FEASIBILITY OF AKOYA PEARL OYSTER CULTURE IN QUEENSLAND

Thesis submitted by

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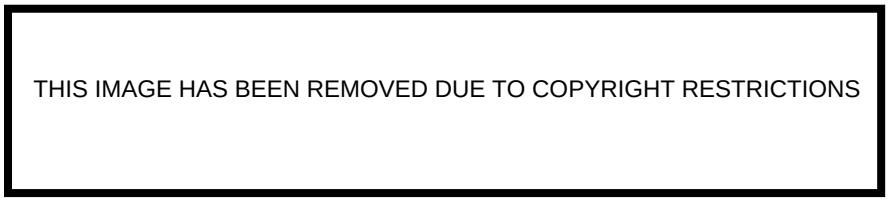
Statement on the Contribution of Others

Financial support was gratefully contributed by Barrier Pearls Pty Ltd and James Cook University.

The following people in addition to my supervisor provided editorial support which aided in the preparation of my thesis: Dr. Brad Evans, Dr. Dean Jerry, Dr. Jens Knauer, Dr. Wayne O'Connor and Andrew Hoey



(Photo: Andrew Beer)

Frontispiece: Pearls and pearl jewellery date back to the Roman Empire where it has been noted by historians that an entire military campaign was financed through the selling of one single pearl (Anon 1998).

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Firstly I would like to thank Assoc. Prof. Paul C. Southgate for his encouragements, support, help and advice throughout the last three years.

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Abstract

Pearl culture is the second largest aquaculture industry in terms of value in Australia. It is currently worth an estimated Aus$300 million annually and it is anticipated that the industry will be worth Aus$500 million by 2010. The Australian pearl industry is currently based on South Sea pearl production from the silver-lip pearl oyster, *Pinctada maxima*, for which it is world renowned. However, there has been recent interest in pearl production from two other major marine pearl oyster species, the blacklip pearl oyster, *P. margaritifera*, and the Akoya pearl oyster, *P. fucata*, which are both abundant in Australian waters.

Production of Akoya pearls, until recently was dominated by the Japanese. However, recent down-scaling of the Japanese pearl oyster industry due to factors that resulted in the death of millions of oysters, has presented an opportunity for other countries to enter the Akoya pearl market. Australia is one such country which has received a lot of interest in Akoya pearl production over the last 5-10 years because of:

(1) its reputation as a quality pearl producing nation;
(2) the clean non-polluted waters around Australia; and
(3) the wide distribution of Akoya oysters along the Australian coastline.

Consequently, there was a need for biological information on which the feasibility of Akoya pearl oyster culture in Australia could be assessed. The major objective of the current project was to develop techniques to determine whether Akoya pearl oyster culture is feasible in tropical north Queensland. The results of this study will compliment the results of research with similar goals conducted in temperate Australia

(New South Wales). The focus of this study was to produce Akoya pearl oysters in tropical Australia for the first time, before optimizing protocols for hatchery and nursery culture. This information was then utilized to suggest possible sites within Queensland which would be suitable for Akoya pearl oyster production, based on 'biological performance'.

The first successful culture of Akoya pearl oysters in Australia under tropical conditions produced 213 000 larvae which were transferred to settlement tanks. A total of 58 000 spat were subsequently transferred from settlement tank and resulted in 48 000 spat ranging in size from 2-30 mm at 3.5 months of age. These spat were produced using established protocols for other pearl oyster species. After 12 months, Akoya pearl oysters had a mean dorso-ventral shell height (DVH) of 56.2 ± 0.2 mm and showed superior growth rate to those reported for this species in more traditional culture regions (i.e. SE Asia).

This project investigated aspects of hatchery production including embryonic and larval development to identify optimal protocols for hatchery culture of Akoya pearl oysters (Chapters 4 and 5). Full orthogonal designs were established to investigate; (1) the effects of water temperature and salinity; and (2) the effects of density and addition of antibiotics on the development of *P. fucata* embryos into D-stage veligers. Maximum development of *P. fucata* embryos into D-stage veligers occurs within a water temperature range of 26-28°C and a salinity range of 28-32‰. Further results suggested that antibiotics are not required during embryonic development of *P. fucata* as development of larvae was not improved in the presence antibiotics. Results have also shown that maximum development of embryos into D-stage veliger occurred when larval stocking densities were low. Suggesting an ideal stocking density is strongly dependant on the individual hatchery and the production

goals. These results have obvious implications for the selection of sites for an Akoya oyster hatchery in Queensland. Ideally, a site should be selected in which water parameters are within the above-mentioned ranges.

A number of pearl oyster culture techniques were investigated during this project to optimise nursery culture of Akoya pearl oysters under Queensland (tropical) conditions. These included the effects of depth, stocking density, culture apparatus and fouling on the growth and survival of pearl oysters.

P. fucata spat were transferred from the hatchery to the long-line and placed in plastic mesh trays at three different depths, 2 m, 4 m, and 6 m. After 8 weeks on the long-line, spat cultured at 2 m were significantly ($p<0.05$) larger in DVH than spat at either 4 m or 6 m, which were not significantly different from each other. Additionally, greater numbers of 'large' spat were recorded when spat cultured at 2 m compared to spat cultured at either 4 m or 6 m.

Hatchery-produced *P. fucata* spat at 3.5 months of age were graded into three size classes, 'small', 'medium' and 'large', which for the purpose of the study were treated as 'slow', 'normal' and 'fast' growers, respectively. This study aimed to determine whether growth rates differed between oysters from the above-mentioned size classes. Results from this study suggest that when oysters are first graded at 3.5 months of age (8 weeks after transfer to the ocean) slow growing oysters should not be discarded (common practice by some pearl farmers within the industry). This is because slow growers, when compared to 'normal' growers, only require an additional 2-4 months before reaching pearl production size. The implications of retaining slow growers is discussed.

Hatchery-produced spat were cultured at different stocking densities to determine optimal growth and survival of *P. fucata*. Stocking densities were

determined on the basis of percentage of total available net area. In Experiment 1 during early nursery culture, spat were stocked at either 25%, 50% or 75% of total available net area. Maximum growth was recorded for spat cultured at the lowest stocking density (25% of total available net area), which were significantly larger that spat cultured at either 50% or 75% of total available net area. Furthermore, spat cultured at 25% of total available net area had significantly greater numbers of spat in the medium and large size classes than spat cultured at 50% or 75% total available net area. In Experiment 2 during late nursery culture, and based on the results from Experiment 1, spat were cultured at four stocking densities (20, 25, 30 and 40% of total available net area). Similar trends to those in Experiment 1 were recorded in Experiment 2 where spat cultured at the lower stocking densities were significantly larger than spat cultured at the other stocking densities. However, the overall growth performance (Φ') was greatest in spat cultured at the highest stocking density (40% of total available net area). Survival was not significantly different between treatments.

Two experiments were conducted with hatchery-produced *P. fucata* spat using four different culture units to determine which culture unit supported maximum growth and survival. In Experiment 1, the four treatments used were 'box', 'tray', 'pearl net' and 'pearl net with noodles'. While maximum growth, was recorded by oysters cultured in pearl nets, there was no significant difference in growth rate to oysters cultured in pearl nets with noodles; however, oysters cultured in the box treatment were significantly smaller than oysters in all other treatments. Survival of oysters in the box treatment was 47%, whereas, survival of spat cultured in the other three treatments was greater than 90%. In Experiment 2, the four treatments included 'pearl net with small mesh', 'pearl net with large mesh', 'panel net with small mesh', and 'panel net with large mesh'. Maximum growth in terms of DVH was recorded for oysters cultured in

panel nets with large mesh, followed by pearl nets with large mesh, pearl nets with small mesh and panel nets with small mesh. Survival was not significantly different between treatments, and all treatments recorded 85% or greater survival.

Site did not affect growth and survival in the present study when *P. fucata* were cultured at Orpheus Island and Magnetic Island for 12 months. Although slight variations in water temperature, salinity and chlorophyll 'a' were recorded between the two sites, no significant differences were recorded in overall oyster growth performance (Φ') of 3.81 and 3.82 for Orpheus Island and Magnetic Island, respectively. Site selection for pearl oyster culture is important if growth and survival are to be maximised during nursery culture. Akoya pearl oysters showed positive growth at all water temperatures experienced throughout this study; however, the range at which optimal growth occurred was between 25.1-28.1°C. Meanwhile, maximum growth occurred within salinity and chlorophyll 'a' ranges of 29-33‰ and 3.5-5.3 μg L^{-1}, respectively.

This project has produced biological information, which will provide a basis for the development of an Akoya pearl oyster industry in Queensland. Establishment of such an industry would compliment the current valuable pearl industry in Australia. While information generated during this study has answered a number of questions in terms of 'biological performance' there is, however, a requirement for further research to appraise pearl production from Akoya oysters in Queensland and factors influencing pearl quality.

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Chapter 1 General Introduction

1.1 General Biology

1.1.1 Taxonomy and Species Description

Pearl oysters belong to the family Pteriidae, which is comprised of three marine genera, *Pinctada, Pteria* and *Electroma* (Gervis and Sims 1992). There are currently three major species utilised for cultured pearl production; the silver- or gold-lip pearl oyster, *Pinctada maxima* (Jameson), the black-lip pearl oyster, *P. margaritifera* (Linnaeus) and the Japanese or Akoya pearl oyster, *P. fucata* (Gould). *Pinctada maxima* is found along the northern coasts of Australia and in Papua New Guinea, the Solomon Islands and throughout S.E. Asia (Gervis and Sims 1992). It is the basis for the Australian cultured pearl industry. *Pinctada margaritifera* has a wide Indo-West Pacific distribution, which includes Australia, French Polynesia, the Persian Gulf, the Red Sea, the south Pacific, Papua New Guinea, Japan and the south-western parts of the Indian Ocean (Anon 1991; Sims 1993). *Pinctada margaritifera* is the focus of the Pacific island pearl industries. *Pinctada fucata* occurs throughout S. E. Asia, Australia, India, Sri Lanka, the Red Sea and the Caribbean (Sims 1993) and is the major species in the Japanese cultured pearl industry (Fig. 1.1). There are also a number of other species used to produce pearls, but production from these species is relatively low. These species include *Pinctada mazatlanica*, and *Pteria sterna* (both cultured on the Pacific coast of Mexico) and *Pt. penguin* (cultured predominantly in Indonesia and Australia). Additionally, large numbers of pearls are produced from freshwater mussels in China (Dan and Ruobo 2003). However, pearls produced from freshwater mussels are typically smaller and less valuable than marine pearls.

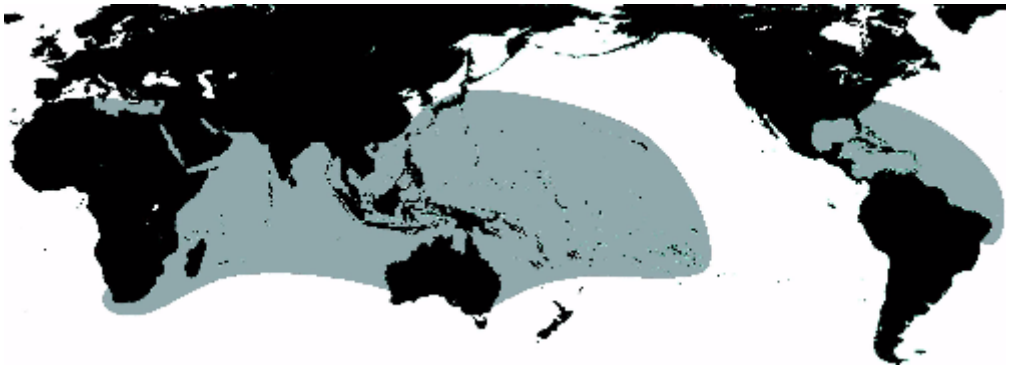


Fig. 1.1. Distribution of "Akoya" pearl oysters from Shirai (1994) and O'Connor *et al.* (2003).

As stated above, *P. fucata* has a wide distribution which has given rise to many names for this species. The generic common name "Akoya pearl oyster" is used loosely by scientists and industry personnel to describe *P. imbricata* (Roding, 1798), *P. radiata* (Leach, 1814), *P. vulgaris* (Schumacher, 1817), *P. fucata* (Gould, 1857) and *P. martensi* (Dunker, 1850). The species name used is most often dependant on location. For example, *P. imbricata* is used in the Caribbean, *P. radiata* in Iran and Sri Lanka, *P. fucata* in Japan, and *P. martensi* in China. While it has been suggested that *P. fucata*, *P. radiata* and *P. vulgaris* are synonymous (Hynd 1955), Shirai (1994) suggested that all the above are one species and should be correctly called *P. imbricata* due to taxonomic precedence. A recent electrophoretic study by Colgan and Ponder (2002) examined specimens from Australia (which they termed *P. imbricata*) and Japan (which they termed *P. fucata*) and found them to be similar and classified them as *P. imbricata*. However, Colgan and Ponder (2002) only examined specimens from 2

locations, Australia and Japan (which is thought to be the original stock for Australia), and did not compare these samples with *P. imbricata* from its type location in the Caribbean. On the basis of the findings of Colgan and Ponder (2002), O'Connor *et al.* (2003) used the name *P. imbricata* to describe work carried out on Akoya pearl oysters in New South Wales. However, Lamprell and Healy (1998) who published the only major work on Australian bivalves to include the Pteriidae, do not recognise *P. imbricata* as occurring in Australia; they refer to specimens of Akoya oysters in Australia as *P. fucata*. Additionally, Beer and Southgate (2000) identified pearl oysters (*Pinctada* spp. and *Pteria* spp.) in north Queensland from spat collection studies. They identified eight species of *Pinctada* and did not recognise *P. imbricata*, but rather identified *P. fucata*. More recently, Yu and Chu (2004) reported on genetic diversity of Akoya oysters from China, Japan and Australia and found them to be similar; they labelled them *P. fucata*. Further work needs to be carried out to determine whether population differences exist between geographical populations of Akoya pearl oysters and to resolve the taxonomic ambiguity associated with *P. imbricata* and *P. fucata*. Until this work is carried out, however, the status quo (e.g. Lamprell and Healy 1998) will be maintained, and *P. fucata* will be used to describe the Akoya pearl oyster throughout this thesis. Where researchers have used a different name, this will be indicated.

1.1.2 Reproduction

Many studies have been conducted on the reproductive biology of different species of *Pinctada* (Tranter 1958a, b, c, d; Rose *et al.* 1990; Saucedo *et al.* 2001). Pearl oysters have been shown to be protandrous hermaphrodites (Gervis and Sims 1992). While Hynd (1957) reported that about 30-40 % of oysters change from male to

female, others have suggested that the sex ratio approaches 1:1 by the age of four to five years (Gervis and Sims 1992; Sims 1993). However, this has been shown to vary, with mature pearl oysters held under culture conditions expressing a male: female sex ratio of 2:1 for *P. maxima* (Taylor 1999), *P. fucata*, and *P. margaritifera* (Pit unpublished data 1998-2003) and even as high as 3:1 for *P. margaritifera* (Pouvreau *et al.* 2000). This later high sex ratio in *P. margaritifera* has been partly attributed to stress caused by husbandry practises (Taylor 1999). Tranter (1958 b, c) suggested that as females have a higher energy requirement for oogenesis compared to spermatogenesis, areas with low food abundance favour 'maleness', while areas of high food abundance generally support a greater proportion of females within a population. Maturation and spawning patterns appear to be influenced mainly by water temperature (Tranter 1958d), but extreme changes in other environmental parameters, including salinity and food availability, have also been shown to stimulate oysters to spawn (Gervis and Sims 1992). Natural spawnings vary in pearl oysters; sub-tropical species show rather discrete spawning periods, while predominantly tropical species show protracted spawnings throughout the year, particularly in the warmer months (Tranter 1958d; Sims 1993).

1.1.3 Life History

Sperm and eggs are released into the water where external fertilisation takes place. Once the irregularly shaped eggs become hydrated and fertilised, they attain a spherical shape (Fig. 1.2)(Tranter 1958d). After 24 h, the embryo develops a shell becoming a 'D-stage' veliger larva of approximately 65-85 μm (depending on the species)(Wada 1991; Doroudi and Southgate 2003). Veligers are able to swim using their ciliated velum. As veligers are phototaxic, they remain close to the water's

surface (Nayar *et al.* 1978). Pearl oyster larvae become globular in shape forming an umbo at 8-10 days when they measure approximately 100-140 μm. Approximately 15-16 days after fertilisation, veligers develop a pigmented 'eye' spot on their dorsal side; they are then termed 'eyed' veligers, measuring approximately 210 μm (Fig. 1.2) (Alagarswami *et al.* 1983, 1989; Taylor 1999). The development of a functional foot is generally seen around 20 days of age when larvae are 220-260 μm in length; they are now termed pediveligers (Gervis and Sims 1992) and are competent to settle (McCue 1992).

The planktonic larval period varies from 2-4 weeks depending on species, environmental conditions, food availability and the presence of a suitable substrate on which to settle (Gervis and Sims 1992). Once settled, oysters undergo metamorphosis and become a 'plantigrade' (Alagarswami *et al.* 1983). At this stage, the velum is resorbed and the post-larval oysters develop labial palps and gill filaments, which eventually develop into gills that capture food and undertake gaseous exchange (Alagarswami *et al.* 1983). The term 'spat' or 'seed', which may be used as a singular or plural noun, is used for young juvenile oysters. Spat bysally attach to natural or artificial substrates and newly settled spat, approximately 25 days after fertilisation, are typically around or greater than 350 μm (Alagarswami *et al.* 1989; Rose 1990). Pearl oyster spat, which resemble the adult shape, continue growing for approximately 18-24 months before they can be used for pearl production. The minimum dorso-ventral shell size (shell height) required for pearl production varies between species (e.g. *P. fucata* >50 mm, *P. margaritifera* >100 mm, and *P. maxima* >120 mm; Gervis and Sims 1992; Sims 1993).

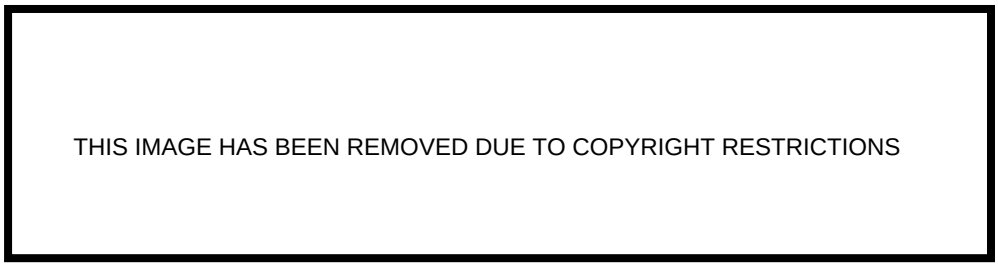


Fig. 1.2. Generalised life cycle of pearl oysters (adapted from Southgate and Lucas 2003).

1.2 Pearl Oysters and their Pearls… A History of Pearling

Records dating back to 4200 B.C. show that the first use of pearl oysters was for their shell, or mother-of-pearl (MOP). They were used for manufacture of items such as buttons and decorations, or were shaped into tools. Pearls were simply a lucrative by-product of the MOP harvest. Although only a by-product (Dybdahl and Rose 1985), pearls which are commonly referred to as the "Queen of the Gems" (Ward 1995) are

quite different to other gems such as diamonds, as they are produced by living animals. Many regions of the world including the Red Sea, the Persian Gulf, the Gulf of Mannar (India) and Sri Lanka had good populations of pearl oysters. However, by the mid 19th century many pearl fishing regions began to show signs of over-exploitation, due to increased harvesting to collect the illustrious pearl (Saville-Kent 1890). This led to a major decrease in wild pearl oyster stocks and provided an impetus for investigations into the possibility of artificial cultivation of pearls (Saville-Kent 1890; Pace 1899).

Artificial pearl culture is believed to have begun in the late 19th and early 20th centuries. However, there is anecdotal evidence that semi-spherical pearls (and small buddha figurines) were produced by placing foreign objects (nuclei) between the shell and the mantle tissue of mussels in the 13th century in China (Taylor 1984). In Japan towards the end of the 19th century, experiments were conducted by Kokichi Mikimoto in which half pearls (mabe) were successfully produced using a similar method (Wada 1991). In 1907, a Japanese government biologist, Tokichi Nishikawa, and a carpenter, Tatsuhei Mise, independently applied for patents for their discovery of pearl production in mantle tissue. They had both discovered that by introducing a piece of mantle tissue into a pearl oyster with a nucleus of shell or metal, a pearl sac formed in which nacre was secreted around the nucleus. After much debate, Nishikawa and Mise were awarded a joint patent for the process known as the Mise-Nishikawa method (O'Sullivan 1998). In 1908, Kokichi Mikimoto also applied for a patent for pearl production using mantle tissue, only to realise that Mise and Nishikawa had already been awarded a patent for this process. Mikimoto altered his patent application to include a technique for 'round' pearl production.

Many believe however, that an English-born Australian fisheries officer, William Saville-Kent, pre-dated the work of the Japanese by two decades with many

successful experiments culturing round pearls in the Torres Strait (George 1978; O'Sullivan 1998). Around the same time as Saville-Kent and the three Japanese, an Indian biologist, James Hornell, was also believed to have mastered the technique of pearl production by producing six free Akoya pearls in 1908 (Silas 2003). Although there is some debate about who was first to develop techniques to produce cultured spherical pearls, Kokichi Mikimoto dominated the industry and has long been regarded as the "founding father" of pearl culture despite releasing his patent and adopting the technique of Mise and Nishikawa. This technique is still used today.

1.3 Australian Pearling: How it began

1.3.1 Wild Shell Collection

The Australian pearl oyster industry began before the first Europeans settled the country. Aborigines harvested pearl shell (*P. maxima*) from the shallow warm waters of tropical northern Australia and had domestic and international trading (with south Pacific Islanders and neighbouring Papuans) in the 17th and 18th centuries (Anon 2000). However, the first pearl discovered in Australia was not documented until 1812 (Taylor 1984). The Australian industry began in earnest in the 1860's in the tropical waters of Western Australia (WA), Queensland (Qld) and the Northern Territory (NT), where the vast majority of pearl shell was collected.

In WA, shell was collected from Shark Bay by wading through the water with collection baskets (Anon 2000). However, through over-exploitation, pearl oysters from the shallow waters became scarce, forcing shell collectors into deeper water. With the introduction of large motorised boats (pearl luggers) in the early 1870's, pearl shell collectors were able to access oyster beds miles off the coastline by free diving. As offshore stocks also became scarce, the fleet of luggers moved north into Roebuck Bay

(in which Broome is now situated) in search of pearl shell (Anon 2000). In the mid 1880's, the introduction of the hard hat helmet revolutionised the industry allowing divers to stay on the bottom for greater lengths of time to search for shell in previously untouched pearling grounds. This also saw the introduction of Japanese and Malay divers as they were far more efficient than the aborigines (Anon 2000).

Concurrently, pearl shell was harvested in 1868 in the Torres Strait of Qld, while pearling began in NT when pearl shell was collected from Darwin Harbour in 1884. The collection of pearl shell in the NT continued throughout the late 1800's and early 1900's on a very small scale. Due to stocks in Qld being fished to almost local extinction, pearl luggers from Qld moved to Roebuck Bay in the 1880's (Anon 2000). By the end of the 1890's, due to decreasing world demand, overfishing, and the introduction of government closures in WA on selected pearling grounds, the Australian wild-collected pearl industry had become depressed. It remained that way until after World War I when it went through a slight recovery. In response to the economic down-turn caused by the "great depression" and the invention of plastic buttons after World War II, the industry finally collapsed (O'Sullivan *et al.* 1995).

1.3.2 Artificial Pearl Production in Australia

1.3.2.1 *Western Australia*

A government scientist allegedly produced the first cultured pearl in WA during the late 19th century (O'Sullivan 1998). When attempts were made to commercialise cultured pearls in the 1920's, the WA government amended the Pearling Act to prohibit production, sale and possession of cultured pearls in an attempt to prohibit the overfishing of potential culture stocks. This unfortunately allowed Japan to dominate the fledgling world pearling industry. However, the Pearling Act was further amended

in 1949, and again in 1956, and the first cultured pearl farm, Pearls Pty Ltd (O'Sullivan *et al.* 1995) owned by Australian and Japanese private companies began in WA and harvested their first cultured pearls in 1957.

The cultured pearl industry in WA, which was traditionally based on collecting adult pearl oysters from the wild (Scoones 1988) continued to flourish from 1960 until 1984 when a bacteria (*Vibrio harveyi*) caused large mortality within the fishing grounds; this bacterium proliferated due to poor husbandry practices, particularly during oyster transportation from offshore fishing grounds to the farms (Dybdahl and Rose 1985). As a result, the pearling industry was regulated to overcome this problem, with an allocation of 16 licences (Ryan and O'Sullivan 2001). Currently, each licence allows collection of 100 000 wild collected pearl oysters per annum, as well as an allocation of up to 20 000 hatchery produced pearl oysters into the farm each year. More emphasis is slowly being placed on hatchery-produced spat as they can be produced in reliable numbers throughout the year and stocks can be potentially selected for commercially desirable traits such as fast growth rates and nacre (MOP) colouration (section 1.4). Western Australia pearl farmers currently generate Aus$200 million annually producing a product, which is marketed as the illustrious and renowned 'South Sea' pearl (O'Sullivan and Savage 2003).

1.3.2.2 *Queensland and the Northern Territory*

The first cultured pearl in Qld was produced by a Qld government scientist, William Saville-Kent, in the mid-late 19th century. While Saville-Kent tried to patent his idea, his death in 1909 ceased any patent actions. The first commercial pearl farm in Qld was a joint Australian/Japanese farm that began in 1950. However, in 1969 the oil tanker "Oceanic Grandeur" ran aground in the Torres Strait causing a catastrophic

oil spill that resulted in massive mortality (~ 90%) of both the wild and cultured pearl oyster stocks. This incident virtually destroyed the developing Qld pearl industry which was valued at around Aus$4.5 million at the time of the spill (Franklin 1973). Despite this set back, the Qld pearl industry continued to grow and, by 1982, was based on six companies with a total farming area estimated to be 2 264 hectares. Production by these six companies was estimated at 12 700 round pearls and 175 000 half pearls per annum (Anon 2003). The number of companies increased to nine in 1997, although total farming area decreased to an estimated 1 820 hectares. Since then, the industry has struggled to expand and currently there are only five pearl producing farms in Qld and four in the NT (Anon 2003).

1.4 Hatchery Production: How and Why it Started

As previously mentioned the Australian pearl oyster industry was traditionally based on collection of adult pearl oysters (*P. maxima*) from the wild (Scoones 1988). In other regions (e.g. French Polynesia), pearls are produced from wild oysters (*P. margaritifera*) collected as spat or juveniles (Friedman and Bell 1996). These wild spat are collected using 'spat collectors' made of natural or synthetic materials, which are placed into the sea to provide a settlement substrate for pearl oyster larvae.

Due to the over-exploitation of adult pearl oyster stocks and the subsequent decrease in spat recruitment, wide variability in wild collection of pearl oysters is increasing in certain countries such as French Polynesia (Dybdahl and Rose 1985). However, gradual development of hatchery techniques to produce pearl oyster larvae and spat has alleviated this problem by decreasing pressures on wild stocks (Alagarswami *et al.* 1983; Rose 1990; Southgate and Beer 1997). As well as reducing reliance on wild pearl oyster stocks, hatchery production allows greater control over

pearl oyster culture systems and perhaps more importantly, manipulation of genetic characteristics of farmed pearl oyster stocks (Gervis and Sims 1992; Wada and Komaru 1994).

Hatchery techniques for pearl oysters have been developed relatively recently (Rose and Baker 1994; Southgate and Beer 1997; O'Connor *et al.* 2003) and have been based on techniques used for other bivalves (e.g. Loosanoff and Davis 1963). As a result, the cultured pearl industry has become less reliant on wild collected stocks with growing interest in hatchery produced spat. Methods currently used for artificial propagation of pearl oysters include induction of adult spawning, larval rearing, nursery culture of spat, and production of micro-algae as a food source and will be discussed separately (see sections 1.4.1-1.4.5)

Hatchery propagation of pearl oysters is currently carried out for *P. maxima* in Australia (Rose and Baker 1994) and throughout S.E Asia (Fassler 1995), for *P. margaritifera* in India (Alagarswami *et al.* 1989), Australia (Southgate and Beer 1997), Hawaii (Clarke *et al.* 1996) Kiribati (Southgate and Beer 1996), the Cook Islands, Tonga and French Polynesia (Southgate 1996), and for *P. fucata* in India (Alagarswami *et al.* 1983), Japan (Wada and Komaru 1994) the Caribbean (Urban 2000a,b) and China (O'Connor *et al.* 2003). A summary of methods employed for hatchery production of Akoya pearl oysters in different countries is outlines in Table 1.1

Table 1.1. Comparison of hatchery production methods used for Akoya pearl oysters

Method	Japan	China	India	Australia
Spawning (induction)	Natural Thermal Chemical-Ammonia	Natural Thermal Stripped	Natural Thermal Chemical-pH	Natural Thermal
Fertilisation		In situ	In situ	In situ
Embryo Tank Size	1000 l	Small tanks 50-60.ml^{-1}	1000 l	500-1000 l 20-50.ml^{-1}
Time to D-stage		24 hrs	20 hrs	20-24 hrs
Larval Density	10.ml^{-1}		2.ml^{-1}	1-10.ml^{-1}
Larval Tank Size	1000 l	20 000 l	1000 l	500-20 000 l
Water Change	Every 3 days	Daily	Every 2 days	Every2-3 days
Feeding rate			D2-10 5 000 cells.ml^{-1} D10-18 10 000 " D18-24 15 000 " D24-30 20 000 " D30-45 30 000 " D45+ 50 000 "	D1-5 5 000 cells.ml^{-1} and increases by 1000 cells.ml^{-1} per day to a maximum of 45 000 cells.ml^{-1} **OR** FR=0.0578 x SL$^{2.3441}$*
Settlement	Water changed daily	Water changed daily	Water changed/2 days	Water changed daily 3 weeks

in different countries.

References: Anon (1991); Guo etal. (1999); Hideki, Barrier Pearls (2000-pers comm.); O'Connor and Wang (2002); O'Connor et al. (2003)
* O'Connor et al. (2003)
FR-feeding rate calculated as number of cells per larvae per ml of culture water.
SL-standard length

1.4.1 Spawning

Spawning begins with the collection and transportation of broodstock, or parent stock, to the hatchery. On arrival at the hatchery the broodstock are cleaned in preparation for spawning. A number of spawning induction methods are used, but the

most common is manipulation of water temperature. Alagarswami *et al.* (1983) induced 87.5% of *P. fucata* to spawn by increasing temperature from 28.5°C to 35°C. More recently, Southgate and Beer (1997) developed 'cold conditioning', whereby *P. margaritifera* were transferred from ambient water temperatures (30°C) and placed into a cooled water bath at 22°C overnight. The following day, broodstock were transferred from the cooled water bath into a spawning induction tray where water temperature was 30°C; this resulted in both males and females spawning within 1 h. This method has been used successfully in our laboratory at Orpheus Island Research Station for the last 6 years (Chapter 2, section 2.2).

Alternatively, oysters can be strip spawned. Stripping involves sacrificing individuals and is considered undesirable in the pearl oyster industry due to the value of the broodstock. Minaur (1969) and Tanaka and Kumeta (1981) strip spawned *P. maxima* with mixed results; fertilisation and subsequent larval growth were greater when oysters spontaneously spawned, compared to those that were strip spawned (Tanaka and Kumeta 1981).

1.4.2 Larval Rearing

Pearl oyster larvae are usually reared in static water systems using tanks of 500 to 10 000 l capacity (Wada 1991), where water is exchanged approximately every two days (Alagarswami *et al.* 1989; Southgate 1995). At each water change, larvae are removed from their tanks and retained on submerged mesh sieves, where growth and survival can be determined (Rose 1990; Southgate 1995). Larvae are then returned to similar tanks, which have been cleaned and refilled with new filtered (1 μm) seawater. Larvae can also be cultured in 'flow-through' tanks where water is exchanged without the removal of larvae (Southgate and Ito 1998). Water flows out of the tank and

through a mesh screen. This prevents larvae escaping and is usually used once the larvae are a minimal of 10 days of age as the larvae are considered more robust. Flow-through designs vary from constant water flow-through to partial water flow-through where an adequate water volume is exchanged; usually 100% per day. Larvae are fed daily with micro-algae (section 1.4.3) and larval rearing lasts for approximately 3 weeks.

1.4.3 Micro-algae Production

Production of micro-algae requires specialised facilities, qualified technical services and is very expensive (Southgate 2003). It has been estimated that the supply of food to bivalve larvae comprises 30-50% of total hatchery running costs (Jeffrey and Garland 1987). Micro-algae used for the culture of pearl oysters is generally a mixture of three genera, the Prymnesiophytes *Isochrysis* spp. and *Pavlova* spp., and the diatoms *Chaetoceros* spp. (Rose 1990; Rose and Baker 1994; Southgate *et al.* 1998a). The importance of using healthy monospecific cultures of micro-algae has been emphasized in order to minimise bacterial outbreaks (Walne 1958; Guillard 1959; Minaur 1969). These three species have been selected based on previous results which have shown them to be successful in the culture of pearl oyster larvae in the tropics.

Micro-algae density is increased with increasing larval age and is dependent on larval density. Southgate and Beer (1997) generated a simplified feeding ration scale for *P. margaritifera* larvae ranging from 1 000 cells mL^{-1} on day 1 through to 35 000 cells mL^{-1} on day 43, assuming an initial larval density of 2 larvae mL^{-1} (section 2.2.6). In more recent research, Doroudi *et al.* (1999a) tried to further simplify this feeding regime, by feeding larvae with a single micro-algae density until larvae had settled. Doroudi *et al.* (1999a) noted that optimum growth occurred in larvae fed at a density of

20 000 cells mL^{-1} and maximum larval survival at a density of 10 000 cells mL^{-1}. However, the authors did not discuss the implications of a high feeding density during the first week of larval culture, which may contribute to poor water quality due to possible uneaten food. Neither did they discuss the implications of a low feeding density at the end of larval development in which density may be sub-optimal.

1.4.4 Settlement

After approximately 3 weeks in larval rearing tanks, larvae are competent to settle (section 1.1.3). Optimal settlement under hatchery conditions occurs using dark collectors such as black plastic or shade cloth (Coeroli *et al.* 1984; Rose and Baker 1989; Rose 1990; Southgate and Beer 1997); however, many other substrates have been used successfully. Rose and Baker (1989) showed that *P. maxima* larvae settled on beige and grey plastic plates, monofilament fishing line and plastic netting. Taylor *et al.* (1998a) found that *P. maxima* larvae prefer polypropylene rope and a combination of rope and PVC slats to monofilament nylon or plain PVC collectors. Settlement of pearl oysters has been greatly improved through the addition of chemicals (i.e. GABA) into larval rearing tanks containing spat collectors (Doroudi and Southgate 2002). Settlement of pearl oyster larvae may last up to a week (Rose 1990) and spat remain in settlement tanks for approximately 2-4 weeks (Southgate and Beer 1997; Pit and Southgate 2000), before being transferred to the nursery site. Therefore, pearl oysters spend approximately 3 weeks in larval rearing systems and 2-4 weeks in settlement tanks prior to being transferred to the ocean for nursery culture.

1.4.5 Nursery Culture

Nursery culture can be subdivided into early nursery culture and late nursery culture (Friedman and Southgate 1999). Early nursery culture usually involves the period immediately following transfer of spat from the hatchery to the ocean. High mortality is often associated with spat transfer due to changes in environmental factors such as water temperature, salinity, currents, and changes in composition and abundance of food sources (Pit and Southgate 2000). In addition, spat are also exposed to predators, which they had previously been protected from during hatchery culture. Pit and Southgate (2000) showed that optimal growth and survival of *P. margaritifera* was achieved with oysters which are transferred from the hatchery 3 weeks after settlement, compared with oysters transferred 5, 7 and 9 weeks after settlement. They attributed this to a better balanced diet available in natural waters compared with a ternary micro-algae diet fed during hatchery culture. Early nursery culture lasts for approximately 3 months or until spat/juveniles attain an approximate size of 30 mm in dorso-ventral shell height. During early nursery culture, oysters are placed in areas of calm conditions on the farm and given additional care in regards to careful handling to minimise stress and subsequent mortality. Oysters are then placed into late nursery culture.

A variety of culture units (Chapter 2, Fig. 2.4) are used for early nursery and late nursery culture of pearl oysters including pearl nets (Gaytan-Mondragon *et al.* 1993) plastic trays (Southgate and Beer 1997) and panel nets (Gervis and Sims 1992). These nets are generally selected on the basis of price, availability and ease of storage (Gervis and Sims 1992). They are generally suspended at depths of 3-10 m from either a floating raft or a long-line (section 2.4). Mesh size of the nursery culture nets is usually increased and the density of pearl oyster juveniles decreased as the oysters

grow; this reduces fouling and increases water-flow through the net ensuring an adequate food supply and more rapid growth (Gervis and Sims 1992). Culture methods other than suspended nets include trestle frames, which are elevated off the seafloor and are usually covered in shade cloth or plastic mesh to exclude potential predators (Dybdahl *et al.* 1990), and plastic trays which are an increasingly popular method for nursery culture of pearl oysters (Fig 2.3).

The nursery stages (early and late) generally lasts for approximately 1-2 years depending on species (Gervis and Sims 1992) or until they reach an appropriate size for pearl production (section 1.1.3). At this point the oysters can be held with the adult oysters and join the rest of the farm stock.

1.5 How Pearls are Produced

Cultured pearls are produced from a modification of the process of natural pearl formation. A natural pearl is formed when a foreign object/body, such as a parasite or a grain of sand, becomes lodged within the tissues of a pearl oyster. If the foreign object is unable to be dislodged, the oyster begins to coat it with MOP or nacre. This is a self-defence mechanism that ensures the object does not harm or kill the oyster. Man has learnt to utilise this process to routinely produce cultured pearls of good quality. The process of producing a cultured pearl is often referred to as 'seeding' or 'grafting' and is also known as the 'operation'. Seeding involves the insertion of a foreign object known as the 'nucleus' (usually machined Mississippi freshwater mussel shell) along with a piece of mantle tissue called the 'saibo' into a recipient oyster.

In order to produce a pearl, two pearl oysters must be used, the donor oyster, which is generally sacrificed to produce the saibo and the recipient oyster from which the pearl is produced (carrier) (Acosta-Salmon *et al.* 2004). One donor oyster,

dependent on size and health, can be used for up to 25 recipient oyster saibo pieces (Scoones 1990). An incision is made into the gonad of the recipient oyster and the saibo is placed into the gonad with the nucleus placed on top (Fig. 1.3). It is very important that the cells which secrete nacre are placed facing the nucleus. Over the next few days, the mantle tissue begins to grow around the nucleus until it creates a pearl sac after approximately 7 days (Kafuku and Ikenoue 1983). Three layers are laid on the nucleus, the periostracum after 15 days, followed by the prismatic layer after 30 days, and finally the nacre layer which begins after approximately 40 days (Kafuku and Ikenoue 1983; Gervis and Sims 1992) (Fig. 1.3). Nacre is then continuously deposited at 0-7 layers per day for approximately 2 years, at which time the nucleus is covered by between 0.5-2 mm of nacre. The rate of nacre production and final thickness is species-specific and dependant on environmental conditions and the health of the oyster (Gervis and Sims 1992). While the established process for pearl production calls for saibo donor oysters to be sacrificed, a recent study has shown that saibo can be removed from donors without killing them (Acosta-Salmon *et al.* 2004). Furthermore, saibo donors regenerate excised saibo tissue which raises the possibility of repeated saibo donations from a single donor or its use as future broodstock (Acosta-Salmon *et al.* 2004).

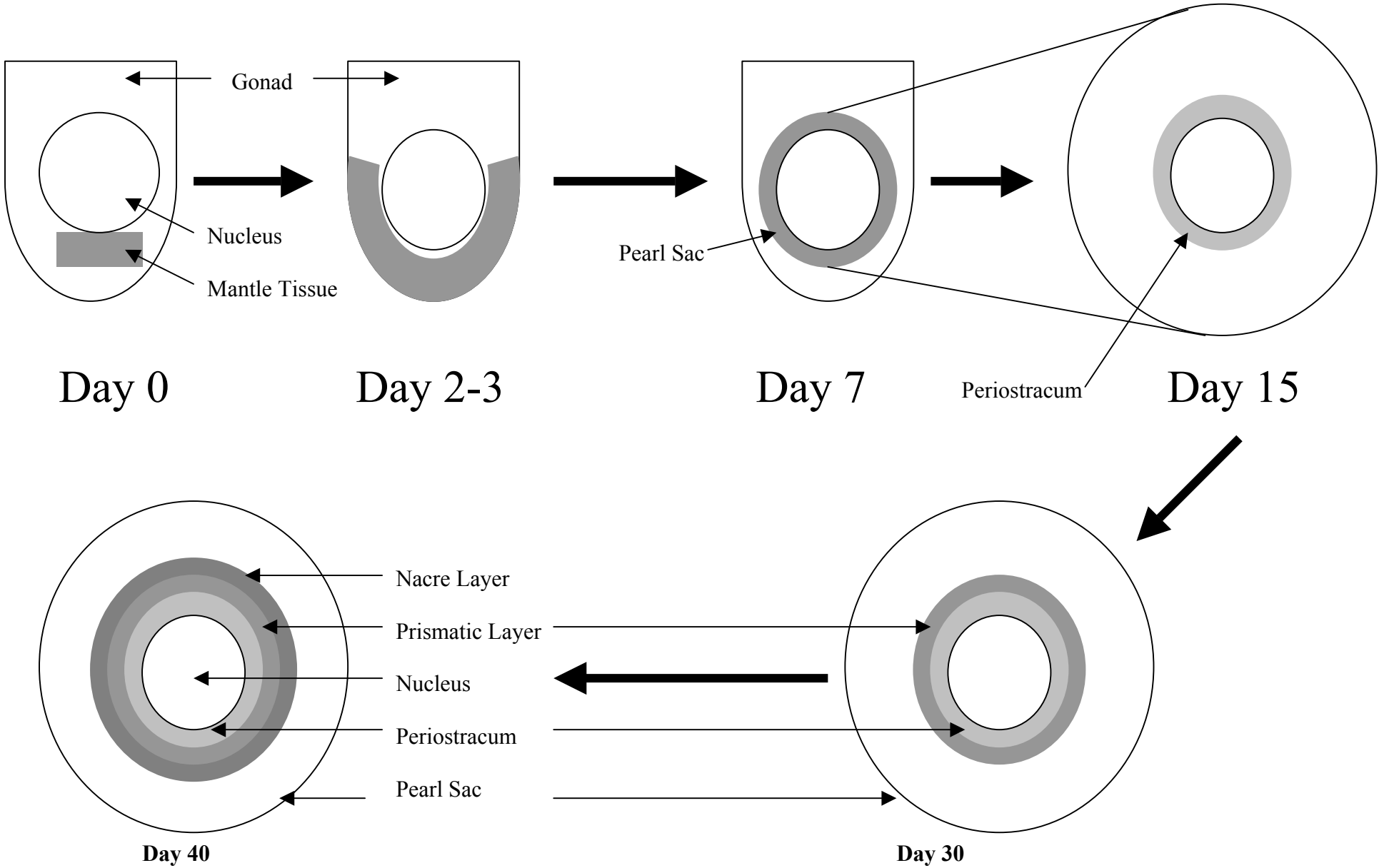


Fig. 1.3. Diagrammatic representation of artificial pearl formation.

1.6 History and Current Status of Akoya Pearl Culture

Akoya pearls are renowned for their lustrous white colour with cream and pink overtones and were once only worn on formal occasions. However, the increase in production of South Sea white pearls, Tahitian black pearls and the availability of lesser value pearls, such as freshwater pearls from China, have made pearls readily available, more affordable, and an item that is worn both casually and formally. Typically, Akoya pearl oysters are seeded at approximately 1-2 years of age when they measure 50-100 mm in dorso-ventral shell height (Victor *et al.* 1995; Guo *et al.* 1999). While up to 3 nuclei can be placed into a single oyster (O'Connor, W., New South Wales Fisheries, pers comm. 2001), usually only 2 are inserted and they are left for approximately 12-24 months until pearls are harvested. Typically, Akoya pearl oysters are only seeded once, and are sacrificed during pearl harvest, whereas *P. maxima* and *P. margaritifera* are re-seeded 3-4 times depending on harvested pearl quality. Harvested Akoya pearls are generally 4-8 mm in diameter with a nacre thickness of 0.5-1.0 mm (Gervis and Sims 1992). Current estimates suggest that only 50% of all seeded Akoya pearl oysters survive the operation and then only 5% of survivors produce high quality pearls (Anon 1998).

1.6.1 Japan

Akoya pearl oyster culture began in earnest in Japan when Kokichi Mikimoto began commercial culture of Akoya pearls in the 1920's. Japan was the world leader in both pearl production and pearl technology until the mid 20th century. However, increasing pollution from post World War II industrialisation, and challenges from other nations entering into pearl production, began to undermine Japan's dominance of the industry. Despite these pressures, Japan maintained a strong market hold producing

approximately 60-90 % of the annual total pearl production until approximately 1980, largely due to the allure of high quality Japanese Akoya pearls (ABARE 2003). Increasing urbanisation and industrialisation in the 1990's along the vast majority of Japan's coastline decreased areas suitable for pearl culture. In response, farms increased stocking density on their sites and the spatial separation between adjacent farms was reduced.

