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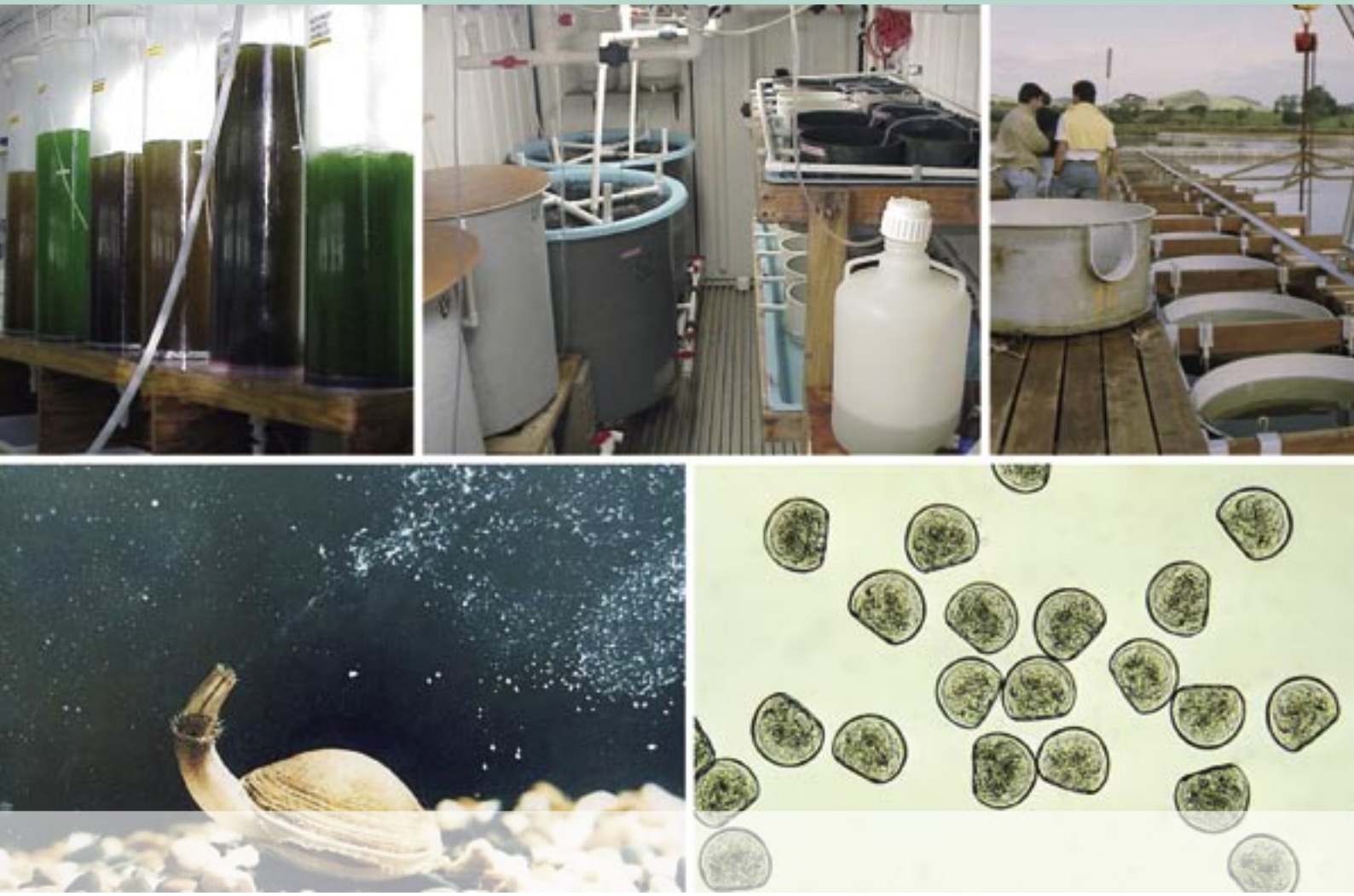
Hatchery culture of bivalves

A practical manual



Cover photographs:

Clockwise from top left – fibreglass cylinders used for microalgae culture; interior of a small bivalve hatchery; raft nursery for bivalve spat; photomicrograph of *Crassostrea gigas* D-larvae (courtesy Michael M. Helm); a spawning female Manila clam (courtesy Brian Edwards).

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 2004

ISBN 92-5-105224-7

Preparation of this document

This manual is part of the publications programme of the Fisheries Department Inland Water Resources and Aquaculture Service of the Food and Agriculture Organization of the United Nations. It is a synthesis of the current methodologies applicable to the intensive hatchery culture of bivalve molluscs covering similarities and differences in approach in rearing clams, oysters and scallops in different climatic regions. All aspects of the culture process are described, together with considerations in choosing a site for hatchery development and in the design of suitable facilities. The manual also includes the post-hatchery handling of "seed" bivalves in land- and sea-based nursery culture preparatory to on-growing. This publication is intended to assist both technicians entering this field as well as investors interested in evaluating the complexity of intensive hatchery production.

The authors bring together a combined 80 years of experience in the biology, management and operation of hatcheries encompassing a range of the more commonly cultured bivalve species in different parts of the world. Preparation of the manual has been under the overall coordination of Alessandro Lovatelli, Fishery Resources Officer (Aquaculture).

The authors wish to acknowledge the contributions of their many colleagues past and present and industry leaders, without which this publication would not have been possible.

Unless otherwise acknowledged, all photographs were taken by the authors.

Distribution:
Directors of Fisheries
FAO Fisheries Department
FAO Fisheries Officers in FAO Regional and Subregional Offices
FI Marine Aquaculture Mailing List

Abstract

Bivalve mollusc culture is an important and rapidly expanding area of world aquaculture production, representing approximately 20 percent of the sector's output at 14 million tonnes in 2000. The majority of production is from natural populations although increasingly stocks are approaching or have exceeded maximum sustainable yields. Stock enhancement through the capture and relaying of natural seed in both extensive and intensive forms of culture is common practice worldwide but the reliability of natural recruitment can never be guaranteed, and conflicts over the use of the coastal zone are becoming ever more pressing. A solution to meeting the seed requirements of the bivalve industry, applicable to the production of high unit value species such as clams, oysters and scallops, is hatchery culture. The production of seed through hatchery propagation accounts at the present time for only a small percentage of the total seed requirement but it is likely to become increasingly important as work continues to produce genetically-selected strains with desirable characteristics suited to particular conditions.

The advent of bivalve hatcheries was in Europe and the United States in the 1960s. Since those early pioneering days, knowledge of the biological requirements of the various species that predominate in worldwide aquaculture production and the technology used to produce them has grown and continues to improve. This manual brings together the current state of knowledge in describing the various aspects of hatchery culture and production from acquisition of broodstock to the stage at which the seed are of sufficient size to transfer to sea-based growout. 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 is not intended as a scientific treatise on the subject. Rather, it 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. Consideration is also given to the culture of tropical bivalves. Methods described are equally as applicable to bivalves of lesser significance in terms of worldwide production.

The authors recognize that bivalve hatchery production is as much an art founded on science as it is a science *per se*. There are as many ways of operating and managing a hatchery as there are hatcheries in terms of the sophistication of the facility and the precision with which each part of production is approached. In this respect, many experienced hatchery managers will consider much of the detailed information as "overkill." However, the authors have considered the need for a thorough grounding for new entrants in this field, not just how the various procedures are done but the biological basis of why they are done in that way. Thus, the content is equally as appropriate to the operation of a closely controlled experimental hatchery as it is to a commercial-scale hatchery.

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 polyploidy, the development of selected strains, cryopreservation of gametes and the need for novel, non-living foods.

Keywords: marine aquaculture, bivalve culture, bivalve hatcheries, bivalve nurseries, bivalve seed production, oysters, clams, scallops

Helm, M.M.; Bourne, N.; Lovatelli, A. (comp./ed.)
Hatchery culture of bivalves. A practical manual.
FAO Fisheries Technical Paper. No. 471. Rome, FAO. 2004. 177p.

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Glossary

Adductor muscle	large muscle (or muscles) that pull the two shell valves together
Algae	aquatic plants that reproduce by spores
Anterior	front or head
Auricle	with respect to scallops, the ear or wing-like projections at the hinge of a scallop (can also refer to the chamber of heart that receives blood from the body)
Axenic	culture of a single species in bacteria-free conditions
Biting	condition where shell margins of two scallops become interlocked, and subsequently damage the inner soft parts
Bivalve	mollusc of the Class Pelecypoda, having a shell of two valves that are joined by a hinge
Byssus	thread-like filaments used by bivalves to attach themselves to a substrate
Cilia	hair-like structures whose rhythmic beat induces a water current in bivalves
Ctenidia	leaf-like appendages that function in respiration and filtration of food from water (used interchangeably with the term gills)
Cultch	material used to collect bivalve spat
Demibranch	single plate or leaf of a bivalve gill
Detritus	fragmented or decomposing organic material from plant and animal remains
Diatom	a single-celled alga of the Class Bacillariophyceae; cells are enclosed in a siliceous shell called a frustule, cells can form chains
Dimyarian	bivalves with two adductor muscles, e.g. clams and mussels
Dioecious	organisms in which male and females reproductive organs occur in different individuals
Diploid	the normal number of chromosomes (2n) in cells
Dorsal	the back or part of an organism away from the ground
Downwelling	in hatchery terminology, a growing system in which the flow of water enters at the top of a spat holding container (compare with upwelling)
D-larva	the early veliger larval stage of bivalves, also known as straight-hinge larva
Embryo	organism in early stages of development; in bivalves, prior to larval stage
Exhalant	area of bivalve where water currents have an outward direction
Exotic	introduced from foreign country or geographic area
Eyespot	simple organ that develops near centre of mature larvae of some bivalves and is sensitive to light

Fertilization	union of egg and sperm
Flagellate	group of single-celled algae characterized by having a locomotory organ called a flagellum
Frustule	siliceous shell-like covering of a diatom
Gamete	mature, haploid, functional sex cell capable of uniting with the alternate sex cell to form a zygote
Gametogenesis	process by which eggs and sperm are produced
Gill	a leaf-like appendage that functions in respiration and filtration of food from water (see ctendia)
Growout	the process of growing seed produced in hatcheries to market size
Halocline	a zone of sharp vertical salinity change
Hinge	dorsal area of bivalve shell where two valves are joined together
HUFA	a highly unsaturated fatty acid, referred to also as polyunsaturated fatty acid (PUFA)
Indigenous	native, not imported
Inhalant	area of bivalve where water current have an inward direction
Larva	a stage of bivalves from the embryo to metamorphosis
Ligament	fibrous spring-like material joining two valves of a bivalve at the hinge
Mantle	the soft fold enclosing the body of a bivalve which secretes the shell
Mean	average
Meiotic Division	process in which normal number of chromosomes (2n) is reduced to the haploid (n) number
Metamorphosis	in bivalves, the period of transformation from the larval to the juvenile stage
Microalgae	small cell-size algae, either single celled or chain forming diatoms, cultured as foods for larvae and spat in a hatchery
Microlitre (μl)	one millionth of a litre or one thousandth of a ml
Micrometer (μm)	one millionth of a metre or one thousandth of a mm
Monoecious	organisms in which both male and female reproductive organs occur in the same individual
Monomyarian	bivalves with one adductor muscle, e.g. oysters and scallops
Natural Set	in bivalves, obtaining spat from spawning of natural populations
Pallial Line	faint circular line on inner surface of shell of bivalves showing location of attachment of mantle to shell
Palp	a sensory appendage near the mouth used to assist in moving food into the mouth
Pedal	pertaining to the foot
pH	a measure of acidity
Plankton	floating or weakly swimming aquatic organisms, can be phytoplankton (plants) or zooplankton (animals)
Planktotrophic	organisms that feed on phytoplankton

Polar Body	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
Polyploid	animals having more than the usual number of diploid (2n) chromosomes
Posterior	the rear, away from the head
Pronuclei	in the egg, the haploid nucleus after completion of meiosis but before infusion with the sperm nucleus
Pseudofaeces	false faeces, waste material not taken into the digestive tract
PSU	a measure of salinity, equivalent to parts per thousand
Resilium	internal portion of the ligament located centrally along the hinge of a bivalve; causes the valves to open when the adductor relaxes
Salinity	the salt content of seawater usually measured in parts per thousand (ppt) or practical salinity units (PSU)
Seed	a hatchery term for spat of a size ready for sale
Settlement	behaviourial process when mature bivalve larvae seek a suitable substrate for attachment
Shell Height	the straight line distance measured perpendicularly from the umbo to the ventral margin of the shell
Shell Length	the straight line distance from the anterior to the posterior margins of the shell
Spat	a newly settled or attached bivalve (also termed post larval or juvenile in bivalves)
Straight-hinge larva	early part of larval stage, sometimes termed D-stage
Tentacle	long, unsegmented threadlike protuberance from edge of mantle that has specialized sensory function
Tetraploid	polyploid animal with twice the normal complement of chromosomes (4n)
Thermocline	a zone of sharp vertical temperature change
Triploid	a polyploid animal with an extra set of chromosomes (3n)
Trochophore	planktonic stage of bivalve embryo
Umbo	beak-like projections at the dorsal part of the shell; it is the oldest part of a bivalve shell (also called the umbone)
Upwelling	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).
Urogenital System	system with organs concerned with excretion (kidney) and reproduction (gonad)
Valve	one of the two parts of a bivalve shell, two valves make up one shell
Veliger Larva	the larval stage of most molluscs, characterized by the presence of a velum
Velum	ciliated locomotory organ of the larva
Ventral	pertaining to the under or lower side of an animal
Zygote	diploid (2n) cell resulting from union of male and female gametes

Abbreviations, acronyms and conversions

BBSR	Bermuda Biological Station for Research
DHA	Docosahexaenoic Acid
DOPA	Dihydroxyphenylalanine
EDTA	Ethylene Diamine Tetraacetic Acid
EPA	Eicosapentaenoic Acid
FAO	Food and Agriculture Organization of the United Nations
FLUPSY	Floating Upwelling System
FSW	Filtered Seawater
GI	Growth Index
GRP	Glass-Reinforced Plastic
HUFA	Highly Unsaturated Fatty Acid
LDR	Light Dependent Resistor
MAFF	Ministry of Agriculture Food and Fisheries
NTM	Net Treatment Mortality
PHCD	Post-Harvest Cell Density
PUFA	Polyunsaturated Fatty Acid
PVC	Polyvinyl Chloride
RSR	Resistance Sensing Relay
SI	Système International
TBT	Tributyltin
TCBS	Thiosulfate Citrate Bile Sucrose
UV	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
inch	inch
ft	foot
yd	yard
mi	mile
ft²	square foot
yd²	square yard
mi²	square mile
m²	square metre
ha	hectare
km²	square kilometre
cc	cubic centimetre (= ml)
m³	cubic metre

ft³	cubic foot
yd³	cubic yard
µl	microlitre
ml	millilitre (= cc)
l	litre
µg	microgram
mg	milligram (milligramme)
g	gram (gramme)
kg	kilogram (kilogramme)
mt	metric tonne (1 000 kg) (also written as tonne)
oz	ounce
lb	pound
cwt	hundredweight [value differs in UK ('Imperial') and US units - see weight conversions]
t	ton [value differs in UK ('Imperial') and US units - see weight conversions]
psi	pounds per square inch
psu	practical salinity units
gpm	('Imperial' = UK) gallons per minute
mgd	million ('Imperial' = UK) gallons per day
cfm	cubic feet per minute
ppt	parts per thousand (also written as ‰)
ppm	parts per million
ppb	parts per billion (thousand million)
min	minute
hr	hour
kWhr	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:

1 µm	0.001 mm = 0.000001 m
1 mm	0.001 m = 1 000 µm = 0.0394 inch
1 cm	0.01 m = 10 mm = 0.394 inch
1 m	1 000 000 µm = 1 000 mm = 100 cm = 0.001 km = 39.4 inch = 3.28 ft = 1.093 yd
1 km	1 000 m = 1 093 yd= 0.621 mi
1 inch	25.38 mm = 2.54 cm
1 ft	12 inch = 0.305 m
1 yd	3 ft = 0.914 m
1 mi	1 760 yd = 1.609 km

Weight:

1 µg	0.001 mg = 0.000001 g
1 mg	0.001 g = 1 000 µg
1 g	1 000 000 µg = 1 000 mg = 0.001 kg = 0.0353 oz
1 kg	1 000 g = 2.205 lb
1 mt	1 000 kg = 1 000 000 g = 0.9842 UK t = 1.102 US t
1 oz	28.349 g
1 lb	16 oz = 453.59 g

1 UK cwt	112 lb = 50.80 kg
1 US cwt	100 lb = 45.36 kg
1 UK t	20 UK cwt = 2 240 lb
1 US t	20 US cwt = 2 000 lb
1 UK t	1.016 mt = 1.12 US t

Volume:

1 µl	0.001 ml = 0.000001 l
1 ml	0.001 l = 1 000 µl = 1 cc
1 L	1 000 000 µl = 1 000 ml = 0.220 UK gallon = 0.264 US gallon
1 m³	1 000 l = 35.315 ft^3 = 1.308 yd^3 = 219.97 UK gallons = 264.16 US gallons
1 ft³	0.02832 m^3 = 6.229 UK gallons = 28.316 l
1 UK gallon	4.546 l = 1.2009 US gallons
1 US gallon	3.785 l = 0.833 UK gallon
1 MGD	694.44 GPM = 3.157 m^3/min = 3 157 l/min

Concentration - dissolving solids in liquids:

1 %	1 g in 100 ml
1 ppt	1 g in 1 000 ml = 1 g in 1 l = 1 g/l = 0.1%
1 ppm	1 g in 1 000 000 ml = 1 g in 1 000 L = 1 mg/l = 1 µg/g
1 ppb	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:

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

Area:

1 m²	10.764 ft^2 = 1.196 yd^2
1 ha	10 000 m^2 = 100 ares = 2.471 acres
1 km²	100 ha = 0.386 mi^2
1 ft²	0.0929 m^2
1 yd²	9 ft^2 = 0.836 m^2
1 acre	4 840 yd^2 = 0.405 ha
1 mi²	640 acres = 2.59 km^2

Temperature:

°F	(9 ÷ 5 x °C) + 32
°C	(°F - 32) x 5 ÷ 9

Pressure:

1 psi	70.307 g/cm^2

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^{-1} in scientific journals. 1 g/kg is written as 1 g kg^{-1}. 12 mg/kg would be written as 12 mg kg^{-1}.95 µg/kg would be written as 95 µg kg^{-1}. A stocking density of 11 kg/m^3 would be written as 11 kg m^{-3}. This system of standardization is not normally used in commercial aquaculture hatcheries and growout units and has therefore not been used in this manual. More information about this topic can be found on the internet by searching for SI Units.

Introduction

Bivalve molluscs (oysters, mussels, clams and scallops) form a significant part of the world's fisheries production. In 2000 landings of bivalves from capture fisheries and aquaculture operations totalled 14 204 152 tonnes (Figure 1). During the decade from 1991 to 2000 there was a continuing increase in production of bivalves, and landings more than doubled from 6.3 million tonnes in 1991 to 14 million tonnes in 2000.

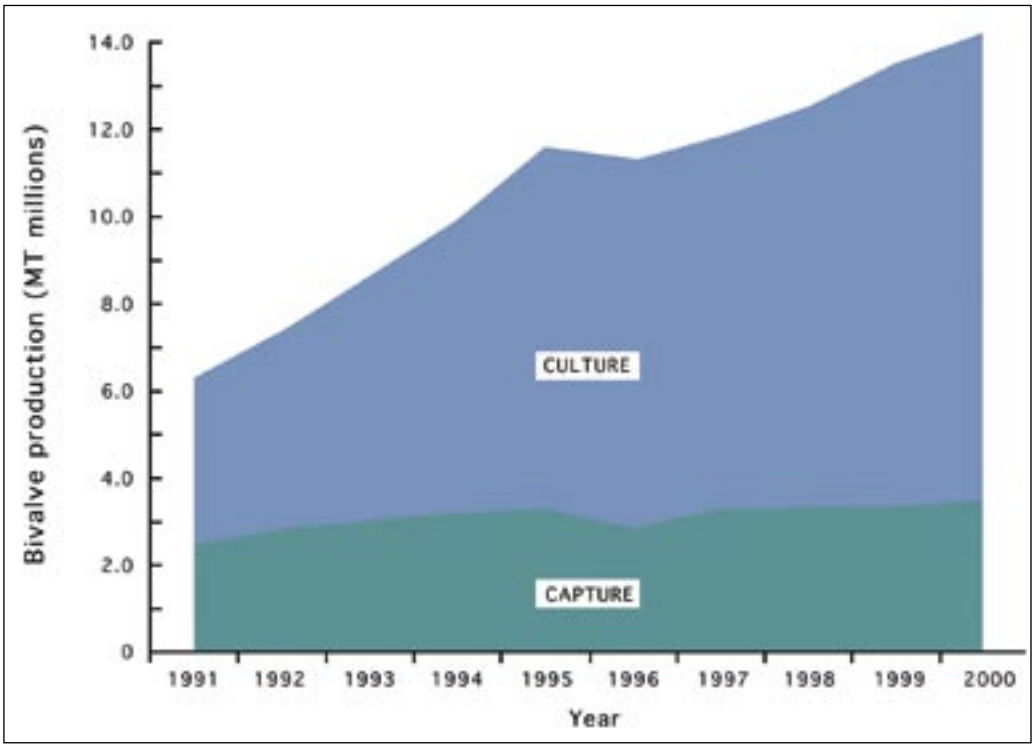

Figure 1: Production (in millions of tonnes) of bivalves from capture fisheries and aquaculture during the decade from 1991 to 2000 (from FAO Yearbooks of Fishery Statistics).

The global trend in the growth of human consumption of seafood will undoubtedly continue. Seafood constitutes an important and essential part of the diet of many people in the world and the need for increased production in these countries will persist as the human population expands. In some countries, seafood is recognized as an important and healthy part of the human diet and demand for seafood products in these countries will also grow. Most of the demand for seafood is and will continue to be for finfish, but the production and harvest of molluscs, particularly bivalves, will also be important in meeting rising demand. While the harvest of natural bivalve stocks will continue to prove significant, many wild stocks are probably already being harvested at or near maximum sustainable limits, in some places and stocks may even be overharvested. Aquaculture is the alternative to the harvest of wild stocks.

During the period 1991–2000, bivalve landings from capture fisheries increased only slightly from about 2.5–3.5 million tonnes, while landings from culture operations more than doubled during this same period, increasing from 6.3–14 million tonnes (Figure 2). About 75% of the world's bivalve production in 2000 resulted from some form of culture operation.

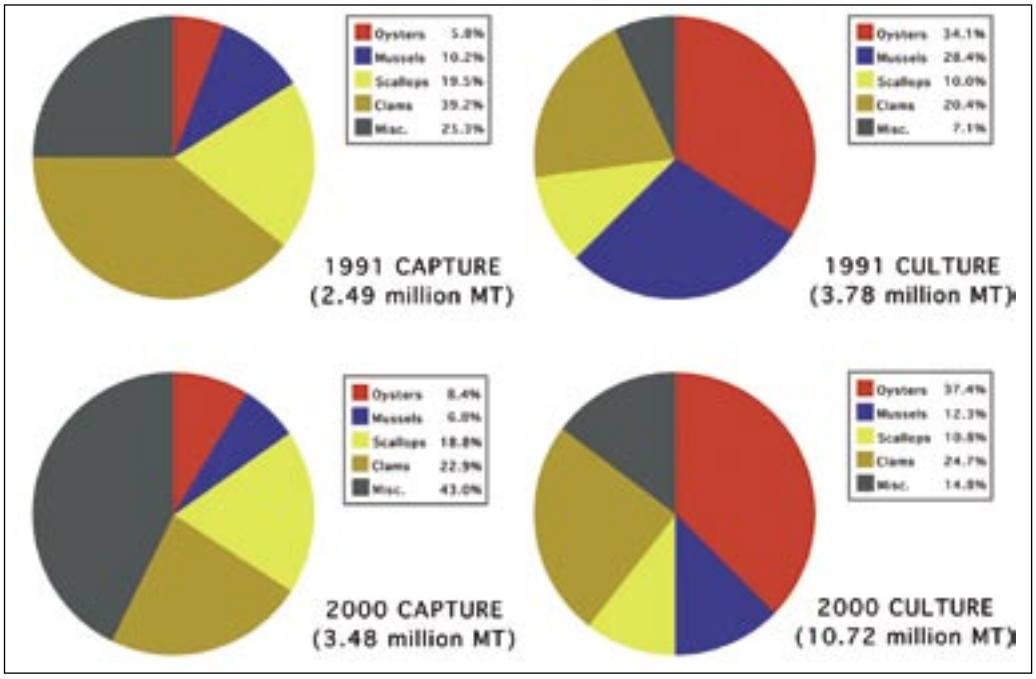

Figure 2: A comparison of production from capture fisheries and aquaculture broken down into the relative contributions of the major groups of bivalves in 1991 and 2000.

Bivalves are ideal animals for aquaculture: they are herbivores requiring no additional feeding apart from the natural algae content of seawater and generally minimum husbandry. Altough they have been cultured for hundreds of years advances in culture technology in recent years have led to significantly higher production. Continued improvements in culture methodology and technology will be required to meet increasing demand and also to make bivalve culture more economically attractive to both investors and people who wish to become shellfish farmers. The need for greater efficiency in culture operations will become even more essential in the future as areas where shellfish farming can be undertaken in the world are limited and may become more restricted as human populations and coastal urbanization grow.

A primary requisite in any culture or farming operation is an abundant, reliable and inexpensive supply of juveniles (seed). At present, most bivalve culture operations in the world obtain their seed by collecting natural sets. A substrate (cultch) is placed in breeding areas to collect metamorphosing larvae and the juveniles collected are transferred to growing areas for culture (growout) to market size. In other operations, juveniles are gathered from areas of natural abundance and are transported to growing areas that may be distant from the source of the seed. Collection of juveniles from areas of natural recruitment will continue to be important in bivalve culture operations worldwide and undoubtedly can be expanded in some areas to meet increased seed requirements for culture operations. The importance of these breeding areas must be recognized and every effort made to conserve them.

In many other culture locations, natural breeding areas do not exist to provide seed or, if they exist, they cannot produce sufficient seed to meet growout requirements, or breeding is erratic and an annually reliable source of seed cannot be guaranteed. There are other considerations that may make collection of natural sets unsuitable as the supply for culture operations. Growers in some areas may wish to develop and culture a particular race or strain of bivalve to suit their individual needs and juveniles for such operations are not available from the local or nearby environment. Another consideration is that growers may wish to introduce a non-indigenous (exotic) species

but a seed source is not available. The alternative to collection of natural sets of bivalves is to produce seed in a hatchery. Bivalve hatcheries have existed for over half a century and are well-established in several countries. They are an integral part of many culture operations and the major or sole source of seed. In the future, bivalve hatcheries will undoubtedly become more important to culture operations as shellfish farming becomes more specialized and the need for seed increases.

Hatcheries have several advantages over collection of natural sets. They are reliable and can supply growers with their seed requirements when it is convenient to them – often much earlier in a growing season than natural sets would occur. They can supply seed that is not available to farmers from natural sets, for example, genetic strains that have improved biological characteristics for farming operations in local areas or a supply of exotic bivalve seed. Cost is the main disadvantage of producing seed in hatcheries: it is more expensive to propagate seed in a facility than collecting it from natural sets. Although economic factors have probably caused the demise of some bivalve hatcheries in the past, recent improvements in technology have greatly enhanced both reliability and financial viability whereby it is possible to produce seed at competitive prices. In some parts of the world hatcheries are the only source of seed for the commercial culture industry but there is ample scope to make them more efficient and more widely accepted as the preferred source of seed.

Constructing and operating a bivalve hatchery is a large and expensive undertaking and careful thought must be given to the developmental phase of a new venture otherwise it will likely end in failure. There is no single plan to construct and operate a bivalve hatchery; indeed many have begun as a small operation and simply grown as markets for their products expanded. Hatcheries vary greatly in their design, configuration and construction from site to site depending on species cultured, target production levels and, most of all, the local conditions and personal preferences of owners/operators. However, the basics for any bivalve hatchery are the same and include a method to condition and spawn adults, rear and set larvae, rear the juveniles to an acceptable size, together with a facility to produce large quantities of food (algae) to feed all stages of the production cycle. Although these essential elements are the same in every hatchery, there are variations in technology and the efficiency of each phase of operation must continually be improved to make hatcheries increasingly more profitable.

This publication is intended to serve as a "how to" manual for bivalve hatcheries. While other documents also describe bivalve hatcheries many are becoming out-of-date and do not include the most recent technological improvements. This manual is intended as a practical introduction to the fundamentals of hatchery operation for new entrants in this field. It will also allow a potential investor to assess whether he/she wishes to build and operate a bivalve hatchery and become involved in the business of producing seed for the culture industry. This is not meant to be a scientific publication in the accepted sense and much of the content is based on the authors' own experience gained over a collective period of 80 or more years. Although there is an extensive literature pertinent to bivalve hatcheries, many of the more practical publications have had limited circulation and are out of print or are only available through specialist library services. Many readers may not be able to access such written material and so efforts have been made to make this manual as comprehensive and available as possible. Rather than including extensive references in the text, a suggested reading list is given at the end of each major section to provide further sources of information on particular subjects and aspects of operation.

Part 1

Site selection, hatchery design and economic considerations

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1.1 SITE SELECTION

1.1.1 Introduction

Selecting the proper site for a bivalve hatchery is the most important consideration when deciding to build a hatchery, yet it is a factor that has often been overlooked when some hatcheries were built. Several factors may have contributed to the inappropriate location of a facility including the lack of one or more of the components of the essential infrastructure, e.g. land availability at reasonable cost, the local availability of electricity and freshwater, a qualified labour force, or good communications. A further consideration has often been that an individual or company may have wished to build a hatchery at a site adjacent to an existing bivalve growout operation. In such cases the hatchery became an add-on feature to an existing operation. Yet, another factor is that an individual or company may own or have rights to a particular location and it proves to be the only place where a hatchery could conveniently be built. While it is true that it may be impossible to build a hatchery at an ideal location, nevertheless certain criteria must be met or a hatchery will likely be doomed to failure.

1.1.2 Considerations

1.1.2.1 Government regulations

The first consideration is to determine if government regulations permit construction of a bivalve hatchery at the desired site. This can be done quickly by making enquiries of local, state, provincial or federal authorities. If regulations do not permit construction of a hatchery at the desired site one must decide if it is preferable to find another location where construction is permitted, or attempt to change existing government regulations to allow construction at the desired site.

It is likely that a number of permits and licences will be required to ensure compliance with local building codes and national and local environmental regulations before any construction is allowed. This can be a lengthy, costly and time consuming process and may require an assessment of the potential impact of the hatchery on the local environment before permission is either granted or not granted to begin the construction phase.

1.1.2.2 Seawater quality

Before committing to what is considered to be a suitable location for a hatchery it is of paramount importance to ensure that good quality seawater exists year-round at the prospective site. This point cannot be overemphasized. If a good seawater source is not available, it will be difficult, if not impossible, to develop an efficient and profitable hatchery operation. For this reason every effort should be made to obtain as much information as possible about the quality of the seawater throughout the year at a potential site - or sites. Information is required not only for surface waters but also for the entire water column, since thermoclines may develop or upwelling may occur periodically. If previous oceanographic surveys have been undertaken in the area, copies of the data should be examined. If such surveys have not been undertaken, one should be prepared to undertake a detailed sampling of the waters at the proposed site for at least a year.

Environmental parameters of seawater that need to be examined will depend in part on geographic location and the intended species for culture. Bivalve larvae as well as juveniles and adults have strict physiological requirements, such as water temperature, salinity and oxygen levels and these must be maintained in a hatchery operation. Water temperatures are higher in the tropics than in temperate regions and indigenous bivalves are well adapted to tolerate these conditions. But in a hatchery situation temperatures must not be allowed to drop too low or larval and juvenile survival and growth will be adversely affected. In temperate areas water temperatures must not be allowed to exceed upper or lower lethal levels to larvae and juveniles. Salinity can vary widely and tolerance to these fluctuations differs among bivalve species. Some require high oceanic levels of salinity while euryhaline (estuarine and brackish water) species exhibit much wider tolerance. Periods of heavy rainfall may not only cause periods of low salinity, but heavy associated runoff can increase quantities of silt and other materials which may lead to problems in a hatchery. Dense concentrations (blooms) of some marine algal and bacteria species may release toxic substances that may cause reductions in both the survival and growth of bivalve larvae or juveniles, or mass mortalities in extreme cases. As much data as possible on these parameters should be collected prior to deciding on the adequacy of a site for a bivalve hatchery. Remedial measures to improve inadequate quality seawater can be extremely costly and may adversely effect the profitability of a venture.

Locations possibly influenced by effluents discharged from industrial plants should be avoided. The lethal and sublethal effects of many industrial pollutants are not completely understood, nor are the additive effects they may exert when several industries are

discharging a range of potentially toxic wastes in nearby waters. Effects of such effluents can be extremely damaging to bivalve larvae. For example, an anti-fouling ingredient added to marine paints, tributyltin (TBT), has been found to be highly lethal to bivalve larvae even at concentrations of a few parts per billion. Drawing a seawater supply from the vicinity of marinas and commercial docks needs to be avoided. If feasible it is advisable to undertake bioassay studies using bivalve embryos to help determine the quality of the water at the potential hatchery site. The presence of deleterious materials may be transitory or seasonal in nature, so sampling for bioassays should be carried out over a period of at least a year and be done preferably on a weekly basis.

Agricultural – forestry included – and domestic sources of pollution should also be avoided. It has recently been shown that runoff from some cultivated lands can carry concentrations of pesticides at levels deleterious to the growth and survival of bivalve larvae. Domestic pollution may not only contain pollutants that are toxic to bivalve larvae but the high organic content can cause depletion of oxygen levels and increased levels of bacteria that could also lead to reduced growth and mortalities of larvae.

Another consideration when deciding upon the location of a bivalve hatchery is whether "civilization" will soon encroach on the site. Urbanization with its ancillary problems is one of the main concerns in bivalve culture. If the site will soon be encompassed by urbanization then every effort must be made to ensure that sources of potential pollution will be kept to a minimum. This will require working closely with planners and developers.

1.1.2.3 Siting the hatchery

The hatchery should be located close to the ocean so that the distance required to pump water is kept to a minimum. This negates the necessity of having to maintain great lengths of pipe. It should also be located as close to sea level as possible to avoid problems of pumping water any great vertical distance. If fluctuations in surface seawater temperature and salinity occur regularly, the intakes for the pipes will need to be located at depth (up to 20 m below the surface) to maintain more constant water temperature and salinity. Depending on the nature of the geological strata, it may be possible to drill wells close to the shore to access seawater aquifers. A water source of this nature will be at a more constant temperature year-round and will already be pre-filtered by percolation through the strata. It may, however, require oxygenating before use. It is always wise to consult with a suitably qualified engineer when making decisions on the best methodology and technology to procure the water supply.

Sufficient area needs to be available at the site to accommodate the hatchery and ancillary buildings and also to allow for any future expansion. The need for adequate surveillance should also be considered.

Other considerations that need to be kept in mind for a site include an adequate supply of electrical power, a source of freshwater and a skilled labour force to operate the hatchery. Good communications should exist so that required materials and supplies can be acquired quickly and larvae and seed can be quickly shipped to their various destinations. The proximity of institutions such as universities, government laboratories and libraries should also be considered since such resources can be of great assistance in operations and in helping towards solutions to problems that may arise.

It is a worthwhile preliminary to prepare a check list of parameters that must be met, or at least reviewed, when considering a site for a bivalve hatchery and work through the list to ensure the site meets as many of the requirements as possible.

1.2 HATCHERY DESIGN CONSIDERATIONS

1.2.1 Introduction

There is no rigid design for a bivalve hatchery. The layout of hatcheries varies from site to site, with species produced, geographic location, funds available, the target production species and personal preferences (Figure 3). Some hatcheries are small and supply seed for their own bivalve on-growing culture operations. Others are large and may only produce seed for sale, or they may produce seed for their own operations and also an excess to sell to other growers. Hatcheries may or may not include a nursery component and some may only produce mature larvae for shipment elsewhere while others may grow and supply seed varying in size from 1 to 12 mm shell length. Much depends upon the nature, requirements and the level of sophistication of the growout operations that collectively make the customer base.

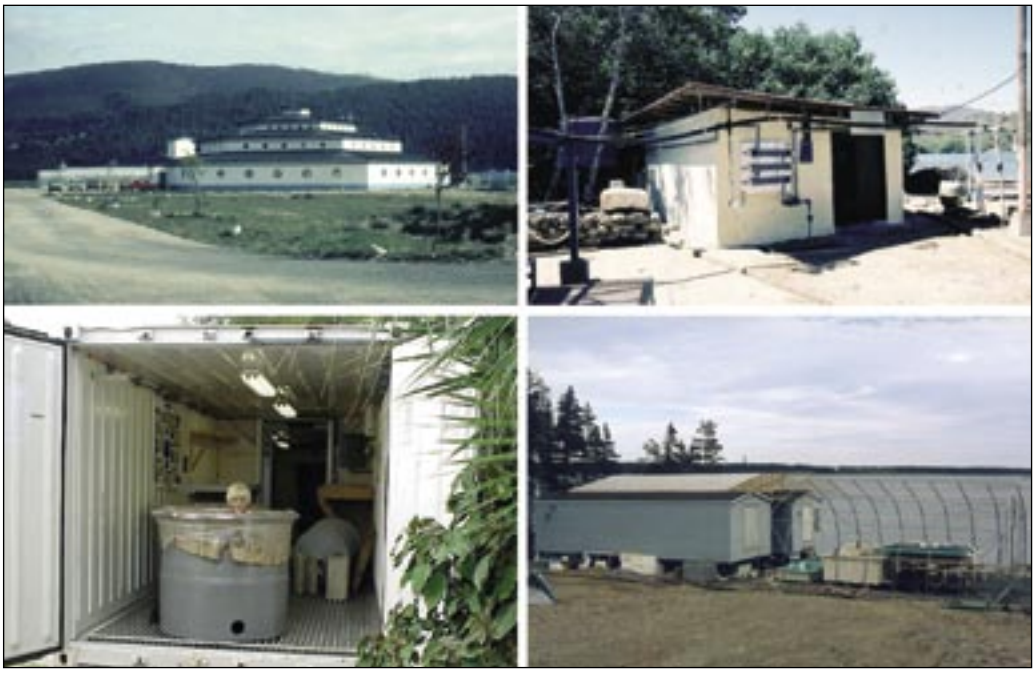

Figure 3: A selection of photographs of hatcheries depicting the variability in size and sophistication of construction that exists around the world. Clockwise from top left: Tinamenor S.A. (Pesues, Spain), Turpiolito hatchery, (Gulf of Cariaco, Venezuela), Bermuda Biological Station's scallop hatchery based on insulated cargo containers and the SMS oyster hatchery (Point Pleasant, Nova Scotia, Canada).

Many hatcheries were built with little advance planning or forethought for possible future development. A hatchery was built to produce a required quantity of seed and when the initial objective was achieved a decision was made to expand and add extra capacity. The resulting facility is often neither efficient nor worker friendly. Other hatcheries were built to produce seed of a single species but other species are produced now and the resulting hatchery is somewhat inefficient in its new role.

Considerable time will be saved and many frustrations avoided if a hatchery is carefully planned before construction begins. Several considerations must be remembered when designing a hatchery and two are of great importance. Firstly, the hatchery operation must be worker friendly and efficient to make the operation as profitable as possible, and secondly, the need for future expansion must be kept in mind.

There are two basic parts to a bivalve hatchery, the salt water system and the physical plant.

1.2.2 Seawater system

The need for a supply of high quality seawater was previously discussed. It is important to ensure that the seawater source and system to pump and treat it is located conveniently close to the hatchery and optimum use made of it to keep capital and operating costs to a minimum.

The hatchery should be located as close to sea level as possible to avoid lifting water. Intakes for the seawater should be as short as possible and conveniently located so they can be serviced and maintained with minimum effort. Intakes for the salt water should be located at depth to avoid fluctuations in temperature and salinity and also to reduce the number of organisms and amount of detritus that will enter the system. In temperate areas, intakes should be located below any thermocline that occurs in summer to reduce temperature variability. In areas where periods of heavy rain occur, the intakes should be deep enough to avoid sudden fluctuations in salinity and heavy siltation that may occur with the rains. Intakes at depth avoid major plankton blooms, some of which may be harmful to bivalve larvae and also greatly diminishes the number of fouling organisms entering the system. Fouling organisms can settle in pipes and greatly reduce water flow into the hatchery. Many of the above sources of variability can be avoided by accessing seawater from drilled wells. This possibility should be investigated before any other solution is considered.

Size of pumps and the diameter of the pipes required will depend on the scale of the operation and the volumes of seawater required to meet all aspects of production. Pumps are available through commercial outlets and the type and size of pump required can be determined after discussions with dealers. It is important to ensure that surfaces that come into contact with the seawater are non toxic. Most plastics, cast iron and certain grades of stainless steel are suitable. Pumps that contain mild steel or brass components should be avoided.

Seawater pumped directly from the ocean is first passed through sand filters that filter out most particulate material greater than 20-40 μm in size (Figure 4). A well maintained sand filter will remove the major portion of detritus and organisms from the water that may interfere with bivalve larvae. It also eliminates many of the fouling organisms that could settle and grow in pipes in the hatchery. They not only can cause problems with water flow but when they die they can produce anaerobic conditions that can be toxic to bivalve larvae. They may also harbour and eliminate bacteria that can be deleterious to larvae. Sand filters are commercially available and are the same or similar to those used to filter water in swimming pools. A series of two or more such filters are generally installed and they are regularly back-flushed to avoid clogging of the filter media. Other types of filters may be used depending on personal preference and cost considerations. Self-cleaning, rotating drum filters offer an alternative to remove larger particulate material and large surface area cartridge or bag filters are available and are extremely effective in removing smaller sized particulates.

Another method to obtain seawater for a hatchery is to pump it from seawater wells. This has become the preferred method for hatcheries to obtain their water supply in recent years. A well is dug or drilled close to the hatchery and is deep enough to provide a sufficient supply of seawater for the hatchery. Water from such wells is of high quality and generally has a constant temperature and salinity. It has already been filtered through sedimentary or porous rock, contains little detritus and few, if any, fouling organisms. Water abstracted in this way requires little if any further filtration. Constructing seawater wells can be expensive initially but the high capital cost is offset by reduced operating costs.

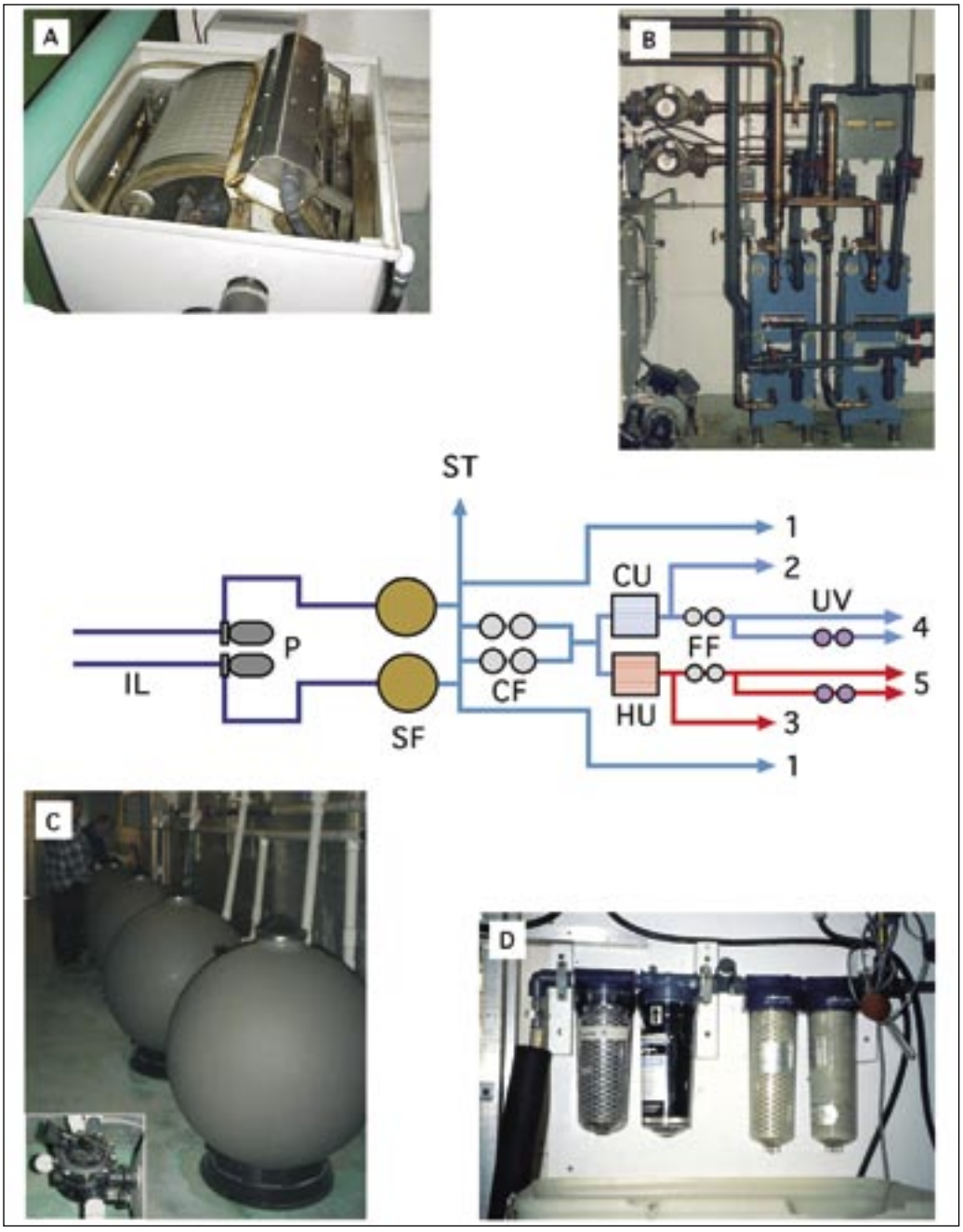

Figure 4: A diagram of the various stages of seawater treatment for hatchery usage from the intake pipes (IL) to the points at which water is used in the different aspects of the operation (1 to 5). Key: P – seawater pumps; SF – sand filters (photograph **C**) or alternatively self-cleaning drum filters (photograph **A**); ST – to storage tanks (if required); CF – cartridge filters of 20 μm and 10 μm; CU – seawater chilling unit (if required); HU – seawater heating unit (if required – photograph **B**); FF – final filtration (5 μm and 1 or 2 μm - photograph **D**); UV – ultra-violet light disinfecting units (if required).

A guide to typical usages (treatment levels vary from hatchery to hatchery):

1 – Unheated, sand-filtered water for broodstock and larger juveniles (if water requires to be heated, then 3).

2 – Chilled seawater filtered to 10 μm for spawning broodstock or for large-scale algal culture of hardy species. Chilled (or ambient temperature) seawater is often mixed with heated seawater to provide intermediate temperatures for a variety of purposes.

3 – Heated seawater filtered to 10 μm for conditioning and spawning broodstock and for growing larger spat. Some hatcheries have a separate heating system for either unfiltered or sand-filtered seawater for broodstock conditioning.

4 – Chilled water filtered to 1 μm and either UV-disinfected or not for algal culture.

5 – Heated water filtered to 1 μm and either UV-disinfected or not for larval culture.

After filtration, all or part of the seawater may be pumped to a storage tank that may be made of either concrete or fibreglass. Use of a storage tank may be a matter of preference and many hatcheries do not have them. They are useful when water can only be obtained at a particular time, e.g. at high tide. Sometimes this method is used in areas where electrical power is unreliable to ensure a supply of seawater is always available. Sufficient water is pumped into the storage tank so it can supply the hatchery until the tank can be refilled. The tank is located at height so that the effect of gravity maintains a sufficient water flow through the hatchery. In other hatcheries, the salt water system is a flow-through system and water is pumped continuously through the hatchery for use where it is needed and then is discharged to waste. Recently, many hatcheries have installed recirculating or partial recirculating systems to reduce operating costs. This is particularly true if seawater is in short supply or if it has been heated or chilled. Recirculated water may be passed over biologically activated filters to remove metabolic wastes of the animals and held before it is reused. If the water has been heated or chilled it may be passed through heat exchangers to partially heat or chill incoming water and thus reduce energy costs.

All piping must be non-toxic, usually PVC (polyvinylchloride) schedule 40 or 80, although ABS or polyethylene pipes and fittings are also sometimes used as alternatives. The diameter of the pipes depends on water demand. In most hatcheries the main distribution lines within the hatchery are 50 mm diameter or less although the main intake pipes may be up to 15 cm diameter. The piping should be well supported and high enough off the ground so that it is out of the way but readily accessible for cleaning. Valves and outlets should be conveniently located. If the water is sufficiently filtered there should be little need to clean the lines frequently. Cleaning may be required periodically, hence, it is important to have clean-out ports or screw unions located conveniently so that the lines can be easily cleaned *in situ* or quickly dismantled for more thorough cleaning.

In most hatcheries in temperate areas there needs to be the capability to heat and sometimes to chill part of the seawater supply. There are commercial units available for this purpose and discussions and calculations on required capacity with dealers will ensure that an adequate supply at the required temperatures is available. Again, it is essential to ensure that surfaces of such units coming in contact with the seawater are non-toxic to bivalve larvae. Most commercially available heat exchange units utilize titanium as the heat transfer surface and this material is preferred by most hatcheries.

Hatchery managers may wish to sterilize (or more correctly, disinfect) all or part of the seawater before use, particularly if disease problems arise. Seawater can be sterilized with either UV (ultra-violet) light or ozone. Commercial units are available and simple calculations will determine the size of unit that is required. Commercial units are normally rated for their performance in sterilizing freshwater. In seawater situations where organic loadings and turbidity caused by colloidal materials are frequently higher than for freshwater, it is recommended that such units are used at half (or less) of the recommended flow rate for satisfactory performance. If UV-light sterilization is used, the water must be filtered to about 1 μm prior to sterilization since UV-light is readily absorbed by particles in the water reducing the efficiency of the unit. Filtration can easily be incorporated into a UV unit and many available units have both filters and the UV lamps combined.

Government regulations may exist in some areas that control the discharge of effluent from a hatchery. Before constructing a hatchery, government regulations controlling discharge of effluents should be reviewed and if they exist they must be followed.

Large floor drains sunk into the floors of wet areas are essential and should be located conveniently throughout the hatchery. Periodically large volumes of water must be discharged, e.g. when emptying tanks, and the drains must be able to handle such discharges.

Some hatcheries may wish to breed exotic species or strains or races of a species that do not occur locally. Depending on government regulations, this may entail installation of a quarantine facility to ensure that pests, parasites and diseases are not introduced with the exotic species or larvae accidentally escape into the natural environment. This will require a separate drainage system in the area of the hatchery designated for quarantine that empties into special holding tanks where the effluent can be sterilized with a strong hypochlorite solution. The sterilized water is then treated with thiosulphate to neutralize any residual chlorine before it is discharged back into the environment. Quarantine facilities may require a separate room to hold, condition and spawn adults. Drains from this room will also empty into the quarantine treatment tanks.

1.2.3 The physical plant

Careful thought should be given to hatchery design to permit convenient and efficient operations. The hatchery should be adaptable so that changes can be made readily without involving major rebuilding. In some hatcheries, tanks have been constructed of concrete and changes cannot be made easily. It is much better to have plastic or fibreglass tanks so they can be easily moved or changed if needed. Floors should be of concrete and have sufficient drains. All surfaces should be covered with a durable, mildew resistant finish to facilitate cleaning. Floor standing cabinets and storage units made from wood should be mounted on concrete plinths to prevent them being damaged by immersion in seawater. Where this is not possible, wood surfaces need to be painted with a good quality epoxy resin.

A hatchery has several areas that are all inter-related. For convenience they have been divided into algal culture, broodstock conditioning and spawning, larval rearing, juvenile culture and service areas (Figure 5).

Figure 5: A generalized floor plan for a purpose-built bivalve hatchery (see the following text for explanation).

1.2.3.1 Algal culture facility

The success of a bivalve hatchery depends on the production of algae. Large quantities of high quality algae must be available when needed. It is a most important part of any hatchery and considerable thought should be given to providing a sufficient and efficient working area for this purpose (AC – Figure 5). Since algae are used in all phases of production, the facility should be located centrally and conveniently. Space required for algal culture depends partly on levels of production, methods of culture and whether algae will be raised entirely inside the hatchery with artificial illumination, or if it will be raised outside under natural light, or a combination of the two. A well ventilated greenhouse is required if algae is grown in natural light and this structure needs to be placed so as to obtain the maximum amount of sunlight. Shading may be need to protect younger, less dense cultures from strong sunlight.

A small room is required to maintain stock (also known as master) cultures of algae (TR). Dimensions vary but it can be as small as 2 x 3 m. The room should be insulated and the temperature kept cool. Shelving is needed with flourescent lights at the back to provide the light source. An air supply is also required. Test tubes with algal slants and small flasks with stock culture that are monospecific and axenic are kept in this room often in a refrigerated, illuminated incubator. Methods are described in a Part 3.

The next phase of culture uses stock cultures from the cool room and grows them in 4 l flasks and 20 l carboys against a bank of fluorescent lamps (SCR). This can be part of the main algal culture area or a small room off it. The space required depends on the number of species and amount of algae being produced. This area requires an air and carbon dioxide supply and needs to be kept at 15 to 18°C. Another adjacent small room (AR) houses an autoclave (a), which is used to heat sterilize medium for the small cultures. Some hatcheries use alternative methods to prepare culture medium and these are described in Part 3.

The size of the main algal culture area depends on the number of species being cultured and the amount of algae required. This area can occupy a substantial part of the hatchery. If most of the algae is raised inside the hatchery by the batch culture method then there must be sufficient space for a series of tanks that can measure up to 3-4 m in diameter and 2 m in depth. If the bag or tall cylinder culture methods are used the amount of floor area required can be reduced. Ballasts for fluorescent lamps used to illuminate cultures need to be of the "cool running" type or isolated in a separate area from which the heat they generate can be dissipated. This area should ideally be maintained at 15 to 20°C.

In many hatcheries, considerable portions of the algae, if not all, are raised in greenhouses. These can be stand-alone structures or attached to one side of the hatchery - preferably the south side in the Northern Hemisphere and northern side in the Southern Hemisphere, so as to receive as much sunlight as possible. The size of the greenhouse depends on the method of culture and quantities of algae that need to be produced.

Sufficient electrical power must be available for artificial lighting when natural sunlight is inadequate. Compressed air and carbon dioxide supplies are essential. There should be adequate ventilation or installed air-conditioning to maintain temperatures at or below 20°C on days when bright sunlight heats the facility. A generator will be required in areas where the electricity supply is unreliable and may be off for several hours or more at a time. Although survival of the algal cultures is not at risk in the absence of light for an hour or two, cultures need to be aerated. Diatoms will settle to the bottom of cultures without aeration and cultures may collapse.

1.2.3.2 Broodstock holding and spawning area

Space is required to hold and condition broodstock (BC – Figure 5). The amount of space needed depends in part on the number of species being held and whether some or most of the conditioning will be undertaken in the open environment rather than in the hatchery. Heated or chilled seawater may be required for this aspect of operation at certain times of the year. The ability to isolate tanks so that photoperiod can be adjusted is desirable since it has been shown that varying periods of light and dark can affect gonadal maturation.

Space is required for spawning trays (sp) but this can be part of the larval rearing area since the space is not require continuously. Spawning trays or dishes can be stored when not in use. Methods for broodstock conditioning, spawning and fertilization are described in Part 4.

1.2.3.3 Larval culture area

Another major part of the hatchery is occupied by the larval rearing facility (LC) and dimensions of this area depend on the scale of production. The space is occupied with tanks, the number needed depending on production levels and the techniques used to rear larvae. On the Pacific coast of North America the tendency has been to raise larvae at low densities of 2-3 per ml in large tanks that measure 3-4 m in diameter, 4-5 m in height and hold 40 000 to 50 000 l. In other hatcheries larvae are raised in smaller tanks of up to 5 000 l in volume at higher larval densities. A manager must decide on required production to meet market demand and the methodology that will be used to rear larvae when planning this part of the hatchery.

Larval rearing tanks are generally made of fibreglass or of a suitable plastic and should be thoroughly leached prior to use. Regardless of the size of tanks used, there should be large sunken floor drains to handle large volumes of water when the tanks are drained. A preparation area in the larvae culture room (P) is required for washing, grading, counting and measuring larvae and for accommodating the equipment used for these purposes. This area requires cupboards and shelves for the storage of equipment when not in use.

1.2.3.4 Juvenile culture area

Once mature larvae have set (settled and begun metamorphosis) they are moved to tanks in the juvenile culture room (JC) for culture until they are of sufficient size to transfer to nursery systems, which may be part of the hatchery or at another location. This is generally when the juveniles (known as spat) exceed 2 mm shell length. The size and types of tanks in terms of volume and surface area used for this purpose vary according to species.

Mature larvae are set in the hatchery or in outside (sometimes remote) facilities. When this procedure occurs within the hatchery it is generally done in the larval culture area, frequently directly in the larval tanks. Space for additional tanks may be required specifically for this process. Spat (early juveniles) are subsequently transferred to tanks systems in a separate area specifically for juvenile culture (JC). The size and types of tanks in terms of volume and surface area used for this purpose vary according to species. They may be upwellers, downwellers or tray systems of varying configuration and the juveniles are grown in these until they exceed 2 mm shell length. To grow spat to a larger size within the hatchery on cultured food is uneconomic since food requirement increases exponentially with size. If the nursery system is located outside the hatchery, sufficient space must be allotted for this operation.

Methods for the culture larvae are described in Part 5 and for spat in Part 6.

1.2.3.5 Other space requirements

Hatcheries dealing with broodstock from outside the immediate region or with exotic species may, as already mentioned, be required to quarantine stock and rear the progeny in isolation. Such hatcheries will include a quarantine room (QR) for this purpose, the effluent from which is discharged into treatment tanks (ET).

Other rooms include a dry laboratory (DL), office (O) and bathroom (BR). The dry laboratory is where algal transfers can be made (if no specific space is allocated elsewhere), chemicals weighed and mixed, microscopes kept for examining cultures, records maintained and for the storage of scientific equipment.

Static machinery such as the main pumps, sand filters and pre-filters (to remove particles down to 10 μm), seawater heating/chilling units, furnaces, the air ventilation system, air blowers/compressors, a standby generator for emergency power supply, together with electrical panels and control equipment, are housed in a soundproof machinery room (MR). Duplication of essential equipment is preferred in the event of electrical or mechanical failure. Compressed air is required in all phases of culture and carbon dioxide is required for algal culture. In many hatcheries the seawater intake pumps and sand filters are located in a separate pump house close to the point of intake and the final filtration of seawater may take place at the point of use rather than at a central, fine filtration unit.

Since storage is always an issue in a hatchery, it is useful to have a large general-purpose area (GPA) that can be used for storing materials and equipment, packing seed and as a workshop. Most of the working areas should be fitted with benches and sinks (s).

It is preferred that the various parts of the hatchery can be isolated in the event of a disease outbreak.

1.3 ECONOMIC CONSIDERATIONS

A bivalve hatchery is a business and like any other business it must be run efficiently and it must be economically viable. Government subsidies or grants may help offset costs particularly during initial stages of operation, but eventually the hatchery must stand on its own and be profitable. The economics of building and operating a bivalve hatchery will vary from business to business, from area to area and country to country but eventually all must turn a profit.

Hatcheries are expensive operations. Considerable capital is required to build a hatchery and finance operations. The owner must have sufficient working capital to carry on operations until income is generated. Before deciding to build a hatchery, one needs to carefully examine all facets of building and operating a hatchery and determine at what level a hatchery will be economically viable. Many costs need to be considered including purchase of the site, construction of the hatchery, installation of the seawater system, equipment needed for all phases of production, maintenance, supplies and utility overheads, loan repayments and the need for a trained staff.

Profitability can vary greatly with other factors including geographic area, the scale of the operation and whether it is part of a fully integrated bivalve culture operation.

In temperate areas a major operating cost is heating (and chilling) seawater, but this cost is generally avoided in tropical areas. This may influence location of a hatchery in temperate areas to sites where warm seawater exists at least for part of the year to help reduce heating costs.

Some hatcheries are small family operated ventures that only produce sufficient seed for their own culture needs. Such hatcheries are generally operated for only a few months a year, production is limited, and costs are much lower than for other larger hatcheries.

Large hatcheries may be part of a fully integrated bivalve culture operation or they may be in business only to supply seed. Where a hatchery is part of an integrated culture operation, the hatchery may be operated to simply break even and show no profit or may even operate at a small loss. Profits for the company are made in other phases of the culture operation. Where the hatchery exists only to produce seed to sell to other growers, a profit must be made solely on the hatchery operation. This emphasizes the fact that before building a hatchery one must make an accurate assessment of the market for whatever seed will be produced and not only the quantity of seed that can be sold but also the price people are willing to pay for seed.

Another consideration in operating a bivalve hatchery is that a critical level of production must be maintained to permit profitability. A hatchery cannot exist by simply producing a few thousand juveniles each year. The cost to do so is too high. In fact the costs associated with producing a few thousand juveniles are almost the same as producing several million – economies of scale apply. A manager must determine the critical level of production that needs to be attained to make the operation profitable and this again points to the necessity of knowing the extent and value of the market for the product.

Accurate records of costs, production and sales must be kept to assess whether the hatchery is being profitably run.

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Part 2

Basic bivalve biology: taxonomy, anatomy and life history

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2.1 TAXONOMY AND ANATOMY

2.1.1 Introduction

Some knowledge of bivalve biology is necessary to understand operations of a bivalve hatchery and to assist in solving problems that arise. It is not the intention here to give a detailed description of bivalve biology but to provide a brief resume of information pertinent to operations of a hatchery. There are several excellent texts on molluscan biology readily available and there are extensive reviews of groups and individual species of oysters, scallops, mussels and clams. The reader is directed to these publications at the end of this section for additional information.

Bivalves belong to the phylum Mollusca, a group that includes such diverse animals as chitons (chain shells), gastropods, tusk shells, cephalopods (squid and octopus) as well as clams, oysters, mussels and scallops. The phylum has six classes of which one is Lamellibranchia or Bivalvia. These animals are compressed laterally and the soft body parts are completely or partially enclosed by the shell, which is composed of two hinged valves. The gills or ctenidia of animals in this class are well developed organs, specialized for feeding as well as for respiration.

2.1.2 External anatomy

The most prominent feature of bivalves is the two valves of the shell that may or may not be equal and may or may not completely enclose the inner soft parts. They have a variety of shapes and colours depending on species. The valves are composed mostly of calcium carbonate and have three layers; the inner or nacreous layer, the middle or prismatic layer that forms most of the shell, and the outer layer or periostacum, a brown leathery layer which is often missing through abrasion or weathering in older animals.

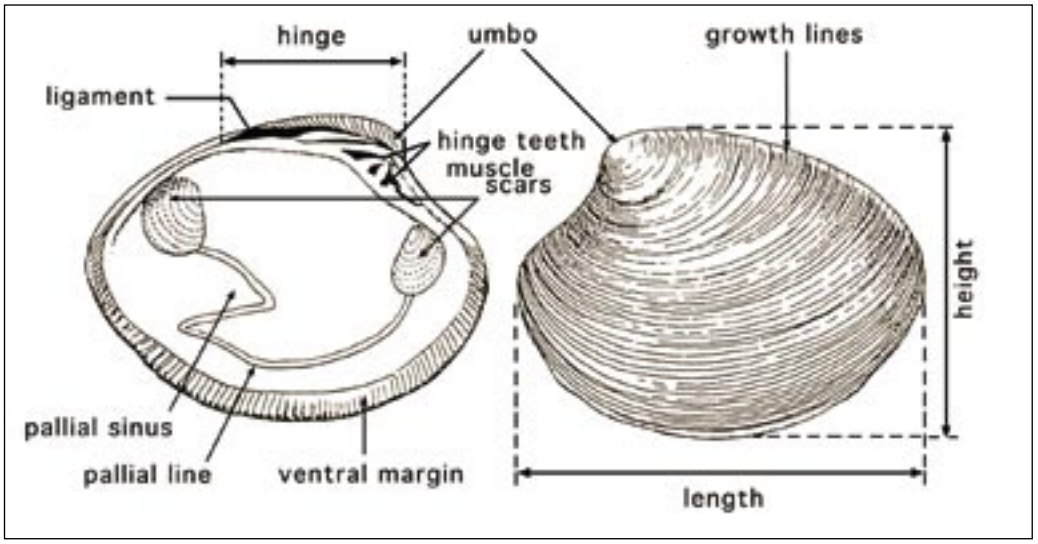

Figure 6: External and internal features of the shell valves of the hard shell clam, *Mercenaria mercenaria*. Modified from Cesari and Pellizzato, 1990.

Bivalves do not have obvious head or tail regions, but anatomical terms used to describe these areas in other animals are applied to them. The umbo or hinge area, where the valves are joined together, is the dorsal part of the animal (Figure 6). The region opposite is the ventral margin. In species with obvious siphons (clams), the foot is in the anterior-ventral position and the siphons are in the posterior area (Figure 7). In oysters the anterior area is at the hinge and in scallops it is where the mouth and rudimentary foot are located.

