

Developing clam aquaculture in Australia: a feasibility study on culturing *Donax deltoides* and *Katelysia rhytiphora* on intertidal and subtidal leases in South Australia



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DEVELOPMENT

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1. NON-TECHNICAL SUMMARY

2009/208 Developing clam aquaculture in Australia: a feasibility study on culturing *Donax deltoides* and *Katelysia* sp on intertidal and subtidal leases in South Australia

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OBJECTIVES

The overall aim of this project was to investigate the aquaculture potential of two local clam species in South Australia. The following were specific objectives;

- 1. Desktop study of existing international clam farming techniques and previous Australian clam research with a view to adopting existing technology where practicable to ensure project efficiency.
- 2. Determine suitable species using field and laboratory based trials.
- 3. Successful production of clam spat from hatchery reared larvae.
- 4. Production of a hatchery manual for possible use by commercial hatcheries wishing to participate in the proposed clam culture industry.
- 5. Undertake field evaluations for identification of likely commercial culture methods and site characteristics.
- 6. Communication and technology transfer between industry participants and researchers in the form of workshops and written reports.

OUTCOMES ACHIEVED

Results from laboratory and field studies completed in this project demonstrate that a species of Vongole (*Katelysia rhytiphora*) is more suitable for culture than the Pipi (*Donax deltoides*) in terms of growth and survival and as such offers the greater potential for commercial culture.

Hatchery production and rearing of *K. rhytiphora* was successful and there is a high likelihood that commercial bivalve hatcheries could readily adopt such technology with little modification to existing equipment.

In field trials, the growth rate of *K. rhytiphora* was far greater than previously reported for wild stock of two other Vongole species, *K. scalarina* and *K. peronii*. The growth and survival of *K. rhytiphora* at a shallow water site in Coffin Bay was greater than at a deep water site in Boston Bay. Fouling was a major issue affecting the use of cages and was far greater at the deep water site than the shallow site. Addressing cage fouling problems might further enhance growth and survival.

Laboratory and field trials demonstrated that the growth and survival of *D. deltoides* and *K. rhytiphora* was better in sand compared to without it. In a raceway experiment, growth of the Pipi, *D. deltoides* increased with current speed across the range assessed 2 cm s⁻¹, 10 cm s⁻¹ and 18 cm s⁻¹. This correlates with this species natural occurrence in high energy environments, which are a challenging environment for aquaculture infrastructure.

Hatchery production and rearing of *D. deltoides* was not successful, the species was naturally spawned and reared through metamorphosis from free swimming larvae to settled spat, but post-settlement survival was poor.

There is continued interest in clam farming by the industry participants in this project and they are currently working with Primary Industries and Regions South Australia (PIRSA) Fisheries and Aquaculture, to secure field sites for commercial clam culture. Based upon this interest in clam farming and results from this and other projects, PIRSA is developing policy for commercial clam aquaculture.

Market demand of Australian clams has increased significantly in recent years due to a decline in wild catch, an increase in quality due to improved post-harvest technology, and increased consumer awareness.

The overall goal of this project was to investigate the aquaculture potential of South Australian clam species to determine their potential to fill the current shortfall in supply to receptive domestic and international markets.

Clam aquaculture is well developed in many countries overseas including Canada, China, England, France, Italy, Mexico, Spain, USA and Vietnam. In 2010, worldwide aquaculture production was 4.9 million tonnes, valued at US\$4.7 billion. In 2010, Manila clam (*Ruditapes*)

philippinarum) production was 3.6 million tonnes valued at \$3.4 billion, the fourth greatest aquacultured species by weight behind grass, silver, and Indian carp and accounted for the greatest volume of any marine/estuarine species. In the same year, clam production was much greater than Pacific Oysters (0.7 million tonnes, \$1.3 billion).

There are four clam species currently harvested by fishers in South Australia (SA) including the Pipi (*Donax deltoides*), known as the Goolwa cockle in SA, and three Vongole species, also known as mud cockles in SA or sand cockles in the eastern States, including *Katelysia rhytiphora*, *K. scalarina* and *K. peronii*. The two clam species studied in this project were *D. deltoides* and *K. rhytiphora*.

The main components of this project were:

- 1) A review of previous research and clam farming techniques used overseas.
- 2) Hatchery production to assess species' suitability for commercial culture.
- Laboratory based trials with various parameters, aimed to help identify suitable species and field sites.
- 4) Field based trials to help identify likely species, parameters and constraints for commercial culture.

K. rhytiphora was successfully cultured at the South Australian Research and Development's Aquatic Science Centre, West Beach, South Australia. Adults were spawned naturally, with high survival rates of larvae through metamorphosis and subsequent nursery culture in upwellers. Growth and survival were also reasonable when cultured in cages on a commercial shellfish lease, suggesting that it may be a suitable candidate for commercial culture.

Results from this project suggest that *D. deltoides* is unsuitable for culture with available techniques and equipment. In the hatchery this species was naturally spawned and reared through metamorphosis from free swimming larvae to settled spat, however post-settlement survival was poor.

More work is needed to identify the optimum characteristics for field sites and evaluate *K*. *rhytiphora* performance over the whole production period. However, favourable results have generated sufficient interest from the private parties involved with this research to pursue the procurement of lease sites where they can continue working towards commercial culture. Primary Industries and Regions South Australia (PIRSA) is also continuing to refine its policies for clam farming in South Australia, based on the outcomes of this project, the FRDC project 2010/233 "PIRSA Innovative Solutions: Investigations to address key policy gaps associated with the development of clam farming in South Australia: genetic and health issues aligned to

translocation and stock identification" and an assessment of likely culture methods and environmental impact.

KEYWORDS

Aquaculture, clam, Pipi, cockle, Eugarie, Vongole, sand cockle, mud cockle.

TERMS USED IN THIS REPORT

<u>Clam</u>: Any edible bivalve mollusc of the taxonomic orders Veneroida and Arcoida, commonly known as cockle, Pipi or Vongole, and including the genera *Donax, Katelysia, Anadara, Glycymeris, Eucrassitella, Notocallista* and others.

<u>Pipi</u>*: *Donax deltoides.* Also known as Goolwa cockle, Eugarie.

<u>Vongole</u>*: *Katelysia* spp. including *K. rhytiphora, K. peronii* and *K. scalarina*. Also known as mud cockles, and sand cockles.

*From Australian Fish Names Standard AS SSA 5300 – 2011. Standards Australia.

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We gratefully acknowledge the Fisheries Research and Development Corporation (FRDC), South Australian Research and Development Institute (SARDI) and SA Clam Aquaculture (SACA) for providing the funds to carry out this project. We also thank Tom Robinson from SACA for his logistical help with Pipi collection and continued interest and support for this project. The assistance and support of FRDC's project management team of Kylie Giles, Carolyn Stewardson and Crispian Ashby has been appreciated. SARDI personnel that have contributed to this project include Gavin Begg, Steven Clarke, Vanessa Beeke, Lynda Phoa, Greg Ferguson, Ben Stobart, John Dent and Damien Matthews. Kate Rodda (PIRSA) has helped with permitting the use of field sites. Field assistance from Lester and Klay Marshall (Coffin Bay Oyster Farm Pty Ltd), Judd Evans (Kiwi's Oyster Smoky Bay) and Mike Thomson, Chris Brooks and crew of the Sanchez J (Clean Seas Aquaculture Pty Ltd) has been greatly appreciated. Laboratory assistance from Yi Bing Liu, Tong Xu and Daqian Zhao (Dalian Ocean University, China) has also been greatly appreciated.

3. BACKGROUND

Clam aquaculture is an innovative concept in Australia, although it is well developed in many countries overseas including Canada, China, France, Italy, Mexico, England, Spain, USA and Vietnam. In 2010, world clam aquaculture production was 4.9 million tonnes, valued at US\$4.7 billion. In 2010, world Manila clam production (3.6 million tonnes, \$3.4 billion) was the fourth greatest aquacultured species behind grass, silver and Indian carp species and accounted for the highest volume of any marine/estuarine species. Production of clams was much greater than that of Pacific Oysters (0.7 million tonnes, \$1.3 billion) (FAO 2010).

There is currently no clam culture industry in Australia although several species are harvested commercially, with the majority being from South Australia (SA), comprising the Pipi (*Donax deltoides*) also known as the Goolwa cockle, and three species of Vongole (*Katelysia rhytiphora, K. scalarina* and *K. peronii*) also known as mud cockles or sand cockles. These species are also harvested in Tasmania and New South Wales (NSW). The SA catch is restricted by quota, and has been declining (Gorman et al. 2010; Rowling 2010; Ward et al. 2010; Ferguson 2012). Species harvested in other States also include *Anadara trapezium, Glycymeris flames, Eucrassitella kingcola, Notocallista kingie* and *Ruditapes largillierti.*

Previous research on Australian clam aquaculture has been undertaken in NSW (Nell et al. 1994, 1995; Paterson and Nell 1997) and Tasmania (Maguire et al. 2005; Bellchambers et al. 2005a, 2005b; Phelps et al. 2008). While growth of clams in Tasmania was considered too slow to be commercially viable, higher growth rates were achieved in NSW (Paterson and Nell 1997) but there was no commercial uptake, possibly due to the relatively low price at the time.

The proposed development of clam aquaculture is strongly market driven with Australian wild catch declining and not able to satisfy a large national and international market prepared to pay high prices. The market for clam products, particularly those from clean Australian waters is substantial, with the price having increased ten-fold in the past decade. The opportunity exists to determine the feasibility of an Australian clam culture industry because there is strong market potential for Australian clam species and techniques have been developed overseas.

The key industry participant in this project, SA Clam Aquaculture Pty Ltd (SACA) is a sister company to Coorong Cockles Pty Ltd which processes over half of the clams harvested in SA in their Australian Quarantine and Inspection Service (AQIS) and European Union (EU) approved processing facility. SACA has long established supply chains and enjoys strong support from other participants in the clam fishery. They have also played a major role in the development of

the Pipi from a product mainly used as fishing bait to one highly valued for human consumption. In recent years the total allowable catch (TAC) of Pipi in SA was reduced from 1150 tonnes to 330 tonnes and is currently 400 tonnes per annum. Further expansion of their business is limited by the level of wild catch and they are actively exploring new opportunities including aquaculture, to realise the potential of the markets they have developed.

SACA was formed to investigate the potential of clam culture via investment in this project. SACA intends to move this project from research and development (R&D) to commercialisation and has formed a partnership with an oyster farming leaseholder with a view to developing *K*. *rhytiphora* culture on that lease.

A number of SA oyster and mussel farmers have also expressed interest in this project, with their interest being in diversification to reduce the business risk associated with their present practice of monoculture. The level of their involvement to date has been via the provision of sites upon which to undertake field research. These farmers' experience and understanding of oyster culture techniques and the environment within their areas of operation may assist with the commercialisation of this project's outputs.

The two clam species investigated in this project, the Pipi (*Donax deltoides*) and a species of Vongole (*K. rhytiphora*) are endemic to southern Australia. In suitable conditions, they grow in high densities and have proven market potential. The Pipi constitutes the large majority of the clam catch in SA, and is the species with which the industry participant in this project (SACA) has the most experience as a fisher, processor and marketer. This species is highly regarded in the market place due to its relatively large size, high meat:shell ratio, good flavour and texture. Demand has increased recently due to improved post-harvest technology and reduced quota. However, it naturally occurs in shallow waters of high energy, wave exposed beaches which are unsuitable for installation of aquaculture infrastructure.

Three species of Vongole are harvested from the wild including *K. rhytiphora*, *K. scalarina* and *K. peronii*. They receive high prices and naturally occur in relatively calm waters ideally suited for aquaculture infrastructure and are found in or near waters currently zoned for aquaculture development in SA. Of the three Vongole species, *K. rhytiphora* was chosen as the most likely for commercial culture for the following reasons:

- Growth studies by Cantin (2010) found that two Vongole species, *K. peronii* and *K. scalarina* grew relatively slowly, taking 5.5 to 6 years to reach a length of 30 mm.
- Bellchambers et al. (2005b) also found that *K. scalarina* grew slowly in Tasmania, with growth rates of 2.1 mm to 4.2 mm over a 10.5 month period.

• *K. rhytiphora* has been shown to grow faster in NSW (Paterson and Nell 1997) than the other *Katelysia* species in Tasmanian and SA studies.

Other as yet unexploited species may have potential for culture but within the time and financial constraints of this project, only two commercially harvested species, *D. deltoides* and *K. rhytiphora* were studied.

4. NEED

The need for development of an Australian clam aquaculture industry comes from the fact that a large international market is prepared to pay a premium price for quality Australian clams. This demand cannot be met by the wild fishery, a range of culture technology is available and clam aquaculture is successful on a large scale overseas.

Some clam fishers are finding that further expansion of their businesses is severely limited by the level of wild catch and as such are actively exploring aquaculture to realise the full potential of the markets they have developed. Post-harvest technology has been improved to the extent that more than 50% of SA harvested clams are now sold for human consumption rather than for fishing bait.

The potential viability of a clam culture industry also appears to compare well with the SA oyster industry which has been successfully developed since the late 1980s and supports a large number of participants directly and indirectly employed. This outlook is supported by the following factors:

- The price per kilogram for clams is currently better than for oysters.
- Infrastructure costs are likely to be less if cultured in the substrate.
- Handling of clams, including rumbling and grading are likely to be less frequent than for oysters.
- The development of a clam culture industry is likely to occur in a shorter time due to the existing technical and resource management experience.

Intertidal waters currently identified as being suitable for oyster culture are close to being fully allocated in SA and therefore opportunities for existing growers to increase business revenue and for new participants are limited. Clams can offer a new income stream from existing oyster leases, from leases that have proven to be unsuitable for oyster culture and from new areas. Diversification of farmed bivalve species is also a risk management strategy in case of a potential OsHV-1 µvar oyster viral disease outbreak.

To assist in the development of an Australian clam aquaculture industry, the following information needs to be determined and provided the focus for this project:

- Appropriate species for culture.
- Optimum site characteristics for culture.
- Collate information from overseas clam culture industries.
- Development of hatchery and nursery technologies.

5. OBJECTIVES

- 1. Desktop study of previous research and international clam farming techniques with a view to adopting existing technology where practicable to ensure project efficiency.
- 2. Determine suitable species using field and laboratory based trials.
- 3. Successful production of clam spat from hatchery reared larvae.
- 4. Production of a hatchery manual for possible use by commercial hatcheries.
- 5. Undertake field evaluations for identification of potential commercial culture methods and optimum site characteristics.
- 6. Communication and technology transfer between industry participants and researchers in the form of workshops and written reports.

6. SUMMARY OF EXISTING CLAM FARMING TECHNIQUES AND AUSTRALIAN RESEARCH

A literature review was undertaken to serve as an introduction to clam farming for prospective participants and is included as Appendix A of this report.

The literature review highlighted the following:

- A process of identification of hardy, fast growing, disease resistant species has occurred.
- The majority of world clam production is based on the Manila clam (*Ruditapes philippinarum*) but hard clams (*Mercenaria mercenaria*) are becoming an important species on the east coast of the USA.
- Selection of farming sites with suitable depth and natural habitat of the target species is critical.
- Development of reliable hatchery technology is important.
- The most common predators of clams are crabs, whelks and rays.
- Clams are most commonly cultured in the substrate with predator control nets placed over the top of them.
- Fouling of predator control nets can be a significant issue.
- Results of previous research on Australian clam culture indicate slow growth of *Katelysia scalarina* in Tasmania but *K. rhytiphora* and *Tapes dorsatus* grew well in NSW.
- To date, commercial clam farming has not been undertaken in Australia.

The literature review was followed by a workshop discussing observations and perspectives of Australian clam culture research and commercial clam culture in Europe, USA and China (Appendix B).

7. HATCHERY AND NURSERY CULTURE OF CLAM SPAT

Hatchery trials were undertaken with the Pipi, *Donax deltoides* and a species of Vongole, *Katelysia rhytiphora*, during 2010 and 2011 at the SARDI shellfish research hatchery, SA Aquatic Sciences Centre, West Beach, Adelaide, South Australia.

7.1. Methods

Pipi (D. deltoides)

Broodstock Conditioning

Prior to the availability of gravid wild broodstock, out-of-season conditioning was attempted with several groups of adult *D. deltoides* collected from the beach of Younghusband Peninsula, approximately 20 km southeast of Goolwa, SA (Figure 1). Broodstock were placed in tanks containing water of the same temperature at the place of collection. Two types of tanks were used in conditioning systems including a 1 m x 1 m x 0.4 m tank and a 3.3 m x 0.35 m x 7 cm shallow raceway tank with a water depth of 4 cm. Water was recirculated through the raceway tank using a submersible pump. New water was provided to both tanks at a rate of 10 exchanges day⁻¹.

Where necessary, water was increased from ambient by 1°C day⁻¹ and maintained at 18°C thereafter. 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 an average density of 50,000 cells mL⁻¹. Broodstock were monitored for mortality at the time of feeding and gonad condition was checked once per week.



Figure 1. Location of clam collection (Goolwa and Coffin Bay) and cage deployment sites (Boston and Coffin Bays)

Spawning

D. deltoides were cleaned with fresh water and placed on a 100 cm x 60 cm x 14 cm shallow black spawning tank. Ideally, sufficient space should be allowed between each clam to easily identify which animal is spawning, however in all spawning attempts, a relatively small proportion spawned so in order to gain a sufficient number of eggs, the spawning table was crowded (Figure 2).

When broodstock were first placed in the spawning tank, water temperature was maintained at the same temperature from where they came, whether that was directly from the wild or the temperature in broodstock conditioning tanks. This was maintained until all clams had opened, after which water temperature was increased to a maximum of 28°C over a period of 1.5 hours in an attempt to induce spawning. If spawning hadn't commenced by then, water temperature was occasionally cycled between 12°C and 28°C within 5 to 10 minutes. Other methods of induction that were used included: (i) adding sperm stripped from a sacrificed male to the water; (ii) completely draining the tank and refilling; and (iii) adding algae to the water.

