Trial farming the akoya pearl oyster, *Pinctada imbricata*, in Port Stephens, NSW

Wayne A. O'Connor, Norman F. Lawler & Michael P. Heasman

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1. NON-TECHNICAL SUMMARY

1.1. Background

The akoya pearl oyster, *Pinctada imbricata* has been fished for pearls for centuries and is amongst the widest spread of the pearl oyster species. Akoya oysters are found on areas of the eastern coastline of North and South America, the east coast of Africa, the Mediterranean, as well as throughout the Indo-Pacific. Most notably, the akoya oyster is found in Japan, where it has formed the basis of a billion-dollar pearling industry.

Recently a dramatic downturn in Japanese pearl production, resulting from a variety of factors including disease, has created an opportunity for NSW to enter the industry. The akoya oyster occurs naturally along much of the NSW coast including the Port Stephens area.

In the early 1990's, Barrier Pearls Pty. Ltd., in association with Japanese pearling interests, began investigating the possibility of establishing a temperate pearl oyster farming industry in NSW. Barrier Pearls with the assistance of the Australian Museum located and surveyed populations of akoya oysters in NSW, which it believed could form the basis for a pearl farming industry. The Company approached NSW Fisheries to conduct the next phase of research, which was to evaluate survival, growth and nacre deposition in this species in NSW waters, primarily Port Stephens. This research was considered consistent with the Department's corporate vision to promote sustainable aquaculture industries and to develop commercial opportunities in aquaculture.

NSW Fisheries subsequently obtained four deepwater lease sites with a total area of 28 ha in Port Stephens. Each of these sites was chosen specifically for pearl culture with the assistance of representatives of Barrier Pearls Pty. Ltd. The Department then called for expressions of interest in a joint venture to evaluate the possibility of pearl culture in Port Stephens. Australian Radiata Pty. Ltd., a company formed by Barrier Pearls and Japanese interests was selected as the most suitable commercial partner in this research.

1.2. Need

A drastic and continuing decline in Japanese production of high quality akoya pearls and pearl shell (e.g. from 118 000 kg in 1993 to 63 000 kg in 1996) due to the degradation of inshore waters and disease has created a large gap in market supply of this class of pearl.

A survey conducted by the Australian Museum revealed the presence and distribution of the akoya pearl oyster in NSW. This species appears to have suitable characteristics for pearl production.

A preliminary evaluation of Port Stephens by NSW Fisheries and Barrier Pearls technical staff identified several potentially suitable sites for farming the akoya oyster.

The real potential of these sites can only be evaluated in pilot farming trials.

This proposal is compatible with research facilities and expertise of staff at the Port Stephens Fisheries Centre and coincides with the availability of the necessary resources.

Pearl farming is a low impact/ high value form of aquaculture compatible with the long-term objectives of NSW Fisheries (sustainable aquaculture).

A pearl farming and jewellery industry would be very compatible with the existing tourist and recreational industries in the Port Stephens area.

1.3. Objectives

Pearl oyster research conducted by NSW Fisheries had three primary objectives: 1) to broaden knowledge of the biology and ecology of akoya oysters in NSW waters; 2) to establish techniques for the production of akoya spat and 3) to commence assessments of aspects of the potential environmental impact of pearl oyster farming in Port Stephens.

Specifically studies of the biology and ecology of *P. imbricata* undertook to determine:

1. Size, age and seasonality in the onset of sexual maturity in akoya oyster stocks.
2. Seasonal changes in the reproductive condition and "spawnability" of captive stocks.
3. Laboratory evaluation of the lethal limits of temperature, salinity and emersion on akoya oysters.
4. Biofouling, shell boring, disease and parasitism in the above stocks.
5. To assess the potential for hatchery production of pearl oyster spat and to monitor post seeding growth survival and nuclei retention of the above stocks.

1.4. Summary of findings

1.4.1. Reproductive Condition

Central to the development of a pearl industry is an understanding of the reproductive biology of the target species. This information assists in timing various aspects of farming including the collection of wild spat, hatchery production and pearl implantation. Regrettably, reproductive studies to date indicate that the behavior of *P. imbricata* varies according to location. Therefore, simultaneous with initial investigations into the farming potential of *P. imbricata* in New South Wales, studies also began to follow the reproductive condition of the oyster.

From May 1998 until August 2000, oysters were collected monthly from Wanda Head, Port Stephens, and observations of reproductive condition were made. Oyster reproductive activity was greatest from late spring to early autumn with oysters in poor reproductive condition during winter. Two annual peaks in condition were observed, the first in November and the second in March-April; however, microscopic examination of the gonad indicated differences between the two peaks. Gonad samples collected following the peak in November showed a high proportion were empty, consistent with spawning, while those taken in April-May suggested the oysters were resorbing the gonad rather than spawning.

In addition to reproductive monitoring, spat collector bags were deployed monthly in Port Stephens to monitor oyster settlement. The numbers of spat settling has varied significantly between years but has been restricted to the summer months, December – February. This is consistent with November spawnings and further suggests that the second, autumnal peak in reproductive activity does not contribute to oyster settlement.

Overall, reproductive patterns in *P. imbricata* are poorly suited to culture in Port Stephens. Farmers desire spat in early spring (Sept) to allow maximum use of the "growing" season (Sept – May). Reproductively capable oysters are not available from the wild until September and natural spatfall is quantitatively too variable and occurs too late in the season (Dec – Jan). As a result

oysters are being conditioned in the hatchery in July, spawned in August and spat are supplied to farmers in mid September.

1.4.2. Growth and Nacre Quality Trials

Concurrent with evaluations of the potential for farming *P. imbricata* at the experimental farm site at Wanda Head, oysters were deployed to a number of sites in Victoria and NSW. In a series of trials extending over three years, the impacts of depth and stocking density were assessed on oyster growth and survival, as well as the impacts of site on growth, survival and nacre quality. In Port Stephens, four experimental locations were evaluated at which the depth of oyster deployment did not affect growth. At three of the sites survival was also independent of depth, although significantly higher mortality was observed in oysters held close to the bottom at Wanda Head. In these early trials, oyster growth differed significantly between the sites within Port Stephens, a prelude for the differences to be observed between sites in later, geographically broader surveys. The density at which oysters were stocked in cages in the early trials also significantly impacted on growth.

When deployed at locations extending from as far south as Port Phillip Bay, Victoria, to the central coast of NSW, oyster growth, survival, nacre quantity and quality all differed significantly between sites. In general, reductions in oyster growth were observed as latitude decreased and was putatively ascribed to declining water temperature; although, significant differences in growth were also found among sites at which temperature was unlikely to have been the predominant factor. Survival was generally high (>90%) but differed between sites, with the poorest performance occurring at sites at which heavy infestations of mudworm (polydora species) were noted.

The shells of oysters deployed at the various locations were sectioned and the thickness of the nacre layer was strongly correlated with growth. The quality of the nacre (colour and lustre) produced at each site was then assessed by an independent panel and scored according to commercial desirability. The scores for colour and lustre of the nacre varied significantly between sites, but neither was correlated with growth. Further, those sites that scored highly for colour did not necessarily score well for nacre lustre. In those sites, which were monitored over successive years, their relative performance with respect to colour and lustre also varied over time.

1.4.3. Temperature and Salinity Tolerance

To assist in establishing protocols for hatchery production and farming of the pearl oyster, *Pinctada imbricata*, an investigation of the responses of embryos and juveniles to variation in temperature and salinity was done. Embryos were held at temperatures in the range 14 to 26^{o}C and exposed to salinities in the range 11 to 35 ppt. Initially, when embryos did develop, the rate of development was affected by temperature. At salinities of 32 and 35 ppt and a temperature of 26^{o}C all embryos had developed to D-veliger stage within 24 h. Development was slower at all other temperature-salinity combinations and the percentage of embryos developing increased between 24 and 40 h post fertilisation. After 40 h, percentage development of D-veligers and D-veliger yield also differed with treatment. Embryos failed to develop to D-veliger stage at 14^{o}C and at salinities of 26 ppt or less. Within the salinity range 29 to 35 ppt, both the percentage development of D-veligers and veliger yield increased significantly with increasing salinity. Within the temperature range 18 to 26^{o}C, the relative percentage of D-veligers among larvae present also increased significantly with increasing temperature and there was a significant interaction with salinity. Temperature, within the range 18 to 26^{o}C, did not however, significantly affect the total numbers of embryos developing to D-veliger stage within 40 h.

Juveniles (17 mm shell height) were held at temperatures in the range of 14 to 24^{o}C and exposed to salinities in the range of 11 to 35 ppt. Initially the rate at which the oysters formed byssal thread

attachments to the walls of the aquaria was monitored and was influenced by both salinity and temperature. The percentage of spat attachment to the aquaria walls increased most rapidly at salinities of 29 and 32 ppt, irrespective of temperature. At these salinities, > 70 % of oysters had attached within 6 h. Outside this narrow salinity range, the rate of byssal attachment decreased. Byssal attachment did not occur at salinities of 17 ppt or less. Temperature also affected byssal attachment, although the impacts were not as pronounced as those of salinity. Within the optimum salinity range (29 to 32 ppt), the rate of byssal attachment was fastest at 18^{o}C, where up to 80% of oysters had attached within 4 h. This rate was slightly faster than that observed at 22^{o}C, which in turn exceeded those observed at both 14^{o}C and 26^{o}C. In the longer term, salinity and temperature also affected oyster mortality. Irrespective of temperature, oyster survival was high at salinities of 32 and 35 ppt, while high mortality occurred at salinities of 23 ppt or less within 7 days. The onset of mortality was most rapid at the two extremes in temperature tested 14^{o}C and 26^{o}C and the greatest overall mortality occurred at these two temperatures.

1.4.4. Emersion Tolerance (Air Exposure)

Regular air exposure of *P. imbricata* during culture prompted an evaluation of their tolerance to emersion. Oysters were held out of water under conditions chosen to simulate the harshest experienced in Port Stephens. Temperatures tested were in the range 12 to 36^{o}C and fans were used to simulate the drying effects of winds. Spat (4.8 mm) and juvenile (12.3 mm) survival was greatest in the range 16 to 24^{o}C. At 20^{o}C oyster survival was greatly affected by oyster size, varying between 4 h for 5 mm spat to 30 h for 37 mm juveniles. Any additional stress that may be imposed by breaking the byssal attachment of the oysters prior to air exposure had no significant effect upon survival. Protection against desiccation was of particular importance and significantly increased oyster tolerance to emersion. For spat (4.8 mm) protection from air-drying provided by storage in plastic bags tripled survival time. Survival was further increased if oysters were wrapped in damp toweling inside the bags, however the replacement of air with oxygen in the bags did not significantly increase survival. As a direct outcome of these findings oysters (12 to 35 mm) are now routinely transported in damp toweling inside plastic bags for up to 30 h without significant losses.

1.4.5. Relaxants

Pearl culture and pearl research both require a number of invasive treatments that can cause "stress" to oysters. To alleviate stress and any complications that may arise, potential anaesthetics and relaxants have been trialed with a number of shellfish species. This study sought to determine the efficacy of one of the more commonly used relaxants, Propylene phenoxetol (PP) with the two species of pearl oyster found in NSW.

The responses of the pearl oysters *P. imbricata* and *P. albina* to PP were similar to those reported for other members of the genus. Both species of pearl oyster opened readily in the presence of PP (2 mL L^{-1} seawater). Relaxation generally occurred within 15 min and, on removal from the relaxant bath, oysters recovered within 10 min without evidence of any ill effects. In general, both relaxation and subsequent recovery times decreased with increasing water temperature. The sizes of oysters had little effect on the time taken to open valves in the presence of PP, the time to relaxation nor the time to recover after exposure. Prolonged exposure to PP (90 min) significantly increased the recovery time, but no mortality or apparent ill effects were observed in the week following exposure.

1.4.6. Predators and Pests

Generally, akoya oysters are particularly robust and in NSW have not been affected by disease; however, they appear to have numerous predators in the wild. Many of the predators such as fish can be effectively excluded in culture, but some have the ability to enter the pearl cages. In

particular, concerns were raised by the occurrence of a predatory flatworm, *Imogine mcgrathi*. Commonly called wafers or leeches these flatworms have been known to attack and consume Sydney rock oysters and have also affected the culture the culture of scallops in Port Stephens. As a result several trials were undertaken to determine if the flatworm was indeed a predator of pearl oysters and to investigate methods for flatworm control.

The flatworm was found to occur at an average of 3.2 individuals per oyster spat collector bag and to eat oysters at a rate of 0.035 to 0.057 day^{-1}. Predation was affected by flatworm size with larger worms capable of eating larger oysters and of eating greater amounts of oyster flesh. Irrespective of flatworm size, predation was generally confined to oysters less than 40 mm in shell height and in the laboratory all predation occurred at night.

To control flatworm infestations, salt, brine baths (250 g kg^{-1}) and freshwater baths were shown to kill *I. mcgrathi*. The ease of use of brine and freshwater baths then encouraged assessments of *I. mcgrathi* salinity tolerance. The flatworms were exposed to solutions ranging in salinity from 0 to 250 g kg^{-1} for periods of from 5 min to 3 h. Despite showing both behavioural and physiological signs of discomfort, *I. mcgrathi* survived the maximum exposure time of 3 h at salinities in the range 7.5 to 60 g kg^{-1}, inclusive. Beyond this range, the duration of exposure tolerated by flatworms decreased until 0 and 250 g kg^{-1}, when flatworms no longer survived exposures of 5 min.

Despite the significant impact of flatworms on other commercial bivalves, *I. mcgrathi* at densities encountered to date in pearl farming trials at Port Stephens constitute a minor pest that can be controlled by freshwater baths. But additional concerns were raised by large numbers of flatworms encountered on mussel culture long-lines in Twofold Bay. This prompted a further study to see if pearl oysters cultured concurrently with mussels were also adversely affected.

The flatworm infesting the mussels was confirmed to be *Imogine mcgrathi* and was found to occur in numbers as great as 386 m^{-1} of mussel culture rope. In the laboratory, these *I. mcgrathi* consumed mussels at a rate of approximately one a month. Despite having previously been confirmed to be a predator of pearl oysters, when held concurrently with mussels and oysters of a similar size, *I. mcgrathi* collected from mussel ropes ate only mussels. When offered only pearl oysters as food, these same flatworms displayed a phenomenon known as “ingestive conditioning”, where they appeared incapable of eating them.

1.4.7. Hatchery Production and Farming

The akoya pearl oyster has been produced in hatcheries in Asia for decades and while it is considered among the most robust of the genus for this purpose (Ito, 1998), there is not a great deal of information available on the techniques used to propagate the species. A description of the techniques used and some of the information garnered over three successive production seasons at the Port Stephens Fisheries Centre is provided. In many regards, the techniques we have chosen to use for akoya production have been modified from those developed for the production of various other bivalves, notably, the Sydney rock oyster and the commercial scallop. As a result they have relied largely upon equipment designed and built for these alternative species. The techniques used though differing in many respects from those traditionally practiced in Japanese or Chinese hatcheries (a description of the later is provided in the Appendices, they have never-the-less been successful in producing millions of akoya spat annually over the past three years.

While this research has focused on aspects of the growout of akoya oysters, development of optimised farming techniques has not been our primary objective. As a result, only an abbreviated description of farming procedures has been provided.

1.4.8. Biodeposition

Preliminary studies were made to assess the environmental impacts of pearl farming. Elsewhere, farming bivalves has been found to be capable of altering the environment through the accumulation of organic material beneath the farms. A survey was made to monitor the levels of three key components, nitrogen (N), phosphorus (P) and total organic carbon (TOC), both beneath the farm and at five other control sites. Each site was sampled on six occasions over the first 21 months of farming activity.

Results to date have shown that the levels of N, P and TOC vary between the sites and over time; however, we have not detected any significant impacts of farming activity on the accumulation of these chemicals beneath the farm. The survey is ongoing and has been expanded to include three additional farming sites. These new sites in Port Stephens will increase the "power to detect significant changes and will, if expanded as planned, provide data on the impact of commercial scale farming in the Port.

1.4.9. Pinctada albina

At the outset of this research an extensive survey of coast of NSW confirmed the presence of two physically similar species of pearl oyster (Ponder and Colgan 1995). The first was *P. imbricata* and the second was later confirmed to be *Pinctada albina*. Although *P. imbricata* rather than *P. albina* were the primary target of this research, a series of useful observations were nevertheless made on *P. albina*. In some instances these observations were made inadvertently or opportunistically, while in others they were targeted to provide useful comparative data with that of *P. imbricata*. For instance in preliminary studies of the effects of relaxants, a shortage of wild *P. imbricata* adults in Port Stephens prompted surrogate use of *P. albina*. During the course of spat fall studies to elucidate natural spawning and recruitment, both species settled on collectors and it was often impossible for divers to discriminate between the species until the oysters were bought to surface and cleaned of fouling. Finally, broodstock of both species were spawned and the resultant larvae cultured to observe the early development of *P. albina.* This was done to reveal possible distinguishing features that would allow young of the two species in wild spat collections to be readily separated thus avoiding the need for laborious and protracted ongrowing procedures.

The reproductive condition of *P. albina* from Port Stephens was monitored over a two-year period and found to have a shorter breeding season than populations in QLD and the Northern Territory. Breeding activity was greatest from late spring to early autumn and oysters were in poor reproductive condition during winter. Peaks in apparent gonad condition (gonadosomatic index) occurred in October 1998, March 1999, January 2000 and April 2000. However, spat collectors deployed at two sites in Port Stephens found spatfall is restricted to the months of November-January, indicating that the autumnal peaks (March & April) in gonad condition did not result in subsequent recruitment.

From a pearl farming perspective, recruitment of wild *P. albina* spat on collectors was so low and variable that it is unlikely that this could provide sufficient spat to support an industry, The observed spring peak in reproductive activity was indicative of the best time for hatchery production in the absence of reproductive conditioning.

P. albina were hatchery reared to monitor larval and spat growth and assess the potential to produce the species in commercial bivalve hatcheries. The early development *P. albina* was very similar to that of *P. imbricata* and to that reported for other pteriids. Broodstock were induced to spawn using serotonin injections, air exposure and temperature shocks. The resultant embryos

developed to D-veliger stage (75.3 µm antero-posterior measurement (APM)) within 24 h. Umbonate larvae (117 µm APM) were present on Day 9 and pediveligers (206 µm APM) on Day 19. Plantigrades (235 µm APM) were first observed in significant numbers on Day 23, but larvae continued to metamorphose over the following week. Larval and spat survival were high, with an overall mean of 27% of D-veligers surviving to plantigrade stage. Given the ease of spawning, the similarities in development with other pteriids and the comparatively high survival, *P. albina* could easily be produced by hatcheries already producing other species of pearl oysters.

Infestations of shell boring organisms can be a problem in oyster culture and were present among the oysters collected to monitor reproductive condition. The degree of shell damage in *P. albina* however, was almost invariably low. Spionid polychaete (mudworms) infestations were the dominant form of borer, being present in 30% of the shells collected. Shell damage typical of boring sponges was also occasionally detected in larger oysters.

2. *PINCTADA IMBRICATA*

2.1. Reproductive condition of the pearl oyster, *Pinctada imbricata* Röding in Port Stephens, New South Wales, Australia

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2.1.1. Introduction and Taxonomy

Pearl oysters form the basis of Australia's most valuable aquaculture industry and are the subject of considerable commercial interest. Largely the focus has been upon the silver-lip pearl oyster, *Pinctada maxima*, although increasing interest is diversifying to include other endemic pteriids. Among these, *Pinctada imbricata* (Röding) is attracting particular attention, however, there is a paucity of information regarding the species in Australian waters.

Considerable pteriid research has been done in the Northern Hemisphere, but the interpretation of published literature has been clouded by a degree of taxonomic confusion. It appears that *P. imbricata* may have been known variously as *Pinctada fucata* (Gould, 1850), *P. radiata* (Leach, 1814), *P. martensii* (Dunker, 1850) and *P. vulgaris* (Schumacher, 1817). Synonymies among these species have been suggested for some time (Jameson 1901, Prashad and Badhuri, 1933) and Hynd (1955), in a study of the genera in Australia, combined *P. fucata*, *P. radiata* and *P. vulgaris*. Subsequently, Shirai (1994) has suggested that *P. martensii* is also synonymous and noted the name *P. imbricata* (Röding, 1798) has taxonomic precedence.

Allozyme electrophoretic studies have been conducted within the species complex. Genetic material from *Pinctada imbricata* collected in NSW, Australia, has been compared with samples collected from southern Honshu, Japan, and are conspecific (Colgan and Ponder, in press). However, Wada (1982, 1984) had previously found genetic discontinuities between *P. fucata fucata* from Okinawa (southern Japan) and *P. fucata martensii* from Northern Honshu (Japan). Thus for the purpose of this discussion we have accepted *P. imbricata* and assumed the cosmopolitan nature of the species (Shirai, 1994), but, *P. fucata fucata* has been treated as a subspecies.

In accordance with the economic importance of *P. imbricata* in Asia, several studies have been done regarding the timing of reproductive events within the species (Table 1). This work is of importance in that an understanding of reproductive biology can assist various aspects of farming including the collection of wild spat, timing hatchery production and the timing of nuclei implantation (Wada et al., 1991, 1995). Reproductive studies to date indicate that the behavior of *P. imbricata* varies markedly according to location.

As initial attempts to establish an industry based upon *P. imbricata* are focusing on the central coast of NSW, studies were undertaken to monitor the reproductive condition of *P. imbricata* stocks native to the area. As such, these observations represent the most southerly record of reproductive condition for the species.

Table 1. Observations of reproductive condition in pearl oysters, putatively *Pinctada imbricata.*

Location	Latitude	Temperature range	Gonad condition peaks	Comments	Author
Kerkennah Islands, Tunisia	35°N	12 –27°C	1	Continuous sexual activity in females with maximum spawning activity in summer.	Zouari and Zaouali, 1994
Nakhiloo, Persian Gulf	27°C	24 - 32°C	2	Bimodal breeding in summer (Apr - July) and autumn (Sept - Dec). Spawning oysters present over nine months of the year.	Behzadi et al. 1997
Zamami Island, Japan	26°N	21 - 28°C	2	Males mature throughout the year but spawning in females unlikely from spring to early summer, major peak in winter, minor peak in summer.	Wada, 1995
Cabo de la Vela, Columbia	11°N	23 - 27°C	2	Mature animals throughout the year, two peaks mid winter to mid summer (Jan - June), minor peak mid autumn (Oct).	Urban, 2000
Isla Margarita, Venezuela	10 – 11°N	22 – 28.5°C		Recruitment possible throughout the year but greatest in early winter (Nov - Dec).	Leon et al., 1987
Gulf of Mannar, Srilanka	7 – 10°N	25 - 31°C*	2	Continuous breeding, peaks mid summer and mid winter.	Pearson et al., 1929
Gulf of Mannar, India	7 – 10°N	25 - 31°C	2-3	Continuous activity, two peaks in 1980, summer – earl autumn (June -Sept) and winter (Dec - Feb), and in 1988, summer (July - Aug) and autumn (Nov) 1981.	Chellam, 1987
Thursday Island, Australia	11°S	24 - 30°C**	2	Mature and spatfall throughout the year reproductive peaks in summer (Jan - Feb) and autumn (Apr - May).	Tranter, 1959
Orpheus Island, Australia	18°S	21 - 31°C	R	Recruitment over eight months of the year with a peak in late summer – early autumn (Feb - Mar).	Beer and Southgate, 2001
Hervey Bay, Australia	23°S		R	Early summer recruitment.	Sumpton et al., 1990
Port Stephens, Australia	32°S	15 - 25°C	2	Poor reproductive condition in late winter and early spring, two peaks in reproductive condition in early summer (Dec - Jan) and autumn (Mar - May). Recruitment in summer.	This study

** From Chellam (1987); ** from Tranter 1958; R = only spat recruitment monitored.*

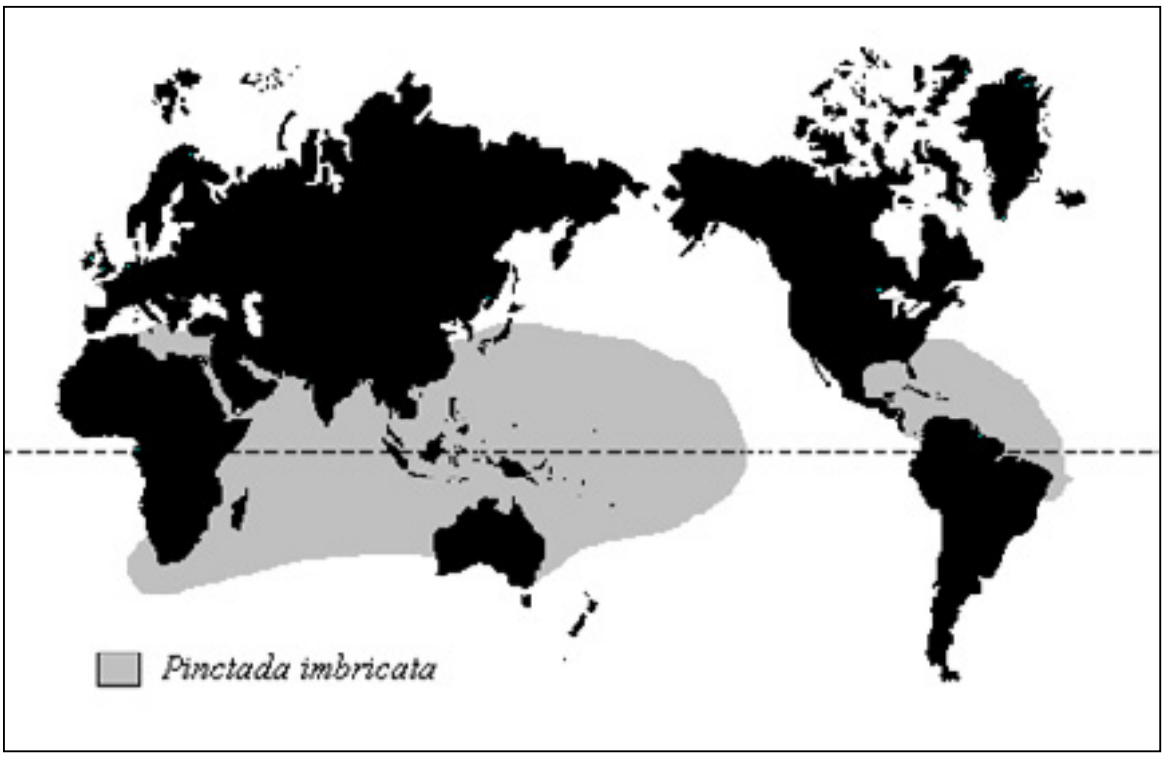

Figure 1. Distribution of *Pinctada imbricata* from Shirai (1994) and amended for the Australian Coastline (after McPherson and Gabrielle, 1962) and New Zealand (B. Marshall, pers. comm.).

2.1.2. Materials and Methods

Populations of *P. imbricata* in Port Stephens and surrounding estuaries are small and confined to areas of suitable habitat, most often sheltered rocky areas subject to moderate to oceanic salinity. One of the larger known populations occurs at Wanda Head, Port Stephens, and was considered one of the few that could support even the modest level of harvest required for this study (ca 10 oysters a month).

Divers gathered oysters from a shallow rock wall adjacent to the shore at Wanda Head and each sample was returned immediately to the laboratory for analysis. Collections began in May 1998 and continued until August 2000. On each sampling occasion, the shell height and total weight of each oyster were recorded to nearest mm and 0.01 g, respectively. The soft body was removed from the shell and its drained wet weight determined to the nearest 0.01 g.

A series of macroscopic observations were then made in which the appearance of gonad and other tissues of each oyster was assessed in the light of a series of criteria thought to be indicative of reproductive and other physiological status factors. For each criteria the oyster was given a score from 1 to 5, with a score of 1 being the base of the scale and indicating poor physiological condition with respect to the criteria.

Macroscopic criteria

Gonad: macroscopic evaluation of gonadal development was based upon criteria described by Tranter (1958) for *Pinctada albina* (Table 2).

Table 2. Criteria for macroscopic scoring of gametogenic stages *.

Stage	Description	Score
Inactive	Gametes are absent. The gonadal area is translucent and the digestive diverticula are visible.	1
Developing 1	Gonads are filling, development is patchy and appears to be emanating from the posterior forward, males and females are indistinguishable.	2
Developing 2	Gonads less patchy in appearance as follicles spread and begin to fill. A pattern of development toward the anterior is less apparent, however the anterior edges of the body remain translucent. Gonad and body thickening	3
Developing 3	Gonad and body turgid and consistent in colour, the development of follicles is no longer apparent with the exception of an occasional translucent strip at the base of the foot. Sex can be generally differentiated on the basis of colour. The digestive diverticula are no longer visible.	4
Ripe	Gonad highly turgid and consistent in colour, follicles not apparent.	5

** Detailed descriptions of the superficial appearance of gonad during development have been given by Tranter (1958a). The criteria for ranking here are based upon the portion of the body exposed when the mantle and gill are folded back, that is the area anterior and ventral to the urogenital papilla.*

Shell growth: In healthy, vigorously growing oysters, the imbricate processes at the distal margin of the shell are long and numerous. At worst these processes are absent (score 1) and at best, several successive layers of finger like projections up to 10 mm extend around the shell margin (score 5).

Byssus: An assessment of the strength of the byssal attachment was based upon the number of byssal threads. Oysters vary in this regard from those with very few (3-4) byssal threads (score 1) to those which have more than 25 byssal threads in the byssus (score 5).

"Fat": A white mucoprotein layer (Tranter, 1958) can frequently be seen in the mantle overlying the gonad. This layer, referred to by some farmers as fat, can vary from translucent, where the underlying gonad and digestive gland are visible (score 1), to a state in which a thick (~ 1 mm) cream coloured layer obscures the organs beneath (score 5).

Mantle: The width and turgor of the mantle edge are reported to vary with oyster condition. Oysters in poor condition have flaccid thin mantles (~ 2 mm; score 1) while those in good physiological condition have turgid thick mantles (~ 6 - 8 mm; score 5). This particular measure is size sensitive and implies a greater degree of subjectivity.

Previous observations during sampling of *P. imbricata* during distribution surveys allowed gonadal changes in oysters to be scored with confidence, however, we had had no prior experience with the scoring remaining criteria. Thus a two-month hiatus occurred as familiarity was acquired and scoring for these criteria commenced in July 1998.

Histology

Following macroscopic assessment, a transverse section of the gonad was excised from each oyster and fixed in Davidsons solution (Shaw and Battle, 1957). Sections were passed through graded alcohol solutions and then xylene before being mounted in paraffin. 6 μm sections were cut and stained with Harris Haemotoxylin. The sections were examined using a microscope (x 200 magnification) and categorised into gametogenic stages using criteria based upon those developed by Tranter (1958b) for *Pinctada albina* and used later with *P. imbricata* (1959). For reasons described previously (O'Connor, 2001), the number of stages was reduced from Tranter's (1958b) nine to five.

The abridged stages of gametogenesis used were as follows. Tranter's (1958b) developing stages 1 and 2, and 3 and 4 were combined to form stages Developing 1 and 2, respectively. Tranter's Developing 5 was retained and called "ripe". The regression stages 1 and 2 have been called spawned/regressing, with the inclusion of the term spawned in acknowledgment of the difficulty in determining whether follicles have been voided as product of partial spawning or autolysis. Finally, the two stages in which the follicles are largely devoid of gametes, "r3" and "inactive", were combined.

Spionid polychaetes

Because shell boring organisms, like spionid polychaetes (*Polydora* and *Boccardia* spp.) can affect the physiological condition of oysters, the valves of each oyster were examined for their presence. Where present, the degree of polychaete infestation was scored on a scale of 0 to 4. Oysters were scored 0 if polychaetes were not detected, 1 if polychaetes were detected in one valve only and had affected < 5% of the surface area of that valve, 2 if polychaetes affected < 5% of both valves, 3 if > 5% of either valve was affected and 4 if > 5% of both valves were affected.

Natural spatfall monitoring

Sets of spat collectors were deployed at 3 – 4 m depth at two sites in Port Stephens where natural spatfall of *P. imbricata* had previously been observed, Wanda Head (152°05'E, 32°43'S) and Tomaree Head (152°11'E, 32°43'S; Fig. 1). Each collector comprised a 0.5 m^2 (1 m x 0.5 m) sheet of semi-rigid black, 6 mm polythene mesh, folded in a concertina fashion and placed inside a 2 mm mesh orange spat bag (500 mm x 800 mm). A polystyrene float was placed in each bag and the bags were anchored such that they were approximately 1 m above the sea floor.

Four replicate collectors were deployed monthly at each site. Sampling began in August 1998 and continued until July 2000 at Tomaree and until April 2001 at Wanda Head. Each set of collectors remained in the water for two months so that, with the exception of the first and last month in the sampling period, two sets of bags were present at any one time. Upon collection, each bag was returned to the laboratory and rinsed gently with seawater to remove silt. The total numbers of spat in each bag were determined and recorded. Due to the lack of morphological differences between the two Pteriid species found in Port Stephens (*P. imbricata* and *P. albina*), particularly when small, we were unable to reliably differentiate between the species in all cases. Thus, twenty spat from each collection were then chosen at random and returned to clean spat bags and ongrown to a size of >30 mm for species identifications.

2.1.3. Results

A total of 232 *P. imbricata* were collected by divers with an average shell height of 63.9 ± 8.4 mm (mean ± s.d.). The majority of oysters collected were between 50 to 70 mm shell height (Fig. 3), but mature oysters ranged from 47 to 85 mm in height and 13.43 to 91.80 g in weight. Water temperatures recorded at the time of oyster collections ranged from 14 - 25°C (Fig. 2), while salinity at the site remained within the range 28 to 35 g kg^{-1}.

Ratios of males to females among collections were influenced by shell height. Approximately 40% of oysters less than 71 mm dorso-ventral measurement (DVM) were female but this ratio was reversed (66% female) for oysters larger than 70 mm (Fig. 3). Overall, 56% of the *P. imbricata* collected were male, reflecting the predominance of oysters less than 71mm shell height among the collections.

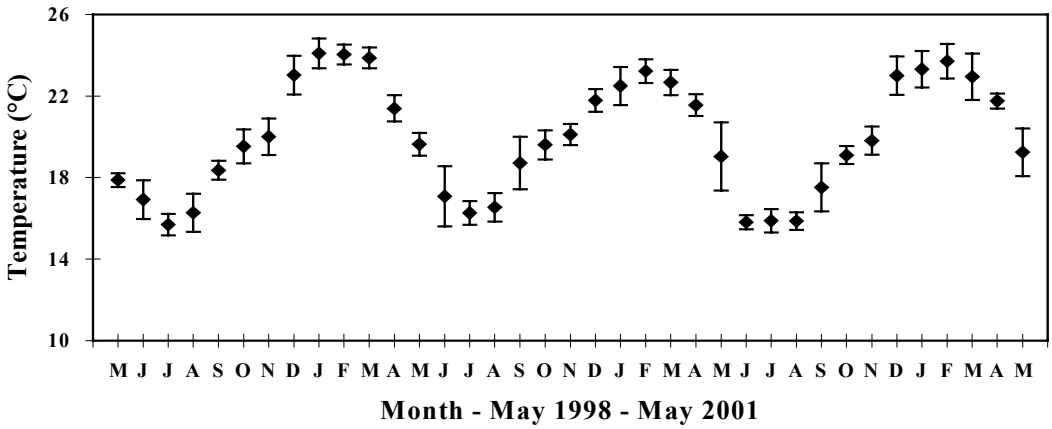

Figure 2. Water temperatures at Wanda Head, Port Stephens. Values are monthly means ± SD.

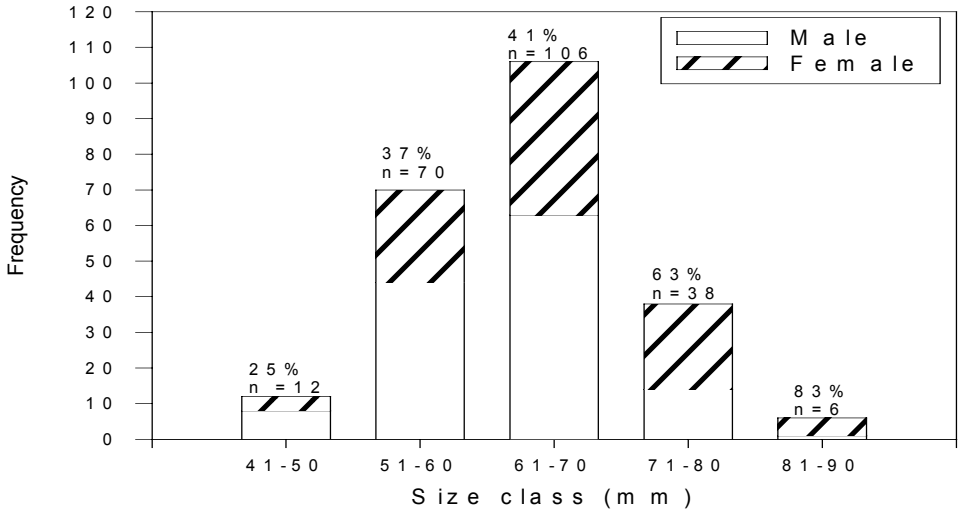

Figure 3. Size frequency and sex ratios of *Pinctada imbricata* collected from Port Stephens, NSW, Australia, from May 1998 to August 2000. Figures above columns indicate percentage female and total numbers for that size class.

Reproductive condition

The macroscopic condition of oysters varied significantly over the two years monitored. During the study, there was evidence of two peaks in reproductive condition annually, followed by troughs suggestive of either spawning or rapid resorption or atresia of gametes (Fig. 4). Oysters peaked in condition in November 1998 and again in March 1999. Reproductive condition was poor through the late autumn and winter (April – Oct.) 1999 before improving gradually to peak again in November 1999. Following a fall in condition in early summer 2000, reproductive condition again increased to peak in April 2000. Gonad condition was found to be significantly ($P < 0.05$) positively correlated with water temperature (Table 3).

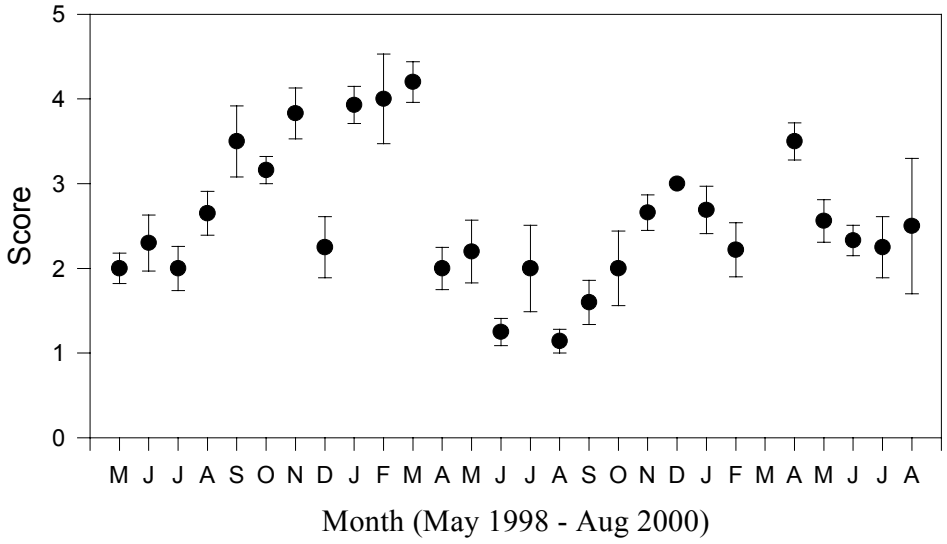

Figure 4. Macroscopic changes in reproductive condition of the pearl oyster *Pinctada imbricata* in Port Stephens, NSW, from May 1998 to August 2000. Values are means ± SE.

Table 3. Correlation coefficients for mean scores on physiological indices and water temperature over the period July 1998 to August 2000.

	Shell growth	Mucoprotein	Mantle	Byssus	Gonad
Mucoprotein	-0.16				
Mantle	-0.25	0.01			
Byssus	-0.42	-0.21		0.48*	
Gonad	0.22	-0.32	-0.24	-0.23	
Temperature	-0.12	-0.44*	-0.04	0.24	0.52*

Values are rank order correlation coefficients.

** Significant at P<0.05, ** Significant at P<0.01*

Gonad histology was generally supportive of the macroscopic observations (Fig. 5). Most peaks in macroscopic condition occurred in collections with high proportions of histologically ripe gonads. However, as observed previously with *P. albina* (O'Connor 2002) macroscopic observations did not allow the clear discrimination between various stages of reproductive development. This can be seen in the collections following the respective reproductive peaks. In November 1998 all

gonads sampled were in a reproductively mature state. In the subsequent December sample, a large proportion of the gonads was devoid of gametes, indicating that spawning had indeed occurred. In contrast, the reduction in macroscopic condition in April 1999 was shown histologically to be largely the product of either partial spawning or regression. In the following breeding season there was small peak in condition in November 1999, although no histologically ripe oysters were found until December and there were no indications that spawning had occurred until January 2000.

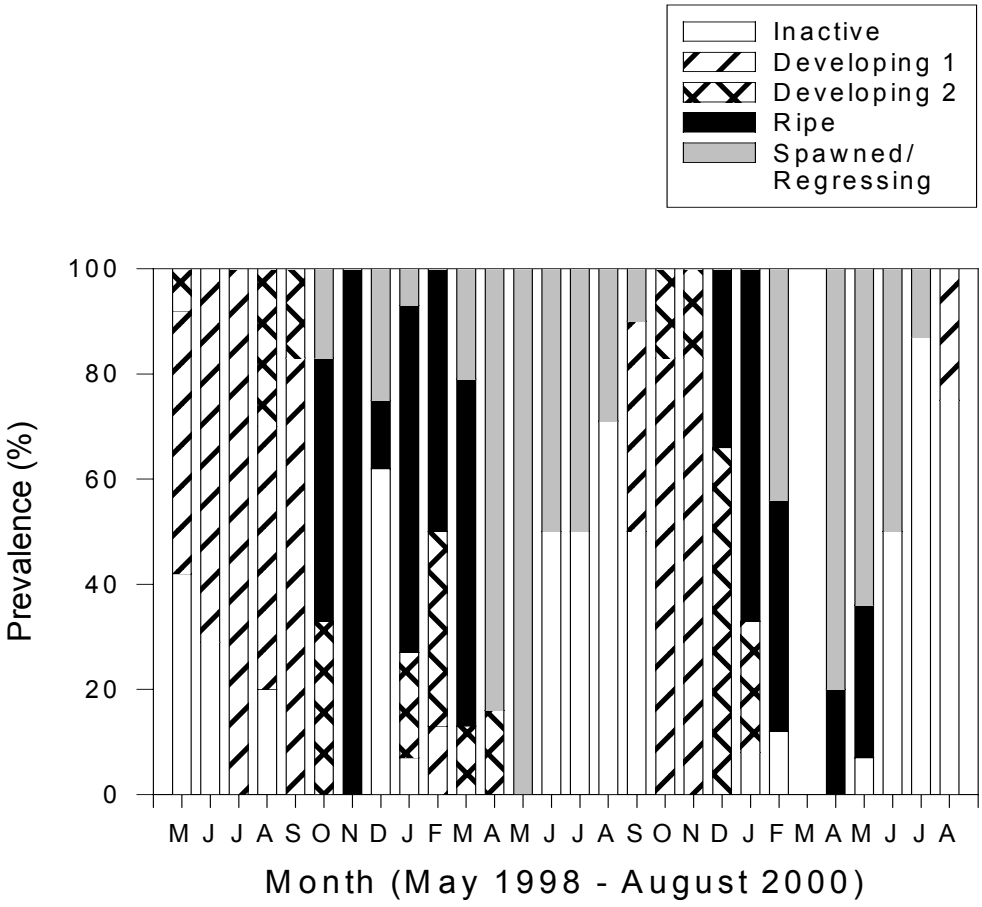

Figure 5. Changes in mean histological rank for of reproductive condition of the pearl oyster *Pinctada imbricata* in Port Stephens NSW from May 1998 to August 2000.

Physiological condition

Of the four physiological indices, both mantle and byssus differed little over the sampling period and both maintained relatively high scores (Fig 6). Shell growth varied, but not with any discernible seasonal pattern and was not significantly correlated with any of the other physiological measures or water temperature (Table 2). The final index, the fat or mucoprotein layers, differed greatly over the sampling period and was significantly ($P < 0.05$) negatively correlated with water temperature (Table 3).

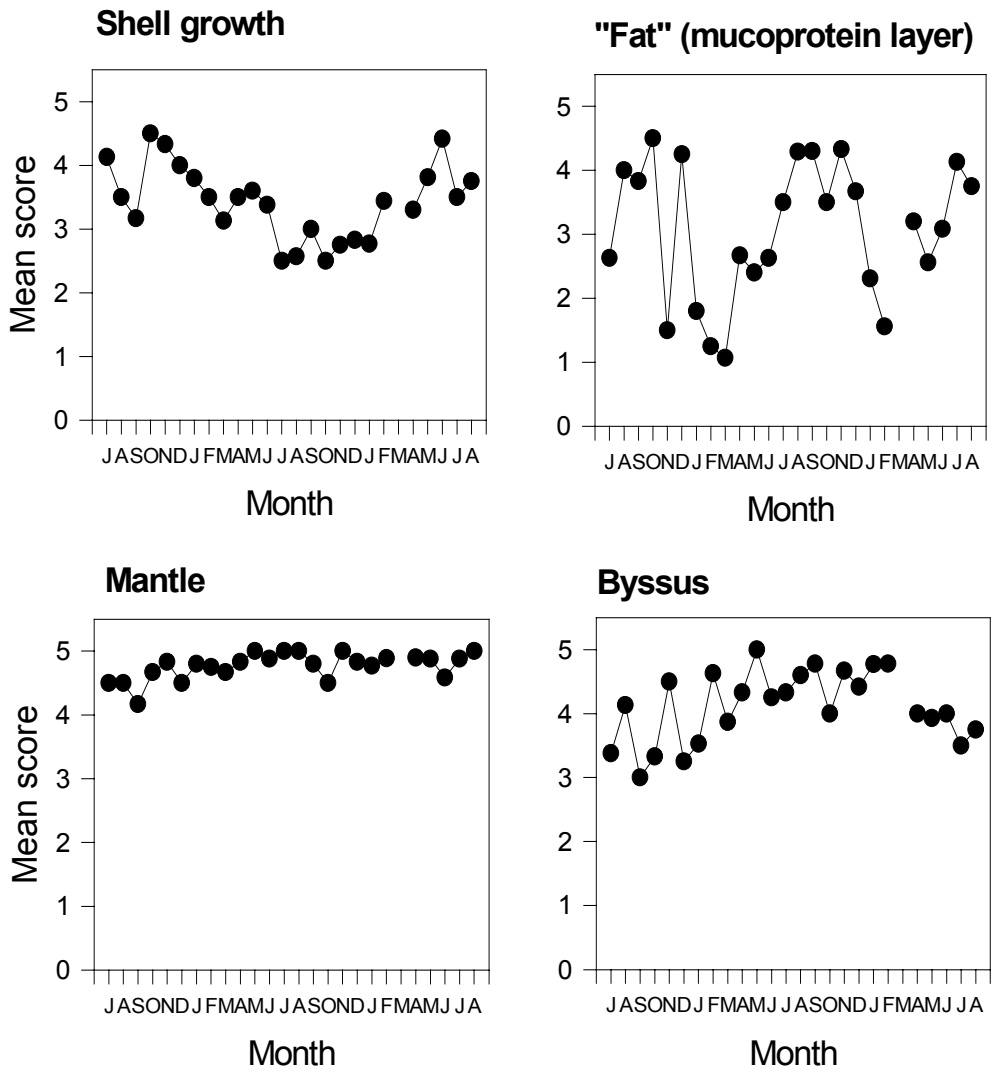

Figure 6. Changes in mean score for four criteria used to assess physiological condition of the pearl oyster *Pinctada imbricata* in Port Stephens NSW from July 1998 to August 2000.

Spat fall

Pinctada imbricata spat settled in collector bags in summer and early autumn but the numbers collected were generally greatest during the months of December and January. Over the three years monitored, the number of oysters collected differed between sites and between years. In 1999-2000, the total number of spat collected at both Wanda Head and Tomaree Head were similar, however spat fall at Wanda Head was confined to the months of December and January, while spatfall at Tomaree continued until March (Fig. 7). At their peak in December 1998, an average of 453 and 241 spat were collected per bag at Wanda Head and Tomaree Head, respectively. In subsequent years spatfall was reduced to and average of less than 20 spat per bag at both sites (Fig. 7).

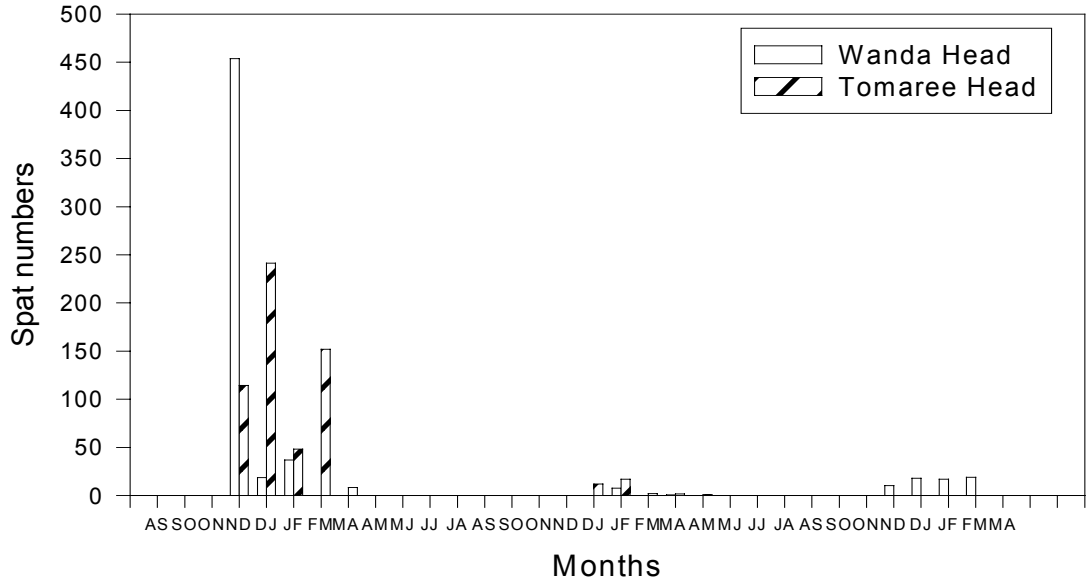

Figure 7. *Pinctada imbricata* spat fall at two sites in Port Stephens, NSW, Australia, from August 1998 to April 2001.

Shell damage

Overall, 23% of *P. imbricata* collected had shell damage (blisters) indicative of spionid infestation. The severity of infestation was low with the great majority (> 95%) of oysters having less than 5% of the surface area of one valve affected. Blister morphologies were similar to those previously observed in *P. albina* collected concurrently from Wanda Head. Damage was not restricted to a particular valve, but blisters were invariably small (< 0.5 cm^2), most commonly ovoid to irregular in shape, with their longer axis oriented toward the shell margin. The numbers of oysters with spionid infestations differed with size class, with a tendency for prevalence to increase with oyster size (Fig. 8). In addition to polychaete damage, shells from larger, presumably older, oysters were also found to have branched pattern of erosion extending through the prismatic layer of the shell consistent with damage caused by boring sponges, *Cliona* spp.