In 1996 the Japanese Akoya pearl oyster industry accounted for 66 % of the total world pearl production. However, due to mass mortality of pearl oysters, in which it was estimated that 150 million oysters died in 1996 alone due to an unidentified disease, Japan's monopoly of world pearl production scene began to decline (Beard and Wade 2002). By 2000, Japanese Akoya pearls only occupied 21% of the total market. Mortality rose a further 10% in 2001 and, by 2002, Japanese Akoya production had fallen to 30 tonnes; 13% of their 1996 production (Dettmar 2002). As a result of increasing urbanisation and industrialisation coupled withy decreasing world Akoya pearl production, there has been increasing interest in development of Akoya pearl production in other countries.

In an attempt to resurrect their Akoya pearl industry, Japan partnered with China to produce a hybrid Akoya pearl oyster by crossing the Japanese Akoya oyster (=*P. fucata*) with the Chinese Akoya oyster (=*P. martensi*) (Beard and Wade 2002). However, the hybrid expressed slower growth and decreased nacre quality and was subsequently not considered commercially viable (Beard and Wade 2002).

1.6.2 Vietnam

The Vietnamese Akoya industry was established in 1972 and bases its production on native pearl oysters, *P. fucata* (Quick 1999). Production to date has been restricted to

relatively small 2-6 mm pearls. However, with the decrease in production from Japan, it is anticipated that Vietnam will begin to supply the world Akoya market once the industry becomes more developed and breeding populations are more advanced. Production in 2001 was expected to reach 1000 kg (Quick 1999), however, no reliable figures are available.

1.6.3 China

The history and production of pearls in China dates back to the 13th century when semi-spherical pearls were produced from freshwater mussels. Freshwater pearls still dominate the Chinese industry, however, China has been producing Akoya pearls since 1970. Akoya pearl oyster culture in China occurs in three provinces, Guangxi, Gaungdong and Hainan (O'Connor and Wang 2002). Pearl production in China escalated from 300 kg 1970 to 15 tonnes by 1995 (Wang A. in O'Connor *et al.* 2003). Their success has been attributed to the healthy stocks of Akoya oysters which are abundant in China, relatively clean water, inexpensive labour, vast areas of sheltered bays for pearl culture and a desire to become a player in world Akoya production. However, production has been largely restricted to relatively small pearls as the Chinese have yet to successfully produce sufficient quantities of good quality Akoya pearls greater than 7.5 mm (O'Connor and Wang 2002).

1.6.4 India

India has a long tradition of pearling, with the Gulf of Mannar being one of the traditional pearling grounds of the world. However, the great pearl grounds of India were declared closed in 1963 due to overfishing. In the early 1970's a team of biologists led by Dr K. Alagarswami investigated the feasibility of Akoya pearl

production in India using *P. fucata*, based on methods developed for Japanese pearl production (Alagarswami 1987). While India has the potential to supplement world Akoya production, there are a number of factors which first need to be overcome including their lack of pearl production technology and the limited area available for pearl production. Most of the Indian coast is either heavily affected by adverse weather conditions or by pollution/industrialisation rendering it unsuitable for pearl production (Kumaraswamy-Achary 2003).

While Japan has dominated the Akoya pearl industry over the past 100 years, the great mortality of pearl oysters and decline in pearl production from the Japanese pearl industry over the last 5-10 years has presented opportunity for other nations, including Australia, China, India, Vietnam and regions throughout the Caribbean, to enter the Akoya pearl producing market (Pit and O'Connor 2003). Of these nations only three, Vietnam, China and India are currently producing high quality Akoya pearls on a commercial scale. It has been suggested that Vietnam and China, and to a lesser extent India and Australia, may become competitors to Japan in the Akoya pearl production market. While this may be the case in the short to medium term, it is difficult to forecast when, and if, the Japanese Akoya industry will recover. On this basis it is essential that other nations with potential for Akoya pearl production continue to develop Akoya pearl industries.

1.7 Akoya Culture in Australia

1.7.1 Source of oysters

Pinctada fucata is distributed throughout northern Australia, southward to Shark Bay in Western Australia and to Sydney in New South Wales (Jameson 1901). However, two specimens housed in the Australian Museum were collected from

'Victoria' and therefore Hynd (1955) extended the eastern distribution to include north eastern Victoria. Additionally, *P. fucata* has been found south of Shark Bay at the Abrolhos Islands (A. Beer, Department of Fisheries, Western Australia data, pers comm.). Large beds of this species have been described from Groote Eylandt in the Gulf of Carpentaria and also from Moreton Bay near Brisbane. While beds in Groote Eylandt were located at a depth of 22 m, specimens in Moreton Bay were associated with grassy intertidal banks just above low water spring tides (Hynd 1955).

1.7.2 Why is there no Australian Akoya Industry?

The decline in world Akoya production has increased demand for pearls in the smaller size range (5-10 mm). Having already been recognised as a quality pearl producing nation, this is a great opportunity for Australia to diversify in an ever-evolving industry. However, there has been a lot of recent interest in diversifying into other species, such as *P. margaritifera* and *Pt. penguin*. Although natural stocks of *P. fucata* are quite substantial in Australian waters, there has been little interest to commercially culture this species in Australia until recently. Researchers are now investigating the feasibility of Akoya culture in Australia. Initially, work was conducted in NSW (O'Connor *et al.* 2003) to identify the potential of Akoya culture in similar temperate conditions to those experienced in Japan; this research was supported by private industry. From the results generated in the NSW study, a commercial pearl oyster venture has begun operation. Approximately 1 year after research began in NSW, a similar project was initiated to determine the feasibility of Akoya pearl culture in tropical conditions within Australia. The latter forms the basis of this thesis.

Work conducted in temperate Australia investigated aspects of larval and nursery culture of Akoya oysters with emphasis on hatchery production and site

selection for early nursery culture. The work of O'Connor *et al.* (2003) was similar to the current study in which they only selected certain aspects of hatchery and nursery culture, with an emphasis on feeding experiments, which is why no feeding trials were conducted in the present study. When determining what aspects of pearl culture to include in this thesis, efforts were made to investigate aspects that had not already been covered by O'Connor *et al.* (2003), however, considerations of the effect of tropical vs temperate were made and some work, such as growth and survival of oysters cultured in different mesh sized nets, was essential due to the varying environmental conditions including fouling and predator compositions.

1.7.3 Benefits of Akoya Pearl Culture in Australia.

Aside from filling the niche market for small pearls, there are a number of advantages in producing pearls from *P. fucata* in Australia. Compared with the two larger species, *P. fucata* can be used for pearl production at a relatively small size (>50 mm), whereas *P. maxima* and *P. margaritifera* need to be a minimum of 100 and 120 mm, respectively. *Pinctada maxima* and *P. margaritifera* therefore require to be a minimum of 2 years of age before being used for pearl production compared to 1 year for *P. fucata*. The industry standard for nacre thickness on cultured pearls usually requires 1.5-2 year's growth. Therefore, pearls produced by *P. fucata* can be harvested from hatchery produced oysters within 2.5-3 years, whereas pearl production from *P. maxima* and *P. margaritifera* takes a minimum of 3.5-4 years. In addition, the shape of *P. fucata* allows for two and sometimes three nuclei to be implanted into a single oyster, whereas only one nucleus is implanted into both *P. maxima* and *P. margaritifera*. On this basis, *P. fucata* is able to produce at least twice as many pearls in a lesser amount of time compared with the other two major pearl producing species.

While numerous studies have investigated different facets of *P. fucata* culture, this has largely been based on wild collected spat, rather than hatchery produced spat. Research to date with hatchery produced *P. fucata* has been very limited to only a few studies (Alagarswami *et al.* 1983; Wada and Komaru 1994; O' Connor *et al.* 2003).

The Australian pearl oyster industry is currently the second largest aquaculture industry in Australia (O'Sullivan and Dobson 2001). It is proposed that the establishment of a *P. fucata* industry in Queensland will enable Australia to diversify in an increasingly competitive world pearl market and maintain its current position and reputation as a leading pearl producing nation in the world. However, for this to occur, research must be conducted to establish baseline information on which an industry can be established and the feasibility and sustainability of such development can be determined. It is anticipated that the results generated from this study will facilitate the establishment of Akoya pearl oyster culture in Queensland and will produce significant economic benefits for the state.

1.8 Objectives

The major objective of this study was to determine the feasibility of Akoya pearl oyster culture in Queensland. Specific areas of research focus for this study were:

(1) to determine whether *P. fucata* can be cultured in Queensland using methods employed for other pearl oyster species (Chapter 3);

(2) to develop pareticular hatchery propagation protocols for *P. fucata* in Queensland (Chapters 4-5);

(3) to develop appropriate nursery culture techniques for *P. fucata* in Queensland (Chapter 6);

(4) to monitor growth rates of *P. fucata* during nursery culture in response to water quality parameters (Chapter 7); and

(5) to select appropriate culture sites based on the above aims, and to make recommendations on the potential of *P. fucata* culture in Queensland (Chapter 8).

Chapter 2 General Materials and Methods

2.1 Study Sites

Two sites were used during this study: (1) Pioneer Bay, Orpheus Island (18° 35' S, 146° 29' E); and (2) Horseshoe Bay, Magnetic Island (19° 07' S, 145° 52' E). Both are close to Townsville in north Queensland (Fig. 2.1). These two sites were selected initially due to their perceived differences in water quality as a result of their inshore and offshore locations and because they already had the infrastructure to conduct bivalve research.

2.1.1 Orpheus Island

Orpheus Island is a member of the Palm Island group approximately 80 km north-east of Townsville, and is located 15 km offshore (Fig. 2.2). Located on the western or leeward side of the island, James Cook University's Orpheus Island Research Station is located in Pioneer Bay which is protected from the predominant SE trade winds. Orpheus Island has a seasonal climate, with a hot and humid 'wet' season (November-April) followed by a warm 'dry' season (May-October). Mean air temperatures in Townsville range from 23.8°C-31.3°C, during the wet season and 13.1°C-24.9°C in the dry season (Ngan and Price, 1980). Mean monthly surface water temperatures range from a maximum of approximately 30°C in February to a minimum of 22°C in August. At a depth of 2-6 m, mean monthly water temperatures range from a maximum of 29°C in March to a minimum of 21°C in August (Beer and Southgate 2000). Salinity ranges from 27.2‰ in March to 36‰ in August at the surface but remain constant (35-36‰) at 3-4 m (Pit 1998).

2.1.2 Magnetic Island

Magnetic Island is a large continental island located 8 km ENE of Townsville (Fig. 2.3). Magnetic Island, like Orpheus Island also experiences a seasonal climate with similar temperatures. The study site, Horseshoe Bay (Fig 2.3), is also protected from the predominant SE trade winds. However, being closer to the coast and part of a large shallow water bay system (Cleveland Bay), it is expected to show differences in temperature, salinity and food availability (chlorophyll level) compared with the more offshore Orpheus Island.

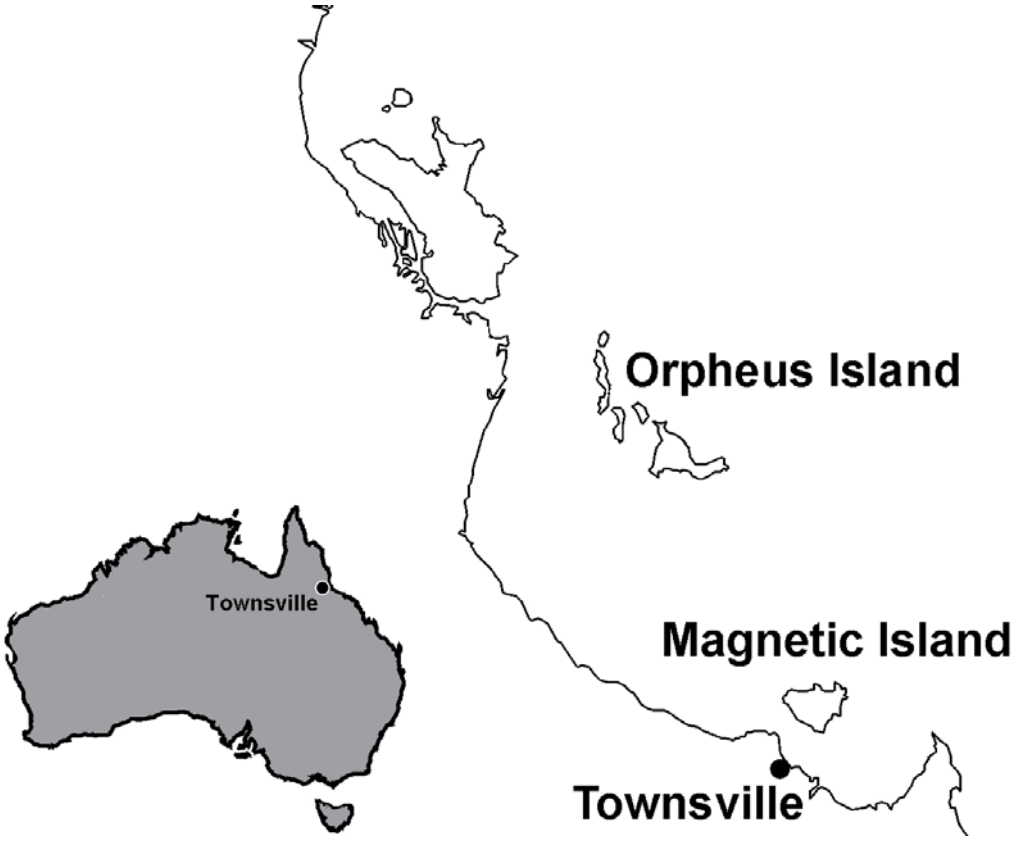


Fig. 2.1. **Location of study sites: (1) Orpheus Island and (2) Magnetic Island near Townsville on the east coast of northern Australia.**

Fig. 2.2. **Location of long-line (▲) at Pioneer Bay on Orpheus Island.**

Fig. 2.3. Location of long-line (▲) at Horseshoe Bay on Magnetic Island.

2.2 Hatchery Production

Pinctada fucata has not previously been cultured in Queensland. As a result the general methods used in the hatchery for this study were initially based on successful methods employed for culture of pearl oysters in tropical conditions in the same hatchery at Orpheus Island Research Station (OIRS) (Southgate and Beer 1997). The original broodstock were collected from spat collection studies at Orpheus Island (Beer and Southgate 2000), however, all oysters used in subsequent experiments throughout this study were produced in the hatchery at OIRS.

2.2.1 Broodstock

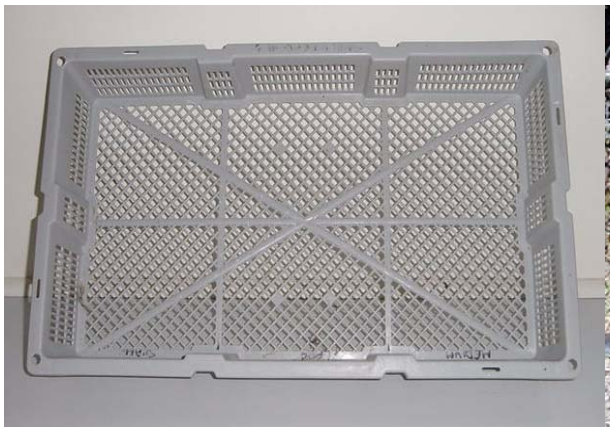
Adult *P. fucata* were maintained on a long-line at OIRS at a depth of 3-4 m in panel nets (Fig. 2.4e). These adults were collected 2-3 years prior to the start of this study from routine spat collection at Orpheus Island (Beer and Southgate, 2000). Spat collectors consisting of 8 pieces of shade cloth (0.5 m^2 each) were placed inside woven 1 mm mesh bags and suspended from the long-line at depths of 2 m, 4 m, 6 m and 8 m. Mesh bags were left suspended for approximately 3-4 months before recruited pearl oyster spat were removed. Spat were then placed into pearl nets and re-suspended from the long-line. Oysters were grown to a size at which they could be used as broodstock (50-60 mm in dorso-ventral shell height-DVH) (Fig. 2.5).

2.2.2 Spawning Induction

P. fucata adults (broodstock) were transported from the long-line (section 2.3) to the hatchery where there were scrubbed and washed with 1μm filtered sea water (FSW) to remove fouling organisms and sediment. Once cleaned, oysters were placed into a 'cold-water' bath at 22°C and held overnight in an air-conditioned room (Southgate and Beer 1997). The following day, oysters were placed into a spawning tank (approximately 300 L capacity) containing pre-heated 1 μm filtered seawater (FSW) at 28-30°C. Oysters were placed in an upright position in racks within the spawning tank. A minimum of 100 oysters were used in every spawning. Once spawning commenced, oysters were removed from the tank and placed individually into separate containers.

(a) (b)

(c)



(d)

(e)

Fig. 2.4. Culture units used for culture of *Pinctada fucata*: (a) pearl net; (b) pearl net with noodles; (c) plastic mesh tray; (d) plastic box; and (e) panel net.

(a)

(b)

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(c)

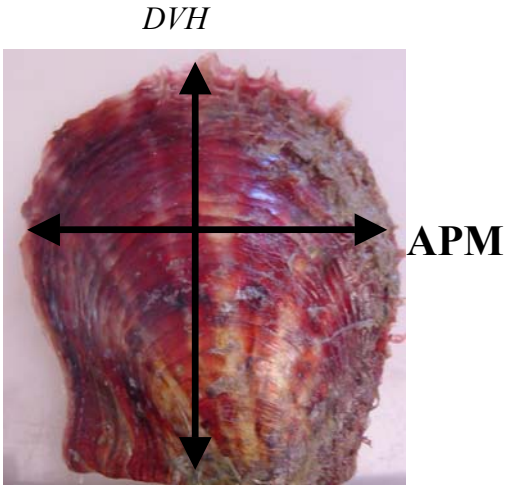


Fig. 2.5. Pearl oyster larvae were measured along the antero-posterior margin (APM) as seen in (a) and (b), while juveniles and adult pearl oysters (c) were measured for dorso-ventral shell height (DVH), APM, shell width and wet weight. (Photo a and b: Erika Martinez-Fernandez)

(containing water at the same temperature and salinity as the spawning tank) and allowed to continue spawning. This allowed eggs and sperm to be inspected separately prior to fertilisation.

2.2.3 Fertilisation and Embryonic Development

Once gametes (eggs and sperm), were collected, eggs were sieved through 88 μm pore-size mesh screen (88 μm square mesh, diagonal measure 124 μm) to remove debris, and the eggs from a number of females were combined into a 20 L container. Motile sperm from a number of males was also combined in a single container. A small sample (approximately 50 mL) of sperm was added to the egg mixture (in a 20 L container) to begin fertilisation. The protrusion of the first polar body, which determines successful fertilisation, occurred approximately 30 mins later (Fig. 1.2). If greater than 70% of eggs were visibly fertilised, then no additional sperm was added. If less than 70% fertilisation rate was observed, then additional sperm (approximately 20 mL) was added. Fertilised eggs were then captured on a 25 μm pore-size mesh screen (25 μm square mesh, diagonal measure 35 μm) sieve allowing excess sperm from the fertilisation container to be removed as the sperm passes through the mesh sieve. Fertilised eggs were then placed into incubation tanks at a density of 30-50 embryos mL^{-1} for 24 h (Southgate and Beer 1997; Southgate *et al.* 1998b). Antibiotics were added to incubation tanks at a concentration of 0.01 g L^{-1} to minimise bacterial effects on the embryos. A combination of two antibiotics were used, Erythromycin and Tetracycline which have been previously shown to improve survival of pearl oysters during embryonic development (Doroudi 2001). Embryos remained in incubation tanks for 24 h.

2.2.4 Larval Rearing

One day old D-stage *P. fucata* larvae were drained from incubation tanks. Incubation and larval rearing tanks were equipped with two draining taps (Fig. 2.6). Larvae were drained from the top tap, ensuring that only healthy swimming larvae were retained and that dead or unhealthy larvae were drained to waste from the bottom tap. Water containing larvae passed through two submerged sieves which retained larvae (Fig. 2.6); sieve size was increased (25, 37, 53, 88, 150μm) with increasing larval size. Once drained, tanks were scrubbed, sterilised with a concentrated chlorine solution, rinsed with fresh water and re-filled with FSW. Larvae were placed into 500 L larval rearing tanks at a density of 1-2 larvae mL^{-1}. Larval rearing tanks were initially set-up as static systems where tank water (FSW) was completely exchanged every two days as discussed above. While tanks were cleaned, larvae were washed from sieves and concentrated in 20 L containers. Three 1 mL sub-samples were taken from this container and the larvae they contained were examined microscopically to determine growth and survival. Prior to sampling, a plunger was used to distribute the larvae evenly throughout the bucket (Braley 1992). Once the sub-samples of larvae were taken, remaining larvae within 20 L containers were returned to larval rearing tanks. On day 10 the larval rearing system was converted to a flow-through system (Southgate and Ito 1998), which allowed 100% water exchange every 24 h. However, every 4-5 days, the larval rearing tanks were completely drained (as detailed above) to enable larval growth and survival to be determined and to thoroughly clean the inner surfaces of the tanks. During larval rearing, oyster larvae were fed twice daily with micro-algae (section 2.2.5).

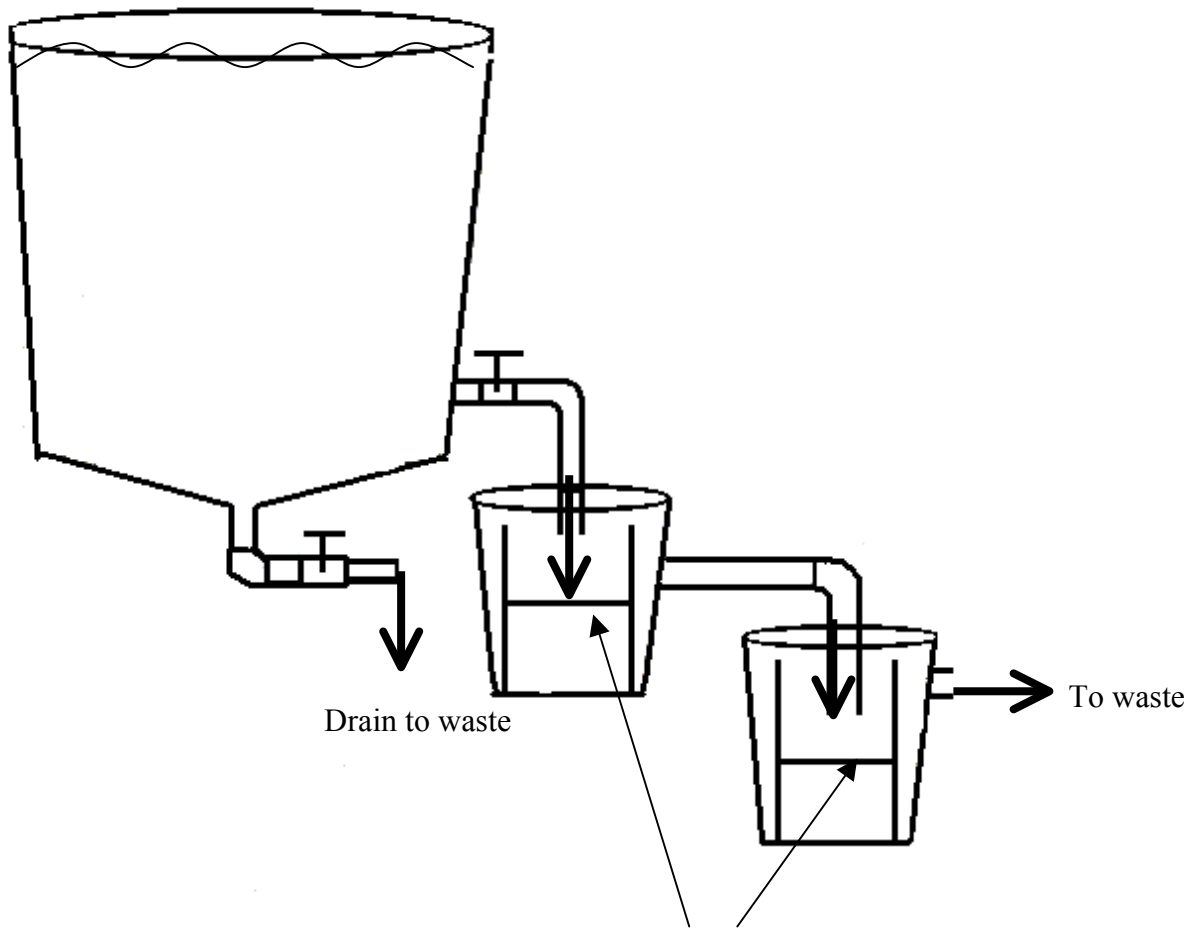


Fig. 2.6. Larval rearing tank (500 L) used during this study showing two drainage taps, the top tap to drain larvae and the bottom tap to drain waste products (dead and unhealthy larvae).

2.2.5 Micro-algae Production

Larvae and spat were fed a mixture of three species of micro-algae; *Isochyrsis* aff. g*albana* clone T-ISO (cs177), *Pavlova salina* (cs 49) and *Chaetoceros muelleri* (cs 176) at a proportional rate of 50:30:20%, respectively, based on cell numbers. Initial micro-algae culture stocks were obtained from the CSIRO Marine Fisheries Division in

Hobart, Tasmania, Australia and CSIRO catalogue codes are shown in brackets. Algae were cultured from 250 mL stock flasks into 500 mL and 3 and 5 L glass flasks containing autoclaved 0.45 μm-filtered and ultra-violet (UV) treated seawater with the addition of Walne's nutrient medium (Walne 1974). Algae cultures were scaled-up (inoculated) from 3 and 5 L flasks (after approximately 5-10 days) into 20 L plastic carboys (Fig. 2.7). All algae were cultured using a 12L:12D photoperiod. Larvae were fed half of their ration in the morning (09:00) and half in the evening (19:00). The algae ration fed increased from 1 000 cells mL^{-1} on day 1 to 45 000 cells mL^{-1} on day 43 (Table 2.1). Algae used to feed larvae was removed from 20 L carboys (Fig. 2.7) and the algae were in the exponential growth phase (Southgate 2003).

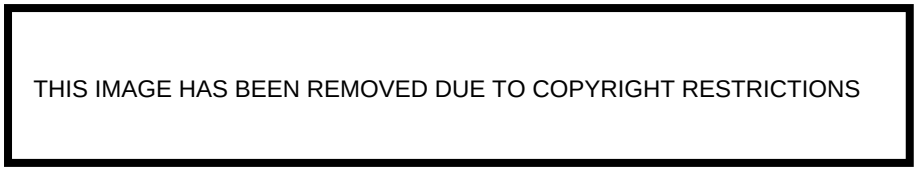


Fig. 2.7. Process of scaling-up micro-algae cultures from 250 mL stock flasks to 20 L carboys and further onto 2 000 L outdoor culture (Southgate 2003).

Table 2.1. Density of micro-algae cells fed to *Pinctada fucata* larvae and spat (modified from Southgate and Beer (1997) and Doroudi *et al.* (1999a)). Larvae were stocked at an initial density of 2 larvae mL^{-1} and placed into settlement tanks on day 21. Larvae were fed a ternary diet of T-ISO, *Pavlova salina* and *Chaetoceros Muelleri* in equal parts.

Age (days post-fertilisation)	**Total Feeding Rate (cells mL^{-1} day^{-1})**
1-2	**1 000**
3-4	**3 000**
5-7	**6 000**
8-14	**15 000**
15-21	**20 000**
22-26	**25 000**
27-32	**30 000**
33-39	**40 000**
40+	**45 000**

2.2.6 Settlement

After approximately after 21 days, oyster larvae reach the 'eyed' stage measuring 210-260 μm (section 1.1.3). Eyed larvae large enough to be retained on a 150 μm-pore-size mesh screen (150 μm square mesh, diagonal measure 212 μm) were removed from larval rearing tanks and transferred into settlement tanks (Southgate and Beer 1997). Settlement tanks of 500 l volume contained suspended spat collectors. Each spat collector consisted of an outer mesh bag (30 x 20 cm), filled with a piece of 0.5 m^2 woven mesh shade cloth. Each settlement tank (500 L) contained 75 spat collectors and was supplied with vigorous aeration through 4 air-lines (Fig. 2.8). Vigorous aeration was supplied to minimise the number of larvae settling on the bottom and sides of the tank. Once settled onto spat collectors, spat remained in settlement tanks for 3 weeks. During settlement, spat were fed twice daily with micro-algae at 09:00 and 19:00 (section 2.2.5) according to the schedule outlined in Table 2.1.

2.2.7 Spat Transfer

Spat were transferred from the hatchery to the nursery approximately 3 weeks after settlement, or 6 weeks after fertilisation. At this stage, spat collectors were removed from settlement tanks and 3 collectors were placed into plastic mesh trays (Fig. 2.4c) with lids (55 x 30 x 10 cm) (Southgate and Beer 1997). Trays were then weighted and placed onto the OIRS surface long-line (section 2.3) at a depth of 3-4 m. Spat remained unattended on the long-line for 2 months or until they were 3.5 months of age (Southgate and Beer 1997).

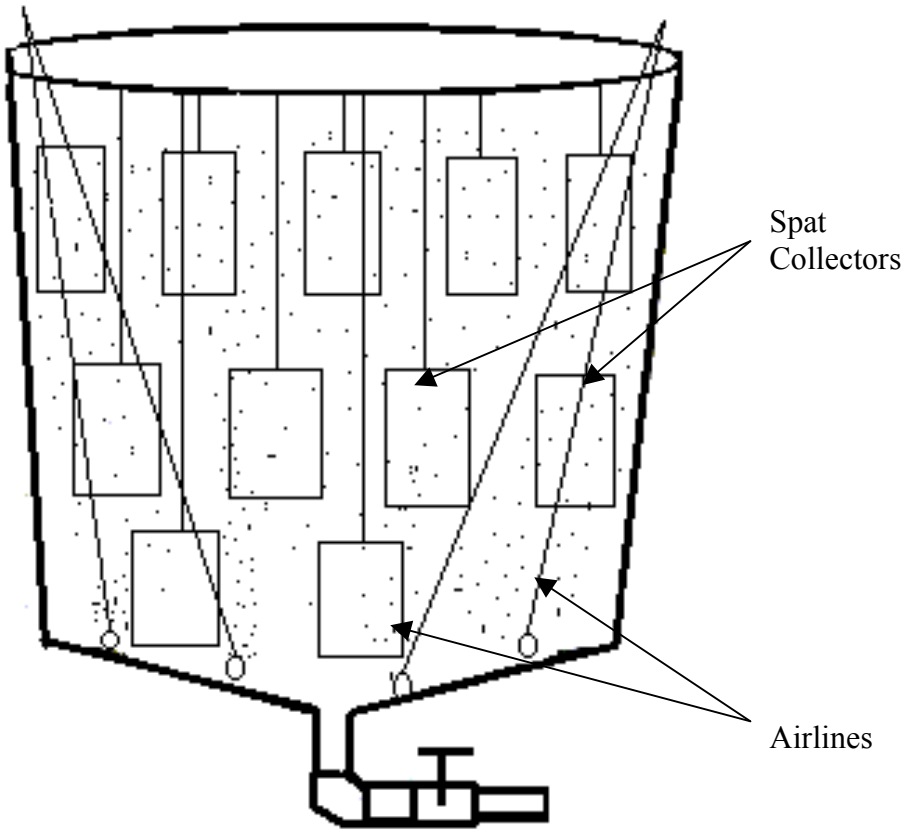


Fig. 2.8. Settlement tank containing spat collectors and vigorous aeration from the tank bottom.

2.2.8 Grading

At 3.5 months of age, spat were separated from the collectors and graded through plastic square mesh sieves (5, 10 and 15 mm) into different size classes, referred to as 'small', 'medium' and 'large', respectively. Given the larger diagonal measurement of these meshes, corrected spat size ranges for each category were small (<7.05 mm), medium (7.05-14.1 mm), and large (>14.1 mm).

2.2.9 Nursery Culture

Once oysters were size graded they were either placed into immediate experiments (Chapter 6) or into pearl nets for use in later experiments (Chapters 6-7). Oysters used for later experiments were placed into pearl nets (Fig. 2.4a) with a square mesh size of 4.5 mm or 9 mm depending on oyster size. As oysters increased in size they were placed into the larger mesh-sized nets. Pearl nets were cleaned every 6 weeks to minimise fouling (Pit and Southgate 2003a). Unless otherwise stated, cleaning involved removing pearl nets containing oysters from the long-line and transferring them to a land-based cleaning station where they were cleaned with a pressure cleaner. Oysters were then placed into raceways prior to being returned to the long-line. Cleaned pearl nets were inspected for predators, which were removed. Every 12 weeks, all oysters were removed from pearl nets and dead shells removed. At this time oysters were individually cleaned and graded so similar sized oysters were placed into the same new pearl nets. The density of the oysters within each net decreased as the oysters attained a larger size. Oysters remained in pearl nets until they reached a size of approximately 50 mm at which time they were transferred to panel nets (Fig. 2.4e).

2.2.10 Measuring Oysters

The antero-posterior measurement (APM; Fig. 2.5a, b) or shell length is perpendicular to the dorso-ventral shell height (DVH) and is generally used to describe larval growth (Minaur 1969, Alagarswami *et al.* 1983, Alagarswami *et al. 1*989, Rose and Baker 1994, Southgate and Beer 1997). Dorso-ventral shell height (Fig. 2.5c) refers to the axis perpendicular to APM and is the preferred dimension for measurement in comparative growth studies of pearl oysters. Dorso-ventral shell

height is also used to determine the size at which oysters can be used for pearl seeding (Gervis and Sims 1992). Shell width is the maximum distance between external surfaces of the two shell valves when they are closed (Gervis and Sims 1992). Shell width is an important parameter as the distance between the valves determines the size of the nucleus that can be seeded into the oyster (Gervis and Sims 1992). Wet weight is also often recorded in the Japanese Akoya pearl industry to determine nucleus size, and to indicate the condition of the oyster (Gervis and Sims 1992). All larval measurements were made along the APM using a binocular microscope with graticule measuring to the nearest 1 μm, while pearl oyster juveniles and adults were measured for DVH, APM, shell width and wet weight, using callipers and a balance, measured to nearest 0.1 mm and 0.01 g, respectively.

2.3 Long-line Culture

Surface long-lines are a commonly used culture apparatus for the culture of pearl oysters. Surface long-lines were used during this project (Fig. 2.9). The long-line consisted of a length of 25 mm rope, 'headline', held in position by floats positioned every 2 m and anchored at each end with three anchors. 'Dropper lines' were attached to the headline at 1 m intervals and were used for attaching pearl nets and panel nets containing oysters. The long-line at OIRS was 120 m long, while the long-line at Magnetic Island was 75 m long.

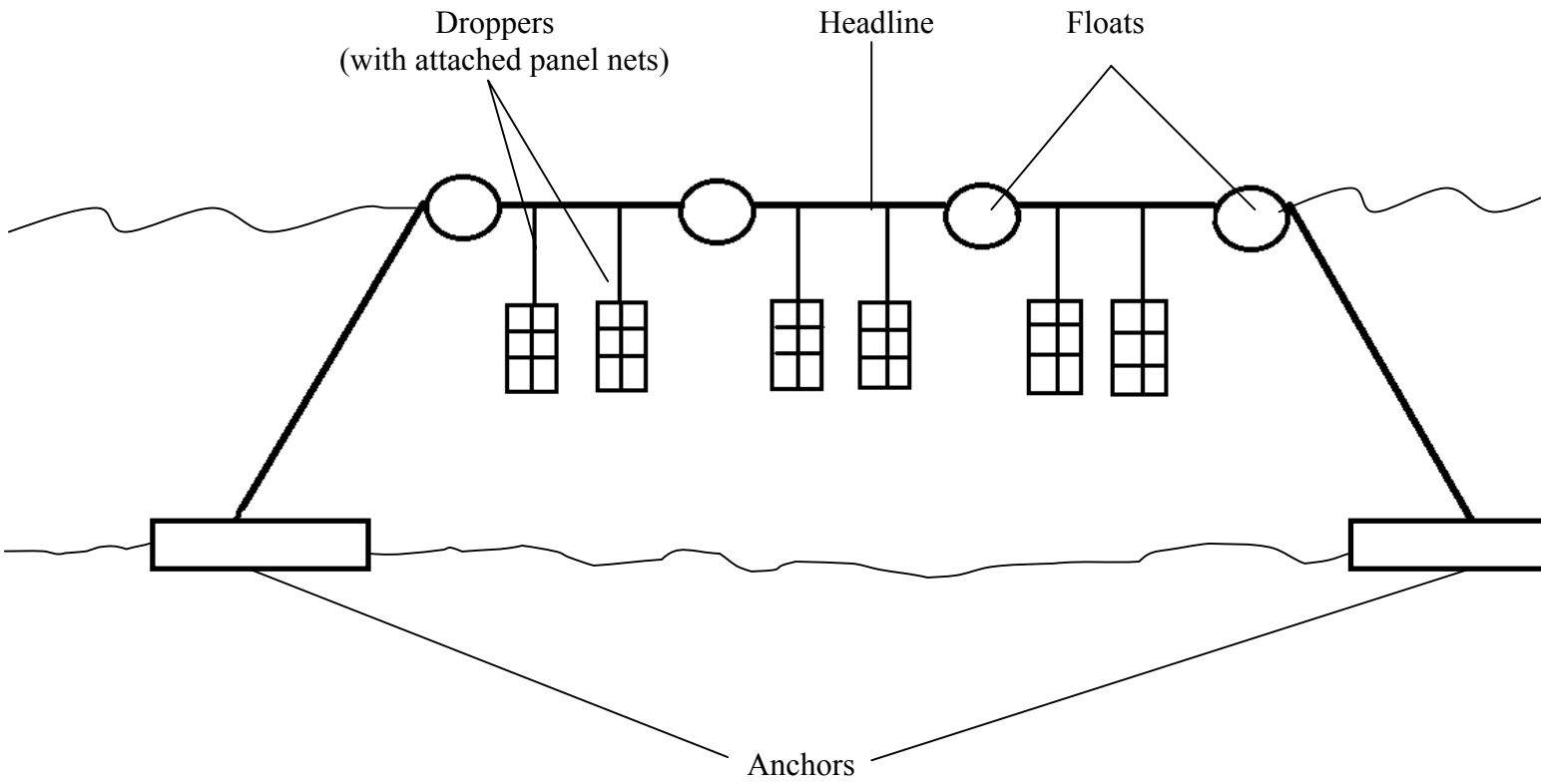


Fig. 2.9. Representation of the surface long-lines used for the culture of *Pinctada fucata* at Orpheus Island and Magnetic Island.

2.4 Water Quality Monitoring

Part of this study included detailed recording of important water quality parameters; water temperature, salinity, and chlorophyll 'a' at both sites (Chapter 7).

A YSI 6600 water quality sonde (John Morris Scientific) was deployed at each site and was set-up to take measurements of the above parameters every 2 h. Water temperature was calculated through the utilisation of a metallic oxide thermistor. Salinity is automatically calculated from water temperature and conductivity data according to the methods described in Glescari *et al.* (1989). Chlorophyll 'a' was automatically calculated by the YSI 6600 by irradiating light (wavelength = 470 nm) through the water column recording a level of fluorescing chlorophyll which is then converted to µg/l. Water samples were also taken to verify the readings obtained *in situ* compared with laboratory techniques (pers comm. J Cook 2003). Water samples were taken randomly on a number of occasions and analysed for chlorophyll content by the Australian Centre for Tropical Freshwater Research at James Cook University.

Chapter 3 Investigation of *P. fucata* culture in Queensland.

3.1 Introduction

Hatchery production of *P. fucata* has occurred throughout Asia for the last 50 years (Guo *et al.* 1999), yet, available information on optimal culture techniques is very limited. However, due to the down-scaling of Akoya pearl production in Japan (Dettmar 2002), research into Akoya pearl oysters is currently being conducted in temperate Australia (O'Connor *et al.* 2003), the Caribbean (Urban *et al.* 2000a, b; Lodeiros *et al.* 2002), China, (Guo *et al.* 1999; O'Connor *et al.* 2003) and India (Jetani *et al.* 2003). Before this species can be cultured successfully on a commercial scale in Australia, basic biological information is required relating to growth and survival rates.

This project focuses on the culture of Akoya pearl oysters in Australia under tropical conditions. This chapter reports on the first successful spawning of *P. fucata* under tropical conditions in Queensland using methods, initially developed in our laboratory for *P. margaritifera*. Optimisation of hatchery conditions for *P. fucata* were further developed later in this thesis (Chapters 4 and 5).

3.2 Materials and Methods

P. fucata were mass spawned according to the general methods as described in Chapter 2, which are based on the culture of *P. margaritifera* (Southgate and Beer 1997). This first attempt to hatchery rear *P. fucata* in Queensland produced sufficient numbers of oysters (100'000) for early nursery and late nursery experiments (Chapters 6-7).

3.2.1 Embryonic Development

Sixty broodstock were removed from the long-line at Orpheus Island and placed into a cool-water bath at 21°C (section 2.2.2). The following day 16 females and 31 males spawned. Egg samples were collected to determine mean sizes (± SE, *n*=50). Fertilised eggs or embryos were placed into two incubation tanks at a stocking density of 4 embryos mL^{-1} containing 0.01 g L^{-1} of antibiotic (Erythromycin and Tetracycline). Samples of embryos were taken every hour for the first 5 h, at 9 h and then every 5 h up to a total of 24 h to determine the major developmental changes seen during embryonic development (Doroudi and Southgate 2003). Embryo samples were viewed using a binocular microscope. A T-test was carried out to ensure that there was no tank effect from the two incubation tanks used.

3.2.2 Larval Development

After 24 h, D-stage larvae were removed from incubation tanks and distributed into larval rearing tanks as outlined in Chapter 2. Larval samples were taken every 2 days to determine growth and survival. Following settlement, spat growth and survival was not monitored. In this baseline study larvae were fed only 2 species of micro-algae, *Isochrysis galbana* (T-ISO) and *Chaetoceros muelleri*, for the first 10 days and were not fed a ternary diet as outlined in Chapter 2 and as used in subsequent Chapters (section 3.4 for explanation). Micro-algae ration remained the same as discussed in Chapter 2 (Table 2.1), and larvae were fed a diet containing 50:50 (micro-algae cell number) of T-ISO and *Ch. muelleri*, respectively. Water temperature throughout the larval culture period ranged from 27.7-28.8°C, while salinity ranged from 28-35‰.

3.2.3 Spat Development

Spat were transferred from the hatchery to the ocean as detailed in Chapter 2. Water temperature within tanks throughout spat development ranged from 28.1°C - 29.0°C. *Pinctada fucata* spat were transferred from the hatchery to the long-line 6 weeks (43 days) after fertilisation with a mean (± SE, *n*=50) dorso-ventral shell height (DVH) of 0.9 ± 0.02 mm.

3.2.4 Juvenile Development

A sample of 2 500 oysters was used to determine general growth during nursery culture at Orpheus Island. Initially, 500 oysters were placed into each of 5 pearl nets and suspended from the long-line. As oysters increased in mean dorso-ventral shell height (DVH), the density was decreased as described in Table 3.1. Once oysters reached approximately 50 mm, they were transferred from pearl nets into 24-pocket panel nets, and then into 15-pocket panel nets at approximately 70 mm. General growth morphometrics were recorded in the form of DVH, APM, shell width and wet weight. Single regression equations were determined with DVH as the dependant variable to describe the relationship between successive morphometrics and to identify changes in growth with age.

Table 3.1. Approximate stocking densities and shell sizes of *Pinctada fucata* cultured at Orpheus Island on a surface long line.

Dorso-ventral shell height (DVH-mm)	Stocking Density (Number of oysters per pearl net)
2-5	500
5-10	400
10-25	200
25-35	150
35-45	100
45+	50

3.3 Results

3.3.1 Embryonic Development

Mean (± SE, *n*=50) egg diameter prior to fertilisation was 48 ± 1.5 μm. Between 30-60 minutes after sperm was added to the egg suspension, the first polar body was observed confirming fertilisation had occurred. Fertilised eggs had a mean (± SE, *n*=30) diameter of 55.8 ± 0.5 μm. A total of 2.1 x 10^6 eggs at a density of 4.0 eggs mL^{-1} were distributed among two incubation tanks. Fertilised eggs first divided into two cells of equal size and then into four cells composed of one large cell and three smaller cells. The cells continued to divide until the gastrula was formed after 5 h in which cilia developed allowing the embryos to move freely throughout the water column in a corkscrew motion. After 9 h, 85% of embryos had reached the trochophore stage and were swimming in a corkscrew motion. The first D-stage larvae

were seen after 14 h and after 19 h, 90% of embryo's had developed into D-stage larvae.

After 24 h, the resultant D-stage larvae had a mean (± SE, *n*=30) APM of 75.8 ± 0.3 μm and there was no significant differences in mean larval size between the two incubation tanks ($p>0.05$). Survival of embryos to D-stage in incubation tanks was 65.1 and 64.6%, respectively (Table 3.2).

3.3.2 Larval Development

Larval growth in the first 10 days was slow reaching a mean of 95±1.5 μm, but increased steadily through to day 20 (200±5.2; Fig. 3.1). Mean (± SE, *n*=3) cumulative survival of larvae on day 13 was 22.6% and 12.4% in tank 1 and 2, respectively (Table 3.2). Due to decreasing larvae density in tanks 1 and 2, larvae were combined into one tank on day 13. Between days 13 and 20 survival declined from 17.5% to 5.2% (Table 3.2).

