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Chapter 3 Planning and Implementing Breeding Programs



Chapter 3 Planning and Implementing Breeding Programs

Important things to know

Abalone broodstock-conditioning facilities are expensive to build, maintain and run. When designing a hatchery and considering issues such as the scale and timing of hatchery production, the amount of capital investment required and ongoing operational costs, it is important to know how many ripe, ready-to-spawn broodstock will be needed and when. If seed (small juveniles) abalone are to be produced for aquaculture, specific breeding lines selected for faster growth may be required. On the other hand, if seed are to be produced for restocking programs, the identity and distribution of genetically distinct populations in the depleted areas must be determined and protected.

To safeguard the genetic diversity of wild populations of abalone, a minimum number of parents (at least 30 to 40 of each sex) need to be used in the production of seed abalone for enhancing depleted wild fisheries. If selective breeding is to be practised over a number of successive generations, each generation must be derived from a large number of mating pairs to avoid inbreeding problems. Such breeding programs impose considerable additional stock management and record-keeping loads and responsibilities. They are also complex and costly. It is therefore strongly recommended that specialist advice be sought when planning and costing selective breeding programs. Such advice can be obtained from the **National Abalone Selective Breeding Program**, coordinated through the Abalone Aquaculture Subprogram of the Fisheries Research and Development Corporation (FRDC).

Reproductive conditioning of broodstock

In the more southern waters of Tasmania and Victoria, relatively predictable seasonal temperatures generally remain below the upper cut-off of about 19°C for breeding in blacklip abalone. As a result, these southern stocks undergo more synchronous and predictable annual breeding patterns in which the gonads start from an initial resting stage of little or no development in autumn and end with full ripeness and spawning during the period of rapidly rising sea temperatures in late spring and summer.

In NSW, by contrast, breeding is subject to less favourable and irregular seasonal temperature regimes. As coastal sea temperatures in central NSW generally rise above the 19°C breeding threshold by November and remain so until as late as July (**Figure 17**), the window for reproductive conditioning can be as short as 4 months (120 days). This is less than the minimum threshold of about 150 days at sub 19°C temperatures required by blacklip abalone to attain ripe, ready-to-spawn condition. Regular and predictable spawning is further disrupted by large ocean eddies. These break away from the East Australian Current around Fraser Island in southern Queensland and then course slowly southward down the NSW coast. Between three and seven such eddies occur each year. If close to shore, the eddies bathe the coast in warm (up to 20°C in winter and 25°C in summer)

subtropical seawater, but if offshore they can bathe the coast with deeper up-welled seawater as cool as 14 to 18°C at any time, including in summer.

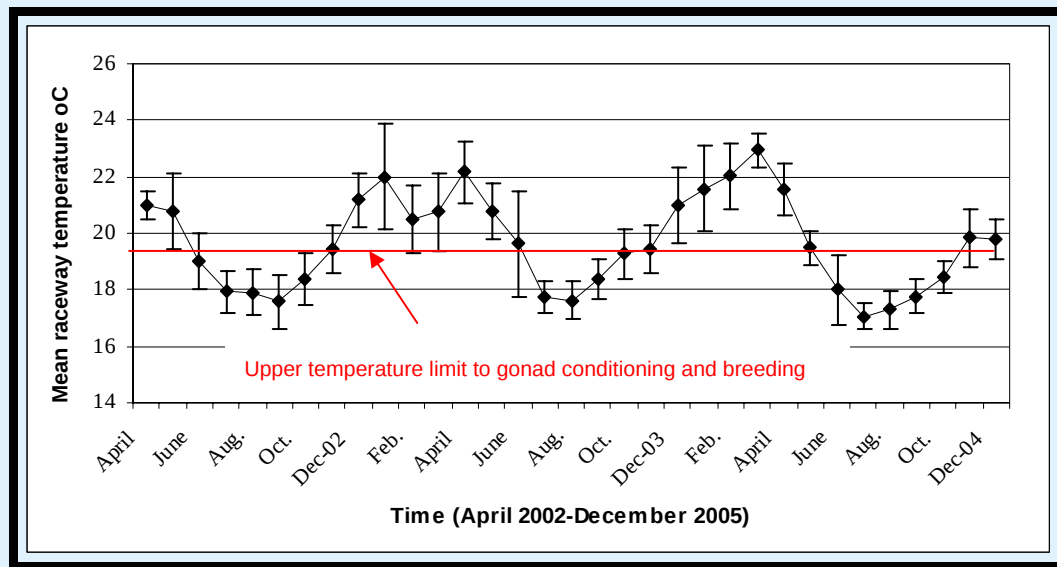


Figure 17 Coastal surface sea temperatures at Port Stephens in central NSW from April 2002 to December 2004. Bars are standard deviations.

An important practical implication for abalone hatcheries in NSW is that for much of the year wild blacklip abalone are asynchronous in their reproductive development. At Port Stephens, near the northern limit of the commercial abalone fishery, wild stock commonly include many females with large gonads swollen with over-ripened eggs. Even after many months of conditioning it is not possible to confidently assess whether such abalone are ripe and ready to spawn or not. In most cases they have to be subjected to repeated spawning attempts at 1 to 2 month intervals before responding. However once they have spawned they can usually be expected to spawn good-quality eggs every 150 to 200 days thereafter when held at optimal temperatures of 16 to 18°C.

It is therefore essential that the conditioning history and spawning activity of individual broodstock be recorded and tracked. Suggested information to be recorded is provided in **Appendixes 3a and 3b**. A simple diary record of the type illustrated in **Appendix 3c** is also recommended. Such records allow rapid easy tracking and collation of the information needed to maximise the spawning success in terms of yields of viable eggs and sperm. The first record-keeping operation is to enter the tag number(s) of each animal onto the spreadsheet, together with where and when it was collected and its sex. The date of each attempt to induce spawning must be linked to this record, together with the outcomes, successful or not. If spawning is successful, additional data on the quantity and quality of eggs or sperm produced and subsequent yields of fertilised eggs and of larvae put to set must also be recorded. This is particularly useful when establishing a new hatchery and for tracking down the causes of spawning failures and other problems contributing to poor or variable yields of eggs, larvae and juveniles.

These issues highlight the importance of tagging and record-keeping practices for determining which broodstock are most likely to spawn at any given time and for choosing the most opportune dates to induce spawning.

To ensure fast, efficient and reliable year-round reproductive conditioning and spawning, broodstock must be maintained with minimal stress and within a relatively narrow temperature range of $16 \pm 2^\circ\text{C}$. This requires controlled temperature-conditioning facilities and an uninterrupted supply of near-oceanic-quality seawater. Open flow-through systems consume large amounts of seawater that equate to at least the biomass of stock every minute. This is necessary to maintain broodstock within optimum ranges of water quality (including those specified in **Table 1**). In NSW, considerable energy may be required to chill incoming ambient seawater to the prescribed temperature of $16 \pm 2^\circ\text{C}$. Indeed, the temperature of incoming ambient seawater may need to be lowered by as much as 9°C in summer. Incorporation of seawater recirculation in reproductive conditioning systems of the type illustrated in **Figure 18 and Appendix 2** therefore offer considerable cost and energy savings. Water recirculation systems also give the broodstock considerable protection from sudden adverse changes in the quality of the source seawater, particularly at sites susceptible to the effects of storms, floods and associated pollutants and to toxic algal blooms.

Table 1 Lethal tolerable and optimal rearing conditions

Environmental / Water quality factor	Lower and upper lethal	Tolerable	Optimal / recommended
Temperature °C			
Fertilisation and Incubation	?	15-21	18
Larval rearing	?	?	18
Settlement Post-larval development	10 and 27	12-25	18-24
Early juvenile	10-and 27	12-24	15-21
Late juvenile and adult	10 and 25	12-23	14-18
Salinity g / kg	25 and 40	30-38	32-36
pH		7.5-8.5	8.0-8.2
Dissolved oxygen		≥ 95% saturation	100% saturation
Free (unionised) Ammonium mg / L	≥0.05	≤0.004	≤0.001

Some useful design and operating protocols for reproductive conditioning facilities are as follows:

- Use two or more independently operated temperature-controlled sub-units to insure against system failure and resultant gaps in year-round hatchery production.
- Operate within stocking densities and seawater exchange rates that promote high reproductive performance and general good health. In the case of the conditioning system described in **Appendix 2**, stocking rates of ≤ 1 kg broodstock biomass per 50 L of standing seawater volume, coupled with a net new seawater exchange rate of 3 to 5 volumes per day, are required, as are recirculation rates that equate to the entire volume of the system every 30 to 60 min. Thus, for every 1000 L of seawater in a system of the type illustrated in **Figure 18** and specified in **Appendix 2**, seawater needs to be continuously recirculated sequentially through a solids-removal filter, a dissolved-nutrients filter (foam stripper) and a biological filter to convert ammonium to nitrite/nitrate at a minimum flow rate of 1000 L/h.
- Provide a dual-cycle air conditioner that can maintain recirculating seawater at $16 \pm 2^\circ\text{C}$ as a back-up in the event of seawater supply or chiller failure.
- Provide a stand-by generator capable of powering the main seawater supply pumps, air compressors, air-conditioning systems and (preferably) seawater chillers.
- Accommodate broodstock in communal tanks that provide ease of observation, access and cleaning.
- Clean and purge conditioning units of uneaten food and faeces, assess the health of abalone, and remove dead or **moribund** (dying, through sickness or injury) individuals at least twice weekly.
- Conduct regular (at least twice yearly) inspections of stock to detect, treat or replace those that are compromised by heavy infections of boring mud-worm and sponges.
- Apply a conditioning temperature of $16 \pm 2^\circ\text{C}$ that promotes rapid conditioning while also providing a reasonable buffer of time for detecting and responding to equipment and power failures. This is particularly important during the warmer half of the year, when power failures can expose broodstock to temperatures of $\geq 20^\circ\text{C}$ that trigger epidemic spawning and/or resorption of developing eggs.