When individuals commenced spawning, they were rinsed of sperm under clean running sea water to minimise the incidence of unintentional fertilisation of eggs before being placed into separate containers for males and females (Figure 3) to continue to spawn, usually taking 20 to 30 minutes. The water temperature of these containers was maintained at the same temperature as the main spawning tank or slightly warmer so that they would continue to spawn after transfer. When sex determination was difficult, the spawning animal was placed into a separate container so that gametes could be checked with a microscope before placement in the appropriate container.



Figure 2. D. deltoides broodstock on spawning tray



Figure 3. Male and female D. deltoides separated while spawning

When broodstock had completely spawned, they were removed from the containers. Eggs were inspected microscopically to ensure uniformity of shape before pouring through a 100 µm screen and combining in a 20 L bucket. If needed, more time was allowed for eggs to gain uniform shape before fertilising within an hour of spawning. Sperm was inspected for motility, combined and screened before being used to fertilise eggs, aiming for less than 5 sperm per egg, which enabled uniform fertilisation but minimised the inclusion of excess sperm which provides a substrate for

undesirable bacterial growth. The sperm of at least ten males was always used to assist in preserving genetic variability.

After the sperm solution was added to the eggs, the bucket was gently mixed with a plunge stirrer to create a homogenous mixture, enabling uniform fertilisation. After fertilisation (Figure 4), eggs were transferred to gently aerated incubation tanks containing 1 ppm ethylenediaminetetraacetic acid (EDTA) and maintained at 18° to 20°C for 40 to 44 hours before draining the tank, retaining straight hinged veligers (or 'D' larvae) on a 45 µm screen.



Figure 4. Early larval stages of *D. deltoides*: fertilised eggs 30 minutes post-fertilisation (A), trochophore 12 hours post-fertilisation (B) and straight hinged veligers, 40 hours post-fertilisation (C)

Larval Rearing

After draining the incubation tank, larvae were rinsed on the screen and microscopic observation was made of condition, including shape, cleanliness, activity and size before counting larvae and transferring to 2,000 L static tanks with densities adjusted approximately to levels described in Table 1. Feed levels were as described in Table 2. Two 300 watt immersion heaters were used in each tank to maintain water temperature at an average of 19.5°C.

Average Length (µm)	Screen Size	S.D. ml ⁻¹
68 (egg)	n/a	10
105	53	5
120	62	4
135	74	3
155	88	3
175	105	2
195	125	1.5

Table 1: Stocking density (S.D. mL⁻¹) used in static larval rearing tanks at an average temperature of 19.5°C for *D. deltoides* larvae.

Table 2: Feeding rates for D. deltoides veligers in a static system

Approx larvae size	Algal species	Background density (cells ml ⁻¹)	Approx. no algal cells consumed per larvae day*
68 (egg)	n/a	n/a	n/a
104	Pav, T-Iso, Cal^	20,000	4,400
124	Pav, T-Iso, Cal	30,000	8,000
148	Pav, T-Iso , Cal, CM	30,000	15,700
170	Pav, T-Iso, Cal, CM	40,000	22,300
190	Pav, T-Iso, Cal, CM	40,000	26,000

*These figures were used as a guide throughout hatchery trials, adapted from Helm et al. (2004) for *Ruditapes philippinarum* at 24°C. [^]Pav = *Pavlova lutheri*, T-Iso = Tahitian strain *Isochrysis* sp., Cal = *Chaetoceros calcitrans*, CM = *C. muelleri*.

Larval rearing tanks were drained daily with larvae being retained and graded using nylon mesh screens, with larval rearing tanks restocked to densities listed in Table 1. Each time tanks were drained, larvae were graded in an effort to retain larvae of uniform development as this enhances settlement and survival rates. When at least 90% of larvae were observed to have developed feet (Figure 5) and 30% were crawling, the group of larvae was transferred to downwellers in the settlement system (Figure 6).



Figure 5. D. deltoides pediveliger

Settlement

The settlement system consisted of two rectangular fibreglass tanks coupled with a 2,000 L reservoir tank and a 200 L 'sump' (Figure 6). Water was recirculated by a pump and all water was drained and refilled daily. Each of the rectangular tanks contained six 25 cm wide x 32 cm long downwellers constructed from sections of PVC pipe, each with 118 μ m mesh adhered to one end upon which the clams sat.

Various settlement methods were trialled including the following:

- Directly on to the downweller screens.
- Sand graded into three sizes (150 to 250 $\mu m,$ 250 to 350 μm and 350 to 450 $\mu m).$
- Crushed and graded oyster shells.
- Crushed and graded clam shells.
- Whole oyster shells.
- Shade cloth mesh.
- Mussel rope.
- Silver rope.
- PVC oyster settlement slats (Zapco Aquaculture Ltd).



Figure 6. Settlement system (plan view)

Downwellers were cleaned daily with 2 µm filtered sea water and clam spat were fed a mixed algal diet of *Chaetoceros calcitrans, C. muelleri,* Tahitian strain *Isochrysis* sp. (T-Iso) and *Pavlova lutheri* to a density of 60,000 cells mL⁻¹. If cell density dropped below 30,000 cells mL⁻¹, a secondary feed was provided later in the day.

Vongole (K. rhytiphora)

Broodstock Conditioning

K. rhytiphora (Figure 7) were in gravid condition when collected from Coffin Bay, SA during March 2011 and spawned soon after without the need for further conditioning. However, when necessary, adult *K. rhytiphora* were maintained in 1 m x 1 m x 0.4 m tanks for extended periods. Water exchange was provided to both tanks at a rate of 10 exchanges day⁻¹.

Water was increased from ambient by 1°C day⁻¹ and maintained at 18°C thereafter. Broodstock were fed twice per day with a diet of *Chaetoceros calcitrans, C. muelleri*, T-Iso and *Pavolva lutheri* to a density of approximately 150,000 cells mL⁻¹ and cleared the water within two hours. Broodstock were monitored for mortality at the time of feeding and gonad condition was checked once per week.



Figure 7. K. rhytiphora broodstock

Spawning and Larval Rearing

Spawning and larval rearing methods were the same as described for *D. deltoides* and several batches were grown commencing March 2011. Larvae took between 12 and 14 days to grow to the advanced pediveliger stage for introduction to the settlement system.

Settlement

Larvae were transferred to a downweller system (Figure 6) and added to 118 μ m screened downwellers at a stocking density of 0.15 million m⁻² of screen area and underwent metamorphosis over a period of one week, attaching directly to the screen mesh of the downwellers. Settling larvae and spat were washed daily with seawater on the screen until reaching a screen size of 350 μ m (spat approximately 500 μ m), after which they were washed with fresh water to minimise bacterial growth.

One week after settlement, spat were changed from a downweller to upweller system by reversing the water flow and adding a screen on the outlet to prevent loss of spat. Spat were graded regularly until large enough to be used in field trials and laboratory-based temperature trials.

More detail on larval rearing methods is provided in the Hatchery Manual for Larval Rearing of Vongole (*Katelysia rhytiphora*) (Gluis and Li 2014).

7.2. Results

Pipi (D. deltoides)

The shallow raceway broodstock holding tanks were more successful than the deeper tanks in keeping adults *D. deltoides* broodstock alive, with adults staying alive for over a month in these, perhaps because the comparatively rapid water flow more closely resembled their natural habitat within the wash zone. However, several attempts at out-of-season conditioning were unsuccessful with overall condition deteriorating and high mortality rates experienced within a month of collection.

Due to the difficulties in conditioning out of season, successful spawning only occurred when gravid wild broodstock were available. Gravid broodstock were collected from the ocean beach at Goolwa or Younghusband Peninsula, SA when they were available.

Prior to commencement of this project, a mass spawning event of wild stock was observed during the second week of October 2009. During the months that followed, reproductive condition was generally poor with small sporadic spawning events occurring. In 2010, the first year of hatchery work with this project, a large widespread spawning occurred in late September, but stock recovered condition to spawn again in late November after which they didn't regain suitable spawning condition that summer (Figure 8).



Figure 8. Intra-annual trends in gonad development of *D. deltoides* on Younghusband Peninsula, SA in 2010, Figure reproduced with permission from Ferguson et al. (2013). See Table 3 for description of gonad stages

Gonad Stage	Gonad Condition		
1	No gonad material visible (Includes immature individuals)		
2	Poorly developed A small amount of gonad material is evident on margins of the viscera Digestive gland completely uncovered upon external observation		
3	Moderately developed Gonad material does not cover an extended area A proportion of the digestive gland is still visible upon external observation		

Gonad material covers large area extending in to the foot Digestive gland completely covered upon external observation

Gonad material 'oozes' out when the body wall is broken Gonad appears 'grainy'; and white to cream in colour

Very tightly packed and body wall hard to touch

Digestive gland completely covered upon external observation

Gonad appears 'grainy' throughout; white to cream in colour

Gonad material covers large area extending in to the apex of the foot

Table 3. Criteria used to determine gonad development (after Gorman et al. 2010)

Well developed

Fully developed

4

5

During artificially induced spawning of gravid wild adult broodstock, a maximum of 20% spawned
and approximately 50% of those were males. Approximately 1 million eggs were produced from
each female.

Larvae grew and survived at reasonable rates (Table 4) compared to other species of shellfish including Pacific Oysters, blue mussels, Manila clams and hard clams (Gluis, unpublished data) and after approximately 12 to 14 days, larvae approached metamorphosis as evidenced by development of a foot, crawling behaviour and early gill development.

Age (days)	Length (µm)	<u>Screen Size</u>	<u>S.D. mL⁻¹</u>
0	68	eggs	10
2	105	53	5
4	120	62	4
6	135	74	3
8	155	88	3
10	175	105	2
12 to 14	195	125	1.5

Table 4: Growth of *D. deltoides* larvae and stocking densities (S.D. mL⁻¹) used in static culture system at an average temperature of 19.5°C

Larvae developed through metamorphosis, however survival of newly settled spat was poor with none surviving beyond a length of 1 mm. Microscopic observation of relentless hyperactive foot activity suggested that the clams were attempting to bury into the substrate. Due to this behaviour, three different grades of sand (150 to 250 µm, 250 to 350 µm and 350 to 450 µm) were added to downwellers so that clams were able to bury. However, survival was no better than with screen mesh only. Additional substrates were compared including crushed and graded oyster and clam shells, whole oyster shells, shade cloth mesh, mussel rope, silver rope, and PVC oyster settlement slats (Zapco, ZAP017-1), with none being successful.

Vongole (K. rhytiphora)

Adult *K. rhytiphora* broodstock were held in tanks and retained gonad condition for up to six months with minimal mortality. The ability to hold broodstock in spawning condition for extended periods is an important consideration for hatcheries. However, this species may be found in gravid condition in their natural environment for extended periods, reducing the need for out-of-season conditioning. In Coffin Bay in 2009, Gorman et al. (2010) found that for 7 months of the year >80% of sampled *K. rhytiphora* were in reproductive condition and >80% of *K. scalarina* were in reproductive condition for 8 months (Figure 9).



Figure 9. Temporal variation in the reproductive condition of *K. rhytiphora* and *K. scalarina*, measured as mean gonad index. Data includes all collected individuals greater than the size of first maturity (i.e. \geq 32 mm shell length (SL) and \geq 27 mm SL, respectively). Figure reproduced, with permission, from Gorman et al. (2010)

Growth (Figure 11) and survival of *K. rhytiphora* larvae was good and no significant problems were experienced. All batches of larvae grew to pediveligers (Figure 10) within 18 days and were transferred to downweller screens in the settlement system (Figure 6). An average of 60% survived through settlement to be retained on a 400 µm screen. Due to delays in procurement of field sites, spat were held for over a year in upwellers without significant loss, providing additional evidence of their suitability for culture.



Figure 10. K. rhytiphora pediveligers on the left showing extended foot and post set, juvenile spat on the right, showing foot and early gill development



Figure 11. Average growth of *K. rhytiphora* larvae. Note: These groups were graded tightly during the larval period

Screen Size (µm)	Spat length (mm)	No L ⁻¹
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

Table 5. Approximate number of K. rhytiphora spat L-1

8. EFFECTS OF KEY ENVIRONMENTAL PARAMETERS ON CLAM PERFORMANCE IN LABORATORY TRIALS

8.1. Methods

This study consisted of three trials:

A: Current speed (2 cm s⁻¹, 10 cm s⁻¹, 18 cm s⁻¹).

B: Temperature tolerance (18°C, 23°C, 28°C).

C: Substrate type (sand, no sand).

The study was carried out in polyethylene raceways measuring 330 cm x 30 cm x 7 cm (Figure 12). Water was introduced to a baffled section at one end before entering the raceway which was divided into four equal 125 cm x 15 cm x 7 cm replicate sections. Effluent water drained into an aerated 150 L water reservoir and a submersible pump circulated water back to the influent end of the raceway and valves were used to control water flow. New water was added to the system at a rate of 10 exchanges day⁻¹. Heating was provided by a 2 Kw titanium immersion heater. Clams were dose fed twice daily with a mixed diet of *Chaetoceros muelleri*, Tahitian strain *Isochrysis* sp. and *Pavlova lutheri* supplied from a continuous algal culture system of 50 L and 500 L polyethylene bags.

The 'substrate' trials (Table 6) were undertaken at the same time as the 'current speed' trial, and the 'temperature' trials were undertaken separately.



Figure 12. Plan view of a raceway divided into four replicate sections

Species	Substrate	Current speed (cm s ⁻¹)	Temperature (°C)	Replicates
D. deltoides	Sand	2	18	4
D. deltoides	Sand	10	18	4
D. deltoides	Sand	18	18	4
D. deltoides	No sand	2	18	4
D. deltoides	No sand	10	18	4
D. deltoides	No sand	18	18	4
K. rhytiphora	Sand	2	18	4
K. rhytiphora	Sand	10	18	4
K. rhytiphora	Sand	18	18	4
K. rhytiphora	No sand	2	18	4
K. rhytiphora	No sand	10	18	4
K. rhytiphora	No sand	18	18	4

Table 6. Substrate and current speed treatments in raceways

Table 7. Temperature treatments in raceways

Species	Substrate	Current speed (cm s ⁻¹)	Temperature (°C)	Replicates
D. deltoides	Sand	18	18	4
D. deltoides	Sand	18	23	4
D. deltoides	Sand	18	28	4
K. rhytiphora	Sand	18	18	4
K. rhytiphora	Sand	18	23	4
K. rhytiphora	Sand	18	28	4

Sand substrate for raceways was collected from above the mean high water level of the beach adjacent Goolwa and autoclaved prior to adding to designated raceways to a depth of 30 mm.

After a one week acclimation period, 60 clams of each species were placed in the relevant replicate sections within raceways and held for 80 days. Growth (change in length and wet weight) and survival were measured on days 0, 40 and 80.

Statistical analyses

All statistical analyses were carried out using SPSS (Version 20, Chicago, USA). A two (temperature and duration) or three (substrate, current speed and duration) factor ANOVA was conducted to assess differences in survival rate, shell length and whole dry weight for each species. When significant differences were detected, Least Significant Difference (LSD) comparisons were used to identify those means that differed (P < 0.05). All percentage data were arcsine transformed before analysis. Assumptions of homogeneity of variances were checked using Leven's equal variance test.

8.2. Results

The laboratory trials showed that both current speed and sand substrate have a significant effect on the growth (length and weight) and survival of *D. deltoides* after 80 days (Figures 13, 14 and 15). Growth of *D. deltoides* held in sand increased significantly with current speed. At each current speed *D. deltoides* grew significantly more in sand substrate compared to no sand. No significant growth of *D. deltoides* was recorded at any current speed without sand substrate (Figures 13 and 14).

Survival of *D. deltoides* was higher in sand compared to those held without it (Figure 15) and in most instances improved with increasing current speed, particularly when cultured in sand.



Figure 13. *D. deltoides* length after 40 and 80 days cultivation with sand (striped columns) and without sand (solid columns) at water current speeds of 2 (blue), 10 (green) and 18 (red) cm s⁻¹. On the same sample day, different upper case letters of the same colour indicate significant differences (*P*<0.05) between different current speed treatments with or without sand. Different lower case letters of the same colour indicate significant at the same colour indicate significant differences (*P*<0.05) between treatments with and without sand at the same current speed. Values are mean \pm SD (n = 4).



Figure 14. *D. deltoides* whole dry weight after 80 days cultivation with sand (striped columns) and without sand (solid columns) at different water current speeds of 2 (blue), 10 (green) or 18 (red) cm s⁻¹. Different upper case letters of the same colour indicate significant differences (P<0.05) between different current speed treatments with or without sand. Different lower case letters of the same colour indicate significant differences (P<0.05) between treatments with and without sand at the same current speed. Values are mean ± SD (n = 4).



Figure 15. *D. deltoides* survival (%) after 40 and 80 days cultivation with sand (striped columns) or without sand (solid columns) at different water current speeds of 2 (blue), 10 (green) or 18 (red) cm s⁻¹. On the same sampling day, different upper case letters of the same colour indicate significant differences (P<0.05) between different current speed treatments with or without sand. Different lower case letters of the same colour indicate significant differences (P<0.05) between treatments with or without sand at the same current speed. Values are mean ± SD (n = 4).
D. deltoides generally grew slowly over the 80 day experimental period. Significantly higher growth was recorded for *D. deltoides* held at 23°C compared to 18°C and 28°C after 80 days (Figures 16 and 17). However, survival declined with increasing temperature (Figure 18). At 40 days, survival was significantly lower for *D. deltoides* held at 28°C compared to those held at 18°C and 23°C, and at 80 days, survival further decreased significantly in all temperature treatments and was significantly lower in raceways held at both 23°C and 28°C compared to the 18° treatment.



Figure 16. *D. deltoides* growth (length) after 40 and 80 days cultivation at different temperatures (18°C, 23°C and 28°C) in sand. Different upper case letters indicate significant differences (P<0.05) between different temperature treatments sampled on the same sampling day. Different lower case letters of the same colour indicate significant differences (P<0.05) between the same temperature treatment sampled on a different day. Values are mean ± SD (n = 4).