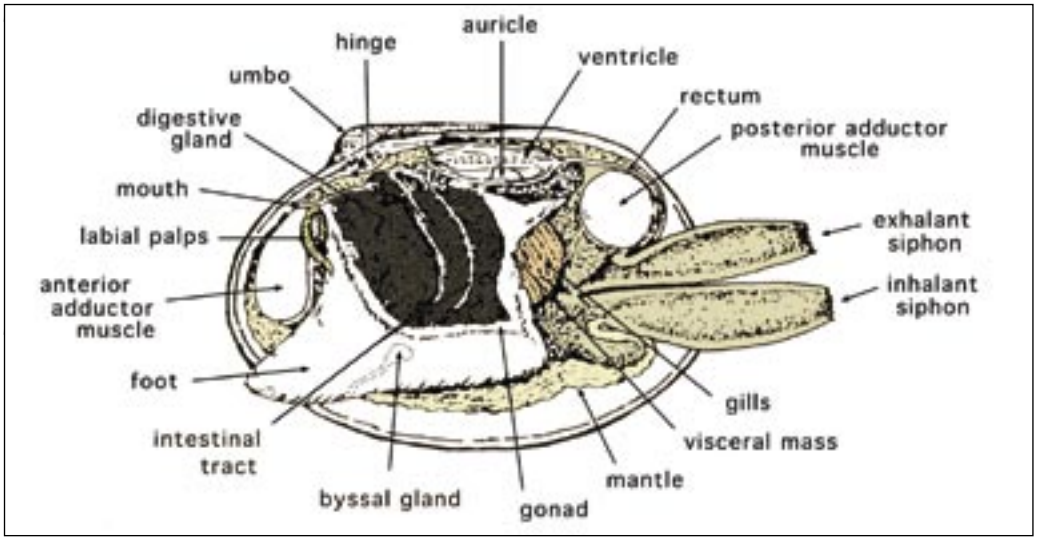

Figure 7: The internal, soft tissue anatomy of a clam of the genus *Tapes*. In this view, the uppermost gill lamellae have been removed to reveal the foot and other adjacent tissues. Modified from Cesari and Pellizzato, 1990.

2.1.3 Internal anatomy

Careful removal of one of the shell valves reveals the soft parts of the animals. The differences in general appearance of an oyster and scallop can be seen in Figure 8.

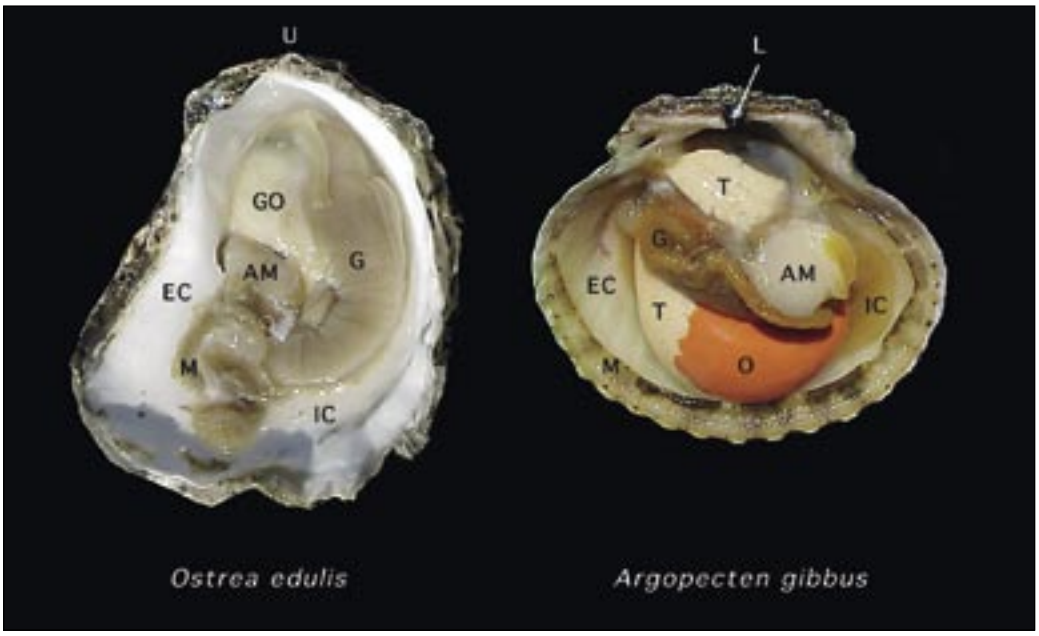

Figure 8: The soft tissue anatomy of the European flat oyster, *Ostrea edulis*, and the calico scallop, *Argopecten gibbus*, visible following removal of one of the shell valves. **Key**: **AM** – adductor muscle; **G** – gills; **GO** – gonad (differentiated as **O** – ovary and **T** – testis in the calico scallop); **L** – ligament; **M** – mantle and **U** – umbo. The inhalant and exhalant chambers of the mantle cavity are identified as **IC** and **EC** respectively.

Mantle

The soft parts are covered by the mantle, which is composed of two thin sheaths of tissue, thickened at the edges. The two halves of the mantle are attached to the shell from the hinge ventral to the pallial line but are free at their edges. The thickened edges may or may not be pigmented and have three folds. The mantle edge often has tentacles; in clams the tentacles are at the tips of the siphon. In species such as scallops the mantle edge not only has tentacles but also numerous light sensitive organs – eyes (Figure 9).

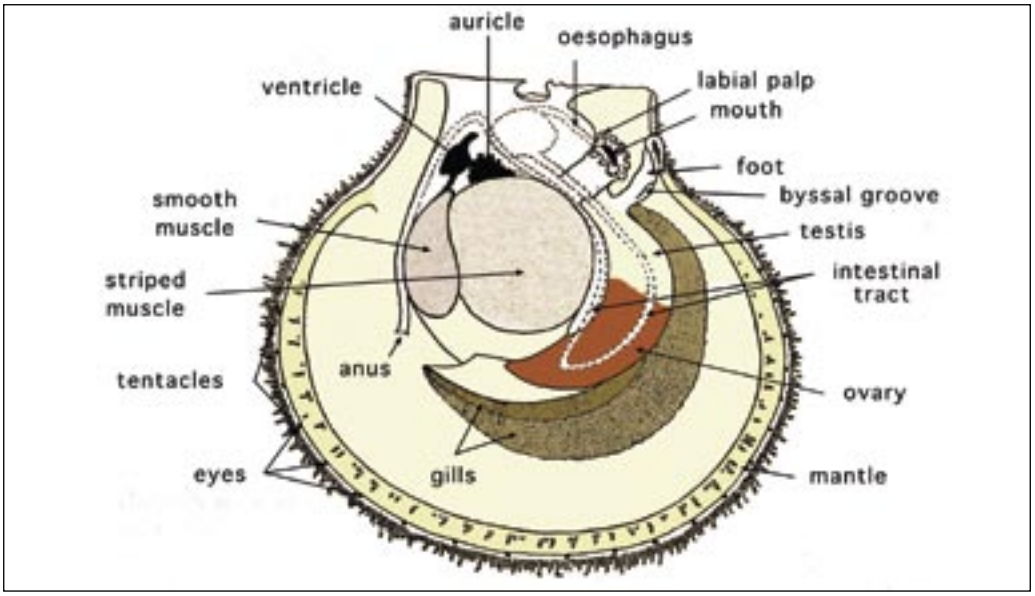

Figure 9: The internal, soft tissue anatomy of a hermaphroditic scallop.

The main function of the mantle is to secrete the shell but it also has other purposes. It has a sensory function and can initiate closure of the valves in response to unfavourable environmental conditions. It can control inflow of water into the body chamber and, in addition, it has a respiratory function. In species such as scallops, it controls water flow into and out of the body chamber and hence movement of the animal when it swims.

Adductor muscle(s)

Removal of the mantle shows the underlying soft body parts, a prominent feature of which are the adductor muscles in dimyarian species (clams and mussels) or the single muscle in monomyarian species (oysters and scallops). In clams and mussels the two adductor muscles are located near the anterior and posterior margins of the shell valves. The large, single muscle is centrally located in oysters and scallops. The muscle(s) close the valves and act in opposition to the ligament and resilium, which spring the valves open when the muscles relax. In monomyarian species the divisions of the adductor muscle are clearly seen. The large, anterior (striped) portion of the muscle is termed the "quick muscle" and contracts to close the valves shut; the smaller, smooth part, known as the "catch muscle," holds the valves in position when they have been closed or partially closed. Some species that live buried in the substrate (e.g. clams) require external pressure to help keep the valves closed since the muscles weaken and the valves open if clams are kept out of a substrate in a tank.

Gills

The prominent gills or ctenidia are a major characteristic of lamellibranchs. They are large leaf-like organs that are used partly for respiration and partly for filtering food from the water. Two pairs of gills are located on each side of the body. At the anterior end, two pairs of flaps, termed labial palps, surround the mouth and direct food into the mouth.

Foot

At the base of the visceral mass is the foot. In species such as clams it is a well developed organ that is used to burrow into the substrate and anchor the animal in position. In scallops and mussels it is much reduced and may have little function in adults but in the larval and juvenile stages it is important and is used for locomotion. In oysters it is vestigial. Mid-way along the foot is the opening from the byssal gland through which the animal secretes a thread-like, elastic substance called "byssus" by which it can attach itself to a substrate. This is important in species such as mussels and some scallops enabling the animal to anchor itself in position.

Digestive system

The large gills filter food from the water and direct it to the labial palps, which surround the mouth. Food is sorted and passed into the mouth. Bivalves have the ability to select food filtered from the water. Boluses of food, bound with mucous, that are passed to the mouth are sometimes rejected by the palps and discarded from the animal as what is termed "pseudofaeces". A short oesophagus leads from the mouth to the stomach, which is a hollow, chambered sac with several openings. The stomach is completely surrounded by the digestive diverticulum (gland), a dark mass of tissue that is frequently called the "liver". An opening from the stomach leads to the much-curled intestine that extends into the foot in clams and into the gonad in scallops, ending in the rectum and eventually the anus. Another opening from the stomach leads to a closed, sac-like tube containing the crystalline style. The style is a clear, gelatinous rod that can be up to 8 cm in length in some species. It is round at one end and pointed at the other. The round end impinges on the gastric shield in the stomach. It is believed it assists in mixing food in the stomach and releases enzymes that assist in digestion. The style is composed of layers of mucoproteins, which release digestive enzymes to convert starch

into digestible sugars. If bivalves are held out of water for a few hours the crystalline style becomes much reduced and may disappear but it is reconstituted quickly when the animal is replaced in water.

Circulatory system
Bivalves have a simple circulatory system, which is rather difficult to trace. The heart lies in a transparent sac, the pericardium, close to the adductor muscle in monomyarian species. It consists of two irregular shaped auricles and a ventricle. Anterior and posterior aorta lead from the ventricle and carry blood to all parts of the body. The venous system is a vague series of thin-walled sinuses through which blood returns to the heart.

Nervous system
The nervous system is difficult to observe without special preparation. Essentially it consists of three pairs of ganglia with connectives (cerebral, pedal and visceral ganglia).

Urogenital system
Sexes of bivalves can be separate (dioecious) or hermaphroditic (monoecious). The gonad may be a conspicuous, well defined organ as in scallops or occupy a major portion of the visceral mass as in clams. The gonad is generally only evident during the breeding season in oysters when it may form up to 50% of the body volume. In some species such as scallops, the sexes can be readily distinguished by eye when the gonad is full since the male gonad is white in colour and the female is red, even in hermaphroditic species. Colour of the full gonad may distinguish the sexes in some species such as mussels. In other species, microscopic examination of the gonad is required to determine the sex of the animal. A small degree of hermaphrodism may occur in dioecious species.

Protandry and sex reversal may occur in bivalves. In some species there is a preponderance of males in smaller animals indicating that either males develop sexually before females or that some animals develop as males first and then change to females as they become larger. In some species, e.g. the European flat oyster, *Ostrea edulis*, the animal may spawn originally as a male in a season, refill the gonad with eggs and spawn a second time during the season as a female.

The renal system is difficult to observe in some bivalves but is evident in such species as scallops where the two kidneys are two small, brown, sac-like bodies that lie flattened against the anterior part of the adductor muscle. The kidneys empty through large slits into the mantle chamber. In scallops, eggs and sperm from the gonads are extruded through ducts into the lumen of the kidney and then into the mantle chamber.

2.2 LIFE HISTORY

2.2.1 Gonadal development and spawning
In most bivalves sexual maturity is dependent on size rather than age and size at sexual maturity depends on species and geographic distribution. Production of eggs and sperm is termed gametogenesis and size of the bivalve along with temperature and quantity and quality of food are undoubtedly important in initiating this process. The gonad is composed of many-branched, ciliated ducts from which numerous sacs, termed follicles, open. Gametes arise by proliferation of germinal cells that line the follicle wall. The gonad undergoes continuous development until it becomes fully mature but this development has been divided into several stages for convenience, e.g. resting, developing, mature, partially spawned and spawned. When the gonads or

gonadal tissue are fully mature they are very evident and form a significant portion of the soft parts of the animal. Gonaducts that will carry the gametes to the body chamber develop, enlarge and are readily observed in the gonad. At this time the animal is frequently referred to as being ripe.

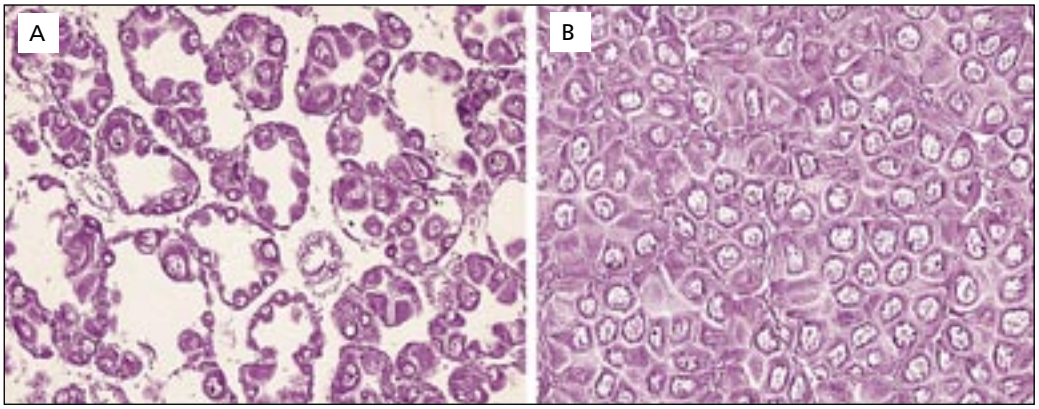

Figure 10: Photomicrographs of histological sections through the ovary of the scallop, *Argopecten gibbus*, during gametogenesis. To the left (**A**), developing eggs can be seen lining the walls of the numerous follicles. The right hand photograph (**B**) shows the follicles filled with mature eggs (courtesy: Cyr Couturier and Samia Sarkis).

Several methods have been used to determine if a bivalve is ripe and ready to spawn. The most accurate method is to make histological slides of the gonad (Figure 10) but this is costly, time consuming and the animal must be sacrificed. Making smears of the gonad or extracting small samples of the gonad from a few individuals of a stock and examining them microscopically is an alternative and the most frequently used technique. In scallops, the gonadal index (weight of the gonad divided by the weight of the soft parts, multiplied by 100) is sometimes used. A rigid routine is generally followed in hatcheries to condition adults for spawning and with practice, most hatchery managers quickly develop the ability to know if the animal is ripe and ready to spawn by examining the gonad macroscopically.

Bivalves that reach the size of sexual maturity and spawn for the first time are sometimes referred to as virgins. Although these animals attain sexual maturity, the number of gametes produced is limited and sometimes not all are viable. During subsequent spawning the number of gametes produced greatly increases.

The period of spawning in natural populations differs with species and geographic location. Spawning may be triggered by several environmental factors including temperature, chemical and physical stimuli, water currents or a combination of these and other factors. The presence of sperm in the water will frequently trigger spawning in other animals of the same species. Some bivalve species in tropical environments have mature gametes throughout the year and limited spawning may occur continuously during the year. In temperate areas, spawning is usually confined to a particular time of the year. Many bivalves undergo mass spawning and the period of spawning may be brief. Virtually the entire contents of the gonad are released over a short period during spawning activity. Other species of bivalves have a protracted spawning period and it may extend over a period of weeks. These species are sometimes referred to as dribble spawners. Limited spawning occurs over a protracted period with one or two major pulses during this time. In some species there may be more than one distinct spawning in a year. In hermaphroditic species, spawning is timed so that either the male or female part of the gonad spawns first. This minimizes the possibility of self-fertilization.

In most bivalve species of commercial interest, gametes are discharged into the open environment where fertilization occurs. Sperm is discharged in a thin, steady stream through the exhalent opening or exhalent siphon. Discharge of eggs is more intermittent and they are emitted in clouds from the exhalent opening or siphon. In species such as scallops or oysters, females frequently clap the valves to expel the eggs. This may be done to clear eggs lodged on the gills. After spawning the gonads of many species are emptied and it is impossible to macroscopically distinguish sex in individuals. The animal is then said to be in the resting stage. In dribble spawners, the gonad may never be completely emptied.

Some bivalves, e.g. the European flat oyster, are larviporous and the early stages of larval development occur in the inhalant chamber of the mantle cavity of the female-phase oyster. Eggs when spawned are passed through the gills and are retained in the mantle chamber. Sperm is taken in through the inhalant opening. The length of time larvae are held in the mantle chamber and subsequently the length of time larvae are free living in surface waters varies with species. In some genera, e.g. *Tiostrea*, larvae may only be part of the plankton for as little as one day.

Occasionally, particularly in temperate areas, spawning may not occur in some years. This can be a consequence of several factors but probably mostly is related to water temperatures, which remain too low to trigger spawning. When this occurs in oysters, the eggs and sperm may be reabsorbed into the gonadal tissue, broken down and stored as glycogen. In clams and scallops the gonad may remain in a ripe condition until the next year.

2.2.2 Embryonic and larval development

These topics are covered more fully in later sections, but a brief description is given here for continuity. Larval development is similar whether initial development occurs in the mantle chamber of the female or completely in the open environment.

Eggs undergo meiotic division at fertilization to reduce the number of chromosomes to a haploid number before the male and female pronuclei can fuse to form the zygote. Two polar bodies are released during meiotic division and when apparent, indicate successful ferilization. Cell division begins and within thirty minutes after fertilization the egg divides into the two-celled stage. The eggs are heavier than water and sink to the bottom of the tank where cell division continues.

The time taken for embryonic and larval development is species specific and temperature dependent (Figure 11). Within 24 hours the fertilized egg has passed through the multi-celled blastula and gastrula stages and in 24 to 36 hours has developed into a motile trochophore. Trochophores are somewhat oval in shape, about 60-80 μm in size and have a row of cilia around the middle with a long apical flagellum and these permit them to swim.

The early larval stage is referred to as the straight-hinge, "D" or Prodissoconch I stage. Shell length of the initial straight-hinge stage varies with species but it is generally 80-100 μm (larger in larviparous oysters). The larva has two valves, a complete digestive system and an organ called the velum that is peculiar to bivalve larvae. The velum is circular in shape and can be protruded from between the valves. It is ciliated along its outer margin and this organ enables the larva to swim but only well enough to maintain itself in the water column. When the larva swims through the water column the velum collects phytoplankton upon which the larva feeds.

Larvae continue to swim, feed and grow and within a week the umbones, which are protuberances of the shell near the hinge, develop. As larvae continue to grow,

the umbones become more prominent and the larvae are now in the umbone or Prodissoconch II stage. Prodissoconch II stage larvae have distinct shapes and with practice it is possible identify larvae of different bivalve species in the plankton. This has been used by biologists to forecast oyster sets in the natural environment for the industry. Duration of the larval stage varies with species and environmental factors such as temperature but it can be 18-30 days. Size at larval maturity also varies with species and can be 200-330 μm.

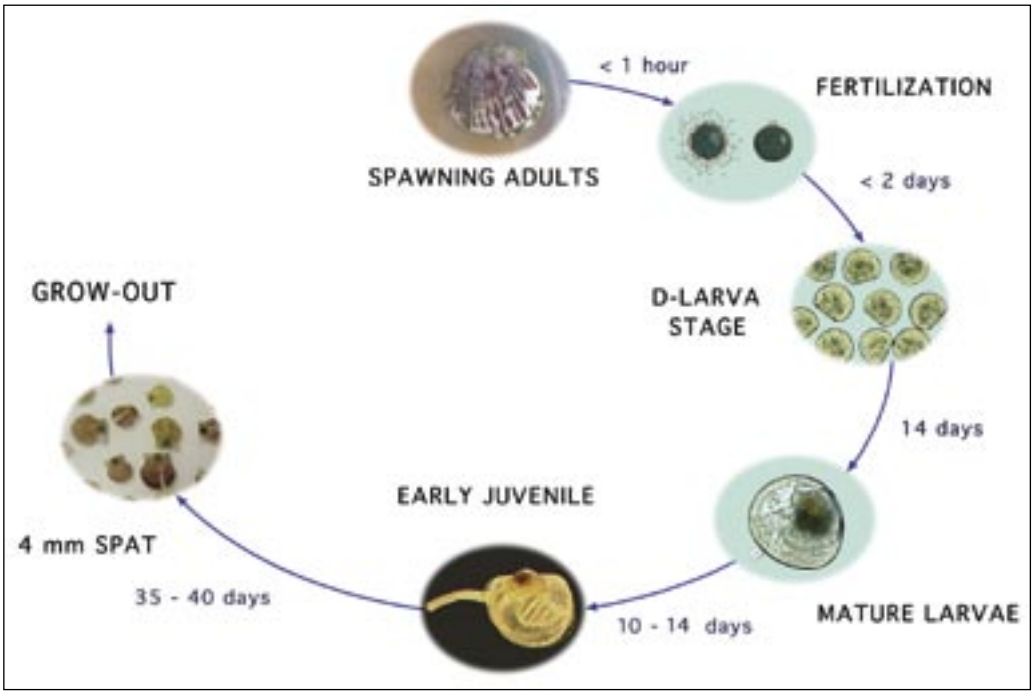

Figure 11: Representation of the developmental stages of the calico scallop, *Argopecten gibbus*, which take place within a hatchery. The duration of the period between the various stages is given in hours or days for this particular species and may differ for other species of bivalves.

When larvae approach maturity, a foot develops and gill rudiments become evident. Small dark circular dots, the eye-spots, develop near the centre of each valve of some species. Between periods of swimming activity, larvae settle and use the foot to crawl on a substrate. When a suitable substrate is found the larva is ready to metamorphose and begin its benthic existence. Mature oyster larvae secrete a small drop of cement from a gland in the foot, roll over and place the left valve in it. They remain attached in that location for the rest of their lives. In other species, the larva secretes byssus from the byssal gland in the foot and this serves as a temporary holdfast to attach to a substrate. The larva is now ready to metamorphose.

2.2.3 Metamorphosis

Metamorphosis is a critical time in the development of bivalves, during which the animal changes from a swimming, planktonic to a sedentary benthic existence. Considerable mortalities can occur at this time both in nature and in hatcheries. The subject is dealt with in detail later since it is an important aspect in the hatchery production of juvenile bivalves.

2.2.4 Feeding

Bivalves are filter feeders and feed primarily on phytoplankton – microscopic plant life. In juveniles and adults, the ctenidia, or gills, are well developed and serve the dual purpose of feeding and respiration. The ctenidia are covered with cilia – tiny vibrating hairs – whose concerted and co-ordinated beat induces a current of water. When resting

on or in a substrate, water is drawn into the animal through the inhalant opening or the inhalant siphon, through the gills and then is returned to the surrounding water through the exhalant opening or siphon. The gills collect plankton and bind it to mucous. Strands of food-laden mucous are passed anteriorly by means of ciliary action along special grooves on the gills to the labial palps whose role is to assist in directing food into the mouth. Bivalves can exercise some selection of their food and periodically the palps reject small masses of food, pseudofaeces, that are expelled from the mantle cavity, often by the vigorous "clapping" together of the shell valves.

What constitutes optimum foods for bivalves remains largely unknown however phytoplankton undoubtedly forms the major portion of the diet. Other sources of food may be important such as fine particles of non-living organic material (detritus) along with associated bacteria and also dissolved organic material.

2.2.5 Growth

Only general statements can be made about growth in juveniles and adults since it varies greatly between species, geographic distribution, i.e. climate, location in the subtidal or intertidal zones, as well as differences in individuals and in their genetic make up. Growth can also vary greatly from year to year and in temperate areas there are seasonal patterns in growth.

Growth can be measured in bivalves by different methods including increments in shell length or height, increases in total or soft body weight, or a combination of all of these factors. In tropical areas growth can vary with season, being faster during or after rainy periods when nutrients are washed into the ocean leading to increased production of phytoplankton. In temperate areas, growth is generally rapid during spring and summer when food is abundant and water temperatures are warmer. It virtually ceases in winter, resulting in annual checks in the shell. These winter checks can be used to age some bivalves. Some species are short lived but others may live to over 150 years.

In culture operations the important considerations in bivalve growth are length of time taken to grow to sexual maturity and to market size. The goal of bivalve culture is to grow bivalves to commercial size as quickly as possible to make the operation as economically attractive as possible.

2.2.6 Mortalities

Bivalves in the larval, juvenile and adult stages can die from a variety of causes, which can be environmental or biological in origin. The subject is much too large to consider in detail here but a brief synopsis is given to highlight a number of pertinent points, which could be important in hatchery operations.

The physical environment can cause severe mortalities to bivalves in all three stages. Too high temperatures or prolonged periods of cold temperatures can be lethal to bivalves as can be sudden swings in temperature. Severe extremes in salinities, particularly low salinities after periods of heavy rain or run off from melting snow, can also cause extensive mortalities. Heavy siltation can smother and kill juveniles and adults.

Pollution, particularly industrial pollution, can cause extensive mortalities in juvenile and adult bivalves. Both industrial and domestic pollution can be problems for hatchery operations and must be avoided. Domestic pollution can increase organic and bacterial loads in water as well as contributing a wide range of potentially toxic materials. Little is known of the combined effects of sub-lethal levels of the wide range of organic and organo-metallic compounds of man-made origin that may be present in such effluents.

Bivalves in the larval, juvenile and adult stages are preyed upon by a wide variety of animals that can cause severe mortalities. In the natural environment plankton feeders probably consume large quantities of larvae. In hatcheries, predation is largely a non-issue since the water used is filtered and any predators are removed.

Bivalves are hosts to parasites that can cause mortalities, particularly in the adult stage. Shell boring worms, *Polydora* sp., and sponges burrow into the shells and weaken them, thus causing mortalities.

Probably the major cause of mortalities in bivalves, particularly of larvae and juveniles in hatcheries, is disease. Considerable research effort has been expended in studying bivalve diseases and trying to develop methods to control them.

Diseases can be devastating to adult bivalves as witness the demise of some populations in the world. A few examples include,

Dermocystidium:
a fungal disease of bivalves caused by *Perkinsus marinus;*

Delaware Bay Disease (MSX):
a disease caused by the haplosporidian protozoan, *Haplosporidium (Minchinia) nelsoni*;

SSO (seaside organism disease):
a disease caused by the haplosporidian protozoan, *Haplosporidium costale*, (which together with *H. nelsoni* has decimated large populations of Virginia oysters on the Atlantic coast of the USA and now extends northwards into Atlantic Canada).

Aber Disease:
A disease caused by the protozoan, *Marteilia refringens;*

Bonamiasis (Haemocytic Disease):
A disease caused by the microcell parasite, *Bonamia ostreae*;
(Aber disease and Bonamiasis have resulted in the virtual demise of the European oyster in some parts of Europe).

Although considerable work has been carried out on these diseases, no practical methods have been developed to control them and restore oyster populations to previous levels. The severity of these diseases points to the care that must be taken when transporting adult bivalve stock into a hatchery.

In hatcheries it appears that diseases which do occur are caused by bacteria and not by protozoans. Bacteria are present to some degree in both algal and larval cultures. Indeed, bacteria may form an important part of the diet of larvae. However, periodically, large groups of larvae will die suddenly and an entire culture is lost. High bacterial counts are almost always associated with such large-scale mortalities. Bacteria may cause mortalities (pathogenic) or they may be simply present as opportunistic bacteria (saphrophytic), feeding on the dying larvae. Bacteria that cause diseases largely belong to the genus *Vibrio* sp. and every precaution must be taken to prevent them from causing epidemics in hatcheries. The best method to prevent such epidemics is to observe strict hygienic procedures and ensure that the larvae are well fed with high quality food. Larvae should be inspected regularly. If a disease occurs or is suspected, tanks and equipment should be sterilized with a bleach solution and rinsed well with

freshwater. To protect larvae from further contamination, tanks should be refilled with UV-irradiated or ozone treated seawater. Use of antibiotics to control diseases is largely avoided in hatcheries. They are expensive and add to cost of operations and also there is the fear of a strain of bacteria developing that will be resistant to the antibiotics, which could lead to even more severe disease problems in the future.

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Part 3

Hatchery operation: culture of algae

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3.1 INTRODUCTION

Unicellular marine microalgae (Figure 12) are grown as food for the various stages in the hatchery culture of commercially valuable shellfish. Until recently living algae constituted the sole food source for bivalve larvae and juveniles. This is now beginning to change as the result of recent research into the development of suitable non-living

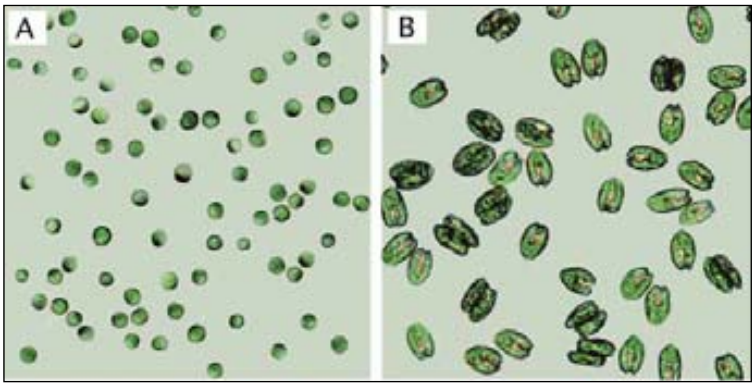

Figure 12: Photomicrographs of two algal species commonly cultured in hatcheries, *Isochrysis* sp. (**A**) and *Tetraselmis* sp. (**B**) showing the relative difference in cell size.

and artificial diets. However, the production of live algae will remain a critically important aspect of successful hatchery management into the foreseeable future, if only as a live food supplement to innovative foodstuffs.

Flagellate and diatom species, among the microalgae, are primary producers at the base of the marine food chain. They manufacture organic cellular components from the uptake of carbon dioxide and nutrients contained in seawater using light as the energy source in a process called photosynthesis. They are normally cultured in hatcheries in suitably treated natural seawater enriched with additional nutrients, which include nitrates, phosphates, essential trace elements, vitamins and carbon dioxide as the carbon source. Synthetic seawater may be used but it is prohibitively expensive except at the small laboratory scale.

The need to culture microalgae arises because the natural phytoplankton content of seawater used in the hatchery is insufficient to support optimum growth of high densities of larvae and juveniles reared. Particularly in the culture of larvae, the water treatments used will remove almost all of the natural phytoplankton which then needs to be replaced from cultures of preferred, high food value species. In this context, and in the provision of suitable food rations for breeding stock and juveniles, few of the very many naturally occurring algae are of good food value to bivalves and not all of these are amenable to artificial culture on a sufficiently large scale. A list of the more commonly used species in bivalve hatcheries is given in Table 1. Parameters of cell size and composition are also shown.

Table 1: The cell volume, organic weight and gross lipid content of some of the more commonly cultured algal species used as foods for bivalve larvae and spat. Species marked * are of relatively poor nutritional value.

Species:	Median cell volume (μm^3)	Organic Wt. ($\mu g\ 10^{-6}$ cells)	Lipid %
Flagellates:			
Tetraselmis suecica	300	200	6
*Dunaliella tertiolecta**	170	85	21
Isochrysis galbana *Isochrysis* (T-ISO) *Pavlova lutherii*	40-50	19-24	20-24
Diatoms:			
Chaetoceros calcitrans	35	7	17
Chaetoceros gracilis	80	30	19
Thalassiosira pseudonana	45	22	24
Skeletonema costatum	85	29	13
*Phaeodactylum tricornutum**	40	23	12

The culture of algae accounts for about 40% of the costs of rearing bivalve seed to a shell length of about 5 mm in a hatchery. For example, 1 million juvenile Manila clams or Pacific oysters of 5 mm shell length will consume 1 400 l of high density, cultured algae each day at the optimum rearing temperature of 24°C. Smaller daily volumes are required to feed broodstock and larvae.

The basic methods of algal culture have changed little over the years and the various steps in the process leading to production-scale cultures are introduced in Figure 13. Hatcheries have either opted for indoor, intensive culture with artificial illumination, usually external to the culture vessels, or outdoor, extensive culture in large tanks

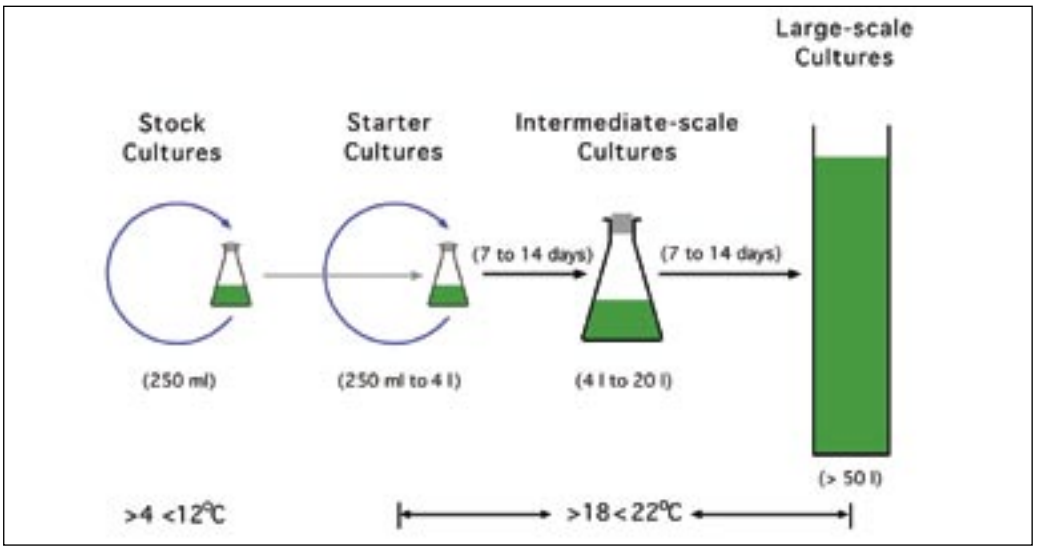

Figure 13: Steps in the production of algae. Stock cultures (250 ml or less) remain in isolation under light and climate control (low temperature) and are only used to inoculate starter cultures when necessary. They are not aerated nor is carbon dioxide added. Starter cultures (250 ml to 4 l in volume) are grown quickly for 7 to 14 days at higher temperatures and light intensity with a supply of carbon dioxide enriched air. When ready, a small portion of the volume is used to start a new starter culture and the main portion to begin an intermediate-scale culture. Intermediate-scale cultures (usually of between 4 l and 20 l in volume) may be used as food for larvae or to start a large-scale culture. Large-scale cultures are generally of a minimum of 50 l and are frequently much greater in volume.

or ponds utilizing natural light. The intensive techniques are satisfactory in terms of reliability and productivity but are expensive in terms of capital outlay and labour, while the extensive methods tend to be less reliable and, sometimes not very productive. Both methods will be considered together with the essential infrastructure and methodologies. A schematic diagram of the process of culturing algae is given in Figure 14 and a floor plan of a hatchery showing the area allocated to algal culture was given earlier in Figure 5 (section 1.2).

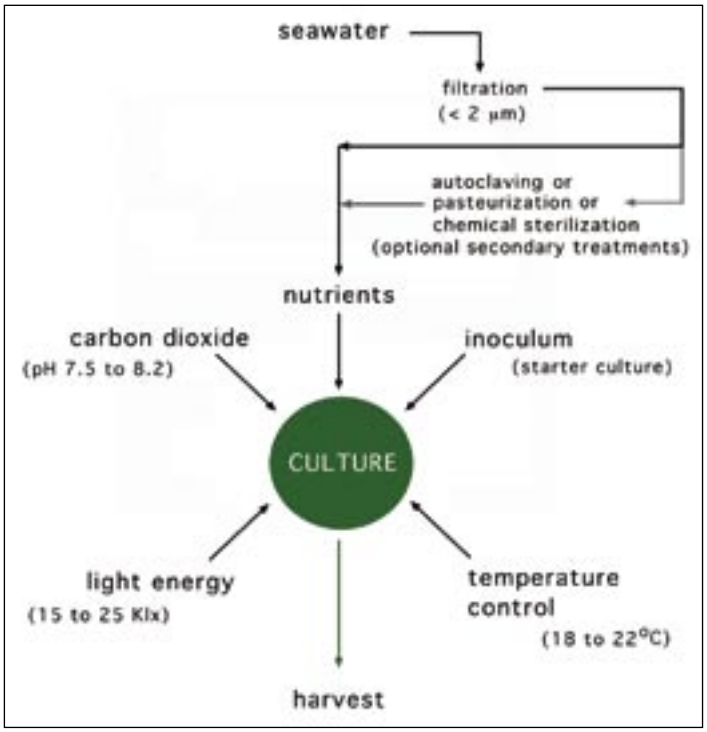

Figure 14: The process of algal culture showing the various required inputs. Whether secondary seawater treatment is necessary or not depends on the extent to which the water is initially filtered.

3.2 MAINTENANCE OF STOCK AND STARTER CULTURES

Stock cultures, otherwise known as master cultures, of the preferred species are the basic foundation of culture. They are normally supplied as monospecific (uni-algal) cultures from reputable culture collections maintained by national institutions or research laboratories. Since they are valuable, they are normally kept in specialized maintenance media, for example, Erdschreiber, or alternatively in F/2 media, or on nutrient enriched agar plates or slopes, under closely controlled conditions of temperature and illumination. A special area or room off the algal culture room is usually allocated to this purpose.

Stock cultures are used **only** to provide lines of starter cultures (also known as inocula) when required. Every effort should be made to minimize the risk of contaminating the stock and starter cultures with competing microorganisms. The sterile procedures described below should be followed to ensure that contamination does not occur.

Stock cultures are kept in small, transparent, autoclavable containers. For example, 500 ml borosilicate glass, flat-bottomed boiling or conical flasks fitted with a cotton wool plug at the neck, suitable for containing 250 ml of sterile, autoclaved medium, are ideal. The composition and preparation of Erdschreiber medium is given in Table 2. Alternate media suitable for the purpose are Guillard's F/2 (see Table 3) and HESAW (see Table 4). Proprietary algal culture enrichment products for addition to suitably treated seawater can also be used according to the manufacturer's instructions. Stock cultures are also often maintained in seawater agar medium impregnated with suitable nutrients in Petri dishes or on slopes in test tubes.

Figure 15: Illuminated, temperature controlled incubators for the maintenance of small algal cultures.

Stock cultures are best kept in a cooled incubator at 4 to 12°C (according to preference), illuminated by two or more 8-watt (W) fluorescent lamps that provide a light intensity of 450 lux measured at the culture surface (Figure 15). Alternatively they can be kept in cool conditions close to a north-facing window (out of direct sunlight), or in a cool room illuminated by fluorescent lamps. The objective is not to allow rapid growth, but to maintain the cultures in good condition. The cultures are not aerated, nor is carbon dioxide introduced.

3.2.1 Procedures for the management of stock cultures

It is necessary to sub-culture stock cultures at monthly intervals to maintain them in a vigorous and healthy state. Following removal of the cotton wool plug from a stock culture flask and flaming the neck of the flask with a Bunsen burner (or butane torch), an inoculum of 20 to 50 ml is decanted into another sterile flask containing autoclaved

medium. The plug is inserted after flaming of the neck of this new flask. Species name and the date are indelibly marked on the flask, which is then returned to the incubator. The original stock culture can be kept for a few weeks in the event that the new stock culture fails to grow. The stock culture transfer procedure is best performed in a cabinet that has been sterilized by ultra-violet light to further reduce the risk of contamination (see Figure 16). Details of the transfer procedure are given in the accompanying box.

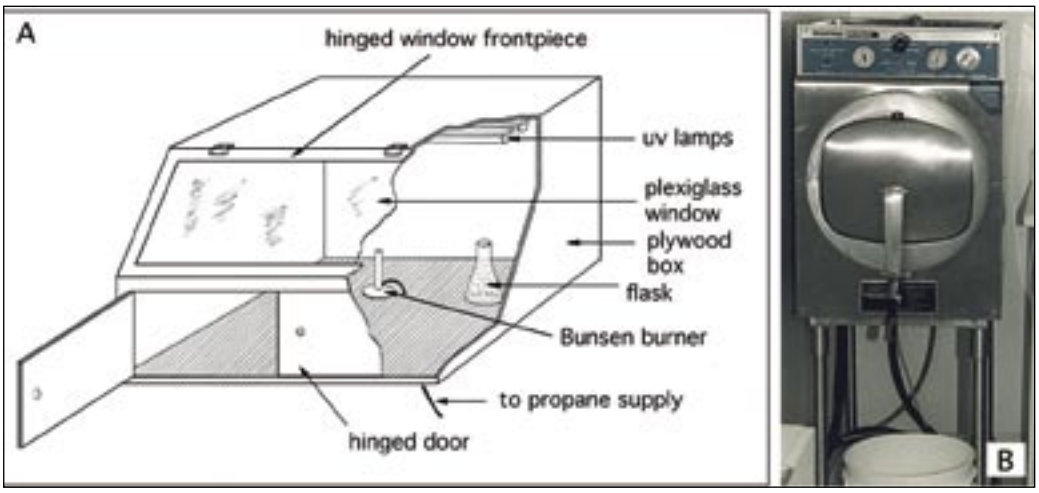

Figure 16: A – schematic diagram of a culture transfer chamber. **B** – an autoclave suitable for the sterilization of small volumes of culture medium.

Table 2: The composition and preparation of Erdschreiber culture maintenance medium.

Constituents:

1. Seawater: Autoclave 2 l in a 3 l borosilicate glass flat-bottomed boiling flask with cotton wool plug at 1.06 kg cm^{-2} for 20 minutes. Stand for 2 days.

2. Soil extract: prepared as follows:
   a) mix 1 kg soil from a woodland or pasture area untreated with artificial fertilizers, insecticides, etc. with 1 l of distilled freshwater;
   b) autoclave at 1.06 kg cm^{-2} for 60 minutes;
   c) decant off the supernatant liquid;
   d) filter supernatant through Whatman No. 1 paper and then through a glass-fibre (GF/C) paper;
   e) autoclave in 1 l aliquots in polypropylene bottles at 1.06 kg cm^{-2} for 20 minutes;
   f) store in deep freeze until required;
   g) autoclave 100 ml in 500 ml borosilicate glass, flat-bottomed boiling flask with cotton wool plug at 1.06 kg cm^{-2} for 20 minutes.

3. Nitrate/phosphate stock solution: Dissolve 40g $NaNO_3$ and 4 g Na_2HPO_4 in 200 ml distilled water. Autoclave in 500 ml flask at 1.06 kg cm^{-2} for 20 minutes.

4. Silicate stock solution: Dissolve 8 g $Na_2SiO_3.5H_20$ in 200 ml distilled water. Autoclave in 500 ml flask at 1.06 kg cm^{-2} for 20 minutes.

Procedure:

Add 100 ml soil extract (2) to 2 l of sterilized seawater (1). With sterile pipette add 2 ml nitrate/phosphate stock solution (3) and 2 ml silicate stock (4). Decant 250 ml into 8 empty autoclaved 500 ml flasks with cotton wool plugs. Use a Bunsen burner or butane torch to flame the necks of the flasks immediately before and after decanting/pipetting. The maintenance medium is now ready to use.

Procedure for transferring algal cultures from flask to flask

(a) Wipe all inner surfaces of inoculating booth with 85% ethanol.

(b) Place all flasks that will be required in the booth; i.e. all flasks to be transferred from (the transfer flask) and flasks containing sterilized media to be transferred into (new flasks).

(c) Close booth and switch on ultra-violet lamp. Leave for at least 20 minutes. (It is not safe to look directly at ultraviolet light, so a dark cover should be placed over the plexi-glass (transparent acrylic plastic) viewing plate when the light is on.)

(d) Switch off lamp. Ignite small burner.

(e) Remove foil caps from one transfer and one new flask. Flame the neck of each flask by slowly rotating the neck through the flame.

(f) Tilt the neck of the transfer flask toward the new flask. In one motion, remove both stoppers and pour an inoculum into the new flask. Transfer approximately 50 ml for diatom species and 100 ml for flagellates. Avoid touching the necks of the two flasks. Never touch the portion of the stopper that is inserted into the flask. Once the inoculum is added, replace the stopper in the transfer flask. Slowly flame the neck of the new flask before replacing its stopper.

(g) Replace foil cap over the neck of the new flask. Using a waterproof marker pen, label the new flask with the algal species inoculated and the date of transfer.

(h) Repeat procedure for all flasks within the booth. Once completed, turn off burner and open booth.

(i) Remove all new flasks and place in the algal incubator or a well-lit area in the algae culture facility.

(j) The remaining inoculum in the transfer flasks can be used to inoculate larger cultures such as 4 l flasks or carboys.

(from: Bourne, Hodgson and Whyte, 1989)

Table 3: Guillard's F/2 media used for culturing algae in bivalve hatcheries from Guillard (1975).

1.	Nitrate	$NaNO_3$	75.0 g per l
2.	Phosphate	$NaH_2PO_4.H_2O$	5.0 g per l
3.	Silicate	$Na_2SiO_3.9H_2O$	30.0 g per l
4.	Trace Metals		
		$FeCl_3.6H_2O$	3.5 g
		Na_2EDTA	4.36 g

Dissolve in 900 ml distilled H_2O

Add 1 ml of each of the following trace metal solutions

$CuSO_4.5H_2O$	0.98 g per 100 ml
$ZnSO_4.7H_2O$	2.20 g per 100 ml

$CoCl_2.6H_2O$	1.00 g per 100 ml
$MnCl_2.4H_2O$	18.00 g per 100 ml
$Na_2MoO_4.2H_2O$	0.63 g per 100 ml

Make up the volume to 1 l with distilled H_20 (pH ca. 2.0)

Add 1 ml per litre FSW of the above solutions (#1-4).

5. Vitamins

Biotin	1.0 mg
B-12	1.0 mg
Thiamine HCl	20.0 mg

Dissolve in 1 l distilled H_20. Store frozen.

Add 1/2 ml of vitamin solution for every 1 l of FSW.

Table 4: HESAW media used for culturing algae in bivalve hatcheries. From Harrison *et al.* (1980).

1.	$NaNO_3$	466.7 g
	Na_2.glycero.$P0_4.5H_2O$	66.7 g

Dissolve in 2 1itres distilled H_2O

2.	$Na_2EDTA.2H_2O$	55.3 g
	H_3BO_3	38.0 g

Dissolve in 1 litre hot distilled H_2O

3.	$FeCl_3.6H_2O$	1.6 g

Dissolve in 100 ml distilled H_20. Add 50 ml to solution #1 and the remainder to solution #2. Mix together solutions #1 and #2.

4.	$MnSO_4.H_2O$	4.1 g, or
	$MnSO_4.4H_2O$	5.4 g

Dissolve in 50 ml distilled H_20. Add to above solution.

5.	$Na_2MoO_4.2H_2O$	1.26 g

Dissolve in 50 ml distilled H_20. Add to above solution.

6.	$ZnS0_4.7H_2O$	7.3 g
	$CuS0_4.7H_2O$	1.6 g

Dissolve in 100 ml distilled H20. Add 10 ml of solution to above solution.

7.	Na_2SeO_3	0.173 g

Dissolve in 1 l distilled H_20. Add 1 ml of solution to 100 ml distilled H_20 to make stock solution. Add 10 ml stock solution to above solution.

Bring volume of solution to 10 l by adding distilled H_20. Autoclave before use. Add 1 ml of solution for every 1 l of FSW.

8.	$Na_2SiO_3.5H_2O$	224.0 g, or
	$Na_2Si0_3.9H_2O$	300.0 g

Dissolve in 1 l distilled H_20. Slowly add 1.5 l of 1 Molar HCl (133.5 ml concentrated HCl in 1.5 L distilled H_20). Bring volume of solution to 10 l by adding distilled H_20. Autoclave prior before use. Add 1 ml of solution for every 1 l of FSW.

9. Vitamins

(Follow directions for vitamins in Table 4.)

3.2.2 Starter culture management

Figure 17: Photographs showing typical facilities for maintenance of starter cultures.

Procedures for the maintenance of starter cultures (inocula) are almost identical to those described above. These cultures are specifically grown to provide inocula to start larger volume cultures needed to produce food.

A line of starter cultures is originally set-up from the stock culture of the required species. Starter cultures, like the stocks, can be grown in 500 ml boiling flasks in 250 ml of culture medium. Because they are needed to provide inocula it is necessary to grow them quickly. They are grown at 18 to 22°C at a distance of 15-20 cm from 65 or 80 W fluorescent lamps, giving a level of illumination at the culture surface of 4 750 to 5 250 1ux (Figure 17). Starter cultures are generally aerated with an air/carbon dioxide (CO_2) mixture.

Starter cultures are grown for variable periods of time prior to use. In the case of diatom species, which have short generation times, this period is from 3 to 5 days. For the majority of flagellates it is 7 to 14 days. When ready for use a starter culture is sub-cultured using sterile techniques, as previously described. Twenty to 50 ml, (depending on species and the density of the culture), is transferred to a fresh 250 ml culture – to maintain the starter culture line. The remainder is used as an inoculum for larger cultures (up to 25 l in volume) to be grown for feeding or as an intermediate step in the process of large-scale culture, where they in turn act as the inocula for much larger cultures.

Larger volume starter cultures may be needed to inoculate large-volume production cultures. For clarity, cultures of between 2 and 25 l volume will be referred to as intermediate-scale cultures. As an example, a 200 l production culture will initially begin with a 250 ml starter of the required species which is then transferred when it has grown to a larger volume 2 to 4 l starter. When a 200 l culture is about to be started, 200 to 400 ml of the 2 to 4 l starter culture is used to start a new 2 or 4 l starter culture and the remainder to start the 200 l production culture.

With larger volume starters it is advantageous to increase the level of illumination and to aerate the culture with an air/carbon dioxide mixture. It is advisable to dilute the medium to grow diatom species to a salinity of 20 to 25 PSU (practical salinity units, equivalent to parts per thousand) to obtain the best possible growth rates. Most flagellate species are best grown at about 30 PSU.

3.3 INTERMEDIATE-SCALE CULTURE

Most laboratories and hatcheries requiring small volumes of algae for food use spherical glass flasks or glass or clear plastic carboys of up to 25 l volume (Figure 18). These are generally operated as batch culture systems or semi-continuously. Batch culture involves the inoculation of the culture medium with the required species. The culture is then grown rapidly until a further increase in cell density is inhibited by the failure of the light to adequately penetrate the culture, The culture is then completely harvested, the container washed and sterilized and started again with a new culture.

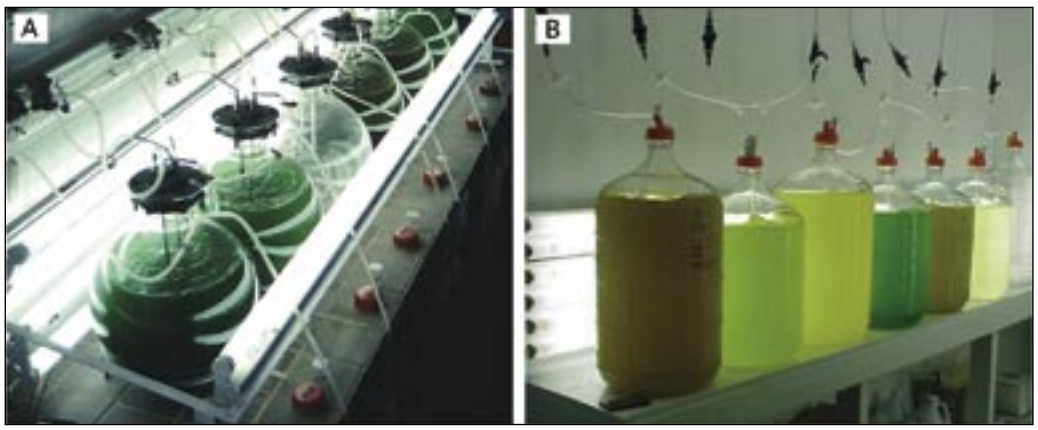

Figure 18: Two different approaches to the intermediate-scale culture of algae: **A** – 20 l volume round flasks; **B** – using equally as effective wine making carboys of 15 to 20 l volume.

The semi-continuous method involves starting the cultures in the same way but instead of completely harvesting them when they have grown, they are partially harvested before the light limiting stage is reached. The harvested volume is then replaced with freshly prepared culture medium and the process repeated 2 or 3 days later. In this

way the life of a culture is extended. With some of the hardier species, e.g. *Tetraselmis suecica*, cultures will last for 3 months or more with harvests of 25 to 50% of the culture volume 3 times each week. Batch culture is generally used for delicate species and the rapidly growing diatoms. Semi-continuous culture is mainly used with hardier species of flagellates.

3.3.1 Growth phases of cultures

Harvesting takes place in semi-continuous culture during the exponential phase of growth. Batch harvests are made generally at the peak of exponential growth as the cultures enter the stationary phase. An illustration of the meaning of these terms is given in Figure 19. In this case the species cultured is the large, green flagellate, *Tetraselmis*.

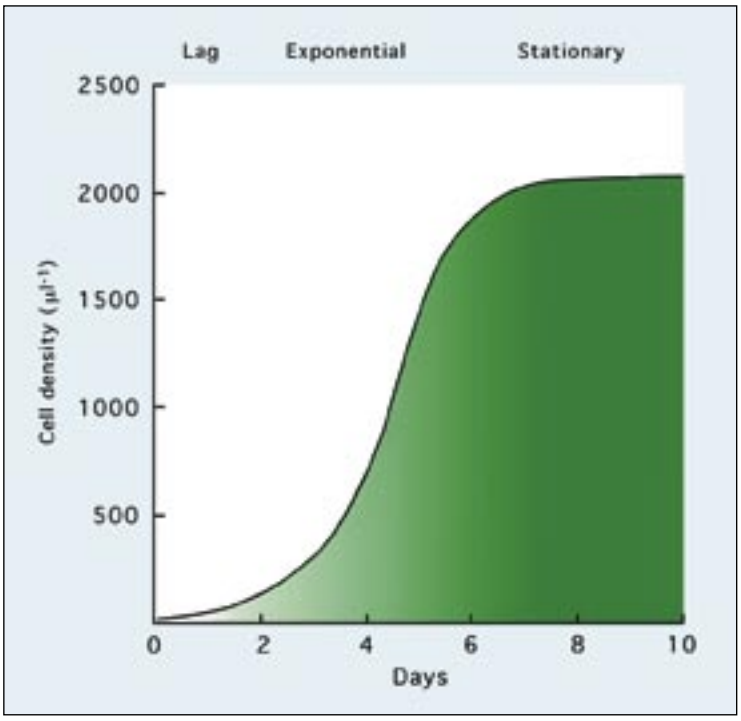

Figure 19: Phases in the growth of algal cultures illustrated by a typical growth curve for the large, green flagellate, *Tetraselmis suecica*.

At inoculation from the starter culture, the starting cell density in the culture is 25 to 50 cells per ml (cells per microlitre). After inoculation these cells grow and divide increasingly rapidly as they acclimatize to the culture conditions. This acclimatization period, which lasts for 2 to 3 days, is called the **lag phase**. Once adapted to the conditions, the rate of cell division accelerates and increase in the number of cells in the culture is logarithmic. This period lasts for 4 to 6 days and is called the **exponential growth phase**. Cell division rate then slows as light penetration through the culture and/or nutrients become limiting. The culture then enters the **stationary phase**, which can last for many days in the case of flagellates or only for a short time for diatoms. Cultures of flagellates remain in this phase by the recycling of nutrients from dead and decaying cells, but in the case of diatoms, which may produce self-inhibiting metabolites, which attract bacterial growth, the culture collapses.

In the example shown in Figure 19, batch cultures of *Tetraselmis* would be harvested at a density of about 2 000 cells per µl and semi-continuous cultures at about 1 500 cells per µl. These densities can be increased, within limits, by increasing the light intensity falling on the cultures, by maintaining the pH at between 7.5 to 8.2 with controlled CO_2 input and by the addition of extra nutrients as the culture density increases.

3.3.2 Details of intermediate-scale culture operation

The complexity of the culture operation depends on the requirement for algae and the cost constraints within which the system needs to operate. In the simplest form the culture system may be just a scaled-up version of the starter cultures, using 2 l to 25 l flat-bottomed, glass flasks or carboys. These are part filled with the culture medium – in this case sterile, nutrient-enriched seawater – and then they are inoculated with the required species and aerated with a mixture of 2% CO_2 carried in compressed air. The carbon dioxide is from a bottled gas source with gas pressure and flow regulation. This is to provide the carbon source for photosynthesis and to control pH within the range 7.5 to 8.2. The air/CO_2 mixture is filtered through a 0.2 µm porosity cartridge or membrane filter to remove the majority of air-borne contaminants and competing microorganisms. Examples of this type of system are illustrated in Figure 18. The culture medium is prepared from filtered or sterilized seawater.

There are various options for culture water treatment:

a) either the seawater is filtered to remove bacteria using 0.22 or 0.45 µm membrane cartridge filters, or,
b) it is batch or continuously pasteurized at 65 to 75°C or,
c) it is autoclaved at 1.06 kg per cm^2 for 20 minutes (After autoclaving the medium must be allowed to stand for 2 days in a suitable container closed from the atmosphere). Or,
d) it is chemically sterilized with sodium hypochlorite solution at 25 mg per l free-chlorine (by adding 0.5 ml of domestic bleach – 5% sodium hypochlorite – per l of filtered seawater). Before use, the residual free-chlorine is neutralized by adding an excess of sodium thiosulphate solution (50.0 mg per l) prepared in distilled water.

Note: Methods (a) and (c) are most commonly used for small-scale culture preparation; (b) and (d), after prior filtration to 1 or 2 µm particle size, for large-scale culture.

After the sterilizing treatment, nutrient additions are made. Details of the nutrient enrichment used at the Ministry of Agriculture, Fisheries and Food, Fisheries Laboratory, Conwy, UK, which is suitable for all of the commonly cultured species, is given in Table 5. Note that diatoms require the addition of silica (Si) to the basic nutrients. The medium is then ready to dispense aseptically to the culture flasks, which are then ready to be inoculated. In recent years, several proprietary brands of algal culture nutrients have become available. These are generally based on the Guillard F/2 formula and provide excellent growth results (see Tables 3 and 4 for the basic formulae).

To obtain the maximum productivity of most species it may be necessary to dilute the seawater with pure (distilled) freshwater (or from an uncontaminated source) before filtration or autoclaving. Growth and cell division rates of *Chaetoceros calcitrans*, *Thalassiosira pseudonana* and *Skeletonema costatum* are optimal at a salinity of about 20 to 25 PSU. Productivity of many of the flagellates is optimal at 25 to 30 PSU.

Illumination for culture growth is provided by fluorescent lamps, usually mounted externally to the culture flasks (see Figure 18). The number of lamps used is determined by the height and diameter of the culture vessels with the object of providing 15 000 to 25 000 lux measured at the centre of the empty culture container. Two 65 or 80 W lamps are sufficient to illuminate 3 l glass flasks, which are about 18 cm diameter, whereas 5 lamps of the same light output are necessary for vessels of about 25 l volume (35 cm diameter). Growth is optimal at 18 to 22°C for most species.

Table 5: Nutrient salt stock solutions for the enrichment of diatom cultures in treated seawater. The addition of stock solution C is omitted in the culture of flagellates.

Stock A

$FeCl_3$. $6H_20$		1.30 g*
$MnCl_2.4H_20$		0.36 g
H_3BO_3		33.60 g
EDTA		45.00 g
NaH_2PO4 .$2H_20$		20.00 g
$NaNO_3$		100.00 g
Trace metal solution*		1.0 ml
Distilled water	to	1000 ml

Add 2 ml Stock A per litre of filtered seawater

*** Trace metal solution**

$ZnCl_2$		2.10 g
$CoCl_2.6H_2O$		2.00 g
$(NH_4)_6Mo_7O_{24}.4H_2O$		0.90 g
$CuS0_4.6H_20$		2.00 g
Distilled water	to	100 ml

Acidify with sufficient conc. HCI to obtain a clear solution.

*Amount for enrichment of autoclaved seawater. For filtered seawater use 3.25 g.

Stock B

Vitamin B_{12} (Cyanocobalamin)		10 mg
Vitamin B_1 (Aneurine hydrochloride)		200 mg
Distilled water	to	200 ml

Add 0.2 ml Stock B per l of filtered seawater

Stock C

$Na_2SiO_3.5H_20$		4.0 g
Distilled water	to	100 ml

Add 2 ml Stock C per l of filtered seawater.

Examples of cell density achieved in the small-scale culture of a number of nutritionally important species are given in Table 6. These are values obtained at the MAFF Fisheries Laboratory, Conwy, and are typical of densities achieved elsewhere in commercial culture enterprises. It is interesting to note that much higher cell densities of *Chaetoceros calcitrans* are obtained in 2 l than in 20 l cultures. This does not necessarily mean that productivity in terms of biomass is lower. In all cultured species the size of cells is variable according to culture conditions and the growth phase. In 2 l cultures of *Chaetoceros* higher cell densities are reached but the individual cells are smaller: 35 μm^3 compared with 50 μm^3 in 20 l cultures. The dry weight content is also lower at about 10 μg per million cells (micrograms per million cells) compared with up to 18 μg per million cells in 20 l cultures. Other species show similar variability in size related parameters depending on cell density and conditions, quite apart from the inherent differences in cell size between species.

Through manipulation of culture conditions of the larger species, such as *Tetraselmis*, it is feasible to alter cell size so that the cells can be more readily ingested by smaller larvae. Small-scale culture systems can be technically improved to increase their

performance by operating them as chemostats. But, if the objective is solely to produce more food, the better solution is to turn to large-scale culture methods.

Table 6: Cell densities at harvest (cells μl^{-1}) achieved in small-scale batch (**B**) and semi-continuous (**SC**) 2 l or 20 l cultures for a selection of nutritionally valuable species. The salinity of the culture medium is also given.

Species	Culture Conditions			Harvest density (cells μl^{-1})
	Volume (l)	Type	Salinity (PSU)	
Isochrysis (T-ISO)	20	SC	25	15 000
Tetraselmis suecica	20	SC	30	2 000
Chaetoceros calcitrans	2	B	20	60 000
	20	B	20	22 000
Thalassiosira pseudonana (3H)	2	B	20	40 000

3.3.3 Estimating algal density

Before discussion of large-scale culture methods, a short description relating to the estimation of the density of cells in cultures at any scale is warranted. Various methods are available to estimate algal cell density including the use of spectrophotometers, fluorometers, haemocytometers, and Coulter counters ("multisizers").

A spectrophotometer or fluorometer measures the chlorophyll A content in an algal culture and this can be used to obtain a quick approximation of cell density. Graphs comparing cell density and readings on either instrument must be prepared for each algal species. However, the chlorophyll A content in an algal cell is not constant and varies with nutritional state of the cell. This will affect the accuracy of cell density estimates derived when using these instruments.

More accurate estimates of cell density can be made using a haemocytometer or a Coulter Counter.

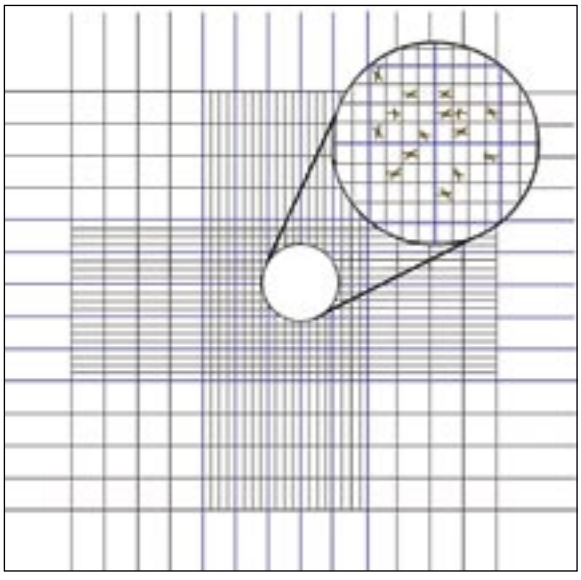

Figure 20: Diagram of the grid marked on a haemocytometer slide.

Haemocytometers are thick glass slides with two chambers on the upper surface, each measuring 1.0 x 1.0 mm. A special coverslip is placed over these two chambers giving a depth of 0.1 mm making the total volume of each chamber 0.1 mm^3. The base of each chamber is marked with a grid to aid in counting cells within the area (Figure 20). Prior to counting motile algal species, 1 or 2 drops of 10% formalin should be added to a 10 to 20 ml sample of the culture to be counted. With the coverslip in position, one or two drops of the algal sample are introduced by means of a Pasteur pipette to fill both chambers.

Cell density is estimated as follows. The central grid of each chamber (outlined in blue in Figure 20) is sub-divided into 25 squares (also outlined in blue in the diagram), each measuring 0.2 x 0.2 mm. Each larger square is further sub-divided into 16 smaller squares measuring 0.05 x 0.05 mm. The numbers of cells in 10 randomly chosen 0.2 x 0.2 mm squares are counted and the average or mean is calculated. This gives the mean number of algal cells per 0.2mm x 0.2mm x 0.1mm, or 0.004 mm^3.

