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Management of a bioeroding sponge on the pearl oyster, pinctada maxima

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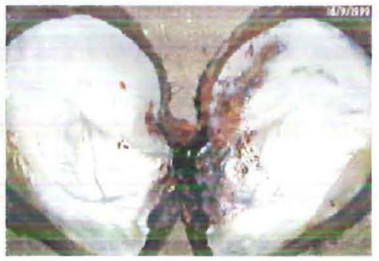

by Luisa Rawlinson

A Thesis submitted in partial fulfilment of the requirement of the award of Bachelor of Science (Environmental Management) with Honours at the Faculty of Communications, Health and Sciences, Edith Cowan University

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Abstract

This thesis examines environmentally and economically viable ways to manage a sponge that is bioeroding the pearl oyster, *Pinctada maxima*, in pearl oyster farms throughout north-western Australia. The sponge is causing a massive loss in revenue to the pearling industry as a result of damage to the half-shell, the pearl and, often, death of the oyster. The information arising from this study is important for pearl producers and the Australian pearling industry, to ensure that the best quality *P. maxima* can be grown in a way that will not have adverse effects on the pristine environment in which these sensitive organisms live. It is of uttermost importance to the pearl oyster farms that solutions to the problem are environmentally appropriate.

Control of the sponge, of the family Clionidae and the genus *Cliona* in addition to other related genera, was based on knowledge of its reproductive cycle, so that a deterrent to egg release can be applied at a time when the sponge is at a vulnerable stage in its life cycle. The reproductive cycle of the sponge was examined using light microscopy, after the sponge samples had been processed using histological methods. The reproductive cycle of the sponge was examined over a 12-month period at five different pearl oyster farms in north-western Australia. Reproductive activity was correlated with environmental parameters, including water temperature and salinity. The results of these studies were integrated and management recommendations based on these results were made.

The study on reproduction of the sponge found no indication of reproductive activity for three of the farms (Morgan Pearl farm and both Paspaley Pearl farms at Vansittart Bay

and Port Bremer) participating in the study. The samples from Maxima Pearl presented some reproductive activity, while Arrow Pearl had relatively high reproductive activity. Additionally, reproduction occurred at two different times of the year.

This study concluded that management of the bioeroding sponge can be improved with knowledge of its reproductive cycle. Other longer-term studies are, however, essential for improved management recommendations. The current management technique recommended, the application of a paint that will smother and kill the sponge infestation, is thought to be environmentally benign and has the potential for pearl producers to reduce the revenue lost as a result of the sponge. This technique should be continued with modifications on the timing of the application to coincide with reproductive activity of the sponge, thereby reducing sponge settlement and consequently reducing farm costs. For the recommended management strategies to be effectively utilised, further research is needed into the origins and reproductive cycle of this bioeroding sponge.

Acknowledgments

This project would not have been possible without the support and assistance of a large number of people. In particular, I thank my supervisors Dr. Jackie Alder and Dr. Jane Fromont (from the Western Australian Museum) for their incredible support, patience, constructive feedback and encouragement.

The pearling industry of Western Australia contributed greatly to this project in many ways. The companies involved: Maxima Pearling Co. Pty Ltd., Paspaley Pearling Co. Pty. Ltd., Arrow Pearl Co. Pty. Ltd. and Morgan & Co. Pty Ltd., provided invaluable information on both pearl farm management and procedures, in addition to details of the 'red arse' problem on their farms. They also provided the samples, environmental data and technical advice for the project. Funding was supplied by the Western Australian Fisheries Industry Council, and the Pearl Producers Association.

In particular, I would like to thank and acknowledge Maxima Pearling Co., for willingly accommodating me during my stay at Maxima Pearl farm, which enabled me to study first hand the sponge problem. Alan Wilmot and Mehdi Doroudi were both especially helpful with my enquires and ensured that I had exposure to as many aspects of pearl farming as possible in the short time I was there.

I would also like to thank the Human Biology Department at Edith Cowan University, for allowing me to use their equipment, especially the microtome on endless occasions.

The Western Australian Museum also contributed to the project, by permitting me to use their equipment in the histological laboratory.

There are many others who provided both support and advice throughout my project, and they have not been forgotten. In particular, Robert Craig, who provided both technical and moral support throughout the project as a technician.

Last but not least I thank my family and partner for providing endless support and being so understanding throughout the past year.

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1 Introduction

Western Australia has a valuable and successful pearling industry that has been operating since the 1880's and is worth around $200 million annually in exports. It is the world's top producer of prized silver-white South Sea pearls (Fisheries Western Australia, 1998). Twelve companies operate 16 licences to fish for pearl oyster stocks and are allowed to harvest 572,000 shells per year. The most sought-after species of pearl oyster is the silver-lip pearl oyster, *Pinctada maxima*, which produces the splendid silver-white South Sea pearl. All sixteen licensees harvest *P. maxima* oysters and these are farmed on the north Australian and northern Western Australian coast. Industry research currently has its main focus on improving pearl quality (Fisheries WA, 1998).

The bioeroding sponge (phylum Porifera) known throughout the pearling industry as 'red arse', attacks the shells of the pearl oyster, *Pinctada maxima*. The shells are infested to varying degrees on farms, causing a minimum of a 2% death rate (A. Morgan, pers. comm., March 2000), although industry wide the cost is likely to run into millions of dollars annually (A. Wilmot, pers. comm., March 2000). The sponge burrows into the shell causing considerable damage and sometimes death of the oyster, rendering the half-shell and occasionally the pearl, unsaleable. It has been a long-term problem within the industry and has devastating effects, as optimal growth conditions for oysters provide the ideal habitat for the sponge.

The sponge has, according to the pearl farms participating in this study, always been a problem for the pearling industry on the north-west coast of Australia. However, the

substantial increase in the presence of this sponge over time is affecting the successful operation of pearl oyster farms and pearl production in the Southern Hemisphere (Moase *et al.*, 1998). Therefore, there is a great need to address the problem of the bioeroding sponge and its incidence on farms so that industry can reduce its impact and improve on its financial success, while minimising the environmental effects of treatment of the sponge.

The origin of the sponge pest is unknown, as is the identity of the species. However, it is possible that the sponge has been introduced into the region via shipping and ballast. Alternatively, the sponge may have been introduced via wild oysters that have been collected from fishing areas and transferred to the pearl farms. These possibilities were explored as part of the study.

The aim of this research was to make recommendations on environmentally and economically appropriate management actions to control bioerosion by the sponge (phylum Porifera), on *Pinctada maxima* in Australia's north-western and northern pearling industry. Three objectives needed to be addressed to meet this aim:

1.) to determine the reproductive cycle of the sponge that bioerodes the pearl oyster, *P. maxima*;
2.) to use the data on reproduction of the sponges to recommend methods to reduce or prevent the infection of bio-eroding sponges; and
3.) to recommend further research that may be necessary to address this problem.

Although there are several aspects to the problem, within the context of this project only a subset of objectives 1, 2 and 3 could be investigated due to time and resource constraints.

This thesis is composed of six chapters. Chapter 2, the background, places this research into context by reviewing the current literature regarding pearl aquaculture, impacts of the bioeroding sponge on pearl oysters, sponge reproduction and environmental impacts of the recommended control measures. The methods for the project are described in Chapter 3. Chapter 4 describes pearl farm operations and is followed by Chapter 5, an outline of the results. Subsequently the results are discussed in Chapter 6 and the implications for the management of the bioeroding sponge in pearl oyster farms provided in Chapter 7. Recommendations for various issues relating to the sponge and its management on pearl oyster farms that arose from this study are also made. The environmental data and the number of eggs noted in each sample are presented in an Appendix.

2 Background

The bioeroding sponge (phylum Porifera) known throughout the pearling industry as 'red arse' attacks the shells of the pearl oyster, *Pinctada maxima.* Prior to this study, it was thought that the sponge belonged to the family Clionidae, a group known to inflict damage on various mollusc fisheries, coral reefs and limestone breakwaters, thereby investing the genus with economic and ecological importance (Warburton, 1958b). The sponge attacks the oysters by boring tunnels within the oyster's calcareous shell, consequently weakening the oyster. This bioerosion, if severe enough, can kill the oyster. It has been a long-term problem within the industry but previously only studied to assess external methods of reducing its incidence on the shell (J. Fromont, pers. comm., August 1999).

Although the specific details of the taxonomy of the sponge were not known prior to this study, it was thought that it belonged to the sponge genus *Cliona. Cliona,* of the family Clionidae, is the most widely reported sponge genus to cause infestation in commercially valuable molluscan species. The single study to date examining boring sponges in molluscs within Australia reported the incidence of two species of *Cliona* from the Sydney rock oyster *Saccostrea commercialis* (Wesche *et al.*, 1997). Both species of *Cliona* were found to be cosmopolitan in their distribution and to have been introduced to Australia in either infected shell or in ballast water (Wesche *et al.*, 1997). Although numerous studies on the various *Cliona* species have been undertaken worldwide, none of these studies concentrated on reproduction of these sponges in the Southern Hemisphere. Other studies on *Cliona* have primarily focused on substrate destruction and sediment production (Acker & Risk, 1985), distribution (Pomponi &

Meritt, 1985), taxonomy (Carballo *et al.*, 1994) and ways in which *Cliona* bioerodes calcium carbonate substrates (Cobb, 1969).

This study was the first attempt in Australia to determine the sponge species that causes major bioerosion problems in the pearl oyster *P. maxima* and to document the life cycle of the sponge to assist with future management of the sponge infestation. Prior to this study, effective environmentally and economically viable methods for reducing the incidence of sponge bioeroders in commercially important molluscan species were not known. It is hoped that this research will improve management of the pearl farms by minimising the impacts of the sponge, a potentially introduced species. By recommending an appropriate time to apply a deterrent to egg release by the sponge, it is anticipated that the pearl farms will benefit economically while reducing any potential environmental impacts from the deterrent. Detailed assessment of the impacts of *Cliona* as a marine pest was outside the scope of the project. However, a literature search was undertaken to explore the possibility. If it was discovered to be imported from ballast water or introduced through transfers between pearl farms, information from this project will assist in controlling the impact of the sponge on commercially important marine fauna such as *Pinctada maxima*.

2.1 *Cliona* – The Bioeroding Sponge

2.1.1 Defining characteristics

It was expected, based on previous studies (Hooper & Wiedenmayer, 1994; Bavestrello *et al.*, 1996; Carballo *et al.*, 1994), that the bioeroding sponge found on the shells of the pearl oysters is of the family Clionidae. *Cliona* is a cosmopolitan genus of marine siliceous sponges (C. Demospongiae, O. Hadromerida, F. Clionidae), remarkable for the habit of living in tunnels and galleries bored into limestone, coral and the shells of molluscs (Warburton, 1958). Most of the tissue of these sponges, therefore, is endolithic, living within the calcium carbonate structure of the shell (Pomponi, 1980). Sponges from this family are obligatory excavating or burrowing sponges that bioerode, partially or completely, calcareous substrata (Hoffman & Kielman, 1992; Carballo *et al.*, 1994; Barbieri *et al.*, 1995; Bavestrello *et al.*, 1996). Members of the family Clionidae penetrate calcium carbonate by a chemomechanical process (Cobb, 1975), utilising filopodial extensions and etching chemical secretions (Rutzler & Rieger, 1973 in Wesche *et al.*, 1997). Settled sponge larvae, following metamorphosis, burrow into the substratum and dwell in burrows for part or all of their lives (Hooper & Wiedenmayer, 1994). These sponges are successful bioeroders and will exploit all available substrate (Mao Che *et al.*, 1996).

The term 'parasite' is used frequently to illustrate the lifestyle of *Cliona*. However, its structure and physiology is consistent with all other free-living sponges (Moase *et al.*, 1998). A parasite, by definition, is an organism that lives in or on the living body of a

plant or animal to obtain its nourishment at the expense of the host (Dorit *et al.*, 1991). In the case of *Cliona*, the sponge does not feed on the oyster or in any way obtain its nourishment from the oyster (Warburton, 1958b), but instead uses the molluscs' calcareous shell to create the tunnel system it requires to survive. Any injury the sponge causes, which often kills the bivalves, is a by-product of their tunnelling activity. It is therefore inappropriate to use the term 'parasite' when referring to the bioeroding sponge.

Apart from *Cliona*'s bioeroding nature, it nevertheless shares similar characteristics to all other sponges in that:

- it is sessile, has no organs, head, mouth or gut cavity;
- its body structure is organised around a system of canals and chambers through which water flows;
- support is provided by internal siliceous spicules; and
- fertilisation can be internal or external and development leads to a free-swimming flagellated larva.

(Dorit *et al.*, 1991).

2.1.2 Taxonomy

It was imperative to identify the sponge s cies to the lowest taxonomic rank possible so that it may be determined whether or not the species is introduced, as was found with the *Cliona* species in the Sydney rock oyster (Wesche *et al.*, 1997), or native to the areas where it is problematic. Alternatively, the sponge infesting the pearl farms in

northern Western Australia may be a new species, as West Australian sponges are in general poorly known and only a small proportion have been described in the taxonomic literature. Knowledge of its taxonomy is needed to ascertain the deterrent procedures that could be used on the sponge. If it is found that the sponge is an introduced species, this will create further issues with respect to environmental management of the species and pearl farms.

It is especially important to determine if only one species is the dominant bioeroder in the shell or if more than one species is attacking the oysters. If more than one species is bioeroding the shell it is likely that the species may have reproductive isolating mechanisms such as differences in timing of reproductive activity and hence differences in the timing of egg release. This information will assist in determining the most appropriate time to apply deterrents to egg release by the sponge. It is possible that throughout the biogeographic range of the study, as well as within farms, different species of this sponge are causing these impacts as opposed to a single species. If this is the case, each sponge species may have different biological and ecological characteristics, once again affecting management of the species.

However, due to time constraints placed on this project it was not possible to investigate all of these taxonomic aspects. Expert advice, therefore, was sought from the Western Australian Musuem (WAM) with regard to the taxonomy of this sponge.

2.1.3 Origin of *Cliona* in north-western Australia

The origin of *Cliona* on the north-western coast of Australia is unknown. The sponge may have been present before pearling began in the 1800's, or it may have been introduced from other areas into the regions concerned. *Cliona* can be observed in reef systems around some pearl farms, for example Maxima Pearl farm, and also in shell which originates from the fishing grounds. Studies have not been done on the extent or the taxonomy of the bioeroding sponge on either the reef systems that surround some of the farms, or the *P. maxima* fishing grounds. However, it is possible, providing the tides and currents around the farms are strong enough and that the farms are in relatively close proximity to the limestone reefs, that when sponge reproduction occurs on the reef, larvae can be carried from the reefs to the *P. maxima* shell in the farms. Additionally, when *P. maxima* shells that are infected with *Cliona* are collected from the fishing grounds and introduced into the farm, this may also provide a possible vector for the introduction of the bioeroding sponge into the farms (A. Wilmot, pers. comm., March, 2000).

If *Cliona* was introduced into the marine environment in north-west Australia, it is likely that it was released unintentionally, such as the Black-striped mussel which was introduced from Central America into many countries, including Darwin Harbour in Australia (S. Slack-Smith, pers. comm., March 2000). However, despite the occasional reports of introductions, an introduction that results in the naturalisation of an organism, which consequently causes severe environmental damage, is a very unlikely outcome (Mack *et al.*, 2000).

If an introduction does result in an aquatic nuisance species, including *Cliona*, becoming established, they can have detrimental effects on human health, commercial fisheries (including marine aquaculture) and the natural environment (Kerr, 1994). Significant changes to resource-based economies can also occur when exotic species are introduced (Olson & Goen, 1998). The anecdotal and preliminary nature of our current understanding of the economics of invasions is poor (Mack *et al.*, 2000). In the case of the pearl oysters of north-western Australia, *Cliona*, a potentially introduced species, is significantly decreasing oyster survival and profits within the pearling industry.

Possible reasons for assuming *Cliona* has been introduced into the regions being studied include that all the farms are in relatively close proximity to seaports. Australia exports a significant amount of bulk commodities and as a consequence of this, 'imports' a considerable amount of ballast water and associated sediment. North-western Australian ports provide the ideal conditions for survival of many imported aquatic organisms. Most marine aquaculture regions in Australia receive ballast water (Jones, 1991), including areas where pearl oysters are farmed. Therefore *Cliona*, supposing it is an introduced species, may have been introduced into Australian waters by ballast. Both ballast water and sediment may contain a wide range of organisms. If the organisms, including *Cliona*, survive the voyage and the de-ballasting process, they have the potential to establish viable populations in the port of discharge (Kerr, 1994). Alien life forms that hitch a ride across the oceans in the ballast water of ships have been creating significant problems for the marine environment, public property (including mollusc aquaculture), tourism and human health (Rigby, 1995).

Globally, it is estimated that about 10 billion tonnes of ballast are transferred each year. That ballast water, probably scooped up and pumped to the ballast tanks in or near the port where the cargo has been delivered, may contain all life stages of aquatic organisms. This may include, in the case of *Cliona* if it is being introduced from temperate regions, gemmules (IMO, 1998).

The survival rate of species after discharge, however, depends upon the conditions of the receiving area, with species more likely to gain a foothold when conditions are similar in terms of, for example, salinity and temperature. Studies indicate that typically less than three percent of the released species actually become established in new regions. However, just one predatory species could seriously harm the local ecosystem (IMO, 1998).

Ballasting of ships is a necessary requirement for the safe operation of shipping when sailing empty to pick up a cargo, or with a light load, and it has been recognised that currently the only effective way to stop the spread of unwanted organisms is to prevent them being dumped in foreign ports (IMO, 1998). Mid-water sea exchange is the current recommended practice to reduce the threat of importing exotic marine pests (IMO, 1998).

Although the risks of invasions are low due to the differences in environmental parameters often found between ports, there is still a significant potential risk that must

be realised and the Australian aquaculture industry is concerned about the potential introduction of a number of serious disease organisms (Jones, 1991).

2.2 Sponge reproduction and life cycle

Although there have been numerous studies on the reproduction and ecology of marine sponges, the complete life history of only a few species is known in any detail (Fell *et al.*, 1984). It is known, however, that sponges reproduce sexually about once per year and also may have the capacity to reproduce asexually. Sexual, as well as asexual components in the form of gemmules were therefore sought to effectively manage the rate of infestation on the pearl farms, although it was not anticipated that gemmules would be found due to the relatively stable environmental conditions in the sampling areas. Gemmules are more likely to be found in temperate regions that experience cooler temperatures, which is the main trigger of asexual reproduction by gemmulation. It is not possible to assess fragmentation and budding by analysis of the sponge samples. This would only be possible by continual monitoring of the sponges *in situ*.

It is generally agreed that Clionids are oviparous (Pomponi & Meritt, 1985) and that the larva is a solid, ciliated parenchymella (Pomponi, 1980). Larvae are known to settle on calcium carbonate substrates that are not heavily encrusted (Pomponi, 1980) and larvae do not normally settle on the surface of encrusting organisms. Hartman (1958) has speculated that *Cliona* larvae have a preference for settling on oysters because these shells are invariably provided with corrugations and ridges which, together with the overhanging areas representing regions of active growth in previous seasons, provide

sheltered locations in which the larvae can find protection from water currents during the critical periods of metamorphosis and establishment of a burrow in the shell.

The sponge may be found in three developmental stages: burrowing into calcareous material (alpha stage); completely encrusting the original objects they have eroded (beta stage); and most conspicuously, in a massive free-living stage leaving no signs of the original excavated material (gamma stage). Normally only the first stage is reached (Rosell & Uriz, 1991 in Wesche *et al.*, 1997).

The time and duration of reproductive effort for coastal marine organisms is generally dependent upon water temperature and other sea or weather conditions (Arakawa, 1986). Most sponges tend to become reproductively active as seawater temperatures increase through spring and summer (J. Fromont, pers. comm., December 1999). Observations on Clionids by Hartman (1958) suggest that these sponges begin their reproductive cycle when the water is warmest in temperate regions. Late summer and early autumn are the seasons of egg and larva production on the coasts of northern France, Britain and in New England for marine sponges (Hartman, 1958). In warm temperate regions, the reproductive cycle begins earlier in the summer or even in late spring and generally lasts longer (Hartman, 1958). Few studies, however, have examined reproduction in sponges in tropical regions where temperature differences are relatively small (Fromont & Bergquist, 1994). Factors other than temperature that may also be involved in controlling the sexual reproductive period (Fell, 1983) include the availability of nutrients, the effect of the lunar phase and the size and age of the sponge

specimens. However, the factors which regulate the occurrence of sexual reproduction in sponges remain poorly understood.

Knowledge of larval settlement patterns is important not only for understanding fouling community development, but also for analysing reproductive strategies (Fell *et al.*, 1984). Larval settlement for some *Cliona* species, for example *C. truitti*, occurs at the same time that new substrates, in this case oyster spat shells (young oysters), are available for settlement (Pomponi & Meritt, 1985). Similarly, the annual growth cycle of *C. truitti* correlates with that of the American oyster *Crassostrea virginica*, into which it bores (Pomponi & Meritt, 1985). This could be because younger oysters have shells that are not as heavily fouled as older oysters, so there is more space available for settlement and survival of sponge larvae.

Information on the life cycle of this sponge should enable a deterrent to egg release to be effectively utilised to eventually prevent, or greatly reduce, fouling by the sponge. The identification of the timing of egg release by the sponge would allow for deterrents to bioerosion to be applied when the sponge is at a vulnerable stage in its life cycle, such as prior to egg release. Prior to this study, neither the sponge species nor the biology of the sponge that bioerodes the valuable pearl oyster beds of north-western Australia were known.

2.3 Bioerosion & impacts of *Cliona* on pearl oysters

2.3.1 Bioerosion

Bioerosion, a term first proposed by Neumann (1966) is described as being the destruction and removal of hard substrates by the direct action of organisms in a wide variety of environments. Many groups of organisms are involved, from bacteria to fishes, and rates of substrate removal may be very rapid (Acker & Risk, 1985). Among the many taxonomic groups involved in internal bioerosion, there is little doubt that the sedimentologically most important ones are the bioeroding sponges. Prodigious amounts of sediment (as characteristically shaped, silt-size chips) are produced by bioeroding sponges and some bottom sediments are dominated by sponge chips (Acker & Risk, 1985), particularly in some coral reefs (MacGeachy, 1977).

