Appendixes

Appendix 1

Description of oocyte developmental stages

Stage 1: **Undifferentiated** – where follicular structure is indistinct and there is no sex cell primordial.

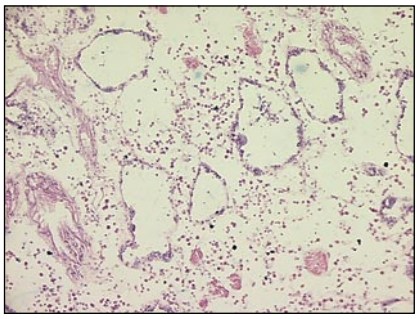
Stage 2: **Differentiated** – where oocyte development commences, primary oogonia are visible, sex cell range from 15–25 µm in diameter.

Stage 3: **Developing** – where there are mostly pre-vitellogenic and vitellogenic oocytes, follicle lumen is open or only partially occluded and oocyte diameter ranges from 30–70 µm.

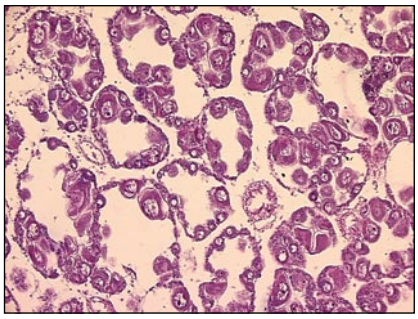
Stage 4: **Ripe** – where gonads show packed follicles, the follicular lumen is occluded, there is no inter-follicular space visible, and oocytes range 60–70 µm in diameter.

Stage 5: **Spawning** – where the follicle lumen is opening, follicle size diminishing and ripe oocytes range 60–70 µm.

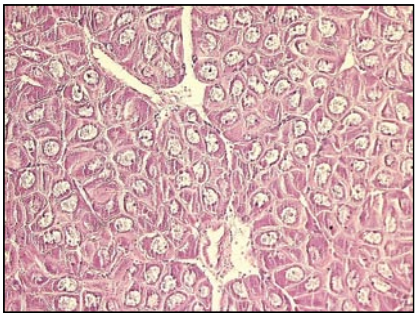
Stage 6: **Spent** – where follicles are empty and frequently filled with haemocyte aggregations on slide preparations and an occasionally atretic oocyte is present.



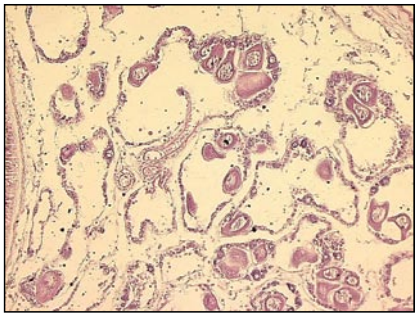
Stage 2: Differentiated



Stage 3: Developing



Stage 4: Ripe



Stage 6: Spent

Appendix 2

Sample data sheet for gonadal and muscle indices

SPECIES:

Scallop No.	Shell height (µm)	Shell lenght (µm)	Total wet weight (gram)	Empty shell weight (gram)	Gonad foil weight (gram)	Muscle foil weight (gram)	Gonad dry weight & foil (gram)	Muscle dry weight & foil (gram)	Gonad dry weight (gram)	Muscle dry weight (gram)	GI	MI

Appendix 3

Broodstock check sheet

YEAR:

MONTH:

Day	Time	B1 T (°C)	Algal food	B2 T(°C)	Algal food	B3 T (°C)/Flow	B4 T(°C)/Flow	COMMENTS
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Appendix 4

Heating unit set-up and take-down

Note: Refer to Technical Drawing – Pg 5A.

Set-up

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

Take-down

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

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

Appendix 5

Maintenance and cleaning of seawater system

Note: Refer to Technical Drawing – Pg 2.

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

End of season

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

Beginning of season

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

Appendix 6

Pump room log

YEAR:			
MONTH:			
Day	Time	PSI pressure	Comments
1			**Check**: Oil pump
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13			
14			
15			
16			**Check**: Oil pump
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Appendix 7