On day 20, larvae large enough to be retained on a 150 μm square mesh sieve with a mean (± SE, *n*=30) APM of 222 ± 2.3 μm were placed into settlement tanks. Mean (± SE, *n*=30) APM of larvae not retained on the 150 μm mesh sieve at this stage and returned to larval rearing tanks was 172 ± 2.6 μm (Fig. 3.2). Larval rearing tanks were drained daily from day 20 to day 25 to remove larvae that were competent for settling (retained on a 150 μm mesh sieve). A total of 213 000 larvae, 16% of D-stage veligers stocked into larval rearing tanks on day 1, were transferred to settlement tanks by day 25.

Table 3.2. Incremental and cumulative survival of *Pinctada fucata* larvae cultured at Orpheus Island.

Day	Tank 1		Tank 2	
	Incremental Survival (e.g. D3-5)	**Cumulative Survival (e.g. D1-5)**	**Incremental Survival (e.g. D3-5)**	**Cumulative Survival (e.g. D1-5)**
1	65.1%	65.1%	64.6%	64.6%
3	88.4%	57.5%	59.4%	38.4%
5	75%	42.9%	75%	28.2%
7	79.2%	33.9%	78.6%	22.2%
9	86%	29.2%	79%	17.5%
11	79%	22.9%	-	-
13	98%	22.6%	66%	12.4%
13*	-	17.5%		
15	89%	15.8%		
20	33%	5.2%		

13* Tank 1 and Tank 2 combined on day 13

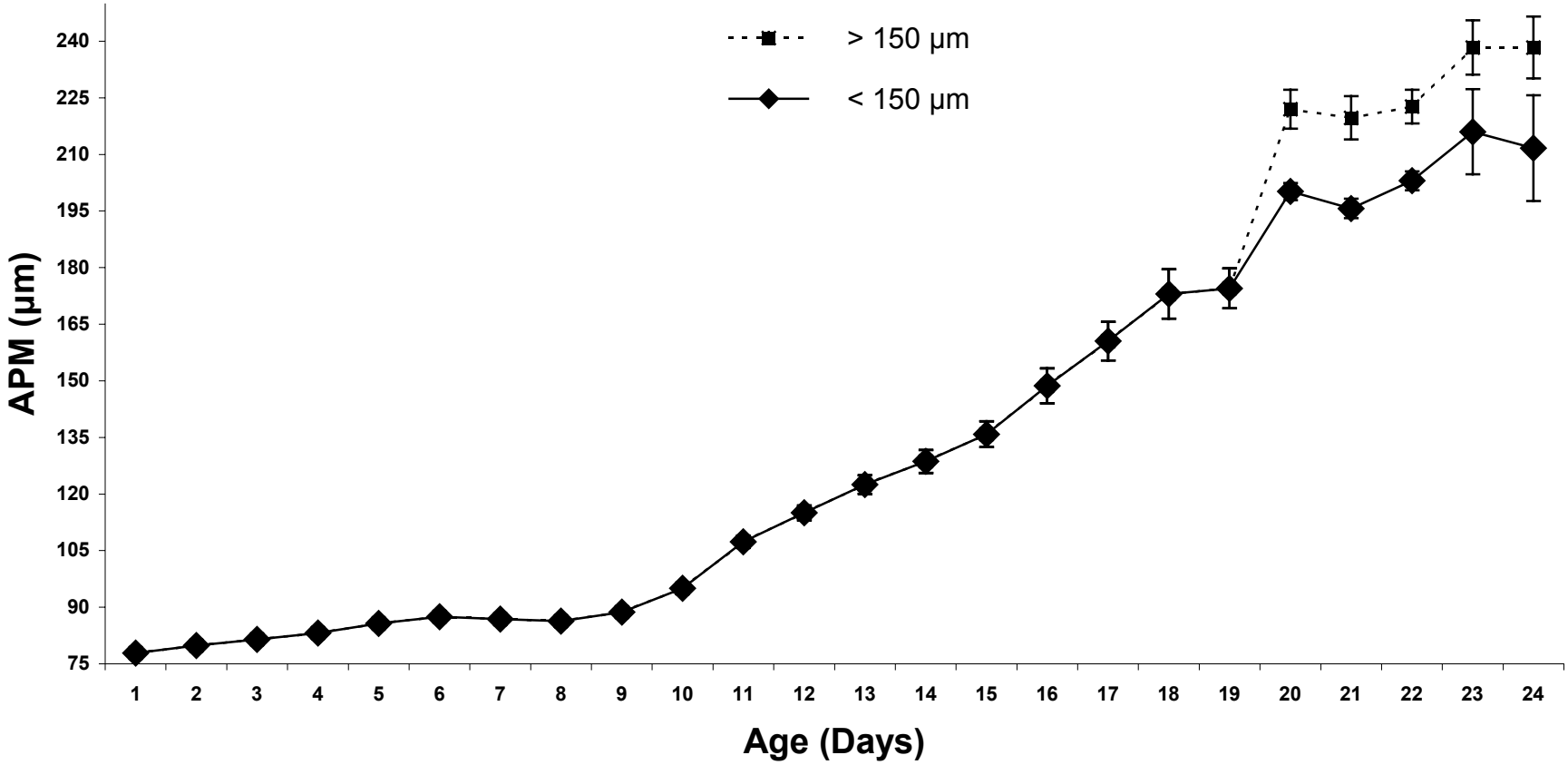


Fig. 3.1. Changes in mean (± SE, *n*=30) antero-posterior shell length (APM) of *Pinctada fucata* larvae cultured at OIRS over a 24-day period. Larvae less than 150 µm (□) remained in larval rearing tanks and those greater than 150 µm sieve (□) were transferred to settlement tanks.

3.3.3 Spat Development

Survival of spat with a mean (± SE, *n*=50) DVH of 0.9 ± 0.02 mm during spat development (25-43 days) was 27% with 58 000 spat transferred to the long-line. At grading, after 3.5 months (99 days post fertilisation), a total of 48 000 (83% survival from transfer-grading) spat ranging in size from 2-30 mm had a mean (± SE, *n*=50) DVH of 12.5 ± 0.4 μm. Spat graded into 'small', 'medium' and 'large' size classes had a mean (± SE, *n*=50) DVH of 10.8 ± 0.2 mm, 14.1 ± 0.3 mm, and 20.8 ± 0.3 mm, respectively. The proportion of spat in each of the three size classes was 63%, 34%, and 3%, respectively.

3.3.4 Juvenile Development

Growth of *P. fucata* returned to the long-line after grading was rapid. Growth of oysters in all dimensions was exponential (Figs. 3.3-3.6). Increases in DVH and APM was rapid in the first 12 months reaching 56.2 ± 0.6 mm and 52.9 ± 0.6 mm, respectively, before reaching maxima of 69.1 ± 0.6 mm and 65.0 ± 0.6 mm, respectively, after 25 months of culture. Maximum shell width and wet weight of oysters at 25 months of culture were 22.6 ± 0.2 mm (Fig. 3.5) and 36.0 ± 1.0 g (Fig 3.6), respectively. Dorso-ventral shell height and APM increased proportionally at a similar rate throughout the 25 months with a b value of 0.93 ($Y= a + bx$; $r^2=0.97$; Fig. 3.7). The proportional increase of shell width compared to DVH was approximately 33% ($r^2=0.95$; Fig. 3.8) while the relationship between shell weight and DVH was exponential ($r^2=0.89$; Fig. 3.9).

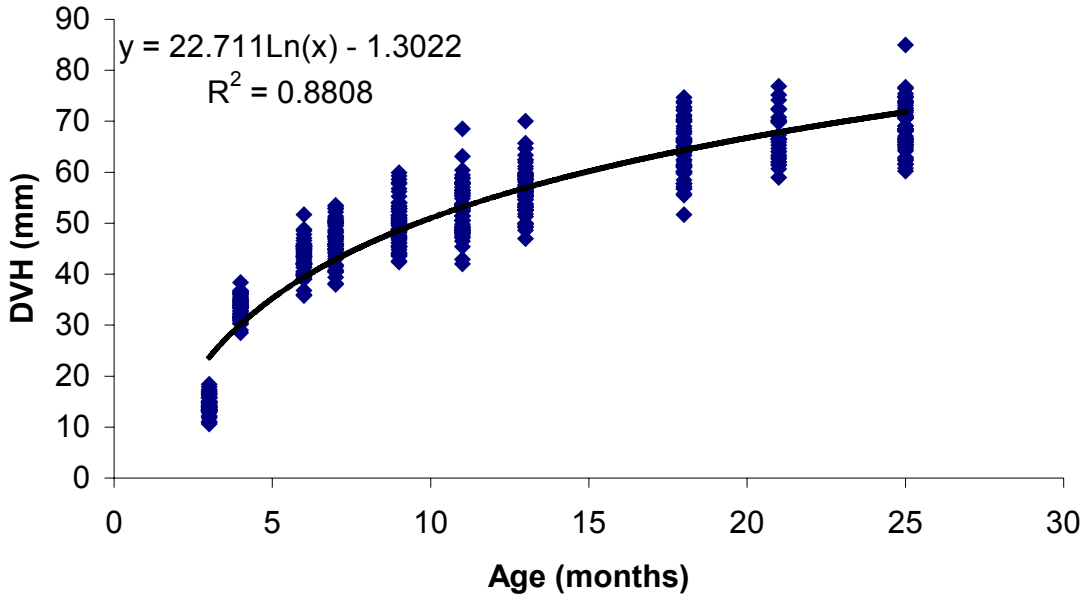


Fig. 3.3. Changes in dorso-ventral shell height (DVH) of *Pinctada fucata* during 25 months of suspended long line culture at Orpheus Island. Data are fitted with a line of best fit.

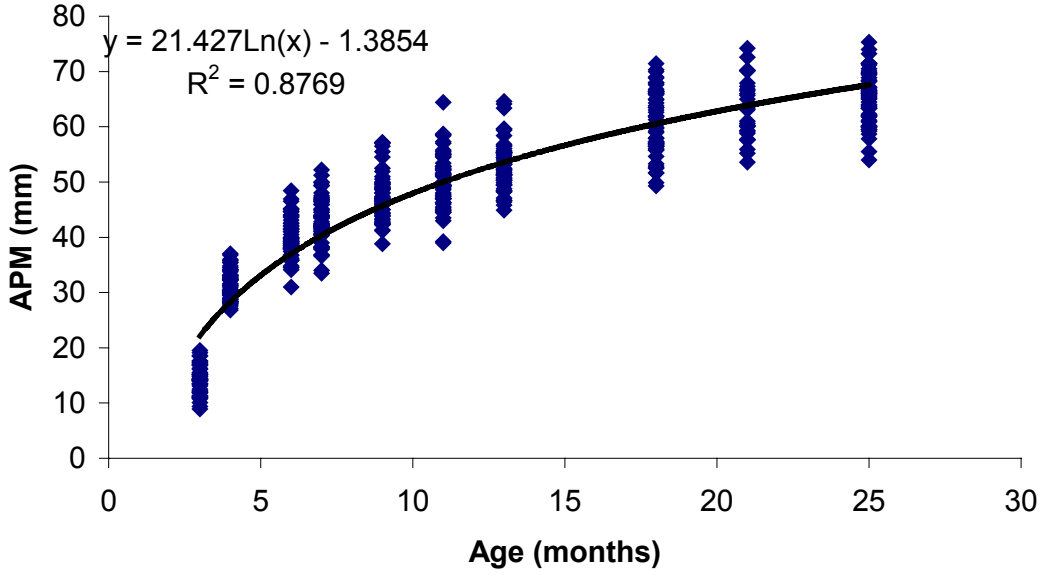


Fig. 3.4. Changes in antero-posterior shell length (APM) of *Pinctada fucata* during 25 months of suspended long line culture at Orpheus Island. Data are fitted with a line of best fit.

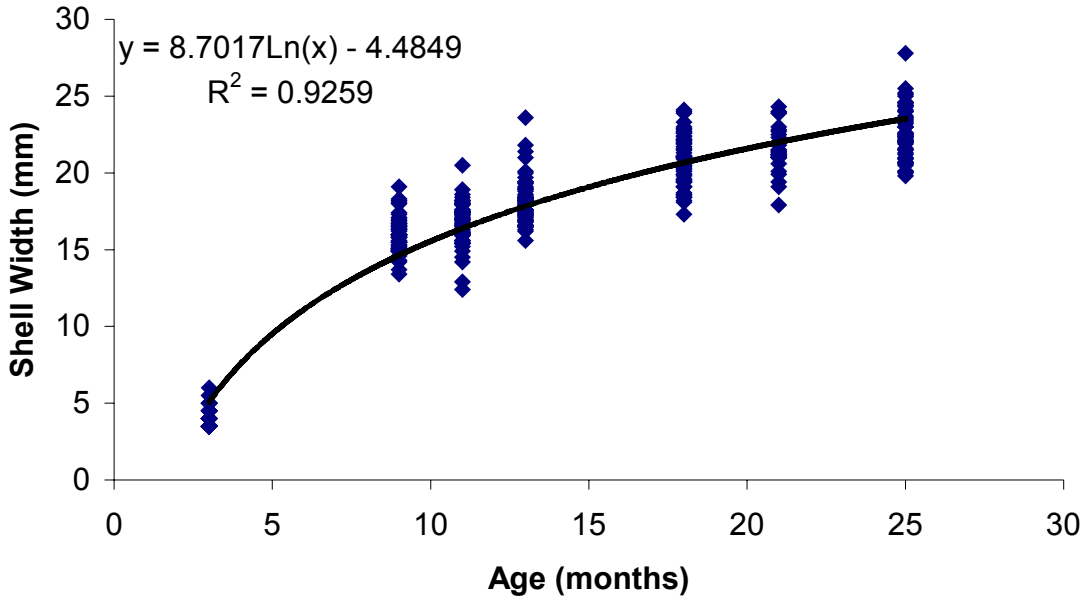


Fig. 3.5. Changes in shell width of *Pinctada fucata* during 25 months of suspended long line culture at Orpheus Island. Data are fitted with a line of best fit.

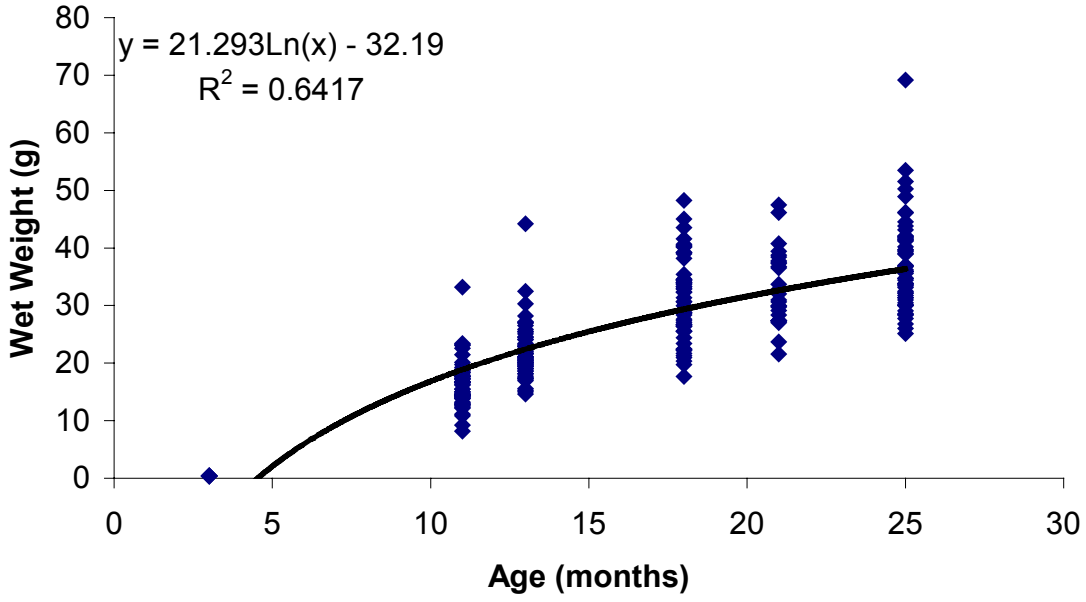


Fig. 3.6. Changes in wet weight of *Pinctada fucata* during 25 months of suspended long line culture at Orpheus Island. Data are fitted with a line of best fit.

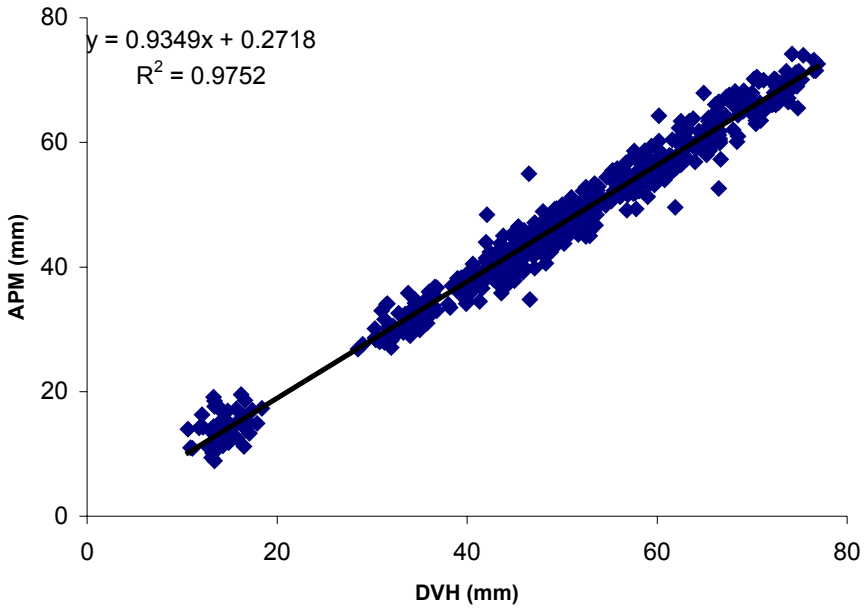


Fig. 3.7. Relationship between dorso-ventral shell height (DVH) and antero-posterior shell length (APM) of *Pinctada fucata* cultured for 25 months at Orpheus Island.

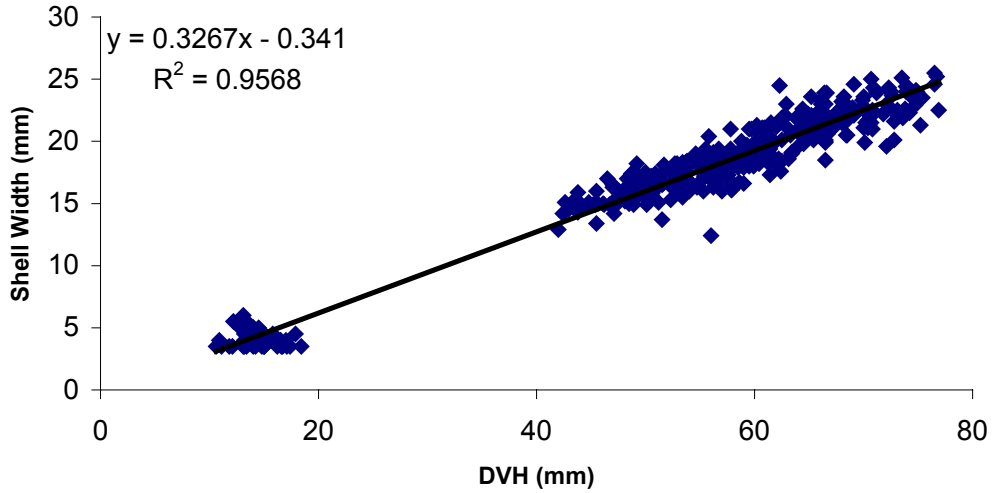


Fig. 3.8. Relationship between dorso-ventral shell height (DVH) and shell width of *Pinctada fucata* cultured for 25 months at Orpheus Island.

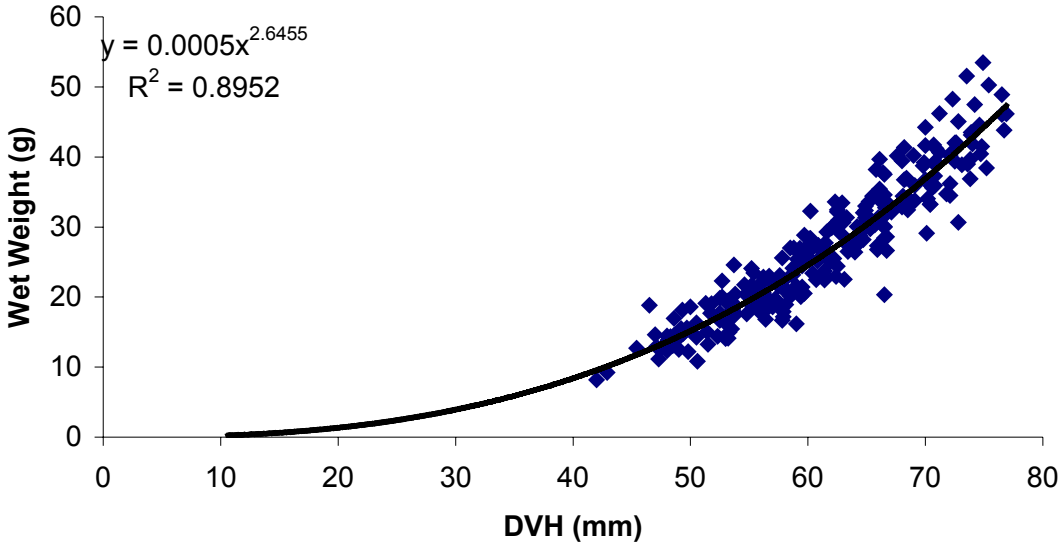


Fig. 3.9. Relationship between dorso-ventral shell height (DVH) and wet weight of *Pinctada fucata* cultured at Orpheus Island.

3.4 Discussion

This study was the first successful spawning of *P. fucata* in Queensland. Over 48 000 (48 250) 3.5 month old spat with a mean (± SE, n=50) DVH of 12.5 ± 0.4 mm were produced. General methods employed to culture *P. fucata* during this study were adopted from methods used in the same hatchery for *P. margaritifera* (e.g. Southgate and Beer 1997; Pit and Southgate 2000; Doroudi and Southgate 2003).

Embryonic development of *P. fucata* was very similar to that described for *P. margaritifera* (Doroudi and Southgate 2003). D-stage larvae were produced after 24 h, which is similar to other works investigating pearl oysters (Alagarswami *et al.* 1983; Rose and Baker 1994; Doroudi and Southgate 2003). In this study, larvae began to develop a shell and attain the D-stage after 14 h with 90% attaining D-stage after 19 h. In similar studies, Ota (1957) and Alagarswami *et al.* (1983) noted that *P. fucata* attain

the D-stage after 20 h and 20 h and 40 mins, respectively. *P. margaritifera* have been shown to reach D-stage after 24 h (Alagarswami *et al.* 1989; Doroudi and Southgate 2003), while *P. maxima* reach the same stage after 18-24 h (Minaur 1969; Tanaka and Kumeta 1981; Rose and Baker 1989; 1994). Variations in embryonic development to D-stage have been attributed to genetic characteristics such as energy levels within eggs passed on from the mother (Rose 1990), egg density (Southgate *et al.* 1998b) as well as water temperature and salinity (Doroudi *et al* 1999b; O'Connor and Lawler 2004), degree of aeration and the use of antibiotics (Doroudi 2001) (Chapter 4).

Survival in both incubation tanks, although relatively low, compared to previous work with pearl oysters at OIRS (Pit and Southgate 2000) was acceptable at 64.6 and 65.1%. This survival level was above the 30-50% survival suggested by Utting and Spencer (1991) as a good survival rate for bivalve larvae at this stage. Within our laboratory and assuming 2 incubation tanks are stocked with 50 eggs mL^{-1} (ca. 25 x 10^6 eggs per tank), only 40% survival would be required in both tanks to have sufficient larvae to fill all larval rearing tanks, which have a total capacity of 20 x 10^6 larvae (Chapter 4). It should be noted that the embryo density used (4 eggs mL^{-1}) was substantially lower than the usual density of 30-50 eggs mL^{-1}, which is routinely used in our hatchery (Southgate and Beer 1997; Pit and Southgate 2000). Consequently, survival observed in this study may have been higher due to lower stocking densities. Southgate *et al.* (1998b) suggested that survival increases with decreasing stocking density during incubation. A detailed discussion on the effects of embryo stocking density on the development of D-stage veligers of *P. fucata* is given in Chapter 4.

Larval development during this study was relatively slow during the first 10 days. Within our laboratory, larval rearing tanks used for *P. margaritifera* are

converted from a static design to a flow-through design on day 10 (Chapter 2). In this study, larval rearing tanks were not converted to flow-through until day 13 because *P. fucata* larvae were slightly smaller on day 10 than *P. margaritifera* larvae. On this basis they were given a few extra days to increase in size. On day 10, larvae had only just reached the early umbo stage (~ 100 μm) and grew on average only 1.8 μm per day. Growth obtained by *P. fucata* larvae in this baseline study was slightly lower than that obtained for the same species in India where larvae reached approximately 130 μm on day 10-12 (Victor *et al.* 1995). However, further optimisation of the culture methods for *P. fucata* in later Chapters of the present study resulted in larvae showing similar growth rates to those reported by Victor *et al.* (1995).

During the first 10 days, larval growth may have been influenced by a number of factors. First and foremost, as mentioned in section 3.2.2, *P. fucata* larvae were only fed two species of micro-algae, (*Isochrysis galbana* (T-ISO) and *Chaetoceros muelleri*) instead of the usual ternary diet (Chapter 2). Previous studies within the James Cook University pearl oyster laboratory have shown that a diet combining three species (the two previously mentioned plus *Pavlova salina*), supports best growth of *P. margaritifera* larvae (Southgate *et al.* 1998a). Unfortunately, cultures of *Pavlova salina* established for this study contained ciliate and bacterial contamination and were unsuitable for use. The use of the three species has been shown to produce good growth throughout the larval cycle for *P. fucata* (see Chapter 5). This suggests that nutrition, and therefore growth, may have been sub-optimal in this first hatchery run.

In addition heavy rainfall was experienced during the first two days of larval rearing. This resulted in a salinity decrease of 6-7‰ over the first two of days of larval rearing which may also have influenced larval growth. Although, adult *P. fucata* are

very tolerant to salinity changes in the range of 12-70‰ (Dholakia *et al.* 1997), studies on the effects of salinity changes on larval growth have not been conducted for *P. fucata*. However, studies have shown that development of pearl oyster embryos only occur within narrow limits (Doroudi *et al.* 1999b; O'Connor and Lawler 2004). Details on the effects of salinity on embryos and embryonic development are discussed in further detail in Chapter 4.

Between day 10 and day 20 larvae grew rapidly averaging 10 μm per day. They reached the eyed stage or settlement size (~ 210 μm, retained on a 150 μm sieve) in 20 days, which is comparable to the results of other studies culturing *P. fucata* (Alagarswami *et al.* 1983; O'Connor *et al.* 2003), *P. margaritifera* (Alagarswami *et al.* 1989; Southgate and Beer 1997; Pit and Southgate 2000; Doroudi and Southgate 2003) and *P. maxima* (Tanaka and Kumeta 1981; Rose and Baker 1994).

A total of 213 000 larvae were placed into settlement tanks three weeks after fertilisation and approximately 60 000 spat were transferred to the long-line at OIRS three weeks after settlement (six weeks after fertilisation). Transferring spat from the hatchery to the ocean three weeks after settlement has been shown to be the optimal time for transfer of *P. margaritifera*, spat (Pit and Southgate 2000). At grading (99 days) 48 000 or 83% of spat transferred from the hatchery were graded into 'small', 'medium' and 'large' size classes. The majority of spat (63%) were considered small (10.8 ± 0.2 mm), while 34% were in the medium size class (16.1 ± 0.3 mm) and only 3% in the large size class (23.8 ± 0.3 mm). While the proportion of oysters in each size class is similar to other results obtained in our hatchery for *P. margaritifera* (Pit and Southgate 2000), it should be noted that there are no published works identifying size proportions on hatchery produced *P. fucata*. Growth of *P. fucata* in this study appeared

to be uniform and quite rapid with oysters reaching pearl production size (ca. 50 mm) in less than a year. This compares favourably to other countries culturing this species in which the time required to reach pearl production size ranges from 12 months in India (Anon 1995) through to 24 months in China (Guo *et al.* 1999). Comparison of oyster growth between this and other studies is discussed in greater detail in Chapters 6 and 7 comparing more detailed results obtained in this study.

Results from this study have shown that Akoya pearl oysters can be cultured in Queensland in commercial numbers (current production from hatcheries in Western Australia is 20 000 oysters per year per farm) and attain pearl production size in a shorter time than in other countries producing *P. fucata*. These data support the assumption that there is a possibility to develop an Akoya pearl oyster industry in Australia. However, it is vital to obtain basic biological information on growth and survival during hatchery and nursery culture. Some of these aspects will be covered in this PhD, but unfortunately, due to the size of such an investigation, only certain aspects can be covered.

Chapter 4 Embryonic Development

4.1 Introduction

Techniques for hatchery production of pearl oysters have been described for *P. fucata* (Alagarswami *et al.* 1983; Anon 1991; Victor *et al.* 1995; O'Connor *et al.* 2003), *P. maxima* (Rose and Baker 1994) and *P. margaritifera* (Southgate and Beer 1997); however, there is limited information on embryonic development (hatching rate of eggs). Embryonic development lasts for approximately 24 hours and is the period where fertilised eggs undergo a number of cell divisions and pass through the free-swimming trochophore stage to become fully shelled D-stage veliger larva.

Successful culture of bivalves is often related to environmental conditions affecting spawning, embryonic development and larval development (Dos Santos and Nascimento 1985). Early life stages of bivalves, especially embryos, are very sensitive to changes in environmental conditions, with growth and development of embryos affected by physical factors such as water temperature and salinity (Kinne 1963; Calabrese 1969). In addition to water temperature and salinity, other factors including embryo density and the presence of antibiotics have been shown to influence embryonic development and survival (Loosanoff and Davis 1963; Lough 1975; Robert *et* al. 1988; Lemos *et al.* 1994; Heasman *et al.* 1996; Southgate *et al.* 1998b; Doroudi 2001). Embryonic development has been related to later growth and survival and therefore it is important to obtain information on factors leading to successful development into D-stage larvae (24 hours of age).

While there have been a number of studies that have identified and discussed embryonic development, in terms of hatching rates, of pearl oysters (Minaur 1969;

Alagarswami *et al.* 1983; Rose and Baker 1994; Araya-Nunez *et al.* 1995; Doroudi and Southgate 2003), only a few studies have looked at the synergistic effects of environmental conditions affecting development. Doroudi *et al*. (1999b) reported that growth and survival of *P. margaritifera* embryos was affected by water temperature and salinity. They found that the optimal water temperature and salinity range for embryos was related to the water conditions at which broodstock underwent maturation and spawning.

Until recently, there was no information on the effects of environmental conditions on the development of Akoya pearl oyster embryos. Due to the cosmopolitan nature of Akoya pearl oysters (section 1.1.1), a wide range of environmental parameters are experienced (O'Connor and Lawler 2004). In Australia, Akoya pearl oysters are found from tropical waters where water temperatures can be as high as 30°C to temperate regions where water temperatures can be as low as 12°C. Akoya pearl oysters are also subject to a wide variety of salinities. In tropical Australia for example, heavy monsoonal rain can cause a decrease in salinity in in-shore waters from 36‰ to 25‰ (Hopley 1982). Consequently, it is important to investigate whether environmental conditions which provide optimal embryonic development differ between tropical and temperate environments. O'Connor and Lawler (2004) investigated the effects of varying water temperature and salinity levels on embryonic development of Akoya pearl oysters from temperate New South Wales. They found that embryos do not undergo normal development at water temperatures lower than 14°C and salinities lower than 29‰. No similar data exists for *P. fucata* from the tropics.

The aim of this chapter was to determine the effects of water temperature, salinity, embryo density and the addition of antibiotics on the development of *P. fucata*

embryos to D-stage veligers. It is essential that this information is gathered to aid in determining suitable sites for *P. fucata* culture in Queensland. This information will compliment the study of O'Connor and Lawler (2004) and will allow a comprehensive analysis of culture conditions required for optimal Akoya pearl oyster culture in temperate and tropical Australia.

4.2 Materials and Methods

Current protocols for the culture of pearl oyster embryos in our hatchery recommend that fertilised eggs are cultured at 28°C and 34‰ (Southgate *et al.* 1998a; Doroudi *et al.* 2002) with fertilised eggs stocked at 30-50 eggs mL^{-1} (Southgate and Beer 1997; Southgate *et al.* 1998b) with 0.01 g L^{-1} of antibiotic (a combination of Tetracycline and Erythromycin) (Doroudi 2001). In order to determine optimal protocols for embryonic development for *P. fucata*, two experiments were conducted to determine: (1) the effects of water temperature and salinity on the development of *P. fucata* embryos into D-stage (24 h old) veliger larvae; and (2) the effects of density and the addition of antibiotics on development of embryos into D-stage veliger larvae. Both experiments were conducted simultaneously and standard protocols (i.e. water temperature and salinity in the density and antibiotic experiment) were based on previous protocols used in our hatchery. Ten females and 20 males were used in this experiment, with all eggs combined and mixed thoroughly prior to fertilisation with fertile sperm. Spawning was carried out at 28°C and 34‰ as detailed in Chapter 2 and fertilised eggs were added to aquaria containing each combination of water temperature and salinity as well as each combination of density and antibiotic (detailed below).

4.2.1 Effects of Water Temperature and Salinity on Development of Embryos

A total of 15 000 fertilised eggs with a mean (± SE, *n*=50) diameter of 57.2 ± 0.5 µm were placed into each 500 mL aquarium containing 1 µm filtered seawater (FSW). A fully orthogonal design was set-up with each combination of water temperature (26, 28, 30 and 32°C) and salinity (28, 31, 34, 37‰) in triplicate. These values were chosen as they represent the natural annual variation in seawater temperature and salinity levels during the spawning months (October-March) throughout Queensland. All replicates were supplied with gentle aeration, stocked at a density of 30 eggs mL^{-1} and given antibiotics (0.01 g L^{-1}). Temperature and salinity was monitored every 2 hours to ensure consistency across replicates and larvae were collected on 25µm sieves to determine growth and survival.

4.2.2 Effects of Density and Antibiotics on Development of Embryos

All replicates contained eggs with a mean (± SE, *n*=50) diameter of 57.2 ± 0.5 µm and were supplied with 1 µm FSW at 28°C and 34‰ and gentle aeration. A fully orthogonal design was set up with each combination of egg density (10, 20, 30, 50, 100 and 150 eggs mL^{-1}) and supplied with or without antibiotics (0.01 g L^{-1}). The antibiotic used was a combination of Erythromycin and Tetracycline which has been shown to be effective with pearl oyster larvae (Doroudi 2001). Each combination was replicated three times.

At completion of both experiments (24 h), all remaining newly hatched larvae were collected on a 25 µm mesh sieve and transferred into 80 ml beakers where samples for each replicate were taken. Larvae were counted and measured using a binocular microscope fitted with a graticule. Percentage development to D-stage

veligers was determined for both experiments, and only larvae that had developed into D-stage veligers were recorded. A two-factor analysis of variance (ANOVA) was used to determine the effects of: (1) water temperature and salinity and (2) density and the addition of antibiotics on the development of *P. fucata* embryos into D-stage veliger larvae. Data were arcsine transformed prior to analysis to normalise the data (Zar 1984). Growth *per se* was not recorded as the variation in size of D-stage veligers for pearl oysters is very minimal and significant differences are hard to identify.

4.3 Results

4.3.1 Effects of Water Temperature and Salinity on Development of Embryos

P. fucata embryos developed into D-stage veligers under most water temperature and salinity combinations used in this study (Table 4.1). While maximum development to D-stage occurred for larvae cultured at 26°C and 28‰, surface response curves indicated greater than 80% development to D-stage veligers cultured at water temperatures of 26-28°C and a salinity range of 28-32‰ (Fig. 4.1). Embryos cultured at a combination of 28°C and 37‰ and at combinations of 32°C and 31‰, 34‰ and 37‰ did not develop into D-stage veligers. Variations in larval development between water temperature, salinity and their interaction were significant ($p<0.05$; Table 4.2). The proportion of embryos developing into D-stage veligers decreased with increasing water temperature and salinity (Fig. 4.1).

4.3.2 Effects of Density and Antibiotics on Development of Embryos

P. fucata embryos developed to D-stage veligers when cultured under all egg densities and antibiotic combinations tested. Development of D-stage veligers was independent of antibiotic addition. Maximum development (82.2 ± 1.5%) to D-stage

occurred for embryos stocked at a density of 10 eggs mL^{-1}, whereas minimum development (53.5 ± 1.9%) occurred for embryos cultured at a density of 150 eggs mL^{-1}. Development of D-stage veligers decreased with increasing stocking density (Fig. 4.2). Variation in larval development due to density and the interaction between density and antibiotic was significant ($p<0.05$), whereas variation due to antibiotic alone was not significant ($p>0.05$; Table 4.3).

Table 4.1. Mean (±SE, *n*=3) survival of *Pinctada fucata* embryos cultured using combinations of four water temperatures (26, 28, 30 and 32°C) and four salinities (28, 31, 34 and 37‰).

Temperature (°C)	Salinity (‰)	Survival (%) (Mean ± SE)
26	28	81.3 ± 7.4
26	31	94.3 ± 0.6
26	34	63.7 ± 5.9
26	37	46.7 ± 1.6
28	28	77.0 ± 8.0
28	31	83.3 ± 7.7
28	34	66.7 ± 3.3
28	37	0.0
30	28	67.3 ± 5.4
30	31	51.7 ± 2.3
30	34	15.3 ± 15.2
30	37	7.0 ± 7.3
32	28	43.7 ± 5.1
32	31	0.0
32	34	0.0
32	37	0.0

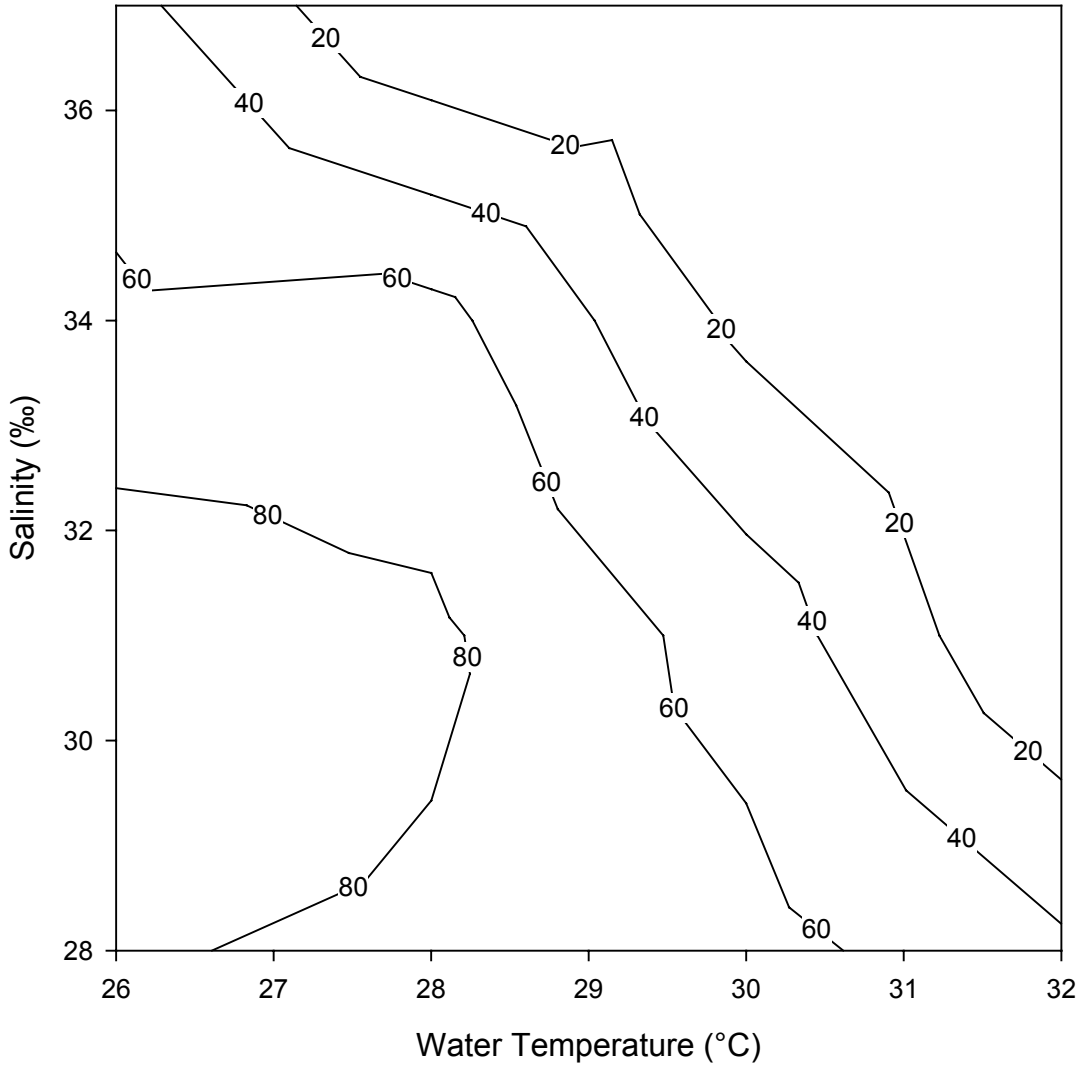


Fig. 4.1. Response surface estimation of percentage development of *Pinctada fucata* embryos to D-stage veligers after 24 h cultured at different water temperature and salinity combinations.

Table 4.2. Analysis of variance of the effects of water temperature and salinity on the development of *Pinctada fucata* embryos, using arcsine transformed data.

Source of Variation	*df*	SS	F	P
Temperature (A)	3	3.77	55.95	0.0001*
Salinity (B)	3	3.06	45.42	0.0001*
A x B	9	1.18	5.86	0.0001*
Error	32	0.72		

* Significant P<0.05

Table 4.3. Analysis of variance of the effects of density and the addition of antibiotics on the development of *Pinctada fucata* embryos, using arcsine transformed data.

Source of Variation	*df*	SS	F	P
Density (A)	5	0.66	23.08	0.0001*
Antibiotic (B)	1	0.009	1.66	0.21
A x B	5	0.15	5.38	0.002*
Error	24	0.14		

* Significant P<0.05

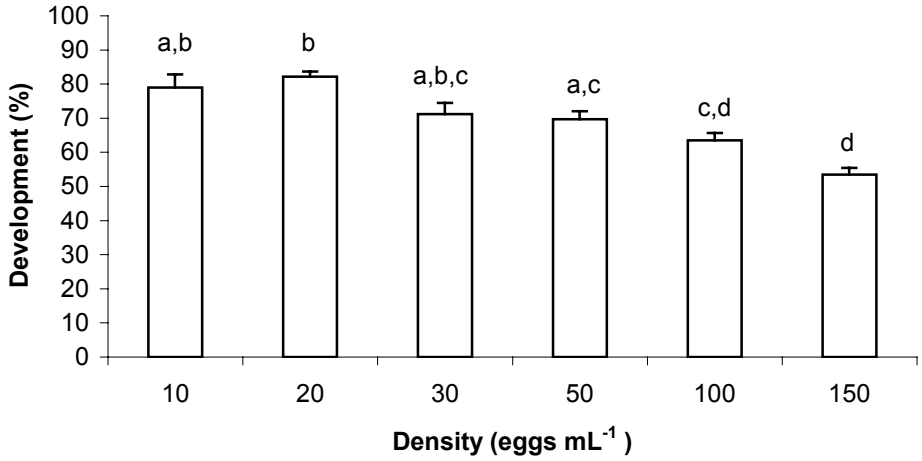


Fig. 4.2. Mean (± SE, n=3) percent development of *Pinctada fucata* embryos to D-stage veligers cultured at various densities (10, 20, 30, 50, 100 and 150 eggs mL^{-1}) using pooled data (with or without the addition of antibiotics). Means with the same superscript are not significantly different (P>0.05).

4.4 Discussion

4.4.1 Effects of Water Temperature and Salinity on Development of Embryos

Most water temperature and salinity combinations used in this study resulted in the development of *P. fucata* embryos into D-stage veligers. Maximum development of embryos occurred under culture conditions of 26-28°C and 28-32‰, which closely resembled conditions at which spawning was conducted (28°C and 34‰). Development of embryos decreased with increasing water temperature above 30°C and salinity above 34‰. In a recent study, O'Connor and Lawler (2004) noted that *P. imbricata* (=*P. fucata*) developed into normal D-stage veligers at 18-26°C and 29-35‰ cultured under temperate conditions. While development of pearl oysters occurred at similar salinities during the present study and that of O'Connor and Lawler (2004), optimal water temperatures in temperate regions were broad at 18-26°C (O'Connor and

Lawler 2004), while in the tropics, optimal water temperatures were narrow at 26-29°C (present study). Doroudi *et al.* (1999b) reported that maximum growth and survival of embryos of *P. margaritifera* (a tropical species) occurred at 26-29°C and 28-32‰, while normal development did not occur at temperatures less that 25°C or above 30°C.

Doroudi *et al.* (1999b) suggested that pearl oyster embryos are only able to tolerate conditions similar to that in which spawning was conducted. It was further suggested that as salinity naturally fluctuates in tropical Australia due to monsoonal conditions that salinity would not affect the culture of *P. margaritifera* to the same extent as temperature, since temperature does not fluctuate as much as salinity during the monsoon season. Other research has suggested that environmental tolerances of embryos are based on the environmental conditions during broodstock maturation and spawning, and has been shown to be true for oysters (Davis and Calabrese 1964), scallops (Tettelbach and Rhodes 1981) and clams (Calabrese 1969; Cain 1973). For example, Tan and Wong (1996) noted that larvae of rock oysters, *Crassostrea belcheri*, which naturally occur in the tropical monsoonal waters of Malaysia, are able to tolerate salinities in the range of 12-30‰; however, optimal development occurs at 24-30‰, which are the normal environmental conditions in which maturation and spawning occurs. Water temperature and salinity ranges of *P. fucata* juveniles are discussed further in Chapter 7.