Figure 17. *D. deltoides* growth (whole dry weight) after 80 days cultivation at different temperatures $(18^{\circ}C, 23^{\circ}C)$ and 28°C) in sand. Different upper case letters indicate significant differences (*P*<0.05) between different temperature treatments sampled on the same day. Different lower case letters of the same colour indicate significant differences (*P*<0.05) between the same treatment sampled on a different day. Values are mean ± SD (n = 4).



Figure 18. *D. deltoides* survival (%) after 40 and 80 days cultivation at different temperatures ($18^{\circ}C$, $23^{\circ}C$ and $28^{\circ}C$) in sand. Different upper case letters of the same colour indicate significant differences (P<0.05) between different temperature treatments, sampled on the same day. Different lower case letters of the same colour indicate significant differences (P<0.05) between the same treatment sampled on a different day. Values are mean ± SD (n = 4).

Growth of *K. rhytiphora* in the substrate/current speed trials was relatively low with no significant increase in length observed after cultivation for 80 days (Figure 19). Whole dry weight, on the other hand increased by more than 15% in *K. rhytiphora* maintained in sand with current speeds of 10 and 18 cm s⁻¹ and without sand with current speed of 18 cm s⁻¹ (Figure 20). They were all significantly greater than other treatments.

Survival was high (>90%) in the low (2 cm s⁻¹) and high (18 cm s⁻¹) flow treatments. However, survival was significantly lower in the medium flow treatments (10 cm s⁻¹) with and without sand (Figure 21).



Figure 19. *K. rhytiphora* length (mm) after 80 days cultivation with sand (striped columns) or without sand (solid columns) at different current speeds (2, 10 and 18 cm s⁻¹). The absence of letters indicates no significant differences between the treatments compared. Values are mean \pm SD (n = 4).



Figure 20. *K. rhytiphora* growth (whole dry weight) after 80 days cultivation with sand (striped columns) or without sand (solid columns) at different current speeds (2, 10 and 18 cm s⁻¹). Different upper case letters of the same colour indicate significant differences (P<0.05) between different current speed treatments with or without sand. Different lower case letters of the colour indicate significant differences (P<0.05) between treatments with or without sand at the same current speed. Values are mean ± SD (n = 4).



Figure 21. *K. rhytiphora* survival (%) after 80 days cultivation with sand (striped columns) or without sand (solid columns) at different current speeds of 2 (blue), 10 (green) or 18 (red) cm s⁻¹). Different upper case letters of the same colour indicate significant differences (P<0.05) between different current speed treatments with or without sand. Values are mean ± SD (n = 4).

In temperature trials, growth (length and whole dry weight) of *K. rhytiphora* increased significantly with temperature except for length from 23°C to 28°C (Figures 22 and 23). At 80 days, survival of the 18°C treatment (>80%) was significantly less compared to higher temperatures of 23° and 28°C (>90%), which were not significantly different from each other (Figure 24).



Figure 22. *K. rhytiphora* length (mm) after 40 and 80 days cultivation at different temperatures (18°C, 23°C and 28°C). Different upper case letters of the same colour indicate significant differences (P<0.05) between different temperature treatments sampled on the same day. Different lower case letters of the same colour indicate significant differences (P<0.05) between the same temperature treatment sampled on a different day. Values are mean ± SD (n = 4).



Figure 23. *K. rhytiphora* growth (whole dry weight) after 80 days cultivation at different temperatures ($18^{\circ}C$, $23^{\circ}C$ and $28^{\circ}C$). Different upper case letters indicate significant differences (*P*<0.05) between different temperature treatments sampled on the same day. Values are mean \pm SD (n = 4).



Figure 24. *K. rhytiphora* survival (%) after 40 and 80 days cultivation at different temperatures (18°C, 23°C and 28°C). Different upper case letters of the same colour indicate significant differences (P<0.05) between different temperature treatments sampled on the same day. Different lower case letters of the same colour indicate significant differences (P<0.05) between the same temperature treatment sampled on a different day. Values are mean ± SD (n = 4).

9. PERFORMANCE OF CLAMS IN FIELD TRIALS

9.1. Methods

Wild-caught juvenile *D. deltoides* were collected from the beach of Younghusband Peninsula, approximately 20 km southeast of Goolwa, SA. Collection was undertaken by loosening sand and associated clams with the feet while outgoing waves carried dislodged clams into framed 1.6 mm mesh nets positioned 0.2 to 0.5 m downstream from the feet of the collector. Clams used were retained on a 5 mm screen mesh, with larger specimens removed by hand, resulting in an average length of 20.2 mm. During the 2 hour period that it took to collect a sufficient number of spat, they were held in 40 L rigid plastic containers filled with sea water. Water exchange was provided by transferring water from the sea in buckets. Spat were transported to the laboratory over a period of 1.5 hours wrapped in a moistened hessian sack and cooled with an ice pack in an insulated cooler.

While juvenile *D. deltoides* of appropriate size were plentiful, insufficient numbers of appropriately sized *K. rhytiphora* could be found after several searches in Coffin Bay, a location known to have high numbers of adults (Gorman et al. 2010). As a result, hatchery reared *K. rhytiphora* spat were produced, taking approximately six months to reach an average size of 11.6 mm for use in field trials.

To comply with PIRSA conditions requiring 100% recovery of clams, cages had to completely envelop the clams. The suitability of a range of commercially produced flat bottomed oyster baskets (Aquapurse*, Aquatray*, Harwood trays* and oyster seed trays) were tested in various configurations for one month in Coffin Bay. None of these cages had a suitable combination of dimensions and mesh size to enable retention of sand and clams as well as adequate water flow so specifically designed flat-bottomed cages were constructed.

Cages were constructed from plant nursery trays measuring 530 mm long x 325 mm wide x 80 mm deep and were lined on the bottom with 3 mm polyethylene oyster mesh and weed mat so as to retain sand. A lid made of 6 mm oyster mesh was fixed using plastic panel clips and cages were tagged to enable easy identification. Cages were fixed to 1.5 m hardwood sticks so that they could be secured to oyster racks and long-lines (Figure 25).

*Manufactured by Tooltech Pty Ltd



Figure 25. Clam cages for shallow water site

On-bottom cages at the deep water site (Boston Bay) were weighted with heavy-walled steel pipe (Figure 26). Mid-water cages were suspended 7 m off the bottom with 250 mm polystyrene buoys, one float for cages with no sand and two floats for cages with sand (Figure 27). Bridles made from stainless steel cable were attached to each corner of the cages, securing the buoys centrally over the top of the cage and ensuring that the trays remained horizontal when suspended. All cages at the deep water site were tethered using 10 mm silver rope.



Figure 26. Weighted cage for on-bottom culture at deep water site

The shallow water site was located within an oyster lease (34°33'39"S, 135°23'01E) in Coffin Bay (Figure 1), lower Eyre Peninsula, SA with a depth of between 0.5 to 2 m over a sand substrate (Figure 28) with current speed up to 15 cm s⁻¹ (Petrusevics 1999). Horizontal timber rails were fixed to posts to hold the mid-water cages, 0.3 m from the substrate, ensuring that the cages remained submerged during low tides. Cages on the bottom were fixed to ropes positioned horizontally at ground level.



Figure 27. Buoyed cages for mid-water culture at deep water site



Figure 28. Oyster racks that clams were fixed to at the shallow water site

The deep water site (34°44'38"S, 135°57'50"E) was within Boston Bay (Figure 1), adjacent Port Lincoln on lower Eyre Peninsula and was 15 m deep. A 50 m horizontal 35 mm rope long-line

was stretched tight along the bottom using three 500 kg anchors and heavy chain (Figure 29) to hold it in position. Cages were attached to this rope with 10mm silver rope.

Prior to positioning the cages at both sites, the underlying substrate was raked by divers to level it and to remove visible fouling organisms.

Sand was collected from the beach at Goolwa, dried, weighed to ensure a uniform volume and bagged so that it could be added to cages under water to achieve a depth of 30mm in the cages. The method of adding sand to cages proved to be the most effective method during preparatory trials. Particle size analysis indicated that it was dominated (59.2%) by particles between 250 μ m and 500 μ m (Table 8).

Size class	Categories	%	
>25µm, < 250µm	very fine sand	15.6	
>250µm, <500µm	fine sand	59.2	
>500µm, <1000µm	medium sand	23.4	
>1000µm	coarse sand	1.8	

Table 8. Particle sizes of sand used in sand substrate cages*

Samples for sediment grain size were analysed at the SARDI Environment and Ecology Laboratory, SARDI Aquatic Sciences Centre. Each of the samples was oven-dried at 90°C, gently homogenised and a 50 g subsample was weighed into a dish. The subsample was then dry sieved through 2 mm and 1 mm sieves with the fraction retained on each sieve weighed to obtain the coarse fractions. The fine fraction (<1 mm) was kept for further analysis using laser diffraction on a Mastersizer 3000 Particle Size Analyser. The samples were stirred in a sonicator with a dispersing agent (50 g L⁻¹ sodium hexametaphosphate in MilliQ water) for 15 minutes before analysis in the Mastersizer.

^{*} Size scale adopted in the GRADISTAT program, modified from Udden (1914) and Wentworth (1922)



Figure 29. Anchors used for securing horizontal 35mm rope

The suitability of sand for *K. rhytiphora* was tested prior to its use in cages. *K. rhytiphora* were held for two weeks in raceways containing sand, with all clams observed to bury in it, with 100% surviving. The sand was the same that wild *D. deltoides* were collected from, so known to be suitable for them. At the deep water site, divers added sand to half of the cages on the bottom and half of the mid-water cages. At the shallow water site, cages were accessed by foot at low tide, allowing sand to be added without diving. In case of possible delays due to cage positioning problems, clams were added one week after deployment of cages at both sites.

D. deltoides were collected from Younghusband Peninsula, SA and held for two days in flowthrough shallow raceways until the day of transfer to cages. On the morning of deployment, clams were counted into numbered containers designated for each cage and air freighted from Adelaide to Port Lincoln. Each specially constructed container consisted of short sections of PVC pipe with fibreglass fly mesh over both ends. The mesh on one end was easy to remove so that divers could insert that end through a small opening in the lid of the cage, 'pouring' the clams in under water, relying on natural movement of the clams to disperse within the cage.

Species	Site	Height	Substrate	Cages
D. deltoides	Deep water (Boston Bay)	bottom	sand	7
	Deep water (Boston Bay)	bottom	no sand	7
	Deep water (Boston Bay)	mid-water	sand	7
	Deep water (Boston Bay)	mid-water	no sand	7
	Shallow water (Coffin Bay)	bottom	sand	7
	Shallow water (Coffin Bay)	bottom	no sand	7
	Shallow water (Coffin Bay)	mid-water	sand	7
	Shallow water (Coffin Bay)	mid-water	no sand	7
K. rhytiphora	Deep water (Boston Bay)	bottom	sand	7
	Deep water (Boston Bay)	bottom	no sand	7
	Deep water (Boston Bay)	mid-water	sand	7
	Deep water (Boston Bay)	mid-water	no sand	7
	Shallow water (Coffin Bay)	bottom	sand	7
	Shallow water (Coffin Bay)	bottom	no sand	7
	Shallow water (Coffin Bay)	mid-water	sand	7
	Shallow water (Coffin Bay)	mid-water	no sand	7

Table 9. List of treatments for cage culture at deep and shallow water sites

The surface area of the bottom of the cage was 0.17 m² and the sand was 30 mm deep. *K. rhytiphora* spat (11.6 mm \pm 1.52 mm long x 9.3 mm \pm 1.43 mm wide; 0.4 g \pm 0.15 g wet weight) were stocked at 175 clams per cage (1030 m⁻²) while *D. deltoides* (20.2 mm \pm 2.68 mm long x 13.7 mm \pm 1.83 mm wide; 1.2 g \pm 0.44 g wet weight) were added at 70 per cage (412 m⁻²). Densities of both species resulted in coverage of 11% of the cage surface area. 11% coverage was chosen as it was thought to be a stocking density below that which would have a significant effect on growth and survival over the trial period and was the same as Paterson and Nell (1997) had previously used so could provide some comparison.

The trial was undertaken over four months, with cages collected at two month intervals. The cages were cleaned in-situ by divers on a monthly basis. Brushes were to remove silt and soft fouling, which could potentially block the mesh and restrict water flow. Although only three cages were to be sampled at each interval, an additional cage was deployed in case any were lost, making seven cages per treatment. When sampled, all clams were removed from the cages for measurements of growth (length, width, depth, whole wet weight, whole dry weight

and dry meat weight) and survival. Temperature was recorded at both sites using Tinytag Plus data loggers (Gemini Data Loggers UK Ltd, West Sussex, UK).

Statistical analyses

All statistical analyses were carried out using SPSS (Version 20, Chicago, USA). A three-factor ANOVA was conducted to assess differences in survival rate, shell length, wet weight and whole dry weight for each species in relation to the fixed factors (duration, substrate and height) respectively. When significant differences were detected, Least Significant Difference (LSD) comparison was used to identify those means that differed (*P*<0.05). All percentage data were arcsine transformed before analysis. Assumptions of homogeneity of variances were checked using Leven's equal variance test.

9.2. Results

During the trial period, the temperature in Coffin Bay ranged from a maximum of 24.7°C in late February to a minimum of 12.6°C in late May. In Boston Bay temperature ranged from 22°C in late February to 15.3°C in late May (Figure 30).



Figure 30. Ambient water temperatures during field work in Boston Bay and Coffin Bay

Survival of *D. deltoides* was low at both trial locations (Figure 31). On average 27% survived to 56 days and 9% survived to 119 days in Coffin Bay. Lower survival was observed in Boston Bay, with 26% surviving to 56 days and <1% surviving to 119 days.

At both the deep water site (Boston Bay) and the shallow water site (Coffin Bay) only *D. deltoides* held in cages containing sand substrate survived to 119 days (Figure 31) although the mid water survivals at Boston Bay were close to zero.

The average size (length) of *D. deltoides* after 56 and 119 days at both the shallow and deep water sites was not significantly different from the size at commencement of the trial (Figure 32).

Within the same substrate type, both growth and survival of *D. deltoides* was not significantly different at different depths at both sites (Figures 31 and 32).

Overall, performance of *D. deltoides* was very poor at both sites and the most significant difference in results was from comparing substrate types, with cages containing sand performing significantly better than those without sand.





Figure 31. *D. deltoides* survival after 56 and 119 days cultivation in Coffin Bay (top) and Boston Bay (bottom) with sand (striped columns) and without sand (solid columns) at different depths (bottom and mid-water). On the same sampling day, different upper case letters of the same colour indicate significant differences (P<0.05) between *D. deltoides* held at differences (P<0.05) between *D. deltoides* held at differences (P<0.05) between *D. deltoides* held with and without sand at the same depth and the same site.





Figure 32. Growth of *D. deltoides* (length) after 56 and 119 days cultivation in Coffin Bay (top) and Boston Bay (bottom) with sand (striped columns) and without sand (solid columns) at different depths (bottom and mid-water). On the same sampling day, different upper case letters of the same colour indicate significant differences (P<0.05) between *D. deltoides* held at different depths with or without sand at the same site. Different lower case letters of the same colour indicate significant differences (P<0.05) between *D. deltoides* held at the same colour indicate significant differences (P<0.05) between *D. deltoides* held with and without sand at the same depth and the same site.

In Coffin Bay, on average 79% of *K. rhytiphora* survived to 56 days and 71% to 119 days (Figure 33) whereas in Boston Bay, only 36% survived to 56 days and 32% survived to 119 days. In Boston Bay, survival of *K. rhytiphora* was highest in cages held on the bottom in sand although this was not statistically significant.





Figure 33. Survival of *K. rhytiphora* (%) after 56 and 119 days cultivation in Coffin Bay (top) and Boston Bay (bottom) with sand (striped columns) and without sand (solid columns) at different depths (bottom and mid-water). On the same sampling day, different upper case letters of the same colour indicate significant differences (*P*<0.05) between *K. rhytiphora* held at different depths with or without sand at the same site. Different lower case letters of the same colour indicate significant differences (*P*<0.05) between *K. rhytiphora* held with and without sand at the same depth and the same site. The absence of letters on the top indicates no significant differences between any of the treatments compared.

Growth of *K. rhytiphora* increased significantly in both Coffin Bay and Boston Bay, with wet weight tripling over 119 days in Coffin Bay (Figure 35). In Coffin Bay, growth (length and wet weight) of *K. rhytiphora* was significantly better for cages held mid-water compared to those on the bottom whereas in Boston Bay, *K. rhytiphora* cultured on the bottom grew better than those held mid-water (Figures 34 and 35).

Statistically, there was no significant difference in the growth (length and wet weight) between

K. rhytiphora held in sand and without sand in Coffin Bay and Boston Bay, except for the length in on-bottom trials in Coffin Bay.





Figure 34. Growth of *K. rhytiphora* (length) after 56 and 119 days cultivation in cages in Coffin Bay (top) and Boston Bay (bottom) with sand (striped columns) and without sand (solid columns) at different depths (bottom and midwater). On the same sampling day, different upper case letters of the same colour indicate significant differences (P<0.05) between *K. rhytiphora* held at different depths with or without sand at the same site. Different lower case letters indicate significant differences (P<0.05) between *K. rhytiphora* held at the same depth and the same site.





Figure 35. *K. rhytiphora* growth (whole wet weight) after 56 and 119 days cultivation in Coffin Bay (top) and Boston Bay (bottom) with sand (striped columns) and without sand (solid columns) at different depths (bottom and mid-water). On the same sampling day, different upper case letters of the same colour indicate significant differences (*P*<0.05) between *K. rhytiphora* held at different depths with or without sand at the same site. Different lower case letters indicate significant differences (*P*<0.05) between *K. rhytiphora* held with and without sand at the same depth and the same site.



