Worldwide, population numbers of *E. ziczac* have been reported to be low, with a decline seen in the 1990's. In Bermuda, it was known to occur in relatively large abundance during the 1940's and 1950's, and was recreationally fished until the early 1970's. It since has been recorded here and there, with no real evidence of a self-sustaining population. To our knowledge, there is currently no existing commercial hatchery for this species.

The calico scallop, *Argopecten gibbus* (L.), is largely restricted to the sub-temperate and tropical waters of the western North Atlantic with major stocks distributed from Cape Hatteras, North Carolina (USA) to the Cape San Blas areas of the northeastern Gulf of Mexico (Waller, 1969). Calico scallops have also been collected from the Greater Antilles, Bermuda, and the western portions of the Gulf of Mexico (Waller, 1969). Commercially important stocks are located off North Carolina and northeastern Florida (USA), where it supports a small and transient fishery (Figure 1.2). The calico scallop has two convex valves, although the right is slightly more convex than the left (Sterrer, 1986). The upper valve is usually mottled with a combination of brown, red, purple, yellow and white. It is found lying on top of the seabed in sandy, rocky and grassy substrates, and has been recorded in Bermuda in several inshore waters, as well as on the more exposed North shore of the Island. It attains a maximum height of 70 mm in Bermuda (Sterrer, 1986). This species, like the sand scallop, was a commonly found bivalve in Bermuda and supported a recreational fishery at one time. Population numbers are at present very low in Bermuda (Sterrer, 1986). There is no commercial hatchery for this species at present.

A generalized diagram of a pectinid is shown below (Figure 1.3) outlining some

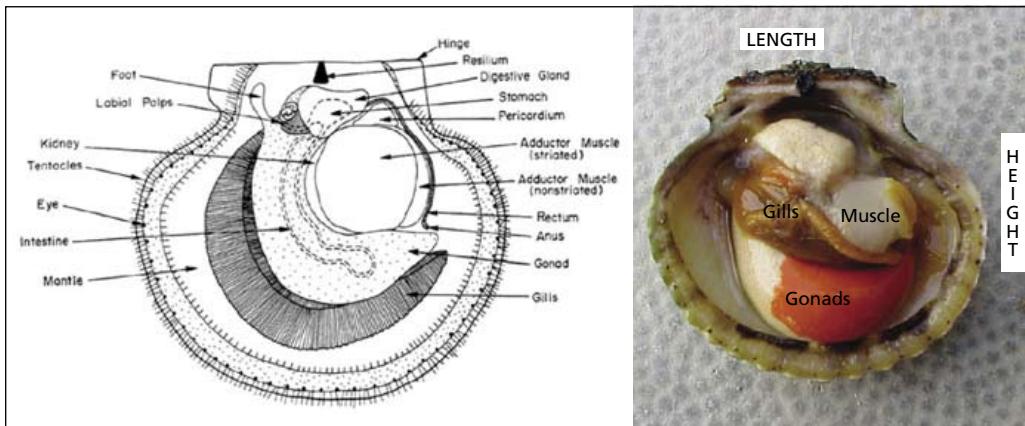


**Figure 1.1:** Photograph of a live *E. ziczac*, the sand scallop or zigzag scallop.



**Figure 1.2:** Map indicating the distribution of the calico scallop, *A. gibbus*.

shell characteristics and scallop organs. A photograph of a dissected calico scallop is also inserted to show the colouration of the gonad when ripe, the muscle and gills.



**Figure 1.3:** Generalized diagram of a pectinid (taken from Bourne, Hodgson and Whyte, 1989) alongside an open calico scallop specimen showing major organs.

## 1.2.2 Reproductive cycle

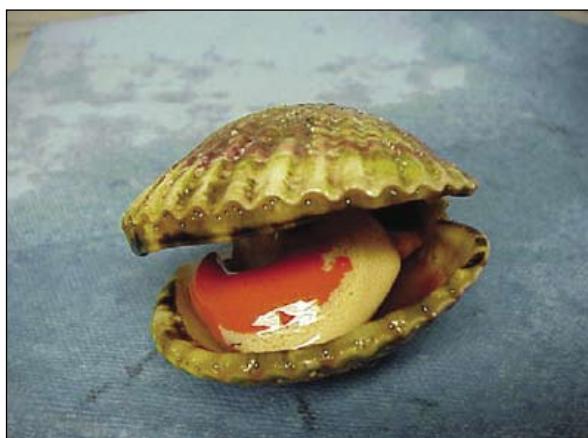
### 1.2.2.1 The sand scallop, *Euvola (Pecten) ziczac*

The reproductive cycle of *E. ziczac* in Bermuda has been determined based on histological analyses, gonadic index determinations and natural spatfall (Manuel, 2001). This species is a simultaneous hermaphrodite with whitish-yellow testis and orange ovaries when mature. According to Manuel (2001), gametogenesis is initiated in autumn and gonads mature during the colder winter months. High gonadic indices were determined by Manuel (2001) when seawater temperature was below 20 °C; this concurs with the observed peak spawning activity at the Bermuda hatchery between December and March. There was no major synchronized spawning recorded for this species in Bermuda waters. Animals tend to spawn sporadically subsequent to any small environmental changes, and very often were seen to release gametes partially rather than completely. This led to their classification by Manuel (2001) as “dribble spawners”.

Lodeiros and Himmelman (1994) found that *E. ziczac* in Venezuela attained full sexual maturity at a shell height of approximately 44 mm; this shell growth is obtained in Bermuda in the first 10–11 months, and allows for a first gametogenic cycle to concur with the first winter period (Sarkis, 1995). Latitudinal differences in the timing of the reproductive cycle are seen with this species as in the more southern region of the Venezuelan coast, where two major spawning events have been recorded for the sand scallop (Velez, Sotillo and Perez, 1987); the first occurring in April/May and the second during August/September, when sea temperatures range from 23–26 °C. Temperature and phytoplankton abundance are two environmental factors which have been cited as influencing gametogenesis and spawning in the sand scallop (Lodeiros and Himmelman, 2000; Velez, Alifa and Freites, 1993). Fecundity of *E. ziczac* when stimulated to spawn in Bermuda at the hatchery averaged 5 million eggs per female; similar results were recorded by Velez, Alifa and Freites (1993).

### 1.2.2.2 The calico scallop, *Argopecten gibbus*

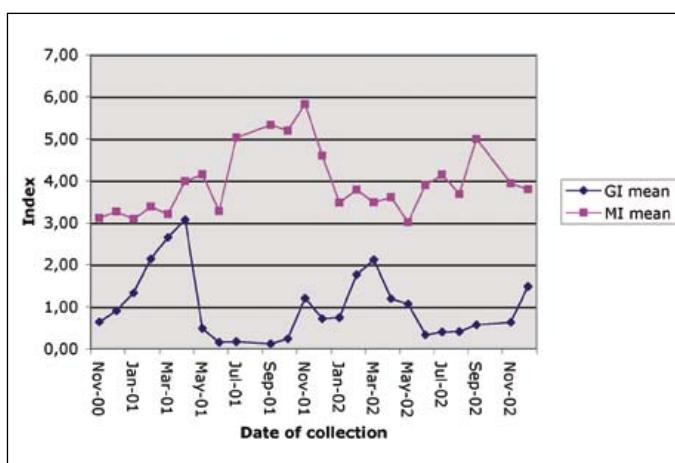
The reproductive cycle of *A. gibbus* in Bermuda was determined using gonadic index and histological analyses. This species is also a simultaneous hermaphrodite with whitish testes and bright orange ovaries when mature (Figure 1.4). For ease of understanding, a description of the oocyte developmental stages is given in Appendix 1.



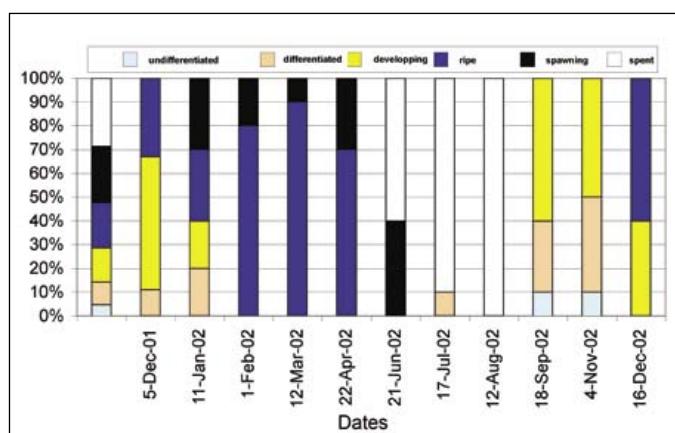
**Figure 1.4:** Calico scallop, *A. gibbus*, showing gonads with both mature ovaries (bright orange) and sperm (white).

with Blake and Moyer (1991) who report, that a large percentage of any sub-population of calico scallops in Florida (USA) waters normally spawns over a 1–3 week period. This definition is reflected in the specific peaks seen in gonad weight between February and April (Figure 1.5) and in the high percentage of ripe cells (70–90 percent) determined in histological sections of the ovary (Figure 1.6). Histological analyses suggest that the spawning period of *A. gibbus* in Bermuda ranges from December to possibly May. In the more southern waters of Florida (USA), Moyer and Blake (1986) reported two spawning periods for the calico scallop, the first in late spring (April to June), and the second in autumn. These latitudinal differences may be explained in part by environmental differences, namely those of food and temperature (Barber and Blake, 1983). The lack of a clear trend in muscle weight determined for *A. gibbus* in Bermuda suggests a direct dependence on food supply for gonadal development and maturation. Blake and Moyer (1991) found that the required threshold temperature for the same species in Florida (USA) was of 19–20 °C. Their

Timing for the initiation of gametogenesis and maturation of gametes is similar to the sand scallop, where gametogenesis is initiated in September and maturation reached during the winter months. The calico scallop in Bermuda exhibits a spawning period over the winter months, as for *E. ziczac*, associated with colder water temperatures and lower food availability. The difference between the two species is a more defined spawning period in *A. gibbus*, where release of gametes is complete and synchronous among the population, as observed in Bermuda. This is in agreement



**Figure 1.5:** Gonadal indices and muscle indices for calico scallop, *Argopecten gibbus* in Bermuda waters (GI= Gonadal Index; MI= Muscle Index).



**Figure 1.6:** Reproductive patterns in cultured *A. gibbus* from Bermuda.

further conclusion that at temperatures above 22 °C maturation apparently stopped and spawning did not occur, concurs with results seen in the histological work and at the Bermuda hatchery, where a lack of gametogenic activity was seen during the warmer months, and spawning terminated in late spring ( $T=22\pm1$  °C).

It has been noted by Blake and Moyer (1991) that an individual scallop normally reproduces for the first time at an approximate age of 6 or 12 months depending upon season of spawn. In Bermuda, 8 month old scallops have been observed to undergo gametogenesis and become reproductively mature within their first year of life (mean shell height of  $47.4\pm2.2$  mm). Each scallop is thought to spawn 2 or 3 times during its 18–24 months life span in Florida (USA) waters (Blake and Moyer, 1991). Although this has not been scientifically assessed in Bermuda, one cohort has been observed to spawn twice a year for a maximum period of three years. Fecundity of *A. gibbus* when stimulated to spawn in the Bermuda hatchery averages 6.26 million (n=5).

### 1.2.3 Life cycle

A generalized diagram of the life history of a scallop is provided below in Figure 1.7. Size shown for each stage is general, and differs among species. As seen in the above section, gamete release from ripe adults into the water, allows for external and controlled fertilization of the oocytes. Fertilized eggs or embryos are allowed to divide and develop into trochophore and early veliger larvae in larval tanks. The early larval stage is often referred to as D-larvae, as they take on a characteristic “D” shape, or straight hinge larvae, or Prodissococonch-I stage. Rearing of larvae is continued in these tanks, for development into umboned or Prodissococonch-II larvae. This hatchery stage lasts until larvae are mature and reach the pediveliger stage. At this time, larvae alternate from a swimming state to substrate-search behaviour by use of a newly developed foot. They may attach to various surfaces by secreting a byssus acting as temporary holdfast. Larvae are then ready to undergo metamorphosis, a critical time in their development. High mortalities may be seen at this time. Metamorphosed larvae settle, and are termed spat. They are reared in a nursery system until they are strong enough to be transferred to the field. Juveniles are reared in enclosures in the natural environment until adult size. Descriptions of techniques for larval, post-larval and juvenile culture are given in Chapters 3, 4 and 5.

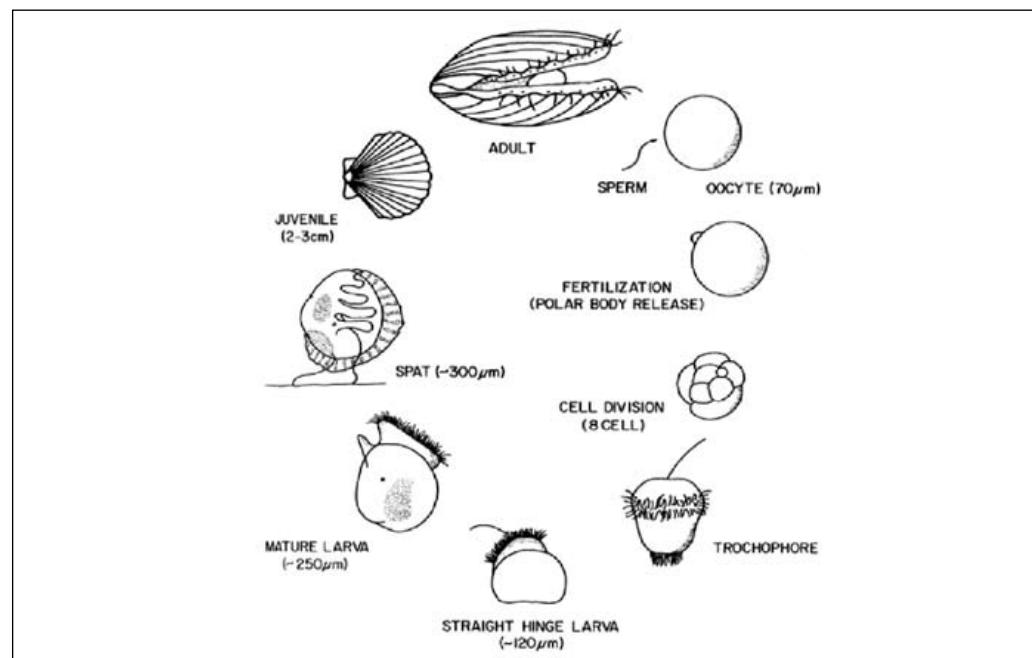


Figure 1.7: Generalized life history of a pectinid (taken from Bourne, Hodgson and Whyte, 1989).

## 1.3 TECHNIQUES – BROODSTOCK

### 1.3.1 Gonadal and muscle indices

The use of gonadal indices as an indication of reproductive activity is a simple, fast and inexpensive procedure in a hatchery. It provides reliable quantitative information required prior to the stimulation of spawning. The verification conducted at the Bermuda hatchery by comparisons with simultaneous histological analyses, confirmed the usefulness of gonadal index determination. Although there are various definitions of gonadal and muscle indices, all are based on the weight change of the tissue with reproductive maturity. Lucas and Beninger (1985) critically reviewed several of these indices, concluding with that best representing bivalve static condition. In the present work, gonadal and muscle indices are calculated separately; the former for reflecting gonadal development, and the latter as an indication of nutrient mobilization and utilization associated with gonadal development (Gabbott and Bayne, 1973). The step-by-step procedure for determining both indices is given in detail below in Protocol-1; a sample data sheet used during analysis is given in Appendix 2.

Gonads and adductor muscles are dissected and dried to constant weight at 80 °C. Indices are calculated as follows:

$$\frac{\text{Dry weight of organ}}{\text{Empty shell weight}} \times 100$$

A high gonadic index is indicative of mature gonads, and a lower index reflects the onset of gametogenesis or spent gonads.

For some pectinid species, visual observation of the gonad is sufficient to determine the spawning condition as for *Patinopecten yessoensis*, the Japanese scallops (Bourne, Hodgson and Whyte, 1989); however, visual observations have been misleading for the calico scallop studied in Bermuda. Although this species exhibits a change in colouration as the gonad matures from whitish-yellow to bright reddish-orange as observed by Roe, Cummins and Bullis (1971), it has been observed in Bermuda that gonads become bright orange prior to maturation and coincidental with sub-maximal gonadic index values. It is therefore advised that gonadic index determinations be made to assess the reproductive status of these species.

### PROTOCOL-1

#### DETERMINING GONADAL AND MUSCLE INDICES

##### Preparation for procedure

1. Collect 15 scallops from the grow-out site. Record date and site of collection.
2. Place scallops in a tank supplied with flowing filtered seawater. Do not feed and allow 24 hours before dissecting to ensure gut clearance.
3. Use a 2 or 3 decimal point balance, if possible. Switch on and leave to warm up for at least 15 minutes.
4. Bring scallops in a bucket of seawater to laboratory.
5. Set up dissection kit with scissors, forceps and scalpel. You will also need Vernier calipers for measuring scallops and a laboratory book to record all information.

6. Take about 1 meter of absorbent paper and lay it on the bench. Number the paper with the number of scallops for dissection, i.e. if there are 10 scallops, Label 1, 2, 3...10, in equidistant spacing along the paper. Lay each scallop by their number on the paper, and the scallop then becomes this number. In this way, it avoids confusion. Also you will find that as scallops start to gape for air they are much easier to work with!
7. Tear small squares of foil paper (6x6 cm); two for each scallop.
8. Record date and site of collection, and date of dissection in lab book.
9. In the lab book, organize columns for scallop number (see step 6), shell height, total wet weight, gonad dry weight, and muscle dry weight.

### The dissection procedure (wet weights)

1. Measure scallop height with Vernier calipers (see Figure 1.3). Record data on the lab book next to appropriate scallop number.
2. Blot dry each scallop with absorbent paper, and place on balance for total wet weight. Record data on the lab book next to appropriate scallop number.
3. As scallops begin to gape open, take one and keep it open with thumb and forefinger. Use scalpel to cut the adductor muscle from shell. This will allow opening the scallop completely, laying it flat for dissection.
4. Take a piece of prepared foil, label as "G" for gonad; write matching scallop number next to it directly on the foil paper. Such that each piece of foil is labelled G1 for scallop no. 1, G2 for scallop no. 2, etc. Tare the balance, and weigh foil. Record as foil weight under proper label.
5. Remove the gonad, using scissors and tweezers. Place the gonad on the matching piece of foil, and weigh. Record as gonad wet weight with foil (gram). Fold the foil around the gonad and place it to one side.
6. Take the next piece of foil for muscle, and label as "M" for muscle; write matching scallop number, such that similar to the gonad, each piece of foil is labelled as M1 for scallop no. 1, M2 for scallop no. 2, etc. Tare the balance and weigh foil. Record as foil weight under proper label.
7. Remove the muscle, carefully removing all the muscle from the ventral shell using a scalpel. Place on foil and record muscle wet weight with foil. Wrap the muscle in the foil and put it to one side.
8. Scrape all other tissues into a waste container.
9. Blot empty shells dry with a piece of absorbent paper. Tare the balance, and weigh and record the weight of the empty shell. Discard shells.
10. Repeat the dissection procedure for all remaining scallops. At least 10 scallops are required for reliable determination of Gonadal Index (GI) and Muscle Index (MI).
11. When all scallops have been dissected, put all labelled gonads and muscles wrapped in foil into a drying oven at 80 °C. Dry until constant weight; this should take 48 hours.
12. Clean all equipment thoroughly, especially dissecting tools and Vernier calipers to prevent any salt corrosion.

### Dry weights

1. After about 48 hours, take gonads and muscles out of the oven. Make sure that constant weight has been reached after this time period.
2. Switch balance on and leave to stabilize according to manufacturer's instructions (usually about 15 minutes).
3. Weigh each organ with foil. Record on lab book.
4. Repeat until all organs weighed.

5. Transfer data into an Excel Spreadsheet (see Appendix 2). This will allow you to calculate gonad and muscle weight without foil, and gonadic and muscle indices as follows:

To calculate Gonadal Index (GI):

$$\frac{\text{Gonad dry weight}}{\text{Empty shell weight}} \times 100$$

To calculate Muscle Index (MI):

$$\frac{\text{Muscle dry weight}}{\text{Empty shell weight}} \times 100$$

Calculate the mean and standard deviation of the GI and MI for each collection date.

6. Keep all gonadal and muscle indices conducted in one spawning season in one Excel workbook. Assess gonad development on a routine basis. Keep yearly records for comparison.

### 1.3.2 Maintenance and conditioning of broodstock

The main goal in maintaining a broodstock in the hatchery is to ensure a healthy stock of reproductively mature and ripe adults. Depending upon species, this goal may be easily achieved or not. Conditioning broodstock, by manipulating environmental factors, such as temperature, food, photoperiod, allows for control of the gametogenic cycle, or parts of, and provides an aquaculturist with a tool for management of spawning periods and larval rearing and production in the hatchery. Again, depending on species, conditioning may be easily achieved or not. It has also been reported that conditioning of the broodstock affects lipid content of the egg, and subsequently larval survival (Gallager and Mann, 1986); these authors found that variations in broodstock conditioning protocol induced large fluctuations in egg lipid levels of two bivalve species, and suggested that strict attention should be paid to conditioning if optimal culture potential is desired. In other cases, difficulty in obtaining ripe broodstock, has prompted development of hatchery conditioning protocols, as for *Pecten fumatus*, in Australia (Heasman, O'Connor and Frazer, 1996); these authors found that the rapid conditioning of *P. fumatus* was possible by controlling water temperature and feeding to satiation; conditioned scallops exhibited a better fecundity than those spawned immediately upon collection from the wild.

For *E. ziczac*, Velez, Alifa and Perez (1993) found that sexual maturation and spawning could be induced out of the regular spawning season, by maintaining a temperature of 26–29 °C up to a total of 400 °C days; they furthermore found that the number of oocytes released was significantly higher in scallops maintained at a higher temperature for a longer number of day degrees. In Bermuda, no attempt was made to condition scallops out of their regular spawning season; however, efforts were made to ensure ripe broodstock at specific periods, thus optimizing use of the available hatchery and nursery space in this compact facility. As is seen below, working with two different species resulted with two different strategies.

In Bermuda, broodstock was maintained in the tanks described on Page 8 of the technical drawings. It was found that 50 calico scallops of mean shell height  $57.2 \pm 2.7$  mm could be comfortably held in one broodstock tank (equivalent to 80 scallops per m<sup>2</sup>). Due to the larger shell size and recessing nature of the zigzag scallop, a maximum of 20 scallops only (approx. 75 mm in shell height) were kept in one broodstock tank (equivalent to 30 scallops per m<sup>2</sup>).

### **1.3.2.1 The sand scallop, *Euvola (Pecten) zigzag***

Unlike results reported by Velez, Alifa and Perez (1993), where conditioning was conducted successfully out of season, it was found that the sand scallop was not easily maintained in the hatchery in Bermuda, even during spawning season. Keeping apparently ripe animals in a broodstock tank with a daily supplement of algae for any length of time, yielded poor spawns in terms of number of eggs released (Fecundity=  $1.4 \pm 1.1$  million eggs per female as opposed to the norm of 5 million eggs per female) and poor subsequent survival to D-larval stages (1.6 percent as opposed to 60 percent). Moreover, this species was found to spawn spontaneously following any type of stress, such as handling on the boat, transport to the hatchery, etc. The strategy for ensuring ripe zigzag scallops thus became one for collection of scallops when ripe, and prevention of spontaneous spawning. Protocol-2 provides a detailed description of the procedure. In short, scallops are collected directly from the field. Their reproductive state is assessed visually on board the boat. Scallops are considered ripe when the following three criteria apply: 1) well rounded gonads 2) female colour orange 3) digestive tubule invisible, covered by gonads. Ripe scallops are transported to the hatchery with great care. In order to avoid release of gametes due to boat movement, scallops are transported “dry”. Zigzag scallops, as most pectinids, gape open when exposed to the air; in order to avoid dehydration, rubber bands are quickly placed around the two shells upon collection to prohibit them from opening their valves. Spawning is induced upon arrival to the hatchery.

### PROTOCOL-2

#### **COLLECTING AND HOLDING OF SAND SCALLOP BROODSTOCK**

##### **Preparation for procedure**

1. Collect 30 specimens from the grow-out site by SCUBA using collector bags.
2. Assess gonadal state on board for as many animals as possible. Place ripest scallops aside (see criteria Section 1.3.2.1).
3. Quickly fasten rubber bands around shell of ripe scallops to prohibit valves from opening.
4. Place scallops gently in a cooler on a bed of seaweed, or layers of polyethylene mesh previously moistened with seawater.
5. Total exposure out of water for this species should not exceed 45 minutes.
6. Upon arrival at the hatchery, transfer scallops to a cold-water bath previously prepared for spawning induction (see Protocol-4).
7. If not possible to induce scallops on the same day, transfer into one of the broodstock tanks at ambient seawater, and induce the following day.
8. Do not feed 24 hours prior to spawning induction if maintained in hatchery.

### **1.3.2.2 The calico scallop, *Argopecten gibbus***

Gonadal indices are determined monthly to assess the stage of reproduction, as visual observation of the gonad was found deceiving in this species (see Section 1.2). Two strategies for ensuring a ripe broodstock in this species are used in Bermuda.

The first strategy relies strongly on the natural gametogenic cycle of the calico scallop. When a gonadal index reaches 2 or above, a sub-sample of scallops is collected from the grow-out sites and brought into the hatchery. Animals are kept in a broodstock tank at ambient seawater temperature, and fed on a daily basis a mixture of algal species. Diets consist of *Isochrysis galbana*, *Chaetoceros muelleri* and *Tetraselmis chuii*. Algal food is

supplied to the broodstock via a 20-litre carboy fitted with a drip-feed. This carboy is filled in the morning and in the evening, such that scallops receive a continuous flow of food amounting to 40 litres of algae for 50 animals or 14 litres of algae per kg total wet weight. This yields levels of  $380 \text{ cells.g}^{-1}$  wet tissue weight. Although, this is a relatively low level of food compared to others (Bourne, Hodgson and Whyte, 1989; Neima and Kenchington, 1997), it seems adequate for development of gonads to maturity as seen in results obtained following a 2-month conditioning period (see below). Feeding is stopped 24 hours prior to spawning induction. Ripe scallops are usually induced to spawn 1 or 2 weeks following collection.

The second strategy involves conditioning of broodstock to accelerate the later stages of gametogenesis, and thus advance the commencement of the spawning period to an earlier date. Several studies were conducted at the hatchery on conditioning of the calico scallop broodstock. Conditioning regime consists of two phases: Phase 1 involves exposure of scallops to a temperature lower than ambient, stimulating the differentiation of gametes; a temperature differential of  $3^{\circ}\text{C}$  was sufficient. Phase 2 involves transition of scallops to ambient temperature, and exposure to a gradual increase in temperature to  $4^{\circ}\text{C}$  higher (for the acceleration of gamete maturation). The evaluation of conditioning is done by routine determination of gonadal indices.

A series of conditioning studies were conducted at the BBSR hatchery, and provides the basis for the standard protocol followed for the calico scallop. These studies demonstrate that the required length of the conditioning regime depends on the gametogenic state of the scallops upon collection. For scallops with an initial gonadal index  $<1$ , both phase 1 and 2 have a duration of 30 days; this regime yields an index approaching 3 by the end of the 60-day conditioning, with 100 percent ripe oocytes (determined by histological analyses) following Phase 1. For scallops with an initially higher gonadal index ( $>1$ ), maintaining scallops in lower than ambient temperature has shown to accelerate gametogenesis within the first two weeks, exceeding the developmental rate of scallops from the wild. Gonadal index doubled in this time. However, for these scallops, Phase 2 should probably be shortened as atretia (or resorption) of oocytes was observed during the latter part of this phase. Spawning induction results show an increased fecundity for conditioned scallops, if atretia is avoided.

The conditioning protocol followed at the Bermuda hatchery, therefore, allows for advancement of the spawning period, by acceleration of the later stages of gametogenesis. Use of gonadal indices is found to be a practical tool supplying reliable information on the reproductive stage of the calico scallops, and may be used as a basis for the timing of spawning induction in the hatchery. In Bermuda, it has been found that a gonadal index of 2 is required for successful spawning induction of calico scallops.

The procedure used in Bermuda is given in detail in Protocol-3.

### PROTOCOL-3

#### CONDITIONING OF CALICO SCALLOP BROODSTOCK

##### **Preparation for procedure**

1. Collect 50 scallops from the grow-out site, and transport back to the hatchery in coolers filled with ambient seawater.
2. Upon arrival at the hatchery, transfer 40 scallops to pre-chilled broodstock tank ( $T= 15^{\circ}\text{C}$ )

3. Remainder 10 scallops are maintained in holding tank at ambient seawater for 24 hours. Use this sub-sample for assessment of gonadal and muscle indices the following day.
4. Feed broodstock with a mixture of two or three algal species, depending on availability. Clean 20-litre carboy with jet of fresh water and chlorine, taking extra care to clean drip-feed set up (stopcock valve and tubing). Fill 20-litre carboy and open drip-feed.
5. Record temperature before leaving the hatchery.
6. Do daily checks, recording temperature, flow rate, algal ration supplied and record in data sheet as sampled in Appendix 3.
7. Two weeks later, collect a second sub-sample of 10 scallops from conditioned broodstock for gonadic and muscle indices. Keep scallops in holding tank, not fed for 24 hours prior to dissection.
8. If gonadic index assessment results in  $GI < 2$ , maintain broodstock at  $T = 15^{\circ}\text{C}$  for another two weeks. Skip to 10.
9. If  $GI > 2$ , transfer broodstock into an ambient seawater tank. Following 24 hours, increase temperature by  $1^{\circ}\text{C}$  every two days, so as to reach  $22^{\circ}\text{C}$  within one week. Maintain high temperature for one week and induce scallops to spawn.
10. From scallops in  $15^{\circ}\text{C}$  tank, collect another sub-sample of 10 scallops after two more weeks of conditioning (or 4 weeks after start of trial) for gonadal index assessment. GI should be close to 2.
11. Transfer the broodstock into an ambient seawater tank. Following 48 hours, increase the temperature by  $1^{\circ}\text{C}$  every two days, so as to reach  $22^{\circ}\text{C}$ .
12. Maintain the broodstock at  $22^{\circ}\text{C}$  for a period of 30 days. Continue the same feeding regime. Following 30 days, collect a sub-sample of scallops for determination of gonadal indices. At this time, gonadal index should be above 2.
13. Stop feeding for 24 hours. Induce spawning.

### 1.3.3 Spawning induction of scallops

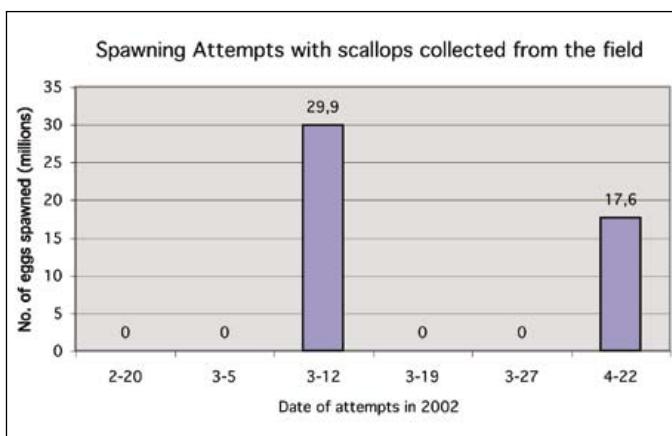
To date, thermo-stimulation is agreed to be the most efficient method for inducing sperm and ova release (Monsalvo-Spencer, Maeda-Martinez and Reynoso-Granados, 1997), especially in pectinids where a critical temperature range or minimum threshold temperature has been most frequently implicated in the initiation of spawning (Moyer and Blake, 1986; Barber and Blake, 1983). This induction of gamete release by temperature proved most efficient for the calico scallop in Bermuda; it yields a relatively rapid (90 minutes following induction) and efficient (70–90 percent) response to release gametes, compared to responses seen in other species Monsalvo-Spencer, Maeda-Martinez and Reynoso-Granados, 1997). This same method proved even more efficient when applied to the sand scallop, when 90 percent of the animals released both male and female gametes within 20 minutes induction.

The protocol utilized at the Bermuda hatchery differs slightly for both scallop species. Differences lie mainly in the degree of thermal shock provided, and length of shock required. *E. ziczac* is more sensitive to stress, and hence requires a lower thermal differential than *A. gibbus*. For both species however, the strategy is the exposure to an initial cold shock, followed by an exposure to a warm shock. Both species release gametes when exposed to warmer seawater temperatures.

As mentioned in the previous section, scallops are induced to spawn when observed to be ripe or close to ripe following methods outlined above. It must be added in this section that the sand scallop responds readily to a thermal shock by releasing gametes; although this response is caused by stress, and release of gametes may occur at times when gametes are not mature. On the other hand, the calico scallop is found to be more

tolerant of stress, such that although gametes may be determined to be ripe ( $GI>2$ ), release does not always occur following thermal stimulation. This was seen on several occasions in the hatchery over a 4-year period. Figure 1.8 outlines the number of attempts made from February 20 to April 22 in 2002, with scallops showing a  $GI>2$ . As can be seen, only 2 out of 6 attempts resulted in release of eggs within 2 hours of induction. It has also been observed that response can also be very slow with the calico scallop, and that a time of up to 5 hours in a warm water immersion may be necessary for gamete release. Nonetheless, this lack of consistent response for animals known to be ripe renders further investigating of what cues initiate spawning, worthwhile. This is especially true for commercial aquaculture applications, where control of timing of spawning events is necessary.

Protocol-4 outlines in point form, the procedure followed for spawning induction of both species in Bermuda. Generally, extreme care is taken to ensure cleanliness of all equipment and seawater lines used during spawning and larval rearing. All equipment is washed with commercial grade bleach, rinsed abundantly with fresh water, and given a final rinse with filtered seawater (1  $\mu\text{m}$ ). Seawater used for spawning baths and for collection of gametes is filtered twice to 1  $\mu\text{m}$ . Set up of the heating system is primordial, as it may take approximately 2 hours to obtain heated seawater for filling of rearing tanks. A saltwater table and bench space are prepared for collection of gametes and counting of eggs prior to distribution into tanks. In both species, sperm release usually occurs first, appearing as a milky white stream. Care must be taken when spawning a hermaphroditic species to avoid self-fertilization. For this reason, attributing a number to a scallop, and keeping the same number as it switches from male to female is important. Scallops are labelled as they begin to spawn; such that the first male is labelled as male 1 on beaker. As soon as egg release is noted (orangey-pink in colour), the scallop is removed from the beaker, the contents of which are discarded, the scallop rinsed, and transferred to a new beaker, labelled as female 1. In this way, sperm from other scallops is taken to fertilize this female. Scallops are changed regularly into new beakers with clean seawater as they spawn, as solutions become very cloudy, and it becomes difficult to observe a change in gamete release (Figure 1.9). As animals are transferred to new beakers, care must be taken to also transfer the label. Sperm or egg solutions are pooled into a larger beaker or bucket for use at a later date. As the spawn continues, initial sperm may be discarded, as it is advised that sperm  $<30$  minutes old be utilized for fertilization. Two or three males may be pooled, and such a pool may be



**Figure 1.8:** Spawning attempts with calico scallops, *A. gibbus*, collected from the grow-out sites.



**Figure 1.9:** Isolating sand scallops, *E. ziczac*, once gamete release is initiated.

used for fertilization, ensuring that the sperm utilized does not correspond to females of same individual. Calico scallops can continue releasing sperm for as long as 3 hours, but normally switch as females after approximately 1 hour.

In order to further avoid self-fertilization, addition of sperm to an egg solution is done quickly; as soon as release of eggs is constant and solution in the beaker appears pink. This precaution is advised as scallops may switch back to male spawning unexpectedly, and result in self-fertilization. The volume of sperm added must be noted, as a lower ratio of sperm to eggs has been found favourable to subsequent fertilization and development rate (Gruffyd and Beaumont, 1972). Fertilization rate is enhanced by gentle mixing, using a homemade plunger. In Appendix 8 details of plungers used are shown. Egg counts are made using a Sedgewick-rafter cell, on pools of fertilized eggs. Development to D-larval stage is optimized, by eliminating debris from the egg solutions and distributing the egg solution to larval tanks prior to multi-division stage. As seen in Figure 1.10 a fertilized egg can be recognized by the presence of a fertilization membrane surrounding the entire egg. When fertilized, the egg undergoes meiotic division, at which time two polar bodies are released. For both the zigzag and calico scallop, a round membrane showing successful fertilization is seen approximately 15 minutes after the addition of sperm. The sequence of events was timed for *E. ziczac*. The first polar body is seen approximately 25 minutes after fertilization. The second polar body is observed 15 minutes later. Cell division, two- and three-celled stages, occurs approximately 75 minutes after the addition of sperm (Figure 1.10). From here on, division continues rapidly. This sequence and timing of events is similar to that reported by Costello *et al.* (1973) for *A. gibbus*, and concurs with the observations made at the BBSR hatchery for this species.

Distribution of eggs in the culture tanks is preferably done at the time of the two-celled division stage. Fertilized egg solutions are passed through a 150 µm sieve prior to suspension to eliminate debris and/or large clumps of eggs. A more complete procedure for removal of debris is the rinsing of eggs within 20 minutes of fertilization; eggs are passed through a 105 µm screen and collected on a 35 µm screen. The eggs are rinsed with filtered seawater while on the smaller screen to remove additional debris and excess sperm. The smaller screen should be submerged in a tray of water, so that the eggs are not pressed against the screen, as they are collected. After the fertilized eggs are rinsed, they are re-suspended in a known volume of filtered seawater. Sub-samples are collected to estimate the number of eggs collected. Care must be taken that the distribution of eggs into the larval tanks must be done prior to the multi-

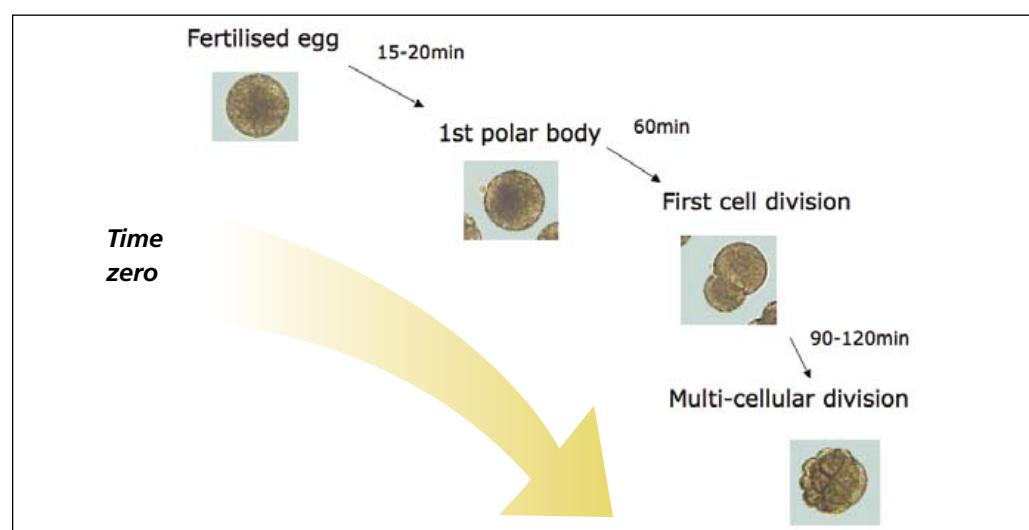


Figure 1.10: Sequence of events following fertilization of *E. ziczac* eggs.

cellular division stage. At this point, eggs become more fragile, and development may be hindered if eggs are passed through a sieve. It is therefore advised that should distribution occur later on, the process of passing eggs through a sieve be eliminated.

Finally, the keeping of records for all procedures, including transfer times between baths, first release of sperm, egg counts, etc., is important in understanding any problems arising at a later date.

#### PROTOCOL-4

#### SPAWNING INDUCTION

1. Set up heating tank (see Appendix 4).
2. Install a second 1 µm filter cartridge and housing inline with 20 mm ID reinforced hose for ease of supply to seawater baths and receiving beakers.
3. Install a 1 µm filter cartridge and housing inline to larval tanks.
4. Fill three trays with double filtered 1 µm seawater.
5. Prepare one cold-water bath ( $14\pm1$  °C for sand scallop,  $11\pm1$  °C for calico scallop). Fill 1 litre zip-lock bags with ice cubes, and place in seawater tray. Once temperature is reached, remove all bags except one to maintain temperature constant.
6. Prepare two warm water baths by filling with 1 µm filtered seawater and immersing 1 250 W aquarium heater in each tray (see Figure 1.11).
7. Prepare beakers for collection of gametes. Fill as many trays as possible (at least 4) to 1/3 with heated 1 µm filtered seawater. Place one heater per tray so as to maintain temperature at 25 °C.
8. In each tray, place a maximum number of 2 and 3 litres beakers filled with heated seawater.
9. 4 litres beakers and/or 1 000 ml graduated cylinders are kept empty for pooling of gametes.
10. Once trays and beakers are ready, start filling larval tanks with double filtered 1 µm seawater.
11. **1<sup>st</sup> cold-water shock** – 30 scallops are selected for spawning. All scallops are placed in the cold-water bath for a period of 30 minutes. Record time and temperature
12. **1<sup>st</sup> warm-water transfer** – Scallops are divided into two and transferred to a warm-water bath for a period of 1 hour. (For sand scallop, response usually occurs within 20 minutes of this first warm water bath).
13. **2<sup>nd</sup> cold-water shock** – Following 1 hour, transfer animals back to cold-water bath. Make sure that water is clean (free of detritus or faeces), and temperature is same to initial cold-water bath. If necessary, prepare a new bath while scallops are in warm water. Record time and temperature. Similarly this cold-water shock only lasts 30 minutes.
14. **2<sup>nd</sup> warm-water shock** – Transfer the batch of scallops to the warm-water bath for a second time, and leave for a period of at least 2 hours. Record time and temperature. Calico scallops usually respond following the first hour of warm-water bath. Should no response be seen, attempt a 3<sup>rd</sup> cold-water shock, and a 3<sup>rd</sup> warm water shock; start flow of water in warm-water bath, and leave scallops. Check for gamete release for next 5 hours.
15. Once a scallop is seen to release gametes, leave for a few minutes in water bath. This may trigger spawning in other individuals. Remove the scallop, rinse with 1 µm filtered seawater to clean off sperm or eggs from shell, and thus preventing self-fertilization. Place scallop in a beaker, label and allow spawning to continue.

16. Once solution is cloudy, transfer scallop to new beaker. Keep sperm until no longer required.
17. Once scallop switches to female, rinse scallop and transfer to new beaker. Discard previous solution if suspecting self-fertilization. Label female with same number as initially given to male.
18. When solution becomes orange-pink, add a mixture of sperm using a Pasteur pipette to egg solution – 1 ml of sperm: 1 litre of eggs.
19. Mix sperm with egg, by a gentle up and down motion with plunger (see Appendix 8 for details).
20. Remove scallop from egg solution, and place into new beaker when solution becomes too thick.
21. Pool two or three egg solutions into a 10 litres bucket.
22. Count eggs from pools – Take a known volume of egg solution with an Eppendorf pipette preferably; 100 µl aliquot is usually sufficient and place on a Sedgewick-Rafter cell. Count using a compound microscope. Start at one end of the cell, scanning up and down, to avoid counting the same egg twice. Do triplicate counts (see Figure 1.12).
23. Calculate number of eggs as follows: (number of eggs.ml<sup>-1</sup> x volume (l) of beaker or bucket) x 1 000.
24. Distribute calico eggs at 15 eggs.ml<sup>-1</sup> and zigzag eggs at 10 eggs.ml<sup>-1</sup> to larval tanks. No aeration is required for the first 24 hours.
25. For distribution to the rearing tank pass fertilized eggs gently through a 150 µm sieve before they reach the multi-division stage.
26. Once all eggs are distributed in tanks, transfer spawning animals to outdoor holding tank. Ensure a high water flow. Allow complete release of gametes.
27. Wash all equipment with commercial bleach solution and rinse with fresh water.
28. Heating tank unit and hatchery pipelines are cleaned following the procedure in Appendix 7.



**Figure 1.11:** Warm water bath set-up for spawning induction of scallops.



**Figure 1.12:** Measuring eggs or larvae on a Sedgewick-Rafter cell.



## Chapter 2

# Algal cultures: facilities and techniques

<b>2.1 ALGAL CULTURE FACILITIES .....</b>	37
2.1.1 Algal culture container .....	38
2.1.1.1 <i>Elevation and connection to outside</i> .....	38
2.1.1.2 <i>Floor plan</i> .....	38
2.1.1.3 <i>Ceiling plan</i> .....	41
2.1.1.4 <i>Details of air supply and 100 l culture vessels</i> .....	41
2.1.2 Chamber unit for master cultures .....	43
<b>2.2 SCIENTIFIC BACKGROUND – LIVE ALGAE AS FOOD .....</b>	43
2.2.1 Algal growth and composition .....	45
2.2.1.1 <i>Selecting algal species</i> .....	45
2.2.1.2 <i>Requirements for algal cultures</i> .....	47
<b>2.3 TECHNIQUES – GROWING ALGAE .....</b>	48
2.3.1 Master cultures .....	48
PROTOCOL-5 – Preparation of culture flasks (125 ml – 500 ml) .....	49
PROTOCOL-6 – Inoculation of 125 ml master cultures .....	50
2.3.2 500 ml batch cultures .....	52
2.3.3 4 litres batch cultures .....	52
PROTOCOL-7 – Inoculation of 500 ml flasks .....	53
PROTOCOL-8 – Inoculation of 4 litres flasks .....	54
2.3.4 100 litres cultures: semi-continuous method .....	55
PROTOCOL-9 – Inoculation and semi-continuous culture of 100 litres vessels .....	56
2.3.5 Monitoring of algal cultures .....	57
PROTOCOL-10 – Estimating cell density using a haemocytometer cell .....	57
2.3.6 Alternate feed for spat .....	58

### 2.1 ALGAL CULTURE FACILITIES

The algal culture facility is a vital part of an aquaculture operation. Extreme care must be taken to ensure the production of healthy monocultures of selected algal species. Algae are harvested from large vessels whose production is reliant on a reservoir of small master or stock cultures. It is crucial to maintain this reservoir free of contaminants and excessive bacteria. Contamination of algal cultures may occur via the seawater supply, air supply and cross-contamination from nearby algal cultures. For this reason, master cultures are maintained in a separate unit from the larger culture containers. Cleanliness and careful transfer techniques cannot be overemphasized in maintaining a functional and healthy algal culture operation. The following section describes the facilities set up for larger cultures, and the smaller incubation unit used for master cultures. The largest

tanks used in Bermuda for algal cultures are 100 l vessels; these are relatively small compared to commonly used tanks of 1 000 to 5 000 l. Techniques, however, remain similar and those used in Bermuda are described in detail in Section 2.2.

### **2.1.1 Algal culture container**

Large scale algal cultures are reared in a 3.7x2.4 m fiberglass container. Four concrete piers make up the supporting corner structures for the container. Steps are constructed with concrete blocks for access to the front door. Coarsely filtered ambient seawater is supplied from the pump house, and passes through an independent filtration system, affixed to the external wall of the algal container.

#### **2.1.1.1 Elevation and connection to outside**

Refer to Technical Drawing – page 9. Sand filtered incoming seawater, diverted from the main supply line by a Y-junction (see technical drawing – 1/Pg4) and regulated by Valve Y, passes through a 40 mm pipe that connects to the filtration system. Valve Q (40 mm), located prior to the 25 µm coarse filter, allows a further control of seawater flow. The fine filtration system depicted here is typical of the system used throughout the complex and described in detail on the technical drawing diagram – 1/Pg5A. It consists of a coarse filter (25 µm) and two in-line cartridge filters of 10 µm and 1 µm; unions are fitted on either side of the cartridge housings, for ease of cleaning and replacement. Seawater supply line is reduced after the 25 µm coarse filter and prior to the finer cartridge filters. Filtered seawater (1 µm) is passed through a 20 mm pipe fitted through the container wall for supply to the algal culture vessels. Elbows in the line are fitted when necessary, and unions are added to facilitate cleaning.

Details of the connection of the incoming water through the container wall and to the interior of the containers are shown in the int-ext connection window on the same page (see technical drawing diagram – 2/Pg9). A 20 mm hole drilled through the container wall is fitted with a thru-hull fitting and made water tight with caulking adhesive. Immediately after entry into the container, a 20 mm union is fitted for ease of cleaning. The incoming seawater pipe is fitted with a 20 mm to 40 mm bushing, leading to a 40 mm coupling and pipe for connection to a Lifeguard vertical UV sterilizer. On the outflow side, the UV sterilizer is fitted with a 25 mm threaded female, connecting to a reducing 25 mm male adapter (25 mm NPT x 20 mm hose barb). In this way a 20 mm flexible hose may be adapted for supply of UV disinfected water to 4 l flasks and 100 l vessels. *Details of the UV connection are illustrated in the technical drawing diagram – 3/Pg10.* It is found that the UV sterilizer improves water quality for algal cultures by eliminating potential bacterial contamination; as efficiency of “sterilization” or disinfection is related to the speed of water flow, care is taken in providing a slow water flow through the UV sterilizer.

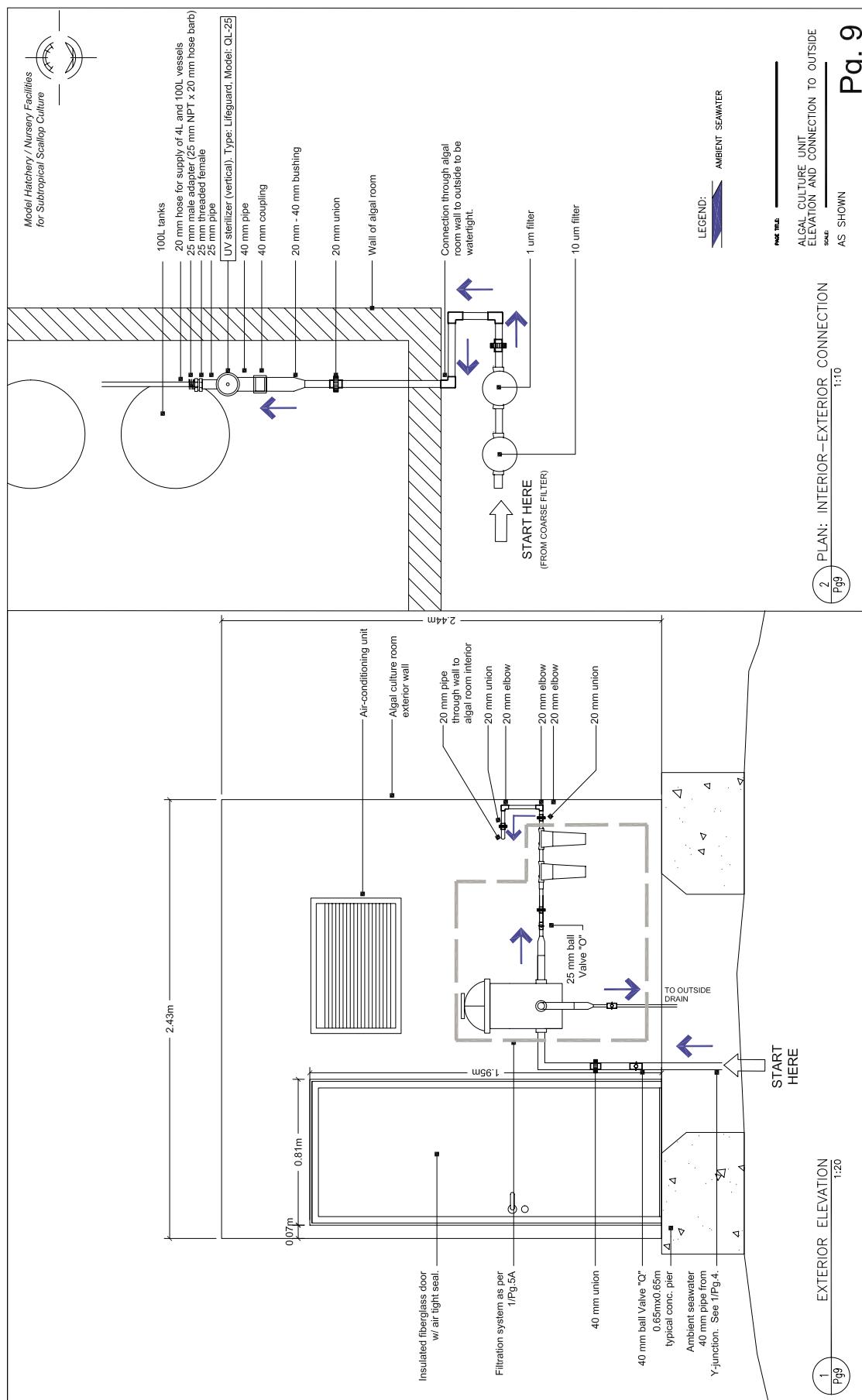
#### **2.1.1.2 Floor plan**

Refer to Technical Drawing – page 10. Climate control in the algal culture facility is maintained using an air condition unit affixed to one of the walls. This area must remain dry at all times to minimize proliferation of bacteria and molds. When required seawater and fresh water are supplied through flexible hoses adapted to their respective outlets. When not in use, both hoses are dismantled, leaving a clean and dry area.

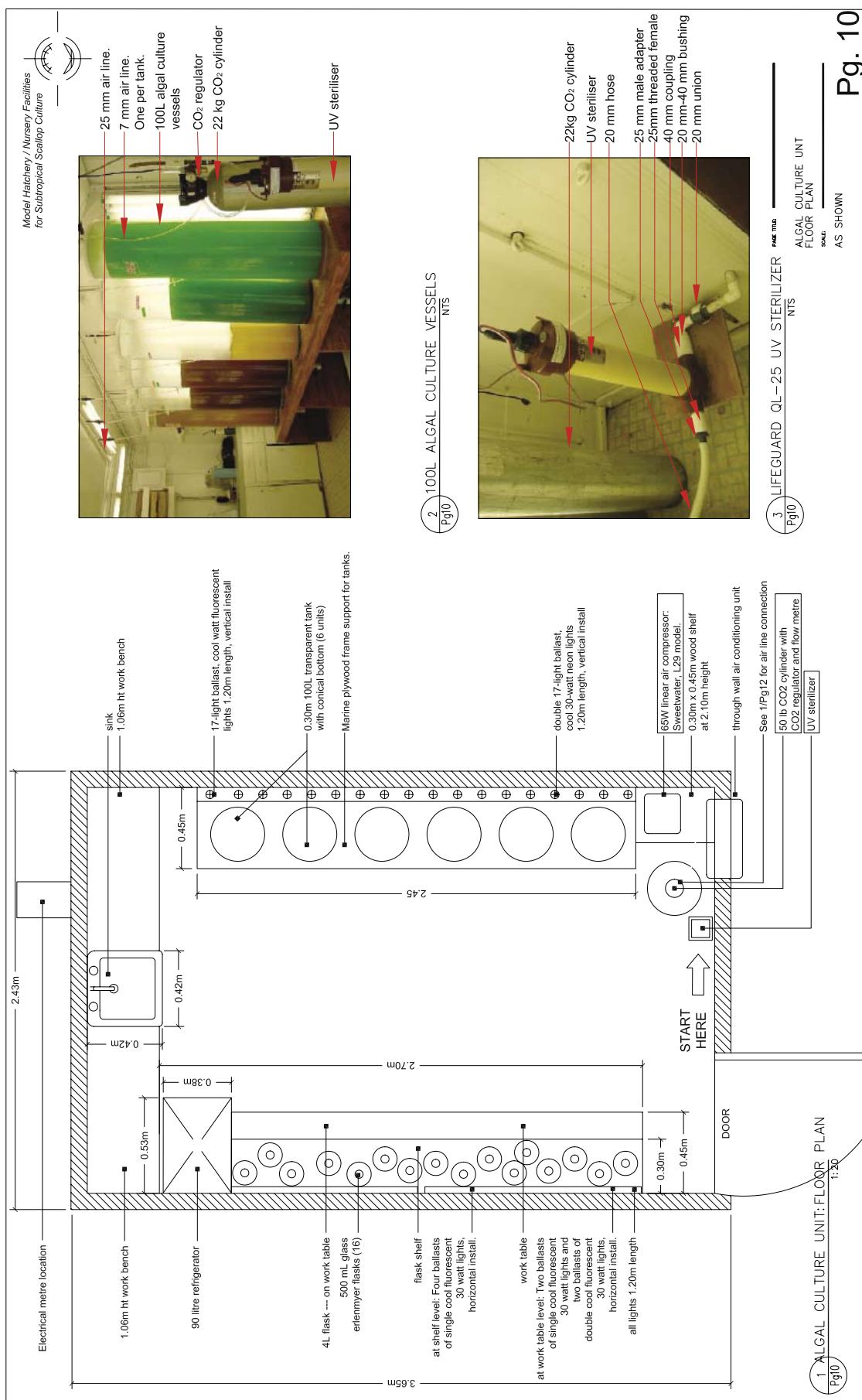
The unit floor plan shows the position of the equipment necessary for serial inoculations from 500 ml cultures to large-scale algal cultures. In brief, 500 ml flasks are used as inoculum to 4 litres flasks, which in turn are used to start-up or boost 100 l cultures. Basic requirements are access to fresh water for cleaning of culture vessels, a sink for draining of fresh water or seawater, and UV sterilized seawater. Various

## Technical drawing, Pg. 9

### Algal culture unit: Elevation and connection to outside



## Technical drawing, Pg. 10



methods are used for algal cultures; at the BBSR hatchery, it is found that aeration of cultures with an addition of CO<sub>2</sub>, for maintenance of constant pH, yields best results. For this reason, an airline is present providing a mixture of air and CO<sub>2</sub> to every algal container.

At the entrance of the facility (on the right) is situated the UV sterilizer affixed on a 0.09 m<sup>2</sup> shelf to the container wall. The flexible hose (20 mm ID) used for supplying incoming seawater should be long enough to provide seawater the entire facility. Above and to the right of the UV sterilizer is a second shelf, supporting an air compressor (Sweetwater, 65 W); it supplies air to the culture vessels in the facility. Below the air compressor, on the floor, is a 22 kg CO<sub>2</sub> cylinder for the addition of carbon dioxide to the air supply. The connection of the CO<sub>2</sub> tank to the airline is shown in detail in technical drawing – page 12. Six 100 litres tanks are located along the right wall of the facility. These transparent tanks with conical bottom are placed on a wooden frame, with access to the drainage valve below the frame (see technical diagram – 2/Pg10). *Details of the drain are in the technical drawing diagram – 3/Pg12.* A work-bench, useful for inoculating small cultures (500 ml and 4 l) is located at the far end of the container. In the middle of the counter is a sink allowing for drainage or overflow of seawater when not in use. Going counter clockwise, a small refrigerator is used for storage of chemicals needed for algal cultures. On the left hand wall, a counter top, with drawers beneath it for storage of small materials, supports 4 l flasks. Above it a 30 cm wide shelf is fixed to the wall for the holding of 500 ml flasks. Lighting is used 24 hours a day for rearing of algal cultures in Bermuda. A light bank is fixed to the wall behind the 100 l tanks, consisting of 17 vertical ballast of cool 30 Watt fluorescent light of 1.2 m length. For the 500 ml flasks, 4 ballasts of single cool fluorescent 1.2 m lights are installed horizontally. For the 4 l flasks, a total of 4 ballasts, 2 with double 1.2 m lights, and 2 with single 1.2 m lights are installed horizontally.

#### **2.1.1.3 Ceiling plan**

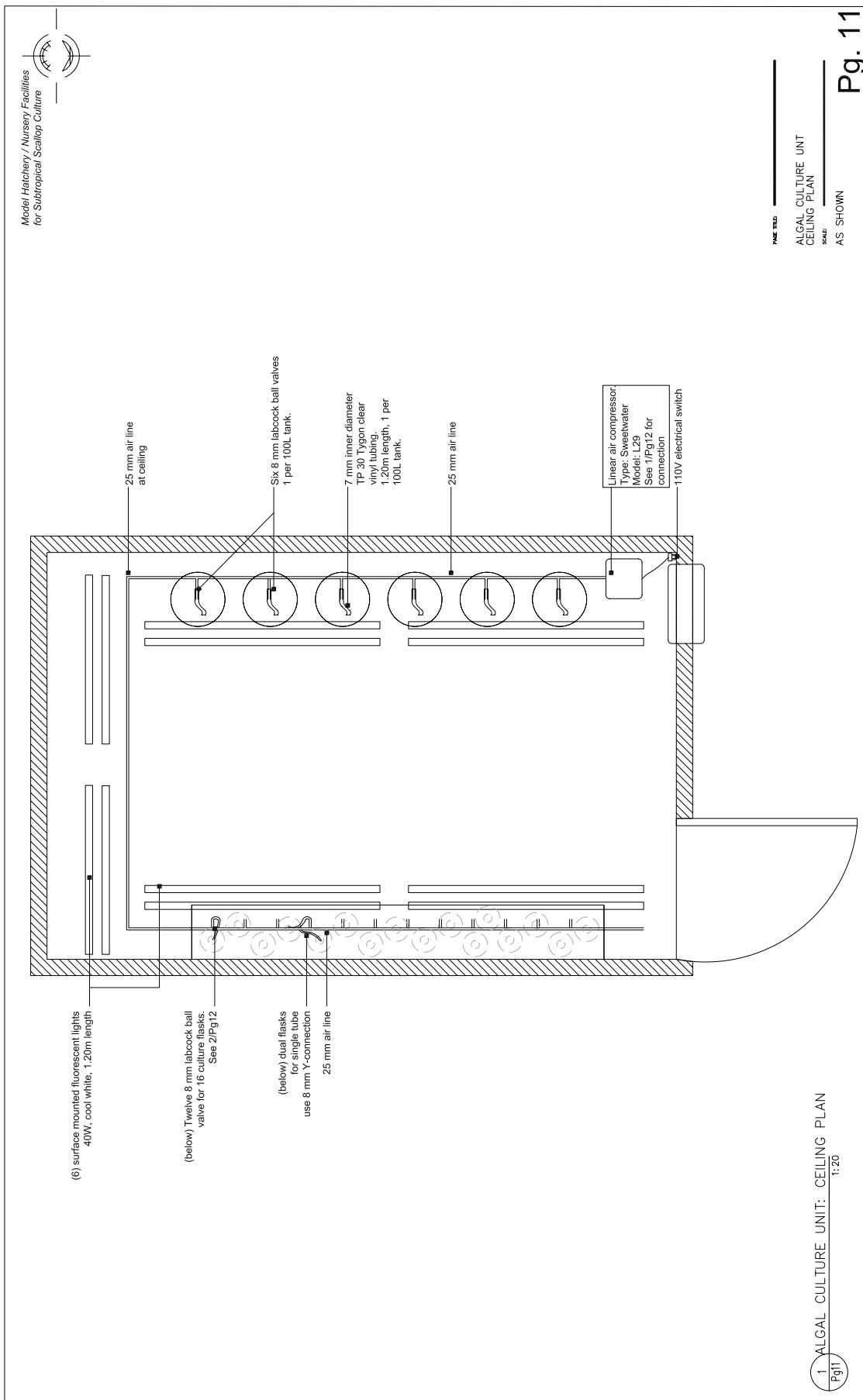
Refer to Technical Drawing – page 11. The ceiling plan outlines the contour of the 25 mm airline. Elbows are glued at the corners of the airline. The airline is secured to the ceiling by plastic clamps. In the airline, 8 mm holes are drilled and threaded with an 8 mm NPT tap drill bit and 8 mm NPT thread cutting tap. To these, an 8x8 mm NPT nipple is connected to an 8 mm labcock ball valve. This is similar to air connections described for the hatchery/nursery complex in Chapter 1 (see technical drawing diagram – 2/Pg7). There is one connection for every 100 l tank; for the 500 ml and 4 l flasks, one connection serves two flasks, joined together using a Y-connection (8–6 mm). *Details of the air connection for the flasks are shown in the technical drawing – page 12.* A total of eighteen air connections are available, allowing for twelve 500 ml flasks, twelve 4 l flasks, and six 100 l tanks, respectively.

#### **2.1.1.4 Details of air supply and 100 l culture vessels**

Refer to Technical Drawing – page 12. All flasks and tanks should be supplied with a mixture of air and CO<sub>2</sub>. In the technical drawing diagram – 1/Pg12, mixing of CO<sub>2</sub> with air is shown, and is further illustrated in technical diagram – 4/Pg12. Fittings for the Sweetwater air compressor are included with this model; the outlet is fitted with 15 mm flexible hose, secured with aluminium rings, and connecting to the 15 mm airline pipe with a 15 mm hose nipple and female fitting into a 15–25 mm bushing. From this bushing, air is directed through to a 25 mm elbow for supply throughout the container via a 25 mm airline. The CO<sub>2</sub> cylinder, located nearby, is fitted with a regulator including a flowmeter, and controls CO<sub>2</sub> input into the airline. From the flowmeter, a 7 mm ID Tygon tube connects to an 8 mm ID. tube leading to a hose barb x 8 mm male adapter fitting. The male adapter is threaded into the air supply via the 25 mm elbow depicted; in this way, CO<sub>2</sub> is mixed with the air supply from the

## Technical drawing, Pg. 11

### Algal culture unit: Ceiling plan



compressor. The technical drawing photo – 4/Pg12 shows the air/CO<sub>2</sub> connection. The general rule is that the air supplied should consist of 0.5–5 percent CO<sub>2</sub>. It is found that one 22 kg bottle of CO<sub>2</sub> lasts 5–7 days of continuous bubbling for all tanks.

Technical drawing diagrams – 2/Pg12 and 5/Pg12 show the air supply to individual culture vessels. Air mixture to each culture vessel is regulated by an 8 mm labcock ball valve (shown in the technical drawing photo – 4/Pg7). From the labcock valve, an 8 mm NPT x 7 mm barb male adapter connects to a 7 mm ID Tygon tube, directly supplying the algal culture. Bacteria filters are placed inline prior to entry into the flask, and help minimize air-borne bacterial contamination. The technical drawing photo – 5/Pg12 shows labels for these connections. There is sufficient pressure from a single valve to supply air to two flasks; the line can be divided if needed, by use of a Y-junction (8–6 mm). To ensure a tight connection, the smaller inner diameter tubing should be heated using a propane torch to fit the Y-junction.

Details of the 100 l vessels in technical drawing – 3/Pg12 describe the fitting of each tank for support, air supply and drainage. A wooden frame, made of marine plywood, is cut for the fitting of all tanks and is supported by concrete blocks on either end, for all tanks. Air is supplied to each tank using a similar connection to that described for the small vessels above, and Tygon tubing is connected to the bottom of the cone into an 8 mm barbed tube fitting. A drainage system is fitted to the bottom of the cones for harvesting of algae, and cleaning of tanks. A 50 mm male adapter is threaded into the base of the tank; an 8 mm hole is threaded into a 50 mm pipe for inflow of air and CO<sub>2</sub> mixture. The 50 mm pipe is reduced to 20 mm and connects to a one-way 20 mm ball valve. A 30 cm diameter lid prevents detritus from falling into the vessel.

### 2.1.2 Chamber unit for master cultures

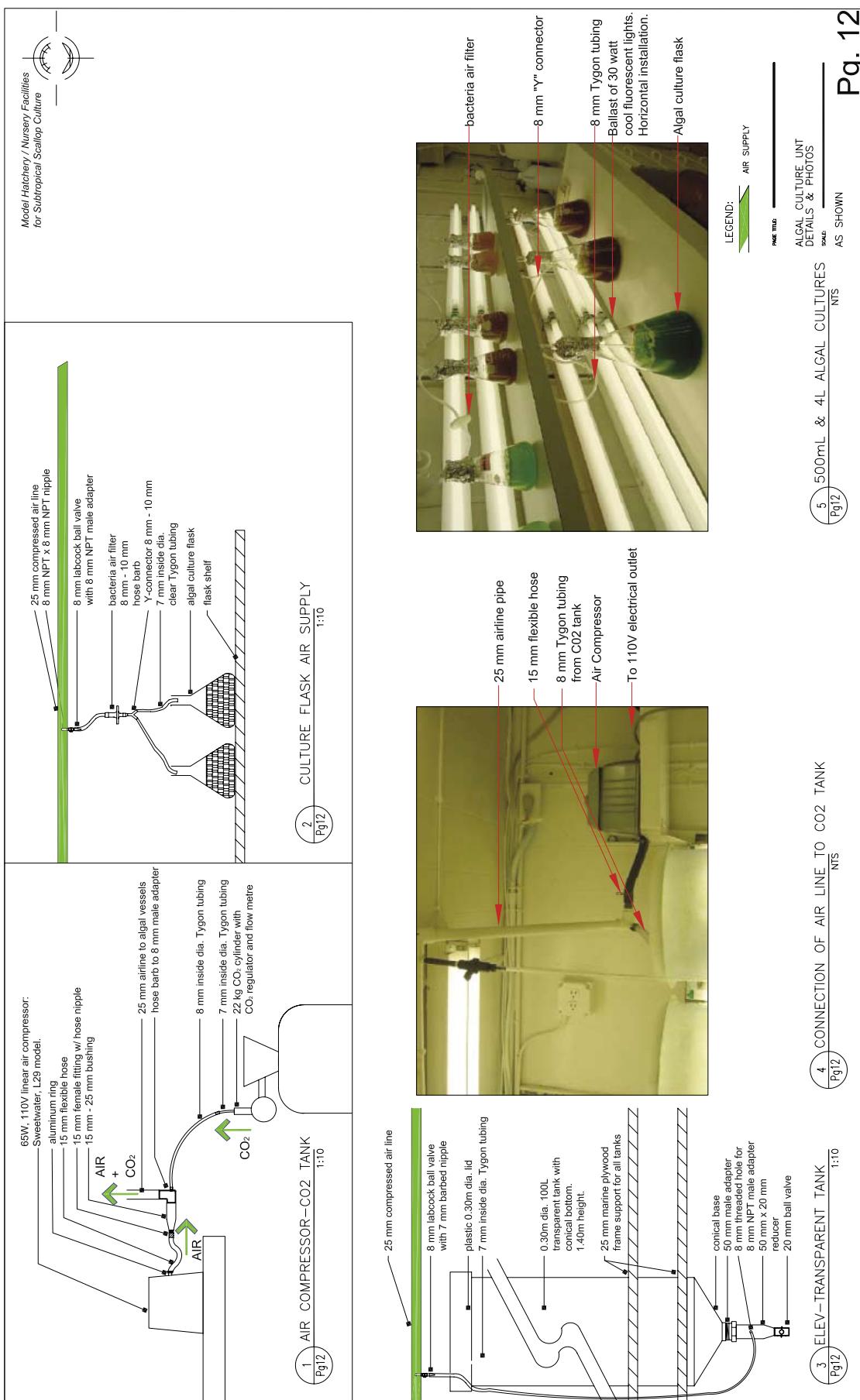
Master or stock cultures received from commercial phytoplankton suppliers arrive in 15 ml test tubes. For start-up of 15 ml test tube cultures and subsequent 125 ml Erlenmeyer flask cultures, an incubation chamber (Dual Program Illuminated Incubator 818 from Precision Scientific) is set at T= 25 °C and placed on a 12 hour illumination cycle. Next to the chamber, is an autoclave used for sterilizing small volumes of seawater, from 125 ml to 500 ml. The incubation chamber is maintained in a separate location from the large-scale culture unit. It allows for maintenance of clean stock cultures, independent of any contamination, which may occur in the larger cultures. Access to a workbench, and fresh water sink nearby allows for inoculation of cultures on a regular basis, and cleaning of culture flasks thereafter. Seawater supply differs for the stock cultures; low nutrient (and in this case “Sargasso”) seawater is used for master cultures, and treated as described below; whereas, 1 µm hatchery filtered seawater is used for 500 ml cultures and above.

## 2.2 SCIENTIFIC BACKGROUND – LIVE ALGAE AS FOOD

The algal culture facility is a most important part of a bivalve hatchery. Adequate quantities of high quality food must be available at all times for successful operation, and a failure in the algal culture facility can be catastrophic to the hatchery. Larval and adult scallops feed on unicellular phytoplankton. Although studies have shown that it is possible to grow bivalve juveniles on a non-algal diet (Langdon and Siefrid, 1984; Chu *et al.* 1987), the manufacture of artificial diets for bivalve hatcheries is still in the experimental stage. Micro-encapsulated diets (Langdon and Onal, 1999; Davis and Campbell, 1998) have been only partially successful in replacing live unicellular algae, which remain the major source of nutrition for filter-feeding organisms. In a compact

## Technical drawing, Pg. 12

### Algal culture unit: Details & photos

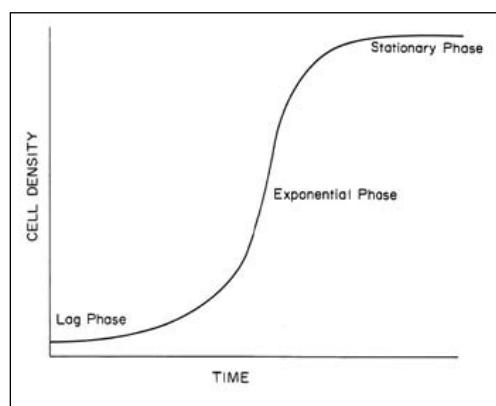


and space-restricted facility such as the one described here, the heaviest load for algal culture production is the broodstock and older spat requirements. For this reason, dry algae are purchased from Reed Mariculture, for the feeding of broodstock and 2–5 mm spat at the BBSR facility. Dry algal use is discussed in Section 2.3. Live algal culture techniques used at BBSR are described below.

### 2.2.1 Algal growth and composition

Growth of unicellular algae is by simple cell division, i.e. a single cell divides to form two cells, which then divide to form four cells, etc. Under normal conditions, an algal culture goes through three phases of growth: lag, exponential and stationary phases (Figure 2.1). The lag phase occurs when the culture is started and little increase in cell density is observed. In healthy cultures this period is quite short. In the exponential phase cell division occurs rapidly and cell density increases geometrically. Growth is limited only by the time required for cell division in this phase. In the stationary phase, the rate of growth (cell division) declines because some factor, such as nutrients or light, has become limiting and cell density remains relatively constant. During these different phases of growth the biochemical content of the algae differs. It has been shown

that higher energy levels are found in the stationary phase for most species, the diatom *Thalassiosira* being an exception (Whyte, 1987). In a hatchery situation, a balance between high cell density and optimal energy content is strived for. For this reason, the strategy at the Bermuda hatchery is to maintain cultures in the exponential phase of growth, thus remaining healthy and continuously dividing, and harvest them at the beginning of the stationary phase, when energy content peaks. Cultures are harvested within 3 days, thus remaining for a short time in the stationary phase, and preventing the presence of a large number of dying cells within the culture.



**Figure 2.1:** Theoretical growth curve of typical algal culture showing lag, exponential and stationary phase (taken from Bourne, Hodgson and Whyte, 1989).

#### 2.2.1.1 Selecting algal species

Unicellular marine algae are widely used as food in the hatchery production of commercially valuable fish and shellfish. Of the many species of algae occurring in the world's oceans, only a handful are routinely used for their nutritive quality in hatcheries. Their success as food species depends not only on their nutritional quality, but also on their tolerance to temperature, salinity and light. Because of this, algal species will exhibit different growth performance depending on the hatchery site. Knowledge of the optimal growth responses of microalgae under local conditions is of great benefit, but a first selection can be based on the literature, as much has been reported on biochemical constituency of microalgae with respect to environmental factors (Brown *et al.*, 1997; Volkman *et al.*, 1989; Brown *et al.*, 1993; Whyte, 1987). The first criteria, in selecting a range of microalgal species is thus dependent on the environmental conditions of the hatchery site itself.

Culture conditions such as, nutrient media used, temperature, light, quality of seawater, and the phase of growth (Brown, 1991; Moal *et al.*, 1987; Wikfors, Twarog and Ukeles, 1984; Dortch, 1982; Fabregas *et al.*, 1986), affect the total concentrations of protein, lipid and carbohydrate in microalgae. Selecting the right algal species is of importance, as the performance of larvae and juveniles in terms of growth and survival is dependent on the content and nature of biochemical constituents in the algal food.

Despite variations among species, protein is usually the major organic constituent, followed by lipid and then by carbohydrate. Whyte, Bourne and Hodgson (1990) stress the importance of carbohydrate in providing a balanced diet for effective conversion of dietary macronutrients to tissue and energy reserves; according to these authors nutritional condition of the larvae correlated in their study with the content of dietary carbohydrate rather than dietary lipid or protein. Microalgal species used in bivalve culture can be divided into two groups: diatoms and flagellates. Whyte (1987) found that *Chaetoceros* sp. and *Thalassiosira* diatoms had a reduced organic content compared to flagellates, such as *Isochrysis* and *Tetraselmis*; on the other hand, diatoms in general do contain higher levels of carbohydrates than flagellates. Considering the total caloric content, Whyte (1987) found that *Isochrysis* species were major sources of energy followed by *Chaetoceros calcitrans*, *Tetraselmis suecica*, *Thalassiosira pseudonana* and finally *Chaetoceros* sp. All of these species have been shown to promote excellent growth for larval and juvenile oysters (Enright *et al.* 1986) and scallops (Bourne, Hodgson and Whyte, 1989). The second main criteria in selecting algal species, is based on the species of bivalve cultured and its specific requirements. Nutritional requirements for bivalves not only vary among species, but also among the various life cycle stages (larval, post-larval, juveniles and adults); for example, size of an algal cell, presence of spines, or cell wall thickness may preclude use of an algal species as larval food. Extensive research has been conducted for some bivalve species (Davis and Guillard, 1958; Epifanio, Valenti and Turk, 1981; Wikfors, Twarog and Ukeles, 1984). Bourne, Hodgson and Whyte (1989) give a detailed account for each life stage for the Japanese scallop, *Pectinopecten yessoensis*. These authors note that young larvae may not be capable of ingesting some of the longer chained algal species, such as *Skeletonema costatum*, or larger algae such as *T. suecica*; the siliceous spines of some *Chaetoceros* species (such as *Chaetoceros gracilis*), have also been shown to be unsuitable for younger larvae. However, *Chaetoceros* sp. are used in larger larvae, as well as juveniles and broodstock; in fact, the biochemical composition of *C. gracilis*, makes it the best single algal diet for conditioning broodstock and juveniles.

Generally, in order to obtain optimum performance in bivalve culture at any stage, a mixed diet of two or three algal species, is found necessary to provide all of the essential constituents. Protein is required for tissue production, carbohydrate for metabolism, and fatty acids for lipid storage and metabolic requirements. The type of fatty acid is also an important consideration, where the polyunsaturated fatty acids (PUFAs) 20:5w3 and 22:6w3 produce the best growth for juvenile oyster and scallops (Enright *et al.* 1986; Whyte, Bourne and Hodgson, 1989). More specifically, Bourne, Hodgson and Whyte (1989) conclude that for complete complements of fatty acids for larval scallops, a mixed diet of any of the diatoms with Tahitian *Isochrysis* is optimal. It therefore becomes clear that algae with more balanced proportions of micronutrients should be of higher food value to bivalves, assuming other nutritional factors are comparable such as, cell size, digestibility, and freedom from toxins or growth inhibitory metabolites.

Species listed in Table 2.1 are commonly used in bivalve hatcheries worldwide and are recognized as suitable species in terms of ease of culture and nutritive value. Coutteau and Sorgeloos (1992) found Tahitian *Isochrysis* used in 72 percent of hatcheries, while only 37 percent of hatcheries reported using *C. calcitrans*. Despite the favourable results in several studies (O'Connor and Heasman, 1997; Peirson, 1983; Nell and O'Connor, 1991), *Pavlova lutheri* was used by only a quarter of hatcheries surveyed (Coutteau and Sorgeloos, 1992). Observations have shown a limited tolerance of this species to temperatures above 27 °C, which may explain its limited use, especially in the tropics.

**Table 2.1:** Commonly used species of micro algae in bivalve hatcheries.

Algae	Cell diameter (µm)
<b>Diatoms</b>	
<i>Skeletonema costatum</i>	6
<i>Chaetoceros calcitrans</i>	2.5
<i>Chaetoceros gracilis</i>	6
<i>Phaeodactylum tricornutum</i>	5
<i>Thalassiosira pseudonana</i>	5.5
<i>Chaetoceros muelleri</i>	3
<b>Flagellates</b>	
<i>Tetraselmis suecica</i>	8.5
<i>Isochrysis galbana</i> or T-Iso	5
<i>Nannochloris occulata</i>	2
<i>Dunaliella tertiolecta</i>	6.5
<i>Pavlova lutheri</i>	5
<i>Tetraselmis chuii</i>	8.5

The algal species cultured at the BBSR hatchery over the course of four years for the rearing of *Euvola ziczac* and *Argopecten gibbus* are: Tahitian *Isochrysis*, *Isochrysis* sp., *C. calcitrans*, *C. gracilis*, *Chaetoceros muelleri*, *T. pseudonana* (clone: 3H), *T. suecica* and *Tetraselmis chuii*. Several studies were conducted at the Bermuda hatchery, evaluating growth performance of algae. From these in-house trials, it was found that *Isochrysis* sp., *C. muelleri* and *T. chuii* were best suited to Bermuda's culture conditions. Furthermore, these species yield satisfactory growth rates for larval and post-larval stages. Due to space limitations in Bermuda, it is found

more manageable to culture a few species, rather than overextend the algal culture facility by rearing a larger number of algal cultures.

### 2.2.1.2 Requirements for algal cultures

Several factors are necessary for algal growth. As with all plants, algae must have sufficient nutrients to support growth. For rearing high concentrations of algae, as in hatcheries, type and amount of nutrients added is crucial. There are several types of nutrient media which may be used; they can either be made using purchased chemicals, or can be purchased ready-made from aquaculture companies. The Culture Centre for Marine Phytoplankton (CCMP) website provides recipes for various culture media (F/2, L1); the Conway medium recipe is provided in Appendix 9 and was initially used at the BBSR hatchery. It is no longer used as the preparation is lengthy, although it does support excellent growth for bivalves (Sarkis, 1987). To minimize the workload, commercially available media is purchased by the BBSR hatchery; F/2 solution is easily obtained from aquaculture suppliers; supplement solutions of vitamins and sodium metasilicate are made at the hatchery. Details for the preparation of all culture media can be found in Appendix 9. Following preparation, culture media is autoclaved when used for smaller volume inoculations. Tris buffer may be added to prevent the precipitation of some chemicals during autoclaving. It also buffers the pH of the culture media during algal growth. *Note: With the F/2 purchased by BBSR, the addition of Tris buffer is not found critical.*

Factors, such as light, temperature, salinity, seawater quality, mixing and cleanliness are essential to algal growth. Attempting to achieve as constant culture conditions as possible will favour optimal algal growth, which will furthermore affect their biochemical composition. As mentioned previously, this is an important consideration in growth and survival of bivalve larvae and juveniles. Ensuring that harvest of algae is conducted at the same phase of growth, is a second constant to be achieved during routine culture procedure. Some of the more important criteria for successful algal growth are reviewed below, but more detailed accounts can be found in the literature.

Light is normally provided by fluorescent lamps. The most commonly used are “cool white”, but little difference has been reported when using others such as gro-lux or full spectrum (Bourne, Hodgson and Whyte, 1989). Increasing the light intensity usually means better growth and faster division of algal cells. Lamps also generate heat, and

hence climate control of the algal culture housing facility is important. Most types of algae grow well between 17–22 °C. Tropical species are chosen at the Bermuda hatchery, as climate control is most easily achieved at 23–25 °C. Above 27 °C, most types of algae will die. Lower temperatures will reduce the growth rate. Ambient salinity is used at the BBSR hatchery (36 ppt), which is relatively high; nonetheless, all algal species, except for diatoms, which are reared at 25 ppt, fare well. Generally salinities between 25–30 ppt are best for the cultures of flagellates, and between 20–25 ppt for the culture of diatoms. Lower salinities can be obtained by diluting seawater with tap water or Q-water (de-ionized water). Salinity can be measured with a hydrometer or refractometer. Seawater used must be clean of unwanted types of algae and other contaminants which may feed or compete with the algae. For this reason, seawater is filtered finely and sterilized or pasteurized. There is a wide range of suitable equipment commercially available for this purpose. Chemical sterilization can be easily achieved for large volumes, if no equipment is available (see Appendix 10). Carbon dioxide is provided to algal cultures for faster growth and maintenance of high densities. Carbon dioxide is supplied from compressed gas cylinders; very little is required (0.5–5 percent) in the air supplied to the culture. The CO<sub>2</sub> should be passed through a flowmeter to facilitate the monitoring of delivery levels; the aim is to maintain a constant pH of 7.5–8.5 in all algal cultures, as algae divide and use up CO<sub>2</sub> in the culture water. The pH can be checked with indicator papers or a pH meter if available. Both the air and the CO<sub>2</sub> should be filtered through an in-line filter unit of 0.3–0.5 µm before entering the culture, as this helps to prevent other, possibly contaminating, organisms from getting into the cultures. Finally, mixing the cultures using air supply, allows all cells to be exposed to light and nutrients. Air can be supplied from a compressor or an air blower, and acts as a carrier gas for carbon dioxide. Not all aquaculturists keep smaller volume cultures mixed by bubbling. However, at the Bermuda hatchery, it is found that best results are obtained with mixing.

## **2.3 TECHNIQUES – GROWING ALGAE**

The following sections provide the protocols used at the BBSR hatchery for culture of algae from stock to 100 litres vessels used for harvest. In order to ensure a contaminant-free culture, sterile microbiological techniques are used during sub-culturing or start-up of solutions. These techniques rely on the flaming of all equipment prior to use, and the continuous working by the flame during addition of nutrients, transfer of cultures, etc. At the Bermuda hatchery, a propane bottle, fitted with a nozzle is used as a flame source. Prior to opening of any culture flasks or nutrient bottles, the torch is turned on, placed on the workbench, and left on throughout the inoculation process. In this way, equipment is continuously flamed and “sterilized” when in use.

### **2.3.1 Master cultures**

All algal culture system require a set of “stock” or “master” cultures; these are usually of about 125–250 ml in volume, and provide the reservoir of algal cells from which to start the larger-scale cultures used for feeding. Several centres, which specialize in the culture of algae can provide inoculum for stock cultures. However, it is also possible to isolate algal species from a specific body of water, and attempt to rear them under controlled conditions for feed. The isolation of single cells of a species from natural live phytoplankton samples can be done using a capillary pipette and/or via a series of dilutions; this allows the separation of a selected species into a culture chamber with nutrient media. Thereafter, algae are cultured as detailed below.

At the BBSR hatchery, master algal cultures are purchased or received from various laboratories. The Culture Centre for Marine Phytoplankton in Bigelow (CCMP) has an extensive list of algal cultures, which may be purchased. Information may be

obtained online at the following address: [www.ccmp.bigelow.org](http://www.ccmp.bigelow.org). Cultures are sent in 15 ml plastic test tubes and shipped by courier, to minimize transport time and ensure a large number of healthy cells upon arrival. The procedure for starting algal cultures and growing them to high density in 125 ml flask is described below. The following section describes the start-up and sub-culturing of 500 ml, 4-litre and 100-litre cultures.

Prior to reception of purchased cultures, 125 ml Erlenmeyer flasks are cleaned and prepared with adequate salinity seawater (see Protocol-5). The seawater used is classified as low nutrient seawater (LNSW), collected from the Sargasso Sea by oceanographers at BBSR. It is collected in 5 percent HCL cleaned 50 litres carboys from a depth of 5 m using Niskin bottles. At the laboratory, it is left to age for about 2–3 weeks to help strip out any inorganic nutrients, and then subsequently filtered through a sterile 0.2 µm mini capsule filter (Pall Corp., Item #12122). Following this procedure, no detectable nitrogen (<0.04 µm) or phosphorus (<0.03 µm) is found. Erlenmeyer flasks (125 ml) are autoclaved with seawater, for sterilizing of both flasks and seawater. Full strength salinity (36 ppt) is used for all cultures, except for the diatoms, *Chaetoceros* species and *T. pseudonana*, which fare best in reduced salinity seawater (25 ppt). Once the autoclaving process is completed, cooled flasks are ready for inoculation. LNSW is used because it is available; however, finely filtered (0.2 to 1 µm) and autoclaved seawater should be adequate.

## PROTOCOL-5

### PREPARATION OF CULTURE FLASKS (125 ml – 500 ml)

1. Wash glass flasks, Pasteur pipettes and rubber stoppers with glass rods in 10 percent HCl (hydrochloric acid) bath.
2. Rinse 3 times with fresh water and do a final rinse with Q-water (de-ionized) and let dry.
3. Fill flask with appropriate volume of filtered seawater using a graduated cylinder.
4. For reduced salinity, dilute seawater with Q-water. Use the following equation to calculate the volume of Q-water required to reduce the salinity:

$$X \text{ (ml seawater)} = Y \times \frac{\text{Total volume (ml)}}{\text{Full strength salinity}}$$

where:

X = volume of seawater added, and

Y = new salinity

Volume of Q-water required = Total volume (ml) - X

so that if full strength salinity = 36 ppt, and total volume in flask is 50 ml, and a new salinity of 25ppt was required

X = 35 ml of seawater, and

Volume of Q-water = 15 ml

5. Label flasks with reduced salinity.
6. Close flask loosely with cotton plug and aluminium foil. Cotton plugs are made with cheesecloth material tied around absorbent cotton.
7. Place flasks in autoclave and start cycle following manufacturer's directions.
8. Wrap all pipettes, stoppers and 4 aeration rods and stoppers separately in aluminium foil. Label clearly on the foil the contents and put an arrow pointing to the fragile thinner end of the pipettes as an indicator. Make sure contents are completely wrapped in foil as they will not be sterile if there are any gaps.

The transfer of purchased stock cultures from 15 ml test tubes to 125 ml Erlenmeyer flasks is described in Protocol–6. Culture media used is F/2, and preparation for small culture volumes is outlined above (Protocol–5). Microbiological sterile techniques are used to transfer master algal stocks from 15 ml test tubes to 125 ml flasks. Depending on the density of each stock culture, one test tube is used to inoculate two flasks, yielding duplicates of each stock. Addition of media to the cultures is done using sterile microbiological techniques. For diatom species, an addition of autoclaved 3 percent sodium metasilicate is added for growth of the siliceous frustule. Flasks are closed with a cotton plug and wrapped with aluminium foil. They are maintained in the incubation chamber at T= 25 °C and on a 12-hour light cycle. Flasks are swirled once a day to prevent settlement and sticking of algae on the bottom; as well as to distribute the culture media throughout the solution. These are hereon referred to as master cultures. These master cultures must be sub-cultured frequently; some hatcheries do this weekly. At the BBSR hatchery sub-cultures are done monthly. Sub-culturing involves inoculating some cells from an old stock culture into fresh culture medium. In this way cells can continue to grow and divide ensuring a healthy culture. If sub-culturing is not carried out, the algal cells in the stock culture will eventually die. It is important to take precautions to prevent contaminants from the air entering the stock cultures when sub-culturing. In this way, master cultures can be maintained indefinitely.

To start a new master culture, 10–20 ml of algae inoculum (depending on density) is taken from a master culture for inoculation of new flasks with new seawater and media. In the first instance, when cultures are inoculated with purchased stock, the lag phase is long, and it is found that an average of 1 month is required to achieve a density capable of inoculating a larger volume of seawater, as density of purchased stock is usually low. Protocol–6 describes the procedure utilized in the first inoculation of 125 ml master cultures with purchased stock, and the subsequent monthly sub-culturing for maintenance of master cultures.

## PROTOCOL–6

### INOCULATION OF 125 ml MASTER CULTURES

#### **Preparation of flasks**

1. Two 125 ml flasks for each start-up culture are cleaned as outlined in Protocol–5.
2. Flasks are filled with 50 ml of seawater with adequate salinity requirement.
3. Flasks and seawater are sterilized using autoclave procedures.
4. Culture media is prepared and sterilized as outlined in Appendix 9.

#### **First inoculation of 125 ml flasks using purchased stock cultures**

1. Upon receipt of stock cultures, open package and let cultures stand upright in incubation chamber, awaiting inoculation. Depending on state of received cultures, they can be left as received for 24 hours.
2. Prepare a work area, with a Bunsen burner close at hand, and F/2 solution mixed with vitamins and sodium metasilicate. Working under a hood is best.
3. Stock cultures usually come in 15 ml test tube with screw caps. Unscrew cap, keeping opening of test tube close to flame; discard cap. With other hand, hold 125 ml flask, remove cotton plug, keeping it in palm of hand.
4. Transfer stock culture to 125 ml flask without mouths of either container touching and remaining close to flame. Place cotton plug immediately back onto flask. Discard tube.

5. Label flask with algal species and date. This will allow you to maintain a tight schedule of re-inoculation.
6. Working close to flame, remove cap of culture media mixed with vitamins. Place cap on clean surface area and maintain media container close to flame. Using a sterile 1 ml pipette, remove 0.5 ml of nutrients from container. Flame mouth, flame cap and close, keeping pipette tip close to flame. Take 125 ml flask with inoculum, remove cotton plug and keep in palm of hand and add 0.05 ml of nutrients to algal inoculum ( $1 \text{ ml.l}^{-1}$ ). Replace cotton plug immediately back onto flask. Discard pipette. Swirl flask to mix nutrients and algae.
7. Using same technique, add sodium metasilicate ( $2 \text{ ml.l}^{-1}$ ) if species is a diatom.
8. Place inoculated flask with nutrients in incubation chamber. Swirl daily.

#### Maintenance of master cultures – monthly sub-cultures

1. Once a month, prepare the same number of 125 ml Erlenmeyer flasks as already inoculated.
2. Fill flasks with 75 ml of seawater adjusted to adequate salinity.
3. Autoclave and let cool.
4. Prepare a workbench as above.
5. Do all transfers using sterile microbiological techniques.
6. Using a sterile 10 ml graduated pipette and bulb, pipette 10 ml of culture, maintaining pipette close to the flame.
7. Pick up one flask (check salinity) with other hand, remove foil and cotton plug, placing it on clean area, and add inoculum close to flame.
8. Quickly close flask with cotton plug after flaming the mouth of the flask and the cotton plug. Be careful not to put cotton plug too close to flame, it will burn.
9. Repeat 2 or 3 times with same culture and same pipette, so as to add an inoculum of 20–30 ml into the new flask.
10. Label with species and date.
11. Add nutrients accordingly.
12. Swirl and store in incubation chamber.
13. Swirl on a daily basis until next inoculation.

Once master cultures are established, larger volumes of algae can be cultured using this reservoir of various algal species. There are many different ways of culturing algae. These can be divided as batch culture, semi-continuous culture and continuous cultures. Batch culture is the most traditional method used for large-scale culture in bivalve hatcheries. Large volumes of algae are grown and harvested fully once desired cell density is achieved. Each new batch is inoculated from working culture flasks. It is a simple method, and a variety of containers can be used, ranging from 20-litre carboys to 3 m diameter tanks. Semi-continuous cultures refer to a system where part of the culture is harvested and used as food, and the amount taken is replaced with fresh culture medium (clean seawater and nutrients). After allowing 2–3 days for the remaining cells to grow and divide, the process is repeated. Semi-continuous cultures may be operated for up to 7–8 weeks. These types of cultures can crash because of a build-up of contaminants, bacteria or mismanagement. Minimizing contamination from any source is critical in semi-continuous cultures. Various containers can be used for semi-continuous cultures. The most common type of container is a sterile polyethylene bag. The bags are sealed and the inside is sterile. They can be inflated with sterile air to form the shape required before filled with seawater. The bags need to be supported by a frame. At the BBSR hatchery, 100 l vessels, described in the facilities Section 2.1 are used for semi-continuous cultures.

Continuous cultures may be maintained by using turbidostat culture, and by chemostat culture. In the former, the number of cells in the culture is monitored, and as cells divide and grow, an automatic system keeps the culture density at a pre-set level by diluting the culture with fresh medium. In the latter, a flow of fresh medium is introduced into the culture at a steady pre-determined rate. The surplus culture overflows into a collecting container, from which it can be taken and used as food. These systems are not commonly used in commercial hatcheries because they are expensive, very sensitive, and difficult to install and maintain.

Depending on the requirement for algae, they can be cultured using closely controlled methods on the laboratory bench top, for a few litres of algae to less controlled methods in outdoor tanks relying on natural light conditions, and producing thousands of litres. At the BBSR hatchery, several steps are taken to ensure a daily harvest of algal food of optimal quality to larvae and post-larvae scallops. Algae are first cultured in batches of 500 ml flasks and 4 l flasks; these 4 l cultures are used in turn to inoculate 100 l vessels, reared in a semi-continuous method and used for daily harvest.

### **2.3.2 500 ml batch cultures**

Once 125 ml cultures achieve higher densities of algae ( $6\,000\text{ cells.ml}^{-1}$ ), inoculation to 500 ml flasks can be performed. These 500 ml cultures are in turn used as inoculum for 4 l cultures. Procedures for preparation, first inoculation, and maintenance of 500 ml flasks are outlined in Protocol-7. Flasks are filled with 250 ml of 1  $\mu\text{m}$  filtered seawater (collected from the hatchery). Similar salinities as to those used for 125 ml flasks are used. Seawater and flasks are autoclaved. All procedures including transfer of inoculum, addition of media, or aeration pipette are conducted using sterile microbiological techniques. The inoculum of 35 ml is transferred using sterile microbiological techniques to the 500 ml flask. The exact amount is dependent on the master culture density. The denser the culture, the smaller the volume of inoculum required. The remaining volume in the master culture is used to re-inoculate a new 125 ml master culture flask (see Protocol-6). Culture media (F/2) and vitamins are added depending on volume of seawater inoculated; sodium metasilicate is added to diatom cultures. In 500 ml flasks, aeration is provided for mixing of culture media and algae, maintenance of algae in suspension and addition of  $\text{CO}_2$  for pH stability. For this reason, sterilized Pasteur pipettes are added prior to closure of flasks with cotton plug and aluminium cover. Flasks of 500 ml are connected to the airline in the algae container. In the first instance of inoculating a 500 ml from a master culture, approximately 2 weeks are required for a high-density culture, as lag phase is longer. At this time, 200 ml of the culture is transferred to a new 500 ml flask filled with 200 ml of sterile seawater. These are allowed to grow until cell density approaches  $10\,000\text{ cells.\mu l}^{-1}$  (1-2 weeks). Thereafter, once cultures are well established and dividing rapidly, 500 ml flasks are re-inoculated twice a week; this high frequency insures a healthy culture in continuous exponential phase. Maintenance of 500 ml flasks is achieved by transferring approximately 50–100 ml of algal culture from one 500 ml flask. The remaining 250–300 ml is used to inoculate a 4 l volume.

### **2.3.3 4 litres batch cultures**

Algal cultures are reared to 4 l volumes for the purpose of obtaining a large inoculum required for the start-up of the semi-continuous 100 l cultures. Four litre Erlenmeyer flasks are too large to be sterilized in the existing autoclave at the Bermuda hatchery. For this reason, 1  $\mu\text{m}$  filtered UV disinfected seawater is used. In the described facility, the seawater supply for the algae room needs to be set up, prior to inoculating 4 l flasks. Appendix 11 indicates the step by step procedure for obtaining 1  $\mu\text{m}$  filtered UV disinfected seawater. *Note: If UV disinfected seawater is not available, it is possible to sterilize seawater chemically, by using sodium hypochlorite (or commercially available*

chlorox), and neutralize it with sodium thiosulfate (1N solution). Appendix 12 provides the protocol for chemical sterilization. This was initially used at BBSR, and yielded satisfactory results.

Flasks are filled to 3.5 litres with UV disinfected seawater and closed with a rubber stopper (no. 10). One half of the 500 ml culture is used to inoculate this volume. Similarly culture media, vitamins and sodium metasilicate (for diatoms) is added to the new solution. For aeration of 4 l flasks, rubber stoppers are fitted with (diameter) glass rods. One rod equates the length of the flask, nearing the bottom used for bubbling, and the other is shorter, acting as a vent. Aluminium foil is used to cover the stoppers. Four litre flasks are connected to the airline in the algal container. Sub-inoculation of 500 ml flasks and 4 l flasks are conducted on the same day at the BBSR hatchery, twice a week (Monday and Thursday). In this way, healthy cultures in the exponential phase of growth are always available for inoculating larger 100 l cultures. Four litre cultures are allowed to grow for a period of 3–5 days before use as inoculum for 100 l cultures.

## PROTOCOL-7

### INOCULATION OF 500 ml FLASKS

#### Preparation of flasks

1. Two flasks for each algal species cultured are cleaned in a 10 percent HCl bath. They are rinsed three times with fresh water and have a final rinse with Q-water.
2. For a first inoculum from 125 ml master cultures, fill flasks to 200 ml. Use 1 µm filtered seawater.
3. Close flask with cotton plug and foil, made as described in Protocol-6.
4. Sterilize flasks and seawater in autoclave.
5. For maintenance and sub-inoculation of 500 ml algal cultures, fill flasks to 350 ml using 1 µm filtered seawater, adjusted to required salinity.

#### First inoculation of 500 ml flasks with 125 ml master cultures

1. When master cultures increase in density, inoculate 500 ml flask (filled with 200 ml of seawater) with 35 ml of master culture. *Note: Remaining volume of master culture is used to re-inoculate a new 125 ml flask. See Protocol-6.*
2. Use microbiological sterile techniques for transfer.
3. Prepare a work area, with a Bunsen burner close at hand, F/2 solution, vitamin solution and sodium metasilicate solution. Keep 5 and 10 ml sterile pipettes and 3-way pipette bulb nearby.
4. Using a 10 ml Pasteur pipette and bulb, transfer 30–35 ml of master culture to 500 ml flask, remaining close to flame at all times. Do not touch mouth of either flask with pipette. Plug flask quickly after transfer.
5. Label flask with algal species and date. This will allow you to maintain a tight schedule of re-inoculation.
6. Working close to flame, remove cap of culture media mixed with vitamins. Place cap on clean surface area, and maintain media container close to flame. Using a sterile 1 ml pipette, remove 0.5 ml of F/2 from container (nutrients added need to equate 1 ml of nutrient per litre of culture). Flame mouth, flame cap and close, keeping pipette tip close to flame. Take 500 ml flask with inoculum, remove cotton plug and keep in palm of hand and add nutrients to algal inoculum. Replace cotton plug immediately back onto flask. Discard pipette. Swirl flask to mix nutrients and algae

7. Using same technique, add sodium metasilicate if species is a diatom at 2 ml of sodium metasilicate per litre of culture. In 500 ml flasks, 1 ml of sodium metasilicate is added.
8. Remove one Pasteur pipette, from autoclaved packet and keep close to flame. Take 500 ml flask in one hand, remove cotton plug and maintain in palm of hand, place Pasteur pipette in flask and plug.
9. Take flask to lightbank shelf and connect Pasteur pipette to airline. Regulate air bubble so as to have good mixing.

### Maintenance of 500 ml cultures

1. 500 ml cultures initially inoculated with master cultures will take some time to reach required densities (2–4 weeks depending on strength of inoculum).
2. Once required densities are obtained, 500 ml cultures are inoculated twice a week, always maintaining cells in exponential phase of growth.
3. Prepare 500 ml flasks as above, but filling with 350 ml of seawater.
4. Transfer inoculum from 500 ml flask directly from flasks using microbiological techniques, working by the flame and avoiding for the mouths of the flask to touch, thus preventing contamination.
5. Label flasks with algal species and date.
6. Add nutrients using techniques described above, but with higher volumes For F/2 add 1 ml.l<sup>-1</sup>, and for sodium metasilicate add 2 ml.l<sup>-1</sup>.
7. Add Pasteur pipette for aeration using techniques described above.
8. Connect Pasteur pipette to aeration. *Tetraselmis* sp. has a tendency to stick to the bottom, if not well aerated, so more vigorous aeration is usually required for this species.

## PROTOCOL-8

### INOCULATION OF 4 LITRES FLASKS

#### Preparation of flasks

1. Clean two flasks for each algal species cultured using commercial grade bleach (5 percent chlorox). If needed, soak in seawater and bleach solution. Rinse well in fresh water.
2. Fill flasks at time of inoculation with 3 litres of 1 µm UV disinfected seawater. If salinity needs to be adjusted, adjust using fresh water (see Protocol-5).
3. Wrap rubber stoppers fitted with glass rods, acting as aerating tubes, in foil and autoclave.

#### Inoculation of 4 l flasks with 500 ml cultures

1. When algal density in 500 ml flasks reach density >10 000 cells.ml<sup>-1</sup>, use 2/3 of culture to inoculate new 4 l flask. Note: Remaining volume of 500 ml culture is used to reinoculate a new 500 ml flask. See Protocol-7.
2. Use microbiological sterile techniques for transfer.
3. Prepare a work area, with a Bunsen burner close at hand, F/2 solution, vitamin solution and sodium metasilicate solution. Keep 5 and 10 ml sterile pipettes, and 3-way pipette bulb nearby.
4. Take 500 ml flask in one hand, remove cotton plug, keeping mouth of flask close to flame. With other hand, flame mouth of 4 l flask.
5. Transfer inoculum from 500 ml flask directly into 4 l flask; avoid the mouths of the flask to touch, thus preventing contamination.

6. After transfer, replace cotton plug quickly on 500 ml, even if discarded later. This ensures you to maintain a clean area, and prevent cross-contamination.
7. Flame stopper (used while cleaning of flask) and mouth of 4 l flask. Close flask quickly.
8. Label flasks with algal species and date.
9. Add nutrients, by pipetting required volume from stock solution, continuously ensuring that pipettes, and flasks are close to the flame. Quickly cap bottles, and transfer nutrients to 4 litres, flaming the mouth of the flask, before addition of nutrients and after. In between addition, keep flask closed with stopper. If you need to put stopper down, make sure it is put on a clean area. For F/2 add 1 ml.l<sup>-1</sup>, and for sodium metasilicate add 2 ml.l<sup>-1</sup>.
10. When nutrient addition is complete, unwrap rubber stopper with aerating rods, and flame. Replace plain rubber stopper with aerating stopper.
11. Connect aerating stopper to aeration tube on shelf by lightbank. *Tetraselmis* sp. has a tendency to stick to the bottom, if not well aerated, so more vigorous aeration is usually required for this species.

Remember:

Always use a different pipette for each algal culture species to avoid cross-contamination.

Use a different pipette for each nutrient solution (F/2, vitamin, sodium metasilicate).

**Note:** For ease of inoculation, it is best to inoculate one species at a time. Do 500 ml sub-inoculation on same day as 4 l. For example: Start with T-Iso, sub-inoculate two new 500 ml and two 4 l. Add nutrients, aeration, connect to air supply. Clean bench, discard pipettes, and work with second algal species.

### 2.3.4 100 litres cultures: semi-continuous method

The volume of algae required in hatchery operations, mainly for larval, post-larval, and broodstock purposes, is harvested daily from 100 l vessels. The set-up at the BBSR hatchery yields approximately 120 l of algae a day.

Preparation and inoculation procedure for 100 l vessels are given in Protocol–9. Similarly to 4 l flasks, 100 l vessels are filled with 1 µm filtered UV disinfected seawater. These large-scale cultures are reared on a semi-continuous cycle. This yields a culture in a continuous exponential phase of growth, and minimizes labour. As a culture is harvested daily, and decreases in volume, new water and nutrients are added; this boosts the culture growth, such that algal densities reach 12 000 cells.ml<sup>-1</sup> within three days. A first inoculum is given initially, and thereafter, addition of new water and nutrients done on a regular basis allows the culture to be maintained for at least one month or more depending on cleanliness of the culture and of the techniques.

Details of the procedure are given in Protocol–9. For a first inoculum, vessels are filled to 25 litres or 50 litres depending on the strength of the inoculum. The lower the strength, the smaller the volume of new seawater inoculated. A 4 l flask is used for inoculum. Culture media, vitamins and sodium metasilicate are added dependent on volume. Algal cultures are allowed to grow for a period of 5 days. At this time, addition of seawater is done, and vessels are filled. Cultures are allowed to grow for a period of 3–5 days; at which time, daily harvest of cultures for the hatchery complex is possible. One hundred litre vessels are harvested down to 25 litres in approximately 4–5 days. When 25 litres of culture remain, 75 litres of new 1 µm UV disinfected seawater is added with culture media. It usually takes approximately 3 days to reach harvest density. In this way, semi-continuous cultures can be maintained on the average 4–6 weeks depending on cleanliness of techniques.

## PROTOCOL-9

### **INOCULATION AND SEMI-CONTINUOUS CULTURE OF 100 LITRES VESSELS**

#### **Preparation of vessels**

1. Allocate two vessels to each algal species.
2. Clean vessels outside using a fresh water hose and commercial grade bleach. If needed, muriatic acid can be used. Care must be taken when using muriatic acid. Special attention is given to the bottom of the cone, valve area, and rim of the cone and other edges when cleaning these vessels. If needed, the bottom of the cone is soaked in seawater and chlorox solution for a few days.
3. Rinse well with fresh water.
4. Fill vessels with UV disinfected seawater prior to inoculation.

#### **Inoculation of 100 l vessels with 4 l cultures**

1. Connect 100 l vessel to airline and turn slight airflow on. It is important to do this, prior to addition of water or algae, as if there is no airflow, water will pass through the Tygon tubing and soak the bacteria filter. If this occurs, the bacteria filter needs to be replaced.
2. For a first inoculation, fill vessel to 25–50 litres, depending on the density of the inoculum. The denser the 4 l culture, the greater an initial volume of water can be used.
3. When density in the 4 l flasks reaches an algal count  $>10\,000 \text{ cells.ml}^{-1}$ , use culture to inoculate 100 l vessel. *Note: Take care not to use the very bottom of the culture, as it often contains some precipitate and detritus.*
4. At this time, sterile microbiological techniques are no longer required. A 4 l culture is simply poured into a 100 l vessel. Also, diatoms appear to grow well in full salinity in these large volumes, and there does not seem any need to adjust the salinity.
5. Label vessel with date and algal species.
6. Add nutrients using 25 ml graduated cylinder or 10 ml pipettes. For 100 l vessels, F/2 solution is taken directly from purchased containers; equal parts of A and B are mixed, according to manufacturer's instructions.
7. When nutrient addition is complete, place lid on top to avoid any detritus from falling and adjust aeration so as to create gentle mixing of nutrients throughout. Again, more vigorous mixing is needed for *Tetraselmis* species.
8. Allow for algal cultures to grow until dense. At this time, fill vessel with UV disinfected seawater. If volume of seawater added is 75 litres, add nutrient volume in accordance to 75 litres volume. For example: in Bermuda, 14 ml of nutrients in total is added to 100 l; during semi-continuous culture, if 75 ml of new seawater is added, only 10.5 ml of nutrients would be added.
9. Allow algal cultures to grow. When densities required are reached begin harvesting using valve at the bottom of the cone. Culture should be harvested within 6–7 days. Do not harvest below 25 l.

#### **Semi-continuous culture method**

1. Once culture volume has decreased to 25 litres, add 75 litres of new UV disinfected seawater and adequate nutrient volumes.
2. Label with date of added water.
3. Allow cultures to grow for 3 days before harvest.
4. Note: Cultures can be boosted at any time; if a culture of 50 litres volume needs to be reboosted, add 50 litres of seawater and according nutrients.
5. Although sterile techniques are not used for these large cultures, cleanliness is a must to avoid cross-contamination between vessels.

### 2.3.5 Monitoring of algal cultures

Algal cultures should be examined daily for clumps or aggregations of cells on the bottom. Colour of the culture is most important, and with experience, one can quickly determine if a culture is healthy. Microscopic examination of the algal culture should be done routinely using a compound microscope. Cultures should have cells of uniform size that are not clumped together, and are actively swimming if the species is motile (for e.g. *Isochrysis* sp., *Tetraselmis* sp.). If the cells are clumped, cell walls broken, more than one species present, or a species other than algae present, or if the culture is badly contaminated with bacteria, it should be discarded. The culture vessel should be well cleaned before next use. A bad odour emanating from a culture vessel usually indicates bacterial contamination. Algal cultures used in bivalve hatcheries are not axenic (bacteria-free). In order to have a healthy algal culture, bacterial levels must be kept under control, since they can depress growth of the algae and cause cultures to crash before reaching harvestable densities. Cultures with high level of bacteria should not be fed to larvae but should be discarded; they could be used for broodstock, if needed. If a severe bacterial contamination occurs in stock cultures, every effort should be made to clean the culture with antibiotics, or a new culture should be ordered from an algal culture centre. There are various methods for determining levels of bacteria contamination. Appendix 12 describes bactopeptone testing.

At the BBSR hatchery, a daily routine check of the algal cultures, giving results of visual inspection, is reported on an “Algal culture check” list (see Appendix 13). Monitoring of algal cultures under the microscope is done during periods of sub-culturing or harvesting. During the latter, algal cell density is estimated to calculate the volume required for feeding. There are two methods used most commonly in hatcheries to estimate algal cell density, haemocytometer and coulter counter. Coulter counters are expensive but useful machines. Sometimes used machines can be purchased from hospitals or factories. The time saved and the accuracy of the counts is superior to that when using the haemocytometer. At the Bermuda hatchery, funds were not available for a coulter counter, and a haemocytometer cell is used. This cell was initially developed to count blood cells, and consists of a thick glass slide with two chambers. A special coverslip is placed over these two chambers giving a total volume of 0.1 mm<sup>3</sup> per chamber. The chambers are divided into a grid, to aid in counting cells within the area (Appendix 14). Before counting motile algal species, 1 or 2 drops of 10 percent formalin should be added to a 50 ml sample. The coverslip is mounted over the chambers, and the chambers are filled with the algal sample using a Pasteur pipette. Care is taken not to introduce any air bubbles, as the number of algae estimated is dependent on the exact volume of the chambers. Protocol-10 outlines the procedure used for estimating the number of algal cells using a haemocytometer cell.

#### PROTOCOL-10

##### ESTIMATING CELL DENSITY USING A HAEMOCYTOMETER CELL

1. Collect 10–20 ml of algal culture in a scintillation vial.
2. Add 2–3 drops of 10 percent formalin to culture if flagellate species to stop it from swimming. Mix sample thoroughly.
3. Mount cover slip on haemocytometer cell.
4. With a Pasteur pipette, retrieve 1 ml of sample, and introduce a drop into the chamber at the edge of the cover slip. Do not force sample in, allow it to run by capillary action. Make sure not to have any air bubbles in cell.
5. Fill grooves of cell completely with algal sample.

6. Allow 1 or 2 minutes for cells to settle out on bottom of counting chamber.
7. Using a counter, count number of algal cells in at least three of the 25 squares. Count all cells lying within the square or overlapping the lines on the right-hand or bottom sides. Each square measures 0.2x0.2 mm.
8. Calculate the average number of cells per square.
9. To obtain the cell density: Average number of cells/square x 250. This gives you number of algal cells per  $\mu\text{l}$ .
10. To obtain the number of cells per ml multiply above number by 1 000.

Explanation:

Each cell is  $0.004 \text{ mm}^3$

Average number of cells is per  $0.004 \text{ mm}^3$

To obtain average number of cells per  $\text{mm}^3$ , multiply by 250.  $1 \text{ mm}^3 = 1 \mu\text{l}$

There are  $1\,000 \text{ mm}^3$  in 1 ml. Multiply average by 1 000 to obtain number of cells per ml.

### 2.3.6 Alternate feed for spat

The production cost of microalgae using conventional phototrophic means, as described above, is high, ranging from 20–50 percent of hatcheries' operating costs (Coutteau and Sorgeloos, 1992). Nutritionally adequate alternatives have been sought that may be more cost-effective than on-site algal production. Some of those tested include spray-dried, heterotrophically grown microalgae (Langdon and Onal, 1999), and microencapsulated artificial diets (Laing, 1987). Although these show potential in future rearing of bivalve spat, they are not commercially available. Other off the shelf alternatives that show more promise include microalgal concentrates; these are produced by centrifugation and refrigerated at 2–4 °C for 1–8 weeks. They have been used successfully as part of mixed or complete diets for larval or juvenile bivalves (Heasman *et al.* 2000). Such microalgal concentrates can either be prepared by hatcheries with existing infrastructure to produce microalgae and concentrates on-site, or by large specialized algal production facilities for sale to hatcheries (Brown and Robert, 2002). It was shown that not all algal species lend themselves to the process of concentration, notably the flagellates, *P. lutheri* and *Isochrysis* sp. (T-Iso) are easily damaged and deteriorate rapidly (Heasman *et al.* 2000). Another alternative is the use of dry, non-live microalgae which can be purchased commercially; studies conducted by several authors (Langdon and Onal, 1999; Davis and Campbell, 1998) found that a mixed live algal diet of T-Iso and *C. calcitrans* supplemented with spray-dried microalgae enhanced juvenile mussel growth; and that a mixed spray-dried algal diet of *Schizochytrium* and *Spirulina* contained the biochemical constituents necessary to satisfy the nutritional requirements of mussels.

In light of the restricted capacity of the algal culture facility at BBSR, replacement of live algal diet with commercially available dry algal mixture was tested on scallop spat. Results of a short study on growth of *E. ziczac* show that food ration composed solely of dry algae meet the nutritional requirements of larger zigzag spat (>3 mm); shell growth was seen to increase by 1.2 mm per week in the first three weeks. This growth was comparable or better to that of spat fed live algae. On the other hand, smaller spat (<3 mm), seemed to fare better in the long term when fed live algae. These results led to the routine use of commercially purchased dry algae for older spat, prior to transfer to grow-out sites. Dry microalgae are purchased from Reed Mariculture ([www.instant-algae.com](http://www.instant-algae.com)).

## Chapter 3

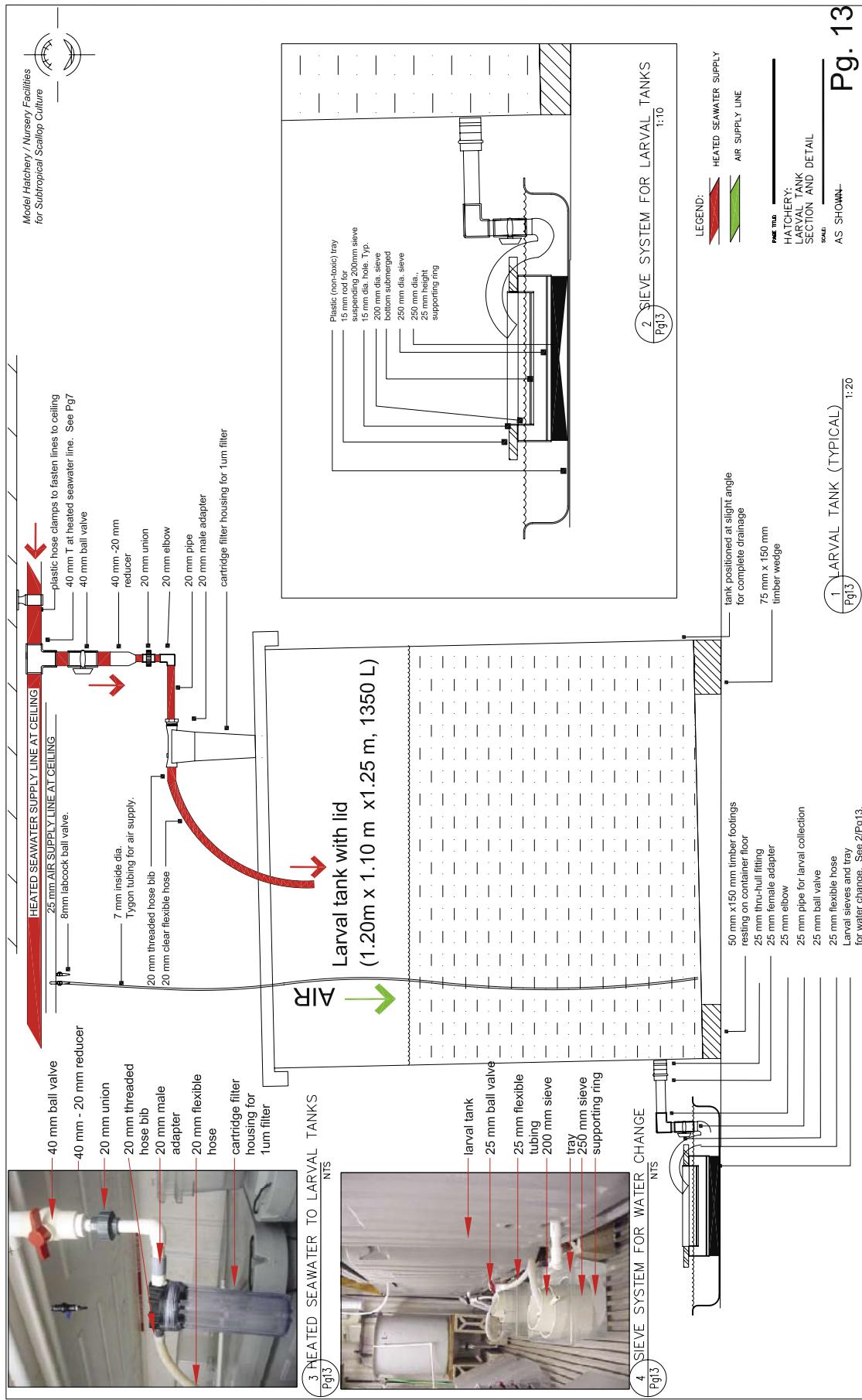
# Hatchery: facilities and techniques for larval culture

<b>3.1 HATCHERY FACILITIES .....</b>	59
3.1.1 Larval tanks .....	61
<b>3.2 SCIENTIFIC BACKGROUND – EMBRYONIC AND LARVAL DEVELOPMENT .....</b>	62
3.2.1 Embryonic development to D-larvae .....	62
3.2.1.1 <i>Fertilized eggs: characteristics and developmental requirements</i> ..	64
3.2.2 Larval development .....	66
3.2.2.1 <i>Veliger larvae</i> .....	66
3.2.2.2 <i>Pediveliger larvae</i> .....	66
<b>3.3 SCIENTIFIC BACKGROUND – FACTORS INFLUENCING LARVAL REARING .....</b>	68
3.3.1 Temperature .....	68
3.3.2 Density .....	69
3.3.3 Salinity .....	69
3.3.4 Food ration .....	70
3.3.4.1 <i>Effect of food ration on calico scallop larvae</i> .....	71
3.3.5 Culture systems: flow-through vs. static .....	72
<b>3.4 TECHNIQUES – STANDARD PROTOCOL FOR REARING CALICO AND ZIGZAG SCALLOP LARVAE .....</b>	74
3.4.1 Larval rearing procedure .....	76
3.4.1.1 <i>Water change</i> .....	76
PROTOCOL-11 – Take-down of larval tanks: larval collection and re-distribution .....	77
3.4.1.2 <i>Standard rearing density</i> .....	78
3.4.1.3 <i>Standard food ration</i> .....	79
3.4.1.4 <i>Counting larvae and determining survival rate and shell growth</i> ..	79
3.4.1.5 <i>Setting of larvae</i> .....	80

### **3.1 HATCHERY FACILITIES**

The goal of the hatchery is to produce quantities of juvenile scallops by inducing spawning of adults, and rearing larvae and post-larvae under controlled conditions. For reliable production of larvae and post-larvae, strict adherence to protocols for each stage of culture and routine chores for daily care is necessary. Throughout larval and post-larval rearing, cleanliness in the hatchery is essential to prevent large-scale mortality and loss. For this reason, details of routine cleaning of seawater supplies and tanks followed in Bermuda are provided in this Chapter. These protocols have proved extremely valuable at the Bermuda hatchery, in ensuring regular maintenance.

## Technical drawing, Pg. 13



This Chapter describes the hatchery facilities necessary for the development of eggs to straight-hinge stage (Figure 1.7), and for the rearing of larvae to the time of settlement.

The overall plan of the facility, showing larval tanks and air compressor is seen on technical drawing – page 6A and described in Chapter 1. A total of four square insulated “BONAR” tanks (1 344 litres total capacity) are used for larval rearing. Larval tanks are supplied with compressed air and heated seawater filtered twice to 1 µm. Description of the heating system and heating lines in the hatchery are provided in Chapter 1 (see Section 1.1.3) and drawings are on technical drawings – pages 5A and 5B.

### 3.1.1 Larval tanks

Refer to Technical Drawing – page 13. In general, the larger the tank the better; it is preferable to minimize the surface area to water volume ratio as surfaces tend to have higher numbers of bacteria. In Bermuda, square tanks with maximum volume of 1 344 litres are used as standard larval rearing vessels. These insulated tanks have double walls filled with foam, and molded legs; they are often used for fish transport, for their insulating capacity, and for this reason, they were selected for scallop larval rearing in Bermuda, as larvae are cultured at a temperature higher than the ambient. Lids are provided with the tanks, and are used for maintaining a constant seawater temperature. For larval rearing, tanks are filled to a maximum of 1 000 litres, and hence referred to throughout this manual as 1 000 litres tanks. Larval tanks are filled with heated seawater, supplied from the heating unit (see Chapter 1). As seen in the technical drawing diagram – 1/Pg13, the seawater line is affixed to the ceiling by plastic hose clamps. A 50 mm T is glued in-line for supply of seawater to two larval tanks. A 50 mm ball valve regulates the flow of seawater to the tank; it is reduced to 20 mm by a 50 mm to 20 mm bushing. A 20 mm union connects the heated seawater supply to a 20 mm elbow fitted to a cartridge containing a 1 µm filter. The 20 mm union above the cartridge allows for dismantling of the cartridge-housing unit for cleaning in-between water changes. This filter system provides additional filtration after heating and prior to delivery to the larval tank. The cartridges used have 20 mm fittings included; on the outflow a 20 mm ID clear flexible hose is used to fill the larval tank. The technical drawing photo – 3/Pg13 illustrates the filter set up in detail.

The air supply to the larval tank is also illustrated in technical drawing – 1/Pg13. Air supply is regulated by labcock ball valves and connected to the airline (see diagram – 2/Pg 7). A 7 mm ID Tygon tube is fitted to a labcock ball valve running down the length of the tank. For larvae, a low volume air flow is provided, equivalent to one bubble at a time, sufficient to oxygenate the tank and prevent algal cells from sinking.

In the hatchery, larval tanks are placed directly on the floor, resting on timber footings at each corner. A gentle slope is provided for complete drainage of the tank, by raising the back side using additional timber wedges. The drain valve is located on the front of the tank. For controlled drainage, necessary for collecting of larvae, a 25 mm thru-hull bulkhead fitting is glued into an existing drain hole. A 25 mm female adapter is threaded into the thru-hull fitting; this is in turn glued to a 25 mm elbow. The flow of water is regulated by a 25 mm one-way ball valve. A 25 mm pipe is dry fitted to the valve and to a 25 mm ID hose for collection of larvae; in this way, a gentle outflow of water is obtained from the larval tank, minimizing any crushing of larvae.

The technical drawing diagram – 2/Pg13, shows, in detail, the equipment utilized for collection of larvae during water transfers. As larvae are culled at every water change, two sieves are placed one within the other; the smaller 20 cm diameter sieve is balanced inside the larger 25 cm diameter sieve, and is supported by a 15 mm transverse pipe, resting on top of the lower sieve. Sieves are kept in plastic non-toxic trays (used in

the restaurant trade), and supported by a 2.5 cm high ring off the bottom; this set up allows collected larvae in both sieves to remain continuously submerged, and for those collected on the bottom sieve to avoid direct contact with the tray and subsequent crushing and damaging of shell. Care is taken that the top of the bottom sieve is above the tray level, so as to not lose any larvae during collection. In this way, water flowing from the larval tank passes through both sieves, where larvae are separated by size and collected; water remaining in the tray, overflows and is discarded without any loss of larvae. Water level in the tray and flow rate from the tank must be monitored through the entire draining process to ensure that the sieve does not become clogged with larvae and overflows; this would cause larval loss. For culling larvae, larger larvae are collected first in the 20 cm sieve (with larger mesh aperture) and smaller larvae are collected in the 25 cm bottom sieve (with smaller mesh aperture). The technical drawing photo – 4/Pg13 illustrates the set-up of trays and sieves for collection of larvae during a water change.

### 3.2 SCIENTIFIC BACKGROUND – EMBRYONIC AND LARVAL DEVELOPMENT

There are many problems which may arise in the reliable hatchery production of bivalve species. Successful hatchery production of larvae and spat is very dependent on the skill and experience of the staff. As mentioned at the beginning of this Chapter, a keen awareness of the importance of hygiene is essential. In general terms, higher mortalities of larvae are often associated with higher temperatures triggering greater bacterial proliferation and infection. Chapter 1 describes the procedures followed for spawning induction of adults and fertilization of the eggs. In the following section, procedures followed for embryonic development and larval rearing are discussed.