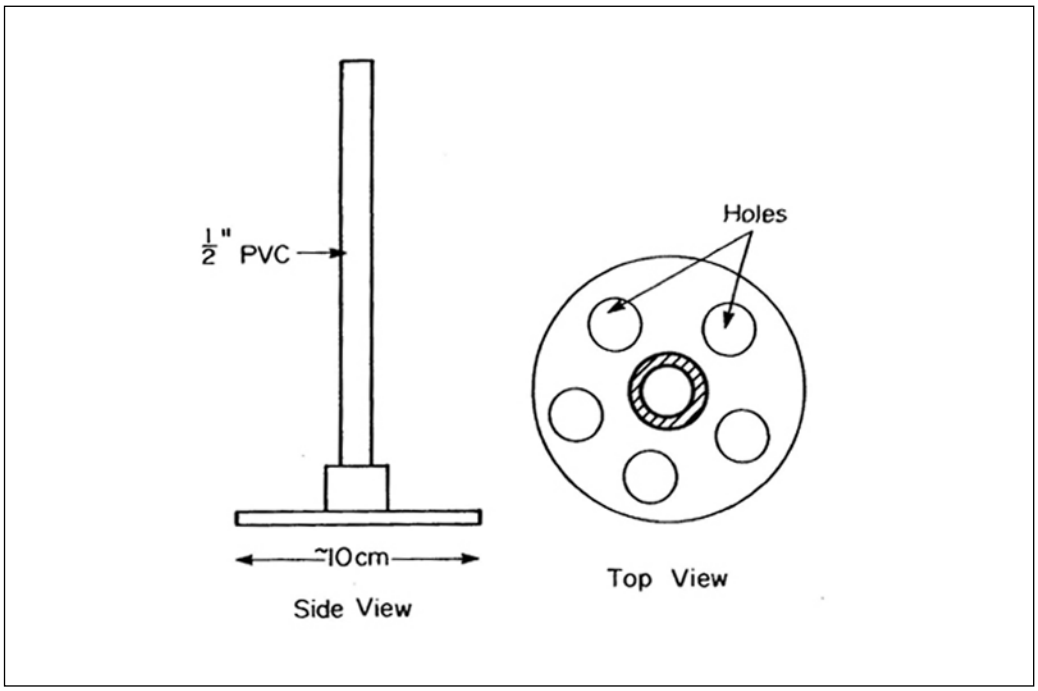
Cleaning hatchery after spawning

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

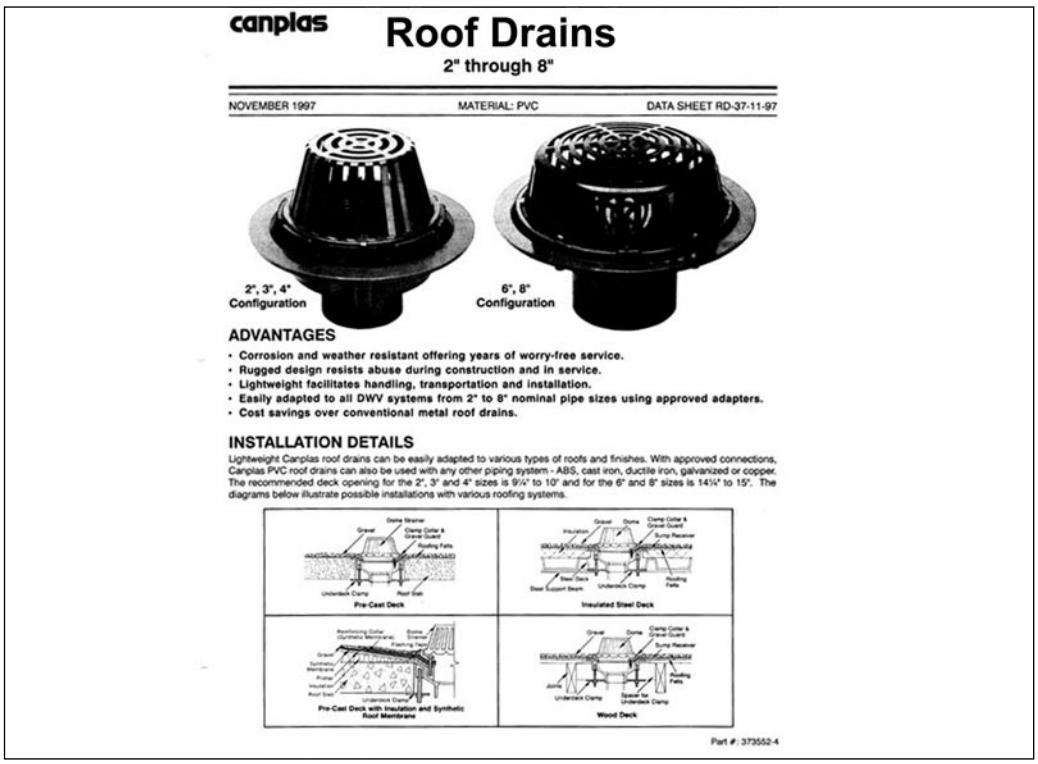
1. Remove all scallops to outdoor tank with high flow of water; allow to spawn completely.
2. Close inflow of water from heating unit.
3. Disconnect cartridge for double 1 µm filter.
4. Open last red valve (heating line) in hatchery; close other valves in heating line.
5. Once chlorinated water has passed,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

