Hatchery Manual for Larval Rearing of Vongole *Katelysia rhytiphora*

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FRDC Project No 2009/208

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EXECUTIVE SUMMARY

This report is part of FRDC Project No. 2009/208, Developing clam aquaculture in Australia: A feasibility study on culturing *Donax deltoides* and *Katelysia rhytiphora* on intertidal and subtidal leases in South Australia.

Pipi (*Donax deltoides*), also known as Goolwa cockle and a species of Vongole (*Katelysia rhytiphora*), were reared in the shellfish research and development hatchery at the South Australian Aquatic Science Centre, West Beach, South Australia. Following hatchery culture, post-settlement nursery rearing, laboratory and field trials, *K. rhytiphora* was determined to be the most promising species for commercial aquaculture development. This manual describes the hatchery culture of this species.

1. INTRODUCTION

1.1. Background

Clam aquaculture has not been undertaken commercially in Australia although is well developed overseas. World production of cultured edible clams was 4.9 million tonnes in 2010, valued at $4.7 billion (FAO 2010). Australian total wild catch is small by comparison and is restricted by quota. Given this and the current high prices, an opportunity exists to develop an Australian clam aquaculture industry.

Previous attempts at clam culture have met with varying degrees of success. Work in Tasmania on *Katelysia scalarina* indicated that the species grew too slowly (4 to 6 years) to be considered for commercial culture (Bellchambers 2005) while trials undertaken in New South Wales (Paterson et al. 1997) showed that *Tapes dorsatus* and *Katelysia rhytiphora* grew at reasonable rates but were susceptible to freshwater inundation and there was no commercial uptake, perhaps due to the relatively lower prices at the time. Since then, the price for clams has increased substantially and there is renewed interest in culture.

With limited numbers of wild spat available and the need to preserve wild stocks, hatchery production will be essential to supply the number required for commercial clam farming in Australia. Hatchery trials were undertaken from 2010 to 2012 at the South Australian Research and Development Institute, West Beach, South Australia on Pipi (*Donax deltoides*) and Vongole (*Katelysia rhytiphora*). Of the two species, hatchery techniques have proven to be more successful with *K. rhytiphora* and this species is also more likely to be successful commercially cultured within sheltered bays. This manual describes the hatchery culture methods for this species.

There is a need to compare the performance of this species with other native clam species that appear to have potential. For example, *Katelysia scalarina* may grow quicker in South Australia than previously reported in Tasmania (Bellchambers 2005) and may tolerate a wider range of conditions, considering that it is mostly found in slightly shallower water than *K. rhytiphora.*

1.2. Objectives

This manual is written for use by operators of shellfish hatcheries that have existing infrastructure and skills. Included in this manual are broodstock conditioning, spawning, larval rearing, settlement and nursery culture techniques that may be different to those used for Pacific Oysters (*Crassostrea gigas*), the most common hatchery cultured bivalve in Australia. General aspects of shellfish hatchery production including site selection, water delivery, water treatment, and algal culture are outside the scope of this project and are not detailed. A shellfish hatchery having suitable water supply, algal production, upweller nursery and skilled personnel will be able to use the information contained within this manual to grow the Vongole clam (*Katelysia rhytiphora*).

2. BROODSTOCK

Broodstock Selection

Previous studies (Gorman et al. 2010) have indicated that gravid *Katelysia rhytiphora* adults are available from October to May. Although at the time of writing, the South Australian Government policy on clam culture is still in developmental stages, Primary Industries and Regions South Australia (PIRSA) requires that broodstock are sourced from nearby the farm site. More detailed information is available from PIRSA Aquaculture. When sourcing broodstock, select clams that appear healthy, clean and fast growing as evidenced by observing shell morphology including widely spaces growth checks, thin undamaged shell margin and relatively flat valves, not rounded and thick-shelled.

Broodstock Conditioning

Generally, growth and survival of larvae from broodstock collected from the wild is superior to those obtained from artificially conditioned broodstock. However, the availability of wild broodstock in spawning condition is seasonal and often artificial conditioning of broodstock is necessary. This is achieved by holding broodstock at a suitable temperature, feeding them a diet high in lipids and keeping them clean in a stable system with good water quality. Research conducted during this project showed good survival and ability to condition *K. rhytiphora* broodstock in land-based holding systems.

Conditioning System

The system used at the South Australian Research and Development Institute (SARDI) consisted of a 350 L reservoir tank that had new seawater continuously added at a rate of 10 exchanges day^{-1}. Using a submersible pump, water was circulated from the reservoir tank through two 80 L shallow raceway tanks within which clam broodstock were contained (Figure 1).