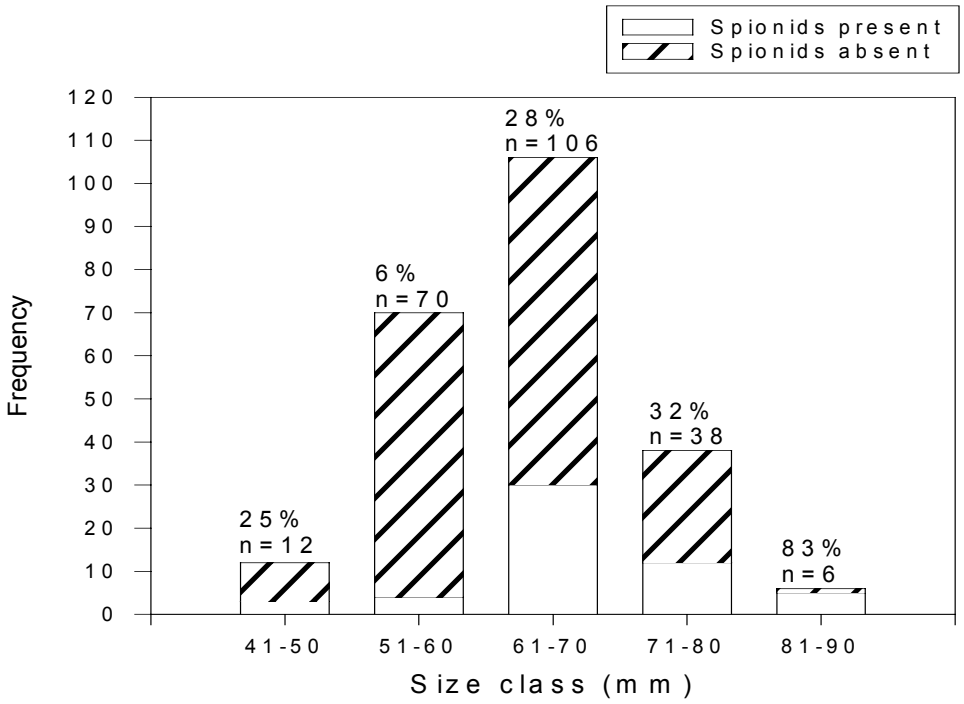

Figure 8. Size frequency and spionid polychaete infestation of the shells of *Pinctada imbricata*. Figures above columns indicate the percentage of oysters with Polydora sp. infestations and the total number of oysters collected within that size class.

2.1.4. Discussion

Macroscopic and histological observations of *P. imbricata* from Port Stephens were indicative of a relatively well-defined reproductive pattern. In both years, oysters were in poor reproductive condition in late autumn and winter before improving in spring. Two peaks in reproductive condition occurred in each seasonal cycle. The first in late spring/early summer (Nov - Dec) and the second in early autumn (Mar - Apr). Of these two peaks the former appears to have been of greater ecological significance. While macroscopic indices were on average greater in the autumnal peak, recruitment in Port Stephens has been largely confined to the summer months and thought to have resulted from the late spring - early summer spawning. The relative recruitment success of each reproductive peak is more accurately reflected in the histological data, in particular within the samples subsequent to each peak. Following the first spawning peak in each season greater proportions of completely voided gonads (apparently inactive) were present. Notably in Dec. 1998 when the greatest recruitment during this study was recorded (Fig. 7). Following each autumnal peak, samples were dominated by partially spawned or regressing gonads, suggesting that the observed reduction in condition may be influenced more by the resorption of gametes than by spawning.

The occurrence of at least two spawning peaks annually appears to be common to populations of *P. imbricata,* although the timing and relative importance of these peaks varies greatly as does the duration of spawning activity (Table 1). Previous attempts to explain this variability have considered the role of water temperature and the impact of latitudinal changes (Tranter 1958, Wada, 1995, Behzadi et al. 1997). This study and the recent observations of Beer and Southgate (2000) and Urban (2000) are consistent if not supportive of these explanations, but are collectively of value because they greatly extend the range over which observations have been made.

A concordance between reproductive condition and temperature in some pteriid populations at higher latitudes and an apparent lack of breeding periodicity among tropical populations led Tranter (1958c) to suggest the existence of a critical temperature for breeding: "this temperature is reached in higher latitudes only during the summer, but in lower latitudes is exceeded all the year round". For the Northern Hemisphere, some evidence for this latitudinal variation has been provided by Wada (1995) and can be seen to an extent in Table 1. In the Southern Hemisphere, observations of variation in *P. imbricata* reproductive behavior conform more closely with the pattern suggested by Tranter (1958). At Thursday Island (11^{o}S), *P. imbricata* recruitment occurs year round with reproductive peaks in early summer and autumn (Tranter, 1959). Some 900 km south at Orpheus Island (18^{o}S), recruitment was limited to eight months of the year with a peak in recruitment in summer (Beer and Southgate, 2000). A similar summer recruitment peak was also observed at Hervey Bay (23^{o}S)(Sumpton et al., 1990), although the duration of recruitment was not recorded. At Port Stephens (32^{o}S), recruitment again peaked in summer, but recruitment was further confined to four months of the year.

In comparison to the Northern Hemisphere, the progressive nature of the latitudinal changes in *P. imbricata* reproductive behavior in the Southern Hemisphere is thought to result from the fact that reports are confined to east coast of Australia and that changes in latitude imply more consistent reductions in temperature. Consistency may also be aided by the possibility of greater genetic similarity among Australian populations. Regardless, temperature is of particular importance to reproduction at higher latitudes, but it is only one of a series of exogenous factors involved. Clearly, there is little correspondence between reproductive peaks and temperature at lower latitudes (11^{o}S - 11^{o}N) with limited number of reports in Table 1 indicating great diversity in timing.

Genetic differences have been shown to be a source of variation in reproductive behaviour among bivalves (Loosanoff, 1969; Barber et al., 1991), and these differences can be the major determinant of breeding season (Cochard and Devauchelle, 1993; Mackie and Ansell, 1993). But as yet there is little evidence of this among pteriids. In comparisons of reproductive timing in two genetically different populations of *P. imbricata* from Japanese waters (*P. fucata fucata* and *P. fucata martensii*), Wada (1995) did find differences, but these can not be solely ascribed to genetic differences. Meanwhile, the importance of exogenous factors in pteriid reproduction is suggested by the close correspondence in reproductive timing in sympatric populations of *P. imbricata* and *P. albina*. At Thursday Island, both species consistently showed peaks in reproductive activity in early summer and autumn (Tranter 1958, 1959), and spat recruited to collectors throughout the year. At Orpheus Island, recruitment was constrained to the eight months of the year and peaks in recruitment occurred in the months of Jan - Feb and Feb - Mar. for *P. albina* and *P. imbricata*, respectively (Beer and Southgate, 2000). In Port Stephens peaks in *P. albina* reproductive indices occurred at similar times (Oct. 1998, Mar.1999, Jan. 2000 and Apr. 2000) and, while spatfall was far more abbreviated (November-January) and sparse, did occur concurrently with that of *P. imbricata*.

Physical condition

In an acknowledgment of the value of practical observations, particularly from Japan where this information has been accumulated over generations, this study attempted to incorporate macroscopic measures suggested by farmers as indicative of physiological condition. With the exception of the "fat" layer, these measures (shell growth, mantle appearance and byssus) either showed little variation or the variation that occurred was not apparently related to seasonal or reproductive changes. This does not discount the value of these observations. We frequently observe dramatic increases in the growth of the imbricate processes of the shell when spat are moved from the hatchery to the field. Similarly, when broodstock collected in winter (< 17^{o}C) are brought to the hatchery for reproductive conditioning marked shell growth is observed. Rather, it would appear that these measures may be more appropriate in highlighting significant or rapid

changes in the environment, such as pollution, red tides or disease outbreak, than reproductive condition in wild populations of *P. imbricata*.

The "fat" or mucoprotein layer was found to be the most variable of this group of measures. The thickness and extent of this layer was negatively correlated with water temperature, with a tendency to peak in spring and trough in late summer and autumn. To an extent this reflected changes in macroscopic condition of the gonad; although the peaks in fat preceded those of gonad condition and supported the suggestion that this material was accumulated and used during gametogenesis. This layer did not however show signs of recovery prior to the second reproductive peak in autumn, although this may simply reflect a continued, high demand for energy for continued gametogenesis.

Shell damage

Reports attributing damage and mortality in *P. imbricata* to spionid polychaetes are common and have arisen in areas such as Sri Lanka (Herdman, 1905), Japan (Mizumoto, 1964), India (Dharmaraj et al, 1987), the Persian Gulf (Doroudi, 1996) and China (A. Wang pers. comm.). Spionids are thought to "fatigue" the host pearl oyster (Wada, 1991) and weaken their shells, increasing their susceptibility to predators and increasing the frequency of shell breakage during operations. Known as the mud worm by NSW oyster farmers, *Polydora websteri* is considered the greatest threat, as it has already been implicated in the mortality of both Sydney rock oysters (Skeel, 1979) and commercial scallops (Dix, 1981). Spionid prevalence in both Sydney rock oysters and *P. imbricata* varies greatly with location. At some sites, mudworm prevalence in *P. imbricata* has reached 90%; however, prevalence in wild stocks at Wanda Head was comparatively low (23%) and more importantly, the damage caused was also low. Overall, total prevalence and size specific prevalence in *P. imbricata* was similar to observations in a sympatric and simultaneously sampled population of *P. albina*. This has suggested that spionid prevalence is independent of pearl oyster species and that the greatest factor affecting prevalence is size of the host. In this case, size is thought to reflect age and that with increasing age comes an increasing (cumulative) chance of exposure to infestation by spionid larvae. Size could also imply a greater surface area on which larvae might settle, however, since prevalence is not proportional to size this might simply be an additional factor.

Culture

Attempts to culture *P. imbricata* in NSW are currently under way and, as a result of this study and surveys by the Australian Museum a picture for the industry in the short term is emerging. Populations of *P. imbricata* are ubiquitous to most NSW estuaries, although the total numbers are comparatively small (Ponder and Colgan, unpublished data) and unlikely to support wild harvest. As a result, culture will depend on the collection of natural spat or hatchery production. Elsewhere attempts to collect *P. imbricata* spat have been promising. Tranter (1959) reported data from J.S. Hynd in which an estimated 149 spat were caught per collector. Sumpton et al. (1990) caught an average 128 spat collector^{-1} and Beer and Southgate (2000) caught up to 324 collector^{-1}. While some care should be taken in comparison of these catches, as collector type and deployment duration varied, they are suggestive that wild catch could be a reliable source of spat supply. Despite a promising start in Port Stephens in 1998 (453 spat collector^{-1}), subsequent catches have been poor (< 20 collector^{-1}). Without a marked improvement in methods, this is likely to discourage a reliance on the collection of spat for seed supply. Reliance on wild spatfall is then further discounted by a preference of farmers in central NSW to obtain spat in September or October to coincide with increasing water temperature and increased food abundance arising from annually recurrent phytoplankton blooms (Hallegraeff and Jeffery, 1993). This preferred time precedes likely spatfall by 2-3 months.

P. imbricata has been artificially propagated in significant numbers (> 10^7 spat) in NSW for experimental purposes, and given the potential for genetic selection in periculture (Wada and Komaru, 1996), this is our preferred means of spat supply. The only inconvenience is that spat are desired in September and October to coincide with a period of increasing water temperature and increased food abundance arising from annually recurrent phytoplankton blooms (Hallegraeff and Jeffrey, 1993). While this is also a disincentive to the use of wild catch that would not be available until early summer, it also occurs at a time at which few "ripe" adult oysters are available. Fortunately, *P. imbricata* are improving in condition in August and September, and their reproductive condition can be improved in the hatchery.

2.2. Growth and nacre quality in the pearl oyster *Pinctada imbricata,* in south-eastern Australia

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2.2.1. Introduction

The pearl oyster, *Pinctada imbricata* Röding, is among the most wide spread of the pteriid species and in Australia, occurs from Shark Bay in the west, around the northern coastline and down the east coast as far south as Victoria (Hynd, 1955; Macpherson and Gabriel, 1962). While *P. imbricata* has been used for pearl culture in Asia for decades, the species has only recently been the subject of commercial interest in Australian waters.

Currently, interest in *P. imbricata* culture is concentrated in NSW. While the environmental conditions appear suitable for pearl production, a lack of protected coastal areas and relatively high population pressure mean that there are very few potential culture sites. Accordingly, the efficient use of the available area will be important in determining the size and profitability of the industry. Two key factors are the effects of depth and stocking density on oyster growth. There is only limited published research on both factors; however, the ability to stock oysters at the optimum density in as much of the water column as possible will be important in the efficient use of the limited area available.

Irrespective of the efficient use of lease area, ultimately, the primary consideration for the selection of sites will be their potential for the production of high quality pearls. Ideally this would be determined by deploying seeded pearl oysters to a number of sites and evaluating the resultant crop. At this point in the development of the industry deploying seeded oysters is particularly costly and security at the various sites would be difficult to maintain. Fortunately, evaluations of the nacre within the shells can provide an indication of the quality of pearls that might be produced.

Initially, two trials were conducted at sites within Port Stephens to assess the effects of depth and stocking density on *P. imbricata* growth. This was followed by two trials to assess growth and nacre quality of oysters deployed at sites ranging from Port Phillip Bay (Victoria) in the south, to Laurieton on the NSW central coast.

2.2.2. Materials and Methods

Oysters used in these trials were hatchery produced progeny of broodstock collected from Port Stephens. Within each trial siblings from the same hatchery batch were deployed at each site. All oysters were held in pyramidal mesh cages known as pearl cages. Stocking densities varied with each experiment. Initially, oysters were stocked at a density of 250 cage^{-1} for the depth trials. This was later reduced to of 100 cage^{-1} for the site trials.

At the beginning of each trail and on each sampling occasion, the shell heights (dorso-ventral height excluding digitate processes) of 30 randomly chosen oysters from each replicate were measured to the nearest 1 mm using vernier calipers and the oysters were weighed to the nearest 0.1 g. A photographic record was kept of the condition of the cages and the shell growth and nacre quality of the oysters from each site. Oysters were sampled at 4 - 6 week intervals when the cages housing the oysters at each site were changed. At the completion of each trial the number of

oysters at each site or in each treatment were counted and percentage survival was determined. In the depth and density experiments, 30 randomly chosen oysters were collected from each replicate and returned to the hatchery where shell height and total weight were determined as previously described. The body of each oyster was then excised and drained before being weighed to the nearest 0.01 g. These procedures were repeated for the site trials; however, only 20 oysters were sampled on each occasion and the shells of each oyster sampled from each site were retained and cleaned for nacre quality assessments.

Nacre quality assessments

A panel of four or five pearl oyster farmers each with a minimum of 5 years experience, including two with pearl wholesale experience, undertook assessments. A sample of 20 randomly chosen shells from each site were provided "blind" to individual members of the panel, who were asked to score them on a scale of 1 to 5 for two characteristics, nacre colour and lustre. A score of 1 indicating the shell was of poor quality with respect to that variable, with a score of 5 awarded to shells of the highest quality. Each panel member undertook the assessments alone and was instructed to ignore shell size and the presence of mudworm blisters within the shell when making assessments. In the first site trial, duplicate samples from two of the sites were included within the evaluation to test for consistency. In the second site trial, two samples from the initial trial were also included in the second evaluation to test for temporal consistency in assessments.

To establish criteria for quantitative assessments of nacre deposition, 5 mm wide transverse sections were cut from both valves of 20 oysters in the size range 45-55 mm. Using a binocular microscope with an eye piece graticule, the thickness of both the prismatic and nacreous shell layers were measured at five equally spaced points across each section (Fig. 1). Based upon these measurements, the assessment of prismatic and nacreous shell thickness for samples from each site were then made at the midpoint of transverse sections cut from the left valve of 20 oysters from each site.

Depth effects

To assess the impact of depth on growth of *P. imbricata* juveniles, oysters were suspended at various depths in the water column in 10 mm mesh pearl cages. Each string of cages began 1 m above the seabed and continued to approximately 2 m below the mean low tide depth. Cages were strung together at intervals of approximately 60 cm and 250 oysters (21.5 ± 0.9 mm; mean ± SE) were placed into each pearl cage. The number of cages in each string varied according to site (Table 1, Fig. 2). Strings of cages remained at each site for three months (March – June 1999) before they were collected.

Stocking density

Pearl cages were stocked with juvenile oysters (25.3 ± 0.9 mm; 1.6 ± 0.1 g; mean ± SE) at one of four densities; 100, 200, 400 or 600 g wet weight cage^{-1}. Four replicate cages at each density were deployed at both Wanda head and Tomaree where they remained for three months (March - June, 1999). On collection, the live weight of oysters per cage was determined to the nearest 1 g and survival and growth were assessed as previously described for depth effects trials.

Upon completion of the first stocking density trial in June, the oysters were cleaned of fouling and restocked in to new pearl cages. On this occasion, juvenile oysters (40.3 ± 1.3 mm; 7.6 ± 0.2 g; mean ± SE) were stocked at densities of either 200, 400, 600 or 800 g cage^{-1}. Again four replicate cages were stocked at each density and placed at both Tomaree and Wanda Head for a further three months (June-September, 1999).

Site trial 1: Port Stephens, Central and South Coasts

Eight experimental sites (listed in Table 2) were selected, ranging from Twofold Bay near the NSW – Victorian border to Providence Bay in Central NSW (Fig. 2). Each of the sites was selected on the basis of a number of criteria. All sites were actively in use for the cultivation of another marine species and thus afforded some measure of security. Each site was in an area not normally encountering extended periods of low salinity.

The initial size and weight of oysters deployed were 15.0 ± 1.0 mm and 0.47 ± 0.08 g, respectively. The oysters were deployed to the sites between 30-Nov-99 and 3-Dec-99 and with the exception of oysters from Jervis Bay, were all collected for final analyses between the 16-Jul-00 and 31-Jul-00. Poor weather conditions prevented the collection of oysters from Jervis Bay until the 11-Aug-00.

Site trial 2: Port Stephens and Central to Mid North Coasts

Nine experimental sites were selected. Two sites, Laurieton and Port Phillip Bay (Victoria) were chosen to extend the geographical range of the evaluations. In addition to Laurieton, a further two sites, Riley's Bay and Hardy's Bay, were selected to provide greater information with respect to the use of existing intertidal oyster (*Saccostrea glomerata*) leases for pearl oyster production. Finally, five sites from the previous evaluation were retained, Lake Berringer, Botany Bay, Wanda Head, Tomaree and Providence Bay.

The initial size of the oysters deployed was 16.44 ± 0.8 mm. The oysters were deployed to all sites in NSW between 25-Jul-00 and 31-Jul-00 and all sites were harvested approximately 9 months later between 26- April-01 and 5-May-01

Statistical analyses

Before ANOVA, homogeneity of variance was confirmed with Cochrans test (Sokal and Rholf, 1981). Where significant differences ($P<0.05$) were detected, treatment means were compared using Student-Newman-Kuels procedure (Winer et al., 1991). For all ANOVAs, $\alpha = 0.05$.

Pearson product moment correlation coefficients were calculated between quantitative variables in the two site trials (shell height, total weight, meat weight, prismatic shell thickness and nacre thickness). Spearman rank correlation coefficients were calculated between the quantitative variables and the two qualitative variables (nacre colour and lustre).

To allow comparisons of growth between sites and with other studies, growth parameters of the von Bertalanffy model were estimated and a "seasonalised von Bertalanffy growth equation" (Pauly and Gaschutz, 1979; Sparre and Venema, 1998) was fitted to age - height data. A seasonalised model was chosen in acknowledgment of the fact that neither site evaluation continued for a full year and that both experiments were done in the warmer months in which greater growth was anticipated.

The equation $$H = H_\infty [1-e^{\{-k*(t - t_0) - (Ck/2\pi)*\sin(2\pi*(t - t_s))\}}]$$

was used, where H is shell height in mm at time t, H_∞ is the asymptotic height in mm, t is the age in years and k is the rate at which the asymptotic height is approached in y^{-1}. C is the "amplitude" of the seasonal oscillation and ts is the "summer point".

Based on four years of observations of the wild population that provided the broodstock for the experimental oysters, our best estimate of H_∞ was approximately 85 mm. However, it has become

clear that in culture a shell height of 85 mm is regularly exceeded. Accordingly, H_∞ was increased to 92 mm to reflect the size achieved by broodstock oysters held in suspended culture. A value of 1 was ascribed to C, which can vary from 0 – 1; 0 when no seasonality in growth occurs and 1 where growth ceases in winter. The model was fitted to each data set using a least squares method.

Having calculated the von Bertalanffy models and the various parameter estimates, this information was used to determine Phi prime (Φ'):

$$\Phi' = Log\ (K) + 2\ Log\ H_\infty$$

Phi prime provides an index of "overall growth performance" for comparisons between sites.

2.2.3. Results

Depth effects

After three months deployment at four sites in Port Stephens, growth differed significantly between sites ($F = 16.11$, $P < 0.001$). The greatest growth was recorded at Tomaree, followed by Wanda Head, Fame Cove and Baromee Point, respectively (Table 1). Growth was not affected by the depth at any of the sites which the oysters had been placed at (Table 1). Average survival for all sites was considered to high (> 93%) but differed significantly between sites ($F = 2.86$, $P <$ 0.05. Survival was greatest at Tomaree followed by Baromee Point, Wanda Head and Fame Cove (Table 1). Survival was unaffected by depth at all sites except Wanda Head. At Wanda Head survival in the bottom cage was markedly reduced due to the presence of predatory gastropods, *Cymatium parthenopeum*, known colloquially as hairy oyster drills. Fouling was considered to be relatively light and although the type of fouling differed between the sites, there were no apparent differences within sites as a result of depth.

Stocking density

The results of the two stocking density trials are shown in Table 2. In both trials and at both sites, the initial stocking density significantly affected oyster growth. Overall there was a trend for growth to be reduced with increases in stocking density. With one exception, survival was not affected by stocking density.

Table 1. The effect of culture depth on growth and survival of the oyster, *Pinctada imbricata* at four sites in Port Stephens, NSW.

Site	Cages/string	Cage position (Depth)*	Shell height (mm) Grand mean : Depth mean		Survival (%) Grand mean : Depth mean	
Baromee Point	4	1	33.6 ± 3.4 [a]	34.6 ± 4.8 [a]	95 ± 2.3 [ab]	96 ± 3.8 [a]
		2		32.3 ± 1.0 [a]		97 ± 1.5 [a]
		3		34.8 ± 4.4 [a]		93 ± 8.9 [a]
		4		32.9 ± 3.1 [a]		95 ± 5.0 [a]
Fame Cove	6	1	34.6 ± 1.3 [a]	34.7 ± 1.0 [a]	93 ± 3.0 [ab]	95 ± 2.0 [a]
		2		33.0 ± 1.7 [a]		91 ± 4.0 [a]
		3		35.3 ± 2.3 [a]		94 ± 2.8 [a]
		4		34.4 ± 1.2 [a]		92 ± 3.4 [a]
		5		34.9 ± 0.7 [a]		92 ± 6.0 [a]
		6		34.8 ± 1.5 [a]		93 ± 4.6 [a]
Wanda Head	5	1	36.9 ± 1.5 [b]	36.9 ± 1.6 [a]	95 ± 4.7 [b]	97 ± 2.1 [a]
		2		36.9 ± 1.3 [a]		99 ± 0.9 [a]
		3		35.7 ± 2.5 [a]		99 ± 1.4 [a]
		4		38.2 ± 1.4 [a]		97 ± 1.9 [a]
		5		37.0 ± 1.9 [a]		84 ± 13.2 [b]
Tomaree Head	7	1	37.7 ± 2.1 [b]	37.4 ± 1.5 [a]	96 ± 4.6 [a]	99 ± 0.5 [a]
		2		38.3 ± 1.7 [a]		99 ± 1.8 [a]
		3		37.9 ± 2.1 [a]		99 ± 0.5 [a]
		4		38.2 ± 3.0 [a]		97 ± 0.5 [a]
		5		36.5 ± 2.9 [a]		99 ± 1.5 [a]
		6		39.1 ± 1.6 [a]		92 ± 9.5 [a]
		7		36.4 ± 1.3 [a]		97 ± 1.4 [a]

** Cages are numbered from the surface down, cage 1 is closest the surface (2 m depth). The final cage in each string is 1 m above the bottom. The number of cages varies according to water depth.*

Values are means ± SD. Grand means within columns with a common superscript do not differ significantly ($P > 0.05$*). Depth means within columns and within sites with a common superscript do not differ significantly (*$P > 0.05$*)*

In the initial trial, growth and biomass increase within respective treatments was similar at both Tomaree and Wanda Head (Table 2). The greatest growth of approximately 16 mm was recorded at the lowest initial stocking density of 100 g cage^{-1}. Subsequent, increases in stocking density resulted in reduced growth, although at both sites, the growth of oysters stocked at the highest tested density of 600 g cage^{-1} were greater than that recorded at 200 g cage^{-1} and 400 g cage^{-1}. Survival at both sites was high (≥ 95%) and although it was significantly lower among oysters stocked at 600 g cage^{-1} at Tomaree, the reductions in numbers were not sufficient to have dramatically altered relative stocking densities.

The second trial was done during the winter months and while growth and biomass increase were again significantly reduced with increasing stocking density, both were markedly lower than achieved during the previous trial (Table 2). The maximum growth recorded at both sites was less than half that of the previous trial and in oysters stocked at the highest tested density at Wanda Head, mean shell height decreased by 2.2 mm. In this trial, growth and biomass increase were greater at Tomaree than at Wanda Head and was thought to be related to warmer mean water temperature encountered during the trial (16.6^{o}C and 17.3^{o}C, respectively). Survival at both sites

was high (≥ 94%) and was not significantly affected by stocking density.

Nacre thickness

Comparisons of prismatic and nacreous shell thickness in *P. imbricata* found no significant difference between left and right valves ($F = 0.74$, $P = 0.39$), however nacre thickness varied across the valves ($F = 0.74$, $P < 0.01$). Nacre was thinnest at the margins of the shell and beneath the muscle scar, and thickest in the mid to anterior region of the shell (Fig 1).

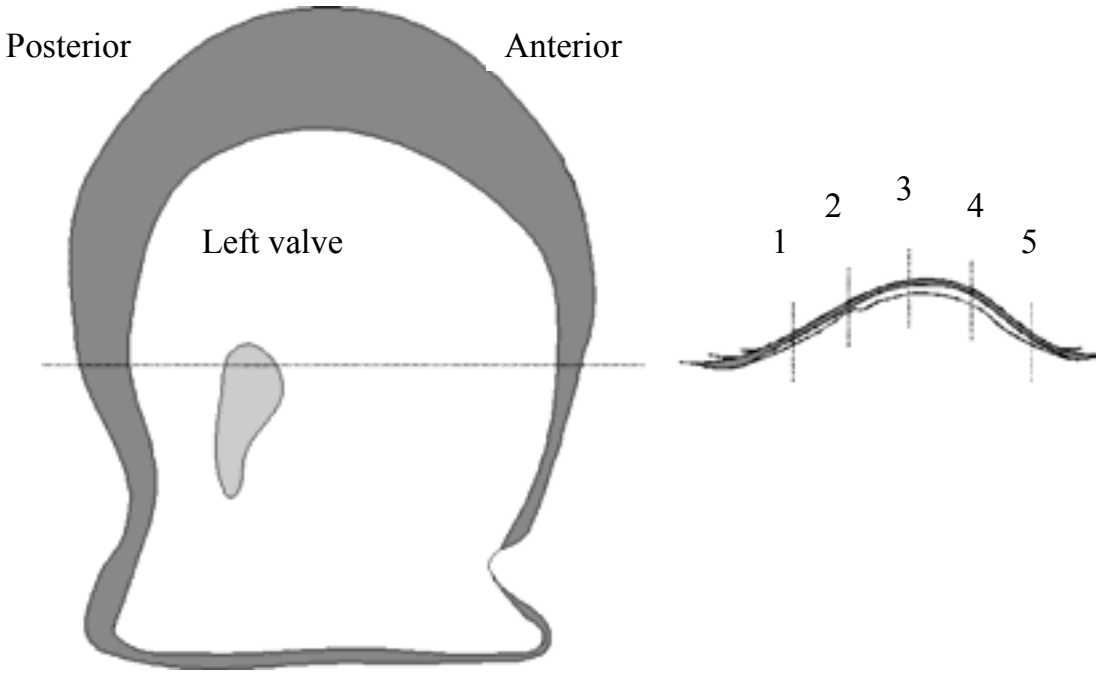

Figure 1. Nacre deposition in the pearl oyster *Pinctada imbricata*.

Trial 1: Port Stephens, Central and South Coasts

Indicators of growth, shell height, total weight and meat weight, were all strongly correlated and all differed significantly between the sites (Tables 3 and 4)**.** In general, the greatest growth rates were recorded toward the northern and warmer extent of the range tested (Tables 2 and 3)**.** Oysters grown at Tomaree Head were the largest and heaviest of the experimental groups and were more than three times the total weight of those grown at the most southerly site, Twofold Bay (Table 2)**.** Growth constants (k) from the von Bertalanffy and subsequent indices of "overall growth performance" (Φ') for each site are given in Table 4. Values for k and Φ' ranged from 0.62 – 0.90 and 3.70 – 3.87, respectively. Survival of the oysters at all sites was relatively high ranging from 88% at Twofold bay to 100% at Providence Bay.

The thickness of the darker prismatic shell layer differed little between sites and was not correlated any of the other variables monitored. This layer was thinnest at Lake Berringer; however, examination of the shell indicated that this may have arisen from the removal of the outer periostracum and abrasion of the underlying prismatic shell. More marked were the variations in nacre thickness between sites, which were correlated with the three measures of growth; height, weight and meat weight. The thickest nacre was found in oysters grown at Tomaree. However,

linear regression of nacre thickness against shell height for all samples collected found shell height explained only 30% of the variation in nacre thickness observed ($r^2 = 0.2956$).

Exact scores for nacre colour and lustre remain commercial in confidence; however the following observations of the "Blind" panel assessments can be made. Panel members were consistent in their ranking of sites with respect to the quality of both nacre colour and lustre. All panel members agreed which were the best and worst sites evaluated for nacre quality and all panel members scored the duplicate samples similarly. Panel members did however differ significantly in the average scores awarded for both nacre colour and lustre. In this respect, some panel members consistently awarded higher average scores. Mean scores for nacre colour and lustre differed significantly between sites and were not significantly correlated with growth or with each other (Table 4).

Table 2. The effect of stocking density on growth and survival of the oyster, *Pinctada imbricata* at two sites in Port Stephens, NSW.

Site	Trial	Initial density (g cage^{-1}; approx. N^{o})	Shell height: (growth increment) (mm)	Survival (%)
Wanda Head	1	100, 62	41.7 ± 1.2 (16.4)^{a}	99 ± 0.9 ^{a}
		200, 125	37.9 ± 1.8 (12.6)^{b}	99 ± 0.9 ^{a}
		400, 250	36.7 ± 1.4 (11.4)^{b}	97 ± 2.5 ^{a}
		600, 375	39.2 ± 2.8 (13.9)^{ab}	97 ± 4.8 ^{a}
	2	200, 26	43.4 ± 0.7 (3.1)^{a}	98 ± 1.1 ^{a}
		400, 53	42.7 ± 0.4 (2.4)^{ab}	98 ± 0.9 ^{a}
		600, 79	40.8 ± 0.9 (0.5)^{b}	97 ± 0.5 ^{a}
		800, 105	38.1 ± 1.0 (-2.2)^{c}	96 ± 1.2 ^{a}
Tomaree	1	100, 62	42.1 ± 2.0 (16.8)^{a}	100 ± 0.0 ^{a}
		200, 125	-	-
		400, 250	36.9 ± 1.6 (11.6)^{b}	100 ± 0.0 ^{a}
		600, 375	38.2 ± 1.3 (12.9)^{a}	95 ± 3.1 ^{b}
	2	200, 26	46.6 ± 1.4 (6.3)^{a}	97 ± 1.8 ^{a}
		400, 53	46.4 ± 0.6 (6.1)^{a}	97 ± 0.9 ^{a}
		600, 79	44.5 ± 2.5 (4.2)^{a}	94 ± 2.3 ^{a}
		800, 105	40.7 ± 2.3 (0.4)^{b}	96 ± 1.2 ^{a}

*Values are means ± SD (*n = 4*). Means within columns and within trials with a common superscript do not differ significantly (*P > 0.05*). Initial spat sizes were 25.3 ± 0.9 mm and 40.3 ± 1.3 mm for Trials 1 and 2, respectively.*

Table 3. Experimental Sites Characteristics (Trials 1 & 2).

Site	Location (Lat., Long.)	Experiment	Depth	Existing use
Port Phillip Bay	38^{o}14”S, 144^{o}48”E	Site 2	22 m	Scallops (*Pecten fumatus*)
Twofold Bay	37^{o}41”S, 152^{o}12”E	Site 1	10 m	Mussels (*Mytilus galloprovincialis*)
Lake Berringer	35^{o}16”S, 150^{o}30”E	Site 1 & 2	2 m	Edible oysters (*Saccostrea glomerata*)
Jervis Bay	35^{o}05”S, 150^{o}42”E	Site 1 & 2	12 m	Mussels(*Mytilus galloprovincialis*)
Botany Bay	34^{o}01”S, 151^{o}12”E	Site 1 & 2	6 m	Snapper (*Sparus auratus*)
Riley’s Bay	33^{o}30”S 151^{o}21”E	Site 2	4 m	Edible oysters (*Saccostrea glomerata*)
Hardy’s Bay	33^{o}31”S, 151^{o}21”E	Site 2	2 m	Edible oysters (*Saccostrea glomerata*)
Wanda Head	32^{o}43”S, 152^{o}05”E	All expts.	5 m	Pearl oysters (*Pinctada imbricata*)
Tomaree Head	32^{o}43”S, 152^{o}11”E	All expts.	6 m	Abalone (*Haliotis rubra*)
Baromee Point	32^{o}40”S, 152^{o}03”E	Depth	5 m	Pearl oysters (*Pinctada imbricata*)
Fame Cove	32^{o}40”S, 152^{o}03”E	Depth	6 m	Pearl oysters (*Pinctada imbricata*)
Providence Bay	32^{o}41”S, 152^{o}12”E	Site 1 & 2	20 m	Snapper (*Sparus auratus*)
Laurieton	32^{o}40”S, 152^{o}04”E	Site 2	2 m	Edible oysters (*Saccostrea glomerata*)

Trial 2: Port Stephens and Central to Mid North Coasts

Several difficulties were encountered during this trial. Initially, oysters held at Tomaree and Providence Bay were lost mid trial during a one-in-ten-year storm, while oysters held in Victoria were disadvantaged by a prolonged quarantine period. Regardless, the results were supportive of those collected previously. Growth differed significantly among sites; however the trend for increasing growth with decreasing latitude was not as clear as in the previous trial. In this instance, growth was reduced at the southern sites, but among the sites on the central coast of NSW from Botany Bay to Laurieton, there was no concordance with latitude (Tables 2 and 5). As observed previously, indicators of growth, shell height and total weight were correlated and differed significantly between the sites (Tables 5 and 6). Oysters grown at Riley's Bay were the largest and heaviest of the experimental groups. Growth constants (k) and indices of "overall growth performance" (Φ') were higher than recorded in the previous trial and ranged from 0.74 – 1.00 and 3.82 – 3.96, respectively. Oyster survival was again high at all sites, ranging from 89% at Hardy's Bay to 95% at nearby Riley's Bay.

The thickness of the prismatic shell layer differed between sites, but on this occasion was correlated with shell height and weight (Table 6). This layer was thinnest at the two southern sites, Lake Berringer and Port Phillip Bay; however, in this trial there was no evidence of excessive shell abrasion at these two sites that could lead to thinner shells. Nacre thickness varied greatly between sites (Table 5) and was correlated with measures of growth.

In nacre quality assessments, panel members showed consistent preferences for nacre from particular sites, although they again differed significantly in the average scores awarded for both nacre colour and lustre. Nacre colour and lustre both differed significantly with site and were not significantly correlated with growth or each other (Table 6). In addition, among the sites evaluated in both trials (Lake Berringer, Botany Bay and Wanda Head) their relative ranking for both nacre colour and lustre changed between trials.

Table 4. Performance of *Pinctada imbricata* spat at eight sites in NSW (Trial 1).

Site	Survival (%)	Shell height (mm)	Weight (g)	Meat weight (g)	Prismatic shell thickness (um)	Nacreous shell thickness (um)	*k*	*Φ'*
Twofold Bay	88	37.3^{f}	8.5^{e}	3.4^{g}	322^{a}	378^{cd}	0.55	3.70
Lake Berringer	97	39.5^{f}	10.1^{de}	4.3^{fg}	278^{b}	290^{f}	0.62	3.75
Jervis Bay	92	50.3^{c}	18.8^{b}	9.6^{b}	313^{ab}	398^{bcd}	0.63	3.76
Botany Bay	96	46.9^{d}	13.8^{c}	6.0^{de}	304^{ab}	402^{bc}	0.62	3.75
Wanda Head	98	53.2^{b}	18.2^{b}	7.1^{d}	302^{ab}	435^{b}	0.80	3.83
Tomaree Head	97	59.5^{a}	29.1^{a}	11.4^{a}	343^{a}	594^{a}	0.90	3.87
Providence Bay	100	50.0^{c}	17.4^{b}	8.3^{c}	322^{a}	349^{de}	0.67	3.77
North Arm Cove	95	43.4^{e}	12.0^{cd}	5.0^{ef}	334^{a}	325^{ef}	0.65	3.75

Means within columns with a common superscript do not differ significantly.

Table 5. Correlation coefficients for various factors across all sites in Trial 1.

	Weight	Meat weight	Prismatic shell	Nacreous shell	Nacre colour	Nacre lustre
Shell height	0.96 **	0.93 **	0.41 ns	0.80 *	0.27 ns	0.14 ns
Weight		0.96 **	0.49 ns	0.87 **	0.27 ns	0.44 ns
Meat weight			0.44 ns	0.73 *	0.47 ns	0.48 ns
Prismatic shell				0.54 ns	-0.41 ns	0.13 ns
Nacreous shell					0.12 ns	0.12 ns
Nacre colour						0.29 ns

ns not significant, * $P < 0.05$, ** $P < 0.01$

Table 6. Performance of *Pinctada imbricata* spat at eight sites in NSW (Trial 2).

Site	Survival (%)	Shell height (mm)	Weight (g)	Prismatic shell thickness (um)	Nacreous shell thickness (um)	*k*	*Φ'*
Port Phillip Bay	-	44.1	-	223[a]	390[c]	-	-
Lake Berringer	94	51.6[d]	13.9[d]	221[a]	380[c]	0.74	3.82
Botany Bay	91	60.7[bc]	23.6[bc]	304[b]	494[b]	0.90	3.91
Riley's Bay	95	65.9[a]	32.3[a]	317[b]	721[a]	1.00	3.96
Hardy's Bay	89	59.4[bc]	25.1[b]	313[b]	679[a]	0.90	3.91
Wanda Head	94	62.5[b]	26.1[b]	310[b]	582[b]	0.91	3.92
Laurieton	92	59.8[c]	20.5[c]	298[b]	584[b]	0.87	3.89

Means within columns with a common superscript do not differ significantly.

Nacre colour and lustre were scored on a scale of 1-5, with higher scores indicating higher quality.

Table 7. Correlation coefficients for various factors within and across all sites in Trial 2.

	Weight	Prismatic shell	Nacreous shell	Nacre colour	Nacre lustre
Shell height	0.95 **	0.91 *	0.81 *	-0.41 ns	-0.02 ns
Weight		0.86 *	0.87 *	-0.63 ns	-0.02 ns
Prismatic shell			0.84 *	-0.49 ns	-0.14 ns
Nacreous shell				-0.31 ns	-0.09 ns
Nacre colour					-0.60 ns

ns not significant, * $P < 0.05$, ** $P < 0.01$

2.2.4. Discussion

The selection of suitable culture sites is essential to all aquaculture ventures and pearl culture is no exception, although in the case of akoya pearl culture, often no one site is ideal. At its simplest, akoya culture requires first rearing an oyster to a size at which a nuclei may be inserted and second the culture of the seeded oyster to produce a quality pearl. The conditions required for these two culture stages and the culture apparatus used can and do vary. For example in the latitudinally wide spread Japanese islands, some farmers operate hatcheries in the warmer southern waters where growth is rapid, but transport oysters to cooler northern waters for pearl production.

The potential dichotomy in requirements for akoya culture, particularly between growth and pearl quality, encourage any assessment of the potential of sites within NSW to include measures relevant to both phases of culture. For growth, this study included measures of shell and somatic growth, and as indicators of pearl quality, nacre thickness, lustre and colour were assessed.

Depth

Depth has previously been shown to have a significant effect on the growth and survival of a number of bivalves and while this also likely to be true for pearl oysters, there is a general paucity of information available. Several studies have compared pearl oyster growth in suspended and bottom culture and found significant differences. Gaytan-Mondragon et al. (1993) found bottom culture to be preferable for the culture of *Pinctada mazatlanica* and *Pteria sterna*; although, Taylor et al. (1997) found both growth and survival in bottom cultured *Pinctada maxima* was reduced. Urban (2000) found little difference between *P. imbricata* cultured in suspended boxes and in those just above (15 cm) the bottom; however as with the two previous studies, the differences between bottom culture and suspended culture are not necessarily indicative of depth per se.

Depth effects on growth and survival of cultured bivalves have been variously ascribed to factors such as food availability and composition, fouling, temperature, salinity and rates of water exchange (Cote et al., 1993). In their studies, Taylor et al. (1997) and Gaytan-Mondragon et al. 1993 highlighted the importance of food availability and fouling, respectively. In this study, oyster growth was unaffected by depth however this is perhaps not surprising as the experiments were all in relatively shallow areas (< 6 m) with good current exchanges that help prevent stratification. As a result there were unlikely to be major differences in any of the previously listed variables. This is of importance to the potential for farming *P. imbricata* in Port Stephens and many of the other estuaries in NSW because they are relatively shallow. Thus the ability to make use of much of the water column may compensate for the small areas available for culture. This is not however to suggest that depth will not be a factor at other sites, particularly deeper sites, where food availability etc may vary.

In general, oyster survival was unaffected by the depth of culture although at Wanda Head, significant mortality was observed in several of the lower cages. This was ascribed to the presence of the predatory gastropod, *C. parthenopeum*, the hairy oyster drill. *C. parthenopeum* is a predator of edible oysters, clams and scallops (Laxton, 1971; Heasman et al., 1997), and members of the genus have previously been implicated in pearl oyster mortality (Chellam et al., 1981; Friedman et al., 1998; Urban, 2000). This particular species is common in Port Stephens and is found naturally along the foreshore at Wanda Head. What is unclear is why predation was restricted to the lower cages.

The occurrence of *C. parthenopeum* in scallop cages at Wanda head has been sporadic. On occasions as many as ten adult drills have been found within one scallop cage in a string of seven cages, while the remaining cages have been unaffected. Given that the mesh size of the cages used

in both scallop and pearl culture is far too small for the direct entry of adult drills, it has been assumed that larvae recruit directly to the cages. If this is the case, the relatively frequent handling and inspection of pearl cages under normal culture conditions is thought to be sufficient to prevent significant predation. Indeed in three years trial farming, *C. parthenopeum* has only been a nuisance in experimental trials.

Stocking density

The stocking density of oysters is of particular importance in optimising the efficient use of leases. Suboptimal stocking density may waste valuable lease space while overcrowded culture can lead to poor growth, reduced survival and poor quality pearls (Chellam et al., 1987). Recommended stocking rates for *P. imbricata* are scarce. Matsui (1958, in Chellam et al., 1981) suggested 70 – 100 oysters m^{-2} at a depth of 5 – 10 m, while Chellam et al. (1981) varied their recommendations on the basis of oyster size. The latter suggested stocking densities of 125, 35-45 mm oysters, 100, 45-55 mm oysters and 75 55-60 mm oysters per cage (40 cm x 40 cm x 10 cm). These rates are slightly higher than those being used in Port Stephens (Table 8), but based on the results of these trials do not appear to be excessive.

Table 8. Initial stocking densities used in experimental long-line cultivation of *P. imbricata* in Port Stephens, NSW.

Shell height (mm)	Stocking Density N°. shell	Culture apparatus
2mm	1,500	1 mm mesh spat bag
7mm	500	Pearl cage
20 – 40mm	100	" "
50mm (18gm)	50	" "
55mm (22gm)	40	" "
60mm (26gm)	112	Panel net
65mm (30gm)	104	" "
70mm (34 gm)	104	" "
75mm (38gm)	96	" "

In the first trial, stocking density was initially very low in comparison to the recommended density of 500, 7 mm oysters (Table 8); however, by the end of the trial the stocking densities were well in excess of those recommended for 20 – 40 mm oysters. Regardless, growth was generally good and although growth did differ significantly among treatments, the variation was not consistent with growth reductions due to increasing stocking density. In the second trial, stocking oysters at a density of 800 g or 105 oysters $cage^{-1}$ did significantly reduce growth at both sites (Table 2). This would suggest that the recommended density of 100, 20 - 40 mm oysters (Table 8) is approaching the maximum density for optimal growth during winter.

Marked differences in growth between the two trials serves to highlight both the impact of season on oyster growth as well to raise the suggestion that optimum stocking density may also vary with seasons. Typically winter is associated with periods of reduced primary productivity during which there may be a reduction in food availability for oysters. Thus, the apparent suitability of the recommended stocking densities at this time suggests they will not have adverse affects at other times when food availability is greater.

Growth potential

The growth of *P. imbricata* was strongly influenced by site and while there are a number of potential variables involved, temperature appears to play a particularly important role. Across all the trials conducted there was a tendency for growth to be reduced with decreasing latitude. The poorest growth recorded in each of the successive site trials was at the most southerly sites, Twofold Bay and Port Phillip Bay, respectively. At these two sites, water temperature was markedly lower than the other sites evaluated. Even among sites within close proximity, growth also varied in a fashion consistent with temperature. Tomaree, the most seaward of the tested sites within Port Stephens maintains higher average temperatures, particularly during winter, which was reflected, in the higher growth during the second density trial. This second density trial, done during winter also clearly demonstrated the seasonality in growth experienced in Port Stephens and indeed observed at all the tested sites.

Although the stock used in the second density trial were larger, and smaller growth increments were expected, overall growth in this trial was on average less than 20% of that in the previous trial.

The estimates of *k* from the von Bertalanffy equations generated and the indices of overall growth performance Φ' from this study, ranged from 0.55 to 1.00 and 3.70 to 3.96, respectively. Both measures closely reflected the final shell heights achieved in both sites trials. These values were also consistent with those previously reported for *P. imbricata.* Urban (2000) reported values of k and Φ' of 0.625 to 0.939 and 3.644 to 3.821, respectively for *P. imbricata* cultured in the Caribbean. Elsewhere, *k* for *P. fucata* (= *P. imbricata*) cultured in Tuticorn Harbour, India, and *k* and Φ' for Japanese *P. fucata* have been estimated to be k = 0.908 and k = 0.76, Φ' = 3.69, respectively (calculated from Chellam, 1988 and Wada 1991, respectively). This consistency indicates that at the very least the growth of *P. imbricata* achieved at many sites in NSW is sufficient to form the basis for pearl culture to occur.

Nacre quantity and quality

Ideally, the potential of a site to produce high quality pearls is best assessed from the pearls themselves. However, this time consuming, logistically difficult and poses security problems when a large number of geographically spread sites are to be evaluated. Fortunately, the characteristics of the nacreous layer of the shells can provide an indication of the pearl quality that might be achieved. The quality of pearls is judged on five factors, lustre (orient), colour, complexion, size and shape. This study provided information pertaining to three of those factors; lustre, colour and size, in order of relative importance.

While discussion of nacre colour and lustre is constrained, several important points were evident in this study.

- Colour and lustre varied significantly between sites.
- Colour and lustre were not correlated with growth.
- Colour was not correlated with lustre.
- Sites vary with respect to colour and lustre over time

While these messages are not necessarily novel to some, it is important to a fledgling industry that they are understood. Investment in culture leases should be made with care. The natural occurrence of oysters in a particular area and the achievement of satisfactory growth are not sufficient to ensure the production of quality pearls at that location.

The rate at which nacre is deposited significantly affects pearl production. In the first instance a minimum thickness of nacre is required to cover the inserted nuclei to achieve a saleable pearl. While this thickness might vary with species and markets, the minimum required for akoya pearls is generally 450 - 500 μm. This can be achieved within a year by oysters at a number of sites in NSW; although it remains to be seen whether nacre deposition continues at the same rate after having achieved a size at which seeding can occur (approx. 50 - 60 mm).

2.3. Salinity and temperature tolerance in the pearl oyster, *Pinctada imbricata*

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2.3.1. Abstract

The responses of embryos and juveniles of the pearl oyster, *Pinctada imbricata*, to variation in temperature and salinity were investigated to assist hatchery production and farming. Embryos were incubated at temperatures in the range 14 to 26°C and salinities in the range 11 to 35 g kg^{-1}. Embryos did not develop to D-veliger stage at 14°C and at salinities of 26 g kg^{-1} or less. Within the salinity range 29 - 35 g kg^{-1}, the percentage embryos developing to D-veliger stage increased significantly with increasing salinity. Within the temperature range 18 – 26°C, increasing temperature increased the rate of development as well as the number of embryos developing to D-veliger stage within 40 h. Juvenile *P. imbricata* (17 mm shell height) held at temperatures in the range of 14 to 24°C were exposed to salinities in the range of 11 to 35 g kg^{-1}. Spat formed byssal attachments most rapidly at salinities of 29 and 32 g kg^{-1}, irrespective of temperature. At these salinities, > 70 % of oysters formed byssal attachments to the aquaria walls within 6 h. Outside this narrow salinity range, the rate of byssal attachment decreased and ceased altogether at salinities of 17 g kg^{-1} or less. Temperature also affected byssal attachment although the impacts were not as pronounced as those of salinity. Within the optimal salinity range (29-32 g kg^{-1}), the rate of byssal attachment was fastest at 18°C, where up to 80% of oysters had attached within 4 h. This rate was slightly greater than that observed at 22°C, which in turn exceeded those observed at 14°C and 26°C. Salinity and temperature also affected survival. Irrespective of temperature, survival was high at salinities of 32 and 35 g kg^{-1}. By contrast high rates of mortality occurred within 7 days at salinities of 23 g kg^{-1} or less. Onset of mortality was most rapid and overall mortality highest at the two extremes in temperature tested, 14°C and 26°C.

2.3.2. Introduction

Autecological study of bivalves has clearly demonstrated that development, growth and survival are affected by physical parameters, in particular, temperature and salinity, which have been described as "master factors" for many marine organisms (Kinne 1964). Accordingly, the effects of these two factors have been described for numerous mollusc species. Equally important, but less researched are the synergistic effects of temperature and salinity. It has long been recognised that the impact of one factor can be modified by the other and that there is a need to study their actions concurrently (Kinne 1964).

Due to the cosmopolitan nature of the pearl oyster *Pinctada imbricata* (= *P. radiata*, = *P. fucata*; Hynd, 1955; Shirai, 1994; Colgan and Ponder, in press) the temperature ranges experienced by populations over its broad geographical range vary considerably. In southern NSW, oysters encounter temperatures of less than 12°C, while in tropical northern Australia, seawater temperatures exceed 30°C (Beer and Southgate, 2000). Given that the temperature range experienced by Port Stephens *P. imbricata* (approx.14° - 26°C) as used in this study of the temperature and halotolerance is intermediate, no mortality was expected as a direct consequence of temperatures being manipulated within this range. However, as *P. imbricata* enter a period of apparent stasis in growth when temperatures fall below 16° – 17°C (W. O'Connor, pers obs.), likely sublethal effects of reduced temperatures were considered pertinent in assessing the farming potential of this species in NSW.

In contrast to temperature, considerable uncertainty surrounded the likely halotolerance of *P. imbricata*. In a comprehensive survey of the distribution of *P. imbricata* in NSW, Colgan and Ponder (in press) noted the occurrence of the species in both coastal and estuarine/lacustrine sites; however, these surveys were done during periods of high salinity (34 g kg^{-1}). Subsequent surveys at several of these sites noted dramatic changes in oyster occurrence suggesting that the penetration of *P. imbricata* in some estuarine and lacustrine environments may only be temporary. For example, a survey by Colgan and Ponder (in press) found significant numbers of *P. imbricata* at Port Macquarie, on the NSW mid north coast. Subsequent surveys by the current author confirmed the earlier presence of *P. imbricata* (frequent observations of recently deceased oysters), but failed to find any live specimens. This subsequent survey followed a period of significant rainfall and the oyster mortality was putatively ascribed to prolonged exposure to reduced salinity.

