Cresko Laboratory Manual

Cresko Lab

2021-05-06

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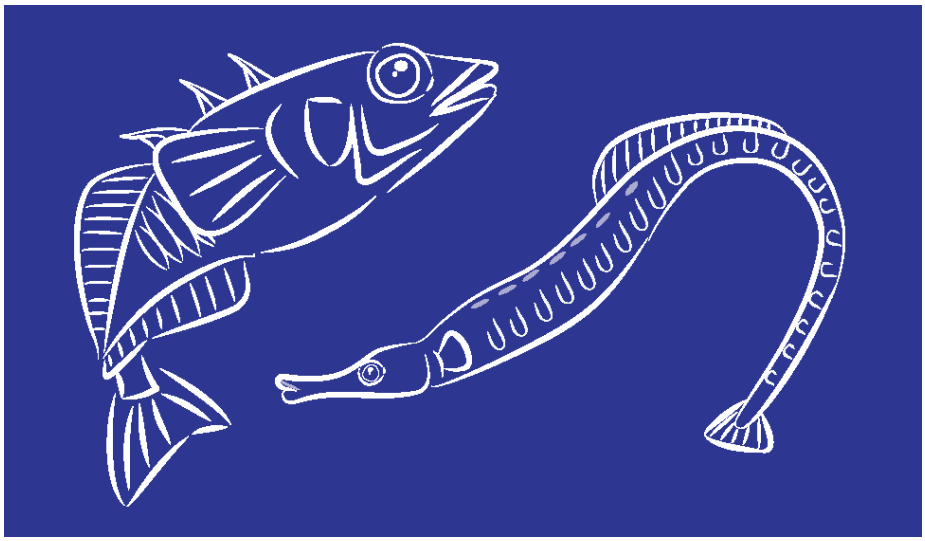

Figure 2.1: Illustration by Dr. Allison Fuiten

We are an intellectual community of geneticists who specializes in quantitative evolutionary genomics. Our laboratory studies the developmental genetic and genomic basis of evolution in natural populations. We use the threespine stickleback and zebrafish as the main animal models in the laboratory, as well as syngnathid. We have produced some of the first work that has helped develop stickleback into a model for dissecting the genetic basis of natural variation. We have developed genomic tools such as sequenced Restriction site Associated DNA (RAD) tags that help geneticists apply Next Generation Sequencing (NGS) technologies to biomedical and evolutionary genetic problems. These techniques

allow for the efficient identification of thousands of single nucleotide polymorphisms (SNPs) throughout the genomes of models and non-model organisms. We produced the first SNP whole genome-scan for selection in the stickleback genome, and we developed novel Maximum Likelihood (ML) analytical tools for NGS data. Computational biologists and computer scientists in our team have produced software packages for genomic analyses that are used by laboratories around the world for the analysis of big data problems. Our laboratory has developed protocols, best practices, and tools for RNA-seq based transcriptomic functional analyses.

Chapter 3

Mission and Vision

Figure 3.1: Willametter River

We describe our methods in this chapter.

Chapter 4

Lab Expectations

4.1 Scientific Ethics and Integrity

- xx
- xx
- xx

4.2 Authorship of Manuscripts

Recommended: At the start of each project, design your plan for authorship of the project so everyone knows the expectations

Authorship criteria:

1) Makes a significant intellectual contribution to research ideas and experimental design

OR

2) Makes a significant contribution to data acquisition, data generation, data analysis, data interpretation, research coordination, and/or financial support of research

AND

3) Contributes to writing part of the manuscript, in addition to editing revisions before submission for publication

AND

4) Remains involved throughout the submission and revision process until final publication

*Research participants not meeting the criteria should be listed in the Acknowledgments section of the final published manuscript

Authorship order:

Generally, the person who had the most significant contribution to the project and who does most of the writing will be the first author. In ecology, the last author is generally the PI of the lab (although not always). The remaining authors are usually listed in their order of contribution. However, if contributions were equivalent, then co-authors can be alphabetized or ordered according to their time since involvement in the project.

Chapter 5

Cresko Lab Safety protocols

FOR YOUR OWN SAFETY AND THE SAFETY OF OTHERS, HEED THE FOLLOWING RULES!

EMERGENCY CONTACT: dial 911 first, *AND* 6-2919 (EHS) | Mark Cell 541-505-0006

Safety Shower, Eyewash, Fire Extinguishers.

Eyewashes must be flushed weekly. *Undergraduate research assistants are responsible for flushing the safety showers each week.* _Each lab member is responsible for knowing the locations of safety showers and fire extinguishers in the lab. Safety showers and fire extinguishers are tested annually by EHS.

Wear a lab coat and closed-toed shoes when working with the following chemicals:

- organics (e.g. phenol/chloroform, Trizol, DNAzol, formaldehyde, formamide, methanol)
- strong acids and bases

Wear eye protection when working with:

- UV light (UV opaque glasses/face shield)
- phenol/chloroform, strong acids/bases, and any splash hazard with anything hazardous in it.

Wear safety gloves when working with ANY of the reagents above.

Heed the "one glove rule": remove one glove when moving between rooms to avoid touching doorknobs with a contaminated glove. Note that glove materials differ in their permeability to different reagents. Standard nitrile gloves are adequate for our lab's standard procedures. However, if you are planning

experiments that involve more dangerous reagents, consult with Luke Sitts at EHS to select appropriate gloves.

Disposal of common hazardous reagents (EHS DISPOSAL: 6-3192)

- E. coli plates and recombinant materials: autoclave buckets or EHS biohazard incineration boxes
- E. coli flasks/liquids: bleach, rinse, drain
- Used alcohols, formaldehyde, and kit waste: waste containers under the thermocyclers.
- organic solvents: waste bottles in hood.

