DEVELOPING ON-GROWING TECHNIQUES AND DISEASE PREVENTION HUSBANDRY OF PEARL OYSTERS IN WESTERN AUSTRALIA (FIRTA Project 87/81).

AND

ON-GROWING MARICULTURE TECHNIQUES FOR THE PEARL OYSTER PINCTADA MAXIMA SPAT IN WESTERN AUSTRALIA (FIRDTF Project 89/60).

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CONTENTS

PREFACE

This document is the final report of research undertaken in FIRTA project 87/81 and in FIRDTF project 89/60. The results reported in the disease prevention segment (FIRTA 87/81) build upon the experience derived from six years of previous FIRTA supported investigation of pearl oyster mass mortalities. The application of preventative measures recommended by these studies and the resulting reduction in the occurrence of such mortalities has helped enable the pearl culture industry to achieve its current economic status as Australia's most lucrative aquaculture industry (the 1989 production figure is in excess of $80 million), and ranking it third in value of production after the rock lobster and prawn fisheries.

This account also reports the findings from research into methods for the on-growing of hatchery propagated pearl oyster spat. Since pilot scale quantities of spat, to facilitate this segment of the project, were not available until the summer of 1987/88 and pearl oysters take slightly longer than a year to grow to a commercially usable size, the completion of the on-growing segment (FIRTA 87/81) was funded by a further one year grant (FIRDTF 89/60).

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EXECUTIVE SUMMARY

DISEASE PREVENTION HUSBANDRY OF FARM STOCK OYSTERS

Infection by marine *Vibrio* bacteria is still considered to be the major opportunistic cause of mass mortalities of pearl oysters (*Pinctada maxima*) on farm lease sites in the north west of Western Australia. Vibriosis of *P. maxima* was found to occur following some form of stressful husbandry practice as similar mortality outbreaks were never apparent in natural populations of pearl oysters throughout this investigation. Potentially harmful *Vibrio* species were found to be associated with pearl oysters in the Broome region throughout the year; therefore vibriosis could occur at any time to oysters subjected to excessive stress.

As treatment with vaccines, chemoprophalaxis and antibiotics is more expensive and has limited effectiveness in the open water aquaculture system used in the pearl culture industry, emphasis should be placed on the prevention of vibriosis through improved husbandry practices.

To alleviate losses of *P. maxima* from vibriosis by, it has been recommended that Industry should:

(1) Handle oysters as little as possible and ensure a period of convalescence between major husbandry operations.

(2) Increase the use of bottom long-lines to hold oysters at the collection grounds while the oysters recover from the stresses of collection.

(3) Avoid transporting oysters during the winter months when minimum water temperatures occur.

(4) Reduce the proliferation of *Vibrio* bacteria during transportation to the lease site by providing adequate water circulation in carrier tanks and practising strict hygiene by ensuring the tanks are kept clean.

(5) Reduce stocking densities to prevent crowding stress on lease sites.

(6) Contain any outbreaks of vibriosis by removing any dead or moribund oysters as soon as detected to prevent further proliferation of bacteria.

To monitor the continued occurrence of the disease, Industry have also been advised to send samples of diseased oysters, together with the history of recent treatment of the oysters, to the W.A. Fisheries Department's Fish Health Section (based at the Department of Agriculture) for diagnosis.

ON-GROWING OF HATCHERY PRODUCED PEARL OYSTER SPAT

This project has demonstrated that cultured spat can be successfully grown to commercially usable sizes (approximately 120mm dorso-ventral length) within 19 months after

fertilization. At this rate of growth artificially cultured oysters could be seeded with nuclei can be during their second year of growth (i.e. as 1^{+} animals). The production of oysters from hatchery produced spat is therefore seen as a potentially viable alternative to the continued reliance on wild caught oysters.

Modified bottom cage on-growing methods were developed to accommodate the high tidal regime in the tropical Broome region, rather than using the more conventional Japanese husbandry system of nets suspended from surface long lines which suits their comparatively calm temperate waters. Bottom cage culture also avoided excessive biofouling problems from algae and barnacles and provided protection from molluscivorous fish. Crabs growing up within the protective mesh and predating on small spat, however, necessitated frequent checks to remove them.

While still requiring the extensive use of divers particularly in the early stages of growth, the above on-growing methods were also developed to utilise some of the more economic existing husbandry practices e.g. the larger juvenile oysters could be cleaned mechanically.

The following recommendations are made based on the results of this research:

(1) Artificial propagation of spat in the hatchery should be timed to occur at the beginning of the pearl oyster

breeding season, that is in September/October, as this optimises the use of the fast growing season over the summer months. As their growth is more rapid during these months of warmer waters temperatures, another benefit is that the juveniles quickly reach a size at which they are much less vulnerable to predation and fouling.

(2) In the Broome region, fewer spat grow-out problems are likely to be encountered using protective cages placed near the sea bottom than by using near surface methods during the first 4-6 months growth.

(3) Biofouling remains one of the greatest obstacles to successful on-growing. The use of antifouling wax on panel nets and "slippery" (polyethylene) plastic mesh in the protective enclosures for spat is therefore recommended. The use of fine mesh (<2mm), to screen out predators is not due to the problem of being too easily clogged with seston.

(4) The hatchery use of flexible settlement surfaces such as monofilament mesh is recommended, so that mesh with the attached spat can be placed directly into protective enclosures. This allows the spat to be on-grown at sea without major disturbance until the spat complete their first 4-6 months growth.

1. INTRODUCTION

The general aim of both projects was to reduce the need for heavy fishing pressure on the natural stocks of pearl oysters (*Pinctada maxima*) in Northwestern Australia. It was considered that this could be most easily achieved by decreasing the high mortality of wild oysters after collection, through the development of a disease prevention programme and secondly by developing the technology necessary to artificially reproduce and grow pearl oysters to culture size as an alternative to continued harvesting of wild stocks.

The specific objectives of the first project (FIRTA 87/89) were:

(1) To monitor the environmental and biological conditions predisposing pearl oysters to mortality when transplanted from the fishing grounds to various farms.

(2) To develop husbandry protocols for preventing and/or containing mortality on farms.

(3) To develop methods for on-growing hatchery produced spat to a size suitable for pearl cultivation

The completion of the on-growing portion was funded by a further one year grant (FIRDTF 89/60).

The specific objectives of this suplementary project were:

(1) To complete the on-growing of the spat produced in 1988, through to commercially usable sizes (120mm plus) - anticipated timing April 1990.

(2) To further test and develop equipment for growing 0^+ year class oysters.

(3) To further develop and document husbandry protocols for 0^+ and 1^+ year oysters (stocking densities, culling routines, cleaning regimes, etc) for future commercial use.

Additional pearl oyster spat to experimentally investigate the third objective were expected to be produced during the austral summer months of 1989/90 by the complementary hatchery project (FIRDTF 89/61). Unfortunately, however, both research staff involved in the project resigned to join private enterprise mariculture concerns about the time that funding was approved. Given the staff resources remaining, this project was scaled down to refining techniques for induction of spawning upon demand (see Final Report FIRDTF project 89/61), and the resulting larvae could not therefore be reared to the settlement stage. As a consequence, the results of the on-growing trials

reported here are based on spat produced in the Broome hatchery during October 1987 and November 1988.

2. DISEASE PREVENTION HUSBANDRY OF FARM STOCK OYSTERS

2.1 MATERIALS AND METHODS

Sindermann's 1979 paper on oyster mortality emphasizes the fact that mass mortalities may have very complex eitiology. As well as investigating the cause(s) of disease it is therefore also considered important to determine whether environmental factors and management practices may increase the oysters susceptibility to disease.

In this project the cause(s) of disease and the predisposing factors were examined simultaneously. Pathological and microbiological studies were conducted on naturally diseased oysters and the findings substantiated by experimental reproduction of disease in the laboratory. Predisposing factors were determined by investigation of environmental factors, particularly water quality and management practices (See Figure 1).

A detailed description of the materials and methods used in this project may be found in Dybdahl and Pass, 1985 and Pass *et al.*, 1987.

2.2 RESULTS

(a) CAUSE(S) OF DISEASE

Throughout this study it was found that infection by marine *Vibrio* species was the predominant cause of mortality of the pearl oyster *P. maxima* after collection and transportation to the farm leases. No other major causative agents were found during the lengthy pathological investigations (Pass *et al.*, 1987; Pass *et al.*, 1988). During that portion of the study *Vibrio harveyi* was used as an example of the *Vibrio* species associated with *P. maxima* and was shown experimentally to induce disease similar to that seen in the field.

Table 1. shows the *Vibrio* bacteria which were isolated from healthy P. maxima from April 1987 to May 1988. Marine *Vibrio* species are a "normal" inhabitant of the bacterial flora of marine animals and of seawater (Austin *et al.*, 1988).

Of the eight species isolated *Vibrio anguillarum* has been associated with disease in bivalve larvae (Hada *et al.*, 1984) while *V. anguillarum* and *V. alginolyticus* have been

reported to be pathogenic for mature edible oysters *Crassostrea gigas* (Grischkowsky and Liston, 1974).

It is well known that *Vibrio* species are pathogens of bivalve larvae (Hada *et al.*, 1984; Elston and Leibovitz, 1980; Jefferies, 1982) but vibriosis of adult bivalves has not been well documented previously.

Potentially pathogenic marine *Vibrio* species were found to be associated with pearl oysters in the tropical Broome region all year (Table 1). In temperate waters however *Vibrio* species arrays have been shown to exhibit seasonal variation in species composition e.g. *V. harveyi* was not detected in the water column during periods of lower temperatures, however, it is known to overwinter in bottom sediments (Ruby and Nealson, 1978).

(b) PREDISPOSING FACTORS

(1) Environmental factors

Water temperature is the environmental factor which has often been associated with outbreaks of vibriosis in fish (Munn, 1977; Thorburn, 1987). Throughout this

study water temperature was also found to be the most important environmental factor associated with pearl oyster mortalities (Dybdahl and Pass, 1985; Pass et al., 1987). The percentage mortality was inversely related to water temperature at the Kuri Bay lease site with the greatest incidence of mortality occurring during months of low ambient temperatures. Oysters were most susceptible to disease after transportation at these low water temperatures. Experiments in the laboratory also showed a significantly greater mortality of oysters inoculated with *V. harveyi* when kept in an aquarium at 19°C compared to oysters kept at 29°C (Dybdahl and Pass, 1985; Pass et al., 1987).