#### 3.2.1 Embryonic development to D-larvae

A generalized life cycle for pectinids is given in Chapter 1 (see Figure 1.7), as well as a brief synopsis of the sequence of events following fertilization, prior to distribution of eggs in culture tanks for both *Euvola ziczac* and *Argopecten gibbus* (see Section 1.3.3). In Figure 3.1, the change in shape of the various stages of development from fertilized egg, through D-larvae to pediveliger for sand scallop larvae is shown. Development of *E. ziczac* and *A. gibbus* larvae is similar to that seen for other pectinids, which has been described in great detail in the literature (Costello *et al.* 1973; Culliney, 1974; Paulet, Lucas and Gerard, 1988; Kasyanov, 1991; Cragg and Crisp, 1991). For the purpose of this manual, a brief generalized description of each stage is given in the appropriate sections as a quick reference. However, as the rate at which larvae develop varies with species, the short section below gives that observed for the sand and calico scallop in Bermuda.

Embryos are left undisturbed for the first 48 hours. During this time period, repeated cleavages lead to formation of the spherical blastula; timing varies among species and was not determined for either the zigzag or calico scallop, but was determined for

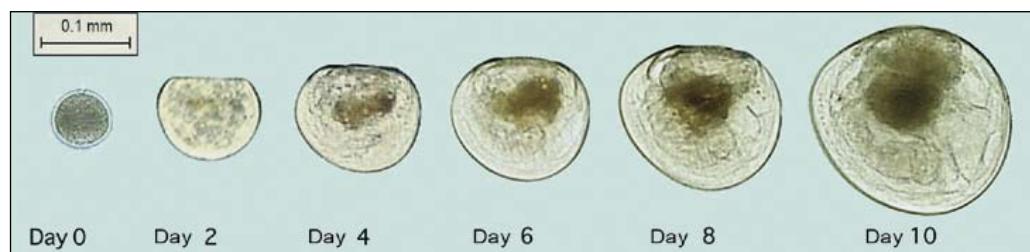


Figure 3.1: Developmental changes of sand scallop larvae to metamorphosis.

a related species, *Aequipecten irradians concentricus*, to be of five hours and fifteen minutes (Sastry, 1965). The rotating ciliated gastrula appears thereafter (nine hours after insemination for *A. concentricus*). Changes from a ciliated gastrula to the top-shaped trochophore are gradual (see Life cycle – section 1.2.3).

First observations at the BBSR hatchery are made 24 hours after distribution of embryos in culture tanks, when samples are taken by skimming the surface of the water with a 20 µm sieve and examined under a compound microscope. At this time, trochophore larvae and often, early veliger larvae are observed for both *E. ziczac* and *A. gibbus* cultures. Pectinid trochophore larvae are characteristic in that the apical end is rounded and surmounted with a tuft of long cilia; the other end of the larva is tapered with indentations on either side of the larva. During this stage, there is little calcification, and shell secretion is initiated at the end of the trochophore stage. Due to this soft-body characteristic they are very fragile; for this reason, 24 hours larval cultures are left undisturbed, as collection on sieves would be damaging.

Figure 3.2 shows veliger larvae of *E. ziczac*, characterised by their velum, a distinct organ emerging during the transition from trochophore to veliger. As these early 24 hours veliger larvae exhibit an active swimming behaviour both for *A. gibbus* and *E. ziczac*, addition of formaldehyde is required for a clear photograph; this also causes the velum to retract, for this reason, only a few of the larvae seen have been caught with the velum extended. Veliger larvae remain uniformly suspended in the water column. To ensure complete development for a maximum number of embryos, a 48-hour time period is allocated prior to the first collection of larvae. This allows time for the developing larvae to take on the D-shaped outline characteristic of the prodissoconch-I shell. Complete development to the D-larval stage (or straight-hinge stage) thus occurs 48 hours after fertilization (Figure 3.3). In contrast to the 24-hour veligers, D-larvae of the sand and calico scallop remain for the most part still and swim occasionally upon being disturbed. Costello *et al.* (1973) report a similar developmental rate for *A. gibbus* D-larvae.

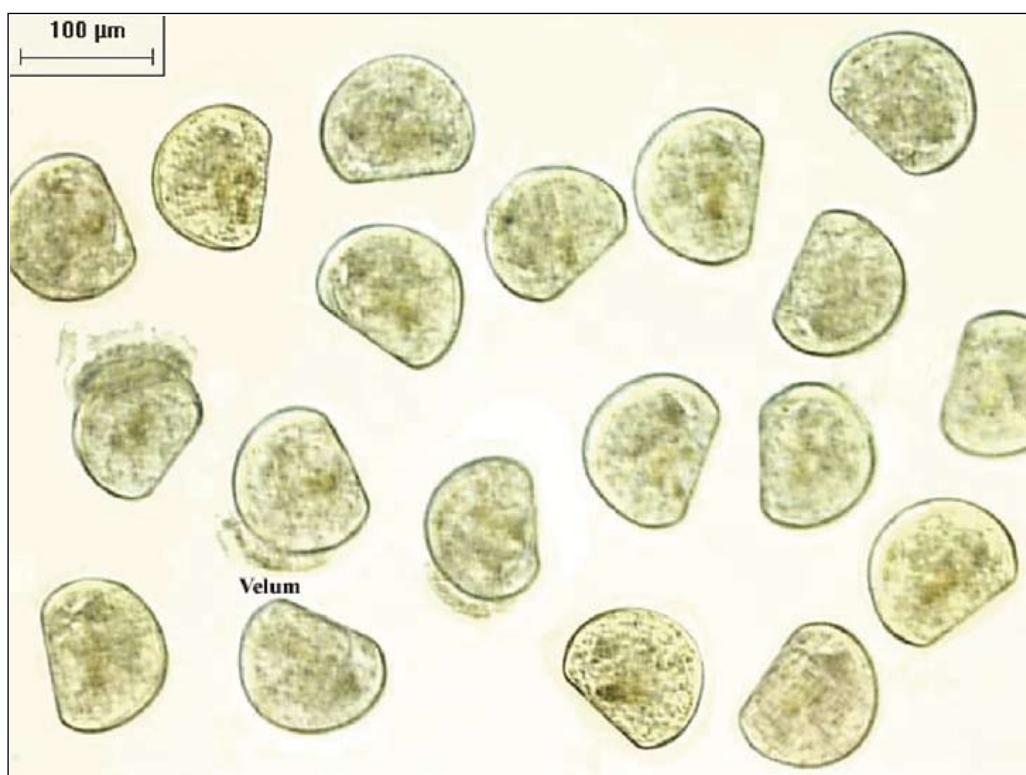
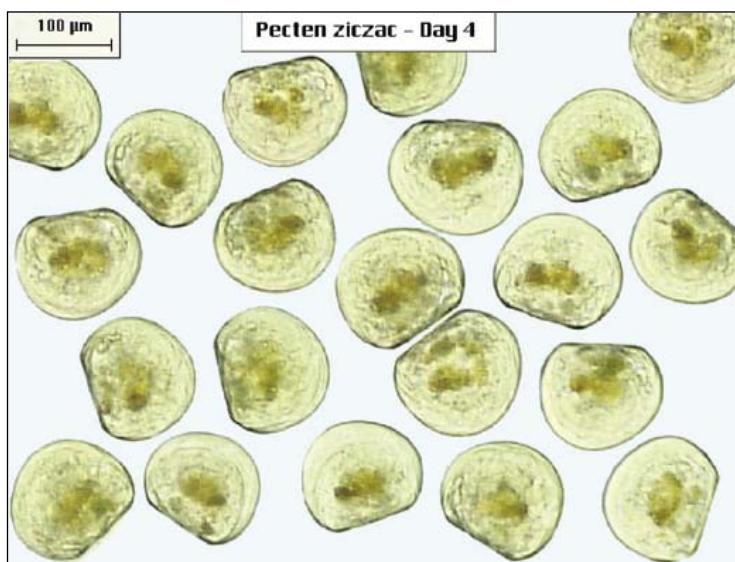


Figure 3.2: One-day old *E. ziczac* veligers showing extended velum.



**Figure 3.3:** Straight-hinge or D-larvae stage of *E. ziczac*.

### 3.2.1.1 Fertilized eggs: characteristics and developmental requirements

Egg diameter for pectinids generally range from 57–79 µm diameter (Cragg and Crisp, 1991). Within a species, egg size, may vary slightly among batches; according to Kraeuter, Castagna and Dessel (1982), this size difference, although slight, may reflect the amount of reserves stored in the egg and impact subsequent development and growth. These authors suggest eliminating smaller size eggs and concentrate on better care of larger egg sizes that are more likely to survive. Reduced survival of certain batches in hatcheries, especially towards the end of the hatchery season (as seen in Chapter 1), is often related to higher temperatures and bacterial contamination; however, a significant portion of these losses may be due to use of smaller eggs with reduced stored reserves. Mean egg size for *E. ziczac* is found to be  $64.8 \pm 6.8$  µm ( $n= 50$ ), and for *A. gibbus*  $55.9 \pm 3.2$  µm ( $n= 50$ ). Size measured for *A. gibbus* in Bermuda is slightly lower than recorded by Costello *et al.* (1973) – 60 µm diameter – for the same species. This difference may be attributed to environmental differences reflected in stored reserves.

Differences among batches of calico scallop eggs are observed at the Bermuda hatchery, namely with respect to buoyancy. Some batches are neutrally buoyant and remain suspended throughout the water column; however, other batches are negatively buoyant and sink to the bottom of the culture tank. This difference in buoyancy among batches has never been associated with a change in developmental success of the embryos or of the larvae thereafter at the Bermuda hatchery. This weight difference may be explained in part by the differences in amount of reserves as reported by Kraeuter, Castagna and Dessel (1982). Negative buoyancy has also been reported for *Patinopecten yessoensis* resulting in a monolayer of eggs at the bottom of the culture tanks (Bourne, Hodgson and Whyte, 1989). For this reason, it is best not to aerate, as embryos will collect in windrows at the edge of the tank because of water circulation. On the other hand, in *E. ziczac*, fertilized eggs are neutrally buoyant remaining suspended throughout the water column.

All fertilized eggs are pooled at the hatchery in Bermuda without any size screening, but with some subjective selection related to shape, development and uniformity. For example, those eggs released as clumps, or potentially self-fertilized, were classified as “bad”. An attempt is made at rearing “good” and “bad” in separate culture vessels. It is uncertain whether this arbitrary selection enhances embryonic development, and batches of “bad” eggs lead at times to surprisingly good D-larval cultures. The density at which eggs are distributed varies with species. Calico scallops have been reportedly

reared at as high a density as 25 eggs.ml<sup>-1</sup> (Costello *et al.* 1973). On the other hand, lower densities (10 eggs.ml<sup>-1</sup>) are used for the queen scallop (Neima and Kenchington, 1997). In Bermuda, it has been observed that a higher survival to Day 2 larvae is obtained for the sand scallop for batches with an initial egg density less than or equal to 10 eggs.ml<sup>-1</sup>.

Egg size and rearing density used are thus two factors, which may affect development success to the D-larva stage. Sperm concentration has also been shown to be a major factor in the production of normal bivalve larvae in the laboratory (Loosanoff and Davis, 1963; Gruffydd and Beaumont, 1970). A ratio of 1:6 (egg:sperm) is a standard objective; in the hatchery 1–2 ml of sperm solution per 1 litre of egg solution is found sufficient for successful fertilization. Beaumont and Budd (1983) have shown that there are significant genetic effects during fertilization and the early larval life in *Pecten maximus*. In their study, the origin of both eggs and sperms independently affected the number of eggs, which produced larvae. For this reason, it is routine procedure to fertilize each female with several sperm solutions, in hopes of enhancing developmental success to D-larval stage. An additional consideration needs to be taken for hermaphroditic species, such as zigzag and calico scallops, to minimize abnormal development. In these species, the probability of self-fertilization during laboratory spawning is enhanced, and self-fertilization has been also associated with detrimental effects in development. Beaumont and Budd (1983) found a severe reduction in growth rate of *P. maximus* veligers in all self-fertilized cultures. Sastry (1965) found similar unsatisfactory results in selfed eggs of *A. irradians concentricus*. It has always been a preventive measure during hatchery procedures in Bermuda to avoid self-fertilization in the laboratory and ensure cross-fertilization using several males.

As seen in Table 3.1, variations in percentage D-larvae obtained among and within batches are large. Variations among batches may be a reflection of one or several of the factors mentioned above, namely egg size (or stored reserves) and density. Variations within batches may also be a reflection of the arbitrary selection conducted during distribution of eggs into culture tanks. Other factors, such as variations in salinity (Gruffydd and Beaumont, 1970), and in dissolved organic matter (Crisp, 1982) have also

**Table 3.1:** Yields of Day-2 larvae obtained for several hatchery seasons following controlled fertilization of *E. ziczac* and *A. gibbus* in the hatchery. Ranges shown indicate yields obtained for all larval tanks in one spawning. Single numbers indicate mean for one spawning.

Species	Year	D-larvae (%)
<b>Sand scallop (<i>Euvola ziczac</i>)</b>		
	1998	7.5–57 % – Spawn 1 <1–20 % – Spawn 2
	2000	66–>90 % – Spawn 1 1.23–50 % – Spawn 2
	2001	31.6 % – Spawn 1 <1 % – Spawn 2 8.2 % – Spawn 3
	2002	1.6–6.1 % – Spawn 1
<b>Calico scallop (<i>Argopecten gibbus</i>)</b>		
	1997	66–86 % – Spawn 1
	1998	35–75 % – Spawn 1
	2000	37.5–>90 % – Spawn 1
	2001	18.4–54.3 % – Spawn 1 22.6–50.3 % – Spawn 2 41.6–49.6 % – Spawn 3
	2002	22.4–39.5 % – Spawn 1 19.7–28.4 % – Spawn 2
	2003	33.4–58.2 % – Spawn 1 43.5–66.9 % – Spawn 2

been associated with variations in yield and normality of *P. maximus* D-larvae. These are most likely not a consideration in Bermuda and have not been investigated.

### **3.2.2 Larval development**

The characteristic “D” shape of early larvae (Figure 3.3) has two valves, a complete digestive system and a velum. The velum is ciliated along its outer margin and enables the larva to swim, enabling it to maintain itself in the water column. As it is swimming, the velum collects unicellular phytoplankton upon which the larva feeds. From hereon, larvae are fed daily a food ration consisting of live algal species, cultured on-site (see Chapter 2).

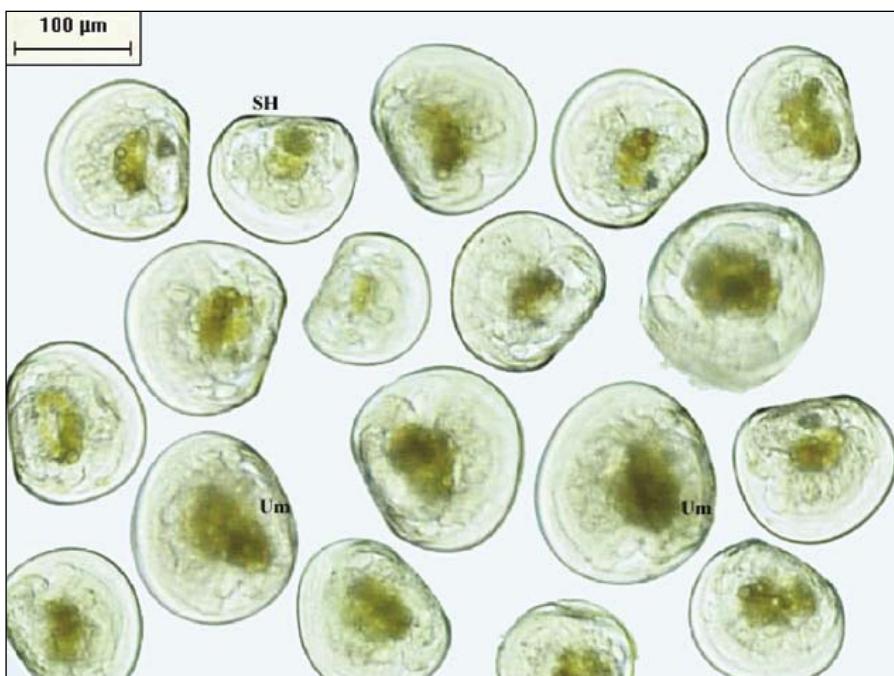
#### **3.2.2.1 Veliger larvae**

As mentioned in the description of embryonic development, the shell appears in the early stages of development (24 hours after fertilization). The veliger shell (prodissoconch-I) is produced by the secretory cells of the shell gland. Kasyanov (1991) gives detailed schematics of the development of the Japanese scallop, *Mizuhopecten yessoensis*. The larval body of the veliger is covered by a semi-transparent shell through which the internal organs are discernible. The digestive system of bivalve larvae consists of the ectodermal fore-gut, the endodermal mid-gut and the ectodermal hind-gut. Food is captured and transported to the mouth opening by ciliary bands on the velum. This function of the velum is as important as the locomotory function. The veliger larva swims actively with the velum extended. The mouth opening situated at the edge of the lower part of the velum leads to the oesophagus, which in turn leads to the stomach. The digestive gland is large and is formed as two stomach pouches. Accumulations of granules of nutritive substances are seen in the cells of the gland. The gland contains digestive enzymes and also opens into the stomach. The stomach leads posteriorly to a short intestine opening to the exterior as anus. The anterior adductor muscle is located dorsal to the anterior attachment of the velum. The respiratory and circulatory systems are absent in the veliger of bivalve mollusc. The influx of oxygen and the excretion of carbonic acid occur by diffusion. The nervous system is considerably developed and is represented by the cerebral ganglion and two pedal ganglia. The main sense organ in the veliger larva is the apical plate of the velum. Straight-hinge larvae vary in length among pectinid species; for example, 78 µm for *A. irradians concentricus*, and 90 µm for *Placopecten magellanicus* (Sastry, 1965; Couturier, Dabinett and Lanteigne, 1996). In Bermuda, shell length for D-larvae of *A. gibbus* ranges from  $92.7 \pm 4.7$  µm to  $101.0 \pm 4.22$  µm, and for *E. ziczac* from  $93.1 \pm 8.3$  µm to  $110.4 \pm 5.0$  µm.

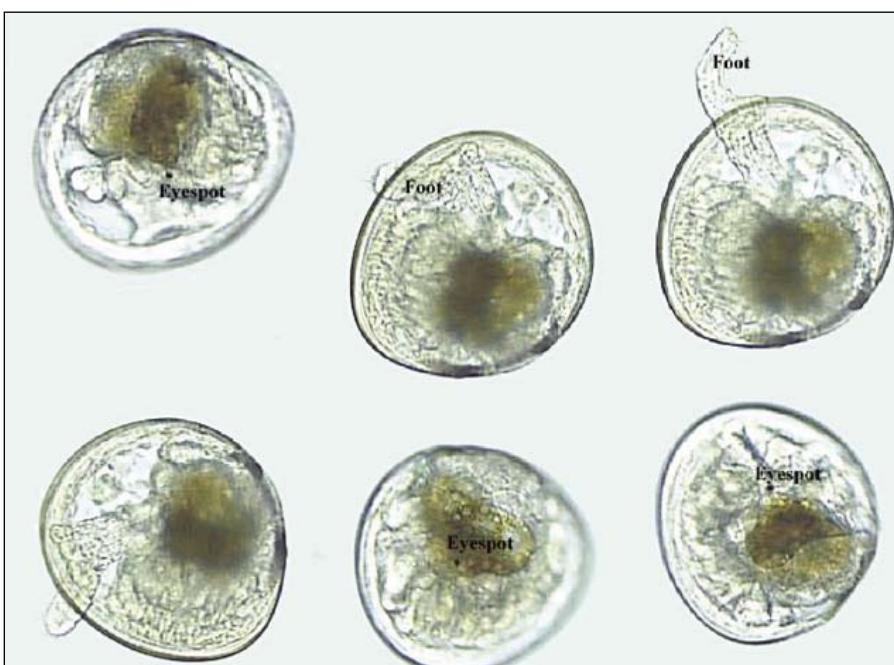
#### **3.2.2.2 Pediveliger larvae**

The shape of the D-larvae gradually changes with age. As veligers develop, a slight reduction in the hinge-length with the extension of umbones over the hinge region is seen. Costello *et al.* (1973) report the appearance of the umbo at 140 µm for calico scallops; in Bermuda, this appearance is seen as early as Day-6 old scallops with a mean size of 115 µm shell length (Figure 3.4). The degree of umbo development varies among species, and is for example more conspicuous in the sand scallop than in the calico scallop. Shell growth in the anterior-posterior direction also takes place. The internal organs, such as the stomach, develop. The foot begins to develop as a small projection ventral to the mouth; it is recognizable especially when the larva has retracted into the shell. In 9 Day old veligers, an “eyespot” (darkly colored spot) becomes conspicuous, especially noticeable in *E. ziczac* (Figure 3.5). This appearance of the eye spots with the statocysts is characteristic of pediveligers. The velum reduces gradually and the foot continues to develop; swimming activity also decreases. By the twelfth day the well grown umbones almost mask the hinge line of the early veliger shell. For this reason, the pediveliger shell is triangular, egg-shape.

Bower and Meyer (1990) give very detailed drawings for *Patinopecten yessoensis*. Pediveliger larvae reach the maximum size and develop a functional foot. The foot is a multi-functional organ; it mainly serves to search for a substrate for settlement and attach the larva to the substrate by byssal threads. Swimming larvae can extend the



**Figure 3.4:** Day-6 sand scallop larvae showing initial development of umbones (Um) compared to straight-hinge characteristic (SH) of D-larvae.



**Figure 3.5:** Day-11 pediveligers of *E. ziczac* showing eyespot and a well-developed foot in and out of the shell.

foot which, when brought into contact with the substrate, facilitates crawling over the substrate. The result of crawling can be that: 1) the larva attaches itself to the substrate, or 2) the larva retracts the foot, leaves this substrate and resumes swimming in search of a more adequate substrate. At this time, larvae are more often collected from the bottom water, rather than throughout the water column as in the earlier stages. The

most important changes in the larvae take place between the twelfth and fourteenth days of development. The umbones are fully grown, overhang the straight-hinge of the early veliger shell, and are directed posteriorly. The shell has a curved appearance at the hinge attachment. This prodissoconch-II stage is identified as such to distinguish it from the early veliger and pediveliger stages. The anatomy of the prodissoconch shows important differences from the earlier stages. The mouth is located in an anterior and dorsal position, bringing it to the adult position. The mantle is well developed. The foot is enlarged and is a wedge-shaped organ with cilia at the free or distal end. The foot has a cleft in the middle region and the byssal gland is located there. A pair of statocysts is present. The gills are developed as a pair of ciliated folds ventral to the foot. With development of new organs and the loss of some larval organs, metamorphosis is completed. The average size of the prodissoconch-II shell for Day-14 calico scallops is of 200 µm shell length, and for zigzag scallops of 215 µm.

Prodissoconch-II larva attaches to the substratum with the byssal threads; in the hatchery, surfaces are provided during this settlement phase. Thereafter, rapid changes in shell morphology and growth of internal organs take place resulting in the adult scallop form. Post-larval development is discussed in Chapter 4.

### **3.3 SCIENTIFIC BACKGROUND – FACTORS INFLUENCING LARVAL REARING**

Larvae can be grown in conical or flat-bottomed vessels. Both types have been tried for rearing *E. ziczac* and *A. gibbus*. Differences in yield between conical and flat-bottomed were not truly tested, but were not apparent. Considering the restriction in space of the model hatchery, it is found that maximal tank capacity is best achieved with flat-bottomed tanks. Square “BONAR” tanks (1 000 litres capacity) are preferred as the main larval tanks mainly due to their insulation characteristic, allowing for rearing of larvae at a temperature higher than ambient. Temperature, salinity, food ration and rearing density are important factors in development of larvae. They are discussed in the following sections in relation to procedures used in Bermuda.

#### **3.3.1 Temperature**

For incubation of fertilized eggs, and larval rearing of both the calico and sand scallop, seawater temperature is increased by 6–8 °C above the ambient; such that rearing temperature is maintained at  $24 \pm 1$  °C for embryonic development to settlement stage. This yields an average larval life of 13 days, when pediveligers are ready for settlement in nursery systems. Costello *et al.* (1973) reared *A. gibbus* under similar conditions ( $T = 23 \pm 2$  °C and  $S = 35$  ppt). Velez and Freites (1993) have also reported successful culture of *E. ziczac* larvae at a salinity of 37 ppt and a temperature of 26 °C, yielding pediveligers within 10–12 days after fertilization.

In their review Cragg and Crisp (1991) found that time to metamorphosis in pectinids is related to temperature. Optimum embryonic and larval development varies with temperature and salinity, dependent on the scallop species and on a specific site. For example, Yamamoto (1968) and Bourne, Hodgson and Whyte (1989) report slight variations in the range of optimal temperatures for *P. yessoensis* ( $T = 10\text{--}15$  °C and  $S = 30\text{--}40$  ppt for the former, and  $15\text{--}18$  °C and 29 ppt for the latter). For the same species, Maru (1985) showed that optimum development of embryos occurred at a higher temperature of 20 °C. However, this does not imply that larval development will be optimal at the same temperature, and consideration to size-specific survival rate must also be given. This was shown for *Pecten fumatus* by Heasman, O’Connor and Frazer (1996). Embryos of this species were seen to develop best at the lowest

temperatures (15–18 °C), whereas larvae initially grew rapidly at 24 °C, but did not survive to metamorphosis; a constant temperature of 21 °C was thus found to yield a maximum number of larvae for settlement. Generally, bivalve larval growth increases with temperature, up to some optimum level, which is species dependent (Bayne, 1983). However, a further increase in temperature causes growth to decline. This was shown for *Chlamys hastata*, where larval growth was faster at 16 °C (5.8 µm/day) than at 12 °C (4.8 µm/day), but was much slower at 19 °C (2.5 µm/day). Similarly, larvae reached a mature stage more quickly when reared at 16 °C, as compared to 12 °C (Hodgson and Bourne, 1988). It is thus of benefit to investigate the highest temperature threshold for larval rearing for the species studies. As a general rule, bivalve larvae reared at temperatures close to their tolerance limits suffer high mortality (Ansell, 1961).

The rearing temperature used for both embryonic development and larvae in Bermuda is similar and was determined through trial and error, rather than a scientific study. The optimal temperature for egg incubation may therefore be worth investigating in these two species, as results in percentage yield of D-larvae varies widely over the years (Table 3.1). However, it has to be noted that bacterial proliferation is associated with high temperatures, and the balance between optimal larval development and low bacterial numbers, needs to be achieved. On the other hand, although scientific investigations may be worthwhile in determining optimal rearing temperatures for larvae, in light of increasing veliger and pediveliger yield, it appears that T= 24 °C yields satisfactory results for both of these scallop species.

### 3.3.2 Density

Density-dependent mortalities have been described by some workers (Loosanoff and Davis, 1963; Gruffydd and Beaumont, 1972). Initial densities of 5–6 larvae.ml<sup>-1</sup> have been described as satisfactory for some bivalve larvae (Jespersen and Olsen, 1982, DiSalvo *et al.*, 1984). Hodgson and Bourne (1988) report that highest survival for *P. yessoensis* was observed when initial density was 2 larvae.ml<sup>-1</sup>. Densities used for *A. gibbus* (15 eggs.ml<sup>-1</sup>) and *E. ziczac* (10 eggs.ml<sup>-1</sup>) fall into the average range used. Velez, Alifa and Perez (1993) maintained the density at 5–10 larvae.ml<sup>-1</sup> for *E. ziczac* throughout its larval life; and do report a low tolerance of this species to high density, especially as they approach settlement. On the other hand, Costello *et al.* (1973) incubated *A. gibbus* eggs at 25 eggs.ml<sup>-1</sup> initially, reducing the concentration to 10 larvae.ml<sup>-1</sup> at the D-larval stage. It is difficult to evaluate the effect of this initial high density as D-larval yields are not reported.

### 3.3.3 Salinity

Most rearing of pectinid larvae has been carried out using the local seawater supply, also used for the maintenance of the adults, with salinities within the range of 30–35 ppt. Reduced salinities adversely affect growth of veligers and severely affect embryonic development (Gruffydd and Beaumont, 1972). The degree to which development is affected is species dependent. Embryonic development of the Japanese scallop can take place over the range of about 14–21.5 ppt salinity with a marked reduction in the rate of development at either end of this range (Maru, 1985). Gruffydd (1976) found that survival of *Chlamys islandica* veligers over a 24-hour exposure period was little affected by salinities as low as about 21ppt, but markedly reduced by salinities of about 14 ppt with salinities of about 7 ppt causing 100 percent mortalities. Culliney (1974) noted that veligers of the queen scallop, *P. magellanicus*, could survive for 48 h at salinities as low as 10 ppt, though there was evidence of tissue swelling and the larvae were incapable of normal swimming. Bourne, Hodgson and Whyte (1989) show little effect of salinity on larval growth of the Japanese scallop; and growth rate averaged 6.9 µm per day at a temperature of 18 °C. Pectinid species showing tolerance

to differences in salinities, such as the bay scallop, are species naturally found in salinity fluctuating environments. The bay scallop, related to the calico scallop studied in Bermuda, is found in bays, sounds and estuaries, where heavy rains will cause salinity reductions at times to as low as 10–12 ppt (Duggan, 1975). It may explain its steady larval growth rate of 10–15  $\mu\text{m}.\text{day}^{-1}$  when reared between 25 and 30 ppt. On the other hand, *A. gibbus* is not normally found in the natural environment in low or fluctuating salinity conditions. It can be assumed that its tolerance to lower salinity or to fluctuating salinity will be low, and be reflected in poor larval growth.

Salinity of ambient seawater in Bermuda is constant at 36 ppt throughout the year. There are no adjustments made to the rearing salinity, as both species of scallops used are well adapted to the ambient conditions. Interest was generated in investigating the larval survival and growth of calico scallop larvae with varying salinities for the potential of culturing this species in Gulf of Mexico waters, where salinities can fluctuate daily from 20 to 35 ppt (Norman Blake, *pers. comm.*). The bay scallop, *A. irradians*, appears to tolerate these fluctuations in salinity; the question was the degree of tolerance of *A. gibbus*. Fluctuations could not be simulated at the hatchery in Bermuda; however, three salinities were tested for rearing of larvae, using 3-litre beakers. It was found that, although *A. gibbus* shows some tolerance to salinity reduced by 8 ppt and even 16 ppt, decreased salinity does seem to have a negative marked effect in both survival rate and growth. The difference in survival to the pediveliger stage for larvae reared at ambient salinity (36 ppt) and at 20 ppt approximates 12 percent. Both shell and tissue growth of calico scallop larvae were also seen to be negatively affected by reduced salinity, especially towards the end of the larval life. This would most probably affect settlement of these larvae and the post-larval yield and growth of surviving scallops.

### 3.3.4 Food ration

Molluscan veliger larvae feed by means of ciliary currents on the velum. Hence, once the straight hinge larval stage is reached, the larvae are planktotrophic and feed on unicellular algae. Rates of clearance of particles from suspension are dependent on particle size, concentration of particles, larval size, density of larvae and temperature (Bayne, 1983). Hence, adequate diet in the hatchery environment needs to be assessed in terms of algal species used (see Chapter 2) and amount of algal cells provided (food ration). The optimal or critical cell concentration provided can be defined as that density where all food cells are taken in and no pseudofaeces are produced (Schulte, 1975). Cary, Leighton and Phleger (1981) showed on video films of *Hinnites multirugosus* larvae (purple-hinge rock scallop) that at high concentrations, mechanical interference was observed, coupled with heavy pseudofaeces production and severe packing of the gut. These authors contend that a finite larval-algal cell encounter/ingestion ratio exists beyond which increasing cell concentrations promote less growth due to the factors previously described. Furthermore, bacterial contamination at high concentrations and the build-up of ectometabolites may render an acceptable diet toxic to developing bivalves (Loosanoff and Davis, 1963).

The protocol used at the hatchery is derived from several in-house trials, as there was little reference in the literature on optimal food ration for calico and sand scallops. Velez, Alifa and Perez (1993) report rearing *E. ziczac* larvae to the pediveliger stage with a food ration of 30 000  $\text{cells.ml}^{-1}.\text{day}^{-1}$  to 70 000  $\text{cells.ml}^{-1}.\text{day}^{-1}$  for a larval density of 5–10  $\text{larvae.ml}^{-1}$ . With this regime and a constant temperature of 26 °C, the pediveliger stage was achieved in 10–12 days after fertilization; survival rate is not provided. Rojas, Velez and Azuaje (1988) recommend an initial density of 5  $\text{larvae.ml}^{-1}$  for *E. ziczac* fed a ration of 10  $\text{cells.ml}^{-1}$  with a diet based on *Isochrysis aff. galbana* (clone: T-Iso) and *T. pseudonana* (clone: 3H), and end with a density reduced to 2–3  $\text{larvae.ml}^{-1}$  with a ration of 70  $\text{cells.ml}^{-1}$ . Preliminary studies in Bermuda (Hohn,

Sarkis and Helm, 2001) on the sand scallop showed that food rations comparable to those of Velez, Alifa and Perez (1993) throughout larval life yielded minimal survival to the pediveliger stage (51 percent of Day-2 larvae), compared to those fed the standard and lower food ration (77 percent of Day-2 larvae). On the other hand, there was no significant difference in shell growth for larvae fed the highest food ration. Costello *et al.* (1973) reported a daily ration of 60 000 cells.ml<sup>-1</sup> for calico scallop larvae throughout their larval life when reared at 10 larvae.ml<sup>-1</sup> and a temperature of 23±2 °C; although growth and development to the pediveliger stage was achieved, survival rate is not given. In a hatchery where maximal production is aimed for, it is worthwhile to investigate more closely the larval requirements with size.

### **3.3.4.1 Effect of food ration on calico scallop larvae**

Preliminary studies in 2-litre culture beakers showed that the standard ration initially used at the hatchery was inadequate at certain times throughout the 13 day larval life of calico scallops, in terms of shell growth (Hohn, Sarkis and Helm, 2001). Based on this preliminary study, where three food rations were tested, a second ration schedule was tested using large scale larval cultures (1 000 l), throughout a complete larval cycle during a hatchery run. This second ration schedule provides double the standard amount of algal cells on a daily basis. Hence, control rations ranged from 10 cells.pl<sup>-1</sup> to a maximum of 21 cells.pl<sup>-1</sup> and tested ration ranged from 20 cells.pl<sup>-1</sup> to a maximum of 42 cells.pl<sup>-1</sup>. Both rations proved adequate in providing nutritional requirements to larvae for growth to the pediveliger stage. The higher food ration seemed to benefit larvae in the middle of the cycle (Day-5 and Day-9), reflected in survival rate only. Nonetheless, a higher yield of pediveligers was obtained from larvae fed the standard (and lower) food ration. Although, differences in food ration were not seen for shell growth, accumulation of reserves reflected in tissue growth was noticeably greater at the end of the larval life in scallops fed the higher food ration. It has been reported that higher reserves in larval life may have an effect on settlement rate, and post-larval growth, influencing the storage of energy reserves and survival of spat (Whyte, 1987). It is therefore advantageous to manipulate food ration to achieve not only high survival rate, but also to ensure enhanced settlement and post-larval growth.

As required food rations are species-specific, consideration must be given to environmental parameters to which the species is acclimated. For example, Bourne, Hodgson and Whyte (1989) reported feeding schedule for *P. yessoensis*, following numerous feeding trials as being best as follows: Initially, *C. calcitrans* is fed to veliger larvae and the algal density in the larval tank is 5 000 cells.ml<sup>-1</sup>. As the larvae grow, additional algal species are fed and the algal density is increased. Such that, by “Day of Setting” (21 days for this species), an algal density of 20 000 cells.ml<sup>-1</sup> is maintained in the larval tank, composed of a mixture of *C. calcitrans*, *Isochrysis* and *T. pseudonana*. These authors had the use of a coulter counter, which provided a daily measure of the amount of algae consumed by larvae. In this way, a supplement of algae is added to equate the desired density. Being able to measure consumption of algae also enables monitoring of the health of a larval culture, since healthy larvae will actively swim and graze the algae. Unfortunately, the hatchery in Bermuda did not have access to a Coulter Counter, and it was simply assumed that larvae consumed all of the algae provided on a daily basis. In general, clearance rates increase with the size of the larva at any one particle concentration (Wilson, 1980); however, if particle concentration (or algal density) is above a certain threshold, larvae may reject particles, interfering with feeding and ultimately growth and survival (Bayne, 1983). For this reason, feeding rations are maintained relatively low in Bermuda, to avoid excess uneaten algae leading to increased rejection of algal cells, bacterial proliferation and contamination of larval culture. Food rations provided are within the range investigated by Lu and Blake (1996) for a related species, *A. irradians concentricus*; these authors determined

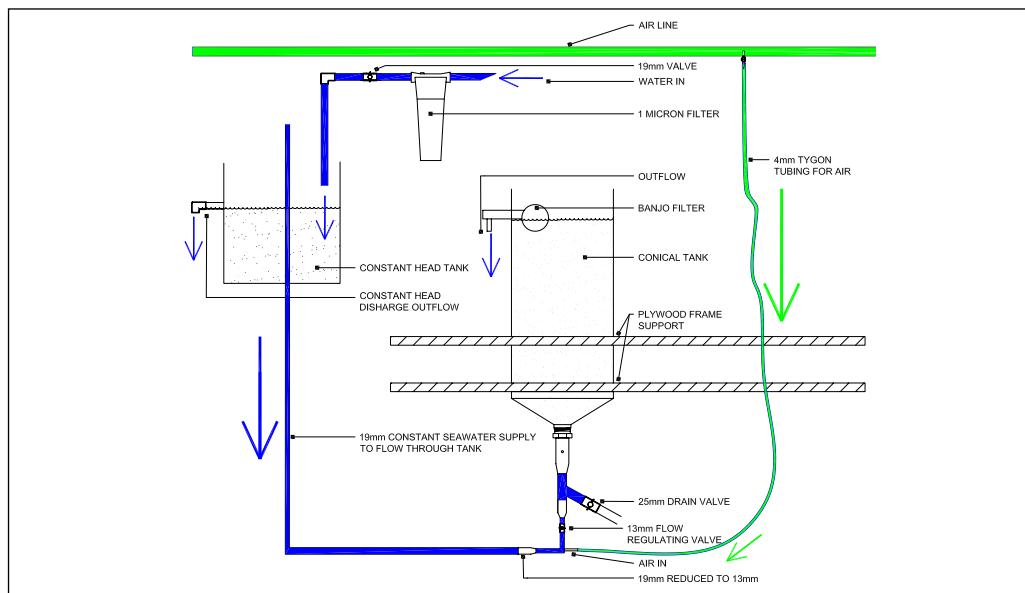
an optimal concentration of 20 cells. $\mu\text{l}^{-1}$  for larvae. Finally, care should also be taken to protect larval tanks from bright sunlight, so that algal blooms do not occur and thus create an overfeeding situation.

### 3.3.5 Culture systems: flow-through vs. static

Traditionally, bivalve larvae are reared in an aerated static system where treated water is changed regularly throughout the larval cycle. Larvae are provided with a daily food supply and are collected prior to every water change. Routine hatchery protocols for the calico and zigzag scallops in Bermuda involve a static system. Although this protocol provides satisfactory results, this method is labour intensive, and requires at times the use of antibiotics to control bacterial contamination within the larval cultures. These two factors play a limiting role in optimizing hatchery rearing of bivalves in general (Southgate and Ito, 1998; Andersen, Burnell and Bergh, 2000). Flow-through systems have been recently attempted with larvae of *Pinctada margaritifera* L. (Southgate and Beer, 1997; Southgate and Ito, 1998), and with *P. maximus* scallop larvae (Andersen, Burnell and Bergh, 2000). Design of the flow-through system appears to be one of the major factors in success of larval rearing. It is worthy of investigation as an adequate flow-through system for rearing of scallop larvae would offer a number of advantages over the conventional static culture systems, including reduced physical handling of larvae, reduced labour demand, and a reduced dependence on the use of antibiotics due to an improved water quality. Additionally, in a space-limited environment such as Bermuda, a flow-through system might provide a greater tank capacity to space ratio.

In-house studies in Bermuda investigated various designs for a flow-through system, using the available 200 litre conical tanks. Comparisons in the larval and post-larval yield of calico scallops reared in a flow-through system are made with scallops reared in static systems for the same larval batches. The flow-through system used is illustrated in Figure 3.6. The differences with the more conventional static system lie in the nature of the seawater flow and the procedure for maintenance. In flow-through systems, seawater flows at a steady rate continually, reducing the necessity for frequent and complete water exchange. For this reason, procedures for maintenance of this system revolve around the daily cleaning of the banjos on the outflow, and ensuring a constant water flow. Sarkis, Helm and Hohn (2006) explain in greater detail these differences and the procedure followed in flow-through rearing. This study also provides results of comparative larval and post-larval growth using a flow-through system and a static system.

The flow-through system described here and tested was developed over the course of three years; many preliminary studies were conducted to ensure an optimal water circulation within the tank, a constant water flow, and a steady supply of algal food. The difficulty in designing a successful flow-through system lies in the minimal handling of the system, and lack of assessment of the culture throughout the larval development, rendering difficult the identification of time periods where the culture performs well or poorly. Preliminary studies focusing on the latter, indicated that Day-8/Day-9 after fertilization, when larvae are pre-metamorphic, was a critical period; in that, high growth and survival were obtained in the flow-through system until this point, but a collapse in culture ensued thereafter, related in part to excessive food ration leading to the clogging of the outflow, restriction in water flow, and accumulation of detritus within the tank. For this reason, one water change was performed at this time to allow for fractionation and culling of larvae, in order to optimize survival rates to metamorphosis; and food ration was decreased in the latter part of the larval life; at this time, extreme care was taken in maintaining a constant water flow by repeated cleaning of the outflow banjo. Southgate and Beer (1997) in their trials of flow-through for oyster larvae utilized a larger surface area allowing for outflow of water. Despite



**Figure 3.6:** Conical tank modified to a flow-through system for larval rearing.

this larger surface area, their results showed a similar trend where larval survival was high during the early part of larval life, and decreased to 7.2 percent survival to the pediveliger stage (Southgate and Ito, 1998). Andersen, Burnell and Bergh (2000) also explained poor larval survival for *P. maximus* reared in a flow-through system by poor water quality caused by overfeeding. Fractionation and culling of larvae during the water change, is likely to enhance growth and survival of larvae. It has been implied that growth of smaller larvae may be inhibited by larger larvae (Bourne, Hodgson and Whyte, 1989); conversely, the presence of dying larvae may have a negative overall effect on the larval culture, as seen in preliminary experiments towards the end of the larval cycle in the flow-through systems. By incorporating a water change towards the latter part of the larval life, and optimizing the daily maintenance procedure, results of this study demonstrate a comparable performance of the flow-through system in terms of pediveliger yield to that of the static system throughout the larval life regardless of density (Sarkis, Helm and Hohn, 2006).

Pediveliger yields are evidently an important assessment of larval rearing conditions, yet, in an aquaculture operation, the true goal is the yield of fixed spat, ensuring an adequate juvenile production. For this reason, the evaluation of the percentage of spat fixed when reared under different larval conditions was determined in the present study. One of the most critical factors is the accumulation of reserves throughout the larval life, related to food ration in both a qualitative and quantitative sense (Farias, Uriarte and Castilla, 1998). It has been shown that rearing conditions affect storage and utilization of biochemical components, and hence metamorphosis and settlement (Gallager and Mann, 1981). Only one diet was provided in the present experiment for all treatments such that nutritional value was not evaluated; the diet chosen was the standard one utilized at BBSR that has proven adequate over the years for ensuring good spat settlement. Quantitatively, food ration was calculated on the basis of water volume within the tank; such that, in the flow-through system, algal ration was calculated based on water flow, equating to approximately 600 litres of water, as opposed to the 200 litres volume of the tank itself. This food ration provided comparable results when larval density was initially at 8 larvae.ml<sup>-1</sup> or 1.6 million larvae in a 200 litre tank; the number of spat fixed approximated 30 percent of the pediveligers for all treatments, in accordance with results obtained for other pectinid species, as for example a 40 percent yield to 1 mm size for *P. magellanicus* (Couturier, Dabinett and Lanteigne, 1996). On the other hand, it appears that food ration may not have been sufficient, once larval

density was increased, for the accumulation of reserves necessary for settlement. The spat yield for flow-through reared pediveligers at a higher density, was much lower than that for those reared in the comparable static system and the standard hatchery system. This density effect was also reflected in the lower shell growth of the fixed spat, as seen when compared to the static system and when compared to the flow-through reared larvae at lower density (Sarkis, Helm and Hohn, 2006).

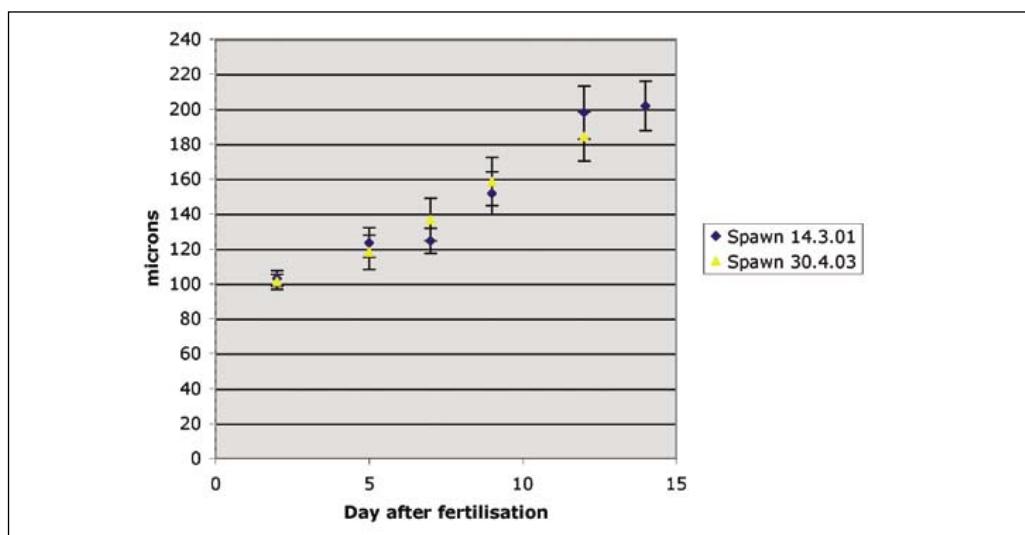
In conclusion, the results obtained in flow through larvae culture with calico scallops suggest that in resource-limited regions this concept is worth investigating. The benefits lie in the reduced labour involved, the absence of antibiotic use, and the optimization of space availability. These three factors are of economical importance, possibly rendering a hatchery operation more cost efficient.

### **3.4 TECHNIQUES – STANDARD PROTOCOL FOR REARING CALICO AND ZIGZAG SCALLOP LARVAE**

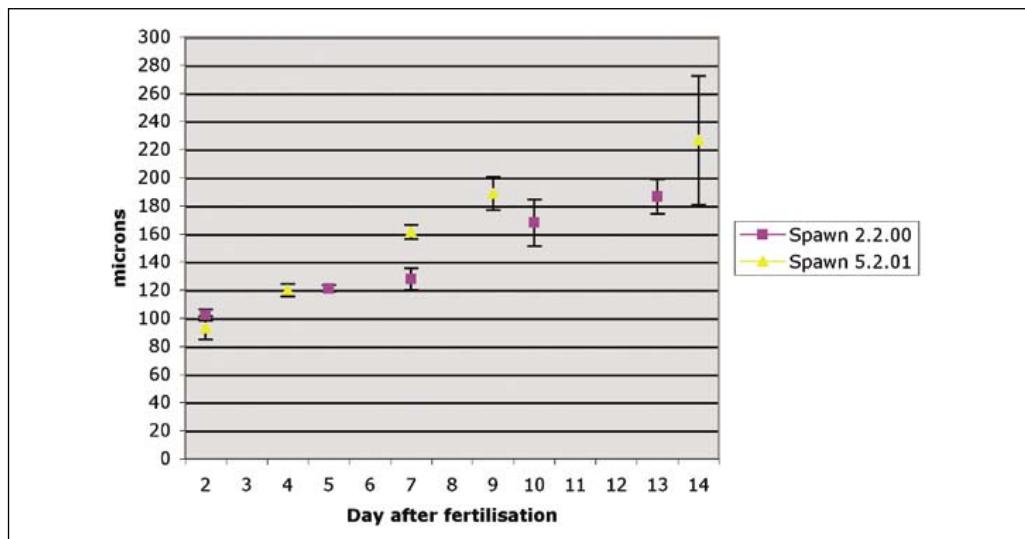
The rearing of calico scallops was developed and well tested at the Bermuda hatchery between 1999 and 2003. The procedures developed give satisfactory results with respect to both larval yields and shell growth rate. In 4 years of hatchery operation yields of D-larvae for calico scallops determined per spawn, ranged from 29.3–58.1 percent, and yields of pediveligers ready for settlement were in the range of 17.8–55.4 percent; pediveliger yields are calculated as percentage of Day-2 D-larvae. (*Note: Throughout the following sections, Day-2 larvae refer to straight-hinge D-larvae developed two days after fertilization*). These yields are in accordance with results obtained by other authors for pectinid species; Rupp (1997) reported a 12.5 percent pediveliger yield for *Nodipecten nodosus*, Uriarte *et al.* (1996) report a range of 17.6–27.8 percent for *Argopecten purpuratus*, and Couturier, Dabinett and Lanteigne (1996), report 50 percent for *P. magellanicus*. On the other hand, zigzag scallop yields are generally lower, and are indicative of the greater sensitivity of this species to handling and bacterial contamination. The range of D-larvae obtained from the number of fertilized eggs was of 0.96 to 49.2 percent per spawn over four years of operation, and for pediveligers of 2.1 to 11.7 percent.

Shell growth rates, vary among pectinid species, ranging from  $4.8 \mu\text{m}.\text{day}^{-1}$  for a cold-water species such as the rock scallop (Bourne, Hodgson and Whyte, 1989) to  $14.8 \mu\text{m}.\text{day}^{-1}$  for the tropical scallop *N. nodosus* (DeLa Roche *et al.*, 2002). A mean growth rate of  $10 \mu\text{m}.\text{day}^{-1}$  is strived for, when rearing both calico and zigzag larvae in Bermuda; this is a relatively fast growth rate when compared to other pectinid species. Throughout the larval life, shell length and height are measured routinely at every water change to assess the state of the larval culture. Figures 3.7 and 3.8 provide shell growth data for calico and zigzag scallops reared in routine hatchery operation. Data shown is a summary of results from the past four years of operation, illustrating the maximal and minimal shell growth curves for batches with average yields. Shell length of Day-2 zigzag larvae range from  $93.1 \pm 83.1 \mu\text{m}$  to  $113.3 \pm 4.3 \mu\text{m}$ . Pediveligers for this species reach shell length of  $161.3 \pm 22.5 \mu\text{m}$  to  $226.6 \pm 45.9 \mu\text{m}$ . For the calico scallop, Day-2 larvae have a shell length of  $92.7 \pm 4.7 \mu\text{m}$  to  $102.9 \pm 4.5 \mu\text{m}$ . Pediveligers for this species reach shell length of  $184.2 \pm 14.0 \mu\text{m}$  to  $203.5 \pm 16 \mu\text{m}$ . Pediveliger shell length is lower than that reported in the literature for the same species (Costello *et al.* 1973). This may also be attributed to the fact that settlement was usually initiated as early as possible to avoid sticking of pediveligers on the sides of the culture tanks. For this reason, shell length provided here, may underestimate maximal pediveliger size.

Daily growth rate for the same larval cycles as in Figures 3.7 and 3.8 are shown in Table 3.2. For both the zigzag and calico scallop, the differences in overall shell growth



**Figure 3.7:** Results of shell growth (length) for calico scallop larvae reared in Bermuda. Two curves show maximal and minimal range obtained over 4 years of operation.



**Figure 3.8:** Shell growth (length) for zigzag scallop larvae reared in Bermuda. Two growth curves show maximal and minimal length obtained over 4 years of operation.

between a “good” run and a “bad run” can be attributed to the slow growth rate at the beginning of larval life between Day-5 and Day-7. Both the shell length curves and the daily growth rate can be used as a standard range for future hatchery rearing of these species.

**Table 3.2:** Daily growth rate for both zigzag and calico scallop larvae reared in Bermuda. Results are shown for two larval batches, one illustrating minimal shell growth and the other illustrating maximal shell growth.

Days after fertilization	Zigzag scallop growth rate (μm per day)		Calico scallop growth rate (μm per day)	
	Minimum	Maximum	Minimum	Maximum
D2-D5	9.5	13.5	6.9	5.7
D5-D7	3.3	13.8	0.6	9.3
D7-D10	13.3	13.7	13.6	10.9
D10-D13	6.2	7.5	15.4	8.6

### 3.4.1 Larval rearing procedure

In light of the in-house studies described above, a standard procedure was developed and adhered to for the rearing of calico and zigzag scallop larvae in Bermuda. The more conventional static rearing system is routinely followed, where square 1 000 litres capacity tanks with lids, are preferred mainly due to their insulation characteristic. This allows for rearing of larvae at a temperature higher than ambient. Temperature fluctuation within larval tanks does not exceed  $\pm 0.5^{\circ}\text{C}$  between water changes. (*Note: The changeover to flow-through system is considered, but the purchase of new tanks is necessary for this.*) Rearing temperature is  $24\pm 1^{\circ}\text{C}$  and salinity is ambient (36 ppt) from the egg stage to settlement stage. Water change is conducted three times a week; at this time, larvae are collected on two sieves of differing mesh size, such that fastest growing larvae are separated from the slower growing or dying larvae. Collected larvae are transferred to temporary containers (10 litres buckets), while tanks are cleaned and re-filled with treated seawater. Any assessment of larval culture is done during this transfer period. Once tanks are ready, larvae are re-distributed, often pooling larvae of similar size into one tank. Beginning Day-2 after fertilization, a small supply of air is given to the larvae, via a small diameter air tube, reaching the bottom of the larval tank (see technical drawing – page 13). The air supply, controlled by a labcock ball valve, is turned on low to only allow one air bubble at a time. Feeding of larvae is provided in a single batch at the same time each day, and after re-distribution of larvae during water change days.

#### 3.4.1.1 Water change

The schedule followed pertains to a working week from Monday to Friday; although feeding and routine checks are conducted daily (including Saturday and Sunday), water change days are avoided over the weekend. Spawns are conducted as outlined in Chapter 1 (Protocol-4), preferably on a Wednesday; the reason for this is related to the length of the larval life (12–14 days). Depending on batches, larvae are ready to be set either on Day-12 or Day-14 after fertilization. Spawning on a Wednesday, results in Day-2 larvae to be distributed on a Friday, and “Setting Day” to be either on a Monday (Day-12), or a Wednesday (Day-14). In this way, any mortality due to delayed set is avoided. Water change days are therefore, Monday, Wednesday and Friday.

Initially Day-2 D-larvae are collected on 40 and 60  $\mu\text{m}$  sieves. Depending on batches, a certain percentage of larvae are large enough to be collected on a 60  $\mu\text{m}$  sieve; however, for the most part, healthy D-larvae are collected on a 40  $\mu\text{m}$  sieve. Some variations are seen among tanks at times. Any abnormal larvae or undeveloped eggs either pass through the 40  $\mu\text{m}$  sieves to drain, or are thereafter discarded should they prove to be the majority of the collected larval culture. Pull-down, or take-down, of tanks is conducted slowly, to avoid crushing of larvae onto the sieves or damage to the shells. For a 1 000 litres tank, 45–60 minutes is allowed for take-down. The model hatchery has four 1 000 litres larval tanks, take-down is initiated at 30-minute intervals to minimize transfer period for each larval batch. Each larval tank is attributed a number (LI to LIV), facilitating the tracking of larval cultures.

Protocol-11 outlines the details for taking-down of larvae; this applies at every water change; the only difference being the increasing mesh size for larvae as they grow and develop. Table 3.3 is a summary of mesh sizes used throughout larval development for calico and zigzag scallops, as well as the relationship between mesh size and larval size. Throughout tank pull-down, larvae are maintained in water, to avoid dehydration; for this reason, sieves are suspended in a tray, and submerged in seawater. Once a tank is completely drained, a thorough rinsing with filtered seawater is done with a gentle flow to ensure that any larvae collected in corners and drain is washed onto the sieves. Once all larvae are collected from the larval tank, immediate transfer into pre-cleaned 10-litre

**Table 3.3:** Relationship between mesh size and larval size retained on it as well as mesh sizes used specifically for the sand scallop and the calico scallop throughout larval development.

Mesh size ( $\mu\text{m}$ )	Size of larvae retained	Days after fertilization	Mesh size used for zigzag and calico scallops
44	D-stage to 110 $\mu\text{m}$	2	40 & 60
120	>113 $\mu\text{m}$	5	40 & 60
150	$\geq 170 \mu\text{m}$	7	60 & 80
160	$\geq 212 \mu\text{m}$	9	80 & 120
180	$\geq 255 \mu\text{m}$	11	80 & 120
200	$\geq 280 \mu\text{m}$	13	150 & 120

buckets allows larvae to remain suspended; larvae collected on different mesh size sieves are transferred into separate containers. Buckets are labeled with the larval tank number and size of mesh upon which larvae are retained. Holding of larvae in buckets should not exceed one hour; this is a stressful period for larvae and diseases that may be present could spread rapidly. Samples are taken at this time for counting of larvae. For re-distribution, larvae of similar “health” and size fractions are pooled; if a culture contains a greater percentage of dead or abnormal larvae than healthy larvae, it is discarded. [Note: Other hatcheries perform bacteriological tests to assess the health of larval cultures; as for example, is reported by Neima and Kenchington (1997), who take samples and plate them on CBS and Marine Agar using standard bacteriological procedures. From these plates, they perform a microbiological screening for a yes/no response. Results of this screening are used to make decisions regarding combining groups of larvae on subsequent change days or discarding larvae]. Larvae are passed through a large mesh sieve (300  $\mu\text{m}$ ) for elimination of debris as they are distributed into larval tanks. To avoid damaging larvae, the previously cleaned sieve is held in the larval tank so that the mesh is submerged, and contents of the transfer bucket are gently poured through it. To ensure that all larvae are transferred, the holding bucket is rinsed with filtered seawater and its contents poured through the sieve; this rinsing process is repeated twice.

At the time of distribution, the number of larvae per tank is recorded along with density and volume of water in tank. On a daily basis, routine checks are made at the very beginning of the day and records are made on the hatchery check sheets provided for each tank. Appendix 15 provides a sample check sheet. During these checks, air supply is verified, ensuring that flow is not too high or stopped; temperature of the tanks is recorded and care is taken to clean the thermometer between each tank to avoid contamination; any observations related to the state of the culture, for example detritus on the bottom, is written on the check sheet and algal food ration and composition provided are recorded for each tank.

Sieves required throughout larval and post-larval cultures can be made using available materials. Large diameter pipes can be cut and transformed into sieves by gluing fine mesh on one end. Details of construction are given in Appendix 17.

#### PROTOCOL-11

#### TAKE-DOWN OF LARVAL TANKS: LARVAL COLLECTION AND REDISTRIBUTION

1. Backwash as per Appendix 6.
2. Do routine checks of broodstock, larvae, post-larvae and algae.
3. Set-up Heating Unit as per Appendix 4.
4. Rinse 1  $\mu\text{m}$  filter, filter housing and 20 mm inner diameter hose with fresh water. Set up second 1  $\mu\text{m}$  filter onto appropriate fittings above larval tanks. Adjust 20 mm ID hose to filter via hose barb (see technical drawing – page 13).

5. Clean sieves and support rings to be used by scrubbing with a cloth soaked in chlorinated fresh water and rinsing well with fresh water.
6. Clean 4 trays similarly (same trays as used for spawning).
7. Remove 4 hose fittings from chlorinated bin and rinse thoroughly with fresh water.
8. Adjust each labeled hose fitting to respective larval tank. Set up one tray per tank.
9. In each tray, place larger sieve on top of ring so that mesh of sieve is not in direct contact with bottom of tray, and that larvae do not get damaged against tray surface.
10. Suspend smaller diameter sieve into large diameter sieve using a 15 mm pipe. Be careful, smaller diameter sieve, has larger mesh size. Large larvae are collected first; small larvae pass through and collected on second large diameter sieve.
11. Fill tray with filtered seawater. Make sure collecting hose is inside smaller diameter sieve.
12. Open valve of larval tank slowly and ensure that a gentle flow of water occurs. There should not be any air bubbles coming out due to vigorousness of flow.
13. Once first tank is 1/3 down, start second tank.
14. Repeat procedure with all larval tanks.
15. Once all water is collected from the larval tank, rinse sides and bottom of tank with filtered seawater. In this way you are ensuring to collect all larvae.
16. Finish off by washing down drain with filtered seawater.
17. Carefully remove the hose from the sieves.
18. From smaller diameter sieve, wash larvae carefully into a previously cleaned bucket with a gentle flow of filtered seawater from a 20 mm ID hose. Preferably use heated seawater (same temperature as larval rearing temperature).
19. Label bucket with larval tank number and mesh size collected.
20. Place in secure place for counting and shell growth determination.
21. Clean larval tank by rinsing with a vigorous jet of fresh water.
22. Close drain valve, fill bottom of tank with approximately 50 mm of fresh water so that drain area and all corners are submerged. Add one capful of commercial bleach and leave for 10 minutes.
23. Use scrub brush and with chlorinated water scrub sides of tanks and bottom thoroughly.
24. Drain chlorinated water and rinse completely with fresh water including rinsing of lid.
25. Do one final rinse with filtered seawater.
26. Start filling tank with heated seawater to desired volume.
27. Adjust airflow so that air is supplied one bubble at a time.
28. Count collected larvae in buckets and determine survival and shell growth of larvae as described below (see Section 3.4.1.4).
29. Once tank is filled pool larvae according to size and health of culture.
30. Re-suspend in larval tanks maintaining an adequate density (see Table 3.4 below).
31. For re-suspension, pass larval culture through a pre-washed 300 µm sieve held at surface of larval tank so as to remove any debris in culture.
32. Feed larvae as required (see Table 3.5 below and Protocol-12). Replace lid.
33. Once all larvae are re-suspended, clean hatchery as outlined in Appendix 7.

### **3.4.1.2 Standard rearing density**

Larval density within one tank, does not exceed 8 larvae.ml<sup>-1</sup> initially, and 5 larvae.ml<sup>-1</sup> towards the end of larval life. Densities gradually decrease with length of larval period as larval mortality naturally occurs. Tank volume is adjusted at times in order to maintain a density of larvae appropriate for growth. Typical densities throughout

larval life are given in Table 3.4. As larvae approach metamorphosis, lower densities (1–2 larva.ml<sup>-1</sup>) are found more favourable to growth and development.

**Table 3.4:** Larval densities in rearing tanks throughout larval life during a typical hatchery cycle.

Days after fertilization	Density (larvae.ml <sup>-1</sup> )	Larval stage
0	12.4	Fertilized eggs
1	12.4	"
2	6.2	D-larvae
3	6.2	"
4	6.2	"
5	4.8	Umbone development
6	4.8	"
7	2.9	"
8	2.9	"
9	1.8	Eyespot appearance
10	1.8	"
11	1.8	"
12	1.8	Set

#### 3.4.1.3 Standard food ration

Food ration and composition given during standard hatchery procedure is given in Table 3.5. The first ration of food is provided at the first appearance of veliger larvae (24–48 hours after fertilization). Consequently to food ration studies conducted in-house (see section 3.3.4.1), algal food ration is increased on Day-9 and Day-10 of larval life.

**Table 3.5:** Food ration and composition used in rearing of calico and zigzag scallop larvae.

Days after fertilization	Algal Species and Ratio	Cells. $\mu\text{l}^{-1}$
0	0	0
1	T-Iso	7
2	T-Iso:Chaeto (1:1)	10
3	T-Iso:Chaeto (1:1)	10
4	T-Iso:Chaeto (1:1)	10
5	T-Iso:Chaeto (1:1)	12
6	T-Iso:Chaeto (1:1)	14
7	T-Iso:Chaeto (1:1)	18
8	T-Iso:Chaeto:Tetra (1:1:1)	18
9	T-Iso:Chaeto:Tetra (1:1:1)	21
10	T-Iso:Chaeto:Tetra (1:1:1)	21
11	T-Iso:Chaeto:Tetra (1:1:1)	18
12	T-Iso:Chaeto:Tetra (1:1:1)	18
13	T-Iso:Chaeto:Tetra (1:1:1)	18
14	T-Iso:Chaeto:Tetra (1:1:1)	18

#### 3.4.1.4 Counting larvae and determining survival rate and shell growth

To determine survival rate, counting of larvae in a sub-sample is done following transfer to the holding buckets. Larvae are gently mixed using a homemade plunger (see Appendix 8). Thorough mixing is obtained by a continuous up and down motion with the plunger, taking care not to touch the bottom of the bucket, to avoid crushing larvae, and staying below the surface of the water, to prevent any splashing or bubbles, which may be damaging to the larvae. During mixing, aliquots of larvae are sampled using an Eppendorf pipette. Aliquots of 1 ml are placed onto a Sedgewick Rafter Cell, and fixed with two or three drops of 10 percent formalin. Counts are made systematically by moving from one end of the grid, scanning the slide up and down to the other end. For larvae located on lines of the grid, care must be taken not to count

them twice. Triplicate aliquots are taken for each larval fraction. To determine survival, the average number of larvae counted in three aliquots is calculated, and used in the following equation:

$$\text{Total number of larvae collected} = \\ \text{Average (larvae per ml)} \times \text{Volume of seawater in bucket (ml)}$$

During routine hatchery procedure, the same aliquot of larvae used for assessing survival is used for assessing shell growth; length is defined as the maximum distance across the shell, parallel to the hinge; height is perpendicular to length, being the maximum distance from the hinge to the edge growth. Measuring 20 larvae, usually gives a good indication of growth rate between water changes. Comparisons are made with growth curves given in Figures 3.7 and 3.8. For any scientific studies however, a minimum number of 50 larvae should be measured. In the hatchery, a compound microscope using an ocular micrometer is sufficient for assessing length; some hatcheries have access to more sophisticated equipment, such as an image analysis program connected to a camera. Although these are extremely useful in detailed scientific studies, they are not necessary for routine assessment of growth.

#### **3.4.1.5 Setting of larvae**

The detailed procedures for setting larvae and post-larval rearing are given in Chapter 4. Determining maturity of larvae and readiness to set is discussed in this section as the conclusion to larval life. As larvae approach metamorphosis, selecting the right day for initiating settlement is crucial. The reason being that if larvae are ready to settle, but maintained in larval tanks, high loss of pediveligers will most probably occur. This loss may be due to two factors. The first is linked to the substrate search behaviour of pediveligers, which will lead them to settle on the sides of the larval tanks; they then, become difficult to dislodge, and become damaged in the process. The second factor is the occurrence of high mortality in metamorphic larvae observed when left too long in larval tanks. In order to avoid this loss of pediveligers, certain criteria are followed at the hatchery in Bermuda to decide whether or not larval batches are ready for settlement.

This set of criteria includes size, morphology and behaviour, and is similar to that used by other aquaculturists for the determination of mature larvae. Bourne, Hodgson and Whyte (1989) found that mature Japanese scallop larvae are 260–280 µm in shell length, and collected on a 180–200 µm screen; this is similar to size criteria for *P. magellanicus* (Neima and Kenchington, 1997). The presence of a well-developed foot, developing gill bars and eyespots approximately 10 µm in diameter, aid in the identification of mature larvae. Neima and Kenchington (1997) calculate percent eyespots and foot activity in samples for a more accurate determination. Eyespots are not as conspicuous in all pectinid species, as is the case for calico scallops, and may be difficult to use as a criteria for these species. A change in larval behaviour, from continuous swimming to periods of swimming interspersed with periods of crawling with the foot on a substrate, is most important. At this time, larvae are often collected from the bottom of larval tanks, rather than from the surface layers, unlike during the earlier stages of larval life. In holding containers, mature larvae will often clump together to form mucous strands in the water. This behaviour is called “rafting” and is a sign of healthy and vigorous larvae that are ready to metamorphose.

Criteria used for initiating settlement at BBSR are:

- 1) Active substrate-search behaviour of larvae, with foot extension and crawling observed when larvae are placed under the microscope.

- 2) Clinging of larvae to each other when they are in the transfer bucket; this “rafting” behaviour creates a very distinct line formation.
- 3) All larvae are collected on 120 µm and 150 µm mesh sieves, with the greatest majority on the latter. Mean shell length ranges from 180–200 µm for calico scallops, and 180–225 µm for zigzag scallops. Because not all larvae develop at the same rate, the smaller size fraction may be kept in a larval tank for a further two days prior to setting. This has occurred several times in Bermuda, with comparable settlement and post-larval development of the smaller size fraction (and delayed set) to the larger size fraction.

If these observations are made, it is best to initiate settlement in larvae and end larval rearing. If not, an additional two days in larval tanks is preferable for a better settlement yield. Chapter 4 describes various methods for setting of mature larvae and their subsequent post-larval requirements.



## Chapter 4

# Nursery: facilities and culture of post-larvae

<b>4.1 NURSERY FACILITIES .....</b>	83
4.1.1 Semi-recirculating raceway system (indoor) .....	84
4.1.1.1 <i>Details of sump tank .....</i>	86
4.1.2 Outdoor raceway .....	86
4.1.2.1 <i>Seawater supply to outdoor raceway .....</i>	89
4.1.2.2 <i>Sieve layout .....</i>	92
4.1.2.3 <i>Outdoor raceway elevations and algal supply .....</i>	92
4.1.3 Circular tanks .....	95
<b>4.2 SCIENTIFIC BACKGROUND – SETTLEMENT AND METAMORPHOSIS .....</b>	97
4.2.1 Factors affecting settlement and metamorphosis .....	99
<b>4.3 SCIENTIFIC BACKGROUND – POST-LARVAL DEVELOPMENT .....</b>	100
<b>4.4 TECHNIQUES – SETTING SYSTEMS AND PROTOCOLS .....</b>	101
4.4.1 Calico and zigzag scallop settlement .....	101
4.4.1.1 <i>Rapid transfer approach .....</i>	102
PROTOCOL-12 – Set of mature larvae in 450 litres tanks – rapid transfer approach .....	104
4.4.1.2 <i>Setting density for raceway system .....</i>	105
4.4.1.3 <i>Raceway set .....</i>	105
PROTOCOL-13 – Setting mature larvae in raceway – maintenance and care .....	106
<b>4.5 TECHNIQUES – POST-LARVAL REARING REQUIREMENTS .....</b>	107
4.5.1 Food ration for spat .....	107
4.5.1.1 <i>Standard food ration protocol for calico and zigzag scallops .....</i>	108
4.5.2 Strategy for efficient use of space in rearing spat .....	108
4.5.2.1 <i>Characteristics of outdoor raceway .....</i>	109
4.5.2.2 <i>Density effect on spat growth .....</i>	110
4.5.3 Raceway weekly maintenance .....	110
PROTOCOL-14 – Rearing spat in outdoor raceway .....	111
4.5.3.1 <i>Maintaining a critical biomass .....</i>	112
PROTOCOL-15 – Weighing and counting of spat for thinning and grading .....	113
4.5.4 Shell growth of calico and zigzag scallop spat .....	114

### 4.1 NURSERY FACILITIES

Two methods of settling larvae are used at the model hatchery/nursery in Bermuda. The first involves the setting of pediveligers on a downwelling system of sieves in a raceway and continued rearing fixed spat until they reach at least 2 mm in shell height;

spat may be kept in this system until they reach 5–10 mm shell height. This method requires continuous monitoring in the nursery and is labour intensive; nonetheless, it maximizes survival of spat to the juvenile stage. The second system, involves the setting of larvae on cultch (or artificial substrate) submerged in a circular tank for a shorter rearing time until spat reach 1 mm shell height. In this latter system, spat are transferred to the natural environment on a suspended culture system of longlines within one month of settlement. This method, maximizes space in the hatchery, however, survival to juvenile stage (5–10 mm shell height) is reduced. The method chosen depends on the specific strategies selected by the hatchery and on conditions prevalent in the field at the time of transfer. Facilities for both methods are shown here as they are both used in Bermuda. However, should one be chosen over the other, modifications can be easily made to the facility.

#### **4.1.1 Semi-recirculating raceway system (indoor)**

Refer to Technical Drawing – page 14. Colour codes are similar to that of other diagrams, in that ambient seawater is coded blue, outflow is coded purple and semi-recirculating flow is coded white.

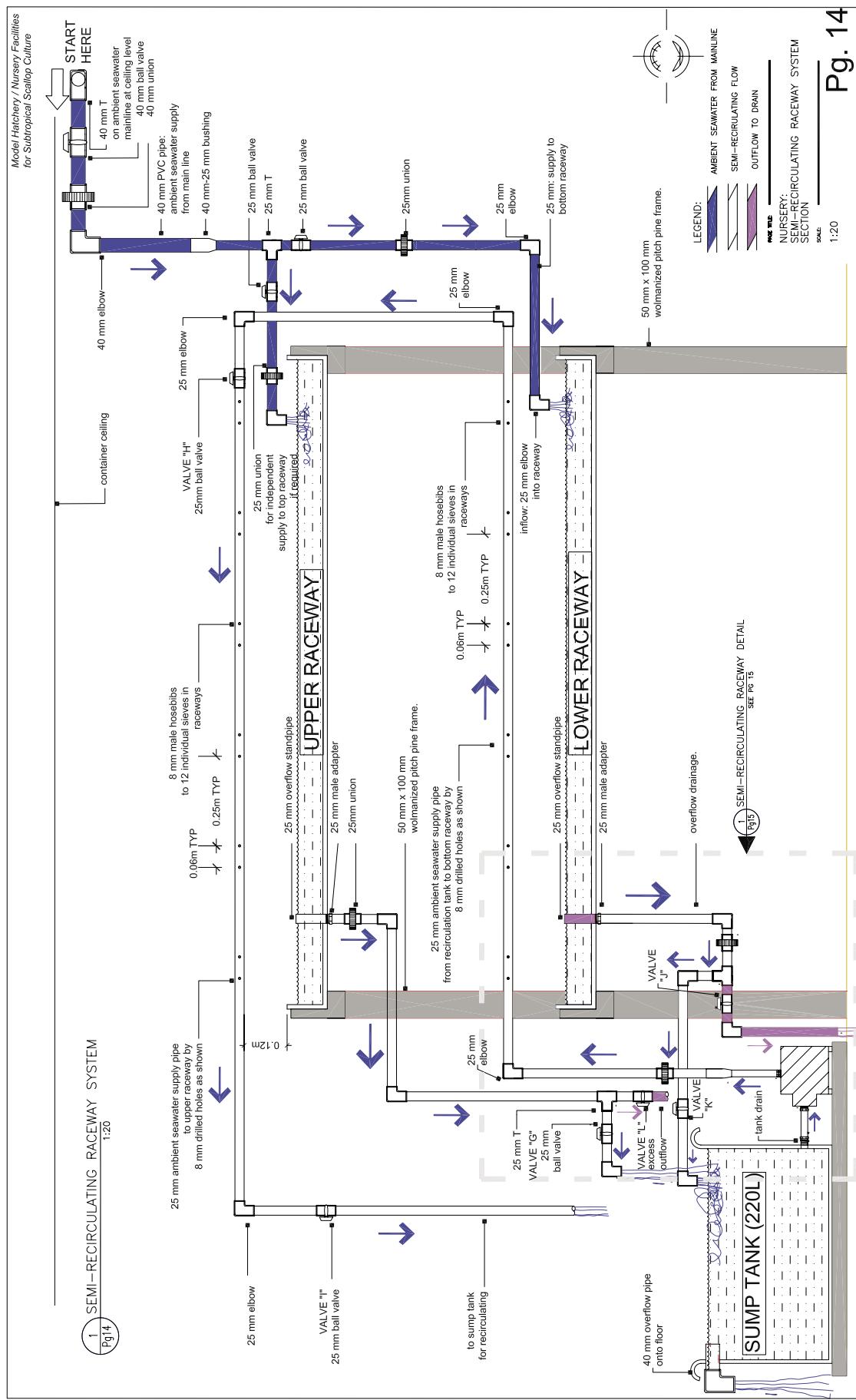
The raceways are two 200x60x15 cm deep fiberglass flow-troughs, with blue gel coated interior and grey pigmented exterior (Red-Ewald Inc.), mounted on a side wall, one above the other, and supported by a wooden frame (60x130 cm). For protection against saltwater the wooden frame is coated with several layers of epoxy. Each raceway has a 25 mm drain hole into which a 25 mm male adapter can be threaded and fitted with an overflow standpipe.

Seawater for the raceway is diverted from the mainline fastened to the ceiling, containing ambient seawater filtered to 1 µm. Diversion is created by a 40 mm T. Flow to the raceway system is regulated by a 40 mm one-way ball valve. A 40 mm union allows for dismantling and cleaning of system. The 40 mm pipe is reduced to 25 mm using a 40 mm to 25 mm bushing. Seawater flow is divided between the top and bottom raceway as it passes through a 25 mm T. Flow is regulated individually to each raceway through a 25 mm ball valve. The fitting of 25 mm unions after each ball valve facilitates cleaning of the pipes at the beginning and end of season. A 25 mm elbow directs the flow into the raceway. This therefore provides ambient seawater to the raceways. For the rearing of spat, the system is usually connected as a semi-recirculating system, where a constant inflow of ambient seawater is provided and partial recirculation of seawater is set. This minimizes the waste of algal food cells provided to the spat and can also be used to control seawater temperature, if needed, during the settlement phase.  
*Note: seawater control can be affected by manipulating seawater temperature in the sump tank.*

For the semi-recirculation system, a 220 litres sump tank is located on the floor next to the bottom raceway. Overflow of the sump tank is at the top through a 40 mm pipe and spills onto the floor. Discharge rate can be easily monitored using this overflow. Seawater is pumped from the bottom of the tank by a quiet vertical pump (P 95V). *Details and dimensions are given in technical drawing – 1/15A.* Water pumped from the bottom of the sump tank passes through a 25 mm pipe affixed to the wall of the container above the raceways. The recirculation pipe follows the length of the lower raceway, bends upward towards the upper raceway through two elbows, and passes through a 25 mm valve H. This valve is an additional control to the flow of water in the raceways. The 25 mm seawater line continues above the upper raceway and runs to the end of the raceway where a 25 mm elbow directs the flow of water through a one-way ball valve (Valve I) to the sump tank. This recirculated water is supplied to the raceways through 8 mm holes drilled into the pipe running the length of the raceway

## Technical drawing, Pg. 14

### Nursery: Semi-recirculating raceway system section



sand fitted with male hose barbs. Tygon tubing connected to these hose barbs are cut in pieces long enough to reach individual sieves suspended in the raceway.

Flow for each raceway is therefore twofold: 1) Incoming flow from the ambient seawater line at the right hand side of the raceway, which flows directly into the raceway trough and empties into the sump tank via the drain; and 2) Seawater flow from the sump tank system supplying each sieve separately. A constant water level is maintained in each raceway by the overflow standpipe. Each standpipe is connected to a 25 mm line fitted under the raceway and connected to a 25 mm union for dismantling and cleaning of pipes. A T-junction diverts the water back to the sump tank for recirculation or directly to the outside through the container wall draining into the external 100 mm drain system. The system is identical for both raceways. For the upper raceway, control of flow to drain is regulated by Valve L; for the lower raceway, it is controlled by Valve J.

The degree of semi-recirculation is controlled by Valves K, G, H and I regulating flow into the sump tank, and by Valves J and L, regulating the degree of water discarded from the raceway overflow. To achieve equilibrium, valves are opened in the following manner: Valves H and I are wide open; Valves G and K are 3/4 opened, and valves J and L are fully closed. Any excess water flows out of the sump tank through the overflow pipe. The last two valves J and L are only opened for cleaning of raceways.

#### **4.1.1.1 Details of sump tank**

Refer to Technical Drawing – page 15A. Details in Diagram 1/Pg15A show a close-up of connections required. The sump tank drain is a thru-hull fitting (20 mm) connected to the vertical pump via a 20 mm pipe and male adapter. This pump is supplied with 20 mm fittings. The outflow of the vertical pump is conducted through a 20 mm female adapter. Water passes through a 20–25 mm bushing increasing the pipe size to 25 mm. A union facilitates the removal of the pump at this end, and the adapter fittings on either end of the pump aid in its replacement when needed.

The seawater line from the pump is taken back to the wall of the container, and runs parallel to the container wall to supply the raceways (see technical drawing photo – 3/pg15A). Once water has passed along raceway contours (as described in the technical drawing – page 14) it is returned to the sump tank. Additional recirculation is provided from the overflow pipe of each raceway, where seawater is passed through a 25 mm T-junction and controlled by Valve G for the upper raceway, and Valve K for the lower raceway. For cleaning of the raceways and complete drainage, Valves G and K are closed, and outflow is diverted through Valves L and J (upper and lower raceway respectively) for discard (see technical drawing photo – 2/pg15A).

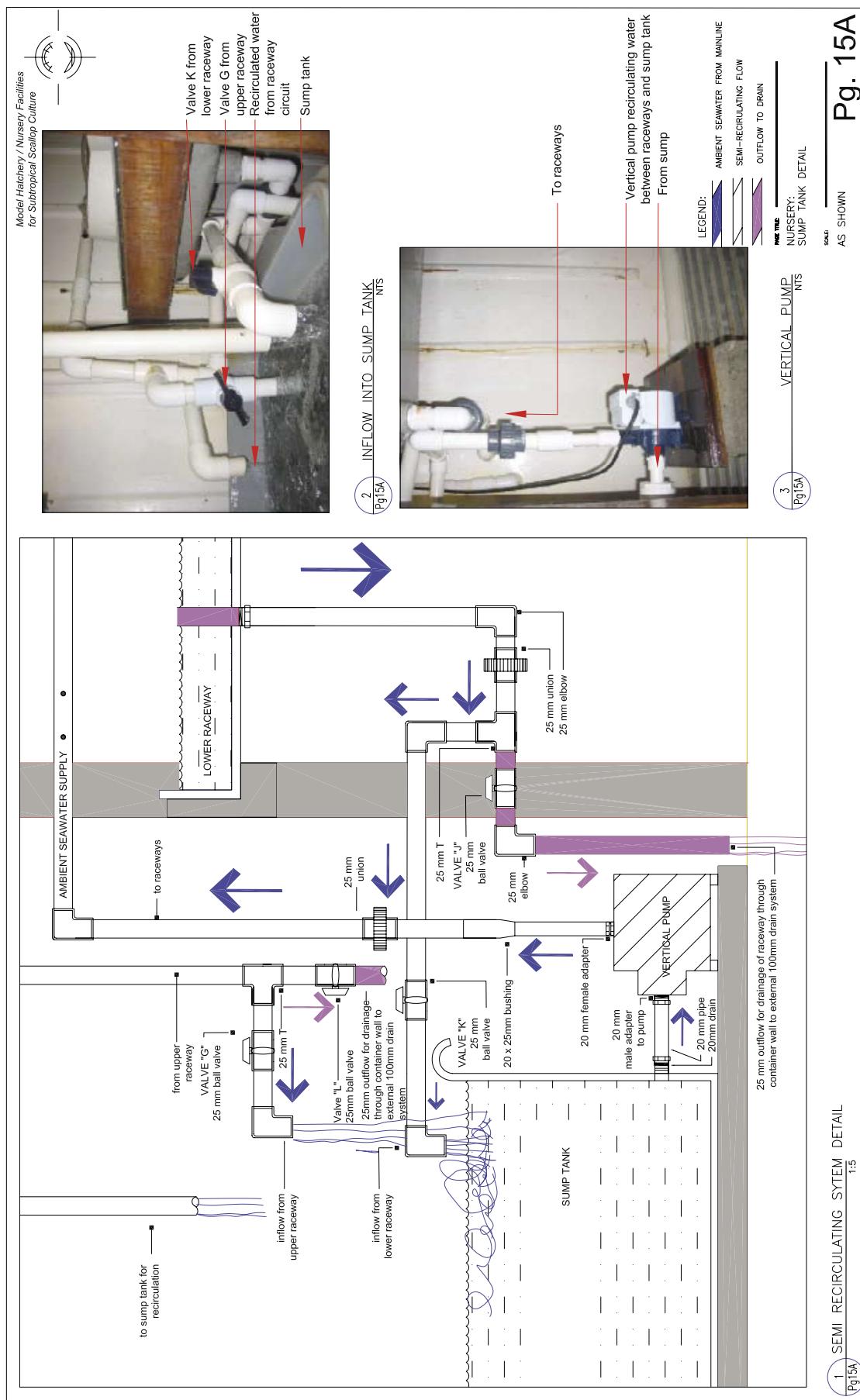
Refer to Technical Drawing – page 15B. The sump tank with overflow discharge and incoming flow is illustrated in Diagram 1/Pg15B. The relative positioning of both raceways and sump tank is seen in Diagram 2/Pg15B. Finally, the sieves used for post-larval rearing and suspended in the raceway, are set as a downwelling system in Diagram 3/Pg15B with incoming seawater flowing into each sieve. Two 25 cm diameter sieves can be placed widthwise and with a total of 12 sieves per raceway. Sieves are suspended by 15 mm transverse pipes resting on the top of the raceway.

#### **4.1.2 Outdoor raceway**

Refer to Technical Drawing – page 16A. An experimental outdoor raceway is used to grow 2 mm spat to >5 mm, a size suitable for transfer to 3 mm mesh pearl nets for growout. This is found beneficial as survival following transfer at sea is observed to be dependent on size of spat at transfer. In this outdoor nursery spat are reared in sieves, similar to the indoor raceway, but of slightly larger diameter (30 cm) and of larger mesh

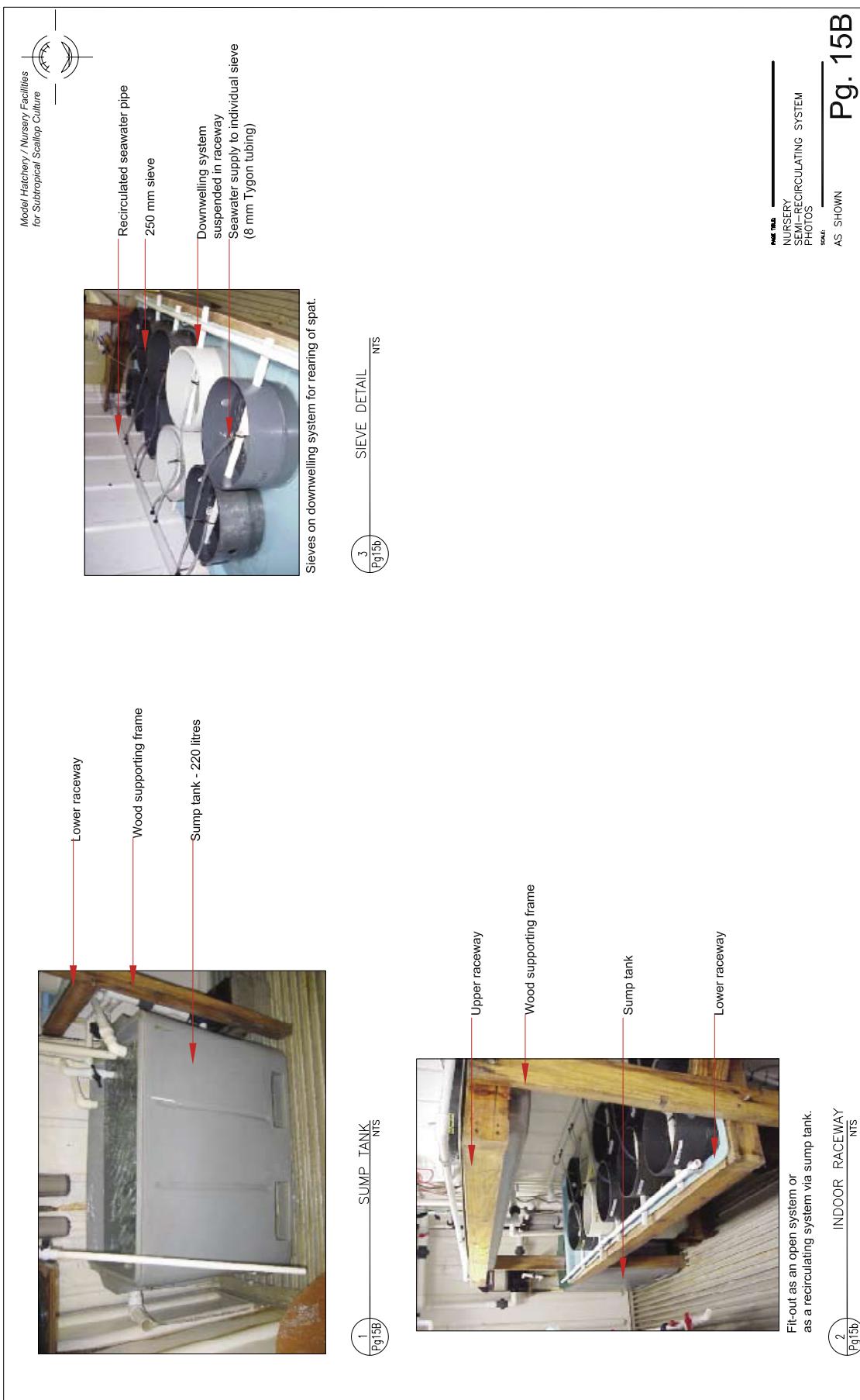
## Technical drawing, Pg. 15A

### Nursery: Sump tank detail



## Technical drawing, Pg. 15B

### **Nursery: Semi-recirculating system photographs**



size (1.2 mm). The system is an upwelling system as opposed to the indoor raceway which is a downwelling system. Seawater and algal supply are supplied at one end of the raceway and passed through the bottom of each sieve flowing out at the top of the sieve. It is found that older spat have better growth and survival when reared in such an upwelling system. In this way spat are not trapped with faecal or detritus material present in the water.

Technical diagram photo 2/Pg16A illustrates the outdoor raceway. It is built on a concrete slab of 3.35x2 m. This support pad is made of a 150 mm reinforced concrete slab on grade. The raceway is constructed of 20 mm, exterior grade plywood coated with polyester resin on all surfaces in contact with seawater. The construction is strengthened by a middle timber brace made of 130x60 cm marine plywood. The entire plywood construction is seated on 4 concrete block series at each corner; raceway channels are thus at waist level and facilitate working. A rooftop, made of corrugated plastic, and fixed onto a wooden frame and 4 wooden posts (130x60 cm), bolted into the trough system, provides shade and protection from debris and rain to the raceway. For further protection for the sides of the raceway, drop-down, fly screen panels are fitted to the canopy.

Refer to Technical diagram – 1/Pg16A. The raceway is 2.56 m in length and is divided lengthwise into 3 sections: a 15 cm wide central drainage channel and two completely separate 43 cm wide raceway channels. These interconnect with the drainage channel via six 2.5 cm diameter drain holes. Coarsely filtered ambient seawater is supplied directly to the raceway from the pump house. T-junction (T7), diverting water from the main line to the exterior raceway is also shown in the overall layout of the facility in the technical drawing – page 1 (Chapter 1). Seawater flow, diverted from the mainline, is regulated by a 50 mm one-way ball valve (Valve M). This point on the line is also one of the lowest points on the entire line and has a 50 mm drain pipe going into the sea for emptying the entire line at the beginning and end of the season. In this drain line, a T-junction diverts the water directly to the outdoor raceway and a 50 mm union valve (Valve N) placed directly afterwards is opened completely when the outdoor raceway is used. The third valve (Valve O) on the other side of the T-junction is only opened for cleaning of the seawater system pipes.

A 50 mm line is laid around the periphery of the cement pad, using 50 mm elbows when required, and clean-out valves are located at the ends to facilitate cleaning of the pipes. On the diagram, clean-out T's shown are associated with bends following the contour of the land not seen in the diagram. Direct inflow of seawater to each raceway channel is provided through a 40 mm pipe. Each raceway channel and central drainage channel has a 40 mm drain, at the opposite end of incoming flow, made of a 40 mm male adapter fitted with an overflow standpipe. Effluent water is then discarded through a 100 mm drain pipe laid on the concrete pad. Seawater supply to the raceway is illustrated and magnified in the technical drawing diagrams – 1Pg16B and 2/Pg16B; for additional clarity a front elevation drawing is given in technical drawing – 2/Pg 17.

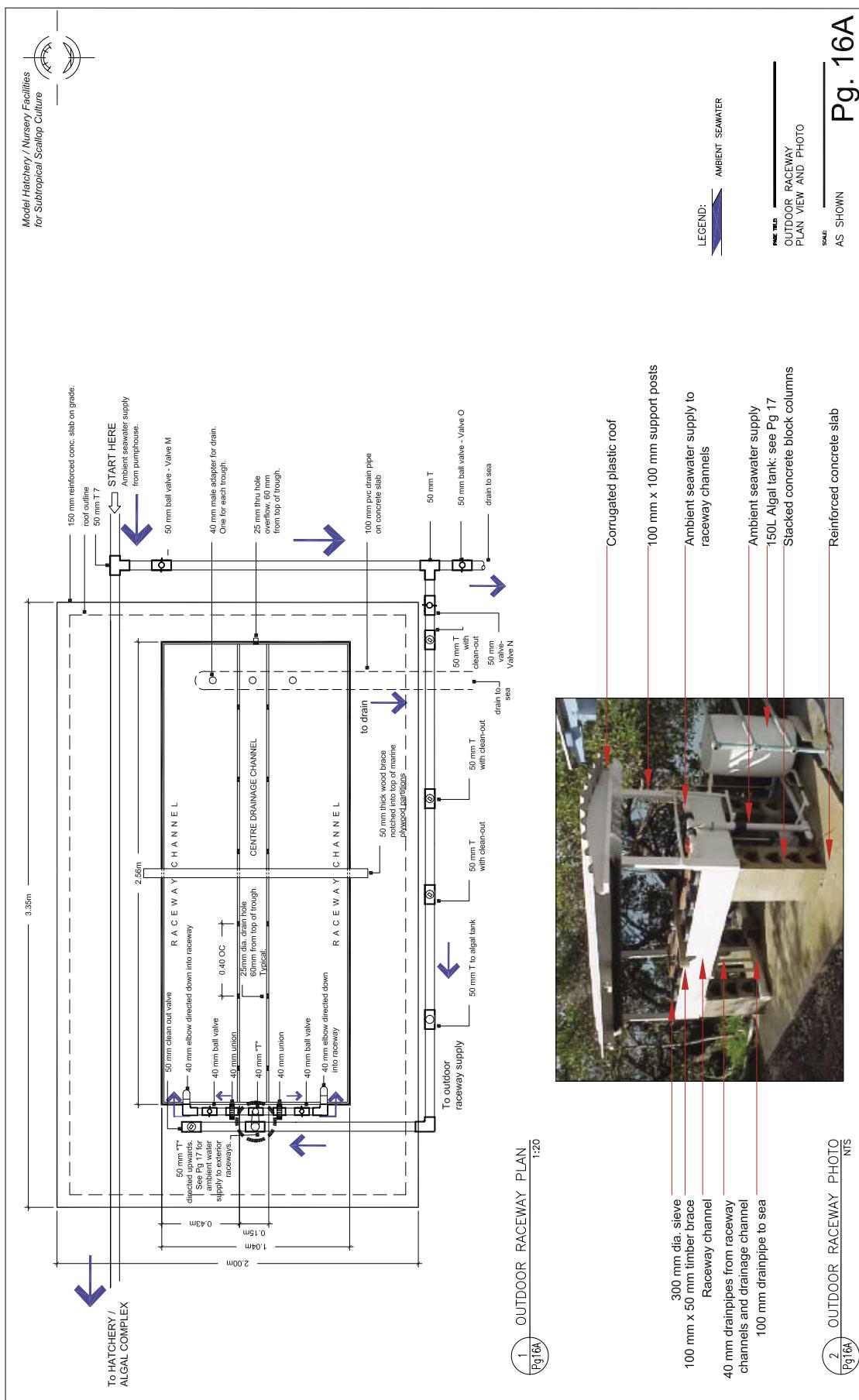
The outdoor raceway is thus an open seawater system, with supply coming from the pump house, flowing into each raceway channel from the left hand side of the diagram and flowing out at the opposite end of the raceway, where it is discharged.

#### **4.1.2.1 Seawater supply to outdoor raceway**

Refer to Technical Drawings – pages 16B and 17. Seawater inflow to raceway is magnified in the technical drawing diagrams – 1/Pg16B and 2/Pg16B. From the T-junction, water is equally distributed to both raceway channels; a 40 mm union is connected on both sides of the T-junction and flow to the raceways is regulated

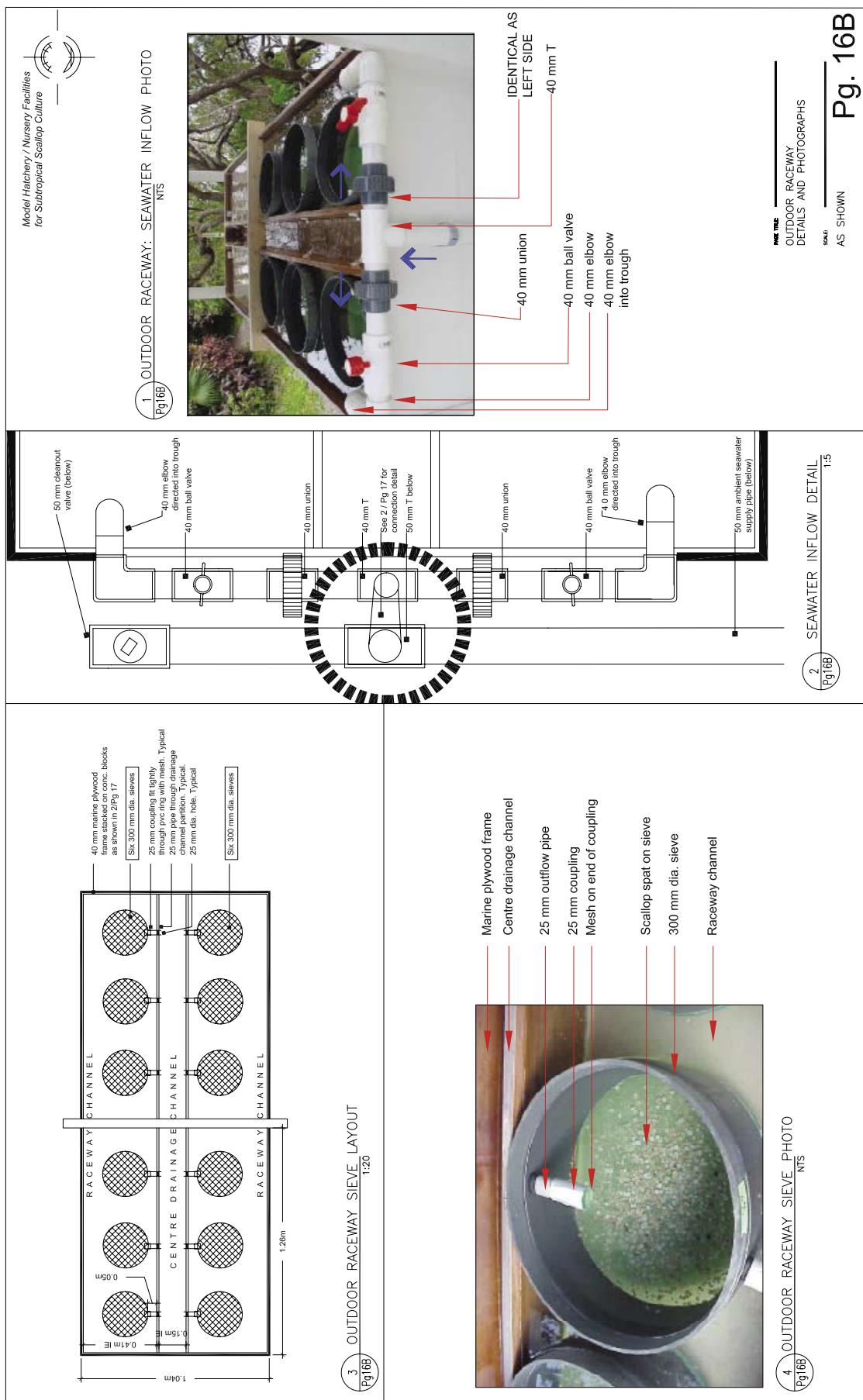
## Technical drawing, Pg. 16A

### Outdoor raceway: Plan view and photos



## Technical drawing, Pg. 16B

### Outdoor raceway: Details and photographs



through 40 mm ball valves. An elbow pointing downwards directs the flow into the raceway. Unions fitted in line allow for replacement of parts and cleaning of pipes.

The front elevation of supply to the raceway is depicted in the technical drawing diagram – 2/Pg17. From the main seawater supply running on the ground along the periphery of the concrete pad, flow is directed upwards via a T-junction capped with clean-out valve for maintenance purposes. A 50 mm union is fitted in-line for ease of dismantling followed by a 50–40 mm bushing reducing the seawater supply line to 40 mm. The line runs in the middle of the raceway frame to the top of the channels; at this point, seawater is equally diverted to both raceway channels via a 40 mm T-junction.

#### **4.1.2.2 Sieve layout**

Refer to Technical Drawing – 3/Pg16B and 4/Pg16B. Each 25 mm drainage pipe connecting the raceway channel to centre drainage channel is fitted with a 25 mm coupling and pipe connected to each sieve. Sieves are 30 cm diameter, 10 cm high with a 1.06x0.72 mm (1.2 mm diagonal) mesh bonded to its base. This provides for a total of 12 spat holding sieves when the raceway is fully utilized.

The procedure for making sieves is similar to that described in Appendix 17. The mesh used to line the bottom is the same as that used for green collector bags with an aperture size of 1.2 mm on the diagonal. Fitting of the sieves to the raceway and outflow system differed from the indoor raceway system. A 25 mm hole is drilled onto the top of the sieve, through which a 25 mm pipe is tightly secured, extending on either side of the sieve wall by 50 mm. A 25 mm coupling is fitted into the 25 mm pipe in the interior of the sieve. The opening of the coupling is closed by a piece of 1.2 mm mesh to prevent any spat from flowing out. The overflow pipe is secured into the wall of the trough by tightening it into the drilled hole (see technical drawing photo – 4/Pg16B). Sieves are suspended off the bottom; and water flow is directed so as to move through the bottom mesh and out at the top of the sieve into the centre drainage channel, causing an upwelling movement.

**Note:** The system can readily be adapted for a downwelling flow by inputting water into the central channel and discharging the waste water through 40 mm diameter drainage pipes at the outflow end of each raceway compartment. In this case, the flow enters the sieve at the top and flows vertically downwards through the mesh of the sieves.

#### **4.1.2.3 Outdoor raceway elevations and algal supply**

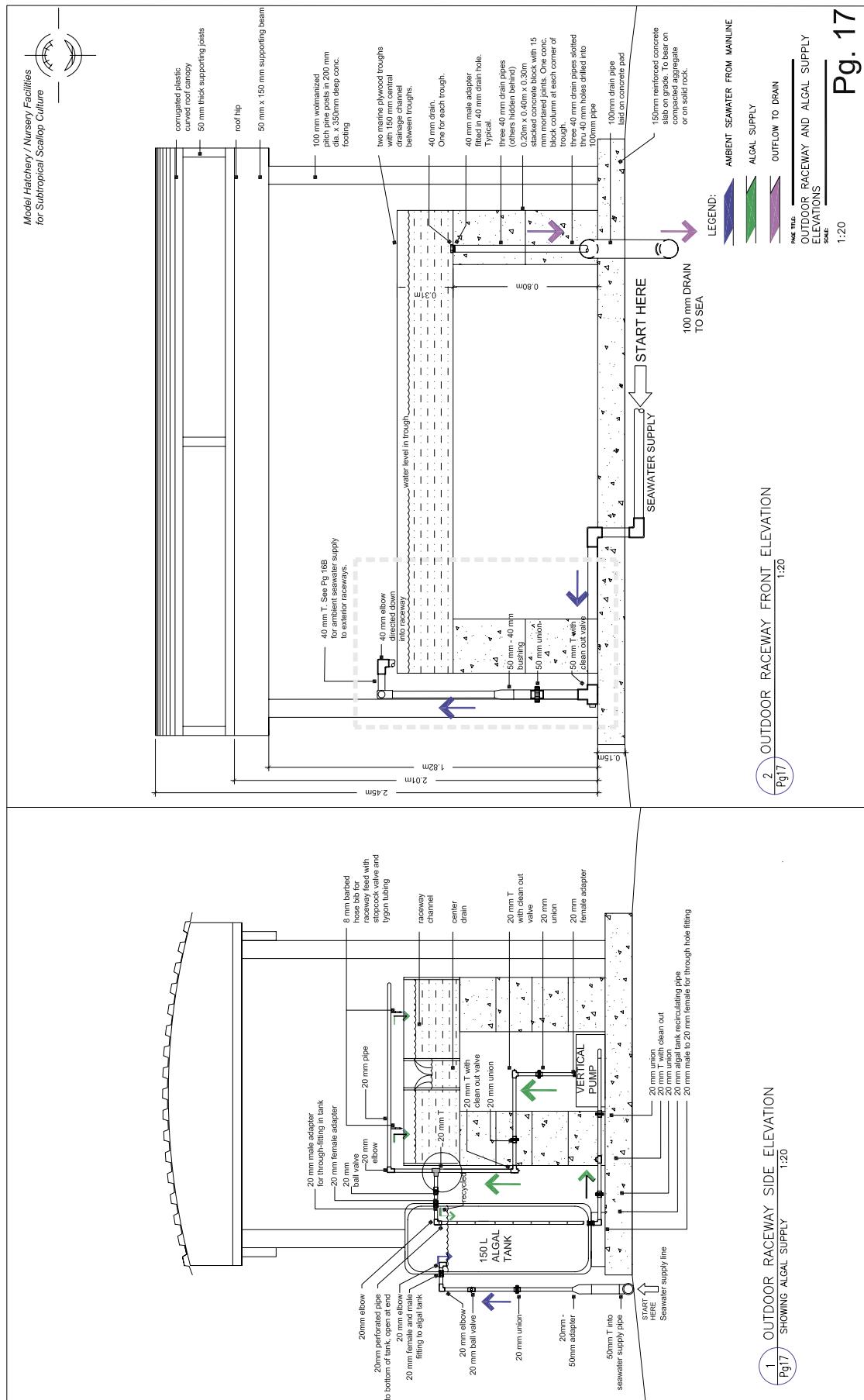
Refer to Technical Drawings – page 17 and page 18). Colour codes for seawater are blue and purple for ambient supply and outflow; additionally, algal supply is coded in green.

The side elevation diagram (see technical drawing – 1/Pg17) depicts the plastic cylindrical tank used as an algal reservoir (56 cm diameter, 75 cm high tank; Volume of 150 l). In order to allow for supply or outflow of algae and seawater, three 20 mm holes are drilled into the wall of the tank. Two holes, on opposite sides of the tank, are close to the top and one is at the bottom. *Position of the holes can be seen in the photos (see technical drawings – 1/Pg18 and 2/Pg18).* The holes are sealed with an O-ring and fitted with a 20 mm female adapter to 20 mm male adapter to make a watertight connection.

There is a small demand for seawater supply to the algal tank on a daily basis for diluting of algal ration and cleaning of tank. For this purpose, the ambient seawater line coming from the pump house is first diverted to fill the algal tank (see technical drawings – 1/Pg17 and 1/Pg18). It is immediately reduced to 20 mm by a 50 mm to

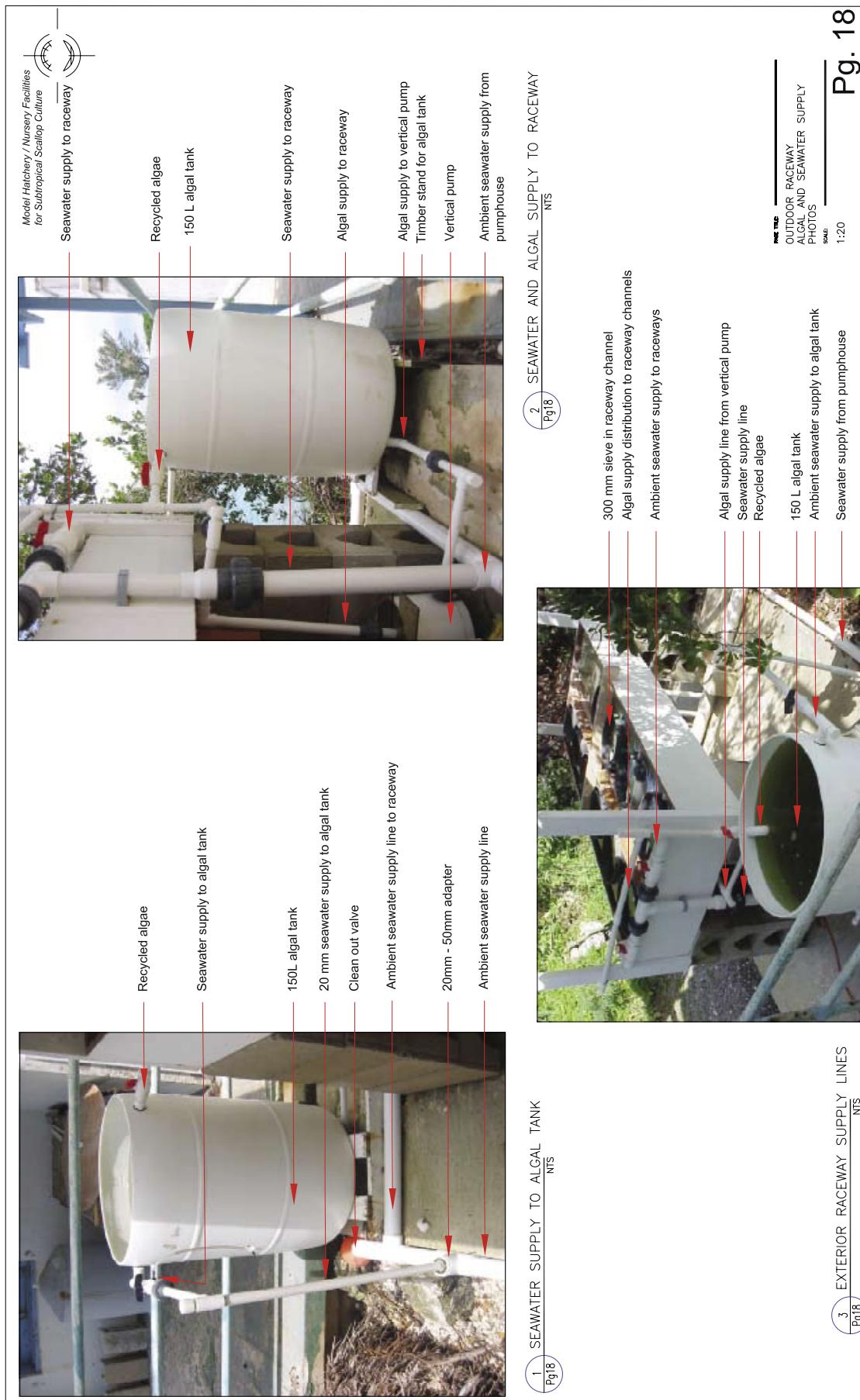
## Technical drawing, Pg. 17

### Outdoor raceway and algal supply elevations



## Technical drawing, Pg. 18

### Outdoor raceway: Algal and seawater supply photographs



20 mm adapter. A 20 mm union fitted in line allows for removal of tank and cleaning of pipes when necessary. Flow is regulated by a 20 mm ball valve; seawater passes through the tank wall, and is directed downward via a 20 mm elbow. *Note: A bleed line (7 mm Tygon tubing) for the seawater supply, is put in as a precautionary measure, but was actually never needed; it is not recorded in these diagrams, but can be seen in the technical drawing photo – 1/Pg18).*

For algal supply to the raceway channels, algal solution is pumped from the bottom of the 150 litres tank and runs through a 20 mm pipe parallel to the ground. Two 20 mm union are fitted in line close to the algal tank for daily cleaning of the tank. A 20 mm T-junction, capped on one end for cleaning, directs the flow to a quiet vertical pump. Fittings for the pump are typical, as described in the technical drawing – page 15, and are supplied with the pump. Algal solution is passed through a 20 mm pipe alongside the raceway wall. Each end is capped by a cleaner valve, facilitating cleaning using a pipe brush. The algal line follows the contour of the raceway channels, running along the width of the channels. For each raceway channel an 8 mm male NPT fitting (hose barb) is threaded into an 8 mm hole. Algal feed is provided through Tygon tubing (7 mm) and regulated by a stopcock valve. Algae are thus continuously injected into the raceway channels at the point of seawater inflow. As pressure from the pump is high, and flow of algae restricted into raceway, an overflow valve is fitted following a T-junction (at the top of the algal tank) in the algal supply line; excess algae is thus passed through a 20 mm pipe through the tank wall for recycling. Any excess algae is mixed into the tank, as it is forced through a 20 mm perforated pipe running the length of the 150 litres tank, stopping 25 mm off the bottom.

The elevation view for the entire raceway also depicts the roof of the raceway and its wooden framework supporting the canopy, the stacked concrete blocks elevating the raceway to waist level, the concrete pad and seawater inflow and outflow. The framework supporting the canopy drawn is a sound engineering design and differs to that quickly constructed at the Bermuda hatchery (see technical drawing photo – 2/Pg16).

Technical drawings photos – 2/Pg18 and 3/Pg18 clearly illustrate the location of the algal supply line relative to the seawater supply line.

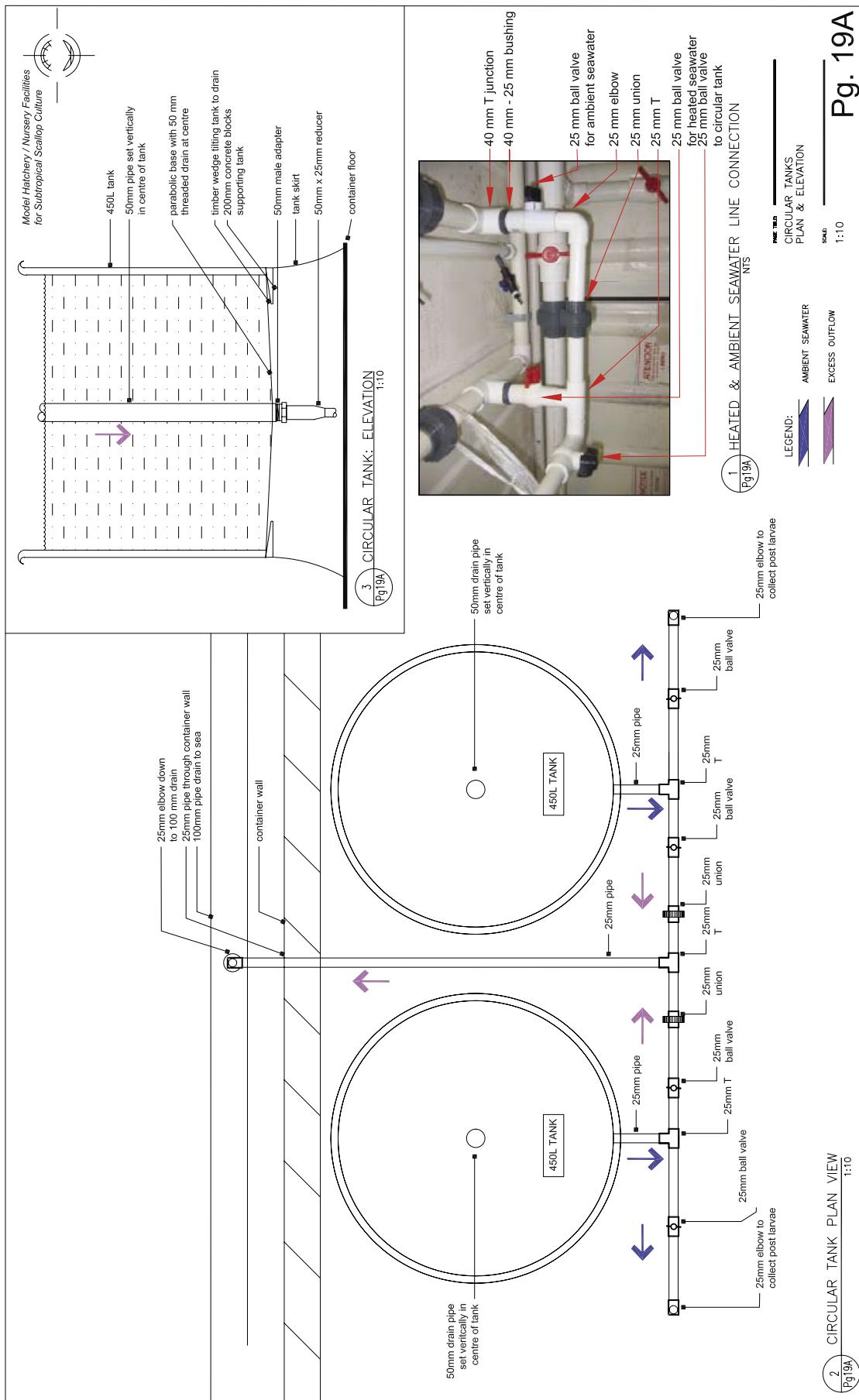
#### 4.1.3 Circular tanks

Refer to Technical Drawings – page 7, 19A and page 19B. The seawater and air supply lines for all tanks including the circular tanks described in this section are shown in a general ceiling plan in technical drawing – page 7. As a reminder, ambient seawater lines (coded blue) and heated seawater lines (coded red) run parallel to each other along the length of the container.

Refer to technical drawing – 1/page 7. Circular tanks are connected to both the heated seawater line and ambient seawater line via three connections. Ambient seawater, filtered to 1 µm, is diverted from the main line via a 40 mm T-junction; for the first connection, seawater is directed to the left, for the other two connections, seawater is directed to the right). The end of the 1 µm filtered seawater line is capped). All connections are similarly fitted, and details of one connection are shown in the technical drawing photo – 1/Pg19A. The connections are identical for both heated and seawater line. In-line of main supply pipes a 40 mm T diverts the flow of water downwards towards the circular tank. A 40 mm to 25 mm bushing reduces the circular tank line to 25 mm. The flow of seawater is regulated by a 25 mm one-way ball valve, glued to an elbow. This elbow allows for ambient seawater to flow into the circular tank. A 25 mm union is glued in line for cleaning and/or replacing of parts. After the union is a 25 mm T-junction directing the water flow to the circular tank and connecting this inflow

## Technical drawing, Pg. 19A

### **Circular tanks: Plan and elevation**



pipe to the heated seawater line. Inflow is regulated by a 25 mm valve and water is distributed at surface of tank.

Refer to technical drawing – Page 19A. The tanks used are of 1 m inner diameter fiberglass with a 10 cm wide top lip, 68 cm high and a 15 mm tapered bottom with a 50 mm centre threaded drain. A 30 cm skirt was added by the company (Red Ewald Inc.). See technical drawing diagram – 3/Pg19A. Interior of tanks was coated with blue gel coat and exterior was of grey pigment. A 50 mm standpipe of 80 cm length is used for overflow. The total capacity of the tank is 450 litres. For drainage a 50 mm drain is fitted with a 50 mm male adapter glued to a 50 mm elbow directing the drain pipe parallel to the floor. The remainder of the line is reduced to 25 mm with a 50 mm to 25 mm bushing. The plan view (see technical drawing – 2/Pg19A) shows the remainder of the connection. The 25 mm pipe extends throughout a ready-made hole in the skirt of the tank and connects to a 25 mm T-junction. This T-junction allows either for collection of swimming non-fixed spat during water exchange, or diverts the outflow to an exterior 100 mm drain system. For the collection of swimming spat, flow is controlled by a one-way 25 mm ball valve fitted with an elbow. A flexible hose is attached to the elbow during collection. For drainage to the outside a mirror system on the opposite side of the T-junction is installed; similarly a one-way 25 mm ball valve regulates the outflow. A 25 mm union allows for cleaning of the system and connects to a T-junction. This junction is the intersection of drainage pipe for two 450 litres tanks. Water is sent to the exterior drain through the container wall.

The 450 litre tanks can be set up as an open, semi-recirculating or recirculating system, useful during spat settlement. Technical drawing photo – 4/Pg19B shows the set-up for an open system where seawater is continuously supplied via a 25 mm pipe; for this, outflow valves are opened completely, and collecting valves closed. Technical drawing photo – 5/Pg19B illustrates the set-up for a semi-recirculating or recirculating system. Four 25 mm pipes of the same height as the tank are vertically placed at opposite ends of the tank and connected together using two T-junctions and four 25 mm transverse pipe lengths in the centre. Additionally, four 100 mm long standpipes are fitted to each vertical pipe via a 25 mm T-junction. The centre 25 mm transverse pipes are drilled with 8 mm diameter holes. An airlift system is achieved by a 7 mm ID Tygon tube connected to the airline and inserted into a vertical pipe for its entire length. In this way recirculation of water is created, where water from the bottom of the tank is driven by air up the vertical pipe, and flow out through the holes of the transverse pipe. It is found that two airlines inserted in two vertical pipes provide sufficient airlift.

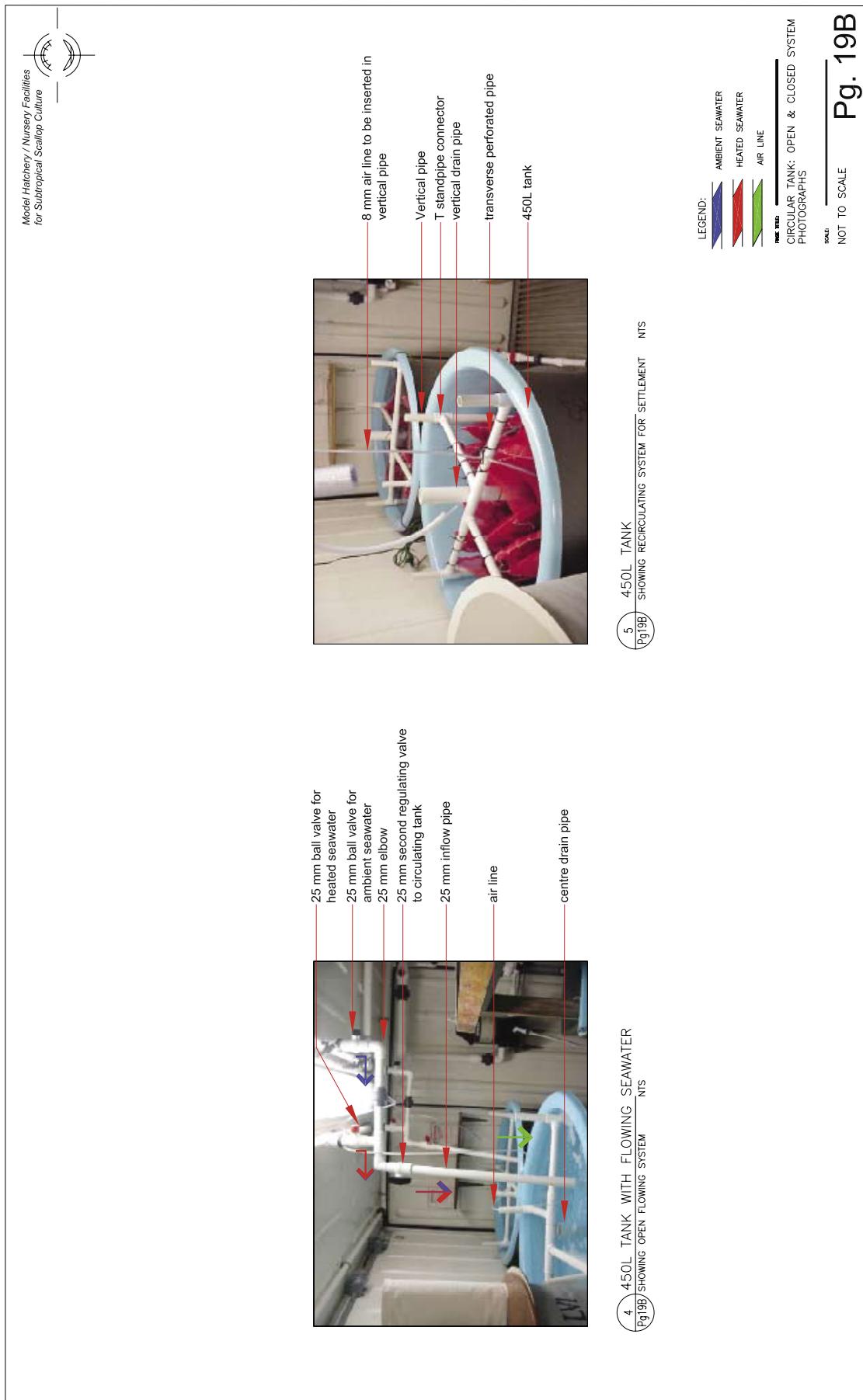
## 4.2 SCIENTIFIC BACKGROUND – SETTLEMENT AND METAMORPHOSIS

The goal of the nursery is to grow large quantities of juvenile scallops quickly from setting size (200 µm) to approximately 5 mm shell height. At this time, they can be transferred to the natural environment for growth to market size (60 mm). The nursery phase in Bermuda can be divided into two stages. The first stage, referred to here as the indoor nursery, involves settlement of mature larvae and post-larval growth of scallops to approximately 2 mm shell height. The second stage, the outdoor nursery, is optional and involves the further rearing of scallops from 2 mm to a minimum of 5 mm and up to 10 mm.

The first nursery stage allows for enhanced survival of juveniles when spat, rather than newly settled larvae are transferred to the field; as has been reported in the literature, survival rate of juveniles in the field is dependent on size at transfer. For example, Bourne and Hodgson (1991) show that immediate transfer of mature larvae to the

## Technical drawing, Pg. 19B

### Circular tanks: Open and closed system photographs



field results in a 2.6 percent survival to 1.5–3.5 cm juveniles. This low survival implies costly hatchery operations and a minimal survival to 10 percent is recommended for economic viability by these authors. The second nursery stage where spat are reared for a longer time period, not only further enhances survival following transfer but also results in a reduction of labour and time required to reach adult/market size; this, therefore maximizes cost-efficiency of a growout operation. In this instance, however, costs in the nursery are increased to rear to a larger size.

#### 4.2.1 Factors affecting settlement and metamorphosis

The process by which marine invertebrate planktonic larvae transform to become bottom dwelling juveniles can be divided into two stages; settlement, a repeatable behavioural stage, and metamorphosis, an irreversible physiological stage. Many mature invertebrate larvae have been induced to metamorphose in response to specific environmental cues – i.e. chemical, photic and tactile cues which may indicate the presence of a substratum or habitat suitable for juvenile life (Crisp, 1974; Hadfield, 1977; Burke, 1983).

In order to develop an efficient nursery system and obtain a reliable number of juveniles per larval batch, optimizing the number of larvae setting and metamorphosing into post-larvae is critical. This phase in the culture cycle is most probably the one where aquaculturists have the least control. It is a critical stage in the life of scallops and high mortalities can occur at this time, as early juveniles are extremely fragile. Bourne, Hodgson and Whyte (1989) report that for the Japanese scallop highest mortalities occur at 0.4–0.6 mm shell height, immediately following metamorphosis. The causes for mortalities seen in the nursery are unknown and are attributed to poor nutrition or improper handling. In an attempt to induce metamorphosis and enhance the number of mature larvae setting, several methods have been investigated for commercially important bivalves. Chemicals, such L-DOPA, norepinephrine and serotonin have been tested on Japanese scallop, *P. yessoensis* mature larvae (Bourne and Hodgson, 1991). Other cues such as cold temperature shock have also been tested on the same species (Kingzett, Bourne and Leask, 1990). Greatest increase in percent metamorphosis (17–19 percent over controls) was obtained with norepinephrine. The procedure is relatively simple and involves exposure of mature larvae to a diluted solution of the chemical ( $10^{-4}$ M– $10^{-6}$ M) for a short time period (60 minutes) prior to settlement. Other authors have obtained complete metamorphosis within 12 h of exposure to glycine and theophylline (Naidenko, 1991 – on *Swiftopecten swifti*). Physical stimuli such as a sudden decrease in water temperature may also influence the onset of settlement, as demonstrated by Bourne and Hodgson (1991) for rock scallop, *Crassadoma gigantea*, larvae when chilled from 15 °C to 5 °C.

