F82/249

No 19.82./.25......

- [ ] NEW PROPOSAL
- [ ] CONTINUING PROJECT
- [x] FINAL REPORT
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FISHING INDUSTRY RESEARCH TRUST ACCOUNT

TITLE OF PROPOSAL/PROJECT: FEASIBILITY STUDIES ON THE ARTIFICIAL PROPAGATION OF PEARL OYSTER SEED

ORGANISATION: WA DEPT OF FISHERIES

PERSON(S) RESPONSIBLE: DR D.A. HANCOCK

FUNDS SOUGHT/GRANTED

YEAR	SOUGHT	GRANTED
1982/83		$58,264
1983/84		$103,087
1984/85		$103,818

RELATED APPLICATIONS:

RECEIVED .31./..1...1986

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Secretary
Fishing Industry Research Council

FEASIBILITY STUDIES ON THE ARTIFICIAL PROPAGATION OF PEARL OYSTER SEED (FIRTA 82/25)

FINAL RESEARCH REPORT

Funded by the Commonwealth Fishing Industry Research Trust Account, this project began in July 1982 and ended 30 June, 1985. The objective of this three-year project was to examine the feasibility of artificially propagating the tropical pearl oyster, *Pinctada maxima* by transferring existing hatchery techniques used in the production of mollusc seed of temperate species such as edible oysters and scallops. Reports on this project appeared during 1985 in "Australian Fisheries" 44(7): 34-36 and "FINS" 18(2):3-6 (please see attached photocopies).

This research was undertaken by the W.A. Fisheries Department in response to predicted long-term requirements of the Western Australian pearling industry, resulting from high rates of pearl oyster mortality at leases, dwindling stocks and escalating costs of collecting natural oysters.

While maximum fishing quotas have been imposed in recent years to prevent over-exploitation of remaining stocks, the industry has emphasised at its annual meetings that not all companies might be able to meet their quotas (Dybdahl and Rose, in press). Moreover, the limited supply of oysters was preventing any expansion in the number of pearl culture farms. A practical solution to this shortage was therefore to supply hatchery-produced spat for nursery and/or on-growing culture at individual farm sites.

Since 1980 the artificial propagation of *P. maxima* has been achieved by several Japanese pearling companies (e.g. Kakuda Australian Pearl Co. in Queensland; Horiguchi Pearl company in both Borneo, Indonesia, and Amami-Oshima, Japan). However, only since 1982/83 has *P. maxima* been cultured through to a suitable size for pearl cultivation by Horiguchi Pearl Company (T. Fuji pers. comm.). As the techniques of these companies are confidential, the W.A. Fisheries Department independently attempted to rear *P. maxima* larvae at its experimental hatchery in Perth.

Construction of an experimental hatchery for both tropical and temperate molluscs at Waterman Laboratories was completed toward the end of October 1983. The hatchery consists of three subsystems: a broodstock and spawning unit; a larval and spat unit; and an algal production unit. The broodstock and spawning unit is capable of maintaining up to 100 pearl oysters at any one time and of processing up to 80 broodstock during a single spawning session. Larvae produced can be grown in either a single 4500 L fibreglass tank or in several smaller fibreglass or PVC plastic tanks (200, 400, 500 L). Spat can be reared in either a raceway or several different types of upwellers. The techniques used for rearing the larvae and spat are based on those developed for scallops by Rose and Dix (1984) and Rose, *et al*. (in prep.). The algal unit has the capacity to grow up to 2000 L of unicellular microalgae. Currently seven species of microalgae (two diatoms and five flagellates) are grown as a food source for broodstock, larvae and spat. The axenic culture techniques used for microalgae production were presented at the annual Australian Society for Microbiology symposium at the University of Western Australia on 15 May 1985 (Rose, et al., 1985).

The lack of successful spawning trials in the past has been partly due to the inability to obtain sufficient ripe oysters and to consistently spawn those few which were ripe. Chemicals used to induce spawning in other bivalves, such as serotonin and hydrogen peroxide, were found to be ineffective both in the field and in the hatchery. Preliminary experiments to fatten broodstock using both natural and artificial foods have also been ineffective.

