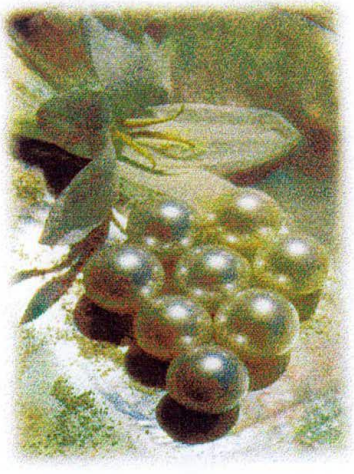
Juvenile Production and Culture of the Silver-lip Pearl Oyster, *Pinctada maxima* (Jameson)



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For the Degree of Doctor of Philosophy in the

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Frontispiece **View over a pearl farm near Bacan Island, Indonesia.**

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Abstract

The silver-lip (or gold-lip) pearl oyster, *Pinctada maxima* (Jameson) is the most highly prized of all pearl oysters. Through production of "south sea pearls", *P. maxima* is the basis of Australia's most lucrative aquaculture industry and is of great economic importance throughout south east Asia. In recent years there has been a rapid expansion in the cultivation of *P. maxima* throughout the region brought about by improvements in hatchery technology. Despite this, there is limited published information concerning rearing of *P. maxima*. This study aims to address this problem by establishing appropriate culture criteria for *P. maxima* in the specific areas of collection (settlement) and maintenance of spat in the hatchery, and the early nursery rearing and the grow-out of *P. maxima* at sea.

In the first of a series of settlement experiments, the influence of pediveliger stocking density on settlement and subsequent growth of spat was investigated. Pediveligers were placed in 15 L vessels at densities of 0.5, 1.0, 1.5 or 2.0 larvae per mL. The number of spat per 100 cm^2 in the 1.0 larva per mL treatment was over double, and significantly greater ($P< 0.05$) than that in the 0.5 larva per mL treatment. Increases in the number of spat were not significant ($P >0.05$) between larval densities of 1.0 and 1.5 larvae per mL and 1.5 and 2.0 larvae per mL. Stocking density did not significantly influence survival ($P>0.05$). By day 44, spat from the 0.5 larva per mL treatment were significantly larger ($P <0.05$) than spat from all other treatments and spat in the 1.0 larva

per mL treatment were significantly larger (P <0.05) than in the 1.5 or 2.0 larvae per mL treatments. Differences in size were most likely due to competition for space and food.

Further settlement experiments assessed artificial substrata for the collection of hatchery-reared *P. maxima*. Pediveliger larvae were settled onto collectors made from: curved PVC slats; polypropylene rope; a combination of PVC slats and polypropylene rope; and mono-filament nylon. Rope and the combined PVC slat and rope collectors had significantly more spat (P <0.05) than either nylon or PVC slat collectors. In a second experiment, significantly more spat (P <0.001) were counted on horizontally positioned PVC slat collectors than those vertically positioned. The concave surface of PVC slats had significantly more (P <0.001) spat than the convex surface, regardless of orientation. In a third experiment, significantly more (P <0.001) spat attached to PVC slats with an epifloral biofilm than clean PVC slats.

An adequate diet for settled spat is essential and seventy-five day-old *P. maxima* were fed for 21 days on the following monospecific micro-algal diets: *Isochrysis* aff. *galbana*, (T-ISO), *Pavlova lutheri*, *Chaetoceros muelleri*, *C. calcitrans*, and *Tetraselmis suecica*. The largest increase in ash free dry weight (AFDW) was for spat fed *C. muelleri*, which was significantly greater (P <0.05) than for any other species. The mean AFDW of spat fed *T. suecica* and T-ISO did not differ significantly from each other, but were significantly greater than for spat fed *C. calcitrans* and *P. lutheri* (P <0.05). The final AFDW of spat fed *P. lutheri* was not significantly different from that of unfed spat (P >0.05).

P. maxima that were ready for transfer to sea were re-settled onto PVC slats at a mean density of 340 per 100 cm^2 and either left exposed (control) or covered with a mesh sleeve of varying aperture sizes (0.75 mm, 1.5 mm or 3.0 mm) before being suspended from a raft. Two weeks later, there was no significant difference (P >0.05) among the number of spat retained on the covered slats; however, all covered slats had significantly greater (P <0.001) spat retention than controls. Spat were significantly larger (P <0.05) with each increase in mesh size. There was no advantage in using sleeves with a mesh size small enough to retain dislodged.

Spat require detaching from their point of attachment during grading. The following stress factors were tested as potential inducers of detachment in *P. maxima*: salinities of 45 $^{o}/_{oo}$, 40 $^{o}/_{oo}$, 30 $^{o}/_{oo}$ and 25 $^{o}/_{oo}$; pH of 10 and 4, and air exposure. High mortality (>80%) resulted from the use of pH 10 and it was abandoned after 1h. Hypersaline sea water (45 $^{o}/_{oo}$) resulted in significantly more spat detaching (92.3 ± 0.6 %, mean ± s.e., P<0.05) than in any other treatment. A pH of 4 resulted in 85.6 ± 2.3 % (mean ± s.e.) detaching after 1 h. Exposure to the treatments beyond 1 h, except in the case of exposure to air, did not yield significant increases (P> 0.05) in numbers of detached spat. Spat that had detached in the treatment baths after the first hour began to re-attach during the second hour. After 24 h exposure to treatments, excluding pH 10 and air exposure, spat had firmly re-attached with 100 % survival and no mortality was recorded 24 h after the spat were returned to normal sea water.

Byssus regeneration following detachment was studied in *P. maxima* of six different age classes. In the first experiment, 75 or 120 day old *P. maxima* were removed from their points of attachment by severing the byssus and byssal thread production and oyster behaviour were monitored for 120 hours. Younger juveniles re-attached faster than older juveniles, but older juveniles produced significantly more (P <0.001) byssal threads after 12 h and significantly more (P <0.001) byssal threads over the 120 h period. Byssus production for the younger juveniles did not increase significantly (P >0.05) after 48 h whereas byssus production from older animals continued to increase significantly (P <0.001). *P. maxima* were observed to eject the byssal apparatus, move and reattach within 24 h. Re-attachment following ejection of the byssus was faster than that following mechanical severing. In the second experiment, older *P. maxima* aged 7, 9, 11 or 13 months were placed in nets in strong (2.5-3.5 knots per h) or mild (<1 knot per h) current. Pearl oysters re-attached faster in the mild current. However, after 5 days, oysters aged 13 and 11 months in strong current had produced significantly more threads (P< 0.05) than oysters in mild current. This trend continued after day 5, but was not significant (P>0.05) for pearl oysters aged 9 and 7 months. By day 11, 9-month-old oysters had produced significantly more byssal threads than any other age class.

P. maxima are grown in tropical regions affected by monsoonal rains which can alter salinity for extended periods. Juvenile *P. maxima* were held over a period of 20 days in the following salinities: 45 $^{o}/_{oo}$, 40 $^{o}/_{oo}$, 34 $^{o}/_{oo}$ (ambient), 30 $^{o}/_{oo}$ and 25 $^{o}/_{oo}$. There was no significant difference (P> 0.05) in survival of spat from the different

treatments; however, growth was significantly depressed ($P< 0.05$) at 45 $^{o}/_{oo}$, 40 $^{o}/_{oo}$ and 25 $^{o}/_{oo}$. The best growth was recorded at 30 $^{o}/_{oo}$, where spat were significantly larger ($P< 0.05$) than those held at ambient salinity.

Several experiments investigated the effects of stocking density directly on growth and survival of juvenile *P. maxima*. Spat were held in suspended nursery culture for six weeks at four stocking densities: 10 juveniles per slat (133 juveniles per m^2); 50 juveniles per slat (670 juveniles per m^2); 100 juveniles per slat (1330 juveniles per m^2) and 150 juveniles per slat (2,000 juveniles per m^2). Best growth and survival was recorded at a stocking density of 10 juveniles per slat (80 ± 4.36%: mean ± s.e.), which was significantly higher than the other densities tested ($P < 0.05$). Survival did not differ significantly between the other densities tested ($P >0.05$). The incidence of growth deformities increased with increasing stocking density.

In a second experiment, juvenile growth was compared at two stocking densities (28 individuals per net: 66 oysters per m^2 or 48 individuals per net: 99 oysters per m^2) with animals held either in suspended or bottom culture. Mean (± s.e.) survival in 28-pocket nets in suspended culture (99.0 ± 1.6 %) was significantly better than any other treatment ($P <0.01$). Survival was also high in the 48-pocket nets in suspended culture (94.8 ± 3.6 %). Mean survival in bottom culture was significantly lower ($P <0.05$), being 15.8 ± 7.8 % and 13.3 ± 3.6 %, respectively, for 28 and 48-pocket nets. Oysters held in suspended culture grew significantly larger ($P <0.001$) than those in bottom culture. In both suspended and bottom culture, *P. maxima* in the 28-pocket nets grew significantly

larger (P <0.001) than those held in 48-pocket nets. The dry weight of suspended solids, and phytoplankton number and diversity were all greater in surface waters indicating greater food availability. In a third experiment, seven month-old *P. maxima* were graded into four size classes (G1 to G4: largest to smallest, respectively). Three replicates for each size class were stocked into 28-pocket nets and 8-pocket nets (19 pearl oysters per m^2). Dorso-ventral shell height (SH), SL and wet weight (WW) were measured monthly for five months. For G2 to G3 no differences in survival or growth were recorded during the experiment. Survival for G1 in 8-pocket panels was 100% and significantly better (P<0.01) than G1 in 28-pocket panels. Further, by the end of the second month, G1 in 8-pocket panels were significantly larger (P<0.001) than G1 in 28-pocket panels. This size advantage was maintained during the course of the experiment. The highest percentage of 'runts' resulted from G1 in 28-pocket panels.

Cleaning is the major activity on pearl farms. A comparison was made of the growth of one-year-old *P. maxima*, cleaned every 2, 4 or 8 weeks or after 16 weeks. The diversity of fouling animals was recorded and their dry weight (DW) estimated. The DW of fouling animals increased steadily over the first 10 weeks of the experiment before declining during weeks 10 to 16. Significant (P< 0.05) differences in the DW of fouling animals between treatments was observed and pearl oyster growth was affected by fouling. SH, SL and WW of pearl oysters cleaned every 2 or 4 weeks was significantly greater (P <0.05) than that of pearl oysters cleaned every 8 weeks or after 16 weeks.

Some pearl oysters that were left uncleaned for 8 or 16 weeks had shell deformities caused by *Pteria* spp. invading the shell margin.

The results of this study provide a guide to *P. maxima* culturists in a number of key areas. In the hatchery, a setting density of no more than 1.0 larva per mL is recommended whilst a spat density of 70 spat per 100 cm^2 gave best growth and survival after settlement. The choice of collector material, the surface orientation of collectors and method of collector preparation further influence settlement. Collectors made from old black polypropylene rope (readily available on pearl farms) resulted in greatest settlement. Safe detachment of *P. maxima* can be achieved through using a salinity of 45 $^{o}/_{oo}$ or reducing the pH to 4 and the diatom *Chaetoceros muelleri* is appropriate as the basis of a diet for settled *P. maxima* whilst still in the hatchery environment.

Spat transferred to the sea were adequately protected by 3.0 mm mesh. Smaller mesh sizes, while not affecting survival, fouled more rapidly and resulted in reduced spat growth. *P. maxima* spat are tolerant of a wide range of salinities. Moreover, reduced salinity may be beneficial to growth. Stocking density and culture system influence growth, survival and the incidence of growth deformity in *P. maxima*. Faster growing *P. maxima* should be separated and placed in larger pocket nets to optimize growth, survival and reduce the incidence of runts. Fouling can further influence the effects of stocking density. Rapidly growing pearl oysters should be cleaned monthly to maximize growth and reduce the risk of growth deformities. More regular cleaning, whilst having no

deleterious effects on pearl oyster growth or survival, appears to be unnecessary and may add to operational costs. These findings should assist *P. maxima* growers and provide a useful background for further research.

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Chapter 1. Introduction

1.1 A Brief History of Pearls and Pearling

The pearl is unique as the only gemstone produced by a living animal. The collection of pearls from pearl oysters is an ancient pastime. Pearls were highly valued in China, India, Persia, Arabia, Egypt and Greece and remain so today. It has even been suggested that the Romans invaded Britain in search of the fabled pearls to be found in fresh water mussels (Farn, 1986). Collecting mother-of-pearl (MOP) and pearls has been practiced around the globe for centuries. MOP ornaments dating back to 4,500 BC were discovered in the Bismaya ruins of ancient Persia (Shirai, 1994). The most important of the ancient pearling grounds were the Gulf of Mannar (India), Ceylon (now Sri Lanka), the Persian Gulf and the Red Sea. Pearls from these regions found their way to other nations via trade or conquest (Farn, 1986). In the 10th and 11th centuries, most fresh water pearls came from sources in the British Isles. Before the 12th century, these pearls were appreciated more for medicinal rather than ornamental value; however, in later centuries, pearls became very popular in jewelry throughout Britain and Europe.

Large scale exploitation of mother of pearl (MOP), particularly in Australia, began in the latter half of the 19th century with the development of diving apparatus (Saville-Kent, 1893; Farn, 1986). The first major pearling centre in Australia was Shark Bay, Western Australia. In 1850 the vessel "Pelsart" surveyed the bay and reported vast quantities of the pearl oyster *Pinctada albina*. Fishing for the oyster commenced the following year (Hancock, 1989). The later discovery of the larger silver-lip (or gold-lip) pearl oyster, *P. maxima* in Broome and Thursday Island marked the decline of the Shark Bay fishery. In the

late 1800s, Thursday Island and Broome dominated as global suppliers of MOP (Saville-Kent, 1893).

The Chinese are believed to be the first to have attempted to produce cultured pearls by placing nuclei prepared from dried mud into the shell cavity of freshwater mussels (Hollyer, 1984; Farn, 1986; Gervis and Sims, 1992). Images of the Buddha, usually cast from lead, were often used as nuclei (Farn, 1986). In 1761, Linnaeus claimed that he could produce pearls from freshwater mussels and offered to publish his method for a suitable reward from the state (Farn, 1986).

The name most closely associated with the development of cultured pearls and pearl farming is the legendary Koichi Mikimoto of Japan. A former vegetable vendor, he produced blister pearls in 1893 using a method he patented the same year. In 1908, Mikimoto obtained the first patent for spherical pearls (Farn, 1986). The reputed founder of this method was a friend of Mikimoto's, the dentist, Otokichi Kuwabara (Hollyer, 1984). The method was to wrap a mother-of-pearl sphere in the mantle tissue from a sacrificed oyster, tie it with a fine silk thread, and implant it into the soft tissue of the recipient. Earlier, in 1907, Tatsuhai Mise unsuccessfully applied for a patent for his technique to produce spherical pearls. At the same time, Dr. Nishikawa was also applying for a patent and, after a series of court battles, a joint patent was awarded (Hollyer, 1984). To further muddy the waters, it has been suggested that the eminent marine biologist, William Saville-Kent, pre-dated the Japanese by almost two decades with a series of successful experiments on round pearl cultivation in north Queensland, Australia, and that both Nishikawa and Mise received knowledge of the technique from Saville-Kent (George, 1994). Regardless of who was the first, Mikimoto quickly dominated the cultured pearl industry and is widely

regarded as the founding father (Hollyer, 1984; Farn, 1986; Gervis and Sims; 1992). Mikimoto gave up the technique he patented and, along with others in the industry, adopted the simpler technique of Mise/Nishakawa which is still used today (Farn, 1986).

The largest and most valuable of pearl oysters is the silver-lip (or gold-lip) pearl oyster, *P. maxima*, which produces the unique south-sea pearls which are often greater than 16 mm in diameter. These large pearls are the basis of a highly lucrative industry. Due to the high value of this oyster, research into its propagation has been a secretive and proprietorial activity. The detailed hatchery manual for *P. maxima* produced by Rose (1990) was, for some time, a restricted publication.

1.2 Pearling and *P. maxima*

Whilst the Australian industry is now based in Western Australia, it has its roots in north eastern Australia, particularly the area around Thursday Island. The average earnings of the Queensland pearl oyster fishery between 1884 and 1888 was £60,000, making it the sixth to eighth top earning primary product in the (then) colony (Saville-Kent, 1893). In later years, the importance of the industry was such that it was exempt from the "White Australia Policy" to allow the continued use of indentured labour from Asia (Ganter, 1994). Queensland (followed by Western Australian) MOP attracted the highest price worldwide (Saville-Kent, 1893). In 1893, the highest quality MOP was worth £177 per imperial ton.

Pearls were a by-product and something of a bonus to MOP collection (Franklin, 1973; Dybdahl and Rose, 1986; Farn, 1986). By the late 1880s a number of companies had moved their pearling fleets from north Queensland to the Kimberley region of Western Australia (Saville-Kent, 1893). By the beginning of the 20th century, some 300 luggers

were operating from Broome, which had already taken over from Thursday Island as the Australian center for pearl oyster fishing. Disruption to the industry during World War II, and the increased use of plastics in peacetime saw the demise of the MOP industry as a major export earner (Dybdahl and Rose, 1986).

Even though a viable technique for culturing pearls had been developed (Hollyer, 1984; Farn, 1986) and round pearls had been produced from *P. maxima* in Sulawesi (Celebes, Indonesia) in the early 1920s (Muller, 1997), pearling companies in Australia actively campaigned against such exploits. This eventually lead to a government ban on the development of pearl culturing techniques (George, 1994). It was not until 1956 that the first pearl culturing farm opened in Australia (Franklin, 1973). This Australian-Japanese joint venture, Pearls Propriety Limited, harvested the first Australian cultured round pearls in June 1958 (Muller, 1997).

During the late 1960s and early 1970s, the Queensland industry suffered a series of major setbacks. In 1969, the oil tanker "Oceanic Grandeur" ran aground spilling approximately 1.3 million L of crude oil near the main pearl oyster fishing grounds of the Torres Straits (Yamashita, 1986). Catastrophic mortality (80-100%) in fished pearl oysters immediately followed this incident. At the time of the accident, the Torres Strait cultured pearl industry was in peak production and valued at 4.5 million dollars per annum; by 1972 this figure had dropped to 3.5 million dollars per annum (Franklin, 1973). Between 1970 and 1976, recruitment in the fishing grounds was extremely poor. Seagrass beds degenerated and, by 1977, the pearl oyster stocks were totally depleted (Yamashita, 1986). Some regeneration of the fishing grounds has occurred since, but the numbers of mature pearl oysters suitable for culturing pearls remain low and Queensland farms still have great

difficulty in accessing healthy stock (Bruce Stevens, Reefarm, Qld., Australia, personal communication, 1996).

Also during the 1970s, the Western Australian industry began suffering mortality in fished oysters following translocation to pearl farms. By 1984, a number of pearling companies were unable to meet their annual quota (Dybdahl and Rose, 1986). *Vibrio harveii* was identified as the pathological agent following research into the cause of pearl oyster mortality (Dybdahl and Pass, 1985). Poor husbandry practices, particularly during transport, caused proliferation of this bacterium; when this was associated with low temperature, the pearl oysters became stressed and disease resulted.

A number of pearl farms on Thursday Island and in Western Australia, particularly those associated with the pioneering company Pearls Pty Ltd., ceased operations during the mid-1980s. Fortunately, changes in husbandry practices led to a turn around in the industry. In Western Australia, *P. maxima* are now fished only during particular times of the year. Translocation from off-shore holding sites to near-shore farm sites is done only when the temperatures of the two sites are similar. Pearl farms have moved away from high density culture systems such as rafts to lower density long-line systems and major improvements have been made in the handling and care of post operative pearl oysters (N. Crane, Dampier Pearling Co., W.A., Australia, personal communication, 1995).

The value of Australian pearls sold in 1995 was $ AUS 252.4 million (O'Sullivan and Kiley, 1996) making it by far the most valuable aquaculture industry in Australia. There are now 16 companies in Western Australia, 4 in the Northern Territory and several in Queensland. Production of cultured pearls from *P. maxima* is now also a major industry

in Indonesia and the Philippines. There are also *P. maxima* pearl farms in Brunei, Myanmar (Burma) China, Vietnam and Malaysia.

1.3 Taxonomy and species description

Phylum:	Mollusca
Class:	Bivalva
Subclass:	Pteriomorpha
Order:	Pterioida or Mytiloida
Family:	Pteriidae
Genus:	*Pinctada*
Species:	*maxima*

Common names Silver-lip Pearl Oyster, Gold-lip Pearl Oyster. Synonyms *Meleagrina margaritifera* (von Martens, 1889). *Meleagrina anomoides* (Melville and Standen, 1899) *Avicula* (*meleagrina*) *margaritifera* (Collett, 1900) *Pteria* (*margaritifera*) *maxima* (Jameson, 1901) *Meleagrina maxima* (Saville-Kent, 1905) *Pinctada maxima* (Hedly, 1916). Source, Gervis and Sims, 1992.

P. maxima is the largest species belonging to the genus *Pinctada*. Mature specimens may measure up to 305 mm in dorso-ventral shell height, with the valves weighing as much as 6.3 kg (Hynd, 1955). Adult *P. maxima* are generally a light brown colour to a darker chocolate. Unlike other pearl oysters, *P. maxima* has no radial markings on the outer shell. The nacreous layers of the shells are silver-white with a distinct silver or gold band at the border of the nacreous and prismatic layers of the shell; hence the common names of silver-

lip and gold-lip. The left valve is more convex than the right and *P. maxima* has no hinge teeth.

Juvenile *P. maxima* display a range of colour morphs (Fig 1.1) which include green, yellow, orange, brown, purple/black, grey, white and a variety of zig-zag patterns. The colour is generally lost after the first 12 to 18 months of life, although the juvenile colour may still be seen on the umbonal region of young adults (Fig 1.2). Juvenile *P. maxima* have very distinct growth processes, which are an aid to identifying this species. These growth processes, commonly referred to as "fingers", are convoluted and wider distally than proximally (Fig 1.1). As the pearl oyster grows, the growth processes become less prominent.

P. maxima occurs naturally in the central Indo-Pacific region from Burma to the Solomon Islands. The most prolific pearl oyster beds are found in the waters of northern Australia, the Philippines, Indonesia and Papua New Guinea (Gervis and Sims, 1992). The range extends north to Hainan off the coast of China to 25°S on the west coast of Australia and 20°S on the east coast (Gervis and Sims, 1992). In Australia there is noticeable genetic sub-division of *P. maxima* from Western Australia to Thursday Island in Queensland (Johnson and Joll, 1993). The Western Australian population has only slight genetic variation over great distances whereas, in the Northern Territory, significant genetic differences were found between populations that were only 320 km apart (Johnson and Joll, 1993).

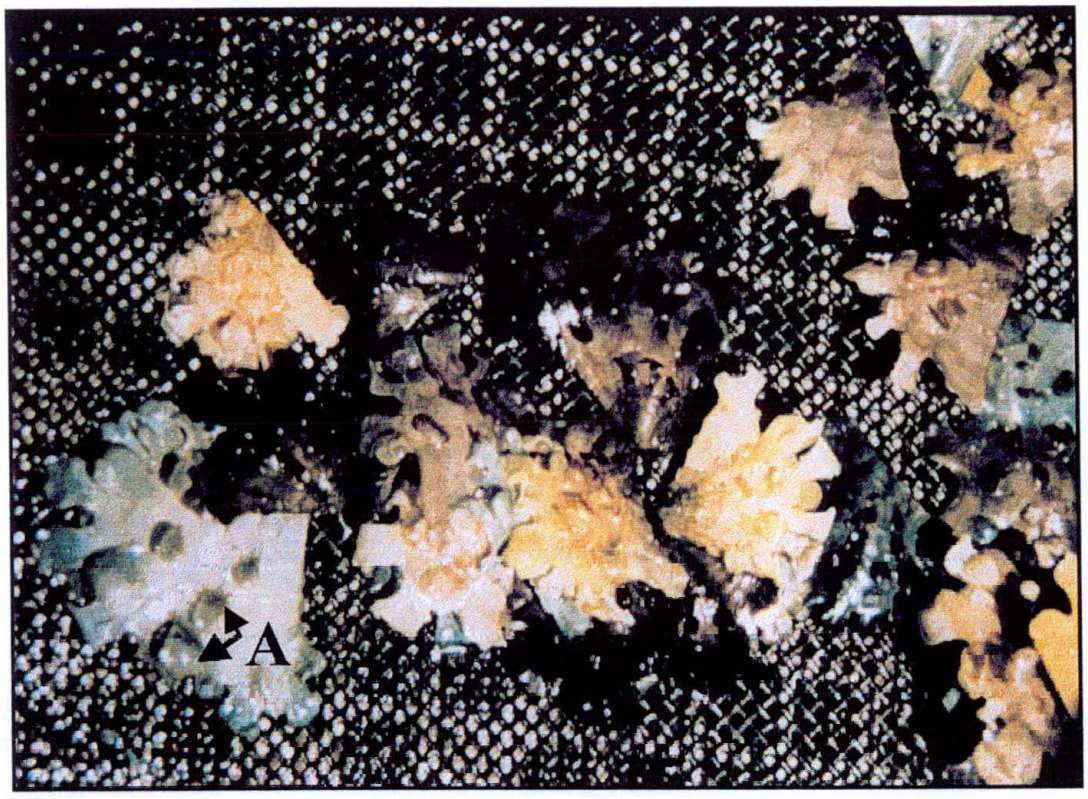


Fig. 1.1 **Juvenile *P. maxima* (3 months old, SHs range from 15 to 35 mm) showing some of the different colour morphs that may occur. Note the "finger-like" growth processes (A).**

Fig 1.2 ***P. maxima*** **(10 months old, SH = 86 mm) showing the colour change from distinct dark green near the umbonal region of the shell to a light brown closer to the growth margin. Note the abrupt change in pigmentation at the border of the two colours.**

1.4 Anatomy

1.4.1 Mantle and Shell

As in all molluscs, the shells of pearl oysters are produced by the mantle (Fig 1.3), the highly specialised organ that lines the inner layer of each valve. The mantle is also the pearl producing organ and, as a consequence, the mantle and pearl formation have been studied by numerous researchers (Tsujii, 1960; Dix, 1972 a,b; Jabbour-Zahab, et al., 1991; Garcia-Gasca et al., 1994; Awaji and Suzuki, 1995). There are three primary layers to the shells of pearl oysters: the periostacum, which forms the thin outer layer, the prismatic layer in the middle and the nacreous or mother-of-pearl layer. The nacre consists of overlapping layers of concholiolin and arogonite (calcium carbonate) which gives the mother-of-pearl its lustre (Hollyer, 1984; Farn, 1986). The periostracum is secreted by the outer margin of the mantle and, once laid down, does not increase in thickness. Similarly, the prismatic layer is secreted by the outer epidermis of the peripheral region of the mantle and is produced once only. The nacreous layer is produced by the pallial and central zones of the mantle and is laid down throughout the life of the pearl oyster (Gervis and Sims, 1992; Garcia-Gasca et al., 1994). If the shell of the pearl oyster is damaged, all three layers are reproduced in sequence. The same is true during pearl formation, the periostracum is laid down first, followed by the prismatic and nacreous layers, respectively. A fourth shell layer, the hypostracum, consists of columnar calcite crystals and is formed where the adductor muscles are attached (Farn, 1986).

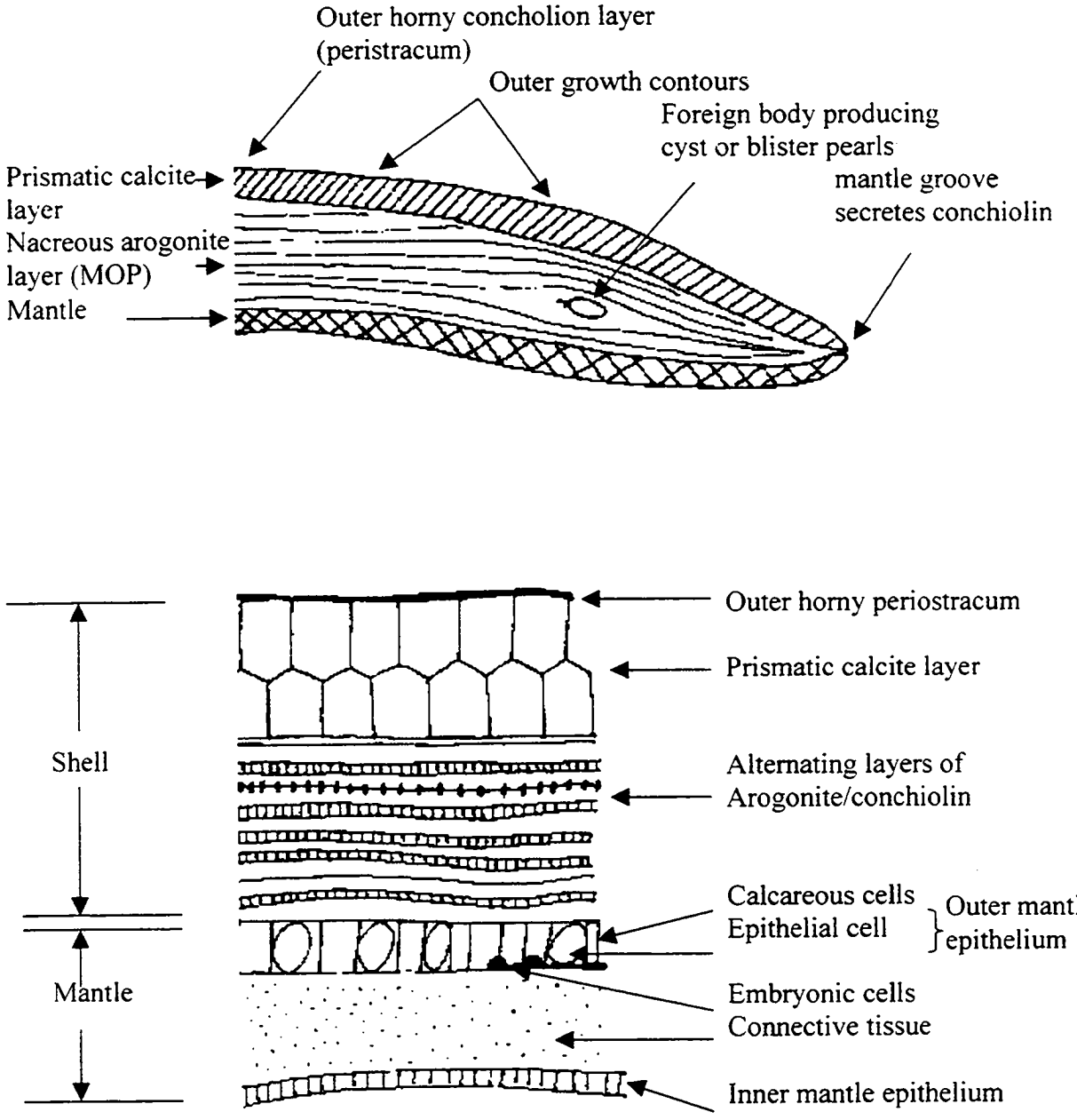


Fig. 1.3 Cross sections through the shell and mantle of a pearl oyster

(source: Farn, 1986)

The mantle has several types of secretory cells. In closely related *P. mazatlanica*, four different types of secretory cells were identified (Garcia-Gasca et al., 1994). In the mantle epithelium were large secretory cells containing carbohydrates, acid proteins, sulfated acid mucopolysaccharides and calcium granules. Found only in the middle mantle fold were small cells that secrete acid mucopolysaccharides. The peristracal groove and shell epithelium contained acidophilic secretory cells that take part in protein synthesis. The central zone had large acidophilic secretory cells associated with glycogen synthesis. Garcia-Gasca et al. (1994) related the specialised secretory cells of the outer mantle epithelium and the presence of alkaline phosphatase and carbonic anhydrase with calcium deposition and suggested that this epithelium is the most suitable as graft tissue in pearl culture.

The shells of pearl oysters have high tensile strength and are resistant to bending and fracture (Currey and Brear, 1984). The shell provides protection from predators and fouling organisms. Nacre production is particularly important in protecting the soft tissues of pearl oysters from intruding parasites or other foreign bodies. The first response of an oyster to a foreign irritant is to expel it. Failing this, the irritant is either immured to the inner shell wall forming a blister or it is enveloped in a cyst. In the case of a parasite, such as a worm, a depression forms on the surface of the mantle, which is eventually sealed over producing a sac of shell forming tissue. This sac, when produced during pearl nucleus implantation is referred to as the pearl-sac. The parasite dies and the remains are coated with conchiolin thus forming a hard nucleus. The epithelial cells of the pearl-sac cover the nucleus with fine layers of nacre. If movement of the nucleus is unrestricted, the result is a spherical pearl

(Farn, 1986). This defense mechanism against foreign bodies has been called "nacrezation" and is common to all bivalves (Malek and Cheng, 1974).

1.4.2 Digestive System

The digestive system of pearl oysters is similar to that of other bivalve molluscs. It has been described in detail for the Indian pearl oyster, *P. fucata* (Velayudhan and Gandhi, 1987) and is abbreviated here. The digestive system consists of a simple digestive diverticular and intestine, which lie within the viscero-pedal mass. Food particles are passed over the labial palps and then into the mouth slit which is found between the anterior levator muscles of the foot. The mouth leads into the short, ciliated oesophagus and on into the stomach. The surface area of the stomach is greatly increased by elaborate folds and depressions. The stomach is connected through a series of alveoli containing ducts to the large green/brown mass of the digestive diverticula (sometimes referred to as the liver). Located at the anterior end of the stomach lies the crystalline style, a gelatinous rod that aids in digestion by grinding food particles against an irregular area of cuticle called the gastric shield.

From the stomach/digestive diverticula, food particles are passed into the intestine via a number of terminal ducts. The intestine is divided into three equidistant portions that fold to form the undulated visceral loop. The anal process and rectum lead away from the main digestive organs following the posterior line of the adductor muscle. From here, waste material is passed out of the shell cavity via an exhalant siphon formed by the folds of the mantle.

1.4.3 Foot and Byssus

The foot and byssal gland of pearl oysters provide mobility and anchorage, respectively. The foot, as in all pearl oysters, is a tongue-shaped organ; the bulk of which is a system of multi-directional fibres. Retractor and levator muscles control foot movement. Extensive blood filled spaces within the foot provide hydrostatic strength and flexibility (Velayadin and Gandhi, 1987). At the proximal end of the foot is the byssal gland, which secretes byssal fibres that pass down the tubular pedal groove. Muscular contractions of the foot cause the formation of the discoid attachment and stem of each byssal thread. Attached to each byssal thread is the byssal root. Attachment takes place as the tip of the foot touches the substratum. Byssal secretions harden quickly in seawater, securing the pearl oyster to the substratum (Herdman, 1903 in Gervis and Sims, 1992; Dharmaraj and Alagarswami, 1987). Further discussion on byssus production is found in Chapter 8 of this thesis.

1.4.4) Reproductive System

The pearl oyster gonad is not discreetly defined: enveloping the visceral mass that encompasses the stomach, associated diverticular and gut loop. In ripe *P. albina*, the gonad completely obscures the dark mass of the gut diverticula (Tranter, 1958a). The gonadal tissue of bivalves arises from primordial cells of the mesoderm (Mackie, 1984). Tranter (1958a) described these cells as "stem cells" in *P. albina*. The thin layered stem cells spread through the connective tissue and attach to associated membranes to become an "early follicle". The follicles elongate, each forming a flattened tubule that branches, infiltrating the connective tissue between the epithelium and viscera. These invasive tubules or

"primary follicles" carry stem cells (primary germ cells) to sites of future gonad development.

The primary follicles continue to expand forming a network around the viscera. Once a follicle is established, gametogenesis begins. Gametes develop from the genital pore and spread laterally along the follicular pathways. Eventually, the interstitial spaces between adjacent follicles fill, coalesce and ramify, forming a dense multi-layered network. At this stage the gonad is fully ripe.

The sequence of events leading to maturation and spawning in bivalves is described in a number stages. A typical scheme is shown in Table 1 for *P. maxima* (Rose, 1990) which is adapted from earlier work by Tranter (1958b and c) on *P. albina.*

1.5 Reproductive Biology

P. maxima is a protandrous hermaphrodite (Wada, 1953; Tranter, 1958a; Rose et al., 1990). Sexual maturity is reached after the first year of age when the majority develop as males. By the time *P. maxima* reach 200 mm dorso-ventral shell height (SH) the female to male ratio is approximately 1:1 (Rose et al., 1990). This is not the case, however, for farm held stock. Five years of sampling data from farms in the Northern Territory, Western Australia, and Indonesia showed a heavy bias towards male development (J. J. Taylor and R. A. Rose, unpublished data, 1993-1999). Most often in the farm situation, the ratio of males to females is greater than 2: 1. This may be in part due to husbandry practices such as cleaning and net-changing, which involve frequent disturbance.

Table 1.1 The reproductive sequence for the silver- lip or gold-lip pearl oyster, *P. maxima* (source: Rose, 1990)

Stage	Description
1	Intermediate or inactive. No gonadal development apart from empty, collapsed follicles and connective tissue containing granulocytes and phagocytes. Early gametogenesis. *Testis* : Stem cells and spermatogonia line the follicles, later primary and secondary spermatocytes proliferate rapidly and fill the follicular lumen. *Ovary* : Follicles are initially small and lined with stem cells and developing oocytes. As oogenesis proceeds, oogonia and young oocytes proliferate along the inside walls with larger oocytes beginning to elongate.
2	Actively developing to near-ripe gametogenesis. *Testis* : Follicles begin to enlarge, spermatogonia and spermatocytes proliferate along the periphery of the lumen, with spermatids and some spermatozoa filling the center of the lumen. Near-ripe follicles are greatly enlarged with developing sperm around the follicular wall which has decreased in thickness. Except for isolated pockets of spermatocytes and spermatids , the follicular lumen is packed with spermatozoa. *Ovary* : Oocytes have begun to accumulate yolk and expand into the lumen, with free oocytes apparent in the center. Near ripe follicles are densely packed with elongated oocytes which are still connected to the follicular wall by a stem of yolk material
3	Spawning ripe. *Testis* : Follicles distended, confluent and almost entirely filled with spermatozoa. Spermatocytes and spermatids are restricted to lining the follicular walls which have become thinner with maturation. *Ovary* : Confluent follicles packed with almost entirely free nucleated oocytes.
4	Partially spawned to spent. *Testis* : Contain partially emptied follicles. Follicles which are still full have a gap between the follicular wall and a mass of spermatozoa. Phagocytes can be found within partially spawned and spent follicles. *Ovary* : Follicles are partially empty to spent. Within spent follicles resorptive tissue, phagocytes and interstitial connective tissue surround isolated, regressing oocytes.

gonad (oogenesis) has a higher energy requirement than the development of a male gonad (spermatogenesis). Individuals with low food reserves reproduce less effectively as females than as males; germ cell rudiments respond to food reserves by differentiating toward "maleness" at low levels and "femaleness" at high levels. Tranter (1958b) postulated that synchronous hermaphroditic bivalves inherit the ability to, firstly, develop either as male or female and, secondly, to respond differentially to prevailing environmental conditions. Dolgov (1991) proposed that sex reversal (females changing to males) observed in a population of *P. margaritifera* was a tactical response to localised environmental stress, in this case oil pollution. An inter-correlation was demonstrated between gonadal development, water temperature and chlorophyll *a* levels. This suggests a relationship between reproduction and environment for *P. maxima* (Rose et al., 1990).

In northern Queensland and Western Australia, the onset of the breeding season is closely correlated with increased water temperature. Wada (1953a and b) reported two peaks in the spawning season of *P. maxima* near Thursday Island. The first and main spawning period was in October/November with a second peak in February/March. Similarly, the beginning of the breeding season in Western Australia corresponds with the annual rapid rise in water temperature during September/October and continues over the southern summer before ending as the temperature declines in March/April (Rose et al., 1990). Near the equator in Indonesia, the breeding season commences in early September and fecund pearl oysters are found into June (R. A. Rose and J.J. Taylor unpublished data, 1989-1990). Hatchery trials in Indonesia suggest that the best spawning condition is during October/November and February/March.

1.6 Historical development of *P. maxima* aquaculture

The earliest attempts to artificial propagate *P. maxima* were those by Wada (1955a), who was able to strip gametes from both male and female adults and fertilize them. Fertilization was facilitated by the use of ammonia hydroxide (NH_4OH). Wada was able to induce males to spawn by manipulating water temperature, but he failed to induce females. The first recorded successful production of *P. maxima* larvae was by Minaur (1969). Artificial fertilization was accomplished by treating gametes stripped from sacrificed pearl oysters with ammonium hydroxide. Although large numbers of larvae were produced, mortality was high and none of the larvae survived beyond pediveliger stage. The first published account of successful spat production was by Tanaka and Kumeta (1981).

Renewed interest in developing hatchery techniques for *P. maxima* followed successive years of declining catch and heavy mortality on Western Australian pearl farms during the late 1970s (Dybdahl and Pass, 1985). In the early 1980s, several Japanese companies, Kakoa Australian Pearls Limited (of Oceangem Pearls Pty Ltd) in Queensland and Western Australia, Anami-Oshimi in Japan and Hamaguchi Pearls in Borneo and Indonesia, invested in hatchery production of *P. maxima*. By 1983, Hamaguchi Pearls had successfully cultivated hatchery propagated *P. maxima* to operable size (Dybdahl and Rose, 1986).

The Western Australian Department of Fisheries and Wildlife, funded by the Fishing Industry Research Trust Account (FIRTA), began to investigate hatchery technology for *P. maxima* in 1982. By 1988, the project had successfully produced several small batches of spat and cultured them to operable size (Rose and Baker, 1989, Rose and Baker, 1994). The techniques developed during this project resulted in the publication of a hatchery manual

(Rose, 1990). Unfortunately, fears of over-production in the pearl industry resulted in limited distribution of the manual and, to this day, it remains a somewhat confidential document. This attitude saw Australian hatchery technology move off-shore to Indonesia where techniques were refined to commercial reality (Bunks, 1992). Fortunately, a gradual change of attitude has developed and there are now a number of commercial hatcheries producing *P. maxima* in Australia. At present, there is one hatchery in Queensland, two in the Northern Territory and five in Western Australia. Indonesia boasts over 12 productive hatcheries (Muller, 1997) and there are also *P. maxima* hatcheries in Vietnam and Thailand (R. Shaw, Pearl Oyster Propagators, N.T., Australia, personal communication, 1997), the Philippines (Doumenge et al., 1991) and Myanmar (D. J. Arbuthnot, Atlas Pacific Ltd., personal communication, 1997).

1.7 Artificial Propagation of Pearl Oysters

The general life cycle for *P. maxima* is similar to that of other bivalves and is summarized in Fig 1.4.