Generally, successful settlement and metamorphosis for bivalves only occur when larvae are “healthy”. One of the most critical factors is the accumulation of reserves throughout the larval life, related to food ration in both a qualitative and quantitative sense (Farias, Uriarte and Castilla, 1998). It has been shown that rearing conditions affect storage and utilization of biochemical components, and hence metamorphosis and settlement (Gallager and Mann, 1981). Rearing techniques described in Chapter 3 for sub-tropical pectinid species have proved adequate in yielding mature larvae with necessary biochemical reserves. This is certainly true for larvae reared in conventional static system, as well as in low-density experimental flow-through system, (Sarkis, Helm and Hohn, 2006). The effect of rearing techniques on post-larval settlement was seen in several instances, namely that of adequate food ration. The standard diet used at the BBSR hatchery has proved adequate over the years for ensuring good spat settlement and food ration is calculated on the basis of water volume within the tank. However, this approach was not found correct for a flow-through system supporting

an increased density of larvae. Sarkis, Helm and Hohn (2006) demonstrated poor survival to post-larval stage for larvae reared in a high-density flow-through; this study has furthermore identified the need to base food ration on biomass rather than water volume for such a system.

#### **4.3 SCIENTIFIC BACKGROUND – POST-LARVAL DEVELOPMENT**

Post-larval development has been well described for some scallop species (Sastry, 1965; Cragg and Crisp, 1991). Following the conspicuous appearance of the foot, the behaviour of the veliger larva changes and may be considered as the beginning of the process of metamorphosis and preparation for settlement. The appearance of the post-larval organs and the actual attachment take place in a relatively short time, but the process of metamorphosis is not an abrupt change from a pelagic larva to an attached post-larval life. In scallop larvae, as in other bivalve larvae, metamorphosis involves changes in the nature of shell secretion, loss of some organs, greater development and/or relocation of others. The principal organs lost at metamorphosis are the velum, the velar retractor muscles and the anterior adductor. Accounts on the loss of the velum vary, but most state that the velum is lost at the end of the pediveliger stage. The veliger actively swims with the extended velum, but as the velum begins to reduce in size the swimming activity decreases. Prior to the conspicuous development of the foot, the larvae alternately swim and rest on the bottom. At this time, veligers are often collected from the bottom of larval tanks rather than the surface.

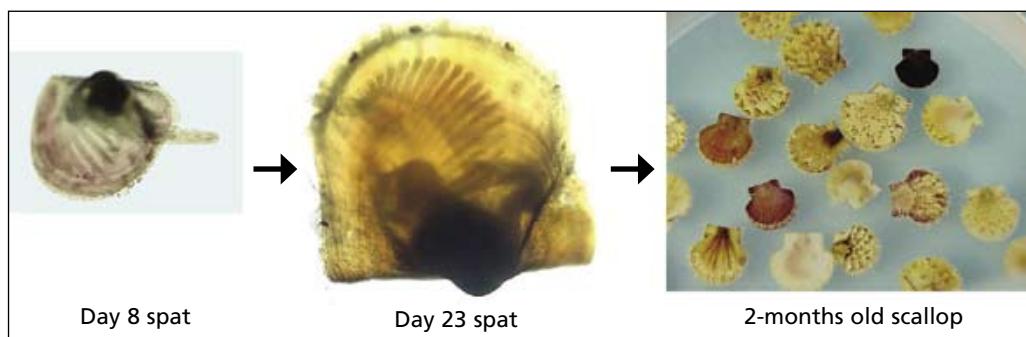
There is a general migration, relative to the axes of the shell of those organs which survive metamorphosis. This results in the mouth migrating from its posterio-ventral larval location to the adult anterior-dorsal position, the foot becomes ventral rather than posterior and the posterior adductor migrates to the centre of the valve. After metamorphosis, the gill filaments increase in length and number. Outer filaments of the adult gill system appear after metamorphosis. The time at which the gill becomes capable of filter feeding is uncertain. Some studies have shown that efficient filter feeding does not occur for spat <460 µm (Bourne, pers. comm.). The shape of the foot gradually changes after metamorphosis, becoming narrow with cilia on the free end. The foot is projected to the outside of shell and acts as a locomotory organ. The glands present in the pediveliger foot become better developed and the byssus secreted changes abruptly to a more sticky form after metamorphosis. The statocysts and eyespots are retained.

Immediately after attachment, the shell grows rapidly. The thin and fragile post-larval shell (dissococonch) is sharply demarcated from the thicker and more homogeneous larval shell. The shell margins extend rapidly, resulting in a complete change in the outward form of the newly settled larval scallop. The most important feature of dissococonch growth is the formation of the byssal notch and the gradual change in the shell to adult form. The byssal notch appears as an indentation on the right valve below the hinge line. The byssal notch narrows to form a groove; teeth appear on the byssal groove and are retained even in the adult shell. Toward the end of dissococonch development, the shell margins are semicircular in outline; the colour appears as small dashes on the shell and spreads over the entire shell by the end of dissococonch stage. Both valves of post-larval and pre-adult scallops show pigmentation.

At the end of the dissococonch stage, the pigmentation has extended over the entire shell. The young spat can now be easily recognized as a scallop. The only difference in the external shell morphology from the adults is the absence of ribs and prominent ears. Furthermore, the plications (ribs) appear on the shell at an average size of 1.175 mm.

The true ears characteristic of adults make their appearance as indentations on the anterior and posterior margins below the hinge line. The only difference at the end of post-larval development with the adults is the absence of gonads.

A series of photos showing the development of *Euvola ziczac* spat is given in Figure 4.1.



**Figure 4.1:** Development of sand scallop, *E. ziczac*, following settlement, showing dissoconch in Day-8 scallops, byssal notch formation and pigmentation in Day-23 scallops and similarity to adults in 2 months old scallops.

#### 4.4. TECHNIQUES – SETTING SYSTEMS AND PROTOCOL

The protocol chosen for setting depends partly on space available in the nursery, the period required to remain in the nursery prior to transfer to the field and of the species itself. Furthermore, setting is influenced by several factors such as, the type of setting system and setting density.

Features in setting systems, such as cultch, bacterial films and water flow are believed to stimulate metamorphosis. Cultch is an artificial substrate which increases the amount of surface area in a setting system; if no material is provided larvae can only settle on sides and bottom of tanks, as scallop pediveligers form a byssus attachment to surfaces. It has been found for some bivalve species that if a suitable substrate is not located, larvae can delay metamorphosis and may die (Bayne, 1965). Scallop larvae are believed to be selective about the type of substrate to which they attach before beginning metamorphosis. Several types of material have been tested as cultch for scallop larvae; monofilament, scallop shells polypropylene line, Kinran and Vexar are a few examples. Bourne, Hodgson and Whyte (1989) found Kinran, an artificial fibre made in Japan, to provide optimal results. In addition, the presence of a biofilm on cultch seems to increase settlement, as demonstrated by Parsons, Dadswell and Roff (1993) on *Placopecten magellanicus*. For this reason, it became standard practice at the Bermuda nursery to soak cultch in filtered flowing seawater for a minimum of 5 days prior to its use for setting.

Other hatcheries use “Chinese hat” collectors for scallop settlement, which are commonly used in the oyster industry (Neima and Kenchington, 1997). These collectors consist of 30 cm plastic disks stacked 6–8 cm apart on a central pipe. A total of 15 collectors can be placed in 1 400-litre tanks. Other hatcheries have devised horizontal panels with laminar flow for the setting of *P. magellanicus* larvae (Dabinett, Caines and Crocker, 1999).

##### 4.4.1 Calico and zigzag scallop settlement

Little work has been conducted on the chemical or physical induction of metamorphosis for *Argopecten gibbus* and *E. ziczac*. In Bermuda, spontaneous metamorphosis is allowed to take its course and setting is initiated by providing a suitable substrate.

Mature larvae are identified as per criteria outlined in Chapter 3 (see Section 3.4). Set is usually initiated on Day-12 or Day-13 after fertilization for both calico and zigzag scallop species. Mature larvae are collected on sieves ranging from 150 µm to 80 µm as conducted throughout larval rearing (see Protocol-11). If any are collected on 80 µm, health of the culture is visually assessed and culture is either discarded (if mortality is high) or maintained for another two days as larval culture. Spat collected on 150 µm and 120 µm sieves are kept separately; they are suspended in 2 litres beakers and counted. At this stage, larvae are very fragile and fix rapidly on the surfaces of the holding beakers; continuous but gentle plunging is required to avoid fixation of larvae on the surfaces and minimize shell damage. A sample of larvae is kept for determination of shell growth. Counts are done microscopically as outlined for eggs and larvae using a Sedgewick-Rafter Cell (see Protocol-4; Chapter 1). Volume required for setting system is calculated as outlined in Protocol-12 and gently siphoned using a 7 mm Tygon tubing fitted with a stopcock valve into graduated cylinder. Mature larvae are placed into setting systems to undergo settlement, metamorphosis and development into juveniles.

Two methods for setting have been tested at the BBSR nursery. The first, referred to as rapid transfer approach, minimizes the time spat are kept in the nursery to allow transfer to the field within one month of setting; this strategy reduces labour required in the nursery and frees up space for subsequent larval batches. This strategy is useful in areas like Bermuda, where physical space is restricted and larval cycle is short (12–14 days); in this way, production is enhanced by conducting frequent spawning inductions, resulting in a quick turnover of larval batches. One important consideration in rapid transfer of spat to the natural environment is that nursery and ambient temperatures coincide, minimizing stress at transfer. The second method of setting is more conventional and used widely, where spat are set in raceways, on individual sieves, and may be reared up to 5 mm in the same system. This approach (Raceway set) facilitates control of food supply and water flow to spat as well as allows for routine monitoring of spat growth and survival. Although it is more labour intensive with respect to nursery work than the first approach, it allows for a more controlled production of older spat and enhances survival of spat following transfer to the natural environment.

A description of each method is given below.

#### **4.4.1.1 Rapid transfer approach**

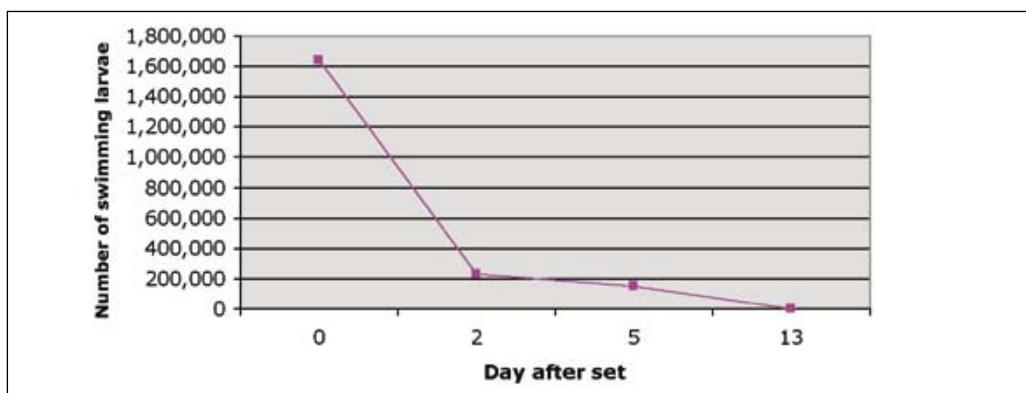
A detailed procedure (Protocol-12) is given below. Circular fiberglass tanks (450 l) are filled with cultch made of black 3 mm polyethylene mesh, acting as substrate for pediveligers. This mesh comes as a roll and is cut off in 30 cm lengths; fifteen of these strips are tied together and soaked for one week in 1 µm filtered flowing seawater



**Figure 4.2:** Cultch made of 3 mm black polyethylene mesh filling 450 litres tanks used for set.

prior to use as settling substrate. All surfaces of the setting tank are covered with bundles of cultch and others are suspended so as to fill the centre of the tank (Figure 4.2). A total of 8 strings are suspended on average. This provides a large surface area for settlement of larvae. Temperature is maintained similar to larval rearing temperature ( $24\pm1^{\circ}\text{C}$ ). Setting (or stocking) density of larvae placed in a setting system is critical. If density is too high, rate of settlement, metamorphosis and subsequent post-larval survival declines. Studies on *P. yessoensis* have shown that low

density yields better settlement; satisfactory results were obtained with densities of 0.5-2 larva.ml<sup>-1</sup> when set in tanks (Bourne, Hodgson and Whyte, 1989). On the other hand, Neima and Kenchington (1997) found satisfactory results when setting 500 000 larvae in a 1 400 litres tank (equivalent to 35 larvae.ml<sup>-1</sup>). Stocking density in tanks was not determined specifically for the two scallop species in Bermuda and densities did not exceed 4 larvae.ml<sup>-1</sup>.



**Figure 4.3:** Evaluation of calico scallop, *A. gibbus*, set in 450 litres tanks.

The duration of the setting period, where the maximal number of pediveligers metamorphoses and becomes fixed, is determined by assessing the culture over time. During this time, setting tanks are treated as static larval tanks. Aeration is provided through 7 mm airlines (2 per tank) and algal ration is provided in batches. Larvae are initially fed a mixed algal diet amounting to 18 cells. $\mu$ l<sup>-1</sup> starting day of set; food ration is gradually increased to 24 cells. $\mu$ l<sup>-1</sup> as the number of larvae metamorphose and settle. A detailed table of daily food ration is given in Table 4.1 (see Section 4.5.1).

Although water is exchanged three times a week as for larvae, the procedure differs in that setting larvae are not removed from the setting tank, and as they need to be continually submerged, the water level in the tank is maintained constant. A 20 mm inner diameter flexible hose is connected to a supply of 1  $\mu$ m filtered heated seawater to provide continuous flow to the setting tank. Water is passed via the outgoing valve at the bottom of the tank and any swimming larvae (or not fixed) are collected on a sieve in a submerged tray as for larvae. The valve is opened to provide a similar outflow to the inflow supplied to the tank; in this way, water level remains constant throughout the water exchange. This water exchange lasts for a period of 1 hour in order to ensure complete water exchange of the whole tank. Larvae collected on the sieve are washed into a beaker, counted using a Sedgewick-Rafter cell and measured with an ocular micrometer using a compound microscope. Larvae collected are for the most part those which had not fixed onto the substrate and are classified as “swimmers”. Collected larvae are re-suspended into the setting tanks, unless a high percentage of dead larvae are seen in the sample. Setting period is considered complete when swimmers are no longer seen in the collected sample.

Towards the end of the setting period (sixth day), temperature acclimation to ambient seawater is achieved by decreasing set temperature by 1 °C every two days. Once maximal number of larvae are set (or 10 days after set for these species), the setting system is changed to an open flow seawater system, where seawater is filtered to 1  $\mu$ m and supplied continually to the tanks; an air-lift driven recirculation system (illustrated in the technical drawing diagram – 5/Pg19B) enhancing water exchange within the tank, is initiated. Algal food supply is provided continuously over 24 hours by drip-feed. Young spat are reared in this way for a further 20 days. After which, they are ready for transfer to growout sites.

For both scallop species reared in Bermuda, a period of 10 days is selected as standard for duration of the setting period. As seen in Figure 4.3, monitoring of 450 litres sets has shown the absence of “swimmers” in the collected sample by Day-13. It is assumed that larvae are either fixed or dead by this time. Note: shell length of larvae collected during setting did not change ( $198 \pm 10.2 \mu\text{m}$ ) implying that larvae collected are those with slow development.

#### PROTOCOL-12

##### **SET OF MATURE LARVAE IN 450 LITRES TANKS – RAPID TRANSFER APPROACH**

1. Five days prior to anticipated setting day, prepare cultch. Cut 30 cm strips of black 3 mm polyethylene mesh and tie in bundles of 15. Soak in 1  $\mu\text{m}$  filtered flowing seawater to develop a biofilm.
2. Fill 450 litres tanks with bundles of 3 mm polyethylene strips. Cover the bottom of tank, and use re-circulation pipe system to suspend bundles so as to fill centre and top of tank.
3. Fill tank with heated double 1  $\mu\text{m}$  filtered seawater. Temperature in 450 litres tank should be same as larval rearing temperature (24 °C).
4. Prepare aeration as for larvae using two 7 mm Tygon tubing connected to airline and reaching to bottom of tank. Place two lines on opposite side of tank.
5. Setting Day referred to as Day-0 of set.
6. Collect mature larvae on 150, 120 and 80  $\mu\text{m}$  sieves. Only set those collected on 150 and 120  $\mu\text{m}$ . Suspend separate fractions in 2 litres beakers. Keep larvae in suspension by using a gentle up and down motion with plunger. Motion for mature larvae has to be continuous during sampling and prior to setting as they will attach to sides of beaker quickly.
7. Count mature larvae using a Sedgewick-Rafter Cell. Calculate number of larvae so as to distribute in 450 litres tank at density not exceeding 4 larvae. $\cdot\text{ml}^{-1}$ .
8. For calculation: Do Triplicate counts and take average count (if culture very dense, take 500  $\mu\text{l}$  aliquots instead of 1 ml).

Example:

- Average count: 151 eyed larvae in 500  $\mu\text{l}$  = 302 larvae in 1 ml
- Total volume of pool (in beaker or in cylinder) = 3 litres = 3 000 ml
- Total number of larvae in pool:  $302 \times 3\ 000 = 906\ 000$  larvae
- Setting in 450 litres tanks at a density of 1.5 larvae per ml:
- Total number of larvae in 400 litres =  $400 \times 1\ 500 = 600\ 000$  larvae
- Since 302 larvae in 1 ml, for 600 000 larvae need to siphon:  

$$600\ 000/302 = 1\ 986\ \text{ml}$$

9. Pass mature larvae over 300  $\mu\text{m}$  sieve. Keep size fractions separate if possible (150 and 120  $\mu\text{m}$ ) and set in different tanks.
10. Maintain temperature in tanks to 24 °C by two immersion heaters.
11. Feed set larvae as per larval rearing, as batch feed.
12. Water exchange conducted every two days as for larvae (Monday, Wednesday and Friday).
13. For water exchange: Prepare shallow tray and sieve (dependent on size set – for 120  $\mu\text{m}$  set use 120  $\mu\text{m}$  sieve; for 150  $\mu\text{m}$  set use 150  $\mu\text{m}$  sieve) to collect any swimming larvae. Connect 20 mm flexible hose to outgoing valve of 450 litres tank and place into sieve. This is same procedure as for collecting larvae outlined in Protocol-11.

14. Connect 20 mm ID flexible hose to treated seawater line (double 1 µm filtered seawater) and start gentle flow.
15. Partially open collecting valve of 450 litres tank and ensure flow of water is into sieve.
16. Ensure to maintain larvae and substrate in water by adjusting incoming flow to be similar to outgoing flow.
17. Exchange water for 40 minutes.
18. At the end of exchange, close collecting valve and stop incoming flow.
19. Count number of larvae obtained in sieve using Sedgewick Rafter Cell; record number of dead and swimmers.
20. If live larvae outnumber dead larvae in collected sample, re-suspend in tank, passing over 500 µm sieve to eliminate any debris. If not, discard.
21. Feed daily as per Table 4.1. Food ration calculated as:  

$$\text{Ration (ml)} = (\text{cells.} \mu\text{l}^{-1} \text{ to supply}/\text{total algal count in cells.} \mu\text{l}^{-1}) \times \text{water volume (l)} \times 1000$$
22. On Day-6, begin decreasing temperature of seawater so as to reach ambient seawater temperature by Day-10. Decrease by 1 °C every two days.
23. Continue rearing in this system until Day-10.
24. When maximal number of larvae is considered fixed (Day-10), begin re-circulating system and change static system to flow-through system. Connect hose to incoming ambient 1 µm filtered seawater supply and open drain valve.
25. Algal feeding supply now provided through 20-litre carboy placed on drip-feed. Carboy cleaned and filled daily.
26. Maintain in open and re-circulating system until transfer to field.

#### **4.4.1.2 Setting density for raceway system**

A second approach to setting makes use of raceway systems. Mature larvae are set on sieves, meshed with 120 and 150 µm Nitex material; sieves are suspended in raceway troughs as shown in the technical drawing diagram – 3/Pg15B. Density of larvae/spat per sieve is critical at all stages of this procedure; it is monitored weekly in terms of biomass (grams wet weight.sieve<sup>-1</sup>) and re-adjusted if needed. In order to optimize percent settlement, initial stocking density for mature larvae set in sieves was investigated for *E. ziczac* at BBSR nursery. Three densities were tested: 25 000, 50 000 and 100 000 pediveligers per sieve. As expected, decreasing initial density results in an increased percentage of fixed spat; it is evident that a balance needs to be achieved at the nursery between an optimal spat yield and usage of space. As a stocking density of 25 000 is not a practical solution in a space-limited hatchery like the BBSR modular hatchery, standard stocking density in Bermuda averages 50 000 larvae per 25 cm diameter sieve. Stocking density never exceeds 60 000 larvae per 25 cm sieve or 30 larvae.cm<sup>-2</sup>.

#### **4.4.1.3 Raceway set**

The standard protocol for setting spat on sieves in raceways is given in Protocol-13. The calculated number of pediveligers is quickly transferred into prepared 150 µm or 120 µm sieve following counting (Figure 4.4). Once again mature larvae are allowed to set with minimal flow and no other disruption for ten days. Food supply is provided from 20-litre carboys, placed on 24 hours drip-feed; this accounts for 50 percent of the ration. The second half of the ration is distributed in the sump tank and distributed through the re-circulating system. Sieves are suspended in the raceway, set on a semi-recirculating system (described in Section 4.1), with inflow of 1 µm filtered seawater at a rate of 3 l.min<sup>-1</sup>. Semi-recirculation ensures that all algae supplied is consumed, and allows for some temperature control of seawater. Similarly to the procedure for 450 litres



**Figure 4.4:** Mature larvae set on meshed sieve suspended as downwelling system in raceway.

tank set, mature larvae are set at a temperature of 24 °C as during larval rearing. Temperature is maintained by two immersion heaters placed in the sump tank used for recirculation.

Monitoring of several larval batches has shown that mature larvae set within 10 days of being provided with a substrate, as seen in section on 450 litres tanks above. Following this time period, any larvae collected are dead or poorly developed.

Following this setting period flow and food ration are increased and the protocol for post-larval rearing in terms of cleaning, monitoring and thinning sieves for optimal post-larval yields is initiated. Food ration throughout this stage in the indoor raceway system is provided in Table 4.1. After Day-10, flow of raceways is set according to biomass in the system; as a rule flow is set between  $25 \text{ ml}.\text{min}^{-1}.\text{g}^{-1}$  of spat (wet weight) to  $50 \text{ ml}.\text{min}^{-1}.\text{g}^{-1}$  of spat. *Note: Flow can be adjusted depending on the amount of live algae available; if fewer algae are available, decrease the flow so as to decrease volume of water.* Keeping a record of biomass and maintaining adequate biomass per sieve and in the system becomes critical towards the third week (Day-22) following set when spat begin a rapid increase in growth. If this biomass per sieve, and per system, is not controlled, high mortalities will ensue coupled with slow shell growth.

Spat are maintained in this system up to 2 months, or until they reach 3.5 mm in shell height. At which time, they are ready for the secondary nursery stage – i.e. transfer to the outdoor raceway for further growth to a minimum of 5 mm in shell height. Adherence to a strict protocol (see later Section 4.5.3) facilitates maintenance of the entire system on a weekly basis, ensuring adequate biomass for growth. Collection of spat for the next stage is simply done by washing them off the sieve with a gentle jet of seawater.

#### PROTOCOL-13

##### SETTING MATURE LARVAE IN RACEWAY – MAINTENANCE AND CARE

Setting Day = Day-0 of set.

1. Clean 30 cm sieves by wiping off sides and mesh with cloth and chlorinated water. Rinse thoroughly with fresh water using a strong jet on mesh to clear all openings.
2. Clean raceway thoroughly following directions in Appendix 19.
3. Fill raceway system (including sump tank) with 1 µm filtered seawater.
4. Once filled, turn off incoming flow and place raceway on re-circulating system only.
5. Place two immersion heaters in sump tank to increase temperature to 24 °C. Monitor temperature.
6. Pool larvae and pass through 150 and 120 µm sieves. Re-suspend each fraction in a graduated container and count as per Protocol-12.

7. Calculate volume required as per Protocol-12.

Example:

Average count: 302 larvae per ml

For Setting in 25 cm diameter sieve at a density of 50 000 larvae per sieve:  
 $50\,000/302 = 165 \text{ ml}$

8. Distribute larvae into 150 or 120 µm raceway sieve dependent on size at a density not exceeding 50 000 larvae per sieve.
9. Partially open incoming seawater flow so as to obtain a flow of 3 l.min<sup>-1</sup>. This should provide a total volume of 1 200 litres for entire raceway system.
10. Restrict flow (drip-like) to individual sieves on setting day so as to leave larvae to settle without any surface agitation.
11. Calculate food ration (Protocol-10 and 12). Distribute 50 percent in sump tank. Distribute remainder in 20-litre of carboy and top up, with 1 µm filtered seawater. Adjust flow of carboy to raceway pipe to create 24 hours drip-feed. Use Table 4.1 for food ration.
12. Fill 1 carboy per raceway. If two carboys are used, total volume of algae required is divided into two.
13. Record number of larvae set in sieve in raceway check sheet (see Appendix 18) and label sieve with date and species.
14. Conduct daily check of flow, temperature and algal ration provided on raceway check sheet.
15. On Day-2 – Increase flow slightly too fast drip.
16. On Day-6 – Start decreasing temperature to ambient seawater temperature (1 °C every two days).
17. On Day-8 – Clean raceways. Transfer sieves to saltwater table filled with heated 1 µm filtered seawater. Clean raceway as detailed in Appendix 19. DO NOT CLEAN SIEVES; DO NOT DISTURB SETTLING LARVAE.
18. On Day-10 – Assess number of swimmers: Prepare a tray (clean with chlorinated water and rinse); fill to 1/4 with filtered seawater. Remove sieve from raceway and gently wash inside mesh and sides into beaker. DO NOT BLAST POST-LARVAE OFF SCREEN; this will damage and cause breakage of shell and mortality. Place sieve in tray so that fixed spat remain submerged. Count swimmers and/or dead larvae using 1 ml aliquots with Sedgewick Rafter Cell. If many swimmers, re-suspend in sieve. Put sieve in raceway and adjust flow to maximum. Collected sample should contain few swimmers and mainly dead larvae. It is difficult to estimate the number of spat set at this time due to small size.
19. From Day-10 on begin routine cleaning of raceway system once a week (see Appendix 19).
20. By Day-22, some crowding may be observed, and spat are seen climbing up the sides of the sieve. Thinning of sieves should be initiated (Table 10).

## 4.5 TECHNIQUES – POST-LARVAL REARING REQUIREMENTS

### 4.5.1 Food ration for spat

Observations suggest scallop spat may use an alternate type of feeding between the time the velum is lost and the gill filaments are able to take over the feeding process. During this time, spat may not be able to filter feed and instead may feed on particulate material adhering to surfaces. The foot may be used for this feeding process. For this reason, culturists differ in their feeding protocols at time of setting. For *P. yessoensis*, Bourne, Hodgson and Whyte (1989) fed setting larvae similarly to other larvae, at a

level of 20–25 cells. $\mu\text{l}^{-1}$  with a mixed diet of algal species (*Isochrysis* sp., *Chaetoceros calcitrans* and *Thalassiosira pseudonana*); these authors report only a slight removal of algal cells from the water column, implying little algal consumption.

Investigating optimal food ration for fixed larvae has been attempted several times at the hatchery in Bermuda. It has proven difficult to assess optimal food ration with newly settled spat. Difficulties lie in the assessment of spat without causing shell damage or mortality, and in setting-up an experimental system, which mirrors a larger nursery system. The method for assessing the effect of food ration in spat was developed over the course of several studies; it was found that due care must be taken for the even distribution of food supply throughout the experimental system. Preliminary studies seem to imply that increasing food ration has little effect on shell growth in the first 14 days of post-larval life. A 14-day experiment, using beakers, was conducted with newly settled spat (Day-10 after set). Some difference was seen, namely in survival rate of post-larvae, with food ration; where, high food ration is associated with low survival rate. On the other hand, no trend was seen between shell growth and food ration. It appears that food ration is not critical to growth of settling post-larvae, however, several subsequent studies indicated that older post-larvae of 1.7–3.0 mm in shell height (approximately 1 and 2 months old spat, respectively) do benefit from an increased food ration. They demonstrated that spat >1.5 mm in shell height benefit from an increased daily food ration, reflected in increased shell and tissue growth, and an associated high survival. The point where food ration supplies exceeds the filtering and ingestion capacity of scallop spat was not reached in these experiments. Nonetheless they provided the basis for the food ration protocol used at the BBSR nursery.

#### **4.5.1.1 Standard food ration protocol for calico and zigzag scallops**

Standard food ration given for calico and zigzag scallops follows the schedule given below. As seen above, food ration does not need to be high in the first 7 days of growth; it is steadily increased as spat grow. An approximative seeding schedule is given in Table 4.1; this is dependent on batches and may vary slightly with different larval cycles.

**Table 4.1:** Standard food ration for rearing of calico and zigzag spat.

Spat size range	No. of cells. $\mu\text{l}^{-1}$	Approximate days after set
<500	18	0–6
500 $\mu\text{m}$ – 1 000 $\mu\text{m}$	40	7–21
1 mm – 1.5 mm	60	22–29
1.5 mm – 2 mm	150	30–39
2 mm and up	220	40

#### **4.5.2 Strategy for efficient use of space in rearing spat**

The main protocol in rearing post-larvae makes use of the raceway system, as opposed to the 450 litres tanks. The reason for this is related to ease of handling of spat as they grew; it proves more labour intensive to remove spat from cultch than to rinse them down from the sieves. Although this is true for both *E. ziczac* and *A. gibbus*, it may not be for other species, which may detach more easily. Furthermore, there are studies showing the successful use of chemicals for detaching of spat from cultch. The setting of mature larvae in 450 litres tanks is therefore done in Bermuda when there is an overflow of pediveligers, which the raceway system cannot accommodate. It is however, anticipated that the nursery in Bermuda will remove the 450 litres tanks and replace them with a second indoor raceway system.

Due to the small size of the hatchery in Bermuda and hence of limited larval production at any one time, the goal of the nursery area is to ensure optimal juvenile production

by enhancing survival rate during the following three stages: 1) From settlement to 3 mm shell height; 2) Transfer to the field; and 3) In the first month of growout in the natural environment. In this way, efficiency is maximized despite the small production capacity of the facility. Survival and growth following settlement to 3 mm size seem to be very dependent on initial stocking density, food ration, available surface area and minimal handling. On the other hand, for older spat, biomass per sieve and associated water flow, appears most important. Finally, in order to minimize mortality during and following transfer to the natural environment, it has been observed that increased size of spat at transfer enhances survival. For these reasons, an additional raceway was built outdoors (see Section 4.1), maximizing available space for growing of spat to a larger size prior to transfer to the field. When spat reach 5–7 mm in shell height, they can be directly transferred to 3 mm pearl nets, and hung on longlines. Survival rate after one month of transfer averages 90 percent. The additional exterior raceway system has for its main purpose the rearing of spat from 2 to 5 mm.

#### **4.5.2.1 Characteristics of outdoor raceway**

The outdoor raceway system differs to that of the indoor raceway system in the following: Sieve characteristics, seawater treatment and algal food composition. Sieves used are of a greater surface area ( $696 \text{ cm}^2$ ) than those used indoor to accommodate a larger biomass of spat. They are meshed with green collector bag mesh (1.2 mm on diagonal), ensuring greater water flow. Sieves are set on an upwelling system, reducing clogging of mesh with detritus.

Seawater supply comes directly from the pump house (see technical drawing – page 16A) and is only filtered through the sand filter; this allows supply of additional nutrients to the spat, such as naturally occurring algal species.

Algal food supply is provided from dry algae cultures, purchased at Reed Mariculture Inc. Three species of algae are provided daily by weight of algae. Appendix 21 gives the procedure for preparation of algal food ration for outdoor raceway. The dry algal mixture is diluted in a small volume of seawater and transferred to a large 150 litres reservoir filled with seawater and connected to the outdoor raceway. Algal supply is administered via the seawater to each raceway channel; algal mixture is supplied to each sieve via an upwelling system. This system most probably does not provide uniform supply of algae to each sieve, dependent on the position of the sieve in the raceway. For this reason, sieves are rotated weekly to ensure uniform supply of food (Protocol-15).

There is less control in this outdoor raceway system than in the indoor raceway, in terms of food composition and seawater treatment. This does not seem a deterrent to successful rearing, as with age spat become more tolerant to varying environmental conditions.

The role of the outdoor raceway is to maximize growth enabling the transfer of spat directly into 3 mm pearl nets and enhance subsequent survival in the natural environment. For this reason, several studies were conducted to investigate the optimal size for transfer of spat to the outdoor raceway, and procedures for optimal shell growth in this system. Zigzag scallop spat of two size fractions (<3.5 mm and >3.5 mm) were reared in the two different raceway systems for a period of 4 weeks. Preliminary results indicate that the smaller size fraction fared best in the indoor raceway system; this system is more controlled in terms of water filtration, distribution of algal ration, temperature and quality of algae provided. On the other hand, as spat grow, they become more tolerant to varying environmental conditions; this was seen in the shell growth rate of older spat reared in the outdoor system. In fact, the indoor system

appeared limiting to the growth of larger spat, and these showed a faster growth rate in the outdoor system. This may be related to nutritional requirements and the lack of fine filtration in the outdoor system, which may provide a nutritional supplement to the spat. On the other hand, the outdoor system did not support good survival of spat >10 mm; this is most probably also due to increasing nutritional requirements exceeding the supply provided in this system.

The efficiency of the raceway system in rearing spat tolerant to subsequent transfer to the natural environment for growout was also seen during this study. No subsequent mortality was recorded following transfer to the field in 3mm Japanese pearl nets.

This preliminary experiment was designed to give an indication of growth and survival of spat in the raceway systems; although it is not comprehensive, it does help in defining a strategy for the overall nursery system and allocate space logically. From thereon, it was concluded that spat larger than 3.5 mm would be either moved to outdoor raceway for rearing to 10 mm prior to transfer to the field, or transferred to the field in a tray system, depending on hatchery capacity at the time.

#### **4.5.2.2 Density effect on spat growth**

Rearing scallops differs from the rearing of other bivalves, namely oysters and mussels, in the required surface area for optimal growth. Scallops do not cluster as some other species do. This explains observations of scallop post-larvae seen “climbing” the sides of the sieve as they grow, distributing themselves in such a way that they are not on top of one another. As space appears to be a factor for growth, effect of density on shell growth of older spat (>3.5 mm) was investigated. Density was expressed as grams wet weight.sieve<sup>-1</sup>, as this is a more practical assessment than counting of spat. The latter can be useful at times, however, and is discussed later. Densities tested were selected on the basis of percent coverage of sieve; three densities were tested 7.2, 21.6 and 43.2 grams wet weight.sieve<sup>-1</sup>. Although survival was not affected within the density range tested, scallop spat reared at lower densities clearly benefit in shell and tissue growth. Practically, low density is difficult to sustain in a compact environment, as is the modular hatchery. As can be seen from Table 4.2, the lowest density translates into 56 spat per sieve at the end of week 4. As it is not cost efficient to culture spat at this density, a balance between space availability and optimal growth needs to be achieved.

**Table 4.2:** Relationship between density tested and number of spat per sieve.

	Initial No. of spat	Initial density spat.cm <sup>-2</sup>	Week-4 No. of spat	Week-4 density Spat.cm <sup>-2</sup>
D1	1 044	1.5	56	0.1
D3	3 132	4.5	450	0.6
D6	6 264	9.0	700	1.0

#### **4.5.3 Raceway weekly maintenance**

The studies conducted on post-larvae and older spat, although not comprehensive, do provide baseline data for management of the nursery area. From these stemmed a protocol, strictly adhered to, in order to maintain correct biomass within raceway systems, thus enhancing survival in the early spat stages, growth rate, and subsequent survival following transfer in the field. Monitoring is done in terms of biomass rather than number of spat, as obtaining wet weight is less time consuming than counting spat per sieve. Nonetheless, at times it is useful to translate production into number of spat, and Protocol-14 gives the procedure used for counting spat directly on a sieve. A grid was made at the BBSR nursery and laminated to be somewhat waterproof; this grid is placed under a sieve of spat for counting (see Appendix 20). Within the nursery

routine, a schedule for cleaning of raceways is incorporated, as cleanliness for post-larvae is as critical as when rearing larvae.

Raceways, associated tanks and sieves are cleaned once a week following the protocol outlined in Appendix 19. The first cleaning following set is done only on the raceway itself (see Protocol-13). Sieves are transferred into a holding area filled with 1 µm filtered seawater and left undisturbed during the period of cleaning. On the second week of set, a regular schedule of cleaning raceways and individual sieves is initiated; sieves are cleaned with a gentle jet of seawater, care is taken to not damage the shells, but detritus is removed to allow for better flow through the mesh. Cleaning of raceways is conducted on Tuesdays allowing for routine hatchery operations on Monday, Wednesday and Friday. Daily monitoring of raceway system and post-larval culture requirements are checked and recorded on a raceway check sheet, a sample of which is given in Appendix 18.

As post-larvae grow, total biomass in the raceway system increases; adjustments in algal food ration, water flow and available surface area are required. Food ration is discussed above and given in Table 4.1 for the indoor raceway. Total seawater volume for the indoor raceway, when used as a semi-recirculated system, is maintained at 2 320 l per day, irregardless of biomass; this is calculated by incorporating incoming flow of 3 l.min<sup>-1</sup>, volume of reservoir (220 l) and overflow discharge of 1.25 l.min<sup>-1</sup>. At this stage, algal food ration is based on volume of water, rather than on biomass. On the other hand, seawater flow for the outdoor raceway, set as an open system, is based on spat biomass in the system (50 ml.min<sup>-1</sup>.g wet weight<sup>-1</sup>, equating 2 l min<sup>-1</sup> for each raceway channel). Care is taken to minimize flow so as to avoid too rapid a discharge of algae. Food ration is provided as commercially purchased dry algae, diluted with seawater in a 150 litres tank and pumped to each raceway channel. Protocol-14 provides details on procedures followed for rearing of spat in the outdoor raceway.

Weekly cleaning of raceway is combined with biomass check starting Day-22 after set. This protocol also allows for monitoring of growth, and size fractionation of spat in the latter stages of the nursery.

#### PROTOCOL-14

##### REARING SPAT IN OUTDOOR RACEWAY

1. Clean raceway once a week (generally on Tuesday)(see Appendix 19).
2. Clean one channel at a time; transferring sieves to other channel during cleaning.
3. Use chlorox and a scrub brush for removing all detritus. Use a bottle brush for cleaning pipes, inserting it through cleaner valves.
4. Clean algal system by chlorinating 150 litres reservoir and pumping chlorinated water through algal supply lines. Be careful not to pump chlorine into raceway channel with spat.
5. Wash sieves gently with saltwater so as to remove silt. Do this twice a week (Tuesday and Friday). System clogs up more quickly as water is not fine filtered.
6. Rotate sieves once a week (Tuesday) so that first three sieves are moved to last three positions to ensure equal supply of food for all.
7. Maintain 25 grams biomass per sieve. Check on weekly basis (Wednesday)(see Protocol-15).

8. Daily, ensure that algal tank does not drain completely and that pump does not run dry. Check algal level before leaving in the evening. It should be 1/3 down. If not, increase algal supply to raceway.
9. For feeding: Turn pump off. Drain algal tank by opening union at bottom of tank (see technical drawing – 1/Pg17) and clean algal tank thoroughly with chlorox and fresh water. Rinse tank with ambient seawater. Close union and fill tank with ambient seawater. Turn pump back on once tank is filled to 1/3.
10. Remove prepared aliquots of dry algae (use a mixture of 3 species) and dilute in 1 litre of seawater. Let sit, and add to 150 litres tank. See Appendix 21 for preparation of dry algae.
11. Adjust supply to raceway using stopcock valves. A fast drip is usually adequate to empty 150 litres tank in 24 hours.

#### **4.5.3.1 Maintaining a critical biomass**

The terms “thinning” and “grading” are used throughout the following section. “Thinning” refers to assessment of biomass (total wet weight) per sieve, and redistribution of spat. “Grading” refers to fractionating sizes of spat using various mesh size.. There are mechanical graders available commercially, and are useful for large operations. In smaller hatcheries, graders can be made using a series of 30 cm diameter sieves, meshed with polyethylene material of various apertures. In Bermuda, collector bags used for natural spat collection in the field, and Japanese pearl nets used for growout were cut and used as mesh for graders. Table 4.3 shows the aperture of each mesh size used; aperture size was measured diagonally as it is the largest opening through which spat may pass.

**Table 4.3:** Aperture size, measured diagonally, for each mesh used in grading of spat.

Mesh type	Aperture size
Green collector bag	1.2 mm diagonal (0.6x0.72 mm)
Red collector bag	2.0 mm diagonal
Black polyethylene mesh	2.7 mm diagonal
Japanese pearl nets	4.9 mm diagonal

A general schedule for thinning and grading of early spat, reared in the indoor raceway system, is given in Table 4.4, and the procedure itself is outlined in Protocol-15. Thinning of spat, or first control of biomass, is usually required by Day-22 after set. Spat are washed off the sieve using a gentle jet of filtered seawater and collected into a tarred mesh square of appropriate size. At Day-22, it may be difficult to detach all spat from sieve; it is best to leave those that stick on, rather than damage by detaching. Collected spat, enclosed in the mesh, are blotted dry and weighed on a Sartorius balance ( $\pm 0.01$  grams) (Figure 4.5). Spat from same set (and size) are divided into

**Table 4.4:** Procedure for maintenance of biomass in raceway system and transfer to outdoor raceway. Sieve size for indoor ( $532 \text{ cm}^2$ ) with 150 or 120  $\mu\text{m}$  mesh. Sieve size for outdoor ( $696 \text{ cm}^2$ ) with 1.2 mm (green) mesh.

Days after set	Procedure	New biomass per sieve (grams)	Sieves used for grading	Raceway
22	Thinning	9		Indoor
29	Grading & Thinning	Small: 9 Large: 25	Red & 120 $\mu\text{m}$	Indoor
36	Thinning	Small: 9 Large: 25	Red & 120 $\mu\text{m}$	Indoor
43	Grading & Thinning	15 to 25	Black & green	Outdoor for larger
50	Grading & Thinning	25	Blue & red	Outdoor and field

9 grams wet weight aliquots and re-distributed into sieves. A 9-gram biomass at Day-22 is equivalent to 60 percent coverage of the sieve; it was found that this provided adequate surface area for growth of spat, and this percent coverage became the standard objective for re-distribution at every grading/thinning session. At Day-22, size fractionation is not found necessary. On the other hand, by Day-29, size range of spat increases, and pooled spat are passed through a red mesh sieve and 120 µm sieve. Those collected on the larger red sieve are ready for transfer to the outdoor raceway. On average, this amounts to approximately half of spat. Spat are weighed and redistributed into sieves; biomass per sieve does not exceed 9 grams for the smaller size fraction (indoor raceway), and 25 grams for the larger size fraction (outdoor raceway). The next thinning and grading session is usually two weeks later (Day-43), where all spat become large enough to be transferred to the outdoor raceway at a density of 15–25 grams per sieve. On Day-50, or <2 months after set, spat are graded using black and red sieves; those spat collected on black mesh are ready for transfer to pearl nets. Others are re-distributed into sieves at a density of 25 grams per sieve.



**Figure 4.5:** Weighing spat on a Sartorius balance ( $\pm 0.01$  gram).

#### PROTOCOL-15

##### **WEIGHING AND COUNTING OF SPAT FOR THINNING AND GRADING**

1. Clean raceway once a week (Tuesday)(see Appendix 19).
2. Thin and grade sieves once a week (Wednesday) starting Day-22.
3. Preparation: Clean 20 mm hose and connect to ambient 1 µm filtered seawater.
4. Clean saltwater table with chlorinated sponge, rinse thoroughly with fresh water and 1 µm filtered seawater.
5. Let filtered seawater flow in table.
6. Place measuring grid (Appendix 20) on saltwater table and place sieve on top.
7. Identify 10 squares (each 1 cm<sup>2</sup>) for counting. Ensure that selected squares represent all areas of the sieve (corners, centre, borders).
8. Count number of spat per cm<sup>2</sup>. Average number of spat per cm<sup>2</sup>. Estimate total number of spat as follows:  
Total number of spat in sieve = Average (spat per cm<sup>2</sup>) x SA of sieve  
(Note: SA for 25 cm sieve = 532 cm<sup>2</sup>; SA for 30 cm sieve = 696 cm<sup>2</sup>)
9. To obtain biomass: Prepare several pieces of 15 cm<sup>2</sup> mesh (aperture size depends on age of spat – see Table 4.4).

10. Tare one piece using a balance ( $\pm 0.1$  gram).
11. Collect sieve from raceway, and using gentle jet of seawater, gather spat to corner of sieve.
12. Pass spat through two sieves for grading (see Table 4.3). Repeat with remaining sieves. Keep two size fractions separate and hold graded spat in two extra sieves sitting in tray with 5 cm of filtered seawater in bottom.
13. When all spat are graded, transfer aliquot of spat into tarred mesh piece using a spatula or small spoon. Wrap spat in mesh and blot dry on a piece of paper towel.
14. Weigh spat on same balance. Record.
15. Clean sieve thoroughly with chlorinated sponge for sides and strong jet of seawater through mesh to remove all debris. Suspend in raceway.
16. Weigh out aliquot of spat required for adequate coverage, dependent on age (see Table 4.4).
17. Transfer spat in cleaned sieve.
18. If require measurement of spat, use ocular micrometer on compound microscope at magnification of 4 until 2.5 mm in shell height. After which, use Vernier callipers ( $\pm 0.1$  mm).
19. Calculate total biomass in raceway and adjust flow based on  $50 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ .
20. Feed according to Table 4.1 and Protocol-13 for indoor raceway and Appendix 21 for outdoor raceway.

#### 4.5.4 Shell growth of calico and zigzag scallop spat

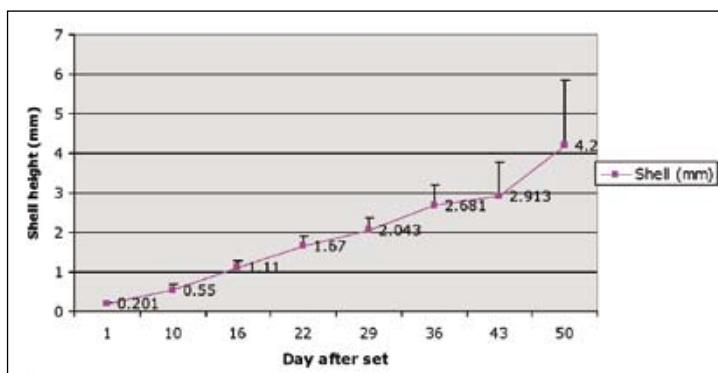
Post-larval shell growth is similar for calico and zigzag scallops. Figures 4.6 and 4.7 show shell height for both species when reared according to the protocols given above. In short, within 2 months of set, >90 percent of spat can be transferred to the field in 3 mm pearl nets. For additional assurance of post-transfer survival, spat can be maintained a further two weeks in the outdoor raceway; thus transfer of spat to pearl nets can be done 2.5 months after set with spat averaging 9 mm in shell height. The total time in hatchery and nursery prior to transfer to the natural environment does not therefore exceed 3 months. As for survival rate, mortality incurred following metamorphosis, setting of larvae and early post-larval growth, results in an average of 20 percent survival of larvae set to time of transfer (Figure 4.8). Growth rate given is associated with temperatures shown in Table 4.5.

In order to estimate total production of hatchery in terms of numbers, it is best to establish a baseline relating wet weight of spat to shell height and age. In this way, for subsequent batches, production can be estimated by extrapolating known biomass. This was done at the BBSR nursery for the calico scallop and is shown in Figure 4.9. In this way, if total biomass of Day-50 spat is known and growing at a known rate, the total number of spat produced can be calculated. This is evidently dependent on rearing conditions and growth and should be established for each nursery.

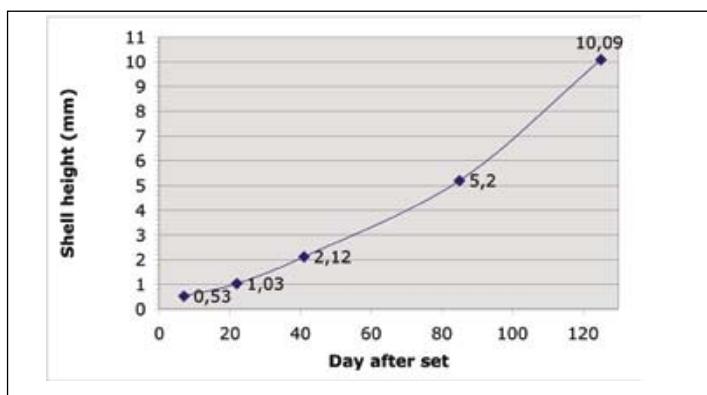
**Table 4.5:** Ambient temperature recorded in raceway systems in Bermuda during post-larval growth. Range represents monthly change.

Month	Temperature (°C)
March	18–2
April	20.5–22
May	22–24
June	24–26
July	26–27.5

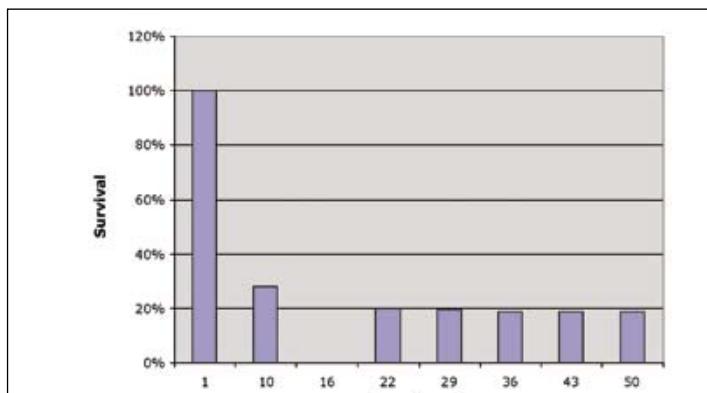
Transfer of spat from the nursery to the natural environment for growout is described in Chapter 5.



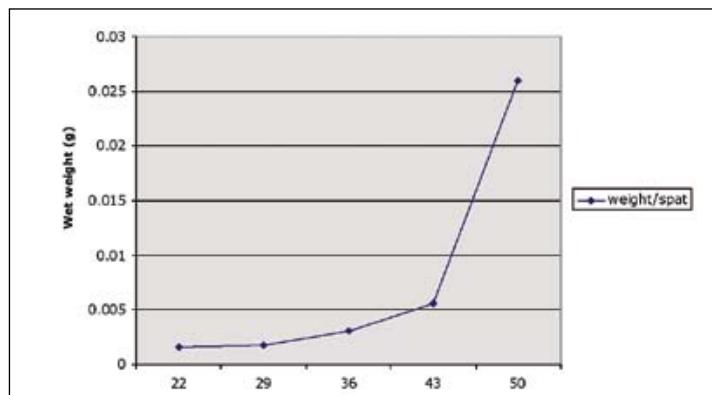
**Figure 4.6:** Shell height for calico scallop, *A. gibbus*, spat reared in raceway system.



**Figure 4.7:** Shell growth (height) of the zigzag scallop, *E. ziczac*, following settlement.



**Figure 4.8:** Survival rate of calico scallop, *A. gibbus*, post-larvae following settlement. Survival rate is calculated from number of larvae set.



**Figure 4.9:** Wet weight of calico scallop, *A. gibbus*, spat (gram per spat) as determined during the nursery stage ( $n= 100$ ).

## Chapter 5

# Growout of juveniles: transfer from nursery to field

<b>5.1 HOLDING AND GROWOUT FACILITIES .....</b>	117
5.1.1 Exterior holding tanks .....	117
5.1.2 Longlines .....	119
5.1.3 Bottom cages .....	120
<b>5.2 TECHNIQUES – TRANSFER OF SPAT FROM NURSERY TO FIELD .....</b>	121
5.2.1 Transfer of 1.5 mm spat from 450 litres tank set .....	121
PROTOCOL-16 – Transfer and retrieval of spat on cultch to field .....	122
5.2.2 Transfer of 2–4 mm spat from raceway to longlines .....	123
5.2.3 Transfer of spat >4 mm .....	123
<b>5.3 TECHNIQUES – GROWOUT OF JUVENILES .....</b>	124
5.3.1 Calico scallop growout .....	124
5.3.2 Zigzag scallop growout .....	125
<b>5.4 TECHNIQUES – TRANSPORT OF JUVENILES .....</b>	126
PROTOCOL-17 – Procedure for long transport periods of juvenile scallops ....	127

### 5.1 HOLDING AND GROWOUT FACILITIES

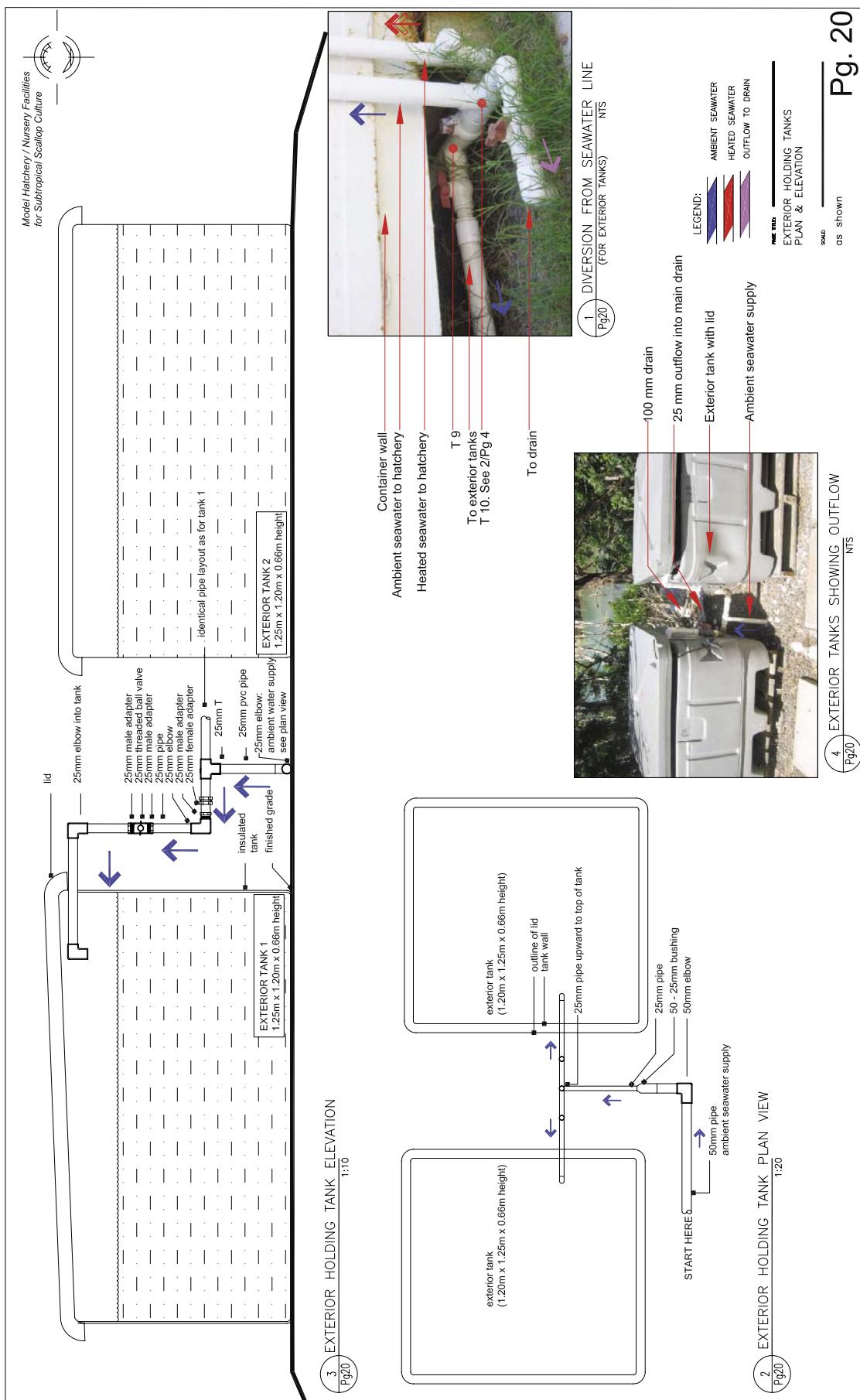
Facilities for holding scallop spat prior to transfer to the field for growout are described below. These exterior facilities can also be used for maintaining adult scallops, or for other purposes. These facilities are found extremely useful when bringing back young juveniles from the field for further sorting on land, if weather conditions do not allow sorting on the boat. The main requirements are cleanliness, as for the rest of the complex, and high water flow. Animals held in these tanks are usually not fed, and for this reason, cannot be kept for extended periods of time. Longline system used in Bermuda is described thereafter; this system is efficient for suspending pearl nets, scallop trays and/or collector bags.

#### 5.1.1 Exterior holding tanks

Refer to Technical Drawing – page 20. Two square insulated “BONAR” tanks (400 litres volume) are installed outside the hatchery on a wooden base. They are supplied with seawater through an open system, with coarsely filtered seawater diverted from the main line, prior to entry into the hatchery complex. Technical drawing diagram – 1/Pg20 illustrates the main valve regulating the flow to the exterior tanks from the pump house. The relative position of the heated seawater line and ambient seawater line supplying the hatchery can also be seen. Finally, any excess flow, pumped in but not used, is diverted to a main drain pipe, buried sub-surface and not seen in this photo. *This photo is also represented in detail in technical drawing diagram – 2/Pg4.*

## Technical drawing, Pg. 20

### Exterior holding tanks: Plan & elevation



Plumbing for these tanks is very simple and done using a 50 mm pipe buried in the ground, running to the tanks. As seen in technical drawing diagram – 2/Pg20, a 50 mm elbow is fitted to the supply line, and points upwards to direct the flow of water to the tanks. From there, the pipe is reduced to 25 mm using a 50 mm to 25 mm bushing, and runs in the centre of the two tanks. A T-junction then divides the line into two. The tank elevation diagram (see technical drawing – 3/Pg20) shows the detailed connections for supply. As these tanks are cleaned, emptied and re-filled frequently, a flexible system, allowing complete drainage of the vertical pipe system, is required. The centre T-junction is fitted with a 25 mm female and male adapter threaded into one another, then glued to a clear elbow. This allows for the entire “arm” to swivel, and allows for draining of the supply pipe. The clear elbow allows to monitor any clogging of the coarsely filtered seawater supply. A 25 mm male adapter threads into a 25 mm threaded ball valve and regulates the flow of seawater into each tank. Once again this allows for turning of the pipe system to divert flow away from tank, required when cleaning. Finally a series of two 25 mm elbow direct the flow into the tank, and flow is supplied via a 25 mm inner diameter re-enforced tubing fitted to the pipe, running to the bottom of the tank.

Outflow is through a standpipe at the opposite end of the tank connected to a bottom drain. The drain is sealed with an O-ring and caulking, fitted with a thru-hull fitting, and connected to a 25 mm pipe draining into the main 100 mm drain pipe. This can be seen in technical drawing photo – 4/Pg20.

Despite high exposure to direct sunlight in Bermuda, the insulating capacity of these tanks, and high water flow generated, maintained an adequate environment for scallops throughout the year.

### 5.1.2 LONGLINES

Calico scallops have shown good shell growth and survival rate, as well as complete reproductive development when reared in suspended cultures. This method is used widely for various scallop species (Bourne, Hodgson and Whyte, 1989; Couturier, Dabinett and Lanteigne, 1989). Animals are reared in nets suspended from longlines in the water column. Longlines consist of two anchor lines and one transverse line with floats. There are various designs in use where transverse lines are maintained at the surface or submerged at various depths. The system in Bermuda consists mainly of a sub-surface transverse line, maintained at approximately 1.5 m depth with 30 cm diameter black floats (Figure 5.1). Each float has a floatation of 14.5 kg; they are spaced every meter and tied securely to the transverse line. The rope used is a floating type

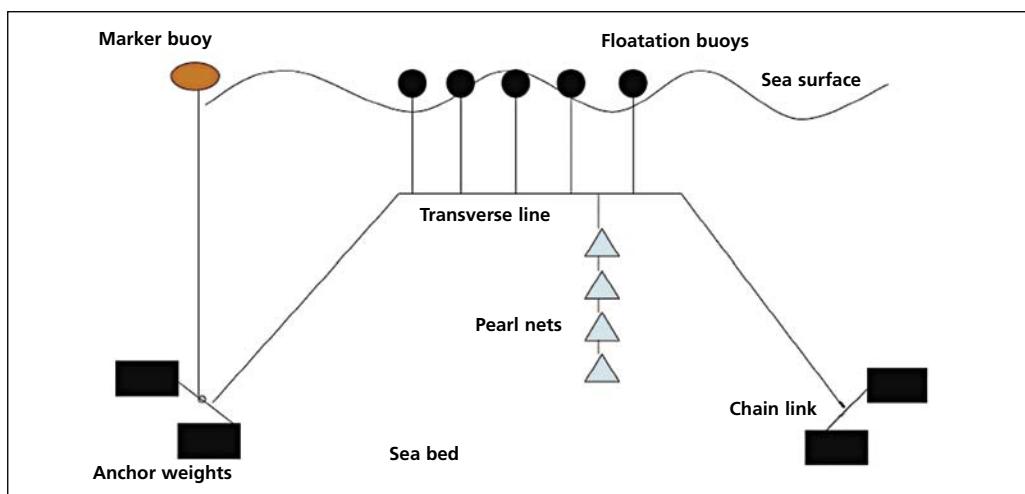


Figure 5.1: Sub-surface longline system used in Bermuda.

rather than sinking type. Maintenance of the line sub-surface serves two purposes: the first is related to the observed better performance of scallops reared below the surface, due most probably to the lack of inhibition from the UV rays. The second reason is related to minimized wave action due to wind, optimizing the longevity of the nets and of the whole system.

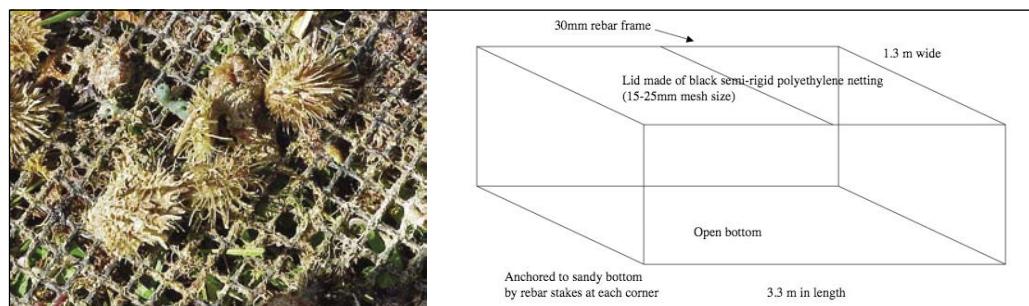
Anchor lines are, as a rule, twice as long as the depth of the water, so that in 10 m of water, each anchor line is 20 m long. They are attached to anchors made of any heavy material retrieved locally; in this case, plastic coated lead weights used for ballasts on boats were used, amounting to 68 kg per anchor. A 2 m chain length is connected to the anchors with shackles to facilitate replacing of the longline when needed. The anchor lines themselves are spliced into loops, protected by plastic tubing. Shackles connect the anchor lines to the chain and the plastic tubing avoids chafing of the rope.

In Bermuda, it is found, that a line needs replacing after two years. Square pearl nets are tied together to form a column of nets, with the last net being one metre off the bottom. As scallops grow, densities are reduced within the nets and nets of increasing mesh size are utilized; such that pearl nets of 3, 6 and 12 mm mesh size are used from juvenile to adult/market stage. Thus, in Bermuda, a 90 m longline set in 8 m of water, can support approximately 20 000–25 000 adult scallops. In the initial set-up, it is recommended to order more than the 12 mm nets needed, as some will tear due to bad weather or to predators.

Prior to transfer of spat to the field, longlines have to be prepared and set in selected sites. When selecting a site for scallop growout, the following considerations should be taken into account. Protection from storms, good water quality, high flow of water, water depth, protection from vandalism and other users, distance from nursery or ease of access.

### 5.1.3 BOTTOM CAGES

As mentioned in Chapter 4, growout of zigzag scallops to 25 mm juveniles is similar to that described for calico scallops. Unfortunately, this species does not fare well in suspended cultures for the rest of its life cycle, and exhibits optimal growth rates when cultured directly on the sandy bottom. Its natural behaviour, recessing in the sand, seems to play a role in limiting fouling on the shell, and enhancing growth. Growout cages used in Bermuda are shown in Figure 5.2. These cages protect scallops from predators as there is mesh on top and sides, but allow scallops to recess on the sandy bottom. Descriptions of the cages experimented with in Bermuda are not given in detail, as this system is not cost efficient. It is extremely labour intensive due to the continuous monitoring of the cages by SCUBA. Survival rate is very dependent on density, and loading density per cage is low (10 scallops per m<sup>2</sup>). In order to make growout of zigzag scallops commercially feasible another growout method needs to be devised.



**Figure 5.2:** Schematic diagram of bottom cages made of rebar and plastic mesh used for protection of sand scallop (*Euovula ziczac*) juveniles and adults. Fouling on meshed lid shown close-up, necessitating retrieval of cages from field.

## 5.2 TECHNIQUES – TRANSFER OF SPAT FROM NURSERY TO FIELD

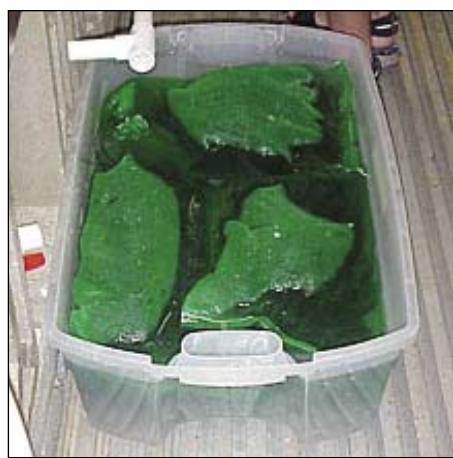
As two methods for set are used at the BBSR nursery (see Chapter 4), and resulting spat are transferred to the growout sites at different stages, procedures in transfer differ. Spat transferred, following the rapid approach set, are younger and smaller. They are more fragile than those transferred from the raceway system mainly due to their age. Although, success has been obtained in using the natural environment as growout site for young spat, survival rate has been enhanced when spat are reared to a larger size prior to transfer.

### 5.2.1 Transfer of 1.5 mm spat from 450 litres tank set

Spat set in 450 litres tanks on cultch are transferred to the field when a shell height of 1.5 mm is reached, or approximately 1 month after set (Figure 5.3). As these are very small and can be easily lost or damaged during handling, care is taken to always keep the spat submerged. Cultch is transferred to green collector bags and held in a container filled with ambient saltwater for transport to the growout sites (Figure 5.4).



**Figure 5.3:** Transfer of one month old calico scallop spat in green collector bags for transfer to growout trays.



**Figure 5.4:** Spat pouches held in seawater prior to transfer to the field.

As outlined in detail in Protocol-16, green collector bags are suspended on longlines using scallop trays. Bags are inserted, one per tray, and trays are stacked one into the other in groups of six (Figure 5.5). The number of trays used in one stack is dependent on water depth, floatation of line, and equipment used for retrieving trays. In Bermuda, all growout handling is done by hand with a small (9 m) outboard motor boat; it is found that as scallops grow and fouling accumulates on trays, groups of six are easiest to handle.

On-site, trays are secured using a 15 mm rope, wrapped around the entire set, in a similar manner to a gift-wrap package. A bridle coming from each corner of the stack

ties in the middle to a stainless steel clip. The clip attaches directly to the longline. Other methods can be used for securing a series of trays, dependent on preference of the grower. Transfer of green collector bags to trays, and securing the trays is done quickly on-site, to minimize the time period in which spat are exposed to the air. Trays are thereafter left for a period of 6 weeks in the field. For collecting of spat, trays are



**Figure 5.5:** Securing of pouches into trays for growout in the field on longlines.

dismantled in the field, collector bags with spat are transferred to containers filled with seawater and transported to the nursery. Upon arrival at the nursery, bags are placed in running seawater in exterior tanks or in 450 litres tanks. Spat are collected by removing mesh from cultch, and shaken into plastic trays filled with seawater. Spat at this time are passed onto a large mesh sieve (3 mm) and distributed by weight to pearl nets. For 3 mm pearl nets, a total of 150 spat (average of 7 mm shell height) are grown for a period of one month. This approximates 23 grams wet weight.

#### PROTOCOL-16

##### TRANSFER AND RETRIEVAL OF SPAT ON CULTCH TO FIELD

1. Prepare cooler or other container for transport on boat to growout site. Clean with fresh water and fill with ambient seawater.
2. Load boat with scallop trays, cut rope for securing, clips and tools. Prepare top trays with bridles and clips (see Figure 5.5).
3. Place green collector bag in 450 litres set tank and, with cultch remaining submerged in tank, transfer gently to collector bag. Fill collector bag to approximately 2/3. Close collector bag with drawstring.
4. Carefully transfer collector bag to transport container.
5. Repeat procedure until all cultch is removed from 450 litres tank.
6. Carry transport container to boat and fill to rim with ambient seawater once on board.
7. Immediately travel to growout site.
8. Upon arrival, prepare 7 trays; bottom tray should have a weight secured for stability (use twine or cable ties for securing 2.5 kg weight). Top tray has ready-made bridle. Begin transfer of green collector bag to tray: place one bag on tray, stack second tray on top, add one collector bag, and stack another tray, etc., until 6 trays have been filled with collector bags.
9. Add top tray with bridle.
10. Wrap rope around stack as for a gift-wrap.
11. Clip tray onto longline.
12. Leave for growth for a period of 6–8 weeks.
13. When ready for collecting and sorting, prepare outdoor tanks or 450 litres with clean seawater. Return to site with transport containers.
14. On-site, remove tray stack. Dismantle and transfer green collector bags to transport container filled with seawater.

15. Upon arrival to hatchery, transfer bags to prepared tanks.
16. For removal of spat, prepare trays with flowing seawater; place bags in tray and remove cultch, shaking off and picking spat.
17. All collected spat is left in trays.
18. Pour water and spat into 3 mm sieve to collect all spat.
19. Sample 150 spat on mesh, blot dry and weigh.
20. Transfer to pearl net.
21. Weigh out sub-samples of same weight for transfer to pearl nets.
22. Maintain pearl nets in flowing seawater (exterior tanks or 450 litres) until transfer to the field.
23. For transport of nets to growout sites, hold nets in containers filled with seawater. Pearl nets are attached in series of six, dependent on water depth, floatation and method of retrieval. Keep at least 1 m distance between last net and seabed.
24. Nets are hung on longline using clips or by a secure knot.

### 5.2.2 Transfer of 2–4 mm spat from raceway to longlines

Procedures for spat set in the raceway and transferred to the field at a size too small to be placed directly into 3 mm pearl nets (2–4 mm) is similar to that described in Section 5.2.1 above. Care is taken not to damage the spat and to maintain them continually submerged during transfer. As they are too small for 3mm pearl nets, insert pouches made of window fly screen material are used as inserts into scallop trays (Figure 5.6). This material is of larger mesh size than the collector bags, allowing for increased seawater flow for larger spat, and reduces the degree of clogging by fouling. Pouches are rectangular in shape with dimensions similar to that of the tray; pouches are sewn on three sides; the fourth side is closed using Velcro for ease of opening and closing. Spat are collected from the sieves as outlined previously. A sub-sample of 400 spat is weighed in a first instance; thereafter, aliquots of similar weight are taken and distributed in each pouch. Remainder of transfer is conducted as described from step 6 on in Protocol–16.

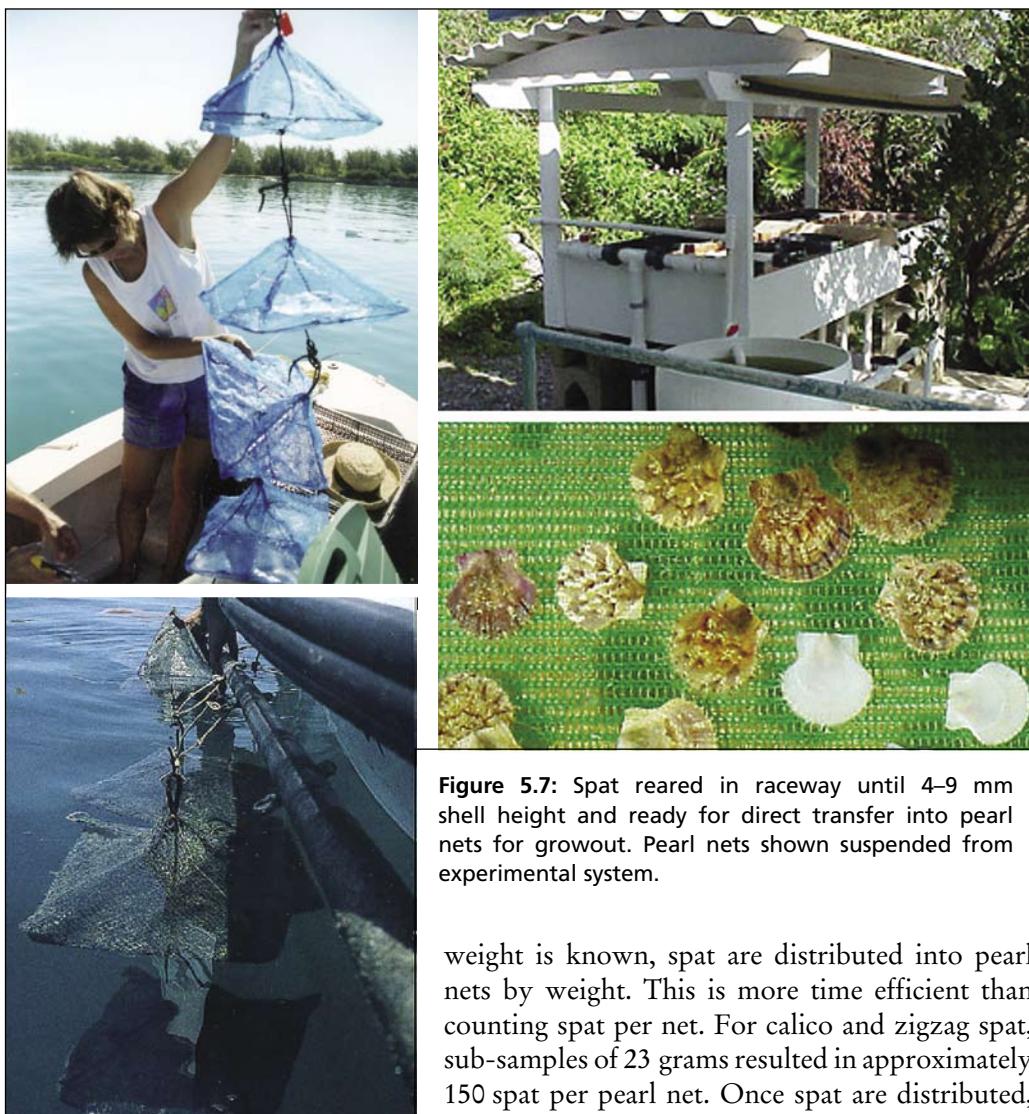


**Figure 5.6:** Transferring 2–4 mm spat into fly-screen pouches used as inserts for growout trays.

The older and larger the spat at transfer from the nursery to the field, the shorter the time period required for growth to 7 mm (and ready for growout in pearl nets). For raceway spat, transferred approximately two months after set, an intermediate growout period of one month is usually required in trays. Following this intermediate growout phase, spat are retrieved from the field and brought to the nursery for assessment of survival, and are re-distributed into 3 mm pearl nets.

### 5.2.3 Transfer of spat >4 mm

Spat reared in the raceway for a longer time period, whether it is in the indoor or outdoor system are ready to be transferred directly to 3 mm pearl nets. The larger the spat become, the easier they are to collect, and the more tolerant they become to handling. Nonetheless, care is still taken to minimize air exposure during handling. Spat from all sieves are pooled. An initial sub-sample of 150 spat is weighed; once the



**Figure 5.7:** Spat reared in raceway until 4–9 mm shell height and ready for direct transfer into pearl nets for growout. Pearl nets shown suspended from experimental system.

weight is known, spat are distributed into pearl nets by weight. This is more time efficient than counting spat per net. For calico and zigzag spat, sub-samples of 23 grams resulted in approximately 150 spat per pearl net. Once spat are distributed, pearl nets are closed and held in flowing seawater until transfer to the field. In Bermuda, pearl nets are usually prepared one day, prior to transfer to the field. On the day of transfer, nets are transported in containers filled with seawater. On site, nets are tied in series of six or seven and connected to the longline using a clip or simply tied. Spat are left in 3 mm pearl nets for a period of one month.

### 5.3 TECHNIQUES – GROWOUT OF JUVENILES

Calico and zigzag scallop juveniles grow quickly at a rate of approximately 5 mm per month. The first few months of growout are therefore labour intensive, in that biomass needs to be regularly controlled to maintain an optimal growth rate. A schedule is given below describing the time between transfers and pearl net size. Once scallops reach a size of approximately 40 mm, at a density of 30 scallops per net, maintenance becomes less labour intensive, involving monthly checks for rips and tears and control of fouling.

#### 5.3.1 Calico scallop growout

Transfer of juvenile scallops to larger size pearl net is conducted on the boat. Large containers of seawater are prepared on board (minimum of two); series of pearl nets are brought on board and scallops removed. Dead scallops are discarded and live animals are transferred to holding containers. Collected scallops are re-distributed into a pearl

net of larger mesh size (6 mm) at a density of 75 scallops per net. Series of nets are then re-suspended on longlines. Scallops remain in these nets for a period of one month. Thereafter, they are transferred to 9 mm nets at a density of 40 scallops per net. They can be maintained up to two months at this density and in this net size; after which they are transferred to 12 mm nets at densities of 30 scallops per net.

Calico scallops average 5 mm per month in shell growth between 3 to 9 months of age; shell growth decreases thereafter allowing for energy utilization during gametogenesis in 10 months old individuals; as spawning period occurs during the winter months (December to May in Bermuda), shell growth remains minimal at this time, but increases once again during the second summer period. Calico scallops have a life span of approximately 2.5 years in Bermuda and can attain a maximal shell height of 65 mm during their second year of growth. The expected growth curve for calico scallops reared on longlines in Bermuda waters is given in Figure 5.8.

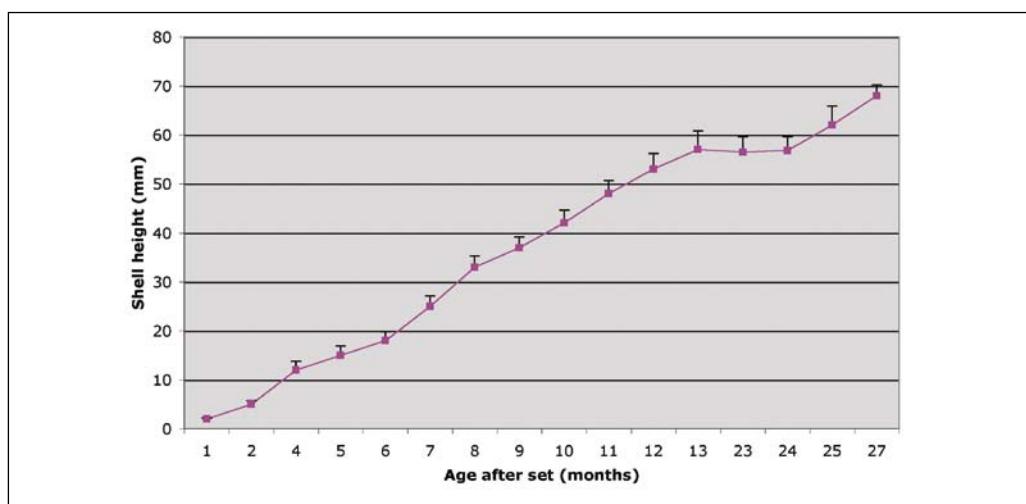


Figure 5.8: Shell growth of calico scallop, *A. gibbus*, juveniles reared on longlines in Bermuda.

For maintenance and control of fouling, monthly checks are made on the longlines. Wear and tear in the line is checked, along with rips in nets. Depending on season and water temperature, nets need to be cleaned monthly (during summer months), and every three months (during winter months). A saltwater power washer (electric motor driven on board) is used for cleaning of nets; it can be connected to the battery of an outboard engine, and nets can be cleaned with scallops remaining inside. This is a much quicker and efficient way of cleaning with no mortality ensued by scallops. Care must be taken to minimize the pressure in order to avoid damaging the scallops when using this method.

### 5.3.2 Zigzag scallop growout

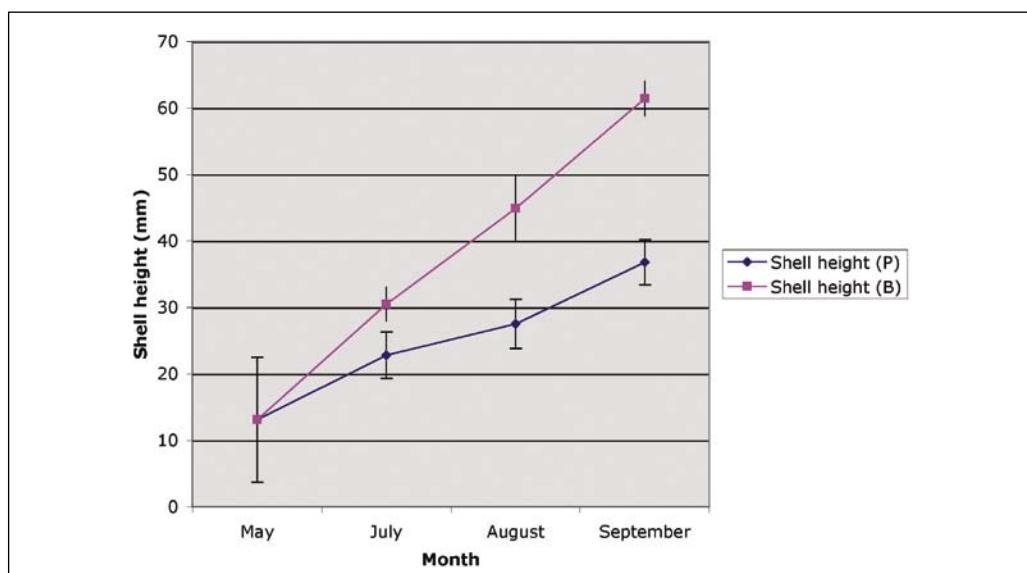
Zigzag spat are treated in a similar manner to calico scallops until the size of 25 mm. The recessive nature of zigzag scallops seen in Figure 5.9 favours bottom rather than suspended culture (Figure 5.10). These results are in accordance with others reported in the literature, notably the investigation on zigzag scallops by Lodeiros and Himmelman (1994). Thus at 25 mm and above, zigzag



Figure 5.9: Adult zigzag scallops, *E. ziczac*, showing natural recessive behaviour in the sand.

juveniles are reared directly on the sandy bottom, enclosed by a cage, protecting scallops from predation. This is a much more labour intensive method of rearing, as all labour needs to be conducted by SCUBA diving. Cages are made of 20 mm rebar, and meshed on top and sides with plastic 15 mm to 25 mm mesh depending on size of scallops. The bottom is left open, allowing scallops to recess directly into the sand. Cages are of 2.5x1.5 m, yielding a surface area of 3.75 m<sup>2</sup>.

The first transfer of 25 mm juveniles to bottom cages (15 mm mesh size) entails the collection of spat from pearl nets. Initial density is of 400 juveniles per cage. Juveniles only remain at such a high density for a period of one month. They then have to be collected by SCUBA, and thinned out to 200 juveniles per cage; again they remain as such for 1-month period, and are then transferred to a 25 mm cage at the final density of 100 per cage. This remains a high density, and periodically, scallops have to be collected and the cage moved. High density of scallops alters the composition of the substrate, leading to hypoxia or anoxia of the sediment, becoming an unfavourable environment for growth. Controlling fouling for cages has to be done monthly by SCUBA using a scrub brush for removal of epiphytes. Although this is intensive, it results in a noticeably faster growth rate than pearl net rearing. The difficulty in commercializing bottom culture lies in developing a time efficient and cost efficient system. To the author's knowledge, such a system for large scale rearing of the sand scallop has not been developed to date.



**Figure 5.10:** Comparative growth between zigzag scallops cultured directly on the sandy bottom (B) and suspended in pearl nets (P).

#### 5.4 TECHNIQUES – TRANSPORT OF JUVENILES

Scallops produced in hatcheries or collected from natural settlements normally require transportation to other sites for continued culture. Scallops are particularly difficult to transport as they are usually sub-littoral organisms, unlike oysters or mussels, and seldom encounter aerial exposure. In part, for this reason, stress associated with transport often causes mortalities. Several methods of transport have been attempted; two of the most common are the use of insulated boxes with material soaked in seawater, referred to as dry transport, (Ventilla, 1982; Maguire, Cashmore and Burnell, 1999; Maeda-Martinez, Siscard and Reynoso-Granados, 2000), and vivier lorry, where animals are transported in seawater with a continuous oxygen flow (Maguire, Cashmore and Burnell, 1999). Although some success is obtained, survival

reported in the literature does not exceed 61 percent immediately following 19 hours of simulated dry transport (Maeda-Martinez, Siscard and Reynoso-Granados, 1999), and increased mortality (up to 51 percent) is often seen in subsequent months during growout (Maguire, Cashmore and Burnell, 1999). As for transporting in seawater, high densities cause a rapid deterioration in quality of holding seawater by reducing dissolved oxygen and increasing ammonia and bacterial levels, leading to gill tissue damage (Maguire, Cashmore and Burnell, 1999).

In Bermuda, due to the small size of the island, transport to growout site can be easily conducted by holding scallops in seawater-filled containers on-board a boat. However, because of interest expressed by other Caribbean islands to rear tropical scallop species and consequent need for transport of spat or adults, procedures for optimal transport were investigated. These procedures were tested for both the sand scallop and the calico scallop, aiming for maximal survival following 24 hours transport time and following transfer to the field.

Details of the experiments conducted are reported in Sarkis, *et al.*, 2005. The procedures are based on methods used for fish transport in the aquarium trade. The methodology developed has been used in two real life situations for the sand scallop. In both cases, scallop spat were taken by air to another site; the total duration of the trip – from packing of scallops to unloading of scallops in the receiving nursery – was of 16 hours. Survival rate upon arrival was >90 percent. It was found that for successful transport of scallop spat, care must be taken to minimize stress during transport, and enhance tolerance of spat to stress by acclimation prior and post transport. Starvation and gradual acclimation to lower temperature, prior to transport, allow for a reduction in metabolic rate, reducing oxygen consumption, amount of excretia and accumulation of acidity, carbon dioxide and ammonia during transport. Low temperatures during transport maintain a low metabolic rate, minimizing oxygen consumption throughout the duration. Following transport, gradual acclimation to ambient temperature minimizes recovery stress, possibly enhancing long term survival. Shell growth of scallops transferred to the natural environment was not affected by simulated transport. The protocol developed is given in detail below (Protocol-17). This procedure should become easily adaptable to various scallops species, with additional insight into some of the species-specific physiological requirements.

#### PROTOCOL-17

##### **PROCEDURE FOR LONG TRANSPORT PERIODS OF JUVENILE SCALLOPS**

1. Do not feed scallops to be transported for 24 hours prior.
2. Acclimatize juvenile scallops to cold water (14 °C) for a minimum period of 6 hours (without feeding).
3. Make a false bottom of polystyrene to fit the cooler, in order to avoid direct contact of scallops with gel packs. Drill several holes in false bottom to allow cooling of entire container.
4. Weigh 160 grams sub-samples of spat (in tarred mesh, blotted dry as in Protocol-14).
5. Fill 1 litre zip-lock bags with 600 ml of 1 µm filtered cold seawater (14 °C).
6. Add scallops to zip-lock bag. Partially close bag.
7. Fill zip-lock bag with oxygen using Pasteur pipette fitted to oxygen regulator and bottle. Close zip-lock bag.

8. Place zip-lock bag into second bag for additional security. Use duct tape to secure closure and avoid any potential tearing or opening.
9. Cool a polystyrene box (60x30x40 cm) to 14 °C using four gel packs. Install false bottom on top of the gel packs and place scallop bags on it.
10. Secure lid of polystyrene box with tape.
11. Following transport do a visual assessment of scallop behaviour and mortality. Record temperature in bag.
12. Transfer scallops into a cold water bath (14 °C) and gradually increase seawater temperature ambient over a 12-hour period.
13. Maintain spat in running seawater once ambient temperature is reached.
14. Following a minimum 3-day acclimation to ambient temperature spat can be transferred to the field.
15. If spat is kept for longer than 3 days, complimentary food supply should be added as described in Appendix 21.

## Chapter 6

# Economic considerations: costs of set-up and labour requirements

<b>6.1 SET-UP COSTS OF A MODULAR HATCHERY .....</b>	<b>129</b>
<b>6.2 OPERATIONAL LABOUR REQUIREMENT .....</b>	<b>130</b>
<b>6.3 FINAL PRODUCT .....</b>	<b>131</b>

### **6.1 SET-UP COSTS OF A MODULAR HATCHERY**

The equipment and materials required for the set-up of the hatchery/nursery complex and for the carrying out of the rearing protocols are given in detail in Appendix 22. This list is given as an aid to assessing the needs for setting-up and operating a modular hatchery and assist in costing out such a hatchery. In-depth economic studies have been conducted on other scallop species (Paquette and Fleury, 1994; Dredge *et al.* 2002), which provide the capacity for modelling. The intent here is to provide baseline information which can thereafter be used in modelling, if required.

For ease of understanding, the list provided has been divided into several sections. Firstly, equipment and materials required for each section of the complex is given with price at the time of publication of this document. It must be noted that all prices are given in US Dollars (USD). Although prices will vary among sites, this can provide a general estimate of the funds required. Additionally, a list of suppliers used for the construction of the Bermuda modular hatchery is given, and may be useful for construction of hatcheries in North and South American regions. Secondly, the list provides small equipment and materials required for conducting the protocols described in this manual. Once again, associated costs (at the time of publication) and suppliers are provided.

Set-up costs and rearing costs involved for this “portable” model hatchery are summarized in Table 6.1 below. Rearing costs refer to requirements for farming scallops as outlined in the protocols of this manual. The price of PVC pipe and parts required for the whole complex is estimated at the price purchased in Bermuda. It has to be kept in mind that Bermuda has a high cost of living and prices given here need to be adjusted to a given region. The amount of PVC required can be estimated from the technical drawings. In total, in Bermuda, it is estimated that USD 16 000 is required for PVC parts. Set-up costs amount to USD 47 500 in Bermuda, and include all equipment outlined in Appendix 22 for set-up. Initial rearing costs amount to USD 25 100 and include all equipment outlined in Appendix 22 for rearing of scallops. The total cost for a turn-key modular hatchery, excluding labour costs, amounted to USD 88 600 in Bermuda. Yearly operating costs are not included here.

**Table 6.1:** Summary of the set-up costs for the hatchery/nursery complex. Set-up costs do not include shipping, PVC pipe/connections, construction materials (cement, concrete blocks, etc.) and electrical components. An estimated figure is given separately for PVC parts based on expenses made in Bermuda (*Note: Table figures taken from Appendix 22 have been rounded off*).

Facility section	Set-up costs (USD)	Rearing costs (USD)	Estimated PVC costs (USD)
Seawater system	3 000		5 000
Heating seawater system	4 000		500
Housing containers (hatchery, nursery, algae)	12 000		
Broodstock	4 000	1 500	500
Algae	10 000	13 000	500
Hatchery	3 500	1 500	5 000
Nursery (indoor & outdoor)	6 000	600	4 000
Transfer	1 000	1 500	500
Growout (for 25 000 adult scallops – 90 m longline)	4 000	7 000	
Grand Total	47 500	25 100	16 000

**Note:**

- Rearing costs for broodstock include materials for spawning procedure (Appendix 22).
- Rearing costs for algae include materials for master and sub-culture, live & dry algae (Appendix 22).
- Rearing costs for hatchery include materials for construction of sieves and miscellaneous equipment (Appendix 22).
- Rearing costs for nursery include materials for construction of sieves for indoor and outdoor raceways (Appendix 22).
- Rearing costs for transfer include transfer materials (Appendix 22).
- Rearing costs for growout include growout materials (Appendix 22).

PVC costs for seawater include main seawater intake line, pump house, main supply to heating unit, algal unit and hatchery complex; as well as main drain pipe system. PVC parts for hatchery include main ambient and heated seawater line to all tanks and air line.

## 6.2 OPERATIONAL LABOUR REQUIREMENTS

Running costs for the entire operation are given in Table 6.2 below. These costs are shown as percentages, as prices and salaries vary widely among countries, and should provide a basis for assessing operational costs for a region. Running costs include electricity, replacement of equipment and consumables. The percent labour column results from an evaluation of the time involved in carrying out the tasks necessary for rearing of scallops at each phase of its life cycle. Both running and labour percentages are given for a 12-month period and are based on the hatchery operating at full capacity, yielding a production of 600 000 to 800 000 spat per year, of 2–9 mm shell height and ready for transfer to the field. It can be seen from Table 6.2 that highest running costs are associated with the larval rearing (hatchery) phase and the growout phase (juveniles to adults). The algal culture phase is least expensive in running costs due in part to the reliance on dry algae for the nursery phase (see Chapter 4). Running costs for the nursery phase are dependent on duration of spat rearing and the strategy followed for transfer to the field. Labour requirements follow a similar trend with the highest requirements during the larval rearing (hatchery) and growout. These requirements are based on rearing larvae in a conventional static system where water is changed every other day; it is anticipated that changing to a flow-through system would reduce the labour requirements for this phase (see Chapter 3). Growout is the most labour intensive part of the operation in Bermuda although needs are not distributed evenly throughout the twelve months and occur in bursts. For example, according to the cycle described in Bermuda, where larvae are reared in the winter months and spat are

transferred to the field in early summer, growout is most intensive during the summer months at the beginning of this phase. By late fall (October/November) growout requirements are much reduced.

**Table 6.2:** Operational costs as a percentage of time for full time aquaculture activities from spawning to growout.

Section	Running costs (in %)	Labour (in %)
Algae	13.7	14
Hatchery	28.3	33
Nursery	18.7	23
Growout	39.3	30

### 6.3 FINAL PRODUCT

This manual has strived to provide step-by-step guidelines for the production of scallop. Scallops are considered a seafood delicacy and the fresh product is, in general, very much appreciated by chefs. Several marketing studies have been conducted in Bermuda demonstrating the high demand for such fresh seafood on this island. Due to the difficulty in developing a cost-efficient growout methodology for sand scallops, large scale rearing is not commercially feasible for this species at this time. However, methods described for the calico scallop have proved successful on a commercial scale. Calico scallops are generally sold at 1.5–2 years old; the whole animal is used and often served in its shell. Scallops, with ripe gonads, are usually preferred by the restaurant trade. A second marketing scheme, selling 30 mm scallops to Italian restaurants, also became successful as young scallops are cooked whole in the shell and mixed in with pasta dishes. The colourful shell of calico scallops and the low price of younger individuals are found to attract this market (Figure 6.1).



**Figure 6.1:** Zigzag and calico scallops (adults and juveniles) ready for market and sold fresh to restaurants in Bermuda.

The rapid growth rate of these tropical species makes them a good candidate for culture, minimizing risks and costs associated with a long growout period.

This work provides an additional technology for the culture of tropical and sub-tropical scallop species. The techniques have been well tested over the past four years and scaling-up production for commercial purposes can be easily done following the Bermuda protocol by simply increasing the tank capacity (the number of tanks or tank volume) for larval and post-larval rearing. This technology can be adapted to other tropical and sub-tropical regions and to other species. Furthermore, the concept of the module hatchery housed in mobile containers provides a relatively inexpensive facility for the rearing of bivalves, minimizing initial capital costs for the set-up of a commercial operation. Due to the flexible nature of the facility, species-specific requirements can easily be accommodated with the current design. Finally, this type of modular or “portable” hatchery can be advantageous for those areas limited in space (like Bermuda), and/or in areas which are potentially prone to pollution issues. The only requirement for such a hatchery is access to clean seawater.

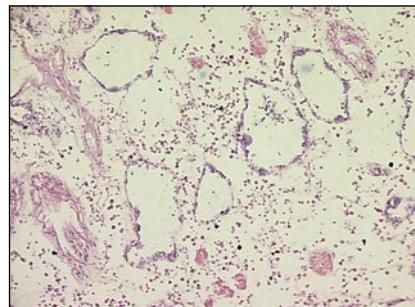
# Appendices

<b>Appendix 1:</b> Description of oocyte developmental stages .....	134
<b>Appendix 2:</b> Sample data sheet for gonadal and muscle indices .....	135
<b>Appendix 3:</b> Broodstock check sheet .....	136
<b>Appendix 4:</b> Heating unit set-up and take-down.....	137
<b>Appendix 5:</b> Maintenance and cleaning of seawater system .....	139
<b>Appendix 6:</b> Pump room log .....	140
<b>Appendix 7:</b> Cleaning hatchery after spawning .....	141
<b>Appendix 8:</b> Details of materials – Plunger and drain .....	142
<b>Appendix 9:</b> Preparation of culture media .....	143
<b>Appendix 10:</b> Chemical sterilization procedure .....	146
<b>Appendix 11:</b> Set-up and take-down of seawater supply in algae container .....	147
<b>Appendix 12:</b> Bactopeptone test .....	149
<b>Appendix 13:</b> Algal culture check list .....	150
<b>Appendix 14:</b> Haemocytometer cell diagram .....	151
<b>Appendix 15:</b> Larval check sheet .....	152
<b>Appendix 16:</b> Determination of dry weight and ash-free dry weight .....	153
<b>Appendix 17:</b> Sieve construction for larval and post-larval collection .....	154
<b>Appendix 18:</b> Raceway check list .....	155
<b>Appendix 19:</b> Cleaning of raceway .....	156
<b>Appendix 20:</b> Counting grid for spat .....	157
<b>Appendix 21:</b> Preparation and ration for dry algae .....	158
<b>Appendix 22:</b> List of equipment: template for costing out set-up of modular hatchery .....	159
<b>Appendix 23:</b> List of selected suppliers .....	166

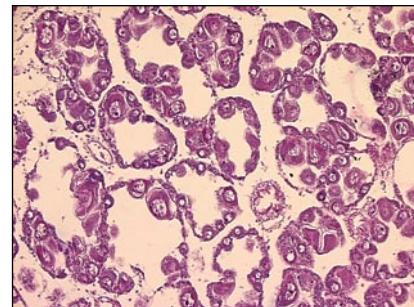
## Appendix 1

### Description of oocyte developmental stages

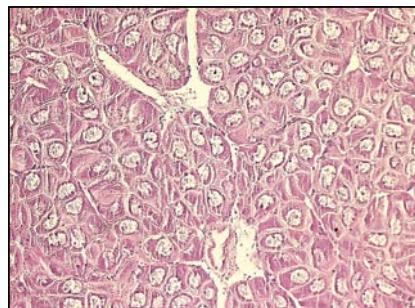
- Stage 1:** Undifferentiated – where follicular structure is indistinct and there is no sex cell primordial.
- Stage 2:** Differentiated – where oocyte development commences, primary oogonia are visible, sex cell range from 15–25 µm in diameter.
- Stage 3:** Developing – where there are mostly pre-vitellogenic and vitellogenic oocytes, follicle lumen is open or only partially occluded and oocyte diameter ranges from 30–70 µm.
- Stage 4:** Ripe – where gonads show packed follicles, the follicular lumen is occluded, there is no inter-follicular space visible, and oocytes range 60–70 µm in diameter.
- Stage 5:** Spawning – where the follicle lumen is opening, follicle size diminishing and ripe oocytes range 60–70 µm.
- Stage 6:** Spent – where follicles are empty and frequently filled with haemocyte aggregations on slide preparations and an occasionally atretic oocyte is present.



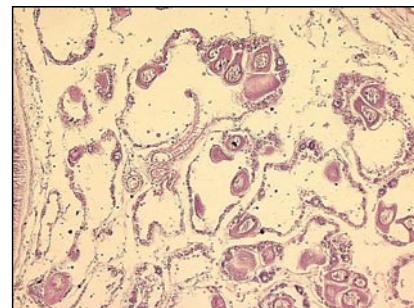
Stage 2: Differentiated



Stage 3: Developing



Stage 4: Ripe



Stage 6: Spent

## Appendix 2

## **Sample data sheet for gonadal and muscle indices**

## Appendix 3

## **Broodstock check sheet**

## Appendix 4

### **Heating unit set-up and take-down**

**Note:** Refer to Technical Drawing – Pg 5A.

#### **Set-up**

1. Backwash entire seawater system first.
2. Rinse coarse filter, 10 µm and 1 µm filters with fresh water.
3. Turn off drain valve for coarse filter; open air vent slightly.
4. Install filters in cartridges.
5. Divert flow of water after 1 µm filter to drain for 15 minutes, clearing any chlorine residue in filters.
6. Open incoming valve to coarse filter halfway and valve to 10 µm filter halfway.
7. Rinse heating tank with fresh water.
8. After 15 minutes, divert 1 µm filtered seawater to fill tank.
9. Open Valves X and E all the way.
10. Make sure valves D, F and W are closed.
11. Once level of water in tank has reached the alarm switch and pump is turned on recycling the water into the tank, make sure plates of titanium heaters are covered with water.
12. Turn heaters and controllers on. Set temperature as desired.
13. A small red dot on some temperature controllers indicates that heaters are heating. Once set temperature is reached, red dot disappears.
14. Close incoming water valve from 1 µm filter to accelerate heating in the tank. So that water is only being heated and recycled.
15. Once desired temperature is reached, open incoming seawater valve from 1 µm filter halfway. You now have inflow of 1 µm filtered seawater and recycled water going into tank.
16. Open valve D halfway to supply hatchery with heated seawater and close valve E to halfway mark.
17. Depending on the supply and degree of heating required, balance may have to be altered by degree of valve closure.

#### **Take-down**

1. Turn off heaters and temperature controllers.
2. In hatchery unit: Open last red valve of heating line; Make sure all other red valves are closed.
3. In heating unit: Close incoming water from filters and Valve D so that water is only recycled within the tank.
4. Pour 100 ml of chlorine into tank and mix by letting water recycle for at least 15 minutes.
5. When hatchery has been cleaned, close valve E and open valve D completely. Chlorinated water will pass through the lines to hatchery.

6. Once water level drops and pump stops, close valve D and open drain valves F and W. Let tank empty completely.
7. Rinse empty tank and heaters with fresh water hose.
8. Remove filters from cartridges and rinse cartridges with fresh water.
9. Place filters in a bucket of fresh water with a capful of chlorine. Let sit for 24 hours.
10. The next day rinse filters with fresh water and let dry.

## Appendix 5

### **Maintenance and cleaning of seawater system**

**Note:** Refer to Technical Drawing – Pg 2.

1. Backwash first thing in the morning every day during months of hatchery operation. If needed (if water has high particulate matter), backwash several times a day.
2. For a continuous pumping system, cleaning of system should be done at the beginning of the season and at the end of the season.

#### **End of season**

1. Pump is turned off. Supply line is drained using drain valves located at lowest point.
2. Empty sand filter by opening drain valve. Remove sand and wash with fresh water. Let dry and store.
3. Clean sand filter and all parts with diluted muriatic acid and fresh water. Dry and store.
4. By SCUBA, remove mesh and drain of anchor. Block inlet with secured reinforced plastic bag. If possible, soak mesh and drain in a bucket of fresh water with a capful of muriatic acid to clean. Store until next season. If too fouled, replace with new mesh and a new drain.
5. Remove check valve, clean and place back in line.
6. Close all drain valves and pour gallons of chlorine into cleaner Y-junction after sand filter to clean supply line to hatchery. Let sit for a few days. Then drain line by opening all drain valves.
7. Once drained, close valves until next season.

#### **Beginning of season**

1. By SCUBA, remove mesh and drain of anchor. Block inlet with secured reinforced plastic bag. If possible, soak mesh and drain in a bucket of fresh water with a capful of muriatic acid to clean. If too fouled, replace with new mesh and a new drain.
2. Remove check valve, clean and place back in line.
3. Pour gallons of chlorine into cleaner T-junction in pump room to allow seepage into the inflow line. Let sit for a few days.
4. Set up sand filter with sand following dealer's instructions.
5. Chlorinate supply lines to hatchery as at the end of the season (see Point 6 above).
6. Remove bag from inlet hole, install cleaned or new drain and mesh.
7. Turn pump on so as to pump water through and send to last drain valve on supply line for 24 hours at least to remove all chlorine residue.

## Appendix 6

### Pump room log

YEAR:			
MONTH:			
Day	Time	PSI pressure	Comments
1			Check: Oil pump
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			Check: Oil pump
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			
29			
30			
31			

## Appendix 7

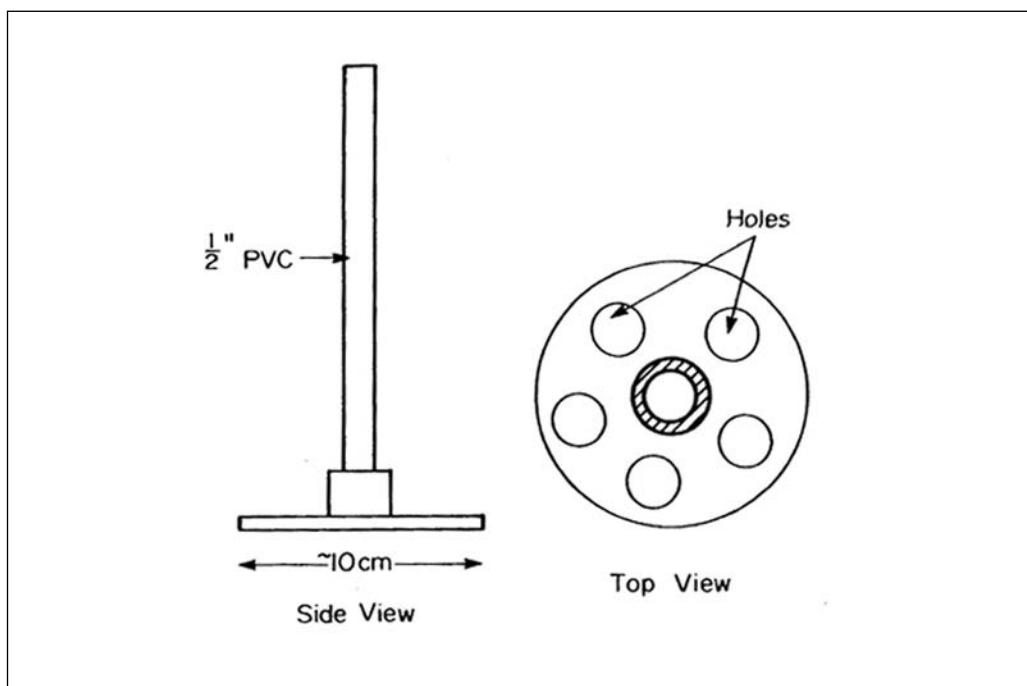
### Cleaning hatchery after spawning

**Note:** Refer to Technical Drawings – 3/Pg 5A and Pg 7

1. Remove all scallops to outdoor tank with high flow of water; allow to spawn completely.
2. Close inflow of water from heating unit.
3. Disconnect cartridge for double 1 µm filter.
4. Open last red valve (heating line) in hatchery; close other valves in heating line.
5. Once chlorinated water has passed, rinse heating line with fresh water hose.
6. Open all red valves for complete drainage of pipes. Once drained close valves.
7. Open valve W at exterior of hatchery to allow for complete draining of heating line.
8. Discard all extra spawn. If species is exotic, discard in a chlorinated tub first.
9. Wash all trays and beakers with chlorine and fresh water.
10. Pass a small piece of chlorinated sponge through flexible hoses with fresh water hose.
11. Dip flexible hose into tub filled with chlorinated fresh water and leave until next water change.
12. All filters are rinsed and placed into tub filled with chlorinated fresh water.
13. Filter housings and immersion heaters are washed with chlorine and fresh water.
13. Store trays, beakers, filter housings and all other materials when dry.

## Appendix 8

## **Details of materials – Plunger and drain**



**PLUNGER** – made of Plexiglas base and a plastic or PVC rod. Used for mixing of egg and larval solutions (taken from Bourne, Hodgson and Whyte, 1989).

**canplas** **Roof Drains**  
2" through 8"

NOVEMBER 1997

MATERIAL: PVC

DATA SHEET RD-37-11-97

#### ADVANTAGES

- Corrosion and weather resistant offering years of worry-free service.
- Rugged design resists abuse during construction and in service.
- Lightweight facilitates handling, transportation and installation.
- Easily adapted to all DWV systems from 2" to 8" nominal pipe sizes using approved adapters.
- Cost savings over conventional metal roof drains.

#### INSTALLATION DETAILS

Lightweight Canplas roof drains can be easily adapted to various types of roofs and finishes. With approved connections, Canplas PVC roof drains can also be used with any other piping system - ABS, cast iron, ductile iron, galvanized or copper. The recommended deck opening for the 2", 3" and 4" sizes is 9 1/4" to 10" and for the 6" and 8" sizes is 14 1/4" to 15". The diagrams below illustrate possible installations with various roofing systems.

Part # .373552-4

**DRAIN** – plastic roof drain used on inlet of seawater pipe as a filter for larger marine organisms.

## Appendix 9

### Preparation of culture media

#### F/2 Nutrient solution

---

This solution was purchased from Aquatic Eco-systems. It is sold in two parts: Part A and Part B.

##### For 125 ml, 500 ml and 4 l cultures:

1. Mix equal volumes of each part into a 500 ml dark glass bottle (some of the chemicals are sensitive to light).
2. Cap the bottle with a cotton plug and autoclave according to manufacturer's instructions.
3. Wrap bottle top with foil and autoclave.
4. Once autoclaved let solution cool.
5. Add vitamin stock solution using microbiological sterile techniques.
6. Close bottle with autoclaved top and label with date.
7. Store at -4 °C.

##### For 100 l cultures:

1. Use solutions directly from purchased containers.
2. Add equal parts of solutions directly to cultures using 25 ml graduated cylinder or 10 ml graduated pipettes.
3. Add algae in accordance with volume of culture. Instructions on volume of F/2 required per volume of culture are given on the purchased bottles.

#### Vitamin stock solution

---

##### Materials:

- 50 ml volumetric flask and top.
- Glass funnel.
- Q-water bottle.
- Vitamin B12.
- Vitamin B1.
- Biotin.

##### Method:

1. Autoclave the volumetric flask and the glass funnel.
2. Weigh out vitamins:  
B12 = 0.005 grams  
B1 = 5 grams  
Biotin = 0.02 grams

3. Transfer vitamins into the flask.
4. Fill up the flask to total volume of 50 ml with Q-water.
5. Insert a magnetic stirrer and stir.
6. Keep the stock solution in the fridge in the algae container.

Vitamin solution is added to the autoclaved F/2 solution as follows:

1 ml vitamin solution per 1 liter F/2 solution

### **Sodium metasilicate solution (3 % w/v)**

---

#### Materials:

- 500 ml dark glass bottle and top.
  - Glass funnel.
  - Q-water.
  - Sodium metasilicate ( $\text{Na}_2\text{SiO}_3$ ).
1. For a 300 ml volume, weigh 9 grams of sodium metasilicate.
  2. Pour 300 ml of Q-water into a 500 ml graduated cylinder.
  3. Fill a squeeze bottle with this water. In this way you'll keep track of the volume of water added for dissolution.
  4. Gradually transfer weighed sodium metasilicate powder into bottle, diluting solution with Q-water from your squeeze bottle progressively. Make sure to add a small amount of powder at a time otherwise it is difficult to dissolve.
  5. Once diluted, cap bottle with cotton plug and autoclave.
  6. Wrap glass top in foil and autoclave.
  7. Once cool, store solution at -4 °C.

**Note:** If there is no dark glass bottle, use clear glass bottle wrapped in foil. Sodium metasilicate is sensitive to light.

Sodium metasilicate is added as a supplement to F/2 and vitamin solution for diatom species. Volume added is 2 ml of sodium metasilicate for 1 l of culture.

### **Convay medium**

---

#### Preparation of Solution C for a 25 l volume:

$\text{KNO}_3$	2 000 grams
$\text{K}_2\text{HPO}_4$	400 grams
EDTA	750 grams
$\text{H}_3\text{BO}_3$	50 grams
TMII	2 000 ml
SWII	125 ml
Vitamin Solution	25 ml

#### TMII Solution (25 l volume):

$\text{Fe}_2(\text{SO}_4)_3$	395 grams
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	37.5 grams

ZnSO <sub>4</sub> .H <sub>2</sub> O	6.25 grams
CuSO <sub>4</sub> .5H <sub>2</sub> O	5.00 grams
CoSO <sub>4</sub> .7H <sub>2</sub> O	0.65 grams
NaMoO <sub>4</sub> .2H <sub>2</sub> O	0.35 grams

SWII Solution (10 l volume):

SrCl <sub>2</sub> .6H <sub>2</sub> O	13.00 grams
AlCl <sub>3</sub> .6H <sub>2</sub> O	0.50 grams
RbCl.6H <sub>2</sub> O	0.20 grams
LiCl.H <sub>2</sub> O	0.10 grams
KI	0.05 grams
KBr	0.65 grams

Vitamin Stock Solution (1 l volume):

B <sub>12</sub>	0.10 grams
B <sub>1</sub>	100.00 grams
Biotin	0.40 grams

**F/2 media**

1. Nitrate	NaNO <sub>3</sub>	75 g/l
2. Phosphate	NaH <sub>2</sub> PO <sub>4</sub> .H <sub>2</sub> O	5 g/l
3. Silicate	Na <sub>2</sub> SiO <sub>3</sub> .9H <sub>2</sub> O	30 g/l
4. Trace Metals	FeCl <sub>3</sub> .6H <sub>2</sub> O	3.5 g
	Na <sub>2</sub> EDTA	4.36 g

Dissolve in 900 ml distilled H<sub>2</sub>O.

Add 1 ml of each of the following trace metal solutions.

- CuSO <sub>4</sub> .5H <sub>2</sub> O	0.98 g/100 ml
- ZnSO <sub>4</sub> .7H <sub>2</sub> O	2.2 g/100 ml
- CoCl <sub>2</sub> .6H <sub>2</sub> O	1.0 g/100 ml
- MnCl <sub>2</sub> .4H <sub>2</sub> O	18.0 g/100 ml
- Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	0.63 g/100 ml

Bring solution to 1 l with distilled H<sub>2</sub>O (pH about 2.0).

Add 1 ml/l of filtered seawater of the above solution (#1-4).

5. Vitamins:	Biotin	1.0 mg
	B <sub>12</sub>	1.0 mg
	Thiamine HCl	20.0 mg

Dissolve in 1 l distilled H<sub>2</sub>O. Store in refrigerator.

Add 0.5 ml of vitamin solution for every 1 l filtered seawater.

## Appendix 10

### **Chemical sterilization procedure**

#### Materials:

- Sodium Hypochlorite (12 %) solution or commercial grade chlorox.
- Sodium Thiosulfate (1 N) solution.
- Glass bottle with screw cap.

#### Procedure:

Prepare 1 N sodium thiosulfate by dissolving 158.1 grams sodium thiosulfate with de-ionised or distilled water. Store in glass bottle.

For sterilizing container with seawater:

1. Fill container or flask with filtered seawater.
2. Add 1 ml of chlorox (or sodium hypochlorite) per liter of seawater.
3. Stir or bubble and allow to sit for a few hours.
4. Close flask with lid or rubber stopper.
5. Neutralize chlorox with 1 ml of sodium thiosulfate per litre of seawater.
6. Stir or bubble.
7. Seawater and container are ready to be used for algal inoculum or other purpose.
8. You will probably need to pour out some of seawater for algal inoculum. But filling the entire container initially allows for complete sterilization.

## Appendix 11

### **Set-up and take-down of seawater supply in algae container**

#### Set-up of seawater supply in algae container:

**Note:** Refer to Technical Drawings – Pg 1 and Pg 9.

1. Remove filters and hose from chlorinated bin.
2. Rinse abundantly with fresh water all filters (coarse, 10 µm and 1 µm) and flexible water hose.
3. Set-up filters in respective cartridges fixed to outside of container, and connect flexible hose to UV sterilizer placing end in sink of algae container.
4. Open drain valve Z by Y-junction and allow chlorinated water to drain. Open union by inflow valve Q to coarse filter and rinse abundantly with fresh water.
5. Close drain valve Z and union.
6. Open inflowing valve Q to coarse filter all the way and close outflowing valve O of coarse filter.
7. Slightly open air vent of coarse filter.
8. Open main valve Y to algal container (from Y-junction) three quarters of the way.
9. Allow coarse filter to fill with water.
10. When water starts to come out of air vent (it takes a few minutes only), open outflow valve O halfway to allow water to pass through 10 and 1 µm filters.
11. Check that water is passing through UV sterilizer and flowing out of flexible hose into sink. Let run for 15 minutes to eliminate all residue of chlorine.
12. Plug UV sterilizer in when ready to use. Control seawater flow with main valve Y and outflow valve O.

#### Take down of seawater supply to algae container:

**Note:** Refer to Technical Drawings – pages 1 and 9.

1. Unplug UV sterilizer.
2. Close main valve K at Y-junction.
3. Close inflow valve I to coarse filter and open drain plug of coarse filter. Allow coarse filter to empty.
4. Remove filters from coarse 10 µm and 1 µm cartridges. Rinse salt off with fresh water and place in chlorinated bin until next use. Do the same with flexible hose.
5. Rinse coarse filter with fresh water allowing to empty via drain plug. Rinse lid as well and place back on coarse filter.
6. Undo union prior to inflow valve I and open drain valve H of pipe adjacent to Y-junction.
7. Blast fresh water through pipe for a few minutes. Close drain valve H. Fill pipe with fresh water and a capful of chlorox. Close union and let sit until next use.

8. Undo union after 10 and 1 µm cartridges and rinse with fresh water hose for a few minutes to rinse line through UV sterilizer with fresh water. Make sure to place a tray under UV sterilizer to collect fresh water and prevent spilling on algal container floor.
9. Allow pipe to drain. Rinse 10 and 1 µm cartridges with fresh water and replace on-line.

## Appendix 12

### **Bactopeptone test**

(from Bourne, Hodgson and Whyte, 1989)

#### Materials:

- Bactopeptone.
- Filtered seawater.
- 10 ml tubes with cap.

#### Procedure:

1. Dissolve 10 g bactopeptone in 1 l filtered seawater.
2. Fill test tubes with 10 ml of media and cap.
3. Autoclave tubes.
4. Inoculate tube containing bactopeptone media with 3–4 drops of algal culture.
5. Store in the dark for up to two weeks..

#### Assessemement:

1. Some faster growing bacteria will become evident within 2–4 days.
2. If bacteria are present, contents of the tube will appear cloudy.
3. Degree of cloudiness reflects degree of bacterial contamination. The cloudier a tube the more contaminated.
4. Discard cultures with heavy bacterial contamination.

## Appendix 13

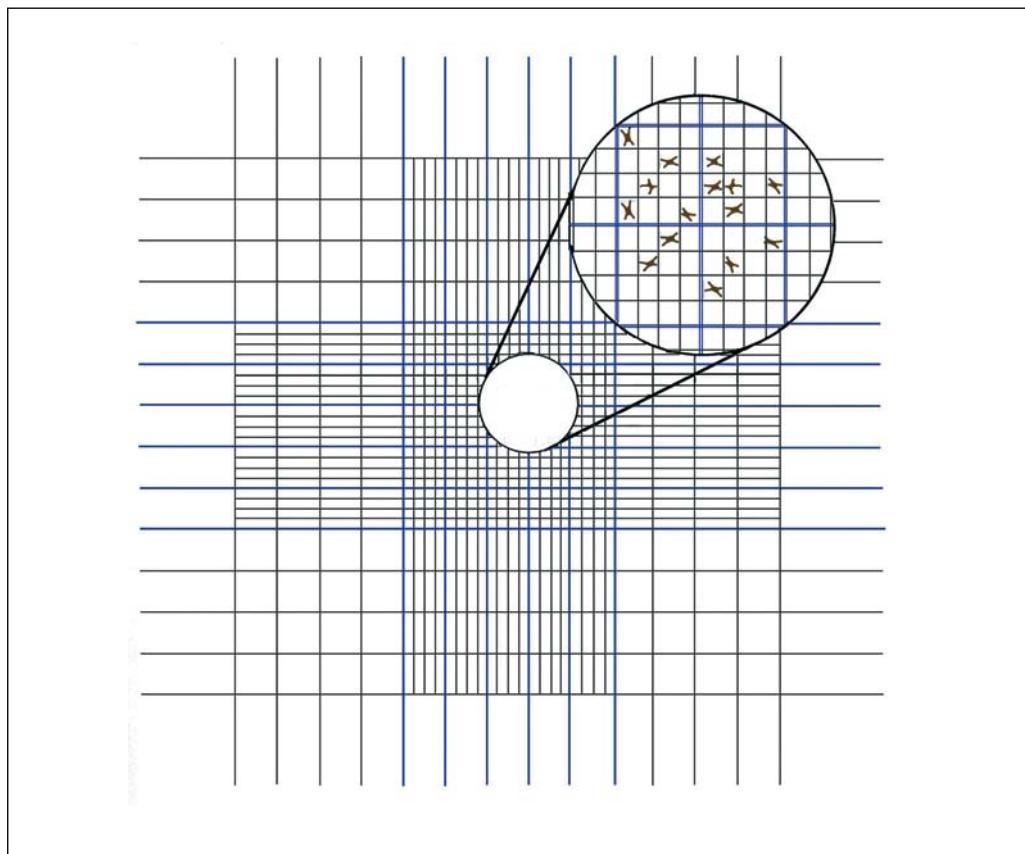
## **Algal culture check list**

YEAR:						
MONTH:						
Day	Time	Temperature	Lights/AC	Cultures bubbling	CO <sub>2</sub> level	Comments
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						
22						
23						
24						
25						
26						
27						
28						
29						
30						
31						

## Appendix 14

### **Haemocytometer cell diagram**

(from Bourne, Hodgson and Whyte, 1989)



## Appendix 15

## Larval check sheet

## Appendix 16

### Determination of dry weight and ash-free dry weight

1. Number (with a pencil) and ash 4.7 cm GF/C filter in a muffle furnace at 450 °C until constant weight.
2. Weigh filters on an electronic microbalance ( $\pm 1 \mu\text{g}$ ). Record weights for each numbered filter.
3. Set-up a Millipore filtering apparatus and connect to a vacuum pump.
4. Place one filter on filtering apparatus.
5. Count larvae using a Sedgewick-Rafter cell.
6. Calculate number of larvae per ml.
7. Using a 7 mm Tygon tube, siphon required number of ml into a graduated cylinder to obtain total number of larvae. For Day 2 larvae collect minimum of 150 000 larvae. For pediveligers collect a minimum of 5 000 larvae.
8. Pour collected larvae through a 20  $\mu\text{m}$  sieve.
9. Wash larvae with isotonic ammonium formate (3 percent) to remove mineral salts.
10. Wash down larvae with ammonium formate onto filter fitted to filtering apparatus.
11. Ensure that all larvae from sieve and sides of filtering apparatus are collected by thoroughly washing with isoosmotic ammonium formate.
12. Remove filter from filtering apparatus and place in a foil disc.
13. Record number of larvae collected and number of filter.
14. Dry filter in an oven at 60 °C until constant weight (approximately 48 hours).
15. Transfer filters to a dessicator to avoid moisture affecting weight.
16. Record weight after drying using same electronic balance.
17. Place filters in a muffle furnace at 450 °C until constant weight.
18. Remove from furnace, transfer into a dessicator, and weigh with electronic balance.
19. Record weight and filter.
20. To calculate larval dry weight (DW):
  - a) Dry weight ( $\mu\text{g}$ ) of filter + Larvae - Initial filter weight
21. To calculate Ash-free dry weight (AFDW):
  - a) Larval ashed weight (inorganic matter)= Ash weight of filter + Larvae - Initial filter weight
  - b) Larval ash-free dry weight (organic matter)= Larval ash weight - Larval dry weight
22. To calculate Condition Index:
  - a) Condition Index= Organic matter/Larval dry weight

## Appendix 17

### **Sieve construction for larval and post-larval collection**

**Note:** Refer to Technical Drawings – Pg 14 and Pg 17.

1. Cut with a circular saw a PVC ring to appropriate height (20 cm high for 20 cm and 25 cm larval sieves, 18 cm high for raceways).
2. Sand surface sieve on both ends to make it as smooth as possible.
3. For a 20 cm sieve for larval collection, drill two 15 mm hole on opposite sides, approximately 40 mm from top. For a 30 cm sieve for outdoor raceway, drill one 25 mm hole 50 mm from top.
4. Cut a square piece of mesh required so as to cover entire surface area of sieve.
5. Lay mesh on top.
6. Adjust hose clamp so that it can be placed on top of mesh and sieve; enough mesh should be available so that it sticks out below hose clamp.
7. Tighten hose clamp along top of sieve with a screwdriver, continuously pulling mesh.
8. Try to eliminate wrinkles in mesh by pulling uniformly around perimeter of sieve.
9. Label size of mesh on side of PVC with a permanent marker.
10. Once mesh is tight and hose clamp secured (approximately 1 cm from top of sieve) use PVC cleaner to prepare surface of sieve for gluing.
11. Glue mesh to PVC using PVC glue (or cement) along surface and side down to hose clamp.
12. Let dry for 24 hours.
13. Once dry, make sure mesh is uniformly glued so that larvae cannot accumulate in small unglued areas.
14. Remove hose clamp and cut unglued mesh with a razor blade.
15. For support rings used in larval collection, cut 25 cm diameter pipe in 2.5 cm height.
16. For larval collection and with 6 larval tanks, two sets of rings (20 cm and 25 cm diameter) of same mesh aperture are used, allowing take down of two larval tanks simultaneously. Approximately 10–15 mm pipes were cut to 30 cm length for suspension of 20 cm sieve onto 25 cm sieve.
17. A total of 16 sieves for post-larval settlement, suspended in indoor raceway are used in the present set-up.
18. A total of 12 sieves for outdoor raceway are used in the present set-up.
19. A range of sieves, with mesh size ranging from 1.2 mm in diagonal to 4.9 mm, are used in the present set-up for grading.

## Appendix 18

### Raceway check list

YEAR:						
MONTH:						
Day	Species	Day after set	Air	Density No./ml	Total No.	Comments
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						
22						
23						
24						
25						
26						
27						
28						
29						
30						
31						

## Appendix 19

### **Cleaning of raceway**

**Note:** Refer to Technical Drawings – Pg 14 and Pg 17.

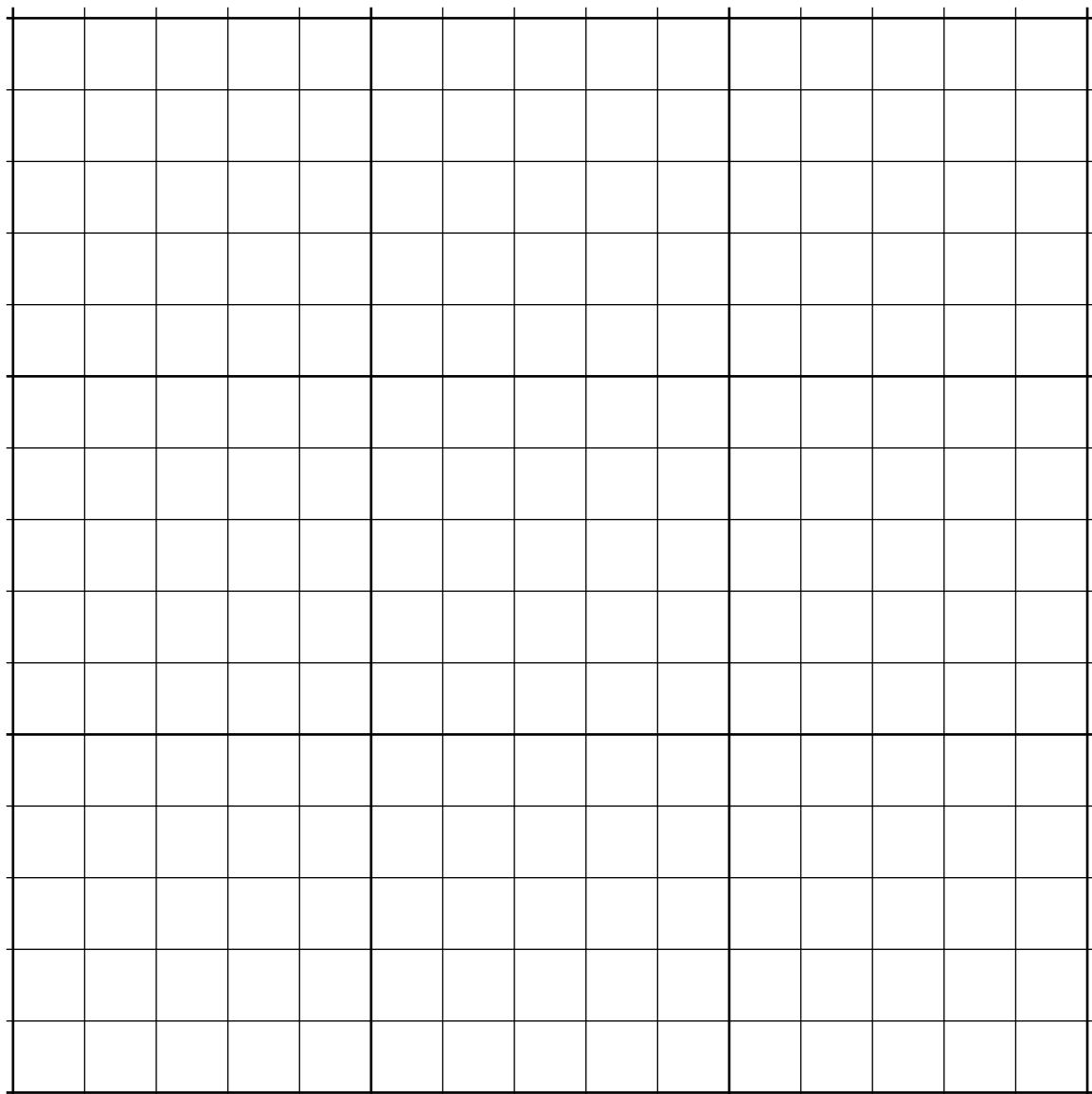
Raceway cleaning involves full chlorination of lines and troughs. The process is similar for both indoor and outdoor raceways. For the indoor raceway, care must be taken to chlorinate all recirculating pipes and sump tank. For the outdoor raceway, algal tank needs to be chlorinated.