Storage of Hazardous Liquids

- Store flammables and strong acids in a latched METAL SAFETY CABINET UNDER THE HOOD.

Heating Liquids in the Microwave Oven**

Triple check that the cap is *very* loose or (better) remove it entirely. Remelting of gels with DNA binding dyes is forbidden.

Bunsen Burners

- Triple check that the gas is shut completely off before you leave the bench/hood.
- keep burners far away from any flammable liquids.

Liquid Nitrogen and Dry Ice

- Use only in well ventilated spaces to avoid asphyxiation.
- Never store in sealed containers to avoid explosions
- Wear lab coat, gloves, goggles. In case of frostbite or burn, soak affected part in tepid water, seek medical attention

Chapter 6

Lone worker guidelines

The UO Laboratory Safety Advisory Committee (LSAC) feels that working alone in laboratories should be discouraged but recognizes that a prohibition would hinder the research and education missions of the UO. To advance personnel safety while also recognizing research needs, the LSAC developed this guidance document to assist lab workers in recognizing dangers and developing appropriate procedures.

The primary danger in working alone is that if an accident should occur, there will be delays in rendering aid.

Before working alone, you should:

1. Ensure that you have been trained on the procedures, reviewed the safety data sheets for all associate materials, and know the emergency procedures for your lab.
2. Consider whether the risk outweighs the benefits of working alone.
3. Consider whether this work can be done at a time when others are around.
4. Consider using a buddy system with individuals in other labs nearby.

If you decide to proceed with hazardous procedures on your own, please use a check-in or text-in system with supervisors or peers, ensuring that they know where and when this work is done and that they have contact information readily available for campus safety personnel.

SPECIFIC GUIDELINES FOR THIS LABORATORY

Examples of materials and procedures in THIS laboratory that should be avoided while working alone are provided below. Should you choose to do lone work of this nature, ensure that others know where and when this work will be performed, and when it is completed.

Building & Room: *Pacific 310 & 324*___ Supervisor: ___Dr. William Cresko_

In this laboratory these chemicals or procedures will not be used or done while working alone:

The use of phenol chloroform and the movement of glass aquariums will not be done while working alone in Pacific 310 or 324.

In this laboratory, these procedures will not be conducted while working alone:

Procedures that require the use of phenol chloroform or the movement of glass aquariums

Other safety considerations for working alone in this laboratory (add pages as needed):

Emergencies – Dial 911 Lab Safety Coordinator: Safety and Risk Services/EHS: 541-346-3192

Chapter 7

Husbandry stickleback crossing

SOP - Fish Density Standards (created by M Currey, August 25, 2011, updated by mcc 151125)

These standards for fish densities are what we in the Cresko lab have determined optimal after 12 years of keeping threespine stickleback in a recirculating aquaculture system.

Fry (9dpf - 2 months):

• Fry are kept in 2.8 L tanks at an ideal density of 20 fish per container. Fry can be kept in densities of up to 40 fish per tank. If fish densities are near 40 per tank fish are transferred or thinned after 1 month of age.

Juvenile (2 months - 4 months) Grow Out:

• Juvenile fish are transferred from fry tanks into 9.5 L tanks and kept at a density of 20 fish per container.

Grow out (4 months – 1 year), Adult (1 year - 1.5 years) and Breeding conditioning:

• Grow out: Juvenile fish are transferred to 20 gallon tanks in the Winter room at an ideal density of 20 fish per container. Fish can be kept at a density of 40 fish per tank if space is needed. • Breeding Conditioning: Once fish are 1 year of age (or older), transfer adult fish to the Summer room. Stickleback males become sexually mature 2-6 weeks after experiencing Summer conditions, females become sexually mature 4-6 weeks after experiencing Summer conditions conditions. Conditioned fish can stay in the Summer room for 5 months.