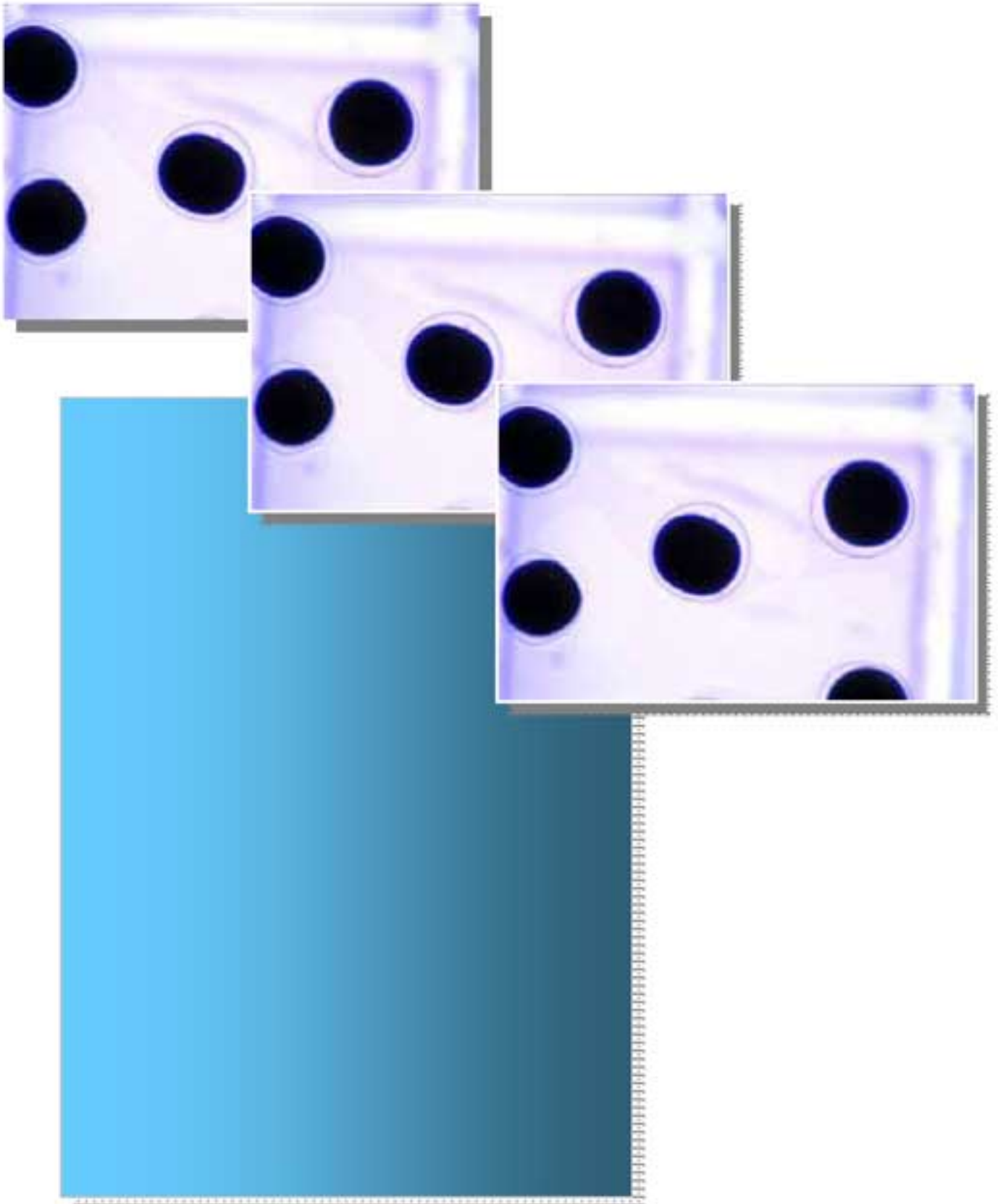


Figure 18 *Controlled temperature reproductive conditioning system that employs recirculation and filtration of seawater to greatly reduce energy costs of heating or cooling (detailed specifications provided in **Appendices 2 & 3**).*

**STEP BY STEP SUMMARY AND CHECK-LIST FOR CONDITIONING BLACKLIP
ABALONE**

- ☑ 1. Handle stock as gently and as quickly as possible during all phases of collection and transportation back to the hatchery.
- ☑ 2. As soon after collection as practicable, measure and double-tag broodstock selected for size, health and gender.
- ☑ 3. Establish a data file for each batch of abalone collected; record data of the type presented in **Appendix 3**.
- ☑ 4. Place animals into a temperature-controlled conditioning facility operated at $16 \pm 2^{\circ}\text{C}$.
- ☑ 5. Maintain the stock under conditions that minimise all forms of stress. This includes all forms of physical disturbance, including handling, background noise, vibration and high or variable light intensity. It also includes maintenance of high water quality (especially pH, ammonium nitrogen and dissolved oxygen), within the optimum ranges specified in **Table 1**.
- ☑ 6. Feed broodstock a conditioning diet to satiation but not to excess.
- ☑ 7. Siphon uneaten food and faeces out of the broodstock-holding tanks and replace with isothermal seawater at least twice weekly, but change the water filter elements and clean out the foam strippers daily.
- ☑ 8. After each attempted induction of spawning (**see Chapter 4**), record the date and the spawning success or failure of individual stock. Confirm their gender, vigour, and health, and especially rates of infestation of the shell by boring sponges and mud-worm.

Chapter 4 Induction of Spawning and Fertilisation of Eggs



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Introduction

The cheapest, yet most reliable, method of inducing ripe abalone to spawn is to bathe them in seawater treated with the oxidant hydrogen peroxide (H_2O_2). An alternative method widely used by commercial abalone farms in Australia is to expose the incoming seawater to a specialised wavelength of UV (ultraviolet) light that converts oxygen dissolved in the seawater to another strong oxidant, called ozone. Both ozone-generating UV lamps and hydrogen peroxide alter the chemistry of seawater by producing free radicals that can stimulate the abalone to release a sex hormone called prostaglandin. This, in turn, triggers a series of chemical pathways that induce final ripening and subsequent spawning of eggs and sperm. We recommend hydrogen peroxide because it acts faster and has a comparable success rate to that of ozone, does not require expensive (\$4,000 to \$10,000) specialised UV equipment, and avoids potentially serious operator safety issues associated with UV light irradiation.

Selecting broodstock for spawning induction

The number and ratio of male to female broodstock needed for each spawning operation depends on the intended use of the juveniles produced. If they are destined for on-farming, a ratio of one male to every two females is adequate. If selective breeding is involved, or if juveniles are to be used as seed to enhance depleted fisheries, then large and equal numbers of male and female broodstock should be used to help ensure the greatest degree of genetic diversity of their offspring.

As previously discussed, sexing blacklip abalone can be difficult, especially in individuals with deeply pigmented skin that obscures the colour of the underlying gonad. Often the only reliable way to sex darkly pigmented individuals is to record their sex when they first spawn.

Spawning induction

A separate hatchery room with controlled temperature and light/dark cycles is required for spawning induction, fertilisation and incubation of eggs and for subsequent rearing of larvae. The room should have a continuous supply of freshly pumped, temperature-controlled ($16 \pm 2^\circ\text{C}$) seawater that is filtered to $1\ \mu\text{m}$ (nominal) and UV-disinfected. Air temperature should also be maintained at $16 \pm 2^\circ\text{C}$ using a conventional dual-cycle air-conditioner.

It is essential to keep hatchery rooms and equipment clean and sterile through chemical disinfection, as described in **Appendix 6**. Two 50 to 100 L squat open-top white plastic bins, one for disinfecting, the second for rinsing off the bulk of residual disinfectant, are required. Fill the first (disinfection) bin with fresh water and make up a 100 mg/L chlorine disinfection solution by adding 1 mL of pool chlorine (10% to 13% w/w sodium hypochlorite solution) per litre. Just before

use it is good practice to hose off all equipment (spawning containers, buckets, beakers and all other containers and utensils) with 1 µm-filtered seawater immediately before dipping and draining through the disinfection and rinsing tubs. The latter will ensure the removal of all vestiges of chlorine and freshwater. These procedures also apply to all air and water supply lines, which can be disinfected by being dismantled and left to soak in the disinfection tub between successive spawning operations or by purging with 100 mg/L chlorine solution and then being allowed to drain and air dry. Always drain the rinsing bin at the end of a day's use and leave to drain and dry before the next use.

During spawning induction it is essential that handling and other forms of disturbance to abalone are kept to a minimum. Only broodstock that meet the following criteria should be selected for spawning induction:

- have been grouped according to gender
- have undergone 150 to 180 days of conditioning at $16 \pm 2^\circ\text{C}$ since their previous spawning
- are in good general health.

Place from one to a maximum of three abalone in small (5 to 10 L) shallow plastic tubs of the type illustrated in **Figure 19**. A single broodstock per tub is recommended, as it gives certainty as to whether or not an abalone actually spawns and, if so, when it spawned and the quantity of eggs or sperm released. A further advantage of individual accommodation is that it avoids the possibility of accidental fertilisation of eggs, as may occur if an abalone is incorrectly sexed.

The spawning tubs should be mounted on shelves 0.3 to 0.6 m above floor level for ease of access, ease of handling and monitoring of broodstock, and ease of siphoning of eggs and sperm (**Figure 19**). The tubs should be supplied with seawater at a flow rate of at least 1 L per abalone per minute. The seawater must be freshly filtered to 1 µm, UV-disinfected, and pre-chilled or heated to the prescribed temperature of 16°C . White or clear plastic tubs are preferable for females, because the dark-coloured eggs can be seen more easily. Conversely, black tubs should be used for males, as the sperm turns the water milky even at low concentrations.

To minimise handling stress on the day of spawning, broodstock should be moved into the spawning-induction tubs on the preceding afternoon and maintained in flowing seawater at 16°C . First thing the following morning, the seawater temperature is raised by 2°C to 18°C and the broodstock left undisturbed for 2 h of 'priming' at the elevated temperature.

The chemical TRIS (MW = 121.14 g), which is used as a pH buffer, is added to the spawning tubs at the rate of 6.6 mL of a 2-molar (M) solution per litre of seawater to adjust the pH to 9.1. After a further 5 to 15 minutes, add hydrogen peroxide (H_2O_2) solution at the rate of 3 mL of a 6% solution per litre of seawater. The abalone are then left undisturbed in the dark for a further 3 h before the tubs are emptied and thoroughly flushed with filtered, UV-disinfected, 18°C seawater to remove residual hydrogen peroxide and TRIS buffer. The tubs

are then refilled with filtered, UV-disinfected seawater at 18°C and aerated to maintain a high level of dissolved oxygen in the absence of water exchange.

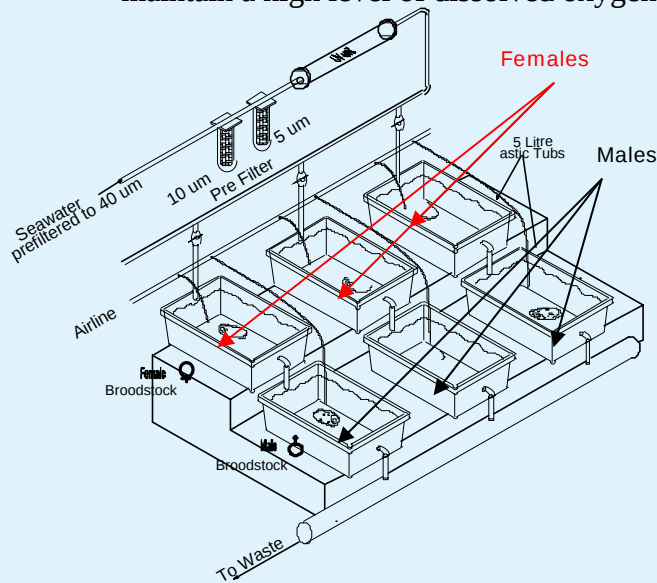


Figure 19 Left: Stacked shelf tray system used to accommodate abalone for induction of spawning. Right: Siphoning eggs into fertilisation container.

The broodstock are again left undisturbed in the dark for a further 1 h before inspections are started at 30 minute intervals for the next 2 h, then every 15 min until spawning commences. Males usually spawn sooner than females (generally within 1 to 3 h) and continue to spawn for a longer time. A compensating delay in the induction of males can be applied to help synchronise the spawning of the two sexes. Sperm is released via the respiratory pores like plumes of white smoke that quickly disperse, turning the seawater milky. The sperm can remain viable for a few hours at a room temperature of 16°C, and for longer if they are stored in a domestic refrigerator at about 4°C. Males often produce copious amounts of sperm that are usually well in excess of that needed to fertilise all the eggs produced.

As the viability of sperm can be substantially reduced if the sperm are stored at high concentration, it is advisable to dilute dense suspensions of sperm. Copiously spawning males should be progressively moved to new tubs of seawater (three tubs may be necessary). Tubs of sperm suspension should be discarded if the time from spawning exceeds an hour. Before fertilising the eggs, estimate the density of the fresh sperm suspensions in the tubs. This can be done with a **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. To count the sperm, take a small (about 1 to 10 mL) sample of sperm suspension and kill the sperm by mixing in one drop of 10% solution of formaldehyde per mL of the sperm suspension. Alternatively, you can use one drop of an iodine disinfection solution per mL of sperm suspension, which both kills and stains the sperm.



Figure 20 Good quality abalone eggs. Note the uniformly dense yolk and well-rounded (spherical) yolk membrane, surrounded by a thin clear vitelline layer.

Place a few drops of killed sperm suspension on the grid of the haemocytometer immediately adjacent to the cover slip and allow it to be drawn under the cover slip by capillary action. Observe the sperm at a magnification of 400x using a compound microscope fitted with a 10x eyepiece and 40x objective

lens. A complete step by step account of the sperm counting procedure is provided in

Appendix 5. If sperm

suspensions are too dense to be

easily counted, dilute the suspension with fresh filtered, UV-disinfected seawater until the appearance of the suspension is reduced to a slight milkiness.