Salinity fluctuations are only likely to occur in in-shore waters and not in off-shore waters. However, in areas of off-shore waters, runoff from continental islands may influence salinity variations. While this study was conducted at Orpheus Island, which has been characterised as off-shore (Chapter 2), plumes of freshwater from the Herbert River reached Orpheus Island on two occasions during this study (Pit pers. obs.). While it is unlikely, that these irregular freshwater plumes will influence the

salinity tolerance ranges of developing embryos of *P. fucata* from Orpheus Island, the high run-off during the monsoonal period may lead to a wider salinity tolerance. As indicated by O'Connor and Lawler (2004), the abrupt salinity changes that embryos were faced with at the beginning of the experiment were severe and do not represent the gradual changes that would occur in the natural environment; however, the information generated from this study can be applied directly to a hatchery environment where water quality parameters are controllable. The aim of this study was to determine the effects of variable water temperature and salinity on the development of *P. fucata* embryos and not the tolerances at which embryos can survive if salinity is slowly changed. The information generated in this study will help in identifying suitable sites for hatchery production of *P. fucata* in tropical areas of Queensland (Chapter 8).

4.4.2 Effects of Density and Antibiotics on Development of Embryos

Embryo density significantly affected development of D-stage *P. fucata* veligers; however, addition of antibiotics did not improve survival when compared to development of embryos without antibiotics. Maximum development of D-stage veligers occurred at a density of 20 eggs mL^{-1} (82.2 ± 1.5%) but was not significantly different to development of embryos cultured at densities of 10 and 30 eggs mL^{-1} (79 ± 3.8% and 71.2 ± 3.3%, respectively). Embryos stocked at a density of 150 eggs mL^{-1} recorded the lowest rate of development into D-stage veligers (53.5 ± 1.9%), however, survival to D-stage was still high. In the only other study on the effects of density on pearl oyster embryos, Southgate *et al.* (1998b) observed that a stocking density of 10 eggs mL^{-1} resulted in the greatest survival of *P. margaritifera* and *P. maxima* embryos

at 93.2 ± 0.55% and 82.5 ± 1.4%, respectively. These values are considerably higher than the results from the current study (maximum 82.2 ± 1.5%). However, Southgate *et al.* (1998b) recorded higher developmental survival rates at all densities compared to the present study (Table 4.4). The differences between studies can probably be attributed to differences between species, variations in egg quality between successive larval runs, and differences in culture conditions. In order to successfully compare between species, experiments would need to be conducted simultaneously in the same laboratory using the same water source.

Table 4.4. Embryonic development of the major pearl producing species and their associated survival rates cultured at different densities.

	Pearl Oyster Species		
Density	*P. fucata* [1]	*P. margaritifera* [2]	*P. maxima* [3]
10	79%	93.2%	82.4%
20	82.2%	90.2%	79.6%
30	71.2%	84.6%	77.5%
40	N/A	N/A	77.2%
50	69.7%	87.8%	76.6%
100	63.5%	79.3%	74.1%
150	53.5%	79.5%	N/A

[1] Present Study
[2] Southgate *et al* (1998b)-Experiments carried out in Australia
[3] Southgate *et al* (1998b)-Experiments carried out in Indonesia

Loosanoff and Davis (1963) noted that the optimal density for fertilisation of oyster, *Crassostrea virginica*, eggs was 40 eggs mL^{-1} while Dos Santos and Nascimento (1985) working with the same species, found that a density of 10-40 eggs mL^{-1} resulted in optimal embryo development. However, densities as high as 100 eggs mL^{-1} resulted in normal development of the embryos of the oyster, *C. gigas* (Helm and Millican 1977). In contrast, Heasman *et al.* (1996) found no significant difference in development of D-stage veligers of scallop, *Pecten fumatus*, cultured at densities of 5-50 eggs mL^{-1}. Heasman *et al.* (1996) further noted that bacteria numbers increased with increased stocking densities, while the presence of *Vibrio* sp., a bacterium, which commonly causes widespread mortality during hatchery production of bivalves, did not differ across different stocking densities. Total bacterial and *Vibrio* counts were not recorded in this study, but may explain the lower developmental success in higher stocking densities (150 eggs mL^{-1}).

Optimal density needs to be determined based on the economics of the hatchery. As stated in section 3.4 our hatchery only requires a survival rate of 40% of stocked embryos to D-stage to allow all larval tanks to be stocked with larvae, assuming a stocking density of 50 eggs mL^{-1}, if two 500 L hatching tanks are utilised. Therefore, it would be more feasible to stock two tanks and achieve 40% survival rather than stock one tank and hope for 80% survival. Furthermore, it has been reported that lower stocking densities not only result in higher survival, but also in healthier larvae (e.g. Southgate *et al.* 1998b; Honkoop and Bayne 2002)

Results from this study suggest that the use of antibiotics will not improve development of *P. fucata* to D-stage veliger larvae. It has been suggested that antibiotics may only benefit during embryonic development when spawning has occurred under unhygienic conditions (pers. comm. J. Lucas). However, Doroudi

(2001) reported the addition of antibiotics improved survival of *P. margaritifera* larvae supplied with Erythromycin and Tetracycline and noted that the antibiotics they used are well suited for pearl oyster culture as they are stable in relatively high water temperatures and are effective against the development of resistant strains of bacteria. The use of antibiotics is often required during larval development of bivalves (Riquelme *et al.* 2001). Bacteria occur naturally in seawater, and those such as *Vibrio* spp., which are opportunistic pathogens (Munro *et al.* 1999), can proliferate rapidly during culture due to inadequate treatment of culture water.

The type of antibiotics used for bivalve culture (if any) depends principally on the water source, the hygiene levels within the hatchery and on environmental regulations which may be present. Some hatcheries routinely use antibiotics, while other hatcheries do not require antibiotics at all. Antibiotics are often used when larvae are weak and therefore more susceptible to bacterial attack. There can however, be severe consequences of continual antibiotic use including the increase in costs of production and the possibility of bacteria becoming resistant to the antibiotic (Uriate *et al.* 2001). Bacterial outbreaks can be minimised through correct management practices. This occurs in the form of adequate treatment of culture seawater by filtration and ultra-violet sterilisation and adequate cleaning of all equipment which comes into contact with the culture seawater or the larvae. Riquelme *et al.* (1997) and Riquelme *et al.* (2001) noted that the use of inhibitor-producing bacteria (pro-biotics) caused no deleterious effects on growth and survival of scallops, *Argopecten purpuratus*, which survived the entire larval stage without the addition of antibiotics.

Unfortunately due to logistics, the current study was only able to look at combinations of two factors at a time. An interesting point, is that the optimal conditions for the development of *P. fucata* embryos (as outlined below) are different

from those used as standard practice in our hatchery at Orpheus Island Research Station (Southgate *et al.* 1998b; Doroudi *et al.* 1999b). For example, in the experiments reported in this chapter, salinity was controlled at 34‰, yet optimal growth and survival in the water temperature and salinity experiment was observed at a salinity of 28-32‰. Both experiments were conducted simultaneously and standard protocols (i.e. water temperature and salinity in the density and antibiotic experiment) were based on previous protocols used in our hatchery. Ultimately, further research is required to look at combinations of many factors to try to identify the synergistic effects of multiple factors.

The results of the two experiments reported in this chapter provide an understanding of the requirements for embryonic development of *P. fucata* and will help establish optimal protocols for hatchery production of Akoya pearl oysters in Queensland. Results from this chapter suggest that embryos should be cultured under the following conditions:

- a water temperature of 26-28°C;
- a salinity range of 28-32‰;
- a density of 10-30 eggs mL^{-1}; and
- without antibiotics.

Chapter 5 Effects of Density During Larval Development

5.1 Introduction

Appropriate biological information relating to growth and survival, is vital in assessing the potential of commercial bivalve culture (Hurley and Walker 1996). Hatchery production of pearl oysters is largely based on the culture methods used for other commercially important bivalves (e.g. Loosanoff and Davis 1963). While some detailed information on hatchery production of pearl oysters is known for some species, there is still a paucity of information for certain aspects of this process for other species. An area that has received little attention to date is the importance of larval density as a factor affecting their growth and survival.

D-stage pearl oyster larvae are typically stocked at densities of 1-10 larvae mL^{-1} (Alagarswami *et al.* 1983; Rose 1990; Victor *et al.* 1995; Southgate and Beer 1997; Pit and Southgate 2000; O'Connor *et al.* 2003). It has been suggested that initial larval densities of 2 larvae mL^{-1} produce optimal growth and survival of *P. fucata* in India (Anon 1991). Further, Alagarswami *et al.* (1987) noted that an initial stocking density of 2 larvae mL^{-1} resulted in maximum growth and settlement of *P. fucata* larvae and spat, compared with larvae cultured at initial stocking densities of 3, 4, 5 and 10 larvae mL^{-1} in India; however, their treatments were not replicated. Dharmaraj *et al.* (1991), however, suggested that initial densities higher than 2 larvae mL^{-1} result in poor growth of pearl oysters, *P. fucata*, in India, while densities as high as 12-14 larvae mL^{-1} have been employed in Japan with no apparent adverse effects on growth and survival of *P. fucata* (Hayashi and Seko 1986).

Rose (1990), suggested that pearl oysters, *P. maxima*, should initially be stocked at a density of 5 larvae mL^{-1} and that stocking density should be decreased to 2 stocked at a density of 5 larvae mL^{-1} and that stocking density should be decreased to 2 larvae mL^{-1} by day 10 and then to 1 larvae mL^{-1} until day 18. They further suggested that larvae should be stocked at densities lower than 0.5 larvae mL^{-1} at settlement. While the above studies have suggested appropriate larval density for pearl oysters, none of them have quantified optimal density and its affects on growth and survival during hatchery production.

Studies investigating the effects of larval density on growth and survival of bivalve larvae have typically fed larvae held at different densities the same ration of algae. Helm and Millican (1977) noted that growth of Pacific oyster, *Crassostrea gigas*, larvae at different stocking densities is a function of food supply, while Loosanoff and Davis (1963) in their review on bivalve rearing noted that sub-optimal food rations lead to decreased developmental rates in a variety of bivalves. This is supported by the findings of His *et al.* (1989) and Hurley and Walker (1996) who found that bivalve larvae held at high stocking densities may have slow development rates due to feed limitation when compared to larvae held at lower stocking densities due to the relatively dis-proportionate amounts of micro-algae that are available to larvae at different densities.

The aim of this chapter was to determine optimal stocking densities for *P. fucata* larvae fed rations proportional to larval density.

5.2 Materials and Methods

Current protocols for culture of pearl oyster, *P. margaritifera*, larvae at the James Cook University hatchery include stocking 1 or 2-day old D-stage larvae at a

density of 1-2 larvae mL^{-1} into larval rearing tanks containing 1 μm filtered seawater ranging from 28-31°C and 33-35‰. As detailed in Chapters 1 and 2, *P. margaritifera* larvae are generally cultured in a static system for 10 days then transferred to a flow-through system until they reach a size at which they are competent to settle (around day 20). Once competent to settle, larvae are transferred to settlement tanks.

A similar protocol was used for *P.* fucata in these experiments, with the following modifications in order to determine optimal density for larval development. Experiments were conducted to investigate the effects of density on growth and survival during the three different phases of hatchery culture from:

(1) static-culture phase (days 2-11);

(2) flow-through culture phase (days 13-20); to

(3) settlement and spat culture (days 20-43).

Spawning and larval rearing were carried out as detailed in Chapter 2 unless otherwise noted.

5.2.1 Effects of Density on Larval Growth and Survival

In order to determine the optimal density for maximum growth and survival of *P. fucata* larvae, two experiments were conducted from: (1) day 2-11 (Experiment 1) and (2) day 11-20 (Experiment 2). Larvae were cultured either using a static system (days 2-11) or a flow-through system (days 11-20). In Experiment 1, two-day old larvae with a mean (± SE, *n*=50) antero-posterior measurement (APM) of 80.8 ± 0.3 μm were stocked at densities of either 1, 2, 5, or 10 larvae mL^{-1}, while in Experiment 2, 11-day old larvae with a mean (±SE, *n*=50) APM of 107.3 ± 1.6 μm were stocked at densities of either 1, 2, or 5 larvae mL^{-1}. Each replicate aquarium (15 L conical based)

contained 10 L of 1 μm FSW supplied with gentle aeration. Flow-through aquaria systems were the same tanks as used for the static system, however, they contained a central standpipe which was fitted with 88 μm mesh to allow a minimum daily water exchange of 100%; the mesh ensured larvae were not washed out of the aquarium (Fig 5.1). Independent of system design (static or flow-through), aquarium were drained every third day and larvae were retained on 37 μm sieves. Larvae were washed from sieves and concentrated into an 80 mL beaker. Duplicate 1 mL aliquots were taken from the beaker to determine growth (by measurement of APM) and survival. In order to ensure that growth and survival was not affected by food availability and that the effects observed were due to larval density, survival was determined for each replicate every 3 days when food ration was altered to ensure larvae across all densities were receiving the same amount of daily ration. The aim of these experiments was to determine the effect of initial stocking density on final growth and survival; density was assessed every three days purely to try to remove dis-proportional feeding as an influential factor.

5.2.2 Effects of Density on Spat Settlement

In order to determine the optimal density for metamorphosis and settlement, and survival of *P. fucata* spat, 20-day old larvae with a mean (± SE, *n*=50) APM of 183 ± 3.6 μm were stocked at densities of 0.1, 0.2, 0.5 or 1.0 larvae mL^{-1}. Three spat collectors (consisting of a 0.5 m^2 piece of woven shade cloth placed inside a mesh bag) were added to each of 3 replicate aquarium (15 L conical based), which contained 10 L of 1 μm FSW supplied with gentle aeration (Fig. 5.1). Aquaria were set-up as a flow-through design as described above (section 5.2.1). Survival and growth data were only

determined on completion of the experiment. On day 43, spat collectors were removed from aquaria. Three replicate spat collectors from each treatment were sampled to determine growth and survival, while the remaining six spat collectors from each treatment were placed into plastic mesh trays (55 x 30 x 10 cm; Fig. 2.4c) and transferred to the long-line at Orpheus Island. Trays remained on the long-line for 14 weeks and were cleaned manually with scrubbing brushes after 8 weeks. After 14 weeks, trays were removed and growth and survival of spat from different stocking densities was determined.

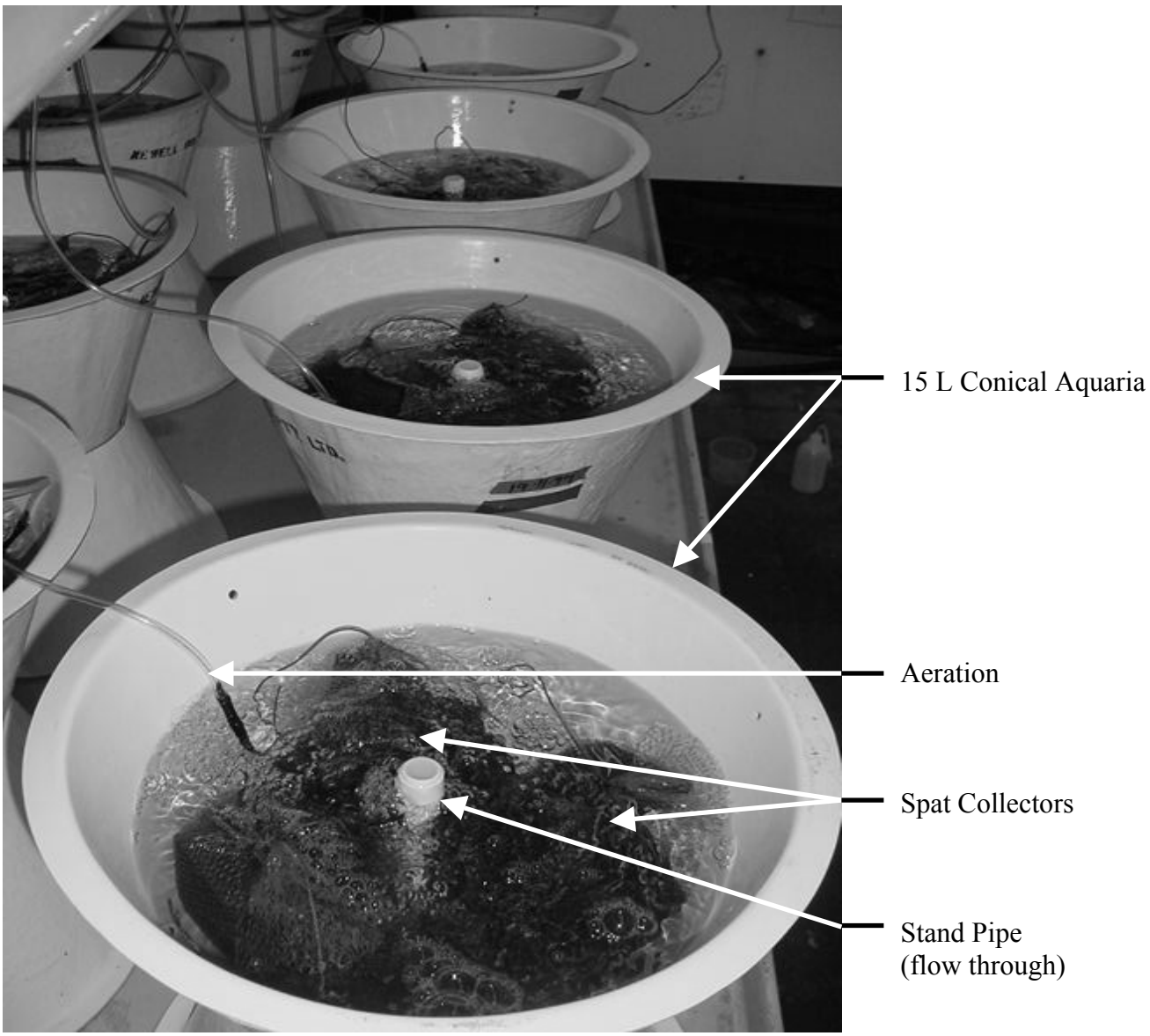


Fig. 5.1. Settlement system used for *Pinctada fucata* 'spat'. This system was also used for experiment 1 and experiment 2.

One-way analysis of variance was used to identify the effects of density on growth and survival of larvae and spat. Assumptions including homogeneity and normality were met (Zar 1984). However, assumptions were violated when determining subsequent juvenile growth as a result of different spat settlement densities and a Kruskal-Wallis analysis was conducted. Means which were significantly different were identified with a Dunnetts T3 post hoc test.

5.3 Results

5.3.1 Effects of Density on Larval Growth and Survival

Initial larval density at day 2 in Experiment 1 had a significant ($F_{3,116}$=23.34, P<0.001) effect on the growth of *P. fucata* at 11 days of age. Maximum mean (±SE, *n*=90) APM (104.7 ± 2.0 μm) at the end of the experiment was recorded in larvae cultured at a density of 1 larvae mL^{-1} and was significantly larger than larvae cultured at all other densities (Fig. 5.2). The mean APM of larvae cultured at a density of 2 and 5 larvae mL^{-1} were not significantly different (p>0.05) at 97.7 ± 1.2 μm and 96.3 ± 1.1 μm, respectively but were greater than that of larvae cultured at a density of 10 larvae mL^{-1} (90.3 ± 0.6 μm) at the end of the experiment.

Survival of *P. fucata* was significantly affected ($F_{3,8}$=11.44, P<0.005) by larval stocking density (Fig. 5.3). Maximum mean (±SE, *n*=3) survival at the end of the experiment was recorded in larvae cultured at a density of 1 larvae mL^{-1} (74.0 ± 9.9%)

but this was not significantly different to that of larvae cultured at stocking densities of 2 and 5 larvae mL^{-1} (51.0 ± 7.9% and 43.9 ± 3.1%, respectively). Lowest mean (±SE, *n*=3) survival was observed for larvae cultured at a density of 10 larvae mL^{-1} (15.0 ± 6.1%), but this was not significantly different from that of larvae cultured at a density of 5 larvae mL^{-1} (Fig. 5.3).

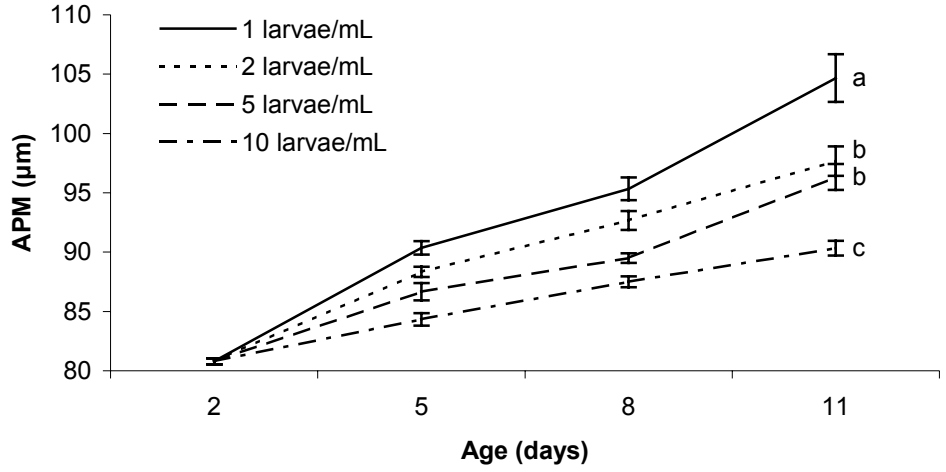


Fig. 5.2. Changes in mean (±SE, *n*=90) antero-posterior shell length (APM) of *Pinctada fucata* larvae (2-11 days of age) cultured at four densities (1, 2, 5, and 10 larvae mL^{-1}). Means with the same superscript are not significantly different (p>0.05).

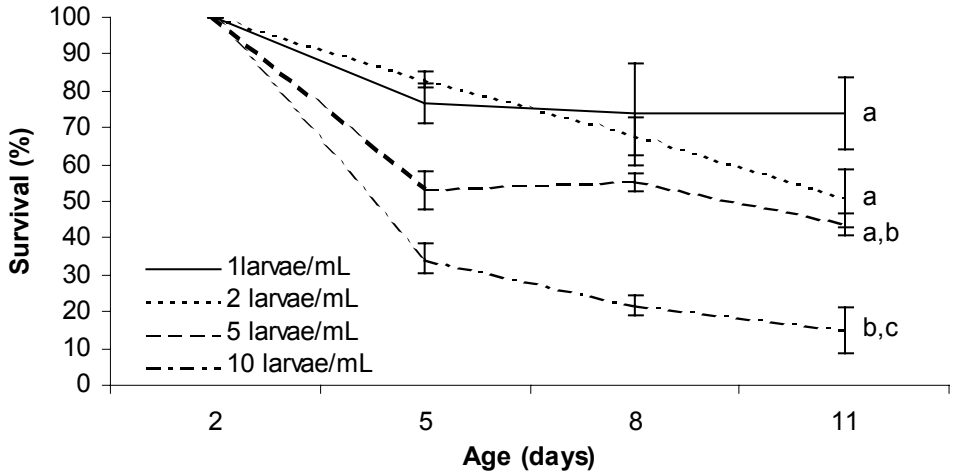


Fig. 5.3. Changes in mean percent (± SE, *n*=3) survival of *Pinctada fucata* larvae (2-11 days of age) cultured at four different stocking densities (1, 2, 5, and 10 larvae mL^{-1}). Means with the same superscript are not significantly different ($p>0.05$).

Mean (±SE, *n*=90) changes in APM for older *P. fucata* larvae (days 11-20) in Experiment 2 cultured at three different densities (1, 2 and 5 larvae mL^{-1}) are shown in Fig. 5.4. Maximum mean APM (223.3 ± 3.3 μm) at the end of the experiment was recorded for larvae cultured at a density of 1 larvae mL^{-1}, which was significantly different ($F_{2,87}$=23.7, $p<0.05$) to that of larvae cultured at densities of 2 and 5 larvae mL^{-1}. Minimum mean APM (190 ± 3.3 μm) at the end of the experiment was recorded for larvae cultured at a density of 5 larvae mL^{-1} and this was significantly different to that of larvae cultured at a density of 2 larvae mL^{-1} (204.3 ± 3.7).

Survival of larvae cultured from days 11-20 was significantly different ($F_{2,6}$=9.18, $p<0.05$; Fig 5.5). Larvae cultured at a density of 2 larvae mL^{-1} recorded the highest survival (53.3 ± 4.4%) at the end of the experiment and this was not significantly different compared to that of larvae cultured at a density of 1larvae mL^{-1} (55.0 ± 2.9%). Larvae cultured at a density of 5 larvae mL^{-1} (37.7 ± 1.5%), had significantly lower survival than larvae cultured at stocking densities of 1 and 2 larvae mL^{-1} (Fig. 5.5).

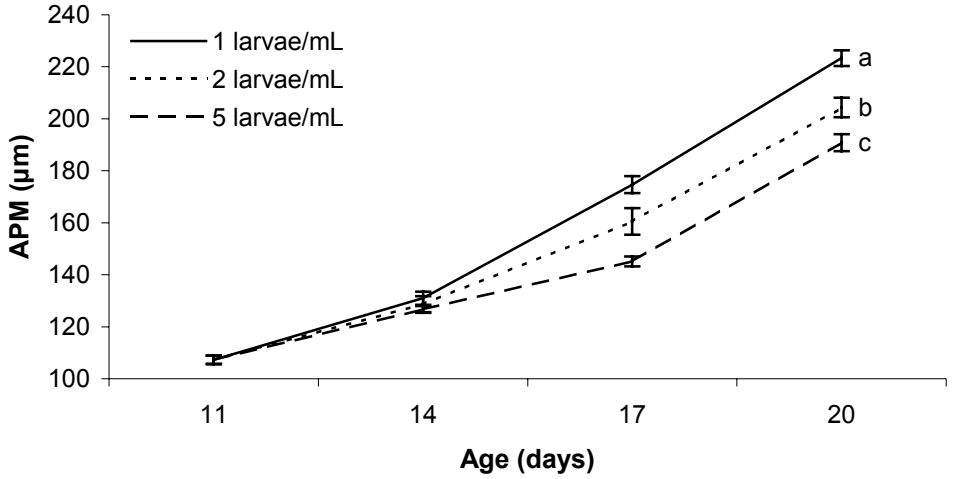


Fig. 5.4. Changes in mean (±SE, *n*=90) antero-posterior shell length (APM) of *Pinctada fucata* larvae (11-20 days of age) cultured three different densities (1, 2, and 5 larvae mL^{-1}). Means with the same superscript are not significantly different (p>0.05).

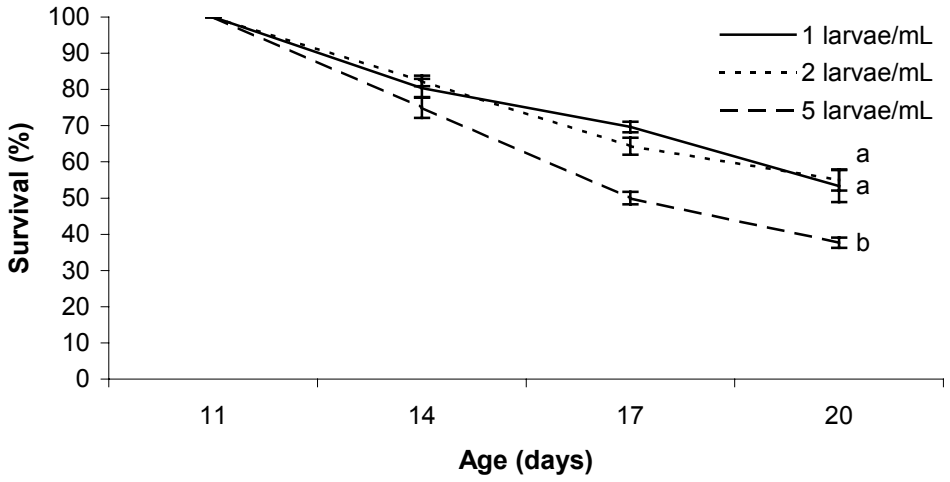


Fig. 5.5. Changes in mean percent (± SE, *n*=3) survival of *Pinctada fucata* larvae (11-20 days of age) cultured at three different stocking densities (1, 2, and 5 larvae mL^{-1}). Means with the same superscript are not significantly different (p>0.05)

5.3.2 Effects of Density on Spat Settlement

The spat of larvae initially placed into settlement tanks at four different densities (0.1, 0.2, 0.5 and 1.0 larvae mL^{-1}) showed significant differences ($F_{3,116}$=78.6, $p<0.001$) in dorso-ventral shell height (DVH) after 23 days. Maximum DVH was recorded for spat resulting from an initial density of 0.1 larvae mL^{-1} (1.71 ± 0.03 mm), but this was not significantly different from that of spat resulting from an initial density of 0.2 larvae mL^{-1} (Fig. 5.6). Spat resulting from an initial density of 1.0 larvae mL^{-1} (1.0 ± 0.03 mm) recorded the lowest DVH after 23 days of culture and were significantly smaller than spat cultured at all other densities.

Spat further cultured from 6 to 20 weeks of age showed similar patterns with those resulting from an initial density of 0.1 larvae mL^{-1} still the largest (42.3 ± 1.3 mm). Spat resulting from an initial density of 1.0 larvae mL^{-1} were significantly smaller than spat cultured at the remaining densities at 6 weeks of age (Fig. 5.6), although this difference was no longer significant at 20 weeks of age (Fig. 5.7).

Maximum mean (±SE, *n*=3) survival was observed for spat resulting from an initial density of 0.1 larvae mL^{-1} (68.1 ± 5.9%) and was significantly greater ($F_{3,8}$=34.2, $p<0.001$) than that for spat cultured at the other densities (0.2, 0.5 and 1.0 larvae mL^{-1}). Spat resulting from initial densities of 0.2 and 0.5 larvae mL^{-1} had lower survival rates (50.5 ± 2.7% and 37.7 ± 1.4%, respectively) that were not significantly different from each other. Lowest survival (20.3 ± 1.8%) occurred for spat cultured at an initial density of 1.0 larvae mL^{-1} and was significantly lower than that of spat cultured at all other densities (Fig. 5.8). Survival from 6 until 20 weeks was not determined.

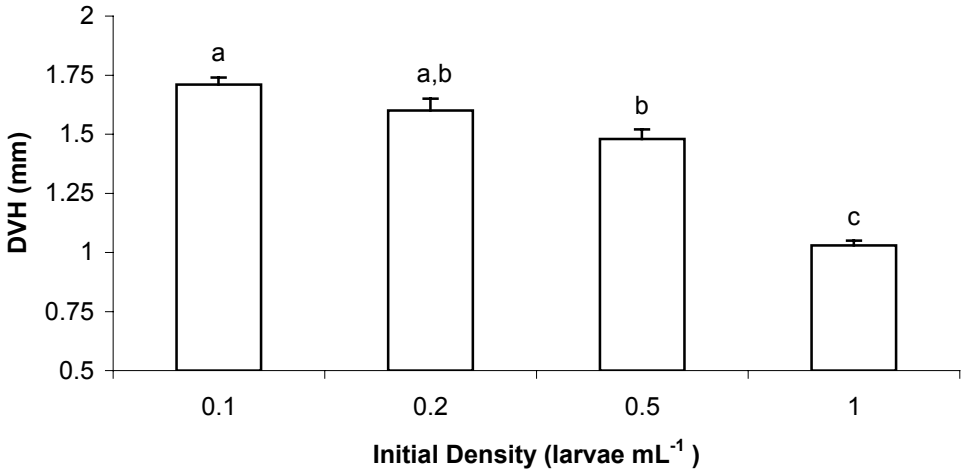


Fig. 5.6. Mean (±SE, *n*=90) dorso-ventral shell height (DVH) of *Pinctada fucata* spat (20-43 days of age) resulting from four initial stocking densities (0.1, 0.2, 0.5 and 1.0 larvae mL^{-1}) at transfer (6 weeks of age). Means with the same superscript are not significantly different ($p>0.05$).

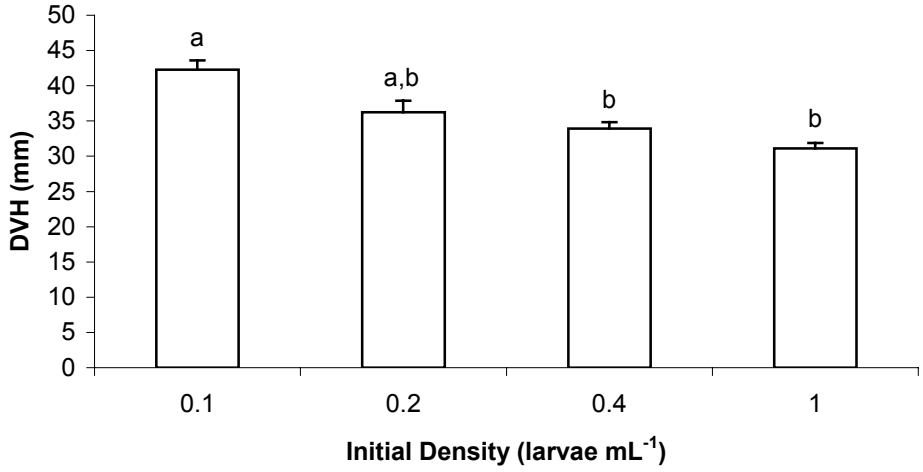


Fig. 5.7. Mean (±SE, *n*=90) dorso-ventral shell height (DVH) of *P. fucata* spat (20-43 days of age) resulting from four initial stocking densities (0.1, 0.2, 0.5 and 1.0 larvae mL^{-1}) at grading (20 weeks of age). Means with the same superscript are not significantly different ($p>0.05$).

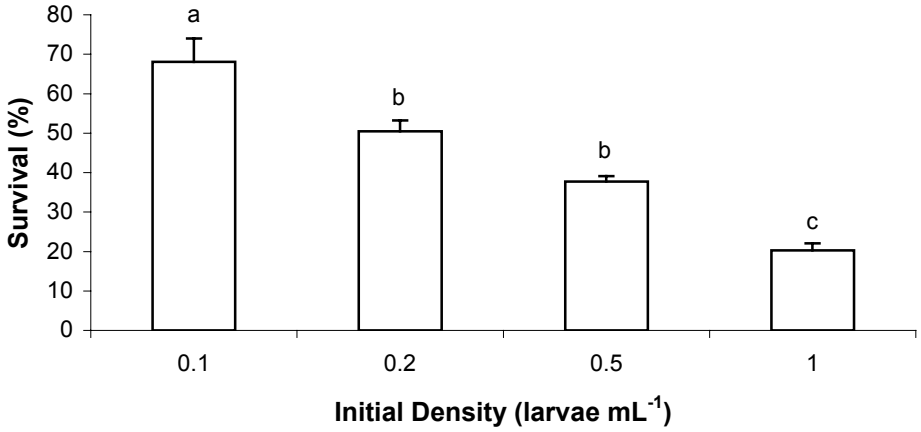


Fig. 5.8. Mean percent (± SE, *n*=3) survival of *Pinctada fucata* spat (20-43 days of age) resulting from four initial stocking densities (0.1, 0.2, 0.5 and 1.0 larvae mL^{-1}). Means with the same superscript are not significantly different ($p>0.05$).

5.4 Discussion

The range of stocking densities used in this study were chosen on the basis of the results of Rose (1990) who suggested that stocking densities should be decreased with increasing larval age. Rose (1990) suggested that *P. maxima* larvae should initially be stocked at a density of 5 larvae mL^{-1} and that this should be decreased to 2 larvae mL^{-1} by day 10 after which larvae should be stocked at 1 larvae mL^{-1} until settlement. *Pinctada fucata* larvae showing the highest growth and survival in the present study were those from the lowest stocking density tested 1 larvae mL^{-1}.

In the only prior study investigating the effects of varying density on *P. fucata* larvae, Alagarswami *et al.* (1987) noted that an initial stocking density of 2 larvae mL^{-1} provided maximum growth and settlement of larvae and spat, compared with larvae cultured at densities of 3, 4, 5 and 10 larvae mL^{-1}; however, treatments in this study were not replicated. In addition, experiments conducted by Alagarswami *et al.* (1987)

were based on initial stocking densities (on day 1) and were not changed during larval development. This would have resulted in varying stocking densities at settlement where fixed algae ration would have been dis-proportionally supplied to larvae (see below). While most of the published research on pearl oyster larvae investigating factors other than stocking density (e.g. algae ration, temperature and salinity tolerances) have suggested optimal stocking densities of around 2 larvae mL^{-1} (Anon 1991; Victor *et al.* 1995; Southgate and Beer 1997; Doroudi *et al.* 1999b; Pit and Southgate 2000), other studies have suggested higher densities ranging from 3-28 larvae mL^{-1} (Alagarswami *et al.* 1983; Hayashi and Seko 1986). Optimal stocking densities for larvae are likely to vary between species and according to environmental conditions (water temperature and salinity) and therefore it is imperative that an economical analysis is conducted on a hatchery-by-hatchery basis (Chapter 8, section 8.2).

Larval density has previously been reported to influence the success rate of settlement, metamorphosis and post-larval survival in bivalves (Bourne and Hodgson 1991). Rose (1990) suggested a stocking density of no more than 0.5 larvae mL^{-1} during settlement for *P. maxima*, whereas Taylor (1998a) suggested that maximum growth and survival of settled spat is obtained using an initial stocking density not exceeding 1.0 larvae mL^{-1} for the same species. Southgate and Beer (1997) stocked *P. margaritifera* larvae into settlement tanks at a lower initial density of 0.2 larvae mL^{-1}, while Rose and Baker (1994) and Taylor *et al.* (1998b) used stocking densities of 0.5-1 larvae mL^{-1} and 0.5-2.0 larvae mL^{-1}, respectively for *P. maxima*. Maximum growth and survival of spat was observed for oysters settled at the lower densities (Taylor *et al.* 1998b).

It has been suggested that mucus secreted by larvae during settlement, may contain a chemical cue which induces settlement of others (Taylor *et al.*1998b). This hypothesis has interesting implications for stocking density at settlement. For example, if this is the case, stocking density of pearl oyster larvae within tanks could be increased prior to settlement to increase rate of settlement, and decreased once settlement has occurred to minimise overcrowding. More research is required on this topic. The idea of naturally produced chemicals in the form of mucous to induce settlement of pearl oysters (Taylor *et al.* 1998b) prompted the work of Doroudi and Southgate (2002), which investigated whether artificial chemicals provided to culture water would induce settlement of pearl oyster larvae. Doroudi and Southgate (2002) reported that the addition of chemicals (such as GABA) into the culture tank increased the rate of settlement of *P. margaritifera* larvae. While they recorded high mortality, they suggested that this was a result of chemical toxicity due to high concentration of the added chemicals. Further work is also required in this area.

Many studies have shown that feeding rate is vital for maximum growth and survival of bivalve larvae (e.g. Helm and Millican 1977; Doroudi and Southgate 2000). Under-fed larvae can show poor growth rates, whereas over-feeding larvae can lead to poor water quality causing decreases in larval growth and survival and increases in hatchery production costs (Doroudi and Southgate 2000). It is important when determining optimal stocking densities that algae ration is proportional to larval density (e.g. His *et al.* 1989; Hurley and Walker 1996). However, the type of algae must also be considered, as ingestion rates are vital. For example, Doroudi *et al.* (2001) reported that *P. margaritifera* larvae show greater ingestion rates for flagellates (*Pavlova* spp. and T-ISO) compared to diatoms (*Chaetoceros* spp.). In a recent study, Doroudi and Southgate (2000) investigated the combined effects of larval density and algae ration on

the growth and survival of *P. margaritifera* larvae. They reported that at suitable densities (≤ 3 larvae mL^{-1}), growth and survival were affected by algae ration; however, their optimal range was quite broad (4.5-11.5 x 10^3 cells mL^{-1}). Doroudi and Southgate (2000) did not account for decreasing larval density on the amount of daily algae fed to each aquarium.

In the present study survival was determined every three days to ensure that differences in growth and survival were a result of larval density and not algae ration. Efforts were made to ensure all larvae, independent of larval density, were supplied with the same food ration, so that differences in growth and survival due to density in the present study resulted from factors other than food ration. It is suggested that other factors such as increases in nitrogenous wastes, carbon dioxide or even oxygen depletion in treatments with greater density may explain differences in growth and survival of *P. fucata*, resulting in competition for space rather than competition for food during the present study. This hypothesis is supported by the results of Wu *et al.* (2003), who reported that increases in nitrogenous wastes and carbon dioxide production, causes decreases in growth of pearl oysters due to a decrease in the availability of oxygen.

In determining optimal density for spat development (days 20-43), growth and survival were not determined during the experiment. While in the other two experiments (days 2-11 and 11-20) algae ration was altered depending on 3-day survival statistics, this was not the case during the spat development experiment. However, similar patterns were seen with respect to maximum growth and survival of larvae and spat in all experiments. This suggests that limited space, rather than limited food supply is the determining factor in the present study. Further research is required in this area.

Mean DVH of spat at 6 weeks of age was lowest when larvae were settled at an initial stocking density of 1.0 larvae mL^{-1} compared to those settled at lower stocking densities. However, after 14 weeks in early nursery culture, there was no significant difference in mean DVH between spat resulting from initial stocking densities of 0.2, 0.4 or 1.0 larvae mL^{-1}. This result is believed to be due to nursery stocking density. As outlined in Chapter 6 (sections 6.1.2 and 6.4.2), stocking density affects growth of pearl oysters during early nursery culture with maximum growth occurring at lower stocking densities. In the present study, the experiment was initially due to be completed at transfer to the ocean (6 weeks of age), but spat were cultured for a further 14 weeks to enable follow-up of growth. However, oysters from each replicate were simply transferred to the ocean and no effort was made to standardise oyster numbers between replicates. As such, spat initially stocked at higher densities (i.e. 1.0 larvae mL^{-1}) had lower numbers of spat after 6 weeks due to high mortality. Due to lower oyster numbers in the initial high stocking densities, growth of spat was rapid. Conversely, there were greater numbers of spat, which were initially stocked at lower densities (i.e. 0.1 larvae mL^{-1}) after 6 weeks and therefore growth of oysters was slower than in replicates with low oyster numbers, which may explain why growth rates observed after 6 weeks were different compared to growth after 20 weeks.

This study has provided valuable information for relating to appropriate stocking densities of *P. fucata* larvae and spat cultured under tropical conditions in Australia. The results support the stocking density suggestions of Rose (1990) for *P. maxima* larvae and spat where stocking densities should be decreased during larval development with increasing age. However, further research is required to identify optimal feeding rations for the optimal stocking densities identified in this study. For example, under commercial-scale culture conditions it may not be feasible to regularly

determine larval survival in individual tanks and to feed different rations to different tanks. Additionally, further research should be conducted to investigate the hypothesis that settling oysters release settlement chemical cues and whether stocking densities of larvae approaching settlement can be increased to increase the rate of settlement.

The results generated in this study suggest that to maximise growth and survival of larvae and spat during hatchery production, larvae and spat should be cultured at a density of:

- 1-5 larvae mL^{-1} for days 2-11;
- 1-2 larvae mL^{-1} for days 11-20; and
- 0.1-0.2 larvae mL^{-1} for days 20-43.

Chapter 6 Effects of Different Culture Methods During Nursery Culture

6.1 Introduction

When oysters are first transferred from the controlled conditions of the hatchery to the natural conditions of the ocean they are met with a number of previously unexperienced factors including fouling, predation, currents and variations in the quality and quantity of food. As such, bivalves are often placed into nursery systems, where they are given additional care to minimise stress. Nursery culture of pearl oysters begins when oysters are either removed from spat collectors (if collected from the wild) or when spat collectors (from the hatchery) are placed into culture units such as pearl nets or trays; this usually occurs when pearl oyster spat are 5-10 mm in size (Gervis and Sims 1992). The nursery phase usually lasts between 12-24 months or until oysters attain a size at which they can be used for pearl production (ca. >50 mm for *Pinctada fucata*, 100 mm for *P. margaritifera* and 110 mm for *P. maxima*; Gervis and Sims 1992).

When pearl oysters first enter the nursery phase they are small and fragile (Monteforte and Morales-Mulia 2002) and nursery culture is often subdivided into early and late nursery culture (Friedman and Southgate 1999). Friedman and Southgate (1999) referred to early nursery culture as 'intermediate' culture and stated that small oyster spat (10-30 mm) need to be nursed through to late nursery culture when they attain a 'size refuge' whereby the pressures of predation and other stressors are decreased. As such, pearl oysters in nursery culture are usually kept in more protected areas than the main farm stock with additional care required.

The Akoya pearl oyster has not previously been cultured in tropical northern Australia. It has however, been cultured in temperate Australia (O'Connor *et al.* 2003). Consequently, research to assess the feasibility of establishing an Akoya pearl oyster industry in tropical Queensland waters is vital. The research carried out in this Chapter looks at a number of factors affecting nursery culture of hatchery produced pearl oyster spat including the effects of depth, stocking density, growth variation, culture method and fouling on oyster growth and survival.