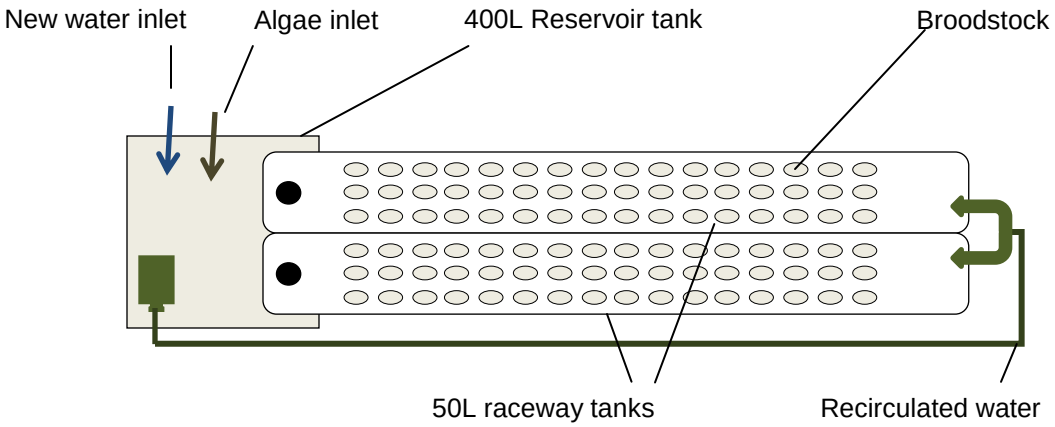

Figure 1. Broodstock holding tank (plan view)

Temperature

When broodstock are first introduced to the conditioning system, water temperature should be raised from ambient at the collection location to the desired temperature at a rate of 1°C day^{-1}. The final holding temperature will vary with the gonad condition of the clam and time until desired spawning date although 18°C is close to the optimum conditioning temperature and was used at SARDI. If the broodstock are not gravid and need to be conditioned quickly, then the feed level and temperature can be raised to no more than 20°C. If broodstock are extremely gravid and a delay in spawning is necessary, they should be held at approximately 14°C, and if necessary, reduce temperature 1° C per day from ambient to this temperature. A small number of broodstock can be sampled approximately once per week to check gonad condition. This assessment is made by checking for the proportion of soft tissue taken up by gonad, the incidence and extent of spawning 'lines', checking how readily gametes are released after making a small incision with a scalpel blade and by microscopic inspection of egg size, shape and uniformity. Sperm activity can also be checked under a microscope, looking for rapidly moving sperm when mixed with sea water.

Diet

A diet consisting of 75% diatoms and 25% flagellates is suggested for broodstock conditioning. It may not be practical to have such a high proportion of diatoms but it is important that they comprise at least 50% of the diet. Broodstock were fed twice per day with a mixed algal diet of *Chaetoceros muelleri, C. calcitrans,* Tahitian strain *Isochrysis* sp. (T. Iso) and *Pavolva lutheri* to a density of 200,000 cells L^{-1} reducing to 0 cells L^{-1} as the clams fed, with some loss due to

water exchange. Broodstock were monitored for mortality at the time of feeding and gonad condition was checked once per week.

Cleaning Broodstock Tanks

Broodstock tanks should be cleaned once per week. When cleaning, it is important to minimise stress and temperature fluctuations as unscheduled spawning may occur. If broodstock are very gravid, they will readily spawn with even slight temperature changes and handling. Keep them out of the water for as short a time as possible. Where possible, place them temporarily into another tank of the same water temperature while their original holding tank is cleaned.

3. SPAWNING AND FERTILISATION

Materials

The following materials should be prepared for broodstock spawning:

- sufficient gravid adults (see note below)
- black spawning tray (approximately 100 cm x 60 cm x 14 cm)
- sufficient trays to separate males and females
- 2 x 20 L graduated buckets
- 150 µm screen
- 53 µm screen
- 45 µm screen
-15 or 20 µm screen
- Ethylenediaminetetraacetic acid (EDTA)
- immersion heater for each incubation tank
- torch
- source of heated water
- plunge stirrer
- 500 mL beaker
- 1 mL pipette
- 0.1 mL pipette
- Sedgwick Rafter slide
- microscope
- hand tally counter

Broodstock

Clam broodstock are relatively small and less fecund than many other bivalves such as oysters, consequently greater numbers of broodstock need to be used to obtain the required number of eggs.

When determining the number of broodstock to use, assume approximately 50% will spawn, 50% of the stock are female, approximately 1 million eggs from female, 80% of eggs will develop to

straight hinge veligers (D larvae), 25% of D larvae to pediveligers, 40% of pediveligers undergoing metamorphosis to settled spat, and 25% of settled spat surviving to 6 mm spat.

For example;

Target number of 6 mm spat	1 million
Newly settled spat	4 million
Eyed larvae	10 million
D larvae	40 million
Eggs	50 million
Female broodstock	50
Male and female broodstock	100
Total broodstock	200

The figures above are provided for a guide only. Percentages are likely to be well above this from a healthy batch of high quality larvae. Larvae can be graded and culled more aggressively if excessive numbers are available; aggressive grading can be undertaken so that only healthy, fast growing larvae of a uniform size are retained.

Before spawning it is recommended that broodstock are not fed for one day to purge the gut in clean water but this is not absolutely necessary.

All spawning equipment should be cleaned with chlorinated water and rinsed well before using.

Spawning

1) Clean the exterior shells of the broodstock with fresh water before placing in the spawning tray.

2) Spread broodstock evenly in the shallow black spawning tray. Do not overcrowd the spawning tray as it will make it difficult to determine which animal is spawning. If, due to insufficient space, it is desirable to have less broodstock in a spawning tray, clams can be sexed by drilling a small hole through the shell (Figure 2) and sampling the gonad with a hypodermic needle. Remove excess males and keep enough females to provide sufficient eggs for the batch.

3) Fill spawning tray with sea water at the same temperature that the broodstock were held in.

4) Maintain the water temperature of the spawning tray until all of the clams have opened, then start increasing water temperature by 1°C every 15 minutes. Raise the temperature to

no more than 28°C over a period of approximately 1.5 to 2 hours, within which time gravid broodstock should spawn.