Female blacklip abalone will start to release eggs from 3 to 5 h after the water change. Like sperm, eggs are also released via the respiratory pores. The colour of blacklip abalone eggs ranges from olive green to maroon but tends toward the latter when the broodstock are fed artificial diets. The released eggs are quite dense and rapidly sink to the floor of the spawning tubs. In preparation for fertilisation, the eggs are siphoned into 20 L white plastic buckets via coarse (1 mm) mesh screens to remove faeces and other foreign matter shed by the broodstock before spawning. Eggs should be siphoned by using a 5 to 10 mm diameter clear food-grade plastic hose.

Check the quality of the eggs by examining a sample of one to several hundred, either on a cavity slide or in a 'weighing boat' under a stereo or compound microscope at 50x to 100x. The eggs should be round and surrounded by a thin clear layer called the vitelline layer (**Figure 20**), and the yolk should be uniformly dark and dense. If the eggs are uneven in size or misshapen, or the yolks are blotchy, they are unlikely to show high levels of fertilisation or yield good quality larvae. Such eggs should therefore be discarded. As eggs do not remain viable for as long as sperm, and their viability steadily decreases over time, they should be fertilised within 1 h of spawning.

Fertilisation of eggs

Before fertilising the eggs, estimate how many good quality eggs have been collected. Next, dilute the eggs in a graduated beaker or bucket with filtered, UV-disinfected, 18°C seawater. Next, disperse the eggs into an even suspension by using a perforated-disc homogeniser, as illustrated in **Figure 21**.

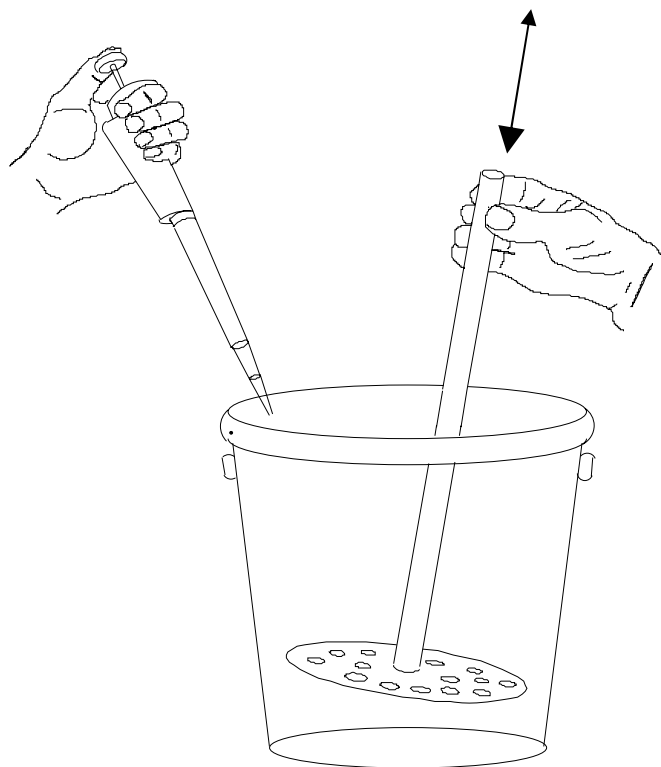


Figure 21 Drawing: Dispersion of eggs into even suspension using a perforated disc plunging homogeniser



Figure 22 Sampling of eggs using an automatic pipette (above) and placing them into small weighing boats (right) for counting by eye or with the aide of stereo microscope at low magnification

The homogeniser must be plunged gently up and down while avoiding contact with the walls or floor of the beaker. While doing so, take from three to five 1.0-mL samples of the egg suspension with an automatic pipette (**Figure 22**). Dispense each sample into a small container such as a weighing boat (**Figure 22**). The container should be white, making the dark eggs easy to see and count. Eggs can be counted with the naked eye or with the aid of a stereomicroscope at 5× or 10×.

If there are too many eggs in the 1.0 mL samples to be easily counted, dilute the egg suspension and repeat the above sampling and counting procedures. Alternatively, dispense 1.0 mL samples onto a **Sedgewick rafter slide**, (a specialist microscope slide with a shallow 1 mL reservoir, the floor of which is divided into 1 × 1 mm grids), where they can be counted grid by grid at 5× or 10× with the aid of a stereomicroscope fitted with a mechanical stage. After counting a minimum of three samples, calculate the average, then multiply this value by the total volume (in millilitres) of egg suspension in the bucket to estimate the total number of eggs.

Once the densities of sperm and eggs have been determined, it is time to fertilise the eggs. Before adding the sperm suspension, quickly check that the sperm are still highly motile by examining them under a microscope at 100× to 400×. If they are not, use more recently shed sperm after again determining the density as described above. For best fertilisation results, the final concentration of sperm should be 10^4 to 10^5 sperm/mL and the density of eggs around 100/mL (**Figure 23**). These egg and sperm densities should yield an optimum ratio of 100 to 1000 sperm for each egg.

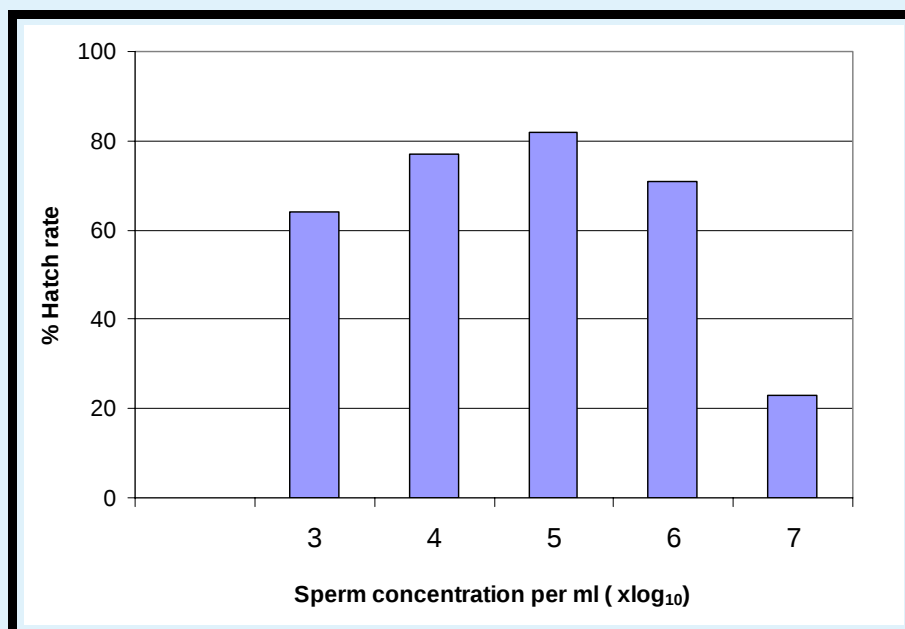


Figure 23 Effect of sperm concentration on fertilisation success of eggs in suspension at 100/ ml (as indicated by yield of trochophore larvae).

A simple way to calculate the amount of sperm suspension needed to optimise fertilisation is to multiply the number of eggs to be fertilised by 1000 to give the required number of sperm and then calculate the volume of sperm suspension (of

known concentration) that will contain this many sperm. Pipette this volume of sperm suspension into the bucket containing the eggs while at the same time keeping the eggs in continuous, even suspension by using a plunging homogeniser, as previously described for egg counting.

After 2 to 3 min, resuspend the eggs uniformly, take a 1.0 mL sample, and examine it under the compound microscope at 100×. Optimal fertilisation of eggs is indicated when, by focusing on the periphery of individual eggs, three to 10 attached sperm can be seen (**Figure 24**). If there are too few sperm, some of the eggs will not be fertilised. However, too many sperm will dissolve the outer protective layer of the eggs or will cause **polyspermy** (i.e. more than one sperm fertilises the egg). Both problems will lead to lower hatch rates.

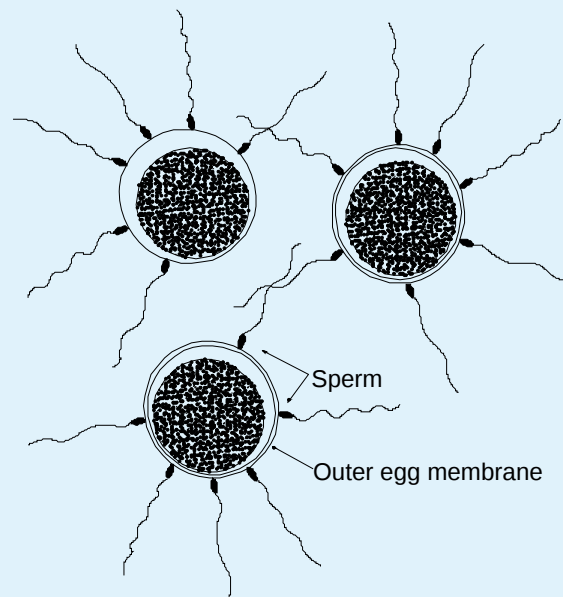


Figure 24 Optimum sperm to egg ratio
(3-10 sperm per egg).



Figure 25 Screen and tub for wet harvesting and rinsing of eggs and larvae. *N.B. Wall height of screen should be substantially greater than the depth of the tub to prevent loss of eggs or larvae through spillage or accidental overflow.*

If there are fewer than three sperm per egg on average, add a further 25% to 50% volume of the sperm suspension, wait a few minutes, and again estimate the average number of sperm attached to individual eggs. If, however, there are more than 10 sperm per egg, rinse the eggs immediately with 1µm filtered and UV-disinfected seawater. Once the optimum number of sperm per egg is observed, leave the eggs and sperm for a further 5 min. Next remove the eggs from further contact with the sperm by rinsing them with filtered, UV-disinfected seawater. To do this, gently pour or siphon the eggs onto an 80 to 100 µm mesh screen mounted in a circular shallow plastic tub pre-filled with 1 µm-filtered and UV-disinfected seawater at 18°C. The screen should have a wall height substantially greater than the depth of the weir of the tub (**Figure 25**) to prevent loss of eggs through spillage or overflow. The mesh floor of the screen should always remain submerged to prevent the delicate eggs from being damaged. Maintain a constant moderate flow of freshly filtered, UV-disinfected 18°C seawater through the screen while gently raising, lowering and swirling the screen for 15 to 30 seconds to ensure the eggs are thoroughly cleansed of residual sperm.

STEP BY STEP SUMMARY AND CHECK-LIST FOR SPAWNING INDUCTION*Preparations the day before spawning:*

- ☑ 1. Rinse and disinfect floors and walls of the spawning facility, spawning tanks, airlines and seawater lines with a dilute chlorine solution (see **Appendix 6**) and set up spawning induction tubs and air lines ready to receive broodstock.
- ☑ 2. Activate reverse-cycle air conditioner set at 16 °C.
- ☑ 3. Prepare stock solutions of the chemicals required, namely:
 - 2M TRIS solution (142 g of TRIS per litre of distilled water)
 - 6% hydrogen peroxide (H₂O₂) solution: dilute 1 volume of off-the-shelf (30% w/w) hydrogen peroxide solution with 4 volumes of distilled water. (Note: This solution should always remain stored in a sealed dark glass bottle to conserve its potency).
- ☑ 4. Fill spawning tubs with 16 °C, 1-µm-filtered and UV-disinfected seawater and continue to supply this seawater at a continuous flow through rate of 1 L/min.
- ☑ 5. Check spawning history records of stock to identify male and female abalone most likely to be ripe and ready to spawn (i.e. those that previously spawned 150 to 180 days earlier) and transfer them into individual aerated spawning tubs to acclimatise overnight.