6.1.1 Does Culture Depth Affect Growth of *Pinctada fucata*?

While some studies have reported that bivalve growth is uniform throughout the water column (e.g. Duggan 1973; Wallace and Reinsnes 1984; Cropp and Hortle 1992), others have shown that depth can be positively or negatively correlated with bivalve growth (e.g. Ventilla 1982; Dadswell and Parsons 1991; Emerson *et al.* 1994). Lodeiros and Himmelman (1996) noted that the scallop, *Euvola* (*Pecten*) *ziczac*, showed increased growth with increased depth. Similar results were also recorded for another scallop, *Amusium pleuronectus*, in which greater growth was achieved at 15 m compared to 5 m (Chaitanawisuti and Menasveta 1991). However, in general, increases in water depth are correlated with decreases in growth which have been attributed too less favourable environmental conditions such as decreased food availability due to decreased primary productivity (Ventilla 1982; Claereboudt *et al.* 1994).

Pearl oyster culture is typically conducted within waters not exceeding 25 m (Gervis and Sims 1992). There is limited information on the effects of depth on pearl oyster growth. There is to date, only one study which has effectively investigated depth effects on pearl oyster growth and survival (O'Connor *et al.* 2003). In their study,

O'Connor *et al.* (2003) reported that depth ranging from 2-6 m did not affect growth of pearl oysters, *P. imbricata* (=*P. fucata*). This suggests that all factors affecting growth of pearl oysters in NSW at 2-6m are identical, in terms of temperature and food composition and abundance. While a number of additional studies have suggested that depth affects growth and survival of pearl oysters, observed differences are more likely to result from different culture method rather than depth *per se* (Gayton-Mondragon *et al.* 1993; Taylor *et al.* 1997a; Urban 2000a). This study will determine the optimal culture depth for spat recently transferred from the hatchery to the ocean.

6.1.2 Does Stocking Density Affect Growth and Survival of *Pinctada fucata*?

Growth and survival of bivalves is known to be density dependent with slower growth and higher mortality observed with increasing stocking density due to competition for resources (Parsons and Dadswell 1992; Holliday *et al*. 1993; Maguire and Burnell 2001). Described as potentially dictating the economic feasibility of a culture operation, optimising stocking density is vitally important (Wildish *et al.* 1988; Mendoza *et al.* 2003). To minimise the negative effects of high stocking density, the number of individual oysters within each culture unit is usually decreased as oysters increase in size. Mesh size (section 6.4.4) of the culture unit it also increased as oysters grow (Gervis and Sims 1992) ensuring that water-flow through culture units, which has been shown to influence growth (Alagarswami and Chellam 1976; Claereboudt *et al*. 1994; Taylor *et al*. 1997a), is maximised.

Information on stocking density of pearl oysters is scarce. Taylor *et al.* (1997a) reported that the number of pearl oysters, *P. maxima*, within each culture unit determines the degree of oyster clumping. They noted that clumping of oyster juveniles caused decreased shell growth and survival due to competition for both food

and space. However, reducing pearl oyster numbers within culture units has been shown to maximise growth and survival (Taylor *et al*. 1997a) It has been suggested that there may be a limit whereby growth and survival does not increase further with decreasing density (Monteforte and Morales-Mulia 2002).

Most previous studies in this field have based stocking densities on the number of individuals per culture unit (e.g. Parsons and Dadswell 1992; Taylor *et al.* 1997a). However, in a recent study, O'Connor *et al.* (2003) suggested that pearl nets should be stocked on the basis of wet weight of oysters to minimise effects due to oyster size. The present study will assess the effects of stocking density in regards to available culture area within the culture unit. Unfortunately, the results of O'Connor *et al.* (2003) were not available at the time this experiment was conducted and it would be interesting to repeat the experiment using a wet weight value compared to the method employed in the present study.

6.1.3 Do Small Oysters 'Catch Up' to their Larger Counterparts?

Growth variation, which is evident for pearl oysters reared under identical conditions, is a major concern in the pearl oyster industry. Oysters need to reach a minimum size before they can be used for pearl production. Therefore, additional time is required to culture slow growers to reach pearl production size. For example, *Pinctada margaritifera* spat have been reported to range in size from 1-5 mm in dorso-ventral shell height (DVH) at 43 days of age (Pit and Southgate 2000), while spat at 106 days of age have been shown to vary in DVH from <2-23 mm (Southgate and Beer 1997). Rose (1990) recommended that continual grading and separation of fast growers from slow growers could alleviate or minimise such size variation in *P. maxima* spat. While this ensures a narrow size range of culture stock, it does not alleviate the

problem of slow growers. In a recent study, Pit and Southgate (2003b) investigated whether slow growing *P. margaritifera* spat were capable of faster growth once provided with good growing conditions. They noted that hatchery produced *P. margaritifera* spat measuring less than 5 mm, which they termed 'runts' at 3.5 months of age, were normally discarded. Although they showed that 'runts' remain relatively slow growers, they did note that some 'runts' were capable of fast growth and attained similar sizes to oysters from larger size classes within a further four months. Pit and Southgate (2003b) conducted their study for only four months before oysters reached pearls production size; however, they estimated that 'runts' required an extra 2-4 months to reach seedable size compared to 'normal' *P. margaritifera* spat.

Previous studies with *P. fucata* in India noted that oysters reach a shell height of ~50 mm (pearl production size) in 12 months (Victor *et al.* 1995), while *P. martensi* (=*P. fucata*) in China requires 24 months to reach the same size (Guo *et al.* 1999). In Columbia, Urban *et al.* (2000a) noted that *P. imbricata* (=*P. fucata*) required more than 12 months of culture, whereas Lodeiros *et al.* (2002) reported that *P. imbricata* (=*P. fucata*) cultured in Venezuela reached pearl production size in only 9 months. In temperate Australia, O'Connor *et al.* (2003) reported that *P. imbricata* (=*P. fucata*) required 12-15 months to reach pearl production size. The present study will determine the time required for different sized *P. fucata* of the same age to reach pearl production size and relate this to growth of Akoya oysters from other regions of the world.

6.1.4 Does Culture Unit Affect Growth and Survival of *Pinctada fucata*?

Culture method has been previously described as a contributing factor to growth and survival of bivalves (e.g. Maguire and Burnell 2001; Mendoza *et al*. 2003), including pearl oysters (Friedman and Southgate 1999; Southgate and Beer 2000;

Teitelbaum 2001). Friedman and Southgate (1999) trialled a number of culture apparatus on the growth and survival of wild-captured *P. margaritifera* juveniles in the Solomon Islands and concluded that apparatus which minimised aggregation resulted in optimal growth and survival of juveniles after 5 months. Similarly, Southgate and Beer (2000) investigated five different methods for nursery culture of hatchery-produced *P. margaritifera* in northern Australia and, supporting the findings of Friedman and Southgate (1999), also found that methods that minimised aggregation achieved optimal growth and survival. Additionally, Urban (2000a) investigated the effects of 3 different culture systems (propylene mesh bags in suspension, plastic boxes covered in propylene net in suspension, and plastic boxes covered in propylene net on the sea-bottom) on the growth and survival of *P. imbricata* (=*P. fucata*) in the Caribbean and reported superior growth in boxes, independent of suspension/bottom culture. Many similar studies have been conducted with scallops. Maguire and Burnell (2001) investigated growth of scallops, *Pecten maximus*, in two types of nets (lantern nets and pearl nets) and they attributed differences in growth to the combined effects of net shape and mesh size. Mendoza *et al.* (2003) reported smaller culture units were superior to large culture units in respect to scallop, *Lyropecten nodosus*, growth. This study investigates the effects of culture unit on growth of *P. fucata* during early and late nursery culture.

6.1.5 What is the Optimal Cleaning Frequency for Maximum Growth and Survival of *Pinctada fucata*?

Fouling is a major problem in suspended culture of bivalves (Claereboudt *et al*. 1994; Lodeiros and Himmelman 1996; Taylor *et al.* 1997b). Negative effects of fouling on growth and survival have been reported for *Pinctada fucata* (Alagarswami

and Chellam 1976; Mohammad 1976), *P. margaritifera* (Pit and Southgate 2003a) and *P. maxima* (Taylor *et al*. 1997b). Fouling of culture units has several drawbacks including:

(1) reduced water-flow through culture units, with a subsequent decrease in food availability (Paul and Davies 1986; Wildish *et al*. 1988; Taylor *et al.* 1997b) and reduced removal of waste products from within the culture unit;

(2) fouling organisms themselves may filter-feed and thus compete with cultured pearl oysters for available food (Claereboudt *et al.* 1994; Côté *et al.* 1994; Teitelbaum *et al.* 2004);

(3) fouling reduces oxygen availability, which has been shown to subsequently affect growth (Wallace and Reisnes 1985; Wu *et al.* 2003) and;

(4) the additional weight from fouling organisms places additional strain on culture systems.

(5) Addition of predators to culture units and subsequent mortality of stock (Pit and Southgate 2003a)

In a recent study, Lodeiros *et al.* (2002) did not observe any negative effects of fouling on growth and survival of *P. imbricata* (=*P. fucata*) in the Caribbean. However, their study, compared growth of oysters from suspended and bottom culture, rather than comparing cleaning frequencies within each of the different culture units; however, different culture units can effect growth and survival of pearl oysters (section 6.4.4).

Damage caused to culture stock, culture units, and the costs involved in regular removal of fouling organisms can result in enormous financial losses to aquaculture industries (Arakawa 1990) including the cultured pearl oyster industry (Taylor *et al.* 1997a). Cleaning protocols within the pearl oyster industry are labour intensive and pearl oyster culture units are generally cleaned every 6-8 weeks (Taylor *et al.* 1997a;

Pit and Southgate 2003a). Minimising cleaning activities will allow pearl oyster farms to substantially decrease farm costs. As a result, it is vitally important to maximise cleaning efficiency and determine optimal cleaning protocols. The aim of this study was to identify a cleaning frequency which does not have any deleterious effects on growth and survival of *P. fucata*.

In summary this Chapter assesses factors which are likely to have a major influence on growth and survival of *P. fucata* and the efficiency of culture systems for this species under Australian conditions in northern Queensland. The aims of this Chapter are to:

(1) determine whether culture depth has an effect on oyster growth when spat are transferred to the ocean;
(2) determine the optimal stocking density during early and late nursery culture;
(3) determine if growth rates of different sized oysters of the same age are similar;
(4) determine the optimal culture apparatus unit for optimal growth and survival during late nursery culture; and
(5) determine the optimal cleaning frequency for maximum growth and survival of oysters during nursery culture.

6.2 Materials and Methods

Experiments were carried out to identify responses in growth and survival of *P. fucata* attributed to the factors listed in section 6.1. All experiments were carried out at James Cook University's Orpheus Island Research Station (Chapter 2) using hatchery-produced oysters as outlined in Chapter 2.

6.2.1 Does Culture Depth Affect Growth of *Pinctada fucata*?

Spat collectors containing forty-three day old spat with a mean (± SE, *n*=50) dorso-ventral shell height (DVH) of 0.9 ± 0.02 mm, were removed from settlement tanks (Chapter 2). Spat collectors had a mean (± SE, *n*=10) number of 775 ± 36 spat per collector. They were tied into plastic mesh trays with lids (55 x 30 x 10 cm, Chapter 2, Fig. 2.4) and transferred from the hatchery to a surface long-line at three different depths (2 m, 4 m and 6 m). Trays are utilised as they are strong and are designed to allow more spat collectors per tray compared to pearl nets. Minimum/maximum thermometers were placed at each depth to monitor variations in water temperature. Three mesh trays, each containing 3 spat collectors, were positioned at each of the three depths. Each tray was positioned randomly over the length of the long-line to ensure independence. Spat remained unattended within trays on the long-line for 8 weeks until they were 3.5 months of age at which time they were removed and growth and survival determined at each depth independently. Spat from each depth were graded separately into small, medium and large size classes. Size classes of <12 mm, 12-18 mm and >12 mm ('small', 'medium' and 'large', respectively) were used.

A one-way analysis of variance (ANOVA) was carried out to determine whether differences in overall size existed due to depth. All assumptions of the ANOVA were met and significant differences were identified using a Tukey post-hoc test. A chi-square homogeneity test was also conducted to determine whether differences between size classes could be attributed to depth.

While optimal growth of transferred spat was shown to occur at 2 m (section 6.3.1), these data were not analysed until after early and late nursery culture. Prior to

analysis, it was decided to that all nursery experiments would be at a standard depth of 4 m (sections 6.2.2, 6.2.3, 6.2.4, and 6.2.5), which has previously been successful for growth and survival of pearl oysters at OIRS.

6.2.2 Does Stocking Density Affect Growth and Survival of *P. fucata*?

6.2.2.1 Early Nursery Culture

Spat, cultured at 2 m and graded into the 'medium' size class (section 6.2.1) with a mean (± SE, *n*=50) DVH, APM and wet weight of 14.1 ± 0.3 mm, 14.2 ± 0.4 mm and 0.4 ± 0.01 g, respectively, were placed into pearl nets (Fig. 2.4) at a density of either 25%, 50% or 75% of total available net area, which corresponded to approximately 400, 800 or 1 200 oysters per net, respectively. Pearl nets were utilised rather than trays section 6.2.1) as trays are typically used when oysters are transferred from the hatchery to the ocean and until spat are graded (3.5 months). Total available surface area of the net was determined as 0.6 m^2. In order to determine the different stocking densities, the number of oysters required to fill a known area (0.01 m^2) as drawn on a piece of paper, was determined 10 times. The mean number of oysters per known area was then extrapolated to determine the approximate number of oysters required to meet 25%, 50%, and 75% stocking rates. Each density was replicated three times and nets were suspended from the long-line at a depth of 4 m with each net allocated a random position on the long-line to ensure independence. The experiment was conducted for 3 months and nets were cleaned externally after 6 weeks to remove fouling organisms. The nets were also internally inspected when cleaned and mortalities were noted and any predators removed and recorded. Mortalities were replaced with oysters of a similar size to maintain stocking densities. At the end of the

experiment, 30 oysters were randomly selected from each net and DVH, APM and wet weight were all measured.

One-way ANOVA's were carried out to determine whether differences in DVH, APM, wet weight and survival existed between treatments. Assumptions of the ANOVA were met for wet weight data, but DVH and APM data were log transformed. Any significant differences in means were identified using the Tukey post-hoc test.

6.2.2.2 Late Nursery Culture

Following the results obtained for the optimal stocking density experiment above (sections 6.2.2.1 and 6.3.2.1) in which 25% was found to be the optimal stocking density for early nursery culture, a narrower range of 4 densities of 20%, 25%, 30% and 40% of total available net area were investigated to optimise stocking density during late nursery culture of *P. fucata*.

Spat with a mean (± SE, *n*=50) DVH, APM, shell width and wet weight of 29.6 ± 0.4 mm, 28.0 ± 0.5 mm, 9.0 ± 0.2 mm and 3.3 ± 0.1 g, respectively, were placed into pearl nets at densities of 90, 115, 135 and 180 oysters per net, corresponding to 20%, 25%, 30% and 40% of total available net area, respectively. The total available net area was determined as described above (section 6.2.2.1). Each treatment was replicated three times and nets were suspended from a long-line at a depth of 4 m, with each net allocated an independent random position on the long-line. The experiment was conducted for 12 months with nets cleaned externally every 6 weeks to remove all fouling organisms by transferring nets (containing oysters) to land and using a high pressure water cleaner before returning the nets to the long-line. Pearl nets were also internally inspected when cleaned; mortalities were noted and any predators removed

and recorded. Mortalities were replaced with similarly sized oysters to maintain stocking density throughout the experiment. At the end of the experiment, 30 oysters were randomly selected from each net and DVH, APM, shell width and wet weight measured.

In order to determine growth characteristics of oysters cultured at different stocking densities, DVH data were fitted to the von Bertalanffy growth model (VBGM), which is an asymptotic logistic model of the form:

$$H_t = H_\infty [1-e^{(-k(t-to))}]$$

Where H_t is the determined height, H_∞ (H-infinity) is a theoretical maximum (asymptotic) height, k is a growth coefficient measuring the rate at which growth approaches H-infinity, t is age and t_o is a theoretical age at zero height (Chen *et al.* 1992).

To determine whether there was any differences between growth models an analysis of the residual sums of squares (ARSS) was carried out (Chen *et al.* 1992). As VBGM is non-linear in formulation, an analysis of covariance (ANCOVA) was unable to be carried out. In addition a growth index, Phi prime (Φ'), as described by Munro and Pauly (1983) was used to determine an overall growth performance to compare between different stocking densities.

$$\Phi' = \text{Log } k + 2 \text{ Log } H_\infty$$

These methods were not employed for early nursery culture experiments because they were only only conducted for 3 months and only sampled twice. Additionally, one-way ANOVA's were carried out to determine whether differences in DVH, APM, shell width and wet weight existed between treatments after 12 months. Assumptions of the ANOVA were met and any significant differences in means were identified using the Tukey post-hoc test. Survival data was not analysed as mortalities were only recorded per treatment, rather than replicates within treatments.

6.2.3 Do Small Oysters 'Catch Up' to their Large Counterparts?

Spat, cultured using the general methods described in Chapter 2, were graded at 3.5 months of age into 3 different size classes. Small, medium and large size classes contained *P. fucata* with a mean (± SE, n=50) DVH of 10.8 ± 0.2 mm, 14.1 ± 0.3 mm and 20.8 ± 0.3 mm and represented runts, normal growers and fast growers, respectively. *Pinctada fucata* from each initial size class differed significantly from each other ($F_{2, 147}$= 426.16, P<0.001). Sixty (60) *P. fucata* spat from each size class were individually fixed to the tops and bottoms of each of three replicate plastic mesh trays (55 x 30 x 10 cm) using a waterproof cyanoacrylate adhesive (Loctite 454 gel, Loctite Australia, Carringbah, New South Wales, Australia). Adhesive was used to minimise aggregation (Friedman 1999; Pit and Southgate 2003), which has been shown to significantly affect growth (Friedman and Southgate, 1999). Each tray was positioned randomly to ensure independence at a depth of 4 m on the long-line. Trays were cleaned every 6 weeks to remove external fouling organisms by removing trays from the water and cleaning them using a high pressure water cleaner. Trays were also inspected internally every 6 weeks to remove dead shells and any predators. At this

time 30 oysters from each replicate tray were measured for DVH and APM. Shell width and wet weight were also measured from 8 months onwards before the oysters were returned to the tray. This experiment was conducted for 22 months.

In order to determine growth characteristics from oysters of three initially different size classes, DVH data were again fitted to the von Bertalanffy growth model (VBGM; section 6.2.2.2). Differences in growth models were identified through the use of ARSS and Phi prime was calculated to ascertain overall growth performance between different treatments (section 6.2.2.2).

One-way ANOVA's were also carried out to determine whether differences in DVH, APM, shell width and wet weight existed when oysters in the smallest size class reached 50 mm (minimum size for pearl production) and also at the end of the experiment. Assumptions of the ANOVA were met and any significant differences in means due to initial size class were identified using the Tukey post-hoc test.

6.2.4 Does Culture Unit Affect Growth and Survival of *P. fucata*?

Two experiments were conducted to determine the optimal culture unit for maximum growth and survival of *P. fucata* during late nursery culture. In Experiment 1 four different types of culture units were evaluated for culturing oysters, which had reached a size suitable to be transferred from early to late nursery culture (ca. 30 mm). In Experiment 2, oysters that had reached a size suitable for pearl production (ca. 50 mm) were either transferred into panel nets of two different mesh sizes or transferred into pearl nets of 2 different mesh sizes.

6.2.4.1 *Experiment 1*

This experiment assessed growth and survival of *P. fucata* juveniles during early nursery culture using four types of culture unit (Chapter 2, Fig. 2.4). The four culture units assessed were:

(1) 'box' -plastic boxes measure 40 x 30 x 15 cm with a lid. They contain horizontal slits (20 x 5 mm) made on the bottom and sides of the box. They are used mainly as conditioning boxes in Japan (W.A O'Connor, New South Wales Fisheries, pers comm.), but also for juvenile culture in India;

(2) 'tray' -plastic mesh trays with lids measuring 55 x 30 x 10 cm have become more popular for pearl culture due to their durability (Southgate and Beer 1997). Trays contained perforations on the top bottom and sides of the tray. They are used mainly for the transportation of frozen prawns;

(3) 'pearl net'-pyramid pearl nets have been used extensively for the culture of pearl oysters (Gervis and Sims 1992)); and

(4) 'pearl net with noodles' -the same as 'pearl net', except that they contained plastic 'noodles' used commercially as spat collectors in Japan.

The above culture units are commercially available for the culture of pearl oysters either in Australia or overseas. 'Box' and 'tray' were selected due to their durability. Pearl nets have been used extensively in the culture of pearl oysters (Gervis and Sims

1992), while 'pearl nets with noodles' were selected on the basis that oysters may attach to 'noodles' allowing for easier maintenance during routine pearl net inspection.

Each different culture apparatus treatment was replicated three times and randomly allocated independent positions on the long-line. All replicates were suspended at a depth of 4 m and stocked at 25% of available area (sections 6.2.2.1 and 6.3.2.1) with *P. fucata* with a mean (± SE, *n*=50) DVH, APM, shell width and wet weight of 29.6 ± 0.4 mm, 28.0 ± 0.5 mm, 9.0 ± 0.2 mm and 3.3 ± 0.1 g, respectively. Replicates were cleaned externally every 6 weeks using a high pressure water cleaner. Mortalities were also noted every 6 weeks and any dead shell and predators were removed from the culture units. At end of the experiment, 30 oysters from each replicate culture unit were randomly selected and measured for DVH, APM, shell width and wet weight. This experiment was conducted for 12 months.

In order to determine growth characteristics from oysters cultured using different nursery culture units, DVH data were again fitted to the von Bertalanffy growth model (VBGM; section 6.2.2.2). Differences in growth models were identified through the use of ARSS and Phi prime was calculated to ascertain overall growth performance between different treatments (section 6.2.2.2).

One-way ANOVA's were also carried out to determine whether differences in DVH, APM, shell width and wet weight existed. Data were all log transformed to meet the assumptions of the ANOVA and any significant differences in means were identified using the Dunnets T3 post-hoc test.

6.2.4.2 *Experiment 2*

This experiment assessed growth and survival of larger (~ 50 mm) *P. fucata*, which had reached a size at which they may be used for pearl production (Chapter 1) with a mean (± SE, *n*=50) DVH, APM, shell width and wet weight of 49.6 ± 0.4 mm, 44.9 ± 0.5 mm, 16.6 ± 0.2 mm and 12.8 ± 0.4 g, respectively. Four culture units were assessed:

(1) panel nets with small mesh (5 mm);

(2) panel nets with large mesh (15 mm);

(3) pearl nets with small mesh (4.5 mm); and

(4) pearl nets with large mesh (9 mm).

Stocking was based on results obtained in section 6.3.2.1 and maintained at 25 % of available area. Each treatment was replicated 3 times and culture units were independently and randomly suspended from a long-line at a depth of 4 m. Culture units were cleaned every 6 weeks by removing culture units from the long-line and transferring them to land where a high pressure water cleaner was used to remove external fouling organisms. At the end of the study, 30 oysters were randomly selected from each net and measured for DVH, APM, shell width and wet weight. This experiment was conducted for 11 months.

One-way ANOVA's were conducted to determine whether culture apparatus effected DVH, APM, shell width or wet weight. Assumptions of the ANOVA were met and any significant differences in means were identified using the Tukey post hoc test. VBGM and ARSS (Section 6.2.4.1) were not utilised during this experiment because

growth parameters were only measured twice, which is insufficient data to generate a growth model.

6.2.5 Does Cleaning Frequency Affect Growth and Survival of *P. fucata*?

This experiment assessed the optimal frequency for cleaning and inspection of culture units to maximise growth and survival of juvenile *P. fucata*. One hundred oysters with a mean (± SE, *n*=50) DVH, APM, shell width and wet weight of 29.6 ± 0.4 mm, 28.0 ± 0.5 mm, 9.0 ± 0.2 mm and 3.3 ± 0.1 g, respectively, were placed into each of 6 treatments:

(1) 'treatment 1'- nets cleaned every 4 weeks, but not changed or inspected;

(2) 'treatment 2'- nets cleaned every 6 weeks, but not changed or inspected;

(3) 'treatment 3'- nets cleaned every 8 weeks, but not changed or inspected;

(4) 'treatment 4'- nets not cleaned, but changed every 4 weeks;

(5) 'treatment 5'- nets not cleaned, but changed every 6 weeks; and

(6) 'treatment 6'- nets not cleaned, but changed every 8 weeks.

These treatments were selected based on previous research suggesting optimal cleaning frequencies for other pearl oyster species (Southgate and Beer 1997; Taylor *et al.* 1997b; Pit and Southgate 2003a). At cleaning, all nets (treatments 1-6) were removed from the long-line and transferred to land based facilities at OIRS and placed into raceways. The treatment nets that needed cleaning were removed from the raceways and cleaned. All nets, those cleaned and non-cleaned, were returned to the long line. This was carried out so that all nets were subject to the same conditions (i.e. removal from water, transferred to land) in order to avoid any effects due to transporting oysters and nets from the long line to the land.

Each net was positioned randomly on the long-line at a depth of 4 m. The experiment was conducted for 6 months (July-January). Oysters were not measured during the course of the experiment, however, when nets were inspected mortalities were noted and predators removed from nets. One-way ANOVA's were carried out to determine whether differences in DVH, APM, shell width and wet weight resulted from different cleaning frequencies. Measures of DVH and APM met ANOVA assumptions, however, shell width and wet weight data violated assumptions and were log transformed. Differences in means were identified with Tukey and Dunnets T3 pot-hoc tests.

6.3 Results

6.3.1 Does Culture Depth Affect Growth of *Pinctada fucata*?

Mean (± SE, n=50) DVH of *P. fucata* transferred from the hatchery to the ocean and cultured at three different depths (2, 4 and 6 m) showed significant differences ($F_{2,87}$=5.947, $p<0.01$; Fig. 6.1). Spat cultured at 2 m with a mean DVH of 13.7 ± 0.6 mm were significantly larger than spat cultured at 4 m and 6 m with mean (± SE, n=50) DVH of 12.1 ± 0.5 mm and 11.4 ± 0.4 mm, respectively (Fig. 6.1).

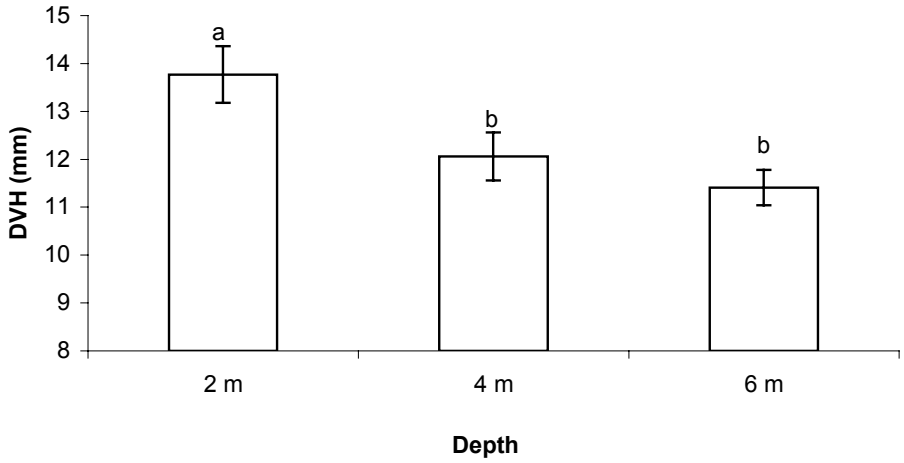


Fig. 6.1. Mean (± SE, *n*=50) DVH of *Pinctada fucata* cultured at 3 different depths (2, 4 and 6 m) during early nursery culture at Orpheus Island. Means with the same superscripts are not significantly different ($p>0.05$).

The number of oysters in each of the 3 different size classes (small, medium and large; significantly different as determined in section 6.2.3) transferred from the hatchery to the long-line was significantly different due to depth ($\chi^2=82.57$, $P<0.001$). Oysters maintained at a depth of 2 m showed the greatest proportion of individuals in both the medium (62.9%) and large (5.7%) size classes and the smallest number of individuals in the small size class (31.4%; Fig. 6.2). Oysters cultured at 6 m, showed the greatest proportion of individuals in the small size class (89.3%) and the lowest proportion of individuals in the large size class (0.4%) compared with oysters transferred to 2 m and 4 m. Survival of oysters was not determined for this experiment. Temperatures at all three depths were similar and varied between a minimum of 26 °C and a maximum of 30 °C.

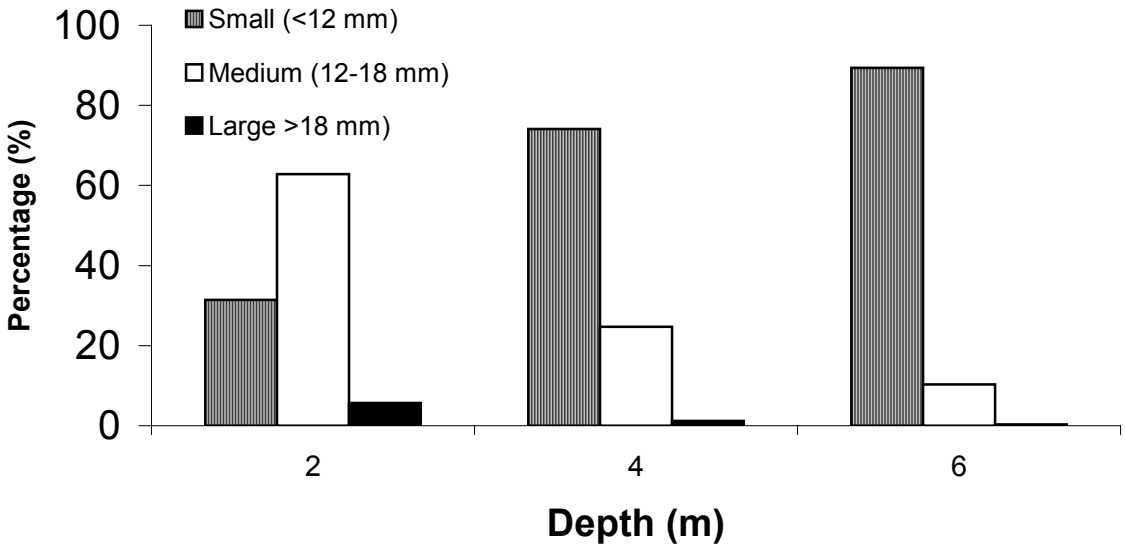


Fig. 6.2. Percentage of *Pinctada fucata* spat in three different size classes (small, medium and large) cultured at three different depths (2, 4 and 6 m) at Orpheus Island and graded after 109 days.

6.3.2 Does Stocking Density Affect Growth and Survival of *Pinctada fucata*?

6.3.2.1 *Early Nursery Culture*

After only 3 months, growth of *P. fucata* was inversely proportional to stocking density. Stocking density had significant effect on DVH ($F_{2, 267}$= 96.03, p<0.001), APM ($F_{2, 267}$=102.35, p<0.001) and wet weight ($F_{2,267}$= 56.58, p<0.001) of *P. fucata* (Figs. 6.3-6.5). Mean (±SE) DVH of oysters cultured at a density of 25% of total available net area was 28.1 ± 0.5 mm and was significantly greater than that of oysters cultured at densities of 50% (23.3 ± 0.4 mm) and 75% (20.4 ± 0.4 mm), respectively, while mean DVH of oysters cultured at 50% and 75% were also significantly different from each other (Fig. 6.3). Similar results were also observed for APM and wet weight with maxima of 25.8 ± 0.5 mm and 3.0 ± 0.02 g, respectively, for 25% stocking density

(Fig. 6.4 and 6.5). Oysters stocked at 75% of total available net area recorded the lowest growth in terms of APM and wet weight with 18.2 ± 0.3 mm and 1.4 ± 0.1 g, respectively. Clumping of pearl oysters was evident at all stocking densities, however individual groups of oysters were not evident in the 50% and 75% stocking densities as oysters formed one large clump in these treatments. In nets with 25% stocking density, clumping was minimised with most individuals attaching to the net rather than other oysters.

Stocking density did not affect survival of *P. fucata* during early nursery culture ($F_{2, 6}$=1.214, p>0.05) and survival of oysters in all treatments was greater than 99%.

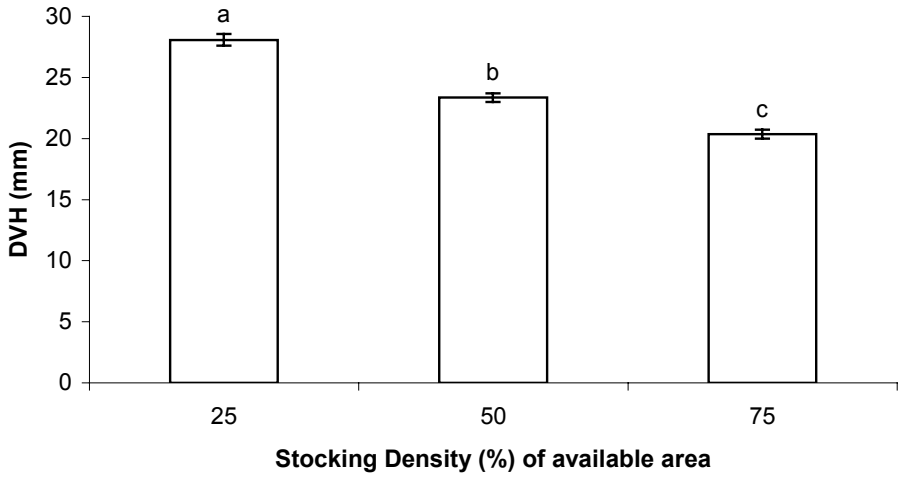


Fig. 6.3. Mean (± SE, *n*=90) dorso-ventral shell height (DVH) of *Pinctada fucata* cultured at three different stocking densities (25, 50 and 75% of available net area) during early nursery culture at Orpheus Island. Means with the same superscript are not significantly different (p>0.05).

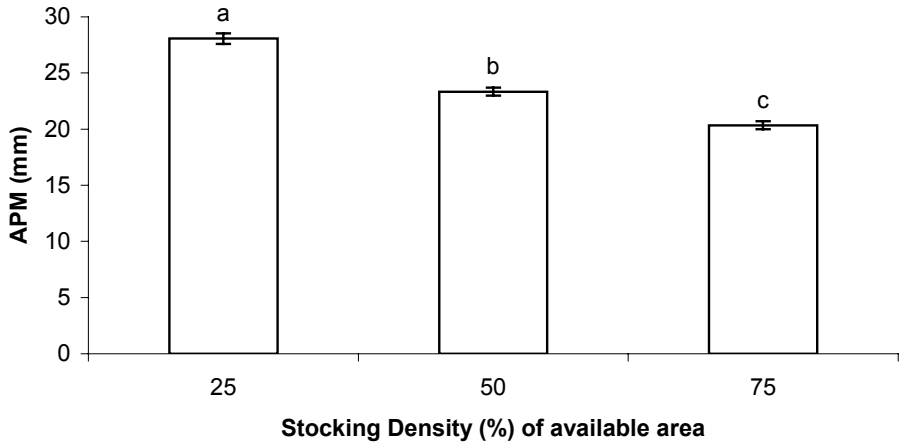


Fig. 6.4. Mean (± SE, *n*=90) antero-posterior measurement (APM) of *Pinctada fucata* cultured at three different stocking densities (25, 50 and 75% of available net area) during early nursery culture at Orpheus Island. Means with the same superscript are not significantly different (p>0.05).

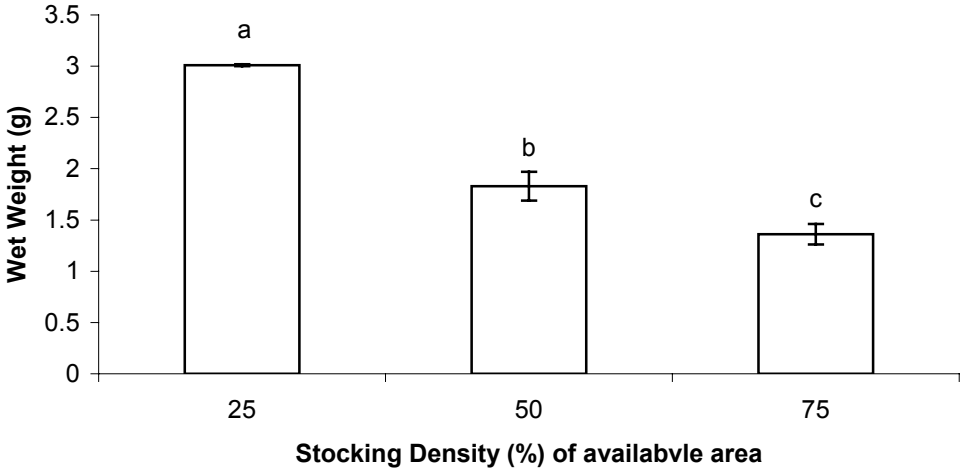


Fig. 6.5. Mean (± SE, *n*=90) wet weight of *Pinctada fucata* cultured at three different stocking densities (25, 50 and 75% of available net area) during early nursery culture at Orpheus Island. Means with the same superscript are not significantly different (p>0.05).

The proportion of oysters in 3 different size classes (small, medium and large) cultured at 3 different stocking densities (25, 50 and 75%) was significantly different (χ^2=74.04, P<0.001) with differences observed within each size class between the different stocking densities. The proportion of small oysters increased with increasing stocking density while the proportion of medium and large oysters decreased as stocking density increased (Fig. 6.6). The maximum proportion of oysters in the large size class at the end of the experiment occurred at a stocking density of 25% of available net area (4%) followed by stocking densities of 50% and 75% of available net area which had 1.2% and 0.1%, respectively of oysters in the large size class (Fig. 6.6). On the other hand, oysters cultured at a stocking density of 75% available net area recorded the highest proportion of small oysters (81%) compared with oysters cultured at stocking densities of 50% and 25% available net area which had 57.6% and 22.3% of oysters in the small size class, respectively (Fig. 6.6).

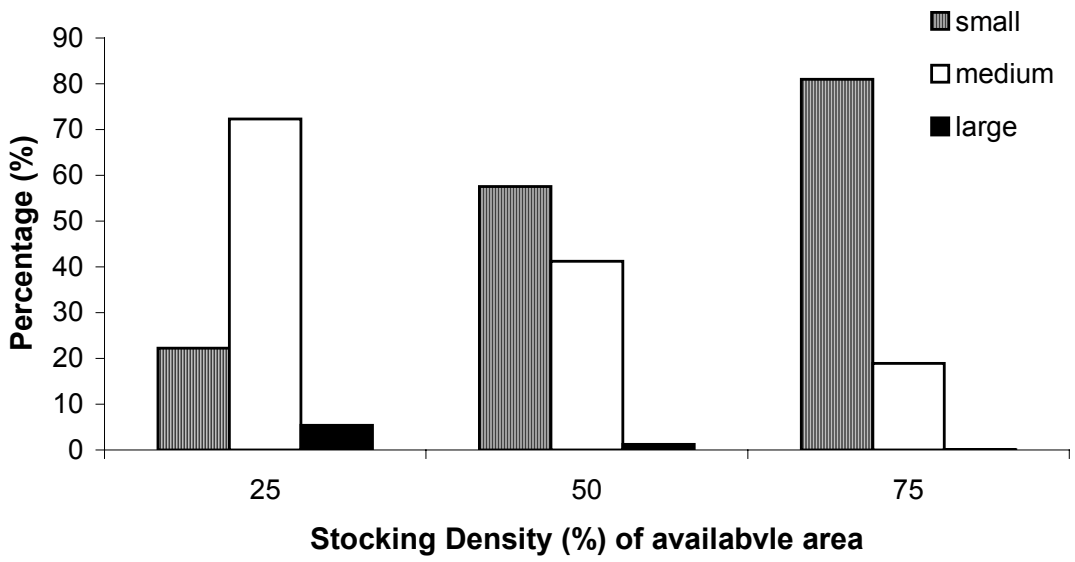


Fig. 6.6: Percentage of *Pinctada fucata* in three different size classes (small, medium and large) when cultured at three different stocking densities (25, 50 and 75% of available net area) during early nursery culture at Orpheus Island.

6.3.2.2 *Late Nursery*

Analysis of sums of squares showed that von Bertalanffy growth models were significantly different for *P. fucata* cultured at densities of 20%, 25%, 30% and 40% of available net area ($F_{9, 1904}$=26.87, p<0.001; Fig. 6.7). The overall growth performance (Φ') was greatest for oysters cultured at 25% total available net area (3.49), while minimal growth performance occurred at 40% total available net area (Φ'=3.83)(Table 6.1).

Table 6.1. Growth characteristics of oysters cultured at four different stocking densities (20, 25, 30, and 40% of available net area) during late nursery culture at Orpheus Island as determined by the von Bertalanffy growth models (VBGM).

	VBGM Growth Parameter			
Stocking Density	H_∞	K	t_o	Φ'
20%	68.34	1.64	0.23	3.66
25%	60.64	2.06	0.25	3.49
30%	61.96	1.52	0.14	3.76
40%	59.33	1.95	0.20	3.83

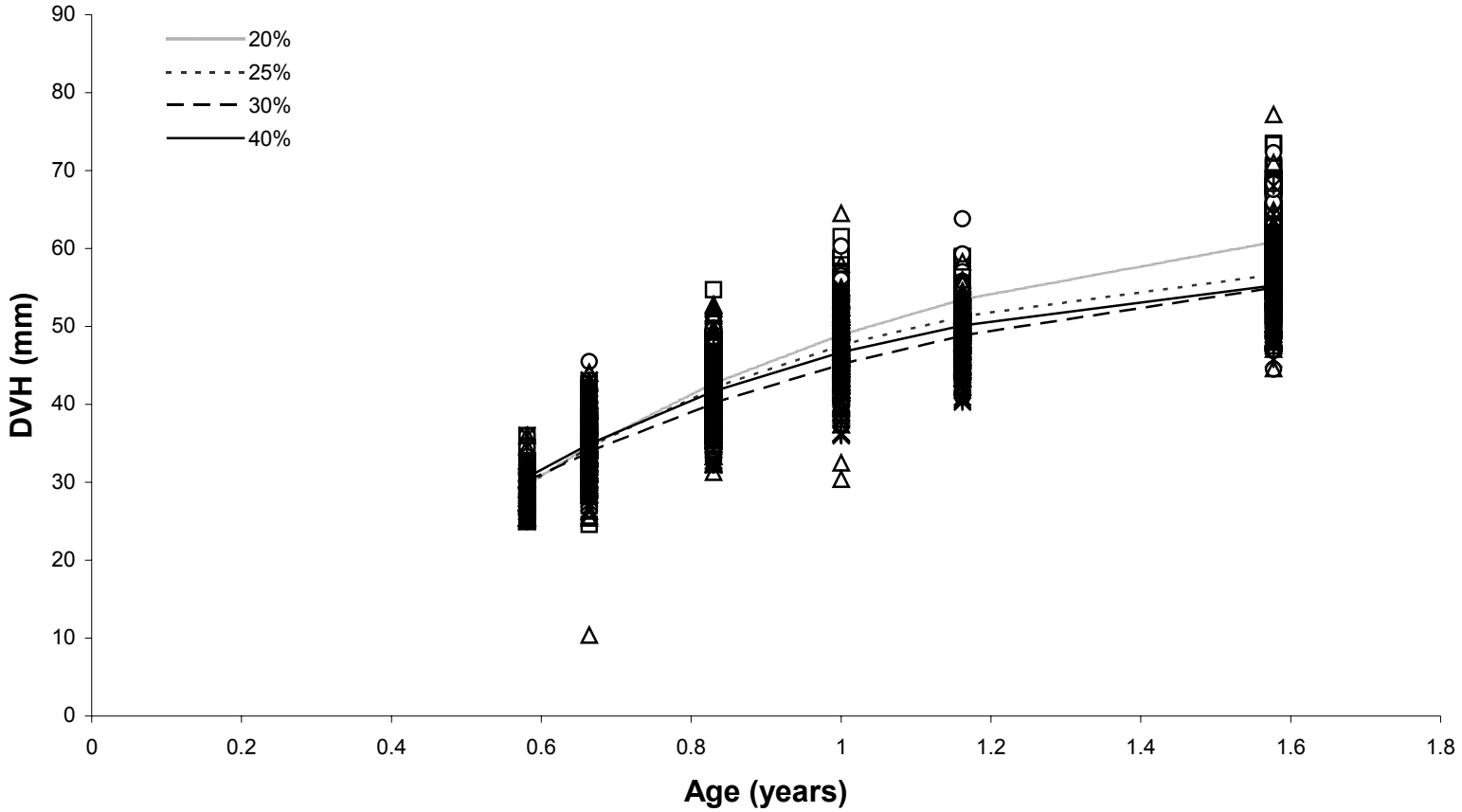


Fig. 6.7. Growth of *P. fucata* cultured at different stocking densities (20, 25, 30 and 40% total available net area) during late nursery culture at Orpheus Island for 12 months.

