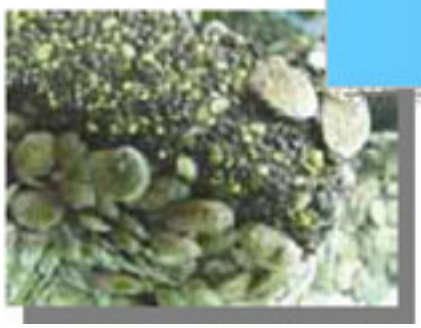
Chapter 7 Management of Nursery Raceways and Weaning



Chapter 7 Management of Nursery Raceways and Weaning

General management procedures

For the first few weeks after they have settled, abalone post-larvae do not have a fully formed gut. During the first week they gain nutrients from the remainder of their yolk stores and by ingesting bacteria mucus and other exudates (mainly sugars) released by crustose coralline algae and diatoms. They are also able to directly absorb soluble organic compounds from seawater through the gills and skin. Beyond the first week, with progressive development of the rasping tongue and the gut, they are able to consume, digest and absorb whole benthic diatoms.

During the nursery phase, the plates have to be regularly (every 1 to 2 weeks) inspected and rotated in the vertical and horizontal planes to ensure even growth of diatoms. This method therefore requires high inputs of labour, equipment and space and consequently has high operating costs. Indeed, to meet such requirements, commercial farms commonly use 10 000 to 25 000 standard plates and nursery site areas of about 1000 m^2. Rearing post-larvae for long periods on diatom plates also brings uncertainty, because the growth of diatoms varies greatly with the temperature, light intensity and levels of nutrients. Most, or all, of these parameters are under only limited control by hatchery staff. Light intensity can be raised to promote greater diatom growth, as in the case of excessive grazing pressure, or lowered to inhibit excessive growth of diatoms and filamentous algae. Such adjustments are made by applying a range of alternative grades (30% to 95%) of shade-cloth that may take the form of covers or canopies.

As discussed above, blacklip abalone post-larvae can be safely harvested from the plates by the time they reach an average shell length of 1.5 mm, with most above 1 mm. On the central coast of NSW this can occur in as little as 30 days through summer but up to 70 days through winter. High-density rearing of post-larvae on the plates makes it essential that their progress is constantly monitored. As also discussed previously, there is a real risk of the post-larvae overgrazing the diatoms, leading to starvation and subsequently to losses or reduced ability to survive handling and weaning.

To avoid these hazards, a small but representative sample of plates should be inspected every few days, beginning a month after seeding **(see Chapter 6 and Figure 40)**. If the film of diatoms covering the plates is grazed below about 10% of the plate surface, the post-larvae should be immediately harvested and transferred to raceways. This should be done even if they have not reached the desired average size of 1.5 mm, because even those with a mean size as small as 1 mm can be successfully weaned provided that they are in good general health. An option for dealing with overgrazing of individual plates before harvesting is to use back-up sets of conditioned diatom plates, batches of which should be initiated at fortnightly intervals as a supplementary food source. These can be clamped to overgrazed plates by using plastic clothes pegs.

Pre-harvest count

From 1 to 3 days before transfer of the post-larvae to raceways, the total number of post-larvae can be estimated by counting those on at least one plate selected at random from each basket of plates. This can be done by two alternative methods. The first is direct counting of undisturbed post-larvae in situ (i.e. still attached to the plates). As already discussed, direct counts should always be conducted on plates fully submerged in seawater in shallow (75 to 100 mm) clear plastic trays **(see Figure 40)** maintained at or below 23ºC. Counting can be facilitated by incorporating a prominent 2 x 2 cm black-line grid on the floor of the trays. Additionally, transparent or translucent plastic trays can be lit from below to silhouette both the counting grids and the post-larvae.

The second option is to pre-harvest and estimate the average numbers of post-larvae per plate (and hence the total numbers of post-larvae available for harvesting) from the representative sub-sample of plates. These sub-sampled plates should be pooled into a single basket before being subjected to the same step-by-step procedures described below for the main harvest. The combined settled volume of these sub-sampled post-larvae can then be determined by transferring them into a seawater-filled measuring cylinder. This volume is finally multiplied by the average count of post-larvae in a minimum of three 1.0-mL settled sub-samples and the result divided by the number of sub-sampled plates to estimate the average count per plate and hence the total number of post-larvae available for harvest. To avoid double-handling, the sub-sampled post-larvae should be immediately transferred to a raceway ahead of the main harvest.

Harvesting and transfer of post-larvae to raceways

Individual baskets of plates are placed in a dedicated tub where the post-larvae are anaesthetised. The tub should be large enough to easily accommodate, submerge and manipulate a full basket of plates in a vigorously aerated anaesthetising solution of benzocaine at 50 mg/L seawater (see step-by-step summary for preparation details). The post-larvae are detached from the plates after 5 to 10 min of anaesthesia by using a soft-bristle paintbrush aided by continuous flushing with seawater. Post-larvae can be detached from the plates into the tub and then drain-harvested onto a submersed screen for transfer to first stage raceways. Alternatively, the post-larvae can be detached plate by plate directly into recipient raceways. The latter is preferable in the sense that double-handling is avoided and the duration of the anaesthesia minimized.

To allow good quality films of diatoms to develop, seawater flow through the nursery raceways should be started about 2 weeks before stocking with post-larvae. To expedite biofilm development, raceways can be inoculated with diatoms scraped from conditioned nursery plates. Daytime hides such as concrete pavers should also be installed in the raceways 1 to 2 weeks before stocking with post-larvae. Although the hides are not used as shelters by the post-larvae for the first few weeks, their initial presence avoids the risk of post-larvae being crushed during their installation. Healthy post-larvae are able to recommence feeding on diatoms the first night after transfer, and the presence of diatom films reduces or

eliminates growth checks otherwise commonly associated with transfer to raceways and weaning onto fine-grained (powdered) formulated diets.

The amount of supplementary formulated food provided over the first week is a matter of trial and error. If there is a lot of uneaten food left on the first morning after transfer, reduce the ration by about 25%, then repeat the exercise until food supply and consumption are matched. If, however, there is no food left over, increase the ration by 25% each night until some residual food is apparent. Avoid over-feeding, which results in both wastage and reduced water quality and hence impaired growth performance and health.

STEP BY STEP SUMMARY AND CHECK-LIST FOR DIATOM PLATE NURSERY REARING AND EARLY WEANING

☑ 1. Once plates have been seeded with post-larvae, inspect regularly to monitor the percentage cover of the diatom bio-film and the growth of the post-larvae. Inspections should be made fortnightly for the first 4 weeks, then on alternate days or daily once plates with a diatom bio-film coverage below about 10% are first detected and/or as post-larvae approach the minimum average weaning size of 1.5 mm.