1. Turn off pump for recycling system and/or algal supply.
2. Drain troughs, pipes and tanks.
3. Rinse with fresh water.
4. Plug troughs and fill with 50 cm of freshwater.
5. Add capful of chlorine and scrub to remove any detritus.
6. Fill sump tank/algal tank with fresh water to 50 cm and scrub.
7. Drain sump tank/algal tank. Fill with seawater and add capful of chlorine. Mix and let sit.
8. Drain troughs and rinsing with strong jet of fresh water.
9. Indoor raceway: start pump for recycling, open recycling valves and let chlorinated seawater pass through pipes. Watch water level in sump tank so that pump does not run dry.
10. When water level in sump tank/algal tank is low, close off pump.
11. Drain sump/algal tank.
12. Rinse with fresh water and saltwater.
13. Rinse troughs with saltwater.
14. Turn seawater supply on, fill sump tank, turn pump on, and let run through troughs and pipes for 15 minutes.
15. Place standup pipes in troughs, fill for use.
16. Reset recirculation system.
17. Outdoor raceway: Turn pump on to recycle chlorinated water through algal lines. Take care not to let pump run dry.
18. When water level low in algal tank, turn off pump. Open union on drain side of tank.
19. Drain tank and rinse with fresh water.
20. Close union, fill tank halfway with seawater. Turn pump on and let run through algal lines.
21. When low, fill tank with seawater and add algae as per usual.
22. Close trough with standup pipes and fill with seawater.

## Appendix 20

### **Counting grid for spat**

(Template to be copied and laminated for use)



## Appendix 21

### Preparation and ration for dry algae

Instant algae comes in 1 l ziplock bag and can be purchased from Reed Mariculture Inc.

- Packs of *Tetraselmis chuii* contain the equivalent of 1 800 l at 2 600 cells. $\mu\text{l}^{-1}$ .
- Packs of others (*Pavlova lutheri*, *Isochrysis galbana* (Clone: T-Iso), *Thalassiosira pseudonana* (Clone: 3H)) contain the equivalent of 3 600 l at 410 cells. $\mu\text{l}^{-1}$ .

They should be refrigerated and have a shelf life of 12 weeks.

To calculate food ration:  $F = (S \times R) / 7$

Where:

$F$ = dry weight of algae per day (mg)

$R$ = ration as dry weight of algae per wet weight of spat (mg algae.mg spat $^{-1}$ )

$S$ = total wet weight of spat (mg)

Example:

Total biomass= 75 g= 75 000 mg.

Ration= 0.4 mg dry weight of algae per mg wet weight of spat.

Diet: Mixture of *Tetraselmis chuii*, *Pavlova lutheri* (or T-Iso) and 3H

$$F = (75\,000 \times 0.4) / 7 = 4285.7 \text{ mg dry wt algae}$$

Daily ration is 4.3 grams dry weight

Prepare aliquots of each algal species. Use at least three different species for daily food ration: In Bermuda, *Pavlova lutheri* and T-Iso is used alternatively. Ration is based on biomass as seen above. For ease of preparation, one week supply of aliquots can be made in advance, assuming an average 350 grams biomass in raceway.

#### Algal preparation and supply:

1. Transfer one 10 ml aliquot of one species to a screw cap 10 ml test tube.
2. Label the vial with species and date.
3. Keep in the refrigerator until use.
4. Daily: Mix 1 vial of 3H and *Pavlova lutheri* (or T-Iso)(10 ml) and 1/2 a vial of *Tetraselmis* (7.5 ml) into 1 l of seawater.
5. Pour into 150 l tank in outdoor raceway when full.
6. This provides an adequate food ration complimentary to any additional nutrition supplied by the raw seawater.

## Appendix 22

### List of equipment: template for costing out set-up of modular hatchery

**Note:** List is drawn by rearing stage (or manual chapter). Costs are based on North American and Bermuda prices, as of 2004, and are given as an indication of expenditures. Costs need to be calculated for each specific project and can be done using this template. PVC parts, shipping of equipment and labour are excluded.

<b>Seawater supply (ambient and heated) and broodstock (Chapter 1)</b>				
<b>QTY</b>	<b>Description</b>	<b>Unit price (USD)</b>	<b>Extended price (USD)</b>	<b>North American/other supplier</b>
<b>Seawater inlet/pump house/heating/broodstock</b>				
2	50mm PVC roof drain	43	86	Local hardware
6	50mm flange (2 flanges, gasket and bolts)	63	376	AES
2	Clear PVC check valve (2")	24	48	AES
1	Concrete anchor	500	500	Homemade
2	50mm flange	50	100	AES
1	Sweetwater centrifugal pump 2 hp	700	700	AES
1	Hayward self-priming super pump (1 1/2 hp)	365	365	AES
1	Jacuzzi sand filter	369	369	AES
2	Sand (22 kg bag)	50	100	AES
<b>Sub-total</b>			<b>2 644</b>	
<b>Heated seawater unit</b>				
1	1 000-litre insulated tank (43" x 48" x 50")	641	641	BONAR
3	Titanium immersion heaters (one-phase) 6 kw	199	597	AES
3	Digital temperature controllers (one-phase)	339	1 017	AES
1	Water level switch	44	44	AES
1	Hayward cartridge filter housing (C250)	240	240	Local pool supplier
2	C250 cartridges	50	100	Local pool supplier
2	25cm filter housings	22	44	AES
20	10µm and 1µm 25cm wound polypropylene filter cartridges	5	100	AES
1	Quiet one centrifugal pump (Vertical) 0.5 amps	117	117	AES
1	Shed (3m x 3.5m)	800	800	Local supplier
1	Shelves and brackets	20	20	Local hardware
<b>Sub-total</b>			<b>3 720</b>	
<b>Containers for hatchery/broodstock complex</b>				
2	6.5m x 3.5m fibreglass insulated containers	2 500	5 000	Local shipping company
	Connection between two containers (concrete and plywood)	1 500	1 500	Local hardware
8	Concrete pads for container corners	200	1 600	Local hardware
2	Doors (PVC with windows)	800	1 600	Local supplier
2	Air conditioners	600	1 200	Local supplier
10	Shelves for storage	20	200	Local hardware
<b>Sub-total</b>			<b>11 100</b>	

<b>Broodstock area</b>				
2	380-litre fiberglass aquarium tanks with viewing window	675	1 350	AES
4	Plexiglass for cover and filtration (8mm)	90	360	Local supplier
1	Sweetwater air pump 1.2 CFM@4 psi (9" x 8" x 9")	279	279	AES
20	Labcock ball valves for air control	5	100	AES
5	Nitex mesh 500µm per yard	12	60	Aquafauna Bio-marine
2	Tygon tubing (7mm ID) in a 18m coil	49	98	AES
1	Aqua Logic drop-in titanium water chiller 1/5 hp	910	910	Aqua Logic
8	250 W submersible heaters	20	160	Aquafauna Bio-marine
2	20-litre carboys	100	200	AES
2	Shelves and brackets	20	40	Local hardware
<b>Sub-total</b>			<b>3 557</b>	

<b>Spawning procedure</b>				
6	Trays (non-toxic)	30	180	Local hardware
5	Graduated 10-litre buckets	10	50	Local hardware
50	Graduated beakers (2 litre and/or 3 litres)	6	313	AES
2	Sedgewick-rafter cell	125	250	AES
1	Plunger			Homemade
1	Eppendorf pipette (0-1 000µl)	350	350	Sigma
1	Eppendorf tips (100µm and 1 000µm)(1box)	50	50	Sigma
1	Pasteur pipettes (1 box of 250)	14	14	AES
4	Bulbs	6	24	AES
10	Recording book (water resistant)	4	35	AES
6	Thermometers	5	30	AES
1	150µm PVC sieve (see Chapter 3)			Homemade
1	300µm sieve (see Chapter 3)			Homemade
<b>Sub-total</b>			<b>1 296</b>	

<b>Algal culture (Chapter 2)</b>				
QTY	Description	Unit price (USD)	Extended price (USD)	North American/other supplier
<b>Algal seawater system</b>				
1	4m x 3.5m fiberglass container	2 000	2 000	Local shipping company
1	Hayward cartridge filter (C250)	240	240	Local pool supplier
1	Replacement cartridge	50	50	Local pool supplier
2	10" Cartridge filter housing	22	44	AES
10	10µm filters (1box)	5	50	AES
10	1µm filters (1box)	5	50	AES
1	Lifeguard vertical UV sterilizer	172	172	AES
1	20mm ID re-inforced vinyl tubing (18m coil)	66	66	AES
1	Shelves and brackets	20	20	Local hardware
<b>Sub-total</b>			<b>2 692</b>	

<b>Air and light requirements</b>				
1	Air conditioning unit	600	600	Local hardware
1	Freshwater hose	13	13	Local hardware
1	Nozzle	5	5	Local hardware

1	Sweetwater air compressor	279	279	AES
1	Shelves and brackets	20	20	Local hardware
2	50lb CO <sub>2</sub> cylinder	75	150	Local beverage supplier
1	CO <sub>2</sub> regulator with flowmeter	188	188	VWR
1	Refrigerator	350	350	Local supplier
17	Double vertical ballasts	96	1 632	Local supplier
48	Coolwhite fluorescent lamps (30watts)	14	672	Local supplier
6	Single horizontal ballasts (1.2m)	70	420	Local supplier
2	Double horizontal ballasts (1.2m)	96	192	Local supplier
1	7mm ID Tygon tubing clear (36m coil)	11	11	AES
20	Male adapter (8mm NPT x 7mm BARB)	<1	6	AES
20	Labcock ball valve (7mm FNPT ports)	4	81	AES
20	Nipples (7mm NPT x 7mm NPT)	<1	7	AES
20	Bacteria filters 0.3µm (8mm hose barb connection)	7	135	AES
1	Soft tubing cutters	12	12	AES
1	Thread cutting tap (8mm NPT)	6	6	AES
1	Drill bit for 8mm NPT tap	4	4	AES
10	Y-connections	<1	4	Sigma
<b>Sub-total</b>			<b>4 787</b>	

**Culture vessels and fittings**

6	100-litre vessels (transparent, cone bottom)	199	1 191	AES
6	Lids for 100-litre vessels	12	74	AES
1	Marine plywood for 100-litre vessel frame	96	96	Local supplier
1	West epoxy resin system	150	150	Local supplier
6	50mm male adapter for 100-litre drain	2	12	Local hardware
6	50mm-20mm reducers for 100-litre drain	3	15	Local hardware
6	20mm valves for 100-litre drain	3	20	Local hardware/AES
8	4-litre Ernlemeyer flasks	45	360	Sigma
3	500ml Pyrex round flasks with flat bottom - 6 per case	69	207	Thomas Scientific
1	125ml Ernlemeyer flasks, screw cap (case of 24)	228	228	VWR
16	3-hole rubber stopper (no. 10)	2	31	AES
1	Glass rod (package)	30	30	Local supplier
3	Cheesecloth material (4 sq. yards)	6	18	Local hardware/Biomarine Aquafauna
1	Cotton (1 roll)	10	10	Local supplier
1	Pasteur pipettes 1.5ml cap (pack of 400)	20	20	VWR
24	5 and 10ml sterile graduated pipettes (packs of 8)	10	240	AES
1	Incubator	500	500	AES
2	Bottle brush	5	10	AES
1	Thermometer	11	11	VWR
1	Utility brush	5	5	AES
<b>Sub-total</b>			<b>3 228</b>	

**Master and sub-culture**

1	Deep plastic reservoir with lid (chemical resistant)	30	30	Local hardware
1	250ml graduated cylinder (glass)	12	12	AES
5	Foil	5	25	Local supplier
1	50ml volumetric flask with cap	8	8	AES

1	500ml glass bottles (dark amber) with cap (case of 12)	30	30	AES
1	Autoclavable funnel	2	2	AES
1	Autoclave	10 000	10 000	
4	Labelling tape	10	38	Sigma
4	Magic marker		0	Local supplier
2	Pipette latex rubber bulb	13	25	VWR
2	Haemocytometer cell	133	266	AES
1	25ml graduated cylinder	4	4	Sigma
1	100 ml graduated cylinder (polypropylene)	5	5	AES
1	F/2 Formula nutrient media (Solution A) (4 litres)	14	14	AES
1	F/2 Formula nutrient media (Solution B) (4 litres)	14	14	AES
1	D-Biotin (1g)	39	39	Sigma
1	Vitamin B12 (250mg)	17	17	Sigma
1	Thiamine hydrochloride (250g)	58	58	Sigma
1	Sodium metasilicate nonahydrate (250g)	25	25	Sigma
1	Scientific calculator	22	22	VWR
1	Propane torch	10	10	Local hardware
1	Propane bottle	7	7	Local hardware
2	10-litre carboy	83	166	AES
6	10-litre graduated buckets	10	60	Local hardware
12	2-litre graduated beaker with handle (polypropylene)	5	54	AES
1	Compound microscope	979	979	AES
5	Muriatic acid (4-litre container)	9	44	Local supplier
50	Chlorox (4-litre container)	4	213	Local supplier
1	Formaldehyde (1-litre)	30	30	VWR
<b>Sub-total</b>			<b>12 196</b>	

**Live and dry algae**

8	stock cultures (15ml volume)	45	360	CCMP
2	Tetraselmis 3 600 (1 litre)	68	136	Reed Mariculture
2	Pavlova 1 800 (1 litre)	38	75	Reed Mariculture
2	Thalassiosira weisflogii 1 800 (1 litre)	30	60	Reed Mariculture
<b>Sub-total</b>			<b>631</b>	

**Larval seawater system (Chapter 3)**

QTY	Description	Unit price (USD)	Extended price (USD)	North American/other supplier
<b>Seawater and air supply (ceiling plan)</b>				
20	50mm plastic pipe hangers	2	40	AES
20	25mm inner diameter clear flexible hose (per 30cm)	2	43	AES
20	Labcock ball valves	4	81	AES
15	8mm FNPT adapter for labcock ball valve	<1	6	AES
20	25mm plastic pipe hangers	1	24	AES
1	Sweetwater 100 V linear compressor	279	279	AES
1	Shelves and brackets	20	20	Local hardware
<b>Sub-total</b>			<b>493</b>	

**Larval tanks**

4	1344-litre square insulated BONAR tanks with lids	536	2 144	BONAR Plastics
4	40mm-20mm reducer	3	12	Local hardware

4	20mm union	6	24	Local hardware
1	20mm elbow	1	1	Local hardware
1	20mm male adapter	1	1	Local hardware
1	Cartridge filter housing	22	22	AES
10	1µm filter	3	25	AES
10	10µm filter	3	25	AES
1	20mm threaded hose barb (20mm NPT x 8mm barb)	1	1	AES
10	20mm mm inner diameter clear flexible hose (per 30cm)	2	15	AES
1	8mm Tygon tubing (90m coil)	13	13	AES
4	25mm thru-hull bulkhead fitting	14	56	Local hardware
4	25mm female adapter	1	3	Local hardware
4	25mm elbow	1	5	Local hardware
4	25mm ball valve	5	18	Local hardware
20	25mm inner diameter clear flexible hose (per 30cm)	2	43	AES
1	Utility brush	6	6	AES
4	Silicon sealant	11	44	AES
<b>Sub-total</b>			<b>2 456</b>	

**Larval sieves**

1	20µm nitex mesh (per metre)	60	60	Aquafauna
2	40µm nitex mesh (per metre)	56	112	Aquafauna
2	60µm nitex mesh (per metre)	42	84	Aquafauna
2	80µm nitex mesh (per metre)	34	68	Aquafauna
6	120µm nitex mesh (per metre)	24	144	Aquafauna
6	150µm nitex mesh (per metre)	20	120	Aquafauna
2	300µm nitex mesh (per metre)	16	32	Aquafauna
6	25cm (10") diameter PVC (per 30cm)	16	93	Local hardware
6	20cm (8") diameter PVC (per 30cm)	13	75	Local hardware
6	15mm PVC pipe (per 30cm)	1	3	Local hardware
2	15cm (6") diameter PVC pipe (per 30cm)	8	15	Local hardware
8	Garden hose clamps (125mm)	2	14	Local hardware
<b>Sub-total</b>			<b>820</b>	

**Miscellaneous equipment**

1	Freshwater garden hose (18m)	25	25	Local hardware
1	Hose nozzle	8	8	Local hardware
1	20-gallon bin for chlorination of small equipment	30	30	Local hardware
20	Chlorine (4-litre containers)	4	85	Local hardware
10	Recording notebook (waterproof)	4	35	AES
2	250ml graduated cylinders	12	25	AES
<b>Sub-total</b>			<b>208</b>	

**450-L and raceway nursery systems (Chapter 4)**

QTY	Description	Unit price (USD)	Extended price (USD)	North American/other supplier
<b>450-litre setting tanks</b>				
4	450-litre tanks with skirt and standpipe assembly	600	2 400	Red-Ewald Inc.
4	Drain pipe assembly (50mm male adapter)	2	8	Local hardware
4	Recirculation assembly (25mm pipe and 25mm T)	120	120	Local hardware
4	20-litre carboys	100	400	AES
2	Shelves and brackets (20cm x 25cm)	20	40	Local hardware

1	3mm black polyethylene mesh (18m roll)	60	60	Atlantic Gulf & Fishing
2	Cable ties (100 per package) - 100mm long	3	5	AES
<b>Sub-total</b>			<b>3 033</b>	

**Indoor raceway system**

1	220-litre tank	350	350	Bonar Plastics
2	Fiberglass troughs (120 litres)	200	400	Red-Ewald Inc.
1	Centrifugal pump (vertical)	120	120	AES
4	50cm x100cm wolmanized wood for frame	12	48	Local hardware
1	West system epoxy resin	150	150	Local hardware
2	Shelves and brackets	20	40	Local hardware
<b>Sub-total</b>			<b>1 108</b>	

**Indoor sieves**

8	25cm (10") pipe for sieves (per 30cm)	16	124	Local hardware
16	Tubing flow valve (for restricting flow)	<1	3	AES
12	Barbed fitting (8mm x 7mm barb)	<1	4	AES
2	20-litre carboys	100	200	AES
	150 and 120µm Nitex mesh - see larval sieves section			
<b>Sub-total</b>			<b>331</b>	

**Outdoor raceway**

1	Cement pad	600	600	Local supplier
5	Untreated wood for frame (50mm x100mm)	12	60	Local hardware
1	West system epoxy resin	150	150	Local hardware
2	Corrugated plastic for roofing (per sheet)	35	70	Local supplier
1	Marine plywood for roof support	96	96	Local hardware
1	150-litre reservoir	60	60	AES
2	8mm stopcock valve	1	2	AES
30	Window screen (per 30cm, 1.2m wide)	2	60	Local hardware
30	1/2" pipe for screen (per 30cm)	1	23	Local hardware
10	Cup hooks for screen	1	7	Local hardware
1	Centrifugal pump (vertical)	120	120	AES
<b>Sub-total</b>			<b>1 248</b>	

**Outdoor sieves**

8	30cm pipe for sieves (per 30cm)	18	140	Local hardware
20	750µm green collector bag	2	35	AES
5	1.5mm red collector bag	2	9	AES
12	25mm coupler and pipe for suspension	2	18	Local hardware
<b>Sub-total</b>			<b>202</b>	

**Transfer phase: exterior tanks and field requirements (Chapter 5)**

QTY	Description	Unit price (USD)	Extended price (USD)	North American/other supplier
<b>Holding tanks</b>				
2	540-litre insulated tank with lid	450	900	BONAR Plastics
2	Drain fittings (bulkhead and female adapter)	17	34	AES
1	Silicone sealant	10	10	AES
10	Reinforced vinyl tubing (25mm inner diameter)(price per 30cm)	2	22	AES
<b>Sub-total</b>			<b>966</b>	

<b>Growout longlines (for 90m line) in 10m depth</b>				
1	15mm twisted polypropylene rope (200m per reel)	70	70	Atlantic Gulf & Fishing
50	30cm diameter black floats with mooring eye (14.8kg buoyancy)	15	750	Go-Deep
6	Marker buoys	9	54	Atlantic Gulf & Fishing
1	Salt water high pressure washer	2 500	2 500	Easy-Kleen
1	Rope cutting gun	40	40	Atlantic Gulf & Fishing
6	20mm shackles (stainless steel)	28	168	Atlantic Gulf & Fishing
<b>Sub-total</b>			<b>3 582</b>	
<b>Transfer materials</b>				
50	window screen and velcro for black insert pouches	3	150	Local hardware
100	750µm green collector bags	2	175	AES
100	Plastic shellfish trays	7	700	Aquaculture Supplier
15	15mm stainless steel snap hooks	10	150	Local hardware
4	Transport coolers	50	200	Local hardware
<b>Sub-total</b>			<b>1 375</b>	
<b>Growout materials</b>				
10	Snap-on connectors (12cm standard) (100 per package)	40	400	Atlantic Gulf & Fishing
220	3mm pearl nets	4	814	AES
440	6mm pearl nets	4	1 540	AES
1000	9mm or 12mm pearl nets	4	3 750	AES
20	Cable ties (150mm long - 100 per package)	4	75	AES
<b>Sub-total</b>			<b>6 579</b>	
<b>TOTAL FOR EQUIPEMENT</b>			<b>68 250</b>	
PVC Estimate	Pipes and parts (Bermuda prices)		16 000	
<b>GRAND TOTAL</b>			<b>84 250</b>	

## Appendix 23

### **List of selected suppliers**

This list is not comprehensive and provides only those suppliers used during the installation and operation of the Bermuda hatchery. The mention or omission of specific companies, their products or brand names does not imply any endorsement or judgement by the Food and Agriculture Organization of the United Nations.

#### **Bonar Plastic Inc.**

125 N. Christopher Court  
Newnan, Georgia 30265  
United States of America  
Tel.: +1 770 2518264 / 800 7686246  
Fax: +1 770 2518275

#### **Red Ewald Inc.**

PO Box 519  
Karnes City, Texas 78118  
United States of America  
Tel.: +1 800 2423524  
Web: [www.redewald.com](http://www.redewald.com)

#### **Aquafauna Bio-marine Inc.**

PO Box 5  
Hawthorne California 90250  
United States of America  
Tel.: +1 310 9735275  
Fax: +1 310 6769387  
Web: [www.aquafauna.com](http://www.aquafauna.com)

#### **Easy-Kleen, Pressure System Ltd.**

26 Eveleigh Street  
Sussex, New Brunswick E4E 2N8  
Canada  
Tel.: +1 506 4333393  
Fax: +1 506 4332443  
Web: [www.easy-kleen.com](http://www.easy-kleen.com)

#### **Aqua Logic Inc.**

8280 Clairemont Mesa Blvd, Suite 127  
San Diego, CA 92111  
United States of America  
Tel.: +1 858 2924773  
E-mail: [aqualogic@aol.com](mailto:aqualogic@aol.com)  
Web: [www.aqualogicinc.com](http://www.aqualogicinc.com)

#### **Reed Mariculture Inc.**

Instant Algae Products  
511 Pamlar Ave, Suite #C  
San Jose, CA 95128  
United States of America  
Tel.: +1 831 7683830  
Fax: +1 831 4012474  
Web: [www.seafarm.com](http://www.seafarm.com)

#### **Aquatic Eco-systems Inc.**

1767 Benbow Court  
Apopka, FL 32703  
United States of America  
Tel.: +1 407 8863939 / 877 3474788  
Fax: +1 407 886 4884  
E-mail: [aes@aquaticeco.com](mailto:aes@aquaticeco.com)  
Web: [www.aquaticeco.com](http://www.aquaticeco.com)

#### **Go Deep International Inc.**

PO Box 493, Station "A"  
Fredericton, New Brunswick E3B 4Z9  
Canada  
Tel.: +1 506 454 5341  
Fax: +1 506 462 9883  
E-mail: [godeep@nbnet.nb.ca](mailto:godeep@nbnet.nb.ca)

## Literature cited

- Andersen, S., Burnell, G. & Bergh, O.** 2000. Flow-through systems for culturing great scallop larvae. *Aquaculture International*, 8: 249–257.
- Ansell, A.D.** 1961. Reproduction, growth and mortality of *Venus striatula* (da Costa) in Kames Bay, Millport. *J. Mar. Biol. Assoc. U.K.*, 41: 191–215.
- Barber, B. & Blake, N.J.** 1983. Growth and reproduction of the bay scallop, *Argopecten irradians* (Lamarck) at its southern distributional limit. *J. Exp. Mar. Biol. Ecol.*, 66: 247–256.
- Bayne, B.L.** 1965. Growth and the delay of metamorphosis of the larvae of *Mytilus edulis* (L.). *Ophelia*, 2(1): 1–47.
- Bayne, B.L.** 1983. Physiological ecology of marine molluscan larvae. In *The Mollusca*. Verdonk, N.H., Van den Biggelaar, J.A.M. & Tompa, A.S., eds. Vol. 3, Development. New York, Academic Press. pp. 299–343.
- Beaumont, A. R. & Budd, M.D.** 1983. Effects of self-fertilization and other factors on the early development of the scallop *Pecten maximus*. *Mar. Biol.*, 76: 285–289.
- Blake, N.J. & Moyer, M.A.** 1991. The calico scallop, *Argopecten gibbus*, fishery of Cape Canaveral, Florida. In *Scallops: Biology, Ecology, and Aquaculture*. S. Shumway, ed. Elsevier, New York. pp. 899–909.
- Bourne, N. & Hodgson, C.A.** 1991. Development of a viable nursery system for scallop culture. In *International Compendium of Scallop Biology and Culture*. S. Shumway & P. Sandifer, eds. The World Aquaculture Society, Louisiana State University, Baton Rouge, LA 70803. pp 273–280.
- Bourne, N., Hodgson, C.A. & Whyte, J.N.C.** 1989. A manual for scallop culture in British Columbia. Canadian Technical Report of Fisheries and Aquatic Sciences. No. 1694. 215 pp.
- Brown, N. & Robert, R.** 2002. Preparation and assessment of microalgal concentrates as feeds for larval and juvenile Pacific oyster (*Crassostrea gigas*). *Aquaculture*, 207: 289–309.
- Brown, M.R.** 1991. The amino-acid and sugar composition of 16 species of microalgae used in mariculture. *J. Exp. Mar. Biol. Ecol.*, 145: 79–99.
- Brown, M.R., Jeffrey, S.W., Volkman, J.K. & Dunstan, G.A.** 1997. Nutritional properties of microalgae for mariculture. *Aquaculture*, 151: 315–331.
- Brown, M.R., Garland, C.D., Jeffrey, S.W., Jameson, I.D. & Leroi, J.N.** 1993. The gross and amino acid compositions of batch and semi-continuous cultures of *Isochrysis* sp. (clone T-Iso), *Pavlova lutheri* and *Nannochloropsis oculata*. *J. Appl. Phycol.*, 5: 285–296.
- Burke, R.D.** 1983. The induction of metamorphosis of marine invertebrate larvae: stimulus and response. *Can. J. Zool.*, 61: 1701–1719.

- Cary, S.C., Leighton, D.L. & Phleger, C.F.** 1981. Food and feeding strategies in culture of larval and early juvenile purple-hinge rock scallops, *Hinnites multirugosus* (Gale). *J. World Maricul. Soc.*, 12(1): 156–169.
- Chu, F.E., Webb, K., Hepworth, D. & Casey, B.** 1987. Metamorphosis of larvae of *Crassostrea virginica* fed microencapsulated diets. *Aquaculture*, 64: 185–197.
- Costello, T.J., Harold Hudson, J., Dupuy, J.L. & Rivkin, S.** 1973. Larval culture of the calico scallop, *Argopecten gibbus*. Proceedings of the National Shellfisheries Association, 63: 72–76.
- Coutteau, P. & Sorgeloos, P.** 1992. The use of algal substitutes and the requirement for live algae in the hatchery and nursery rearing of bivalve molluscs: an international survey. *J. Shellfish Res.*, 11: 467–476.
- Couturier, C., Dabinett, P. & Lanteigne, M.** 1996. Scallop culture in Atlantic Canada. In Cold-Water Aquaculture in Atlantic Canada. A.D. Boghen, ed. Second Edition. University of Moncton, Moncton, N.B., Canada. pp. 297–340.
- Cragg, S.M. & Crisp, D.J.** 1991. The biology of scallop larvae. In Scallops: Biology, Ecology and Aquaculture. S. Shumway, ed. pp. 75–127.
- Crisp, D.J.** 1974. Factors influencing the settlement of marine invertebrate larvae. In: Chemoreception in marine organisms. Grant, P.T. & Mackie, A.M., eds. Academic Press, London. pp. 177–277.
- Culliney, J.L.** 1974. Larval development of the giant scallop *Placopecten magellanicus* (Gmelin). *Biol. Bull. (Woods Hole)*, 147: 321–332.
- Dabinett, P., Caines, J. & Crocker, K.** 1999. Hatchery production of sea scallop spat (*Placopecten magellanicus*) in Newfoundland, Canada. In Book of Abstracts. 12<sup>th</sup> International Pectinid Workshop 5–11 May 1999. Bergen, Norway. pp. 63–64.
- Davis, H.C. & Guillard, R.R.** 1948. Relative value of ten genera of microorganisms as foods for oyster and clam larvae. *Fish. Bull. U.S.*, 58: 293–304.
- Davis, J.P. & Campbell, C.R.** 1998. The use of a *Shizochytrium* based HUFA enriched dry feed for culturing juvenile mussels (*Mytilus galloprovincialis*) and the comparative routine costs of producing live algae in a commercial bivalve hatchery. European Aquaculture Society, Special Publication No. 26, Oostende, Belgium. pp. 64–65.
- DeLa Roche, J.P., Marin, B., Freites, L. & Velez, A.** 2002. Embryonic development and larval and post-larval growth of the tropical scallop *Nodipecten* (= *Lyropecten*) *nodosus* (L. 1758) (Mollusca: Pectinidae). *Aquaculture Research*, 33: 819–827.
- DiSalvo, L.H., Alarcón, E., Martinez, E. & Uribe, E.** 1984. Progress in mass culture of *Chlamys (Argopecten) purpurata* Lamarck (1819) with notes on its natural history. *Revista Chilena de Historia Natural*, 57: 35–45.
- Dortch, Q.** 1982. Effect of growth conditions on accumulation of internal nitrate, ammonium, amino acids, and protein in three marine diatoms. *J. Exp. Mar. Biol. Ecol.*, 61: 242–264.

- Dredge, M., Duncan, P., Heasman, M., Johnston, B., Joll, L., Mercer, J., Souter, D. & Whittingham, T. 2002. Feasibility of scallop enhancement and culture in Australian waters. Project Report Q002010. Department of Primary Industries. Queensland Government, Australia.
- Duggan, W.P. 1975. Reactions of the bay scallop, *Argopecten irradians* to gradual reductions in salinity. *Chesapeake Science*, 16(4): 284–286.
- Enright, C.T., Newkirk, G.F., Craigie, J.S. & Castell, J.D. 1986. Evaluation of phytoplankton as diets for juvenile *Ostrea edulis* L. *J. Exp. Mar. Biol. Ecol.*, 96: 1–13.
- Epifanio, C.E., Valenti, C.C. & Turk, V.L. 1981. A comparison of *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* as foods for the oyster, *Crassostrea virginica*. *Aquaculture*, 23: 347–353.
- Fabregas, J., Herrero, C., Cabezas, B. & Abalde, J. 1986. Biomass production and biochemical composition in mass cultures of the marine microalga *Isochrysis galbana* Parke at varying nutrient concentrations. *Aquaculture*, 53: 101–113.
- Farias, A., Uriarte, I. & Castilla, J.C. 1998. A biochemical study of the larval and postlarval stages of the Chilean scallop *Argopecten purpuratus*. *Aquaculture*, 66(1-2): 37–47.
- Gabbott, P.A. & Bayne, B.L. 1973. Biochemical effects of temperature and nutritive stress on *Mytilus edulis* L. *J. Mar. Biol. Assoc. U.K.*, 53: 269–286.
- Gallager, S.M. & Mann, R. 1981. Use of lipid-specific staining techniques for assaying condition in cultured bivalve larvae. *J. Shell. Res.*, 1: 69–73.
- Gallager, S.M. & Mann, R. 1986. Growth and survival of larvae of *Mercenaria mercenaria* (L.) and *Crassostrea virginica* (Gmelin) relative to broodstock conditioning and lipid content of eggs. *Aquaculture*, 56: 105–121.
- Gruffydd, I.L.D. 1976. The development of the larva of *Chlamys islandica* in the plankton and its salinity tolerance in the laboratory (Lamellibranchia, Pectinidae). *Astarte*, 8: 61–67.
- Gruffyd, L.K. & Beaumont, A.R. 1972. A method for rearing *Pecten maximus* larvae in the laboratory. *Marine Biology*, 15: 350–355.
- Gruffyd, L.D. & Beaumont, A.R. 1970. Determination of the optimum concentration of eggs and spermatozoa for the production of normal larvae in *Pecten maximus* (Mollusca, Lamellibranchia). *Helgolander wiss. Meeresunters*, 20: 486–497.
- Hadfield, M.G. 1977. Chemical induction in larval settling of a marine gastropod. *Marine Natural Products Chemistry*. Faulkner, D.J & Fenical, W.H., eds.) Plenum Press, New York. pp. 403–413.
- Heasman, M., Diemar, J., O'Connor, W., Sushames, T. & Foulkes, L. 2000. Development of extended shelf-life micro-algae concentrate diets harvested by centrifugation for bivalve molluscs – a summary. *Aquacult. Res.*, 31: 637–659.
- Heasman, M.P., O'Connor, W.A. & Frazer, A.W. 1996. Temperature and nutrition as factors in conditioning broodstock of the commercial scallop *Pecten fumatus* Reeve. *Aquaculture*, 75–90.

- Hohn, C., Sarkis, S. & Helm, M.** 2001. The effect of algal food rations on growth and survival of *Pecten ziczac* and *Argopecten gibbus*. Proceedings of the 13<sup>th</sup> International Pectinid Workshop, April 18–24, 2001, Coquimbo, Chile.
- Hodgson, C.A. & Bourne, N.** 1988. Effect of temperature on larval development of the spiny scallop, *Chlamys hastata* Sowerby, with a note on metamorphosis. *J. Shell. Res.*, 7(3): 349–357.
- Huguenin, J.E. & Colt, K.** 2002. Design and operating guide for aquaculture seawater systems- second edition. Elsevier Science B.V. The Netherlands. 328 pp.
- Jespersen, H. & Olsen, K.** 1982. Bioenergetics in veliger larvae of *Mytilus edulis* L. *Ophelia*, 21(1): 101–113.
- Kasyanov, V.L.** 1991. Development of the Japanese scallop *Mizuhopecten yessoensis* (Jay 1985). In An International Compendium of Scallop Biology and Culture. Shumway, S.E. & Sandifer, P.A., eds. World Aquaculture Society, Louisiana State University, Baton Rouge, LA 70803.
- Kingzett, B.C., Bourne, N. & Leask, K.** 1990. Induction of metamorphosis of the Japanese scallop *Patinopecten yessoensis* Jay. *J. Shell. Res.*, 9(1): 119–124.
- Kraeuter, J.N., Castagna, M. & Van Dessel, R.** 1982. Egg size and larval survival of *Mercenaria mercenaria* (L.) and *Argopecten irradians* (Lamarck). *J. Exp. Mar. Biol. Ecol.*, 56: 3–8.
- Laing, I.** 1987. The use of artificial diets in rearing bivalve spat. *Aquaculture*, 65: 243–249.
- Langdon, C.J. & Siegfried, C.A.** 1984. Progress in the development of artificial diets for bivalve filter feeders. *Aquaculture*, 39: 135–153.
- Langdon, C. & Onal, E.** 1999. Replacement of living microalgae with spray-dried diets for the marine mussel *Mytilus galloprovincialis*. *Aquaculture*, 180(3-4): 13–22.
- Lim, L.C., Dhert, P. & Sorgeloos, P.** 2003. Recent developments and improvements for ornamental fish packaging systems for air transport. *Aquac. Res.*, 34: 923–935.
- Lim, L.C., Wong, C.C., Koh, C.H., Dhert, P. & Sorgeloos, P.** 2000. A stress resistance test for quality evaluation of guppy (*Poecilia reticulata*) (Abstract). In Agrifood & Veterinary Authority of Singapore, Singapore, Abstract Book of First AVA Technical Seminar 1 September 2000. pp. 4–5.
- Lodeiros, C., Freites, L., Fernandez, E., Velez, A. & Bastardo, J.** 1989. Efecto antibiotico de tres bacterias marinas en la supervivencia de larvas de la vieira *Pecten ziczac* infectadas con el germe *Vibrio anguillarium*. *Bol. Inst. Oceanog. Venezuela, Univ. Oriente*, 28: 165–169.
- Lodeiros, C.J. & Himmelman, J.H.** 1994. Relations among environmental conditions and growth in the tropical scallop *Euvola (Pecten) ziczac* L. in suspended cultures in the Golfo de Cariaco, Venezuela. *Aquaculture*, 199: 345–358.
- Lodeiros, C.J. & Himmelman, J.H.** 2000. Identification of factors affecting growth and survival of the tropical scallop *Euvola (Pecten) ziczac* (L.) in Golfo de Cariaco, Venezuela. *Aquaculture*, 182: 91–114.

- Loosanoff, V.L. & Davis, H.C.** 1963. Rearing of bivalve mollusks. *Adv. Mar. Biol.*, 1: 1–136.
- Lu, Y.T. & Blake, N.J.** 1996. Optimum concentrations of *Isochrysis galbana* for growth of larval and juvenile bay scallops, *Argopecten irradians concentricus* (Say). *J. Shell. Res.*, 15(3): 635–643.
- Lucas, A. & Beninger, P.G.** 1985. The use of physiological condition indices in marine bivalve. *Aquaculture*, 44: 187–200.
- Maeda-Martinez, A.N., Siscard, M.T. & Reynoso-Granados, T.** 2000. A shipment method for scallop seed. *J. Shell. Res.*, 19(2): 765–770.
- Maguire, J.A., Cashmore, D. & Burnell, G.** 1999. The effect of transportation on juvenile scallops *Pecten maximus* (L.). *Aquac. Res.*, 30: 325–333.
- Manuel, S.** 2001. Reproduction and spat settlement of *Euvola ziczac* around Bermuda. Ph.D. thesis. University of Liverpool, U.K.
- Maru, K.** 1985. Tolerance of scallop, *Patinopecten yessoensis* (Jay) to temperature and specific gravity during early developmental stages. *Sci. Rep. Hokkaido. Fish. Expl. Stn.*, 27: 55–64.
- Moal, J., Martin-Jezequel, V., Harris, R.P., Samain, J.F. & Poulet, S.A.** 1987. Interspecific variability of the chemical composition of marine phytoplankton. *Oceanol. Acta*, 10: 339–346.
- Monsalvo-Spencer, P., Maeda-Martinez, A.N. & Reynoso-Granados, T.** 1997. Reproductive maturity and spawning induction in the Catarina scallop *Argopecten ventricosus* (= *circularis*) (Sowerby II, 1842). *J. Shell. Res.*, 16(1): 67–70.
- Moyer, M. & Blake, N.J.** 1986. Fluctuations in calico scallop production (*Argopecten gibbus*). Proceedings of the Eleventh Annual Tropical and Subtropical Fisheries Conference of the Americas. pp. 45–58.
- Naidenko, T.** 1991. The laboratory culture of two scallop species from the sea of Japan: Development and induction of metamorphosis. In IFREMER, Actes de Colloques. No. 17: 107–110.
- Neima, P.G. & Kenchington, E.** 1997. Report on commercial scallop hatchery design. *Canadian Technical Report of Fisheries and Aquatic Sciences*, No. 2176. 55 pp.
- Nell, J.A. & O'Connor, W.A.** 1991. The evaluation of fresh algae and stored algal concentrates as a food source for Sydney rock oyster *Saccostrea commercialis* (Iredale and Roughley) larvae. *Aquaculture*, 94: 65–78.
- O'Connor, W.A. & Heasman, M.P.** 1997. Diet and feeding regimens for larval doughboy scallops, *Mimachlamys asperrima*. *Aquaculture*, 158: 289–303.
- Paquette, P. & Fleury, P.-G.** 1994. Analyse technique et financière d'un projet d'élevage de coquilles Saint-Jacques de l'éclosé jusqu'à la recapture des semis. IFREMER. RIDRV-94.13/SEM Paris –R.A. Brest. 37 pp.

- Parsons, G.J., Dadswell, M.J. & Roff, J.C.** 1993. Influence of biofilm on settlement of sea scallop, *Placopecten magellanicus* (Gmelin, 1791), in Passamaquoddy Bay, New Brunswick, Canada. *J. Shell. Res.*, 12(2): 279–283.
- Paulet, Y.M., Lucas, A. & Gerard, G.** 1988. Reproduction and larval development in two *Pecten maximus* (L.) populations from Brittany. *J. Exp. Mar. Biol. Ecol.*, 119: 145–156.
- Peirson, W.M.** 1983. Utilization of eight algal species by the bay scallop, *Argopecten irradians concentricus* (Say). *J. Exp. Mar. Biol. Ecol.*, 68: 1–11.
- Pezzuto, P.R. & Borzone, C.A.** 1997. The scallop *Pecten ziczac* (Linnaeus, 1758) fishery in Brazil. *J. Shell. Res.*, 16: 527–532.
- Roe, R.B., Cummins Jr., R. & Bullis Jr., H.R.** 1971. Calico scallop distribution, abundance, and yield off eastern Florida, 1967–1968. *Fish. Bull.*, 69: 399–409.
- Rojas, L.M., Velez, A. & Azuaje, O.** 1988. Efecto individual y combinado de la densidad larval y la racion de alimento sobre la supervivencia y crecimiento de la vieira, *Pecten ziczac*. *Bol. Inst. Oceanogr. Venezuela, Univ. Oriente*, 27(1&2): 57–62.
- Rupp, G.S.** 1997. Desenvolvimento de tecnologia de producao de sementes *Nodipecten nodosus* (Linnaeus, 1758) (Bivalvia: Pectinidae). Informe Final. Program RHAE/PIBIO-UFSC. 57 pp.
- Sarkis, S.** 1987. Gross biochemical composition and shell growth of *Crassostrea gigas* spat, fed seven types of algal diets. *Haliotis*, 16: 413–425.
- Sarkis, S.** 1995. Scallop culture in Bermuda: A saga. *Actes de Colloques IFREMER* 17: 115–121.
- Sarkis, S., Boettcher, A., Ueda, N. & Hohn, C.** 2005. A simple transport procedure for juvenile scallops, *Argopecten gibbus*, (Linnaeus, 1758). *J. Shell. Res.* 24(2): 377–380.
- Sarkis, S., Helm, M. & Hohn, C.** 2006. Larval rearing of calico scallop, *Argopecten gibbus*, in a flow-through system. *Aquacult. Int.*
- Sastray, A.N.** 1965. The development and external morphology of pelagic larval and post-larval stages of the bay scallop, *Aequipecten irradians concentricus* Say, reared in the laboratory. *Bull. Mar. Sc.*, 15(2): 418–435.
- Schulte, E.H.** 1975. Influence of algal concentration and temperature on the filtration rate of *Mytilus edulis*. *Marine Biology*, 30: 331–341.
- Southgate, P.C. & Beer, A.C.** 1997. Hatchery and early nursery culture of the blacklip pearl oyster (*Pinctada margaritifera* L.). *J. Shell. Res.* 16(2): 561–567.
- Southgate, P.C. & Ito, M.** 1998. Evaluation of a partial flow-through culture technique for pearl oyster (*Pinctada margaritifera* L.) larvae. *Aquacultural Engineering* 18(1): 1–7.
- Sterrer, W.** 1986. Marine Fauna and Flora of Bermuda. A systematic Guide to the identification of Marine Organisms. John Wiley & Sons, New York. 742 pp.

- Uriarte, I., Farias, A. & Munoz, C.** 1996. Cultivo en hatchery y pre-engorde del ostion del norte, *Argopecten purpuratus* (Lamarck, 1819) en el sur de Chile. *Rev. Biol. Mar. Valparaiso* 31(2): 81–90.
- Velez, A., Sotillo, F. & Perez, J.** 1987. Variacion estacional de la composicion quimica de los pectinidos *Pecten ziczac* y *Lyropecten nodosus*. *Bol. Inst. Oceanog. Venezuela, Univ. Oriente*, 26: 67–72.
- Velez, A. & Lodeiros, C.J.** 1990. El cultivo de moluscos en Venezuela. In *Cultivo de Moluscos en America Latina*. A. Hernandez, ed. Red Regional de Entidades y Centros de Acuicultura de America Latina. CIID-Canada. pp. 345–369.
- Velez, A., Alifa, E. & Freites, L.** 1993. Induccion de la reproducion en la vieira *Euvola (Pecten) ziczac* (Mollusca: Bivalvia) maduracion y desove. *Carib. J. Sc.*, 29: 209–213.
- Ventilla, R.F.** 1982. The scallop industry in Japan. *Adv. Mar. Biol.*, 20: 309–382.
- Zar, J.H.** 1984. Biostatistical Analysis. Second Edition. Prentice-Hall, Inc. Englewood Cliffs, NJ.
- Volkman, J.K., Jeffrey, S.W., Nichols, P.D., Rogers, G.I. & Garland, C.D.** 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *J. Exp. Mar. Biol. Ecol.*, 128: 219–240.
- Waller, T.R.** 1969. The evolution of the *Argopecten gibbus* stock (Mollusca: Bivalvia) with emphasis on the tertiary and quarternary species of eastern North America. *J. Paleont.*, 43(Suppl.): 1–125.
- Whyte, J.N.C.** 1987. Biochemical composition and energy content of six species of phytoplankton used in mariculture of bivalves. *Aquaculture*, 60: 231–241.
- Whyte, J.N.C., Bourne, N. & Hodgson, C.A.** 1989. Influence of algal diets on biochemical composition and energy reserves in *Patinopecten yessoensis* (Jay) larvae. *Aquaculture*, 78: 333–347.
- Whyte, J.N.C., Bourne, N. & Hodgson, C.A.** 1990. Nutritional condition of rock scallop, *Crassadoma gigantea* (Gray), larvae fed mixed algal diets. *Aquaculture*, 86: 25–40.
- Wikfors, G.H., Twarog, J.W. & Ukeles, R.** 1984. Influence of chemical composition of algal food sources in growth of juvenile oysters, *Crassostrea virginica*. *Biol. Bull. (Woods Hole, Mass.)*, 167: 251–263.
- Wilson, J.H.** 1980. Particle retention and selection by larvae and spat of *Ostrea edulis* in algal suspensions. *Mar. Biol.* 57: 135–145.



Limiting factors such as minimal capital investment, lack of technical support or expertise and available physical space may put severe restrictions on setting up a hatchery. Not all investors have the means or the will to take the risk to support a large commercial aquaculture operation without substantial proof of its production capacity. For these reasons, the setup of an inexpensive modular hatchery may be a simpler option to the start-up of a large commercial operation, or may be sufficient to the needs of a smaller operation. This manual was written for those interested in establishing a bivalve hatchery, with minimal experience in this activity, limited technical support and restricted access to information. The manual stands as an entity, providing not only the technicalities of setting up and operating a hatchery, but also makes some of the scientific background, deemed useful to the aquaculturist, readily accessible. The manual is divided into chapters for each stage of rearing: broodstock conditioning, algal culture, hatchery, nursery, growout and economic considerations. The first five chapters include both the physical requirements and culture considerations and procedures for the relevant rearing stage. The final chapter on economic considerations provides an insight into the labour involved for each stage of production, along with a list of equipment and supplies, which may be used as a template for a new installation.

**FULL PAPER**

## Breeding and Larval Rearing of Asian Moon Scallop *Amusium pleuronectes* in Eastern Samar, Philippines

Nonita S. Cabacaba<sup>1,2,\*</sup>, Ed-Marie B. Boiser<sup>1</sup>, Kimberly A. Badocdoc<sup>1</sup>, and Cristan Joy M. Campo<sup>1</sup>

<sup>1</sup>National Fisheries Research and Development Center – Marine Fisheries Research and Development Center (NFRDI-MFRDC)

<sup>2</sup>Bureau of Fisheries and Aquatic Resources – Guiuan Marine Fisheries Development Center (BFAR-GMFDC), Guiuan, Eastern Samar 6809

### A B S T R A C T

Asian moon scallop, *Amusium pleuronectes*, is among the most common commercially harvested scallop in the Philippines. This study investigated suitable conditioning methods, induced spawning, and documented scallop larval development at the Marine Fisheries Research and Development Center (MFRDC) Guiuan, Eastern Samar. Scallops held in tanks with flow-through water system without substrate yielded high survival rate of  $48.06 \pm 5.95\%$ . Optimum water temperature was 28-29°C for maintaining scallops. *Isochrysis galbana* and *Chaetoceros calcitrans* were suitable microalgal feed for *A. pleuronectes* consumed at 7,388,888 cells min<sup>-1</sup> per scallop. These significant results were applied in maintaining scallops at the hatchery. As a result, scallops with an average 19.8 mm shell length (SL) reached 37.5 mm SL within four months with a survival rate of  $96.57 \pm 2.04\%$ , average daily growth rate (ADGR) of  $0.13 \pm 0.04$  mm.day<sup>-1</sup>, and specific growth rate (SGR) of  $3.92 \pm 1.31\% \text{ d}^{-1}$ . Natural spawning was successful under controlled conditions while induced spawning trial through thermal stimulation, food shock, sexual stimulation, and serotonin injection resulted unsuccessful release of sperm and eggs. The estimated number of fertilized eggs per spawning ranged from 0.22-1.4 million. Fertilized eggs appeared spherical and dark in color with 54.2-62.57 µm in diameter. After nine hours, the larvae developed into trophophore stage with 59.08-84.4 µm in length. D-veliger with 120.37-157.07 µm shell length developed after 24 hours. Development of the early umbone stage was reached on day 5; and on day 7, the umbo larvae become well-developed with shell length of 135.45-173.36 µm. On day 9, pedi-veligers were observed in the culture. Spat grew 312.41-509.48 µm on day 16 and survived until four months with final shell length of 4-10 mm. For the larval rearing, stocking density of *A. pleuronectes* larvae observed highest survival rate of  $0.04 \pm 0.03\%$  at 200 larvae/L, while no larvae survived at 800 larvae/L due to contamination of protozoans in the culture medium.

\*Corresponding Author: *nitz\_sur@yahoo.com*

Received: August 1, 2019

Accepted: May 15, 2020

**Keywords:** *Amusium pleuronectes*, conditioning methods, spawning, larval development

### 1. INTRODUCTION

**A** *musium pleuronectes* is a bivalve species which belongs to the Pectinidae family and commonly known as Asian moon scallop. It has thin, smooth, biconvex white and brown colored valves. *A. pleuronectes* lies recessed in the substrate, the white colored valve positioned lying on the seabed while brown colored valve is on top and the umbral region is submerged in the substrate (Morton 1980).

This species naturally occur in soft substratum such as sandy-mud to muddy bottom at a depth of 18-40 meter (Gabral-Llana 1980; Del Norte 1988) where it feeds on phytoplankton, detritus, and other suspended particulates. Within two-year period, it can rapidly grow up to 106 mm shell length (Belda and Del Norte 1988; Del Norte 1988) and reach sexual maturity at a shell length of 54 mm (Gabral-Llana 1980). The spawning of *A. pleuronectes* was observed all year round with peaks during the colder months at

temperatures ranging 24.6 to 26°C (Llana and Aprieto 1980; Del Norte 1986).

Asian moon scallop has a very wide distribution, being recorded from the Indo-Pacific countries like Ryuku Islands of southern Japan, China, Thailand, and Indonesia (Habe 1964). In the Philippines, *A. pleuronectes* has been reported to occur in Lingayen Gulf (Del Norte 1986) and Visayan Sea (Llana 1983). It is also found in Eastern Visayas and locally known as "tipay" (Cabacaba and Salamida 2017, unpublished).

In Eastern Visayas, *A. pleuronectes* is a commercially important species. The adductor muscles are basically traded for consumption and shells are utilized as raw materials for handicraft production. The fresh shell meat produced is sold to Metropolis such as Ormoc, Tacloban, and Cebu's seafood restaurants for 100-200 pesos per kilo and shells were utilized for decoration ornaments and souvenir items in the region. Because of increasing demand for scallops, trawl gears are used to maximize the collection (Cabacaba and Salamida 2017, unpublished).

Based on the study of Cabacaba and Salamida (2017, unpublished) conducted in Eastern Visayas, this species is primarily harvested through bottom trawling by the use of trawl gear or the "baby trawl" in commercial and municipal waters. Trawl gears are active gears that is characterized by the pursuit of the target species by towing or dredging. Bottom trawling and the frequent discarding of non-target sizes and species both of fish and non-fish species threatens the seabed ecosystem (FAO 2001). Likewise, high unregulated trawl fishing consequently leads to overfishing, depletion of the resource, and eventual loss of species diversity (Bobiles and Soliman 2018). Thus, this fishing practice is now banned by the amended Philippine Fisheries Code Republic Act 10654.

To provide an alternative solution about the banning of trawl gears, scallop aquaculture has been established to support production and sustainability of scallop stocks. In the Philippines, no farming attempt has been reported; however, interests about the scallop species *Amusium pleuronectes* have been previously observed. There are published works that have been reported and these were mainly about the population dynamics, spawning, breeding, and culture of the commodity (Llana 1983; Llana and Aprieto 1980; Del Norte 1988; Belda and Del Norte 1988; Chaitanawisuti and Menasveta 1992; Rice et al. 1994; Wang et al. 2009; Cabacaba and Salamida 2017, unpublished).

Recently, there has been an interest in the cultivation of *Amusium pleuronectes* because of its relatively fast growth and utilization of low food chain phytoplankton, detritus, and other suspended particulates (Rice et al. 1994). To address that interest, this study was conducted to determine the applicability of breeding and larval rearing of *A. pleuronectes* in Guiuan, Eastern Samar. The determination of conditioning methods, spawning technique, early development stages, and larval rearing are essential for the hatchery operation of *Amusium pleuronectes*. Therefore, this study also aims to contribute to the growing knowledge necessary for the hatchery rearing of commercially important scallops. Moreover, the technology on aquaculture of *Amusium plueronectes* will serve as a solution to the sustainability of scallop in the fishery industry as total implementation of the banning of trawl gears will be strengthened.

## 2. MATERIALS AND METHODS

This study was conducted at the Marine Fisheries Research and Development Center (MFRDC), Brgy. Sto. Niño, Guiuan, Eastern Samar. Materials and facilities used for the setup of experiments were composed of filtered seawater lines, conditioning tanks and plastic containers, aeration system, and a laboratory space equipped with a microscope for counting, monitoring, and data collection. For the larval rearing, the use of different size of sieves are necessary for the collection of larvae. The sieves vary in size from 40 µm-120 µm depending on the developmental stages of scallop.

### 2.1 Microalgal Culture

Microalgae *Tetraselmis sp.*, *Chaetoceros calcitrans*, and *Isochrysis galbana* were initially grown and collected at the Phycology laboratory of the Bureau of Fisheries and Aquatic Resources - Guiuan Marine Fisheries Development Center (BFAR-GMFDC), Guiuan, Eastern Samar. Cylindrical transparent tanks with 100-L capacity were used for the mass culture of microalgae. Starter culture (8 L) of microalgae *Tetraselmis sp.* *Chaetoceros calcitrans* and *Isochrysis galbana* from the phycology laboratory were scaled-up to 50 liters in an outdoor culture system at the hatchery prior to feeding of scallops and larvae. Mass cultured microalgae were ready to harvest after three consecutive days of culture.

## 2.2 Collection of *Amusium pleuronectes*

*Amusium pleuronectes* were collected at the Biliran Strait and Leyte Gulf (Figure 1) through the use of trawl gears. Monthly collection was done to secure enough number of scallops to be used in conditioning experiments and as broodstock for spawning. Scallops collected from the trawl gears were kept alive in plastic bags (8-10 scallops per bag) with approximately 2 liters of seawater and inflated to full capacity with pure oxygen. The plastic bags were placed in styrofoam fish transport boxes for over-land transport to the study site at the MFRDC. The scallops were evaluated to classify its gonad stages and determine its size. The size (shell length) of *Amusium pleuronectes* were measured

from the dorsal hinge to the ventral margin of the shell using a caliper with graduation of 0.01 mm. Gonad stages were classified by opening the valves wide enough to allow visual grading of the gonad without sacrificing the animals. Scallops identified as mature have bulgy/full, bright orange ovary, and cream-colored testes (Appendix B, Figure 8). Scallops in spawning stage have hollow “channels” on the surface of the gonad, which appear shrunken. Those that are in spent stage, their gonads appear dull, sunken, and flaccid. After the classification, mature scallops were used as broodstock and tested for experimental spawning technique trials, while non-mature scallops were subjected to experimental conditioning set-ups at the hatchery in MFRDC.

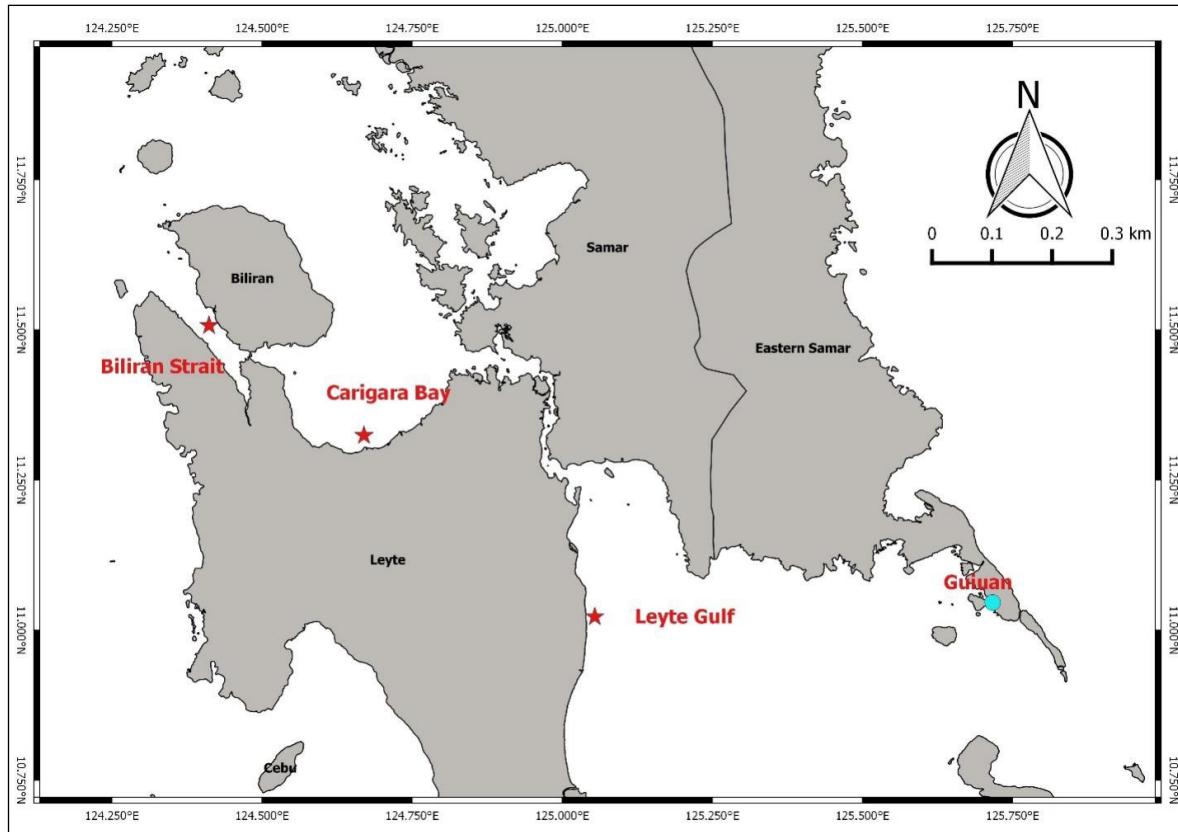


Figure 1. Collection sites of *Amusium pleuronectes* in Eastern Visayas.

## 2.3 Experimental set-ups on conditioning methods for *Amusium pleuronectes*

Difficulties in obtaining ripe broodstock from the wild populations in Leyte Gulf and Biliran Strait prompted the development of hatchery conditioning protocols. Conditioning methods such as holding

technique, holding temperatures, microalgal diets, and clearance rates of individual scallop favorable for survival were identified. Then, significant findings from the previously mentioned conditioning methods were applied to scallops maintained in the hatchery in order to assess its effectiveness in terms of growth, survival, and gonad development.

#### Experiment I: Different holding systems for *A. pleuronectes*.

The healthy stocks collected from the wild population were transported and kept in the hatchery to ensure suitable condition ready for spawning. To evaluate suitable holding techniques, experimental set-up included two holding systems: a.) Flow-through water system with muddy substrate; and b.) Flow-through water system without substrate. The substrate used prior to the experiment underwent a series of sun drying and washing to ensure minimal contamination and eliminate other organisms. A total of 48 *A. pleuronectes* with shell length of 31-52 mm from the same batch of collection were randomly chosen for the experiment. Scallops were kept in an aerated 100-L round flat-bottom plastic containers filled with filtered seawater and maintained at a temperature of 28-29°C and salinity of 34 ppt. Three replicates in each treatment group were set up containing eight scallops. The scallops were supplemented daily with 2 liters of either *I. galbana*, *C. calcitrans*, or *Tetraselmis sp.* every morning and afternoon. During feeding, flow-through were temporarily turned off for about an hour or until microalgae diets were consumed, to prevent the microalgae from escaping. Monitoring and maintenance involved cleaning and daily removal of fecal materials or dead scallops usually prior to feeding. After the eight-week period, assessment for the survival rate of *A. pleuronectes* was conducted.

#### Experiment II: Different holding water temperatures for *A. pleuronectes*.

Establishing the optimum temperature was also seen as a critical step in obtaining and ensuring survival of *A. pleuronectes* in the hatchery. In this study, different water temperatures of 26-27°C (ambient temperature at the collection site), 28-29°C (ambient temperature in Guiuan), and 30-31°C (above ambient temperature) were tested. The experimental temperature under 26-27°C was attained by a static water system. For the water temperature under 28-29°C, flow-through water system has been set-up, and the 30-31°C set-up was achieved by the use of a heating device. A total of 27 *A. pleuronectes* with shell length ranging 69-83 mm were used in the experiment. Prior to experimentation, visual grading of gonad stages of scallops was conducted in order to serve as a basis of possible gonadal development within

the period. Each temperature treatment contained 3 scallops which were held individually in a 10 L aquaria without substrate. The temperature treatments were triplicated, and the 10 L aquarium were placed inside a 1 x 1 x 0.33 m wooden tanks (water bath) for 8 weeks. The scallops were fed twice daily in the morning and afternoon with 2 L of *I. galbana* and *C. calcitrans* at 10,000-15,000 cell per ml. Then after every feeding, wooden tanks were covered with black net to regulate the desired temperatures and minimize activity of scallop due to exposure of light. After 8 weeks of maintenance, scallops were evaluated for survival rate and gonad changes.

#### Experiment III: Different microalgae diets for holding *A. pleuronectes*.

The experiment on the determination of clearance rate of scallops was conducted following the previous work of Rice et al. (1994); however, different microalgae diets were used in this study. Clearance rates of individual scallop per experimental diets were determined by monitoring depletion of microalgae species from the medium. The microalgae diets used in the experiment were *Isochrysis galbana*, *Tetraselmis sp.*, and *Chaetoceros calcitrans*.

A total of nine scallops with shell length of 60-70 mm were stocked in 10-L aerated static aquarium at 28-29°C. Prior to the experiment, all scallops were starved for 24 hours in order to fully digest all food item in their gut and allow the release as fecal materials. After 24 hours, feces and pseudofeces were removed while replacing old seawater with new filtered seawater. Then, total algal concentration of  $2.28 \times 10^9$  cells of microalgae (approximately 2 L per scallop) was added to the aquarium containing the scallops.

Monitoring of depletion of microalgae diets in the water was done by taking 1 mL water samples every 2 h, 4 h, 6 h, 8 h, 10 h and 24 h from each of the aquarium. These samples were brought to microscopy room and was counted using a hemacytometer. At the completion of experiment, fecal samples were collected and examined under microscope at 100x magnification to evaluate the degree of digestion of algal cells. Lastly, clearance rate was calculated by the equations of Jorgensen (1943) or Coughlin (1969) as cited by Rice et al. (1994), and expressed as mL per min or cells filtered per unit time:

$$F = V [\ln(C_0/C_t)/t]$$

where, F - the filtration rate in ml.hour<sup>-1</sup>  
 V - the volume of the experimental vessel in ml,  
 $\ln(C_0/C_t)$  - the natural log of the cell concentration at time zero divided by the concentration;  
 t - time in hours

#### Experiment IV: Conditioning of *A. pleuronectes*

Conditioning of *A. pleuronectes* was attempted using protocols based on the findings of previous experiments. Experimental animals used in the experiment were small scallops measuring 18-23 mm shell length. These scallops were placed in a 1-ton fiberglass tank filled with 250 liters filtered seawater and provided with moderate aeration. The tanks were covered with black net since scallops respond to light and vibration by closing their shell valves and drawing in their mantles. The set-ups were placed in a separate and isolated area to avoid frequent disturbances such as sound of the water pump and power blower engines. This was done to lessen the disturbance they receive and allow more time spent on feeding.

This experiment assessed the growth and survival of scallops using the significant results from the experimental conditioning methods (optimum water temperature, suitable algal concentration of microalgae given, and appropriate holding method in the hatchery). Specifically, the water temperature was maintained at 28-29°C. These scallops were fed twice daily to satiation at algal concentration of  $2.28 \times 10^9$  cells per scallop (approximately 2 liters of microalgal diet) as determined in the experiment of clearance rate. The best mixed algal species *Isochrysis galbana* and *Chaetoceros calcitrans* were given to scallops equally at 570,000 cells/ml. At monthly interval, scallops were assessed for growth and survival. The growth of the scallops were determined using average daily growth rate (ADGR) and specific growth rate (SGR) which can be calculated through the following formulae:

$$\text{ADGR} = \frac{\text{final length} - \text{initial length}}{\text{No. of days interval}}$$

$$\text{SGR} = \frac{\ln(\text{final length}) - \ln(\text{initial length})}{\text{No. of days interval}} \times 100$$

The survival rate was determined using the following formula:

$$\text{Survival rate} = \frac{\text{total number of individuals survived}}{\text{total no.of individuals initially stocked}} \times 100$$

#### 2.4 Spawning of *A. pleuronectes*

The selection of broodstock used for spawning trial should consider a well-formed shell with complete mantles extending to the shell margin and plump healthy-looking soft tissue (Appendix B, Figure 9). Larger scallops produce more eggs, thus a good size of shell length 80-90 mm were used. In this study, several spawning techniques were observed and tested using different methods such as food shock, thermal stimulation, sexual stimulation, and serotonin injection. On the other hand, observation of natural spawning for *A. pleuronectes* was also conducted at the hatchery during days of full or new moon.

##### a. Food Shock

The use of microalgae for food stimulation in induced spawning of bivalve species was tested based on the study of Breese and Robinsons (1981). In this study, eight hermaphroditic mature scallops (shell length 84-87 mm) were placed in a clear round tank filled with 20 L filtered seawater and provided with moderate aeration. *Amusium pleuronectes* were induced to spawn by adding excessive amount of microalgae to the holding tank. Twenty (20) liters of either *I. galbana* or *C. calcitrans* with a dense concentration of  $6.0 \times 10^9$  cells were given to the broodstock scallops. Scallops were bathed in the tanks with microalgae for 45 minutes. After 45 minutes, scallops were transferred to another clear round tank with 10 L filtered sea water for observation of possible spawning activity.

##### b. Thermal Stimulation

Based from the study of Belda and Del Norte (1988), thermal stimulation was done by raising the temperature of the water by 10°C from ambient temperature of about 27°C. However, results from this study was not successful. In this experiment modification was made, scallops were exposed to temperature cycles wherein water temperature from ambient 24°C was increased gradually up to 30°C over a period of 6 hours. Initially, *Amusium pleuronectes* were placed individually in 10 L aerated aquaria and preconditioned at 24°C for 24 hours.

After preconditioning, temperature cycling was done by gradual increase of the water temperature using a water heater. Once the temperature reached 30°C, the water heater was constantly set and maintained for 45 minutes. Within 45 minutes, scallops were observed for possible release of either sperm or egg. However, failure of spawning warranted further cycle of temperature adjustment. This was done by adding pre-cooled water into the container holding the broodstock scallop. Then, water temperature was decreased gradually from 30°C to 24°C at one degree per hour. Four to five temperature cycles was done until spawning or release of sperms and eggs was observed.

c. Sexual Stimulation

Sexual stimulation was accomplished by introducing freshly emitted spawn, strip, or extract of ripe gonad. Briefly, gonads of hermaphroditic scallops were dissected out and placed in a petri plate with 10 mL filtered sea water. Gonads were cut up into small chunks and was agitated to release the gametes. The sperm suspension or egg suspension was filtered at 60 µm to remove the large chunks of gonad and diluted into 1000 mL beaker. Five hundred (500) mL sperm suspension was added to 60 L capacity rectangular plastic containers filled with 50 L seawater containing ten (10) mature scallops. Waiting time of 4-5 hours was allotted for the observation of possible release of sperm or eggs.

d. Serotonin Injection

A total of five (5) mature scallops were selected for serotonin treatment. In each case, 0.4 mL of a 3mM solution was injected into the anterior portion of the abductor muscle and in the gonads of both sexes. Then, each scallop was placed in a white plastic basin filled with 10 L filtered seawater and moderate aeration. A maximum of 5 hours observation period was allotted for the surveillance of possible spawning activities after the injection of stimuli.

e. Natural Spawning

Observation of natural spawning was done based on the lunar phase. Timing of collection was conducted prior to the full moon phases or new moon phase of the month at Biliran Strait. Ripe scallops collected from the wild population were stocked in a plastic barrel filled with 100 L filtered seawater and moderate aeration in static water system. The setup

was fully covered with black net to ensure minimal disturbance. If spawning did not occur before or on full moon, continuation of observation was done in three consecutive days after the moon phase. The monitoring of spawning was done every day between 4 am to 5 am in the morning during which successful release of sperms and eggs occur. The fertilization was also indicated by the presence of foamy bubbles on the surface of water in the tank.

## 2.5 Embryonic and Larval development of *A. pleuronectes*

After successful fertilization, fertilized eggs were collected from the tank using a series of sieves piled from smaller mesh size to larger mesh size (40µm - 60µm - 120µm). Larvae were transferred into the 60 L incubation tank with minimal aeration. Prior to counting and collection of samples, an improvised plunger was used to mix the water in the incubation tank in order to obtain even distribution of fertilized eggs. The total fertilized egg count was estimated by taking 5 mL samples from the incubation tank. The fertilized eggs were then distributed into several 100 L capacity culture vessels. Each culture vessel was filled with 70 L aerated filtered seawater at stocking density of 500 larvae per liter. The temperature of the cultures ranged from 27°C to 28°C and salinity was maintained at 33 ppt.

The embryonic and larval development of *Amusium pleuronectes* was monitored daily in the laboratory. Samples from the culture were taken and examined under microscope at 100x magnification. Using digital camera, photographs of each larval stages were taken for the description of major features of the larval development of *A. pleuronectes* reared in the laboratory. A maximum of 10 samples of larvae were taken daily and measured using digital microscopy. The larvae were given with *I. galbana* at an initial concentration of 10,000 cells/ml once daily, and increased by 5,000 cells/ml on each succeeding day.

## 2.6 Larval Rearing: Survival *A. pleuronectes* larvae in different stocking density

The purpose of larviculture is to optimize rearing factors and increase production per unit in a limited water volume at minimal cost (Liu et al. 2006). In this study, experiment on stocking density was designed to determine optimal conditions giving the highest survival rates in hatchery culture of *Amusium pleuronectes* and produce healthy spats.

To determine the optimal larval density of the scallop *Amusium pleuronectes*, experiments with the stocking densities of 200, 300, 500, and 800 larvae/L were tested, which included the developmental stages from D-larvae to a one month settled spat. Experimental set-up were carried out in a 60-liter rectangular plastic containers without flow-through, and each treatment group comprised three replicates. During the rearing period, 50% of seawater was replenished everyday prior to feeding. Water temperature was at 28-29°C, while salinity was at 34 ppt with continuous supply of aeration. Larvae were fed with *Isochrysis galbana* every morning and afternoon daily. Concentration of *I. galbana* were proportionately increased with stocking density to supply larvae in each treatment with the same amounts of microalgal feed (Appendix A, Table 1.)

After one-month stocking, survival rate and shell length of spat were recorded. Larvae were sampled from the bottom of the rectangular plastic containers and survival rate was recorded as percentage of surviving individuals. It is important to handle the spats with care when collecting them since their shells break easily.

## 2.7 Data and Statistical Analysis

Data processing and analysis were conducted using MS Microsoft Excel 2013 and SPSS version 21 programs. Data were presented as means and standard error of the mean in graphs and tables. Significance testing were done using ANOVA and t-test, and Tukey test for post hoc test. Survival rate data were arcsine-transformed prior to statistical analysis. Significance levels for all analysis were set at 95% confidence interval.

## 3. RESULTS AND DISCUSSION

### 3.1 Different Holding Systems

Results have shown that in flow-through water system without substrate, scallops obtained higher survival rate ( $48.06 \pm 5.95\%$ ) compared to scallops maintained in flow-through water system with muddy substrate ( $45.49 \pm 3.38\%$ ) (Figure 2) but with no significant difference ( $p > 0.05$ ). This implies that either of the two treatments can be used in holding scallops at the hatchery. According to Helm et al. (2004), the basic methods for broodstock conditioning are much the same for all bivalves. Adults from the sea that were brought in to the hatchery were kept in a best

possible condition according to the characteristics of broodstock species. Clams and scallop species are normally buried in substrate at the natural habitat; thus it is maintained in hatchery provided with substrate. On the other hand, species such as oysters and mussels do not require a substrate therefore it is maintained in tanks without substrate.

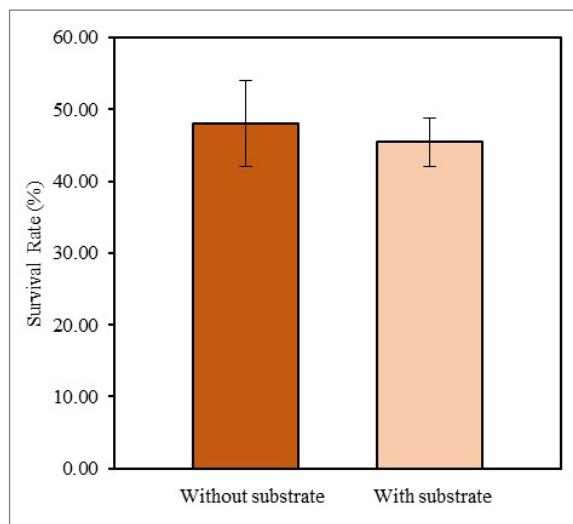


Figure 2. Mean survival rate of *A. pleuronectes* maintained in different holding techniques: flow-through water system with muddy substrate and flow-through water system without substrate.

However, in this study, holding scallops in flow-through water system without substrate has some advantages such as reduced physical handling of scallops, reduced labor demand, and reduced possible dependence on the use of disinfectants to improve water quality. The sediments also caused turbidity upon agitation. On top of all that, cleaning and siphoning fecal materials from the tanks without the toil of removing and washing the sediments daily was also convenient.

### 3.2 Different Holding Temperatures

Figure 3 shows the mean survival rate of *Amusium pleuronectes* maintained in different water temperatures. ANOVA results have shown that the survival rates of scallops were significantly different among different holding temperatures. *A. pleuronectes* maintained at 28°C-29°C obtained the highest survival ( $90 \pm 0.00\%$ ) while the lowest survival ( $48 \pm 6.48\%$ ) was observed in the temperature range of 30-31°C. Furthermore, post hoc test show that there was no significant difference observed between 26-27°C and 28-29°C holding temperatures.

Being in the tropics, little variation in seawater temperature and food availability is observed during the year. Thus, the temperature 30-31°C is an extreme temperature that caused low survival for *Amusium pleuronectes*. According to Chaitanawisuti and Menasveta (1992), 30-31°C is relatively higher from the ambient temperature (26°C-28°C) recommended for the tropical species *A. pleuronectes*. The increased temperature could have caused low survival in scallop due to energy losses associated with active behavior. *Amusium pleuronectes* are known to be among the most active swimming scallops (Morton 1980) and it is reasonable to expect that interactive energy losses occur in this species as well (Rice et al. 1994). Kinne (1970) suggested that the scallops should be conditioned by lowering the temperature to lower the metabolic activities, and energy is spent on feeding that promotes growth and gamete maturation. Thus, optimum holding temperature for *Amusium*

*pleuronectes* in the hatchery must not exceed 28-29°C and that temperatures range from 26°C-29°C, which resulted in relatively high survival, was the optimum water temperature in holding scallops in the hatchery.

### 3.3 Different Microalgal Diets for holding

#### *A. pleuronectes*

The experimental trial on the clearance rate of individual *Amusium pleuronectes* on the consumption of different species of microalgae is presented in Table 1. Clearance rates of scallops were observed to range from 278.74–446.99 mL·min<sup>-1</sup> for *Isochrysis galbana*, 267.47–401.21 mL·min<sup>-1</sup> for *Chaetoceros calcitrans*, and 69.58–74.50 mL·min<sup>-1</sup> for *Tetraselmis sp.* Moreover, results have shown that the clearance rate of *Isochrysis galbana* and *Chaetoceros calcitrans* was not statistically different at  $p>0.05$ .

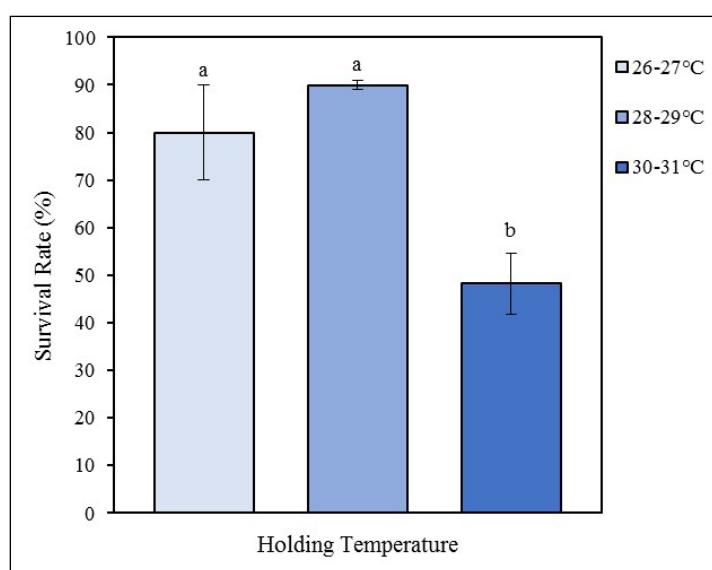


Figure 3. Mean survival rate (%) of *Amusium pleuronectes* maintained in different water temperatures: 26-27°C, 28-29°C, and 30-31°C. Note: Mean  $\pm$  SE of different water temperature with the same letters show no significant difference ( $p > 0.05$ ).

Table 1. Clearance rate of different microalgae at algal concentration of  $2.28 \times 10^9$  cells in individual *Amusium pleuronectes* (n=3)

Clearance Rate	<i>I. galbana</i>	<i>C. calcitrans</i>	<i>Tetraselmis sp.</i>
Mean clearance rate (ml per min.)	341.24	322.22	71
Range clearance rates (ml per min.)	278.74 – 446.99	267.47 – 401.21	69.58 – 74.50
Mean clearance rate (cells per min.)	7,388,888	7,388,888	1,583,333

The microalgae *I. galbana* and *C. calcitrans* were rapidly consumed compared with *Tetraselmis* sp. Scallops fed with *Isochrysis galbana* and *Chaetoceros calcitrans* have a clearance rate of 7,388,888 cells.  $\text{min}^{-1}$ , which was significantly higher compared to *Tetraselmis* sp. (Table 1). After 6 hours, the consumption of the algal cells differed significantly with the algal species *Isochrysis galbana*, *Chaetoceros calcitrans*, and *Tetraselmis* sp. (Figure 4). The number of algal cells consumed after 6-8 hours was greatest in *Isochrysis galbana* and *Chaetoceros calcitrans*, and low consumption of *Tetraselmis* sp., was observed for 24 hours. After 8-10 hours, microalgae species *Isochrysis galbana* and *Chaetoceros calcitrans* were almost consumed from the water column as indicated by clearer water in the aquaria, and their cell count was already negligible. The microscopic examination of fecal samples from scallops fed with *Tetraselmis* sp. revealed the presence of many undigested cells, but not in the case of *Isochrysis galbana* and *Chaetoceros calcitrans* sp.

The results in this study indicate that size

of food particles was a limiting factor that resulted in relatively poor consumption of microalgae *Tetraselmis* sp. Additionally, *Tetraselmis* sp. belongs to the chlorophyte microalgae, which is characterized by a thick cell wall or theca. Thus, poor digestion of *Tetraselmis* sp. may have resulted from difficulties encountered in digesting the theca of this alga (Epifanio 1979).

Moreover, according to Bricelj and Shumway (1991), the suggested size of food particles with effective retention is about 5-7  $\mu\text{m}$ . Likewise, poor retention of food particles with size below 5  $\mu\text{m}$  is due to the poor development of the latero-frontal ciliary tracts of scallops. Based from the previous work of Nell and O'Connor (1991), the average cell dimension of *Chaetoceros calcitrans* is 4 x 3.1  $\mu\text{m}$ , *Tetraselmis* sp. and *Isochrysis galbana* have 14 x 8  $\mu\text{m}$  and 4 x 3  $\mu\text{m}$ , respectively (Creswell 2010). Thus, inference of the present study is that *Isochrysis galbana* and *Chaetoceros calcitrans* can be used as microalgae diets, and *Tetraselmis* sp. may not be appropriate as feeding diets for *Amusium pleuroenectes*.

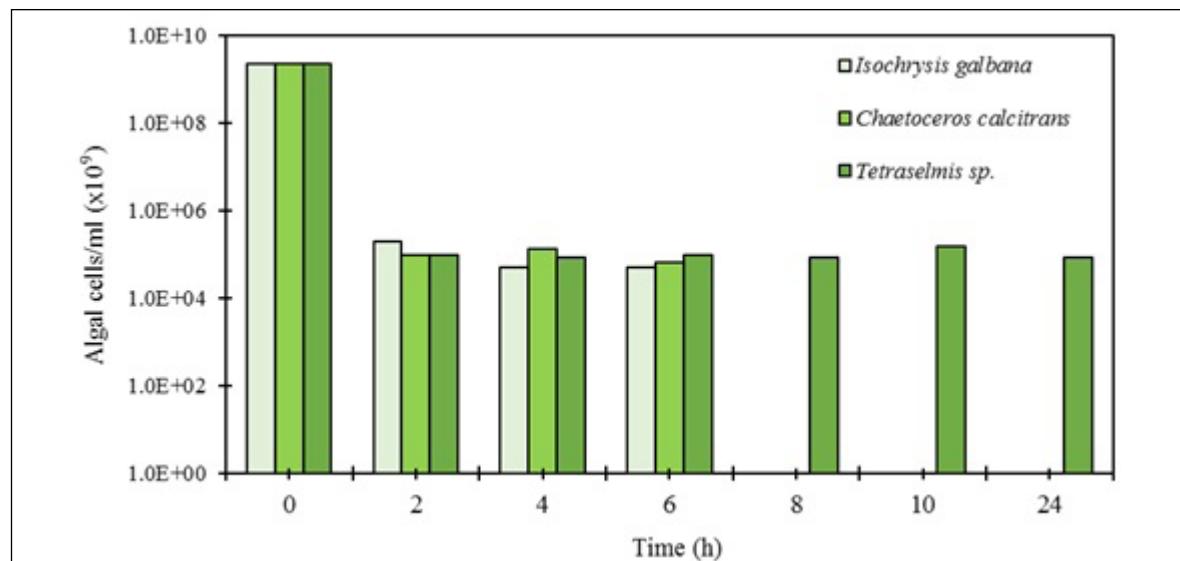


Figure 4. Average algal cells count of *Isochrysis galbana*, *Chaetoceros calcitrans*, and *Tetraselmis* sp. at total algal cell concentration of  $2.28 \times 10^9$  within 24 hours.

### 3.4 Conditioning of *A. pleuronectes*

Experimental conditioning of *Amusium pleuronectes* in the hatchery yielded high survival and growth rate under the following conditions: the setup was carried out in tanks without substrate, water temperature maintained at 28-29°C, and fed equally with *Isochrysis galbana* and *Chaetoceros calcitrans* total cell count of  $2.28 \times 10^9$  cells.

Figure 5 below shows the growth rate of *A. pleuronectes* in monthly interval. The highest growth rate was observed within four months at 35.7 mm shell length and survival rate of  $96.57 \pm 2.04\%$ . Growth parameters such as average daily growth rate (ADGR) and specific growth rate (SGR) was recorded at  $0.13 \pm 0.04 \text{ mm day}^{-1}$  and  $3.92 \pm 1.31\% \text{ d}^{-1}$  respectively. These results only illustrate the application of the significant conditions based on the previous

experiments conducted. The data have shown that these conditions (holding technique and microalgae diets and ratio) promote high survival and growth rate of *A. pleuronectes* maintained in the hatchery.

On the other hand, four months stocking of scallops in the hatchery did not guarantee rapid conditioning. Although important hatchery factors

such as holding techniques, diet, and ration were determined in this present study, the requirement time of conditioning the scallops in order to reach spawning readiness was not determined. Therefore, conditioning experiments on scallops already undergoing gametogenesis rather than conditioning with sexually undifferentiated scallops warrant future investigation.

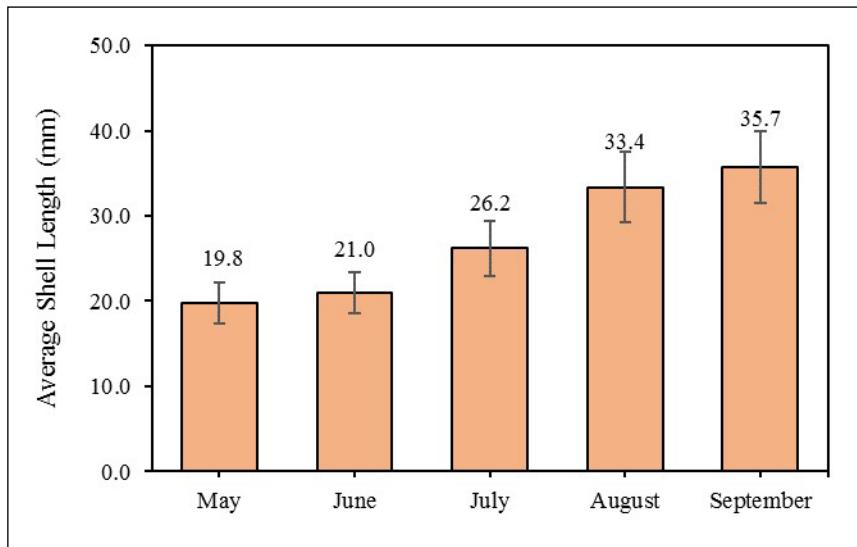


Figure 5. Average shell length (mm) of *Amusium pleuronectes* from May to September 2017

### 3.5 Spawning of *A. pleuronectes*

In this study, successful spawning of *Amusium pleuronectes* was observed naturally in the hatchery, while stimulation methods used such as food shock, thermal stimulation, sexual stimulation, and serotonin injection yielded unsuccessful release of eggs or sperms.

There were four separate natural spawning events from different batches of scallop

(Table 2). Spawning occurred either on, before, or after lunar phases and was easily noted according to the appearance of foamy bubbles on the water surface. Mature scallops exhibited synchronized spawning in every spawning trial. As shown in Table 2, a number of broodstock scallops partially released either sperm or egg while only one or two scallops fully spawned as evident by their empty gonads. The estimated number of fertilized eggs per spawning event ranged from 220,000-1.48 million.

Table 2. Summary of successful spawning of *Amusium pleuronectes* during April, August, and November 2017.

Date of Spawning	Lunar Phase	Total no. of scallop	No. of scallop fully spawned	Fertilized Egg Count
April 1, 2017	New moon	21	2	1,480,000
April 9, 2017	Full moon	19	2	450,000
August 6, 2017	Full moon	8	1	220,000
November 17, 2017	Full moon	9	2	775,000

Various stimuli can be applied to induce spawning of *Amusium pleuronectes*; however, this study demonstrated successful spawning through natural spawning in relation to the lunar cycle. According to Barber and Blake (1991), one of the parameters potentially triggering spawning includes lunar phase. Lunar phase influenced the fluctuation of tide. This phenomenon allows movement of water and steering of nutrients which are very favorable for larval dispersal, development, and survival. Being conditioned in the wild, mature scallops that were brought into the hatchery responded to the lunar cue, which allowed them to spawn even under controlled condition.

The previous work of Jimenez et al. (2011) was an evidence that bivalve species is capable of spawning naturally under controlled environment. The study reported successful natural spawning of *Lutraria* sp., an edible and commercially important bivalve collected in Taguines Lagoon, Camiguin, Philippines. Natural spawning under laboratory conditions was attained two months after broodstock conditioning and occurred either on the first quarter, full moon, or last quarter phase of the moon. Therefore, natural spawning as spawning method of *A. pleuronectes* in order to produce viable larvae in the hatchery would likely be successful.

Different induction methods resulted to unsuccessful spawning of either eggs or sperms. In this study, thermal stimulation was not effective in inducing *A. pleuronectes* to spawn. According to Belda and Del Norte (1988), it is possible that *A. pleuronectes* must be "cold-conditioned" first to be receptive to thermostimulation. The study of Young (1978) reported cold-conditioning of the tropical mussel *Perna viridis* before inducing it to spawn by thermostimulation was found effective. However, this study showed that cold conditioning the scallops at 24°C for 24 hours and gradually exposing to 30°C was still ineffective. Possibly, a sudden decrease of temperature from the ambient 24°C could effectively induce *A. pleuronectes* to spawn rather than sudden increase in temperature since the spawning peak for this species was observed to occur during colder months of the year at temperatures ranging from 24.6-26°C in the Philippines (Belda and Del Norte, 1988). This aspect also warrants future research undertaking.

Induced spawning of *Amusium pleuronectes* using microalgal feeds *Isochrysis galbana* and *Chaetoceros calcitrans* was also found ineffective. Perhaps, the concentration of *I. galbana* and *C. calcitrans* was not enough to induce *A. pleuronectes* to spawn. Also, the study was limited from conducting

further verificatory trials on this aspect. Nevertheless, the study of Breese and Robinson (1981) support this claim since concentration of marine algae used was between 2 to 2.5 million cells/ml and found effective in inducing the razor clam *Siliqua patula* to spawn. Aji (2011) also suggested that the microalgae diet is good for conditioning bivalves and stated that the reproductive performance, gonad maturation, and the quality of broodstock are mainly influenced by food availability. Martinez et al. (2000) also reported gonadal recovery and conditioning of the *Argopecten pupuratus* fed by microalgae. *Ostrea edulis* produced more broods of larvae when fed with a mixture of microalgae (Helm et al. 1973).

In this study, sexual stimulation resulted no eggs or sperms emitted in all scallops tested while in the study of Belda and Del Norte (1988), the method was successful but not for release of eggs. Moreover, the study of Barber and Blake (1991) reported effective stimulation of spawning in *C. varia* using the combination of thermal stimulation with extract of gametes. The researchers of this present study speculate that the induction time and internal physiological conditions were possibly not met, hence, the failure of spawning.

The use of serotonin injection as spawning method was also ineffective. According to Aji (2011), serotonin injection is mostly used and effective to giant clams and some scallop species. Gibbons and Castagna (1984), reported six bivalve species that showed success in induced spawning using serotonin injection. These are the surf clam *Spisula solidissima*, the bay scallop *Argopecten irradians*, American oyster *Crassostrea virginica*, ribbed mussel *Geukensia demissa*, the ocean quahog *Arctica islandica*, the hard clam *Mercenaria mercenaria*, and *Mactra chinensis* that have injected by serotonin in their foot also spawn (Fong et al. 1996).

Moreover, some scallops in this present study expired after serotonin injection, probably because of stress. According to Aji (2011), handling should be carefully done so as not to puncture any visceral organs of the scallop which could lead to mortality.

### 3.6 Embryonic and Larval Development of *Amusium pleuronectes*

The description and sequence of development after fertilization of *A. pleuronectes* larvae is presented in Figure 6 and Table 2. Development of *A. pleuronectes* larvae is similar to that as seen with other pectinids (Belda and Del Norte 1988; and Wang et al. 2009).

Fertilized eggs of *A. pleuronectes* appeared

Table 2. Summary of the larval development of *Amusium pleuronectes* held at the MFRDC hatchery, Guiuan, Eastern Samar.

Stage	Age	Size ( $\mu\text{m}$ )
Fertilized egg	0	54.2 - 62.57
Trochophore	9 hrs.	59.08 - 84.4
D-veliger	24 hrs.	120.37 - 157.07
Umbone stage	7 days	135.45 - 173.36
Pedi-veliger	9 days	160.63 - 375.29
Spat	16 days	312.41 - 509.48
4-month old spat	120 days	4 - 10 mm

spherical and dark-colored, and the egg diameter ranged from 57-79  $\mu\text{m}$ . Embryos were left undisturbed for the first few hours. During this period, repeated cleavages led to the formation of the spherical blastula. Timing varies among species and was not determined for *Amusium pleuronectes*, but was determined for related species, *Aequipecten irradians concentricus*, to be of five hours and fifteen minutes (Sastry 1963). After nine hours, top-shaped trochophore appeared. Trochophore larvae were characterized with its apical rounded shape end and cilia.

Complete development of a straight-hinge veliger appeared in less than 24 hours and is characterized by its D shape. It has two valves, visible organs, and a velum. The velum is ciliated along its outer margin, and enables the larva to swim and maintain itself in the water column. The D-veliger shell length ranged from 120.37-157.07  $\mu\text{m}$ .

Development of the early umbone stage reached five days after fertilization. The shape of the D-larvae gradually changed as it developed. There was a slight reduction in the hinge-length with the extension of umbones over the hinge region. At seventh day, the umbo become well developed, appearing as a conspicuous, broadly rounded knob, which ranged from 135.45-173.36  $\mu\text{m}$  in size. On the ninth day, pedi-veligers were seen in the culture. Pedi-veliger larvae developed a functional foot, which can extend when brought into contact with the substrate. The shell length of pedi-veliger that was observed in the laboratory ranged from 160.63-375.29  $\mu\text{m}$ . On the 16th day, spats appeared at the bottom of the tanks and can be seen visibly as minute fan-like, white colored shells. The shell length of these spats ranged from 312.41-509.48  $\mu\text{m}$  and have foot used for crawling at the bottom of the tank.

Different batches of larvae from different spawning events were monitored, and the development of larvae varies in each spawning. Spats produced from the successful spawning lasted until four months in the hatchery with shell length ranged from 4 mm to 10 mm, while other batches of larvae were until pedi-veliger stage only or some died as early as D-larvae. Therefore, further study and experimentation under improved conditions is necessary and crucial. Factors like stocking density, rearing temperature, and microalgae diets affect optimal growth, survival, and development of *Amusium pleuronectes* larvae under control conditions are still for investigation.

### 3.7 Larval Rearing

The majority of larvae in every treatment, except for 800 larvae/L stocking density, developed into spat after one month. Mass mortality was observed in 800 larvae/L on day 18 pedi-veliger stage. The survival rates ranged from  $0.00 \pm 0.00\%$  to  $0.04 \pm 0.03\%$  (Figure 7) and significant differences was observed among 200 larvae/L, 300 larvae/L, and 500 larvae/L ( $p < 0.05$ ).

High mortality in larvae may be due to poor water quality or disease. This has been observed in the setup for 800 larvae/L stocking density, wherein contamination of protozoans was seen. This was also similar in the previous work of Avila et al. (1997) where the stocking density of *H. crassicornis* larvae reared at 0.5-4 individuals/mL showed decreased survival but a density of 15 larva/mL resulted in larval mortality. Moreover, according to Orensanz et al. (1991), the decreased survival rate at high densities is possibly due to food and oxygen depletion, predation, or other environmental stresses.

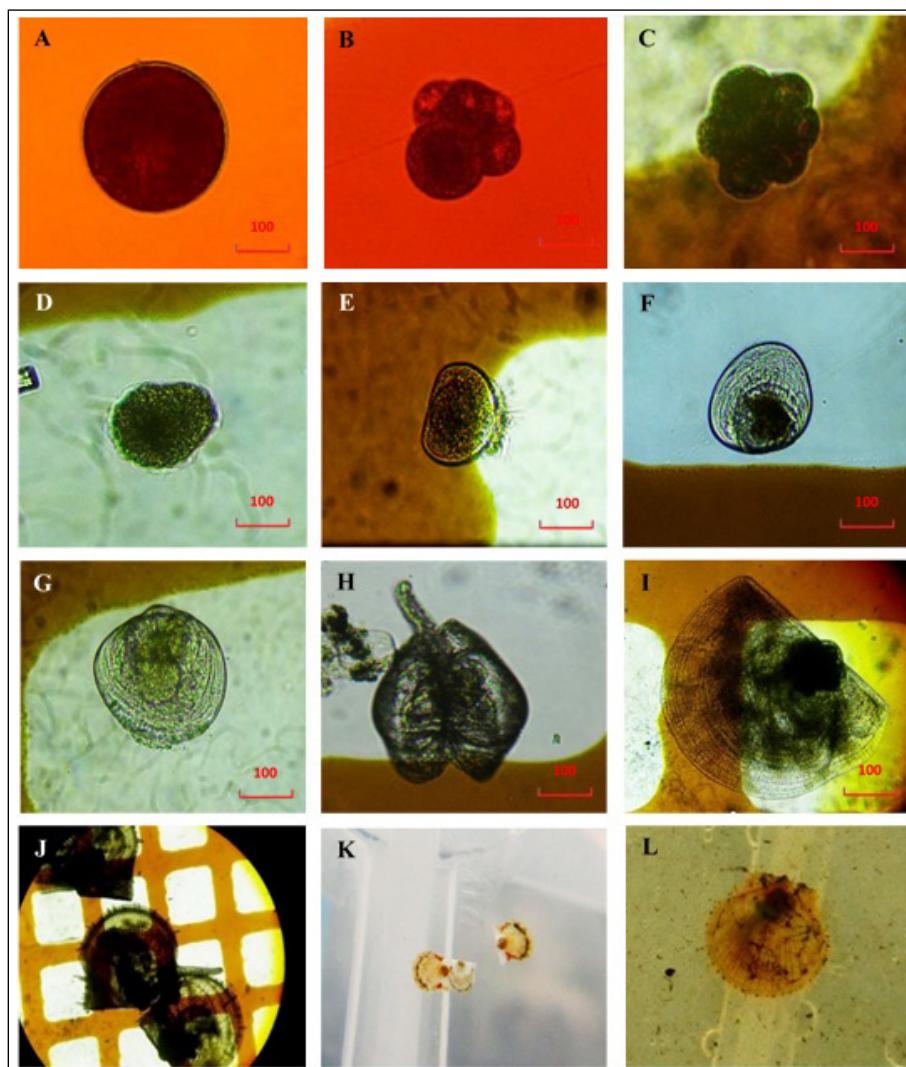


Figure 6. Embryonic and larval development of *Amusium pleuronectes*: (A) fertilized egg with the expulsion of polar body; (B) 4-cell embryo; (C) multi-cell stage; (D) trocophore larva; (E) Straight-hinge larva; (F) eyespot larva or the umbo stage; (G) early pediveliger larva; (H) hinge of pediveliger stage larva; (I) spat on day 16; (J) one-month old spat; (K) two-month old spat; and (L) 10 mm 4-month old spat. Scale bar: 100  $\mu$ m

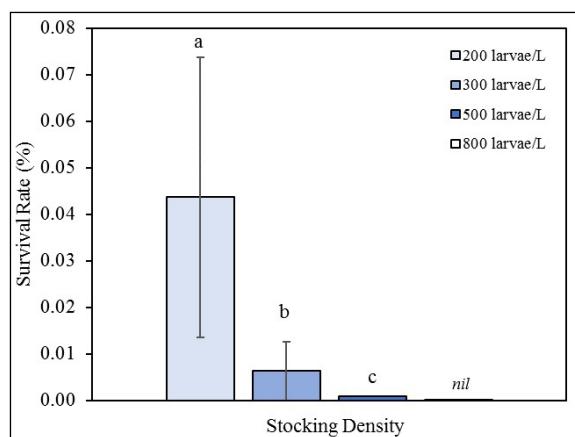


Figure 7. Mean survival rate of *Amusium pleuronectes* larvae at different stocking densities. Note: Mean ( $\pm$  SE) values of different stocking densities with different letters show significant difference ( $p < 0.05$ ).

In this study, algal concentrations were adjusted accordingly with different larval densities to ensure larvae received an equal diet. Thus, food is not a limiting factor in the survival of larvae. The mass mortality of larvae in 800 larvae/L stocking density could be possibly due to abrupt sinking of larvae. Unremoved dead larvae could contribute to the contamination of protozoans and build-up of bacteria in the rearing container. Therefore, water exchange management and other factors were taken into strict consideration in rearing the larvae. Being in static water system, culture medium must be renewed to prevent build-up of bacteria or other metabolites. The experimental densities tested in the present study cannot conclude or recommend a density-dependent survival rate. Thus, it is suggested that in laboratory conditions, water treatment used in the experiment should be considered such as sand filtered, UV treatedm and chlorinated seawater.

#### 4. CONCLUSION

Generally, preliminary data and information were presented in the attempt of breeding and larval rearing of *Amusium pleuronectes* in Guiuan, Eastern Samar. These include broodstock conditioning, spawning technique, larval development stage, and larval rearing. *A. pleuronectes* is best conditioned in a flow-through water system without substrate at water temperature of 26-29°C. The suitable microalgal diets were *Isochrysis galbana* and *Chaetoceros calcitrans* at 7,388,888 cell.min<sup>-1</sup> given twice daily. These promoted higher survival of scallop in the hatchery, but required time of conditioning to reach spawning readiness is still unknown. Moreover, *A. pleuronectes* spawn naturally, and the subsequent development of the larvae under laboratory condition was recorded up to 4-month old juveniles with very low survival rate. Further studies on the induction of spawning is warranted since the present study was hindered with limited number of experimental trials. Moreover, different aspects may also be considered in the future to improve larval rearing techniques.

#### 5. ACKNOWLEDGMENT

The authors are grateful for the support of the National Fisheries Research and Development Institute and the technical assistance of the staff of Guiuan Marine Fisheries Development Center (GMFDC) and Guiuan Marine Research Development Center (GMRDC), including Mr. Mheljay D. Burlaza, Clifford

O. Relator, Michael Gayoso, Alicia Lacdo-o, and Denis R. Salameda. Also, the authors extend their sincere gratitude to the agriculture technicians of the Local Government Units of Biliran, Biliran and Mayorga, Leyte for their logistic help.

#### 6. REFERENCES

- Aji LP. 2011. Review: Spawning Induction in Bivalve. *Jurnal Penelitian Sains*, Vol. 14, p. 33-36.
- Avila C, Grenier S, Tamse CT, Kuzirian AM. 1997. Biological factors affecting larval growth in the nudibranch mollusk *Hermisenda crassicornis* (Eschscholtz, 1831). *J. Exp. Mar. Biol. Ecol.* 218: 243-262.
- Barber BJ, Blake NJ. 1991. Reproductive Physiology, In: S.E. Shumway (Ed.), *Scallops: Biology, Ecology and Aquaculture*. Elsevier, Amsterdam, pp. 394-407.
- Belda CA, Del Norte AGC. 1988. Notes on the Induced Spawning and Larval rearing of the Asian Moon Scallop, *Amusium pleuronectes* (Linne), in the Laboratory. *Aquaculture*. Vol. 72, pp. 173-179.
- Breese WP, Robinson A. 1981. Razor clams, *Siliqua patula* (Dixon): Gonadal development, induced spawning and larval rearing. *Aquaculture*. 22: 27-33.
- Bricelj VM, Shumway CE. 1991. Physiology: Energy Acquisition and Utilisation. In: SE. Shumway (Editor), *Scallops: Biology, Ecology and Aquaculture*. Elsevier, Amsterdam. pp. 305-346.
- Cabacaba NS, Salamida MT. 2017. Distribution, Abundance and Reproductive Biology of Asian moon Scallop, *Amusium pleuronectes*, in Eastern Visayas Philippines (unpublished data).
- Chaitanawisuti N, Menasveta P. 1992. Preliminary studies on breeding and larval rearing of the Asian moon scallop (*Amusium pleuronectes*). *J. Aquaculture Trop.*, 7: 205-218.
- Creswell L. 2010. Phytoplankton Culture for Aquaculture Feed. Southern Regional Aquaculture Center, No. 5004.

- Del Norte AGC. 1988. Some aspects of the growth, recruitment, mortality and reproduction of the Asian moon scallop *Amusium pleuronectes* (Linne) in Lingayen Gulf, Philippines. *Ophelia.* 29(2): 153-168. DOI: 10.1080/00785326.1988.10430826.
- Del Norte AGC. 1986. Some aspects of the growth, recruitment, mortality and reproduction of the Asian moon Scallop *Amusium pleuronectes* (Linne) in Lingayen Gulf, Philippines. Master's Thesis, University of The Philippines (Diliman), 114 p.
- Epifanio CE. 1979. Growth in bivalve molluscs: nutritional effects of two or more species of algae in diets fed to the American oyster *Crassostrea virginica* (Gmelin) and the hard clam *Mercenaria* CL. *Aquaculture.* 18: 1-12.
- FAO. 2001. Fishing Gear types. Bottom pair trawls. Technology Facts Sheets. FAO Fisheries and Aquaculture Department [internet]. Rome. Updated 13 September 2001. [Cited 28 September 2018]. <http://www.fao.org/fishery/>
- Fong PP, Deguchi R, Kyozuka K. 1996. Serotonergic ligands induce spawning but not oocyte maturation in the bivalve Mactra chinesis from central Japan. *Biol. Bull.* Vol. 191, p. 27-32.
- Gabral-Llana ME. 1980. A Contribution to the Biology of the Asian moon scallop, *Amusium pleuronectes* (Linnaeus, 1758). Master's Thesis, University of The Philippines.
- Gibbons MC, Castagna M. 1984. Serotonin as an inducer of spawning in six bivalve species. *Aquaculture* 40: 189-190.
- Habe T. 1964. Notes on the species of the genus *Amusium* (Mollusca). - *Bull. natn. Sci. Mus. Tokyo* 7(1): 1-5.
- Helm MM, Bourne N, Lovatelli A. 2004. Hatchery culture of bivalves. A practical manual. FAO Fisheries Technical Paper. No. 471. Rome, FAO. 177 p.
- Helm MM, Holland DL, Stephenson RR. 1973. The effect of supplementary algal feeding of a hatchery breeding stock of *Ostrea edulis* L. on larval vigour. *J. Mar. Biol. Assoc.UK* 53. pp. 673-684.
- Jimenez CR, Dejarme HE, Jimenez JU, Gaid RD. 2011. Habitat Characteristics, Spawning, Relative Fecundity and Larval Development of *Lutraria* sp. (Bivalvia: Mactridae). *Journal of Environment and Aquatic Resources.* 2: (11-20).
- Kinne O. 1970. Temperature - animals, invertebrates. In: O. Kinne (Ed.). *Marine Ecology, a Comparative Treatise on Life in Oceans and Coastal Waters Environmental Factors.* Wiley-Interscience. New York. Volume 1. pp. 821-995.
- Liu B, Dong B, Tang B, Zhang T, Xiang Z. 2006. Effect of stocking density on grow settlement and survival of clam larvae, *Meretrix meretrix*. *Aquaculture* 258: 344-349.
- Llana MEG. 1983. Size composition, occurrence, distribution and abundance of scallops in the Visayan Sea. – *Philipp. J. Fish.* 16(2): 75-94.
- Llana MEG, Aprieto VL. 1980. Reproductive biology of the Asian moon scallop *Amusium pleuronectes*. *Fish. Res. J. Philipp.*, 2:1-10.
- Martinez G, Aguilera C, Mettifogo L. 2000. Interactive effects of diet and temperature on reproductive conditioning of *Argopecten purpuratus* broodstock. *Aquaculture.* 183: 149-159.
- Morton B. 1980. Swimming in *Amusium pleuronectes* (Bivalvia: Pectinidae). *Journal of Zoology* 190: 375-404.
- Nell JA, O'Connor WA. 1991. The evaluation of fresh algae and stored algal concentrates as a food source for Sydney rocks, *Saccostrea commercialis* (Iradale and Roughley) larvae. *Aquaculture.* 99: 277-284.
- Orensanz JM, Parma AM, Iribarne OO. 1991. Population dynamics and management of natural stocks. In: Shumway, S.E. (Ed.), *Scallops: Biology, Ecology and Aquaculture.* Elsevier, New York, pp. 625-713.

- Rice MA, Rheault RR, Perez MS, Perez VV. 1994. Experimental Culture and Particle Filtration by Asian Moon Scallops, *Amusium pleuronectes*. Asian Fisheries Science 7: 179-185.
- Sastray AN. 1963. Reproduction of the bay scallop, *Aequipecten irradians* Lamarck. Biol. Bull (Woods Hole) 125: 146-153.
- Wang Y, Ye L, Yang Q, Chen X, Wen W, Wu K. 2009. A preliminary research on artificial breeding of the Asian moon scallops *Amusium pleuronectes*. South China Fisheries Sciences 5(1): 36-41.
- Young AL. 1978. Larval and post-larval biology of the tropical green mussel *Smaragdinus Chemnitz*. Master's Thesis, University of The Philippines (Diliman), 114 p.
-

## APPENDIX A

Table 3. Daily food ration for the larvae at different stocking densities during the experimental period.

Day No.	Daily algal ration (x 104 cells/mL)			
	200 larvae per Liter	300 larvae per Liter	500 larvae per Liter	800 larvae per Liter
Day 0	1.0	2.0	4.0	8.0
Day 1	2.0	4.0	8.0	16.0
Day 2	3.0	6.0	12.0	24.0
Day 3	4.0	8.0	16.0	32.0
Day 4	5.0	10.0	20.0	40.0
Day 5-30	6.0	12.0	24.0	48.0

## APPENDIX B

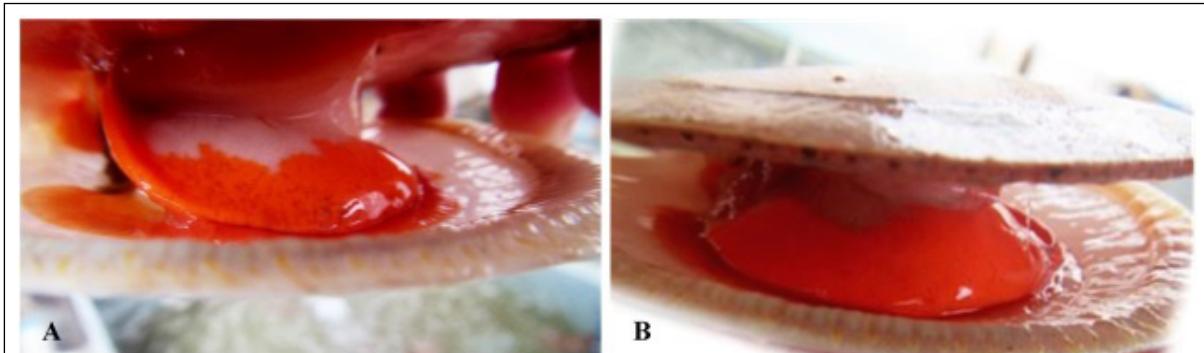


Figure 8. Gonad of Asian moon scallop reveals a bright orange ovary and cream-colored testes. A) Partially spawned gonads; B) Gonad in its mature stage



Figure 9. Asian moon scallop with well-formed shells and mantles extending to the shell margin and shell length ranged from 80-90 mm.

# Hatchery-based seed production of the Japanese scallop, *Mizuhopecten yessoensis*



**Cover photographs:**

Semi-continuous batch culture vessels of microalgae (top left); Yesso scallop D-larvae (top centre); conical tanks used for flow-through rearing of scallop larvae (top right); adult Yesso scallop specimens ready to spawn (bottom left); and Yesso scallop seed (bottom right) (Courtesy of Samia Sarkis).

# Hatchery-based seed production of the Japanese scallop, *Mizuhopecten yessoensis*

FAO  
FISHERIES AND  
AQUACULTURE  
TECHNICAL  
PAPER

683

**Samia Sarkis**  
Living Reefs Foundation

Edited by  
**Alessandro Lovatelli**  
Food and Agriculture Organization of the United Nations

Required citation:

Sarkis, S. and Lovatelli, A. (ed.). 2022. *Hatchery-based seed production of the Japanese scallop, Mizuhopecten yessoensis*. FAO Fisheries and Aquaculture Technical Paper No. 683. Rome, FAO. <https://doi.org/10.4060/cc0535en>

The designations employed and the presentation of material in this information product do not imply the expression of any opinion whatsoever on the part of the Food and Agriculture Organization of the United Nations (FAO) concerning the legal or development status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. The mention of specific companies or products of manufacturers, whether or not these have been patented, does not imply that these have been endorsed or recommended by FAO in preference to others of a similar nature that are not mentioned.

The views expressed in this information product are those of the author(s) and do not necessarily reflect the views or policies of FAO.

ISBN 978-92-5-136411-6

© FAO, 2022



Some rights reserved. This work is made available under the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 IGO licence (CC BY-NC-SA 3.0 IGO; <https://creativecommons.org/licenses/by-nc-sa/3.0/igo/legalcode>).

Under the terms of this licence, this work may be copied, redistributed and adapted for non-commercial purposes, provided that the work is appropriately cited. In any use of this work, there should be no suggestion that FAO endorses any specific organization, products or services. The use of the FAO logo is not permitted. If the work is adapted, then it must be licensed under the same or equivalent Creative Commons licence. If a translation of this work is created, it must include the following disclaimer along with the required citation: "This translation was not created by the Food and Agriculture Organization of the United Nations (FAO). FAO is not responsible for the content or accuracy of this translation. The original [Language] edition shall be the authoritative edition."

Disputes arising under the licence that cannot be settled amicably will be resolved by mediation and arbitration as described in Article 8 of the licence except as otherwise provided herein. The applicable mediation rules will be the mediation rules of the World Intellectual Property Organization <http://www.wipo.int/amc/en/mediation/rules> and any arbitration will be conducted in accordance with the Arbitration Rules of the United Nations Commission on International Trade Law (UNCITRAL).

**Third-party materials.** Users wishing to reuse material from this work that is attributed to a third party, such as tables, figures or images, are responsible for determining whether permission is needed for that reuse and for obtaining permission from the copyright holder. The risk of claims resulting from infringement of any third-party-owned component in the work rests solely with the user.

**Sales, rights and licensing.** FAO information products are available on the FAO website ([www.fao.org/publications](http://www.fao.org/publications)) and can be purchased through [publications-sales@fao.org](mailto:publications-sales@fao.org). Requests for commercial use should be submitted via: [www.fao.org/contact-us/licence-request](http://www.fao.org/contact-us/licence-request). Queries regarding rights and licensing should be submitted to: [copyright@fao.org](mailto:copyright@fao.org).

# Preparation of this document

This manual focuses on hatchery seed production of the Japanese or Yesso scallop, *Mizuhopecten yessoensis*, and is intended as a practical compilation of techniques for all stages of seed culture. Scallops are a high value lower trophic marine bivalve species with minimal impacts to the natural environment when cultured. Producing this species in a consistent and sustainable manner is best achieved through hatchery seed production.

The information presented and the techniques described are a result of scientifically-based experimental studies on the culture of several scallop species tested at pilot-scale and implemented at commercial-scale for the Japanese scallop in various parts of the world. The manual also discusses the transfer of seed out of the nursery and methodologies for transport to the farm sites.

This manual differs from others in that it pertains specifically to the Japanese scallop, a species with a proven potential for commercial culture, and provides a range of hatchery methodologies including low and high technologies applicable to small and large-scale operations.

The overarching goal for the publication of this manual is to contribute to the growth of aquaculture worldwide and improve its performance in the production of nutritional food as well as export products to generate foreign exchange. The larger sized Japanese scallop yields one of the most sought-after shellfish food products; by lending itself to hatchery seed production and suspended farm culture of market-sized animals, it is most likely one of the best suited species of scallops to commercial aquaculture and one of the lower trophic species with an impactful contribution to global aquaculture growth.

This document is one of three technical guides on shellfish culture produced as part of a project funded and implemented by the Food and Agriculture Organization of the United Nations (TCP/DRK/3803) in enhancing coastal livelihoods and food security in the Democratic People's Republic of Korea.

## Abstract

This guide is intended as a standalone practical manual for the culture of the Japanese or Yesso scallop, *Mizuhopecten yessoensis*. It is written for hatchery staff as a reference for daily operating procedures and for developing a site-specific and resource-specific seed production strategy. To that end, the whole production cycle is addressed, from broodstock conditioning to transport of seed to the farm. It is the aquaculturist's decision as to whether all stages are required to achieve the target production in a given site and hatchery facility. Standard and more recent emerging techniques are included where possible, for the equal benefit of low and high technological operations.

The manual starts with a brief overview of the anatomy and morphology of the scallop and describes the main organs of the adult specimen and the stages of its life cycle; the anticipated development time between each stage throughout its culture is added for the aquaculturist's benefit. This is followed by a chapter on the culture of live microalgae for food; different approaches to culturing large-scale microalgae are given, including traditional batch culture to the more recent newly designed photobioreactors. Protocols are given from stock to intermediate microalgal cultures for the inoculation of large-scale vessels. The integration of probiotic bacteria as an alternative to standard antimicrobial drugs is described in a separate chapter; this is a critical component of this manual as it is a current and important shift in sustaining optimal larval and spat performance. The need for biosecurity in a full cycle hatchery operation is emphasized and conceptually illustrated in Chapter 4; as much has been written on the design of bivalve hatcheries, the reader is directed to further readings for details.

Culture protocols for scallops start in Chapter 5 with the holding and conditioning of broodstock; assessments of the gametogenic stage, the manipulation of holding temperature to maintain and/or enhance gametogenesis and food requirements are all discussed to ensure the supply of broodstock for spawning when needed. Larval culture is one of the longest chapter of this manual and describes rearing in both static and flow-through systems with the expected growth and survival rate for the Yesso scallop spat. Post-larval culture in the nursery chapter is divided into early post-set up to Day-14, rearing of 1 mm spat and raising seed up to 5 mm or more in a land-based environment; as for larvae, expected performance for the Japanese scallop is given based on real-life experience. The final chapter discusses different strategies affecting the time and size at which seed are transferred out of the nursery facility to either intermediate outdoor nurseries (such as ponds) or to the farm sites. Considerations to be taken into account when selecting a strategy are given. The alternative "remote setting" approach, which bypasses the land-based nursery stage, is also discussed; this is especially useful to large-scale aquaculture operations.

The manual ends with a list of suggested readings providing further details on procedures at various stages, and appendices of working templates for recording data by hatchery staff.

**Keywords:** Japanese scallop, hatchery, seed production, broodstock conditioning, remote setting, nursery, seed transport

# Contents

Preparation of this document	iii
Abstract	iv
Acknowledgements	xi
Acronyms and abbreviations	xii
Conversions	xiii
<b>1. The Japanese scallop</b>	<b>1</b>
1.1 Behaviour and distribution	1
1.2 Morphology and anatomy	1
1.3 Life cycle	3
<b>2. Microalgal culture</b>	<b>7</b>
2.1 Microalgal culture overview	8
2.2 Microalgal protocols	10
2.2.1 Protocol: Daily algal checks	11
2.2.2 Protocol: Inoculating and maintaining starter algal cultures	12
2.2.3 Protocol: Counting algae and estimating algal culture density	14
<b>3. Probiotic bacteria culture</b>	<b>15</b>
3.1 Probiotic bacteria culture equipment	16
3.2 Probiotic bacteria culture protocols	17
3.2.1 Protocol: Preparing thiosulfate citrate bile salts sucrose (TCBS) agar plates	18
3.2.2 Protocol: Preparing marine broth for probiotic bacteria cultures	19
3.2.3 Protocol: Culturing probiotic bacteria – seed culture to starter culture	20
3.2.4 Protocol: Streak plate method to identify <i>Vibrio</i> or bacteria in a culture	21
3.2.5 Protocol: Preparing marine agar plates for assessing bacterial counts	22
3.2.6 Protocol: Determining colony-forming units in bacteria cultures	23
3.3 Co-cultivation of probiotic bacteria and microalgae	24
3.3.1 Protocol: Scaling up probiotic bacteria culture, harvesting and co-culturing with microalgae	26
3.3.2 Protocol: Determining bacteria counts in co-cultures	28
3.3.3 Notes on identifying probiotic bacteria colonies	28
3.4 Integrating probiotic bacteria to larval and post-larval culture systems	30
<b>4. Hatchery facility layout</b>	<b>31</b>
4.1 Seawater treatment per sector	31
<b>5. Broodstock holding and conditioning</b>	<b>33</b>
5.1 Reproductive cycle overview	34
5.1.1 Determining gonadosomatic indices	34
5.1.2 Visual assessment	34
5.1.3 Protocol: Determining gonadic and muscle indices	36
5.1.4 Protocol: Assessing reproductive status visually	39
5.2 Holding broodstock	39
5.3 Conditioning broodstock	41
5.3.1 Overview of temperature cycles for broodstock conditioning	41

5.4 Feeding broodstock	43
5.4.1 Protocol: Calculating algal food ration for broodstock	45
5.5 Spawning induction	46
5.5.1 Protocol: Spawning Japanese scallop in saltwater table and in large round larval tanks	49
<b>6. Larval rearing</b>	<b>53</b>
6.1 General larval rearing procedures	54
6.1.1 Protocol: Making sieves and banjos for larval and spat collection	55
6.1.2 Protocol: Takedown of larval tanks for water change	59
6.1.3 Larval tank aeration	61
6.1.4 Larval food ration and distribution	61
6.1.5 Use of antibiotics and probiotics	64
6.2 Larval growth and survival	64
6.2.1 Protocol: Counting and measuring larvae	65
6.3 Grading larvae	67
6.4 Setting pediveligers	70
6.4.1 Raceway setting	71
6.4.2 Protocol: Setting and rearing mature Yesso scallops in a semi-recirculated raceway system from Day-0 to Day-14 after set	73
6.4.3 Tank setting	73
6.4.4 Preparing a tank for setting	74
6.4.5 Protocol: Assembling an airlift system for nursery tanks	74
6.4.6 Protocol: Grading and setting larvae for round nursery tank system	77
<b>7. Nursery</b>	<b>79</b>
7.1 Seawater system for early post-set nursery phase	79
7.1.1 Raceway set: keeping sieves clean from Day-0 to Day-14	79
7.1.2 Raceway set: growing spat Day-14 to 2+ mm seed	80
7.1.3 Tank set: water exchange and feed from Day-0 to Day-14	80
7.1.4 Protocol: Spat water exchange using a tank setting system	82
7.1.5 Tank set: continuous flow for Day-14 spat to 2+ mm seed	82
7.1.6 Protocol: Monitoring settlement rate and growth for spat <1 mm shell height	83
7.1.7 Protocol: Changing from static to continuous spat culture tank system	85
7.2 Feeding spat in land-based nursery	86
7.2.1 Spat food ration	86
7.2.2 Feeding spat in raceway setting system	86
7.2.3 Feeding spat in tank setting system	87
7.2.4 Food ration and composition for spat in raceway and tank systems	88
7.2.5 Protocol: Calculating food ration using commercial diets	89
7.2.6 Stocking density and adjusting flow for seed biomass	91
7.2.7 Calculating spat biomass in a tank setting	93
7.2.8 Protocol: Estimating spat biomass on substrate	93
<b>8. Nursery to farm</b>	<b>97</b>
8.1 Remote nursery	97
8.1.1 Use of spat bags for remote nursery approach	97
8.2 Retrieving nursery-reared >2 mm seed for transfer at sea	99
8.2.1 Protocol: Grading seed	103
8.2.2 Protocol: Thinning seed	104
8.2.3 Expected survival and growth rates post-transfer at sea	105

8.3 Transporting spat to farm	105
8.3.1 Dry and wet transport of bags or nets	105
8.3.2 Protocol: Packing spat for transport (dry and wet)	107
8.3.3 High density transport of seed	107
8.3.4 Protocol: High density transport of seed, the “coffee filter” method	108
8.3.5 Seed monitoring on the farm	108
8.3.6 Protocol: Seed monitoring on the farm	109
<b>Further readings</b>	<b>111</b>
<b>Glossary</b>	<b>113</b>
<b>Appendices</b>	<b>115</b>
Appendix I – Datasheet sample for gonadic and muscle indices	117
Appendix II – Broodstock feeding record	119
Appendix III – Preparing measuring scoops for thinning	121

# Figures

<b>1. The Japanese scallop</b>	<b>1</b>
1.1 Specimens of <i>M. yessoensis</i> – (a) left and right valves showing anterior and posterior regions of the right valve; and (b) live scallop sitting on its right valve and showing eyes	1
1.2 Anatomy of the Japanese scallop, <i>M. yessoensis</i>	2
1.3 Series of blue-green “eyes”, distinct in the Yesso scallop, are clearly captured here in a specimen of the Atlantic bay scallop, <i>Argopecten irradians</i>	3
1.4 Life cycle of <i>M. yessoensis</i> showing all stages from spawning to larval and post-larval development and juvenile	4
1.5 Larval stages of scallops – (a) early veliger larva; (b) D-larvae; and (c) umboone larvae	5
1.6 Post-set stages of <i>M. yessoensis</i> – (a) early spat with dissoconch; and (b) older spat >1 month post-set translucent	6
1.7 Juveniles of <i>M. yessoensis</i> – (a) 8 to 15 mm seed with semi-translucent shell; and (b) robust 30 mm juveniles with opaque shell	6
<b>2. Microalgal culture</b>	<b>7</b>
2.1 Progressive batch culture for live microalgal species	8
2.2 Master cultures received from a commercial laboratory	8
2.3 Starter algal cultures in 500 ml flasks, aerated – (a) illumination with standard cool white fluorescent lamps; starter cultures top shelf, intermediate cultures (4 L) bottom shelf; and (b) illumination with Gro-Lux® LED strip lights and mirror shelves	9
2.4 Intermediate algal cultures (10 L carboys), aerated – (a) grown with CO <sub>2</sub> and cool white fluorescent light; and (b) using natural light	9
2.5 Large-scale algal cultures – (a) 100 L semi-continuous batch cultures; and (b) 500 L continuous cultures, showing nutrient line and harvest line	10
2.6 (a) Photobioreactors for growing live microalgal cultures; and (b) close up computer monitor for data entry and nutrient bottles	11
<b>3. Probiotic bacteria culture</b>	<b>15</b>
3.1 Seed culture received from commercial supplier in 30 ml vial	15
3.2 Stock cultures of probiotic bacteria used to inoculate 500 ml starter cultures	17
3.3 Stepwise procedure for streak plate method	21
3.4 Stepwise dilution procedure for determining CFU in probiotic bacteria culture	24
3.5 Colony-forming units of probiotic bacteria on TSA plates – (a) too many colonies to count; top 1:10, bottom 1:1 000; (b) co-cultivation in continuous culture (500 L) showing probiotic bacteria colonies and naturally occurring bacteria in algae; top post-inoculation (left), Day-1 after inoculation (right); and (c) top: pure probiotic culture 1:10 000 dilution; bottom: co-cultivated in 20 L and 3 L batch culture full concentrations (left and right)	29
<b>4. Hatchery facility layout</b>	<b>31</b>
4.1 An illustrated hatchery complex housing all sectors for land-based production of scallop seed	32

<b>5. Broodstock holding and conditioning</b>	<b>33</b>
5.1 Stages of Japanese scallop gonad – (a) male partially spent Stage 6; (b) female partially spent Stage 6; (c) female Stage 3 with intestinal loop; (d) male Stage 3 with intestinal loop; (e) female Stage 4, bright orange and turgid, some flaccidity along tip edge; and (f) Stage 5 female gonad with gonoducts	35
5.2 Dissecting gonad and muscle from mature scallop to determine gonadic and muscle indices	38
5.3 Broodstock tank with airlift pipe, stacked trays and a dedicated batch-feeding tub	40
5.4 Two temperature cycles for conditioning Yesso scallops – (a) 55-day temperature Cycle-1 for scallops at the start of gametogenesis; and (b) 35-day temperature Cycle-2 for scallops which have partially spawned or with a starting gonadic index close to 16	42
5.5a Step-by-step illustration of spawning set-up for scallops, collecting gametes, and distributing fertilised eggs in larval tank – saltwater table spawn	47
5.5b Step-by-step illustration of spawning set-up for scallops, collecting gametes, and distributing fertilised eggs in larval tank – tank spawn	48
<b>6. Larval rearing</b>	<b>53</b>
6.1 Larval tanks – (a) conical tanks used for flow-through rearing; and (b) illustrated series of larval tanks in hatchery layout with series of flow-through tanks	53
6.2 Schematic of a larval flow-through tank suitable for scallop rearing, showing direction of incoming seawater flow	54
6.3 Take down larval tank for water exchange with larvae collected on meshed sieve	54
6.4 Re-distributing collected larvae into a new clean flow-through tank by passing through a large mesh sieve to remove detritus	61
6.5 Collecting and measuring larvae	65
6.6 Expected trend for shell growth for larvae reared in a static system at standard densities (n = 50)	66
6.7 Average percent survival of <i>M. yessoensis</i> larvae determined during water changes in a 5 000 L static tank (T = 13.5 °C)	67
6.8 Grading and collecting larvae during a full water exchange	69
6.9 Setting pediveligers in sieves suspended off the bottom in a downwelling raceway system. Algal food reservoir and incoming seawater lines are also shown	72
6.10 Settling larvae – (a) rafting behaviour of larvae in holding bucket; (b) close up of valve regulating incoming flow in downwelling sieve	72
6.11 Setting tank (a) airlift system assembled; and (b) tank filled with substrate	76
<b>7. Nursery</b>	<b>105</b>
7.1 Downwelling sieve system showing centre channel receiving outflowing water from each sieve going to drain	80
7.2 Settled scallops - (a) seed attached to settling substrate (3 mm mesh); (b) close up of fixed spats	81
7.3 Expected shell height for Yesso scallop fed live microalgae from Day-1 to Day-55 after set (n = 50)	90
7.4 Growth rate for <i>M. yessoensis</i> spat cultured in a setting tank system and fed a mix of live microalgae and commercial diet (n = 50)	90
7.5 Scallop seed (3–5 mm) reared in upwelling sieve in raceway, showing partial coverage of the meshed surface area with spat one layer deep	91
7.6 Varying seed density for Yesso scallops on setting substrate suspended in nursery tanks – (a) high seed biomass on top section of substrate; and (b) low and medium seed biomass on substrate	92

---

7.7	Relationship between Yesso spat shell height (mm) and weight (mg.spat <sup>-1</sup> ) from newly settled spat (<1 mm) to 7 mm seed reared in nursery tanks (n = 50 for height; n = 15 for 100 spat subsample weight)	95
<b>8.</b>	<b>Nursery to farm</b>	<b>97</b>
8.1	Remote nursery approach: spat bags suspended in nursery tank for setting and rapid transfer to longlines	98
8.2	Retrieving spat from settling substrate and scooping known density into spat bags	100
8.3	Collecting seed from substrate onboard the farm boat for thinning and transfer into new nets	101
8.4	Grow-out of Yesso seed in spat bags – (a) sewing 2 mm seed in spat bags; (b) seed naturally distributed in bags; (c) 30 days later retrieved from longline (>5 mm)	102
8.5	Collecting seed from spat bags – (a) spat bag bundles with substrate and seed; (b) pool of seed collected in tub; (c) lantern net with good seed stocking density	103
8.6	Packing spat bags for “dry” transport using absorbent pad layering	106
8.7	Transporting seed at high density using the “coffee filter” method – (a) seed in filter; and b) top view of styrofoam transport container showing ice packs laid over insulated material and covering series of coffee cone filters with seed	108

## Tables

1.1	Description and timeline for development of early life stages of <i>M. yessoensis</i> , reared at 13±0.5 °C	4
3.1	List of small equipment specific to probiotic bacteria culture, additional to that used for microalgal cultures	16
3.2	Compatibility of standard hatchery algal species with probiotic bacteria <i>Pseudoalteromonas</i> sp. “C” is compatible; “IC” is incompatible, “NT” is not tested	24
5.1	Description of arbitrary visual index assessing reproductive status of adult scallops	35
5.2	Level of condition required for Yesso scallops collected at different gametogenic stages	41
6.1	Water exchange schedule scenarios for static and flow-through tanks	58
6.2	Datasheet for food ration for Yesso scallops cultured at 13±1 °C	63
6.3	Percentage of larvae retained on sieves of increasing mesh size (µm) according to their respective shell length, throughout the larval life	68
6.4	Criteria for setting Yesso scallop larvae	70
7.1	Food ration for spat from day of set (Day-0) to >2 mm shell height	87
7.2	Food ration for <i>M. yessoensis</i> spat from Day-1 after set to Day-100	88
7.3	Increasing a 10 000 L tank capacity with increasing incoming seawater flow rate to accommodate spat growth	92
8.1	Stocking densities for seed of increasing size adapted from various sources. Densities are given for 50 cm round lantern nets	102

# Acknowledgements

A practical manual such as this one could not be written without the hard work of many researchers, hatchery managers and technicians; they cannot all be named here, but their dedication, long hours and culturing skills are the backbone of this manual. Thank you to those who provided their photos of larval and early spat stages.