Example:

A. Counts of algal cells: 40 + 30 + 50 + 60 + 55 + 65 + 70 + 45 + 40 + 70 = 525

   Average = 52.5 cells per 0.004 mm^3

B. Multiply the average by 250 to give the average number of cells per mm^3.

C. Since there are 1000 mm^3 in 1 ml, multiply the value calculated in B by 1 000. In this example, the cell density would be 52.5 x 250 x 1 000 = 13.1 million (13.1 x 10^6) cells per ml.

Note: 1 cell per ml (cells ml^{-1}) = 1 000 cells per µl (cells μl^{-1})

An easier and more accurate method of estimating cell density is to use a Coulter Counter (now called a "multisizer" – see Figure 21). This instrument was developed primarily for counting blood cells.

Several models are available and all operate on the same principle. A small electrical current is passed between two electrodes. Each time a cell passes between them, the current is impeded and the cell is counted. The size of the aperture tube is important, and for counting algal cells of between 2 to 10 µm an aperture of 50 or 100 µm in diameter is required. A known volume of water is drawn through the opening in the aperture tube and the cells are counted. More detailed explanations of the operation of a Coulter Counter are available and are included in the suggested reading material at the end of this section.

Since algal cultures are often dense, samples must be diluted to a density that can be counted accurately when using an electronic counter – approximately 50 000 cells per ml (50 cells per µl). Algal samples are usually diluted with a 3% solution of sodium chloride (by dissolving table salt in distilled water) or with 0.45 µm membrane filtered seawater.

Example:

Add 0.2 ml of algal culture to 20 ml 3% NaCl. Mix well.

Count 3 times and obtain a mean value. Individual counts = 5 280; 5 336; 5 120.

If the volume of the solution sampled by the Coulter counter is 0.1ml, then the average = 5 245 cells per 0.1 ml.

Multiply 5 245 by 10 to obtain the number of cells in 1 ml of the sample, and multiply by 100 to correct for the dilution factor.

In this example, cell density would be 5 245 x 10 x 100 = 5.2 million (5.2 x 10^6) cells per ml.

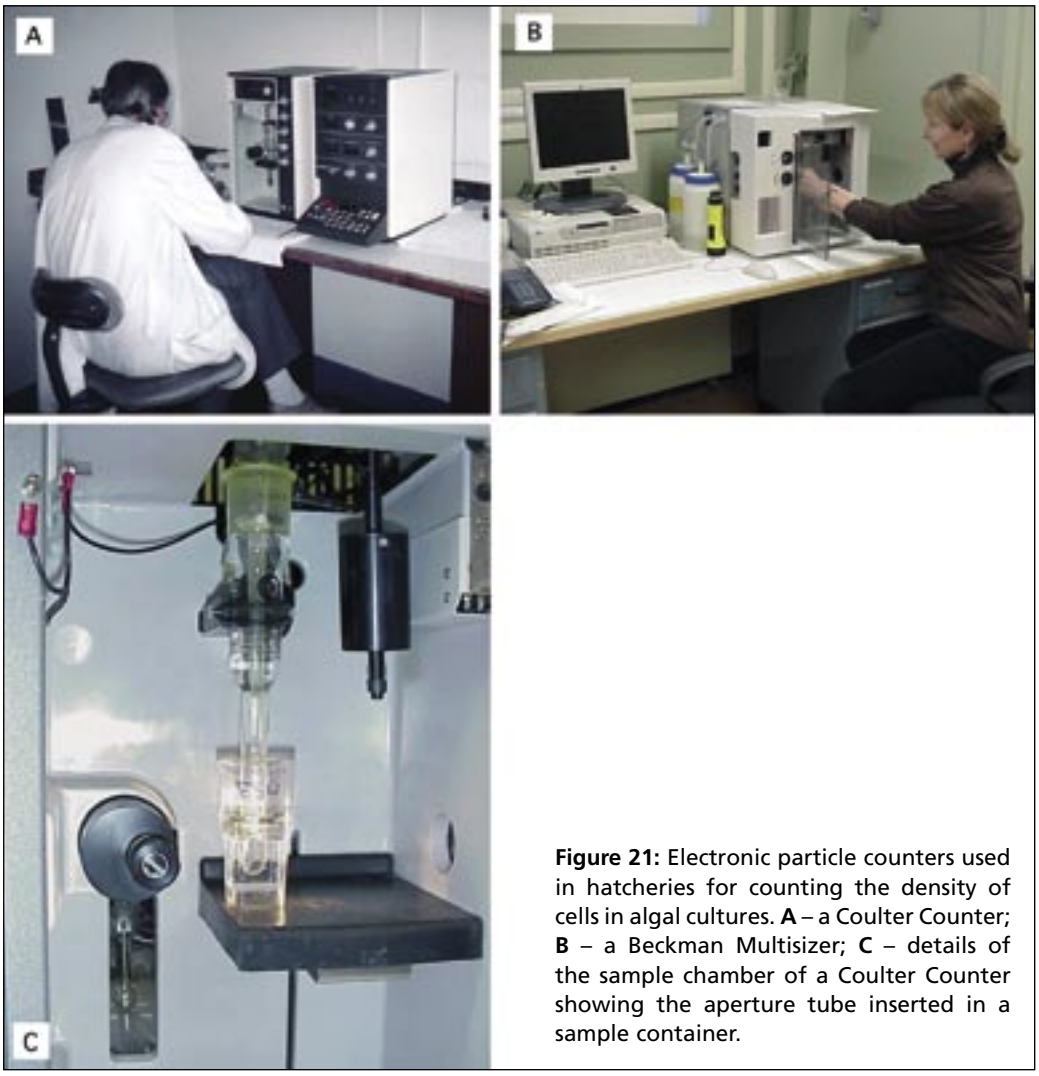

Figure 21: Electronic particle counters used in hatcheries for counting the density of cells in algal cultures. **A** – a Coulter Counter; **B** – a Beckman Multisizer; **C** – details of the sample chamber of a Coulter Counter showing the aperture tube inserted in a sample container.

Electronic counters and particle sizers are expensive but used machines can be purchased for a reasonable price. The cost of purchase is quickly offset by the time saved and the accuracy of the counts.

3.4 LARGE-SCALE CULTURE

Figure 22: Large-scale culture was often in large, circular or rectangular tanks with overhead illumination. This format has been largely superceded by the use of tall, cylindrical vessels.

Commercial bivalve hatcheries need to produce large volumes of good quality, high-food-value algae daily to support economic-scale seed production. Examples of some of the systems currently in use in Europe and North America are described in this section. They range from simple polyethylene bags either suspended, or supported by a cylinder of plastic coated or galvanized steel mesh, to sophisticated electronically operated turbidostats. All have the common feature that the culture is contained in a tall, narrow cylinder, this being

the most efficient configuration. Culture in rectangular (Figure 22) or circular tanks with overhead illumination is largely obsolete. The exception is in hatcheries, mainly on the west coast of North America. They continue to use large, circular tanks illuminated by high output, metal halide lamps. Highest productivity is achieved by mounting the illuminating lamps internally in the cultures (Figure 23) rather than externally as a bank of fluorescent lamps.

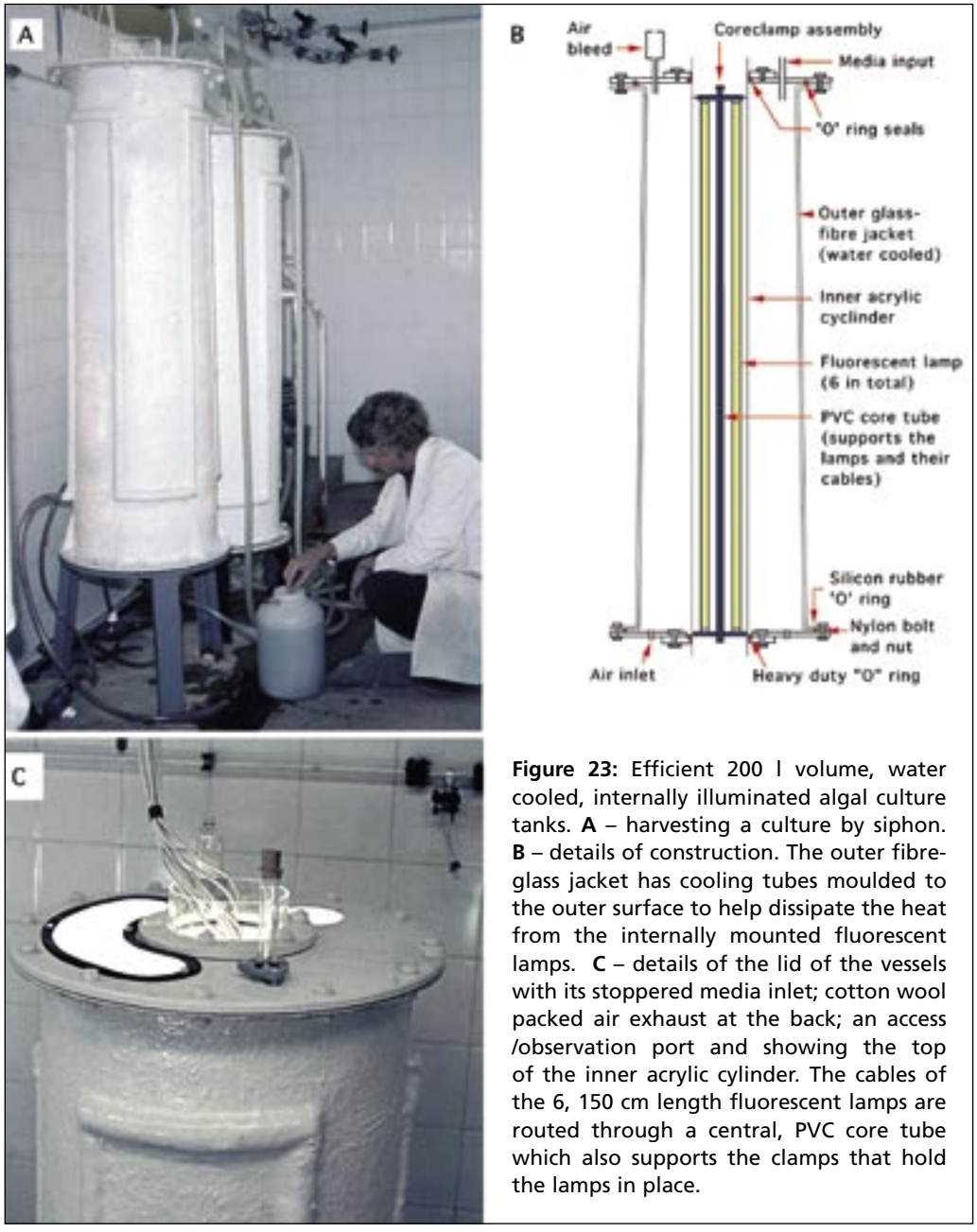

Figure 23: Efficient 200 l volume, water cooled, internally illuminated algal culture tanks. **A** – harvesting a culture by siphon. **B** – details of construction. The outer fibre-glass jacket has cooling tubes moulded to the outer surface to help dissipate the heat from the internally mounted fluorescent lamps. **C** – details of the lid of the vessels with its stoppered media inlet; cotton wool packed air exhaust at the back; an access /observation port and showing the top of the inner acrylic cylinder. The cables of the 6, 150 cm length fluorescent lamps are routed through a central, PVC core tube which also supports the clamps that hold the lamps in place.

3.4.1 Bag and cylinder cultures

Polyethylene can be purchased as heavy gauge, "lay-flat" tubing in various widths and in rolls containing convenient lengths. By cutting a suitable length and heat-sealing one end a sterile, flexible culture container can be formed, either as a cylinder or as an oblong bag. Containers formed in this way can be strengthened by supporting them within a plastic or plastic coated steel mesh frame. Alternatively, the cylinders can be suspended, with or without lateral support mesh, if the diameter of the bag is less than 30 cm and the height less than 200 cm. Examples are shown in Figure 24.

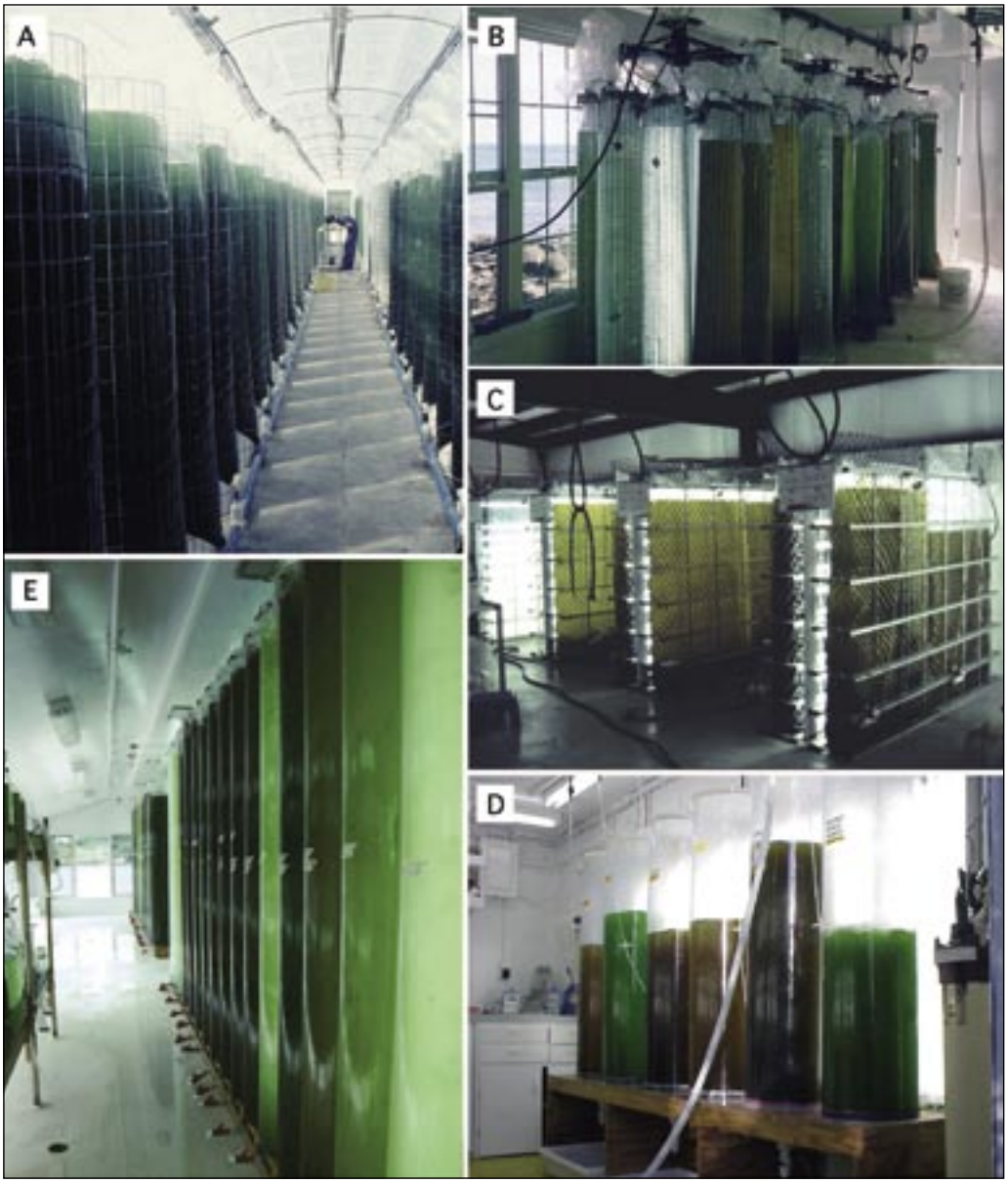

Figure 24: Examples of polyethylene bag and solar grade, fibreglass cylinder algal culture systems: **A** – 480 l polyethylene bags supported inside steel mesh frames and illuminated by natural light within a greenhouse. **B** – 80 l bags suspended around a circular central framework on a rotating, ceiling mount. Fluorescent lamps are attached to the central framework. **C** – plastic mesh supported, oblong polyethylene bags mounted either side of banks of fluorescent lamps. **D** – 100 l solar grade, fibreglass cylinders against a bank of vertically mounted fluorescent lamps. **E** – 2.4 m high, 0.3 m diameter fibreglass cylinders, externally illuminated by vertically mounted 2.4 m length fluorescent lamps.

Bags are the least expensive way of constructing large-scale culture vessels and such containers can be used indoors with artificial illumination or outdoors taking advantage of natural light. The bags illustrated in Figure 24A are formed from lengths of 10 000 gauge, extra heavy duty, 90 cm width polyethylene tubing. They are supported by welded steel mesh frames and have a capacity of 480 l with a large surface area of 3.2 m^2 for light penetration. Large cultures of this type can be illuminated by vertically mounted 1.8 m long, 80 W fluorescent lamps or can be sited outdoors, out of direct sunlight. Bag systems shown in Figure 24B and C are formed from the same material but are supported by sturdy, plastic mesh.

In general, the larger the diameter of the culture vessel, the lower is the maximum cell density possible with a fixed level of illumination. Nevertheless, these bags are superior in productivity to similar volume rectangular, fibreglass or plastic tanks sometimes still used for mass culture. They are, however, inefficient when compared with internally illuminated cultures as can be seen in yield data given in Table 7.

Polyethylene bag cultures have a relatively short life because the internal surface attracts culture debris and bacteria, which collectively reduce light penetration and are a source of contamination. At the end of a culture run it is necessary to renew the bag. Large diameter bags are inefficient but those less than 30 cm diameter can be effective because the surface area to volume relationship for light penetration is improved.

A more permanent solution to the same principle is offered by solar grade, transparent fibreglass sheet that can be formed into a cylinder and solvent welded or purchased in cylindrical form. The light penetration qualities of this material are excellent and the vessels so constructed are durable. Cylinders that are 150 to 240 cm high and 30 to 50 cm in diameter are commonly used in North American hatcheries (Figure 24D and E).

Table 7: Comparison of yields of *Tetraselmis and Phaeodactylum* from various large-scale culture systems. Yield is calculated as litres per day at a standard cell density per litre of culture volume. (* Internally illuminated systems). References quoted are given in full in the suggested reading list at the end of this section.

***Species*/System**	**Reference**	**Yield**
Tetraselmis		
80 l turbidostat*	Laing & Jones, 1988	1.25[^]
200 l vessels*	Laing & Helm, 1981	0.40
340 l tanks	Griffith *et al*., 1973	0.12
Phaeodactylum		
200 l vessels*	Helm & Laing, 1981	0.35
20 l flasks	Ukeles, 1973	0.33
480 l polyethylene bags	Baynes *et al*., 1979	0.15
195 l cylinders	Wisley & Purday, 1961	0.06

[*] A yield value of 1.25 indicates an average daily harvest of 100 l at standard cell density from an 80 l volume culture.

3.4.2 Internally illuminated cultures

Internally illuminated culture vessels are costly to construct but inexpensive to operate. By mounting the lamps inside a glass or clear plastic cylinder, as in Figure 23, the effective distance for the light to penetrate the culture is greatly reduced. In the example shown, the culture vessel is 150 cm high by 40 cm diameter. The internally mounted light cylinder is 15 cm diameter, therefore, the light energy emitted by 6, 80 W, 150 cm length fluorescent lamps travels only about 14 cm to the perimeter of the culture. In a later development, this distance was further reduced in smaller 80 l culture vessels and yet the same total productivity was achieved as in 200 l cultures.

Productivity (or yield) is determined as the total number of algal cells harvested from a culture each day. Internally lit cultures have a long life, some lasting for more than 100 days with the hardier species. When a culture is finished, the vessel is sterilized by filling with 20 to 50 mg per l (free-chlorine) hypochlorite solution, allowing it to stand

for at least an hour, before thorough rinsing with culture quality, filtered seawater, after which it is drained and re-started.

Basic conditions of culture are essentially the same as previously described. The main difference is in the treatment of water to be used as the culture medium. Autoclaving or sub-micron filtration is too expensive for the large volumes necessary. Seawater filtered to 1 or 2 μm particle size by cartridge filtration is acceptable for some of the larger celled species, e.g. *Tetraselmis* and *Skeletonema.* Otherwise, pasteurization or chemical sterilization is recommended. Control of salinity and pH is required and, to obtain maximum productivity, the intensity of illumination must be carefully calculated for the diameter of the culture vessel.

3.4.3 Principles of large-scale culture management

The objective in culture management is to obtain the greatest possible daily yield of algae so that the culture systems are operated cost effectively. This yield must be sustained for long periods of time to maintain the hatchery output of juveniles. Ineffective management of algal culture greatly influences the potential for production and ultimately the selling price of the bivalve seed.

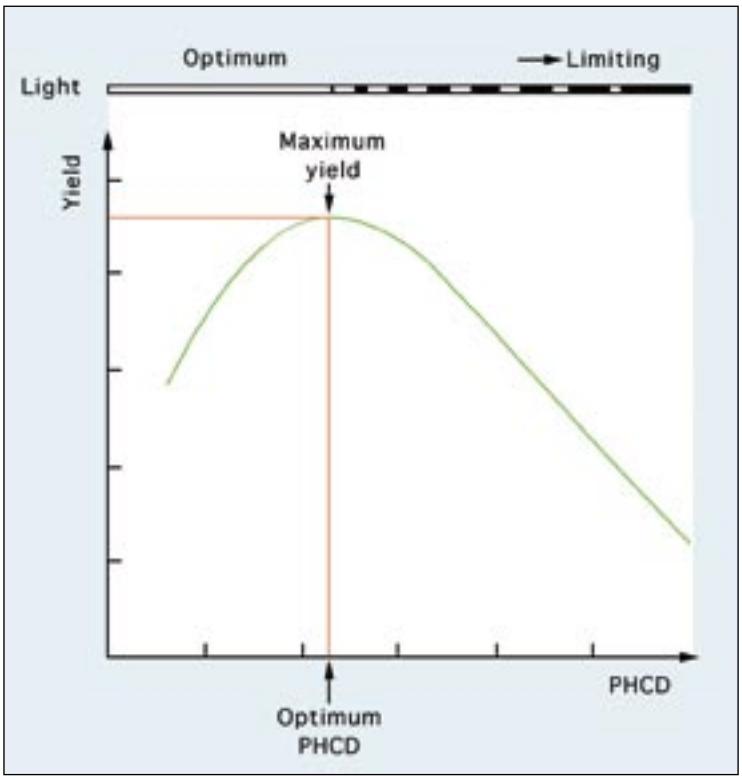

Figure 25:
The relationship between the productivity of a culture system (yield) and light energy input. See text for explanation.

The operation of semi-continuous, internally illuminated cultures will be considered in this section. The general principles are applicable to any culture facility and at any scale of production. The basic yield relationship with light energy input is shown in Figure 25. Yield is calculated as the number of litres of algae harvested per day at a standard cell density per μl.

The use of the term *standard cell density* requires explanation. In order to compare yields of different species in a culture system, a common factor based on dry weight biomass of harvested algae is applied. Different alga species vary widely in linear dimensions and in weight per cell, as already seen in Table 1. Knowing the weight per

cell, an equivalent number of cells can be calculated for each species to provide a given biomass. For some of the important species this approximates to:

250 cells of *Chaetoceros calcitrans* = 100 cells of *Isochrysis galbana* = 60 cells of *Skeletonema costatum* = 10 cells of *Tetraselmis suecica*, on a dry weight basis.

Thus, for *Skeletonema* and *Tetraselmis* the standard cell densities used in yield calculation are 6 000 and 1 000 cells per µl respectively (alternatively, 6 million and 1 million cells per ml).

Another term requiring explanation is the concept of *post-harvest cell density* (PHCD).

PHCD = cell density per unit volume (cells per µl) immediately after daily harvesting and replacement of the volume removed with fresh culture medium.

It is the cell density (following harvesting and replenishment of the culture volume with new medium) relative to light intensity that will largely dictate growth of the culture in the following 24-h period. Reference to Figure 25 shows that yield is at a maximum at the optimum PHCD when light energy input is not limiting. At PHCD values below the optimum, cell division rate (K), described by the equation:

$$K = \frac{1.443}{t \text{ (days)}} \times \frac{\log_n N_t}{\log_n N_0}$$

(N_t = cells per µl at harvest)
(N_0 = PHCD)

is at its maximum but the PHCD is too low for maximum productivity. Above the optimal PHCD, light becomes increasingly limiting due to the self-shading effect of cells at higher culture densities. Photosynthesis decreases, therefore, cell division rate and daily yields decrease. Yield is maximal at a particular light intensity and can be increased or decreased by altering the light energy input.

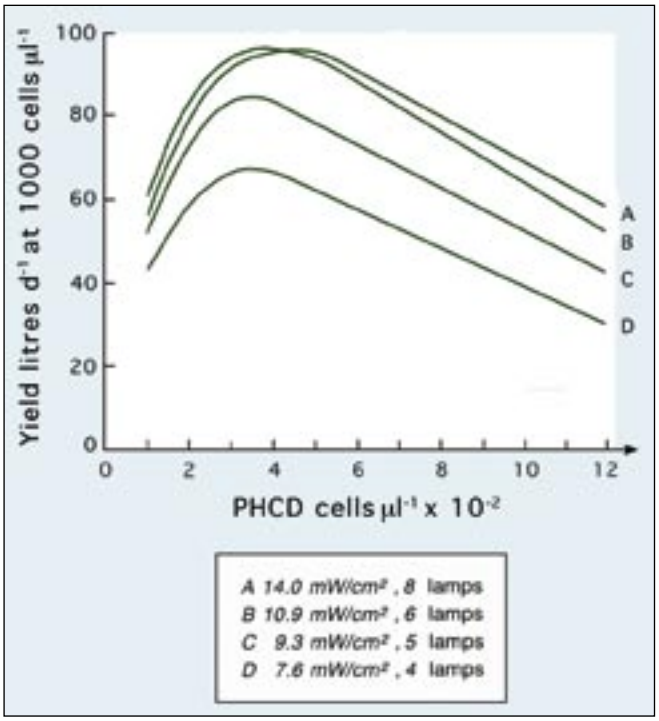

Figure 26: The effect of light intensity on yield of *Tetraselmis* in 200 l internally illuminated culture vessels.

The effect of increasing light intensity in 200 l cultures of *Tetraselmis* by increasing the number of 80W fluorescent lamps from 4 to 8 is shown in Figure 26. Four lamps provide an illumination intensity of 7.6 mW per cm^2 (7.6 milliwatts per centimetre squared which provides an illumination intensity of 28 000 lux) and 8 lamps, 14.0 mW per cm^2 (52 000 lux). Maximum yields, increased from 67 l per day at 1 000 cells per µl at 28 000 lux to 96 l per day at the same cell density at the highest illumination intensity. Improvements in yield result from an accelerated cell division rate and, because of the greater

light energy input, cultures can be operated at a higher PHCD. Yields from 8 and 6 lamp units are similar. This is because the cultures approach light saturation at the highest illumination level, therefore, yield relative to cost of the extra energy input decreases with 8 lamp units.

The influence of PHCD on cell division rate (K) in *Tetraselmis* in 200 l cultures is shown in Figure 27A. Increasing PHCD values result in exponentially decreasing K values, as light becomes progressively more limiting. Data in Figure 27B and C show that values of K decrease, therefore, yield decreases with increasing pH and salinity. This highlights the need to control these parameters by (a), increasing carbon dioxide input in the case of increasing pH and (b), by diluting the culture medium in the case of elevated salinity. Devices to control pH by varying the rate of input of carbon dioxide are available from suppliers of aquaculture equipment.

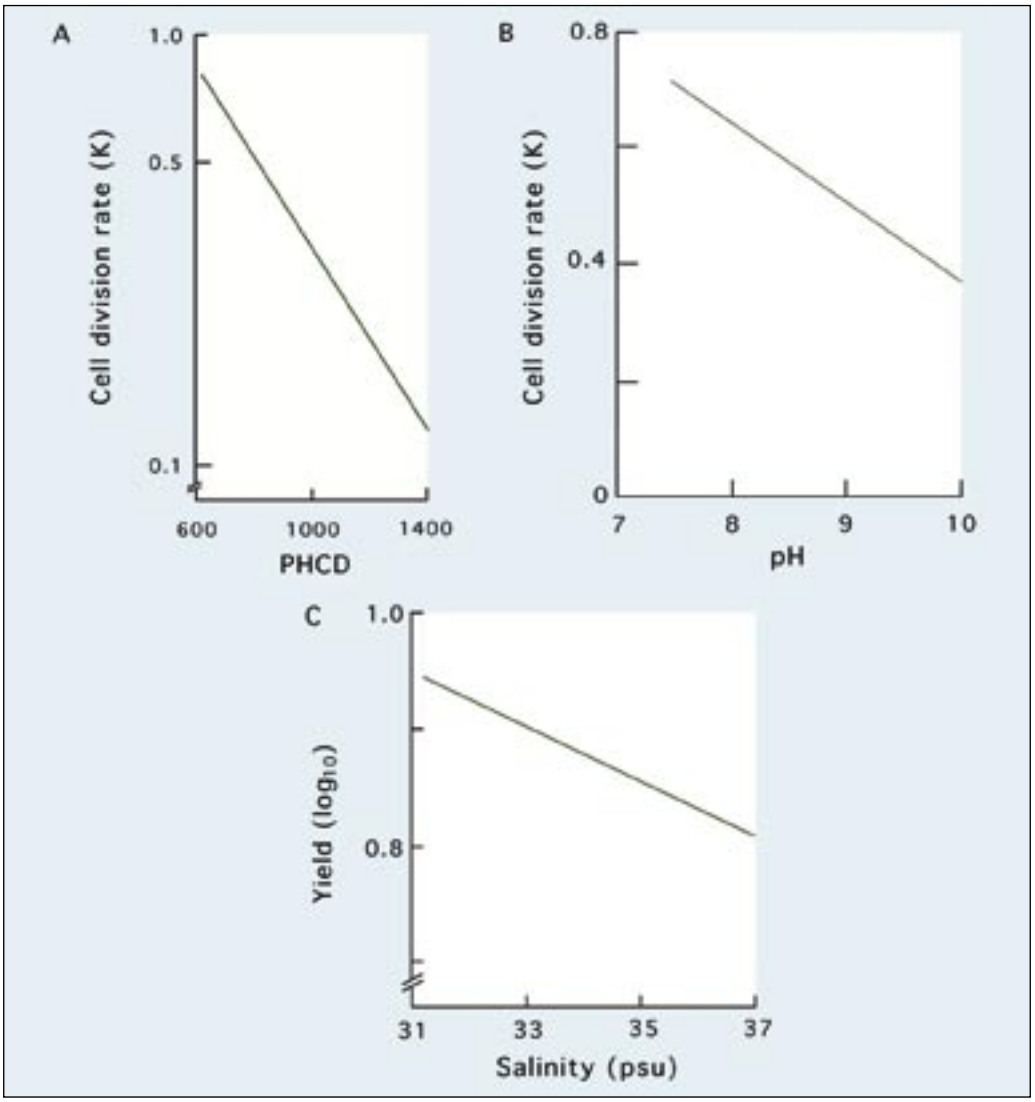

Figure 27: Effects of **A** – post-harvest cell density (PHCD) and **B** – pH on cell division rate, and the influence of salinity on the productivity of cultures of *Tetraselmis suecica* – **C**.

Culture techniques that improve maximum yield can also have the effect of altering the size of cells at harvest (Figure 28). With increasing PHCD and onset of light limitation, cells decrease in size measured as either dry or organic weight. However, within normal operating limits of PHCD the overall effect on maximum yield, on a biomass basis, is small.

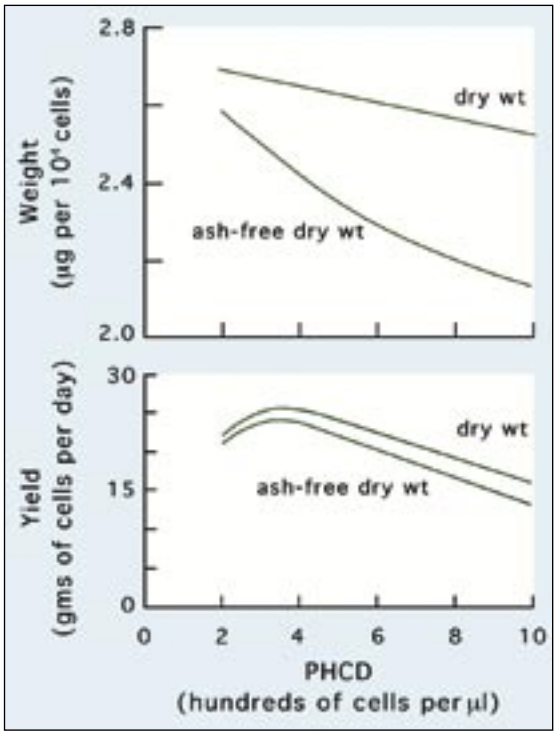

Figure 28: Relationships between post-harvest cell density (PHCD) and the size of cells in terms of weight and the productivity of semi-continuous culture of *Tetraselmis suecica*.

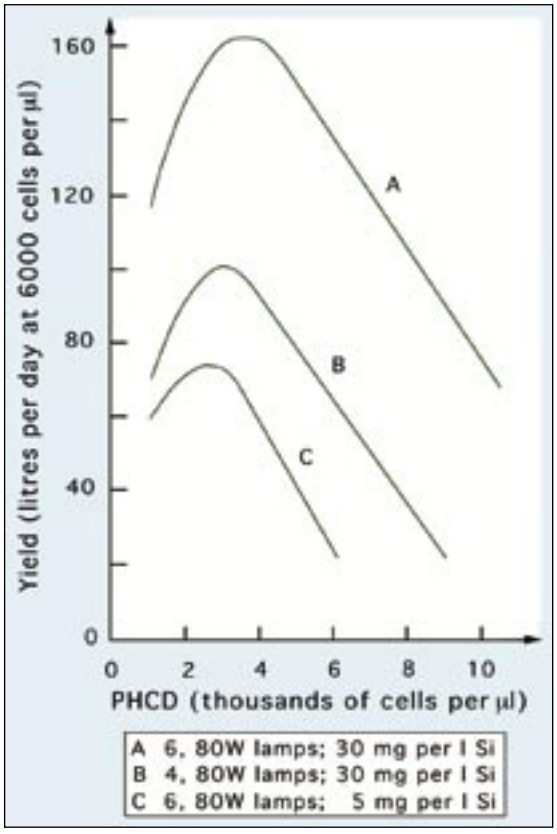

Figure 29: Relationships between post-harvest cell density and yield at standard cell density of *Skeletonema costatum* cultures operated semi-continuously at two light intensities and silicate concentrations.

The nutrient content of the culture medium also has an important effect on the maximum yield achievable in large-scale culture systems. This is shown in Figure 29, which provides data on the culture of the diatom, *Skeletonema costatum*. Diatoms require silica, which is provided in the form of SiO_3-Si, to allow development of the silicaceous frustrules that enclose the cytoplasm. If silica is limiting, cell growth and division rates decrease and yields diminish. This is clearly shown in the comparison of 6, 80 W fluorescent lamp units at 30 mg per l Si (Figure 29A) and at 5 mg per l Si (Figure 29C). Cultures at 30 mg per l Si provided a maximum daily yield of 160 l (from a culture volume of 200 l at 6 000 cells per µl), whereas at 5 mg per l the maximum yield was only 74 l – less than from a 4 lamp unit at the highest Si level (Figure 29B). The maximum yield (Figure 29) is considerably greater than was obtainable from efficiently operated cultures of *Tetraselmis* and reflects the much higher cell division rates hence, productivity achievable with diatoms.

3.4.4 Automated large-scale culture

So far discussion has centered on semi-continuous culture methods. Although less labour intensive than batch harvested systems, the manpower component in operating a daily harvesting schedule is still relatively high. As a consequence it is usual to decrease the harvesting rate to 48-h intervals. This is achieved by operating the cultures at a lower PHCD. Nevertheless, the point of maximum yield can be reached within the 48-h interval so that light limitation will influence overall productivity. The solution is to operate the cultures continuously, i.e. continuous harvesting. This solution is feasible using opto-electronic control of cell density. A diagram of an automated system developed and used at the Fisheries Laboratory Conwy, UK is shown in Figure 30.

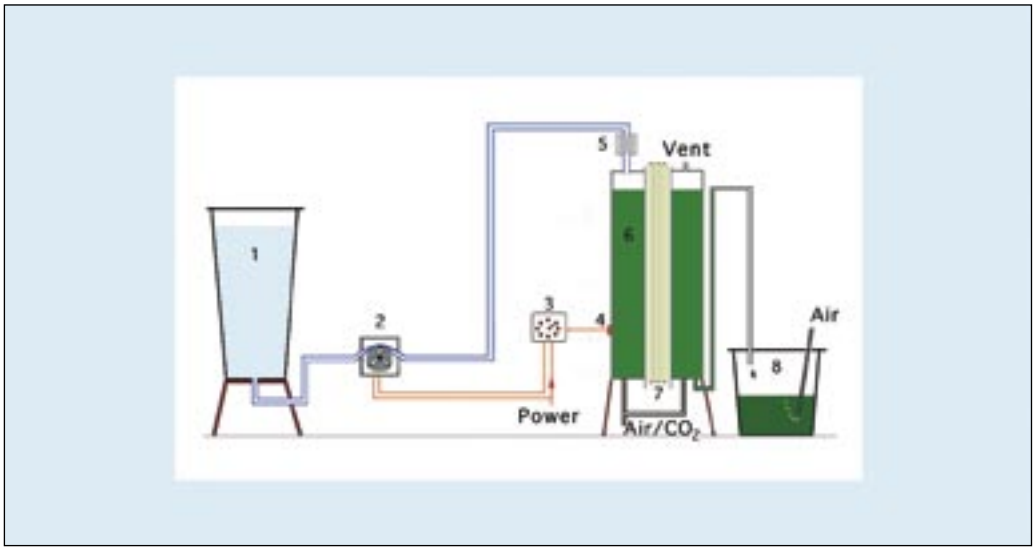

Figure 30: Schematic of a continuous ("turbidostat") culture system (not drawn to scale). **Key**: **1**, reservoir of seawater medium (200 l volume); **2**, peristaltic pump; **3**, resistance sensing relay (50 to 5 000 ohms); **4**, light dependent resistor (ORP 12); **5**, cartridge filter (0.45 μm); **6**, culture vessel (80 l volume); **7**, six, 80 W fluorescent lamps; **8**, collecting tank to receive the harvest (125 l volume).

The key component in this system is a Light Dependent Resistor (LDR) clamped onto the outer surface of a transparent culture vessel. Light falling on the LDR after penetrating the culture varies depending upon the density of cells in the culture. Internal illumination is used, as in the semi-continuous, large-scale systems previously described. As cell density increases the light transmittance through the culture decreases and this increases the resistance value of the LDR. This increase is detected by a Resistance Sensing Relay (RSR) which is set to activate a peristaltic pump when a certain pre-set resistance value is reached. The RSR is adjusted to operate at the light intensity at which cell division is at a maximum. When activated, the peristaltic pump supplies fresh medium to the culture vessel and this displaces an equal volume of the culture into a receiving container. As the culture in the vessel becomes increasingly diluted, the transmittance of light through the culture, detected by the LDR increases, the resistance of the LDR decreases, and the RSR switches off the peristaltic pump.

This apparatus is inexpensive to construct with modern electronics and is very effective in maintaining cultures at peak productivity. Yields from an automatic, 80 l system for *Isochrysis galbana* (Clone T-Iso) and *Tetraselmis* are similar to those from the larger, 200 l units operating semi-continuously. A maximum yield of *Tetraselmis* of about 100 l per day at 1 000 cells per μl is achievable by operating the automatic system at about 2 000 cells per μl. Yields of about 90 l per day at 10 000 cells per μl have been obtained with *lsochrysis* operating at a culture density of 16 000 cells per μl.

The principle of automatic operation is not new. Chemostats or turbidostats using external light sources for the production of microalgal species have previously been described. The Conwy system described above is an updated and more efficient version of the concept. Continuous culture systems based on either vertically or horizontally arranged, polyethylene bag units are commercially available.

3.4.5 Troubleshooting

Cultures will fail to grow, will become overly contaminated with competing micro-organisms or will crash even in the best-run hatcheries. Below are some pointers to check to determine the source of such failures.

1. Air supply. Is there adequate air entering the cultures? Are the cells sedimenting to the bottom of the culture vessel? This may happen when culturing certain diatoms, in which case the air flow rate should be increased. It should not happen in the case of commonly cultured flagellates. If it does, then the problem lies elsewhere.

2. Temperature. Check min/max thermometer. Were there any increases or decreases in the temperature of the algal culture facility over the past 24 hours? Most of the commonly cultured algal species cannot tolerate temperatures above 26°C for extended periods – or temperatures below 12°C. Temperatures in the range 18 to 22°C are ideal.

3. pH. Check CO_2 supply. Is the CO_2 cylinder empty? Check pH of the algal cultures using a pH probe. Is the pH too high (above 8.5)? Is the pH too low (below 7.5)? Adjust the CO_2 supply accordingly.

4. Nutrients. Check records for the last time the cultures received nutrients. This is particularly important for semi-continuous cultures.

5. Contamination. Are the walls of the culture container, particularly at the water/air interface, visibly foaming or fouled with what appears to be detritus? If so, the culture is at the end of its useful life and needs to be replaced. If this is a continuing problem in the early stages of the culture cycle with a particular species, then check the starter cultures for signs of contaminating organisms and replace them as necessary.

Not all species can be cultured successfully during the entire season. Some have particular "windows of opportunity" when they can be grown reliably. However, there is no consistency between hatcheries as to when a named species will grow well or will not. This has to be learned through on-site experience and highlights the importance of maintaining thorough records.

3.4.6 Extensive outdoor culture

Intensive culture systems, described above, are closely controlled and highly productive, providing food for larvae, small juveniles and hatchery-held broodstock. An alternative, particularly suited to the provision of food for larger juveniles, is extensive outdoor tank culture, which makes use of natural light (Figure 31). This involves the fertilization of a large volume of seawater with the basic nutrients necessary for production, namely nitrogen, phosphorus and silica in one form or another. Here, the objective is not necessarily to induce a monospecific bloom, but a mixed flagellate and diatom population at a density greater than would normally occur in the sea.

It is possible to induce monospecific blooms by prior fine (<2 μm particle retention) filtration of the impounded seawater and the introduction of an inoculum of the required species, as long as it is hardy and vigorous. The use of seawater, or suitably saline brackish water, drawn from wells will also serve this purpose. However, it is difficult to maintain such blooms for long periods because they rapidly become contaminated with other microorganisms.

Multispecific blooms are more easily managed and rely on the natural phytoplankton content of the seawater utilized as the inoculum. While the species composition will vary from one blooming to another, according to season and environmental conditions, algae produced in this manner is nutritionally valuable in growing juveniles and also for maintaining broodstock.

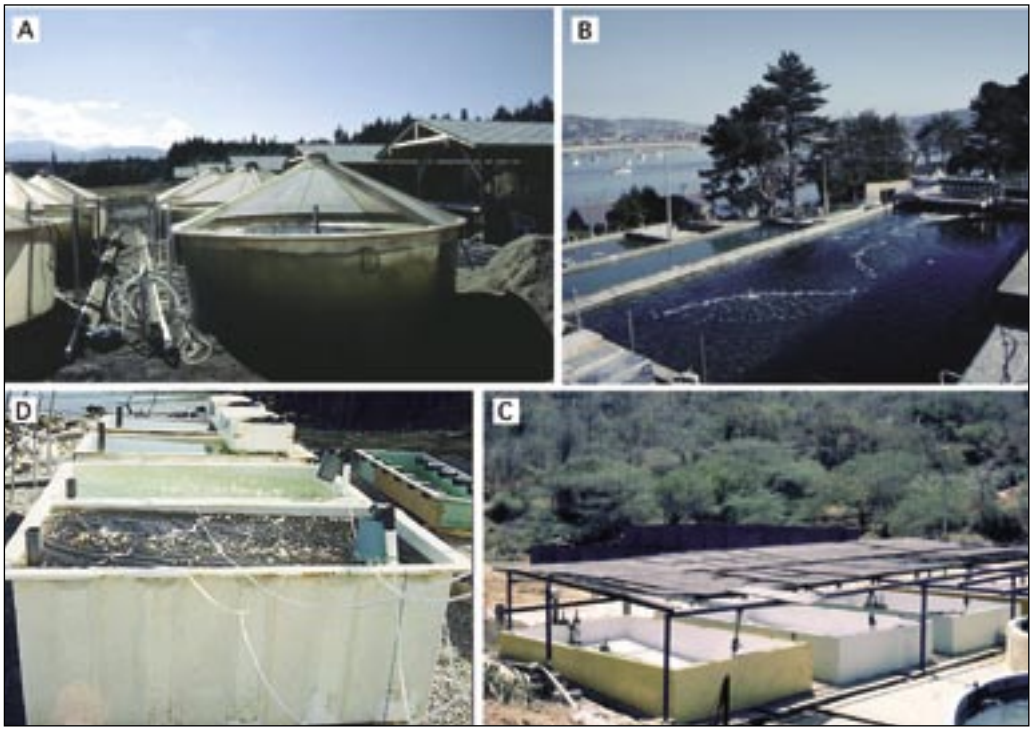

Figure 31: Examples of large-scale, outdoor algal production. **A** – circular, covered, semi-transparent, fibreglass tanks at a hatchery in British Columbia, Canada; **B** – 450 000 l concrete tanks used to bloom natural phytoplankton in support of spat culture at the Fisheries Laboratory, Conwy, UK; **C** – large concrete tanks with sloping bases used for monospecific algal production at Turpiolito, Venezuela: **D** – 2,500 l fibreglass "fish boxes" at a hatchery in Nova Scotia, Canada.

At the Fisheries Laboratory, Conwy, large, outdoor concrete tanks ranging in volume from 60 m^3 to 450 m^3 have been used for extensive algal production in support of the nursery culture of bivalve seed. These tanks are filled with seawater of 28 to 32 PSU salinity from the adjacent estuary at approximately 2-week intervals. In this form of culture, fertilizers are added 3 days before the tank is needed to produce algae as food for juvenile bivalves. The chemicals added are:

Urea NH_2CONH_2	(46% N)	1.50 g per m^3
Triple superphosphate P_2O_5	(20% P)	1.56 g per m^3
Sodium metasilicate $Na_2SiO_3.5H_20$	(13% Si)	10.60 g per m^3

Concentrations of NH_2N are 50 µg atoms per l; PO_4-P, 10 µg atoms per l and of SiO_3-Si, 50 µg atoms per l. More crudely, the application of poultry or other animal manure at 500 kg per hectare for tanks and ponds of about 1 m depth can be an effective and less costly source of nutrients.

The rate of development of a bloom is related to the initial species composition and density of algae in the seawater, day length, the amount of incidental illumination falling on the surface of the water, nutrient levels and temperature. The surface area/volume relationship of the tank or pond is important. Shallow tanks and ponds of about 1 m depth are more effective than deeper water, permitting better light penetration. Aeration of the tanks or ponds is beneficial to production.

The duration of the bloom depends on a number of factors related to the species of algae that develop in the prevailing conditions and the rate at which the algae are grazed by the bivalves. Usually, a bloom of useful density for feeding purposes can

be maintained for 7-10 days after which the tank is drained, cleaned and refilled with fresh seawater.

The species composition of blooms can be manipulated to some extent by altering the types of fertilizers added. For example, by omitting Si, flagellate species may dominate because the natural Si content of the water upon which diatoms depend will rapidly become depleted. In smaller tanks it is possible to inoculate the fertilized water with a species grown in intensive culture systems. Whether or not this species will become dominant in the bloom depends on environmental conditions and the presence or absence of competing species. In general, the use of artificial fertilization of impounded seawater is a valuable technique in bivalve culture, particularly in nursery systems for juveniles. It is often possible to improve phytoplankton production by a factor of 5 or more compared with open sea conditions. The cost in fertilizers is small per 1 000 l of seawater compared with the considerable benefits in the increased commercial value of the faster growing juveniles.

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Part 4

Hatchery operation: broodstock conditioning, spawning and fertilization

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4.1 BROODSTOCK CONDITIONING

4.1.1 Introduction

Conditioning broodstock is essential in the provision of larvae for culture (Figure 32). It is the procedure by which hatcheries are able to extend their production season, removing reliance on the relatively brief period in the year when adults of the desired species are bearing mature gametes in the sea. In the case of hatcheries in marginal climates, there is distinct advantage in producing seed early in the year – often months before stock have developed and matured in nature.

Early season production in colder climates ensures that seed have a maximum growing period prior to their first over-wintering. Thereby, they are larger and more resistant to low temperature. This may be relevant in the culture of exotic species where small

seed may not be as fully cold hardy at a small size as are similarly sized seed of native species. Hatchery conditioning of stock is also relevant in circumstances where an exotic species has been introduced for farming purposes but will not recruit reliably in its new habitat.

Figure 32:
A typical broodstock conditioning system.

Many bivalves will mature in their first year of life as males. As they age, year by year, an increasing percentage may switch sex and become females. This is known as protandric hermaphroditism. Among the commonly cultured species in hatcheries exhibiting this form of sexual development are clams of the genus *Tapes*, *Mercenaria*, *Mya* and *Spisula*, oysters of the genus *Crassostrea* and the many types of mussel including *Mytilus* sp. *and Perna* sp.

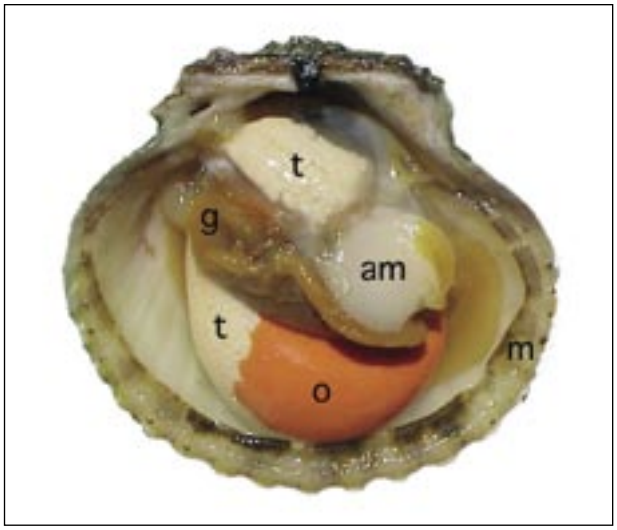

Figure 33: The anatomy of a fully mature calico scallop (*Argopecten gibbus*): **am** – adductor muscle; **g** – gills (raised to reveal the gonad); **m** – mantle; **o** – ovary; **t** – testis.

Some species of bivalves are truly functional hermaphrodites. They mature both male and female gonads simultaneously (Figure 33). Gametes are spawned sequentially, usually sperm first followed by the eggs, with later reversal to sperm again within the spawning cycle. This group of monoecious species includes the northern European King scallop, *Pecten maximus*, the (Brazilian or Caribbean) sand scallop, *Pecten (Euvola) ziczac*, the bay scallop, *Argopecten irradians*, the calico scallop, *Argopecten gibbus*, the Chilean scallop, *Argopecten purpuratus*, and some species of *Chlamys*. Sexes are separate (dioecious) in other large sea scallops, e.g. *Placopecten magellanicus* and *Patinopecten yessoensis*.

Flat oysters of the genera *Ostrea* and *Tiostrea* exhibit alternate sexuality. They switch sex at the end of each reproductive cycle. A single European oyster (*Ostrea edulis*) can go through two or three sex reversals each spawning season when sufficient food is available and during an extended warm water period.

Conditioning case history - the Manila clam, *Tapes philippinarum*

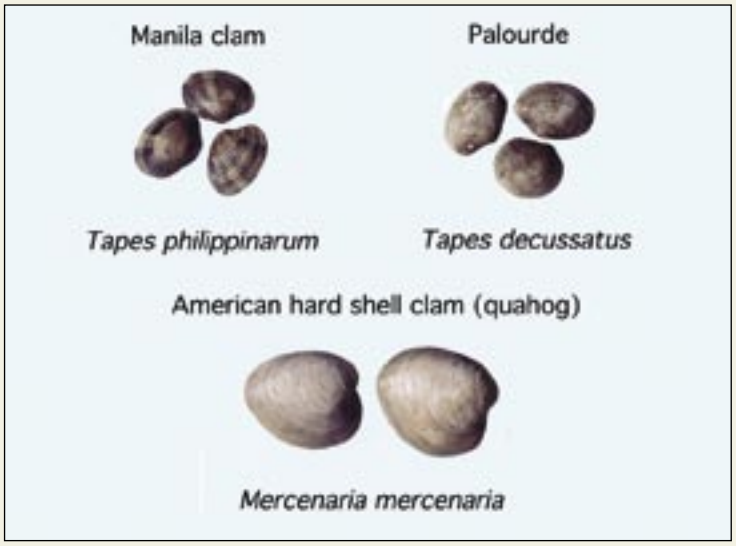

Figure 34: A selection of clams commonly cultured in hatcheries. Note that the nomenclature of the genus *Tapes* is synonymous with *Venerupis* and *Ruditapes* in European hatcheries, thus Manila clams may be referred to as *Tapes* or *Venerupis* or *Ruditapes philippinarum* (with *semidecussatus* or *semidecussata* being other alternative specific names). Nomenclature is equally as confusing in some other commonly cultured bivalves).

In the Manila clam (Figure 34), as in other bivalves, egg production increases with increasing adult size. Mature females of 10–20 g live weight will spawn 5–8 million eggs on average depending on their condition and time of year they are brought into breeding condition.

Populations of 2 and 3-year-olds show close to a 50:50 sex ratio. For example, of 138 conditioned clams subjected to spawning stimuli in trials at the MAFF Fisheries Laboratory, Conwy, UK, in 1987, 54 spawned as females and 55 as males. The remaining 29 clams, that were mainly in earlier season spawning attempts, failed to liberate gametes and were probably 'under-ripe.'

Sexual development starts in the sea when the water temperature exceeds 10°C. Gametes develop during late May or June and mature in July or August to be retained until spawning is stimulated by high temperatures (>20°C) or by a series of thermal or handling shocks. In northern European waters, where temperatures are rarely high enough to stimulate spawning, mature gametes are retained into the early winter and are then resorbed.

Maturity can be accelerated in the hatchery by holding the clams at elevated temperatures and providing them with a suitable food ration. It is possible to mature adults in the winter and early spring, before clams in the sea commence sexual development, thereby extending the period hatcheries have access to larvae. Clams in spawning condition can, therefore, be made available for most of the year. To obtain spawning in the autumn it is possible to mature juveniles from early same season spawning by conditioning them at high temperature and high food rations.

Temperate climate bivalves generally have two spawning periods within a year following spring and autumnal peaks in phytoplankton production. Tropical species exhibit less well defined spawning periods. Spawning takes place during most of the year with a low percentage of adults maturing at any point in time. This habit presents problems for hatcheries in the tropics since many of the individuals will be spent (i.e., have recently spawned) or be in the early stages of gamete development when stock is brought into the hatchery. This is wasteful in terms of time, space and food resources. However, there are ways of bringing broodstock into greater synchronicity of reproductive development (see section 4.1.3).

4.1.2 Conditioning methods

4.1.2.1 Tank systems and water treatment

The basic methods for broodstock conditioning are much the same for all bivalves. It is usual for a hatchery to maintain its own stocks for production purposes in local, sea-based growout. These stocks are kept in the best possible conditions of high water flow and at low density in well maintained growout equipment. They are often the offspring of previous hatchery-reared generations, selected for desirable characteristics such as growth rate, shell shape and colouration.

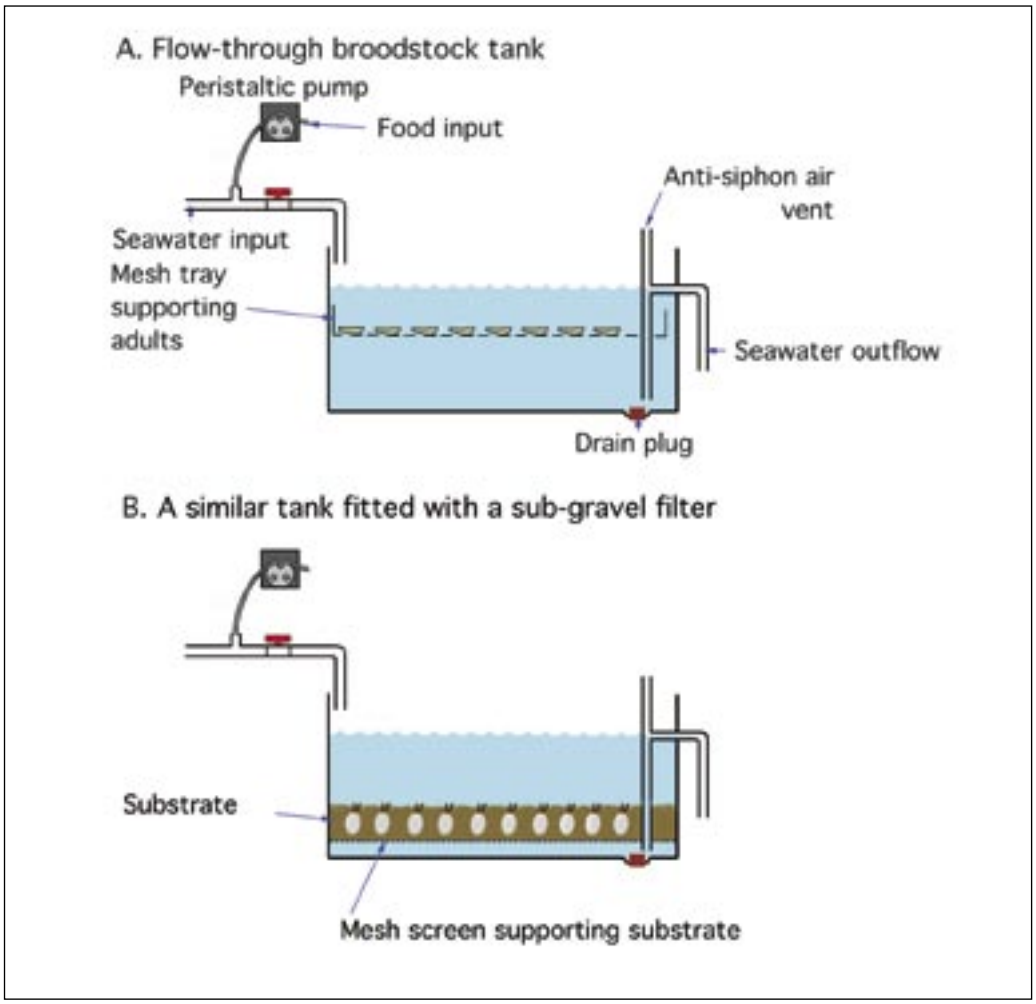

Figure 35: Diagrammatic representation of **A** – a flow-through broodstock tank in which adults are suspended off the bottom in a mesh tray with large apertures in the base so as not to retain faeces and detritus; **B** – a similar tank fitted for sub-gravel filtration. Systems of type A are suitable for most species that do not require a substrate. Clams and some scallop species often condition better in tanks of type B.

Adults taken from the sea are brought into the hatchery, their shells thoroughly scrubbed and rinsed to remove epifaunal (fouling) organisms and sediment, and then placed in tanks similar to those shown in Figure 35 (see also Figure 32). Clams and also scallop species (e.g. *Pecten ziczac*) which are normally partially buried in the substrate in nature, feed more efficiently if they are kept in a suitable substrate. In tanks of the type illustrated, clams or scallops are allowed to bury in 10 cm deep trays filled with coarse sand or shell gravel, or in a sufficient depth of substrate over a sub-sand filter (Figure 35B). Trays are supported off the bottom of the conditioning tank when stocked with bivalves that do not require a substrate, e.g. oysters, mussels and some scallop species (Figures 35A and 36).

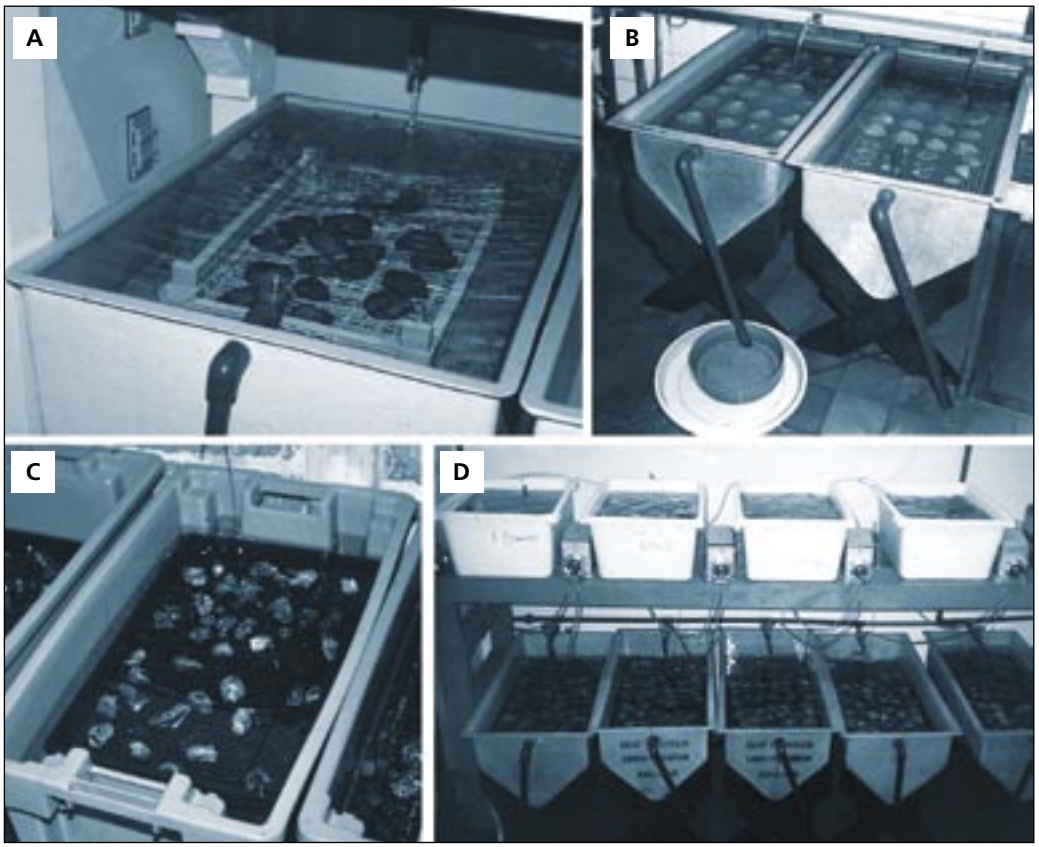

Figure 36: A to **D** – Examples of various types of flow-through tanks used for broodstock conditioning. The tray under the outflow in **B** – contains a mesh-based sieve, used to retain European flat oyster larvae that may otherwise be lost in the tank discharge when liberated by the adults. **C** – is an experimental system with each broodstock tank supplied a different diet by peristaltic pump from the reservoir tanks alongside.

Seawater used need not be filtered: the diversity of food species present in unfiltered seawater is beneficial in the conditioning process. While it is possible that the broodstock may be exposed to the infective stages of parasites or potentially pathogenic micro-organisms present in the incoming water supply, the cost benefit in not filtering the water often outweighs the risks. In most cases, conditioning takes place in flow-through systems, which may or may not include an element of water recycling to conserve cultured algae added as feed.

It is also feasible to condition bivalves in recirculation systems where the total live weight biomass of adults (the collective weight – shells included – of all the animals in the tank) does not exceed 2 or 3 g per l. In this case, it is advisable to drain and refill the total volume of water in the system at least twice each week to prevent build-ups of bacteria and metabolites.

Both salinity and temperature should be appropriate to the species being conditioned. Most commonly cultured bivalves will undergo reproductive development and mature gametes at salinities greater than 25 PSU (practical salinity units, equivalent to parts per thousand) and temperatures of between 16 and 24°C. However, each species will have optima for both of these parameters. Manila clams and Pacific oysters for example, respond best to water temperatures between 22 and 24°C. Pacific oysters will condition at a wide range of salinities (15 to 34 PSU) while Manila clams prefer higher salinities of between 25 and 34 PSU with an optimum of around 30 PSU. The American (Virginia) oyster, *Crassostrea virginica*, will condition at much lower salinities. As one would expect, offshore, deeper water species require cooler conditions and near oceanic salinity.

Water flow rate through conditioning tanks should exceed 25 ml per minute per adult and no more than 5 kg live weight biomass of stock should be held in a tank of 120 to 150 l volume (Figure 37). The water should not be recycled and reused in such small tanks when heavily stocked. When bivalves from outside the immediate area are used as stock, the effluent water discharged from the tanks should be diverted to a treatment tank to prevent the transfer of pathogens and parasites to the local environment. The effluent needs to be treated with >100 mg per l free-chlorine or a similarly effective disinfecting/sterilizing agent (e.g. ozone) for a minimum 24-hour period (preferably 48 hours) before it is returned to the sea.

Figure 37: A 120 l broodstock tank stocked with 55 oysters averaging 80 g live weight. The minimum flow rate of cultured food supplemented seawater through the tank at this stock density is 1.375 l per minute.

Hatcheries usually have a separate broodstock conditioning room or locate the conditioning tanks in a quiet area of the plant where stock will not be subject to frequent disturbance. Most species respond to shadows and vibration by closing their shell valves. The less disturbance they receive, the more time they will spend feeding.

Small and medium size hatcheries usually have between 5 and 20 conditioning tanks to accommodate the various species reared and to permit the regular introduction of new stock to maintain a rotation and ensure a continuous supply of larvae. Large hatcheries may have more of the smaller tanks or fewer that are larger. When the steady production of spat of a particular species is required over an extended period of the year, new stock is brought in to start the conditioning process on a weekly or two-weekly basis. In this way, adults are available for spawning every week.