1.7.1 Spawning

There are various means for inducing spawning in pearl oysters and other bivalves; the most common is manipulation of water temperature. In early experiments with *P. maxima* (Wada, 1955a; Minaur, 1967; Tanaka and Kumeta, 1981) eggs were stripped from the gonads of sacrificed adults and fertilization was achieved by treating those gametes with ammonium hydroxide. However, only in the study of Tanaka and Kumeta (1981) was there any successful spat settlement from larvae produced in this manner.

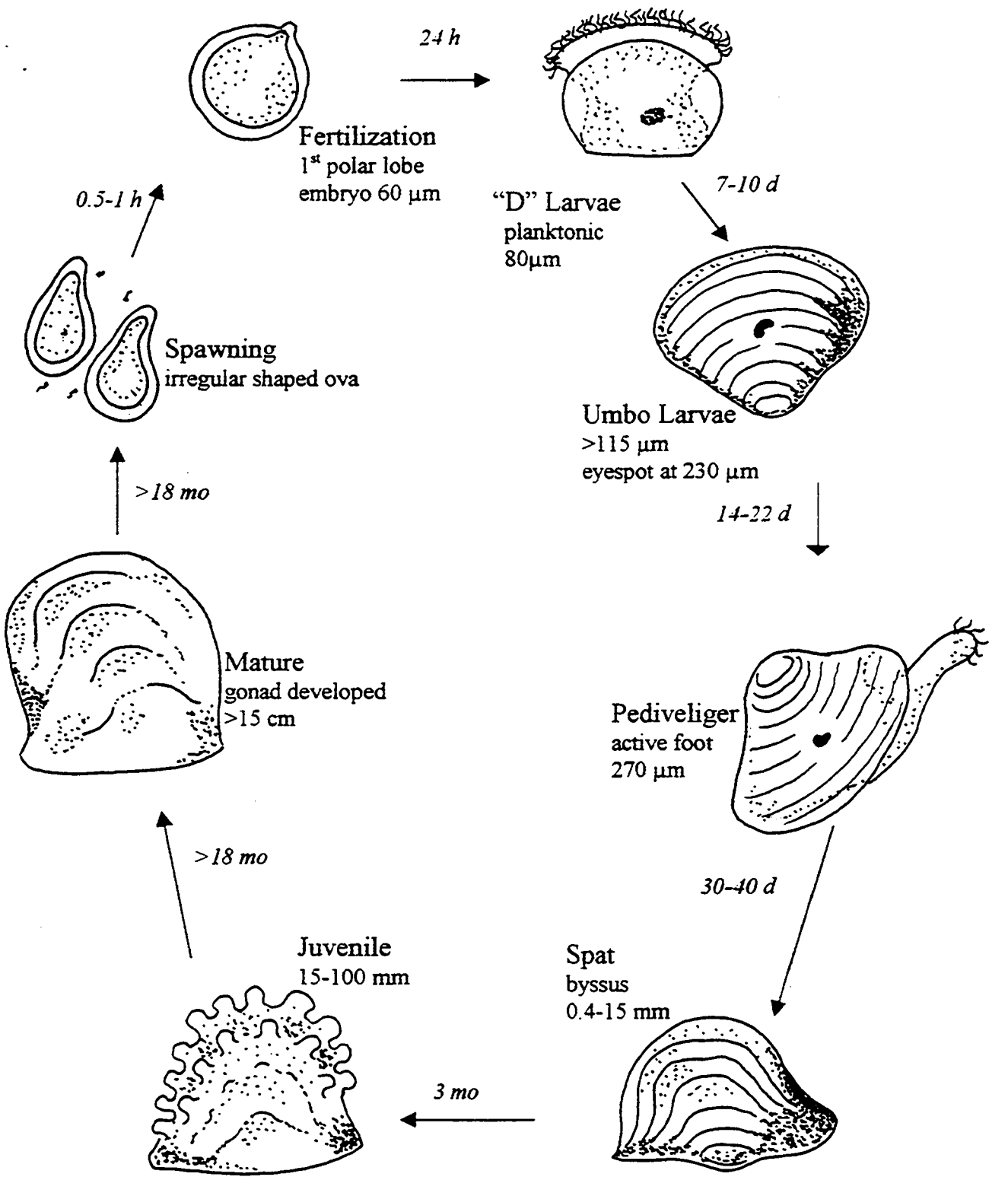


Fig. 1.4 Major stages in the life cycle of *P. maxima*

Tanaka and Kumeta (1981) were far more successful with larvae that resulted from a spontaneous spawning in the hatchery.

Alagarswami et al. (1983) had mixed success in attempts to induce *P. fucata* to spawn using hydrogen peroxide, Tris-buffer, sodium hydroxide (NaOH) and combinations of hydrogen peroxide and Tris/NaOH. Increasing alkalinity of seawater to a pH of 9.0 with Tris-buffer successfully induced 78.6% of pearl oysters to spawn. Similarly, seawater with a pH adjusted to 9.5 by adding NaOH induced spawning in 68 % of oysters. Other treatments were less effective. In one experiment 87.5 % of the pearl oysters spawned after the water temperature was raised from 28.5°C to 35.0°C. However, temperature manipulation on other occasions gave either a poor response or none at all.

Serotonin has been used as a successful inducer of spawning in a number of bivalves. Gibbons and Castagna (1984) used intra-gonadal injection of 2 m*M* serotonin solution at a dose rate of 0.4 mL to induce spawning in the bay scallop (*Argopecten irradians*) the American oyster, (*Crassostrea virginica*) and the surf clam (*Spisula solidissima*). Additionally, injection of serotonin into the adductor muscle of the ocean quahog (*Arctica islandica*) the ribbed mussel (*Geukensia demissa*) and the hard clam (*Mercenaria mercenaria*) induced spawning. Intra-gonadal injection of serotonin has been successfully used to promote spawning in a number of giant clam species: *Tridacna derasa*; *T. gigas*; *T. squamosa*; *T. derasa*; *T. maxima* and *Hippopus hippopus* (Braley, 1985) and the scallops, *Pecten ziczac* (Vélez et al., 1990) and *P. fumatus* (Heasman et al., 1996). Heasman et al. (1996) suggested that serotonin-induced spawnings may be advantageous for genetic selection as individuals can be spawned in isolation allowing control of gamete mixing.

In contrast to successes detailed above, serotonin and hydrogen peroxide were found

to be ineffective in inducing spawning in *P. maxima* both in the field and in the laboratory (Rose et al., 1986). Spawning in *P. maxima* was best induced by raising the temperature of ultra-violet irradiated and aerated filtered seawater 3-5°C above ambient. Spawning was enhanced if suspensions of sperm or eggs were added to the spawning tank and if the water was completely replaced periodically (Rose et al., 1986).

1.7.2 Larval Culture

Larval development in *P. maxima* is similar to that of other pearl oysters (Table 1.2). As for both *P. fucata* (Alagarswami et al., 1983) and *P. margaritifera* (Alagarswami et al., 1989); *P .maxima* reach the straight hinge or D-shape larval stage within 24 h. The umbo of *P. maxima* begins to form as early as 5 days after fertilization (J. J. Taylor unpublished data, 1993-1998), with the majority of larvae being fully umbonal by day 10 (Rose and Baker, 1994). A major difference between *P. maxima* and other species of pearl oyster is the colour of the eye spots. *P. maxima* develop eye spots from day 12 onward and, unlike *P. margaritifera* and *P. fucata*, which have darkly pigmented eye-spots (Alagarswami et al., 1983, 1989), the eye-spots of *P. maxima* are red (Rose and Baker, 1994).

Table 1.2. Larval size for age data for three species of pearl oyster. Adapted from Alagarswami et al. (1989) with additional data from Rose and Baker (1994).

Stage	*P. fucata*				*P. margaritifera*	
	Alagarswami et al. (1983)		Ota (1957)		Alagarswami et al (1989)	
	Size (µm)	Age	Size (µm)	Age	Size (µm)	Age
Egg spherical	47.5	-	-	-	45	-
D-shape	67.5 x 52.5	20 h 40 m	72 x 60	20 h	75 x 60	24 h
Early Umbo	100 x 95	-	96 x 87	d 8	110 x 90	d 9
Umbo	135 x 130	d 10 - 12	-	-	140 x 130	d 12
Eye spot	210 x 190	d 15	170 - 200	-	210 x 200	d 16
Pediveliger	230 x 200	d 20	209 x 195	d 25	220 x 210	d 20
Plantigrade	250 x 240	d 22	200 - 300	-	260 x 240	d 23

Stage	*P. maxima*					
	Minaur (1969)		Tanaka and Kumeta (1981)		Rose and Baker (1989; 1994)	
	Size (µm)	Age	Size (µm)	Age	Size (µm)	Age
Egg spherical	59 - 60	-	-	-	60	-
D-shape	75 x 70	24 h	57 - 77	20 h	79 x 67	18 - 24 h
Early Umbo	96 x 87	d 5	110 - 125	d 10	110 x 100	d 8 - 9
Umbo	125 x 112	d 12	-	-	114 x 103	d 10
Eye spot	-	-	-	-	230	d 12 -15
Pediveliger	180 x 160	d 14 - 21	234	d 19	270 x 220	d 14 - 22
Plantigrade	-	-	233 - 281	d 21	268 x 222	d 25

Note: Where two measurements are separated by the symbol "x" the first is antero-posterior shell length (SL) and the second dorso-ventral shell height (SH). Time from fertilization is given in min (m) hours (h) and days (d).

1.7.3 Settlement.

Following the development of eye spots, larvae begin to exhibit foot activity and locomotion. At this stage, usually between days 14 and 22 for *P. maxima* (Rose and Baker, 1989, 1994) larvae are referred to as pediveligers. In the hatchery, pediveligers are removed from the larval rearing tanks and placed in settlement systems containing settlement substrata or "collectors". Collectors for *P. maxima* and other pearl oysters larvae have been produced from a variety of natural and synthetic products (Table 1.3). Ideally, collector materials should be inexpensive, durable, light-weight and easily handled when transported to the farm (Alagarswami et al., 1987). The efficiency of different settlement substrata varies enormously and is the subject of experimental work for this thesis.

Settlement and metamorphosis are influenced by a variety of chemical and physical factors. Settlement in bivalve larvae can be induced through exposure to a range of specific chemicals (Keck et al., 1971; Coon et al., 1985; Bonar et al., 1990; Zimmer-Faust and Tamburri, 1994). Aging or seasoning collector materials (Gunn, 1984; Roland and Broadley, 1990) and encouraging the development of biofilms on collector surfaces (Weiner et al., 1989; O'Foighil et al., 1990) have been shown to improve bivalve settlement. Wiener et al. (1989) were able to improve larval settlement on materials which are unattractive substrata (for example, mylar) by encouraging certain bacteria to colonize the surfaces of substrata. Physical factors such as surface texture and contour (Alagarswami et al., 1983; Holliday, 1996), illumination (Ritchie and Menzel, 1969; Alagarswami et al., 1987) and collector orientation (Thomson, 1950; Cranfield, 1970; Holliday, 1996) also affect the settlement of bivalve larvae. However, very little of this type of research has been done with pearl oysters.

The density of larvae at settlement can influence the rate of settlement, metamorphosis and post larval survival of bivalves (Bourne et al., 1989; Bourne and Hodgson, 1991). Bourne et al. (1989) recommended a settlement density of 0.5 larvae per mL for the Japanese scallop (*Patinopecten yessoensis*) after testing a range of densities up to 14 larvae per mL. Rose and Baker (1994) used stocking densities of 0.5 - 1.0 larva per mL or less at settlement for *P. maxima*.

P. maxima like other pearl oysters such as *P. margaritifera* (Southgate and Beer, 1997), are aggregate settlers and occur in clusters of 2-8 individuals (Rose and Baker, 1994). *P. maxima* are highly mobile and are able to detach from their original settlement site and crawl to new sites using their muscular foot (Saville-Kent, 1890 and 1893). Rose and Baker (1994) observed live spat floating near the water surface in settlement tanks with the aid of long mucous strands.

1.8 Nursery Rearing of Pearl Oysters

1.8.1 Hatchery Phase.

Of major importance to hatchery-based nursery rearing of bivalve spat is the provision of an adequate diet. Cultured micro-algae remain the main source of nutrition for commercial rearing of bivalves (Brown and Jeffrey, 1992) despite continuing efforts to develop cost effective artificial alternatives (Langdon and Siegfried, 1984; Chu et al., 1987; Southgate et al., 1992; Southgate et al., 1998).

There is little published information on the nutritional requirements of pearl oysters and, as a result, most producers of pearl oyster spat rely largely on micro-algae reported as having a high nutritional value for other bivalves. Unfortunately for

Table 1.3 Materials used as collecting substrata for pearl oysters (adapted from Gervis and Sims, 1992)

Species	Material	Reference
*P. fucata martensii**	• cedar sprigs	
Akoya pearl oyster	• mollusc shells	
	• old fish nets	Shirai (1970)
P. fucata	• split bamboo	
Indian pearl oyster	• fibreglass plates	
	• mono-filament nylon	
	• old fish nets	
	• coconut shells	Alagarswami et al. (1987)
	• oyster baskets	Victor et al. (1987)
	• nylon mesh	Nayar et al. (1978)
	• nylon frills	Achari (1980)
P. maxima	• glass	
Silver-lip pearl oyster	• shade cloth	
	• PVC plates	
	• artificial rope	Rose and Baker (1994)
	• mono-filament nylon	Rose and Baker (1994); Knuckey, (1995)
P. margaritifera	• hyzex film	Cabral et al. (1985) and Passfield (1989)
Black-lip pearl oyster	• *Pemphis acidula*	
	• artificial rope	Passfield (1989)
	• shade cloth	Friedman and Bell (1996); Southgate and Beer (1997)
	• wooden boards	
	• split bamboo	Crossland (1957)
P. mazatlanica	• black polyethylene sheets	Gayton-Mondragon et al. (1993)
American pearl oyster		

* *P. fucata martensii* has been formerly referred to as both *P. fucata* (Mizumoto, 1976) and *P. martensii* (Shirai, 1970). Shirai (1994) stated that the new correct name for all Akoya type pearl oysters is *P. imbricata*. For consistency, however, the name *P. fucata martensii* is used throughout this thesis.

producers of *P. maxima* and other tropical pearl oysters, the majority of micro-algal isolates available for mass culture are of temperate origin and are often difficult to grow under tropical conditions. Problems can result when micro-algae grown at a temperature below ambient are introduced into culture vessels used for larvae or spat. Tanaka and Inoha (1970) recommended against using *Pavlova lutheri* (a temperate golden-flagellate) in tropical hatcheries producing *P. margaritifera* because it rapidly perished when taken from an optimum growing temperature of 20°C and introduced into culture vessels at 28-30°C. Despite this, *P. lutheri* is still used (Tanaka and Kumeta, 1981; O'Sullivan, 1994).

Typical micro-algal diets used for rearing pearl oyster spat consist of *Isochrysis* spp., *Pavlova* spp. and *Chaetoceros* spp. (Alagarswami et al., 1983, 1987; Rose and Baker, 1994; Southgate and Beer, 1997).

The stocking density of spat within a nursery system affects growth and survival through competition for resources such as space and food (Holliday et al., 1993). There is also an effect on the build up of waste material and bacterial levels which in turn have implications for water exchange rates, nursery husbandry and hatchery hygiene. No information is presently available on the optimum stocking density for hatchery based spat rearing.

The length of time spat are held in the hatchery varies enormously between operations and is largely dependent on factors such as food availability, health of the spat, proximity to sea leases and economics. For *P. fucata*, Alagarswami et al. (1987) recommended that spat attain a minimum size of 3 mm before being transferred to sea to reduce stress related mortality. Rose and Baker (1994) and Southgate and Beer (1997)

transferred *P. maxima* and *P. margaritifera* spat, respectively, approximately three weeks after completion of settlement.

Spat that have settled onto the surfaces of tanks or that require removal from collectors are generally removed by scraping or by using of water jets (Alagarswami et al., 1987; Rose, 1990; Rose and Baker, 1994). Spat removed in this way are allowed time to re-attach to other substrata and placed in down-weller systems or transferred to sea (Alagarswami et al., 1987; Rose, 1990; Rose and Baker, 1994).

1.8.2 Sea Phase

In general, spat are removed from the hatchery still attached to collectors on which they originally settled. They are then suspended at various depths from long-line or raft systems (Gervis and Sims, 1992; Rose and Baker, 1994). In order to protect spat from predators, wave action and certain types of fouling, and to help retain dislodged spat, the collectors are housed in mesh or netting (Alagarswami et al., 1987; Gervis and Sims, 1992; O'Sullivan, 1994; Rose and Baker, 1994; Southgate and Beer, 1997). Mesh size influences water exchange, and therefore growth and survival of bivalves (Holliday et al., 1991). The mesh must be kept clean and requires regular replacement as the spat grow to ensure adequate water exchange.

On most pearl oyster farms, mesh sleeves and collectors are cleaned using water jets. Typically, spat enclosed in fine mesh (≤ 1 mm aperture size) must be cleaned weekly to prevent clogging (J.J. Taylor, unpublished data, 1993-1998). There is considerable conjecture in the pearling industry over the appropriateness of different mesh sizes. Many growers fear high spat losses if mesh with an aperture size greater than that of the spat is

used. A secondary protective cage or basket may also be used to house the collectors. This method has been adopted in India for *P. fucata* (Chellam et al., 1987) and tested in Australia for *P. margaritifera* (Southgate and Beer, 1997). Caging of collectors has been used on a number of Australian and Indonesian pearl farms for *P. maxima* with varying degrees of success (S. Arrow, Arrow Pearling, WA, Australia, personal communication, 1993; D. J. Arbuthnot, Atlas Pacific Ltd., WA, Australia, personal communications, 1996; J. Taylor, unpublished data, 1994).

Spat are maintained on collectors until they are large enough to be removed from their point of attachment and graded. This is typically when they have reached a dorso-ventral SH of 10 mm (Gervis and Sims, 1992). Juvenile pearl oysters are removed from their point of attachment by breaking the byssal attachment. In most cases by severing the byssus with a scalpel or fine sharp blade.

1.9 Grow-out

1.9.1 Culture Systems

Grow-out or on growing commences when juvenile pearl oysters are removed from collectors and placed in secondary culture systems. A number of different containing systems are used for juvenile pearl oysters (Fig 1.5) including lantern nets, pearl nets, box nets, plastic or steel baskets and pocket nets or panels (Gervis and Sims, 1992; Gaytan-Mondragon et al., 1993). Gayton-Mondragon et al. (1993) used pearl nets, lantern nets and pocket nets suspended to a depth of 10 m and plastic cages held off-bottom at 10 m to compare growth and survival of juvenile *P. mazatlanica* in different

structures. No significant differences were recorded in growth between treatment but survival was highest in the plastic cages.

A variety of grow-out systems are used in Australia and Indonesia. A popular system is to place juveniles into plastic mesh envelopes or "inserts" that can then be placed into the pockets of adult size pocket nets and/or pocket nets with varying pocket dimensions and mesh sizes (refer to section 2.6). The suitability of the various grow-out systems available has not been studied in detail for *P. maxima*.

In nearly all cases, juvenile oysters are suspended from long-lines or rafts (Fig 1.6) or are placed on trestle or fence systems in near-bottom culture. Gervis and Sims (1992) describe systems for pearl cultivation in detail. The position of a bivalve within the water column affects factors such as water exchange, food availability, seston levels, temperature, salinity and the level of fouling (Leighton, 1979; Wilson, 1987; Brown and Hartwick, 1988a, 1988b; Cote et al., 1993; Smitasiri et al., 1994).

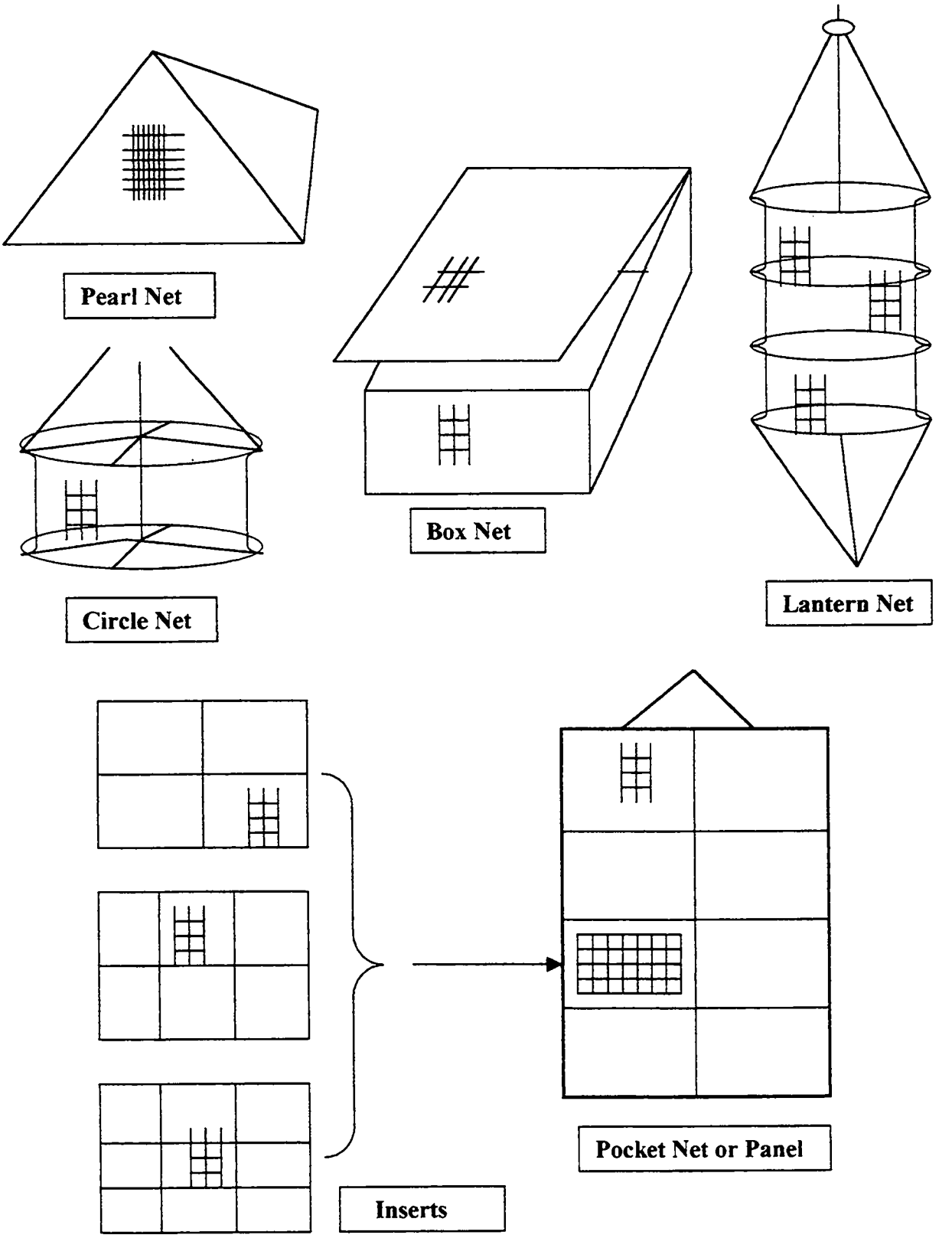


Fig. 1.5 Examples of nets and containers used in the grow-out of pearl oysters.

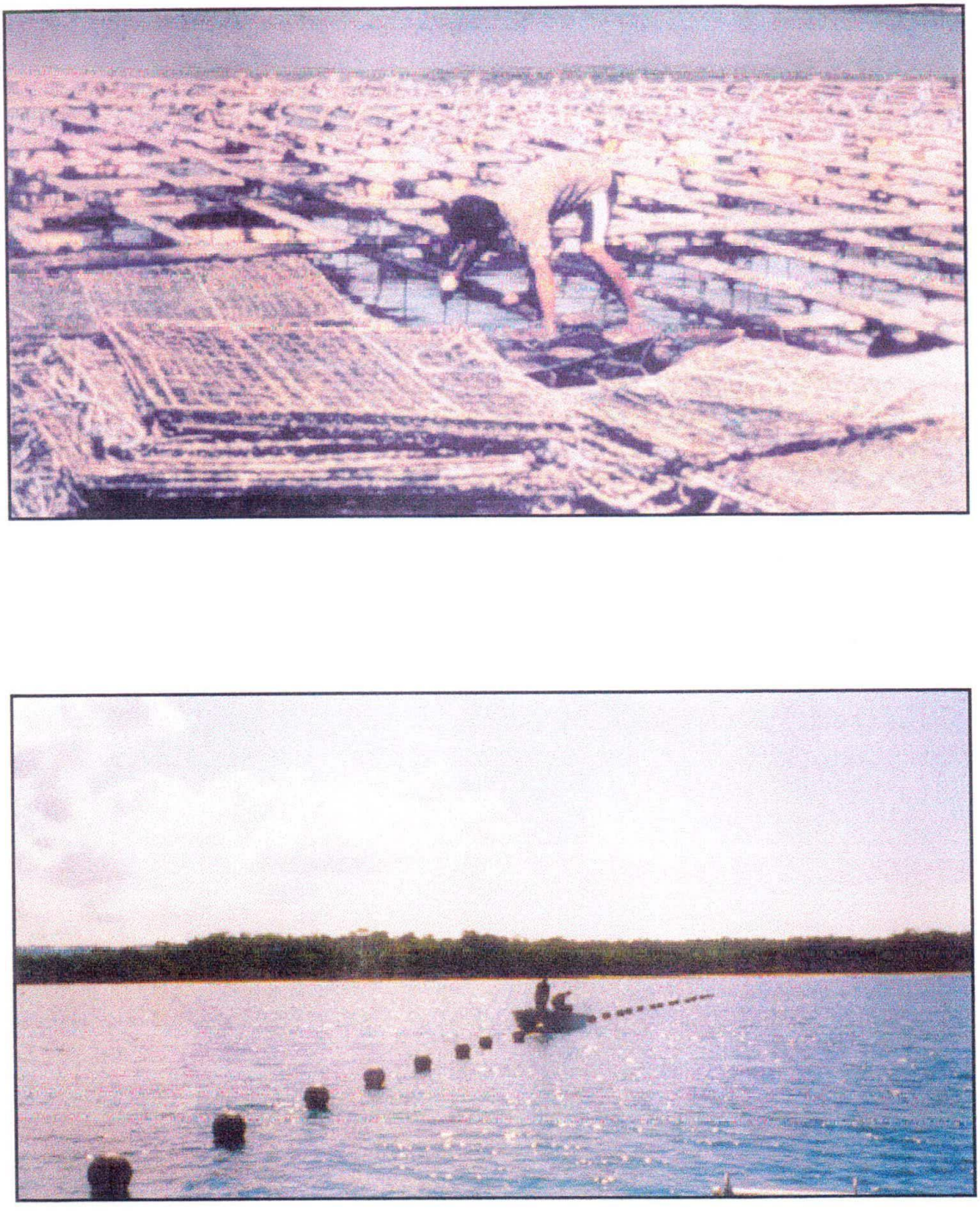


Fig. 1.6 **Different culture systems used for growing pearl oysters: raft above and long-line below.**

1.9.2 Stocking Density and Grading

Stocking density influences growth and survival of pearl oysters and other bivalves as well as the cost of production (Duggan, 1973). Benchmarks for appropriate stocking densities have been established for a variety of bivalves including clams (Hadley and Manzies, 1984; Hurley and Walker, 1994) edible oysters (Jaryaband and Newkirk, 1989; Arakawa, 1990; Roland and Albrecht, 1990; Holliday et al., 1991; Holliday et al., 1993; Rheault and Rice, 1996) and scallops (Duggen, 1973; Parsons and Dadswell, 1992; Gaudest, 1994).

By contrast, very little work has been done on appropriate stocking densities for pearl oysters. Survival of *P. maxima* was similar at stocking densities of 400 individuals per m^2 and 2,500 individuals per m^2 (cultured in down-wellers) and 300 individuals per m^2 and 700 individuals per m^2 (cultured at sea); however, growth was slower in both cases at higher densities (Rose and Baker, 1994). More detailed research into stocking density is required.

Regardless of stocking density, there is a recognized need for bivalves to be graded at regular intervals during grow-out to separate faster growers from slower growers and to cull poor quality individuals. At each grading, equipment requires modification (particularly mesh size) to ensure adequate water exchange and the supply of sufficient food and oxygen (Gervis and Sims, 1992). This is especially the case for pearl oysters as the timing of pearl nucleus implantation or seeding is directly related to pearl oyster size. Slower growing *P. maxima* take up to 30 months to reach operable size as opposed to only 18 months in faster growing individuals (Scoones, 1990). Slower growing individuals have a longer non-productive culture period and increase production cost.

1.9.3 Fouling and Fouling Control

Fouling impacts greatly on growth and survival of bivalves (Claeroboudt et al., 1994). Excessive fouling results in slower growth of pearl oysters and increased mortality. Of particular danger to pearl oysters are boring organisms such as sponges (*Cliona* spp.) polychaetes and some species of molluscs (Alagarswami and Chellam, 1976; Mohammad, 1976; Chellam, 1978; Dharmaraj and Alagarswami, 1987). Pathological effects of borers include shell deformation, formation of blisters on the internal surfaces of shells, hinge imbalance, shell fragility and the staining of nacreous surfaces with dark organic material (Dharmaraj and Alagarswami, 1987; Doroudi, 1996). Gastropods such as *Murex* spp. and *Cymatium* spp. are predatory and are a source of pearl oyster mortality (Crossland, 1957; Southgate and Beer, 1996; Friedman and Bell, 1996). In a feeding rate experiment, 2 specimens of *M. virgeneus*, 54 mm in size, consumed 20 *P. fucata* in 49 days whilst 20 *P. fucata* were consumed by 2 specimens of *C. cigulatum* within 37 days. Twenty *P. fucata* were consumed in 20 days by *C. cigulatum* of 40.5 mm and in 19 days by *C. cigulatum* of 61.8 mm (Anon, 1991).

Invasion of the shell opening of pearl oysters by barnacles or other bivalves can lead to shell deformation (Dharmaraj and Alagarswami, 1987). If left unchecked, barnacles can cement the valves of a pearl oyster closed or become so large on the hinge-line that they prevent the pearl oyster from opening: both cases result in death of the oyster (Dharmaraj and Alagarswami, 1987). Growth of hard fouling from within the shell opening can eventually prevent pearl oysters from closing rendering them vulnerable to attack from predators such as fish and crabs (J. Taylor, unpublished data, 1993-1998).

The constant removal of fouling from bivalves and culture equipment and the damage caused to cultivated bivalves is a major cost to bivalve culturists (Arakawa, 1990). Cleaning regimes for *P. maxima* vary with site and season but are generally conducted every 4-6 weeks. Cleaning involves mechanical or manual scrubbing and scraping of shell surfaces with knives and brushes (M^{c}Guinness, 1994; Gervis and Sims, 1992) or the use of high pressure water jets (Scoones, 1990; Gervis and Sims, 1992). Control measures for the removal of fouling other than strictly physical have been used with varying success on smaller species of pearl oyster such as *P. fucata martensii* (Shirai, 1970) and *P. fucata* (Dharmaraj and Alagarswami, 1987; Chellem et al., 1987). Immersion in brine for several hours will kill most external fouling organisms (Shirai, 1970; Dharmaraj and Alagarswami, 1987). Treatment with 1.0% formalin and immersion in fresh water will also kill external fouling organisms (Chellam et al, 1987; Dharmaraj et al.,1987). Air exposure for 2-3 hours was also successful in killing newly settled fouling organisms (Chellam et al., 1987). To date, *P. maxima* producers have not adopted these treatments.

Removal of fouling is not only costly but could also expose pearl oysters and other bivalves to handling stress. In several studies, repeated handling contributed to mortality of juvenile scallops (Ventilla, 1982; Dadswell and Parsons, 1991; Parsons and Dadswell, 1992). However, other studies have demonstrated significant improvement in bivalve growth and survival through regular cleaning (Jakob and Wang, 1994; Claereboudt et al., 1994.

1.10 Pearl Cultivation

Pearl cultivation is possible because of the ability to implant or graft spherical nuclei, together with a piece of donor mantle tissue, into the gonad of a recipient pearl oyster. If successful, the epithelial cells of the outer epidermal layer of the donor mantle tissue grow around the nucleus to form the nacre producing body termed the pearl-sac (Farn, 1986). The majority of the world's pearl technicians, responsible for the delicate operation of implanting the nucleus, are Japanese (Hollyer, 1984; Gervis and Sims, 1992; Fassler, 1995). However, there are growing numbers of competent Australian, American, Chinese, Indian, Polynesian and Philippine technicians (Fassler, 1991; Gervis and Sims, 1992). For *P. maxima*, the current cost of implanting nuclei is around $AUS 10 per oyster and some Australian farms pay as much as $AUS 13 per oyster (L. Petersen, Atlas Pacific Ltd., personal communication, 1998). By the time wild *P. maxima* have been implanted with nuclei, the estimated production cost to the farmer is $AUS 50 per oyster (M^{c}Guinness, 1994).

1.10.1 Pre-operative Care and Conditioning

Western Australian fishing regulations require wild *P. maxima* to be a minimum SH of 120 mm before they can be taken for pearl cultivation (M^{c}Guinness, 1994). For hatchery produced *P. maxima*, a minimum SH of 105 mm is recommended before attempting to implant pearl nuclei (Scoones, 1990). Approximately 20 to 25% of a given population of hatchery produced *P. maxima* will attain a SH of 120 mm by 19 months of age (Rose and Baker, 1994).

It has been recognized that the condition of pearl oysters prior to implanting pearl nuclei is critical to the success of the operation (Alagarswami,1970; Mizumoto, 1976;

Hollyer, 1984; Alagarswami, 1987a; Gervis and Sims, 1992). Pre-operative conditioning has two aims: (1) to rid the gonad of gametes, thus providing space for the nuclei and avoiding the oozing of gametes that would obscure the view of the technician during operation; and (2) to reduce metabolic activity, in particular the strength of the adductor and retractor muscles that can cause expulsion of the nucleus (Alagarswami et al., 1987a).

Pre-operative conditioning can be achieved in various ways. When and where possible, pearl oysters are placed in cooler water (Mizumoto, 1976; Alagarswami, 1987a; Gervis and Sims, 1992). This may be achieved using seasonal variation in seawater temperature or by lowering pearl oysters into deeper, cooler water (Alagarswarmi, 1987a). Alternative methods for pearl farmers in tropical waters, where there is little seasonal or depth related differences in water temperature, include crowding the pearl oysters in baskets and/or covering the pearl oysters in a fine mesh (Alagarswami, 1987a). The result in all cases is reduced food availability, a loss of reproductive condition and a lowering of metabolic rate.

Growers of *P. maxima* in Australia take advantage of lower winter water temperatures and generally operate only during the cooler months of June through to September (Scoones, 1990). *P. maxima* farmers in Indonesia and the Philippines use alternative methods, in particular the covering of pearl oysters with fine mesh. The period of pre-operative conditioning varies but is usually one to two months (Gervis and Sims, 1992).

A variety of chemicals have been assessed as anaesthetics for pearl oysters prior to operation (Alagarswami, 1987a; Norton et al., 1996), the aim being to reduce the risk of

damage to the mantle and adductor muscle when oysters are forced open prior to operation. Propylene phenoxetol has shown particular promise as it induces rapid anaesthesia in *P. margaratifera*, *P. albina* (Norton et al., 1996) and *P. maxima* (Mills et al., 1997) with short recovery times. In tests with *P. albina* and *P. margaritifera*, no mortalities were observed seven days after recovery (Norton et al., 1996). Large scale testing with *P. maxima* resulted in only negligible mortality (Mills et al., 1997).

1.10.2 Pearl Nuclei

Pearl nuclei are almost exclusively produced from shells of American fresh water mussels. In particular the pigtoe, washboard, butterfly, three ridge and dove shells of the Family Unionidae provide ideal raw material for nuclei manufacture (Alagarswami, 1970; Fassler, 1991). The shells of these mussels have very thick nacreous layers with a hardness, specific gravity and thermal conductivity that make them particularly suitable for use as pearl nuclei (Gervis and Sims, 1992). The process of bead manufacture involves cutting the shells into cubes, followed by tumbling in a lapping machine to form spherical beads. A final treatment in hydrochloric acid produces a polished finish (Gervis and Sims, 1992). Beads of up to 13.5 mm in size can be produced from these fresh water mussels (Roberts and Rose, 1989).

Unfortunately, stocks of American fresh water mussels are under serious threat due to loss of habitat and the spread of the introduced zebra mussel which has displaced the larger indigenous species in many areas (Fassler, 1995). The threat to the American fresh water mussels is now so great that a total ban on commercial fishing may be imposed by the year 2,000 (Fassler, 1995). As a result, there has been considerable interest in

alternative materials for the manufacture of pearl nuclei. Roberts and Rose (1989) produced nuclei from giant clam shells; however, difficulties were encountered in the drilling of resulting pearls due to differences in thermal conductivity. Scoones (1990) reported that nuclei made from *P. maxima* MOP were a suitable alternative to traditional nuclei. Pearl industry representatives raised concerns, however, due to the darker colouration of the MOP beads. Several patents exist in Japan for artificial nuclei produced from materials such as ceramics (Fassler, 1995). Recently the Biron Corporation Ltd in Australia produced artificial nuclei from a calcium carbonate based material with the same attributes as the American mussel nuclei (M. Snow, Biron Corporation Ltd., WA, Australia, 1996). These "bironite" nuclei have now been implanted in *P. maxima* in a series of experiments to test nacre adhesion and other properties (J.J. Taylor, unpublished data, 1998).

1.10.3 Nucleus Implantation

Oysters for operation are brought to the operating area (pontoon, platform or boat) cleaned, and placed in a standing position in "pegging" baskets with the hinge down. The pegging baskets, similar to dish racks, are placed in tanks usually equipped with running seawater. *P. maxima*, are often left in the tanks overnight to allow easier shell opening the following day. As the pearl oysters relax, a pair of shell openers or "speculum" (similar to reverse action pliers with flat, paddle like blades) is inserted into the gaping oyster. By applying pressure with the shell openers, a wooden wedge or "peg" can be inserted to hold the oyster open whilst awaiting operation.

Donor mantle tissue is prepared by sacrificing a healthy pearl oyster. Pearl oysters used to provide mantle tissue are carefully selected as the donor tissue will influence the colour and quality of the resulting pearls (Wada, 1985; Wada and Komura, 1996). The mantle is cut and trimmed from the donor shell. A thin strip of mantle is removed from each valve and later cut into small square pieces, usually around 2.5 mm^2. For *P. maxima*, 30 to 50 recipient pearl oysters can receive mantle grafts from a single donor. The donor tissue must be kept moist to prevent dehydration of the nacre producing cells prior to implantation.

Recipient pearl oysters are passed to the technician and held in a clamp (the pearl oyster stand) with the right valve uppermost. The foot is held and any byssal threads are cut away. Whilst holding the foot, an incision is made into the gonad just above the foot. Using a specially crafted scalpel, a tunnel like cut is made under the gonad surface. After removal of the scalpel, either the donor mantle tissue followed by the nucleus or alternatively, the nucleus followed by the donor mantle tissue, is inserted into the recipient pearl oyster. For the operation to be successful, the mantle tissue must be in contact with the nucleus. The Japanese Akoya pearl oyster, *P. fucata martensii* can be seeded with multiple nuclei; however, the larger species of *P. margaritifera* and *P. maxima* are seeded with a single nucleus only (Gervis and Sims, 1992). Once the operation is complete, the pearl oysters are returned to the water to recuperate.

1.10.4 Pearl Formation

The process of pearl formation is identical to that of shell formation (Farn, 1986). During the first weeks of recuperation the pearl-sac develops. Initially, the inner

epidermis and mesodermal layers of the mantle tissue graft degenerate leaving only the outer epidermal layer (Kawakami, 1952a, 1952b). The pearl-sac is the result of the epithelial cells of the mantle epidermis growing around the nucleus to completely encase it. After two weeks, the pearl-sac begins to lay down peristracal material followed later by the prismatic layer. After approximately 40 days, the nacreous layers begin to form (Farn, 1986).

1.10.5 Post-operative Care

P. maxima are returned to the sea and placed in a horizontal position either in pocket nets (net panels) on or near the sea floor or in rectangular "turning" baskets suspended from rafts or long-lines. Turning baskets for *P. maxima* are rectangular with a series of dividers to separate the oysters and each basket holds 10 pearl oysters. Generally, *P. maxima* are initially placed right valve uppermost in the same position used for operating. After an initial resting period of about a week, oysters are turned so that they lie left valve uppermost (Scoones, 1990). This process is repeated every two or three days for up to two months. It is thought that the turning aids development of the pearl-sac and helps to produce rounder pearls. However, Scoones (1990) was unable to experimentally confirm the value of turning. At the completion of turning, pearl oysters are placed in the main farm system.

Four to 6 months after implantation, *P. maxima* are X-rayed using a machine similar to that used at airports. Pearl oysters that have rejected the nucleus can be separated from the rest and, if suitably healthy, offered to the technicians for re-operation (Gervis and Sims, 1992).

1.10.6 Culturing and Harvest

Depending on species and location, pearls are cultured for between 1.5 and 3.5 years before harvest (Gervis and Sims, 1992). As with grow-out, it is essential to maintain a strict cleaning programme in order to promote the health of the pearl oysters. Most Australian and Indonesian pearl farms use suspended culture from rafts or long-lines. Some farms do, however, favour bottom farming. Scoones (1990) reported better quality pearls produced using bottom farming practices in Roebuck Bay, Broome, Western Australia.

Harvesting is usually done at the coolest time of the year. This is to promote a finer finish and better lustre to the pearls as nacre deposition is slower in cooler water (Alagarswami, 1987b). In Indonesia, pearl growth is sometimes slowed during the last months prior to harvest by lowering the pearl oyster to greater depths or by crowding them on culture rafts. Careful monitoring of such activity is essential to maintain good health of the oysters in these last critical stages before harvest.

Smaller species of pearl oyster, such as *P. fucata martensii*, are sacrificed at harvest and no repeat operation is performed (Gervis and Sims, 1992). For the larger species, *P. maxima* and *P. margaritifera*, the pearl is surgically removed and, depending on the quality of the harvested pearl and the health of the pearl-sac, a second nucleus is implanted. The second nucleus is much larger and generally results in a larger more valuable pearl (up to 18 mm diameter for *P. maxima*) than the first pearl produced.

