FIRDTF PROJECT 89/61 - FINAL REPORT

JUNE 1990

STUDIES ON THE DEVELOPMENT OF HATCHERY AND NURSERY CULTURE OF THE SILVER-LIPPED PEARL OYSTER (PINCTADA MAXIMA).

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PREFACE

This document is the final report of research undertaken in FIRDTF project 89/61. The results reported here on broodstock selection and spawning methods of the pearl oyster, *Pinctada maxima*, build upon and complete the development of pilot scale technology which has been derived from six years of previous FIRTA supported investigation into the artificial propagation of pearl oysters. This technology has now been developed to the point where the pearl culture industry can keep abreast of bivalve mariculture developments and use this information to enhance 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 commercial rock lobster and prawns fisheries.

The pearl oyster is an ideal mariculture candidate as artificially propagated spat can be used by the pearl culture industry during their second year of growth (i.e. as 1^+ animals).

ACKNOWLEDGMENTS

This study was financed by a grant from the Australian Government Department of Primary Industry's Fishing Industry Research and Development Trust Fund (FIRDTF project No. 89/61). Spat produced by this project have been used in on-growing trials to provide culture size pearl oysters in a separate (but complementary) FIRDTF project, 89/60. The authors wish to acknowledge W.A. Fisheries Department biologists Dr R.A. Rose and Mr S. Baker for their concerted efforts in establishing the hatchery facility and the extensive technical assistance of Mr R. Allison throughout the final year's field work phase of this project. While interest was shown by the whole of the pearl culture industry, the personnel of Broome Pearls, especially Messrs R. Scoones and C. Richards, should be accorded special recognition for their many contributions towards the successful completion of this project. Thanks are expressed to the Master of R.V. Flinders, Mr T Berden and crew for their assistance in field collection and to the staff of the Western Australian Marine Research Laboratories for support during the computing and report writing phase of this project.

EXECUTIVE SUMMARY

The Australian cultured pearl industry currently produces pearls valued in excess of $80 million (1989 production value) making it Australia's most valuable aquaculture venture and third most lucrative commercial fishing activity after rock lobsters and prawns. The industry at present relies almost entirely on catches from the Western Australian natural stock of the silver-lipped pearl oyster *Pinctada maxima* for pearl culture. Hatchery propagated oysters would be a useful alternative to wild stock oysters, in alleviating the effects of variable recruitment and for allowing future economic expansion of cultivated pearl production.

(i) Objectives

The purpose of this project was therefore to reduce some of the technical and biological constraints affecting hatchery production of pearl oyster spat. The primary objective of this research was "to establish artificial propagation techniques for the silver-lipped pearl oyster to provide an alternative source of oysters for the pearl culture industry". More specifically the project in 1989/90 was to concentrate on refining techniques for the field selection and maintenance of pearl oyster broodstock for induction of

spawning which had been the major constraint on hatchery production up to 1988/89.

(ii) Broodstock Selection prodedures

Broodstock oysters for the spawning trials were selected from three different locations in the Broome region. The oysters were maintained on a holding site in Roebuck Bay and later suspended under the jetty at the Broome hatchery for convenience during the trials. A total of 27 spawning trials were carried out between the 11th of October and the 1st of December 1989 resulting in the successful release of gametes on 10 occasions. During these trials broodstock from all three collection sites were induced to spawn. It was also found that the same oysters could spawn more than once and that females were capable of releasing several million eggs per spawning. The larvae produced from the successful spawnings were kept alive without feeding for up to five days until reaching the straight hinged veliger developmental stage ("D-shape") at a shell length of approximately 90µm.

(iii) Spawning induction technique

On the basis of this sequence of 10 successful spawnings and a limited number of other successful spawnings over the previous six years (1982-1988), the effectiveness of various

combinations of induction methods can be ranked in decreasing order, as follows: (i) temperature manipulation in combination with gamete suspensions and ultraviolet (UV) irradiated seawater, (ii) temperature manipulation combined with gamete suspensions, (iii) temperature manipulation combined with UV irradiated seawater, (iv) temperature manipulation, (v) gamete suspensions, (vi) UV irradiated seawater, (vii) cleaning/disturbance and (viii) chemical stimuli.