4.1.2.2 Feeding broodstock

Cultured marine algal species are used most frequently as the principal food supply during conditioning. Alternative sources are natural phytoplankton bloomed extensively in outdoor tanks or ponds, or commercially available algae pastes.

Useful algal species that can be cultured intensively on a large-scale are *Tetraselmis* (various species, including *T. chuii*, *T. tetrahele* and *T. suecica*), *Isochrysis galbana* (and the T-Iso clone), *Pavlova lutherii*, *Chaetoceros muelleri* (previously named *C. gracilis*), *Thalassiosira pseudonana* and *T. weisfloggii* and *Skeletonema costatum*. (This list is by no means exhaustive). A mixture of these species, on a proportional basis, is more beneficial than a single species diet. Care should be taken not to feed relatively indigestible species (e.g. *Chlorella* sp.) or, species known to be deficient in the more highly unsaturated fatty acids (e.g. *Dunaliella tertiolecta*).

An example of the consequences of feeding a deficient diet is the reduced production of larvae from *Ostrea edulis* adults when held in filtered water and fed only *Dunaliella tertiolecta* (Table 8). *Dunaliella* is known to be lacking in the C20 and C22 highly unsaturated fatty acids considered to be nutritionally essential. In this trial, groups of 60 adults were kept in tanks provided with a through-flow of either unfiltered seawater or seawater filtered to 2 µm particle size. (The experimental tank system is shown in the bottom right photograph of Figure 36C). A daily 3% ration based on the initial dry meat weight of the oysters was provided to three of the groups as *Dunaliella* alone or in combination with either *Tetraselmis suecica* or T-Iso. Control groups were kept in both flowing filtered and unfiltered seawater without the addition of cultured algae.

Table 8. Effect of diet on the production of *Ostrea edulis* larvae. **Key**: Seawater (**SW**) treatment, **F** and **UF** refer to filtered and unfiltered seawater respectively; Diet, **Dt** – *Dunaliella tertiolecta*, **Ts** – *Tetraselmis suecica*, **T-Iso** – *Isochrysis galbana* (Clone T-ISO). Days - refers to the number of days from the start of conditioning until larvae were first released. Total larvae is the number of larvae produced by each group of adults in a 70-day period and when this value is divided by the number of adults in the group provides larvae/oyster. From Millican and Helm (1994). See text for further details.

SW Treatment	Diet	Days	Total larvae	Larvae/oyster
F	None	35	1.16	19 367
F	Dt	49	0.65	10 280
F	Dt + Ts	31	3.00	49 950
F	Dt + T-Iso	32	4.70	78 250
UF	None	33	8.12	135 317

The elapsed time from the beginning of conditioning to the first release of larvae in each group was noted and daily counts of larval released were made during the 10-week duration of the trial. Results given in Table 8 show that the single species diet of *Dunaliella* delayed both the onset of larval production and reduced overall production in comparison with the alternative treatments tested. Interestingly, considerably greater numbers of larvae were released by adult oysters held in unfiltered seawater without cultured algae addition than from the other treatments. This reinforces the previously made point that there may be a cost benefit in not filtering seawater for conditioning.

The duration of the above trial encompassed the spring phytoplankton bloom when chlorophyll a in the unfiltered control seawater averaged 1.68 mg per m^3 compared with 0.35 mg per m^3 in the filtered control seawater. Particulate lipid averaged 62 ng per l (nanogram per l) compared with 9.7 ng per l respectively.

Methods for both intensive and extensive algal culture are described in Part 3 of this manual. Steps in the calculation of the required food ration are described below in section 4.1.2.3. The calculation does not, however, apply to extensively grown phytoplankton where species diversity, abundance and overall nutritional value will vary day by day. In this case, an approximation of abundance can be made by determining the ash-free dry weight of particulate matter per unit volume, or by organic carbon analysis. Alternatively, the operator can dilute the bloomed water "by eye" to provide an adequate ration.

Algal pastes of the various nutritionally preferred species are convenient to use and suppliers provide information on the equivalent number of cells per unit volume of the product. Many of these products also bear quantitative details of nutritionally important components on the packaging. Once opened, the non-living product has a relatively long shelf life when the supplier's instructions are closely followed. Such pastes are probably best used in flow-through conditioning and attention must be paid to the hygiene of the tanks.

Provision of a satisfactory ration of nutritionally valuable food species during conditioning has a marked beneficial effect on egg production.

4.1.2.3 Calculating food ration for conditioning

The required food ration for conditioning is based on the dry meat weight of the adults. It is usually between 2 and 4% of the mean dry meat weight of the adults at the start of the conditioning period in dry weight of algae fed per day. Rations exceeding 6% are not conducive to successful conditioning. Rather, the bivalves will grow strongly in response to high feed levels and the high temperature of conditioning at the expense of reproductive development.

It is a simple procedure to determine the dry meat weight of a stock of bivalves of known live weight brought to the hatchery for conditioning. Opening a random sample of 10 or 12 individuals, removing the soft body tissues and weighing the meats after drying them to constant weight in an oven (60 to 80°C for 48 to 72 hours) will provide data for the calculation of ration. The equation below is to determine the dry weight of algae per adult required for a 3% daily ration.

$$\text{Ration g per day per adult} = 3 \times \text{mean dry meat weight (g)}/100$$

Thus, a 3% ration for an adult of 0.75 g dry meat weight amounts to 0.0225 g dry weight of algae per day. Reference to the dry weight data given for the various algae species (see Table 1 – Part 3.1) shows that 1 million *Tetraselmis* cells have a dry (organic) weight of about 0.2 mg.

Assuming that 50% of the 3% daily ration (= 1.5%) is to be provided to the broodstock as *Tetraselmis* and the total dry meat weight biomass of the stock is 50 g (converted to mg in the equation below), then:

$$\text{Ration (1.5\%) per day (in millions of cells)} = [(1.5 \times (50 \times 1\,000))/100]/0.2$$
$$= 3\,750 \text{ million cells}$$

If, for example, the harvest density of *Tetraselmis* on a particular day is 1.5 million cells per ml, then the volume required to feed the stock a 1.5% ration will be 3 750/1.5 = 2 500 ml, or 2.5 l. Calculation of the remainder of the ration is similar for the other component species of the diet. If instead of, or in addition to *Tetraselmis*, *Chaetoceros muelleri* is to be fed at a harvest density of 7 million cells per ml, then the volume

required for a 1.5% ration will be 3.57 l. *Chaetoceros muelleri* has a dry weight approximating to 0.03 mg per million cells.

4.1.2.4 Adjusting ration for flow-through systems

In calculating ration, account needs to be taken of the configuration of the tanks and system in which the adults are conditioned. This is not of particular concern in closed systems where algal cells, as yet uneaten, are not lost other than through sedimentation or settling on surfaces. In flow-through systems and tanks of the type shown in Figures 32, 36 and 37, however, a proportion of the algae fed will inevitably be uneaten and will be lost in the outflow. For this reason, adequately stocked tanks of 100 to 150 l with slow rates of water exchange are preferred.

From experience, a total water exchange rate in excess of 90 minutes minimizes cultured algae loss, giving the stock adequate time to filter and consume 60 to 80% of the food. For example, a tank of 150 l volume stocked with 50 oysters or scallops of 75 to 100 g live weight requires a flow of 1.25 l per minute at 25 ml per minute per adult. At this rate of flow, tank volume exchange rate is 120 minutes. Where smaller bivalves, eg. Manila clams, are stocked, numbers of adults per tank should be increased correspondingly on a live weight biomass basis.

It is also preferred that the ration is dosed continuously into the water delivery line to the tank by peristaltic pump to effect better mixing. In some hatcheries, the daily food ration is divided into several batch feeds. The seawater supply is turned off for an hour or so after each addition. This can be problematic in terms of contamination with the waste products of metabolism if the water is inadvertently not switched back on in good time.

In the absence of the means to determine percentage particle removal between the in-flow and out-flow from a flow-through tank, it is recommended that food supply be calculated as a 4% ration to allow for losses discussed above. If the operator has access to an electronic particle counter and sizer (e.g. a Coulter Counter – refer to Figure 21), adjustments to ration can be made based on hard data.

4.1.2.5 Two-stage early season conditioning

Conditioning can be a two-part procedure. Early in the season in temperate and cold-water climes, when adults in the wild have yet to start gamete development, it is often advantageous to provide conditions of high food availability at a temperature intermediate between the ambient and that required for conditioning. The objective is to boost the levels of food reserves in the adults that will later be mobilized in gamete development. This is more important for females than it is for males because egg development and maturation is considerably more energy intensive. Following 4 to 6 weeks of a high ration: moderate temperature regime, temperature is gradually raised (1 to 2°C per day) and food ration is somewhat reduced (from 4 to 6% to 2 to 3% per day).

Food supply for the first stage, which can be called the pre-conditioning stage, can be in the form of algal pastes, bloomed natural phytoplankton (from extensive algal culture, Part 3.4.6) or, intensively cultured algal species. It is important to bear in mind that during this stage, principally the structural lipid (phospholipid) composition of the early stage oocytes will be influenced by the diet and ration available to the broodstock. Thus, a diet deficient in highly unsaturated fatty acids (HUFAs) of known importance, including EPA (eicosapentaenoic acid, 20:5n-3) and DHA (docosahexaenoic acid, 20:6n-3), will be reflected in eggs with cell membranes with reduced content of these components. For this reason, the ration should contain nutritionally valuable diatoms

(e.g. *Chaetoceros muelleri* or *Thalassiosira* sp.) and flagellates such as *Pavlova lutherii* or *Isochrysis galbana*, all of which are rich in one or other of these HUFAs.

Triacylglycerols – the neutral lipids that are laid down as reserves in maturing eggs – are accumulated during the later stages of the second, warm water phase of conditioning. These lipids are drawn upon as sources of energy during embryo and larval development. Their composition appears to be more dependent upon lipids being mobilized directly from the food ingested by the adult than it is from maternally derived reserves.

4.1.3 Conditioning bivalves in the tropics

Mention was made earlier in this chapter of the habit of many tropical species to spawn intermittently throughout most of the year. This presents problems in obtaining sufficient numbers of larvae to support production requirements of hatcheries in tropical and sub-tropical climates.

When there is little variation in seawater temperature and food availability during the year, bivalves do not to have a quiescent period – as do temperate and cold water species – that triggers synchronicity in reproductive development within a stock. This cooler period can be provided in tropical hatcheries by holding stock in water chilled to between 5 and 10°C below ambient with an adequate food ration for a period of 4 to 6 weeks. After this period they are gradually warmed to ambient conditions when a greater percentage of adults will mature gametes synchronously. This is a similar approach in many ways to that described in section 4.1.2.5.

The technique has been used with the mangrove oyster, *C. rhizophorae*, in Cuba. Similar methodology has also been applied successfully in conditioning Pacific oysters, *C. gigas*, in parts of Brazil. The problem is somewhat different in the latter case. Pacific oysters (an introduced exotic species) grow extremely well in the more southerly states of the country but they do not undergo reproductive development to the extent that they will spawn.

4.2 SPAWNING AND FERTILIZATION

4.2.1 Introduction

A summary of information pertinent to conditioning and egg/larval production for a number of commonly cultured bivalves is given in Table 9.

Many temperate and cold water climate bivalves will require 4 to 8 weeks of conditioning to reach spawning readiness during late winter and early spring (Figure 38). A progressively shorter period will be required as the natural breeding season approaches. Precise timing depends on the species being conditioned, the initial condition of the broodstock, stage in gametogenesis when the bivalves begin conditioning and hatchery-related factors, the most important of which are temperature, diet and ration. Hatchery operators will normally use stock already undergoing gametogenesis when returned from the sea, rather than begin conditioning with sexually undifferentiated adults. Advantage can be taken during the natural spawning period of the generally better quality of eggs in terms of important reserves (particularly lipids) from adults brought into the hatchery directly from the sea. These adults may only require 7 to 12 days at conditioning temperature with an adequate food ration to mature their gametes.

When given an adequate food supply, many coastal and estuarine temperate water bivalves will require between 350 to 650 degree-days (deg d) from the start of conditioning in late winter/early spring to the time they are ready to spawn. The

hatchery operator needs to know the temperature at which reproductive development starts in the sea for the species in question. This is often between 8 and 12°C – the "biological zero" (b_0) for gametogenesis – for commonly cultured species such as *Crassostrea gigas*, *Ostrea edulis*, *Pecten maximus* and *Tapes philippinarum*. Knowing what the effective b_0 temperature is for reproductive development and the water temperature during the conditioning period, calculation can be made of the number of

Table 9: Summary of information relevant to the conditioning and egg (or larval) production for a number of commonly cultured bivalves. A key to the meaning of the symbols under sex type is given at the bottom of the table. Conditioning times are for adults brought back to the hatchery early in the season (* time in days will vary considerably according to the stage in gametogenesis the adults are in when brought to the hatchery). Fecundity values are a guide only and will vary according to the size of adult spawned, its condition and other factors. The average shell lengths of fully developed, early-stage D-larvae (2–3 days after fertilization) are also given for comparative purposes.

Group / Species	Sex Type	Conditioning Period (days*)	Temp (°C)	Fecundity (millions)	D-larva size (μm)
Oysters:					
C. gigas	O–D	28 – 42	20 – 24	50+	70 – 75
C. virginica	O–D	28 – 42	20 – 22	50+	60 – 65
C. rhizophorae	O–D	21 – 35	20 – 22	7 – 12	55 – 60
O. edulis	L–A	28 – 56	18 – 22	1 – 3	170 – 190
T. lutaria	L–A	28 – 56	18 – 20	0.02 – 0.05	450 – 490
Clams:					
T. philippinarum	O–D	28 – 42	20 – 22	5 – 12	90 – 100
M. mercenaria	O–D	28 – 42	20 – 22	10 – 20	90 – 100
Scallops:					
P. yessoensis	O–D	14 – 21	7 – 8	20 – 80	100 – 115
P. magellanicus	O–D	28 – 42	12 – 15	20 – 80	80 – 90
P. maximus	O–M	35 – 56	10 – 15	20 – 80	90 – 100
P. ziczac	O–M	14 – 28	20 – 22	7 – 15	90 – 100
A. gibbus	O–M	14 – 28	20 – 22	4 – 7	90 – 100
A. irradians	O–M	21 – 35	20 – 22	4 – 7	90 – 100
Mussels:					
M. edulis	O–D	28 – 35	12 – 16	5 – 12	90 – 100

Key to sex-type: **O** – oviparous (gametes shed into the water); **L** – larviparous (adults brood larvae which are then shed into the water); **D** – Dioecious (sexes are separate); **M** – monoecious (hermaphroditic - both sexes in the same animal); **A** – alternate sexuality (sex switches in the same animal after each spawning).

Figure 38: A spawning female Manila clam (photograph courtesy Brian Edwards).

days of conditioning required. For example, if the mean conditioning temperature is 20°C and the b_0 temperature for reproductive development is 10°C, then every day that passes the number of degree-days will increment by 20 minus 10 = 10. Thus, a 30-day conditioning period at 20°C will accrue 300 deg d and the same period at 22°C will amount to 360 deg d. This represents the likely minimum time period later in spring before stock will be ready to spawn. Obviously, when new stock brought back to the hatchery for conditioning have already started gametogensis, fewer degree-days will be required before the adults are spawning ready.

In cold water scallops, such as *Pecten maximus* and *Placopecten magellanicus*, the number of degree-days from the time adults begin conditioning to spawning readiness is within the same range. But the duration of the conditioning period for cold water bivalves can be much longer (sometimes more than 8 weeks) because the maximum temperature of conditioning is no higher than 15 or 16°C and may be as low as 10 to 12°C. Colder water bivalves are often gradually acclimated to the required conditioning temperature by raising temperature from the ambient at a rate of 1 or 2°C per week. This also extends the overall conditioning period.

Spawning is the hatchery procedure by which conditioned bivalves are induced to liberate their mature gametes in response to applied stimuli. In the case of clam and scallop species, viable embryos cannot be obtained from "stripped" gametes (see the next section below for explanation of the term "stripped"). Eggs need to undergo a maturation process during passage down the oviducts before they can be successfully fertilized.

4.2.2 Gamete stripping

Fully mature gametes can be "stripped" (removed) from the Pacific oyster, *Crassostrea gigas*, the American (Eastern) oyster, *Crassostrea virginica*, the mangrove oyster, *Crassostrea rhizophorae*, and from other similar oviparous oyster species. This is a common and convenient way of "spawning" these species following a suitable period of conditioning.

Figure 39: Stripping and transferring gametes from Pacific oysters to a beaker of filtered seawater using a Pasteur pipette.

This procedure involves sacrificing a number of ripe adults when larvae are required (Figure 39). Removing the flatter shell valve reveals the soft body tissues of the oysters. The gonad overlies the digestive tissues towards the umbone and hinge of the shell and when very ripe will extend around the adductor muscle. Either the gonad can be cut repeatedly with a scalpel and the exhuding gametes washed with filtered seawater into a part-filled beaker or bucket, or a clean Pasteur pipette can be inserted beneath the overlying gonad epithelium and the gametes removed by exerting gentle suction. The pipette contents are then transferred to a beaker or bucket containing seawater at culture temperature. In both cases, a small sample is first removed from each of the number

of opened oysters. These samples are examined microscopically under x40 to x100 magnification to determine sex and the appearance of the gametes. The sperm should be motile and the eggs, which are normally pear-shaped when first removed, should round off in contact with seawater within 20 minutes. The top shell valves should be replaced on the oysters to await stripping in order to prevent dessication.

Assuming the gametes are fully mature, the process is continued to remove gametes from the opened oysters – whose sex is now known – starting first with the females. *Crassostrea* oysters are extremely fecund. Seventy to 90 g females may each be carrying 80 to 120 million eggs, not all of which need to be stripped.

Care needs to be taken to prevent puncture of the digestive gland during stripping. This is to avoid contamination of the gametes with tissue and bacteria and other micro-organisms of gastro-intestinal origin. Either the eggs of individual females can be collected separately in clean 2 to 5 l glass beakers or they can be pooled together in 10 to 20 l plastic buckets that are 75% filled with filtered, ultra-light disinfected seawater at the required temperature (usually 24±2°C).

After completion of egg stripping, the males are dealt with in a similar manner. The exception is that it is more common to pool small samples of the sperm from each of the males in a 1 l glass beaker, part filled with filtered ultra-light disinfected seawater at the same temperature, making sure that the final sperm density is not too great. As a guide, one should just be able to see nearby objects through the beaker and its contents. The gametes are then ready for fertilization.

4.2.3 The special case of flat oysters

Before considering spawning in clams, scallops and mussels the special case needs to be noted concerning oysters of the genera *Ostrea* and *Tiostrea*. These, unlike other commonly cultured bivalves, do not need to be stimulated to spawn. They will spawn of their own accord during the conditioning process and will brood larvae within their mantle cavities for varying periods of time depending on species and temperature. This group of oysters, including the European flat (or "Belon") oyster, *Ostrea edulis* (Figure 40), the New Zealand ("Bluff" or mud) oyster, *Tiostrea lutaria*, and the closely related Chilean flat oyster, *Tiostrea chilensis*, are referred to as larviparous. The latter two species release their larvae into the surrounding water after about a

Figure 40: Anatomy of a developing flat oyster, *Ostrea edulis*; **am** – adductor muscle; **g** – gonad tissue overlying the digestive gland; **gl** – gills; **h** – hinge; **ic** – inhalant chamber of mantle cavity. At spawning, eggs pass through the gills into the inhalant chamber of the mantle cavity where they develop to fully shelled larvae over the course of a week or more, depending on species. The parent releases larvae when they are able to ingest and digest algae. (The anatomy of oysters of the genera *Tiostrea* and *Ostrea* is essentially similar).

20-day brooding period when the larvae are between 450 and 490 μm shell length and are almost ready to set. In contrast, the European flat oyster releases its larvae, after a brooding period of 6 to 8 days at normal conditioning temperatures, when they are 170 to 190 μm shell length and require a further 10 to 12 days of culture before they reach maturity and are ready to set. Eggs of the New Zealand and Chilean flat oyster are 350 μm diameter compared with 150 μm in the European flat oyster.

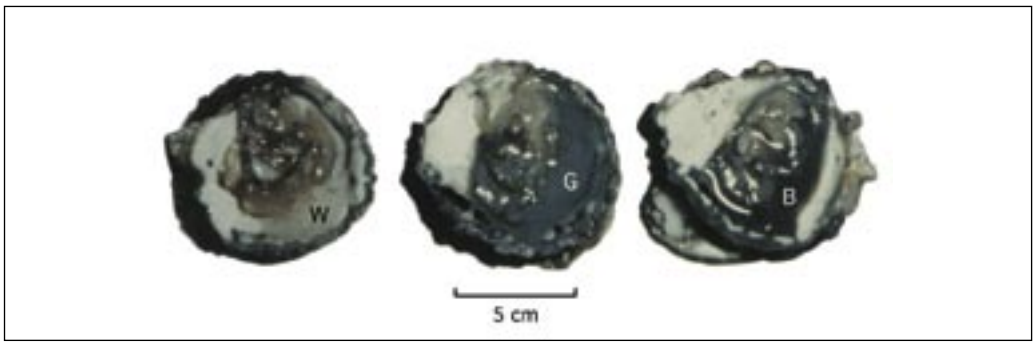

Figure 41: Brooding stages of the European flat oyster, *Ostrea edulis.* **W** – the "white sick" stage shortly after eggs are passed to the inhalant chamber of the mantle cavity; **G** – the "grey sick" stage, beyond the trochophore stage, when the shell valves are well developed but the larval organs not yet fully developed (3 to 5 days after spawning); **B** – the "black sick" stage at which larvae are almost fully developed and are ready to be released. White, grey and black "sick" are traditional terms applied to brooding oysters in Europe.

The above species are not mass spawners. Rather, stocks of adults produce larvae over an extended period. It is extremely rare to see mature males liberating sperm into the surrounding water and it is assumed that they do so periodically in small quantity. Adjacent female-phase oysters (these species exhibit alternate sexuality) draw in sperm in their inhalant current, in the same way as food particles, and in response, release their eggs into the exhalant chamber of the mantle cavity – as do oviparous species. However, the eggs are not expelled into the surrounding water. Instead they are passed through the gill filaments into the inhalant chamber of the mantle cavity where they are fertilized and develop over an extended period (Figure 41), to be fully motile, completely shelled veligers at the time of release (Figure 42).

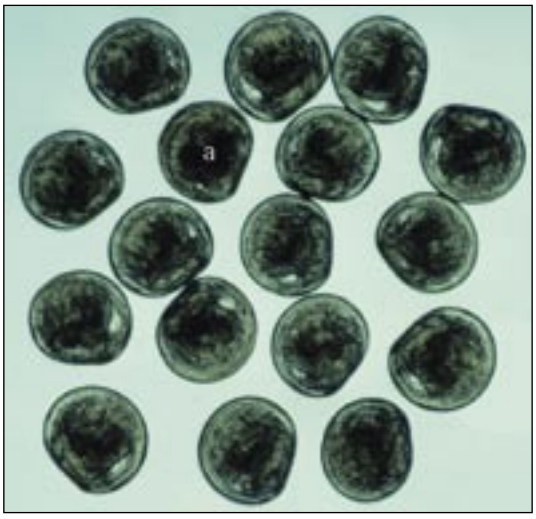

Figure 42: The appearance of *Ostrea edulis* veliger larvae (175 μm mean shell length) at release from the adult. All larvae are normally formed except for - **a** - which exhibits incomplete development of one shell valve.

Hatchery technicians experienced in rearing these species, can often identify spawning and brooding female-phase oysters from small quantities of eggs that escape mantle cavity retention and settle on the upper shell valve, adjacent to either the inhalant or exhalant mantle apertures. Brooding oysters also tend to be inactive, retaining only a minimal shell gape for long periods.

When larvae of the larviparous oysters are liberated into the water they either swim to the surface forming visible "rafts" in the case of *O. edulis,* or they immediately seek a surface upon which to settle and undergo metamorphosis in the *Tiostrea* sp. In the latter case, suitable settlement surfaces need to

be added to the broodstock tanks in advance of larvae liberation. The surfaces can either be shell or plastic cultch materials or plastic mesh (see later section dealing with settlement).

When the expected liberation period is reached in the case of *O. edulis*, tanks should be checked every 2 or 3 hours for signs of larval release. Swimming larvae can be skimmed from the water surface of the conditioning tanks with a beaker or a small 90 μm mesh sieve and transferred to a bucket of water. Alternatively, they can be allowed to flow in the tank discharge into a larger sieve of the same mesh aperture, which is partially submerged in a tray of water (Figure 43). It is always best to collect the larvae as soon after release as possible to avoid larvae becoming contaminated by adult faecal matter in the water, or being filtered out of the water by the filtration activities of the adults.

Figure 43: Experimental broodstock conditioning of *Ostrea edulis.* Note the green coloured sieves immersed in shallow trays to catch and retain larvae.

Once a brood has been collected they are counted (see later) and distributed between culture tanks at the appropriate density. Female-phase European flat oysters of 70 to 90 g (the size of oysters in Figure 41) will liberate broods of between 1 and 2.5 million larvae. In contrast, female-phase *Tiostrea* oysters, which produce considerably larger eggs, will liberate much smaller broods of 20 000 to 50 000 larvae.

Larvae can be removed from adults identified as brooding either from the conditioning tanks, or in stock brought back from growout – or from wild populations – during the natural breeding season. The steps in this procedure are illustrated in Figure 44. It is sometimes used as a method to obtain larvae before they have developed a functional gut in the later stages of brooding. This can be relevant in the summer when potentially pathogenic bacteria are prevalent. There is evidence to suggest that brooding larvae begin feeding while still in the parental mantle cavity and, thereby, may be exposed to high loads of bacteria and other micro-organisms accumulated and defaecated both by the parent and adjacent stock.

Whether larvae are liberated naturally by the stock or are removed prior to release, they are grown following the standard methodology described later in the larval culture sections of this manual. Best results are with broods that have developed to the fully shelled, motile, D-larva stage. If removed at an earlier developmental stage, food is withheld until larvae have developed a fully functional alimentary system – visible through the transparent shell valves as a darker S-shaped structure, which can be seen

in Figure 42. This may take 2 or 3 days from the time of removal. Prior to this stage, the soft body tissues are a densely, granular, grey colour and the larvae only weakly motile (see Figure 41 – "grey sick" larvae).

Figure 44: A – Stripping *Ostrea edulis* larvae from a brooding adult. **B** – The top (flat) shell valve is removed, then the brooding larvae are washed through a 90 μm sieve balanced over a bucket of filtered seawater (**C**). **D** – Most of the larvae swim rapidly to the water surface where they aggregate (raft) together. They are then ready to be sampled for counting and for size determination. Photographs were taken at the Harwen Oyster Farm hatchery in Nova Scotia (courtesy John and Krista Harding).

4.2.4 Induced spawning of oviparous bivalves

Other commercial species reared in hatcheries are known as oviparous as compared with the larviparous oysters discussed above. Oviparous species shed their eggs and/or sperm into the surrounding water where fertilization takes place.

Various stimuli can be applied to induce spawning; the most successful being those that are natural and minimize stress. The description that follows is of a technique known as thermal cycling, which is the most widely used method for oviparous species. As a general rule of thumb, if stock do not respond to thermal stimuli within a reasonable period of time, the gametes they carry are most likely not fully mature.

The use of serotonin and other chemical triggers to initiate spawning is rarely beneficial. Eggs liberated using such methods are often less viable than are those produced in response to thermal cycling.

4.2.4.1 Thermal cycling procedure

Mature bivalves taken from broodstock conditioning tanks are cleaned externally to remove any adhering debris and fouling organisms from their shells and then are thoroughly rinsed with filtered seawater. After cleaning they are placed in a spawning trough or tank. The preferred tank-type is a shallow, fibreglass tray of approximately 150 x 50 x 15 cm depth – 10 cm water depth (Figure 45). It needs to be of a size that can be viewed by two or more operatives who are experienced in detecting the onset of spawning by adults (an important point in the spawning of monoecious species – see later).

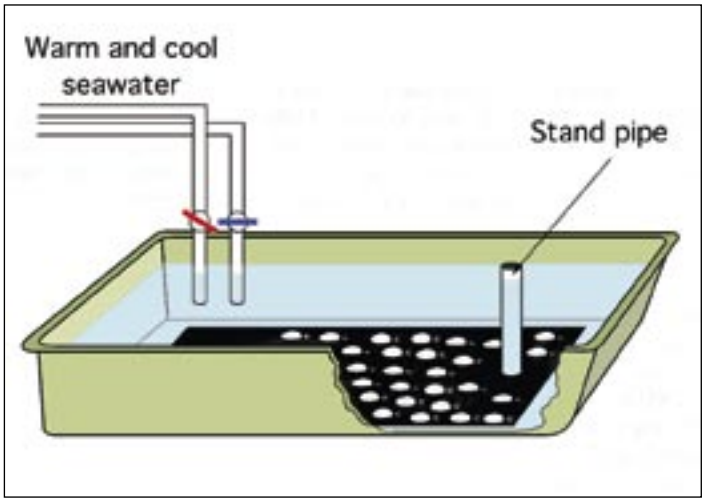

Figure 45: Diagram of a tray arrangement widely used for the spawning of oviparous bivalves. (After Utting and Spencer, 1991)

The trough is often fitted with a standpipe drain and two filtered seawater supplies, one heated or chilled to 12 to 15°C and the other at 25 to 28°C (e.g. for *Crassostrea* species and Manila clams). Lower temperatures apply to cooler water species. The importance is the differential between the lower and higher temperature, which will normally be about 10°C.

The base of the trough is painted matt black or is covered by black plastic sheet to provide a dark background against which gametes being liberated can be readily seen (Figure 45).

The trough is part filled with the **cooler** water to a depth of about 10 cm and a small amount of cultured algae is added to stimulate the adults to open and start pumping activity. After 30 to 40 minutes the water is drained and replaced with water at the **higher** temperature, again with a small addition of algae. This water is drained after a similar time period and replaced with **cooler** water and the procedure is repeated.

The number of cool/warm cycles that are required to induce spawning depends on the state of maturity of the gametes and the readiness of the adults to spawn. In summer the adults may spawn within an hour of induction, but earlier in the season it may take 3 or 4 hours of cycling before the first animal spawns. Generally, if the adults do not respond within a 2 to 3-hour period they are returned to the conditioning tanks for a further week. Adults may start spawning on either the cool or warm part of the cycle, most commonly the warm. Although it is generally the case that males will spawn first, this cannot be guaranteed.

Additional stimuli can be provided in the form of stripped eggs, or sperm from an opened male. The gonad is located at the base of the foot in clams. In scallops it is a separate organ and can be seen when the mantle and gill tissues are lifted. If the gonad is carefully punctured with a Pasteur pipette and suction applied, quantities of gametes can be withdrawn which can then be mixed in a small volume of filtered seawater before adding to the seawater in the tray. In clams with discrete siphons, the diluted gametes are directed towards the inhalant siphon of active clams with a Pasteur pipette so that they are drawn into the mantle cavity by the pumping action of the adults. The inhalant siphon is the siphon furthest away from the hinge and has the largest diameter aperture. When spawning occurs in clams, gametes are expelled through the exhalent siphon as shown in Figure 38. The thermal shock during the second warm water cycle almost always elicits a spawning response in ripe clams and in other fully mature, oviparous bivalves within 1 to 2 hours.

4.2.4.2 Spawning dioecious bivalves

In dioecious species (refer to Table 9), in which the first adults to spawn are almost invariably males, it is good practice to remove them from the trough and leave them out of water until sufficient eggs have been collected from spawning females. The reason is that sperm ages more quickly than eggs and if more than 1-hour-old at the time fertilization is made, fertilization rate may be reduced.

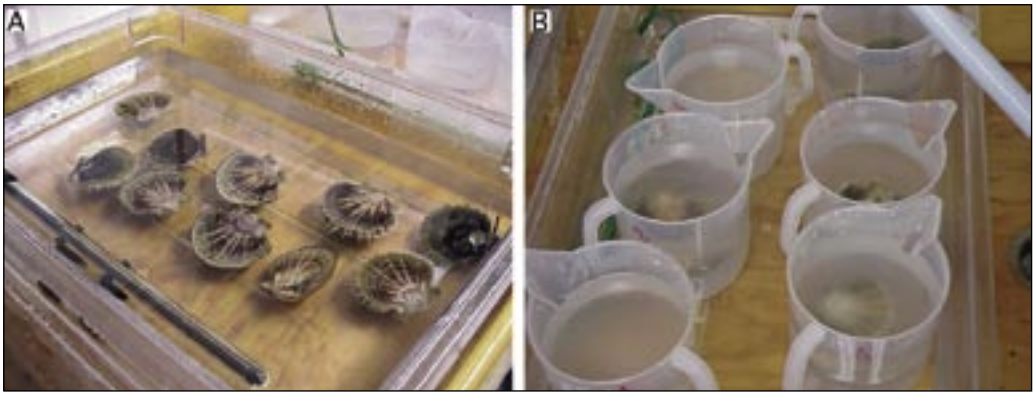

Figure 46: A – *Pecten ziczac* adults undergoing thermal cycling in a spawning tray. Note the aquarium heater used to maintain the elevated temperature. A similar tray of water is chilled with ice packs to provide the cold shock. **B** – Individual scallops spawning in 3 l plastic beakers immersed in a constant temperature water bath. While this species is not dioecious, the illustration applies to procedures used in spawning any species.

As each female begins to spawn it is necessary to remove it from the spawning trough and transfer it to an individual spawning dish or beaker part-filled with filtered seawater at 24-26°C (Figure 46). The dishes/beakers are contained in a heated water-bath to maintain the temperature. The same procedure applies to spawning males, which can be identified as such by the continuous stream of milky fluid escaping from the exhalant siphon compared with the granular appearance or clumps of eggs shed by a female. Females may start spawning as much as 30 to 60 minutes after the first male begins to liberate sperm.

Time to completion of spawning for an individual is variable but gamete liberation rarely lasts for more than 40 to 60 minutes, often a shorter period in females. It may, however, be necessary to remove a spawning female from its container and place it in a fresh one when large numbers of eggs have been liberated. The presence of dense concentrations of eggs in the water inhibits pumping activity and hence the expulsion of further eggs. In addition, the female may start to filter the eggs out of suspension.

Eggs may be liberated in clumps that will eventually settle to the base of the dish or beaker. These clumps are separated when spawning is completed by carefully pouring the dish contents through a 90 μm nylon mesh sieve (eggs will not be retained by this mesh size), retaining the separated eggs on a 20 to 40 μm mesh sieve. The eggs are then gently washed into a clean glass or plastic container with filtered seawater at the required temperature. "Clumpy" eggs often do not fertilize well. The best success is most often obtained when females liberate streams of well-separated eggs that remain in suspension for longer periods than do the clumps.

When first spawned the eggs are pear-shaped but they rapidly hydrate and assume a spherical shape when in contact with seawater. Eggs from different females are collected separately to provide opportunity to visually assess quality using a microscope at about

x100 magnification. Batches of eggs that do not round off after about 15 to 20 minutes in seawater should be discarded. Reproductive development in the females of oviparous bivalves is not completely synchronous so that at any point in time eggs spawned by different females will be at slightly different stages of maturation. When separation and examination of the eggs is complete, batches of eggs that appear good can be pooled in a larger volume container.

Sperm from the various males that spawn are similarly pooled. It is good practice to use eggs from at least 6 females and sperm from a similar number of males to provide larvae for a production run. This ensures satisfactory genetic variability among the offspring, the extent of which will depend on the degree of heterozygosity of the parents. Small volumes of the pooled sperm suspension are mixed with eggs during gentle agitation of the contents of the container in the proportion 1 to 2 ml per l of egg suspension.

4.2.4.3 Spawning monoecious bivalves

The procedure to spawn hermaphroditic species, including many species of scallops, in which individual adults mature both eggs and sperm synchronously, is more complex. Here, the objective is to minimize chances of eggs being fertilized by sperm from the same individual (self-fertilization). It is rarely the case that an adult will spawn both eggs and sperm at the same time. More usually, sperm will be liberated first followed by the eggs. Individuals will often revert to liberating sperm once their eggs have been spawned.

There are two approaches to maximize chances of cross-fertilization. Large numbers of adults can be spawned in large-volume, deep tanks. These are fitted for flow-through so that the contribution of sperm from a particular individual is a small proportion of the total and the overall amount of sperm is continuously being diluted by water flow. When individuals switch to female production, the denser eggs are retained in the tank and chance dictates that the eggs of that individual will more likely be fertilized by the sperm of other individuals than by its own sperm. This method – applicable also to the large-scale spawning of dioecious species, where self-fertilization is not an issue – is used in mass production facilities for *Argopecten purpuratus* in Chile and is also used in the pond culture of bivalves in Asia.

Alternatively, permitting closer control of fertilization, each adult is transferred to a small container of filtered seawater at the required temperature once it begins to spawn (Figure 47). The container is labelled with the time and a reference number that will follow the progress of that particular adult throughout its spawning activities. As the adult spawns and clouds the water with its gametes, it is moved to a fresh, clean container after first being thoroughly rinsed with filtered water. This container is labelled with the time of transfer and the same adult-specific reference number. Careful observation is maintained on each beaker containing an adult liberating sperm in order to detect the onset of egg liberation, which is usually a sudden change. As each adult switches to egg production it is immediately removed and transferred to another container after rinsing, carrying with it the same adult-specific reference number and the time of the switch. Once sufficient eggs have been spawned, the adults are removed from the beakers before they revert back to sperm production. Thus, eggs and sperm are separately accumulated from each adult, identified as to origin by the different adult-specific reference numbers and time of production.

Mature adults with ripe gametes obtained directly from the sea can be induced to spawn in the hatchery in the same way.

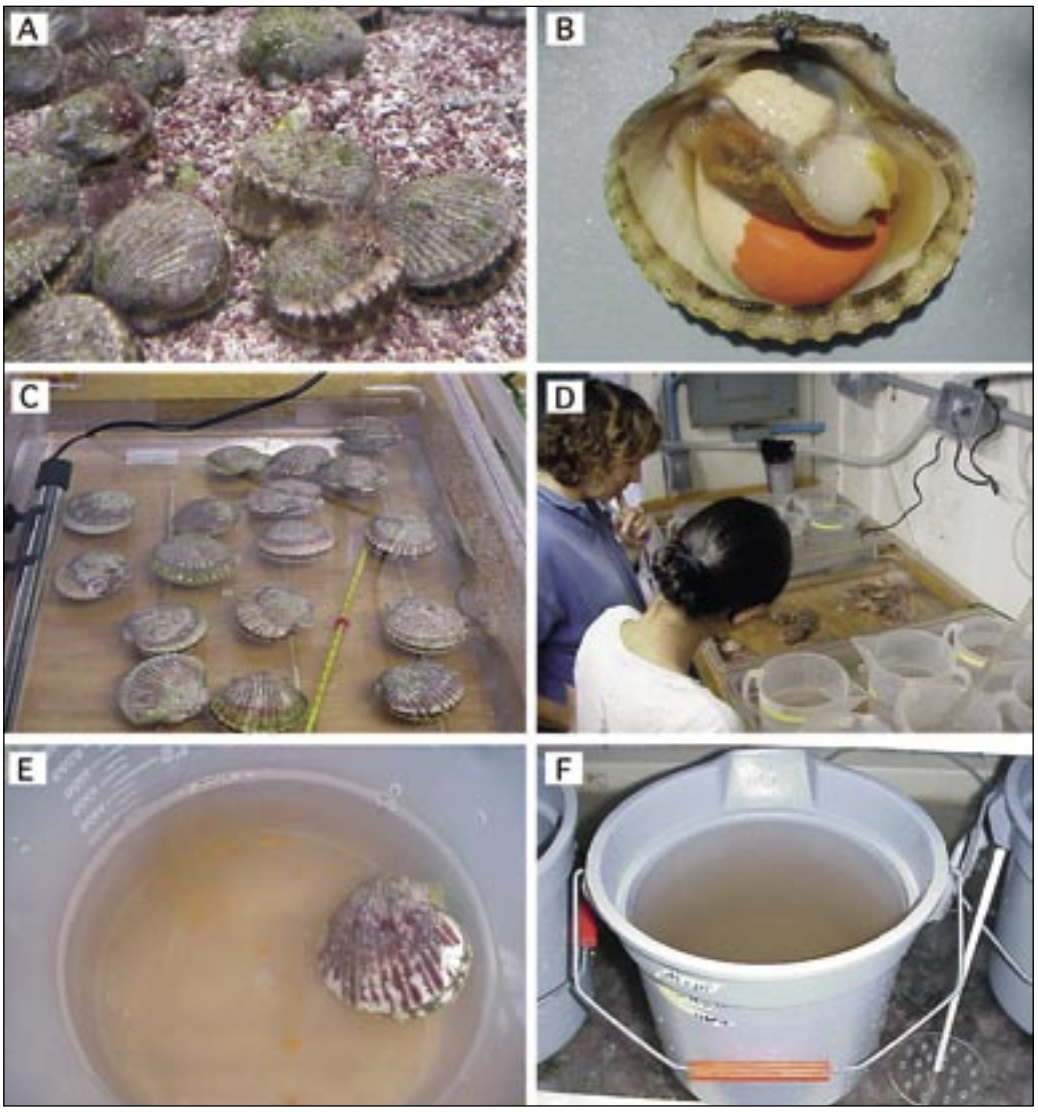

Figure 47: This sequence of photographs illustrates the spawning of the monoecious calico scallop, *Argopecten gibbus,* at the Bermuda Biological Station for Research, Inc. (BBSR).

A – Broodstock are conditioned in the hatchery at 20-22°C for 2 to 4 weeks during late winter, early spring. A constant flow of seawater is maintained through the tank and food is added daily.

B – The appearance of a fully mature scallop; the orange coloured ovary and white testis occupy the distal and proximal parts of the gonad respectively. The adductor muscle is centre right and the brown-coloured tissue includes the gills and mantle, which have been raised to expose the gonad.

C – Up to 20 scallops are spawned at a time in transparent plastic trays of approximately 75 x 45 x 5 cm water depth. The trays contain sufficient 1 μm filtered seawater to fully cover the scallops. One is chilled to 12°C with ice packs and the other is heated to 25 to 27°C with a 150W aquarium heater. The scallops are cycled between the two temperatures as explained in the text.

D – Staff keep careful watch to identify scallops as they begin to spawn in the warm water tray. Spawners are rinsed with filtered seawater and transferred individually to labelled plastic beakers containing 0.5 to 1 l of seawater in other trays acting as warm water baths at the spawning temperature.

E – After liberating sperm, scallops will suddenly switch to spawning orange coloured eggs. It is important that as soon as the switch is made scallops are removed, rinsed and returned to clean beakers containing filtered seawater to continue egg liberation. If egg production is swift and prolific, sperm from other scallops will often be added at this time.

F – Eggs of good quality, determined by microscopic examination, are pooled in 10 l buckets. Note the perforated plastic plunger used to gently agitate the bucket contents to keep the fertilized eggs in suspension. The bucket may contain between 5 and 10 million eggs – judged "by eye".

4.2.5 Fertilization procedures

Before fertilization, if not already done, egg suspensions should be gently filtered through a suitable mesh-size sieve (90 μm aperture or greater) held so that the mesh is below water level in a larger volume bucket or container. This step is to remove contaminating faecal pellets from the adults prior to the addition of the sperm to reduce risk of the subsequent proliferation of bacteria and other micro-organisms during the next stage in the culture process.

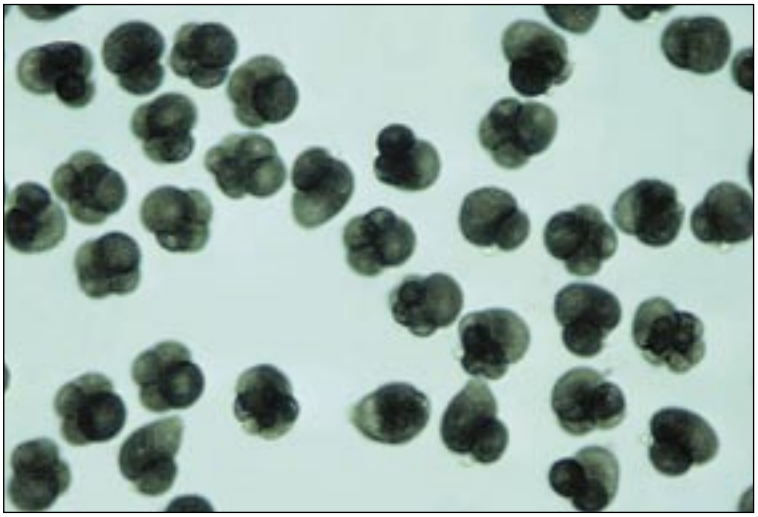

Figure 48: Dividing *Crassostrea gigas* eggs about 50 minutes after fertilization. Most of these eggs are developing normally and are at the 2 and 4-cell stage.

The method used to fertilize eggs is essentially the same whether for monoecious or dioecious species. The one exception in hermaphroditic bivalves is to ensure that eggs are cross-fertilized with sperm from adults other than the one that provided the particular batch of eggs. For this reason, batches of eggs from the different adults are kept separate and are separately fertilized with recently shed sperm from 3 or 4 males in the ratio 2 ml of sperm per l of egg suspension. Following sperm addition, they are allowed to stand for 60 to 90 minutes before pooling – if required – with the fertilized eggs from other adults.

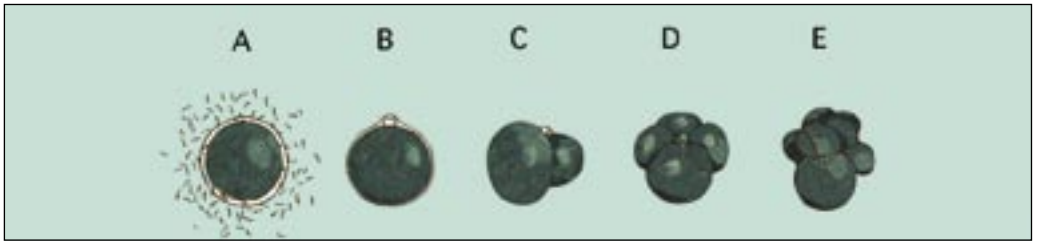

Figure 49: Stages in the early development of eggs; **A** – sperm swarming around a rounded-off egg; **B** – extrusion of the first polar body following fertilization; **C** – two-cell stage also showing the second polar body; **D** – four-cell stage; **E** – eight-cell stage. The eggs of most oviparous bivalves range in size from about 60 to 80 μm, depending on species. The time from fertilization to the various developmental stages is species and temperature dependent.

Within this time period, at the appropriate temperature for the species, the fertilized eggs will begin to divide, first almost equally into two cells and then unequally into 4 cells where one large cell will be observed capped by 3 much smaller cells. The first sign of successful fertilization, however, before cell division starts, is the extrusion from the egg of a small, transparent, dome-like structure, which is the first polar body (Figures 48 and 49). Assessment of the percentage of eggs developing normally can be made using a relatively low power microscope (x20-40 magnification). Fertilization rates almost invariably exceed 90% assuming the eggs are fully mature.

It is desirable to estimate egg numbers prior to or within 20 to 30 minutes of fertilization since development will be impaired if the density of embryos per unit volume beyond the early stages of cleavage exceeds certain specified limits. This density is specified later and the method to determine both egg and larval numbers is described in section 5.1.2.3.

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Part 5

Hatchery operation: culture of larvae basic methodology, feeding and nutrition, factors influencing growth and survival, and settlement and metamorphosis

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5.1 BASIC METHODOLOGY

5.1.1 Introduction

The hatchery culture of bivalves is as much an art as it is a science and the old adage applies that "there are many ways to skin a cat". In a similar vein, the success of a hatchery is related more to the skill and intuitive "feel" for the work of the manager and technicians than it is to the location, the scale and quality of the physical structure and the sophistication of the available equipment. Every hatchery is different in the way it is managed and in the nuances of the manner in which the various aspects of culture are approached and the work done. There is no standard methodology as such but there are common denominators that relate to the need to fulfill the biological requirements of the different bivalve species through their early developmental stages.

This section of the manual synthesizes the various approaches and the methods used in the culture of larvae from the fertilized egg to settlement with emphasis on some of the more commonly cultured species.

5.1.2 Methods for embryo development

5.1.2.1 Tanks for embryos and larvae

Fertilized eggs are permitted to develop to the fully-shelled "D" veliger stage in tanks of the type shown in Figures 50 and 52. This early veliger stage is known as the D-larva stage because of the characteristic "D" shape of the shell valves (Figure 51). D-larvae of the various, commercially cultured bivalves are similar in appearance.

Figure 50: Fertilized eggs can be incubated in various types of tanks in filtered seawater for a period of 2 to 3 days, depending on species and temperature.

A wide range of circular or semi-square (square with rounded corners) tanks can be used for embryo development and also for larval rearing (Figure 52). They should be constructed from preferably either "virgin" (new, non-recycled) polyethylene or fibreglass (alternatively known as GRP – glass reinforced plastic or fibreglass). Previously unused vessels should be filled with seawater and allowed to soak with weekly changes of water for 2 to 4 months before use. Soaking removes toxic substances that leach from the surface of new plastics which may be harmful to larvae. Steam curing of fibreglass tanks substantially reduces the period the tanks need to be seawater soaked.

Flat-bottomed or steeply tapering conical tanks (i.e. almost flat bottomed) are most commonly used for embryo development (Figure 52). Shallowly tapering conical tanks (shaped like an ice cream cone) are less satisfactory because the early embryos are immobile and will tend to aggregate together at the bottom of the cone.

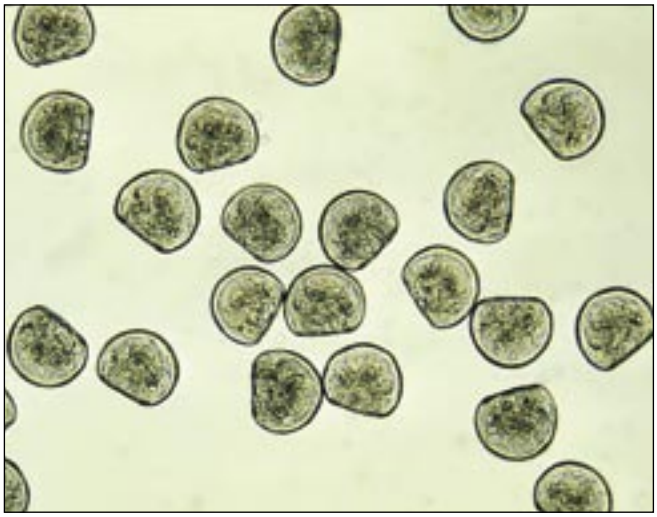


Figure 51: Photomicrograph of *Crassostrea gigas* D-larvae (48-h after fertilization). Mean size is 75 μm shell length.

Surface area of the tank base rather than water depth is more important. Aeration during this early stage is not recommended. The mechanical effects of the disturbance it creates can lead to abnormal development.

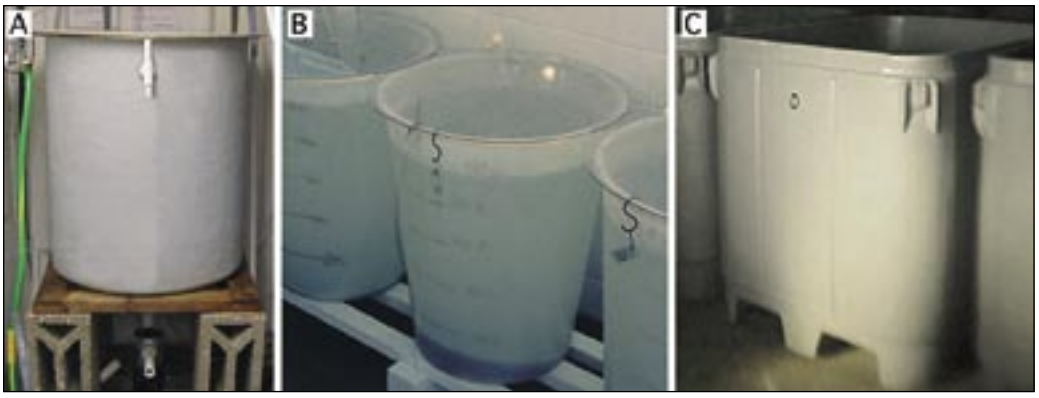


Figure 52: Suitable rearing vessels for embryo (and larval) development. **A** – 200 l steeply tapering conical fibreglass tank with bottom drain; **B** – 125 l polyethylene flat-bottomed tank; **C** – 1 000 l insulated polyethylene, square tank with rounded corners.

5.1.2.2 Water treatment

Culture tanks are filled with seawater filtered to 1 to 2 μm particle size (Figure 53A) and heated to the required temperature (usually 18 to 24°C; cooler for cold water species). Some hatcheries disinfect the water following fine filtration by passing it through an ultra-violet light (UV) unit (Figure 53B), the value of which is questionable unless it is used properly and with discretion.

UV units should be maintained according to manufacturer's recommendations and a record kept of hours of lamp usage. Lamps must be replaced as they reach the specified hours of use at which time the quartz silica sheath that separates the lamp from the water flow needs to be cleaned with a soft cloth soaked in alcohol. Moreover, these units are designed to disinfect freshwater and are not as efficient in killing or immobilizing marine bacteria and other micro-organisms.

As a rule of thumb – if UV disinfection is considered necessary – it is best to pass water through two or three similar units, connected in series, at half the flow-rate recommended for a single unit (Figure 53). It should be remembered that limiting the diversity of bacteria in a culture of embryos or larvae may reduce competition

and thereby permit potentially harmful bacteria to predominate. Modern thinking is that the probiotic approach is the better option. This approach entails controlling the density of larvae carefully, feeding them properly with only the best cultured algae available and, paying attention to the hygienic operation of both the cultures and equipment.

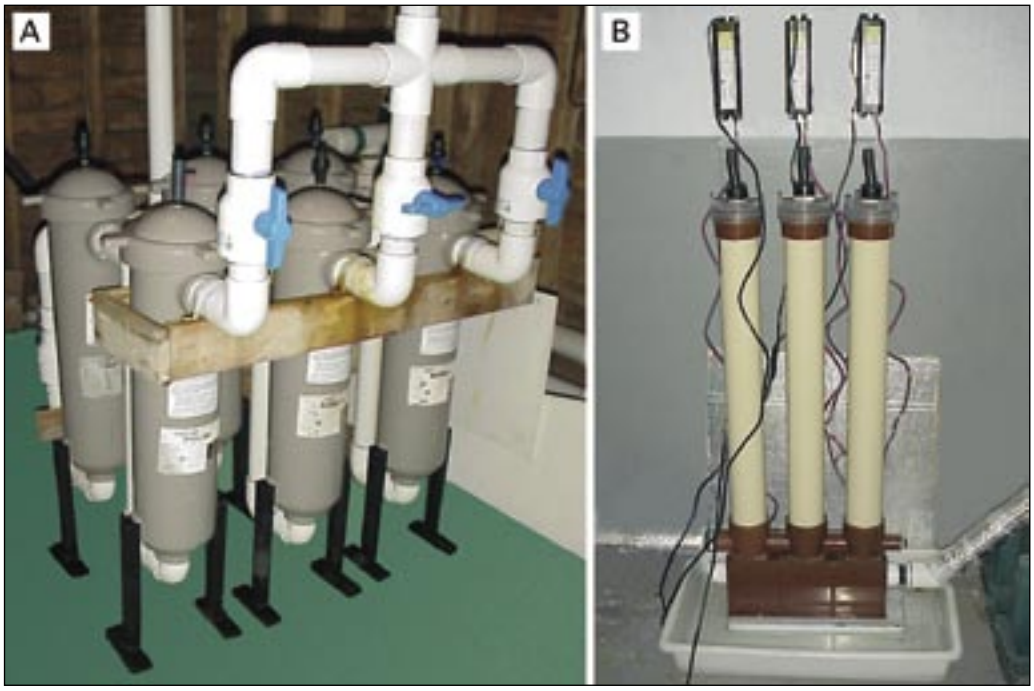


Figure 53: Examples of suitable equipment for water treatment. The multiple bag filtration unit (**A**) is arranged for fine water filtration. One bank of 3 filters is in use while the second bank is serviced and readied for use. These filtration units contain bags that progressively remove particulate matter from 10 μm down to 2 μm in three stages. The uv disinfection unit (**B**) consists of 3 lamp units arranged in series and is designed to treat a continuous flow of previously filtered seawater. This is the recommended arrangement in seawater treatment rather than relying on the water being treated by a single lamp unit.

It is sometimes beneficial to filter the water and to fill the culture tanks 24 hours before they are needed. This is more applicable in hatcheries located adjacent to estuaries contaminated by industrial or domestic wastes, or by the leachings from richly metaliferous geological strata (and mine workings) in the catchment area, which may contain elevated quantities of heavy metals. The water is then treated by adding 1 mg per l of EDTA (sodium salt – as used in the preparation of algal culture medium) and 20 mg per l of sodium metasilicate and is vigorously aerated for 24 hours. Pre-treatment helps to complex heavy metals and render them non-toxic to the particularly vulnerable early stages in development of bivalve larvae. The water does not need to be re-filtered after pre-treatment but aeration is switched-off during embryo development.

5.1.2.3 Culture of embryos

Embryos are stocked in the culture tanks about 2 hours after fertilization and at the appropriate density. Fully developed D-larvae are recovered 24 to 48 hours later, depending on species and water temperature (Figure 54). Either no or very low aeration is used during embryo development.

Embryo stocking densities for many of the commonly cultured oviparous oysters and clams can be as high as 50 000 to 80 000 per l of culture, although 20 000 per l is more generally considered to be the safe upper limit (Table 10). In contrast, similarly high

initial embryo densities of many of the scallop species leads to abnormal development and numbers are usually restricted to 10 000 to 15 000 fertilized eggs per l of culture tank volume in warmer water species. Egg densities are more commonly based on the surface area of the tanks rather than on tank volume in cold water scallop species, where maximum density should not exceed 1 000 per cm^2 (Table 10).

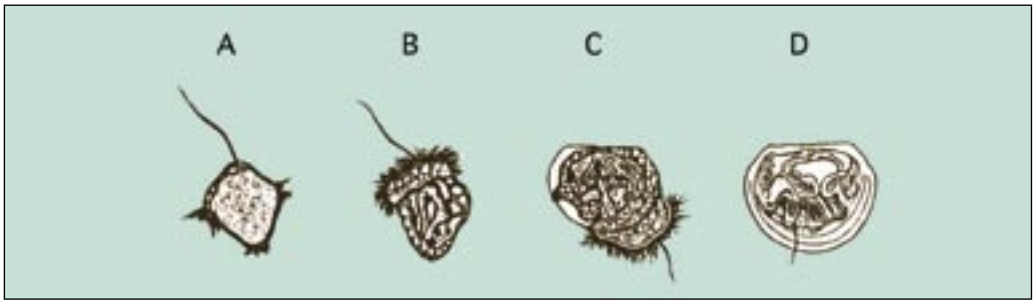


Figure 54: Development of embryos from the early trochophore (**A**) to the fully shelled D-larva stage (**D**). The ciliated swimming feeding organ (velum) can be seen in **B** and early shell valve formation in **C**. Fertilized eggs will develop to fully formed D-larvae in less than 2 days in many warm water species but the entire developmental process can take 4 or more days in cold water species.

Table 10: Summary data of typical embryo densities (thousands per l), initial D-larva size (shell length, μm), densities of D-larvae (thousands per ml) and culture conditions in terms of suitable temperature (± 2°C) and salinity (± 5 PSU) for the culture of embryos and early larvae of a number of bivalves. Notes: N/A – not applicable: embryo development takes place within the mantle cavity in *Ostrea edulis*. * Embryo densities in cold water scallops are calculated as embryos per unit area of the base of tanks rather than per unit volume. Maximum density should not exceed 1 000 fertilized eggs/embryos per cm^2.

Group/ Species	Embryo density (thousands per l)	D-larva size (mm)	D-larva density (thousands per l)	Temp (°C)	Salinity (PSU)
Oysters:					
C. gigas	15 – 20	75	10 – 20	25	28
C. virginica	15 – 20	65	10 – 20	25	28
C. rhizophorae	15 – 20	60	10 – 20	25	35
O. edulis	N/A	175	5 – 10	22	30
Clams:					
T. philippinarum	20 – 40	95	10 – 20	25	30
M. mercenaria	15 – 25	95	10 – 20	25	28
M. arenaria	15 – 25	95	10 – 20	19	30
Scallops:					
P. yessoensis	*	105	1 – 2	15	30
P. magellanicus	*	90	1 – 2	15	30
P. maximus	*	95	1 – 2	14	30
P. ziczac	10 – 15	95	2 – 5	25	32
A. gibbus	10 – 15	95	5 – 10	24	30
A. irradians	10 – 15	95	5 – 10	23	30
Mussels:					
M. edulis	15 – 25	95	10 – 20	16	30

A recovery of from 30% to 85% perfectly formed D-larvae from the initial number of embryos stocked is normal in large-scale rearing. Imperfectly formed D-larvae – those with incomplete or misshapen shells – rarely develop further.

Fully shelled D-larvae have a mean shell length of 90 to 100 μm in most species of clams, scallops and mussels, and 55 to 75 μm in oviparous oysters of the genus *Crassostrea* (Table 10). *Crassostrea gigas* has larger D-larvae than either *Crassostrea virginica* or *Crassostrea rhizophorae*.

A special case is the larviparous oysters of the genera *Ostrea* and *Tiostrea*, which have considerably larger eggs. They brood larvae until they are released into the surrounding water at 170 to 200 μm shell length (retained by a 90 μm mesh screen) in the case of *Ostrea edulis* and an average length of 490 μm in *Tiostrea* species (see part 4.2.3). *Tiostrea* larvae are liberated at the pediveliger stage (the pre-settlement and metamorphosis stage) and are ready to set almost instantaneously (<1 hour after release).

Shell length is best measured with a monocular microscope (x100 magnification) fitted with an eye-piece graticule calibrated against a micrometer slide (Figure 55).

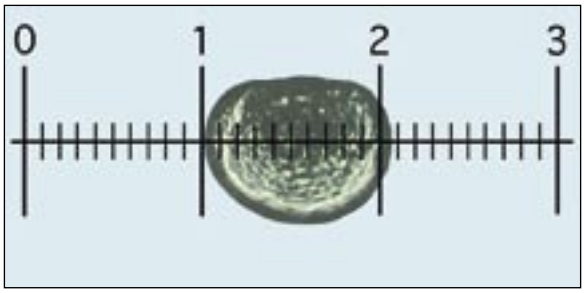


Figure 55: Measuring larvae: each larva is oriented and lined-up with the calibrated eye-piece graticule, as shown, and the number of small sub-divisions it spans on the scale, equivalent to shell length, is recorded. In this case, at x100 overall magnification (x10 eye-piece and x10 objective), each small division is 10 μm. Thus, the D-larva shown measures approximately 105 μm.

Normal D-larvae are retained by a 45 μm nylon mesh-based sieve (35 μm in the case of D-larvae of *Crassostrea gigas*, or 25 μm for *C. rhizophorae* and *C. virginica*) and the number recovered is estimated as described later in section 5.1.2.3.

Recovering D-larvae

Tanks containing newly developed D-larvae are drained 2 days after fertilization. There is merit in adding a small quantity of food to the tank on the day prior to draining, i.e. 24-h to 36-h after fertilization. While embryos develop to the D-larvae stage utilizing maternally derived reserves, fully developed D-stage larvae are able to ingest algal cells of the smaller food species and benefit from uptake of dissolved nutrients.

Figure 56: The arrangement of sieves to capture D-larvae from a tank where a smaller diameter, 60 μm mesh sieve is suspended over a larger diameter 40 μm sieve which is partially immersed in a shallow tray containing the discharge seawater. This arrangement permits grading of larvae by size at the point of collection and ensures larvae are not allowed to dry out.

The method employed to catch and retain larvae while tanks are being drained is illustrated in Figure 56. When the tank is full, the drain valve is opened a fraction to allow a slow flow of water into a sieve, or series of sieves, contained in a shallow tray. This arrangement ensures the mesh of the bottom sieve is always immersed in seawater, which minimizes damage to the shells of the fragile D-larvae during draining. As the tank drains down, the valve can be opened further making certain that the flow is not sufficiently violent so as to cause excessive turbulence. Any larvae retained in the tank once it is empty are flushed out with a flow of filtered seawater. Tanks without drains can be emptied by siphoning the water through a similar arrangement of sieves using a length of flexible hose.

D-larvae can be graded during tank emptying by suspending a slightly larger aperture sieve over one with a smaller mesh aperture, as in Figure 56. This is often of benefit and can help separate the better, larger D-larvae from those that are imperfectly formed and abnormal (Figure 57). Once the tank has been completely emptied, filtered seawater is gently sprayed over larvae retained in the upper sieve to wash any smaller individuals into the lower sieve. The contents of larvae in both sieves are washed into separate, graduated containers and are then estimated for numbers and examined as described below. At the same time small sub-samples are often taken to be subsequently measured for shell length.