The importance of temperature, salinity and their interactions in hatchery rearing and in the selection of sites suitable for pearl farming with *P. imbricata* prompted an investigation of tolerance in. Tolerances of embryos to salinity and temperature were initially assessed as a guide to appropriate conditions for hatchery rearing of larvae. These investigations were also prompted by the fact that embryos are easily obtained, their tolerance to stressors can be rapidly assessed, and also by experimentation with other bivalves (O'Connor and Heasman, 1998) that had shown embryos to be more sensitive to changes in temperature and salinity than other ontogenetic stages. For the purpose of selecting farm sites, hatchery reared juvenile *P. imbricata* were exposed to various salinities at temperatures including the approximate minimum and maximum encountered in outer Port Stephens (Fig. 1).

2.3.3. Materials and Methods

All *P. imbricata* used in this study were progeny of broodstock collected from Wanda Head, Port Stephens. Replicate treatments were held in individual water baths and temperatures were maintained with thermostatically controlled immersion heaters. Salinities were measured using a temperature/salinity bridge (Yeo-kal, Sydney, Australia) and hyposaline solutions (< 35 g kg^{-1}) were generated by the dilution of seawater with rainwater.

Experiment 1: Embryo salinity and temperature tolerance

Embryos were collected from a single mass spawning of 40 hatchery-conditioned broodstock. A minimum of five males were observed to have spawned and the total number of eggs collected (> 50 $x10^6$) indicated a contribution from more than five females. Thirty minutes after spawning commenced, embryos were collected on a 20 μm nylon mesh sieve and resuspended in 10 l of seawater ($21^oC \pm 0.5^oC$, 35 g kg^{-1} salinity).

A fully orthogonal experiment was designed in which replicate sets of four 100 ml containers were maintained at each of nine salinities (11, 14, 17, 20, 23, 26, 29, 32 or 35 g kg^{-1}) and each of four temperatures (14, 18, 22, or $26^oC \pm 0.5^oC$). Less than 1 h-old embryos were then stocked at 10 ml^{-1} into each replicate.

D-veliger larvae can be observed as early as 20 h post fertilisation, although the rate of development is thought to be temperature dependent and thus replicates were sampled after 24 h post-fertilisation and again after 40 h. When sampled, the water in each container was thoroughly mixed and a 10 ml sample was collected. The relative number of zygotes having developed to D-veliger stage was determined by dispersing each sample on a Petri dish, fixing the embryos and larvae with formalin and counting larvae with the aid of a dissecting microscope (40x magnification). In each replicate, the percentage of D-veligers among the first 50 embryos/larvae observed was recorded and is referred to hereafter as percentage development. This procedure was

repeated after 48 h, but on this occasion, the total number of D veligers within each 10 ml sample was also recorded and is referred to as D-veliger yield.

The remaining 80 ml of seawater in each replicate was pooled across the treatments in which embryos developed to D-veliger stage. These pooled samples were then passed over a 20 µm sieve and the D-veligers were collected. The larvae were placed on a sedgewick-rafter slide and the shell length (antero-posterior measurement) of 50 D-veligers from each replicate was then measured with the aid of a microscope (100 x). Pooling was necessary to gather sufficient larvae for sizing in treatments in which the D-veliger yield was low.

Experiment 2: Juvenile salinity and temperature tolerance

A total of 1440 hatchery-reared *P. imbricata* juveniles (16.7 ± 1.5 mm shell height, mean ± sd) were collected from a hanging culture facility at Wanda Head and cleaned of biofouling. These oysters were divided at random into 144 groups of ten and each group was placed in an separate 8-l aerated aquarium. The aquaria were divided into four sets of 36 with each set held at one of four temperatures (14, 18, 22 and 26^{o}C ± 0.5 ^{o}C). The oysters remained in the aquaria for 3 days to acclimatise before the salinities were adjusted. The salinity of the water in four replicate aquaria within each temperature was then adjusted to one of nine salinities (11, 14, 17, 20, 23, 26, 29, 32 or 35 ± 1 g kg^{-1}). The four replicates of each salinity within temperature sets were randomised for position.

The oysters were fed a mixture Tahitian *Isochrysis* aff. *galbana* and *Chaetoceros muelleri* daily. Every second day, the water in each aquarium was drained and replaced with fresh, temperature and salinity equilibrated seawater. Temperatures and salinities were monitored daily throughout the experiment to ensure that they did not vary outside the prescribed ranges. The experiment was run for 10 days. Initially, the oysters were monitored after 0.5, 1, 2, 4 and 6 h to determine the number of oysters that had begun to form byssal attachments to the walls of the aquarium. On each occasion oysters were gently nudged with an artists brush to determine if byssal attachment had occurred. Thereafter, oysters were monitored daily for survival. Oysters were deemed to have died when they failed to respond to gentle probing of the mantle and gills by either closing their valves or withdrawing the mantle.

Statistical analysis

Percentage development data from embryo trials was arcsin transformed to satisfy a requirement for homogeneity of variance before being analysed using two factor ANOVA. Where significant differences (P<0.05) were detected, treatment means were compared using Student-Newman-Kuels procedure (Winer et al., 1991). For all analyses, $\alpha = 0.05$. Surface response plots were generated with the aid of Sigmaplot (SPSS Science, Chicago, IL).

2.3.4. Results

Embryos

Both temperature and salinity significantly affected the development of *P. imbricata* embryos. Initially, when embryos did develop, the rate of development was affected by temperature. At salinities of 32 and 35 g kg^{-1} and a temperature of 26^{o}C, the percentage development of embryos to D-veliger stage had approached 100% within 24 h. At all other temperature-salinity combinations, percentage development increased between 24 and 40 h post fertilisation (Fig. 1).

After 40 h, percentage development of D-veligers and D-veliger yield also differed with treatment. Embryos failed to develop to D-veliger stage at 14°C and at salinities of 26 g kg^{-1} or less (Fig. 1). Within the salinity range 29 - 35 g kg^{-1}, both the percentage development to D-veliger and D-veliger yield decreased significantly as salinity was decreased (F = 18.31; df 2/27; P <0.001 and F = 68.63; df 2/27; P <0.001, respectively). Within the temperature range 18 – 26°C, the relative percentage of D-veligers among larvae present also decreased significantly as temperature was decreased (F = 4.92; df 2/27; P <0.05) and there was a significant interaction with salinity (F = 3.71; df 2/27; P <0.05). Temperature, within the range18 – 26°C, did not however, significantly affect D-veliger yield within 40 h (F = 2.66; df 2/27; P > 0.05)(Fig. 2a).

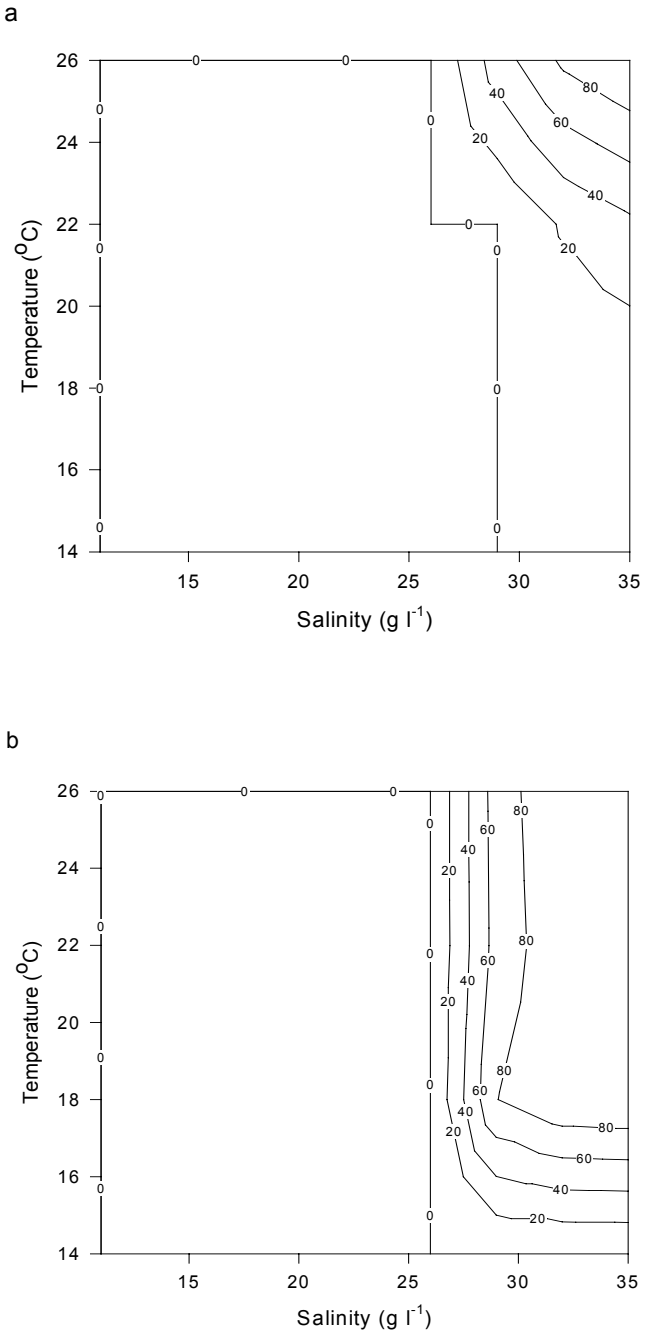
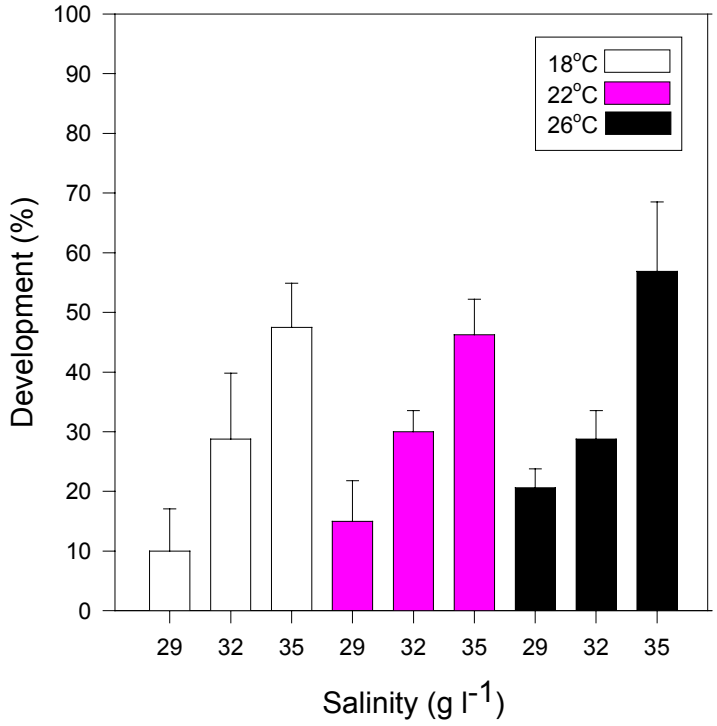


Figure 1. The effect of temperature and salinity on the development of *Pinctada imbricata* embryos after 24 h (a) and 48 h (b). Isopleths indicate the percentage of embryos present that had reached D-veliger stage.

a



b

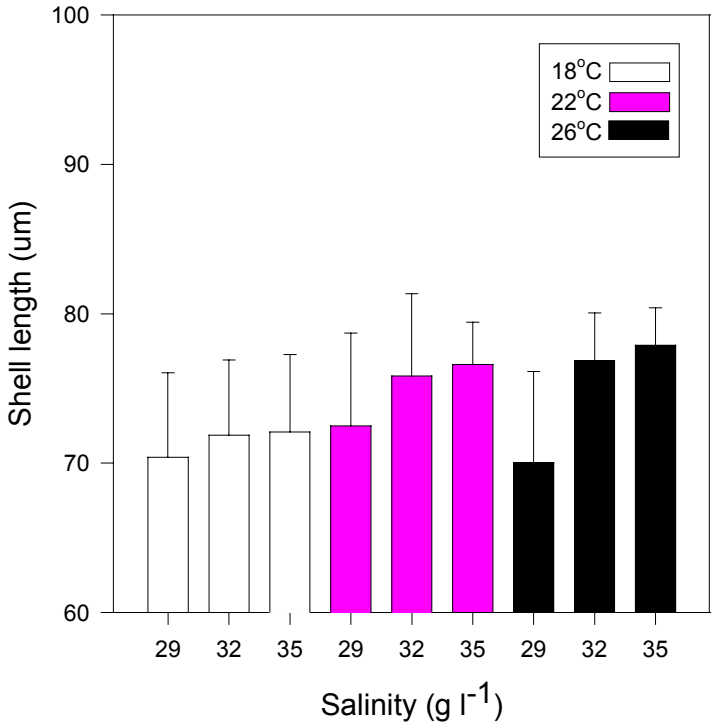


Figure 2. Percentage of *Pinctada imbricata* embryos initially stocked that developed to D-veliger stage within 40 h (a) and the mean larval size of those veligers when incubated at one of three salinities and one of three temperatures (b). Values are means ± SD.

Due to the small numbers of D-veligers present in some replicates, replicates were pooled within treatments to provide sufficient larvae for measurements of larval growth. While this precluded statistical analysis, the results have been presented in Figure 2b. While there appears to be a trend for growth to have been reduced with both reduced temperature and salinity, further investigation is required to validate these observations.

Juveniles

Both salinity and temperature influenced byssal attachment of juvenile pearl oysters (Fig. 3). The percentage of spat forming byssal attachments was highest at salinities of 29 and 32 g kg^{-1}, irrespective of temperature. At these salinities, > 70 % of oysters attached to the aquaria walls within 6 h. Outside this narrow salinity range, the rate of byssal attachment decreased. Byssal attachment did not occur at salinities of 17 g kg-1 or less. Temperature also influenced byssal attachment, although the effects were not as pronounced as those of salinity. Within the optimum salinity range (29-32 g kg-1), the speed of byssal attachment was fastest at 18^{o}C, where up to 80% of oysters had attached within 4 h. This was slightly faster than that observed at 22^{o}C, which in turn exceeded those observed at 14^{o}C and 26^{o}C.

Salinity and temperature also affected survival (Fig. 4). Irrespective of temperature, survival was high at salinities of 32 and 35g kg^{-1}, while high mortality occurred at salinities of 23 g kg^{-1} or less within 7 days. The onset of mortality was most rapid, and the overall mortality greatest at the two extremes in temperature tested, namely 14^{o}C and 26^{o}C.

2.3.5. Discussion

Embryos

It is clear that both temperature and salinity affect the speed and success of early development in *P. imbricata*. Embryos have little tolerance of reduced salinity (< 29 g kg^{-1}) and do not develop at the lower extremes in temperature (≅14^{o}C) experienced in Port Stephens. Two measures of the impact of these factors were used. Initially the number of embryos that had developed to D-veliger stage in each sample was expressed as a percentage at two times (24 and 40 h). This indicated that both temperature and salinity affected the rate of development and that these factors acted synergistically to slow development under suboptimal conditions. Similarly, the total number of D-veligers present after 40 h was affected by temperature and salinity; however, no synergistic affects were noted. In this instance, the numbers of D-veligers decreased with decreasing salinity, but at temperatures at which larvae developed, the yield of D-veligers did not vary (Fig. 2).

Our results indicate that reductions in salinity cause an incremental reduction in both the rate of development and embryo yield, while temperature affects the rate of development, but having reached some critical lower threshold, D-veliger yield is unaffected by further increases in temperature. It may be that yield is unaffected across a broad range of temperature, but that incremental reductions to zero yield occur as temperatures fall from the lower threshold of about 18^{o}C to about 14^{o}C.

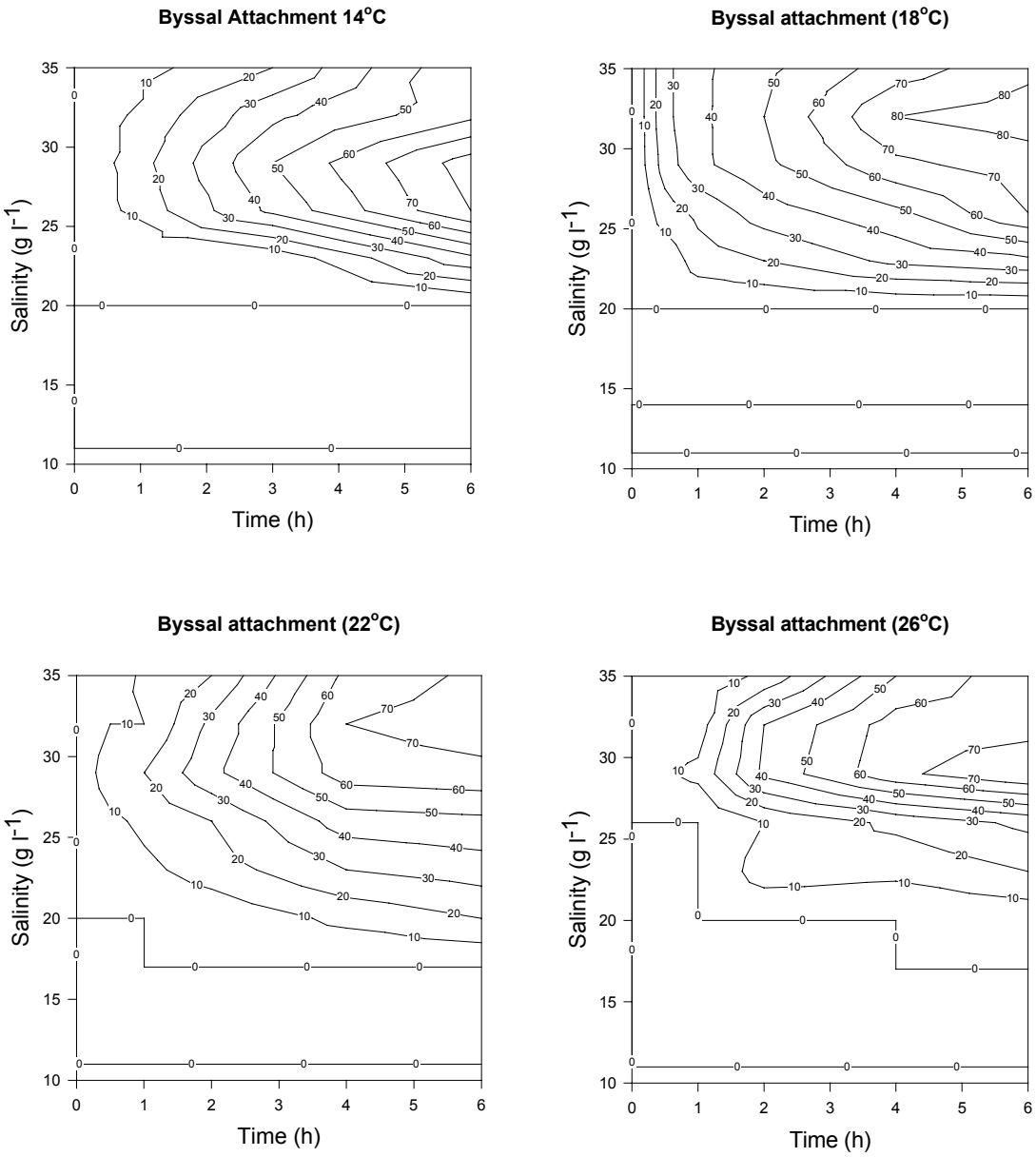


Figure 3. Byssal attachment of juvenile *Pinctada imbricata* during the initial 6 h of exposure to temperatures and salinities within the ranges 14 –24°C and 11 – 35 g kg^{-1}, respectively. Isopleths indicate percentage byssally attached.

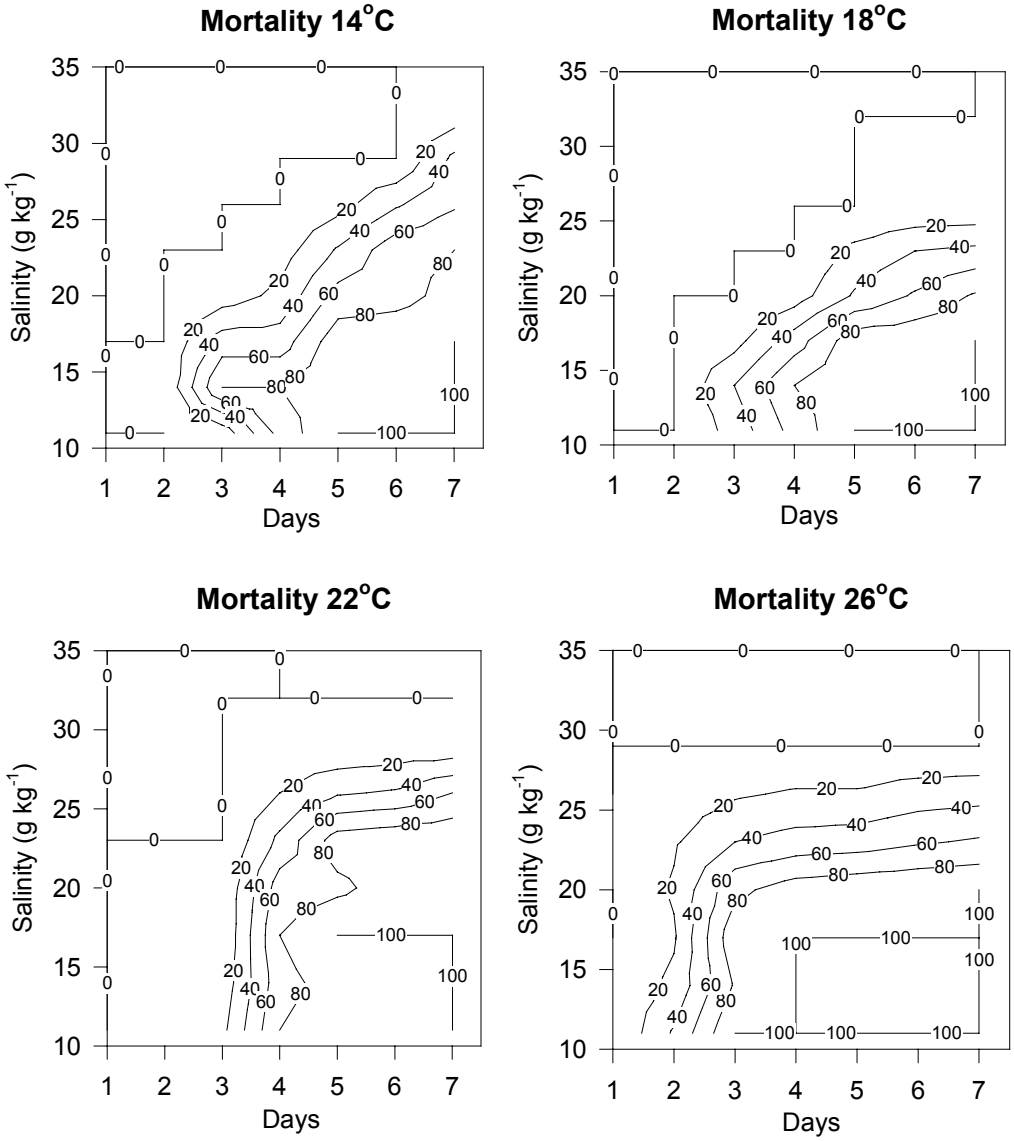


Figure 4. Mortality of juvenile *Pinctada imbricata* during seven days exposure to temperatures and salinities within the ranges 14 –24°C and 11 – 35 g kg^{-1}, respectively. Isopleths indicate percentage mortality.

From a hatchery perspective, two things should be noted. First, the highest D-veliger yields achieved of 50 – 60% in the small scale rearing apparatus used in this study are low in comparison to 95% yields commonly achieved in the hatchery. Despite this, these results were considered sufficiently robust to demonstrate ontogenetic changes in tolerance between embryos and later developmental stages such as juveniles. Further, they provide valuable information for the establishment of hatchery protocols for embryo incubation. However, care should taken before they are used to predict the yield of D-veligers expected under particular environmental temperature and salinity conditions in the field. Second, while salinity optima do not appear to differ greatly with ontogeny, temperature optima do. Although the size of larvae after 40 h varied little between 22° and 26°C, subsequent larval growth within the hatchery is markedly greater at 26°C (W. O'Connor pers. obs.).

Despite the presence of *P. imbricata* in Port Stephens, embryo development does not occur across the full range of temperatures naturally encountered. Rather development appears to be limited to a narrower temperature range that more closely reflects those encountered during the breeding season of *P. imbricata* in Port Stephens. Latitudinal variation in the breeding season of *P. imbricata* on the Australian east coast has been recorded (O'Connor and Lawler, this report) with oysters in Port Stephens in particular limiting reproductive activity to the warmer months of the year. Two peaks in reproductive condition have been recorded in each seasonal cycle. The first in late spring/early summer (Nov - Dec) and the second in early autumn (Mar - Apr). Of these two peaks the former appears to have greater ecological significance, as the later has not lead to subsequent spat recruitment. Regardless, both of these peaks occur when water temperatures in Port Stephens are in excess of 18^{o}C and thus permit embryonic development.

Juveniles

Given the distribution of *P. imbricata,* it is not surprising that at a salinity close to oceanic (32-35 g kg^{-1}) juveniles within this study survived well within the temperature range commonly encountered in Port Stephens (14 – 24^{o}C, Fig. 4). These results are consistent with those obtained elsewhere. In Japan, favourable temperatures for adult oysters have been reported to lie within the range 13 –25^{o}C, while temperatures outside the range 7 - 29^{o}C are considered critical (Wada, 1991). Interestingly, the later (29^{o}C) is routinely exceeded in many areas in which *P. imbricata* occurs in Australia and thus, as noted by Wada (1991), differences in temperature tolerance may occur with geographic area. Differences in temperature tolerance also occur during ontogeny, not only between embryos and juveniles as observed here, but also between spat and adults, particularly at low temperatures. In Japan, spat of 3 mm were found to be intolerant of temperatures less than 17.5^{o}C, with a suggested lower limit of 15^{o}C (Numaguchi and Tanaka, 1986a). Juveniles (17 mm) in this study, and indeed in Port Stephens, have survived well at 14^{o}C, albeit for relatively short periods of time. This study finished after 7 days and although temperatures can fall to14^{o}C at the areas farmed in Port Stephens, they do not do so for protracted periods. Accordingly, 14^{o}C may be approaching the tolerance limit for juveniles and until such time as tolerances below 14^{o}C are assessed, great care should be taken when handling stock during the coldest winter months? In the case of routine pilot farming operations being staged in Port Stephens, spat are not deployed to the farm at temperatures less than 17^{o}C, indeed handling oysters, irrespective of size, is avoided at or below this temperature.

Salinity tolerances reported for *P. imbricata* also appear to be a function of a number of factors including geographic location, ontogeny, and experimental methods. In this study, oysters were generally tolerant of salinities as low as 27 - 25 g kg^{-1}, with high mortality (> 50%) commencing within the range 26 - 22 g kg^{-1}. In India, *P. imbricata* has been found to be tolerant of salinities within the range 24 – 50 g kg^{-1} for 2-3 days (Alagarswami and Victor, 1976; Dharmaraj et al., 1987). While this range includes a lower salinity minimum, it should be noted that the bulk of mortality in this study occurred after 2-3 days and that at most temperatures, oysters survived well for 3 days at 24 g kg^{-1} (Fig. 4). In Japan, longer-term studies reported the minimum salinity optimum for spat to be 22.7 g kg^{-1} (Numaguchi and Tanaka, 1986b).

Other than the work of Doroudi et al. (1999) with *P. margaritifera* embryos and larvae, there is little information regarding interactions of temperature and salinity on pteriid oysters. With scallops, several studies have shown a significant interaction between temperature and salinity (Paul 1980a, Tettelbach and Rhodes 1981, Mercaldo and Rhodes 1982, Hodgson and Bourne 1988, Strand et al. 1993, O'Connor and Heasman, 1998). In general, extremes in one factor reduce tolerance to variations in the other (synergism), although one factor can influence responses more rapidly than the other. With *P. imbricata*, the onset of mortality was more rapid at extremes in temperature tested and tolerance to reduced salinity decreased markedly at 14^{o}C (Fig. 4).

Ecological and farming implications

Despite the paucity of published accounts of the impacts of temperature and salinity on pteriid oysters, the value of short-term studies like this has attracted some debate. It has been argued that abrupt salinity changes are not representative of the changes encountered in the natural environment as they provide little opportunity for animals to acclimatise (Davenport et al., 1975). Alternatively, it has also been suggested that while such results are likely to be a "severe " indication of tolerance they can still be of particular value to aquaculture. Optima derived from this study are immediately applicable in hatcheries where these parameters are controllable and can be manipulated during early ontogeny. Similarly, even starkly conservative estimates of tolerance are of value in selecting sites for nursery rearing and ongrowing pearl oysters.

An additional factor that might have amplified treatment responses and therefore led to conservative estimates of tolerable ranges of temperature and salinity was that the byssal attachments of all spat were severed just before the start of the experiment. This could have encouraged the oysters to open their valves to reform attachments and thus unnecessarily expose themselves to suboptimal conditions. Moreover, byssogenesis places additional energy demands on bivalves, estimated to be between 4-14% of the energy budget for somatic production in some scallops (Vahl 1981). These additional demands may exacerbate the effects of suboptimal temperature or salinity by accelerating the onset of morbidity, or by increasing morbidity under otherwise marginal conditions.

Despite the potential for the estimates obtained in this study to be conservative, these results, together with observations of the natural distribution of *P. imbricata* in NSW, indicate that areas of relatively high and stable salinity are best suited to pearl oyster culture. In NSW, deep embayments and other protected areas of water with high and stable salinity are not common. Thus, it has been suggested that subtidal sites currently allocated to farming the Sydney rock oyster, *Saccostrea glomerata*, could be used for pearl production. The decline of the Sydney rock oyster industry (Nell, 1993) has seen the dereliction of large areas of oyster lease which have been suggested as sites for alternative mariculture. Although current trial farming of pearl oysters on Sydney rock oyster leases at several sites in NSW is producing encouraging growth rates, the majority of oyster farming leases in NSW are subject to extended periods of reduced salinity (Table 1). Accordingly only the lower reaches of some estuaries such as the outer Port Stephens sites used in this study are likely to be appropriate, The “take home message” is that great care should be exercised when selecting areas for pearl oyster farming.

The impact of salinity upon *P. imbricata* demonstrated in this study is to an extent evident within the species’ natural distribution. Stable populations of the *P. imbricata* are known to exist in estuaries and embayments where high salinity is maintained, such as outer Port Stephens. Elsewhere, these oysters are most commonly found toward the mouths of estuarine systems where any perturbations in salinity due to flooding are relatively short lived. In some situations tidal flows are sufficient to restore salinity while in others they may drive wedges of saline water back into the estuary sufficient to negate the effects of freshwater. There is no doubt, *P. imbricata* can frequently penetrate well into estuarine and lacustrine environments, and several Sydney rock oyster farmers have reported significant spatfalls. However, we have no knowledge of persistent populations in NSW that would bring into question the salinity tolerances suggested by this study.

Table 1. Summary of temperature and salinity data for major oyster bearing estuaries of NSW (1966-1973).

Location	Temperature (°C)			Salinity (g kg^{-1})		
	mean	max	min	mean	max	min
Tweed R.	22.1	32.0	13.0	29.6	38.1	0
Richmond R.	21.5	29.0	9.0	27.8	38.7	0
Clarence R.	20.2	30.0	9.0	27.0	38.0	0
Wooli R.	20.8	31.4	13.0	31.3	39.6	0
Macleay R.	20.4	29.0	9.0	15.4	36.1	0
Hastings R.	19.3	27.0	12.0	28.3	38.9	0
Manning R.	19.9	31.6	11.8	24.4	38.7	0
Camden Haven	20.5	30.0	13.0	30.7	39.4	0
Wallis Lake	20.0	28.0	11.0	30.5	38.4	0
Port Stephens	19.4	30.0	8.0	29.9	39.0	0
PSRC	19.1	28.5	8.6	29.4	35.4	7.0
Karauh R.	19.2	31.0	9.8	27.4	39.2	0
Brisbane Waters	21.5	33.0	10.5	33.1	39.0	19.7
Hawkesbury R.	20.0	32.4	9.0	26.1	35.8	0
Georges R.	17.8	27.0	7.4	32.5	40.3	7.1
Clyde R.	18.6	29.0	11.0	30.8	38.2	0
Tuross Lake	17.2	25.0	9.0	31.0	39.9	0
Wagonga Inlet	18.5	29.0	10.0	28.7	35.4	0
Wapengo Lake	17.7	30.0	6.0	34.6	41.9	0
Merimbula Lake	17.0	29.8	6.3	34.7	38.6	0
Pambula R.	16.8	28.0	7.0	33.2	38.7	0

All data has been summarised from Wolf and Collins (1979). Where multiple sites were monitored within an estuary, data for the site with the highest minimum salinity has been presented.

Behavioural responses

Behavioural or physiological changes are a useful indicator of stressors within the environment of bivalves (Davenport et al. 1975, Roberts 1973 cited in Paul 1980b, Heasman et al. 1996) and have been used to detect environmental preferences in pearl oysters (Numaguchi and Tanaka 1986; Dharmaraj et al., 1987; Wada, 1991). Most bivalves close their valves in response to salinity change to allow isoosmotic intracellular regulation to commence (Hawkins and Bayne 1992). This was observed previously by Alagarswami and Victor (1976) with *P. imbricata* and was evident in this study. At reduced salinities, juveniles maintained closed valves significantly longer and thus the rate of byssal attachment was significantly slower. Ultimately, byssal attachment over the first 6 h reflected juvenile temperature and salinity tolerances. While temperature had only a minor impact, the rate of attachment slowed markedly at salinities in the range 23 –25 g kg^{-1}; the range at which significant mortality was later observed. Interestingly, in common with some scallops (Paul 1980b), byssal attachment also occurred under conditions in which juveniles did not survive. For instance, some *P. imbricata* juveniles formed byssus at salinities of 17 ppt although none survived at this salinity for more than one week. Regardless, behavioural observations provide an additional tool in the evaluation of potential culture environments, particularly for species such as *P. imbricata* that continue byssal secretion into adulthood.

A final cautionary note to be sounded in the interpretation of byssal attachment observations is that the rate of byssal attachment in *P. imbricata* is affected by oyster size and time of day, with larger oysters taking longer to form byssal attachments and preferring to do so at night (Dharmaraj et al., 1987; Ghorbani, 1997).

2.4. Emersion tolerance of pearl oyster, *Pinctada imbricata* Röding, spat and juveniles

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2.4.1. Abstract

Regular air exposure of spat and juvenile pearl oysters, *Pinctada imbricata*, during culture prompted an evaluation of their tolerance to emersion. Oysters were emersed under conditions chosen to simulate the harshest experienced in Port Stephens, NSW Australia. Temperatures tested were in the range 12 to 36°C and fans were used to simulate the desiccative effects of winds. Spat (4.8 mm) and juvenile (12.3 mm) survivals were greatest in the range 16 to 24°C. At 20°C survival was size dependent, varying between 4 h for 5 mm spat to 30 h for 37 mm juveniles. Any additional stress that may be imposed by breaking the byssal attachment of the oysters prior to emersion had no significant effect on survival. In attempts to increase oyster tolerance to emersion, protection against desiccation was of particular importance and significantly increased oyster tolerance. Protection of spat (4.8 mm) from airflow by placing them in plastic bags increased survival times three-fold. Tolerance was further increased if oysters were wrapped in damp toweling inside the bags, but the replacement of air with oxygen in bags did not significantly increase survival. With the practical application of these results Oysters (12 to 35 mm) are now routinely emersed and transported for up to 30 h without significant loss by the industry.

2.4.2. Introduction

The pearl oyster, *Pinctada imbricata* Röding, is among the most widespread of the Pteriid species (Shirai, 1994) and, in Australia, occurs from Shark Bay in the west, around the northern coastline and down the east coast as far south as northern Victoria (Hynd, 1955). While *P. imbricata* has been used for pearl culture in Asia for decades, the species has only recently been the subject of commercial interest in Australian waters.

Although predominantly subtidal in nature, *P. imbricata*, is occasionally subject to periods of emersion. In wild populations, oysters are emersed relatively briefly and infrequently by spring low tides (Hynd, 1955), but, it is in culture that the frequency and duration of emersion can increase greatly. Oysters are regularly taken from the water for procedures such as cleaning, grading and nuclei insertion. As optimal conditions for successive stages of pearl production commonly occur in different parts of particular estuaries or in different estuaries, extended emersion associated with transportation cannot be avoided.

Given the need for farmed *P. imbricata* to endure protracted exposure to air and the paucity of information regarding its impacts on any *Pinctada* spp., this study was done to provide an indication of the tolerances of both *P. imbricata* spat and juveniles to emersion. Oysters were emersed for periods of up to 60 h at temperatures in the range 12 to 36°C and emersion experiments were done under conditions that varied from those considered to be harsh, to those thought to be conducive to oyster survival.

2.4.3. Materials and Methods

All oysters used in this study were produced in the hatchery at Port Stephens Fisheries Centre and, in the case of juveniles, grown in nursery facilities located on Port Stephens. Prior to each experiment, oysters were brought to the hatchery and divided into groups of ten. Each group was placed on an individual mesh screen (90 mm diameter) and the screens were stacked so that spat were confined to the screen on which they had been placed. Screen stacks containing the groups of oysters were then placed in a 200 L seawater bath at 24°C. An airlift pump was used to ensure water flow through the stacks (Fig. 1) and the oysters were allowed to acclimatise to their surroundings for a minimum of 24 h before experimental emersion. Oysters held in the 200 L bath were fed mixtures of Tahitian *Isochrysis* aff. *galbana*, *Pavlova lutheri* and *Chaetoceros muelleri* ad libitim before and after experimental emersion.

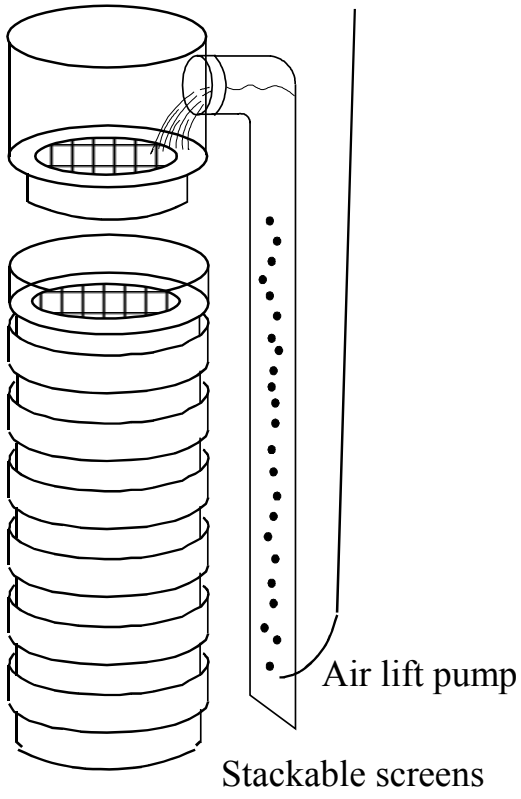

Figure 1. Screen and stack arrangement used to hold spat and juveniles.

Experiment 1: The effect of duration of emersion and temperature on oyster survival

Spat (4.8 ± 1.0 mm, dorso-ventral shell height ± SD) were emersed at one of seven temperatures {12, 16, 20, 24, 28, 32 or 36^{o}C (± 0.5^{o}C)} in temperature-controlled, incubator cabinets with fan forced airflows to promote even temperature. In each case, six screens were placed in an incubator. At intervals of 2 h over a 12 h period, one screen was removed from the incubator and returned to the 200 L seawater bath. A seventh screen of spat remained in the seawater bath as a control. The procedure was repeated three times with different groups of spat so that three replicate results were recorded for each time and temperature combination. Due to the limited number of incubators available the treatments were randomised in order and with respect to incubator, and the emersion series were done four at a time. Two days after emersion, each replicate was removed from the water bath and each screen was inspected with the aid of a binocular microscope to determine the survival of spat.

The previously described experimental procedure was repeated using juveniles (12.3 ± 2.1 mm, mean ± SD). However additional replicate screens were used on each occasion so that emersion times could be extended to 20 h.

Experiment 2: Extending emersion tolerance

Seventy-two groups of *P. imbricata* (n = 5) were divided among two temperature controlled incubators set at 20^{o}C - the temperature found to be most conducive to oyster survival in Experiment 1. The 36 groups in each incubator were then divided equally among four treatments. Nine groups were held in air filled plastic bags so that the oysters were protected from the fan forced airflows. Nine were placed in plastic bags filled with medical grade O_2 to increase partial pressures for O2 diffusion across the gill surface. Nine groups were wrapped in damp absorbent paper to keep the air moist and placed in air filled bags and the remaining nine groups remained on mesh screens (as in Experiment 1). After 12 h, one group of oysters from each treatment was removed from each incubator and returned to a seawater bath to recover. Every 6 h thereafter, up until 60 h, an additional group of oysters from each treatment was removed and returned to seawater. An additional two groups of oysters remained on screens in the seawater baths as controls.

The experimental procedure was repeated on three occasions using either spat (4.8 ± 1.0 mm; x ± SD) or juveniles of one of two size classes (15.5 ± 2.6; 36.7 ± 1.8 mm; x ± SD). For juveniles the number of groups initially placed in each incubator was reduced to 28 because the minimum time for emersion was increased to 24 h.

Experiment 3: Byssal attachment and emersion tolerance

In contrast to Experiment 1, spat used in the investigation of protection from desiccation in Experiment 2 were detachment from the screens on which they had been acclimated. This raised concerns that the additional stress imposed by breaking the byssus to remove the oysters from the screens could affect emersion tolerance. This possibility was investigated by emersing both byssally attached and detached juvenile oysters (25.1 ± 2.7 mm; x ± SD) for periods of either 24, 30, 36, 42, 48 or 54 h at a temperature of 20^{o}C. Three replicate screens of juveniles for both treatments (attached or detached) were emersed for each time period. Another three screens of attached oysters and three screens of detached oysters remained in the seawater bath as controls.

2.4.4. Results

Wile no mortality occurred among oyster spat or juveniles held in non-emersed control screens in any of the trials done, the emersion tolerance of *P. imbricata* was affected by temperature, oyster size and the conditions under which the oysters were held. In Experiment 1, the survival of both spat and small juveniles were greatest in the range 16 to 24°C and poorest at the highest temperature tested, 36°C (Fig 2). Spat (4.8 mm DVH) were capable of surviving up to 4 h in the incubator at 20°C without mortality, while 12 mm juveniles could survive 10 h at the same temperature. This trend for increasing tolerance to emersion continued in Experiments 2 and 3, where 16 mm, 25 mm and 37 mm juveniles survived 24 h, 24 h and 30 h, respectively, at 20°C without mortality (Fig. 3).

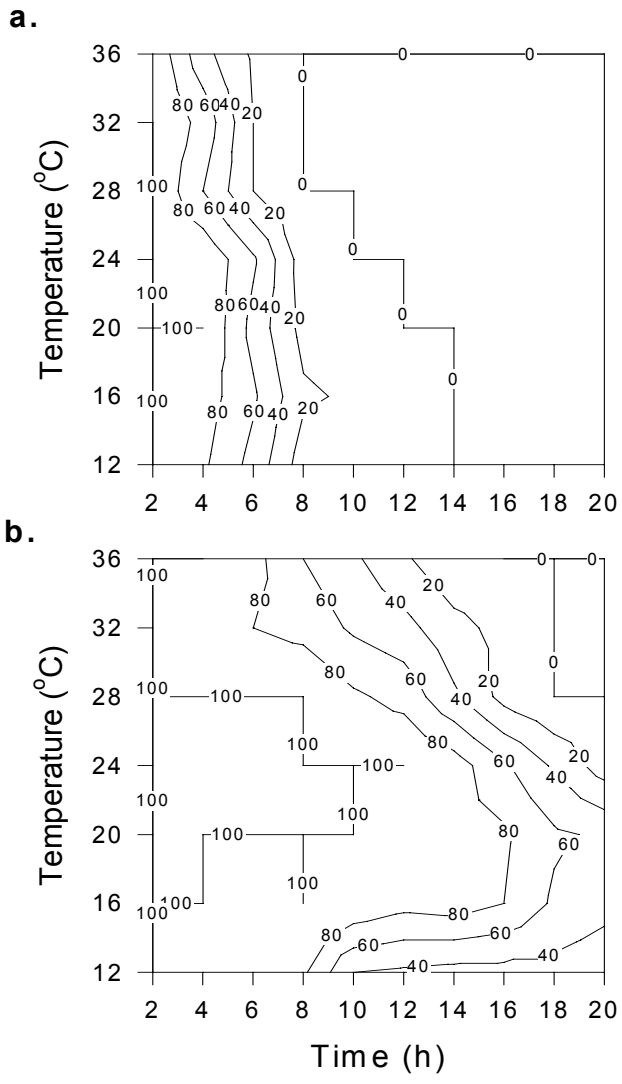

Figure 2. The effect of temperature and duration of emersion on survival of *Pinctada imbricata* a) spat (4.8 ± 1.0 mm) and b) juveniles (12.3 ± 2.1 mm). Isopleths indicate the number of live oysters expressed as a percentage of the initial number emersed.

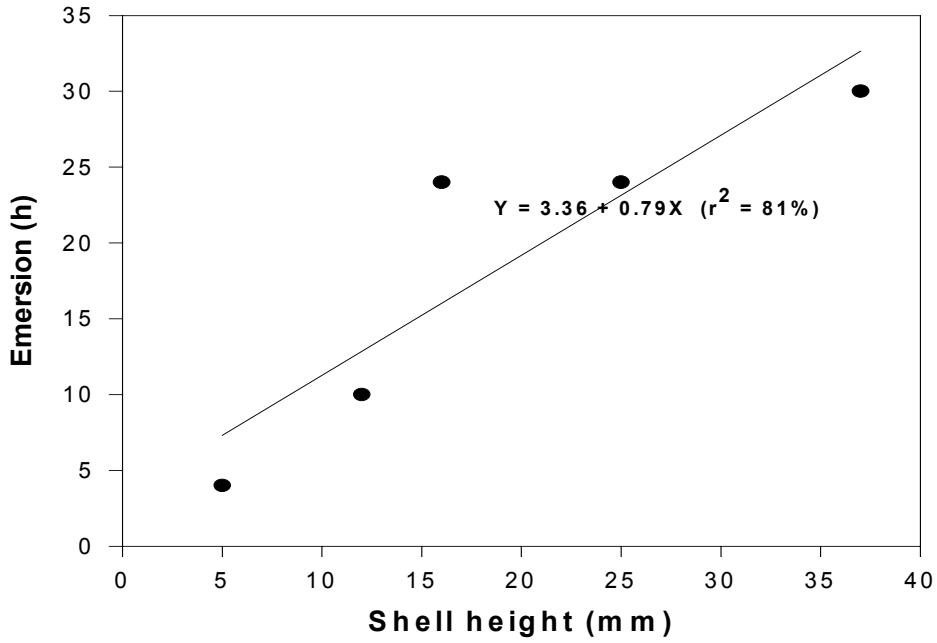

Figure 3. Emersion tolerance of *Pinctada imbricata* spat and juveniles as a function of size. Points represent the maximum duration of emersion without mortality at 20°C.

The effects of treatments to increase emersion tolerance (Experiment 2) were compared using three-way-Anova (Sokal and Rohlf, 1981). To permit a balanced design, survival data for spat prior to 24 h were not included in the analysis. Emersion treatment differences were evaluated using the Student-Newman-Kuels procedure (Winer et al., 1991). Oyster tolerance of emersion increased significantly as the size of the oysters increased and as the time of emersion decreased (F = 345.02; df 2/84; $P < 0.001$ and F = 146.28; df 6/84; $P < 0.001$, respectively; Fig. 4). The treatment used during emersion also significantly affected survival (F = 75.72; df 3/84; $P < 0.001$). Oysters protected from airflow with plastic bags showed significant improvements in emersion tolerance (SNK, $P < 0.05$), with those wrapped in moist toweling inside the bags showing significantly greater tolerance than any other treatment (SNK, $P < 0.05$). The use of oxygen to fill the bags did not significantly improve survival (SNK, $P > 0.05$).

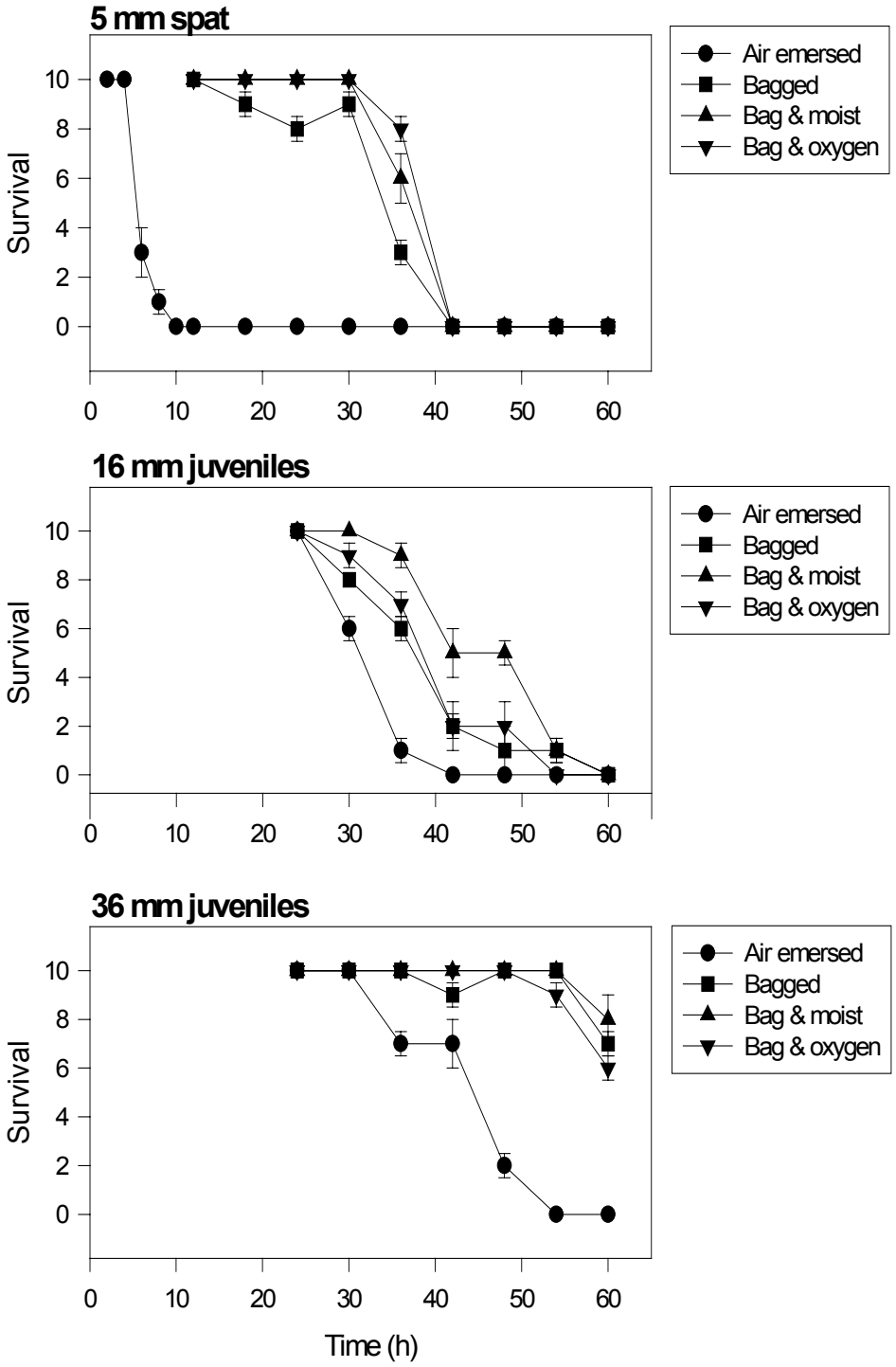

Figure 4. A comparison of survival of three size classes of *Pinctada imbricata* emersed at 20°C using one of four treatments: 1) emersed and exposed to airflow; 2) emersed in a protective plastic bag; 3) emersed wrapped in moist paper in a plastic bag, 3) emersed an oxygen filled plastic bag. Survival data for 5 mm spat emersed and exposed to airflow has been drawn from Experiment 1. Values are means ± SE.