Figure 36. Indicative sample of *K. rhytiphora* showing wide growth margin (indicated by arrows) after rapid growth in cages in Coffin Bay

Despite monthly cleaning of the outside of all cages, those held on the bottom in Coffin Bay became fouled with drift algae while mid-water cages in Boston Bay were heavily fouled on the inside of cages. Fouling organisms included ascidians, tube worms, scallops, oysters and sea urchins (Figure 37). Compared to the fouling on the inside of cages in Boston Bay, there was much less fouling on the outside, consisting mainly of silt and some fine algae. Amongst other organisms living within the cages, there were crabs and flat worms (Figure 38) that are known to be predators of bivalves.



A. Fouling inside of a cage held mid-water in Boston Bay (deep water site)



B. Fouling in a cage held mid-water in Boston Bay (deep water site)



C. Less fouling on the outside of cages held on the bottom in Boston Bay (deep water site)



D. Less fouling on the inside of cages held on the bottom in Boston Bay (deep water site)



E. Fouling on the outside of cages held in Coffin Bay (shallow water site)



F. Tubeworm fouling on the outside of cages held in Coffin Bay (shallow water site)

Figure 37. Indicative fouling on representative clam culture cages in Boston Bay and Coffin Bay



A. Crab (Nectocarcinus integrifons)



B. Flatworm (family Stylochidae)

Figure 38. Examples of organisms living within the cages.

10. DISCUSSION

In summary, seven steps were originally designed in this project to investigate the aguaculture potential of SA clam species (Table 10). It was anticipated that after the completion of the literature review, the workshop on clam aquaculture, and the preliminary field trials to compare the performances between Pipi and Vongole (the first three steps), only one species would be selected for the subsequent trials (Steps 4, 5 and 6). However, the preliminary field trials at Step 3 were delayed substantially, mainly due to the non-availability of wild Vongole spat and the subsequent need for hatchery production of this species, taking approximately 9 months to complete. In an effort to avoid delaying Steps 4, 5 and 6, both Pipi and Vongole were included in the hatchery (Step 4) and key environmental parameter trials (Step 5) so that these could be achieved on time, doubling the amount of work. In Step 6 (see Table 10) the project aimed to undertake a wider range of field-based research to further optimise farming methods of the preferred species, including the evaluation of stocking density, growth and survival at different environmental conditions/locations. It was anticipated that at least one suitable location would be used for pilot scale trials and if successful, commercial development would follow after completion of the project. However, due to the unforeseen time needed for the relevant government agencies to assess site applications, non-availability of wild Vongole spat and lack of resources in the original budget for production of distinct batches of spat for each site (a regulatory requirement only identified during the project), the full scope of proposed work could not be completed within the time frame of the project.

Steps	Original Plan	Activities and/or Requirements	Variations	Reasons	Delivery of Original Plan
1	Literature review	Desktop study			Achieved
2	Workshop on clam aquaculture and research	Presentations			Achieved
3	Preliminary field trial to identify a species and/or environmental parameters to be evaluated in the subsequent steps	 Two species; Wild spat; Shallow and deep water localities; Three water depths 	Use of hatchery reared Vongole spat, which took at least 9 months to produce	 Unavailable of wild Vongole spat 	Achieved with substantial delay and additional work
4	Development of hatchery technique	 One of the two species selected in Step 3 	Two species	 Requirement of hatchery produced Vongole spat in Step 3; Substantial delay in the completion of Step 3 	Achieved with doubled amount of work
5	Laboratory trials to determine tolerant ranges of key environmental parameters in a selected species to help identify suitable field sites	 One of the two species selected in Step 3; Three parameters (temperature, water flow rate and substrate) 	Two species	 Due to the substantial delay in the completion of Step 3 	Achieved with doubled amount of work
6	Pilot field trials to identify likely commercial culture methods and site characteristics	• Further evaluate the species, culture methods and parameters optimised in the previous laboratory and field trials	Omitted	 An anticipated lengthy lease application process; Requirement of locally derived spat at each experimental region due to current policy, which 	No

Table 10. Summary of project original plan, variations and deliveries

			will need additional time and cost	
7	Communications	 Literature review; Workshop; Milestone and final reports; Emails, phone calls, and face to face discussions; Hatchery manual 		Achieved

10.1. Pipi (*D. deltoides*)

Hatchery production must be achieved reliably before the development of a *D. deltoides* culture industry. Although spawning and larval rearing through metamorphosis have been successful with this species, out of season broodstock conditioning was not, and this is usually necessary to achieve sustained high levels of production over an extended period in a commercial hatchery. Furthermore, determination of suitable post-settlement culture techniques was not achieved within this project. Newly metamorphosed spat were observed to be continuously attempting to bury within the substrate but despite trialling a range of substrates, all died before reaching 1 mm in length. O'Connor and O'Connor (2011) found the same problem in hatchery trials undertaken with this species in NSW.

Improved understanding of the natural settlement conditions for *D. deltoides* is needed in order to develop suitable artificial systems to enhance the likelihood of post settlement survival. It is likely that *D. deltoides* settles on beaches considering that 2 to 3 mm juveniles are found there, but the presence of spat below this size has not been verified. Laudien et al. (2001) found that the incidence of juvenile surf clam *Donax serra* on beaches in Namibia was not predictable in relation to spawning times suggesting the possibility of settlement in other areas. Donn (1987) reported that juvenile *Donax serra* of 2 to 5 mm were most abundant close to a river mouth in South Africa but declined over distances up to 10 km from it, suggesting possible settlement near river mouths and later migration along the beach. If they do settle on beaches, they may bury deep into the substrate for protection and consume a diet of organisms within it including benthic algae and bacteria. The Baltic clam (*Macoma balthica*) has been found to be able to adapt from consuming benthic particles as a juvenile to a diet higher in suspended particles as an adult (Rossi et al. 2011).

Considering their 2+ week larval period in the sea, *D. deltoides* larvae would drift and settle in a range of conditions similar to other bivalves, including Manila clams (Hamaguchi and Tezuka 2007), and may move towards the beach as juveniles. The New Zealand Pipi *Paphies australis* has been found to drift in the water column, mainly as juveniles (<15 mm) but also as adults (Hooker 1995). Wild 5 mm *D. deltoides* have been observed to 'glide' in water currents by fully extending their foot and syphons (Gluis, unpublished data). Also, in the aforementioned project, 5 mm *D. deltoides* were successfully held in upwellers for 6 weeks with minimal mortality (11%)

and reasonable growth (17%). After completion of that project, spat were held in upwellers for a further 6 months, indicating that it is possible to hold this species in captivity beyond settlement. Nevertheless, the limiting factors to the successful development of a *D. deltoides* culture industry are;

- 1. Inability to condition broodstock out of season.
- 2. Lack of an effective nursery system including suitable substrate for post-settled spat.
- 3. Intolerance of low water flow.
- 4. Intolerance of elevated temperatures naturally found in shallow bays currently used for shellfish aquaculture in SA.

The low growth and survival in field and laboratory trials suggest that *D. deltoides* is unsuitable for culture using the systems and locations trialled in this study. Considering the natural high energy environment where they occur, it is likely that *D. deltoides* is intolerant of relatively low water flow and high summer water temperatures experienced in the protected bays where shellfish aquaculture is currently undertaken in SA. Although probably not insurmountable, the difficulty of *D. deltoides* hatchery culture is also currently an impediment to commercial production.

In laboratory trials, *D. deltoides* growth was highest at 23°C but survival decreased with increasing temperature over the three temperatures used (18°, 23° and 28°C). Survival in field trials was poor, and may have been affected by water temperatures reaching 22°C in Boston Bay and 24°C in Coffin Bay, although mortality was also high in the last two months of the trial when water temperatures were ≤ 20 °C at both sites. Laboratory trials also indicated that *D. deltoides* prefer high water movement. Murray-Jones and Johnson (2003) reported that mass mortalities of *D. deltoides* had been observed during periods of high temperature combined with low water movement. The combination of high water temperature and low flow during tidal cycles at the trial sites, exacerbated by fouling, may have contributed to the high mortality.

10.2. Vongole (*K. rhytiphora*)

Considering the high rates of growth and survival, successful hatchery and nursery culture and good market acceptance, there appears to be potential for commercial culture of *K. rhytiphora*,

Results from hatchery trials indicate that hatchery rearing of *K. rhytiphora* is relatively easily achieved compared to *D. deltoides*, with high survival of larvae and spat. In previous work by the author, *K. scalarina* was also able to be cultured successfully to settlement. Reliable hatchery production is an important consideration in the establishment of an aquaculture species that will need to rely on hatchery reared stock to reach a high level of production. Wild stock in SA is currently harvested by fishers and is unavailable for stocking of clam farms.

Growth of *K. rhytiphora* in field trials was greater than those previously recorded for the two other species of Vongole, *K. scalarina* and *K. peronii* in SA (Cantin 2010) and Tasmania (Riley et al. 2005) and was similar to rates previously recorded for this species in NSW (Paterson and Nell 1997). The growth rate in Coffin Bay from February to June 2012 averaged 2.1 mm month⁻¹ which is at least 17 times faster than the estimates for other Vongole species (*K. peronii* and *K. scalarina*) of 0.12 and 0.05 mm month⁻¹, respectively (Cantin 2010).

Overall, survival rates of *K. rhytiphora* (74% in Coffin Bay and 33% in Boston Bay) over the relatively short term of this study were lower than the survival rates of other cultured shellfish such as oysters (Li 2008). However, considering the sub-optimal conditions, including heavy fouling of cages and the use of a deep water site, it is likely that these figures could be improved by development of better culture systems and operating methods. The survival rates in Coffin Bay were similar to those (72% and 75%) previously reported over four months with the same species by Paterson and Nell (1997) in NSW.

In Coffin Bay, there was not a large difference in survival averaged over all treatments between days 56 and 119 (79% and 71% respectively), suggesting that most mortality occurred during the first 56 days, possibly due to the effect of initial translocation or high summer temperatures, although further work would be needed to verify these suggestions.

Although the growth rate of *K. rhytiphora* in cages at field sites was relatively high during months where water temperature averaged 19°C in Boston Bay (range 15.3°C to 22°C) and 18.1°C in Coffin Bay (range 12.6°C to 24.7°C), it is likely to be less during colder months. Paterson and Nell (1997) found that growth of *K. rhytiphora* was greatest during late summer

and early spring, declining towards winter, a trend also likely in SA. Additionally, as the clams used in these experiments were immature, growth is likely to decline with the onset of maturity when energy is diverted to gonad production, as has been shown to occur with other species including Manila clams, *Ruditapes philippinarum* (Kanazawa and Sato 2007) and smooth clams *Callista chione* (Moura et al. 2009).

In a 2009 survey, Gorman et al. (2010) found that maturity occurred at 31 mm (L_{50}) in *K. rhytiphora* and 26.1 mm (L_{50}) in *K. scalarina* in Coffin Bay. However, Edwards (1999) found that the length at first maturity in the Port River populations was 24 to 28 mm (L_{50}) for the three species present (*K. rhytiphora, K. scalarina* and *K. peronii*), indicating that size at first maturity differs according to location. This is likely due to environmental influences considering that other bivalves such as Pacific Oysters (*Crassostrea gigas*) and Native oysters (*Ostrea angasi*) are known to grow rapidly in Coffin Bay, but genetics may also be a contributing factor. There is a need to better understand the growth rate of *K. rhytiphora* over the entire size range. Further work is also needed to determine the most cost-effective harvest size. Clam marketers suggest that there is demand for small clams under the size at first maturation. If growth is rapid to that size it may be more cost-effective to harvest prior to maturation rather than holding them longer if subsequent growth rate is lower.

Fouling was a problem at both Boston Bay and Coffin Bay despite monthly cleaning of the outside of all cages. While there was a considerable amount of fouling on the inside of cages in Boston Bay, there was much less on the outside, consisting mainly of fine silt and algae (Figure 37A and 37C). This was possibly due to (i) monthly cleaning of the outside surfaces and (ii) protection from predation of fouling organisms on the inside of the cages. Those held on the bottom in Coffin Bay became fouled with drift algae while mid-water cages in Boston Bay were heavily fouled on the inside of cages by non-photosynthetic organisms including ascidians, tube worms, scallops, oysters and sea urchins (Figure 37A and 37B). There was less fouling on cages held on the bottom in Boston Bay compared to those held mid-water (Figure 37C and 37D).

Although not quantified, siltation appeared higher on cages held on the bottom and this may have had an effect on settlement of fouling organisms. After four months, mid-water cages had approximately 8 times the volume of fouling compared to those held on the bottom. Typically, fouling decreases with depth (Head et al. 2004), however a study by Cronin et al. (1999) on fish

cage fouling undertaken within Boston Bay, only 2 km away from the deep water site used in this clam study, found that non-photosynthetic fouling didn't change with depth to 12 m.

Fouling can cause reduced growth through competition for food particles and reduction of water flow. It is usually worse on off-bottom structures and can effect survival (Adams et al. 2011). Heavy silting and fouling of cages are likely to have been major contributing factors for the slower growth rate of *K. rhytiphora* in Boston Bay than Coffin Bay. Ascidians were one of the dominant fouling organisms found in clam cages trialled in Boston Bay and are known to be one of the most common and devastating biofoulers in the aquaculture industry (Adams et al. 2011).

Although not as serious as in Boston Bay, fouling was also a problem in Coffin Bay. Fouling in Coffin Bay not only consisted of silt, tubeworms and attached algae, but also drift algae that became caught on the outside of cages, particularly on those held on the substrate. The source of this fouling seemed to be from the array of algal growth and seagrass on the substrate surrounding the oyster racks where the trial was undertaken.

Oyster growers commonly position their stock within the intertidal zone to reduce fouling. Similarly, clam farmers overseas often plant clams in an area that is exposed briefly at low tide, reducing fouling on predator exclusion nets and enabling relative ease of maintenance and harvesting of clams (Manzi 1985). Observations while collecting broodstock suggest that *K. rhytiphora* may naturally grow in slightly deeper water than *K. scalarina*. If the optimum depth of *K. rhytiphora* is entirely subtidal, there is likely to be a high incidence of fouling on nets or cages. Work is needed to identify the optimum depth of these species and which is more suitable for culture at a depth that doesn't encourage fouling. Regardless, predator exclusion nets would need to be changed when fouled, but the incidence may be reduced when positioned within the intertidal range to allow exposure to air and sun to prevent most algae from growing. Exposure can also be an impediment to parasites and predators (Hadley et al. 1997).

Overall, field and laboratory trials indicated that for both species, clam growth and survival was generally higher when held in sand compared to without sand. In their natural environment, living within the substrate offers clams: (i) secure positioning against the influences of currents and waves; (ii) protection from predators; and (iii) a more thermally moderate environment (Pariseau 2007). Living within the substrate also prevents attachment of fouling organisms due to the lack of light, and sand abrasion would also undoubtedly clean the shell. Most commonly, cultured clams are held in the substrate and protected by predator exclusion nets (Ayers 2006).

However, at the time of writing, PIRSA have not developed policy that allows for in-substrate farming of clams in SA.

Considering the time to reach a size suitable for introduction to field sites, it is likely that the price of *K. rhytiphora* spat would be similar to oysters, which currently is approx \$20 per 1000 for 6 mm spat, retained on a 3.5 mm grading screen. Manila clam spat, grown on the west coast of the USA are generally less expensive than oysters, whereas the spat prices of hard clams and oysters on the east coast of the USA are similar. The difference seems to be that Manila clams are relatively easy to culture, fast growing and the spat market is much larger compared to the east coast.

One dozen oysters weigh approximately 1 kg, whereas the number of clams per kg will range between 40 and 120 (average 80 per kg). Taking into account these estimates on weight and price and ignoring mortality, the spat price per kg of oysters is \$0.24 compared to \$1.60 for clams, which is a comparatively large portion of the likely cost of production. However, depending on which clam farming system is used, the cost of infrastructure can be considerably less than oysters. Clearly, mortality levels would need to be minimal or the seed price per animal could be even higher.

Because clams grow within the sediment and with a uniform shape, they do not need to be brought ashore for rumbling, grading and removal of fouling as is done with Pacific Oysters and can stay at the same location from spat planting until harvest of market-sized product if the simple method described below is adopted in Australia. They will grow at different rates but can be graded at harvest and either sold as different size grades as with hard clams in the USA (topnecks, littlenecks, cherrystone and chowder) or returned to the farm for later sale. Other than the price of spat, establishment costs are likely to be far less than other shellfish species including oysters and mussels. A simple and relatively inexpensive method of clam containment and predator exclusion consists of a single layer of plastic mesh over the top of clam beds (Ayers 2006). Maintenance costs will include replacing predator nets when fouled or damaged and the frequency of this would depend on conditions at the site.

10.3. Other Species

Although this project concentrated on the Pipi *D. deltoides* and a species of Vongole, *Katelysia rhytiphora*, there are many other native clam species found in Australian waters and some of these may prove to be suitable for culture providing they are a high quality product and have a good market price, both are essential for Australian aquaculture due to comparatively high production costs compared to other countries. Other southern Australian clams that grow to a suitable size include *Katelysia scalarina*, *Katelysia peronii* (both currently harvested commercially), *Venerupis galactites, Circe rivularis, Bassina pachyphylia,* and *Dosinia diana.* It should be noted that there is no known information on the suitability of these for culture other than for *K. scalarina*, which has been found to grow slowly in Tasmania (Maguire 2005), and wild populations of both *K. scalarina* and *K. peronii* have also been found to grow slowly in SA (Cantin 2010).

11. BENEFITS AND ADOPTION

Favourable results have encouraged the private investor in this project (SACA) to form a partnership with an oyster farming leaseholder, and apply for inclusion of *K. rhytiphora* on that lease with a view to developing commercial culture of this species. Ongoing discussions between SACA, PIRSA and other interested parties are aimed at developing policy that will enable commercial clam farming to commence in SA. PIRSA are currently collating a range of information that will assist in policy development including outputs from this FRDC 2009/208 project, another FRDC funded 2010/233 project looking at genetic variation of *K. rhytiphora*, an assessment of likely clam farming methods and potential environmental impacts.