☑ 2. If the biofilm on a majority of plates drops below 10% coverage, wean the post-larvae immediately, whether or not an average size of 1.5 mm has been reached.

☑ 3. When the post-larvae reach an average shell-length of 1.5 mm (a process that will take from as little as 30 days in summer but as long as 70 days in winter), wean them regardless of the remaining biofilm coverage.

☑ 4. Set up the nursery raceways with flow-through seawater up 2 weeks before the anticipated first date of harvest and weaning to promote development of diatom films on the floor and walls of the raceways.

☑ 5. Estimate the total numbers of post-larvae 1 to 3 days before transferring them to raceways, by counting those on a plate randomly selected from each basket to be harvested. There are two alternative ways of doing this:

- Count the numbers of post-larvae on the plates in situ. This should always be done on plates fully submerged in seawater in shallow (75 to 100 mm) clear or translucent plastic trays maintained at or below 23 ºC. This method can be facilitated by incorporating a prominent 2 x 2-cm black-line grid on the floor of the trays and/or by under-lighting them to silhouette both the grids and the post-larvae; or
- Harvest and count post-larvae from a representative sub-sample of plates by using the techniques described below.

☑ 6. On the day of harvesting and transfer of post-larvae to the raceways, fill a tub large enough to accommodate a full basket of plates with 10-µm-filtered seawater and add anaesthetic stock solution as follows:

- Make up a stock solution of benzocaine by adding 50 g of ethyl-p-amino-benzoate in 500 mL of 95% ethyl alcohol (0.4 M solution).
- Add 0.5 mL of this solution per litre of seawater to the tub[1].

☑ 7. Leave the plates in this solution for about 5 min, after which time you will able to easily dislodge the post-larvae with seawater by rinsing aided by gentle use of a soft-bristle brush. Post-larvae from plates can either be:

- dislodged into the tub for collective harvesting into a flooded 10-µm-mesh screen and then transferred into a nursery raceway; or preferably
- dislodged as described, but directly off the plates into prepared raceways.

☑ 8. In either case, raceways must be supplied with flowing seawater and vigorous aeration to speed the recovery of the post-larvae from anaesthesia[2].

[1] Do not attempt to handle or to anaesthetize post-larvae or small juveniles when heat-stressed at temperatures above 23°C.
[2] Total time of exposure of post-larvae to anaesthesia should be kept as brief as possible and less than 15 min.

Chapter 8 Intensive on Rearing in Shallow Raceways

Chapter 8 Intensive On-Rearing in Shallow Raceways

General

Shallow raceway nursery rearing of post-larvae from 1 to 2 mm to juveniles in the range 7 to 15 mm and suitable for on-farming or fisheries reseeding operations is best completed in two stages. First-stage raceways of the type illustrated in **Figure 41** may be as small as 1 m^2 (2 × 0.5 m) and no more than 15 cm deep.

Cascade systems can be used to improve floor space efficiency, but the minimum seawater exchange needed ensure maintenance of optimum seawater quality (≥ 1 L seawater/kg biomass/min) must be adjusted accordingly. Likewise, care needs to be taken to ensure that the longer seawater flow-paths do not result in the abalone being exposed to temperatures outside their tolerable range of 10 to 23°C.

Stocking rates

First-stage raceways can be stocked with 1 to 2 mm ex-plate post-larvae at initial densities of up to 50 000/m^2. However, even with good husbandry, numbers of juveniles are likely to progressively fall by an average of 30% to 50% over the subsequent 2 to 3 months. By this time they should have grown to an average size of 4 to 5 mm, ready to shift to second-stage raceways. A second important criterion for adjusting stocking density is that the collective shell area of stock should not occupy more than about 60% of the floor area of the raceways. The exponential shell growth of post-larvae reared on diatom plates immediately shifts to a constant (linear) rate **(Figure 42)** following transfer of the post-larvae to shallow raceways and weaning onto formulated diets.

At 4 to 5 mm, juveniles are stocked at initial densities up to 20 000/m^2 into the larger second-stage raceways of the type illustrated (**Figure 43)**. Here they soon adopt a strictly nocturnal pattern of activity **(Figure 44),** remaining cryptic and inactive by day**.** As in first stage raceways, densities commonly fall by 30% to 50% as the juveniles grow through to a 'button' size range of 7 to 15 mm over the ensuing 2 to 3 months. At this size they are suitable either for fisheries seeding operations or for on-farming to a final market size, most commonly in the range 80 to 90 mm.

In raceways the average monthly shell growth of seed from the time of weaning to a final mean size of 7 to 15 mm can vary from 45 to 80 μm/day, depending mainly on the seasonal temperature. Farming methods include offshore cages located either on the seabed or suspended from mid-water long-lines, or (more commonly) land-based raceway tank systems. These shallow raceways or ponds are provided with either hides or riffle ridges incorporated into the floor. Various types of newer-generation grow-out tanks commonly used throughout southern Australia are illustrated **(Figure 45)**.

Stocking rates up to harvest size (80 to 90 mm and 80 to 100 g) continue to be limited to 60% of the available tank floor area, which amounts to 200 abalone/m^2 or a biomass of about 12 kg/m^2.