1.11 Aims

The major aim of this study is to enhance our limited knowledge of spat (juvenile pearl oysters) production, nursery rearing and grow-out of the silver-lip (or gold-lip) pearl oyster, *P. maxima*, in order to optimize production and survival of hatchery produced seed. Specifically, the study investigated the following:

1) Collection and maintenance of *P. maxima* spat in the hatchery environment:
   - to determine appropriate larval stocking density at settlement;
   - to evaluate a variety of substrata and cues for collecting settled spat;
   - to evaluate a variety of micro-algae as food for spat;
   - to determine an appropriate method for inducing detachment of spat; and
   - to determine the effects of changing salinity growth and survival of spat.

2) Early nursery rearing of *P. maxima* spat at sea:
   - to determine an appropriate mesh size to protect *P. maxima* spat in nursery culture;
   - to determine an appropriate stocking density for *P. maxima* spat on collectors;
   - to determine the time for regeneration of byssus in *P. maxima* following severing.

3) Grow-out or rearing of *P. maxima* at sea:

- to determine appropriate stocking densities for different age classes;
- to evaluate suspension and bottom culturing for juvenile *P. maxima*;
- to determine the effects of hard fouling on growth and survival of *P. maxima* and the optimal interval for cleaning;

A major goal was to transfer the findings of the study directly to commercial production of *P. maxima*. All experimental work was completed at commercial facilities in Australia or Indonesia. Locally available materials and equipment were used at all times and experiments were designed to conform with the production targets and activities at the relevant facility.

Chapter 2. General materials and methods

2.1 Study Sites

The studies for this thesis were conducted in Australia and Indonesia. In Australia, the Darwin Hatchery Project (DHP) facility was used. This hatchery is located at the old Stokes Hill power station, Darwin (Fig 2.1). The hatchery draws water from Darwin Harbour. During primary, treatment seawater was pumped through a sand filter and nominal 10 μm and 5 μm paper filters before entering an elevated 22,000 L temporary storage/header tank. This tank supplied water to the larval and algal production units. Larval water was filtered to nominal 1 μm using cartridge filters before passing through a UV sterilizing unit. Water used for micro-algal production was filtered to 0.2 μm. Micro-algae were produced in glass flasks, polycarbonate carboys, polyethylene plastic bags and fibreglass tanks. Methods used to culture algae were as developed by the WA Fisheries Department (Rose, 1990) and CSIRO in Hobart (Brown et al, 1989). In general, f or f/2 media (Guillard, 1972) were used as a nutrient source for micro-algae.

The hatchery in Indonesia was located near the island of Bacan (lat. 0.5^{o} S, long. 127^{o} E) Maluku Utara (Fig 2.1). Water was drawn from the surrounding sea, and primary and secondary treatment was the same as that described for DHP. In Indonesia the seawater was not UV treated.

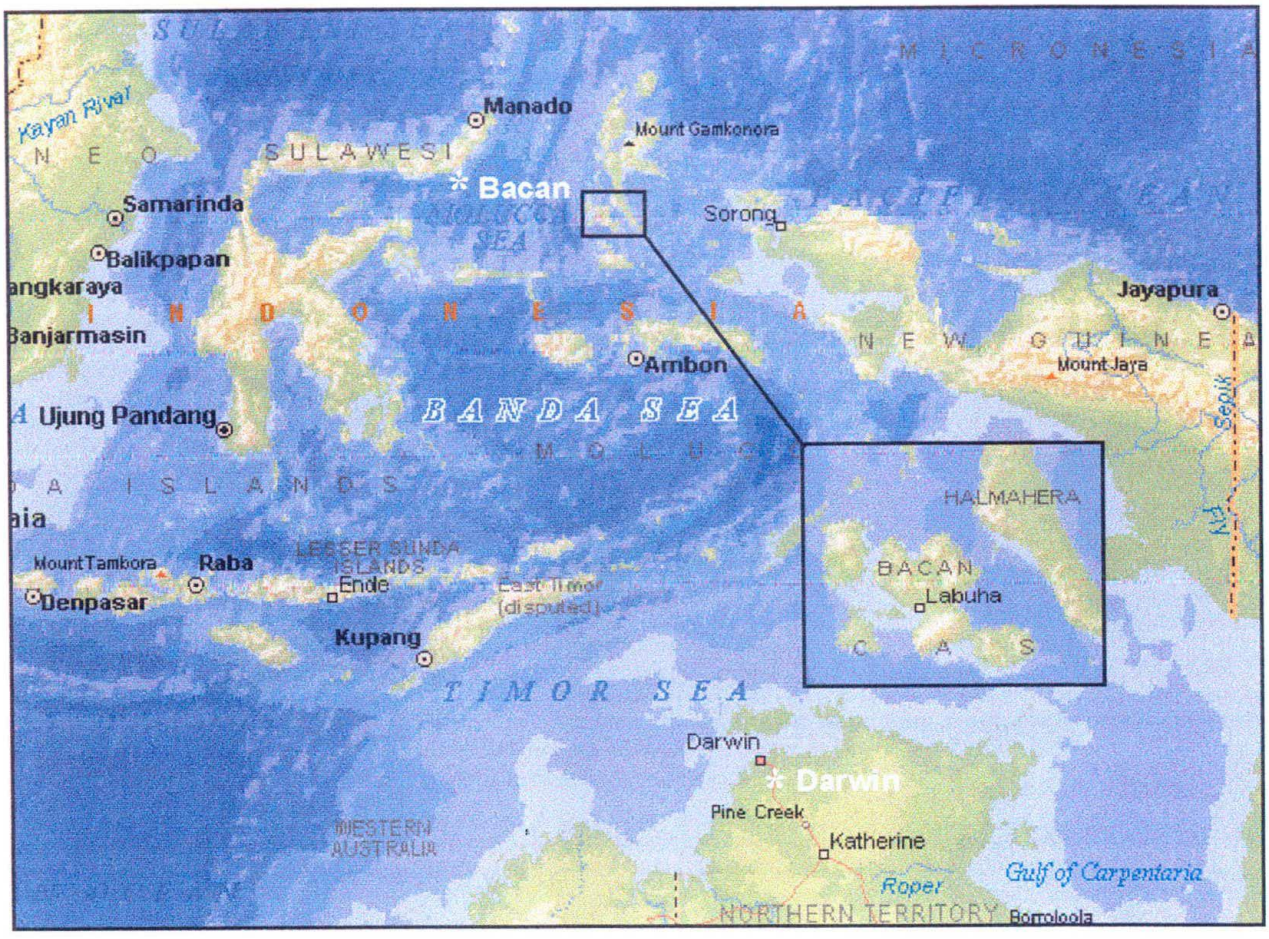


Fig. 2.1 Location of study sites

2.2 Broodstock

In all cases, whether propagation trials were attempted in Australia or Indonesia, broodstock were obtained from general farm stock held on long-lines. In Australia, broodstock were originally obtained from Western Australia before being relocated to Bynoe Harbour, near Darwin (Fig 2.1). During the spawning season (mid-late September to early May) broodstock selection was based on visual scoring of the gonad (Table 2.1). It was rare that oysters were found on the farms in the fully ripe stage 3 condition. Most pearl oysters used in spawning trials were in stage 2 or 2^+ condition.

The situation was similar in Indonesia, oysters originally fished in Bacan or the Aru Islands (lat. 6^oS, long. 132^oE) were held on long-lines near the hatchery until required. The spawning season in Indonesia is generally longer than that in Northern Australia, and begins in September and finishes in May-June (R. A. Rose and J. J. Taylor, unpublished data 1990-1998).

2.3 Spawning

Once sexed and graded, broodstock were brought to the hatchery where they were carefully cleaned to remove external fouling. Rose (1990) described the general methods used for spawning induction. Once cleaned, the male oysters were cooled in an air-conditioned room (22-24°C) for up to 1 h to lower body temperature. Females were placed in a flow-through holding tank at ambient temperature. Males were removed from the air conditioned room and placed in gently aerated spawning tanks (usually 250-500 L) after which the water temperature was raised in a series of cycles to stimulate

spawning. Usually, the water temperature was greater than 30°C before males began to spawn. On some occasions, males were sacrificed and sperm was extracted to be used as a further spawning stimulus. This sperm was activated by the addition of ammonium hydroxide using the technique described by Wada (1953a) before administering to the male spawning tank. Spawning males were transferred to spawning tanks (500-1,000 L) containing 1 μm filtered seawater together with females. Females usually began to spawn once active sperm was present in the water. Each time spawning stopped, pearl oysters were transferred to fresh tanks to encourage further spawning. Additional males were added to the spawning tanks as they began to release sperm. Up to 6 spawning tanks were utilised in any one trial. On average, females produced between 20 and 30 million eggs, although on occasions some females produced greater than 40 million eggs.

Once pearl oysters had ceased spawning they were removed from spawning tanks. Thirty min after spawning had ceased, zygotes were carefully collected using a series of nylon sieves, the smallest of which had a 20 μm mesh. Collected zygotes were washed in 1μm filtered seawater and placed in gently aerated hatching tanks at a stocking density between 10 and 30 zygotes per mL.

Table 2.1 Scoring system used for *P. maxima* broodstock based on the visual appearance of the gonad.

Score	Description
0	Gonad flaccid and sex indeterminate
1^-	Sex can be determined based on colour (cream-white for males; yellow-orange for females) but development is slight and patchy, the gonad is still flaccid and the colour is somewhat dull
1	Development has progressed, with the gamete patches becoming larger and more closely associated. It is now quite easy to distinguish the sexes
1^+	Patches of gametes are more confluent and have taken on a cloudy appearance
2-	Gonad turgid, general the gonad is well developed on one side but less so on the other
2	Gametes now appear as dark bands of colour with both sides of the gonad well developed
2^+	The gonad is enlarged with development on either side of the gonad now confluent. The strong colouration has almost reached the distal end of the gonad. The gonad is turgid with a glossy appearance
3	The gonad is fully developed, glossy in colour and full from the proximal to the distal end of the gonad. There are no breaks or light patches in the colouration, the oyster is now fully ripe.

2.4 Larval culture

After 24 h, straight hinge or D-stage veliger larvae were collected on 45 μm sieves and stocked into larval tanks at a density of 5-7 larvae per mL. Larval tanks varied in capacity from 1,000 L to 5,000 L. In Darwin, larvae were fed a mixture of *Isochrysis* aff. *galbana* (clone T.-ISO, CS-177), *Pavlova lutheri* (CS-182) and *Chaetoceros calcitrans* (CS-178). In Indonesia, larvae were fed a mixed diet of T. ISO, *Pavlova salina* (CS-49) or *P. lutheri*, and *C. calcitrans* or *Thalassiosira pseudonana* (CS-173). Once larvae in the Indonesian hatchery had completed umbonal development (after day 7), *Chatoceros muelleri* (formerly *C. gracillis*, CS-176) was gradually introduced to replace the aforementioned diatoms. This was required because of the difficulties in culturing large volumes of either *C. calcitrans* or *T. pseudonana* at the Indonesian hatchery. In all cases, stock cultures of micro-algae originated from the CSIRO Marine Laboratories, Hobart and CSIRO catalogue codes are shown in brackets .

The time between water changes varied from two to five days, depending on water quality and the health and developmental stage of the larvae. By the time larvae were fully umbonal (usually 7 to 8 days after fertilization) water was changed every two days. During water changes, larvae were graded and small larvae were culled. By the time larvae had developed eye-spots (day 12 to 21) stocking density was reduced to 2 larvae per mL.

2.5 Settlement and Nursery

Once larvae had well developed eye-spots and were showing signs of foot activity, they were placed in settlement tanks with spat collectors. Settlement techniques are detailed in Chapter 4.

After settlement was complete, water was changed daily using a combination of full batch-exchange and flow-through. During standard nursery culture of spat, fountain or air-lift pumps were placed in the settlement tanks and aeration was vigorous to promote water circulation. Spat left the hatcheries between 30 and 50 days after fertilization, depending on the number of spat held, the availability of space and food, and the destination. At DHP, spat tended to be held longer in the hatchery than in Indonesia due to differences in management practices and government regulations concerning the release of hatchery spat in Australia. Except for a specific feeding trial detailed in Chapter 5, spat were fed a mixed algal diet, similar to that of the larvae but with a greater emphasis on *C. muelleri* and the occasional use of *Tetraselmis sueicica* (CS-187).

2.6 Nursery and Grow-out

All grow-out experiments were conducted at locations in the Bacan area of Indonesia. Specific details of grow-out experiments are described in Chapters 6, 10, 11 and 12. In the first instance, spat were generally cultivated on the collectors on which they had settled whilst in the hatchery. Spat were removed from the collectors between

days 65 and 120 depending on size, density of spat on collectors, availability of equipment and the health of the animals. Depending on size, spat were placed in net panels of varying pocket arrangements (Fig 1.5) and mesh sizes or into mesh envelopes with either 4, 6 or 9 pockets (inserts) that were then placed into adult sized net panels (Fig 1.5). Juvenile oysters were graded into progressively larger nets as they grew.

Except for a specific experiment (Chapter 11), juvenile *P. maxima* were held in suspended culture from either long-lines or rafts (Fig 1.6). Rafts in this study were of timber construction and had an overall dimension of 10 m x 20 m. Rafts were anchored on all 4 corners using admiralty-type steel anchors. The rafts were buoyed using plastic covered polystyrene drum-shaped floats with a length of 110 cm and a diametre of 60 cm. Long-lines were constructed from polypropylene, ultraviolet (UV) resistant, black rope. Long-lines were buoyed every 3 m using polystyrene filled black polypropylene floats, with a diametre of 36 cm (all floats and rope supplied by U. D. Abadi, Surabaya, Indonesia). Each long-line had 100 "droppers" or ropes for hanging nets which were spaced every metre to give a total long-line length of 100 m.

Once pearl oysters were nearing a size suitable for pearl nucleus implantation (usually >85 mm HL) they left the grow-out system and became part of the farm stock. No experimental work for this thesis was carried out on pearl oysters once they became farm stock.

2.7 Measurements

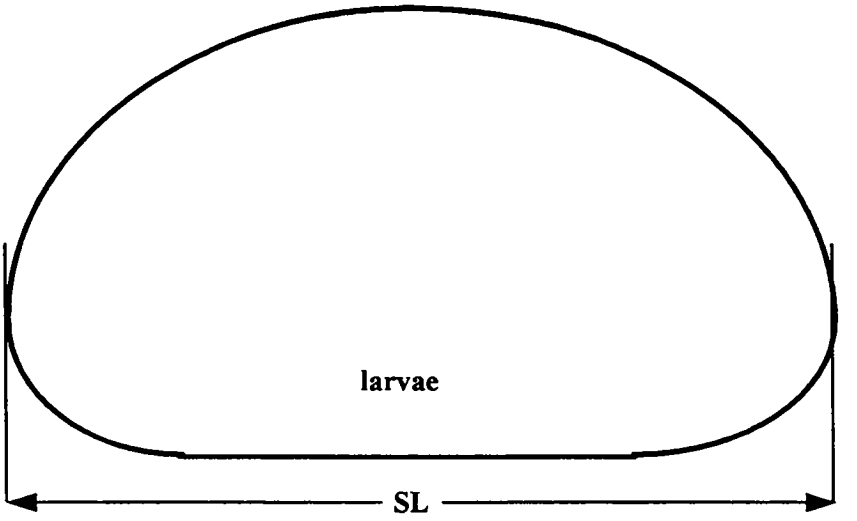
Larvae and spat were measured along the antero-posterior or shell length (SL) axis at the widest point parallel to the hinge (Fig 2.2). Juvenile pearl oysters were measured along the dorso-ventral or shell height (SH) axis and either the SL axis or along the hinge length (HL). Before weighing live oysters for wet weight (WW) they were dabbed dry with absorbent tissue to remove excess moisture. Methods for dry weight (DW) and ash free dry weight (AFDW) determination are described in the relevant chapters.

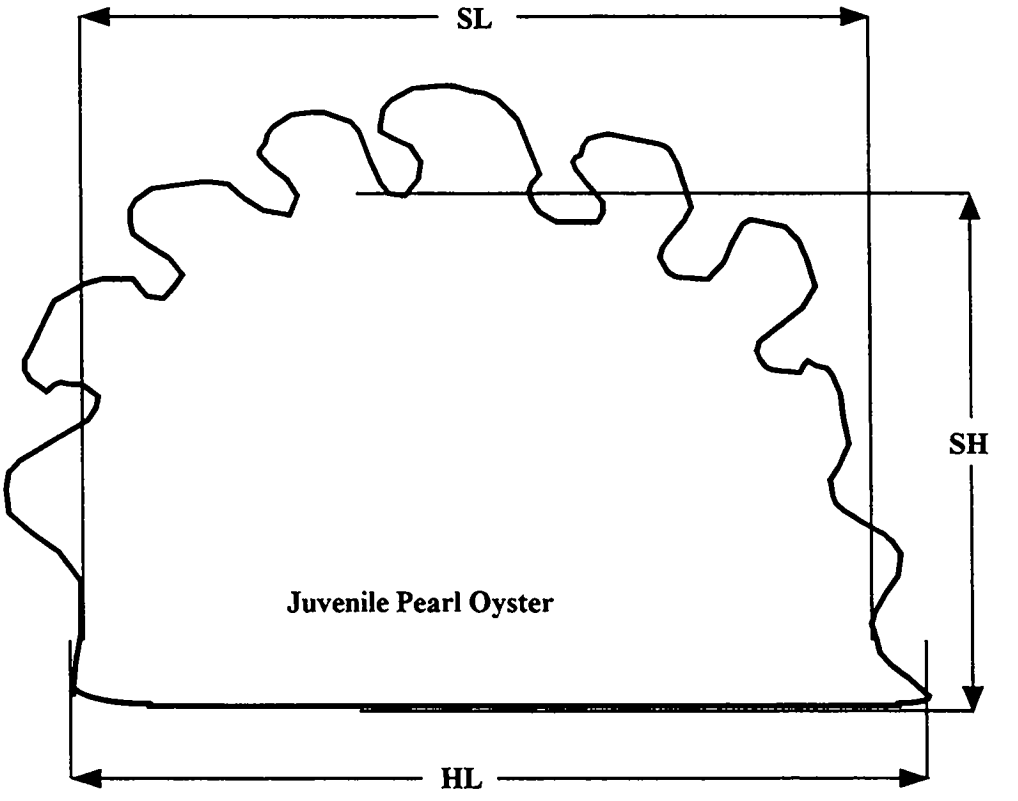
2.8 Origin of Experimental Animals

Spat used in the collector orientation experiment in Chapter 4, Chapter 5 and the first experiment on byssal production in Chapter 8 were produced during 1994 at the Darwin Hatchery Project and resulted from three different hatchery cohorts. *P. maxima* used in all other experiments were produced in the Indonesian hatchery in Bacan from October 1994 to February 1996. The same oysters were never used in more than once.

2.8 Statistical Analysis

In all cases statistical analysis was performed using the StatView statistical program, version 4.5, for Windows 95 (Abacus Concepts, StatView, 1996).





SL = Antero-posterior measurement
SH = Dorso-ventral measurement
HL = Hinge length

Fig 2.2 System of measurement used in this study

Chapter 3. Effects of larval set density on subsequent growth and survival of *Pinctada maxima*

3.1 Introduction

A number of factors affect settlement of bivalve larvae including the availability of suitable substrata (Butler, 1955; Cranfield, 1970), the presence of appropriate physical and chemical cues (Keck et al., 1971; Weiner et al., 1982; Coon and Bonar, 1985; Bourne et al., 1989; Weiner et al., 1989; Soniat et al., 1991) and the health and competency of larvae (Loosanoff and Davis, 1963; Chevolot et al., 1991). For hatchery operators, the number of larvae introduced into a setting system is another important factor. Larval density effects the rate of settlement, metamorphosis and post-larval survival of bivalves (Bourne et al., 1989; Bourne and Hodgson, 1991). The density of settled spat on collectors, in turn, effects growth and survival through competition for space and food (Holliday et al., 1993). If spat are to be left attached to collectors during early nursery grow-out, then the density of settled spat has an important influence on growth and survival (see Chapter 10). Too few spat per unit collector is not cost effective whereas very heavy set numbers may effect survival and reduce growth rates of spat (Askew, 1978).

The effects of crowding during the larval cycle are well known. The number of larvae in a given volume has an optimal level beyond which increasing larval population cannot be compensated for by proportional increases in food availability (Loosanoff and Davis, 1963). In view of this, and the costs associated with producing bivalve spat, there is surprisingly little information on optimal larval set densities for bivalves. Bourne et al.

(1989) evaluated the effects of set densities of 0.5 to 14 larvae per mL on the Japanese scallop (*Patinopecten yessoensis*) and recommended a set density of 0.5 larvae per mL. No similar information is available for *Pinctada maxima*, even though there have been published accounts of *P. maxima* settlement in hatcheries (Rose, 1990; Rose and Baker, 1994). Densities of between 1 and 3 larvae per mL are commonly used in commercial hatcheries propagating *P. maxima* (R. A. Rose and J. J. Taylor, unpublished data 1989-1998). However, it seems that increasing the number of larvae placed in a settlement system does not necessarily result in a proportional increase in the number of resulting spat. The aim of this study was to test this hypothesis and to determine an optimal set density for larvae of *P. maxima* .

3.2 Materials and Methods

P. maxima larvae were reared as described in Chapter 2 (section 2.4). Twelve 15 L aquaria, which had been soaked in seawater for 2 weeks, were used as settlement containers. The inner surfaces of the aquaria provided settlement substrata. In addition, two easily retrieved flat PVC slats (100 mm long x 75 mm wide x 1.5mm thick) were placed in each aquarium to facilitate counting and observations of settled spat. Aquaria were filled with 10 L of seawater filtered to 1 μm (nominal). Sixteen day-old pediveliger larvae from a routine production trial were isolated on a 200 μm sieve and added to the aquaria at different densities. Four density treatments, each with four replicates, were assessed: these were; 0.5 larva per mL; 1.0 larva per mL; 1.5 larvae per mL and 2.0 larvae per mL. Seawater in each aquarium was changed every 2 days and larvae that had not yet settled were retained on a 200 μm sieve and returned. After the third water

change, seawater filtered to 5 μm (nominal) was used. Larvae were fed twice daily with a mixture (on an equal dry weight basis) of *Chaetoceros muelleri*, *Isochrysis* aff. *galbana* (T-ISO) and *Teraselmis suecica*. Algal dry weights were obtained from literature values for the three micro-algal species (Nell and O'Connor, 1991; O'Connor et al., 1992). Spat were initially fed 30,000 cells per mL per day; this amount was increased by 5,000 cells per mL per day during the course of the experiment to approximate feeding regimes used for commercial production.

On days 23 and 44, the number of spat per 100 cm^2 was estimated by removing the two PVC slats from each aquarium, placing a piece of clear plastic over the upper surface of each slat and counting the number of spat in a 100 cm^2 grid drawn on the plastic. The counts from the two slats were averaged. SLs of 10 randomly selected spat from each aquarium were measured weekly from day 23 to 44 using a light microscope fitted with a calibrated eyepiece.

All experimental data were compared using one-way ANOVA (Sokal and Rohlf, 1981) with means compared using Fisher's Protected Least Significant Difference (PLSD) test. Homogeneity of variances was confirmed with Cochran's test (Snedcore and Cochran, 1967).

3.3 Results

The mean (± s.e., n=4) number of spat per 100 cm^2 on day 23 and day 44 at each of the four larval densities is shown in Fig 3.1. After day 23, the lowest number of spat on the PVC slats (67 ± 7 spat per 100 cm^2) was shown in the 0.5 larva per mL treatment. This value was significantly lower than in all other treatments (P <0.05). Larvae stocked

at a density of 1 per mL resulted in spat at a mean density of 220 ± 18 per 100 cm^2 while those stocked at 1.5 per mL produced spat at a mean density of 296 ± 50 per 100 cm^2. There was no significant difference in the density of spat between these two treatments. Larvae stocked at a density of 2 per mL resulted in a spat density of 321 ± 31 per 100 cm^2 at day 23. While this value was significantly greater than that of the 1.0 per mL treatment (P <0.05) it did not differ significantly from that of the 1.5 per mL treatment (P >0.05). This pattern was the same on day 23 and day 44.

The high level of variation between replicates other than 0.5 larva per mL treatment made statistical analysis of survival data between the two sampling periods difficult. However, the highest survival was 84.43 ± 2.69 % (mean ± s.e.) at a set density of 0.5 larvae per mL. The mean survival of spat from the 1.0 larvae per mL, 1.5 larvae per mL and 2.0 larvae per mL treatments were 72.9 ± 10.3 %, 63.7 ± 10.5 % and 65.7 ± 9.5 %, respectively.

There were, however, significant differences (P <0.05) in SL between treatments (Fig 3.2). Differences in size were apparent on day 23. By day 44, the spat in the 0.5 larva per mL treatment were significantly larger (P <0.05) than spat in any other treatment. Spat in the 1.0 larva per mL treatment were significantly larger than those in either the 1.5 or 2.0 larvae per mL treatments. There was no significant difference (P >0.05) in the SL of spat in the 1.5 and 2.0 larvae per mL treatments.

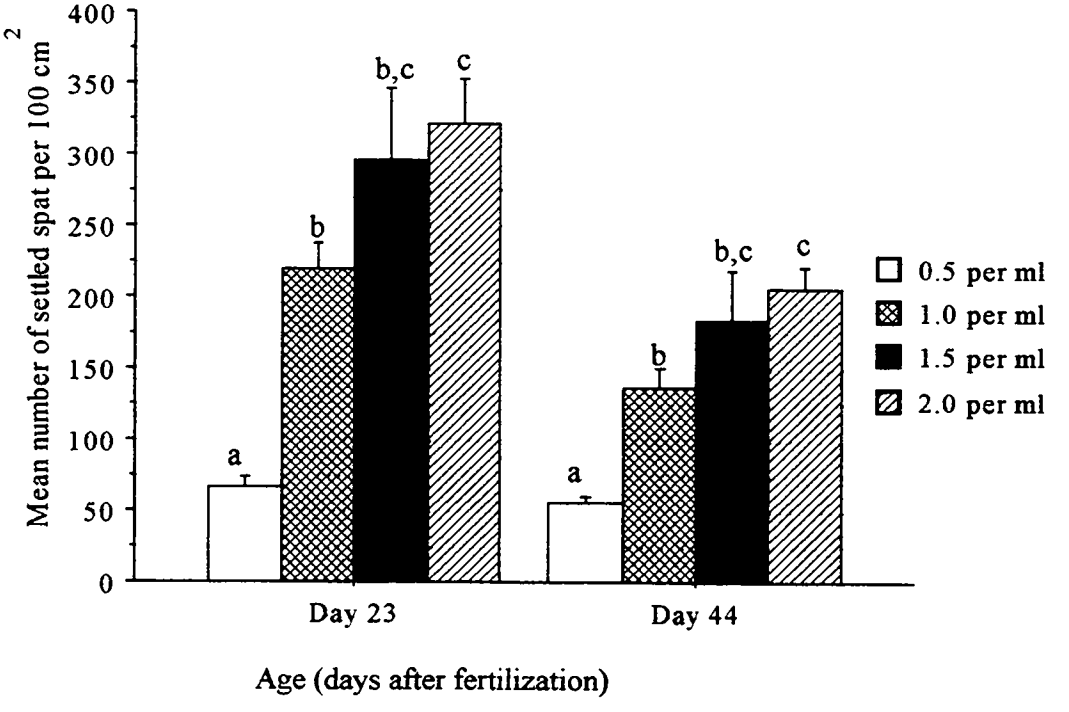


Fig 3.1 **Mean (± s.e., n=4) spat density on day 23 and 44 resulting from different larval stocking densities. Values with the same superscript for each day do not differ significantly (P>0.05).**

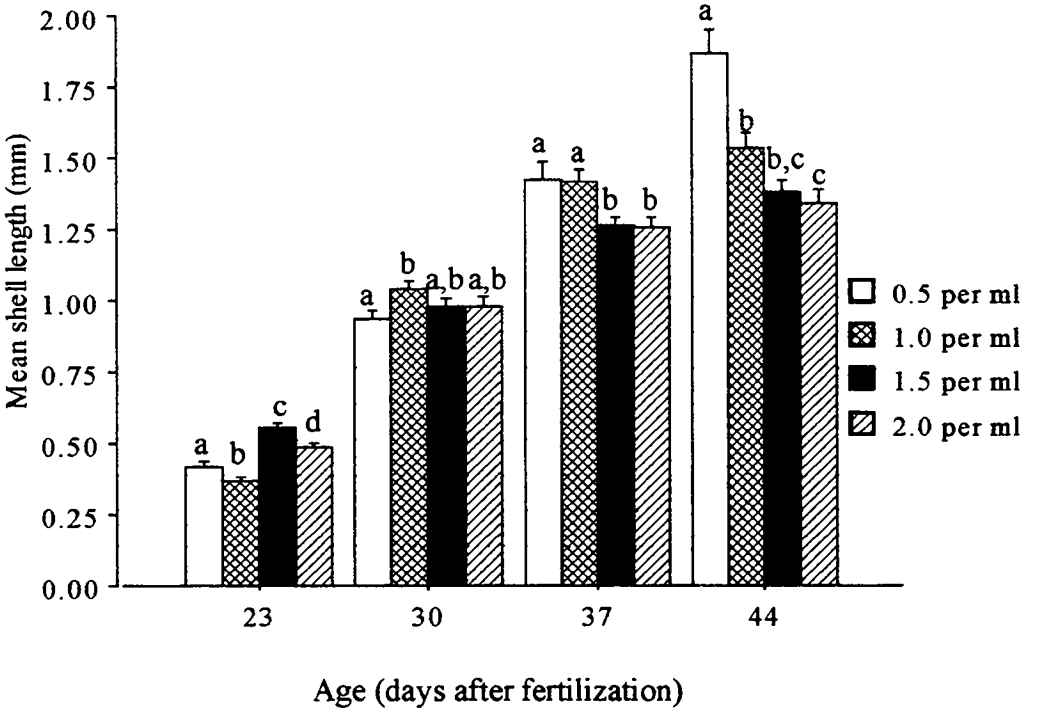


Fig. 3.2 **Mean (± s.e., n=4) antero-posterior shell length (SL) of *P. maxima* spat set at different stocking densities at 23, 30, 37 and 44 days after fertilization. Values with the same superscript for each day do not differ significantly ($P>0.05$).**

3.4 Discussion

Although the lowest larval density (0.5 larva per mL) produced the smallest number of spat per 100 cm^2, it resulted in the best growth in terms of shell length and the highest survival. The increase in the number of spat per 100 cm^2 from a larval density of 1.0 larva per mL was over double, and significantly greater, than that for a larval density of 0.5 larva per mL. Further increases in larval density to 1.5 larvae per mL and 2.0 larvae per mL did not bring about a proportional increase in the number of resulting spat. This implies that setting *P. maxima* larvae at densities greater than 1 per mL is not an efficient means of increasing spat production. Bourne et al. (1989) and Bourne and Hodgson (1991) reported similar results with the scallop, *Patinopecten yessoensis*, when survival of larvae during metamorphosis was higher at a density of 1.0 larva per mL than at a density of 0.5 larva per mL; however, further increases in larval density at settlement had a negative effect on survival. Early studies by Bourne et al. (1989) recommended that set densities for *Pat. yessoensis* should be no higher than 2 larvae per mL; however, this was later reduced to 0.5 larva per mL following further research (Bourne and Hodgson, 1991).

The large variation in survival within treatments for larval densities of 1.0, 1.5 and 2.0 larvae per mL, suggests that the ability to predict settlement numbers becomes increasingly more difficult above a setting density of 0.5 larva per mL. In commercial setting operations with *P. maxima* larvae, large variations in spat recovery were observed at larval densities between 2.0 and 3.0 larvae per mL with heavy mortality experienced in some settlement tanks but not others (J. J. Taylor, unpublished data, 1994-1996). Excessively heavy settlement may predispose spat to mortality following slight

environmental changes that in normal circumstances would not be a problem. This may result from the increased population pressure on limited resources such as food, space and water quality.

Increasing the numbers of spat on collectors detrimentally affects growth and survival of *P. maxima* in sea-based nursery systems and produced higher incidences of growth deformity (see Chapter 10). In Chapter 10, growth and survival of *P. maxima* declined when stocking density was increased from 1.3 spat per 100 cm^2 to 20 spat per 100 cm^2. Both these densities are well below that resulting from larval set densities used in this study. Similarly, heavy natural settlement of Sydney rock oysters, *Saccostrea commercialis*, has been shown to contribute to mortality during nursery rearing (Holliday et al., 1993).

P. maxima spat settled at the two highest densities (1.5 and 2.0 larvae per mL) were significantly larger than those in the two lower densities (0.5 and 1.0 larva per mL) on day 23. This difference in size may be due to differences in the rate of settlement between treatments. Larvae at higher densities may have settled and metamorphosed more rapidly than those at lower densities, possibly as a result of a higher concentration of a settlement triggering substance. During settlement, a mucus is produced by *P. maxima* larvae which becomes most obvious when they are held in higher densities. This mucus may contain chemical cues that help induce settlement. A variety of natural and synthetic chemicals that trigger settlement in bivalves have been described (Keck et al., 1971; Weiner et al., 1982; Coon and Bonar, 1985; Bourne et al., 1989; Weiner et al., 1989; Soniat et al., 1991; Chevolot et al., 1991; Pascual and Zampatti, 1995). As the experiment progressed, the differences in spat density began to affect growth and, by day

44, spat at the lowest density (0.5 larva per mL) were significantly larger than spat in all other treatments. As all treatment received the same micro-algal ration, competition for food could account for much of the observed differences in size at the end of the study.

This is the first study on larval set density for *P. maxima* and the results will provide a useful guide for commercial hatcheries. For efficient production of *P. maxima* spat, a larval density at settlement of no more than 1.0 larva per mL is recommended. However, based on these results, a spat density approximating 70 spat per 100 cm^2 is suggested to maximize growth and survival in the hatchery.

Chapter 4. Assessment of artificial substrata for collection of hatchery-reared *Pinctada maxima* spat

4.1 Introduction

Efficient hatchery culture of bivalve molluscs requires a simple, reliable and cost-effective means of collecting and deploying juveniles (spat). Attachment to substrata by bivalve and other marine larvae is not a completely random event and, in order for larvae to metamorphose, suitable substrata must be available. Larvae are attracted to particular substrata and set in particular patterns on a given surface to maximize survival (Wethey, 1986). A number of physical, chemical and behavioural factors have been shown to influence larval settlement in bivalves (Butler, 1955; Hidu, 1969; Ritchie and Menzel, 1969; Hidu and Haskin, 1971; Ajana, 1979; Phelger and Carey, 1983; Weiner et al., 1989; Fitt et al., 1990; O'Foighil et al., 1990; Tritar et al., 1992; Turner et al., 1994; Zimmer-Faust and Tamburri, 1994). Chapter 3 demonstrated that larval set density influenced settlement with 1 larva per mL being an appropriate stocking density.

Many studies have investigated the use of collectors for setting bivalve larvae. This research has included investigation into: substratum or collector material (Hidu et al., 1981; Phelger and Cary, 1983; Soniat et al., 1991; Holliday et al., 1993; Holliday, 1996); collector orientation and surface contour (Hopkins, 1935; Bonnot, 1937; Thomson, 1950; Cranfield, 1970; Ajana; 1979; Holliday, 1996) and conditioning of collectors with biofilms (Weiner et al., 1989; Fitt et al., 1990; O'Foighil et al., 1990; Tritar et al., 1992).

It has been shown that specific chemicals associated with settlement surfaces can induce settlement and metamorphosis of oyster larvae (Keck et al., 1971; Coon et al,

1985; Bonar et al., 1990; Zimmer-Faust and Tamburri, 1994). Wiener et al. (1989) showed that biofouling on the surfaces of collectors by certain bacterial colonies positively influenced larval settlement, and made generally unattractive substrata more attractive to settling larvae. Encouraging the growth of diatomaceous films on collector surfaces increased settlement and early growth of the scallop, *Patinopecten yessoensis*, (O'Foighil et al., 1990). O'Foighil et al. (1990) suggested that as well as providing a settlement cue, diatoms may have provided nutrition to metamorphosing larvae by means of "deposit feeding". Clearly, greater understanding of how these factors influence settlement and post-settlement survival of a particular species will allow improvement in the efficiency of setting operations in commercial bivalve hatcheries.

Previous studies on settlement and post-settlement survival *Pinctada maxima* have been inconclusive. Minaur (1969) offered the following substrata to crawling *P. maxima* pediveligers: glass, glass wool, byssus threads from juvenile *P. maxima*, string, nylon thread, grooved, bamboo, sand grains, beach stones and bivalve shells. He also treated replicate substrata with "oyster extract" prepared by macerating adult *P. maxima* tissues in seawater. Although some pediveligers began to settle, metamorphosis failed in all cases and no spat resulted, regardless of the type of substratum or the use of "oyster extract". Consequently, Minaur (1969) could not make any recommendation on collector type or collector preparation. Rose (1990) and Rose and Baker (1994) used a variety of materials (glass, PVC plates, artificial rope, shade cloth and mono-filament nylon) as settlement substrata for *P. maxima* larvae. However, these studies were unable to demonstrate that one type was better than another. Although the number of hatcheries propagating *P. maxima* in Australia and south east Asia has increased dramatically over

recent years, there is a paucity of information on the use of spat collectors for hatchery reared *P. maxima*. The aim of this study was to generate information on the efficiency of a variety of collector materials and on the effects of collector orientation and the presence of a biofilm on collectors.

4.2 Materials and Methods

All experiments were conducted in commercial silver-lip pearl oyster hatcheries during routine spat production trials. The number of larvae and the age of larvae at settlement varied with location and season. In all cases, aeration was moderate during settlement and vigorous after settlement was completed. Water exchange following settlement was achieved using constant flow-through with at least a 100% water exchange each day. Experiments 1 and 3 were conducted in Indonesia (see Chapter 2) where the water temperature and salinity were 28 ± 0.5°C and 34 $^{o}/_{oo}$, respectively. Experiment 2 was conducted in Australia where the water temperature and salinity during the experiment were 28 ± 1°C and 36 $^{o}/_{oo}$, respectively. It was important to conduct the experiments under commercial conditions and this limited the number of tanks available for experimental use. In all experiments, spat culture conditions were based on those described by Rose (1990) and Rose and Baker (1994).

4.2.1 Experiment 1: Collector-type

For this experiment, 100 nine-pocket net panels made from polypropylene rope netting stitched to a powder coated steel frame (560 mm x 560 mm) were used as the basis for the 4 types of spat collectors examined (Fig 4.1). Each type of collector varied

in the substratum it contained. The substrata used were: 1) white PVC slats (used in all three experiments) with a rough-textured (grooved) concave surface and a smooth convex surface (Fig 4.2); 2) black polypropylene rope; 3) translucent mono-filament nylon; and 4) a combination of 1 and 2. These materials were chosen on the basis of availability and ease of collector construction. PVC slats were woven through the collector netting while the rope and mono-filament nylon were simply placed into the pockets of the net. Twenty-five replicate collectors were made for each of the following collector types:

1) six PVC slats (500 x 75 mm) per collector;
2) polypropylene rope, in each pocket of the collector;
3) mono-filament nylon, in each pocket of the collector;
4) six PVC slats, plus polypropylene rope in each pocket of the collector.

All materials used for making the collectors were soaked in seawater prior to the experiment to leach out any potential toxins (Gunn, 1984).

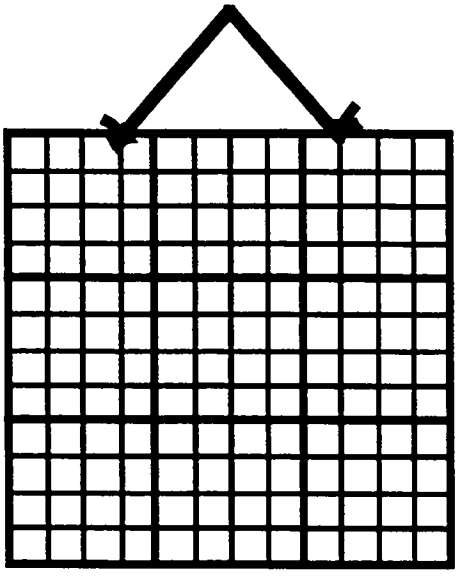


Fig 4.1 **A net panel (560 x 560 mm) used in experiment 1 to form a supporting frame for different types of collector material: PVC slats were woven through the netting; rope and mono-filament nylon were placed into each of the 9 pockets.**

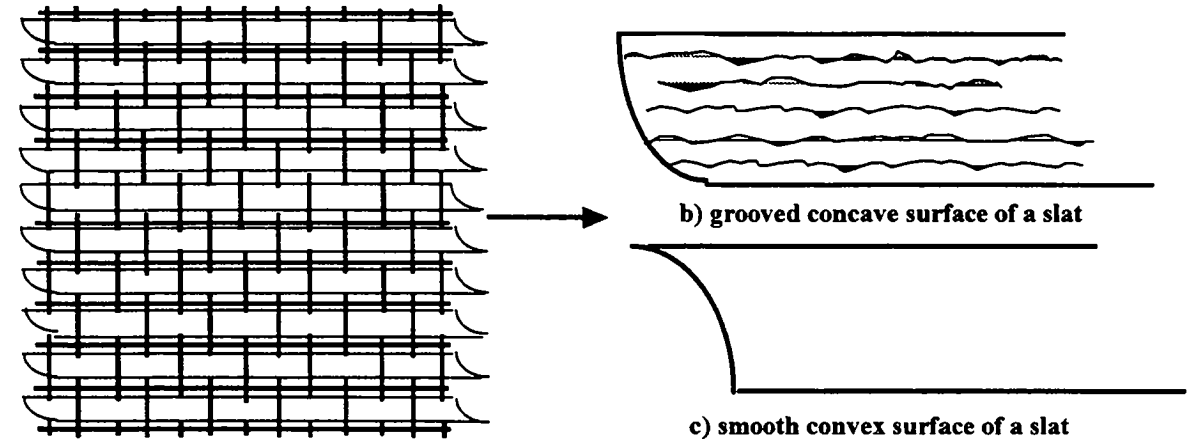


Fig 4.2 **The arrangement of PVC slat collector units (a) used to set *Pinctada maxima* pediveliger larvae. The slats were white with a grooved concave surface (b) and a smooth convex surface (c). The units were either placed in the tanks such that the slats were horizontally oriented (as shown) or the units were rotated 90° so that the slats were vertically oriented. When oriented horizontally, the concave surface of the PVC slats was uppermost.**

The finished collectors were acid washed, pressure cleaned with fresh water and sun dried before being placed in a 2,000 L fibreglass tank. The tank was filled with seawater filtered to 1 μm before 16-day old pediveliger larvae were added at a density of 1 per mL. Thirty-seven days later, 6 replicate collectors of each type were removed from the tank and the number of spat on each counted.

Due to difficulties in accurately estimating the surface area of rope and mono-filament nylon, different collector types were not made up to present the same surface area for larval settlement. Rather, the aim was to look at different materials that could be used to make up collector units (based on the 9 pocket net panel) that would take up the same space within a settlement tank.