(iv) Spawning seasonality

Most of the successful spawnings during the 1989/90 programme occurred during November, that is, when the ambient seawater temperature was rising rapidly. This confirmed previous observations that, hatchery spawnings were most often achieved during spring/early summer period. It is there suggested that the best months for attempting spawning are as follows: November, December, October=March, February, January and April. Given the small number (approximately half a million per year) of oysters required by the culture industry, however, it is neither necessary nor cost effective to attempt induce spawning outside these months. Therefore it is recommended for the successful artificial propagation of *Pinctada maxima* that spawning for hatchery production is confined to the peak breeding season of November/December.

1. INTRODUCTION

The Western Australian cultured pearl industry is worth in excess of $A 80 million per annum, making it Australia's most valuable aquaculture venture and third most valuable commercial fishing industry after rock lobsters and prawns. Currently the industry relies almost entirely on the wild stocks of the tropical, silver-lipped (or gold-lipped) pearl oyster, *Pinctada maxima* (Jameson) on the NW Shelf coast for pearl culture. Total reliance on wild stock does impose some economic risks for industry which involves large fixed capital investments for pearl production (Dybdahl and Rose, 1986). The risks related to the possibility of recruitment failure will increase if industry is permitted to fish more wild stock to increase pearl production or if unexpectedly high levels of mortality occurs amongst oysters after collection, as recorded in Western Australia during the last decade (Pass *et al.*, 1987). In addition, operating costs associated with the collection and transportation of wild oysters for pearl production are likely to continue to increase, above the current cost of $A 12 to $A 16 per oyster. For all of these reasons, artificial propagation has been investigated via a number of FIRTA-funded projects since 1982, as the above factors suggested hatchery produced pearl oysters were needed to provide for future economic expansion of cultivated pearl production and to alleviate effects of variable recruitment in the wild stock fishery.

The previous FIRDTF funded hatchery programme (Project 87/82) had succeeded in producing pilot scale quantities of spat (thousands) for the first time last year (1988/89). Additional funds were sought to complete this research and to improve spawning techniques which had yet to be developed to a stage where direct industry operation of a hatchery was possible.

The original objectives of the subsequent FIRDTF Project 86/61 were to perfect hatchery culture techniques for the silver-lipped pearl oyster to provide an alternative source of oysters for the pearl culture industry. More specifically the project was to attempt to:-

(1) refine the techniques developed in 1988/89 for the field selection and maintenance of pearl oyster broodstock for hatchery spawning.

(2) further develop and improve the culture methodologies for optimising growth and survival and settlement of pearl oyster larvae.

(3) further improve the newly developed handling protocols for nursery stage pearl oyster spat.

After the project was funded, a variation to the original objectives was deemed necessary when both research staff involved in this programme submitted their resignations to join private enterprise mariculture concerns. As a

consequence, the project was scaled down with the emphasis remaining on the first objective of refining spawning techniques for broodstock pearl oysters. The inability to induce spawning on demand had been found to be the greatest constraint on hatchery production of spat and was therefore considered to have the highest priority for our understanding of the artificial propagation of pearl oyster spat.

The revised objective of this research was "to establish artificial propagation techniques for the silver-lipped pearl oyster to provide an alternative source of oysters for the pearl culture industry. More specifically the project was to concentrate on refining the techniques developed in 1988/89 for the field selection and maintenance of pearl oyster broodstock for consistant induction of spawning."

Therefore this project would be considered successful if it met the following criteria:-

(1) provides tested (i.e. over two seasons) procedures for the field collection and selection of ripe broodstocks in the Broome region and,

(2) provides tested methodologies for broodstock maintenance and the induction of spawning in ripe pearl oysters.

2. MATERIALS AND METHODS

Previous FIRTA projects (82/85, 87/82) have indicated that broodstock oysters selected from the wild were usually the most reliable spawners. Broodstock maintained on farm leases in Roebuck Bay often showed arrested or delayed gonad development, making them unsuitable for use as spawners compared with those obtained directly from the wild.