Despite bioerosion being a widespread phenomenon, relatively little quantitative research has been devoted to this important process. Much of what is known comes from research on coral reefs (Bergman *et al.*, 1982). Therefore it is anticipated that this study on reproduction and environmental factors surrounding these sponges, will further our knowledge of bioeroding sponge species.

2.3.2 Impacts of *Cliona* on pearl oysters

Sponges from the family Clionidae have been known to cause problems in commercial shellfish stocks (Schleyer, 1991) since they were discovered at the beginning of the

1800's in French oyster beds, where they caused 'spice bread disease' (Rutzler, 1975; Thomas, 1981 in Wesche *et al.*, 1997). Bioeroding sponges are capable of attacking shells of molluscs, causing considerable damage or even death (Schleyer, 1991; Thomas *et al.*, 1993; Mao Che *et al.*, 1996). *Cliona* penetrates the outer prismatic and inner nacreous layers of *P. maxima* (Figure 2.1), resulting in high mortalities over a relatively short period of time (Moase *et al.*, 1998).

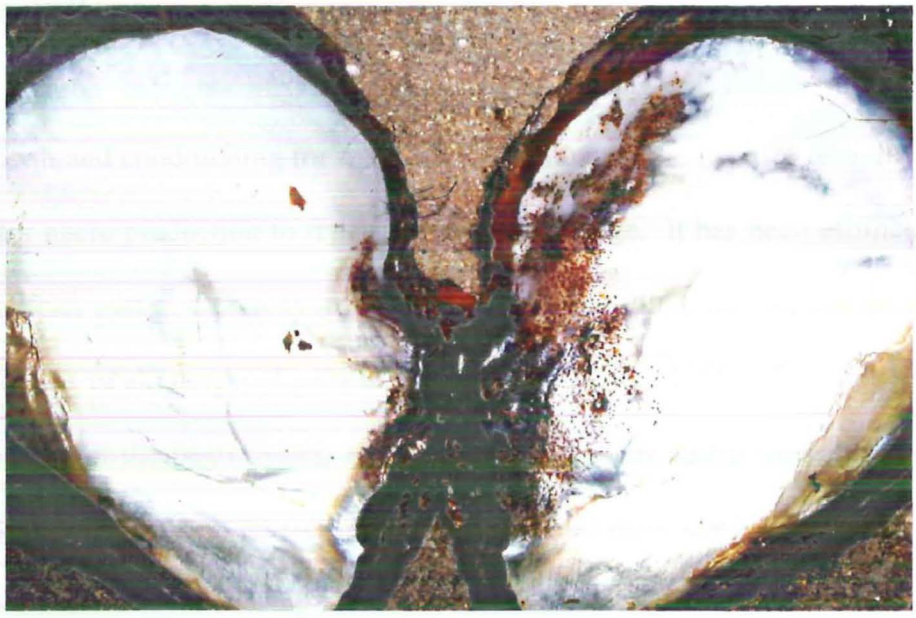


Figure 2.1. Shell of dead *P. maxima* infected by *Cliona*.

When these sponges burrow into the living shells of commercial shellfish stocks they become a pest (Wesche *et al.*, 1997). Clionid sponges penetrate the periostracum forming holes in the outer surface and a tunnel network throughout the shell. Chronic invasion may result in penetration of the conchiolin layer through to the inner surface of the shell. The oyster may, or may not, successfully wall-off the nacreal opening made by the sponge, preventing entry of sand, mud or other irritants (Bower & McGladdery, 1996). If unsuccessful in producing sufficient nacre to wall-off the sponge, structural

support may be compromised, thereby weakening the oyster, eventually leading to death. Often large proportions of the shells are excavated, leaving the shells fragile and weak (Wesche *et al.*, 1997). Interference with abductor muscle attachment impedes feeding and causes mortality (Wesche *et al.*, 1997). Sponge tunnels may become inhabited by other organisms, such as polychaete worms, which may reduce market value. However, these organisms rarely impact directly on oyster health (Alagarswami & Chellam, 1976).

Oyster growth and conditioning for market may be stunted due to extra resources being allocated for nacre production to repair the sponge damage. It has been estimated that, in the American oyster, *Crassostrea virginica*, shell deposition may require as much as one-third of the total energy of growth (Pomponi & Meritt, 1985). It is not known if this is the case for the pearl oyster, *Pinctada maxima*. This added stress on the oyster may result in mortality in severe sponge infestations, particularly where the sponge penetrates the nacre layer causing adhesions of the mantle (Wesche *et al.*, 1997). This is due to physical exhaustion by the oyster in cases of extreme attacks by the sponge (Alagarswami & Chellam, 1976). Often *P. maxima*'s method of defence becomes futile as infestation of the shell reaches a capacity far greater than the oyster can handle. At that stage, the shell becomes weak, brittle, the mantle retracts and the animal dies (Moase *et al.*, 1998).

The compromised structure of the oyster may also cause processing of oysters for the half shell trade difficult. When holes etched by the sponge reach the pearl,

consequently discolouring it and removing the sheen, the infected oysters may lose their commercial longevity, resulting in a decrease in pearl productivity (Doroudi, 1993).

Although larvae are known to settle at the same time that new substrates are available for settlement (Pomponi & Meritt, 1985), visual evidence suggests that *Cliona* displays a preference to infestation of larger pearl oysters, many of which have entered the operation phase of their lifecycle on the farm (Bower & McGladdery, 1997). However, due to the rapid growing phase of juvenile oyster shells, the sponge may still be present, but not appear to have penetrated its host. Once mature, the shell growth decreases, with the sponge continuing development at a faster rate (Moase *et al.*, 1998).

The result of infestation is discernible both internally and externally. Externally the shell becomes excavated with holes forming a 'honeycomb' pattern, often bright red or orange in colour. Internally, the oyster deposits thickened nacre around visible darkened lesions beneath nacreous layers when penetration into the muscular cavity appears inevitable. As *P. maxima* concentrates its energy on fighting the sponge, it neglects to deposit nacre on the previously inserted nuclei. From this stage forward the pearls display physical imperfections and discolouration, resulting in a substantial decrease in quality. This sponge, therefore, in advanced stages of infestation, renders the pearl shell unsaleable or of a much poorer quality than uninfected shell (Doroudi, 1994).

2.4 *Cliona* as a problem in aquaculture

There are a number of possible reasons for *Cliona* posing problems in aquaculture and, in this case, pearl farming. The predominant reason is the high density that the shell is held at in the pearl farms in a monoculture-style farming practice.

It is well known that the spread of infections and disease can be much more prolific in a monoculture than a polyculture and that ecological stability correlates directly with ecological diversity (Rappaport, 1976 in Phanthong & Patterson, 1996). The benefits of using more than one species have been convincingly established (Reay, 1979). In the wild, several species of fish live together as a community. Typically, however, most aquaculture systems will simplify this community to an extreme by utilising one species, in this case *P. maxima*, and eliminating the rest, which may include predators and competitors.

Running a monoculture farm is a high risk strategy, being more prone to adverse weather conditions and promoting the rapid spread of disease, pests and invasions by exotic species (Meadows *et al.*, 1992), such as the bioeroding sponges. Diverse populations, created through mixing species and varieties can withstand infections and environmental problems better than a single species can, as different species are prone to different problems. This is due to the greater variation in the genetic make-up of a polyculture, as opposed to a monoculture.

In addition to monoculture farming practices, a higher density of shell will promote the spread of *Cliona* due to there being a greater amount of calcareous substrate for the larvae to settle on. These practices are sure to have promoted the spread of *Cliona* in the pearl oyster farms throughout north-western Australia. Animals and plants grown in aquaculture are more vulnerable to pests and diseases than wild organisms because they are kept at high stocking densities which enables rapid spread of disease if there is a disease outbreak (Jones, 1991).

2.5 Economic loss to pearl oyster farms resulting from *Cliona*

The damage inflicted by *Cliona* on pearl farms is intense, although an exact figure of loss has not been calculated. Although the sponges do not attack living tissue, the damage they inflict on the mollusc shells can kill the bivalves. Therefore they are an important economic problem in these fisheries (Pomponi, 1980). Morgan Pearl, which suffers the least amount of infestation of all the pearl farms in this study, has a death rate of 2% as a result of *Cliona* (A. Morgan, pers. comm., March 2000). In most instances the loss suffered by the individual pearling companies as a result of the sponge is not publicly acknowledged, although it is estimated that industry wide the cost runs into millions of dollars annually (A. Wilmot, pers. comm., March 2000).

One standard bucketful of pearls for most farms equates to approximately a third of a year's harvest for a company, with a wholesale value of about $2 million and worth $4 million retail. An "average pearl" is worth approximately $2000 to $3000 wholesale.

Consequently, the loss of even one pearl in a harvest is a substantial loss to a pearling company.

Perhaps even more important than damage to the pearl is the damage inflicted on the half-shell, which is one of the main products of pearling in north-western Australia. The half shell, once used to make buttons, is now used primarily for ornamental purposes and to manufacture jewellery because of its beautiful lustre and colouring (Taylor, 1985). In terms of the industry, the half shell is worth approximately $170 million (Fisheries WA, 1999). Therefore, to improve the quality of the product by 10%, an increase of $1.7 million in value, would be worth the equivalent value of the state's abalone fisheries between Cape Leeuwin and the Northern Territory border (Fisheries WA, 1999). The meat of the abductor muscle, considered a delicacy, is another product of pearl farming and was sold for up to A$300 per kilogram (dry weight) in 1988, although this price fluctuates annually (Fisheries WA, 1999). The sponge has the capacity to destroy abductor muscle attachments in the oyster.

In addition to the costs suffered by the pearl farms as a result of imperfect pearls and damage to the half shell, the cost of labour required to clean the shell of fouling on the farms is very high. The oyster shells are individually scrubbed approximately once per month (although this varies between farms) to rid the shell of sponges, barnacles and other fouling organisms. This is a very labour intensive and time-consuming process. *Cliona*, therefore, significantly decreases oyster survival and profits from oyster beds (Olson & Goen, 1998).

2.6 Previous management options

Little information exists on the reproductive cycle of the sponge and therefore management of the problem has been to take preventative action year round by periodically cleaning the oysters (Figure 2.2). As noted above it involves eradicating the sponge by individually brushing and chipping each shell monthly, to remove the sponge from the shells. An extremely labour intensive, time-consuming and cost ineffective method, shell cleaning is also a dirty, monotonous job that produces a high turnover of workers (Aquilina & Reed, 1997).

Figure 2.2. Workers on Maxima pearl farm cleaning shell (Anderson, 1996).

Consequently an urgent need developed for effective remedial measures against the bioeroding sponges infesting the pearl oysters. Because the quality of the pearls is directly related to water quality, any remedial measure must not degrade the quality of the environment surrounding the pearl farms. Pearl farmers were therefore seeking appropriate environmentally benign and economically viable solutions to the problem.

Bailey-Brock and Ringwood (1982) investigated various ways in which to eliminate the spionid worm, *Polydora websteri*, from the edible oyster, *Crassostrea gigas*, in the Hawaiian Islands. A number of control procedures were investigated for both adults and larvae of *P. websteri* and their effects were assessed on oyster vitality, without jeopardising human consumption of oysters or polluting the surrounding environment. Toxic and non-toxic methods were considered. Control methods included dipping the oysters in brine and solutions containing diclorobenzene, phenol, DDT and Victoria blue to kill adult and larval stages (Bailey-Brock & Ringwood, 1982). These methods were generally found to be effective in killing the worm.

Results of their experiments showed that low concentrations of acetic acid or chlorox effectively killed the larvae and adults of *P. websteri* that were removed from their burrows (Bailey-Brock &. Ringwood, 1982), however this treatment may be somewhat toxic. Treatment with saline solutions was marginally successful, although only very healthy oysters are tolerant of high salinity levels. The strong brines require large quantities of salt and constant mixing, which would be expensive to install on a large scale (Bailey-Brock & Ringwood, 1982). Fresh, heated water appeared to be most promising as a dipping treatment, with heated water treatment resulting in low oyster mortality. Adult oysters responded with some variability to these treatments. However, heating the water has a high energy cost which may be prohibitive for operations on isolated areas, such as the pearl farms, where power is generated on site.

Topsent (1900) in Hartman (1958) also suggested immersing the edible oyster, of the *Crassostrea* species, for a short time in fresh water as a control method for the sponge. Experiments by Hartman (1958) carried out in a temperate region, indicate that at temperatures near 23°C, a period of exposure to fresh water between one and two hours would be necessary to kill the sponges. However, it is quite probable that gemmules would arise from the cells which were hidden in the recesses of the excavations and eventually regeneration would ensue (Hartman, 1958).

A paint recently developed by the Australian Cooperative Research Centre (CRC) for Aquaculture to kill the sponge and prevent egg release, has proven to be the most effective control method for *Cliona* on *P. maxima* shell (Maxima employees, pers. comm., Sept 1999). No other situation has been found where the description of the life cycle of a marine invertebrate pest has allowed a simple intervention to prevent infection of the host.

Previously, it was thought that growing shellfish off the bottom in a hanging culture (Bower & McGladdery, 1996) would most easily reduce shell damage as a result of this sponge. However, personal observations and reports from farms participating in the study indicate that this is not the case. All farms participating in the study operate by surface long-lines, yet all have *Cliona* damaging their shell.

Aside from studies, many of them quite old, attempting to address the issue of bioeroding sponges in edible oysters (Warburton, 1958) and other bioeroding organisms such as the worm in Bailey-Brock & Ringwood's (1982) study, few previous attempts

have been made to address the issue of bioeroding sponges in pearl oysters. Velayudhan (1983) suggested possible control measures, such as brushing the external surface of the shells with 1% formalin and immersing the shells in brine solution. However, this work was done on the Japanese pearl oyster, *Pinctada fucata,* and the control methods were semi-effective. Methods of control based on reproduction were not attempted. No previous studies have attempted to control *Cliona* on the pearl oyster, *P. maxima.*

2.7 Current management options

Although previously research into controlling sponge infestations may not have been considered profitable to the industry, the significance of the problem recently, the current high value of the product and the importance of pearling to the Western Australian economy have made it worthwhile more recently. The Australian CRC for Aquaculture based in New South Wales is currently researching paints that can be used to retard growth of fouling organisms on pearl oyster shells. The most recent and viable option for the management of *Cliona* in the pearl oyster farms is the application of a paint that would prevent egg release by the sponge, in addition to killing other organisms, on the oyster shell. Extensive trials are currently being undertaken to determine the effectiveness of this paint.

Two paint products are being trialed for use on the pearl oyster farms to kill *Cliona.* PearlSafe is currently under full registration by the national registration authority (R. De Nys, pers. comm., Feb 2000). This paint smothers the sponge and is a non-toxic coating

developed by the CRC. It is applied to the infected shell by dipping the hinge of the shell, plus any other infected area, into the coating. After approximately two weeks, the coat erodes, falls off the shell and the sponge has been killed. There are no residues and the shell is able to regrow and function as it did prior to the infection (R. De Nys, pers. comm., Feb 2000).

PearlClear is still being trialed on pearl farms, including Paspaley Pearl and Maxima Pearl farms. It is designed to protect the shell from fouling - particularly hard foulers such as barnacles (R. De Nys, pers. comm., Feb 2000). PearlClear is also free of toxins and safe for handling and for the environment (R. De Nys, pers. comm., Feb 2000). The product will become commercially available when the formulation of the product, which aims to prevent a wide range of organisms from settling on the shell, is complete.

If it is known when the sponge is reproductively active, this should assist in determining the most appropriate time to apply the paint. Therefore, although it is difficult to estimate the operational costs of using the paint, as applying the paint is still a labour-intensive process, if the time when the paint should be applied to prevent egg release is determined, the cost should decrease. As stated in Arakawa (1986), effective means of prevention and removal must be based on knowledge of the biology of the species in question.

2.8 Environmental management implications

In Australia, most pearl oysters live in areas remote from human and industrial pollution. The pearl oyster farms participating in this study depend on a pristine environment to sustain their mother of pearl shell. The shell is delicate and for it to produce the world's finest pearls it needs to be in a nutrient-rich and pollution-free site (Paspaley, 1999). All care is taken to provide a natural habitat for the shell at each of the pearl farms. Many Australian oyster farms are in remote bays, where their lines and buoys present no obstruction to boat traffic. Their location also reduces wind and wave action, which can decrease pearl quality. Oysters, like most shellfish, are sensitive to water quality and if stressed they produce poor quality pearls and become susceptible to disease (Fisheries WA, 1998). All pearling companies participating in the study believe that they place enormous emphasis on providing the best environmental conditions in order to produce potentially the world's finest South Sea Pearls.

The pearling industry and the rapidly increasing development of other shellfish aquaculture around the world with a concomitant increase in demand for the introduction and transfer of different shellfish species and stocks has increased the risks of spreading parasites and diseases around the world (Bower & McGladdery, 1997). This has escalated the need for vigilance against the spread of shellfish diseases. The risks associated with uncontrolled transfer and introduction of live aquatic organisms have long been recognised (ICES, 1988 in Bower & McGladdery, 1997).

In the last 10-20 years the frequency of shellfish transfers has increased due, in part, to the development of hatchery-based seed production and the remote setting of culture facilities, as well as to the increasing use of non-indigenous species in aquaculture. The development of hatchery technology within the pearling industry highlights the issues associated with translocation of pearl oysters from one area to another. Such issues are particularly important if the hatchery being used to produce spat is located in another area, either interstate or overseas.

Aquatic nuisance species may be released or "introduced" into the marine, freshwater or terrestrial environment intentionally or unintentionally. If such species become established and thrive, they will influence the native flora and fauna and their habitats, and may affect the local economy. Non-native species often out-compete, prey upon or bring diseases or parasites to economically and ecologically valuable native species, often adversely changing the ecosystem in the process (Olson & Goen, 1998).

An example of this is the accidental introduction of the Black-stripe mussel, *Mytilopsis sallei*, into Darwin Harbour in Australia (S. Slack-Smith, pers. comm., March 2000). This mussel is native to Central America where it attaches to stones and algal mats in Mexico and occurs in coastal lagoons in Belize and Venezuela. It has been shown in various studies that it is a rapidly growing, fast maturing opportunist (Huang & Morton, 1983; Morton, 1980). *Mytilopsis sallei* is a harbour species and was most likely introduced by wooden-hulled vessels entering the marina. Fortunately Darwin Harbour is a 'locked' harbour, in which water can be retained within the marina when the tides are low. Therefore, the mussel was found only within the marina and was easier to

treat. The mussel infestation within this harbour was treated by pouring bleach and copper sulphate into the water of the marina. Although this killed the mussels, every other organism within the marina was also killed (S. Slack-Smith, pers. comm., March 2000).

The WA government recognises the risks associated with developing an aquaculture industry and to reduce these risks it introduced regulations (Fisheries Regulations, rr. 1996, Division 1) to control the transfer of fish. These regulations deal with movement between water catchment areas or separate water bodies, empowering the Director of Fisheries to prohibit taking of species to minimise the risk of contamination or disease to other fish in other areas, movement of contaminated or diseased species, establishing quarantine areas and the release of exotic species (Fisheries Regulations, rr. 12-26).

Recognition of the correlation between shellfish transfers and disease-spread has been reflected by global development of regulations and guidelines to control live imports of shellfish (Bower & McGladdery, 1997). The Ministerial Policy Guidelines (FDWA, 1997a) and the Pearl Oyster Translocation Protocol (FDWA, 1997b) provide a detailed series of requirements for the handling of hatchery grown pearl oysters to reduce the risk of transferring diseases.

There are a number of practices used to manage the pearl oyster farms, such as regulations on the transfer of wild stock pearl oyster quotas, foreign ownership and use of pearl oysters for research by the industry, detailed in FDWA (1997b) to help reduce the spread of infestations. However they provide little guidance once an area is

infected. To avoid the accidental introduction of infectious disease agents, information on known parasites and diseases must be readily available (Bower & McGladdery, 1997).

The control methods proposed in this study have a minimum effect on the environment. Although derived from chemicals, the paint suggested for use operates by smothering the sponge, as opposed to emitting toxins. Therefore, in this case, the option of controlling the sponge chemically is environmentally (and economically) viable, due to the low toxicity of the paint.

2.9 Summary

Pearling in Australia is, economically, an extremely important industry, generating hundreds of millions of dollars annually in exports and employing over 1,000 people in WA alone. Based in the Kimberley, the WA pearling industry operated initially as a source of mother-of-pearl and more recently as Australia's largest and most successful aquaculture venture, which produces quality pearls worth about $153 million each year (Fisheries WA, 1999). It is also an industry that is highly dependent on pristine waters. The industry is therefore environmentally aware with respect to water quality and conditions.

Unpolluted waters and high tidal movement are necessary to give a good nutritional flush (Scourfield, 1997). Oysters, like most shellfish, are sensitive to water quality and if stressed they produce poor quality pearls and become susceptible to disease (Fisheries

WA, 1999). Considering that colour, shape and weight determine the value of a pearl, *Cliona* infestation on a large scale within a farm's lease (and even from wild caught shell) can cost a pearling company millions of dollars, resulting in a massive loss of revenue each year (Moase *et al.*, 1998). Managing or reducing the impact of this bioeroding sponge from pearl oyster shells with minimal or no environmental damage in Australia is therefore imperative.

Little literature is available on the reproduction and life cycle of this sponge, or on ways in which to manage sponge pests in mollusc aquaculture. Clearly, research is required in a number of areas including the ecology and reproduction of the sponge and ways to prevent spreading of the sponge via transfers of shell in aquaculture and shipping (via ballast). The research conducted here contributes to environmentally responsible management of a bioeroding organism that is having adverse effects on the pearling industry in north-western Australia.

3 Pearl farm siting and management

The economic success of a pearl farm relies on two main factors – siting of the farm and management practices. These two factors are not mutually exclusive and indeed, if good environmental management practices are not followed, the farm will not succeed irrespective of the site's position. Poor management practices will not produce a quality product and therefore will reduce the profitability of the farm. Clearly, maintaining a healthy environment through good management practices, including the control of *Cliona*, is linked to the appropriate siting and management of the farm.