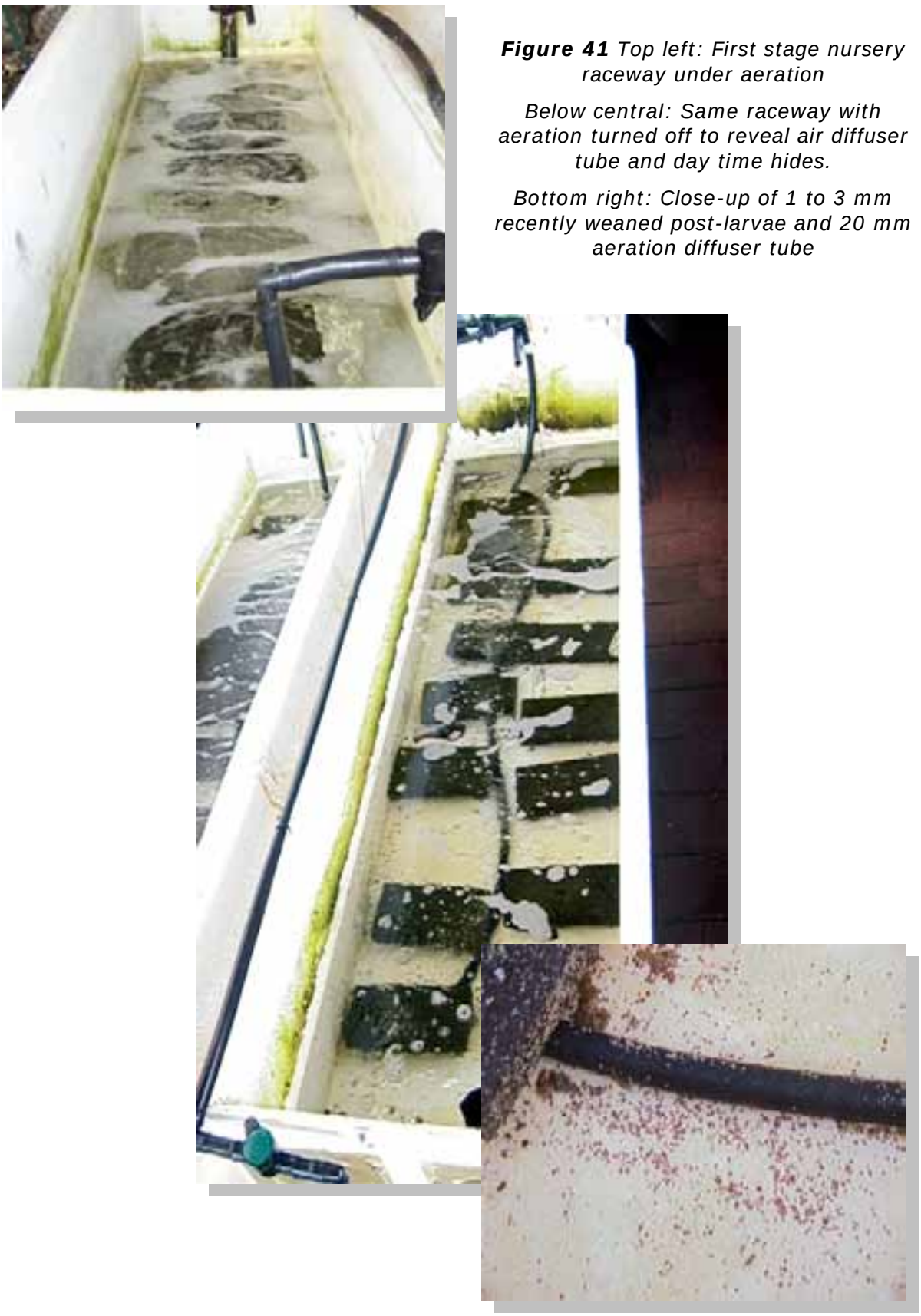


Figure 41 *Top left: First stage nursery raceway under aeration*

Below central: Same raceway with aeration turned off to reveal air diffuser tube and day time hides.

Bottom right: Close-up of 1 to 3 mm recently weaned post-larvae and 20 mm aeration diffuser tube

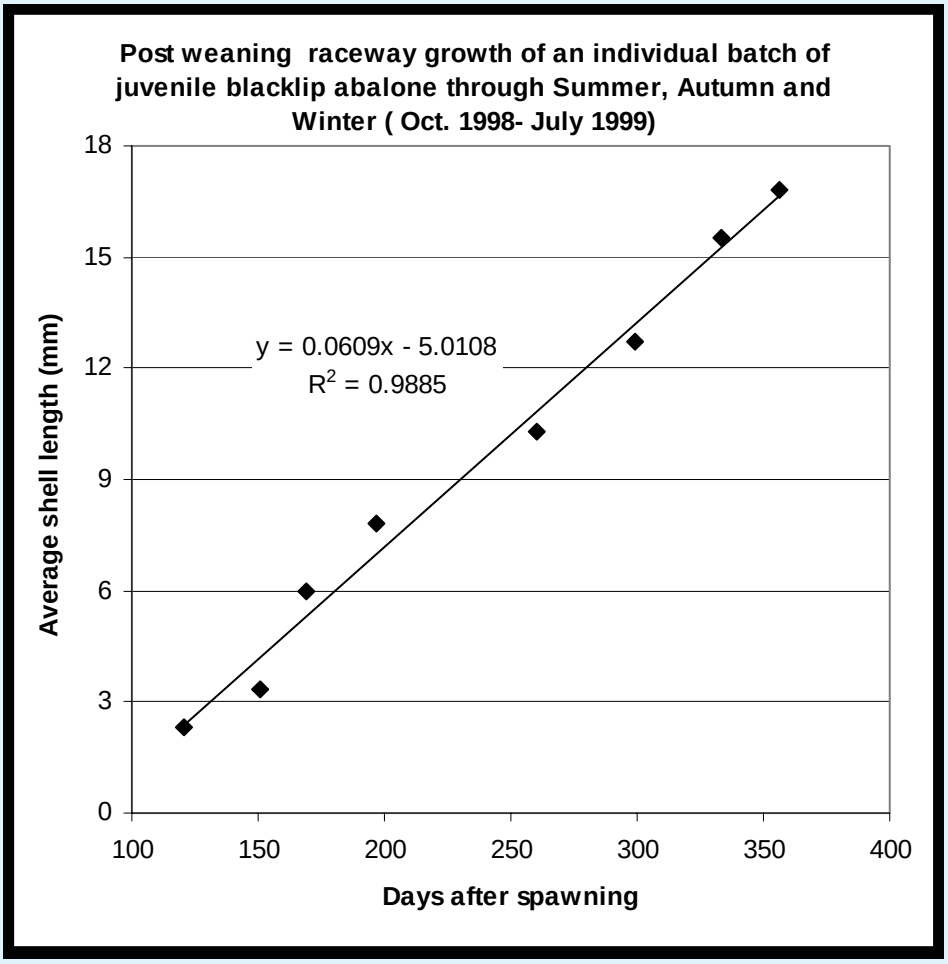


Figure 42 *Constant (linear) shell growth of weaned raceway reared juveniles. (From Heasman et al., 2004) Note the contrast with earlier exponential growth pattern of diatom plate reared post-larvae (**see Figure 37**).*

Figure 43 *Larger (3 to 4 m^2) second-stage raceways operated at a minimum recommended depth of 5 cm. Note: These are used to on-rear the juveniles from 4 to 5 mm up to 7 to 15 mm at densities of 20 000/m^2 (initial) to 10 000/m^2 (final).*

Figure 44 *Top: Daytime photograph of secondary nursery system with aeration off to show juveniles inactive and sheltering under artificial hides*