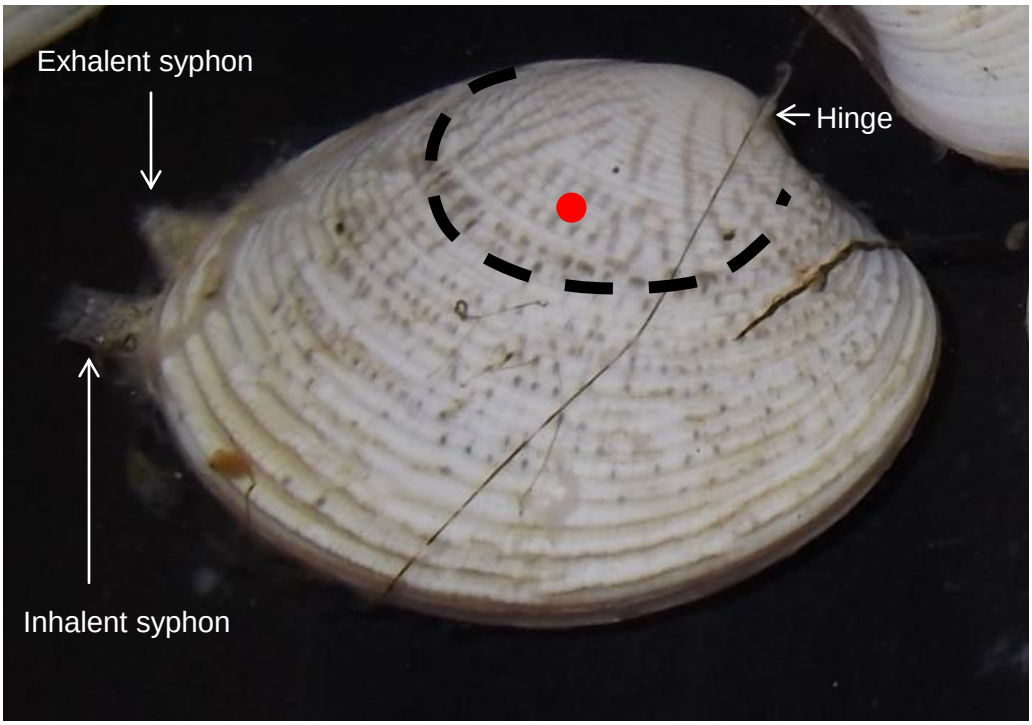

Figure 2. *K. rhytiphora* showing approximate location of gonad (dashed line) and hole (red dot) that can be drilled in shell to enable gamete sampling.

If the clams have not spawned by then, there are several measures that can be taken to help induction of spawning, including;

- draining and refilling of spawning tank with the same temperature water (if this is done, take the opportunity to rinse the tank of faeces).
- dipping clams in cold water briefly before replacing in heated water.
- adding microalgae to the water.
- sacrificing and stripping a clam and adding sperm (works best) or eggs to the spawning tank.
- recirculating ultra violet (UV) irradiated water through the spawning tank.

Separation of Spawning Males and Females

Both sperm and eggs are ejected from the exhalent siphon and can be difficult to distinguish from one another. Although sperm can come out of the siphon in a thick stream, it will usually 'dissolve' to appear milky in the water. Eggs tend to come out thicker and are more inclined to sink to the bottom and are also granular upon close inspection. If in doubt, place the spawning animal in its own container and check gametes with a microscope before mixing with other spawning male or female clams. To reduce the chance of unwanted fertilisation, rinse females with clean sea water before placing in the container to finish spawning. A good source of light such as a torch, as well as a magnifying glass, helps differentiate between eggs and sperm and minimises the chance of placing a male in with the females. Often males will spawn first, and if it appears as though females will spawn much later than males, cover a beaker of sperm solution and place it in the fridge until required (in our studies the sperm were not kept for longer than 4 hours).

When clams start spawning, place males and females in separate containers, at a density of approximately 1 female L^{-1}, ensuring that there is not an excessive volume of water as it can make screening of eggs difficult. When transferring broodstock between spawning tanks, ensure that the water temperature is the same in each container; otherwise there is a risk of females stopping spawning when transferred to water of a different temperature. Males generally continue to spawn even if the water temperature is different.

It is desirable to have uniformly developed eggs at fertilisation so that subsequent development is uniform. This is especially important when estimating fertilisation rate and screening eggs over fine screens (15 or 20 µm), which is occasionally necessary.

When spawning is complete, to remove faeces and other debris, pour the eggs through (approximately) 150 and 53 µm screens to catch debris, retaining the eggs in a bucket. Do the same with approximately 500 mL of sperm solution, retaining sperm in a beaker.

Gamete Counts

Count the number of eggs and sperm cells. This is done by the following procedure:

Fill screened egg bucket to a known volume (e.g. 10 L), mix the contents with a plunge stirrer to achieve a homogenous suspension, simultaneously taking a 0.1 mL sample using a pipette. Transfer this sample to a Sedgwick Rafter slide and count eggs using a microscope, multiplying the result by 10 and then the number of millilitres of the original solution to determine the number of eggs (e.g. n x 10 x 10,000 for a 10 L volume). This can be repeated to ensure accuracy.

With experience, a diluted sperm mix can be counted in a similar manner.