4.2.2 Experiment 2: Collector Orientation

PVC slats were used as the settlement substratum. Collector units were made by weaving ten slats, each measuring 500 mm x 75 mm, through sheets of black plastic mesh (50 mm^2 aperture). Each collector unit held 10 individual PVC slats with the slats spaced approximately 25 mm apart. A total of 40 collector units were made. As in experiment 1, the units were soaked in seawater before use. After soaking, the units were pressure cleaned then dipped in a bath of 10% hydrochloric acid (HCl) for 15 min. and again pressure cleaned with fresh water.

The units were placed in a 1,000 L fibreglass tank with the mesh holding the slats positioned vertically. All collector units were placed at the same height. Twenty units had the slats oriented vertically and 20 units had the slats oriented horizontally (Fig 2). When oriented horizontally, the concave surface of the PVC slats was uppermost. Horizontally

oriented slats within each collector unit were at different heights within the water column, from close to the water surface to near the tank floor. The tank was filled with seawater filtered to 1 μm before pediveliger larvae were added. To take account of different rates of larval development, larvae were added to the tank over a 5 day period to give a final density of 1 larva per mL. The first pediveligers added to the tank were 21 days old. Forty-five days after addition of the first larvae, 5 vertical PVC slat units and 5 horizontal PVC slat units were removed from the tank and the number of spat on each slat in each unit were counted. Spat were counted on both the convex and concave surfaces.

4.2.3 Experiment 3: Conditioning Collectors with a Biofilm

Short PVC slats measuring 100 mm long x 75 mm wide x 1.5 mm thick were used as the settlement substratum. Two treatments were examined. In the first, 9 slats were acid washed in 10% HCl and pressure cleaned with fresh water. They were then placed in a 10 L aerated culture of the diatom *Chaetoceros muelleri* for 72 h to allow development of an epifloral biofilm. Seventy-two h has previously been shown to be the time required for bacterial biofilms to become most attractive to oyster larvae (Weiner et al., 1989). In the second treatment, 9 slats were acid washed and pressure cleaned before being placed in 10 L of chlorinated water for 12 h. They were then rinsed with fresh water and dried.

The slats from both treatments were placed randomly in a 1,000 L fibreglass tank. The tank was filled with 1 μm filtered seawater before 19 day-old *P. maxima* pediveligers were added at a density of 1 per mL. After 20 days, when spat were clearly visible, slats were removed and the number of spat on each counted. Ten spat from each PVC slat were measured with a calibrated eyepiece fitted to a light microscope.

4.2.4 Statistical Analysis

Data from experiment 1 were compared using one-way ANOVA (Sokal and Rohlf, 1981) with means compared pairwise using Fisher's protected least significant difference (PLSD) test. The number and size of spat from experiment 2 were compared using ANOVA with the variables for surface contour (convex or concave) nested within slat orientation (horizontal or vertical). For horizontally oriented slats, the numbers of spat on the slats at different heights within the water column were compared using one-way ANOVA. The number and size of spat from experiment 3 were compared using *t*-tests. Homogeneity of variances was confirmed with Cochran's test (Snedcore and Cochran, 1967).

4.3 Results

4.3.1 Experiment 1: Collector-type

At the end of the experiment, spat had a shell length of 3.7 ± 0.7 mm (mean ± s.e., n=50). The number of spat counted per collector varied depending on the type of collector used (Fig 4.3). The rope collectors had the highest number of spat (2,819 ± 327) followed by slat + rope (2,612 ± 439) slat (2,612 ± 439) and nylon (1,419 ± 155). The number of spat on the rope collectors was significantly higher than on either the slat or nylon collectors (P <0.05) but did not differ significantly from the number of spat on slat + rope collectors (P >0.05). Slat + rope collectors had a significantly higher number of spat than the nylon collectors (P <0.05). There was no significant difference between slat + rope and slat collectors or between slat and nylon collectors (P >0.05).

4.3.2 Experiment 2: Collector Orientation

Spat were 66 days old with a shell length of 4.6 ± 2.2 mm (mean ± s.e., n=50) when collectors were removed from the tank. Orientation of collectors and surface contour greatly affected the distribution of pearl oyster spat on the collectors. The number of spat was significantly higher (P <0.001) on horizontally positioned PVC slats (662 ± 62 spat per slat unit: 0.06 spat per cm^2) than on vertically positioned PVC slats (240 ± 59 spat per slat unit: 0.03 spat per cm^2). Regardless of orientation, surface contour also significantly influenced the number of resulting spat (P <0.001). The concave surface (uppermost in the horizontally positioned slats) received significantly more spat than the convex surface (Fig 4.4). The concave surface of the horizontal slats had a mean (± s.e., n=6) of 529 ± 58 spat per slat unit (0.14 spat per cm^2). The convex surface had 135 ± 11 spat per slat unit (0.04 spat per cm^2). Similarly, the concave surface of the vertical slats had 171 ± 48 spat per slat unit (0.05 spat per cm^2) and the convex surface had 72 ± 13 spat per slat unit (0.02 spat per cm^2).

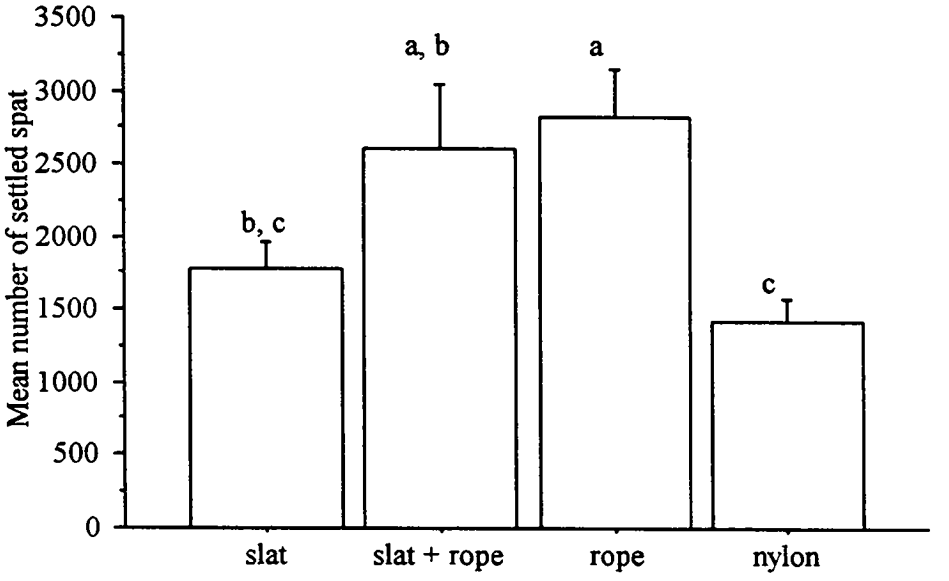


Fig 4.3 **Mean number (± s.e., n=6) of *Pinctada maxima* spat on five types of collectors used in experiment 1. Means with the same superscript are not significantly different (P >0.05).**

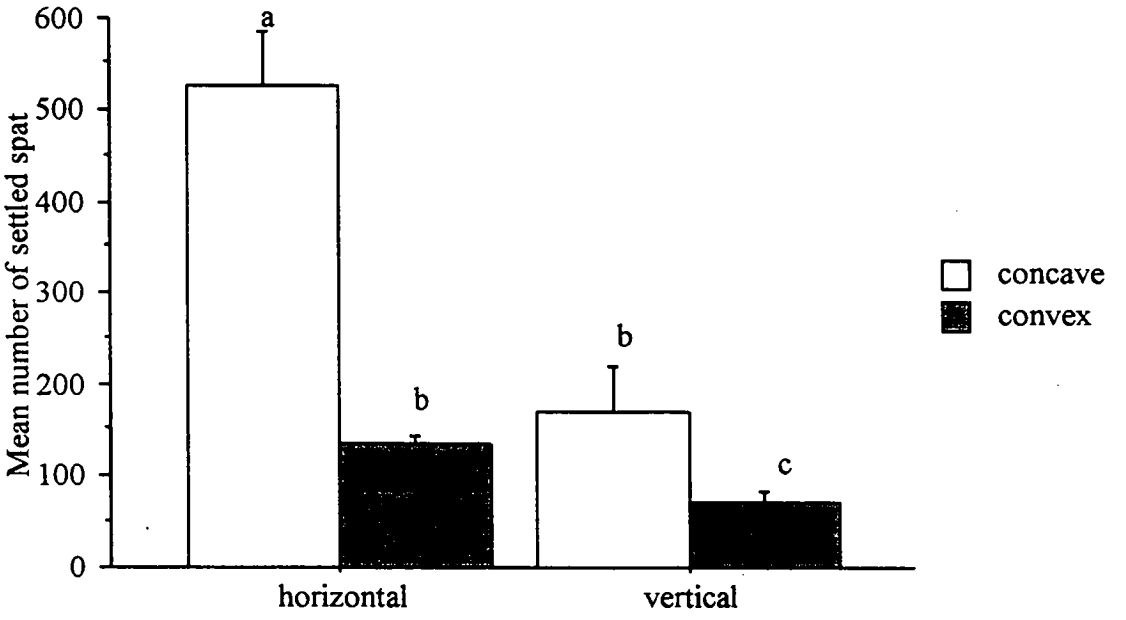


Fig4.4 **Mean number (± s.e., n=5) of *Pinctada maxima* spat on the concave and convex surfaces of PVC slat collectors positioned either horizontally or vertically. Means with the same superscript are not significantly different (P >0.05).**

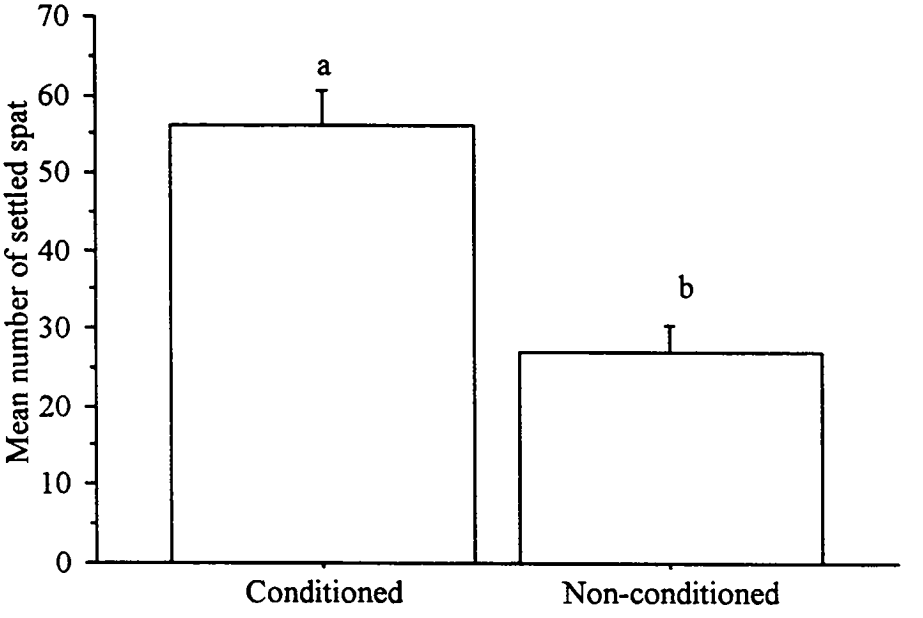


Fig 4.5 **Mean number(± s.e., n=5) of *Pinctada maxima* spat on conditioned and non-conditioned PVC slat collectors. Means with the same superscript are not significantly different (P >0.05).**

Differences in the number of spat on horizontally positioned slats at various heights in the water column were non-significant ($P > 0.05$).

4.3.3 Experiment 3: Conditioning Collectors with a Biofilm

When spat were removed from the set system for counting, there was no significant difference in size between treatments ($P > 0.05$: shell lengths 0.97 ± 0.03 mm and 0.98 ± 0.03 mm for conditioned and non-conditioned collectors, respectively). However, there was a significant difference ($P < 0.001$) between the numbers of spat on conditioned and non-conditioned collectors with more than twice the mean number of spat present on the conditioned collectors (Fig 4.5). Conditioned collectors had a mean of 56 ± 5 spat per slat (0.04 spat per cm^2) compared to 27 ± 3 spat per slat (0.02 spat per cm^2) on non-conditioned collectors.

4.4 Discussion

Net panel collectors filled with either rope strands or a combination of rope and PVC slats collected greater numbers of *P. maxima* spat than those composed of PVC slats alone or mono-filament nylon. In commercial rearing trials, up to 69,000 *P. maxima* spat have been observed on rope collectors of the type used in this study (J.J. Taylor unpublished data, 1996). Rope is also cost-effective and practical as old or damaged rope is readily available on pearl oyster farms. Although PVC slats were not as effective at catching spat as rope, they may offer advantages in grow-out. The large stable area of attachment offered by PVC slats allows spat to place byssal threads in a radial pattern providing a strong anchorage (see Chapter 8). On ropes, the area for attachment becomes

reduced as the animals grow which increases the chance of being dislodged in strong currents. Rose and Baker (1994) suggested that glass or plastic plates are more suitable for on-growing spat in areas of strong currents and large tides, whereas mono-filament nylon and shade cloth are better suited to areas with mild currents.

Mono-filament nylon, commonly used to collect bivalve spat, performed poorly under the conditions of this study. Phleger and Cary (1983) reported mono-filament nylon to be a "good" collector for the scallop, *Crassadoma* (formerly *Hinnites*) *gigantea* and Rose and Baker (1994) recommended its use for setting *P. maxima* larvae. The fibrous nature of rope and the roughened texture of the PVC slats may have provided better tactile stimuli for crawling pediveligers and/or spat than the comparatively smooth surface of mono-filament nylon. Fibrous substrata consistently resulted in higher numbers of scallop (*Crassadoma gigantea* and *Patinopecten yessoensis*) spat (Bourne and Bunting, unpublished data). Cranfield (1970) reported the greatest number of settled oysters (*Ostrea lutaria*) on plastered asbestos plates (which had the roughest surface and were completely opaque); fewer settled on sand blasted glass (which had a regular, smoother surface and were only slightly opaque) and the lowest number settled on smooth, clear glass.

The results of Experiment 2 showed that surface texture had a clear effect on the number of spat collected. Spat in this experiment overwhelmingly preferred the rough, concave surface of PVC slats than the smooth, convex surface when the collectors were oriented vertically. Factors such as water turbulence around the collectors, created as result of vigorous aeration, may have influenced the results. For example, Wethey (1986) showed that contour is an important cue in the settlement of barnacle larvae, with larvae

seeking out particular contours, especially where shear force and subsequent turbulence were low. Holliday (1996) reported significantly higher numbers of Sydney rock oyster, *Saccostrea commercialis*, spat on the concave surface of horizontally positioned PVC slats compared to the convex surface, but did not offer an explanation for this. In Holliday's experiment, the convex surfaces of horizontally positioned slats were uppermost (in contrast to this study); however, there was no significant difference between the number of spat on the concave and convex surfaces of slats deployed vertically (Holliday, 1996). Alagarswami et al. (1983) found that larvae of *Pinctada fucata*, were more attracted to the concave surface than to the convex surface of split bamboo collectors. Differences in the mechanics of attachment may also play a role in determining settlement patterns of bivalves. Spat of *P. maxima* and other pearl oysters, and those of scallops and mussels, byssally attach to substrata. In contrast, attachment in most Ostreidae ("edible" oysters) is achieved by cementing the left valve of the shell to the substratum (Cranfield, 1973). This fundamental morphological difference may influence settlement behaviour and choice of substratum by different species of bivalves.

The higher number of spat on horizontally placed collectors compared to those placed vertically is in general agreement with similar field and laboratory studies with edible oysters (Thomson, 1950; Cranfield, 1970; Holliday, 1996). The upper, concave surface of the horizontal PVC slats had the highest number of spat in the present study. However, reports of settlement on the upper or lower surfaces of collectors vary. Hopkins (1935) reported that *Ostrea lurida* preferred the underside of cardboard "egg crate" collectors. Negative phototactism was ruled out as the explanation for this behaviour as greater numbers of spat were collected on the lower surfaces of both clear and black

painted glass. It was suggested that because the larvae of *O. lurida* characteristically swim with the velum and ciliated foot uppermost, it was easier for pediveliger larvae to grasp the under-surface of settlement substrata, particularly when orientated horizontally. By contrast, Bonnot (1937) also studying *O. lurida*, found that spat preferred the upper-surface of layered collectors. It was concluded that because the flat surfaces of the collectors were closely spaced resulting in considerable turbulence, larvae would be unable to maintain a swimming position favouring settlement to the under-surfaces of collectors and the effect of gravity would therefore favour settlement on the upper-surfaces of the collectors (Bonnot, 1937). Cranfield (1970) also cited turbulence caused by collector orientation and arrangement (either singly or in a series) as a major factor in spatfall variation. Cranfield (1970) proposed that variation in experimental technique is largely responsible for conflicting reports on the settlement pattern of oyster larvae.

The level of illumination affects the settlement pattern of American oysters (*Crassostrea virginica*) with larval settlement preference being associated with light avoidance behaviour (Ritchie and Menzel, 1969). Similarly, larvae of the mangrove oyster (*Crassostrea gasar*) preferentially settled on shaded collectors (Ajana, 1979). Alagarswami et al. (1987) noted improved settlement of pearl oyster (*P. fucata*) larvae when dark-coloured tanks were used. The number of *P. maxima* spat on the horizontally oriented slats did not vary significantly with height in the water column, even though the number of spat attached to tank surfaces tends to be greatest on or near the tank floor, with progressively fewer spat towards the top of the tank (J. J. Taylor, unpublished data, 1996). As there were no differences in settlement of *P. maxima* on horizontally placed PVC slats at different heights, it is difficult to infer a strong influence of either light or

gravity; both would have resulted in greater numbers of spat on the lower parts of settlement units regardless of orientation. The recruitment pattern of *P. maxima* spat in this study would appear to have been most influenced by surface contour and texture. O'Connor et al. (1994) found doughboy scallops (*Chlamys* (*Mimachlamys*) *asperrimus*) settled preferentially on the floor of settlement tanks.

Promoting development of an epifloral biofilm by exposing PVC slats to the diatom *Chaetoceros muelleri* and associated bacteria, positively affected the number of spat on PVC slats. Double the number of spat were present on conditioned PVC slats compared with non-conditioned PVC slats. Newly settled bivalves may be unable to filter feed due to regression of the velum and the absence of a functional gill-based filtering mechanism. As such, nutrient reserves built-up during larval development must, therefore, provide nourishment to newly settled bivalves (O'Foighil et al., 1990). Some newly settled bivalves may be able to use the foot as a secondary feeding organ by gathering detritus, benthic algae and other material from the surfaces of substrata. King (1986) described this as "pedal-palp feeding". O'Foighil et al. (1990) found that the Japanese scallop (*Patinopecten yessoensis*) preferentially settled on collector material conditioned with an epifloral film of diatoms. During the first week after settlement, growth of newly settled scallop spat on "fouled cultch" was significantly greater than spat on "clean cultch". However, there was no evidence that the presence of an epifloral film enhanced subsequent spat growth. Additionally, the importance of an epifloral film was much less than that of suspended micro-algae for early juvenile growth (O'Foighil et al., 1990). The present study did not test the effects of epifloral biofilms on the early growth of *P. maxima* spat; however, 20 days after the start of settlement, there was no significant

difference in mean shell length between spat on the conditioned slats and those on non-conditioned slats. This suggests that even if there was some nutritional benefit from the presence of an epifloral biofilm during the first few days after settlement, it quickly loses importance as the spat become competent filter feeders.

Biofilms may provide important chemical cues for larval settlement. Weiner et al. (1989) demonstrated that biofilms of the marine bacterium, *Alteromonas colwelliana*, increased settlement of edible oysters (*Crassostrea gigas* and *C. virginica*) on glass, polystyrene and mylar surfaces; substrata not generally conducive to larval settlement. Bacterial exopolysaccharide (EPS) or a molecule bonded to EPS appear to provide a chemical cue to larval settlement. Supernatants from cultures of the bacteria, *A. colwelliana* and *Vibrio cholerae*, promoted settlement behaviour in *C. gigas* veliger (Fitt et al., 1990). In a similar study, biofilms of the bacterium, *Shewanell colwelliana*, promoted settlement of *C. gigas* and *Ostrea edulis*, but did not stimulate settlement behaviour of scallop (*Pecten maximus*) larvae (Tritar et al., 1992). Tritar et al. (1992) suggested that the bacterium might not have been effective in promoting settlement of *Pec. maximus* as the culture temperature was sub-optimal (16^{o}C) for the growth of *S. colwelliana*. More recently, low molecular weight peptides with arginine at the C terminal, were identified by Zimmer-Faust and Tamburri (1994) as natural inducers of oyster, *C. virginica*, settlement. Similar chemical cues may have been responsible for promoting the greater number of *Pinctada maxima* spat on collectors with a biofilm in this study.

The materials used to make collectors, the orientation of collectors and the presence or absence of an epifloral biofilm clearly influenced recruitment of *P. maxima*.

Based on the results of this study, old rope is recommended for collector construction. If materials such as PVC slats are to be used then they are best oriented horizontally and encouraging the development of an epifloral biofilm may further enhance their performance.

Chapter 5. The nutritional value of five species of micro-algae for *Pinctada maxima* spat

5.1 Introduction

Providing an adequate diet for the early life stages of bivalves is essential to hatchery success. Despite efforts to develop artificial alternatives (Langdon and Siegfried, 1984; Chu et al., 1987; Southgate et al., 1992; Southgate et al., 1998) cultured micro-algae remain a critical resource for commercial rearing of marine animals (Brown and Jeffrey, 1992). The nutritional value of a given of micro-alga depends on the nature and composition of its biochemical constituents (Whyte et al., 1990) and its physical suitability (Rose and Baker, 1994). There is considerable variability in the ability of different algal species to support adequate growth of bivalves (Brown and Jeffrey, 1992).

Whilst hatchery production of *Pinctada maxima* is firmly established in Australia and south east Asia (Gervis and Sims, 1992; O'Sullivan, 1994; Rose, 1994) there is very little published information on the nutritional requirements of *P. maxima* and the suitability of different species of cultured micro-algae for this species. Minaur (1969) reported on comparative feeding trials with larvae of *P. maxima* and, although there are a number of reports on rearing *P. maxima* spat (Tanaka and Kumeta, 1981; Rose, 1990; Rose and Baker, 1994), no information on the nutritional value of various micro-algal species has yet been published. There is, however, a plethora of information regarding the nutritive value of micro-algal species for larvae and spat of other bivalves such as the Pacific oyster, *Crassostrea gigas* (Langdon and Waldock, 1981; Laing and Verdugo,

1991; Laing and Millicen, 1992; Thompson et al., 1993) the European flat oyster, *Ostrea edulis* (Walne, 1963 and 1970; Laing and Verdugo, 1991) the Sydney rock oyster, *Saccostrea commercialis* (O'Connor et al., 1992); the clams, *Mercenaria mercenaria* (Laing and Verdugo, 1991; Wikfors et al., 1992) and *Tapes phillipinarum* (Laing and Millican, 1991; Laing and Verdugo, 1991) mussels, *Mytilus galloprovincialis* (Fidalgo et al., 1994) and the scallops, *Patinopecten yessoensis* (Whyte et al., 1989) and *Crassadoma gigantea* (Whyte et al., 1990).

Tropical aquaculture hatcheries are still largely reliant on species of micro-algae isolated from cooler, temperate waters (Jeffrey et al., 1992). Hatchery propagation of *P. maxima* in Australia typically uses isolates from temperate waters cultured at cooler than ambient temperature (O'Sullivan, 1994). This in itself may be a problem when micro-algae cultured at a relatively low temperature are fed to bivalve larvae or spat cultured at a higher water temperature. For example, Tanaka and Inoha (1970) did not recommend the use of *Pavlova lutheri* as a food black-lip pearl oysters (*P. margaritifera*) because when the micro-alga was taken from an optimum growing temperature of 20°C and introduced into larval culture vessels at 28-30°C it quickly died. However, this alga is still widely used in commercial tropical pearl oyster hatcheries (Gervis and Sims, 1992) and was recommended as a food for *P. maxima* larvae by Minaur (1969).

This study assessed five species of micro-algae for their nutritional value *P. maxima* spat. The five species were selected on the basis of their ease of culture under tropical conditions and their reported nutritional values; they were the golden-brown flagellates, *Isochrysis* aff. *galbana* (clone T-ISO) and *Pavlova lutheri*, the diatoms, *Chaetoceros muelleri*, and *C. calcitrans* and the green flagellate, *Tetraselmis suecica*. T-

ISO and *C. muelleri* are rated as good sub-tropical to tropical species (temperature range 15-30 °C) *P. lutheri* as a good sub-tropical species (10-25 °C) and *C. calcitrans* and *T. suecica* as excellent universal species (10-30 °C) (Jeffrey et al., 1992). All five species have previously been used at 25 ± 1 °C with moderate to excellent results with Sydney rock oyster (*Saccostrea commercialis*) spat (O'Connor et al., 1992).

5.2 Materials and Methods

Batches of 13 hatchery reared *P. maxima* spat were held in 1 mm mesh baskets (100 mm x 70 mm x 30 mm) and individual baskets were suspended in 750 mL aerated plastic aquaria. Each aquarium contained 1 µm filtered UV sterilized seawater (36 $^{o}/_{oo}$). At the start of the growth trial, spat were 75 days old with a mean SL and WW of 4.48 ± 0.03 mm and 5.59 ± 0.11 mg (mean ± s.e., n = 312) respectively. Fifteen individuals were dried at 50 °C for 48 h and then heated at 500 °C for 4 h to determine initial ash content. The ash free dry weight (AFDW) was calculated as the difference between dry weight and ash weight. The mean AFDW at the start of the experiment was 0.25 mg.

Starter cultures for the five species of micro-algae assessed in this (*Isochrysis* aff. *galbana* {clone T-ISO, CS-177} *Pavlova lutheri* {CS-182} *Chaetoceros calcitrans* {CS-178} *C. muelleri* {formerly *C. gracillis* , CS-176} and *Tetraselmis suecica* {CS-187}) were obtained from the CSIRO, Hobart, Australia and codes in brackets refer to the CSIRO catalogue codes. Algae were cultured in 2 L borosilicate flasks using f medium (Guillard, 1972) in 0.2 µm filtered seawater. Cultures were maintained at 24 ± 0.5 °C using a 14:10 h light: dark cycle. Cultures were harvested for feeding to spat during the exponential or log growth phase. Each species was fed on an equal dry weight basis,

using previously published dry weight values (Nell and O'Connor, 1991; O'Connor et al., 1992) at an initial daily ration equivalent to 80,000 T-ISO cells per mL per day. This amount was increased every four days by 5,000 cells per mL per day to approximate feeding regimes used for commercial production of *P. maxima* spat. An unfed control treatment was also included and each treatment was assessed in triplicate.

The experiment was terminated after 21 days. Shell length was determined for individual spat. WW and AFDW were determined by weighing each replicate group of spat and dividing by the number of surviving individuals. Length and weight data were compared using one-way ANOVA (Sokal and Rohlf, 1981) with means compared pairwise using Fisher's protected least significant difference (PLSD) test. Homogeneity of variance was confirmed with Cochran's test (Snedcore and Cochran, 1967).

5.3 Results

Survival was generally high during the experiment (> 73%) with the exception of one replicate fed *C. calcitrans* . This replicate suffered high mortality (62%) in the final week of the trial with only five live animals retrieved. The reason for this mortality was unclear. Disregarding this replicate; there were no significant differences (P >0.05) in survival between treatments.

Growth (SL, WW and AFDW) of spat fed *C. muelleri* was significantly greater (P <0.05) than for any other species tested (Fig 5.1). SL and WW, did not differ significantly (P >0.05) between spat fed either *P. lutheri*, *C. calcitrans*, T-ISO or *T. suecica* and the unfed control group. There was no significant difference (P >0.05) in final AFDW between the unfed control and spat fed *P. lutheri*. Spat fed any of the other species had significantly greater AFDW than the unfed controls (P <0.05). Final AFDW of spat did not differ significantly (P >0.05) between *C. calcitrans* and *P. lutheri* fed spat or between T. ISO and *T. suecica* fed spat (Fig 5.1).

5.4 Discussion

Of the five species of micro-algae species assessed, *C. muelleri* supported the greatest increase in SL, WW and AFDW of *P. maxima* spat. These results support those of similar growth trials with bivalves. For example, Enright et al. (1986) ranked *C. gracillis* (=*C. muelleri*) as the best single species diet when compared with 16 other micro-algal species.

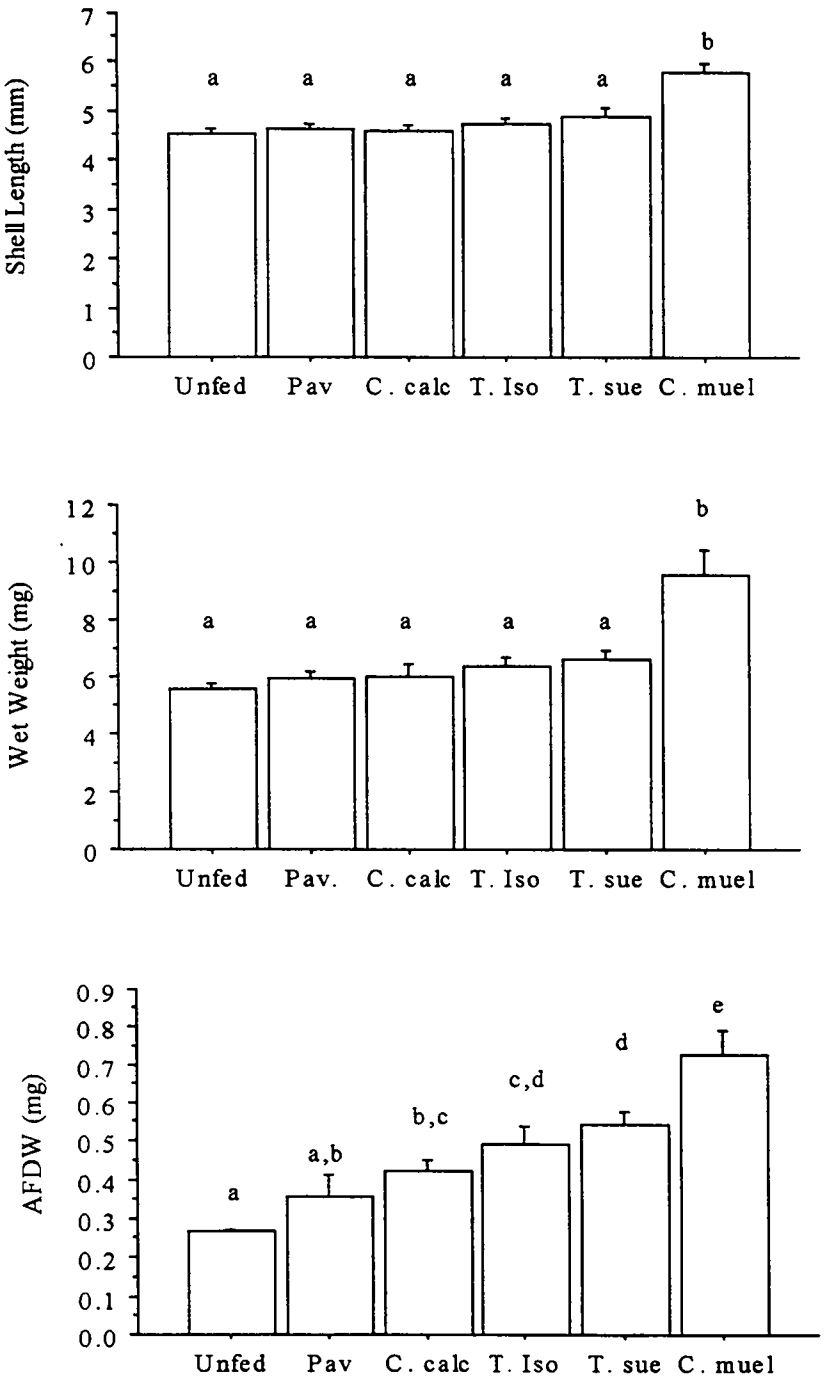


Fig 5.1 **Mean (± s. e., n=4) shell length, wet weight, and ash free dry weight (AFDW) of *P. maxima* spat fed five different species of micro-algae after21 d growth trial. Initial = AFDW before growth trial, Unfed = unfed control, Pav = *P. lutheri*, C. calc. = *C. calcitrans*, T. Iso = T-ISO, T. sue = *T. suecica* and C. muel. = *C. muelleri*. Values different superscripts are significantly different ($P< 0.05$).**

O'Connor et al. (1992) rated *C. muelleri* very highly as a diet for Sydney rock oyster (*Saccostrea commercialis*) spat, and not significantly different ($P > 0.05$) from the best performing mono-specific diet, *Skeletenoma costatum* .

The result obtained for *T. suecica* is interesting as there are conflicting reports of its nutritional value. Walne (1970) reported that "...species of *Tetraselmis* are of outstanding value as food for juvenile bivalves"; however, Langdon and Waldock (1981) and Laing and Verdugo (1991) described *T. suecica* as being of "moderate food value" for juvenile bivalves. Epifanio (1979) and Laing and Verdugo (1991) suggested that the food value of *T. suecica* is enhanced when fed as part of a mixed species diet. Poor performance of *T. suecica* in other feeding trials has been ascribed to difficulties met by spat in digesting the theca of this alga (Epifanio, 1979). Indeed, Heasman et al. (1996) found that *T. suecica* was poorly ingested and not digested by adult commercial scallop (*Pecten fumatus*). Similar feeding difficulties associated with *T. suecica* were not apparent in the present study. Clearly, the experimentally derived nutritional rating of a micro-algal species depends to a great degree on what it is compared with and to which species it is fed.

The remaining species produced moderate to poor growth. The low growth rates of spat fed *C. calcitrans* and *P. lutheri* are surprising, as both are generally highly rated foods for bivalves (Brown et al., 1989). In a similar trial with Sydney rock oyster spat, *P. lutheri* performed moderately well and not significantly different to *T. suecica*. *C. calcitrans* performed as well as *S. costatum* , which was the best species trialed (O'Connor et al., 1992) slightly better, although not significantly so, than *C. gracillis* (=

C. muelleri) and a great deal better than *T. suecica* (O'Connor et al. , 1992). As the seawater temperature was 25 ± 1 °C in both trials, it would seem that there may be differences in the nutritional requirements of *Pinctada maxima* spat or with the palatability of *P. lutheri* and *C. calcitrans*. As the nutritional value of micro-algae varies according to growing conditions (Walne, 1970) local conditions may have influenced the nutritional quality and subsequent performance of both species. The use of *P. lutheri* for the black-lip pearl oyster, *Pinctada margaritifera* was questioned by Tanaka and Inoha (1970); problems were associated with algal morbidity due to differences between algal and larval culture temperatures. However, in this study the temperature difference between the algae and the spat culture vessels was slight (1-2 °C). Minaur (1969) rated *P. lutheri* and T-ISO as the best two species to feed to *Pinctada maxima* larvae after comparing larval growth rates when fed 11 different mono-specific diets. Growth of larvae was further enhanced when equal numbers of cells of *P. lutheri* and T-ISO were fed as a mixture. As has been suggested for other bivalves (O'Connor et al., 1992), there may well be differences in the nutritional requirements of *Pinctada maxima* larvae and spat.

There are conflicting reports on the relationship between the gross biochemical composition of micro-algae and their nutritional value. In a feeding trial with scallop (*Patinopecten yessoensis*) larvae, the level of carbohydrate in the diet was highly correlated with larval quality (Whyte et al., 1989). *T. suecica* has more than double the carbohydrate content of *C. calcitrans* and T-ISO, and a third more than *P. lutheri* (Volkman et al., 1989). However, Wikfors et al. (1992) found that the importance of dietary carbohydrate was far less than that of protein and lipid, which were found to be

the most important gross biochemical constituents for clam nutrition. *T. suecica* has relatively low amounts of dietary lipid but similar amounts of protein compared to the other species trailed (Volkman et al., 1989).

Bivalves have a dietary requirement for the long-chain highly unsaturated fatty acids (HUFA) eicosapentaenoic acid (20: 5n-3; EPA) and docosahexaenoic acid (22: 6n-3; DHA) (Trider and Castell, 1980; Langdon and Waldock, 1981; Enright et al., 1986), and species of micro-algae rich in these HUFA are generally assumed to be of high nutritional value (Brown et al., 1989). The fatty acid profiles of a number of species of micro-algae originating form the same laboratory as the stock used in this study (CSIRO, Hobart) have previously been determined (Volkman et al., 1989). Volkman et al. (1989) reported significant levels of EPA in *C. calcitrans*, *C. gracilis* (= *C. mulleri*) and *P. lutheri*, and low levels in T-ISO and *T. suecica*. The same study also reported trace amounts of DHA in *T. suecica*, small amounts (<0.8% total fatty acids) in the *Chaetoceros* species and significant levels of DHA in T-ISO and *P. lutheri* . Thus, of the species assessed in the present study, only *P. lutheri* would be expected to contain high levels of both EPA and DHA. Nevertheless, this species supported the lowest mean weight gain of *Pinctada maxima* spat. Additionally, *T. suecica* , which would be expected to contain only trace amounts of DHA, supported a significantly better growth rate than *P. lutheri* .

Thompson et al. (1993) suggested that the nutritional value of EPA and DHA has been somewhat over-rated and that there are threshold levels of these fatty acids beyond which growth does not improve. Thompson et al. (1993) reported that the fastest growing oyster (*Crassostrea gigas*) larvae contained relatively higher levels of the 14: 0 and 16: 0

fatty acids and it was suggested that the nutritional value of micro-algae was more closely related to saturated fatty acid composition than to HUFA content. The C16 fatty acids form a relatively high percentage (>20.0%) of the total fatty acids for *T. suecica* (Volkman et al., 1989).

This is the first study to assess single species diets for *P. maxima* spat and give an indication of suitable micro-algal species to use as feed. Undoubtedly a mixed diet will produce far better growth in *P. maxima*, as has been previously demonstrated with other bivalves (Whyte et al., 1989; Whyte et al., 1990; O'Connor et al., 1992; Fidalgo et al., 1994). The results presented here are an indication of the importance of testing algal diets experimentally rather than relying solely on previously published data when selecting suitable species of micro-algae as food. Finally, cultured micro-algae fed to *P. maxima* spat in the hatchery are, as yet, unable to match the growth recorded in spat held at sea (Rose and Baker, 1994; Taylor, unpublished data) and considerable work is required before a truly adequate diet is developed for *P. maxima* spat. Further experimentation should focus on blended algal diets of differing composition and ration.

Chapter 6. Effects of mesh covers on the growth and survival *Pinctada maxima*, spat

6.1 Introduction

Pinctada maxima and other bivalves, are often housed in mesh or netting during nursery and grow-out phases (Walne and Davies, 1977; Alagarswami et al., 1987; Bourne and Hodgson, 1991; O'Sullivan, 1994; Rose and Baker, 1994). The mesh provides a degree of protection from the elements, such as excessive wave action, and predation, and helps retain dislodged individuals (Walne and Davies, 1977; Holliday et al., 1991). The size of the mesh used for this purpose is important as it influences growth and survival of spat. Excessively small mesh, particularly once fouled, restricts water exchange and the availability of food particles (Holliday et al., 1991). Small mesh also requires replacement as bivalves grow and careful cleaning, which increases labour and equipment costs; however, a mesh which is too large allows predators easier access to the juveniles (Walne and Davies, 1977) and potential for spat to escape.

In hatcheries propagating *P. maxima*, larvae settle onto substrata placed into settlement tanks or directly onto the tank surfaces (Rose, 1990; O'Sullivan, 1994; Rose and Baker, 1994). Commonly used substrata or collector materials include PVC slats, mono-filament netting, shade cloth and glass plates (Rose and Baker, 1994; Chapter 4). Spat attached to tank surfaces are removed using water jets or by scraping before being re-settled onto a suitable substratum for nursery culture (Rose, 1990; Rose and Baker, 1994). Spat are transferred to ocean-based nursery attached to collectors, which are

covered in protective mesh. Producers of *P. maxima* spat use a variety of different mesh sizes during nursery culture. Many pearl oyster growers fear that large numbers of spat will be lost through mesh coverings or sleeves with an aperture larger than the spat themselves. Some growers have blamed inappropriate mesh size for low retention of spat during nursery culture; however, when mesh sleeves have a small aperture, large numbers of dead spat are often found in the corners of the sleeves.

The aims of this study were: 1) to determine the effects of various mesh sizes on growth and survival of *P. maxima* spat during the first two weeks of suspended culture at sea and; 2) to determine if there are benefits in using a mesh small enough to retain spat that detach or become dislodged from spat collectors.

6.2 Materials and Methods

P. maxima larvae were cultured as described in Chapter 2 (section 2.4). Forty-five days after fertilization, spat were removed from the surfaces of a settlement tank by severing the byssus with a sheet of thin plastic. Spat had a mean (± s.e., n=50) SH and SL of 1.2 ± 0.3 mm and 1.8 ± 0.5 mm, respectively.

Spat were re-settled onto 28 curved white PVC slat collectors (500 mm long x 75 mm wide x 1.5 mm thick), which were emersed in aerated seawater and left undisturbed. Four days later, the number of settled spat per 100 cm^2 area of slat was estimated. This was done by placing a piece of clear plastic, which was the same length as the slats, over each slat and counting the number of spat in each of four 25 cm^2 grids drawn on the plastic. The total number of spat in the four grids was tallied to estimate the number of spat per 100 cm^2. This was done for both sides of the slats and the mean spat density per

100 cm^2 for each slat was calculated. The mean (± s.e., n=28) number of spat per 100 cm^2 was 340 ± 12.

Individual slats were selected at random and placed into black mesh sleeves. Three sizes of nylon/polyester mesh were used in this experiment with mesh apertures (side measurement) of 0.75 mm, 1.5 mm and 3.0 mm. One PVC slat was placed in each mesh sleeve and 7 replicates were used for each mesh size. Four slats, in sleeves, were tied horizontally to each of 7 plastic coated steel frames (850 mm x 500 mm). Each frame contained one slat in each of the 3 mesh sizes and a control without a mesh sleeve. The slats were placed on the frames 150 mm apart and the position of each slat on the frame was assigned randomly. Frames, with attached PVC slats, were suspended vertically from a raft at a depth of 3 m in waters near the island of Bacan, Maluku Utara, Indonesia (see Chapter 2). Frames were positioned across the prevailing current at intervals of 1 m. The PVC slats were cleaned twice weekly using a light spray of seawater to remove fouling. This method of cleaning was not observed to dislodge spat.