Huge thanks to the many hatcheries which enabled research for the advancement of scallop aquaculture and for the preparation of this manual; special thanks to the Canada Blossom Team who shared their knowledge on Japanese scallop broodstock temperature requirements, to the staff at Coastal Shellfish Corporation for their assistance in applying known techniques to the large-scale culture of this species, and to Nova Harvest for their development in probiotic integration to shellfish culture.

A special thanks to Jaramar Balmori (Mowi Canada West) for her microbiological expertise and assistance for the “probiotic” chapter. Guilherme Rupp (Center for Development of Aquaculture, Santa Catarina, Brazil) is gratefully acknowledged for generously taking the time to scientifically review the entire manual. Massimiliano Lipperi (@studiotwildart) applied his artistic talent to producing beautiful illustrations of various scallop culture stages. Graphic design and layout was carried out by Jose Luis Castilla Civit. Last but not least, appreciation is also due to the staff at the FAO Country Office in the Democratic People’s Republic of Korea for their support and project coordination.

This manual is dedicated to Neil Bourne, former researcher at the Pacific Biological Station in Nanaimo, British Columbia, Canada, whose work on the larval rearing of the Japanese scallop continues to be relied upon by many to this day and which is the scientific basis for the techniques related in this document.

## Acronyms and abbreviations

<b>CFU</b>	colony-forming units
<b>CO<sub>2</sub></b>	carbon dioxide
<b>DI</b>	de-ionized water
<b>GI</b>	Gonadic Index
<b>HCl</b>	hydrogen chloride
<b>LED</b>	light-emitting diode
<b>MI</b>	Muscle Index
<b>PVC</b>	polyvinyl chloride
<b>RPM</b>	revolutions per minute
<b>SI</b>	Système International
<b>SW</b>	seawater
<b>TCBS</b>	thiosulfate citrate bile salts sucrose
<b>TSA</b>	tryptic-soy agar
<b>UV</b>	ultraviolet

<	less than
>	greater than
<b>µm</b>	micrometer (micron)
<b>mm</b>	millimetre
<b>cm</b>	centimetre
<b>µl</b>	microlitre
<b>ml or mL</b>	millilitre
<b>L</b>	litre
<b>g</b>	gram

# Conversions

1 µm	$0.001 \text{ mm} = 0.000001 \text{ m}$
1 mm	$0.001 \text{ m} = 1 000 \mu\text{m} = 0.0394 \text{ inch}$
1 cm	$0.01 \text{ m} = 10 \text{ mm} = 0.394 \text{ inch}$
1 µg	$0.001 \text{ mg} = 0.000001 \text{ g}$
1 mg	$0.001 \text{ g} = 1 000 \mu\text{g}$
1 g	$1 000 000 \mu\text{g} = 1 000 \text{ mg} = 0.001 \text{ kg}$
1 µl	$0.001 \text{ ml} = 0.000001 \text{ l}$
1 ml	$0.001 \text{ l} = 1 000 \mu\text{l} = 1 \text{ cm}^3$
1 L	$1 000 000 \mu\text{l} = 1 000 \text{ ml} = 0.264 \text{ US gallons}$
1 m <sup>3</sup>	$1 000 \text{ l} = 264.16 \text{ US gallons}$
1 cm <sup>3</sup>	$0.001 \text{ l} = 1 \text{ ml}$

## Scientific units

Scientists have a different way of writing some of the units described in this glossary. They use what is called the Système International (SI). The units are referred to as SI units. For example: 1 ppt, which can be written as 1 g/l is written as  $1 \text{ g.l}^{-1}$  in scientific journals, 1 g/kg as  $1 \text{ g.kg}^{-1}$ , 12 mg/kg as  $12 \text{ mg.kg}^{-1}$  and 95 µg/kg as  $95 \mu\text{g.kg}^{-1}$ . A stocking density of 11 kg/m<sup>3</sup> would be written as  $11 \text{ kg.m}^{-3}$ . This SI system is not normally used in daily hatchery records, however for the purpose of standardization, it is used throughout this publication.



# 1. The Japanese scallop

**IN THIS CHAPTER –** General characteristics of the Japanese scallop, its distribution and life cycle.

## 1.1 BEHAVIOUR AND DISTRIBUTION

The Japanese scallop, *Mizuhopecten yessoensis* (Jay, 1857), is a cold water marine bivalve species, in the family of Pectinidae. It is synonym with *Patinopecten yessoensis* (Jay, 1857), and also referred to as Yesso or Ezo scallop. Scallops begin their lives as byssally attached juveniles, but rapidly grow into free-living adults; they are one of the few groups of bivalves capable of rapidly swimming short distances, and in some cases even migrate across the ocean floor. The Yesso scallop does not recess into the substrate but lies on top of the seabed; if faced with a predator, it may attempt to escape by swimming swiftly but erratically through the water by clapping its shells together.

Yesso scallops naturally occur in sheltered, shallow bays and inlets adjacent to rocky shores (4–10 m) and up to depth of 40 m. Inshore distribution is limited by ice depth during the winter. Seawater salinity ranges from 32–34 ppt, optimal growth temperature is 4–8 °C with a wide tolerance range between 2–26 °C.

The species is common along the coast of northern Japan. Its native distribution ranges from the Sea of Japan (Democratic People's Republic of Korea, Republic of Korea, and Russian Federation) to the Pacific coast of Japan. The species was introduced for culture purposes in the Northeast Atlantic, the Mediterranean and the Pacific coast of Canada.

## 1.2 MORPHOLOGY AND ANATOMY

A scallop shell consists of two valves, left and right, and the Yesso scallop rests on its right valve. The left valve is flat or slightly concave, and the right or bottom valve is slightly more convex (Figure 1.1). Both valves are similar in size and are ridged in the Yesso scallop. These “ribs” result in a relatively strong and heavy shell. The shell is streamlined to facilitate ease of movement during swimming. The shells radiate from a winged or eared umbo, seen on either side of the shell’s midpoint. Each valve is regarded as having a dorsal, ventral, anterior and posterior margin. The hinge serves as a reference point and is considered the “dorsal” region; the underside is the “ventral”

FIGURE 1.1

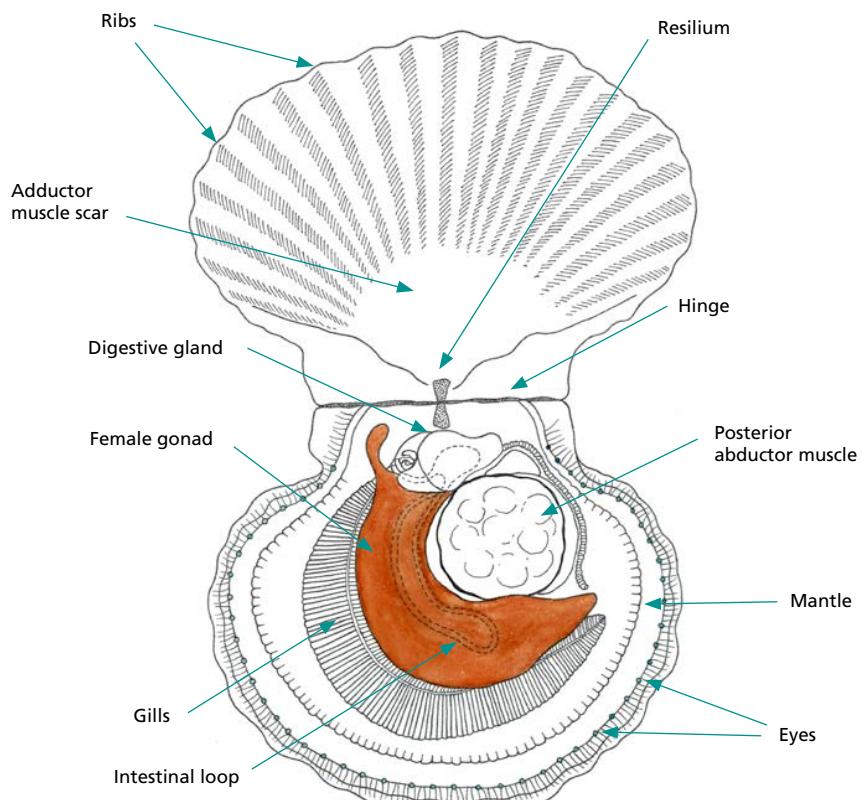
Specimens of *M. yessoensis* – (a) left and right valves showing anterior and posterior regions of the right valve; and (b) live scallop sitting on its right valve and showing eyes



region; the “anterior” region is that closest to the extreme point of the shell margin and the “posterior” is opposite.

As the scallop grows, it lays down distinct growth lines as concentric circles around the perimeter of the shell; these originate at the centre of the hinge (or beak), surrounded by the umbo. Growth rings increase in size downwards until they reach the curved ventral edge of the shell. Slower growth during the winter months concentrates the lines into an “annulus” and can be used as an indicator of age; although, any disruption in feeding, or rough handling, high stocking density in culture operations, might also result in the formation of non-annual concentrations of lines. The main organs of an adult Yesso scallop are illustrated in Figure 1.2.

**FIGURE 1.2**  
**Anatomy of the Japanese scallop, *M. yessoensis***

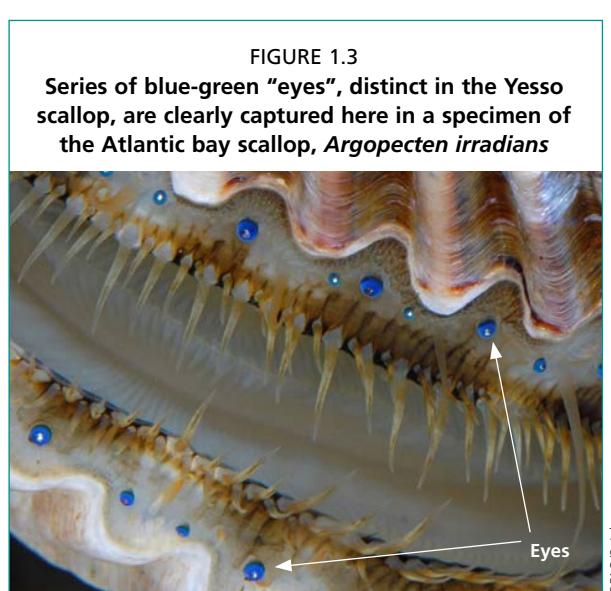


**The mantle** – It consists of three folds, each serving a separate function. The first fold lies immediately interior to the shell and is responsible for the synthesis of the shell along the thickened outside margin. Attached next is the sensory fold with its tactile organs (tentacles) and eyes (ocelli). The third fold is the velar fold, a muscular flap that plays an important role in swimming. Acting as a flapper valve, it extends to seal the valve margins and is used while swimming. The scallop’s movements are achieved with rapid flushing of the water from its mantle cavity; its configuration controls to some degree the direction and distribution of the expelled water. These behaviours are termed coughing, jumping and swimming.

**Adductor muscle** – Scallops have a single central well-developed adductor muscle, and inside of each shell there is a characteristic central scar, which is the point of attachment for the muscle (Figure 1.2). Scallops have a well-developed nervous system and have a

ring of simple eyes (about 1 mm) situated around the edge of their mantles (Figures 1.2 and 1.3). The nervous system of scallops is controlled by three yellowish paired ganglia, located at various parts throughout their anatomy; the cerebral or cerebropleural ganglia, the pedal ganglia, and the largest visceral or parietovisceral ganglia. The visceral ganglia connects to the circum pallial nerve ring, which loops around the mantle and connects to all of the scallop's tentacles and eyes. The cerebral ganglia controls the scallop's mouth and the statocyst (sensing its position). The pedal ganglia controls movement and sensation in its muscular foot. The foot is most prominent during the early life stage (pediveliger) at the time of metamorphosis and settlement; it is used for crawling and manipulating or attaching threads secreted by the byssal gland located at its base. In adults, the foot becomes vestigial and useless.

**Eyes** – The characteristic scallop eyes focus and retro-reflect light (Figure 1.3). They rely on concave, parabolic mirror of guanine crystals and possess a double-layered retina, with the outer retina responding most strongly to light and the inner to abrupt darkness. This grants scallops contrast definition and the ability to detect changing patterns of light and motion. Scallops primarily rely on their eyes as an “early-warning” threat detection system, scanning around them for movement and shadows, which could potentially indicate predators. In some cases, they may alter their swimming or feeding behaviour based on the turbidity or clarity of the water, as they detect the movement of particulate matter in the water column.



**Gills** – Gills are large leaf-like organs located on each side of the visceral mass and used for respiration and feeding (Figure 1.2). Water flows through the mantle cavity and across the gill plates, driven by the synchronous beating of ciliated regions on both the ventral mantle surface and the gill filaments. There is no siphon region; the inhalant current flows in across the circumference of the shell margin and the exhalant current flows out of a narrow post-dorsal region of the shell.

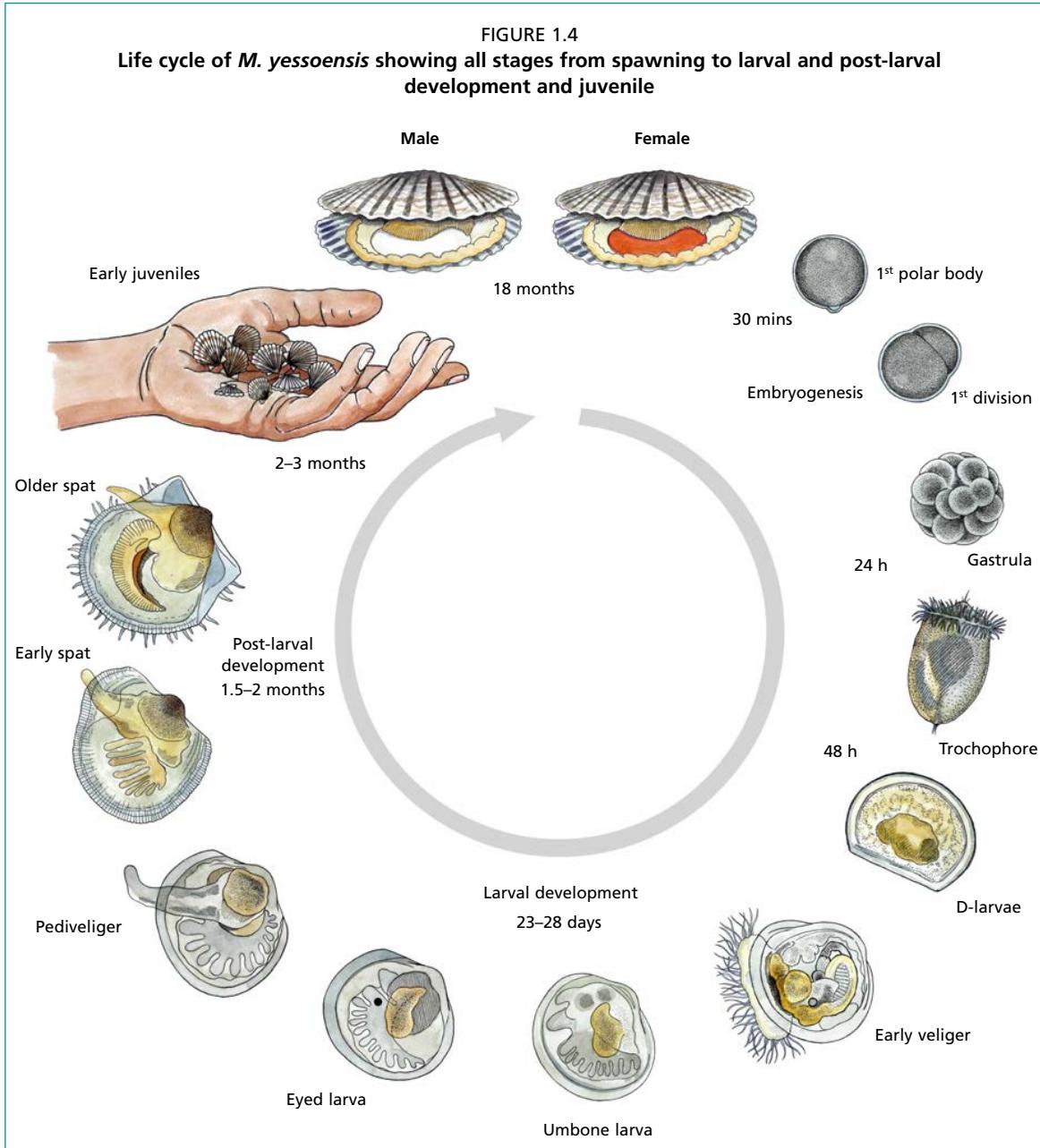
**Digestive gland** – Scallops are filter feeders. The cilia-driven water delivers food particles to the gill surface; particles become trapped in mucus and then digested in the digestive gland (Figure 1.2). This organ includes the oesophagus, stomach, intestine, rectum and anus. Other particles are rejected as pseudofaeces and flushed into the excurrent flow.

**Gonad** – Yesso scallops are dioecious and sexes are separate. The gonad is distinct crescent-shaped and lies antero-ventral to the adductor muscle and over the visceral mass. Female gonads become bright orange as they mature, and male gonads are white. Yesso scallops are protandrous hermaphrodites initially maturing as males and changing sex to female as they age.

### 1.3 LIFE CYCLE

The Japanese scallop releases eggs and sperm in the water column, and fertilisation of oocytes follows. Development of fertilised embryos into subsequent larval, post-larval and juvenile stages is illustrated in Figure 1.4. Development rate is dependent in great

part on rearing temperature and the expected time period between stages is given in Table 1.1.



**TABLE 1.1**  
**Description and timeline for development of early life stages of *M. yessoensis*, reared at 13±0.5 °C**

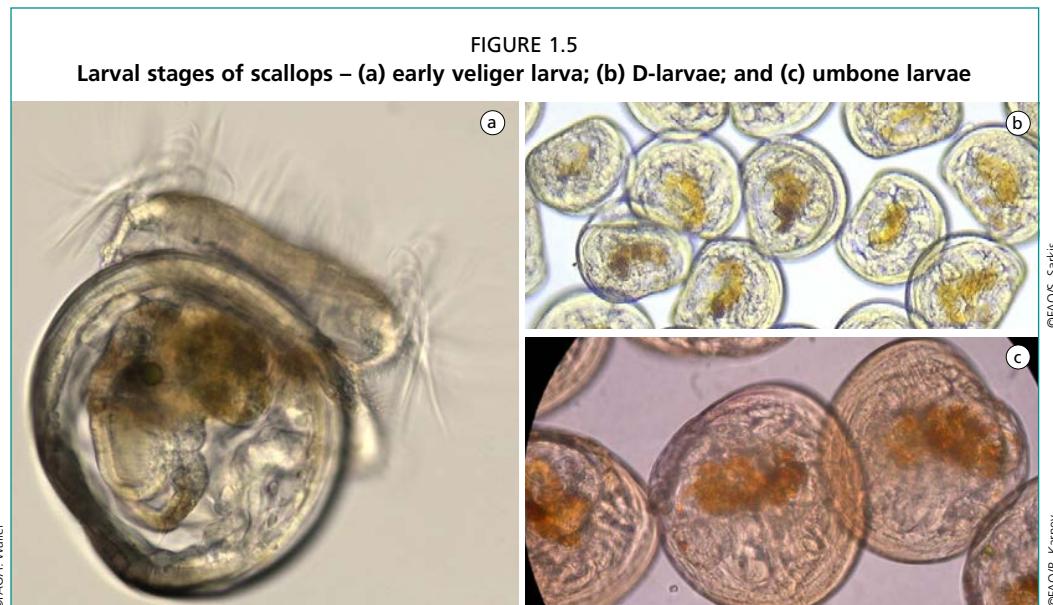
Description	Expected time after addition of sperm
First polar body	30 mins
First mitotic division (2-celled "snowman")	>1 h
Trochophore	24 h
First D-larvae	48 h
D-larvae	72 h
Umboned veliger larvae	Day 12
Eyed veliger larvae	Day 20-22
Pediveliger (foot development)	Day 23-28

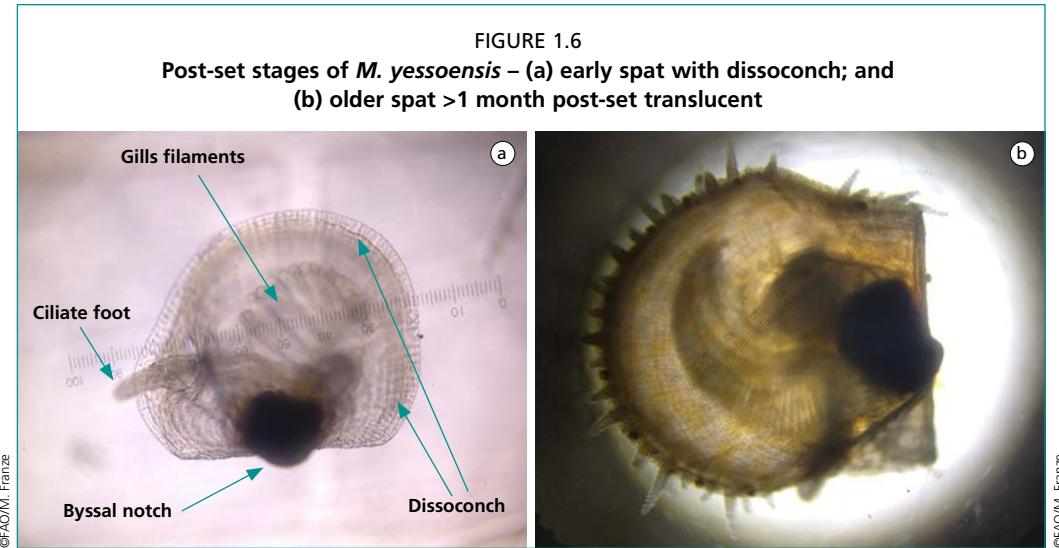
**Embryonic development: fertilised egg to D-larva** – The first polar body appears within 15–30 minutes of fertilisation followed by the second polar body (Figure 1.4). Expulsion of both first and second polar bodies occurs, and the first division or blastomere (pear-shaped) can be observed within 45 minutes of fertilisation. Multi-cellular division occurs during the next 2 hours, and the gastrula stage develops within the first 12 h following fertilisation. Gastrulae develop into active trochophores in 24–36 h. Trochophores are characterised by the apical tuft – a cilia banding around the zygote (Figure 1.4).

**Early larvae** – At a rearing temperature of  $13\pm0.5$  °C, first D-larvae of the Yesso scallop can be seen as early as 48 h, but more frequently at 72–96 h after fertilisation, averaging  $115\pm5$  µm in shell length, and larvae with prodissoconch II develop shortly after (Figures 1.4 and 1.5). This veliger stage is characterised by the swimming and feeding ciliated organ, called the velum; it consists of two large ciliated lobes developed from the prototroch (Figure 1.5). When the velum is retracted, larvae appear under the microscope as transparent semi-circles or D-shaped, hence referred to as “D-larvae” (Figure 1.4). Tissues can be seen, and consumed phytoplankton can be seen in the digestive gland appearing as a prominent dark brown area. Larvae have a shell made of aragonite (calcium-based mineral) as do the adults. As the larva grows, the umbo becomes more prominent and triangular, and the resemblance to a capital D decreases (Figure 1.4).

**Mature larvae** – Umbonate larvae occur around Day-11 after fertilisation. Eyespots, indicating the approach to metamorphosis and settlement, are frequently observed around Day-21. The pediveliger stage, characterised by the development of a ciliate foot, is first seen in Yesso scallops around Day-22, with a shell length averaging  $234\pm15$  µm. Length/height relationship changes towards the end of the larval life, and pediveligers and post-set stages exhibit a higher rate of height growth than earlier D-larval stage. Metamorphosis occurs between Day-24 and up to Day-28 (dependent on rearing conditions) for pediveligers ranging in size from  $243\pm15$  µm to  $280\pm14$  µm; a thickening of the shell at the end of metamorphosis indicates an early fixed dissoconch.

**Early post-set spat (<1 month)** – Following settlement, early spat are found attached within the first day; spat grow quickly and are well formed following ten days of settlement. At this time, the dissoconch appears as a colourless band with growth lines

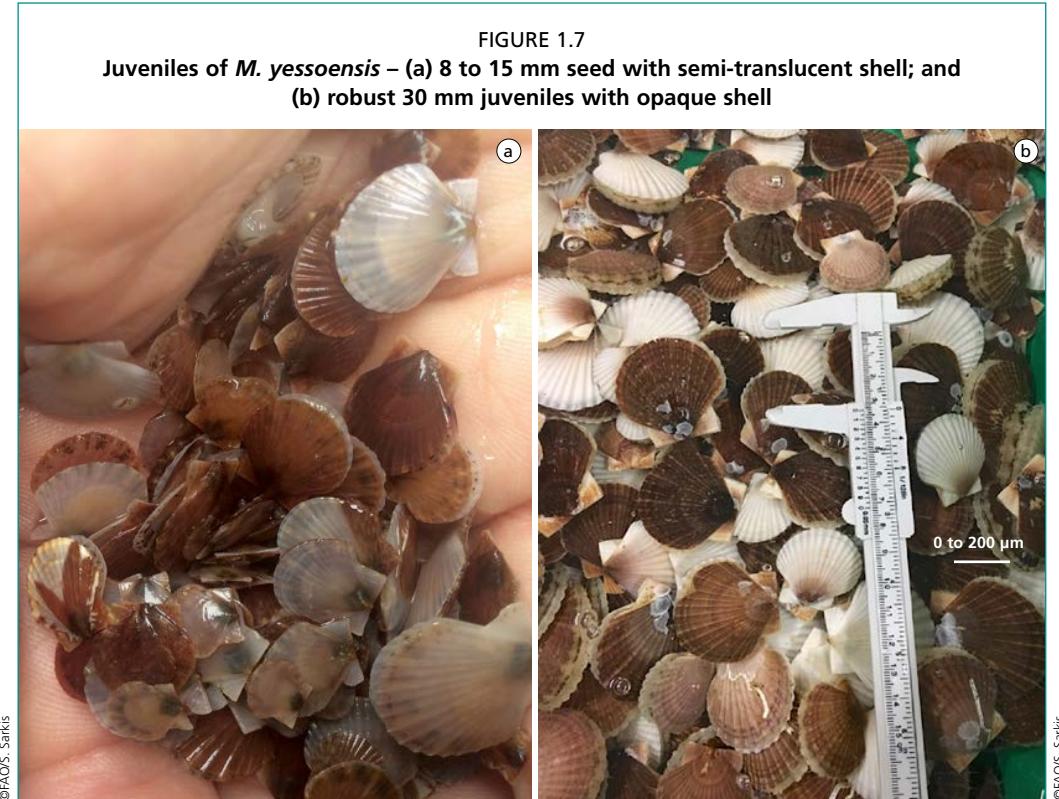




(Figure 1.6a). Post-larvae (2–3 weeks) show almost complete internal development with a ciliate foot, an outline of the byssal notch, and a group of rudimentary gill filaments.

**Older post-set spat (>1 month)** – Older spat (1.5–2 months) begin to resemble adult morphology and are no longer attached to the substrate (Figure 1.6b). *M. yessoensis* attains 4–8 mm shell height (growth determined by height rather than length), 4–5 months following settlement (Figure 1.7a).

**Juveniles** – Once transferred at sea, juveniles growth rate averages 8 mm.month<sup>-1</sup>, reaching 40 mm within 6 months of transfer at sea (Figure 1.7b). Market size adults (120±10 mm shell height) are obtained 18 months following transfer at sea.



## 2. Microalgal culture

**IN THIS CHAPTER – How to produce a live high quality microalgae food source.**

Scallops filter feed on phytoplankton throughout their lives as larvae, juveniles and adults. The microalgal culture hatchery unit is vital to successful seed production; without high quality live microalgae, survival and growth of larvae and post-larvae will be poor.

**Goal of microalgal facility** – Produce large volumes of healthy monocultures of selected algal species, grown from a reservoir of starter stock cultures; this is referred to as the “Progressive batch culture” process described in detail in Section 2.1.

**Microalgal species suited for the Yesso scallop:**

Flagellate species - *Isochrysis galbana*, *Pavlova lutheri*, *Tetraselmis* sp.

Diatom species - *Chaetoceros muelleri* or *calcitrans*, *Thalassiosira pseudonana*

**Feeding larvae, seed and broodstock** – The variety of algal species grown serves different needs throughout the production cycle with respect to size and digestibility, culture characteristics and nutritional value. Live diets for scallops are composed of at least three algal species, which will provide all of the essential constituents suited to the requirements of the life stage. For the algal culture facility, this means the large-scale culture of 4–6 different algal species in total.

**Note:** The use of commercially available diets as substitutes to live microalgae for broodstock conditioning and older spat can alleviate the demands on the algal culture unit. Details are given in Chapters 5 and 7.

**Microbiological techniques** – Extreme care is needed when culturing microalgae, as a failure in algal culture production has repercussions on the entire hatchery. Contamination of algal cultures may occur via the seawater supply, air supply, and cross-contamination from nearby algal cultures. Cleanliness and careful microbiological transfer techniques are to be applied daily and to all culture steps without fault to maintain algal cultures free of contaminants and excessive bacteria.

**Algal culture facility components** – Capacity for sterilisation and filtration (1 µm) of seawater for all vessels, from stock to large harvestable cultures.

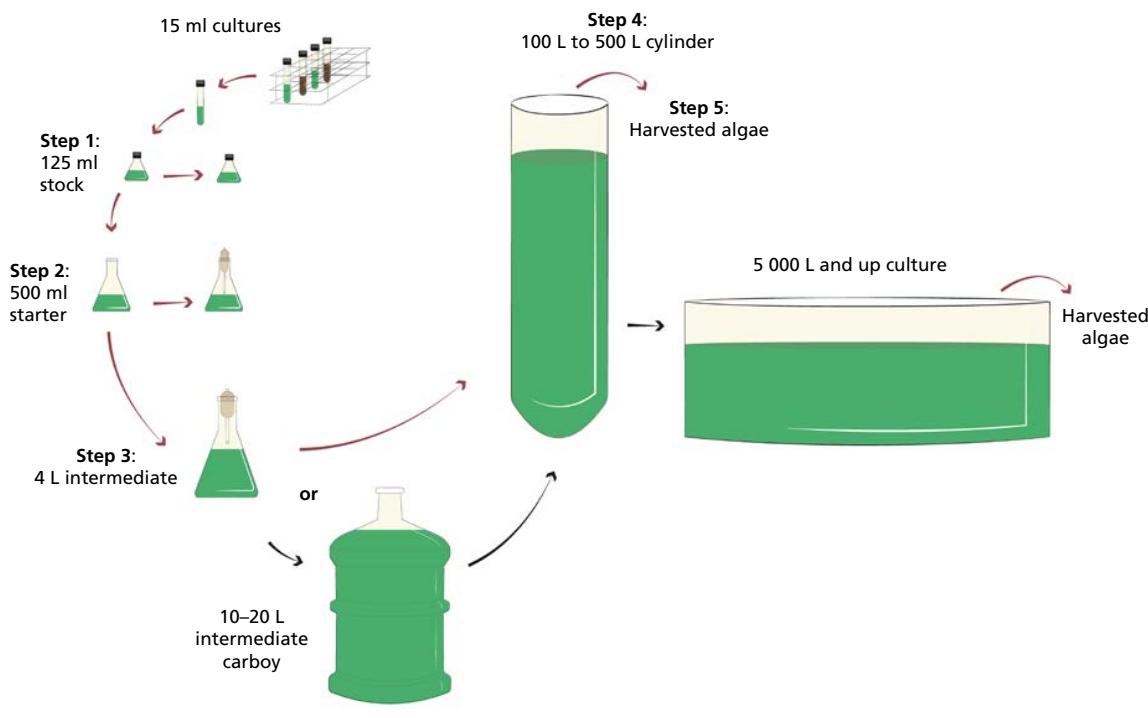
- Seawater temperature control ( $T = 24^{\circ}\text{C}$ ).
- Light banks.
- Filtered aeration.
- Carbon dioxide ( $\text{CO}_2$ ) input for pH control.
- Freshwater for cleaning.

The design, installation and operation of an algal culture facility for bivalves is described in detail in other technical publications (see Further Readings section).

## 2.1 MICROALGAL CULTURE OVERVIEW

**Progressive batch culture process** – is the growing of algae to large harvestable volumes through a series of culture vessels increasing in volume at every stage; small-volume cultures of concentrated algae inoculum are transferred to larger volumes of treated enriched seawater (Figure 2.1). All algal vessels are maintained at constant temperature (typically  $T = 23 \pm 1^\circ\text{C}$ ), lighting regime (24 h illumination; or 12 h illumination) and pH (7.5–8.5); nutrients are added to sterile seawater for algal growth. Smaller volumes (stock starter and intermediate cultures) are usually reared as batch or static cultures; larger volumes can be cultured as semi-continuous or continuous cultures.

FIGURE 2.1  
Progressive batch culture for live microalgal species



### STEP 1

**Stock cultures** – Algal master cultures can be isolated from natural seawater or purchased by the aquaculturist. These are typically received in 15 ml test tubes, when purchased (Figure 2.1). They can be maintained in similar 15 ml test tubes or transferred to 125–250 ml flasks (Figure 2.2), loosely closed with a screw cap; this allows some oxygen in culture, and there is no need for aeration.

The use of flasks is often preferred over test tubes, as it ensures the stocking of a larger volume of inoculum. Stock culture flasks are kept in an isolated section of the dry laboratory area, in order to prevent contamination from other flasks, or in an incubator. They are maintained through monthly subsampling and re-inoculations as a backup, in case of failure in the starter, intermediate or larger algal culture system.

FIGURE 2.2  
Stock cultures maintained in 250 ml Erlenmeyer flasks

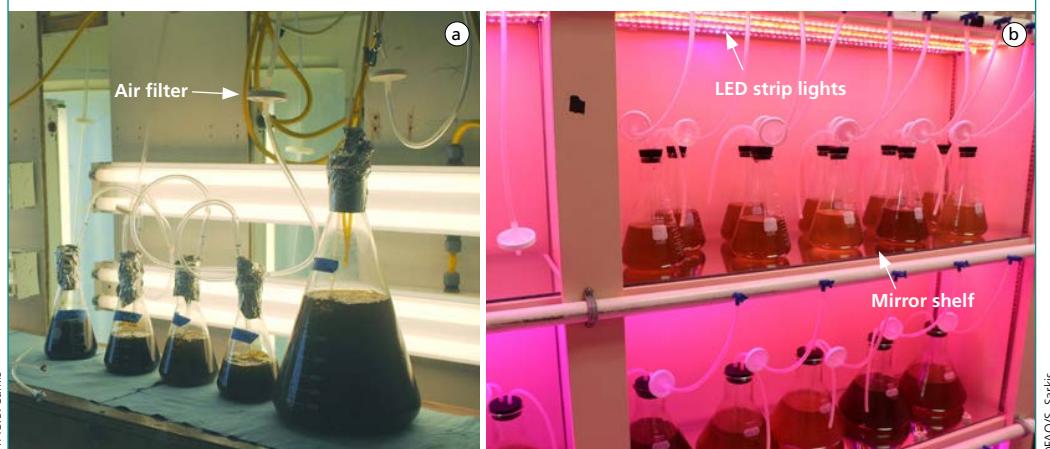


## STEP 2

**Starter cultures** – Stock cultures are used to inoculate larger starter cultures (500 ml flasks); these can be aerated or not. Aeration maintains cells in suspension, especially useful for some species such as *Tetraselmis* sp. (Figure 2.3). Aeration is also the means by which carbon dioxide (CO<sub>2</sub>) is added to the microalgal cultures; air supply is supplemented with CO<sub>2</sub> (2–5 %) to stabilise the pH of the culture, which ensures that sufficient carbon is available for photosynthesis as cells divide and the density of the culture increases. Standard lighting is by cool white fluorescent lamps (40 watts) (Figure 2.3a); Gro-Lux® LED strip lights are also used by some aquaculturists (Figure 2.3b). To increase illumination, algal flasks can be placed on a mirror shelf as illustrated in Figure 2.3b.

FIGURE 2.3

**Starter algal cultures in 500 ml flasks, aerated** – (a) illumination with standard cool white fluorescent lamps; starter cultures top shelf, intermediate cultures (4 L) bottom shelf; and (b) illumination with Gro-Lux® LED strip lights and mirror shelves



## STEP 3

**Intermediate cultures** – In turn, starter cultures inoculate intermediate cultures (4 L flasks to 20 L carboys); these are typically aerated, and densities are operator-dependent, but average 15 000 cells·μl<sup>-1</sup> and can reach up to 30 000 cells·μl<sup>-1</sup> (Figure 2.4).

FIGURE 2.4

**Intermediate algal cultures (10 L carboys), aerated** – (a) grown with CO<sub>2</sub> and cool white fluorescent light; and (b) using natural light



## STEP 4

**Large cultures** – Intermediate cultures are used to inoculate large harvestable size cultures (Figure 2.1); typically 100–500 L translucent fiberglass cylinders or polyethylene bags. Round fiberglass tanks (5 000 L and up) can also be inoculated and grown as batch cultures. Algae can be harvested 4–5 days after inoculation of a large culture vessel. Large-scale cultures can be grown as semi-continuous batch cultures, or as continuous cultures (Figure 2.5). Semi-continuous batch cultures follow the same inoculation protocol as intermediate cultures; the difference is that when the culture is harvested down to  $\frac{1}{3}$  of the vessel, new sterile seawater and nutrients is added to the vessel and the culture re-grows to full volume. Semi-continuous cultures can be maintained in the same vessel for up to 30 days (Figure 2.5a). Continuous cultures can be grown in polyethylene bags supported by a wire frame (Figure 2.5b); these receive a continuous input of seawater and nutrient and can be harvested on a continuous daily basis once suitable densities are reached. Details on protocols for large-scale cultures of both batch and continuous systems are available in other technical guides (see Further Readings section).

FIGURE 2.5

Large-scale algal cultures – (a) 100 L semi-continuous batch cultures; and (b) 500 L continuous cultures, showing nutrient line and harvest line



**Alternative large cultures** – Other systems for growing algae in large volumes include the more recent technology of photobioreactors (Figure 2.6). Photobioreactor systems provide higher algae densities, more efficient space usage (a smaller footprint), and lower labour requirements. The disadvantages are that they are costly and rely on computer software for good functioning; technical support should be easily accessible in case of breakdown. Training by the manufacturer is needed.

## 2.2 MICROALGAL PROTOCOLS

**Daily monitoring** – Constant monitoring of algal cultures is critical and must become part of an automatic check at the beginning and end of the day. Protocol 2.2.1 is an example of daily tasks to ensure the availability of healthy and “clean” algal cultures free of ciliates or other undesirable microorganisms for high quality algae production.

**Inoculations** – The protocols used for preparing algal vessels and inoculating them is similar for all steps in the progressive batch culture (see Protocol 2.2.2). An increasing larger volume of inoculum is used at every step of the process, in order to obtain sufficient harvestable algal volumes to supply the hatchery needs. Rigorous use of microbiological techniques is required throughout. Differences in large algal vessel types are at the discretion of the aquaculturist and are dictated by the scale of algal production required.



## 2.2.1 PROTOCOL: Daily algal checks

**Note:** Keep the algae laboratory as clean as possible. Wash hands with antibacterial soap before working with algae cultures. Wear laboratory gloves to prevent contaminating surfaces and cultures.

### Beginning of day

- Swirl stock cultures to resuspend algae and record room/incubator temperature.
- Check small and intermediate cultures and note if any are growing poorly or crashed; check that all are correctly aerated (if using air); if not, swirl gently without moistening the plug. Record air temperature and check lighting.
- Check all large vessels similarly. If any crashed cultures, drain tank immediately, and freshwater rinse before preparation for new inoculate. Do not leave crashed cultures in tank, and do not leave dirty algal tanks to avoid any cross contamination to healthy cultures.
- Check air supply line for condensation build-up and purge, if required.
- Correct any aeration or temperature problems.
- Check air filter and replace, if needed.
- Record CO<sub>2</sub> tank pressure; always have two tanks for back up. Order new tank as soon as one is empty.
- Replace any non-working light bulbs or LED strip lights.
- Add bleach in chlorine bath at entrance of algal room to clean boots, if needed.
- Remove items from autoclave and place on cooling rack or storage shelves.
- Reload and start autoclave, if needed.

### Rest of day

- Collect algal samples for checks and counts for feeding.
- Check pH if there is concern of fluctuations.
- Calculate amount of algae required for feeding and harvest.
- Distribute algae for feeding immediately after harvest. If algae are held in harvest containers for any length of time, make sure they are well aerated.
- Do new inoculations.

### End of day

- Walk through facility, re-check aeration, lighting and temperature level.

## 2.2.2 PROTOCOL: Inoculating and maintaining starter algal cultures

### MATERIALS

- 10 % hydrogen chloride (HCl) bath
- De-ionized water
- Flasks: 125 ml and 500 ml
- Sterile graduated pipettes: 5 ml and 10 ml
- Bunsen burner (or small propane torch) and lighter
- Pipette bulb (3-way) for dispensing nutrients
- Algal nutrient media: F/2 commercially purchased or other, vitamin solution, sodium metasilicate solution (3 % W/V)
- Fume hood
- Cotton plugs or steri-stoppers
- Algal stocks

### METHOD

#### Preparing flasks

1. Clean 2 flasks for each algal species in a 10 % HCl bath; rinse twice with freshwater and one final rinse with distilled water.
2. Fill 500 ml flask with 350 ml of 1 µm filtered seawater.
3. Close flask loosely with plug (e.g. cotton plug and foil).
4. Sterilise in autoclave.



©FAO/R. Kamey

New sterile flasks under fume hood

#### Inoculating 500 ml starter cultures with 125 ml stock cultures

5. Work under the fume hood.
6. Wear gloves and spray with ethanol to sterilise.
7. Use microbiological sterile techniques for all transfers.
8. Prepare work area, with a Bunsen burner or small propane torch nearby, nutrient solution (F/2<sup>(1)</sup>, vitamin and sodium metasilicate).
9. Use a 10 ml pipette, transfer 30–35 ml of stock culture to 500 ml flask, staying close to flame at all times. Do not touch mouth of either flask with pipette. Plug flask quickly after transfer.
10. Label flask with species and date of transfer.

## 2.2.2 PROTOCOL (continued)

11. Working close to flame, remove cap of nutrient solution and place on clean surface area, maintain mouth of nutrient media container close to flame.
12. Using a sterile pipette, collect nutrient media. Dosage is 1 ml.l<sup>-1</sup> of F/2 nutrients to algal inoculum; for e.g. for a 500 ml algal flask, 0.5 ml nutrient solution will be required.
13. Close nutrient container immediately after pipetting by holding close to flame, flaming mouth and cap before closing.
14. Always keeping pipette tip with nutrients close to flame.
15. Hold algal flask in one hand, and pipette in the other close to flame.
16. Remove flask plug and keep in palm of hand; add nutrients to algal inoculum.
17. Close flask immediately with cotton plug in hand. Discard pipette. Swirl flask to mix nutrients and algae.
18. Using a second sterile pipette, add 2 ml.l<sup>-1</sup> of sodium metasilicate for diatom species. For e.g. for 500 ml flaks, add 1 ml in total.
19. Flame aerating tube and insert into 500 ml flask, holding close to flame.
20. Move completed flask to light bank, and connect to airline; bubbling ensures homogeneous mixing.

### Maintenance of 500 ml starter cultures

21. 500 ml cultures will be ready for transfer within 3–4 days of inoculation.
22. 500 ml culture is used as inoculum for new 500 ml flask, and for new intermediate flask.
23. For 500 ml flask, transfer 100–200 ml of inoculum (from old 500 ml flask) to new autoclaved 500 ml flask containing seawater. Add nutrients using same dosage as above.
24. For intermediate culture, transfer remaining of old 500 ml flask. Take care not to pour the bottom of the culture as it may contain some detritus.
25. Add nutrients and aeration line. Plug and move to light bank for growth.

(1) There are several nutrient solutions suitable for microalgal culture and dosage may differ with nutrient composition. F/2 is a common, commercially available solution.

**Determining algal culture density** – Subsamples of algal cultures ready for harvest are checked under the microscope to make sure that they are free of ciliates and other foreign and undesirable organisms. Clean cultures are counted on a daily basis for feeding of larvae and spat. Algae can be counted using sophisticated equipment such as a Coulter Counter, or less costly tools such as an XpertSea digitized bucket (XperCount), and more typically a haemocytometer cell under a compound microscope. Assessing density of microalgal culture is needed to calculate the food ration for scallops; this is dependent on age (for larval/post-larval stage), stocking density (for broodstock/older spat) and volume of culture tank.

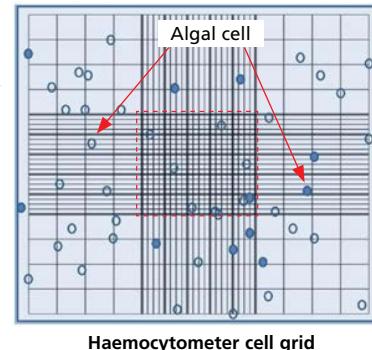
### 2.2.3 PROTOCOL: Counting algae and estimating algal culture density

#### MATERIALS

- Haemocytometer cell
- Small vial or test tube or well plate
- 10 % formaldehyde
- Pasteur pipette and bulb
- Clicker

#### METHOD

1. Collect a subsample of algal culture and transfer to vial.
2. Add 2–3 drops of 10 % formalin if using a flagellate species to halt swimming and movement. Mix.
3. Mount cover slip on haemocytometer cell.
4. Collect 1 ml of algal sample using a Pasteur pipette and introduce a drop into chamber at edge of cover slip. Do not force sample in, allow it to run by capillary action. Make sure not to have any air bubbles in cell.
5. Fill grooves of cell completely with algal sample.
6. Using a clicker (counter), count number of algal cells in at least three of the 25-square grid in the centre of the cell (highlighted in red in photo).
7. If algal culture is not very dense (as in photo), count all of the cells in 25-square grid.
8. Calculate average number of cells per square.
9. To obtain cell density: Average number of cells  $\times 250$ . This gives the number of algal cells per  $\mu\text{l}$ .
10. Multiply by 1 000 for number of cells per ml.



#### Explanation

Each cell is  $0.004 \text{ mm}^3$

By multiplying by 250, you obtain number of cells per  $\text{mm}^3$

$1 \text{ mm}^3 = 1 \mu\text{l}$

**Note:** To avoid counting the same algal cell twice, select top border and right border of each small square (within 25-square grid); count only algal cells sitting on top and right border.

#### Example

1. Count algal cells in 3 squares within 25-square grid.  
Count in square 1 = 56 algal cells  
Count in square 2 = 48 algal cells  
Count in square 3 = 51 algal cells
2. Calculate average = 51.6 algal cells per square.
3. Calculate culture cell density =  $51.6 \times 250 = 12\,916 \text{ cells.}\mu\text{l}^{-1}$  or  $12\,916\,000 \text{ cells.ml}^{-1}$ .

### 3. Probiotic bacteria culture

**IN THIS CHAPTER –** Steps for culturing and introducing probiotic bacteria in larval and spat culture systems.

Probiotics should be considered in any new hatchery operation as a biocontrol agent. Shellfish aquaculturists worldwide are increasingly turning to the use of probiotic bacteria for controlling pathogens as an alternative to that of antimicrobial drugs. Bacterial balance has been shown to be important in maintaining healthy larval cultures. Reports are available for probiotic use in oyster larval culture; however, little is reported for scallop culture. Preliminary trials have been successfully conducted on spat rearing of the Japanese scallop; more trials are needed to optimise procedures for administering probiotics to larval and spat cultures. This section provides a brief overview on probiotics in general, but focuses on procedures specific to probiotic bacteria. The culture and monitoring of probiotic bacteria, co-cultivation of probiotic bacteria with microalgae used as feed, and a general approach on their integration to larval and spat cultures are described.

**Types of probiotics** – Probiotics generally refer to organisms which beneficially affect the host; benefits are often achieved by improving microbial balance of the host or of the ambient environment.

Some microalgae species have probiotic properties; for example, *Skeletonema costatum*, a commonly used microalgae, produces an extract inhibiting growth in several *Vibrio* species and can potentially act in a manner beneficial to shellfish larviculture. It is reported that when *S. costatum* is added to the developing *M. yessoensis* trochophores at a ratio of 4 000 cells.ml<sup>-1</sup>, it reduces bacterial contamination during this early vulnerable stage. Other microalgae species, *Phaeodactylum tricornutum* and *Tetraselmis suecica* also inhibit *Vibrio* sp. and are used as probiotics.

A diverse range of Gram-positive bacteria is used worldwide as probiotics, known to have beneficial effects on the digestive system of bivalve larvae. *Pseudoalteromonas* sp. is one species, which is commercially available and successfully used for commercial oyster culture for its antibacterial and antivirulent compound production.

**Selecting a probiotic bacteria species** – Selecting a probiotic bacteria species for scallop culture is at the trial and error stage, and a screening process by the aquaculturist will most likely be necessary to find a species specific probiotic. Potential probiotics may be obtained from various sources such as: a) isolates from local seawater, b) commercial suppliers, and c) intestinal tract of the aquatic species itself. The probiotic selected needs to fulfil the greatest number of the following properties:

- a) harmless to the host;
- b) accepted by the host through ingestion, colonisation and proliferation within the host;
- c) reach target organs where it can perform; and
- d) contain no virulent resistance or antibacterial genes.

The first property (a) is key and the aquaculturist must ensure that the probiotic species does not cause any toxic side effects to the host. As this is species specific, it

is an essential part of the screening process and should be tested at small scale before introducing to the entire operation. For example, *Pseudoalteromonas*, used for oyster culture, is reported to be toxic to other invertebrate species.

Probiotics can be used singly or in combination; those based on mixed strains are found to be more effective. For example, a co-culture of *Roseobacter* BS107 and *Vibrio anguillarum* enhances the survival of larval scallop (*Pecten maximus*). The aquaculturist will need some time to conduct trials to determine the most suited species and best protocols; the ultimate goal is to ensure a continuous presence of probiotic bacteria in the scallop cultures at a density sufficient to provide larvae with antimicrobial defence mechanism.

**Administering a probiotic** – A proper administration method creates favourable conditions in which the probiotic performs well. Probiotic bacteria have shown compatibility with several microalgae species used as standard scallop food; probiotic bacteria can be co-cultivated with live microalgal food and introduced to larvae with the food ration. This method improves the ingestion of probiotics by scallop larvae and spat.

Alternatively, probiotics can be introduced directly to the culture, by adding them as a bacterial suspension into larval and post-larval tanks.

### 3.1 PROBIOTIC BACTERIA CULTURE EQUIPMENT

**Dedicated space** – Probiotic bacteria can be cultured in the algal dry laboratory area of a hatchery complex. Ideally, stock and starter cultures should be kept separate from those of microalgae; but practically, these can be maintained in the same climate-controlled room on separate shelves; adherence to strict protocols to eliminate the risk of cross-contamination is a must throughout all steps of culture.

**Equipment and materials** – A fume hood is essential for inoculation and transfers of probiotic bacteria. Additional small equipment is required for the culture of probiotics; a list is given in Table 3.1. The agar media used depends on the probiotic bacteria species cultured and on the need for differentiation of *Vibrio* species, Gram-positive and Gram-negative bacteria.

TABLE 3.1  
List of small equipment specific to probiotic bacteria culture, additional to that used for microalgal cultures

Description	Minimum quantity
BD Difco™ dehydrated culture media: Marine Agar 2216 or Difco™ Marine Broth 2216 powder	1 (500 g)
Thermo scientific Remel Simmons Citrate Agar dehydrated <sup>(1)</sup> or Thiosulfate Citrate Bile Salts Sucrose (TCBS) Agar	1 (500 g) Package of 10 (15 x 100 mm plate)
Tryptic-Soy Agar (TSA) plates – purchased ready to use <sup>(2)</sup> (Merck)	
Dispenser for Marine Broth to stocks: Scilogex SCI-Spense Bottletop Chemical Dispenser, 1–10 ml volume	1
Premiere Benchtop centrifuge 1 000–4 000 revolutions per minute (RPM) holding 50 ml test tubes	1
Sterile centrifuge tubes with flat caps (50 ml) RCF (relative centrifugal force) 12 000/g	Packs of 25
Magnetic stirrer hot plate (to hold 250 ml <sup>-1</sup> L flasks)	1
Shaker table (able to hold up to 1 L Erlenmeyer flask and set 25 RPM)	1
Fume hood	1
Eppendorf pipette and tips (up to 1 ml)	1
Glass spreader	2
Petri dishes	As required

<sup>(1)</sup> TCBS agar is selective and used to assess presence of *Vibrio* species; plates can be made or purchased.

<sup>(2)</sup> TSA is a universal medium, which supports the growth of both Gram-positive and Gram-negative bacteria.

### 3.2 PROBIOTIC BACTERIA CULTURE PROTOCOLS

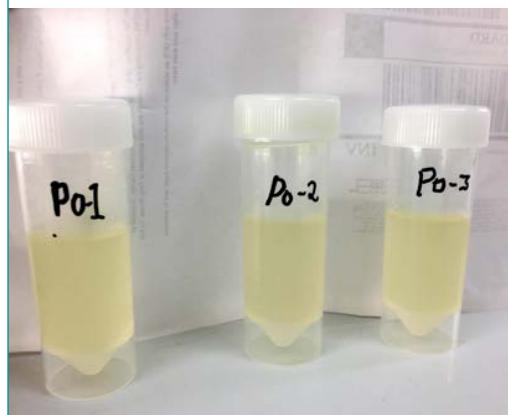
**Seed cultures** – Probiotic bacteria are received in small containers, such as 30 ml vials (Figure 3.1). Cultures are removed from the shipped box as soon as they are received and placed under the fume hood until they are ready for use. These seed cultures are used to inoculate the aquaculturist's own stock cultures as a backup for the probiotic bacteria cultured on site. The first step is to check the cleanliness of seed cultures received by checking for *Vibrio* (see Protocol 3.2.4). A subsample of seed culture is plated on TCBS agar; if *Vibrio* are present, they will grow on the plates. A clean culture will not show any growth on TCBS.

**Batch culture** – Once seed cultures are verified as free of undesirable organisms, they are grown using bacteria specific nutrient media. All materials and media are autoclaved prior to use, similar to the procedure for algal cultures. The progressive batch approach described for microalgae is also used for the culture of probiotic bacteria; seed cultures received are sub-sampled to ensure a stock culture as back-up and to inoculate starter cultures (Figure 3.2); from these, probiotic bacteria are harvested for co-cultivation with algae in larger volumes or introduced directly into larval and post-larval tanks. Note that the total volume of probiotic bacteria produced is orders of magnitude smaller than that of food microalgae and requires substantially less space in the algal facility.

**Harvestable target** – As a guide, culturing a total of approximately 2 L of probiotic bacteria every 3.5 days should suffice a hatchery/nursery of 150 000 L continuous algal capacity. It is recommended that new stock cultures be received from the supplier every 4–6 months.

**Protocols** – Six protocols follow, outlining procedures from the time the probiotic bacteria shipment is received to the production of starter cultures. Two types of agar plates are prepared: a) TCBS agar is highly selective for *Vibrio*, undesirable in an isolated probiotic bacteria seed culture (see Protocol 3.2.1). The streak method is applied to the seed cultures received (see Protocol 3.2.4); b) Marine agar or TSA are used to monitor probiotic bacterial growth (see Protocol 3.2.5). One common nutrient medium used for growing bacteria is Marine Broth or Marine Agar, and is prepared by the algologist (see Protocol 3.2.2). The density of bacteria is estimated based on the number of colony-forming units (CFU), determined through a series of dilutions (see Protocol 3.2.6). Probiotic bacteria are cultured independently of microalgae initially as starter cultures (see Protocol 3.2.3); these are grown to densities high enough for harvest used for co-cultivation with microalgae or for direct addition to tanks (see Protocol 3.3.1).

FIGURE 3.1  
Seed culture received from commercial supplier in 30 ml vial



©FAO/S. Sarkis

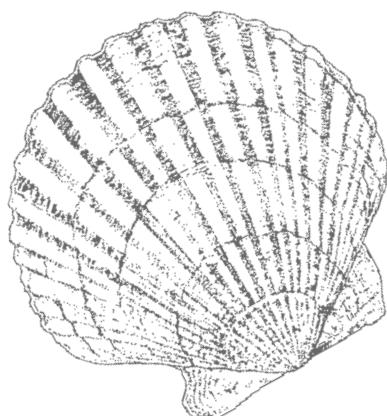
FIGURE 3.2  
Stock cultures of probiotic bacteria used to inoculate 500 ml starter cultures



©FAO/S. Sarkis/Nova Harvest

### 3.2.1 PROTOCOL: Preparing thiosulfate citrate bile salts sucrose (TCBS) agar plates

MATERIALS
<ul style="list-style-type: none"> <li>- Scale</li> <li>- TCBS</li> <li>- 1 L flask</li> <li>- De-ionized water (DI)</li> <li>- Seawater (SW)</li> <li>- Magnetic stirrer</li> <li>- Magnetic stirrer hot plate</li> <li>- Steri stoppers</li> <li>- Aluminium foil</li> <li>- Graduated cylinder</li> <li>- Parafilm</li> </ul>
METHOD
<p><b>Note:</b> TCBS plates are used to check for <i>Vibrio</i> sp. in cultures. Manufacturer's instructions should be on TCBS container.</p> <ol style="list-style-type: none"> <li>1. Weigh the amount of TCBS needed for the required volume (<math>88 \text{ g.l}^{-1}</math> DI water).</li> <li>2. Pour TCBS Agar into a 1 L flask.</li> <li>3. Measure required volume as per manufacturer's instruction, using 50 % SW and 50 % DI water.</li> <li>4. Pour DI water into the flask sides, making sure all the powder falls to the bottom of the flask.</li> <li>5. Put one magnetic stirrer into the flask.</li> <li>6. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder (take care not let it boil over the flask).</li> <li>7. Cover with a steri stopper and aluminium foil.</li> <li>8. Do NOT autoclave.</li> <li>9. Leave to cool to 40–45 °C.</li> <li>10. Pour into plates, filling around half the plate.</li> <li>11. Leave for a day, making sure there is no water condensation on the top of the plate.</li> <li>12. Next day, check for condensation and contamination. If there is none, make packages of 5 using parafilm.</li> <li>13. Keep the plates upside down and in the fridge until use.</li> </ol>



### 3.2.2 PROTOCOL: Preparing marine broth for probiotic bacteria cultures

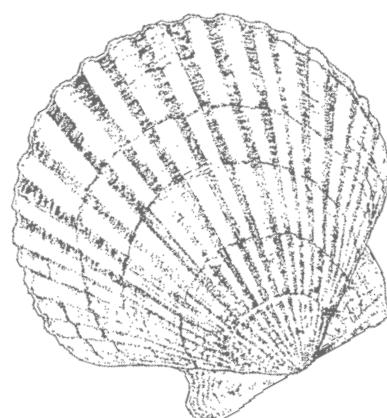
#### MATERIALS

- |   |  |
|---|--|
| - Difco™ Marine Broth 2216 powder         | - Six 1 L Ernlemeyer flasks                              |
| - Scale $\pm 0.01$ g                      | - Magnetic stirrer                                       |
| - Magnetic stirrer hot plate              | - Steri stoppers or cotton plugs (made with cheesecloth) |
| - Graduated cylinder (500 ml or 1 000 ml) | - Aluminium foil   |
| - De-ionized water (DI)                   | - Autoclave  |

#### METHOD

**Note:** Manufacturer's instructions should be on the side of the container.

1. Prepare a 1 L flask with 500 ml purified (or DI water if you have it); add one magnetic stirrer to flask.
2. Weigh 37.4 g Difco™ Marine Broth powder.
3. Pour into a 1 L flask filled with 500 ml DI water.
4. Pour an additional 500 ml of purified water onto the flask sides, making sure all powder falls to the bottom of the flask.
5. Transfer flask to a magnetic heated stir plate.
6. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder (take care not to let it boil over the flask).
7. Transfer to a new 1 L Ernlemeyer flask, leaving the magnetic stirrer in the old flask.
8. Plug flask with a steri stopper (or cotton plug and aluminium foil similar to that used for algal culture growth flasks).
9. Repeat Steps 1–8 two additional times to have  $3 \times 1$  L flasks of Marine Broth ready for autoclave.
10. Autoclave at 121 °C for 15 minutes.
11. Remove from autoclave and leave to rest for one day before use.



### 3.2.3 PROTOCOL: Culturing probiotic bacteria – seed culture to starter culture

#### MATERIALS

- Fume hood
- Shaker table
- Autoclaved Marine Broth
- Autoclaved flasks (250 ml and 500 ml)
- Steri stopper or cotton plugs
- Aluminium foil
- Sterile pipettes
- Bunsen burner or propane torch
- Three 30 ml seed cultures

#### METHOD

##### Example: Receiving 3 × 30 ml seed cultures

1. As soon as cultures are received, open box, loosely open cap to allow some oxygen in but avoid contamination.
2. Clear fume hood of any materials, keeping only Bunsen burner and clean surface.
3. Seed cultures received should be cloudy. Note state of cultures once removed from shipping box.
4. As a further check collect one drop of culture from each vial, place on slide, and check motility under the microscope. Plate 1 µl from each vial to check that cultures are viable (see Protocol 3.2.1 for TCBS plates and Protocol 3.2.4 for rapid streak plating procedure).
5. Place seed cultures under fume hood until ready to transfer into Marine Broth.
6. You will first inoculate 250 ml flasks as stock cultures; these will be kept as back-up and used for inoculating 500 ml starter cultures.
7. Working under the fume hood, and using sterile microbiological techniques, transfer 100 ml autoclaved Marine Broth into 3 × 250 ml flasks.
8. Using 1 of 3 seed cultures received – Transfer seed culture into one 250 ml flask with Marine Broth. Close with steri stopper. Label starter culture with probiotic reference number and date.
9. Repeat Steps 1–8 for second and third seed culture.
10. Place 250 ml flasks on a shaker table (25 RPM) and maintain overnight at 25 °C for cultures to grow. The agitation keeps the solution aerobic. Cultures on shaker can be kept in a dark incubator or lighted room.
11. Once agitated for one night, 250 ml cultures of probiotic bacteria can be kept in a small incubator in the algae room at 25 °C.
12. Monitor probiotic bacteria growth by subsampling once a week for CFU (see Protocol 3.2.6 for CFU assessment).
13. Once CFU in stock cultures reach  $10^6$ , prepare 3 × 500 ml flasks for stock cultures.
14. Transfer 250 ml of (autoclaved) Marine Broth into each 500 ml flask.
15. Using sterile microbiological techniques, working by the flame, transfer 100 ml stock culture to 500 ml flask containing Marine Broth. These are now your starter solutions for growing probiotic bacteria for inoculum to larger flasks or harvest.
16. Label new 500 ml starter cultures accordingly with date of inoculation.
17. Transfer remaining stock culture into new 250 ml flask to maintain stocks.
18. Initially, CFU is monitored weekly to assess growth; afterwards, monitoring every 2 weeks should be sufficient. A minimum density of  $10^6$  is required for harvest.
19. Establish a sub-culture schedule to maintain stocks every 2 weeks or when CFU reaches  $10^6$ .



Shaker and probiotic bacteria stock cultures

©FAO/S. Sarkis

### 3.2.4 PROTOCOL: Streak plate method to identify *Vibrio* or bacteria in a culture

#### MATERIALS

- Marine agar or TCBS plates (as required)
- Samples
- Inoculating loop

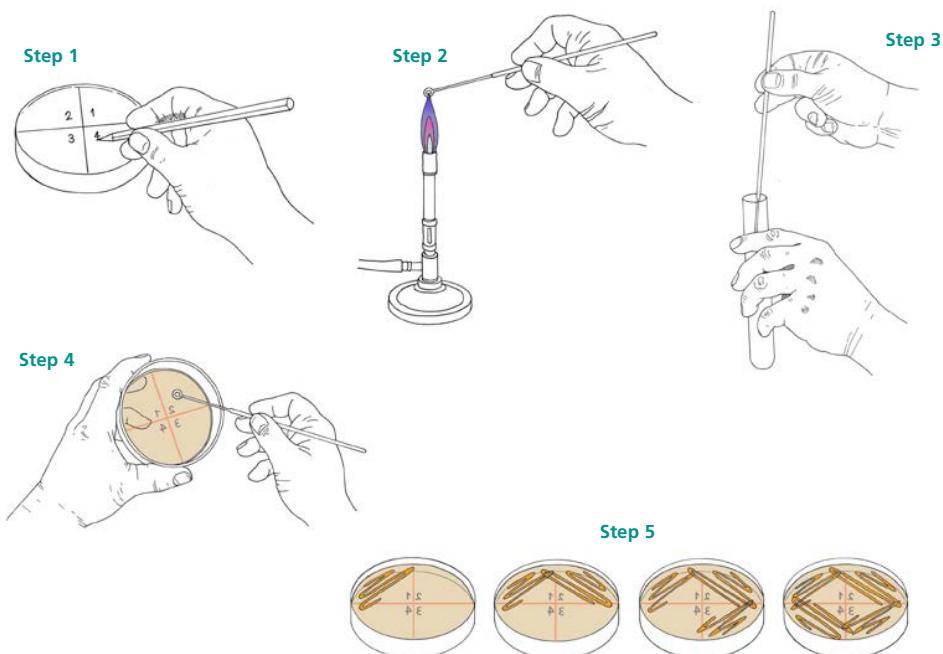
#### METHOD

Streaking is a rapid method used to identify presence of *Vibrio* or bacteria in a culture.

Routine checks using TCBS plates are commonly done in algal cultures and larval/nursery tanks to assess presence of *Vibrio*.

1. Take the number of plates required from the fridge and place into the fume hood until they are at room temperature.
2. Divide and mark plates, as needed. Label each plate with the sample that will be plated and the date (Step 1 in Figure 3.3).
3. Sterilise the inoculating loop using a Bunsen burner (or propane torch) by keeping the loop into the flame until it is red hot. Allow it to cool (Step 2 in Figure 3.3).
4. Insert sterilised loop into tube containing sample and collect a drop of culture (Step 3 in Figure 3.3).
5. Immediately streak the inoculating loop very gently over the space of the plate designated for the sample with a single zig-zag streak (Step 4 in Figure 3.3).
6. Flame the loop again and allow to cool before taking the next sample.
7. Repeat until all your samples have been plated (Step 5 in Figure 3.3).
8. Flame your loop once more for a final sterilisation.
9. Close plate with lid, invert plate and place at 26 °C for 48–72 h in a small countertop incubator.

**FIGURE 3.3**  
Stepwise procedure for streak plate method



### 3.2.5 PROTOCOL: Preparing marine agar plates for assessing bacterial counts

#### MATERIALS

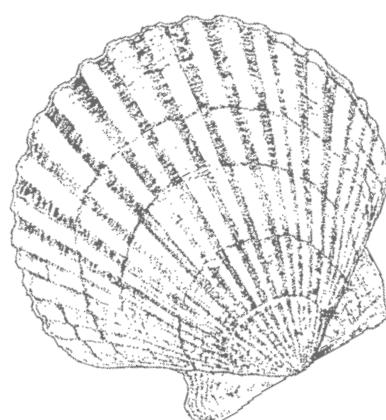
- Scale
- Marine Agar or TSA
- 1 L flask
- De-ionized water (DI)
- Magnetic stirrer
- Magnetic stirrer hot plate
- Steri stoppers or cotton plug
- Aluminium foil
- Graduated cylinder
- Sterile Marine Agar/TSA plates
- Parafilm

#### METHOD

Marine Agar plates supports bacteria growth and is used to assess CFU in probiotic bacteria cultures. Tryptic (trypticase) soy agar (TSA) can also be used.

Manufacturer's instructions should be on Marine Agar container.

1. Weigh the amount of Marine Agar needed for your required volume ( $55.1 \text{ g.l}^{-1}$  DI water).
2. Pour Marine Agar into a 1 L flask.
3. Measure volume of DI water needed.
4. Pour DI water into the flask sides, making sure all the powder falls to the bottom of the flask.
5. Place one magnetic stirrer into the flask.
6. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder (do NOT let it boil over the flask).
7. Plug with steri stopper or cotton and cover with aluminium foil. Label with autoclavable tape.
8. Autoclave at  $121^\circ\text{C}$  for 15 minutes.
9. Leave to cool to  $40\text{--}45^\circ\text{C}$ .
10. Pour into plates, filling around half the plate.
11. Leave for a day, making sure there is no water condensation on the top of the plate.
12. Next day, check for condensation and contamination. If there is none, make packages of 5 using parafilm.
13. Keep the plates upside down and in the fridge.



### 3.2.6 PROTOCOL: Determining colony-forming units (CFU) in bacteria cultures

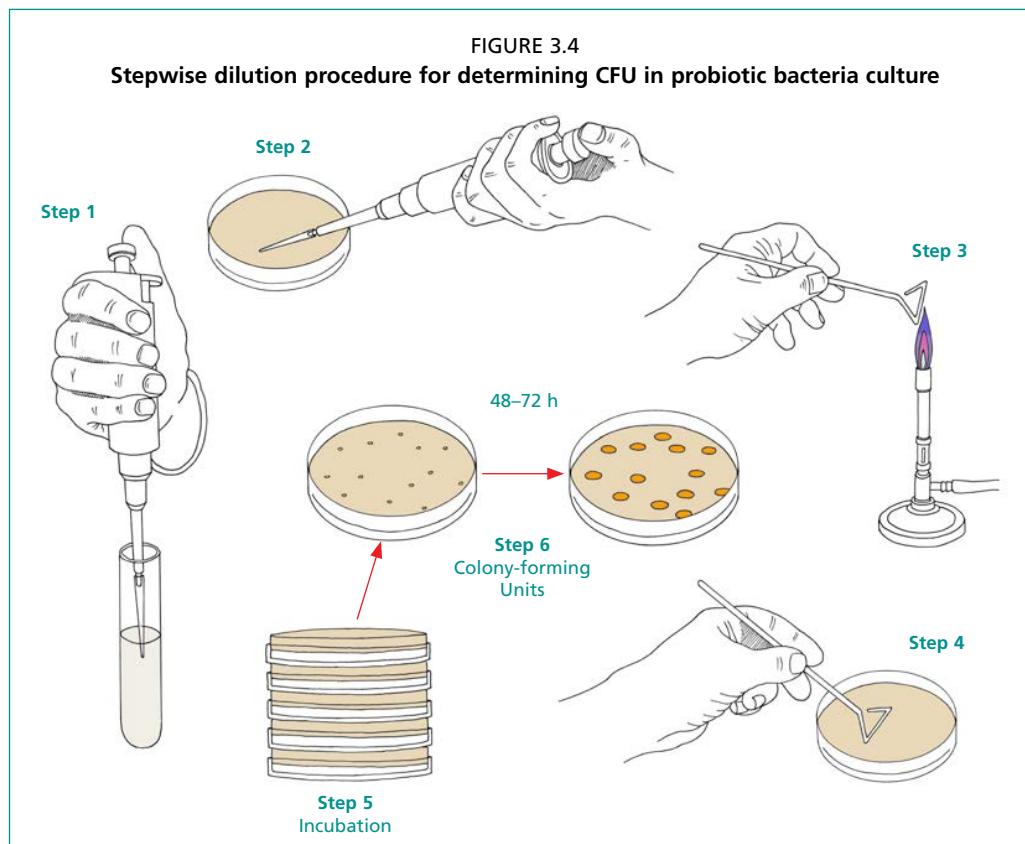
#### MATERIALS

- Fume hood
- Bunsen burner or propane torch
- Four 15 ml test tubes with caps
- 60 ml of Marine Broth
- 10 ml sterile pipettes, 1 pipette bulb
- Marine Agar or TSA plates
- Glass spreader
- Eppendorf pipette and tips
- Autoclaved seawater

#### METHOD

This protocol uses the serial dilution technique where the sample is diluted enough so that bacterial colonies grown on the marine agar plates are differentiated and can be counted.

1. Prepare fume hood and materials. All materials, Marine Broth and seawater is autoclaved prior to subsampling for CFU.
2. Label test tubes: 1:10, 1:100, 1:1 000, 1:10 000.
3. Label TSA plates: 1:1 000, 1:10 000 with date of plating.
4. Add 9 ml of Marine Broth to each test tube with a 10 ml sterile pipette. Always work close to flame, using microbiological techniques. Discard pipette.
5. For 1:10 dilution – add 1 ml of probiotic solution to 15 ml test tube with new sterile 10 ml pipette and mix.
6. For 1:100 dilution – add 1 ml of 1:10 dilution to 15 ml test tube. Screw cap on and mix by inverting test tube gently a few times.
7. For 1:1 000 dilution – add 1 ml of 1:100 dilution to 15 ml test tube. Screw cap on and mix.
8. For 1:10 000 dilution – add 1 ml of 1:1 000 dilution to 15 ml test tube. Screw cap on and mix.
9. Remove probiotic culture and any other material from fume hood for next steps; keep only dilutions (Steps 6–9) and TSA plates.
10. Mix 1:1 000 dilution by inverting test tube a few times and collect 100 µl aliquot using Eppendorf pipette (Step 1 in Figure 3.4).
11. Remove lid from TSA plate, and dispense aliquot in the middle of the TSA plate with matching label (Step 2 in Figure 3.4).
12. Discard Eppendorf tip.
13. Sterilise the glass spreader by dipping it into 70 % ethanol, and then flame it using Bunsen burner (Step 3 in Figure 3.4).
14. Let spreader cool by placing gently on TSA (not directly on probiotic aliquot).
15. Spread the 100 µl aliquot on the plate by moving the spreader around the surface of the plate making a circular motion until all the sample has been absorbed in the plate (Step 4 in Figure 3.4).
16. Close plate with lid. Do not invert plate right away. Let dry 5 minutes to make sure all probiotic sample is absorbed in agar.
17. Once sample is absorbed, place TSA plate upside down in incubator (26 °C) for 48–72 h (Step 5 in Figure 3.4). Temperature for incubation can vary dependent on probiotic bacteria species used.
18. Sterilise glass spreader and repeat Steps 11–18 with 1:10 000 dilution.
19. After incubation, there should be no more than 30–300 colonies on plate (Step 6 in Figure 3.4). Identify colonies of probiotic bacteria and make sure to differentiate with naturally occurring colonies.
20. Count probiotic colonies with a clicker and record.
21. Calculate CFU.ml<sup>-1</sup> as follows: Number of colonies counted per plated volume × dilution factor. Dilution factor for 1:1 000 is 1 000. Dilution factor for 1:10 000 is 10 000.



### 3.3 CO-CULTIVATION OF PROBIOTIC BACTERIA AND MICROALGAE

The compatibility of probiotic bacteria and microalgae species used for food must be assessed prior to introduction of probiotic to entire algal culture system.

#### STEP 1

Assess the compatibility of probiotic bacteria with the microalgal species used in the hatchery, if unknown.

Table 3.2 illustrates the importance of this assessment before integrating probiotic bacteria in algal lines. Information for two strains of *Pseudoalteromonas* is given, and incompatibility with some microalgal species and the difference between strains is seen.

**TABLE 3.2**  
**Compatibility of standard hatchery algal species with probiotic bacteria *Pseudoalteromonas* sp.**  
“C” is compatible; “IC” is incompatible, “NT” is not tested

Algal species	<i>Pseudoalteromonas</i> sp. strain compatibility	
	P02-1	P02-45
<i>Tahitian isochrysis</i>	C	C
<i>Chaetoceros calcitrans</i>	C	C
<i>Chaetoceros gracilis</i>	IC	C
<i>Rhodomonas</i>	NT	C
<i>Tetraselmis</i> sp. (Plat P)	NT	C
<i>Tetraselmis</i> sp. (429)	C	C
<i>Thalassiosira pseudonana</i> (3H)	C	IC
<i>Skeletonema costatum</i>	NT	C
<i>Pavlova lutheri</i>	NT	C

Source: Aquatechnics supplier, 2018.

**STEP 2**

Harvest the probiotic bacteria by removing the Marine Broth in which it is grown. This is done by centrifuging a known concentration of bacteria culture; the bacteria is concentrated, the supernatant is discarded and the remaining bacterial pellet used for inoculating. Bacterial pellets are dissolved in filtered, autoclaved seawater.

For co-cultivation, pellets are used to inoculate intermediate algal cultures using microbiological techniques or are introduced via the algal nutrient pumping system for large-scale culture systems (see 3.3.1 for Protocol on harvest and co-cultivation)

For direct addition to larval and spat culture tanks, pellets are resuspended in seawater prior to addition to tanks to ensure adequate mixing in the tanks.

**How much probiotic bacteria is needed?** – The bacterial isolate will co-cultivate with algae when transferred at concentrations of  $10^6$ – $10^8$  CFU; optimal concentration is dependent on the species of algae. Resulting co-cultures are monitored daily, algal cell density is assessed ( $\text{cells}.\mu\text{l}^{-1}$ ) (see Protocol 3.3.2 for bacteria counts in co-cultures).

During the initial research and development phase, co-cultivated algal culture growth is compared to a standard algal culture without probiotic addition. At least three species of microalgae used in food ration should be tested simultaneously with the same bacteria culture, in order to avoid any uncertainties as to results obtained. Ultimately, all microalgal species used in the hatchery and/or nursery should be assessed for compatibility.

**How often is probiotic bacteria added?** – The goal is to maintain a CFU of  $10^6$  on a daily basis in the co-culture; and this will most likely be achieved through routine injections of probiotic bacteria in the co-culture at least twice a week. Typically, in the 3 days following injection, *Pseudomonas* CFU remains steady around  $250 \times 10^6$ ; and in some cases increases up to  $10^9$  CFU; if there is no additional injection, a rapid decrease in CFU is seen, with no probiotic bacteria detected 5 days post-injection. This has been reported in the co-culture of three algal species (*Tetraselmis* sp., *Chaetoceros muelleri* and *Pavlova lutheri*) during preliminary trials.

To achieve success, the emphasis is on the cleanliness of the algal culture; for algal cultures where naturally occurring bacteria level is high, co-cultivation of probiotic bacteria will most likely be unsuccessful, and natural bacteria will prevail.

**Differentiating bacteria species** – The aquaculturist will establish a database with photos to facilitate the identification of probiotic bacteria. Identification is based on shape and colour; photos are useful to differentiate with any naturally occurring bacteria species in microalgal cultures and/or seawater (see Section 3.3.3).

### 3.3.1 PROTOCOL: Scaling up probiotic bacteria culture, harvesting and co-culturing with microalgae

MATERIALS
<ul style="list-style-type: none"> <li>- Fume hood</li> <li>- Centrifuge</li> <li>- 1 L flasks (autoclaved)</li> <li>- 500 ml flasks (autoclaved)</li> <li>- Marine Broth (autoclaved)</li> <li>- 12 centrifuge tubes and lids (50 ml)</li> <li>- Clean and healthy 4 L and 20 L algal culture</li> <li>- Steri stoppers or cotton plugs</li> <li>- Aluminium foil</li> <li>- Sterile pipettes (25 ml)</li> <li>- Bunsen burner or propane torch</li> <li>- 1 L sterile 1 µm filtered seawater</li> <li>- 1 L sterile harvest flask (dry)</li> <li>- 500 ml probiotic stock cultures</li> </ul>
METHOD
<p><b>IMPORTANT</b></p> <p><b>Marine Broth in probiotic culture has to be discarded when harvesting bacteria and before transfer to algal culture for co-cultivation</b></p> <p><b>Note:</b> Probiotic cultures can be used to inoculate individual algal vessels (4 to 20 L) or a continuous culture system by injection into nutrient supply. As is recommended for algal cultures, the greater the volume of the inoculum, the faster the growth of the culture. A 1-week old 500 ml culture can reach <math>1.9 \times 10^7</math> CFU in one week, compared to <math>5.2 \times 10^6</math> CFU in two weeks with a lower volume inoculum.</p> <p><b>Scaling up probiotic bacteria cultures from 500 ml to 1 L</b></p> <ol style="list-style-type: none"> <li>1. Prepare previously autoclaved 1 L flasks with 600 ml Marine Broth.</li> <li>2. Working under the fume hood near the flame, and using sterile microbiological techniques, transfer 200 ml of starter culture into 1 L flask containing Marine Broth. Close and label. Grow for 2 weeks or until CFU reaches <math>10^6</math></li> <li>3. Divide remaining starter culture into two. Transfer half into a new 500 ml flask containing Marine Broth. Close and label accordingly. Repeat with second half; these are your new duplicate starter cultures; they are used for re-inoculating 1 L harvest cultures if these become contaminated.</li> <li>4. Harvest cultures are sub-cultured for co-cultivation with algae.</li> </ol> <p><b>Harvesting probiotic bacteria (from 1 L flask)</b></p> <ol style="list-style-type: none"> <li>5. To maintain 1 L cultures for continuous harvest, re-inoculate new 1 L flasks with 600 ml Marine Broth at every harvest.</li> <li>6. Place 1 L new flasks with 600 ml Marine Broth under the fume hood.</li> <li>7. <b>Re-inoculate new 1 L flask:</b> Working near the flame, collect 50–100 ml of probiotic culture (<math>10^6</math> density at 10–14 days old) from old 1 L starter cultures.</li> <li>8. Transfer inoculum to new 1 L flask with Marine Broth. Grow for 10–14 days or until CFU reach <math>10^6</math>. This will be used for the next harvest.</li> <li>9. Remaining probiotic culture from old 1 L flask will be used for co-cultivation with algae.</li> <li>10. <b>Harvest bacteria:</b> Collect 45 ml of probiotic bacteria culture from remaining 750 ml harvest culture using a graduated 50 ml pipette.</li> <li>11. Dispense 45 ml into centrifuge tube and close tube with screw cap. Repeat to centrifuge entire harvest culture. The number of centrifuge tubes to be filled depends on available spaces in the centrifuge.</li> <li>12. It is important to have equal measures in each centrifuge tube in order to keep centrifuge balanced.</li> </ol>

### 3.3.1 PROTOCOL (continued)

13. Alternatively, if probiotic culture is very concentrated, collect 17.5 ml of probiotic culture and dilute each test tube with 17.5 ml Marine Broth. This will allow for better precipitation and pellet formation during centrifuging.
14. Place tubes at opposite ends of centrifuge to keep it balanced.
15. Set centrifuge to 4 000 RPM for 10 minutes.
16. Once done, remove tubes from centrifuge.
17. Decant the supernatant, making sure not to disturb the probiotic bacteria pellet or precipitate.
18. Re-suspend the pellet in the same tube using sterile seawater (if <95 % of supernatant has been discarded, an additional rinse with sterile seawater will eliminate any remaining Marine Broth).

#### Inoculate single vessel microalgal culture

19. Working close to the flame, transfer bacteria/seawater solution into selected algal culture vessel.
20. Grow co-cultivated bacteria and algae under same conditions as algal cultures.
21. Monitor daily, collect a subsample to determine CFU and assess algal density. This will be an indicator of growth for both probiotic bacteria and microalgae.
22. Compare to algal growth in vessels with no probiotic addition.
23. Steps 7–23 can be followed as part of the screening process to assess the performance of the probiotic bacteria species, and the quantity of bacteria needed in co-cultivation. As a starting point, for a single 500 L bag, approximately 250 ml of probiotic bacteria culture is centrifuged.

#### Introduce probiotic bacteria to continuous large-scale microalgal system

24. Wash bacteria/seawater suspension (Step 19) into a 2 L sterile harvest flask. This is sufficient for a 150 000 L algal production system.
25. Pour harvested probiotic bacteria in nutrient reservoir supplying all algae.
26. Once both bacteria and algae reach optimal densities, feed to larvae or spat as per standard procedures.
27. Monitor CFU in larval and spat tanks three times a week.
28. Harvest probiotic bacteria when required and add to algal system to maintain CFU in co-cultivated algae. For a 150 000 L continuous algal system, this is done every 3.5 days.
29. Compare scallop growth and survival when fed co-cultivated probiotic bacteria/algae to standard techniques and assess effectiveness of probiotic bacteria.



### 3.3.2 PROTOCOL: Determining bacteria counts in co-cultures

MATERIALS
<ul style="list-style-type: none"> <li>- 15 ml test tubes</li> <li>- TSA plates</li> <li>- Eppendorf pipette and tips</li> <li>- Sterile seawater</li> </ul>
METHOD
<p><b>Note:</b> Several TSA plates are prepared, comparing full concentration of the co-culture and diluted co-culture samples. If concentration of probiotic bacteria is high, it may be too difficult to count the colonies in a fully concentrated sample. The aliquot volume subsampled can also be adjusted dependent on the expected probiotic concentration.</p> <ol style="list-style-type: none"> <li>1. Collect a sample of algal culture in a sterile 15 ml test tube, labelled with algal culture/Date plated/Day after injection of probiotic/Full (“Full” indicates full concentration of co-cultivated sample).</li> <li>2. Label one more test tube similarly for a 1:10 dilution of co-cultivated sample.</li> <li>3. Label three plates adjusting the aliquot volume plated, resulting in labels. For example: <ul style="list-style-type: none"> <li>- Algal culture/Date plated/Day after injection of probiotic/0.1 ml/Full (for 0.1 ml aliquot of fully concentrated co-cultivated algae).</li> <li>- Algal culture/Date plated/Day after injection of probiotic/0.1 ml/1:10 (for 0.1 ml aliquot of diluted co-cultivated algal sample).</li> </ul> </li> <li>4. Add 9 ml of sterile seawater to test tube labelled “1:10”.</li> <li>5. Add 1 ml of co-cultivated algal culture into 1:10, 15 ml test tube and mix.</li> <li>6. Collect 100 µl from co-cultivated algal test tube labelled “Full”.</li> <li>7. Remove lid from TSA plate and dispense aliquot on TSA plate labelled accordingly.</li> <li>8. See Protocol 3.2.6 for details on plating.</li> <li>9. Repeat collecting 100 µl from co-cultivated algal test tube labelled “1:10”.</li> <li>10. Once all plates are completed and probiotic bacteria samples are absorbed by agar, place TSA plate upside down in incubator (26 °C) for 48–72 h. Incubation temperature depends on probiotic species and can be as high as 32 °C.</li> </ol>

### 3.3.3 Notes on identifying probiotic bacteria colonies

Bacteria colonies differ in appearance, size and colour, and the aquaculturist will have to learn to differentiate probiotic bacteria from naturally occurring bacteria. Here is an example on the approach used for the identification of bacteria colonies using a *Pseudoalteromonas* probiotic seed culture.

1. This seed culture has two distinct bacteria: Large orange and large creamy.
2. Algal culture bags have naturally occurring bacteria, which can be whitish pinhead size colonies.
3. To determine CFUs in probiotic cultures: A series of dilutions in Marine Broth is needed.
4. To determine CFUs in algal culture, a Fully concentrated sample and 1:10 dilution is required until the standard operating procedure is established.
5. A description of the probiotic bacteria colonies based on size, shape, colour, texture assists the technicians in the identification. In our example: probiotic bacteria colonies can be described as large, round, slightly raised, opaque, and a buttery surface.

6. The time of incubation varies among bacteria species. It takes 48 h of incubation for *Pseudoalteromonas* colonies to fully grow (at 26 °C), but 24 h for naturally occurring pinhead size bacteria.
7. Photos can be used as a reference to identify CFU in algal cultures and larval tanks (Figure 3.5).

FIGURE 3.5

**Colony-forming units of probiotic bacteria on TSA plates – (a) too many colonies to count; top 1:10, bottom 1:1 000; (b) co-cultivation in continuous culture (500 L) showing probiotic bacteria colonies and naturally occurring bacteria in algae; post-inoculation (left), Day-1 after inoculation (right); and (c) top: pure probiotic culture 1:10 000 dilution; bottom: co-cultivated in 20 L and 3 L batch culture full concentrations (left and right)**

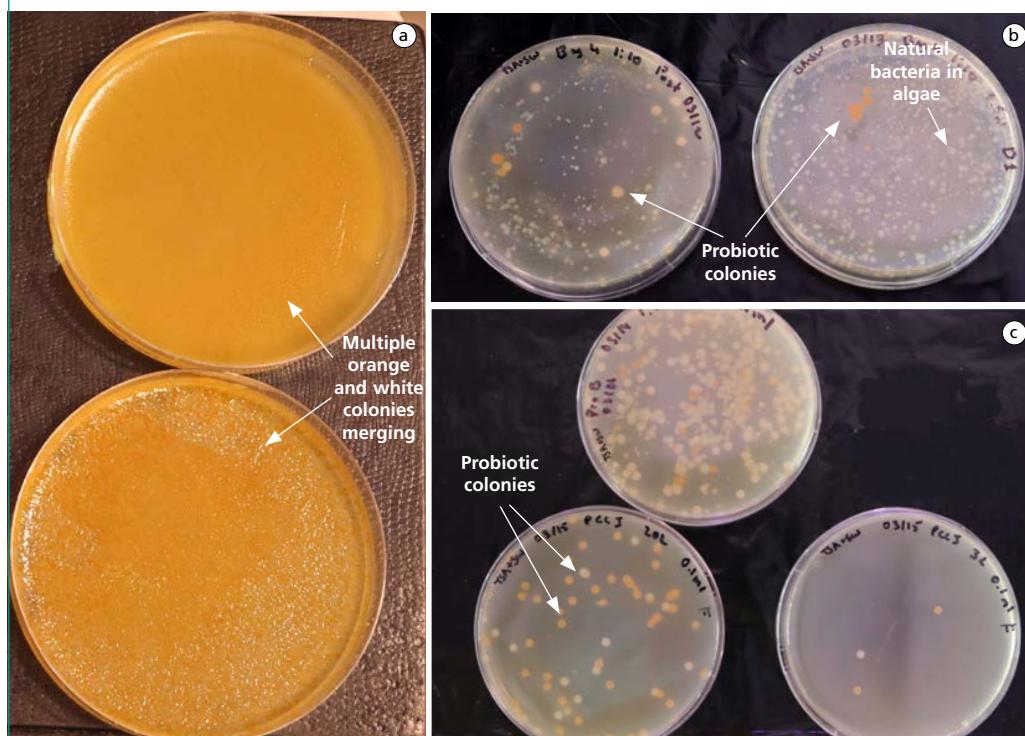


Figure 3.5 illustrates the challenge in assessing the dilution factor which enables differentiation of colonies and assessment of CFU; and in distinguishing between probiotic bacteria and naturally occurring bacteria found in algal cultures. Figure 3.5a shows plates with complete coverage of probiotic bacteria colonies illustrated by a uniform orange plate; even a dilution of 1:1 000 (bottom plate) results in too many colonies, which have merged and make it impossible to count. Figure 3.5b illustrates probiotic bacteria co-cultivated in continuous 500 L algal vessel plated post-inoculation of bacteria (see left plate) and Day-1 after inoculation (see right plate) both at 1:10 dilution. Probiotic bacteria colonies are distinct in post-inoculation plate (Figure 3.5b left) with some pinhead-size natural bacteria seen; this indicates the presence of naturally occurring bacteria in algal culture. In Day-1 plate, naturally occurring bacteria are seen in increasing numbers whereas probiotic bacteria colonies numbers decreased. This potentially indicates an unfavourable environment for probiotic bacteria overtaken by the naturally occurring bacteria. On the other hand, probiotic bacteria co-cultivated in small batch cultures illustrate a cleaner culture with undetected naturally occurring bacteria (Figure 3.5c). Top plate in Figure 3.5c is a pure probiotic bacteria culture illustrating a high CFU but possible to count at a dilution of 1:10 000.

### **3.4 INTEGRATING PROBIOTIC BACTERIA TO LARVAL AND POST-LARVAL CULTURE SYSTEMS**

**Initiate trials** – Dedicate replicate larval and post-larval tanks for first trials. Add co-cultivated microalgae on daily basis as per standard feeding, monitor growth and survival of scallops, and compare with culture tanks fed standard microalgae. The goal is to achieve comparable, if not better, growth and survival without the use of antimicrobial drugs.

Probiotic bacteria species will differ in their effect on *M. yessoensis* early stages. Switching to a different probiotic bacteria species is recommended if co-cultivation and/or larval and spat responses are poor.

As a starting point, real life preliminary trials starting with Day-6 *M. yessoensis* larvae using *Pseudoalteromonas* indicated comparable growth and survival rate between probiotic and control batches with a survival rate >95 percent for larvae fed probiotic-enriched algae.

## 4. Hatchery facility layout

**IN THIS CHAPTER –** A conceptual design for a hatchery complex including all sectors of seed production is illustrated.

A brief overview of the hatchery complex is illustrated in Figure 4.1. Coarsely filtered seawater is pumped to the hatchery complex and treated according to the needs of the various scallop life stages. Any large equipment dedicated to seawater treatment, such as heating or cooling, protein skimmers and/or UV sterilisation are maintained separate from scallop and algal culture vessels. Smaller filtration and/or sterilisation units are frequently installed inline in relevant hatchery sectors. There are four main sections to the hatchery facility: broodstock, hatchery (larval culture), nursery (post-larvae and spat culture), and the algal culture areas. All are isolated from one another in order to avoid cross-contamination; biosecurity in a hatchery complex is a major component of successful seed production. Within the algal sector, additional precautions are taken to isolate stock, starter and intermediate cultures from large algal cultures. A dry laboratory area is needed in both the larval and algae sector. Design and construction for scallop or bivalve hatcheries are given in great detail in other manuals (see Further reading list).

### 4.1 SEAWATER TREATMENT PER SECTOR

**Broodstock** – Seawater is coarsely filtered. Temperature is ambient or according to conditioning cycle selected. This requires chilling and heating the incoming seawater at different stages of the conditioning.

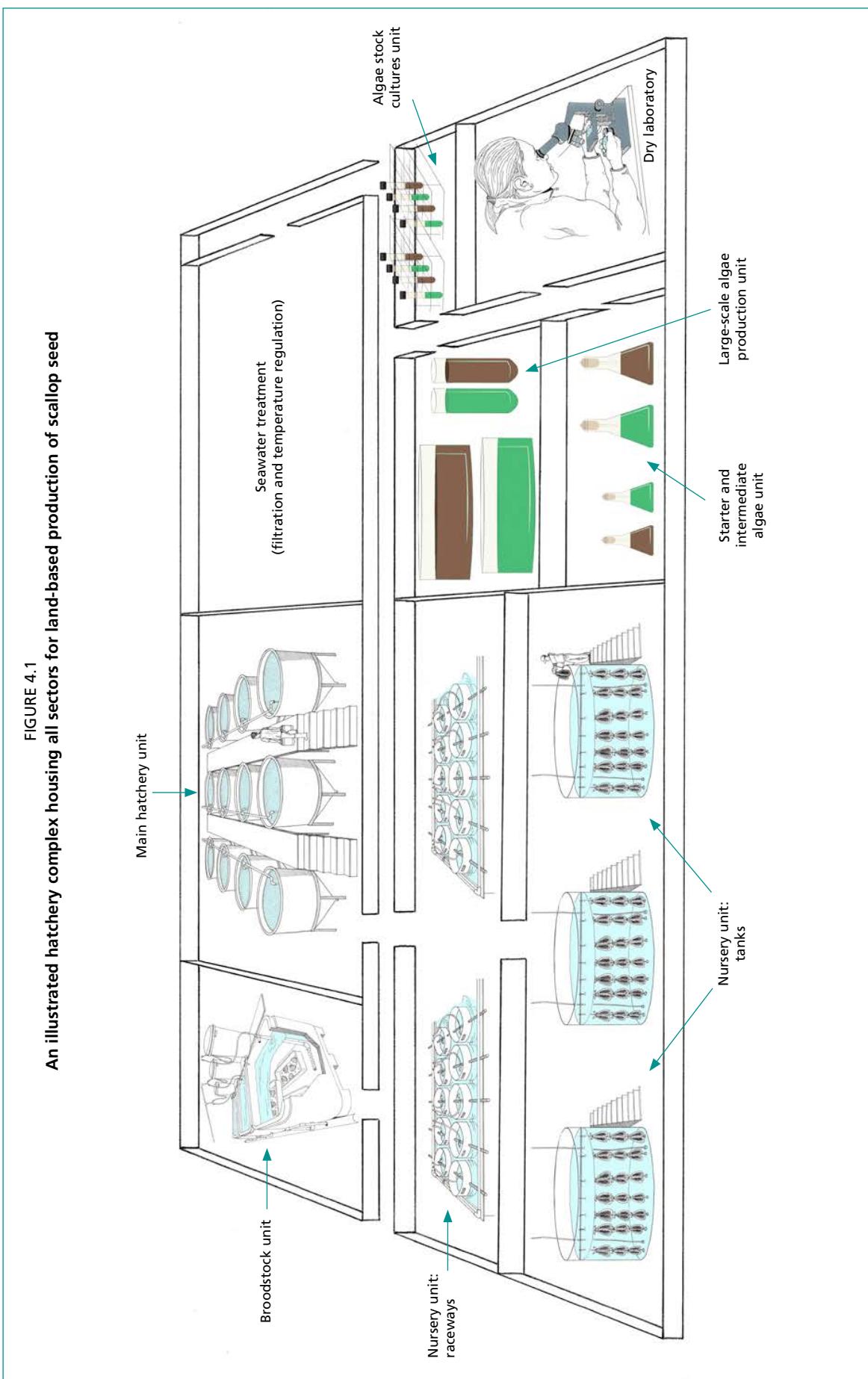
**Hatchery** – Seawater is at minimum filtered to 1 µm and in some cases sterilised using UV or equivalent. Rearing temperature for *M. yessoensis* ranges from 12–14 °C and requires heating of the incoming seawater.

**Nursery** – Seawater is initially filtered as for the hatchery; filtration is reduced to 10 µm within one month of settlement. Seawater temperature is as for larvae, and requires heating of the incoming seawater; the capacity to reduce temperature by 1 °C in tanks with a continuous flow of seawater to ambient is needed for acclimatation at the end of the nursery period.

**Algal cultures** – Seawater is sterilised for all stages (stock to large-scale); UV is used for all; chemical sterilisation can be used for larger round batch tanks. Optimal temperature range for culturing algae is 22–24 °C.

**Intermediate nursery** – This is not shown in Figure 4.1, but seawater for intermediate outdoor nursery tanks or ponds is coarsely filtered using a sand filter or equivalent. Temperature is at ambient.

All sectors require a continuous air supply; this is further filtered by inline filters to individual vessels for the algal sector and for the hatchery. If air quality is poor, inline air filters are also added to the nursery sector.



## 5. Broodstock holding and conditioning

**IN THIS CHAPTER –** How to maintain or condition broodstock to ensure reliable spawning in the hatchery and how to implement a controlled spawn.

Mature scallops, both male and female, are required for spawning under controlled conditions, producing gametes and D-larvae for the commencement of the larval rearing cycle.

**Yesso scallop gametogenic cycle** – The seasonality of the gametogenic cycle is site and temperature dependent. Generally, Japanese scallops show active gametogenesis at seawater temperatures of 4 °C, increasing with temperatures of 6 °C.

**Obtaining mature broodstock** – There are two main approaches to obtaining mature broodstock ready for spawn.

1. *The aquaculturist relies on the natural gametogenic cycle.* This is supported by favourable environmental factors and is the simplest and less costly approach. It is used for species, which exhibit a distinct reproductive cycle and spawn naturally several times a year. This is achieved by collecting naturally ripened broodstock from the farm one week prior to natural spawn.

To facilitate collection by farm personnel, the hatchery manager identifies a healthy stock of reproductively mature scallops; these are maintained on a dedicated longline with labelled nets. Ripe broodstock can be held at T = 6 °C in the hatchery for up to 14 days before spawning if fed daily. A few days before spawning, seawater temperature in the broodstock tank is gradually increased by 1 °C every two days until it reaches 8 °C; scallops can be maintained at this temperature for up to one week before spawning, but careful monitoring is required at this stage to detect and/or prevent spontaneous spawning. Although raising the temperature to 8 °C does not result in a marked increase in gonadic weight, it seems to enhance egg quality and larval viability.

Feeding is stopped 24 h prior to spawning; this minimises the amount of faecal material excreted during spawning and yields a cleaner solution of eggs; in turn, this reduces the risk of bacterial contamination during embryogenesis.

2. *The aquaculturist controls the reproductive cycle.* This is most useful for species, which only spawn naturally once a year, have unpredictable spawning timing, and/or inhabit inconsistent natural environmental conditions. Conditioning broodstock enables the timing of spawning and allows to extend the natural spawning season; the disadvantages of conditioning are that it requires additional labour, space and feed volumes. Conditioning broodstock is achieved by collecting broodstock individuals which have started gametogenesis from the farm.

In the hatchery, the aquaculturist manipulates temperature and food ration to accelerate the gametogenic process. Details are given in Section 5.3.

A mix of the two approaches is usually the strategy adopted by most commercial hatcheries.

## 5.1 REPRODUCTIVE CYCLE OVERVIEW

**Gonad development** – Gonad weight in adult scallops increases to a maximal value pre-spawn; this requires a substantial amount of energy, especially for female gametes (or eggs). Energy is initially obtained directly from the external food source through the digestive gland; as gametes mature – or when food supply is scarce – energy for gonad development is obtained from the reserves stored in the adductor muscle. If the natural reproductive cycle is not known, it is strongly advised to assess the gonadic and muscle indices on a monthly basis for the first 12 months. Indices are based on gonad and tissue weight; their relative fluctuations on a yearly basis provides a trend as to the changes in reserves for both tissues, and an indication of gamete ripeness. A high gonadic index is indicative of mature gonads, and a lower index reflects the onset of gametogenesis or spent gonads. A simultaneous monthly assessment of gonadosomatic indices and visual stages over a 1-year period will validate the latter; this will allow the aquaculturist to select ripe broodstock based on rapid visual assessments in subsequent years. For a more in-depth scientific analysis, cytological changes can be examined in gametes using histology to assess gamete development in a definitive manner. This is not usually conducted in a commercial hatchery environment.

### 5.1.1 Determining gonadosomatic indices

**Gonadic index (GI) and muscle index (MI)** – Using GI and MI to assess the gametogenic cycle is a simple, fast and inexpensive procedure which provides reliable quantitative information on gonad stage and mobilisation of reserves. Expressing indices in terms of shell weight is recognized to be most accurate; such that gonadic and muscle indices can be expressed as:

$$\text{Tissue wet weight} \div \text{dry shell weight} \times 100$$

or

$$\text{Tissue dry weight} \div \text{dry shell weight} \times 100$$

Dry weight is more accurate; however, a comparable trend can be obtained using wet weight, if a drying oven is not available. Other methods can be used where indices are determined based on “soft tissue weight” rather than shell weight; however it is recognized that using shell weight is more accurate. See Protocol 5.1.3 for determining GI and MI.

Seawater quality at site of broodstock sampling is determined during collection; this information assists in understanding seawater conditions favourable to natural gametogenesis, namely temperature and serves to implement and adapt a conditioning programme, if needed. A professional multiparameter meter (e.g. YSI™ Pro Plus meter) is sufficient for obtaining basic data such as temperature, salinity, pH and oxygen in the field.

### 5.1.2 Visual assessment

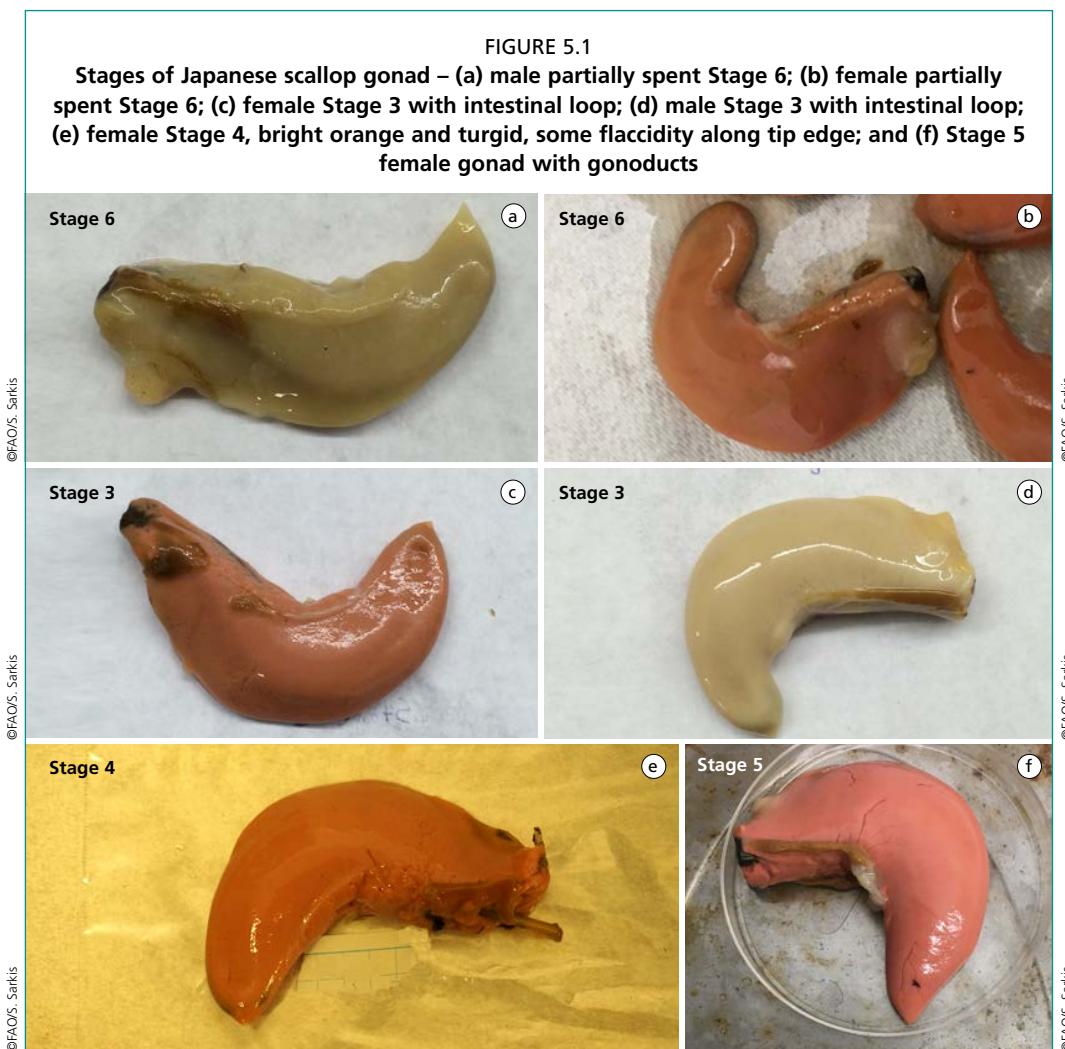
**Gonad visual assessment** – Visual assessment of gonads is common practice in hatcheries, because it is a non-destructive, rapid method and can provide a rough estimate of the gonadal status when conducted by an experienced technician. However, this is a subjective method and is not as accurate as tissue indices. Ideally, for the selection of broodstock for a scheduled spawn or for a conditioning regime, tissue indices are conducted on a subsample for an overview of the gametogenic stage of the whole stock and visual assessment is used to separate the broodstock according to level of ripeness. Table 5.1 provides the description of stages in a visual scale; the number

of stages within a scale and consequently its defining characteristics may vary slightly according to the preference of the aquaculturist. Photos of the Yesso female gonads are given in Figure 5.1 with visual stages subjectively attributed by an aquaculturist. See Protocol 5.1.4 for steps to assess gonad stages visually.

**TABLE 5.1**  
**Description of arbitrary visual index assessing reproductive status of adult scallops**

Visual Index	Description	Gonad condition
0	Gonad is completely empty. Gametes are absent or largely so. Not possible to differentiate sex. Gonad is small, thin, flaccid and translucent. Intestinal loop is clearly visible.	Immature or spent
1	Gonad is translucent, white to grey. Slightly possible to differentiate sex.	"
2	Gonad is small, but pale. Sex is clearly differentiated: male (white) and female (orange). Intestinal loop is visible.	Active
3	Gonad is larger than visual grade 2 and increasing in turgor. Gonad is less granular in appearance. Intestinal loop is partially obscured.	"
4	Gonad is large and turgid. Ovarian tissue appears brighter, uniform in colour and texture. Very little of intestinal loop is visible (usually only a small portion at the distal extremity of the gonad).	"
5	Gonad is very large and thick as if ready to burst. Ovarian tissue is bright, uniform in colour, glossy and highly turgid. Gonucts are usually large and conspicuous. Intestinal loop is not visible.	Ripe
6	Gonad is reduced in size compared to visual grade 4 and 5 and has lost turgor. Ovary appears mottled or lattice-like (as if partially emptied). Intestinal loop is usually visible but not always.	Partially spent

Source: Adapted from Williams, 2005.



### 5.1.3 PROTOCOL: Determining gonadic and muscle indices

#### MATERIALS

- Dissecting kit
- Scale ( $\pm 0.1$  g)
- Absorbent paper
- Petri dish
- Vernier caliper

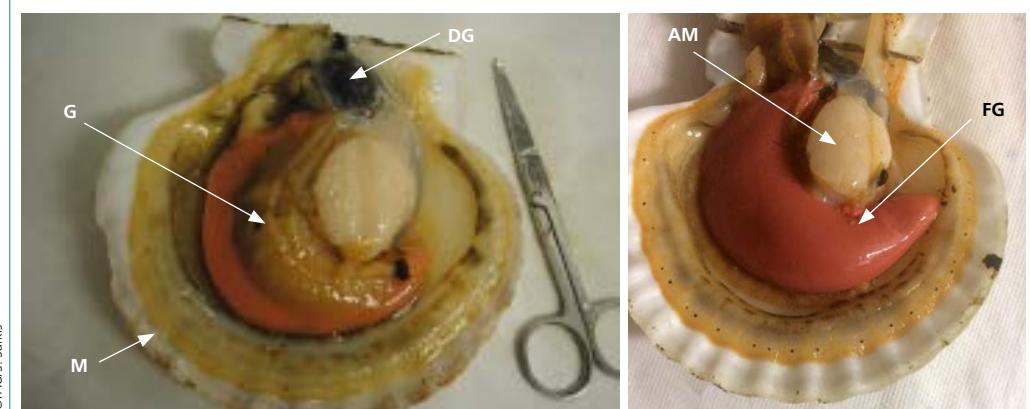
#### METHOD

##### Preparation of dissection

1. Set up balance, dissection kit with scissors, forceps and scalpel, and Vernier caliper for measuring scallops. Use a waterproof notebook to record all information.
2. A minimum of 10 females from each site or conditioning tank should be dissected. Males are only dissected, if there are any uncertainties or for a more scientific and comprehensive analysis.
3. Keep track of scallop source; attribute each scallop a number and label according to site or tank from which it is collected.
4. Record date of collection and dissection in laboratory book (or “lab book”).
5. In the lab book, organize columns for scallop number, collection site or tank number, total shell weight (g), wet gonad weight (g), wet muscle weight (g), rest (rest of tissue wet weight in g), empty shell weight (g), and shell height (mm).

##### Dissection procedure (wet weight)

1. Work with 2 or 3 scallops at a time.
2. Place scallops convex bottom valve on a counter to allow valves to open.
3. Tare balance to read zero.
4. Blot dry each scallop with absorbent paper, wipe off any detritus accumulated on shell, and place on balance for total wet weight. Record data in lab book next to appropriate scallop number.
5. When a scallop is seen to gape, slide the scalpel in the opening close to hinge of the scallop, and staying as close to upper flat shell as possible, cut the adductor muscle from upper shell.
6. Separate the two valves and lay the convex bottom valve on counter for dissection.



**G** = gills; **DG** = digestive gland; **M** = mantle; **FG** = female gonad; **AM** = adductor muscle

### 5.1.3 PROTOCOL (continued)

7. Cut the adductor muscle from the bottom shell, scraping as closely to shell as possible for a clean cut (Figure 5.2 – Step 1).
8. Drain excess water before weighing and blot dry.
9. Lift the gills and using forceps hold the gonad.
10. Separate the gonad from the muscle by cutting around the muscle using scissors (Figure 5.2 – Step 2).
11. Cut gonad from digestive gland. Cut vestigial foot from gonad. Cut the gill from gonad.
12. Record if male or female (male-white, female-orange). If gonad is not developed and sex cannot be assessed, record as “undifferentiated”.
13. Tare clean petri dish on balance. Balance reads 0.00 g.
14. Blot gonad dry with paper towel. Place gonad on petri dish and record weight.
15. Wipe petri dish clean, tare again.
16. Cut the mantle around the muscle to remove the muscle. Make sure you have all muscle tissue from ventral (bottom) and dorsal (upper) shell (Figure 5.2 – Step 3).
17. Blot muscle dry with absorbent paper.
18. Place muscle on petri dish and record weight.
19. Wipe petri dish clean, tare again.
20. Scrape all other tissue from both shells. Blot as dry as possible.
21. Place on petri dish and record weight under the “Rest” column to obtain total soft tissue weight.
22. Take both ventral and dorsal shells, dry with paper towel.
23. Tare balance to zero and weigh empty shells.
24. Reconstruct scallop (flat shell on bottom), and measure height (hinge to opposite side) with a Vernier caliper to  $\pm 0.1$  mm. Record.
25. Repeat procedure for remaining scallops in sample.
26. **For conditioned scallops:** The number of scallops sub-sampled must be large enough to give the aquaculturist an indication of the conditioned stock, but does not have to be statistically representative, as this would deplete the broodstock available for spawning.
27. Subsample a few scallops from each conditioned tank and repeat Steps 1–24.
28. Clean all equipment thoroughly, especially balance, dissecting tools and Vernier caliper to prevent any salt corrosion.
29. Enter data as shown in the worksheet template (Appendix I).
30. Dissected gonads and muscles can be placed in a labelled ziplock bag and stored in a freezer to weight at a later date, if needed.



©FAO/S. Sarkis



©FAO/S. Sarkis

### 5.1.3 PROTOCOL (continued)

#### Calculating indices

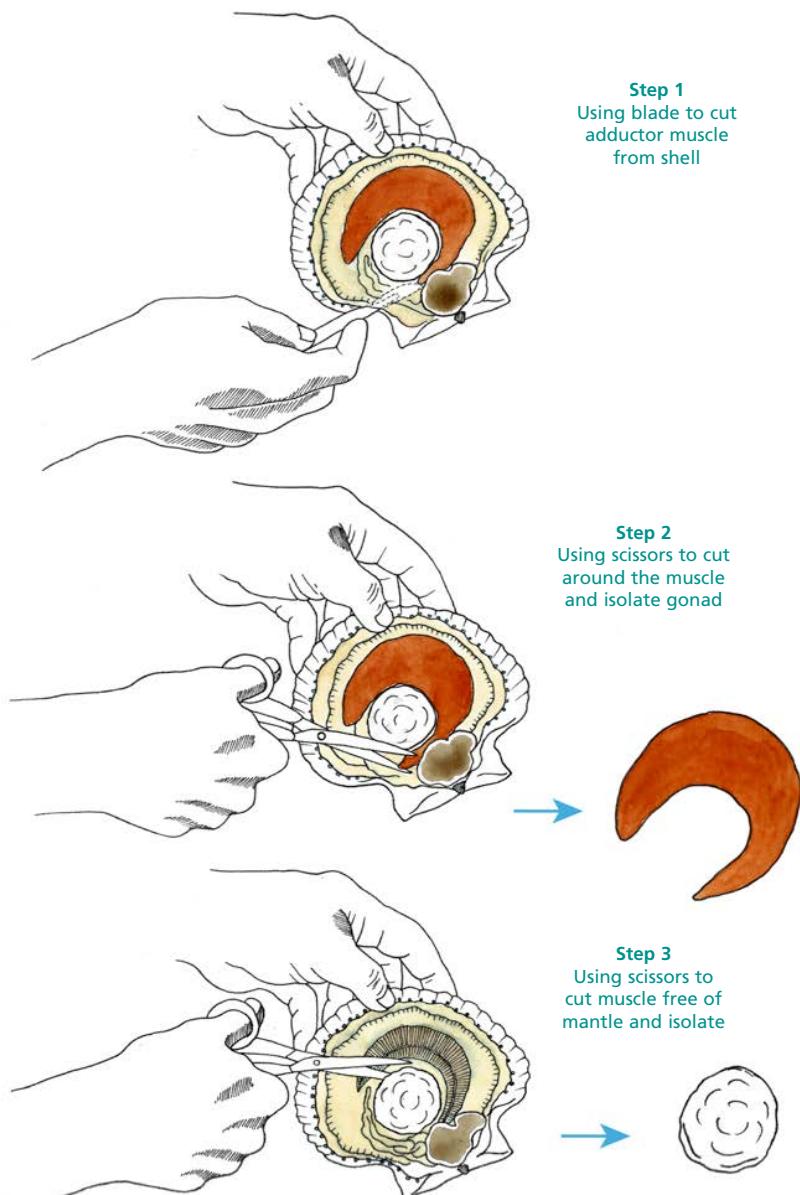
1. Data is entered in Excel sheet
2. Wet Gonadic Indices (GI) are calculated as:  

$$\text{gonad weight (g)} \div \text{empty shell weight (g)} \times 100$$
3. Wet Muscle Indices (MI) are calculated as:  

$$\text{muscle wet weight (g)} \div \text{empty shell weight (g)} \times 100$$

**Note for dry weight indices:** The same procedure is followed, but organs are dried in a drying oven at temperature of 60–80 °C for 48 h or until constant weight, before weighing.

**FIGURE 5.2**  
**Dissecting gonad and muscle from mature scallop to determine gonadic and muscle indices**



### 5.1.4 PROTOCOL: Assessing reproductive status visually

#### MATERIALS

- Light torch
- Zip tie to maintain valves opened
- Sub-sample of scallops

#### METHOD

1. Collect a subsample of the broodstock (females >85 mm shell height).
2. Expose to air.
3. When valves open, place a round smooth object between the two valves (a zip tie) to maintain valves opened long enough for a visual inspection of the gonad.
4. Use a light torch to shine onto the gonad.
5. Check the gonad against photos in Figure 5.1 and inspect the gonad for:
  - Intestinal loop – progressively less clear as gonads ripen.
  - Uniformity in colour – if gonad appears mottled or lattice-like or interspersed with isolated specs of translucent sections (or acini), it has likely partially spawned or atrophied due to poor conditions. If it is uniform in colour but pale, it is in early development stage.
  - Colour, brightness of gonad – the deeper red in colour and brighter, the closer is the female gonad to ripeness.
  - Size of gonad – as it ripens, gonad will appear increasingly rounder and more swollen; a flaccid, flat or thin gonad is not close to ripeness stage.
  - Gonucts – these are very obvious in ripe gonads.
6. Give a visual stage number to gonad as per the visual scale in Table 5.1.



Male Yesso scallop

©FAO/S. Sarkis



Female Yesso scallop

©FAO/S. Sarkis

**Note:** A visual stage of 3 represents a GI of 18 (based on wet tissue weight/dry shell weight). Spawning is induced with a minimum visual stage >3.5 (GI > 20) to obtain viable larvae.

## 5.2 HOLDING BROODSTOCK

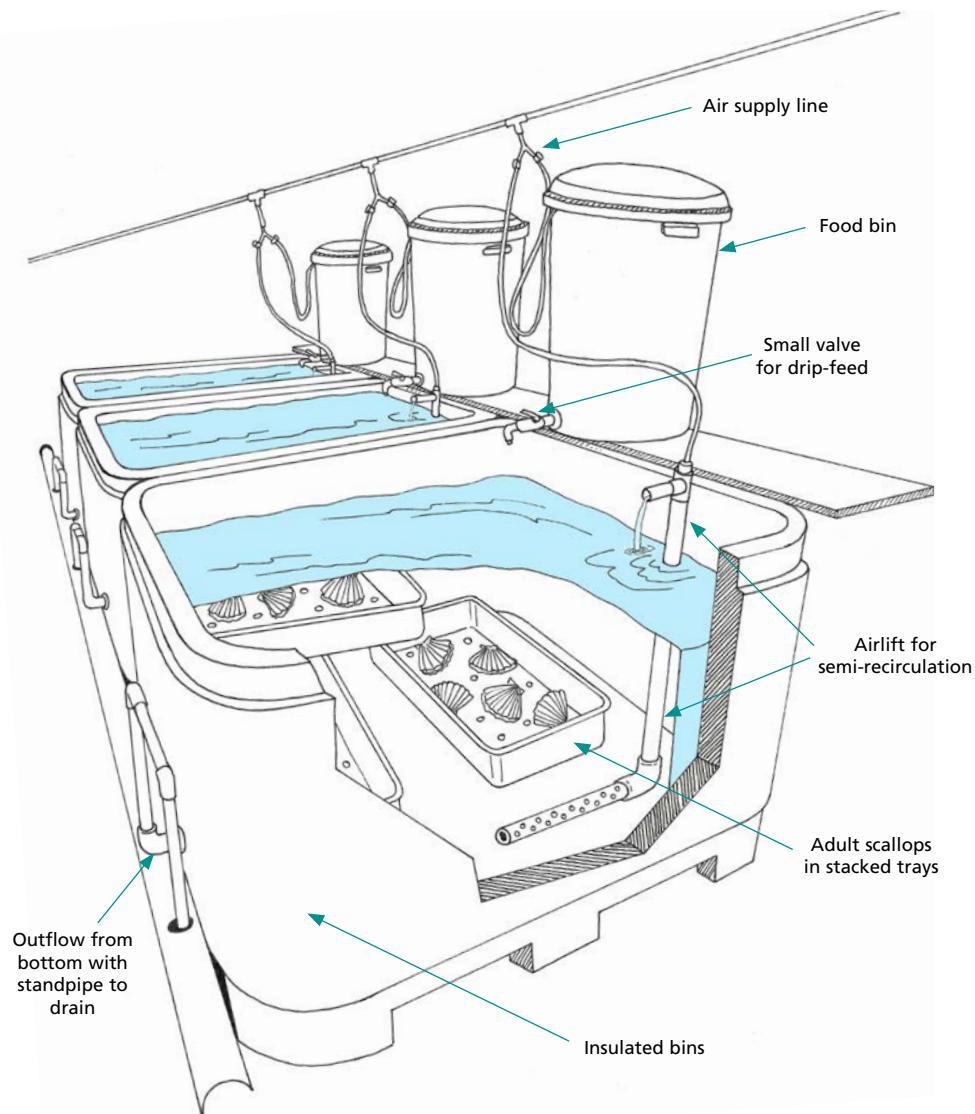
**Water flow and temperature** – Broodstock is kept in coarsely filtered seawater, typically in an open flow-through or semi-recirculated tank system. The key to an efficient broodstock tank system is to ensure a) a good seawater flow providing a 100 percent exchange of seawater every 2 h, b) the ability to control the seawater temperature to  $\pm 1^{\circ}\text{C}$ , and c) the ease of maintaining bottom of tank clean through removal of faecal material produced by broodstock feeding.