While ARSS identified a difference between VBGM's, ANOVA's were carried out to identify final size differences. *P. fucata* cultured at four different stocking densities (20, 25, 30 and 40 % of available net area) were significantly different in DVH, APM, shell width and wet weight after 12 months (Table 6.2). Oysters cultured at a stocking density of 20% available net area had a mean (± SE, *n*=90) DVH of 61.2 ± 0.5 mm and were significantly larger than oysters cultured at stocking densities of 25%, 30% and 40% available net area, which had mean (± SE, *n*=90) DVH of 57.0 ± 0.5, 55.5 ± 0.5 and 55.2 ± 0.5 mm, respectively (Fig. 6.8). Similar patterns were observed for APM, with a maximum mean (± SE, *n*=90) APM of 56.7 ± 0.5 mm for oysters cultured at a stocking density of 20% available net area. This was significantly different to oysters cultured at stocking densities of 25%, 30% and 40% available net area which had mean (± SE, *n*=90) APM of 52.8 ± 0.5 mm, 51.8 ± 0.7 mm, and 51.21 ± 0.5 mm, respectively (Fig. 6.9). Mean APM at these densities were not significantly different. While oyster shell width and wet weight were greatest in oysters cultured at a stocking density of 20% available net area with 20.5 ± 0.2 mm and 28.0 ± 0.7 g, respectively, oysters cultured at stocking densities of 25% and 30% available net area were significantly greater in shell width and wet weight than oysters cultured at a stocking density of 40% available net area (Fig. 6.10; 6.11). Mortality of oysters cultured at stocking densities of 20%, 25%, 30% and 40% available net area was, 4.8%, 6.4%, 4.7% and 5.9%, respectively, and did not show any apparent patterns. Unfortunately, data were not collected to enable statistical analysis.

Table 6.2. Significance levels from analysis of variance on the effects of four stocking densities (20, 25, 30 and 40% of available net area) on DVH, APM, shell width and wet weight of *Pinctada fucata* during late nursery culture at Orpheus Island.

Parameter	*F-value*	**p-value**
DVH	29.4	< 0.001
APM	20.4	< 0.001
Shell width	42.0	< 0.001
Wet Weight	40.8	< 0.001

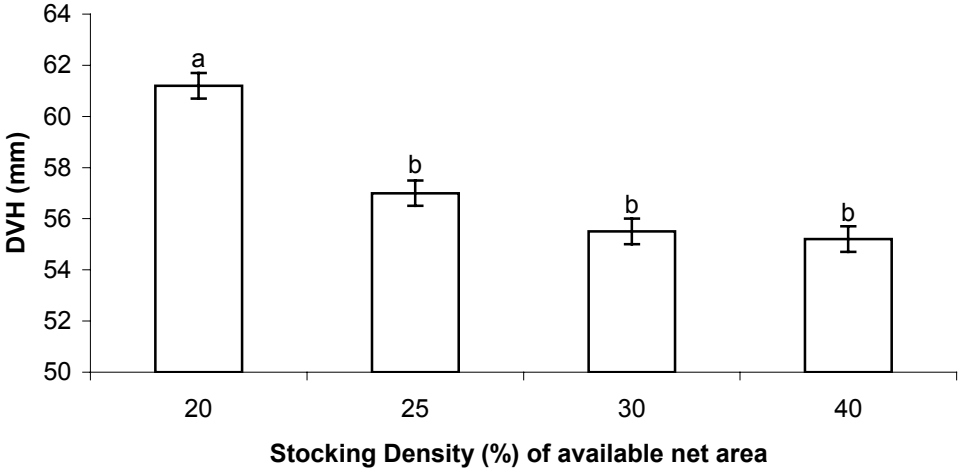


Fig. 6.8. Mean (± SE, *n*=90) dorso-ventral shell height (DVH) of *Pinctada fucata* cultured using four different stocking densities (20, 25, 30 and 40% of available net area) during

late nursery culture at Orpheus Island. Means with the same superscript are not significantly different (p>0.05).

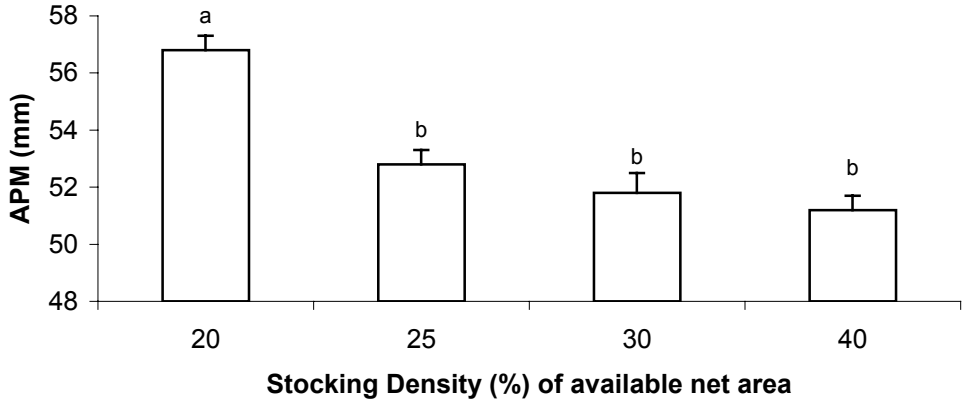


Fig. 6.9. Mean (± SE, *n*=90) antero-posterior measurement (APM) of *Pinctada fucata* cultured using four different stocking densities (20, 25, 30 and 40% of available net area) during late nursery culture at Orpheus Island. Means with the same superscript are not significantly different (p>0.05).

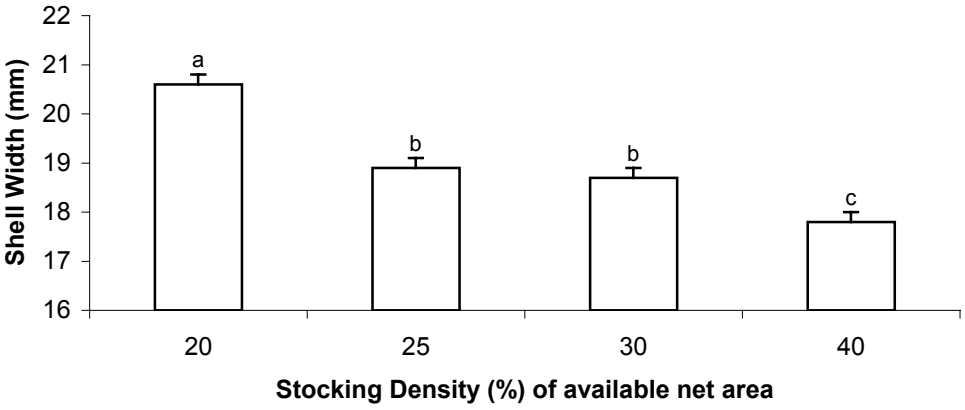


Fig. 6.10. Mean (± SE, *n*=90) shell width of *Pinctada fucata* cultured using four different stocking densities (20, 25, 30 and 40% of available net area) during late nursery culture at Orpheus Island. Means with the same superscript are not significantly different (p>0.05).

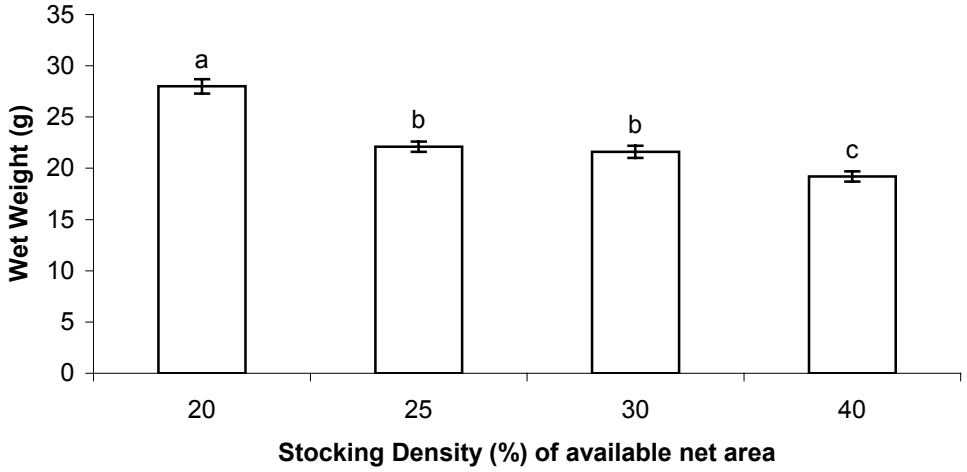


Fig. 6.11. Mean (± SE, *n*=90) wet weight of *Pinctada fucata* cultured using four different stocking densities (20, 25, 30 and 40% of available net area) during late nursery culture at Orpheus Island. Means with the same superscript are not significantly different (p>0.05).

6.3.3 Do Small Oysters 'Catch Up' to their Larger Counterparts?

Analysis of sums of squares has shown that von Bertalanffy growth models are significantly different for *P. fucata* growth (DVH) cultured from three different size classes of small (<12 mm), medium (12-18 mm) and large (>18 mm)($F_{9, 1665}$ =66.26, p<0.001; Fig. 6.12) at 25 months of age. While H_{∞} was relatively similar between treatments (Table 6.4) at 25 months of age, it appears that there is a difference between oysters from the large size class compared with those from small and medium size classes

(Fig. 6.12). However, at a size at which oysters are utilised for pearl production (11 months of age; ca. 50 mm) it appears that the oysters from the three size classes are still different (Fig. 6.12) suggesting that small runt oysters are not able to catch up to their larger counterparts within 11 months. Meanwhile, the overall growth performance (Φ') increased with increasing initial size with Φ' values of 3.93, 3.98 and 4.02 for oysters from small, medium and large size classes, respectively (Table 6.3).

Table 6.3. Growth characteristics of oysters cultured from three different size classes (small, medium and large) during nursery culture at Orpheus Island as determined by the von Bertalanffy growth models (VBGM).

	VBGM Growth Parameter			
Stocking Density	H_∞	k	t_o	Φ'
Small <12 mm	68.19	1.85	0.108	3.93
Medium 12-18 mm	67.57	2.13	0.07	3.98
Large >18 mm	69.31	2.18	0.02	4.02

Oysters from the small size class reached pearl production size (ca. 50 mm) after 7 months of culture (11 months of age). At this time these oysters, which had a mean (± SE,

n=90) DVH of 50.9 ± 0.4 mm, were significantly smaller than oysters in the medium and large size classes which had means (± SE, *n*=90) for DVH of 53.0 ± 0.5 mm and 55.9 ± 0.5 mm, respectively, and were significantly different from each other (Table 6.4; Fig. 6.13). Similar trends were also observed for APM (Fig. 6.14). In terms of shell width and wet weight, oysters from the small size class were significantly different from oysters in both the medium and large size classes, while oysters from the medium and large size classes were not significantly different after 7 months of culture (Table 6.4; Figs. 6.15-6.16).

Table 6.4. Significance levels from analysis of variance on the effects of different size classes (small, medium and large) on DVH, APM, shell width and wet weight of *Pinctada fucata* after 7 and 21 months of culture at 11 and 25 months of age, respectively.

Age	*Growth*	*F-value*	**p-value**
11 months	DVH	34.97	<0.001
	APM	24.66	<0.001
	Shell width	27.59	<0.001
	Wet weight	22.75	<0.001
25 months	DVH	7.06	P=0.001
	APM	6.69	P=0.002
	Shell width	3.29	P=0.039
	Wet weight	3.3	P=0.039

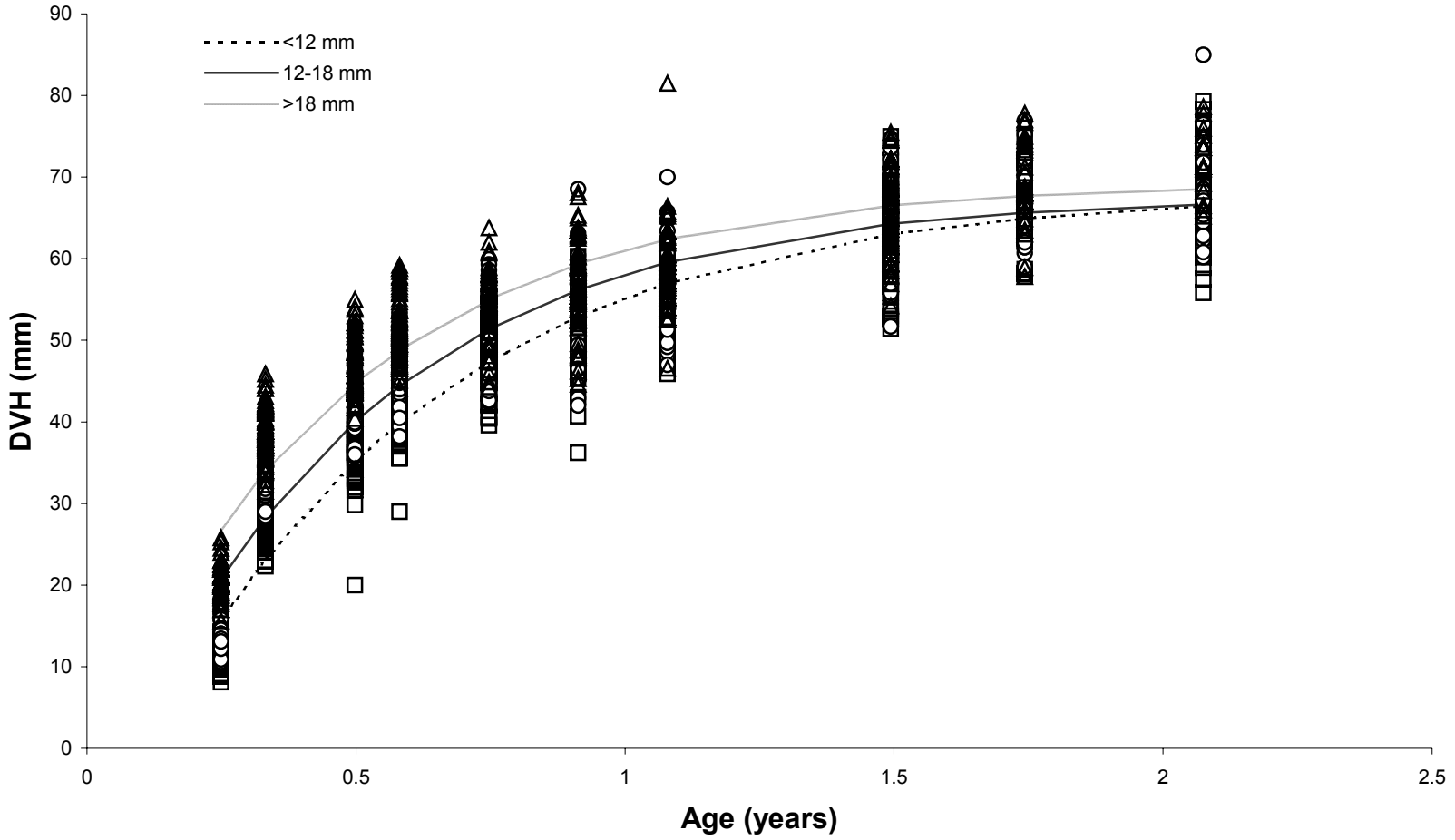


Fig. 6.12. Growth of *Pinctada fucata* cultured from three different size classes (small, medium and large) during nursery culture at Orpheus Island for 25 months.

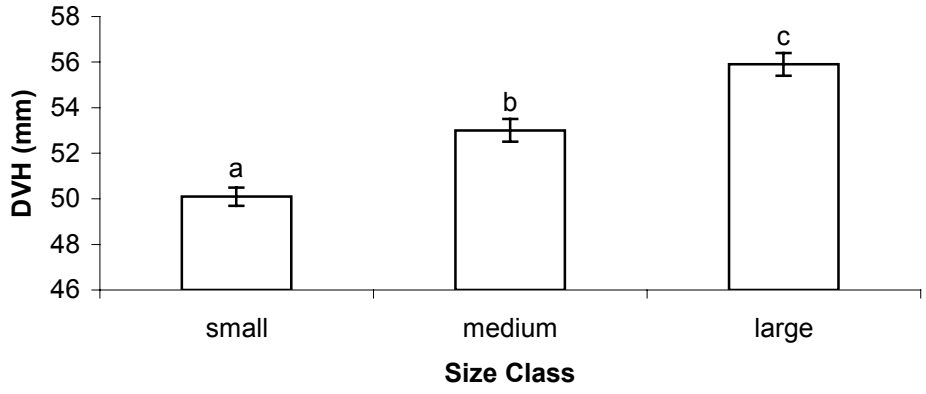


Fig. 6.13 Mean (± SE**,** *n*=90) dorso-ventral shell height (DVH) of *Pinctada fucata* from three different size classes (small, medium and large) cultured for 7 months (11 months of age). Means with the same superscript are not significantly different (p>0.05).

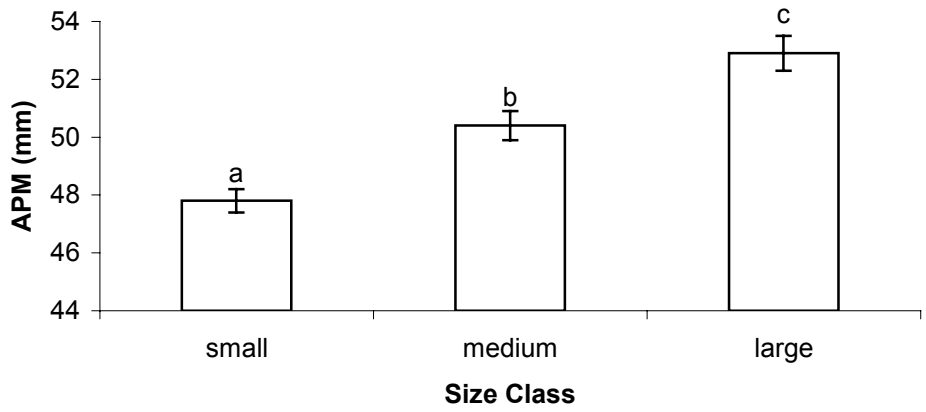


Fig. 6.14 Mean (± SE, *n*=90) antero-posterior measurement (APM) of *Pinctada fucata* from three different size classes (small, medium and large) cultured for 7 months (11 months of age). Means with the same superscript are not significantly different (p>0.05).

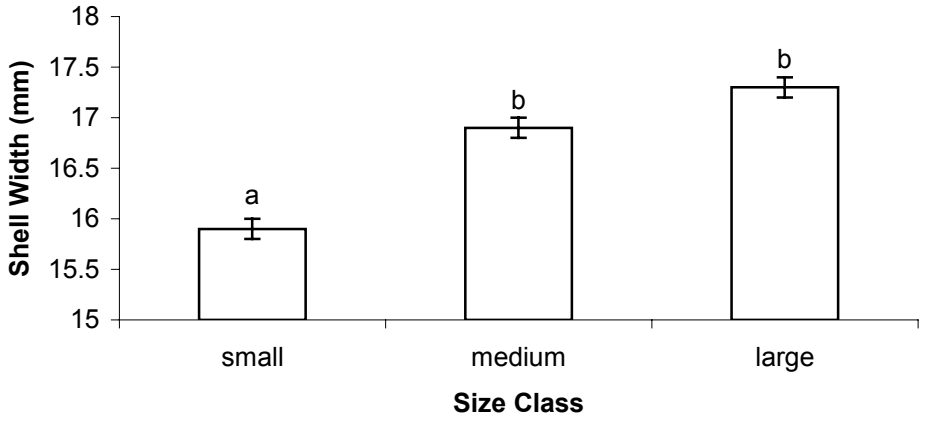


Fig. 6.15. Mean (± SE, *n*=90) shell width of *Pinctada fucata* from three different size classes (small, medium and large) cultured for 7 months (11 months of age). Means with the same superscript are not significantly different (p>0.05).

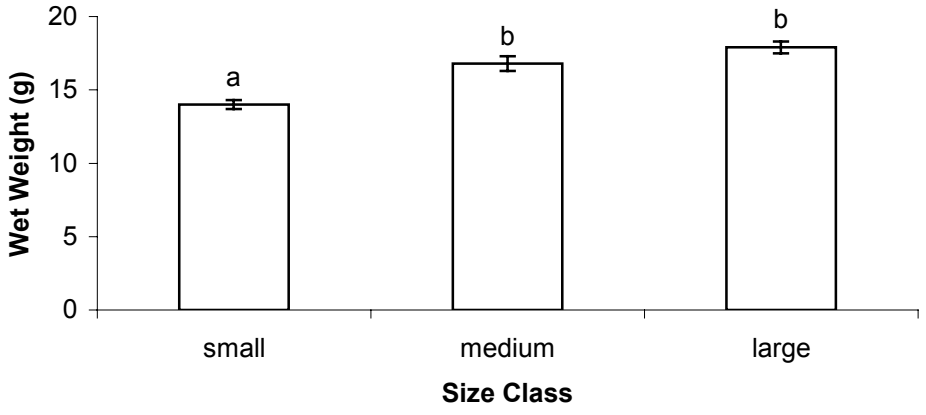


Fig. 6.16. Mean (± SE, *n*=90) wet weight of *Pinctada fucata* from three different size classes (small, medium and large) cultured for 7 months (11 months of age). Means with the same superscript are not significantly different ($p>0.05$).

However, with a further 14 months of culture (25 months of age) oysters from the small size class did not differ significantly in DVH ($p>0.05$) from oysters in the medium size class with means (± SE, *n*=90) for DVH of 67.1 ± 0.8 and 69.1 ± 0.6 mm, respectively (Fig. 6.17). Oysters from the large size class were still significantly larger than those in the other two size classes with a mean (± SE, *n*=90) DVH of 71.1 ± 0.8 mm. In terms of APM, shell width and wet weight, there was no significant difference in mean (± SE, *n*=90) oyster size between those in the large and medium size classes, nor was there any significant differences in mean oyster size between small and medium size classes (Table 6.2; Figs. 6.18-6.20), although smallest and largest size classes were significantly different. Survival of *P. fucata* throughout the experiment was very high at 97 ± 0 % for all 3 treatments.

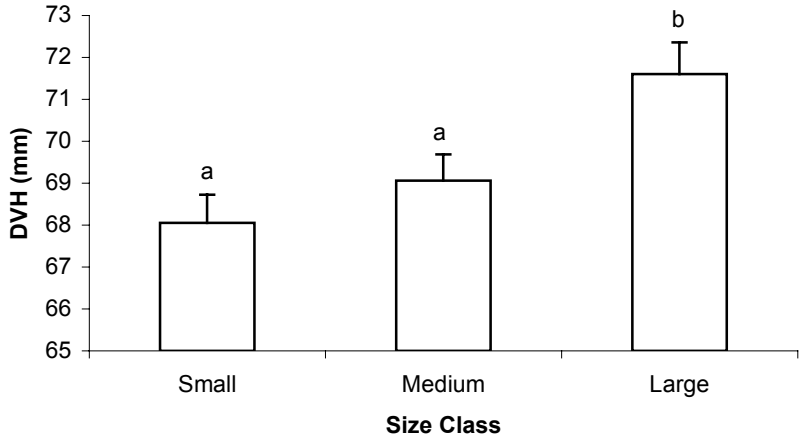


Fig. 6.17. Mean (± SE, *n*=90) dorso-ventral shell height (DVH) of *Pinctada fucata* from three different size classes (small, medium and large) cultured for 21 months (25 months of age). Means with the same superscript are not significantly different (p>0.05).

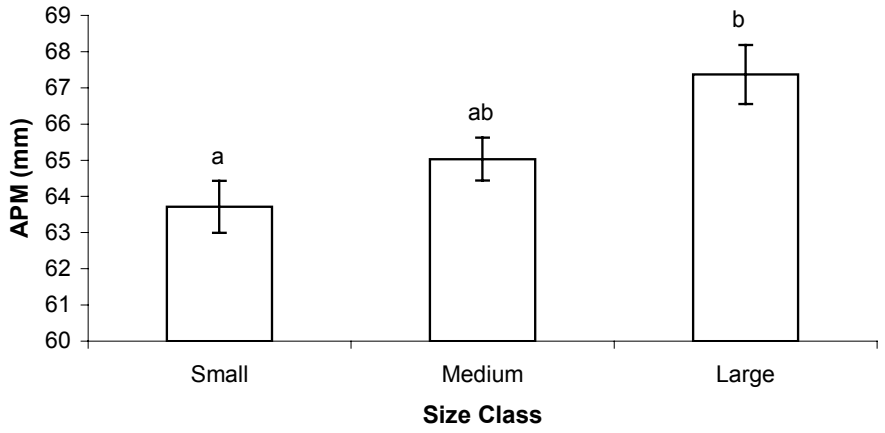


Fig. 6.18. Mean (± SE) antero-posterior measurement (APM) of *Pinctada fucata* from three different size classes (small, medium and large) cultured for 21 months (25 months of age). Means with the same superscript are not significantly different (p>0.05).

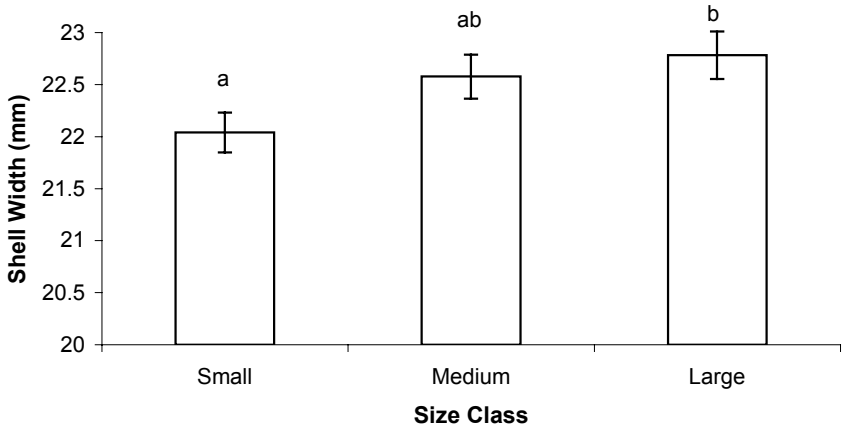


Fig. 6.19. Mean (± SE) shell width of *Pinctada fucata* from three different size classes (small, medium and large) cultured for 21 months (25 months of age). Means with the same superscript are not significantly different (p>0.05).

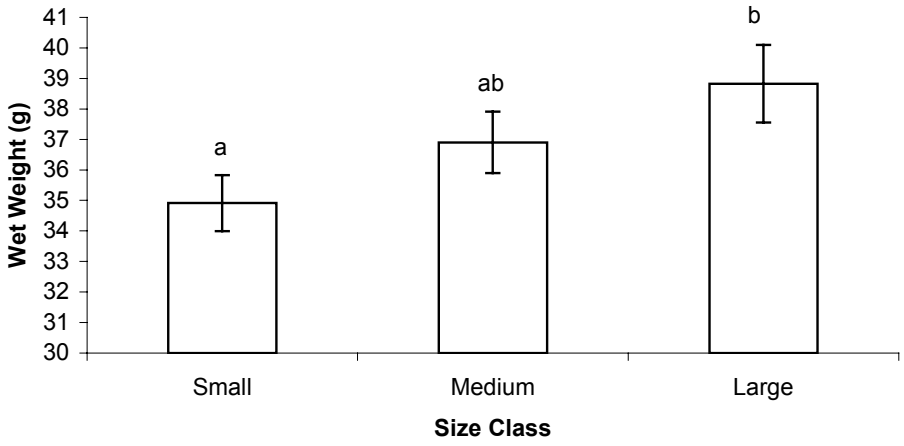


Fig. 6.20. Mean (± SE) wet weight of *Pinctada fucata* from three different size classes (small, medium and large) cultured for 21 months (25 months of age). Means with the same superscript are not significantly different (p>0.05).

6.3.4 Does Culture Unit Affect Growth and Survival of *Pinctada fucata*?

6.3.4.1 *Experiment 1*

Analysis of sums of squares showed that von Bertalanffy growth models were significantly different for DVH of *P. fucata* cultured using four different culture apparatus units (box, tray, net and noodles) ($F_{9, 1772}$=420.45, p<0.0001; Fig. 6.21). Oysters cultured using pearl nets and pearl nets with noodles showed very similar growth models, whereas oysters cultured using box culture grew extremely slowly and did not express the 'typical' von Bertalanffy growth model. This is supported by values generated for overall growth performance (Φ'), which were very similar for oysters cultured using pearl nets and pearl nets with noodles at 3.76 and 3.77, respectively, while the growth performance of oysters cultured in the box treatment was poor (Φ'=3.16; Table 6.5). These observations can be supported through the use of ANOVA's (see below).

Culture unit significantly affected DVH, APM, shell width and wet weight of *P. fucata* (Table 6.6). Oysters placed in box culture were significantly smaller in mean (± SE, *n*=90) DVH (35.2 ± 0.4) compared to all other treatments Figs. 6.22-6.25). However, there was no significant difference in mean (± SE, *n*=90) DVH of oysters cultured in pearl nets or in pearl nets with noodles which measured 59.9 ± 0.6 and 59.5 ± 0.7 mm, respectively and were significantly greater than other treatments (Fig. 6.22). Similar patterns were also observed for APM, where oysters cultured in pearl nets and pearl nets with noodles showed significantly greater means (± SE, *n*=90) for APM with 55.7 ± 0.6 and 57.0 ± 0.6 mm, respectively (Fig. 6.23). However, shell width and wet weight, were significantly different for oysters in all culture apparatus (Fig. 6.24; 6.25). In both cases

(shell width and wet weight), oysters cultured in pearl nets were significantly larger with 20.0 ± 0.3 mm and 27.3 ± 0.8 g for shell width and wet weight, respectively. Oysters cultured in boxes were significantly the smallest with means of 11.7 ± 0.1 mm and 4.8 ± 0.1 g for shell width and wet weight, respectively (Fig. 6.24; 6.25).

At the start of the experiment, different numbers of oysters were added to the different types of culture unit to remove any possible density effects due to varying culture unit sizes. Unfortunately, mortality was only recorded per treatment and not per replicate at each time interval. Therefore data is only available for total mortality and analyses could not be conducted. However, the data are quite convincing with greatest mortality (47%) for oysters cultured in boxes. Mortality was lowest for oysters cultured in pearl nets (4%) and pearl nets with noodles (5%), while only 10% of oysters died in the tray treatment.

Table 6.5. Growth characteristics of oysters using four different nursery culture apparatus (box, tray, pearl net and pearl net with noodles; see text for definitions) at Orpheus Island as determined by the von Bertalanffy growth models (VBGM).

	VBGM Growth Parameter			
Culture Apparatus	H_∞	k	t_o	Φ'
Box	36.82	1.09	-1.11	3.16
Tray	64.61	1.03	-0.12	3.63
Pearl Net	77.83	0.97	0.08	3.76

Pearl Net with Noodles	74.90	1.07	0.10	3.77

Table 6.6. Effects of four nursery culture apparatus (box, tray, pearl net and pearl net with noodles; see text for definitions) on the growth (DVH, APM, shell width and wet weight) of *Pinctada fucata* cultured at Orpheus Island for 12 months.

Growth	*F-value*	**p-value**
DVH	519.4	< 0.001
APM	578.1	< 0.001
Shell width	480.3	< 0.001
Wet weight	656.2	< 0.001

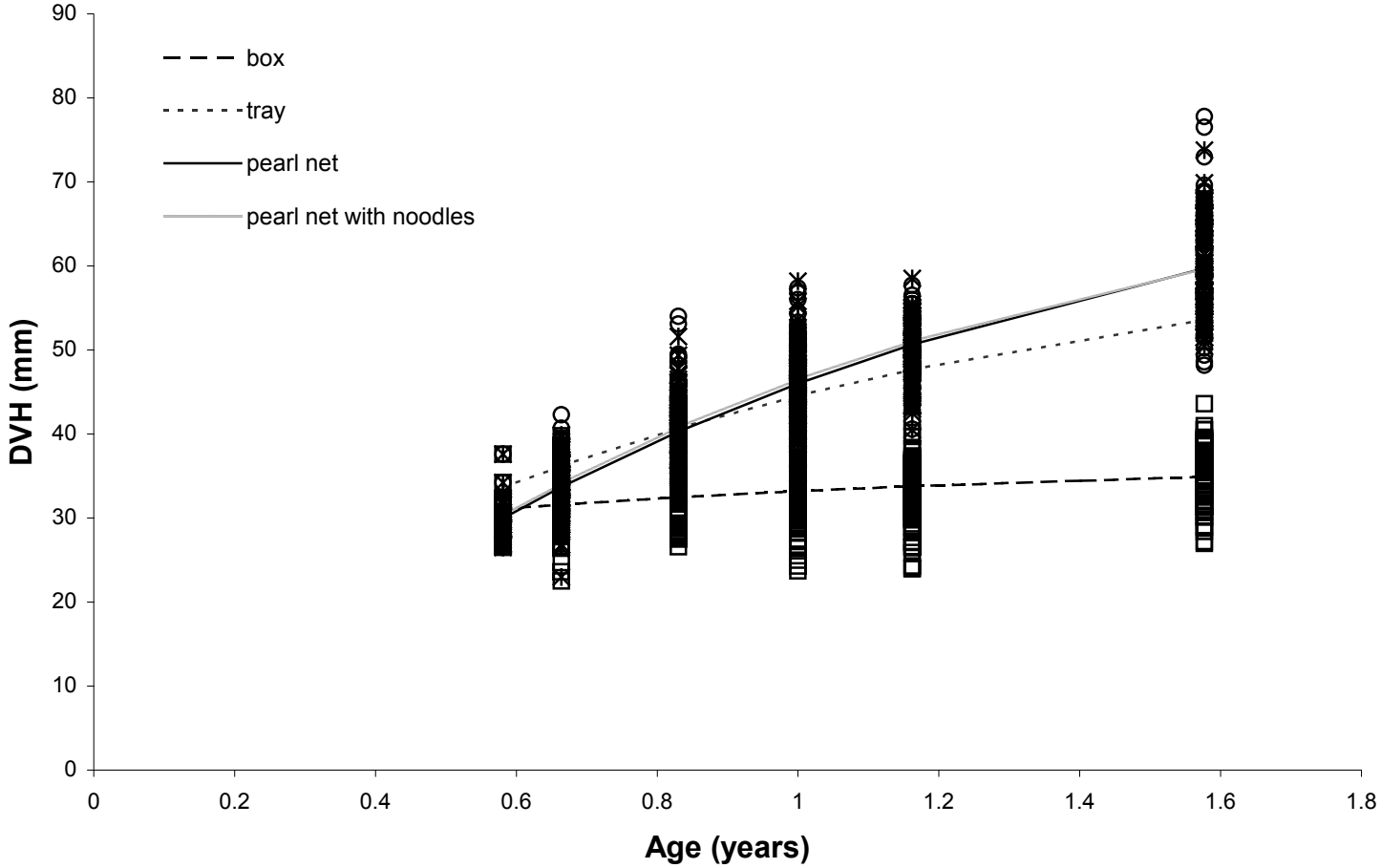


Fig. 6.21. Growth of *Pinctada fucata* using four different nursery culture apparatus (box, tray, pearl net and pearl net with noodles; see text for definitions) cultured for 12 months at Orpheus Island.

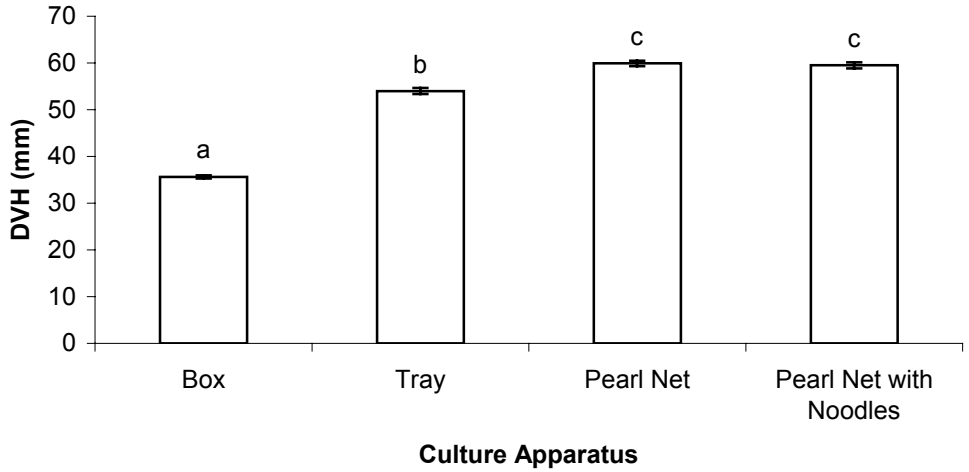


Fig. 6.22. Mean (± SE, *n*=90) dorso-ventral shell height (DVH) of *Pinctada fucata* cultured using four different nursery culture units (box, tray, net and noodles; see text for definitions) for 12 months at Orpheus Island. Means with the same superscript are not significantly different (p>0.05).

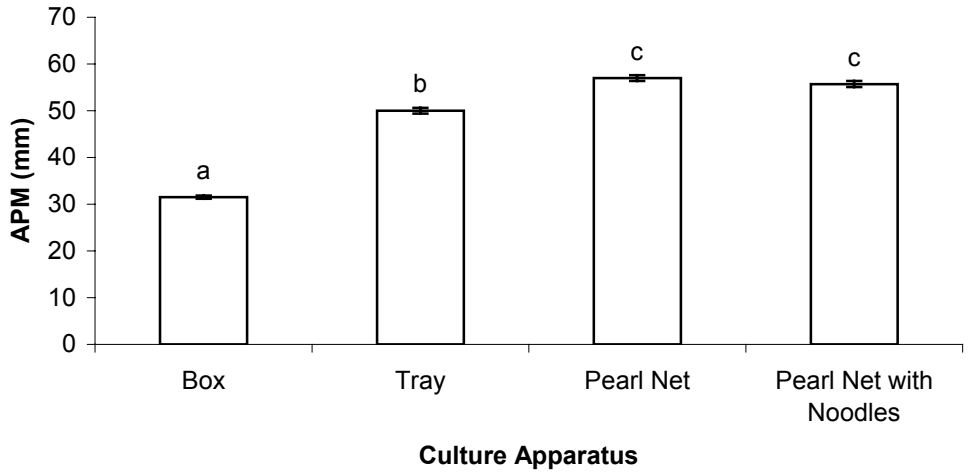


Fig. 6.23. Mean (± SE, *n*=90) antero-posterior shell length (APM) of *Pinctada fucata* cultured using four different nursery culture units (box, tray, net and noodles; see text for definitions) for 12 months at Orpheus Island. Means with the same superscript are not significantly different (p>0.05).

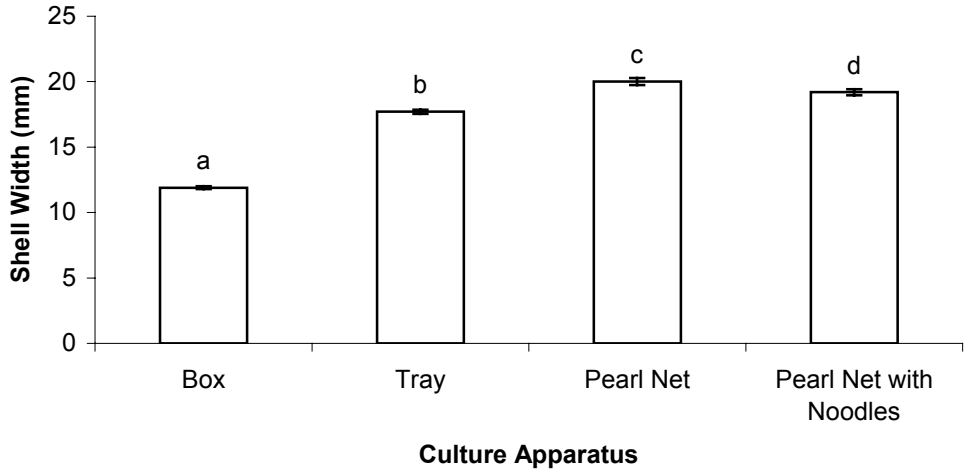


Fig. 6.24. Mean (± SE, *n*=90) shell width of *Pinctada fucata* cultured using four different nursery culture units (box, tray, net and noodles; see text for definitions) for 12 months at Orpheus Island. Means with the same superscript are not significantly different ($p>0.05$).

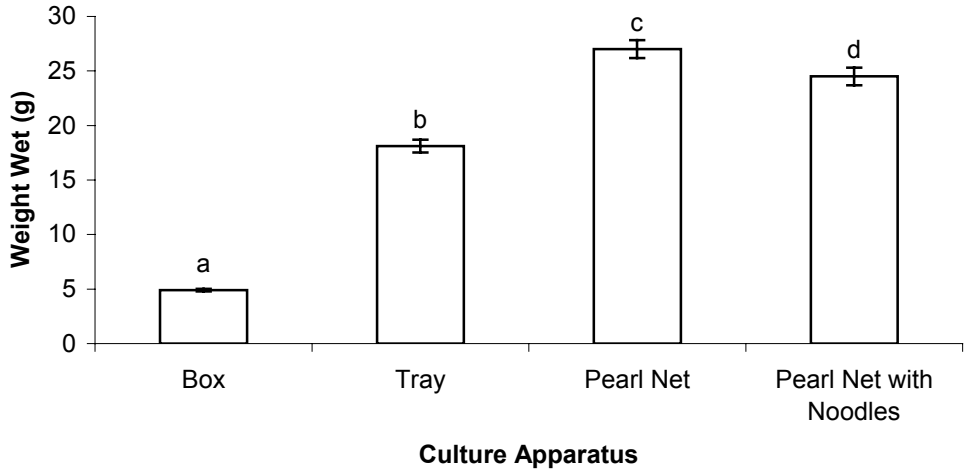


Fig. 6.25. Mean (± SE, *n*=90) wet weight of *Pinctada fucata* cultured using four different nursery culture units (box, tray, net and noodles; see text for definitions) for 12 months at Orpheus Island. Means with the same superscript are not significantly different ($p>0.05$).

6.3.4.2 *Experiment 2*

Similar to Experiment 1, oysters cultured using different nursery culture apparatus showed significant differences in DVH, APM, shell width and wet weight (Table 6.7). Mean (± SE, *n*=90) DVH of *P. fucata* was greatest (73.8 ± 0.9 mm) when cultured in panel nets with large mesh but this did not differ significantly from oysters cultured in pearl nets with large mesh (71.9 ± 0.8 mm). Oysters cultured in pearl nets with small mesh had a mean (± SE, *n*=90) DVH of 70.0 ± 0.7 mm but were not significantly smaller than oysters cultured in pearl nets with large mesh. The smallest mean (± SE, *n*=90) DVH (67.0 ± 1.1) was recorded for oysters cultured in panel nets with small mesh but this was not significantly different to that of oysters cultured in pearl nets with small mesh (Fig. 6.26).

The highest mean (± SE, *n*=90) APM (68.1 ± 0.7 mm) was recorded for oysters cultured in large meshed panel nets, but this was not significantly different to that of oysters cultured in pearl nets with small or large mesh which had a means (± SE, *n*=90) for APM of 65.4 ± 0.7 mm and 67.3 ± 0.8 mm, respectively (Fig. 6.27). Smallest mean (± SE, *n*=90) APM was recorded for oysters cultured in panel nets with small mesh. Mean (± SE, *n*=90) shell width and wet weight was greatest for oysters cultured in large meshed panel nets with 25.2 ± 0.3 mm and 53.2 ± 1.5 g, respectively (Fig. 6.28 and 6.29). Oysters cultured in panel nets with small mesh had the smallest mean shell width and mean wet weight with 22.0 ± 0.3 mm and 35.5 ± 1.4 g, respectively, but these values were not significantly different to those of oysters cultured in small meshed pearl nets. Though both differed from oysters cultured in large meshed panel and pearl nets.

Predation in the first 6 weeks (believed to be the result of an octopus) killed approximately 50% of all oysters contained within panel nets. These oysters were replaced (and an octopus removed) and subsequent survival which was above 85% was non-significant between all treatments ($p>0.05$).

Table 6.7. Significance levels from analysis of variance for the effects of four different culture apparatus (pearl nets and panel nets of small and large sizes) on DVH, APM, shell width and wet weight of *Pinctada fucata* cultured at Orpheus Island for 11 months.

Parameter	*F-value*	**p-value**
DVH	10.66	<0.001
APM	26.09	<0.001
Shell width	23.46	<0.001
Wet weight	35.56	<0.001

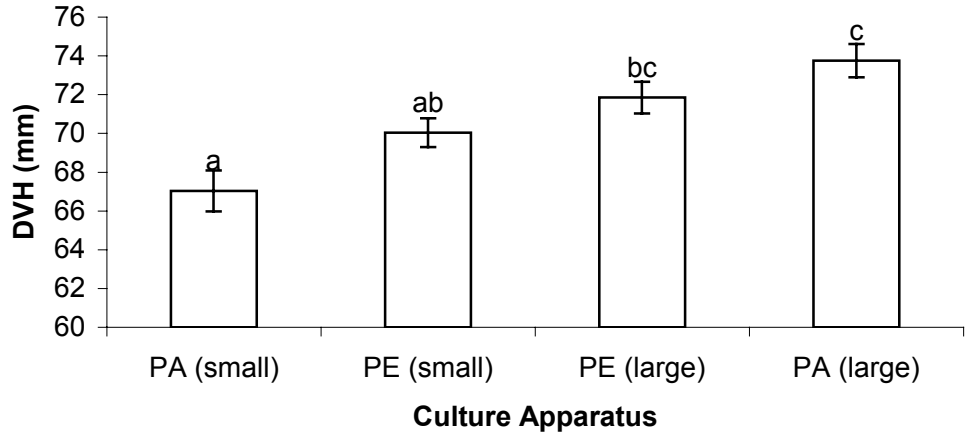


Fig. 6.26. Mean (± SE, *n*=90) dorso-ventral shell height (DVH) of *Pinctada fucata* cultured using four different nursery culture units (pearl nets- PE and panel nets- PA of two different mesh sizes; see text for definitions) for 11 months at Orpheus Island. Means with the same superscript are not significantly different (p>0.05).