The pearling industry has four basic procedures: collection of wild oysters, production of oysters in hatcheries, seeding of nuclei, growing-on of the oysters to produce pearls and marketing of the final product. The first three functions are closely linked to farm management practices, while the fourth is usually linked to business management strategies and is outside the scope of this project. Although pearl companies generally follow similar farm management procedures, there are small differences in farming techniques between the farms, which vary according to the company that is operating them and the location of the farm lease. The siting of the farm, collection of the oysters and growing-on can impact on the environmental management of the farm and surrounding areas and are discussed below.

3.1 Selection of farm site and effects of environmental parameters

The selection of an ideal farm site is of paramount importance. The selection should be based on an appraisal of the life history and habits of *P. maxima* and the ambience of the environmental parameters (Chellam *et al.*, 1987). Factors such as proximity to markets, transportation and a labour force are secondary to these.

The site should provide congenial conditions in the form of protection from rough sea conditions in case of cyclones and storms, sufficient tide and current flows to flush water around the oysters, sufficient depth, clarity, optimum salinity, temperature and adequate amounts of phytoplankton (Chellam *et al.*, 1987) to provide an ideal growing environment. Australia's north-west coastline provides farm sites with these fundamental environmental requirements which prevail for most parts of the year.

A sheltered bay with protection from wind and wave action offers an ideal site for farming the pearl oysters by giving protection to the long-lines. Also, pearl oysters open their valves for feeding only when water is calm and undisturbed. The big tides in the pearl farm areas mix the water, bringing a rich soup of organic particles to the oyster (L. Joll, 1992 in Doubilet, 1992). Food is therefore abundant in these fertile areas and replenished daily.

When selecting a suitable site for pearl oyster farming, the depth of water should generally be greater than five metres and proximity to a river mouth should be avoided due to prolonged reduced saline conditions and possible sediment loading during floods.

A mild current, which brings in food and removes faeces and detritus from the farm site, enhances the growing conditions of the oyster (Chellam *et al.*, 1987). The farm and adjacent areas should be free from any form of pollution, including antifouling compounds such as TBT.

The growth of pearl oysters and the size and colour of the pearl is strongly affected by water temperature, the physiological state of the pearl oyster and the condition of culture grounds. The latter seems to depend principally on the difference in chemical constituents of the seawater as well as on the kind and amount of plankton present (Chellam *et al.*, 1987).

The thickness of the layers of the pearl are affected by minute changes in water temperature during the day and vary considerably according to the seasons of the year. The deposition of calcium is stopped at water temperatures of 13° C or lower and the oyster perishes at 6° C (Chellam *et al.*, 1987). Consequently, sites in tropical and subtropical areas are ideal.

Pearl oysters seem to prefer high salinities, but oysters raised in such water produce pearls with a golden tint, which are possibly of lower value. The effect of salinity on the growth of pearl oysters is not clear (Chellam *et al.*, 1987).

3.2 Collection of oysters

The source of *P. maxima* for pearl culture is either the natural population in the pearl oyster beds, the hatchery or both the hatchery and wild stock. The collection of wild stock is managed in Western Australia by Fisheries WA and is discussed below. Spat (young oyster) collection in the sea is done to augment the supply of oysters, although since the introduction of hatchery technology, it occurs less frequently. The relatively recent achievement in the controlled production of pearl oyster seed by hatchery method has opened up a new chapter in pearl oyster production. Millions of pearl oyster seed are produced in the hatchery and reared in the farm to adult size and are used in the production of pearls. Therefore, although there is still a large reliance on wild stock, dependence on the natural populations for culture has been reduced. Production of pearl oysters in hatcheries is more dependable and the required quantities of oysters can be produced and supplied for pearl culture (Chellam *et al.*, 1987).

Wild stocks are presently found in the pristine areas of Western Australia's north-west, and collection of shell occurs from four management zones along the north-west coast. (Figure 3.1). These zones include:

- Pearl Oyster Zone 1: NW Cape (including Exmouth Gulf) to longitude 119°30'E – 5 licensees;
- Pearl Oyster Zone 2: East of Cape Thouin (118°10'E) and south of latitude 18°14'S – 11 licensees;

- Pearl Oyster Zone 3: West of longitude 125°20'E and north of latitude 18°14'S – 2 licensees (plus 11 Zone 2 licensees); and
- Pearl Oyster Zone 4: East of longitude 125°20'E to WA/NT border (all licensees have access)

(Penn, 1999).

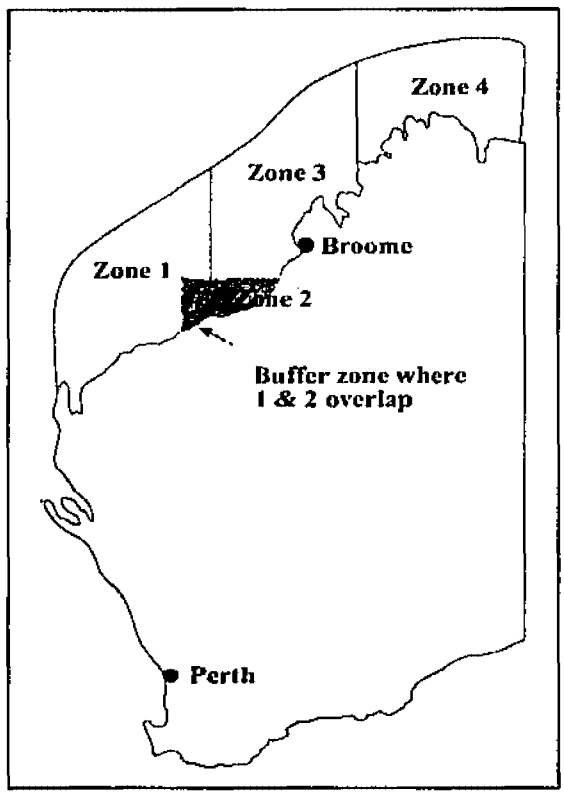


Figure 3.1. **Map of shell collection area (Fisheries WA, 1998).**

Pearl oysters are collected by skin diving or using SCUBA from the oyster beds. Fisheries WA designate collection zones and shells must be a minimum size of 120 mm. Oysters over 160 mm are generally not collected, as they are not suitable for round pearl production.

3.3 Methods of rearing

Once *P. maxima* shells have been introduced into the farm from either wild stock or the hatchery, there are many possible methods of rearing them. These methods may include raft culture, collapsible rafts, on-bottom culture and also the long-line method of farming (Chellam *et al.*, 1987). All farms participating in this study operate by hanging culture, also known as the long-line method, and therefore this is the only method that will be discussed here.

The most common system used in Australia today involves suspending pearl oysters, held in netting panels, from dropper-lines attached to massive long-lines (Figure 3.2). The 24-28 mm diameter long-lines are buoyed by numerous plastic floats and held in place with large steel or cement anchors which are anchored up to two metres deep in the mud or sand of the sea floor. Long-lines are always under tension to maintain stability of the structure. The long-lines are generally held at least 20 to 30 metres apart to avoid entangling adjacent lines if one breaks. An average line is 100 metres long with panels every metre for a total of 600 pearl oysters on the line (EMEC, 1998). Vertical lines, called droppers, with panels containing pearl oysters are hung from the buoys and are maintained well off the bottom (approximately 2 metres below the surface) in the hope that it will avoid fouling by organisms such as *Cliona* (EMEC, 1998).

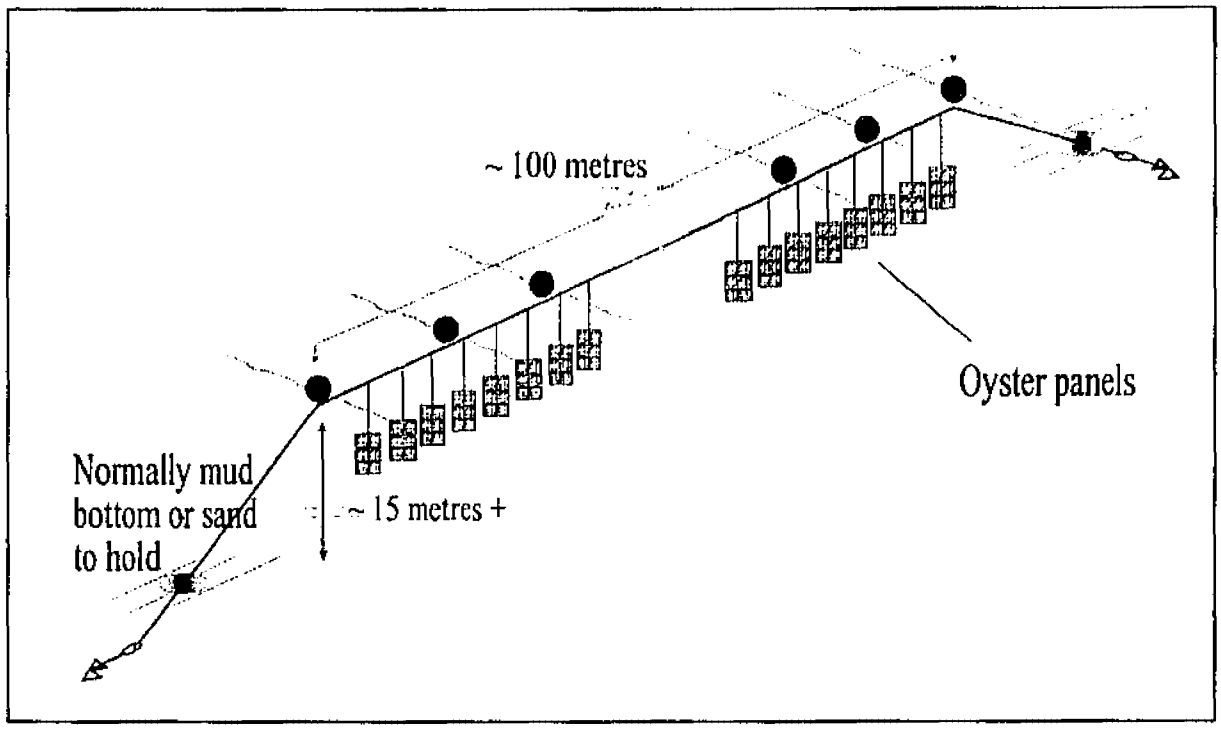


Figure 3.2. The long-line farming method (EMEC, 1998).

With the long-line system, the oysters hang in the water where maximum food is available (EMEC, 1998). This method also has the advantage of avoiding the use of divers, minimising interactions with crocodiles and allowing the use of less skilled workers for routine work.

3.4 Shell density on farms

The density of shells – the number per panel, the number of panels per long-line and the number of long-lines per farm, may influence the local environmental conditions and the levels of *Cliona* infestations. If *Cliona* can reproduce asexually via growth, then the likelihood for infestation spreading when the densities of shell are high is increased if the shells are close together. Generally the long-lines are sufficiently far enough apart

to prevent *Cliona* spreading. If long-lines are close together, there is a potential to alter current patterns which could ultimately affect local environmental conditions.

Density of shell varies between farms and location. However, the density of the pearl oysters in the culture grounds should be kept at optimum level. This density level will vary from farm to farm according to the size of the farm site, in addition to a number of other secondary factors. Overcrowded culture conditions can have such adverse effects as retardation of growth, poor quality of pearls, slow formation of the pearl layer and spread of diseases or parasites, including *Cliona*, causing severe and heavy damage to the pearl oysters. Too low a density will reduce the number of animals and therefore reduce profits unnecessarily. The oyster load per unit surface area is dependent on the depth of the farm and various other factors such as physical conditions and primary production of the area (Chellam *et al.*, 1987).

3.5 Farm maintenance and cleaning

Many undesirable organisms, such as *Cliona*, settle on the pearl oyster during farming. Since these have a direct bearing on the formation of low quality pearls, retarded growth and mortality in oysters, they are removed periodically depending on their intensity and seasons of settlement. This is done by regular cleaning of lines and shell to control fouling and is essential on every farm.

Shell cleaning is done on custom-built aluminium workboats six to ten metres long (see Figure 2.2) and the following cleaning technique is relatively standard throughout all farms in the study. The time between cleaning varies from farm to farm, but generally

occurs once every four to five weeks and more regularly in the wet season when the growth of fouling organisms is faster. Of the farms participating in this study, the Paspaley Pearl farms cleaned most regularly (approximately once every 10 days), and Morgan cleaned least regularly (approximately 6 weekly). The panels and their oysters are hauled up into the boats and cleaned on a regular basis. Cleaning machines have been developed which use high-pressure water to mechanically remove as much fouling as possible. The water hits the panels of oysters from both the top and the bottom. This is generally enough to dislodge seaweed, but encrusting oysters, barnacles, sponges (including *Cliona*) and sea squirts must be removed by hand when the panels emerge from the machine (Aquilina & Reed, 1997). In some cases, it is impossible to remove all of the *Cliona* from infected shells. In these cases, either as much of the sponge is removed as possible, or the shell is discarded, depending on the severity of the infection and whether or not it is treatable. Dead shells are also removed during cleaning. The time that the animals are out of the water is kept to a minimum (EMEC, 1998) and care is taken during the cleaning process not to damage the shell margins (Chellam *et al.*, 1987).

Most pearl farms in the study clean their oysters at least monthly, such as Morgan Pearl. In some places, including the Paspaley Pearl farms, and at certain times of the year, it is necessary to clean every second week. Maxima Pearl cleans their shell on a three-weekly basis. Shell cleaning is a dirty, monotonous job that produces a high turnover of workers (Aquilina & Reed, 1997) and therefore the industry is seeking methods to reduce this activity.

By adopting appropriate management techniques, the survival rate of pearl oysters in the farm can be enhanced. Periodic maintenance of the oysters and culture containers and removal of fouling and predatory organisms, including *Cliona*, from the pearl oyster panels and oysters, assists in minimising the mortality rate. If pests or predators of the oysters are introduced accidentally (such as during collection of young oysters from natural beds), the mortality rate can increase dramatically (Chellam *et al.*, 1987) which may have been the case with the introduction of *Cliona* into the pearl oyster farms of north-western Australia.

3.6 Pearl culturing

Pearls are created by the laying down of a lustrous nacre around a nucleus which is causing an irritation to the oyster. Under normal conditions, the nucleus is natural – a piece of sand or shell. However, cultured pearls are created by implanting a small piece of Mississippi mussel shell to create an artificially large pearl (Anderson, 1996b). The results of pearl culturing are three pearl types: cultured irregularly shaped pearls (baroque), half-round pearls (mabe) which are made by fixing hollow plastic shapes to the oyster's shell wall, and irregularly-shaped natural pearls with no artificial nucleus (keshi).

Prior to seeding an oyster, the shells are carefully cleaned and the oysters are allowed to rest in nets in their natural grounds to recover from any stress of being moved. Two to three months later, they are opened a maximum of 2cm and "seeded". A skilled technician places a tiny Mississippi clamshell bead into the oyster's pearl sack, plus a

small piece of mantle tissue from another oyster into a small surgically created pocket in the animal's gonad (Scourfield, 1997).

The oyster then begins coating the nucleus while it is cared for by farm workers. After three months, the oysters are X-rayed to check whether or not the nucleus has been rejected. Those that are not growing a cultured pearl, either because they have rejected the nucleus or because the graft tissue was not in proper contact with it, are put aside for another operation attempt (Aquilina & Reed, 1997).

Two years must pass before the crop is harvested. Oysters are out of the water for just two hours as the pearl is removed and healthy oysters are re-seeded (Scourfield, 1997). Theoretically, the longer a pearl has to develop, the thicker and deeper the coating of nacre and the higher the quality of the pearl. However, there is a limit to what is practical and profitable.

The best pearls come from healthy oysters. The presence of *Cliona*, for example, can severely tarnish the pearl. Producers expect approximately four year's production from a good oyster, seeding it first at about two years of age, and in its last year it may be used for half-shell production. The temperature of the surrounding seawater has an important effect on the lustre and colour of the pearl. These features are best in winter, so the pearls are harvested during July and August (Scoones, 1991 in EMEC, 1998). During harvest, suitable pearl oysters are reseeded with a new nucleus to begin the two-year process of producing a new pearl.

3.7 Industry controls

Fisheries WA now issues pearling licences with 20,000 hatchery options each. That is, each farm can use 20,000 oysters from a hatchery for round pearl production in addition to their wild quota. This measure is designed to encourage the development of, and interest in, new technology in the industry without destabilising production and possibly affecting pearl prices (Fisheries WA, 1999). Quotas for wild stock have been introduced as an industry control to ensure sustainability of the stock and to optimise the value of pearls to the community by maintaining prices. Fisheries WA also allocates a quota of wild shell to each licensed company. There are currently sixteen licences issued to companies harvesting *P. maxima* shell. In 1998-99, 565,000 pearl shells were collected from WA waters from a total allowable catch of 572,000 (Penn, 1999). However the tight controls that exist today are relatively recent. Quotas for wild stock were introduced in 1982 (Penn, 1999). Prior to having pearl quotas imposed on the industry, the sustainability of wildstock was at risk. Oyster stocks have since recovered to the point where divers no longer need to descend to dangerous depths to find shell and take hours to surface safely (Anderson, 1996a).

Most of the basic techniques for pearl growing are established, but research and development continue, either within company laboratories funded by the Pearl Producers Association (PPA) or at Fisheries WA which concentrates on continuous monitoring of the oyster stocks using surveys and logbooks kept by fishers.

3.8 Typical annual pearl farm operating schedule

The typical work schedule of a pearl oyster farm is a busy one and operates on a 12-month cycle. To further assist in understanding pearl farm operations and management, a typical schedule for a pearl farm is summarised and outlined below (Table 3.1). It is clear that timing of tasks undertaken on the farm is of paramount importance in obtaining a good final product.

As evidenced from the schedule, cleaning of shell, which includes controlling *Cliona*, occurs often and therefore if this work can be reduced, farms should save money and substantially lower the risk of shell damage. It is clear that effective management of pearl farms, which involves the maintenance of a pristine environment combined with aiming for the optimal yield, is of paramount importance if each farm is to obtain the best possible final product.

Table 3.1
Pearl farm operating schedule (Maxima Pearls (1996) in Anderson, 1996b)

Month	*Tasks*
January	- prepare for wild shell collection
	- organise dive crews, fishing gear, paper work and licence fees
February	- begin fishing for 20,000 wild shells (fishing linked to tide patterns)
March	- collected shell is 'dumped' on the seabed or site leased by the company and allowed to rest.
	- maintenance of dumped shell includes turning and cleaning
	- shells seeded in the previous year are x-rayed and checked to see if implanted nuclei have been rejected
	- oysters that reject nuclei are re-seeded
April	- water temperature begins to drop as winter approaches (this is a rest period for the shells)
May	- ongoing farm work
	- turning and cleaning of the previous two year's seeded oysters that are suspended in wire panels in the water column
June	- prepare for operating on oysters to implant nuclei.
	- seeding and harvesting begin
July	- normal operating time for pearls
	- seeding new oysters
	- re-seeding those which have rejected nuclei
	- oysters that produce acceptable pearls are also reseeded
August	- harvest of previous year's seeded shells continues
	- two-month turning program of seeded shells follows operations. Oysters turned to encourage production of round pearls
September	- turning of operated shell
October	- turning of shells
	- cleaning of shells
November	- transportation of operated shells to grow-out areas
December	- oysters introduced into long-line system
	- clean gear

Note: cleaning is a continuous process throughout the year

4 Methodology

The study consisted of four major components: sampling of *Pinctada maxima* to collect sponges for processing using histological methods; determining the reproductive stages of the sponges using thin sections and light microscopy; surveying the appropriate literature for information on sponge reproduction and pearl farm management; and also discussing with pearl farmers and other researchers current and proposed methods of management of *Cliona*.

4.1 The sites

Four pearl oyster companies with five pearl farms participated in the study. The sites were located throughout north-west and northern Australia in the Kimberley coast near-shore waters (Figure 4.1). The pearl oyster farms were operated by Maxima Pearling Co. Pty Ltd., Paspaley Pearling Co. Pty. Ltd., Arrow Pearl Co. Pty. Ltd. and Morgan & Co. Pty Ltd. Paspaley Pearling had two farms participating in the research, located in Vansittart Bay (13°57'S, 126°10'E) which has a total area of 3.2 square nautical miles (NM) and Port Bremer (11°15'S, 132°15'E), with an area of 2.5 square NM. Maxima's farm lease, of 5.9 square NM is located in Cone Bay (16°29'S, 123°31'E). Arrow's pearl farm lease is located in Beagle Bay at (16°50'S, 122°30E) and Morgan's farm lease is 9.1 square NM and is located at the Monte Bellos Islands (22°24'S, 114°07'E). All farms employ the long-line farming method, whereby panels of oysters are hung on long-lines approximately 1 – 2 metres below the surface of the water (see Figure 3.2). Similarly, all farms harvest the "silver-lipped mother of pearl" shells, or *P. maxima*.

Cliona has been a constant problem in these pearl oyster farms most likely since the beginning of pearling in the late 1800s.