**Tank systems** – Dependent on the level of conditioning and broodstock selection, broodstock tank systems can be simple flow-through tanks, or more complex compartmentalised systems. As a guide, broodstock tanks approximating 600 L, with a 100 percent exchange of water every 90 minutes, are suitable to hold 40–50 scallop broodstock (90–120 mm shell height). Water exchange can be optimised throughout the tank with an airlift system, which partially recirculates the seawater and enhances food distribution. The difference between other bivalves and scallops is their tendency

to move and swim away from disturbance. This causes “clamping”—as they move or swim, they tend to clamp down on one another’s shells and damage both shells and mantle. Figure 5.3 illustrates the use of trays, stacked to increase available surface area and increase capacity to hold broodstock. This is not ideal as faecal material from scallops in top trays will fall through lower trays, but it is space efficient; monitoring of scallops and changing tray placement is required in this system to ensure comparable water quality for all.

Broodstock held for longer than four days need to be fed on a daily basis. Feed can be distributed by batch and drip-fed over a period of 24 h. A rigorous cleaning protocol must be implemented to avoid a high deposition of faecal matter by fed scallops and consequently a decline in water quality.

**FIGURE 5.3**  
**Broodstock tank with airlift pipe, stacked trays and a dedicated batch-feeding tub**



### 5.3 CONDITIONING BROODSTOCK

**Goal** – Generally, several spawns a year are required to attain targeted seed production. A continuous conditioning programme can provide readily available broodstock to accommodate the spawning demands on a when needed basis or is implemented when natural seawater parameters do not support consistent gonad development.

**Conditioning** – Conditioning a broodstock is labour intensive, it requires the capacity to control seawater temperature in large volumes and demands a high volume of feed. For these reasons, a strategic approach to conditioning and obtaining ripe broodstock is necessary to be as cost-effective as possible.

**Role of temperature in conditioning** – Conditioning of broodstock relates mainly to the manipulation of the seawater temperature; the aquaculturist simulates the seawater temperature cycle to which scallops are exposed in their natural environment, in an accelerated fashion. Dependent on the gametogenic stage of scallops when collected, the temperature cycle used for conditioning will differ.

**Gametogenic stage at start of conditioning** – The time at which the broodstock is brought into the hatchery depends on whether the objective is to trigger or enhance gametogenesis activity or ripen the oocytes in the final stages. However, the gametogenic stage at which broodstock are collected will affect the duration of the conditioning period. A broodstock collected at the beginning of the gametogenic cycle (visual stage <2.5) will require a longer conditioning period (minimum three months), starting at a lower temperature (2 °C). On the other hand, broodstock collected at a more advanced stage of gametogenesis is conditioned more quickly; for example, animals with a visual stage of 3, can be conditioned within one month starting at a temperature of 4 °C. Table 5.2 provides some guidance on the expected duration of conditioning for scallops of different levels of maturity and shows the equivalence between gonadic index (GI) and visual stage in each case.

TABLE 5.2  
Level of condition required for Yesso scallops collected at different gametogenic stages

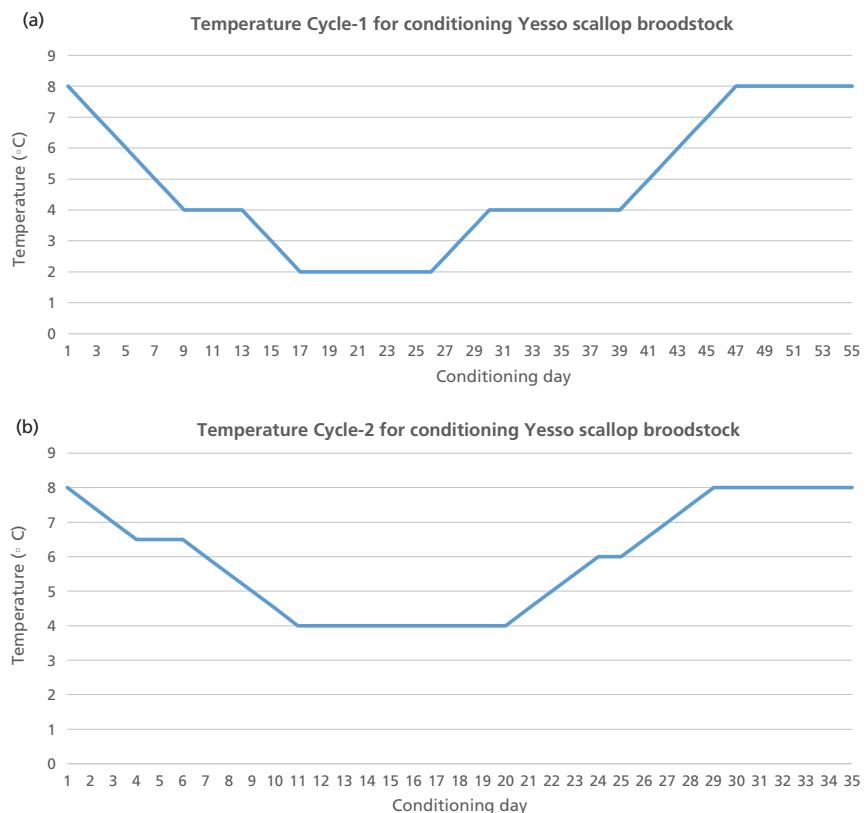
Duration of conditioning	Visual stage (1–6)	Gonadic Index (GI) (wet weight ÷ shell weight × 100)	Initial conditioning temperature (°C)
4–5 months	1.5	13.5	2±0.1
80 days	2.5	16	2±0.1
40 days	3	>16	4±0.1
7 days	6	>19.5	4±0.1

#### 5.3.1 Overview of temperature cycles for broodstock conditioning

**Temperature cycles** – A full conditioning regime consists of a 2-part conditioning programme, preceded by a gradual stepwise acclimation from the ambient seawater (e.g. 8 °C) to the start of the conditioning temperature cycle (2 °C or 4 °C) (Figure 5.4). The first part of the temperature regime, when temperature is at a minimum, stimulates broodstock to accumulate reserves necessary to oocyte production; this occurs at 2±0.2 °C for scallops at the beginning of gametogenesis (Figure 5.4a), or at 4±0.2 °C for scallops which are at a more advanced gametogenic stage (Figure 5.4b). The second part of the cycle subjects scallops to a gradual increase in temperature for oocyte maturation. The longer conditioning period at colder water temperatures (2 °C) is referred to as Cycle-1 in the rest of this document; the shorter temperature regime initiated at 4 °C is referred to as Cycle-2.

FIGURE 5.4

**Two temperature cycles for conditioning Yesso scallops adapted by S. Sarkis – (a) 55-day temperature Cycle-1 for scallops at the start of gametogenesis; and (b) 35-day temperature Cycle-2 for scallops which have partially spawned or with a starting gonadic index close to 16**



Source: adapted by S. Sarkis (unpublished).

**Duration of temperature cycle** – The more advanced the scallops are in the gametogenic cycle, the shorter the duration of conditioning and the easier the process. Broodstock selected for conditioning should at minimum have differentiated gonads with a visual stage of 1.5–2.

**Visual stage 0:** Conditioning scallops with undifferentiated gonads (visual stage 0) is difficult; it is a long and costly process and may result in sub-optimal egg quality. Cycle-1 will have to be repeated several times before reaching a broodstock ready for spawning. Hence, the recommendation above to start with a visual stage of 1.5–2.

**Visual stage <2.5:** Scallops exhibiting visual stage 2 or thereabouts will require a prolonged exposure to the 4 °C phase in Cycle-1 before increasing temperatures to 6 °C and completing the cycle. Evaluation of the gonadic and muscle indices mid 4 °C phase will assist the aquaculturist in assessing the need for a prolonged cold phase.

**Visual stage >3:** Scallops exhibiting close to visual stage 3 (or GI approaching 18) will be ready for spawning within a 40-day conditioning cycle, following Cycle-2 (Figure 5.3b), and should reach a GI of 27 at this time.

**Relationship between GI, MI and conditioning** – Yesso scallops release viable eggs developing into viable larvae when mean gonadic index is >20.

1. Exposure to 2 °C during conditioning, results in increased muscle reserves, reflected as a high muscle index (MI).

2. Exposure to 4 °C results in a gradual increase in gonad weight and yields a higher gonadic index (GI) with input from muscle reserves. This reduces muscle weight and gives a lower MI.
3. Subsequent exposure to 6 °C results in continued increase in gonad weight with reliance on external food.
4. Maintaining scallops at 8 °C for one week prior to spawning, does not cause any change in GI or MI; however, this further ripens the oocytes, and possibly improves egg quality and larval viability.
5. Ripe scallops can be maintained at optimal gametogenic stage until spawning at a sub-optimal T = 5–6 °C, to prevent spontaneous release of eggs and sperm.
6. Partially spawned broodstock can be re-conditioned rapidly for a subsequent spawn within a 7-day period using Cycle-2.

Broodstock induced to spawn and spent should be routinely replaced by backup broodstock from the farm, such that broodstock are collected from longlines and/or natural beds on a regular basis throughout the spawning season. The aquaculturist can also save time by re-conditioning partially spent broodstock.

For successful conditioning, seawater quality must remain constant, with the seawater temperature cycle strictly adhered to and a high food ration given daily.

#### 5.4 FEEDING BROODSTOCK

**Food ration** – Daily feeding is a must for adequate conditioning and for maintaining scallops at optimal gametogenic stage in the hatchery. During conditioning the diet given aims to provide the essential nutrients specific to egg development; standard practice is to calculate food ration as four percent per day based on dry weight of broodstock. Using 100 percent live microalgae as food supply places a high labour demand on the algal sector. Commercially available diets can supplement part of the daily feed requirements; rations are calculated according to the manufacturer's instructions. A detailed protocol for calculating live microalgae food ration is given in Protocol 5.4.1.

**Food composition** – A diverse diet will enhance scallop gonadic development and scallop health. The following has been used successfully for the Yesso scallop:

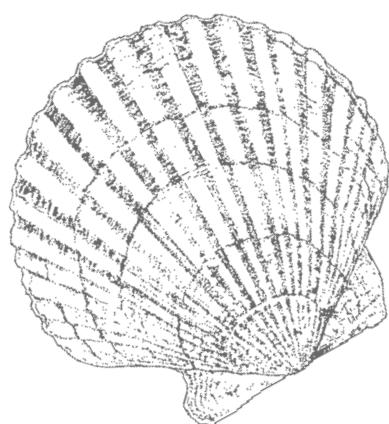
1. Live microalgae – includes standard diatom species (*Chaetoceros muelleri*, *Chaetoceros calcitrans*, *Thalassiosira pseudonana* and *Phaeodactylum tricornutum*), and flagellates (*Isochrysis galbana*, *Pavlova lutheri* and *Tetraselmis* sp.).
2. Shellfish Diet 1800® – a commercially available product, consisting of 6 microalgal species (*Isochrysis*, *Pavlova*, *Tetraselmis*, *Chaetoceros calcitrans*, *Thalassiosira weissflogii*, and *Thalassiosira pseudonana*). This algal diet is often used as a supplement to the live microalgae for all life stages of bivalves.
3. AlgaMac-Enhance – a spray dried formulation that is high in DHA (docosahexaenoic acid – required by maturing scallops) and astaxanthin (A carotenoid). It contains intact cells of two algae, *Schizochytrium* and *Haematococcus*, non-genetically modified organisms (GMOs), known to improve the growth of juvenile bivalves. The product also contains inactivated yeast, which serves as an additional source of amino acids, nucleic acids, vitamins and beta-glucans. It is sold as a supplement for maturation and conditioning in aquaculture.

**Mixing live and commercial foods** – Live microalgae undoubtedly provides the best nutrition, but costs in terms of space and labour need to be assessed by the aquaculturist; this has to be balanced with the purchase costs of commercial diets. Commercial feeds used as partial substitutes for live microalgae, result in successful gonad development,

comparable egg fertilisation rate and high larval viability. The relative contribution of live to commercial can vary with live microalgae quality and quantity; the following proportions are suitable for conditioning of the Yesso scallop:

- Shellfish Diet 1800® can be used for up to 50 percent of total broodstock diet.
- AlgaMac Pro Plus or Enhance can be used for up to 35 percent of total broodstock diet.

A daily feeding record is kept by the aquaculturist to ensure appropriate food ration throughout the conditioning period; a template is provided in Appendix II.



### 5.4.1 PROTOCOL: Calculating algal food ration for broodstock

#### METHOD

Food ration for broodstock is calculated on a percentage tissue weight basis. Total tissue weight in a broodstock tank can be estimated as follows:

1. Count the number of scallops per broodstock tank.
2. Estimate total wet weight per tank (approx. 80 g per scallop for the Japanese scallop).
3. Estimate total dry weight per tank (approx. 8 g per scallop).
4. Create an Excel spreadsheet.

#### To calculate live algae ration based on dry weight food ration

Standard food ration is given at 4 % dry weight of animal tissue.

Average weight for microalgae 0.02 mg dry weight per cell.

- except for *Tetraselmis* sp. (0.2 mg dry weight per cell) and
- *Chaetoceros calcitrans* (0.007 mg dry weight per cell).

For ease of calculation: Use 0.02 mg for all flagellates and diatoms and 0.2 mg for *Tetraselmis* per cell.

5. Give a mixed 1:1:1 ratio of flagellate:diatom: *Tetraselmis*.

Diet equivalent to:

- $\frac{1}{3}$  mix of flagellates and diatoms = 2.7% of total diet and
- $\frac{1}{3}$  *Tetraselmis* = 1.3 % of total diet

#### 6. Calculate flagellate/diatom mix:

ml of algal culture to be given =  $(2.7 \times \text{mg dry weight biomass}) \div 100 \div (\text{cell density} \times \text{dry weight microalgae})$

##### *Example:*

For an algal cell density of 10 million cells.ml<sup>-1</sup>,

dry weight of microalgae =  $10 \times 0.02 = 0.2 \text{ mg}$

Scallop dry biomass in broodstock tank = 60 g = 60 000 mg

ml of microalgae to give =  $(2.7 \times 60\,000) \div 100 \div (10 \times 0.02) = 1620 \div 0.2 = 8\,100 \text{ ml}$  or 8.1 L (for 24 h period)

#### 7. Calculate *Tetraselmis* 1.3%:

ml of algal culture to be given =  $(1.3 \times \text{mg dry weight biomass}) \div 100 \div (\text{cell density} \times \text{dry weight microalgae})$

##### *Example:*

For a cell density of 5 million cells.ml<sup>-1</sup>

dry weight of microalgae =  $5 \times 0.2 = 1 \text{ mg}$

Scallop dry biomass in broodstock tank = 60 g = 60 000 mg

ml to be given =  $(1.3 \times 60\,000) \div 100 \div (5 \times 0.2) = 780 \text{ ml}$  or 0.78 L (for a 24 h period)

**Total food ration to give (sum of 6 and 7):** 8.1 L (flagellate/diatom mix) + 0.78 L (Tetra) = 8.88 L total microalgae administered over 24 h period.

## 5.5 SPAWNING INDUCTION

Broodstock is spawned under controlled conditions, eggs and sperm are collected, eggs are fertilised and embryos are distributed into larval tanks for development.

**Inducing spawning** – Techniques for spawning scallops are well known. In general, spawning of scallops can be induced using thermal shock, air exposure, increased supply of food, and chemical exposure. Yesso scallops respond to an air exposure of 2–4 h and release oocytes within one hour following transfer to a warm water bath.

**Overview spawning protocol** – In brief, a GI taken the week of spawning dictates the spawning date for the hatchery staff. Scallops are collected and the ripest are selected by doing a rapid visual assessment of the gonads (see Table 5.1). The aquaculturist aims for a spawning stock with a ratio of 1:4 male:female, in order to have an adequate pool of sperm to egg ratio. Scallops are scrubbed clean, any epiphytes are removed and they are exposed to air in a clean area of the hatchery for 2 hours; there should be minimal disturbance of scallops during this time. Following air exposure, scallops are transferred to a warm water bath ( $T = 13 \pm 1^\circ\text{C}$ ), approximately 4–5 °C higher than that held prior to induction. Females and males are kept separate; this enables controlled addition of sperm to egg solution. Females can be placed in a saltwater table (Table spawn) or in lantern nets (5 scallops per layer) suspended in a tank (Tank spawn). Using a saltwater table will allow for a more careful monitoring and fertilisation procedure; however, the tank approach allows for a larger scale induction and yields comparable results in terms of larval production and percentage of pediveligers.

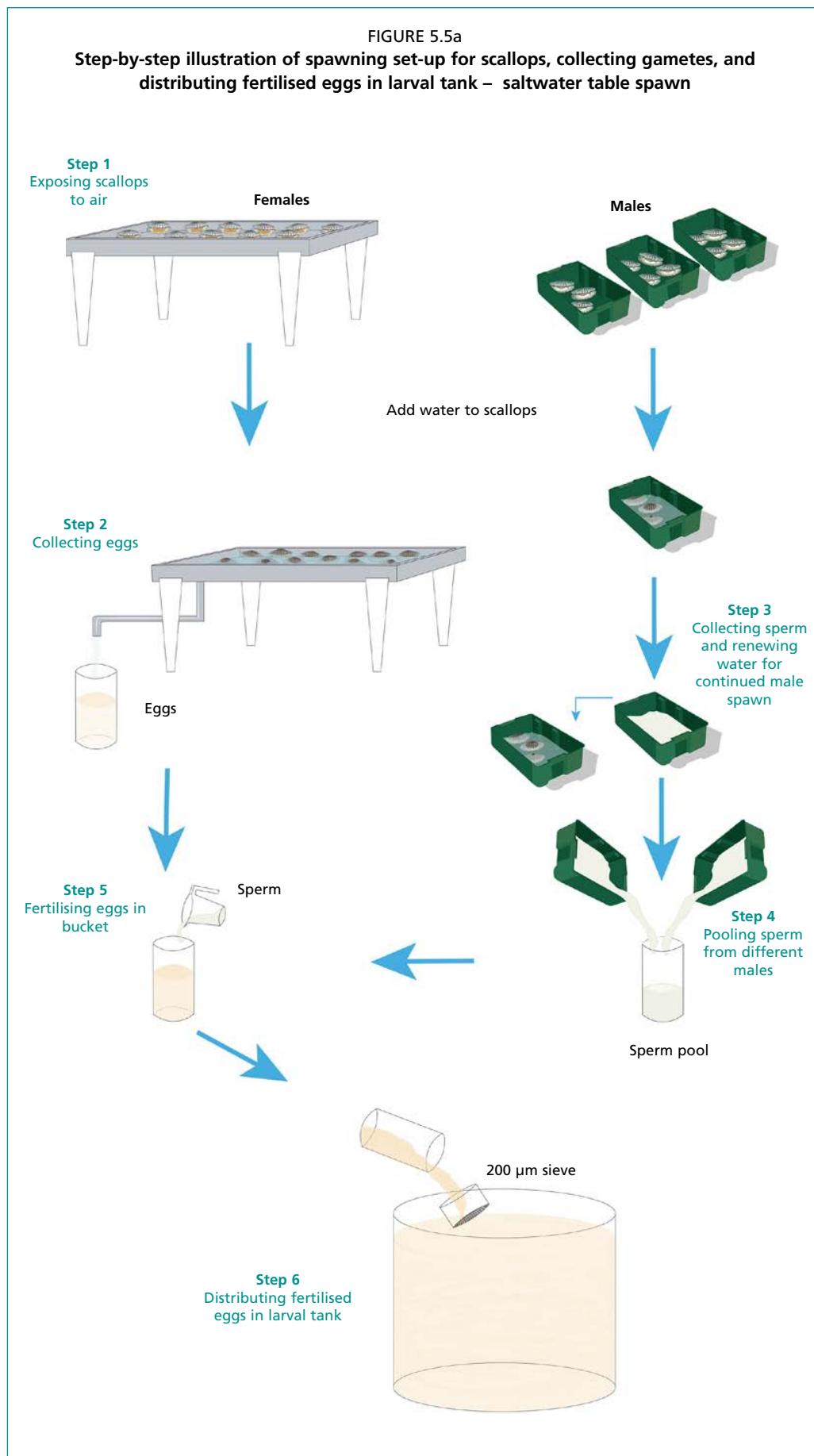
Protocol 5.5.1 describes a mixed approach of saltwater table and of the larger scale method using round larval tanks (5 000 L and up). The saltwater table method facilitates the monitoring of the spawn, allows for an accurate count of eggs released and ensures that the correct ratio of sperm:egg is added; this optimises the fertilisation rate and maximises the D-larval yield. A Table spawn should at the very least be carried out during the first spawns of the hatchery to obtain a better understanding of scallop fecundity and fertilisation rate. The larger scale spawning method (Tank spawn) using round tanks makes it difficult to accurately assess the number of eggs released and suitable volumes of sperm solution for fertilization. A high sperm:egg ratio increases the risk of abnormal development and/or mortality, as does a high egg density. When carefully executed with previous knowledge obtained during a Table spawn, spawning directly in tanks does provide a simpler large-scale approach to spawning.

**Spawning timeline** – First response is usually seen by males within 45 minutes following transfer to warm water bath; females usually first respond within 1–1½ hour after transfer to warm water.

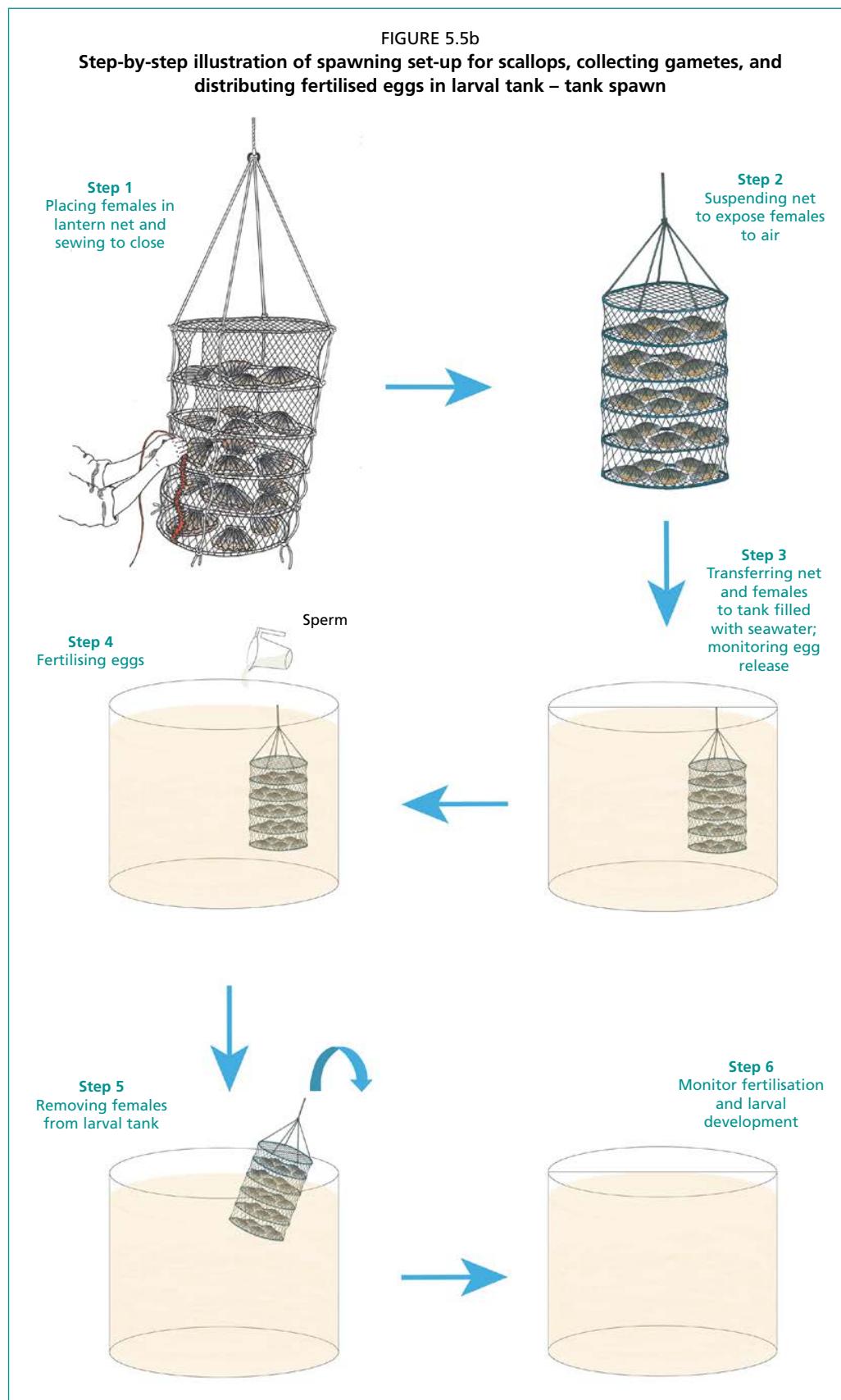
**Sperm collection** – As males spawn and water in tray becomes milky, sperm from various males is collected. It is important to collect sperm at frequent short intervals and keep track of the time each pool is created. As sperm lose viability with time, the newest pools are used to fertilise the eggs.

**For the table spawn** – Eggs are collected at regular intervals as they pass through the saltwater table drain and are fertilised within 30 minutes of collection. Evidence of fertilisation is checked before distribution into larval tanks (Figure 5.5a).

**For the tank spawn** – Sperm is added into the tank itself as eggs are released; it is important to keep track of the volume of sperm to avoid a high ratio of sperm:egg leading to abnormal embryo development. The total sperm volume is added over a



2-hour interval, with the last addition made once females are removed from the tank (Figure 5.5b).



Approximately 2–3 h after warm water immersion, the rate and intensity of egg release is seen to decrease; at this time, female scallops are removed from the spawning table, and final egg collection is done. The first three hours of spawning yield the highest number of eggs; however, scallops can continue to release eggs and sperm for 12 h or more. To halt spawning, broodstock is transferred to a cold water tank. Average number of eggs released per female is expected to approximate 30 million eggs; this can most likely be improved through conditioning and spawning techniques.

Scallop eggs are distributed in relatively low densities compared to other bivalves; standard densities for scallops range from 5–20 eggs.ml<sup>-1</sup>. For the Yesso scallop, densities ranging from 5–8.5 eggs.ml<sup>-1</sup> result in a 42–55 percent D-larval development; higher densities should be tested as they may prove suitable.

Flat bottom tanks with a very slight aeration are used for the development of embryos to prevent the aggregation of the early immobile embryos at the bottom of the tank.

### 5.5.1 PROTOCOL: Spawning Japanese scallop in saltwater table and in large round larval tanks

#### MATERIALS

- Hatchery logbook
- Saltwater table with open-flow capacity; outflow connected to clean hose
- 5-layer lantern nets or equivalent
- Buckets (10–20 L)
- Plungers for tanks and buckets
- Cleaning agent: Bleach, Virkon™ or equivalent
- 150 µm and 500 µm sieves
- Shallow trays or equivalent to isolate males
- Sedgewick-Rafter cell
- Eppendorf pipette
- Compound microscope with ocular micrometre or with a digital camera for measuring

#### METHOD

CHECK gonadic state of the scallops 3–4 days prior to spawn and isolate scallops selected for spawning. Visual index has to be >3.5; Gonadic index has to be ≥20 (GI based on shell dry weight).

SEAWATER used at all stages of spawning and egg fertilisation is preferably sterilised (UV or other); if sterilisation is not possible, double filter seawater to 1 µm.

#### Day before spawn: preparation

1. Do not feed selected broodstock the day before inducing spawn. This will minimise the amount of pseudofaeces produced during spawning and result in a cleaner egg solution.
2. Clean all tanks identified for D-larval development with a bleaching agent, scrub and rinse three times with freshwater. If used immediately, rinse one more time with filtered/sterilised saltwater.
3. Check that all airlines to tank are clean; each with an inline air filter. For large tanks, more than one airline may be needed to ensure a homogeneous and gentle flow in tank.



Stage 5 female Yesso scallop ready for spawn

©FAO/S. Sarkis

### 5.5.1 PROTOCOL (continued)

**Option to use tank spawning for large-scale, add Step 4 to preparation:**

4. Clean number of lantern nets required (5 scallops per layer), by immersing in a bin filled with a solution of freshwater and bleach (1 ml.l<sup>-1</sup> of bleach per litre of water) for up to 24 h before rinsing with freshwater. Suspend to dry until spawning the next day. If used immediately, rinse one more time with filtered/sterilised saltwater.

#### Spawning day

5. Start preparing spawn early in the day.
6. Clean saltwater table using freshwater and bleach (or equivalent); make sure to scrub corners and clean outflow drain and hose.
7. Select scallops keeping males isolated from females.
8. Remove any epiphytes from scallops using a metal scraper; scrub scallops vigorously using a brush and rinse well in 1 µm filtered seawater.
9. Distribute females on dry saltwater table for exposure to air; distribute males in smaller trays (4 per tray) or in a second saltwater table, if available (Figure 5.5a). If scallops originate from different conditioning regimes, keep batches separate throughout spawning and label each batch.
10. Record initial time of air exposure; leave for 2–4 h. After 2 h, test one scallop by pushing down gently on its valve; if scallop responds slowly, terminate air exposure. If reaction is quick, leave exposed to air and test every 45 minutes.
11. During air exposure prepare all equipment/materials and continue filling tanks for fertilised eggs. Final temperature in tanks should be at 13±1 °C.
  - a. Set up clean filters for filling larval tanks.
  - b. Fill tanks with double filtered 1 µm seawater.
  - c. Monitor water temperature as tanks fill; record final temperature and salinity once filled.
  - d. Set up 1 µm filter for saltwater table.
  - e. Clean 10–20 L buckets for sperm and egg collection.



Stage 5 female Yesso scallop ready for spawn

©FAO/S. Sarikis

#### Following air exposure

##### Table spawn

12. Fill saltwater table with 13±1 °C (UV or double 1 µm filtered seawater) and distribute females in table.
13. Fill trays (or separate saltwater table) with saltwater as for females (Step 12) and distribute males.

##### Tank spawn

14. Place females in lantern net (5 per layer) and suspend in hatchery tank (Figure 5.5b). Record female origin per tank. Aerate tank gently. Record time of immersion.

**Note:** For Tank spawn, scallops can be distributed in nets and exposed to air; or left on a table for this period.

### 5.5.1 PROTOCOL (continued)

#### Egg and sperm release and collection

15. Leave scallops undisturbed following immersion, minimising movement around saltwater table and males and dimming lights in spawning area.
16. Males usually release first; it is expected that they start to spawn within 30–45 minutes after immersion in warm water; earliest response known is 1 minute after immersion. If males do not release sperm as planned, change water (they shouldn't stay in the same water for too long). If after 1 hour, males are still not seen to spawn, dissect one male from broodstock, collect sperm from gonad using a Pasteur pipette, dilute in a 50 ml beaker with filtered seawater and distribute into spawning trays.
17. Record time males first seen to spawn. Once males start to spawn, leave in tray until water becomes quite milky.
  - a. Collect sperm from tray into a bucket when water gets milky.
  - b. Add new UV or 1 µm heated seawater (leaving some sperm in tray to keep triggering release).
  - c. Label the bucket with the time of collection.
  - d. Pool sperm from various trays into one bucket.
18. Females should release eggs within 1–1.5 h following immersion in warm water; earliest response known is 25 minutes after immersion. Record time of first response and allow eggs to be released for approximately 30 minutes before first collection into bucket.
19. Collecting eggs from saltwater table: Place drain hose into 150 µm sieve over a bucket. Crack one-way valve to allow for a gentle flow of egg solution in sieve. Care must be taken not to break the eggs. Fill bucket up to  $\frac{2}{3}$  of total capacity.
20. Collect eggs every 30 minutes or when you see too high a density in table. After each collection, re-fill spawning table with new  $13\pm1$  °C 1 µm filtered and/or UV disinfected seawater.



Bucket with pool of sperm collected from all males (top); spawning males showing milky water (bottom)

©FAO/S. Sarkis

#### Fertilisation

##### Table spawn

21. Count and fertilise eggs in bucket as follows:
  - a. Count number of eggs per bucket by sub-sampling 1 ml aliquots with an Eppendorf pipette as described in Protocol 6.2.1.
  - b. Measure a sub-sample by recording the diameter under the microscope.
  - c. Add 1 ml of sperm per 1 L of egg solution. Use newest pool of sperm released; try not to use sperm older than one hour.
  - d. Mix sperm by plunging with gentle and long up and down motions. Plunger should not hit bottom of bucket (otherwise crushing the eggs) and should not come out of the surface water (also damaging eggs).
  - e. Leave eggs to fertilise in bucket for 20 minutes or until first polar body is seen.
22. To distribute eggs in larval tanks; pass eggs through a 500 µm sieve to remove any detritus or faeces (Figure 6.4).
23. Distribute fertilised eggs at a minimum density of 10 eggs.ml<sup>-1</sup>.

### 5.5.1 PROTOCOL (continued)

#### Tank spawn

24. Check for eggs using flashlight. Females should spawn approximately 1–1.5 h after immersion in warm water. If egg release is not seen, add some sperm as a trigger.
25. Record time when eggs first observed.
26. After approximately 20 minutes of spawn, add a first batch of sperm into tank; total volume of sperm should be equivalent to 1 ml of sperm per 1 L of water in tank.
  - a. Collect a first sample of sperm into a graduated beaker and pour into tank.
  - b. Mix sperm gently in tank using long and slow up and down motions with tank plunger.
  - c. Make sure to mix gently and thoroughly entire volume, going around the tank.
27. Label tank; record number of litres of sperm added and time of addition in order to keep track of total volume added throughout spawn.
28. Allow females to spawn in tank for approximately 1.5 h after the first egg release is noticed; a good proportion, if not all, eggs should be released by that time.
29. Gradually add sperm solution as eggs are released in tank.
30. Plunge slowly after each addition, so that eggs are not disturbed.
31. Record volume of sperm added after each addition.
32. Estimate egg density in tank; if it exceeds 8 eggs.ml<sup>-1</sup>, remove females.
33. Once females are removed from tank, record total amount of sperm added. Total amount of sperm in a 5 000 L tank should be between 5–8 L of sperm (or 1–1.5 L of sperm per 1 000 L of seawater).
34. To continue spawn, transfer nets with spawning females to new larval tanks filled with seawater. Record time of second immersion.
35. Make sure total amount of sperm is added to first set of tanks, as these will most likely be the largest volume and best eggs of the spawn.
36. Fertilise the second set of tanks as described in Steps 25 to 31.
37. Once egg release seems to slow down or stop (or 2 hours after second immersion) remove nets with scallops from tanks.
38. Remove females from nets and transfer to a holding broodstock tank filled with flowing coarsely filtered seawater at 6 °C. To completely stop egg release, decrease the temperature to 4 °C.
39. Repeat Step 38 for males, placing them in a separate tank to avoid continued induction and fertilisation.
40. Record time that spawning is terminated.
41. Gently aerate all hatchery tanks with fertilised eggs – a very slow stream of rising bubbles (one bubble at a time) coming to the surface should be visible. Too much aeration will trap the fertilised eggs on edge of the tank. Many aquaculturists do not aerate at this stage; however, this helps to keep embryos in suspension and prevent accumulation at the bottom of the tank.
42. Leave eggs undisturbed with airline until D-larvae development.
43. Sub-sample for the following two or three days to monitor division and stages. Record in hatchery logbook.
44. Add the first food ration at the late trochophore/early D-larvae stage. See Table 6.2.
45. A D-larvae culture should be ready for take down on Day-3 after fertilisation; it can extend to Day-4 dependent on time of day when eggs initially fertilised and rearing temperature.

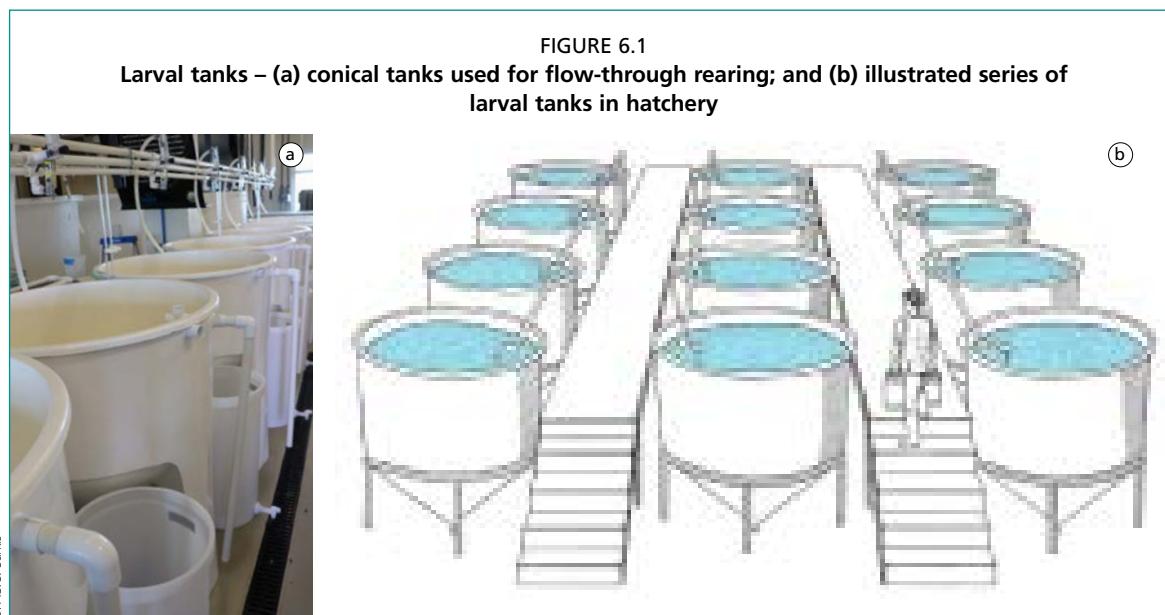
**Note:** Scallops often only partially spawn and can be re-conditioned subsequently.

## 6. Larval rearing

**IN THIS CHAPTER –** How to culture larvae for successful metamorphosis into spat by managing tank seawater flow systems, seawater requirements, food composition and ration.

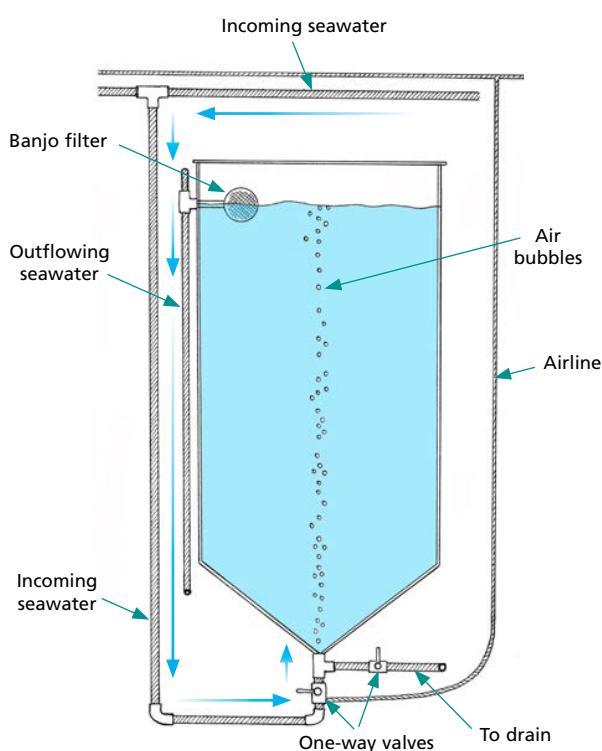
**Larval cycle** – Larval rearing refers to the culture of D-larvae to the metamorphosis stage, where larvae change from a planktonic to a benthic stage. The larval stage for the Japanese scallop ranges on average between 23 and 28 days (see Table 1.1) when reared at  $T = 13\text{--}14\text{ }^{\circ}\text{C}$ . The first sign of metamorphosis is the presence of an “eye” on D-larvae; this is followed by the appearance of a foot. Substrate search behaviour can be seen under the microscope as larvae extend the foot out of the shell; this leads to “rafting” of competent larvae (or larvae ready for settlement) and can be observed during water exchanges in holding containers. “Rafting” appears as filaments of larvae in the water column. Techniques for larval culture of scallops are well known and a 40 percent survival from D-larvae of pediveligers is standard.

**Larval tanks** – Larvae can be reared in static or flow-through systems, or a combination of both. Larval tanks are typically flat-bottom round or semi-round tanks for static systems, ranging from 200–20 000 L and up. Flow-through larval tanks are typically cone-shaped and smaller up to 1 000 L in volume (Figures 6.1 and 6.2). Static systems require 100 percent water changes 2–3 times a week to maintain “clean” seawater culture conditions. Flow-through systems rely on a continuous low flow of incoming seawater; this improves water quality and requires minimal water change throughout the duration of the larval life. Consequently, labour requirement in the hatchery is reduced and larval cultures are less prone to bacterial contamination.

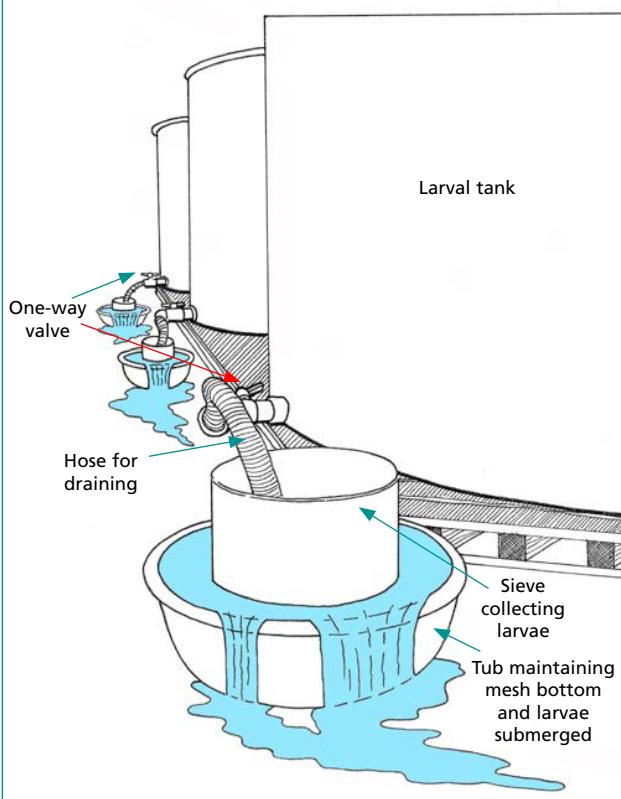


A general flow-through larval tank system is illustrated in Figure 6.2. Incoming seawater is filtered twice (1 µm) and often collected in a small header tank prior to the larval tank; this ensures a constant flow rate of seawater into the larval tank, minimising

**FIGURE 6.2**  
Schematic of a larval flow-through tank suitable for scallop rearing, showing direction of incoming seawater flow



**FIGURE 6.3**  
Take down larval tank for water exchange with larvae collected on meshed sieve



surges occurring in the main seawater line. In turn, the level of seawater in the header tank is maintained constant. Incoming seawater flows down the tank supply line to the bottom of cone, through gravity. Influx of seawater into the bottom of the tank is regulated by a valve. Air supply is mixed with incoming seawater, resulting in a slow stream of air bubbles rising to the surface of the larval tank. Outflow of water occurs at the top of the tank, through a banjo filter which prevents larvae from being discharged with the outgoing seawater. Suction pressure at the outflow must be considered and is minimised by fitting an appropriate diameter banjo. Outflow discharge rate of  $0.8 \text{ ml} \cdot \text{min}^{-1}$  for every  $\text{cm}^2$  of banjo mesh surface area prevents trapping of larvae on mesh. Further details on setting up a flow-through system are available (see Further Readings).

## 6.1 GENERAL LARVAL REARING PROCEDURES

**Use of sieves** – The entire larval culture is collected by draining a tank through an appropriate mesh sieve (Figure 6.3). Larvae are collected to monitor growth, survival of the culture, and for transfer to new high quality seawater especially needed for static systems. Mesh size of sieve increases as larvae grow, to ensure that larvae are retained when draining of the tank, and range from 40–200  $\mu\text{m}$ . Following monitoring, larvae are re-distributed in clean tanks filled with filtered seawater. Sieves are made in-house in PVC (polyvinyl chloride), fiberglass, or with hard plastic baskets (see Protocol 6.1.1); mesh used must be uniform to ensure that larvae are not lost. Sieves should have some support on the bottom so that mesh is not in direct contact with surfaces; if there is no bottom ring, always store sieve upside down so that mesh does not touch surface. Any small hole in mesh will result in a loss of larvae or spat. Storage must be in a dedicated area to avoid any spillage or tearing of mesh. If well taken care of, sieves can last several years. Care must also be taken when cleaning sieves to avoid tear. At every use, check sieve for any holes. At the beginning of every hatchery season, check all sieves and re-mesh, if needed.

### 6.1.1 PROTOCOL: Making sieves and banjos for larval and spat collection

#### MATERIALS

##### Glue material

- PVC frames – PVC cement glue and cleaner
- Fiberglass frames – Epoxy West System resin or cyanoacrylate superglue and accelerator

##### Frames

- PVC, fiberglass or plastic
- Permanent marker for labelling sieve
- Nitex® mesh 40–200 µm
- Hose clamps
- Razor blade or cutter knife
- Disposable brushes for epoxy resin

#### METHOD

##### PVC frames

###### To make sieve for larval and spat collection:

1. Cut PVC ring to appropriate height.
2. Sand surface of PVC frame on both sides to make it as smooth as possible.
3. If sieve is to be used as an inset, drill two 15 mm holes in opposite sides approximately 40 mm from the top. This will be used for suspending the sieve into a larger one.
4. Cut a square piece of mesh, so that entire surface of sieve is covered, with 3–4 cm of extra mesh hanging on side.
5. Lay mesh on top of frame.
6. Adjust hose clamp so that it can be placed on top of mesh and frame; some mesh will extend below clamp.
7. Tighten hose clamp along top of frame with a screwdriver, while pulling on hanging mesh.
8. Try to eliminate wrinkles in mesh by pulling uniformly around perimeter of sieve.
9. Mesh should be tight on surface of frame.
10. Label size of mesh on side of PVC frame with a permanent marker.
11. Once mesh is tight and hose clamp secure (approximately 1 cm from top of sieve) use PVC cleaner to prepare surface of frame for gluing.
12. Glue mesh to PVC using PVC cement along surface and side down to hose clamp.
13. Let dry for 24 h.
14. Once dry, make sure mesh is uniformly glued so that larvae cannot accumulate in small unglued areas (pay special attention to the rim on the inside of the sieve; larvae can become trapped between mesh and frame if not glued properly).
15. Remove hose clamp and cut unglued mesh with a razor blade.



Pulling mesh tight with hose clamp



©FAO/S. Sarkis



PVC sieves stored upside down

©FAO/S. Sarkis

### 6.1.1 PROTOCOL (continued)

To make banjo filters for flow-through tanks:

16. Cut PVC ring to appropriate height.
17. Drill a 15–25 mm hole on side to fit PVC connectors. This will secure banjo filter to larval tank.
18. Follow same steps as for PVC frame sieves (4–12) to mesh one side
19. Repeat on other side of the frame.
20. Let dry for 24 hours and follow Steps 14–15.



Banjo filters

### Fiberglass frames

21. Smooth surface of frame.
22. Follow Steps 3–10 above.
23. Make up a small volume (60 ml) of Epoxy West System (mixing hardener with resin using manufacturer's instructions).
24. Using a disposable paint brush, fix mesh to fiberglass dabbing top of mesh so that resin fills any uneven surface, gaps or holes.
25. Continue with paint brush on sides, taking care not to have the epoxy resin dripping onto clamp as this will also glue the clamp to the frame.
26. Once the first coat is completed, apply a second coat paying special attention to uneven surfaces.
27. Leave sieve to dry overnight in a dry and heated area.
28. Once dry, remove the clamp.
29. Cut excess mesh with a razor blade or cutter knife.



Fiberglass sieve with independent support

### 6.1.1 PROTOCOL (continued)

#### Plastic sieves

Plastic sieves can be made with cheap plastic tubs purchased in department stores

30. Cut out bottom of container.
31. Cut a piece of mesh large enough to cover the bottom of container.
32. Clean plastic edge upon which mesh will be glued.
33. Using cyanoacrylate glue, secure mesh on plastic edge by gluing along edge.
34. Spray with the cure accelerator for instant gluing.
35. Continue gluing mesh by going around whole perimeter of container.



Plastic sieve

©FAO/S. Saitki

#### Cleaning sieves

Before use for larval or spat collection, sieves have to be cleaned to avoid contamination of culture. Sieves are also cleaned after use to prevent any build-up of detrital material.

36. Use a damp soft cloth or sponge dipped into a cleaning agent diluted with freshwater.
37. Wipe around sieve frame inside and outside, and on mesh inside and outside with damp cloth.
38. Hose down thoroughly three times with freshwater.
39. Store in a dedicated area for drying until next use.

#### Water quality, temperature and density

**Water quality** – Water quality is the key factor in successful larval rearing. It is maintained through frequent water changes, when larvae are provided with new seawater. A complete water exchange involves the draining of the whole culture tank and the collection of all larvae for transfer into a new culture tank. This enables a comprehensive assessment of the growth and survival rate of the larval batch, and the aquaculturist can make any adjustments to culture parameters to improve larval performance at this time.

**Flow-through systems** – Continuous treated seawater flows into the larval tank at a rate equivalent to 2–3 times a full water exchange over a 24 h period. This maintains optimal water quality; in this case, manual water exchanges are not needed as frequently as for static systems. The cone shape in a flow-through tank results in the “trapping” of dead larvae or detritus when the incoming flow is stopped; occasionally, the drain can be opened briefly to remove accumulated matter. The flow-through tank will need to be thoroughly flushed and cleaned some time during the larval life to eliminate all detrital matter and prevent subsequent bacteria or *Vibrio* contamination. A manual water exchange can be scheduled every 7–10 days or more frequently dependent on incoming seawater quality and the health of the larval culture. The most critical period is as larvae approach the vulnerable pediveliger stage, and water exchange is a must at this time to successfully complete larval development.

Maintaining water quality on a daily basis in a flow through tank involves mostly the daily cleaning of the banjo filters. Unconsumed algae and/or pseudofaeces accumulate on banjo filters, and if not cleaned, the filter will clog and affect water flow. Banjos of increasing mesh size (40–150 µm) are used on the outflow as larvae grow to reduce clogging by the increasing amount of detrital matter associated with increasing food ration.

Daily checks for a flow-through tank system include:

- monitoring flow rate;
- cleaning banjo on outflow; and
- monitoring temperature – this is especially important as rearing temperature will be affected if incoming flow is inconsistent.

**Static systems** – Seawater is oxygenated via a gentle aeration. daily checks for static tanks include:

- visual check of tank and larval culture – bottom of tank should be clear of detritus or any trace of bacteria or *Vibrio* (e.g. *Pseudomonas* leaves red streaks on bottom of tank);
- monitor temperature, oxygen and other key parameters, if needed; and
- check aeration produces a slow stream of bubbles rising to the surface.

The hatchery manager sets a water exchange schedule to ensure that there is a complete (100 %) water exchange of larval culture at least three times a week. A detailed protocol for water exchange is given in Protocol 6.1.2.

The schedule utilised depends on the hatchery capacity, namely the:

- size of the larval tanks;
- available supply of seawater at the appropriate temperature and filtration; and
- number of hatchery staff available at any one day.

Table 6.1 below gives a few examples of water exchange schedules; these can be adapted dependent mainly on the seawater quality and on the health of the larval batch. For static tanks, 30 percent of the water can be exchanged between full 100 percent exchanges to optimise seawater quality if considered poor. For flow-through systems, a 100 percent exchange every 7–10 days is often sufficient; if there are concerns with respect to water quality or to larval batch, the number of water exchanges per week can be increased. A complete water exchange is a must if there is any evidence of contamination in the larval tank.

TABLE 6.1  
Water exchange schedule scenarios for static and flow-through tanks

Water exchange scenarios	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
	% <b>Static</b>						
<b>Schedule A</b>	100	30	30	30	100	-	30
<b>Schedule B</b>	100	-	100	-	100	-	-
<b>Flow-through</b>							
<b>Schedule A</b>	100	-	-	-	-	-	-
<b>Schedule B</b>	100	-	-	100	-	-	-

**Temperature** – Larvae of Yesso scallops are successfully reared in 1–5 µm filtered seawater, at a temperature of 13–14 °C; this will result in a 23 to 25-day larval life.

**Density** – Larval density in the culture tank is reduced as larvae grow; initial density is 5–5.5 larvae.ml<sup>-1</sup> and is usually decreased to an optimal density of 1–2 larvae.ml<sup>-1</sup> at the pediveliger stage. However, comparable metamorphosis and settlement occurs for Yesso scallop pediveligers reared at densities of 3 larvae.ml<sup>-1</sup> prior to settling.

In a flow-through system, larval densities are calculated based on the incoming flow rate; this results in a high-density system. For example: For a flow-through tank

with a 3-fold water exchange per 24 h, the initial stocking density is also 3-fold or 15 larvae.ml<sup>-1</sup> (3 × 5 larvae.ml<sup>-1</sup> as for static tanks), and final stocking density is 6 larvae.ml<sup>-1</sup> (or 3 × 2 larvae.ml<sup>-1</sup> as for static tanks).

Care must be taken to adjust larval densities in flow-through tanks as they grow; this is monitored during manual water changes.

### 6.1.2 PROTOCOL: Takedown of larval tanks for water change

#### MATERIALS

- Log book
- Cleaning agent (clorox bleach or Virkon™)
- Soft cloth for sieves and buckets
- Scrubbing brushes for tanks
- Holding buckets (10–20 L)
- UV and 1 µm filtered seawater supply (to wash sieves and fill holding buckets)
- Plungers for buckets
- Appropriate sieves
- Sedgewick-Rafter cell
- Eppendorf pipette
- Compound microscope with ocular micrometer, or with digital camera and computer set up for measuring

#### METHOD

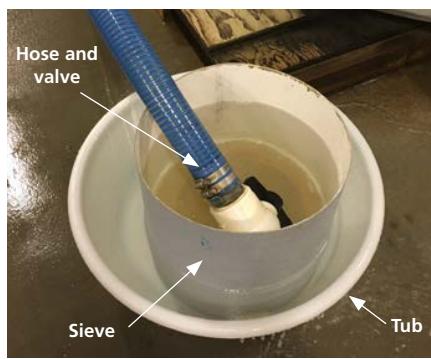
##### Day before 100 % take-down preparation

1. For any empty larval tanks, clean tank as per standard protocol.
2. Make sure airlines are clean with bacteria filters inline.
3. Make sure any cartridge filters are clean and air dried before use.

**Note:** If larval tanks are >10 000 L, draining the tank and collecting larvae with one hose only will take a few hours. PVC siphons with a one-way valve can be placed inside the tank, 30 cm off the bottom, to collect additional water and larvae; this accelerates collection, monitoring and transfer of the larval culture to a new tank, and prevents any damage to the shells by maintaining a suitable outflow rate while draining.

##### Day of take-down

4. Start as soon as possible in the morning.
5. Backwash and set up filtration system for double filtered 1 µm seawater supply and UV treated, if possible, to larval tanks.
6. Do routine checks of larval tanks before takedown (temperature; ~but also salinity, pH, oxygen if there are concerns with seawater quality).
7. Clean hoses, sieves, tubs, buckets using bleach, a freshwater rinse (3×) and one saltwater rinse (use same heated and filtered seawater as for larval culture).
8. Set up sieves in tubs, with hose connected to drain valve of tank.
9. Fill tub with treated seawater (filtered/sterilised and heated).
10. Place sieve in tub filled with seawater; this ensures that larvae collected always remain immersed in seawater. Make sure the sieve has a supporting base to maintain screen off the bottom of the tub; this can be a 5 cm high PVC ring of the same diameter. This maintains larvae in suspension off the hard bottom of the tub and avoids damaging larval shells during take down.



Collecting larvae in sieve

### 6.1.2 PROTOCOL (continued)

11. Place hose at an angle alongside sieve wall, to ensure that larvae flow out gently and are not damaged against sieve walls or pressed into the screen.
12. Open valve of larval tank slowly and ensure a gentle flow. There should not be white foam or bubbling during take-down due to vigorous flow (see photo).
13. Once first tank is  $\frac{1}{3}$ – $\frac{1}{2}$  empty, start the second tank. Timing takedown for a series of hatchery tanks depends on availability of sieves, seawater supply, rate of filling of new clean tanks and the numbers of tanks to take down on the day.
14. Once larval tank level is above drain, stop flow from hose. Carefully remove hose and siphon from sieve.
15. Keep the bottom of the larval culture separate from the rest, as it will contain detrital material from pseudofaeces, faeces and any dead larvae and/or unconsumed algae.
16. **To separate remaining bottom fraction of culture** – place a new sieve in tub and collect remainder.
17. Sides and bottom of tank should be washed with filtered and heated seawater to make sure that all larvae are collected (if the culture is healthy).
18. Gently wash larvae collected on sieves using heated filtered seawater supply into a clean 10–20 L bucket (make sure water used for washing larvae is the same filtration and temperature as larval rearing water).
19. Label bucket with tank number, sieve mesh size and record time when transferred in bucket. Larvae should not stay in the bucket for more than 1 h.
20. Set up an airline in the bucket with a low airflow, such that bubbles are seen rising in a steady stream to the surface of the bucket.
21. Examine both fractions of the culture (suspended and bottom). If the bottom fraction does not look healthy, with much detritus or moribund larvae sitting on the bottom, discard.
22. If the bottom fraction is healthy and consists of live swimming larvae, it can be added to the initial larval collection. If in doubt, it is better to discard, or pool all bottom cultures and re-distribute in a separate larval tank.
23. Clean larval tanks once empty.
24. Refill empty tanks as soon as they are clean, and adjust airflow in larval tanks, such that bubbles are seen to rise to the surface of the tank and spread throughout entire surface. Bubbling is not vigorous.
25. Count collected larvae in bucket and determine survival and shell growth of larvae (see Protocol 6.2.3).
26. Re-distribute larvae into filled tanks; dependent on numbers of larvae available, pool larvae according to size and health of culture into one tank.
27. Day-3 to Day-8 larvae are re-distributed at densities of 4–5.5 larvae.ml<sup>-1</sup>; larvae older than Day-9 are re-distributed at densities  $\leq$ 3 larvae.ml<sup>-1</sup>.



Vigorous flow during tank take down  
damages larvae

©FAO/S. Sarkis



Larvae held in bucket with gentle aeration

©FAO/S. Sarkis

### 6.1.2 PROTOCOL (continued)

28. To re-distribute – pass larval culture through a clean 500 µm sieve. Lower sieve below the surface so that half of the mesh is submerged. Pour the larval culture from holding bucket gently into the sieve so that any large debris or foreign larvae in culture are removed (Figure 6.4).
29. Feed larvae, as required (see Table 6.2).

Once all larvae are re-suspended:

- Clean all materials and filters used.
- Drain all hatchery lines so that no stagnant water remains overnight.
- Hose down hatchery floor with freshwater and cleaning agent.

### 6.1.3 Larval tank aeration

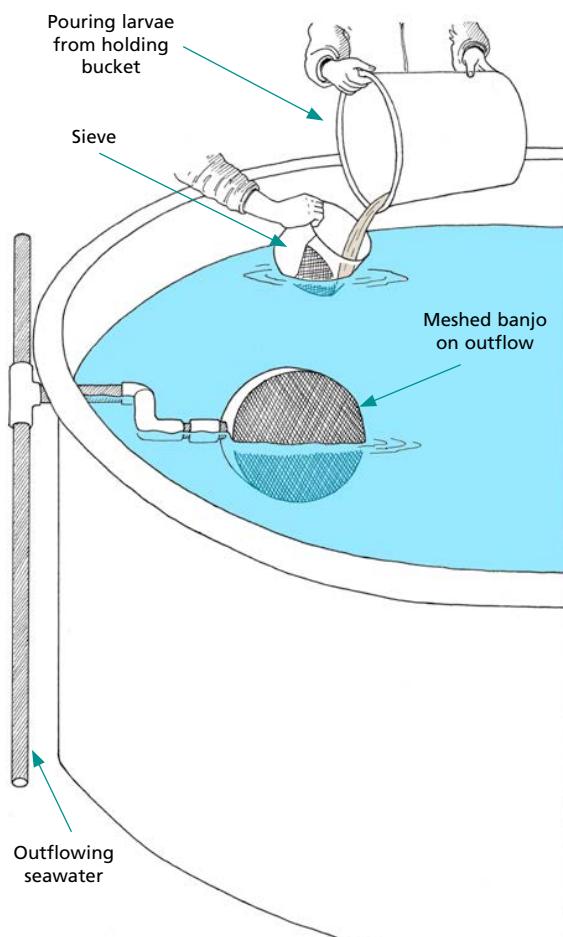
Aeration is provided throughout the larval life as this maintains algal food cells and larvae in suspension. Embryo development into D-larvae is conducted in a static system. During the first 48–72 h of embryo development, minimal aeration is provided (one bubble at a time) to avoid disturbance and damage. Throughout larval life for static systems, aeration of the tank ensures a homogeneous distribution of algal food and suitable oxygenation to larvae; one to several airlines is used per tank dependent on the size of the tank. Air is often filtered using a 0.45 µm bacteria filter. Aeration throughout the larval life is gentle, and air is regulated by stopcock valves such that air bubbles can be seen rising to the surface from each line and joining at the surface. Aeration lines are weighed down but NOT fitted with air stones, as small air bubbles generated by air stones trap larvae.

### 6.1.4 Larval food ration and distribution

Live microalgae cultured at the hatchery typically supplies 100 percent of the food administered to scallop larvae and <1.5 mm spat.

**Distributing algal food** – Dependent on the scale of the hatchery, algae can be drip-fed over 24 h into larval tanks from a 20–100 L bin. Alternatively, harvested algae are pumped to a food reservoir and diluted with filtered seawater; algal ratio is administered continuously over 24h and regulated via a dosage pump.

**FIGURE 6.4**  
Re-distributing collected larvae into a new clean flow-through tank by passing through a large mesh sieve to remove detritus



**Food ration and composition** – Both ration and composition change throughout the duration of larval life, to suit the metabolic requirements of larvae. Egg viability, embryogenesis, development rate and survival rate of early larvae depend in great part on egg quality. This in turn, depends on the condition of the broodstock and its gametogenic state. During the first few days of larval life, bivalves rely partly on the lipid reserves in the eggs; food ration is maintained low. Throughout larval life, the larvae's lipid-based metabolism slowly changes into a carbohydrate-based metabolism, which the aquaculturist satisfies by altering the algal composition. In general, scallops do not require as high an algal ration as other bivalves.

**Daily larval food ration guide** – The food ration for the Yesso scallop is well-tested and given in Table 6.2. A first ration of 5 000 cells.ml<sup>-1</sup>, composed of a smaller algal-celled species (*Chaetoceros calcitrans/muelleri*; *Isochrysis* sp.) is given to larvae as soon as early D-larvae are observed, usually Day 2 after fertilisation. Food ration is slowly increased as larvae grow, to a maximum of 21 000 cells.ml<sup>-1</sup> as they approach competency. There is a transition stage during metamorphosis when larvae lose their velum and do not feed. It is recommended to reduce feeding of the larval culture as larvae develop into pediveligers to prevent an excess of unconsumed algae and accumulation of detrital matter in the larval tank. Once pediveligers are observed in the culture, food ratio is slightly reduced to 18 000 cells.ml<sup>-1</sup>, especially if setting of the whole larval batch spans over a few days.

Calculating algal food ration for larvae is based on larval tank volume. Algal density of algal vessels to be harvested is determined as described in Protocol 2.2.3. The amount of algae to feed each larval tank is calculated as shown in the example below:

#### EXAMPLE – How to determine volume of algae to feed larvae

Tank volume = 5 000 L  
 Algal density = 10 000 cells.ml<sup>-1</sup>  
 Food ration = 10 000 cells.ml<sup>-1</sup>

#### Volume of algal culture to give (ml):

$$\text{Food ration (cells.ml}^{-1}\text{)} \div \text{algal density (cells.ml}^{-1}\text{)} \times \text{tank volume (L)}$$

$$\frac{10\ 000}{10\ 000} \times 5\ 000 = 5\ 000 \text{ ml}$$

or 5 L of algae to distribute over 24 h to larval culture in 5 000 L larval tank

Calculating volume required for *Tetraselmis* sp. *Tetraselmis* is 10 times bigger than the other microalgal species used.

For a 5 000 L tank, an algal density of 10 000 cells.ml<sup>-1</sup> and a food ration of 3 000 cells.ml<sup>-1</sup>

#### Volume of Tetra culture to give (ml):

$$\text{Food ration (cells.ml}^{-1}\text{)} \div \text{algal density (cells.ml}^{-1}\text{)} \times \text{tank volume (L)}$$

$$\frac{3\ 000}{10\ 000} \times 5\ 000 = 1\ 500 \text{ ml}$$

or 1.5 L of algae

To compensate for the larger algal cell size for *Tetraselmis*:

1 500 ml ÷ 10 = 150 ml to distribute over 24 h to larval culture in 5 000 L tank

Table 6.2 is intended to be used as a datasheet to record actual food ration and composition given, dependent on availability. It should be posted in the algal laboratory next to the algal counting station; this will help the algal culturist to plan the algal production based on the daily harvest volume required. Columns for the actual food ration given are useful in the case of a change of ration or composition due to insufficient algae.

The algal composition assumed for Table 6.2 is initially an equal mix of 2–3 algal species consisting of flagellates and diatoms. Ration is usually composed of a 1:1 ratio of flagellates to diatoms. Flagellate species often used are *Isochrysis galbana* (clone T-Iso) and *Pavlova lutheri*; standard diatom species are initially *Chaetoceros muelleri* or *Chaetoceros calcitrans*. *Thalassiosira pseudonana* (clone 3H) is added to the diatom mix around Day-9. As larvae grow and approach the umbone stage, the larger flagellate *Tetraselmis suecica* or *Tetraselmis chuii* is added as a third part of the ratio for its high lipid content.

As mentioned above, food ration is decreased during metamorphosis when larvae lose their velum. This is reflected in Table 6.2, starting Day-23 or when pediveligers are first observed.

**TABLE 6.2**  
**Datasheet for food ration for Yesso scallops cultured at 13±1 °C**

Larval stage and size (µm)	Days after fertilisation	Planned ration (cells.ml <sup>-1</sup> )	Planned algal ratio	Actual food ration given (cells.ml <sup>-1</sup> )	Actual algal composition
Early D-larvae 110 µm	Day-3	5 000	Chaet or Iso		
D-larvae	Day-4	6 000	1:1 Chaet:Iso		
	Day-5	7 000			
	Day-6	8 000			
	Day-7	8 000			
	Day-8	10 000			
	Day-9	10 000	1:1:1 Chaet:Iso:3H		
	Day-10	12 000			
	Day-11	12 000			
Umbone 170 µm	Day-12	14 000	Add Tetra 1:1:1 Flagellates:Diatoms:Tetra		
	Day-13	14 000			
	Day-14	15 000			
	Day-15	15 000			
	Day-16	15 000			
	Day-17	18000			
	Day-18	20 000			
	Day-19	20 000			
220 µm	Day-20	21 000			
Eyed	Day-21	21 000			
	Day-22	21 000			
Pediveligers 235–250 µm	Day-23	18 000–20 000			
	Day-24	18 000–20 000			
Day of set- based on behaviour	Day-25	18 000–20 000			

**Note:** Abbreviations in Table 6.2 are as follows: *Chaetoceros muelleri* or *calcitrans* (Chaet), *Isochrysis galbana* or Tahitian clone (Iso), *Thalassiosira pseudonana* (clone 3H) and *Tetraselmis* sp. (Tetra). *Pavlova lutheri* (Pav) can be substituted for Iso.

### 6.1.5 Use of antibiotics and probiotics

**Antibiotics** – Minimising bacterial contamination is inherent to the hatchery biosecurity operational mode. Lids on static larval tanks, cleanliness of all materials used, dedicated equipment to the larval section are such examples. For static systems, antibiotics can be used during water exchanges to reduce the risk of contamination; a small antibiotic dose can be added to the holding buckets post-drain and prior to re-distribution. Florfenicol (an alternative to chloramphenicol) is often used in large-scale operations (also in veterinary medicine and the salmon industry); a dose of 2 mg.l<sup>-1</sup> mixed into the holding buckets for a period of 1 h enhances the larvae's immunity to harmful bacteria.

**Probiotics** – Probiotics is the more ethically acceptable and sustainable alternative to the use of antimicrobial drugs for the control of bacterial contamination in hatchery cultures; their use is expanding in the shellfish industry and are routinely used in some commercial oyster; however, little is reported for scallop culture. Preliminary trials on Yesso larvae and spat are promising. There are two methods of integrating probiotics in larval culture. Chapter 3 provides further details on this including a description of probiotic culture techniques.

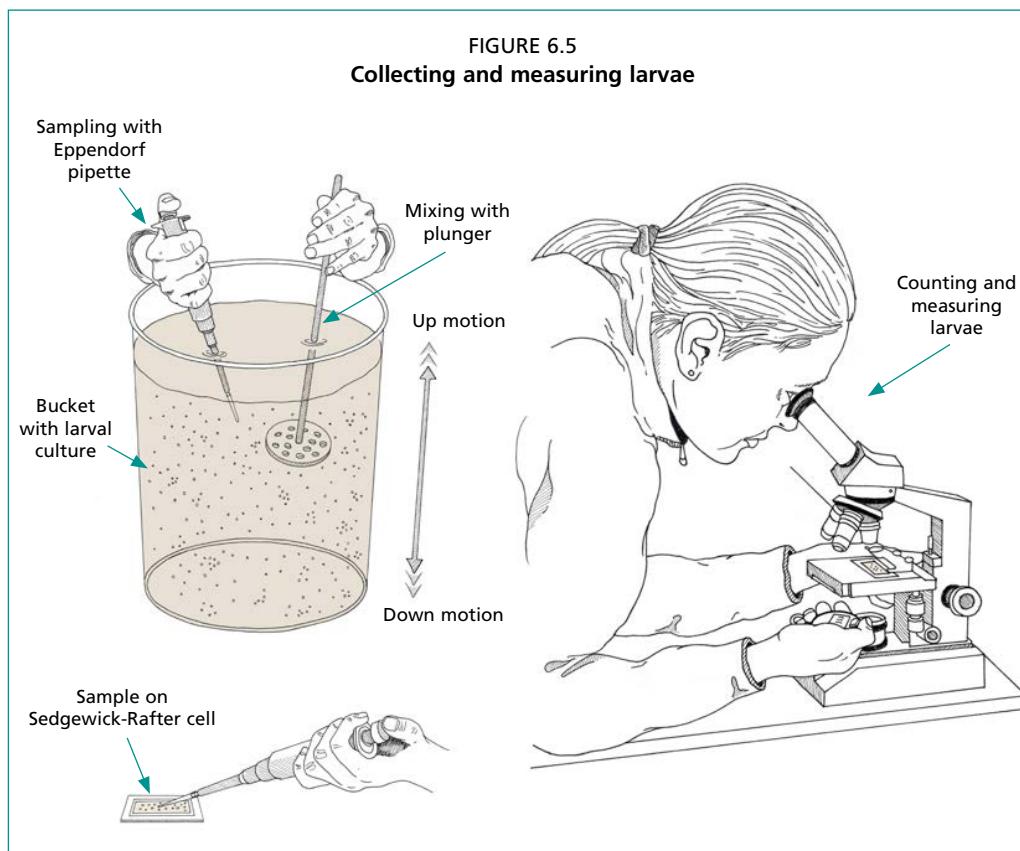
## 6.2 LARVAL GROWTH AND SURVIVAL

Larval cultures are routinely monitored for growth and survival. This provides the aquaculturist with a good understanding of the performance of the larval batch and an early warning of potential issues due to handling, poor seawater quality, poor algal food quality and bacterial/*Vibrio* contaminations.

**Measuring growth** – Assessing growth throughout larval life is based on shell length. An accurate assessment is obtained following a 100 percent water change, when the entire larval culture of a given tank is collected on a sieve prior to transfer to a new clean tank. For flow through systems, where 100 percent water change is less frequent, a subsample for shell length can be obtained by “scooping” a sample from the surface using a small sieve. This is a quick and basic assessment of growth but will not accurately reflect survival of the whole culture, as only healthy larvae suspended in the water column will be scooped.

Length can be measured on a subsample of 30–50 larvae using an ocular micrometre on a compound scope. The microscope is calibrated when first used; generally, with an objective of  $\times 10$ , 1 unit equates 9.6  $\mu\text{m}$  (often rounded off to 10  $\mu\text{m}$  for ease of calculation), and with an objective of  $\times 4$ , 1 unit equates 25  $\mu\text{m}$ . For round eggs, the diameter is recorded as measurement of size; for D-larvae, length is equivalent to the straight hinge of larvae; as larvae grow and develop an umbo, the largest distance parallel to the hinge is taken as “length”.

**Estimating survival** – Assessing survival is based on larval counts. Counts of live and dead larvae per ml are made on subsamples of the whole larval culture collected from one tank following water exchange. At this time, all larvae are collected from a rearing tank and washed into a holding bucket. Larvae are gently mixed for subsampling; triplicate samples for each larval fraction are taken and the average number of larvae calculated (Figure 6.5). This provides a “production yield” value and is used to assess the performance of the larval batch and its contribution to the annual production target. Standard procedures used for counting larvae are given in Protocol 6.2.1.



### 6.2.1 PROTOCOL: Counting and measuring larvae

#### MATERIALS

- 10 to 20 L bucket
- Homemade plunger
- Eppendorf pipette
- Sedgewick-Rafter cell
- 10 % formalin
- Compound microscope with ocular micrometre or with digital camera

#### METHOD

1. Once larvae are collected from a rearing tank and washed into a bucket, mix using a homemade plunger: mix thoroughly using a continuous up and down motion with the plunger, taking care not to touch the bottom of the bucket, to avoid crushing larvae, and staying below the surface of the water to prevent any splashing or bubbles which may damage larvae.
2. During mixing take an aliquot (100 µl) of larvae using an Eppendorf pipette.
3. Place sample onto a Sedgewick-Rafter cell and fix with two or three drops of 10 % formalin.
4. Count larvae in a systematic fashion by moving from one end of the grid, scanning the slide up and down to the other end. For larvae located on lines of the grid, care must be taken not to count the same larvae twice.
5. Measure larvae using an ocular micrometre along the straight edge of the shell; this will give you shell length.
6. Record the number of units on the micrometre and convert to µm according to your initial calibration.
7. Triplicate aliquots are taken for each larval fraction.
8. To determine survival, the average number of larvae counted in three aliquots is calculated, and used in the following equation:

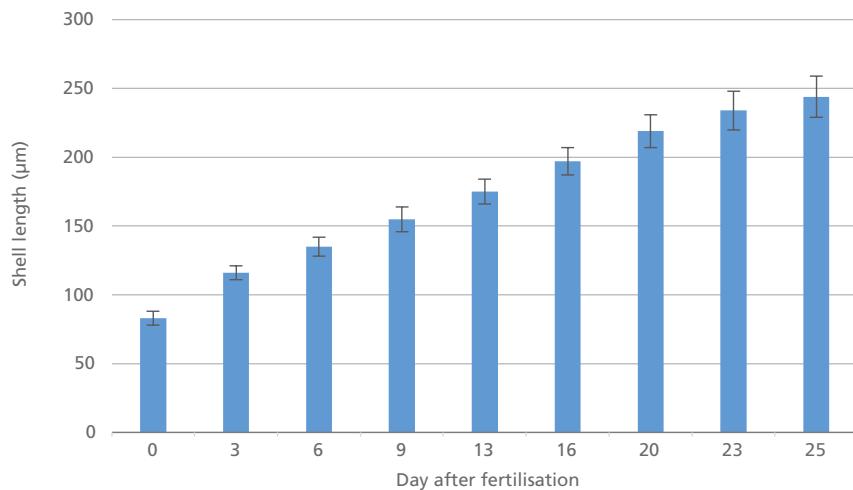
### 6.2.1 PROTOCOL (continued)

**Total number of larvae collected = Average (larvae.ml<sup>-1</sup>) × Volume of seawater in bucket (ml)**

9. Survival is calculated from previous water change throughout larval life. A good culture will have a survival >90 % between water changes.
10. Overall performance of the larval batch is expressed as survival rate of pediveligers; this is calculated from Day-3 D-larvae (or Day-4 if development is slow).

**Yesso larval growth** – Yesso scallop D-larvae average 110 µm in shell length, growing to 244–280 µm prior to settlement. A larval growth rate of 5–10 µm.day<sup>-1</sup> can be expected for a healthy larval batch. Competent larvae ready for settlement are recorded between Day-25 and Day-31 after fertilisation. The expected average shell growth for *M. yessoensis* larvae is given in Figure 6.6. Rearing temperature, water quality, algal supply and quality, air supply and circulation in tank are all key factors affecting the duration of larval life and survival rates. Inconsistency in the implementation of rearing techniques results in poor survival, and strict adherence to protocols is a must to achieve production targets.

**FIGURE 6.6**  
**Expected trend for shell growth for larvae reared in a static system at standard densities (n = 50)**

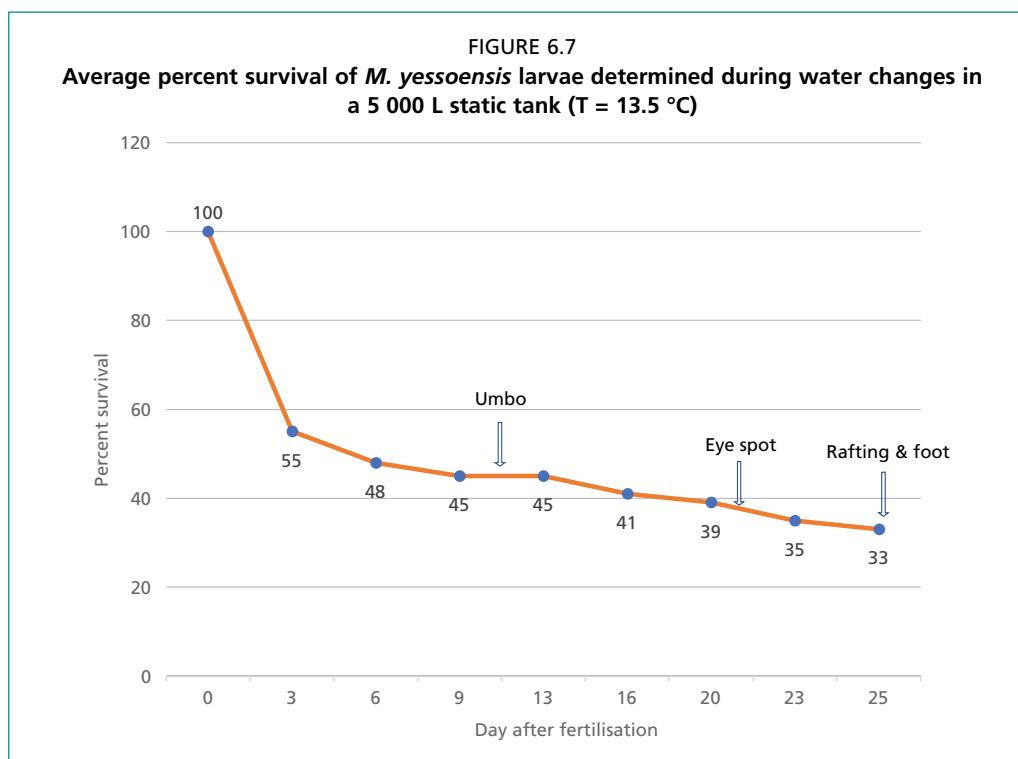


As larvae approach Day-23 of culture, signs of readiness for set or competence of larvae for metamorphosis can be observed; this is an indication to the aquaculturist to prepare settlement conditions for the next rearing stage.

**Expected Yesso survival rate** – Survival rate from D-larvae to pediveliger stage is a standard measure of the performance of a larval batch. A 40 percent survival of pediveligers from D-larvae can be expected for a successful larval batch.

Survival rate between 100 percent water changes provides an indication of the larval health on a weekly basis. A healthy larval batch which remains clean of bacterial or other contamination has an expected survival rate >90 percent between 100 percent water changes. Survival rate decreases with larval life, with the highest mortality rate generally seen shortly before metamorphosis.

Figure 6.7 illustrates a typical survival trend when rearing Yesso scallop larvae at a  $T = 13.5^{\circ}\text{C}$ . There are three critical points in larval rearing to which the aquaculturist must pay extra attention. The first is during embryogenensis where highest mortalities are encountered during development to the D-larval stage (Day-3 or Day-4). Thereafter, if protocols are strictly adhered to, high survival between water changes can be expected between Day-3 and Day-21. As eyed larvae approach metamorphosis, they become more vulnerable; this is the second critical point during the larval hatchery phase, as an increased mortality rate between water changes is likely. If optimal conditions are maintained, the aquaculturist can expect a 40–60 percent survival of pediveligers from D-larvae. Collecting and setting pediveligers in a timely fashion is the third critical point. As rafting and substrate search behaviour with foot extension is observed, setting must be initiated by the hatchery staff; if pediveligers are kept in larval tanks too long, the aquaculturist can lose more than 50 percent of the larval batch, as pediveligers will set on the sides and bottom of the larvae tank. At this point, it is very difficult to retrieve set larvae without damage to the shell. Note that in a large-scale operation, portions of the larval culture can be set at different times. This is done by grading larvae, setting those that are ready, and re-distributing the slower growing ones in the larval system for a few more days. The next section discusses grading larvae.



### 6.3 GRADING LARVAE

Larvae are graded according to size throughout the duration of the larval life. This has a threefold purpose: 1) It allows for selection of faster growing larvae; 2) it allows the slower growing larvae to catch up in shell growth by removing the larger-sized larval fraction; and 3) it helps in controlling bacterial contamination by separating the larval size fractions and removing smaller deformed or dead larvae.

**Mesh size for grading** – Meshed sieves are used for separating different size fractions of the larval culture. Day-3 D-larvae with an average shell height of 115 µm are collected from the tank and passed through a 60 µm and 40 µm mesh; the 40 µm portion often consists of undeveloped embryos or detritus and is generally discarded. As larvae

grow, they are collected on 80 µm, 120 µm, 150 µm mesh and 180 µm sieves. While grading, care must be taken not to damage the larvae as they are poured through the sieve; best practice is to have the sieve partially submerged in a tub of water, so that larvae accumulating on the mesh remain in water at all times (see Protocol 6.1.2).

Table 6.3 provides a guide on mesh size used for grading as larvae grow. The grading guide given is compiled from several sources; ranges may differ slightly, dependent on mesh type used and sieve characteristics. Shell length will vary with Day after fertilization from batch to batch. Setting is from Day-23 on depending on shell growth rate.

**TABLE 6.3  
Percentage of larvae retained on sieves of increasing mesh size (µm) according to their respective shell length, throughout the larval life**

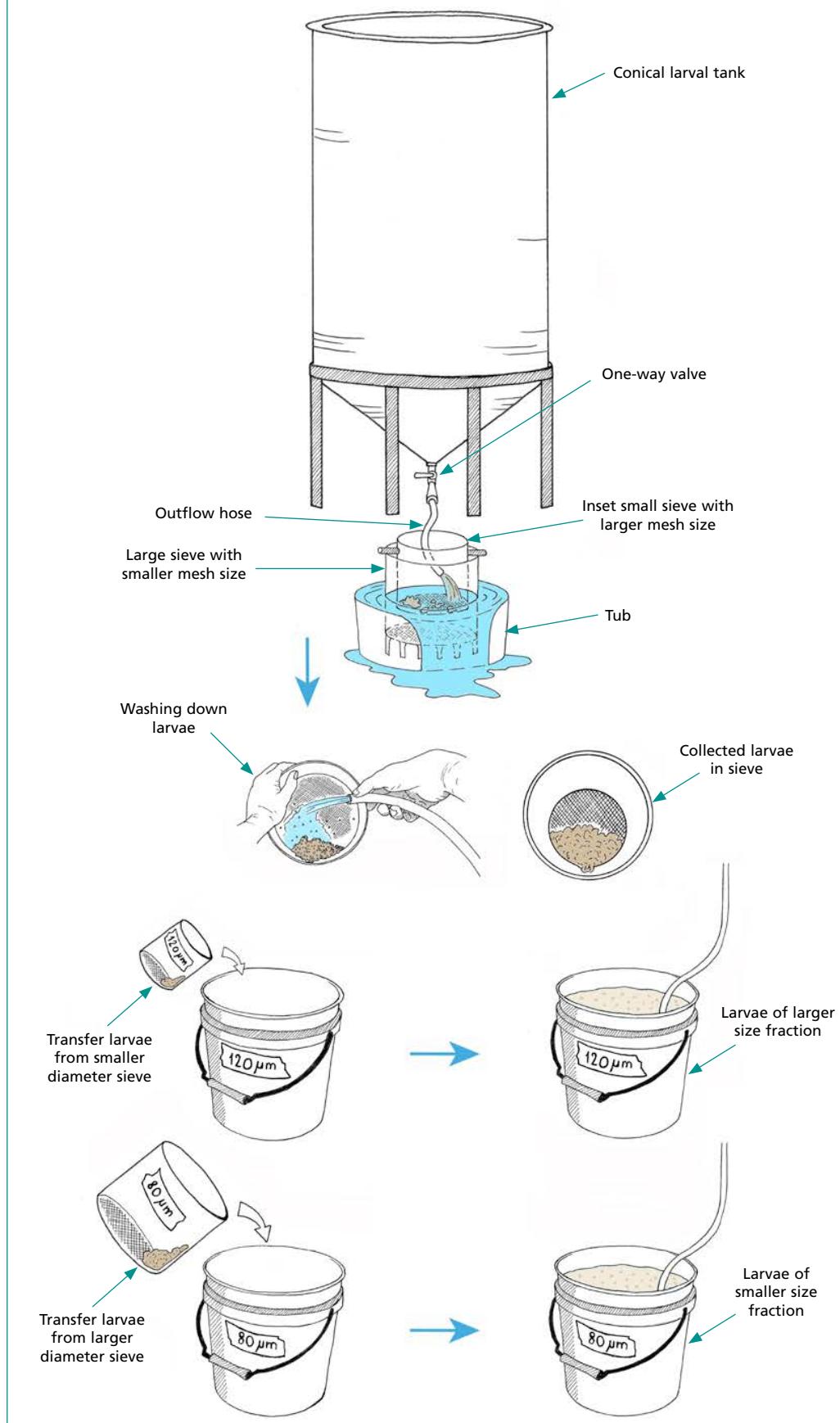
Day after fertilisation	Larvae retained on mesh size in µm (%)						Shell length range (µm)
	40	60	80	120	150	180	
0	100						78–88
3	0	100					108–118
7		70	30				126–144
9		7	93				131–155
13			60	40			160–170
16			20	80			175–190
21				45	55		185–200
25				12	88		190–220
28				15	82	3	200–260
31					35	65	240–275

**Grading methods** – Grading can be done either during takedown of larval tanks or after collection into a bucket through a grading sieve.

- A. Grading during takedown involves the use of an inset sieve of larger mesh size placed inside a larger diameter sieve with smaller mesh size. Larvae are automatically graded as they are collected, with larger larvae retained in the inset sieve and small larvae retained by the bottom sieve. Larvae are thereafter washed into two separate buckets (Figure 6.8).
- B. Alternatively, grading can be done once larvae are collected in the holding bucket; they are passed through a grading sieve placed over a bucket; larger larvae are retained in the grading sieve and smaller larvae are passed through. The disadvantages to this method are that it requires an additional step in handling, and in most cases, when collected on the grading sieve, there is a risk of damaging larvae retained on the mesh out of water.

**Re-distributing larvae** – Standard practice is as per Protocol 6.1.2 and Figure 6.4. Larvae are passed through a large mesh sieve held partially submerged in the new tank. Larvae are poured gently from the bucket to avoid crushing of larvae against the mesh while passing through.

FIGURE 6.8  
Grading and collecting larvae during a full water exchange



#### 6.4 SETTING PEDIVELIGERS

The settlement period is one of the most critical stages in a hatchery operation. The setting system and process are designed to obtain a maximum survival of pediveligers to 1 mm spat. Dependent on the system used, the timing of when to introduce competent larvae for set will differ slightly.

**Setting systems – Scallop pediveligers can be set using two different systems, shallow raceways or round nursery tanks.**

In raceways, competent larvae are set on meshed sieves installed as a downwelling system; such systems yield high settlement rates but require intensive labour and a large surface area within the infrastructure of the hatchery complex. If space or labour are insufficient to accommodate a raceway setting system for the targeted production, a small-scale raceway system is useful to monitor settlement and survival rate to post-set during the first year of operation; this enables the aquaculturist to adapt the nursery tank system for maximal survival and growth.

In tanks, individual mesh substrates are used to fill the tank vertically. Round tanks enable the setting of a higher number of pediveligers within a given area, but yield a typical lower settlement rate. This system is less labour intensive during post-set rearing but becomes tasking when it is time for transfer of seed to the sea-based farm.

**Knowing when to set –** Larvae change as they become competent to set. Larvae will develop at different rates, and grading of larvae into larger and smaller size fractions is necessary to differentiate competent and non-competent larvae. The assessment is similar regardless of the setting system used. Criteria for set are based on the distinct changes in morphology and behaviour (described in Table 6.4), and involve an increasing number of “eyed” larvae, the development of a foot, “rafting” in holding buckets, substrate search behaviour with extension of the foot, and a thickening of the shell. The latter indicates the disappearance of the prodissoconch larval shell and formation of spat dissoconch shell. Table 6.4 guides the aquaculturist as to the readiness of larvae to settle. It also describes the morphological changes observed during and post settlement.

TABLE 6.4  
Criteria for setting Yesso scallop larvae

Larval stage	Description	Behaviour	Observations in holding bucket
“Eyed” larvae	An eyespot appears at the back of the digestive gland; this spot is inconspicuous and irregular at first but becomes in 2–3 days more regular in shape (round) and conspicuous with a dark brown colour.	Larvae are still swimming, but eyespot indicates end of swimming stage and approach of metamorphosis. Not always easy to see the eyespot, and lack of observation does not necessarily mean its absence.	Swimming larvae suspended in water column.
Early pediveliger	Development of a foot. Use of both velum and foot alternatively.	Swimming, but can be occasionally seen under the microscope to extend foot out of shell, in “substrate-search” mode.	Rafting of larvae and suspended in water column, with some “crawlers” exhibiting substrate search behaviour on bottom of bucket.
Thickening of shell margin	In preparation for development of dissoconch shell.		
Metamorphosis	Disappearance of velum. Retention of functional foot. Beginning of dissoconch shell.	Spat can attach and detach dependent on favourable conditions.	
Settled larvae	Foot, velum and eyespot degenerate. Gills and adductor muscle develop. Appearance of dissoconch shell.	Spat are fixed to substrate.	

Source: Adapted from various sources by S. Sarkis.

The most reliable and easily assessed criterium for setting is the rafting behaviour for larvae retained on a 150–180 µm sieve. This is easily identified by untrained eyes and is a sure sign of larvae approaching metamorphosis.

**Setting density** – Setting density is calculated differently for a tank and a raceway system.

In a tank system, densities are calculated as per larvae, on a volume basis. A density range of 1–8 larvae.ml<sup>-1</sup> for setting of Japanese scallop larvae has been tried in tank systems. Scientific recommendations for an optimal settlement rate are in the range of 1–1.5 larvae.ml<sup>-1</sup>. On a large-scale, setting at densities of 2–2.2 larvae.ml<sup>-1</sup> are known to yield up to 14 percent survival to 1 mm spat (1 month post-set) if protocols are strictly implemented. Higher setting densities may result in a high settlement, but subsequent survival and shell growth of post-set will most likely be poor, due to overcrowding. This author recommends a setting density for *M. yessoensis* larvae of 1–2.5 larvae.ml<sup>-1</sup> when using a tank system.

For raceways, setting density is based per surface area of the meshed sieve. Competent larvae are distributed at an initial density of 30 larvae.cm<sup>-2</sup> of sieve; surface area is the main limiting factor in scaling up the raceway methodology. A minimum settlement rate of 16 percent is expected for Yesso scallops set in raceways (Day-14 after set).

**Duration of set** – Generally, competent larvae are fixed following 10–14 days after setting. Numbers of swimming larvae are monitored and recorded during this period and decline to zero, as settlement phase is completed. Settlement rate refers to the number of fixed larvae counted on Day-10 or until “swimmers” are no longer observed.

#### 6.4.1 Raceway setting

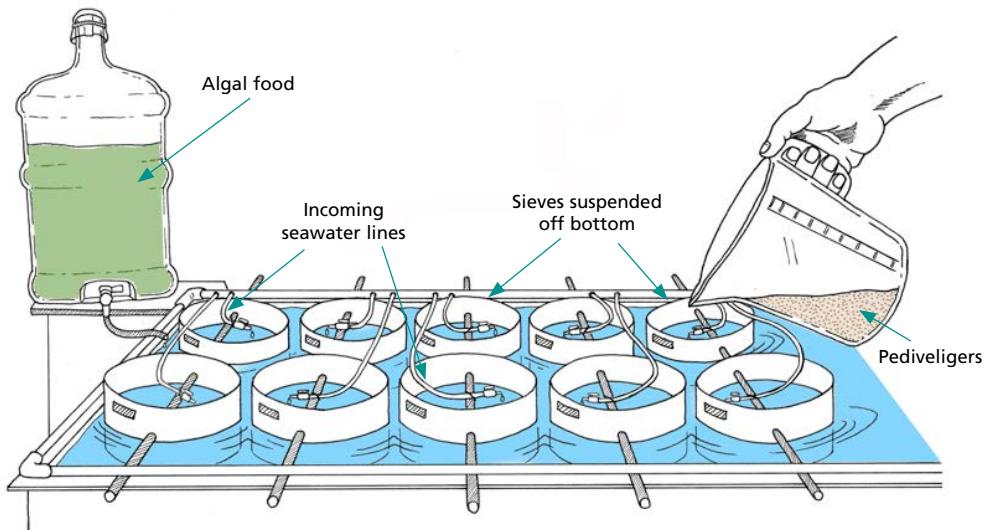
Setting in a raceway system is used for settlement of pediveligers by some commercial scallop hatcheries and for rearing seed up to 5 mm. A brief overview of the procedures used is given in this manual; details are available in other technical guides (see Further Readings).

A known number of larvae is poured gently directly into the sieve used for setting (Figure 6.9). Sieves with scallop pediveligers are suspended in a shallow raceway (a minimum of 15 cm deep) and receive continuous filtered seawater and algae; the latter is pumped from an external sump tank to the raceway. Seawater characteristics are similar to those used for larvae (double filtered to 1 µm and T = 13–14 °C).

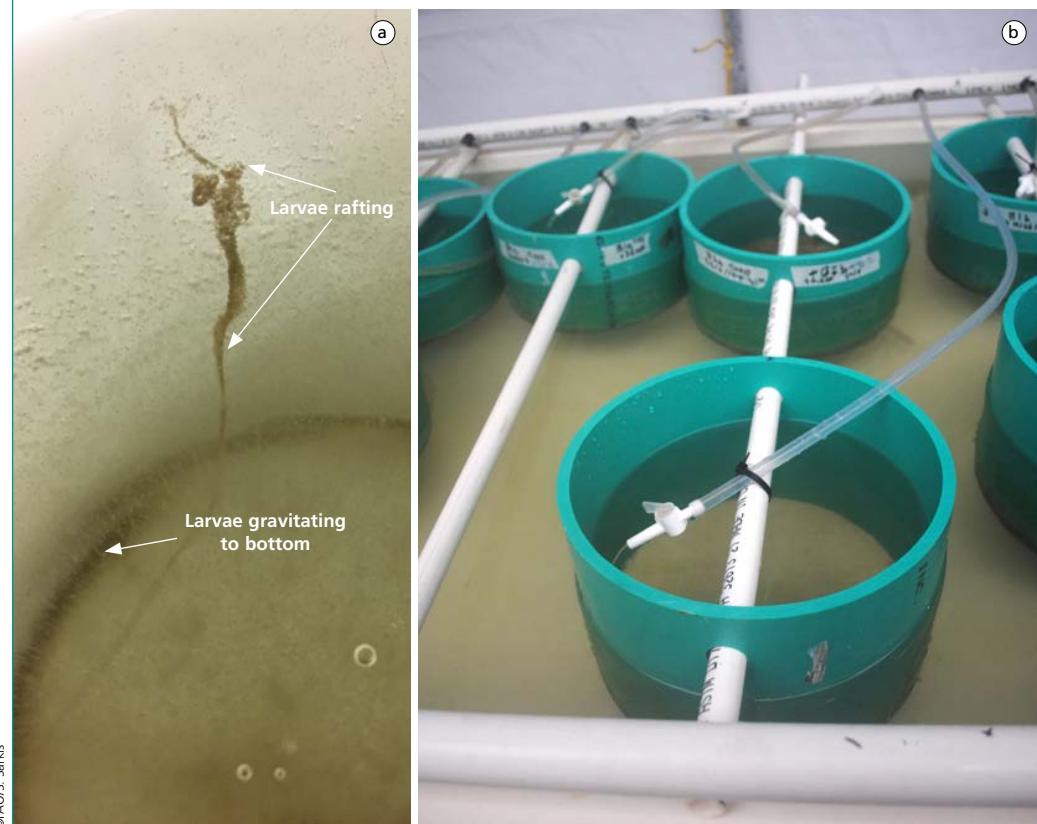
**Downwelling system** – A downwelling system is used to enhance settlement. Incoming seawater and algae from the sump tank are distributed into the raceway through a PVC line drilled at regular intervals and fit with adapters; these connect to Tygon® tubes which supply incoming seawater and algae directly into each sieve (Figures 6.9 and 6.10). Incoming water and algae pass from the top of the sieve through the mesh to flow out of the raceway through a drain valve. For the first 48 h, the system operates as a recirculated system. Thereafter, new water flows into the sump tank, and the system is changed to semi-recirculated; part of the water flows back into the sump tank; the other part drains out. This increases the residence time for the algae in the raceway, as a portion of unconsumed algae are recirculated to raceway and maintains the temperature constant. Spat are drip-fed continuously over a 24 h period. Settlement yield and growth rate is often higher in a raceway system, mainly because monitoring stock density is relatively easy.

The downwelling system is continued until spat reach approximately 2 mm shell height; after that, spat growth benefits from an upwelling system (see Section 7.1.2).

**FIGURE 6.9**  
**Setting pediveligers in sieves suspended off the bottom in a downwelling raceway system. Algal food reservoir and incoming seawater lines are also shown**



**FIGURE 6.10**  
**Settling larvae – (a) rafting behaviour of larvae in holding bucket; (b) close up of valve regulating incoming flow in downwelling sieve**



## 6.4.2 PROTOCOL: Setting and rearing mature Yesso scallops in a semi-recirculated raceway system from Day-0 to Day-14 after set

### MATERIALS

- Sieves 120–150 µm
- 10–20 L buckets
- Saltwater table or extra raceways

### METHOD

#### Setting day = Day-0 of set

1. Clean sieves on sides and mesh, inside and outside.
2. Clean raceway.
3. Fill raceways and sump tank with double filtered 1 µm seawater at  $T = 12 \pm 1^\circ\text{C}$ .
4. Once filled, turn off incoming flow and place raceway on re-circulating system only.
5. Pool larvae and pass through 150 µm and 120 µm sieves.
6. Resuspend each fraction in a 10–20 L bucket and count (see Protocol 6.2.1).
7. Calculate volume to set per sieve as per example below:
 

Larval density: 400 larvae per ml  
 Sieve diameter = 25 cm  
 Target setting density = 30 larvae per  $\text{cm}^2$   
 Total number of larvae to set = 70 000 larvae  
 Volume of larval pool to distribute per sieve =  $70\,000 \div 400 = 175\,\text{ml}$
8. Distribute larvae into sieve.
9. Partially open incoming seawater flow to obtain a total flow of  $3\,\text{L}\cdot\text{min}^{-1}$  in the raceway.
10. Restrict flow to individual sieves on setting day, to leave larvae settle without any surface agitation.
11. Calculate food ration (see Table 7.1). Distribute 50 % in the sump tank. Distribute remainder in 20 L carboy and top up with 1 µm filtered seawater.
12. Adjust flow of carboy to drip-feed to distribute total algal solution over 24 h.
13. Record number of larvae set per sieve and label sieve with date of set.
14. Do a daily check of flow, temperature and algal ration.
15. On Day-2 – increase flow slightly to increase drip.
16. On Day-8 – clean raceways. Transfer sieves to clean raceway or saltwater table with spat in the sieve. Do not clean sieves. Do not disturb larvae.
17. On Day-10 – Assess the number of swimmers. Wash spat off the mesh with a gentle stream of filtered seawater into a holding bucket.
18. From Day-10 on, begin routine cleaning of raceway system once a week.
19. Thinning of sieves is started when crowding is observed and spat are seen climbing up the sides of the sieves.
20. For thinning, collect the spat as in Step 17. Divide the culture into two and re-distribute into two sieves.

### 6.4.3 Tank setting

This system is useful for round tanks of 1 000 L and up.

**Substrate type** – Substrate type can affect settlement rate and it is recommended to use a substrate providing optimal surface area for settlement as well as allowing for good water flow in the setting tanks. Various substrates specific to scallop settlement have been tested, such as Kinran and Netron™. Less costly versions of a similar mesh are a 3 mm black polyethylene mesh often used in the mussel industry, which can be “fluffed” to increase volume and water flow.

#### 6.4.4 Preparing a tank for setting

Once larvae are metamorphosed and are fixed on the substrate, they are reared in the same tank until they are large enough to be transferred to a pond or farm. For this reason, the settling tank has to be prepared to allow a change from static to continuous flow system, with a semi-recirculating airlift system; this will ensure homogeneous water circulation and distribution of food for fixed spat. Such a system can be used for spat up to 5 mm shell height.

**Preparing the substrate** – The mesh selected as substrate is usually cut into lengths of approximating 50 to 70 cm in length, or a suitable length for the tank depth. Substrate is cleaned. Standard practice is to soak cleaned substrate in continuous filtered seawater for a period of one week prior to setting; this produces an organic film on the substrate and is believed to favour settlement.

**Airlift and setting installation** – For setting, tanks and airlines are cleaned as for larvae. The airlift system for recirculation is cleaned and installed (see Protocol 6.4.5; Figure 6.11). Substrate is suspended in the setting tank, filling the entire tank volume, once the airlift is in place. The aquaculturist must consider the best design for suspending the substrate in the setting tank to facilitate retrieval of the substrate and spat when they are ready for transfer out of the nursery to ponds or to the farm. One method is to make sets of 20–25 substrate pieces tied in line on a rope; the rope is in turn attached to opposite sides of the tank (essentially creating a longline within the tank). It may be necessary to weigh the substrate to maintain it vertical in the water column.

Once the airlift and the substrate are in place, the setting tank is filled with filtered and heated seawater (as for larvae); any gaps in tank are filled with single pieces of mesh.

#### 6.4.5 PROTOCOL: Assembling an airlift system for nursery tanks

##### MATERIALS

Dimensions below are for an airlift system to fit a round tank with diameter approximating 300 cm and height of 200 cm (see Figure 6.11).

##### For ONE airlift system:

- 15 m of 25 mm PVC pipe (schedule 40)
- Drill and 8 mm bit
- Hacksaw or PVC pipe cutters
- Four 25 mm “90° elbows”
- 25 mm “T” connector
- Sand paper

##### METHOD

**Note:** DO NOT GLUE ANY PART – there is no need and system can be easily disassembled and cleaned between spawning seasons.

##### Bottom pieces

1. Cut four 76 cm pieces of 25 mm pipe.
2. Using magic marker, mark every 8 cm.
3. Flip pipe, and mark every 8 cm on opposite side.
4. Drill marks using 8 mm drill bit.
5. Use sand paper to smooth and clean edges.

#### 6.4.5 PROTOCOL (continued)

##### Transverse top pieces

1. Cut two 134 cm pieces of 25 mm pipe.
2. Cut two 152 cm pieces of 25 mm pipe.
3. Mark holes every 8 mm on pipe.
4. Mark another set of holes at a slight angle – water will recirculate back at an angle, rather than parallel to surface of water.
5. Drill holes at marks with 8 mm bit.
6. Using sand paper, smooth edges and remove bits of PVC.
7. Cut a 5 cm long piece of 25 mm PVC pipe as a connector.
8. Sand and connect two Ts using the 5 cm piece (with each T facing in opposite direction).

##### Vertical pieces

1. Cut four 152 cm pieces of 25 mm pipe.

##### Before assembly

1. Clean all pipes and PVC parts.

##### Assembly

1. Put aside, four elbows and six Ts for each airlift system.
2. Fit one elbow to each of the 76 cm pipes.
3. Connect one 152 cm vertical to elbow.
4. Fit a T at end of 152 cm pipe.
5. Repeat for each of 76 cm bottom pipes.
6. Inside tank – place assembled vertical and bottom pipes at opposite sides (as for a clock at 12, 3, 6, and 9 o'clock positions), with bottom 76 cm pipes facing towards the centre.
7. Outside of tank – connect one top 133 cm pipe to connector T-section, and one top 152 cm pipe to other side.
8. Take the whole top section and fit to Ts of vertical pipes already in tank, connecting vertical pipes on opposite sides (e.g. 3 and 9 o'clock).
9. Repeat Steps 7 and 8 using remaining two other transverse pipes (connecting 12 and 6 o'clock vertical pipes).

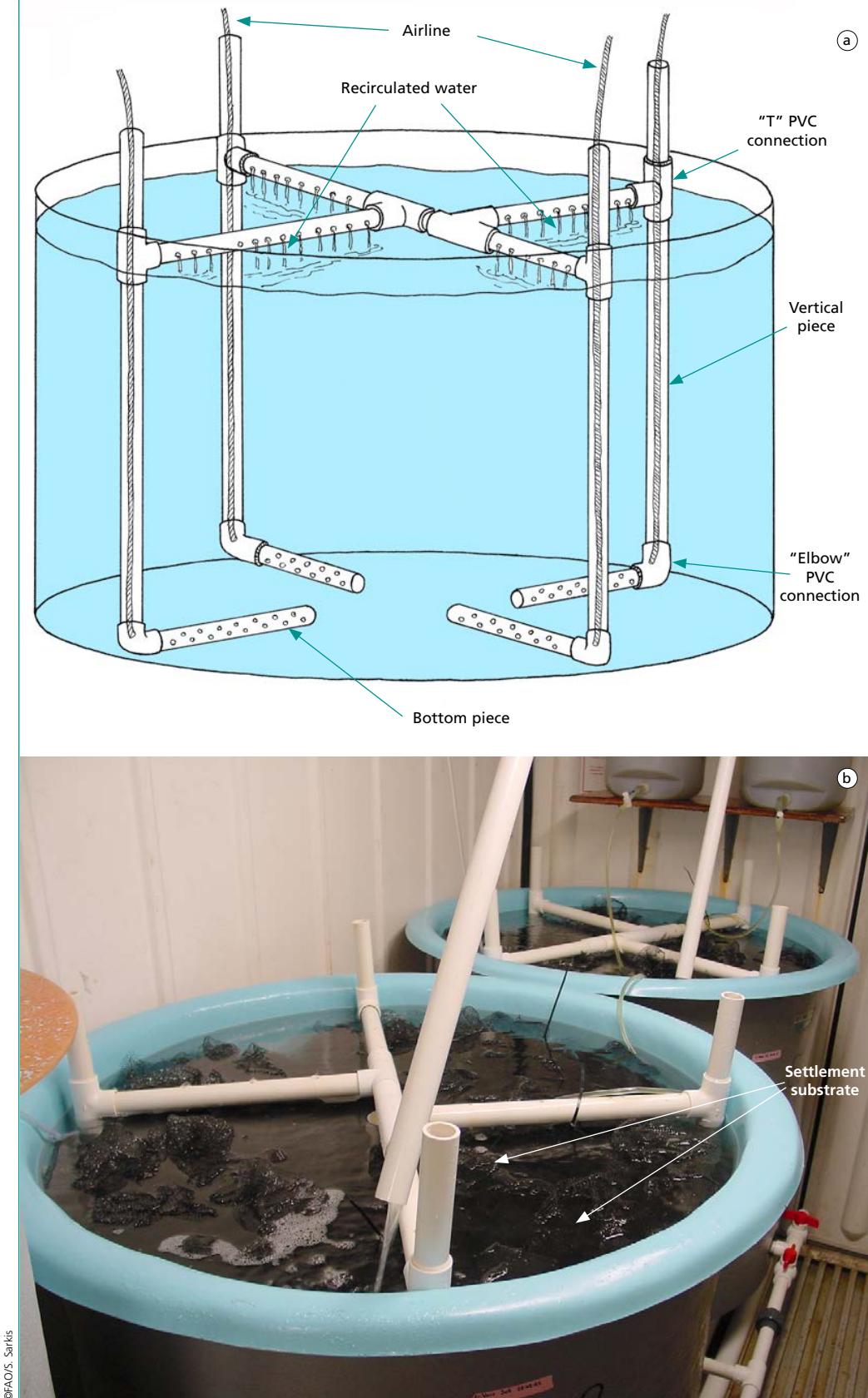
##### Airline

1. Cut four 7 mm inner diameter tubing to fit from manifold to bottom of each vertical pipe.
2. Cut tubing at top of vertical T and fit with a one stopcock valve in order to adjust airflow when tank is full.

#### ASSEMBLE AIRLIFT IN TANK BEFORE SETTING LARVAE

**Note:** For first 10–14 days of setting (until no more swimmers are seen in drain samples) – weigh down airlines and use as regular airlines outside of airlift system.

FIGURE 6.11  
Setting tank (a) airlift system assembled; and (b) tank filled with substrate



#### 6.4.6 PROTOCOL: Grading and setting larvae for round nursery tank system

##### MATERIALS

- Sieves: 120 µm, 150 µm, 180 µm, 500 µm
- Buckets
- Plunger for buckets
- Sedgewick-Rafter cell
- Eppendorf pipette and tips

##### METHOD

Day of set ranges from Day-25 to Day-28 after fertilisation

Average size of larvae set ranges from  $224 \pm 15.6$  µm to  $247 \pm 20.9$  µm

Range of settlement rate 4–16 % dependent on larval batches and setting procedures

1. Prepare setting tanks with airlift systems and substrate prior to collecting competent larvae.
2. Takedown larval tanks as in Protocol 6.1.2.
3. Larvae are separated into three fractions (120 µm, 150 µm, 180 µm). Larvae observed to raft in buckets or extend a foot with or without substrate search behaviour under the microscope are considered ready for set.
4. Count and measure larval fractions as per Protocol 6.2.1.
5. Assess the “health” of each larval culture. Larvae of similar health and size fractions are pooled.
6. Healthy smaller size fraction not seen to raft are often pooled with smaller fraction of other tanks and placed back in larval tank for further rearing. Care must be taken to monitor this larval batch carefully, as readiness to set will occur quickly (within 2 days).
7. For setting: pool larvae of same size fractions if they are healthy.
8. Count larvae in each pool. Set at a density of 1.5–2.5 larvae.ml<sup>-1</sup>
9. Distribute larvae from holding bucket to tank:
  - using a 500 µm sieve, lower sieve halfway into surface of tank water; and
  - gently pour larvae through mesh into tank.
10. Leave larvae undisturbed until Day-2 with no exchange of seawater.
11. A small food ration is given to setting larvae (see Table 7.1). Algae is distributed as for larvae as a batch in a drip-feed system over 24 h.



## 7. Nursery

**IN THIS CHAPTER –** How to rear post-set scallops until transfer out of nursery – changing seawater system from static to continuous, managing food demand and stocking density.

**Terms for post-set larvae** – The terms “spat” and “seed” are commonly used in the nursery phase. The word “spat” relates to bivalve larvae that have set and undergone metamorphosis. The word “seed” is to describe juvenile products supplied by hatcheries to shellfish farmers. The broader term “post-set” is applied to spat and seed. Post-set maturity is referred to as Day after set, with Day of set being 0.

**Nursery goal** – The goal of the nursery is to produce scallops of size suitable to farm conditions, enabling them to grow and survive at optimal rates post-transfer. The nursery phase involves a) setting larvae until they are fixed spat (10–14 days), b) rearing post-set spat ( $300\text{ }\mu\text{m}$ ) up to  $>2\text{ mm}$  shell height, or until ready for transfer to intermediate nurseries, such as ponds or sea-based farm sites.

**Nursery phases** – Nursery culture is typically divided into two phases: an early post-set period to  $<2\text{ mm}$  shell height, and a subsequent juvenile phase to  $>5\text{ mm}$ . The size to which an aquaculturist will grow seed is dictated by several factors related to: a) infrastructure of the operation – mainly the scale of the land-based nursery facility; b) the cost-effectiveness of the nursery; and c) the natural conditions prevailing at the farm site.

In other words, size of spat at transfer and time of year at transfer are key to subsequent grow-out performance and to achieving market production target. A good understanding of the physical, chemical and biological characteristics of the farm environment are needed to assess the most cost-effective “Transfer to sea” schedule.

### 7.1 SEAWATER SYSTEM FOR EARLY POST-SET NURSERY PHASE

This phase is generally an extension of the hatchery larval/setting system where temperature and filtration requirements are similar to that of larvae and food is composed of live microalgae. For the Yesso scallops, seawater is double filtered to  $1\text{ }\mu\text{m}$  and  $T = 12 \pm 1\text{ }^{\circ}\text{C}$ .

#### 7.1.1 Raceway set: keeping sieves clean from Day-0 to Day-14

**Water flow** – In the initial 10–14 days post-set, spat are not disturbed from the sieve. During the first 24 h, supply minimal flow to each sieve to allow the larvae to settle. By Day-2 after set, flow is gradually increased to each sieve but remains gentle to allow spat to fix.

**Keeping raceway clean** – Extra care is taken during the first clean. As larvae have not fully settled by then, sieves containing spat are transferred to a saltwater table filled with filtered and heated seawater (as per culture requirements). Sump tank and raceways are drained, cleaned and re-filled with seawater. Sieves with settling larvae are placed back into the raceway undisturbed. As of Day-14, spat in sieves are well fixed and gently rinsed with seawater to remove accumulated detrital matter.

### 7.1.2 Raceway set: growing spat Day-14 to 2+ mm seed

**Flow and food ration change** – Starting Day-14 after set, seawater flow and food ration are increased, and the protocol for post-larval rearing in terms of cleaning, monitoring and thinning to maintain an optimal biomass per sieve is initiated. Food ration is given in Table 7.1. Flow of raceway is set according to total biomass in the system; as a rule flow is set between  $25 \text{ ml}.\text{min}^{-1}.\text{g}^{-1}$  of spat (wet weight) to  $50 \text{ ml}.\text{min}^{-1}.\text{g}^{-1}$  of spat. Keeping a record of biomass and maintaining adequate biomass per sieve become critical around the third week after set, as spat begin an exponential increase in growth. If biomass per sieve is not controlled, high mortalities and slow shell growth will ensue.

**Changing to upwelling system** – Seed measuring 2+ mm continue to grow exponentially requiring an increasing volume of food and space. At this time, they benefit from additional nutrients naturally occurring in seawater. Filtration is reduced to  $10 \mu\text{m}$  for incoming seawater. Increasing faecal production per sieve can lead to clogging and an accumulation of detrital matter within the sieve. At this time, the downwelling system is changed to an upwelling system to improve water quality per sieve. Water flow is reversed; incoming water flows directly into the raceway, regulated by a ball valve; water flows up from the bottom of the raceway through

the mesh of the sieve and flows out at the top of the sieve through an outflow pipe drilled at the top of the sieve. The outflow of the sieve is collected in a centre drainage channel (Figure 7.1). Reversing the flow counteracts the increased faecal deposition by spat.

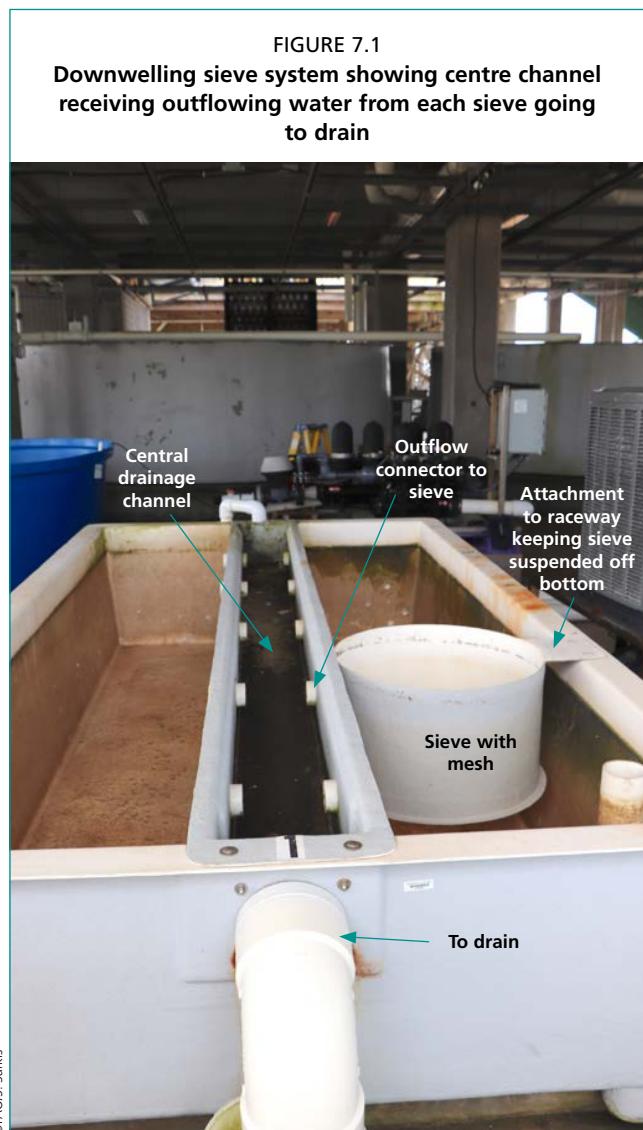
Details of all protocols for rearing scallop spat in raceway are available in other technical guides referred to in Further Reading section.

**Expected Yesso set in raceway** – Setting on sieves is a more controlled approach and high percentage of sets can be obtained. The 16 percent settlement rate reported at Day-14 for Yesso scallops can most likely be improved through additional adjustments in temperature and flow.

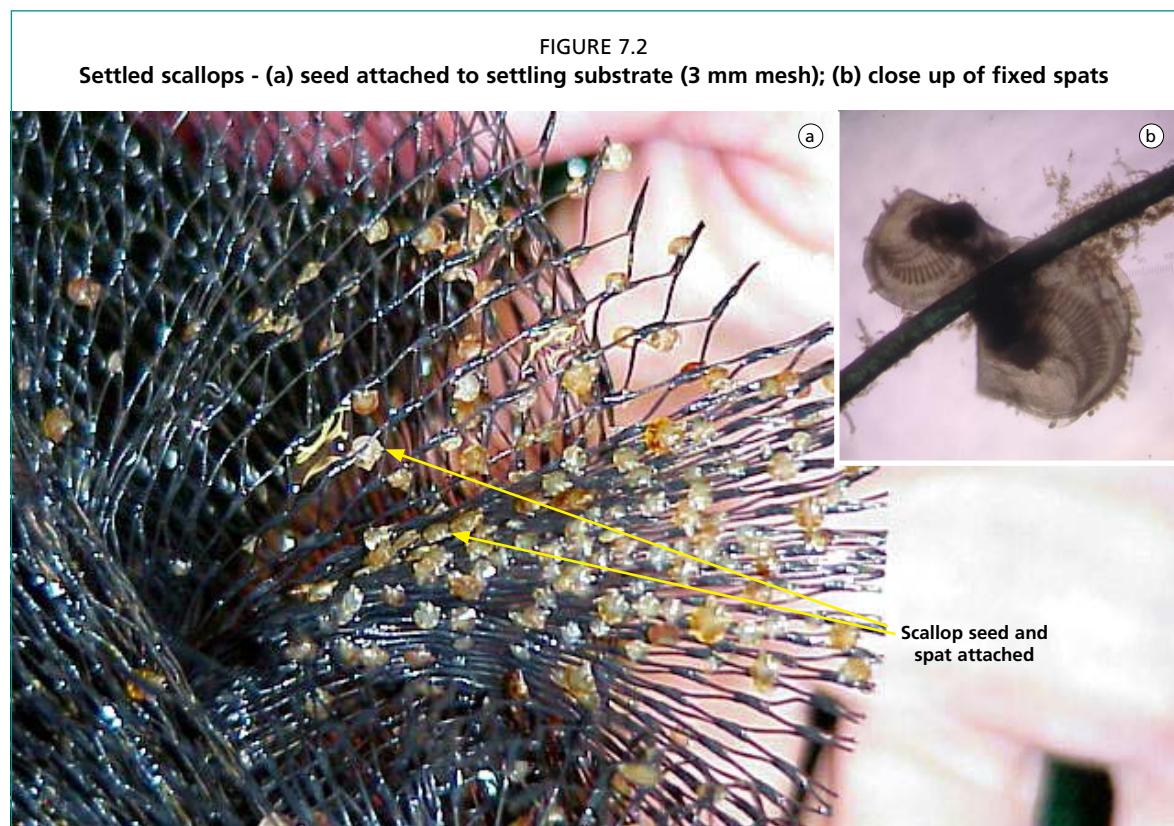
The disadvantage in using raceways is the high demand for space in the nursery to accommodate scallop biomass as they grow. Scallop spat do not perform well if they are crowded or sit on the mesh in more than one layer, unlike oysters or mussels.

### 7.1.3 Tank set: water exchange and feed from setting day to Day-14

**Water exchange** – Spat are reared as for larvae in a static system from Day-2 to Day-4 after set, where water is exchanged



several times a week. The difference between a larval and spat water exchange is that spat exchange is done without exposing the substrate (and set scallops) to air. Figure 7.2 shows a close up of spat attached to the substrate; in order to ensure their survival, a monitored volume of new water flows into the top of the tank while an equal volume is drained from the bottom. The water level in the tank remains the same at all time and the substrate and spat remain always submerged. The duration of the water exchange depends on the water flow and size of the tank. The objective is to exchange 100 percent of the water in the tank at a minimum of three times a week. A water exchange schedule is established as for larvae. Outflowing water is drained through a sieve and any non-set larvae or “swimmers” and dead larvae or spat, are collected; this gives an initial estimate of settlement rate and of the state of the culture (see Protocol 7.1.4).



### 7.1.5 Tank set: continuous flow for Day-14 spat to 2+ mm seed

**Changing flow system** – Following Day-14 after set, any “swimmers” collected are not worth keeping, and are discarded through flushing of tank. The static closed system is changed to a low continuous flow of 1 µm filtered seawater with new incoming seawater filtered and heated similarly to the first 14 days (Protocol 7.1.7). Aeration in the tank is changed from a gentle aeration via airlines to a pre-assembled airlift system. This semi-recirculating system is a combination of continuous flow and airlift and provides a good water circulation among the suspended substrate pieces. This ensures homogeneous water quality and algal distribution throughout the tank. A rearing temperature for Yesso scallop of  $12-13\pm1^{\circ}\text{C}$  provides rapid growth and high survival of spat and should be maintained for as long as possible until 1 week prior to the time for transfer at sea.

### 7.1.4 PROTOCOL: Spat water exchange using a tank setting system

MATERIALS
<ul style="list-style-type: none"> <li>- Hose and/or siphons</li> <li>- Sieves of various mesh size (&gt;120 µm)</li> <li>- Graduated beaker (3–4 L)</li> <li>- Clicker</li> <li>- Compound and dissecting microscope</li> <li>- Slides</li> <li>- Ocular micrometre</li> </ul>
METHOD
<ol style="list-style-type: none"> <li>1. Check tanks every morning as for larvae, adjusting air or temperature when required. Record on daily check sheet.</li> <li>2. For Day-2 to Day-14 after set, nursery tanks follow a similar water exchange schedule as static larval tanks; seawater filtration and temperature are also similar (UV/double filtered 1 µm; T = 13–14 °C).</li> <li>3. Adjust valves so that outflow rate is equivalent to incoming flow rate.</li> <li>4. Setting tanks often have drains at the top and bottom. For Day-2 to Day-14, outflow is from the top as a precaution; it avoids lowering the water level inadvertently should incoming water flow stop. It also leaves metamorphosing larvae gravitating to the bottom of the tank undisturbed.</li> <li>5. Make sure water level in tank does not change; all substrate and spat are continually immersed in seawater.</li> <li>6. During 100 % water exchange, clean sieve (mesh size &gt;120 µm), hose and tube, and place under bottom drain.</li> <li>7. Once 100 % water exchange is complete, wash larvae collected into a 3 L beaker (there should not be enough for a 20 L bucket); any live swimming larvae which has not set is referred to as “swimmers”.</li> <li>8. Count and measure larvae; record any mortality.</li> <li>9. Calculate average shell length and record all data.</li> <li>10. If there are live larvae in sample and culture is in good health, return larvae to tank, passing through a 500 µm mesh.</li> <li>11. If there are few larvae or a mortality &gt;10 %, discard.</li> <li>12. Feed tank AFTER water exchange is complete.</li> <li>13. Day-0 to Day-14 after set: Live microalgal food is distributed by batch as for larvae until a continuous seawater system is started.</li> <li>14. Food ration for spat is given in Table 7.1.</li> <li>15. Early spat are fed by batch; transfer daily algal ration in a food reservoir for each setting tank: <ul style="list-style-type: none"> <li>a. distribute volume of algal species as calculated and dilute with 1 µm filtered seawater (same filtration and temperature as spat tank) to fill food bin;</li> <li>b. Drip-feed over 24 h (this should be fast enough to avoid clogging of spigot by overnight and stop algal distribution).</li> </ul> </li> </ol>

**Keeping culture clean** – As spat grow, the food ration requirement increases exponentially and generates an increasing amount of detrital matter; this along with dead larvae or spat accumulate on the bottom of the culture tank. Spat tanks are flushed weekly using the bottom drain or a siphon; outgoing water is checked for spat mortality and/or input of other invertebrates from natural seawater.

### 7.1.6 PROTOCOL: Monitoring settlement rate and growth for spat <1 mm shell height

#### MATERIALS

- Sieve with 120 µm mesh
- Dissecting microscope
- Petri dish with 1 cm<sup>2</sup> grid drawn on bottom of dish
- Lab wash bottle
- Scale ( $\pm 0.01$  g)
- Laminated grid (20 × 28 cm) of 1 cm<sup>2</sup>
- Ocular micrometer or digital camera fitted to microscope

#### METHOD

**Note:** Scallop spat <1 mm are fragile and difficult to handle.

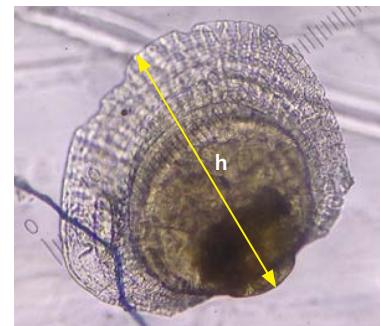
Monitoring shell growth and spat numbers is conducted weekly; alternative tanks can be sampled daily to provide an estimate for the whole batch and minimise handling for any one setting tank.

#### During 100 % water exchange

1. Collect any dead or live spat washed into sieve during water exchange.
2. Record number of live and dead spat under the microscope.
3. Record any dead larvae seen.
4. Make observations on dissoconch development, presence of detritus, evidence of feeding by appearance of digestive gland (full and brown in colour).