Bottom: Same system photographed 1 h after nightfall, revealing active nocturnal foraging

Figure 45 *Various types of land based abalone grow-out farm systems used in Australia*

Left and above: Greenlip abalone in "Slab" tanks

Middle left: Large outdoor circular tanks

Bottom left: Maze tanks

Below & bottom right: Intermediate pipe tanks being used to rear greenlip abalone

Water quality and temperature

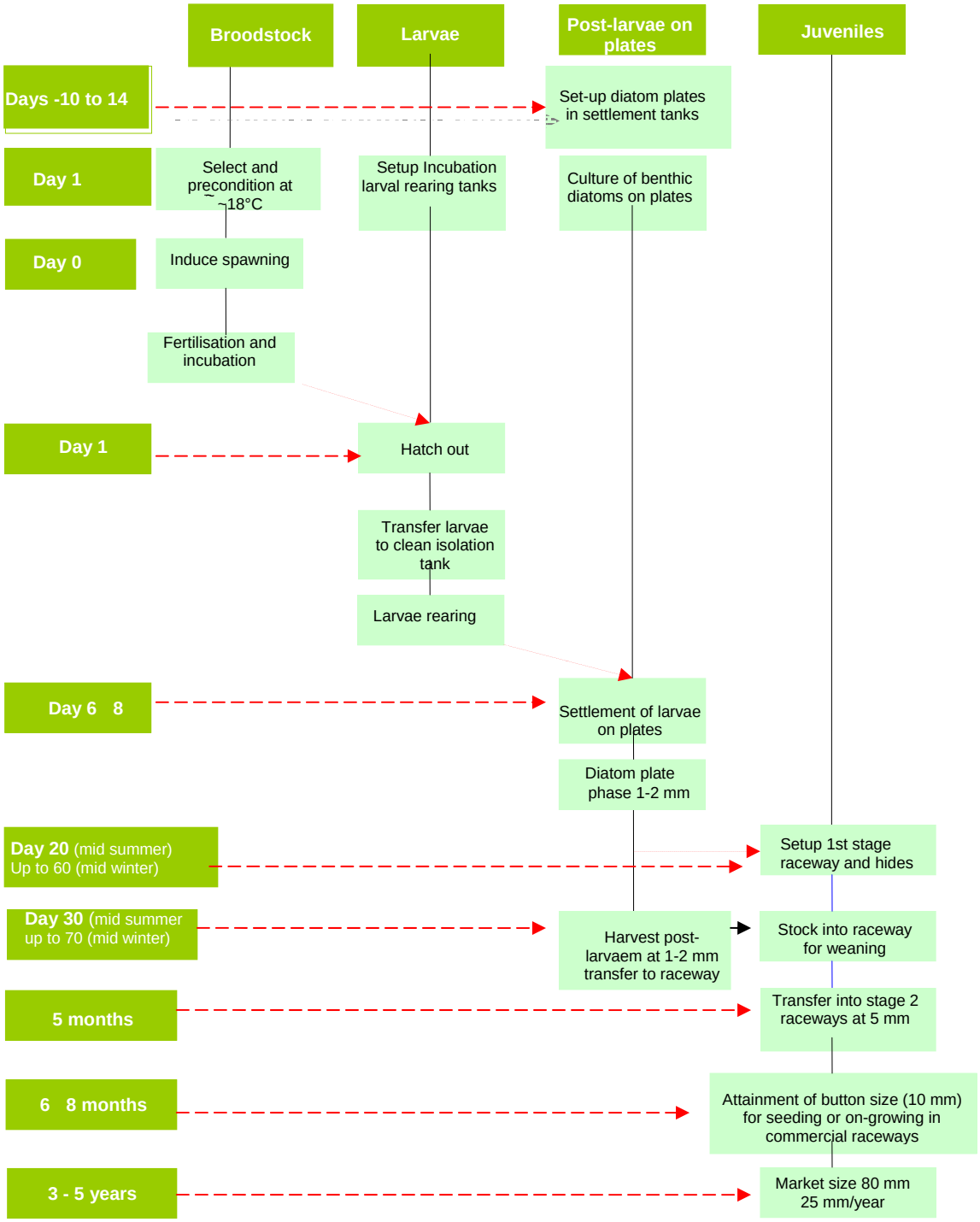
Juvenile blacklip abalone, especially in the larger 1+ to 3+ year old age-classes, should always be maintained at temperatures below 24 ºC and in continuously flowing, oxygen-saturated seawater. As already stated, a useful guide to the minimum seawater exchange rates required to maintain optimum growth and health is that they should match or exceed the combined body weight of abalone every minute (1 L of seawater for every kg of abalone). Assessment of density for blacklip abalone should be made during the period of peak activity 1 to 4 h after nightfall. In this regard it is important to know that the older and larger the juveniles, the less tolerant they are to common forms of stress, including reduced seawater quality parameters such as pH, salinity, dissolved oxygen and temperature extremes (especially high temperatures). It is also important to appreciate that any form of stress will lower resistance to other stress factors, including infectious diseases, starvation and nutritional deficiencies.

Feeding

Dry formulated feeds should be ordered in regularly (monthly) to avoid extended storage times and associated deterioration. Even then, they are best stored deep-frozen (-18°C) to preserve their nutritional integrity—especially that of the volatile/labile components, including some essential vitamins and fatty acids. Frozen storage also minimises microbial spoilage, including the production of fungal-generated toxins (aflatoxins). Food consumption and feed application rates should be monitored and matched on a routine basis to achieve satiation feeding. Satiation feeding maximises growth and water quality (and hence health) while minimising the cost of wasted food. This is a simple process of making small (5%) upward or downward adjustments in the weight of formulated food rations fed to each raceway. Thus at the beginning of each week the ration fed to particular raceways will be raised if very little or no residual (uneaten) food has been logged over the previous seven mornings. Likewise, raceway rations should be left unchanged if near-satiation feeding has been achieved, or reduced by 5% if low to moderate levels of uneaten food have been consistently observed over the previous week.

Appendices

Appendix 1 Schedule of hatchery operations



Appendix 2 Design and operating specifications of a relatively simple and inexpensive abalone broodstock conditioning system

As insurance against system failure at least two independent units of the type illustrated in this Appendix should be used for year-round temperature-controlled reproductive conditioning of abalone. Each unit needs to be separately housed within an insulated room. The units illustrated in **Figure A1** are hybrid systems incorporating both continuous low-level exchange (1 to 3 total system volumes/day) with a much higher rate of seawater recirculation (1 to 1.5 total system volumes/h). Recirculation needs to incorporate continuous or semi-continuous removal of solids by physical filtration, removal of dissolved organics—especially proteinaceous and oily (lipid) materials—by using foam (fractionation), and removal/detoxification of excreted ammonia by using biological and/or denitrification filtration.