Multivariate analysis of the effects of the duration of emersion on attached and detached 25 mm juveniles found survival decreased significantly with time (F = 37.45; df 5/29; P < 0.001; Fig. 5), however severing the byssal attachment prior to emersion did not significantly affect survival (F = 0.04; df 1/29; P > 0.05).

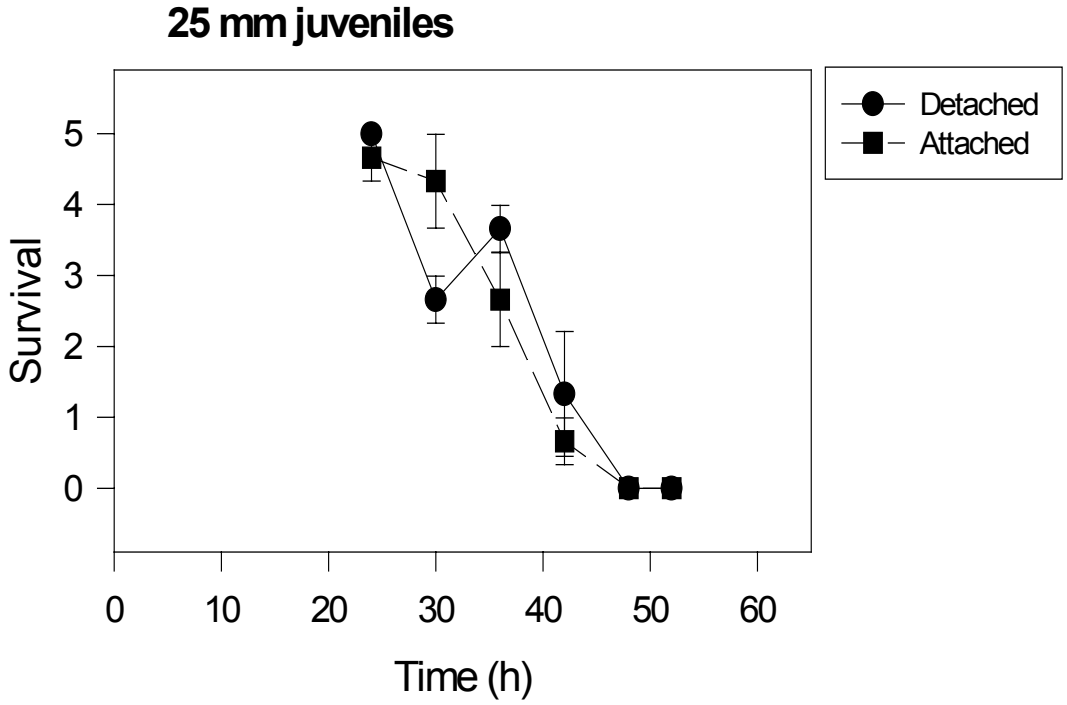

Figure 5. Emersion tolerance of byssally attached and detached *Pinctada imbricata* juveniles (25 mm shell height) at 20°C. Values are means ± SE.

2.4.5. Discussion

Within its wide geographic range, farmed *P. imbricata* may be exposed to a variety of atmospheric conditions. In Port Stephens the most harsh conditions are probably in summer when temperatures can rise to the mid thirties and hot dry winds from the west can persist for weeks. Under these circumstances, emersed oysters, particularly spat and small juveniles encounter high temperatures and an increased risk of desiccation; both factors which have been identified in reducing the viability of emersed bivalves (Davenport and Wong, 1992; McMahon and Payne, 1992). For this reason we felt it necessary to conduct emersion tolerance trials under conditions that simulate among the most harsh likely to be encountered. Therefore, oysters were emersed in incubators in which fans were used to circulate the air. As a result, the tolerance of the various sized oysters used was frequently less than we had expected from our experiences in the field, but was thought to provide useful baseline data for safe emersion under most circumstances.

Regardless of the severity of the conditions, responses of *P. imbricata* were in accordance with those of other bivalves in several respects. Initially, *P. imbricata* commonly responded to emersion by gaping as has been observed with other bivalves such as the oyster *Crassostrea rhizophorae* (Littlewood and Young, 1994) and has been reported in other Pteriid oysters (Hancock, 1973). Second, *P. imbricata* emersion tolerance was influenced by temperature, with the mortality rates increasing at elevated temperatures (Davenport and Wong, 1992; McMahon and

Payne, 1992). Third, like emersed clams, *Tapes philippinarum* (Richardson, 1988), *P. imbricata* that survive prolonged emersion show a distinct layer in the shell that has been reported by farmers to be typical of the response to stressful events. Finally, but less frequently reported in bivalves, the size of *P. imbricata* significantly affected their tolerance to emersion. Tolerance increased from 4 h for spat to 30 h for 37 mm juveniles (Fig. 3) without signs of a significant plateau in survival, suggesting larger oysters may be capable of withstanding even greater periods of emersion.

As noted earlier, the risk of desiccation in these trials was likely to be high and thought to be similar to that experienced by farmed oysters under the harshest conditions in Port Stephens. It is therefore not surprising to find that treatments that reduced the risk of desiccation greatly increased emersion tolerance. Simply protecting oysters from airflow was particularly effective in this respect, trebling the tolerance of spat. Smaller, but significant improvements in tolerance were achieved by wrapping spat in moist substrates within the bag, which was thought to assist in the maintenance of humidity and thus prevent desiccation.

The use of an oxygen rich environment is common during the transport of aquatic organisms and was thought to be potentially useful with bivalves by increasing partial pressures for diffusion across the gill surfaces. However the inclusion of O_2 failed to infer any advantage. The reasons for this are unclear, although it may serve to further reinforce the importance of preventing desiccation. Any advantage that O_2 might provide may be ameliorated by the fact that when it was supplied in bottled form it is moistureless and may have exacerbated desiccation. On the other hand the amount and diffusion rate oxygen in air may simply not have been a limiting factor under the experiment conditions applied. Further trials could be done to test if there is some synergistic advantage in the use of a moist, oxygen enriched environment.

In practice we have combined techniques to enhance survival of juvenile *P. imbricata* during transport. Oysters have been placed upon several layers of wet absorbent paper in a plastic bag that is subsequently filled with oxygen and sealed with elastic bands. Oxygen has been used, as the use of wet paper is considered sufficient to offset any initial reduction in humidity. Oyster numbers are such that they occupy no more 30% of the total volume of the bag and are in a layer two to three oysters deep. The bag is placed in a polystyrene box for transport and if temperatures are likely to increase beyond 20^{o}C, a frozen block is taped inside the lid of the polystyrene container. Under these conditions, juvenile oysters (12 to 35 mm) have been emersed during transport for up to 30 h without significant mortality.

2.5. Propylene phenoxetol as a relaxant for the pearl oysters *Pinctada imbricata* and *Pinctada albina*

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2.5.1. Abstract

The responses of the pearl oysters *Pinctada imbricata* and *Pinctada albina* to the relaxant, propylene phenoxetol (PP) were similar to those reported for other members of the genus. Wedges to hold open the valves of oysters were unnecessary as most opened readily in the presence of PP (2 mL L^{-1} seawater). Relaxation generally occurred within 15 min and, on removal from the relaxant bath, oysters recovered within 10 min without evidence of any ill-effects. In general, both relaxation and subsequent recovery times decreased with increasing water temperature. The size of oysters had little effect on the time taken to open valves in the presence of PP, the time to relaxation nor the time to recover after exposure. Prolonged exposure to PP (90 min) significantly increased the recovery time, but no mortality or apparent ill effects were observed in the week following exposure.

Key words: Propylene phenoxetol; Relaxant; Pearl oyster.

2.5.2. Introduction

In both pearl culture and pearl oyster research, the invasive nature of some procedures has led to the evaluation of potential relaxants to reduce stress to oysters (Tranter 1957, Hildemann et al. 1974, Dev 1994, Norton et al. 1996, 2000). In particular, relaxants have been suggested as a means of reducing oyster mortality and increasing pearl quality by: preventing muscle damage during nuclei insertion operations; reducing muscularly induced haemolymph loss and increasing the ease and accuracy of surgery and biopsy by preventing muscular contractions (Norton et al. 1996). Among the relaxants tested, propylene phenoxetol (PP) is particularly useful to relax the oysters *Pinctada margaritifera* (Hildemann et al. 1974, Norton et al. 1996), *P. albina* (Norton et al. 1996) and *P. maxima* (Norton et al. 1996, Mills et al. 1997). In these species, 1.5-2.5 mL L^{-1} PP produces relatively rapid relaxation (generally < 15 min, Mills et al. 1997) with a short recovery period, although its effectiveness was reduced at lower temperatures (Norton et al. 1996).

Pinctada imbricata is native to the New South Wales coast (Australia) and is the subject of research that would benefit from the availability of a suitable relaxant, notably during experimentation where handling could reduce growth rates or induce spawning. PP was suggested on the basis of its success with other pearl oyster species, but the possibility of species-specific differences in responses to relaxants (Runham et al. 1965, Kaplan 1969, Heasman et al. 1995) required the evaluation of PP prior to routine use on *P. imbricata*. *P. albina* is also native to NSW and the efficacy of PP has previously been evaluated with this species. However, PP had only previously been used on larger oysters (> 90 mm or >120 g) at higher water temperatures (>21°C). The *P. imbricata* and *P. albina* of interest were routinely between 40 and 90 mm shell height (20-120 g total weight) and commonly required relaxation at temperatures of < 20°C.

2.5.3. Materials and Methods

All pearl oysters used in this study were collected from Port Stephens, New South Wales (32°44'S, 152°08'E). To prepare relaxant baths, PP (1-phenoxy-propan-2-ol ($C_9H_{10}O_2$), Nipa Laboratories,

UK) was added to a small quantity (100-200 mL) of seawater in a bottle and shaken vigorously to aid dispersion before being added to 4 L of seawater (35 g kg^{-1} salinity). The term "relaxant" has been used in preference to anaesthetic" to acknowledge the difficulties in differentiating between muscular paralysis and anaesthetisation in pearl oysters (after Norton et al. 1996).

In all experiments, the concentration of PP was 2 mL L^{-1} of seawater and unless otherwise specified the temperature of baths was 18°C. Pearl oysters were placed in the baths vertically on their hinge and lent against the wall of the bath. To recover following relaxation, oysters were placed into a 200 L aerated tank of seawater that was held at the same temperature as the relaxant bath.

Oysters were defined as being "relaxed" when they gaped and gentle probing of the mantle failed to induce the oyster to either withdraw the mantle or close the shell and when the oyster could also be removed from the relaxant bath without it closing its shell (after Heasman et al. 1995, Norton et al. 1996). Oysters were considered to have recovered when any handling or disturbance induced the shell to close. All oysters were maintained within the hatchery for at least 24 h after experimentation to monitor any resultant mortality.

Experiment 1: Valve wedges and relaxation

Wedges are commonly used in the pearl industry to prevent oysters closing their shells prior to nuclei insertion and can be experimentally useful to ensure an oyster is immediately exposed to the relaxant (Norton et al. 1996). However, wedges can also be difficult to use with smaller oysters and are considered on occasions to be impractical and potentially stressful to the oyster. In particular, we wished to avoid using wedges and inducing any associated stress to oysters during routine assessments of reproductive condition. As a result, the time to relaxation and subsequent time to recovery of 12 oysters with their valves wedged open were compared with those of 12 oysters placed directly in the relaxant baths. In the latter treatment, the time to relaxation was defined as the time elapsed between when the oysters first opened their valves and when they subsequently relaxed.

Experiment 2: Prolonged exposure and oyster survival

Ten oysters were placed in individual baths of PP solution and retained in the bath for 90 min after relaxation. Each oyster was then removed and placed in a recirculating holding system to recover. Individual response times (opened, relaxed and recovered) were recorded and oyster survival was monitored for one week following exposure to PP.

Experiment 3: The effect of temperature on relaxation and recovery

Oysters were held in the hatchery in 200 L tanks of seawater for 24 h at one of four temperatures, 14, 18, 22 or 26°C. A mixture of *Pavlova lutheri* and *Chaetoceros calcitrans* was added to each tank to encourage the oysters to gape and filter feed. Each oyster was then placed in an individual PP bath and the times taken to open the valves, to relax and to recover following removal from the bath were recorded. The response times (opened, relaxed and recovered) of ten oysters were recorded for each temperature.

Experiment 1 used only *P. albina*, while Experiments 2 and 3 were repeated using both *P. imbricata* and *P. albina*.

Statistical analysis

The effects of wedging oyster valves on response times (Exp. 1) and of prolonged exposure on response times (Exp. 2) were evaluated using ANOVA (Sokal and Rholf 1981) after homogeneity

had been confirmed with Cochran's Test (Winer et al. 1991). For Experiments 1, 2 and 3, the relationships between shell height and response times were investigated using Pearson product-moment correlations (Sokal and Rholf 1981). Due to the number of correlation coefficients calculated (33), α was set *a priori* at 0.01 to reduce the possibility of Type I error (Sokal and Rholf 1981). The effects of temperature on response times (Exp. 3) were analysed using linear regression analysis (Sokal and Rohlf 1981).

2.5.4. Results

Experiment 1: Valve wedges and relaxation

For *P. albina* without wedged valves, the time taken to open the valves ranged between 0.5-33 min, but generally occurred between 3-6 min after immersion. Having opened their valves, the time taken for oysters to relax (5.5 ± 0.6 min) did not differ significantly ($F = 2.199$, df 1/22, $P > 0.05$) from the time taken for relaxation in oysters with valves wedged open (8.8 ± 2.1 min). The time taken to recover was, however, significantly faster for oysters with valves wedged open (3.31 ± 0.4 min and 5.45 ± 0.8 min, respectively; $F = 5.423$, df 1/22, $P < 0.05$). No significant correlations were found between the shell height of oysters and either the time taken to open in the bath, to relax in the presence of PP nor to recover following exposure to the relaxant (Table 1).

Experiment 2: Prolonged exposure and oyster survival

For both *P. imbricata* and *P. albina*, the time taken to open in the presence of PP and the time to relaxation did not differ significantly from those observed at the same temperature (18°C) in Experiment 3. However, recovery following prolonged exposure to PP was significantly protracted (*P. imbricata*, $F = 10.372$, df 1/18, $P < 0.05$; *P. albina*, $F = 4.755$, df 1/18, $P < 0.05$). There were no significant correlations between shell height and response times for either species (Table 1) and no mortality occurred in the week following prolonged exposure. Indeed, throughout these experiments, all oysters were retained for a minimum of 24 h after exposure to PP, during which no mortality occurred.

Experiment 3: The effect of temperature on relaxation and recovery

The responses of both *P. imbricata* and *P. albina* to immersion in PP baths were similar with the exception of the time taken for oysters to open when initially placed in the bath. *P. imbricata* generally opened valves within 10 min irrespective of the temperature of the bath (Fig. 1). *P. albina*, however, showed a significant reduction in the time taken to open their valves as water temperatures increased (Fig. 2).

Both *P. imbricata* and *P. albina* commonly relaxed within 15 min of valve opening and both species showed significant reductions in the time taken to relax as water temperatures increased (Figs. 1 & 2). Recovery for both species was rapid with a tendency for reduced recovery times with increasing water temperature, although this trend was significant only in the case of *P. albina* (Figs. 1 & 2).

Response times were again independent of shell height with the exception of time to relaxation of *P. imbricata* at 18°C, where a significant positive correlation was found (Table 1).

Table 1. Correlation coefficients for oyster shell height and response times during and after exposure to propylene phenoxetol baths (2mL L^{-1}) at one of four temperatures.

	Experiment 1	**Experiment 2**		**Experiment 3**				**Experiment 3**			
Species	*P. albina*	*P. albina*	*P. imbricata*	*P. albina*				*P. imbricata*			
Size range	39-115 mm	49-83 mm	54-88 mm	46-115 mm				49-95 mm			
Temperature	18°C	18°C	18°C	14°C	18°C	22°C	26°C	14°C	18°C	22°C	26°C
Time to open	-0.33	-0.34	-0.36	0.00	-0.34	-0.36	-0.49	-0.41	-0.35	-0.07	0.45
Time to relax	0.00	0.31	0.52	-0.28	0.64	-0.14	0.64	0.15	0.85*	0.02	0.66
Time to recover	-0.38	-0.14	-0.43	0.05	0.34	0.35	-0.24	0.36	0.04	-0.22	0.25

Values are Pearson product-moment correlation coefficients.

** Significant at P<0.01.*

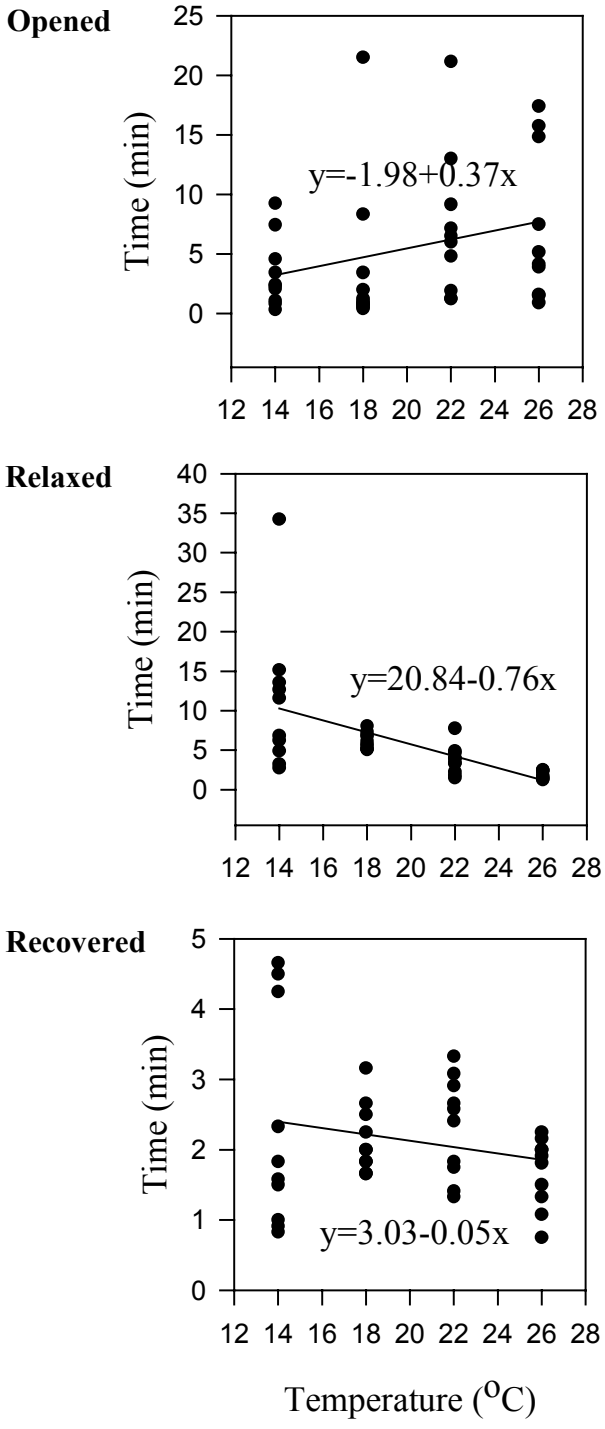

Figure 1. The time taken for valve opening, relaxation and subsequent recovery of *Pinctada imbricata* exposed to a 2 mL L^{-1} propylene phenoxytol solution.

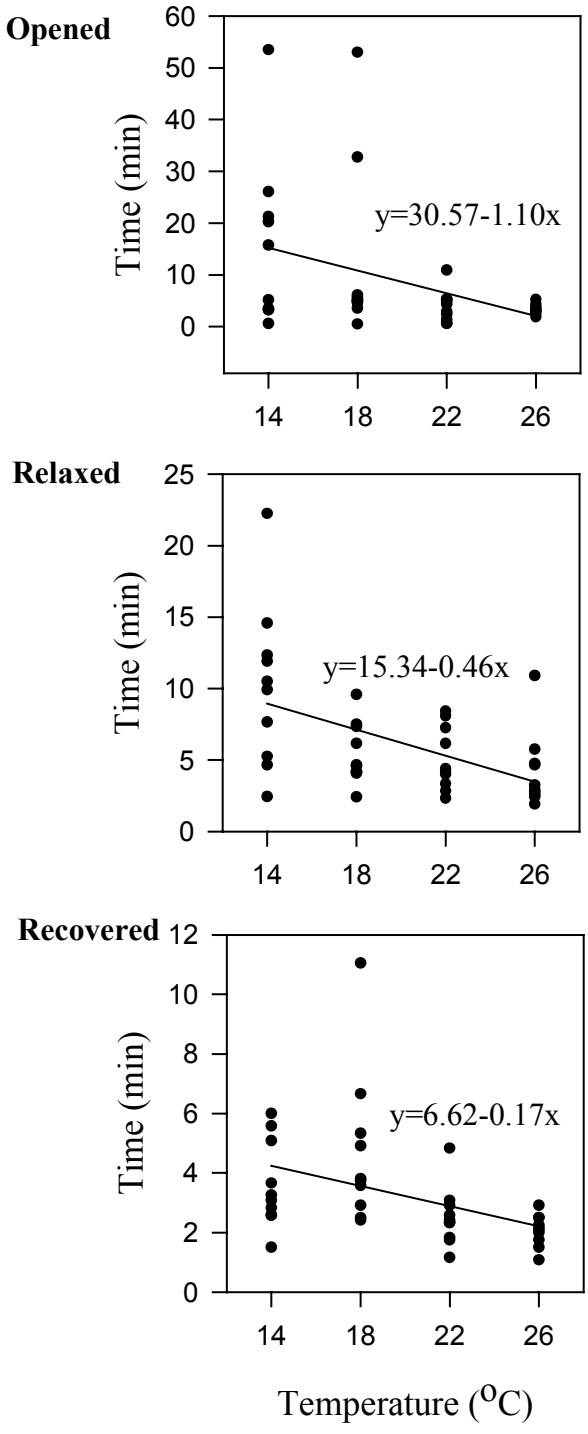

Figure 2. The time taken for valve opening, relaxation and subsequent recovery of *Pinctada albina* exposed to a 2 mL L^{-1} propylene phenoxetol solution.

2.5.5. Discussion

While the response of other molluscs to PP varies, there would appear to be a degree of uniformity in response among members of the genus *Pinctada*. In all species evaluated thus far, relaxation and recovery from the influence of PP are relatively rapid and without obvious negative consequences (Table 2). For all species tested, response times are unaffected by oyster size, but can vary according to water temperature (Norton et al. 1996, Mills et al. 1997, this study). In all cases, reductions in temperature can extend the time taken to relax and in most cases protract the time taken to recover.

Stress in pearl oysters has provided both the impetus for relaxant studies and has been identified as a factor influencing the efficacy of PP induced relaxation (Mills et al. 1997). For this reason, we have tried to avoid the use of wedges as they are considered to be both stressful and a source of damage to the shell and mantle. Although no significant differences were found between the time taken to relax in *P. albina* with wedged valves and those introduced directly to the bath, relaxation times in the latter treatment were, on average, faster (8.8 min versus 5.5 min, respectively). This is thought to reflect the PP solution entering the shell cavity, mostly through the byssal notch and initiating relaxation, prior to the shell first opening. Given that both oyster species will open in the presence of PP, we have found that the time, difficulty and potential stress and damage to experimental oysters associated with wedging is unwarranted.

In previous studies, response times for other pearl oysters have been independent of shell height and this was the case for *P. albina*. However, a significant correlation was found between time to relaxation at 18^{o}C and the shell height of *P. imbricata.* Rather than being indicative of any underlying trend, this result is thought to have arisen as product of the number of comparisons made (33) and to have occurred by chance alone (Type I error). Relaxation times for *P. imbricata* at other temperatures were not significantly correlated with shell height, nor were relaxation times for oysters held at 18^{o}C in Experiment 2. Notably, the oysters used in Experiment 2 were also representative of a larger size range and were therefore considered more likely to exhibit size-related effects if such an effect did exist.

Overall this study has reflected the findings of previous researchers, particularly those of Norton et al. (1996). Approximately 2.0 mL L^{-1} PP is an effective relaxant for use with pearl oysters which induces rapid relaxation with short recovery periods and without subsequent mortality. However, while this study and that of Mills et al. (1997) found PP to be useful in the laboratory, particularly with reproductive studies, care should be taken with certain applications. One of the proposed uses of relaxants has been to reduce stress during nuclei insertion for pearl production (Norton et al., 1996; Mills et al., 1997). Recent observations with *P. margaritifera* have suggested that the use of PP in this regard is not without adverse effects on oyster survival, and pearl weights (Norton et al. 2000). While we have not reached stage in our research where we can comment on the ultimate effects of the use PP in operations, we do note that seeding technicians have commented that PP makes pearl insertion more difficult. In particular, technicians noted that the mantle of the oyster occasionally collapsed or retracted obstructing the body of the oyster and, second, that speculums would occasionally fall out of the relaxed oysters. These physical problems are not insurmountable, but warrant acknowledgment.

Table 2. The response of pearl oysters of the genus *Pinctada* to the relaxant propylene phenoxetol.

Pinctada species	Size	Water temperature	Concentration (mL L^{-1})	Time to Time relaxation		Mortality recovery	Author
P. margaritifera	--	--	2.5	10-20 min	10-15 min	0	Hildemann et al. (1974)
	90-170 mm	21-31°C	2.0-3.0	1-48 min	4-57 min	0	Norton et al. (1996)
	100-150 mm	--	2.0	--	--	18-22%*	Norton et al. (2000)
P. albina	70-100 mm	21-31°C	2.0-3.0	1-40 min	6-39 min	0	Norton et al. (1996)
P. maxima	120-2000 g	24-32°C	1.5-2.5	6-15 min	--	"negligible"	Mills et al. (1997)
	"large"	--	2.5	<15 min	--	--	Norton et al. (1996)
P. imbricata	49-95 mm (20-100 g)	14-26°C	2.0	2-15 min	1 - 5 min	0	This study
P. albina	39 -115 mm (20-120 g)	14 - 26°C	2.0	2-15 min	1 - 8 min	0	This study

-- *Data unavailable*

* *Mortality when anaesthetised in conjunction with nuclei insertion.*

2.6. Halotolerance of the oyster predator, *Imogine mcgrathi*, a stylochid flatworm from Port Stephens, New South Wales, Australia

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2.6.1. Abstract

The stylochid flatworm, *Imogine mcgrathi* was confirmed as a predator of the pteriid oyster *Pinctada imbricata*. Occurring at an average of 3.2 per oyster spat collector bag, the flatworms were found to consume oysters at a rate of 0.035 to 0.057 day^{-1} in laboratory trials. Predation was affected by flatworm size with larger worms capable of consuming larger oysters and of consuming greater dry weights of oyster flesh. Irrespective of flatworm size, predation was generally confined to oysters less than 40 mm in shell height. Although all predation occurred at night, shading flatworms during the day did not significantly increase the rate of predation, but there were significant increases in the dry weight of oyster meat consumed.

As a means of controlling flatworm infestations, salt, brine baths (250 g kg^{-1}) and freshwater baths were effective in killing *I. mcgrathi*. The ease of use of hyper- or hyposaline baths then encouraged assessments of *I. mcgrathi* halotolerance. The flatworms were exposed to solutions ranging in salinity from 0 to 250 g kg^{-1} for periods of from 5 min to 3 h. Despite showing both behavioural and physiological signs of stress, *I. mcgrathi* survived the maximum exposure time of 3 h at salinities in the range 7.5 to 60 g kg^{-1}, inclusive. Beyond this range, the duration of exposure tolerated by flatworms decreased until 0 and 250 g kg^{-1}, at which the flatworms no longer survived the minimum tested exposure of 5 min. Thus, despite the significant impact of other stylochids on commercial bivalves, at their current prevalence, *I. mcgrathi* can be controlled by exposing them to hyper- and hyposaline baths for the culture of *P. imbricata* in Port Stephens, NSW, Australia.

2.6.2. Introduction

The oyster, *Pinctada imbricata* Röding, is among the most cosmopolitan of its genus, being found on areas of the eastern coastline of North and South America, the east coast of Africa, the Mediterranean, the Red Sea, as well as throughout the Indo-Pacific (Shirai, 1994). In Australia, *P. imbricata* occurs from Shark Bay in the west, around the northern coastline and down the east coast as far south as northern Victoria (Hynd, 1955).

Endemic also to the Australian east coast are a variety of acotylean polyclad flatworms of the family Stylochidae (Platyhelminthes, Polycladida) which have long been implicated as significant predators of commercial bivalves. Known colloquially as "oyster leeches" or "wafers" (Stead, 1907; Dakin, 1952; Newman et al., 1993; Jennings & Newman, 1996), these flatworms have been found to kill and consume mussels (Galleni et al., 1980), scallops (Heasman et al., 1998), giant clams (Newman et al., 1993), edible oysters (Littlewood & Marsbe, 1990; Chintala & Kennedy, 1993; Jennings & Newman, 1996) and pearl oysters (Newman et al., 1993). As a result, concerns arose over the occurrence of a native stylochid flatworm, *Imogine mcgrathi* Jennings & Newman, in association with *P. imbricata* culture in Port Stephens, New South Wales.

Initially, this study was undertaken to assess the potential for predation of *P. imbricata* by *I. mcgrathi* and then to estimate predation rates. Preliminary laboratory observations indicated that *I.*

mcgrathi remained inactive during the day and that predation only occurred at night. This led to the suggestion that the increased exposure to light in the experimental systems may restrict predation. Hence an additional trial was done using shading to reduce light to a level similar to that occurring during the day at the farm site in Port Stephens. Having confirmed predation occurred and that shading did not significantly increase predation, the efficacy of three treatments used to assist in the removal of fouling organisms from *P. imbricata* were evaluated with respect to their impact on *I. mcgrathi*.

In practice, fouling organisms on *P. Imbricata* are treated with course salt or with either hyper- or hyposaline baths. The latter involving immersing both the oysters and their cages in either freshwater (0 g kg^{-1}) or a brine solution (250 g kg^{-1}) for 3 h or 15 min, respectively. As the baths are used repeatedly, seawater and salt associated with the oysters progressively alters the salinity of the baths. Thus further trials were undertaken to determine the point at which dilution of the baths permitted *I. mcgrathi* to survive the process. In doing so, it was also acknowledged that a greater understanding of the autecology of *I. mcgrathi* would be of value in the management of the impact of the species.

2.6.3. Materials and Methods

Between 4 February and 19 March, 1999, 104 spat bags were taken from the hatchery and deployed in the field, each containing approximately 5000, 5 mm *P. imbricata* spat. The bags were suspended 1 m above the bottom in approximately 5 m of water off Wanda Head, Port Stephens, NSW (32°45'S, 152°10' E). Between 29 July and 5 August 1999, 24 of the bags were chosen at random and returned to the laboratory. Each bag was sorted and the number of *I. mcgrathi* present in each bag counted.

Several specimens were collected to confirm species identification and were fixed by the frozen polyclad fixation method outlined in Newman & Cannon (1995). Species identifications were made by examination of gross morphological features of preserved specimens and by comparison of serial sections of the reproductive system with those of the holotype at the Queensland Museum (see Jennings & Newman, 1996).

The flatworms used in these trials were gathered from oyster culture apparatus deployed at Wanda Head. Initially flatworms were taken from collector bags returned to the hatchery between 29 October and 12 November, 1999, and subsequently from cages and collectors of oysters returned between 18 November 1999 and 16 March 2000.

To estimate the size of *I. mcgrathi,* the animals were assumed to be elliptical in shape and dorsal surface area was calculated from the length and width measurements.

During predation it appears that a single flatworm consumes the entire soft body of the oyster. Thus to gain an estimate of the dry weight of oyster meat consumed a regression equation was derived from measurements made on a sample of oysters taken from the collector bags. The shell height of 56 oysters in the size range 10 to 50 mm was measured to the nearest 1 mm before the meat was removed and dried at 100°C for 24 h. The relationship between flesh dry weight (DW; g) and shell height (H; mm) was described by the following equation:

$$DW = e^{(-5.4029 + H \times 0.1047)}, (r^2 = 0.89).$$

Effects of I. mcgrathi size, oyster size and shading upon predation

Eight mesh screens were stocked with three 10, 20, 30, and 40 mm *P. imbricata* per screen and placed in a downwelling system (Fig. 1). Two large *I. mcgrathi* (625 ± 44 mm^2, mean ± SE, n = 8) were then placed on each of four screens, the remaining four screens were used as controls. Each screen was checked daily for spat mortalities and when dead spat were found, the shell was removed and measured and then replaced with a similar sized live oyster. Seawater (33 g kg^{-1} salinity) in the downwelling systems remained at ambient temperatures and within the range 18-20°C. Water in the system was changed thrice weekly and the experiment ran for four weeks.

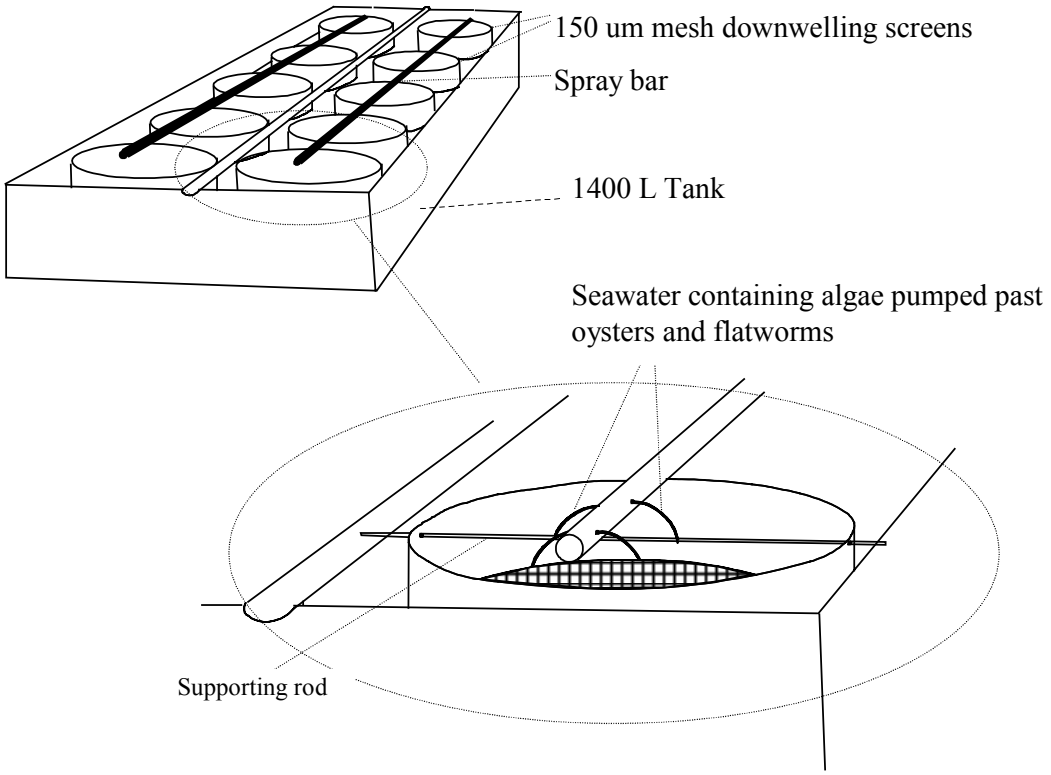

Figure 1. Downwelling systems used to house flatworms and oysters.

Using the equipment and protocols described for the first experiment, two further experiments were done in which only the size of the flatworms and the shading of screens varied, respectively. First, instead of the two large flatworms in each replicate, two smaller *I. mcgrathi* (311 ± 32 mm^2, mean ± SE, n = 8) were used to investigate the impact of flatworm size upon predation. Second, all mesh screens were covered to provide shading during the daylight hours. Light levels in the field were measured inside spat bags using a Gossen Mastersix light meter (Gossen, Nurnburg, Germany) in a waterproof housing and found to range between 2 and 48 lux according to depth and position. On the basis of these observations, black polythene sheeting was chosen to reduce light levels to <10 lux. For this experiment, two large flatworms (681 ± 591 mm^2, mean ± SE, n = 8) were placed on each screen.

Effects of P. imbricata cleaning method on survival of I. mcgrathi.

The removal of fouling from the shells of *P. imbricata* involves either one or both of two processes depending on the nature of the fouling. "Hard" fouling, such as serpulid worms, barnacles and other oysters, are removed mechanically. "Soft" bodied fouling, such as algae and ascidians, are treated with either salt, brine or freshwater. In practice, the most common approach has been to pressure clean the cages containing the oysters and then use one of the treatments for soft-bodied fouling organisms.

Salt cleaning involves spreading oysters out in a layer on a table and coating them with salt. The quantity of salt applied was approximately 0.1 the volume of oysters to be treated. The oysters and salt were mixed thoroughly by hand and then allowed to sit for 15 min before the salt was rinsed from the shells. Brine cleaning required the oysters to be immersed in a 250 g kg^{-1} brine solution for 15 min before they were removed and rinsed with seawater. Both salt and brine treatments used ground refined salt (Cheetham Salt, South Australia). Freshwater treatments involved immersing oysters in rainwater for 3 h.

Experimentally, each procedure (salt, brine and freshwater) was done with four replicate groups of 100 oysters that had recently been removed from mesh collectors. On each occasion two *I. mcgrathi* were included with each group of oysters. For both freshwater and brine treatments, oysters replicates were immersed in individual 1-L beakers of the respective fluids. At the culmination of the procedures, the flatworms from each replicate for each treatment were removed and placed in individual 1-L beakers of seawater for 24 h.

Halotolerance in I. mcgrathi.

All *I. mcgrathi* used in these trials ranged in from 393 to 1201 mm^2. Prior to experimentation, the worms were held on mesh screens (Fig. 1). Seawater was held at 20 ± 1°C and salinity remained within the range 32 ± 0.5 g kg^{-1}.

To test salinity tolerance, the flatworms were removed from the screens and placed in individual 500 ml food grade plastic containers, holding 250 ml of the test solution. The tested salinities included 0.0, 2.5, 5.0, 7.5, 30, 60, 90, 120 and 250 g kg^{-1} and the flatworms were exposed for periods of 5, 15, 30, 60, 120 or 180 min. Three worms, in individual containers were exposed to each of the tested time/salinity combinations. At the completion of each exposure the water was removed from the container and replaced with seawater (32 g kg^{-1}). Flatworm survival was then monitored in situ for 24 h.

Solutions were made by either diluting seawater with rainwater or by the addition of ground refined salt until the desired salinity was reached. Salinity was measured using an active conductance salinometer (Yeokal Instruments, Sydney, NSW). Surface response curves were generated with the aid of Sigmaplot, Scientific Graphing Software (Jandel Corporation, San Rafael, CA).

2.6.4. Results

The mean number of *I. mcgrathi* present in collector bags after 3 months deployment at Wanda Head was 3.2 ± 1.7 bag^{-1} (mean ± SD, n = 24). The average length, width, and size (approximate dorsal surface area) were 39.7 ± 4.4 mm, 19.7 ± 2.5 mm and 628 ± 140 mm^2 (mean ± SD, n = 30), respectively. A number of other polyclads were also observed at this time including unidentified cotyleans *Thysanozoon sp.* and *Cycloporus sp*. and planocerid acotyleans.

Irrespective of trial, no mortalities occurred among oysters used in control treatments.

Effects of flatworm size, oyster size and shading upon predation

Predation of juvenile *P. imbricata* occurred at a rate of 0.035, 0.039 and 0.057 oysters day^{-1}, for large, large shaded and small sized *I. mcgrathi*, respectively. Differences in size of oysters consumed were observed on the basis of flatworm size (Fig. 2). On average, large flatworms consumed larger oysters (χ^2 = 35.00, $P < 0.0001$), particularly in the shaded trials (χ^2 = 65.1, $P < 0.0001$), although care should be taken in the interpretation of these results as in some cases the observed frequencies are small (< 5). This variation in prey selection is reflected in differences in the dry weight consumption of oyster flesh among the three groups tested, with large, large shaded and small *I. mcgrathi* consuming an average of 2.2, 4.9 and 1.4 mg day^{-1}, respectively.

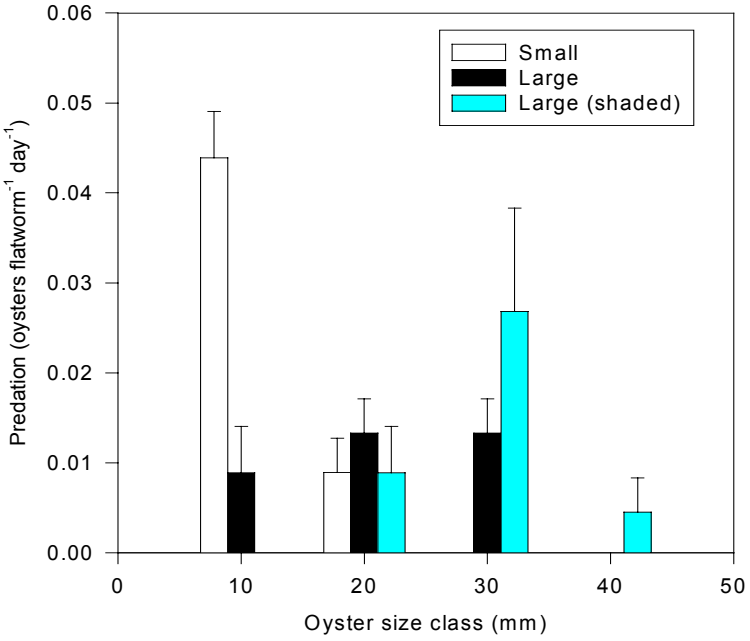

Figure 2. Predation of various size classes of *Pinctada imbricata* by two size classes of the flatworm *Imogine mcgrathi*, small (311 ± 32 mm^2) and large (625 ± 44 mm^2).

Despite several large *I. mcgrathi* used in this experimentation being among the largest we have collected, on only one occasion was an oyster greater than 40 mm shell height consumed. In this case, the mortality occurred in the presence of the largest *I. mcgrathi* we have collected from Port Stephens (67 x 52 mm, surface area 2736 mm^2).

Observations of predation were limited as predation was shown to occur at night. Typically, *I. mcgrathi* were observed to spend the day either beneath the oysters on the screen or wrapped around the hinge of an oyster. Oyster mortalities were evident in the morning. The shells of the oyster, still connected at the hinge, were found with all the tissue removed. Frequently, eggs were found on the internal shell surfaces of recently consumed oysters. Eggs were 124.9 $\pm$ 5.7 μm (mean $\pm$ SD, n = 30) in diameter in a single layer covering an area of up to approximately 2 cm^2.

On one occasion, a single *I. mcgrathi* was found inside the shell of an oyster that was alive the previous evening. The body of the flatworm was markedly distended and upon removal and examination, the contents of the stomach were regurgitated and found to contain the entire soft body tissue of the oyster.

Effects of cleaning P. imbricata on survival of I. mcgrathi.

All three cleaning methods tested (salt, brine and freshwater) were effective in killing *I. mcgrathi* (100% mortality). In the presence of salt and brine solutions, *I. mcgrathi* immediately exhibited signs of physical discomfort by writhing and, on occasions, by everting its pharynx. The salt cleaning procedure appeared particularly stressful with all flatworms showing signs of physical damage. This was presumed to have occurred as a result of abrasion during the salt mixing process. Following exposure to both salt and brine solutions, some flatworms were noted to have released eggs. *I. mcgrathi* immersed in freshwater quickly became immotile and after 2 h were moribund and had swollen and lightened in colour.

Halotolerance in I. mcgrathi

Upon introduction to both the hyper- and hyposaline solutions tested, the flatworms initially writhed vigorously, but made no attempt to adhere to the walls of the container nor indeed to assume normal orientation if they had fallen on their dorsal surface. The worms all very quickly excreted a layer of mucus that gave the appearance of a marked lightening in colour. The amount of mucus and the corresponding reduction in colour were greater at lower salinities. Shortly after excreting the mucus the worms became motionless and largely remained so until returned to seawater. Upon return to seawater, the worms generally became more motile and shed the layer of mucus as though it were a second skin. This discarded mucus did not dissolve rapidly and was often clearly visible after 24 h. At the salinity extremes tested (<5 and >90 g kg^{-1}) and longer exposure times (≥30 min), worms began to disintegrate prior to removal from the solutions.

The responses of the worms in terms of survival (Figs 3 & 4) were generally either complete survival or mortality at each time/salinity combination. Flatworms were unable to tolerate freshwater for periods of 15 min or more, but as salinity increased, so to did tolerance. At 5 g kg^{-1} salinity, *I. mcgrathi* survived exposures of up to two hours and some individuals survived an exposure of 3 h when salinity reached 7.5 g kg^{-1}.

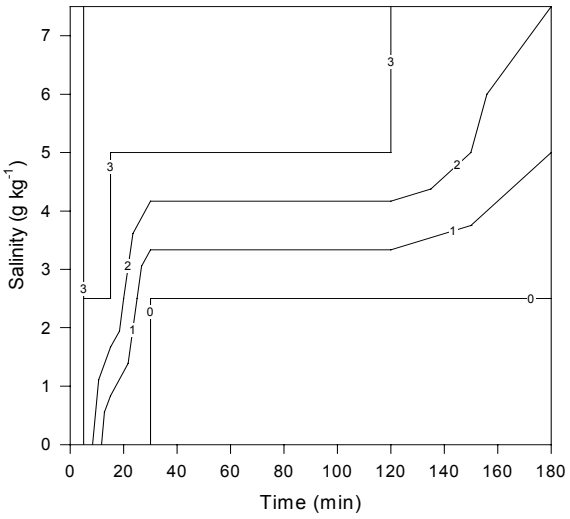

Figure 3. The effect of reduced salinity and exposure time upon survival of the flatworm Imogine mcgrathi. Isopleths indicate the number of flatworms surviving. Three flatworms were exposed at each exposure time and salinity combination tested.

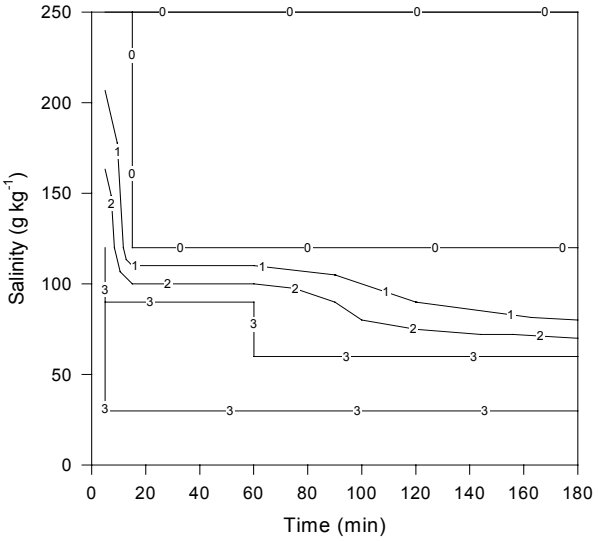

Figure 4. The effect of increased salinity and exposure time upon survival of the flatworm *Imogine mcgrathi*. Isopleths indicate the number of flatworms surviving. Three flatworms were exposed at each exposure time and salinity combination tested.

2.6.5. Discussion

Although *I. mcgrathi* has only recently been described (Jennings & Newman, 1996), its association with commercial bivalves has been noted for some time. Oyster farmers in NSW are familiar with flatworms, which are known colloquially as oyster leeches (Stead, 1907; Dakin, 1952) and Jennings & Newman, (1996) found that *I. mcgrathi* fed on oysters in the laboratory. Prior to confirmation of its identification, flatworms thought to belong to this particular species were associated with increases in mortality among juvenile scallops, *Pecten fumatus* (Heasman et al., 1998) and more recently significant mortalities among juvenile mussels, *Mytilus galloprovincialis* (O'Connor & Newman, unpublished data). Despite this, the feeding strategy of *I. mcgrathi* is not fully understood as worms have not been observed entering oysters.

This study showed that predation occurred at night, with the flatworms spending the daylight hours avoiding light, commonly laying under or around the byssally attached *P. imbricata*. Shading during the daylight hours did not increase predation, which remained in the range 0.035 to 0.057 oysters day^{-1}. This feeding rate was much lower than observed for *Stylochus mediterraneus* predation of mussels (0.07 to 0. 33 day^{-1}) (Galleni et al., 1980) and more akin to predation of edible oysters by *S. ellipticus* (0.014 to 0.1 day^{-1}) collected by Landers & Rhodes (1970) from Milford Sound, USA.

Size dependent differences in predation were noted between the two size classes of *I. mcgrathi* used (Fig. 2). Presumably as a result of physical limitations, smaller *I. mcgrathi* consumed smaller oysters while larger flatworms were capable of consuming larger prey. This may in turn have lead to the slightly elevated frequency of predation observed with smaller flatworms as they attempted to compensate for the significantly lower flesh weights of smaller oysters. Galleni et al. (1980) found an inverse relationship between the frequency of attack by *S. mediterraneus* and mussel size, however, *I. mcgrathi* showed little difference in attack frequency. Both size classes of flatworms consumed oysters at a similar rate with larger *I. mcgrathi* showing a slight preference for larger oysters. This preference however did not generally extend to oysters of 40 mm shell height or greater, despite the fact that some of the flatworms used during experimentation were among the largest we have observed in Port Stephens and are consistent with the upper size limit suggested for this species by Jennings & Newman (1996). In these experiments and in general observations from the field, oysters greater than 40 mm shell height have been largely unaffected by the presence of flatworms.

I. mcgrathi were occasionally found within the shells of recently consumed oysters but there were no physical signs of damage to the shell and the hinge remained intact. It has been suggested that the polyclad mucus may be toxic (Hyman, 1951; Lin et al., 1998). Although *I. mcgrathi* spent by far the bulk of the time in these lengthy trials in close proximity to the oysters, in many cases resting on the oysters, there did not appear to be any indiscriminate mortality. Furthermore, the size dependent predation observed suggests prey selection rather than a random process of waiting for an oyster to succumb to a toxin and then attacking it.

Several treatments have been suggested to control flatworms including emersion (Littlewood & Marsbe, 1990), dipping in hypersaline solutions (Espinosa, 1981) and calcium hypochlorite solutions (Yang, 1974). As *P. imbricata* is a subtidal organism we have avoided extended periods of emersion. However, each of the three commonly used procedures for removing soft-bodied fouling organisms from *P. imbricata* were effective in killing *I. mcgrathi* and in the case of the freshwater and 250 g kg^{-1} salinity baths, have considerable margins of safety. Both bathing techniques remain effective following some degree of dilution of the bath and allow for reductions in the immersion times if required.

For freshwater baths, in which oysters and equipment are immersed for 3 h, salinity may be permitted to rise to 5 g kg^{-1} while retaining their ability to kill *I. mcgrathi*. Alternatively, immersion times can be reduced to as little as 30 min, provided salinity remains below 2.5 g kg^{-1}. Depending upon the number of oysters to be treated, there may be major advantages in ensuring salinities remain below 2.5 g kg^{-1} so that immersion times can be kept to a minimum and larger numbers of stock treated.

In general the responses of *I. mcgrathi* to salinity reductions are in many respects quite similar to those reported for other stylochids. *Stylochus frontalis* can tolerate salinities as low as 6 g kg^{-1} (Pearse & Wharton, 1938), while Stylochus *ellipticus* can survive abrupt salinity reductions from 27 g kg^{-1} to 7.5 g kg^{-1} (Landers & Toner, 1962). With *S. ellipticus* a 20% mortality occurred at 5 g kg^{-1} and 100% mortality at 2.5 g kg^{-1} (Landers & Toner, 1962). However, on a cautionary note, if *S. ellipticus* is acclimatised slowly it can survive exposure to 2.5 g kg^{-1} (Landers & Toner, 1962), and thus circumstances in which acclimation may occur should be avoided.

For brine baths, salinity can be permitted to fall by over 50% and retain lethality or alternatively exposure times at 250 g kg^{-1} may be reduced to five minutes. Of the two alternatives, brine baths offer the most rapid means of treatment if time is of paramount importance. Although it is not a great financial burden, purchasing salt is an additional cost associated with the use of brine baths. If this cost is significant the quantity of salt used may be reduced or freshwater baths considered.