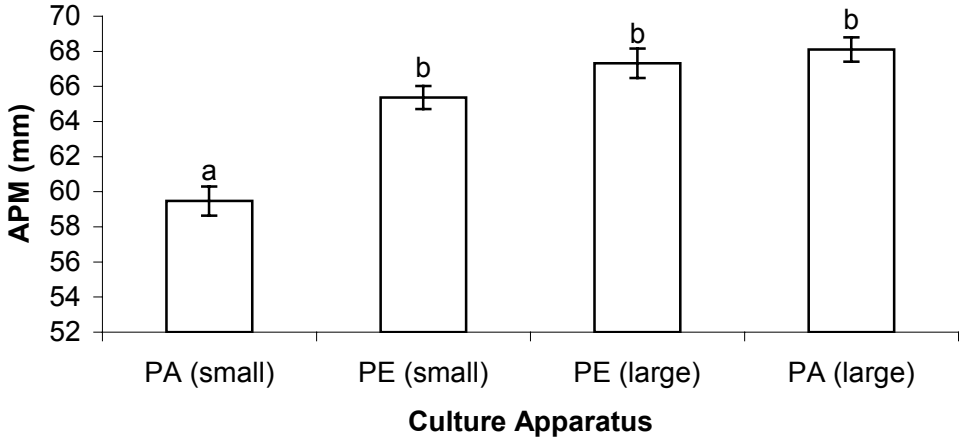


Fig. 6.27. Mean (± SE, *n*=90) antero-posterior shell length (APM) of *Pinctada fucata* cultured using four different nursery culture units (pearl nets- PE and panel nets- PA of two different mesh sizes; see text for definitions) for 11 months at Orpheus Island. Means with the same superscript are not significantly different (p>0.05).

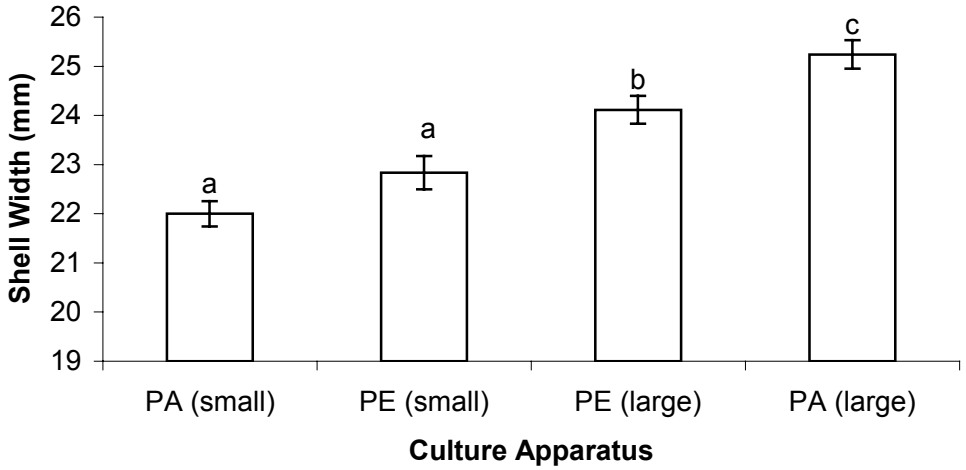


Fig. 6.28. Mean (± SE, *n*=90) shell width of *Pinctada fucata* cultured using four different nursery culture units (pearl nets- PE and panel nets- PA of two different mesh sizes; see text for definitions) for 11 months at Orpheus Island. Means with the same superscript are not significantly different (p>0.05).

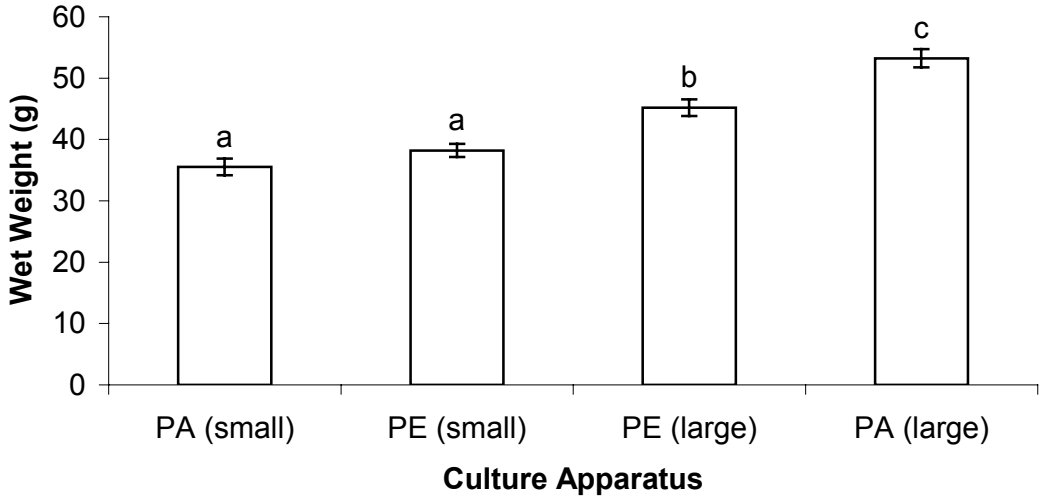


Fig. 6.29. Mean (± SE, *n*=90) wet weight of *Pinctada fucata* cultured using four different nursery culture units (pearl nets- PE and panel nets- PA of two different mesh sizes; see text for definitions) for 11 months at Orpheus Island. Means with the same superscript are not significantly different (p>0.05).

6.3.5 What is the Optimal Cleaning Frequency for Maximum Growth and Survival of *Pinctada fucata*?

Mean (± SE) DVH of *Pinctada fucata* was greatest (49.2 ± 0.3 mm) in oysters that underwent net changes every 6 weeks (Table 6.8). However, there were no significant differences within any of the six cleaning frequencies used in this study. Interestingly mean (± SE) DVH was greatest for oysters in pearl nets which were changed and from predators removed, compared to oysters in nets which were simply cleaned with the high pressure water cleaner. There were no other apparent patterns in the data for the other growth parameters (APM, width and wet weight).

Survival was not significantly between treatments ($p>0.05$) and ranged from 83-100% for all replicates. However, aside from one replicate net, which contained a single *Cymatium muricinum* measuring 50.5 mm in shell length which had consumed 17 oysters (resulting in 83% survival), all replicates had survival rates greater than 99% (Table 6.8).

Table 6.8. Mean (± SE with ranges in parentheses, *n*=90) DVH, APM, shell width, wet weight and survival of oysters cultured under different cleaning protocols (see text for definitions) at Orpheus Island for 6 months. Means with the same superscript in the same column are not significantly different (p>0.05).

Treatment*	DVH	APM	WIDTH	WET WEIGHT	Survival
1	48.5 ± 0.4 mm [a] (39.6-64.8 mm)	46.3 ± 0.4 mm [a] (38.5-61.5 mm)	14.8 ± 0.1 mm [a] (12.5-19.8 mm)	13.7 ± 0.4 mm [a] (12.8-14.6 mm)	94 ± 3.0 % [a] (83-100 %)
2	48.5 ± 0.3 mm [a] (42.0-57.5 mm)	45.9 ± 0.4 mm [a] (38.9-56.1 mm)	15.2 ± 0.1 mm [a] (12.7-17.8 mm)	12.9 ± 0.3 mm [a] (12.3-13.4 mm)	99 ± 0.6 % [a] (97-100 %)
3	48.3 ± 0.3 mm [a] (41.2-60.0 mm)	45.2 ± 0.4 mm [a] (38.2-55.5 mm)	15.0 ± 0.1 mm [a] (13.0-19.6 mm)	13.5 ± 0.3 mm [a] (12.9-14.2 mm)	99 ± 0.6 % [a] (97-100 %)
4	48.6 ± 0.3 mm [a] (41.4-58.5 mm)	46.2 ± 0.4 mm [a] (38.9-55.5 mm)	15.0 ± 0.1 mm [a] (12.8-18.0 mm)	13.5 ± 0.3 mm [a] (12.9-14.1 mm)	99 ± 0.0 % [a] (99 %)
5	49.2 ± 0.3 mm [a] (41.7-60.4 mm)	46.7 ± 0.4 mm [a] (38.0-57.7 mm)	15.2 ± 0.1 mm [a] (13.0-18.0 mm)	13.8 ± 0.3 mm [a] (13.3-14.3 mm)	99.5 ± 0.3 % [a] (99-100 %)
6	49.0 ± 0.3 mm [a] (41.4-63.2 mm)	46.3 ± 0.3 mm [a] (39.1-59.2 mm)	15.1 ± 0.1 mm [a] (12.1-20.0 mm)	13.5 ± 0.2 mm [a] (13.2-13.7 mm)	100 ± 0.0 % [a] (100 %)

* ‘treatment 1’- nets cleaned every 4 weeks, but not changed or inspected;

‘treatment 2’- nets cleaned every 6 weeks, but not changed or inspected;

‘treatment 3’- nets cleaned every 8 weeks, but not changed or inspected;

‘treatment 4’- nets not cleaned, but changed every 4 weeks;

‘treatment 5’- nets not cleaned, but changed every 6 weeks; and

‘treatment 6’- nets not cleaned, but changed every 8 weeks.

6.4 Discussion

6.4.1 Does Culture Depth Affect Growth of *Pinctada fucata*?

Results from this study show that depth was a significant factor influencing the growth of *Pinctada fucata* when first transferred from the hatchery to the ocean. Maximum growth and a greater percentage of larger individuals were observed in spat cultured at 2 m compared with those grown at 4 m and 6 m. In a similar study, O'Connor *et al.* (2003) reported that there was no significant difference in growth of *P. imbricata* (=*P. fucata*) juveniles after three months of culture at depths ranging from 2-6 m; however, survival was low in pearl nets close to the bottom due to the presence of *Cymatium parthenopeum* which caused widespread mortality. Species of the genus *Cymatium* have previously been shown to prey on pearl oysters (Pit and Southgate 2003a). O'Connor *et al.* (2003) attributed the lack of significant difference in growth to good water circulation, which they suggested limited any environmental stratification. This is further supported by Gervis and Sims (1992) who suggest that reduced growth rates of pearl oysters at greater depths may be a result of lower water temperatures and decreased food availability. Similarly, Lodeiros and Himmelman (2000) showed that scallop, *Euvola ziczac*, spat transferred from the hatchery to the ocean expressed greater growth at the shallower depths of 8 and 21 m compared to 34 m, which they attributed in part to environmental conditions (food availability and water temperature). However, they noted that fouling (which is related to environmental factors) were probably more influential on growth compared to environmental conditions alone. Similarly, Lodeiros *et al.* (1998) reported that maximum growth of the scallop, *Lyropecten nodosus*, occurred at 8 and 21 m compared to 34 m. They attributed the differences in growth to changes in phytoplankton density, which decreased with depth

and, while water temperature varied between depths, temperature variation was assumed to be too slight to have caused the observed growth differences. Furthermore, Emerson *et al.* (1994) noted that phytoplankton density is positively correlated with growth of scallops, *Placopecten magellanicus.* While the above studies show similar patterns to the present study, in which growth was significantly different at the three depths, unfortunately chlorophyll was not measured in the present study as we only had access to one chlorophyll data logger. Therefore growth differences can only be suggested to have been influenced by chlorophyll levels.

Due to the long-line position and its close proximity to the reef flat (approx. 200 m) on a high continental island (Orpheus Island), differences observed in oyster growth as a function of depth may be related to high productivity as a result of run-off, resulting in high chlorophyll and phytoplankton compared to 4 and 6 m. However, it is very apparent that spat cultured at shallow depths (2 m) at Orpheus Island are significantly larger than spat at greater depths (4 m and 6 m). As maximising oyster size is a goal of pearl oyster culture (section 8.4), results from this study suggest that oysters transferred from the hatchery to the long-line at Orpheus Island be placed at 2 m. However, further research is required to understand why these differences were observed. Additionally, further research must also be conducted for early and late nursery culture to identify if similar patterns are observed, and whether depth effects pearl quality. Cahn (1949) suggested that nacre deposition is maximised under blue light, which resembles that of deeper water, while Kafuku and Ikenoue (1983) suggest that oysters cultured at depths greater than 5 m produce high quality ‘pink’ pearls.

6.4.2 Does Stocking Density Affect Growth and Survival of *Pinctada fucata*?

Pinctada fucata stocked initially at 25% of total available net area of the culture unit showed maximum growth during early nursery culture after 3 months compared to oysters cultured at 50% and 75% of available net area. Furthermore, during late nursery culture, oysters cultured at 20% of total available net area were significantly greater in size than oysters cultured at 25%, 30% and 40% of total available net area. Growth of DVH was shown to vary with stocking density during late nursery culture. While the results of one-way analysis of variance indicated no significant difference in final DVH, APM, shell width and wet weight for oysters cultured at 25% and 30% of total available net area, overall growth performance (Φ') indicated a difference. For more detailed comparisons of growth performances from other studies and within this project refer to Chapter 7 (section 7.4) and Chapter 8 (section 8.3), respectively. In a recent study, Urban (2000a) reported no significant differences in growth of pearl oysters, *P. imbricata* (=*P. fucata*), cultured at two different stocking densities (20% and 30% of available net area) in suspended and bottom culture in Columbia. The work of Urban (2000a and b) is the only work to the authors knowledge aside from the current study investigating Akoya pearl oyster culture under tropical conditions, and while the current study is quite detailed, that of Urban (2000a) simply looked at growth and survival of Akoya oysters in suspension and bottom culture. Similar to the Australian position, there is a large amount of research which needs to be carried out before Columbia can begin commercial culture of Akoya pearls.

A similar study on stocking density based on total available net area has suggested that when culturing scallops, areal coverage should not exceed 33% (Imai 1977). This is supported by others (e.g. Paul *et al.* 1981) who suggested that densities greater than 33% may affect growth and survival of cultured scallops, *Chlamys*

opercularis and *Pecten maximus*. However, Parsons and Dadswell (1992) who were the first to test this hypothesis found that stocking densities as high as 50-60% still supported good growth and survival of the scallop, *Placopecten magellanicus*.

Traditionally, research with pearl oysters has reported densities in relation to the number of oysters per culture unit, which has then been converted to a density of oysters per area. For example, Taylor *et al*. (1997a) looked at the stocking density of *Pinctada maxima* during nursery culture utilising four stocking densities of 10, 50, 100, and 150 individual oysters per PVC slat (75 x 500 mm^{-2}) or 13, 67, 133, and 200 juveniles per 1 m^{-2}. The authors made no mention of the percentage of available area used by the oysters. Similarly, Monteforte and Morales-Mulia (2002) reported stocking densities as a function of area with no concern of oyster size. The disadvantage of this approach is that growth will vary with different sized oysters even if stocked at the same density (numbers per unit area). Monteforte and Morales-Mulia (2002) cultured *P. mazatlanica* at densities ranging from 132-400 individuals m^{-2}; however, when they transferred oysters from early to late nursery culture, they increased the density from 132-148 individuals m^{-2} up to 400 individuals m^{-2} before decreasing the density gradually to 200-240 individuals m^{-2}. The idea of increasing the density between early and late nursery culture goes against the published work of others which suggests that density should be decreased as oyster size increases.

Recently, O'Connor *et al.* (2003) investigated optimal stocking density of pearl oysters, *P. imbricata*, (=*P. fucata*) as a function of wet weight. They conducted two experiments assessing the effects of stocking density by stocking pearl nets at densities of either 100, 200, 400 or 600 g per net for oysters measuring 25.3 mm in DVH (1.6 g) and at densities of 200, 400, 600 and 800 g for oysters measuring 40.3 mm in DVH (7.6 g). A general trend of decreased growth and survival was recorded with increased

stocking density (O'Connor *et al.* 2003). Similar to the present study, the research of O'Connor *et al.* (2003) accounted for varying oyster size with both studies suggesting, that stocking density (of available net area and wet weight) should be decreased with increased oyster size.

Many studies have identified growth to be inversely proportional to stocking density (e.g. Parsons and Dadswell 1992; Taylor *et al.* 1997a; Maguire and Burnell 2001). Competition for resources and the phenomenon of clumping (which is common in pearl culture), are thought to be the major factors causing differences in growth as a factor of stocking density. Space and food are the two most important resources in bivalve culture. Parsons and Dadswell (1992) noted that observed differences in growth as a function of stocking density are more likely due to food depletion rather than competition for space. They attributed this to the fact that at high stocking densities no obvious signs of stress or 'misshapen' shells were observed which would be expected if space was a limiting factor. Additionally, Côté *et al*. (1994) found that increased density had no affect *per se* on growth and survival of the giant scallop, *Placopecten magellanicus*. Côté *et al.* (1994) placed living and non-living ('dummy') scallop shells into nets to determine the effect of density and found that at higher densities where 'dummy' scallops were used, growth was increased. They attributed differences in growth and survival to food depletion and not a function of space. However, unlike pearl oysters, which form clumps and restrict the amount of water and food that the innermost individuals within the clump are able to access (Friedman and Southgate 1999), some scallops have the ability to move within the culture unit by clapping (opening and closing their shells quickly propelling themselves) which minimises the chances of forming large bysally attached aggregations or clumps. Additionally, most scallops cease byssus production once they attain 10-15 mm in shell

height (pers. comm. P. Duncan 2004). Similar results have also been noted for the mussel, *Mytilus galloprovincialis*, which is also able to move around and reorientate itself to obtain food on culture ropes (Fuentes *et al.* 2000).

While oyster clumping was minimal to non-existent in the present study at the lowest stocking densities (20% and 25% of total available net area), oysters in the 40%, 50% and 75% stocking densities all but formed one clump. The gregarious behaviour of oysters is well documented and was first reported by Crossland (1957). Crossland (1957) reported *P. margaritifera* readily formed clumps which resulted in stunting and mortality to the innermost individuals which may explain why treatments with greater stocking density recorded significantly smaller oysters. This behaviour has since been confirmed for *P. margaritifera* (Southgate and Beer 1997) and reported for *P. maxima* (Taylor *et al*. 1997a). Taylor *et al.* (1997a) noted that increasing stocking density of *P. maxima* to greater than 1.3 juveniles per 100 cm^{-2} resulted in decreased DVH and APM, and increased the number of oysters in a clump. They also found that increasing stocking density increased the amount of deformities in pearl oysters. Deformed pearl oyster shells are undesirable as the oysters are unable to be used for pearl production (Taylor *et al.* 1997a).

Survival in the present study was independent of stocking density, which suggests that the upper and lower stocking density limits were not tested in this study. Taylor *et al.* (1997a) noted that maximum survival for *P. maxima* occurred at the lowest stocking density tested (13 oysters m^{-2}). However Taylor *et al.* (1997a) recorded that there were no significant differences in survival of oysters cultured at densities ranging from 67-200 oysters m^{-2}, and they suggested that the upper limit for stocking density was not tested. Rose and Baker (1994) reported similar results to those of Taylor *et al.* (1997a) in that no significant differences in survival were

recorded for oysters cultured at stocking densities ranging from 30-70 oysters m^{-2}. Furthermore, Honkoop and Bayne (2002), noted that survival of rock oysters, *Saccostrea glomerata* and *Crassostrea gigas*, was not affected by stocking density.

While stocking densities in the present study of 50% and 75% of available net area appear too high during early nursery culture as shown by low growth and survival, stocking densities of 25% promote good growth and high survival. In terms of late nursery culture, 20% stocking density provides optimal growth and survival, while densities any lower than this would possibly lead to economical pitfalls. This study has provided information on optimal stocking densities for Akoya pearl oysters for the possible establishment of an Akoya pearl industry in Queensland. However, similar research to that of Monteforte and Morales-Mulia (2002), who reported that decreasing density beyond a certain lower limit would not increase growth and survival of *P. mazatlanica* needs to be conducted for *P. fucata*.

6.4.3 Do Small Oysters 'Catch Up' to their Larger Counterparts?

After 7 months of culture, (11 months of age), oysters from the small size class were large enough to be utilised for pearl production (ca. 50 mm). Oysters in the small size class (51 mm) were, however, significantly smaller than oysters from the medium (53 mm) and large (56 mm) size classes, which were significantly different from each other. While oysters in the small size class reached pearl production size at 11 months of age, oysters from the medium and large sizes classes only require 9 and 7 months, respectively, before they could be utilised for pearl production. In the only other study investigating whether small pearl oysters 'catch up' to large pearl oysters, Pit and Southgate (2003b), noted that some *P. margaritifera* individuals from the small size class, termed 'runts' can catch up to oysters from the medium and large size classes.

They suggested that rather than discarding potential pearl producing animals at 3 months of age measuring less than 5 mm, that oysters should be retained until they are 5-6 months of age, when slow growers can be discarded. The work of Pit and Southgate (2003b) however, was only conducted until juveniles were 7.5 months of age and pearl production size had not been obtained. In the current study, oysters from the three size classes had significantly different growth parameters after 7 months of culture, however, with a further 14 months of culture, there was no significant difference between oysters from the small and medium size classes with regard to DVH, APM, shell width and wet weight. Additionally oysters from the medium and large size classes showed no significant difference in DVH, APM, shell width and wet weight. However, overall growth performance was greatest for oysters in the large size class (see Chapter 7 and 8 for discussion comparing growth performance of other pearl oysters).

The smallest oysters in the current study reached pearl production size after only 11 months. Previous work in India with *P. fucata* noted that a shell height of ~50 mm requires 12 months (Victor *et al.* 1995), while *P. martensi* (=*P. fucata*) in China requires 24 months to reach pearl production size (Guo *et al.* 1999). Urban *et al.* (2000a) noted that *P. imbricata* (=*P. fucata*) required more than 12 months of culture to reach pearl production size in Columbia. However, in Venezuela, Lodeiros *et al.* (2002) recorded growth of 55 mm in *P. imbricata* after only 9 months. Both of these South American studies used wild collected spat, not hatchery produced spat. Results from this present study are very encouraging, as the slowest growers reach pearl production size (ca. 50 mm) in a time quicker than most other published *P. fucata* producing nations. However, further research is required to identify whether there are any possible implications (i.e. decreased pearl quality or increased farm costs) with

retaining slow growing oysters and producing pearls from slow growers. Possible implications are discussed further in Chapter 8 (section 8.3).

6.4.4 Does Culture Unit Affect Growth and Survival of *Pinctada fucata*?

The use of different culture methods for *Pinctada fucata* in the present study has shown that pearl nets and large mesh panel nets provide maximum growth and survival during nursery culture for oysters measuring 30 mm and 50 mm, respectively.

During early nursery culture (initially 30 mm DVH), there was no significant difference in DVH of oysters cultured in pearl nets and pearl nets with noodles; however, pearl nets with noodles would be preferred because during routine maintenance, oysters are more easily removed from pearl nets (with noodles) as they prefer to attach to the noodles rather than the sides of the pearl net (Fig 6.30). Maximum growth was achieved by oysters cultured in the smaller culture units (pearl nets compared with boxes and trays). While stocking density was maintained at 25% of available net area, it has been suggested that smaller culture units promote greater flow of water and subsequently greater food availability than larger culture units and therefore result in increased growth (Mendoza *et al*. 2003). Additionally, water-flow through the box culture and tray culture units would have been restricted due to the size of the slots (mesh size). Mesh size has previously been shown to be influential in growth of pearl oysters (see below).

During nursery culture (initially 50 mm DVH), pearl nets and panel nets with large mesh promoted superior growth of *P. fucata* when compared with pearl nets and panel nets with small mesh. Similarly, in a recent study with pearl oysters, Teitelbaum (2001) noted that oysters cultured in large mesh (9 mm) pearl nets grew significantly larger than oysters cultured in pearl nets with smaller mesh (1 and 4.5 mm). He

attributed observed differences to the combined effects of fouling and water-flow; fouling was greater on small mesh nets which drastically decreased water-flow and promoted predator settlement with *Cymatium* (a known pearl oyster predator-Teitelbaum *et al.* 2003) was recorded in 1 mm meshed nets but not in larger meshed nets.

Fig. 6.30. Attachment of *P. fucata* to 'noodles' from within pearl nets.

Similar results have also been reported for scallops. Côté *et al* (1994) noted that culture units with smaller mesh attract more fouling than culture units with large mesh. They suggested that the increased fouling on the small mesh nets decreased water velocity through the nets and that the fouling organisms may also compete for resources which would subsequently affect oyster growth. Additionally, Maguire and Burnell (2001) noted superior growth of the scallop, *Pecten maximus*, in nets with large mesh (15 mm) compared with scallops in small mesh (6 mm) nets. Similar results were also observed for *Argopecten irradians concentricus* in which large meshed nets (9 mm) recorded greater growth than oysters cultured in small mesh nets (3 mm). Walker and Heffernan (1990) had mixed results with superior growth observed in large mesh

nets for surf clams, *Spisula solidissima*, whereas small mesh nets provided superior growth of hard clams, *Mercenaria mercenaria*; however, survival of oysters in small meshed nets resulted in over 80% mortality in 4 months.

When oysters were cultured in units which exclude aggregation, (panel nets compared with pearl nets), growth was superior for large mesh culture units. It has been suggested by many authors that aggregation in bivalve culture units decreases the availability of food to all individuals evenly (e.g. Mendoza *et al.* 2003). Southgate and Beer (1997) noted that individual pearl oysters, *P. margaritifera*, in the centre of the aggregation are smaller than individuals on the outermost part of the aggregation presumably due to decreased food availability. Meanwhile Friedman and Southgate (1999) and Southgate and Beer (2000) noted that higher growth was recorded for *P. margaritifera* cultured in units in which aggregation was minimised or not possible. Southgate and Beer (2000) also found that 8-month old *P. margaritifera* (41.5 ± 0.6 mm DVH) showed superior growth when cultured separately in panel nets or ear-hung compared to oysters which were able to aggregate in tray culture.

Lodeiros *et al.* (2002) recorded superior growth of *P. imbricata* (=*P. fucata*) in suspended culture compared to bottom culture in Venezuela. This contradicts the findings of Urban (2000a) who noted that no significant difference in growth of *P. imbricata* (=*P. fucata*) occurred between suspended and bottom culture in Columbia. Meanwhile, Gayton-Mondragon *et al.* (1993) reported superior growth for *P. mazatlanica* and *Pteria sterna* in bottom culture compared to suspended culture. Superior growth in suspended culture compared to bottom culture has been attributed to greater food availability (Lodeiros *et al.* 2002), whereas decreased growth in suspended cultured compared to bottom culture has been attributed to stress as a result of less than optimal environmental conditions, decreases in fouling of culture units or culture

systems used (Freites *et al.* 2000; Urban 2000a). The above studies illustrate the fact that sites, which vary in their physical and biological characteristics, are different. Therefore culture protocols that provide optimal growth and survival in one location may be less than optimal at another location (Southgate and Beer 2000).

Predation by fish, molluscs and crustaceans, is a major cause of mortality of commercially important bivalves (Freites *et al.* 2000), including pearl oysters (Alagarswami and Qasim 1973; Wada 1973; Pit and Southgate 2003a). In the present study, predation caused major mortality at the beginning of the experiment. While the predator was not observed, remnants of cultured shell were located on the sea floor in a pile, characteristic of an octopus lair (Steer and Semmens 2003). Octopi are known predators of pearl oysters (Steer and Semmens 2003) and once the octopus was captured, mortality of pearl oysters decreased to near zero. In a recent study, Pit and Southgate (2003a) noted that routine inspection and removal of predators will minimise loss of stock and should be incorporated into regular husbandry protocols for pearl oysters.

Results from this study suggest that *P. fucata* growth can be maximised using culture units which prevent aggregation and allow maximum water-flow through the use of large mesh. However, as with all sites, frequent inspection of culture units is required to minimise stock loss due to predation.

6.4.5 What is the Optimal Cleaning Frequency for Maximum Growth and Survival of *Pinctada fucata*?

The six cleaning protocols employed in this study did not significantly affect pearl oyster growth. It is therefore suggested that *P. fucata* cultured at Orpheus Island can be left unattended for 8 weeks (the longest period between cleaning nets during this

study) before nets are changed. However, results suggested that the upper time interval limit between cleaning was not reached. It is not fully understood why there was no significant difference in oyster growth rates between the different treatments during this study. Pit and Southgate (2003a) recently reported that to maximize growth, *P. margaritifera* cultured in plastic mesh trays should be cleaned every 8 weeks compared with cleaning every 4 or 16 weeks to maximize growth. Taylor *et al*. (1997b) noted that cleaning and removing fouling organisms every 2 and 4 weeks promoted growth of *P. maxima* when compared to those cleaned every 8 and 16 weeks. This was attributed to increased fouling, reduced water-flow and food availability causing subsequent decreases in growth.

While fouling has been previously shown to cause major problems with pearl oyster culture at Orpheus Island (Pit and Southgate 2003a), it should be noted that fouling assemblages are complex, vary both spatially and temporally and are influenced by local conditions such as water temperature and rainfall (Dharmaraj *et al*. 1987; Arakawa 1990; Claereboudt *et al.* 1994). Arakawa (1990) noted that some rock oyster growers believe that a small amount of fouling is beneficial to oyster development. Tanita *et al*. (1961 in Arakawa 1990) reported that fouling organisms can often 'excite' oyster shell margins, which they suggested stimulates growth. Similarly, Ross *et al*. (2002) showed that fouling might not actually reduce the availability of food to the culture organism but rather increase it by creating mini ecosystems within culture units. They also stated that fouling might actually increase the amount of available food for the cultured organism, which supports the findings of Arakawa (1990).

The process of cleaning pearl oysters and their culture units generally involves mechanical or manual scrubbing with brushes and removal of hard fouling with knives (Gervis and Sims 1992), or use of high pressure water cleaners (Scoones 1990). Many

of the common fouling organisms of pearl oyster culture include barnacles, sponges, worms and other bivalves, which can interfere with shell opening, cause shell deformities and may cause death (Alagarswami and Chellam 1976; Wada 1991; Taylor *et al.* 1997b). The removal of fouling is labour intensive, causing subsequent increases in farm costs (Arakawa 1990; Parsons and Dadswell 1992). Minimising the time interval between successive cleaning and/or net changes would provide significant economical gains to the pearl industry (Taylor *et al.* 1997b). However, compromises must be made between increased growth with clean nets, or slightly decreased growth and decreased profitability due to the costs involved with routine cleaning and maintenance.

The results of this study are promising for the development of an Akoya pearl oyster industry in Queensland as the expensive and time consuming activity of pearl net cleaning may be minimal. Further is required to identify whether similar patterns in terms of cleaning frequency occur in other Queensland sites.

6.4.6 Conclusion

In summary, based on the results of this study, the recommendations for the culture of *Pinctada fucata* during nursery culture at Orpheus Island include:

(1) transferring spat from the hatchery to the long-line at a depth of 2 m;

(2) retaining 'runts' or slow growers to seeding size;

(3) stocking oysters at a density of 20-30% of available net area;

(4) culturing oysters in pearl nets or pearl nets with noodles until they attain a DVH of 50 mm;

(5) culturing oysters in panel nets or pearl nets with large mesh once they attain a size greater than 50 mm; and

(6) changing culture units no frequently than every 8 weeks to minimise fouling and maximise growth and survival.

Chapter 7 Site Dependent Growth

7.1 Introduction

Aquaculture sites are generally selected on the basis of practical considerations such as property availability and access, security, position in relation to markets as well as protection from wave exposure (Grant 1996). Furthermore, biological and physico-chemical factors that influence growth and survival of cultured aquatic animals are also taken into account when selecting appropriate sites (Silvany *et al.* 1999). Environmental parameters are site-dependent and not all sites can support high-density aquaculture due to unfavourable environmental conditions. Although selecting an 'ideal' site is generally not possible, a site selection process can determine whether or not an aquaculture operation is feasible (Haws 2002).

Bivalve aquaculture is typically restricted to calm protected coastal waters such as embayments, rivers and estuaries (Kaiser *et al.* 1998). These sites are limited in number and are primarily selected in areas with high incidence of natural spat of the selected species. However, advancements in bivalve hatchery technology coupled with less reliance on natural spat has enabled site selection to be based upon factors which optimise growth and survival (i.e. environmental parameters) of the target bivalve, rather than being limited to sites which have high levels of natural spat of the selected bivalve. The objective of any bivalve aquaculture operation is to maximise growth and minimise the time required for the animal to reach market size; in the case of the pearl oyster industry, the target size is the size at which oysters can be seeded for pearl production. Pearl farms are best suited to sheltered bays, which offer protection from the prevailing winds (Anon 1991).

Whilst a number of factors affect bivalve growth and survival including depth, stocking density and culture apparatus (Chapter 6), several environmental water quality parameters have also been shown to affect growth and survival (Bayne and Newell 1983, Sims 1993, Lodeiros *et al.* 1998). Many studies have looked at the influence of environmental parameters on the physiology and growth of bivalves. Most have concluded that water temperature and food availability are the primary factors influencing bivalve growth (Bayne and Newell 1983; Toro *et al.* 1995, 1999; Tomaru *et al*. 2002a). Stirling and Okumus (1995) noted that water temperature, food availability and, to a lesser extent salinity, can explain the differences in growth rate observed between spatially close populations of mussels (*Mytilus edulis*). Although survival, like growth, can be affected by environmental conditions, it is largely determined by genetic factors (Brown and Hartwick 1988).

High correlation between mean annual water temperature and annual growth rate of bivalves have been observed (Emerson *et al.* 1994), however, Bayne and Newell (1983) suggested that food availability has a greater effect on bivalve growth than water temperature. Other studies including those of Toro *et al.* (1995) and Toro (1996) have suggested that seasonal phytoplankton blooms/abundances and concentration of chlorophyll 'a' covary with water temperature and that trying to differentiate between the two makes it hard to identify which is the driving force under natural conditions. While it would be possible to carry out such an experiment in the laboratory, the problem would be the possible synergistic effects that may contribute to patterns seen in nature. However, the uptake of phytoplankton by *Pinctada martensii* (=*P. fucata*) has been shown to enhance assimilation of calcium from seawater (Kuwatani 1964), suggesting that a measure of phytoplankton such as chlorophyll 'a' level could be correlated with oyster shell growth.

The aim of this study was to identify whether environmental parameters including water temperature, chlorophyll 'a' level, and salinity affect growth of *P. fucata* and to identify how growth of *P. fucata* varies temporally at two sites in north Queensland.

7.2 Materials and Methods

In order to look at variation in growth resulting from environmental parameters, two sites in north Queensland were chosen; Orpheus Island and Magnetic Island (Fig. 2.1-2.3). These sites were chosen for two reasons: (1) differences in location and environmental factors; Orpheus Island is typically referred to as a mid-reef island, and Magnetic Island is considered to be an inner-reef island (i.e. closer to the coast; Chapter 2); and (2) both sites have been used successfully for pearl oyster culture in the past (Southgate and Beer 1996). These sites were therefore compared to determine the best site for culture of Akoya pearl oysters in the Townsville region and to identify suitable ranges of water quality parameters to assist selection of appropriate sites to be used for Akoya pearl oyster culture in north Queensland.

One hundred oysters (cultured according to the general methods described in Chapter 2) with a mean (± SE, n=50) dorso-ventral shell height (DVH), antero-posterior shell length (APM), shell width and wet weight of 30.4 ± 0.5 mm, 28.5 ± 0.4 mm, 9.4 ± 0.2 mm and 3.5 ± 0.2 g, respectively, were placed into each of five replicate pearl nets. Nets were placed independently in a random position on a single long-line at a depth of 4 m at both sites (Orpheus Island and Magnetic Island). Nets were cleaned externally every 6 weeks to remove fouling organisms. This involved removing nets from the water and manually scrubbing with brushes, while hard fouling,

(oysters/barnacles) was removed with the use of knives (Pit and Southgate 2000). During each cleaning period, nets were also inspected internally and dead oyster shells and predators were recorded and removed and replaced with live oysters to maintain densities. Approximately every 6-12 weeks, 20 oysters from each replicate net were measured for DVH, APM, shell width and wet weight. This experiment was conducted for 12 months from July 2001-July 2002.

At each site a 'YSI-6600' water quality data logger was deployed at a depth of 4 m to monitor water temperature, salinity, and chlorophyll 'a' levels. The data logger made constant measurements every 2 h; data were uploaded every 6 weeks when the logger was cleaned to remove any fouling which had settled on the surface of the unit. Calibrations were carried out every 3 months to ensure consistency in data collection. However, the conductivity probe, which is used to calculate salinity, malfunctioned during the final deployment of the data logger at Orpheus Island. Consequently, salinity data are missing for this period. To overcome this, a simple regression was calculated to estimate a value for salinity during the final deployment.

Principal component analysis (PCA) was carried out to determine whether sites could be distinguished according to environmental parameters. In order to determine growth characteristics of oysters at both sites, data were fitted to the von Bertalanffy growth model (VBGM; section 6.2.2.2). Differences in growth models were identified through the use of ARSS, and Phi prime was calculated to determine whether overall differences in growth occurred at the two sites (section 6.2.2.2).

While the ARSS determined differences as a result of growth models over the year, T-tests were also carried out to determine whether final growth differed at the two sites. Simple regression was carried out to determine the relationship between

environmental parameters and oyster growth and the data met analysis assumptions (Zar 1984).

7.3 Results

Mean water temperature, salinity and chlorophyll 'a' values were determined for the duration of the experiment (12 months) at both Orpheus Island and Magnetic Island and followed very similar trajectories for each water quality parameter (Fig. 7.1). Mean (± SE) water temperature at Orpheus Island at a depth of 4 m during this study ranged from 23.0 ± 0.01°C at the start of the study (July) to a maximum of 29.6 ± 0.01°C in November (Fig. 7.1a). Meanwhile mean water temperatures at Magnetic Island at a depth of 4 m ranged from a minimum of 22.3 ± 0.02°C in July to a maximum of 30.5 ± 0.05°C in November (Fig. 7.1a). During the dry season (May-October) mean water temperatures at Magnetic Island were typically lower than those at Orpheus Island except in May and October while during the wet season months, water temperatures were higher at Magnetic Island compared to Orpheus Island.

Levels of chlorophyll 'a' were higher at Magnetic Island than at Orpheus Island (Fig. 7.1b). At Orpheus Island there appears to be two distinct peaks or blooms in chlorophyll 'a' levels (maximum of 4.66 $\mu g.l^{-1}$ at the completion of the study in July), whereas at Magnetic Island levels peak at a maximum of 7.06 ± 0.3 $\mu g.l^{-1}$ in November and then remained above 2.5 $\mu g.l^{-1}$ for the remainder of the study. The minimum recorded levels of chlorophyll 'a' at Magnetic Island were 2.04 $\mu g.l^{-1}$, whereas at Orpheus Island, the minimum was 0.95 $\mu g.l^{-1}$ (Fig. 7.1b).

Salinity at both sites was very similar during the first 6 months of the study at which time slight variations were recorded (Fig. 7.1c). Maximum salinity at Orpheus and Magnetic Islands of 36.3 ± 0.01‰ and 36.8 ± 0.02‰, respectively, were recorded

in July (the middle of the dry season). Lower levels of salinity were recorded in November at both Orpheus and Magnetic Islands of 25.2 ± 0.1‰ and 26.4 ± 0.1‰, respectively.

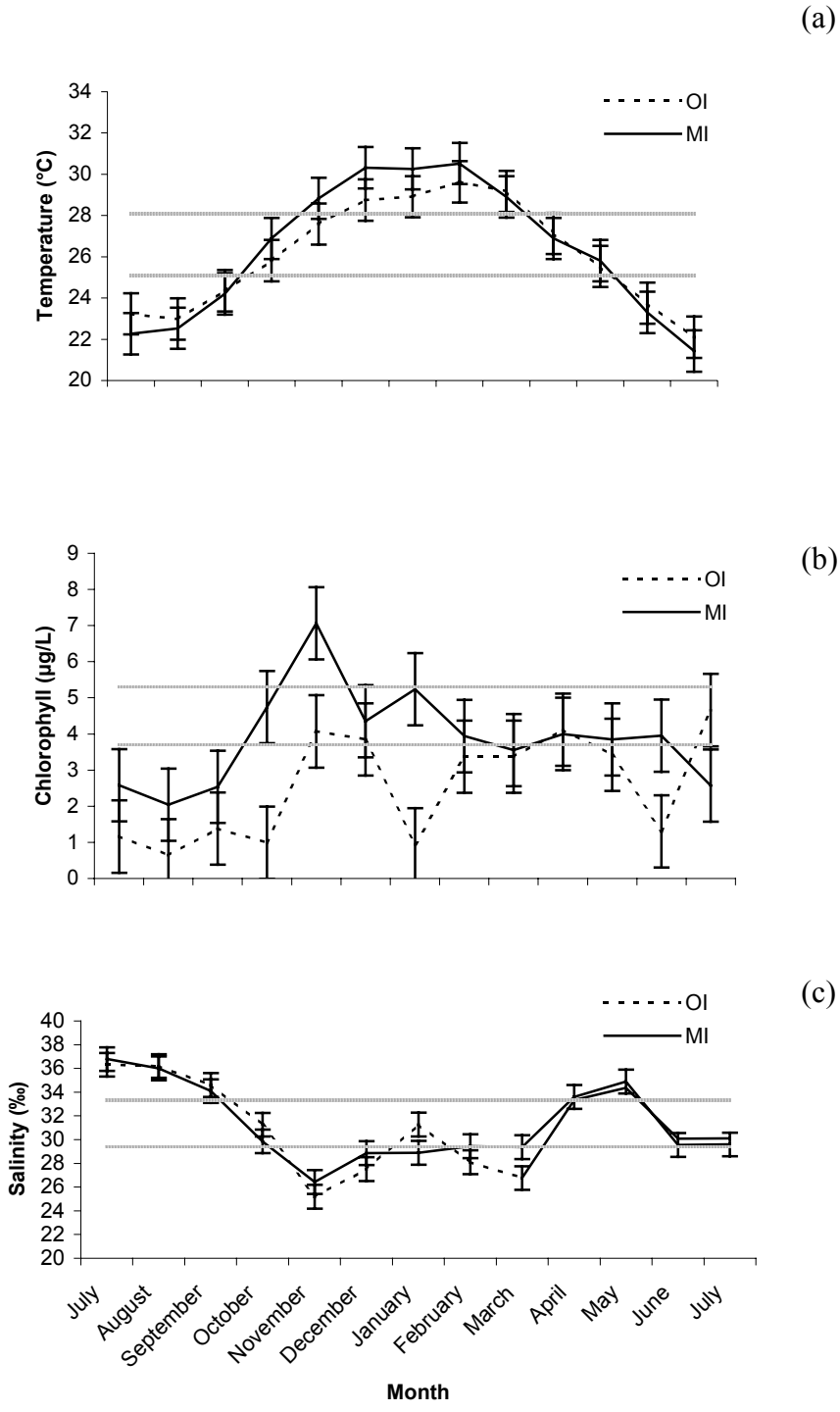


Fig. 7.1. Changes in mean (±SE) annual water quality parameters: (a) water temperature; (b) chlorophyll 'a'; and (c) salinity, at Orpheus Island (OI) and Magnetic

Island (MI). Error bars are 95% confidence limits, dashed horizontal lines refer to optimal growth conditions (refer text).

Oyster measurements at Orpheus Island and Magnetic Island occurred during September, November, December and February and at completion of the experiment in July. In order to determine if and what factor was driving growth of pearl oysters at the two sites, further characterisation of water quality data was required to identify water quality patterns between successive oyster measurements (Fig. 7.2). Water temperature increased from a mean of 23.1 ± 0.01°C at Orpheus Island and 22.5 ± 0.01°C at Magnetic Island during July-August through to a maximum in December-January (Orpheus Island, 30.0 ± 0.02°C and Magnetic Island, 30.3 ± 0.02°C) and then decreased again in February-July (Fig. 7.2a). The water temperature during February-July was relatively high, but this was primarily influenced by high water temperature during February and March and to some extent April (Fig. 7.2a).

Mean chlorophyll 'a' levels were higher at Magnetic Island compared to Orpheus Island (Fig. 7.2b). At both sites, chlorophyll 'a' levels were low in July-August compared to a peak in November (Orpheus, 3.96 ± 0.2 $\mu g.l^{-1}$; Magnetic Island 7.06 ± 0.29 $\mu g.l^{-1}$) before decreasing during February-July.

At the beginning of the experiment, salinity was similar to oceanic conditions (36‰) at both sites measuring 36.3‰ and decreased to a low of approximately 26‰ in November before increasing to approximately 31‰ in February-July (Fig. 7.2c). The salinity for Orpheus Island during February-July was estimated through simple regression analysis. Principal component analysis separated the two sites with chlorophyll 'a' being the major environmental parameter influencing the difference between sites. Although only slightly different chlorophyll 'a' levels at Magnetic

Island were higher than those at Orpheus Island (Fig. 7.3). Salinity and water temperature do not appear to have any significant site influence (Fig. 7.3), and this assumption is supported by general temporal changes (Fig. 7.2a, c).