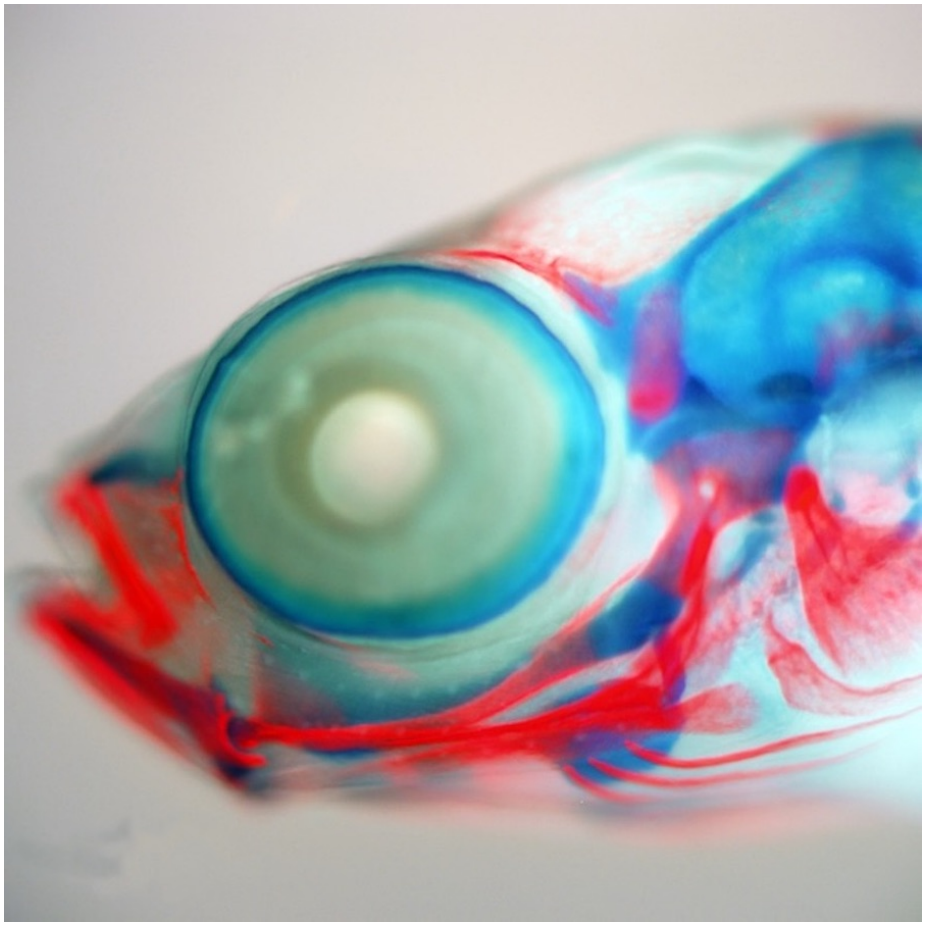

Figure 7.1: Photo by Mark Currey

Chapter 8

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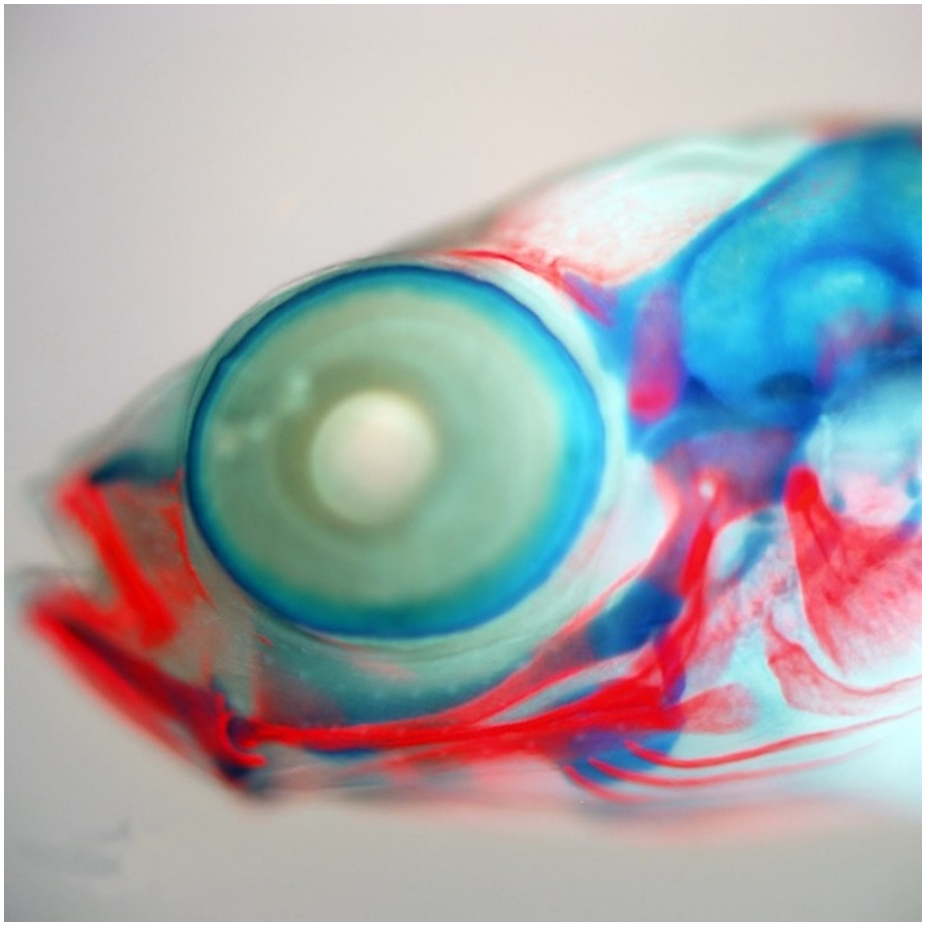

Figure 8.1: Photo by Mark Currey

8.1 Embryo Bleaching for Disinfection

(created by M Currey, August 25, 2011, updated 151201 by mcc)

8.1.1 Purpose:

This protocol allows the removal of ecto-parasites from embryos produced from wild-caught stickleback. Use this protocol whenever new fish are added to a pre-existing stickleback rearing system, or whenever stocks are transferred between labs.

8.1.2 Materials needed:

• Wash bottle and petri dishes • Timer

8.2 Solutions

• 6% sodium hypochlorite (standard bleach) • Working stock of bleach – 500µl of bleach into 1 liter of embryo medium (see rearing protocols)

8.3 Procedure:

1. Raise embryos according to standard crossing and rearing protocols (see husbandry SOP)
2. At 48-60 hours post-fertilization (@ 20C – at this point most organogenesis is complete) remove embryo medium from petri dishes and rinse embryos once with fresh embryo medium.
3. Fill petri dish with working stock solution of bleach. Swirl and let sit for 1.5 min. Do not bleach embryos for longer as chorions can thicken and it becomes difficult for embryos to hatch.
4. Drain bleach solution from embryos, and wash them three times with fresh embryo medium. This is really important.
5. Replace embryo medium daily. If crosses are done in the field, the embryos can be shipped from day 3 to day 6 post-fertilization. To ship embryos, rinse once with embryo medium, place embryos into a 50ml conical tube (~100 embryos/tube) and fill with fresh embryo medium. Place tubes in a shipping container with wet ice. Separate the tubes from the ice with bubble-wrap and newspapers. Ship the embryos overnight.
6. When the embryos arrive at the lab, rinse the outside of the containers well before moving into the lab. Allow them to equilibrate to room temp

(~20C). Place the embryos into petri dishes, rinse once, and add new embryo medium. Rear according to standard protocol.