Fertilisation

After gamete counts, it is usually easier to estimate the required amount needed to achieve approximately 5 sperm egg^{-1}, taking care not to add too much, and checking several times with a microscope (Figure 3), adding sperm where necessary. It may be necessary to adjust contrast on the microscope to ensure good visibility of sperm. It is important to have no more than 10 sperm egg^{-1}. Ideally, there would be only one viable sperm for each egg, but more is usually used to achieve uniform fertilisation within a reasonable time. It is important to fertilise eggs with the sperm of at least five male broodstock (preferably 10+).

Although the amount of sperm needed will vary greatly with the number of eggs, the following serves as a guide;

- dense sperm solution ~30 mL
- medium sperm solution ~100 mL
- light sperm solution ~200 mL

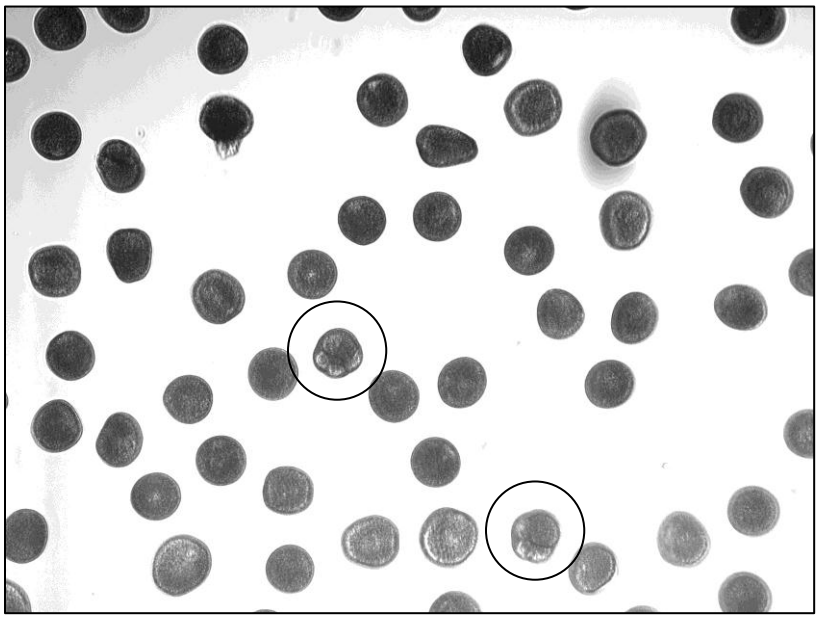

Figure 3. Eggs showing undesirable range of development with some that are fertilised (circled) and others that are not well ‘rounded’.

Figure 4. Fertilised egg showing first polar body extrusion (approximately 20 minutes post fertilisation at 20°C). Figure reproduced with permission from Wallace et al. (2008).

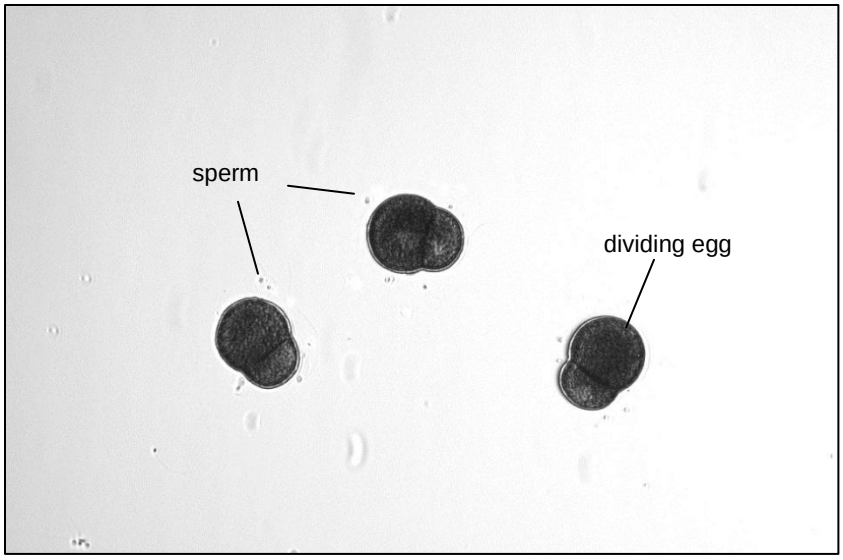

Figure 5. Fertilised, dividing eggs with adjacent sperm.

Look for first polar body movement (Figure 4) as an early sign of fertilisation. When it is obvious that good fertilisation has been achieved (80%+), pour these eggs over a 20 µm screen, retaining them on the screen. Although not essential, the screen can be submerged to help prevent blockage and this may also assist in minimising damage to the eggs. Look for first polar body extrusion rather than waiting for eggs to divide (as shown in Figure 5) before rinsing, as dividing eggs are more likely to pass through the screen and be lost. This is another reason for trying to achieve synchronous fertilisation. Rinse well and gently pour in to another bucket. Gently swirl and

take a 0.1 mL sample for counting the eggs. Rinsing eggs is not absolutely necessary but helps to remove excess sperm and bacteria, often resulting in a healthier group of larvae.

Add 1ppm ethylenediaminetetraacetic acid (EDTA) to each incubation tank. Calculate the volume of eggs required to stock the eggs at approximately 5 to 8 eggs mL^{-1} and transfer the fertilised eggs to the incubation tanks, which should only be supplied with weak aeration. If necessary, place an immersion heater directly over the air bubbles to maintain the temperature at 18°C to 20°C.