#### Measure newly settled spat (<1 mm)

5. Measurements are made once a week on live spat.
6. Spat are measured along their shell height (h), not shell length (unlike larvae). See photo.
7. Cut an Eppendorf tip at the end, so that aperture is larger to collect spat.
8. Collect a 1 ml sample using an Eppendorf pipette with newly cut “spat” tip, and place on Sedgewick-Rafter cell. To spread sample evenly, place cover glass over chamber top.
9. Measure a minimum of 30 spat under compound or dissecting scope using an ocular micrometer or digital camera. Note objective size or scale and record number of units.
10. Convert number of units to microns according to magnification or scale used (objective size).
11. A petri dish marked with a grid of 1 cm<sup>2</sup> is used for viewing subsample under the dissecting scope.
12. For the petri dish subsample:
  - collect spat in a small diameter sieve (15 cm), and wash into petri dish using a lab wash bottle; and
  - count spat under microscope, using a clicker and working your way across and down the squares.



Spat height (h)

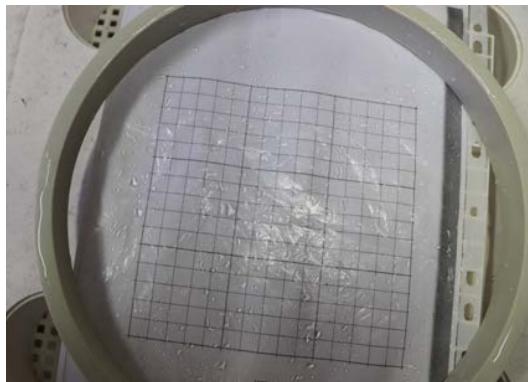
©FAC/M. Franze

**Note:** The ocular micrometer or digital imaging must be calibrated for each objective lens and recorded. This is necessary only once for each magnification and each microscope.

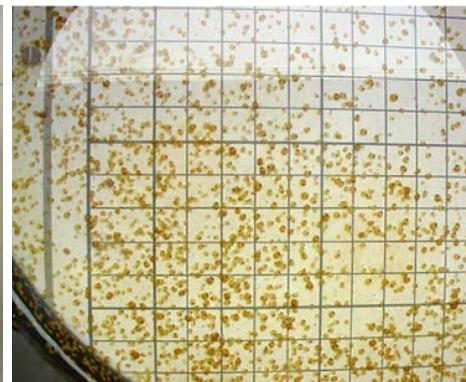
### 7.1.6 PROTOCOL (continued)

#### Measuring spat >1 mm

13. Spat close to 1 mm shell height are visible with the naked eye as brown spots.
14. Collect one piece of substrate with spat or a subsample (if spat density is too high) from setting tank, and place into a 30–40 cm 120 µm sieve.
15. Working on the saltwater table, wash down spat from substrate using a gentle jet of filtered seawater (<10 µm) into a sieve.
16. Place sieve on top of a laminated 1 cm<sup>2</sup> grid.



Laminated grid



Measuring spat on 120 µm sieve placed on laminated grid

17. Using clicker, work your way across the squares of the grid (as you would with a Sedgewick-Rafter cell) and count.
18. Take care not to count the same spat twice.
19. Measure a minimum of 30 spat.
20. Alternatively, counting devices such as XperCount digitized bucket can be used.



Counting spat

#### Weigh >1 mm spat

21. Using the same subsample measured, record weight by following the same procedure as Steps 1–12 in Protocol 7.2.7.
22. Estimate stocking density (see Section 7.2.6).

**Expected Yesso set –** The expected percentage set is as follows:

- Tank system – From Day-0 to 1 mm spat:  
Percentage set can range from 3 % to approximately 14 % when set at an initial average setting density of 2.8 larvae.ml<sup>-1</sup>.
- Tank system – From Day-0 to 2.5 mm:  
2 % to approximately 5 % with same setting density.
- Raceway system – From Day-0 to 1 mm spat:  
16 % with initial setting density of 30 larvae.cm<sup>-2</sup>.

The percent set and survival varies with respect to the seawater system and seawater quality, handling and food composition. Setting rates of up to 40 percent are reported for other species and the expected percentage set for the Yesso scallop may be increased through improved husbandry.

### 7.1.7 PROTOCOL: Changing from static to continuous spat culture tank system

#### MATERIALS

- Sieves for bottom drain
- Sedgewick-Rafter cell
- Compound or dissecting microscope

#### METHOD

**Note:** Using continuous flow vs static system is dependent on the heating capacity of the nursery. If heated seawater is not sufficient to remain constant during continuous flow of all spat tanks, post-larvae can be reared in a static system until 1.5 mm shell height (Day-50). Spat >1.5 mm are difficult to rear in a static system.

#### Changing to semi-recirculation system

1. Once the number of swimmers collected during water change has declined to zero, or those obtained are moribund, the setting period is ended; all remaining fixed larvae are referred to as spat.
2. Start the airlift system by transferring airlines in tank into vertical airlift pipes. Airlines should be all the way down to bottom of vertical pipe. Water should be seen to come out of ALL the holes in the transverse pipe. This recirculates water from bottom to top.
3. Filtration remains at 1 µm until 1.5 mm size.

#### Static tank system

4. Water exchange schedule is the same as that of larvae
5. Feeding is by batch or it can be introduced to all tanks simultaneously by pumping from a common reservoir with known amount of algae.

#### Continuous tank system

6. Outflow is from the bottom; a stand-up pipe is used to make sure there is no loss of larvae if incoming flow stops.
7. For added control, outflow can be additionally regulated using a top drain valve.
8. Incoming seawater is regulated by one-way valves and flows at the top of the tank. If there is concern of accumulated detritus, the valve for incoming flow can be connected to a pipe extending vertically close to bottom of tank.
9. For spat >1.5 mm, water is filtered to 10 µm. This supplements the food ration given by supplying naturally occurring algae and dissolved organic matter.
10. Adjust incoming flow rate according to spat biomass. Flow rate is increased as spat grow and stocking density increases, as follows:
  - Initial incoming flow rate is adjusted to provide a 50 % water exchange;
  - Incoming flow rate is gradually increased to 70 %, 100 % and >100 % throughout larval life based on stocking density; and
  - Care must be taken to ensure that residence time is long enough for food to be consumed by spat and NOT to be flushed out.

**Daily morning checks:** monitor water level, flow rates, oxygen, temperature and any other relevant parameters. Record values.

### 7.1.7 PROTOCOL (continued)

**Flushing bottom detritus from tank system** (frequency once a week)

11. Water is collected from the bottom of the tank. Use existing drain or siphon to flush bottom water.
12. Incoming water is regulated by a one-way valve connected to a 25 cm pipe extending to the bottom of the tank.
13. Do a water exchange for a period exceeding 100 %. For example, for a 10 000 L tank with an incoming flow of  $6 \text{ l}.\text{min}^{-1}$ , water exchange lasts 2 h.
14. Place a sieve with mesh size ranging from 80–120  $\mu\text{m}$  under bottom drain valve to collect dead spat or debris.
15. Once flushing is complete, contents of sieve are washed into a 2 L beaker, and examined under microscope.
16. Record observations. Note other invertebrate larvae or organisms, state of culture, or dead spat.

#### If there are dead spat

17. Count and measure a subsample of 20. This will provide an indication as to timing of mortality and the cause may be explained by factors occurring during this period.
18. Record dead spat height and calculate mean.

#### Acclimating spat to ambient

19. Spat are to be acclimated to ambient prior to transfer to ponds or to sea, or if heating of seawater is not possible.
20. Acclimation takes place over a 5–10 day period.
21. Rearing temperature is decreased gradually by  $1^\circ\text{C}$  per 24–48 h period.
22. Filtered ambient seawater is added to tank such that temperature decreases by  $1^\circ\text{C}$ .
23. This process is repeated daily thereafter until ambient temperature is reached. Spat are kept at ambient for at least 48 h before transfer.

## 7.2 FEEDING SPAT IN LAND-BASED NURSERY

Providing a suitable amount of food to spat as they grow can prove challenging. Food is supplied in the form of live microalgae, commercially available diets and naturally occurring plankton in incoming seawater.

### 7.2.1 Spat food ration

Early spat (<2 mm) are fed microalgae, using the same species as for larvae. As spat grow and become more robust, commercially available substitutes to live microalgae are known to result in good growth and survival rates. Table 7.1 shows the food ration, expressed as algal cells. $\text{ml}^{-1}$  from day of set (Day-0) until >2 mm shell height. Thereafter, ration is based on dry algal weight and live (wet) weight of spat. The algal culturist will calculate the volume of algae to harvest based on the food ration; records of the quantity and composition of food are kept daily.

### 7.2.2 Feeding spat in raceway setting system

**Day-0 to Day-14:** The raceway seawater system is closed; live microalgae is mixed into the sump tank, and enriches the seawater supplied to each individual sieve.

**Day-14 onwards:** Once the system is changed to semi-recirculating with the input of new seawater, food ration is divided into two; half of the food ration is distributed via a

TABLE 7.1  
Food ration for spat from day of set (Day-0) to >2 mm shell height

Spat size	Day after set	Food ration (cells.ml <sup>-1</sup> )	Food composition
<500 µm	0	19 000	1:1:1 (Flagellates:Diatom:Live Tetra)
	1–6	20 000	
	7–11	25 000	
	12	27 000	
	13	30 000	
	14–15	32 000	
	16	35 000	
	17	38 000	
	18	40 000	
	19	45 000	
600 µm	20	50 000	
	21	55 000	
	22	60 000	
	23–36	60 000	<b>Option:</b> Substitute ½ of ration with Instant Algae 1:1:1 (Flagellates:Diatom:Instant Tetra)
	37	65 000	
	38	70 000	
	39	75 000	
	40	80 000	
	41	85 000	
	42	90 000	
>2 mm	43	95 000	
	44	100 000	
	45	120 000	
	46	150 000	
	47–50	190 000	<b>Option:</b> Substitute with Instant Algae™ up to 60 % of total food ration
	51–61	220 000	
	62	4–6 % wet weight of spat	
<b>Note:</b> "Tetra" refers to <i>Tetraselmis</i> sp.			<b>Option:</b> Substitute up to 85 % of total food with Shellfish Diet/Instant Algae or equivalent

carboy which drip-feeds into the raceway, and the other half mixed into the sump tank. In this way, spat receive an adequate density of live microalgae, which is maintained over 24 h.

### 7.2.3 Feeding spat in tank setting system

**Day-0 to Day-14:** Microalgal ration is drip-fed over 24 h period from a carboy dedicated to each tank; live microalgae are kept in suspension in the tank through a gentle aeration to avoid disturbance to larvae which would limit settlement.

**Day-14 onwards:** Once the semi-recirculated system is started, a continuous supply of food is pumped from a food reservoir to the spat tanks. All live and commercial food is calculated and diluted with seawater in the food reservoir on a daily basis; the food reservoir often consists of a round flat bottom tank and is cleaned daily prior to the addition of new food.

### 7.2.4 Food ration and composition for spat in raceway and tank systems

Live microalgal ration for spat is initially calculated as for larvae, based on algal cell density and volume of rearing tank. Food ration for spat starts at 19 000 cells.ml<sup>-1</sup> increasing to 120 000 cells.ml<sup>-1</sup> by Day-45 after set; by Day-60 or when spat average 2 mm shell height, food ration totals 220 000 cells.ml<sup>-1</sup>. Live microalgae is best suited for optimal scallop spat growth and is especially important in scallop spat <2 mm shell height.

Beyond 2 mm, spat biomass starts to increase exponentially, and maintaining growth requires a rapidly increasing amount of feed. At this stage, food ration is based on spat biomass (mg wet weight) per tank or per batch and averages 4 percent. High volumes of live microalgae are required at this time to satisfy the growing spat, equivalent to a daily ration of 4–6 percent dried algae per mg wet weight of spat; this can put substantial pressure on the algal production unit of the hatchery. In order to alleviate the demand for high quality microalgae, commercially available substitutes can be used, such as instant algae, or Shellfish Diet.

**Food composition for spat >2 mm** – Keeping a portion of the food ration as live microalgae benefits scallop spat growth; a minimum 15 percent of total food ration is recommended as live microalgae. Commercial substitutes are introduced gradually over a 7-day period to reach the targeted proportion of diet.

An example of food composition for Yesso spat reared in a land-based nursery is given in Table 7.2. Shellfish Diet can be substituted as early as Day-50, replacing Instant Algae, as shown in the example. If the operation's capacity for live microalgae is sufficient, there is no need for commercially available diets. See 7.2.5 for calculating rations when using commercial diets.

TABLE 7.2  
Food ration for *M. yessoensis* spat from Day-1 after set to Day-100

Day after set	Live microalgae (%)	Instant Algae (%)	Shellfish Diet (%)
Day-1 to Day-45 (minimum)	100 1:1:1 Iso/Pav:Chaet/3H:Tetra	0	0
Day-46 to Day-66	40–50 % (minimum)	50–60 % (maximum) 1:1:1 Iso/Pav:3H:Tetra	0
Day-67 to Day-100	15	0	85 Shellfish Diet 1800®

## 7.2.5 PROTOCOL: Calculating food ration using commercial diets

### MATERIALS

- Shellfish Diet 1800®
- Instant Algae
- L graduated beaker or graduated cylinder
- 20 µm sieve

### METHOD

1. Use Table 7.2 as a guide for supplementing commercial diet.
2. Provide 100 % live microalgae at least until Day-45, preferably until Day-55.
3. Estimate biomass of batch on a weekly basis.
4. Calculate volume of Shellfish Diet as shown below.

#### Live microalgae

5. Estimate maximum capacity for live microalgae production for spat in facility.
6. Estimate maximum volume of live microalgae available per spat tank.
7. Count algae and calculate to provide food ration as per Table 7.2 until maximum capacity for live microalgae per spat tank is exceeded.
8. Once live microalgal capacity exceeds the requirements for 100 % live microalgae ration, give each spat tank calculated maximum live microalgae and supplement with commercial diet.

#### Calculating Shellfish Diet (SFD) to supplement food ration

9. Estimate total wet weight (or biomass) in grams for tank, batch or raceway to be fed.
10. See example below for calculations.

#### *Example:*

Live algal production has maximum capacity to supply 40 000 cells.ml<sup>-1</sup> per spat tank  
 $40\ 000\ \text{cells.ml}^{-1} = 15\ \% \text{ of total food ration}$

SFD supplements 85 % of total food ration

Total spat biomass per tank is 750 g wet weight/tank

#### Calculate as follows:

for 100 % ration

ml of SFD needed =  $750\ \text{g} \times 0.036$  (manufacturer's instructions)

for 85 % ration

ml of SFD needed =  $85 \times (750 \times 0.036) \div 100 = 23\ \text{ml}$

#### Preparing and distributing Shellfish Diet

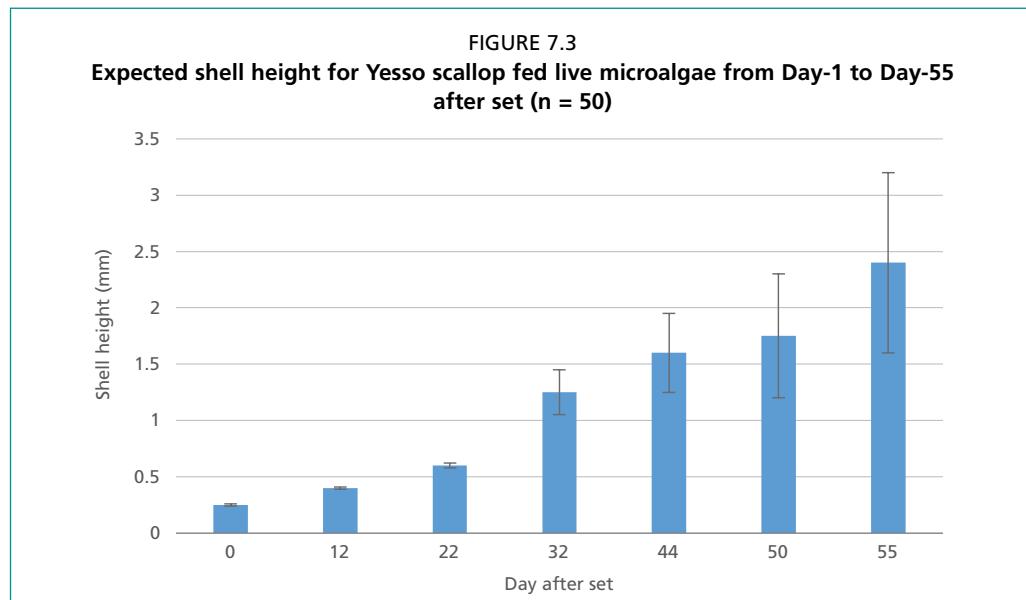
11. Collect amount calculated in a graduated cylinder or beaker.
12. Pass through a 20 µm sieve before distributing into food reservoir.
13. **For batch feed:** pour SFD and dilute with 1 µm filtered seawater to top of food reservoir.
14. **For continuous feed tank:** divide food ration into 3 batches, administered over the 8 h day.
15. Fill feed tank to half with filtered seawater.
16. Pass  $\frac{1}{3}$  of Shellfish Diet required through sieve directly into tank.
17. Make sure tank is aerated vigorously and recirculate water to mix well.
18. Add live microalgae portion at this time and fill tank with filtered seawater.
19. Distribute food to spat tanks by pumping.
20. Repeat over the 8 h day with last  $\frac{1}{3}$  of Shellfish Diet added before end of day.

#### Calculating Instant Algae

Calculate as per live microalgae using manufacturer's information for cell density.

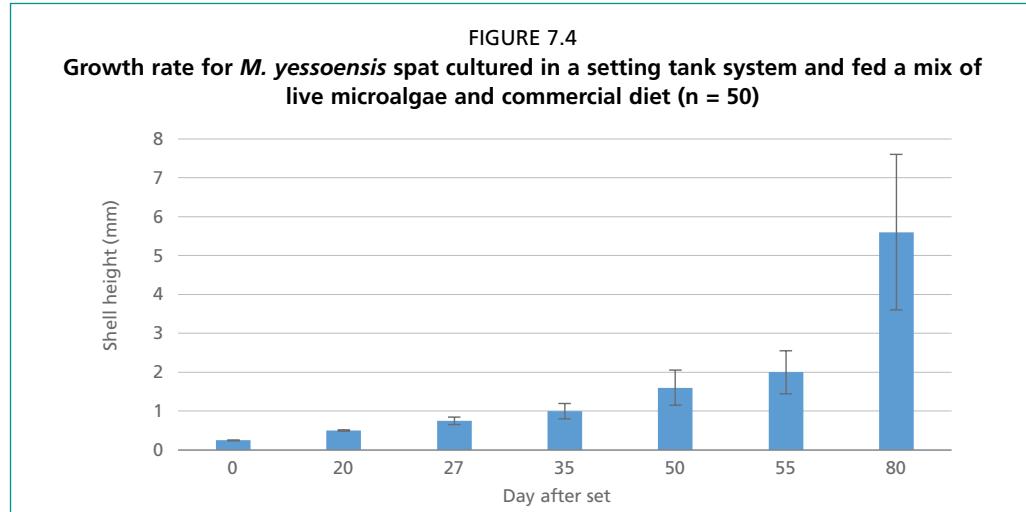
**Expected Yesso spat growth** – Spat growth for a good batch of *M. yessoensis* larvae set in 5 000 L round tanks at a density of 2 larvae.ml<sup>-1</sup> at T = 13±1 °C and fed 100 percent live microalgae is illustrated in Figure 7.3. Average shell height >1 mm is attained by the first month of setting. Within two months, spat should grow to 2 mm and beyond. As a rule of thumb, spat grow approximately 1 mm per month. The increasing error bars in Figure 7.3 reflect the wide variation seen in growth rate among spat.

If spat are reared in a raceway setting, grading is done routinely to separate the larger and smaller size fractions.



Shell height for Day-55 spat is slightly higher but comparable when fed a 100 percent live microalgae ration (Figure 7.3) and a mixed food ration of live microalgae and SFD as illustrated in Figure 7.4. Food ration in these examples is as given in Tables 7.1 and 7.2. For spat in Figure 7.4, by Day-46, more than 50 percent of the diet consisted of commercially available food; by Day-67, 85 percent of the diet consisted of instant algae and Shellfish Diet. Total food ration by Day-70 is equivalent to a live microalgal ration of 350 cells.ml<sup>-1</sup>.

Spat kept in the nursery tanks until Day-100 after set with the same diet (15 % live microalgae, 85 % SFD) reached a maximum shell height of 9 mm.



### 7.2.6 Stocking density and adjusting flow for seed biomass

Maintaining an optimal stocking density in a raceway or tank system is critical to successful seed production. Critical seed density in a nursery system is largely a function of the available surface area to the growing spat and the inflow rate of new seawater. At this stage, biomass becomes the measuring criteria rather than shell height.

**Raceways** – In raceways, scallops are reared one layer deep in sieves, at a density covering approximately  $\frac{1}{3}$  of the surface area of the sieve (Figure 7.5). As spat grow, the bottom of the sieve becomes fully covered and crowding occurs. To maintain an optimal biomass for continued growth, spat must be re-distributed at lower densities; this is referred to as “thinning”. The objective is to provide sufficient space for spat as they grow. This becomes especially critical starting Week-3 after set when spat begin a rapid increase in growth.

Thinning to the appropriate stocking density in sieves can be estimated visually by maintaining a 60 percent coverage on the surface area. If stocking density per sieve is not controlled, high mortalities and slow shell growth will ensue.

The size and volume of the raceway system dictates the total maximum stocking density supported by the raceway. Incoming flow to the raceway can be increased to adjust to a higher biomass in conjunction with semi-recirculation, but should not exceed  $50 \text{ ml}.\text{min}^{-1}.\text{g}^{-1}$  of spat (wet weight); increasing the flow beyond the maximum will not retain added food long enough in the system to be consumed by spat.

The longer the spat are kept in the nursery, the higher the stocking density and the greater the number of raceways required to accommodate the increasing biomass.

The main limiting factor to stocking density in a raceway system is the total surface area of the meshed sieves. For larger operations, raceways may not prove sufficient to achieve the target seed production, if the goal is to rear seed  $>2 \text{ mm}$  in size. In this case, the aquaculturist may achieve the target seed production by combining the raceway system with the nursery tank system. Alternatively, the aquaculturist can choose to transfer seed out of the nursery at a smaller size to an intermediate nursery.

**Nursery tanks** – Rearing spat in tanks may prove more advantageous for larger scale seed production or when space in the facility is limited. The use of substrates (bundles of mesh) increases the surface area available to spat (see Figure 7.2) and allows for increasing biomass as spat grow; in turn, the increasing biomass can be supported by increasing the incoming seawater flow.

FIGURE 7.5  
Scallop seed (3–5 mm) reared in upwelling sieve in raceway, showing partial coverage of the meshed surface area with spat one layer deep



©FAOM Helm

In general, recommended stocking density for scallops is equivalent to 200 mg of biomass for 1 000 L volume of seawater. As spat biomass increases, incoming flow is increased to maintain the recommended stocking density; this gradually converts the spat tank up to twofold its volume. Table 7.3 provides an example of increasing flow rate in a 10 000 L nursery tank, and its effect on the resulting volume of seawater passed through spat. Accordingly, food supply rate is also adjusted to provide a continuous supply over a 24 h period. Residence time of food in the tank is partially ensured by semi-recirculation and by not exceeding a threshold in incoming seawater rate.

At the same time, food supply is supplemented by additional nutrients with incoming seawater when filtered to 10 µm (not 1 µm as during early set).

**TABLE 7.3**  
**Increasing a 10 000 L tank capacity with increasing incoming seawater flow rate to accommodate spat growth**

Weeks after set	Mean shell height (mm)	Incoming seawater flow (L·min <sup>-1</sup> )	Tank capacity as L·h <sup>-1</sup> (tank volume plus additional flow )
Week 4–5	1	7	10 420
Week 8–9	2	38	12 280
Week 12	5	380	22 800

“Thinning” to reduce spat biomass per surface area of substrate cannot be realistically implemented in a nursery tank system, as is done for sieves in a raceway. Maintaining growth and survival rates as spat grow in the nursery tanks, relies on an adequate initial setting density and on adjusting flow rate. A higher mortality rate can be expected in a nursery tank system compared to a raceway system and attaining production target relies on a higher volume of seed produced per given surface area of the nursery facility.

Initial setting density is a major factor in the resulting biomass per substrate in a tank system. An initial high setting density can result in a high yield of 2 mm seed, as is shown in Figure 7.6a. However, this will subsequently result in a high mortality rate, if young seed are not collected from the substrate and provided with a larger surface area in due time. Setting at lower densities will provide spat with more substrate area as they grow (Figure 7.6b); this facilitates the managing of stocking density and will allow to raise to a larger size in the nursery. This is a management decision and depends on production scale, personnel and space resources available.

**FIGURE 7.6**  
**Varying seed density for Yesso scallops on setting substrate suspended in nursery tanks – (a) high seed biomass on top section of substrate; and (b) low and medium seed biomass on substrate**



### 7.2.7 Calculating spat biomass in a tank setting

In order to calculate biomass for spat reared in a tank, total wet weight per tank is estimated. The following information is needed:

- number of substrate pieces used for setting per tank;
- average shell height; and
- average spat weight per substrate piece.

In the first year of operation, collecting data to determine the relationship between shell height and weight of spat throughout the nursery phase is recommended; this enables a rapid assessment of biomass for future batches and in the years to come. A subsample of substrate is collected, from which spat are washed onto a pre-weighed mesh, measured and weighed; data is graphed and used to estimate total spat weight in a tank. Protocol 7.2.8 describes the steps used for estimating spat settled on substrate; the procedure is the same for spat set on raceway sieves.

### 7.2.8 PROTOCOL: Estimating spat biomass on substrate

#### MATERIALS

- |   |  |
|---|--|
| - Screen with mesh of appropriate size  | - Pieces of mesh (various aperture size) |
| - Sedgewick-Rafter cell   | - Analytical scale ( $\pm 0.001$ g)      |
| - Ocular micrometer with compound microscope or digital camera fitted onto microscope for imaging | - Petri dishes                           |
| - Eppendorf pipette and tip   | - Dissecting Scope                       |
|   | - Scale ( $\pm 0.01$ g)                  |
|   | - 10 % formalin                          |

#### METHOD

##### Weighing spat 1–1.5 mm

1. Prepare several pieces of 25 cm<sup>2</sup> 700 µm mesh.
2. If spat are seen to be relatively dense on substrate; take a subsample of substrate piece (¼).
3. Make sure to carry substrate and spat in holding beaker or bucket.
4. Wash down spat from substrate into a small sieve using a gentle jet of filtered seawater.
5. Collect spat in 100 ml containers.
6. Cut an Eppendorf tip at the end, so that aperture is larger to collect and measure 30 spat using a Sedgewick-Rafter cell or a camera fitted on the microscope.
7. As spat grow, subsamples are collected on petri dish lined with a grid and measured under a dissecting scope.
8. Collect a sample of a minimum of 100 spat in a 100 ml container.
9. Tare 25 cm<sup>2</sup> mesh using analytical scale.
10. Carefully wash sample through mesh.
11. Blot mesh dry by placing on a piece of absorbent paper; paper only touches mesh not spat.
12. Weigh mesh and spat. Record value.
13. Calculate spat weight for 100 spat.
14. Repeat Steps 2–13 for 2 other pieces of substrate in the same tank, and for two other tanks.
15. Enter in Excel datasheet for plotting height vs weight graph.

### 7.2.8 PROTOCOL (continued)

#### Weighing spat >2 mm

16. Follow Steps 1–4 to collect spat in a small container.
17. Tare one piece of 25 cm<sup>2</sup> mesh on scale.
18. Place tared mesh over measuring cup and carefully pour your entire spat sample through the mesh, so that you collect all spat on the mesh.
19. Measure 30 spat under the dissecting microscope or with Vernier caliper if spat are >2.5 mm.
20. Blot mesh and spat dry with absorbent paper.
21. Weigh and record. If only ¼ piece of substrate was used for weight, multiply recorded weight by 4 to obtain total weight per 1 piece of substrate.
22. Repeat Steps 16–21 with two more samples taken from different pieces of settling substrate from same tank.
23. Repeat Steps 16–22 for two other setting tanks.
24. Enter in Excel datasheet to graph height vs weight.



Weighing spat on mesh

#### Estimate spat biomass per tank

25. Calculate:

$$\text{Average biomass/substrate} \times \text{No. of substrate per tank}$$

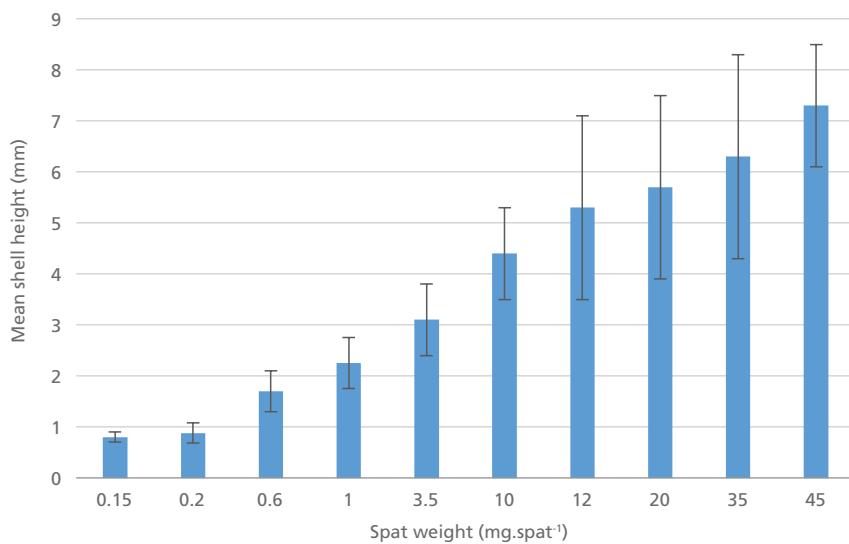
#### Estimate spat biomass for the whole batch

26. If calculated biomass per tank is similar among replicate tanks (Step 22), assume equivalent biomass for all tanks (this assumes equal number of substrate pieces per tank).
27. Calculate average biomass per tank × number of tanks for the batch.

**Yesso spat height and weight** – Data for height and weight of spat reared at 12±1 °C in a nursery tank system is given in Figure 7.7. At this temperature, 7 mm seed is expected to have a mean weight approximating 45 mg.spat<sup>-1</sup>. Variations in shell height increase as spat grow reflecting a marked difference in growth within a spat batch. This relationship is dependent on nursery conditions, namely food composition and ration, and data given in Figure 7.7 should be validated against a new nursery system. Example below describes how to calculate total biomass per tank or per batch using the height/weight graph. The biomass data is also used for: a) calculating the food ration for the nursery tanks for seed >2 mm (see Section 7.2); b) distributing seed in bags and/or nets for transfer at sea (see Section 8.2); c) assessing total seed production per batch; and d) anticipating the number of bags, nets and longlines required for grow-out on the farm.

FIGURE 7.7

**Relationship between Yesso spat shell height (mm) and weight ( $\text{mg} \cdot \text{spat}^{-1}$ ) from newly settled spat (<1 mm) to 7 mm seed reared in nursery tanks ( $n = 50$  for height;  $n = 15$  for 100 spat subsample weight)**



### EXAMPLE – Using a height vs weight graph to calculate stocking density

#### Using the height vs weight graph in Figure 7.7

Mean shell height = 800  $\mu\text{m}$  (or 0.8 mm)

Total weight of all spat collected from 1 piece of substrate = 225 mg

Number of pieces of substrate in one tank = 50

From graph - Average weight per 0.8 mm spat is 0.15 mg

#### Calculation:

Total number of spat on 1 piece of substrate = Total weight (mg)  $\div$  Average weight per spat ( $\text{mg} \cdot \text{spat}^{-1}$ )

$$= 225 \text{ mg} \div 0.15$$

$$= 1\,500 \text{ spat on substrate}$$

For a tank with 50 pieces of substrate

$$\text{Total number of spat in tank} = 1\,500 \times 50 = 75\,000 \text{ spat}$$

### 7.2.9 Rearing spat >2 mm

As spat grow greater than 2 mm shell height, they become increasingly tolerant to natural environmental conditions; they also become more costly to maintain and raise in a land-based nursery as their demand for higher food ration and seawater supply continues to grow exponentially. The rearing system in the land-based nursery remains similar; a 10  $\mu\text{m}$  filtration is recommended, incoming flow rate is increased according to biomass and resulting stocking density, and there is often an increased reliance on commercially available food.

**When to transfer spat out of nursery** – The hatchery manager assesses the optimal time for transfer to outdoor intermediate nurseries (such as ponds, larger outdoor nursery tanks or upwelling raceways), or directly to the farm.

The size until which young seed is kept in a controlled land-based nursery is dependent on nursery parameters, but also on the physical, biological and chemical seawater characteristics of the farm site to which they need to be transferred. These will fluctuate seasonally and affect distinct spat batches differently. Acclimating nursery-produced spat to ambient conditions prior to transfer out of the nursery is a must; the duration of acclimation depends in great part on the temperature difference between the nursery and ambient conditions. For a continuous 12-month production, the aquaculturist will utilise several strategies to optimise timing and size of seed at transfer, based on seasonal conditions.

## 8. Nursery to farm

**IN THIS CHAPTER –** How to maximise cost-effectiveness in the nursery and how to transport seed from nursery to farm.

The nursery is costly in terms of food production, labour, seawater volume and treatment requirements and space. Ideally, the aquaculturist strives to transfer seed as soon as possible to the farm; but a balance has to be achieved between reducing the work load on land and optimising the growth and survival of seed transferred. The key is to coordinate spat or seed size at transfer with natural physical, chemical and biological conditions at the farm; if conditions are not suitable for the size of seed ready for transfer, it is recommended to hold the seed longer in the nursery or make use of an intermediate nursery. Successful rearing of Yesso scallops >2 mm and up to 10 mm shell height is doable in intermediate nurseries, if optimal stocking density is maintained.

**Farm conditions for seed** – The time of year and size at which spat are transferred is dependent on many environmental factors, including: temperature, primary production and natural recruitment of potential predators. These will affect the survival and growth of transferred seed. For example, moving young scallop seed to the farm during a crab recruitment period, can result in the trapping of crab larvae in scallop nets and subsequent predation of fast growing crab on the scallop seed. Information relating to the environmental processes at the farm is valuable and is important to obtain prior to setting up a farm site.

**Production scale considerations** – The production volume of spat is also a factor in selecting a strategy for transfer to farm. Hatchery operations which produce spat on a large-scale can opt to move very young spat (<1 month old) out of the nursery and transfer to the farm; they know that the survival rate will be low but this is counteracted by the large volume transferred. This approach is often referred to as a “remote nursery”. On the other hand, smaller scale hatcheries will benefit from a longer nursery period to produce spat >2 mm as seed survival following transfer to farm increases with seed size.

### 8.1 REMOTE NURSEY

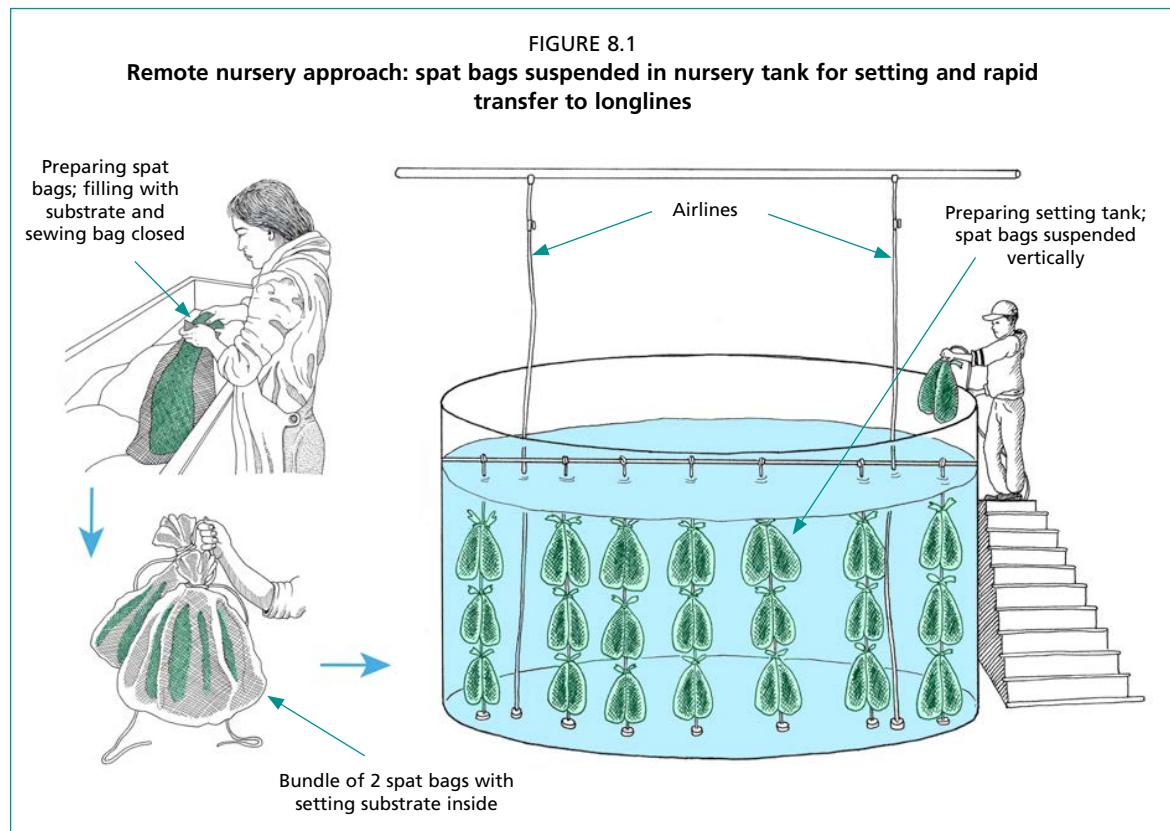
In cases where nursery operations are not cost-effective and high volumes or an excess volume of pediveligers are produced during the hatchery cycle, a “remote nursery” approach is an alternative. Using this methodology frequently results in high losses of competent larvae and/or young spat within the first month of grow-out on longlines; this subsequently leads to a reduced survival of spat to larger seed size. For this reason, it is only under circumstances described above, or if there is an excess of spat settled, that the culturist can afford to adopt such a cost saving protocol for the nursery operations.

The general concept of a remote nursery is to minimise the nursery load by transferring spat recently set (<800 µm) from the land-based facility to the farm.

#### 8.1.1 Use of spat bags for remote nursery approach

**Setting for remote nursery approach** – This approach is used at the time of writing in large-scale hatchery operations, routinely producing >50 million pediveligers per

spawn. Setting substrates are placed into fine meshed spat bags and suspended in a static aerated round or conical tank with gentle aeration (Figure 8.1). Spat bags come in various mesh sizes and sizes of 425–700 µm are used for the Yesso scallop. Sets of two spat bags are attached at regular intervals along a piece of floating rope tied to opposite sides of the setting tank; bags are suspended vertically at 1 m intervals to fill the tank with substrate from the surface to the bottom. Another option is to lay spat bags horizontally in lantern nets (3–4 mm mesh size). Two spat bags can be placed per layer of lantern net to maximise space on the longline at the farm. Competent larvae are introduced in the tank and allowed to set into the bags. The smaller the spat bag mesh size, the more likely larvae and young spat will be retained, and the higher the resulting density of spat settling per bag. Scallops will settle both inside the bag and on the outside of the bags; this means that an unknown percentage of scallops is additionally lost during transfer as spat dislodge easily during handling and will fall off the bags.



**Size of spat at transfer** – Spat settled in substrate and bags are cultured at  $T = 12 \pm 1^\circ\text{C}$  to attain desirable sizes in 2–4 weeks. Minimum shell height for spat retained in 425 µm mesh bag is 600 µm and is attained within two weeks of nursery culture; for retention of spat in 700 µm bags, spat must exceed 1 mm in shell height, expected to be reached in 4 weeks of nursery culture.

Spat are gradually acclimated to natural seawater temperatures one week prior to transfer. Once ambient seawater is reached in the nursery tanks, spat bags are moved to longlines moored in a protected site for on-growing to juveniles.

**Controlling fouling** – Once transferred to the farm, maintenance of bags is critical to optimising survival and growth. Most importantly is the control of fouling on the spat bags; excessive fouling will reduce the water flow, poor growth and spat mortality will follow. For this reason, waiting an additional two weeks in the nursery and transferring

spat in the larger 700 µm spat bags is advisable; a large size mesh reduces the time in which fouling clogs the mesh aperture and the water flow supplying nutrients to young spat.

Routine maintenance includes scrubbing bags on a regular basis. Depending on the primary productivity level at the farm, scrubbing may be required monthly or more frequently; it will most likely cause additional loss of spat by removing and damaging spat fixed on the bag itself. This procedure can be very labour intensive, and ineffective in the operation of the farm. It is most suited to regions where labour is readily available and fouling is minimal.

**Transfer to nets** – Based on seed growth rates obtained for the Yesso scallop in northern temperate areas, it is anticipated that spat will be large enough to be transferred to 1 mm mesh bags within 1 month of culture on the longline. This can be a relatively simple and rapid procedure, dependent on the level of fouling of the substrate itself. In case of minimal fouling, the settling substrate with attached spat can be directly removed from a 700 µm bag into a 1 mm bag, without disturbing young seed.

For a substrate with heavy fouling and if seed are 2 mm+, scallops are washed off the substrate onto sieves and “thinned” for re-distribution in appropriate size mesh bags. A minimum 2 mm shell height is the advised size for handling spat in this way as smaller spat are too delicate and will incur high mortality if the aquaculturist attempts to remove these from the settling mesh.

The procedures used for retrieving seed from the settling substrate, thinning and re-distributing are from here on similar to those used for nursery-reared seed (see Section 8.2).

## 8.2 RETRIEVING NURSERY-REARED >2 MM SEED FOR TRANSFER AT SEA

The larger the seed, the harder and more tolerant to handling it becomes; this minimises any potential mortality associated with stress during handling and transfer at sea. From the aquaculturist point of view, larger seed facilitates the retrieval of seed from the settling substrate and its distribution in grow-out bags or nets. This means a more labour and cost-effective process.

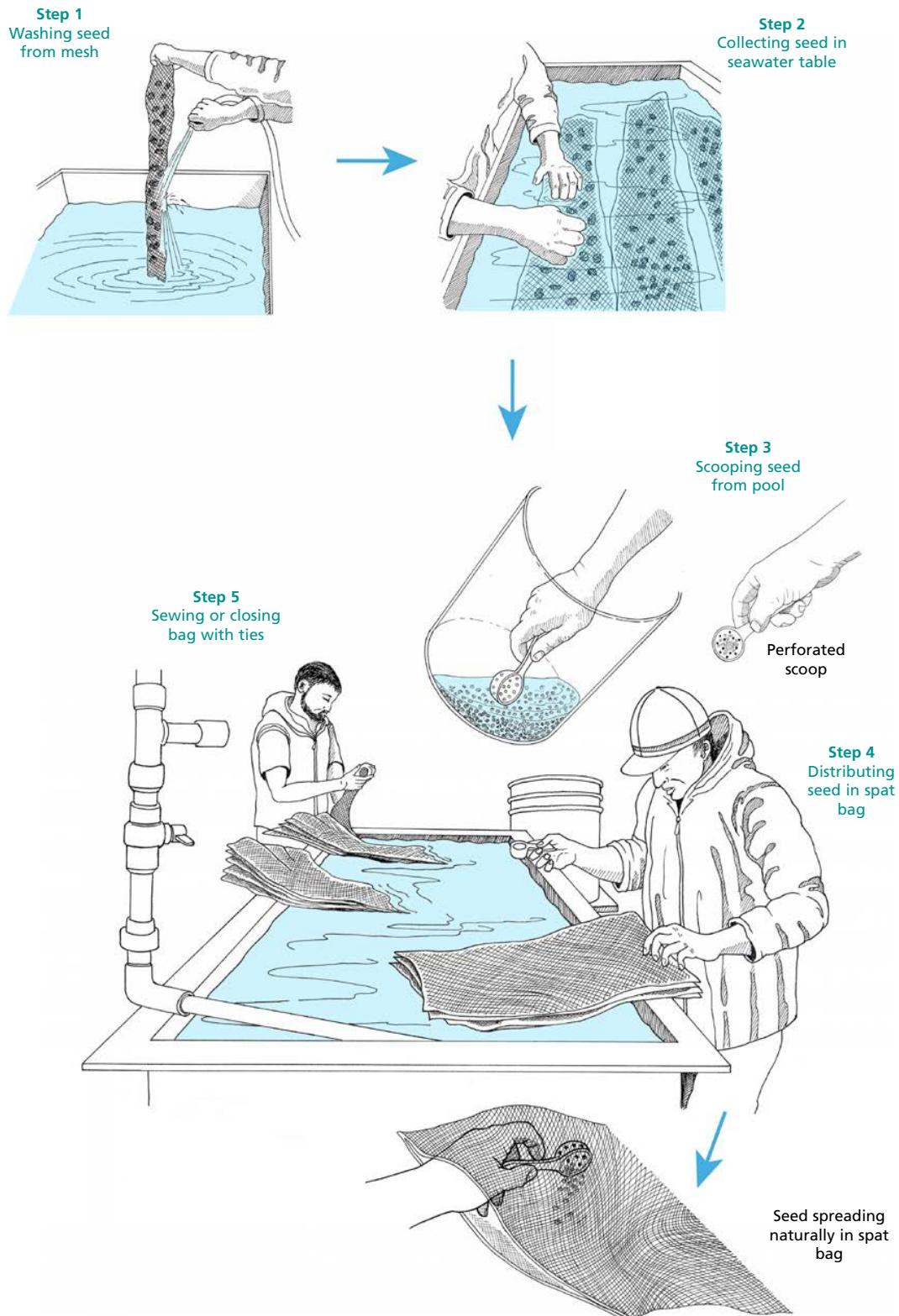
**Raceway set** – Spat set in sieves on raceways are easily washed off using a gentle jet of seawater into a holding bucket for distribution into pearl or lantern nets, prior to transfer to the farm.

**Tank set** – For spat set on substrate in a tank system, thinning is more easily and rapidly achieved when seed is free from substrate and collected from spat bags or nets. For this reason, if reared in the nursery, >2 mm seed can be washed off the settling substrate and distributed into spat bags in the nursery prior to transfer to the farm (Figure 8.2). It will be done more efficiently and thoroughly in the nursery rather than onboard the farm boat; it also reduces the workload of the farm crew for subsequent thinning at the farm by eliminating the handling of setting substrate and working with nets only.

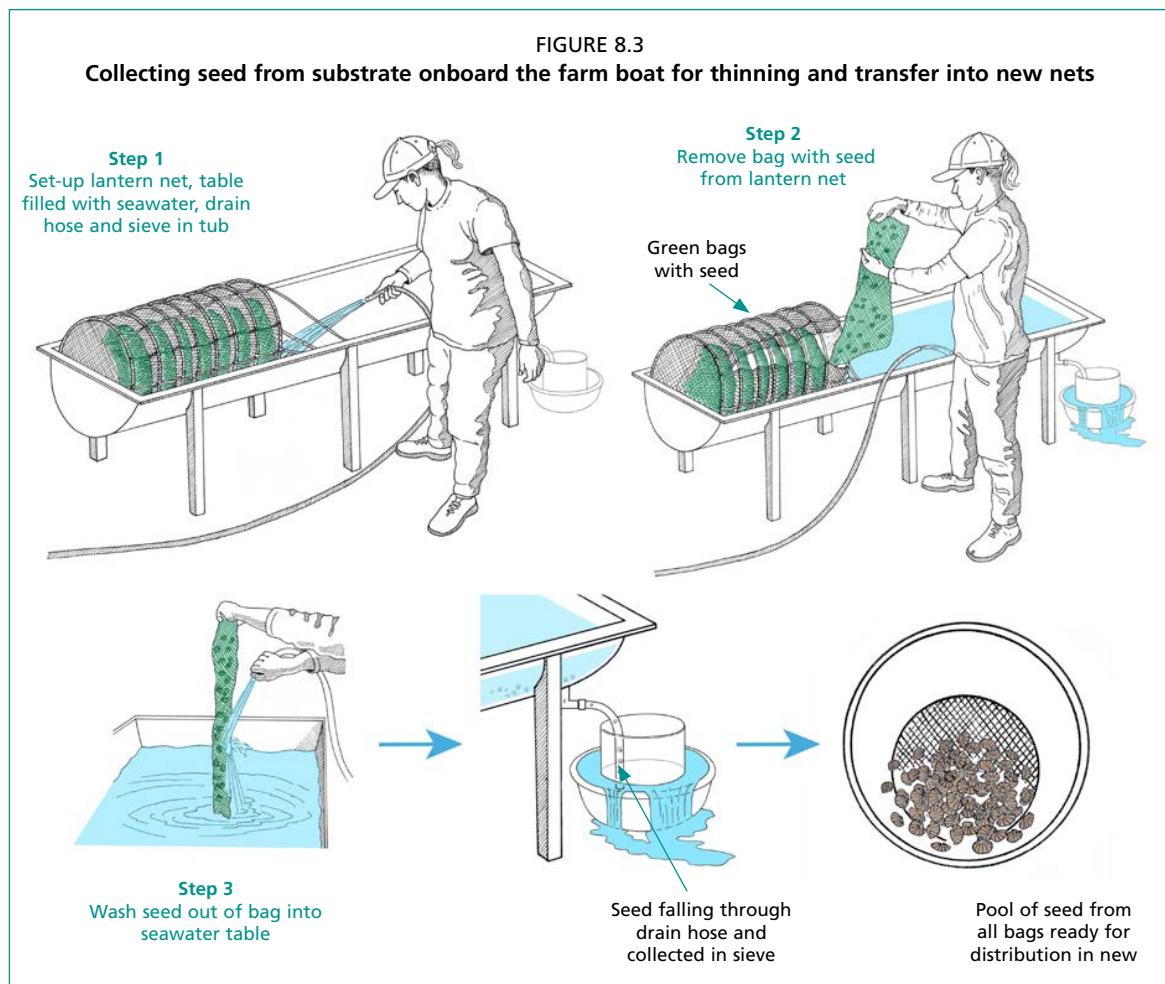
**Types of grow-out nets** – There are three main methods for suspending seed in the water column: Pyramid-shaped pearl nets, round lantern nets, and square shallow scallop trays. Minimum spat shell height for use of nets is 2.8 mm (2 mm mesh). Pearl nets have a smaller surface area than lantern nets ( $0.12 \text{ m}^2$ ); they are generally used for 3 mm seed and up to 30 mm scallops. Unlike pearl nets, lantern nets are not tied one below the other but one lantern net is multi-tiered extending vertically. Round

lantern nets consist of 5, 10 or 15 tiers with one seam running along side the entire net; diameters commonly are 30, or 50 cm (0.07 and 0.19 m<sup>2</sup> surface area respectively). Square shallow scallop trays approximate 30 × 30 × 10 cm (l × w × h); they can be tied in a stack and clipped on to a longline using a single point of attachment. As spat grow, they are gradually transferred to larger size mesh bags and nets.

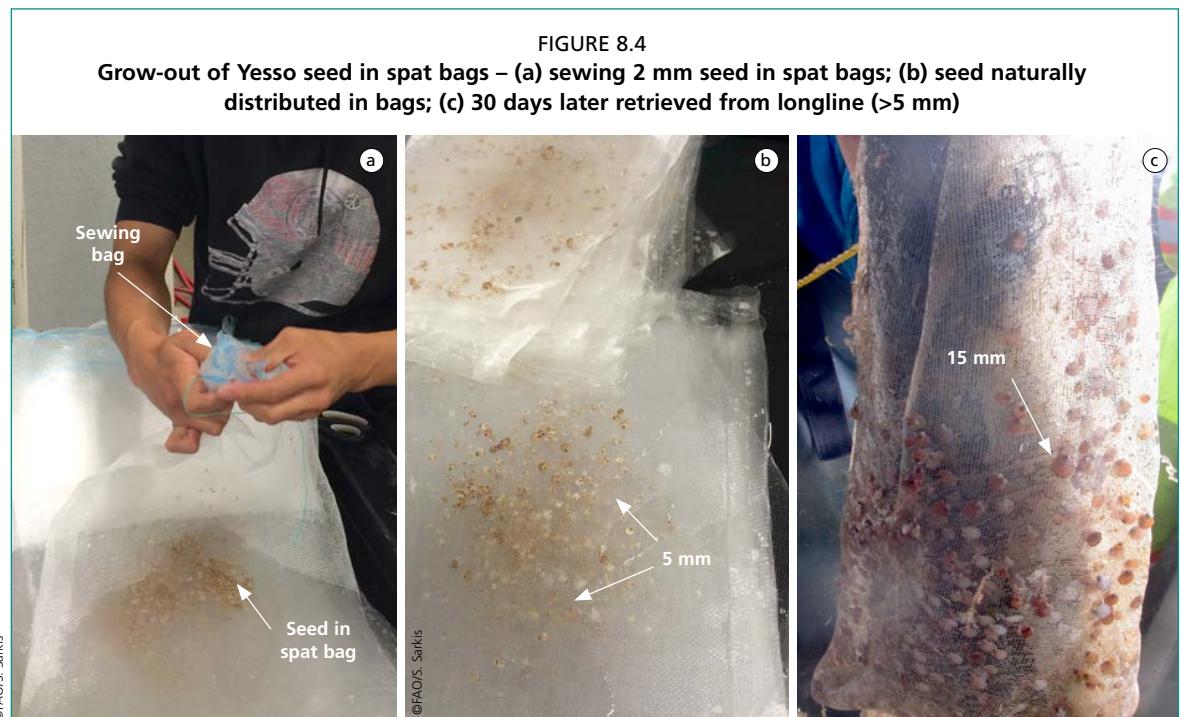
**FIGURE 8.2**  
Retrieving spat from settling substrate and scooping known density into spat bags



**Thinning seed and stocking densities** – The concept of “thinning” is similar to that applied to raceway setting in Section 7.2.6. It provides additional surface area to the scallops as they grow and it is applied throughout various stages of seed production: in the land-based nursery prior to transfer at sea, in the retrieval of seed from the remote nursery and during the juvenile farm stage of production. Methods used are similar whether they are implemented on land in the nursery or onboard the farm boat (Figure 8.3). Thinning involves the grading of seed according to size and methods are described in Protocols 8.2.1 and 8.2.2. The aquaculturist adapts his resources to facilitate the collection, grading and thinning of scallop seed. Protocols given are suggestions and other methods can be devised. The key is to minimise handling of seed, work quickly and reduce the exposure of seed to air.



Because Yesso seed grow at a rate of 5–10 mm per month, thinning needs to be conducted every month for 5–6 months following transfer of seed from nursery to farm. The rate of thinning after this depends on the seawater temperature and the associated growth rate. The faster the growth rate, the more frequent the transfer into new nets at lower stocking densities, until the final density for market size scallops is reached. The reduced densities in nets provides the space required by scallops for optimal growth; this is one of the limiting factors in large-scale scallop culture; unlike other cultured bivalves such as mussels and oysters, free-swimming scallops suffer high mortalities and stunted growth if reared at high densities. They will spread out naturally in the bag or net (Figure 8.4). Table 8.1 gives suitable stocking densities in spat bags and nets for optimal growth during the first few months.



**TABLE 8.1**  
**Stocking densities for seed of increasing size adapted from various sources. Densities are given for 50 cm round lantern nets**

Mesh size and type of net	Number of spat/bag	Spat shell height (mm)
700 µm bag	400	1
1 mm bag	400	1.5
1.5 mm bag	200	2
2 mm pearl net	150	2.8
3 mm pearl net	100–150	4
4 mm lantern net	100	5.5
6 mm lantern net	60	8

**Preparing scoops for thinning** – Scoops and plastic cups are identified for specific spat size and volume according to shell height and weight of spat. They are re-used at every routine thinning operation by nursery and farm crews. A procedure and example are given in Appendix III.

**Grading seed on a large-scale** – Aquaculturists devise various ways to simplify the grading of seed, especially in larger scale operations. A grading ‘table’ can be constructed which allows for grading a larger volume of seed at any one time than possible using a series of sieves. This grading table is a multi-layered structure with mesh frames secured one above the other. The largest mesh aperture is on top, and frames with decreasing mesh size secured underneath. The last mesh frame sits off the bottom, such that collected on the mesh do not touch a hard surface but are submerged in a shallow seawater tray.

Once scallops attain 8 mm shell height, they enter the farm system for juvenile grow-out to market size.

### 8.2.1 PROTOCOL: Grading seed

#### MATERIALS

- Grading sieves (400 µm to 4 mm)
- Seawater table
- Clean empty spat bags and lantern nets
- Labelling tape and magic marker
- Tubs for holding bags with seed

#### METHOD

Procedures described here apply to the land-based nursery or onboard the farm boat if it has a large enough deck (Refer to Figure 8.3).

##### Collecting spat from spat bags

1. Set up a spat collection station. This will be the area for washing spat from bags; each station has flowing seawater, a saltwater table, a space to stack new clean bags/nets.
2. Clean saltwater table and all equipment used.
3. Fit a draining hose to the drain plug of the saltwater table.
4. Place a sieve under the draining hose; with the sieve sitting in a tub filled with seawater. Seed will be washed from the table through the hose and collect on an appropriate mesh size.
5. Collect spat bags from longlines. Temporarily store in a large bin filled with seawater.
6. Start seawater flow in saltwater table.
7. Place one spat bag on saltwater table, remove mesh from bag
8. For seed <2 mm – Place mesh directly into a sieve; and wash spat into the sieve
9. For seed >2mm – Seed is washed into saltwater table and flushed through the drain hose.
10. Wash seed from bag using a gentle flow of seawater.
11. Wash spat bag thoroughly to remove any seed fallen to the bottom of the bag.
12. Repeat with all bags.
13. Seed can be pooled into one sieve until such time that there are too many layers of scallops in one sieve.
14. Grade collected seed by passing through 2 or 3 sieves stacked with larger mesh size at the top and lowest at the bottom.
15. Collect each size fraction in a separate tub (Figure 8.5). Label each tub with size fraction.
16. See Protocol 8.2.2 for distributing seed in appropriate densities.

FIGURE 8.5

Collecting seed from spat bags – (a) spat bag bundles with substrate and seed; (b) pool of seed collected in tub; (c) lantern net with good seed stocking density



## 8.2.2 PROTOCOL: Thinning seed

### MATERIALS

- Seawater table
- Meshed bags (425 µm to 1.5 mm)
- Pearl or lantern nets (2 to 4 mm mesh)
- Labelling tape and permanent marker
- Tubs for holding bags with spat
- Perforated measuring scoops (for spat)
- Plastic cups (50 ml) or similar (for larger seed)

### METHOD

This method is used for all spat sizes ready for transfer to bags or nets and is applied to the nursery or onboard the farm boat.

#### On-board thinning

Prepare all materials on land; this includes labelled scoops and cups for distributing seed (see Step 6).

#### Collecting seed for appropriate stocking density

##### Method 1 – Using a height:weight graph

1. Measure 30 spat from a subsample of each size fraction previously graded (see Protocol 8.2.1).
2. Check new stocking density (Table 8.1).
3. Pour spat in appropriate scoop or cup as previously identified (see Appendix III).

If you do not have a height:weight graph, use Method 2 below:

##### Method 2 – Counting spat

4. Collect a subsample of each size fraction graded previously.
5. Count the number of spat in each size fraction for the appropriate stocking density (Table 8.1).
6. Pour into appropriate size measuring scoop or cup and mark level to fill.
7. Label scoops and cups used for size fraction for future record.
8. Counting the number of spat to attribute a spoon or cup for each size fraction is only done once; the same spoons and cups are re-used in future thinning.



©FAO/S. Sarkis

#### Distributing spat in new bags or nets

9. Work in saltwater table with running filtered seawater.
10. Place new bags and tub of collected seed nearby.
11. Using the appropriately labelled scoop or cup for new stocking density, collect seed from holding bin; fill to the level marked (as per Steps 4 to 6).
12. Empty the scoop or cup into the new bag or net; make sure bag is lying flat in flowing seawater; this makes it easier for smaller spat to distribute in the new bag. Procedure is similar to Steps 4 and 5 in Figure 8.2.
13. Sew or close bags and nets, and place in clean holding bin with flowing seawater dedicated to new bags.
14. Repeat until all seed for this fraction is distributed.
15. Bags and nets are either prepared for transport to the farm or are suspended directly on longlines if work is carried out at the farm site.

### 8.2.2 PROTOCOL (continued)

#### Securing bags to longlines

16. Place two bags per lantern net compartment, laying them flat.
17. Suspend lantern nets on longlines.
18. **Alternative:** Bags are suspended directly on longlines in single lines; they are tied to alternating side of a rope secured with an anchor and held vertical and taut by buoys.

### 8.2.3 Expected survival and growth rates post-transfer at sea

High survival rates can be expected when:

- environmental conditions are favourable,
- spat densities are maintained according to size; and
- nets are cleaned monthly.

Survival rates are given for a 30-day grow-out period between thinning and net change:

- Remote nursery: Survival rate of spat <1 mm on longlines is <2 %
- In 1.5 mm mesh bags: Survival rate of 2 mm spat averages 90 %
- In 2 mm nets: Survival rate of 3 mm seed averages 90 %
- In 4 mm nets: Survival rate of 5.6 mm averages 100 %

In order to achieve a 90 percent survival in 1.5 mm mesh bags, spat must be re-distributed after 4 weeks of grow-out; if not, clogging of the mesh will occur quickly. Survival rate can rapidly decrease from 90 to as low as 30 percent if transfer is delayed for 8 weeks and to a further 17 percent if transfer is delayed 10 weeks.

## 8.3 TRANSPORTING SPAT TO FARM

Scallop seed can be transported in bags or lantern nets using 1) a moist, insulated container; tolerated duration of transport is species specific (referred to as “dry transport”); or 2) a seawater-filled insulated container with adequate aeration and preferably continuous flow (referred to as “wet transport”). Alternatively, 3) if space onboard is limited, seed is transported in high densities and distributed in bags/net at the farm site; this is done using small moist pouches such as coffee filters. Dry transport success depends on maintaining a constant and suitable temperature in holding bin for the duration of transport. The wet transport method minimises stress as scallops are maintained in well oxygenated water, but this requires a larger vessel. The “coffee filter” alternative minimises space required for transport.

### 8.3.1 Dry and wet transport of bags or nets

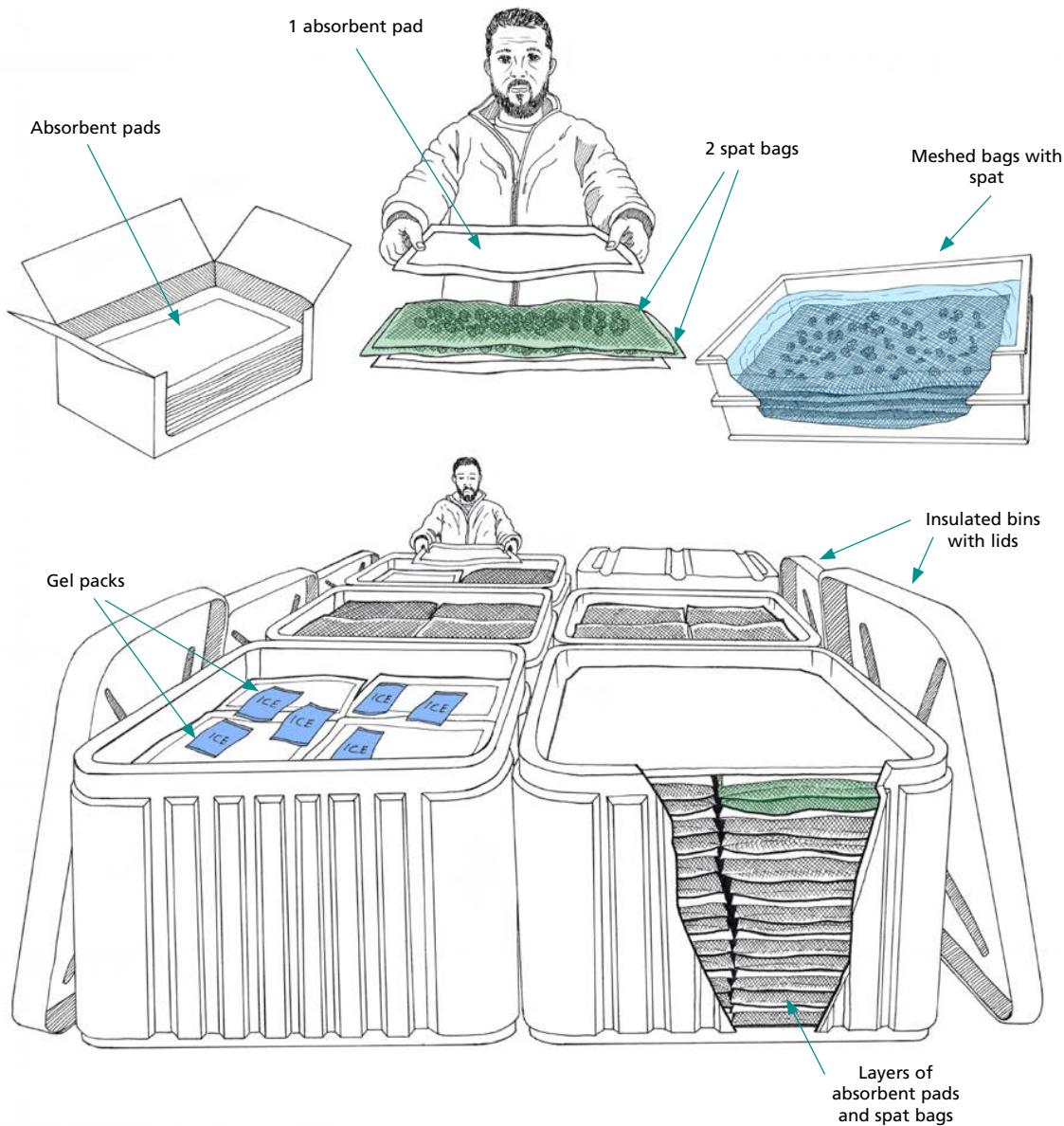
Both methods of packing and transporting (dry and wet) result in live spat, with no mortality seen at destination for transport durations of 6–8 h. Both methods use insulated bins, where nets or bags are packed loosely. A stepwise procedure is given in Protocol 8.3.2.

**Dry transport** – Dry transport is in fact the layering of spat bags in-between absorbent pads moistened with filtered seawater. Using an insulated bin, absorbent pads dampened with seawater sandwich spat bags and maintain seed moist. The layering pattern is repeated until the bin is nearly full; temperature is maintained cool for the duration of transport by the use of gel packs. The lid is placed tightly on for insulation and ready for transport (Figure 8.6).

Simulated nursery experiments showed that spat survival was near 100 percent during this type of transport, for a simulated transport period of 4 h. Post-transport stress was also monitored by recording survival of spat in nursery tanks one week after the simulated transport. No mortality was recorded for Yesso spat.

**Wet transport** – Wet transport also involves the layering of spat bags or lantern nets in an insulated container. The difference is that the container is filled with seawater and is supplied with continuous seawater flow throughout the duration of transport. This is possible with larger vessels, which have the ability to pump large volumes of water for an extended period of time while moving. For a shorter duration of transport, a static system of well-oxygenated insulated bins filled with seawater is suitable.

FIGURE 8.6  
Packing spat bags for “dry” transport using absorbent pad layering



### 8.3.2 PROTOCOL: Packing spat for transport (dry and wet)

#### MATERIALS

- Insulated bins with lids
- Absorbent pads
- 20 L buckets

#### METHOD

##### Preparation

1. Hatchery manager to coordinate with Boat captain for transport of spat packed in bins.
2. Clean insulated bins used for transport.
3. Packs of absorbent pad and 2 or 3 buckets half-filled of filtered seawater are prepared close to packing bins for moistening pads.

##### DRY transport for spat bags

4. Two layers of spat bag are placed on bottom of tank.
5. Absorbent pad is moistened with seawater and laid over bags.
6. A second layer of absorbent pad is placed on top of first pad.
7. Repeat Steps 4–6 until bin is full.
8. On top of last absorbent pad, add an insulator material and gel pack(s) to maintain temperature cool (Figure 8.6). Make sure gel pad does not come in direct contact with scallop seed.
9. Close bin tightly with lid to maintain temperate constant (transport temperature must be assessed by aquaculturist).
10. Load bins onto farm boat.
11. Once at farm site, spat bags can be transferred into lantern nets before suspension on longline.

##### WET transport for bags or nets

12. Bags or nets with spat are placed flat in transport bin.
13. Cover with lid.
14. Load bins on farm boat.
15. Once onboard connect the bin with flowing seawater and fill.
16. If seawater cannot be continuously pumped during transport, aerate bin with onboard air compressor throughout transport.

### 8.3.3 High density transport of seed

Experimental studies show that Yesso scallop seed with an average shell height of  $5.3 \pm 1.6$  mm can be transported for up to 8 h in moist coffee filters, with no apparent stress observed and a survival rate of 100 percent following transport (Figure 8.7). Further monitoring after 30 days of grow-out on the farm, shows growth rate to be comparable to seed transported using more standard methods and a 100 percent survival. There are no published reports for transporting seed of smaller sizes using this method but the likelihood of success is high. This method is very space efficient and can be used for operations which have a farm boat limited in deck space, or if seed needs to be transported by air or by road. Procedures are given in Protocol 8.3.4.

FIGURE 8.7

Transporting seed at high density using the “coffee filter” method – (a) seed in filter; and b) top view of styrofoam transport container showing ice packs laid over insulated material and covering series of coffee cone filters with seed



### 8.3.4 PROTOCOL: High density transport of seed, the “coffee filter” method

#### MATERIALS

- Conical coffee filters
- Seed scoop or measuring cup
- Insulated container (e.g. styrofoam box)
- Air bags for insulation
- Ice packs

#### METHOD

1. Scoop up to 1 800 seed using pre-measured spoon or cup (seed >4 mm).
2. Dampen coffee cone with filtered seawater and lay flat.
3. Transfer seed to cone.
4. Lay cone flat into insulated container one on top of the other.
5. Fill the container until all seed is used.
6. Make sure container is closely packed.
7. On top layer, add air bags or insulating separator.
8. Place ice packs on top to maintain temperature in container at 6 °C; make sure not to have direct contact with ice packs and coffee filters with scallops.
9. Secure lid on top of insulated container.
10. Once at destination, scoop out seed and distribute in appropriate densities in bags or nets.
11. Monitor survival and growth following 30 days of culture on farm site.

### 8.3.5 Seed monitoring on the farm

A comprehensive seed monitoring programme is advised during the first year of operation; this is valuable to both the nursery and farm managers. In subsequent years, a basic routine seed monitoring is sufficient to have a good understanding of the performance of the farm and allows to address any issues prior to high mortality.

A comprehensive seed monitoring programme involves monthly subsampling of seed from selected lantern nets. A vertical profile of water quality at the farm site is recorded at the same time. Procedures are given in the Protocol 8.3.6.

Growth rate of 2 mm seed during the first 4 months of transfer at sea attains 10 mm per month. Shell height of 8 mm is considered the size at which scallops enter the farm management protocols. Assuming a good farm management and favourable

environmental conditions, Yesso scallops grow from 8 mm to 40 mm shell height within 4 months; at which point they are robust with minimal mortalities seen until market size, assuming a good farm management and favourable environmental conditions.

### 8.3.6 PROTOCOL: Seed monitoring on the farm

#### MATERIALS

- Several shallow trays for collection of spat
- Two 10–20 L buckets
- 2–3 L beakers for counting/measuring
- Vernier caliper
- Sampling vials/formalin
- Data sheets/clipboard/field notebooks
- Seawater table or deep rubber bins for holding bags

#### METHOD

1. Create an Excel sheet to record seed batch growth and survival with following fields:
  - Date transferred at sea
  - Site and Longline
  - Mesh size of spat bag and/or net from which sample is taken
  - Date collected
  - Label of vertical spat bag line or net
  - For each vertical line or net record data from each depth, for e.g. in a 10-tiered net:
    - > Layer 1: no. of live scallops/no. of dead scallops
    - > Layer 1: shell height (mm) for 50 scallops
    - > Layer 5: no. of live scallops/no. of dead scallops
    - > Layer 5: shell height (mm) for 50 scallops
    - > Layer 10: no. of live scallops/no. of dead scallops
    - > Layer 10: shell height (mm) for 50 scallops

#### At the farm site

2. Collect a minimum of two vertical spat bag lines (remote setting) or two nets (lantern or pearl) representing each spat batch transferred to sea for sampling.
3. Label vertical spat bag lines and nets as follows:
  - batch number
  - date transferred
  - initial stocking density
4. From each vertical line, collect and label bags or nets from 2 or 3 different depths to reflect any changes in seawater chemistry within the water column. For e.g. if using a 10 or 15-tiered lantern net, sample top, middle and bottom layers.
5. Wash all of spat from bag or net into a dedicated small tray, keeping each sample separate. Label.
6. Count live and dead scallops for each sample. Record live and dead.
7. Record site, number of seed per bag and per layer.
8. Pool all seed from same batch collected for all layers into one tray.
9. Measure shell height of 50 seed using Vernier calipers (see photo).



Measuring seed with Vernier calipers

### 8.3.6 PROTOCOL (continued)

10. If there is no time to measure onboard, fix sample with formalin for later analysis.
11. Record any wear and tear of nets/bags, fouling, predation by crabs or other organisms and clamping (see photo).
12. Re-distribute seed in new net, keeping the same label; see Table 8.1 for stocking densities based on size and Protocol 8.2.2 for procedure on “Distributing spat in new bags or nets”.
13. Return bag lines or nets to longline.
14. Repeat for two other bags or nets of same batch for triplicate measurements of growth and survival.
15. Do vertical water quality profile using YSI Pro meter (from 1m below surface to 1 m off the bottom).



Juvenile scallops clamping

©FAO/S. Sarkis

#### In the office

16. Enter all data in Excel sheet.
17. Calculate mean and standard deviation (SD) of shell height for each sample.
18. Calculate survival rate from previous monitoring.
19. Record all observations on state of culture.

## Further readings

- Beninger, P.G. & Le Pennec, M.** 2006. Chapter 3 – Scallop structure and function. *Developments in Aquaculture and Fisheries Science*, 40: 85–159. [https://doi.org/10.1016/S0167-9309\(06\)80030-X](https://doi.org/10.1016/S0167-9309(06)80030-X)
- Bourne, N., Hodgson, C.A. & Whyte, J.N.C.** 1989. A manual for scallop culture in British Columbia. *Canadian Technical Report of Fisheries and Aquatic Sciences*. No. 1694. 215 pp. <https://waves-vagues.dfo-mpo.gc.ca/Library/112003.pdf>
- De La Roche, J.P., Marin, B., Freites, L. & Velez, A.** 2002. Embryonic development and larval and post-larval growth of the tropical scallop *Nodipecten* (=*Lyropecten*) *nodosus* (L. 1858) (Mollusca: Pectinidae). *Aquaculture Research*, 33: 819–827. <https://www.academia.edu/19674800>
- Hai, N.V.** 2015. The use of probiotics in aquaculture. *Journal of Applied Microbiology*, 119(4): 917–935. <https://doi.org/10.1111/jam.12886>
- Helm, M.M., Bourne, N. & Lovatelli, A.** 2004. *Hatchery culture of bivalves: a practical manual*. FAO Fisheries Technical Paper No. 471. Rome. FAO. <https://www.fao.org/3/y5720e/y5720e00.htm>
- Kim, Y.D., Lee, C., Shim, M.J., Kim, M., Kim, G.S., Choi, J-S., An, W.G. & Nam, M-M.** 2014. A study on the growth of juvenile *Patinopecten yessoensis* from different aquaculture regions. *Korean J. Malacology*, 30(4): 321–334. (also available at <https://doi.org/10.9710/kjm.2014.30.4.321>).
- Leavitt, D., Surier, A. & Karney, R.** 2010. *Grow-out culture of the bay scallop*. NRAC publication No. 216. 10 pp. <http://agrilife.org/fisheries2/files/2013/09/NRAC-Publication-No.-216-2010-%E2%80%93-Grow-out-Culture-of-the-Bay-Scallop.pdf>
- Ruiz-Ponte, C., Samain, J.F., Sanchez, J.L. & Nicolas, J.L.** 1999. The benefit of a *Roseobacter* species on the survival of scallop larvae. *Marine Biotechnology*, 1: 52–9. <https://doi.org/10.1007/pl00011751>
- Sarkis, S., Karney, R. & Creswell, R.L.** 2021. Chapter 12, Design and construction considerations for a molluscan hatchery. In: *Molluscan Shellfish Aquaculture: A Practical Guide* (Ed.: S.E. Shumway). 5M Books Ltd., Great Britain. Pages: 305–383.
- Sarkis, S. & Lovatelli, A.** 2007. *Installation and operation of a modular bivalve hatchery*. FAO Fisheries Technical Paper No. 492. Rome. FAO. <https://www.fao.org/3/a0797e/a0797e00.htm>
- Sarkis, S., Helm, M. & Hohn, C.** 2006. Larval rearing of calico scallop, *Argopecten gibbus*, in a flow-through system. *Aquaculture International*, 14: 527–538. <https://doi.org/doi:10.1007/s10499-006-9052-3>
- Supan, J.** 2014. High density rearing of oyster larvae in flow-through systems. *SRAC Publication*, No. 4311. 6 pp. [https://shellfish.ifas.ufl.edu/wp-content/uploads/High-Density-Rearing-of-Oyster-Larvae\\_SRAC-4311.pdf](https://shellfish.ifas.ufl.edu/wp-content/uploads/High-Density-Rearing-of-Oyster-Larvae_SRAC-4311.pdf)
- Uddin, M.J., Park, K-I., Kang, D-H., Park, Y-J. & Choi, K-S.** 2007. Comparative reproductive biology of Yezo scallop, *Patinopecten yessoensis*, under two different culture systems on the east coast of Korea. *Aquaculture*, 265: 139–147. <https://doi.org/10.1016/j.aquaculture.2007.01.047>
- Williams, J.R.** 2005. Reproductive ecology of the scallop *Pecten novaezealandiae*. University of Auckland, Auckland, New Zealand. PhD dissertation.
- William, J.R. & Babcock, R.C.** 2005. Assessment of size at maturity and gonad index methods for the scallop *Pecten novaezealandiae*. *New Zealand Journal of Marine and Freshwater Research*, 39: 851–864. <https://doi.org/10.1080/00288330.2005.9517357>



## Hatchery culture of bivalves – A practical manual

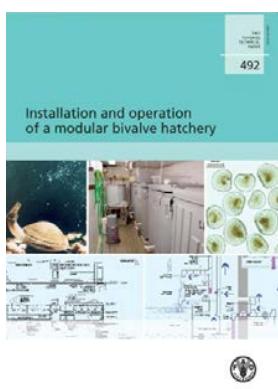
**Michael M. Helm, M.M., Bourne, N. & Lovatelli, A.**  
FAO Fisheries Technical Paper 471, Rome, FAO, 2004

This manual describes the various aspects of bivalve hatchery culture and production from acquisition of broodstock to the stage at which the seed are of sufficient size to transfer to sea-based grow-out. Focus is on intensive methodology in purpose-built hatchery facilities rather than on more extensive methods of seed production in land-based pond systems. For a complete view, the intermediate nursery phase of production, which is the interface between the hatchery and sea-based growout, and the concept of remote setting are also described and discussed in some depth.

This manual provides the reader with a practical insight as to what is required in the way of resources and details of how to handle and manage the various life history stages of bivalves in the hatchery production cycle. Examples are largely drawn from the more commonly cultured temperate climate species including the Pacific oyster, *Crassostrea gigas*, the American (Eastern) oyster, *Crassostrea virginica*, the European flat oyster, *Ostrea edulis*, the Manila clam, *Tapes philippinarum* and a range of scallop species. Methods described are equally as applicable to bivalves of lesser significance in terms of worldwide production.

In addition to explanations of culture technology and methodology, the manual includes a brief discussion of the processes of identifying a suitable site for locating a hatchery and considerations in planning and designing the hatchery. It also includes advances that are likely to improve the reliability and economic viability of the hatchery industry in the near future, featuring topics such as polyplodity, the development of selected strains, cryopreservation of gametes and the need for novel, non-living foods.

Link English version: [www.fao.org/3/a-y5720e.pdf](http://www.fao.org/3/a-y5720e.pdf)  
 Link Spanish version: [www.fao.org/3/a-y5720s.pdf](http://www.fao.org/3/a-y5720s.pdf)



## Installation and operation of a modular bivalve hatchery

**Sarkis, S. & Lovatelli, A.**  
FAO Fisheries Technical Paper 492, Rome, FAO, 2007

Limiting factors such as minimal capital investment, lack of technical support or expertise and available physical space may put severe restrictions on setting up a hatchery. Not all investors have the means or the will to take the risk to support a large commercial aquaculture operation without substantial proof of its production capacity. For these reasons, the setup of an inexpensive modular hatchery may be a simpler option to the start-up of a large commercial operation, or may be sufficient to the needs of a smaller operation. This manual was written for those interested in establishing a bivalve hatchery, with minimal experience in this activity, limited technical support and restricted access to information. The manual stands as an entity, providing not only the technicalities of setting up and operating a hatchery, but also makes some of the scientific background, deemed useful to the aquaculturist, readily accessible. The manual is divided into chapters for each stage of rearing: broodstock conditioning, algal culture, hatchery, nursery, grow-out and economic considerations. The first five chapters include both the physical requirements and culture considerations and procedures for the relevant rearing stage. The final chapter on economic considerations provides an insight into the labour involved for each stage of production, along with a list of equipment and supplies, which may be used as a template for a new installation.

Link English version: [www.fao.org/3/a0797e/a0797e.pdf](http://www.fao.org/3/a0797e/a0797e.pdf)

# Glossary

<b>Adductor muscle</b>	Large muscle near centre of scallop that pulls the two valves together.
<b>Algae</b>	Aquatic plants that reproduce by cell division or spores.
<b>Anterior</b>	Front or head.
<b>Banjo filters</b>	In hatchery terminology, a ring meshed on both sides affixed to the outflow of a tank preventing larval loss through drain.
<b>Bivalve</b>	Mollusc of the Class Pelecypoda, having a shell of two valves that are joined by a hinge.
<b>Byssus</b>	Thread-like filaments used by bivalves to attach themselves to a substrate.
<b>Diatom</b>	A single-celled algae of the Class Bacillariophyceae; cells are enclosed in a siliceous shell called a frustule, cells can form chains.
<b>Dioecious</b>	male and female reproductive organs in separate individuals.
<b>D-larva</b>	The early veliger larval stage of bivalves, also known as straight hinge larva.
<b>Downwelling</b>	In hatchery terminology, a growing system in which the flow of water enters at the top of a spat holding container (compare with upwelling).
<b>Embryo</b>	Organism in early stages of development; in bivalves, prior to larval stage.
<b>Eye spot</b>	Simple organ that develops near centre of mature larvae of some bivalves and is sensitive to light.
<b>Fertilization</b>	Union of egg and sperm.
<b>Flagellate</b>	Group of single-celled algae characterized by having a locomotory organ called a flagellum.
<b>Gamete</b>	Mature, haploid, functional sex cell capable of uniting with the alternate sex cell to form a zygote.
<b>Gametogenesis</b>	Process by which eggs and sperm are produced.
<b>Gastrula</b>	The embryonic stage of development consisting of two layers of cells enclosing a sac-like central cavity with a pore at one end.
<b>Gill</b>	A leaf-like appendage that functions in respiration and filtration of food from water.
<b>Gonadal Index</b>	In this case the relationship of gonad weight to shell weight, reflecting gonad growth or depletion.
<b>Gonads</b>	The primary sexual organ: testis producing sperm or ovary producing eggs.
<b>Grow-out</b>	The process of growing seed to market size.
<b>Inoculum</b>	Culture of an organism (alga, rotifer), which is used as a starting point for another culture.
<b>Larva</b>	A stage of bivalves from the embryo to metamorphosis.
<b>Mantle</b>	The soft fold enclosing the body of a bivalve that secretes the shell.
<b>Meiotic division</b>	Process in which normal number of chromosomes ( $2n$ ) is reduced to the haploid ( $n$ ) number.

<b>Metamorphosis</b>	In bivalves, the period of transformation from the larval to the juvenile stage.
<b>Microalgae</b>	Small cell-size algae, either single celled or chain forming diatoms, cultured as foods for larvae and spat in a hatchery.
<b>Muscle Index</b>	In this case, the relationship of muscle weight to shell weight, reflecting muscle growth or depletion.
<b>Oocyte</b>	Cell, which develops into an ovum.
<b>Pediveliger</b>	Larval stage of molluscs that still has the swimming ciliated organ (velum) and sensitive foot needed for settlement and attachment.
<b>pH</b>	A measure of acidity.
<b>Polar body</b>	Minute cells released during meiotic division of the egg after the sperm has penetrated the egg; contains excess chromosomal material to produce a haploid egg.
<b>Posterior</b>	The rear, away from the head.
<b>Prodissococonch</b>	Bivalved shell formed by larva prior to metamorphosis. It may be possible to distinguish an earlier, smaller prodissococonch-I from a later, larger prodissococonch-II that encloses the entire animal.
<b>Pseudofaeces</b>	False faeces, waste material not taken into the digestive tract.
<b>Seed</b>	A young scallop with no specific definition to size.
<b>Settlement</b>	Behavioural process when mature bivalve larvae seek a suitable substrate for attachment.
<b>Shell height</b>	In scallops, the straight-line distance measured perpendicularly from the umbo to the ventral margin of the shell.
<b>Shell length</b>	In scallops, the straight-line distance from the anterior to the posterior margins of the shell.
<b>Spat</b>	A newly settled or attached bivalve (also termed post larval or juvenile in bivalves).
<b>Spawning</b>	Release of ova, fertilized or to be fertilized.
<b>Trochophore</b>	The first free-swimming planktonic stage of a mollusc larvae or bivalve embryo.
<b>Umbo</b>	Beak-like projections at the dorsal part of the shell; it is the oldest part of a bivalve shell (also called the umbone).
<b>Upwelling</b>	In hatchery terminology, a growing system in which a flow of water is induced through the base of a spat holding container (compare with downwelling).
<b>Veliger larva</b>	The larval stage of most molluscs, characterized by the presence of a velum.
<b>Velum</b>	Ciliated locomotory organ of the larva.
<b>Ventral</b>	Pertaining to the under or lower side of an animal.
<b>Vitellogenesis</b>	Formation of the yolk of an egg.

## Appendices

Appendix I – Datasheet sample for gonadic and muscle indices	117
Appendix II – Broodstock feeding record	119
Appendix III – Preparing measuring scoops for thinning	121



## Appendix I – Datasheet sample for gonadic and muscle indices

Site: .....

Date: .....

**Rest** = remaining soft tissues  
(less gonad and muscle)

**GI** = Gonad wet weight/shell dry weight × 100  
**MI** = Muscle wet weight/shell dry weight × 100

#### **Notes:**



## Appendix II – Broodstock feeding record

### **Notes:**



## Appendix III – Preparing measuring scoops for thinning

1. Measure 30 spat from a subsample of each size fraction previously graded (Protocol 8.2.1).
2. Estimate the weight based on height, using the height:weight graph.
3. Check new stocking density (see Table 8.1).
4. Weigh spat for required new stocking density. Note: this is only done once for each size fraction.
5. Collect in a measuring scoop of appropriate size or mark the level to which scoop needs to be filled.
6. For larger seed, use a plastic cup and mark the level filled by spat as in photo.
7. Repeat steps 1–6 each time spat growth is monitored.
8. Label scoops and cups for each size fraction; use in nursery or on the boat during future thinning such that weighing in Step 2 is no longer needed.

### Example as per steps above:

- Average shell height of spat = 1.5 mm
- Weight of 1.5 mm spat averages 0.5 mg per spat (see Figure 7.7)
- New stocking density = 400 spat per bag (see Table 8.1)
- Weight of 400 spat =  $400 \times 0.5 = 200$  mg
- Weigh 200 mg of spat
- Pour 200 mg of spat into most suitable size container. Spat should fill the scoop or cup; if not, mark the level on container showing for 200 mg

## Notes and observations







Scallop culture at a commercial scale lags behind that of clams and oysters in terms of number of operations and production volumes. The main reasons revolve around the natural free-swimming seabed bottom preference of scallops, their requirement for low-density culture, their sensitivity to seawater chemistry parameters and their short shelf life when sold live. These limiting factors translate into aquaculture challenges especially in large-scale farming or “grow-out” of market-size animals because of physical space demand, labour and cost. The Japanese scallop is one of the most attractive scallop culture candidate and offers several advantages; it generates a live and processed product, yields one of the largest scallop muscles highly prized as both a food and export product and more importantly, it attains market size in suspended cultures. The latter enables large-scale farming and harvesting, eliminates SCUBA-based labour as for bottom cultures, and allows for seed monitoring and harvesting of market-size scallops from a boat or raft. This manual describes well-tested hatchery-based techniques implemented at large-scale for all stages of seed production. It provides the potential to extend the hatchery cycle with a broodstock conditioning strategy, to rear and set pediveligers using different methodologies, to produce up to 10 mm seed on a large-scale in a land-based nursery and to achieve a 100 percent survival and growth for seed transported up to 9 hours to farm sites. This is a roadmap to the technical success of a commercial operation of the Japanese scallop. The financial sustainability of the operation will depend on a well-developed strategy, a sound business plan, a realistic target for production and on its management.

ISBN 978-92-5-136411-6 ISSN 2070-7010



9 789251 364116  
CC0535EN/1/06.22



# Manual for Hatchery Culture of the Bay Scallop



**Sea Grant**  
Connecticut



National Marine Fisheries Service  
Northeast Fisheries Science Center  
Milford Laboratory  
Milford, Connecticut 06460

# **MANUAL FOR HATCHERY CULTURE OF THE BAY SCALLOP, *ARGOPECTEN IRRADIANS IRRADIANS***

James C. Widman Jr., Joseph Choromanski,  
Richard A. Robohm, Sheila Stiles,  
Gary H. Wikfors and Anthony Calabrese

National Oceanic and Atmospheric Administration  
National Marine Fisheries Service  
Northeast Fisheries Science Center  
212 Rogers Avenue  
Milford Laboratory  
Milford, Connecticut 06460

## FOREWORD

This manual is dedicated to those Milford Laboratory scientists who earlier worked in the disciplines described in this manual and who were instrumental in the development of shellfish culture techniques now being used worldwide and from which the bay scallop culture techniques described here are derived. Dr. Victor Loosanoff, along with his associate Mr. Harry Davis, originally described culture techniques for many species of bivalves. Dr. Ravenna Ukeles first established protocols for the culture of microalgae which she then used in shellfish nutrition studies. Mr. Haskell Tubiash was one of the first individuals to study diseases of shellfish larvae in culture. Dr. Arlene Longwell was one of the first geneticists to study selective breeding in marine bivalves, particularly the oyster. These individuals made significant contributions to the field of shellfish culture as we know it today and we thank them for their contributions.

### *NOTE*

This manual describes methods used at the National Marine Fisheries Service's Milford Laboratory to culture bay scallops in the northeastern U.S. from eggs to the point at which they can be placed in nets or cages in the environment. As you go through the manual, certain questions may arise regarding particular techniques. The laboratory can be contacted at (203) 579-7000 for further clarification. Individual training in techniques discussed here can be provided at the Milford Laboratory at no cost.

Cover design: Joseph Choromanski, National Marine Fisheries Service, Milford  
Interior design: Peg Van Patten, Connecticut Sea Grant Communicator

This manual was published by the Connecticut Sea Grant College  
Program in collaboration with the NOAA National Marine  
Fisheries Service.



© 2001 Connecticut Sea Grant      CTSG-01-03

## TABLE OF CONTENTS

I.	Introduction .....	1
II.	Facilities .....	5
III.	Broodstock Management.....	7
	A. Broodstock Selection .....	7
	B. Size and Appearance. ....	7
	C. Broodstock Numbers .....	8
IV.	Hatchery Production.....	9
	A. Broodstock Conditioning.....	9
	B. Condition/Ripeness .....	11
	C. Spawning .....	12
	1. Mass Spawning.....	12
	2. Individual Spawning.....	14
	D. Determining Egg and Larval Counts. ....	15
	E. Rearing Containers .....	19
	F. Larval Rearing .....	19
	G. Genetic Considerations and Selective Breeding.....	23
	H. Juvenile Nursery .....	24
V.	Algal Foods .....	29
	A. Diets for Pre-set Larvae .....	29
	B. Diets for Larvae during Metamorphosis.....	29
	C. Precautions Producing Microalgae.....	31
	D. Seed Cultures .....	31
VI.	Disease Problems .....	32
	A. Procedures to Control Introduction of Pathogens .....	32
	1. Equipment and Materials .....	32
	2. Seawater .....	33
	3. Broodstock .....	33
	4. Algal Food .....	33
	5. Scallop Stocks from New Sources.....	34
	B. Larval Disease .....	35
	1. Diagnosis. ....	35
	2. Treatment .....	35
	C. Grow-out Disease .....	37
	1. Diagnosis .....	37
	2. Treatment .....	37
VII.	Acknowledgments .....	38
VIII.	References .....	39

## TABLE OF CONTENTS, continued

IX.	Useful Citations .....	42
X.	Suppliers Guide .....	43
Appendix A - Quantifying Microalgal Feeds .....		44
Appendix B - Step-wise Procedures for Testing Water or Food for Presence of <i>Vibrio</i> .....		47
Appendix C - Common Celsius to Farenheit Equivalents .....		50

## LIST OF FIGURES

Fig. 1. Life cycle of the bay scallop . . . . .	3
Fig. 2. Bay scallop with fully ripe female and male sex organs . . . . .	7
Fig. 3. Bay scallop with black integument covering immature gonad . . . . .	8
Fig. 4. Conditioning scallops in static culture . . . . .	9
Fig. 5. Conditioning scallops in flowing seawater trays . . . . .	10
Fig. 6. Scallops that have partially spawned with traversing "rivers" running through the gonad . . . . .	11
Fig. 7. Mass spawning of scallops . . . . .	13
Fig. 8. Spawning of scallops individually . . . . .	14
Fig. 9. Separation of eggs from debris by passage through a 100- $\mu\text{m}$ screen . . . . .	15
Fig. 10. Mixing of egg suspension with a perforated plastic plunger . . . . .	15
Fig. 11. Dispensing a 1-mL sample of egg suspension on a Sedgwick-Rafter counting cell . . . . .	16
Fig. 12. "Standard" 400-liter fiberglass tanks for culture of embryos and larvae . . . . .	18
Fig. 13. Small plastic containers for culture of embryos and larvae. . . . .	18
Fig. 14. Passing larval culture water through an appropriate-size screen to collect larvae . . . . .	20
Fig. 15. Larval-collection screen placed in seawater to prevent damage . . . . .	20
Fig. 16. Size of larvae (in $\mu\text{m}$ ) from about 48 hours to about 10 days of age . . . . .	22
Fig. 17. Scallops with distinct striped markings . . . . .	23
Fig. 18. Raceway system for culture of juvenile scallops . . . . .	25
Fig. 19. Tray system for culture of juvenile scallops . . . . .	25
Fig. 20. Downwellers for the culture of scallops up to 10 mm. . . . .	26
Fig. 21. Spat bags for the culture of juvenile scallops in the natural environment . . . . .	27
Fig. 22. Pearl nets for the culture of juvenile scallops in the natural environment . . . . .	28
Fig. 23. Bag filter systems for filtration of seawater . . . . .	34
Fig. 24. Ultraviolet (UV) light systems for sterilizing seawater . . . . .	35
Fig. 25. TCBS agar plate showing bacterial colonies . . . . .	36

## LIST OF TABLES

Table 1 - Recommended stocking densities of eggs and larvae based on age and size .....	17
Table 2 - Recommended mesh sizes of screens for larvae based on age and size .....	17
Table 3 - Microalgal strains tested for feeding larval bay scallops through metamorphosis .....	30

## I. INTRODUCTION

The native New England bay scallop, *Argopecten irradians irradians*, is a high-value seafood species with an existing market but an unstable yield from the wild-caught fishery. Wide inter-annual fluctuations in bay scallop populations can be attributed largely to two factors: 1) the short life history of the bay scallop, i.e., most live less than two years and spawn only once (Orensanz *et al.*, 1991), and 2) their sensitivity to environmental stresses (Tettlebach *et al.*, 1985), particularly winter mortality (Mercaldo & Rhodes, 1982; Bricelj *et al.*, 1987). The bay scallop is, however, an extremely fast-growing bivalve, reaching a minimum market size of 40+ mm (millimeters) (one mm equals 0.03937 inches) in less than one year (Oesterling & Rose, 1996). For comparison, oysters require 3-5 years to reach market size (Burrell, 1985), and quahogs grow even slower, requiring 4 years or more to reach littleneck size in New England waters (Menzel, 1989). When available, New England bay scallop adductor muscles (the only portion of this animal currently marketed widely) sell for \$9-16 per pound or more. The high market value, coupled with limited wild-fishery yield and rapid growth, make the bay scallop an attractive candidate for aquaculture (Gates *et al.*, 1974; Webber & Riordan, 1976).

An aquaculture strategy for the bay scallop must address the basic biological constraints that limit the wild fishery; in fishery biology terms, these constraints are recruitment and survival to market size. Recruitment is the addition of new individuals to the population. In shellfish aquaculture, "recruits" are referred to as "seed."

There are two ways to obtain bivalve seed: one is collection of wild "spat," or seed, in natural waters, usually employing an artificial substrate deployed in the water. The other source of seed is through hatchery production. Spat collection is dependent upon a sufficient population of "spawning stock," i.e., reproductive adults, and conditions suitable for development and retention of planktonic larvae in the estuary. Efforts to collect bay scallop spat in southern New England estuaries have met with limited and inconsistent success (Tammi *et al.*, 1997). We, therefore, have based our strategy for bay scallop aquaculture development in New England upon hatchery production of seed scallops.

Practical methods for artificial spawning of bivalve mollusks and rearing of larvae and young spat were developed at the Milford Laboratory over four decades ago (Loosanoff & Davis, 1963). Preparation of adult bivalves for induced reproduction (referred to as "conditioning" or "ripening") can be accomplished at almost any time of year by increasing or decreasing water temperature incrementally over several weeks to the desired conditioning temperature and providing sufficient microalgal food to fuel the energy-intensive process of gamete (eggs and sperm) formation. "Ripeness" is easier to determine in bay scallops than in other bivalves because the gonads (reproductive organs) can be inspected visually without sacrificing the animal. Conditioned scallops held at 17-20°C (see Appendix C for conversion of °C to °F) can be stimulated to spawn by increasing the temperature of the water to 25°C over a period of one hour. Gametes (both eggs and sperm) may be released from the same

individuals (hermaphroditism). Fertilization occurs immediately and embryos may be collected, counted, and dispersed into suitable containers. Embryos require no supplemental feeding for 24-48 hours, deriving their nutritional needs from storage reserves of the egg. For this reason, it is important that broodstock be nutritionally robust.

When larvae reach the veliger stage (characterized by the presence of a locomotory and feeding organ called the velum), after about 24 hours at 25°C, they are about 70 µm (one µm or micrometer equals one thousandth of a mm) in size; at this time they are fed a diet of cultured microalgae. Effective microalgal diets for larval bay scallops must be: 1) non-toxic, 2) sufficiently small to be ingested, 3) digestible, and 4) nutritionally complete. Whereas toxicity and size of potential microalgal diets are evaluated easily, digestibility and nutritional composition are more difficult to ascertain. For bay scallop larvae, we have shown that algal diets high in specific, "essential" fatty-acids and sterols support more rapid growth and result in a higher percentage of larvae successfully undergoing metamorphosis (a physiological change from one form to another) to the post-set stage (Alix *et al.*, 1996).

After 7-14 days of larval development and growth to about 200 µm, scallops undergo metamorphosis, also called "setting," when adult morphological characteristics are established. As this process includes loss of the velum and development of a new, gill-feeding apparatus, feeding may be interrupted or become less efficient. Thus, nutritional condition of larvae at the pre-setting stage may be important in determining setting success.

Metamorphosing scallops attach to surfaces by means of byssal threads similar to those of mussels. It is thought that byssal attachment to

eelgrass and other living and non-living structures in nature serves to keep scallops off the bottom to avoid crustaceans and other predators and to ensure a current-mediated supply of phytoplankton food (Brand, 1991). In the hatchery, scallops are set on mesh "onion bags" filled with nylon monofilament line, plastic strips or burlap, fiber-glass, or directly on the container sides. Setting success in the hatchery generally ranges from less than 1% to about 10%; this variation offers a potential area for improvement, but from a practical standpoint, does not constrain hatchery production seriously because of the large number of larvae (millions) that can be obtained from spawning several individuals. Post-set scallops then enter a stage of culture referred to as the "nursery." Traditionally, young, post-set scallops, as well as other bivalves, are reared in tanks through which coarse-filtered seawater is pumped (Rhodes & Widman, 1980) or in protected enclosures placed in the natural environment (Widman & Rhodes, 1991). In either case, young animals are exposed to ambient seawater temperatures and depend upon natural phytoplankton, not cultured microalgae, for food.

In nature, the bay scallop in northeastern U. S. waters usually spawns in July, although peak spawning may occur from June through mid-August (Belding, 1910), with a lighter secondary spawning possible in the fall (Tettlebach *et al.*, 1999). The life cycle of the bay scallop (Figure 1) begins when the fertilized egg develops into a D-shaped larva, known as a veliger or prodissoconch, within 24–48 hours. The veliger has two valves (or shells) and a ciliated velum which it uses for feeding and locomotion. After 7 to 14 days, the larva undergoes metamorphosis, a physiological change whereby it develops into a juvenile scallop, or dissoconch, which is similar in appearance to an adult. Just prior to metamorphosis, the larva develops a foot which it uses for crawling, while maintaining its ability to swim.

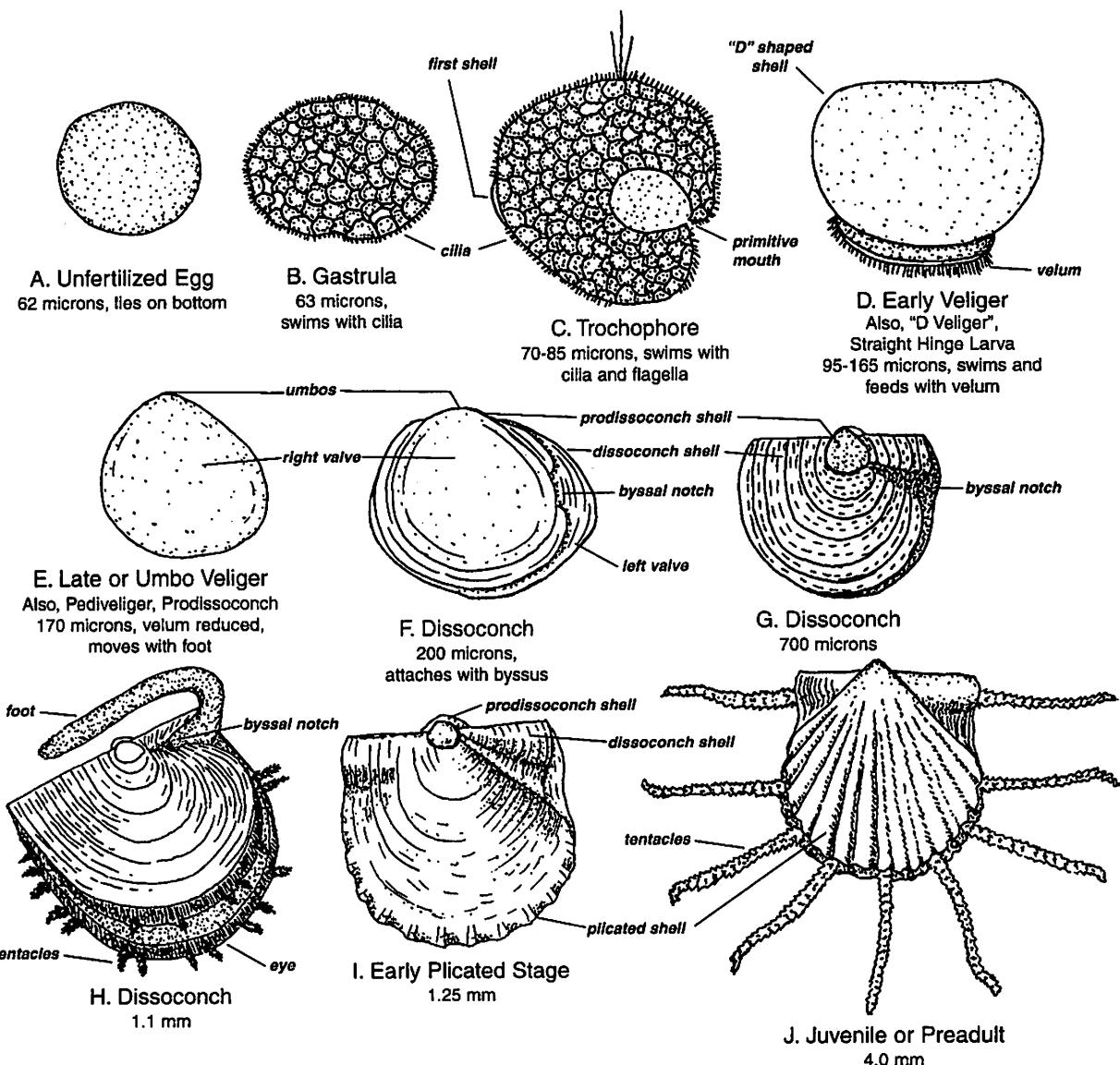


Figure 1. Early life cycle of the bay scallop. [Bottom (right) valve is shown uppermost in E-J, A-D after Sastry, 1965; E-J after Belding, 1910].

During this period the juvenile scallop tests various substrate materials to determine their suitability as places for attachment or settlement. A preferred substrate is eelgrass, *Zostera marinus*, although seaweed, pilings, rocks, etc., are used as well. The scallop grows rapidly at this stage, as long as the water temperature remains warm and sufficient food is available. Juvenile scallops typically attach and detach a number of times because of the growth and shedding of the eelgrass frond. As they get larger, they settle to the bottom. By late November to early December they grow to a shell height of 20 to 40 mm, although growth can range from 7 to 60 mm depending on environmental conditions. As water temperature decreases to below 15°C, growth slows considerably and eventually stops. There appears to be no growth at temperatures

below 7 to 10°C. As temperature increases the following spring, growth resumes. Considerable energy is needed for maturation of the gonads during this period. Adults mature at approximately one year of age when spawning normally occurs, and resume growing until late fall when water temperatures cool. The majority of scallops appear to live for only 20 months, although this is not likely due to aging alone but to environmental stresses as well. Some scallops have been observed to be at least two years old, and some even three.

Citations for further reading on the life history of the bay scallop are presented in an Indexed Bibliography of the Bay Scallop (*A. irradians*) by Sabo & Rhodes (1987), which is available at the Milford Laboratory.

## II. FACILITIES

Facilities for the culture of the bay scallop require access to a continuous supply of high-quality seawater. The salinity of the water should range from 25 to about 33‰ (parts per thousand). In the northeastern U.S., wide fluctuations of ambient seawater temperature necessitate the heating and chilling of the seawater supply. Conditioning broodstock, spawning, and culture of larvae all require temperature-regulated seawater. Heat exchangers can be used to raise or lower the temperature in flow-through systems. Electric aquarium heaters can be used in static systems by placing them directly into the holding systems. It is most likely that any facility that draws seawater from a natural source and discharges waste waters into a natural water body would require permits from the appropriate state and/or local government agencies.

The size of the facility would be dependent upon the seed production anticipated. The facility should be of such size to house the proper numbers of hatchery and nursery tanks needed, seawater pumps, heating and chilling units with associated energy sources, as well as storage areas and lab space. Associated facilities for production of microalgal feed cultures should be conveniently located adjacent to or within the hatchery installation.

The facilities at Milford described in this manual are just one design of a shellfish hatchery. Numerous hatcheries throughout the U.S., both public and private, provide alternative design ideas.

All new materials to be used in the culture of larvae should be cleaned with a biodegradable soap solution and household bleach; i.e., we routinely scrub with 5 milliliters (mL) of Liquinox® (or any similar biodegradable liquid detergent) plus 5 mL of bleach in 10 liters (L) of tap water. Then the material should be submersed in seawater for a minimum of 24 hours prior to use. After the submersion period the materials should again be washed in a soap and bleach mixture. After washing, materials should be rinsed three or more times with hot fresh water and stored dry. Prior to contact with larvae, the materials should be rinsed with 10-µm filtered seawater to remove any chemical residues.

References to use of seawater in this hatchery manual imply 10-µm cartridge-filtered seawater at 25°C unless otherwise noted. While cartridge filtration is recommended, it should be noted that there have been great strides made in bag filtration methods, and these may be acceptable alternatives.

No metallic items (except titanium) that would come in contact with seawater should be used. Copper and brass are extremely toxic to shellfish, and should never come in contact with the seawater system. Seawater is extremely corrosive; it has been our experience that stainless steel #316 still corrodes in these environments. For most needs, PVC, fiberglass, polycarbonate, plexiglass, most plastics, and glass are acceptable for use. If metal items are used, they should be coated prior to use; one acceptable coating is Teflon®.

Hatchery culture typically uses elevated seawater temperatures, thus requiring scallops to undergo an acclimation period before they are placed in warmer or cooler waters. Normally, in the hatchery, scallops can tolerate temperature changes, up or down, of 1°C/day very well.

Changes above that rate may increase mortalities. During this phase, follow the normal standard operating procedure of periodic water changes and feedings. As temperatures decrease, scallops feed less, hence water changes can be made less frequently.

#### **FACILITY REQUIREMENTS**

- Seawater source
- Size dependent upon production goals
- Must accommodate tanks, pumps  
heaters/chillers, lab work counter space
- Room for associated algal culture
- No metal in contact with seawater

### III. BROODSTOCK MANAGEMENT

## A. BROODSTOCK SELECTION

The main objective of broodstock management is selection of good quality broodstock in adequate numbers to ensure genetic diversity, especially over the long term. Selection of good quality broodstock is critical to the success or failure of any cultured species, including scallops. The best broodstock often is found at a site where scallops occur naturally.

Knowledge of life history characteristics of bay scallops, such as growth and survival, is useful in predicting the expected performance of hatchery broodstock. For example, scallops are hermaphroditic, i.e., contain both male and female sex organs (Figure 2). Accordingly, under

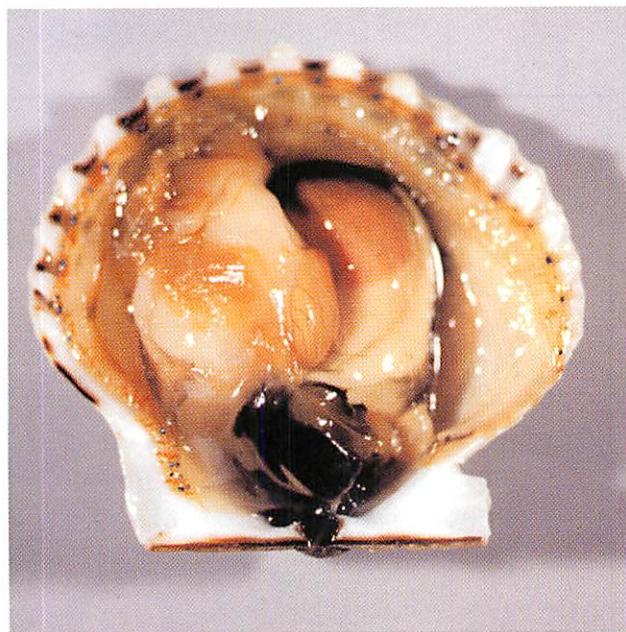


Figure 2. Bay scallop with fully ripe female (pinkish-orange) and male (white) sex organs.

hatchery conditions, it is possible that some inbreeding through self-fertilization will occur when the scallops are spawned; therefore, maintain sufficient numbers of scallops in the hatchery to allow for genetic diversity. Inbreeding depression manifested as reduced growth and survival can occur.

## B. SIZE AND APPEARANCE

Generally, larger animals produce greater quantities of gametes. Typically, scallop populations in the wild consist of only two year classes, as the normal life span is only about 20 months. A mixed-size group of both year classes could be used for spawning stock. Maturing scallops generally display a black integument (tissue) (Figure 3) that covers the immature gonad which is quite distinct prior to gametogenesis (the development of eggs and sperm). As gametogenesis progresses, the integument disappears and the discrete white-colored male and pinkish-orange female gonads ripen and become prominent, indicating spawnability. When scallops are collected in the wild, shells should appear well formed without indentations or blunt or abnormal margins. Healthy scallops usually close their valves when touched. Scallops with valves that remain open or are gaping are probably dying. Hinges should close tightly, and should fit together evenly.

A check of tissues should be made to see if they are plump and healthy rather than flaccid. If assessment of tissue quality and gonad cannot be made adequately from external observations while holding the valves open, 2 to 3 scallops can be shucked for examination of tissue quality. If



Figure 3. Bay scallop with black integument covering immature gonad.

most of the scallops in a broodstock tank recently have died, it is better to seek a new source of broodstock or use scallops from a separate tank where few mortalities are evident.

## C. BROODSTOCK NUMBERS

An adequate supply of broodstock should be available to initiate spawning at appropriate times of the year. At a minimum, 300 adult scallops should be collected and maintained in the hatchery. When mass-spawning scallops, 40 to 50 animals are used since only about half will spawn. Of those that spawn, only a few contribute most of the eggs and sperm.

## BROODSTOCK MANAGEMENT

- Local stock is best to use
  - Select large & healthy individuals
  - Use sufficient numbers to ensure genetic diversity; about 300

#### IV. HATCHERY PRODUCTION

## A. BROODSTOCK CONDITIONING

**C**onditioning of broodstock is the process of artificially ripening the gonad so that mature gametes will be released upon spawning. The conditioning process is required when attempting to spawn scallops outside of their normal spawning period (June - August). When spawning scallops just prior to their natural cycle, no conditioning usually is required.

Scallops typically are conditioned at 17-20°C in the northeastern United States, specifically Connecticut, during the winter/spring months. Conditioning temperatures will vary with location (latitude); it may be done statically in a container that provides sufficient bottom area for the adults to spread out (Figure 4), with daily or every-other-day water changes. Scallops also can be conditioned in flowing seawater (Figure 5), but heating or chilling of flowing seawater can be expensive. If the salinity of the incoming seawater is below 17‰ and water temperature is above 15°C, mortalities will occur (Mercaldo & Rhodes, 1982). Nutrition is crucial in the successful conditioning of scallops out-of-season. A drip system or peristaltic pump (metering pump to deliver liquid at precise doses) can be used to feed the scallops from an algal source. Numerous daily feedings will improve effectiveness of feeding. We recommend that at least two groups of broodstock be conditioned at the same time so that at least one group is still available in the event that one group spawns inadvertently during the ripening process. Spawning may occur if the water temperature varies too

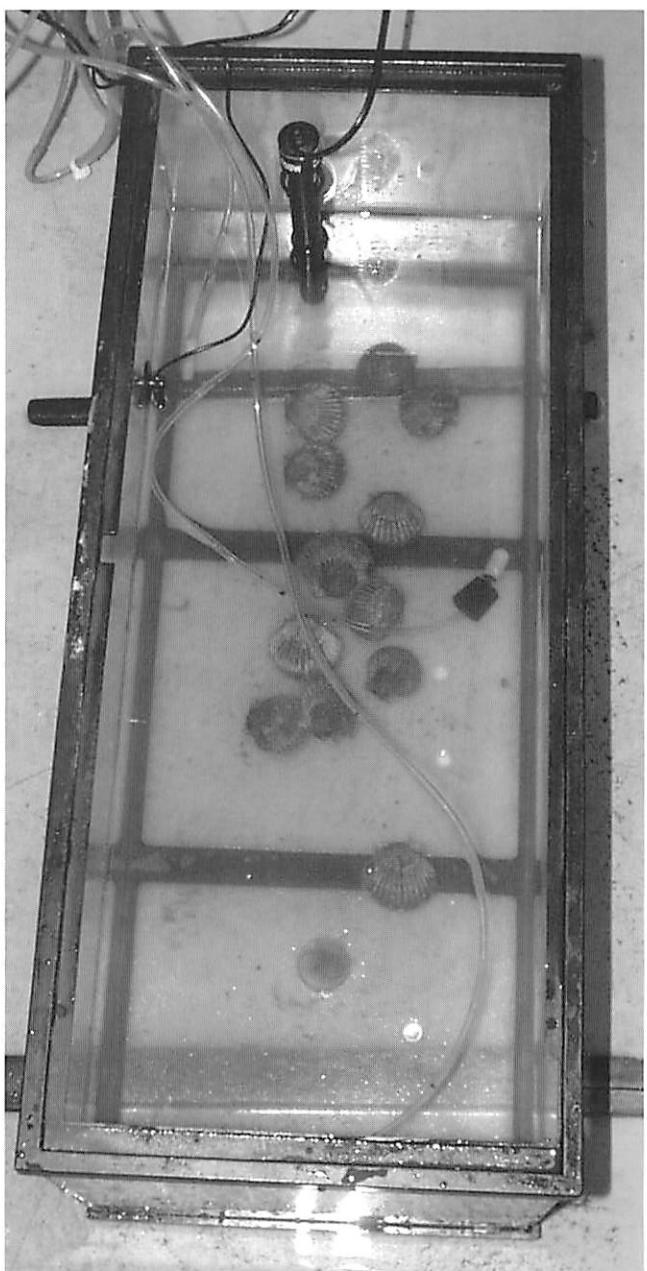


Figure 4. Conditioning scallops in static culture.

much during the conditioning process. In New England, the process of ripening the gonads takes approximately 6-8 weeks when starting in December. Closer to the natural spawning sea-

son, less time is required to condition the gonads. Spawning also can be delayed by holding the broodstock in cooler ( $10^{\circ}\text{C}$ ) water during the warmer months.



Figure 5. Conditioning scallops in trays of flowing seawater.

## TEMPERATURE CONTROL

Providing a stable temperature environment (plus or minus 1° Celsius) is important for both conditioning broodstock and larval rearing. Lower temperature variations usually provide better and more consistent results. Heating can be as simple as using aquarium immersion heaters and water baths or as complex as a titanium heat exchanger. Many hatcheries, including this one, rely on multiple heating methods. There are numerous digital controllers on the market that provide excellent temperature control. Even some inexpensive (less than \$50) immersion heaters come with fairly sophisticated controllers now. Do not use non-submersible aquarium heaters as these usually fail rather quickly in a seawater environment. Cooling can also be done by immersion titanium heat exchangers, water baths or special chillers made for seawater.

gonad are observed, normally the scallop can be spawned successfully. Scallops that have spawned, but are still ripe (capable of producing sperm and eggs), will appear to have "rivers" traversing the colored parts of the gonad (Figure 6). Gonads that are spent or depleted typically have a translucent beige coloration, and usually do not release eggs or sperm. More vivid coloration of the gonad usually indicates a riper gonad, with the potential for more gametes to be released as well as a greater 48-hour larval survival of the released gametes. Scallops having a white-colored gonad only usually indicates a nutritional deficiency traceable to the microalgae being fed to the broodstock.

## B. CONDITION/RIPENESS

Visual observations can determine whether the scallop appears to be ripe enough to spawn. With the scallop held open, peer in between the valves and observe the coloration of the gonad. The scallop, as mentioned earlier, is a functional hermaphrodite, i.e., contains both male and female sex organs. The pinkish-orange area produces eggs, whereas the whitish area produces sperm. It also is common to find a black integument covering the gonad during the conditioning process. This integument usually disappears as the gonad ripens, but can be scraped away gently to reveal the coloration of the gonad. If both colors (pinkish-orange and white) of the



Figure 6. Scallops that have partially spawned with traversing “rivers” through the gonad.

### BROODSTOCK CONDITIONING

- Necessary to spawn out of season
- Temperature varies; usually 17-20°C
- Salinity > 17 ‰
- Up to eight weeks required
- Ripeness evaluated by color of gonad

## C. SPAWNING

We use two approaches to spawning, both of which rely on increased water temperature to stimulate the scallops: 1) mass spawning, i.e., spawning a number of individuals collectively to induce spawning of the group more easily, and 2) individual spawning, i.e., scallops are spawned in individual containers and the gametes are kept separate, which is particularly important when scallops with special traits are desired. As the bay scallop is hermaphroditic, spawning must be monitored closely during individual spawning to prevent self-fertilization. See Section VI.A.3 for cleaning of scallops prior to spawning.

For both spawning methods, scallops are placed into the spawning container(s) with water at the same temperature from which the scallops were removed (usually 17-20°C). They are allowed to acclimate to this new environment for one hour and then the temperature is increased gradually to approximately 21°C over a 20-minute period. Usually, no spawning occurs during this time, but if the scallops are very ripe, even a temperature change of 1°C can be enough to induce spawning.

Once the temperature reaches 21°C the temperature is held steady for 20 minutes; it is then increased to 25°C over another 20-minute period. After the temperature reaches 25°C, it should be maintained for at least one hour (**do not exceed 26°C**). Typically, spawning will begin during this time. If the scallops still have not started spawning, the temperature can be lowered to 17-18°C and later increased following a 20-minute temperature change and 20-minute holding cycle.

1. **Mass spawning** (Figure 7). Two methods of mass spawning are described here. One method is to place 40-50 scallops into a large, flat-bottomed, dark-colored tray or tank and allow the scallops to spawn by increasing the temperature as described above. (Black is preferred to determine visually when gametes are released.) When an individual scallop spawns, it is removed and placed in a separate container with seawater at the same temperature. (Removing the scallop will minimize any self-fertilization that may take place.) The spawner is allowed to continue spawning in this container, but must be watched closely



Figure 7. Mass spawning of scallops.

to prevent self-fertilization from occurring should the scallop start releasing gametes of the other sex. To help prevent self-fertilization, we recommend the spawner be placed in a new container of sea-water every 7-10 minutes, or when the scallop appears to have ceased spawning. As the culturist becomes more adept at controlling (or observing) the spawning process, he or she will be able to determine the type of gamete being released into the water column. A thin milky stream is an indication of sperm, whereas a grainy appearance indicates eggs have been released.

A second method of mass spawning is to place scallop broodstock (in nets) into a conical tank or other large container in which the larvae are to be reared. (This method may result in increased rates of self-fertilization.) The temperature of the water in these containers should be pre-heated to 25°C to allow spawning to take place. Periodic sampling of the spawning container is required to determine the number of eggs produced; this is done so that the spawning process can be stopped when the desired number of eggs is obtained. The embryos/larvae resulting from this spawn should

not be handled for 48 hours unless the numbers of larvae are too great and need to be reduced or separated into additional containers.

Using either of these methods, it is possible to stop the scallops from further spawning so as to be able to re-spawn this broodstock a number of times (typically four). To stop the spawning process, the broodstock are removed and typically placed in chilled seawater (17-18°C) - although sometimes cooler water is needed.

2. Individual spawning (Figure 8). Individuals are placed into separate containers (Pyrex® bread pans work well) placed over a dark background and the temperature is increased (as described above), usually by flowing warm tap water around the outside of these containers, until spawning takes place. This method of spawning offers considerable control, and it is possible to cross

individual animals if desired. This process is more laborious and usually slower than mass spawning. During individual spawning, one must exercise care so that individuals do not self-fertilize by releasing both sperm and eggs in the same spawning container. Self-fertilization may be prevented by switching each scallop to a new container periodically.

## SPAWNING

- Two methods: mass or individual
- Temperature increase to 21°C, then to 25°C in two stages
  - **do not exceed 26°C**



Figure 8. Spawning of scallops individually.

#### D. DETERMINING EGG AND LARVAL COUNTS

Eggs spawned in the containers are separated from debris by passing the egg suspension through a 100- $\mu\text{m}$  or larger screen size (Figure 9). Seawater and eggs that pass through the screen are collected in an appropriately-sized container. If the egg suspension is too dilute during mass spawning, it may be necessary to concentrate the eggs on a 20- $\mu\text{m}$  screen; however, frequent screening of the eggs may be detrimental to normal development. Another approach to concentrate the eggs is to siphon out the excess liquid in the container by covering the inlet of the siphon with a 20- $\mu\text{m}$  screen. To count the eggs, it is necessary to evenly distribute them in the collection container by mixing with a perforated plastic plunger (Figure 10) in an up-and-down motion.

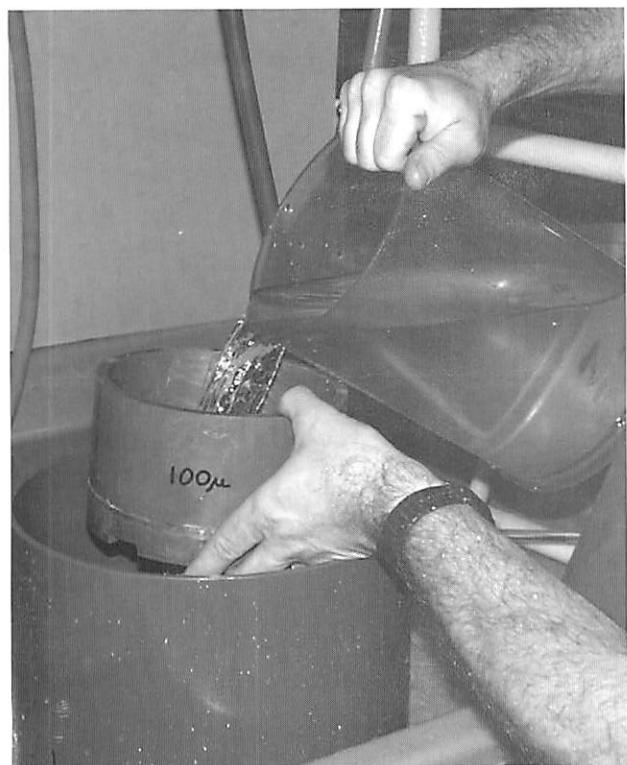


Figure 9. Separation of eggs from debris by passage through a 100- $\mu\text{m}$  screen.

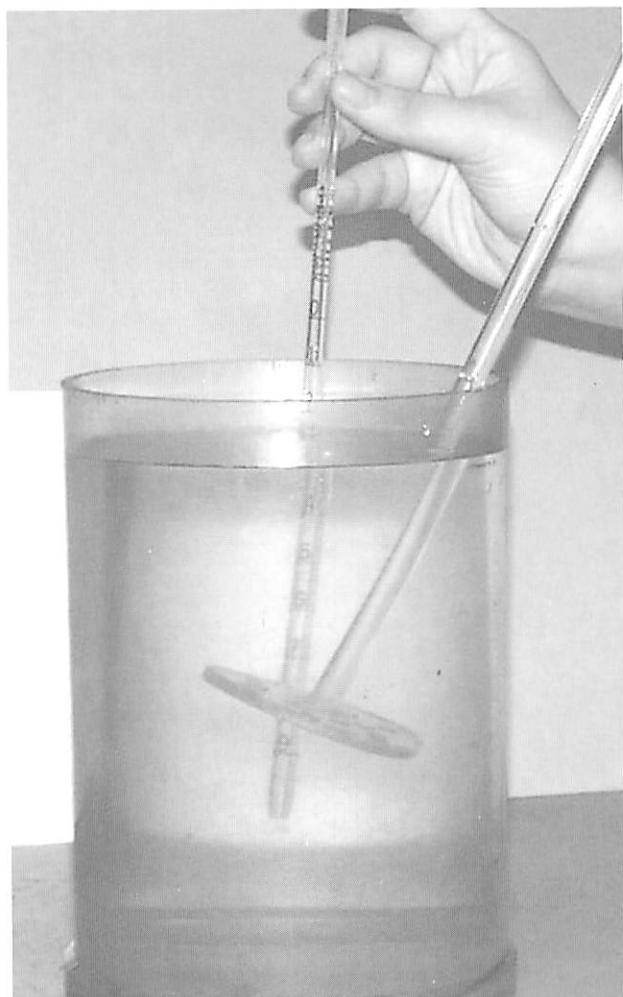


Figure 10. Mixing of egg suspension with a perforated plastic plunger.

This must be done in a predetermined volume. If the mixing motion is circular, the eggs can become concentrated at the bottom and not evenly distributed, resulting in an erroneous count. Once the eggs are mixed in a known volume such as a 250-mL or 1,000-mL graduated cylinder, a 1-mL pipette with a large bore opening is used to remove eggs from the container to count them (Figure 10). This sample is placed on a Sedgwick-Rafter counting cell (Figure 11). This must be done quickly; no more than 7-10 seconds from pipette to slide to count the eggs under a



Figure 11. Dispensing a 1-mL sample of egg suspension on a Sedgwick-Rafter counting cell.

compound microscope. By scanning the entire slide at 60 X, the number of eggs in 1 mL is determined. By multiplying the number of eggs in 1 mL by the total number of mL in the container, you obtain the total number of eggs in the container. This number then is used to determine the volume of egg suspension to add to each rearing container.