When choosing a suitable protocol, some considerations should be given to both the species cultured and to other fouling organisms. In these and other trials, the oysters, *Crassostrea glomerata* and *Pinctada imbricata,* are not adversely affected by either the freshwater or brine (250 g kg^{-1}) treatments used, however this may not be so for other bivalves. In addition, the effects of these treatments were initially tested because they were already in use as a means to reduce fouling and there was a desire to ensure they were effective in killing flatworms. Thus care should be taken to ensure manipulations of the protocols do not adversely affect their efficacy in fouling reduction.

Given its current, low prevalence, the frequency with which *P. imbricata* is handled and the relative effectiveness of routine methods for fouling removal in killing the flatworm, *I. mcgrathi* has not yet been as a significant impediment to pteriid oyster farming in Port Stephens. However it is important not to underestimate the potential for impact upon *P. imbricata*, or the impact upon other bivalves in Port Stephens. Anecdotal reports by oyster farmers of sporadic events in which "thousands" of flat worms settle upon Sydney rock oysters (G. Diemar, pers. comm.) raise the possibility of dramatic increases in prevalence and possibly impact of *I. mcgrathi*. Further, these sporadic increases in *I. mcgrathi* prevalence have resulted in significant economic loss to both mussel and Sydney rock oyster farmers in NSW.

2.7. Predation of cultured mussels, *Mytilus galloprovincialis*, by stylochid flatworms, *Imogine mcgrathi,* from Twofold Bay, New South Wales, Australia

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2.7.1. Abstract

The Stylochid flatworm, *Imogine mcgrathi*, was found to be a predator of the mussel *Mytilus galloprovincialis*. These flatworms consumed mussels at a rate of 0.032 day^{-1} or 12.6 mg day^{-1} in laboratory trials and occurred at numbers as great as 386 m^{-1} of mussel culture rope in Twofold Bay. Despite having previously been confirmed to be a predator of the oyster, *Pinctada imbricata*, when held concurrently with mussels and oysters of a similar size, *I. mcgrathi* collected from mussel ropes restricted their predation solely to mussels. When offered only *P. imbricata* as a food source, these same *I. mcgrathi* appeared incapable of eating the oysters. *I. mcgrathi* have the potential to pose a significant threat to mussel culture and their abundance in culture warrants carefully scrutiny.

2.7.2. Introduction

Often called the Mediterranean mussel, *Mytilus galloprovincialis* is far less parochial than the name might suggest. Found in Europe, Asia, southern Africa and Australia (McDonald et al., 1991), *M. galloprovincialis* forms the basis of several significant mussel culture industries. In Australia, mussel culture is in its infancy, particularly in New South Wales (NSW) where currently approximately 31 tonnes of mussels are cultured annually (ABARE, 2000). However, proposals are in place that could see a significant increase in the production of *M. galloprovincialis* in the near future.

In Europe, one impediment to the culture of *M. galloprovincialis* is predation by *Stylochus mediterraneus*, an acotylean polyclad flatworm of the family Stylochidae (Platyhelminthes, Polycladida) (Galleni et al., 1980). Fortunately this flatworm has not been reported in NSW waters although the Australian east coast is host to a number of other stylochids (Jennings and Newman, 1996a, b), some of which have already been implicated as predators of commercial bivalve species. Despite a general paucity of information regarding Australian polyclad flatworm fauna, one species in particular, *Imogine mcgrathi* Jennings and Newman, has been associated with mortalities among edible oysters (Jennings and Newman, 1996a), scallops (Heasman et al. 1998) and pearl oysters (O'Connor and Newman, in press).

Small numbers of the pearl oyster, *Pinctada imbricata*, were taken to mussel farms in Twofold Bay, NSW (Fig. 1), to assess both the potential for oyster culture in southern NSW and the possibilities for polyculture with other commercial molluscs. At the time of deployment (January 2000) large numbers of small *I. mcgrathi* were observed in association with cultured mussels. This raised concerns for both the cultured mussels and the oysters, as *I. mcgrathi* had previously been found to eat *P. imbricata* cultured in Port Stephens, NSW (O'Connor and Newman, in press).

This study was done to determine the possibility of *I. mcgrathi* eating cultured mussels and to estimate potential predation rates. Having confirmed predation, an assessment was made of the likelihood of *I. mcgrathi* posing an immediate threat to other molluscs being cultured concurrently

at the mussel farm sites.

2.7.3. Materials and Methods

On the 22 March 2000, four 20 cm samples of culture rope were taken at random from longer sections collected by divers from a mussel farm in Twofold Bay, NSW (Fig. 1). The samples were transported back to the laboratory and the mussels were stripped from the culture rope. The numbers of live and dead mussels were determined and the shell height (umbo to distal margin) of the various groups were measured to the nearest mm using vernier calipers. Ten live mussels were chosen at random and the total weight and wet tissue weight were determined to the nearest 0.01 g. The mussel tissue was then dried at 100^{o}C for 24 h to determine tissue dry weight.

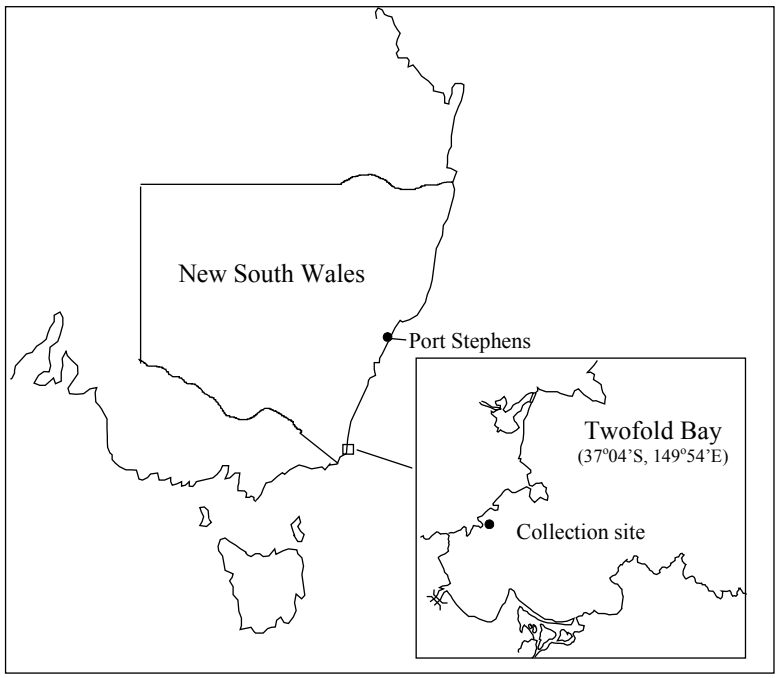


Figure 1. Map of southeastern Australia, showing flatworm collection and mussel cultivation site.

The number of *I. mcgrathi* found in and on the shells of the mussels on each rope sample was recorded and a randomly selected sample (n = 20) of flatworms was measured to determine average size. *I. mcgrathi* were assumed to be elliptical in shape and surface area was calculated as follows.

Surface area = π [(L/2)*(W/2)], where L and W are length and width, respectively.

Approximately 60 mature *I. mcgrathi* were kept alive in 400-L aerated aquaria for predation trials. Several additional specimens were collected to confirm species identification and were fixed by the frozen polyclad fixation method outlined by Newman and Cannon (1995). Species identifications were made by examination of gross morphological features of preserved specimens and by comparison of serial sections of the reproductive system with those of the holotype at the Queensland Museum (see Jennings and Newman, 1996a).

All mussels and flatworms used in these trials were taken from the samples collected from Twofold Bay in March 2000. The oysters used were collected from a group of hatchery reared juveniles being held in Port Stephens.

Flatworm predation

Eight, 160 μm mesh screens were held in a downwelling system (O'Connor and Newman, in press). Four of the screens were each stocked with 10 *I. mcgrathi*. Each of the eight screens was then stocked with 10 mussels and left for a period of one week. After one week, five mussels were removed from each screen and replaced with five oysters of a similar shell height (30 to 40 mm). Together, the flatworms, mussels and oysters were held for a further two weeks before the five remaining mussels were removed and replaced with oysters. The flatworms and oysters were then held concurrently for a further two weeks.

Throughout the trial, each screen was checked daily and the number of dead mussels or oysters was recorded. Predation was considered to have occurred only when the soft tissue had been removed from the shells. The shells of dead bivalves were removed and measured before being replaced with a similar sized animal of the same species. Seawater (33 g l^{-1} salinity) in the downwelling systems was held at 22°C and a mixture of three algal species was added daily to feed mussels and oysters. Water in the system was changed thrice weekly.

2.7.4. Results

On average, samples from Twofold Bay held the shells of 725 mussels m^{-1} of culture rope. The shell length of live mussels was 44.1 ± 0.64 mm (mean ± SE) and mean dry weight of mussel flesh was 0.391 ± 0.03 g (SE). At the time of collection 36% of the mussels were dead (260 ± 25.6 m^{-1} of culture rope, mean ± SE). These dead shells (38.3 ± 0.62 mm; mean ± SE) showed no signs of physical damage and in most cases the valves remained attached at the hinge. Size frequency distributions for live and dead mussels are shown in Figure 2. The mean number of *I. mcgrathi* present was 386 ± 23.1 m^{-1} of culture rope. The average length, width and size (approximate surface area) were 24.9 ± 1.3 mm, 13.5 ± 0.6 mm and 266.0 ± 20.3 mm^2 (mean ± SE), respectively.

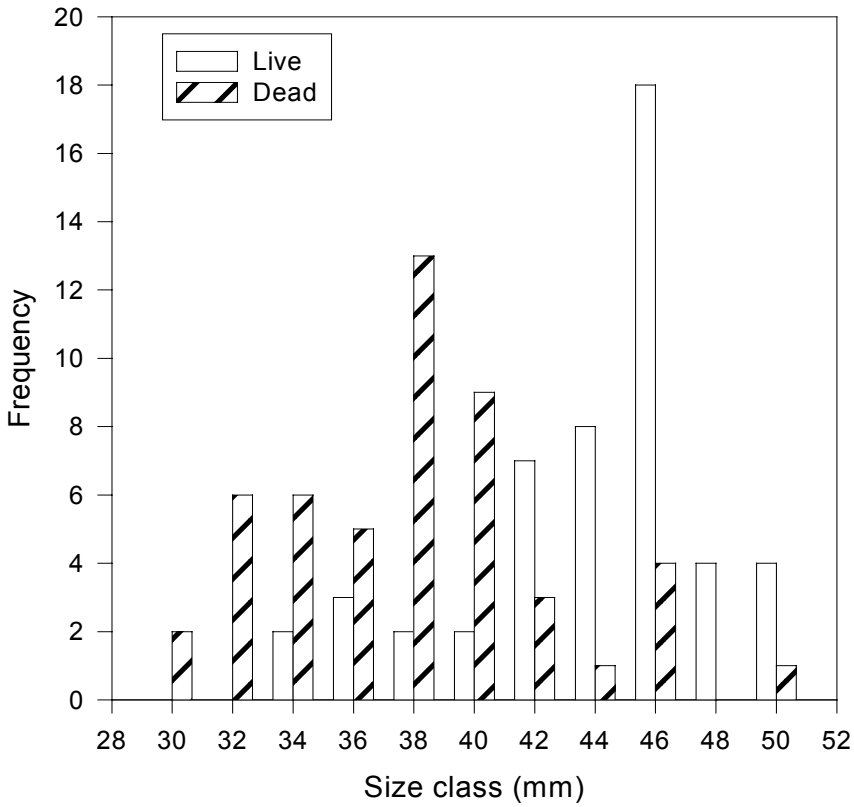


Figure 2. Size frequency distributions for live and dead mussels collected from Twofold Bay, NSW, Australia.

Flatworm predation

I. mcgrathi was shown to be a predator of *M. galloprovincialis*, with each flatworm consuming an average of 0.032 mussels day^{-1} or a dry weight of 12.6 mg mussel flesh day^{-1}. When held concurrently with oysters, the rate of mussel predation by flatworms was not significantly affected (X^2 = 1.43, P = 0.23) and no predation of oysters occurred. Upon removal of mussels from the upweller screens, all predation ceased (Fig. 3). Throughout this trial no mortality occurred among oysters and mussels held in control screens, however, four dead mussels with flesh intact were taken from the screens containing flatworms during the trial.

Mussel predation occurred at night with the flatworms spending the day sheltering beneath oysters or mussels on the screen (pers. obs.). Despite the preference for mussels as prey, the flatworms showed a preference for sheltering beneath oysters rather than mussels when held with both species concurrently. Commonly two to four flatworms were found within the shells of eaten mussels, however inspection of the flatworms during the removal of the shells suggested a single worm had consumed the entire mussel. On two occasions during the removal and replacement of dead mussels, the flatworm assumed to be responsible for the attack regurgitated a relatively intact mussel when disturbed. It was also common to observe a single layer of eggs on the inner surfaces of mussel shells the morning following predation.

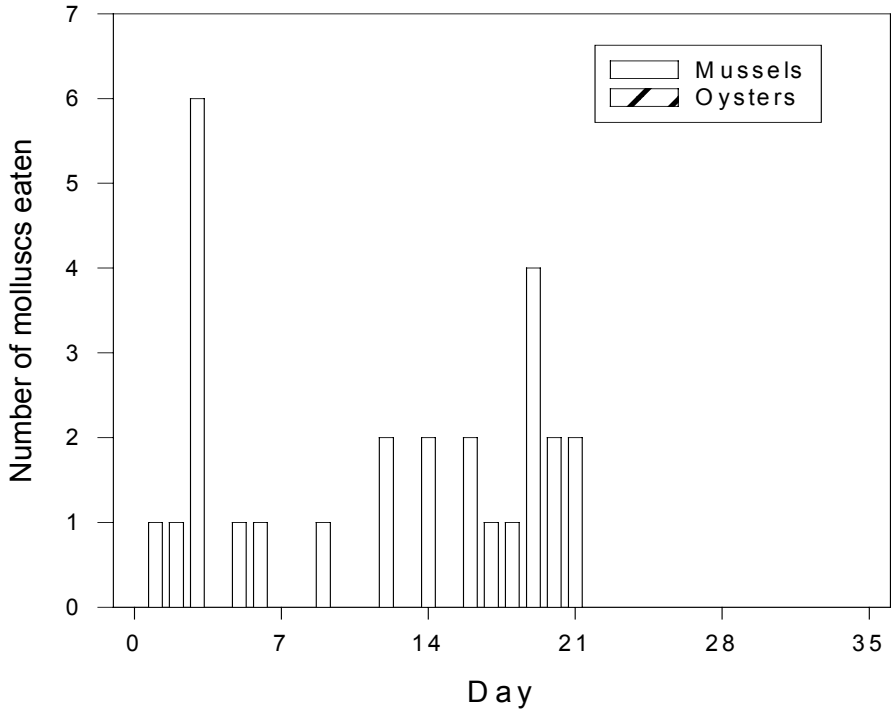


Figure 3. Predation by *Imogine mcgrathi* when held concurrently with mussels (days 0-21) and oysters (days 7-35).

2.7.5. Discussion

The occurrence of *M. galloprovincialis* on the east coast of Australia has not precluded it from stylochid flatworm predation, indeed, predation by *I. mcgrathi* was found to be similar to that reported for *S. mediterraneus* on the Italian coast (Galleni et al., 1980). In laboratory trials, *S. mediterraneus* demonstrated a preference for smaller mussels (< 25 mm) and consumed them at a rate of between 0.07 and 0.33 day^{-1} (Galleni et al., 1980). Here, *I. mcgrathi* were only offered larger mussels (approx. 44 mm) and predation rates were comparatively low, 0.032 day^{-1}. However, dry weights of mussel flesh consumed by *S. mediterraneus* (26-35 mm in length) and *I. mcgrathi* (mean length 24.9 mm) were similar, at 11.2 and 12.6 mg day^{-1}, respectively.

It is possible that *I. mcgrathi* may increase predation rates in the presence of smaller mussels as observed by Galleni et al. (1980) with *S. mediterraneus*. But in previous trials in which *I. mcgrathi* were offered oysters of various sizes, there was a tendency for larger flatworms to eat larger oysters rather than increasing predation on small oysters (O'Connor and Newman, in press). In these earlier trials, *I. mcgrathi* was found to prey upon oysters at a similar rate to *S. mediterraneus*, approximately 0.035 to 0.057 day^{-1}. However, *I. mcgrathi* consumed much larger dry weights of mussel flesh than that of oysters (12.6 and 4.9 mg day^{-1}, respectively). The reason for this is unclear, but may in part be due to water temperature. Landers and Rhodes (1970) found predation by *S. ellipticus* reduced by as much as 72% over a 5 - 6^{o}C temperature range. Water temperatures in this study were on average 3^{o}C greater than in the earlier study of oyster predation (22^{o}C and 18 - 20^{o}C, respectively). In common also with *S. ellipticus, I. mcgrathi* showed a first,

marked peak in predatory behaviour after three days (Fig. 3). Landers and Rhodes (1970) observed these "first peaks" in all of their trials within 2 to 4 days, but were unsure of the cause. The possibility that the absence of food during the pre-experimental period may have accentuated the initial burst of predatory behaviour was suggested (Landers and Rhodes, 1970). Yet in this trial, flatworms could only have been considered to be without food during transport to the laboratory. At this time, the flatworms were still on the mussel samples but may have been unable to feed while emersed.

The behaviour of *I. mcgrathi* in the presence of mussels was similar to that described previously for oysters although we had been unable to observe the method used by *I. mcgrathi* to consume the bivalve. With mussels, *I. mcgrathi* were seen on two occasions to slip between the valves of the mussel without evoking any obvious response from the mussel. When the mussel was opened some 30-45 minutes later, the posterior adductor muscle had been detached from the shell and the flatworm had everted its pharynx and begun to engulf the mussel flesh from the distal margin inwards.

As observed with *S. ellipticus* (Landers and Rhodes, 1970) and suggested for *S. mediterraneus* (Galleni et al., 1980), *I. mcgrathi* collected from mussels demonstrated what has been called "ingestive conditioning": a behaviour in which predation is limited to the species with which the predator had been associated in nature. *I. mcgrathi* collected from Port Stephens have been demonstrated to eat *P. imbricata* but their conspecifics from Twofold Bay failed to do so in these trials. This was despite the likelihood that at the culmination of the trial many of the worms had not fed in over a month.

I. mcgrathi is thought to have the potential to be a major pest to the cultured mussel industry. It eats mussels in the laboratory and the large numbers of dead intact shells on the culture ropes are consistent with flatworm predation, however, the size of the empty shells raises several questions. Assuming *I. mcgrathi* is responsible for the observed mortality, the mussel size frequency data (Fig. 2) suggests that predation is a relatively recent phenomenon. Given the absence of empty shells less than 30 mm long, a mussel growth rates of 1 - 1.5 mm a week and the smallest live mussels present being 34 mm long, it is likely that there has been some 3-4 weeks since mussels of 30 mm shell length were present and thus 3-4 weeks since predation commenced. Assuming that predation began progressively and that flatworms consumed mussels at approximately the same rate as observed in the laboratory ($\approx$ 1 month^{-1}), 260 empty shells for 386 flatworms is not inconceivable for one month's predation. However, the impetus for this study arose from observations of *I. mcgrathi* two months earlier and given their ability to eat 10 mm *P. imbricata*, they should be capable of consuming small *M. galloprovincialis*. This would suggest that the shells of predated mussels are dislodged from the ropes or decompose within weeks and that the overall losses from *I. mcgrathi* may be much greater than the 36% calculated from dead shell numbers.

The presence of empty shells in the largest mussel size class suggests that predation is ongoing and that mussels have not grown to a size at which they are beyond attack. Thus, if flatworm numbers remain constant and they eat mussels at $\approx$ 1 month^{-1}, they have the ability to consume the standing crop within six weeks. Subsequent to this study, the mussels present were harvested and no further observations were possible but, so dire are the potential consequences, that flatworms warrant close attention. Farmers reported the harvest was extremely poor, although large numbers of mussels were lost in heavy seas (M. Bamford pers. comm.), thought to be exacerbated by the increasing numbers of dead shells that were no longer byssally attached to the culture rope. Strategies for the control of *I. mcgrathi* have been discussed with mussel farmers, including freshwater or brine baths (O'Connor and Newman, in press), but the efficacy of these treatments with respect to the tolerances of *M. galloprovincialis* and the farming practices used need to be evaluated.

Whether the flatworms present on mussel culture ropes pose a threat to other molluscs remains to be seen. The occurrence of "ingestive conditioning" augers well for the survival of *P. imbricata* in Twofold Bay in the short term, however, it was assumed that given the size of the population, juvenile flatworms may recruit to the oyster cages. Ultimately this did not occur and the oysters were harvested several months later without significant losses. There are many potential ecological explanations for this such as unsuitable environmental conditions for flatworm recruitment; dispersion of flatworm larvae to other areas by prevailing currents; etc, but the threat that flatworms may recruit to other bivalve culture systems in coming seasons remains a concern.

3. TECHNIQUES FOR HATCHERY PRODUCTION AND GROWOUT OF THE AKOYA PEARL OYSTER, *PINCTADA IMBRICATA*

3.1. General Introduction

The akoya pearl oyster, *Pinctada imbricata*, has been produced in hatcheries in Asia for decades and while it is considered among the most robust of the genus for this purpose (Ito, 1998), there is comparatively little detailed information available on the techniques used to propagate the species. The following describes the techniques used and some of the information garnered over three successive production seasons at the Port Stephens Fisheries Centre.

In many regards, the techniques for production of *P. imbricata* have been modified from those developed for the production of various other bivalves, notably, the Sydney rock oyster (Saccostrea glomerata) and the commercial scallop (Pecten fumatus). As a result they have relied largely upon equipment designed and built for that purpose. Thus the techniques differ in many respects from those that might be encountered in either Japanese or Chinese hatcheries. Doubtless there will be a number of modifications over the coming years, never-the-less the techniques described have been successful in producing millions of *P. imbricata* spat annually over the past three years.

While this research has focused on aspects of the growout of *P. imbricata*, it has not been one of our primary objectives. As a result, only an abbreviated description of procedures has been provided to give an overview of culture requirements.

3.2. Hatchery and Nursery Culture

Seawater treatment

Of paramount importance in the production of bivalves are access to good quality seawater and the subsequent methods of seawater storage and treatment. All seawater used during the conditioning of broodstock and for culture of larvae and juveniles at the PSFC is collected from a coastal site and trucked to the centre. Upon arrival it is transferred to clean 45 000 L storage tanks where it remains for a minimum of seven days settlement. Outlets of these tanks are positioned such that only approximately 40 000 L can be pumped to the hatchery. The residual seawater in the bottom of the tank contains what ever has settled out and is either discarded or used in less critical applications.

Treatment of seawater following settlement varies according to its intended use. Broodstock systems use water directly from the storage tanks without filtration. Seawater used for embryo culture is filtered using 1 µm nominal cartridge filters, while larval rearing water is filtered using either 1 µm nominal filters or 5 µm filter bags, depending on the volume of seawater required.

While UV sterilisation and ultra-filtration equipment (0.2 µm absolute filters) are available in the hatchery we have previously found it ineffectual or detrimental to embryo and larval cultures.

Fortunately we have never found it necessary to contemplate the use of this equipment with *P. imbricata* larvae, spat or broodstock.

NB: Care should be taken if UV equipment is to be used in association with *P. imbricata* broodstock, this may induce spawning.

3.3. Broodstock and husbandry

P. imbricata is generally single sexed but has the ability to change sex. Bisexual individuals, with apparently functional eggs and sperm have been found (possibly an interim state in the alternation between sexes). Individual oysters can mature sexually within the first year and at relatively small sizes (< 35 mm shell height), but neither age nor shell height ensure maturity. There are tendencies for smaller animals to be male and as the oysters grow and age for the percentage of females to increase.

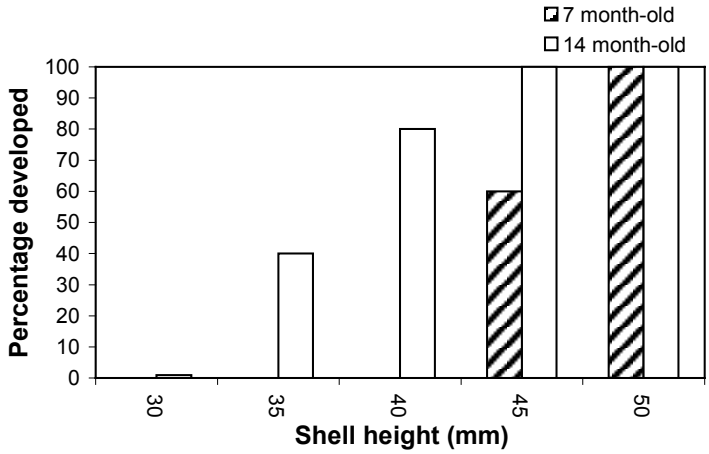


Figure 1. A comparison of the numbers of *Pinctada imbricata* with gametes present in various size classes of oysters of two ages collected simultaneously.

The gonad is readily observable in *P. imbricata* and is wrapped around the digestive gland. The reproductive condition of the oyster can be inferred from the appearance of the gonad, however, unlike some other pearl oyster species, colour is not necessarily a good guide to the sex of the animals (Tranter, 1958).

Broodstock selection

Selecting appropriate broodstock is of extreme importance in two regards. First, animals in suitable reproductive condition should be selected to save the time and expense associated with conditioning. Second, a great deal of emphasis is placed upon the physical traits (phenotype) of the broodstock to be used.

Ideally, ripe, ready-to-spawn broodstock are collected from the wild when required, however, this is not always possible. To an extent, hatchery operations can be extended by collecting broodstock from a number of areas in which the breeding seasons do not coincide, but in NSW this unlikely to encompass the entire year.

It is worth noting that genetic surveys have indicated the *P. imbricata* populations of NSW are relatively homogeneous and that the threats of the dilution of the genetic characteristics of

individual populations do not appear to be great. In other areas and also with other Pteriid species considerable care should be taken before translocations occur.

With regards to physical characteristics of the broodstock, it can be argued that in comparison to general bivalve culture (scallops, edible oysters, mussels), pearl oyster culture is particularly complex. Rather than simply selecting large, healthy, rapidly growing animals, two types of brood stock are chosen. These two types reflect two sets of phenotypic traits required for pearl production. The first and most numerous of the oysters required are those that are to be nucleated to produce pearls ("host" or "mother" oysters). Broodstock for these animals in our trials have been selected primarily upon shell shape and there are equations to quantify suitability. Primarily these equations express a measure of convexity of the shells. That is, shells with broader shell cavities can allow the insertion of larger nuclei, which have the potential to produce larger, and consequently, more valuable pearls.

The second group of oysters are those that provide the mantle tissue that will accompany the nuclei in the seeding process ("donor" or "saibo" oysters). In this instance it is the colour and quality of the nacre produced by the oyster that is of interest. To evaluate this, an area of the darker, prismatic, outer layer of the shell is removed using sandpaper to expose the pearly nacre layer.

Transportation

The collection of oyster broodstock from the wild may necessitate protracted periods of transport and while broodstock used for spat production at the PSFC have been collected from Port Stephens, we acknowledge the potential for remote collections. Such collections may seek to take advantage of particular characteristics that may differ between populations or simply to access stock in better states of reproductive condition.

P. imbricata is not normally considered to be an intertidal bivalve, but it is relatively robust and with care will tolerate reasonable periods of air exposure (emersion). We recommend holding oysters in a moist, protected environment at temperatures of 16 – 20^{o}C. Commonly we hold stock for transport in wet jute (hessian) sacks or towels inside polystyrene boxes with lids. If emersion is to exceed 12 h, the oysters are placed in oxygen filled plastic bags inside the polystyrene box and a small frozen block is taped to the lid of the box to help keep the container cool. In all cases, survival is strongly affected by oyster size, with larger oysters tolerating longer emersion, however in a moist, oxygen enriched environment oysters should tolerate a transport duration of at least 24 h.

The use of anaesthesia to reduce handling stress

The invasive nature of procedures such as nuclei implantation, reproductive examination and gonad biopsy have led to the evaluation of potential relaxants for pearl oysters with one of the more useful being propylene phenoxetol (PP)(Hildemann et al., 1974; Norton et al., 1996; Mills et al., 1997). With *P. imbricata*, PP generally induces relaxation within 15 min and, on removal from the relaxant bath, oysters recover within 10 min without evidence of any ill-effects. In general, both relaxation and subsequent recovery times decrease with increasing water temperature. The size of oysters has little effect upon the responses to PP and prolonged exposure to PP (90 min) was not found to cause any apparent ill effects.

Recent work with *P. margaritifera* has indicated PP may have sublethal effects, particularly when combined with other sources of stress such as nuclei implantation (Norton et al., 2000) and both more research and care are required in the application of relaxants.

Feeding broodstock

P. imbricata of 55 - 75 mm shell height, are fed to satiation by adding the equivalent of approximately 6 x 10^9 cells of T. *Isochrysis*/oyster/day.

Choosing the best composite of algae to condition broodstock is complex question and for pteriids we do not have the advantage of a great deal of literature on which to make recommendations. We can however make the following general recommendations based upon observations among other bivalve genera:

1) That the diet comprise a minimum of two (preferably three or more) different species of microalgae.
2) That the species used include a diatom such as *Thalassiosira pseudonana*, S*keletonema costatum*, or *Chaetoceros muelleri* {the latter having been found to be of particular value in conditioning the doughboy scallop *Mimachlamys asperrima* (O'Connor and Heasman, 2000)}. In addition, a species rich in highly unsaturated fatty acids (HUFA's) such as *Pavlova lutheri* is recommended.
3) *P. imbricata* may have difficulty in digesting some microalga such as *Tetraselmis suecica* and the quantities of this species within the diet should be limited.
4) While recirculating broodstock holding systems necessitate the use of drip feeding to prevent large quantities of algae being lost in the biological filter. There has been evidence to suggest that food supplied in this fashion is more effectively utilised by *P. imbricata* than a similar quantity of food added at one time.

Broodstock assessment and conditioning

Reproductive condition can be determined by various means, but for hatchery broodstock this is generally based upon a visual inspection of the gonad. Oysters will occasionally gape when taken from the water, or speculums may be used to open the shell. Prolonged emersion can trigger spawning so a relaxant might be considered if you are attempting to monitor condition rather than eliciting spawning. In assessing reproductive condition, the physical extent of the gonad, its turgor, its colour and the degree to which the gonad is filled with gametes, are all used. Gonads are ranked on a scale of between 1 and 5, with 1 representing gonads which have recently spawned or have no gametogenic development, through to 5 indicating ripe, ready to spawn oysters. For strip spawnings, only oysters ranked 4 or 5 are used, however for natural spawnings we are less prescriptive. The assumption being that animals that can be induced to spawn using natural cues are more likely to provide eggs of reasonable quality.

P. imbricata have been conditioned in the hatchery out of season in 1400 L modular recirculating systems (Heasman et al., 1995) held in a temperature controlled room and stocked at a rate of 30-40 oysters per unit. Recirculating systems reduce the demand upon seawater supplies which must be temperature matched to within 0.5^{o}C before addition to the system. Under such conditions, water changes should be made thrice weekly, such that a 100% exchange occurs each week. The alga used to feed broodstock is drip fed into the container housing the oysters. This practice ensures that food is used efficiently and that food consumption is not affected by rapid increases in food concentration.

The time taken to condition broodstock has been determined experimentally for Japanese stocks of *P. imbricata* and is estimated to be between 700 – 800 degree days (Wada 1991). This is calculated by summing the number degrees over 13^{o}C per day. For instance, oysters held at 23^{o}C for 10 days have accumulated 100 degree days.

$$(23^oC - 13^oC) \times 10 \text{ days} = 100 \text{ degree days}$$

While the utility of this equation has not been empirically evaluated with *P. imbricata* from NSW waters, our observations have indicated that it does provide a reasonable estimation of the time required for reproductive conditioning.

Having conditioned broodstock, care should be taken to avoid any stimuli that could encourage spawning. Variations in temperature, oyster emersion and handling can all pose threats, particularly during routine water exchanges. It should also be noted that we avoid trying to maintain broodstock in conditioning systems for periods longer than required for oysters to reach suitable reproductive condition. On several occasions in which broodstock have been held for protracted periods we have observed slow larval development and poor survival.

3.4. Spawning and Fertilisation

We have used three alternative techniques to induce spawning of ripe *P. imbricata*; thermal stimulation, chemical induction and stripping.

Thermal stimulation

Our preference has been to spawn *P. imbricata* using temperature fluctuation techniques based on those of Loosanoff and Davis (1963).

- Remove ripe broodstock from the conditioning systems (21 - 22^{o}C), scrub them clean and expose them to air for a period of 30 - 60 min.
- Place the oysters in a 400-L bath of seawater at their former conditioning temperature and allow them to acclimatise to their new surrounds. After approximately 30 min the oysters have generally opened their valves and begun filtering. With some species we have added algae to the water to encourage filtering, but this has not been necessary with *P. imbricata*.
- Raise the temperature of the bath 4^{o}C above a base temperature of 21^{o}C, over the next 60-90 min using an immersion heater.
- If spawning does not commence, repeat the cycle by returning the oysters to cooler water (20 - 21^{o}C) for 30 min before raising the temperature again. (The success of these techniques can vary and is to a large extent dependent upon the reproductive condition of broodstock).
- In our experience males are often the first to commence spawning and are allowed to remain in the bath. Polyspermae has not been a problem we have encountered and the animals are allowed to remain in the spawning tank until spawning has ceased or sufficient eggs have been gathered for the purpose at hand.
- If a spawning has been particularly intense we will often siphon all the water possible from the spawning tank without disturbing or emersing the broodstock. This water is passed through a 20 μm filter to retain the zygotes, which are then stocked into tanks for incubation. The water in the spawning tank is then carefully replaced with filtered water of the same temperature. If done with care this water exchange does not halt the spawning process.

Chemical induction

Intragonadal injections of serotonin solution (0.05 ml 10^{-3} N creatinine sulphate complex, $C_{14}H_{21}N_5O_6S.H_2O$, Merck, Darmstadt, Germany) have been used to induce spawning but are generally unnecessary. On those occasions on which we have used serotonin, males have been selected and injected so that sperm will be released to elicit female spawning.

Other reported chemical alternatives for spawning induction include the use of hydrogen peroxide or ozone. However techniques involving these alternatives appear to be used in situations in which

attempts are being made to reduce the reproductive condition of the oyster rather than to provide gametes for larval production.

Strip spawning

Strip spawning is widely used as a means of obtaining gametes from *P. imbricata* and offers a number of advantages. It is quick and inexpensive and allows the gametes from known individuals and/or a known numbers of individuals to be used for particular spawnings. Its disadvantages in our experience have been that firstly it requires killing the oyster. This may not be a problem elsewhere, however, in NSW where populations of wild stocks occur in low numbers and even fewer of these oysters meet the phenotypic selection criteria for broodstock, we are often reluctant to sacrifice broodstock populations. A second concern has been that the quality of strip spawned gametes is lower than those released in response to exogenous cues.

The procedures for strip spawning are simple but require a little practice. Suitable oysters are collected and cleaned of fouling. In China this is accompanied by a brief soak (approx. 5 min) in a disinfectant solution, commonly potassium permanganate. The oysters are then opened and a pipette is inserted into the gonad near the base of the foot to draw gametes from the body of the oyster. Great care is taken not to puncture the digestive gland beneath the layer of gametogenic tissue. The eggs are pipetted into a beaker of seawater and allowed to settle to the bottom. After standing for 10 -15 minutes, the seawater is siphoned off and the eggs are again resuspended in seawater. Eggs can remain in the seawater for up to 2 h before use without ill-effect (Yan Bing, pers. comm.).

Both eggs and sperm are "activated" with an ammonium solution. In practice, 2 drops of a 25% ammonium are added per L of gamete suspension. The frequency spawning attempts for larval production between August 1998 and August 2001. Fill patterns indicate either spawnings that failed to produce sufficient gametes to warrant attempting larval production (Unsuccessful), spawnings from stock gathered directly from the field (Natural) or stock gathered and held in the laboratory under regimens designed to improve reproductive condition (Conditioned).

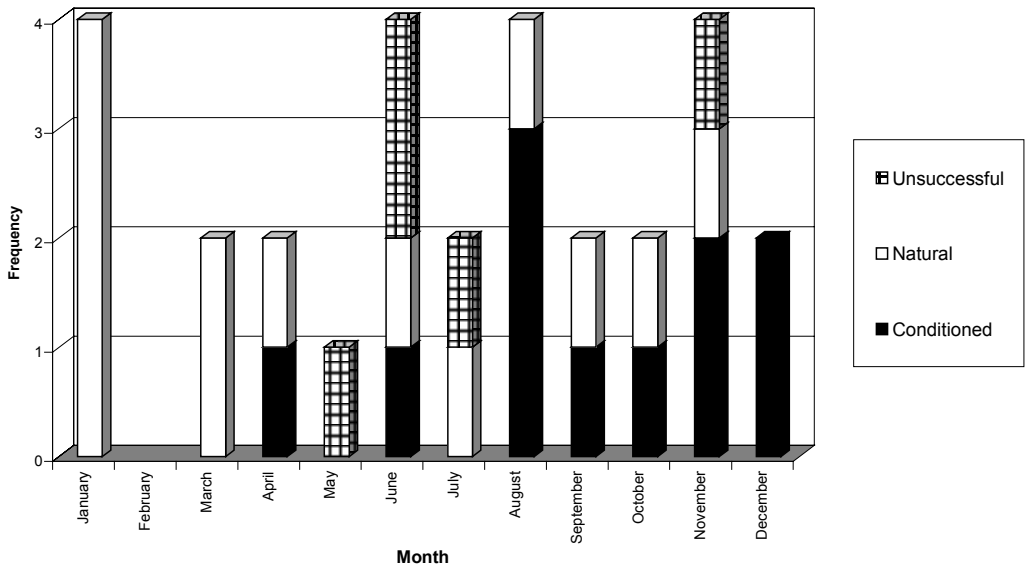


Figure 2.

Despite variability in the reproductive condition of *P. imbricata*, we have been able to obtain viable gametes throughout most of the year. While our greatest difficulties with spawning have been encountered in late autumn and early winter, production at this time remains possible.

Fertilisation

For temperature induced spawnings with or with out the additional use of serotonin, fertilisation takes place in situ. For strip spawnings, volumes of sperm suspension are added to egg suspensions in the ratio of approximately 1:100, respectively. The gamete suspension is then gently mixed and a sample is taken and examined to ensure that there is a minimum of 1-5 sperm present at the surface of each egg.

3.5. Embryonic Development

Rinsing embryos

Following fertilisation, embryo suspensions commonly contain large numbers of excess sperm, unfertilised eggs, faeces and pseudofaeces from broodstock, and occasionally, clumps of eggs. To avoid adding this material to culture vessels, *P. imbricata* embryos are rinsed by pouring onto a flooded 20 μm mesh screen held in a basin of filtered seawater (embryos are not dry screened) before additional seawater is added to flush excess sperm. The embryos are then passed through a 160-200 μm mesh screen suspended in the culture vessel to remove larger debris.

Incubation

Recommended procedures and equipment for incubating mollusc embryos are highly species specific. *P. imbricata* have been relatively robust and are stocked at densities of up to 15 ml-1 in 1000-L tanks. The tanks are gently aerated and an immersion heater is used to hold water temperatures within the range 24-26^{o}C. Development rates for *P. imbricata* embryos, vary with the means of spawning, but are generally high (>90%). The time taken to develop to D-veliger stage varies with water temperature, but is routinely about 24 h.

3.6. Larval Rearing

Larval *P. imbricata* have been successfully reared in both 1000-L polyethylene tanks and 20 000-L fibreglass tanks. While previously we have preferred larger rearing vessels, there has been no evidence to suggest that the performance of *P. imbricata* is enhanced.

Following incubation, D- veliger larvae are drained from the tanks and collected on nylon mesh screens. These screens are held in baths of water such that the larvae remain suspended in water and are not “dry screened”. The D-veligers are counted and are returned to the culture tanks at a reduced stocking density of 5-7/mL. Culture tanks are used in pairs. While one is in use, the other will be cleaned with a providone iodine solution, rinsed and allowed to dry. Immediately prior to use, the clean tank is filled with filtered seawater and an immersion heater is used to raise the water temperature to the desired level. A length of clean food grade plastic tubing is lowered to a position near the centre of the tank just above the bottom and sufficient air is supplied to gently aerate the larval suspension.

The larvae are transferred to new tanks of seawater on every second day or third day. For *P. imbricata* larvae, the mesh screens used for water changes range from 45 μm (63 μm diagonal) to wet harvest D-veligers to 160 μm (226 μm diagonal) used to harvest ready-to-set pediveligers. Commonly two mesh screens of different sizes are used in series at each water change so that larger faster growing larva can be separated. Smaller, slower growing larvae can then be checked

microscopically for signs of disease and if necessary discarded. It is also common practice to drain all but the last few inches of the tank so those larvae actively swimming in the water column are kept separate. The walls of the tank are then rinsed so that any adherent larvae are removed and the remaining water is drained. Again the larvae from the tank floor and walls can be assessed for viability and if necessary, discarded. It is worth noting that we very rarely had any reason to discard larvae other than to reduce stocking densities. Survival of *P. imbricata* larvae can be quite high and it is common practice to either discard smaller or non-swimming larvae. It has been our practice to reduce larval densities by at least half during larval culture.

Counting and measuring larvae

At each water change larvae are collected in a 20-L bucket and are gently suspended uniformly through the water column using a perforated plunger. Four replicate 1-mL samples are taken using an automatic pipette and each is placed in a separate Sedgwick-Rafter chamber for counting. Using a binocular microscope fitted with an ocular micrometer (40 x magnification) the number of larvae within each sample is determined and the antero-posterior shell lengths of 30 or more larvae are measured.

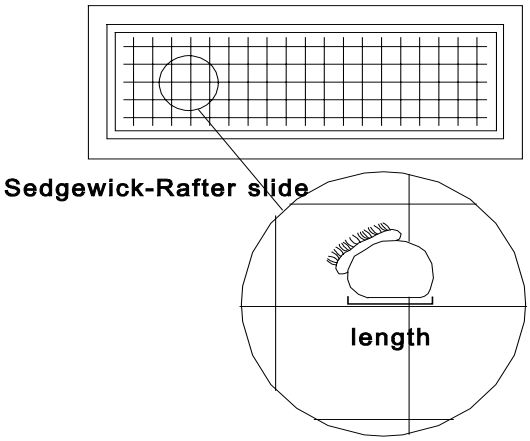


Figure 3.

A sample of larvae should be examined live to assess apparent vigour, however samples usually need to be fixed with a drop of formalin to allow rapid accurate counting and sizing. These counts and sizes are used to determine feed rates and to adjust larval densities when required.

Growth

Despite the relative reliability of larval batches of *P. imbricata,* larval growth has varied markedly and so to has the size at which metamorphosis has occurred. For example, the time to commencement of metamorphosis for *Pinctada imbricata* varies from 13 to 25 days (Wada, 1991) and in our laboratory, under relatively uniform conditions, has ranged from 16 and 23 days. Temperature has a major affect on growth, with increases in temperature within the range 21-28^{o}C leading to faster growth. For convenience within a multi-species hatchery, larval runs are routinely done at 26^{o}C. At this temperature larvae have generally been put to set by Day 20, when they will have routinely achieved a mean shell length of approximately 200 µm.

P. imbricata larvae have proved to be relatively robust with greater than 60% of larvae surviving to metamorphosis in early culture attempts. More recently these percentages have been deliberately reduced. With the ease of conditioning and spawning, there has been a tendency to produce larger numbers of embryos than required and to progressively cull slower growing larvae

throughout each larval run. This has lead to faster larval runs, but more importantly has led to more rapid settlement with greater percentage survival.

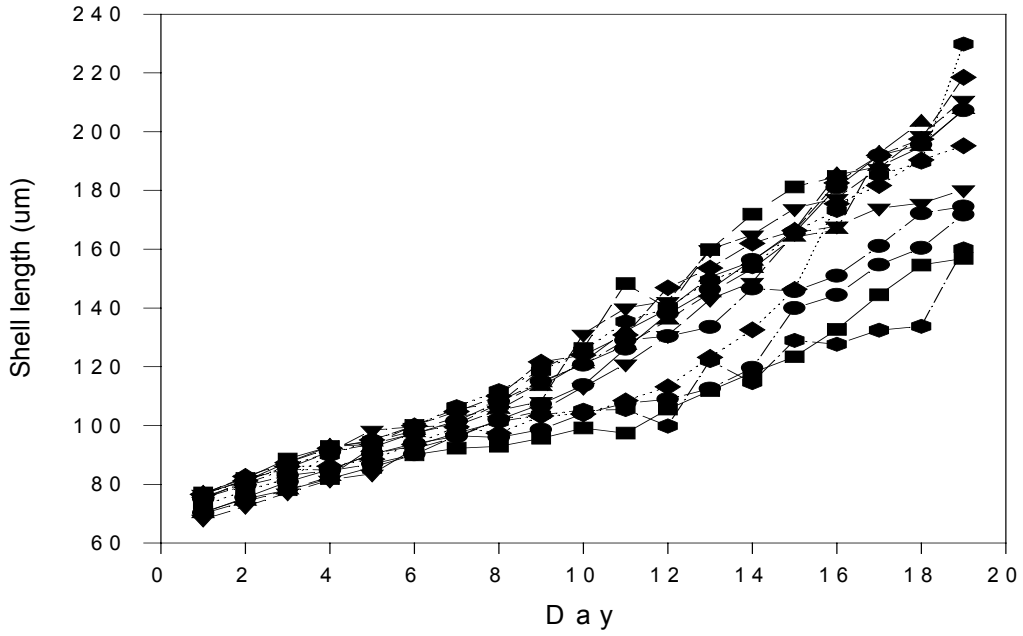


Figure 3. Examples - *Pinctada imbricata* larval growth at Port Stephens Fisheries Centre.

Diet and feeding rate

Assessment of several algal species available at the PSFC has led to the use of a ternary diet comprising *Pavlova lutheri*, Tahitian *Isochrysis* aff. *galbana* and *Chaetoceros calcitrans* (see table below). Larval feeding begins shortly before development to D-veliger stage, when larval numbers and mean size have been determined. Initially, larvae were fed according to a feed curve developed for scallops (O'Connor and Heasman, 1997) and with each successive larval run the feed rate was adjusted according to the amount of food consumed by larvae. The curve provides an estimate of the total number of algal cells to be fed per larvae per day, increasing as larvae grow. "Cell numbers" in the curve relate to T. *Isochrysis*, other alga such as *P. lutheri* and *C. calcitrans* are included on an equal dry weight basis. Effectively, the total number of cells to be fed is determined from the curve. One third of the total is fed as T. *Isochrysis* and to account for the differing dry weights of the algal cells of each species, one third the total x 0.8 is fed as *P. lutheri* and one third the total x 1.2 is fed as *C. calcitrans*. (See Appendix 1 for example).

Table 1. Growth and survival of day-old pearl oyster, *Pinctada imbricata*, larvae fed one of 11 algal diets for seven days.

Diet		Growth* (μm)	Survival** (%)
Mono	*Pavlova lutheri*	28 ± 1.1^{b}	99 ± 2^{a}
	Tahitian *Isochrysis*	26 ± 1.0^{bc}	99 ± 2^{a}
	Chaetoceros calcitrans	19 ± 2^{f}	92 ± 1^{d}
	Chaetoceros muelleri	19 ± 1^{f}	92 ± 1^{d}
	Tetraselmis chui	18 ± 1^{f}	91 ± 1^{de}
	Nannochloris oculata	17 ± 1^{f}	90 ± 1^{e}
Binary	*P lutheri* & T. *Isochrysis*	32 ± 2^{a}	100 ± 0^{a}
	P. lutheri & C. calcitrans	22 ± 1^{e}	96 ± 3^{c}
	P. lutheri & C. muelleri	23 ± 3^{de}	97 ± 3^{bc}
	P. lutheri & N. oculata	23 ± 2^{de}	97 ± 1^{bc}
	P. lutheri & T. chui	25 ± 2^{cd}	99 ± 1^{a}
Unfed		17 ± 1^{f}	90 ± 1^{de}

** Values are means ± SD. Means within columns with a common superscript do not differ significantly (*P > *0.05). Initial larval length 73.41 ± 3.71 μm (n = 30).*

*** proportion of live larvae at the time of sampling.*

After 16 larval runs the equation to calculate feed rate according to larval size was:

$$FR = 0.0578 \times SL^{2.3441}$$

Where FR is feed rate in cells larvae-1 day-1 and SL is shell length (antero-posterior measurement) in microns.

This feed rate should be used as a guide only and is altered in accordance with daily observations of the larval cultures, especially degree of uniformity of apparent gut fullness. Excess food in the culture water may lead us to reduce the feed rate while observations of the amount of food in the gut of larvae may warrant increases. In general the total daily allowance of food is divided and fed in equal portions in the morning and evening.

3.7. Settlement

Challenges in optimising settlement of *P. imbricata* larvae have mirrored those experienced with other byssal attaching species. The most cost effective settlement strategy, and the approach used in Japan and China, is to place pediveligers in tanks containing settlement substrates and then after larvae have settled, transfer them directly to the longline. We have used this approach and have evaluated a variety of potential settlement substrates; however, this approach has several limitations.

Notably:
- Larval settlement can be protracted.
- Large numbers of larvae can avoid the mesh substrates and choose to settle on the floors and walls of the tank
- Settlement density varies greatly between bags.

Screen settlement

In response to similar challenges in the culture of the scallop, *Pecten fumatus,* a method was trialed in which pediveligers were held on screens for metamorphosis. Ready-to-set larvae, usually > 200 µm in shell length, are retained on 45 cm diameter 160 µm mesh screens. Up to 100 000 larvae are added to each of 10 screens, which are then placed in a 1700 L downwelling unit. Seawater in these units is fully exchanged every second day, when the units are scrubbed clean. Larvae are fed twice daily. Initially, 5 000 cell ml^{-1} algae is added on each feeding occasion to the downwelling unit. After two weeks the feed rate is increased to 10 000 cells ml^{-1}. The diet remains the same as that used for larval culture until the rate of metamorphosis has peaked and begins to decline; usually after a week to ten days. At this time, *Chaetoceros muelleri* is substituted for *C. calcitrans* in the diet.

Great care should be taken when handling larvae and spat on screens. Before a downweller is drained and cleaned, a similar unit is filled with temperature equilibrated seawater so that the spat may be directly transferred to a clean unit without prolonged emersion (< 1 min). Screens are normally cleaned with a gentle spray of seawater although the frequency of cleaning is kept to an absolute minimum. In order to prevent screen clogging, larvae are transferred to larger mesh screens as soon as possible. It is during the time spat are on 160 µm in which the greatest losses are observed. By the time spat have reached a size sufficient to be retained by 350 µm mesh (>500 µm shell height) mortality rates fall.

Direct settlement

The most widely used means of settling Pinctada larvae involves the deployment of settlement substrates into the larval culture tank. A variety of settlement substrates have been used, including used monofilament mesh, rigid black polythene mesh, plastic sheets, knotted rope and several commercial mussel collecting rope types. We have assessed a number of substrates for use with *P. imbricata* and have had particular success with rigid 6 mm polythene mesh, although, we have now chosen to use sections of acrylic sheet suspended at intervals on ropes that are hung in the larval tanks.

The acrylic sheet is cut into sections 3 x 10 cm in size and threaded onto 3 mm polythene rope at intervals of approximately 5 cm. The sections are then suspended in the larval tank when approximately 80% of the larvae have reached the eyed stage. The water in the tank is changed thrice weekly and any larvae remaining in the water column are retained on 150 µm screens and returned to the tank. The tanks are fed at the same rate as the downwelling systems (initially 10 000 cells ml^{-1} day^{-1}).

After approximately 10 days in the settlement tanks, the collectors can be transferred to new tanks if required. Any spat attached to the walls of the tank are gently rinsed off and can either be placed on settlement screens or moved with the collectors to the new tank. Seawater temperatures for settlement are maintained at 25 – 26^{o}C for both collector and downwelling systems. In both cases larvae are expected to have achieved a mean size of approximately 1.5 mm shell length within a month and are ready for deployment to the field.