4. LARVAL REARING

Draining Tank and Inspection of Larvae

The tank can be drained when the larvae have passed through their first larval stage, called trochophores (Figure 6) and have developed into straight hinged veligers (D larvae) (Figure 7). This is often 30 to 40 hours after fertilisation. As veligers, the larvae can be drained slowly over a 150 µm screen to catch debris, a 53 µm screen to catch good larvae, and a 45 µm screen to catch 'pass throughs', which may be discarded depending on their condition. A plastic frame or empty screen frame should be positioned under the 45 µm screen to enable water to run away. Switch off any heaters prior to draining tanks.

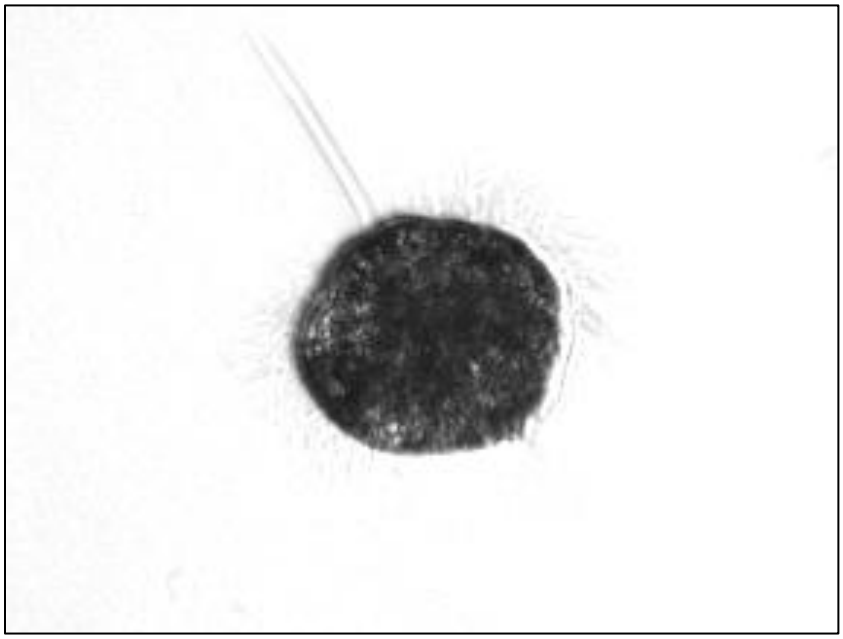

Figure 6. Trochophore, approximately 10 hours post-fertilisation at 20°C.

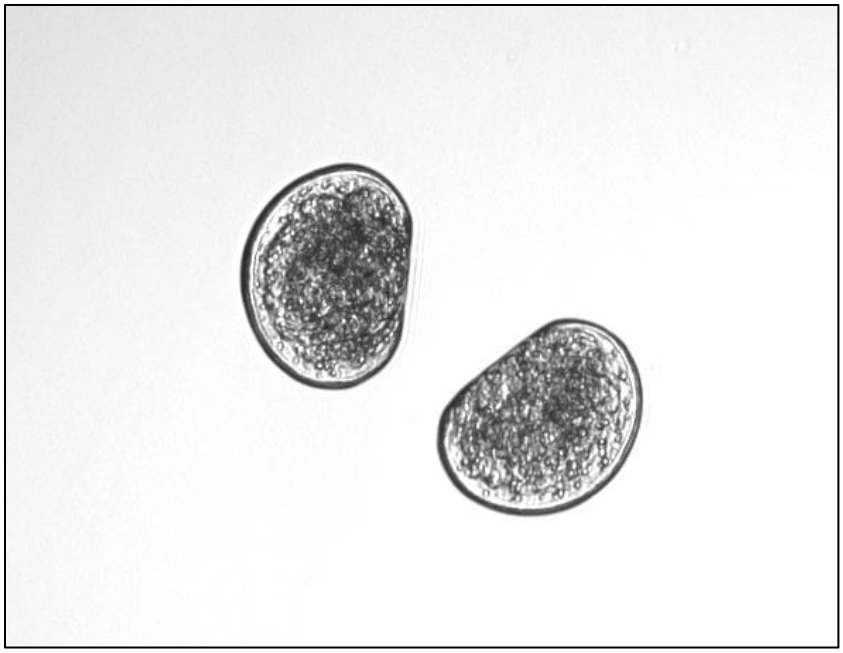

Figure 7. Straight hinge veliger (D-larvae), approximately 40 hours post-fertilisation at 20°C.

Although larvae can be inspected when the tank is completely empty, it is preferable to do this when the tank is approximately half drained so that decisions can be made on which tanks to transfer them to, which screens to grade them on, and how much microalgae to add to the new tank, etc. so that the larvae are not left sitting on a screen while these tasks are undertaken.

Having a good range of well-maintained nylon screens is an important tool in larval culture. They enable the development of a uniform batch of larvae, maintenance of optimum stocking densities while retaining the best larvae, and removal of inferior larvae. Suggested sizes for clam larval and nursery culture are as follows (figures in µm):

Larval culture: 15, 20, 45, 50, 63, 71, 85, 100, 112, 118, 125, 132, 140, 150, 160, 170, 180, 200µm

Nursery culture: 250, 300, 355, 400, 500, 750, 1000, 1500, 1800, 2000, 3150, 3500, 4000, 5000µm.