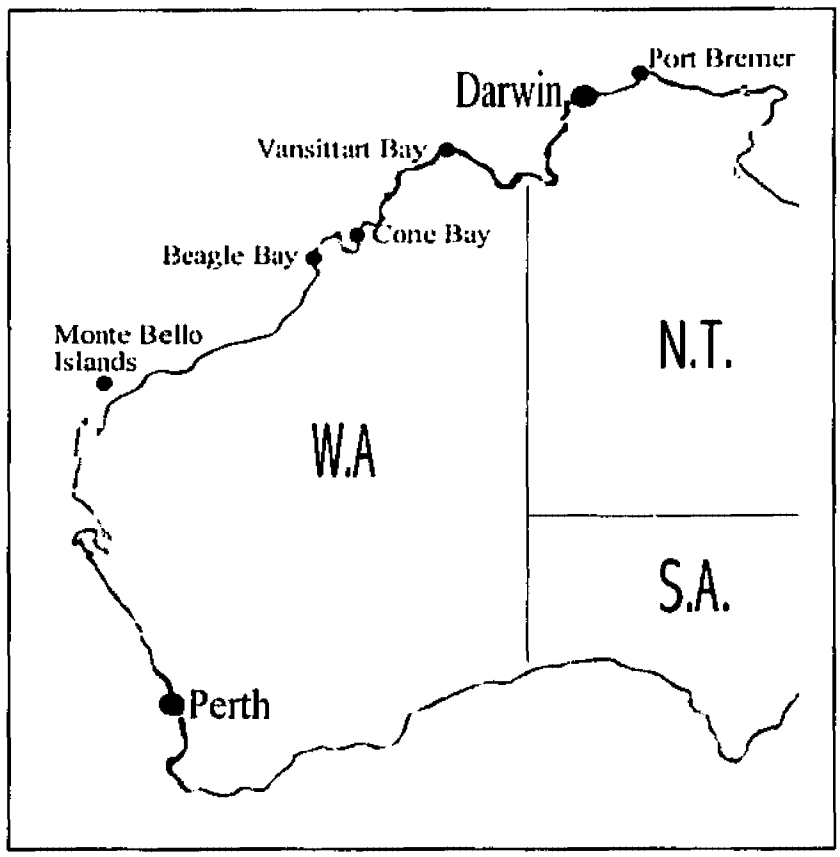


Figure 4.1. Map of the study sites

In order to gain an understanding of pearl farming operations and field methods in maintaining good quality oyster and shell, the sea-based Maxima Pearl farm, located in Cone Bay with a base on Turtle Island, WA, was visited for one week in September 1999. This field trip ensured that the design and layout of the farm and the way in which a surface long-line pearl farm operates was completely understood. Farming procedures were discussed with farm staff, including the shell-cleaning process. The hatchery and its operations were also investigated, in order to gain a full understanding of all aspects of pearl oyster farming. Research officers at Maxima pearl farm provided an insight into work on a farm, by allowing the researcher to participate in many of the

roles of employees on a farm. These included cleaning the *P. maxima* shell and oyster panels, assisting with the spawning of *P. maxima* in the hatchery and investigating fouling, including fouling by bioeroding sponges, on the shell. Additionally, the nature and extent of sponge infestations was discussed with staff, and the sponge infestation was observed *in situ*.

4.2 Environmental monitoring of sites

Environmental monitoring of the farms was undertaken by research staff and farm workers on each of the pearl farms. When the sponges were sampled, environmental data was recorded. Data including salinity, secchi depth, water temperature, and turbidity were collected, however, not all farms were able to collect all environmental data requested. Environmental data collected by each of the farms has been summarised in Table 4.1. The farms collected temperature data with a dataflow logger and salinity data by refractometer. The Arrow Pearl and Morgan Pearl farms were unable to collect data other than temperature. Maxima provided data on dissolved oxygen, pH, salinity, temperature and turbidity; and both Paspaley farms (Vansittart Bay and Port Bremer) collected data on salinity and secchi depths in addition to temperature.

Table 4.1
Summary of data collected from each of the 5 pearl farms

	PARAMETER					
FARM	**Temp (°C)**	**Salinity**	**DO**	**PH**	**Turbidity**	**Secchi depth**
Arrow	✓					
Maxima	✓	✓	✓	✓	✓	
Paspaley (PB*)	✓	✓				✓
Paspaley (VB*)	✓	✓				✓
Morgan	✓					

* PB = Port Bremer; VB = Vansittart Bay

The temperature data for Morgan Pearl farm were taken on the day of collection of the samples at 1 metre below the surface. All data for Maxima Pearl farm were collected at a three-metre depth, similar to the depth the shell are kept when cultured on surface lines. The temperature for Maxima Pearl was recorded weekly. Both Paspaley Pearl farms collected their water quality data at a 2-metre depth, and these were supplied as a monthly average. Morgan collected their temperature data at 1 metre depth on the day of sampling. Arrow also recorded environmental data on the day of sampling.

4.3 Sampling of sponge specimens

A 13-month sampling program was initiated at the five pearl oyster farms throughout north-western Australia to ensure that reproductive development within the sponges would be captured. Sampling was undertaken by qualified research officers employed at each farm. It was considered beneficial for sampling to occur for a minimum of 13 months to ensure that a full annual cycle of sponge development was monitored, with a 1-month overlap. Unforseen circumstances such as cyclones, however, prevented

sampling every month at some sites. Nevertheless, in most cases the sampling took place over 12 months, from October 1998 to November 1999.

When deciding upon which shells to sample, six live mature shells of *Pinctada maxima* with extensive sponge infection, and large enough to sample over 13 months, were selected at random from a farm and placed into a panel (Figure 4.2). The shells were randomly sampled, in order for the study to be considered appropriate in meeting the assumptions of a repeated measure-balanced single factorial design with the farms representing the factors. The assumption of randomness was met since samples were taken at random throughout the farms. The farms were geographically distant from each other and therefore independent of each other in terms of egg development. Six samples in each sampling period provided for sufficient degrees of freedom in the error mean square term used to test for significant difference between farms. Six samples also did not impact heavily on the normal farm workload and meant a minimum loss of shell to the farms.

Live shells were selected, as they are considered to be more applicable to farm management than dead shell and also Pomponi and Meritt (1985) found that sponges may have different bioeroding rates in live shell than in dead shell. Their study also found that *Cliona* is most commonly associated with live oysters. Each individual oyster was identifiable by a pocket number, and the panels were held at a depth of 2-3 metres below the water surface (Figure 4.2), which was the normal place for shell to be held on the farms. In the case of oyster death, a new oyster with infected shell was randomly selected from the farm and introduced into the sampling regime and a new

pocket number (Figure 4.2) was assigned to this oyster. The sampling program was then continued. In the case of both the Paspaley Pearl farms, 12 shells were used and were sampled bimonthly, as it was not expected that the shells were large enough to survive 12 months of sampling. Shells containing sponges with the same colour morph (i.e. orange) were selected to reduce any variability due to differences in species.

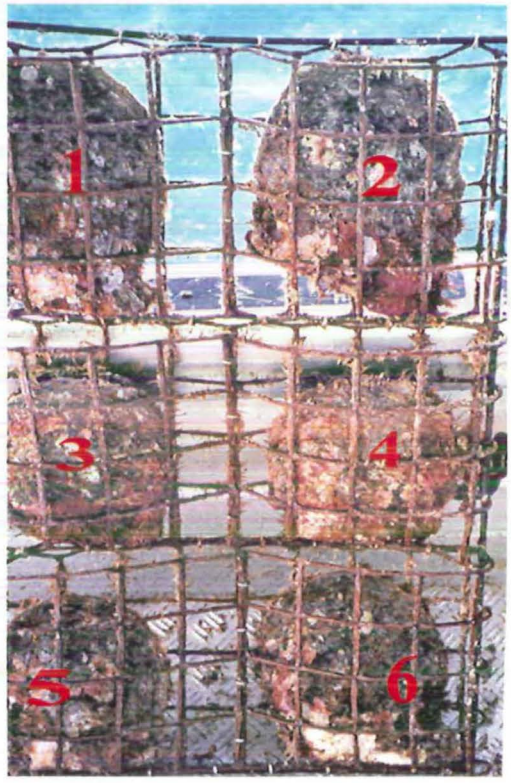


Figure 4.2. Diagram of a panel of oysters. Each oyster is in a pocket (numbered 1-6).

Although cleaning of the shell is usual on the farms, the shells being sampled were gently hand-cleaned only so that fouling by organisms other than the sponge (such as polychaetes, barnacles and oysters) did not cause oyster mortality. This gave maximum sponge available for sampling. Undamaged and unimpeded sponge was required for maximum likelihood of collecting sponge reproductive products. However,

unnecessarily different procedures from normal on the farms were avoided in this project to reflect, as far as possible, usual farm procedures.

Sampling began in November 1998. Where possible, the monthly samples were collected at each farm at the same time each month. Two incisions were made into each of the six shells and the piece of shell between the slits was collected as a monthly sample. The samples were generally a piece of shell no smaller than approximately 1 cm, although the amount of sponge found on the shells for the different months varied between 5 mm to 2 cm. Similarly, the depth of the shell sampled varied from 1-2mm thickness to approximately 1 cm, depending on the farm. Monthly sampling occurred at the same time each month on the farms and the date of collection was recorded as well as the pocket number and farm identification.

The six pieces of shell were placed individually in vials in gonad fixative (FAACC's)[1] to stabilise the structure of the tissues. The major aims of fixation are:

- to prevent autolysis or decomposition due to bacterial and osmotic changes;
- to preserve the tissue as near to its original form as possible;
- to prevent loss of tissue constituents and change in spatial relationships between organelles and macromolecules;
- to protect the tissue against subsequent changes during processing and embedding;

[1] Two litres FAACC contains:
- 37-40% formaldehyde solution (full strength commercial solution) (200ml);
- glacial acetic acid (100ml);
- calcium chloride dihydrate (26 gm); and
- tap water (1700 ml)

- to give the tissue a texture that facilitates sectioning; and
- to render the various tissue constituents reactive to proposed stains.

(Winsor, 1978).

The vials were then labelled with their pocket number, farm site, company and date of collection. After 48 hours, the shell pieces were transferred to 70% ethanol or denatured alcohol for storage (Winsor, 1978). Samples were then stored in a cool place and periodically checked for evaporation of ethanol.

At intervals, the shell samples were packaged and sent to the Western Australia Museum (WAM) for processing and analysis.

4.4 Histological methods

Sponges were processed for examination via light microscopy using histological techniques. The histological methods comprised three main procedures:

- Blocking – preparation of material from 70% ethanol storage into wax blocks for thin sectioning;
- Cutting – sectioning of material at 8 μm thickness and mounting onto glass scribed microscope slides; and
- Staining – processing slides through haematoxylin – eosin to stain cellular structures for later interpretation using light microscopy.

Blocking

The six pieces of sponge tissue collected monthly from each experimental shell were, if possible, carefully separated from the shell material. If in some samples the shell could not be removed, the samples were processed with the minimal amount of shell possible. The samples were put into labelled histological cassettes for processing into wax blocks. The sponge tissue was processed through an ethanol and xylene series (Figure 4.3) using an automatic tissue processor (Figure 4.4), which transfers the tissues mechanically from reagent to reagent both by day and night. Continual agitation in an automatic tissue processor reduces the time required for penetration into tissues in each fluid. The time in each solution is regulated by a clockwork mechanism operating from a notched disk (Winsor, 1978). The tissues finished in two warm wax baths.

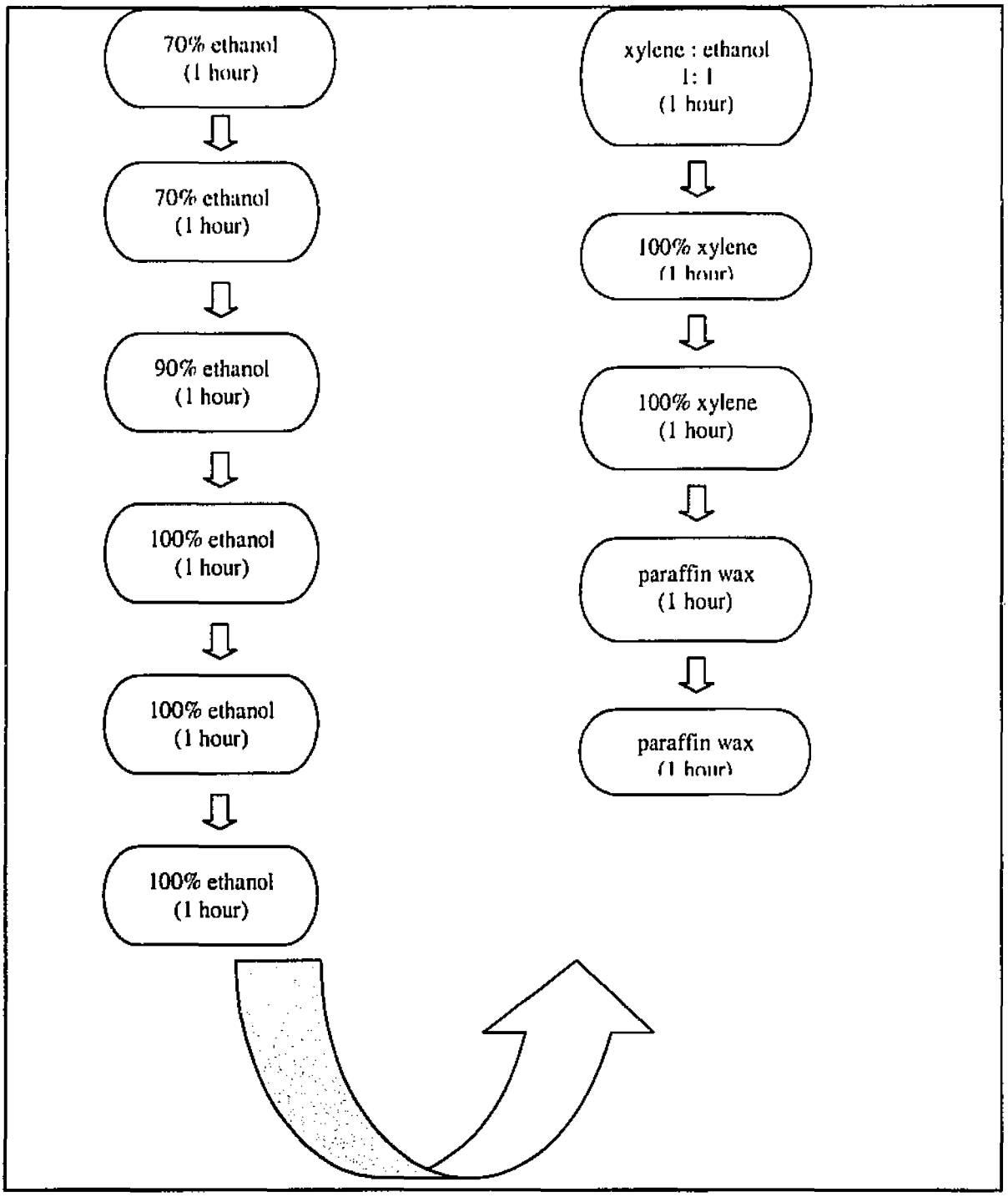


Figure 4.3. **The ethanol and xylene series.**

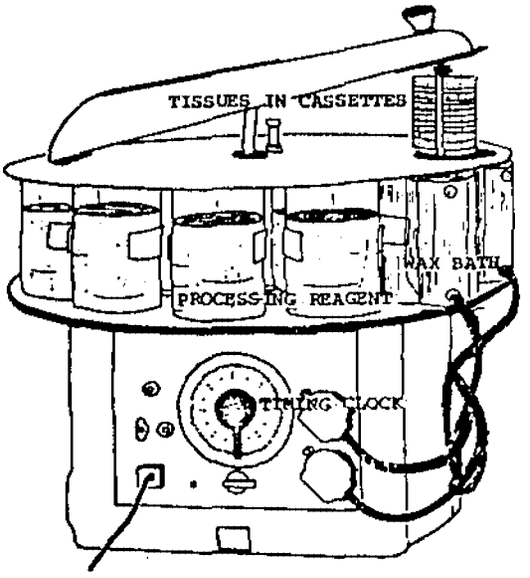


Figure 4.4. **An automatic tissue processor (Winsor, 1978).**

Once processed through the tissue processor, the tissue was infiltrated with paraffin wax using a vacuum pump for 30 minutes at 25 mmHg, to remove any air from the tissues. The fully impregnated tissues were then embedded in wax blocks by pouring molten wax into a warmed mould. Forceps were used to orientate the tissue correctly in the molten wax with the surface of the shell containing sponge placed on the base of the mould, to more easily cut thin sections from the sponge. Once the wax had cooled, the blocks were set in a freezer and then removed from their embedding trays. The blocks were then replaced in the freezer to be kept chilled to aid sectioning.

Cutting

The wax blocks were sectioned in a rotary microtome (Figure 4.5) at 6-8 μm. Prior to cutting, the blocks were placed, sponge downwards, onto a block of ice, as it is easier to cut cold blocks than warm blocks. When the block was cold, it was locked onto the

microtome for cutting. The block was trimmed using the microtome until the sponge tissue was exposed. When a section of sponge tissue had been cut, it was gently laid in a warm water bath. A microscope slide was labelled with a diamond scribe and smeared with egg albumin to act as a section adhesive. The thin section was then transferred from the water bath onto the prepared microscope slide and placed in a slide box for storage until staining. For each block, two slides were made so that the best possible thin section was obtained. This process was repeated for all samples.

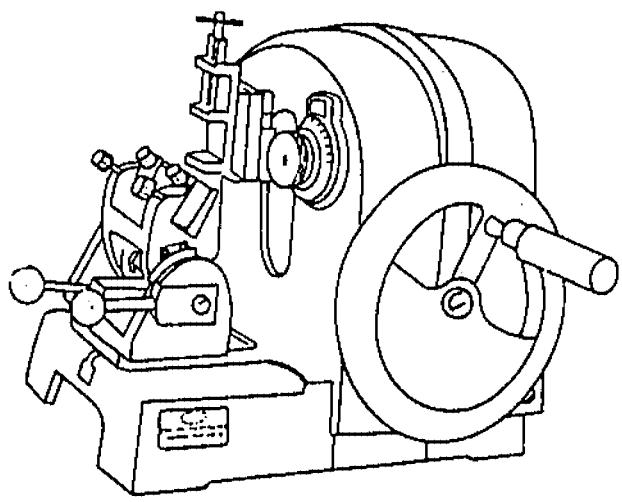


Figure 4.5. A Swift Rotary Microtome (Winsor, 1978).

Staining

Prior to staining, the slides were warmed to assist with wax removal. The slides were then treated with xylene and hydrated with graded alcohols to water and then stained with haematoxylin and eosin (Figure 4.6). Following staining, the samples were dehydrated through graded alcohols and cleared in xylene. Haematoxylin and eosin is the most popular and important routine staining sequence in the histological laboratory (Winsor, 1978). In a properly differentiated haematoxylin and eosin section, cell nuclei,

cytoplasm and connective tissue are clearly distinguishable. Nuclear chromatin stains blue and other structures stain various shades of pink and blue. This stain enables reproductive products, namely eggs, to be clearly visible using light microscopy. Following staining, the thin sections were mounted with Shandon Consul mountant, coverslipped and left to dry. Slides were then re-labelled with a self-adhesive label.

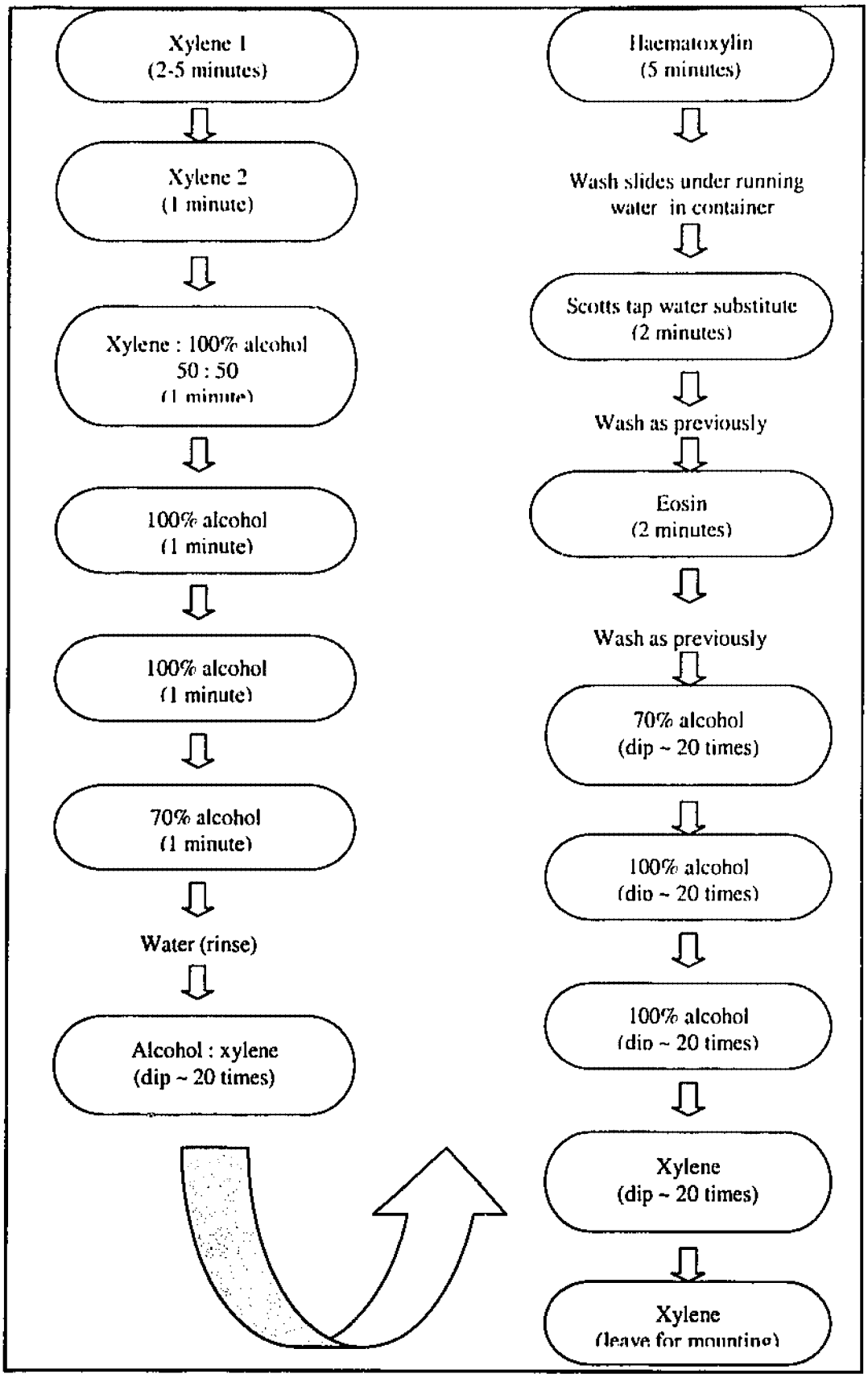


Figure 4.6. **Details of the staining procedure**

4.5 Reproductive analysis

The times when sponges were reproductively active was determined by light microscopy. Slides were examined and checked for the presence of eggs, sperm and asexual products (Figure 4.7). Fecundity was estimated using average densities of gametes in a 0.5-cm² area of tissue. Data from the slide analysis were entered directly into an Excel spreadsheet for initial storage. Time when eggs were present was then compared between farms to assess whether or not the sponges were reproducing at the same time each year throughout the biogeographical range of the study.