8.4 SOP – Testes Storage

(created by M Currey, August 25, 2011, updated 151201 by mcc)

8.4.1 Purpose: Storage of stickleback testes for fertilization up to 1-2 months post extraction.

Materials needed: • Testes solution • 15 ml falcon tube • 4 C refrigerator

8.4.2 Procedure:

1. Dissect testes (see Stickleback crossing SOP).
2. Place testes in 15 ml tube with ~ 10 ml of testes solution.
3. Label tube with stock number and date of testes dissection and place tube with testes in 4 C refrigerator.
4. Change testes solution once per week.

Note: Testes can be used up to ~ 1 month when stored this way.

8.5 Egg Storage

(created by M Currey, August 25, 2011, updated 151201 by mcc)

8.5.1 Purpose: Storage of eggs for fertilization up to 24 hours post stripping.

Materials needed: • NaCl • KCl • CaCl2 • NaHCO3 • Tris, pH 7.2 • npH2O • Streptomycin & Penicillin Solution (PenStrep) from Sigma (P-0906) Stock – 100% • 50 ml sterile polypropylene conical tubes • 90 mm petri dish

8.5.2 Procedure:

1. Make stock of Holtfreiter's Solution, store at 4C

   Mix solids into 750ml of npH2O 3.5 gm NaCl 0.05 gm KCl 0.1 gm CaCl2 0.02 gm NaHCO3 1.0 ml Tris Bring to 1 liter with npH2O

2. Add PenStrep to a final concentration of 1% (10ml into 1 liter), store at 4C.

3. Strip eggs from ripe female into a conical tube with 25ml cold Holtfreiters.

4. Eggs can be transported on ice and fertilized up to 24 hours later (maybe longer).
5. To fertilize, move eggs to 90mm petri dish and allow temperature to equilibrate to 20C.
6. Remove Holtfreiter's solution, and rinse eggs once with embryo medium (important to. make sure that salt concentration is low enough for sperm to be optimally activated).
7. Add macerated testis as in general husbandry SOP and cover eggs with embryo medium. SOP - Live Food Culture, Moina and Mysid Shrimp: (updated 151201 by mcc)

Chapter 9

Husbandry_stickleback_food

9.1 Stickleback Feeding

(created May 6, 2008 by m currey, revised October 21, 2014 by mcc)

9.1.1 Materials:

- Fish food mix or dry fry food
- Decapsulated Artemia (see artemia decapsulating SOP)
- Color coded feeding Spoon

9.1.2 Fish foods for fry and juvenile/adults:

- Fry - newly hatched baby brine shrimp (see hatching brine shrimp SOP) and Zeigler Larval Diet (Larva "Z" Plus 250-450 Microns).
- Juvenile and Adult fish food mix (see fish food mix SOP).

Dry Foods Are Stored in freezer in the stickleback facility

Procedure: - Fry are located along the south wall of the summer room. - AM feeding: Feed fry once per day with newly hatched brine shrimp (this should happen during the opposite feeding of larval diet) - PM feeding: Feed fry once per day with Ziegler larval diet 1/8 spoon full. - Juvenile and Adults are located in the 20 gallon tanks in the Summer and Winter rooms. Additionally, individualized adults are sometimes located on the fry rack in the Summer room. Please check white board for notification of adults on fry rack. - Feed juveniles and adults twice per day with dry food mix. Using the color coding on spoon, food mix, and tags on tanks. (e.g. for adults: use the red spoon, red food mix, and