(2) Management practices

During previous studies (Pass et al., 1987) it was found that the greatest mortality problem was related to transporting oysters in high densities on vessels with inadequate water circulation which allowed a massive build up of *Vibrio* bacteria in the carrier tanks. These

conditions were believed to be conducive to outbreaks of vibriosis.

Experimentally mortality was found to increase significantly with increased stocking densities in carrier baskets. These results are consistent with Sneiszko's hypothesis that if the occurrence of stress coincides with the presence of pathogenic micro-organisms it is logical to assume that outbreaks of disease are more likely to take place (Sneiszko, 1974).

The most important handling innovation which reduced mortality was found to be the use of bottom long-lines to hold oysters on the fishing grounds until the oysters had recovered from the stress of collection and until water temperatures were more favourable for transport to the lease sites. In addition to improving water circulation in carrier tanks, the above procedures were found to reduce mortality from vibriosis substantially. More detailed results from this study may be found in Dybdahl and Pass, 1985 and Pass et al.,1987.

2.3 DISCUSSION

In an aquaculture system there is a close interaction between the cultured shellfish, its environment and any potential pathogens. If there is an imbalance during the interaction of the cultured shellfish, the environment and the pathogen, disease is more likely to occur (Bower, 1988). When considering disease control all three of these factors must be thoroughly examined and the relationship between them determined.

In this study pathological and microbiological studies were conducted on naturally diseased oysters and the environmental factors (particularly water quality and management practices) were examined. It was found that the high level of pearl oyster mortalities experienced in the north west of Western Australia were a result of infection by marine *Vibrio* species.

Vibriosis, as defined by Egidius, 1987 is an infection caused by a bacterial species of the genus *Vibrio*. *Vibrio* species are found to occur in marine and estuarine waters and the accompanying sediments (Colwell and Grimes, 1984). *Vibrio* species are therefore ubiquitous and usually coexist well with the surrounding marine animals. It is only when the balance, between the environment, the potential

pathogen and the cultured shellfish, is upset that disease may become a problem.

Vibrio species are considered to be opportunistic pathogens which often infect shellfish secondarily following stress from other factors (Bower, 1988). The relationship between stress and bacterial disease is well documented (Pearce, 1989; Thorburn, 1987; Sniesko, 1974). Stress is believed to lower an animals resistance to disease and predispose to infection by opportunistic pathogens such as *Vibrio*. By minimizing stress the animals resources for disease resistance are maximized (Pearce, 1989). In an aquaculture system stress may be minimized by developing husbandry practices which provide optimal conditions for the cultured animal.

There was no evidence of excessive mortality of *P. maxima* when they were observed in their natural environment. In their subtidal habitat pearl oysters are presumably buffered by the relatively constant conditions which exist in the ocean. They may therefore exhibit little tolerance to rapidly changing conditions. When the oysters are collected and transported to the lease site they are subjected to an unnatural and stressful environment due to being handled, crowded and confined for up to five days on the carrier vessels.

Mortality and morbidity of the pearl oysters occurred after they were transported to the lease sites (mainly between 7 and 40 days after arrival). The number of *Vibrio* bacteria was found to rise dramatically in the carrier tanks during the transportation of the pearl oysters and this was believed to be the time when infection occurred. It was therefore thought to be the combination of *Vibrio* bacteria and environmental stress that eventually kills cultured pearl oysters on a large scale.

Increased mortality was observed during months of low ambient temperature. Presumably this was because the pearl oysters were further stressed by exposure to the low temperatures making them more susceptible to invasion by *Vibrio* bacteria. These temperatures are still ideal, however for the rapid multiplication of *Vibrio* species (Munn, 1977).

The major predisposing factors associated with high mortalities were cold water temperatures, concentration of oysters during transportation and inadequate water circulation in carrier tanks.
It was therefore concluded that the high levels of mortality of the pearl oysters in the north west of Western Australia were caused by marine *Vibrio* bacteria which acted as opportunistic invaders causing disease in *P. maxima* which had decreased

resistance due to stress caused by environmental and management factors. It was suggested that vibriosis of *P. maxima* could be avoided or greatly reduced by corrective husbandry methods.

During annual meetings to report research findings, the pearl culture industry was advised to minimise transportation of oysters during the winter months, to improve carrier tank hygiene and water circulation and to reduce the stocking density of the oysters at the lease sites by using long lines until the oysters had recovered from the stresses of collection and transportation. The industry have since implemented these recommendations and modified their transport and husbandry practices and as a result losses of *P. maxima* due to infection by marine *Vibrio* bacteria have been considerably reduced to the point that no major mass mortalities have been reported within the industry since 1987.

The prevention of disease rather than its treatment should be emphasized. There are major problems associated with treatments such as vaccines, chemoprophylaxis and the use of antibiotics particularly in an open ocean aquaculture system such as pearl oyster culture. Vaccines are available for a limited number of pathogens but they are expensive and have limited effectiveness. Chemoprophalaxis has problems concerning both

environmental and personal safety and the use of antibiotics is expensive and likely to result in antibiotic resistant bacterial pathogens.

Although *Vibrio* bacteria have been shown to contribute to the major mass mortalities of transported oysters in Western Australia, there may be other causes of mortality for which the risk can be most easily reduced by continuing the policy of limiting the transfer of oysters from other localities into Western Australia. Unregulated transportation of bivalves in other parts of the world have resulted in the introduction of epizootic diseases which have devastated some important mariculture industries (Bower, 1989).

2.4 CONCLUSIONS

As a result of this investigation it was concluded that the high mortality of collected pearl oysters after transportation to the lease sites in the north west of Western Australia was associated with cold water temperatures, crowding of oysters during transport and inadequate water circulation in carrier tanks. This is thought to have resulted in the oysters being stressed and therefore more susceptible to potentially pathogenic bacteria. Mortality was found to be primarily due to infection with marine *Vibrio* bacteria and in general death

occurred following some form of stressful husbandry practice.

During this study potentially pathogenic bacteria of the species *Vibrio*, however, were frequently isolated from healthy pearl oysters. Also it should be emphasized that pearl oysters in the Broome region are exposed to *Vibrio* species throughout the year and may therefore be susceptible to vibriosis at all times.

2.5 RECOMMENDATIONS

As a result of this investigation the following husbandry practices, to minimize the mortality of *P. maxima* by vibriosis, are recommended.

(1) Stress should be minimized by:

(a) Handling oysters as little as possible

(b) Avoiding overcrowding during transportation and by using lower stocking densities at the lease site.

(c) Allowing a period of convalescence between major husbandry operations

(d) Keeping transportation of *P. maxima* to a minimum during the winter months.

(2) The number of *Vibrio* bacteria which are in contact with *P. maxima* should be kept to a minimum by:

(a) Providing adequate water circulation in the carrier tanks e.g. a flow through rate such that the water volume is completely replaced approximately every twenty minutes.

(b) Practicing stricter hygiene to reduce marine bacteria by flushing the tank with clean fresh water before transporting the oysters and/or cleaning each carrier tank with a biodegradable chlorine compound such as "Chlorofos" (Nightingale chemicals).

(c) Keeping the carrier tanks free of large amounts of organic material from faeces and loosened fouling organisms which provides an ideal environment for the proliferation of bacteria.

(3) To provide information to assist in the diagnosis of disease should it occur, comprehensive records detailing observations on

mortality outbreaks as well as the corresponding history of oyster husbandry operations including stocking densities and easily measured water quality parameters such as water temperatures should be kept.

(4) If a disease problem is suspected it may be contained by alleviating the stress on the oyster population, e.g. by reducing density, thereby decreasing their susceptibility to disease and by removing dead or moribund oysters to prevent further proliferation of bacteria.

(5) Oysters which are suspected to be diseased may be sent to the W.A. Fisheries Department's Fish Health Section based at the W.A Department of Agriculture for examination (see Appendix 1). A proper diagnosis of any disease outbreaks is essential for the ongoing improvement in husbandry techniques.

3. ON-GROWING OF HATCHERY-PRODUCED PEARL OYSTER SPAT

3.1 MATERIALS AND METHODS

Tropical bivalve mariculture is hindered by the comparative lack of an adequate research base since

most aquaculture techniques have been developed for temperate species such as the Japanese pearl oyster *Pinctada fucata* (Ikeoue, 1983, Ohwada and Uemoto, 1988). The on-growing equipment used in Japan e.g. pearl and lantern nets suspended from surface long-lines, were found to be difficult to use in the high tidal regime at Broome unless they could be monitored and cleaned of fouling organisms on a regular basis. Modifications to these existing on-growing culture methods to suit pearl oysters in the Broome region therefore had to be made to take into account the strong tidal regime, abrading effects of bottom sand movements, excess silting, intense fouling, predation and damage from severe weather conditions such as cyclones. To counteract most of these specific problems which had already been encountered in the maintenace of adult broadstock pearl oysters, the eventual solution appeared to be to on-grow spat in robust metal cages which were held slightly off the sea floor by star pickets driven into the substrate.

Successful spawnings in the Broome jetty hatchery facility in November 1988 (for further details see Rose and Baker, 1989) produced sufficient artificially propagated *P. maxima* spat for on growing trials on industry lease sites in Roebuck Bay, Broome (Figure 2). The diving depth on the holding site where these trials were conducted

ranged between 11m to 15m during the low neap tidal cycle.

During neap tidal periods in February and March 1989, small spat (average size approximately 6mm dorso-ventral length) were scraped off glass settlement surfaces in the hatchery nursery tanks. These spat were resettled on layers of black polypropylene knitted mesh ("Treeguard", Rheem) (Figure 3); and batches of approximately 200 spat were then enclosed in black polyethylene mesh "onion" bags (Boral Cyclone). The bagged spat were transported in water filled drums approximately 4 nautical miles to a nearby pearl farm lease within Roebuck Bay where the spat were placed by divers in on-growing cages positioned 0.5 m above the sea floor i.e. above the abrading effects of sand movements.

Initially the bagged spat were placed in fine polypropylene 2mm mesh ("Shadecloth", Rheem) covered metal framed pyramids in an attempt to reduce losses of the small unattached spat (Figure 4). This fine mesh retained too much seston and the spat were subsequently transferred to larger 17mm diameter mesh (Polyethylene) covered metal framed cylindrical or rectangular tray cages (Figure 5) which were fashioned from pool safety fence panels (Smorgon ARC). During this transition, spat density was

reduced to lots of 25 by rebagging them in weighted paired orange polypropylene mesh tubing expanded with loose bundles of monofilament shark mesh. (Figure 6).