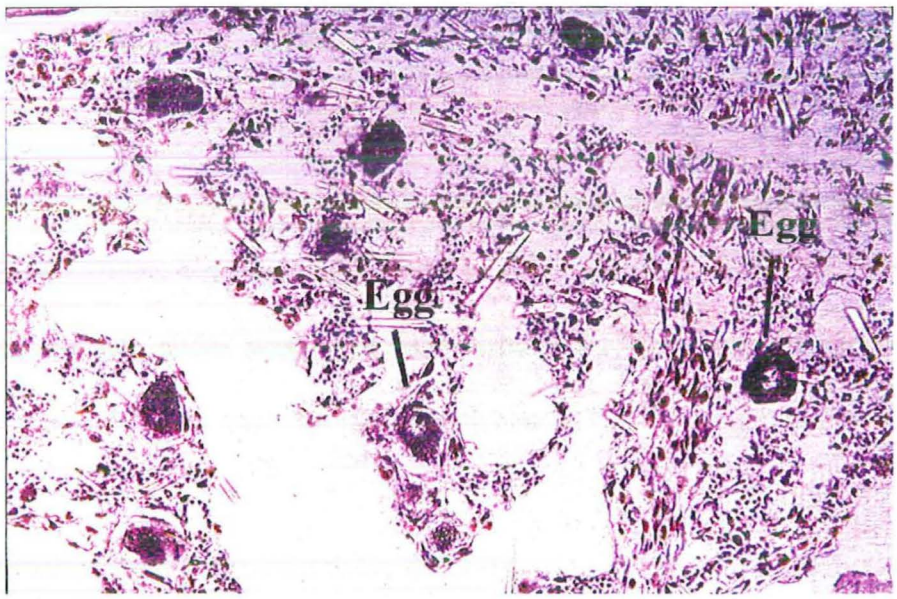


Figure 4.7. Photograph of a slide (Arrow Pearl, May 13th 1999) with eggs.

This methodology is standard practice for determination of reproductive timing in sponges (Fromont & Bergquist, 1994). However, it is the first time these techniques have been used on the sponges that bioerode pearl oyster shells in Australia.

4.6 Taxonomy

Expert advise was sought from the Western Australian Museum to investigate whether there was only one sponge species infesting *P. maxima* in the samples being used. Taxonomy was determined with excess pieces of sponge that were not used for the reproductive processing.

4.7 Data analysis

In preparation for the analyses, the data was entered into a Microsoft Excel (Windows platform) spreadsheet for initial storage, verification and editing. It was then analysed using SPSS version 10.0 for Windows.

Because two slides were generally taken of each sponge sample, the maximum egg count for the two slides was taken to determine the maximum fecundity. It could therefore be determined that a sponge sample had 'at least' a certain number of eggs.

The data were initially analysed using descriptive statistics for each farm x time period, to show the variability and distribution of the data. They were then analysed as a repeated measure single factorial Analysis of Variance (ANOVA) in time, crossing the number of eggs observed with time (months) and site (farm). Before commencing with this analysis, the data were checked for homogeneity of variance by calculating the coefficient of variation. The egg counts were square root transformed, in line with the Poisson distribution, to control variability in the data. The 95% confidence intervals

were calculated for the means and the means were then graphed over time and compared visually.

A regression using egg production with water temperature and salinity was carried out. If a relationship existed, they were then used as covariates in the Analysis of Covariance (ANCOVA), in order to test for differences between timing of reproductive development of the sponge and the influence of environmental factors.

Adjustments in the data analysis, by either removing months or coding these as missing values, were made when samples for some months could not be taken due to unforseen and unfavourable weather conditions.

5 Results

In total, 273 samples were analysed from the five sites (Table 5.1). Not every farm was able to sample for every month, due to unavoidable and unfavourable weather conditions, including cyclones. Although overall the data indicated relatively low fecundity of bioeroding sponges on the pearl oyster farms participating in the study, the presence of synchronously developing eggs within two of the farms, Maxima and Arrow, indicates that the sponge species infecting the pearl oysters are oviparous (the sponge has synchronous development and release of eggs). Previously, this has not been studied in Australia, or the Southern Hemisphere for sponge species bioeroding *Pinctada maxima*.

Table 5.1
Summary of samples collected from each site for each month

Site	Month														
	Nov '98	Dec '98	Jan '99	Feb '99	Mar '99	Apr '99	May '99	Jun '99	Jul '99	Aug '99	Sep '99	Oct '99	Nov '99	Dec '99	Total
Ma	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔			72
A	✔	✔	✔	✔	✔	✔	✔	✔		✔	✔	✔		✔	72
M		✔		✔	✔			✔	✔	✔	✔	✔			42
PB	✔	✔		✔	✔	✔	✔	✔	✔	✔	✔				51
VB				✔	✔	✔	✔		✔	✔	✔				42
															279

Ma = Maxima; A = Arrow; M = Morgan; PB = Port Bremer; VB = Vansittart Bay;
Total = the total number of samples collected from each farm

Examination of the sponge samples from Morgan Pearl farm and both Paspaley Pearl farms (Vansittart Bay and Port Bremer) presented no signs of reproductive activity. Sponge samples from Arrow Pearl farm demonstrated signs of a relatively high level of sexual reproductive activity. Arrow had four out of the six specimens showing signs of reproductive activity in samples collected on 13th May, 1999 (Appendix 1). Maxima had a lower level of reproductive activity, with only one specimen with developing

eggs. These were found on 4th September 1999 (Appendix 1). In both cases, numbers of eggs per sample were similar and these dates appear to be times when this species of *Cliona* is developing eggs for spawning. Another researcher who is familiar with sponge reproduction and who examined the samples confirmed these findings (J. Fromont, pers. comm., March 2000).

The low fecundity of the samples limited the statistical (univariate and multivariate) analysis that could be done on the data. It should also be noted that there were a large number of missing values in the data set for both the collection of sponge samples and the collection of environmental data. There are several reasons for these missing values. Primarily, the collection of samples did not occur in some months due to adverse weather conditions, such as cyclonic events. Additionally, the collection of environmental data, such as salinity, did not always occur at every site because of the malfunction of equipment, including a data flow logger at Arrow Pearl farm. This further restricted the statistical analysis that could be done. The non-normal distribution of the data, the minimal amount of data from the farms, and a lack of any relationship between the environmental variables and egg development, meant that multivariate testing was not warranted.

Although the results indicate low fecundity in the sponge, in which egg counts were taken from an area of 0.5 cm^2, the coefficients of variation (Table 5.2) demonstrate high variability in the egg counts when the sponge is at a reproductively active stage in its life cycle. The graphs of the means (Figure 5.1) further demonstrate the variability when the eggs are present. The mean number of eggs for Maxima Pearl appears

particularly low on the graph, as this is the mean number of eggs for all six samples, and eggs were only observed in one sample from this month. Descriptive statistics for the data from Paspaley Pearl farms and Morgan Pearl farm have been left out, due to the lack of reproductive activity at these farms.

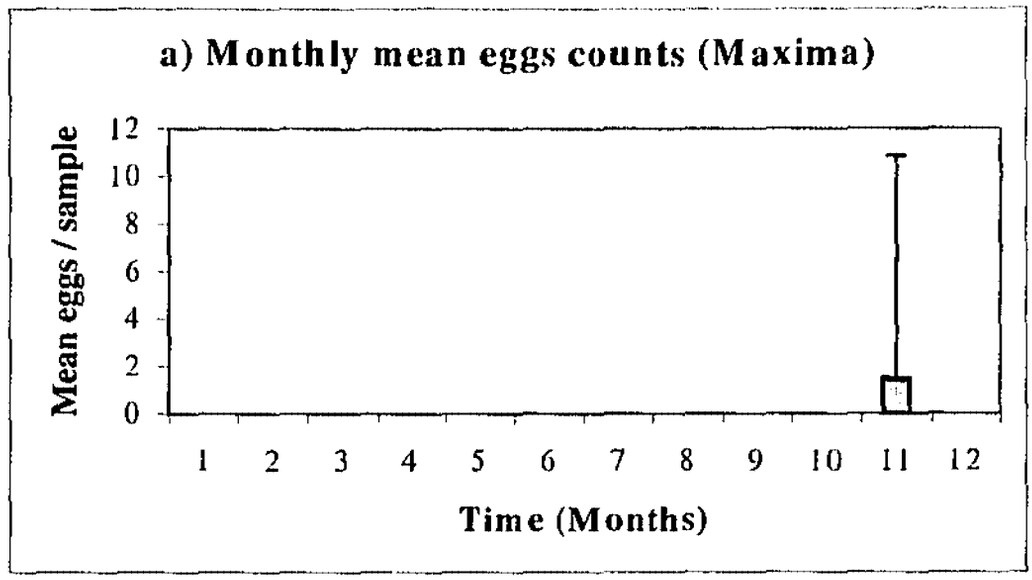
The high variability, as shown by the coefficients of variation, may be due to the non-normal distribution of the data, which is indicative of a Poisson distribution. The number of eggs noted in the sponge samples for Arrow and Maxima tends the data towards a Poisson distribution (Table 5.2). This is further justified by the significant (alpha < 0.05) results of the Chi-square goodness of fit for a Poisson distribution of 18.3930 (d.f. = 1) for Arrow and 7.4543 (d.f. = 1) for Maxima. Due to the Poisson distribution, the data were square root transformed (using $\sqrt{x + \frac{1}{2}}$) according to Zar (1984). Non-parametric methods were an alternative analysis. However, these tests are not considered as appropriate or as powerful. Similarly, the non-normal nature of the data combined with a lack of environmental data precluded multivariate analysis.

Table 5.2

Maxima and Arrow means, standard error of mean, standard deviations and coefficients of variation for the number of eggs (N=144)

	Maxima				Arrow			
Sampling Time	mean	std error of mean	stdev	CV (%)	mean	Std error of mean	stdev	CV (%)
Nov '98	0	0	0		0	0	0	
Dec '98	0	0	0		0	0	0	
Jan '99	0	0	0		0	0	0	
Feb '99	0	0	0		0	0	0	
Mar '99	0	0	0		0	0	0	
Apr '99	0	0	0		0	0	0	
May '99	0	0	0		4	1.43	3.52	88.03
Jun '99	0	0	0		0	0	0	
Jul '99	0	0	0		0	0	0	
Aug '99	0	0	0		0	0	0	
Sep '99	1.5	1.5	3.67	244.95	0	0	0	
Oct '99	0	0	0		0	0	0	
Total	0.13	0.13	1.06	848.53	0.33	0.17	1.45	436.05

CV = coefficient of variation; std error of mean = standard error of mean; stdev = standard deviation



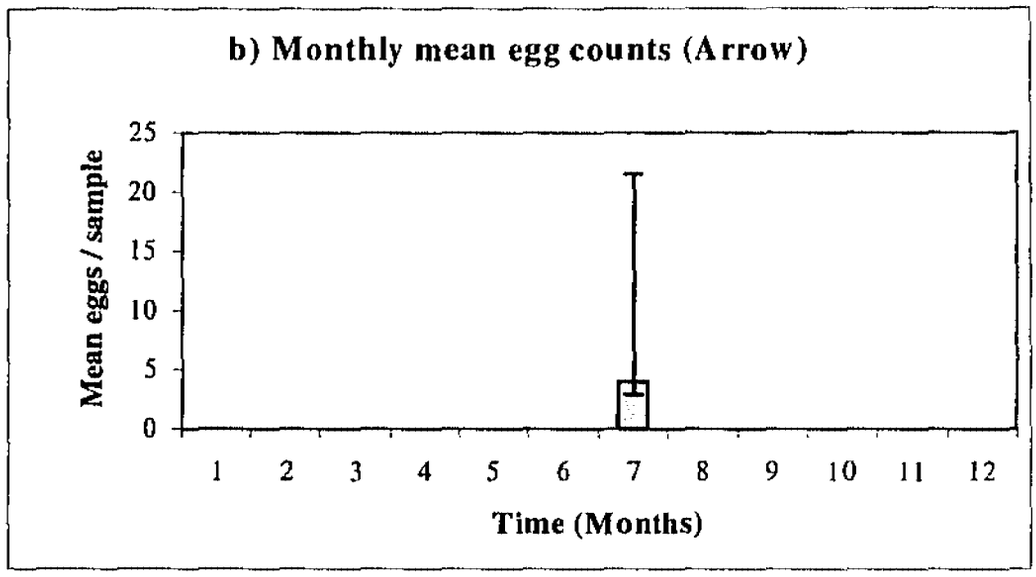


Figure 5.1a-b. Mean monthly egg counts for Maxima Pearl and Arrow Pearl farms. The error bars represent the 95% confidence intervals, based on the Poisson distribution.

The data from the egg counts for Maxima and Arrow were analysed using a one-way Analysis of Variance (ANOVA). Statistical significance was assessed at $P<0.05$. These tests demonstrated, for both the raw and transformed data from Maxima pearl farm, no significant difference in the number of eggs between months (time periods). Arrow Pearl farm, however, had a highly significant result for the number of different eggs observed between time periods ($p<0.001$) on the transformed and untransformed data

(Table 5.3), indicating that at this farm, time of year has an influence on egg production by the sponge.

Table 5.3
Results of ANOVA carried out on variation in monthly egg counts in Arrow and Maxima samples

	Between groups	
	F (11,60)	**Sig**
Maxima		
Data not transformed	1	ns
Data transformed	1	ns
	F (11,60)	**Sig.**
Arrow		
Data not transformed	7.742	0.000
Data transformed	10.737	0.000

ns = not significant

An ANOVA was also carried out on the entire data set (Table 5.4), to investigate variations in egg counts between farms and over time. All results from this two-way ANOVA using both transformed and raw data are significant to some degree. However, all results show a higher significance on the transformed data, as opposed to the untransformed data. The farm*time interaction is highly significant (p=3.86E-14), indicating that the differences between time periods depends on the farm in question. That is, some farms produced eggs at different times compared to other farms. As a result of this interaction, it is difficult to look at differences between months (time) or farms as main effects.

Table 5.4
Results of ANOVA for all farms participating in the study (egg count). The numbers in brackets indicate the degrees of freedom to test the factor.

Untransformed data

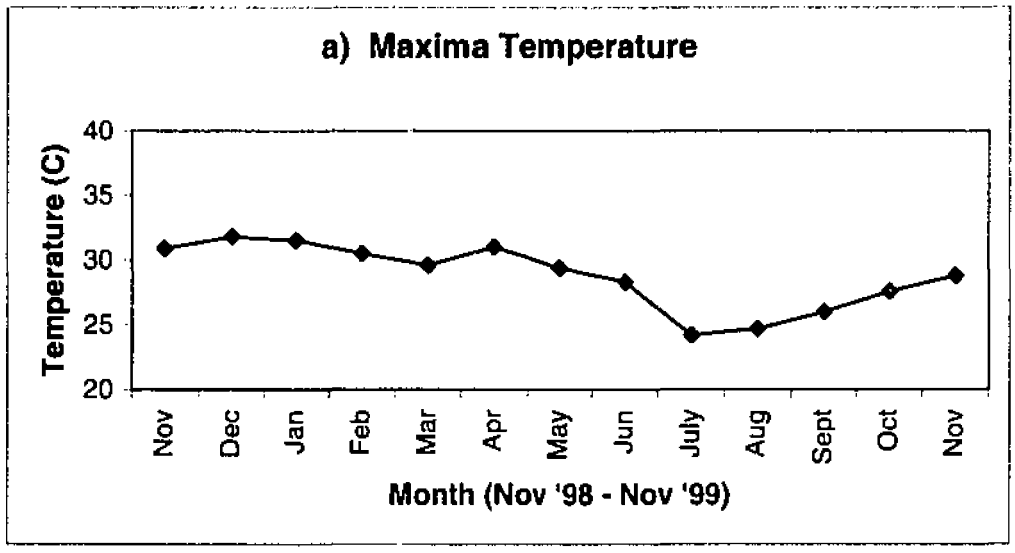
Factor	F	d.f.	Significance
Farm	3.5311 (4, 25)	4	0.0204
Residual	1	25	0.4672
Time	1.7157 (11, 275)	11	2.2E-05
Time*Farm	4.2910 (44, 275)	44	3.86E-14
Error		275	

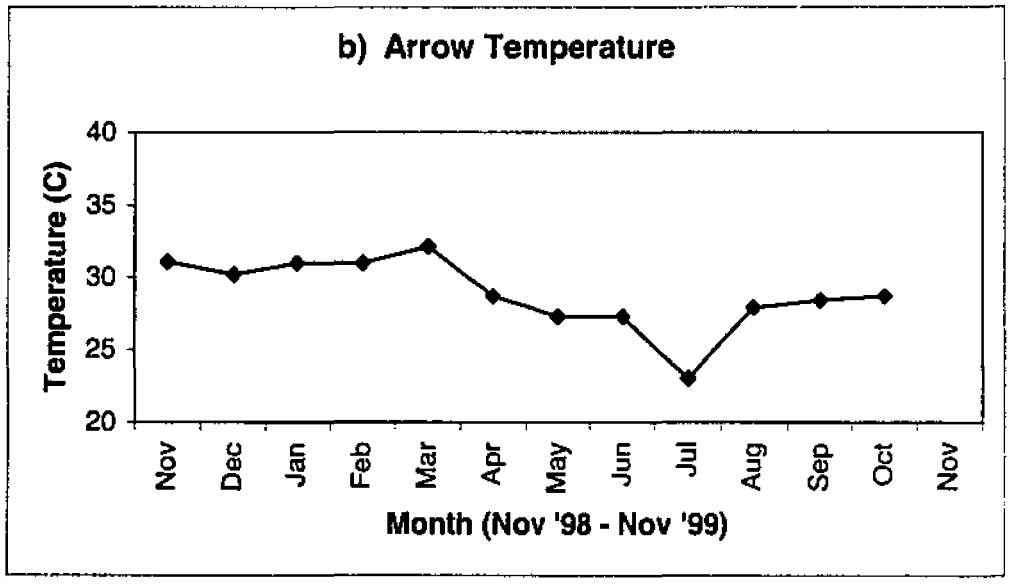
Transformed data

Factor	F	d.f.	Significance
Farm	4.6000 (4, 25)	4	0.0064
Residual	1	25	0.4672
Time	5.0878 (11, 275)	11	3.01E-07
Time*Farm	5.4281 (44, 275)	44	0.0000
Error		275	

5.1 Environmental monitoring data for each site

Regression analyses were also undertaken for egg production with the environmental data salinity and temperature (Table 5.5), so it could be investigated as to whether or not a relationship exists between these environmental parameters, and the reproductive timing of the sponges. The graphs of temperature and salinity for all the pearl farms demonstrate few fluctuations in these parameters for all study sites (Figure 5.2), further supporting the lack of correlation between salinity and water temperature and egg production by the sponge.