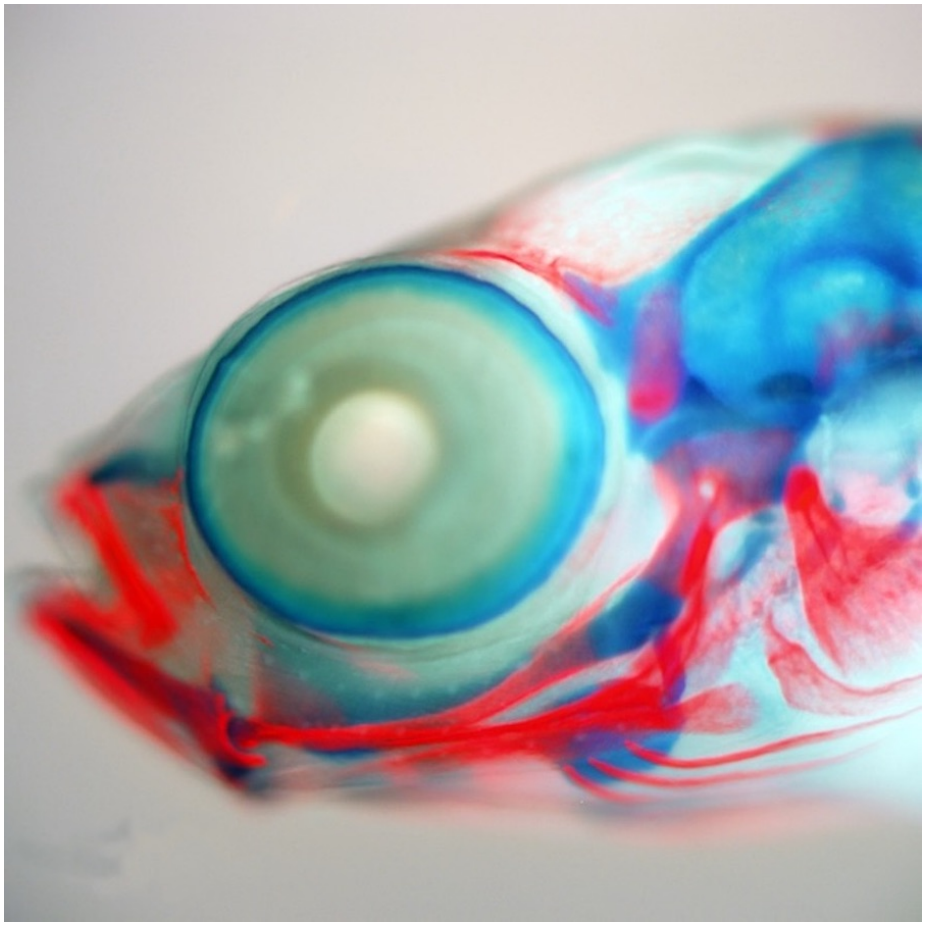

Figure 9.1: Photo by Mark Currey

feed the tanks with a red tag). - Unused brine shrimp can be fed to juvenile and adult fish.

9.1.3 Food Storage and Handling:

- See Fish food storage SOP.

Initial daily check list after you have fed.

9.2 Fish Food Mix

(created August 25, 2011 m currey)

9.2.1 Materials:

- Freezer
- Fridge
- 1 gallon bucket with a tight seal
- plastic measuring cup

9.2.2 Dry Mix:

- Mix the following dry foods in 1 gallon bucket

9.2.3 Juvenile Mix:

- 4 cups Pentair finfish starter ZC1
- 1 cup New Life Spectrum optimum saltwater flake
- 1 cup Ziegler AP100

9.2.4 Adult mix:

- 4 cups Pentair finfish starter ZP1
- 4 cups Pentair finfish starter ZC1
- 1 cup New Life Spectrum grow
- 1 cup New Life Spectrum optimum saltwater flake
- 1 cup Hikari micro pellet
- 1 cup Hikari marine S
- 1/8 cup golden pearls

9.2.5 Storage:

- Label with expiration date (6 months after making) and store at -20°C.

9.3 Fish Food: Storage and Sources

(created by M Currey, August 25, 2011)

Label all foods with received date and expiration date (see below for how to determine expiration date).

9.3.1 Brine Shrimp:

Good indefinitely if frozen in tightly sealed container

1 Upon receiving label with received date. Store unopened tins in -20°C freezer. After de-capsulation label with date de-capsulated and date of expiration (30 days from de-capsulation). Store de-capsulated shrimp at 4°C.

Source – Brine Shrimp Direct, www.brineshrimpdirect.com

9.3.2 Golden Pearl Larval Diet:

1. 800 – 1000 micron

Good 3-5 years if kept in freezer

1. Store in -20°C freezer.
2. Unopened label with expiration date 3 years from receiving date.
3. Upon opening change expiration date to 6 months from date opened.

Source – Brine Shrimp Direct, www.brineshrimpdirect.com

9.3.3 Hikari dry foods:

• Marine S • Micro Pellets

If un-opened, expiration date is labeled on container by manufacturer. Once opened the food is good for 6 months.

1. Keep out of direct sunlight, high heat, and humidity.
2. Store at 4°C.
3. Upon opening change expiration date to 6 months from date opened.

Source – Pet Mountain, www.petmountain.com That Pet Place: http://www.thatpetplace.com

New Life Spectrum dry foods:

2. Optimum saltwater flakes
3. Growth Formula

If un-opened, expiration date is labeled on container by manufacturer. Once opened the food is good for 6 months.

• Keep out of direct sunlight, high heat, and humidity. • Store at 4°C. • Upon opening change expiration date to 6 months from date opened.

Source – Jehmco, www.jehmco.com

9.3.4 Zeigler Larval dry food:

1. AP100 (150-250 microns)

Good 2 years if kept unopened and in freezer.

• Upon receiving label with received date and expiration date (2 years from received date). • Store at 4°C. • Upon opening change expiration date to 6 months from date opened.

Source – Aquatic Ecosystems, http://pentairaes.com Pentair Finfish Starter dry foods:

2. ZP1 - 1.5 mm slow sinking pellet
3. ZC1 – 0.6 – 0.85 mm #1 crumble

If un-opened, expiration date is labeled on container by manufacturer. Once opened the food is good for 6 months.

• Keep out of direct sunlight, high heat, and humidity. • Store at 4°C. • Upon opening change expiration date to 6 months from date opened.

Source – Aquatic Ecosystems, http://pentairaes.com

9.3.5 Selcon (brine shrimp supplement):

Good 1 year if kept unopened and in fridge.

1. Upon receiving label with received date and expiration date (1 years from received date).
2. Store at 4°C.

Source – Aquatic Ecosystems, http://www.aquaticeco.com/

9.3.6 Frozen Mysid and Blood Worms:

Expiration date printed on front label.

- Upon receiving label with received.
- Store at -20°C.

Source – Nautilus Tropical Fish – Springfield Oregon, 727 Main St. 541-344-3474

9.4 Hatching and Feeding Brine Shrimp

(created by M Currey 5/6/08, updated 151201 mcc)

9.4.1 Materials Needed:

- De-capsulated Brine Shrimp
- Rock Salt
- 105 m mesh shrimp collector
- Baking Soda
- Squirt Bottle

9.4.2 Procedure:

1. Collect Brine Shrimp: Drain entire cone into 105 m mesh shrimp collector (located on shelf near cone). Rinse and pour shrimp into squirt bottle. Feed fish. Rinse squirt bottle and place on shelf to dry.
2. Reset Brine Cone: Fill cone with DI water to 10 L. Put airline and heater (set at 80°F) into cone. Add 300 ml of rock salt and 1 scoop (5 ml) of baking soda. Obtain de-capsulated brine from refrigerator and shake to homogenize solution. Measure out 150 ml* of de-capsulated brine and add it to the cone.
3. Wait 24 hours and repeat….