STEP BY STEP SUMMARY AND CHECK-LIST FOR SPAWNING INDUCTION [CONT'D]

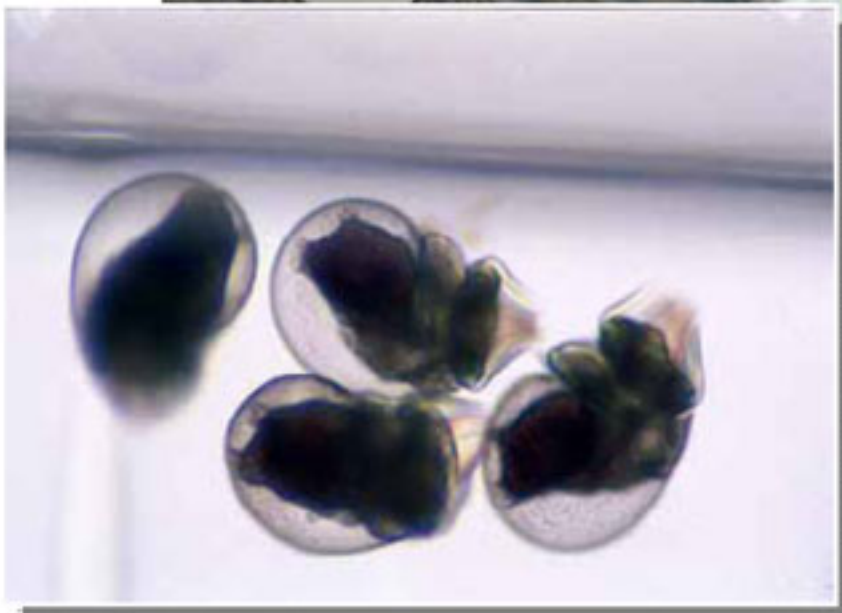
On the of morning spawning induction:

- ☑ 1. Drain and rinse tubs clean of faeces, then resume flow of 1-µm-filtered and UV-disinfected seawater at the rate of 1 L/min.
- ☑ 2. Raise water and room air temperature by 2 °C to 18 °C (but no higher) and leave abalone undisturbed for 2 h.
- ☑ 3. Stop seawater flow but provide aeration to the tubs.
- ☑ 4. Adjust the pH of the seawater in the tubs to 9.1 by the addition of TRIS stock solution at the rate of 6.6 mL of 2 M TRIS solution/L of seawater, then wait for 5–15 min before adding hydrogen peroxide stock solution at the rate of 3 mL of 6% peroxide (H₂O₂) solution/L of seawater.
- ☑ 5. Leave the abalone in darkness and do not disturb them.
- ☑ 6. After 3 h, empty and wash out each tub, then re-fill with freshly pumped 1-µm-filtered and UV-disinfected seawater at 18 °C.
- ☑ 7. Leave the abalone in the dark, undisturbed for another hour.
- ☑ 8. Start checking the abalone every 30 min over the next 2 h and then, if they have not started to spawn, check every 15 min until they do. (Pre-spawning abalone commonly crawl upwards close to, or breaking, the surface, then exhibit a characteristic rearing and waving posture just before and during spawning.)
- ☑ 9. Collect, rinse and fertilise eggs as described in the sub-routine below.
- ☑ 10. At the end of spawning, record for each broodstock whether they spawned or not, and, if they did, a) the start and finish times of spawning and b) the quality (degree of clumping, and uniformity of shape and yolk density) and quantity of eggs and of sperm (% motility and amounts). Note the tag numbers of abalone that spawned, confirm the gender, and (in the case of females) record the quantity and quality of eggs.

STEP BY STEP SUMMARY AND CHECK-LIST FOR SPAWNING INDUCTION [CONT'D]*Fertilisation sub-routine:*

- ☑ 1. After the abalone stop spawning (or sooner if you already have the required number of eggs and sperm), check the quality of each batch of eggs under a microscope at 40x. Good quality eggs suitable for fertilising are round, not irregular. They have a clear outer vitelline layer that is distinctly separated from the margin of the yolk, which should be uniformly dark (high density) and not mottled.
- ☑ 2. Inspect sperm under the microscope at 100x to 400x. Sperm suitable for fertilising the eggs must be highly motile (> 90% wriggling vigorously). If not, use fresher sperm that are.
- ☑ 3. Collect and mix approximately equal contributions of fresh sperm suspension from as many males as available in a clean beaker. Estimate the sperm density with a haemocytometer (cell counter) as described in **Appendix 5**.
- ☑ 4. Estimate the number of eggs collected (commonly 0.5 to 3 million per spawner), as follows. Harvest all good quality eggs into a single 200-L bucket and make up to a known volume with 1- μ m-filtered UV-irradiated seawater.
- ☑ 5. Gently agitate the eggs into an even suspension with a perforated plunger/homogeniser and take three to five 1.0-mL samples.
- ☑ 6. Dispense each 1.0-mL sample onto a weighing boat and count by eye, or if need be, onto a Sedgewick rafter slide, and count the eggs at low magnification.
- ☑ 7. Multiply the average count by the volume (in millilitres) of the egg suspension to estimate the number of eggs in the bucket.
- ☑ 8. Calculate how much of the mixed sperm suspension is needed to provide a sperm to egg ratio of 1000:1.
- ☑ 9. Add the sperm suspension while continually mixing with the homogeniser (perforated plunger) to maintain an even dispersion of eggs and sperm.
- ☑ 10. After a few minutes examine a small sample of eggs at 40x or 100x. If there are too few (average less than three) sperm attached to the periphery of eggs, add 25% to 50% more sperm suspension, but if there are too many wash the eggs immediately in clean 1- μ m-filtered and UV-disinfected seawater.
- ☑ 11. About 5 min after adding the sperm, transfer the fertilised eggs to a flooded 80- to 100- μ m screen and rinse them for about 1 min with 1- μ m-filtered, UV-disinfected seawater that has been temperature matched (18 °C).
- ☑ 12. Transfer the fertilised eggs from the screen back into a clean bucket before gently pouring or ladling them into a cylindro-conical larval rearing vessel pre-filled with 1- μ m-filtered, UV-disinfected and temperature-matched (18 °C) seawater.

Chapter 5 Incubation of Eggs and Larval Rearing



Chapter 5 Incubation of Eggs and Larval Rearing

On average, about 40% of fertilised eggs spawned by well-conditioned blacklip abalone broodstock can be expected to yield 6- to 8-day-old **competent** (ready and able to settle) larvae. In turn, 10% to 30% of competent larvae put to set can be expected to reach a minimum weaning size of 1 mm if the hatchery and plate nursery techniques recommended below are used.

Abalone larvae are easy to rear because their planktonic larval stage is brief and does not require feeding. The most important factor for successful rearing of the larvae is strict hygiene. As described for spawning and fertilisation, all equipment used for incubating **embryos** (fertilised eggs undergoing successive cell divisions and cell differentiation) and rearing larvae should be washed, disinfected and thoroughly rinsed before and after use. Good survival and significant growth of post-larvae are confined to temperatures ranging from 10 to 26°C. Maximum growth rate occurs at about 22°C (**Figure 26**), while highest survival of post-larvae occurs at a lower temperature of about 19°C (**Figure 27**). At a compromise temperature of 18°C, recommended for fertilisation and incubation as well as for larval rearing, larvae are best put to set after 6 to 8 days of rearing. The final decision is based on their 'stickiness' that is the tendency to cling to surfaces and to each other.

Although abalone embryos and larvae can be successfully reared by using a range of alternative batch and flow-through systems described in an earlier companion manual (Hone et al. 1997), we describe only a flow-through system that has proved reliable under the stewardship of a number of hatchery managers over a total period of 7 years. Regardless of design, rearing tanks and associated equipment such as banjo screens should be thoroughly washed and disinfected with dilute (100 mg/L) chlorine solution and then thoroughly rinsed with fresh water, drained and air-dried the day before induction of spawning.

Using the flow through hatchery equipment illustrated in **Figures 28, 29 and 30**, initial incubation and hatching can be conducted under static or low rates of water exchange. In the latter case, fresh 1 µm filtered and UV-disinfected seawater at 18°C is continuously supplied at a rate of about **one vessel volume exchange every 4 to 6 h**. A **banjo filter** screen fitted with fine (60 µm) polyester mesh is connected to an overflow mounted through the upper wall of the vessel to prevent loss of developing embryos. The appropriate diameter of banjo screen is 200 mm for 150 to 200 L rearing vessels and 300 mm for larger vessels up to 500 L. Overflow/wastewater discharge outlets should have an internal diameter of 20 or 25 mm.

Regardless of whether incubation and hatching are conducted under static or low flow-through conditions, aeration must be provided from the base cone of the culture vessel as moderately sized (5 to 20 mm diameter) 'slug flow' bubbles. This is to prevent any dead spots from developing and to ensure that the embryos and larvae are continuously and evenly dispersed through the full depth of the water column. Slug flow aeration also helps to prevent the fine-mesh banjo screens from blocking in the case of flow-through exchange and also eliminates

fine air-bubble entrapment of embryos or larvae. Slug air-flow is regulated by using a simple thumb-screw clamp on the clear silicon-rubber air supply hose at the base of the tank; this hose doubles as a drain (**Figure 30**).

A sample of embryos should be taken and checked at 40× to 100× under a compound microscope 18 to 20 h after fertilisation. By this time they should have completed incubation, hatched and reached the first trochophore larval stage (see **Figure 6, Chapter 1**). At this time the air and seawater supply should be temporarily stopped for 10 to 15 min. This is to aid the separation of unhatched embryos, empty egg cases, non-viable larvae and other organic debris from active healthy trochophore larvae. In still water, trochophores are **photopositive** and will swarm to the surface in pursuit of the brightest available light.

At this point the surface-swarming trochophore can either be directly ladled off and transferred into a new, clean larvae-rearing tank pre-filled with temperature-equilibrated seawater, or left for a further 24 h in the same tank operated as before, except that the original banjo screen is exchanged with a clean screen and seawater flow-through at about one exchange every 2 to 3 h is imposed.

Drain harvesting (see below) should not be practised until the larvae have reached the fully shelled veliger stage (36 to 48 h after fertilisation at 18°C; see **Figure 6, Chapter 1**). To harvest the larvae, a polyester 60-µm mesh flooded harvesting screen (**Figure 31**) is first set up as previously described for washing fertilised eggs. Immediately before harvesting, aeration is shut off by tightening the bottom drain/aeration-line clamp and allowing dead larvae and other debris to settle out for a few minutes. The clamp on the drain/aeration-line is then loosened and about 20% of the seawater and associated debris within the conical base of the rearing vessel is drained to waste. Next the larvae are gently drain-harvested onto the flooded screen.

Once all the larvae have been collected on the screen they are gently rinsed into a 20-L bucket. The bucket is then topped up with 1 µm filtered, UV-disinfected 18°C seawater to one of several reference volume points marks (5, 10, 15 or 20 L) clearly marked on the inside. The larvae are next gently agitated into suspension by using the plunging homogeniser (as described above for egg counting) until they are evenly dispersed. While they are evenly dispersed, three to five 1.0 mL samples of larval suspension are randomly collected with an automatic pipette. Larvae within these samples are counted by following the same detailed procedure, involving the use of a Sedgewick rafter slide and microscope, already described in the preceding chapter for estimating numbers of eggs.