To assess the health of the larvae, the following steps should be undertaken:

1) While on the screens, wash the larvae thoroughly with clean sea water to remove any debris and so that it is a true representative sample of graded larvae. Wash gently up until the larvae are approximately 150µm in length and slightly more vigorous thereafter.
2) Using a blunt pipette so as not to damage the screen, take a sample of the larvae and place it on a clean counting slide (e.g. Sedgwick Rafter), transferring it to a microscope.
3) Start draining the tank again.

Often when draining and handling larvae, they will close their shells. Where time permits, allow enough time for the larvae to swim on the slide, noting the following factors:

Activity: Generally, high activity suggests healthy larvae. However this can be affected by the temperature and handling larvae.

Velums: Should be clean and well formed, with clean cilia.

Add 1 drop of alcohol to the slide to stop larvae swimming and note the following:

Gut content: Assess the colour of gut and whether microalgal cells are being digested, if there is any inflamed gut tissue (an indication of *Vibrio* infection).

Development: Comparing previous notes, assess the rate of development and the change of shape as they grow.

Shell: The cleanliness of the shell, there should be nothing sticking to it.

Lipid reserves: Note the amount of lipid reserves, usually seen as small oil droplets, especially in the umbo when approaching metamorphosis.

Size: Measure the size of the larvae. When compared with previous records, this enables determination of growth rate, adjustment of stocking density, and allows the operator to check that the correct screen size has been chosen and helps to determine the best size to use next time the larvae are drained. Record the size of a representative sample of larvae (under the microscope at 100x magnification) to determine and record the size range and mean.

Once the size and condition of the larvae is known, the correct stocking density can be applied. Suggested stocking densities for static systems are provided in Table 1. Flow-through tanks can have considerably higher densities.

It is important to have plenty of larvae in the batch (without overstocking tanks) so that culling of poor quality larvae can be undertaken while retaining adequate numbers of healthy larvae. Larvae should be counted each time the tank is drained. To count larvae, the following can be carried out either separately or in conjunction with the sampling steps 1 to 3 above.

4) Add approximately 2 or 3 L to a bucket. Repeat steps 1 and 2 above;
5) Wash the contents of the screen into the bucket, filling to a known volume (e.g.10 L);
6) After mixing the contents of the bucket using a plunge stirrer, take a 0.1 mL sample;
7) Using a microscope and a Sedgwick Rafter slide, calculate the number of larvae.

Growth rate will vary with a range of factors including water temperature, food quality, stocking density and overall health of the batch. Towards metamorphosis, larvae can get very sticky and accurate numbers can be difficult to determine. It is important to have confidence in counts so that correct stocking densities can be maintained.

Table 1. Larval growth at 20°C and recommended stocking densities in a static system

Age (days)	Developmental Stage	Length (µm)	Screen Size (µm)	No. per mL^{-1}
0	eggs	68	20	5 to 8
2	straight hinged veligers	105	53	5
4	early umbonate veligers	120	62	4
6	umbonate veligers	135	74	3
8	umbonate veligers	155	88	3
10 to 12	umbonate veligers	175	105	2
14 to 16	pediveligers	195	125	1.5

Do not leave the larvae in the bucket for longer than 5 minutes before pouring the contents into a clean larvae tank, preferably with microalgae previously added. The water temperature of larval tanks should be maintained within the range of 18 to 21°C from D larvae to metamorphosis, avoiding sudden changes in temperature.

Feeding Larvae

Microalgal species are selected for the following characteristics.

- size of cell (preferably below 10 µm).
- nutritional qualities.
- ease of culture.

The species used to culture *K. rhytiphora* were:

Diatoms

Chaetoceros calcitrans (Cal) 5 µm

Chaetoceros muelleri (CM) 7 x 9 µm

Flagellates

Tahitian strain *Isochrysis* sp. (T. Iso) 6 to 7 µm

Pavlova lutheri (Pav) approximately 6 to 7 µm

A 50:50 ratio of diatoms:flagellates is desirable, although not essential. *C. calcitrans* is able to be fed from early veligers and *C. muelleri* added approximately 4 days before settlement.

Maintaining background microalgal density is important in ensuring ready availability of feed to larvae (Rico-Villa et al. 2008). Microalgal density in larvae tanks should be maintained between 10,000 cells mL^{-1} for early stage veligers to 40,000 cells mL^{-1} for larger larvae approaching metamorphosis. Algal density needs to be monitored closely to ensure that it remains within this range as it is constantly being consumed by larvae. To avoid large fluctuations in density, if practicable it is preferable to add algae to larvae several times throughout a 24 hours period rather than adding it once per day. Table 2 below serves as a guide but hatchery personnel need to check background densities. Note that eggs and trochophores are not fed.

The sum of background feed levels and likely feed ingestion can be used to calculate the total feed budget. A method of determining feed ingestion can be found in Helm et al. (2004).

Table 2. Feeding rates for veligers in a static system

Screen size	**Approx. min. larvae size (µm)**	**Algal species**	**Background density (cells mL^{-1})**	**Approx. no of Iso equivalent algal cells consumed per larvae per day***
eggs	70	None	None	
74	104	Pav, Iso, Cal	20,000	4,400
88	124	Pav, Iso, Cal	30,000	8,000
105	148	Pav, Iso, Cal	30,000	15,700
125	176	Pav, Iso, Cal, CM	30,000	26,000
150	212	Pav, Iso, Cal, CM	40,000	29,100

*These figures are only a guide, from Helm et al. (2004) for *Ruditapes philippinarum* at 24°C.