The monofilament also provided a resettlement surface to on-grow the juvenile pearl oysters to a size of approximately 20mm DVL when they were large enough to be retained within pockets (each about 115mm high X 110mm wide) fashioned by weaving twine through 10 or 15mm mesh sized black polyethylene "onion" bags (Figure 7). These tagged "individual pocket mesh" panels containing measured pearl oysters were placed in protective 17mm mesh sized black polyethylene tubes (Boral Cyclone) and suspended from the Broome jetty (Figure 8) or simply placed inside the protective on-growing cages on the holding site. Size increment measurements were made also every six to eight weeks on slightly larger oysters (initial size approximately 45mm DVL) placed within individual pockets of 25mm square mesh versions of standard panel nets (Figure 9) used in farm husbandry by the pearling industry.

3.2 RESULTS

(a) 1987 Spawned Spat Trials

A small number of *P. maxima* pearl oyster larvae were successfully reared to settlement stage in the Broome hatchery during late October 1987. Preliminary attempts to on-grow spat from these larvae in the cages began in late April 1988 (Table 2, Batch A). The growth rate for these spat (initially, N=16) for the first 24 months after fertilization is shown in Figure 10. By February 1989, the largest surviving juveniles were approaching the legal minimum size collected by the pearling industry i.e. 120 mm dorso-ventral length (DVL). At the latest measurement check in May 1990, (i.e. 24 months after fertilization) their mean size was 122.9mm (± S.D.=10.9mm) with a cumulative mortality of 50% (Table 2).

(b) 1988 Spawned Spat Trials

Some 14,000 pearl oyster spat resulted from successful November 1988 spawnings and were used for more comprehensive on-growing trials. Representative spat (Table 2, Batch B; initially, N=120) held in individual pocket meshes inside cages on the sea bottom almost

doubled in size during the first on-growing period of 44 days (between measurements on 17 March and 30 April 1989) with the mean DVL increasing from 14.8 mm to 26.6 mm (Figure 10). At the latest measurement check in May 1990 (e.g. 19 months after fertilization) their mean DVL was 68.0mm (± S.D.=12.2). The growth rate of these measured spat averaged 3.8mm DVL per month during the 18 months since settlement. The average daily growth rate for these spat was greater during the warmer austral autumn months e.g. March-April 1989 = 0.28mm/day than the daily growth rate during periods of cooler water temperatures e.g. July-October 1989 = 0.11mm/day. The cumulative mortality between March 1989 and May 1990 for this batch of measured spat was 32% (Table 2).

Similar sized spat (Table 2, Batch D; Tags: W1 and Y1, initially, N=66) held in individual pocket meshes that were suspended from the Broome jetty grew to a mean size of 81.1mm (S.D. ± 12.2) by their latest measurement in May 1990. Even though these oysters were held two per pocket, their growth was significantly greater than the above spat (Batch B) grown near the sea bottom on the lease site (Analysis of variance, $F=36.05$, 1df, $P<0.001$). The spat on-grown near the sea surface, however, were

subjected to more intense fouling and required much more cleaning effort before they could be measured. These spat also showed a greater daily growth rate during periods of warmer seawater temperatures e.g. December 1989 - February 1990 = 0.19mm/day compared to austral winter months e.g. July - October 1989 = 0.08mm/day. The cumulative mortality between May 1989 and May 1990 was 20%.

Spat suspended from the jetty (Table 2, Batch D; initially, N=140) were also used to check the effect of increasing the density of spat per pocket on their growth rate. An analysis of covariance was used to test for different growth rates by doubling the number of spat from two oysters to four oysters per pocket. The growth rates however, were found to be the same (i.e. similar graphic slopes) even though there was a significant difference between the initial sizes of the oysters used ($P < 0.001$). The tagged panel with four per pocket, however, did have a higher cumulative mortality of 28% verus 20% in panels with two oysters per pocket.

3.3 DISCUSSION

In order to produce pearls larger than can be produced by the competing Japanese industry the Australian pearl culture industry requires *P. maxima* of shell size greater than approximately 120 mm dorsal-ventral length. Preliminary growth studies and ageing techniques indicate that a size of 120 mm may be achieved in as little as 15 months, therefore *P. maxima* is considered a suitable candidate for aquaculture. Although these field trials have successfully demonstrated that pearl oyster spat can be on-grown to commercially usable sizes within 19 months after fertilization in the Broome hatchery, several modifications to our grow-out methods are considered important enough to warrant mention in the expectation that artificially propagated spat in the future could be on-grown to usable sizes faster and with fewer problems.

(a) Seasonality

The first modification is that hatchery production should be timed to occur at the beginning of the pearl oyster breeding season in September/October to enable the settled spat to be on-grown in the sea over the maximum number of summer months. As spat appear to grow at a faster rate during the months of

warmer seawater temperature, this timing also would enable them to be on-grown over two summer periods which should enable most oysters to reach a size large enough to be seeded for pearls during their second winter (the pearling industry prefers to undertake these options during the cooler months of the year).

(b) Predation

If the hatchery produced spat can be placed in the sea during the summer months initially, a greater percentage of them also will grow quickly to a size large enough (approximately 20mm DVL) to be less vulnerable to the major source of predation within the grow-out enclosures i.e. from crabs (Figure 11). Until the majority of the on-growing spat are greater than 20mm DVL, frequent checks e.g. at least monthly must be made to remove crabs growing up within the protective mesh and predating on small spat.

(c) Biofouling

While biofouling was found to be less of a problem overall during the experimental period than expected e.g. major barnacle settlement on spat did not eventuate (Figure 12), much less

cleaning effort was expended upon spat on-grown near the sea bottom than on those spat suspended from the hatchery jetty near the sea surface. Once the spat are large enough (> 20mm DVL), however, they were robust enough to be cleaned mechanically (albeit, with much less water pressure than is used on the adult culture oysters) and therefore could be incorporated into existing surface long-line farm husbandry practices. The use of a non-toxic antifouling wax ("Easynet", Bullivants) on half the number of panel nets used for on-growing seems to have reduced biofouling (Figure 13) without affecting the pearl oyster spat and is therefore recommended.

(d) On-growing gear

The extensive hand labour as well as the physical loss of spat involved during grow-out transitions (Figure 14) could be greatly reduced in future hatchery work by settlement directly onto monofilament mesh so that spat could complete their first 4-6 months growth near the sea bottom within protective enclosures without major disturbance. At the end of this initial period of growth, the majority of spat will be greater than 20mm DVL in size (see Figure 4); and they will be robust

enough to be cleaned and culled to a recommended density not to exceed approximately 250 spat/m^2 surface area of the protective enclosures (Figure 15). Still in these cages, the spat can be on-grown further to a size large enough to be placed in the individual pockets of panel nets and then be integrated directly into the standard pearl culture farm husbandry procedures.

3.4 CONCLUSIONS

This project segment has successfully demonstrated that spat can be on-grown to commercially usable sizes within 19 months of fertilization. Table 2 illustrates the initial growth rate for some measured batches of spat resulting from the November 1988 spawning. From this same spawning at least 5 specimens measured > 120mm DVL in May 1990 (i.e. approximately 19 months after fertilization) thus demonstrating that it was indeed feasible to on-grow pearl oyster spat that could be used commercially in their second year of growth (i.e. as 1^+ animals).

3.5 RECOMMENDATIONS

The following recommendations are made based on the results of this research:

(1) Artificial propagation of spat in the hatchery should be timed to occur at the beginning of the pearl oyster breeding season in September/October, as this enables them to be on-grown over the maximum number of summer months. As their growth rate is more rapid during months of warmer water temperatures, another benefit is that a greater percentage of the juveniles quickly will reach a size at which they are much less vulnerable to predation and fouling.

(2) In the Broome region, fewer spat grow-out problems are likely to be encountered using protective cages placed near the sea bottom than by using near surface methods during the first 4-6 months growth.

(3) As biofouling remains one of the greatest obstacles to successful on-growing, the use of antifouling wax on panel nets and "slippery" (polyethylene) plastic mesh in the protective enclosures is to be commended. Too fine of mesh (<2 mm), however, could not be cleaned frequently enough to prevent being clogged with seston.

(4) The hatchery use of flexible settlement surfaces such as monofilament mesh are preferable to the glass plate used in 1987 and 1988. Mesh with the attached spat could be placed directly within protective enclosures and left to on-grow at sea without major disturbance until the spat complete their first 4-6 months growth.

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Appendix 1. SUBMISSION OF PEARL OYSTER SPECIMENS TO FISH HEALTH SECTION, W.A. DEPARTMENT OF AGRICULTURE

The Fish Health Section requires live affected specimens, very fresh chilled specimens, or a combination of fixed specimens plus fresh chilled or fresh frozen specimens. Fixed tissue is used for histopathological studies, and fresh tissue is used for detection of bacteria, viruses, fungi and toxins. Three is a good minimum number of each kind of specimen. Any specimens are better than no specimens, but good specimens are the first step in obtaining a useful diagnosis. The ideal stage of disease to sample is when the pearl oysters are alive but affected or moribund. With shellfish from remote locations, however, this option is inherently difficult as observed sick oysters are likely to be in an advanced state of putrefaction (and therefore almost completely useless for disease investigation) by the time they can be transported to the Fish Health Section in Perth.

Option A: Fixed plus fresh chilled or frozen specimens (where delays in transport exceed 12 hours).

Small pearl oysters may be fixed whole in the shell, but break the edge of the shell to allow penetration of the fixative into the viscera body. The viscera mass from larger pearl oysters may be removed whole from the shell (to reduce shipping weight) before fixing. The best field fixative is 10% formalin in seawater, 10 volumes of fixative to 1 volume of tissue. Formalin can be obtained from some chemists, farm suppliers or the Fisheries office in Broome. After fixing the viscera for three days, the volume of fixative can be greatly reduced for shipping. Sample containers still must be well sealed to prevent leakage of formalin. Include 3 or more chilled or frozen specimens in plastic bags, on at least 3 times as much ice, in a small foam esky sealed to prevent leakage.

Option B: Very fresh chilled specimens (for overnight delivery and where formalin is unavailable).