Appendix 8

Details of materials – Plunger and drain



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


canplas

Roof Drains

2" through 8"

NOVEMBER 1997 | MATERIAL: PVC | DATA SHEET RD-37-11-97

2", 3", 4" Configuration

6", 8" Configuration

ADVANTAGES

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

INSTALLATION DETAILS

Lightweight Canplas roof drains can be easily adapted to various types of roofs and finishes. With approved connections, Canplas PVC roof drains can also be used with any other piping system - ABS, cast iron, ductile iron, galvanized or copper. The recommended deck opening for the 2", 3" and 4" sizes is 9¼" to 10" and for the 6" and 8" sizes is 14¼" to 15". The diagrams below illustrate possible installations with various roofing systems.

Pre-Cast Deck

Insulated Steel Deck

Pre-Cast Deck with Insulation and Synthetic Roof Membrane

Wood Deck

Part #: 373552-4

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

Appendix 9

Preparation of culture media

F/2 Nutrient solution

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

For 125 ml, 500 ml and 4 l cultures:

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

For 100 l cultures:

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

Vitamin stock solution

Materials:

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

Method:

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

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

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

1 ml vitamin solution per 1 liter F/2 solution

Sodium metasilicate solution (3 % w/v)

Materials:

- 500 ml dark glass bottle and top.
- Glass funnel.
- Q-water.
- Sodium metasilicate (Na_2SiO_3).

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

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

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

Convay medium

Preparation of Solution C for a 25 l volume:

KNO_3	2 000 grams
K_2HPO_4	400 grams
EDTA	750 grams
H_3BO_3	50 grams
TMII	2 000 ml
SWII	125 ml
Vitamin Solution	25 ml

TMII Solution (25 l volume):

$Fe_2(SO_4)_3$	395 grams
$MnSO4.H_2O$	37.5 grams

$ZnSO_4.H_2O$	6.25 grams
$CuSO_4.5H_2O$	5.00 grams
$CoSO_4.7H_2O$	0.65 grams
$NaMoO_4.2H_2O$	0.35 grams

SWII Solution (10 l volume):

$SrCl_2:6H_2O$	13.00 grams
$AlCl.6H_2O$	0.50 grams
$RbCl.6H_2O$	0.20 grams
$LiCl.H_2O$	0.10 grams
KI	0.05 grams
KBr	0.65 grams

Vitamin Stock Solution (1 l volume):

B_{12}	0.10 grams
B_1	100.00 grams
Biotin	0.40 grams

F/2 media

1.	Nitrate	$NaNO_3$	75 g/l
2.	Phosphate	$NaH_2PO_4.H_2O$	5 g/l
3.	Silicate	$Na_2SiO_3.9H_2O$	30 g/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.

- $CuSO4.5H_2O$ 0.98 g/100 ml
- $ZnSO4.7H_2O$ 2.2 g/100 ml
- $CoCl2.6H_2O$ 1.0 g/100 ml
- $MnCl2.4H_2O$ 18.0 g/100 ml
- $Na_2MoO_4.2H_2O$ 0.63 g/100 ml

Bring solution to 1 l with distilled H_2O (pH about 2.0).

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

5.	Vitamins:	Biotin	1.0 mg
		B_{12}	1.0 mg
		Thiamine HCl	20.0 mg

Dissolve in 1 l distilled H_2O. Store in refrigerator.

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

Appendix 10

Chemical sterilization procedure

Materials:

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

Procedure:

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

For sterilizing container with seawater:

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

Appendix 11

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

Set-up of seawater supply in algae container:

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

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

Take down of seawater supply to algae container:

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

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

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

Appendix 12

Bactopeptone test

(from Bourne, Hodgson and Whyte, 1989)

Materials:

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

Procedure:

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

Assessement:

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

Appendix 13

Algal culture check list

YEAR:
MONTH:

Day	Time	Temperature	Lights/AC	Cultures bubbling	CO_2 level	Comments
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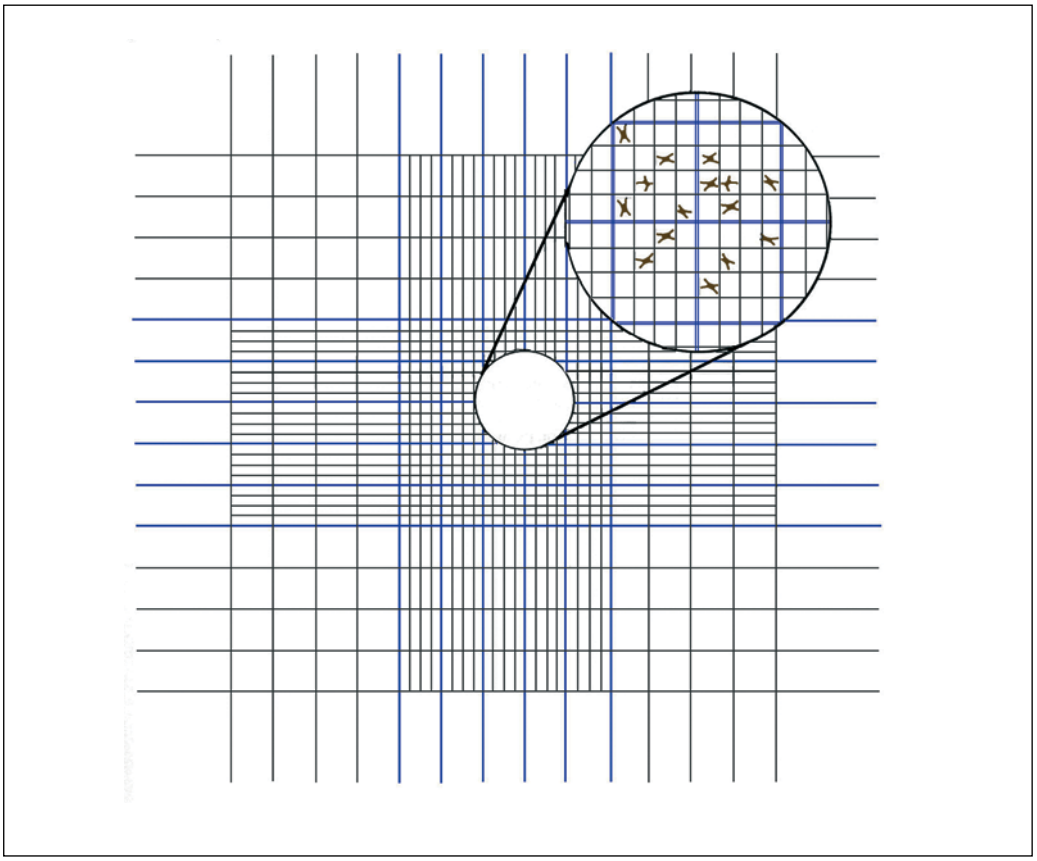
Appendix 14

Haemocytometer cell diagram

(from Bourne, Hodgson and Whyte, 1989)



Appendix 15

Larval check sheet

YEAR:											
MONTH:											
Day	Species	Day after set	T(°C)	Air	Density No./ml	Total No.	Volume	Rations and species	Comments		
1											
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Appendix 16

Determination of dry weight and ash-free dry weight

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

Appendix 17

Sieve construction for larval and post-larval collection

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

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

Appendix 18

Raceway check list

YEAR:						
MONTH:						
Day	Species	Day after set	Air	Density No./ml	Total No.	Comments
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Appendix 19