When selecting microalgal feed, only use cultures that are clean, growing well and not sticking to the surface of the culture vessel. Regular testing of algal cultures for *Vibrio* spp. on thiosulphate citrate bile salts sucrose (TCBS) agar is recommended to identify contamination of cultures.

5. SETTLEMENT

In order to achieve uniform settlement, larvae should be graded tightly so that a uniform group of faster growing larvae is retained.

Even a tightly graded group of larvae will have a slightly uneven range of development. Larvae will be approaching settlement size when retained on a 118 µm screen, but may not be sufficiently developed until they are retained on a 125 µm screen. It is important to judge the optimum time for settlement. If set too early, they will not develop much further when transferred to the settlement system but if too late, a large number will set on the bottom of tanks. With clams, this is not as critical as oysters, because clams do not adhere strongly to the bottom and can be rinsed off. Once at least 40% are advanced crawling pediveligers (Figure 8), they can be placed into downwellers.

As clams do not remain permanently attached to the substrate, they can be set on to the screen mesh of a downweller. Larvae should be added to downwellers at a density of approximately 1 million per 0.15 m^2. The downweller screen mesh size should be slightly smaller than the larvae grading screen. For example, use a 110 µm downweller screen for larvae sitting on a 118 µm screen.

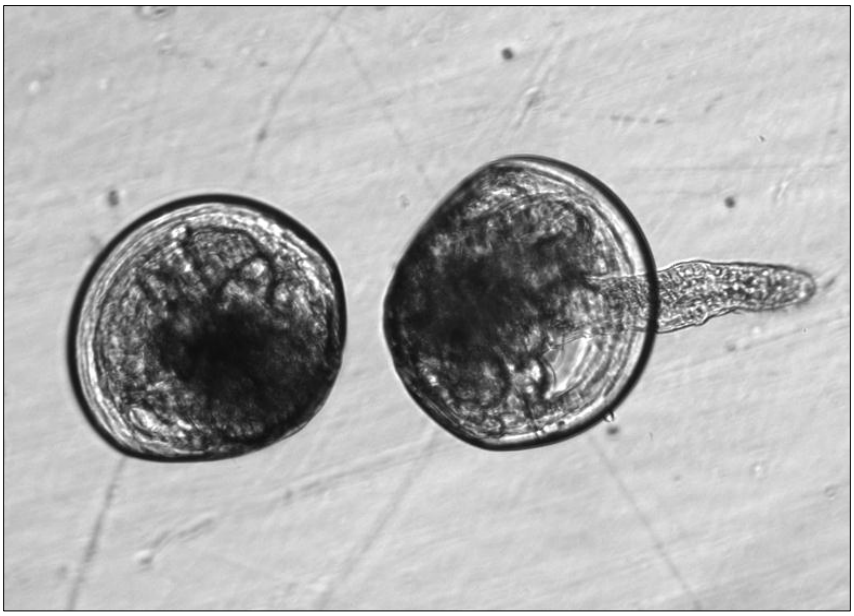

Figure 8. Pediveligers (approximately 12 to 14 days post fertilisation).

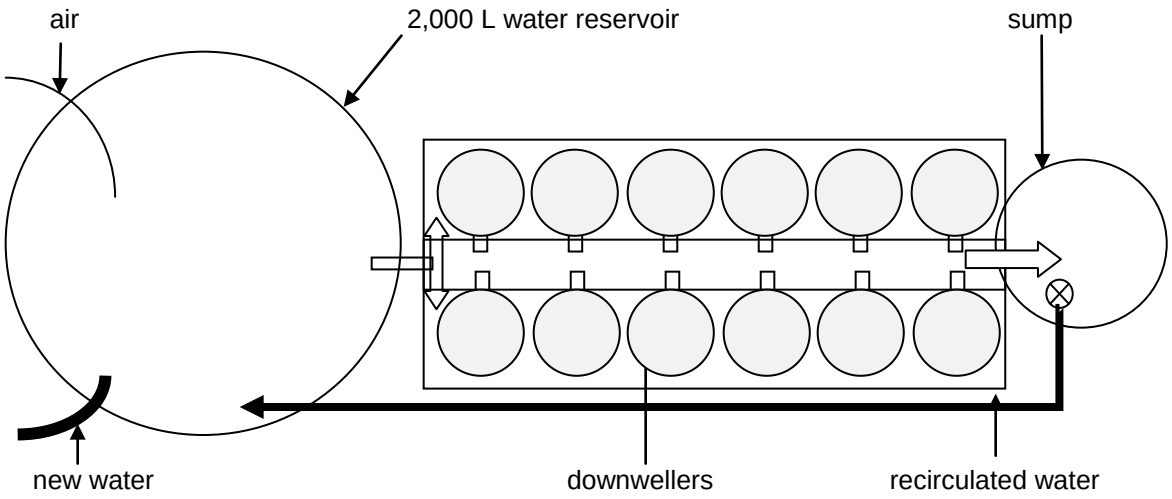

Figure 9. Plan view of combined upweller/downweller tank.