Upon development of clam culture policy by PIRSA, it is anticipated that approval of at least one site will occur. Upon the successful development of that farm, it is likely that there will be interest from a range of parties and further development of sites. The key beneficiaries targeted by this project are new clam growers. It is expected that development of this industry will occur in parallel with domestic and international market development over and above current demand so that prices can be maintained. The establishment of a successful clam culture industry that mostly targets an international market has potential to provide significant financial return to Australian producers and ancillary businesses from the transport, processing and marketing sectors.

The development of a clam industry reliant upon cultured spat presents an opportunity for a commercial hatchery(s) to diversify into clam production although the market will remain very small until clam farming is more widespread. Until commercial hatcheries become involved, research hatcheries such as the one at SARDI would be able to provide stock to support expansion of commercial clam aquaculture and continued relevant research.

In SA, areas that are likely to benefit include the Yorke and Eyre Peninsulas. There is also a high probability that clam culture will be undertaken in other Australian States upon commencement of the industry in SA.

12. FURTHER DEVELOPMENT

High priorities for further work identified to assist commercial development of clam culture in SA include:

- Identification and procurement of suitable farm sites.
- Assessment of growth over the full range of sizes from spat to market and determination of the most cost-effective harvest size.
- Identification of optimum culture depth, stocking density, biofouling minimisation and predator exclusion methods.

At the same time the following matters should also be addressed:

- Government policy development on clam farming in South Australia.
- Uptake of technology by commercial hatcheries to enable supply of seed stock.

13. PLANNED OUTCOMES

The overall aim of the project was to provide private companies with sufficient information to demonstrate the potential of clam farming and use this information to proceed with the development of a new Australian clam culture industry. Although the extent of field work was less than originally planned, results from hatchery, laboratory and field work have been sufficiently promising for private enterprise to continue the momentum of the project via on-going efforts to gain farm sites through forming alliances with existing shellfish farmers and by working with PIRSA to develop a clam culture policy.

A likely species, *K. rhytiphora* has been identified for culture and another, *D. deltoides* eliminated from current consideration, although future work should also look at other species not studied within this project.

A key outcome of this project is the successful hatchery production of *K. rhytiphora* spat, which is essential for undertaking further work and development of a commercial scale industry. Hatchery production of bivalve shellfish has inherent challenges with maintaining water quality, larval and algal cultures and minimising bacterial contamination. However, production of *K. rhytiphora* was readily achieved in the SARDI research hatchery and has a high chance of being successfully produced in a commercial hatchery.

A hatchery manual has been published as a standalone report (Gluis and Li 2014). A literature review and workshop notes have also been produced and are included as appendices to this report.
14. CONCLUSION

The results from this project through literature reviews, and laboratory and field experiments show that:

- D. deltoides is not suitable for culture in SA using the systems and locations currently available as this species exhibited poor growth and survival in laboratory and field trials and although they were reared through metamorphosis in the hatchery, post-settlement survival was poor. Other suitable equipment and techniques may become available in the future but considering its natural occurrence in high energy environments, it is likely that the species may be intolerant of lower water flow and high summer water temperatures experienced in shallow bays in SA where shellfish aquaculture has been established and can most easily expand.
- *K. rhytiphora* seems to have potential as a suitable species for culture in SA considering its reasonable rates of growth and survival, successful hatchery culture and high market acceptance. Growth of *K. rhytiphora* in field trials was much greater than those previously recorded for wild stock of the two other species of Vongole, *K. scalarina* and *K. peronii.*
- Favourable results have encouraged the private investor in this project to form a partnership with an oyster farming leaseholder, and an application has been made to PIRSA for inclusion of *K. rhytiphora* on that lease with a view to undertaking more research before commercial culture commences.

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A1. INTRODUCTION

Worldwide, there has been a steady increase in clam farming over the past 20 years, with aquaculture production increasing four-fold in the 10 years from 1989 (Gosling 2003). In six years from 2004 to 2010, clam culture has increased from 3.6 to 4.9 million tonnes, accounting for 86% of world clam production. In 2010 the worldwide value of cultured clams was US\$4.7 billion which was greater than the value of oysters (US\$3.6 billion) in the same year (FAO 2010).

Clam aquaculture is well developed in many countries overseas including China, USA, Canada, England, Spain, France, Italy, Mexico and Vietnam. In 2010, worldwide aquaculture production was 4.9 million tonnes, valued at US\$4.7 billion. In 2010, worldwide Manila clam (*Ruditapes philippinarum*) production was 3.6 million tonnes valued at \$3.4 billion, and was the fourth greatest volume behind grass, silver, and Indian carp, and was the greatest of any marine/estuarine species. The volume and value of clam production was much higher than Pacific Oysters (0.7 million tonnes, \$1.3 billion).

In 1999, as a proportion of the world's clam production, Manila clams (*Tapes philippinarum*) comprised 67% of world clam production while hard clams (*Mercenaria mercenaria*) comprised 29%. Other taxa include *Anadara granosa*, *Arca* sp. and razor clams (Gosling 2003).

Interest in Australian clam culture precedes this report, with research having been undertaken since 1991 although there has been no commercial uptake of clam farming. Steadily increasing demand of clams as a food item in domestic and international markets, coinciding with a reduction of wild catch from New South Wales (NSW) and South Australia (SA) has resulted in increasing prices for Australian clams. This presents an opportunity for clam culture as a potential method to bridge the gap between supply and demand.

This report reviews published literature on the biology of southern Australian clams, in particular, species of Vongole (*Kataleysia* spp.) and Pipi (*Donax deltoides*). Also included is a review of clam culture experiences within Australia and overseas with a summary on the main issues concerning the potential establishment of clam culture in SA.

A2. BIOLOGY OF SOUTHERN AUSTRALIAN COMMERCIAL SPECIES

A2.1. Vongole (Katelysia spp.)



Figure A1. A species of Vongole, K. rhytiphora.

A2.1.1. Nomenclature

There has been some confusion regarding the number of species represented within the genus *Katelysia*. In 1857 Edward Romer described four Australian species, *Venus corrugate, V. scalarina, V. peronii* and *V. strigosa* (Smith 1974). Pritchard and Gatliff in 1903 named three species, *Chione strigosa,* C. *scalarina* and C. *peronii* (Smith 1974). Cotton (1934) identified only two species (*K. peronii* and *K. corrugata*). In 1935 Lamy proposed that *V. corrugata* be named *Katelysia rhytiphora* (Smith 1974) and in 1938 Cotton and Godfrey named them *K. scalarina, K. peronii*, and *K. corrugata*, disregarding *C. strigosa* and *K. rhytiphora* (Smith 1974). Subsequently, there was general agreement on the classification of *K. scalarina* and *K. peronii* but confusion continued to exist around the identification of *K. corrugata, K. strigosa* and *K. rhytiphora*. Smith concluded that there were three species in southern Australia, *K. scalarina, K. peronii* and *K. corrugata*. The latter has since been clarified as *K. rhytiphora* and the name remains in use.

Edwards (1999a) described a method to identify the three species, with *K. rhytiphora* being distinguishable under 10 x magnification by fine lines radiating out from the umbo. *K. scalarina's* concentric rings are sharper, there are no radial lines and the interior of the shell does not have yellow patches. *K. peronii* has no radial line and is usually a smaller rounder shape and has flat concentric ridges.

A2.1.2. Distribution

Katelysia rhytiphora and *K. scalarina* are naturally distributed in temperate waters around the southern Australian coast from Augusta in Western Australia (WA) to Port Jackson in NSW (Gorman et al. 2010) while *K. peronii* inhabits waters from WA to Victoria but does not extend into NSW (Soh et al. 1998). The three species occupy a similar habitat, often occurring at the same locations within the intertidal zone and the top 5 cm of the substrate (Edwards 1999a).

In SA *K. scalarina* was found to be the most abundant *Katelysia* species at the Section Bank, Port River, Adelaide (Edwards 1999a) and *K. rhytiphora* was found to be the most abundant *Katelysia* species at Coffin Bay, Eyre Peninsula (Gorman et al. 2010).

A2.1.3. Growth

Estimates of growth rate of *Katelysia* clams vary, with estimates of four to six years for *K. scalarina* to reach approximately 30 mm shell length (maximum distance between dorsal and ventral margins of shell) in Tasmania and SA (Riley et al. 2005; Cantin 2010). However, in trials in NSW, *K. rhytiphora* grew to 15.4 mm in 10 months in sediment trays on an oyster lease and after a further 4 months of in-sediment culture grew to 25.7 mm. It was expected that *K. rhytiphora* would reach 38 mm in 3 years (Paterson and Nell 1997).

A2.1.4. Maturation

Edwards (1999a) found that all *K. rhytiphora* and *K. scalarina* less than 20 mm in shell length were immature in the Port River SA, while all *K. peronii* less than 22 mm were immature. The length at first maturity (L_{50}) of the Port River populations was 24.0 to 27.5 mm for the three species present (Edwards 1999a). The L_{50} of the Coffin Bay *K. scalarina* and *K. rhytiphora* populations was 26.1 and 31.1 mm, respectively (Gorman et al. 2010).

Edwards (1999a) found that;

• 62.5% of K. peronii were mature in the size class of 25 mm to 27.5 mm

- 87.5% of K. rhytiphora were mature in the size class of 25 mm to 27.5 mm
- 68.8% of K. scalarina were mature in the size class of 24 mm to 26 mm

The onset of sexual maturity is likely to result in a decline in growth rate as the animal puts more energy into gonad development (Sato 1994).

Smith (1974) reported that spawning of *Katelysia* clams in temperate Australia appears to commence in September and October with a possible secondary spawning in March. Edwards (1999a) also reported animals in spawning condition from September to March at the Section Bank, Port River, SA. In 2009 at Coffin Bay, Gorman et al. (2010) found that for 7 months of the year, >80% of sampled *K. rhytiphora* were in reproductive condition, while >80% of *K. scalarina* were in reproductive condition for 8 months (Figure A2).



Figure A2. Temporal variation in the reproductive condition of *K. rhytiphora* and *K. scalarina*, measured as mean gonad index. Data includes all collected individuals greater than the size of first maturity (i.e. \geq 32 mm SL and \geq 27 mm SL, respectively). Figure reproduced with permission from Gorman et al. (2010).

A2.1.5. South Australian Fishery

In South Australia, the fishery commenced in Coffin Bay in 1985/86 and currently occurs in the Port River, Coffin Bay, Venus Bay, Streaky Bay and Smoky Bay, with the latter three bays

comprising the West Coast Zone (Gorman et al. 2010). Previously, fishing has also occurred at the Bay of Shoals and American River on Kangaroo Island (Edwards 1999b).

Since an export market was established from Melbourne in 1986, higher prices were realised and there has been increased interest in Vongole as a food item. This has resulted in new markets and interest in the product and hence more effort going into the fishery (Edwards 1999a).

In 1999, the most significant fishery was at Section Bank, Port River comprising 97% of the total SA catch (Edwards 1999a), although the largest catch currently comes from Coffin Bay (Gorman et al. 2010).

Prior to commencement of the 2008/09 season, a quota system was introduced and the total allowable commercial catch (TACC) currently stands at 22.6, 56 and 15 tonnes in the Port River, Coffin Bay and West Coast zones respectively (Gorman et al. 2010).

At Coffin Bay in 1989, densities were up to around 900 clams m⁻² (Edwards 1999a). Length frequency at three sites in Coffin Bay had a mode of 35-40 mm in length. Estimates of L_{50} were 35, 39 and 40 mm respectively at the three sites studied. Upon this information, the legal size for Vongole harvested from Coffin Bay was increased from 30 to 38 mm in 1990 (Edwards 1999a).

A2.2. Pipi (Donax deltoides)



A2.2.1. Distribution and Habitat

Known as Pipi on the east coast of Australia and Goolwa cockles in SA, *Donax deltoides* naturally occur between southern Queensland (Qld) to Point Sinclair on the west coast of SA (from South Australian Museum samples). Previous work by Murray-Jones (1997) showed that there was a high level of gene flow in east coast *D. deltoides* sampled at various locations between Fraser Island, Qld to south of Batemans Bay, NSW, a distance of over 1200 km, although similar studies are yet to be undertaken in SA.

D. deltoides occurs on high energy wave exposed beaches around the lower intertidal zone although they are highly mobile, migrating up and down the beach depending upon tide and weather conditions. Highest abundance is just below the low tide level (Murray-Jones and Johnson 2003). *D. deltoides* natural occurrence within the swash zone means that they live within a naturally highly oxygenated environment and are reported to experience high levels of mortality when oxygen levels decline below 5.0 mg L⁻¹ at 17°C (Murray-Jones and Johnson 2003).

A2.2.2. Maturation

D. deltoides matures at approximately 13 months of age at a length of approximately 36 mm (King 1976). It is thought that the fishery is based mainly on 1 and 2 year old animals (Murray-Jones and Johnson 2003).

Based on oocyte size and number Murray-Jones (1999) concluded that reproductive activity occurs throughout the year in NSW. King (1976) reported that spawning commenced in October 1973 and September 1974 and continued for approximately six months at Goolwa, SA. Observations at Goolwa suggest that a single complete spawning occurred in October 2009 and subsequent monitoring until August 2010 revealed slow and incomplete gonad development, suggesting the possibility of a single distinct annual spawning event in that year (Gluis, unpublished data). Ferguson et al. (2013) found a high level of gonad development in late September followed by a spawning event in October and redevelopment and mass spawning in November of the same year (Figure A4).



Figure A4. Intra-annual trends in gonad development of *D. deltoides* on Younghusband Peninsula, SA in 2010. Figure reproduced with permission from Ferguson et al. (2013). See Table A3 for description of gonad stages.

Table A1. Criteria used to determine gonad development (after Edwards 1999a; Gorman et al. 2010).

Gonad Stage	Gonad Condition				
1	No gonad material visible (Includes immature individuals)				
	Poorly developed				
2	A small amount of gonad material is evident on margins of the viscera				
	Digestive gland completely uncovered upon external observation				
	Moderately developed				
3	Gonad material does not cover an extended area				
	A proportion of the digestive gland is still visible upon external observation				
	Well developed				
	Gonad material covers large area extending in to the foot				
4	Digestive gland completely covered upon external observation				
	Gonad material 'oozes' out when the body wall is broken				
	Gonad appears 'grainy'; and white to cream in colour				
	Fully developed				
	Digestive gland completely covered upon external observation				
5	Gonad material covers large area extending in to the apex of the foot				
	Very tightly packed and body wall hard to touch				
	Gonad appears 'grainy' throughout; white to cream in colour				

A2.2.3. South Australian Fishery

The South Australian fishery operates on the beaches of the Younghusband Peninsula, south east of the mouth of the River Murray. Although commercial fishing has occurred along the beach at Goolwa this area is now mainly used by recreational fishers. Commercial fishers use rakes to collect *D. deltoides* (Figure A5) and are not permitted to use mechanical harvesting techniques in order to minimise damage to the ecology of the area and undersized *D. deltoides*.



Figure A5. D. deltoides harvesting in SA.

To promote sustainability of the resource, Primary Industries and Regions SA (PIRSA) together with the licensed fishers, manage the fishery by imposing closure periods and catch quota based upon stock assessments conducted by the South Australian Research and Development Institute (SARDI) (Figure A6). In recent years the total allowable catch (TAC) of the Pipi in SA was reduced from 1150 tonnes to 330 tonnes and is currently 400 tonnes per annum.

In the 2009/10 season 28% of the South Australain *D. deltoides* harvest was sold for human consumption (L. Triantafillos, unpublished data) and this is expected to increase in coming years. The South Australian Shellfish Quality Assurance Program (SASQAP) monitors waters from which *D. deltoides* are harvested to ensure their safety as a food item.



Figure A6. Total annual catch of *D. deltoides* in SA from 1984 to 2009. LCF: Lakes and Coorong Fishery; MSF: Marine Scale Fishery; Rec: Recreational Fishery. Figure reproduced with permission from Ferguson (2010).

A2.2.4. New South Wales Fishery

The NSW *D. deltoides* catch increased from below 100 tonnes in the 1984/85 financial year to over 600 tonnes in 2000/01 (Figure A7). The majority of the catch comes from the central and mid-north coasts of NSW between Tuggerah to Crowdy Head (Phelps et al. 2008). Figures reported by the Newcastle Fishermans Cooperative indicate that catch has been reducing in this area with 145, 127 and 39 tonnes landed in years 2005, 2006 and 2007 respectively (Phelps et al. 2008).



Figure A7. Commercial landings of *D. deltoides* for NSW from 1984/85 to 2006/07 (reproduced from Scandol et al. 2008)

A3. AQUACULTURE

A3.1. Hatchery Culture

The ability to rear commercial quantities of clams in a hatchery is a major contributing factor towards development of a successful clam culture industry (Castagna and Manzi 1989). European farms are largely dependent on hatchery reared seed (Gosling 2003) and although the United States of America (USA) industry has previously used natural spatfall, since the recent rapid expansion of clam farming on both the east and west coasts, it is also almost totally reliant on hatcheries. There are also clam hatcheries in all other major cultured clam producing countries including Canada, China, France, Ireland, Italy, Spain and the United Kingdom (UK) (Gosling 2003).

Establishment of hatchery production techniques including algal culture and water purification have been critical in the development of hatcheries and the expansion of the clam culture industry (Castagna and Manzi 1989).

Commercial hatchery production of clams has occurred since 1956 when Richard Kelly started a hatchery in Virginia, USA, based upon methods established by Victor Loosanoff and colleagues at the USA Fish and Wildlife Service, in Milford, Connecticut (Manzi and Castagna 1989).