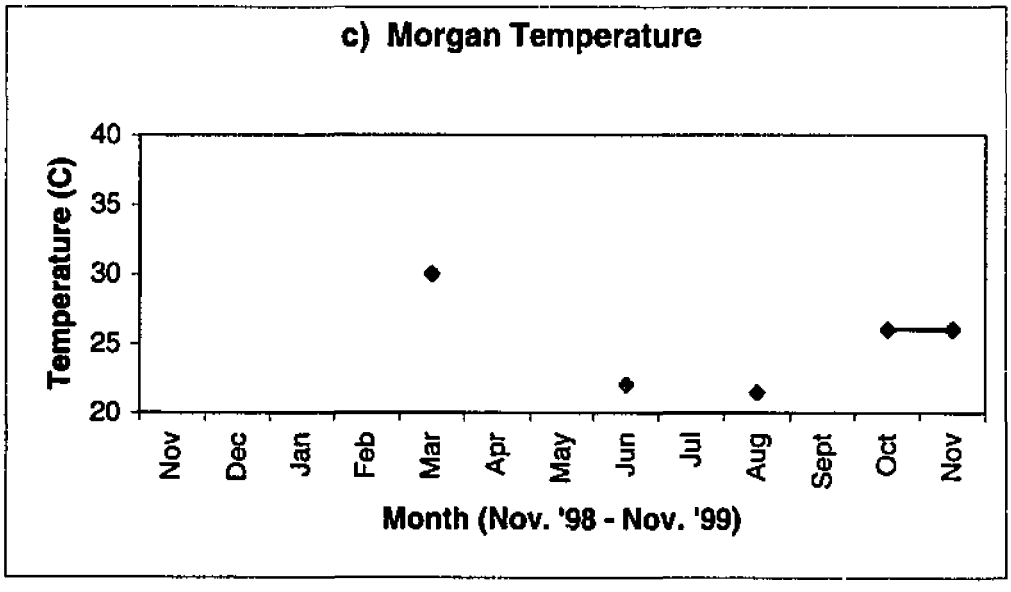
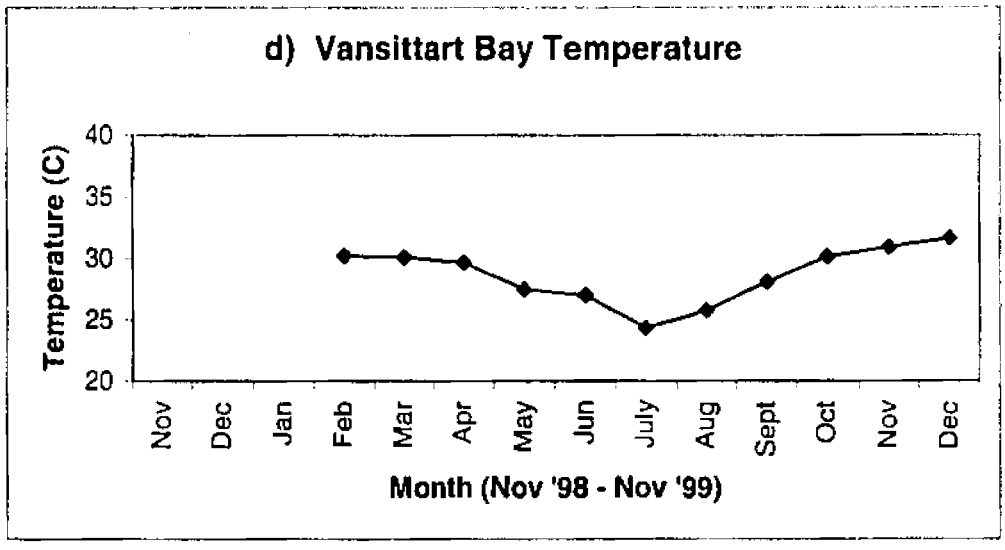


Figure 5.2 a-c. **Plots of temperature data for Maxima Pearl, Arrow Pearl and Morgan Pearl.**



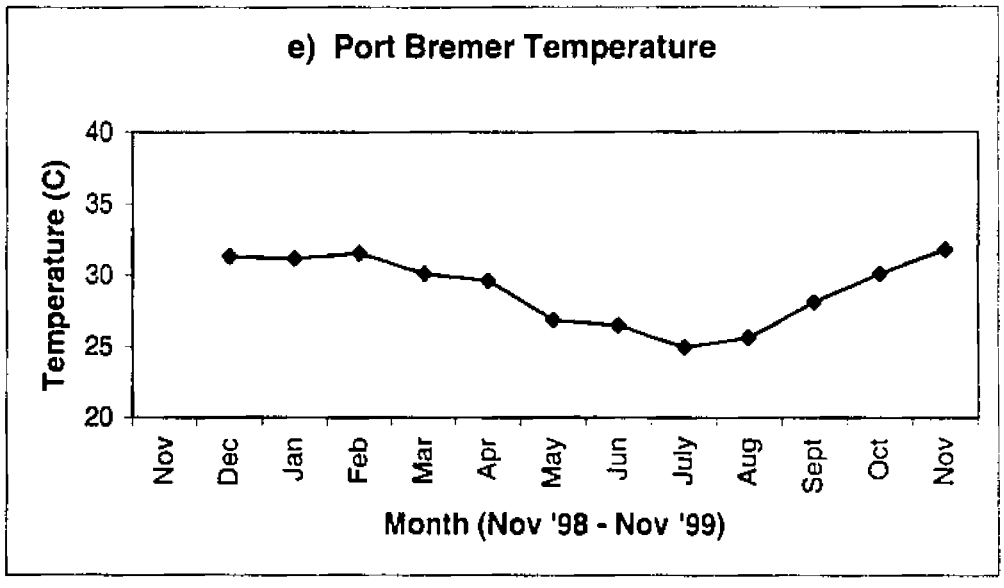
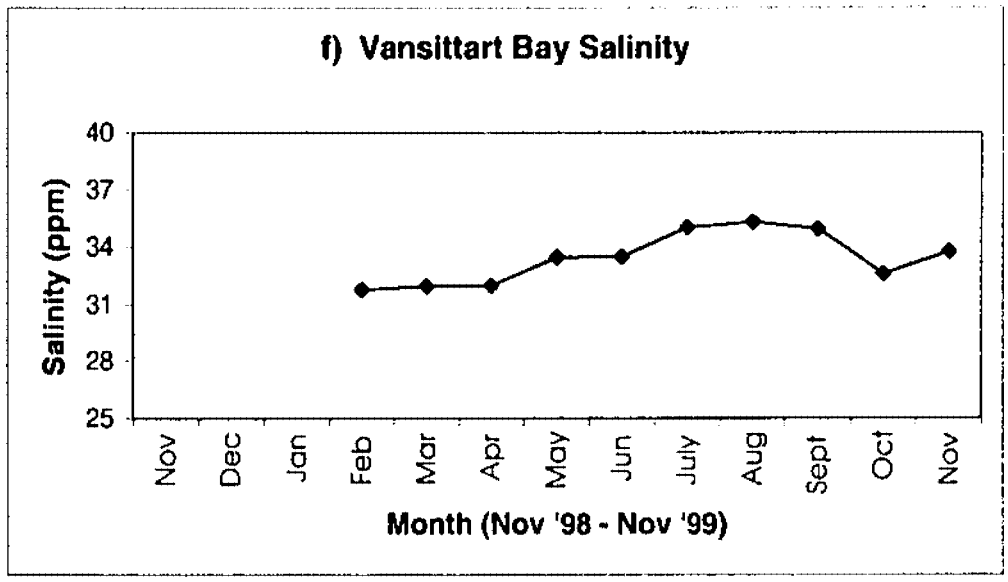
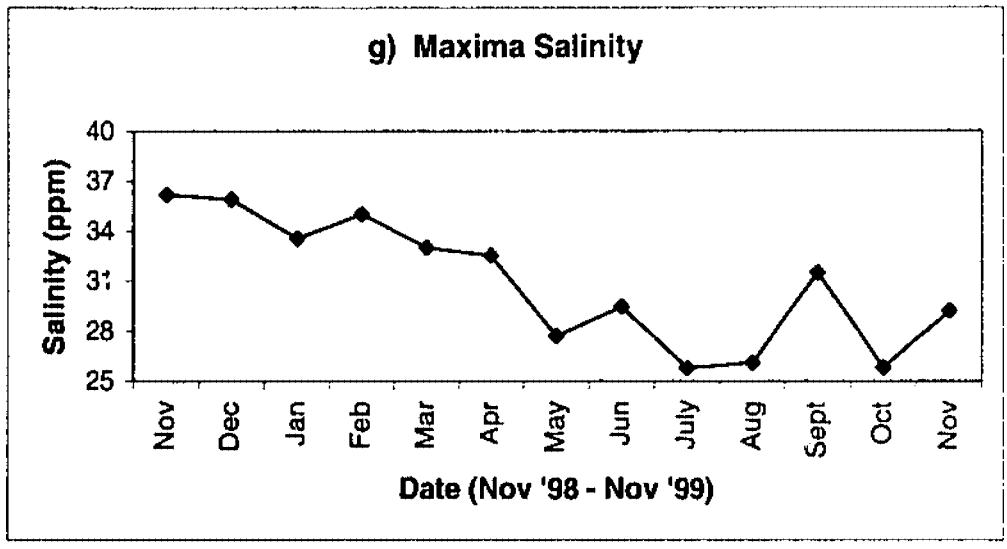


Figure 5.2 d-e. **Plots of temperature data for Vansittart Bay (Paspaley Pearl) and Port Bremer (Paspaley Pearl)**





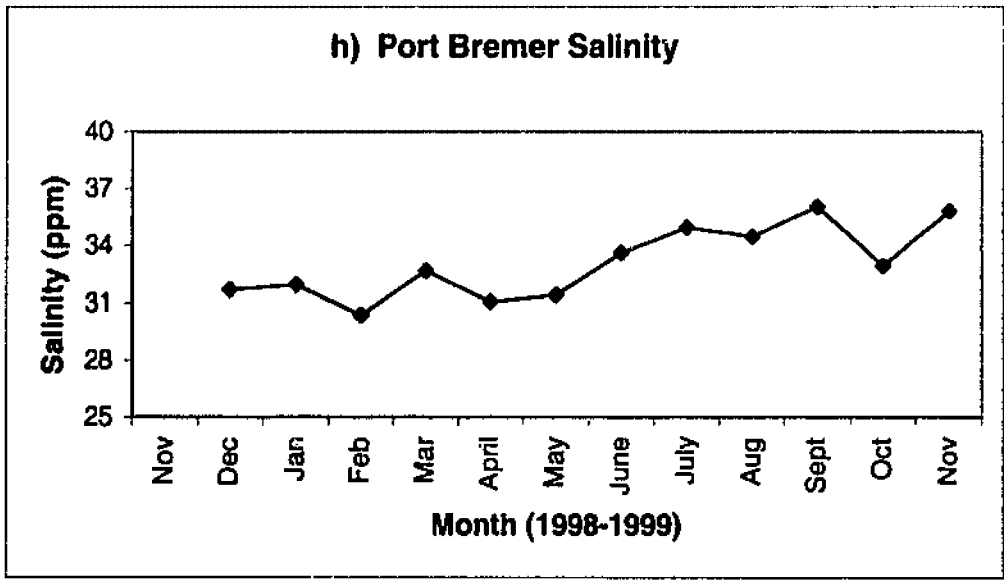


Figure 5.2 f-h. **Plots of salinity data for Vansittart Bay (Paspaley Pearl), Maxima Pearl and Port Bremer (Paspaley Pearl).**

Table 5.5
Regression analysis for transformed and raw data for egg production with temperature and salinity

Environmental Factor	Slope	Significance
Temperature		
Untransformed data	-0.0224	0.2639
Transformed data	-0.0062	0.2675
Salinity		
Untransformed data	0.0045	0.1925
Transformed data	0.0011	0.1956

The regressions (Table 5.5) demonstrated no significant relationships between egg production and temperature and salinity. Therefore, neither temperature nor salinity appears to affect egg production or density of eggs. Due to the lack of any relationship between these covariates and egg production in the sponges, analyses of covariances (ANCOVAs) to test for the significance of these relationships were not necessary.

Additional data that were collected on dissolved oxygen (DO), turbidity, secchi depth and pH were ignored, as this data was not consistently collected from all study sites. Additionally, preliminary comparisons of reproductive timing with these environmental factors revealed no obvious correlations. Likewise, the literature mentions no other environmental parameters other than temperature, salinity and lunar phase (for synchronicity of spawning) as having an effect on the reproductive cycle of marine sponges.

5.2 Taxonomy

Expert advice on the taxonomy of the species infecting the pearl oysters indicate that there is at least three different species of sponges from the family Clionidae bioeroding

the pearl oyster shells in the sponge samples collected in this study (J. Fromont, pers. comm., March 2000). The species names of the sponges have still not been determined. However, it is likely that more than one sponge genus is represented within the family Clionidae. The three different species found infecting the shell specimens are currently identified as Species 1, Species 2 and Species 3 (J. Fromont, pers. comm., March 2000). These species have so far been identified via spicule examination (Figure 5.3) and are currently being compared to type material for confirmation of species identifications. Species 1 was identifiable by the three different spicule types it contained: tylostyles, spirasters and acanthoxea (spiny oxea); Species 2 by one spicule type: tylostyles; and Species 3 was identified by the presence of tylostyles and spirasters. Tylostyles are characteristic of the family Clionidae. Although the initial taxonomic study indicates that the sponges sampled are different species, they are all close relatives and are therefore likely to have similar life histories.

It is thought, although it has not yet been confirmed, that the sponges found to be reproducing at both Arrow Pearl and Maxima Pearl farm belong to the same species - Species 2 (J. Fromont, pers. comm., March 2000). This was the most common species on the shell from all farms. Species 3 was the least common of the three species observed.

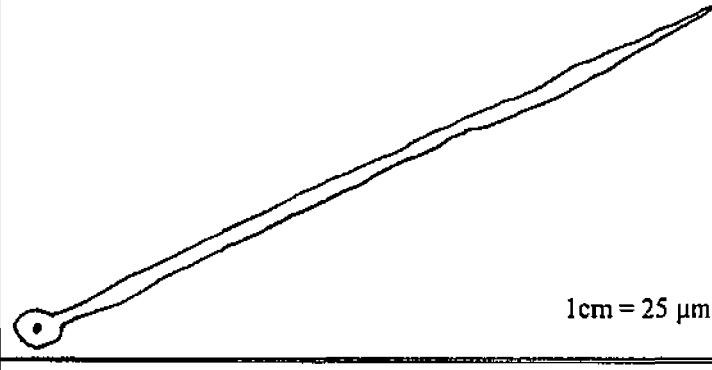
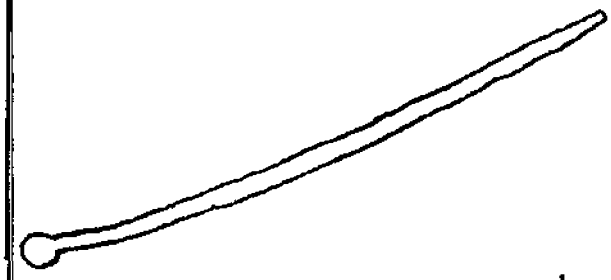
Species	Spicule Type	
Species 1	Tylostyle	 1cm = 21.65 µm
	Spiraster	 1cm = 46 µm
	Acanthoxea	 1cm = 49.07 µm
Species 2	Tylostyle	 1cm = 25 µm
Species 3	Tylostyle	 1cm = 36.8 µm
	Spiraster	 1cm = 36.8 µm

Figure 5.3. **Spicules of Species 1, Species 2 and Species 3**

5.3 Form of reproductive activity observed

As anticipated, no asexual activity was observed in any of the samples for this study, most likely due to reasons that were outlined in the background section – that gemmulation generally only occurs in temperate regions and fragmentation and budding cannot be assessed by analysis of the sponge samples. However, it is possible that the sponge did reproduce via fragmentation. In order to view fragmentation occurring, continual monitoring of the sponges *in situ* would have been necessary, as it is not possible to assess fragmentation away from the farm sites.

In the Arrow Pearl and Maxima Pearl samples, eggs were sighted, indicating that the sponges do reproduce via sexual reproduction. The mode of reproduction for the other three sites, Paspaley – Vansittart Bay, Paspaley - Port Bremer and Morgan, cannot be deduced due to the absence of eggs in their samples.

6 Discussion

This is the first study of the reproductive biology of sponges that bioerode the pearl oyster, *Pinctada maxima*. It has been established that within two pearl oyster farms in north-western Australia that the common bioeroding species, which belongs to the family Clionidae, has synchronous development of eggs. What is most interesting is that for these two farms (Maxima Pearl and Arrow Pearl) which are close together latitudinally and are thought to have the same reproductively active species of Clionidae, the development of eggs occurred in May at one farm and September at the other.

The majority of sponge species with oviparous development (broadcasters) have discrete, recurring, annual periods of sexual reproduction, with usually one cycle per year (Simpson, 1979). Recent studies have clearly demonstrated that sponges display sexual reproductive activity which, in a majority of cases, is cyclic, and it can be assumed that all sponges are sexually active during some portion of the year (Simpson, 1979). Some species that brood and incubate larvae (brooders), have no such cycles and produce gametes all year (Simpson, 1979). In the case of the sponge bioeroding pearl oysters in north-western Australia, reproductive activity was only noted at two farms. It should, however, be noted that this level of reproductive activity occurring in the sponges is a result of the synchronous egg developmental cycle that was found in these oviparous sponges. Such synchronous development can be missed, when environmental conditions prevent consistent monthly sampling. The low level of replication in sampling may also have contributed to the possibility of missing

reproductive events. These results have significant implications for the strategy of preventing the sponges from attacking the pearl oysters and will be discussed further.

Information on sexual differentiation and sexual behaviour of sponges is very poor. It consists mainly of descriptions of the reproductive processes and scarcely refers to the related regulatory mechanisms (Sara, 1983). This situation is above all due to a lack, at the moment, of procedures for maintaining sponges under controlled conditions for extended periods of time (Sara, 1983) so that reproduction can be studied in detail. A precise analysis of the factors regulating sponge reproduction will depend upon the development of such procedures (Sara, 1983).

Furthermore, there is a great need for an experimental approach to investigations of sexual processes in sponges. The conspicuous absence of these approaches to investigations in sponges is due to several factors: the difficulty in maintaining sponges in controlled laboratory conditions, the lack of external characters with which to determine species and therefore to select individuals for study, the lack of localised discrete gonads in this group and the limits of local population sizes for repetitive sampling (Reiswig, 1983). This study highlights the need for further work in both areas of research: the factors that regulate sponge reproduction and experimental approaches.

6.1 Low fecundity of the bioeroding sponge

As outlined in the results section, sponges in the study from the Paspaley Pearl farms and Morgan Pearl farm, did not show signs of reproductive activity. At Maxima Pearl farm, one of the six specimens sampled was reproductively active. Arrow Pearl farm

had a relatively high level of reproductive activity, with four out of the six samples found to be reproductively active. However, it should be noted that in those sponges that did reproduce, low numbers of eggs were observed (Figure 5.1). The number of eggs found in this study (5-10 eggs per 0.5 cm^2) compared to others (Fromont & Bergquist, 1994), which found between 37-255 reproductive elements per 0.5 cm^2 (in *Xestospongia),* were very low.

There are many possible reasons for such low fecundity of the bioeroding sponges found on the *P. maxima* shells at the sites sampled. It should be noted that, in general, the great variability in almost all but the most general aspects of reproduction makes it impossible to predict detailed patterns of reproductive behaviour in any group of sponge which has not been studied specifically and in detail (Bergquist, 1978). *Cliona* is one genus that has not been studied in detail and no studies of the reproductive characteristics of *Cliona* in tropical regions have been published. The possible reasons for the observed low reproductive activity include sampling limitations, lack of reliance on sexual reproduction, sponge recruitment outside of the sampling sites, male dominance, reproduction anomalies and environmental factors. These are outlined below.

Sample area was not large enough to see eggs

The first possible reason for this result is that the area of the sponge that was sampled was not large enough to see significant numbers of eggs. This study has established that these species have synchronous development indicating that in north-western Australia, *Cliona* is a broadcast spawner, whereby eggs are released into the water canals of the sponge, carried out with the water stream and undergo development in the sea water

(Fell, 1974). Typically, when a sponge that is a broadcaster is reproductively active, the eggs are plentiful. A square centimetre is generally all that is needed to locate many eggs (Fromont & Bergquist, 1994).

However, species of the family Clionidae are obligate bioeroders of calcium carbonate. In their alpha (colonisation) stage, few contiguous areas of sponge mesohyl of 0.5 cm^2 exist. Much of the eroding part of the sponge is found in minute pinholes within the shell and the collection of shell along with the sponge tissue is unavoidable. Therefore, due to the large amounts of shell contained in the samples collected, the area of sponge that could be observed was greatly reduced. Egg development was found in the sponge areas overgrowing the surface of the shell in a thin encrusting layer. A comparison with *Chondrilla*, a non-bioeroding sponge with a large sponge mesohyl area (the layer of mesenchyme that lies between the pinacoderm and the choanocytes), with sponges in this study is shown below (Figure 6.1). This picture clearly demonstrates that in a sample of sponges from the family Clionidae, there is much less area of sponge to observe than there would be for other sponge species that are not obligate bioeroders of calcareous substrates.

a.) **b.)**

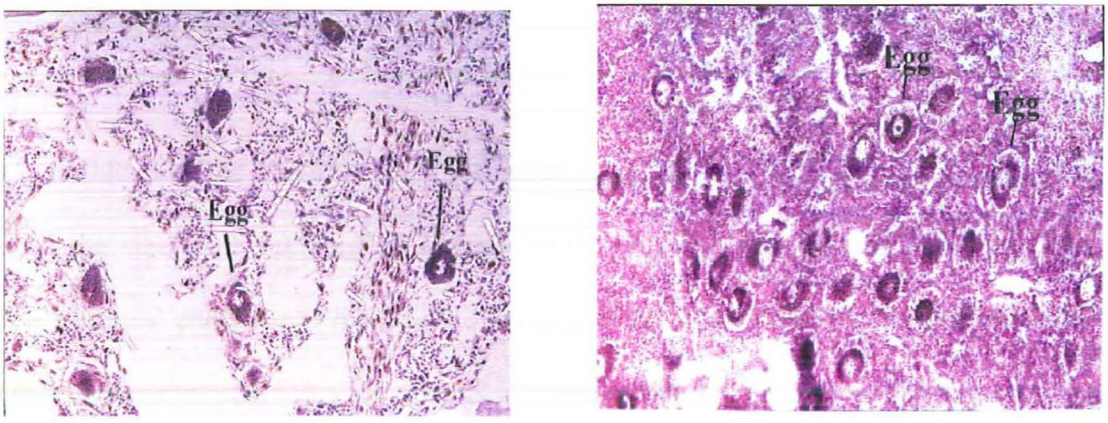


Figure 6.1 a-b. a.) A slide with eggs of a sponge collected from Arrow Pearl farm (13th May, 1999), compared to b.) a slide of *Chondrilla* (17th February, 1998).

Similarly, in some cases other organisms, including algae and polychaetes were sampled instead of, or as well as, the sponge. As mentioned in the Results section, more than one sponge species was sampled and analysed for reproductive activity. Both these factors decreased the sample size, as only samples collected from Species 2 were in large enough numbers at all farms for reproductive analysis.

The sponges were too small to reproduce (from previous months' sampling)

Another possible reason for the low reproduction rate determined in this study is that the sponges sampled may have been too small to reproduce. This is likely to be the case for Morgan Pearl farm, in which most of the samples collected had little sponge cover, and it was difficult to see the sponge microscopically, let alone any reproductive products. It is possible that sponge individuals on this farm were too small to be reproductively active. Generally in sponges, reproductive elements of oviparous (broadcasting) species are scattered throughout much of the mesohyl (Dorit *et al.*, 1991), and total fecundity is thought to increase with the size of individuals (Fell, 1983). There have been a number of reports of large individuals producing greater numbers of female reproductive elements (Fell, 1983). For example, in *Mycale sp.* (a brooder), the female sponge was only found to be reproductively active when it reached a net volume greater than 200 ml (Reiswig, 1973).

It is possible that the monthly samples taken from each of the farms damaged and reduced the size of the remaining sponge, so that further reproduction was inhibited. All the sponges' energy may have been allocated to recovery from the sampling process and maintenance of individual health. However, it should be noted that if this was the case, sponges throughout the entire farm would cease to reproduce sexually, due to the

vigorous cleaning that the shell is subjected to each month. In comparative terms, the sponges being sampled in this study, which were chosen for the extensive cover they had over the oyster shells, were not brushed each month and were subjected to minimal disturbance. Therefore, the sampled sponges should have been healthier than other sponges infecting the oysters on the farm. In summary, standard methods for assessing reproductive activity were followed in this study, except that the sponge mesohyl region was unavoidably reduced in size in most samples due to the bioeroding nature of the sponge.

The sponges are not reliant on sexual reproduction

As expected, there was no asexual reproduction via gemmulation noted in any of the sponge samples. Surveys of the literature reveal no records of gemmulation in tropical marine species of sponges, as this mode of asexual reproduction is largely reserved for withstanding conditions that may result in freezing and desiccation of the sponges (Bergquist, 1978). Therefore, it is conceivable that these tropical sponges do not rely on gemmulation to overwinter and reproduce.