- The amount of brine shrimp needed will vary depending on the number of juvenile fish that need to be fed. If there is a need for more brine shrimp add more de-capsulated brine to cone and leave a note for the next person. Increase the amount in 50 ml increments. *

9.5 Artemia Decapsualtion

(Adapted by M Currey 4/3/08, 151201 updated by mcc)

9.5.1 Materials:

- 15 oz can of dried Artemia cysts (approximately 430 g)
- 4.3 L ~6% laundry grade bleach
- Rock Salt (NaCl)
- 125 ml 40% Lye (NaOH) solution
- 30.0 g Sodium thiosulfate (Na2S2O3)
- 16 L Hatching Cone with aeration
- 125 m mesh bag (Aquatic Eco-Systems PMB3, 125 micron x 18”)
- Several 3-5 L beakers
- (1-2) Squirt bottles - squeeze type

9.6 Solutions:

- Solutions should be prepared in advance *

- Bleach, ~6% laundry grade
- 25 ppt Salt Solution:

1. Combine: 50 g Rock Salt (NaCl) To 2.0 L with tap water
2. Stir to dissolve completely.

- 40% Lye (NaOH) solution

1. Combine: 200 g Lye (NaOH) To 500 mL with tap water
2. Stir to dissolve completely.
3. Store in refrigerator (4°C)

- Buffered Salt Solution

1. Combine: 2L, 25 ppt Salt Solution
2. 125 mL 40% Lye Solution, pre-chilled to 4°C

- 1.0% Sodium Thiosulfate

1. Combine: 30 g sodium thiosulfate To 3.0 L with tap water
2. Stir to dissolve.

- Saturated Brine

1. Combine: ~25g Rock Salt to 4.0 L with tap water
2. Aerate to dissolve.

9.6.1 Procedure:

1. Cyst hydration: Hydrate one full can of dried cyst in 5 L of tap water in a hatching cone with aeration for 1 hour at room temp. Examine the cyst under a dissecting scope with top lighting before proceeding. Dry cysts are dimpled, resembling a deflated basketball, whereas fully hydrated cysts are completely spherical in shape. The cysts must be fully hydrated prior to the de-capsulation step. If cysts are not completely spherical after 1 hour, continue the hydration process (for a maximum of 2 hours), checking the progress of the cysts under a microscope every 15 min.
2. Filter and rinse cysts: Collect the hydrated cyst in a 125 um mesh bag and rinse with cool tap water.
3. Transfer cysts back to the cone: Add the Buffered Salt Solution to the cone and aerate (save back a filled squirt bottle of salt solution to help transfer cysts to cone). Transfer cysts into cone.
4. De-capsulation: Add the bleach (4.3 L) to the cone and continue aeration. Watch the cysts turn from brown to grey to orange, When the cysts are 90% orange, stop the reaction by quickly siphoning the cysts through a 125 um mesh bag and rinsing well with cool tap water.
5. Neutralization residual chlorine: To neutralize any residual chlorine transfer the mesh bag to a clean 4 L beaker and pour the 1.0% Sodium Thiosulfate (3L) into the bag. Soak the cysts in the sodium thiosulfate solution

for ~1 min, then rinse the cysts with de-ionized tap water. Rinse until discharge turns clear.

6. Dehydration for long-term storage: Transfer the cysts back to the cone with 4 L of saturated brine and aerate until salt is dissolved. Transfer dehydrated cyst to (5 or 6) 1 L Nalgene bottles filled with 200 - 300 grams of salt. Add enough salt so that it does not dissolve when de-capsulated brine is added. Fill the bottles with de-capsulated brine. Store in refrigerator. The de-capsulated brine will store for at least 1 month. Hatch brine as you would capsulated brine (see Hatching and Feeding Brine SOP).

Chapter 10

Recipes

10.1 Embryo Medium

10.1.1 Material Needed:

- Instant Ocean Salt
- Baking Soda
- npH2O

10.1.2 Embryo Medium solution:

1. Add 8g Instant Ocean to 2 liters of npH2O
2. Add ~0.5g baking soda
3. Check pH and adjust to 7.0 – 8.0
4. This makes 2 liters

- Salt and baking soda are located in containers near the dissecting scope. Rinse 2 liter flasks with DI water between uses. _____

10.2 MESAB

Tricaine must be pharmaceutical-grade. We use tricaine purchased from Pentair, manufactured by Western Chemical and FDA approved. Tricaine (3-amino benzoic acid ethyl lester also called ethyl m-aminoboenzoate) comes in a powdered form. Purchase the smallest amount possible because tricaine expires quickly.