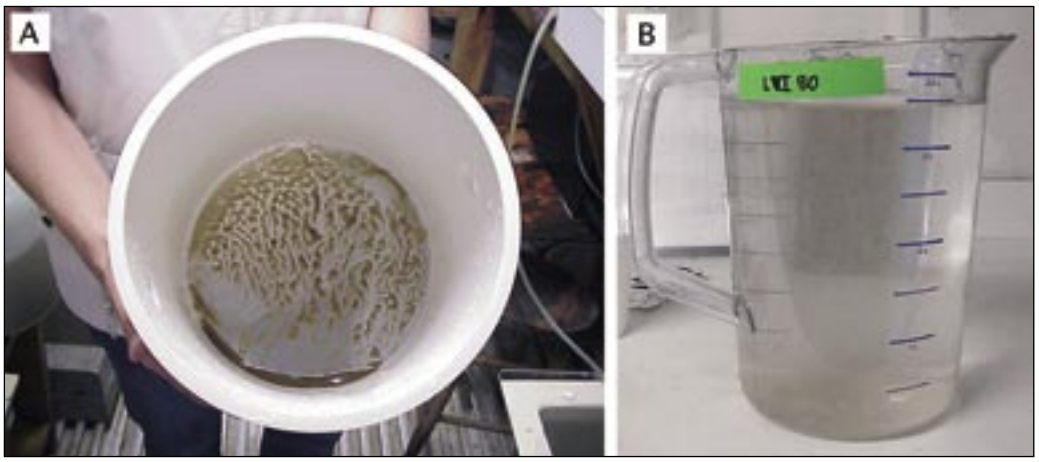


Figure 57: The appearance of almost 5 million calico scallop, *Argopecten gibbus*, larvae concentrated in a 20 cm diameter sieve (**A**) and after transferring to a 4 l graduated jug, preparatory to estimation (**B**).

Estimating Egg, Embryo and Larval Numbers

Care needs to be taken in handling eggs and larvae. When transferring eggs, embryos or larvae from one container to another through a mesh-based sieve, always ensure that the mesh of the sieve is submerged below the surface of the receiving container. All equipment used in transfers of this kind and in the estimation of numbers should be thoroughly cleaned beforehand and rinsed with filtered seawater.

(i) equipment required

Much of the equipment used for the purpose of estimating larval numbers needs to be specially made. For example, sieves are prepared from PVC pipe or high-impact, rigid styrene garden plant pots or horticultural containers. (Proprietary screens/sieves constructed of metal should be avoided).

To make suitable sieves, the bases of plastic containers are removed and nylon monofilament mesh is fixed tautly in place at the cut end with suitable solvent cement. Alternatively, 15 cm sections of suitable diameter PVC pipe (20 to 30 cm diameter is

convenient) are cut and nylon mono-filament mesh fitted to one end in the same way. Sieves should be indelibly marked with mesh size for easy identification.

It is useful to make a number of sieves for each of a wide range of mesh sizes ranging from 20 μm to 250 μm for the various purposes of embryo, larvae and early juvenile culture. Useful sieve sets for clam and scallop larvae are 40, 60, 80, 120 and150 μm mesh aperture (Table 11). The range needs to be extended upwards for larvae of the various commonly cultured oyster species.

Perforated plunger agitators and counting slides can be made in the workshop from plexiglass or transparent PVC pipe, sheet, and rod. Proprietary sedgewick rafter slides, available from scientific and aquaculture suppliers are useful for counting purposes (Figure 58).

Table 11: The relationship between the mesh aperture of sieves (screens) and the minimum size of larvae they will retain. This information is for guidance only and differs from species to species according to the shape of larvae. Experienced hatchery technicians can estimate the mean size of larvae from a culture by their distribution and retention on a range of mesh sizes at grading.

Mesh aperture (μm)	Minimum size of larvae retained shell length (μm)
45	75
80	120
120	145
150	170
160	210
180	255
200	280
220	300

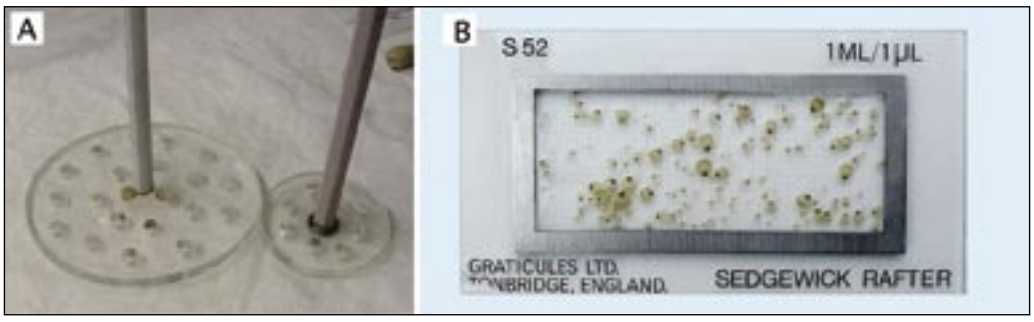


Figure 58: Equipment used in estimating numbers of larvae. **A** – perforated plungers for evenly suspending larvae in containers from which known volume sub-samples are taken in the estimation of larval numbers. **B** – a sedgewick rafter microscope slide, which has a chamber designed to take a 1 ml sample. The chamber has a grid marked on the base for easy tracking while viewing and counting larvae or spat in the sample. Similar slides can be made from transparent plastic.

Specific equipment that needs to be purchased includes adjustable volume automatic pipettes (0.1 to 1.0 ml and 1.0 to 5.0 ml ranges are useful); various measuring cylinders from 25 ml to 2 l volume and wash bottles.

Where budget permits, an electronic particle counter performs the same task as described below and saves time. It is also extremely useful for counting cell density in algal cultures and in determining food cell consumption at all stages in the hatchery process (refer back to Figure 21).

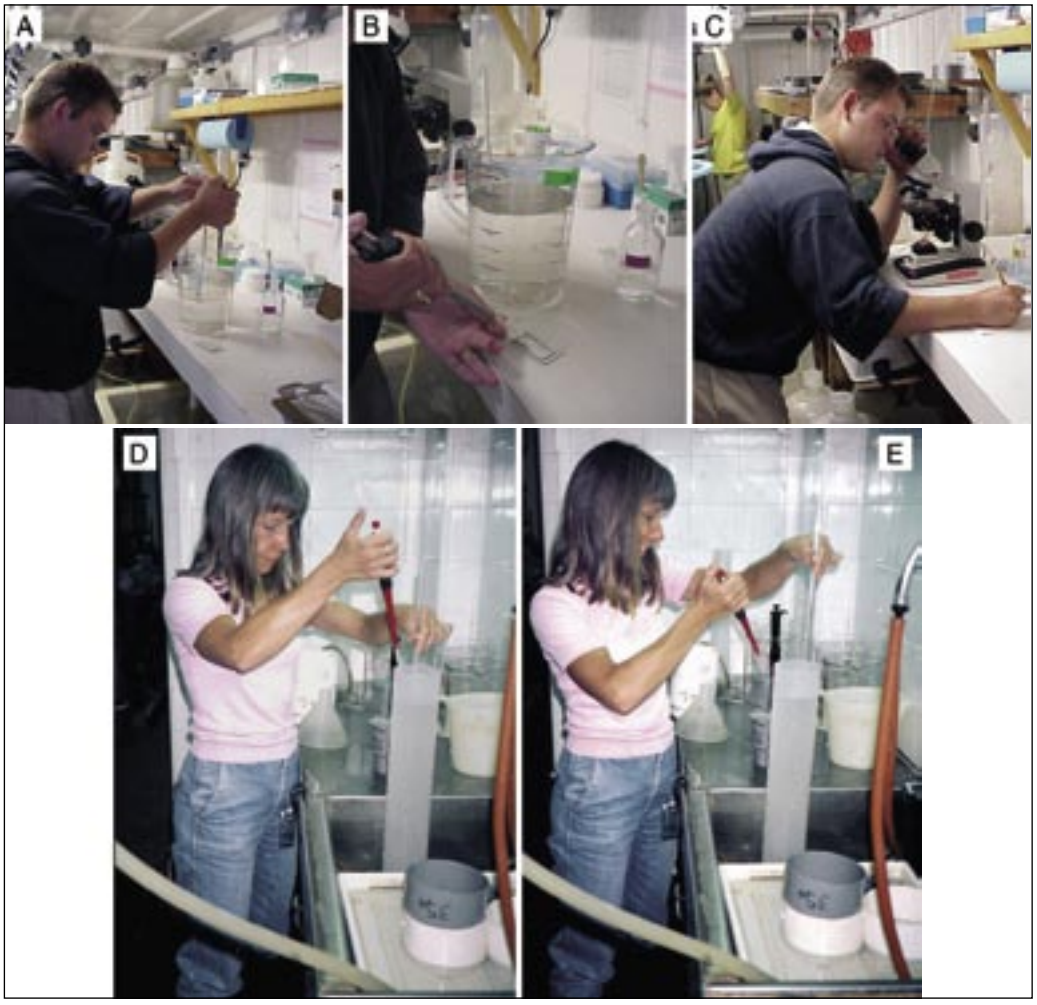


Figure 59: The steps in taking sub-samples of larvae for counting in the estimation of total numbers. **A** – taking a sub-sample with an automatic pipette while agitating the contents of the jug containing the pool of larvae; **B** – transferring the sub-sample to a sedgewick rafter slide; **C** – counting and recording the number of larvae in the sub-sample. The lower photographs (**D** and **E**) show a similar technique where larvae, concentrated in a 2 l graduated cylinder are sampled by automatic pipette during agitation.

*(ii) estimating procedure (*Figure 59*)*

a) After sieving and rinsing eggs, newly fertilized embryos or larvae, transfer them to a graduated measuring cylinder (1 or 2 l volume) or, if numbers are anticipated to exceed 5 to 10 million, to a bucket or similar larger volume container graduated in l, pints or gallons.

Note: For greater accuracy when using larger volume containers, use an accurately calibrated measuring cylinder or jug to fill the container to about 8 cm below the rim. Note the volume added and mark a calibration line on the inside of the container at the waterline with an indelible marker.

b) Add filtered seawater to the container to the graduation mark. (The total volume needs to be known).

c) With an automatic pipette set at 0.5 ml (volume may vary – see later) take 3 replicate sub-samples of the contents while agitating the contents of the measuring cylinder, or other container, with a suitable diameter perforated plunger. Make sure that the eggs or larvae are evenly distributed in the water column during sub-sampling (Figure 59A).

Note: The diameter of the plunger should be slightly, but not considerably, smaller than the diameter of the container to be sampled. Agitation should be sufficient to lift eggs or larvae off the bottom of the container and evenly suspend them, but not vigorous enough to cause excessive turbulence. A slow, rhythmical up and down motion at about 1 complete cycle per 4 seconds is recommended.

d) Transfer the sub-samples to the compartments of the counting slide (Figure 59B). The compartments should be etched with a suitable grid, as illustrated in.

Note: Take smaller volume sub-samples when numbers of larvae are anticipated to be large, or use a larger volume measuring cylinder/graduated container, or a combination of the two. In the case of eggs, which are very delicate, it may be easier to transfer the egg suspension to a large container, for example, a 10 l polyethylene bucket. Make up the volume to the calibration line and withdraw sub-samples while gently agitating the bucket contents with a large diameter, perforated plastic plunger.

e) Count the eggs or larvae in each sub-sample using a microscope (x40 magnification – Figure 59C).

Note: In the case of eggs and newly fertilized embryos, separate counts can be made of the total number per sub-sample and the number that have not rounded-off and appear abnormal. The same procedure applies to D-larvae where calculation can be made, based on the counts, of the percentage that have developed normally. Similarly, mortality rate estimation can be made on later larvae as part of the counting procedure by counting the live and dead or moribund larvae separately.

f) Calculate the total number as in the following example:

Example:

Larvae counts in the three sub-samples = 414; 389; 402.

Mean = 414 + 389 + 402/3 = 402

Volume of sub-sample = 0.5 ml.

Total volume of cylinder = 2 000 ml.

Total number of larvae = 2 000/0.5 x 402 = **1 608 000**

Eggs and larvae can also be counted using an electronic particle counter (e.g. Coulter Counter) fitted with a suitable aperture, sampling head. While this method is quick and convenient, it is impossible to distinguish between normally and abnormally developing larvae or between dead and live larvae. There is no substitute for visual examination and discrimination of the quality of a culture by an experienced hatchery technician.

5.1.3 Methods for rearing larvae

D-larvae recovered are counted as described above. They are now at the stage where they need feeding with unicellular, cultured algae. Species of good nutritional value include the diatoms,

Chaetoceros calcitrans,
Chaetoceros muelleri,
Thalassiosira pseudonana (3h)

and the flagellates,

Isochrysis galbana (or the 'T-Iso' clone),

Pavlova lutherii, and one of the
Tetraselmis species (but only for larvae >120 μm length).

Details of diets, rations and how to calculate them are described in part 5.2

Larvae can be grown in the same flat-bottomed tanks used for embryo development or in conical-based fibreglass tanks fitted with bottom drains (see Figure 52). Tanks may be of relatively small volume (200 to 1 000 l) for experimental purposes and small-volume production or much greater in both size and volume in high volume output commercial hatcheries. They may be operated as static systems or on flow-through. Water is changed in static systems on a periodic basis, whereas in flow-through culture, it is continuously introduced, a fixed volume being exchanged and replaced daily. This topic is discussed in depth in section 5.1.4.2.

D-larvae of hardier species (including *Crassostrea* and *Tapes*) can be grown at densities of 15 000 to 20 000 per l, but growth and survival are generally improved at lower densities (Table 10). Reduced densities are recommended for scallop species of the genera *Pecten*, *Patinopecten*, *Placopecten* and species of *Chlamys* and *Argopecten*, where between 5 000 and 10 000 early-stage larvae per l is appropriate. The larviparous flat oyster, *Ostrea edulis* is generally grown at 2 000 to 5 000 larvae per l because of the large size of the intial D-larvae. Some species can be successfully grown more intensively than above using high-density culture techniques (see section 5.1.4.1).

Rearing tanks are aerated – most commonly by a single, central air outlet located just off the tank bottom – at flow rates ranging from a slow bubble rate for D-larvae increasing to 200 l per hour for later stage larvae. The source of pressurized air needs to be free of carbon and oil. Low pressure, high volume, regenerative air blowers are ideal for the purpose. The air is filtered at source to 0.22 or 0.45 μm particle size by a series of cartridge filters of decreasing porosity. This is to reduce air-borne contaminants which may include harmful micro-organisms. It is also advisable in humid conditions to dry the air before it enters the tanks by passing it through a sealed unit, such as filter-cartridge housings, containing either anhydrous calcium chloride or silica gel. These drying agents need to be replaced as they become saturated to be effective.

5.1.3.1 Starting a new culture

Larval culture tanks and all equipment to be used must be thoroughly cleaned and then rinsed with either freshwater or filtered seawater. Mild liquid detergents added to hot water, or suitably diluted sterilizing/disinfecting agents such as bleach (sodium hypochlorite solution) at 20 mg per l free-chlorine can be used for cleaning purposes. The process of starting a new culture is as follows:

a) Fill the required number of clean larval rearing tanks with seawater filtered to 1 or 2 μm at the required temperature and salinity.

Note: It may be beneficial to reduce near oceanic salinities when rearing euryhaline species such as the American eastern oyster, *C. virginica*, by adding finely filtered freshwater from a clean, unpolluted source. A salinity of between 20 and 25 psu is recommended for this and other *Crassostrea* species).

b) If problems have been experienced with abnormal mortality of larvae in the recent past, bacteria may have been responsible. In this situation UV-light treatment of the water before filling the tanks is used by some hatcheries and may help. As a **last resort** if mortalities persist, a broad-spectrum antibiotic such as chloramphenicol at 2-5 mg per l of seawater may be used experimentally under veterinary prescription.

c) Add D-larvae to the vessel at the appropriate density.

d) Calculate, following the procedure given in part 5.2.3.2, The volumes of harvested algae to add to the vessels to provide the required food ration.

e) Turn on the flow of air so that there is a good turnover of water to suspend and evenly mix both the larvae and food.

f) The culture is then left for 24 hours before further husbandry is necessary.

5.1.3.2 Husbandry of larval cultures

Cultures of larvae require daily maintenance. They are operated most commonly as static water systems, i.e. without a continuous exchange of water, although some hatcheries operate flow-through culture systems (see section 5.1.4.2). Food cell concentrations need to be maintained at levels conducive to efficient feeding activity.

To prevent the accumulation of potentially harmful, metabolites, tanks require complete water changes at regular intervals throughout larval development from the D-stage to the onset of metamorphosis. The frequency with which this is done depends on the number and mean size of larvae being cultured. Water is changed either at 48-hour intervals or 3 times each week:

- at higher densities of early-stage larvae (15 000 to 20 000 per l at <120 μm),
- at low densities of late-stage larvae (<5 000 per litre at 150 to 200 μm) or ,
- at about 2 000 per litre at 250 to 300 μm).

Note: The values given are a rough guide to applicable densities related to mean shell length. More accurately, if a culture requires feeding less than 200 cells per μl *Isochrysis* equivalents per day then it is regarded as low density – see part 5.2.3.1). Water changes need to be daily at higher daily additions of food or alternatively, the tanks operated on the flow-through principle.

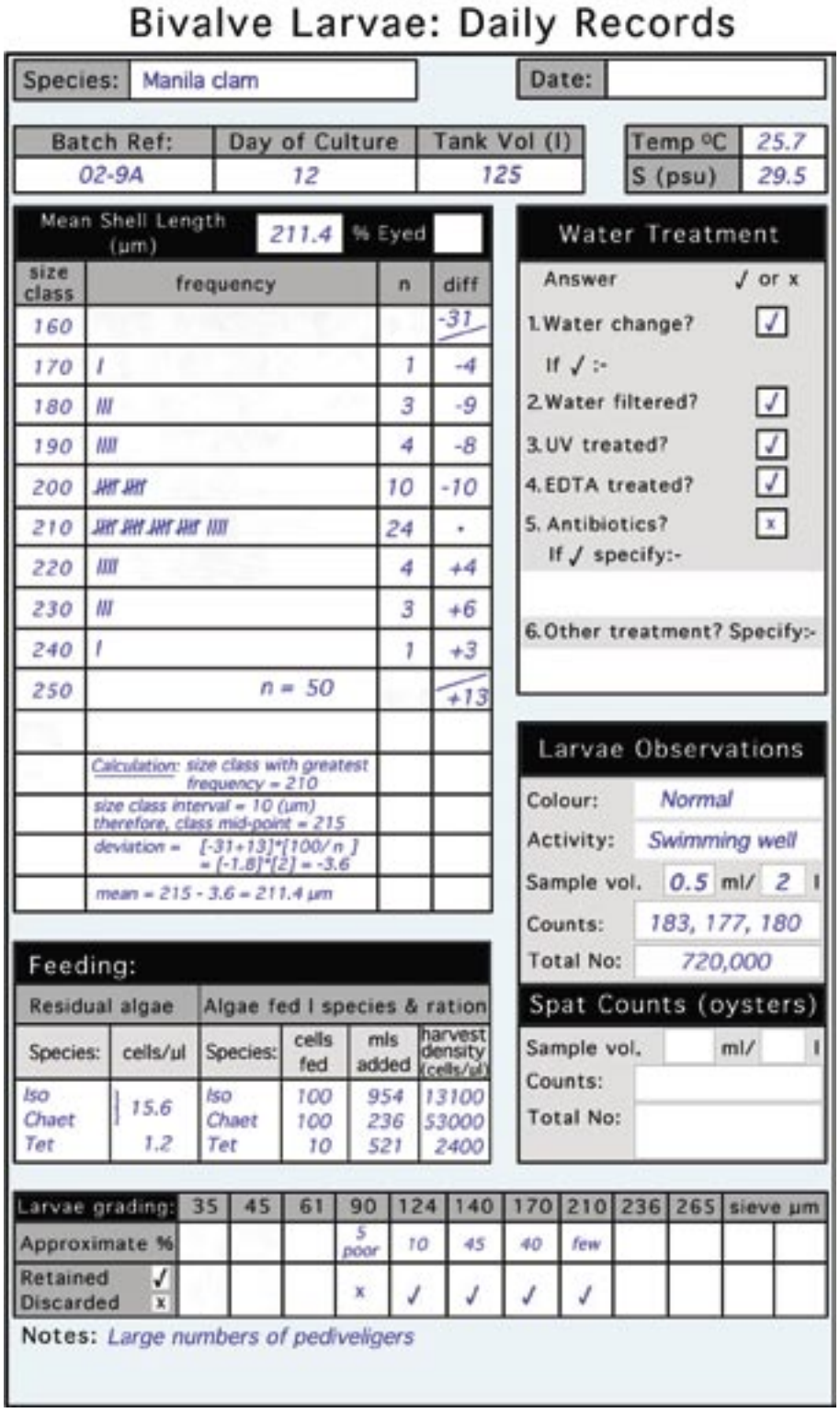
(i) Husbandry on non water change day

Husbandry on days between water changes consists of restoring the food cell concentration to compensate for cells eaten in the previous 24-h period by adding sufficient freshly harvested algae. A sample of water is taken from each vessel and the remaining algae cells (residual algae) per unit volume are counted, either by using a microscope with a haemocytometer slide at x100 magnification, or, more easily, by a coulter counter or similar particle counter. Where it is impractical to determine residual algae, either a full or partial ration of food can be added on intermediate days between water changes based on the previous day's ration fed.

Daily records should be kept of culture temperatures, residual algae and any additions of food to restore optimum food cell concentrations. An example of such a record form is shown in Figure 60. Volumes of additional algae are calculated as described in part 5.2.3.2.

(ii) Husbandry on water change days

The procedure is similar to that described and illustrated in the previous section on embryo development (Figure 56). The tank is emptied by means of a siphon or from a bottom drain, delivering the discharge flow into a sieve which is of sufficient aperture to retain large debris but not the larvae – a 250 μm sieve is ideal (Figure 61). The larvae are retained on the mesh of a lower sieve of suitable mesh aperture.



Bivalve Larvae: Daily Records

Species:	Manila clam	Date:	

Batch Ref:	Day of Culture	Tank Vol (l)	Temp °C	25.7
02-9A	12	125	S (psu)	29.5

Mean Shell Length (µm): 211.4 % Eyed:

size class	frequency	n	diff
160			-31
170	I	1	-4
180	III	3	-9
190	IIII	4	-8
200	卌 卌	10	-10
210	卌 卌 卌 卌 IIII	24	•
220	IIII	4	+4
230	III	3	+6
240	I	1	+3
250	n = 50		+13
	Calculation: size class with greatest frequency = 210		
	size class interval = 10 (µm) therefore, class mid-point = 215		
	deviation = [-31+13]*[100/ n] = [-1.8]*[2] = -3.6		
	mean = 215 - 3.6 = 211.4 µm		

Water Treatment

Answer	✓ or x
1. Water change?	✓
If ✓ :-	
2. Water filtered?	✓
3. UV treated?	✓
4. EDTA treated?	✓
5. Antibiotics?	x
If ✓ specify:-	
6. Other treatment? Specify:-	

Larvae Observations

Colour:	Normal
Activity:	Swimming well
Sample vol.	0.5 ml/ 2 l
Counts:	183, 177, 180
Total No:	720,000

Feeding:

Residual algae		Algae fed l species & ration			
Species:	cells/ul	Species:	cells fed	mls added	harvest density (cells/ul)
Iso	15.6	Iso	100	954	13100
Chaet		Chaet	100	236	53000
Tet	1.2	Tet	10	521	2400

Spat Counts (oysters)

Sample vol.	ml/ l
Counts:	
Total No:	

Larvae grading:	35	45	61	90	124	140	170	210	236	265	sieve µm	
Approximate %				5 poor	10	45	40	few				
Retained ✓				x	✓	✓	✓	✓				
Discarded x												

Notes: Large numbers of pediveligers

Figure 60: An example of a daily record sheet and the type of information that is useful to record in order to follow the progress of a batch or a tank of larvae. The steps in calculating the mean shell length of larvae from size/frequency plots are also shown.

Figure 61: Draining static larval tanks on water change days.

The procedure is as follows:

a) Wash any remaining larvae from the vessel into the sieve.

b) Clean the vessel with a sponge and hot detergent or bleach solution and rinse well.

c) Refill the vessel with appropriately treated seawater at the required temperature and salinity.

d) Grade the larvae by washing them through a stack of sieves of descending aperture size with filtered seawater. A guide to suitable mesh sizes for larvae of different shell lengths is given in Table 11.

e) Take small samples from each sieve upon which larvae have been retained and observe the appearance and activity of the larvae with the aid of a microscope. Discard any sieve fraction containing predominantly dead or slow-growing larvae.

Note: Sieve fractions containing mainly empty shells and larvae with decomposing tissues need to be discarded. The tissues of healthy larvae are of golden-brown colouration with a well-defined and darkly coloured digestive gland. Moribund larvae tend to be more darkly and uniformly granular in appearance.

f) Wash fractions containing healthy larvae into a measuring cylinder.

g) Take sub-samples as described before and determine the total number surviving. Measure a sample of 50 to 100 and calculate mean shell length.

Note: The addition of a few drops of formalin (10% formaldehyde solution, neutralized over calcium carbonate in the form of limestone or marble chips) will immobilize the larvae. Discard the counted sample(s).

h) Return the larvae to the culture tank and restore the aeration.

i) Repeat this procedure at 48-hour intervals.

5.1.4 Growing larvae more efficiently

Mention has been made earlier in this section of methods that can be employed to improve the efficiency of larval culture, either by operating the culture tanks on a flow-through of seawater or by rearing larvae at higher densities in static water tanks. In fact, the two methodologies can be combined to good effect in increasing production where space is limited with the added benefit of reducing the labour component in husbandry.

Although some hatcheries are beginning to turn to flow-through, the practice is not yet widespread. There is, however, ample scope to improve productivity by raising the density at which larvae are reared either by using existing equipment more efficiently or by investing in electronic devices to control feeding. The usual larval densities can be doubled or trebled by feeding to the number of larvae in a tank and their size, rather than by adding food to the water volume to a certain food cell density per unit volume, irrespective of the number and size of larvae. But if larval densities are further increased then the rate at which the feed is supplied becomes critical and needs to be continuously monitored. This approach is more suitable for the hardier species. If mortalities do occur for one reason or another, the effects in terms of lost productivity can be drastic. Most hatcheries prefer to opt for the more cautious approach.

5.1.4.1 High density culture

The rate at which food cells are ingested by larvae of different sizes (or weights) of the bivalve species being cultured needs to be known. This information is shown later in Table 12 (part 5.2.3.2) For three commonly cultured species when grown at 24±1°C. Where such information is lacking it will need to be determined experimentally or by the "trial and error" principle.

Knowing the relationship between larval size and food cell ingestion rate, it is a simple matter to calculate how much food needs to be added to the tank during the next 24-h period for a given number of larvae in culture of a particular mean shell length. Details of the calculation with worked examples and an explanation are given in the next section (part 5.2.3.2). At higher densities than the norm it will be necessary to provide part of the ration as a bulk feed at the beginning of the day; the remainder being dosed at a constant rate by drip feed or peristaltic pump over the next 24 hours.

At more than 20 000 larvae per l, particularly as they approach metamorphosis, feeding rate becomes more critical. It is more detrimental to over-feed larvae than it is to under-feed them. The amount of faecal waste and metabolites will accumulate in the culture water and can give rise to great increases in bacterial numbers. This can reduce feeding rate and result in more food being added to the tank than can be filtered by the larvae when added at a constant fixed rate. The solution has been tackled experimentally by the use of electronic sensors and control equipment to continuously monitor the food cell density in the culture tank (Figure 62). [A full explanation is given in Higgins *et al.* (1987) – see suggested reading list].

Figure 62: Experimental automatic control of food cell density in high-density cultures of bivalve larvae. **AR** – chilled, aerated algal reservoir containing the daily food ration; **P** – peristaltic pump which delivers the required amount of algae upon demand; **C** – control equipment containing a relay that switches the pump on when the sensor (**S**) detects a decrease in food cell concentration in the larvae tank (**LT**) below a certain pre-set threshold. This device utilizes an infra-red transmitter and receiver and could be greatly improved with modern electronics.

A summary of comparative results is given in Table 12, which includes data from trials with European flat oyster and Pacific oyster larvae.

Table 12. The average number of larvae stocked initially (N_o) and surviving immediately prior to settlement (N_p) in 5 comparisons of high and normal density rearing with the European flat oyster, *O. edulis*, and 3 comparisons with the Pacific oyster, *C. gigas*. The number of days to the onset of settlement and information on average spat yields (both as % of the initial number of larvae and as spat per l of water used during culture) are also shown.

	Larvae per l		Days to	Yield	
	N_o	N_p	set	% set	spat per l
O. edulis					
High density	9 954	5 942	9.8	40.5	512
Normal density	1 440	1 083	10.0	40.3	161
C. gigas					
High density	56 667	24 900	20.7*	21.6	735
Normal density	5 333	2 766	19.0*	25.0	202

* Days to set from the D-larva stage allowing a 4-day settlement period from the time settlement began.

5.1.4.2 Flow-through culture

The impetus to develop flow-through methods of larval culture stem from a number of objectives. Larvae of some species are less tolerant than others to generally used methods of culture in hatcheries. Those of the various pectinid species are a good case in point. They usually exhibit higher mortality rates and are not as amenable to high-density culture in static systems.

Other hatcheries are testing the potential of flow-through technology to make better and more efficient use of available resources. There may be the need for greater production within physical space constraints or to reduce labour costs and time spent in larval husbandry. Flow-through culture does offer these benefits. Time can be saved in raising the density of larvae without the need to drain statically operated tanks 3 or 4 times each week. The method may be wasteful in the use of cultured algae in that water is continuously being exchanged, albeit at a slow rate, and is run to waste. But

the food is relatively inexpensive to produce at the required volumes at this stage in the production cycle.

Tank design is important when considering flow-through. Larvae need to be retained within the tank and the volume large enough to ensure that added food has a sufficient residence time to be eaten. The exchange rate needs to be sufficient to prevent metabolic wastes and debris from accumulating, yet there may still be the need to flush out the tank periodically after cleaning the interior surfaces. The general approach is commonly used in culturing the early pre-feeding stages of marine fin-fish, e.g. Halibut, where purpose made tanks are available and can be adapted with minimal alteration. Rather than the flat-bottomed or steeply tapering conical tanks generally used in bivalve larval culture, these conical tanks have shallowly tapering, long cones (Figure 63).

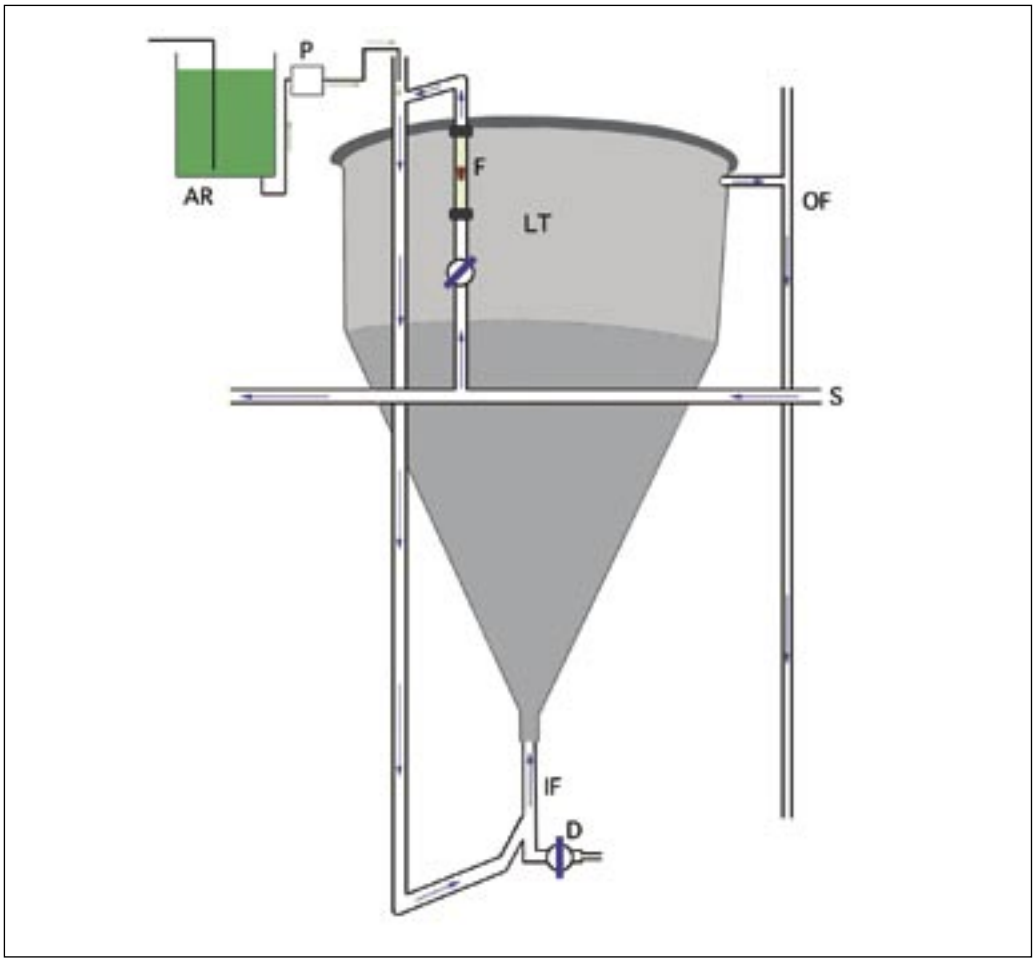


Figure 63: A typical arrangement for flow-through larval culture. See text for a description. Arrows show the direction of flow of both algae and seawater.

The rate of flow of suitably treated seawater (from the delivery pipe, S) is controlled and adjusted by a diaphragm valve and flowmeter (F). Depending on the density of larvae, the flow is adjusted such that the total daily throughput (IF – in-flow, OF – out-flow) is the same or greater than the total tank (LT – flow-through larval tank) volume that the number of larvae would require when cultured at normal densities. If, for example, larvae are normally cultured at 5 000 per l in a 500 l tank, then 20 000 larvae per l in a flow-through tank of the same volume will require a minimum throughput of 2 000 l per day. Larvae are retained in the tank by a large diameter "banjo" filter fitted with a suitable aperture mesh screen (see Figure 64 for details).

The tank is fitted with a drain valve (D), which also serves as the input port for a "salt-plug" of saturated brine solution. When the in-flow is switched off, this "salt plug" of 2 or 3 l volume is gravity-fed into the drain and the valve closed. Living larvae will swim to the water surface and thereby avoid the dense salt solution, which traps the dead and moribund. After a few minutes, the drain is opened a fraction to eliminate the "salt plug" and the dead larvae, much in the same way as dead marine fish eggs are accumulated and eliminated from incubators.

Larvae are supplied with the required food ration by peristaltic pump (P) from a cooled and aerated algal reservoir (AR). The quantity and rate at which the ration is supplied depends on the number of larvae, the size they are and the flow rate through the tank (refer to parts 5.1.4.1 and 5.2.3.2). Mean shell length can be determined by taking daily samples, but survival is more problematic. An estimate of mortality can be obtained by sub-sampling the volume of saturated brine collected after a "salt plug" and counting the dead larvae it contains. This can be done at 2 to 3 days intervals to keep track of survival.

Internal surfaces of flow-through tanks need to be cleaned with a soft-bristle brush, attached to a pole of suitable length, at least once during the culture period of a batch of larvae. The flow rate should be increased during cleaning to flush out the dislodged debris.

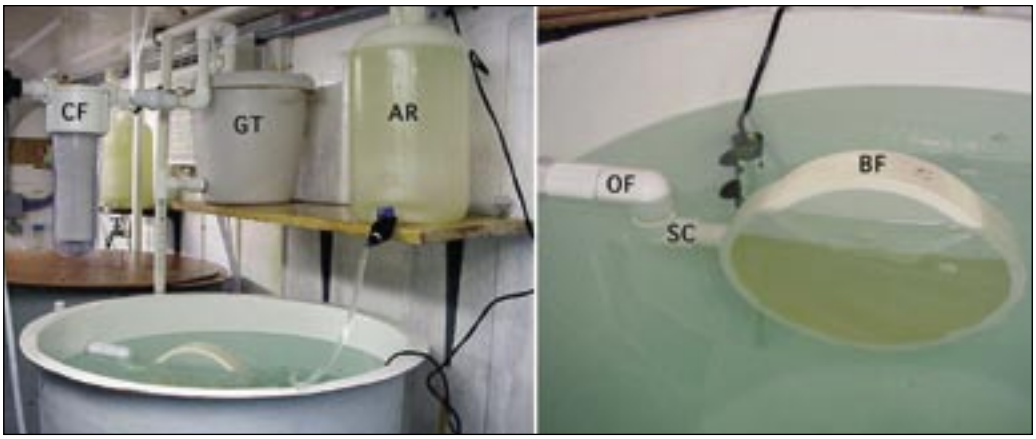


Figure 64: Detail of the top of an experimental flow-through tank showing the "banjo" filter (**BF**) attached to the out-flow pipe (**OF**). In this example, seawater filtered to 1 μm by a filter cartridge (**CF**) is supplied to a gravity tank (**GT**) from which it flows at a controlled, constant rate into the base of the larvae tank. Food is drip-fed into the tank from an algal reservoir (**AR**). The "banjo" filter is made from a 20 cm diameter section of PVC pipe and is fitted both sides with 60 μm nylon mesh screen, solvent cemented to the cut faces of the cylinder. A short length of suitable diameter PVC pipe is welded into a hole drilled through the plastic to connect with the out-flow pipe. Large diameter "banjos" are recommended to reduce the per unit area force generated by the out-flow. They should be totally, or almost totally, submerged and need to be cleaned daily. For this purpose, the "banjo" is a push-fit into a swivel coupling (**SC**) made from a pair of 90° PVC elbows. This coupling can be swivelled upwards to raise the filter above the water level for removal and cleaning, or replacement.

Rearing larvae in flow-through tanks does have disadvantages but they are potentially more than outweighed by the benefits. Since larvae are not graded on a regular basis as in static culture, considerable variability in size will develop over time. Also, larvae will need to be transferred to settling tanks when they reach the pediveliger stage. This is standard practice in many hatcheries but not in others, where larvae are allowed to set on the sides and bases of the larval rearing tanks from which they are later removed.

Removing attached pediveligers and spat from the internal surfaces of shallowly tapering cones will be extremely difficult. Settlement tanks will be needed, particularly for late-stage larvae of the various oyster species that cement themselves to surfaces (see 5.4.3).

5.1.5 Growth and survival of larvae

The growth and stages in development of larvae of the Pacific oyster, C*rassostrea gigas*, and the sand scallop, *Pecten ziczac*, from the D-stage to metamorphosis are shown in Figure 65.

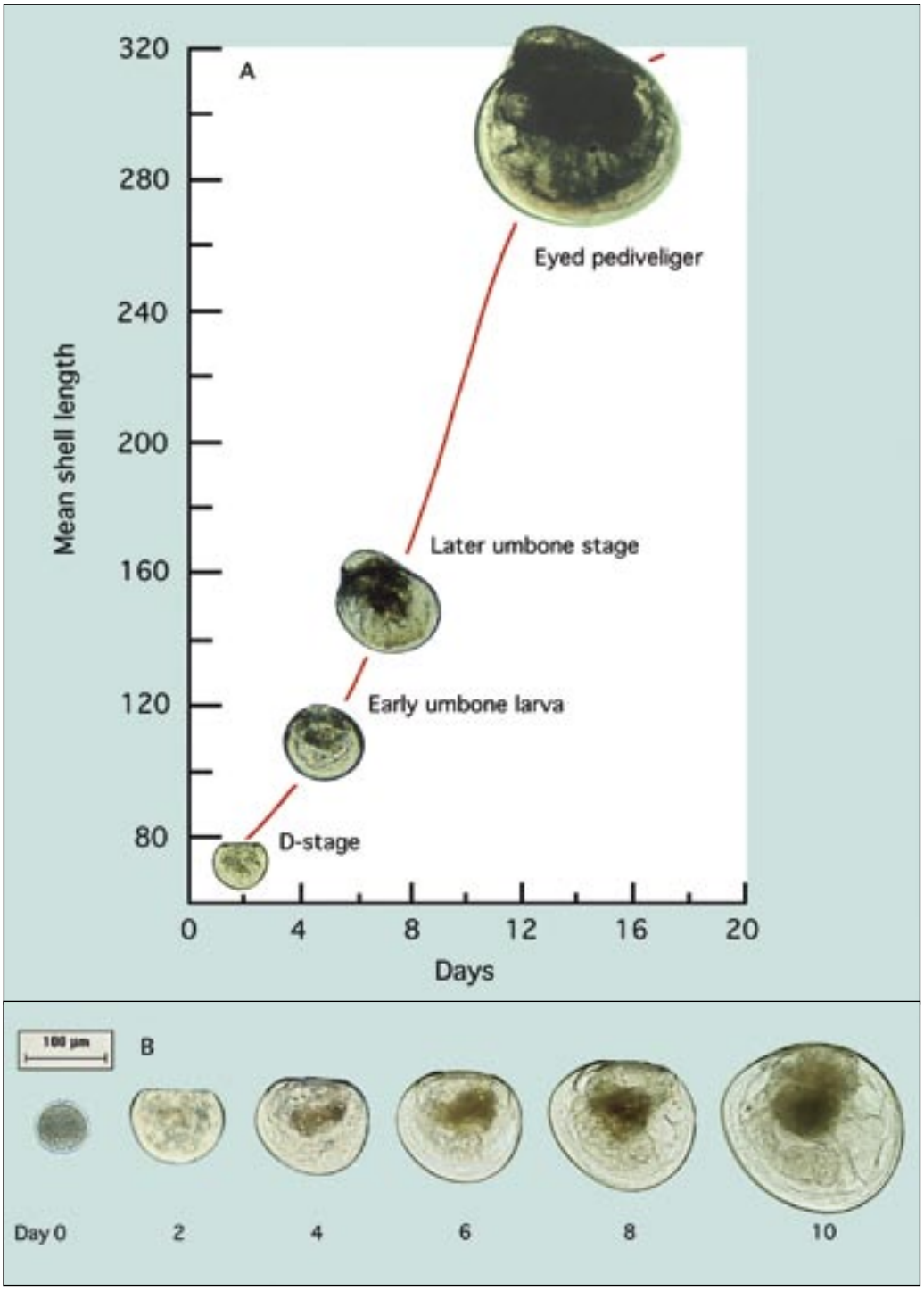


Figure 65: Photomicrographs of the growth and development of Pacific oyster, *Crassostrea gigas*, (**A**) and sand scallop, *Pecten ziczac*, (**B**) larvae.

Larvae of the different groups of bivalves grow at different rates. Those of scallops and clams have initially larger D-larvae and reach settlement size and metamorphosis at a considerable smaller shell length (210 to 230 μm) than do larvae of the oviparous oysters (320 to 340 μm). Comparative growth rate of a number of species cultured at 24±2°C is shown in Figure 66. Some species, including the calico scallop, are slower in reaching an exponential rate of growth. They tend to have a lag phase before rapid growth commences. Others like the Manila clam and Pacific oyster grow rapidly from the initial D-stage. Growth rate slows in all species as they approach the settlement stage.

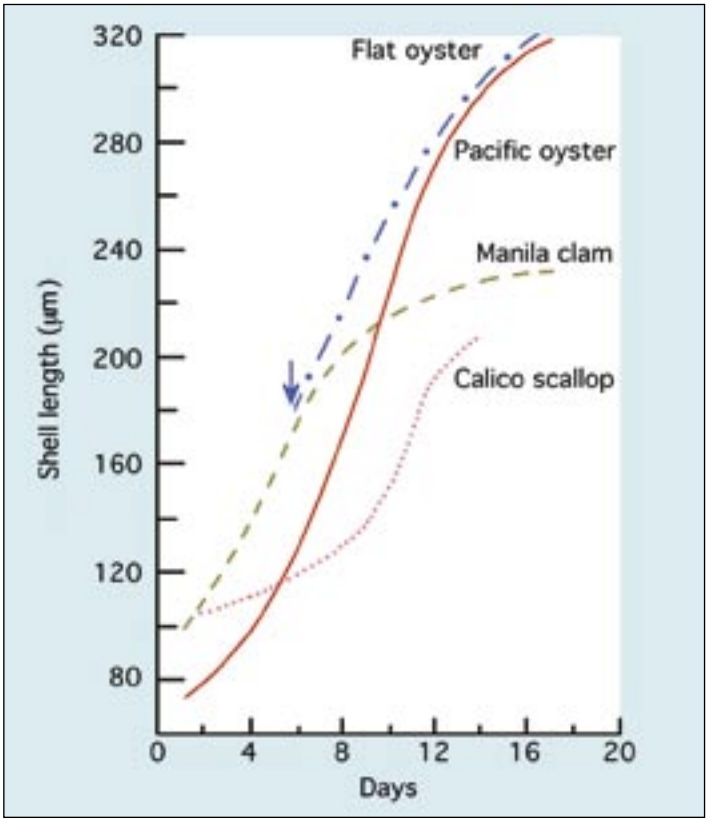


Figure 66: Comparative growth of larvae of some warmer water bivalve species (European flat oyster, *Ostrea edulis*, Pacific oyster, *Crassostrea gigas*, Manila clam, *Tapes philippinarum* and the calico scallop, *Argopecten gibbus*) from the D-larva stage to metamorphosis when cultured at 24±2°C. Day 0 denotes the day that eggs were fertilized. The blue arrow shows the day that flat oyster larvae were liberated by brooding adults.

Similarly, survival of larvae from the D-stage to metamorphosis is variable among different species. It may be as high as 50 to 70% on average in some of the oyster and clam species and as low as 15 to 30% in scallops. Much depends on culture protocols and the extent of the culling (removal) of slower growing larvae during the rearing process. A considerable part of losses in larval numbers through the culture period is often associated more with culling and discarding of slower growing individuals than it is to the death of larvae. The proportion of larvae that reach metamorphosis is also related to culture conditions including diet and ration, temperature and salinity and to relatively uncontrollable factors such as seawater quality and disease (see 5.3).

5.2 FEEDING AND NUTRITION

5.2.1 Introduction

Feeding commences as soon as larvae become fully shelled and their organs including the digestive system have developed. Prior to that time, energy for respiration and development is derived from reserves laid down during egg development (oogenesis) by the maturing females (see 5.3.4). It is also likely that developing embryos are able to absorb organic nutrients from the surrounding seawater. Indeed, there is often benefit in adding a little cultured algal food to tanks containing embryos 12 hours before they

have reached the D-larva stage and are capable of ingesting particulate food. It may not be the algal cells themselves that are important but rather organic nutrients in solution in the algal cultures. In this respect, the addition of small quantities of diatoms (e.g. *Chaetoceros muelleri* at 10 to 20 cells per µl) from cultures nearing the stationary phase appears to be most effective.

Once the velum is developed at the D-larva stage and the fully shelled larvae are swimming, the beat of the velar cilia direct food particles towards the mouth as well as providing the motive force for swimming activity (Figure 67). At this point – Day 0 as it is usually referred to – the quality (diet composition) and quantity (ration) of food added to culture tanks becomes important.

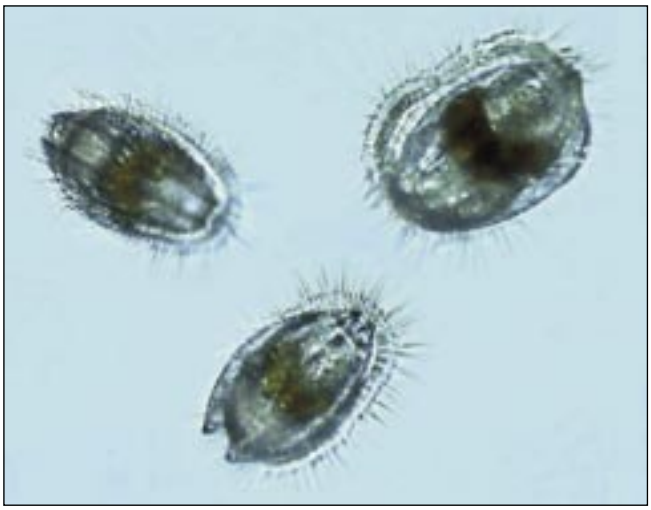


Figure 67: Larvae feed as they swim. Beating of the cilia of the swimming organ, the velum, also directs food particles towards the mouth. The three Day 8 scallop larvae shown are swimming on a collision course. Their darkly coloured digestive glands are clearly visible.

5.2.2 Dietary considerations

Mixed algal diets are beneficial. A combination of two or three high nutritional value species including a suitably sized diatom and a flagellate invariably provide improved rates of larval growth and development than do single species diets (Figure 68). They also improve spat yields and influence the subsequent performance of spat in terms of both growth and survival.

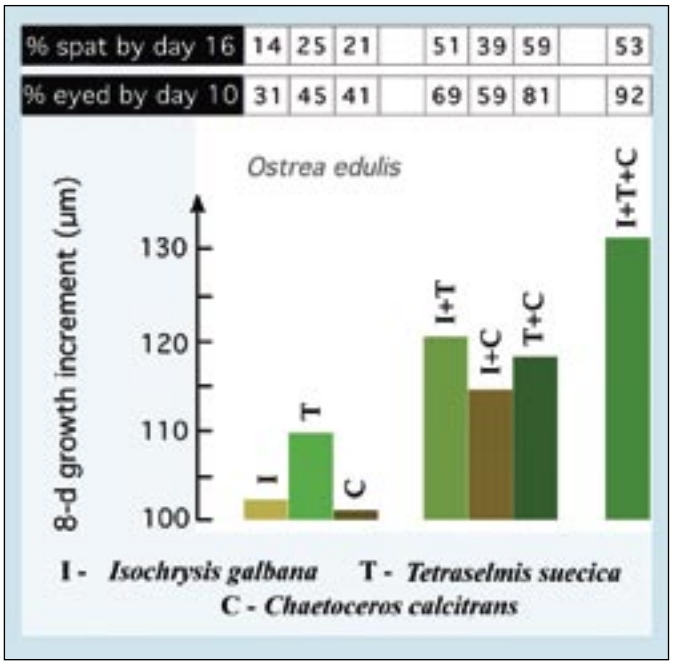


Figure 68: Growth (in an 8-day period), development (% eyed larvae by Day 10) and settlement (percentage spat of the initial larval number at Day 0) of *Ostrea edulis* larvae fed various single and mixed diets of the three algal species indicated. Values are the means of a large number of trials.

Not all of the suitably sized and readily cultured algal species available from culture collections are of good food value to larvae. But generally those that are valuable foods for the larvae of one species will be of similar value to others. There are exceptions to this rule as will be explained later. The food value of a particular alga is determined not only by its biochemical composition but also its "ingestability" and digestibility. For example, diatoms with long, silicaceous spines may be difficult to ingest and be an irritant to be expelled by larvae closing their shell valves. Some varieties of *Phaeodactylum* are a good case in point. Other species, such as *Chlamydomonas coccoides* have thick cell walls that render them almost indigestible. Yet others, including *Dunaliella tertiolecta*, lack certain essential highly unsaturated fatty acids (HUFAs) required for larval development and while digestible, have little or no nutritional value.

A comparison of the HUFA profiles of a number of algal species that are either good or poor foods for larvae is given in Figure 69. Also shown are values of total lipid content as a percentage of ash-free dry weight.

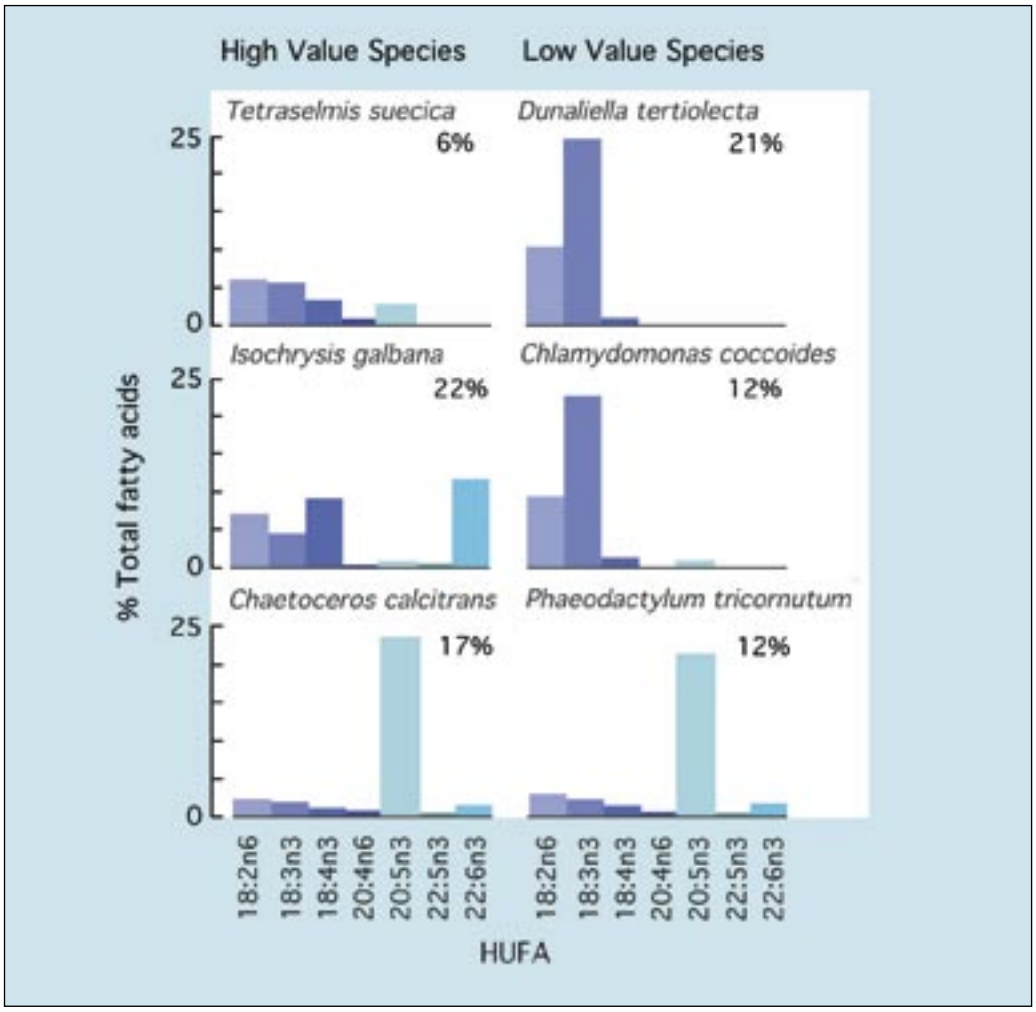


Figure 69: Comparison of total lipid as a percentage of ash-free dry weight and the relative abundance of various highly unsaturated fatty acids (HUFAs) in a number of algal species of both high and low nutritional value to bivalve larvae.

High food value species tend to have relatively high proportions of either 20:5n3 (EPA – eicosapentaenoic acid) or 22:6n3 (DHA – docosahexaenoic acid) compared with many of the poor value species. If these components are lacking in the diet, it appears that the larvae of most bivalves have either no or only a limited ability to synthesise them

from less highly unsaturated precursors. It is the case for many of the diet fastidious species that feeding a combination of species rich in either EPA or DHA (or both) will provide the best results. Larvae of clams tend to be less dependent in this respect than those of oysters or scallops.

The relative proportions of HUFAs and the overall lipid content of species of algae useful for hatchery production vary according to phase of the culture cycle and also culture conditions, which differ from hatchery to hatchery. However, species that are of good nutritional value in one hatchery situation will always be of similar value elsewhere given reasonably good attention to details of the culture conditions.

The various diatom species commonly grown in hatcheries have very similar HUFA profiles, all being rich in EPA. Total quantities of particular fatty acids in the different diatom species are somewhat variable. They tend to be higher in cultures entering the stationary phase than during exponential growth.

Among the small cell-size brown flagellates, *Pavlova lutherii* has a similar HUFA profile to *Isochrysis galbana* (Figure 69) but tends to have more DHA. In contrast, the T-ISO clone of *Isochrysis* has only 50 to 70% of the DHA of *Isochrysis galbana* when grown in side-by-side culture in the same conditions of light and nutrients. T-ISO tends to be grown in more hatcheries than its near relatives because it is easier to culture year round and is tolerant to higher temperatures. Useful substitutes for *Tetraselmis* are species of *Pyramimonas* (e.g. *P. obovata* and *P. virginica*). They have HUFA profiles intermediate between *Tetraselmis* and *Isochrysis* but can be difficult to culture at certain times of year.

5.2.3 Diet composition and ration

A suitable starter diet for D- and early-stage larvae (<125 μm shell length) of most commonly cultured bivalves is a mixture of:

One of the following diatoms:
Chaetoceros calcitrans or *Thalassiosira pseudonana* (for larvae >55 μm) or *Chaetoceros muelleri* (for larvae >90 μm),

combined with:

One of the following flagellates:
Isochrysis galbana or 'T-Iso' or *Pavlova lutheri,* in equal proportion by cell numbers.

When the mean size of larvae exceeds 120 μm shell length, the larger cell-size flagellate, *Tetraselmis* spp. (*T. chuii*, *T. suecica*, *T. tetrahele*, etc.), can usefully be added to the diet.

Food rations are usually quoted as the total number of algal cells per microlitre (cells per μl) or per millilitre (cells per ml) of the culture tank volume. Note that 100 cells per μl are equivalent to 100 000 cells per ml.

Account needs to be taken of the fact that the cells of the different algal species vary widely in mean size and, thereby, in volume and mass (see Table 1, Part 3.1). In calculating a ration for a diet incorporating two or three species, the representation of each in the ration is calculated on a cell volume equivalency basis, where (in approximate terms):

1.0 cell of *Isochrysis galbana*, T-Iso or *Pavlova lutherii* =
0.1 cells of *Tetraselmis* sp., or
1.0 cells of *Thalassiosira pseudonana*, or
2.25 cells of *Chaetoceros calcitrans*, or
0.75 cells of *Chaetoceros muelleri*

Thus, a suitable food ration for early-stage *Crassostrea* or *Tapes* larvae (and for most other species), where the target food cell density is equivalent to 100 cells *Isochrysis* per µl, can be satisfied by the following dietary combinations:

125 cells per µl *C. calcitrans* + 50 cells per µl *I. galbana*, or
37.5 cells per µl *C. muelleri* + 50 cells per µl *P. lutherii*, or
50 cells per µl *T. pseudonana* + 50 cells per µl *P. lutherii*

Any of these mixed-species diets are excellent for larvae of bivalves most commonly cultured in hatcheries, although the ration as cells per µl will vary both with species and density of larvae in the culture. Cell densities quoted above are ideal for larvae of the various *Crassostrea* sp., *Ostrea edulis*, the clams *Tapes philippinarum*, *Tapes decussatus*, *Mercenaria mercenaria*, *Mya arenaria* (and many others), and the mussels *Mytilus edulis* and *Perna perna* at previously quoted larval densities (refer to Table 10, Part 5.1.2.3). In contrast, larvae of many scallop species show better overall performance when fed the same diets but at lower rations. For example, larvae of the scallops, *Pecten ziczac* and *Argopecten gibbus,* express maximum growth rates at a total ration of between 5 cells per µl at the D-larva stage, increasing to 18 cells per µl prior to the pediveliger stage. Other hatcheries use rations two to three times greater with larvae of different scallops, but rarely do they use rations as high as for oysters, clams and mussels, over a similar range of larval sizes.

It will be noted in the example of dietary combinations given above that T-Iso has been omitted. While T-Iso is a perfectly good species to feed to larvae of clams, mussels and scallops, there are reservations to its use in diets for the early larvae of *Crassostrea* species (Figure 70). Compared with *Isochrysis galbana* and, more so, *Pavlova lutherii*, levels of the important highly unsaturated fatty acid (HUFA) DHA are considerably lower. When T-Iso is fed as a single species diet to larvae of the various *Crassostrea* sp., both larval growth and development are severely retarded beyond a shell length of 110 µm. For this reason, it is recommended that hatcheries should concentrate their small flagellate, algal culture on well proven strains of *Isochrysis galbana* and *Pavlova lutherii.*

Larvae can be grown from D-larvae to metamorphosis on two-species combination diets, such as those shown above. However, once mean larval shell length exceeds 120 µm, it is advantageous to add a third species in the form of one of the smaller *Tetraselmis* sp. Evidence shows that growth rate and the proportion of larvae that successfully complete metamorphosis improves when *Tetraselmis* is included in the diet (refer to Figure 68).

Either *Tetraselmis* can be used as a direct substitute for *Isochrysis* or *Pavlova* in the diet or, better still, it can be used as an additional species in formulating a three-species diet. It should not, however, be substituted for the diatom in the diet. Each of the three recommended diatoms, mentioned above, contains another important HUFA (EPA) of known nutritional and developmental significance.

When substituted for *Isochrysis* or *Pavlova* in a two-species diet, *Tetraselmis* is fed at 10% of the cell density appropriate to the smaller flagellates, thus:

37.5 cells per µ l *C. muelleri* + 50 cells per µl *P. lutherii*
becomes:-
37.5 cells per µ l *C. muelleri* + 0.5 cells per µl *T. suecica*

When used as an additional species to make a three-species combination, each of the component species is provided at 33.3% of the target cell density, which may be 100 cells per µ l *Isochrysis* equivalents. Thus:

Two-species combination:
37.5 cells per µ l *C. muelleri* + 50 cells per µl *P. lutherii* =
100 cells per µl *Isochrysis* equivalents;

Three-species combination: 25 cells per µl *C. muelleri* + 33.3 cells per µl *P. lutherii* + 3.33 cells per µl *T. suecica* =
100 cells per µ l *Isochrysis* equivalents

The overall amount of algae fed in terms of cell volume/mass is approximately the same for these two example diets, both of which are suitable for larvae >120 µm shell length.

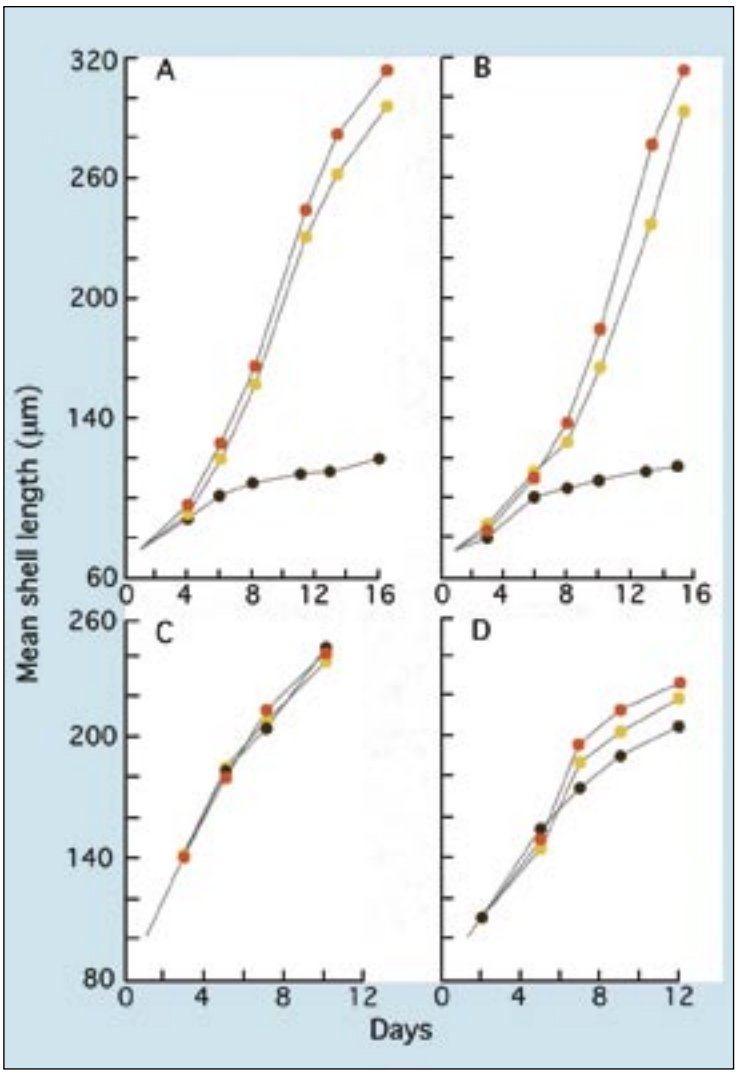


Figure 70: The growth of **(A)** *Crassostrea gigas,* **(B)** *Crassostrea rhizophorae*, **(C)** *Mercenaria mercenaria* and **(D)** *Tapes philippinarum* larvae fed T-Iso (brown circles), *Chaetoceros calcitrans* (yellow circles) and a two species mixture of these two algae (orange circles).

Attention so far in this section has focussed on general guidelines for diets and rations for larvae. In many small owner operator hatcheries run on the "wet thumb" principle – and often on a very tight budget – little or no regard is paid to counting the density of algae at harvest, or in formulating a combination of species to feed with any great degree of precision. An experienced operator rearing one or more of the hardy and tolerant bivalve species will judge which algae cultures look to be the best on a particular day and will add enough of each to the larvae tanks until the water colour looks about right.

At the other end of the spectrum – in large hatcheries supplying seed at industrial-scale either for their own growout, or in support of private growers within a region – responsibility and the scale of financial investment dictate that husbandry is maintained under closer control. Here, the priority is to maximize cost-efficient output of the seed products and make a profit. How this can be achieved by making the most effective use of cultured algae in feeding larvae is discussed next.

5.2.3.1 Feeding strategies

There are two basic strategies used in hatcheries to ensure that a sufficient food ration is provided to larvae. The first is to add algae to the seawater volume contained in the larval culture tanks with the objective of raising food cell density to a concentration that supports maximum larval growth rate. The second strategy involves feeding the ever-increasing biomass of larvae, as development proceeds, according to known food-cell ingestion rates of larvae of different mean shell lengths. This latter approach has already been briefly mentioned in discussion of high density larval culture in Part 5.1.4.1.