The settlement system used at SARDI was a relatively small research system (Figure 9) and was used for containing metamorphosing spat in downwellers (Figure 10A) and for metamorphosed spat in upwellers (Figure 10B). Water was introduced to the top of the downwellers via an airlift mounted on the side of the downweller, whereas in upwellers water was introduced from the bottom of the upweller and exited via a screened outlet attached to the central drain.

A. Airlift downweller

B. Upweller

Figure 10. Settlement and early spat culture containers used in settlement system.

Settling larvae in downwellers need to be cleaned once each day with sea water. After draining the downweller tank, use sea water to spread the larvae on the screen, cleaning the screen and larvae at the same time. The most common larval pathogen, *Vibrio* spp., are generally surface colonising bacteria, so it is important to thoroughly clean all downweller and tank surfaces. If care is taken to avoid downweller screens, other surfaces should be cleaned with fresh water, being careful not to splash it on to the larvae.

Fresh water should only be used to clean spat after they have reached a size sufficient to be retained on a 350 µm screen. An algal feed density of at least 50,000 cells mL^{-1} is preferred but spat can tolerate up to 200,000 cells mL^{-1} for short periods of approximately 4 to 6 hours.

Grading From Downwellers

After 5 to 7 days, competent spat will have undergone metamorphosis and the group can be graded over 150 or 180 µm screens to separate settled spat from unsettled larvae. Larvae that passed through these screens should be checked for condition and discarded if they are not healthy (active, clean, etc.), otherwise they can be returned to a separate clean downweller (Figure 10).

Sub 250 µm screen spat can remain in downwellers, while anything larger than this can be placed in upwellers. Upwellers should also have a screen on the outlet to prevent escape of spat that may get caught on air bubbles and lifted out of the upweller.

The condition of the spat should be regularly monitored under a microscope, looking for growth, cleanliness, gill development (Figure 11) and activity. Stalked ciliates (*Vorticella* spp.) and fungal growth, particularly around the umbo are signs of poor hygiene which may be caused by over-feeding, over-stocking or inadequate cleaning procedures. If there are any fouling organisms, the spat can be vigorously sprayed with, or dipped in fresh water, but only after they are large enough to be retained on a 350 µm screen.

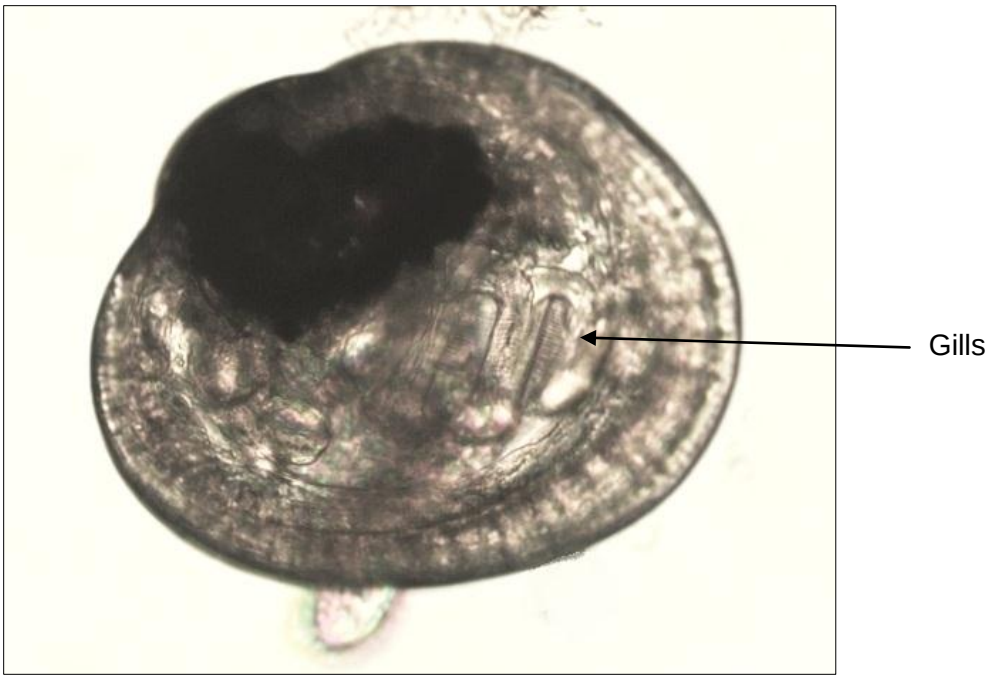

Figure 11. Gill development in early spat.

After settlement, clam spat need to be graded regularly to maintain good growth and to facilitate good water flow through upwellers. Grading can be a very labour-intensive task and depending on growth rate, each upweller may need to be graded every two months. It is important to ensure that sufficient space is allowed for containing spat and not overstocking. The volume of 1 million 1 mm screen spat is approximately 7 L, whereas 1 million 6 mm screen spat is approximately 400 L (Table 3) so nurseries must be adequately designed to accommodate these. Consideration must be given to the number of upwellers and tanks, feed availability, water supply, plumbing, aeration, electricity availability and required labour force.

Table 3. Approximate volumes of *K. rhytiphora* spat at different sizes

Screen Size (µm)	Spat length (mm)	No. per Litre
1000	1.5 to 2.5	138,000
2000	3 to 4.5	55,200
3000	4.5 to 6	25,000
4000	5.5 to 8	8,000
6000	8 to 10	2,500

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