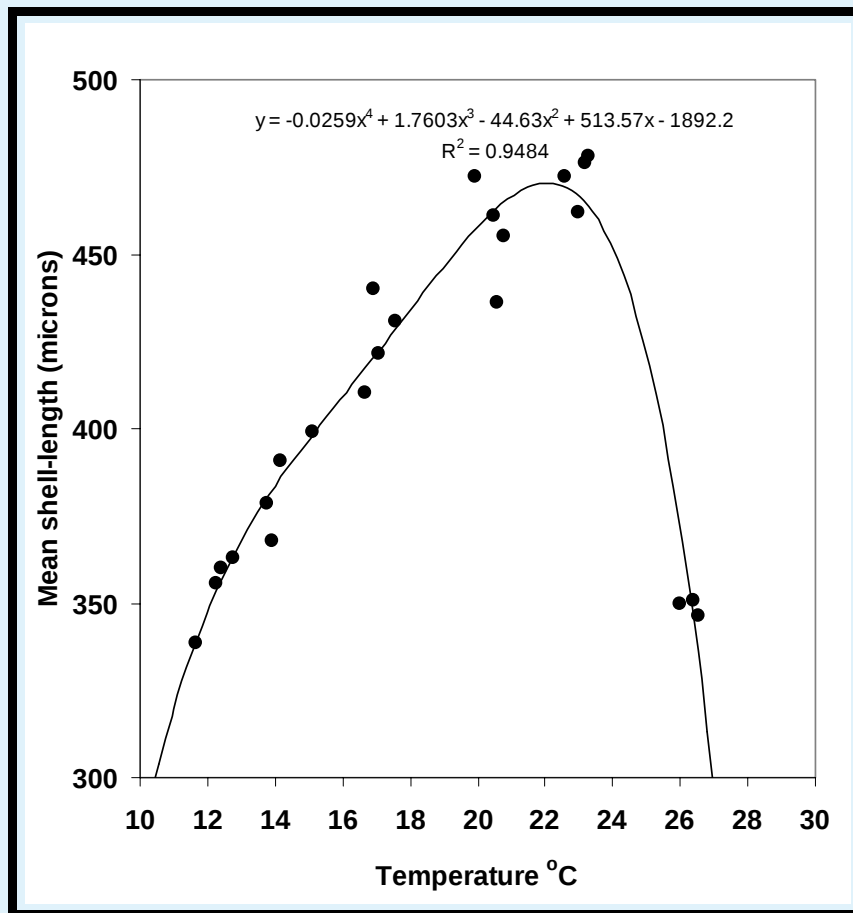


Figure 26 Effect of temperature on the growth (shell length) of 6-day post-settlement blacklip abalone post-larvae. From Heasman et al., 2006.

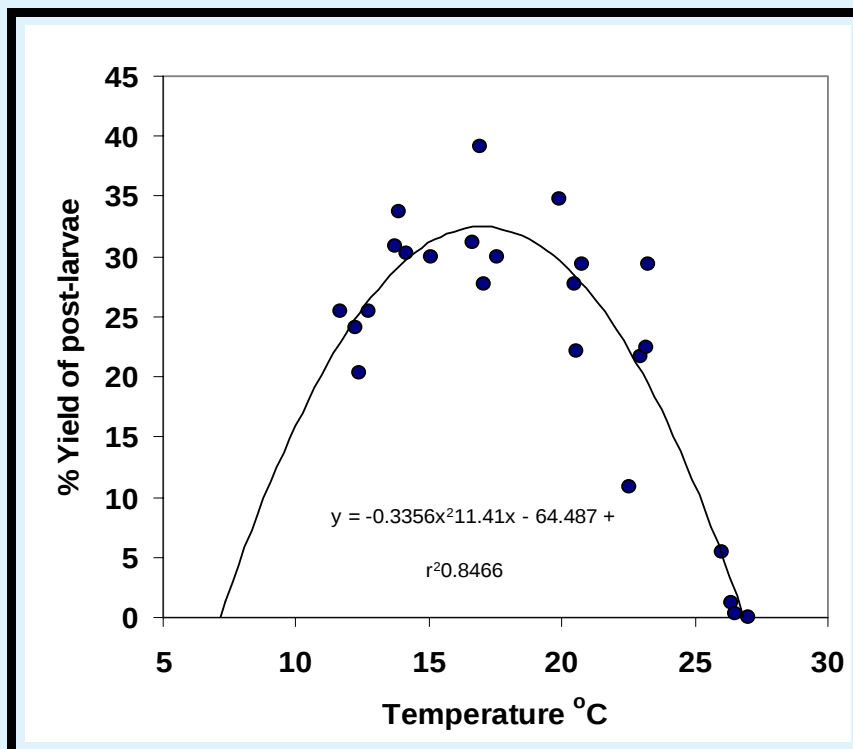


Figure 27 Effect of temperature on survival (% yield) of 6-day post-settlement blacklip abalone post-larvae. From Heasman et al., 2006.

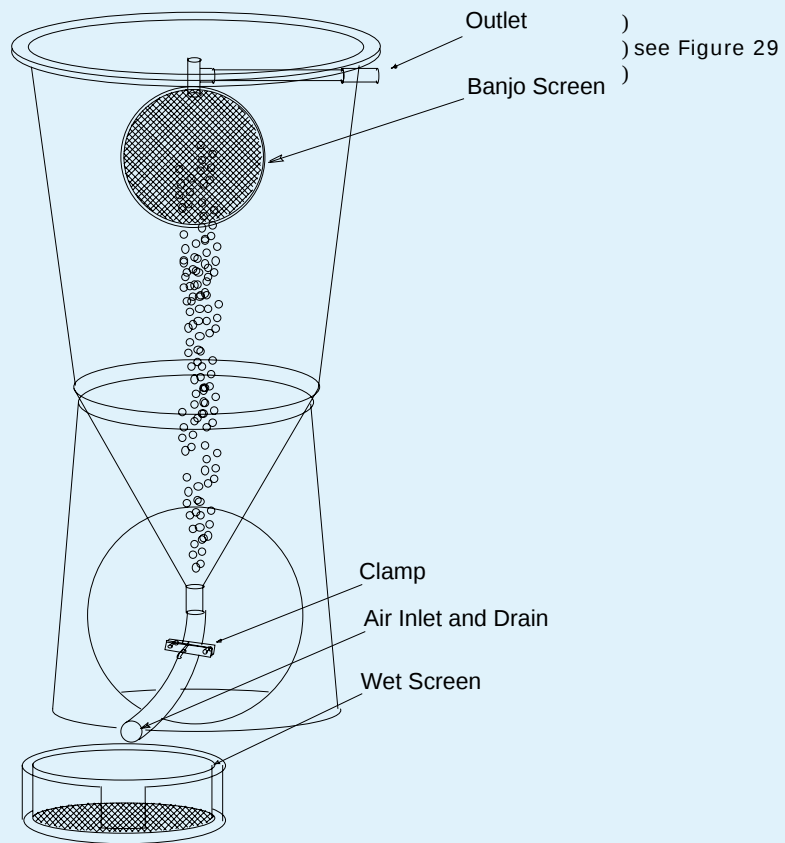


Figure 28 Sketch of egg incubation and larval rearing vessels comprising 150 to 500 L cylindrical fibreglass or moulded plastic vessels with steeply sloped (45° to 60°) conical bottoms.



Figure 29 Overhead view of incubation/larval rearing vessel showing the internal surfaces that must be smooth and scratch-resistant, and the base, that should be conical and white to aid hygiene and visual assessment of the condition of developing larvae.



Figure 30 A thumb-screw clamp attached to a clear silicon-rubber air input and drainage hose is the simplest way to provide regulated slug air-flow within larval rearing vessels.



Figure 31 Alternate-day wet-screen harvesting of shelled veliger larvae. Once collected in a flooded screen, they are rinsed and stockpiled in a bucket awaiting re-counting and transfer to a clean rearing vessel.

Finally, the larvae are restocked at up to 20 larvae/mL into a new sterile rearing vessel pre-filled with 1 μm filtered, UV-disinfected, 18 °C seawater. Continuous seawater exchange of about **one vessel volume exchange every 2 to 3 h** and gentle slug aeration are then resumed. These harvesting, counting and re-stocking procedures are repeated on alternate days until the larvae are ready (competent) to settle and metamorphose.

The larvae are competent to settle after 6 to 8 days of rearing, over which time they become progressively more ‘sticky’, adhering to objects they encounter, including each other, to form chains and rafts, particularly at or near the surface of the rearing vessel. However, some may begin to crawl on the base and sides of the vessel before this. Larvae should be routinely inspected in the rearing vessel and under the microscope at least once a day to assess developmental stage, level of activity, the extent of malformations and any obvious dropout of larvae from the water column or unusual aggregations. The most important malformation to look for is a breakdown of attachment of the twin retractor muscles to the inside (integument) of the larval shell (**see Figures 6 and 7 Chapter 1**).

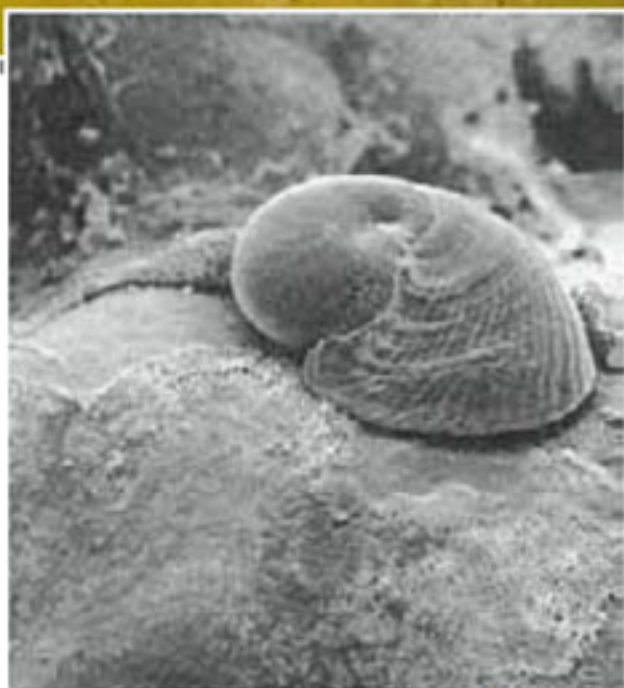
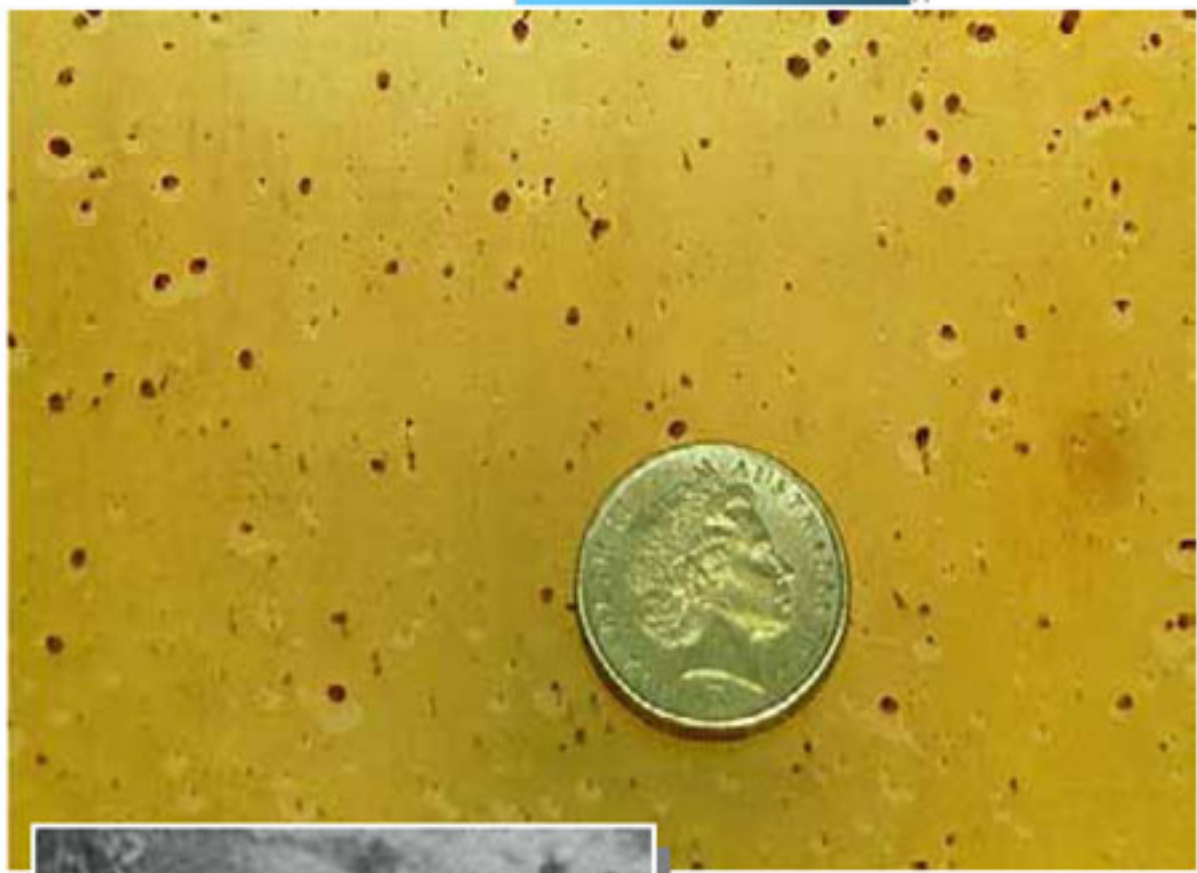
Loss of one or both attachments is commonly referred to as **IDS** (integument/muscle detachment syndrome). This is thought most likely to be caused by a bacterial infection arising from either poor seawater quality or inadequate hygiene. The procedures recommended here place total reliance on high flushing rate and good hygiene. In particular, daily changes of banjo screens, changes of rearing vessels on alternate days, and physical filtration and UV disinfection of seawater will keep potentially harmful bacteria (especially those belonging to a group called *Vibrio*) at bay and will prevent IDS. In our experience the most common primary cause of IDS is malfunction or inadequate maintenance of the UV disinfection units.