Rather than rely upon opportunistic collection of broodstock oysters from commercial fishing vessels as has been done in past years, the Western Australian Fisheries Department research vessel "Flinders" was used to collect oysters in the Broome area over the neap tidal periods during October 1989. Large oysters with a shell dorso-ventral length (DVL) in excess of 150mm were specifically sought to ensure a plentiful supply of females for the spawning trials. Work on the reproductive cycle during the previous six years (Rose *et al.*, 1990) has confirmed *P. maxima* to be a protandrous hermaphrodite, reaching maturity as a male in the first year of it's life (110-120mm DVL) with the incidence of female sexuality increasing with age or size. Oysters from the wild had a sex ratio approaching 1:1 when their shell size was 200mm or greater in DVL.

During the first neap tidal period (7-13 October) oysters were collected by diving with hookah in shallow water (10-18 m) on the main fishing grounds off Eighty Mile Beach (between Lat. 18^0 30'S; Long. 120^0 41'E and Lat. 19^0 50'S; Long. 120^0 51'E). After visually examining the gonad sexual development stages (see below) of the collected broodstock, two preliminary spawning trials were made onboard within 24 hours of collection. These wild oysters were then taken to the holding site on the pearl oyster lease in Roebuck Bay (Lat. 18^0 5'S; Long. 122^0 15'E as shown in Figure 1.), where additional large oysters had been left undisturbed since the previous year's (1988/89) spawning trials, (see final report, FIRTA project 87/82).

During the second neap tidal period (20-27 October) wild oysters were collected in deeper water (> 20m) 14 nautical miles north-west (Lat. 17^0 48'S; Long. 121^0 58'E) of Gantheaume Point. These oysters were maintained in standard commercial panel nets on bottom longlines with the other oysters on the holding site in Roebuck Bay. From this lease site some oysters were removed and suspended from the Broome jetty, for convienience during hatchery spawning trials and "conditioning" (i.e. gonadal development can be stimulated to a ripened condition by holding adult oysters in the slightly warmer surface waters and by allowing the suspended oysters greater access to phytoplankton food than oysters maintained on the sea bottom).

Oysters to be used as broodstock were visually staged for their sexual development of gonads as follows:-

Stage 0: gonad tissue flaccid or invisable, sex indeterminate.

Stage 1: gonad visable but proliferation to gut loop was slight and proximal, gonad appeared granular and difficult to sex by colour (male-white and female-yellow).

Stage 2: sex easily determined by colour, tissue had proliferated distally along lateral walls of gut loop and appeared semi-confluent (at this stage spawning could occur but gametes were usually immature or non-viable).

Stage 3: gonad ripe and bulging, gonad tissue extended over the surface of stomach, gut loop and digestive gland; gonad appeared confluent and when pierced diffused profusely.

During the initial collection of broodstock, oysters which were visually determined as having the most advanced sexual development i.e. near-ripe gonads (stage 2) were used in preliminary spawning trials onboard. From past experience ripe (stage 3) oysters sometimes spawn in the carrier tanks after being disturbed and cleaned during collection within their breeding season (September to March). These two

preliminary spawning trials were conducted in square plastic tubs containing 80 litres of seawater heated several degrees above ambient seawater temperature.

Artificial induction of spawning in temperate bivalves e.g. edible oysters, has become standard practice in hatcheries where exact control of temperature and chemical stimulation is possible. Chemicals used with other bivalves to induce spawning such as hydrogen peroxide and serotonin (5-hydroxytryptamine), however, have been found to be ineffective in inducing spawning in *P. maxima* in previous trials (Rose *et al.*, 1986). In the Broome hatchery tropical pearl oysters have been induced to spawn previously using aerated, slowly circulating, ultraviolet irradiated, filtered seawater raised 3-5^{o}C above ambient (29-30^{o}C) seawater temperature. The induction of spawning has been enhanced by chemicals released by the actual gonadal products i.e. suspension of gametes added to the spawning tank. Gametes can also be stripped (excised) from the gonads of sacrificed broodstock oysters. Unless the gonads are suitably ripe, however, the gametes released are invariably less viable and the percentage of fertilisation is low (<30%) with subsequent high abnormal larval development (>85%).