See Table 1 (page 17) for stocking densities and Table 2 for screen sizes to be used. Counting eggs is relatively simple because they are not motile.

## **EGG COUNTING**

- Screen to concentrate and clean
  - Mix thoroughly with plunger
  - Subsample 1 mL with wide-tip pipette
  - Count in Sedgwick-Rafter Cell at 60 X magnification
  - Multiply number in 1 mL X total volume in milliliters to calculate total number of eggs (embryos)

**Table 1.****Recommended stocking densities of eggs and larvae based on age and size.**

<u>Age/Size*</u>	<u>Density</u>
embryos/fertilized eggs	30/mL
48 hours	17.5/mL
150 µm	10/mL
175 µm	1.25/mL - near setting size

\*Sizes may vary with variations in the nutritional value of the microalgae being fed.

**Table 2.****Recommended mesh sizes of screens for larvae based on age and size.**

<u>Mesh Size*</u>	
20 µm -	retains eggs (usually only need a 6" diameter screen)
54 µm -	retains 48-hour-old larvae
75 µm -	retains 4-to 5-day-old larvae
100 µm -	retains 6-to 7-day-old larvae
135 µm -	retains 7-to 14-day-old metamorphosing & metamorphosed larvae

\*Screens generally used are 10" diameter, although other sizes can be used. At our facility we use 10" screens when draining 400-L conical tanks.



Figure 12. "Standard" 400-liter fiberglass tanks for culture of embryos and larvae.



Figure 13. Small plastic containers for culture of embryos and larvae.

## AERATION

Aeration is vital in a hatchery when rearing shellfish in recirculating or static systems. Small, fine bubbles diffuse more air into the water than larger bubbles because of the greater surface area. The longer the bubble is in contact with the water the better, hence an air diffuser should be placed as deep in the water column as possible. Adding air to the water does a number of things: it provides oxygen which is consumed by the shellfish, mixes the water, and helps in the removal of various waste compounds. Aeration can be provided by a small aquarium-style air pump, an oil-less compressor or air blower. The method chosen should be based on total usage. Since air is so vital to the shellfish, a backup system is recommended.

## **REARING CONTAINERS**

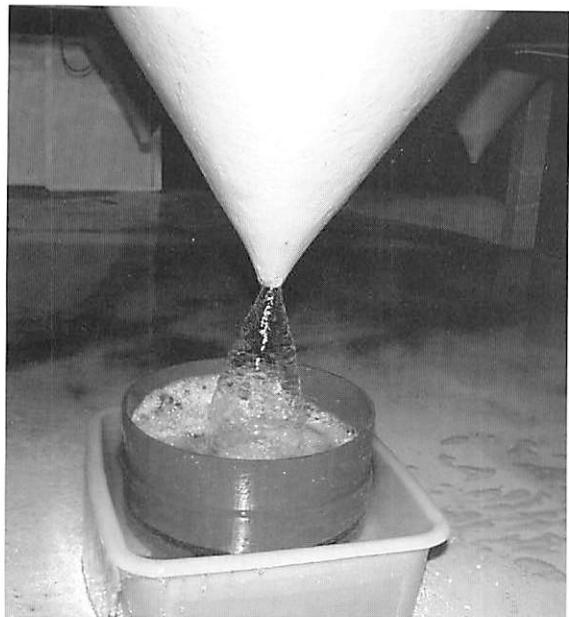
- Size variable
  - Smooth surface
  - Light color
  - Aeration

## F. LARVAL REARING

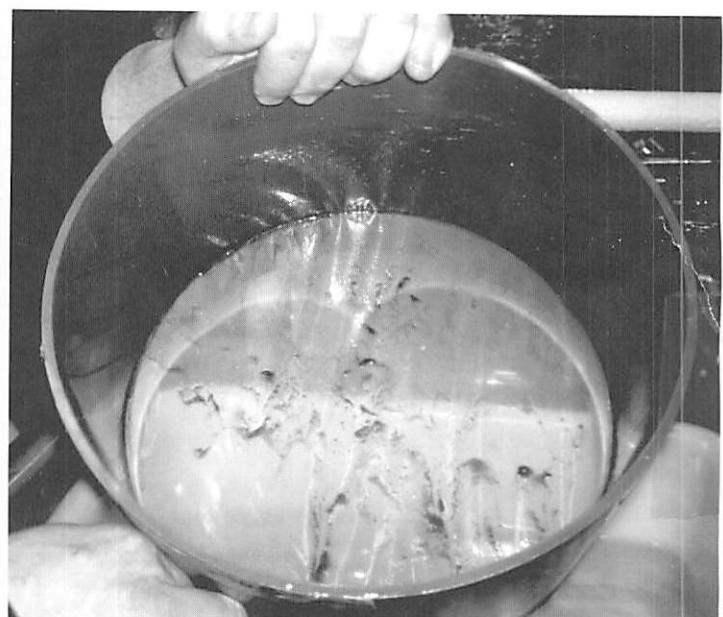
Optimal larval rearing conditions (See Table 1 on page 16 for stocking densities and Table 2 for screen sizes to be used) are at a water temperature of  $25\pm1^{\circ}\text{C}$  and a salinity of 25‰ (Tuttlebach & Rhodes, 1981). Temperatures should not exceed  $26^{\circ}\text{C}$ . All items that are used in spawning and larval rearing should be washed immediately prior to use with a biodegradable soap and chlorine bleach solution (see section VI.A.1.). Then wash with at least three hot water (as hot as tolerable) rinses, followed by a rinse with 10-µm filtered seawater. During the first 24 hours of life, the fertilized egg develops into the following stages: gastrula, trophophore, and, finally, a swimming veliger (Figure 1). Larvae are first fed 24 hours after spawning and daily thereafter. Water in the culture containers remains static for the first 48 hours to allow the fertilized eggs to develop into "D-shaped larvae" (veligers). At 48 hours, the larvae have already developed shells and are able to swim by moving their cilia. Movement of the cilia causes water to move within the larval shell cavity, maintaining an oxygen-rich environment and providing food to the larvae. Healthy larvae can be identified under a microscope at 60 X by their circular swimming pattern and their dark, rich gold/tan coloring (although the coloring can differ depending on the microalgae being fed). Occasionally, after the larvae are disturbed—possibly by changing the water—they become "shocked"

## E. REARING CONTAINERS

Shellfish hatcheries use a variety of containers to rear shellfish larvae. Some hatcheries use containers as large as swimming pools, others use the "standard" 400-L fiberglass conical tanks (Figure 12), and smaller operations successfully use plastic garbage containers (Figure 13). When selecting a container, choose one with a smooth surface to help reduce bacterial growth and allow easier cleaning (we prefer light-colored containers) and rigid enough to support the volume of water held. Gently aerate the seawater in these containers to maintain uniform temperatures and provide oxygen to the larvae.

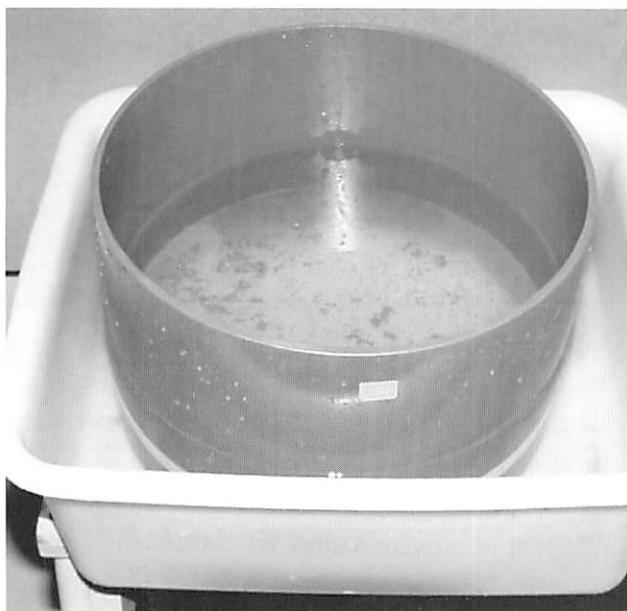


a. Screening culture water

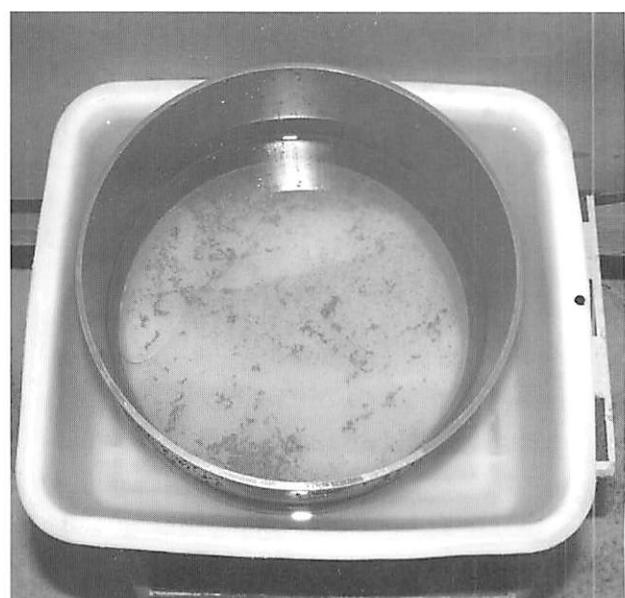


b. Larvae on screen

Figure 14a,b. Passing larval culture water through an appropriate-size screen to collect larvae.



a.



b.

Figure 15a,b. Larval-collection screen placed in seawater to prevent damage.

and are not very mobile. It is then important to view the larvae under a microscope at 120 X power or higher to determine whether the cilia are moving within the shell. Another method to determine how well the larvae are doing is to keep the larvae on the slide for a longer period of time (minutes) in order to observe whether they begin to exhibit normal swimming behavior.

**NOTE:** Do not keep the microscope light on during this time as you may heat-stress the larvae.

Water changes are required to maintain a healthy growing environment and remove the waste products that accumulate in the larval culture. Water for larvae should be changed on a daily basis after the first 48 hours, although some shellfish hatcheries change every other day. To change the larvae, the container is drained through an appropriate-sized mesh-screen (mesh size varies with the size of the larvae-Table 2) (Figure 14a) so that the larvae are retained on the screen (Figure 14b). Depending upon the length of time it takes to drain the larval container, it may be necessary to place the screen in seawater to help prevent damage to the larvae (Figure 15a,b). Increasing the mesh size of the screen as the larvae grow aids in the removal of debris that would otherwise accumulate in the culture and provide additional substrate for bacteria. As an example, we typically use a 54- $\mu\text{m}$  screen after 48 hours and a 75- $\mu\text{m}$  screen after four to five days. If you are trying to maximize genetic diversity, we recommend using a 36- $\mu\text{m}$  screen at 48 hours to retain all larvae.

Occasionally, you may want to separate the culture based on larval size. This can be accomplished by passing the culture water through a series of mesh screens, a process known as

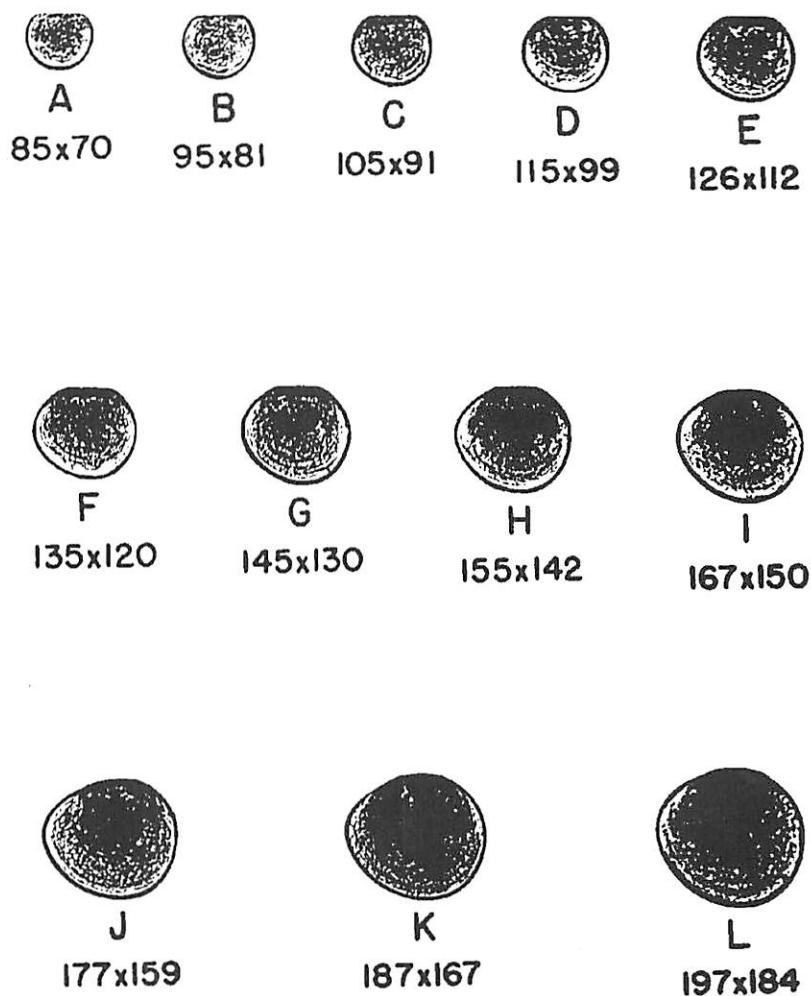
culling. It is called culling because some hatcheries discard the runts (slowest growers), especially if there is a shortage of phytoplankton or larval rearing containers. Always discard larvae that you are unable to feed or rear, rather than starve or crowd them, which usually results in higher mortalities.

Sampling of the larvae in the culture containers should be done on a daily, or at least every other day, schedule. When larvae are sampled, a preservative that immobilizes the larvae on the slide must be used to obtain an accurate count (3 to 5 drops of ethanol or a 10% household chlorine bleach will suffice). Generally, it is a good idea to concentrate/dilute larval suspensions to obtain counts of larvae in the 250-300 per mL range.

It is important to note that even if the larvae are not counted and measured on a daily basis, they at least **SHOULD** be observed under the microscope on a daily basis. (This cannot be emphasized enough!)

## LARVAL REARING

- Temperature 25°C; salinity 25‰
  - Start feeding after 24 hours
  - Change water after 48 hours
  - Sample and observe under a microscope daily
  - Feed 400-liter conical 1liter of algae at packed-cell-volume of 0.010 daily
  - Record survival and growth



## **ARGOPECTEN IRRADIANS**

x 112

Figure 16. Size of larvae (in  $\mu\text{m}$ ) from about 48 hours to about 10 days of age. Largest dimension is in a line parallel to the hinge.

Larvae are fed microalgae at a rate equivalent to 1000 mL at a packed-cell volume (PCV) of 0.010 per 400 L of larval culture per day (See Table 3 and Appendix A). As an example, we normally feed a 400-L larval culture  $15 \times 10^9$  cells of *Isochrysis* sp. strain T-ISO or  $13.5 \times 10^9$  cells of *Pavlova* strain 459. To provide a good nutritional mix, at least two types of microalgae are recommended as a food source.

Records of survival and growth should be maintained for each spawning batch. By tracking growth of the larvae (Figure 16), one can get a feel for the robustness of a particular spawn and any possible problems. Growth of the larvae can be measured with an ocular-micrometer placed in one of the eyepieces of the binocular microscope. Normally, a decrease or cessation of growth indicates some type of problem. Two common problems are bacterial contamination and nutritional deficiencies.

## G. GENETIC CONSIDERATIONS AND SELECTIVE BREEDING

In most hatcheries, faster-growing larvae are selected for further grow-out. It is common practice in a hatchery to screen-out and discard bottom material, or "dregs", and slow-growing or dying larvae. Doing this may prevent a buildup of harmful microorganisms in the culture. If possible, however, it is wise to save some of the moderately-growing larvae. It is important to realize that inadvertent selection and, therefore, narrowing of the gene pool, occurs with standard hatchery practices such as spawning small numbers of broodstock and screening or culling larvae and juveniles for size. Some unexplained "crashes" (near total mortality) of larval cultures may be attributable to a narrow gene pool. These crashes can be reduced by mass spawning more diverse broodstock.

If some inbreeding from self-fertilization does occur, one can minimize effects by taking certain steps. If possible, maintain sufficient numbers of adult broodstock in the hatchery so as to be able to spawn several times. Also, spawn as many scallops as possible at one time. Split a batch of larvae into several containers to minimize loss of the entire population from one mistake or malfunction. This is based on the practical advice not to "put all of your eggs in one basket".

In some hatcheries, certain characteristics may be desired to improve or increase production. Crossing of selected scallops could increase the frequency of certain traits like faster growth, disease resistance, or shell color or markings (Stiles *et al.* 1998 a, b). Some scallops have obvious genetic markings or color patterns on their shells, such as stripes (Figure 17), which could be used in identifying stocks from a particular hatchery. For example, dark red vertical bands on shells of the hard clam, called Notata clam, are used for this purpose in the clam industry (Stiles & Choromanski, 1995). More detailed



Figure 17. Scallops with distinct striped markings.

discussion of genetics and selective breeding is beyond the scope of this manual.

# **GENETICS AND SELECTIVE BREEDING**

- Moderate culling removes dregs, but preserves genetic diversity
  - Replicate spawnings minimize risk
  - Crosses may be made to select for desired traits.

handling is important. Scallops metamorphose within 1-2 weeks inside the larval containers and juveniles can be found attached to the bottom and sides. Scallops that are not attached can be rinsed away with a gentle stream of seawater (soft rinse); the metamorphosed scallops remain attached to the sides. To remove the attached metamorphosed scallops, a hard stream of seawater (hard rinse), similar to that formed by restricting the flow of water from a hose, is used to dislodge the scallops from the container/conical tank. From here, the scallops normally are transferred to either static or flow-through tanks of seawater. Static cultures are drained and refilled every day or less often.

Once the scallops have set they can be moved to various systems, including placement in the natural environment. The circumstances chosen for this stage of culture will depend upon final product/harvest size and natural growth cycle/temperature regime. When transporting scallops, keep them moist with seawater, cool, and out of direct sunlight. One useful method is to keep them covered with moist burlap or newspaper in a cooler with a cooling block.

Scallops in static tanks are fed daily and water is changed every other day. To improve growth rates, microalgae can be added on a semi-continuous basis (manually, drip-feed, or use of a peristaltic pump) over a 20-22 hour period. This assures that very little algae is wasted when the water is changed. The available microalgal production will determine how long and how many scallops can be reared in this way.

Post-set scallops attach to substrates by means of byssal threads and the substrates take on a textured appearance. By taking advantage of this attachment phenomenon, scallops can be allowed to set on various materials. Typically, used gill net in "onion" bags, plastic sheets, or strips of burlap are placed in the larval containers for scallop attachment. Scallops, unlike oysters, can readily detach from these setting materials, so gentle

Flow-through systems such as raceways (Figure 18) or trays (Figure 19) also can be used (Rhodes & Widman, 1980). Scallops can be placed in these systems (more vertical attachment areas) as long as the ambient water temperature is greater than 15°C. Stocking densities are based on



Figure 18. Raceway system for culture of juvenile scallops.



Figure 19. Tray system for culture of juvenile scallops.

the microalgal food supply at each hatchery site. See Section VI.C.1. and 2. for disease diagnosis and cleaning of trays or raceways. Downwellers (where water flows from the top down through the scallops and the mesh bottom) are used successfully to grow post-set scallops up to shell heights of 10 mm (Figure 20). Upwellers can be used as well, but must be modified to prevent scallops from swimming out the drain.

Another technique for culturing juvenile scallops involves suspension culture in the

natural environment, typically employing either spat bags (Figure 21) and/or pearl nets (Figure 22). Spat bags are fine-mesh bags filled with a substrate, e.g., gill netting, on which scallops have set. Larger scallops (>4 mm) can be placed directly in pearl nets which utilize a larger mesh and are pyramid-shaped. Scallops can be placed out in natural waters successfully at temperatures as low as 5°C in the spring in the northeastern United States in these types of gear. Little growth occurs until the water temperature reaches about 15°C (Widman & Rhodes, 1991).

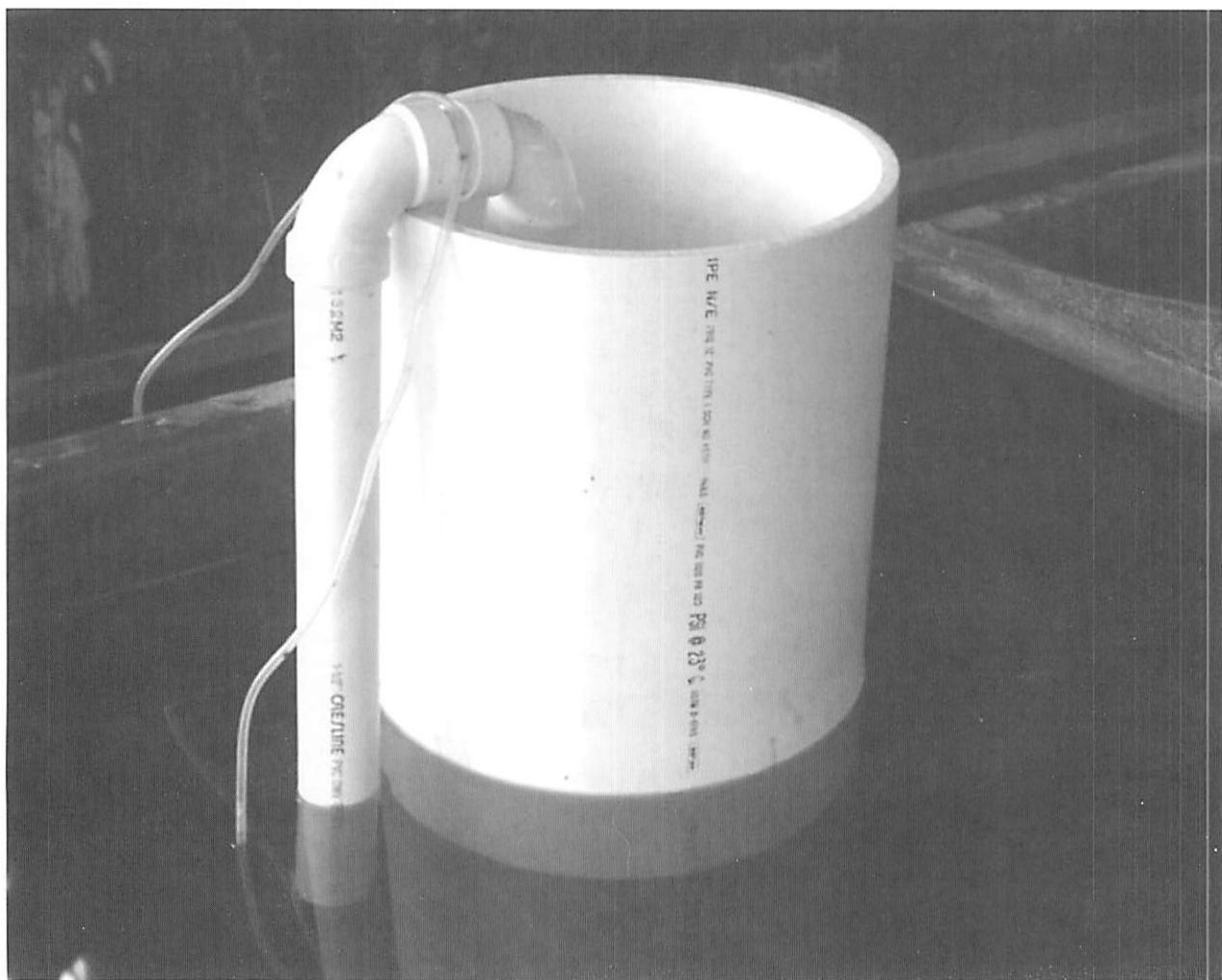


Figure 20. Downwellers for the culture of scallops up to 10 mm.

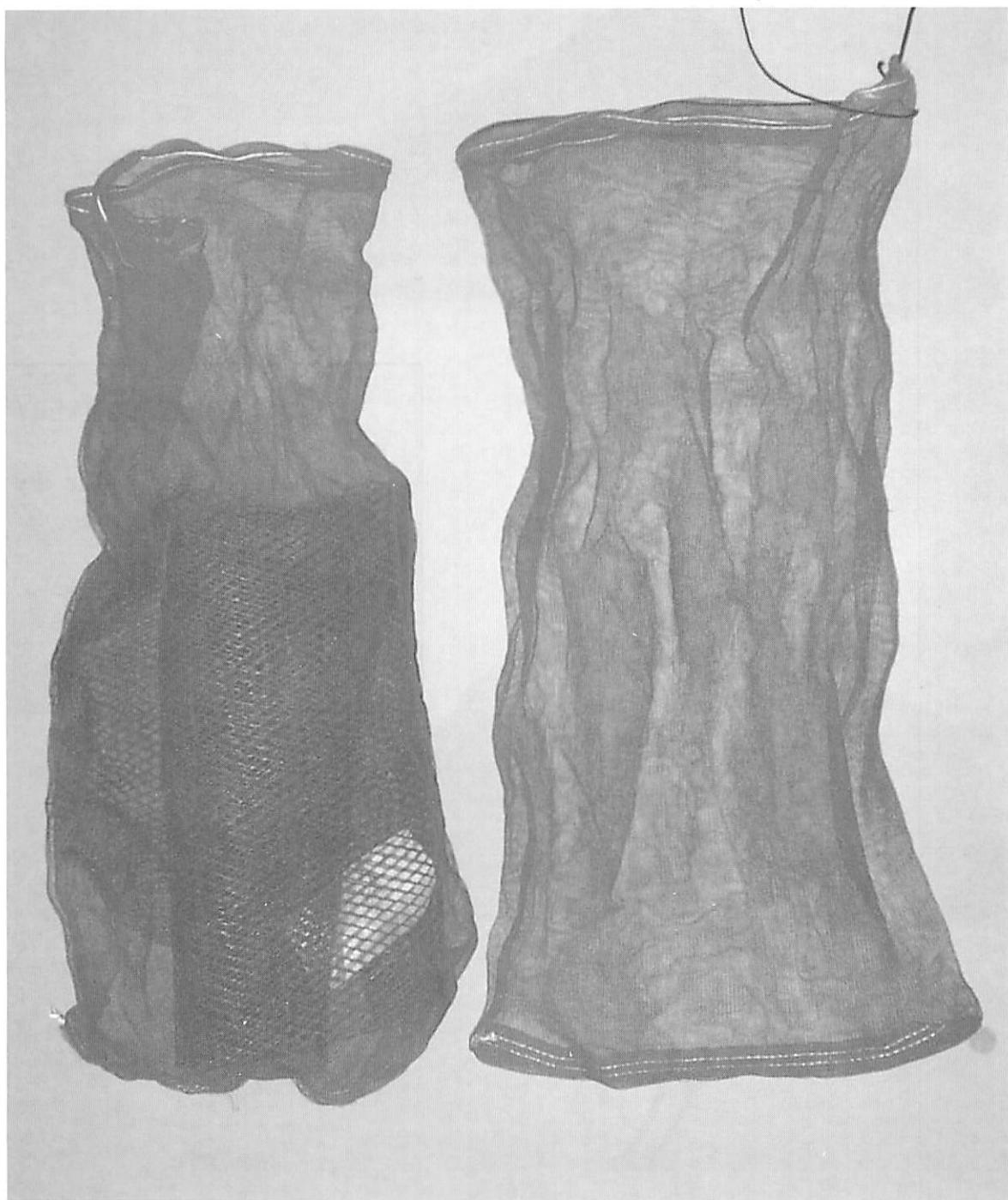


Figure 21. Spat bags for the culture of juvenile scallops in the natural environment.



## JUVENILE NURSERY

- Do not allow post-set to dry out
  - Static tanks with supplemental algal food
  - Flow-through with raceways, downwellers, or upwellers
  - Nets in the sea

Figure 22. Pearl nets for the culture of juvenile scallops in the natural environment.

## V. ALGAL FOODS

#### A. DIETS FOR PRE-SET LARVAE

To feed any animal, one must know three things: what, how much, and how often to feed. Feeding rations and regimes—how much and how often—are covered elsewhere in this manual. To answer the first question—what to feed—dietary items must be found that are not toxic, are ingested and digested, and supply the energy and materials needs of the animal being fed. As with larvae of other molluscan shellfish reared in captivity, living cultures of selected microalgae have proven to be most successful in practical application. Not all algal strains, however, satisfy the criteria listed above for a suitable diet. Further, not all algal strains satisfy an additional criterion for success in the hatchery—ease of culture. Table 3 summarizes findings of controlled feeding studies conducted at Milford to compare growth and metamorphosis of larval bay scallops on unialgal diets of some commonly-cultured microalgal strains, ranked in order of nutritional value with the best diets at the top.

It is clear from Table 3 that many easily-cultured microalgal strains are not particularly good diets for larval scallops. Reasons for poor larval growth on diets ranked as "awful" appear to be related to either size (PLY429 is too big to be ingested by first-feeding larvae), indigestibility (UTEX2341, Sticho-GSB, and 580), or biochemical composition (3H and perhaps Chaet cal). Several *Pavlova* strains appear to be the best diets, but these can be difficult to culture in large scale; nevertheless, some hatcheries have used these strains successfully. T-ISO combines attributes of a good larval diet and dependable growth in industrial settings; therefore, this strain is recommended for those inexperienced in microalgal culture. Another strain of *Isochrysis* – strain

C-ISO – appears to have similar nutritional characteristics to T-ISO and may grow better than T-ISO under some conditions. *Pavlova* strains may offer substantial benefits if the facility's conditions are conducive to their growth; at present, we do not know what these conditions are. Significance of the final column, involving relationship between cell size and packed-cell volume is described in Appendix A..

## B. DIETS FOR LARVAE DURING METAMORPHOSIS

In addition to feeding through the larval stage, suitable diets must be provided as larvae are undergoing metamorphosis. As scallops set, capture of food particles shifts from the larval feeding organ, the velum, to the developing gills. Particles in the size range suitable for larval ingestion, including the useful larval diets listed in Table 3, are too small to be retained efficiently by the gill-feeding structures of post-set larvae. Hence, it is critical to include algal feeds larger than about 6  $\mu\text{m}$  during the time that scallops are setting. We have achieved best setting success by beginning to add one or more strains of *Tetraselmis*, PLAT-P (*T. striata*, about 9  $\mu\text{m}$ ) and/or PLY429 (*T. chui*, about 12  $\mu\text{m}$ ) between days 5 and 7 of larval life, first as about a third of the ration and then shifting to 100% of the feed after setting is complete. These two *Tetraselmis* strains contain extremely high levels of essential fatty acids and dietary sterols that are also associated with rapid growth in post-set scallops and oysters. Broodstock conditioning also can be done using these *Tetraselmis* strains; however, inclusion of a diatom or a larger brown flagellate (e.g., *Pavlova* #93) may be necessary to provide fatty-acids not found in *Tetraselmis*.

**Table 3.** Microalgal strains tested for feeding larval bay scallops through to metamorphosis.

Algal Strain	Identification	Nutritional Value	Ease of Culture	Size (μm)	$10^6$ cells/mL @ pcv=0.010*
CCMP459	<i>Pavlova</i> sp.	Excellent	Very difficult	6x5	13.5
CCMP609	<i>Pavlova</i> sp.	Excellent	Difficult	6x5	13.5
MONO	<i>Pavlova lutheri</i>	Very good	Difficult	5x4	15
DICRAT	<i>Dicrateria inornata</i>	Very good	Difficult	5x4	15
T-ISO	<i>Isochrysis</i> sp.	Very good	Easy	6x5	13.5
GBF	<i>unident. prymnesiophyte</i>	Good	Easy	3x2	17
Chaet cal	<i>Chaetoceros calcitrans</i>	Poor	Easy	7x6	9.5
PLY429	<i>Tetraselmis chui</i>	Awful	Easy	12x10	2.5
3H	<i>Thalassiosira pseudonana</i>	Awful	Easy	3-5	12
UTEX2341	<i>Nannochloropsis</i> sp.	Awful	Easy	2-3	20
Sticho-GSB	<i>Nannochloropsis salina</i>	Awful	Easy	3-4	17
580	<i>Chlorella autotrophica</i>	Awful	Easy	3-4	18

\*pcv (packed-cell volume units are mL of packed cells per 10 mL of culture.

How to use the last column of this Table to calculate daily rations for larvae from cell counts:

1. Divide the volume of your larval culture (in liters) by 400 to determine the liters of algal food at 0.010 PCV needed (ex.  $400 \text{ L} / 400 = 1\text{L}$ ).
  2. Find the cells per mL @ 0.010 on this Table (ex.  $13.5 \times 10^6$  for T-ISO)
  3. Multiply the result of step 1 by the result of step 2 and divide by the cell count in your culture (ex.  $1,000 \text{ mL} \times 13.5 / 6 = 2,250 \text{ mL}$ )

**Note:** This calculation assumes linearity between pcv and cell count, a reasonable assumption between  $10^5$  and  $10^7$  cells per mL.

### C. PRECAUTIONS IN PRODUCING MICROALGAE

Detailed instructions on how to culture microalgae are beyond the scope of this manual; the first volume of *The Handbook of Phycological Methods* (Stein, 1973) is a good place to obtain such basic information. Nevertheless, a few comments are in order about precautions that should be taken to ensure that microalgal cultures provide a wholesome and nutritional diet for young scallops. Although it is impractical to produce bacteria-free feed cultures in a farm setting, care must be taken to minimize the potential for infection of microalgal cultures with pathogenic microorganisms. Saline water from a well may be the best option for algal-culture water; generally, microbial numbers are very low, salinity is stable, and further treatment prior to inoculation with seed algal cultures usually is unnecessary. If coastal surface water is used, some combination of filtration, pasteurization, or other treatment methods may be necessary. Nutrients that must be added to seawater to support algal growth should be sufficiently refined to avoid introducing chemical contaminants that may harm both algae and shellfish. Reagent chemicals can be combined according to recipes listed in algal-culture manuals (e.g., Stein, 1973); however, pre-mixed nutrient products (usually described as 'f/2' mixes) available from aquaculture supply companies are suitable, convenient, and widely-used.

for re-starting production cultures after the initial starter is obtained from an outside source. Beyond the expense or potential delays of repeatedly sending for new starter cultures from an outside source, there are advantages to maintaining one's own seed cultures. First and foremost, inocula for production cultures are available as needed. In addition, algal strains may become adapted to local salinity and/or other seawater chemistry and out-perform new starters. Guidelines for maintaining small cultures of microalgae under aseptic conditions can be found in *The Handbook of Phycological Methods* mentioned previously or adapted from bacteriological procedures. The hatchery operator should be prepared to accomplish all practical aspects of microalgal culture before attempting to rear animals.

## ALGAL FOODS

- Best larval diets are nutritious and easy to culture — T-ISO and other flagellates
  - Provide setting larvae and post-set with larger cells — PLAT-P or PLY429
  - Broodstock conditioned on mix of *Tetraselmis* and diatoms or “brown” flagellate
  - Microalgae culture precautions
  - **Do not** attempt to grow larvae until you are able to feed them!

#### D. SEED CULTURES

Starter, or "seed" cultures of microalgae are available from the Milford Laboratory, as well as from commercial aquaculture supply companies and a few research institutions that maintain collections of microalgal strains. Hatcheries need to maintain their own seed cultures, usually in test tubes or small flasks that are "transplanted" periodically using aseptic microbiological techniques,

## VI. DISEASE PROBLEMS

**B**ay scallops are relatively resistant to infectious diseases. Infections, however, may be introduced into aquaculture systems by several routes; when they become established, they can spread rapidly and may kill most of the scallop larvae or juveniles within the system. Likely routes for pathogen entry into the hatchery or nursery are through:

- (1) contaminated equipment or materials,
- (2) the seawater source,
- (3) the broodstock,
- (4) algal-food stocks, and
- (5) addition of scallop stocks from new sources.

Even when diseased scallops are removed, the pathogen may be maintained in the system when it grows within a biofilm on pipes, grading screens, or tank walls.

These biofilms have been found to consist of layers of microorganisms bound together with a polysaccharide “glue” that is resistant to cleaning and to penetration of chemical agents. Although they are protected within this film, pathogenic microorganisms can be shed back into the system. Therefore, maintenance of hygienic conditions is very important in controlling pathogenic agents in the hatchery.

Although the literature about pathogens of bay scallops is sparse, bacteria (primarily *Vibrio* species), rickettsiae, *Chlamydia*, and fungi have been described. Regardless of the type of infecting agent, the recognition of a disease condition and the procedures for limiting disease problems in the hatchery are similar. Information in the following sections is offered as “best practice”, with the

recognition that individual hatchery operators will make decisions about available resources, and relative need for control of disease in the hatchery.

### DISEASE PROBLEMS

- Potential pathogens are everywhere
- Hygiene is critical

#### A. PROCEDURES TO CONTROL INTRODUCTION OF PATHOGENS

**1. Equipment and Materials.** Routine washing and drying of all equipment and materials may be the best single means to control disease. It is, however, also wise to use separate handling equipment for each group of scallops so that a non-apparent disease (in its early stages) is not transferred between groups. Equipment and tanks should be washed with a biodegradable detergent (we routinely scrub with 5 mL of Liquinox® plus 5 mL of household bleach in 10L of tap water); multiple rinses (preferably with hot tap water and once with filtered seawater) are needed to remove all traces of detergent. Items should be allowed to dry completely—this includes screens and hoses which may allow growth of microbes if left damp. Water delivery and holding containers should be designed to avoid pockets where debris and moisture can collect. Also, pipes and valves should be large enough so that a brush can be used in cleaning if disease problems occur. Damp filters used for filtering the incoming seawater provide excellent substrates for microbial growth;

therefore, filters should be washed and dried. (Old filters should be bagged carefully prior to disposal, to contain any disease organisms.)

Even the most heat-resistant bacteria will be killed when exposed to water at 71°C (160°F) for 60 seconds. On the other hand, bacterial spores may survive boiling water for several hours; however, spore-forming bacteria are not known to be pathogenic for molluscs. Screens, sponges, brushes, and other equipment can be sterilized effectively by dipping them in hot water for one to two minutes, provided that they are clean. Presence of organic material may protect microbes from the penetration of heat; therefore, items either should be cleaned first or the heat treatment should be longer (i.e., 5-10 minutes).

**2. Seawater.** Filtration will remove most microorganisms from the seawater used during the rearing of scallop larvae to the 10- to 12-day stage. Water filtration becomes impractical for larger, post-set animals which require greater amounts of natural foods in flowing systems. A useful first step is to pass raw seawater through a 10-µm bag-filter (Figure 23) to remove large particles and most fouling organisms from the water as it enters a holding tank. Water pumped from the holding tank through two 10-µm nylon filters provides further clean-up of the water. Usually it is not necessary to sterilize the water with ultraviolet (UV) light (Figure 24) after the filtration step; in fact, some experience suggests that complete removal of competing microorganisms will allow unrestricted growth of microbial pathogens in the system. However, if incoming seawater is determined to be the source of high levels of disease organisms, the pre-filtered water should be UV-treated by passing it through a commercial UV unit before it is added to larval scallops. Filtration must occur before UV treatment because particulate matter in raw seawater can block the penetra-

tion of light, and silt can damage light-conducting surfaces of most UV units.

**3. Broodstock.** Bay scallop broodstock should be healthy. If many scallops in a broodstock tank recently have died, it is better to seek a new source of broodstock or use animals from a separate tank where no mortalities are evident. Although the surface of other molluscs such as clams or oysters (which close tightly when confronted with stressful agents) may be chemically disinfected, this is not possible with bay scallops. Therefore, removal of surface films and organisms must be done by scraping any hard deposits from the shell with a blade followed by scrubbing it with a clean brush. The scallop should be rinsed well with clean, filtered seawater before placing it in a holding-tank of clean seawater. Although not routinely practiced, it may be possible to reduce transfer of pathogens to gametes even further by allowing the cleaned broodstock to purge for 24 hours in 17-20°C filtered seawater, with several water changes, before the scallops are used in spawning.

**4. Algal food.** Food used in rearing larval scallops may be delivered in several forms. The most controllable feeding is done with a suspension of selected, cultured microalgae (a single species grown in seawater filtered or otherwise treated to eliminate microorganisms) that is known to have good nutritional qualities. In some high-volume aquaculture operations, it may be necessary to feed larvae a mixture of natural algae in bag-filtered (25 µm) seawater supplemented with cultured algae. This mixture will include many natural bacteria, which may be benign or even helpful in maintaining the health of scallops. However, *Vibrio* spp. often grow in association with natural marine algae; some of these *Vibrio* spp. are pathogenic to bay scallop larvae. Surprisingly, even carefully-controlled algal cultures may become contaminated with bacteria despite careful efforts of

the culturist; this often includes *Vibrio* spp. which seem to grow better in association with algae.

If the resources are available, food and tank-water containing larvae can be checked for *Vibrio* spp. by counting bacterial colonies which grow on TCBS agar (Difco Laboratories) (Figure 25). The step-wise procedure for counting *Vibrio* is given in Appendix B.

**5. Scallop stocks from new sources.** If you plan to bring in scallop stocks from new sources (larvae, juveniles or adults), the first consideration should be whether the source (natural or hatchery) is known to be disease-free. There should be an initial period of quarantine (i.e., complete separation from existing stocks for a period long enough to determine whether scallops are growing normally and show no evidence of disease). Imported

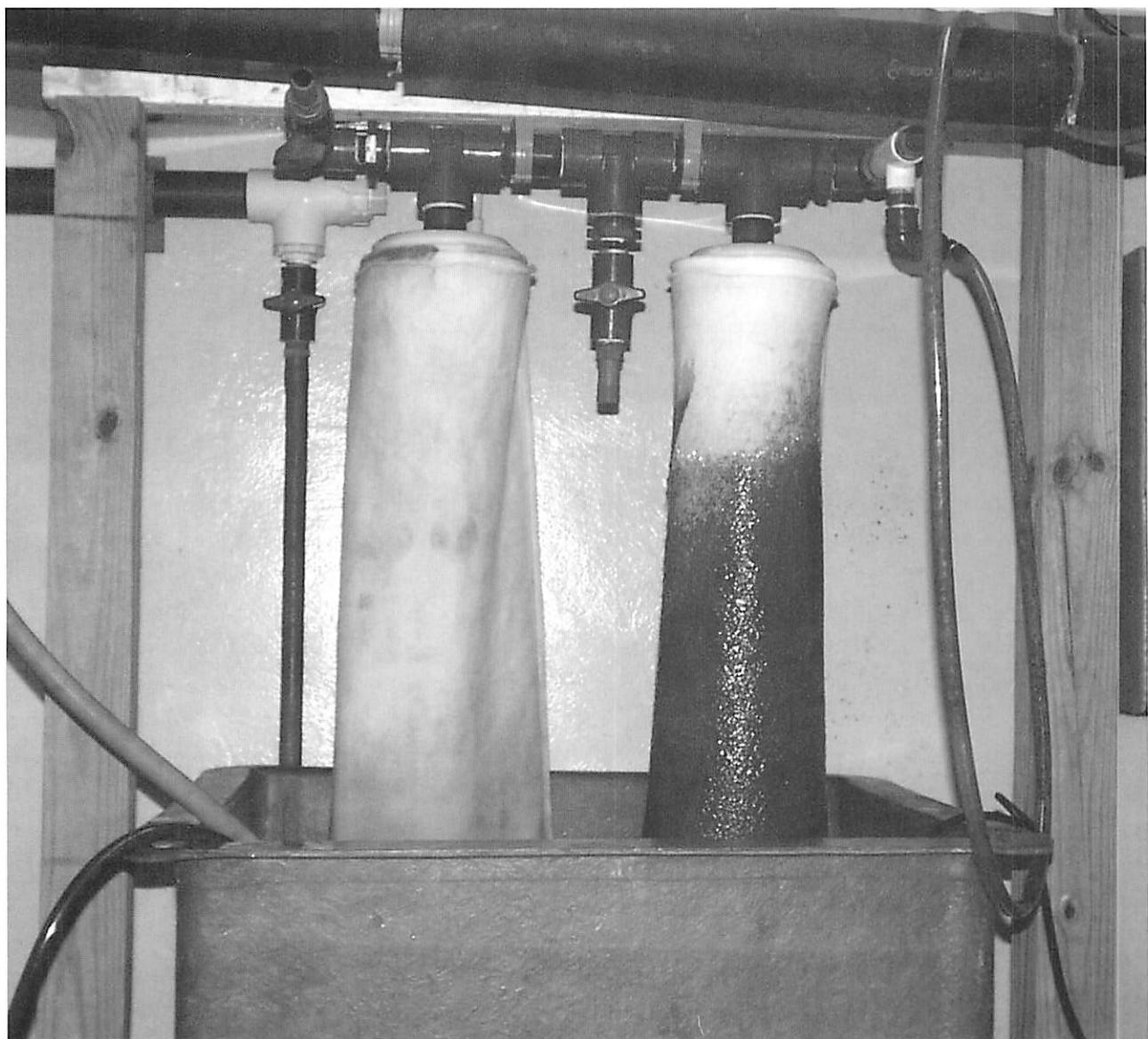


Figure 23. Bag filter systems for filtration of seawater.

larvae can be tested for disease by grinding 500–1,000 larvae in a tissue grinder or between two pieces of clean glass and then placing the ground mash in an isolated culture vessel containing about 1,000 one-day-old, healthy, native larvae. Compare larvae in the test vessel with healthy native larvae in a second vessel containing no ground mash; look for disease symptoms as in Section VI.5.B.1., below, or evidence that the shells do not develop in the normal D-shape. If no evidence of disease is seen in 48 hours, then serious pathogens probably are not present. Whole larvae or ground mash also can be placed on TCBS agar to test for presence of *Vibrio* spp. as described in Appendix B. Juvenile and adult animals should be observed in isolation for several weeks before being introduced into water systems in common with those of native stock.

## B. LARVAL DISEASE

**1. Diagnosis.** Once per day inspect larvae with a microscope (60 X to 100 X total magnification). Healthy scallop larvae actively swim, have good (not weak or transparent) color, and clean vela. Healthy larvae should grow about 10  $\mu\text{m}$  per day when held above 20° C. Sick larvae exhibit slow growth, poor color, and little or no swimming activity (finally sinking to the bottom); also they may have debris attached to the velum, detached velar cells, and protozoans swimming around them. Bacterial infection may show up as swarms of bacteria and protozoans around a gaping shell.

If you have the resources, the water in tanks containing scallop larvae can be monitored daily for evidence of increased *Vibrio* counts as described in Appendix B. Counts increasing to 10,000 per mL (possibly as low as 1,000 per mL) indicate a problem with vibriosis. If the food source is free of *Vibrio* spp., then increased counts in water indicate either a larval infection or insufficient removal of the biofilm during the cleaning process.



Figure 24. Ultraviolet (UV) light systems for sterilizing seawater.

**2. Treatment.** Very mild infections may not be evident except that some larvae may exhibit poor growth (disease is only one of several factors that can cause slow growth). Therefore, it is good practice to size the larvae daily (or at least every other day) through several screens. Discard larvae on the smallest screen after first soaking the larvae and screen in hot >71°C (160°F) water for 10 minutes to kill any pathogens. To maintain clean conditions in hatchery conical tanks, scrub them daily with mild detergent and bleach and rinse them thoroughly as in Section VI. A.1. If possible, place the larvae in another, clean tank of seawater and allow the first tank to dry for at least 24 hours.



working with other molluscan species rely instead on rigorous scrubbing of system surfaces with abrasive pads, thorough rinsing with freshwater, and then allowing the system to stand dry for several days.

The use of antibiotics to treat or prevent disease is not recommended for several reasons. First, it is illegal (FDA has not approved any antibiotics for use in seafood), and, second, routine use of antibiotics promotes the emergence of antibiotic-resistant strains of bacteria. Health studies surrounding the use of antibiotics in cattle feeds show that antibiotic-resistant strains which emerge in cattle can transfer their resistance to bacterial strains which are pathogenic in humans (for example, *Salmonella*, *Campylobacter*, *E. coli* O157, and some strains of *Enterococcus*).

high percentage of gaping scallops or empty shells, a severe disease problem may exist.

**2. Treatment.** Once scallops reach a size where they are placed in trays receiving flowing seawater as a food source, measures that can be taken to prevent disease become limited. Complete removal of biofilms from trays would require detachment and removal of scallops; this seems to retard scallop growth. Bi-weekly cleaning of the trays by washing away fecal material and other detritus with a mild stream of water is the preferred route. If fouling is a problem, the incoming seawater can be passed through a 50- $\mu\text{m}$  bag-filter which will remove most of the fouling organisms, but allow algal food to pass through. If a severe disease problem occurs, the remaining scallops should be disinfected and discarded and the trays cleaned and disinfected with bleach as described in Section VI.5.B.2.

### C. GROW-OUT DISEASES

### **1. Diagnosis.**

Post-set scallops may be affected by the same disease organisms as larvae; however, recognition of a problem is more difficult. A sample consisting of 15 - 30 scallops should be examined under a microscope several times each week to determine general health, percent survival, and growth. Pre-cleaned medicine droppers and other sampling equipment should be kept separate for each grow-out table and sterilized by heat or disinfectant after use. Notes should be maintained on each group of scallops on each grow-out table. Slow growth or the presence of red stain around the scallop are indicators of stress or death - possibly caused by disease (a red bacterium, a pseudomonad, may grow on the increased ammonia being released by a dying scallop, but the bacterium is not in itself pathogenic). If inspection reveals a

## **PATHOGEN CONTROL**

- Routine washing and drying
  - Separate equipment for each population
  - Hot-water dip effective bactericide
  - Filter and/or UV-treat seawater
  - Spawn only healthy broodstock
  - Check for *Vibrio* in algal food with TCBS agar if materials and expertise are available
  - Quarantine any new scallops until health can be evaluated
  - Discard diseased populations and disinfect all materials in contact

## **VII. ACKNOWLEDGMENTS**

The authors thank the Connecticut Sea Grant College Program for providing funds to publish this manual. We are especially grateful to Ms. Nancy Balcom, Ms. Peg Van Patten, and Ms. Tessa Simlick of the Connecticut Sea Grant College Program for their support and assistance. Nancy encouraged us to prepare the manual and, along with Tessa, provided critical review and editing. Peg provided the design and preparation of the manual prior to publication.

We also thank Dr. Christopher Martin, Michael Oesterling, Allan Jacques, Loy Wilkinson, and Philip Curcio for their critical reviews of the manual.

## VIII. REFERENCES

- Alix, J.H., M.S. Dixon, B.C. Smith, and G.H. Wikfors. 1996. Scallop larval feeding experiments: Some surprises and unanswered questions. *J. Shellfish Res.*, 15:451 (abstract)

Belding, D. L. 1910. A report upon the scallop fishery of Massachusetts, including the habits, life history of *Pecten irradians*, its rate of growth and other factors of economic value. Spec. Rep., Comm. Fish and Game, Mass. 150 pp.

Brand, A.R. 1991. Scallop ecology: Distribution and behavior. In: Shumway, S.E. (Ed.), *Scallops, Biology, Ecology, and Aquaculture*, Elsevier, New York. Pp. 517-584.

Bricelj, V.M., J. Epp, and R.E. Malouf. 1987. Comparative physiology of young and old cohorts of bay scallop *Argopecten irradians irradians* (Lamarck); mortality, growth, and oxygen consumption. *J. Exp. Mar. Biol. Ecol.*, 112: 73-91.

Burrell, V.B., Jr. 1985. Oyster culture. In Huner, J.V. and E.E. Brown (eds.), *Crustacean and Mollusk Aquaculture in the United States*, AVI Publishing Company, Inc., Westport, CT. Pp. 235-274.

Gates, J.M., G.C. Matthiessen, and C.A. Griscom. 1974. Aquaculture in New England. Univ. Rhode Island Tech. Rpt. Ser. No.18, Kingston, RI. 77 pp.

Loosanoff, V.L. and H.C. Davis. 1963. Rearing of bivalve mollusks. In: Russell, F.S. (Ed.), *Advances in Marine Biology I*, Academic Press, London. Pp. 1-136.

Menzel, W. 1989. The biology, fishery and culture of quahog clams, *Mercenaria*. In: Manzi, J.J. and M. Castagna (eds.), *Clam Mariculture in North America*, Elsevier, New York. Pp. 201-242.

Mercaldo, R.S. and E.W. Rhodes. 1982. Influence of reduced salinity on the Atlantic bay scallop *Argopecten irradians* (Lamarck) at various temperatures. *J. Shellfish Res.* 2: 177-181.

Oesterling, M.J. & L.A. Rose. 1996. Bay scallop culture in a Virginia saltwater pond. *J. Shellfish Res.*, 15: 458-459 (abstract).

Orensanz, J.M., A.M. Parma, and O. Iribarne. 1991. Population dynamics and management of natural stocks. In: Shumway, S.E. (Ed.), *Scallops, Biology, Ecology and Aquaculture*, Elsevier, New York, Pp. 625-713.

Rhodes, E.W. and J.C. Widman. 1980. Some aspects of the controlled production of the bay scallop (*Argopecten irradians*). *Proc. World Maricult. Soc.*, 11: 235-246.

- Sabo, B.D. and E.W. Rhodes. 1987. Indexed bibliography of the bay scallop (*Argopecten irradians*). NOAA Tech. Memo. NMFS-F/NEC 48, 85 pp.

Sastray, A. N. 1965. The development and external morphology of pelagic larval and post-larval stages of the bay scallop, *Aequipecten irradians concentricus* Say, reared in the laboratory. *Bull. Mar. Sci.* 15(2): 417-435.

Stein, J.R. (Ed.). 1973. *Handbook of Phycological Methods*, Culture Methods and Growth Measurements. Stein, J.R. (Ed.) Vol. 1, Cambridge Univ. Press, New York, NY, 448 pp.

Stiles, S. and J. Choromanski. 1995. Inbreeding studies on the bay scallop, *Argopecten irradians*. Aquaculture '95 Meeting. San Diego, California. *J. Shellfish Res.* 14(1): 278 (Abstract).

Stiles, S., J. Choromanski and C. Cooper. 1998a. Selection studies on growth and survival of bay scallops (*Argopecten irradians*) from Long Island Sound. *J. Shellfish Res.* 17(1): 363 (Abstract).

Stiles, S., J. Choromanski, C. Cooper and Q-Z Xue. 1998b. Genetics and breeding investigations of bay scallops (*Argopecten irradians*). 18th International Congress of Genetics Proceedings. Beijing, China. P.173 (Abstract).

Tammi, K.A., E. Buhle, and W.H. Turner. 1997. Making the perfect spat bag for collection of the bay scallop, *Argopecten irradians*. *J. Shellfish Res.*, 16: 295 (abstract).

Tettlebach, S.T. and E.W. Rhodes, 1981. Combined effects of temperature and salinity on embryos and larvae of the northern bay scallop *Argopecten irradians irradians*. *Mar. Biol.* 63(3):249-256.

Tettlebach, S., P.J. Auster, E.W. Rhodes and J.C. Widman. 1985. A mass mortality of northern bay scallops, *Argopecten irradians irradians*, following a severe spring rainstorm. *The Veliger*. 27(4): 381-385.

Tettlebach, S.T., C.F. Smith, R. Smolowitz, K. Tetrault, and S. Dumais. 1999. Evidence for fall spawning of northern bay scallops, *Argopecten irradians irradians*, (Lamark 1819) in New York. *J. Shellfish Res.* 18(1): 47-58.

Ukeles, R. 1973. Continuous culture - a method for the production of unicellular algal foods, pp. 233-254. In: Stein, J.R. (Ed.), *Handbook of Phycological Methods*. Cambridge Univ. Press, New York, NY.

Webber, H.H. and P.F. Riordan. 1976. Criteria for candidate species for aquaculture. *Aquaculture*, 7: 107-123.

Widman, J.C. and E.W. Rhodes. 1991. Nursery culture of the bay scallop, *Argopecten irradians irradians*, in suspended mesh nets. *Aquaculture*, 99: 257-267.

## IX. USEFUL CITATIONS

- Bourne, C.A., A. Hodgson and J.N.C. Whyte. 1989. *A manual for scallop culture in British Columbia*. Can. Tech. Rep. Fish. Aq. Sci. No. 1694, 215 pp.
- Castagna, M. 1975. Culture of the bay scallop, *Argopecten irradians*, in Virginia. *Mar. Fish. Rev.* 37(1):19-24.
- Castagna, M. and W. Duggan. 1971. Rearing the bay scallop, *Argopecten irradians*. *Proc. Nat. Shellfish. Assoc.* 61:80-85.
- Clemon, W.F., R.J. Neves and G.B. Pardue. 1983. Species profiles: life history and environmental requirements of coastal fishes and invertebrates (mid-Atlantic). *Bay Scallop*. Fish & Wild. Ser., U.S. Dept. Int. FWS/OBS-82/11.12.TR EL-82-4.
- Duggan, W.P. 1973. Growth and survival of the bay scallop, *Argopecten irradians*, at various locations in the water column and at various densities. *Proc. Nat. Shellfish. Assoc.* 63:68-71.
- Gutsell, J.S. 1930. Natural history of the bay scallop. *Bull. U.S. Bur. Fish.* 46:569-632
- Kirby-Smith, W.W. 1972. Growth of the bay scallop: the influence of experimental water currents. *J. Exp. Mar. Biol. Ecol.* 8:7-18
- Kirby-Smith, W.W. and R.T. Barber. 1974. Suspension-feeding aquaculture systems: effects of phytoplankton concentration and temperature on growth of the bay scallop. *Aquaculture* 3:135-145.
- Osterling, M.J. 1998. Bay scallop culture. *Virginia Sea Grant Marine Resource Advisory No. 67*, VASG-97-10, 6 pp. Virginia Sea Grant Marine Advisory Program.
- Risser, J. 1901. Habits and life-history of the scallop (*Pecten irradians*). *31st Ann. Rep. Comm. Inland Fish.*, State of Rhode Island and Providence Plantations. p. 47-55.

## X. SUPPLIERS GUIDE

*Aquaculture Magazine.* 2000. Buyer's Guide and Industry Directory 2000. 29th Annual Edition. Aquaculture Magazine, P. O. Box 2329, Asheville, NC 28802.

This guide provides the names of hundreds of vendors providing supplies, equipment, and services to the aquaculture industry.

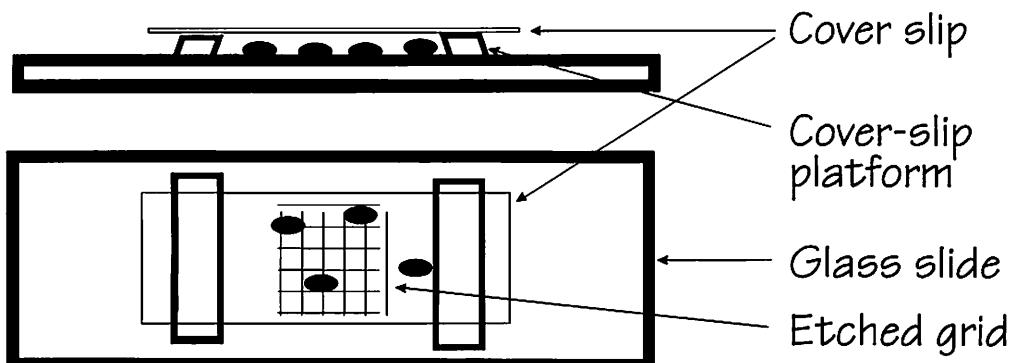
## APPENDIX A

# QUANTIFYING MICROALGAL FEEDS

Unlike cells of metazoan plants and animals, microalgal cells separate from each other after dividing; therefore, as algal cultures “grow,” you get more rather than bigger algae. For this reason, microalgal feeding rates for scallops must take into account the volume of algal culture fed to a population of scallops, how many cells are in that volume, and also how big cells of the algal species are. Three practical methods have been developed for quantifying how much algal food is in a given volume of culture: 1) microscope cell counts using a hemocytometer; 2) determination of packed-cell volume in a centrifuged, hematocrit-type tube; and 3) percent transmittance of light through a culture measured with a colorimeter/spectrophotometer. All three of these methods require collection of a sub-sample from the culture and determination of the number of cells in that sub-sample; therefore, it is critical that the culture be mixed thoroughly before sampling so that the subsample measured will be representative of the whole culture. Information provided in this manual facilitates use of cell counts or packed-cell volume to adjust feeding rates on a daily basis.

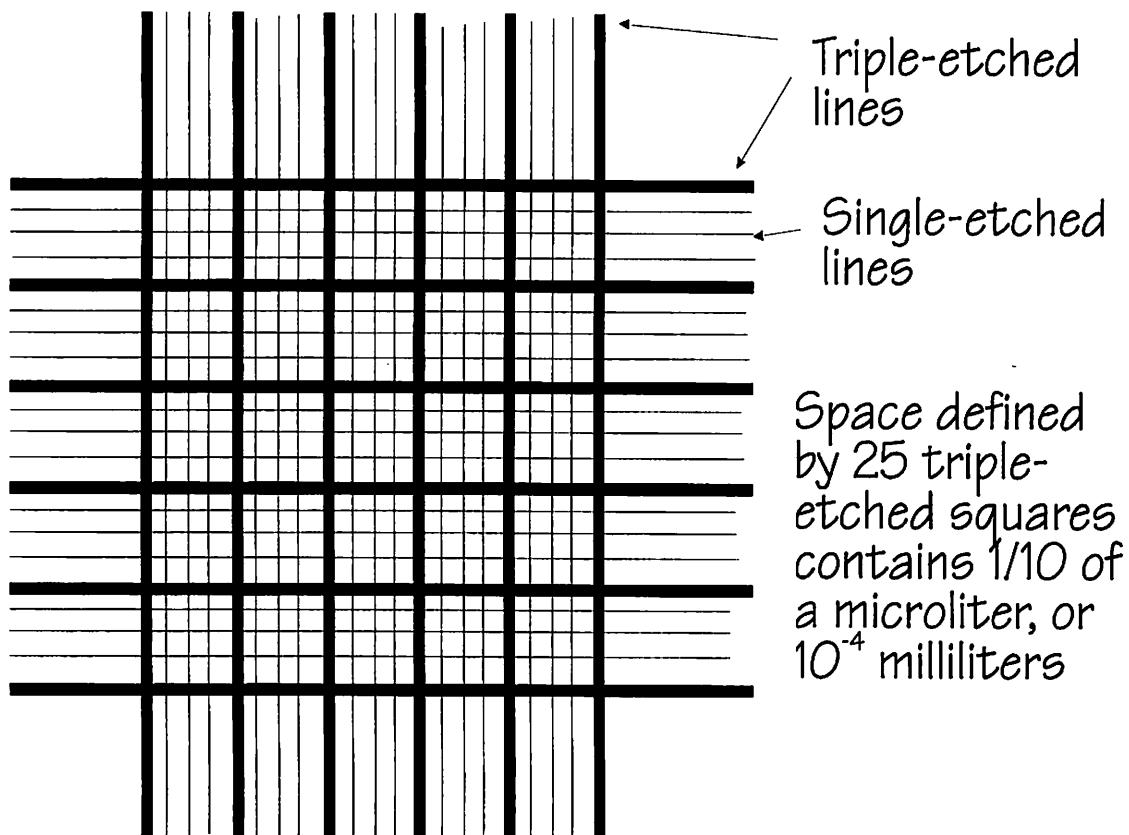
**1) Cell Counting:** a hemocytometer is a microscope slide designed for clinical counting of human blood cells. The principle of the hemocytometer is to define a three-dimensional space (volume) in which individual cells can be counted with a microscope; this volume is defined by a cover slip resting upon a platform above a polished glass surface with an etched grid. The etched grid defines the portion of the slide that is counted, and the cover slip limits the “depth” of water above the grid. A subsample is removed from the culture to be counted, killed with a small volume of formalin, Lugol’s iodine solution, or iodine crystals (which we use so that dilution by fixing solution need not be calculated), and loaded by pipette into the hemocytometer. A compound light microscope is used to count cells at 100-400X total magnification.

## Diagram of Hemocytometer



In the Improved Neubauer hemocytometer design that we use, a grid of 25 squares delineated by triple-etched lines contains 10 mL; each of these 25 squares is further subdivided by single lines into 16 squares to facilitate counting at higher magnifications. If all cells within the 25 squares are counted, then the

## Hemocytometer Grid

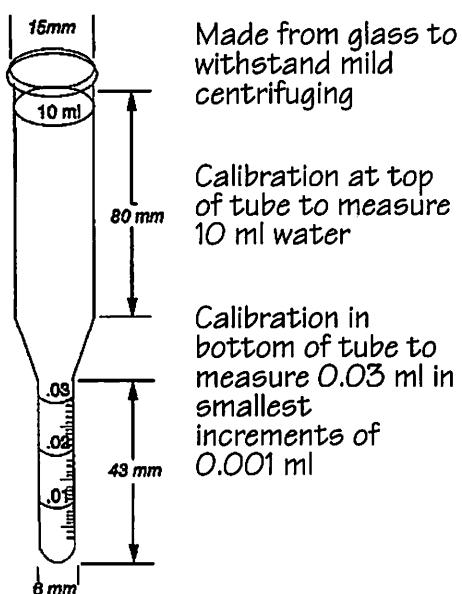


count is multiplied by 10,000 ( $10^4$ ) to obtain an estimate of the number of cells in one milliliter. In dense cultures, hundreds or thousands of cells may be present in  $10^{-4}$  mL; in these cases, five of the 25 squares are counted and the count is multiplied by  $5 \times 10^4$  to obtain the number of cells per milliliter. Counts between 30 and 300 recorded with a hand-held counter are considered valid. Advantages of this method include a high degree of precision and accuracy, forced microscopic evaluation of the culture for contaminants, and the ability to compare the culture with much of the published literature which generally reports algal densities in terms of cell number. The main disadvantages of counting cells are that the method is labor intensive (ca. 10 minutes per count), it requires a compound microscope, and hemocytometers are fragile and expensive to replace. Regardless of the method selected for daily quantification of algal feeds, it would be to the advantage of a hatchery operator to be able to do cell counts.

2) Packed-cell volume (pcv): when scallops feed on a relatively dilute suspension of microalgal cells in seawater, they essentially concentrate the cells ingested from the volume of water "filtered" to the volume of their digestive systems. Bivalve larvae will feed actively until their guts are full, spend some time digesting the "meal," expel undigested parts of the microalgae as feces, and then begin feeding again. Thus, the parameter that really defines how much a larva will eat is the volume of cells that it can pack

into its digestive system. Measuring packed-cell volume (pcv), which we define as mL of packed algal cells per 10 mL of algal culture, is the method that is most consistent with this view of its food. We employ a centrifuge tube developed to measure the volume of blood cells within a blood sample. This tube consists of a thin, capillary section with etched graduations attached to the bottom of a larger tube that holds 10 mL total volume.

Modified Hopkins tube, after Ukeles, 1973, for determining packed-cell volumes of phytoplankton cultures



10-mL of algal suspension is measured into the tube, and the tube is centrifuged (600XG for 5 min.) so that all algal cells are "packed" into the capillary portion of the tube. The volume of cells, distinct in appearance from the now-clear medium, is read from the scale on the capillary portion of the tube. Packed algal cells are washed from the capillary using a wide-bore needle and syringe, and the tube is re-used indefinitely. Advantages of this method include the aforementioned relevance to the scallops, low labor as six or more samples can be centrifuged and read in less than 10 minutes, and an "automatic" adjustment for cell-size differences between algal strains. Disadvantages include the high cost of pcv tubes (which are no longer in medical use and must be custom-made), need for a centrifuge (although a small, inexpensive, table-top unit will do), and the inability of the method to differentiate between the alga being cultured and any particulate contaminants that may be present in the cul-

ture. We use packed cell volume measurements to adjust daily feeding rates for both larval and post-set scallops at Milford, chiefly because of the low labor required and the ability to equalize rations of algal strains of different size.

3) Spectrophotometer/colorimeter: it is easy to see that a culture containing more cells is "darker" than a less dense culture; light coming through a culture is absorbed and scattered by the algal cells in suspension. A spectrophotometer or colorimeter is an analytical instrument that measures the amount of light (usually a defined wavelength range) that passes through a cuvette (a specialized test tube). The simplicity of this principle is seductive, but practicality of application to production feed cultures tempers attractiveness of the method. Calibration of the instrument, calculation of useful values from numbers read on the instrument, and very high potential for interference from contaminants are drawbacks that make this method of quantifying algal-feed production cultures on a daily basis the least suitable. Those wishing to pursue this method further should consult Volume 1 of the *Handbook of Phycological Methods* (Stein, 1973) for detailed procedures.

## APPENDIX B

### STEP-WISE PROCEDURE FOR TESTING WATER OR FOOD FOR PRESENCE OF *VIBRIO* (SEE ALSO THE BOXED DIAGRAM)

(The procedure is based on the use of a selective medium which suppresses growth of most other seawater bacteria.)

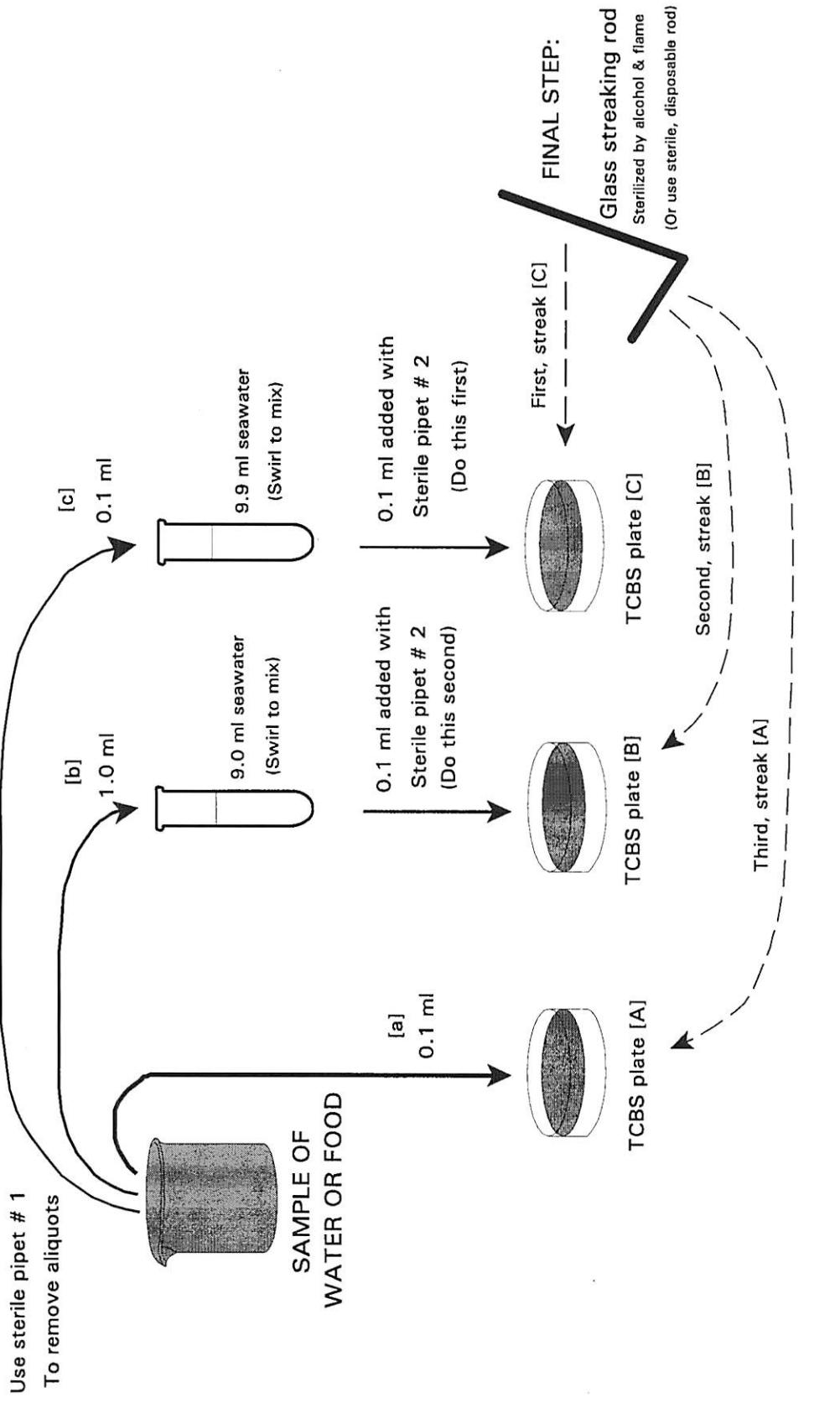
1. Prepare TCBS agar (Difco Laboratories) by dissolving the powder in distilled water that is heated to boiling according to the manufacturer's instructions; dispense into sterile Petri plates (about 10 mL per plate).
2. Allow the surface of the plates to dry overnight in a protected area, then stack the plates upside down in sealed, plastic bags and store in a refrigerator (this prevents condensation of water droplets on the lid of the plate).
3. With a sterile, 1.0-mL pipet, remove three samples (a), (b), and (c) of food or water according to the following: (a) add a 0.1-mL sample to the surface of a TCBS plate [A], (b) also add 1.0 mL to a sterile tube #1 containing 9.0-mL sterile seawater and mix the contents, and (c) add a 0.1-mL sample to a sterile tube #2 containing 9.9 mL of sterile seawater and mix the contents.
4. With a second sterile pipet, remove 0.1 mL from the tube #2 and place on the surface of a TCBS plate [C]; then with the same pipet remove 0.1 mL from tube #1 and place on the surface of a TCBS plate [B] (this is done with tube #2 first and then tube #1 because tube #2 has 10-fold fewer bacteria than tube #1); wetting the pipet first with the dilution in tube #2 will not bias the results when you then wet the same pipet with the higher number from tube #1.
5. Sterilize a bent, glass streaking-rod by dipping it in 70 - 95% ethanol and then burning it off by flaming it.
6. Cool the rod by touching it to the surface of plate [C] in an area free of the sample and then streak the sample smoothly across the surface of the plate while turning the plate. With the same rod, streak the sample on plate [B] and then on plate [A]. Note: If steps 4 and 5 (above) are not completed swiftly, the drop of sample on plate [A] may be absorbed into the medium and the bacteria will not be spread around the plate; if this happens, it is better to spread the drop on this plate with a sterile streaking rod immediately after placing it on the plate (i.e., during step 3).

**STEP-WISE PROCEDURE FOR TESTING WATER OR FOOD FOR PRESENCE OF *VIBRIO***  
*continued from previous page*

7. Cover the plates with their lids, and place them upside down in a plastic bag. After a 24-hour incubation at 68 - 78°F (20 - 26 °C), count the bacterial colonies on any plate having fewer than 300 colonies (statistically accurate counts are found only on plates containing 20 to 300 colonies). Count the clearly visible yellow or green colonies (ignore any tiny, nearly invisible colonies because these are non-*Vibrio* bacteria that are able to grow slowly on the medium despite its growth-suppressive ingredients).
8. Multiply the counts on plate [A] by 10, or plate [B] by 100, or plate [C] by 1000 to determine the number of bacteria per mL of food source.
9. Any TCBS plates containing bacteria should be autoclaved or boiled in water before they are discarded.

**Note:** If the counts from step 7 (above) exceed 10,000 per mL, the food source or water may cause mortalities in scallop larvae. Some studies with other scallop species suggest that as few as 1,000 *Vibrio* per mL may cause problems. However, our experience in establishing open-tank, algal cultures suggests that initially vibrio counts in the food may reach as high as  $4 \times 10^5$  per mL for several weeks. After the algal cultures become well-established, the counts drop to very low levels. Mortalities in scallops being fed *Vibrio*-contaminated food depends on the *Vibrio* strain that is present in the food. If mortalities occur, it is best to suspend feeding with that food source until *Vibrio* counts are low.

## PROCEDURE FOR VIBRIO TESTING



**APPENDIX C****COMMON CELSIUS TO FAHRENHEIT EQUIVALENTS**

°C	°F	°C	°F
40.0 -----	104.0	20.0 -----	68.0
39.0 -----	102.2	19.0 -----	66.2
38.0 -----	100.4	18.0 -----	64.4
37.0 -----	98.6	17.0 -----	62.6
36.0 -----	96.8	16.0 -----	60.8
35.0 -----	95.0	15.0 -----	59.0
34.0 -----	93.2	14.0 -----	57.2
33.0 -----	91.4	13.0 -----	55.4
32.0 -----	89.6	12.0 -----	53.6
31.0 -----	87.8	11.0 -----	51.8
30.0 -----	86.0	10.0 -----	50.0
29.0 -----	84.2	9.0 -----	48.2
28.0 -----	82.4	8.0 -----	46.4
27.0 -----	80.6	7.0 -----	44.6
26.0 -----	78.8	6.0 -----	42.8
25.0 -----	77.0	5.0 -----	41.8
24.0 -----	75.2	4.0 -----	39.2
23.0 -----	73.4	3.0 -----	37.2
22.0 -----	71.6	2.0 -----	35.6
21.0 -----	69.8	1.0 -----	33.8
		0 -----	32.0

\*Equivalents not shown can be calculated as follows:

$$^{\circ}\text{F} = (^{\circ}\text{C} \times 9/5) + 32$$

RECEIVED  
NATIONAL SEA GRANT LIBRARY

JUL 21 2003

Pell Bldg. URI Bay Campus  
Narragansett RI 02882 USA

## The Influence of Temperature - Food Availability on the Tissue Growth of Sea Scallop *Placopecten magellanicus*

**Adi Santoso**

Department of Marine Science, Faculty of Fisheries and Marine Science,  
Diponegoro University, Semarang - Indonesia  
E-mail : santoso\_undip@yahoo.co.id

### **Abstrak**

Studi terhadap pertumbuhan kerang simping *Placopecten magellanicus*, yang dibudidayakan dengan metode "suspended culture" telah dilakukan selama tujuh bulan di lokasi budidaya di Graves Shoal, Mahone Bay, Nova Scotia, Kanada. Benih scallop muda dipelihara dalam pearl nets dengan kepadatan 30-35 ekor dan ditempatkan pada empat lokasi yang mewakili perairan permukaan (7 m), dasar perairan (14 m), di luar lokasi budidaya (outer edge), di tengah-tengah lokasi budidaya (centre). Pertumbuhan jaringan lunak (whole tissue weight) diamati setiap bulan sekali. Monitoring terhadap suhu dan ketersediaan pakan pada permukaan dan dasar perairan juga dilakukan. Hasil penelitian menunjukkan bahwa tingkat pertumbuhan pada jaringan lunak lebih besar di permukaan perairan dibandingkan dengan di dasar perairan, tetapi tidak ada perbedaan nyata antara antara pertumbuhan di luar lokasi budidaya dengan di lokasi budidaya. Pertumbuhan jaringan lunak sendiri tidak ada korelasinya dengan suhu dan ketersediaan pakan di perairan.

**Kata kunci :** suhu, ketersediaan pakan, berat total jaringan lunak, kerang simping

### **Abstract**

A study of the growth of the sea scallop, *Placopecten magellanicus*, under suspended culture conditions was carried out over a seven month period at a culture site in Graves Shoal, Mahone Bay, Nova Scotia - Canada. Scallop spat were cultivated in pearl nets at a density of 30-35 per net set at four locations corresponding to the surface (7 m) and bottom (14 m) at the outer edge and the center of the site. Whole tissue weight was measured at monthly intervals. Environmental conditions represented as temperature and food availability at the surface and bottom over the same period were also monitored. The result showed that the mean values of whole tissue weight at the surface sites were greater than that at the bottom sites, but there were not significantly different between the outside sites and the inside sites. Growth in whole tissue weight was not to correlate to temperature - food availability.

**Key words :** temperature, food availability, whole tissue weight, sea scallop

### **Introduction**

The potential for scallop culture remains high in many countries but it will require a firm commitment by governments and industry to achieve this goal (Bourne, 2000). The sea scallop, also known as the giant scallop or smooth scallop, *Placopecten magellanicus* (Gmelin, 1791) it self is one of the most economically important species of shellfish on the east coast of Canada and the northern United States (Beninger, 1987). The environmental factors surrounding a site determine the water quality in providing food supply, proper temperature and salinity, and current velocity for growth of scallops (Grecian et al., 2000). More specifically, temperature and food availability have been considered the main

factors affecting growth and production (Bayne and Newell, 1983; Shumway, 1991; Sokolova and Portner, 2001; Heilmayer, 2003). An investigation carried out in eastern Newfoundland and around St. Andrews, New Brunswick, Canada concluded that the more favorable temperature and food conditions are usually found in shallow water and result in greater somatic growth than in deeper water (MacDonald and Thompson, 1985). In the Gulf of Maine, Schick et al. (1988) found that in shallow water (15 and 25 meter depths) the scallop growth was greater than the growth of the deep water scallops (170 and 174 meter depths). But, interestingly in Passamaquoddy Bay, where the water column is thoroughly mixed by tidal forces, there is no difference in either shell growth or somatic growth of scallops from various depths

(MacDonald and Thompson, 1985). Comparisons of growth rates at five depths ranging between 55–144 m in the Bay of Fundy resulted in similar conclusion (Caddy et al., 1970).

There is lack information on the specific food items preferred by bivalve species in their natural habitats (Shumway et al., 1987). For *Placopecten magellanicus*, phytoplankton may be the primary sources of nutrition. Detritus alone is apparently a poor alternative but can be utilized as an additional food source when phytoplankton concentrations are low (Cranford and Grant, 1990). Others have reported that *P. magellanicus* is an opportunistic filter feeder that ingests a wide spectrum of pelagic and benthic organisms and detritus ranging in size from 10 to 350 µm (Shumway et al., 1987). As pointed out by Levinton (1972), not only is the food supply constantly fluctuating, it is unpredictable and these suspension feeding organisms must maintain an adaptive strategy which maximizes the generality of their food requirement.

The objective of this study was to determine both the quality and quantity of temperature and food availability for the sea scallop, *P. magellanicus*, and their relation to the tissue growth at a culture site in Graves Shoal, Mahone Bay, NS Canada.

## Material and Methods

Juvenile giant scallops 9–12 mm in shell height (summer spat cohort) were placed into pearl nets and deployed at a grow-out site located at Graves Shoal in Mahone Bay (Figure 1). Approximately 3,000 scallops were transferred to 84 pearl nets at a density of 30–35 individuals per net: 21 at a site located at a depth of 7 m and on the outside margin of the site (SUROUT); 21 at a depth of 7 m and located within the interior of the site (SURIN); 21 at depth of 14 m and on the outer margin (BOTOUT); and 21 at depth of 14 m located within the interior of the site (BOTIN). The depth used in this study was in accordance with the depth for *P. magellanicus* studied by MacDonald and Thompson (1985). At each site there were 7 arrays each of which contained 3 pearl nets representing 3 replicates.

Growth parameters were measured at monthly intervals over a seven month period. For shell growth (shell height), it was presented in separated paper (Santoso, 2004). Whole tissue weight in term of dry weight was obtained after dissecting whole tissue (consisting of meat, viscera and gonad) of each scallop, then drying at 90°C for 24 h in a vacuum oven.

During May to December the following environmental factors were monitored on a weekly basis; water temperature, chlorophyll a concentration and particulate matter concentration. One l water samples for determination of chlorophyll a and particulate matter concentrations were taken at depths corresponding to the surface and bottom sites. Water samples for chlorophyll a were filtered through Whatman GF/C glass fiber filters under gentle vacuum (<20 mmHg) and the filters stored frozen until analysis. Chlorophyll a measurements were made spectrophotometrically (Strickland and Parsons, 1972) after extracting the pigment in 15 ml of 90% acetone for 24 h at 4°C in the dark.

Three measurements of particulate matter were made: Total Particulate Matter (TPM), Particulate Inorganic Matter (PIM), and Particulate Organic Matter (POM). TPM was determined by filtering 1 l of water onto previously combusted and tarred Whatman GF/C filters. The filters were then dried at 60–70°C for 24 h in a vacuum oven and reweighed. For PIM determination, the dried filters were combusted at 450°C for 24 h in a muffle furnace and then reweighed. POM was calculated as the difference between TPM and PIM measurements.

A variety of statistical procedures were used to analyze the data set. These included Pearson correlation analysis and analysis of variance (ANOVA). For ANOVA analysis, pairwise mean differences and comparison probability matrices (based on Bonferroni probability levels) were presented to facilitate interpretation of results.

## Results and Discussions

### Water temperature

During the study period water temperature ranged between 3–19 °C at 3 m depth (representing the surface site) and 2–17 °C at 14 m depth (representing the bottom site) (Figure 2).

At both surface and bottom sites temperature peaked in mid-August. Up to this period stratification also increased, and at maximum stratification the mean difference between surface and bottom site was about 4 °C. Near the end of August a mixing event caused stratification to break down, but this was reestablished shortly afterwards and lasted until about mid-October when the system became destratified and remained so for the remainder of the study period.

### Food availability

Phytoplankton chlorophyll a at the surface site



Figure 1. Map showing location of the study site at Graves Shoal, Mahone Bay, Nova Scotia - Canada

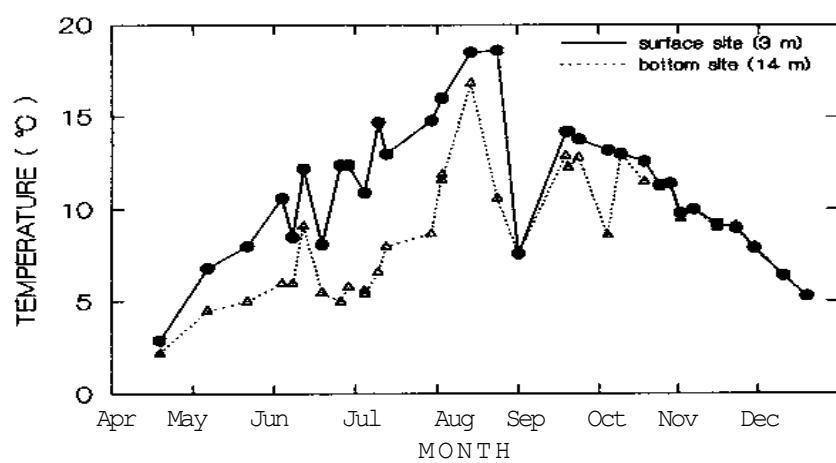


Figure 2. Seasonal variation of temperature at the surface site and bottom site at Graves Shoal, Mahone Bay

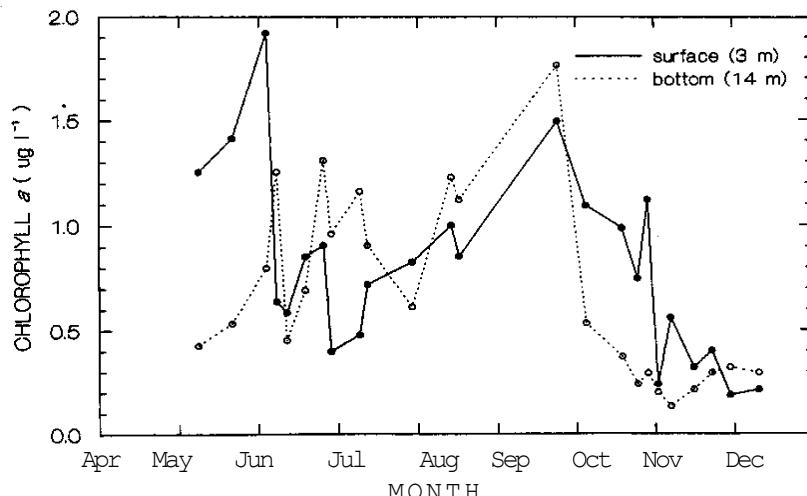
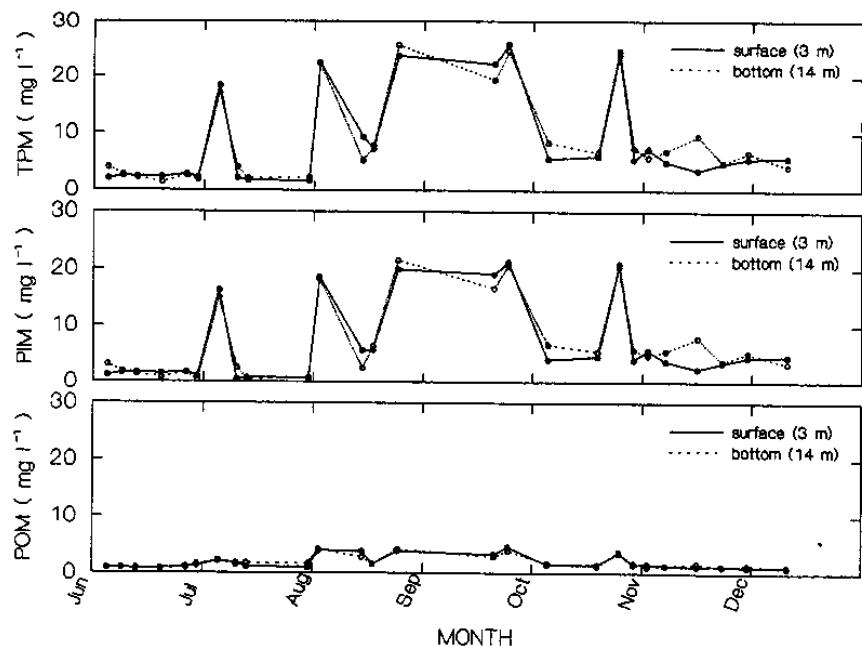
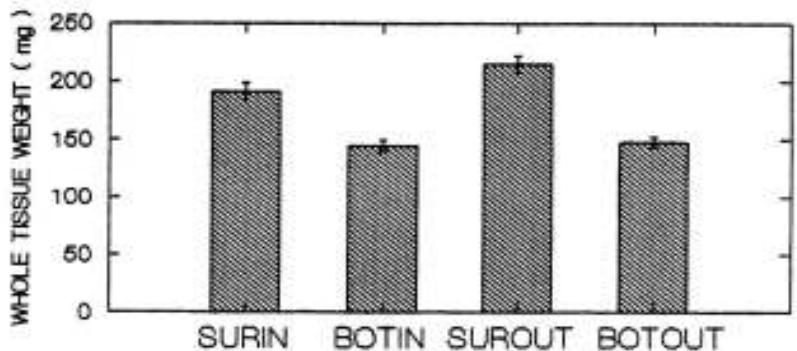


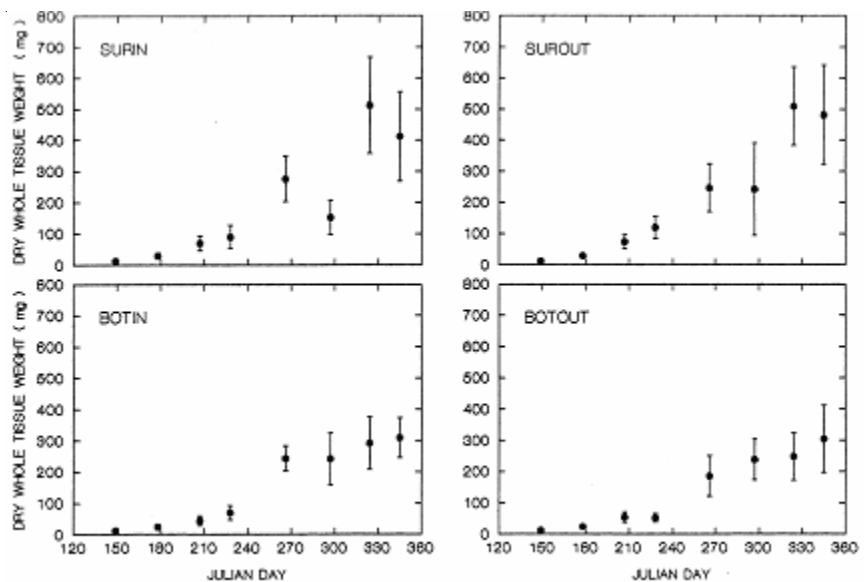
Figure 3. Seasonal variation in chlorophyll a concentrations at the surface site and bottom site at Graves Shoal, Mahone Bay



**Figure 4.** Seasonal variation in Particulate Organic Matter (POM), Particulate Inorganic Matter (PIM) and Total Particulate Matter (TPM) concentrations at surface site and bottom site at Graves Shoal, Mahone Bay



**Figure 5.** Comparison of the mean and standard error of Whole Tissue Weight at each site



**Figure 6.** The change in mean value of Whole Tissue Weight over the study period (error bars are one standard deviation of the mean)

ranged between 0.19–1.92 mg l<sup>-1</sup> with a mean of 0.78 mg l<sup>-1</sup>. Bottom site chlorophyll a concentrations were slightly lower than those at the surface ranging between 0.13–1.86 mg l<sup>-1</sup> with a mean of 0.71 mg l<sup>-1</sup> (Figure 3). Seasonally chlorophyll a values peaked during early June and late September. Between mid-June and early August chlorophyll a levels were generally low with surface values being slightly less than bottom values.

Total Particulate Matter Concentration over the study period ranged between 1.6–25.6 mg l<sup>-1</sup> with a mean of 8.5 mg l<sup>-1</sup> (Figure 4). There was little difference between concentrations at the surface and bottom. The seasonal variation in TPM was very erratic. Peaks occurred in early July and August, and in late October. Between the end of August and late October TPM values remained relatively constant and high. There was no clear relationship between TPM concentration and mixing events although the peak in late September did occur at the period of fall destratification.

POM concentrations at both depths were always much lower than PIM concentrations and ranged between 0.6–4.6 mg l<sup>-1</sup> with a mean value of 1.7 mg l<sup>-1</sup>. In general, POM accounted for about 20 percent of TPM indicating that most of the particulate matter present was inorganic. In addition, POM showed very little seasonal variation compared to that exhibited by PIM.

### **Whole tissue growth**

The mean values of whole tissue weight at the surface sites were greater than that at the bottom sites (Figure 5). But, ANOVA analysis indicated that means of whole tissue weights were not significantly different ( $p < 0.05$ ) at the outside sites and at the inside sites. Figure 6 presents the value of Whole Tissue Weight at each sampling time.

### **The influence of temperature - food availability on the whole tissue growth**

In most studies, there has been a general agreement that growth of sea scallop is depth dependent; with increasing depth representing deteriorating environmental suitability (Shick *et al.*, 1986). Therefore, in stratified systems, growth in the upper mixed layer is generally greater than that in the bottom waters. In this study, the difference in growth rate (in term of whole tissue weight) between the surface and bottom was significant (Figure 5). The greater growth rates of *P. magellanicus* at surface sites was probably a result of more favorable environmental

conditions within the water column as opposed to bottom sites, especially during the system stratified up to the mid October. In this case surface waters tended to have higher temperatures (Figure 2), but there was little difference between food availability as measured by phytoplankton chlorophyll a (Figure 3) and POM (Figure 4) concentrations. The mean chlorophyll a level was 0.7 mg l<sup>-1</sup>, whereas the mean POM level was 1.8 mg l<sup>-1</sup>. Bacher *et al.* (2003) measured that the both values were 4.3 mg l<sup>-1</sup> for chlorophyll a and 1.5 mg l<sup>-1</sup> for POM in developing a model for the scallop growth in Sungo Bay, China.

Attempts to relate scallop growth in whole tissue weight to temperature - food variables showed that there was no significant relationship occurred. This result was different compared to shell growth (measured in shell height) as previously reported (Santoso, 2004). According to Santoso (2004), between shell height and chlorophyll a there was a significant relationship exist. It also suggests that in general chlorophyll a may be a better indicator of food availability than variables related to particulate matter concentration, although this phenomenon did not happen for tissue growth as in this study. Particulate matter concentrations include both inorganic and organic materials and there is some evidence that the ratio of these components, in addition to their absolute concentration, may be important in determining their ability to be utilized. Vahl (1980), in a study on the Iceland scallop, *Chlamys islandica*, reported that POM could not be absorbed as food when PIM comprised more than 80 percent of the seston. In another study on the same species, Wallace and Reiness (1985) showed that growth was seriously reduced when the ratio of PIM to POM in seston exceeded a critical value of 3.5. In the present study the ratio of PIM to POM averaged about 4 (Figure 4) and this may indicate relatively poor food quality, especially if *P. magellanicus* exhibits the same response to the relative proportions of PIM and POM as does *C. islandica*.

Chlorophyll a concentrations peaked during late May and late September (Figure 3) while temperature peaked during early August (Figure 2). As result, the increase in filtration rates would have occurred at a time when food concentrations were low and the benefits of increased filtration rates would not have been realized.

Variation in tissue growth between scallops located on the outside edge of the site relative to those located within the interior of the site were relatively minor. Scallops located near the margins of a culture site, compared to those located within the

interior, are less likely to be affected by depletion of food materials as water flows through the site. The lack of any clear difference in growth rates suggests that food depletion was not a problem at the scallop densities used in this study.

### Acknowledgements

Personally I would like to thank Dr. Michel Brylinsky who from the beginning of this project always welcomed me with his tremendous patience, support, expertise and advice. A special thank to Dr. Michael J. Dadswell who facilitated this study by providing field equipment and time during field work. I am grateful to Dr. Graham R. Daborn, the Director of Acadia Centre for Estuarine Research (ACER) Acadia University, for providing advice, instruments and laboratory facilities.

### References

- Bacher, C., J. Grant, A.J.S. Hawkins, J. Fang, M. Zhu, and M. Besnard. 2003. Modeling the effect of food depletion on scallop growth in Sungo Bay (China). *Aquat. Living Resour.* 16: 10-24.
- Bayne, B.L. and R.C. Newell. 1983. Physiological energetics of marine mollusks. In Saeluddin A.S.M. and K.M. Wilbur (Eds.) *The Mollusca: Physiology Part 1*. Academic Press, Boston.
- Bourne, N.F. 2000. The potential for scallop culture - the next millennium. *Aquaculture International* 8 (2-3) : 113-122. Springer, Berlin.
- Chandler, R.A., G.J. Parsons, and M.J. Dadswell. 1989. Upper and Northern Bay of Fundy Scallops Surveys, 1986-1987. *Can. Tech. Rep. of Fish and Aquat. Sci.* 1665: 37p.
- Cranford, P.J. and J. Grant. 1990. Particle clearance and absorption of phytoplankton and detritus by the sea scallop *Placopecten magellanicus* (Gmelin). *J. Exp. Mar. Biol. Ecol.* 137(2) : 105-121.
- Dadswell, M.J., and G.J. Parsons. 1991. Potential for aquaculture of sea scallop, *Placopecten magellanicus* (Gmelin, 1791) in the Canadian Maritimes using naturally produced spat, p. 300-307. In S.E. Shumway and P.A. Sandifers (eds.) *Scallop Biology and Culture*. The World Aquaculture Society, Baton Rouge, LA.
- Grecian, L.A., G.J. Parsons, P. Dabinett, and C. Couturier. 2000. Influence of season, initial size, depth, gear, type and stocking density on the growth rates and recovery of sea scallop, *Placopecten magellanicus*, on a farm-based nursery. *Aquaculture International* 8 (2-3) : 183-206. Springer, Berlin.
- Grizzle, R.E. and P.J. Morin. 1989. Effect of tidal currents, seston, and bottom sediments on growth of *Mercenaria mercenaria*: results of a field experiment. *Mar. Biol.* 102: 85-93.
- Heilmayer, O. 2003. Environment, adaptation and evolution: Scallop Ecology across the latitudinal gradient. PhD Thesis. The University of Bremen, Bremen.
- MacDonald, B.A. 1986. Production and resource partitioning in the giant scallop *Placopecten magellanicus* grown on the bottom and in suspended culture. *Mar. Ecol. Prog. Ser.* 34: 79-86.
- MacDonald, B.A., and R.J. Thompson. 1985. Influence of temperature and food availability on the ecological energetics of the giant scallop, *Placopecten magellanicus*. I. Growth rates of shell and somatic tissue. *Mar. Ecol. Prog. Ser.* 25: 279-295.
- Santoso, A. 2004. The Influence of Food Availability on the Shell Growth of Sea Scallop *Placopecten magellanicus* (Gmelin, 1791). *Majalah Ilmu Kelautan* 9(1): 8-13.
- Schick, D.F., S.E. Shumway, and M.A. Hunter. 1988. A comparison of growth rate between shallow water and deep water populations of scallop, *Placopecten magellanicus* (Gmelin, 1791), in the Gulf of Maine. *Amer. Malac. Bull.* 6(1): 1-8.
- Seed, R. 1980. Shell growth and form in the bivalvia, p. 23-67. In D.C. Rhoads and R.A. Lutz (eds.) *Skeletal growth of aquatic organisms*. Plenum Press, New York.
- Shumway, S.E., R. Selvin, and D.F. Schick. 1987. Food resources related to habitat in the scallop *Placopecten magellanicus* (Gmelin, 1791): A Qualitative Study. *J. Shellfish Res.* 7(1): 77-82.
- Shumway, S.E. 1991. *Scallops: Biology, Ecology and Aquaculture*. Elsevier, Amsterdam.
- Sokolova, I.M. and H.O. Portner. 2001. Temperature effects on key metabolic enzymes in *Littorina saxatilis* and *L. obtusata* from different latitudes and shore levels. *Mar. Biol.* 139: 113-126.
- Strickland, J.D.H., and T.R. Parson. 1972. A practical handbook of seawater analysis. *Bull. Fish. Res. Bd. Can.* 167.

- Val, O. 1980. Seasonal variations in seston and the growth rate of the Iceland Scallop, *Chlamys islandica* (O.F. Miller), from Balsfjord, 70° N. *J. Exp. Mar. Biol. Ecol.* 48: 195-204.
- Wallace, J.C. and T.G. Reinsnes. 1985. The significance of various environmental parameters for growth of the Iceland scallop, *Chlamys islandica* (Pectinidae), in hanging culture. *Aquaculture* 44: 229-242.

# PEMANFAATAN RUMPUT LAUT *Sargassum* sp. SEBAGAI ADSORBEN LIMBAH CAIR INDUSTRI RUMAH TANGGA PERIKANAN

***Utilization of Seaweed Sargassum sp. as an Adsorbent for Fisheries Home Industry Liquid Waste***

Bustami Ibrahim\*, Dadi R. Sukarsa, Linda Aryanti

Departemen Teknologi Hasil Perairan, Fakultas Perikanan dan Ilmu Kelautan, IPB

Diterima 5 Agustus 2011/Disetujui 14 Oktober 2011

## **Abstract**

Seaweed *Sargassum* sp. is able to adsorb organic materials in fisheries liquid waste because it contain polysaccharides, protein and lipid in cell wall surface that consist of functional groups like amino, hydroxil, carboxyl and sulfate. This research aims were to determined the utilization of *Sargassum* as an adsorbent, the effect of chemical modification of *Sargassum* and adsorbent's weight in ability to adsorb fisheries liquid waste. Some parameters were tested in this research. Colour of liquid waste was observed visually, pH of liquid waste was tested by pH meter, turbidity was tested by turbidimeter, total suspended solids (TSS) was tested by gravimetry method and chemical oxygen demand (COD) was tested by closed reflux with titrimetry method. Reduction COD value of fisheries home industry liquid waste optimally can be achieved by *Sargassum* modified by acid. Optimal performance was obtained when the weight value of adsorbent between 1.0-2.0 gram in 100 ml liquid waste. Generally, optimal weight of adsorbent in order to reduce organic compounds was 1.0 gram in 100 mL fisheries home industry liquid waste. Using 1.0 gram *Sargassum* with acid modification made the liquid waste colour almost clearly, reached a pH level of 4.84, decreased turbidity level of 245 NTU, decreased TSS level of 851.7 mg/L and COD level of 720 mg/L.

Keyword: acid modification, adsorb, adsorbent, fisheries liquid waste, *Sargassum* sp.

## **Abstrak**

Rumput laut *Sargassum* sp. mampu mengadsorbsi bahan organik pada limbah cair perikanan karena mengandung polisakarida, protein atau lipid pada permukaan dinding selnya yang terdiri dari gugus fungsional, seperti amino, hidroksil, karboksil, dan sulfat. Penelitian ini bertujuan untuk menentukan pengaruh penggunaan *Sargassum* sebagai adsorben, pengaruh modifikasi kimia pada rumput laut *Sargassum* dan bobot adsorben yang digunakan terhadap kemampuan mengadsorbsi limbah cair perikanan. Beberapa parameter diuji selama penelitian. Warna limbah cair diamati secara visual, nilai pH diuji menggunakan pHmeter, kekeruhan dengan turbidimeter, total padatan tersuspensi (TSS) secara gravimetri dan COD dengan metode refluks tertutup secara titrimetri. Modifikasi optimum yang mampu menurunkan kadar COD limbah cair industri rumah tangga perikanan adalah modifikasi menggunakan asam. *Sargassum* sebagai adsorben limbah cair industri rumah tangga perikanan berfungsi dengan baik pada bobot 1,0-2,0 gram dalam 100 mL limbah cair. Bobot adsorben secara umum yang paling efektif dalam menurunkan beban limbah cair industri rumah tangga perikanan adalah pada bobot 1,0 gram dalam 100 mL limbah cair. Penggunaan 1,0 gram *Sargassum* modifikasi asam dapat menjadikan warna limbah cair menjadi agak bening, menurunkan pH menjadi 4,84, menurunkan nilai kekeruhan menjadi 245 NTU, nilai TSS sebesar 851,7 mg/L dan nilai COD sebesar 720 mg/L.

Kata kunci: adsorbsi, adsorben, limbah cair perikanan, modifikasi asam, *Sargassum* sp.

## **PENDAHULUAN**

Perkembangan industri perikanan saat ini menimbulkan permasalahan berupa pencemaran limbah cair yang dihasilkan

---

\*Korespondensi: Jln. Lingkar Akademik, Kampus IPB Dramaga 16680. Telp. +622518622915, Fax. +62251 8622916. E-mail: [bustamibr@yahoo.com](mailto:bustamibr@yahoo.com)

selama proses produksi. Limbah cair industri perikanan mengandung bahan organik dengan konsentrasi yang sangat tinggi. Kandungan nutrien organik yang tinggi pada limbah cair perikanan apabila berada dalam badan air akan menyebabkan eutrofikasi pada perairan (Aloui *et al.* 2009). Eutrofikasi dapat menyebabkan kematian organisme yang hidup di dalam air tersebut, pendangkalan, penyuburan ganggang dan menimbulkan bau yang tidak nyaman (Ibrahim 2005).

Proses adsorpsi merupakan salah satu metode yang efektif untuk menghilangkan komponen organik dari limbah buangan. Karbon aktif adalah adsorben yang banyak digunakan karena memiliki kemampuan yang sangat baik dalam mengadsorbsi komponen organik (Velmurugan *et al.* 2010). Penggunaan karbon aktif sebagai adsorben sangat terbatas dan biayanya tidak ekonomis. Penggunaan biomaterial sebagai adsorben merupakan alternatif yang sangat potensial untuk menggantikan metode tersebut. Banyak penelitian yang telah membuktikan bahwa ganggang atau alga merupakan salah satu adsorben yang efektif pada pengolahan limbah (Antunes *et al.* 2003). Alga laut memiliki kemampuan mengadsorbsi karena mengandung polisakarida, protein atau lipid pada permukaan dinding selnya yang terdiri dari gugus fungsional, seperti amino, hidroksil, karboksil dan sulfat (Kannan *et al.* 2010). Pemanfaatan *Sargassum* dalam pengolahan limbah tekstil diharapkan juga bisa dimanfaatkan dalam pengolahan limbah cair perikanan.

Tujuan penelitian ini adalah menentukan pengaruh penggunaan *Sargassum* sebagai adsorben, pengaruh modifikasi kimia pada rumput laut *Sargassum* dan bobot adsorben yang digunakan terhadap kemampuan mengadsorbsi limbah cair perikanan.

## MATERIAL DAN METODE

### Bahan dan Alat

Bahan-bahan yang digunakan adalah rumput laut coklat *Sargassum* sp., limbah cair

industri rumah tangga perikanan, indikator Ferroin, dan larutan Ferrous Amonium Sulfat (FAS).

### Metode Penelitian

Penelitian meliputi beberapa tahapan, yaitu preparasi adsorben, analisis limbah cair industri rumah tangga perikanan, penentuan modifikasi adsorben, penentuan lama waktu pengadukan, penentuan selang bobot adsorben optimum serta penentuan bobot adsorben optimum. Rumput laut *Sargassum* yang telah dikeringkan digunakan sebagai bahan baku utama pembuatan adsorben. Limbah cair yang digunakan pada penelitian ini adalah limbah cair dari proses perebusan bakso ikan salah satu unit industri rumah tangga. Air sisa perebusan bakso ikan tersebut tidak dimanfaatkan dan langsung dibuang ke perairan. Penumpukan limbah cair tersebut menyebabkan perairan berwarna hijau dan menimbulkan aroma yang tidak sedap.

Perlakuan modifikasi adsorben yang dilakukan berupa modifikasi asam ( $HCl$  0,1 M), kalsium ( $CaCl_2$  0,2 M) dan aldehid ( $CH_2O$  36% dan  $HCl$  0,1 M). Analisis yang dilakukan pada penentuan modifikasi adsorben optimum adalah analisis warna secara visual dan analisis nilai COD, sedangkan pada penentuan lama waktu pengadukan dan penentuan selang bobot adsorben optimum dilakukan analisis nilai pH dan kekeruhan serta warna secara visual. Beberapa parameter uji yang diteliti pada analisis limbah cair industri rumah tangga perikanan dan penentuan bobot adsorben optimum adalah parameter warna secara visual, nilai pH dengan menggunakan pH meter, nilai kekeruhan dengan turbidimeter, nilai TSS secara gravimetri dan nilai COD dengan metode refluks tertutup secara titrasi.

## HASIL DAN PEMBAHASAN

### Preparasi Adsorben

Kadar air rata-rata dari *Sargassum* kering yang digunakan sebagai adsorben adalah 12,37%. Adsorben yang dihasilkan berwarna

coklat dengan ukuran  $\pm 80\text{ mesh}$ . Adsorben merupakan suatu bahan (padatan) yang dapat mengadsorbsi adsorbat (Fransiscus *et al.* 2007). Adsorbsi merupakan suatu gejala permukaan terjadi penyerapan atau penarikan molekul-molekul gas atau cairan pada permukaan adsorben (Yun *et al.* 2001).

### **Analisis Limbah Cair Industri Rumah Tangga Perikanan**

Hasil analisis limbah cair industri rumah tangga perikanan disajikan pada Tabel 1. Nilai parameter pH, kekeruhan, TSS dan COD limbah cair melebihi nilai baku mutu limbah cair yang telah ditetapkan oleh keputusan Menteri Negara Lingkungan Hidup Nomor KEP-51/MENLH/10/1995. Nilai pH limbah cair industri rumah tangga perikanan yang diuji adalah 5,95; sedangkan menurut baku mutu limbah cair industri, limbah yang bisa dibuang langsung ke perairan adalah yang memiliki pH 6-9. Parameter kekeruhan tidak dijadikan sebagai baku mutu dalam daftar peraturan pemerintah Republik Indonesia tentang baku mutu limbah cair bagi kegiatan industri karena kekeruhan terkait secara langsung dengan kandungan total tersuspensi (TSS). Nilai COD limbah yang sesuai dengan baku mutu limbah berkisar antara 100-300 mg/L, sedangkan nilai COD limbah yang digunakan pada penelitian ini sebesar 3487-3713 mg/L. Nilai TSS limbah cair yang digunakan berkisar antara 4146,2-4240,2 mg/L, nilai tersebut lebih besar dibandingkan nilai mutu baku limbah yang telah ditetapkan, yaitu 200-400 mg/L.

Tabel 1 Karakteristik limbah cair industri rumah tangga perikanan

Parameter	Nilai
Warna	Keruh
pH	5,95 $\pm$ 0,02
Kekeruhan (NTU)	785,00 $\pm$ 63,60
COD (mg/L)	3600 $\pm$ 113,0
TSS (mg/L)	4193,2 $\pm$ 47,0

Keterangan : Data dari rata-rata dua kali ulangan  
NTU : Nephelometric Turbidity Unit

### **Modifikasi Adsorben**

Hasil analisis limbah cair industri rumah tangga perikanan pada perlakuan modifikasi adsorben disajikan pada Tabel 2. Perlakuan modifikasi asam menyebabkan warna limbah yang semula keruh menjadi agak bening. Perlakuan adsorben dengan modifikasi asam juga mampu menurunkan nilai COD limbah cair industri rumah tangga perikanan, yang semula 3600 mg/L menjadi 960 mg/L. Alga laut mengandung komponen organik dalam jumlah yang tinggi, seperti karbohidrat, protein, lipid dan pigmen. Sebagian dari komponen tersebut akan larut dalam air selama proses adsorbsi, hal tersebut terlihat dari perubahan warna yang terjadi pada air setelah adsorbsi. Warna air berubah menjadi kekuningan atau kehijauan (Kleinubing *et al.* 2010). Modifikasi permukaan merupakan salah satu alternatif untuk mengatasi hal tersebut. Beberapa modifikasi yang dilakukan terhadap *Sargassum* sebagai adsorben, yaitu modifikasi asam, modifikasi aldehid dan modifikasi kalsium (Rubin *et al.* 2005).

### **Penentuan Lama Waktu Pengadukan**

Beberapa perlakuan yang diujikan pada penelitian ini adalah 15 menit, 30 menit, 45 menit dan 60 menit. Hasil analisis limbah cair industri rumah tangga perikanan pada perlakuan lama waktu pengadukan disajikan pada Tabel 3. Perlakuan lama waktu pengadukan selama 15 menit menyebabkan perubahan warna limbah cair industri rumah tangga perikanan yang semula keruh menjadi agak bening. Penurunan pH disebabkan oleh adanya tumbukan antara partikel koloid limbah yang terikat dengan adsorben yang mengandung asam sehingga terjadi pelepasan ion  $H^+$  ke dalam larutan (Parubak *et al.* 2001). Semakin lama waktu pengadukan yang dilakukan maka semakin banyak ion  $H^+$  yang dilepaskan sehingga pH limbah mengalami penurunan.

Tingkat kekeruhan limbah cair perikanan yang terbaik terlihat pada perlakuan selama 15 menit, yaitu 320 NTU. Lama

Tabel 2 Hasil analisis limbah cair industri rumah tangga perikanan pada perlakuan modifikasi adsorben

Perlakuan	Warna	COD (mg/L)
Kontrol	Keruh	3600
Adsorben tanpa modifikasi	Kecoklatan	1600
Adsorben modifikasi asam (HCl 0,1 M)	Agak bening	960
Adsorben modifikasi kalsium(CaCl <sub>2</sub> 0,2 M)	Kekuningan	1920
Adsorben modifikasi aldehid (CH <sub>2</sub> O 36% dan HCl 0,1 M)	Kecoklatan	2080

Tabel 3 Hasil analisis limbah cair industri rumah tangga perikanan pada perlakuan lama waktu pengadukan

Lama waktu pengadukan (menit)	Warna	pH	Kekeruhan (NTU)
0 (Kontrol)	Keruh	5,95	785
15	Agak bening	5,69	320
30	Agak keruh	5,36	410
45	Agak keruh	5,33	470
60	Agak keruh	5,31	540

Tabel 4 Hasil analisis limbah cair industri rumah tangga perikanan pada perlakuan selang bobot adsorben optimum

Bobot adsorben (gram) dalam 100 mL limbah cair	Warna	pH	Kekeruhan (NTU)
Kontrol (0)	Keruh	5,95	785
0,1	Keruh	5,78	540
0,5	Keruh	5,57	520
1,0	Agak Bening	4,94	129
1,5	Agak Bening	4,63	143
2,0	Agak Bening	4,56	167

waktu pengadukan yang melebihi 15 menit menyebabkan kekeruhan pada limbah cair industri rumah tangga perikanan, hal ini sesuai dengan penelitian Raghuvanshi *et al.* (2004) yang menyatakan bahwa efisiensi penyerapan berbanding lurus dengan waktu sampai pada titik tertentu, kemudian mengalami penurunan setelah melewati titik tersebut.

#### Penentuan Selang Bobot Adsorben Optimum

Beberapa perlakuan yang dicoba pada penelitian ini adalah adsorben dengan bobot

0,1 g, 0,5 g, 1,0 g, 1,5 g dan 2,0 g. Hasil analisis limbah cair industri rumah tangga perikanan pada perlakuan selang bobot adsorben optimum disajikan pada Tabel 4. Perlakuan bobot adsorben mampu mengurangi tingkat kekeruhan limbah. Penambahan adsorben dengan bobot yang berbeda-beda juga mempengaruhi pH. Semakin besar bobot adsorben yang ditambahkan, maka semakin rendah nilai pH limbah cair industri rumah tangga perikanan tersebut, hal ini disebabkan adanya pengaruh modifikasi asam yang digunakan pada adsorben. Penelitian

menghasilkan tiga perlakuan bobot adsorben terbaik dalam mengurangi nilai kekeruhan limbah cair industri rumah tangga perikanan. Perlakuan yang dijadikan sebagai perlakuan utama adalah adsorben dengan bobot 1,0 g; 1,5 g dan 2,0 g. Penentuan perlakuan tersebut didasarkan pada hasil pengamatan parameter warna limbah dan nilai kekeruhan.

### **Penentuan Bobot Adsorben Optimum Perubahan warna**

Secara visual warna limbah cair industri rumah tangga perikanan yang digunakan pada penelitian ini berwarna keruh. Penambahan adsorben pada limbah cair industri rumah tangga perikanan tersebut, menyebabkan terjadinya perubahan warna yang nyata, limbah yang semula berwarna keruh menjadi berwarna agak bening. Perubahan warna limbah perikanan dari yang berwarna keruh menjadi agak bening pada semua perlakuan disebabkan adsorben mengikat partikel-partikel koloid yang mengakibatkan warna

keruh pada limbah cair perikanan. Rumput laut *Sargassum* merupakan alga laut coklat yang mempunyai kemampuan absorpsi yang tinggi dikarenakan dinding selnya mengandung polisakarida (Kleinubing *et al.* 2010).

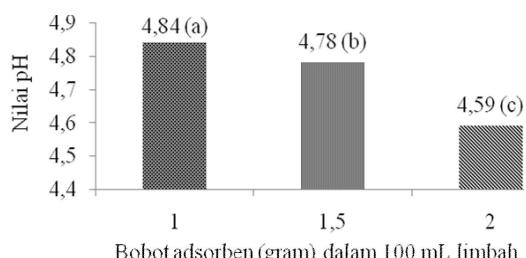
#### **Nilai pH**

Peningkatan bobot adsorben yang digunakan sebanyak 1,0 ,1,5 dan 2,0 g dalam 100 mL limbah cair memberikan pengaruh terhadap nilai pH limbah cair industri rumah tangga perikanan. Pengaruh bobot adsorben terhadap nilai pH limbah cair industri rumah tangga perikanan disajikan pada Gambar 1. Jumlah bobot adsorben yang ditambahkan berbanding terbalik dengan pH limbah cair industri rumah tangga perikanan. Semakin besar bobot adsorben yang ditambahkan maka akan semakin rendah nilai pH limbah cair industri rumah tangga perikanan, hal ini disebabkan adsorben yang digunakan telah mengalami modifikasi kimia dengan asam, yaitu asam klorida (HCl 0,1 M).

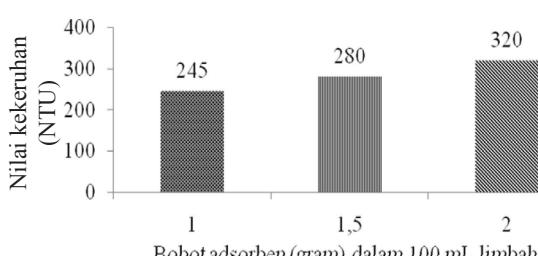
Aktivasi dengan asam klorida menyebabkan keasaman pada rumput laut. Keasaman rumput laut disebabkan adanya proton yang dapat terdisosiasi atau terlepasnya ion-ion H<sup>+</sup> dari gugus-gugus karboksilat (-COOH) dan gugus hidroksil (-OH) yang terdapat pada adsorben rumput laut *Sargassum*. Peningkatan keasaman permukaan yang terjadi pada adsorben tersebut disebabkan oleh pembentukan situs-situs aktif karena diaktivasi diberi perlakuan dengan asam sehingga meningkatkan kapasitas adsorbsinya. Aktivasi adsorben dengan asam klorida juga dapat mengurangi pengotor-pengotor yang terdapat pada adsorben (Sudiarta 2009).

#### **Nilai kekeruhan**

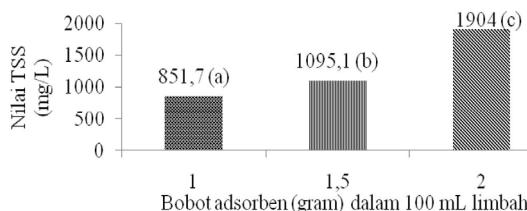
Peningkatan bobot adsorben yang digunakan (1,0 ,1,5 dan 2,0 g dalam 100 mL limbah cair) tidak memberikan pengaruh terhadap kualitas limbah cair industri rumah tangga perikanan. Pengaruh bobot adsorben terhadap nilai kekeruhan dalam 100 mL



Gambar 1 Pengaruh bobot adsorben terhadap nilai pH dalam 100 mL limbah cair industri rumah tangga perikanan. Angka-angka pada diagram batang yang diikuti huruf (a,b,c) menunjukkan bobot adsorben memberikan pengaruh berbeda nyata ( $p<0,05$ ) terhadap nilai pH.



Gambar 2 Pengaruh bobot adsorben terhadap nilai kekeruhan dalam 100 mL limbah cair industri rumah tangga perikanan.



Gambar 3 Pengaruh bobot adsorben terhadap nilai TSS dalam 100 mL limbah cair industri rumah tangga perikanan. Angka-angka pada diagram batang yang diikuti huruf (a,b,c) menunjukkan bobot adsorben memberikan pengaruh berbeda nyata ( $p<0,05$ ) terhadap nilai TSS.

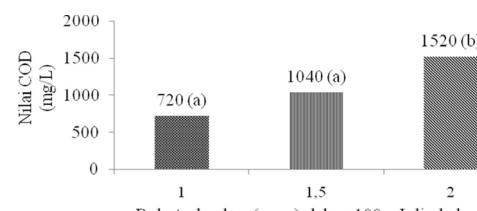
limbah cair industri rumah tangga perikanan disajikan pada Gambar 2. Semua pelakuan bobot adsorben yang ditambahkan mampu menurunkan nilai kekeruhan limbah cair industri rumah tangga perikanan.

#### Nilai total padatan tersuspensi (TSS)

Peningkatan bobot adsorben pada konsentrasi 1,0 ,1,5 dan 2,0 g dalam 100 mL limbah cair memberikan pengaruh terhadap nilai TSS limbah cair industri rumah tangga perikanan. Pengaruh bobot adsorben terhadap nilai TSS limbah cair industri rumah tangga perikanan disajikan pada Gambar 3. Semakin besar bobot adsorben yang ditambahkan, maka semakin lemah kemampuan adsorben dalam menyerap padatan tersuspensi. Penambahan adsorben sampai kondisi tertentu tidak akan memberi dampak pada peningkatan kapasitas adsorbsinya, namun akan menyebabkan terjadinya desorpsi (Yu *et al.* 2003). Padatan tersuspensi merupakan bahan-bahan yang melayang dan tidak larut dalam air. Padatan tersuspensi sangat berhubungan erat dengan tingkat kekeruhan air (MetCalf dan Eddy 2003).

#### Nilai COD

Peningkatan bobot adsorben (1,0, 1,5 dan 2,0 g dalam 100 mL limbah cair) memberikan pengaruh terhadap nilai COD limbah cair industri rumah tangga perikanan. Pengaruh bobot adsorben terhadap nilai COD limbah cair industri rumah tangga perikanan disajikan pada Gambar 4. Perlakuan adsorben dengan



Gambar 4 Pengaruh bobot adsorben terhadap nilai TSS dalam 100 mL limbah cair industri rumah tangga perikanan. Angka-angka pada diagram batang yang diikuti huruf (a,b,c) menunjukkan bobot adsorben memberikan pengaruh berbeda nyata ( $p<0,05$ ) terhadap nilai TSS.

penambahan bobot 1,0 g dan 1,5 g dalam 100 mL limbah cair memberikan hasil penurunan nilai COD yang berbeda nyata dibandingkan dengan penambahan 2,0 g adsorben. *Chemical oxygen demand* (COD) atau kebutuhan oksigen kimia merupakan jumlah oksigen yang dibutuhkan oleh oksidator (misal kalium dikhromat) untuk mengoksidasi seluruh material organik yang terdapat di dalam air (MetCalf dan Eddy 2003).

Bobot adsorben mempengaruhi kapasitas adsorbsi. Pada saat bobot adsorben dinaikkan, sedangkan volume limbah cair industri rumah tangga perikanan tetap, maka akan terjadi penurunan kapasitas adsorbsi, selain itu, sisisi aktif yang tersisa pada adsorben juga akan mengalami kejemuhan selama proses adsorpsi (Yahaya *et al.* 2011).

#### KESIMPULAN

Modifikasi optimum yang mampu menurunkan kadar COD limbah cair industri rumah tangga perikanan adalah modifikasi dengan menggunakan asam. Secara umum bobot adsorben yang paling efektif dalam menurunkan beban limbah cair industri rumah tangga perikanan adalah pada bobot 1,0 g dalam 100 mL limbah cair.

#### DAFTAR PUSTAKA

- Aloui F, Khoufi S, Loukil S, Sayadi S. 2009. Performances of an activated sludge process for the treatment of fish processing saline wastewater. *Journal of Desalination* 248:68-75.

- Antunes WM, Luna AS, Henriques CA, Costa ACA. 2003. An evaluation of copper biosorption by a brown seaweed under optimized conditions. *Journal of Biotechnology* 6(3):174-184.
- Fransiscus Y, Hendrawati L, Esprianti MA. 2007. Proses biosorpsi Cu dan phenol dalam kondisi tunggal maupun simultan dengan menggunakan lumpur aktif kering. *Jurnal Purifikasi* 8(1):67-72.
- Ibrahim B. 2005. Kaji ulang sistem pengolahan limbah cair industri hasil perikanan secara biologis dengan lumpur aktif. *Buletin Teknologi Hasil Perikanan* 8(1):31-41.
- Kannan RR, Rajasimman M, Rajamohan N, Sivaprakash B. 2010. Brown marine algae *Turbinaria conoides* as biosorbent for malachite green removal: equilibrium and kinetic modeling. *Journal of Environmental Science Engineering* 4(1):116-122.
- Keputusan Menteri Lingkungan Hidup. 1995. *Baku Mutu Limbah Cair Bagi Kegiatan Industri*. Jakarta: KEP-51/MENLH/10/1995.
- Kleinubing SJ, Vieira RS, Beppu MM. 2010. Characterization and evaluation of copper and nickel biosorption on acidic algae *Sargassum filipendula*. *Journal of Materials Research* 13(4):541-550.
- MetCalf, Eddy. 2003. *Wastewater Engineering: Treatment, Disposal and Reuse*, 4<sup>th</sup> ed. New York: McGraw Hill Book Co.
- Parubak AS, Sugiharto E, Mudjirin H. 2001. The effect of salinity on the release of copper (Cu), lead (Pb) and zinc (Zn) from tailing. *Indonesian Journal of Chemistry* 1(1):16-22.
- Raghuvanshi SP, Singh R, Kaushik CP. 2004. Kinetics study of methylene blue dye bioadsorption on baggase. *Journal of Applied Ecology and Environmental Research* 2:35-43.
- Rubin E, Rodriguez P, Herrero R, Cremade J, Barbara I, Manuel. 2005. Removal of methylene blue from aqueous solutions using biosorbent *Sargassum muticum*: An invasive macroalga in Europe. *Journal of Chemical Technology and Biotechnology*: 1-16.
- Sudiarta IW. 2009. Biosorpsi ion Cr(III) pada rumput laut *Eucheuma spinosum* teraktivasi asam sulfat. *Jurnal Kimia* 3(2):93-100.
- Velmurugan P, Kumar R, Dhinakaran G. 2010. Dye removal from aqueous solution using low cost adsorbent. *Journal of Environmental Science* 1(7): 1492-1503.
- Yahaya EM, Pakir MF, Abustan I, Bello OS, Ahmad MA. 2011. Adsorptive removal of Cu (II) using activated carbon prepared from rice husk by ZnCl<sub>2</sub> activation and subsequent gasification with CO<sub>2</sub>. *International Journal of Engineering and Technology IJET-IJENS* 11(01):207-211.
- Yu LJ, Shukla SS, Dorris KL, Shukla, Margrave JL. 2003. Adsorption of chromium from aqueous solutions by maple sawdust. *Journal of Hazardous Materials* 100 (1-3):53-63.
- Yun YS, Park D, Park JM, Volesky B. 2001. Biosorption of trivalent chromium on the brown seaweed biomass. *Journal of Environmental Science and Technology* 35:4353-4358.