Holding tank:
FRP (fibreglass) -constructed rectangular tank, 1500 L nominal capacity.
Dimensions: 2700 × 1000 base × 500 mm tapered wall.
BU1: Open tank with hides of fibre cement sheets.
BU2: Buckets. Abalone held in fifteen to eighteen 30 L drums with five to eight abalone per drum. (The drum system follows that used by Moss [pers. comm.], Mahunga Bay, New Zealand for *H. australis*.)

Header tank: 1000-L nominal capacity.

Physical filter: Dacron (Aquahort) or similar.

Biofilter: Layer of Dacron over 10 L of coral debris over 100 L of bioballs.

Pumps: Davey Models XF171 and XF221.

Foam fractionator (**Figure A2**):
Diameter: Downtube 150 mm PVC class-18 pressure pipe.
Height: 1890 mm.
Foam effluent: Transparent cone with 19 mm poly waste line.
Venturi: Mazzei injector model 484 (20 mm).
Flow-through fractionator, 1 L/s.

Temperature control:
Air-conditioner, Email GM155H dual cycle, 1.5 kW or similar
Chillers. Carrier 30ZQ024 on header tank. Ozsea 2 hp on recirculation.
Temperature maintained at 15.0 or 16.0°C ± 1.0°C, monitored by temperature dataloggers (Hastings).
The header tank also supplies temperature-controlled water to the spawning/larvae-rearing unit.

Aeration:
Gentle aeration through 3-mm CVT (clear vinyl tube) to each bucket.

Flow rates:
New water throughput, 2 to 4 L/min. (2 to 4 total system volume exchanges/day.)
Recirculation through chiller, foam fractionator and biofilter is 0.5 to 1 L/s.
(using Davey XF 171 pump), representing 1 to 2 total volume turnovers/h.

Stocking rate:
Each system is designed to accommodate a total of about 100 adult abalone (total biomass 30 kg)

Feed rates:
0.75% to 1% of body weight/day of formulated feed (Adam and Amos).

Ammonia levels:
Routinely less than 0.2 mg/L total ammonia nitrogen, pH range 7.9 to 8.2

Light levels:
Dim light. Constant photoperiod of 10 h light, 14 h dark.

Maintenance:
Daily. Feeding, manual temperature check and visual check of system and abalone.
Each 2 to 3 days. Full drain-down, water change and cleaning of holding tank to remove detritus and surface biofilms. Cartridge filter cleaned.
Foam fractionator and Dacron solids-removal filter cleaned.
Each month. Wash half of biofilter. Test for ammonium (Merck, Aquamerck ammonium test) and pH.

Alarm system:
Notification of system failures via phone link (Telstra SMS).
Temperature alarm if outside ± 2.0°C.
Header tank low-water level alarm.
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Abalone broodstock conditioning unit
NSW Fisheries / FRDC, Abalone enhancement project.

Nick Savva, June 2000.