In the Broome hatchery (for details see Taylor, 1986) spawning trials were conducted using more systematic methods. Oysters were brought up from the jetty, cleaned of fouling organisms and placed in the broodstock tank containing 500 litres of seawater (Figure 2.). Spawning was usually induced in ripe oysters by raising the temperature 2-4^{o}C above ambient by using heated ultraviolet irradiated seawater. If oysters were sufficiently ripe, temperature manipulation by itself would usually stimulate them to spawn. After an hour if this treatment was unsuccessful, gamete suspensions or chemicals were tried as additional stimuli.

After a complete spawning (i.e. when both sexes have released gametes) an hour was allowed to ensure fertilisation. The water containing fertilised eggs was passed through a fine mesh sieve to remove large particles of debris and the eggs were transferred into a 20 litre bucket to facilitate monitoring larval development. The number of eggs were quantified and their size and shape were determined microscopically as an indication of their viability. Fertilised eggs were kept in the buckets to develop to at least the straight hinged veliger developmental stage ("D-shape") as further confirmation of spawning success. Given the staff resources available, it was beyond the scope of the revised objective to rear the larvae to the settlement stage. Therefore the larvae were

not fed and were discarded when they succumbed to starvation.

3. RESULTS

The collection of broodstock oysters using the "Flinders" was successfuly completed in October 1989 with 118 oysters (DVL > 150 mm) collected south of Broome off Eighty Mile Beach. From the deeper water location north of Broome, a total of 303 broodstock oysters were also collected. After two unsuccessful spawning trials onboard (see Table 1, trial numbers 1 and 2), all the broodstock oysters were placed in panel nets on bottom longlines with approximately 350 large oysters which had been left undisturbed on the Roebuck Bay holding site from the previous year's (1988/89) spawning trials.

In addition to the onboard trials, 25 spawning trials were conducted in the Broome hatchery between the 20th of October and the 15th of December 1989. Ten of these trials were considered successful with one or both sexes releasing gametes (Table 1). Broodstock from all three collection sites were induced to spawn.

On the basis of these and previous successful spawnings (Rose *et al.*, 1990) the effectiveness of induction techniques could be ranked in decreasing order, as follows:

(i) temperature manipulation in combination with gamete suspensions and ultraviolet (UV) irradiated seawater, (ii) temperature manipulation combined with gamete suspensions, (iii) temperature manipulation combined with UV irradiated seawater, (iv) temperature manipulation, (v) gamete suspensions, (vi) UV irradiated seawater, (vii) cleaning/disturbance and (viii) chemical stimuli.

Most of the successful spawnings during the 1989/90 programme occurred in November (Table 1). As in previous years *P. Maxima* were able to be spawned during the November\December period when the ambient seawater temperature was increasing rapidly from annual minimums during July/August (Figure 3). This confirmed previous observations that hatchery spawnings were most often achieved during the spring early summer period. It is therefore suggested that the best months for attempting spawning (ranked in decreasing order) are as follows: November, December, October=March, February, January and April (Rose *et al.*, 1990).

This study also confirmed that the same oysters spawned more than once e.g. Table 1, trial numbers 12 and 13; 26 and 27. Although it was sometimes difficult to ascertain the number of spawning females in each successful trial (i.e. released gametes clouded the water), the total number of eggs produced ranged between 1.6×10^6 and 4.1×10^6. The larvae

produced from these successful spawnings were kept alive without feeding for up to five days reaching the straight hinged veliger developmental stage and a shell length of approximately 90 μm.

4. DISCUSSION AND RECOMMENDATIONS

In bivalve breeding programmes for such temperate species as edible oysters and scallops, the primary objective is to produce large numbers (e.g. millions) of spat whereas the pearling industry has an underlying concern to prevent the overproduction of high quality pearls. At present the Western Australian pearling industry only requires approximately 0.5 million culture oysters each year. For this reason artificial propagation of *P. Maxima* outside the main breeding period i.e. September/October to March/April, (Rose *et al.*, 1990), is neither necessary nor cost effective given the difficulty involved with conditioning oysters to induce spawning outside this period. Effective broodstock management should be concerned with maximising larval survival per spawning, (Lannan *et al.*, 1980). Therefore spawning trials are recommended for the successful artificial propagation of *P. maxima* during the peak breeding period of November/December. From the mariculture perspective of being able to on-grow the resulting spat to commercially usable sizes (approximately 120 mm dorso-ventral length) within the shortest time period

(approximately 19 months), hatchery production, if possible, should be timed to occur even earlier at the beginning of the breeding season in September/October. This earlier timing would enable the spat to be on-grown over the maximum number of summer months (their growth rate is more rapid during months of warmer water temperatures - cf. final report FIRDTF Project 89/60).