There are five main processes in the hatchery rearing of clams;

- 1. Broodstock conditioning
- 2. Spawning
- 3. Larval rearing
- 4. Post-set culture
- 5. Food production

The ability to condition broodstock out of season allows for increased larval production and better use of production facilities. Spat that have been produced early are able to make full use of optimum natural growing conditions once transferred to field sites, all contributing to higher production. Broodstock clams can be conditioned to spawn outside of their natural spawning period by manipulating water temperature and feeding regime (Matias et al. 2009). However, the response to various conditioning treatments varies greatly between species (Matias et al. 2009). In laboratory based experiments, *Donax deltoides* has proven difficult to keep alive long

enough to condition out of season (Gluis, unpublished data). Its survival was improved when held in sand but more research needs to be undertaken on this aspect.

Kent et al. (1998) successfully conditioned *Katelysia scalarina* in Tasmania in August, December and January, although the latter two months are witjin their natural spawning period. The conditioning process is also greatly affected by the time at which the broodstock is collected (Matias et al. 2009) with the most difficult period being immediately after spawning when energy reserves are low. It is very difficult to condition clams that have recently spawned and so for spawning early in the season it may be more effective to maintain unspawned animals for long periods in cooler water (Hadley et al. 1997).

Hard clams are induced to spawn by alternately raising and lowering cooling water temperature (Hadley et al. 1997). Gametes may be excised from sacrificed animals (usually males) and added to water in spawning tanks to help stimulate spawning.

In Australia the following clams have been spawned experimentally *Katelysia rhytiphora* (Nell et al. 1994), *K. scalarina* (Kent et al. 1998), *Anadara trapezia* (Nell et al. 1994), *Tapes dorsatus* (Nell et al. 1995), *Ruditapes largillierti* (Kent et al. 1999) and *Donax deltoides* (W. O'Connor, pers. comm.).

In NSW Nell et al. (1994) was able to spawn *K. rhytiphora* within its natural spawning period by raising the water temperature from 19°C to 22°C over 2 hours. Between 0.2 and 2.7 million eggs were produced per female and larvae were placed in settlement downwellers on day 12 at an average size of 198 µm. In Tasmania, Kent et al. (1999) spawned *Ruditapes largillierti* by increasing water temperature from 13°C to 16°C.

Although there are several commercial bivalve hatcheries in Australia that are capable of producing commercial quantities of clams, they are unlikely to do so until there is sufficient interest in the development of a large scale clam culture industry and the associated need for spat. Bacterial problems also remain a problem in larval rearing and post-set culture. In many case bacterial infection is secondary, due to other sub-optimum contributing factors (Jones 2006).

A3.2. Triploidy

Typically, the growth rate of bivalves reduces upon maturation when energy, previously used for growth is devoted to reproduction (Baron et al. 2004). This is likely to occur in *Katelysia rhytiphora* and *Donax deltoides* that have both been reported to have relatively fast growth prior

to maturity (Paterson and Nell 1997; Murray-Jones and Johnson 2003). Triploid clams do not produce gonad and may be a viable method to maintain a faster growth rate through the age where maturity would normally occur.

In Australia, triploidy rates of 56% to 85% of *Tapes dorsatus* pediveligers were achieved using Cytochalasin-B (Nell et al. 1995). EI-Wazzan and Scarpa (2009) achieved up to 94% triploidy of hard clams (*Mercenaria mercenaria*) also using cytochalasin-B. Another technique used was heat shock of fertilised eggs for 10 minutes at 32°C which produced 55% triploid embryos in *Ruditapes philippinarum* (Gosling et al. 1989).

El-Wazzan and Scarpa (2009) found that triploid juvenile hard clams (*Mercenaria mercenaria*) grew slower than diploids of the same age (14 to 18 weeks), although they surmised that the benefits of triploidy could be expressed when maturation would normally occur in diploids. Eversole et al. (1996) found little difference in the growth of diploid and triploid hard clams (*Mercenaria mercenaria*) until nearly four years, when the triploids were significantly larger than diploids.

A3.3. Settlement

Settlement (or metamorphosis) occurs when free swimming pediveliger larvae (larvae with a 'foot') attach to the substrate. In the case of clams, settlement can be a protracted event, taking up to seven days to complete. This is a critical period with regard to bacterial contamination. Bacteria of the genus *Vibrio*, several of which are highly pathogenic, colonise surfaces. During settlement, pediveligers are in contact with screen mesh with a high surface area, often with algal cells, faeces and dying larvae and the incidence of *Vibrio* infection can be very high and result in high mortalities. To reduce the incidence of mortality, every effort must be made to keep larvae, tanks, screens, feed and water very clean.

Settlement rate (both percent and time) can be enhanced by addition of a small amount of adult pallial fluid (Hidu and Newell 1989). Garcia-Lavandeira et al. (2005) reported that the percentage of metamorphosis of the clam *Venerupis pullastra* was improved by the addition of GABA (γ -aminobutyric acid) and epinephrine while settlement of *Ruditapes philippinarum* was improved by the addition of GABA but not epinephrine. Kent et al. (1999) found that epinephrine and norepinephrine had no effect on settlement percentage of *Ruditapes philippinarum* when compared to non-treated larvae.

A3.4. Nursery Culture

Even with predator control, clams below 8-10 mm have relatively poor survival (Manzi and Castagna 1989). This has necessitated the development of nurseries in which smaller clams are provided with sufficient water flow, feed, cleanliness and prevention of predators to enable enhanced survival and growth compared to the natural environment.

There are two phases of nursery culture;

- 1) Post-settlement culture of recently settled spat prior to introduction to ambient conditions.
- From the size purchased from the hatchery (this can vary considerably) to a size suitable for planting/stocking for grow-out.

Nurseries take two forms, land based and sea-based. In land based nurseries, spat are contained in upweller or raceway tanks. Upwellers consist of a cylinder with mesh fixed to the bottom upon which spat are placed, with water entering below the screen and moving up through the clams before exiting through an outlet in the top of the upweller. Upwellers have various advantages over raceways in that they take up a relatively small area for the amount of spat they can hold and are relatively easy to clean (Hadley et al. 1997).

Manzi et al. (1986) used upwellers with natural waters and no supplementary feed in South Carolina and stocked 4 mm clams at various densities of 2.5, 5, 10, 20, 30 and 40 kg m⁻² of screen area. Most rapid growth was in autumn when water temperatures were at 18 to 22°C. Growth positively correlated with flow rate. A flow of 15 times the spat volume per minute resulted in the biomass doubling after 30 days whereas a flow of 30 times the spat volume tripled biomass over the same period.

Raceways are also used in the USA. In raceways, water is introduced to one end of a shallow tank where it then runs over the clams before exiting from the other end. Some nurseries are established adjacent waters that are naturally high in feed particles such as microalgae, while others require cultured algae to supplement naturally available food. In some instances a 'flupsy' (floating upweller system) is used within natural waters or ponds with either tidal flow or an on-board paddle-wheel to move water through the spat.

Hadley and Manzi (1984) undertook raceway culture trials to find the optimum density of hard clams (*Mercenaria mercenaria*) as measured by growth and survival. In early spring, 3.9 mm clams were stocked at densities of 740, 2220, 6660 and 19980 seed m⁻² until the end of summer. Growth was greatest at the inlet end of the lowest density raceways and lowest at the outflow end of the highest density raceways. Water flow was 2 L min⁻¹ for a 0.07 m² raceway.

Growth was recorded by volumetric increases of the clams using a measuring cylinder. Maximum growth was when water temperature was between 20 and 24°C. It was found that the growth limiting density was 9 litres of clams m^{-2} .

Algal cultures are an important and often costly aspect of most land-based nurseries, although where there is sufficient natural food, they are not necessary.

In field nurseries clam spat are seeded to the bottom and protected from predation by nets. Nursery plots are usually seeded with a high stocking density and later transferred to growout plots after removal and grading. Field nurseries are generally a low cost method but they have a disadvantage in that there is no control over environmental conditions, and predation and storm damage are risks (Manzi and Castagna 1989).

Trays are sometimes used and can be placed either in the substrate or above it, fastened to racks. Trays are made from wooden or plastic frames with mesh attached top and bottom and are occasionally stacked in tiers. Trays require regular maintenance to remove fouling and silt so as not to impede water flow, a situation that can result in death and/or poor growth (Hadley et al. 1997).

Trays are sometimes left in/on the substrate for a while to accumulate sand before planting (Hadley et al. 1997). Attention is needed to ensure that excessive silt doesn't smother the trays and clams within them. If sand is added to trays, this should be done prior to planting (Hadley et al. 1997). Paterson and Nell (1997) found that a higher level of mortalities occurred when clams were added to sediment filled trays prior to deployment in the field rather than adding the clams after the sediment and allowing them to bury themselves.

In Italy, 4 to 5 mm seed are placed in 1 m x 5 m trays with a 2 mm or 4 mm mesh screen and stacked underwater until the seed reach 10 to 12 mm after 3 to 4 months, depending upon seas (Gosling 2003). A similar system is used in Mexico and some locations on the West Coast of the USA with Manila clams (*Ruditapes philippinarum*). Castagna (1984) found that small hard clam seed of 3 to 4 mm grew well in trays and other screened containers. Trays of 2.1 m x 1.2 m x 0.2 m have been successfully used.

In Ireland, seed trays of 3 m x 1 m are used for a total of up to 12 months. The stocking density of 2 mm clams is initially about 100,000 individuals m⁻² but they are then graded from time to time with the density adjusted to 10,000 individuals m⁻² when they are 6 mm in size. It takes approximately one year for the seed to grow to 9 to 10 mm. Another method involves the use of 1.5 m x 2 m x 4 mm mesh bags placed on trestles positioned at the low spring tide level or

directly on the substrate at a density of approximately 3,000 spat m⁻². Clams are removed at 13 - 15 mm before being spread over prepared beds at a density of about 300 m⁻² (Gosling 2003).

Despite seed trays and rafts having been used for many years in the USA, the trend is away from these to bottom culture techniques as they are relatively expensive to build and maintain, and can be damaged by storms.

Ponds are sometimes used in France (approx 400 m⁻²). These ponds use pumped water and cultured food to sustain growth (Gosling 2003). In China, a range of pond practices are used where ponds are drained, dried, limed, filled and fertilised to encourage algal blooms before adding clam larvae or recently metamorphosed spat. Clams remain in these ponds until they are large enough for harvest (2nd author, pers. comm.).

Hatchery rearing costs of the soft shell clam *Mya arenaria* was estimated at US\$12 per thousand in 1981 (Hidu and Newell 1989). This price will differ greatly with the size of the spat and the capacity and location of the hatchery but is similar to current USA clam prices. Depending on size, Australian oyster spat prices currently range from \$20 to \$40 per thousand and it is likely that the costs of producing clam spat will be within this range as the systems required are very similar.

A3.5. Growout Methods

A3.5.1. On-Bottom Culture

In most cases, clams grow better in sediment (Hadley et al. 1997). There are likely to be nutritional advantages to clams living within the sediment as they are known feed on particles both within the sediment and the water column (King 1976; Bricelj and Malouf 1984).

On-bottom culture involves 'planting' clam spat (or 'seed') directly on to suitable natural substrate at a sustainable and profitable density that allows for maximum return while still allowing for good growth. Clams are then covered with netting to prevent predation. Most clam culture is undertaken this way as this is their natural environment. On-bottom culture requires less infrastructure and as a result is more cost-effective when compared to other methods. The intertidal zone is most often used for clam farming due to ease of equipment maintenance and lesser infrastructure and operating costs when compared to subtidal culture (Kraeuter and Castagna 1989).

Paterson and Nell (1997) compared growth of *Tapes dorsatus* in a range of culture systems including within natural sediment, in floating baskets, baskets on racks containing a range of sediments and baskets without sediment. Not only did clams grow faster when in sediment, but growth was faster in sand/shell sediment than in finer sediment or shell grit alone.

A3.5.2. The Parc System

The Parc system consists of a fenced off area of the shore. Fences are about 80 cm high and designed to prevent predators from entering. Baited traps inside the Parcs are used to capture crabs. The floor of the Parc is covered with a mesh to prevent predators such as oyster catcher birds (Gosling 2003).

Seeding is mostly done in spring to make full use of warmer water (Gosling 2003). To prepare the site, the substrate is loosened with rakes and aggregate of gravel or crushed shell may be added to provide protection from crabs. A light mesh of 6 to 12 mm is placed over the seed and anchored around the edges, using leadline, steel rods or sandbags. Floats may be placed under the netting to reduce siltation of the netting. Net covers do not work very well in soft substrates (Hadley et al. 1997).

The Parc system is expensive and difficult to maintain and so has been superseded by the 'Plot' system (Gosling 2003).

A3.5.3. The Plot System

Strips of 4 - 20 mm mesh are placed over the seeded clams, with the edges buried to prevent incursion of crabs and predation from above by crabs, birds and rays. Mesh is typically 1 - 4 m wide, up to 300 m long and is placed on the substrate, often from a reel on the back of a tractor and positioned parallel to wind and water movement (Gosling 2003). Planting and mesh laying is done by machine, ploughing in the sides of the mesh to a depth of approximately 100 mm and seeding the clams simultaneously (Gosling 2003). In some European farms, a 0.5 m gap is maintained between rows of clam beds to allow access for tractors (Gosling 2003).

Published recommendations on clam stocking density vary, most likely due to different environmental conditions and characteristics of the clam species (Table A2). Densities that are too high will result in clams that are stunted and with poor survival (Hadley et al. 1997). Edwards (1999a) found that the influence of competition in *Katelysia* only occurred at 320 clams m⁻² at the Section Bank, Port River, SA.

Species	Country	Size clam	Stocking density (m ⁻²)	Reference
Manila clams	Italy	To market	200	Gosling 2003
	USA	Juvenile	700 to1000	Becker et al. 2008
	USA	To market	300 to 500	Becker et al. 2008
Hard clams	USA	5 mm	3000 to 4000	Menzel 1989
	USA	7 to 8 mm	Up to 5000	Hadley et al. 1997
	USA	20 mm	Up to 500 to 650	Hadley et al. 1997
	USA	20 mm	Up to 500 to 650	Hadley et al. 1997
	USA	To market	400	Menzel 1989
	USA	8 mm	250 to 1000	Castagna 1984

Table A2. Stocking densities in clam culture

A3.5.4. Off-Bottom Culture – Suitable for South Australia?

Although the majority of clam culture is undertaken in the sediment, there are some potential advantages in culturing clams in the water column in trays or baskets such as the lack of sediment particles which may otherwise need to be purged prior to human consumption. Access to larger areas suitable for culture also becomes available because factors such as depth and sediment type become less important (Paterson and Nell 1997).

Paterson and Nell (1997) found that clams (*Tapes dorsatus* and *Katelysia rhytiphora*) cultured on racks and in floating baskets didn't grow as well as those grown in the sediment but clams held in floating baskets grew better than those grown on top of the sediment.

Shell shape was different for tray cultured as compared to substrate cultured *Mya* clams, with tray cultured individuals being much wider and fatter than bottom cultured clams, however, tray cultured clams ceased to grow in length after 35 mm but grew much fatter (Hidu and Newell 1989).

Where clams are held in the water column, fouling organisms are likely to be a significant factor in the maintenance of submerged equipment and may colonise shells, potentially having a detrimental effect on appearance and marketability. Fouled clams will require cleaning and grading is more difficult (Gosling 2003). Common fouling organisms include algae, tunicates, vorticellae, amphipods, polychaetes and boring sponges (Gibbons et al. 1989).

Filamentous algae can clump seed together (Gosling 2003). In Ireland, nets are inspected for holes and cleaned every spring tide with a type of static broom or brush mounted on the back of a tractor (Gosling 2003). Other drawbacks to in-water culture include possible slower growth, increased potential for theft, conflicting uses and visibility (Hadley et al. 1997). Added costs include the need for a substantial boat, relatively expensive equipment such as cages, long-lines, anchors etc and the increased workload involved with cleaning equipment and clams.

A3.6. Growth rates

The growth rate of clams varies greatly with species, location, season and culture method as well as fed levels and stocking density (Manzi and Castagna 1989). Table A3 shows various growth rates of clams.

Species	Size Range	Seasons	Growth Rate (mm month ⁻¹)	Reference
Manila Clam	From larvae	All	0.67 - 2.25	Chew 1989
		All	2.25 - 3.1	Chew 1989
	From larvae	All	0.83 - 1.06	Gosling 2003
	Planted seed to market	All	2.2	Gosling 2003
Hard Clam	Larvae to 50 mm	All	0.8 - 1.7	Menzel 1989
	Larvae to 50 mm	All, average growth from Canada to Florida	1.25	Menzel 1989
	Early spat to 50 mm	Growing season	1.5 - 2	Hadley et al. 1997
	Early spat to 50 mm	Spring, summer	1.8 - 2.2	Manzi and Castagna 1989
	Larvae to 50 mm	All	2	Castagna 1984
European cockle	Larvae	All	0.68	Jensen 1992
NZ Pipi	13 to 37 mm	All	1.4	Hooker 1995

Table A3. Monthly growth rates for various clam species

It has been shown that clams grown under nets grew faster than those that were not. An explanation given was that the clams didn't have to expend so much energy on maintaining position as the net stabilised the substrate (Chew 1989).

In the experiments conducted by Kent et al. (1999), hatchery-produced *Ruditapes largillierti* clam seed raised in trays and baskets on the substrate in Tasmania grew to 32 mm shell length over 28 months from spawning, with an average growth rate of 1.3 mm month⁻¹ over this period. While growth of clams in the study by Kent et al. (1999) was less than optimum, they did observe relatively uniform growth rates within their samples.

Investigations of the effects of clam size and density on growth of *Ruditapes largillierti* (Kent et al. 1999) showed that after 8 months (April - December) in partially-buried plastic mesh cages on a shallow subtidal flat, larger clams (43.5 mm) exhibited slower growth than small ones (27.4 mm), with average growth rate being 1.5 and 0.5 mm month⁻¹ respectively. Rates of growth observed in the warmer months (April - June) were 2.7 and 1.1 mm month⁻¹ for small and large clams respectively, and 1.8 and 0.4 mm month⁻¹ in the cooler months (October - December). This study confirms that growth rate declines with increasing size and varies between seasons.