Strategy 1 is the easier option, since the volume of water in a tank is unlikely to be varied during the culture period. It is the appropriate strategy where lower densities of larvae are maintained. In effect, the food ration is delivered once per day. Over the course of the next 24-hour period the food will be grazed to a low level. It is only for a brief period in the 24-hour period that food cell concentration is optimal. However, the strategy can be modified, and often is, by feeding an additional 50% (or more) ration 8 to 12 hours after the main ration. The intention is to maintain food cell density nearer the optimum for the greater part of the day. At low mortality rates, the numbers of larvae may need to be reduced as development proceeds by division between two or more tanks so that the ration is not grazed too quickly.

Strategy 2 requires knowledge of the rate at which food cells are depleted from the water by a known number of larvae at all shell lengths (or weights) for all stages in development from the D-larva stage to metamorphosis. Having determined mean size and the number of larvae surviving at successive tank water changes the operator can calculate how much feed needs to be added to the tank to support maximum growth rate for the biomass of larvae at that time. In this way, higher densities of larvae can successfully be maintained in a given tank volume.

However, over-feeding is equally if not more damaging to the performance of larvae than is under feeding. As mentioned previously, at higher larval densities it may be necessary to feed twice each day as two separate rations at the recommended optimum cell density for the larvae of most species, i.e. at close to 100 cells per µl *Isochrysis* equivalents. Providing a double ration as one bulk feed per day to a culture of larvae will exceed the density and volume of food cells at which feeding activity of larvae is at its most efficient. Overfeeding may lead to bacterially related disease in situations where larvae are already stressed. In this case, the required ration is divided into two equal parts. The first part is added directly to the tank and the remaining half is dosed or drip fed over the following 24-h period.

A logical development in ensuring proper feeding of larvae is the use of modern, sophisticated opto-electronics. Some progress has been made with more primitive devices that shine an infra-red light beam through a culture to a detector, comparing the turbidity caused by the presence of the optimal food cell density in the culture volume with a reference signal. When food cells are grazed by the larvae, turbidity of the water decreases. At a certain preset value, a relay is triggered which activates a small peristaltic pump that adds more algae to the tank from an aerated reservoir until the desired turbidity is restored. The reader is referred to Part 5.1.4 for further information.

5.2.3.2 Calculating food rations

Feeding Strategy 1: Volumes of the algal species necessary for addition to larval rearing vessels to achieve the required cell densities are calculated from the following equation:

$$\text{Volume (l) to feed} = \frac{\text{required cell density [cells per µl]} \times V}{\text{cell density of harvested algae [cells per µl]}}$$

where V = volume of the larvae culture tank in litres.

Example:

Basic Information:

Diet and cell density to be fed:

37.5 cells per µl *C. muelleri* + 50 cells per µl *P. lutherii*

Cell densities of harvested algae:

C. muelleri	4 800 cells per µl
P. lutherii	8 900 cells per µl

Volume of larvae culture = 800 l

Calculation:

Volume of *C. muelleri* required = 37.5x800/4 800 = **6.25** l
Volume of *P. lutherii* required = 50.0x800/8 900 = **4.49** l

Feeding Strategy 2: Calculation involves determining the number of food cells additional to an initial daily ration equivalent to 75 cells per µl *Isochrysis* required during the following 24 hours to maintain the algal cell density constant. Steps in the calculation are detailed in the following example that applies to *Crassostrea gigas* larvae:

Example:

Basic Information:

Larvae culture tank volume	-	1 000 l
Number of *C. gigas* larvae	-	22.5 million
Mean shell length of larvae	-	170 µm
Algal diet provided	-	equal mixture by cell volume of: *P. lutherii*, *C. muelleri*, *T. suecica*
Harvest densities of algae	-	*P. lutherii* = 15 000 cells per µl
		C. muelleri = 7 400 cells per µl
		T. suecica = 1 200 cells per µl

Calculation:

a) Provide an initial ration of 25 cells per µl *P. lutherii*, 18.75 cells per µl *C. muelleri* and 2.5 cells per µl *T. suecica* = **1.67**, **2.53** and **2.08** l respectively at the harvest cell densities given above (see **Feeding Strategy 1** for the method of calculation).

b) Read the number of cells consumed by a 170 µm Pacific oyster larvae in 24 hours from Table 13 = 30 100

c) Divide 30 100 by the number of algae species in the diet = 10 033 cells per µl *Pavlova* (1 003 cells in the case of *Tetraselmis* and 7 525 cells in the case of *C. muelleri* to account for cell volume differences).

d) Calculate the volume of harvested algae of each species required to maintain the optimum food cell density in the 1 000 l tank stocked with 22.5 million larvae:

$$\text{Vol. of } \textit{Pavlova} = \frac{\text{value in (c) x number of larvae (millions)}}{\text{cell density of algal culture [cells per µl]}}$$

$$= 10\,033 \times 22.5 / 15\,000 = \mathbf{15.04}\ \text{l}$$

Similarly, the volume of *C. muelleri* required is: 7 525x22.5/7 400 = **22.88** l
and for *T. suecica* it is: 1 003x22.5/1 200 = **18.81** l

e) Add the volumes calculated in (a) above directly to the larval tank. The remainder (**15.04** minus **1.67** l for *Pavlova*, etc.) is mixed in a cooled, aerated reservoir of sufficient volume. Dose this volume at a constant rate over the 24-hour period. From the practical point of view, it is advisable to make up the algae contained in the reservoir with filtered seawater to the volume pumped in 24 hours by the peristaltic pump.

Note: Data given in Table 13 applies to larvae grown at 24±1°C. At a fixed rearing temperature, the growth of larvae is generally predictable so that daily measurements of shell length are not essential. Measurements should, however, be made at 48-hour intervals and can be estimated on the intermediate days based on experience.

Table 13: The number of algal cells ingested per larva per day by three commonly cultured bivalves relative to the mean shell length of the larvae. Values are shown as cells equivalent in size to *Isochrysis galbana*.

	Cells (*Isochrysis* equiv.) ingested per larva per day		
Mean Shell Length (mm)	*C. gigas*	*O. edulis*	*T. philippinarum*
100	2 800		4 400
110	6 700		6 000
120	10 600		8 000
130	14 500		10 200
140	18 400		12 800
150	22 300		15 700
160	26 200		18 900
170	30 100	19 200	22 300
180	34 000	28 200	26 000
190	37 900	37 300	29 900
200	41 900	46 300	29 100
210	45 800	55 400	21 900
220	49 700	64 500	14 900
230	53 600	73 500	
240	57 500	82 600	
250	61 400	91 600	
260	65 300	100 600	
270	69 200	109 800	
280	73 100	118 800	

Growth rates of larvae are not significantly different whether they are grown at low density using **Feeding Strategy 1** or at high density using **Feeding Strategy 2**. The advantage of the latter strategy is in operational cost efficiency, both in terms of labour and the better use of hatchery space. When using opto-electronic control of feeding (as in Part 5.1.4.1) estimated volumes of the required food species are calculated as in **Feeding Strategy 2.**

5.3 FACTORS INFLUENCING GROWTH AND SURVIVAL

5.3.1 Introduction

Effects of diet and food ration were specifically dealt with in the previous section. This section provides useful background information in discussing other aspects of culture conditions and how they influence the performance of both embryos and larvae. Topics include temperature and salinity, seawater quality, egg and larval quality and disease. Much of the information included has not previously been published and in contrast to other sections of this manual references have been cited in the text to enable the reader to pursue topics of interest in greater depth.

5.3.2 Effects of temperature and salinity

Of all the factors that impinge on growth, development and survival of larvae in culture, temperature is one of the most important since metabolic rate is dictated by temperature of the water in which they swim. Larvae of many of the commonly cultured bivalves exhibit a wide tolerance to both temperature and salinity, often well beyond conditions they may be exposed to in their natural environment. It should not be assumed when one is dealing with a species normally inhabiting cooler, offshore habitats, that larvae will necessarily show optimum performance within the temperature range to which the wild stock is exposed. Often, larvae grow best at higher temperatures than they would experience in nature. Similarly, tolerance limits of larvae to salinity is often much wider than might be anticipated. For example, calico scallop, *Argopecten gibbus*, larvae from stock adapted to an almost invariable salinity of 36 PSU in Bermuda are able to grow and develop to settlement at 20 PSU. Growth and development are slower, but survival to settlement is little different than in cultures raised at the higher salinity.

The growth of larvae of the Japanese scallop, *Patinopecten yessoensis*, larvae at various salinities and temperatures is shown in Figure 71. Tolerance exhibited to temperature by this species is quite typical of growth rates that apply to other cooler water scallop species including *Placopecten magellanicus* and *Pecten maximus,* which are normally cultured at 14 to 16°C. Growth, development and survival are adversely affected at higher temperatures. Offshore scallops such *as Placopecten magellanicus* and *Pecten maximus* have a higher salinity requirement (>30 PSU). In contrast, larvae of *Argopecten* species, e.g. the calico scallop *(Argopecten gibbus*) and the Bay scallop (*Argopecten irradians concentricus*) can be successfully cultured at temperatures as high as 26 to 28°C.

Oysters of the genus *Crassostrea* are extremely tolerant to both temperature and salinity in the culture environment. Interactions of these two factors on growth are shown in Figure 72. In both the Mangrove oyster, *Crassostrea rhizophorae*, and the Pacific oyster, *Crassostrea gigas* – and the same is true for the American oyster, *Crassostrea virginica* – growth, development and survival are near optimal at 28°C and at a salinity of 25 PSU. Larvae will also tolerate salinities as low as 10 PSU but survival suffers at 5 PSU. Survival exceeded 80% in all treatments over the duration of the trials with the two species.

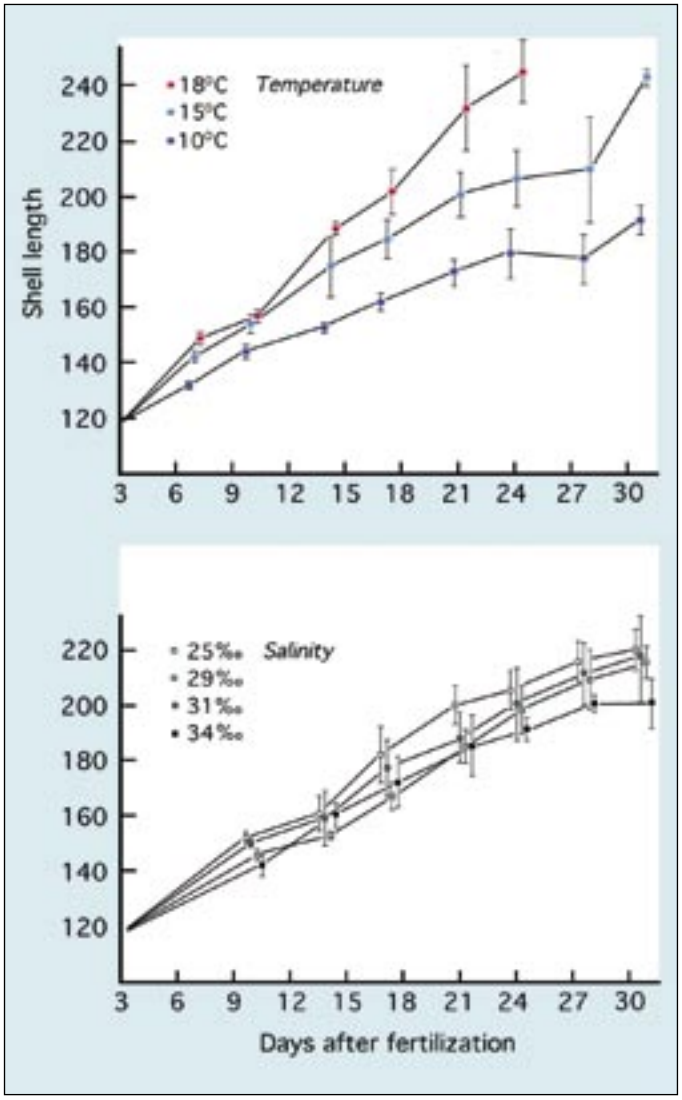


Figure 71: Effects of temperature and salinity on the growth of larvae of the Japanese scallop, *Patinopecten yessoensis*. Larvae were grown at a salinity of 29 PSU in the temperature trial and at 15°C in the salinity trial. From Bourne *et al.* (1989)

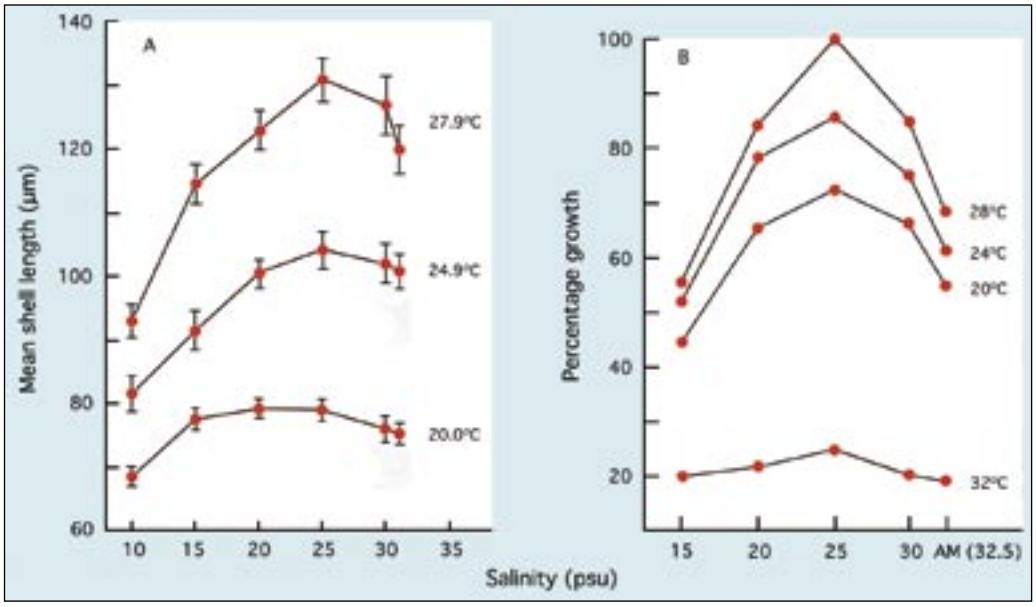


Figure 72: The growth of **A**, Mangrove oyster, *Crassostrea rhizophorae*, in a 7-day period from D-stage (initial mean length 65 µm) and **B**, Pacific oyster, *Crassostrea gigas*, larvae in a 10-day trial at various temperatures and salinities. Pacific oyster results are expressed as a percentage of the growth of larvae in the best treatment (28°C at 25 PSU). AM denotes ambient salinity which was 32.5 PSU in B.

Larvae of the European flat oyster, *Ostrea edulis*, are as tolerant to temperature as the *Crassostrea* species, but they are not as widely tolerant to lower salinities. While they will survive brief exposure to 20 PSU, growth and development rates in culture are near optimal at 28 to 32 PSU.

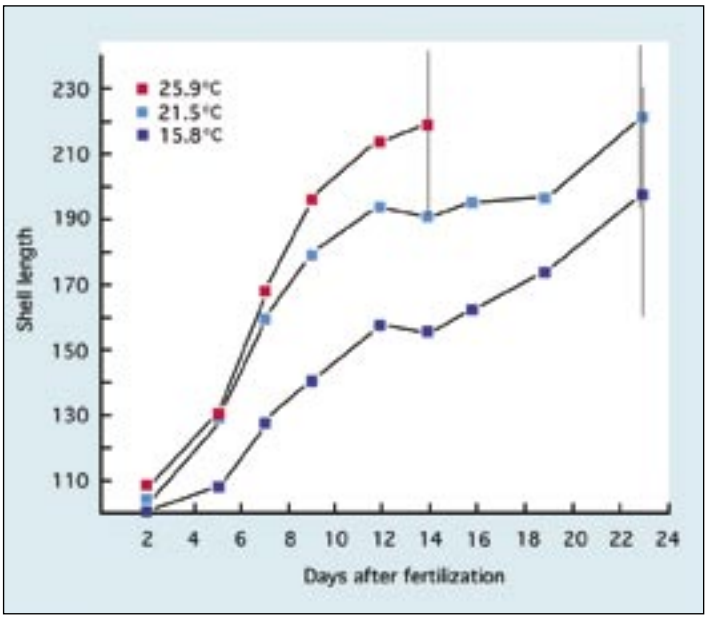


Figure 73: The growth of Manila clam, *Tapes philippinarum*, larvae from D-stage to metamorphosis at 3 temperatures. Vertical bars denote the range of larval shell lengths (μm) when pediveligers were first noted in cultures (A. Lovatelli, MSc thesis).

The growth of larvae of commercially cultivated, inshore and estuarine clams including the Manila clam, *Tapes philippinarum*, American hard shell clam, *Mercenaria mercenaria*, and the soft shell clam, *Mya arenaria*, also show tolerance to wide-ranging temperature and salinity conditions. Except for *Mya arenaria*, which is normally cultured at 18 to 20°C, larvae are generally grown at 25±2°C and at salinities ranging from 25 to 34 PSU. The effect of temperature on the growth of Manila clam larvae is shown in Figure 73.

5.3.3 Seawater quality

It is generally the exception for a hatchery to operate at a constant rate of production throughout the year. Factors of a seasonal nature that cannot easily be controlled may bring about periods in the year when the performance of larvae in terms of growth rate and survival is significantly poorer than at others. In the absence of a technical explanation, such as filter failure or the corrosion of equipment – among other possibilities – or the use of poor quality algal cultures that may have become contaminated, or lapses in husbandry (human error), seawater quality may be responsible.

It has long been established that seawater varies seasonally in its ability to support the growth and survival of embryos and larvae. This may not happen globally, but adverse conditions do occur on both sides of the Atlantic Ocean, particularly when the sea begins to warm in spring and coincident with periods of intense phytoplankton blooming both in spring and in early autumn. The precise reasons for deterioration in seawater quality during these times are not completely understood and they may not recur annually. Some years are better than others in that respect.

By comparing the development of embryos or the growth of larvae week by week in normally treated hatchery seawater and in an artificial seawater control medium using standardized bioassay techniques it is possible to detect and quantify variations in seawater quality. The methodology for bivalve embryo bioassays is detailed in Utting

and Helm (1985). Adaptation can be made at the beaker or bucket scale to determine variability as it effects larval growth and survival. Artificial seawater can be prepared according to various recipes from analytical grade chemicals or be purchased as proprietary brands from laboratory suppliers and from fish hobbyist stores. It must always be prepared in the same way as the constant quality, control medium.

An example of seawater quality variability as it influences development of Pacific oyster embryos in the hatchery is given in Figure 74. The development of fertilized eggs to perfectly formed D-stage larvae is expressed as net treatment mortality (NTM), where

$$NTM = 1-\left\{\frac{\text{Mean yield of D-larvae in 2 ml of normally treated SW}}{\text{Mean yield of D-larvae in 2 ml of artificial SW}}\right\} - 100$$

A net treatment mortality value of 0 indicates as great a number of fertilized eggs surviving to the D-stage in both media and an NTM of 100 denotes a total failure in development in the normally treated seawater. Negative values indicate that the hatchery water was superior to the artificial seawater.

In the early part of the year in northern temperate latitudes of the Atlantic Ocean, when sea temperatures are cool and day length short, seawater quality is relatively stable. As the coastal waters warm and day length increases towards and during spring and early summer, seawater quality becomes increasingly variable. NTM values begin to climb unpredictably and in some years there will be periods when it is difficult to produce D-larvae from apparently good quality eggs – eggs will develop normally in the artificial control medium but not in the hatchery seawater. The phenomenon applies equally to a wide range of bivalve species, not just to the Pacific oyster.

Unstable seawater quality is generally coincident with intense phytoplankton production in coastal waters during spring bloom conditions. There is no direct evidence that it is the metabolites or break down products of the phytoplankton that cause deterioration in water quality. Rather, it may be the bacteria associated with blooms or the metabolites, including the exotoxins, they produce.

A situation of this kind is illustrated in Figure 74 at a hatchery location where the dominant algal species towards the end of the spring bloom in coastal waters was the colonial flagellate, *Phaeocystis pouchetti*. Comparison of the NTM values for the different years shows that the quality of hatchery seawater was at its poorest when numbers of bacteria forming colonies on TCBS agar were at their highest. Bacteria that form colonies on this agar include species of the genus *Vibrio*. They include known opportunistic pathogens, such as *V. anguillarum*, which have frequently been reported as among the more dominant bacteria in hatchery systems at that time of year. They are often implicated in disease situations. *V. anguillarum* is known to produce potent exotoxins including a low molecular weight ciliostatic toxin that inhibits the beating of cilia of the velum in larvae and gills in juveniles.

Seawater quality for embryo development can be improved by various chemical pre-treatments for 24-hour periods before use. This is in addition to the usual filtration and UV disinfection measures. Frequently effective treatments include the addition of 1 mg per l EDTA (ethylenediamine-tetraacetic acid) and 20 mg per l sodium metasilicate ($Na_2SiO_3.9H_2O$) to tanks containing filtered seawater which are then vigorously aerated until required for use 24 hours later. The percentage of eggs that develop to the D-larva stage can often be significantly improved by such treatment. For example, in 28 trials with Pacific oyster embryos, yields of D-larvae from weekly spawnings

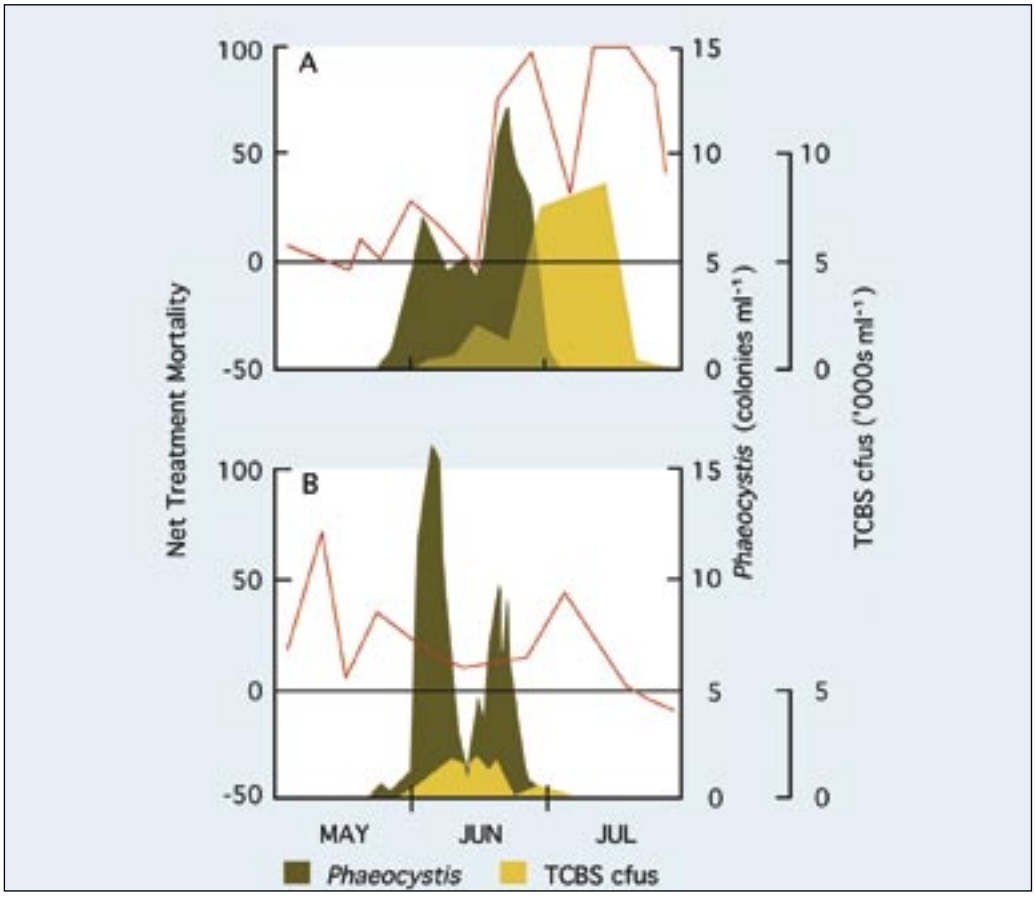


Figure 74: The relative survival (as net treatment mortality – red line) in bioassays comparing development to D-stage larvae of fertilized Pacific oyster eggs in artificial and in normally treated hatchery seawater during the period May to July, 1977 (**A**) and 1978 (**B**). The horizontal black line, equivalent to a net treatment mortality of zero, denotes equality in survival in both the tested seawater and the control medium. The blooming of the colonial flagellate, *Phaeocystis pouchetti*, (as colonies per ml) and the number of bacteria colonies (cfus – colony forming units – as thousands per ml) growing on TCBS agar in samples taken from the adjacent coastal waters are superimposed. Adapted from Utting and Helm (1985) including previously unpublished data.

throughout the hatchery season (March to September) were improved from a mean of 36.6% to 52.9%. The improvement through use of chemical pre-treatment compares with a mean yield of 54.6% in the artificial control medium.

The growth rate of larvae from the D-stage is also influenced by variability in seawater quality in much the same way and for the same reasons as is embryo development. Again, effects on growth have been evident in all species of bivalves tested. Comparative growth of Pacific oyster larvae over a 6-day period from the D-stage when cultured at the beaker scale at 25°C in normal hatchery water and in an artificial seawater preparation (Lyman and Fleming formula, from Sverdrup *et al.* (1942)) is shown in Figure 75. Differences in growth rate are expressed as Growth Index (GI), where:

$$GI = \frac{\text{6-d growth increment (µm) in hatchery seawater}}{\text{6-d growth increment (µm) in the artificial seawater control}}$$

Growth indices >1.0 are indicative of periods when growth was superior in hatchery water; a GI of 1.0 shows parity in performance between the two media and GIs of <1.0 denote times when growth rate was inferior in hatchery water compared with the artificial medium.

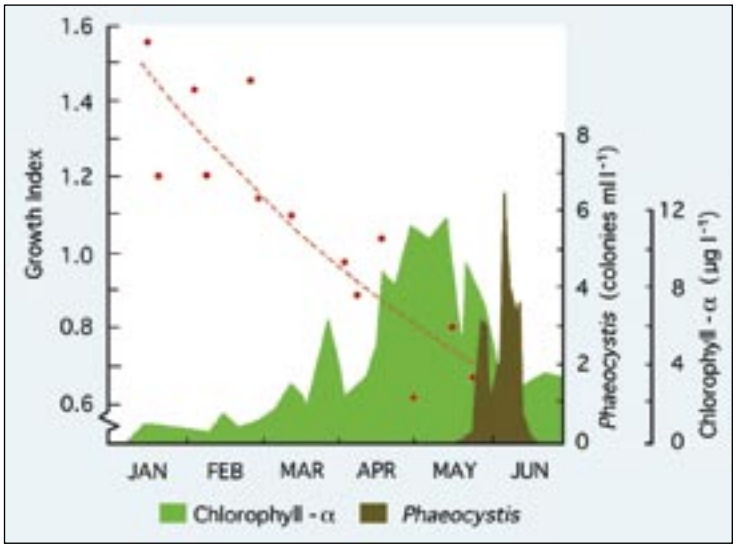


Figure 75: The comparative growth of Pacific oyster larvae over a 6-day period at 25°C in normal hatchery seawater and artificial seawater calculated as a growth index (see text). Chlorophyll–α and the numbers of *Phaeocystis pouchetti* colonies at the hatchery intake are shown as indicators of phytoplankton production in the coastal waters adjacent to the hatchery. (M.M. Helm, unpublished)

Results shown in Figure 75 imply a progressive deterioration in seawater quality from the beginning of the hatchery season in January until trials ended in late May when – for a period of approximately 6 weeks – larvae failed to survive the 6-day experimental period in either medium. Until the end of April, batches of larvae reared for spat production developed normally to settlement in hatchery seawater and provided good yields of spat. Large-scale culture was problematic beyond that time with poorer survival and eventual failure of larvae to reach settlement. The same trend is apparent for European flat oyster, *Ostrea edulis*, larvae where deteriorating performance both in terms of growth (Figure 76) and survival often culminated in disease and the total mortality of broods of larvae in culture in May and June. Again, seawater quality was more variable in some years than in others.

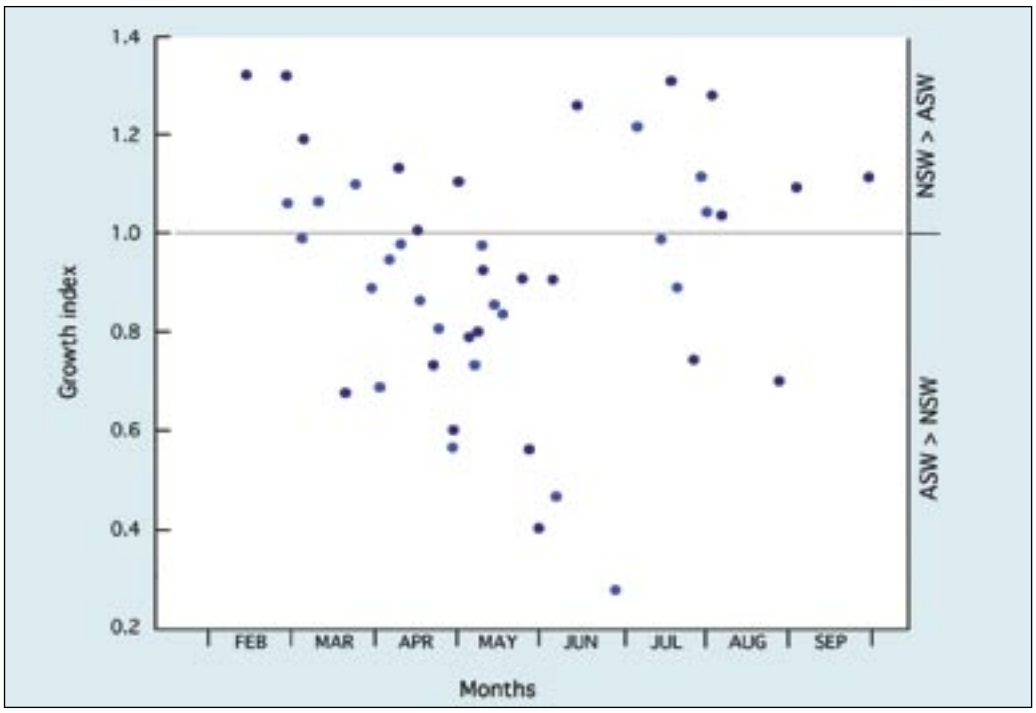


Figure 76: Growth indices of samples of broods of European flat oyster, *Ostrea edulis,* larvae grown at the beaker scale in hatchery and artificial seawater for a 4-day period from the time of release at 24±1°C over a hatchery season. Results cover a 2-year period – differentiated by the shade of the data points. From M.M. Helm (1971) and previously unpublished data.

5.3.4 Egg and larval quality

The quality of eggs in terms of their quantitative and qualitative biochemical composition also has a bearing on subsequent performance of larvae. Studies on this topic have mainly focussed on lipid content and, in particular, on the importance and role of highly unsaturated fatty acids (HUFAs) either donated by the female during oogenesis or mobilised directly from the diet during the period of egg maturation prior to spawning.

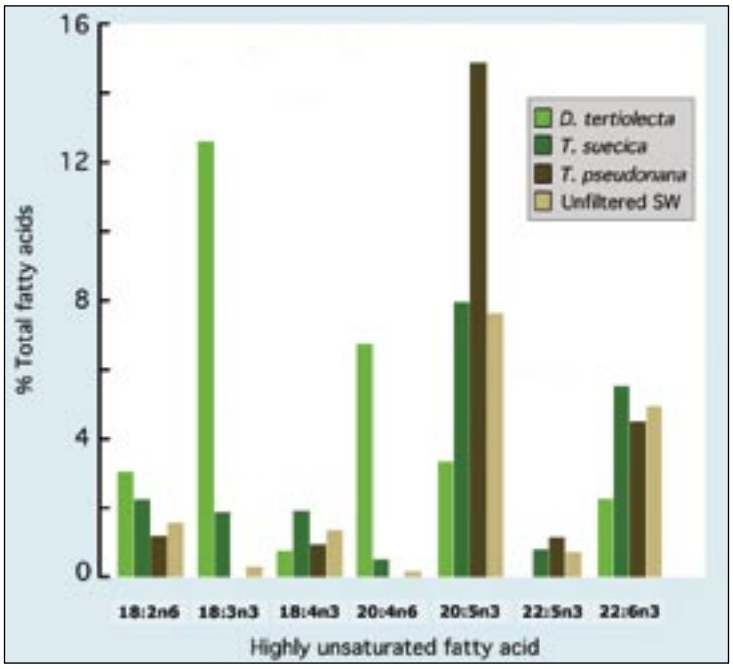


Figure 77: The highly unsaturated fatty acid composition of Manila clam, *Tapes philippinarum*, eggs from a hatchery-held broodstock provided with different diets during conditioning. Control clams were kept in unfiltered seawater while others were supplied a 3% ration of either *Dunaliella tertiolecta, Tetraselmis suecica* or *Thalassiosira pseudonana* in 2μm filtered seawater. (I. Laing, A. R Child and M. M. Helm – previously unpublished data).

The conditions to which females are exposed during oogenesis and egg maturation can have a profound effect both on fecundity and the quality of eggs subsequently spawned. Diet composition and abundance of food are of great importance and apply equally to stocks in their natural habit and in the hatchery broodstock conditioning environment. The HUFA composition of freshly spawned eggs can be significantly altered by the diet provided during conditioning (Figure 77) but there is no evidence that such changes exert a readily discernable effect on the viability and vigour of the larvae developing from the eggs. Neither is there any evidence to suggest that larvae from wild stock European flat oysters are any more or less viable than from a hatchery conditioned stocks although their HUFA profiles may be very different (Figure 78). The differences may be too subtle to be obvious in the context of hatchery culture where efforts are made to provide near optimal conditions for the larvae.

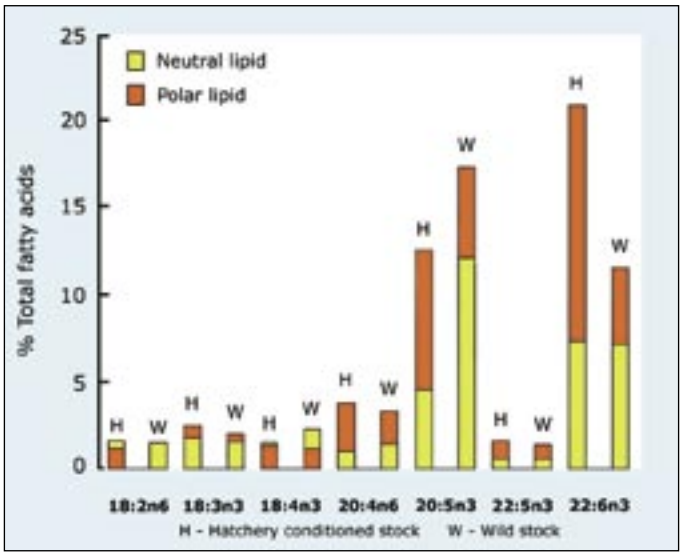


Figure 78: A comparison of the highly unsaturated fatty acid composition of hatchery conditioned and wild stock European flat oyster, *Ostrea edulis*, larvae. Fatty acids are differentiated into neutral (triacylglycerols) and polar (structural) components. Modified from M.M. Helm *et al.* (1991).

What tends to be more important is the total lipid content of newly spawned eggs, or of newly liberated larvae in the case of the European flat oyster. Total lipid as a proportion of the ash-free dry (organic) weight of Pacific oyster eggs is positively correlated with the percentage that develop to the D-larva stage (Figure 79). Even when using a standard broodstock conditioning protocol, lipid content can vary widely according to time of the year and from year to year (Figure 80A). This may be explained by the quantity, diversity and nutritional value of food present in unfiltered seawater supplied to conditioning tanks before cultured algae is added (Figure 80B). It may also help to explain why some years in the hatchery are more productive and suffer fewer disruptions than others.

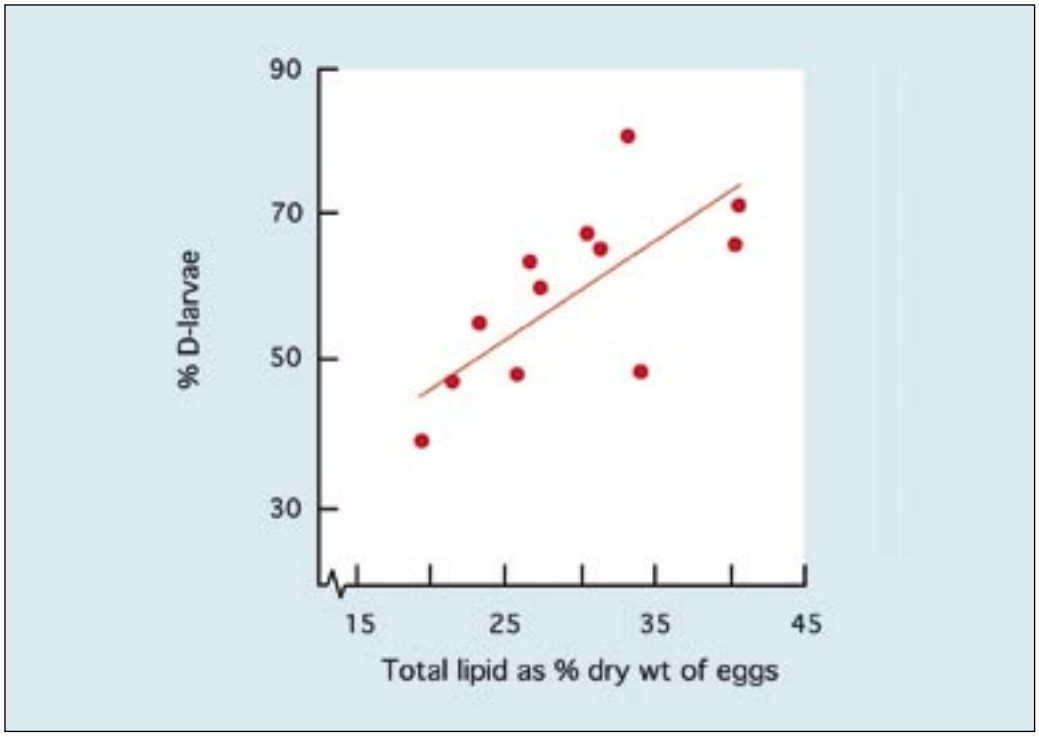


Figure 79: Relationship between total lipid as a percentage of dry weight and the percentage of Pacific oyster, *Crassostrea gigas*, eggs that develop to the D-larva stage. From S.D. Utting and M.M. Helm (1985) and previously unpublished data.

In larviparous oysters, e.g. *Ostrea edulis*, the growth increment of larvae in the 4-day period following liberation from adults is significantly correlated with lipid content at the time of release, suggesting the importance of maternally donated reserves during early larval development (Figure 81). Again there are indications of seasonality and differences between years. However, such effects become less pronounced as larvae continue to develop when diet and ration fed day by day are of overriding influence.

There is close similarity in the relationship between shell length and the ash-free dry weight of larvae amongst most of the bivalve species commonly cultured when they are each grown in near optimum conditions (Figure 82A). The exception among species investigated is the larviparous oyster, *Ostrea edulis*, where there is increasing divergence in shell length related ash-free dry weight in later stage larvae compared with larvae of *Crassostrea* sp. This is explained by the more than 3-fold difference in the rate at which lipid is accumulated as larvae grow towards metamorphosis (Figure 82B). Studies suggest that lipid is much more important as an energy source during metamorphosis in *Ostrea edulis* than it is in the oviparous oysters.

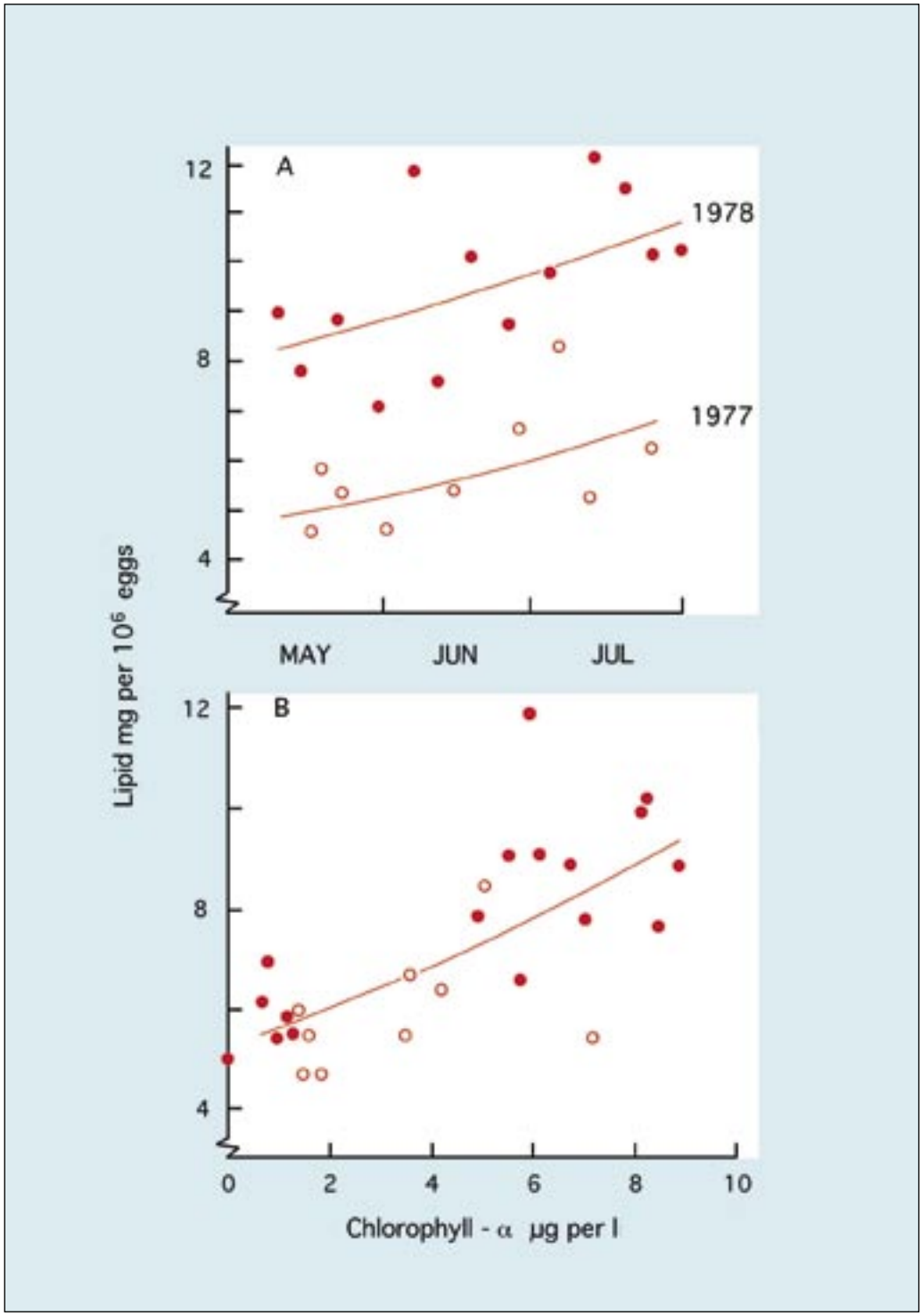


Figure 80: Relationships between the total lipid content of freshly spawned Pacific oyster eggs (expressed as lipid per million eggs) and, (**A**) months of the year in two different years and (**B**), the chlorophyll – α content of unfiltered seawater supplied to broodstock in a hatchery when employing a standard conditioning protocol. From Utting and Helm (1985) and previously unpublished data.

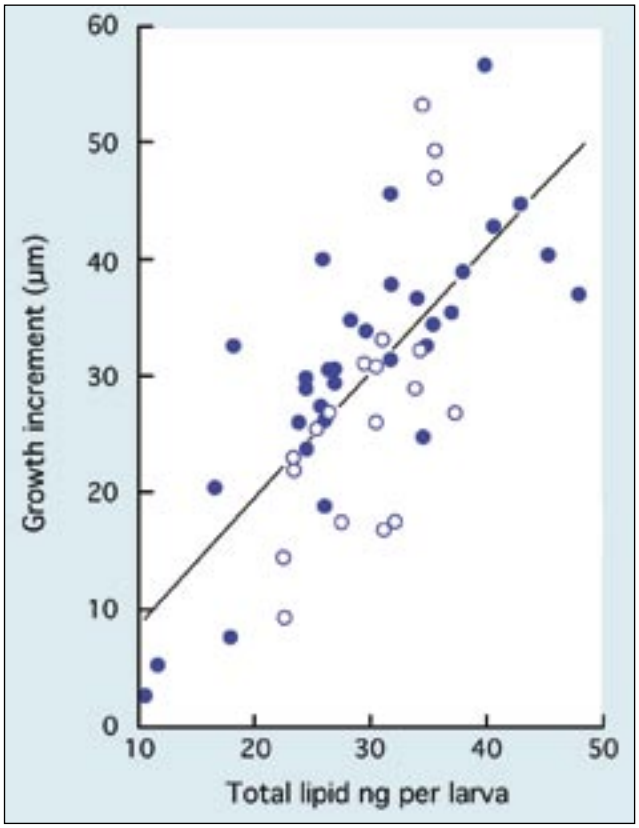


Figure 81: The relationship between the growth increment of *Ostrea edulis* larvae in a 4-day period from liberation and total lipid content at liberation from hatchery conditioned broodstocks. Each data point represents a specific brood of larvae over a 2-year period – each year differentiated according to the shading of the data point. Larvae were cultured on the beaker scale in artificial seawater and provided with the same diet and ration to provide standardized conditions throughout the sequence of trials. From M.M. Helm (1971) and previously unpublished data.

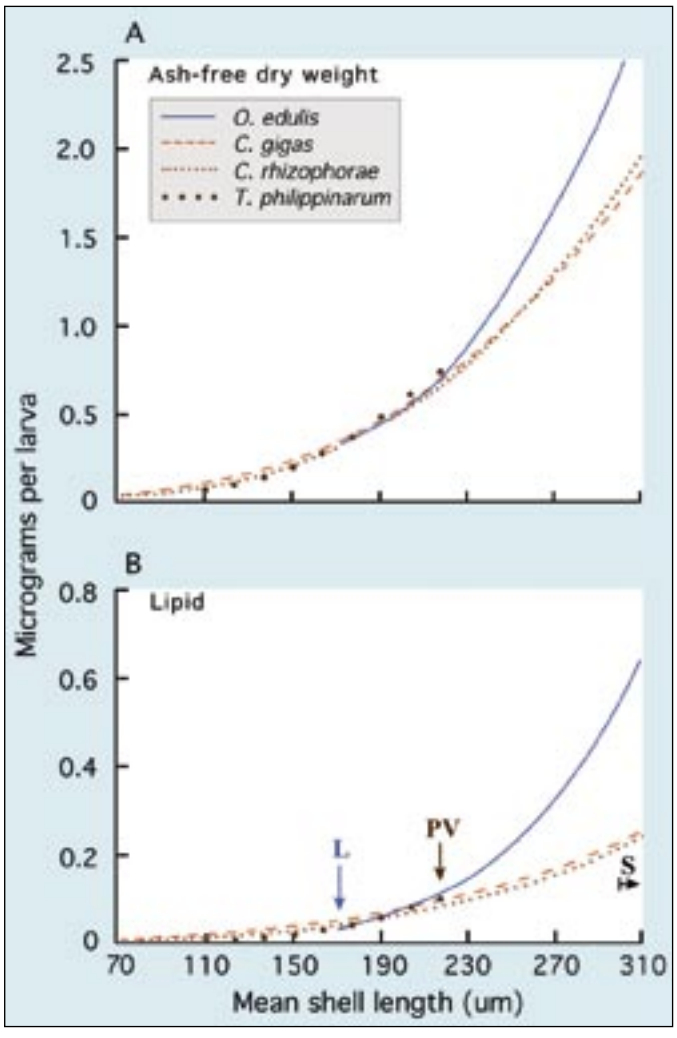


Figure 82: A comparison of increases in both (**A**) ash-free dry (organic) weight and (**B**) lipid content per larva relative to mean shell length in larvae of four bivalve species. **L** – denotes mean size at liberation of *Ostrea edulis* larvae; **PV** – the onset of the pediveliger stage in *Tapes philippinarum* and **S** – the onset of settlement in the three oysters. Source: M.M. Helm – previously unpublished data.

5.3.5 Disease

Mention was made in 5.3.3 of the implication of bacteria of the genus *Vibrio* in mass mortalities of larvae, which occur from time to time in the best run hatcheries. *Vibrio* sp. may not always be the direct cause of abnormal rates of mortality, nor are they the only group of opportunistic or obligate pathogens that can contaminate cultures and present problems. Potentially pathogenic species are present within the hatchery environment year round but are for the most part held in check by being only a minor part of the bacterial flora. At other times of the year, as indicated in 5.3.3, they may proliferate and dominate the microbial flora, posing a serious threat to production.

Before ascribing mass mortalities of larvae to a disease outbreak other potential causes need to be investigated. For example, the cleanliness of pipelines and filters needs to be inspected. Likewise, equipment such as pumps and air blowers that may have corroded or be leaking oil needs to be thoroughly examined. Algal cultures may have become badly contaminated or a technician may have made an error of judgement or miscalculation and grossly overfed a culture, or have forgotten to switch on the air flow to a tank or tanks, or not rinsed a tank after bleach treatment. Only after every avenue has been investigated and discounted should the possibility of disease be considered.

Unlike disease in larval fish, the onset of disease in bivalve larvae is swift and catastrophic. Larvae rarely show protracted symptoms leading to a mass mortality situation. They might appear perfectly normal in terms of colouration and behaviour the night before, but by the next morning be on the bottom of the tank, either dead or moribund with their shells almost devoid of tissue and filled with ciliate protozoans as opportunistic scavengers. Often there is prior warning in terms of larvae not grazing the food to the extent that would normally be expected the day before a mass mortality occurs. This highlights the importance of maintaining thorough records.

Once larvae have dropped to the bottom of a tank there is little the hatchery operator can do other than add a powerful sterilizing agent such as bleach to the tank. Even though a small percentage of the larvae may still be active and appear normal, they will invariably die before they reach metamorphosis if a pathogen is implicated. The objective is to try and contain the disease and eliminate the source of infection. This may mean closing the hatchery for thorough fumigation, ensuring that all equipment is cleaned and sterilized. The hatchery is then allowed to lie fallow for a week or two before production is resumed. The use of antibiotics is inadvisable during such outbreaks. They rarely improve the situation and there is always the risk that the pathogens will gain resistance to them.

Many hatcheries concentrate their production during periods of the year when mass mortalities are unlikely to occur. In temperate regions the most reliable period is winter and early spring, i.e. before the onset of phytoplankton blooming. Late June through to the end of September is often suitable for uninterrupted production.

Further reading on disease in bivalve larvae is to be found at the end of Part 5.

5.4 SETTLEMENT AND METAMORPHOSIS

5.4.1 Introduction

Larvae swim freely in the water column for much of the larval phase (Figure 83A). Typically, they will swim upwards to the water surface and then retract the swimming/feeding organ (the velum), close their shell valves and sink towards the bottom to resume swimming activity again. As they develop towards the end of the larval phase,

feeding activity slows, less food is consumed, and larvae spend an increasing period of time towards the bottom and on the bottom of the tank. This marks the beginning of metamorphosis, a critical stage in development during which extensive mortalities can occur. Considerable anatomical changes take place during metamorphosis. Successful transformation and survival to the juvenile form is dependent on a number of factors, not least of which is the availability of energy reserves accumulated during the larval phase. The importance of producing healthy larvae with large energy reserves cannot be over stressed.

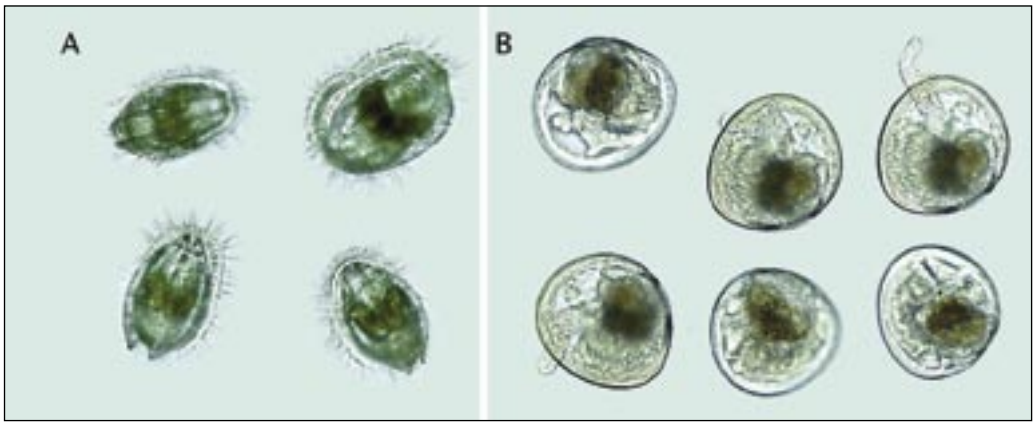


Figure 83: Photomicrographs of (**A**) swimming *Argopecten gibbus* larvae showing the ciliated swimming/feeding organ, the velum, and (**B**) eyed pediveligers of the same species. The foot can be seen extending between the shell valves in three larvae and the small, black eye-spot is visible below the digestive gland, particularly clear in the upper left larva in (B).

Metamorphosis can be divided into two stages, settlement which is reversible (except in oysters) and metamorphosis which is irreversible.

Settlement is the initial stage of metamorphosis. Larvae begin to drop out of the water column onto a substrate, crawl around on the substrate using their foot with the shell upright and search the surface for a suitable place to settle (Figure 83). If the surface is unsuitable they will move off or swim away and seek a more suitable location. This process can be repeated several times and metamorphosis can be delayed for some time if a suitable surface is not found.

Metamorphosis is the second stage and it is irreversible. Factors that trigger it remain unknown but type of substrate along with physical, chemical and biological cues are undoubtedly important. Considerable morphological and physiological changes occur in the animal at this time as it changes from a swimming larva to a spat. Metamorphosis can occur quickly but can be delayed if suitable conditions are not met. In hatcheries it can sometimes be delayed if water temperature is reduced.

5.4.2 Maturation of larvae

Indication that substrate searching behaviour, preparatory to settlement (sometimes termed fixation or setting) and metamorphosis has, or is about to begin, is the appearance – in many species – of a pair of darkly pigmented eye-spot, one on either side between the surface of the digestive gland and the shells valves (Figure 83). What role the eye-spot actually plays is a matter for conjecture. The appearance of the eye-spot is size-related (see later) and coincides with "stringing" or "funneling" behaviour of the larvae *en masse* when they aggregate together with mucous secretions when transferred from screens to buckets at water change (Figure 84). These are clear signs that larvae are ready to set.

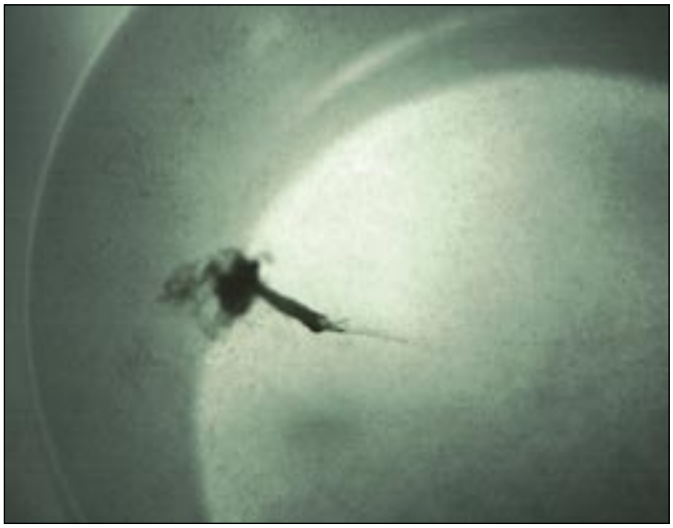


Figure 84: "Stringing" (or "funneling") behaviour of mature larvae prior to settlement. The black mass is numerous larvae aggregated together at and just below the water surface in a bucket.

At this time, or a day or two later, larvae will also be seen probing a newly developed foot between the shell valves (Figure 83B). This foot has a ciliated tip and numerous sensory receptors and is used in substrate searching when larvae are seeking a suitable niche to settle and either form a byssus or cement attachment with the chosen location. The foot provides them with the mobility to crawl across surfaces and may also have a feeding ("pedal feeding") function in some species. It is also the site of byssus or cement glands, depending on species. Oysters form cement attachments with the surface while other bivalves attach with byssus threads. Larvae are referred to as pediveligers at this stage.

There has been considerable controversy as to whether bivalve – and other invertebrate larvae – settle and metamorphose on a rigid schedule or if they select a particular substrate and require a specific cue(s) before the process begins. At present the general belief is that environmental cues influence settlement and metamorphosis and that larvae require specific chemical stimuli before the processes of settlement and metamorphosis are initiated. Studies show these cues are chemicals called neurotransmitters and they must be present to initiate settlement and metamorphosis.

5.4.3 Setting larvae

5.4.3.1 Settlement stimuli

Strategies to facilitate and enhance settlement of pediveligers vary widely among hatcheries according to species and to the methods that will be employed to grow the early juveniles. Hatchery managers want larvae to settle on a convenient substrate (cultch – see 5.4.3.2) and to begin metamorphose as quickly as possible. Studies have shown that several methods, including both physical and chemical stimuli help initiate these processes. The most common physical method used is temperature shock, chilling mature larvae (sometimes in a refrigerator) and then placing them in warm water in setting tanks. Results have been variable but there is an indication that the success rate of metamorphosis can be improved when this method has been used.

A common method to stimulate and increase the success of metamorphosis is the use of chemicals. Several have been tried including ammonia and a group of chemicals known as neurotransmitters including L-DOPA (L-3-4-dihydroxyphenylalanine), epinephrine, norepinephrine and yohimbine.

Many hatchery managers question the use of chemicals to stimulate and increase the success of metamorphosis and they are not used at many hatcheries. Managers believe

that high rates of successful metamorphosis of hatchery produced bivalve larvae can be attained at either a hatchery or a remote setting site if the larvae are of high quality with good food reserves and are handled properly. They believe addition of neurotransmitters may initially yield higher rates of successful metamorphosis than untreated larvae, but little if any difference is observed in the number of juveniles that grow to 5 to10 mm in treated or untreated larvae. Neurotransmitters have permitted some larvae to metamorphose that ordinarily would not have been able to do so but they do not have sufficient reserves to develop further into juveniles.

5.4.3.2 Suitable settlement substrates

The material used to settle larvae on at hatcheries or remote setting facilities is termed cultch and it can be a variety of materials. Two important criteria for cultch are that it must be a suitable surface for larvae to settle on and it must be easily handled.

Oyster hatcheries on the West Coast of North America do not always settle the pediveligers themselves but supply growers with eyed larvae to be set remotely at locations adjacent to the oyster farms (Figure 85). This methodology is dealt with in Part 6.2

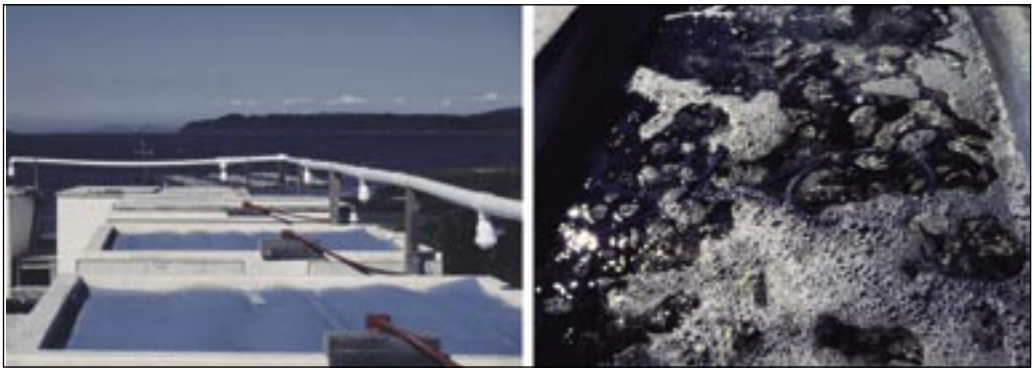


Figure 85: A remote, oyster setting system located on Vancouver Island, British Columbia, Canada. Eyed Pacific oyster, *Crassostrea gigas*, larvae are received from West Coast hatcheries and are set in concrete tanks packed with net bags filled with clean, aged Pacific oyster shells. Once the shells have a sufficient set – a few days later – they are moved to nursery growout at the farm.

The following is a synopsis of the more common methods employed to set mature, eyed larvae of the various bivalve groups.

(i) Oysters

Surfaces are provided for settlement either in the tanks in which the larvae are grown – directly in the broodstock tanks in the special case of *Tiostrea* larvae – or in special-purpose settlement tanks. This is when 50% or more of the larvae are at the eyed stage and applies equally to *Ostrea* and *Crassostrea* species. Hatcheries will often grade out the largest larvae in a batch on a 240 μm mesh (retaining larvae of 300 to 340 μm shell length) for settlement, leaving the remainder to grow and develop further. Appropriate densities of oyster larvae per unit volume at settlement are within the range 2 000 to 5 000 per l although the area of settlement surface is the more important criterion. Types of materials in common usage to provide large surface areas for settlement include the following:

a) Sheets of slightly roughened PVC, which may be vertically stacked in the water column with each sheet separated by a spacer, or be a single sheet lying on the base of the tank. (PVC sheet formed in the shape of semi-cylindrical roof tiles are also sometimes used).

b) Layers of shell chips and particles prepared by grinding aged, clean oyster shell spread over the base of settlement trays or tanks. The particulate material is graded so that only pieces that pass through a 500 μm but are retained by a 250 μm screen are used.

c) Bundles, bags or strings of aged clean oyster shells dispersed throughout the water column, usually in settlement tanks.

d) Various plastic or ceramic materials coated with cement (lime/mortar mix). For example, stacks of cement-coated, plastic Chinese "hat" collectors are sometimes used to settle oyster spat in large tanks. Once grown to a suitable size spat can be removed by bending and flexing the collectors to break apart the cement coating.

Figure 86: A and **B** – In this example, matt surfaced PVC sheets used as settlement substrate for oyster spat are placed on the base of larval culture tanks (A). The tanks are illuminated by overhead, tungsten filament lamps to aid rapid settlement. Spat collectors are checked several times each day (B) and when the set density is sufficient, the newly settled spat are gently removed with a razor blade. **C** and **D** – Staff at a Cuban oyster hatchery stringing mangrove oyster shells on lengths of nylon twine (C). These strings are placed in concrete settlement tanks with sufficient eyed larvae to provide the required set density (D). Large-volume larval culture tanks can be seen behind the settlement tanks in photograph D.

Larvae tend to settle and attach more prolifically on the shaded under surface of substrate materials in shallow tanks. A low intensity tungsten filament lamp (60W), mounted above deeper tanks, will also encourage larvae to settle towards the bottom

in the more shaded areas (Figure 86A and B). The attractiveness of large surface area collectors can be enhanced by painting them with an aqueous extract of homogenized oyster flesh. They are then allowed to air-dry before being placed in the settlement tanks. The reason is that larvae exhibit gregarious behaviour and will tend to attach where others have attached before. PVC collectors improve in their ability to attract settlement with usage over time. When sufficiently "aged" they do not require coating with the aqueous extract.

Methods (a) and (b) above are used to produce what are known as "cultchless" spat. Cultchless oyster spat (spat that are no longer attached to a substrate or are attached to a shell particle) can then be grown as separate individuals through to marketable size to service the half-shell trade. In contrast, survivors of those set on whole shells will eventually grow together, their shells fuse and form clusters, and are suitable only for meat extraction when harvested.

To provide cultchless spat when using PVC collector sheets, the newly set spat need to be removed from the surfaces with a razor blade within 24 hours of attachment. This is done by immersing the sheet in a shallow tray of seawater and gently scrapping the razor blade, mounted in a suitable holder, across the surface while spraying the blade with a jet of seawater. The number of removed spat can be estimated using the same method as for larvae (section 5.1.2.3). They are then moved to the within hatchery, early juvenile culture system.

As previously mentioned, eyed oyster larvae can be encouraged to undergo metamorphosis without cementing themselves to a substrate by the use of the neuro-transmitter, epinephrine. This involves dissolving 0.1832 g of epinephrine (adrenalin) in a little 10% hydrochloric acid and then diluting it in 10 l of filtered seawater, which is a sufficient volume to treat 2 million eyed larvae. Larvae of settlement size are exposed to this treatment for 60-90 minutes and are then returned to the culture tanks. At the next water change, larvae that have metamorphosed and have begun growth as spat are graded from those that are still larvae by retaining them on a 270 μm mesh sieve. Only larvae ready for imminent settlement will respond to this treatment and will complete metamorphosis without fixation. Larvae that do not respond are unharmed and can be treated again one or two days later. This method of treatment can be used with or without the provision of settlement surfaces (usually with).

Post-settlement survival rates in oysters are generally high with 50–70% of those that set reaching 2 mm shell length.

(ii) Scallops

In contrast to oyster larvae, eyed scallop pediveligers form a byssus attachment to surfaces upon which they settle. They will attach to filamentous red algae, hydrozoans, bryozoans and polychaete tubes in nature, amongst other suitable living and non-living substrates. Polyethylene netting, nylon mesh and a variety of other similarly filamentous materials provide satisfactory substitutes in the hatchery. Eyed pediveligers can be set in larval tanks or in purpose-provided settlement tanks, either in static water conditions or in flow-through. In the latter, a mesh screen on the out-flow is essential to retain larvae within the tank. Since scallop larvae, pediveligers and early juveniles are particularly fragile and delicate the tendency is to remove them from the larvae tanks when eyed and to transfer them to settlement tanks. This is at a considerably smaller shell length than for oyster larvae – 220 to 240 μm compared with 300 to 340 μm. Examples of suitable types of settlement tank and collector materials are shown in Figures 87 and 88.