Cleaning of raceway

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

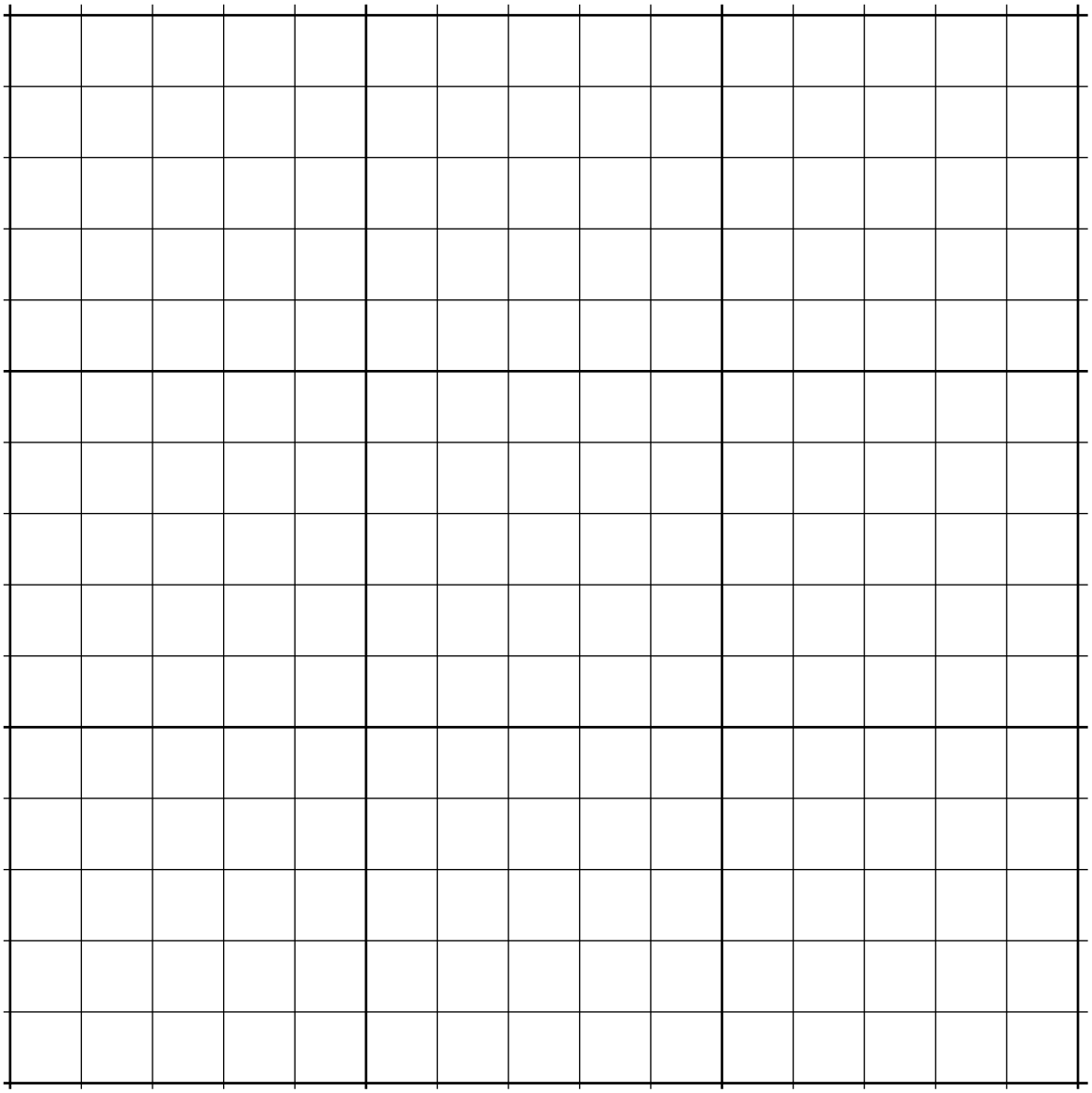
Raceway cleaning involves full chlorination of lines and troughs. The process is similar for both indoor and outdoor raceways. For the indoor raceway, care must be taken to chlorinate all recirculating pipes and sump tank. For the outdoor raceway, algal tank needs to be chlorinated.

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

Appendix 20

Counting grid for spat

(Template to be copied and laminated for use)



Appendix 21

Preparation and ration for dry algae

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

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

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

To calculate food ration: F= (SxR)/7

Where:

F= dry weight of algae per day (mg)

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

S= total wet weight of spat (mg)

Example:

Total biomass= 75 g= 75 000 mg.

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

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

F= (75 000x0.4)/7= 4285.7 mg dry wt algae

Daily ration is 4.3 grams dry weight

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

Algal preparation and supply:

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

Appendix 22

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

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

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

Broodstock area				
2	380-litre fiberglass aquarium tanks with viewing window	675	1 350	AES
4	Plexiglass for cover and filtration (8mm)	90	360	Local supplier
1	Sweetwater air pump 1.2 CFM@4 psi (9" x 8" x 9")	279	279	AES
20	Labcock ball valves for air control	5	100	AES
5	Nitex mesh 500µm per yard	12	60	Aquafauna Bio-marine
2	Tygon tubing (7mm ID) in a 18m coil	49	98	AES
1	Aqua Logic drop-in titanium water chiller 1/5 hp	910	910	Aqua Logic
8	250 W submersible heaters	20	160	Aquafauna Bio-marine
2	20-litre carboys	100	200	AES
2	Shelves and brackets	20	40	Local hardware
Sub-total			**3 557**	
Spawning procedure				
6	Trays (non-toxic)	30	180	Local hardware
5	Graduated 10-litre buckets	10	50	Local hardware
50	Graduated beakers (2 litre and/or 3 litres)	6	313	AES
2	Sedgewick-rafter cell	125	250	AES
1	Plunger			Homemade
1	Eppendorf pipette (0-1 000µl)	350	350	Sigma
1	Eppendorf tips (100µm and 1 000µm)(1box)	50	50	Sigma
1	Pasteur pipettes (1 box of 250)	14	14	AES
4	Bulbs	6	24	AES
10	Recording book (water resistant)	4	35	AES
6	Thermometers	5	30	AES
1	150µm PVC sieve (see Chapter 3)			Homemade
1	300µm sieve (see Chapter 3)			Homemade
Sub-total			**1 296**	