No significant difference in growth rates was found between clams held at 200 and 400 m⁻². Condition index, maintained at either low or high density showed a similar trend throughout this study, although varied significantly different between size groups and seasons. Survival was not

significantly affected by both initial size and density, and overall survival was 84% (Kent et al. 1999).

Hatchery produced *Katelysia rhytiphora* spat used in a study by Paterson and Nell (1997) were 15.4 mm in length when 10 months old. After 6 months cultivation (December - July) in 6 mm plastic mesh baskets buried in a sand/shell substrate they reach a size of about 28.6 mm at Port Stephens, NSW at a growth rate of 2.3 mm month⁻¹ and an average survival rate of 84%.

Tapes dorsatus reach a maximum shell length of 100 mm (Paterson and Nell 1997). The 3.5 month old, 14.5 mm hatchery reared spat of this species were grown for 6 months from July to December in 6 mm plastic mesh baskets that were buried in a sand or sand/shell substrate. They reached a size of 39.5 mm at Port Stephens, NSW at a growth rate of 3.1 mm month⁻¹ and survival rate of 97% (Paterson and Nell 1997).

Pilot farming trials of *T. dorsatus* were also conducted by Paterson and Nell (1997) over 48 weeks from March 1996 to February 1997 at four locations within NSW. The clams were held in intertidal grounds characterized by sandy substrate and high salinity. The growth rates among sites were highly variable, attaining an average shell length of 27 to 38 mm at the completion of the study from an initial size of 15.3 mm at a stocking density of 700 clams m⁻².

The survival rates were highly variable among sites (17.6% to 76.9%). Clams grown at Brisbane Waters achieved the best growth (38 mm shell length) and second highest survival (69.1%). The authors attributed this growth to warm water, lower growing height and the inclusion of two growing seasons. Although it has been identified as having potential for culture, *Tapes dorsatus* is a tropical/sub-tropical species and its natural range does not extend to SA.

Trials conducted by Bellchambers et al. (2005a) on *Katelysia scalarina* investigated the effect of intra-specific competition on the survival, growth and condition index of this species. Plastic mesh cages initially stocked with 20 - 25 mm juveniles were stocked range of densities (57.2 - 1886.9 clams m⁻²) were held in the intertidal zone of Moulting Lagoon, Coles Bay, Tasmania for 10.5 months from March 1996 to January 1997. None of the measured parameters displayed a significant response to density manipulations. Survival at this site was very high (usually greater than 95%). Growth rate was however, very low, being approximately 0.4 mm month⁻¹ (Bellchambers et al. 2005a). Comparable studies on this species in Princess Royal Harbour, WA reported growth rates of 0.31 mm month⁻¹ over a period of 8 months (Peterson and Black 1993).

Investigations on the effects of tidal position and density on *Katelysia scalarina* were also conducted by Bellchambers et al. (2005b). The experiments involved three density treatments (172 - 686 clams m⁻²) and five tidal positions over a 10.5 month period from February 1996 to January 1997. Tidal height and density had a significant effect on the survival of *K. scalarina*. The major effect was depressed survival at the highest level on the beach and at this position mortality was exacerbated by increasing density. At high tidal positions, shell growth was approximately half that of those lower on the shore where growth at the highest density was usually only reduced by less than 10% compared to the lowest density. Meat ratio (% of total weight consisting of meat weight) displayed a significant response to tidal position. At higher tidal heights shell growth was more depressed than meat growth and hence the meat to shell ratio improved (Bellchambers et al. 2005b).

Preliminary results from age determinations of wild *K. scalarina* indicate it takes 4 to 6 years for them to grow to commercial size of approximately 32 mm in Tasmania, depending on locality (Riley et al. 2005).

A3.7. Predators

Predation is widely reported to be the dominant factor controlling clam abundance in their natural environment (Kraeuter and Castagna 1989) and can account for mortality of 100% of stock (Craig et al. 1998).

There are many predators that feed on clams including crabs, drilling gastropods (whelks), prawns and shrimps, finfish, polychaetes, rays, starfish, lobsters, octopus, birds such as oyster catchers and ducks (Schwind 1977; Gibbons et al. 1989; Hidu et al. 1989; Hadley et al. 1997; Craig et al 1998; Gosling 2003).

In the USA, the most serious of these predators are blue crab and whelks (Menzel 1989), where crabs are able to open clam shells up to 30% of their carapace length and can dig holes up to 10 cm deep in search of clams. However, their presence is usually seasonal (Gosling 2003).

A3.7.1. Predator Control

Reducing the impact of predation involves the initial removal of as many predators from the site as possible. Exclusion is the method most commonly used and involves the prevention of predators coming in direct contact with clams. Efforts to exclude predators with the use of plastic mesh, trays or within mesh 'pillows' was found to be effective and allowed for further increase in production (Gibbons and Blogoslawski 1989).

Regular inspection for crabs is essential as they may enter through holes in the mesh. Clams inspected weekly have twice the survival of those inspected monthly (Hadley et al. 1997). It is also important to stock clams for growout that are of sufficient size to prevent predation (Gibbons and Blogoslawski 1989). The larger the clam, the less susceptible they are to predation. Clam seed of 7 - 10 mm can be planted directly to the substrate (Hadley et al. 1997; Gosling 2003). Examples of Manila clam survival (Gosling 2003) when planted at various sizes in the UK are;

3 mm clams34% survival10 mm clams60% survival24 mm clams77% survival.

Mechanical methods such as the 'crab killer' are sometimes used. It consists of a drum with 75 mm vertical tines that penetrate into the substrate as the drum is rolled along the bottom and is usually mounted on the back of a tractor. The drum can also have the added benefit of loosening the substrate to allow clams to bury in it (Gosling 2003). Other mechanical methods

include hand operated crab spikes, ploughs/harrows, traps and hand collection Gibbons and Blogoslawski 1989).

Baited traps have also been used within clam culture sites to remove crabs before and after planting (Gosling 2003). Biological methods have also been used including the use of toadfish to control crabs, and periwinkles to control fouling on nets (Gibbons and Blogoslawski 1989).

In some countries, chemicals including quicklime, salt, copper sulphate, chlorinated hydrocarbons, and insecticides have been used to control predation. The use of chemicals has however been restricted as they are deemed too harmful for endemic species (Gibbons and Blogoslawski 1989).

Exposure to air dries out the site and if for long enough can prevent most algae from growing. It can also be an impediment to parasites and predators such as borers (Hadley et al. 1997).

A3.8. Diseases

Several diseases pose a risk to clam culture. Brown Ring Disease (BRD) of Manila clams has been a significant problem in France, UK, Ireland, Spain, Portugal and Italy (Gosling 2003; Drummond et al. 2007). BRD is caused by *Vibrio tapetis* (Kim et al. 2008) and the name is derived from the brown conchiolin deposits on the inner shell of diseased clams (Kim et al. 2008). It first appeared in 1987 in France, 15 years after Manila clams were introduced to Europe, and later in Spain where mass mortalities occurred in 1989 (Drummond et al. 2007). There are now strict controls of clam movements within the UK and around Europe to prevent spread of disease (Gosling 2003).

Clam larvae cultures can be affected by *Vibrio* infections passed from adult broodstock to progeny (Gibbons and Blogoslawski 1989). *Perkinsus olseni* (also known as *P. atlanticus*) is also a pathogen of the Manila clam (Kim et al. 2008), infecting connective tissues of organs (Hegaret et al. 2007).

Nuclear inclusion X (NIX), an intracellular prokaryote parasite has been found within the nuclei of gill epithelial cells and interferes with gill function. Secondary bacterial and mycotic infections occur after NIX infection (Gibbons and Blogoslawski 1989).

Hard clams (*Mercenaria mercenaria*) on the east coast of the USA are frequently infected with the protistan parasite 'Quahog Parasite Unknown' (QPX) that can cause mortality (Perrigault et al. 2009; Hegaret et al. 2010).

Herpes virus infections in Tasmanian *Katelysia scalarina* were found to have caused mortalities in larvae (Handlinger 2005). The same virus was found in the parent stock, suggesting transfer from them to the larvae. Handlinger (2005) sampled Tasmanian *Katelysia scalarina* clams each month over a four year period and, as well as the Herpes virus mentioned above, found several species of flukes, infecting gills, gonad, foot muscle and other tissues. Intracellular bodies typical of *Rickettsia* or *Chlamydia* were seen infrequently and were considered low-grade pathogens that occur in other aquaculture species without ill effect.

Other clam pathogens (Gibbons and Blogoslawski 1989; Hooker 1995) include;

- Fungal infections of larvae (Sirolpidium zoophthorium in Mercenaria mercenaria cultures)
- Papova like virus in connective tissues of Mya arenaria
- Birnaviruses in Mercenaria mercenaria
- Chlamydia like infections
- Amoeba and protozoans
- Parasitic copepod Mytilicola porrecta in Mercenaria mercenaria
- Cancerous like growths in Mercenaria mercenaria
- Digenetic trematode fluke.

A3.9. Site Selection

There are many factors that contribute to a successful site for culturing clams (Kraeuter and Castagna 1989; Hadley et al. 1997) and these include;

- Temperatures within the species natural range
- Suitable water depth for the clams and to enable cost-effective maintenance of clams and structures
- Suitable substrate that allows the clams to bury
- Tidal flow sufficient to deliver food particles, oxygenated water and removal of waste products but not so great that the substrate is unstable and not so slow that siltation occurs, which has the potential to bury clams and/or become anaerobic
- Suitable feed levels
- Free from bacterial and chemical pollution
- Accessible

- Suitable land-based site nearby for grading, packaging, equipment maintenance and freight forwarding
- Staff available in the area
- No major conflicting uses.

Mostly, sites are chosen that are within and just below the intertidal zone. It is advantageous for the clams to be under water as much as possible for optimum growth but good access is needed at low tide for the considerable amount of work needed while maintaining sites and harvesting clams (Schwind 1977).

It is important to consider both the physiological requirements of clams and the characteristics of the waters in which clam farming is proposed. Generally, tolerance of environmental stresses is greater in species found in soft substrates (Manzi and Castagna 1989). A harder substrate is typical of an area with higher water flow whereas a softer substrate suggests lower water flow. Animals naturally occurring in these environments would be adapted to corresponding high and low water quality conditions. Many clams are able to withstand conditions of low dissolved oxygen (Sobral and Widdows 1997). In Australia, the natural occurrence of *D. deltoides* within the swash zone means that they live in highly oxygenated environment and in laboratory trials have been reported to experience high levels of mortality in oxygen levels below 5.0 mg L⁻¹ at 17°C (Murray-Jones and Johnson 2003).

One indication of a good site is to look for fast growing native clams as indicated by a white growth shell margin, which will be more noticeable in small clams. This type of inspection is likely to be less useful winter when growth rates may be naturally low due to cold water temperatures (Hadley et al. 1997).

The natural distribution of a species is not always a good indicator of whether the species will live in that environment. For instance, Pacific Oysters do not naturally occur in some colder waters, perhaps because the larvae do not tolerate this. However, if conditions are manipulated to produce larvae and spat within a hatchery, then these can do well when transferred to colder growout sites (Manzi and Castagna 1989). Another example, the surf clam *Spisula solidissima* is a coastal species that does not naturally occur in sheltered bays and estuaries, but which has been shown to grow very well in these environments when held in trays with no substrate (Manzi and Castagna 1989). In addition, other factors such as predation may play an important role in determining a species natural distribution (Castagna and Manzi 1989).
The absence of clams may be an indication of other problems (Hadley et al. 1997). Seasonal hypoxia has been a problem (Manzi and Castagna 1989). Events with a dissolved oxygen level below 3 ppm have have killed Atlantic surf clams (*Spisula soldissima*) on several occasions (Cargnelli et al. 1999). Low dissolved oxygen this may occur during very dense algal blooms and/or during periods of very high temperature and low tidal flow (e.g., during dodge tides).

Gosling et al. (2003) recommends monitoring the site for at least a year prior to development to ensure that the quality of the substrate and water is known.

Shallow bays with muddy sand or firm mud can be good sites for Manila and hard clams (Kraeuter and Castagna 1989). A mixture of sand and mud that is firm enough to be walked on but soft enough to move a rake through is suitable (Hadley et al. 1997).

The effect of suspended sediment varies according to its type and volume and the biology of the clam itself. Depending on the level of suspended sediment, the effects can be positive or negative (Kraeuter and Castagna 1989).

Seagrass sites can be excellent as they are stable (Kraeuter and Castagna 1989), but may not be approved as culture sites due to concerns within most government regulatory agencies concerned about seagrass loss. However, aquaculture structures can have a positive effect upon seagrasses due to the stabilising and shading effects of structures. Examples of this have occurred in SA where seagrasses have become established since installation of oyster farming racks (L. Marshall, pers. comm.).

Locations with a sediment of 50-80% gravel, sand, broken shells and a small amount of mud (4-5%) are suitable for Manila clams. This may be due to the protection from predators and stability that gravel provides (Chew 1989). Such a coarse sediment is also likely to be a sign that there is a reasonable amount of water flow, which tends to be beneficial in that it delivers food to the clams along with good water quality.

The experience of SA oyster farmers has been that growth, meat condition and survival are much better in areas of high water flow compared to low flow environments. This is likely to be the same for clams and has also been reported for hard clams in the USA (Craig et al. 1988).

A3.10. Species Selection

Species that have proven to be successfully farmed, typically have the following characteristics (Gosling 2003; Kraeuter and Castagna 1989);

- High price and market demand
- Ease of hatchery culture
- Market demand for the hatchery product
- Tolerance of a wide variety of environmental conditions
- Shallow burial in the substrate
- Disease resistance
- Fast growth
- Able to be handled without significant mortality.

Attempts have been made to farm many clam species in the USA, but only the hard clam and Manila clam have proven to be successful on a commercial scale (Kraeuter and Castagna 1989) although more recently, geoduck clams (*Panopea abrupta*) have been successfully cultured in north-west of the USA and Canada.

The Manila clam which makes up the majority of global farmed clam production, is a native of Japan, Korea and the Philippines (Gosling 2003) and is cultured in many countries across the northern hemisphere including the USA, Canada, UK, Ireland, Spain, Portugal, France, China, Korea and Japan and accounts for the majority of cultured clam production. It was accidentally introduced to the west coast of the USA along with Pacific Oysters in the 1930's (Becker et al. 2008) and to Europe in 1972 (Drummond et al. 2007), and has proven to be a hardier and faster growing species than the European native clam *Ruditapes decussates* (Gosling 2003).

The hard clam (or quahog) is grown on the east coast of the USA from Maine to Florida, and has proven to be a fast growing and hardy species. Its production is fast increasing along with the development of hatcheries and growout techniques.

A3.11. Harvesting

In Europe, most clam farmers use mechanical harvesters mounted on the back of tractors. In the UK, where plots are under water, growers use suction or elevator dredges before being mechanically graded on land (Gosling 2003).

In the USA, farmers use various mechanical methods as well as collection by hand. Manila clams in the north west of the USA are harvested year round from intertidal beds by contract diggers, often using forks and rakes, although recently some mechanical harvesting methods have been developed (Figure A8).

Harvesting usually occurs when about 80% of clams reach market size with sub-market size clams are returned to the farming area for further growout (Hadley et al. 1997). Prior to harvest, clam stock is checked for size. Where mesh bags are used the whole bag is lifted out, with sediment washed from the bag, so that only clams are left (Gosling 2003).



Figure A8. A mechanical clam harvester used on the west coast of the USA.

A3.12. Summary in Relation to Australian Species

Clams currently harvested from southern Australia present challenges for culture. *Katelysia* spp. are comparatively slow growing (Maguire 2005), yet seem to be hardy and naturally occur in protected bays and estuaries typical of those that are used for culturing other bivalves such as oysters, suggesting that infrastructure development will be possible. *D. deltoides* on the other hand, appears to be a relatively fast growing species (Murray-Jones 1999) but occurs naturally in exposed high energy coastlines, making the establishment of culture systems difficult. There may be other species that are currently not commercially harvested that may be suitable for aquaculture.

Before the establishment of a clam culture industry occurs, important factors need to be addressed such as species selection, hatchery and nursery culture, growth rates under different conditions, site selection, culture techniques, equipment, susceptibility to disease, etc.