Figure 87: Scallop pediveligers can be set at a density of up to 2 000 per l in cultch filled tanks equipped for static water, recirculation or flow-through. The system illustrated is at the Bermuda Biological Station of Research, Inc. and is used for both *Argopecten gibbus* and *Pecten ziczac*. See text for an explanation of the steps involved.

Scallop pediveligers can be set at densities of between 1 000 and 2 000 per l in cultch-filled tanks equipped for static water, recirculation or flow-through. The example shown in Figure 87 utilizes circular, fibreglass fish tanks of 450 l volume (**A**) fitted with bottom drains and standpipes. Bundles of plastic mesh netting (**B**) are loosely packed in the tank(s) (**C**) or are enclosed in fine mesh "onion" bags suspended in the water column (**D**). Spat set mainly on the black mesh (**E**). The plastic pipe arrangement above the water surface, clearly visible in D, is part of an air-lift driven upwelling system. Each vertical limb has an air-line fitted at the base. With the air flow turned on, water is lifted from the base of the tank to be sprayed from drilled holes in the above-water delivery pipes and back into the tank. In operation the water level in the tank half covers the delivery pipes.

Settlement tanks are treated as larval culture tanks for the first 6 to 8 days once pediveligers have been added. Water is changed 3 times during this period by draining water through a sieve to retain the remaining swimming larvae (note the drain valves

visible in Figure 87A). At the same time, filtered seawater is added at a rate to balance with the out-flow in order to maintain the water level constant, which prevents the cultch and attached larvae from being exposed to air. This water exchange is continued for 30 to 45 minutes. Numbers of larvae retained on the sieve, their survival and the numbers of metamorphosed but unattached spat are estimated before returning them to the tank. Tanks are gently aerated during this period and are supplied with food in exactly the same way as in larval culture.

After this first week, the air-lift driven upwelling system is switched on and the temperature of the water in the tank is gradually lowered over a period of days to the ambient. The tanks are then operated on flow-through by turning on a continuous and sufficient in-flow of ambient temperature seawater to exchange the tank volume 3 or 4 times each day. The air-lift driven upwelling is maintained and food is added continuously. Three weeks after introduction of the pediveligers the largest attached spat measure 2 mm shell height (Figure 87E).

In essence, the process described above is a hatchery adaptation of the widespread use of plastic mesh contained in "onion" bags to capture natural spat in the sea. A different approach is to set pediveligers in trays or cylinders with suitable aperture mesh bases (120 or 150 μm aperture). The trays are held in shallow tanks through which food supplemented water is recirculated or flows continuously to waste (Figure 88).

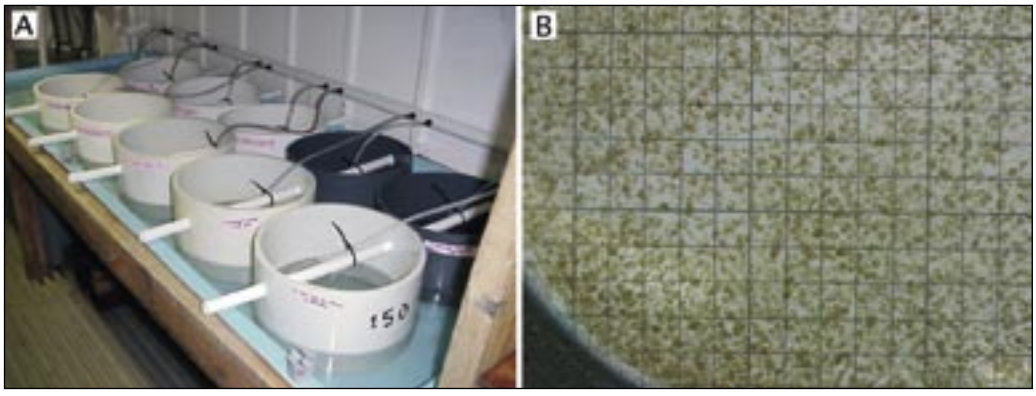


Figure 88: A – cylindrical, nylon mesh based trays used to set scallop pediveligers at the Bermuda Biological Station for Research, Inc. The trays are partially immersed in shallow raceway tanks through which seawater can either be recirculated or flow to waste. Each tray receives a downwelling flow of cultured food supplemented seawater. **B** – the appearance of 3-week old *Argopecten gibbus* spat growing attached to the mesh base of the tray. A grid marked in 1 cm squares has been placed beneath the mesh to indicate the density of set and enables estimates of numbers to be determined.

Pediveligers are stocked in the trays at a density no greater than 100 per cm^2 of the base area. For example, a 25 cm internal diameter cylinder has a bottom mesh area of approximately 500 cm^2 and can be stocked with up to 50 000 pediveligers. Space availability for settlement of the pediveligers and for the growth of those that attach and metamorphose to juveniles is critical in determining stock density. Spat are mobile and will respond to overcrowding by detaching their byssus attachment and swimming in search of a less crowded area to re-attach. Soft tissue damage resulting in mortality will occur if spat collide with their neighbours and interlock their shell valves.

Various adaptations of the concept of setting scallop pediveligers in shallow, mesh-based trays are used in Europe for *Pecten maximus*.

Post-settlement survival rates in scallops are usually not very high; 15 to 30% from the initial number of pediveligers to 2 mm shell height is considered normal. Survival tends to be greater using the set in trays method (Figure 88) but growth rate is superior when using bundles or bags of mesh (Figure 87). This is probably because spatial separation of the attached spat is much improved over the large surface area provided throughout the volume of the tank in the latter case.

(iii) Clams and mussels

Clam larvae begin substrate search behaviour at a similar shell length to scallop larvae (at 220 to 240 μm). They also attach to surfaces and to each other, with byssus threads. A convenient way to handle them at this stage is to transfer them to a settlement tank, such as in the example shown in Figure 88, until metamorphosis is complete. Otherwise, they can remain in the larval tanks until settlement is complete. Since they are of similar size and behaviour to scallop pediveligers, similar densities can be set per unit area of the trays. Although adult clams bury themselves in the substrate in nature there is no need to provide substrate until the spat exceed 7 mm shell length. Settled spat can be removed from surfaces with a water jet.

Mussels also attach by means of byssus threads, but more strongly than scallops and clams and they retain their ability to form such attachments throughout their life. Because of their low per unit value compared with oysters, scallops and most of the commercial clams, hatchery culture is less common. Mussel spat are usually collected in nature although interest in hatchery production is now being shown on the West Coast of the USA and in New Zealand. Panels or coils of the same materials used to catch wild spat can be used in the hatchery, including rope, netting and panels of plastic mesh. The type of system with deeper tanks shown in Figure 87 is equally as appropriate for the settlement of mussel larvae as it is for scallops.

How spat are grown once they have settled is dealt with in the next section.

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Part 6

Hatchery operation: culture of spat in remote setting site, in the hatchery and in nurseries

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6.1 INTRODUCTION

The word "spat" is an old English term applied to the early juvenile stage of bivalve development and is perhaps the most commonly used term applied to juveniles in hatcheries. It relates to bivalve larvae that have set and undergone metamorphosis.

Another frequently used term for early juveniles is "seed" and this word is used to describe juvenile products supplied by hatcheries to shellfish farmers.

The extent to which hatcheries are involved in growing spat beyond pediveliger stage larvae varies considerably and relates to the preferences of the growout industry. Provision of eyed, pediveliger Pacific oyster larvae for remote setting at oyster farms is common practice on the Pacific coast of North America. Hatcheries provide the mature larvae and the farmers themselves set them and grow the spat for seeding oyster beds or in suspended culture. Details of the methodology are given in 6.2.

In other parts of the world hatcheries set the larvae and grow the spat to a size that growers are comfortable to handle and grow. This may be when spat are 1 to 2 mm in shell length or often larger. The size that spat is supplied is largely dictated by the requirements and maturity of the growout industry. Hatcheries would prefer to deliver them at the smallest size possible because the economic implications of growing them further within closely controlled conditions are significant. It takes only a relatively small tank volume and a comparatively small quantity of algae to grow larvae and set a million spat but once they are set costs associated with growing them escalate rapidly.

Consider the requirements of 1 million oyster spat. At 1 mm shell length, individual live weight (shell and body) is approximately 0.3 mg. Clam and scallop spat are about 30% lighter than oyster spat for a given shell length within the size range grown in hatcheries. The biomass (total live weight) of one million oyster spat is therefore 0.3 kg. Growth rate of spat in closed seawater systems (systems without continuous water exchange) is biomass dependent. To ensure commercially acceptable (not maximum) growth rates, spat need to be grown at a maximum of 200 g live weight biomass per 1 000 l (0.2 kg per m^3). This is the biomass at the beginning of a weekly period irrespective of the size spat are and allows for significant growth during the course of the week. Biomass is reduced at the end of a weekly period by distributing the spat at 0.2 kg per m^3 in a greater tank volume – either more tanks of the same size or larger tank systems.

Growth rate decreases significantly as stock density per unit volume increases. At 0.4 kg per m^3, for example, newly metamorphosed Manila clam spat will grow to only about 0.5 mm in a 6-week period compared to a mean shell length of 1.4 mm at 0.2 kg per m^3. This is at the same temperature and with food ration calculated on the basis of biomass (section 6.4). It is not important to know the numbers of spat at this stage. Total live weight biomass is the criterion upon which food ration is based, i.e. the weight of shell, body and water contained between the shells. Section 6.3.5. describes protocols for grading and estimating seed.

Returning to the example, one million 0.3 mg – 300 g in total – oyster spat will need a minimum culture tank volume of treated and heated seawater of 1 500 l. By the time they reach 5 mm shell length individual live weight has risen to approximately 32 mg. The biomass of one million 32 mg spat has increased to 32 kg and the volume of treated and heated water required to grow them is now 160 000 l (Table 14). Food requirements increase proportionately (section 6.4). For example, 1 million 0.3 mg spat require 17 g dry weight of algae per day, which is equivalent to 85 700 million cells of *Tetraselmis suecica*, or 85.7 l of harvested culture at 1 million cells per ml. At 5 mm shell length, food requirement for the same number of spat has risen to 9 130 l *Tetraselmis* at the same harvest cell density (Table 14). The 4 mm increase in shell length is associated with more than a 100-fold increase in biomass and the same increase in food is required. Clearly, there is a limit to the size hatcheries can grow the spat in terms of

spatial requirements to accommodate them, the need to treat and heat seawater and the volumes of food required to feed them.

Table 14: Tank water volume and daily food requirements for bivalve spat of different sizes when grown at a biomass of 200 g live weight per 1 000 l (0.2 kg per m^3). Data are for oysters but relate to other bivalves where clam and scallop spat are approximately 70% of the weight of oyster spat for a given shell length.

Length (mm)	Weight (mg per spat)	Number per 200 g	Tank volume (l) per million spat	Daily food (l* per million spat)
0.3	0.01	2.0×10^7	50	2.9
0.5	0.07	2.9×10^6	350	20.0
1.0	0.30	666 700	1 500	85.7
2.0	2.2	90 900	11 000	628.5
3.0	7.0	28 700	34 840	1 999.0
4.0	17.0	11 765	85 000	4 856.0
5.0	32.0	6 270	160 000	9 130.0

*Daily food requirement calculated as l of *Tetraselmis* at 1×10^6 cells per ml

Various solutions and approaches are adopted to overcome cost limitations to growing spat within the hatchery. These are described in section 6.3. Most commonly, spat are grown in closely controlled conditions to a size at which they will be retained by either a 1 or 1.5 mm mesh screen at 2 to 3 mm shell length. They are then transferred to outdoor nursery systems, which may be part of the hatchery operation or belong to a farmer or group of farmers. Or such nurseries may be part of a vertically integrated company operating a hatchery and producing seed for its own growout requirements. Outdoor nurseries are designed to protect small spat from predators while growing them at high density to a size at which they can be transferred to sea-based growout. Key features of outdoor nurseries are that they operate on the flow-through principle, utilizing natural phytoplankton productivity to provide the food supply (section 6.6). They may be land or sea-based and if they are located on land the source of seawater may be from artificially dug or natural ponds that can be emptied and re-filled from the sea. Measures are often taken to enhance algal productivity of the ponds by the application of fertilizers (see 3.4.6).

The following section deals with the special case of procedures for setting mature larvae at remote sites and growing them from the time they set to the time they begin growout to market size. Subsequent sections follow the various methods in common usage to grow recently set spat to suitable sizes within a hatchery until they are sold directly to farmers or transferred to land- or sea-based nursery systems.

6.2 REMOTE SETTING

The technique by which eyed larvae are supplied by hatcheries to farmers who set and grow them on the Pacific coast of North America is described here. This is a special case and its use is commercially confined to the Pacific oyster, *Crassostrea gigas*, although it is equally as applicable to other oyster species in other parts of the world.

6.2.1 Background

On the Pacific coast of North America most oyster production is from intertidal bottom culture and more recently floating culture. Originally juvenile oysters were imported annually from Japan and spread on a grower's lease for growout. Juvenile oysters were set on bivalve shell, usually old scallop shell, but this supply of seed ended

when it became too expensive. Breeding areas were located along the Pacific coast and used to augment seed imported from Japan and eventually to replace it. Oyster larvae were generally set on bivalve shell, mostly old oyster shell, and allowed to grow on the shell in breeding areas until the juveniles attained a shell length of about 1 cm at which time the cultch with attached juvenile oysters was transported to a grower's facility. In intertidal bottom culture the seed was spread either directly in growout areas or held on seed ground for upwards of a year and then spread in growout areas. In floating culture the cultch with juveniles could be strung on wires or ropes and suspended from floats or longlines. The method was generally effective for reliably supplying growers with their seed requirements, but there were disadvantages with the system. The main disadvantage was that failures or insufficient breeding occurred in breeding areas in some years. Consequently growers did not have sufficient seed for growout operations. Cost was another problem. Shell is bulky and heavy and it was expensive to move large quantities of juveniles attached to oyster shell. Another disadvantage was that the seed could generally only be moved during the cooler, wetter months, October and November, and this was frequently inconvenient for a grower who wanted the seed at other times, particularly during spring and early summer. It was also impossible to select for a particular strain or race of oyster in natural breeding areas.

Studies showed that mature Pacific oyster larvae with well developed eye spots could be held out of water in a damp but cool condition (5-10°C) for upwards of a week. Thus it became possible to ship mature Pacific oyster larvae considerable distances, literally anywhere in the world. A grower could purchase mature oyster larvae from a hatchery whenever it was convenient, have them shipped to his/her facility and set them on the preferred type of cultch used in their growout operation. The disadvantages of previous techniques including reliability of a seed supply, cost of handling bulky cultch with attached juveniles and not being able to obtain seed when desired, could be averted. Further, a grower did not have the expense nor time consuming effort to build and operate a bivalve hatchery. The method, now widely used by growers along the North American Pacific coast, provides a convenient and efficient way to ensure a reliable and plentiful supply of oyster seed for culture operations.

6.2.2 Preparing larvae for shipment

The method, developed in the 1980's, has been refined over the years and is straightforward. It provides good results if proper procedures are followed. Oyster larvae are produced in a hatchery and a grower will make arrangements with the hatchery to deliver the amount of larvae required at his/her facility at convenient times. Larvae are filtered on screens at the hatchery and placed in a piece of nylon mesh to form a bundle that is kept damp, a bundle about 5 cm in diameter contains about 2 million mature oyster larvae (Figure 89). The bundle of larvae is placed in a chilled styrofoam container with ice packs to maintain a temperature of 5-10°C. The container with larvae is then shipped to the grower.

Figure 89: Receiving a consignment of eyed Pacific oyster larvae wrapped in nylon mesh at a remote setting site in British Columbia, Canada.

6.2.3 Preparations at the remote site

An important consideration for a grower is site selection for the remote setting operation. Of primary concern is water quality and the criteria used in selecting a site for a hatchery apply equally to a remote setting operation. Areas with known sources of pollution need to be avoided. Salinity must be within an accepted range (greater than 20 PSU for Pacific oysters), the water should be well oxygenated and the temperature close to 20°C or above during summer months to eliminate the need to heat water. Water should be pumped from at least 2 m below the surface to reduce variations in salinity in areas of high rainfall. The water should be phytoplankton rich so that it can be used as a source of food for juveniles and reduce the need for adding food. The site must have electrical power, sufficient space for tanks and other parts of the operation, good communications so that larvae can be readily received, and the site should be close to the intertidal beach area where juveniles will be transferred and held after they are removed from setting tanks.

Tanks are constructed at a grower facility for setting the larvae. There are no set dimensions for the tanks, it depends partly on the type of cultch used, size of the operations, methods used to handle juveniles and individual preference (Figures 85 and 90). Cultch used on the Pacific coast is either old bivalve shell – mostly oyster shell – or grooved plastic pipes of about 2 cm in diameter. Bivalve shell is placed in plastic mesh ("vexar") bags that are 1 to 2 m in length and 50-70 cm in diameter. Each bag holds 100 to 200 pieces of shell. Grooved plastic pipe is usually cut in 2 m lengths. Smaller tanks may measure 1.5 x 2.5 x 2.5 m in size but may be much larger and hold 40 000 l.

Figure 90: Setting tanks at a site in British Columbia, Canada. Note the loose cultch and vexar bags filled with cultch stacked on the bank behind the tanks. Refer also to Fig. 85, Part 5.4.3.2.

The tanks can be constructed of a variety of materials, including concrete, fibreglass, or fibreglass coated wood. Regardless of the material used, tanks should be well leached before use. In temperate areas the walls of fibreglass tanks are generally insulated with styrofoam to help maintain water temperature. In some instances the tanks are also fitted with a lid to further improve insulation. A 2-cm plastic pipe with holes drilled at regular intervals is placed around the inner circumference of the bottom of the tank and serves as an air line. The water may need to be heated at certain times of the year

in temperate regions. A hot water line can be run from the main facility or individual electrical heaters placed in each tank. Tanks should be constructed so they can be readily cleaned and be fitted with drain valves.

The first step when a remote set is planned is to add the cultch to the tanks so that they are as completely filled with settlement material as possible. Vexar bags with bivalve shell are stacked one on top of the other or plastic pipes bundled together in modules. The cultch, whether plastic pipes or old bivalve shell, is generally not conditioned in seawater for a sufficient period of time to acquire a biofilm. Plastic pipes are well leached before use. Shell is generally air dried and exposed to the elements for at least six months before use and then it is washed so that the surfaces are clean.

The amount of cultch required depends on size of the tanks. Generally, between 16 and 20 vexar bags of cultch will occupy about 1 m^3. The tanks are filled with seawater filtered to about 50 μm either through a sand filter or by individual filter bags on each of the tanks. Seawater is heated to the desired temperature which is generally between 20 and 25°C for Pacific oysters.

6.2.4 Receiving the eyed larvae

Mature larvae are shipped from the hatchery to the remote setting site. Two million mature Pacific oyster larvae form a ball about 5 cm in diameter when wrapped in mesh (Figure 89). Once received, they are placed in a plastic bucket with 10 l of water at 20 to 25°C and allowed to acclimate for fifteen to thirty minutes. The contents of the bucket are then poured into the tank. The number of larvae added per tank depends on size of the tank and the amount of cultch but as a "rule of thumb" 1 300 to 2 200 larvae are added per 2 m length of plastic pipe and about 100 larvae are added per piece of shell cultch. Air is turned on for about thirty minutes to ensure thorough mixing of larvae in the tank and is then turned off to allow the larvae to attach to the cultch. If pipe cultch is used, only half the larvae may be added originally and after one day the pipe modules are flipped and the remainder of the larvae are added. This helps create an even set on all surfaces of the pipe.

6.2.5 Setting the larvae and growing the spat

Oyster larvae attach to the cultch and metamorphose into spat usually within 24 hours from the time larvae were added to the tanks. Some setting can occur on the bottom and lower part of the sides of the tank but this can be avoided by painting these parts of the tank with liquefied wax (paraffin). Loose shell can also be scattered over the bottom of the tank to catch larvae that may settle there.

Once larvae have metamorphosed to spat they must be fed. When remote setting began, hatcheries supplying eyed larvae often also supplied algal paste for use as food. The algal paste was algae grown in a hatchery and centrifuged down to form a disc of concentrated algae about 12 cm in diameter and 3 cm in thickness. A portion of the paste was broken off, placed in a bucket with seawater, stirred briskly to break up the clumps and then added to the tanks. Air was turned on to ensure adequate mixing of the food in the tanks. Species used to make the algal paste were the same as those grown in the hatchery to rear larvae. Algal paste is still used by some growers but it is not as common as was the case previously. Most hatcheries now require their entire algal production for their own use and have none to ship to remote setting sites. There are companies that grow algae for sale as a concentrated slurry and this can be used as food. Many growers now culture their own algal food using standard methods as described previously. Species used vary from site to site but are the same as those used in hatcheries to feed larvae.

Water in the tanks is not exchanged for the first two or three days after setting but after that a slow flow-through of coarsely filtered seawater is begun. The objective is to acclimate the spat to local environmental conditions and also provide additional natural food. If algal food is added to the tanks, the water flow from the open environment is turned off for a short period so that as little as possible of the added food is lost.

The length of time spat are held in the tanks is variable. In early spring and late fall it may be upwards of a month but in summer if can be as brief as one week. It also depends on the schedule used at a grower's facility as the following example illustrates.

Example:

The grower has 18 tanks.

a) Larvae are added to each of six tanks at the beginning of the week,

b) Another six tanks hold spat from larvae received last week. They are being acclimated ready to transfer to growout at the end of the week.

c) The remaining six tanks are being cleaned and prepared for the next batch of larvae which will arrive at the beginning of the next week.

d) Thus, six tanks of cultch with attached oyster spat are being produced regularly each week. (Spat are kept in the tanks for a minimum period since it is costly to feed them with artificially produced food).

The spat are usually 2 to 3 mm in size when they are transferred to growout. Bags of cultch with spat are placed in the mid- to lower intertidal zone on pallets to keep the cultch out of the substrate and reduce mortalities. In summer, transfer from the tanks to growout generally occurs in early morning or late evening when temperatures are lower. The time taken for the transfer should be kept to a minimum to reduce stress and mortalities. Bags can be stacked to a height of 2-3 m, depending on tidal range. Tarpaulin covers are placed over the bags to keep the spat out of direct sunlight and to reduce settlement of fouling organisms. Bags with spat are left in the intertidal area for varying periods of time and then the cultch with spat is spread either on good growing ground or is strung on ropes or wires for floating culture.

As with hatchery operations, it is important that growers keep accurate records of each set. With acquired experience they can determine optimum conditions to maximise the production of seed from larvae.

The remote setting concept was developed and perfected as a relatively inexpensive way to produce Pacific oyster seed but it could be used for clams, scallops and mussels. To date it has not been used widely for species that do not attach firmly to cultch as do the oysters.

The technology has opened new opportunities for bivalve culture worldwide. If a grower wishes to culture a species of bivalve and cannot obtain sufficient seed from local natural sources or prefers to use hatchery seed, he no longer needs to build an expensive hatchery. Arrangements can be made to produce larvae at any hatchery and ship them to the grower's site. It is important to realize that the hatchery can be located anywhere in the world since larvae can be shipped great distances and arrive in a healthy state. Hence large, efficient hatcheries can be located at ideal sites rather than at locations that may be politically expedient but are not ideally suited for the purpose.

A distinct advantage in shipping mature larvae rather than juveniles is that larvae are grown in water that is finely filtered and may also have been sterilized with either UV-light or ozone. The danger of spreading diseases or parasites is much reduced compared to shipping juveniles that are generally grown to the desired size in the sea and may have acquired local diseases or parasites.

6.3 METHODS FOR GROWING SMALL SPAT

6.3.1 Introduction

Constraints to growing spat to a large size under closely controlled conditions in a hatchery have been dealt with in general terms in 6.1. Space, the supply of treated, heated water and the large volumes of cultured algae required are major cost considerations. Hatchery managers will know the production cost factors that need to be taken into account when fixing the price of seed. Prices will increase exponentially as size in terms of mean shell length increases and a point will be reached when growers are no longer prepared to pay for spat in the larger size categories. In developed countries with mature industries this point is generally reached when spat are 3 to 4 mm and very often when they are somewhat smaller.

Methods commonly used to handle and grow newly set scallop and clam spat were introduced in 5.4.3.2. Procedures for oysters are different but before describing what these differences are it is relevant to begin with the various tank system options for this part of the hatchery process, beginning with those used for spat set on cultch.

6.3.2 Growing systems for spat set on cultch

Tanks systems – essentially similar to those described for remote setting in the previous section – are commonly used in the hatchery for the initial stages of the growth of oyster, scallop and mussel spat set on cultch (Figure 91). They may be closed systems, i.e. with a static volume of water changed two or three times each week, or open systems operated on flow-through, depending on the extent to which the water needs heating. Very often they will be a combination of the two with aeration to mix and circulate the water and the daily food ration throughout the tank volume. Food will be added continuously in the case of flow-through. Oyster spat may spend as little as a week in these systems whereas the slower growing scallops and mussels will stay longer before transfer to the sea.

Sand-filtered water or water filtered to about 20 μm particle size is usually used at this stage so that spat can benefit from the diversity of naturally occurring algal species in the water in addition to the added ration of cultured food. Feeding is not usually closely controlled in terms of species composition of the diet and ration. Enough food will be added to the tanks to colour the water sufficiently. If the algae is grazed quickly then more will be added. If the water is heated then spat will be gradually acclimated to ambient sea temperature before leaving the hatchery.

Figure 91: Simple tank systems are used for growing spat set on cultch. They are either closed or flow-through systems or a combination of both. **A** – growing tanks mainly used for scallop spat set on cultch at a British Columbia hatchery. **B** – note that the lined plywood tanks are situated outdoors and are roofed over to shade the water surface. **C** – scallop spat may be set on filamentous cultch packed in onion bags, initially in tanks of the type shown in A and B at the hatchery site. **D** – detail of spat set on the filamentous material after a period of floating culture in the sea. **E** – growing mangrove oyster spat set on strings of oyster shell cultch at a Cuban hatchery. **F** – when the spat are 2 or 3 mm in size the strings of cultch are hung from mangrove poles on rafts located in productive waters.

6.3.3 Growing systems for unattached spat

Unattached spat (i.e. spat grown free of cultch – "cultchless") are grown in large volume tanks equipped for recirculation – often with a gradual continuous exchange of water – or they are grown in open, flow-through systems. Whichever method is used depends on species and the size of spat. Smaller spat may be grown in recirculation systems until 1 or 2 mm in size and then be transferred to flow-through to grow to 3 or 4 mm before being sold or transferred to an outdoor nursery.

The spat growing area of a hatchery may contain a number of different growing systems for spat of different sizes and species. Most commonly the systems will utilize oblong concrete, fibreglass or lined or epoxy painted plywood tanks to use space as efficiently as possible. The large tanks that act as the reservoirs have drains plumbed directly into the hatchery's main drain since large volumes of water will be discharged periodically.

Hatchery managers have their own preferences as to the best way to handle spat of the species they produce according to cost factors and what suits the particular requirements of the local industry. As with larval culture many different approaches are taken but there are a number of common factors that apply in the basic methodology.

Oysters are completely sedentary and clam and mussel spat are mostly so once they have settled and completed metamorphosis – scallop spat are the exception. Scallop spat retain the ability to detach their byssus attachment and briefly swim in the water column to find a different location to attach. Food needs to be carried to spat of any species in the water currents. How to manage them in a convenient way and the manner in which the water – as the carrier of food – is delivered to the spat become important considerations.

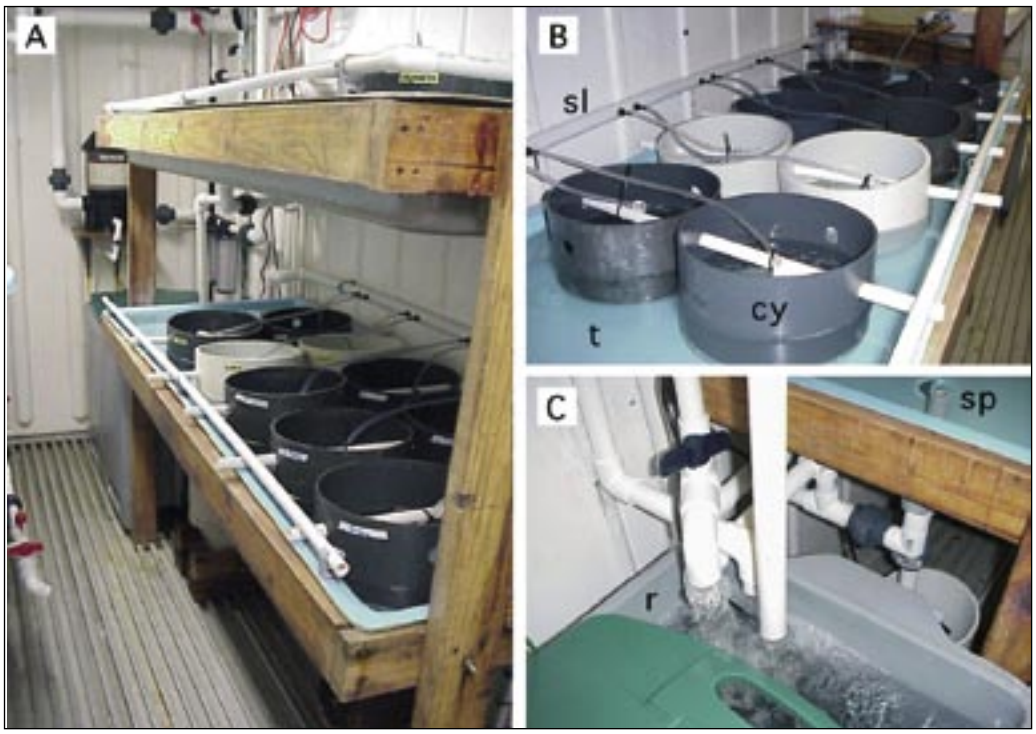

Figure 92: A closed tank system designed for holding scallop spat in cylinders with a downwelling flow of water. **A** – spat holding cylinders are held in shallow troughs (**t**) stacked one above the other. **B** – water flows into each cylinder (**cy**) through a flexible tube connected to the supply line (**sl**). **C** – water returns to the reservoir tank (**r**) via a stand-pipe (**sp**) fitted in each trough which maintain the water depth in the troughs. Water is pumped from the reservoir back to the troughs. Systems of this type are also suitable for clam spat.

Spat are almost always contained in mesh based trays or cylinders in a holding tank which, if not of sufficient volume itself, is connected with a large-volume reservoir tank. Containment of spat in trays or cylinders facilitates ease of management in cleaning and grading the animals. Water with algal food is circulated by electric pump or air-lift from the reservoir to the holding tank, passed by the spat and then returns to the reservoir. Examples suitable for growing scallop and clam spat have previously been given in Figures 87 and 88. Figure 92 shows the delivery of water to each of the cylinders in the holding tank by a flexible hose attached to nipples in the delivery pipe. Water flows in from above the water surface within the cylinder at a controlled rate, downwards passed the spat and out through the mesh base of the cylinder to be returned to the reservoir by a stand-pipe or an overflow which maintains the water level constant in the holding tank. This flow pattern is called downwelling. The other approach used for oysters and clams is to reverse the direction of flow so that it enters

at the base of the cylinder (or tray), passes upwards through the bed of spat and is discharged at the top, from wich it flows back into the reservoir. This is referred to as an upwelling circulation. Both of these principles are illustrated in Figure 93.

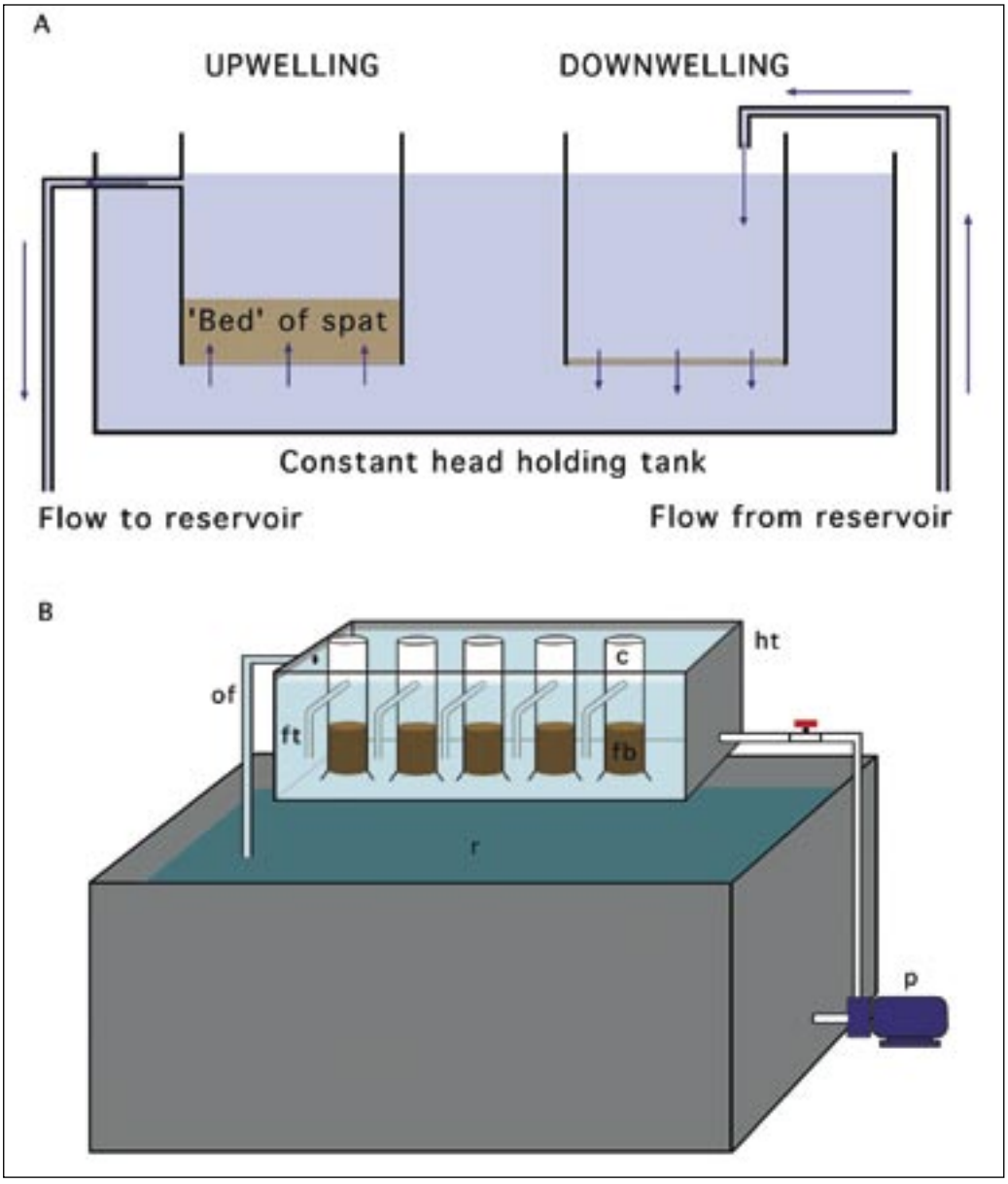

Figure 93:

A – diagram illustrating the difference in flow circulation in upwelling and downwelling spat systems. Arrows show the direction of water flow. Upwelling systems are used for oyster spat from set size upwards and for fully metamorphosed clams. Downwelling systems are used for clam pediveligers (until they have completely lost the ability to swim) and for scallops from the pediveliger stage onwards. Only rarely are upwellers used for scallops and then at a much lower biomass per unit area than for oysters and clams.

B – diagram of an upwelling tank system showing the reservoir (**r**) from which water is pumped (**p**) to a holding tank (**ht**) maintained at a constant water level (head) by an overflow pipe (**of**) through which excess water is discharged back to the reservoir. The holding tank contains a number of tall, narrow cylinders (**c**) with mesh bases in which the spat are held as a fluidized bed (**fb**). Holes are drilled in the holding tank below water level to take flexible tubes (**ft**) that interconnect with the cylinders. Thus, there is a head difference between the water level in the holding tank and the water level that can be maintained within the cylinders. Water flows through the mesh bases of the cylinders, up through the bed of spat and then back to the reservoir through the flexible tubes. The extent to which the spat bed is fluidized (i.e. the spat lifted by the flow) can be changed by altering flow rate.

It is quite common to use inverted, plastic soft drinks bottles of 1 to 3 l volume as upwelling cylinders. Instead of a mesh screen containing the spat, a ball or large marble is placed inside to cover the opening of the neck. This serves as a non-return valve. The flow of water from the bottom keeps the juveniles suspended in the water column inside the cylinder but if water pressure is lost the ball or marble seals off the neck so that no juveniles are lost. Discharge water from a series of upwelling bottles is passed over a screen to collect any juveniles that may accidentally float away.

6.3.4 Operation of closed upwelling systems

Upwelling is particularly useful in the culture of post-settlement oysters. Small spat are amenable to stocking in depth at high density, i.e. layered one above the other. The same applies to clam spat once they approach 0.5 mm in size. Holding the small oysters in this way with a sufficient flow of water to fluidize the "bed" of spat prevents adjacent spat from fusing together to form clusters as they grow. Cluster formation can be a problem in *Crassostrea* species if the spat are not kept moving – for example, if they are grown in trays with a downwelling flow. This habit is more pronounced at the high water temperature, which is generally between 22 and 25°C for oyster spat growth. An upwelling flow is also more efficient in keeping the spat free of faecal deposits than is downwelling, where faeces tends to accumulate on and around the spat. This can result in blockage of the mesh, which is less of a problem in upwelling containers.

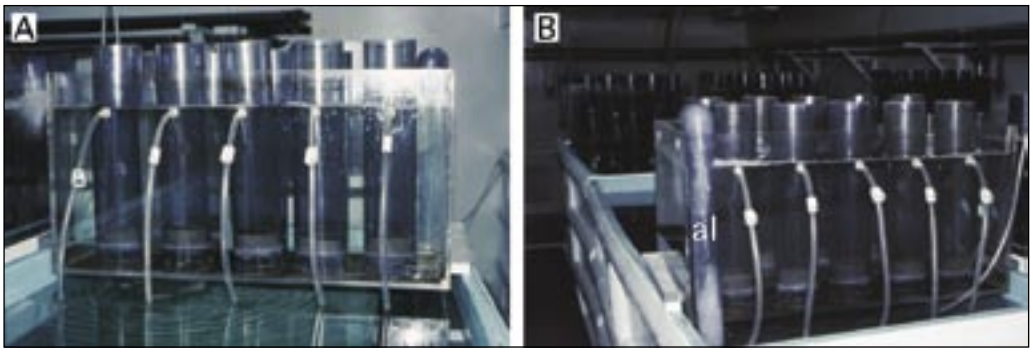

Figure 94: A and **B** – Closed upwelling systems in use for the growing of small oyster spat. The total volume of each tank unit is approximately 3 m^3 and the spat holding tanks hold 10 cylinders each stocked with 60 g live weight of spat at the beginning of a weekly period. The flexible outflow tubes from each cylinder are fitted with an adjustable clamp to permit individual control of flow rate. **B** – water is lifted from the reservoir to the holding tank by air-lift (**al**). This is a 5 cm diameter pipe with an airline fitted into the base. The flow of air into the bottom of the pipe lifts a sufficient volume of water to operate the system without the need for an electric pump.

Upwelling containers (referred to as cylinders or tubes) can be of varying diameter and they are made from sections of PVC or acrylic pipe fitted with nylon mesh bases of different aperture size for the range of sizes of spat grown. They do not need to be transparent as in Figure 94, but transparency is an advantage in gauging the flow rate required to fluidize the biomass (bed) of spat contained. The flow rate needed to fluidize, i.e. lift and circulate, the bed will depend on the size/weight of the spat and on the diameter of the pipe section. The larger the spat are the more flow will be needed to fluidize the bed. Lower flows are required in narrower cylinders. Typically, a flow of 1 or 2 l per minute through 5 or 10 cm diameter cylinders will fluidize a bed of 1 to 3 mm oyster spat. A flow of between 25 and 40 ml per minute per g of spat is ideal. Beds of clam spat, which embyss together, will not fluidize. Nevertheless the method works just as well as it does with oysters. Possibly the effect of spat being aggregated together is advantageous in that it simulates conditions when they are buried in substrate. Beds of clam spat – tightly embyssed together – when held in downwelling conditions tend to act as sediment traps and meshes soon become blocked.

The numbers of spat that can be held in an upwelling tank system depends on their size/weight (Table 14). Take, for example, the system shown in Figure 94 in which the combined volume of each reservoir and spat holding tank unit is close to 3 000 l. There are 16 similar units in the hatchery. Each unit is suitable for growing a live weight biomass of 600 g. Assuming the spat to be grown are 2 mm shell length, reference to Table 14 indicates that 272 700 spat of this size will make up the initial biomass. The holding tank shown in Figure 94 contains 10, 10 cm diameter cylinders. At the beginning of a 7-day period, each cylinder is stocked with 600/10 = 60 g of spat. These will have been graded using a 1.5 mm mesh screen over another screen of 1 mm aperture upon which they will have been retained (2mm spat will not be retained by a 1.5 mm mesh). In this context, the relevance of accurately knowing the numbers of spat stocked in a unit is superseded by the importance of knowing their biomass. For further explanation see section 6.3.5.

Seawater to fill the tank units is filtered and heated to larval culture standards for spat in their first week after set. After that time, they are filled with either sand-filtered or 10 or 20 μm cartridge filtered water and the temperature is decreased by 1 or 2°C per week to start acclimation to prevailing conditions in the nursery or sea.

At the end of the 7-day period, during which time the tank volume will have been changed twice and the spat and system cleaned at each water change, the spat are graded and redistributed again. The 600 g biomass at the beginning of the week will have doubled or even trebled for oysters by the end of the 7-day period and so will need to be redistributed between two or three 3 000 l units to grow for a further week. Spat will not have grown evenly in size during the previous week. By grading through a stack of screens production from the tank unit can be size fractionated (section 6.3.6). The growing process functions more efficiently if spat of the different size fractions (grades) are grown in separate tank units so that those in any one unit are of the same size grade.

6.3.5 Operation of closed downwelling systems

Downwelling tank systems without continuous water exchange are dealt with following the same procedures as described above. The only major difference is that the biomass of spat is spread over a much greater surface area than in upwelling systems because the juveniles – most commonly scallops – are sensitive to overcrowding. Thus, they are maintained with sufficient spatial separation to permit growth as a single layer so that individuals are not in immediate contact with adjacent spat.

Methods to maintain spatial separation vary from hatchery to hatchery and where the spat have been set on cultch procedures outlined in section 6.2.2 apply. If not set on cultch but on the mesh bases of cylinders or trays as shown in Figure 88 (section 5.4) and Figure 92 then system design and operational management details are different. The holding tanks supplied from the reservoir will need to have a large enough area to fit the numbers of trays or cylinders required to hold the biomass of spat appropriate to the total tank unit volume. For this reason, holding tanks of the kind shown in Figure 92 are shallow and are often stacked one above the other.

As in closed upwelling systems, water quality is maintained by complete water changes twice or three times each week. The trays or cylinders containing the spat are removed and each is sprayed with a seawater jet to dislodge and remove detritus adhering to the spat and to the mesh of the containers. The reservoir and holding tanks are cleaned and refilled before returning the spat containers. Seawater used may be finely or coarsely filtered depending on the size of spat. It is usually filtered to 1 to 2 μm for early-stage spat and is sand-filtered for larger spat about to be transferred to the sea. Spat are gradually acclimated to ambient sea temperature before they are transferred.

Scallop spat are not as amenable to removal from the containers for grading and size determination. Their shells are more fragile and care needs to be exercised not to damage their byssus gland or displace their shell valves and damage the resilium during removal. Gentle water jets may be used but it is more appropriate to count them if necessary *in situ*. This can be done as shown in Figure 88B by using a plastic sheet marked with a grid (1 cm squares) under the mesh base of a random selection of trays or cylinders. Means calculated from counting the numbers per cm^2 in random squares over 10% of the surface area of a selection of containers multiplied by the total area occupied by spat will give a good approximation of total numbers. Small samples can be removed to weigh and measure to track growth and biomass.

6.3.6 Grading and estimating spat

Mechanical graders are available from specialist equipment suppliers and they are applicable when millions of seed are being handled on a routine basis. Otherwise hand graders are used in the majority of cases. These can easily be made as a series of large diameter (>30 cm) fibreglass or PVC pipe sections with nylon or plastic meshes of various aperture sizes fixed to one cut face.

Spat grading is best done in water. The grading screens, each marked with the size of mesh, should fit comfortably within a plastic tray fitted with a stopper or drain valve at one end. The tray is part-filled with seawater when in use. Small numbers of spat are added to a screen of a mesh size slightly smaller than the largest individuals. The sieve is then shaken from side to side and up and down in the water until no more spat escape through the mesh (Figure 95). More spat are added periodically until all have been graded through that screen. Those retained in the sieve will need to be removed from time to time to maintain efficiency of the process. They are transferred to a screen of known (tare) weight with the same mesh size and are left dry to await estimation. The tray is then emptied and the smaller seed recovered for further grading. The procedure is then repeated with a screen of the next smaller mesh aperture, and so on.

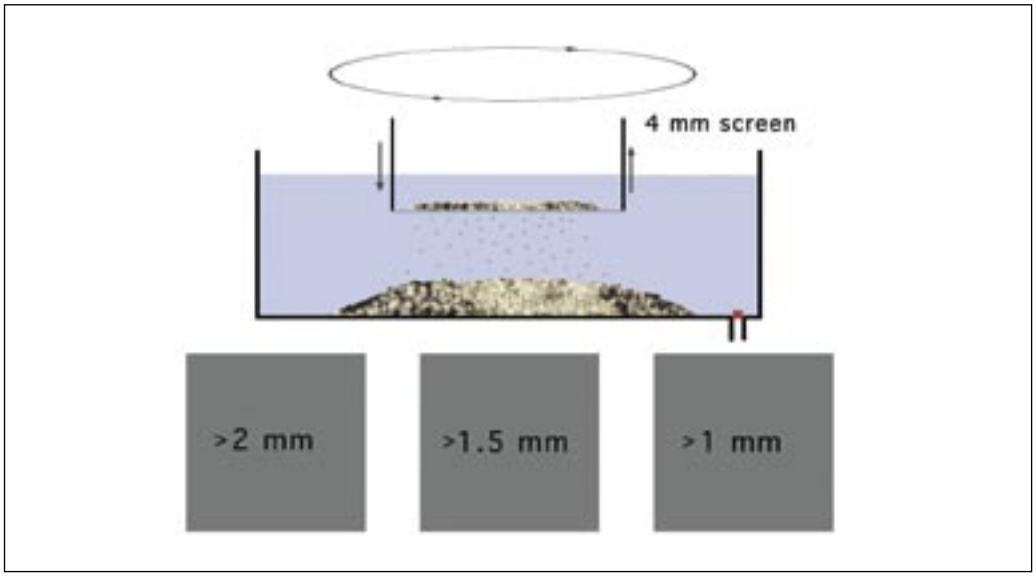

Figure 95: Grading of spat with hand-held sieves (screens) in shallow tanks. The grading screen is rotated from side to side and up and down in the tank until all spat smaller than can be retained on the mesh fall through and collect at the base of the tank. Once grading with a particular screen is complete, the tank is drained into a receiving screen of appropriate mesh size, marked with the grade size of the spat. In this example, spat smaller than 4 mm mesh retention size will be collected in a 1 mm receiving container (of small enough mesh size to collect all that remain). The process continues with grading screens of decreasing mesh size until all spat have been fractionated into the various grade sizes.

Once separated by grade, the next task is to determine the biomass of spat in each grade. Screens containing the different grades need to be allowed to thoroughly drain until the contained spat are "damp dry." Draining can be accelerated by dabbing the bottom mesh of the screens with dry clothes or paper towelling until excess water has been removed. The screens are then weighed and the weight of the screen itself subtracted to provide the weight of spat it contains. This is the biomass of spat of that particular grade.

At the same time, numbers of spat and a check on survival can be made. Numbers can be estimated either by weight or volumetrically. The former method requires accurate balances while the latter can be achieved with simple apparatus, e.g. small plastic containers of between 1 and 5 ml volume to hold sub-samples. This method will be described.

From the screen containing the largest sized seed, fill three sub-sample containers to the brim. Empty one into a shallow white tray containing a little seawater. A Petri dish marked with a grid, observed under a low power microscope is useful for counting very small spat. Count the total number of seed in the sub-sample. If there is no dark spot within the shells (the digestive system) or if the shells are gaping open, place to one side. Record the total number of seed and the number dead. Repeat for the second and third sub-sample. Determine the total volume of spat of that grade by transferring them to graduated containers and reading the volume they occupy. From this information, the total number alive and percentage mortality can be calculated as follows:

Example:

Basic information:

Sub-sample volume = 2 ml
Sub-sample 1: 865 total, 33 dead;
Sub-sample 2: 944 total, 41 dead;
Sub-sample 3: 871 total, 33 dead.

Total volume of seed (including the 3 sub-samples) in the grade = 1 850 ml

Calculation:

Average number of seed (live & dead) per 2 ml sub-sample
= (865 + 944 + 871)/3 = 893

Average number of dead per 2 ml sub-sample
= (33 + 41 + 33)/3 = 36

Mortality = (36/893)x100 = 9.6%

Estimated total number alive = (893 – 36)x(1 850/2) = 792 725

Numbers in the other grade fractions are estimated in the same way. Smaller volume sub-samples will be necessary for the smallest seed sizes.

Estimating numbers by weight follows the same basic method with small sub-samples taken for accurate weighing from the bulk of spat in the particular grade(s). Numbers in the weighed sub-samples are counted. Once the total weight of spat in the grade has been determined, total numbers can be calculated as above.

Spat of the various clam species are more difficult to grade than oysters because of their habit of attaching to one another and to the meshes and internal surfaces of the containers in which they are grown by byssus threads. Nevertheless they are handled in similar manner using water jets from a pressurized hose to separate them during the grading process.

6.3.7 Operating systems on flow-through

Tank systems of the various kinds described above are often operated with a partial exchange of water each day or on a continuous open flow. Partial or total flow-through systems are used for growing larger spat when maintaining a temperature higher than the ambient is not an important consideration, i.e. when ambient water temperature is sufficiently high to support good growth. Two advantages of flow-through are a) the increased biomass of spat that can be held and grown in the spat holding tanks and b) spat can benefit from the natural or enhanced productivity of the exchange seawater. The diversity of algal species in the generally coarsely filtered exchange water more closely resembles natural conditions as spat are gradually acclimated in preparation for transfer to growout.

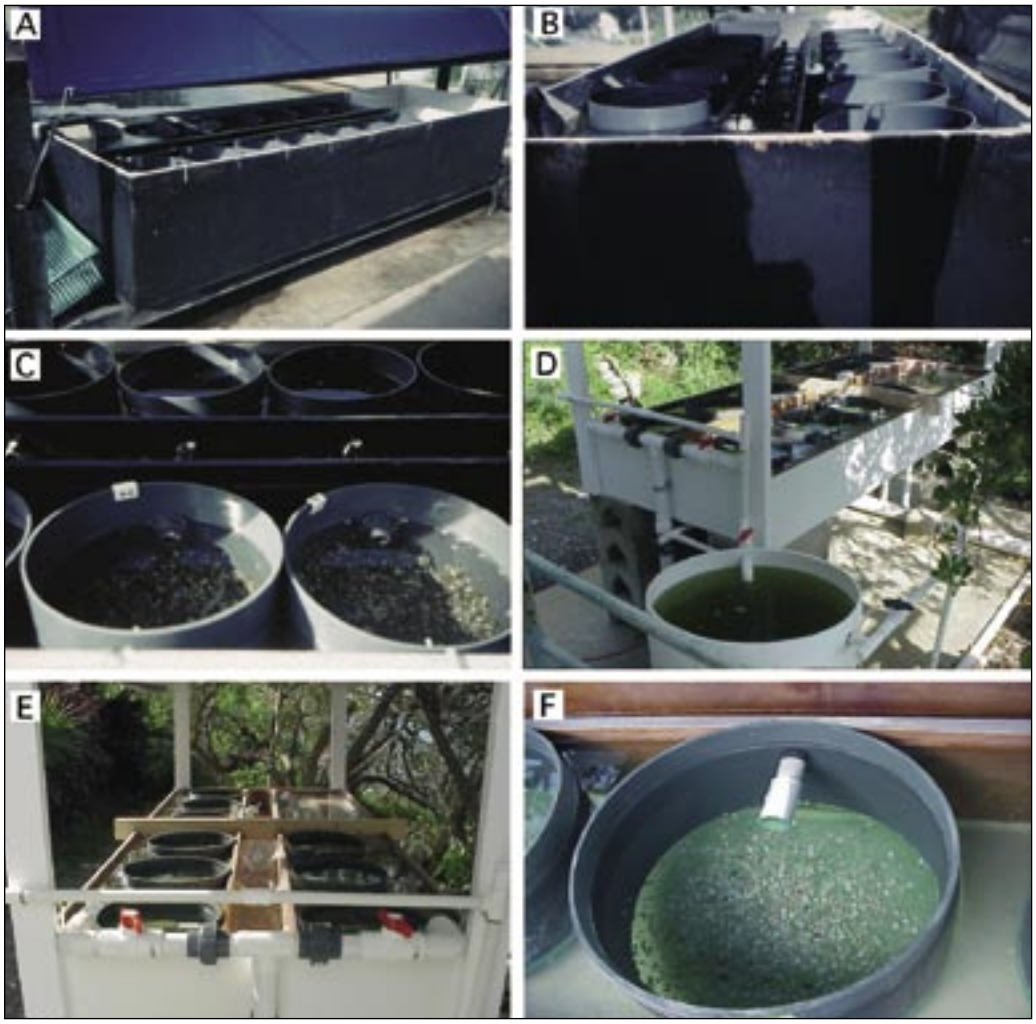

Figure 96: Upwelling tank units for larger size spat operating on flow-through. **A**, **B** and **C** – a system for growing clam spat at high density. This system is in circuit with a 90 m^3 outdoor concrete reservoir in which naturally occurring algae is "bloomed" by adding nutrients. Note the central collecting channel which carries the upwelling water flow from the cylinders back to the reservoir. This is a pumped system. **D**, **E** and **F** – a system for growing scallop spat at lower density. This unit is plumbed directly into the hatchery's main seawater supply and is fed continuously from a reservoir containing diluted algal paste, which can be seen in D. Otherwise, the configuration of the system is similar to A with cylinders either side of a central water collecting channel.

It was pointed out in section 6.2.3 that the optimum biomass for growing spat in closed systems is 200 g per m^3 of the total volume of the reservoir and spat holding tank combined. Consider the example of a 3 000 l tank unit given in section 6.2.4 in which 600 g live weight biomass of spat will grow at a satisfactory rate. When total tank volume is completely exchanged in the course of a 24-h period then the biomass can be approximately doubled. At this rate of water exchange – equivalent to 125 l per hour – and assuming that cultured algae is the principal food source, little food will be lost especially if it is added directly to the spat holding tank of the unit. The food ration will need to be doubled because the biomass of spat has been increased by a factor of two. At the higher spat density and the increased quantity of food, tank fouling with faeces and detritus is greater and needs to be taken into account during routine husbandry. The tank units may need to be drained and cleaned three times a week instead of twice.

Spat holding tanks operating on total flow-through are usually configured differently. Instead of being in circuit with an adjacent reservoir tank they are stand-alone units, each being plumbed directly to the seawater supply (Figure 96D). They may be located inside the hatchery or outdoors. Many hatcheries operating flow-through units plumb a water supply from outdoor shallow ponds or very large volume tanks located adjacent to the hatchery premises. They are used to bloom algae. Furthermore, the temperature of the water in these ponds will be elevated above ambient sea temperature by solar heat gain for much of the year, particularly in temperate latitudes (see section 6.6). Seawater discharged from the spat holding tanks is returned to the ponds. In this way algae is conserved.

In essence, flow-through units are little different to the concept of nursery culture, which is described in greater detail in section 6.6. Hatchery-based flow-through units tend to be used for spat in the smaller size grades and many hatcheries will also have a nursery for the further growth of larger seed in close proximity. Staff can thereby manage the entire production process from egg to larger seed with the hatchery infrastructure of equipment, laboratory space, etc., available in support.

6.4 DIETS AND FOOD RATIONS FOR SMALL SPAT

6.4.1 Species composition of diets

Foods suitable for growing small spat in closely controlled conditions within hatcheries are the same as those used in larval culture (section 5.1). When spat are in their first week after settlement they are usually fed the same diet they were fed before settlement occurred. As they increase in size it may not be possible to produce sufficiently large quantities of some of the more delicate and difficult to grow algae. Diets for larger spat tend to be made up of the hardier species such as *Tetraselmis* sp. and the diatoms *Chaetoceros muelleri*, *Thalassiosira weissflogii* and *Skeletonema costatum*.

The highly unsaturated fatty acid (HUFA) DHA (22:6n3) does not appear to be as important in the development of spat as it is during larval development so that *Isochrysis galbana* and species with a similar HUFA profile – while useful as minor components of the diet – are not essential. Typically, diets will be approximately a 50:50 ratio of a *Tetraselmis* species and one of the above named diatoms. Part of the ration may be in the form of an algal paste rather than freshly grown live algae (Figure 97). Some products support satisfactory growth rates. The suggested reading list at the end of this section includes papers on recent research with a range of non-living foodstuffs.

Figure 97: An example of a proprietary algal paste product suitable as a partial or complete replacement for hatchery grown live algae in the culture of bivalve spat. Packs of *Tetraselmis* and *Thalassiosira* contain the equivalent of 3 600 l at 410 cells per μl and 1 800 l at 2 600 cells per μl respectively. When refrigerated, shelf life is 12 to 14 weeks. A range of useful species is available.

6.4.2 Calculating food ration

Ration is calculated on the basis of the biomass of spat held in a tank unit whether it is a closed downwelling or upwelling system or a system operated with partial water exchange. Spat of most bivalves have similar requirements in terms of the quantity of food required per unit biomass. Thus, a ration calculated for a given biomass of oyster spat will be equally as suitable for the same biomass of clams and mussels although growth responses may be very different. For example, clams will initially grow more slowly than oysters even in the best possible conditions. Scallops are again the exception and respond best to lower rations per unit biomass.

Ration in terms of the dry weight of algae required is calculated from the equation:

$$F = (SxR)/7$$

where, **F** = the dry weight of algae per day (mg); **R** = ration as dry weight of algae (mg) per mg live weight of spat per week and **S** = the live weight of spat (mg) at the beginning of each week.

A worked example is given below together with an extension of this equation to calculate the volume of harvested algae required for the daily ration.

Example:

Basic Information:

Live weight biomass of spat at the beginning of the week = 600 g = 600 000 mg
Ration = 0.4 mg dry weight of algae per mg live weight of spat per week
Diet: *Tetraselmis suecica* at a harvest cell density of 1 500 cells per μl

Calculation:

F=(600 000x0.4)/7 = 34 286 (mg dry wt of algae)

Therefore, the daily ration fed to 600 g of spat will be 34 286/1 000 = 34.286 g dry weight of algae.

Reference to Table 1 (section 3) shows that 1 million cells of *Tetraselmis suecica* weighs 0.2 mg.

The volume of *Tetraselmis* required to provide the daily ration is calculated from the equation:

$$V=(Sx0.4)/(7xWxC)$$

Where, V= the volume of harvested algae (l) required to provide the daily ration
W = the weight of 1 million algal cells of the required species, and
C = the harvest cell density of that species (cells per µl)
Thus,

$$V = (600\,000x0.4)/(7x0.2x1\,500) = 114.3\ l$$

Therefore, 114.3 l of *Tetraselmis* at a harvest cell density of 1 500 cells per µl provides the daily ration for 600 g biomass of spat.

Note: A ration of 0.4 is satisfactory for oyster and clam spat of any size within the range likely to be grown on the hatchery premises.

A diet made up of *Tetraselmis* and *Skeletonema* in a 50:50 ration by dry weight will be 57.2 l of the former at 1 500 cells per µl and 76.5 l of *Skeletonema* at a harvest cell density of 7 000 cells per µl. The dry weight of one million cells of *Skeletonema* is 0.032 mg.

A biomass of 600 g of oyster or clam spat will need to be grown in a 3 000 l volume. Adding the above ration will result in an initial algal cell density within the system of 57 cells equivalent in size to *Tetraselmis* per µl (57 000 cells per ml). This algal cell density is too high to support optimum growth if it is delivered as a single batch feed. The optimum food cell density in this respect is 10 000 cells per ml. The solution is to add (10/57x114.3) l = 20 l of food as a batch feed and the remainder by drip feed or dosing pump over the 24-h period.

A ration of 0.4 mg algae per mg live weight of spat per week is towards the upper limit for spat of warm water scallops, such as *Argopecten* species, which are grown at the same temperature as the oysters and warm water clams (i.e. $23\pm2^{o}C$). Ration needs to be reduced for cold water scallop species.

Calculations given in the example above apply equally to systems operated with a partial daily water exchange. Ration is calculated for the biomass of spat held and not the water volume in which they are grown.

When spat systems are operated on flow-through and food supply is from a nutrient enhanced pond or tank it is not possible to accurately assess the species composition of the food supply or the ration that needs to be provided. It will vary from day to day according to the state of the bloom. An experienced technician will be able to judge whether the pond water will need to be diluted with non-bloomed seawater in order to keep the daily ration within reasonable bounds. Excessive pseudofaecal production by spat indicates that the food supply is too high.

6.5 GROWTH AND SURVIVAL

Assuming spat are being cultured at reasonable density, their growth rate is largely influenced by the quality of food given in terms of the nutritional value of the component species of the diet, the ration of food provided and water temperature. Other factors play a part, such as salinity and genetics, but their effects are relatively minor in comparison. The effects of the biomass of spat per unit volume of the system

in which they are grown have already been discussed. A density of 200 g live weight per m^3 of tank volume represents a good compromise between density for maximum growth, which occurs at below 25% of that biomass, and economic considerations such as the space needed to accommodate tanks and the volumes of heated, treated seawater required.

6.5.1 Variability in spat growth between species

The different bivalve species commonly grown in hatcheries have widely differing growth rates when grown at reasonable densities on an adequate diet and ration and at close to the optimum temperature. Oyster spat grow considerably more quickly to a saleable seed size than do spat of the various commercial clams and scallops. Cold water scallops grow more slowly than warmer water species. Partly this is related to the larger larval size of oysters at settlement and partly the fact that there is no lag phase while metamorphosis takes place.

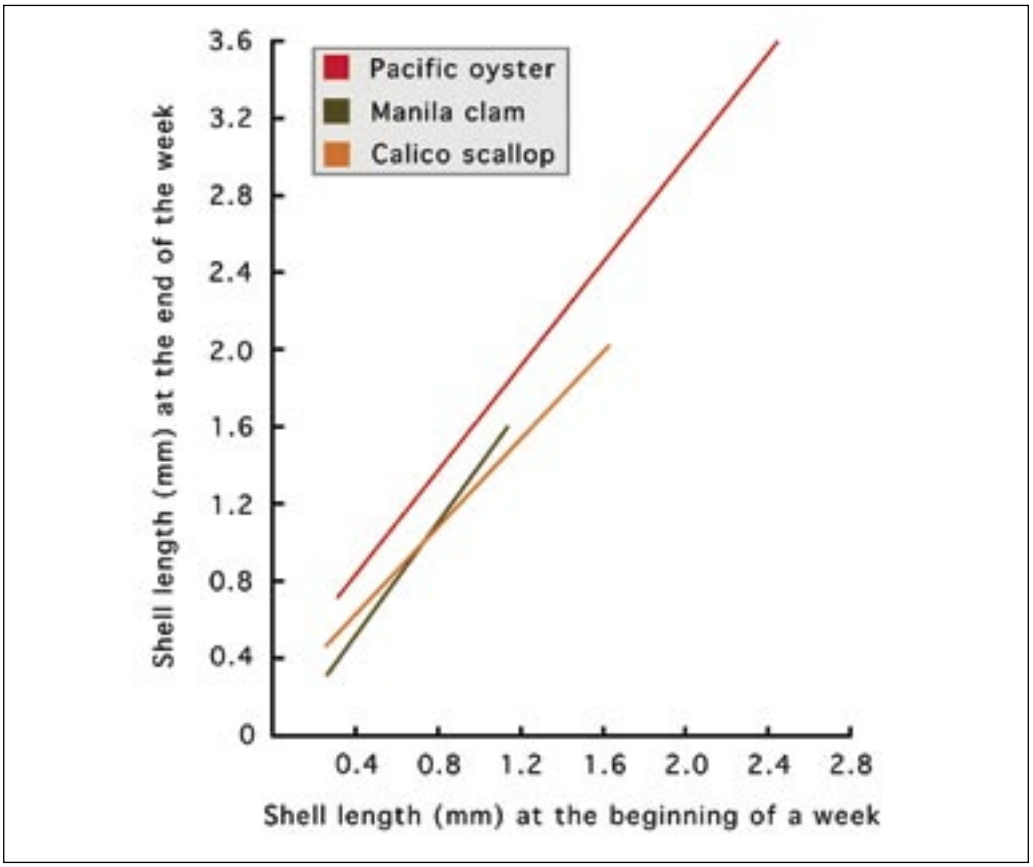

Figure 98: Comparison of the growth of Pacific oyster, Manila clam and calico scallop spat in similar conditions. Growth is shown as mean shell length (shell height in the case of calico scallop spat) at the beginning and end of a 7-day period.