Algal culture (Chapter 2)				
QTY	**Description**	**Unit price (USD)**	**Extended price (USD)**	**North American/other supplier**
Algal seawater system				
1	4m x 3.5m fiberglass container	2 000	2 000	Local shipping company
1	Hayward cartridge filter (C250)	240	240	Local pool supplier
1	Replacement cartridge	50	50	Local pool supplier
2	10" Cartridge filter housing	22	44	AES
10	10µm filters (1box)	5	50	AES
10	1µm filters (1box)	5	50	AES
1	Lifeguard vertical UV sterilizer	172	172	AES
1	20mm ID re-inforced vinyl tubing (18m coil)	66	66	AES
1	Shelves and brackets	20	20	Local hardware
Sub-total			**2 692**	
Air and light requirements				
1	Air conditioning unit	600	600	Local hardware
1	Freshwater hose	13	13	Local hardware
1	Nozzle	5	5	Local hardware

1	Sweetwater air compressor	279	279	AES
1	Shelves and brackets	20	20	Local hardware
2	50lb CO_2 cylinder	75	150	Local beverage supplier
1	CO_2 regulator with flowmeter	188	188	VWR
1	Refrigerator	350	350	Local supplier
17	Double vertical ballasts	96	1 632	Local supplier
48	Coolwhite fluorescent lamps (30watts)	14	672	Local supplier
6	Single horizontal ballasts (1.2m)	70	420	Local supplier
2	Double horizontal ballasts (1.2m)	96	192	Local supplier
1	7mm ID Tygon tubing clear (36m coil)	11	11	AES
20	Male adapter (8mm NPT x 7mm BARB)	<1	6	AES
20	Labcock ball valve (7mm FNPT ports)	4	81	AES
20	Nipples (7mm NPT x 7mm NPT)	<1	7	AES
20	Bacteria filters 0.3µm (8mm hose barb connection)	7	135	AES
1	Soft tubing cutters	12	12	AES
1	Thread cutting tap (8mm NPT)	6	6	AES
1	Drill bit for 8mm NPT tap	4	4	AES
10	Y-connections	<1	4	Sigma
Sub-total			**4 787**	
Culture vessels and fittings				
6	100-litre vessels (transparent, cone bottom)	199	1 191	AES
6	Lids for 100-litre vessels	12	74	AES
1	Marine plywood for 100-litre vessel frame	96	96	Local supplier
1	West epoxy resin system	150	150	Local supplier
6	50mm male adapter for 100-litre drain	2	12	Local hardware
6	50mm-20mm reducers for 100-litre drain	3	15	Local hardware
6	20mm valves for 100-litre drain	3	20	Local hardware/ AES
8	4-litre Ernlemeyer flasks	45	360	Sigma
3	500ml Pyrex round flasks with flat bottom - 6 per case	69	207	Thomas Scientific
1	125ml Ernlemeyer flasks, screw cap (case of 24)	228	228	VWR
16	3-hole rubber stopper (no. 10)	2	31	AES
1	Glass rod (package)	30	30	Local supplier
3	Cheesecloth material (4 sq. yards)	6	18	Local hardware/ Biomarine Aquafauna
1	Cotton (1 roll)	10	10	Local supplier
1	Pasteur pipettes 1.5ml cap (pack of 400)	20	20	VWR
24	5 and 10ml sterile graduated pipettes (packs of 8)	10	240	AES
1	Incubator	500	500	AES
2	Bottle brush	5	10	AES
1	Thermometer	11	11	VWR
1	Utility brush	5	5	AES
Sub-total			**3 228**	
Master and sub-culture				
1	Deep plastic reservoir with lid (chemical resistant)	30	30	Local hardware
1	250ml graduated cylinder (glass)	12	12	AES
5	Foil	5	25	Local supplier
1	50ml volumetric flask with cap	8	8	AES