At least 3 affected and if possible 3 unaffected pearl oysters in separate plastic bags, on at least three times as much ice, in a foam esky sealed to prevent leakage.

Option C: Live specimens (on occasions suspected moribund oysters may be available for examination alive if they can be transported to Perth rapidly).

At least 3 affected and if possible 3 unaffected pearl oysters, shipped in separate plastic bags and kept moist and cool inside a foam esky or plastic containers.

Delivery Address:

Fish Health Section
Atten: Dr Jeremy Langdon
WA Department of Agriculture
Baron - Hay Court
SOUTH PERTH WA 6157

Carriers: Courier Australia, Ansett, Australia Post, Comet, etc.

Notification: Please ring (09 368 3351) or send facsimile (09 474 1881) to give us advance notice of specimens arrival and to discuss how to send them.

TABLE 1: *Vibrio* bacteria isolates from *P. maxima* (April 1987-May 1988)

	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC
V. alginolyticus *	+	+	+	+	+	+	+	+	+	+	+	+
V. anguillarum/tubiashii *	+	+	+	+	+	+	+	+				+
V. harveyi *	+	+	+	+	+	+	+			+		+
V. splendidus							+		+	+	+	
V. pelagius						+			+		+	
V. natriegens					+		+					
V. damsela								+	+			

* Potential pathogens

TABLE 2 Growth measurements of tagged batches of juvenile pearl oysters on-grown from spat produced in the Broome hatchery in October 1987 and November 1988. Dorso-ventral shell lengths are given as mean ± standard deviation and range of values in millimetres at various measurement dates. The percentage of cumulative mortality for each batch of oysters is given also at the curtailment of measurements in May 1990.

MEASUREMENT DATE	BATCH DESIGNATION						
	A	B	C	D	E	F	G
April 1988	39.2 ± 8.0 (17 - 46)						
July 1988	47.6 ± 9.6 (28 - 65)						
February 1989	71.6 ± 19.5 (39 - 95)						
March 1989		14.8 ± 2.3 (10 - 21)					
May 1989		26.8 ± 4.3 (17 - 38)	38.2 ± 2.3 (33 - 41)	22.2 ± 4.1 (12 - 33)			
July 1989		37.2 ± 5.9 (21 - 52)	38.0 ± 2.3 (34 - 42)	34.6 ± 6.4 (16 - 50)			
September 1989		45.2 ± 7.1 (30 - 63)	46.7 ± 4.3 (37 - 58)				
October 1989	100.0 ± 13.8 (65 - 110)		53.5 ± 7.1 (40 - 75)	43.6 ± 8.2 (21 - 62)	41.7 ± 8.6 (22 - 65)		
December 1989		52.9 ± 9.0 (31 - 74)	66.1 ± 8.1 (44 - 87)	52.0 ± 10.3 (28 - 74)	51.9 ± 10.2 (25 - 77)	52.2 ± 5.5 (40 - 67)	44.3 ± 11.0 (19 - 65)
February 1990	109.3 ± 7.6 (96 - 122)	55.4 ± 9.5 (38 - 74)	70.6 ± 13.9 (40 - 100)	61.9 ± 10.8 (36 - 84)	60.2 ± 13.0 (26 - 90)	55.7 ± 6.7 (42 - 72)	53.3 ± 10.7 (32 - 78)

March 1990	111.4 ± 8.6 (102 - 124)	59.7 ± 10.4 (35 - 86)	80.4 ± 15.0 (44 - 112)	70.7 ± 12.3 (40 - 98)	67.7 ± 14.0 (36 -99)	61.7 ± 7.5 (42 - 75)	66.9 ± 13.1 (35 - 97)
May 1990	122.8 ± 10.9 (104 - 141)	68.0 ± 12.2 (39 - 92)	90.1 ± 14.4 (57 - 125)	77.6 ± 12.5 (44 - 102)	74.1 ± 14.3 (38 - 102)	76.6 ± 8.6 (55 - 92)	74.5 ± 14.2 (42 -102)
Cumulative mortality							
Initial No. oysters/batch	16	120	153	140	302	90	74
Final No. (survivors)	8	82	136	103	224	54	62
% dead	50	32	11	26	26	40	16

NB A summary of the on-growing history of each of the above batches of oysters is given below. Details are provided as to net tag designations stored in computer files held at the Waterman Laboratories; final type of on-growing net used (IPM = individual pocket mesh panels, PN = panel nets); and the on-growing location (J = Jetty, H = holding site). Only Batch A oysters were derived from spat settled in the hatchery in November 1987; all other on-grown oysters were derived from hatchery spat which settled in December 1988. Oysters were normally on-grown in a density of one oyster per pocket, however, Batch D oysters were on-grown in higher densities (its nets tagged W_1 and Y_1 had 2 oysters/pocket; Y_2 had 4 oysters/pocket; unfortunately, Y_3 which had only one oyster per pocket as a control was lost from the jetty after only initial measurements and was not included in the above growth measurements for Batch D).

Batch A Tag: JH; PN, H.

B Tags: GR 1, 3 and OR 2,4; IPM; H.

C Tags: EN 1, 2, 3, 6, 7, 9 and SN 1, 2, 6, 7; PN; J.

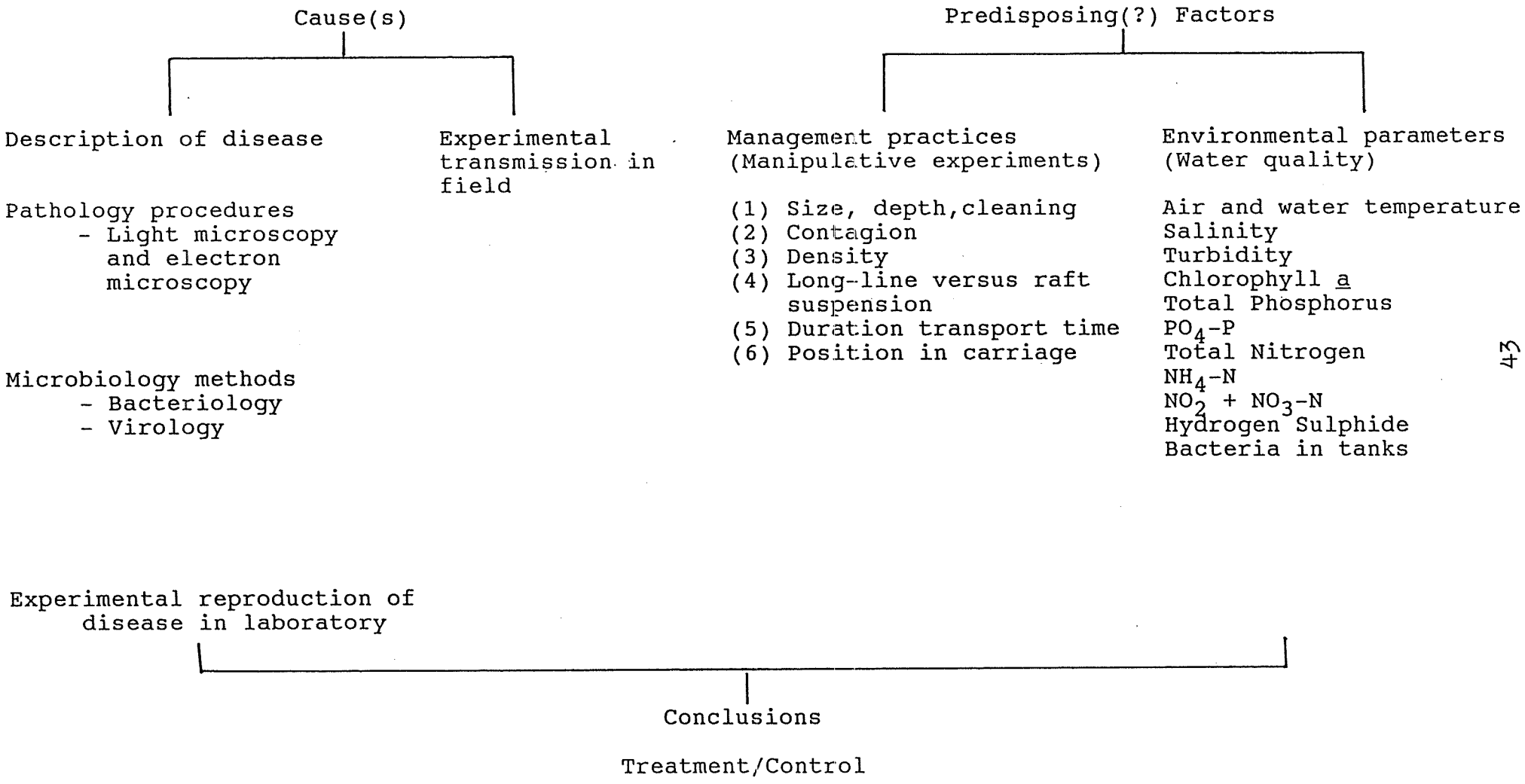
D Tags: W1 and Y 1, 2; IPM; J.

E Tags: T1-4 and B1-3; IPM; J.

F Tags: SNA, C, D and EN B, E, F; PN; H.

G Tags: O and P, IPM: J.