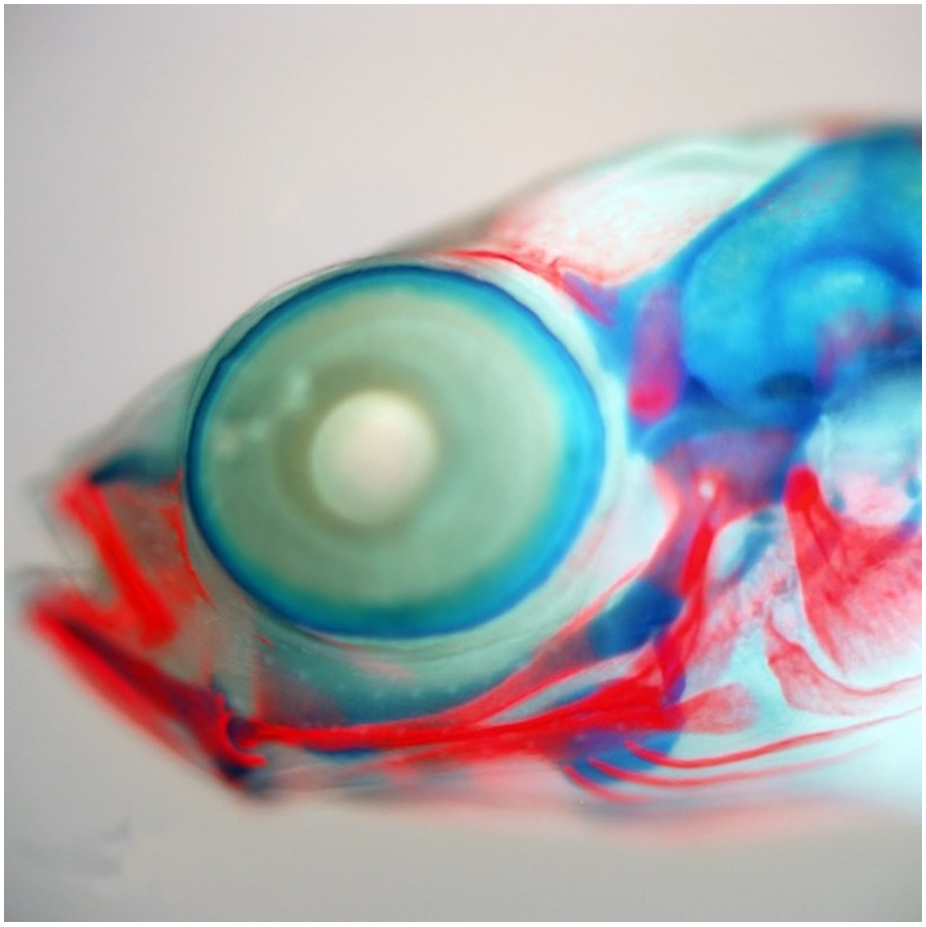

Figure 10.1: Photo by Mark Currey

10.2.1 Material Needed:

- Mesab, a.k.a. MS222, tricaine, or 3-aminobenzoic acid ethyl ester
- 1 M Tris (pH 9)
- DD water

10.2.2 Mesab Stock Solution (4g/L) (tris buffered):

- 1. 4 g tricaine powder
- 2. 979 ml DD water
- ~21 ml 1 M Tris (pH 9)
- Adjust pH to ~7
- Aliquot in 50ml tubes, label with MESAB Stock Solution 4g/L, and store in a -20 freezer
- This makes 1 liter of solution.

10.2.3 Euthanasia Solution (300 mg/L):

1. Make a solution of tris buffered Stock Solution as described above. (Or obtain an aliquot from the freezer)
2. Combine 7.5ml of stock solution into 100 ml of fish water.

10.2.4 Anesthesia (168 mg/L):

1. Make a solution of tris buffered Stock Solution as described above. (Or obtain an aliquot from the freezer)
2. Combine 4.2 ml of stock solution into 100 ml of fish water.

10.3 Testes Storage Solution

10.3.1 Materials Needed:

1. NaCl
2. KCl
3. CaCl2
4. NaHCO3
5. npH2O
6. Gentamycin (antimycotic) (Stock – 10mg/ml)*
7. Cell Culture anti-biotic/mycotic from Gibco-BRL (15240-096) 100x Concentration*.

- Both of these reagents are located in separate boxes in Mark's space in the
- 20° C freezer. They are partitioned into 100 l aliquots.

10.3.2 Solutions:

Ginzberg's Ringers - Mix solids into 750 ml of npH2O - 6.6g NaCl - 0.25g KCl - 0.3g CaCl2 - 0.2g NaHCO3 - Bring to 1 liter total volume with npH2O. - Store at 4° C.

10.3.3 Testes solution (100ml)

• Add 100 l of Gentamycin and 100 l of Anti-biotic/mycotic to 100ml of Ginzburg's Ringers solution. • Store at 4° C.

Chapter 11

Pipefish Husbandry Protocols

Figure 11.1: Photo by Mark Currey

11.1 Pipefish Feeding

(created by M Currey 7/23/09)

Materials Needed:

- Decapsualted Brine Shrimp (see artemia decapsulations SOP)

- Adult Brine Shrimp
- Live Moina
- Frozen myisid Shrimp
- Live mysid shrimp
- Shrimp collector
- Squirt Bottle
-

11.1.1 Fish foods for fry, juvenile and adults:

- Fry - newly hatched baby brine shrimp (see hatching brine shrimp SOP), salt water copepods. Fry are fed once per day
- Adult – newly hatched brine shrimp, Adult brine shrimp, Moina. Adults are fed once per day. Feed adult brine shrimp when we have them. Use moina when we are out of adult brine shrimp. Adult brine shrimp are from a local fish store and are only available every tow weeks. They last ~ one week and therefore adult pipefish are fed adult brine shrimp for one week and moina the next.

Fry:

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Fry tanks are designated with an orange dot.
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1. Newly hatched brine: Collect newly hatched brine and place into a squirt bottle (see brine shrimp SOP). Feed all tanks with an orange dot.