To fulfil the requirements of the industry only a small number of female broodstock are required as the eggs produced during all of our spawning trials over a six year period ranged from 0.5 - 12×10^6 ova released per female (Rose *et al.*, 1990).

Using a scallop species with a slightly higher average fecundity (7×10^6 eggs per spawning), Cropp and Frankish (1989) projected it would require a minimum of only 30 broodstock to produce 100 million fertilised eggs per spawning in order to have 15 million "eyed" larvae remaining at settlement (assuming a generous estimate of mortality of 85%). This number of "eyed" larvae would be required to produce their target of 1 million spat on-grown to 10-15 mm shell height. Spat of this size can be easily on-grown to adults with only very small losses due to mortality. By using similar projections, the Western Australian pearling industry's annual requirement of oysters to culture pearls could easily be met with an equally small number of ripe

broodstock. Therefore only several hundred broodstock would need to be maintained in reserve to ensure spawning success rather than the several thousand recommended previously (cf. final report, FIRTA project 87/82).

As broodstock from all three locations were induced to spawn the collection site is now considered less important as a factor in spawning success than previously suggested (cf. final report, FIRTA project 87/82).

Given our present understanding of the breeding season of *P. maxima*, it is recommended that broodstock be collected for spawning trials by September at the latest each season. When monitored for their gonadal development, broodstock were seen to require a minimum of 5 weeks to mature from indeterminate/early developmental stages (stage 0/1) to the spawning ripe stage (stage 3) regardless of sex (Rose *et al.*, 1990). During gonadal development, disturbance should be minimised as females rarely developed beyond the near ripe stage (stage 2) under the intensive husbandry conditions on farm leases. Oysters to be used as broodstock during the summer breeding season therefore should be cleaned of fouling organisms during the preceeding winter months when their gonad development is minimal (Rose *et al.*, 1990). It is recommended that the oysters then be left undisturbed on the holding site as their gonads ripen as the seawater temperature rises during spring (see Figure 3).

Past experience has shown that attempts to condition unripe oysters to spawn outside the main breeding season (i.e. April to September) are unlikely to be successful. During the peak spawning period, however, oysters can be induced to spawn almost routinely using any of the main induction methods. The suggested induction procedure is therefore temperature manipulation enhanced with gamete suspensions and UV irradiated water none of which harm the oysters and allow for multiple spawning.

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TABLE 1

TRIAL NUMBER	DATE	TOTAL NUMBER OF BROODSTOCK IN TRIAL	BROODSTOCK COLLECTION SITE	INDUCTION METHOD USED	SUCCESSFUL SPAWNING	NUMBER OF MALES SPAWNED	NUMBER OF FEMALES SPAWNED	NUMBER OF EGGS PRODUCED
1	11:10:89	24	EMB[1]	T[a]				
2	12:10:89	24	EMB	T				
3	20:10:89	40	RB[2]	T				
4	30:10:89	32	GP[3]	T				
5	1:11:89	21	EMB	T	X	1	0	
6	2:11:89	21	EMB	T				
7	3:11:89	23	EMB	I[b]	X	4	>3	1.6×10^6
8	11:11:89	12	GP	Stripped Gonads				
9	11:11:89	22	GP	Stripped Gonads	X	1	1	4.0×10^6
10	13:11:89	32	GP	UV[c] + T	X	8	3	3.0×10^6
11	14:11:89	12	GP	UV + T				
12	14:11:89	17	RB	UV + T	X	3	1	3.2×10^6
13	16:11:89	20	GP & RB	GS[d] + T	X	8	1	3.7×10^6
14	17:11:89	12	GP	C[e] + T	X	1		
15	18:11:89	18	GP	UV				
16	20:11:89	18	GP	C + T + UV				
17	23:11:89	6	GP	GS + T				
18	24:11:89	24	GP & RB	GS + T				
19	28:11:89	16	GP	T				
20	29:11:89	16	GP	T				
21	30:11:89	13	GP & RB	I	X	3	3	4.1×10^6
22	7:12:89	18	GP	T				
23	11:12:89	22	RB	T				
24	12:12:89	37	RB	GS + T				
25	13:12:89	39	GP & RB	UV + T				
26	14:12:89	62	GP & RB	GS + UV + T	X	?[f] (TOTAL=7)	?	2.6×10^6
27	15:12:89	62	GP & RB	GS + UV + T	X	3	0	