Figure 1. **Outline of Study during original three year investigation of pearl oyster mortality**



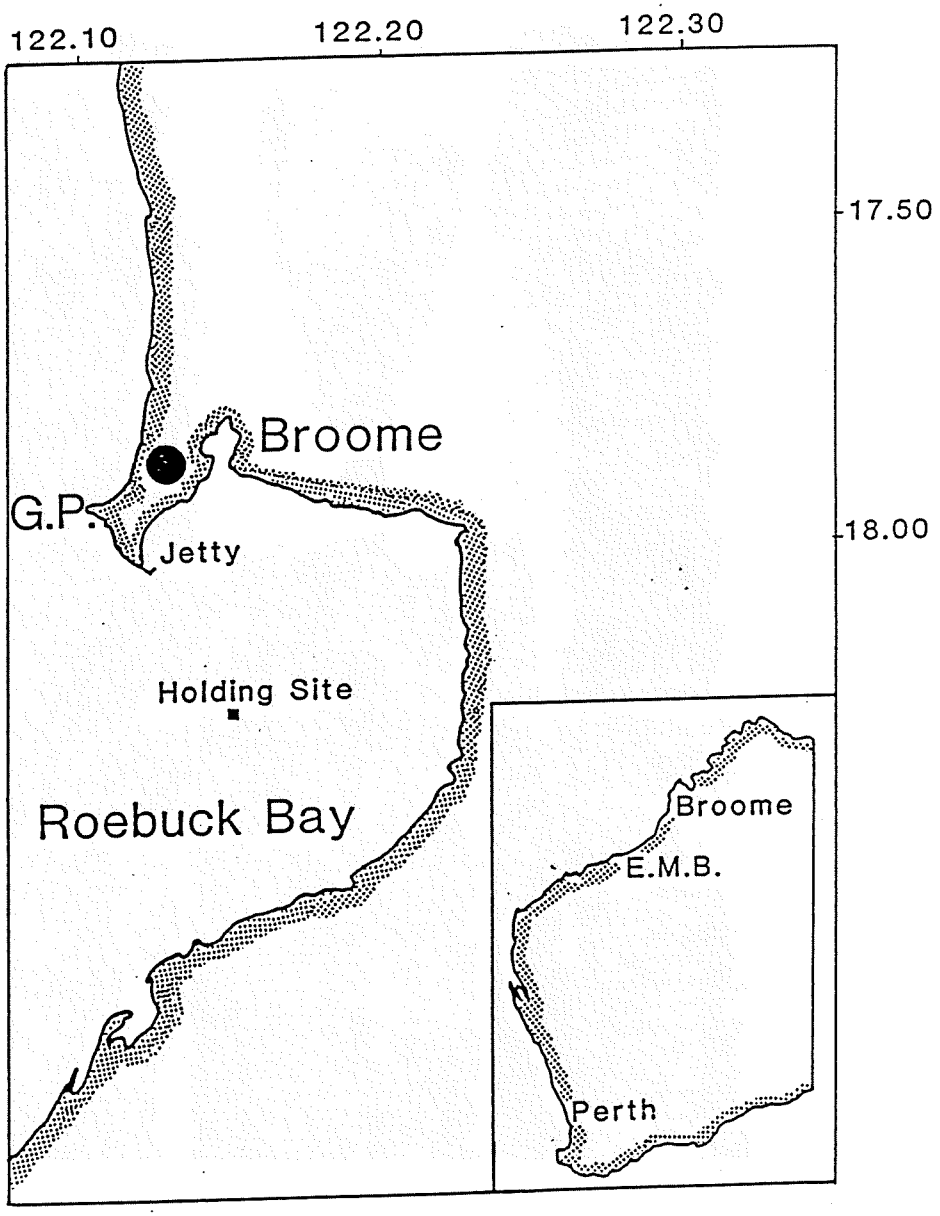


Figure 2 Location diagram of the Broome area in the Northwest of Western Australia showing hatchery jetty and pearl oyster holding site referred to in the text. Abbreviated locations are as follows: (G.P.) Gantheaume Point and (E.M.B.) Eighty Mile Beach.

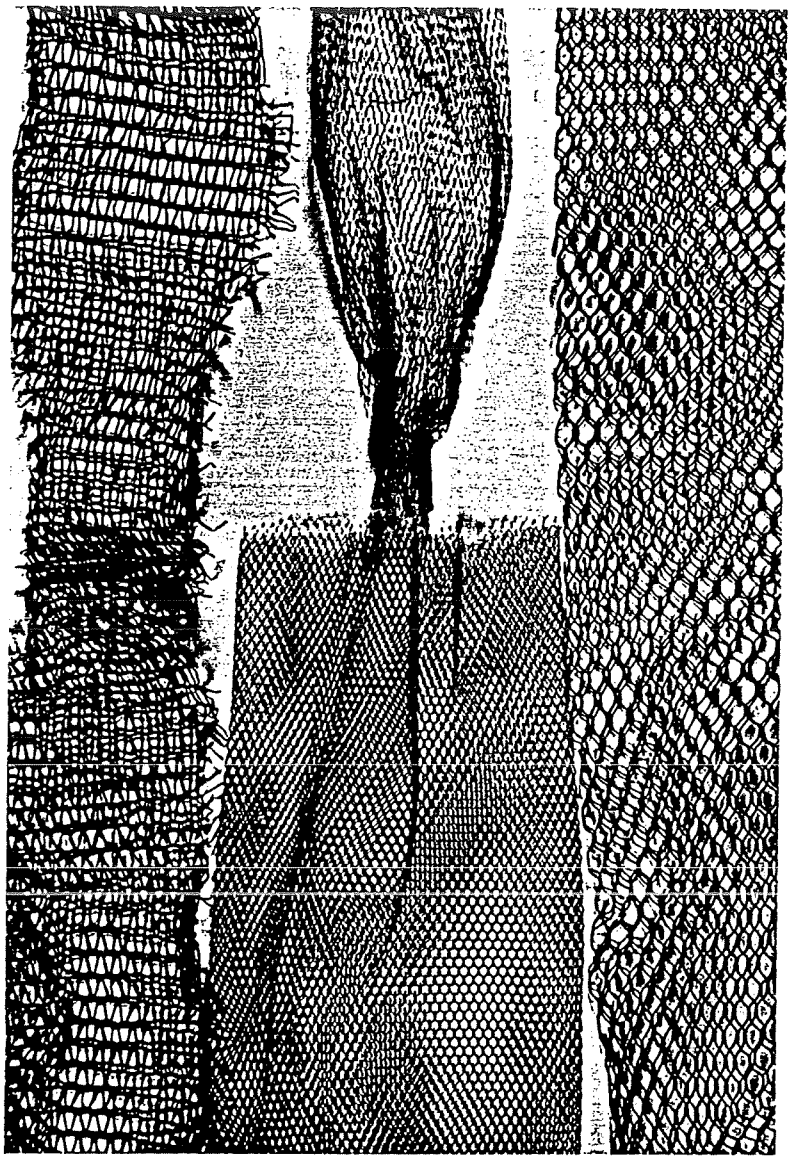


Figure 3 Various plastic meshes to used to on-grow juvenile oysters. *Left*: black polypropylene knitted 10mm mesh ("Treeguard"); *top middle*: orange polypropylene 12 mm mesh tubing; *bottom middle*: black polyethylene 6 mm mesh "onion" bag; *right*: black polyethylene 15 mm mesh "onion" bag.

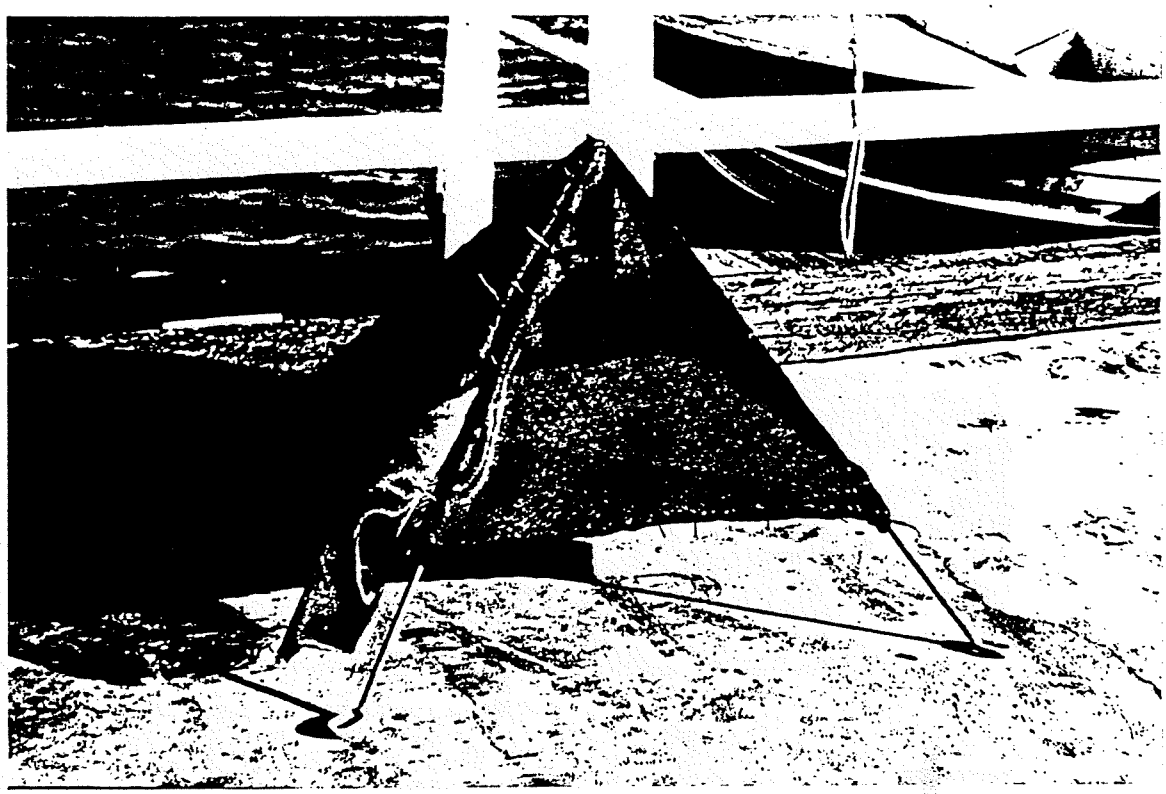


Figure 4 On-growing pyramid cage constructed with a mild steel rod frame and covered with "shadecloth" mesh. The extended legs raise the caged juvenile oysters above the sea floor and the abrading effects of sand movements.