The most probable form of asexual reproduction that the sponge may have used is by fragmentation, whereby fragments may break off sponges as a result of physical and biological disturbance and are then recruited as independent individuals (Maldonado & Uriz, 1999). One method by which the sponges may have fragmented, is via the cleaning process, whereby fragmentation of the sponges occurs as the encrusting phase (alpha stage) of the sponge is chipped off the oysters. If the sponge fragments were reproductively active, and were not smothered by mud or silt on the bottom of the sea floor, it is possible that these fragments could release eggs. Maldonado & Uriz (1999)

found sponge fragments with larvae were still able to release these sexual products after fragmentation. It is not known whether this is possible for oviparous species with mature eggs. Alternatively, the sponge fragment could survive and attach to the substrate. At all farms in the study, bottom type was described by the farms as silt or mud, suggesting that sponge fragments falling to the substrate may not find attachment points and will be smothered.

Fragmentation may be involved in sponge reproduction if fragmentation of the sponge occurred at the top of the panel of oysters and if fragments were held for a sufficient length of time to become attached. Fragmentation of sponges occurs constantly as bioeroding sponges are scrubbed and chipped from the shell along with other fouling organisms and washed back into the farm site. It is possible that these fragments may remain viable if they find a suitable attachment site, such as the panels or shell held within them. The size of sponge fragments and their ability to survive is presently unknown. Probably all species are capable of regenerating viable individuals from fragments, but whether this is the case for fragments generated by shell cleaning was beyond the scope of this study.

An alternative to fragmentation as a mode of population increase is growth. In many panels of oysters on the pearl farms, the *P. maxima* shells are held so that they are touching each other within the panel. This is the case especially for the older, larger oysters. When the oysters are touching one another within the panel, the sponge could potentially grow across onto the neighbouring oyster shell. Therefore the sponges on the farm, where there is a high density of calcareous substrates, may rely on spreading by growth. Although growth *per se* does not lead to an increase in the number of

individuals and is therefore not technically reproduction, continuing, somatic growth, has a direct relationship to fragmentation (amongst other forms of asexual reproduction) and gemmule formation and is itself asexual (Simpson, 1979).

Somatic growth is a widespread phenomenon among sponges (Simpson, 1979). The tactic of placing more emphasis upon somatic growth has clear advantages (Simpson, 1979). Coupled with very substantial regenerative capabilities, somatic growth may represent the most important means of maintaining population size and biomass in at least some sponge species (Simpson, 1979). The greater the capacity for continuing, somatic growth, the less necessity there is for efficiency in sexual reproduction, since inefficiency, such as sporadic gamete production, can be quantitatively compensated for through an increase in biomass by somatic growth (Simpson, 1979).

However it is unlikely that the sponge spreads via this form of growth in the pearl farms of north-western Australia. The position of the sponge infection on the shell suggests that this does not happen. Infestations of sponge are generally found around the hinge of the bivalved oyster shell, and spread outwards from this region. This was reported by Thomas (1981) on *P. margaratifera* shells with *Cliona* infestations in the Indian Ocean. If the sponge were to grow across onto neighbouring shells, it would spread from the hinge across to the lip of the oyster and onto the lip of the adjacent shell. However, a sponge bioeroding the shell is rarely, if ever, observed on the outer regions of the shell moving towards the umbo. Generally, the hinge of the oyster shell is infected first and the sponge spreads outwards.

Sponges may be recruiting from outside the pearl farms

An alternative to reproduction occurring on the shell in the farms is that recruitment of the sponges may be occurring in areas outside the farm area and the larvae enter the farms on the currents and tides. This recruitment may be occurring on limestone or coral reefs or fishing grounds located in relatively close proximity to the farms. Recruitment from outside the farms is a possibility particularly for Maxima Pearl farm, which has a reef system within the bay in which the farm is located. *Cliona* has been observed on these reefs, although the population of *Cliona* is not large (A. Wilmot, pers. comm., March 2000). Morgan Pearl farm is also located in close proximity to a limestone reef (0.2 NM) (A. Morgan, pers. comm., March 2000).

Samples of nearby reefs in this study may have confirmed these possibilities, however this was beyond the scope of the project – both in terms of the companies sampling outside of their farms and the cost of processing the samples. Nevertheless, the results of this project suggest that future research include studies of the wild sponges.

The paint that is being trialed for treatment of the sponge on the pearl oysters has been used on some farms and *Cliona* still appears to infest the shell. This is a further indication that recruitment of the sponge maybe occurring outside the bay in limestone reefs or fishing grounds.

The wrong area and depth of the shell was sampled

The lack of any organised gonads or gonoducts in sponges means that there is no clear target area for study of sponge reproduction, as the gametes can be dispersed throughout large areas of the sponge mesohyl (Bergquist, 1978). Therefore, the area of the shell

that was sampled may not have been reproductively active. It is possible, although unlikely, that reproductive activity is confined to a particular area of the oyster shell, such as the hinge region, where the oldest area of the sponge occurs. In addition the sponge may not be reproductive at its actively bioeroding areas within the shell, where cells mainly used for bioeroding occur. To prevent missing reproductive activity, samples were consistently collected away from the growing edge of the sponge and in bioeroding areas.

Sectioning of the sponge samples occurred from the outer-most edge of the sponge to approximately 3 mm. into the shell. The eggs that were noted in both the Maxima Pearl and Arrow Pearl samples occurred on the surface of the sponge, indicating that sampling did occur at the correct depth. Additionally, it is likely that it would be easier for a sponge to broadcast eggs when they are located closer to the surface, or near the canal regions responsible for outgoing water exchange. Sponges that broadcast eggs consistently have eggs in their upper mesohyl regions, particularly when spawning is imminent (Fromont & Bergquist, 1993).

The sponge is not an annual reproducer

Although it has never been recorded before, the sponge bioeroding the pearl oysters may not be an annual reproducer. There are no studies that indicate that some sponges are biannual reproducers. More often tropical sponge species reproduce annually if they are oviparous, or for long periods through summer if they are brooders (Sara, 1983). At three of the five study sites there was no sign of reproductive activity, yet all farms were in the tropical zone. Sampling was interrupted during the year and this may have occurred when the sponges were reproductively active.

All the shells sampled contained male sponge

It is possible, although this would constitute an unlikely coincidence, that all the sponges at Morgan Pearl farm and Paspaley Pearl farm selected for sampling were male. There was no way of knowing the sex of the sponges prior to the commencement of this study. If all the sponge samples (minus those at Maxima and Arrow which had eggs) were male, the chances of observing sperm is very low, as sperm generally only appears 1-2 days before spawning, and sampling occurred monthly.

Cyclone impacts

Four cyclones passed through the north-west of Western Australia during the period of this study: Cyclone Billy (3^{rd}–6^{th} December, 1998), Cyclone Thelma (6^{th}-12^{th} December, 1998), Cyclone Vance (17^{th}-24^{th} March, 1999) and Cyclone Gwenda (5^{th}-8^{th} April, 1999). Although the environmental data collected from the farms did not suggest that these cyclones resulted in diminished or changed water quality, the cyclones may have potentially stressed the sponges by causing short term changes in the environment that could not be detected in the monthly monitoring. This may have delayed reproductive activity in some cases. The weather conditions also precluded sampling in some months, thereby disrupting the monthly sampling program and potentially missing development of reproductive products.

6.2 Reproductive variations shown with time of year and locality

In addition to the low levels of eggs in the samples, this study has indicated that the same sponge species has reproduced at two different times of the year: May and September. The results of this study indicated a strong farm*time interaction for the

ANOVA, suggesting that the timing of egg production varies with the farm and over time.

Arrow and Maxima are in relatively close proximity to one another and these results were highly unexpected. There are few reasons to explain the varying reproductive times and the close proximity of the two farms (Maxima Pearl and Arrow Pearl).

One possible reason for the difference in timing of reproduction within the species at Arrow and Maxima is that the year in which sampling occurred (1999) was a blue moon year, in which there were 13 instead of 12 moons in the year. Although blue moons tend to impact on spawning times of marine invertebrates and have been known to induce split spawning (J. Fromont, pers. comm., March 2000), it is unlikely to have impacted on this study, as there was a large time gap in between spawning times of the sponges. If a blue moon were to have impacted on the reproductive activity of the sponges, a much smaller gap between spawning times of the sponges would have been noted. In Scleractinian corals, for example, some individuals of a population spawn on one moon and the remainder of the population on the moon after (Babcock, 1986). Therefore, spawning is separated by one month and is not months apart.

Another possible reason is that the variations in timing of reproductive activity between the two sites may be due to differences in local environmental conditions. Although the two sites are located, geographically, very close to one another, they may experience some slight differences in, for example, temperature. Even if the sponges that are reproducing at different times at Maxima and Arrow are the same species, it is possible that, if the sponges have been on the farms for a long period of time, the species may

become entrained to the local environmental conditions. Most shell had been on the farms for approximately four to five years, so this is a possibility. Additionally, Arrow Pearl farm is more exposed to the ocean than Maxima Pearl farm. This would again have an effect on the environmental characteristics of the farm. It was not possible to study this aspect in detail, however, due to the lack of environmental data provided by the farms.

Although not part of the original methods, each farm collected their shell from a different fishing ground, or management zone, and one farm (Maxima Pearl) used hatchery shell for the study. Although studies on the *Cliona* infestation in the fishing grounds has not been carried out, it is possible that differences in reproductive timing of the sponges may be due to the different origins of the shell (and therefore the sponge). Each fishing ground may contain different species of the bioeroding sponge. It is unlikely that the sponges becoming entrained to local environmental conditions within the fishing grounds would affect reproductive timing, as the experimental shell on each farm had been there for a minimum of four years prior to the study.

The final possibility for reproduction of the sponges occurring at different times of the year at the two different farms is that the sponges belong to different species. Taxonomic studies to date, however, indicate that they are the same species. If the two sponges are shown to be different species, the differences in reproductive timing may be the result of reproductive isolation between the sponge species.

6.3 Reproductive variations shown with changing environmental factors

There are a number of environmental factors which possibly influence reproduction in marine sponges, however in many studies the predominant parameters which appear to influence gametogenesis are water temperature and salinity. Studies on these environmental factors have generally been undertaken in temperate regions, so may not apply to this study, which was carried out in a tropical region.

Very little is known concerning the factors that influence gametogenesis. Some studies have shown that water temperature may be an important factor (Fell, 1974; Reiswig, 1983; Cobb 1969). Ecological factors, especially temperature, have considerable importance in triggering sexual maturation (Sara, 1983). Water temperature is generally considered the most important among the exogenous factors influencing sponge gametogenesis. In this study, however, water temperature was found to have no relationship to sponge reproduction. This may be due to the relatively small fluctuations in water temperature in these regions compared to temperate regions. All the study sites had water temperatures in the range of approximately 24° C to 32° C.

Similarly, it is unusual in this study that salinity does not appear to have had any influence on the reproductive activity of the sponges. Other studies on *Cliona* have shown salinity to have an effect on the numbers of eggs in the sponge (Fell, 1983).

The factors effective in controlling sexual processes in highly synchronised species in habitats devoid of large environmental variables may be very subtle in intensity and unusual in nature (Reiswig, 1983). Sponges inhabiting less variable environments

generally either continuously apportion resources to growth, reproduction, and maintenance simultaneously or utilise subtle exogenous cues to ensure synchrony of gametogenesis and reproductive success (Reiswig, 1983).

Other environmental influences

Environmental influences have also been shown to have an effect on rates of bioerosion. At Morgan Pearl farm, it has been speculated that the low amount of sponge bioerosion experienced may be due to a number of factors, including the location of Morgan's farm. Morgan Pearl farm is situated much further south than all the other farms and therefore has different environmental conditions, including cooler temperatures (Morgan pearl farm is located at 22°24'S, 114°07'E and Port Bremer (Paspaley Pearl) is the furthest north, located at 11°15'S, 132°15'E). The water at Morgan pearl farm is very silt-laden and the shell at this farm are held at a much lower density, therefore providing less opportunity for larvae or asexual products to spread (A. Morgan, pers. comm., April 2000). The shells are held closer to the bottom and the oysters and the sponge are subjected to less sunlight. Additionally, Morgan has a more moderate cleaning regime, with cleaning of shell occurring once every six weeks, resulting in a smaller chance of damaging the oyster shell.

5.4 Dominant form of reproduction – sexual or asexual?

No asexual reproduction was encountered. Little is known of the factors influencing asexual reproduction in marine sponges (Fell, 1974), but this is most likely due to the fact that sponges only tend to reproduce asexually, via methods such as gemmulation, when faced with adverse environmental conditions (Rosell, 1993). These

environmental conditions may include low water temperatures as are found in temperate regions. Gemmulation provides a mechanism for dispersal and survival under these conditions. As all the farms that participated in the study are located in tropical areas, and have relatively constant environmental conditions, including only a small fluctuation in water temperature (see Figure 5.2), the sponge did not die off during winter and require an overwintering stage such as gemmulation. The only form of asexual reproduction which may have occurred and escaped observation, is reproduction via fragmentation or budding. This, however, could not be observed, as in order to see fragmentation, constant monitoring of the sponges *in situ* is required.

The reproduction observed was sexual and therefore suggests that if sponges reproduce on the farms sampled it is likely to be by sexual means.

6.5 Possible modes of entry of *Cliona* in to the pearl oyster farms

It is important, as part of the proposed management strategy for the prevention of the *Cliona* infection, to identify ways in which it may have been introduced into the pearl oyster farms. It is possible that it has "always been there" (A. Morgan, pers. comm., March 2000; A. Wilmot, pers. comm, September 1999) and occurs naturally in the bays, however alternative modes of entry must also be investigated. The natural levels of *Cliona* in wild stock have not been studied. Further research on the incidence and species of *Cliona* in tropical limestone and coral reef systems adjacent to farm localities is essential to examine this hypothesis.

An alternative route, in which *Cliona* may have been introduced into the pearl oyster farms, is via infected wild stock from the fishing grounds from which the shell is collected and taken to the farms. *Cliona* has been found on shell in the fishing grounds, although the extent of the infestation at the fishing grounds has not been investigated. To make further recommendations on control measures, such a study should be undertaken.

Another possibility, as outlined in the background section, is that *Cliona* has been introduced into the farms from ballast water. If it is found that the sponge is an introduced species at the study sites, this will need to be investigated further.

6.6 Limitations

This study was limited to six samples for each monthly sampling period at each farm. Although more replicates would have statistically improved the results of this study and increased the power of the ANOVAs, a compromise was necessary with the pearl oyster farms. The minimum number of replicates was used, so that the project would be of minimal labour and production costs to the companies involved.

The finding, as part of this study, of more than one species of *Cliona* means that the minimum sample size (six sponge samples per farm) necessary to monitor the reproduction of all the species was not obtained. There are no results available on the reproduction of two of the species attacking the pearl oysters and in some cases, the dominant bioeroding species was not sampled six times per month as originally planned.

7 Implications, Conclusions and Recommendations

7.1 Implications for management of *Cliona* on *Pinctada maxima*

The analysis and discussion of the project outcomes have highlighted several implications for the management of the bioeroding sponge in pearl oyster farms. However, these implications may only be applicable to particular farms or a particular region and even then may vary with different environmental conditions and pearl farm procedures.

Benefits to Pearl Producers

The most important benefit to the pearl producers will be evident for Maxima Pearl and Arrow Pearl. These farms should benefit from the results of the project in terms of being able to apply environmentally and economically appropriate management techniques to the sponge at the most effective (vulnerable) phase of its life cycle. Therefore, the paints presently being trialed on the farms should be applied when the sponge is reproductively active, thereby smothering both the adult sponge and the developing eggs. These farms should experience a reduction in the percentage of shell normally seriously infected each year by the bioeroding sponge if egg release from the adult sponges on the shell is prevented. The pearl producers will also know whether they are dealing with a single bioeroding sponge species or more than one, with consequent alterations to implacement of deterrents. This will result in an increased proportion of shell surviving to produce quality pearls rather than the usual poor quality or non-saleable pearls resulting from sponge infection.

The refinement of management techniques will also reduce maintenance costs to pearl farms. As it has now been established that there is one month in the year in which the paint should be applied to the shell at Maxima Pearl and Arrow Pearl farms, the cost of painting the shell has been reduced by approximately 25 to 30 percent.

The paints are expensive and therefore the results of this study so far suggest that costs can be reduced by painting the shells in the middle of September for Maxima and at the beginning of May for Arrow.

Implications of low reproductive activity for controlling the bioeroding sponge

The low rate of reproductive activity at Maxima Pearl and the lack of reproductive activity on the Morgan Pearl farm and Paspaley Pearl farms, indicate the need for additional management strategies to battle *Cliona* to be devised on these three farms. In addition, further research must be undertaken to determine the reasons for the lack of reproductive activity by the sponges at these sites.

If it is found that recruitment of the sponges is occurring outside the pearl oyster farms, the appropriate control measure for minimising the infestations would be to seek a method of preventing larval settlement as an alternative to preventing egg release. The paint presently being trialed smothers the adult sponge. This paint should be examined to determine if it could also act as a deterrent to larval settlement, thus preventing recruitment from nearby reef systems.

Other non-reproductively linked strategies for combating this bioeroding sponge include individually brushing and chipping at each shell on a regular basis to remove as much of

the fouling as possible. This is the current management practice at the pearl farms. However it has been found that removing the sponge via this method is only a short-term strategy that is rarely effective in the long term due to the difficulties of removing all the sponge. A longer-term strategy is therefore required that will reduce the chance of sponge survival.

It should also be noted that it is essential to prevent further introductions of the sponge into the pearl farm environment if the infestation is to be permanently eliminated. The source of the infection must be determined. This may be done by inspecting shells from the fishing grounds where the *P. maxima* shells are collected and discarding those that are infected with *Cliona*. Alternatively, if it is found that the sponge is an introduced species in the region, control over such things as introductions of exotic species via ballast should be considered.

Ultimately, a combination of all the above-mentioned management practices may be necessary to more effectively manage this sponge in the pearl farms. It has been found that two strategies that are environmentally or economically feasible are either to prevent egg release of the sponge, and therefore prevent sponge reproduction in the immediate farm locality, or to prevent settlement of the sponges on the shell. The latter was not investigated as part of this project.

Effects of deterrents to egg release on the environment

It is of upmost importance to all pearl oyster farms participating in the study, that environmental conditions at the farms do not deteriorate as a result of pearl farming practices. All pearl farms are reliant on the waters in which the pearl oysters are kept to

remain in a pristine condition, so that superior products from the pearl oysters are produced. However, if environmental conditions are close to natural, populations of sponges reach their full potential, as they are sensitive to water quality and are indicators of low pollution levels (Nicol & Reisman, 1976). The high infection rate of *Cliona* on these farms suggests that good environmental conditions for sponge growth are a further indication of the environmental conditions of these farms. Methods to control fouling of any kind on the *Pinctada maxima* shells, therefore, must be environmentally benign.

Two paints, PearlSafe and PearlClear, have been trialed for use in the pearl oyster farms. Both PearlSafe and PearlClear are said to be environmentally benign and emit no toxins into the water (R. DeNys, pers. comm., Feb 2000). The paint essentially works by suffocating the sponge, and chemicals from the paint are not released into the water. Utilising these paints as deterrents to egg release is therefore a suitable method for control of the sponge, as they are not expected to have any adverse impacts on the pristine environment in which the sensitive *P. maxima* oysters live.

Marine invader management

This study has highlighted that, in order for the bioeroding sponge to be managed completely on the pearl oyster farms, the potential pool of recruits in the region must be reduced. This may require either further study into ballast water if this sponge is also found in ballast water entering the region, research into the extent of the sponge infestation on wildstock from which the oysters for the farm are collected, and assessment of *Cliona* infestions on adjacent limestone reef systems which may harbour larval recruits.

7.2 Conclusions and Recommendations

In addition to the implications for management of *Cliona*, a variety of conclusions can be drawn from the results of this study. These are closely associated with the recommendations that can be made and therefore will be combined. Once again, the following conclusions and recommendations may only be applicable to conditions similar to those of the study sites.

Recommendations on translocation of Pinctada maxima shells

If it is found that *Cliona* has been introduced into the pearl farms via wild stock from the fishing grounds, new management recommendations should be formed to prevent this practice. It may be beneficial for translocation of pearl oysters from the fishing grounds to the pearl oyster farms to be monitored for the bioeroding sponge. Currently, there are no regulations that prohibit shell that contains the sponge to be introduced into the pearl farms from the fishing grounds. This is most likely due to the lack of research that has been done on the extent of the *Cliona* infestation in the fishing grounds. If *Cliona* could be prevented from entering the pearl farms in the first instance, this may prevent further sponge infestations from occurring after the sponge has been eliminated from the farm region following the initial recommended management strategy: killing the sponge and preventing egg release via the application of a paint.

Recommendations for alternative treatment of wastage from the cleaning process

During the cleaning process on the farms, the sponge (in addition to many other fouling organisms) is put through a vigorous cleaning process whereby the shells are subjected

to a cleaning machine and the remaining fouling organisms are removed via chipping and brushing. All wastage from the cleaning process is discharged back into the water. As a result, the cleaning process does not remove the fouling completely from the environment in which the oysters are kept. If the sponge is introduced back into the water via the cleaning process to settle on the bottom, provided it is not covered by mud (which is the bottom substrate on most of the farms), the sponge may reproduce sexually and release eggs. Cleaning of the shells could therefore cause the production of viable fragments of the sponge for asexual propagation, thereby assisting the sponge in spreading throughout the farm.

Additionally, if sponge fragments are washed back into the water over the panels of oysters, a sponge fragment may easily become lodged within the panel, and continue to remain viable. It is therefore highly recommended that farms ensure sponge fragments, and all other fouling organisms, are not washed back into the water, over the panels of oysters.

It would be beneficial as a management precaution for the material removed from the shells not to be returned to the water at all, at least in its natural state. Ideally, all waste from the cleaning process should be caught and disposed of on land. Alternatively, the wastage could be treated in an environmentally appropriate manner, such as by subjecting the waste to high water temperatures so as to sterilise the fouling material. This should ensure that the sponge is dead and incapable of reproducing. It is not known, due to minimal information obtained on the resources available at each farm, if it is possible for the pearl farms to carry out these recommendations.