[1] EMB = Eighty Mile Beach
[2] RB = Roebuck Bay
[3] GP = Gantheaume Point

[a] T = Temperature manipulation
[b] I = Immediate spawning after cleaning disturbance
[c] UV = UV irradiated water
[d] GS = Gamete suspension
[e] C = Chemical (e.g. Serotonin)
[f] ? = Sex undetermined as gametes clouded water

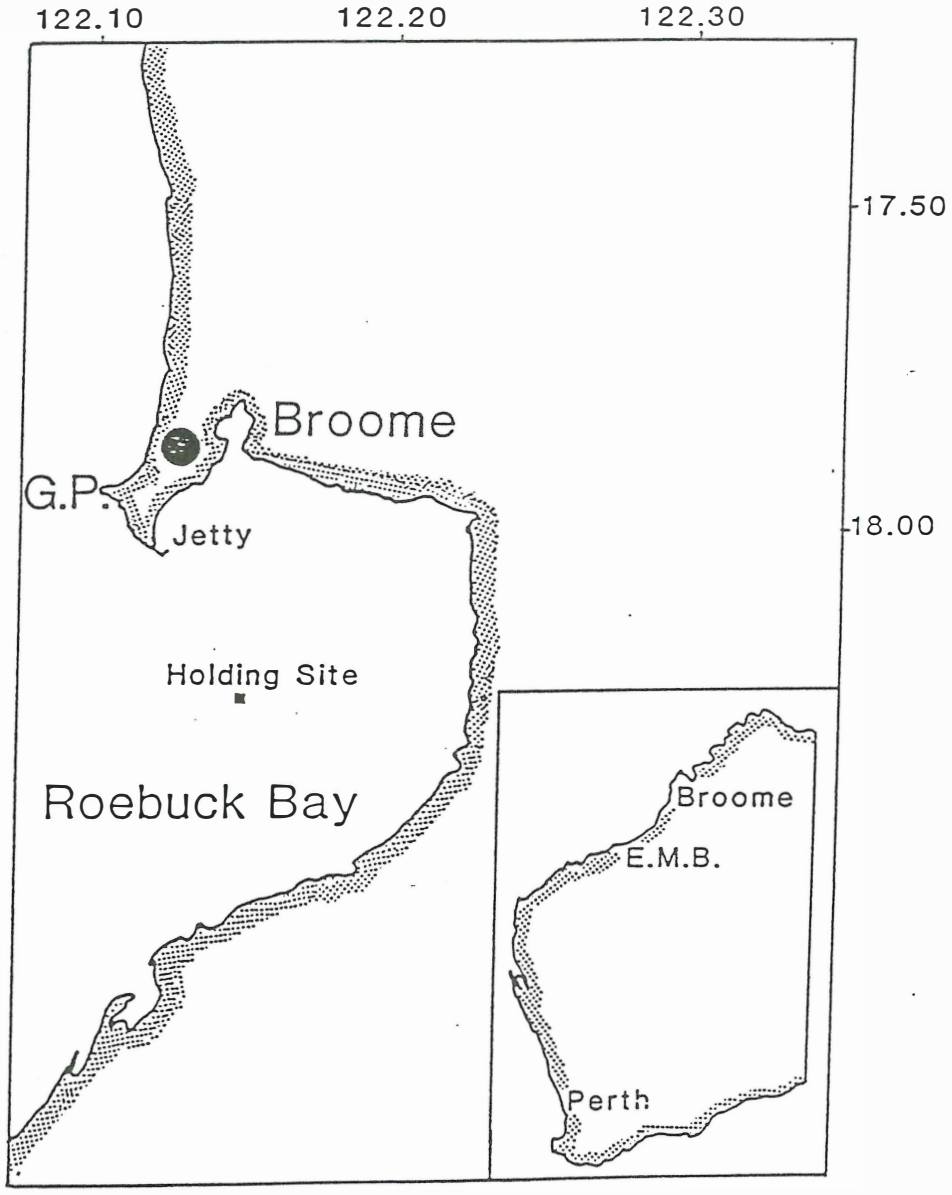

Figure 1. Location diagram of the Broome area in the Northwest of Western Australia showing hatchery jetty and pearl oyster holding site referred to in the text. Abbreviated locations are as follows: (G.P.) Gantheaume Point and (E.M.B.) Eighty Mile Beach.

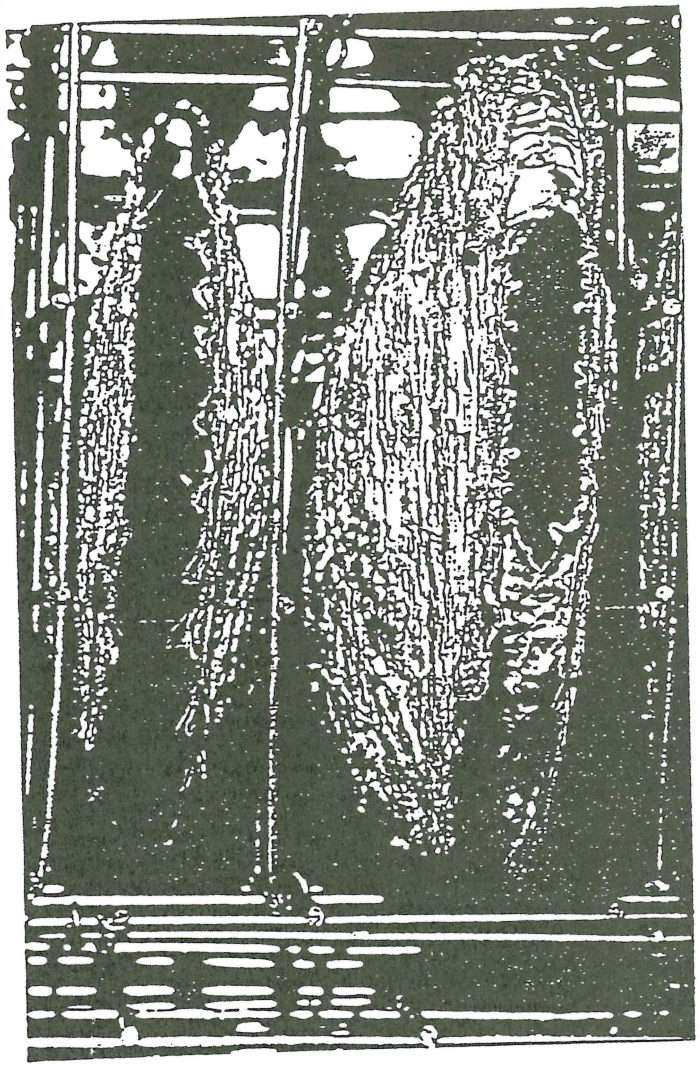

Figure 2. Large pearl oysters held in metal baskets within the broodstock tank during a spawning trial.

Figure 3. The annual breeding season of Pinctada maxima derived from six years of recorded field and hatchery spawnings (horizontal bars - the solid portion signifies the major spawning period). The surface sea-water temperatures (°C) (o--o) at the Broome Jetty, is plotted also for the period April 1986 to April 1989. Cyclical temperature curve plotted was determined by the following equation: °C = 26.95 + 2.46 sin θ + 4.09 cos θ - 0.78 sin (2θ) - 0.96 + cos (2θ) when θ = 2 (day of year)/365, r^2 = 0.93 and n = 150. This figure is adapted from Figure 10 in Rose et al. (1990).