1	500ml glass bottles (dark amber) with cap (case of 12)	30	30	AES
1	Autoclavable funnel	2	2	AES
1	Autoclave	10 000	10 000	
4	Labelling tape	10	38	Sigma
4	Magic marker		0	Local supplier
2	Pipette latex rubber bulb	13	25	VWR
2	Haemocytometer cell	133	266	AES
1	25ml graduated cylinder	4	4	Sigma
1	100 ml graduated cylinder (polypropylene)	5	5	AES
1	F/2 Formula nutrient media (Solution A) (4 litres)	14	14	AES
1	F/2 Formula nutrient media (Solution B) (4 litres)	14	14	AES
1	D-Biotin (1g)	39	39	Sigma
1	Vitamin B12 (250mg)	17	17	Sigma
1	Thiamine hydrochloride (250g)	58	58	Sigma
1	Sodium metasilicate nonahydrate (250g)	25	25	Sigma
1	Scientific calculator	22	22	VWR
1	Propane torch	10	10	Local hardware
1	Propane bottle	7	7	Local hardware
2	10-litre carboy	83	166	AES
6	10-litre graduated buckets	10	60	Local hardware
12	2-litre graduated beaker with handle (polypropylene)	5	54	AES
1	Compound microscope	979	979	AES
5	Muriatic acid (4-litre container)	9	44	Local supplier
50	Chlorox (4-litre container)	4	213	Local supplier
1	Formaldehyde (1-litre)	30	30	VWR
Sub-total			**12 196**	
Live and dry algae				
8	stock cultures (15ml volume)	45	360	CCMP
2	Tetraselmis 3 600 (1 litre)	68	136	Reed Mariculture
2	Pavlova 1 800 (1 litre)	38	75	Reed Mariculture
2	Thalassiosira weisflogii 1 800 (1 litre)	30	60	Reed Mariculture
Sub-total			**631**	

Larval seawater system (Chapter 3)				
QTY	**Description**	**Unit price (USD)**	**Extended price (USD)**	**North American/other supplier**
Seawater and air supply (ceiling plan)				
20	50mm plastic pipe hangers	2	40	AES
20	25mm inner diameter clear flexible hose (per 30cm)	2	43	AES
20	Labcock ball valves	4	81	AES
15	8mm FNPT adapter for labcock ball valve	<1	6	AES
20	25mm plastic pipe hangers	1	24	AES
1	Sweetwater 100 V linear compressor	279	279	AES
1	Shelves and brackets	20	20	Local hardware
Sub-total			**493**	
Larval tanks				
4	1344-litre square insulated BONAR tanks with lids	536	2 144	BONAR Plastics
4	40mm-20mm reducer	3	12	Local hardware

4	20mm union	6	24	Local hardware
1	20mm elbow	1	1	Local hardware
1	20mm male adapter	1	1	Local hardware
1	Cartridge filter housing	22	22	AES
10	1µm filter	3	25	AES
10	10µm filter	3	25	AES
1	20mm threaded hose barb (20mm NPT x 8mm barb)	1	1	AES
10	20mm mm inner diameter clear flexible hose (per 30cm)	2	15	AES
1	8mm Tygon tubing (90m coil)	13	13	AES
4	25mm thru-hull bulkhead fitting	14	56	Local hardware
4	25mm female adapter	1	3	Local hardware
4	25mm elbow	1	5	Local hardware
4	25mm ball valve	5	18	Local hardware
20	25mm inner diameter clear flexible hose (per 30cm)	2	43	AES
1	Utility brush	6	6	AES
4	Silicon sealant	11	44	AES
Sub-total			**2 456**	
Larval sieves				
1	20µm nitex mesh (per metre)	60	60	Aquafauna
2	40µm nitex mesh (per metre)	56	112	Aquafauna
2	60µm nitex mesh (per metre)	42	84	Aquafauna
2	80µm nitex mesh (per metre)	34	68	Aquafauna
6	120µm nitex mesh (per metre)	24	144	Aquafauna
6	150µm nitex mesh (per metre)	20	120	Aquafauna
2	300µm nitex mesh (per metre)	16	32	Aquafauna
6	25cm (10”) diameter PVC (per 30cm)	16	93	Local hardware
6	20cm (8”) diameter PVC (per 30cm)	13	75	Local hardware
6	15mm PVC pipe (per 30cm)	1	3	Local hardware
2	15cm (6”) diameter PVC pipe (per 30cm)	8	15	Local hardware
8	Garden hose clamps (125mm)	2	14	Local hardware
Sub-total			**820**	
Miscellaneous equipment				
1	Freshwater garden hose (18m)	25	25	Local hardware
1	Hose nozzle	8	8	Local hardware
1	20-gallon bin for chlorination of small equipment	30	30	Local hardware
20	Chlorine (4-litre containers)	4	85	Local hardware
10	Recording notebook (waterproof)	4	35	AES
2	250ml graduated cylinders	12	25	AES
Sub-total			**208**	