Antibiotics should never be used, nor should they ever need to be.

**STEP BY STEP SUMMARY AND CHECK-LIST FOR INCUBATING EMBRYOS AND
LARVAL REARING OPERATIONS**

- ☑ 1. Wash and disinfect all equipment with dilute chlorine solution (**see Appendix 6**) and rinse with 1- μ m-filtered, UV-disinfected seawater.
- ☑ 2. Stock fertilised eggs into the larvae-rearing container filled with 1- μ m-filtered and UV- disinfected seawater at 18 °C.
- ☑ 3. Apply gentle slug aeration and either operate as a static system or apply a low seawater flow of one exchange every 4 to 6 h (about 0.5 to 1 L/min) of 1- μ m-filtered, UV-disinfected 18 °C seawater.
- ☑ 4. After 18 to 24 h, check that trochophore larvae have hatched, stop aeration and allow 10 to 15 min to allow non-viable eggs and larvae, egg capsules and other organic debris to sink and separate from photopositive trochophores at the tank surface.
- ☑ 5. Either:
 - drain off the bottom 20% of seawater to remove settled debris, refill the tank and resume aeration and apply/re-apply low water exchange for a further 12 to 24 h to allow development to the fully shelled veliger larval stage
 - ladle-off surface swimming trochophores and transfer them to a new, clean rearing vessel pre-filled with 1- μ m-filtered, UV-disinfected seawater at 18 °C.
- ☑ 6. After 36 to 48 h, drain harvested larvae into a flooded screen and rinse veligers, then transfer to a clean bucket and top-up to known volume reference mark.
- ☑ 7. Estimate the number of larvae by counting those in three to five 1.0-mL sub-samples and then multiplying the average larval count per millilitre by the volume of the bucket in millilitres.
- ☑ 8. Restock the larvae at a maximum density of 20/mL into a clean and sterile rearing vessel pre-filled with 1- μ m-filtered, UV-disinfected seawater at 18 °C.
- ☑ 9. Repeat flooded screen harvests, counts and tank changes on alternate days. Observe larvae in the tank and examine a small sample microscopically, recording general appearance and behaviour, development, and health status every day.
- ☑ 10. At day 6 start checking the larvae under the microscope for competence.

Chapter 6 Nursery Production



Chapter 6 Nursery Production

Overview of settlement of larvae on diatom plates

There is much published information on how to settle and grow abalone post-larvae. In practice, successful settlement of larvae and rearing of post-larvae are relatively easy. As discussed in **Chapter 1**, competent abalone larvae settle in response to chemical cues given off by substrates that constitute their preferred habitats that can support early juvenile survival and growth. It is believed that larvae first detect the chemicals in the water column and then inspect the bottom substrate, possibly by tasting it. If the substrate has the right properties the larvae will stay attached and metamorphose. Otherwise they resume swimming and continue their search. In the case of blacklip abalone, wild competent larvae settle on rocky surfaces overgrown by crustose coralline algae, especially in shallow (1 to 5 m deep) exposed boulder areas subject to high wave energy and disturbance.

To successfully settle larvae and on-rear them as post-larvae in the nursery, settlement substrates must stimulate initial settlement and metamorphosis and also provide adequate food for early growth. Diatom-dominated biofilms are excellent for this. There are four key procedures that will ensure reliable and efficient settlement and metamorphosis of larvae and subsequent rapid growth and high survival of blacklip abalone post-larvae to a stage at which they can be reliably weaned onto formulated diets.

These procedures are:

1. Initial inoculation and colonisation of plastic nursery plates with the alga *Ulvella lens*. This green alga can be easily induced to develop and release motile spores that immediately attach to the surfaces of the plates and rapidly form thin plaque-like colonies of cells (**Figure 12e, Chapter 1**).
2. In turn, these colonies, like those of the much slower-growing crustose coralline algae, attract and promote rapid and consistently high rates (40% to 80%) of settlement and metamorphosis of post-larvae.
3. Follow-up preparation and management of plastic nursery plates to ensure that post-larvae can feed to satiation at all times through to a minimum average weaning size of about 1.5 mm on nutritious diatom-dominated biofilms.
4. Larvae are seeded onto the plates within the prescribed range of density and at favourable temperatures that help ensure consistently high yields of weanable post-larvae.
5. Post-larvae are carefully harvested off the plates and weaned onto formulated diets while they are still in peak growth and health and before they have fully exhausted the diatom-dominated biofilm on the plates.

Nursery facilities equipment and operation

In most abalone hatcheries in Australia, larval settlement plates are 600 x 300 mm and constructed from clear 1 to 1.5 mm thick PVC. These plates are mounted vertically on their long side in plastic coated or stainless steel wire baskets that hold 14 to 18 plates (**Figure 32**). The baskets are fully submerged in partially shaded shallow tanks commonly located under enclosed or semi-enclosed green-house like canopies (**Figure 33**).



Figure 33 Standard 600 x 300 x 1.5 mm clear PVC nursery settlement plates. These plates are mounted on their long edge in plastic coated or stainless steel wire baskets that hold 14 to 18 plates.



Figure 32 A small-scale abalone nursery comprising baskets of plastic plates submerged in partially shaded tanks located within enclosed or semi-enclosed shade houses.



Figure 35 Nursery tank filter element being changed.

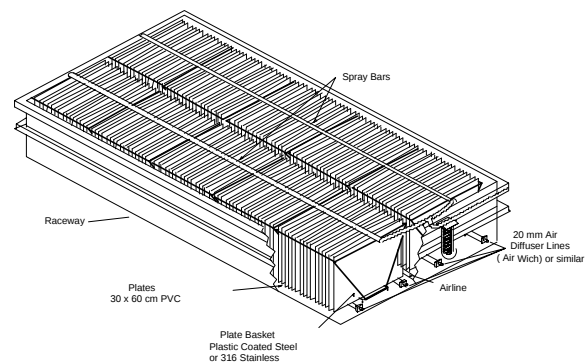


Figure 34 Cutaway drawing of nursery tank

The tanks are fitted with standpipes to maintain a water depth of about 0.4 m. Seawater sprinkler bars are mounted centrally over each row of baskets. A high level of aeration is maintained across the surfaces of all plates continuously via floor-mounted 16 mm air diffuser hoses. Two rows of diffuser hose are anchored to the floor of the tanks beneath each row of baskets (**Figure 34**). Seawater entering the nursery tank is pre-filtered to 10 µm (**Figure 35**) to prevent the entry of zooplankton, especially tiny crustaceans called **copepods** that rapidly multiply and directly compete with the abalone post-larvae for food and space on the plates. Unrestricted entry of plankton will also lead to the settlement and proliferation of an undesirable **epi-biota** (mixed layer of plant and animal life) on the surfaces of plates. Such undesirable epi-biota commonly include filamentous and thin sheet (thallus) forms of colonial algae and an array of other invertebrates, including oysters, mussels, limpets, tube-worms, barnacles, sponges and hydroids, as well as the above-mentioned copepods.

Preparation of diatom plates

(For a comprehensive description of these procedures see **Table 2** and the companion manual by Daume 2004).

Between 3 weeks (in summer) and 6 weeks (in winter) before starting a new hatchery/nursery production cycle, nursery plates already in use that have developed large mature patches of *Ulvella lens* are selected. Juvenile abalone, if present, are removed by using the standard anaesthesia techniques described below. One mature *Ulvella* plate is required for every basket of new plates to be seeded with abalone larvae.

The mature *Ulvella* plates are wiped clean with a sponge to remove diatom films and other unwanted biota before being stored for 2 weeks (summer) to 4 weeks (winter) in a separate, heavily shaded tank, where they are supplied with 1 µm filtered flow-through seawater and light aeration. This conditioning period encourages spore formation within the mature *Ulvella* colonies.

One week (summer) to 4 weeks (winter) before the proposed start of a hatchery/nursery production cycle, all nursery tanks and plates to be used are scrubbed clean. The tanks are filled with fresh 1-µm-filtered seawater and then chlorinated at 10 mg/L (10 ppm) active chlorine (**see Appendix 6**) overnight. The next morning, sodium thiosulfite solution is added and vigorous aeration applied to remove any residual active chlorine (**also see Appendix 6**). Next, the mature *Ulvella* plates are removed from the shaded conditioning tank and placed singly between each basket of new clean plates. The new tanks are then left static for 4 days without shading to enable solar heating sufficient to raise the temperature up to a range of 20 to 25°C. The resultant sudden increase in light intensity and temperature triggers spore release by mature *Ulvella* colonies. Spore release generally peaks after about 4 days (summer) to 8 days (winter) and starts daily 1 to 2 h after sunrise. Normal flow-through of 1 µm filtered seawater can then be resumed, the *Ulvella* spores having settled and germinated on the new plates.

The next critical step is to promote the establishment and growth of nutritious types of **adventitious** (naturally occurring) benthic diatoms (**see Figures 12a, b and c Chapter 1**) on the new plates. Diatom productivity on settlement plates determines their ‘carrying capacity’ in relation to abalone post-larvae. This carrying capacity in turn is determined by temperature, light and availability of nutrients. Varying grades of shade-cloth, providing from 90% down to 50% shade, can be used to adjust light intensity and hence the growth of diatoms to help match the grazing rates of the abalone post-larvae. The ideal diatom biofilm looks like a thin scum covering the surfaces of the plates (**Figure 36**). An accumulation of brown scum will collect on a fingertip when dragged across such plates. If the diatom film is too thin initially and the post-larvae are at particularly high densities, they can prematurely graze out the diatoms and starve before reaching minimum weaning size. Conversely, if the biofilms are too mature they will form a thick, three-dimensional layer containing unwanted and nutritionally poorer chain forming microalgae and/or seaweeds (macroalgae) (**Figure 36**)—especially opportunistic species such as sea lettuce (*Ulva*) or its filamentous counterpart, mermaids hair (*Enteromorpha*).