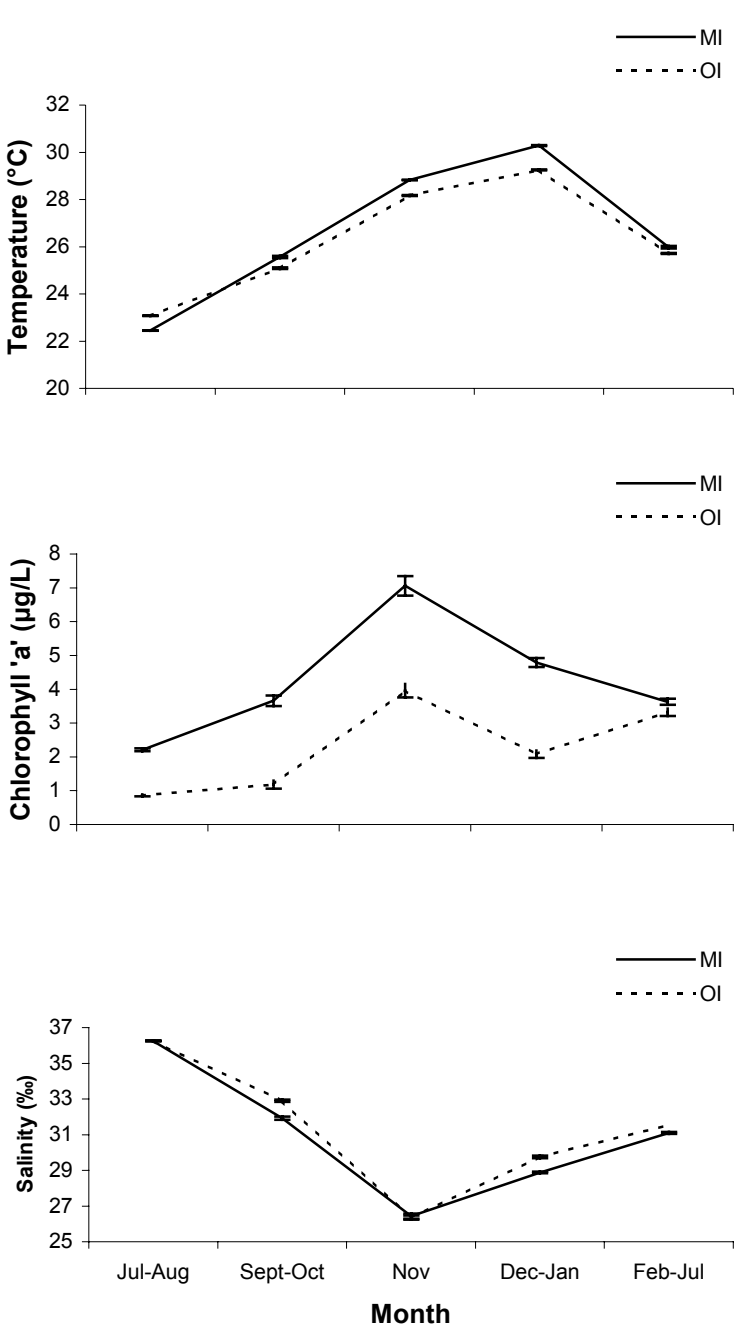


Fig. 7.2. Changes in mean (±SE) water quality parameters of: (a) water temperature; (b) chlorophyll 'a'; and (c) salinity, at Orpheus Island (OI) and Magnetic Island (MI) over successive measurement periods in 2001 and 2002 (Jul-Aug, July-August; Sept-Oct, September-October; Nov, November; Dec-Jan, December-January; Feb-Jul, February-July).

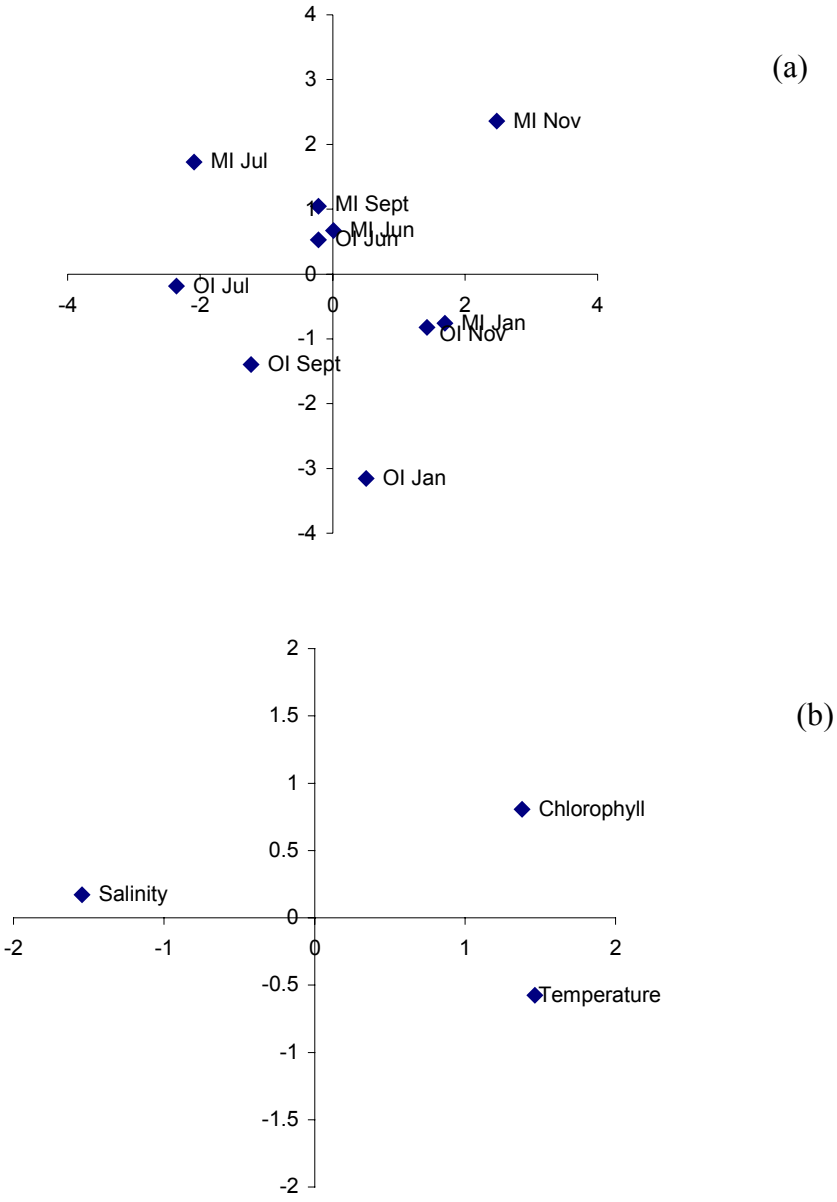


Fig. 7.3. Principal component analysis of (a) water quality parameters at Orpheus Island (OI) and Magnetic Island (MI) during different seasons (Jul: July-August; Sept: September-October; Nov: November; Jan: December-January and Jun: February-June) and (b) environmental vectors driving the processes.

Growth of oysters in terms of increases in DVH was fitted to VBGM (Table 7.1; Fig. 7.4). ARSS showed that growth models were not significantly different ($F_{3,894}$=1.21, p>0.05). Oysters cultured at Magnetic Island had a greater theoretical maximum shell height (H_∞), and overall growth performance (Φ') of 87.8 mm and 3.82, respectively, which is greater than oysters cultured at Orpheus Island (H_∞=79.6 mm, Φ' =3.81). Furthermore, the K value (or the rate at which maximum growth is approached) for Magnetic Island (0.86) was lower than the K value for Orpheus Island (1.02).

DVH of *P. fucata* increased from a mean (± SE, *n*=100) of 30.4 ± 0.5 mm to 62.1 ± 0.5 and 62.8 ± 0.5 mm at Orpheus Island and Magnetic Island, respectively, during this study, and final size after 12 months was not significantly different (Table 7.2; t=-0.996, df=198, p>0.05). No significant differences were observed between oysters cultured at Orpheus Island and Magnetic Island in terms of mean APM (Table 7.2; t=0.895, df=198, p>0.05), mean shell width (Table 7.2; t=7.553, df=198, p>0.05) or mean wet weight (Table 7.2; t=1.299, df=125, p>0.05) after 12 months of culture at both sites.

Growth rates of *P. fucata* at both sites were similar and mean daily growth rates based on changes in DVH ranged from a mean minimum of 37 μm day^{-1} to a mean maximum of 99 μm day^{-1}. Daily growth rates could be calculated because the time interval between successive oyster measurements was known. Additionally, mean values for water quality parameters were calculated between successive oysters measurements. Regression equations were fitted to daily growth rates and individual water quality parameters to identify optimal levels of each parameter for growth (Fig. 7.5). To determine the optimal range of each water quality parameter, an arbitrary

value of 80% of maximum observed daily growth during this study (99 μm day^{-1}) was selected. Water parameter levels were considered optimal if growth rate was greater than 79.2 μm day^{-1} (80% of maximum). Based on regression analysis, the optimal conditions for *P. fucata* growth in north Queensland are:

(1) a water temperature range of 25.1-28.1°C;

(2) a chlorophyll 'a' range of 3.7-5.3 μg L^{-1}; and

(3) a salinity range of 29.4-33.3‰ (Figs. 7.1 and 7.5).

Table 7.1. Growth characteristics of *Pinctada fucata* cultured at Orpheus Island and Magnetic Island for 12 months as determined by the von Bertalanffy growth model (VBGM).

	VBGM Growth Parameter			
Site	H_∞	K	t_o	Φ'
Orpheus Island	79.6	1.02	0.05	3.81
Magnetic Island	87.7	0.86	0.04	3.82

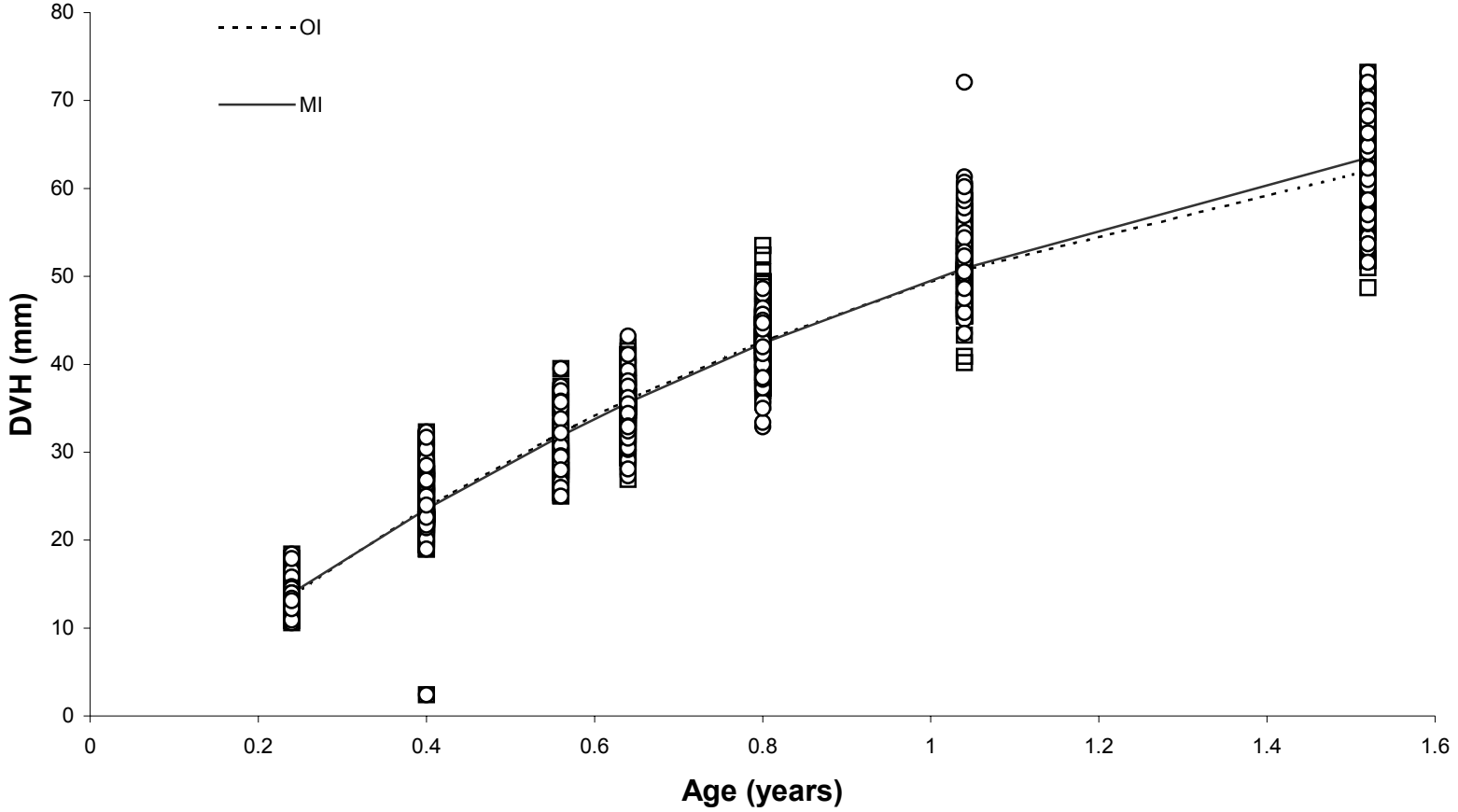
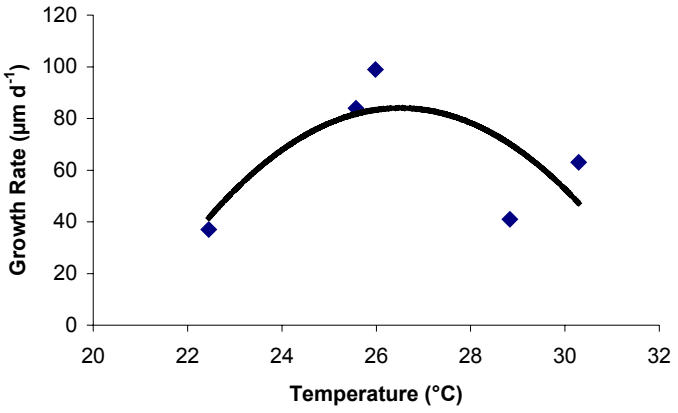


Fig. 7.4. Growth of *Pinctada fucata* cultured in pearl nets at Orpheus Island and Magnetic Island for 12 months.

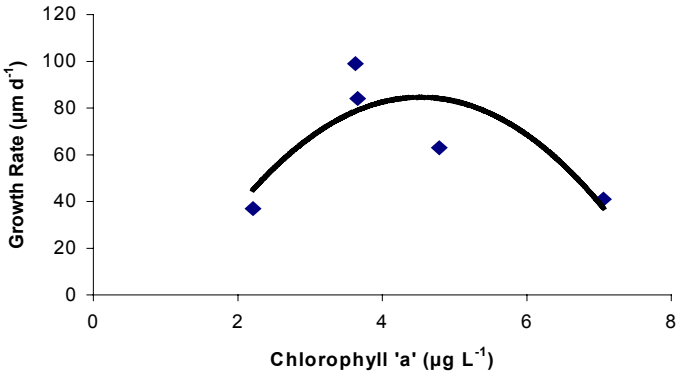
Table 7.2. Changes in *Pinctada fucata* shell parameters (DVH, APM, shell width and wet weight) at Orpheus Island and Magnetic Island between July 2001 and July 2002. Values represent means ± SE (*n*=100). Means with the same subscript are not significantly different between sites (P>0.05).

Growth Measure	Island	Start	End (12 months)
DVH (mm)	Orpheus	30.4 ± 0.5^{a}	62.1 ± 0.5^{m}
	Magnetic	30.4 ± 0.5^{a}	62.8 ± 0.5^{m}
APM (mm)	Orpheus	28.5 ± 0.4^{b}	58.3 ± 0.5^{n}
	Magnetic	28.5 ± 0.4^{b}	57.9 ± 0.6^{n}
Shell Width (mm)	Orpheus	9.4 ± 0.2^{c}	20.1 ± 0.2^{o}
	Magnetic	9.4 ± 0.2^{c}	20.7 ± 0.4^{o}
Wet Weight (g)	Orpheus	3.5 ± 0.2^{d}	27.9 ± 0.6^{p}
	Magnetic	3.5 ± 0.2^{d}	26.1 ± 1.2^{p}

(a)


Growth Rate (µm d-1)
120
100
80
60
40
20
0
20
22
24
26
28
30
32
Temperature (°C)

(b)


Growth Rate (µm d-1)
120
100
80
60
40
20
0
0
2
4
6
8
Chlorophyll 'a' (µg L-1)

(c)

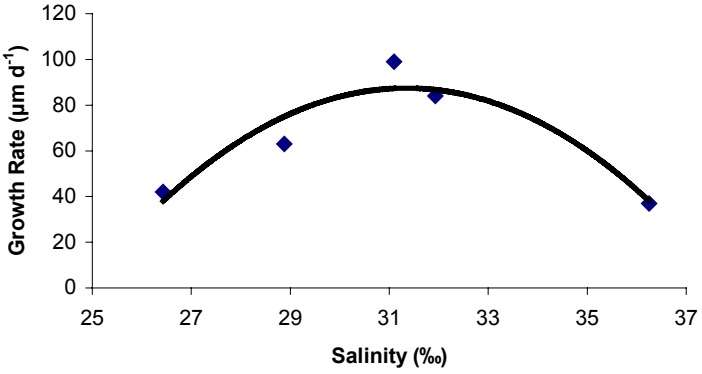

Growth Rate (µm d-1)
120
100
80
60
40
20
0
25
27
29
31
33
35
37
Salinity (‰)

Fig. 7.5. Relationship between combined (Orpheus Island and Magnetic Island) water quality parameters of: (a) water temperature; (b) chlorophyll 'a'; and (c) salinity, against daily growth rate of *Pinctada fucata*. Lines represent 'best fit'.

7.4 Discussion

Growth of oysters during this study occurred throughout the range of water temperatures experienced during the study (21-32°C). However, optimal growth of *P. fucata* in north Queensland occurred within a water temperature range of 25.1-28.1°C. These conditions occurred for 4 months at Orpheus Island and for 3 months at Magnetic Island during the 12-month study. This is similar, but slightly higher, to the optimal water temperature range for maximum growth of *P. fucata* in India, which has been reported as 23-27°C (Anon 1991). Schone *et al.* (2003) noted that the water temperature ranges supporting optimal growth of the bivalve, *Phacosoma japonicum*, (24.6-27.2°C) is very narrow; although, water temperature ranges in which growth occurs are relatively broad. If water temperatures fall below or rise above critical limits, then growth ceases. *P. martensii* (=*P. fucata*) has been shown to cease growth in Japan when water temperature falls below 13°C (Kobayashi and Tobata 1949, in Tomaru *et al.* 2002a). Numaguchi (1994a) showed that 28-30°C is the critical upper water temperature limit at which physiological condition is lowered in *P. fucata* in Japan.

In the present study the critical low water temperature limit (13°C) reported by (Kobayashi and Tobata 1949, in Tomaru *et al.* 2002a) was not encountered, while the critical upper water temperature limit (28-30°C), as reported by (Numaguchi 1994a), was reached. However, these limits are based on results obtained for this species sub-tropical

conditions in Japan. It is inappropriate to speculate too heavily on their implication for *P. fucata* originating from the tropics. It would perhaps be reasonable to expect lower and upper water temperature limits in the present study to be higher than those experienced in sub-tropical Japan; however, critical limits were not investigated during this study. Schone *et al*. (2003) noted that bivalve growth may cease at optimal water temperatures if no food is available, while MacDonald and Thompson (1986) reported that sea scallop, *Placopecten magellanicus*, growth may be independent of water temperature if sufficient food is available.

P. martensii (=*P. fucata*) has been shown to increase filtration rate with increasing water temperature between 13-22°C with maximum filtration rate occurring at 22-28°C and decreasing substantially at water temperatures > 28°C (Numaguchi 1994b). This may explain the results obtained in this study in which incremental growth, while positive at all water temperatures, was slightly lower at water temperatures greater than 28°C. However, these water temperatures occurred during times that are typical of reproduction for Akoya oysters in north Queensland (Tranter 1958d) and therefore caution must be taken when relating decreased growth to elevated water temperatures as oysters may have been partitioning more energy into gametogenesis rather than somatic growth. Lodeiros *et al.* (2002) noted a decrease in growth of *P. imbricata* (=*P. fucata*) during gametogenesis, while Mendoza *et al.* (2003) reported similar findings for scallops, *Lyropecten nodosus*.

Optimal growth of *P. fucata* during this study occurred at chlorophyll 'a' levels of 3.7-5.3 μg L^{-1}. These conditions occurred over 4- and 7-month periods during the study at Orpheus and Magnetic Islands, respectively. It is interesting to note that oysters

cultured at Magnetic Island experience almost twice the length of time of optimal chlorophyll 'a' levels compared to oysters at Orpheus Island, yet growth of oysters at the two sites was similar after 12 months. Growth rate based on increases in wet weight ranged from a daily mean minimum of 140 mg d^{-1} to a mean maximum of 210 mg d^{-1} at chlorophyll 'a' levels of 0.95 – 7.06 μg L^{-1}. Growth rates of 1-year-old *P. martensi* (=*P. fucata*) in Japan of 157 and 138 mg d^{-1} (wet weight) were recorded in separate experiments with chlorophyll 'a' levels of 2.5 and 3.1 μg L^{-1}, respectively, which are below the optimum ranges found during this study (Numaguchi 1994a). In a recent study at Orpheus Island with *P. margaritifera*, Southgate and Beer (2000) recorded growth rates of 27.7-164.4 mg d^{-1}, but did not record chlorophyll data.

There were no observed differences in growth of oysters cultured for 12 months at Orpheus and Magnetic Islands although chlorophyll 'a' levels at Magnetic Island were higher than chlorophyll 'a' levels at Orpheus Island. While Kuwatani (1964) suggested that chlorophyll 'a' information alone would be sufficient to determine oyster growth, Cranford *et al.* (1998) suggested that to effectively predict and manage bivalve growth, it is essential to have an understanding of the phytoplankton community available to cultured bivalves. It has also been suggested that phytoplankton quality and to a certain extent particle size are more important than quantity, as quantity alone is not a good indication of food availability (Tomaru 2002b; Schone 2003). Fukushima (1970) for example, noted that when a phytoplankton community was dominated by the bacillariophyceae, *Nitzschia*, mortalities of *P. fucata* increased; however, when dominated by *Chaetoceros*, *Thalassionema*, *Bacteriastrum*, and *Rhizosolenia*, mortalities of oysters did not occur. This is further supported by the findings of Tomaru *et al.* (2001)

who recorded mass mortalities of pearl oysters due to algal blooms of *Nitzschia* spp., which are inedible for pearl oysters. Additionally *Nitzschia closterium* has been shown to be unfavourable in the culture of *P. fucata* spat (Numaguchi 2000). Furthermore, Lodeiros *et al.* (2002) reported higher growth of pearl oysters *P. imbricata* (=*P. fucata*) when cultured in suspension compared to bottom culture even though chlorophyll 'a' level was greater at bottom culture areas; they suggested that chlorophyll quality is more important than chlorophyll quantity.

Numaguchi (1994a) noted that during phytoplankton algae blooms, or red tides when chlorophyll levels are high (30-40 μg L^{-1}), that *P. martensii* (=*P. fucata*) reduced filtration rates compared to times with low chlorophyll levels. This was further supported by the results of Tomaru *et al.* (2002c) who noted that under conditions of high food levels, respiration and absorption efficiencies decrease in *P. martensii* (=*P. fucata*), suggesting that growth in eutrophic or turbid environments may be less than optimal. Therefore information relating to the phytoplankton community as well as information on ingestion and digestion rates of food particles by *P. fucata* is required to allow the selection of appropriate sites for the establishment of an Akoya pearl oyster industry in Queensland.

Optimal growth of *P. fucata* during this study occurred at a salinity range of 29-33‰, however, oysters encountered salinities from a mean minimum of 25.2‰ to a mean maximum of 36.8‰. Numaguchi and Tanaka (1986) reported optimal salinity ranges for *P. fucata martensi* (=*P. fucata*) in the range of 22.7-37.9‰, while Victor (1983) suggested that *P. fucata* are able to tolerate salinities of 15.5-37‰. However, Dholakia *et al.* (1997) reported that *P. fucata* are able to tolerate a salinity range of

12-70‰, and are able to survive quick salinity decreases and increases of 16‰ and 18‰, respectively, as long as these conditions are not sustained (no time frame was given). In a recent study, Taylor *et al.* (2004) investigated the effect of salinity on growth and survival of *P. maxima* juveniles and determined that while survival was not affected at a salinity range of 25-45‰, growth was suppressed at salinities of 20‰ and >40‰. The optimal salinity range for *P. maxima* juveniles during nursery culture has been estimated at 30-34‰, which is very similar to that experienced by *P. fucata* in the current study (29-33‰).

Although the maximum theoretical shell heights for *P. fucata* are less than 90 mm in the present study, oysters measuring greater than 110 mm in shell height have been recorded at both sites. Urban (2000b) suggested that if growth data are not collected from the oysters whole growth range, that H_∞ (L_∞) can be under- or over-estimated. This may explain the underestimation of oyster shell height in the current study as oyster growth was only monitored for 12 months. Oysters cultured at Magnetic Island had a higher H_∞ and overall growth performance (Φ') value (87.8 mm and 3.82, respectively), than oysters cultured at Orpheus Island (H_∞=79.6 mm, Φ'=3.81); however, results show that oyster growth and final size are not significantly different between the two sites. These results are very similar to those recorded by O'Connor *et al.* (2003) who found that *P. imbricata* (=*P. fucata*) in temperate New South Wales have an overall growth performance (Φ') of 3.7-3.96, however, they used a H_∞ of 92 mm based on observed broodstock sizes, which are slightly smaller than broodstock observed in tropical Queensland which achieve shell heights of greater than 110 mm. Growth rates in terms of increases in DVH of *P. fucata* recorded during this study are very promising and

encouraging as they exceed general growth performances when compared to other studies with *P. fucata* (Table 7.3). This further supports the possibility of successfully establishing an Akoya pearl oyster industry in Queensland.

Table 7.3. Reported growth performance of Akoya pearl oysters (=*P. fucata*).

Oyster	Location	VBGM Growth parameter		Reference
		K	Φ'[2]	
P. fucata	Magnetic Island	0.86	3.82	Present Study
	Orpheus Island	1.02	3.81	Present Study
P. fucata	India	0.91	3.75	Chellam (1988)
P. imbricata[1]	Port Stephens (New South Wales)	0.55-1.0	3.7-3.96	O'Connor *et al.* (2003)
P. imbricata[1]	Columbia	0.63-0.94	3.64-3.82	Urban (2000a)
P. fucata	India	1.04-1.15	3.54-3.69	Velayudhan *et al.* (1996)

[1] (=*P. fucata*)
[2] Ranked based on minimum Φ' values

While *in situ* studies can be very complex to ensure that all parameters have been taken into account, they are often more beneficial than *in vivo* studies where individual parameters are isolated due to the synergistic effects of all environmental parameters. This study has identified that both Orpheus Island and Magnetic Island could be used for the culture of *P. fucata*. While annual mean water temperatures and salinities were similar at both sites, chlorophyll 'a' was shown to vary between sites. Further research is

required to identify what component of the phytoplankton community is utilised by *P. fucata* for nutrition and how much of the non-phytoplankton (other organic matter) is utilised for growth. Additionally, research to determine whether decreased growth rate of oysters at water temperatures greater than 28°C is a result of gametogenesis or due to changes in metabolic rate.

Research also needs to be conducted on the quality of pearls produced by *P. fucata* at these locations. While maximising growth and survival to minimise the time required for oysters to reach pearl production size is a general goal, the overall objective of pearl oyster industries is pearl production. While fast growth of pearl oysters is desired, if nacre quality and resulting pearl quality is poor, then pearl oyster culture is simply not feasible. Anecdotal evidence suggests that pearl quality in turbid in-shore waters is superior to that of pearls produced in non-turbid off-shore waters. However, Tomaru *et al.* (2000c) suggested that oyster growth in turbid waters would be less than optimal. While this study showed no significant differences in growth of *P. fucata* over 12 months from in-shore and mid-shore locations, it is vital that research is conducted on the quality of pearls produced at both these locations before commercial culture of Akoya oysters is initiated.

The results of this study showed that spatially separate sites did not result in significant differences in growth of *P. fucata*, while maximum growth is achieved within the following parameter ranges:

- a water temperature range of 25.1-28.1°C;
- a chlorophyll 'a' range of 3.7-5.3 $\mu g\ L^{-1}$;
- a salinity range of 29-33‰.

Chapter 8 General Discussion

8.1 Introduction

Akoya pearl oysters have been artificially cultured since the early 19th century and Japan has dominated the pearl industry since. In the early 1990's, pearl production in Japan drastically decreased resulting in a reduction of Akoya pearls on the world market and presenting an opportunity for a number of countries to enter the Akoya pearl industry. Australia initially received a lot of interest because: (1) it has a reputation for producing high quality cultured pearls and (2) Akoya pearl oysters are distributed along the majority of its vast coastline. However, there was a need for biological information on which the feasibility of Akoya pearl oyster culture in Australia could be assessed. In the mid 1990's a research project was established in New South Wales to assess the potential of an Akoya pearl oyster industry in temperate Australia (O'Connor *et al.* 2003). The project in NSW investigated growth, survival and nacre deposition of Akoya pearl oysters and resulted in a trial pearl harvest in 2003 (O'Connor, W.A. NSW Fisheries, pers. comm.). Initial results from this project regarding growth and survival of Akoya pearl oysters were comparable to those in more traditional culture regions for this species (i.e. SE Asia).

The major pearling activities within Australia occur in the tropical waters of Western Australia, Northern Territory and Queensland. As previously mentioned, Akoya pearl oysters occupy a vast amount of the Australian coastline; however, prior to this project there was no information on the culture of Akoya oysters in tropical Australia.

The major objective of the current project was to generate information on which the feasibility of Aoya pearl oyster culture in north Queensland could be assessed. The results of this study will compliment the results of research with similar goals conducted in temperate Australia (O'Connor *et al.* 2003). This Chapter highlights the major outcomes of this project and the results are summarised schematically in Table 8.1.

8.2 Hatchery Production

This project investigated aspects of hatchery production of *P. fucata* including embryonic and larval development, to identify the optimal protocols for hatchery culture of Akoya pearl oysters (Chapters 4 and 5). Biological information generated will provide information which is helpful in the facilitation and establishment of an Akoya pearl oyster hatchery in Queensland which could potentially lead to a new industry.

Results generated from this project showed that maximum development of *P. fucata* embryos into D-stage veligers occurs within a water temperature range of 26-28°C and a salinity range of 28-32‰. These results have obvious implications for the selection of sites for an Akoya oyster hatchery in Queensland. Ideally, a site should be selected in which water parameters are within the above-mentioned ranges. Further results from this study suggest that antibiotics are not required during embryonic development of *P. fucata* as development of larvae was not improved when antibiotics were added to culture water. Use of antibiotics would ultimately depend on individual hatchery hygiene practices and the protocols used for spawning and during hatchery production. However, the recent introduction and use of probiotics has decreased the

need for antibiotics within hatcheries (Riquelme *et al.* 2001), although research on the use of probiotics with pearl oysters has not been conducted.

Table 8.1. Schematic overview of the results and impacts of this study.

Stages of Culture	Major Findings/Recommendations
Hatchery	
Embryonic Development	Culture embryos at a temperature of 26-28°C and a salinity of 28-32‰. Embryos should be cultured at a density of 10-30 embryo's mL^{-1} while the addition of antibiotics is not required.
Larval Development	Larvae should be cultured at densities of: (1) 1-5 larvae mL^{-1} at 2-11 days of age; (2) 1-2 larvae mL^{-1} at 11-20 days of age; and (3) 0.1-0.2 larvae mL^{-1} at 20-43 days of age.

Nursery Early nursery culture	At transfer from hatchery to nursery, place spat at a depth of 2 m. 'Runts' or small oysters should not be discarded.
Late nursery culture	Oysters should be cultured at a density of 25-30% of total available space. Oysters should be cultured in pearl nets or in pearl nets with 'noodles'. Oysters can be left for up to 8 weeks before cleaning and net changes. Oysters should be cultured at 25-28 °C, 29-33‰ and 3.5-5.3 μg L^{-1} chl'a'.

Results have also shown that maximum development of embryos into D-stage veligers occurred when larval stocking densities were low. This suggests an ideal stocking density is strongly dependant on the individual hatchery and the production goals. For example, if a hatchery aims to produce 1 000 000 competent settling larvae (day 20) then, based on the results from this study, they may achieve this by stocking three 500 L tanks at a density of 1 larvae mL^{-1} (requiring in 74% survival) or one 500 L tank at a density of 5 larvae mL^{-1} (requiring in 44% survival). While both scenarios would produce approximately 1 000 000 larvae (based on the results of this study), ultimately the choice culture strategy will depend on the size of the hatchery, the available tank space and the available labour. For example, if the number of tanks within

the hatchery is increased, there will be a requirement for additional labour which will elevate overall hatchery costs.

While the above information indicates that hatchery conditions require to be within the optimal range for oysters in order to maximise larval development, there are a number of other factors which also must be considered in site selection (section 8.4). However, with the advancements of techniques such as remote setting for bivalves (Gosling 2003), whereby larvae are transferred from a land-based hatchery site to a distant ocean-based nursery site, the importance of finding a site which is suitable for both hatchery and nursery culture is perhaps less important. Further research needs to be conducted in this area for pearl oysters. In Western Australia, a number of pearl farms use a common hatchery to minimise costs. A similar method could be employed in Queensland, whereby one hatchery may supply numerous pearl farms with Akoya pearl oyster spat.

8.3 Nursery Culture

A number of pearl oyster culture techniques were investigated during this project to optimise nursery culture of Akoya pearl oysters under Queensland (tropical) conditions (Chapter 6). Aspects of pearl oyster culture investigated during this study included the effects of depth, stocking density, culture apparatus and cleaning on the growth and survival of pearl oysters. A major objective in pearl oyster culture is to minimise the time required for oysters to reach a size at which they can be used for pearl production. However, unlike most other cultured bivalve industries (i.e. edible oyster and scallop industries) where market size is the end of production, pearl oysters are only part of the

way through the production cycle at this stage; they must be grown for a further 1.5-2 years for pearl production.

Current practice among many commercial pearl hatcheries/farms is to discard slow growing pearl oyster spat to minimise the time required for culture stock to reach pearl production size. However, results from this study and a similar study with *P. margaritifera* (Pit and Southgate 2003b), suggest that when oysters are first graded at 3.5 months of age (8 weeks after transfer to the ocean) slow growing oysters should not be discarded. This is because slow growers, when compared to 'normal' growers, only require an additional 2-4 months before reaching pearl production size. It is likely to be more cost effective to rear these slow growers, rather than producing extra larvae and spat in the hatchery or purchase them; however no specific data were collected to support this in the present study.

Growth of oysters during nursery culture can be affected by a number of factors including depth, stocking density, water-flow, food composition and abundance and fouling. While this study identified the effects of depth, stocking density and cleaning, it did not look at food composition and abundance or the effects of water-flow on pearl oyster growth and survival. The above factors have been shown to act synergistically. For example, shallow water has been suggested to improve growth of pearl oysters (Gervis and Sims 1992), which in part, is believed to result from increased food availability. However, such areas are also usually associated with high fouling assemblages (Urban 2000a). There is a trade-off therefore, between increased growth and increased cleaning frequencies (higher labour costs) to ensure fouling does not impede water-flow and subsequent food availability to the oysters. Lowering pearl oyster

culture units to greater depths can decrease fouling (Urban 2000a); however, higher growth rates are often sacrificed with increasing depth (Gervis and Sims 1992).

Culturing oysters at depth also often increases initial farm set-up costs associated with the resources required. As stated above, increased stocking density results in decreased growth and survival of Akoya pearl oysters. However, higher survival at a lower stocking density may produce lower numbers of oysters than systems with higher stocking densities resulting in lower survival. For example, in the present study during late nursery culture, oysters cultured at a stocking density of 20% of total available net area had survival of 90%, which was significantly greater than survival of oysters cultured at 40% of total available net area (75%). There is therefore a trade-off between greater numbers of oysters and a slower growth rate, which is observed at higher stocking densities as stocking oysters at twice the density (40%) did not result in twice as many mortalities. There are additional factors, however, aside from growth and survival, which can affect the pearl farm. As stated above decreasing stocking density increases growth rate; however, a decrease in stocking density would result in an increase in the number of pearl nets which require maintenance in the form of cleaning. Pearl farmers may therefore decide that the slightly slower growth rates are not worth the additional costs associated with cleaning extra nets. Further research would be required on a site specific and operational specific basis to determine the most economic culture protocol.

Growth performance (Φ') of *P. fucata* cultured during this project varied from 3.16-4.02 (Table 8.2). This variation was, however, recorded across a number of experiments. Aside from the culture unit experiment (section 6.3.4), where growth of oysters cultured in the 'box' treatment was very poor (Φ'=3.16), growth performance did

not vary greatly between treatments within experiments. However, growth performance of oysters in the present study include the highest ever recorded for Akoya pearl oysters (Chapter 7, Table 7.3). While these values, which are very encouraging, have been obtained under experimentation, the next step is to take all the results, combine them into a trial and investigate further areas such as pearl production. The high growth performances generated in both this study and the study of O'Connor *et al.* (2003) suggest that eastern Australia has a significant opportunity to establish Akoya pearl culture in both temperate and tropical waters. While information gathered on nursery culture of Akoya oysters will be invaluable in the establishment of an Akoya pearl oyster industry in Queensland, it must be used as a guide only. As individual sites vary considerably in biological and physical parameters (Southgate and Beer 2000), site-specific information is required (section 8.4).

Table 8.2. Growth performance characteristics from different experiments conducted during this study and analysed with von Bertalanffy growth models.

Chapter	Experiment	Growth Performance (Φ')
6	Stocking Density (6.3.2)	3.49-3.83
6	Runts (6.3.3)	3.93-4.02
6	Culture Apparatus (6.3.4)	3.16-3.77
7	Different Sites (7.1)	3.81-3.82

While emphasis with pearl oyster culture has been to minimise the time required for oysters to reach a size in which they can be used for pearl production, there may be

implications with subsequent pearl quality. The current industry standard is to harvest pearls during the cooler months or during periods of slow growth. This is because pearl nacre is deposited at a slower rate during cooler months. Slower nacre deposition is known to produce pearls with a higher lustre and subsequent higher value (Anon 1998). Discarding 'runt' or slow growing oysters may therefore result in the loss of oysters capable of producing high quality pearls. This hypothesis assumes that slow growing oysters also deposit nacre at a slower rate. Further research is required to determine whether slow growing oysters produce high quality pearls and, if they do, whether the increased value of the pearl produced outweighs the costs involved in rearing slow growers to pearl production size. While biological information gathered from this project are very encouraging with regard to establishing an Akoya pearl oyster industry in Queensland, further information is required in relation to pearl production and factors affecting pearl quality, before the feasibility of such an industry can be established.

8.4 Site Selection

Site selection for pearl oyster culture is important if growth and survival are to be maximised during hatchery and nursery culture. Akoya pearl oysters showed positive growth at all water temperatures experienced throughout this study; however, the range at which optimal growth occurred was within the range of 25.1-28.1°C. Using water temperature alone as a criteria for site selection, it appears possible to culture Akoya pearl oysters along the entire Queensland coast (Table 8.3). Mean water temperatures fall within optimal water temperature levels at Night Island (Cooktown) for a greater time frame compared to other sites in which water temperature data were available. Optimal

water temperature occurred for 6 months at Night Island (Cooktown), for 5 months at Thursday Island, Moore Reef (Cairns), Hay Point (Mackay) and Moreton Bay (Brisbane), 4 months at Halfway Island (Rockhampton) and at Orpheus Island (study site for this project), while Magnetic Island only experiences optimal water temperature levels for 3 months of the year. It should be noted, however, that there is a temperature gradient along the Queensland coastline with far northern sites (Cairns and north) having warmer water temperatures with a 5°C difference between minimum and maximum mean values, whereas, more southern sites (Mackay and south) are generally cooler with a difference between minimum and maximum mean values of approximately 10°C.

In regards to chlorophyll 'a' levels, data collected along the Great Barrier Reef during 1993-1996 (Steven *et al.* 1998) suggests that only Keppel Bay (Rockhampton) along with Orpheus Island and Magnetic Island (results from this project) experience chlorophyll levels suggested in the present study as promoting maximum growth of Akoya pearl oysters. However, site selection cannot be based purely on 'biological performance'. Factors such as availability of land and water resources, power, security and a readily available labour force must all be taken into consideration when selecting sites for culture of pearl oysters. Furthermore, when selecting sites it is also important to make the decision based on factors that affect pearl quality.

While a particular site may support good growth and survival of oysters, resulting pearls may be of poor quality. For example, there is anecdotal information that oysters cultured in areas of high water temperature produce yellow coloured nacre, which is an undesirable pearl colour. Yellow colouration has also been anecdotally suggested to occur in older shells, with a high proportion of *P. fucata* older than 7 years expressing

yellow colouration (Koichi Ohara pers. comm.). While yellow colouration was observed in some wild collected stocks during this project, it was decided to remove these individuals during spawning events. Less than 1% of subsequent hatchery-produced oysters had yellow coloured shell. This suggests that selection trials to minimise yellow coloured stocks is possible. Information on broodstock selection to improve nacre colour is scarce; however, nacre colour has been shown to be highly heritable (Wada and Komaru 1994) and further research is required in this area. Additionally, anecdotal information suggested that oysters cultured in turbid in-shore waters provide better quality pearls than oysters cultured in non-turbid off-shore waters. However, growth compared to oysters in these turbid waters has been shown to be less than growth of oysters in non-turbid waters (Tomaru *et al.* 2000c). This suggests, that pearl farms should perhaps utilise a number of sites to maximise the perceived advantages of each:

(1) a hatchery site with water quality parameters that maximise larval development;

(2) a site with calm conditions for early nursery culture;

(3) a site with high food availability to maximise growth for nursery and grow-out culture; and

(4) an area with high turbidity for pearl production.

The present study has provided information on general growth and survival of oysters at different sites, but no difference in shell growth was observed from an in-shore and a mid-shore site; there also did not appear to be any differences in nacre colour between oysters from both sites.

Further research is required to:

(1) produce pearls from Akoya oysters in tropical Australia and

(2) identify factors influencing nacre quality (i.e. turbid waters) for Akoya oysters in Queensland.

Table 8.3. Annual water temperatures and chlorophyll 'a' ranges for a number of sites along the Queensland coast.

Location	Water Temperature (°C)	Chlorophyll 'a' ($\mu g\ L^{-1}$)
Torres Strait	25.1-29.9[1]	
Night Island (Cooktown)	23.8-29.5[1]	
Lizard Island (Cooktown)		0.07-1.44[5]
Moore Reef (Cairns)	23.6-29.5[1]	0.03-0.45[5]
Orpheus Island (Townsville)	23.0-29.6[2]	0.95-4.66[2]
Magnetic Island (Townsville)	22.3-30.5[2]	2.04-7.06[2]
Hay Point (Mackay)	18.8-28.0[3]	
Halfway Island (Rockhampton)	19.6-29.1[1]	0.11-6.09[5]
Moreton Bay (Brisbane)	21.5-26.5[4]	

[1] Ray Berkelmans AIMS/CRC Reef, 2002 data-Unpublished
[2] Present study
[3] Ray Berkelmans AIMS/CRC Reef, 2000 data-Unpublished
[4] Queensland Department of Primary Industries-Unpublished
[5] Steven *et al.* (1998)

8.6 Future Directions

One area of future research which is required and has just recently started focuses on population genetics of Akoya pearl oysters. As already stated, there is much debate in regards to the commonly used 'Akoya' oyster which currently describes 5 species names (Chapter 1). Work has already begun investigating the relationship of different populations of Akoya oyster from a number of locations around the world (Dr B. Evans, James Cook University, pers. comm.). Information on different populations is extremely important, particularly if oyster farms wish to transfer stocks to different locations.

Despite their economic value, there has been surprisingly little interest in genetic improvement of pearl oysters in the form of ploidy manipulation, selective breeding and the use of cryopreservation (freezing gametes of improved lines). While information on the production of triploids is widely available for edible oysters (Nell 2002), there is limited information available for pearl oysters (e.g. Wada and Komuru 1994; O'Connor *et al.* 2003). O'Connor *et al.* (2003) reported on some early results of ploidy manipulation for Akoya pearl oysters in China. They noted that while replication was less than optimal, tetraploid embryos were produced, although, survival was low.

Induction of triploidy has been shown to produce sterile oysters. The benefit of this to the edible oyster industry is that triploid oysters grow larger than their diploid counterparts because energy utilised for gametogenesis in diploids is used for somatic growth in triploids, which reach market size at an earlier age. Similar benefits could be utilised in the pearl oyster industry, however, there are a number of other advantages that would arise from manipulating ploidy levels in pearl oysters. Seeding or operating on pearl oysters to produce pearls requires them to be spent (empty gonads). Prior to the

operation, oysters need to be induced to spawn or stressed to inhibit gametogenesis. Inducing pearl oysters to spawn or stressing them is time consuming, however, triploid or sterile animals would always be ready for operating. Current methods for the production of triploid oysters are not 100% guaranteed (Nell 2002). However if triploids are produced then it is possible to inhibit normal development to produce tetraploids in a similar way as triploids are produced from diploids (Nell 2002). The benefit of producing tetraploids is that, in theory, they can produce 100% triploid offspring when crossed with diploids.

This project has produced biological information which will provide a basis for the development of an Akoya pearl oyster industry in Queensland. Establishment of such an industry would compliment the current valuable pearl industry in Australia and bring significant economic benefits for Queensland. While information generated during this study has answered a number of questions in terms of 'biological performance' there is, however, a requirement for further research to appraise pearl production from Akoya oysters in Queensland and then determine factors influencing pearl quality. This information, as well as information on a comprehensive economical analysis, which is currently being developed by Queensland Department of Primary Industries (QDPI), is required to fully assess the feasibility of establishing an Akoya pearl industry in Queensland. The encouraging results generated from research conducted on Akoya pearl oysters in Australia in this study and in New South Wales (O'Connor *et al.* 2003) have already prompted the establishment of an Akoya pearl culture operation in Moreton Bay in southern Queensland. Interest in similar operations have also been vested in Hervey Bay (Qld) and a number of locations in New South Wales (Wayne O'Connor, NSW

Fisheries, pers. comm.). Western Australia and Victoria have also shown interest in developing pearling industries based on Akoya pearl oysters.

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