Adults:

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Adult tanks are designated with a yellow dot.
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1. Newly hatched brine: Collect newly hatched brine and place into a squirt bottle (see brine shrimp SOP). Feed all tanks with an orange dot.
2. Frozen Mysis: Obtain a quarter-sized piece of frozen mysis from the freezer. Place into squirt bottle and add water. Wait until mysis thaws and feed to all adult tanks.
3. Adult Brine shrimp: Scoop out adult brine shrimp with net. Wash into a ball and place over the top of squirt bottle. Wash ball of brine into squirt bottle and feed all adult pipefish.
4. Moina: Scoop out with net and wash into a ball. Invert ball over collection beaker and wash moina into beaker. Pour moina into squirt bottle and feed.
5. Live Mysid: See live foods SOP

11.2 Live Food Culture, Monia and Mysid Shrimp:

Moina

Materials:

- 10 gallon glass tanks
- corner sponge filter
- Air supply
- Rotifer diet
- Powdered nannochloropsis

Procedure:

- Fill 10 gallon tank 3/4 full of stickleback system water
- Add corner filter and activate with air.
- Add Moina
- Change water once every 2-3 weeks by removing half of the water and replacing with stickleback water.
- DO NOT break tank down and clean as moina do not respond well to this.

Feeding:

- Add 15 drops of rotifer diet and 1/8 scoop of powdered nannochloropsis each day.

11.2.0.0.0.1 Collection and feeding to fish

- See pipefish feeding SOP

11.2.0.0.0.2 ***Mysid Shrimp*** For a description of the mysid generator please visit:

http://www.mblaquaculture.com/assets/docs/MBL_AQ_Mysid_Generator.pdf

Materials:

- 10 gallon tank generator system
- Salt water

Feeding:

- Feed newly hatched brine shrimp daily to both adults and juveniles.

Water Change:

- 2-3 times per week empty 5 gallons of water from the system and replace with new make up water.

- Make new water in 5 gallon bucket by adding DI water and 2 scoops of salt.

Juvenile Collection (Daily):

- Turn off water to tanks.
- Remove collection cup, using mysid system water, rinse juveniles into plastic container.
- Pour juveniles into grow out tank.
- Replace collection cup.
- Turn water on and start siphon.

Adults collection and feeding to pipefish:

Juvenile will reach adult size in three weeks. At three weeks these new adults will replace old breeding adults. The old breeding adults that are being replaced are feed to the pipefish.

- Let juveniles grow to three weeks at which point they reach adult stage
- Siphon adults through a net and collect in a container.
- Siphon old adults out of one of the 10 gallon tanks and feed to pipefish
- Clean tank, fill with water and add new adult.

11.3 Live Food Culture, Monia and Mysid Shrimp:

Moina

Materials:

- 10 gallon glass tanks
- corner sponge filter
- Air supply
- Rotifer diet
- Powdered nannochloropsis

Procedure:

- Fill 10 gallon tank 3/4 full of stickleback system water
- Add corner filter and activate with air.
- Add Moina
- Change water once every 2-3 weeks by removing half of the water and replacing with stickleback water.
- DO NOT break tank down and clean as moina do not respond well to this.

Feeding:

- Add 15 drops of rotifer diet and 1/8 scoop of powdered nannochloropsis each day.

11.3.0.0.0.1 Collection and feeding to fish

- See pipefish feeding SOP

11.3.0.0.0.2 ***Mysid Shrimp*** For a description of the mysid generator please visit:

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- 10 gallon tank generator system
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- Siphon adults through a net and collect in a container.
- Siphon old adults out of one of the 10 gallon tanks and feed to pipefish
- Clean tank, fill with water and add new adult.

Chapter 12

Histological Protocols

12.1 Alizarin Staining

12.1.1 PURPOSE: Alizarin staining of fixed adult stickleback.

12.1.2 MATERIALS:

- 0.5% Alizarin red S Stock: To make 50 mls add 0.25g alizarin red S powder to 50 ml water.
- 0.025% Alizarin Stain: To make 100 mls: Add 500µl 0.5% alizarin red S (stock) to 99.5ml 1% KOH
- 1 Liter: Add 5ml 0.5% alizarin red S (stock) to 9950ml (1 liter) 1%KOH
- 3% H202/0.5%KOH: Mix and keep at 4C; Before using, bring to room temperature to hold down
- introducing bubbles under the skin: 0.5ml 6%H202 & 0.5ml 1%KOH.
- MESAB: Tricaine: 3-amino benzoic acid ethyl ester from Sigma (Cat # A-5040). Mix in fish safe container with a stir bar:
  - 400 mg tricaine powder
  - 800 mg Na2HPO4 (anhydrous)
  - 100 ml glass distilled water

Adjust to ~pH 7 with a drop at a time of 1N NaOH or 1N HCl if needed but it's usually right if you weigh the sodium phosphate carefully and measure the water with a graduated cylinder.

For storage: Aliquot into 6 x 25 ml fish safe plastic bottles and store at 4C. Label with date made and use within a couple of weeks.

8% PFA: {#pfa .subhead2}

- 8 g Pelleted PFA (Ted Pella, Inc.; cat# 18501)

- 90 ml dH2O
- 25 drops 1N NaOH

1. Heat at very low heat and stir until solution clears.
2. Add 25 drops 1N HCl. pH should be 7.0-7.2.
3. Filter and store at 4C not more than 1 week.
4. Use as 4% PFA: dilute 1:1 with 2X PBS, do not store solution more than a few hours.

2X PBS {#x-pbs .subhead2}

- 1.6% NaCl
- 0.04% KCl
- 0.04 M PO4 pH 7.0- 7.3

12.1.3 Procedure:

Day

Step

Time for Step

Date and Time

1

2h-8h at R/T depending on size on shaker.

1h or longer at R/T on shaker.

Without agitation and with lid open until eyes start to lighten and all skin pigment is gone (usually about an hour or more)

2

2 h to O/N at R/T on shaker

2 h to overnight O/N at R/T on shaker.
Check for bone staining.

R/T on shaker until excess stain in tissue is gone.

Without agitation

Wild caught specimens are put in 100% EtOH in the field and then rehydrated and put into 4% when back in the lab.

Chapter 13

IACUC

descriptions of animal care IACUC protocols