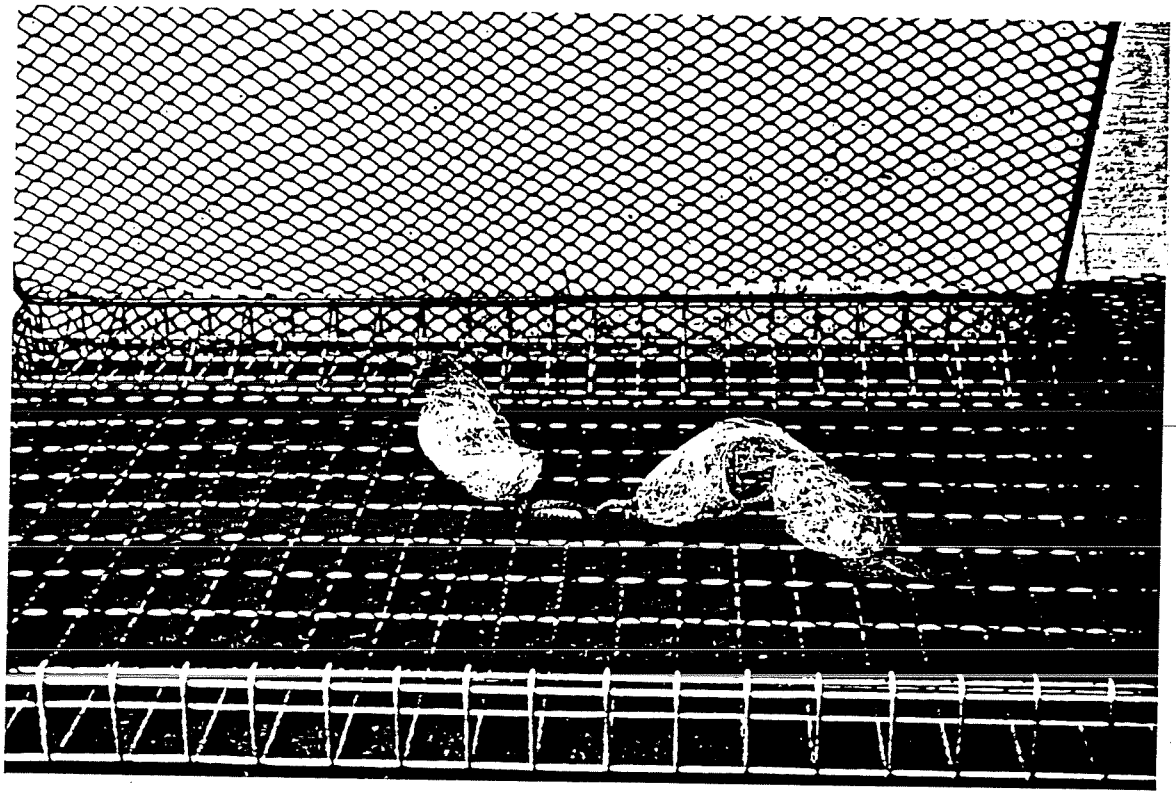


Figure 5 On-growing tray cage fashioned from a metal fence panel (2m x 0.9m x 0.1m) lined with polyethylene 17mm mesh. Also shown is a paired orange polypropylene mesh tubing containing juvenile pearl oysters with a lead weight in the middle to restrict movement within the cage.

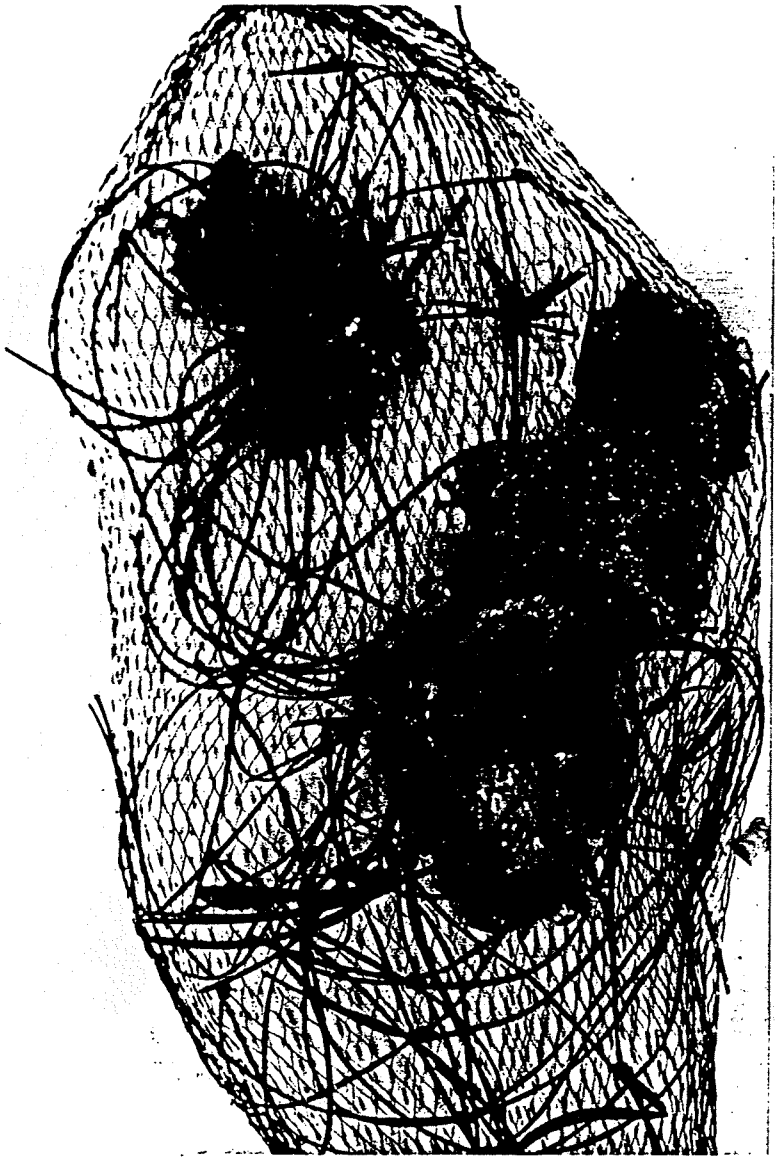


Figure 6 Half of a paired on-growing "orange" bag. The heavy monofilament keeps the mesh tube expanded and provides a reattachment surface for the pearl oyster's byssal threads.

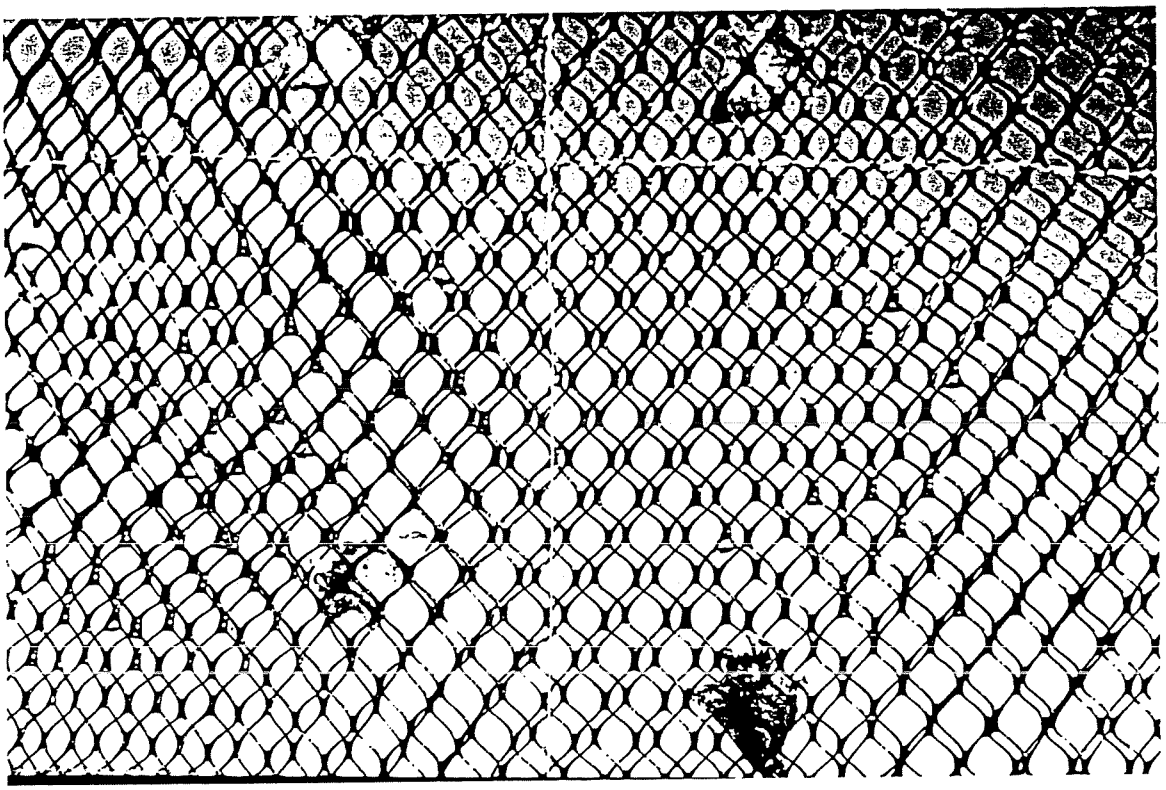


Figure 7 Juvenile pearl oysters (0.67 x actual size) enclosed within individual pockets of net panel fashioned from 15 mm mesh black polyethylene "onion" bags.

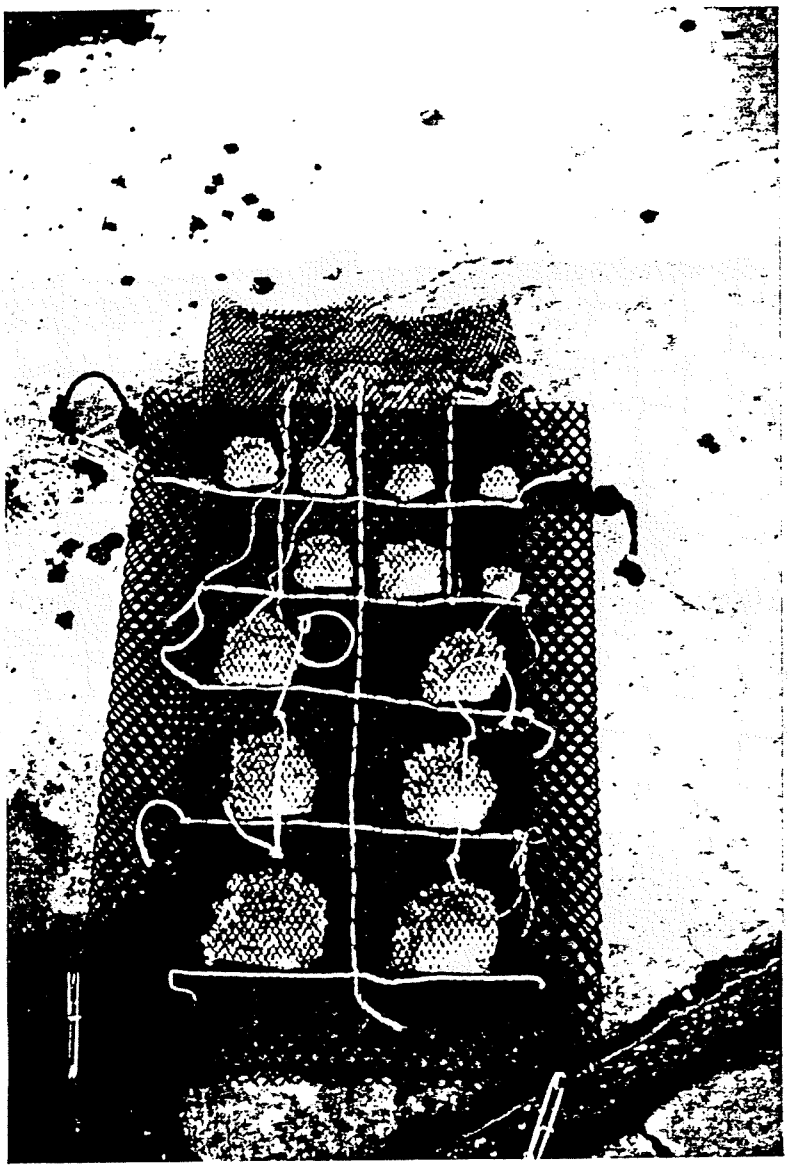


Figure 8 Juvenile pearl oysters from Batch A (see Table 2) before their new "individual pocket mesh" panel was placed inside a protective black polyethylene 17 mm mesh tube and suspended from the jetty with ropes using shark clips. The dorso-ventral length for these 14 month old juveniles ranged between 35-95 mm when measured in February 1989.