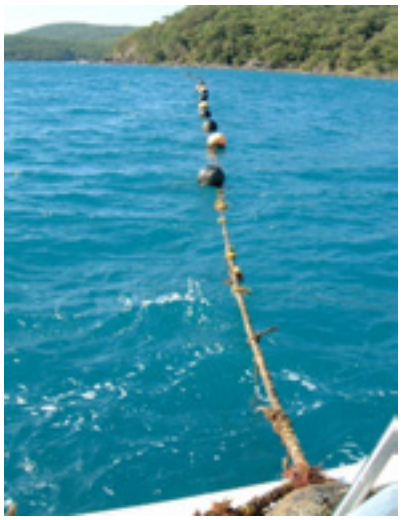
3.8. Farming *Pinctada imbricata*

Culture of *P. imbricata* post hatchery/nursery can be arbitrarily divided in to several stages; juvenile rearing, growout, operation, ongrowing and harvest. Each of these stages has particular infrastructure, environmental and cultivation requirements. Throughout this study, each of these stages has been done using longlines within Port Stephens; however, in the commercialisation phase a broader range of equipment and techniques are proposed.

Cultivation equipment

Longlines

Conventional long-line systems used to culture marine bivalves, can be altered and adapted to suit wide variety of environmental and location constraints. Basic long-line systems consist of a single, main horizontal rope (backbone) anchored at each end and suspended from the surface by buoys. Most commonly, long-line systems set the backbone on the water surface; however, the backbone can be set at any depth in the water column to fulfil conditions or constraints applied to the cultivation process. Inherent advantages of this system include low capital cost to build and install, ease of maintenance and ready access to stock. Positioning the backbone below the surface can increase both the aesthetic acceptability and the degree of access afforded to boating traffic.



Surface longline

Within Port Stephens, farming has been done on 200 m subsurface long-lines set at depth of 4 m below the surface at low tide. The long-line is constructed from 20 mm polypropylene/polyethylene blend rope and anchored at both ends with 10 m to 14 m of 16 mm chain and a 80 kg fixed fluke, Danforth anchor. Additional weights are positioned along the line to maintain the backbone at the preset depth in the water column. One large weight (70 kg) is used near each end of the line and small intermediate weights (approx. 15 kg), spaced at 20 m intervals. Flotation varies with stocking rates; however a maximum of nine buoys are visible on the surface. A 30 cm buoy marks each end of the line (above the 70 kg weight) and 22 cm black intermediate buoys occur at intervals of approximately 25 m. The sole purpose of these surface buoys is to show the position of the line. All other flotation is attached to the main line 3 m to 4 m below the surface. Flotation on the line is adjusted to compensate for changes in crop weight and fouling.

Long-lines are installed parallel to one another and can be linked by breast lines. The orientation of the lines can vary with location. At the experimental farm off Wanda head, the lines have been installed in the direction of current flow. This serves the dual purpose of minimising the load placed on the culture apparatus and minimising any impact on current velocity; the latter helps to prevent sedimentation beneath the lease. Other factors that may influence line orientation include food availability within the water column, prevailing wind direction and the shape of the lease area.

The bags, cages and panels used to cultivate the oysters are attached on short (~ 1 m) dropper lines tied at intervals of 1 –1.5 m along the backbone. Generally there is a cluster of bags (1 – 4) or a single panel per dropper line, but cages can be strung in series. The number of cages will depend on the depth of water beneath the line and the lifting equipment available to remove the cages for cleaning. We have strung up to 14 cages in series in previous research; however, strings of cages at Wanda Head have been limited to five (2.25 m long). With a minimum water depth of 9 m and

a backbone set at 4 m below the surface, this gives the cages a minimum clearance from the bottom of 2.75 m.

Rafts

While rafts have not been used in this research program they have been proposed for limited use in the commercial scale farm. Constructed from hardwood timber and fiberglass covered foam buoys, the rafts can be used for pre-operative "conditioning" and post-operative recovery process.

Access

Aluminium work punts similar to those used by Sydney rock oyster farmers have been used to service the lines. These punts provide the stability needed to lift the lines and have sufficient space to house the pressure cleaners and equipment used to reduce fouling. Each punt is fitted with an electric winch and roller system that assists in lifting the line and in moving along the backbone.

Juvenile rearing

Having reached a shell length of approximately 1.5 mm, spat are transferred from the nursery systems to long-lines in 0.5 mm nylon mesh bags, 0.4 m x 0.8 m in size. Each bag contains 0.5 m of 6 mm black plastic mesh as attachment substrate and is stocked at a density of 5000 bag $^{-1}$. When the spat have achieved a mean shell height of 5mm they are transferred to 2mm nylon mesh bag and the stocking densities are reduced to 1,500 bag^{-1}. Due to biofouling and siltation the spat bags are pressure cleaned on a weekly basis. This ensures a more consistent water flow (algal food and dissolved oxygen) through the spat bags. Spat are reared to a mean size of 20 mm, before transfer to the smallest meshed pearl cage for growout.

Spat bags

Growout

The growout phase of production encompasses the rearing of the 20 mm spat through to a size at which they are suitable for nucleus implantation. Upon removal from the spat bags the 300 – 500 juvenile oysters are placed in each 4 mm mesh pearl cage. Oysters will be sorted and graded for the first time when they have attained a mean size of 30 mm to 35 mm, in approximately 3 to 6 months depending on environmental conditions. At each grading the stocking densities are reduced and the mesh size of the pearl cages is increased. During this farming phase, slow growing and damaged oysters are culled and removed from the cages. When

Pearl cage

oyster have attained a size of 50 mm or greater they are transferred to panel nets or large mesh pearl cages. Oysters are then graded by weight until they achieve operable size. Oysters during this phase of farming are referred to as "mother oysters".

During growout, the oysters and cages are cleaned every 2 - 4 weeks to maintain oyster growth rates and minimise levels of biofouling. Bi-annually, all oysters are collected and transported to the land base site for additional cleaning, treatment and inspection. Cages containing oysters are pressure cleaned to remove biofouling and then undergo a hyposaline treatment (immersion in fresh water) or hypersaline treatment (immersion in high salinity water). Oyster age, operational status and the environmental conditions experienced at the cultivation locations determine which process is used. The water from the hypo-saline treatment is reused until salinity has increased above 5 ppt.

Care is taken to ensure the temperature of the freshwater does not increase greatly above that of the ambient seawater as this can induce gaping (shell opening) and lead to osmotic damage to the oyster.

Table 2. Shell size and stocking densities used for long-line cultivation of *P. imbricata* at Port Stephens, NSW

Shell size mm	**Stocking Density No. Shell cage^{-1}**
2 mm	1,500
7 mm	500
20 – 40 mm	100
50 mm (18 g)	50
55 mm (22 g)	40
60 mm (26 g)	112
65 mm (30 g)	104
70 mm (34 g)	104
75 mm (38 g)	96
100 mm (50 g)	112

Predators pests and parasites

In keeping with their comparative vigour in the hatchery, the survival of *P. imbricata* during growout is high. To date there has been no evidence of disease affecting pearl oysters cultured in Port Stephens; however, their numbers in the wild are small. This has been presumed to be the product of limitations in the areas suitable for their growth, but is also undoubtedly affected by predation. Studies of spat settlement have shown that at least in some years, *P. imbricata* has the potential to recruit to rocky shores in Port Stephens in large numbers, although this has not occurred. This is thought to be the result of fish predation, although evidence has also been found for both mollusc and flatworm predation.

Observations by divers have indicated that a number of fish species eat spat and juvenile *P. imbricata*. These fish include bream, snapper, leatherjacket and wrasse. Trials have been done to investigate the nature of the predation. Spat have been allowed to byssally attach to trays and cement blocks and have then been placed along subtidal rocky shores in areas in which settlement naturally occurs. In some instances the spat are covered with various size meshes to see what protection they afford. Typically, those spat that are not protected have been completely removed (presumed eaten) often within two days, always within a week. When protected spat survival is

significantly higher. In instances in which the spat can not escape the mesh cage survival is typically greater than 90%. Where the mesh is large and the spat can escape survival falls to approximately 40 to 50%.

Along the foreshore, naturally occurring adult oysters and those held in panel nets are eaten by octopus, but when held on longlines this has not occurred. Predation in culture tends to arise from species that can settle in the cages and then grow to a size at which they can eat oysters. Examples of these species include some crabs, boring whelks such as the hairy oyster drill and flatworms. Crabs and whelks have only occurred on occasions when the cages have been neglected for some time and simply require vigilance. Flatworms, have been potentially the greatest problem and thus methods for their control have been investigated and detailed elsewhere.

Mudworm is problem faced by all mollusc farmers in Port Stephens and while it also affects *P. imbricata*, it has not severely affected stocks in Port Stephens. It is likely that the severity of mudworm infestation varies with location and that regular cleaning of the oysters and their cages reduces its potential impact.

Operation procedures

Pre-operative preparation (conditioning), operation and post operative recovery periods are an involved process that can span 3 to 4 months from initiation.

The "conditioning" process, prior to operation, results in a reduction of the physiological state of the oyster. The main objectives are reported to be the removal of the gametes from the gonad for nucleus implantation and lowering the metabolic activity of the oyster to minimise the reaction during the operative and postoperative periods. Lowering of physiological condition is achieved by stocking the pearl oysters at high densities in a "conditioning boxes", which are plastic boxes designed to restrict water flow and reduce the amount of food available to the pearl oyster. Areas of water or leases with reduced current velocity are often selected for this process to further reduce the availability of natural feeds.

The operation process involves the implantation of the nucleus and graft tissue within the gonad of a recipient oyster. The nuclei are generally spherical beads of mussel shell, although a number of composite and artificial nuclei are now available. Graft tissue, commonly called "saibo", is a small segment of mantle tissue excised from a donor pearl oyster that has been selected for desirable nacre traits (nacre = the pearly layer of the shell). If this procedure is successful the donor mantle tissue will grow and form a "pearl sac" surrounding the nucleus. The "pearl sac" is responsible for nacre deposition coating the nucleus surface. All facets of this procedure require specialist training. The only chemicals used in this process are a small amount of an antibiotic (tetracycline) used for nuclei insertion and eosin used to stain the graft tissue. The antibiotic increases operative hygiene and the stain assists the operator to position the graft tissue.

Oysters can be operated on when they have achieved a shell weight of approximately 30 gm (around 60 mm in shell height). As a rule, the larger the oyster, the larger the nuclei and potentially the greater the value of the pearl.

Post-operative care necessitates the return of the oyster to an area of minimal wave action and low current velocity. Oysters are allowed to recover slowly over an extended period before being returned to an area of high current flow for ongrowing to harvest. From hatchery to harvest is approximately 2 to 3.5 years depending on the time of year the spat are produced, when the operation procedure is done and the thickness and quality of nacre desired.

To maximise nacre deposition and quality, the oysters are retained at low stocking densities and cleaned regularly during the ongrowing stages (every 2-3 weeks).

Harvest

At harvest the pearl oyster is sacrificed to remove the pearl from the gonad. This process is done seasonally. The time of year to harvest and obtain the best quality nacre can vary from year to year (depending on environmental variables experienced during the previous winter period). The premium time for harvest is thought to be toward the end of the winter months when nacre deposition is slowest giving better color and luster.

APPENDIX 1

Sample feeding calculation

Total number of larvae 5×10^6 mean size 150 µm shell length

Feed curve value for 150 µm larvae = 7500 cells/larvae/day of T *Isochrysis*

Culture densities

T. *Isochrysis*:	10×10^6 cells mL^{-1}
Pavlova lutheri:	8×10^6 cells mL^{-1}
Chaetoceros calcitrans:	20×10^6 cells mL^{-1}

Dry weight factors (From Nell and O'Connor, 1991)		Factor
T. *Isochrysis*	19 pg	1.0
Pavlova lutheri:	23 pg (23x 0.8= 19 approx.)	0.8
Chaetoceros calcitrans:	15 pg (15 x 1.3 = 19 approx.)	1.3

Total number of cells required:

5×10^6 (No larvae) x 7500 (cells/larvae) = $37.5 \times 10^{9 \text{ cells}}$

T. *Isochrysis* required:

$37.5 \times 10^9 \times 0.33$ (ie one third of the total requirement) = 12.375×10^9

$12.375 \times 10^9 / 10 \times 10^6$ = 1238 mL of culture

Pavlova lutheri required:

$37.5 \times 10^9 \times 0.33 \times 0.8$ (dry weight factor) = 9.9×10^9

$9.9 \times 10^9 / 7 \times 10^6$ = 1414 mL of culture

Chaetoceros calcitrans required:

$37.5 \times 10^{9\,x} 0.33 \times 1.3$ (dry weight factor) = 16.0875×10^9

$16.0875 \times 10^9 / 20 \times 10^6$ = 804 mL of culture

APPENDIX 2

Larval Record Sheet

Batch Number.....**7**..... Date.....**17/3/98**..... Day....**9**.....
Salinity.....**35 ppt**..... Temperature....**25°C**.....

Sizes

19	20	21	22	23	24	25	26	27	28	29	30	31	32
								I	II	III	IIIII IIII	IIII I	IIIII
33	34	35	36	37	38	39	40	41	42	43	44	45	46
IIIII I	III	I	I										

Larval Size.....31.27 x 5.1136* = 159.9 μm..... Screen size..90 μm.....
Observations.....ie larval appearance (gut colour), motility, developmental stage- umbo developing, eyespot developing, foot etc.....
.....
Larval Number.....5.6 x 10^6.....
Feed rate (Cells/larvae/day).....7675.....
Total number of cells.....42 979 x 10^6.....

Species	Weight factor	Date	Count	Proportion	Volume (am)	Volume (pm)
Pavlova	0.8	10/3	6.26x10^6	33%	915 ml	915 ml
Isochrysis	*1.0*	10/3	7.31x10^6	33%	980 ml	980 ml
Calcitrans	1.2	12/3	21.25x10^6	33%	405 ml	405 ml

* Sizes are in eyepiece micrometer units which are later converted to microns. For this example the conversion factor is 5.1135.

4. AN EXAMINATION OF THE POTENTIAL FOR BIODEPOSITION FROM PEARL CULTIVATION IN PORT STEPHENS

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4.1. Introduction

Bivalve culture has the capacity to impact on the environment in a numbers ways. Bivalves are filter feeders that consume substantial quantities of particulate matter from the water column (i.e. phytoplankton, yeast, bacteria, and detritus etc.). So much so that in some instances, species such as mussels, are capable of consuming between 35 - 60% of the food material available in the surrounding water (Figueras, 1989; Hickman, 1989). This food consumption can then lead to significant biodeposition; the benthic accumulation of wastes from the shellfish themselves. The accumulation occurs in the form of faeces and pseudofaeces, but may be significantly augmented with organic material arising from stock losses and through cleaning and harvest operations that dislodge fouling. In some instances the process of biodeposition is exacerbated by hydrological changes induced by the bivalve culture apparatus. If the ropes, floats and cages used to culture bivalves sufficiently retard currents flowing through the lease, the settlement of heavier particles is triggered. Collectively, biodeposition and sedimentation often lead to the accumulation of fine, organically-rich materials beneath shellfish leases (Dahlback and Gunnersson, 1981; Mattson and Linden, 1983; Kaspar et al., 1985; Hatcher et al., 1994). This process can significantly alter benthic environment which may cause significant changes in the macrofaunal assemblages in the sediment (Mattson and Linden, 1983; Kaspar et al., 1985; Weston, 1990; Hatcher et al., 1994; Grant et al., 1995; Weston, 1991).

A monitoring program was initiated to investigate the potential for impact of pearl farming in Port Stephens and to establish protocols for future environmental monitoring. The measures used to detect environmental impact in this study were chosen to reflect the nature of potential impacts. In the first instance sediment samples from the surface of the benthos were collected to investigate accumulation of organic material beneath the lease site. Specifically, total organic carbon (TOC), nitrogen (N) and phosphorus (P) levels were analysed. In addition, samples of benthic fauna were collected to determine species presence and abundance, although, these faunal samples have been preserved and archived for later analysis.

4.2. Materials and Methods

Port Stephens is an estuary (drowned river valley) approximately 275 km north of Sydney, NSW. Covering an area of 126 km^2, the estuary is divided into an inner and outer region. Following an evaluation of a number sites in and around Port Stephens, a 9 ha area in 14 m of water off Wanda Head in the outer port was chosen as the first site to trial pearl oyster farming (see Fig. 1).

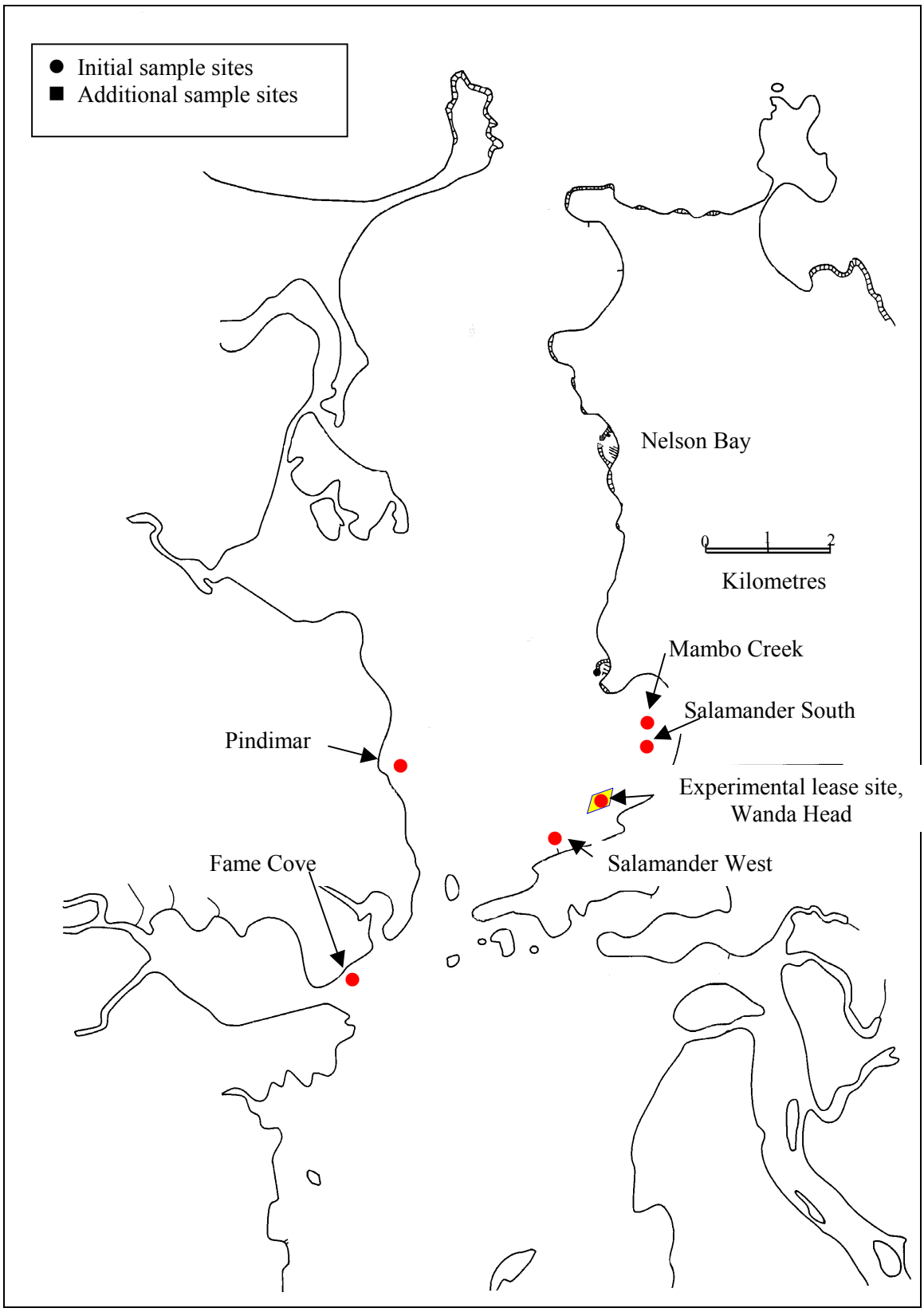

Figure 1. Map of Port Stephens, NSW, Australia, showing the location of the oyster farm and additional sites monitored.

Three potential lease sites (including the current Wanda Head site) have been identified as potential commercial pearl oyster sites within Port Stephens. These sites vary in depth from 7 to 17 m (Lowest Astronomic Tide (L.A.T.)). The bottom substratum at each location is composed of soft mud with a small amount coarse shell grit and sand.

At the time of the initial sediment sampling program in February 2000, the farm had been in operation at the Wanda Head site for 4 months. At this time the farm consisted of four 200 m long-lines that were predominantly stocked with spat and as a consequence had a low overall biomass. With the decision to select Wanda head as the initial farm site, the number of lines was progressively increased to 15 by the time of the third sediment sampling program in November 2000. Each long-line extends over a distance of approximately 200 m and incorporates a main line of 20 mm diameter polyethylene rope strung between two 70 kg anchors (Fig. 2). The main line is held 4 m below the surface and floats tied directly to the line provide buoyancy. Oysters are housed in nets and cages hung from the main line. Surface floats are deployed at intervals of 30 m to allow the depth of the line to monitored visually from the surface. No more than five cages are strung in series so that at Wanda head oysters remain a minimum of 7 m above the seabed. The maximum stocking density in the trial lease at Wanda Head can not exceed 6.9 tonnes ha^{-1}.

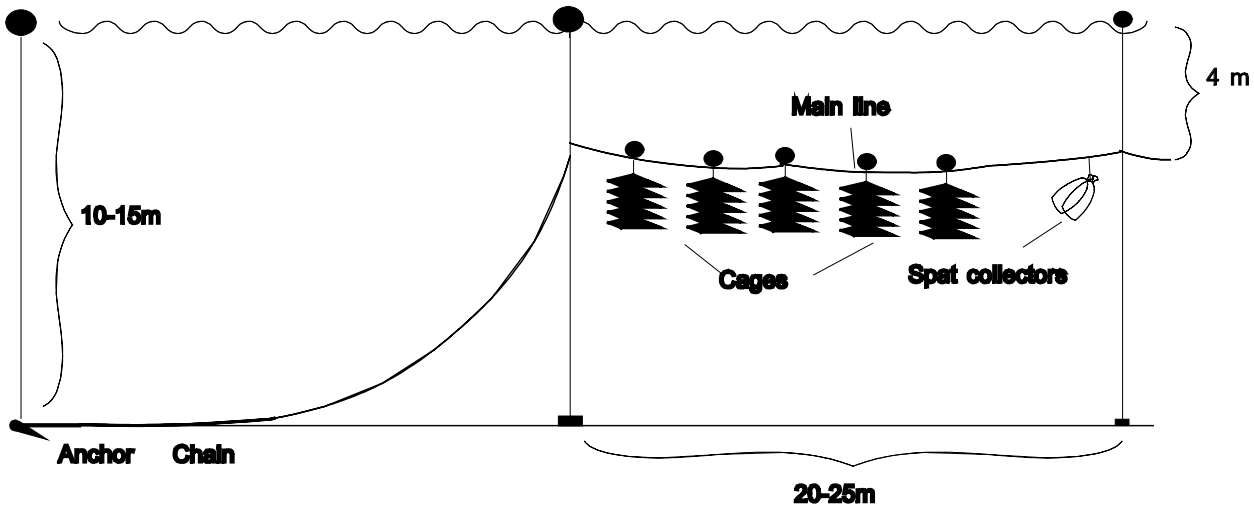

Figure 2. Design of long-line bivalve culture facilities installed at Wanda Head, NSW, Australia. A single line is depicted, however farms typically have a number of these lines laid in parallel.

In addition to Wanda Head, five sites were initially chosen as control locations (Fig.1). Each of these sites are in similar depths of water to those found at Wanda Head (10 – 15 m), and with the exception of Fame Cove were all located in the outer port. The Fame Cove site was situated in the narrows that separate the inner and outer ports.

Field procedures

Divers collected sediment samples from each site on six occasions, February, August and November with additional samples being collected in July, September and November 2001. With the intention of continuing the sampling beyond the period of the current study, a deliberate decision was made to avoid regular sampling intervals. In lieu, samples were to be collected an average of four times annually. On each occasion and at each site, eight sediment cores were collected. Three 500 ml samples of the surface sediment were taken for chemical analyses and a further five, 10 cm diameter 10 cm long sediment cores were collected to monitor benthic fauna.

The latter were processed and any fauna fixed (10% formalin and rose bengal stain) for later analysis. The samples for chemical analyses were placed in insulated containers held at 4^{o}C and transported directly to commercial laboratory for analysis.

Laboratory procedures

TOC: Organic carbon was determined by digestion using a strong acid solution in the presence of excess potassium dichromate, followed by titration with iron sulphate (BCRI, 1987).

Total Phosphorus: P was determined photometrically following oxidation to orthophosphate by digestion with ammonium persulphate under acidic conditions. The orthophosphate produced was reacted with ammonium molybdate and antimony potassium tartrate in acid medium to form phosphomolybdic acid, which was reduced to molybdenum blue by ascorbic acid. The absorbance was measured at 880 nm (APHA, 1992).

Nitrogen: Initially total nitrogen was determined from the sum of oxidised nitrogen and total Kjeldhal nitrogen, however the contribution of oxidised nitrogen was so small (<0.5%) that it was excluded from later samples to reduce costs.

Statistical analyses

Recent reviews (e.g. Hurlbert, 1984) have shown that a large proportion of recent marine research is compromised through poor experimental design. The common problems encountered were little or no replication, pseudoreplication, confounding of variables, and errors in computation or interpretation of analyses. A more recent study (Fairweather, 1989) has identified these problems are the same as those that beset the field of environmental impact assessment. Consequently, the discovery of studies with adequate replication, no pseudoreplication, and the use of multiple controls are often the exception rather than the rule. The net result of poor experimental design is an inability to critically assess the degree and/or magnitude of an impact. The inability to detect an impact that has occurred would have serious ramifications when formulating further monitoring studies.

It is crucial from scientific and economic points of view to ensure that any monitoring program is based on the best possible sampling design given the prevailing financial and logistic constraints. To this end, an adequate, cost-effective sampling design should be the first consideration in any major monitoring program as this will form the basis for all subsequent statistical analyses that test a range of hypotheses. More recently, Peterman (1990) has addressed the question of "adequacy" and emphasised the need to consider the statistical power of the proposed sampling design in any cost-benefit decision. For example, a design may be adequate from the point of view of cost-benefit, but the same design may not have sufficient power to detect the effects that are of primary interest (in this case the effects of the pearl oyster culture) and the sole reason for doing the monitoring.

This section describes the statistical technique that has been used to analyse the results from the monitoring program established to assess the impacts of pearl oyster culture. It is important to note that the design discussed below is complex and requires a substantial understanding of statistical theory for its full appreciation. Consequently, there are a number of issues that are deliberately not discussed. These include the derivation of the mean square estimates using the Cornfield and Tukey algorithm (Cornfield and Tukey, 1956), the designation of fixed and random factors, the assumptions underlying analysis of variance, and the theory underlying the calculation of statistical power.

Ideally, an assessment of the impacts of pearl oyster culture would have been based on replicated sampling before and after the commencement of the trial program. As the initial number of

longlines and stocked biomass were low and as any accumulation of organic matter would be slow if at all, results from the first sampling period in February 2000 are considered to be indicative of the pre-commencement condition. As a result, there is a very low potential that the analysis is confounded in that it is not possible to be absolutely sure that any changes detected are unequivocally due to pearl oyster culture. Thus the possibility of another external source still remains, however as shown by the data, the natural variance at the site and at the control sites is high.

In addition, as there is only one presumed impacted site (i.e. the pearl oyster lease), the analysis design becomes asymmetrical with respect to impact sites. Control sites should be and are replicated in this design and can have a great influence on the power of the tests for impact. Table 1 shows the design that will be used to analyse the temporally-replicated data obtained after the pearl oyster culture commences. Note that all terms denoted with an asterisk involve an asymmetry in the levels of the particular factor.

While this design lacks sampling prior to the commencement of the disturbance (i.e. pearl oyster culture), there are several important features that assist in overcoming problems that have beset approaches to impact assessment in the past (Otway, 1995; Otway et al., 1996a, b). First, the design incorporates spatial and temporal replication thus overcoming problems of pseudo-replication (Hurlbert 1984). Second, temporal replication after the disturbance is done at several times to identify temporal trajectories and this will be ongoing. Third, the design can infer whether the disturbance causes a detectable change in the variable of interest at the presumed impacted site. Last, the design can infer impacts that occur at different temporal scales (i.e. as a short-term 'pulse' or sustained (longer-term) 'press' changes (Bender et al., 1984)).

Table 1. Asymmetrical analysis of variance assessing the impacts of pearl oyster culture in the absence of data collected before the putative disturbance. Design involves one putatively impacted site and multiple control sites contemporaneously sampled (with replication) through time after the disturbance has commenced.

Source of variation	**Denominator for *F***
Times	MS Times X Among Controls
Lease vs Controls	MS Among Controls
Among Controls	MS Residual
Times X Lease vs Controls*	MS Times X Among Controls
Times X Among Controls	MS Residual
Residual	
Total	

MS = mean squared

The repartitioning of the asymmetrical analysis of variance in Table 1 provides for temporal interactions with an *a priori* orthogonal contrast between the single presumably impacted site and the control sites after the disturbance begins. It is this feature that permits tests for presumed impact. The detection of impact depends on the duration of the changes caused by the disturbance and the space-time interactions that occur naturally, i.e. in the absence of an anthropogenic disturbance. The detection of impacts at different temporal scales requires a sequence of tests and these are described below.

A sustained (long-term) impact can be detected (inferred) using the *F*-ratio of MS (Mean Square) Times X Lease vs Controls / MS Times X Among Controls and consistent results from *post-hoc* comparisons among means. *Post-hoc* tests such as the Student-Newman-Keuls (SNK) test (Winer, 1971; Snedecor and Cochran, 1980) or Ryan's test (Ryan, 1960) are most commonly used. To infer sustained (long-term) impacts requires that the presumably impacted location consistently differs from the control sites over all times of monitoring. Alternatively, if there is no significant temporal variation among the control sites i.e. the *F*-ratio of MS Times X Among Controls / MS Residual is not significant at $P = 0.25$, then the MS Times X Among Controls term can be eliminated (i.e. pooled with the Residual) from the analysis. This then results in a test for impact with substantially more power as the MS Residual and its associated degrees of freedom are then used.

Short-term impacts can also be assessed (inferred) from a significant ($P < 0.05$) MS Times X Lease vs Controls / MS Times X Among Controls and by using *post-hoc* comparisons among means. To infer short-term impacts from such tests requires that the putatively impacted location (i.e. the lease site) differ intermittently from the control sites. This can, however, occur naturally and have nothing whatsoever to do with the pearl oyster culture. Hence the potential confounding and thus additional supporting information is needed for impacts to be identified. Finally, it is important to note that these *post-hoc* tests are less powerful than analyses of variance and may, on occasions, give inconclusive or equivocal results.

Asymmetrical analyses of variance were done with the assistance of Statgraf 4.1 (Statistical Graphics Corporation).

4.3. Results

Total Organic Carbon (TOC)

Quantities of TOC measured over the course of this investigation varied from 0.2% to 2.7% (dry weight) of the sediment (Fig. 3). As a whole, the quantity of sediment TOC did not differ significantly over the six sampling occasions, although the TOC did vary significantly with time at individual sites (Table 2). The average quantity of TOC at particular sites also varied significantly (Table 2). Sites such as Pindimar Channel exhibited a low but variable TOC, while sites such as Salamander South and Wanda Head had comparatively high and stable TOC quantities. Regardless, the average TOC observed at Wanda Head did not differ significantly to that observed among the control sites. Further the changes in TOC observed over time did not differ significantly to those observed among the control sites.

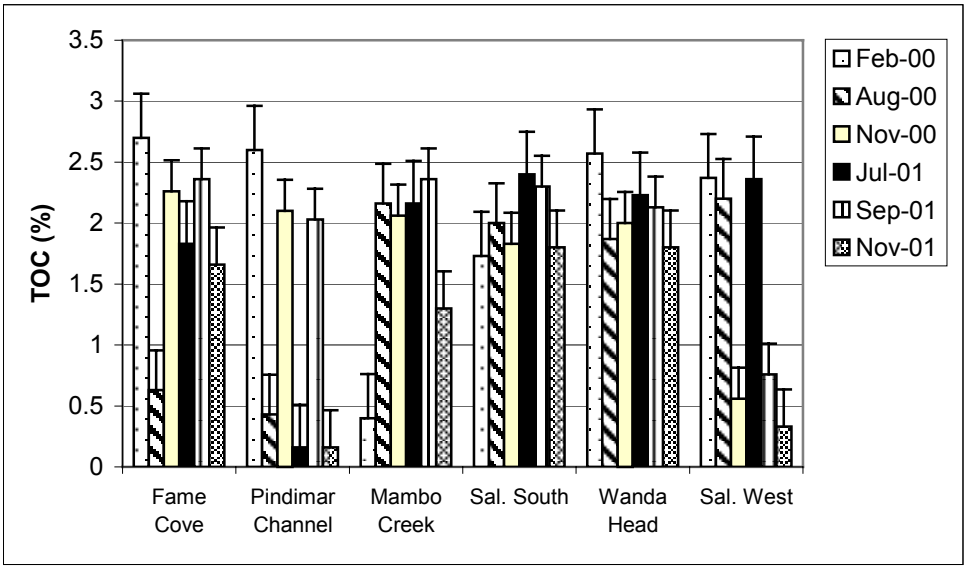

Figure 3. Variation in total organic carbon (TOC) in the sediment at six sites in Port Stephens, sampled on six occasions from 2000 to 2001.

Table 2. Asymmetrical analysis of variance of total organic carbon (TOC) in the sediment at one impacted and five control sites in Port Stephens, NSW, Australia.

Source of variation	SS	df	MS	*F*	*P*
Time	9.7933	5	1.9587	0.9371	NS
Impact vs Control	2.7307	1	2.7307	1.5221	NS
Among Controls	7.1760	4	1.7940	15.2441	***
Times x Impact vs Controls	0.6133	5	0.1227	0.0587	NS
Times x Among Controls	41.8000	20	2.0900	17.7592	***
Residual	8.4733	72	0.1177		
Total	70.5866	107			

NS not significant, * P < *0.05,* ** P < *0.01,* *** P < *0.001.*

Nitrogen (N)

Sediment N demonstrated far greater variability than observed for TOC, with measurement ranging from approximately 200 to 10 000 mg kg^{-1}. Unlike TOC, the average quantity of sediment N across all the sites did differ significantly over the six sampling occasions, in particular in July 2001, when at five of the six sites monitored the quantity of N present was the highest observed during this study (Table 3, Fig. 4). Similarly there were also significant differences in the average quantity of sediment nitrogen among the sites. Pindimar Channel and Salamander west had low average nitrogen levels, Wanda Head had an intermediate and relatively stable N level and Salamander south had the highest average sediment N (3161 mg kg^{-1}). The pattern of change in sediment N at individual control sites over time also differed (Table 3), although in this respect the changes in sediment N over time at Wanda Head did not differ significantly from the controls.

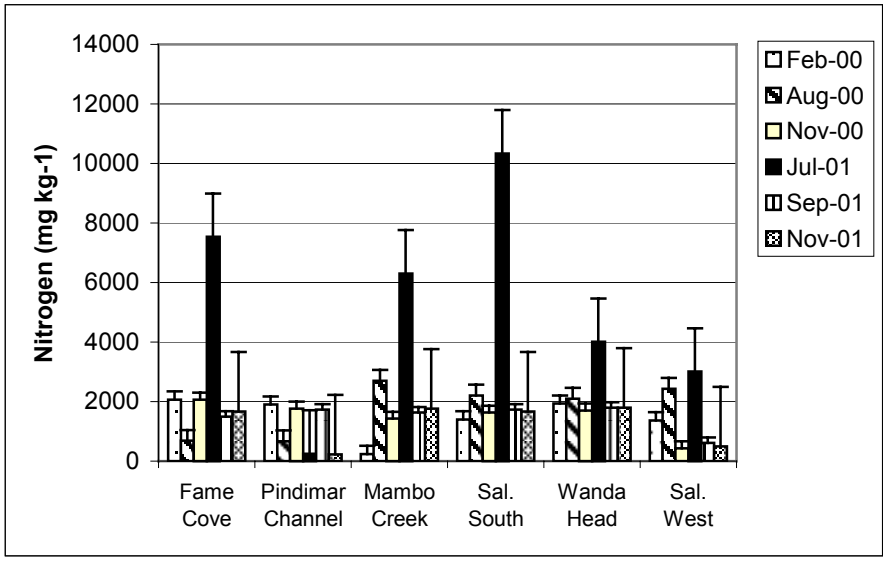

Figure 4. Variation in sediment nitrogen at six sites in Port Stephens, sampled on six occasions from 2000 to 2001.

Table 3. Asymmetrical analysis of variance of sediment nitrogen at one impacted and five control sites in Port Stephens, NSW, Australia.

Source of variation	SS	df	MS	*F*	*P*
Time	211000000	5	42161000	5.1038	***
Impact vs Control	459700	1	459700	0.0349	NS
Among Controls	52620900	4	13155225	2.9007	**
Times x Impact vs Controls	7518000	5	1503600	0.1820	NS
Times x Among Controls	165000000	20	8260700	1.8215	*
Residual	327000000	72	4535125		
Total	763000000	107			

NS not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Phosphorus (P)

Mean P levels at each location are shown in Figure 5 and like sediment N show considerable variation, ranging from approximately 2 to 130 mg kg^{-1}. Like N, the average quantity of sediment P across all the sites differed significantly over the six sampling occasions (Table 4). The highest levels of P at each site were generally recorded in the first sample collected in February 2000, following which there was a trend for sediment P to reduce with time. Overall, the average quantity of P did not differ between sites and no significant differences were found over time between individual sites (Table 4). The putatively impacted site, Wanda Head, had intermediate quantities of sediment P that exhibited a steady decline with time (Fig 5).

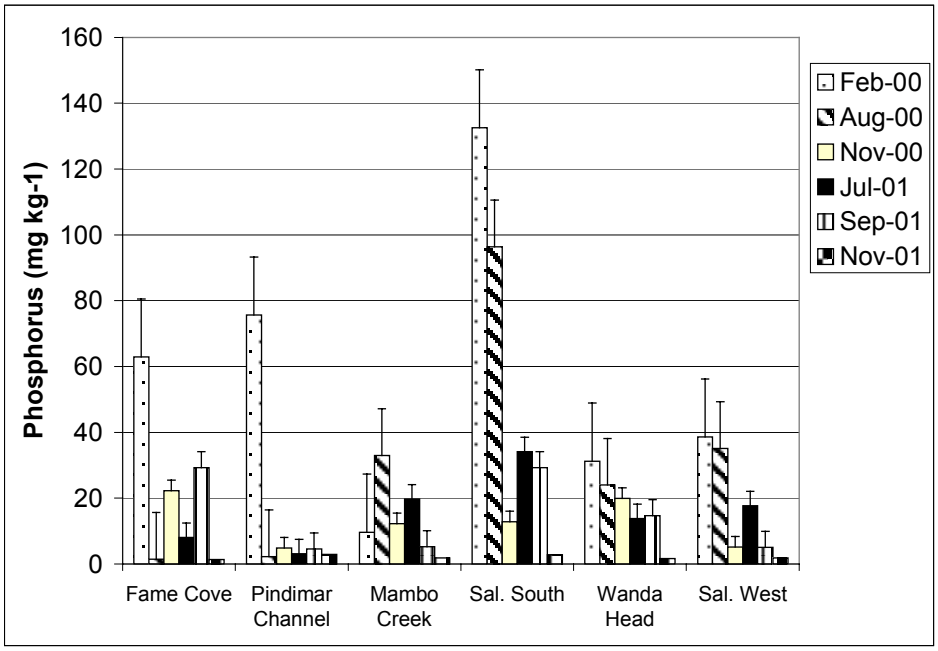

Figure 5. Variation in phosphorus in the sediment at six sites in Port Stephens, sampled on five occasions in 2000 - 2001.

Table 4. Asymmetrical analysis of variance of data for phosphorus in the sediment at one impacted and five control sites in Port Stephens, NSW, Australia.

Source of variation	SS	df	MS	*F*	*P*
Time	70688.1	5	23562	4.7854	***
Impact vs Control	2912.7	1	2912	0.1340	NS
Among Controls	51332.9	4	12833	1.5777	NS
Times x Impact vs Controls	7099.4	5	2366	0.3375	NS
Times x Among Controls	54176	20	4514	0.5353	NS
Residual	193629	72	4033		
Total	379838.1	107			

NS not significant, * P < *0.05,* ** P < *0.01,* *** P < *0.001.*

4.4. Discussion

Initial results from environmental impact monitoring studies have been encouraging, particularly in that the composition of the sediment underneath the experimental farm at Wanda Head has not changed significantly over time relative to the control sites. Indeed, levels of TOC, P and N have remained within the range recorded at the surrounding control sites at all times. This suggests that the farming activities at Wanda Head have not altered the environment beyond the level of naturally occurring variation or beyond the ability of the environment to rapidly assimilate any additional nutrients. This does not however completely preclude the possibility of impact. There remain shortcomings with this evaluation. First, a lack of prior sampling, and second, the need for

continued sampling. While it may be unlikely that either of these alter the conclusions, both warrant further consideration.

As noted briefly in the description of statistical methods, the absence of prior ("before") sampling prevents the conclusive exclusion of the possibility of impact. Commonly three types of impact have been identified, press, pulse and catastrophic. Briefly, press impacts arise from a chronic or sustained disturbance to a population; pulse impacts are acute or short-term disturbances, while catastrophic impacts cause a major irreversible disturbance. In this instance, and in the absence of prior sampling, it is possible that the initiation of farming at Wanda Head has caused either a press or catastrophic impact that has led to either an immediate increase or decrease in sediment nutrient levels so that the site now conforms with the levels found at the control sites. This however is unlikely. At the time of the first sediment sampling (February 2000) only four longlines were in place and they were stocked predominantly with spat (5 - 10mm oysters). By the time the fourth sampling was done (July 2001), the number of longlines had been increased to 15 and the size of the oysters had increased to comprise largely 40 to 60 mm adults. These changes progressively produced an almost fourfold increase in the potential for sedimentation (4 to15 longlines) and greater that a tenfold increase in biomass available to cause biodeposition. These increases would suggest that a progressive increase in sediment nutrient levels might be observed, rather, both TOC and N levels remained comparatively constant during this period while P levels fell.

The second difficulty encountered in this study arises from the "power" associated with the analyses used to detect impact. In cases such as this, where the potential impact is small (discussed later) and occurs in an environment of high variability, tests of great power are required to detect significant changes. Due to the variability spatially and/or temporally in TOC, Phosphorus and Nitrogen concentrations in the sediment, that has been established as part of this sampling program, the power of the analyses reported here are inadequate to preclude the possibility of impacts. A larger data set comprising more sites and a longer sampling period will be required to preclude the potential for impacts. It is proposed that both these measures be taken to increase the ability of this ongoing sampling regime to detect change. The number of sites now sampled has been increased from six to nine, and includes the three proposed impacted sites and six control sites in Port Stephens. In addition, the monitoring program will be continued to provide a longer and larger data set.

The difficulty in detecting small impacts over and above natural changes in the physical and chemical composition of the sediment beneath the trial farm site was expected. This was due to the comparatively low stocking densities to be used the farming practices adopted. Prior to farming evaluations, the stocking density for oysters was limited to a maximum of 6.9 t ha^{-1}. This is comparatively a very low stocking density. Elsewhere, bivalves in hanging culture have been stocked at densities as great as 48 t ha^{-1} yr^{-1} for mussels and 22.9 t ha^{-1} yr^{-1} for *P. imbricata* in Japan (Table 5).

Table 5. A comparison of stocking densities used for pearl culture in Port Stephens, NSW, Australia with those used for suspended culture of bivalves at other locations.

Location	Species	Suspended culture method	Density (tonnes ha^{-1} yr^{-1})	Author
Port Stephens, NSW, Australia	Pearl oysters *Pinctada imbricata*	Mid-water long-line	6.9	This study
Ago Bay, Japan	Pearl oysters *Pinctada imbricata*	Raft Culture	22.9	Uyeno et al., 1970
Kyushu, Japan	Pearl oysters *Pinctada imbricata*	Surface long-line	22.9*	Y. Suto, pers. comm.
Twofold Bay, NSW, Australia	Mussels *Mytilus galloprovincialis*	Surface long-line	28.8	Forteath et al., 1996
Twofold Bay, NSW, Australia	Mussels *Mytilus galloprovincialis*	Raft culture	26.4	Forteath et al., 1996
Ria de Arousa Spain	Mussels *Mytilus galloprovincialis*	Raft culture	48.0	Comacho et al., 1991
Tjärnö, Sweeden	Mussels *Mytilus edulis*	Raft culture	47.5	Dahlback and Gunnarson, 1981

** Assumes average oyster weight is 50 g.*

The low stocking density for pearl farming is but one of a number of farming practices that have been adopted to reduce any potential for impact. In mussel culture, it has been suggested that the accumulation of mussels and the associated fouling organisms beneath the leases does more to influence the environment than the biodeposition of faeces and psuedofaeces (Tenore et al., 1982; Jaramillo et al., 1992; Grant et al, 1995; Stenton-Dozey et al., 1999). There is little scope for this type of accumulation to occur at Wanda Head. The oysters are cleaned on a regular basis, every 2 to 4 weeks preventing the growth of larger fouling organisms. Those fouling organisms that do occur are removed, collected on the boat and disposed of on shore. The oysters themselves are considerably more valuable than mussels and are retained in cages, preventing accidental losses.

In NSW, long-line culture of bivalves has only been done at several locations with one in particular, mussel farming in Twofold Bay, attracting scrutiny for potential ecological effects. In this instance, investigations of TOC content of sediment at the mussel farm found no evidence for increased TOC levels above those occurring naturally (NSW Fisheries, 1996). Several years later after more than a decade of mussel farming, surveys of the benthic fauna below the farm found only a small amount of evidence for ecological impact and only within the bay in which the mussel farm was located (Underwood and Hoskins, 1999). This is despite the mussels in Twofold Bay being farmed at densities approximately four times greater than those used for pearl oysters at Wanda Head (Table 5). Further, greater impacts would be expected at the Twofold Bay site as the area chosen for mussel farming is in approximately 10 m of water and experiences current flows of the order of 0.1 m s^{-1} (Forteath et al., 1997). At Wanda Head the farm is located in an area with a minimum depth of 14 m and experiences tidally dependent currents that range between 1.5 m s^{-1} to 0.1 m s^{-1} (McOrrie, 1984; P.W.D. NSW, 1987). The greater depth increases the time required for

sedimentation to occur and coupled with the additional current flow, acts to disperse the sediment over a wider area, reducing any potential for impact.

4.5. Conclusions and future studies

Ideally, studies of this nature would involve monitoring before and after the installation of the farm in what has been called a "Before/After, Control/Impact" or "BACI" design (Underwood 1995). These designs are particularly useful as they incorporate some estimate of the state of the environment and its variability at the particular site of interest, before the impact occurs. This information is then compared with the changes occurring at a number of similar sites (controls) over the same experimental period. Unfortunately this was not possible in this case. Farming *P. imbricata* in NSW is in its infancy and sites could not be reliably identified at the outset of the study. As discussed, initial samples were taken shortly after the longline equipment was installed and when the stocking biomass was low. An example of the value of the BACI design can be seen with respect to the high TOC levels found at Wanda Head. Despite the high likelihood that the elevations in TOC are naturally occurring, it is not fully possible to eliminate that an impact had already occurred prior to the time of the first sampling and that the benthos had then reached a steady state. Based on the low stocking levels and experience at other more heavily stocked sites, this possibility is however extremely low. With the proposal for additional farming areas at Mambo Creek and Pindimar, both sites have been added to the current sampling regime so that should the applications be successful, "before" data will be available for later analysis.

5. *PINCTADA ALBINA SUGILLATA*

5.1. General Introduction

At the outset of this research an extensive survey of coast of NSW confirmed the presence of two morphologically similar members of the genus *Pinctada* (Ponder and Colgan 1995). Allozymic comparisons with specimens from Queensland and Japan confirmed one of species represented to be *Pinctada imbricata*. The second Pteriid specie was simply referred to as "Type II" and awaited identification. Ultimately, allozymic comparisons with oysters from Western Australia, the Northern Territory, Queensland and NSW, confirmed theat Type II oysters were members of the *Pinctada albina* species complex.

Although *P. albina* was not the primary target of this research, a series of observations regarding the specie were made. In some instances these observations were inevitable, while in others they were made to directly benefit research with *P. imbricata*. For example, in studies of the effects of relaxants, a shortage of *P. imbricata* adults encouraged the use of *P. albina* as a surrogate in initial evaluations. In spatfall studies both species recruited to collectors and thus observations for both species were recorded. During collections for reproductive monitoring it was often impossible for divers to discriminate between the species until the oysters were bought to surface and cleaned of fouling. Finally, broodstock were spawned and the resultant larvae cultured to observe early ontogeny in *P. albina*. This was done in the hope that there may be features that would allow us to distinguish spat of the two species and thus avoid a laborious ongrowing procedure required to identify which of the species were recruiting to collectors.

The pearl oyster, Pinctada albina sugillata.

5.2. Latitudinal variation in reproductive behavior in the pearl oyster, *Pinctada albina sugillata*

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5.2.1. Abstract

Increased interest in periculture in New South Wales (NSW), Australia, encouraged an assessment of the changes in reproductive condition of pearl oyster, *Pinctada albina sugillata*. Over a two-year period, macroscopic and histological observations were made of oysters collected monthly from Port Stephens. In contrast to more northerly populations, *P. a. sugillata* in NSW were found to have a truncated breeding season. Reproductive activity was greatest from late spring to early autumn with oysters in poor reproductive condition during winter. Peaks in reproductive indices occurred in October 1998, March 1999, January 2000 and April 2000. Spat collectors deployed at two sites in Port Stephens found spatfall to be restricted to the months of November-January, indicating that the autumnal peaks (March & April) in reproductive activity did not result in subsequent recruitment. These results supported previous observations of latitudinal change in the reproductive behavior of pearl oysters in which populations at higher latitudes have truncated breeding seasons that tend to occur during the warmer months. Recruitment to spat collectors was low and variable, discouraging the collection of wild spat for culture, yet the spring increase in reproductive activity was coincident with the likely time for hatchery propagation. Infestations of shell boring organisms were found among wild oysters, although the degree of shell damage was almost invariably low. Most common were infestations of Spionid polychaetes, present in 30% of the shells collected, with several larger oysters showing shell damage typical of boring sponges.

5.2.2. Introduction

Known locally by the unfortunate colloquialism "the bastard oyster", *Pinctada albina* occurs in many areas of the Indo-Pacific (Shirai 1994) and is found around Australia's northern coastline from the Houtman-Abroholos Islands in the west to central NSW in the east (Fig. 1). At various times *P. albina* has been of minor commercial importance. From the 1850's the oyster was harvested in Shark Bay for its small but colourful pearls before the industry eventually collapsed in 1939 (Morton et al., 1998). More recently, the success of pearl culture ventures with other pteriid oysters has prompted investigations of the potential of *P. albina* for cultured pearl production and one farm has been set up in Shark Bay, Western Australia.

Two subspecies of *P. albina* occur in Australian waters; *Pinctada albina albina* (Lamarck, 1819) in northern Western Australia and *Pinctada albina sugillata* (Reeve, 1857) down the northeastern coastline from the Torres Strait Islands to New South Wales (Lamprell and Healy, 1998). While *P. a. albina* has been the focus of commercial interest in Australia (Morton et al., 1998), *P. a. sugillata* possesses several morphological characteristics that offer potential advantages in pearl culture. *P. a. sugillata* grows to a greater size and is more convex than its conspecific (Lamprell and Healy, 1998), offering the potential to produce pearls of a greater size and thus greater value.