Numbers of surviving spat per 100 cm^2 on each slat were estimated after 2 weeks at sea (the time at which 0.75 mm mesh sleeves were normally changed) using the method described above and 10 randomly selected spat from each PVC slat were measured for SH and SL using a pair of vernier calipers calibrated to 0.1 mm. The possibility of an effect due to the position of frames on the raft (i.e. block effect) on survival, SH and SL was assessed using two-way ANOVA, which showed position to have a non-significant effect on survival (P=0.88) SH (P=0.46) and SL (P=0.46). As a result, data were then compared using one-way ANOVA (Sokal and Rohlf, 1981) and treatment means were compared using Fisher's protected least significant difference

(PLSD) test. Homogeneity of variance was confirmed with Cochran's test (Snedcore and Cochran, 1967).

6.3 Results

Survival, at the end of the experiment, was not significantly (P >0.05) affected by mesh size (Fig 6. 1). Regardless of the mesh size used, greater than 60 % of the spat were lost from the PVC slats; however, all covered PVC slats had significantly higher (P< 0.001) spat retention than the control (Fig 6.1). The mean (± s.e., n=7) numbers of spat retained in the 0.75 mm, 1.5 mm and 3.0 mm mesh sleeves were 120 ± 17, 127± 6 and 113 ± 5 spat per 100cm^2, respectively. The control PVC slats had fewer than 1 spat per 100cm^2 with 3 of the 7 PVC slats in this treatment having no spat at all. Size differed significantly (P <0.05) between all treatments (Fig 6.2). The largest spat were those held in the 3.0 mm mesh sleeves which had mean (± s.e.) SH and SL of 2.5 ± 0.1 mm and 3.7 ± 0.1 mm, respectively. Due to the very low retention of spat in the control treatment, all remaining spat were measured. Mean (± s.e.) SH for control slats, those in 0.75 mm mesh sleeves and those in 1.5 mm mesh sleeves were 1.0 ± 0.1 mm, 1.6 ± 0.1 mm and 2.3 ± 0.1 mm, respectively and the mean (± s.e.) SL for the same treatments were 1.6 ± 0.1 mm 2.5 ± 0.1 mm and 3.5 ± 0.1 mm, respectively. The mean size of spat that remained on the control slats was smaller than the mean size of spat at the start of the experiment; however, this difference was not significant (P>0.05).

Box fish (Ostraciidae) and file fish (Monacanthidae) were observed pecking at both exposed and covered PVC slats. These fish possibly ate spat.

The 0.75 mm sleeves fouled more quickly than either the 1.5 mm or 3.0 mm sleeves and were more difficult to clean. Silt and algae heavily fouled the 0.75 mm mesh sleeves after only 3 or 4 days at sea. Spat in the 0.75 mm and 1.5 mm sleeves that were dislodged from the PVC slats, collected in the sleeve corners where the majority perished. Very few spat accumulated in the corners of the 3 mm mesh sleeves as most were small enough to pass through the mesh. Several spat were attached to the outside of the 3 mm mesh sleeves and appeared healthy.

6.4 Discussion

The use of mesh sleeves to cover and protect spat greatly improved survival on PVC slats; however, there was no significant difference in survival between the three mesh sizes used. The size of mesh did, however, effect growth of *P. maxima* spat. Spat were significantly larger with each increase in mesh size. These size differences were probably due primarily to the effects of mesh size on water exchange with smaller mesh reducing the flow of water through the sleeve. Flow-rate of water is a major influence on the availability of food to bivalves and, as such, is an important influence on growth (Kirby-Smith, 1972; Walne, 1972; Spencer and Gough, 1978; Rodhouse and O'Kelly, 1981; Hadley and Manzi, 1984). Once fouling occurred on the mesh sleeves, water exchange would have been further restricted.

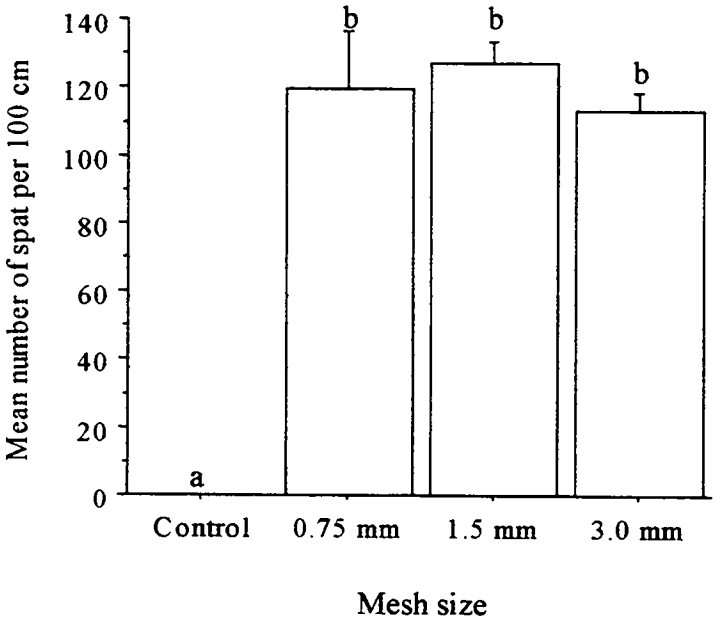


Fig 6.1 **Numbers of spat (mean ± s.e., n=7) per 100 cm^2 counted on PVC slats (500 mm X 75 mm) placed in different size mesh sleeves or left exposed (control) after two weeks in suspended culture. Values with the same superscripts are not significantly different (P>0.05).**

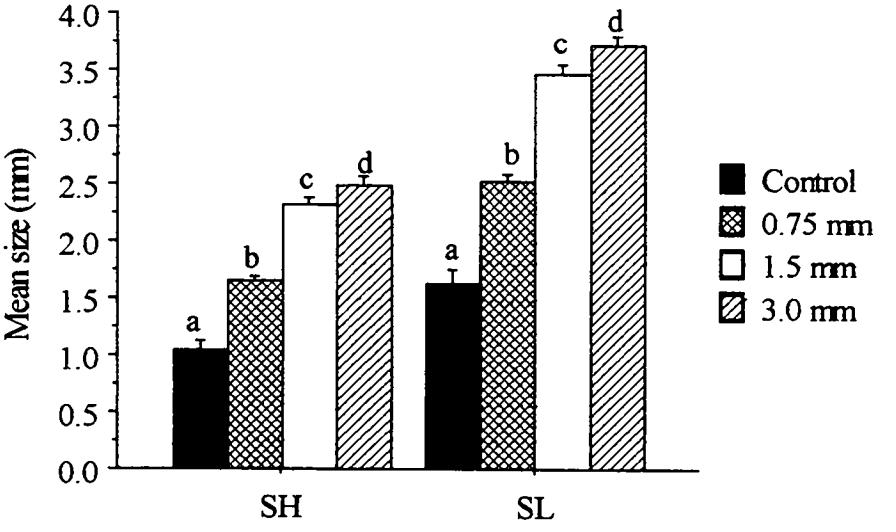


Fig 6.2 **Mean (± s.e., n=7) SH and SL of spat cultured on PVC slats (500 mm x 75 mm) placed in different size mesh sleeves or left exposed (control) after two weeks in suspended culture. Values with the same superscripts are not significantly different(P>0.05).**

The most obvious explanation for the near total loss of spat on the exposed PVC slats was predation by fish. If spat had simply detached, then spat losses would have been as high from slats held in 3 mm mesh sleeves; this was not the case. Similar results have been obtained using collectors on a commercial scale (J. J. Taylor, unpublished data, 1996). Collectors with attached spat, placed in 3 mm mesh sleeves had far higher spat retention than those without sleeves, even though spat were small enough to fall through the mesh when first placed in nursery culture (J. J. Taylor, unpublished data, 1996). The small size of the few spat remaining on the exposed PVC slats suggests that the larger individuals were eaten by predators first, presumably because they were the most obvious. The white PVC slats would have provided a contrasting background to the darker spat and probably aided visual identification of spat by predators. In a similar study, Walne and Davies (1977) reported that unprotected oyster (*Crassostrea gigas*) spat were smaller than those protected by mesh.

There was no advantage in using sleeves with a mesh small enough to retain dislodged spat. The majority of dislodged spat in the 0.75 mm and 1.5 mm mesh sleeves perished when they fell into the corners of the sleeve. Presumably these spat died due to excessive crowding and insufficient food. Sick and dying spat accumulating inside sleeves with small mesh aperture could also lead to disease amongst healthy individuals and increased mortality. A number of spat in the 3.0 mm mesh sleeves passed through the mesh before attaching to the outside where they continued to grow. It is impossible to say if these spat suffered predation; however, it is likely that the mesh provided some degree of camouflage, making those spat less obvious to predators than those on the exposed

PVC slats.

In summary, a 3.0 mm mesh was appropriate to house spat of the size used in this study. Smaller mesh sizes, while not affecting survival, reduce growth and are more difficult to keep clean. The choice of mesh size will depend on circumstance. It may be best to use a finer mesh when there are low numbers of spat on collectors and a larger mesh when there are high numbers of settled spat.

Chapter 7. Inducing detachment of *Pinctada maxima* spat from collectors

7.1 Introduction

In hatcheries propagating *Pinctada maxima*, larvae settle onto substrata placed into settlement tanks or directly onto the tank surfaces (Rose, 1990; O'Sullivan, 1992; Rose and Baker, 1994). Spat that attach to tank surfaces are physically removed by scraping or by using of water jets before being re-settled onto other substrata suitable for nursery culture (Rose, 1990; Rose and Baker, 1994). Spat at this stage are small (<5 mm shell length) and fragile. Mechanical detachment spat is time consuming and can result in mortality due to shell and tissue damage. If large rearing tanks are used, hatchery operators must climb into the tank, which further increases risk of injury and mortality to spat. The expense associated with producing *P. maxima* and their high value (Up to US$0.15 per mm hinge length-Aboosally and Sheung, 1998) make such losses costly.

A safe means of inducing voluntary detachment of spat would alleviate these problems and improve efficiency when dealing with large numbers of spat. Bucaille and Kim (1981) induced pediveligers of mussels (*Mytilus edulis* and *M. galloprovincialis*) to detach without consequent harm by exposing them to low concentrations of available chlorine (0.5 ppm). Bourne et al. (1989) found that exposure to a 500 ppm solution of sodium hypochlorite induced scallop (*Patinopecten yessoensis*) spat to detach from cultch after only 5 min while a 250 ppm solution was effective after 30 min. Scallop spat also detached when exposed to a 10% solution of sodium chloride and all the spat used in these treatments were alive and healthy 48 h later (Bourne et al., 1989). However, air

exposure for 30 min, followed by agitation in seawater, was the method adopted by Bourne et al. (1989) to induce detachment of large numbers of *Pat. yessoensis* spat during production trials. Heasman et al. (1994) found that exposure to sodium chloride (115 ppm of available chlorine) resulted in high mortality of *Pecten fumatus* spat, whereas exposure to air, hypersaline ($45^{o}/_{oo}$) baths, addition of magnesium chloride (27 g per kg) to seawater and reduction of the pH of seawater to 2, promoted detachment with non-injurious results. Hypersalinity and air exposure were particularly effective, resulting in more than 95% of *Pec. fumatus* spat detaching after 2 h.

The aim of this experiment was to assess the effectiveness of changes in salinity, changes in pH and exposure to air as a means of inducing detachment of *P. maxima* spat.

7.2 Materials and Methods

Hatchery propagated spat that had settled onto PVC slats (see Fig 4.2) were exposed to elevated salinity, reduced salinity, elevated pH and reduced pH for 24 h, or to air for 6 h. Spat used in the experiments had a mean (± s.d., n=25) SH and SL of 3.3 ± 0.8 mm and 4.5 ± 0.9 mm, respectively. The mean (± s.e., n=10) number of spat per PVC slat was 153 ± 70. Experiments were conducted in 20 L plastic basins, which contained seawater made up according to the treatments, described below. The number of spat attached to collectors was counted prior to immersion in the basins (time 0) and again after 0.5, 1, 2, 3, 6 and 24 h of exposure to the treatments. For the air exposure treatment, slats were simply placed in the experimental basins without water; spat in this treatment were counted at the same intervals as other treatments but only up to 6 h. Treatments were randomized among experimental basins and each treatment was replicated in 3

basins at 28.5°C. Spat that dropped off the PVC slats into the basins were left there to determine the effect of the treatment on detached spat. However, smaller samples of approximately 10-15 detached spat from each replicate were collected after 1 h and placed in petri dishes containing control seawater to monitor health and re-attachment. Experimental treatments were as follows:

7.2.1 Control

Seawater nominally filtered to 5 μm with a salinity of 34 $^{o}/_{oo}$ and pH 8.0 was used as the control.

7.2.2 Salinity

Salinity of the control seawater was raised to 40 $^{o}/_{oo}$ and 45 $^{o}/_{oo}$ by adding natural sea salt or reduced to 30 $^{o}/_{oo}$ and 25 $^{o}/_{oo}$ by adding rainwater. Salinity was measured using a refractometer (Atago Co. Ltd., Japan).

7.2.3 pH

The pH of control seawater was adjusted to pH 4 by adding hydrochloric acid (HCl) and pH 10 by adding Tris (hydroxymethyl) aminomethan (Tris-buffer, E. Merck, Darmstadt). Measurements of pH were made using a pH Scan 2 waterproof meter (Activon, Sydney).

7.2.4 Air

Spat exposed to air were left at room temperature (28.5°C) for 6 h and rinsed as previously described. After this period, spat that had not detached were returned to control seawater and monitored for any ill effects of exposure to air.

7.2.4 Statistical Analysis

Due to variations in the number of spat on each PVC slat at the start of the experiment (range 85 to 350 spat per PVC slat) the results for each treatment were compared on the basis of the percentage of spat that detached. Percentage data at each time interval were arcsin transformed before being compared by one-way ANOVA (Sokal and Rohlf, 1981). Means compared using Fisher's Protected Least Significant Difference (PLSD) test. Homogeneity of variances was confirmed using Cochran's test (Snedcore and Cochran, 1967).

7.3 Results

All treatments induced significantly greater ($P<0.001$) percent detachment of spat than the control treatment (Fig 7.1). The highest rate of detachment (98.6 ± 0.2 %, mean ± s.e.) recorded during the first hour was in the pH 10 treatment. However, spat exposed to pH 10 suffered catastrophic mortality (86 %) within the first hour and the treatment was abandoned (Fig 7.2). From a sample of 50 spat removed after 1 h exposure to pH 10, only 7 were alive. These spat re-attached when placed in control seawater and were still alive 48 h later. Samples of spat from other treatments returned to control seawater after 1 h exposure had 100 % survival and were observed crawling and beginning to re-attach. Survival of spat which remained exposed to treatments for 24 h was 100 % as was

survival of spat exposed to air for up to 6 h; no mortality was recorded for any treatment 24 h after the spat were returned to control seawater.

The majority of spat detached in the first hour of the experiment. The pH 10 treatment aside, the highest rate of detachment during the first hour (92.3 ± 0.6 %, mean ± s. e.) occurred in spat exposed to a salinity of 45 $^{o}/_{oo}$ (Fig 7.1). The rate of detachment for this treatment was significantly greater ($P< 0.05$) than for any other. The pH 4 treatment induced the next highest detachment rate with 85.6 ± 2.3 % of the spat detaching after 1 h. The mean (± s.e.) percentage of spat detached after 1 h exposure to air, and salinities of 40 $^{o}/_{oo}$, 30 $^{o}/_{oo}$ and 25 $^{o}/_{oo}$ were 75.9 ± 0.6 %, 63.6 ± 0.6 %, 49.5 ± 3.8 % and 59.7 ± 4.0 %, respectively (Fig 7.1).

Exposure beyond 1 h, except in the case of exposure to air, did not yield significant increases ($P> 0.05$) in the number of detached spat (Fig 7.2). The rate of detachment for spat exposed to air continued to rise until 6 h when the slats were returned to control seawater. In the salinity treatments and the pH 4 treatment, spat began to move on and off the slats after 3 h. Because of this, the number of spat counted on some slats increased between sampling intervals (Fig 7.2).

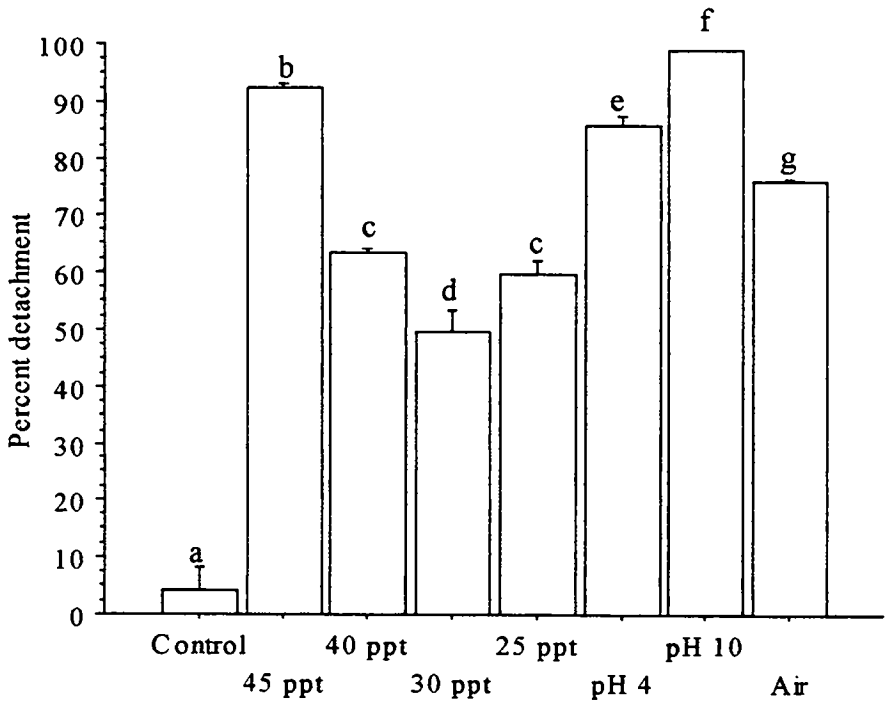


Fig 7.1 **Mean (± s.e., n=3) percentage of *P. maxima* spat that detached after 1 h when exposed to a variety of salinity and pH treatments or exposed to air. Control = seawater with a salinity of 34‰. Values with the same superscripts are not significantly different(P>0.05).**

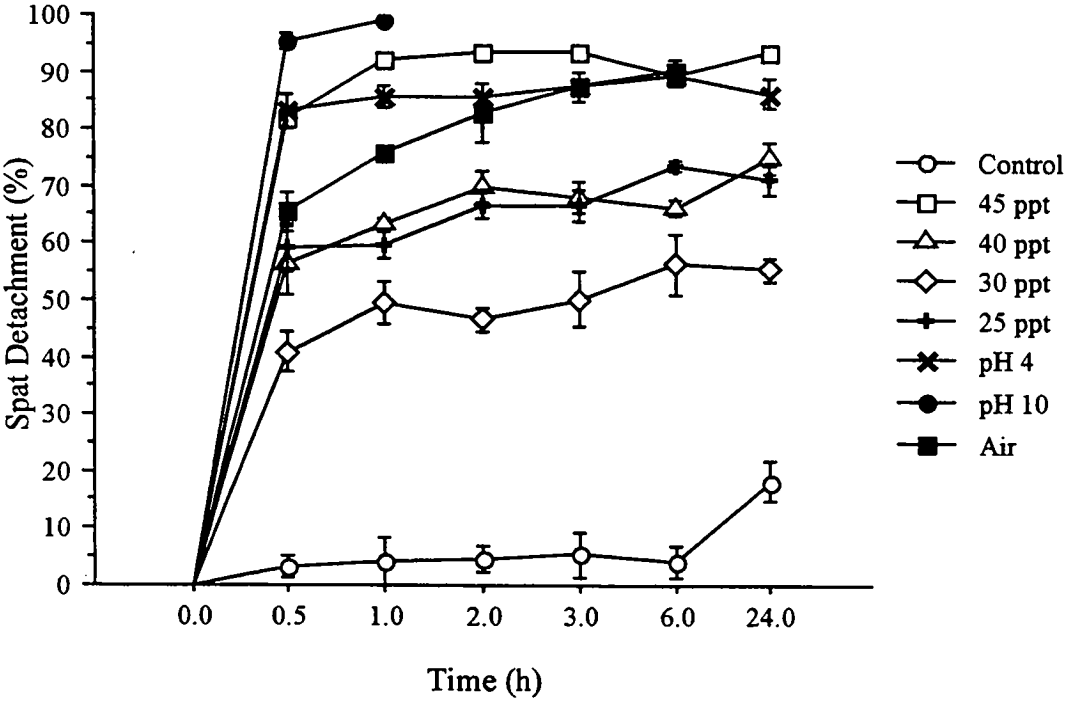


Fig 7.2 **Mean (± s.e., n=3) rate of detachment of *P. maxima* spat when exposed to a variety of salinities, changes in pH and air. Control = sea water with a salinity of 34 ‰ and a pH of 8.0. Note that the "x" axis is not to scale**

Spat that detached from slats and fell into the treatment basins were observed crawling and re-attaching during the second hour of the experiment. In the salinity and pH 4 treatments, spat were observed climbing the walls of the basins. In most cases, spat that had detached in the first hour had re-attached to the floor or wall of the treatment basins during the second hour. After 24 h exposure to the treatments, spat in the basins had firmly attached and had apparently adapted to the new environmental conditions.

7.4 Discussion

Exposure of *P. maxima* spat to a salinity of 45 $^{o}/_{oo}$ was found to be an effective and safe means of inducing a high percentage of spat to detach from collectors. Exposure to a pH of 4 was also effective. Exposure beyond 1 h did not significantly improve detachment. Apparently, spat became accustomed to their new environment within 1 to 2 h. In the salinity and pH 4 treatments, spat had firmly re-attached after 24 h exposure indicating that they were tolerant of the conditions of the experiment. In a similar experiment, spat and adults of the scallop, *Pec. fumatus* exhibited stress responses (shell gaping and mantle retraction) when exposed to salinities above 40 $^{o}/_{oo}$ for 24 h and mortality resulted when exposure was prolonged to 48 h (Nell and Gibbs, 1986; Heasman et al., 1994). This was certainly not the case with *P. maxima*. In Chapter 8, prolonged exposure of *P. maxima* spat to elevated and reduced salinity is assessed.

Further experimentation with elevated pH was abandoned following the severe mortality that resulted from the Tris-buffer treatment (pH 10). Alagarswami et al. (1983) used Tris-buffer to raise the pH of seawater to induce spawning of Indian pearl oysters, *P. fucata*. In that study, adult pearl oysters were exposed to a pH range from 8.5 to 10.0

for up to 2 h. No mortality was reported. Kuwatani and Nishii (1968) used Tris-buffer to maintain the pH of culture water above 8.0 in longer term (35 days) laboratory rearing experiments with one-year-old Japanese pearl oysters, *P. fucata martensii*. Although the pearl oysters decreased in weight during the course of the experiment, no deaths were reported (Kuwatani and Nishii, 1968). The results reported here for *P. maxima* suggest that there are differences between *Pinctada* species in their tolerance to such treatments and/or differences in tolerance due to age.

The results of this study are similar to those reported by Heasman et al. (1994). They found that a salinity of 45 $^{o}/_{oo}$ was a safe means of inducing detachment in scallop (*Pec. fumatus*) spat; however, there were differences in the rate of detachment of *Pec. fumatus* spat, depending on the brand of artificial sea salts used (Heasman et al., 1994). In the present study, natural sea salts were found to be effective and other types of salts were not assessed. Reducing the pH in the present study induced a higher rate of detachment in *P. maxima* than reported for *Pec. fumatus* by Heasman et al. (1994); however, air exposure was more effective in inducing *Pec. fumatus* to detach than was found in the present study with *P. maxima*.

The fact that sudden salinity change caused detachment of *P. maxima* spat has implications for the nursery rearing of this species. Areas in the Northern Territory of Australia and the Kimberley region of Western Australia experience flash flooding during the summer monsoon. For example, in Bynoe Harbour (Northern Territory, Australia) salinity can drop rapidly from a high of 37 $^{o}/_{oo}$ to as low as 29 $^{o}/_{oo}$ during the first week or two of heavy rain (J. J. Taylor, unpublished data, 1993). Where the mesh aperture of nursery culture equipment is sufficiently large to allow small spat to fall

through (see Chapter 6), it is possible that such flooding may cause spat to detach from collectors resulting in significant losses. Alternatively, if the mesh aperture of nursery equipment is too small, mortality may occur when detached spat fall to the bottom of nursery containers and smother each other (see Chapter 6). Similar problems could result from prolonged air exposure during transport. If there is insufficient moisture retained around spat during transportation, the detachment that is likely to result may lead to losses once the animals are returned to seawater.

Increasing the salinity of seawater to 45 $^{o}/_{oo}$ by the addition of natural sea salts is a safe and effective means of inducing a high percentage of *P. maxima* spat to detach from collectors. Reducing the pH of seawater to 4, although less effective, is a useful alternative where sea salts are not readily available. These methods may prove beneficial in hatchery operations by reducing the risk of injury to small spat that require removal from rearing tank surfaces and other points of attachment.

Chapter 8. Byssus production in six age classes of *Pinctada maxima*

8.1 Introduction

The foot and byssal gland of *Pinctada maxima* provide mobility and anchorage, respectively (see Chapter 1). At the proximal end of the foot is the byssal gland, which secretes byssus fibres that pass down a tubular pedal groove (Farn 1986). Muscular contractions of the foot cause the formation of the discoid attachment and stem of each byssal thread. Attachment takes place as the tip of the foot touches the substratum. Byssal secretions harden quickly in seawater securing the pearl oyster to the substratum (Herdman 1903, Dharmaraj and Alagarswami 1987).

P. fucata (Kafuku and Ikenoue 1983), *P. margaritifera* (Nichols 1931) and *P. maxima* (Saville-Kent 1890, 1893) juveniles are able to sever their byssal attachment, move position, and re-attach. *P. maxima* ceases to use the byssus as a point of anchorage at about three years of age, when it is sufficiently heavy to avoid being moved by ocean currents. However, large (3-5 kg) wild *P. maxima* have been found with byssal threads attached to rubble (Rose, unpublished data 1984-1988). In contrast *P. fucata* and *P. margaritifera* maintain byssal attachment as an anchorage system for life (Gervis and Sims 1992).

In aquaculture facilities, regular grading increases grow-out efficiency by separating faster growers from slower growers and removing individuals that are not growing at a profitable rate. This is particularly important in pearl oyster culture because the timing of the implantation of the pearl nucleus depends on the size of the oyster. As with other byssally attached bivalves, grading requires breaking the byssus to remove

animals from their point of attachment (Bourne et al. 1989, Heasman et al 1994). Generally, the byssus of *P. maxima* is severed with a scalpel or razor blade before grading. For commercial rearing of *P. maxima*, the period required for re-establishment of the byssus is important because of the common practice of using pressurized water for routine cleaning of pearl oysters. If pearl oysters have not re-established a firm anchorage, this method of cleaning may prove harmful resulting in possible losses or fatality.

P. maxima growers recognize weak byssal attachment or detachment by juvenile pearl oysters as a sign of ill health (J. Jorgensen, Atlas Pacific Ltd; M. Pieper, Cahaya Cemerlang; S. Arrow, Arrow Pearling Co., pers. com. 1993-1997). Detachment, accompanied by other symptoms such as mantle retraction, have been observed prior to and during mass mortality incidents (losses of up to 75 % of the population) in juvenile *P. maxima* (J. J. Taylor and R. A. Rose unpublished data 1993-1997). Knowing how long it takes for byssus to regenerate and if the time required varies as pearl oysters grow, would therefore aid grow-out management and provide valuable data for general health monitoring in commercial operations. To this end, this study determined the time required for re-attachment for six age classes of *P. maxima* juveniles, following mechanical severing of the byssus.

8.2 Materials and Methods

8.1.1 Experiment 1

Plastic petri dishes were used as experimental settlement substrata. The petri dishes provided a clear substratum through which attachment was observed and individual byssal threads could be counted over time. Byssus were observed by inverting the petri dishes under a dissecting microscope. Juveniles of two age classes were used: 75 day old juveniles, with mean (± s.e., n=25) SH and SL of 6.7 ± 0.5 mm and 10.9 ± 1.0 mm, respectively and 120 day old juveniles, with mean (± s.e., n=25) SH and SL of 14.9 ± 1 mm and 20.7 ± 1.4 mm, respectively. Fourteen juveniles of each age class were used for the experiment. At time 0, juveniles were removed from their point of attachment by severing the byssus with a scalpel blade. Two juveniles of the same age class were placed in each of 14 petri dishes. Juveniles were monitored every 30 min for the first 3 h. Juveniles were inspected for the following six days, and attached byssal threads were counted.

8.1.2 Experiment 2

This experiment was conducted with pearl oysters of 13, 11, 9 and 7 months of age. Prior to the start of the experiment, the number of byssal threads of 25 randomly selected individuals from each age class were counted before the oysters were removed from their grow-out net panels (see Chapter 1). The same animals were also measured and weighed. Oysters of 12 month of age were held in 8-pocket net panels (Gervis and Sims 1992) while all other age classes were held in 28-pocket net panels. Only 10 pearl oysters were placed in each net and 16 nets were used for each age class. Half of the pearl oysters from

each age class were placed in either an area of strong current (2.5-3.5 knots per h) or mild current (<1 knot per h) in Indonesia (see Chapter 2). The number of byssal threads produced by each pearl oyster were counted daily for 7 days after the start of the experiment and again on days 9 and 11.

8.1.3 Statistical Analysis

Data were compared using ANOVA (Sokal and Rohlf 1981) and means were compared using Fisher's Protected Least Significant Difference (PLSD) test. Homogeneity of variances was confirmed using Cochran's test (Snedcore and Cochran 1967).

8.3 Results

8.3.1 Experiment 1

Fig 8.1 shows the number of byssal threads produced by pearl oysters, in each of the two age classes, at intervals during the 120 h experiment. After only 2 h, six of the 14 younger individuals (75 days old) were able to hold position when inverted and washed gently with seawater. However, no byssal threads were evident at this time and position appeared to be maintained by the foot alone. Younger individuals showed significantly greater byssal thread production than older individuals (120 days old) during the first 3 h of the experiment (P <0.001: Fig 1). Within 12 h, all pearl oysters, in both age classes, had formed a byssal attachment. After 12 h, older individuals had produced significantly more byssal threads than younger individuals (P <0.001: Fig 8.1). The total number of threads produced over the 120 h period also differed significantly (P <0.001); the older

pearl oysters produced 21.9 ± 1.1 threads (mean ± s.e.) and the younger pearl oysters produced 11.3 ± 0.8 threads. Byssal thread production for the younger juveniles did not increase significantly (P >0.05) after 48 h whereas older individuals produced significantly more threads each day from 24 h onwards (P <0.001).

New and emerging byssal threads appeared pinkish. Within a few hours they began to change colour, initially becoming translucent before gaining a greenish hue. The colour darkened and the threads thickened over time. The point of attachment was splayed (Fig 8.2 and 8.3) and the fibres of the byssal threads were obvious at the point of attachment. On flat surfaces, byssal threads were arranged in a radial pattern (Fig 8.2). Where juveniles had moved to the edge of the petri dish and attached to the dish wall the threads were attached predominantly in a single direction (Fig 8.3).

In many instances, byssal threads were ejected from the byssal gland and were observed with one end still attached to the petri dish and the other end floating free (Fig 8.4). In some cases the entire byssus was jettisoned and the oysters had moved some distance before reattaching. This loss and replacement of byssal threads occurred within 24 h.

8.3.2 Experiment 2

The mean (± s.e., n=25) SL, SH, WW and the number of byssal threads for each age class at the start of the experiment are shown in Fig 8.5. The 13 month old *P. maxima* had significantly fewer ($P< 0.01$) byssal threads (8.9 ± 0.7) than any other age class. The number of byssal threads counted from 11 month old *P. maxima* did not differ significantly ($P >0.05$) from the 9 or 7 month old individuals but the 7 month old individuals had significantly fewer byssal threads ($P <0.05$) than 9 month old oysters (Fig 8.5). Significant differences resulted when the ratios of WW to number of byssal threads (BT) were compared (Fig 8.6). The WW/BT ratio for 13 month old individuals was significantly greater ($P< 0.01$) than for any other age class. The WW/BT ratio became significantly less ($P<0.01$) with each age class with the exception of the 9 month old and 7 month old pearl oysters where the WW/BT ratio did not differ significantly ($P >0.05$).

Following mechanical severing of the byssal threads, differences in byssus production were noted due to both age and current strength. Regardless of age, oysters in the mild current area produced significantly more byssal threads during the first day of the experiment (Table 8.1). This was also true after day 2 for all but the 13 month old oysters. From day 5 onwards there were generally more threads produced by pearl oysters in strong current compared to mild current; however, these differences were not significant ($P>0.05$) for pearl oysters aged 9 and 7 months. By the end of the experiment, pearl oysters aged 9 months had produced significantly more threads ($P<0.01$) than any other age class and differences in the number of threads produced were significant ($P<0.01$) between all age classes.

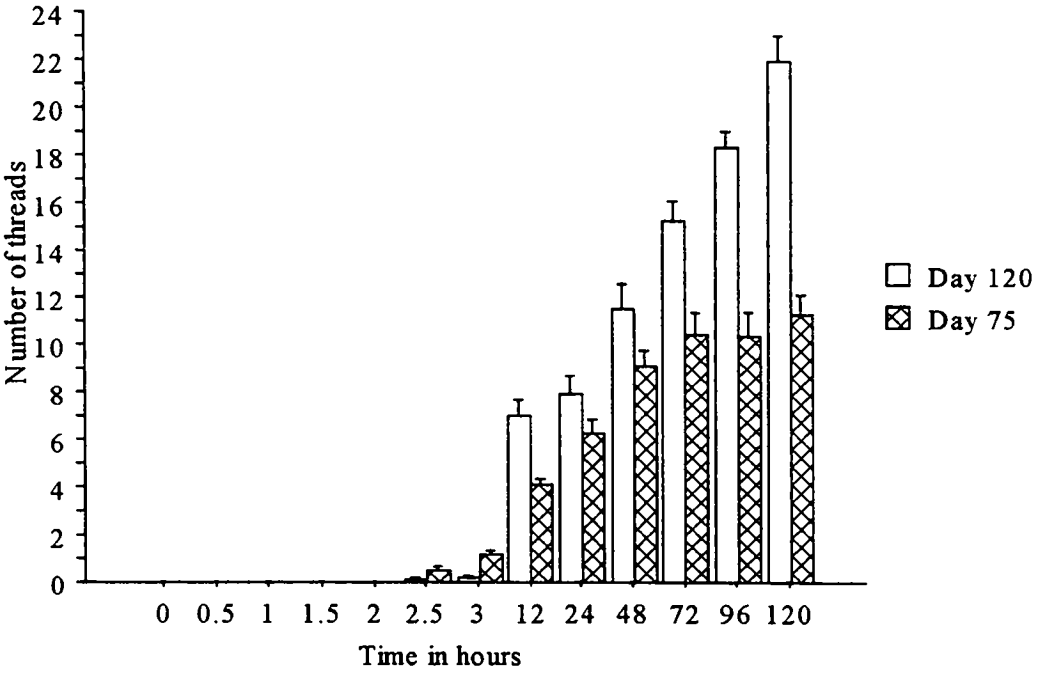


Fig 8. 1 **Byssus thread production over time (mean ± s.e., n=14) in 75 day old and 120 day old *P. maxima* juveniles.**

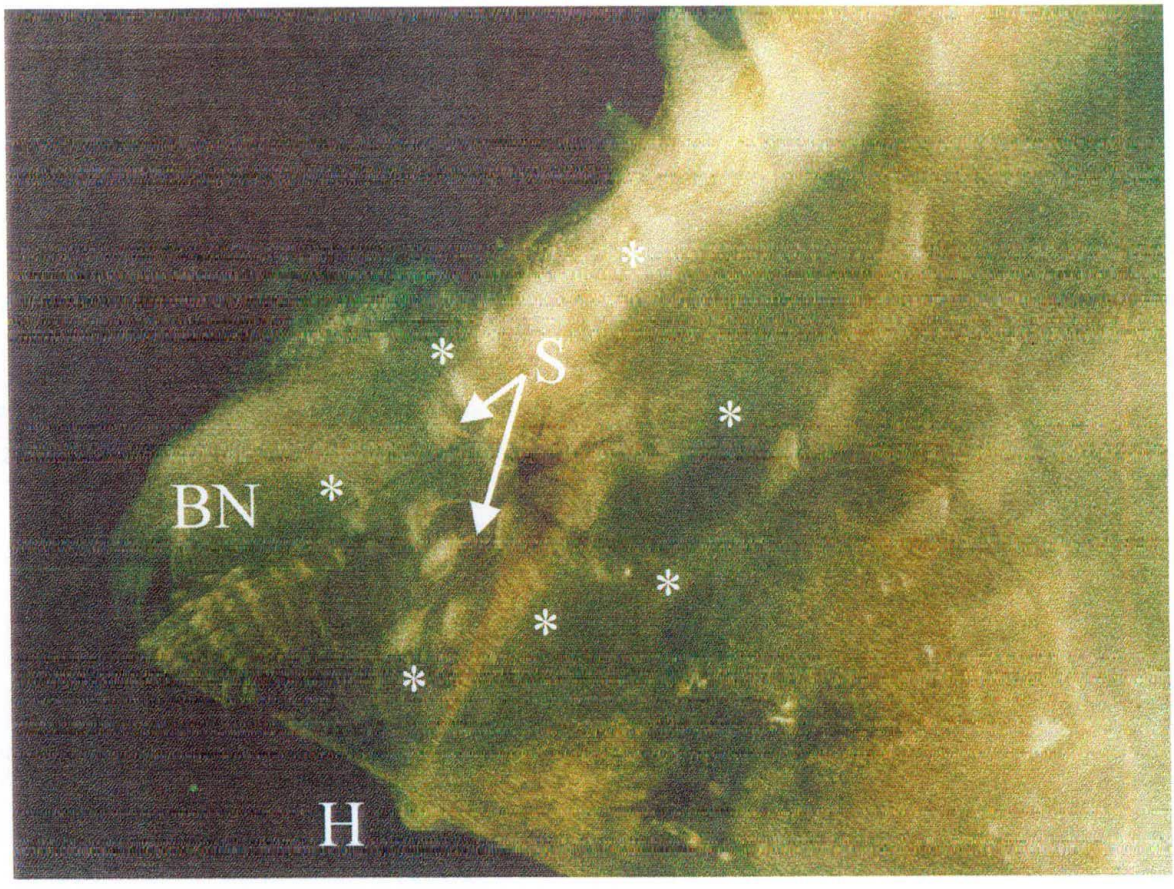


Fig 8.2 **Byssal threads of a juvenile *P. maxima* attached to the flat surface of a petri dish and arranged in a radial pattern. This juvenile attached to the flat surface in the centre of a petri dish. Note:H = the hinge of the oyster; BN = the byssal notch; S = the splayed end of the byssal threads at the point of attachment; * indicates the perimeter of the byssal threads.**

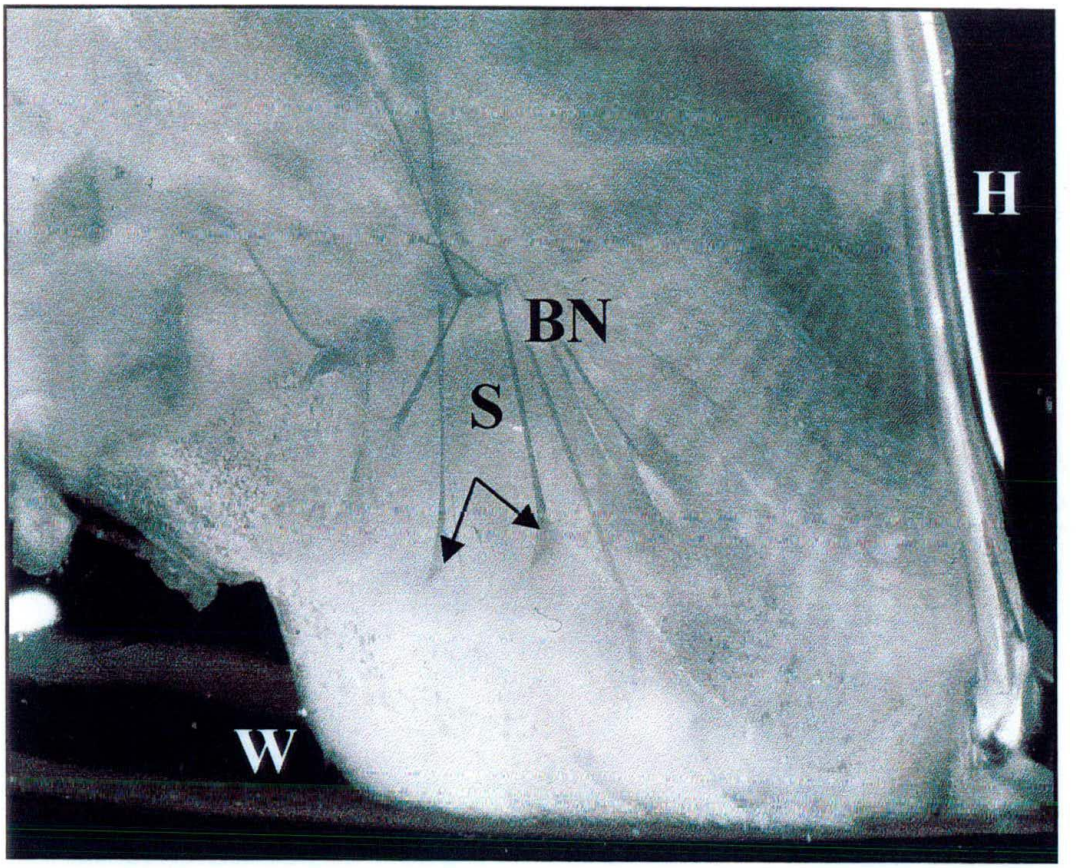


Fig 8.3 **Byssal threads of a juvenile *P. maxima* attached near to the wall of a petri dish with the threads predominantly in a single direction. Note: H = the hinge of the oyster; BN = the byssal notch; S = the splayed end of the byssal thread at the point of attchment; W = the wall of the petri dish.**