Appropriate time in which to apply deterrents to egg release and treatment of Cliona

It is recommended that the paint, if it is to act as a deterrent to egg release, be applied at the times when the sponges were found to be reproductively active. For Maxima Pearl, it is highly recommended that they apply the paint just prior (approximately 2 weeks) to 4th September. Therefore, late August is the suggested time period to apply the treatment, so that the reproductively active sponge and the eggs can be killed before the release of eggs occurs.

Similarly, Arrow Pearl is also recommended to apply the paint just prior to the eggs being released, most appropriately in late April, to early May.

It is not possible to base management recommendations for control of the bioeroding sponges at Morgan Pearl and Paspaley Pearl farms on a lack of data. Therefore, further research is needed into the reproductive biology of the sponge at these pearl farms. Until the reproductive cycle and modes of population increase of this sponge has been determined, it is highly recommended that these three farms continue the current cleaning regimes that have been adopted, although release of fouling material into the water column is not recommended. It is also recommended that the paint developed by the CRC for Aquaculture be applied to the oysters so the sponge will be killed, although the appropriate time in which to apply this paint so as to prevent egg release is not currently known.

Marine invaders should be prevented from entering pearl farm waters via ballast and other infected shell

If we do not work to prevent biotic invasions, from both ballast and other sources, we risk impoverishing and homogenising the very ecosystems on which we rely to sustain our fisheries and other resources (Mack *et al.*, 2000). Therefore, it is essential to implement effective strategies to curb the most damaging impacts of invaders. These imperatives also apply in the pearl oyster industry. *P. maxima* shell should only be transferred from the fishing ground to the pearl farms if it can be certified that the pearl shell to be transferred is sponge free or it has been treated so that any sponge that was present is killed.

In the last decade, the International Maritime Organisation (IMO) has been working through its Member States to tackle the problem of the transfer of unwanted organisms. *Guidelines for Preventing the Introduction of Unwanted Organisms and Pathogens from Ships' Ballast Waters and Sediment Discharges* were initially adopted in 1991 and IMO is now working towards adopting mandatory regulations on the management of ballast water (IMO, 1998).

If it is found that *Cliona* was introduced into the study area via ballast, ballast water treatment immediately becomes an issue with respect to control of this sponge. There are many methods of ballast water treatment, however ballast water exchange in deep sea (depths of 2000 metres or more) is generally seen as the most effective and practical method of minimising risk of transfer of unwanted species. Deep ocean water contains few organisms and these are unlikely to survive transfer to coastal or freshwater environments.

It is unknown if this practice would be effective in preventing the introduction of the bioeroding sponge species into the pearl farms. International shipping is not a likely source in the future if shipping companies comply with the guidelines and Australia's Quarantine Service is vigilant in monitoring ballast water management. However, domestic shipping still needs to be considered and management measures including the following should be considered.

- The baseline status of biological communities and water quality needs to be established and monitored
- All ballast water should be sampled and an inventory of tests should be kept. These tests should be confirmed at the entry point to Australia.
- Research should also be carried out into microfiltration of intake water, treatment through heat exchange units, use of fresh water and sterilisation. Additional funding must be provided for research.

Recommendations for further research

The results of this study have revealed many areas for further research with regards to the control of marine pests, particularly *Cliona*, in aquaculture. At this stage, it appears difficult to control the sponge pest at three of the five pearl farms by utilising knowledge of its reproductive cycle. Further studies are therefore needed to build on this knowledge, or offer alternative management strategies.

In order to draw definite conclusions about the reproduction of these sponges, the population must be studied over an extended period of time, so that a pattern may be determined in the reproductive cycle of these sponges over a number of years. It may

be possible that these sponges reproduce less often than annually. A variety of approaches should also be used, such as aquarium studies in addition to the field approach, so that any potential errors in methodology may be eliminated.

Further research on the extent of the sponge infestation in the fishing grounds that the pearl farms use should indicate if *Cliona* is being introduced into the farms via wild stock. Similarly, studies should be carried out on the extent of *Cliona* infestations on surrounding limestone reefs or other calcareous substrates outside the pearl farm. In general, sponge epidemiology on farms and in the wild needs to be studied and specifics of its reproduction, in terms of timing and mode of reproduction, should be further assessed. This would give an indication of whether or not the sponge is recruiting from outside the pearl farm and then settling on the *P. maxima* shells after being introduced into the farm region with the tides and currents.

If it is found from this study that recruitment is occurring from areas outside the pearl farm, a paint should be developed that will prevent larval settlement on the shells and not just suffocate gametes.

As the results indicate that more than one sponge species is attacking the pearl oysters, further study is needed into the reproductive cycles of the other sponges that also bore into these shells. This is because the deterrents to egg release will not be as effective if it is applied only when one of the sponge species is at a vulnerable stage in its life cycle. In order to impact on all species, the application of the paint will need to occur just prior to gamete release of each of the three species. This may mean that the paint will need to be applied three times during the year, as opposed to once.

As the sponge could potentially be reproducing as a result of sections of the sponge being chipped off during routine cleaning of the shell on the farm, studies are required to determine whether or not the material cleaned from the shell is able to form viable sponge. This would need to be done under laboratory conditions in aquaria systems. Developments of methods of maintaining marine sponge species in controlled laboratory conditions is required for obtaining a breakthrough in determination of factors controlling spermatogenesis and general biological processes (Rieswig, 1983). Results from this study may raise further management recommendations with regards to cleaning of the shell, and disposal of waste from cleaning, on pearl farms.

Finally, a much better understanding of the epidemiology of marine invasions is clearly required, especially of the bioeroding sponge on pearl shell. It would be highly beneficial to develop innocuous experimental releases of organisms that can be manipulated to explore the enormous range of chance events to which all immigrant populations may be subjected. Additionally, a comprehensive cost-benefit analyses that accurately and effectively highlights the damage inflicted on the pearl economy by these sponge infestations would be beneficial. At a higher level, greater public and governmental awareness of the chronic and global effects of invasive organisms and the tools available to curb their spread and restrict their ecological and economic impacts is needed. It is essential, for both the conservation of our waters and for the effective management of the pearling industry in Western Australia, that future research is carried out into the control of marine pests that are invading our waters.

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Appendix 1

Number of eggs, salinity (ppm) and temperature (C) for each sample
(- = missing data)

MAXIMA

Sample	Date	No. of eggs (per 0.5 cm²)	Salinity (ppm)	Temperature (°C)
Ma 1	1/11/98	0	36.20	30.90
Ma 2	1/11/98	0	36.20	30.90
Ma 3	1/11/98	0	36.20	30.90
Ma 4	1/11/98	0	36.20	30.90
Ma 5	1/11/98	0	36.20	30.90
Ma 6	1/11/98	0	36.20	30.90
Ma 1	3/12/98	0	35.93	31.80
Ma 2	3/12/98	0	35.93	31.80
Ma 3	3/12/98	0	35.93	31.80
Ma 4	3/12/98	0	35.93	31.80
Ma 5	3/12/98	0	35.93	31.80
Ma 6	3/12/98	0	35.93	31.80
Ma 1	2/1/99	0	33.55	31.50
Ma 2	2/1/99	0	33.55	31.50
Ma 3	2/1/99	0	33.55	31.50
Ma 4	2/1/99	0	33.55	31.50
Ma 5	2/1/99	0	33.55	31.50
Ma 6	2/1/99	0	33.55	31.50
Ma 1	3/2/99	0	35.03	30.50
Ma 2	3/2/99	0	35.03	30.50
Ma 3	3/2/99	0	35.03	30.50
Ma 4	3/2/99	0	35.03	30.50
Ma 5	3/2/99	0	35.03	30.50
Ma 6	3/2/99	0	35.03	30.50
Ma 1	1/3/99	0	33.00	29.60
Ma 2	1/3/99	0	33.00	29.60
Ma 3	1/3/99	0	33.00	29.60
Ma 4	1/3/99	0	33.00	29.60
Ma 5	1/3/99	0	33.00	29.60
Ma 6	1/3/99	0	33.00	29.60
Ma 2	1/4/99	0	32.53	31.00
Ma 3	1/4/99	0	32.53	31.00
Ma 4	1/4/99	0	32.53	31.00
Ma 5	1/4/99	0	32.53	31.00
Ma 6	1/4/99	0	32.53	31.00
Ma 7	1/4/99	0	32.53	31.00
Ma 2	23/4/99	0	27.70	29.40
Ma 3	23/4/99	0	27.70	29.40
Ma 4	23/4/99	0	27.70	29.40
Ma 5	23/4/99	0	27.70	29.40

Ma 6	23/4/99	0	27.70	29.40
Ma 7	23/4/99	0	27.70	29.40
Ma 2	2/6/99	0	29.45	28.30
Ma 3	2/6/99	0	29.45	28.30
Ma 4	2/6/99	0	29.45	28.30
Ma 5	2/6/99	0	29.45	28.30
Ma 6	2/6/99	0	29.45	28.30
Ma 7	2/6/99	0	29.45	28.30
Ma 1	28/6/99	0	25.78	24.20
Ma 2	28/6/99	0	25.78	24.20
Ma 3	28/6/99	0	25.78	24.20
Ma 4	28/6/99	0	25.78	24.20
Ma 7	28/6/99	0	25.78	24.20
Ma 8	28/6/99	0	25.78	24.20
Ma 1	2/8/99	0	26.10	24.70
Ma 2	2/8/99	0	26.10	24.70
Ma 3	2/8/99	0	26.10	24.70
Ma 4	2/8/99	0	26.10	24.70
Ma 7	2/8/99	0	26.10	24.70
Ma 8	2/8/99	0	26.10	24.70
Ma 1	4/9//99	0	31.50	26.00
Ma 2	4/9/99	0	31.50	26.00
Ma 3	4/9/99	0	31.50	26.00
Ma 4	4/9/99	0	31.50	26.00
Ma 7	4/9/99	0	31.50	26.00
Ma 8	4/9/99	9	31.50	26.00
Ma 1	3/10/99	0	25.80	27.60
Ma 2	3/10/99	0	25.80	27.60
Ma 3	3/10/99	0	25.80	27.60
Ma 4	3/10/99	0	25.80	27.60
Ma 7	3/10/99	0	25.80	27.60
Ma 8	3/10/99	0	25.80	27.60

ARROW

Sample	Date	No. of eggs (per 0.5 cm^2)	Salinity (ppm)	Temperature (°C)
A 1	10/11/98	0	-	31.1
A 2	10/11/98	0	-	31.1
A 3	10/11/98	0	-	31.1
A 4	10/11/98	0	-	31.1
A 5	10/11/98	0	-	31.1
A 6	10/11/98	0	-	31.1
A 1	16/12/98	0	-	30.2
A 2	16/12/98	0	-	30.2
A 3	16/12/98	0	-	30.2
A 4	16/12/98	0	-	30.2
A 5	16/12/98	0	-	30.2
A 6	16/12/98	0	-	30.2
A 1	16/1/99	0	-	31
A 2	16/1/99	0	-	31
A 3	16/1/99	0	-	31
A 4	16/1/99	0	-	31
A 5	16/1/99	0	-	31
A 6	16/1/99	0	-	31
A 1	18/2/99	0	-	31
A 2	18/2/99	0	-	31
A 3	18/2/99	0	-	31
A 4	18/2/99	0	-	31
A 5	18/2/99	0	-	31
A 6	18/2/99	0	-	31
A 1	15/3/99	0	-	32.1
A 2	15/3/99	0	-	32.1
A 3	15/3/99	0	-	32.1
A 4	15/3/99	0	-	32.1
A 5	15/3/99	0	-	32.1
A 1	13/4/99	0	-	28.7
A 2	13/4/99	0	-	28.7
A 3	13/4/99	0	-	28.7
A 3	13/4/99	0	-	28.7
A 4	13/4/99	0	-	28.7
A 5	13/4/99	0	-	28.7
A 6	13/4/99	0	-	28.7
A 7	13/5/99	10	-	27.3
A 8	13/5/99	9	-	27.3
A 9	13/5/99	0	-	27.3
A 10	13/5/99	6	-	27.3
A 11	13/5/99	5	-	27.3
A 12	13/5/99	0	-	27.3
A 1	17/6/99	0	-	27.3
A 2	17/6/99	0	-	27.3

A 3	17/6/99	0	-	27.3
A 4	17/6/99	0	-	27.3
A 5	17/6/99	0	-	27.3
A 6	17/6/99	0	-	27.3
A 2	15/8/99	0	-	27.9
A 3	15/8/99	0	-	27.9
A 4	15/8/99	0	-	27.9
A 6	15/8/99	0	-	27.9
A 13	15/8/99	0	-	27.9
A 14	15/8/99	0	-	27.9
A 7	22/9/99	0	-	28.4
A 8	22/9/99	0	-	28.4
A 9	22/9/99	0	-	28.4
A 10	22/9/99	0	-	28.4
A 11	22/9/99	0	-	28.4
A 12	22/9/99	0	-	28.4
A 2	19/10/99	0	-	28.7
A 3	19/10/99	0	-	28.7
A 4	19/10/99	0	-	28.7
A 6	19/10/99	0	-	28.7
A 13	19/10/99	0	-	28.7
A 14	19/10/99	0	-	28.7

MORGAN

Sample	Date	No. of eggs (per 0.5 cm^2)	Salinity (ppm)	Temperature (°C)
Mr 2	20/12/98	0	-	-
Mr 3	20/12/98	0	-	-
Mr 4	20/12/98	0	-	-
Mr 5	20/12/98	0	-	-
Mr 6	20/12/98	0	-	-
Mr 1	2/99	0	-	-
Mr 3	2/99	0	-	-
Mr 4	2/99	0	-	-
Mr 7	8/3/99	0	-	30.00
Mr 8	8/3/99	0	-	30.00
Mr 11	8/3/99	0	-	30.00
Mr 12	8/3/99	0	-	30.00
Mr 1	3/6/99	0	-	22.00
Mr 2	3/6/99	0	-	22.00
Mr 3	3/6/99	0	-	22.00
Mr 5	3/6/99	0	-	22.00
Mr 6	3/6/99	0	-	22.00
Mr 7	7/7/99	0	-	-
Mr 8	7/7/99	0	-	-
Mr 9	7/7/99	0	-	-
Mr 11	7/7/99	0	-	-
Mr 12	7/7/99	0	-	-
Mr 1	5/8/99	0	-	21.50
Mr 3	5/8/99	0	-	21.50
Ma 4	5/8/99	0	-	21.50
Mr 6	5/8/99	0	-	21.50
Mr 7	5/8/99	0	-	21.50
Mr 7	10/9/99	0	-	-
Mr 8	10/9/99	0	-	-
Mr 10	10/9/99	0	-	-
Mr 11	10/9/99	0	-	-
Mr 12	10/9/99	0	-	-
Mr 1	6/10/99	0	-	26.00
Mr 2	6/10/99	0	-	26.00
Mr 3	6/10/99	0	-	26.00
Mr 4	6/10/99	0	-	26.00
Mr 5	6/10/99	0	-	26.00
Mr 6	6/10/99	0	-	26.00
Mr 7	10/11/99	0	-	26.00
Mr 2	9/12/99	0	-	27.00
Mr 4	9/12/99	0	-	27.00
Mr 6	9/12/99	0	-	27.00

VANSITTART BAY

Sample	Date	No. of eggs (per 0.5 cm^2)	Salinity (ppm)	Temperature (°C)
V 1	16/1/99	0	-	-
V 1	15/2/99	0	31.80	30.20
V 2	15/2/99	0	31.80	30.20
V 3	15/2/99	0	31.80	30.20
V 4	15/2/99	0	31.80	30.20
V 5	15/2/99	0	31.80	30.20
V 6	15/2/99	0	31.80	30.20
V 1	23/3/99	0	31.97	30.09
V 2	23/3/99	0	31.97	30.09
V 3	23/3/99	0	31.97	30.09
V 5	23/3/99	0	31.97	30.09
V 6	23/3/99	0	31.97	30.09
V 1	18/4/99	0	32.00	29.63
V 2	18/4/99	0	32.00	29.63
V 3	18/4/99	0	32.00	29.63
V 4	18/4/99	0	32.00	29.63
V 5	18/4/99	0	32.00	29.63
V 6	18/4/99	0	32.00	29.63
V 1	16/5/99	0	33.48	27.45
V 2	16/5/99	0	33.48	27.45
V 3	16/5/99	0	33.48	27.45
V 4	16/5/99	0	33.48	27.45
V 5	16/5/99	0	33.48	27.45
V 6	16/5/99	0	33.48	27.45
V 1	17/7/99	0	35.00	24.30
V 2	17/7/99	0	35.00	24.30
V 3	17/7/99	0	35.00	24.30
V 4	17/7/99	0	35.00	24.30
V 5	17/7/99	0	35.00	24.30
V 6	17/7/99	0	35.00	24.30
V 1	17/8/99	0	35.29	25.76
V 2	17/8/99	0	35.29	25.76
V 3	17/8/99	0	35.29	25.76
V 4	17/8/99	0	35.29	25.76
V 5	17/8/99	0	35.29	25.76
V 6	17/8/99	0	35.29	25.76
V 1	16/9/99	0	34.93	28.07
V 2	16/9/99	0	34.93	28.07
V 3	16/9/99	0	34.93	28.07
V 4	16/9/99	0	34.93	28.07
V 6	16/9/99	0	34.93	28.07
V 4	23/9/99	0	34.93	28.07

PORT BREMER (PASPALEY)

Sample	Date	No. of eggs (per 0.5 cm^2)	Salinity (ppm)	Temperature (°C)
Pb 2	22/11/98	0	-	-
Pb 3	22/11/98	0	-	-
Pb 2	11/12/98	0	31.69	31.31
Pb 3	11/12/98	0	31.69	31.31
Pb 6	11/12/98	0	31.69	31.31
Pb 1	20/2/99	0	30.36	31.51
Pb 3	20/2/99	0	30.36	31.51
Pb 4	20/2/99	0	30.36	31.51
Pb 6	20/2/99	0	30.36	31.51
Pb 1	18/3/99	0	32.67	30.10
Pb 2	18/3/99	0	32.67	30.10
Pb 3	18/3/99	0	32.67	30.10
Pb 4	18/3/99	0	32.67	30.10
Pb 5	18/3/99	0	32.67	30.10
Pb 6	18/3/99	0	32.67	30.10
Pb 1	17/4/99	0	31.07	29.61
Pb 2	17/4/99	0	31.07	29.61
Pb 3	17/4/99	0	31.07	29.61
Pb 4	17/4/99	0	31.07	29.61
Pb 5	17/4/99	0	31.07	29.61
Pb 6	17/4/99	0	31.07	29.61
Pb 7	17/4/99	0	31.07	29.61
Pb 1	20/5/99	0	31.43	26.85
Pb 2	20/5/99	0	31.43	26.85
Pb 3	20/5/99	0	31.43	26.85
Pb 4	20/5/99	0	31.43	26.85
Pb 5	20/5/99	0	31.43	26.85
Pb 1	23/6/99	0	33.62	26.48
Pb 2	23/6/99	0	33.62	26.48
Pb 3	23/6/99	0	33.62	26.48
Pb 4	23/6/99	0	33.62	26.48
Pb 5	23/6/99	0	33.62	26.48
Pb 6	23/6/99	0	33.62	26.48
Pb 1	15/7/99	0	34.96	24.95
Pb 2	15/7/99	0	34.96	24.95
Pb 3	15/7/99	0	34.96	24.95
Pb 4	17/7/99	0	34.96	24.95
Pb 5	15/7/99	0	34.96	24.95
Pb 6	15/7/99	0	34.96	24.95
Pb 1	15/8/99	0	34.50	25.59
Pb 2	15/8/99	0	34.50	25.59
Pb 3	15/8/99	0	34.50	25.59
Pb 4	15/8/99	0	34.50	25.59
Pb 5	15/8/99	0	34.50	25.59

Pb 6	15/8/99	0	34.50	25.59
Pb 1	12/9/99	0	36.04	28.08
Pb 2	12/9/99	0	36.04	28.08
Pb 3	12/9/99	0	36.04	28.08
Pb 4	12/9/99	0	36.04	28.08
Pb 5	12/9/99	0	36.04	28.08
Pb 6	12/9/99	0	36.04	28.08

Honours Thesis submitted in partial fulfilment of the requirement of the award of Bachelor of Science (Environmental Management) with Honours at Edith Cowan University

L. Rawlinson, 2000. Management of a bioeroding sponge on the pearl oyster, *Pinctada Maxima*

ERRATA

Location/Page	Error	Correct
2.5 / 21	Consequently, the loss of even one pearl in a harvest is a substantial loss to a pearling company. Perhaps even more important than damage to the pearl is the damage inflicted on the half-shell, which is one of the main products of pearling in north-western Australia. The half shell, once used to make buttons, is now primarily used for ornamental purposes and to manufacture jewellery because of its beautiful lustre and colouring (Taylor, 1985). In terms of the industry, the half shell is worth approximately $170 million (Fisheries WA, 1999). Therefore, to improve the quality of the product by 10%, an increase of $1.7 million in value, would be worth the equivalent value of the state's abalone fisheries between Cape Leeuwin and the Northern Territory border (Fisheries WA, 1999).	Consequently, the loss of even one pearl in a harvest is a substantial loss to a pearling company. Therefore, even to reduce the morbidity of the shell by 2%, resulting in an increase of approximately $5 million in value, would be worth more than the value of the state's abalone fisheries between Cape Leeuwin and the Northern Territory border (Fisheries WA, 1999). Perhaps even more important than damage to the pearl is the damage inflicted on the half-shell, which is one of the main products of pearling in north-western Australia. The half shell, once used to make buttons, is now used primarily for ornamental purposes and to manufacture jewellery because of its beautiful lustre and colouring (Taylor, 1985).
3.2 / 36	Pearl oysters are collected by skin diving or using SCUBA from the oyster beds.	Pearl oysters are collected from the oyster beds by using SSBA (Surface Supplied Breathing Apparatus).
3.6 / 41	The results of pearl culturing are three pearl types: cultured irregularly shaped pearls (baroque), ...	The results of pearl culturing are four pearl types: round pearls, cultured irregularly shaped pearls (baroque), ...
3.7 / 43 Last sentence of page	... either within company laboratories funded by the Pearl Producers Association ...	... within companies or funded by the Pearl Producers Association ...