Central to periculture is an understanding of the reproductive biology of the species to be used, either to allow hatchery production or to optimise the timing of nuclei implantation (Wada et al., 1995). Within Australia a number of studies have been done on reproductive condition of pteriids including *P. a. sugillata*, but intraspecific changes in reproductive condition have been reported (Tranter, 1958a; Wada et al., 1995). Indeed, Tranter (1958a, 1959) suggested latitudinal variations

in condition for several pteriids in which populations at higher latitudes have truncated breeding seasons that tend to occur during the warmer months. While there is evidence to support this generalisation with species such as *Pinctada imbricata* (Wada et al., 1995), there is a paucity of information with respect to *P. albina*.

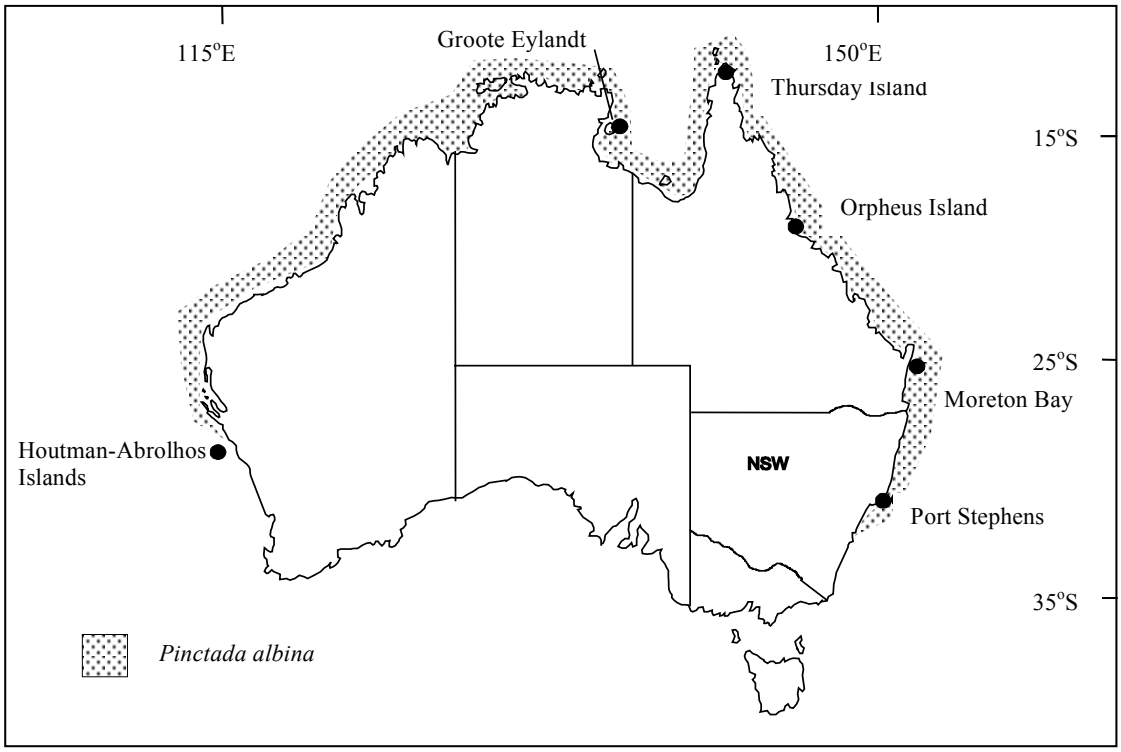

Figure 1. Distribution of *Pinctada albina* in Australian waters.

Pinctada albina sugillata is hermaphrodite with a tendency to protandry (Tranter, 1958c). Off Thursday Island, northern Australia (Lat. 10° 35" S, Fig. 1), where water temperature rarely falls below 25°C, *P. a. sugillata* breeds throughout the year, but most actively in April and May when water temperatures are falling (Tranter 1958c). In accordance with this breeding activity, spatfall is heaviest from June to August but also occurs throughout the year (Tranter 1958c). Approximately 900 km further south at Orpheus Island (Lat. 18° 37' S) observations of pteriid spat fall found *P. a. sugillata* recruitment had been limited to 10 months (Beer and Southgate, 2001). This suggested latitudinal variation in breeding and raised the possibility of further variation toward the southern extent of the species range. In particular in Port Stephens, NSW (Lat. 32° 43" S), some 1600 km further south of Orpheus Island, where water temperatures rarely exceed the minimum experienced at Thursday Island (25°C).

Latitudinal variation in the reproductive condition of bivalves is not rare (Sastry 1970, Hesselman et al. 1989, MacDonald and Thompson, 1988), but the importance of any variation, particularly in bivalves with potential for periculture, warrants investigation. The regular presence of small numbers of *P. a. sugillata* in collections of pteriids from Port Stephens and their occurrence on spat settlement collectors provided an opportunity to monitor reproductive condition in one of the most southerly populations of *P. a. sugillata*. We have confirmed that variations in the breeding season of *P. a. sugillata* do occur and that they are consistent with the changes suggested by Tranter (1958c).

5.2.3. Materials and Methods

Pinctada albina sugillata were present in small numbers among collections of *Pinctada imbricata* taken by divers at Wanda Head, Port Stephens (152°10'E, 32°45'S). This site has a gently sloping rock wall that extends from the shore for up to 5 m and to a depth of approximately 3 m.

In total, 183 mature *P. a. sugillata* larger than 40 mm dorso-ventral measurement (DVM) were collected over a two year period. A minimum of six *P. a. sugillata* were collected each month with the exception of March 2000, when none were found among the oysters collected. Each sample was returned to the laboratory for immediate analysis. Collections began in June 1998 and continued until May 2000.

On each sampling occasion the shell heights and total weights of each oyster were recorded. The soft body was removed from the shell and its wet weight was determined to the nearest 0.01 g. The mantle and gill were then folded back to expose the body of the oyster adjacent to the foot to permit a macroscopic assessment of reproductive condition. Each oyster was given a score from 1 to 5 (Table 1), with a score of 1 being the base of the scale and indicating poor reproductive condition.

As a result of the potential for shell boring organisms, such as spionid polychaetes, to affect the physiological condition of oysters, the valves of each oyster were examined for their presence. The degree of polychaete infestation was scored on a scale of 0 to 4. Oysters were scored 0 if polychaetes were not present, 1 if polychaetes were present in one valve only and had affected < 5% of the surface area of that valve, 2 if polychaetes affected < 5% of both valves, 3 if > 5% of either valve was affected and 4 if > 5% of both valves were affected.

The soft body of each oyster was taken and a transverse section was excised starting at the base of the foot. The sections were fixed in Davidsons solution for 24 h (Shaw and Battle, 1957) before being passed through graded alcohol solutions and then xylene. Finally, samples were mounted in paraffin and sections 6 μm thick were cut and stained with Harris Haemotoxylin. The sections were examined using a microscope (x 200 magnification) and categorised into gametogenic stages using criteria based upon those of Tranter (1958b). The number of stages was reduced from Tranter's (1958b) nine to five for several reasons. First, as acknowledged by Tranter (1958b), the stages are not always clear cut, and there is a degree of subjectivity in their assignment. Second, follicles within a gonad can differ in their stage of development or regression. Finally, a simplification of the system aids in the presentation and interpretation of the data.

The abridged stages of gametogenesis used were as follows. Tranter's (1958b) developing stages 1 and 2, characterised by the predominance of either spermatogonia and spermatocytes or oogonia and oocytes, were combined to form Developing 1. Stages 3 and 4, where the oocytes increase in size and detach from the follicle wall or the numbers of spermatids or spermatozoa increase, were combined to form Developing 2. Tranter's Developing 5, in which the follicles contain predominantly spermatozoa or large, free oocytes, was retained and called "ripe". The regression stages 1 and 2 have been called spawned/regressing, with the inclusion of the term spawned in acknowledgment of the difficulty in determining whether gamete numbers in the follicles have been reduced as product of partial spawning or autolysis. Finally, the two stages in which the follicles are largely devoid of gametes, "r3" and "inactive", were combined and called "inactive".

Table 1. Criteria for macroscopic scoring of gametogenic stages*.

Stage	Description	Score
Inactive	Gametes are absent. The gonadal area is translucent and the digestive diverticula are visible.	1
Developing 1	Gonads are filling, development is patchy and appears to be emanating from the posterior forward, males and females are indistinguishable.	2
Developing 2	Gonads less patchy in appearance as follicles spread and begin to fill. A pattern of development toward the anterior is less apparent, however the anterior edges of the body remain translucent. Gonad and body thickening	3
Developing 3	Gonad and body turgid and consistent in colour, the development of follicles is no longer apparent with the exception of an occasional translucent strip at the base of the foot. Sex can be generally differentiated on the basis of colour. The digestive diverticula is no longer visible.	4
Ripe	Gonad highly turgid and consistent in colour, follicles not apparent.	5

** Detailed descriptions of the superficial appearance of gonad during development have been given by Tranter (1958a). The criteria for scoring used here are based upon the portion of the body exposed when the mantle and gill are folded back, that is the area anterior and ventral to the urogenital papilla.*

Natural spatfall monitoring

Sets of spat collectors were deployed at 3 – 4 m depth at two sites in Port Stephens where natural spatfall of *P. a. sugillata* had occurred, Wanda Head (32°43'E, 152°05'S) and Tomaree (32°43'E, 152°11'S, Fig. 1). Each collector comprised a 0.5 m^2 (1 m x 0.5 m) sheet of semi-rigid black, 6 mm polythene mesh, folded in a concertina fashion and placed inside a 2 mm mesh orange spat bag (500 mm x 800 mm). A polystyrene float was placed in each bag and the bags were anchored such that they were approximately 1 m above the sea floor.

Four replicate collectors were deployed monthly at each site, beginning in August 1998 and continuing until July 2000. Each set of collectors remained in the water for two months so that, with the exception of the first and last month in the sampling period, two sets of bags were present at any one time. Upon collection, each bag was returned to the laboratory and rinsed gently with seawater to remove silt. The total numbers of spat in each bag were determined and recorded. Due to the considerable morphological similarities between the two pteriid species found in Port Stephens (*P. a. sugillata and P. imbricata*), particularly when small, we were unable to reliably differentiate between the species in all cases. Thus, twenty spat from each collection were then chosen at random and returned to clean spat bags and cultured to a size of > 30 mm for species identification.

5.2.4. Results

A total of 183 *P. a. sugillata* were collected by divers with an average shell height of 69.3 ± 8.8 mm (mean ± s.d.). The majority of oysters collected were between 60 to 80 mm shell height (Fig. 2), but mature oysters ranged from 41 to 112 mm in height and 15 to 144 g in weight. Water temperatures recorded at the time of oyster collections ranged from 14 - 25°C (Fig. 2), while salinity at the site remained within the range 28 to 35 g kg^{-1}.

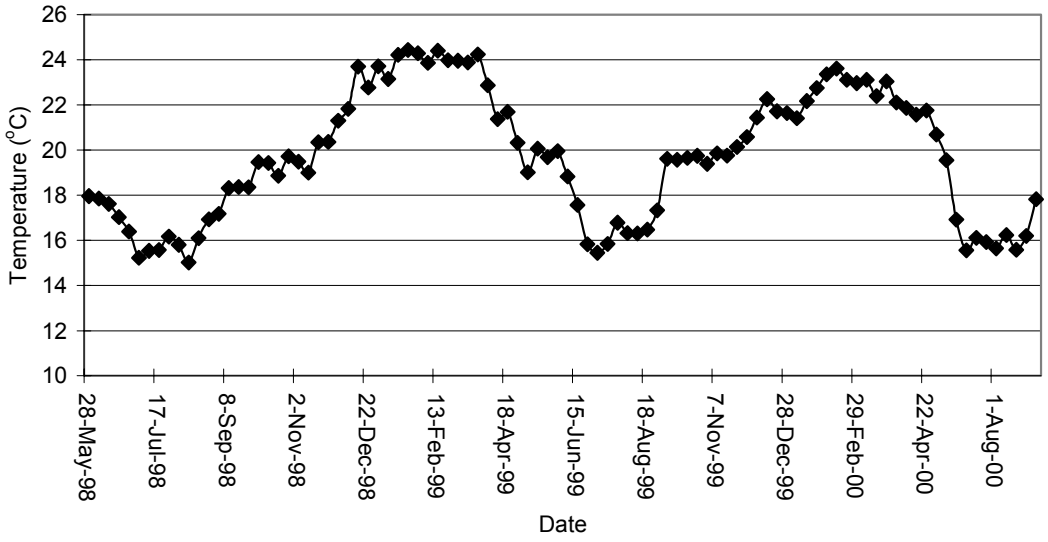

Figure 2. Water temperatures at Wanda Head, Port Stephens.

Ratios of males to females among collections were influenced by shell height. Approximately 65% of *P. a. sugillata* less than 71 mm dorso-ventral measurement (DVM) were male while this ratio was reversed for oysters larger than 70 mm (Fig. 3). Overall 55% of the *P. a. sugillata* collected were male, reflecting the predominance of oysters less than 71mm shell height among the collections. Two of the oysters examined were hermaphrodites with DVM of 75 and 78 mm. In both cases the majority the oocytes remained attached to the follicle wall while spermatocytes were in later stages of development, suggesting a transition from sperm to egg production.

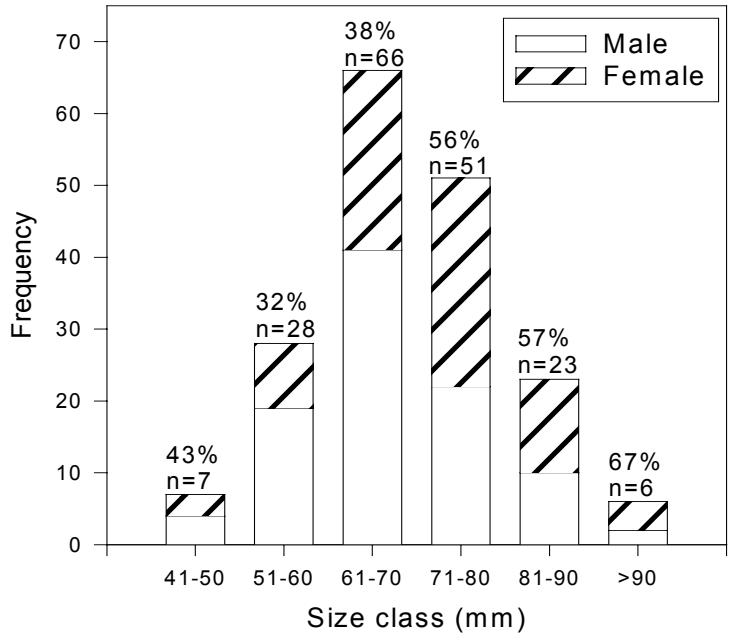

Figure 3. Size frequency and sex ratios of *Pinctada albina sugillata* collected from Port Stephens, NSW, Australia, from July 1998 to May 2000. Figures above columns indicate percentage female for that size class

Reproductive condition

The macroscopic condition of oysters varied significantly over the two years monitored. In both years there were two peaks in reproductive condition followed by marked troughs indicative of either spawning or rapid resorption of gametes (Fig. 4). In 1999, oysters climaxed in condition in October and again in March 2000. Condition was poor through the late winter and early spring 2000 (Apr. – Oct.) before improving to peak in December. As in the previous season there was a second smaller peak in condition in autumn, on this occasion one month later in April 2001.

Histological changes in gonad condition were in general supportive of the macroscopic observations (Fig. 5). Peaks in macroscopic condition occurred in collections with high proportions of histologically ripe gonads, however macroscopic observations were unable to distinguish clearly between ripe gonads and those that had entered the first regressive stages. This led to a tendency for the maintenance of high macroscopic scores in situations where gonads had begun to regress. The histological condition of oysters during the troughs following each peak differed greatly. Following the peak in October 1999, histology showed oysters in either spent or partially spawned condition, consistent with a major spawning event. In months following the three remaining peaks, gonads in a partially spawned or regressing state dominated histological samples. This was particularly so for the two autumnal peaks in March 1999 and April 2000, where more than 60% of the gonads had entered a regressive stage.

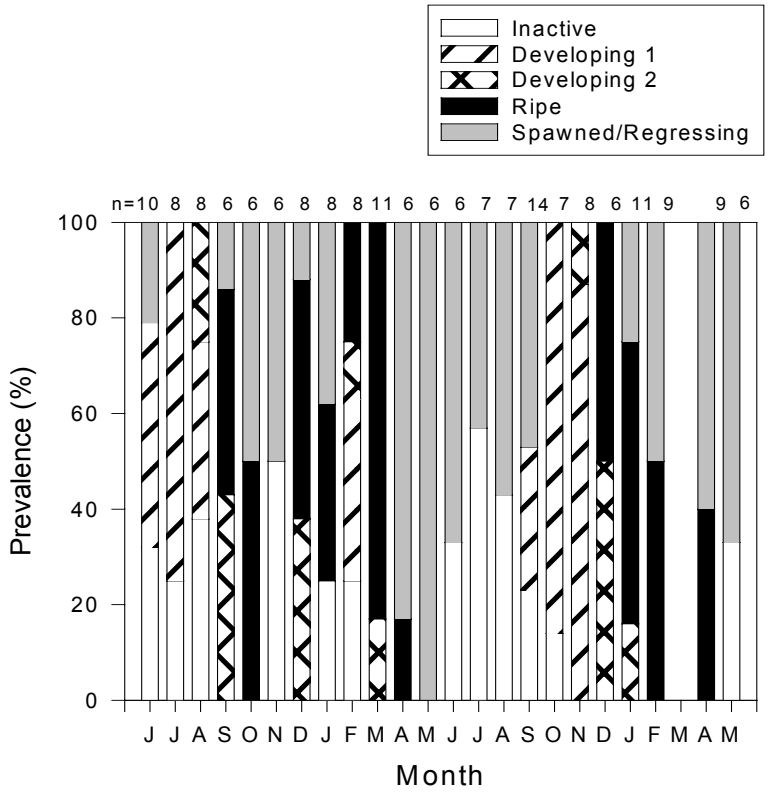

Figure 4. Changes in macroscopic scoring of reproductive condition of the pearl oyster *Pinctada albina sugillata* in Port Stephens, NSW from July 1998 to May 2000. Values are means ± SE, numbers adjacent to points indicate sample sizes.

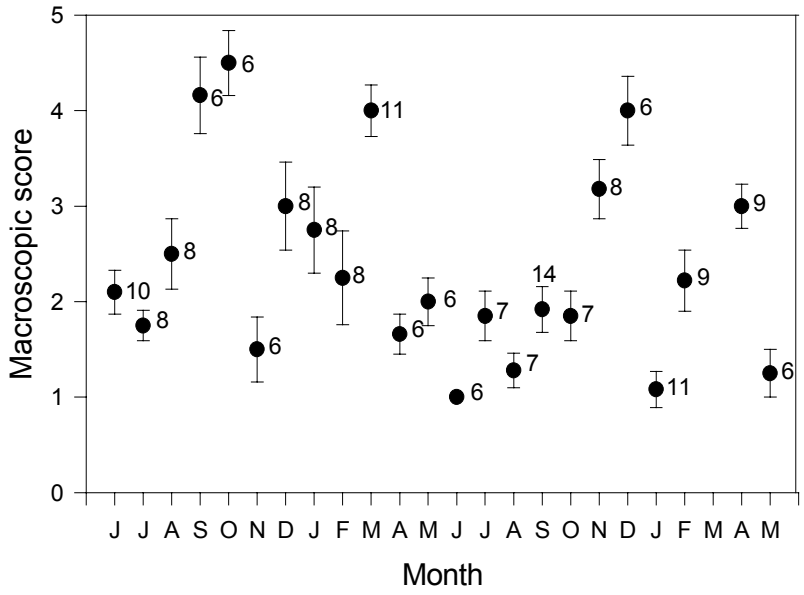

Figure 5. Changes in mean histological scoring of reproductive condition of the pearl oyster *Pinctada albina sugillata* in Port Stephens NSW from July 1998 to May 2000.

Spat fall

Pinctada albina sugillata spat settled on collector bags at both Wanda Head and Tomaree in both 1999 and 2000, but settlement was confined to the months of November, December and January. In 1999, spat fall at Wanda Head preceded that of Tomaree, occurring predominantly in November, and the numbers of spat collected were almost five-fold greater than at Tomaree, approx. 45 - 50 bag^{-1} and 8 - 13 bag^{-1}, respectively. In 2000, spatfall occurred concurrently at both sites in January with similar low numbers of spat collected (< 10 bag^{-1}). The potential for post settlement loss of spat from the collector bags is unknown; however, no evidence of mortality (dead or damaged shells) was found.

Shell damage

Upon inspection, 30% of *P. a. sugillata* shells had blisters indicative of spionid infestation. The degree of infestation was relatively low with by far the majority of oysters (> 95%) having less than 5% of the surface area of one valve affected. Blisters varied in their position on the shell and in morphology, but, were generally small, (< 0.5 cm^2) ovoid to irregular in shape, with their longer axis oriented toward the shell margin. Occasionally, the blisters had tubes extending to the shell margin, but more commonly an entrance to the blister could be found on the external surface of the shell. In the latter case the tube would enter the shell obliquely as if to have occurred between the periostracal layers rather than having been bored vertically through the shell directly to the site of the blister. The numbers of oysters with spionid infestations differed with size class, with a tendency for prevalence to increase with oyster size (Fig. 7).

Several shells from larger, presumably older, oysters were found to have branched pattern of damage extending through the prismatic and nacreous layers of the shell. The pattern of the perforations was consistent with those caused by boring sponges, Cliona spp. in *Pinctada maxima* and suggested that the infestation had begun near the hinge, possibly at a site at which the periostracum had been removed.

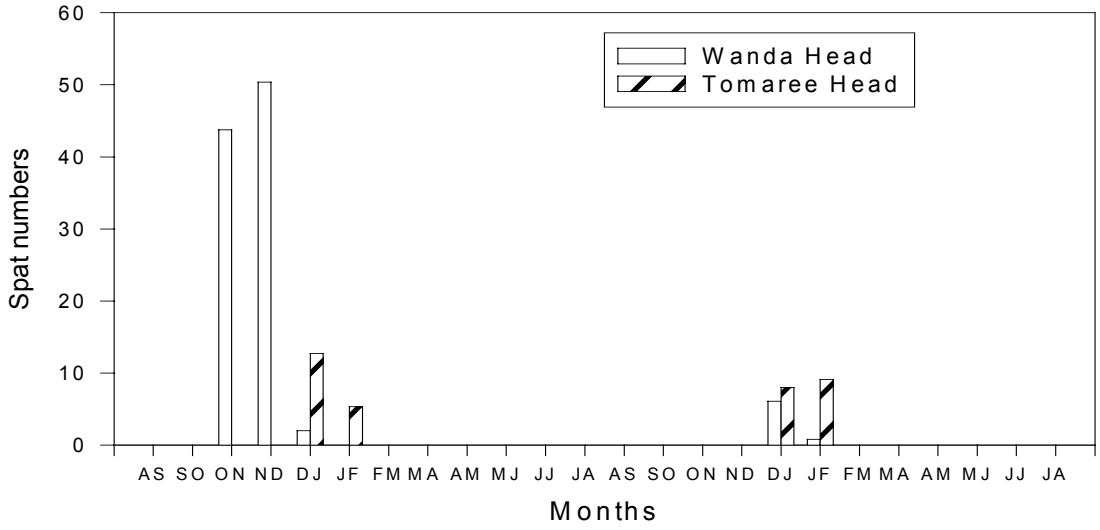

Figure 6. *Pinctada albina sugillata* spat fall at two sites in Port Stephens, NSW, Australia, from July 1998 to August 2000.

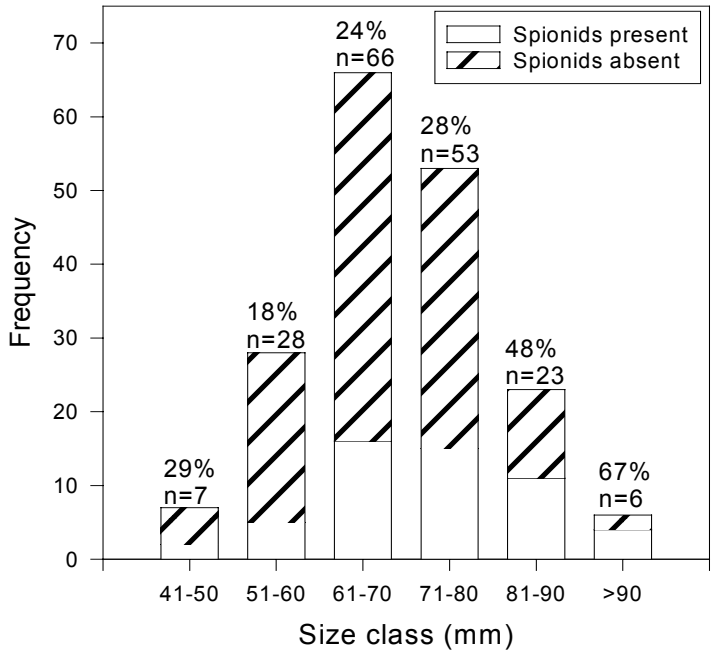

Figure 7. Size frequency and spionid polychaete infestation of the shells of *Pinctada albina sugillata*. Figures above columns show the percentage of oysters in that size class with Polydora sp. infestations.

5.2.5. Discussion

In many respects *P. a. sugillata* collected from Port Stephens exhibited characteristics that were similar to those described by Tranter (1958a, b, c) for more northerly populations. Oysters from Port Stephens were capable of reaching sexual maturity by the time they had reached 41 mm DVM. Based upon observations of the spat retained for species identification, this size is achieved within their first year, although this is not likely to have been reached within 6 months as thought possible at Thursday Island (Tranter, 1958a). Several observations were made that were consistent with protandric hermaphroditism. Notably, there was a tendency for maleness among smaller oysters that decreased progressively with increasing oyster size and two hermaphrodites were found.

It is within the timing and duration of breeding activity that the greatest differences between the *P. a. sugillata* populations of Thursday Island and Port Stephens lie. Although the numbers of animals available to this study were small, a distinct seasonal pattern in the reproductive activity of *P. a. sugillata* was evident, a pattern that appears to be strongly influenced by water temperature. In both years studied, oysters from Port Stephens were in relatively poor reproductive condition in late winter and early spring when water temperatures were at their lowest. Unlike Thursday Island, no oysters in ripe condition were found in the winter months of June, July or August (Fig. 5). As temperatures increased so too did the reproductive condition until a spawning is thought to have occurred in October 1999 and December 2000. Evidence for this spring/summer spawning could be seen in both the fall in reproductive indices in the following month (Fig. 4) and in the recruitment recorded in November - December 1999 and January 2000. Following each of these putative spawning events, water temperatures remained high and the oysters regained reproductive condition to show a second, autumnal peak in reproductive condition. Although concurrent with the major spawning peak observed at Thursday Island (Tranter 1958c), this second reproductive peak in Port Stephens differed from the first. In this case the oysters were in macroscopically poorer condition despite the potential over-scoring of early stage regressing gonads. The histological condition of gonads following the peak was more frequently indicative of gonadal regression than spawning; and there were no subsequent recruitments.

The pattern of reproductive behavior in *P. a. sugillata* in Port Stephens is in contrast to the observations of continuous breeding and recruitment at Thursday Island (Tranter, 1958a, b, c), but is consistent with patterns of latitudinal change seen in other pteriids (Tranter 1958c, Wada et al., 1995). As suggested by Tranter (1958c) the breeding season of *P. a. sugillata* in higher latitudes has been confined to the warmer months. This would suggest that at locations between Thursday Island and Port Stephens some intermediate variant of this pattern should occur. While there have been no other reports of long term monitoring (> 12 months) of reproductive condition in *P. a. sugillata*, recruitment studies of pteriids in general have been done which support a progressive reduction in the duration of the breeding season. Recruitment of *P. a. sugillata* at Orpheus Island occurred over a 10 month period (Beer and Southgate, 2001). A duration that is intermediate to the year round recruitment at Thursday Island and the 1-2 months observed in Port Stephens.

In addition to supporting a progressive truncation in breeding seasons, observations at Orpheus Island are also suggestive of a transition in the relative importance of season in recruitment. At Thursday Island, peak recruitment occurred in winter (June-Aug.; Tranter, 1958c). At Orpheus Island recruitment occurred in winter, but was greatest in the summer months of Jan.-Feb. (Beer and Southgate, 2000). In Port Stephens recruitment occurred only in the months of Nov-January.

Reports of concordance between reproductive condition and temperature in some pteriid populations at higher latitudes and an apparent lack of breeding periodicity among tropical populations led Tranter (1958c) to suggest the existence of a critical temperature for breeding. "*this temperature is reached in higher latitudes only during the summer, but in lower latitudes is*

exceeded all the year round". Subsequently the notion of degree-days has been used more commonly to describe the time required for bivalves to reach reproductive condition. For *P. imbricata* in Japan the critical temperature has been found to be 13^{o}C and that 700 – 800 degree-days are required to achieve spawnable condition (Wada 1991). In the case of *P. a. sugillata*, reproductive condition appears to improve as temperatures exceed 17 - 18^{o}C; however, their decline in autumn begins prior to temperatures falling to this level.

Shell damage

Infestations of spionid polychaetes, known locally as mudworms, are an impediment to the culture of numerous bivalves and pearl oysters are no exception. Shell boring spionids are thought to "fatigue" the host pearl oyster (Wada, 1991) and to weaken their shells, increasing their susceptibility to predators and increasing the numbers of shells broken during nuclei insertion operations. Three spionid species have been reported in NSW, *Polydora websteri*, *P. haswelli* and *Boccardia chiliensis* (Skeel, 1979). Despite moderate prevalences, the extent of spionid infestation in individual *P. a. sugillata* was so low that it was thought to pose little threat to the physiological condition of the oyster.

Culture

The absence of the economically more important pteriid species *P. maxima and P. margaritifera* from NSW waters has meant any potential for periculture is reliant upon the native species *P. a. sugillata* and *P. imbricata*. While both these species are attracting some interest, these findings have a number of implications for any potential that may exist for the culture of *P. a. sugillata*. At the outset of this research an extensive survey of *P. a. sugillata* in NSW (Ponder and Colgan, unpublished data) had shown that native populations were small and thus unlikely to support wild harvest. Therefore any attempts to establish periculture with this species are likely to be reliant upon the collection of natural spat or hatchery production. This study has shown, in Port Stephens at least, that while spat fall is confined to a short period within the year, its intensity is low and variable between years. Without a marked improvement in methods, this is likely to discourage a reliance on the collection of spat for seed supply. Fortunately, *P. a. sugillata* has been artificially propagated in significant numbers in NSW (W. O'Connor, unpublished data) and given the potential for genetic selection in periculture, this may well be the preferred means of spat supply. It is possible the restricted breeding season could inconvenience hatcheries; however, the spring-summer peak in reproductive activity coincides with the most desirable time to place spat in the field. At this time water temperatures are increasing and phytoplankton blooms occur off the NSW coast, which combine to promote rapid growth rates in many local bivalve species.

In addition to any culture potential, *P. sugillata* has been used to produce hybrids with *P. fucata* (= *P. imbricata*; Shirai, 1994) in India (Velayudhan, 1987). While the exact relationship between *P. sugillata* and *P. a. sugillata* is unknown, an understanding of the reproductive behavior of *P. a. sugillata* would be of value should any attempt arise to artificially propagate pteriid hybrids.

5.3. Early ontogeny and nursery culture of pearl oyster, *Pinctada albina sugillata*

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5.3.1. Abstract

Larvae and spat of the pearl oyster, *Pinctada albina sugillata* were cultured to assess their potential for production in commercial bivalve hatcheries and to compare early ontogeny with that of a sympatric species, *Pinctada imbricata.* Adults were induced to spawn using intragonadal serotonin injections, emersion and temperature shocks. Eggs averaged 54 µm in diameter. At 26°C, first and second polar bodies were evident after further 18 and 35 min, respectively, while first cleavage occurred within 50 min of fertilisation. Embryos reached trochophore stage within 8 h before developing to D-veliger stage (75.3 µm antero-posterior measurement (APM)) within 24 h. Umbonate larvae (117 µm APM) were present on Day 9 and pediveligers (206 µm APM) on Day 19. Plantigrades (235 µm APM) were first observed in significant numbers on Day 23, but larvae continued to metamorphose over the following week. Early ontogeny in *P. a. sugillata* was both temporally and morphologically similar to that reported for other members of the genus. Larval and spat survival was high, with an estimated 27% of D-veligers surviving settlement. Post settlement survival was also high with > 90% of settled spat surviving to deployment size of 5 mm after 12 weeks. The ease of spawning, the similarities in development with other pteriids and the comparatively high survival of *P. a. sugillata* suggest this oyster can readily be propagated in commercial bivalve hatcheries.

5.3.2. Introduction

Ontogenetic studies of commercially valuable molluscs have been done for a various reasons. Often to establish protocols for artificial propagation and/or to assist in the identification of early developmental stages of molluscs collected from the field. Such studies have been done for several pteriids (Minaur 1969, Alagarswami et al.1983, 1989, Rose and Baker 1994), but there is a paucity of information regarding the pearl oysters of the *Pinctada albina* species complex.

Two subspecies of *Pinctada albina, Pinctada albina albina* (Lamarck 1819) and *Pinctada albina sugillata* (Reeve, 1857) are found in Australian waters. *P. a. albina* in northern Western Australia and *P. a. sugillata* down the north eastern coastline from the Torres Strait Islands to New South Wales (Lamprell and Healy 1998). *P. a. albina* has attracted commercial interest (Morton et al. 1998) and is currently farmed in Western Australia. *P. a. sugillata* has been of little importance despite possessing several morphological characteristics that offer potential advantages in pearl culture. *P. a. sugillata* grows to a greater size and is more convex than its conspecific (Lamprell and Healy1998) providing the potential to produce pearls of a greater size and thus potentially greater value.

Pearl culture in NSW is in its infancy and if *P. a. sugillata* is to be exploited it is essential to establish hatchery techniques to provide seedstock. Alternative strategies of harvesting adults or spat from the wild are both impractical. The numbers of adult *P. a. sugillata* in NSW waters are low (Colgan and Ponder) and will not support large-scale harvest. Natural recruitment has also been low (O'Connor 2002), negating deployment of spat collectors as a routine source of seed.

The regular presence of *P. a. sugillata* in collections of pteriid oysters from Port Stephens allowed

the propagation the species and thus an opportunity to observe development, settlement and spat growth. Further, this study allowed an assessment to be made of the potential for existing hatchery facilities to produce *P. a. sugillata* spat.

5.3.3. Materials and Methods

Broodstock and spawning

Two separate spawnings were done using *P. a. sugillata* (58-102 mm shell height) gathered from Wanda Head, Port Stephens, NSW (32°45'S, 152°10' E). In the first spawning, 18 oysters were cleaned of fouling organisms and held in a recirculating system in the hatchery at Port Stephens Fisheries Centre. After two days, six oysters were taken from the broodstock conditioning system and relaxed in a seawater bath containing propylene phenoxytol (2 mg l^{-1}). Four of the relaxed oysters were males and were injected intragonadally with serotonin solution (0.05 ml, 10^{-3} M creatinine sulphate solution). Concurrently, 12 oysters were emersed for 45 min at approximately 30°C. All 18 oysters were then placed in an aerated 200 l bath of seawater. Once spawning had commenced the oysters remained in the 200-l bath for a further 30 minutes before they were removed and returned to a conditioning system. The zygotes from this spawning were collected and used for larval rearing trials.

Due to the imprecision of group spawnings with regard to the exact time of fertilisation, a second spawning was done in an attempt to document development during the first 24 h. In this instance, gametes from four male and four female oysters were collected and were "activated" in a 0.5% ammonium hydroxide solution for 20 min (after Wada 1953, Minaur 1969). Four individual fertilisations were done, each with the gametes from one male and one female. Progeny of each cross were stocked in an individual 8-l aquarium and maintained at 26°C.

Larval rearing

Techniques for rearing *P. albina* larvae were similar to those described for other pteriids, notably by Minaur (1969), Alagarswami et al. (1983, 1994) and Rose and Baker (1994). The contents of the spawning bath were siphoned through a 118 µm nylon mesh sieve and retained on a 20 µm sieve to attempt to remove debris and excess sperm. The zygotes were then stocked at a density of 18.5 ml^{-1} into a 1000-l aerated, polyethylene tank of seawater held at 26 ± 1°C. All seawater (34 g l^{-1} salinity) used was filtered with 1 µm (nominal) cartridge filters. After 24 h, the tank was drained and D-veliger larvae were collected on a 45 µm sieve. A total of 5.1 x 10^6 larvae were then stocked into fresh seawater in a second 1000-l tank. Larvae were sampled daily, from which 30 larvae were chosen at random for size determinations. Seawater was changed thrice weekly at which time larvae were placed in a clean tank of freshly filtered seawater (26 ± 1°C). Larval survival was determined at each water change.

Larvae were fed a mixture of Tahitian *Isochrysis* aff. *galbana*, *Pavlova lutheri* and *Chaetoceros calcitrans* on an equal dry weight basis in accordance with the feed curve described by O'Connor and Heasman (1997). The daily algal ration was divided equally over a morning and afternoon feeding.

Nursery culture

Nursery culture techniques used for the production of *P. albina* were a departure from those described elsewhere for pteriids. In preference to the settlement of spat directly to some form of mesh or plate collector, spat were settled using techniques more akin to those developed for the scallop *Pecten fumatus* (Heasman et al. in press). When the bulk of larvae had reached pediveliger

stage they were collected on a sieve and put to set on 450 mm diameter, 150 µm mesh screens (without culch) in downweller units (Utting and Spencer 1991). Each screen was stocked with approximately 2 x 10^5 larvae and placed in a 1 700-l settling/nursery system, as used for oyster larvae (Bayes 1981). Water in the downwelling systems was maintained at 25 ± 1°C and was changed thrice weekly with temperature equilibrated, 1 µm filtered seawater. Each screen was rinsed with seawater at each water exchange.

After 26 days in the downweller system, spat were gently removed from the screen surface with a soft-bristle paintbrush and washed into 350 µm mesh screens (~ 5 x 10^4 spat screen $^{-1}$). These screens were placed in upweller units at Wanda Head, Port Stephens, which were supplied with raw seawater drawn from a depth of 2-3 m. The upweller units were drained thrice weekly and the spat were gently rinsed to remove silt.

5.3.4. Results

As previously reported (Tranter 1958), the gonads of male and female *P. albina* are creamy white and pale yellow, respectively. When injected with serotonin males began spawning within 20 min, followed by several males from the emersed group. The first of the females commenced spawning 35 min after immersion in the spawning bath and the eggs averaged 54 ± 0.5 µm in diameter (mean ± SD, n = 30). In the initial mass spawning, first and second polar bodies were evident after a further 18 and 35 min, respectively, while first cleavage resulting in two unequal blastomeres occurred within 50 min of fertilisation. Subsequent cleavage was spiral and morulas developed within 3 h. The embryos then progressed through a ciliated gastrula stage to become trochophores after 8 h. In strip spawned, ammonia activated, eggs, development was far more variable. Polar bodies were not observed within 20 min of fertilisation and were uncommon within 30 min. First cleavage in stripped eggs occurred after 80 min and, although it was not recorded for normally released eggs, it was apparent that the percentage of stripped eggs undergoing cleavage (55 ± 19%) was markedly reduced. Approximately 10% of zygotes appeared to be developing abnormally and the percentage of zygotes developing to D-veliger was relatively low (21 ± 13%).

After 24 h, 92% of the fertilised eggs from the mass induced spawning had developed to D-veliger stage and the prodissoconch I shell had a mean antero-posterior measurement (APM) of 75 µm and dorso-ventral measurement (DVM) of 63 µm. By Day 9, larvae had reached an average of 117 ± 7 µm APM and had entered the umbonate stage. On day 15, eyespots were first observed and development of the foot was evident among larger larvae (175 - 180 µm APM). On Day 21 larvae had reached a mean shell length of 202 ± 16 µm APM. At this time 36% of larvae were crawling pediveligers (> 206 µm APM) and the entire batch was put to set.

The growth of larval *P. a sugillata* as indicated by increases in shell length was found to be largely linear, with a tendency to increase slightly toward settlement (Fig. 1). The growth rate of larvae was described by the equation: SL = 61.4 + 6.5D (r^2 = 0.98), where SL is the shell length of larvae and D is the age of the larvae in days. The relationship between shell width and height was best described by the equation: APM = 5.7729 + 1.0276 DVM (r^2 = 0.99). Larval survival remained high throughout the larval rearing period, so much so, that on Day 17, almost half the larvae were discarded so that densities were reduced from 4.66 to 2.76 larvae ml^{-1}. The remaining larvae were cultured for a further five days before 1.88 million larvae were placed on settlement screens on Day 21. Overall percentage survival to settlement, corrected to account for larvae discarded, was 58%.

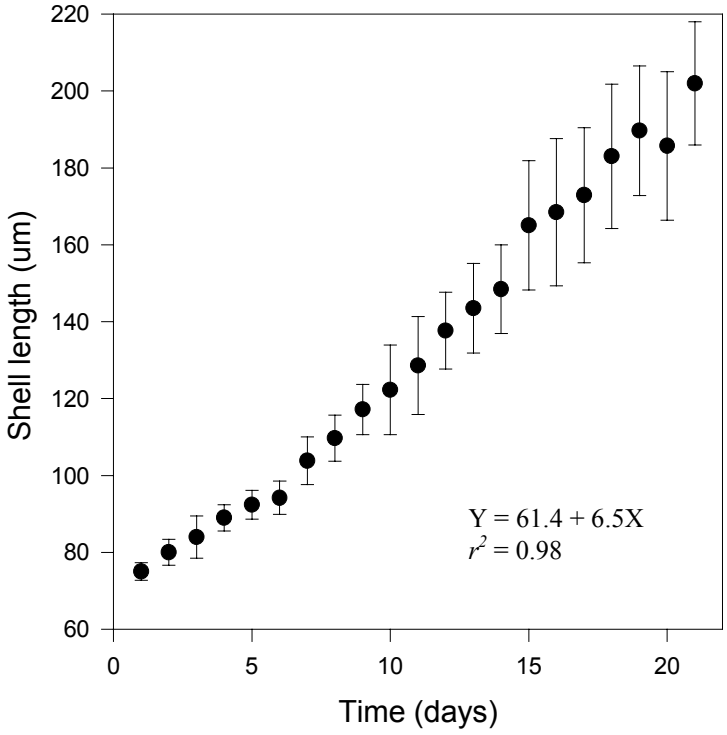

Figure 1. Growth of *Pinctada albina sugillata* larvae.

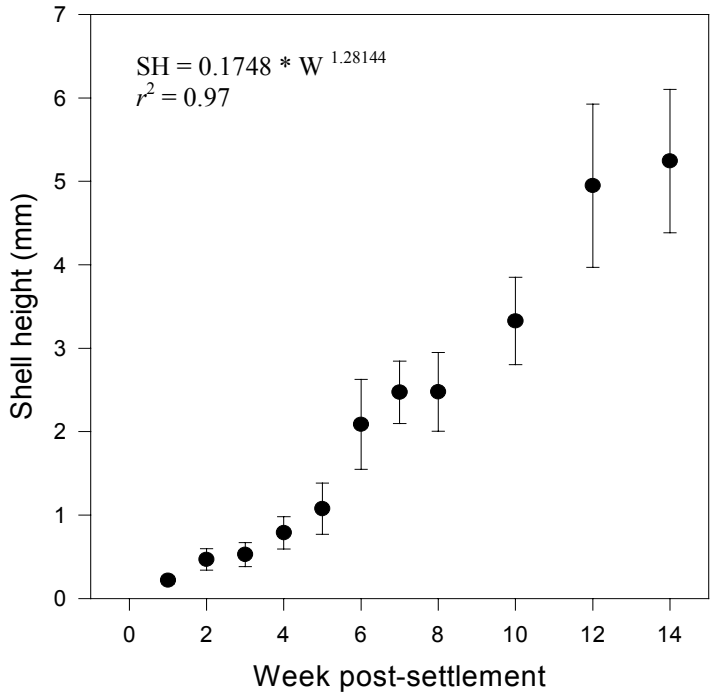

Figure 2. Growth of *Pinctada albina sugillata* spat.

The majority of larvae that were ultimately to metamorphose did so between Days 23 and 25. However, residual larvae continued to metamorphose over the following week until approximately 47% had entered the plantigrade stage. The remaining larvae died progressively over the following two weeks, apparently incapable of settling. The mean maximum size of prodissoconch II shells were 235 µm x 220 µm (APM x DVM). The delineation between the darker prodissoconch II and the subsequent dissoconch shell was strongly marked. Initially, deposition in the dissoconch appeared as a thin, less convex, transparent reticulated layer, which increases slightly in opacity as spat grow. Later, the shell becomes a light golden brown with the prismatic pattern of the columnar shell clearly visible in spat of up to 6 mm. As spat exceed 2 mm, the first in what was to become a row of imbricate shell processes radiating outward from the umbo could be seen to develop. As the spat grew further the number of rows increased, however these processes were delicate and often destroyed by handling or cleaning.

Spat held on mesh screens tended to spread out more or less uniformly across the settlement screen surface and to a lesser extent would also attach to the walls of the screens. With time, both in the hatchery and at the remote nursery site, spat also climbed the walls of the screen to beyond the water surface, requiring the use of brushes or plastic spatulas to detach them and return them to the water. From observations made in earlier emersion tolerance trials (W. O'Connor pers. obs.), these self-emersed spat survived for considerably longer than expected (2-3 days). Samples of these spat located to separate screens showed that this behavior did not result in significant mortality.

Post-metamorphic shell growth was exponential in nature and described by the equation $SH = 0.1748 * W^{1.28144}$, where SH is shell height (DVM) and W is the number of weeks post settlement. After 26 days in the downweller system, spat had reached a mean shell height of 790 ± 190 µm and were transferred to 350 µm mesh screens. Spat were maintained until they had reached a mean shell height of approximately 5.5 mm before they were discarded due to competing demands on nursery facilities. Observations of spat for approximately 14 weeks post settlement found that spat mortality was low, less than 10%.

5.3.5. Discussion

In all respects the early ontogeny in *P. a. sugillata* is similar to that reported for other pteriids both morphologically and temporally. Morphologically most bivalves develop through trochophore and veliger stages before metamorphosing from the plankton into the adult form. For *P. a. sugillata* the dimensions of the various ontogentic stages are broadly similar to those reported for other pteriids, especially during the planktonic veliger stages (Tables 1 and 2). In particular, the ratios of dimensions, notably DVM/APM that has been used to assist larval mollusc identification, are very similar (Table 2). Indeed, so much so that they precluded the use of shell of measurements to reliably differentiate between the two species (*P. a. sugillata and P. imbricata*) found in Port Stephens (Fig. 3). While this prevents larval differentiation on a size basis, it is of some assistance to those who may be interested in pteriid culture. The similarities allow the use of common equipment such as sieves and setting screens without modifications or additions. Importantly it implies that hatcheries such as those in Western Australia, that are increasingly looking toward the production of pteriids other than *P. maxima*, can do so with existing equipment.

Table 1. Comparison of characteristics during early ontogeny in the larvae of four pteriid species.

	P. a. sugillata		*P. maxima*		*P. imbricata*		*P. margaritifera*	
	Size (µm)	**Time**	**Size (µm)**	**Time**	**Size (µm)**	**Time**	**Size (µm)**	**Time**
Egg	54	-	60	-	47.5	-	45	-
D-veliger	75.3	20-22 h	79	18-24	67.5	20 h 40 min	75	24 h
Umbonate larvae	117	Day 9	114	Day 10	135	Day 10-12	110	Day 9
Pediveliger	206	Day 21	211	Day 22-24	230	Day 20	220	Day 20
Plantigrade	235	Day 23-25	270	Day 24-25	250	Day 22	260	Day 23
Author		This study	Rose and Baker (1994)		Alagarswami et al. (1983)		Alagarswami et al. (1983)	

Table 2. Comparison of shell dimensions of larvae and spat in four pteriids.

Species	**Larvae**	**Spat**		**Spat**	
	DVM/APM	**Size**	**APM/hinge**	**Size**	**DVM/hinge**
P. a. sugillata	0.921	0.4–2.5 mm	1.08	0.4–2.5 mm	0.82
P. margaritifera	0.896	3.8 mm	0.7 - 0.9	3.8 mm	0.7
P. imbricata	0.904, 0.890*	1.0 –3.0 mm	0.96	1.0 –3.0 mm	0.76
P. maxima	0.885				

Data: P. a. sugillata *from this study,* P. margaritifera *from Alagarswami et al. (1989),* P. imbricata *from Alagarswami et al. (1983) and * Ota (1957),* P. maxima *from Rose and Baker (1994).*

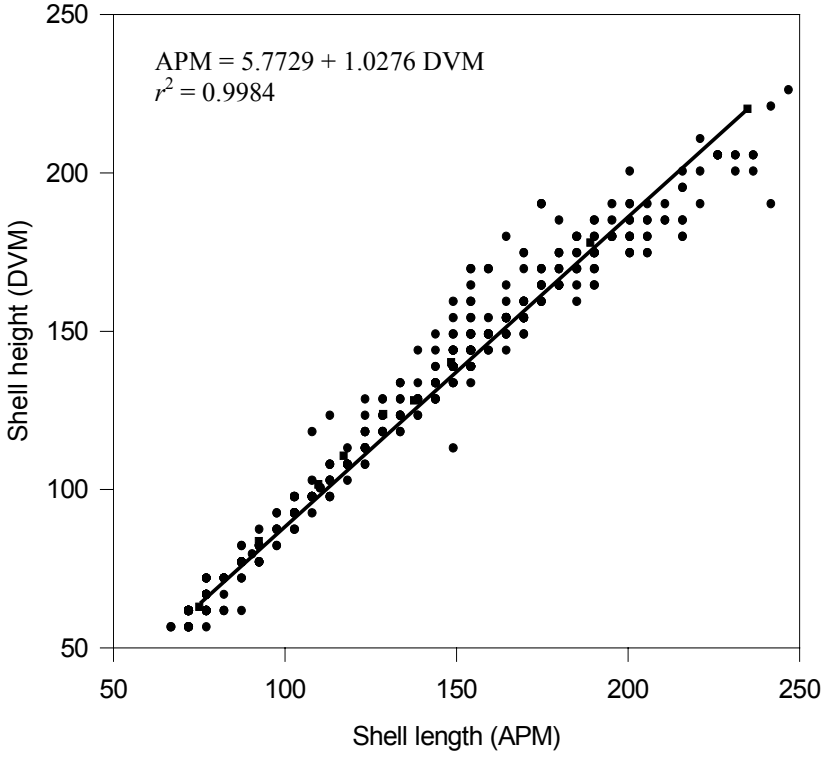

Figure 3. The relationship between shell height (dorso-ventral measurement) and shell length (antero-posterior measurement) for *Pinctada albina sugillata* (line) superimposed observations of shell height and length for *Pinctada imbricata* larvae (n = 468).

Temporally, it has been common to compare the rate of development among pteriids and in this respect *P. a. sugillata* also follows a similar pattern; D-veligers within 24 h, umbonate larvae on Day 9, pediveligers on Day 19 and plantigrades on or about Day 23 (Table 1). However, some care should be taken in the interpretation of this and similar ontogenetic comparisons. In some instances the published observations arise from early attempts to produce the particular species, as is the case here, and have not benefited from refinement of techniques and protocols for rearing. Further, many of those experienced in pteriid larval culture report far greater variations between larval batches within species than would be suggested to exist between species by Table 1. For example, the time to commencement of metamorphosis for *Pinctada imbricata* varies from 13 to 25 days (Wada 1991) and in our laboratory, under relatively uniform conditions, has ranged from 16 and 23 days (O'Connor unpub. data). In addition, reports of early ontogeny can also give the impression of homogeneity in development to particular stages, which of course is not the case. As highlighted by Rose and Baker, metamorphosis to plantigrade, is often protracted and in the case of *P. a. sugillata* occurred over more than a week. Thus the times chosen to reflect the rate of development in a species (see Table 1) require some arbitrary characteristic to be used, often the date on which the particular stage was observed. Despite these caveats temporal comparisons are also of value to those contemplating hatchery culture in that they provide an indication of the time demands and scheduling required for production runs of a particular species.