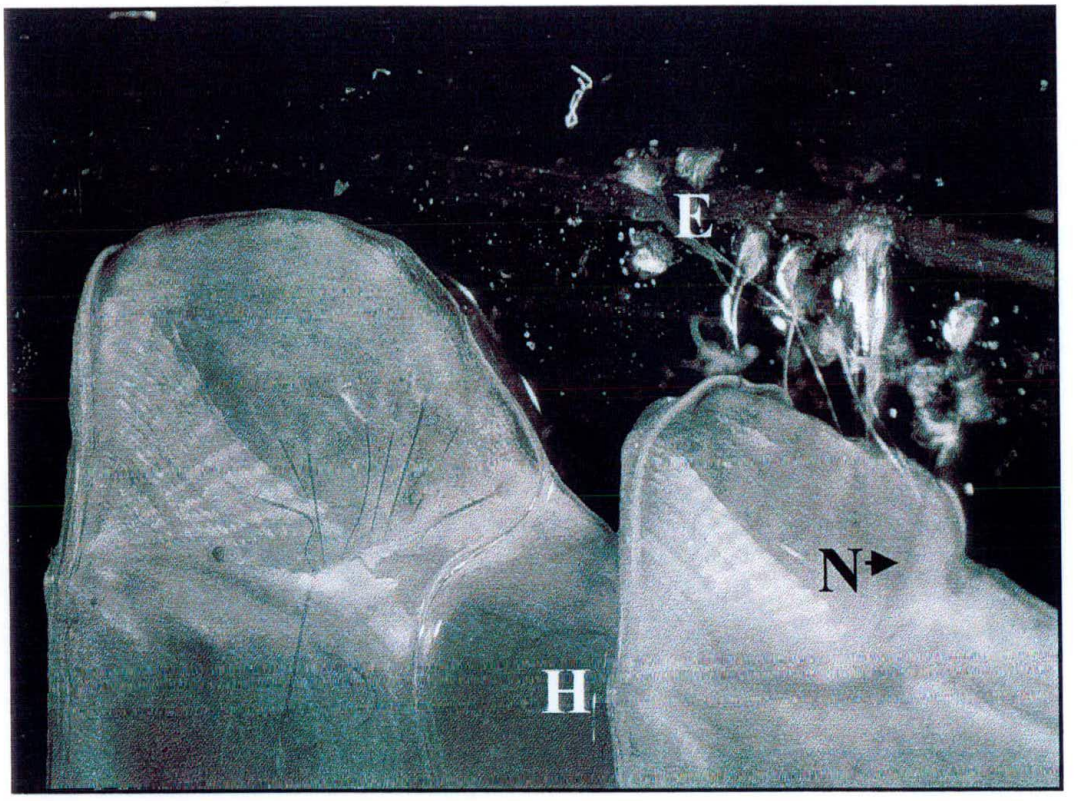


Fig 8.4 **A juvenile *P. maxima* (far right) that has detached, moved position and reattached leaving ejected byssal threads behind. Note: H = the hinge of the oyster; N = new byssal threads; E = ejected byssal threads that are still attached to the surface of the petri dish.**

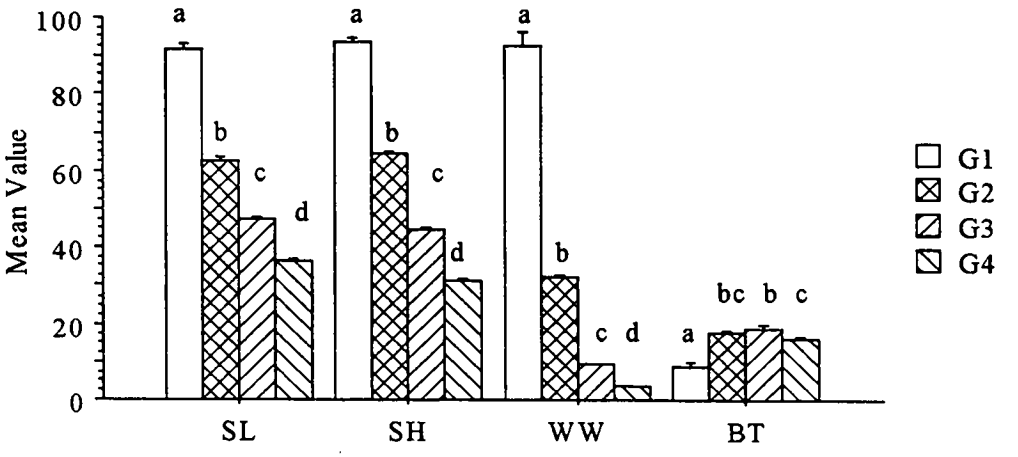


Fig 8.5 **Mean (± s.e., n=8) shell height in mm (SH) shell length in mm (SL) wet weight in g (WW) and number of byssal threads (BT) of four age classes of *P. maxima*. G1 = 13 months old, G2 = 11 months old, G3 = 9 months old, G4 = 7 months old. Values with the same superscript for each variable are not significantly different ($P > 0.05$).**

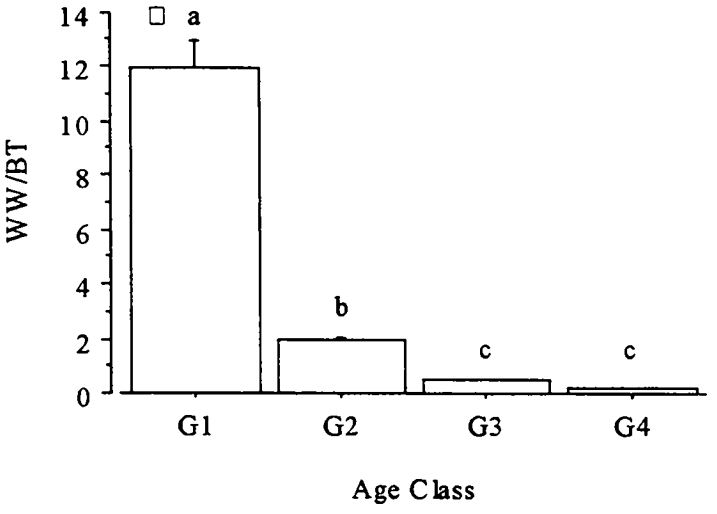


Figure 6. **Mean (± s.e., n=8) ratio of wet weight (WW) to number of byssal threads (BT) of four age classes of *P. maxima*. G1 = 13 months old, G2 = 11 months old, G3 = 9 months old, G4 = 7 months old. Values with the same superscript for each variable are not significantly different ($P > 0.05$).**

Table 8.1 **Mean (± s.e., n=8) byssal thread production of four age classes of silver-lip pearl oyster, *Pinctada maxima*, placed in either an area of strong current (SC) or an area of mild current (MC). G1 = pearl oysters aged 13 months, G2 = pearl oysters aged 11 months, G3 = pearl oysters aged 9 months and G4 = pearl oysters aged 7 months. Means with the same superscript (alphabetical across rows, numerical down columns) are not significantly different ($P > 0.05$).**

Day	Current	G1	G2	G3	G4
1	SC	0.2 ± 0.1^{a1}	0.5 ± 0.1^{b1}	1.1 ± 0.1^{c1}	1.3 ± 0.1^{c1}
	MC	0.5 ± 0.1^{a2}	$0.9 \pm .01^{b2}$	1.8 ± 0.1^{c2}	2.1 ± 0.1^{c2}
2	SC	1.0 ± 0.2^{a1}	1.1 ± 0.2^{b1}	2.6 ± 0.2^{c1}	2.9 ± 0.2^{c1}
	MC	1.1 ± 0.2^{a1}	1.9 ± 0.2^{b2}	3.3 ± 0.2^{c2}	3.3 ± 0.2^{c2}
3	SC	1.8 ± 0.2^{a1}	2.2 ± 0.2^{b1}	4.3 ± 0.3^{c1}	4.5 ± 0.2^{c1}
	MC	1.5 ± 0.2^{a1}	2.4 ± 0.2^{b1}	4.1 ± 0.3^{c1}	4.5 ± 0.2^{c1}
4	SC	2.7 ± 0.3^{a1}	3.1 ± 0.3^{b1}	5.6 ± 0.3^{c1}	5.7 ± 0.2^{c1}
	MC	1.9 ± 0.3^{a2}	3.1 ± 0.3^{b1}	5.4 ± 0.3^{c1}	6.0 ± 0.3^{c1}
5	SC	3.8 ± 0.4^{a1}	4.9 ± 0.3^{b1}	7.1 ± 0.4^{c1}	7.1 ± 0.3^{c1}
	MC	2.5 ± 0.4^{a2}	3.6 ± 0.3^{b2}	7.2 ± 0.4^{c1}	6.8 ± 0.3^{c1}
6	SC	4.8 ± 0.4^{a1}	5.6 ± 0.4^{b1}	8.7 ± 0.4^{c1}	8.6 ± 0.4^{c1}
	MC	3.0 ± 0.4^{a2}	4.5 ± 0.3^{b2}	8.2 ± 0.4^{c1}	8.1 ± 0.3^{c1}
7	SC	5.0 ± 0.4^{a1}	6.4 ± 0.4^{b1}	9.7 ± 0.4^{c1}	8.7 ± 0.3^{d1}
	MC	3.5 ± 0.4^{a2}	5.9 ± 0.3^{b1}	9.6 ± 0.4^{c1}	8.6 ± 0.4^{d1}
9	SC	7.0 ± 0.4^{a1}	8.6 ± 0.4^{b1}	11.9 ± 0.5^{c1}	9.8 ± 0.4^{d1}
	MC	5.0 ± 0.4^{a2}	7.4 ± 0.4^{b2}	11.2 ± 0.5^{c1}	10.1 ± 0.4^{d1}
11	SC	7.8 ± 0.4^{a1}	9.8 ± 0.5^{b1}	13.0 ± 0.6^{c1}	11.0 ± 0.4^{d1}
	MC	6.2 ± 0.5^{a2}	9.0 ± 0.4^{b2}	12.0 ± 0.5^{c1}	10.1 ± 0.4^{d1}

A number of individuals from each age class ejected the original byssal plug from the shell cavity. After 11 days, no oyster in any of the age classes had produced the number of threads that were counted at the start of the experiment and some of the older individuals did not re-attach at all.

8.4 Discussion

Juveniles of 75 days of age began reattaching faster than 120-day-old juveniles. However, after the first 12 h, older *P. maxima* produced significantly more threads than the younger individuals and significantly more threads over the 120 h experiment. Moreover, byssal thread production for the younger juveniles did not increase significantly after 48 h whereas production from older animals continued to increase significantly. This suggests that younger pearl oysters regain maximal anchorage after a shorter period than older pearl oysters. The maximum number of threads produced by a single individual in the older age class was 30 compared to 16 in the younger age class. A stronger anchorage may have been required by the older individuals to compensate for greater resistance to water currents due to larger surface area. Saville-Kent (1890, 1893) reported that juvenile *P. maxima* of a size range between 8 and 65 mm had 30 to 40 byssal threads. Rose and Baker (1994) reported the average number of byssal threads in 10-15 mm *P. maxima* juveniles to be approximately 20; neither study reported differences in byssal production with age or between size classes.

Juvenile *P. maxima* have the ability to sever the byssus, move and re-attach (Saville-Kent 1890, 1893). This behaviour was observed in this study with juveniles

moving and re-attaching with the same or a greater number of threads within a 24 h period. One younger individual moved twice within a 24 h period and produced a total of 20 new byssal threads. This suggests that when a pearl oyster voluntarily ejects the byssus, it can re-attach more rapidly than following mechanical severing. *P. maxima* juveniles can also eject individual threads which will allow minor positional changes, perhaps to adjust to water flow without losing the security of the entire byssus.

The number of byssal threads counted at the start of the second experiment shows reduction in byssal thread production as *P. maxima* ages. Presumably, a point is reached where the increased water resistance, due to greater surface area, is offset by greater stability resulting from increased mass. Eventually, the weight of the pearl oyster is such that byssal attachment loses importance as the main means of maintaining position. Large differences in the ratio of WW to the number of byssal threads supports this notion. Even in very large specimens of *P. maxima* (> 1 kg WW) the byssus may still be observed even though there is no attachment to substrata and therefore no byssal anchorage (Rose, unpublished data 1984-1988).

In the second experiment, *P. maxima* initially re-attached with a greater number of threads when placed in relatively calm water with little current. It appears that stronger current made initial re-attachment more difficult. This may be because the net holding the oysters was less stable under these conditions. After initial attachment, greater numbers of threads were produced by pearl oysters in the strong current indicating additional threads were required to secure the pearl oysters in the nets. This was particularly the case for older and larger individuals which, in some cases, failed to re-attach during the 11 day experiment. The results of this study indicate that byssus production in *P. maxima*

may be the result of a subtle relationship between stability of substratum, resistance of a pearl oyster to a given current and the size and weight of the individual. In this experiment, the pearl oysters were maintained under typical farm conditions; vertically orientated in net panels. Results may have differed had the pearl oysters been placed flat on the sea bed where resistance to current would have been reduced.

The results of these simple experiments provide useful information on the time required for re-attachment following mechanical severing of the byssus of *P. maxima*. Where possible, it is advised that newly graded pearl oysters, or oysters that have been transferred to new nets, should be placed in areas with calm water for a minimum of 24 h to allow a reasonable degree of re-attachment before moving them into areas of higher current or wave action.

Chapter 9. Effects of salinity on growth and survival of *Pinctada maxima* spat

9.1 Introduction

Salinity is a major environmental factor determining the distribution of bivalve molluscs (Hummell, 1980; Fuersich, 1993). Salinity tolerances of a number of commercially important bivalves such as the scallops *Pecten fumatus* (Nell and Gibbs, 1984) *Argopecten irradians* (Mercaldo and Rhodes, 1982) and *A. ventricosus-circularis* (Singnoret-Brailovsky et al., 1996); oysters, *Ostrea angasi*, *Saccostrea commercialis* (Nell and Gibbs, 1984) *O. edulis* (Castagna and Chanley, 1973) and *Crassostrea virginica* (Anderson and Anderson, 1975) and mussels *Mytilus edulis planulatus* (Nell and Gibbs, 1984) have been determined. Similar research has also been conducted on pearl oysters such as *Pinctada fucata* (Alagarswami and Victor, 1976) and *P. fucata martensii* (Kafuku and Ikenoue, 1983).

Changes in salinity have been shown to affect filtration rate (Riva and Masse, 1985; Villiers et al., 1989), oxygen consumption (Bernard, 1983) and electrolyte balance (Natochin et al., 1979) of bivalves as a result of osmoregulation. It can also affect the rate of particle transport over the gills (Paparo, 1981; Paparo and Dean, 1982). Physiological responses to changing salinity affect the health of bivalves and, consequently, growth and survival.

P. maxima is found predominantly in oceanic tropical waters of the Indo-Pacific where salinity approximates 35 $^{o}/_{oo}$ (Gervis and Sims, 1992). However, *P. maxima* are often cultured in near-shore estuarine areas that are subject to fluctuating salinity as a

result of heavy seasonal rains. For example, pearl oyster cultivation sites in the Northern Territory of Australia experience heavy annual rain fall that depresses salinity to less than 30 $^{o}/_{oo}$ for periods of two weeks or more (J. J. Taylor and R. A. Rose, unpublished data, 1993-1996). There is scant information on the effects of environmental fluctuations, such as salinity, on growth and survival of *P. maxima*. This is particularly the case for juveniles, which may be more sensitive to environmental change. Tanaka and Kumeta (1981) expressed concerns about the detrimental effects of fluctuating salinity on survival of *P. maxima* spat during the rainy season in north Queensland, Australia. Chapter 7 showed that increased (to 45 $^{o}/_{oo}$ and 40 $^{o}/_{oo}$) and reduced (to 20 $^{o}/_{oo}$ and 25 $^{o}/_{oo}$) salinities were an effective means of promoting detachment of *P. maxima* spat. Exposure of spat to all four salinities resulted in significantly greater detachment than recorded at ambient salinity (34 $^{o}/_{oo}$) and exposure to these salinities for up to 24 hours did not result in any mortality. Although affecting byssal detachment over short periods, the effects of prolonged exposure to reduced or increased salinity on growth and survival of *P. maxima* spat have not been studied. This Chapter determines the effects of prolonged exposure to elevated and reduced salinity on the growth and survival of *P. maxima* spat.

9.2 Materials and Methods

After two weeks in nursery culture, 45 day-old spat were returned to the hatchery. They were placed in a 500 L fibreglass tank with a flow-through seawater supply; nominally filtered to 5 μm. Spat were fed a mixed micro-algal diet composed of *Chaetoceros muelleri*, *Pavlova salina* and *Isochrisis* aff. *galbana* (T. ISO) for seven days

prior to the commencement of the experiment. Micro-algae were cultured at a salinity of 34 $^{o}/_{oo}$ using techniques described in Chapter 5 (section 5.2).

Four treatments and a control, each with 5 replicates, were prepared using gently aerated 1 L plastic aquaria. All seawater was nominally filtered to 5 μm. Ambient salinity (control) was 34 $^{o}/_{oo}$. In two of the treatments salinity was increased to either 40 $^{o}/_{oo}$ or 45 $^{o}/_{oo}$ by adding natural sea salts to seawater. In another two treatments salinity was decreased to either 30 $^{o}/_{oo}$ or 25 $^{o}/_{oo}$ by adding rain water to seawater.

Five individual spat were placed in each aquarium. The mean (± s.e., n=25) SH and SL of spat was 3.2 ± 0.3 mm and 4.6 ± 0.4 mm, respectively. Spat were fed daily on the mixed micro-algal diet described above. The initial ration was 100,000 cells per mL per day, which was gradually increased to 150,000 cells per mL per day by the end of the experiment. Ten and 20 days after commencing the experiment, spat were measured for SH and SL and the numbers of survivors were counted. On day 20, surviving spat were dried in an oven at 50°C for 48 h prior to DW determination.

SH, SL, DW and survival data were compared using ANOVA (Sokal and Rohlf, 1981) with the means compared using Fisher's Protected Least Significant Difference (PLSD) test. Homogeneity of variances was confirmed using Cochran's test (Snedcore and Cochran, 1967).

9.3 Results

There were no significant differences ($P > 0.05$) in survival between treatments on either day 10 or day 20 (Table 9.1). There were, however, significant differences in size between treatments on both occasions. On day 10, spat with the largest mean (± s.e.) SH and SL were those cultured at either 30 $^{o}/_{oo}$ or 34 $^{o}/_{oo}$. At these salinities, there were no significant differences in size ($P > 0.05$); however, both groups of spat were significantly larger ($P < 0.01$) than spat in any other treatment (Fig 9.1). By day 20, spat cultured at 30 $^{o}/_{oo}$ had a SH significantly greater ($P < 0.01$) than spat in any other treatment where SH did not differ significantly (Fig 9.1). The mean SL of spat cultured at 30 $^{o}/_{oo}$ was significantly larger ($P < 0.01$) than that of spat cultured at 45 $^{o}/_{oo}$, 40 $^{o}/_{oo}$ or 25 $^{o}/_{oo}$ but did not differ significantly ($P > 0.05$) to spat cultured at 34 $^{o}/_{oo}$ (Fig 9.1.). The mean SL of spat held at 34 $^{o}/_{oo}$ did not differ significantly ($P > 0.05$) to those cultured at 45 $^{o}/_{oo}$, 40 $^{o}/_{oo}$ or 25 $^{o}/_{oo}$. However, the DW of spat cultured at 30 $^{o}/_{oo}$ was significantly greater ($P < 0.05$) than that of spat held at any other salinity (Fig 9.2). Spat cultured at 34 $^{o}/_{oo}$ had a significantly greater ($P < 0.05$) mean DW than those cultured at 45 $^{o}/_{oo}$, 40 $^{o}/_{oo}$ or 25 $^{o}/_{oo}$, which did not differ significantly ($P > 0.05$) from each other.

9.4 Discussion

The salinities tested in this study had no significant effect on survival of *P. maxima* spat; however, reducing or increasing salinity significantly effected growth. Reducing salinity to 30 $^{o}/_{oo}$ significantly improved spat growth, while a further reduction to 25 $^{o}/_{oo}$, or an increase to 40 $^{o}/_{oo}$ or 45 $^{o}/_{oo}$, reduced growth. The tolerance of *P. maxima* to fluctuating salinity appears to be greater than that suggested for some

Table 9.1 Mean (± s.e., n=5) number of surviving spat on days 10 and 20 after being cultured at different salinities. Values for each column with the same superscript do not differ significantly (P >0.05).

Salinity ($^{o}/_{oo}$)	Number of Surviving Spat	
	Day 10	Day 20
45	2.6 ± 0.4^{a}	2.2 ± 0.7^{a}
40	3.8 ± 1.6^{a}	3.0 ± 1.0^{a}
34	3.6 ± 0.5^{a}	3.2 ± 0.8^{a}
30	3.2 ± 0.4^{a}	2.2 ± 0.4^{a}
25	3.4 ± 0.3^{a}	3.2 ± 0.4^{a}

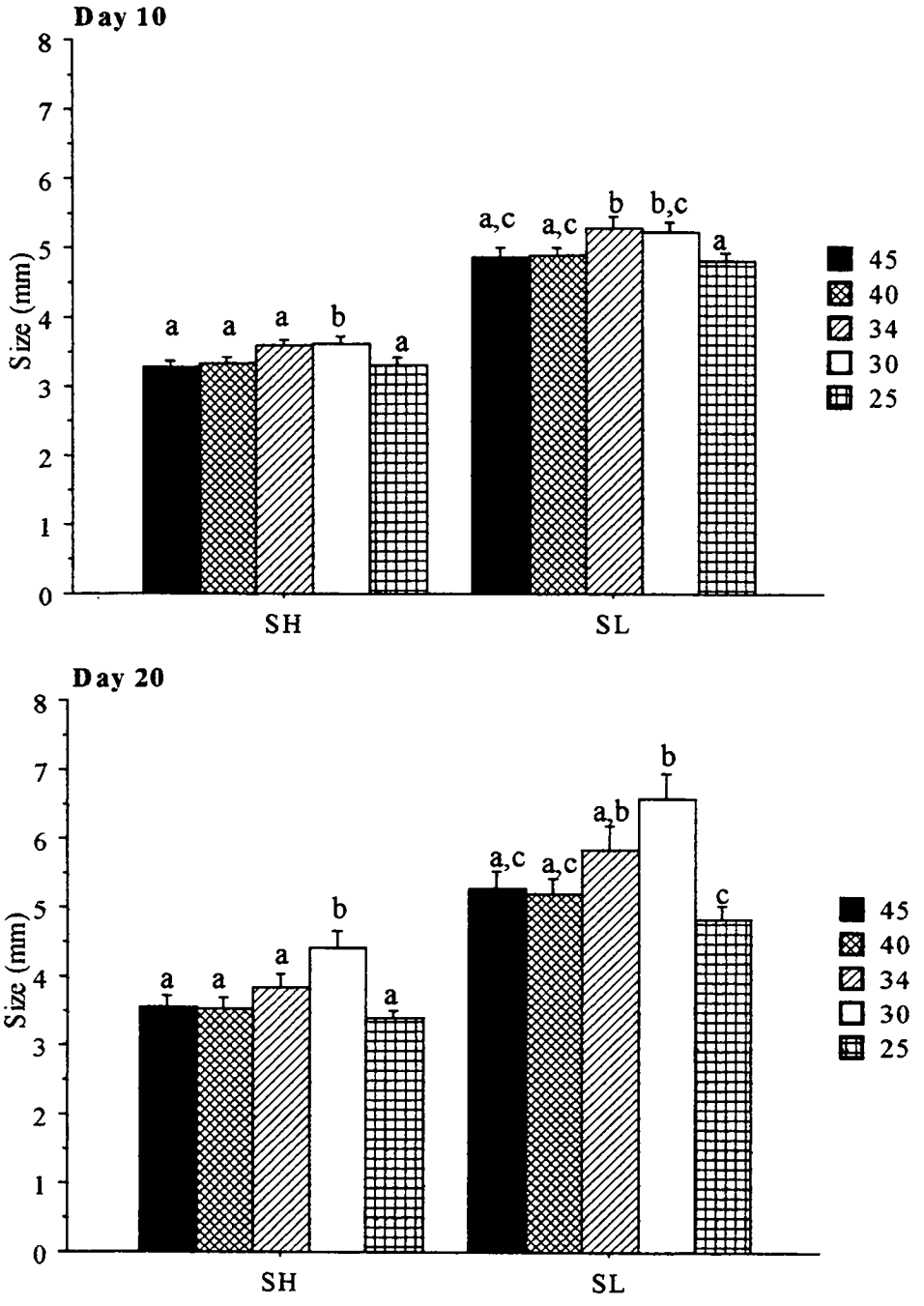


Fig 9.1 **Mean (± s.e., n=5) shell height (SH) and shell length (SL) of *P. maxima* spat after 10 and 20 days at different salinities (25‰, 30‰, 34‰, 40‰ and 45‰). Values with the same superscripts are not significantly different(P>0.05).**

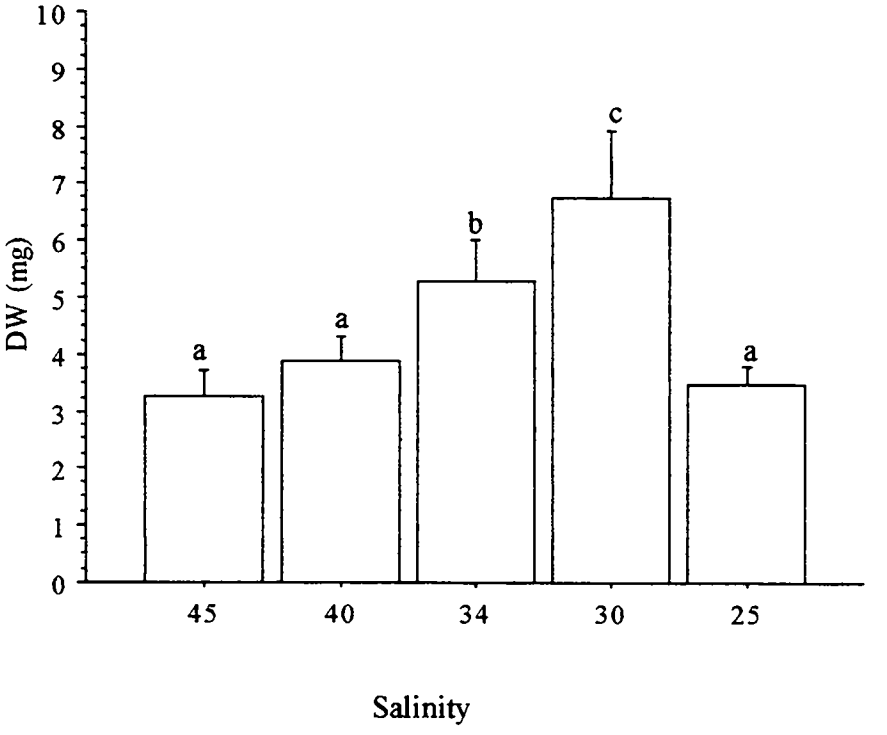


Fig 9.2 **Mean (± s.e., n=5) dry weight (DW) of *P. maxima* spat after 20 days at different salinities (25‰, 30‰, 34‰, 40‰ and 45‰). Values with the same superscripts are not significantly different(P>0.05).**

other oceanic bivalves. The scallop *Pecten fumatus*, for example, has limited tolerance to hypersaline seawater. Within 24 h of exposure to salinities above 40 $^{o}/_{oo}$, mantle retraction and gaping have been reported (Heasman et al., 1992) and death occurred in both spat and adult *Pec. fumatus* exposed to such salinities for 48 h (Nell and Gibbs, 1986; Heasman et al., 1992).

The best indicator of growth in this trial was dry weight. The clearer result provided by dry weight measurements of spat possibly result from spat preferentially producing shell material over body tissue. This has been reported for other bivalves grown in less than favourable conditions (Brown and Hartwick, 1988a).

As the micro-algae used in this study were cultured at 34 $^{o}/_{oo}$, it is possible that the rapid change in salinity, when introduced into experimental aquaria, may have affected algal viability. However, *P. maxima* spat grew better at 30 $^{o}/_{oo}$ than at 34 $^{o}/_{oo}$, suggesting that changes in algal viability were not the major cause of the observed results. Moreover, Jeffrey et al. (1992) reported the three species of micro-algae used in this study to have a broad salinity tolerance within the range 7 - 35 $^{o}/_{oo}$.

Rapid decline in growth rate between spat cultured at 30 $^{o}/_{oo}$ and 25 $^{o}/_{oo}$ suggests a sharp cut-off point between a reduced salinity that improves growth and one that retards growth. It may be that salinities just below 30 $^{o}/_{oo}$, for example those occurring in northern Australia during the rainy season, detrimentally effect growth (R. A. Rose and J. J. Taylor, unpublished data, 1993). Such effects are most likely compounded by silt-laden run-off into estuarine culture sites.

Reduced growth of spat cultured at salinities of 25 ‰, 40 ‰ and 45 ‰ may partially result from physiological stress caused by osmotic imbalance. Bernard (1983) reported a decrease in ventilation function in oysters (*Crassostrea gigas*) at around 18 ‰. Altered salinity effects the speed of particle transport across the frontal cilia, which in turn, effect feeding efficiency (Paparo, 1981; Paparo and Dean, 1982). Low salinities (9 ‰ and 18 ‰ compared to 30 ‰) have been reported to cause changes in the angle of beat of the latero-frontal cirri of the gills and reduce the rate of particle transport across the gills of the mussel, *Mytilus edulis* (Paparo and Dean, 1982). Presumably, this would reduce the rate of ingestion, which in turn, is likely to effect growth. This may, in part, explain some of the negative effects of salinity variation on growth found in the present study. Salinity also effects electrolyte balance and consequently the energy budget of bivalves. Matsushima et al. (1984) reported changes in the "energy charge" in clams (*Corbicula japonica*) when exposed to osmotic stress. It was suggested that changes in the energy charge may regulate activity of key enzymes involved in amino acid metabolism. Salinity change affected retention of potassium (K) in the cells of *M. edulis* and the gastropod *Littorina littorea* (Natochin et al., 1981). Retention of K levels at high salinity and removal of K ions at low salinity maintained cell volume. Grinberg and Diton (1981) reported that salinity or osmotic blood pressure affects some cardiac functions in bivalves. In Chapter 7, short term exposure to elevated or reduced salinity induced rapid (1 h) detachment in *P. maxima* spat.

Salinity changes affect a range of metabolic functions in bivalves that influence growth and survival. This study demonstrates that *P. maxima* spat are capable of survival for prolonged periods in salinities from 25 ‰ to 45 ‰. However, results indicate that

the most appropriate salinity range for nursery rearing *P. maxima* spat is between 30 $^{o}/_{oo}$ and 34 $^{o}/_{oo}$ and this range should be targeted during site selection.

Chapter 10. Effects of stocking density on growth and survival of early juvenile *Pinctada maxima* held in suspended nursery culture

10.1 Introduction

Bivalve research has shown that stocking density affects growth and survival of spat and juveniles (Hadley and Manzi, 1984; Rolland and Albrecht, 1990; Holliday et al., 1991; Parsons and Dadswell, 1992; Holliday et al., 1993). Research of this nature has established bench-mark figures for appropriate stocking densities for commercial operations (Hadley and Manzi, 1984; Holliday et al., 1991). However, there has not yet been a detailed study on the effects of stocking density on the growth and survival of *Pinctada maxima* spat. Rose and Baker (1994) published preliminary findings that stocking densities of 400 individuals per m^2 and 2,500 individuals per m^2 (cultured in down-wellers) and 300 individuals per m^2 and 700 individuals per m^2 (cultured at sea) resulted in similar survival; however, growth was slower in both cases at higher densities.

Optimizing stocking density to maximize growth and survival of *P. maxima* during nursery culture, would be a major benefit for the pearl oyster industry. As such, the objective of this study was to determine the effect of various stocking densities on the growth, survival and the incidence of growth deformities for *P. maxima* spat held in suspended nursery culture.

10.2 Materials and Methods

P. maxima were hatchery reared according to the general methods of Rose (1990) and settled onto spat collectors (Chapter 2). At 45 days of age, when spat had a mean shell length of approximately 3 mm, collectors were removed from the hatchery, placed into 1 mm mesh sleeves and suspended from a long-line at a depth of 2.5 m. At 63 days old, spat from two collectors were graded by size and spat with a mean (± s.e., n=25) SH, SL and WW of 5 ± 1.5 mm, 6.2 ± 1.8 mm and 0.02 ± 0.01 g, respectively, were used for the experiment. Four stocking density treatments, each with seven replicates, were established by re-setting spat onto PVC slats (500 mm x 75 mm). The following densities were used: 10 juveniles per slat (133 juveniles per m^2), 50 juveniles per slat (670 juveniles per m^2), 100 juveniles per slat (1,330 juveniles per m^2) and 150 juveniles per slat (2,000 juveniles per m^2). Spat density was calculated using both sides of the PVC slats. Spat were well spaced after re-attaching. The PVC slats with pearl oysters attached were placed inside 3 mm mesh sleeves (one sleeve per slat) to prevent loss of experimental animals, wired to standard pearl oyster net panels and suspended from a raft at sea at a depth of 3 m.

The pearl oysters were cleaned with seawater using a pressure cleaner every three to four days. After six weeks, the PVC slats were returned to the hatchery and the number of surviving animals were counted. Samples from each replicate were taken for measurements of SL, SH and WW as follows: all of the surviving animals from the 10 juveniles per slat replicates, 25 oysters from the 50 juveniles per slat replicates and 50 from each of the 100 and 150 juveniles per slat replicates. The number of animals with

growth deformities (exaggerated length to height ratio, thickened shell margins, twisted shell growth) in each sample were also counted.

Size and weight data were compared using a one-way ANOVA (Sokal and Rohlf, 1981) with the means compared using Fisher's Protected Least Significant Difference (PLSD) test. Percent survival and percentage growth deformity data were arcsin transformed before analysis (Sokal and Rohlf, 1981). Homogeneity of variances was confirmed using Cochran's test (Snedecore and Cochran, 1967).

10.3 Results

A considerable number of fouling organisms were present on the retrieved slats and on the pearl oysters themselves at the end of the experiment. Fouling organisms included sponges, ascidians, crustaceans, polychaete worms and gastropod and bivalve molluscs; however, the regular cleaning schedule kept fouling by algae to a minimum. During their time in the sea, many individuals had moved together to form large groups, with only one or two individuals in the group being anchored to the PVC slat. This gregarious behaviour was more pronounced at higher stocking densities. Table 10.1 shows the mean numbers of grouped individuals for each treatment. The maximum number of individuals observed in a single group was 25.

Best survival (80 ± 4.36%: mean ± s.e.) was found at a density of 10 juveniles per slat. This was significantly higher (P <0.05) than at all other densities (Fig 10.1). However, there were no significant differences in survival between densities of 50 juveniles per slat, 100 juveniles per slat and 150 juveniles per slat (P >0.05). Mean

survival for stocking densities of 50 juveniles per slat, 100 juveniles per slat and 150 juveniles per slat were 65.43 ± 3.8%, 64.43 ± 3.66% and 64.38 ± 4.53%, respectively.

Stocking density affected growth of the juvenile *P. maxima*. Each of the measured variables decreased as stocking density increased. Mean WW of individuals was significantly different at each stocking density (P <0.05: Fig 10.2). SH and SL varied significantly (P <0.05) between all stocking densities except the 10 juveniles per slat and 50 juveniles per slat treatments where there were no significant differences between mean SH (P >0.05: Fig 10.3). The SH: SL ratios differed significantly between all stocking densities (P <0.05: Table 10.2) except between the 100 juveniles per slat and 150 juveniles per slat treatments where there was no significant difference (P >0.05).

The occurrence of growth deformities increased with increasing stocking density (Fig 10.4). Deformed individuals had abnormal shell shapes; typically growth along the SH axis was exaggerated compared to the SL axis. Growth processes were distorted or lacking, while growth margins were unusually thick (Fig 5). The incidence of growth deformities was significantly different (P <0.05) between all stocking densities apart from the 100 juveniles per slat and 150 juveniles per slat treatments (P >0.05). The lowest incidence of growth deformity (5.26 ± 2.52%: mean ± s.e.) was seen in the 10 juveniles per slat treatment. In the 50 juveniles per slat, 100 juveniles per slat and 150 juveniles per slat treatments, the percentage of deformed individuals was 12.00 ± 1.23%; 20.86 ± 2.22% and 26.86 ± 1.30%, respectively.

Table 10.1 The maximum, minimum and mean (± s.e., n=7) number of individual early juvenile *P. maxima* within groups stocked at different densities on PVC slats (500 x 75 mm) after six weeks in suspended culture. Means with the same superscript(s) do not differ significantly (P >0.05).

Stocking Density/PVC slat	Maximum	Minimum	Mean
10	2	1	1.12 ± 0.05^{a}
50	12	1	2.60 ± 0.29^{b}
100	19	1	$3.43 \pm 0.36^{b, c}$
150	25	1	3.67 ± 0.46^{c}

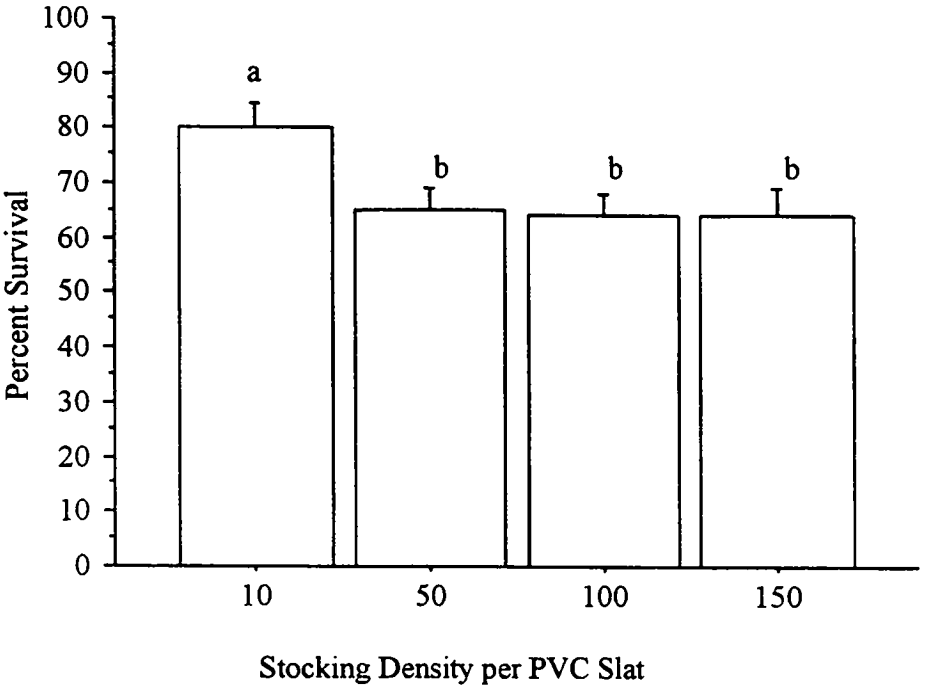


Fig 10.1 **Percent survival (mean ± s.e., n=7) of juvenile *P.maxima* stocked at different densities on PVC slats (500 x 75 mm) after six weeks in suspended culture. Means with the same superscript do not differ significantly ($P > 0.05$).**

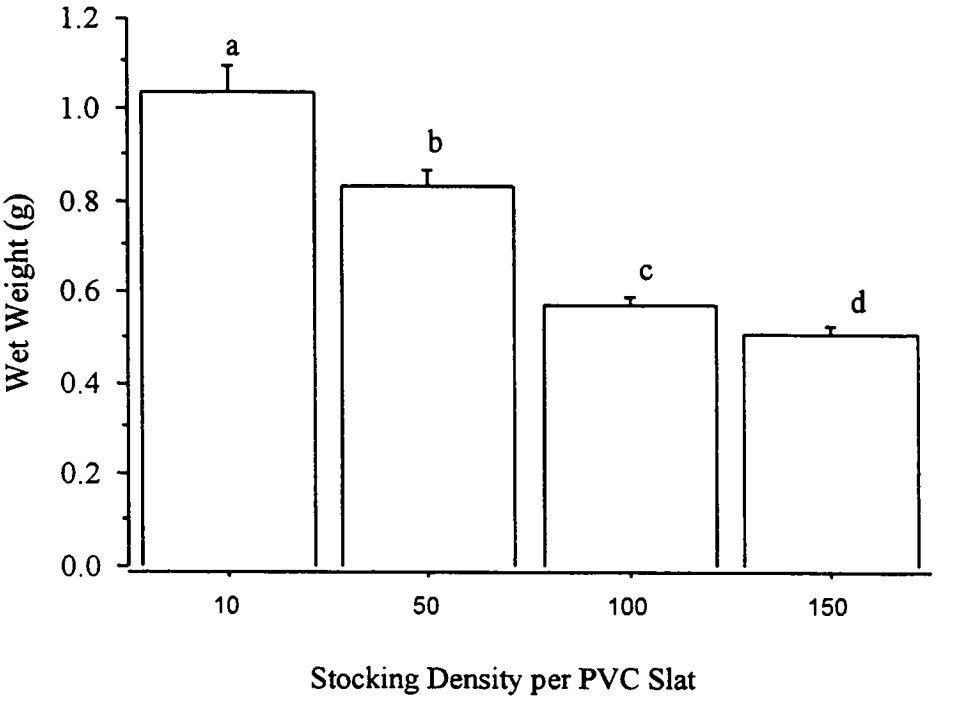


Fig 10.2 **Wet weight (mean ± s.e., n=7) per individual for early juvenile *P. maxima* stocked at different densities on PVC slats (500 x 75 mm) after six weeks in suspended culture. Means with the same superscript do not differ significantly ($P>0.05$).**

Table 10.2 The ratio of shell height over shell length (mean ± s.e., n=7) for early juvenile *P. maxima* pearl oysters stocked at different densities on PVC slats (500 x 75 mm) after six weeks in suspended culture. Means with the same superscript for each column do not differ significantly (P >0.05).