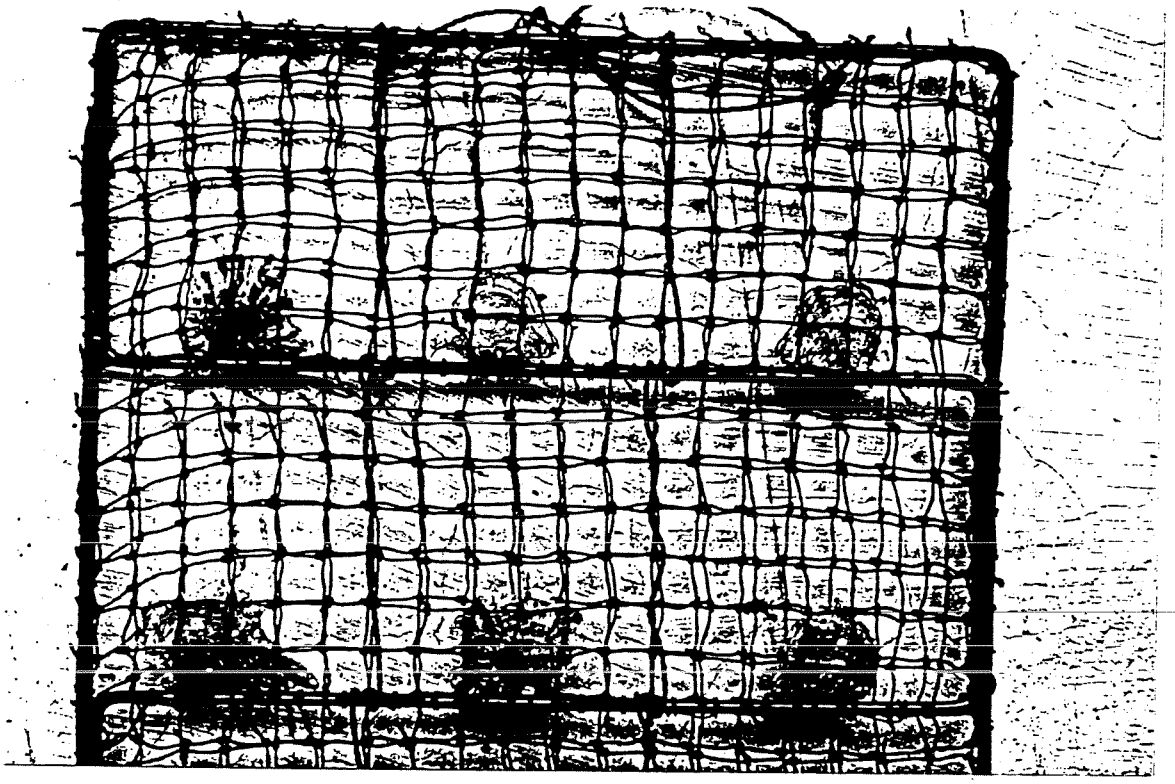


Figure 9 Top half of a 25 mm square mesh version of a standard panel net. The dorso-ventral length of these on-growing juveniles pearl oysters is approximately 50mm.

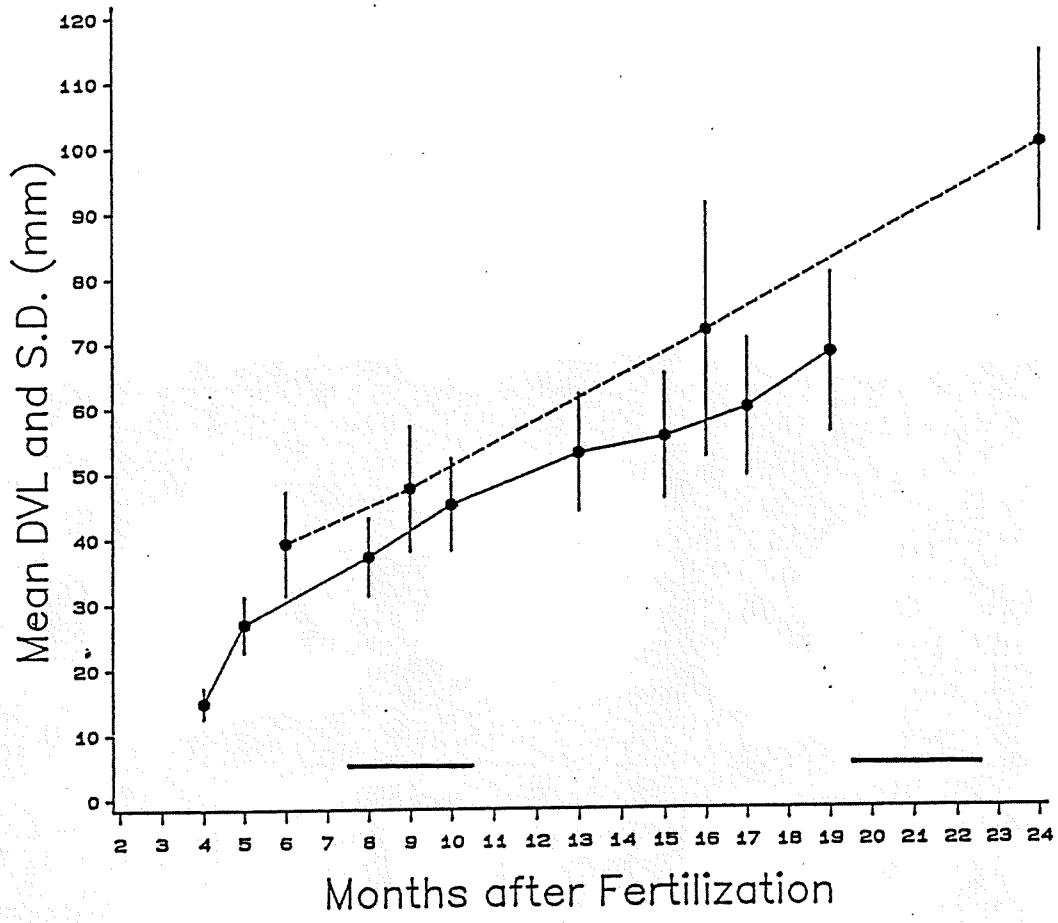


Figure 10 Growth rates of *Pinctada maxima* from spat produced in the Broome hatchery in October 1987 (o--o) and November 1988 (o—o). The solid horizontal bars denote months of cooler seawater temperatures i.e. June to September.

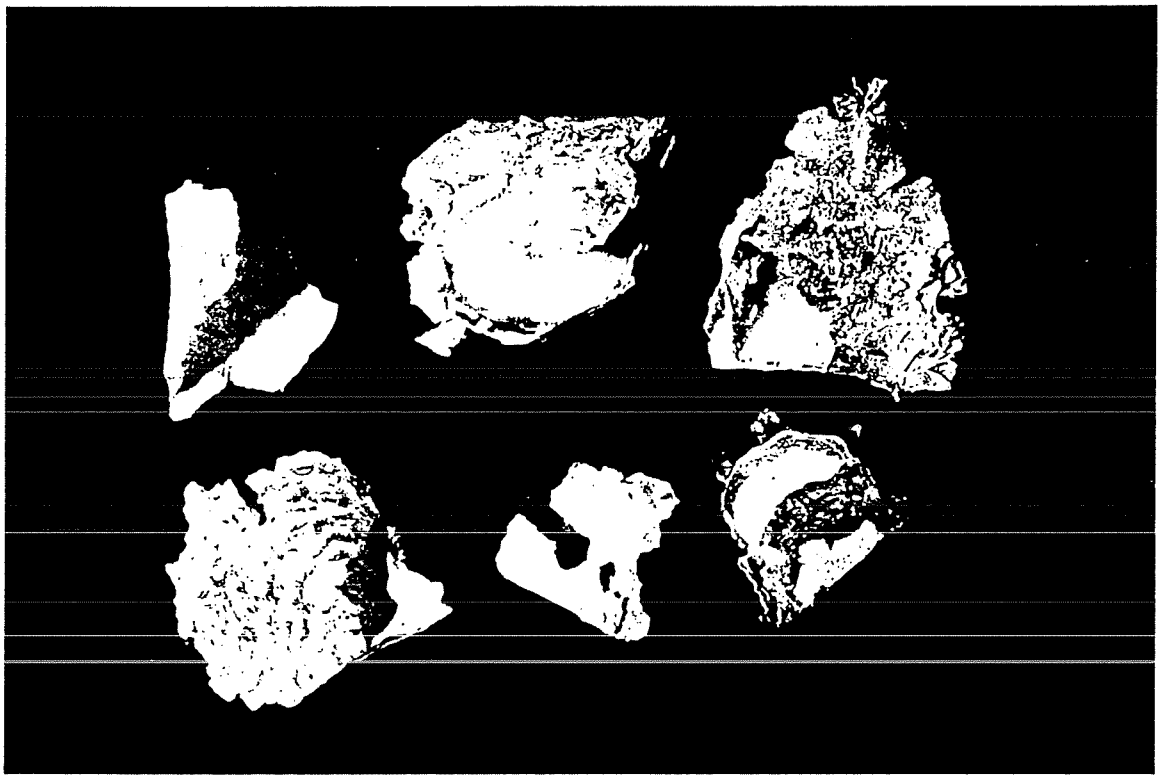


Figure 11 The broken remains of dead juvenile pearl oysters (1.3 x actual size) that have been predated upon by crabs within the protective cages.