With the exception of strip spawned zygotes, the overall survival of *P. a. sugillata* larvae and spat have been outstanding. In the studies of Alagarswami et al. (1989) and Southgate and Beer (1997) the percentage of *P. margaritifera* larvae to metamorphose was 6.3% or less. For *P. maxima*, Rose

and Baker (1994) reported an average survival of larvae to plantigrade of less than 4%, although more recent reports have indicated percentages of greater than 13% can be achieved (Ito 1998). In contrast, a survival of 27% with *P. a. sugillata* is a promising result and one which is comparable with the 15 – 35% expected with *P. imbricata,* a species considered to be among the most robust of the cultured pteriids (Ito 1998).

Post settlement spat survival on screens remained high and, beyond providing further support for the robust nature of *P. a. sugillata,* also supported the utility of screen settlement systems for nursery rearing. As with scallops, this system was adopted because of the additional control it provides in the rearing process. Quantification of settlement success in collector bags or plates can be difficult and settlement densities often vary greatly among collectors. Known quantities of larvae are placed on each screen allowing control of densities and easy assessment of numbers at hand.

In general, strip spawning as a means of obtaining bivalve gametes has met with mixed success. While reports of poor results are common (Loosanoff and Davis 1963, Chanley 1975, Hooker 1995), there are also accounts such as that of Debrosse and Allan (1991) in which initial survival of *Crassostrea virginica* larvae from stripped eggs exceed that of larvae from natural spawnings. For *P. a sugillata*, development was slower and survival was reduced in embryos produced following stripping. While success may be species specific, in this case stripping is further complicated by the requirement for ammonia activation of eggs. Regardless, *P. a. sugillata* gametes can be stripped and thus the uncertainties surrounding the timing and control of natural spawnings can be reduced.

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7. APPENDICES

Farming the Pearl Oyster, *Pinctada imbricata,* in Southern China

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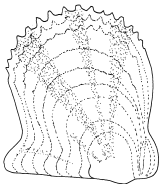
CONTENTS



GENERAL INTRODUCTION

Attempts by NSW Fisheries to develop a temperate pearl oyster industry in NSW are currently under way and have benefited greatly from the considerable expertise of the joint venture partners in this exercise, Australian Radiata Pty Ltd. The focus of this industry is to be placed upon the akoya oyster, *Pinctada imbricata*, which is native to NSW. This oyster however is also probably the most cosmopolitan of the Pinctada species, also being found in both Japan and China where it forms the basis of a billion dollar pearl industry.

The purpose of the visit to China and the province of Guangxi in particular (Figure 1), has been to gain an over view of the Chinese akoya pearl industry in order to look for equipment and techniques that may be of direct use or capable of modification for use in NSW. Techniques for culturing the akoya oyster in China have to an extent developed independently to those in Japan and have made use of quite diverse culture conditions.

The development of pearl oyster culture in Guangxi has benefited from the research conducted by staff of the Guangxi Institute of Oceanography (GIO). The Institute was established in 1978 and houses approximately 90 research staff. Projects range from resource assessments to biotechnology with a total funding of approximately AUD $300,000 annually.

At the forefront of pearl oyster research in Guangxi has been Wang Aimin, Director of the Centre for Biotechnology at the GIO. Research conducted by Mr Wang and his colleagues has been broad ranging including in vitro maintenance of saibo (mantle tissue) and in the development of tetraploid induction techniques. The latter has implications both for pearl oyster production and for tetraploid induction in other species such as the Sydney rock oyster, *Saccostrea glomerata*.

Pearl culture in China: An overview

Pearl culture in China is centuries old and has traditionally been based upon a freshwater mussel species cultured in the eastern central region of China. Although the history of the exploitation of saltwater pearls extends as far back as 200 BC in China, it has only been for the last 30 - 40 years that the interest has extended to farming pearls in the marine environment. Culture has been limited to the southern provinces of Guangxi, Gaungdong and Hainan (Figure 1).

Initially pearl production in China was low (150 kg annum^{-1}) but began to increase in 1970 (300 kg annum^{-1}; Yakushi, 1991). In 1986, the first private farms were established and production rose rapidly. By 1989 pearl production had risen to 4 tons annum^{-1} and although exact production figures are not available, was thought to have risen to about 15 tons annum^{-1} in 1995 (Wang A., pers. comm.). More recently production is thought to have decreased dramatically, purportedly due to environmental degradation, and is thought to be around 10 tons annum^{-1}. Production in the coming season is also expected to be reduced to approximately 7 tons annum^{-1}. Despite some notable exceptions, the overall quality of Chinese pearls is lower than that of its major competitor, Japan.

The bulk of high quality pearls produced are sold overseas, particularly the US, which has recently experienced a 19% increase in the sales of Chinese pearls (AJN, 1999). There is also a thriving local market for pearls and pearl products. Little is wasted, pearl shells are used for decorative work and are popular as a base for cosmetics. Pearl meats are also consumed fresh and dried or are used as chicken or duck feed (Guo et al., 1999).

Chinese researchers and farmers are particularly interested in culturing alternative pearl oyster species found in China and elsewhere. Foremost among those species are the silver lip oyster *Pinctada maxima* and the winged oyster *Pteria penguin*. However production is in its infancy and has not been covered in this report.

HATCHERY PRODUCTION

Introduction

The Chinese pearl industry is founded almost exclusively upon hatchery produced spat which are cheap and readily available. Farmers purchase spat from the hatcheries by weight. Spat are sold when they reach about 1 mm in shell height and number around 2000 g^{-1}. Initially, spat were sold for approximately 200 yuan g^{-1} (AUD$ 7), however, with the proliferation of small hatcheries (about 50 in the Beihai area) the price has fallen to 2 yuan g^{-1}.

Hatchery rearing generally commences from March to May to make spat available for the spring-summer growing season, however, both the GIO hatchery and some private hatcheries were still in operation in October at the end of the visit.

Algal production

Algal production for *P. imbricata* in is based upon two species, *Dicrateria zhanjiangenis* and *Tetraselmis* (formerly *Platymonas*) *subcordiformis*. The former is a prymnesiophte related to *Isochrysis galbana* and is similar in size (5-7 μm) and appearance. *T. subcordiformis* is larger (11-16 μm) and similar in appearance to *Tetraselmis tetrathele*.

At the GIO, both *D. zhanjiangenis* and *T. subcordiformis* are reared in 500 mL flasks and 10 L glass jars holding approximately 100 mL and 3 L of culture, respectively. Seawater for these cultures is transported to the GIO in 50 L drums and is boiled prior to use. When cool, growth media (Table 1) and an algal inoculum are added and the culture is capped with brown paper held in place with an elastic band.

Figure 1. *Algal culture facilities at a private hatchery, Beihai, Guangxi.*

Equipment suitable for counting cell densities was not available, but cultures were active and estimated to have densities of approximately 5 $x10^6$ cells mL^{-1} (*D. zhanjiangenis*) and 1 $x10^6$ cells mL^{-1} (*T. subcordiformis*).

Media used to culture algae at farms varies, but is largely based upon fertilisers and urea. Culture vessels include plastic bags, glass aquaria, earthenware pots and concrete tanks up to approximately 1500 L in volume.

Table 1. Algal growth medium (Guangxi Institute of Oceanography)*

$NaNo_3$	60g L^{-1}
NH_4NO_3	60g L^{-1}
Urea	60g L^{-1}
KH_2PO_4	10g L^{-1}
$NaSiO_3$**	10g L^{-1}
Ferric citrate***	1g L^{-1}
Fish juice#	

* The media is added to seawater at a rate of 1 mL L^{-1}
** NaSiO3 is used despite the fact that neither species is a diatom
*** Ferric citrate is boiled to dissolve and is not accompanied by a chelating compound.
# Fish extract is made by boiling dried fish to produce a pungent brown liquid that is added in small quantities to the medium.

Algal production is occasionally unreliable, particularly for *D. zhanjiangesis*, and thus a substitute is often used. In china, *Saccaromyces cerivisae* (Bakers yeast) is commonly available in a dry powder or tablet form and is often used as a medicinal digestive aid. *S. cerivisae* is used as a substitute or addition to larval diets during the first 4-10 days of development. Researchers from the Ocean University of Zhanjiang also reported farmers using a local "beer" as a food substrate, again presumably a source of yeast. These yeasts are only substitutes for algae and are inferior feeds, possibly contributing to the slow larval growth rates observed in Chinese hatcheries.

Spawning

Natural spawning
Researchers and hatchery managers are aware of simple temperature and emersion techniques for the initiation of spawning in *P. imbricata,* however these are not commonly used. The convenience of strip spawning is preferred despite an acknowledgment that the quality of the resultant eggs is more variable.

Strip spawning
Oysters for strip spawning are selected largely from farm stock and are transported to the laboratory where they are cleaned of fouling and soaked briefly (approx. 5 min) in a disinfectant solution, commonly potassium permanganate. The oysters are then opened and a pipette is inserted into the gonad to draw gametes from the body of the oyster. Great care is taken not to puncture the digestive gland beneath the layer of gametogenic tissue. The eggs are pipetted into a beaker of seawater and allowed to settle to the bottom. After standing for 10-15 minutes, the seawater is siphoned off and the eggs are again resuspended in seawater. Eggs can remain in the seawater for up to 2 h before use without ill-effect (Yan Bing pers comm.).

Both eggs and sperm are "activated" with an ammonium solution. In practise, 2 drops of a 25% ammonium are added per L of gamete suspension. Prior to fertilisation eggs are passed through several layers of wet gauze and sperm is then added by eye. Polyspermae is not considered a significant problem with *P. imbricata* embryos, although microscopic inspection of newly fertilised egg suspensions showed the volumes of sperm used did not result in excessive numbers of sperm present at the surface of the eggs.

Figure 2. *Strip spawning Pinctada imbricata*

Larval rearing

At the GIO, embryo development occurs in glass flasks or small tanks and is conducted at high density (50-60 ml^{-1}). For the first 4-5 hr, prior to the development of trochophore larvae, the eggs are not aerated and permitted to settle to the bottom of the container. Eggs that remain in the water column are considered inferior and are siphoned from the container at regular intervals.

As larvae develop to the trochophore stage they begin to swim and move to the surface of the container. As they "raft" or gather at the surface, "lines" of sinking trochophores can also be seen descending down the walls of the glass containers and are considered to be a sign of good development.

As farmers pay a premium for spat produced early in the season, larval rearing commences in late winter. Water temperatures at this time are suboptimal and so wood fired heaters are used to raise temperatures to a minimum of 20^{o}C. Commonly, the heated water is circulated through a closed system of pipes that are immersed in the larval rearing tanks.

In most hatcheries larvae are cultured in large rectangular concrete tanks, commonly approx. 2 m deep and holding around 20 000 L. Stocking density is not determined accurately, rather a number of females are strip spawned per m^3 of seawater. Larval rearing water is changed daily by placing a mesh-covered siphon into the tank and draining the bulk of the culture water. The larvae remain in the same tank throughout culture.

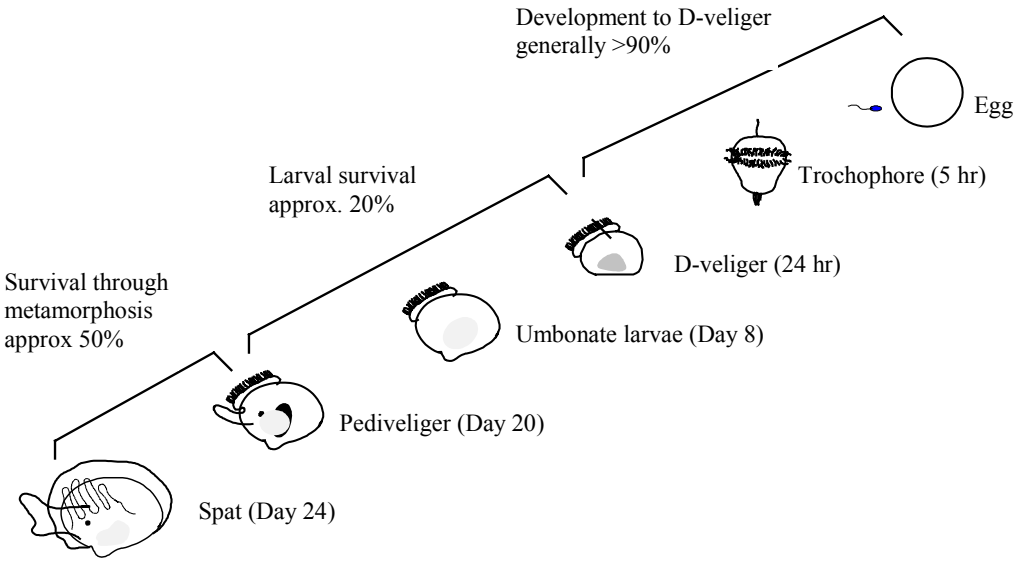


Figure 3. *Development and survival of larval Pinctada imbricata in Chinese hatcheries*

Facilities at hatcheries are simple by Australian or Japanese standards and equipment such as microscopes are not always present. As a result, larvae are fed by eye or by experience. Equipment used in conjunction with larval rearing is cleaned using a potassium permanganate solution.

Figure 4. *Larval culture tank (20 000 L)*

Settlement

As larvae develop to pediveliger stage, settlement collectors are introduced to the larval culture tank. These collectors are made of thin red plastic plates (20 x 20 cm) strung together at intervals of approximately 10 cm (Fig. 5). Each string holds 10-15 plates and approximately 200 strings are placed in each tank. Larvae also frequently settle on the walls of the culture tank where they are allowed to remain.

When spat have reached an average size of about 1 mm they are gently removed from the plates and the walls of the tank using a sponge and sold to farmers.

Nursery culture

Hatchery produced spat are ongrown in the field in fine mesh (approx 1.5 mm) bags similar to those currently used for the collection of scallop spat in Australia. When they have reached a size of 5-8 mm (Guo et al., 1999) they are transferred to cages.

Figure 5. *Collectors for Pinctada imbricata spat*

FARMING TECHNIQUES

Two farming areas were visited in Guangxi which demonstrated markedly different farming practices; Fang Cheng Gang and Beihai.

Fang Cheng Gang

Fang Cheng Gang lies approximately 80 km to the west of Beihai close to the Vietnamese border. The area has been farmed for pearls for almost 30 years however the bulk of farms began in the 80's. The particular farm visited was 4 years old and considered by researchers to be a model farm among the 300 farms in the area.

Figure 6. *Oyster cages*

The farms all lie within a large embayment that is occasionally adversely affected by freshwater intrusions. The tidal range at FCG is approximately 5 m and the temperatures are usually within the range 18-30^{o}C. Each lease is covered at high tide and the reportedly low current flow in the area was a largely product of tidal movement.

Figure 7. *Concrete and timber oyster culture racks*

Farms in FCG were constructed of concrete pylons that are made on site and then driven into the muddy substrate. Local timber from a conifier is placed between the pylons to produce a lattice from which the cages are hung. Most farms have at least one hut positioned on timber struts above the leases for security.

Oysters were grown in two level lantern cages, with approx 70, 50 mm oysters per 35 cm diameter cage. Cages were strung from the timber lattice at a level such that oysters remained submerged at low tide. The cages appear to be quite dense with at least one cage per m^2.

Long lines were present but were not popular, reportedly because of the quality of rope and equipment available for their construction. There was however an acknowledgment from some farmers and researchers that there use is likely to increase in order to permit the expansion of farming in certain areas.

Triploid *P. imbricata* were under evaluation at FCG and farmers reported to them to grow faster, but noted no other characteristics that distinguish them from diploids. At the time of the visit, triploids had not yet been operated (nuclei inserted).

One of the most striking initial impressions of FCG was that the stocking densities used in culture were much higher than expected and that the general condition of the oysters was poor. The finger like extensions of the shell were small and byssal attachments were weak. Gut content was low and the crystaline style was smaller than expected in wild stocks from Australia. Both the development of the glyco-protien layer (referred to as glycogen in Japan) and gonadal development were poor.

Beihai

Beihai has a long history of wild pearl harvests and remains one of the better known pearl farming areas in China. The area surrounding the city currently hosts 500 - 600 farms, mostly small (< 1 ha) and all in relatively shallow water.

Figure 8. *Bottom culture of Pinctada imbricata (intertidal exposure only on spring low tides).*

Given the geographical proximity of Beihai and Fang Cheng Gang, many of the techniques used and the timing of key procedures were in essence the same. The major difference arose from the use of large areas of tidal flats.

Oysters are grown in square aluminium framed cages which are strung between posts driven into the sand (Fig. 8). Each cage houses between 80-100, 50 mm oysters.

As at FCG, the condition of oysters at Beihai appeared to be poor in comparison to Japanese and Australian stocks.

Mortality of pearl oysters in FCG and Beihai was reported to be between 20-50% per annum, with FCG considered to be the better of the two farming areas. The poorer survival in Beihai was ascribed to the remarkably high incidence of a polychaete, referred to in Australia as mud worm. Indeed, of the approximately 300 oysters from Beihai opened during the visit, all showed extensive mudworm damage. Farmers reported mudworm to be a problem and suggested that salting the oysters was effective in reducing its impact. However the condition of the oysters opened suggested that salt was either of limited value or used too infrequently. The mudworm problem is wide spread in Guangxi although severity is site specific.

Figure 9. *Mudworm blisters in Pinctada imbricata shells*

Other causes of mortality included an un-named and poorly understood disease and several predators including marine snails (Latin name unavailable but resembled a Thaid)

Nuclei insertion

Oysters were seeded at 2yrs of age and 5 cm in shell height. The technique is similar to that used by Japanese farmers, however oyster pre-treatment to prepare for nuclei implantation was reportedly uncommon.

Mantle tissue is immersed in dye solution before insertion with the nuclei. Both nuclei and mantle tissue can also be treated with a proprietary solution that was suggested to increase the quality of pearls, reduce nuclei rejection rates and post operative mortality. These solutions are known to contain antibiotics, which is probably small compensation for the standard of the implantation sheds. Commonly two nuclei are inserted however there is the opinion that quality is greater from single inserts.

Nuclei are most commonly inserted in oysters in spring and harvested at the end of the following winter. This allows for a period of rapid nacre deposition over summer, which is then followed by the deposition of a slower, but superior quality, layer of nacre in winter. Some farmers are attempting to increase the quality of their pearls by extending the duration of insertion to include two winters.

The nuclei used were all produced in China from mussel shells from Jiangxi province. Nuclei range in size from 5 to 8 mm but the vast majority of those inserted are 6-7 mm. Good farmers take great care in the selection of nuclei for which they pay around 500 yuan kg^{-1}.

Figure 10. *Nuclei insertion operations*

Pearl harvests most commonly took place in late winter to optimise period of thin nacre growth. Most pearls are sold to processors although some farmers also own their own retail outlets. Prices at the farm gate vary from 2000 to 19 000 yuan /kg depending upon quality. Quality varies according to region, with pearls from Fang Cheng Gang in Guangxi province reported to be of the highest quality in China (Wang Aimin, pers comm.). Pearls from Guangxi and Guangdong were, in general, preferred to those from Hainan.

Management practices vary and were thought by researchers to be the major problem facing many farmers. This is particularly apparent in preparation for nuclei insertion, insertion techniques and post operative care. In contrast to the regimentation of farming techniques demonstrated by Japanese farmers, Chinese farmers can neglect or truncate procedures considered by both Japanese farmers and Chinese researchers to be essential.

GENERAL DISCUSSION

Hatchery production

While it is difficult not to admire the achievements of Chinese hatchery operators under the conditions that prevail, it is also possible to see numerous avenues for improvement that could be done at little to no cost.

Growth and survival of larval *P. imbricata* in China are both low and are likely to benefit from improvements in diet. Firstly, the use of yeast should be limited and more appropriate algal species should be selected. The almost universal condemnation of Tetraselmis spp as a bivalve larval diet would suggest that this species might be more appropriate in only the later stages of culture (post settlement). Similarly the lack of reliability of *D. zhanjiangesis* may warrant the investigation of alternative species. Research institutes such as the GIO could make a positive contribution through the selection of more appropriate algal species. Either species that are of greater nutritional quality or are more robust to the conditions faced in Chinese hatcheries. There is a range of species readily available including some diatom species used for prawn larvae that may be appropriate for *P. imbricata*.

It is common practice to use two or more species for the culture of bivalve larvae, some efforts should be made to broaden the pool of available algal species. Notable inclusions might be *Chaetoceros calcitrans*, Tahitian *Isochrysis* aff *galbana* and *Pavlova salina*.

With the proliferation of small hatcheries, mostly operated by farmers, there is an adequate supply of spat at an almost incomprehensibly low price. In contrast to the price of Sydney rock oyster spat in NSW (approx 2 cents each), *P. imbricata* spat are sold at approximately 0.02 cents each. This is, in one sense, an advantage for Chinese pearl oyster producers, however this has come about through such strong competition that only the most rudimentary facilities and techniques can be afforded. There is no profit margin to accommodate research and there are strong disincentives to any improvements that would increase production costs.

Initially, the limitations on hatchery production have severely limited broodstock selection procedures. In the hatcheries visited, broodstock were collected from the farms in the immediate vicinity, wild stocks were rarely used and there was a lack of understanding of the genetic implications of these practises. On a broader scale, researchers were largely unaware of the state of wild populations in particular in relation to stock variability. It is hoped that study proposed by the GIO involving the use of microsatellites may provide a far greater understanding. It will remain to be seen how rapidly and extensively this knowledge will be adopted in practice.

Industry

The Chinese pearl industry is fortunate in that it has an abundance of cheap labour and materials and has the input of businessmen of great acumen and experience in pearl culture and sales. However, Chinese production has been reportedly falling and faces a number of difficulties.

Without doubt the greatest challenge facing the Chinese pearl industry is the continued degradation of the environment. Pearl production is decreasing and both population and farming pressure are increasing. Despite an acknowledgment that this is occurring the attitude of most sectors associated with the industry was fatalistic. Government is reportedly powerless or disinterested in controlling pearl farming to the degree that they are largely unaware of the numbers of farmers in the industry and have no clear estimates of their production capacity.

Pearl farmers show far greater concern for the environment; however, their response has in many cases been to attempt to grow more oysters to replace falling production. A response previously

shown by Japanese experience to be inappropriate. In certain quarters, large and influential farmers realised the need for rationalisation in the industry and that at that time there would be the opportunity to reduce the density of farms and address some of the issues affecting pearl quality and quantity.

One positive response to the pressures placed upon the environment would be for researchers and farmers to enter into a collaborative arrangement to investigate the carrying capacities of the various farming areas. Many examples of apparent overcrowding were observed and can not be effectively addressed with background information.

While the response by Chinese farmers to the opportunity to discuss pearl culture during the visit was extremely encouraging and there were clearly strong associations between farmers and the researchers at the GIO, the potential for science to assist farmers in the challenges facing the industry were limited. Funding of marine science is so poor that facilities such as the GIO do not have the capacity to adequately respond to industry needs. Further there does not appear to be a coordination of research efforts and thus what little funding is available may to some extent be squandered. There is an important role for a body within the industry to encourage investment in research, to coordinate the disparate programs currently in progress and to represent the interests of farmers to government.

TETRAPLOID INDUCTION

Introduction

The rationale given by Chinese researchers for the production of polyploid pearl oysters is similar to those given for the production of other polyploid bivalves. Primarily, triploid oysters are desired for farming because of the expected increase in growth that results from functional sterility. While sterility remains questionable, the growth advantages of triploid induction have been clearly demonstrated with a number of species. Chinese researchers also suggest that just as growth is reduced during gametogenesis, so to is nacre deposition and that triploid pearl oysters will be capable of producing pearls more rapidly.

Triploidy also confers an additional benefit to pearl farming. The timing of the insertion of pearl nuclei centres around the reproductive condition of the host oyster. Insertions are only made when the oyster is spent or is in the early stages of gametogenesis. Thus triploid oysters allow a prolonged period for nuclei insertion and reduce the need for the packing procedures used to prepare oysters for nuclei insertion.

The interest in the production of tetraploid oysters has arisen from the desire to produce batches exhibiting 100% triploidy. Previous attempts to produce triploids with both 6DMAP (6 Dimethlyaminopurine) and CB have met with mixed results. (Wang et al., 1999).

Materials and Methods

The techniques used were based upon those introduced by Dr Stan Allen (Virginia Institute of Marine Science). Oysters collected from a farm in the Beihai area were cleaned and rinsed in a potassium permanganate $KMn0_2$ solution. Gametes were obtained by strip spawning (described earlier) and were activated with the use of an ammonia solution.

Experiment 1: Timing of CB exposure

The eggs from five females were collected and were inspected microscopically for quality. The eggs from two females perceived to be of the highest quality were pooled and divided into 100 mL aliquots in six beakers.

Following the addition of a small, but equal quantity sperm suspension to each beaker, one beaker was treated with 1 mL of DMSO (Dimethyl sulfoxide). The remaining five beakers were treated with 1 mL Cytochalasin B dissolved in DMSO (effective dose rate 0.5mg CB/L) at either 3, 5, 7, 9 or 12 min after fertilisation. In each case, eggs were exposed to DMSO or DMSO + CB for 18 min.

At the completion of each treatment, eggs were decanted and resuspended in 400 mL of 0.5 mL/L DMSO in seawater solution, twice. Water temperature during experiment was 27^{o}C and polar body extrusion in controls commenced 5 min after fertilisation.

Ploidy in experiment was determined by flow cytometry 6, 8 and 10 h after fertilisation.

Experiment 2: Duration of CB exposure

The techniques used for this trial a repeat of those used in Expt.1. The exception being that the in all cases CB was introduced 3 min after fertilisation and duration of exposure to CB was varied; either 12, 15, 18, 21 or 25 min.

Experiment 3: CB concentration

The experimental protocol was again similar to the previous two trials; however, the time of introduction of CB and the duration of exposure were fixed at 3 min and 18 min, respectively. In this experiment, fertilised eggs were treated with differing concentrations of CB, either 0.3, 0.5, 0.7 or 1.0 mg/l.

Ploidy was determined using a Partec PA flow cytometer. Sperm and untreated embryo cells were used as haploid and diploid standards in association with each trial. Cells were stained using DAPI.

Results

Experiment 1: Timing of CB exposure

Observation of developing embryos 1 h after treatment showed normal extrusion of polar bodies by control (DMSO) treated animals. Eggs treated with CB showed a reduction in the number of polar bodies present (Table 2).

Table 2

Treatment	*Eggs with 1 polar body*	*Eggs with 2 polar bodies*
Control		~ 80%
3 min	~ 20%	0
5 min	~ 30-40%	~ 5%
7 min	~ 30-40%	~5%
9 min	~ 40-50%	~10-15%
12 min	~ 40-50%	~10-15%

After 24 h the number of embryos developing to D-veliger stage was clearly reduced in those treatments exposed to CB, ranging between 36 and 51% (Table 3). In contrast, 82% of DMSO treated embryos had reached D-veliger stage.

Table 3

Treatment	*Trochophores*	*D-veligers*	*% Development*
Control	4	18	82%
3 min	21	19	48%
5 min	23	13	36%
7 min	27	18	40%
9 min	22	18	45%
12 min	22	23	51%

Ploidy evaluations were complicated by several factors. Initially, a pronounced peak occurred in control samples in the region in which tetraploid cells would be expected (Figure 11, Control). This second peak was ascribed to the presence of doublets (double cells) and restricted assessment of the tetraploid percentage in experimental treatments. This was further hampered by the inability of the Partec flow cytometer to allow peak selection. In several instances what was thought to be a triploid peak was included within the tetraploid peak (see Figure 11, 3A) where a triploid peak appears to be present and yet only two peaks are acknowledged). This prevented accurate quantification of peaks and thus statistical analysis of results was not undertaken.

Despite these difficulties several observations can be made. Importantly, tetraploid peaks were significantly larger that doublet peaks observed in control treatment, suggesting the presence of a significant number of tetraploids, possibly 12-15 %. The tetraploid peak was largest in eggs treated 3 and 5 min post fertilisation. Tetraploids were reduced in eggs treated after 7 min and not apparent in 9 and 12 min treatments. Mr Yan reported that in previous experiments, 3 min post fertilisation has been found to be the optimum time for CB introduction.

Mr Yan also reported that development in tetraploids was slower than that of other embryos. This was to some extent apparent in the percentage triploidy in eggs treated at 3 min after fertilisation but this trend was not at all clear in tetraploid peaks for 5 and 7 min post fertilisation treatments.

Experiment 2: Duration of exposure to CB

As in Experiment 1, exposure to CB led to reductions in the percentage development of D-veligers. Prolonged exposure to CB further reduced percentage development, although this was not clearly apparent until exposure times exceeded 18 min (Table 4).

Table 4

Treatment	*Trochophores*	*D-veligers*	*% Development*
Control	6	21	78%
12 min	21	26	55%
15 min	22	36	62%
18 min	17	24	59%
21 min	20	17	46%
25 min	21	9	30%

Flow cytometry results for this trial again showed evidence of the presence of tetraploid embryos (Fig. 12). Controls showed the presence of doublet cells, however the ratio of the area beneath diploid peaks to tetraploid peaks shows an increase in treated embryos, indicative of tetraploidy.

Experiment 3: CB concentration

Inspection of embryos from each treatment 1.5 h after fertilisation noted the development of embryos in the control treatment was proceeding more rapidly than that of the treated embryos. Differences between treatments were small and were most notable in embryos exposed to 1.0 mg/l CB.

Flow cytometry results for this trial were inconclusive and were more indicative of a high triploidy percentage than tetraploidy (Fig. 13).

Discussion

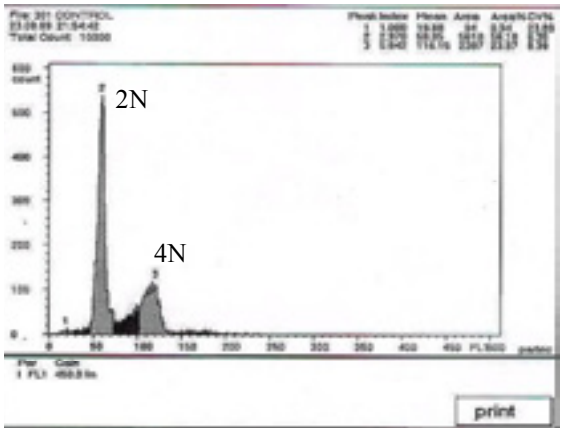
In previous experiments conducted at the GIO, the optimum CB dose, the duration of exposure and the time at which exposure to CB should commence were determined to be 0.5 mg/L, 18 min and 5 min, respectively. These experiments were designed to demonstrate these optima. These experiments were unreplicated for the sake of expedience given the brief duration of the visit and the limited facilities available.

Although this experimentation did not clearly support the previous findings there is sufficient evidence to establish that this regimen does successfully induce tetraploidy in embryos and that the suggested regimen is likely to be close to optimal.

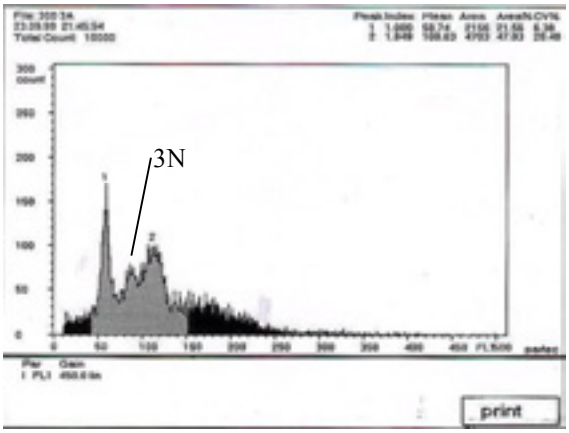
Replicated trials, using the suggested regimen, were conducted at the hatchery facility run by the GIO during the visit and an opportunity to observe the 2-day-old larvae was provided. It was immediately apparent that number of treated larvae was significantly lower than the suggested stocking density (at least 10 fold) and that a significant number of the larvae present were deformed (approx. 50%). This suggested the possibility that the use of CB to prevent the extrusion of the first and second polar in *P. imbricata* has effects similar to those observed at PSRC. That is although tetraploid embryos are produced their survival may be negligible.

Figure 11. Experiment 1 Timing of CB exposure

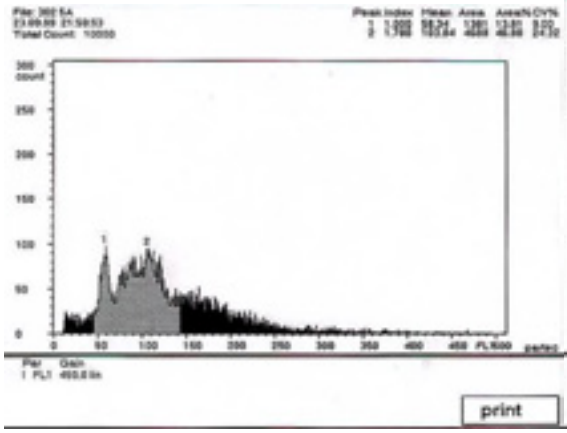
Control



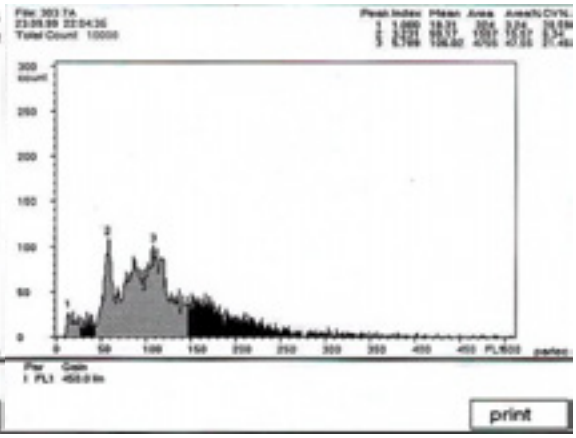
3A



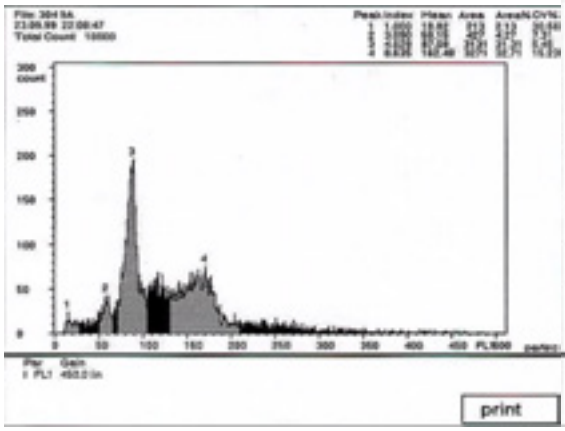
5A



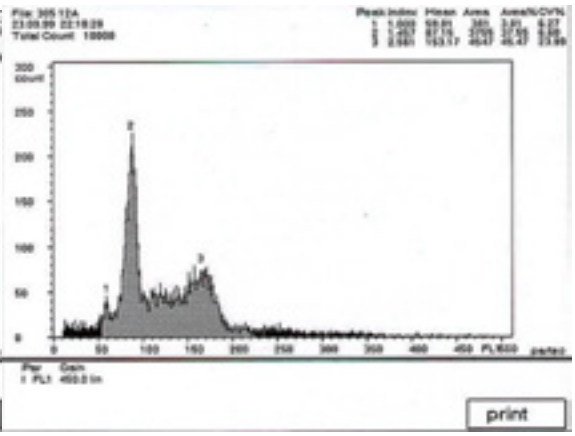
7A



9A



12A



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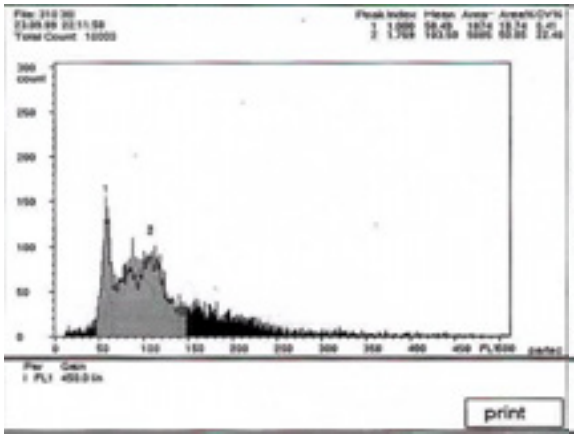

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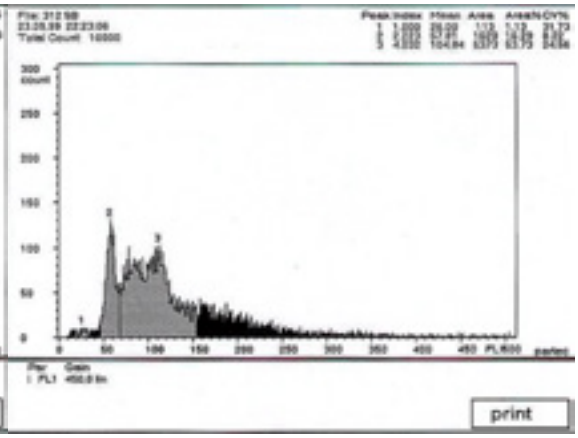

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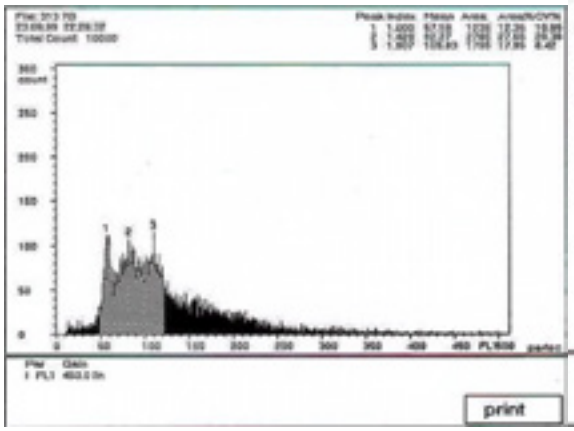

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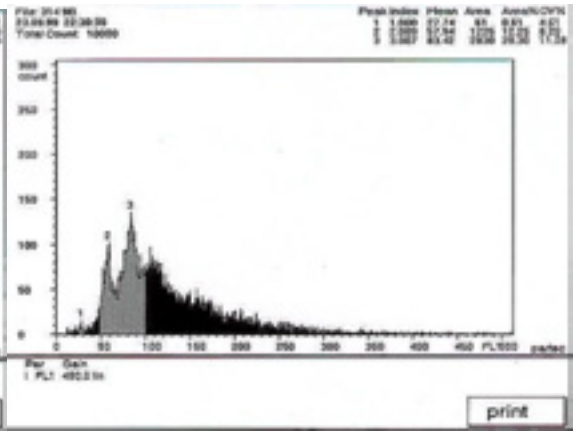
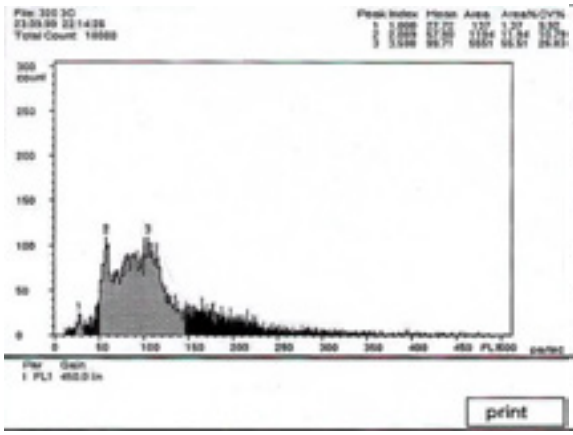


3C



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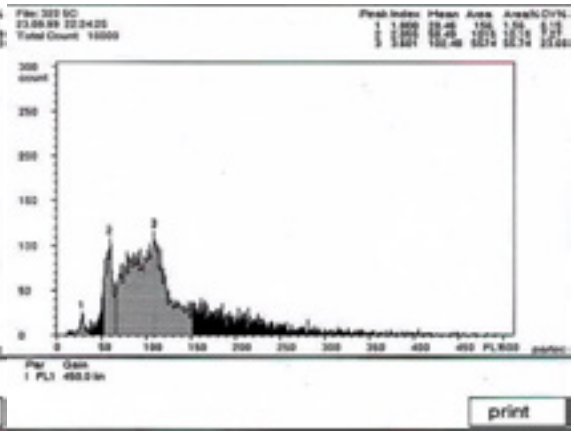

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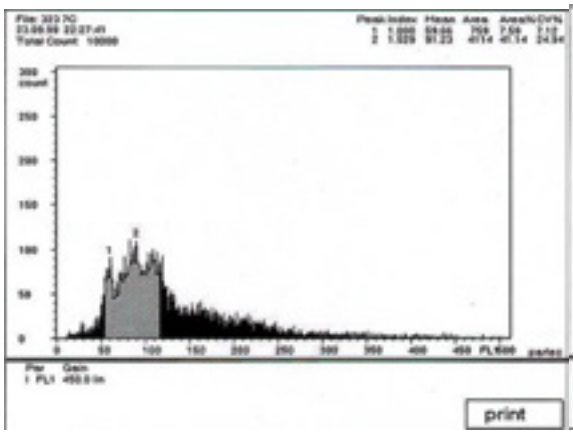

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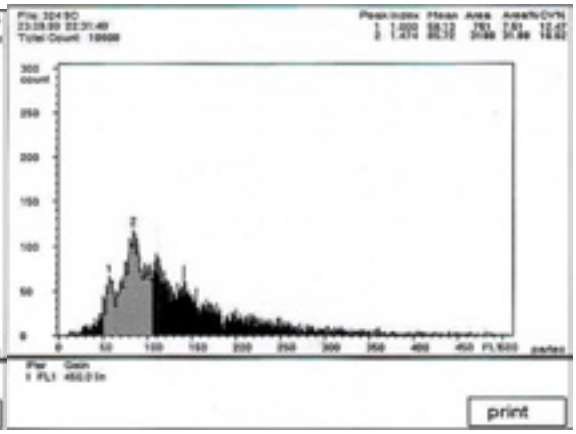

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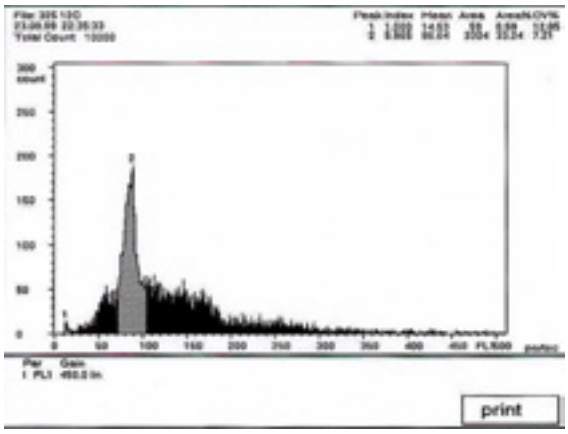
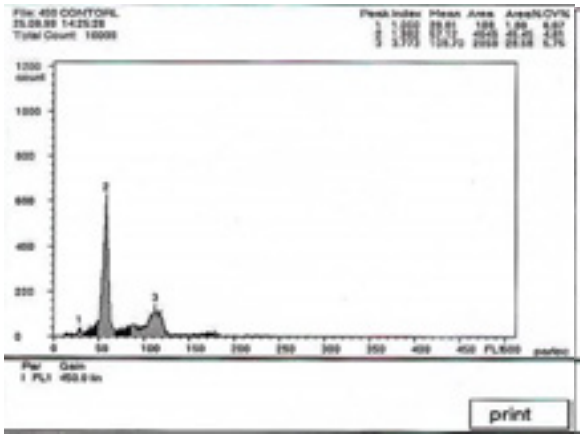
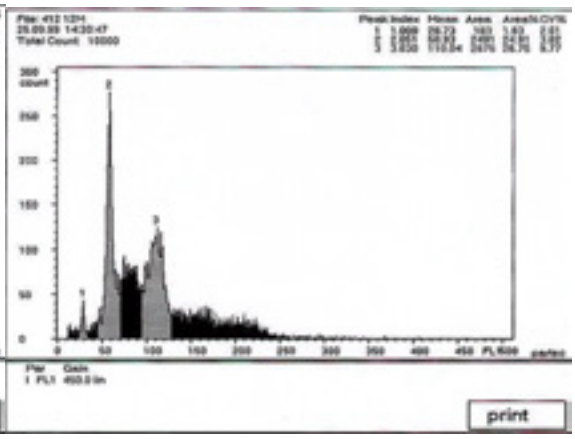


Figure 12. Experiment 2 Duration of CB exposure

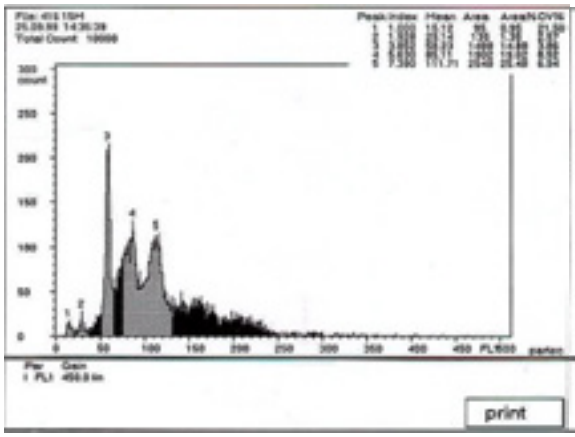
Control



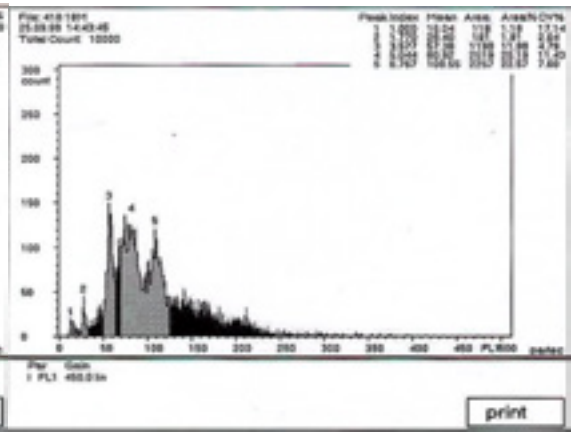
12 min



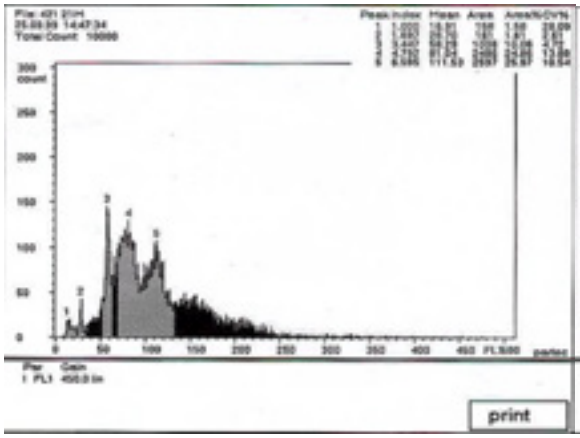
15 min



18 min



21 min



25 min

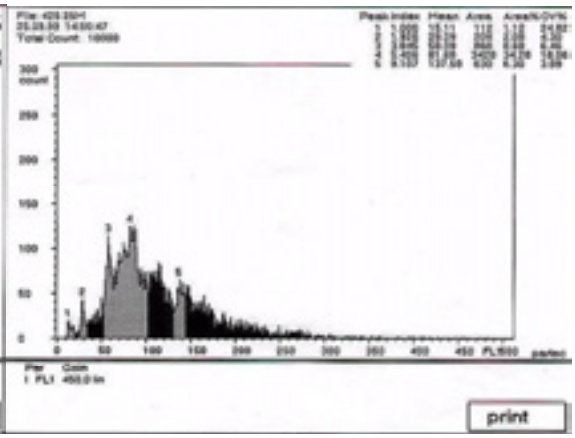
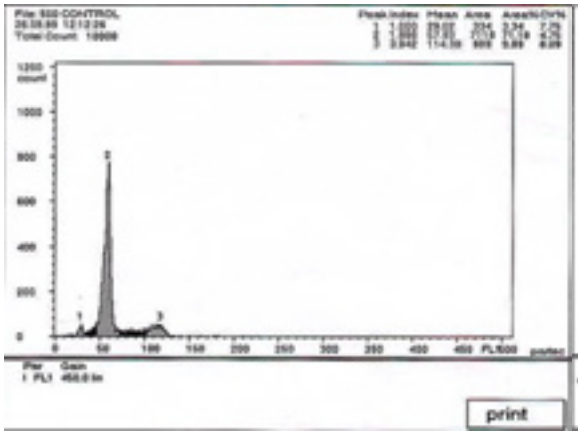
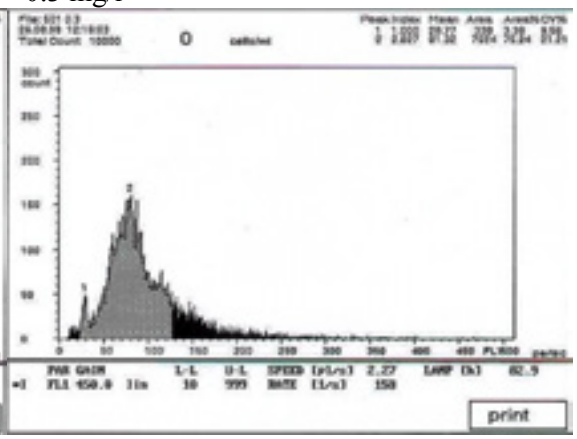


Figure 13. Experiment 3 CB concentration

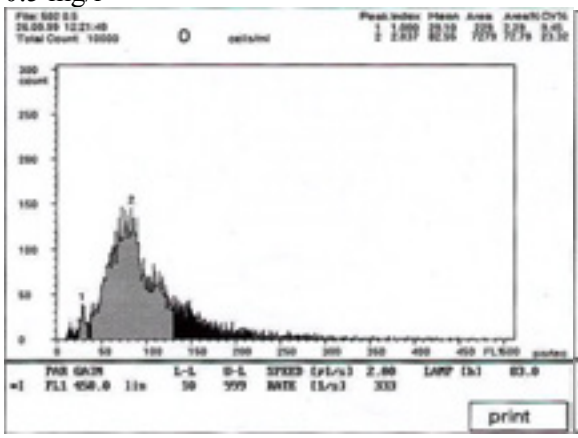
Control



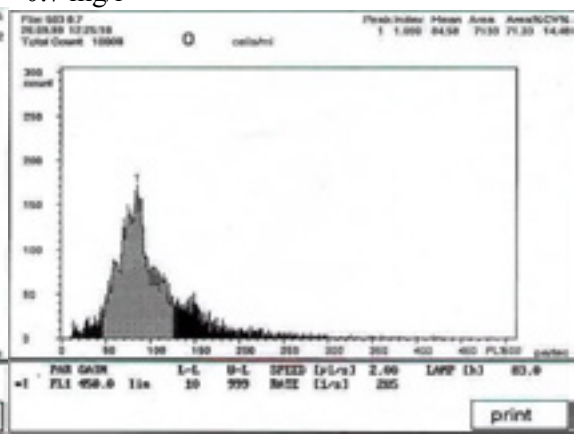
0.3 mg/l



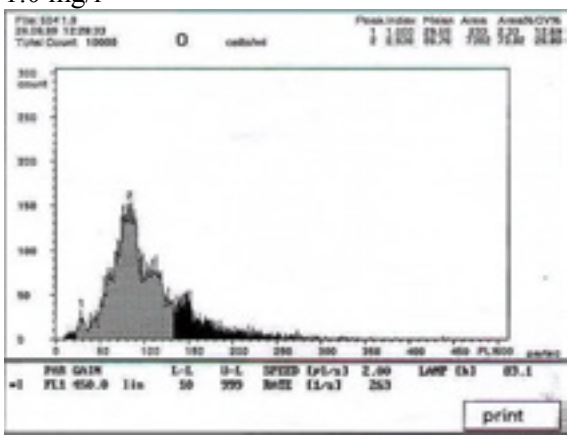
0.5 mg/l



0.7 mg/l



1.0 mg/l



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APPENDIX 1

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