Stocking Density/PVC slat	Ratio: Shell Height/Shell Length
10	0.85 ± 0.02^{a}
50	0.90 ± 0.01^{b}
100	0.93 ± 0.01^{c}
150	0.93 ± 0.01^{c}

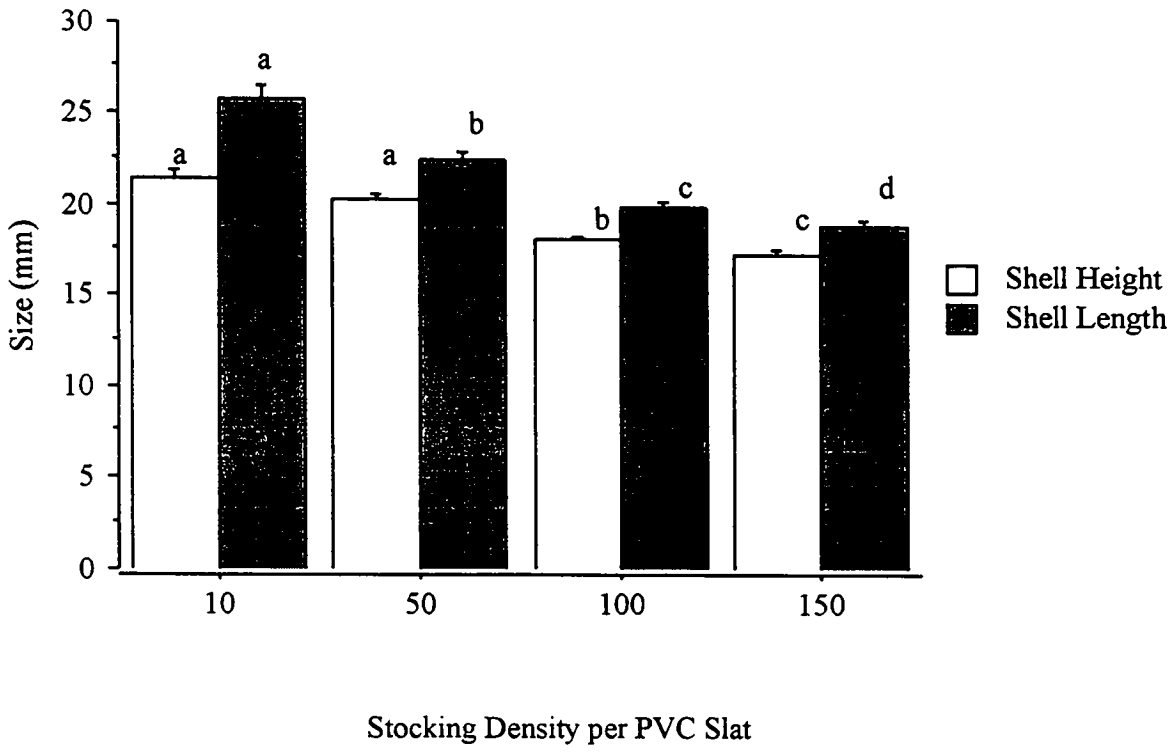


Fig 10.3 **Shell height and shell length (mean ± s.e., n=7) of early juvenile *P. maxima* stocked at different densities on PVC slats (500 x 75 mm) after six weeks in suspended culture. Means for shell height or shell length with the same superscript do not differ significantly ($P > 0.05$).**

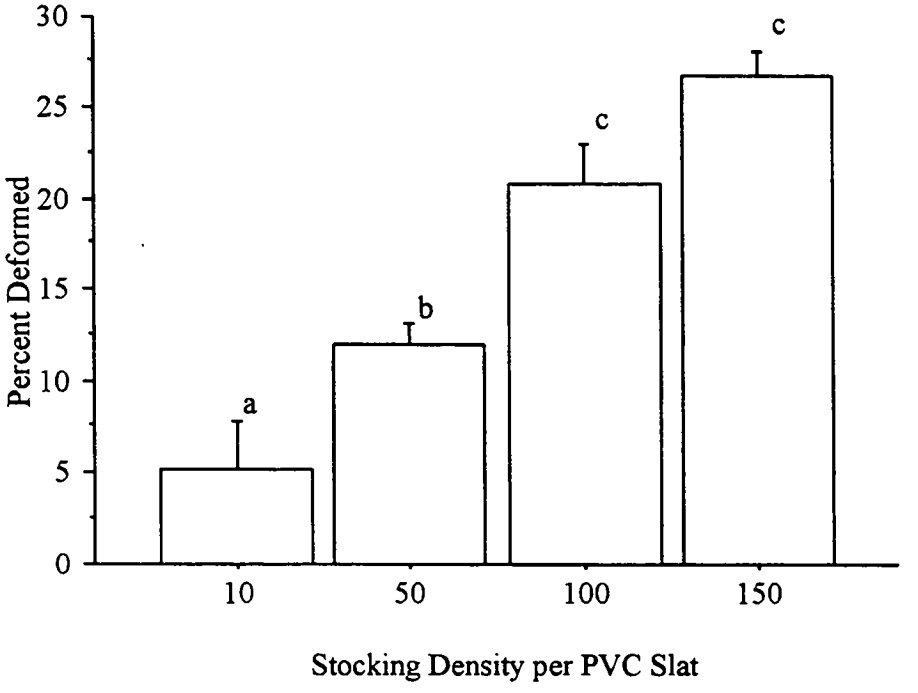


Fig 10.4 **Percentage (mean ± s.e., n=7) of early juvenile *P. maxima* with growth deformities stocked at different densities on PVC slats (500 x 75 mm) after six weeks in suspended culture. Superscripts relate to values after arcsin transformation of the data: means with the same superscript do not differ significantly ($P > 0.05$).**

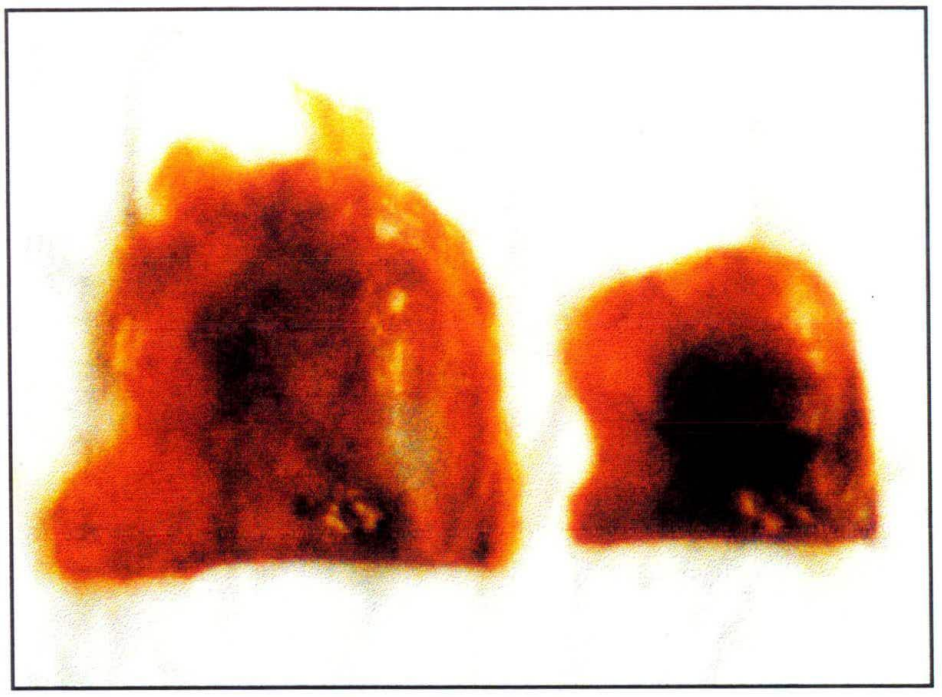


Fig 10.5 **The appearance of normal and deformed juvenile *P. maxima*. The pearl oyster on the left is typical of normal juvenile *P. maxima* shape, the specimen on the right shows growth deformity, in particular an exaggeration of growth along the height axis compared to the length axis.**

10.4 Discussion

Early juvenile *P. maxima* exhibited fastest growth, highest survival and lowest incidence of growth deformity at a density of 10 individuals per PVC slat (133 individuals per m^2). There were no significant differences in survival at densities greater than 10 individuals per PVC slat. This suggests that the upper-limit for stocking density effects on survival was not tested during this study. Similarly, Rose and Baker (1994) found no difference in survival for stocking densities of 400 individuals per m^2 and 2,500 individuals per m^2 (cultured in down-wellers) and 300 individuals per m^2 and 700 individuals per m^2 (cultured in sea-cages). However, survival in both cases, 98-99% (down-wellers) and 88-91% (sea-cages) was higher than in this study and may reflect differences in site, season and methods of cultivation.

The difference between growth along the SH axis and growth along SL axis decreased with increasing density indicating that stocking density not only affected the general growth rate of individuals but also the manner in which they grew. Moreover, the incidence of growth deformity increased at higher stocking densities. The increasing prevalence of deformed individuals at higher density is most likely the result of space limitation. To grow, individuals had to conform to the space available. In a similar study with the giant scallop (*Placopecten magellanicus*), Parsons and Dadswell (1992) found no difference in the proportion of deformed or stunted scallops between stocking densities ranging from 136 to 818 spat m^{-2} (15-90/pearl net). However, Gervis and Sims (1992) cited localised overcrowding as a cause of growth deformity and slow growth rate in juvenile Japanese pearl oysters, *Pinctada fucata martensii*. The gregarious behaviour exhibited by pearl oysters in this study may have exacerbated the effects of increasing

density. In the higher stocking densities, up to twenty-five individuals were found attached together as a group. Similar gregarious behaviour is reported in *P. margaritifera* (Crossland, 1957; Southgate and Beer, 1997) where spat aggregate in dense clusters which, if not separated, can lead to stunting or mortality in the innermost individuals.

Water flow-rate is an important influence on bivalve growth (Kirby-Smith, 1972; Walne, 1972; Spencer and Gough, 1978; Rodhouse and O'Kelly, 1981; Hadley and Manzi, 1984; Wilson, 1987). Food availability is equally important and closely linked to flow-rate. Under prolonged periods of low food availability, oysters preferentially partition energy resources into increasing shell weight and thickness over body tissue weight (Wilson, 1987; Brown and Hartwick, 1988). Increased stocking density and fouling no doubt, reduced localised water exchange and the availability of food for the juvenile pearl oysters. This could partly explain the deleterious effects on pearl oyster growth seen in this study. Leighton (1979) reported similar effects on growth for the rock scallops (*Hinnites multirogosus*) cultured at depths with a high level of fouling. Claereboudt et al. (1994) reported a 68% increase in muscle mass for giant scallops (*Plac. magellanicus*) kept in clean nets as opposed to those in fouled nets. Fouling by barnacles, oysters and other molluscs has been shown to reduce growth of pearl oysters (*P. fucata*) due to the combined effects of reduced food availability (caused by a decrease in water flow) and increased competition (Alagarswami and Chellam, 1976; Mohammad, 1976). Additionally, fouling by barnacles and species of *Crassostrea* interfered with the filtration efficiency of *P. fucata*; in some cases, fouling organisms cemented the two valves of the pearl oysters together, preventing them from opening and leading to starvation (Alagarswami and Chellam, 1976).

When determining appropriate stocking densities, non-biological factors as well as biological factors require consideration. Of primary importance to commercial bivalve production is the cost of nursery rearing (Spencer et al., 1985) the time between investment and return and the frequency of returns (Askew, 1978). According to Holliday et al. (1993) for edible oysters, "if initial costs of spat are high then the appropriate criteria for optimum stocking density is survival, followed by weight gain". *P. maxima* spat are expensive to produce and, as such, lower stocking density deserves consideration in the context of scale of operation and/or production targets. On this basis, the results presented above clearly indicate the commercial advantage of stocking at lower densities during nursery culture.

P. maxima juveniles are usually removed from collectors after a few months in nursery culture. The oysters are graded and re-stocked into net panels with varying numbers of pockets depending on the size of the oysters (Gervis and Sims, 1992; O'Sullivan, 1994). In most cases, there are three net size configurations: a first stage net panel (32 to 48 pockets); an intermediary size net panel (24 to 28 pockets) and a final grow-out panel suitable for oysters ready for pearl nucleus insertion (6 to 8 pockets). A lower initial stocking density may reduce the number of net changes required subsequently because oysters could stay longer on collectors and reach a greater size before re-stocking to net panels is required. If juvenile *P. maxima* were placed directly into an intermediary size net panel this would reduce the number of net changes during the culture process from three to two. While this protocol would increase the cost of nursery rearing, the cost of later grow-out would be reduced. Similarly, Parsons and Dadswell (1992) advocated a lower initial stocking density to reduce the cost of later

handling for the culture of *Plac. magellanicus*. Moreover, the production of *P. maxima* is often inconsistent, with boom and bust patterns occurring from year to year and even within a single season (J.J. Taylor, unpublished data, 1993-1998). Reduced stocking densities would optimize growth and survival when spat numbers are low. Alternatively, heavier densities may be appropriate when there are large numbers of spat. In this case, an operation may be able to select the best animals from a given collector for on-growing and return the "graded" collector to the nursery for future use if required. In this way, the collector remains a useful and valuable source of future seed.

Chapter 11. Effects of stocking density on growth and survival of juvenile *Pinctada maxima* in suspended and bottom culture and *P. maxima* of different size classes in suspended culture

11.1 Introduction

The effects of stocking density on growth and survival is well documented for bivalves such as clams (Hadley and Manzi, 1984; Hurley and Walker, 1994), edible oysters (Jaryaband and Newkirk, 1989; Arakawa, 1990; Roland and Albrecht, 1990; Holliday et al., 1991; Holliday et al., 1993; Rheault and Rice, 1996) and scallops (Duggan, 1973; Parsons and Dadswell, 1992; Gaudest, 1994). The type of system used for commercial bivalve culture also influences growth and survival and the cost of production (Duggan, 1973; Spencer and Gough, 1978; Toro and Varela, 1988; Gayton-Mondragon, et al. 1993). In particular, the position of a bivalve within the water column influences factors such as food availability, seston levels, rate of water exchange, temperature, salinity, and the level of fouling (Leighton, 1979; Wilson, 1987; Brown and Hartwick, 1988a, 1988b; Cote et al., 1993; Smitasiri et al. 1994).

The results of Chapter 10 demonstrated that stocking density is a major factor affecting growth, survival and the level of growth deformity during the nursery phase; however, nursery culture is only the first stage of rearing juvenile pearl oysters. Following nursery culture on collectors, spat are removed and transferred into grow-out

(Chapter 1, section 1.9). There is a paucity of published information concerning grow-out techniques and the effects of stocking density on young *P. maxima* during grow-out.

Pearl oyster farmers often use bottom culture systems to hold adult pearl oysters. These may be simple "shell dumps", where newly-fished animals are placed in a particular spot on the sea floor before entering the farm, or "bottom-line" systems where pearl oysters are held in pocket nets tied to a rope anchored along the sea floor. Pearl farmers often use a bottom-line system to hold pearl oysters during the "pearl turning" program adopted following pearl operation (section 1.10.5). In this system, nets holding newly operated pearl oysters are regularly turned such that the side previously face down is face up. Most operators believe that this encourages better development of the pearl-sac (Scoones, 1990; Gervis and Sims, 1992). One advantage of this system is that it reduces the need for regular cleaning as the turning process itself helps reduce fouling. Additionally, anecdotal evidence suggests that survival of post-operative pearl oysters is higher when placed on the sea-floor than in suspended culture near the sea surface. Scoones (1990) reported better quality pearls produced from *P. maxima* cultured in bottom systems.

Grading is an essential part of the grow-out of bivalves, allowing faster growers to be separated from slower growers. The final stage of grow-out for *P. maxima* is grading oysters into adult size net panels, which in most cases, have 6 or 8 pockets. This represents a dramatic reduction in stocking density from nets used in earlier grow-out which usually have 24 or 28 pockets. Determining the most appropriate size for pearl

oysters placed in these nets will effect the economics of the farm. Obviously, reducing stocking density requires additional equipment. On a standard 100 m long-line, where one net is hung every metre, only 800 pearl oysters can be held if 8-pocket nets are used. This is a huge reduction from the 2,800 pearl oysters that can be held per 100 m long-line when 28-pocket panels are used. Using more equipment also requires greater labour input to maintain it. Both these factors increase the cost of grow-out. In Australia and Indonesia, *P. maxima* are between 18 months and two years of age before they are ready for pearl nucleus implantation. Determining the size at which transfer of young oysters into adult nets is most appropriate would allow more efficient use of valuable resources during the grow-out phase.

The aim of this study was to assess the effects of both stocking density and culture method (suspended or bottom culture) on the growth and survival of juvenile *P. maxima* and to determine an appropriate age or size for transfer to adult size pocket nets.

11.2 Materials and methods

11.2.1 Experiment 1

Spat that had settled onto collectors were placed in suspended culture at sea once they had reached an SL of approximately 3 mm (at between 35 and 40 days of age). Juvenile *P. maxima* were removed from collectors after 10 weeks at sea by severing the byssal attachment with a scalpel blade; they were then graded by size. Individuals with mean (± s.e., n=25) SH and HL of 30.5 ± 2.1 mm and 34.1 ± 1.9 mm, respectively, were

selected for the study. Individuals were either stocked into nets (frame size: 500 mm x 850 mm, Fig 11.1) with 48 pockets (pocket dimensions 8 x 10 cm; 99 oysters per m^2) or 28 pockets (pocket dimensions 12 x 12 cm; 66 oysters per m^2). Seven of each net type were suspended at 1 m intervals from a surface long-line to a depth of 2.5 m (suspended culture) and a further seven of each net type were placed flat on the sea-floor (coarse sand) at a depth of 20 m (bottom culture). Nets held on the surface were cleaned using a high-pressure seawater jet approximately every 10 days. Nets on the sea-floor were turned weekly to minimize fouling.

Water temperatures near the sea surface and on the sea floor were recorded weekly. Water samples were taken every two weeks and 2 L of water from each site were filtered using a pre-weighed Whatman GF/C filter fitted to a vacuum flask. The filter was then dried for 24 h at 40°C and re-weighed to determine the dry weight of suspended solids. To measure food availability, two 1 L samples of seawater from each site were filtered using the above equipment. Phytoplankton collected on the GF/C filters was resuspended in 1 mL of seawater (previously filtered to 1 μm) and the numbers of morphologically different phytoplankters were counted using a compound microscope and a Sedgwick-rafter counting cell.

During the fifth week of the experiment, heavy mortality occurred in pearl oysters held in bottom culture. During the sixth week the trial was terminated and the numbers of surviving animals from each system were counted. SH and HL measurements were taken

for all the surviving individuals held in bottom culture and the same measurements were taken for 20 randomly selected animals from each net in suspended culture.

11.2.2 Experiment 2

Seven month-old hatchery propagated *P. maxima* that were graded into four size classes based on SL by passing them through a series of plastic plates with different sized holes. The diametres of the holes were: 75 mm, 65 mm, 55 mm and 45 mm (Fig 11.1). The oysters graded in this way were coded in decreasing size from G1 (< 45 mm) to G4 (< 75 mm). The mean (± s.e., n=25) SL, SH and WW for the four size classes are shown in Table 11.1.

Three replicates were used for each stocking density within each size class. Pearl oysters were either stocked into 28-pocket net panels (66 pearl oysters per m^2) or 8-pocket net panels (19 pearl oysters per m^2). Net frame dimensions were 850 mm x 500 mm (Fig 11.2).

Each month from July to October 1996 the pearl oysters were removed from their nets, and cleaned and measured for SL and SH using vernier calipers calibrated to 0.1 mm. Following removal of excess moisture with absorbent tissue, WW was measured to 0.01 g using a Sartorius (Japan) balance. The numbers of surviving pearl oysters were counted and the nets were thoroughly cleaned before the oysters were returned to the culture site. At the end of the fifth month, the percentage of *P. maxima* that were

undersized (runts) for each group was calculated. *P. maxima* that had not attained a minimum size of 75% of the mean size for the group were considered runts.

11.2.3 Statistical Analysis

SH and HL data were compared by one-way ANOVA (Sokal and Rohlf 1981) and means were compared using Fisher's Protected Least Significant Difference (PLSD) test. Percent age data were arcsin transformed before analysis (Sokal and Rohlf 1981). Homogeneity of variances was confirmed using Cochran's test (Snedcore and Cochran 1967).

11.1 Results

11.3.1 Experiment 1

Both stocking density and culture system affected survival. Large numbers of dead pearl oysters were observed in the bottom culture system at the end of the fifth week and the trial was terminated during the sixth week. No evidence of attack on the juvenile pearl oysters by predatory animals was observed in either surface or bottom culture systems. Survival of pearl oysters at the end of the experiment is shown in Fig 11.2. Mean (± s.e., n=7) survival of oysters held in 28-pocket nets in suspended culture was 99.0 ± 1.6%, and was significantly higher than in any other treatment (P <0.05). Survival was also high in the 48-pocket nets in suspended culture (94.8 ± 3.6 %). Mean survival of pearl oysters held in bottom culture was significantly lower than in either stocking density in

suspended culture (P <0.05) being 15.8 ± 7.8 % and 13.3 ± 3.6 %, for 28 and 48-pocket nets, respectively. These values did not differ significantly (P >0.05: Fig 11.2).

Shell growth (SH and HL) was also affected by culture system (Table 11.2). Juvenile *P. maxima* held in suspended culture were significantly larger (P <0.001) than those in bottom culture and those held in the 28-pocket nets were significantly larger (P <0.001) than those in the 48-pocket nets. Additionally, pearl oysters held in bottom culture had noticeably thinner shells and brittle shell margins compared to those held in suspended culture.

The total phytoplankton count per L of seawater (Fig 11.3) and the diversity of phytoplankton species (Table 11.3) was always greater in surface waters than in water adjacent to the bottom. Additionally, the dry weight of suspended solids was always higher in surface water samples (Table 11.3). Mean water temperature 2.5 m from the surface over the 6 week period was 29.5 ± 0.2°C (± s.e.); mean water temperature on the sea-floor was 28.8 ± 0.1°C (± s.e.).

Experiment 2

P. maxima in the G4 size class stocked into 28-pocket panels suffered heavy mortality during the first two weeks of the study. As a result, size and survival data were collected for these animals but they were not included in any of the analyses comparing stocking density and data from the G4 group stocked into 8-pocket panels was used to compare growth rates between size classes.

Table 11. 1 Initial mean (± s.e., n=25) HL, SH and WW of seven month-old *P. maxima* graded into four size classes.

Size Class	HL (mm)	SH (mm)	WW (g)
G1	66.3 ± 0.7	65.9 ± 0.7	23.18 ± 0.73
G2	57.8 ± 0.6	59.5 ± 0.6	17.32 ± 0.53
G3	50.5 ± 0.8	52.7 ± 0.7	13.10 ± 0.53
G4	38.4 ± 0.8	38.4 ± 0.7	5.57 ± 0.26

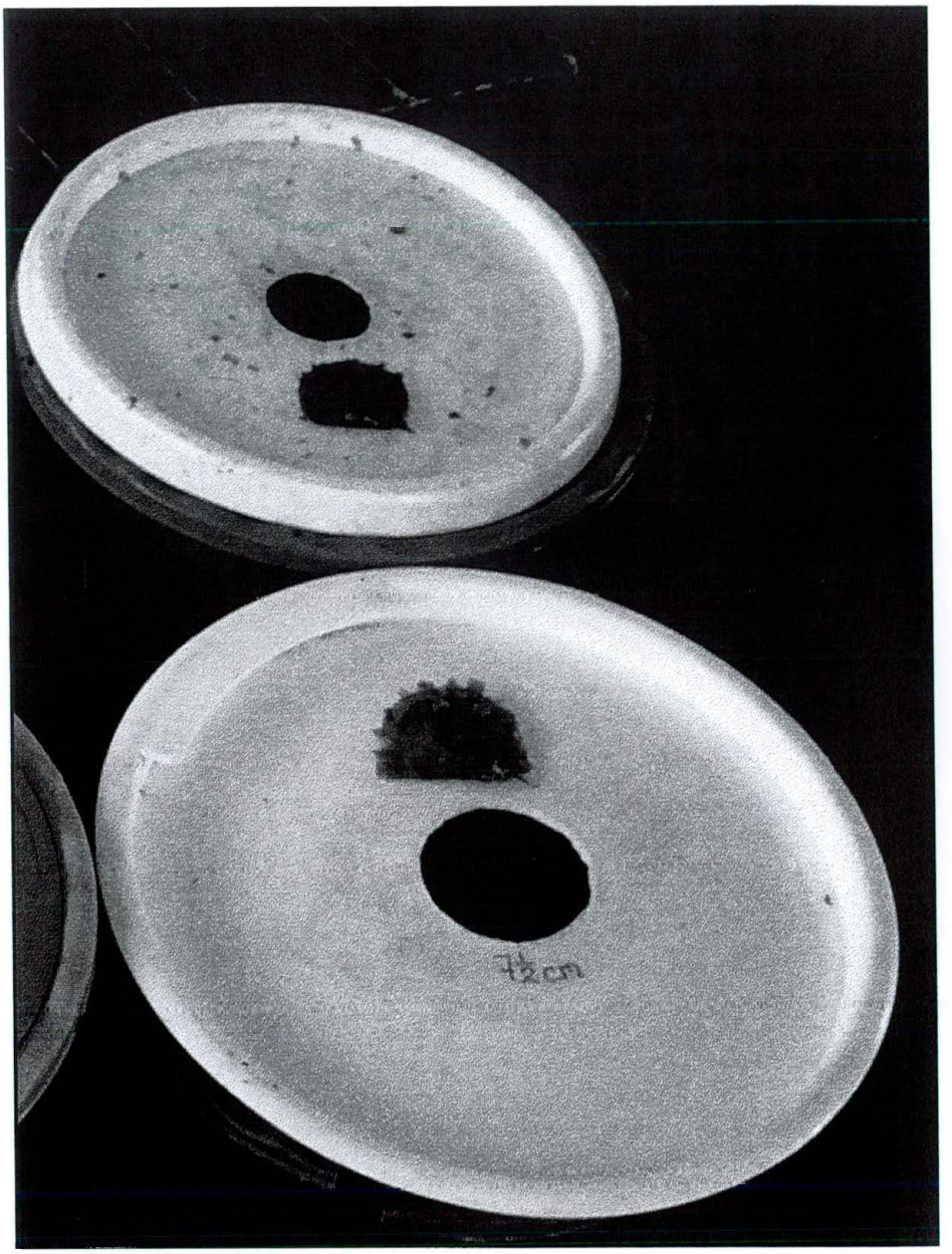


Fig 11.1 Grading system adopted for *P. maxima*

One hundred percent survival was recorded for the G1 size class stocked to 8-pocket nets (Table 11.4). There were no significant differences ($P>0.05$) in survival between the G2 and G3 size class oysters stocked in either 8 or 28-pocket nets. There were also no significant differences in survival ($P>0.05$) between size classes stocked in the same type of net.

Mean monthly SH, SL and WW data are presented in Table 11.5. Monthly increases in WW are presented in Figs. 11.4 and 11.5. *P. maxima* in the G1 size class in 8-pocket nets grew significantly faster ($P< 0.001$) in the first month following grading than any other group (Table 11.5, Fig 11.4, 11.5). This growth advantage was maintained throughout the course of the study (Fig 11.4). In the fourth month, *P. maxima* in the G1 size class stocked into 28-pocket nets, recorded the smallest increase in WW (Fig 11.4).

By the end of the study, *P. maxima* in the G1 size class in 28-pocket nets were significantly smaller ($P<0.05$) than *P. maxima* in the G2 size class stocked in either 8 or 28-pocket nets (Table 11.5).

By the end of the fifth month, there were oysters in each size class that had completely filled the available growing space in the 28-pocket panels (Fig 11.6). At this stage all of the oysters from the 28-pocket nets were removed, graded and stocked into 8 or 28-pocket nets. No "runts" were recorded in *P. maxima* of the G1 and G2 size classes in 8-pocket nets. Runts were observed in all size classes in 28-pocket panels and in the G3 and G4 size classes in 8-pocket nets (Table 11.6). The greatest percentage of runts was recorded from the G1 size class stocked in 28-pocket nets (Table 11.6). The

Table 11.2 The mean (± s.e., n=7) shell heights and hinge lengths of juvenile *P. maxima* cultured for six weeks in either suspended (SC) or bottom culture (BC) in pocket nets holding either 48 or 28 individuals Values for shell height or hinge length with different superscripts are significantly different (P <0.001).

No. of pockets per net	Shell Height		Hinge Length	
	SC	BC	SC	BC
48	43.9 ± 0.3^{a}	34.9 ± 0.8^{b}	43.8 ± 0.4^{a}	39.1 ± 1.0^{b}
28	48.1 ± 0.3^{c}	38.0 ± 0.7^{d}	49.5 ± 0.4^{c}	42.6 ± 0.9^{d}

Table 11.3. The dry weight of suspended solids (DWSS) and the number of morphologically different phytoplankters (NMDP) counted from water samples taken 2.5 m from the sea-surface (SS) and at a depth of 20 m on the sea-floor (SF).

Week	DWSS (g • per 2 L)		NMDP (per L)	
	SS	SF	SS	SF
1	0.02	0.01	57	44
3	0.02	0.01	69	53
5	0.02	0.01	87	58

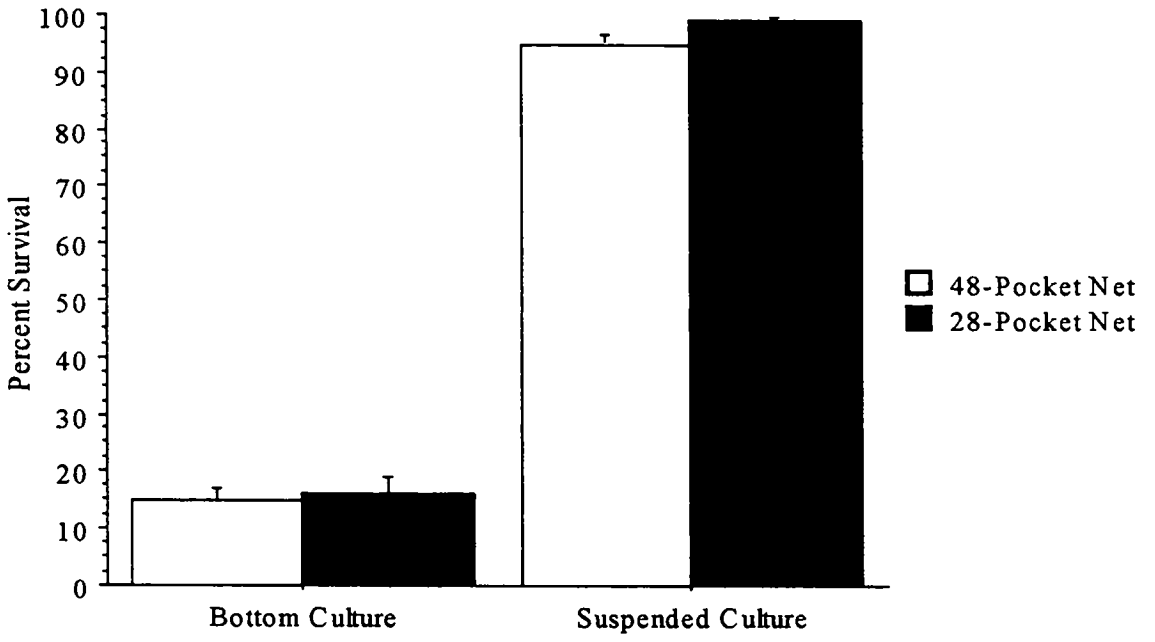


Fig 11.1. **Percent survival (mean ± s.e., n=7) for juvenile *P. maxima* cultured for six weeks in either suspended culture or bottom culture in pocket nets holding either 48 or 28 individuals**

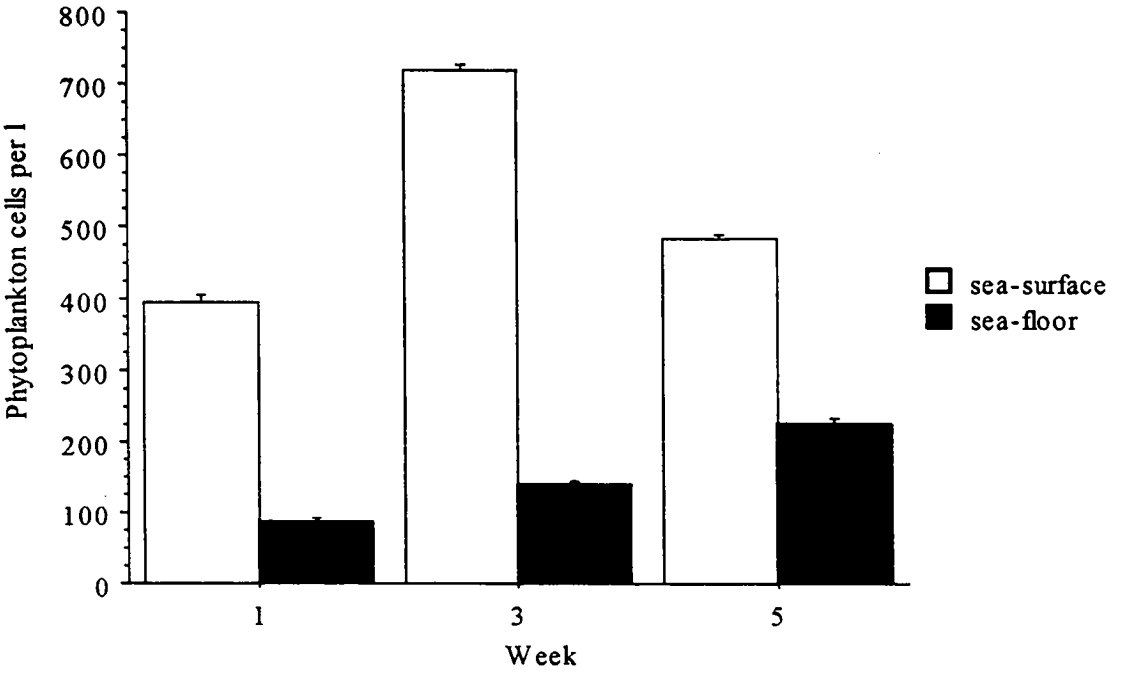


Fig 11.3 **The number of phytoplankton cells (mean ± s.e., n=2) per L of seawater sampled 2.5 m below the sea surface and at a depth of 20 m on the sea-floor**

Table 11.4 **The monthly mean (±„ n=3) survival (%) for *P. maxima* of 4 different size classes (G1-G4) held in pocket nets containing either 28 or 8 individuals for 5 months. Values with different superscripts are significantly different categories of net size and size class for each month.**

Month	Net	Percent Survival			
		G1	G2	G3	G4
1	28	91.7 ± 1.2^{a}	91.7 ± 4.3^{a}	90.5 ± 1.2^{a}	
	8	100.0 ± 0.0^{b}	100.0 ± 0.0^{b}	$95.8 \pm 4.2^{a,b}$	$95.8 \pm 4.2^{a,b}$
2	28	91.7 ± 1.2^{a}	$90.5 \pm 4.8^{a,b}$	90.5 ± 1.2^{a}	
	8	100.0 ± 0.0^{b}	$95.8 \pm 4.2^{a,b}$	$95.8 \pm 4.2^{a,b}$	$95.8 \pm 4.2^{a,b}$
3	28	90.5 ± 1.2^{a}	90.5 ± 4.8^{a}	89.3 ± 0.0^{a}	
	8	100.0 ± 0.0^{b}	$91.7 \pm 8.3^{a,b}$	$95.8 \pm 4.2^{a,b}$	$95.8 \pm 4.2^{a,b}$
4	28	90.5 ± 1.2^{a}	90.5 ± 4.8^{a}	84.5 ± 1.2^{a}	
	8	100.0 ± 0.0^{b}	$91.7 \pm 8.3^{a,b}$	$95.8 \pm 4.2^{a,b}$	$91.7 \pm 4.2^{a,b}$
5	28	90.5 ± 1.2^{a}	90.5 ± 4.8^{a}	84.5 ± 1.2^{a}	
	8	100.0 ± 0.0^{b}	$91.7 \pm 8.3^{a,b}$	$95.8 \pm 4.2^{a,b}$	$91.7 \pm 4.2^{a,b}$
6	8	100.0 ± 0.0^{b}	$91.7 \pm 8.3^{a,b}$	$95.8 \pm 4.2^{a,b}$	$91.7 \pm 4.2^{a,b}$

Table 11.5 **Monthly mean (± s.e., n=3) wet weights (WW) shell heights (SH) and shell lengths (SL) for 4 different size classes of *P. maxima* (G1-G4) held in pocket nets containing either 28 or 8 individuals for 5 months. Initial mean WW, SH and SL are given in Table11.1. Values with different superscripts are significantly different between categories of net size and size class for each month.**

Month	Net	Wet Weight				
		G1	G2	G3	G4	
1	28	31.07 ± 0.84^{a}	26.25 ± 0.61^{b}	21.84 ± 0.94^{c}		
	8	33.67 ± 0.89^{a}	26.89 ± 0.94^{b}	20.51 ± 0.66^{c}	15.39	±
0.81^{d}						
2	28	33.73 ± 1.16^{a}	34.83 ± 0.83^{c}	28.83 ± 0.71^{d}		
	8	50.03 ± 3.31^{b}	36.90 ± 1.58^{c}	28.60 ± 1.03^{d}	23.08	±
1.28^{e}						
3	28	52.43 ± 1.64^{a}	$46.50 \pm 1.13^{a,c}$	39.43 ± 1.02^{d}		
	8	63.45 ± 3.18^{b}	50.50 ± 2.17^{a}	40.13 ± 1.38^{d}	34.04	±
2.44^{e}						
4	28	67.84 ± 2.24^{a}	64.53 ± 1.66^{a}	56.13 ± 1.44^{c}		
	8	84.59 ± 3.54^{b}	69.21 ± 2.15^{a}	57.01 ± 2.44^{c}	47.43	±
3.51^{d}						
5	28	80.47 ± 2.70^{a}	75.35 ± 1.87^{a}	67.53 ± 1.81^{c}		
	8	100.12 ± 3.90^{b}	80.72 ± 2.59^{a}	66.87 ± 2.58^{c}	57.87	±
4.83^{d}						

Table 11.5 continued.

Month	Net	Shell Height			
		G1	G2	G3	G4
1	28	68.1 ± 0.6^{a}	62.7 ± 0.5^{b}	58.2 ± 0.6^{c}	
	8	69.5 ± 1.3^{a}	62.5 ± 0.8^{b}	56.9 ± 0.8^{c}	50.7 ± 0.9^{d}
2	28	70.9 ± 0.8^{a}	67.2 ± 0.7^{c}	63.2 ± 0.7^{d}	
	8	75.7 ± 1.3^{b}	$69.3 \pm 1.3^{a, c}$	63.2 ± 0.7^{d}	57.1 ± 1.4^{e}
3	28	79.7 ± 1.1^{a}	76.9 ± 0.9^{a}	73.2 ± 0.9^{d}	
	8	85.7 ± 1.4^{b}	80.3 ± 1.1^{a}	73.8 ± 1.0^{d}	67.0 ± 1.9^{e}
4	28	84.9 ± 1.2^{a}	82.8 ± 1.1^{c}	80.2 ± 1.0^{d}	
	8	90.8 ± 1.4^{b}	$84.8 \pm 1.3^{a, c}$	79.3 ± 1.3^{d}	71.9 ± 2.1^{e}
5	28	$88.7 \pm 1.4^{a, c}$	86.0 ± 1.0^{a}	83.4 ± 1.0^{d}	
	8	95.9 ± 1.5^{b}	89.5 ± 1.0^{c}	84.5 ± 1.4^{d}	76.3 ± 2.5^{e}

Month	Net	Shell Length			
		G1	G2	G3	G4
1	28	68.9 ± 0.6^{a}	64.17 ± 0.6^{c}	59.0 ± 0.6^{d}	
	8	72.0 ± 1.0^{b}	64.23 ± 0.8^{c}	58.9 ± 1.3^{d}	52.6 ± 1.1^{e}
2	28	73.6 ± 0.9^{a}	69.4 ± 0.9^{c}	65.2 ± 0.8^{d}	
	8	76.4 ± 1.3^{b}	69.7 ± 1.3^{c}	65.1 ± 1.4^{d}	57.9 ± 1.6^{e}
3	28	78.4 ± 1.1^{a}	76.3 ± 1.0^{a}	72.4 ± 1.0^{c}	
	8	83.0 ± 1.3^{b}	78.2 ± 1.5^{a}	73.5 ± 1.7^{c}	64.7 ± 2.2^{d}
4	28	83.7 ± 1.4^{a}	83.9 ± 1.0^{a}	79.6 ± 1.0^{c}	
	8	89.6 ± 1.3^{b}	84.2 ± 1.4^{a}	80.4 ± 2.3^{c}	72.7 ± 2.6^{d}
5	28	86.4 ± 1.5^{a}	87.2 ± 1.0^{a}	83.4 ± 1.1^{c}	
	8	94.0 ± 1.1^{b}	88.5 ± 1.6^{a}	85.2 ± 2.1^{c}	76.4 ± 3.1^{d}

Table 11.6 **The mean (±s.e. , n=3) percentage of *P. maxima* runts of five different size classes cultured for 5 months in 28 or 8-pocket nets. Values with different superscripts are significantly different ($P<0.01$)**

Net	Percentage of Runts			
	G1	G2	G3	G4
28	17.2 ± 2.8^{a}	5.7 ± 5.7^{b}	10.6 ± 5.9^{c}	
8	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	$7.3 \pm 7.3^{b,c}$	$7.3 \pm 7.3^{b,c}$

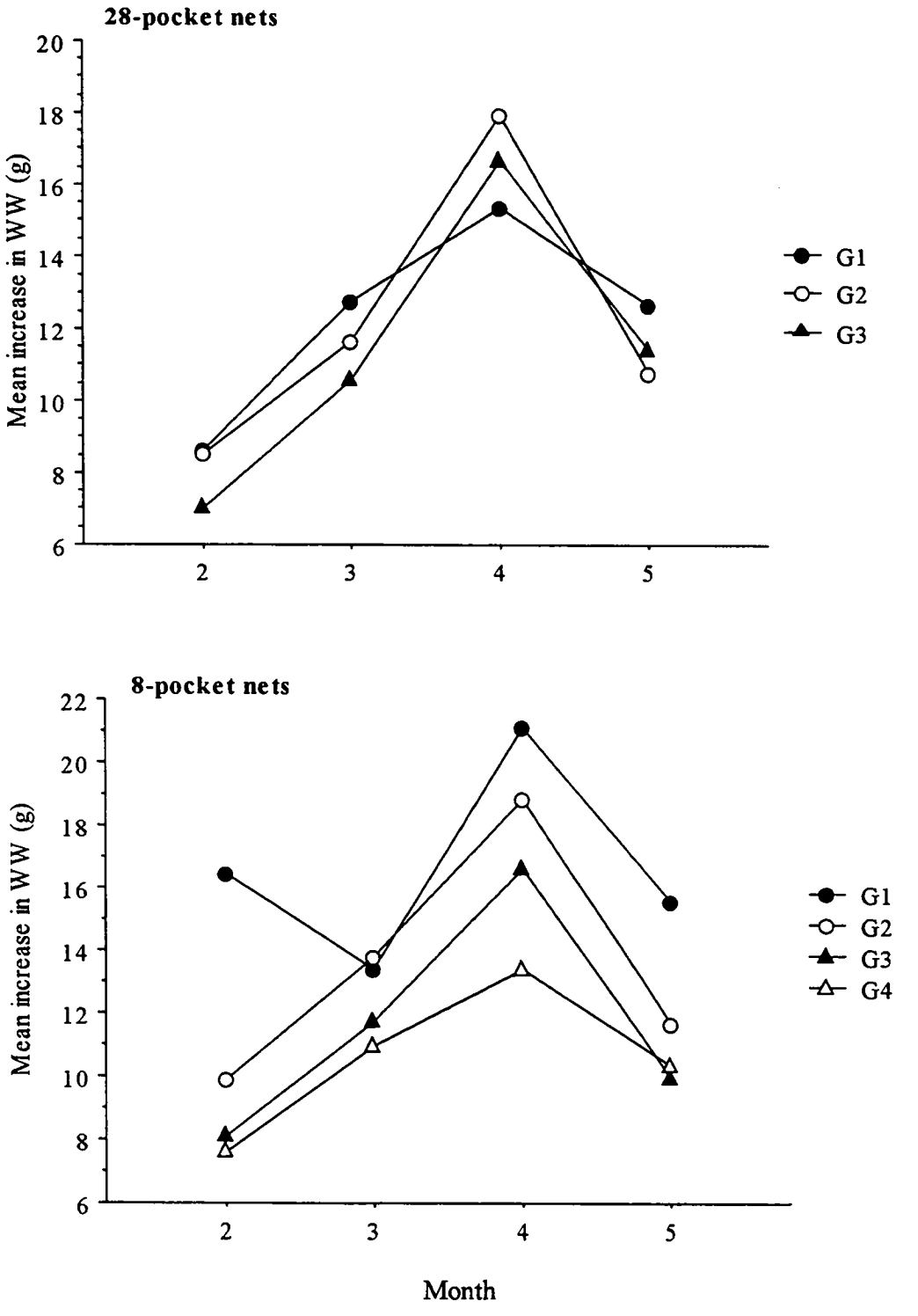


Fig 11.4 **Mean monthly increases in WW of *P. maxima* of different size classes held in 8 or 28-pocket nets.**

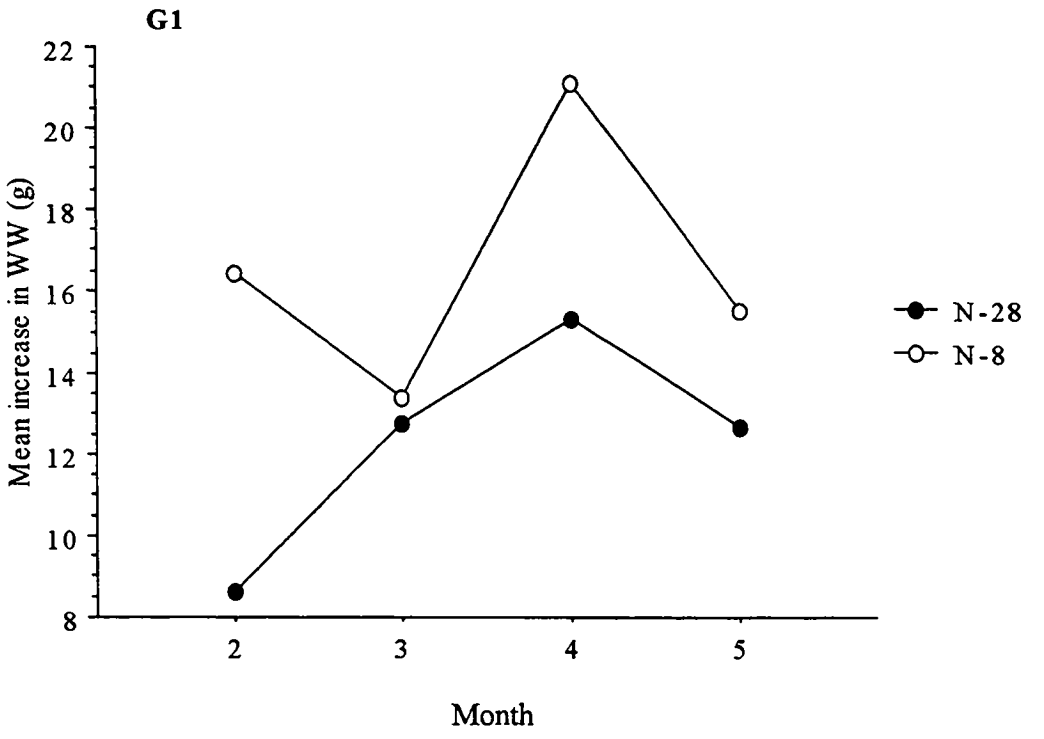


Fig 11.5 **Mean increases in WW of G1 *P. maxima* held in 8 and 28-pocket nets for 5 months.**

Fig 11.6 **One year-old *P. maxima* cultured for 5 months in a 28-pocket net. Three of the survivors are runts.**

percentage of runts for G1 in 28-pocket nets was significantly greater than for any other group ($P<0.01$). No runts were recorded in the G1 and G2 *P. maxima* held in 8-pocket nets.