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APPENDIX A1. CLAM INFORMATION ON THE INTERNET

Hard Clams (Mercenaria mercenaria)

http://aquanic.org/species/shellfish/documents/fs745.pdf

http://www.fao.org/fishery/culturedspecies/Mercenaria_mercenaria/en

http://www.sms.si.edu/IRLSpec/Mercen_mercen.htm

http://www.thefishsite.com/articles/279/introduction-to-infectious-diseases-in-hard-clams

http://ian.umces.edu/imagelibrary/displayimage-82-5612.html

http://www.youtube.com/watch?v=qX9anEK8WI4

Manila Clams (Ruditapes philippinarum)

http://www.seafish.org/pdf.pl?file=seafish/Documents/ClamCultivation.pdf

http://www.seafoodchoices.org/archived%20smartchoices/species_clams.php

http://www.taylorshellfishfarms.com/files/Manila%20Clams%201.pdf

http://www.bcsga.ca/about/industry-encyclopedia/clams

http://www.lib.noaa.gov/retiredsites/korea/main_species/manila.htm

http://www.ipmcenters.org/cropprofiles/docs/WAbivalve.pdf

http://www.fao.org/fishery/culturedspecies/Ruditapes_philippinarum/en

http://www.penncoveshellfish.com/Farming/farm_clams.html

Irish Clam Farming

http://staffweb.itsligo.ie/staff/bcrowe/bill/styles/frames/research/clamfhis.htm

Hatchery and nursery

https://srac.tamu.edu/index.cfm/event/getFactSheet/whichfactsheet/198/ http://nsgl.gso.uri.edu/njmsc/njmsch98001.pdf http://www.card.com.vn/news/Projects/027VIE05/MS8_Manual%20of%20hatchery%20culture%

20of%20Clam.pdf

http://www.fao.org/docrep/007/y5720e/y5720e07.htm

Clam Harvesting

http://shellfish.if as.ufl.edu/pdf/Harvesting%20 and%20 Handling%20 Clams%20 PPT%20 for%20 with the standard standard

eb.pdf

http://shellfish.ifas.ufl.edu/pdf/Harvesting%20and%20Processing%20Equipment%20Suppliers% 202003.pdf http://www.reeis.usda.gov/web/crisprojectpages/193193.html

http://www.youtube.com/watch?v=uG6Eaql2_vc

http://www.youtube.com/watch?v=tfszb2vPEoM

Environmental Effects

http://www.cefas.co.uk/publications/techrep/Clam%20cultivation.pdf

http://www.deq.virginia.gov/Portals/0/DEQ/CoastalZoneManagement/task11-07-04a.pdf

Mortalities

http://www.biosecurity.govt.nz/media/21-08-09/cockle-death-whangateau-estuary http://www.springerlink.com/content/u0r3714xu24730p2/

Markets/Marketing

http://www.fl-

seafood.com/pubs/pubform/pdf/Market_Research_Report_Farm_Raised_Clams.pdf http://www.agmarketing.ifas.ufl.edu/pubs/2000s/Blood%20Ark%20Clams%20Marketing.pdf https://www.was.org/Documents/MeetingPresentations/AQUA2006/WA2006-797.pdf http://www.ngaitahu-seafood.com/pdf/clams.pdf http://www.ngaitahu-seafood.com/pdf/fish-clams.pdf

Pipi (D. deltoides)

http://www.abc.net.au/landline/content/2008/s2736426.htm

http://publications.frdc.com.au/presentation.php?version=10&quality=low&cache=4&publication

=Fish17-2_Beach_rescue_for_Pipi

http://new.dpi.vic.gov.au/notes/fish/research--and--education/fn0609-pipi-now-and-forever---

venus-bay-to-host-new-research-project

APPENDIX A2. DONAX SPECIES WORLDWIDE

Donax anatia	Venezuela
D. bipartitus	South Africa
D. brazieri	Australia (SA)
D. burnupi	South Africa
D. californicus	USA (California)
D. carinatus	Mexico
D. columbella	Australia (WA)
D. deltoides	Australia (Qld, NSW, Tas, SA, WA)
D. denticulatus	Mexico, Panama, Venzuela
D. erythraeensis	Seychelles
D. faba	Australia (WA), Indonesia, Philippines, Kenya
D. fossor	USA (New Jersery, New York)
D. gouldii	USA (California)
D. hanleyanus	Argentina, Brazil
D. kiusiuensis	Japan
D. lubricus	South Africa
D. madagascariensis	South Africa, Madagascar
D. panamensis	Mexico
D. peruvianus	Peru
D. punctatostriatus	Mexico, Peru
D. rugosus	Angola, Senegal
D. scortum	India, Taiwan, Thailand
D. semistriatus	Italy
D. serra serra	South Africa
D. serra auriantica	South Africa
D. simplex	South Africa
D. sordidus	South Africa
D. striatus	Mexico
D. townsendi	India
D. trunculus	France, Morocco, Portugal, Spain
D. tumidus	Mexico
D. variabilis	Mexico, USA (Florida, Texas)
D. variegatus	France, Italy, Spain
D. venustus	France, Italy
D. vittatus	Belgium, France, UK

APPENDIX A3. KATELYSIA SPECIES WORLDWIDE

Katelysia. hiantina	Indonesia, Philippines
K. japonica	Japan, Philippines, Indonesia, Australia (Qld)
K. opima	India
K. peronii	Australia (NSW, Vic, Tas, SA, WA)
K. rytiphora	Australia (NSW, Vic, Tas, SA, WA)
K. scalarina	Australia (NSW, Vic, Tas, SA, WA)
K. virginea	Taiwan

APPENDIX B: CLAM CULTURE WORKSHOP

20 April 2011

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B1. WELCOME

Mark Gluis and Steven Clarke

B2. WORKSHOP AIMS

- Inform people about progress in current SA clam project
- Enhance communication amongst people with clam culture experience
- Identify key findings/issues confronting clam culture in Australia, with particular emphasis on grow-out culture.

B3. ATTENDEES AND APOLOGIES

Present

Crispian Ashby (FRDC) Steven Clarke (SARDI) Mark Gluis (SARDI) Greg Kent (Southern Cross Oysters, NSW) Dr Xiaoxu Li (SARDI) Dr Greg Maguire (Edith Cowan University, WA) Mr Doug McLeod (Glenelg Aquaculture Consulting) Kristy McQueen (formerly Kristy Paterson; Coastal Ecology, NSW) Dr Wayne O'Connor (NSW Fisheries) Tom Robinson (SA Clam Aquaculture)

Apologies

Dr Lynda Bellchambers (WA Fisheries) Kylie Giles (FRDC) Greg Kessegian (SA Clam Aquaculture) Dr Peter Lauer (PIRSA) Dr Kate Rodda (PIRSA)

B4. DESCRIPTION OF CURRENT PROJECT

Funding agencies	FRDC, SA Clam Aquaculture and SARDI	
Term of project	Three years from 1 st April 2010 to 31 March 2013	
Species	Donax deltoides and Katelysia sp	
Reason for project	Reduced wild catch; high market demand and price; occurrence of successful overseas culture industries	

Project Objectives

- 1. Desktop study of prior Australian research, overseas clam farming techniques and their application for use with Australian species and conditions
- 2. Using wild collected spat, undertake field trials to facilitate the selection of suitable species as well as controlled experiments in the laboratory to determine optimum environmental parameters and assist with later site selection
- 3. Hatchery and nursery production of clam spat. It is expected that these will be used to undertake field studies at different locations and using different farming systems
- 4. Undertake field evaluations for identification of likely commercial culture methods and site characteristics
- 5. Communication and technology transfer between industry participants and researchers

B5. OBSERVATIONS FROM PREVIOUS CLAM GROW-OUT EXPERIENCE

B5.1. New South Wales Clam Culture

Kristy McQueen and Wayne O'Connor

- Hatchery work was conducted at Port Stephens with a few different clam species including *Tapes dorsatus*, *Katelysia rhytiphora*, *Anadara trapezia*, and more recently *Donax deltoides*
- Had some early batches of larvae batches fail but have been better at the hatchery since improvements to the water quality were made.
- *Katelysia rhytiphora* and *Tapes dorsatus* trialled for over 4 ½ years. *Tapes* generally did better and are a larger, subtropical species.
- Tapes dorsatus were grown on screens up to 2mm and grew to 7 to 8 mm in 15 weeks.
 Diploids and triploids were grown and there wasn't much difference in growth between them. Triploidy was induced using Cytochalasin B and achieved 50% triploidy.
- Anadara trapezia were very slow growing as spat
- Tried different salinities, growing heights, settlement substrates including various eel grasses and shells, in and out of sediment.
- Grew and survived better in sediment so then tried different types of sediment
- Density trials were undertaken but weren't very successful so ended up sticking with 750 per square metre
- In salinity below 20 ppt all died in lab trials below 20ppt. Katelysia rhytiphora preferred 25 to 35 ppt
- Had problems excluding predators when clams were not held in baskets. Borers and crabs were a big problem.
- *Tapes dorsatus* and *Katelysia rhytiphora* did well in ponds at Port Stephens Research Centre (tidally flushed ponds) when predators were excluded.
- Spat were planted at 10 mm and harvested at 38 mm 6 months later
- At other sites, clams where grown intertidally at 0.3 m and did well, perhaps due to less predation. Kristy believes that subtidall culture is best if predators can be avoided.
- Katelysia would likely take 3 years to market size
- Tapes were grown to market size in approximately two years
- Farming trial with Tapes was done with oyster farmers
- Tried all sorts of baskets but concluded that growing in baskets off the bottom didn't work all that well.
- By the end of their trials they just used the substrate. In-substrate culture was undertaken using oyster mesh laid over the top and folded down the sides to a depth of 150 mm but still had predation problems.

- Wild clam spat weren't found so hatchery production is likely to be essential for farms.
- Lots of seagrass under oyster racks which couldn't be dug up to seed clams so there is a limit of suitable substrate in NSW estuaries where seagrass is common.
- Pipis were strip spawned. Didn't have many larvae because of this.
- 35% of Pipi larvae survived through metamorphosis but did not survive after that.
- Small Pipi spat were extremely active and seemed to find the settlement substrate unsuitable. Half had died to 500 µm and continued to deteriorate, with none surviving beyond 800 microns. Would like to try other substrates such as sand.
- A reseeding proposal on Pipis was recently lodged with FRDC and an economic assessment of reseeding done with QDPI, using \$17.50/kg as the projected price.
- NSW Pipi harvest was previously 110 to 120 tonnes.

B5.2. Tasmanian Clam Culture: Reporting on Findings of FRDC Project 93/232, Enhancing Tasmanian Clam Resources

Greg Maguire and Greg Kent

- The species cultured in this project was Katelysia scalarina.
- Katelysia scalarina grew slowly.
- High mortalities were occasionally experienced and is a concern for commercial culture.
- A virus in larvae was found by Judith Handlinger.
- Density trials had little effect so that is encouraging. They seemed reasonably tough.
- Extended settlement period. Were pediveligers for a long time without putting on growth.
- Growth seemed to slow at about 800 to 900 microns.
- Growth and survival was better in coarse substrate.
- Orientation of the shell seemed to be very important to growth rate.

B5.3. USA and European Clam Farming

Mark Gluis

Washington State, USA

Production

4500 tonnes of Manilas clams (Ruditapes philippinarum) grown in Washington State.

Introduction of Manila Clams

Manila clams have become widespread on the USA west coast after they were introduced with Pacific Oysters shortly after World War II.

Growout

Spat are seeded at an average of 5 to 7 mm and also up to 20mm. Much depends on winter conditions and predators.

Clams grow better in sand compared to gravel. Manila clams are found naturally in gravelly areas but this may be because the gravel offers some protection from predators rather than being necessary for good growth. Sand offers the potential for mechanised harvesting whereas this is not done where gravel is because the digger (a person) has to sort through and differentiate between clams and gravel. Tractor-mounted equipment from agricultural industries can sometimes be modified for digging clams.

Often tractor serviced farms have comparatively narrow rows of clams so that tractor wheels can drive either side without damaging the clams. In this situation, predator net consists of a heavy plastic 1.2 m x 50 m long 'oyster mesh' rather than wider, lighter panels that are often used with hand harvesting. The ground is tilled before rolling out the net. The outer 125 mm of the net is buried at a 45° angle and covered with sand by the tractor. Steel reinforcing bar (rebar) is used make 'staples' with 450 mm legs. The same tractor is used for brushing weed off the predator exclusion net by sweeping with a street sweeper mounted on the back and also has equipment that is used to roll out and retrieve the mesh when needed. If strong enough, the mesh can be re-used after fouling is removed.

Fouling can grow quickly on the plastic mesh so need to be cleaned frequently. Clams grown in deeper water grow well but need to tended more frequently, are more susceptible to fouling and predation. It is good to have some exposure to air to dry out fouling algae and also to enable much of the work using a tractor.

Clams dig 75 to 100 mm into the substrate in the summer and 125 mm in the winter.

Where possible, it is advisable to use hardy native species suited to local conditions.

Planting

Spat are preferably planted in spring after winter rain, when there is plenty food in the water, usually at a size of 3.5 mm, spread over 6 mm mesh at a rate of 850 clams per square metre with a survival rate to harvest of about 50%, harvested in the 3rd summer. It is advisable to seed farms early in optimal growing seasons so that the seed can be as large as possible going into the cold Washington winter, optimising survival.

Clams are spread over the top of the net, within a few days of rolling out the mesh, and bury down under the net and into the substrate below. Mesh is large enough for clams to bury through it but too small for predators.

The clams are fairly uniform after the 3rd year so all are harvested and graded, with small ones returned to the farm for further growth. The annual harvest from 2.5 acres is 32 to 45 tonnes.

Predators

Crabs and scooter ducks are the main predators. Crabs use their pointed legs to feel through the sand for clams. Some protection can be provided by gravel. In exposed areas where wind generated waves occur, some people just use gravel with no nets, although harvesting by hand is needed. Gravel is sometimes added to the site for this purpose.

Other methods

There is no off-bottom culture because Manila clams don't do well in this situation. Nurseries do use upwellers and raceways to a size but if they are held too long they will become stunted.

Market

Manila clams have a good shelf life, although can be only a couple of days if dug up immediately after spawning whereas otherwise they will last 1 to 2 weeks. When steamed they all open up at the same time (3-4 minutes) whereas the native littleneck will not and so if you continue to steam until they all open, then many will end up very tough and rubbery.

Clams are purged of sand in tanks prior to selling to market.

Recently a problem with cheap frozen Manila clams imported from China selling for less than half the price of the local product. Much of the USA market is price sensitive so this has had a considerable effect on local growers.

European Clam Farming

Clam farming in Sligo, Ireland is undertaken using mechanisation where possible. Clams are grown in rows under nets. Spat are grown to 1 mm on land based nurseries and then transferred to wooden spat trays about 100 mm deep with three compartments. Axles are put on the bottom of the trays and concrete filled rubber tyres used for wheels. The trays are towed out to the growout sites on the back of tractors and brought back in when ready to grade. Clams are planted at a slightly larger size than generally undertaken in the USA (1/2 to 3/4" in length) to prevent predators, even though they are planted under nets. Irish have been using a once only usable net which they can't dump and so is building up as waste in their yards.

Diseases

Washington does not have clam disease problems. Occasionally mortalities are experienced with excess fresh water and ice. The effect of these often takes 3 or 4 months to show up. There are occasional heat kills but these are very rare.

Irish farmers have a problem with brown ring disease and possibly a herpes like virus.

B6. PRESENTATIONS

B6.1. FRDC 2009/208 Project (Mark Gluis)







- Desktop study of prior Australian research, overseas clam farming techniques and their application for use with Australian species and conditions
- Using wild collected spat, undertake field trials to facilitate the selection of suitable species as well as controlled experiments in the laboratory to determine optimum environmental parameters and assist with later site selection
- Hatchery and nursery production of clam spat. It is expected that these will be used to undertake field studies at different locations and using different farming systems
- 4. Undertake field evaluations for identification of likely commercial culture methods and site characteristics
- 5. Communication and technology transfer between industry participants and researchers









B6.2. Clam and Cockle Aquaculture in China (Xiaoxu Li)



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Main Clam/Cockle Species Farmed in China

Family Arcidae:

Tegillarca granosa (Linnaeus)

Shandong, Zhejiang, Fujian, Guangdong, Hainan, etc (from cold to tropical costs)



Scapharca subcrenata (Lischke)

Hebei, Tianjin, Liaoning, Shandong, etc. (from cold and temperate costs)

S. Broughtonii (Schrenck)

Hebei, Tianjin, Liaoning, Shandong, etc (from cold and temperate costs)







2. Nursery stage (10-15mm)

Coastal pond (shrimp pond) for Veneridae & Tegillarca granosa



*

Shallow water or lower tidal bags for Scapharca subcrenata & S. Broughtonii





Grow-out Settings

	Species	Methods
	Ruditapes philippinarum	1. Lower dam and its maintenance;
	R. Variegata	2. Chemical/herb treatments
	Meretrix meretrix	a. Lower end protection nets;
		b. Horizontal substrate lines to prevent animal movements
	Cyclina sinensis	A. Intertidal with protection net "fence";
		B. Coastal pond
	Tegillarca granosa	a) Midtidal shallow pond with or without protection net "fence"
		b) Mid to high tidal pond
	Scapharca subcrenata	Subtidal; avoiding macroalgae
*	S. Broughtonii	Cages <4cm (waste nets); ranching >4cm
		of South Australia

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Farming Duration & Harvest Size

Species	Duration (yr)	Size (cm)
Ruditapes philippinarum	1.5-2	>3
R. Variegata	1.5-2	>3
Meretrix meretrix	>3	>5
Cyclina sinensis	~1.5	>3.5
Tegillarca granosa	2-3 (S); 3-4 (N)	>2.5
Scapharca subcrenata	1-2	>4
S. Broughtonii	>4	>5.5





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B6.3. Clams: Here, There – Everywhere (Doug Mcleod)

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Site Selection

- Critical parameters for on-growing:
 - Substrate (whether inter- or sub-tidal): Firm mud/sand mixture preferred
 - Salinity: Avoid periods of low salinity (as experienced in estuaries)
 - Presence/absence of indicator/predator species can be helpful in evaluating suitability
 - Space for pre-growing seed from spat (e.g. bags on trestles)
 - Access for seeding, management, harvesting
 - Space/licences for shore facilities (launching vessels, landing harvest, processing harvest)





European Cultivation Practices

- Post-hatchery nursery (spat to seed): oyster bags on trestles (inter-tidal) or trays in stacks (sub-tidal)
- Majority of production (IT, ES) located in shallow lagoons, broadcast sown; using hand or boat operated dredges for harvest
- Elsewhere, mostly inter-tidal on sandy/mud beaches, under mesh; using manual and mechanical dredges for harvesting












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- Commercial Sub-Tidal cultivation: depths vary dramatically (1 -10 metres); seed 'broadcast' on to benthos either manually or hydraulically; can be harvested:
 - 'manually', using hand operated dredges
 - 'mechanically', using powered, vessel-based equipment
- Manual example from NZ, harvesting Littleneck Clams (A.stutchburyi) from Otago, for local and international (USA, EU) markets:





















- Effects of high density on-growing on local ecosystem:
 - Sedimentation (incl. lowered redox)
 - Removal of nutrients and phytoplankton





- Alien species:
 - Strong opposition to the introduction of any exotic species (even UK, despite tidal action from European mainland waters)
- Visual:
 - Inter-tidal farms can be perceived as visually intrusive in remote/tourism valued regions
- Denial of habitat:
 - Displacement of natural beach fauna, in particular birds