A comparison is given of the growth of Pacific oyster, Manila clam and calico scallop spat in Figure 98. This contrasts growth of the three species from settlement size by comparing mean shell length at the beginning of a 7-day period with mean shell length 7 days later. Spat of the three species were grown at pilot-scale in systems of the types described previously at commercial densities and with adequate diets, rations and at $23 \pm 1^{o}C$. In this graph, the more steeply the growth curves are inclined to the left, the faster is the growth rate. Manila clams are in fact growing faster than Pacific oyster spat but they are starting from a smaller size. At the end of a 3-week period from settlement, Pacific oyster spat will grow to approximately 3.4 mm mean shell length compared with Manila clam spat which will reach 1.14 mm. This is mean shell length

and the distribution about the mean is much greater in clam spat than it is in oysters. Calico scallops grow more slowly with an equally as large-size distribution about the mean. After 5 weeks growth they will reach approximately 1.5 mm mean shell height (where height is almost the same as length at this stage). Manila clam spat will exceed this size in 4 weeks (1.6 mm).

Cold water scallops such as the Japanese scallop, *Patinopecten yessoensis*, will take 4 or 5 months to reach 5 mm shell height even when grown in ideal conditions.

6.5.2 Effect of ration on growth

The ration given in sections 6.3 and 6.4 for the purposes of explanation of spat culture methodology is 0.4 mg dry weight of algae per mg live weight of spat per week (R 0.4). It has proved to be a practical ration in hatcheries because it is not excessive in terms of algal food production requirements and it is adequate in providing satisfactory growth rates of most species. Better growth rates can be achieved by feeding higher rations. As an example, the growth of Pacific oyster spat is given in Figure 99 when experimentally fed rations ranging from R 0.1 to R 1.0 at a mean temperature of 24°C. The graph shows growth in a 7-day period for spat of different mean live weights at the beginning of the week. Clearly, growth continues to increase when spat are provided with higher rations than R 0.4. Spat of 2 mg at the beginning of a week will reach almost 7 mg by the end of the week when fed R 0.5 and 9 mg when fed R 1.0.

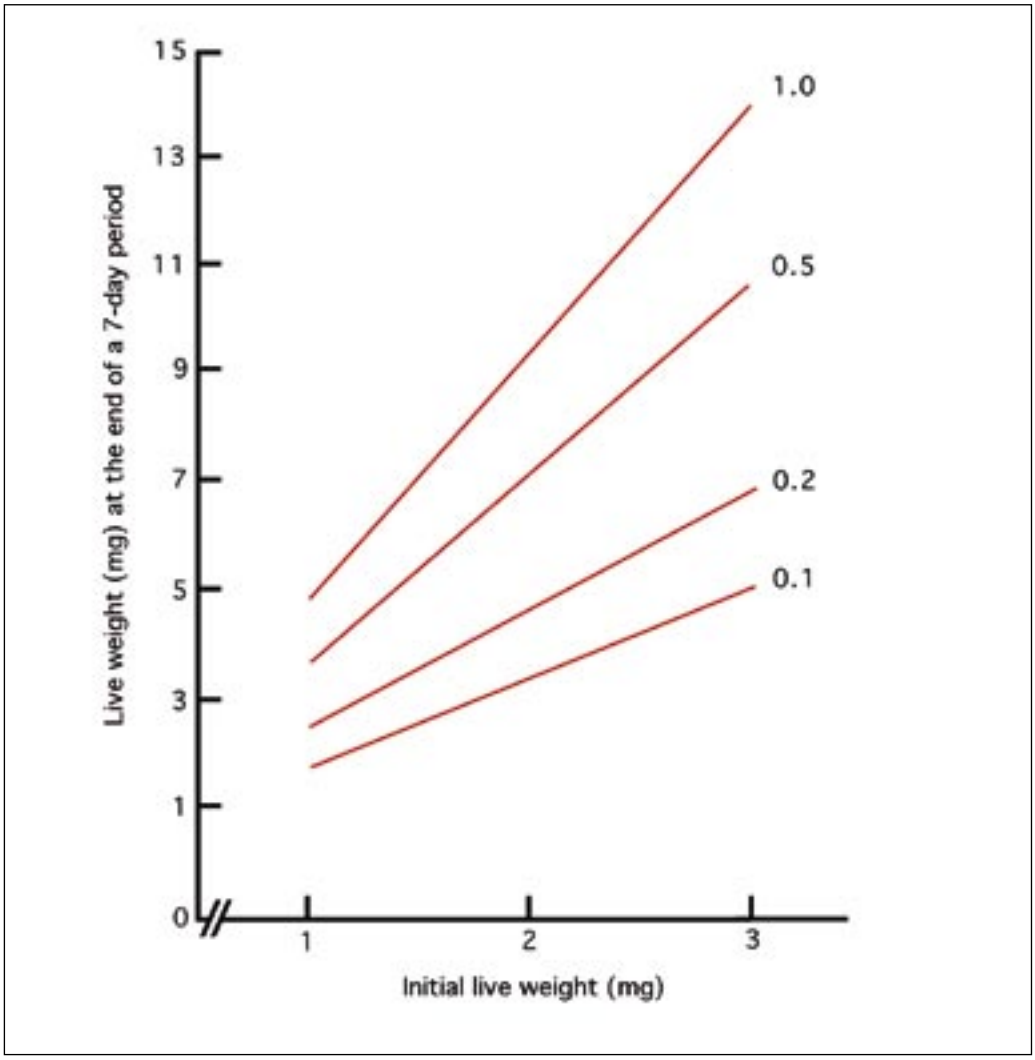

Figure 99: The relationship between food ration and growth for Pacific oyster spat.

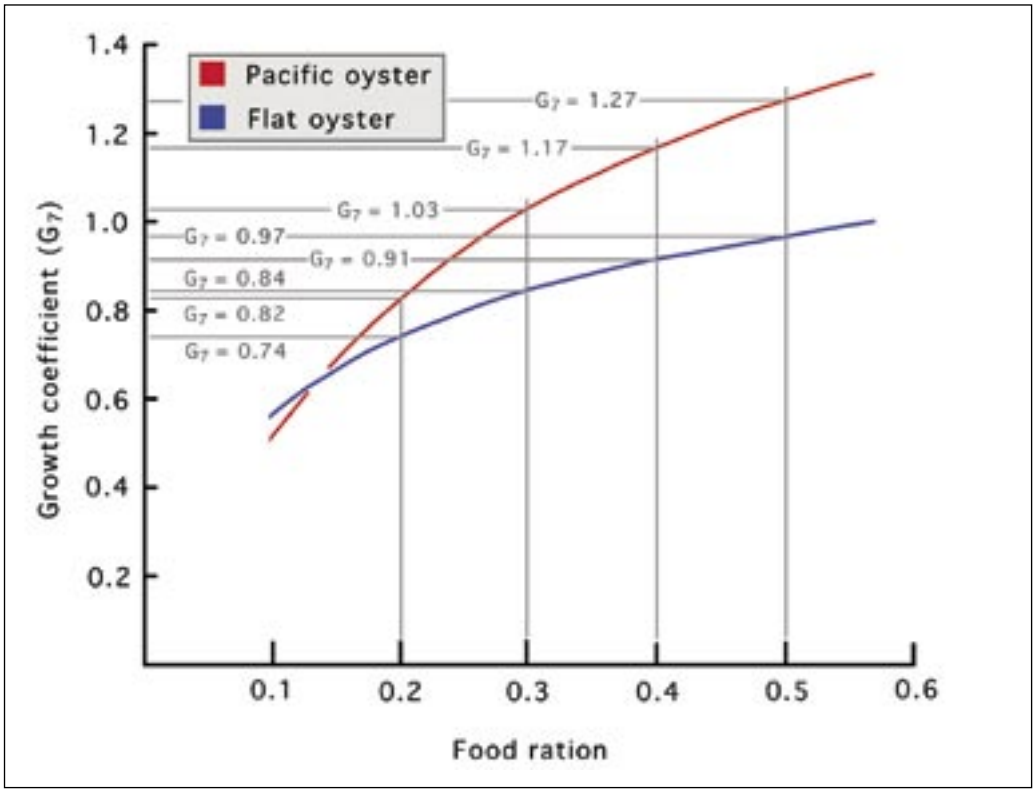

Figure 100: Comparison of the growth of European flat oyster and Pacific oyster spat at 24°C when fed various rations of a mixed diet of *Isochrysis* and *Tetraselmis.*

Among the oysters cultured in hatcheries, the various *Crassostrea* species respond very similarly in terms of growth rates for given rations. Spat of the European flat oyster, *Ostrea edulis*, do not grow quite as rapidly when provided the same conditions. Comparative growth in live weight of European and Pacific oysters is shown as the growth coefficient G_7 when fed rations ranging from R 0.1 to R 0.5 at 24°C in Figure 100. G_7 is calculated from the following equation:

$$G_7 = \ln wt_7 - \ln wt_1$$

where wt_7 is the mean live weight of spat at the end of a 7-day period and wt_1 is the mean live weight at the beginning of the period (ln denotes natural logarithm).

The size spat will grow to at the end of a week when they begin the week at a specified size can be calculated from the equation. Growth coefficients are marked on the graph for both species when fed the same rations per unit live weight biomass. What this means for spat of both species when they begin a week at 2 mg mean live weight is shown in Table 15. Spat at least doubled their weight by the end of the week on all rations and Pacific oyster spat more than trebled their weight on rations R 0.4 and R 0.5.

Table 15: Mean live weight of *Ostrea edulis* and *Crassostrea gigas* spat at the end of a 7-day period when beginning the week at an initial mean live weight of 2 mg and fed rations ranging from R 0.2 to R 0.5 at 24°C. Ration is as dry weight of algae (mg) per mg live weight of spat per week. The diet was *Isochrysis* and *Tetraselmis* in a 50:50 ratio by dry weight.

Ration:	*O. edulis*	*C. gigas*
0.2	4.19	4.54
0.3	4.63	5.60
0.4	4.97	6.44
0.5	5.28	7.12

6.5.3 Combined effects of ration and temperature

The effects of growing, for example, European flat oyster spat on different rations each at a range of temperatures are shown in Table 16. These data were calculated from similar growth curves to those shown in Figure 100 and apply to spat beginning a weekly growth period at 2 mg mean live weight.

Table 16: The combined effects of temperature and food ration on *Ostrea edulis* spat beginning a weekly growth period at 2 mg mean live weight. Rations provided are lower than in Table 15 and range from R 0.05 to R 0.2. The diet supplied was Isochrysis. ND – no data.

Ration:	0.05	0.10	0.15	0.20
Temperature (^{0}C):				
16	2.52	2.63	2.67	ND
18	2.65	2.82	2.89	ND
20	2.80	3.06	3.22	3.29
22	2.92	3.27	3.53	3.68
24	2.95	3.52	3.87	4.17

The lowest ration tested (R 0.05) was still adequate to support growth at the highest temperature although growth rate at this ration was declining rapidly as temperature increased. Food supply must be sufficient to support metabolism, the rate of which increases as the temperature rises, with energy remaining for growth. Low food rations at high temperatures result in spat that may increase in size in terms of shell growth but at the expense of the soft body. Spat that leave the hatchery in poor condition are more likely to die during early growout. Much information exists in the literature and the reader is directed to the suggested reading at the end of Part 6 to pursue this topic further.

6.5.4 Survival

The percentage of spat that will survive to be sold is extremely variable between species, within and between years and between hatcheries. As a general rule spat are not as vulnerable as larvae to pathogenic micro-organisms but, occasionally, abnormal rates of mortality will occur in smaller-size spat coincident with mass larval mortalities.

The survival of oysters is usually in the region of 50 to 70% from set to 2 to 4 mm shell length. For clams and scallops it may be in the 10 to 20% range (Figure 101). Much of the mortality takes place in the first week following settlement in oysters and during the first two weeks for clams and four weeks for scallops. Many larvae that set fail to survive metamorphosis, presumably because they have insufficient food reserves to complete this critical life history stage. Early mortality does not appear to be as much of a problem with the oysters which set and complete metamorphosis within a day or two. However, it has frequently been observed within hatcheries that a higher than average set does not necessarily mean that more viable spat will be obtained. Conditions may favour a good set but they do not necessarily improve the levels of reserves in larvae that may not be fitted for survival through metamorphosis.

When spat are set on cultch, survival is dependent on the density of set. This applies mostly to oysters that cement themselves to the substrate. Clams and scallops are able to change their position relative to their neighbours if overcrowding occurs. Where density of set is intense in oysters the stronger will overgrow the weaker which will inevitably die.

Mortalities will occur if oyster spat are grown at too high a biomass per unit volume in closed systems. The first symptoms are when the shells of the spat gradually or

suddenly turn pale in colouration. If they are not reduced in density at this time then the calcium carbonate crystals in the shell will dissolve. This only happens when biomass grossly exceeds the recommended or if a water change has been missed. A check of the water contained in the tank system with a pH meter will show that the pH level has dropped sharply. It normally decreases between water changes from pH 8.2 to pH 7.6 or thereabouts, but if for the reasons mentioned above husbandry has been neglected, it may drop to below pH 7.0. The reason is partly the build-up in CO_2 in the system from the respiration of the biomass of spat and the numerous bacteria in the water. The only remedy if the problem is recognized soon enough is to change the water and reduce the biomass of spat.

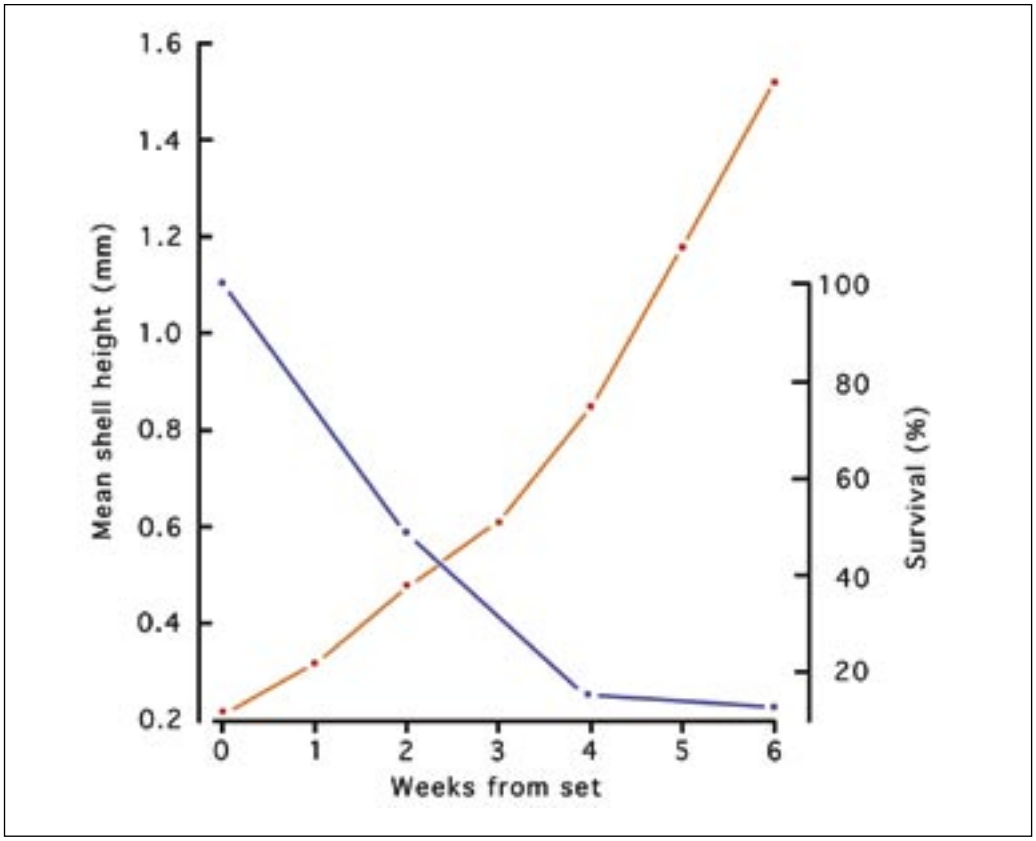

Figure 101: The survival (blue line) and growth (orange line) of calico scallop, *Argopecten gibbus*, spat during a 6-week period post-settlement. Estimates were made of survival at 2-weekly intervals.

6.5.5 Hatchery production

Before considering the nursery culture of spat output from the hatchery it is relevant to consider the process of hatchery production as an entity. When designing a new hatchery the various parts of the operation need to be assessed in relation to expectations in terms of the targeted output of seed. For example, the larval facility may be capable of setting 100 million larvae per year, therefore the capacity to grow spat needs to be equally matched to handle that production to whatever size the market requires. Likewise, the algal unit needs to be designed to reliably produce the daily volume of the required food species to feed the broodstock and the maximum numbers of larvae and spat at the various stages in development which will be in production at any point in time. These factors will vary from hatchery to hatchery according to the species to be cultured and the anticipated volume of sales.

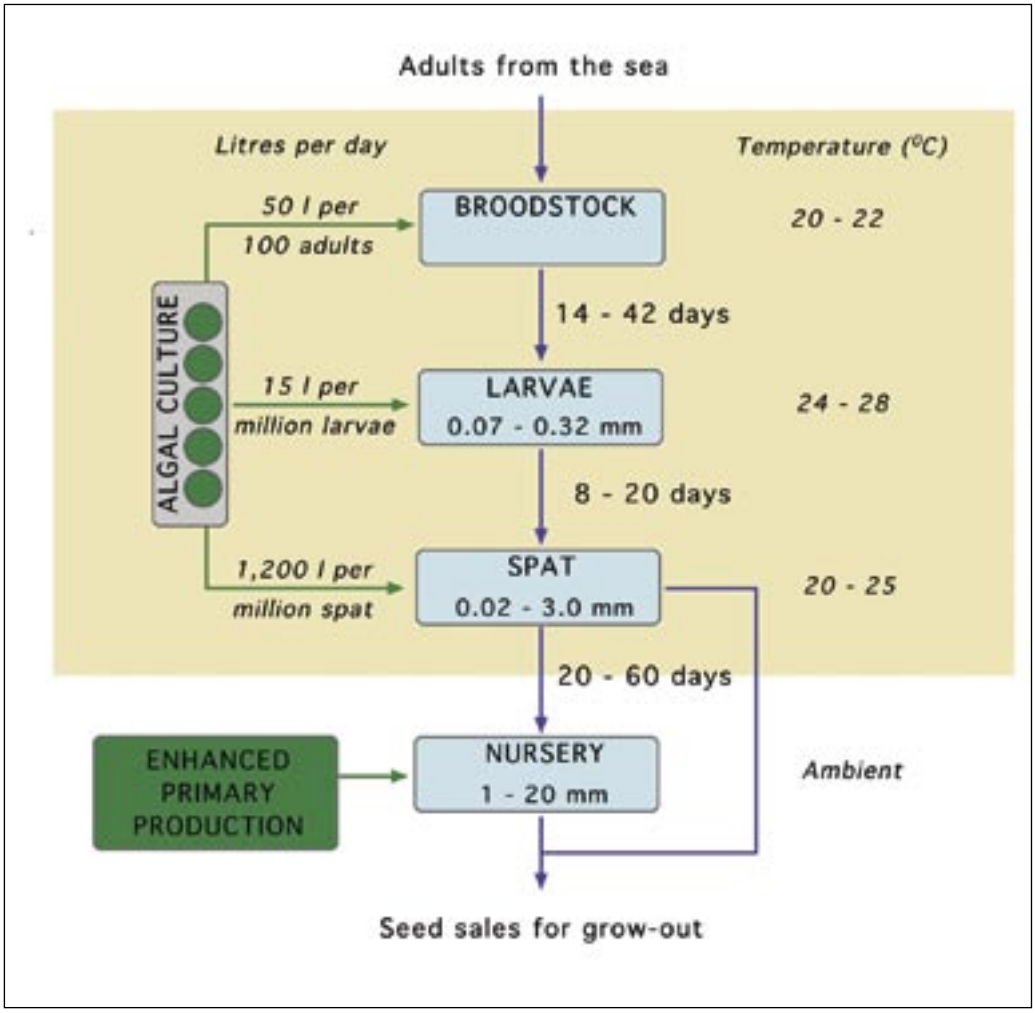

Figure 102: A summary flow diagram of the various aspects of hatchery production showing the temperature range and the daily food requirement per unit number of animals at each stage. This diagram is applicable to most warm water bivalve species.

As a general guide, a summary of the various facets of culture operation and requirements in terms of the temperature of the water and daily food rations is given in Figure 102. Also shown is a range in days that each stage in the production cycle will take which encompasses most warm water bivalve species. Food requirements have been calculated for the average size of larvae and spat that will be in culture on any given day when the hatchery is functioning at maximum capacity. It is assumed that spat will be grown to 3 mm shell length before sale or transfer to a nursery

6.6 NURSERY CULTURE

Bivalve nurseries serve as an interface between hatcheries and the growout phase, i.e. the culture of bivalves in suspension or off-bottom in the sea. They are cost-efficient systems that eliminate the necessity of growing very small seed in fine-mesh nets such as Pearl nets, whose meshes readily clog with floating seaweed, sediment and the settlement of fouling organisms. The purpose of nurseries is to rapidly grow small seed at low cost to a size suitable for transfer to growout trays, bags, or nets with mesh apertures of 7 to 12 mm. Larger mesh size growout trays are not as prone to rapid clogging and require less maintenance.

Nursery systems evolved in Europe and the USA in the 1970s and early 1980s as a natural adjunct to hatcheries. They can be regarded either as the final stage in hatchery production or the first stage in growout. The most efficient nurseries stock seed at high density in upwelling containers. Others may consist of floating or submerged tray units in productive waters with or without an element of forced as against passive flow, but these systems are more akin to growout and will not be considered here.

Nursery spat holding containers may be mounted on rafts or barges moored in productive estuaries or saltwater lagoons. Others are placed in troughs adjacent to or on upwelling rafts floating in natural or artificially constructed seawater ponds (Figure 103). Primary production can be enhanced in ponds and lagoons as already explained by the application of natural or artificial fertilizers to encourage blooms of algae, usually of naturally occurring species. In this respect, they are more amenable to management than sea-based nursery systems because the quantity and to some extent the quality of the available food supply can be manipulated and controlled.

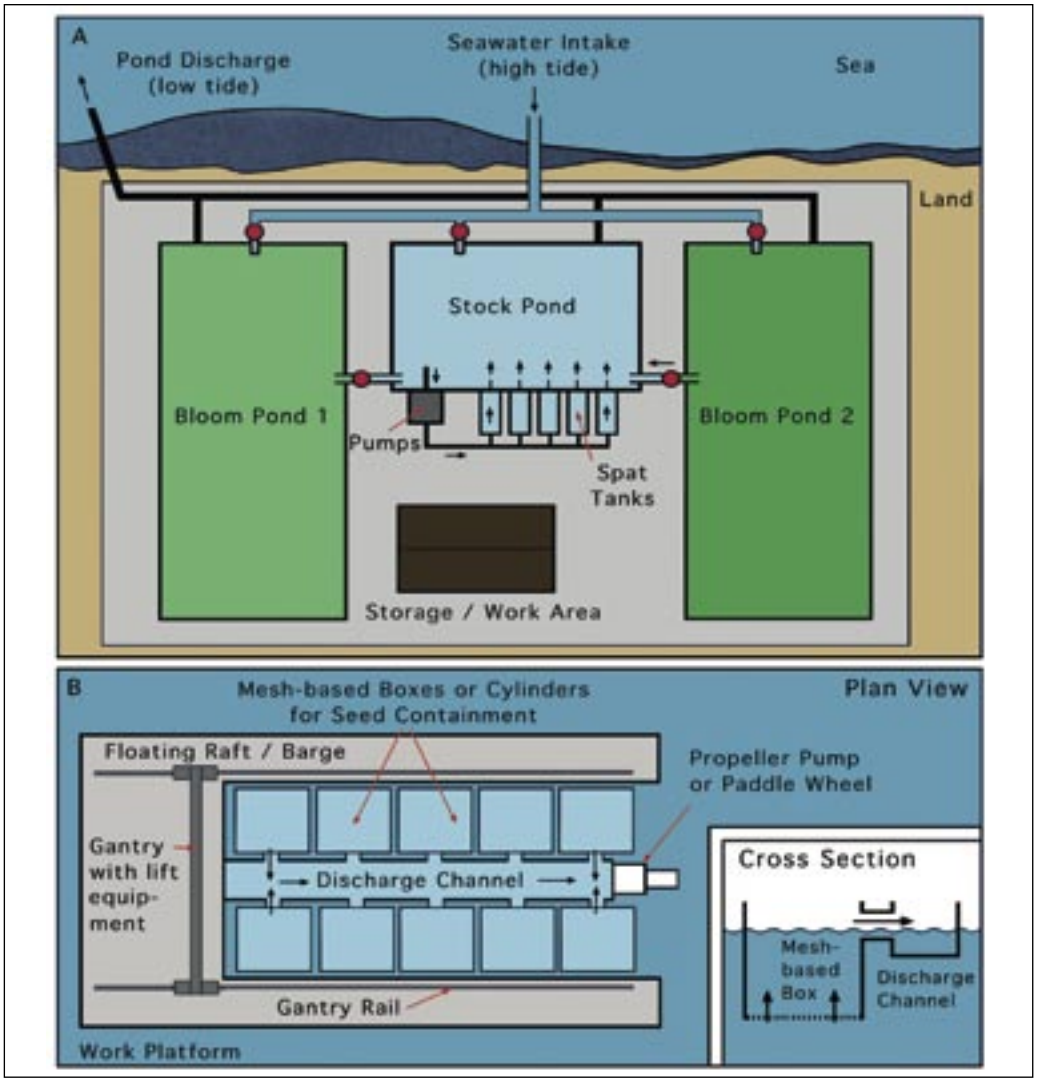

Figure 103: A – a land-based nursery with food supplied by a pair of blooming ponds that are filled and fertilized at different times to promote a succession of blooms. Food is controlled by allowing a flow of water from the most productive pond – Pond 2 in the diagram – into the stock pond from which the troughs holding containers of spat are supplied. **B** – a floating barge or raft nursery that may be moored in a productive estuary or in a large coastal lagoon or system of ponds. Small floating nurseries may be powered by a low-head propeller (axial flow) pump and larger versions by a paddle wheel, both of which drain water from the discharge channel generating upwelling through the mesh bases of the spat holding containers.

6.6.1 Land-based nurseries

Land-based pond nurseries are generally located on low-lying land close to the sea. The ponds are flooded at high tide via a sluice, through a culvert with flap valves that opens to the sea, or by low-head pumping. They can be drained by gravity at low tide (see Figure 103). A land-based nursery system usually comprises a number of shallow, large surface area ponds or tanks interconnected by channels or pipes with sluices or valves. Most of the ponds are used to bloom naturally occurring microalgae species present in the water at the time of filling. Blooming can be controlled and enhanced by the application of agricultural-grade nitrogen and phosphorus fertilizers and a soluble form of silica (section 3.4.6) although reliance on the natural fertility of the water is the more common approach. These algal ponds are used in rotation to supply "bloomed" water to a pond adjacent to the upwelling seed containment unit. Excess water from the pond drains back to the sea and in many cases there is a regular or continuous partial replacement of the water direct from the sea to control the food density and to flush out waste and metabolites. Water is pumped from the supply pond to the upwelling unit, which operates according to the same basic principle as upwellers in the hatchery. Alternatively, if the upwelling unit is a floating structure, water flow is generated by propeller pumps or paddle wheels. Examples of land-based nurseries are shown in Figures 104 and 106.

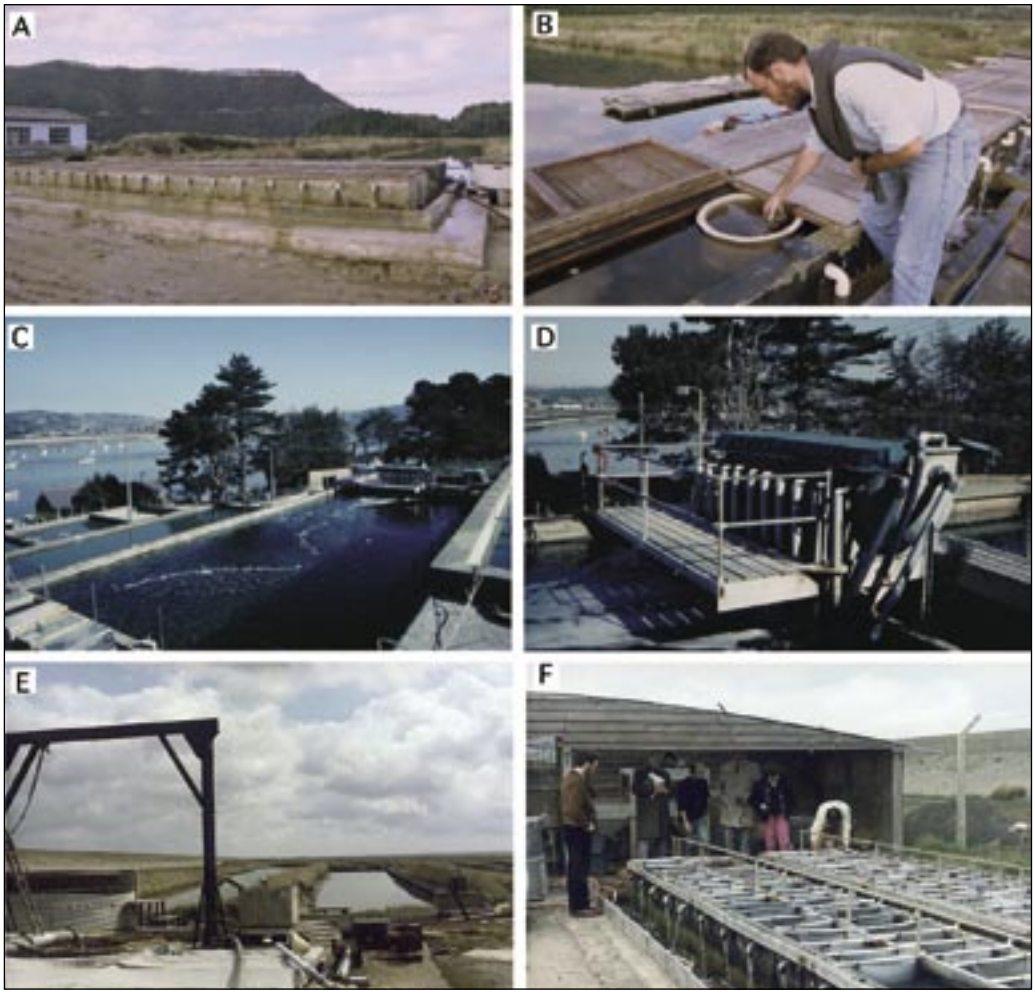

Figure 104: Examples of land-based nurseries. **A** and **B** – concrete spat holding tanks containing upwelling cylinders of spat (Tinamenor S.A., Pesues, Spain). Water is pumped from ponds into the tanks and is discharged into a drainage trough at the base of the tanks. **C** and **D** – a upwelling nursery system supplied from a nutrient enriched 450 m^3 concrete tank at the Fisheries Laboratory, Conwy, Wales, UK. Water is delivered to the spat holding unit (D) by a high capacity submersible pump. **E** and **F** – the progenitor of most European bivalve nurseries developed by Seasalter Shellfish at Reculver, Kent, England.

The biomass of spat stocked in a land-based nursery is dependent upon the productivity of the ponds or tanks and this can be influenced by such factors as temperature and salinity as well as nutrient levels. Shallow pond systems of large surface area and volume act as heat sinks and will gain temperature from solar irradiation. They will often be at a significantly higher temperature than the adjacent seawater, which is beneficial for the growth of warm water species but requires careful management since blooms may be sudden and short-lived (Figure 105). There is always the risk that excessive blooming of algae will result in oxygen depletion of the pond water. Algae, which normally output oxygen as a by-product of photosynthesis, switch to a net uptake of oxygen for respiration during the hours of darkness when unable to photosynthesise. During intense blooming, sufficient oxygen is withdrawn from the water by the algae that the level of oxygen saturation can drop to as little as 20% over the course of a few hours, usually reaching a low point in the early hours of the morning. This can give rise to unexpected mass mortalities of the small bivalves. It is a wise precaution to have oxygen monitoring equipment connected with an alarm installed in the system. Careful management is exercised to control blooming by water exchange between ponds – assuming that there is more than one – and by diluting blooms with water drawn directly from the sea. If the sea is at a lower temperature than the ponds then it will have a higher oxygen content. Aeration equipment is often used to help maintain oxygen levels in pond systems.

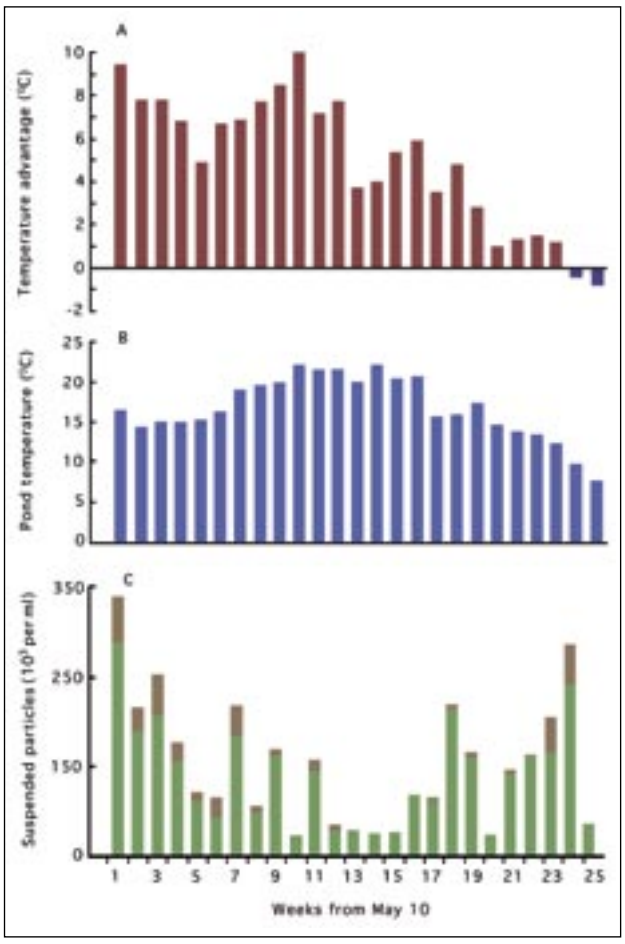

Figure 105: Data from a land-based nursery pond system in Nova Scotia, Canada, operated from early May to the end of October: **A** – the temperature advantage of the ponds over ambient sea temperature; **B** – mean weekly temperature of the pond system; **C** – mean weekly suspended particulate matter (as thousands of particles per ml) in the size range 2.5 to 5.0 μm diameter (green histograms) and 5.0 to 10.0 μm (brown histograms). Particulates were determined using a Coulter Counter. Samples were examined by microscopy to ascertain that the particles were mainly of algal origin.

Salinity in the ponds can be lowered by heavy rainfall and by unexpected sources such as freshwater seepage through the ground or by springs or streams that may be seasonal in nature. As in site selection for hatcheries, careful research needs to be undertaken before committing to the development of a nursery at an unknown location.

Determining the biomass of spat that can be held in a pond system is largely a matter of trial and error. A general rule is that 1 hectare surface area of shallow pond will support the production of between 1 and 3 tonnes biomass of seed, depending on levels of algal productivity, over the course of a growing season. This represents the maximum sustainable biomass that can be maintained with careful management. The areas covered by many European nurseries can be measured in the tens of hectares. Spat are managed in much the same way as in hatcheries. They are regularly graded and redistributed so that any spat container will hold spat of a particular grade. Grading is usually accomplished with mechanical graders (Figure 106). Management also

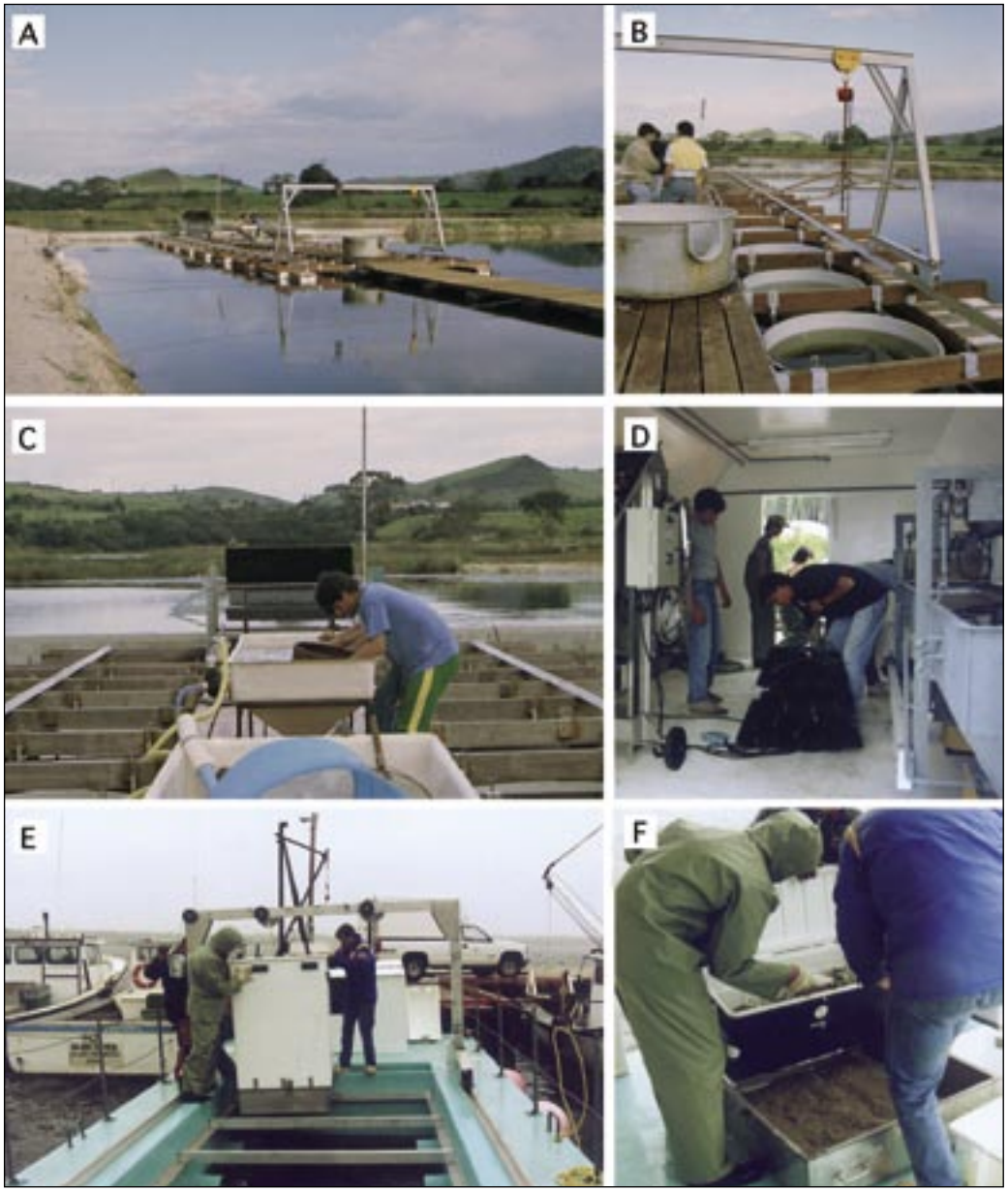

Figure 106: Examples of raft or barge-type nurseries: **A** to **C** – raft floating in a man-made pond connected to a large network of blooming ponds with interconnecting channels (Tinamenor S.A., Pesues, Spain); **B** – detail of the raft showing the cylindrical spat holding cylinders and the lifting device; **C** – the same raft with detail of the paddle wheel which drives water from the raft's discharge channel into the pond on the other side of the dam. Clam spat are being hand-graded on the work platform. **D** – a mechanical seed grader (right) as part of an oyster hatchery/ nursery operation in Atlantic Canada. **E** – a barge operating on the same upwelling principle but in an estuary in Prince Edward Island, Canada. **F** – loading the base of a spat container with small oysters from an insulated "cooler" in which they were transported from the hatchery. In this example the stainless steel base is detachable from the fibreglass body of the container.

involves controlling the blooming of algae and this requires regular observation on some parameter or parameters connected with algal production i.e. determinations of suspended particulate material, either as numbers per unit volume (Figure 105C) or as weight per unit volume, chlorophyll determination, or by microscopy. A reference to methodologies can be found in the suggested reading list at the end of Part 6 (Strickland and Parsons, 1968).

While it is generally possible to raise primary production in ponds to levels significantly higher than those prevailing in the sea it cannot always be guaranteed that the types of algae growing are of the size, digestibility, and nutritional value appropriate to the seed in culture. On occasion it may be necessary to alter the mix of fertilizers being used and "spike" a pond with a sufficient quantity of cultured algae to promote a bloom of the required composition (see section 3.4.6).

6.6.2 Barge-type nurseries

Water flow in barge-type nurseries is generated by low-head (axial flow), propeller pumps, or electrically driven paddle wheels mounted in channels which receive the discharge from the upwelling containers (Figures 103 and 106). The pumps or paddle wheels force water out of the channel(s) back to the surrounding water. This causes a head difference between the level of the surrounding seawater and the lower water level in the discharge channel, with the result that water flows through the mesh bases of the upwelling containers from the outside. The water passes through the bed of contained spat and is discharged into the channel from which it is driven back to the sea or pond.

Regardless of the technology used, careful management must be exercised to match the total biomass of seed held in the unit with the continuity, quantity, and quality of available food. This is dependent on whether the barge is moored within a pond system (Figure 106A–C) or it is floating in an unmanaged saltwater lagoon or in an estuary (Figure 106E–F). The operator can choose between producing large numbers of small seed grown to a moderate size or a smaller number of seed grown to a larger size. Assuming the case of a barge moored in a productive estuary, a flow of between 10 and 20 l per minute per kg of spat should carry a sufficient supply of food to the animals. Each spat holding container (1 m^2 base area), of which there may be up to 32 in a unit, will hold up to 120 kg of seed at the maximum biomass loading, requiring a flow per container of >1 200 l per minute. The total flow per 32-container unit will therefore be in excess of 38 400 l per minute (38.4 m^3 per minute). A paddle wheel is more energy efficient in inducing a flow of this magnitude than is an axial flow, propeller pump. Driving a paddle wheel with an electric motor connected via a gearbox provides scope to vary the total flow according to the size of spat and the total biomass held. Lower flow rates per unit biomass than those quoted above may be appropriate in a managed land-based pond system where levels of algal productivity are higher.

Nurseries of the various types described above are in common usage in Europe and North America as part of well-established regional shellfish industries. There are, however, occasions when smaller nurseries are applicable, for example when a new industry is in the early stages of development or as part of a small owner-operated, vertically integrated business. Small, floating nursery units can be home-built or purchased direct from manufacturers without major financial investment (Figure 107). The operating principle is exactly the same as the large-scale commercial units. They are generally powered by an axial flow pump of about 1 m^3 per minute capacity.

Nursery systems as shown in Figures 104 and 106 require access to a reliable supply of electricity. If power is not available at a remote site or on a barge floating in a

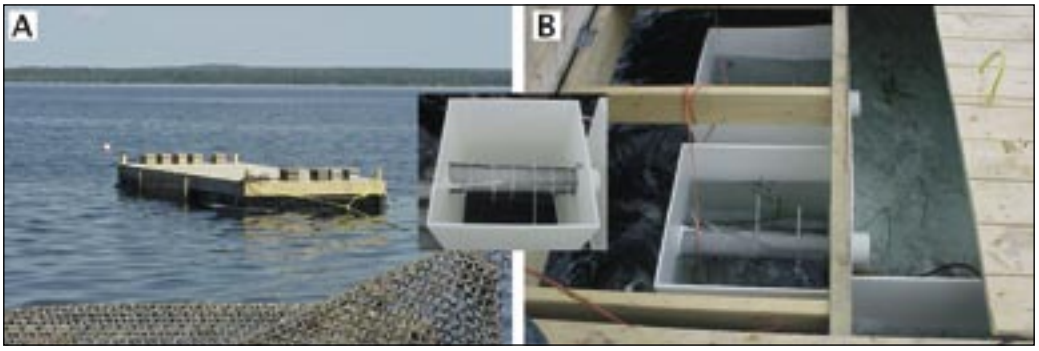

Figure 107: A small, commercially manufactured upwelling nursery powered by an axial flow pump in use at Harwen Oyster Farm, Port Medway, Nova Scotia, Canada. Information on this and similar types using solar power to provide electricity for the pump is available on the internet. The operation of this nursery is exactly the same as previously described.

tidal estuary then tidal power can be harnessed to operate an upwelling system. The principle is known as "FLUPSY" – floating upwelling system – and is illustrated in Figure 108. FLUPSYS require a tidal flow of at least 50 to 100 cm per second to function efficiently.

Figure 108: Tidal powered, floating upwelling systems – "FLUPSYS": **A** – a small experimental unit showing the various components. The unit floats on the water surface buoyed by styrofoam filled flotation pipes (**f**). It swings about a single point mooring (**m** – one of two mooring brackets) to face the direction of the tide so that water is forced into the throat (**t**) of the device and up through the spat container (**sc**). The spat container has a mesh base and may contain a bed of spat or a stack of trays. The forced water flow is discharged at the rear of the spat container with provision to prevent the escape of spat. **B** – a commercial application of the principle where several large "FLUPSYS" are mounted in a raft.

Land-based nurseries have advantages compared with sea-based systems. They function at higher temperatures during the growing season and food supply can be manipulated. The disadvantage is that they are less stable than sea conditions and can be prone to eutrophication if not properly managed. The concept of managed productive seawater pond systems offers much potential for development beyond the application to the bivalve seed nursery. In the foreseeable future, fertilized natural- or artificial-pond systems or coastal inlets enclosed by dams with sluices could be effectively used for the semi-natural production of bivalve seed, in this way bypassing the need for hatcheries. This approach has been used successfully by Atlantic Shellfish Ltd in Ireland and by various companies in Norway.

6.7 SUGGESTED READING

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Part 7

The future of hatcheries: developing technologies

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7.1 GENETICS

Until recently, people simply grew bivalves. In spite of the example of agriculture where over the past several thousand years selective breeding and genetics have produced plants and animals that are far superior to the original wild plants and animals, little selective breeding has been undertaken in bivalve culture operations. This has been due in large measure to the method of culture. Juveniles for most bivalve culture operations are obtained from natural sets and collected from natural breeding areas. They are planted in areas selected for good growth and then the crop is harvested when it reaches commercial size. Bivalves cultured over an extensive area essentially come from the same source and form one large genetic pool. Seed bivalves, whether produced in hatcheries or from natural sets, are frequently transported over considerable distances and even to different countries so that the same gene pool can extend over a wide geographic range. Any regionally distinctive strains or races that might have developed in the past have quickly disappeared in the general gene pool. Development of genetic strains under such circumstances was difficult if not impossible and attempts made to undertake local breeding work were minor.

Studies of population genetics of some species of bivalves have been made. A focus of these studies has been to determine if different sub-populations, races or strains of these species exist throughout the animal's distribution. Results indicate that sub-populations of some bivalves do exist within their range and this raised the question of whether juveniles from one sub-population should be transferred into areas with a different sub-population. It also raises the question of whether animals from one sub-population would perform better if transferred to the area of another sub-population. Population genetic studies have also included assessment of some bivalve populations that over time have become isolated from the parent stock to determine if significant differences now exist in the two populations. A good example is populations of Pacific oysters along the west coast of North America compared to those in Japan where the North American stock originated. Results of these studies indicate that little if any genetic drift has occurred in these widely separated populations.

Our knowledge and interest in the field of bivalve genetics and the potential it has in culture operations has increased greatly in the past twenty years because of two factors;

development of hatcheries and the advent of technology in the field of genetics, e.g. electrophoresis used to examine genetic variation. With the development of bivalve hatcheries it has become possible to undertake selective breeding programs to develop strains or races of bivalves. There is considerable interest in developing strains of bivalves that are better suited to particular growout conditions than the original stock. A further impetus for development of bivalve genetic programs has been the production of strains of oysters that are resistant to the devastating diseases that have decimated stocks in North America and Europe.

The field of bivalve genetics is highly complex and technical and a thorough discussion of work being done currently in the field is not appropriate for this publication. The intent here is to briefly mention the scope of work being done and its implications for hatchery production in the future. A list of reading material is given in section 7.3 to provide the reader with further information on the subject.

7.1.1 Polyploidy

One area of bivalve genetics that has been investigated and is now widely practiced is polyploidy, particularly the production of animals that are triploids (3n). Although triploid scallops, clams and mussels have been produced, most work has centred on the production of triploid oysters and in particular triploid Pacific oysters.

Interest in developing the technology for the production of triploid oysters on the Pacific coast of North America arose for two reasons. First there was the desire by industry to have an oyster that was of good eating quality throughout the year in order to maintain and extend the marketing season. Gonads of Pacific oysters can occupy up to 50% of the weight of the soft body parts. When glycogen is converted into gametes in the spring the oysters become unpalatable and after spawning the soft parts become emaciated and watery. Both states render the product unsuitable for marketing. Secondly, if spawning could be avoided there was possibility of reducing mortalities due to the so called “summer disease” which is believed to result in part from physiological stress at the time of breeding. If transformation of glycogen into gametes can be prevented by growing triploid oysters, it is conceivable that mortalities could be significantly reduced.

Triploids are produced by preventing the egg undergoing meiosis so that it remains in the diploid (2n) state. When such an egg is fertilized by sperm in the 1n (haploid) stage the result is a triploid (Figure 109).

Bivalve eggs can be prevented from undergoing meiosis to the 1n state by subjecting them to pressure, heat or chemicals. Originally most triploids were produced by treating eggs with a chemical, cytochalasin B. Eggs were stripped from females and fertilized with sperm. Gametes were kept separate until ready to fertilize so that the process could be closely controlled. After the first polar body appeared, the fertilized eggs were treated with cytochalasin B preventing the eggs from undergoing meiosis. Thus, the eggs remained in the 2n state and with the contribution of the male chromosome set, the result was a triploid embryo. The technique was perfected over time so that success rate in producing triploids was about 90%.

There are two problems in producing triploids by this method. The first is that it does not produce 100% triploids. The second is that the chemical cytochalasin B is carcinogenic and although it is only used in the fertilization of the animals and hence poses little possibility of carrying over a toxic effect, there has been concern of repercussions from the public. The chemical method to produce triploid oysters is no longer generally used in hatcheries.

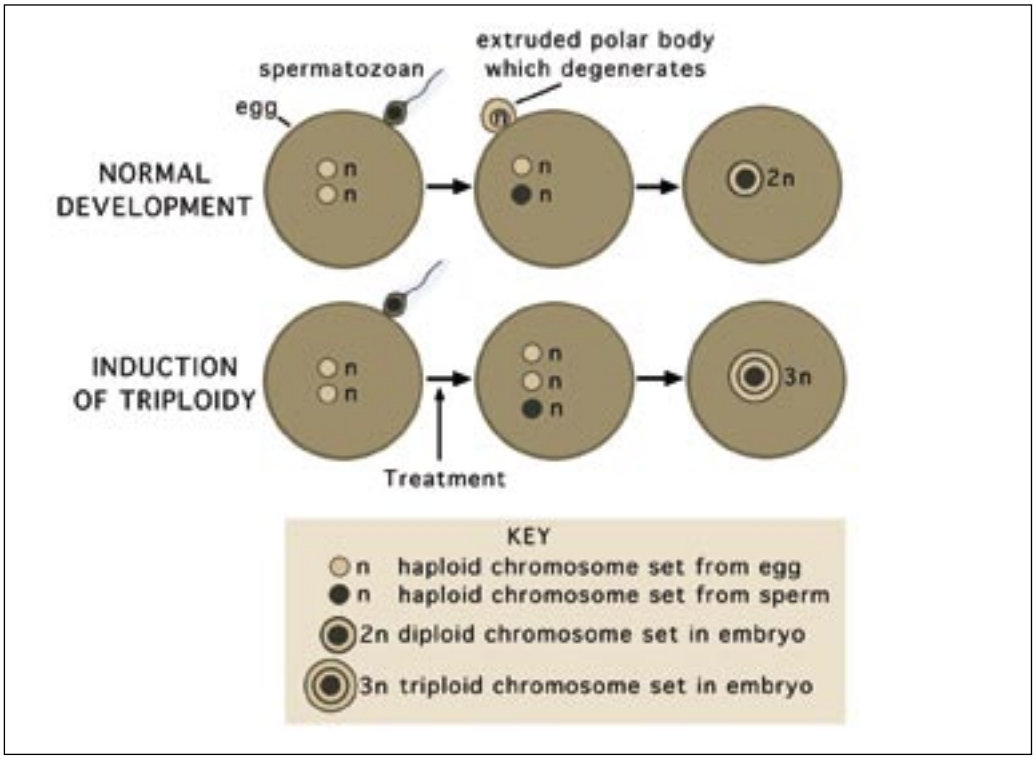

Figure 109: Representation of the process of triploidy induction.

The method now used by some hatcheries is heat shock. Fertilized eggs, normally held at 25°C, are suddenly subjected to a temperature of 32°C for two minutes and then are returned to 25°C. The temperature shock is applied after the emission of the first polar body, about twenty minutes after fertilization. Again this method has been perfected and the success rate in producing triploids is about the same as with the chemical method, i.e. averaging about 90%.

Both the chemical and heat shock methods are effective but the main disadvantage of both is that 100% triploids are rarely, if ever, achieved. A method was needed that could consistently produce 100% triploids with each breeding.

Research in both Europe and the United States has led to development of methods to produce tetraploid (4n) oysters. To date only male tetraploids have been produced and the method is proprietary so few details of the methods can be given. Arrangements can however be made with companies that produce tetraploids to obtain them for use in hatcheries as broodstock. When mated with diploid oysters they always produce triploids. The method is effective and will probably be employed widely by the hatchery and growout industry as tetraploids become more readily available.

On the Pacific coast of the United States a major portion of the production of juvenile Pacific oysters in hatcheries is now triploids.

7.1.2 Quantitative and molecular genetics

Results of polyploidy work have been significant and work in the field will continue, but the real advantage to hatcheries will be in other fields of genetics, e.g. quantitative genetics, which includes selective breeding, and molecular genetics, focussing on the actual genotype of the individual animal. Most people in the industry are expressing interest in the potential arising from selective breeding programmes. The possibility exists to develop disease resistant strains and bivalves which grow faster, produce

more meat per animal and are able to grow quickly at higher or lower temperatures. It should now be possible in aquaculture to approach the example of agriculture where it is estimated there has been a 30% increase in the efficiency in producing protein since 1900 from genetic improvements alone.

Research work in bivalve genetics is being carried out at several institutions in different parts of the world. Most studies involve oysters since this is the animal of most concern to the industry, but research is also being undertaken with other bivalve species. These studies not only focus on producing improved strains of bivalves but they are also concerned with the preservation of the gene pool of original natural populations in the event that such stocks are required for future work.

The goal of much of the research is to improve both the yield per recruit and survival including resistance to disease. There have already been encouraging results. Improvements in the live weight of mass selected Sydney rock oysters, *Saccostrea commercialis*, have been 4% and 18% after one and two generations of selection compared to non-selected controls. A 16% to 39% increase in growth rate was found after one generation of mass selection in the eastern oyster, *C. virginica* and a 21% to 42% increase in growth rate of the European flat oyster, *O. edulis*, compared with non-selected controls. Similarly an increase of about 10% was found in live weight of Pacific oysters, *C. gigas*, after one generation in selected lines compared with non-selected controls. Increases in the resistance of eastern oysters to MSX (*Haplosporidium nelsoni*) have also been reported through selection.

Selected brood-lines of some species of oysters are now established in some countries in the world and work continues to improve them. It is not unrealistic to believe that further selection with these lines will lead to even greater improvements and that eventually the selected stocks will become generally available to hatcheries for use in producing seed stock. One institution on the west coast of the USA is now actively seeking input from industry as to what characteristics industry wishes to have in oysters so that they can begin to incorporate them in specific brood-lines. The possibility of producing a brand name oyster is now not beyond the realms of possibility.

An interesting development in oyster breeding occurred in a programme on the Pacific coast of the United States. The Kumomoto oyster, *Crassostrea sikamea*, was virtually exterminated in its original location in southern Japan. Populations of this species were imported to the west coast of the United States but their gene pool had become contaminated with the Pacific oyster, *C. gigas*. Breeding work at a hatchery facility has enabled production of Kumomoto oyster stocks that breed true and can be used for culture in the USA. They could also be used to re-introduce the species to Japan.

Research in the field of molecular genetics and in modifying specific genes is in its infancy with bivalves. It is a more controversial field compared with selective breeding but advances made in molecular genetics in agriculture are impressive and similar results with bivalves could lead to important advances in production. Research on genetically modified bivalves is being undertaken at several institutions in the world but it will probably be many years before results in this field are considered for application in commercial bivalve hatcheries.

Most research in bivalve genetics is currently being undertaken at university or government facilities. The research is expensive, requires highly trained personnel, considerable space for holding selected lines and may take many years to yield results. Genetic programs must be carefully planned and proper protocols observed or serious problems can arise. Sufficient broodstock must be used in breeding otherwise problems

with inbreeding depression may occur. Before any breeding work is undertaken in the field of genetic improvement, goals must be set and mating schemes and proper broodstock selected. Most commercial hatcheries don't have the time or resources to undertake such long-term programmes, however, they can be active participants.

Improved strains could be developed at commercial hatcheries jointly with research institutes, which could then be mass produced for sale to growers. Certainly, in planning the construction of a hatchery, the need for facilities to carry out genetic work should be kept in mind and incorporated in building plans. With the ability to ship eyed larvae successfully over great distances, larvae of improved strains could be transported anywhere in the world for remote setting and subsequent growout.

The role of genetics in bivalve culture is in its infancy and undoubtedly will become more important to culture operations in the future. Bivalves with faster growth rates, resistance to disease, variously coloured soft parts, oysters with deeper cups, etc. will become a reality in the near future. It will no longer be common practise to simply culture a species of bivalve. Carefully selected strains or breeds will be farmed to produce a specific product to be marketed as a particular brand. The field of bivalve genetics probably offers the best scope to increase production in culture operations throughout the world and every opportunity should be given to encourage research and development in this exciting field.

7.2 THE FUTURE

The increasing demand for seafood, including bivalves, will undoubtedly continue in the future and production will need to be increased to meet this demand. Supply is unlikely to increase significantly from traditional bivalve fisheries since most natural stocks are being harvested at or near maximum rates. Any significant increases in supply will likely come from aquaculture. Indeed, the present goal of many culture operations is to restore populations to levels that existed prior to over-harvesting. Future culture operations will need to be as efficient as possible, not only for reasons of economical viability, but to make optimum use of production areas that will come under ever increasing pressure from human activities and may even be reduced as human populations continue to increase.

Any future increases in bivalve production will require an increase in seed supply that must be reliable, plentiful and inexpensive. Collection of juveniles from natural sets will continue to be important but such areas are limited. Major increases in seed supply will be from hatcheries. There are added advantages in producing seed in hatcheries over the collection of natural sets including reliability, the capability to supply to meet demand and the ability to provide seed of selected strains, along with seed of exotic species.

Continuing research and development will improve hatchery technology and make them more efficient and hence more profitable. There are many areas where research is needed and some have already been mentioned in the text.

Improvements in nutrition are needed to produce healthy larvae that will metamorphose into healthy spat and can be grown quickly and economically to market size. Producing algae to feed the larvae and juveniles is a major cost in operating a hatchery. This expense could be greatly reduced if artificial diets of equal nutritional value to the best algal species could be formulated. Studies have been made but to date, although progress has been made, a satisfactory product is not available for sale. One

of the obstacles is the size of market for such products which, at this time, is not large enough to attract investment in development by the major feed manufacturers.

For bivalve aquaculture to fully achieve its potential it must follow the methods of agriculture. This will require extensive research programmes for all phases of production. One of the most important fields for future research already discussed in section 7.1 is genetics where perhaps the greatest gain will be from the development of strains and varieties of bivalves that are suited to particular environments. This requires extensive research in the selection of brood-lines. Once stains are established they can only effectively be maintained by breeding them in hatcheries. A major goal for hatcheries will be to improve technology so that seed from such strains can be supplied to growers on demand and as inexpensively as possible.

Some developments in the field of genetics such as the production of triploid oysters have already been of major benefit to the industry, particularly the oyster industry on the west coast of North America. Continued improvements in polyploidy will ensure that a reliable supply of triploid seed of any desired bivalve species is available to industry.

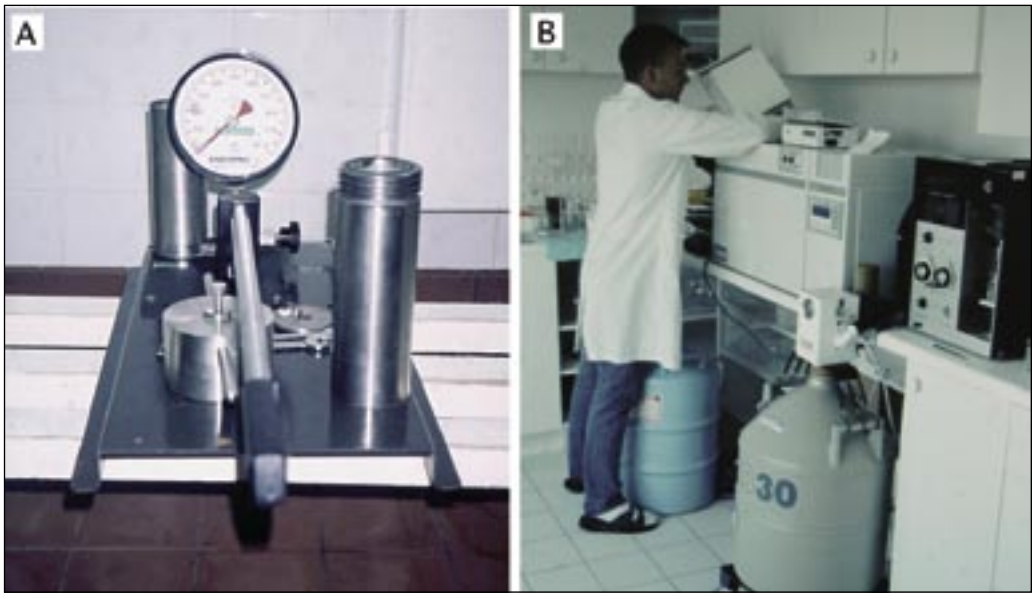

Figure 110: A – a device for exerting pressure on eggs to prevent chromosomal reduction through the suppression of meiosis. **B** – experiments in the cryopreservation of bivalve gametes and larvae.

Developments in cryopreservation technology for male and female gametes and even larvae will be of great benefit to hatcheries since gametes could be obtained when adults are in prime condition and stored for future use. Space and time needed to condition adults and the requirement to produce large quantities of food to keep adults in prime breeding condition could all be eliminated. Fertilization of thawed gametes could be effected in a short time period whenever desired. Progress has been made in this field but at present it is costly and beyond the scope of hatcheries to utilize the technology in-house (Figure 110B).

Siting of hatcheries will assume greater importance in the future. The advent and success of remote setting methods demonstrates that hatcheries do not need to be situated close to growout operations. With modern trade networks they can be located where ideal conditions exist to rear larvae and juveniles and then be transported over great

distances to growout sites with virtually 100% survival. A case in point is provided by the practice of some hatcheries in the State of Washington in the USA. They have transferred part of their hatchery operations to Hawaii where a source of nutrient rich water requiring little if any heating is available year round. The abundant sunshine in Hawaii is used to culture algae. It is cheaper to transport mature larvae and juveniles from Hawaii to Washington State than it is to heat water and grow algae there.

Large hatcheries with highly trained staff can be operated efficiently and produce seed more economically than smaller ones. Economies of scale apply. If hatcheries are equipped with quarantine facilities they can produce seed of any commercially valuable species from any part of the world without major risk of introducing exotics to the local environment. Since larvae are generally cultured in water filtered to 1 μm, which could be treated with UV-light or ozone, the danger of transferring pests, parasites and diseases from one area to another is greatly reduced. This applies to the shipping of eyed larvae compared to shipping juveniles that have been exposed to the open environment in the area of origin.

Large hatcheries could supply metamorphically competent larvae of any bivalve species anywhere it is needed in the world. This is the practice that agriculture has adopted. Seed required in many growing operations is often produced at great distances from where it is eventually planted. Similarly, many juvenile animals are often not produced where they are eventually raised.

It is necessary to get over a parochial attitude in bivalve culture and realize that the industry exists in a global economy. It is no longer essential for every area or even every country to have a bivalve hatchery to supply seed needed to meet local growout requirements. One well-placed, well equipped and well staffed hatchery can supply the seed requirements for many culture operations in many different parts of the world.

A possible major problem for hatcheries will be diseases as it is when any organism is mass cultured intensively. Future research needs to include the development of methods to control diseases in hatcheries so as to minimize instances of mass mortalities caused by either obligate or opportunistic pathogens. Results of genetic research are likely to be of value in selecting strains of bivalves that are more resistant to disease. Research is also required to develop inexpensive and effective treatments should diseases occur in a hatchery situation.

Future bivalve landings will undoubtedly continue to increase to meet the demands of an ever increasing human population. Most of this increase in production will be from culture operations and this will require the availability of large quantities of juveniles (seed) to meet culture demands. While collection of seed from natural sources will remain important, most of the seed needed for increased production will be from hatcheries. This is particularly true as the industry begins to demand strains or races of bivalves that are developed to grow in specific areas. Hatcheries will eventually become the mainstay of seed production for bivalve growout operations. In the future every effort must be made to improve hatchery technology to enable them to supply an abundant, reliable and inexpensive supply of juvenile bivalves for the culture industry.

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