Figure 36 Left: Inspection of plates being prepared for seeding. Top Right: Photomicrograph of benthic diatoms on well-prepared nursery plate at 500x. Below Right: Close-up view of diatom plate over grown by invasive filamentous algae

Table 2 Summarised Schedule of activities for the preparation and operation of nursery diatom plates, integrating the use of *Ulvelia* to enhance rate and degree of successful settlement and metamorphosis of blacklip abalone larvae seeded at high densities (4,000-6,000 larvae per standard 300 x 600 mm plate)

Initiation time and duration of activity conducted at warmer seasonal temperatures of 19 to 23 °C	Activity	Initiation time and durations of activity conducted at cooler seasonal temperatures of 14 to 18 °C
Day –21 to Day –8 (3 weeks before seeding of plates with competent larvae)	Select from among nursery plates in current use those extensively covered with mature colonies of <i>Ulvelia</i>. One such <i>Ulvelia</i> inoculation plate is required for each rack of new plates to be prepared for seeding with abalone larvae. Remove residual juvenile abalone and wipe off all other extraneous biota from <i>Ulvelia</i> inoculation plates before placing in a deeply shaded nursery tank with low aeration and low flow input of 10-µm-filtered seawater. Maintain this status for 4 days (warm period) to 28 days (cold period) to promote development of spores.	Day –42 to Day –15 (6 weeks before seeding of plates with competent larvae)
Day –7 to Day –2 (1 week before seeding of plates with competent larvae, and coincident with initiation of spawning induction and hatchery production of abalone competent to set larvae)	Cease seawater exchange and remove shading to promote solar heating of seawater up to the range 20 to 25 °C. Stock <i>Ulvelia</i> seeding plates at the rate of one per basket among new, clean disinfected plates in nursery tanks. Spore release by <i>Ulvelia</i> is promoted by temperature rises, with peak releases occurring after about 4 to 7 days, depending on temperature. New disinfected plates with settled and germinated spores soon begin to proliferate across the plates. Addition of fertilisers (Guillard's F/2 or Aquasol) will enhance these developments.	Day –14 to Day –6 (2 weeks before seeding of plates with competent larvae)
Day –1 (1 day before seeding of plates with abalone larvae)	Resume low exchange rate of 1-µm-filtered ambient seawater and maintain low aeration. Add plant fertiliser (Guillard's F/2 media or Aquasol) to promote further proliferation of <i>Ulvelia</i> colonies and adventitious benthic diatoms. Inoculate with pure strain cultures of high performance diatoms (if available).	Day –5 (5 days before seeding of plates with competent larvae)
Day 0 (induction of spawning of abalone broodstock in ripe condition and initiation of hatchery rearing cycle)	Continue as above to encourage proliferation of diatoms, adding shading once a complete film of low-profile benthic diatoms (as indicated by finger-swipe test and/or microscopic inspection of plates) is established.	Day 0 (induction of spawning of abalone broodstock in ripe condition and initiation of hatchery rearing cycle)
Day +1 to Day +6 (hatchery production cycle)	Continue as above.	Day +1 to Day +6 (hatchery production cycle)
Day +7 (seeding of plates with competent larvae)	Maintain low aeration in nursery tanks and cease seawater exchange before adding competent larvae at the rate of 4000 to 6000/plate Allow larvae 12 to 24 h to achieve full settlement and metamorphosis of larvae to post-larvae.	Day +7 (seeding of plates with competent larvae)
Day +8 onwards (on-growing of post-larvae on plates)	Resume low exchange rate of 1-µm-filtered ambient seawater and apply moderate aeration.	Day +8 onwards (on-growing of post-larvae on plates)

The speed of development and productivity of biofilms vary with the season, being greatest in the warmer and higher light-intensity months from late spring to early autumn (November to March) and lowest in winter and early spring (July to August). Biofilm productivity may also vary among different settlement tanks according to exposure to sunlight and solar heating. The most common and nutritious naturally occurring (adventitious) diatoms are single-celled species belonging to the groups *Navicula*, and *Nitzschia*. Each type of diatom is easily recognised by its distinctive shape as illustrated in **Figure 12, Chapter 1**.

As already stated, installation and conditioning of settlement plates pre-seeded with *Ulvela* spores should precede spawning induction by about a week. If spawning is delayed, the nursery plates can be shaded to prevent the diatom films becoming too dense and/or rangy and overgrowing the young colonies of *Ulvela*. The net effect of the latter is reduced ability of the biofilms to attract initial settlement and metamorphosis of the abalone larvae and to support high subsequent growth and survival of the post-larvae.

As growth of diatoms is much higher on the upper surfaces of the plates (especially the sides with highest exposure to sunlight), the plates should be alternately rotated in both the horizontal and the vertical planes once a week to ensure the best and most even productivity. If the growth of diatom biofilms surges ahead of consumption by post-larvae, additional shading of the plates may be required to prevent them from becoming overgrown by filamentous algae, which may also restrict the movement of the post-larvae.

Each abalone farm site and nursery configuration has its own particular adventitious diatom production characteristics that vary with the season and weather. Optimised management of nurseries leading to consistently high and reliable yields of weaning-size juveniles within these farms is therefore likely to entail several years of keen observation and fine-tuning by technical staff.

As indicated above, a much more comprehensive description of how to manage and optimise diatom productivity on nursery plates, including the use of specially selected highly productive strains of diatoms with or without the addition of one or a combination of supplementary plant nutrients, heating and illumination of tanks, is provided in a companion manual (Daume 2004).

Seeding of larvae onto plates at optimum densities and favourable temperatures

As discussed above, competent blacklip abalone larvae are able to successfully settle and metamorphose over a relatively broad range of temperature (see **Figure 27**). At temperatures of 12 to 24°C, which span the annual range of sea temperature on the central and southern coasts of NSW, survival of blacklip abalone larvae through settlement and metamorphosis remains at or above 75% of the peak rate at 18°C. However, subsequent growth rates of early-stage post-larvae (see **Figure 26**) are much more affected by temperature (especially low temperature), rising steadily from zero at 12°C to a peak at 23°C, then rapidly back to zero with a further small temperature rise to 27°C. Such temperature effects render the NSW Central Coast (Sydney to Port Stephens) the most

suitable region for year-round nursery production of blacklip abalone, with little or no need to heat or cool ambient seawater at any time. However, even within this region, the periods required by post-larvae to reach a minimum average weaning size of about 1.5 mm can vary markedly from about 30 days in the warmest summer and early autumn months up to 70 days or more during winter and early spring (**Figure 37**).

Seeding density

Blacklip abalone post-larvae consistently show **exponential growth** (**Figure 37**), even when seeded on diatom plates over a wide range of densities throughout the year. Exponential growth implies that the time taken to double in size from any starting point remains constant. Depending mainly on seasonal temperature, blacklip abalone post-larvae reared on the Central Coast of NSW double their shell length every 12 to 36 days and their weight every 6 to 12 days. An important implication of exponential growth for managing diatom plate nurseries is that daily food consumption of post-larvae increases at the same proportional rate as their weight, which equates to a massive factor of 64 times between the time of first feeding (from the first week after settlement) to the time they reach an average minimum weaning size of about 1.5 mm, 30 to 70 days later.

An important consequence of exponential growth is that if the initial larval seeding density is too high then the resultant post-larvae may run out of food and stop growing before they reach the minimum weaning size. Conversely, if post-larvae that have reached weaning size are inadvertently left and allowed to graze out the diatoms, they also will stop growing and, if left for extended periods, will begin to suffer serious consequences of starvation. As illustrated in **Figure 38**, stalled growth and substantial (sometimes catastrophic) losses of post-larvae can result if starvation is allowed to continue for several weeks. Such losses may be a direct consequence of starvation. However, they may also reflect an inability of nutritionally compromised post-larvae to endure the combined stresses of anaesthesia, handling, and exposure to the air and sun during transfer from nursery plates to first stage raceways for weaning onto formulated diets. Generally speaking, the higher the seeding density of larvae on diatom plates, the higher the risk of stalled growth and starvation before the larvae can attain minimum weaning size.

In commercial hatcheries, the key to achieving consistently high yields of post-larvae from diatom plates is to seed them as larvae at densities that will allow the greatest number to maintain normal exponential growth through to the minimum average weaning size of 1.5 mm but without posing undue risk of them grazing out the diatoms and starving. This task is not difficult to achieve, providing that the following conditions are met:

- The larvae are healthy and competent to settle.
- The larvae are seeded onto diatom plates at densities of 1 to 1.5/cm² (= 4000 to 6000 per standard 300 × 600 mm nursery plate).
- Temperature remains within the range 15 to 23 °C.

Our experience is that, regardless of the initial larval seeding density, after about a month the survival of post-larvae usually stabilises at 10% to 30% of the larvae originally seeded. Provided the post-larvae on diatom plates are in good health and growing normally (exponentially), they can be successfully harvested off the plates, restocked into shallow raceways and immediately weaned onto formulated diets with relatively few losses. This can be done at an average shell length as small as 1.0 mm, but, as already stated, it is best applied at an average shell length of 1.5 mm. As illustrated in **Figure 39**, well prepared and managed diatom nursery plates will consistently support normal exponential growth of up to 1000 post-larvae per plate ($0.3/\text{cm}^2$) up to the recommended average weaning size of 1.5 mm.

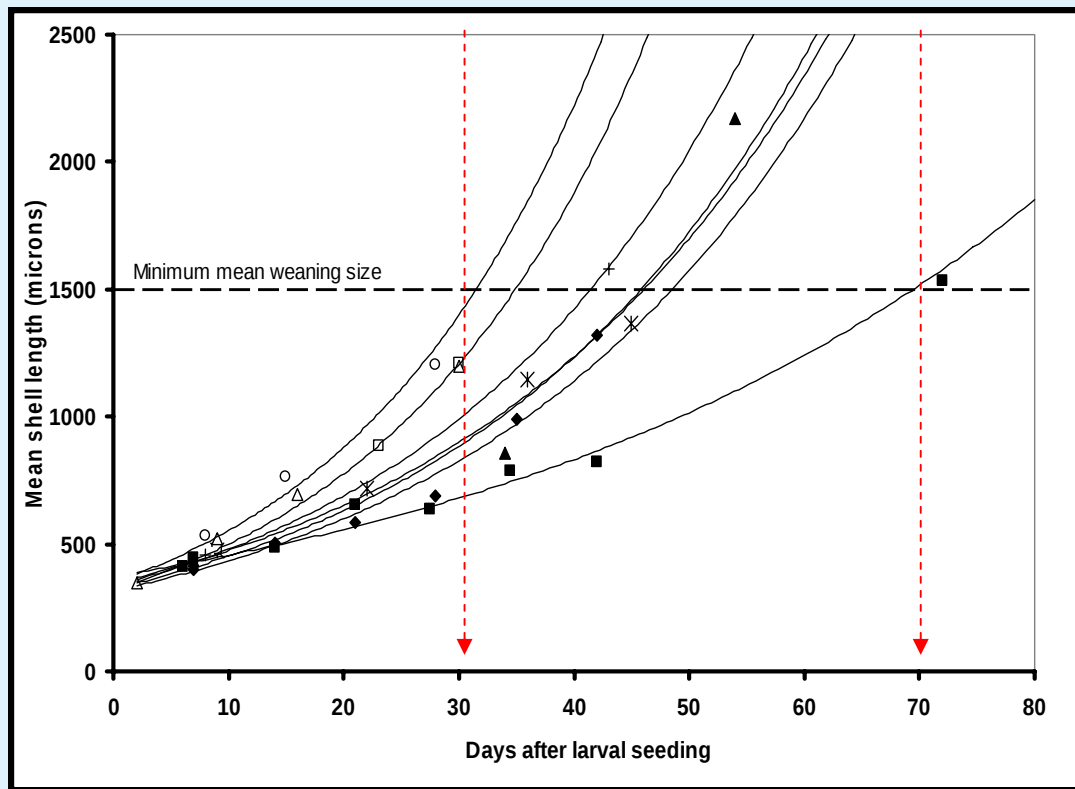


Figure 37 Usual exponential growth pattern of post-larvae reared diatom plates on the central coast of NSW (100 microns = 1 mm). Note: Periods required by post-larvae to reach a minimum average weaning size of 1.5 mm can vary markedly from 30 days in summer and early autumn to more than 70 days in winter and early spring (Heasman et al., 2004).