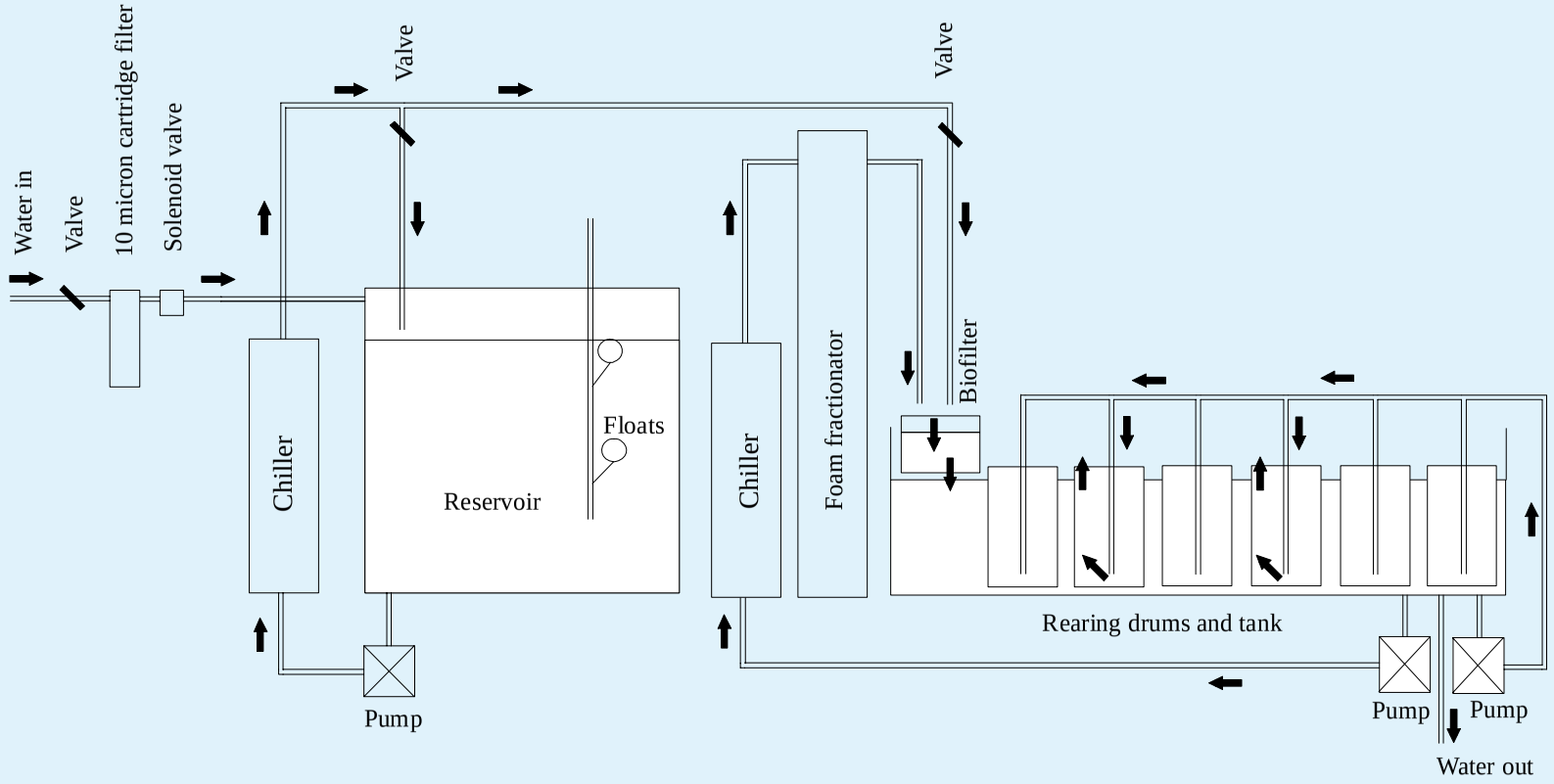

Figure A1 Example of the broodstock conditioning unit – schematic design

Not to scale. All dimensions in mm. Motive flow, Davey XF 171. Flow rate, 1

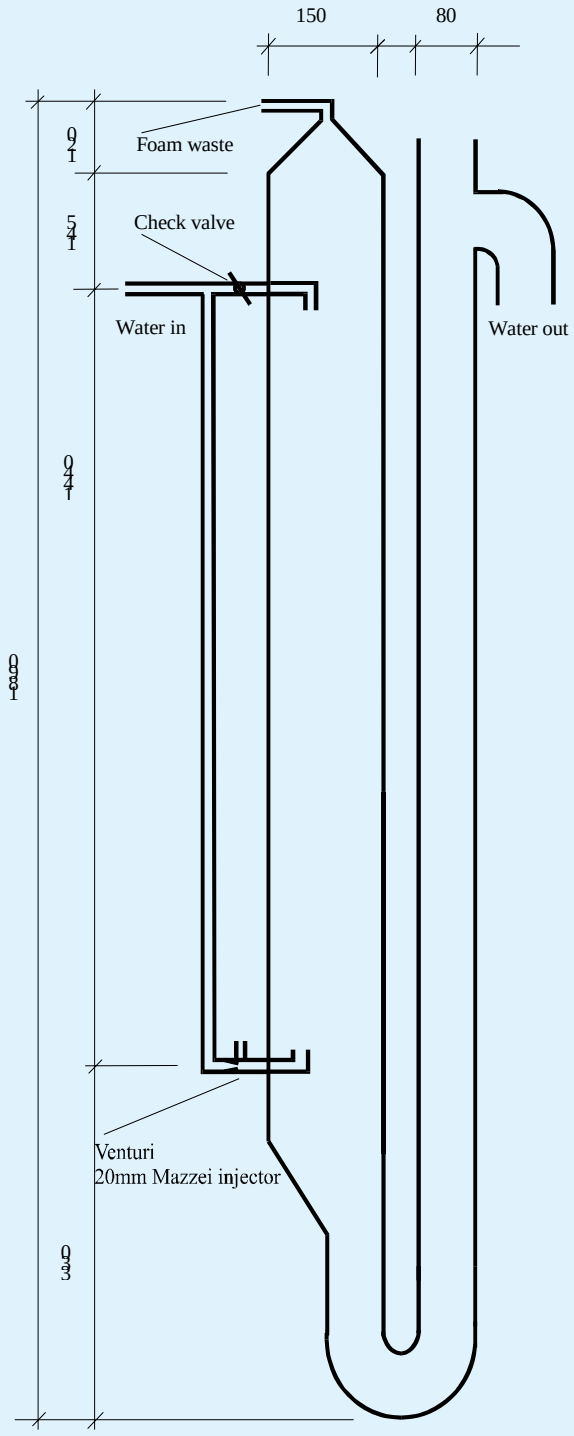

Figure A2 Detailed design of foam fractionator

Appendix 3(a) Data to be recorded for broodstock, their collection and conditioning history and spawning performance

Collection:

- Where collected
- Depth
- Time/date/ambient sea temperature
- Allocated Tag and Code numbers
  e.g. .***Tag number*** *a-012 =* ***Code number*** *BH -230203-(2F)* where: BH is letter code of collection site (Boat Harbour); 230203 is date (ddmmyy) of collection and 2F is designated number and sex of individual
- Conditioning tank number allocated to after quarantine

Conditioning protocol (per tank):

- Temperature (maximum /minimum monitored daily)
- O_2 (monitored weekly)
- N as ammonia and nitrite (monitored weekly)
- Net exchange and recirculation of flow rates (monitored weekly)
- Date of cleaning
- Mortality(monitored daily)
- Feeding rate recorded daily (normally 1% body weight per day)

Animal condition and performance:

- Weight and length when initially transferred to conditioning unit and on all subsequent spawning induction occasions
- Gonad index when initially transferred to conditioning unit and on all subsequent attempted spawning induction occasions
- Spawning response including egg numbers and quality in the case of females

Appendix 3(b) Data to be recorded for all attempted inductions of spawning

Batch code/ identification:
- date
- person responsible for spawning

Spawning:
- tank setup- including how many abalone per tank
- time UV was turned on
- time males spawned
- time females spawned
- temperature and any alterations to temperature
- comments on egg quality (colour, form/shape, clumped/uniform/layer)

Fertilisation:
- number of females and males spawned fully
- approximate number of eggs per female
- sperm concentration and motility
- time fertilised
- time washed

Hatchout phase:
- system setup
- temperature of water
- time to hatchout
- percent hatched

Larval phase:
- system setup
- water temperature
- water flow/exchange rate
- larval density each day
- cleaning routine
- time to shell development
- time to settlement

Juvenile/ adult phase:
- growth rate
- survival rate

Appendix 3(c) Example of data collection for spawning operations

Spawning Attempt 5/4/01

Broodstock collected from Broughton Is. 15/4/99					
Assessment of broodstock by N.S and C.B. on 5/4/01.					
Rivet Tag	**Glued Tag A**	**Visual gonad index**	**Tub**	**Eggs?**	**Comments**
22 FEMALES					
1001	502	3	7	0.91	
1002	504	2	9	1.0	NB. Except for 1001, nos. of eggs are averaged across multiple spawners.
1004	506	1.5	5		
1005	507	1	8		
1006	509	1	10		
1009	512	1	8	1.0	
1010	513	2	6	1.0?	Unsure which or both in tub 6 spawned.
1011	514	2	2		
1012	515	2	1	1.0	
1013	518	2	7		
1014	519	2	1	1.0	
1015	520	2	2	1.0	lost tag, to be retagged
1016	521	1	6	1.0?	Unsure which or both in tub 6 spawned.
1017	523	1	10	1.0	
1018	524	2.5	3		
1019	525	2	4		
1020	526	2	4		
1021	527	1.5	5		
1022	528	1	9		
1023	529	2	3		
7 MALES					
1024	500	2	11		
1025	511	1.5	11		
1026	503	2.5	12		
1027	508	3	11		
1028	516	3	12		
1029	517	1.5	12		
1030	522	1	11		

Appendix 3(c) cont'd. Example of Companion Diary Notes of Spawning Operation

0850 Abs transferred from BU2 to spawning containers in hatchery.

0905 Tris added at 6.6ml/L
.
0915 Peroxide added at 3ml/L.

1015 +1hr. All quiet
.
1115 +2hrs. All quiet, plenty of bubbles.