11.4 Discussion

Both the density and the type of culture system affected growth and survival of juvenile *P. maxima*. However, differences in growth and survival were influenced more by culture system than stocking density. Differences in survival between the stocking densities tested within each culture type were not great and, for pearl oysters cultured on the sea-floor, the difference was not significant. Best survival and growth was shown by juveniles held in 28-pocket nets (66 oysters per m^2) suspended from a surface long line. Mean shell heights and hinge lengths of *P. maxima* held in suspended culture were greater than those in bottom culture regardless of stocking density. Juvenile pearl oysters held in 28-pocket nets in suspended culture were, on average, approximately 5 mm longer along the height and length axes than those in 48-pocket nets (99 oysters per m^2) in suspended culture. However, the same animals were almost 10 mm longer along the height and length axes than those in 28-pocket nets held on the sea-floor. The effect of culture system on growth was therefore far greater than that of density.

A major difference between surface seawater and that near the sea-floor was the amount of food available to juvenile *P. maxima*. Water samples from near the surface always had higher phytoplankton counts, diversity of species and level of suspended

solids, than samples taken from the sea floor. The results suggest that growth and survival of *P. maxima* held on the sea floor was influenced by reduced food availability. Similar results have been shown in a number of growth studies with bivalves (Brown and Hartwick 1988b, Leighton 1979, MacDonald 1986, Numaguchi 1994). Numaguchi (1994) attributed slower than normal growth of *P. fucata martensii*, in Ohmura Bay, Japan, to low food abundance. Similarly, slower growth rates of giant scallops (*Placopecten magellanicus*) cultured on the bottom, compared to those in suspended culture, reflected lower food levels between sites (MacDonald 1986). Rock scallops (*Hinnites multirugosus*) showed suppressed growth at depths equal to or greater than 60 m compared to scallops at shallower depths (30 m or less); the biomass of phytoplankton was much less at depths greater than 50 m (Leighton 1979). The same study showed that scallops held at the greater depths had thin fragile shells; this was also the case in the present study where *Pinctada maxima* held in bottom culture developed brittle shell margins and thin shells. Wilson (1987) suggested that low food availability and the reduced growth rates of *Ostrea edulis* and *Pecten maximus* that resulted, were worse where tidal currents were low. Wilson (1987) suggested that low tidal flow does not allow renewal of the food resources depleted by bivalves as they feed. This may have influenced the results in the present study as currents were reduced near the sea-floor.

The results strongly indicate that a major factor influencing growth of pearl oysters in this study was food availability. However, other factors such as disease and/or disturbance from fish and benthic animals may have also influenced results. At the time

of this study, commercial trials of bottom culture were attempted at other sites in Indonesia with similar results. Bottom culture was clearly not suitable for juvenile *Pinctada maxima* at the site used in this study even though it is widely used for adult silver-lip pearl oysters in Australia (Gervis and Sims 1992).

In the second experiment, growth and survival advantage was only observed in the largest size class and only after the second month of cultivation. The results suggest that unless *P. maxima* have reached a mean SL of 70 mm or greater (in this case by 8 months of age), there is no advantage to drastic reduction in stocking density.

Differences in growth and survival between *P. maxima* in the G1 size class are most likely the result of intra-specific competition for space and food. This was apparently the case for giant scallops reared under different stocking densities (Parsons and Dadswell, 1992).

Larger size classes of *P. maxima* maintained a size advantage over the smaller size classes during the five month period. The pattern of growth was similar for all groups, with faster and slower growth occurring in the same months Presumably, better growth during these months was the result of favourable environmental conditions and may represent a cyclic or seasonal condition. The best growth was recorded in September, which coincides with the beginning of the breeding season for mature *P. maxima* at the study location (Chapter 2, section 2.2). The percentage of oysters within each size class that could be considered undersized or runts was low (5-10 %, except for G1 in 28-pocket nets) and non-existent for *P. maxima* in the G1 and G2 size classes in 8-pocket nets.

These results underline the importance of regular grading in order to maintain discrete size groupings; however, some caution is required when culling oysters at a young age. Even in the smallest size class, G4, many *P. maxima* were of good size by the end of the experiment and would certainly reach minimum operational size of 105 mm SH (Scoones, 1990) by 18 months of age. Similarly, Newkirk (1981) reported data supporting the notion of "catch up" effect whereby slower growing *Ostrea edulis*, which could have been considered runts during the first season of growth, were able to reach marketable size in subsequent seasons. At the end of this experiment, *P. maxima* were 12 months old and only 6 months from operation. This may be an opportune time to cull the slowest growers.

Culture system and stocking density are major factors influencing the economics of bivalve aquaculture (Askew 1978, Roland and Albrecht 1990, Holliday et al. 1991, Parsons and Dadswell 1992, Holliday et al. 1993). Based on the results of this study, suspended culture at the lower stocking density in 28-pocket nets (66 oysters per m^2) is appropriate for juvenile *P. maxima*. Moreover, *P. maxima* can be held in 28-pocket nets until 12 months of age providing that the fastest growers are regularly graded out and moved into 8-pocket nets. An appropriate strategy for oysters less than 12 months of age is to grade and reduce stocking density of size classes rather than the total of one batch of similar age. This could minimize the number of nets required during grow-out and reduce operational costs. Furthermore, regular grading of oysters allows for discrete size groupings in the months leading to operation. Most pearl technicians working with *P.*

maxima implant between 500 and 600 pearl oysters with nuclei per day (Fassler, 1995). As the size of nuclei is directly influenced by the size of the pearl oyster, providing discrete size classes is likely to increase operator efficiency.

Chapter 12. Fouling animals and their effect on growth of *Pinctada maxima* in suspended culture

12.1 Introduction

Cultivation of *Pinctada maxima* from hatchery produced seed is expanding throughout Australia and the south east Asian region (O' Sullivan, 1994; Rose, 1994); however, there is little published information on appropriate grow-out techniques for this species. To date, most published accounts have concentrated on larval rearing and spat production (Minaur, 1969; Tanaka and Kumeta, 1983; Rose et al., 1986; Rose and Baker, 1994).

Fouling studies on wild *P. maxima* by Takemura and Takashi (1955, 1958) described various species of fouling animals that attach to wild adult *P. maxima*. However, these studies were concerned with identification of fouling species rather than determining any effects they may have had on *P. maxima*. Fouling, and its effect on bivalve growth and survival, is a major concern to aquaculturists (Claereboudt et al., 1994). Fouling organisms cause considerable damage to cultivated marine animals and may result in enormous financial losses for the aquaculture industry (Arakawa, 1990). Fouling has been shown to negatively affect growth and survival of the related pearl oysters, *P. fucata* (Alagarswami and Chellam, 1976; Mohammad, 1976; Chellam, 1978), and *P. margaritifera* (Doroudi, 1994) and other commercially cultivated bivalves (Duggan, 1973; Leighton, 1979; Arakawa, 1990; Claereboudt et al., 1994). In Chapter 10, the results suggested that fouling may have exacerbated the negative affect of increasing stocking density through increased competition for resources. Additionally, fouling

places additional weight and drag on culture equipment increasing the need for greater buoying and stronger anchoring systems (Claereboudt et al., 1994; Taylor and Rose, unpublished data).

Removal of fouling organisms requires cleaning of both the cultured bivalves and the structure in which they are housed. Removing fouling organisms from pearl oysters (and other bivalves) requires manual handling that may place some degree of stress on the animals. For example, repeated manual handling of juvenile scallops, grading and net changing can contribute significantly to their mortality (Ventilla, 1982; Dadswell and Parsons, 1991; Parsons and Dadswell, 1992). By contrast, a study assessing the effects of manual handling on oysters (*Crassostrea virginica*) in land-based cultivation concluded that bi-weekly cleaning promoted oyster growth (Jakob and Wang, 1994). Similarly, regular net changing to remove fouling organisms was shown to yield a 68% increase in muscle weight for the giant scallop *Placopecten magellanicus* (Claereboudt et al., 1994).

Observations on *Pinctada maxima* during grow-out in Indonesia and Australia suggest that, providing that pearl oysters are in good health, regular careful cleaning and handling (e.g. physical removal of fouling, grading and net changing) promoted vigorous growth (J. J. Taylor, unpublished data, 1993-1996). In this chapter, the effects of accumulated fouling by marine animals attached to 1 year-old *P. maxima* and removal of fouling animals, were assessed. A major goal was to determine the longest interval between cleaning that would provide best growth and survival and reduce labour costs associated with cleaning.

12.2 Materials and Methods

Two hundred 1 year-old *P. maxima* were selected by size and individuals were placed into single pockets in ten-pocket net panels (net dimension: 450 mm x 850 mm)(Fig 12.1). At the start of the experiment, pearl oysters had a SH, HL and WW (mean ± s.e., n=25) of: 59.2 ± 0.5 mm, 56.0 ± 0.4 mm and 21.40 ± 0.35 g, respectively. There were five replicate net panels for each treatment. The nets were suspended to a depth of 3 m from a surface long-line. The experiment ran for 16 weeks from late September through to the beginning of January, a period corresponding with regular heavy rainfall and a sea-surface temperature range of 27.5 to 29°C.

Cleaning and handling consisted of: severing the byssal attachment; removing individuals from the nets; cleaning shells to remove fouling; measuring and weighing the pearl oysters and returning them to clean nets. Pearl oysters were cleaned either every 2 weeks, every 4 weeks, every 8 weeks or after 16 weeks. The diversity of fouling species was recorded each time cleaning took place. Fouling animals removed from the 10 pearl oysters in each net were sorted and counted. Fouling animals from the pearl oysters within each net were then combined and dried at 50°C for 72 h before weighing. In all treatments, nets were cleaned fortnightly with a seawater pressure-pump to remove accumulated silt and algae; because algae were easily removed in this way, it was not included as part of this experiment. In this chapter, unless otherwise stated, cleaning refers only to the removal of fouling animals. The single pearl oyster that died during the experiment was replaced. The replacement animal was not measured for growth but the fouling on it was recorded.

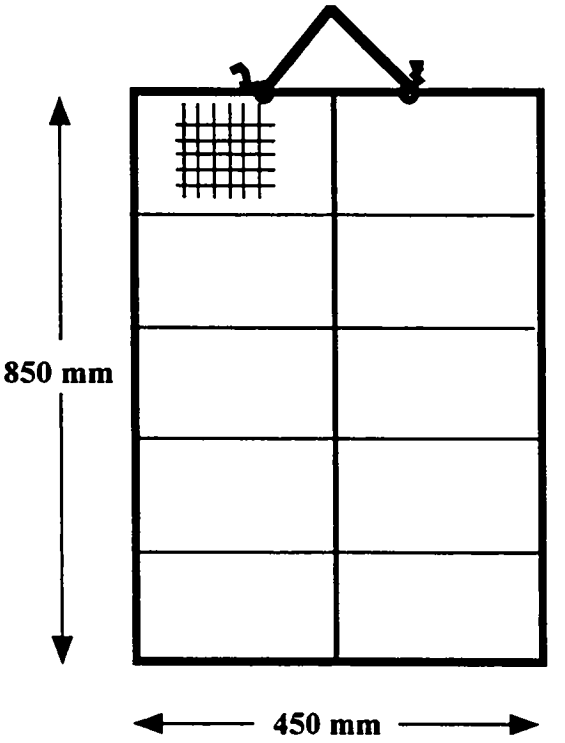


Fig 12.1 A 10-pocket net panel used to hold *P. maxima*.

Size and weight data between treatments were compared using a one-way ANOVA (Sokal and Rohlf, 1981) with means compared using Fisher's Protected Least Significant Difference (PLSD) test. Homogeneity of variances was confirmed using Cochran's test (Snedcore and Cochran, 1967).

12.3 Results

Handling, did not appear to have any detrimental effect on either growth or survival of *P. maxima*. Survival was 100%, with the exception of a single death in one replicate, which was cleaned every 4 weeks. The dry weight of fouling recorded from each sampling varied over time and with the period of exposure (Table 12.1). For pearl oysters cleaned every 2 weeks, there was a steady increase in the amount of fouling up to week 10, when the dry weight of fouling reached a maximum. After week 10, there was a rapid decline and, at week 14, no fouling organisms were found on the pearl oysters. The same trend was seen in pearl oysters cleaned every 4 or 8 weeks when significantly (P <0.05) more fouling was recorded during the middle part of the experiment.

The WW of pearl oysters was affected by the amount of fouling (Table 12.2). There was no significant difference (P >0.05) in WW between pearl oysters cleaned every 2 or 4 weeks; however, pearl oysters cleaned every 8 weeks and after 16 weeks had significantly (P <0.05) lower WW than those in other treatments (Table 12.2). The WW of pearl oysters cleaned at 8 or 16 week intervals did not differ significantly (P >0.05). The same was true for SH and HL where there were no significant differences (P >0.05) between pearl oysters cleaned every 2 or 4 weeks or between those cleaned every 8 or 16

Table 12.1 **The mean (± s.e., n=5) dry weight (DW) of fouling animals removed from groups of *P. maxima* cleaned every 2, 4 and 8 weeks and after 16 weeks. Means with common superscripts are not significantly different (P>0.05).**

Week	DW (g) of fouling per cleaning interval			
	2 weeks	4 weeks	8 weeks	16 weeks
2	$0.79 \pm 0.09^{a,e}$	-	-	-
4	0.91 ± 0.07^{a}	3.43 ± 0.65^{a}	-	-
6	$1.31 \pm 0.15^{a,b}$	-	-	-
8	$1.77 \pm 0.12^{b,c}$		10.17 ± 0.54^{b}	42.66 ± 4.64^{a} -
10	2.11 ± 0.37^{c}	-	-	-
12	1.20 ± 0.32^{a}	3.24 ± 0.69^{a}	-	-
14	0.00 ± 0.00^{d}	-	-	-
16	$0.29 \pm 0.08^{d,e}$	0.46 ± 0.09^{c}	13.95 ± 0.72^{b}	97.57 ± 5.35

Table 12.2 **Final mean (± s.e., n=5) wet weight (WW) hinge length (HL) and shell height (SH) for *P. maxima* from which fouling organisms were removed every 2, 4, and 8 weeks and after 16. Means with common superscripts are not significantly different ($P>0.05$).**

Frequency of Cleaning	WW (g)	HL (mm)	SH (mm)
2 weeks	72.51 ± 1.53^{a}	82.4 ± 1.1^{a}	83.1 ± 0.8^{a}
4 weeks	72.14 ± 1.74^{a}	84.3 ± 1.1^{a}	84.7 ± 1.0^{a}
8 weeks	66.40 ± 2.06^{b}	78.3 ± 1.2^{b}	79.9 ± 1.3^{b}
16 weeks	66.24 ± 1.57^{b}	78.1 ± 1.1^{b}	80.3 ± 1.1^{b}

weeks; however those cleaned every 2 or 4 weeks were significantly larger ($P<0.05$) than those cleaned every 8 or 16 weeks (Table 12.2).

The following bivalve species were regularly found attached to *P. maxima*: *Pteria* spp.; *Crassostrea* spp.; *Mytilus* spp. and *Pinctada* spp. Two razor clams (*Pinna* spp.) were removed from shells of pearl oysters cleaned after 16 weeks. Polychaete worms and barnacles were also common. Table 12.3 shows the relative abundance of different species of fouling animals at the different cleaning intervals over the 16 week study. Variation in recruitment intensity and composition were noted over time and between cleaning intervals (Table 12.3). By the end of week 16, the differences in the fouling on the pearl oysters were obvious (Fig 12.2).

Pteria spp. and *Pinctada* spp., although not the most prolific fouling animals, were the most obvious. This was particularly the case when pearl oysters were left uncleaned for 8 or 16 weeks (Fig 12.2). On occasion, these large bivalves, particularly *Pteria* spp., caused growth deformity by settling on the inside of the wide growth margin between the two valves of *P. maxima* (Fig 12.3). Fig 12.3 shows a *Pteria* invading the shell opening of *P. maxima* and the resulting deformity left after its removal. In this and other cases, the invading fouling animal had grown such that it was in contact with the inner nacreous layer of the *P. maxima*. Deformities as a result of invasive fouling were recorded from 6 pearl oysters cleaned after 16 weeks and 2 pearl oysters cleaned every 8 weeks. There were no similar deformities in pearl oysters cleaned either every 2 or 4 weeks.

Table 12.3 **The mean (± s.e., n=5) numbers of different species of fouling animals removed from groups of *P. maxima* pearl oysters cleaned at different intervals over 16 weeks; n = 5 nets x 10 pearl oysters. Note: Barn. = Barnacles; Crass. = *Crassostrea* spp.; Mytil. = *Mytilus* spp.; Pinc. = *Pinctada* spp.; Poly. = Polychaetes and Pter. = *Pteria* spp.**

Week	Species					
	Barn.	Crass.	Mytil.	Pinc.	Poly.	Pter.
Cleaned every 2 weeks:						
4	0.6 ± 0.2	17.8 ± 4.6	1.6 ± 1.0	3.2 ± 0.6	0.2 ± 0.2	3.6 ± 1.2
6	0.6 ± 0.4	19.8 ± 2.8	0.2 ± 0.2	2.0 ± 0.4	6.8 ± 1.2	2.0 ± 0.9
8	1.0 ± 0.6	4.6 ± 0.5	0.2 ± 0.2	5.6 ± 2.4	4.8 ± 2.3	2.2 ± 0.4
10	6.4 ± 1.5	4.8 ± 1.0	0.0 ± 0.0	1.4 ± 0.5	6.4 ± 1.5	0.4 ± 0.2
12	0.0 ± 0.0	2.4 ± 1.5	0.0 ± 0.0	1.4 ± 0.5	9.4 ± 3.7	0.0 ± 0.0
14	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Cleaned every 4 weeks:						
4	7.8 ± 3.0	61.2 ± 7.0	2.8 ± 1.0	27.4 ± 2.8	5.0 ± 1.4	43.6 ±4.3
8	1.6 ± 0.5	17.6 ± 3.0	0.0 ± 0.0	15.0 ± 2.2	10.0 ± 2.1	15.6 ±1.2
12	0.0 ± 0.0	6.0 ± 1.2	2.4 ± 0.5	0.0 ± 0.0	6.6 ± 2.9	1.4 ±0.7
16	9.4 ± 2.1	7.0 ± 2.4	4.4 ± 1.0	0.4 ± 0.2	6.0 ± 0.8	0.6 ±0.4
Cleaned every 8 weeks:						
8	6.4 ± 3.3	72.0 ± 7.1	1.2 ± 1.2	43.4 ± 4.3	14.2 ± 4.1	39.8 ±4.9
16	4.2 ± 0.9	7.4 ± 0.5	0.8 ± 0.5	6.8 ± 1.5	9.6 ± 1.6	0.6 ±0.4
Cleaned after 16 weeks:						
16	3.8 ± 1.3	47.4 ± 3.7	2.0 ± 0.7	19.0 ± 2.9	12.2 ± 1.9	16.6 ±2.9

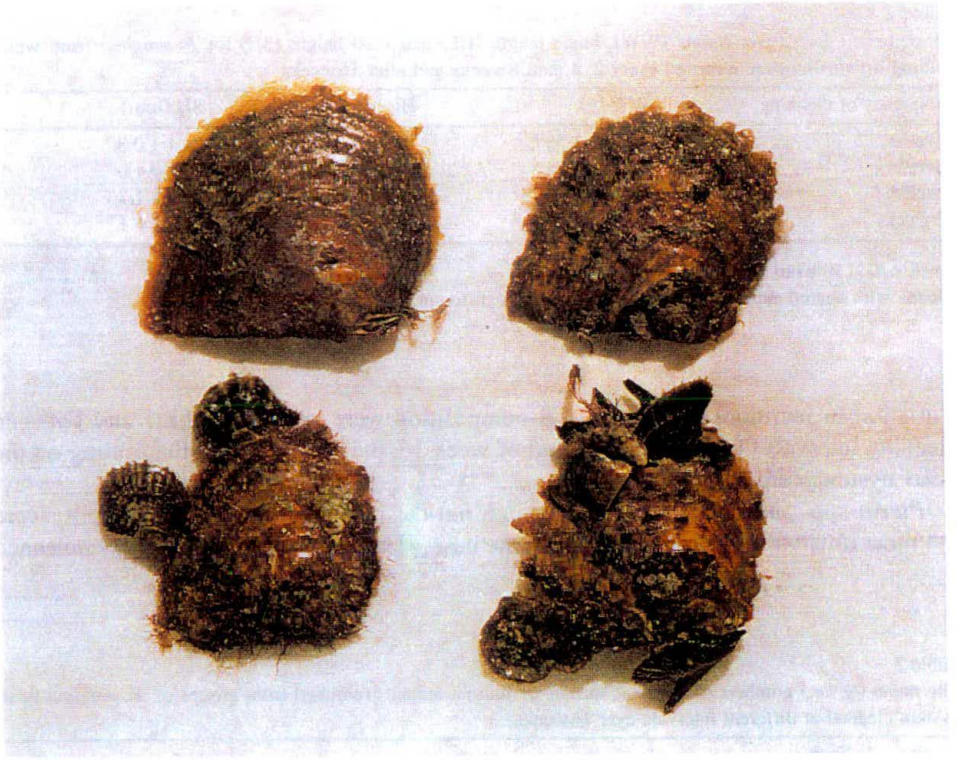


Fig 12.2 **Appearance of *P. maxima* under different cleaning regimes after 16 weeks in suspended culture: top left - cleaned every 2 weeks; top right - cleaned every 4 weeks; bottom left - cleaned every 8 weeks; bottom right - cleaned after 16 weeks.**

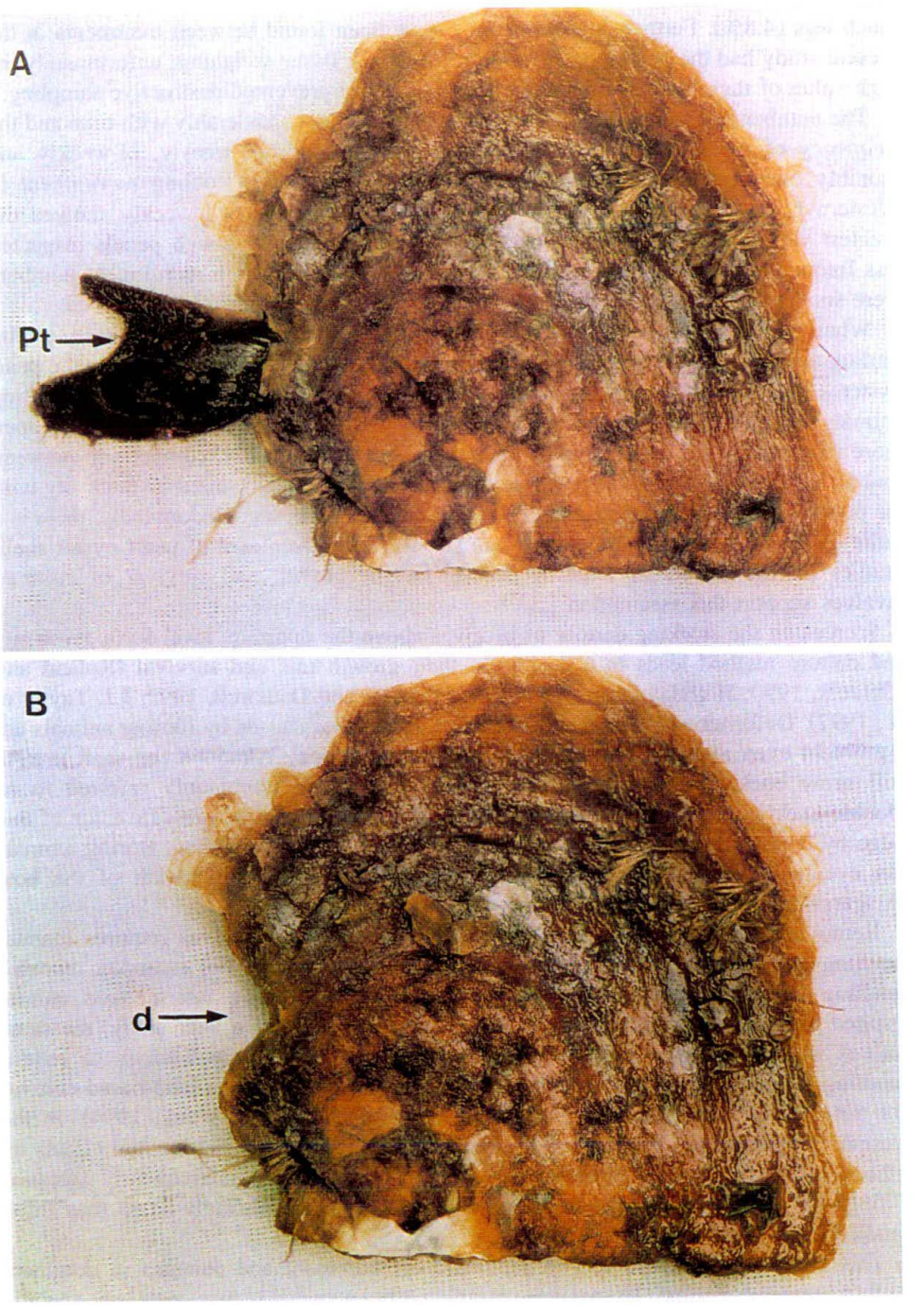


Fig 12.3 A. *P. maxima* with *Pteria* sp. (Pt) invading the shell margin. B. The deformity (d) left after the removal of the invading *Pteria* sp.

12.4 Discussion

Fouling did not affect survival of 1 year-old *P. maxima* but regular removal of fouling animals at 2 or 4 week intervals promoted growth and reduced shell deformity. Similarly, Mohammad (1975) found an inverse correlation between growth of *P. fucata* and diversity of fouling organisms. Claereboudt et al. (1994) reported a 68% increase in muscle mass for giant scallops (*Placopecten magellanicus*) kept in clean nets as opposed to those in fouled nets, even though the difference in shell height was much less (4.8 %). Further differences may have been found between treatments in the present study had pearl oysters been sacrificed for tissue weighing; unfortunately the high value of these animals ($AUS 10 -15.00 ea.) prevented destructive sampling.

The number and composition of fouling species varied considerably with time and the frequency of cleaning. Judge (1995) using settlement panels inspected weekly, bi-weekly and monthly over a 4 month period, found similar differences in fouling recruitment in Western Long Island Sound, USA. Panels inspected and cleaned weekly showed the greatest variations in recruitment over the 4 month period. Although panels inspected less frequently showed similar seasonal variability, the changes in recruitment numbers were smaller.

When pearl oysters were left for 16 weeks before cleaning, further recruitment by fouling animals may have been inhibited by those animals already attached to pearl oysters. This would, in part, explain the relatively low numbers of individual fouling animals recorded from pearl oysters cleaned after 16 weeks compared to those cleaned more regularly in the earlier part of the experiment. Additionally, competition between fouling animals as their number and size increased may have resulted in mortality with

the population reaching a maximum biomass and then declining. Undoubtedly, there is a finite biomass of fouling animals that can survive on a given area of pearl oyster shell. Studies on the effects of stocking density on growth and survival of cultured bivalves support this assumption.

Increasing the stocking density of bivalves above the optimum level for a given site and culture method leads to a decline in their growth rate and survival (Roland and Albrecht, 1990; Holliday et al., 1991; Parsons and Dadswell, 1992; Holliday et al., 1993; Chapters 10 and 11). Deformities of the shell margins of *P. maxima* caused by fouling animals are a problem even after the removal of the offending animal. Although the shell margin will grow back, this usually results in a distinct ridge (commonly referred to as "double-back") that develops between the old and new growth margins. Invasion of this ridge by boring animals, such as sponges and polychaetes, is common. Boring animals can eventually invade soft tissues causing stress and finally death of the host (Alagarswami and Chellam, 1976).

Removing fouling animals from commercially important bivalves requires manual handling that may be, in certain cases, detrimental to survival. For example, repeated handling of giant scallops (*Placopecten magellanicus*) during intermediate culture resulted in a 23% mortality (Parsons and Dadswell, 1991). In a later study, the same authors recommended using low stocking densities for *Plac. magellanicus* to reduce handling. In contrast, regular handling of oyster (*Crassostrea gigas*) spat in land based cultivation resulted in higher rates of growth and survival (Jacob and Wang, 1994). In the present study, repeated handling of *P. maxima* did not have any detrimental effects on either growth or survival. Indeed, those animals handled most frequently (cleaned

fortnightly) along with those cleaned every 4 weeks, were significantly larger than those handled every 8 weeks or after 16 weeks.

This chapter demonstrated the cyclic nature of settlement and changes in dominant fouling species over time. To maximize cleaning efficiency and reduce costs, site specific knowledge of fouling is required. For example, pearl farmers in northern Australia expect (and prepare for) a heavy settlement of barnacles that generally follows the onset of monsoonal rains. The time spent removing fouling animals is a major cost in pearl farm operations. For the purposes of this experiment, pearl oysters were removed from their nets before being cleaned. In practice, pearl oysters can be cleaned *in situ* by sliding a solid steel scraper or blunt chisel into the pocket and chipping off fouling animals. Based on the above results, fouling animals should be removed monthly to maximize growth rate and reduce the risk of growth deformities. More regular cleaning, whilst having no deleterious effects on pearl oyster growth or survival, appears to be unnecessary and may add to operational costs.

Chapter 13. General Discussion

The aims of this study were to improve our knowledge of spat production, nursery rearing and grow-out of *Pinctada maxima* in order to optimize growth and survival of hatchery produced seed and to relate the findings directly to industry. These goals have been achieved. The salient points of the study are schematically summarized in Fig 13.1.

This study demonstrated that larval settlement density influenced subsequent growth and survival. To this end, a settlement density of no more than 1 larva per mL was recommended, whilst a spat density of 70 spat per 100 cm^2 gave best growth and survival following settlement. The type, conditioning and orientation of collectors further influence settlement. Collectors manufactured from old black polypropylene rope were shown to result in greatest settlement. Further, orienting collectors horizontally in tanks and conditioning them with a biofilm resulted in improved settlement. Pearl farms by their very nature, are a great source of materials for collector manufacture. By adopting a collector design similar to the one used here, old rope and pocket nets can be utilised for spat settlement thus extending their useful life. This information allows commercial *P. maxima* producers to standardize settlement technique and thereby minimize equipment costs and optimizing efficiency.

Unfortunately, large numbers of spat do not settle on the provided collectors, preferring instead to settle on the surfaces of rearing tanks. The problem of removing small spat from their point of attachment without causing undue stress or damage was resolved in this study. Spat can be safely induced to detach from substrata by raising the salinity of culture seawater to 45 $^{o}/_{oo}$ or by reducing the pH of culture seawater to 4. These simple measures circumvent the need to scrape or otherwise physically remove

spat from their point of attachment and greatly reduce the risk of damage or death to the spat. Furthermore, the data collected cautions about the effects of sudden environmental changes on small spat, particularly during transfer from hatchery to sea, which could result in losses of spat.

Prior to this study, there was no published comparative feeding trial using micro-algae for spat of *P. maxima*. The literature for pearl oysters on this subject is somewhat grey and often contradictory. For this reason a number of mono-specific micro-algal diets were compared. Of the species tested, *C. muelleri* promoted best growth in *P. maxima* spat indicating that a diet based on *C. muelleri* is appropriate for *P. maxima* spat. This work underlined the importance of assessing the nutritional value of micro-algae experimentally rather than relying solely on published data.

Mesh sleeves are commonly used in bivalve nursery culture to protect small and often fragile spat. This study showed that the use of protective mesh sleeves greatly improved retention of spat on collectors. Moreover, mesh in the range 0.75-3 mm is appropriate to protect small spat from predators such as fish. The size of mesh influences growth with significantly larger oysters recorded at each increase in mesh size. The choice of mesh size will depend on site and circumstance but, in general, smaller mesh is perhaps best used when settlement numbers are low with larger mesh being more appropriate at higher spat densities.

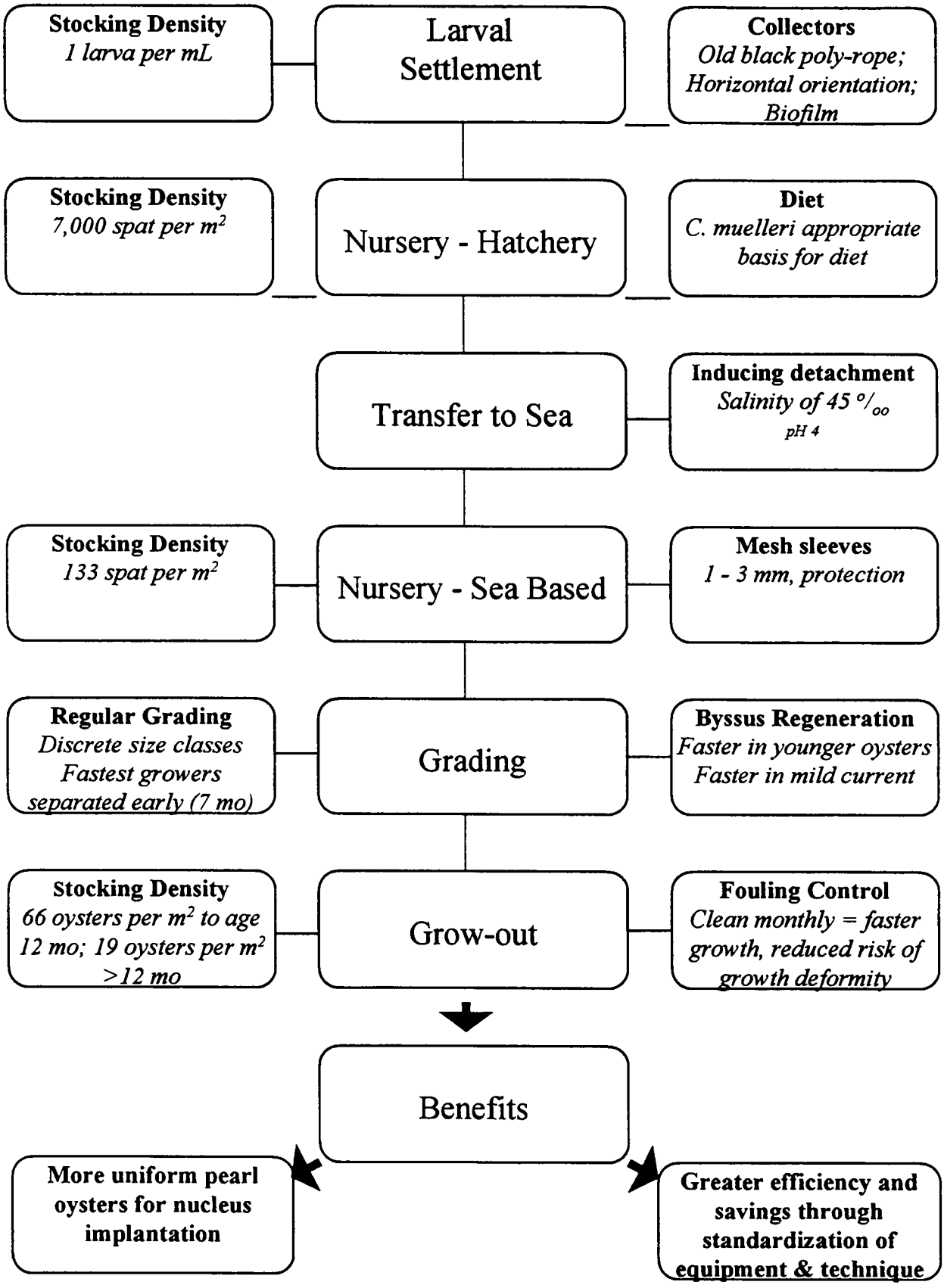


Fig 13.1 **A schematic overview of this study**

Regeneration of the byssus requires considerable energy expenditure on the part of pearl oysters (Yukihira, 1998). This study shows the differences in time required for byssal regeneration of different age classes of *P. maxima* and the influence of current speed. Ideally, newly-graded *P. maxima* should be placed in areas of moderate current to promote faster regeneration of the byssus. Once anchorage is established, the pearl oysters can be moved to areas of stronger current.

Many *P. maxima* pearl farms, especially in northern Australia, are located in estuarine areas subject to fluctuations in salinity. This study indicated that *P. maxima* spat were tolerant of salinities within the range 25 $^{o}/_{oo}$ to 45 $^{o}/_{oo}$ but that best growth occurred within the narrow range of 30 $^{o}/_{oo}$ to 34 $^{o}/_{oo}$. This information can be of some assistance in determining sites for nursery rearing of *P. maxima*.

Stocking density has a proven effect on bivalve growth and survival as well as influencing the economics of bivalve cultivation. This study showed that juvenile *P. maxima* grew best and had the highest survival and lowest rate of growth deformity when stocked at 133 oysters per m^2 on collectors during nursery culture. Furthermore, Chapter 11 suggests, once juveniles are removed from collectors and placed into pocket systems for grow-out, the density should be reduced to 66 oysters per m^2. At this particular stocking density in 28-pocket nets, *P. maxima* can be maintained until they are 12 months old providing that the fastest growers are regularly graded-out and placed in lower density systems (i.e. 8-pocket nets: 19 individuals per m^2). These results can be used as the basis for determining which nets to use when culturing *P. maxima*. Essentially, only two types of nets (or two pocket sizes) are required for grow-out leading to nucleus implantation.

Fouling and fouling control is a major problem in suspended bivalve culture causing considerable losses to the industry in general and more specifically to pearling. This study determined the effects of fouling on growth of *P. maxima* and showed that fouling should be removed monthly to maximize growth potential and minimize the risk of growth deformity as a result of hard fouling.

Prior to this study there was a paucity of published material on scientific experimentation relating to *P. maxima* cultivation. The south sea pearling industry, particularly in Australia, has largely developed behind closed doors and this had prevented rapid evolution of appropriate rearing systems and strategies. This study and the publications resulting from it will serve as a bench-mark for future studies on *P. maxima* cultivation and provide a guide for commercial growers. As it stands, many of the findings of this thesis have already been incorporated into hatchery, nursery and grow-out practices at the commercial facilities used during the study.

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