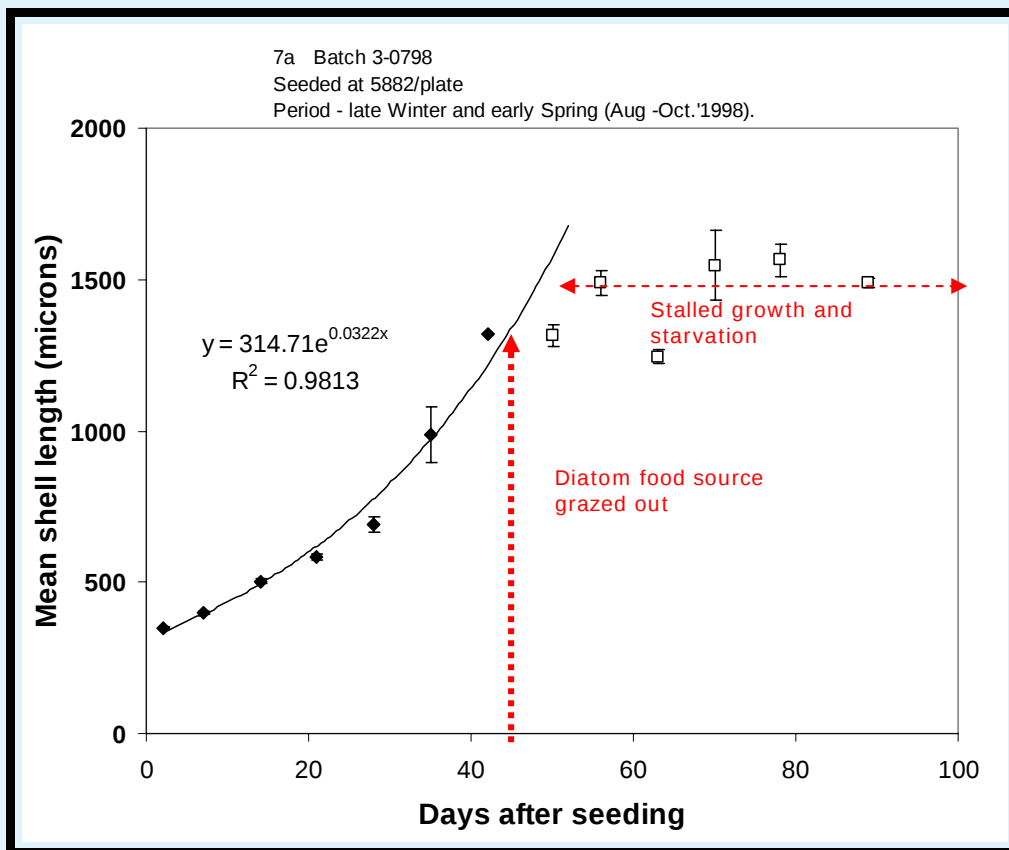


Figure 38 Effect of over-grazing of diatom plates and subsequent protracted starvation on growth of blacklip abalone post-larvae. Bars indicate standard error of means. (Heasman et al., 2004)

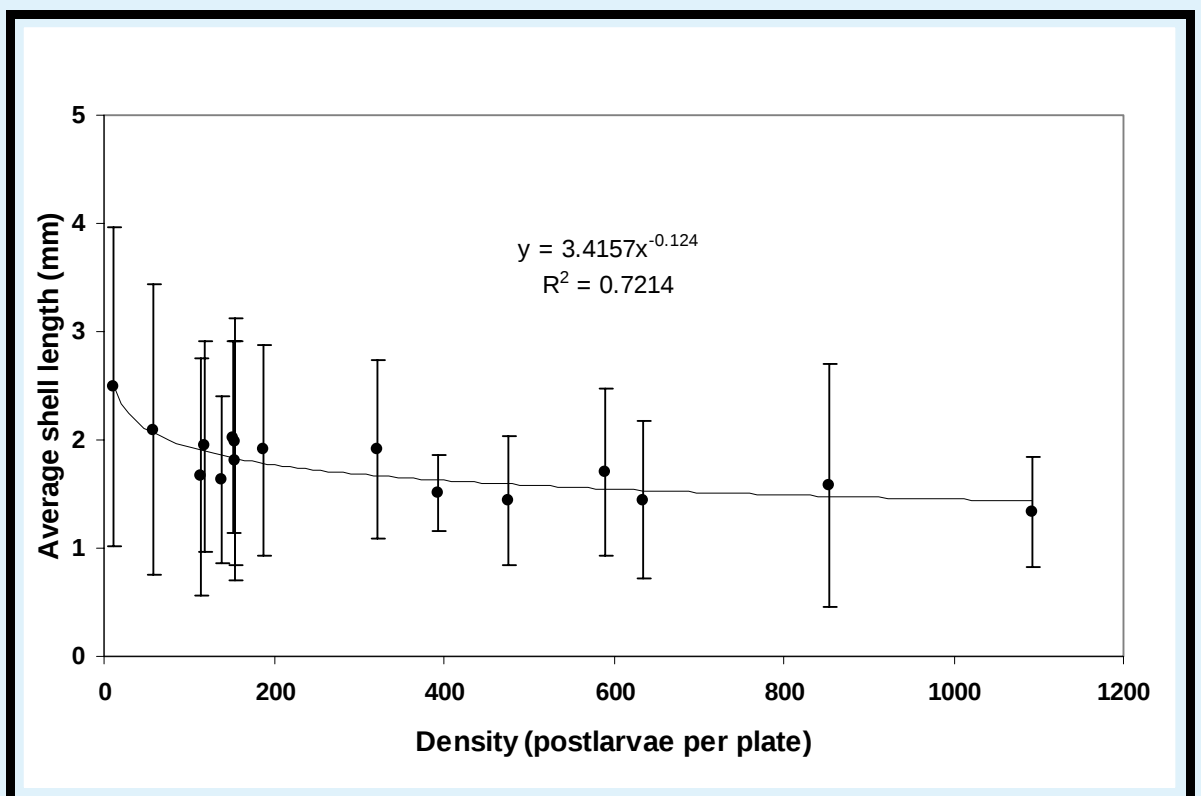


Figure 39 Effect of density on growth of weaning size. Bars indicate common size range (95% confidence limits). (Heasman et al., 2004)



Figure 40 Above: section of heavily grazed nursery plate. Note: Blacklip abalone post-larvae exhibiting “grazing fronts” in relation to remnant patches of benthic diatoms. Inset: Recommended method of inspecting nursery plates

Another factor critical to achieving consistently high nursery plate yields of post-larvae is to avoid exposing them to the air while assessing their size, number and health and/or the status of diatom films. The reason is that the upper and lower short-term lethal thermal tolerance temperatures of about 30 and 5°C, respectively, of tiny (0.3 to 2 mm) post-larvae can be attained in a matter of seconds if they are exposed to air temperatures that in southern Australia can reach 35 to 45°C during summer and 0 to 5°C during winter. Plates should therefore be visually inspected in accordance with the recommendations in **Table 3**.

Table 3 Recommended out-of-water inspection times for nursery plates

Air temperature (°C)	Max recommended air exposure times for post larvae on nursery plates
<10	Nil
10-25	1 minute
20-25	30 seconds
25-30	5 seconds
>30	nil

Even then, such brief visual inspections should be confined to the early morning or night-time. This is because the post-larvae will sustain even more extreme temperature and/or associated osmotic shock if additionally exposed to high velocity and/or low-humidity wind and/or direct sunlight.

A much more preferable practice for the safe inspection of plates is to keep them submerged in shallow (75 to 100 mm) trays filled with ambient seawater at a minimum depth of 50 mm. Such trays should be equipped with a thermometer to ensure that the temperature is always maintained within the safe limits of 10 to 25°C for blacklip abalone post-larvae.

These simple precautions will enable protracted inspection, non-invasive counting, and even measurement of post-larvae in situ at any time of the day and under most weather conditions. Fast and accurate counting and measurement of post-larvae can be made with the aid of a stereomicroscope (**Figure 40**) mounted on an adjustable boom and fitted with calibrated eyepiece graticules.

If the above guidelines are adhered to, then standard 300 × 600 mm nursery plates will consistently yield an average of 200 to 1000 weaning-size post-larvae (**Figure 39**). As previously stated, for a hatchery located on the Central Coast of NSW, the minimum average weaning size of 1.5 mm will be attained in as little as 30 days in summer at temperatures of 20 to 24°C, but in up to 70 days in winter at temperatures of 14 to 16°C. For hatcheries located farther south in NSW and elsewhere, production of weanable post-larvae during the coldest months will require heating of incoming seawater when the temperature falls below about 14°C.

**STEP BY STEP SUMMARY AND CHECK-LIST FOR PREPARING AND SEEDING SETTLING PLATES
(SEE ALSO TABLE 2)**

- ✓ 1. Three to 8 weeks before a planned spawning and hatchery cycle, depending on the season, select sufficient nursery plates bearing large areas of mature *Ulvelva* colonies from among an ongoing nursery operation to provide one such plate per basket of 15 to 18 plates to be seeded.
- ✓ 2. Remove any abalone juveniles in accordance with the procedures described in **Chapter 7**. Gently rub and rinse off diatom films and other extraneous biota while preserving as much of the generally tougher and strongly adhering mature *Ulvelva* colonies as practicable.
- ✓ 3. Collect the plates into a separate, fully shaded tank supplied with 10-µm-filtered seawater and light aeration for 2 to 4 weeks to encourage the development of *Ulvelva* spores.
- ✓ 4. Five days before attempting to induce the *Ulvelva* colonies to release spores, set up new larval settlement tanks by placing air diffuser tubes on the bottom of the tank and starting flow of seawater filtered to 10 µm.
- ✓ 5. The following morning, neutralise any residual active chlorine by first adding sodium thiosulfate solution (**see Appendix 6**) and then apply vigorous aeration to mix and distribute the thiosulfate before using a simple test kit to verify the complete neutralisation of chlorine (**also see Appendix 6**).
- ✓ 6. Insert one mature *Ulvelva* bearing nursery plate for every basket of new non-seeded plates, and operate the tank unshaded and static with gentle aeration for 4 days (up to and including the day of induced spore release) to facilitate solar heating and high light-intensity-mediated induction of *Ulvelva* spore release.
- ✓ 7. Restore flow-through of 10-µm-filtered ambient seawater and apply moderate aeration for a further 7 to 21 days to allow inoculation and preliminary proliferation of adventitious diatoms.
- ✓ 8. Regularly inspect the diatom growth on the plates. A good diatom biofilm appears as a thin brown scum on the plate. The quality of the diatom film in terms of dominant types of single-cell benthic diatoms (common species of *Navicula* and *Nitzschia* as illustrated in **Figure 12**) can be confirmed by examining the surface of a small but representative sample of plates under a dissecting microscope. Development of a good covering of benthic diatoms generally takes from 2 to 3 weeks in summer at 20 to 24 °C but as long as 4 to 6 weeks in winter at 12 to 16 °C. These times can also be shortened by one or a combination of the following procedures:
 - run the tanks statically (temporarily stop seawater flow) with low to moderate aeration
 - heat the tanks by using clear covers, such as common bubble-film packing, that allow solar heating by day but act as heat-retaining blankets at night (capable of raising ambient temperature by 2 to 4 °C), and/or use submersible heaters. Well-insulated tanks will require only about 100 to 200 W of heating/m³ of tank volume.
 - add plant nutrients to promote propagation of diatom films
 - inoculate the plates with specially selected pure cultures of highly productive benthic diatoms, available from the CSIRO (see Daume et al. 2004).

STEP BY STEP SUMMARY AND CHECK-LIST FOR PREPARING AND SETTLING LARVAE

- ☑ 1. Once the larvae are confirmed as being competent to settle and metamorphose into post-larvae, wet-screen harvest them (**see Figure 31**).
- ☑ 2. Gently rinse the larvae from the wet screen into a bucket and top up the bucket to a known volume with fresh 10-µm-filtered, UV-disinfected 18° C seawater.
- ☑ 3. Mix the larvae well in the bucket with a hand-operated homogeniser (as for counting eggs; **see Figure 21**) and while doing this collect three to five 1.0-mL samples of the larval suspension with an automatic pipette (**see Figure 22**) and dispense onto a rafter counting slide.
- ☑ 4. Calculate the average number of larvae per millilitre and multiply this number by the total volume of the larval suspension in millilitres to determine the total number of larvae.
- ☑ 5. Turn off the water flow to the settlement tank and decrease the airflow to a trickle.
- ☑ 6. Add between 4000 and 6000 larvae for each settlement plate (1 to 1.5/cm²) in the nursery tanks, making sure the larvae are evenly distributed among the plates.
- ☑ 7. After 24 h, resume water flow and full aeration.