1215 +3hrs. FWC. Flow on. Water temp.

1310 Flow off. Air on. 17.0°C.

1320 Sperm released in tubs 11 and 12 (only light so far.) very small # of eggs in Tubs 7 and 2. Eggs from tub 7 are of poor quality and are possibly atretic. Eggs from tub 2 appear fine for fertilisation. Will wait for hopefully more eggs before acting.

1345 Rapid succession of spawning females over past 35mins
Coll'n 1. Tub 1, #1014 and 1012. 1.05 million.
Coll'n 2. Tub 2, #1015(lost tag). 1.4 million.
Coll'n 3. Pooled tubs. 1,2,6,8. 1.38 million.
Coll'n 4. Tub, 7. #1001. 0.91 million, questionable quality.
Coll'n 5. Tubs, 1,2,6,8,9. 2.64 million.
Coll'n 6 Tubs, 1,2,6,8,9. 2.84 million.
All eggs except Tub 7, to LT2. 9.3 million.
Eggs from tub 7, #1001 to LT100:2. 0.91 million.

TOTAL EGGS SPAWNED 10.2 million.

Abs returned to BU1.

Batch designated as 02-0401.

Appendix 4 Prevention and treatment of mud-worm infestation (adapted from M. Lleonart, 2001).

Mud-worm infestation of the shells of conditioning broodstock can and does seriously reduce productive performance of and indeed can cause the death of these valuable stock if left untreated. The threat to broodstock abalone is particularly high because of the ability of mud-worms to breed asexually, and hence to rapidly and heavily re-infect both their hosts and other previously un-infested broodstock in close proximity within controlled temperature conditioning systems. The first and most important way of preventing and controlling mud-worm infestation is therefore to cull heavily infested stock at the time of their collection from the wild. Effective treatment of new lightly infected stock can be accomplished by use of either shell drying shell salting or shell waxing. For shell drying, the abalone need to be removed from water and their shells allowed to dry out in accordance with the following conditions.

- 2-4 hours out of water
- Minimum temperature of 15°C & recommended maximum of 21-22 °C
- Air humidity of less than 64%

If shells of stock are not apparently drying within ~30minutes of air exposure the conditions are probably unsuitable. Merely removing abalone from water, even for longer periods of time and/or higher temperatures, will not kill mudworms if shell surfaces do not dry properly. Air humidity can be measured simply and cheaply with a wet & dry bulb hygrometer and accompanying conversion tables (scientific equipment supply companies ~$30). Typically, days with minimal cloud cover will be sufficiently dry for treatment in southern Australia. Abalone should be dried in shade, not direct sunlight. Depending on-rearing vessel design and degree of fouling of these, it may be possible to dry stock within rearing vessels. If not, abalone will have to be removed and placed on a suitable substrate such as sheet plastic. It may be appropriate to combine treatment activities with grading, thinning or other activities requiring handling.

Mud-worm kill rates of up to 90% may be achieved by shell drying provided the worms treated are small (less than 6 months old). Reduction in worm numbers may be possible after this time but the result will vary with period of infestation, extent and depth of blisters, size of abalone and degree of shell fouling. The treatment drying conditions referred to are generally safe for blacklip abalone. However drying can significantly suppress growth and probably gonad development as well. Moreover as exposure of conditioning broodstock to temperatures of 19°C or higher will either promote unplanned spawning of ripe eggs or atresia (regression) of developing eggs, drying should not be applied to conditioning broodstock except for individual stock in the period immediately following successful induction of spawning.

The alternative treatment of shell waxing involves the manual application of a thick (2 to 5mm) layer of pliable non-toxic surfboard wax. Shell waxing, which is best preceded by blot drying with paper toweling and swabbing with 90% ethanol, is far more labour intensive and therefore expensive than shell drying. However it has a number of major advantages over shell drying namely:

- it is capable of suffocating all mud-worms and their young if applied diligently and left for a minimum of 2 days
- broodstock suffer much lower stress in the absence of desiccation and much shorter periods of air exposure (less than 30 minutes)
- it can also be safely applied to conditioning stock at air temperatures of 10-15°C and the broodstock immediately returned to the conditioning system thereby eliminating risk inadvertent induction of spawning of ripe eggs or regression of developing eggs

Shell salting, the simplest and cheapest of the three alternative treatments. It is also the quickest entailing a one hour application of copious amounts of damp sticky rock salt to the drained damp shell of broodstock while strongly attached to a smooth flat horizontal surfaces such as those provided by clean diatom nursery plates. Salt coating has the same advantages of waxing in application to conditioning broodstock in being able to be applied within the safe temperature range of 10-15°C and without the risk of undue physiological stress associated with shell drying. However the killing efficiency of salting is probably lower than both drying or waxing as mudworm occurring on the curved peripheral margin of the shell and adjacent to the respiratory pores may escape exposure to lethal levels of osmotic stress. This is because of the poorly adherence characteristics of the salt and the need to prevent salt from coming in direct contact with soft delicate tissues of the foot and mantle on the underside of the abalone and with the gills and neighboring tissues in the mantle cavity via the respiratory pores.

Appendix 5 Determination of sperm concentrations using haematocytometer

Haemocytometer slides have 2 rafters allowing for 2 sub-samples to be examined. The sampling and counting procedures are as follows:

1. dilute sperm sample if needed (use formalin 4% to fixate moving sperm) clean slide and cover-glass with Kleenex-paper
2. press cover glass onto the slide until the Newton diffraction rings appear
3. fill both slides of the counting chamber under the cover-glass with a single smooth flow of suspension using a Pasteur pipette (avoid air bubbles)
4. count sperm in, 20 small squares under a microscope (objective 40 x). Count cells which touch the upper and left border but not those which touch the lower and right borders (see schematic diagram)
5. the sub-sample on the other side of the chamber is counted in the same way

- **calculation of density per ml.**

numbers of cells per ml = $(n_1 + n_2)/(2\times20) \times250\times10^3\times d = (n_1 + n_2)/160\times10^6\times d$

where:

n_1 = number of sperm counted in upper rafter
n_2 = number of cells sperm counted in lower rafter
d = dilution factor

NB For greater accuracy make 3 duplicate counts (3 separate dilutions each counted in two rafters).