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

1	3mm black polyethylene mesh (18m roll)	60	60	Atlantic Gulf & Fishing
2	Cable ties (100 per package) - 100mm long	3	5	AES
Sub-total			**3 033**	
Indoor raceway system				
1	220-litre tank	350	350	Bonar Plastics
2	Fiberglass troughs (120 litres)	200	400	Red-Ewald Inc.
1	Centrifugal pump (vertical)	120	120	AES
4	50cm x100cm wolmanized wood for frame	12	48	Local hardware
1	West system epoxy resin	150	150	Local hardware
2	Shelves and brackets	20	40	Local hardware
Sub-total			**1 108**	
Indoor sieves				
8	25cm (10") pipe for sieves (per 30cm)	16	124	Local hardware
16	Tubing flow valve (for restricting flow)	<1	3	AES
12	Barbed fitting (8mm x 7mm barb)	<1	4	AES
2	20-litre carboys	100	200	AES
	150 and 120µm Nitex mesh - see larval sieves section			
Sub-total			**331**	
Outdoor raceway				
1	Cement pad	600	600	Local supplier
5	Untreated wood for frame (50mm x100mm)	12	60	Local hardware
1	West system epoxy resin	150	150	Local hardware
2	Corrugated plastic for roofing (per sheet)	35	70	Local supplier
1	Marine plywood for roof support	96	96	Local hardware
1	150-litre reservoir	60	60	AES
2	8mm stopcock valve	1	2	AES
30	Window screen (per 30cm, 1.2m wide)	2	60	Local hardware
30	1/2" pipe for screen (per 30cm)	1	23	Local hardware
10	Cup hooks for screen	1	7	Local hardware
1	Centrifugal pump (vertical)	120	120	AES
Sub-total			**1 248**	
Outdoor sieves				
8	30cm pipe for sieves (per 30cm)	18	140	Local hardware
20	750µm green collector bag	2	35	AES
5	1.5mm red collector bag	2	9	AES
12	25mm coupler and pipe for suspension	2	18	Local hardware
Sub-total			**202**	

Transfer phase: exterior tanks and field requirements (Chapter 5)				
QTY	**Description**	**Unit price (USD)**	**Extended price (USD)**	**North American/other supplier**
Holding tanks				
2	540-litre insulated tank with lid	450	900	BONAR Plastics
2	Drain fittings (bulkhead and female adapter)	17	34	AES
1	Silicone sealant	10	10	AES
10	Reinforced vinyl tubing (25mm inner diameter)(price per 30cm)	2	22	AES
Sub-total			**966**	

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

Appendix 23

List of selected suppliers

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

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

Aquafauna Bio-marine Inc.
PO Box 5
Hawthorne California 90250
United States of America
Tel.: +1 310 9735275
Fax: +1 310 6769387
Web: www.aquafauna.com

Aqua Logic Inc.
8280 Clairemont Mesa Blvd, Suite 127
San Diego, CA 92111
United States of America
Tel.: +1 858 2924773
E-mail: aqualogic@aol.com
Web: www.aqualogicinc.com

Aquatic Eco-systems Inc.
1767 Benbow Court
Apopka, FL 32703
United States of America
Tel.: +1 407 8863939 / 877 3474788
Fax: +1 407 886 4884
E-mail: aes@aquaticeco.com
Web: www.aquaticeco.com

Red Ewald Inc.
PO Box 519
Karnes City, Texas 78118
United States of America
Tel.: +1 800 2423524
Web: www.redewald.com

Easy-Kleen, Pressure System Ltd.
26 Eveleigh Street
Sussex, New Brunswick E4E 2N8
Canada
Tel.: +1 506 4333393
Fax: +1 506 4332443
Web: www.easy-kleen.com

Reed Mariculture Inc.
Instant Algae Products
511 Pamlar Ave, Suite #C
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Tel.: +1 831 7683830
Fax: +1 831 4012474
Web: www.seafarm.com

Go Deep International Inc.
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