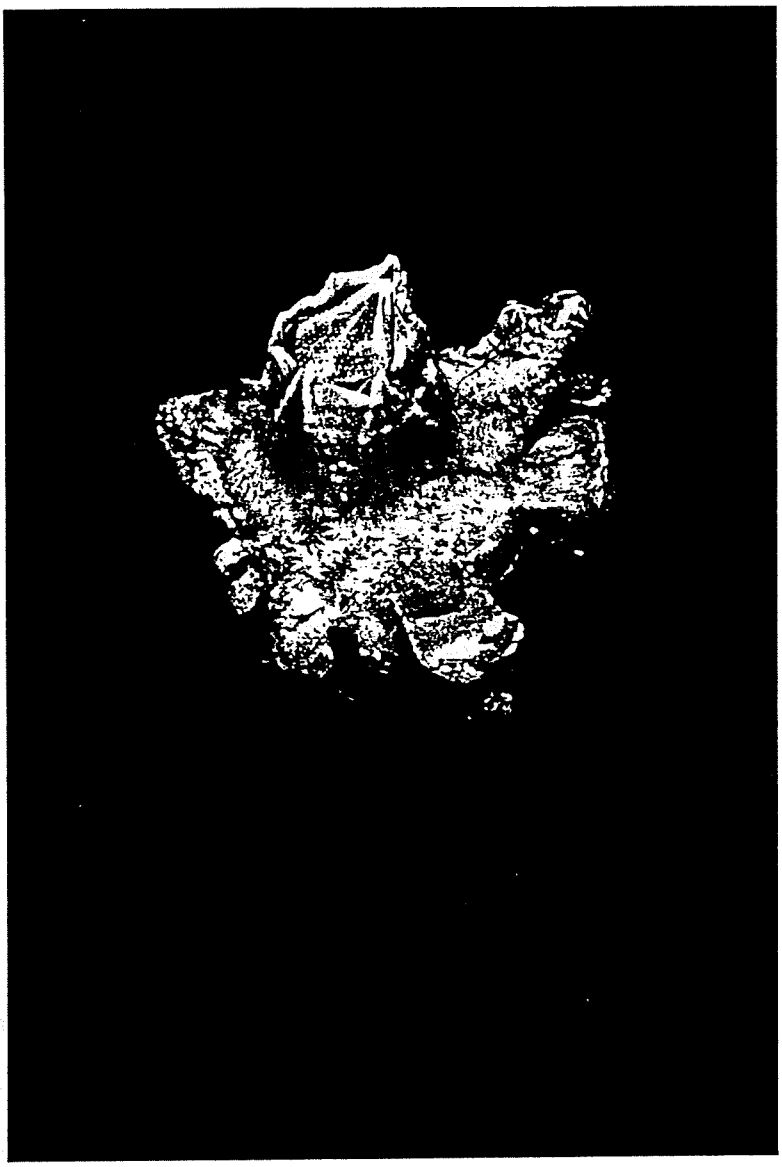


Figure 12 A barnacle (1.5 x actual size) that has cemented itself on an on-growing juvenile pearl oyster. This fouling organism grows more rapidly than the small oyster and its forced removal is likely to break the thin fragile shell of the juvenile oysters.

Figure 13 The use of an antifouling wax on the right panel net has noticeably reduced biofouling (from algae, sponges, bryozoans) compared to the standard panel net on the left during the two month period between growth measurements of the encased juvenile pearl oysters.

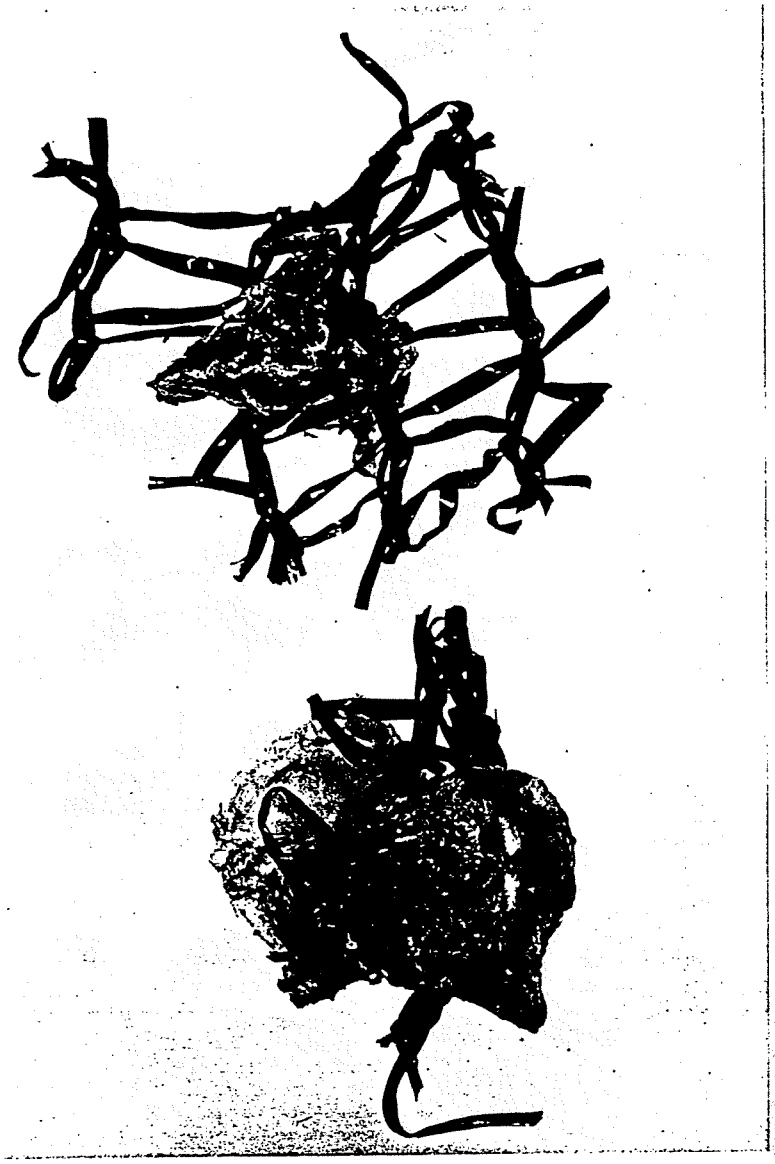


Figure 14 Deformed juvenile pearl oysters (2 x actual size) that have become enmeshed in "Treeguard" during grow-out partially as a consequence of having scraped the spat from glass settlement surfaces in the hatchery onto the several layers of mesh required to retain the loose spat while they reattach to another surface.

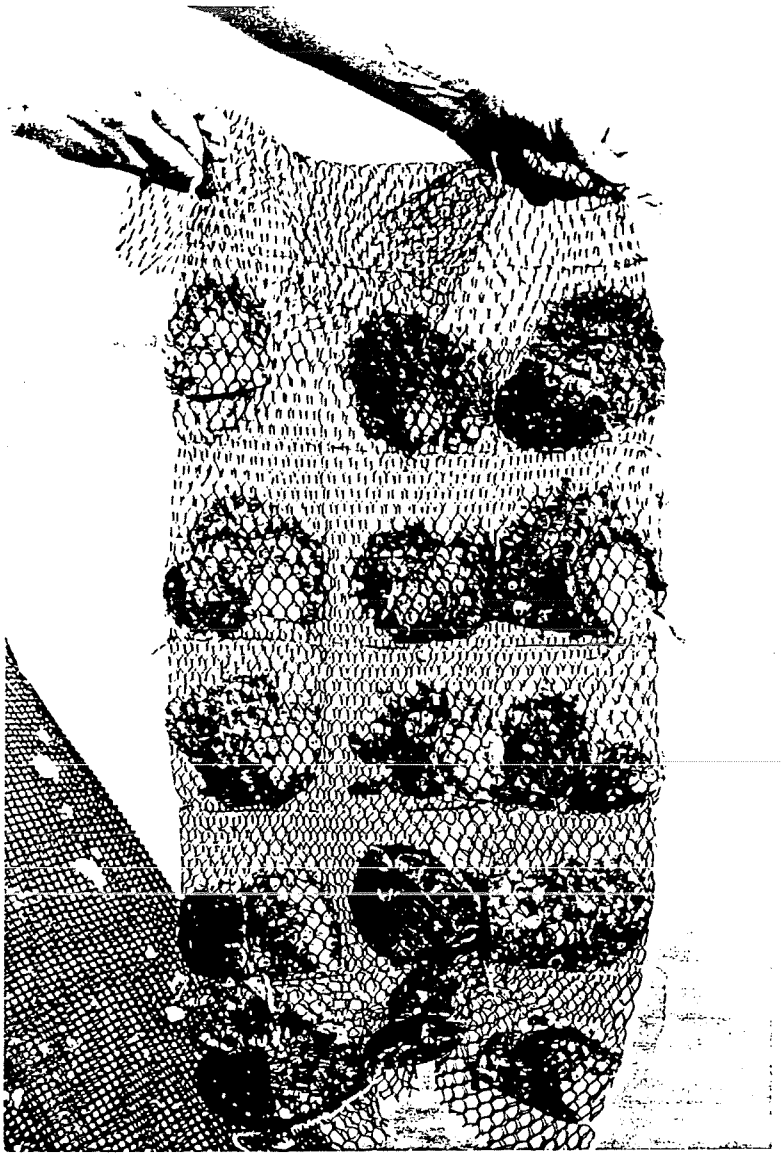


Figure 15 An example of November 1988 spat held in an on-growing panel with a density of three oysters per individual pocket. As each pocket is approximately 144cm^2, the overall density of this panel extrapolates to 207 oysters/m^2 surface area. The dorso-ventral length of the measured oysters ranged between 46 and 102mm when the picture was taken in May 1990 i.e. 19 months after fertilization.