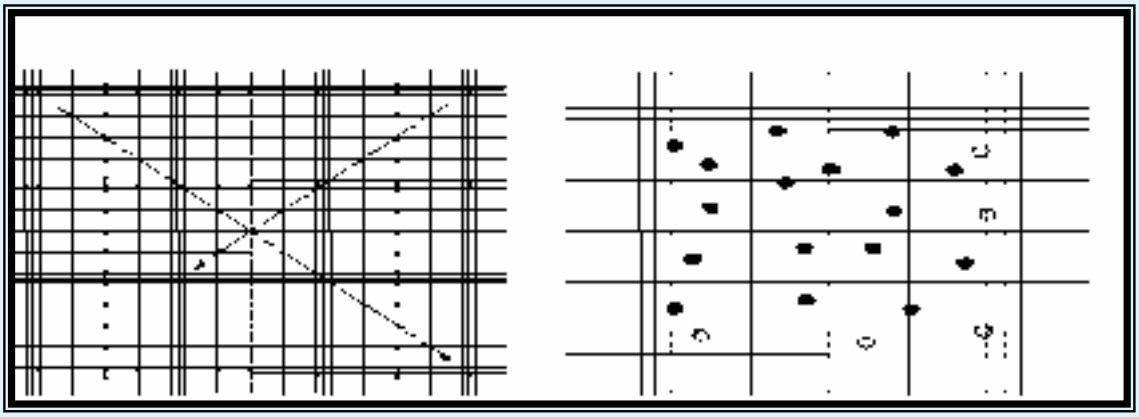

Figure A3 *Counting directions (follow arrow), count the sperm cells in the square and those which touch the top and left borders (•). Do not count the ones touching the right and bottom borders (o).*

Appendix 6 Chlorine disinfection and neutralization protocols

Disinfection Task	Procedures	Amount of stock chlorine to use
Nets/buckets/boots:	0.25ml **stock chlorine solution**[1] per litre of freshwater for ≅ 24hours	25ml/1000L
Transport containers and	0.8ml **stock chlorine solution** per litre of freshwater for ≅ 24hours	80ml/1000L
Rearing vessels	1.25ml **stock chlorine solution** per litre freshwater for ≅ 24hours	125ml/1000L
Nursery plates and seawater prior inoculation with *Ulvella* spores	0.8ml **stock chlorine solution** per litre of 1∂m filtered seawater for ≅ 24hours followed by the addition of 0.02 ml/L of **stock *thio***[2] solution and heavy aeration (air stripping) and finally test for traces of **residual chlorine**[3]	80ml/1000L

[1] **Stock hypochlorite solution** contains 10-12% (100 to 120 g/L) active chlorine

[2] **Stock *thio*** (chlorine neutralising) **solution** 2 molar (316g/L) solution of sodium thiosulphite in freshwater

[3] **Residual chlorine test** -add 1 DPD No 4 chlorine test tablet per 10-20ml sub-sample of thio neutralised and air stripped seawater

Glossary

adventitious, naturally occurring; used here to describe types of wild microalgae and macroalgae that opportunistically colonise bare substrates

banjo filter, a filter used in flow-through larval culture to stop loss of larvae via the outflow

barrens, bare (or, more commonly, crustose coralline algae-coated) rock areas commonly created by dense aggregations of grazing animals, especially sea urchins and (in NSW) the black (or purple) urchin (*Centrostephanus rodgersii*)

benthic, bottom-living

benzocaine, a chemical (ethyl-p-amino-benzoate) used to anaesthetise abalone before handling or examination for health and breeding status

biofilms, thin films of micro-organisms (initially bacteria and fungi) that colonise bare substrates

competence, the ability and urge of larvae to actively seek out and settle on suitable habitats and to be able to *metamorphose* (transform) into reef-dwelling *post-larvae*

copepods, very small planktonic and surface-living crustaceans

crustose coralline algae, colonial algae that form a tough, limestone depositing skin on rocky surfaces that constitute the principal settlement substrate and early juvenile habitat of blacklip abalone; commonly known as 'pink rock'

diatoms, a special group of microalgae with an outer cell-coat of silica

demersal, found near or on the sea bed

drift, broken-off and drifting pieces of seaweed, including common red seaweeds such as nori (*Porphyra* spp.) and *Gracilaria* and green seaweeds such as sea lettuce (*Ulva* spp.)

embryos, fertilised eggs undergoing successive cell divisions and cell differentiation

epi-biota, mixture of plants and animals attached to surfaces

exponential growth, implies that the time it takes to double in size from any starting point remains constant

gonads, the reproductive organs of abalone and other animals, being the egg-producing ovaries in females and sperm-producing testes in males

haemocytometer, a simple cell counter consisting of a thick glass slide with a very fine set of gridlines etched on the surface, over which a special heavy-duty glass cover-slip is laid

IDS (integument/muscle detachment syndrome), the most important affliction of abalone veliger larvae, whereby there is a breakdown of attachment of the twin retractor muscles to the inside (integument) of the shell. This loss of one or both attachments is commonly considered a symptom of bacterial infection (usually by *Vibrio*) arising from either poor quality of seawater or inadequate hygiene.

macroalgae, large filamentous or thallus-forming seaweeds

metamorphose /metamorphosis, transformation of planktonic veliger larvae into reef-dwelling *benthic* post-larvae

microalgae, microscopic single or chain-forming algal cells

moribund, sick, weakened and dying as consequence of injury, disease, malnutrition or age

photopositive, attracted to light

plankton(ic), small aquatic animals (zooplankton) and plants (phytoplankton) that live permanently in the water column

polyspermy, deleterious effects of very high sperm to egg ratio during fertilisation, when more than one sperm fertilises an egg

post-larvae, name given to the juvenile form of animal that in abalone spans the period after metamorphosis up to 3 mm shell length

radula, rasping tongue of the abalone

Sedgewick rafter slide, a special type of microscope slide with a shallow 1-mL reservoir, the floor of which is divided into 1 × 1-mm grids where numbers of small particles such as larvae or microalgae sampled from a dispersed suspension can be systematically counted and measured grid by grid at 5× or 10× with the aid of a stereomicroscope fitted with a mechanical stage.

trochophore, first-stage swimming but non-feeding planktonic larval stage of abalone that develops about 16 h after fertilisation and lacks a shell

veliger, second and final fully-shelled larval stage of abalone and many other types of mollusc