During the 1984-85 breeding season, however, broodstock from deepwater fishing grounds were observed to spawn regularly in their holding tanks, while being transported back to Broome. In the laboratory, these oysters were induced to spawn using slowly circulated, aerated filtered seawater irradiated with ultraviolet light and gradually raised to 3°C above ambient (29-30°C). Spawning appeared to be enhanced if suspensions of sperm or egg were added to the spawning tanks and if the oysters were submerged at least 0.3 m below the surface.

Over the last two breeding seasons (1983-84 and 1984-85) only four spawnings have been achieved out of 36 trials, involving more than 865 oysters. The results of these four spawnings are summarized in Table 1. Except for the December 1984 spawning, none of them produced sufficient quantities of viable larvae (straight-hinged veligers) required for large-scale culture. The spawning in January 1985 was the most successful as it produced rapidly growing larvae which reached the most advanced developmental stage (pediveliger) before failing to settle after 28 days (Figure 1). Because of the low numbers of larvae produced during this spawning, routine culling of small, slower-developing individuals from the population was not possible. Failure to do this appeared to have prevented the larger, faster developers from metamorphosing and settling out of the water column. Work done on culturing larvae of edible oysters and scallops in Tasmania and New Zealand has shown that culling slower growing individuals from the population is necessary for successful settlement.

According to Japanese researchers, P. maxima larvae settle three weeks after fertilization and range from 233 to 281 μm in shell length. Although larvae produced in January 1985 did not settle, many of the faster growing individuals at this time were greater than 400 μm in shell length (Figure 1). This indicated that the feeding regime provided was more than adequate.

Thus our most significant obstacle to rearing the larvae on a pilot scale has been the lack of experience that can be gained only from repeated trials in which large numbers of viable larvae are produced from ripe healthy oysters. Stockpiling deepwater broodstock onto leases located near Broome and the spawning techniques developed have helped to ensure that a greater number of spawnings can be achieved. However, the logistics of supplying large numbers of ripe oysters still remains a problem as experience has shown that up to 70% of the broodstock held for some time on the leases and then airfreighted to Perth will be unsuitable for spawning. A small duplicate experimental hatchery and nursery is now being set up in Broome in an attempt to overcome this problem (FIRTA 85/58).

Detailed analysis of the gametogenic cycle of P. maxima from monthly samples is still in progress but preliminary histological findings and field observations suggest that breeding occurs from September to March.

P. maxima is a protandrous hermaphrodite which changes sex according to environmental conditions, such as food. Maturity is usually reached when the dorso-ventral (DV) shell length is approximately 120 mm. The sex ratio of wild stock approaches 1 to 1 when their DV shell length is 200 mm or greater, i.e. when they weigh 1 kg or more. Out of 224 deepwater oysters (170 to 234 mm DV shell length) used as broodstock in 1984-85, the sex ratio was: 47% female and 53% male.

Prior to the 1984-85 breeding season, gonad sampling was insufficient during the non-fishing months of the year (December 1984 to March 1985). This problem was solved by collecting gonads over these months from surplus broodstock stockpiled on to farms near Broome. Preliminary analysis of these gonads does not alter earlier findings that Western Australian pearl oysters breed from September to March. Unlike Queensland oysters which may have two peaks during their breeding season (October-November and February-March), the presence of one or more peaks has not been detected from gonad samples analysed to date.

The development of nursery and on-growing culture techniques has followed similar strategies used for other commercially farmed bivalves. Modifications that have occurred have taken account of the intense fouling and strong tides which typify tropical W.A. waters. Research on developing technology for on-growing spat (e.g. upwelling tanks and raceways) has been hampered by the lack of hatchery-produced spat from our spawning trials. Wild juveniles collected from shells of adults fished by the Industry were substituted to test a variety of cages which had been constructed for on-growing juveniles 15 mm in length or greater. In May 1983, bottom trays like those used for edible oyster cultivation were tested with tagged juveniles. Over a four month period the oysters grew only 2 cm or less and had a 47 percent survival rate. The poor results were attributable to an excessive amount of silt accumulating inside the trays, which retarded growth, and to the accidental deaths of a number of spat due to rough weather overturning the trays. These preliminary findings suggest that modified edible oyster trays raised off the sea floor are practical provided they are lined with the appropriate size plastic mesh to prevent silt from building up inside the tray. A more detailed appraisal of these types of systems is needed before the feasibility of on-growing pearl oysters can be demonstrated.

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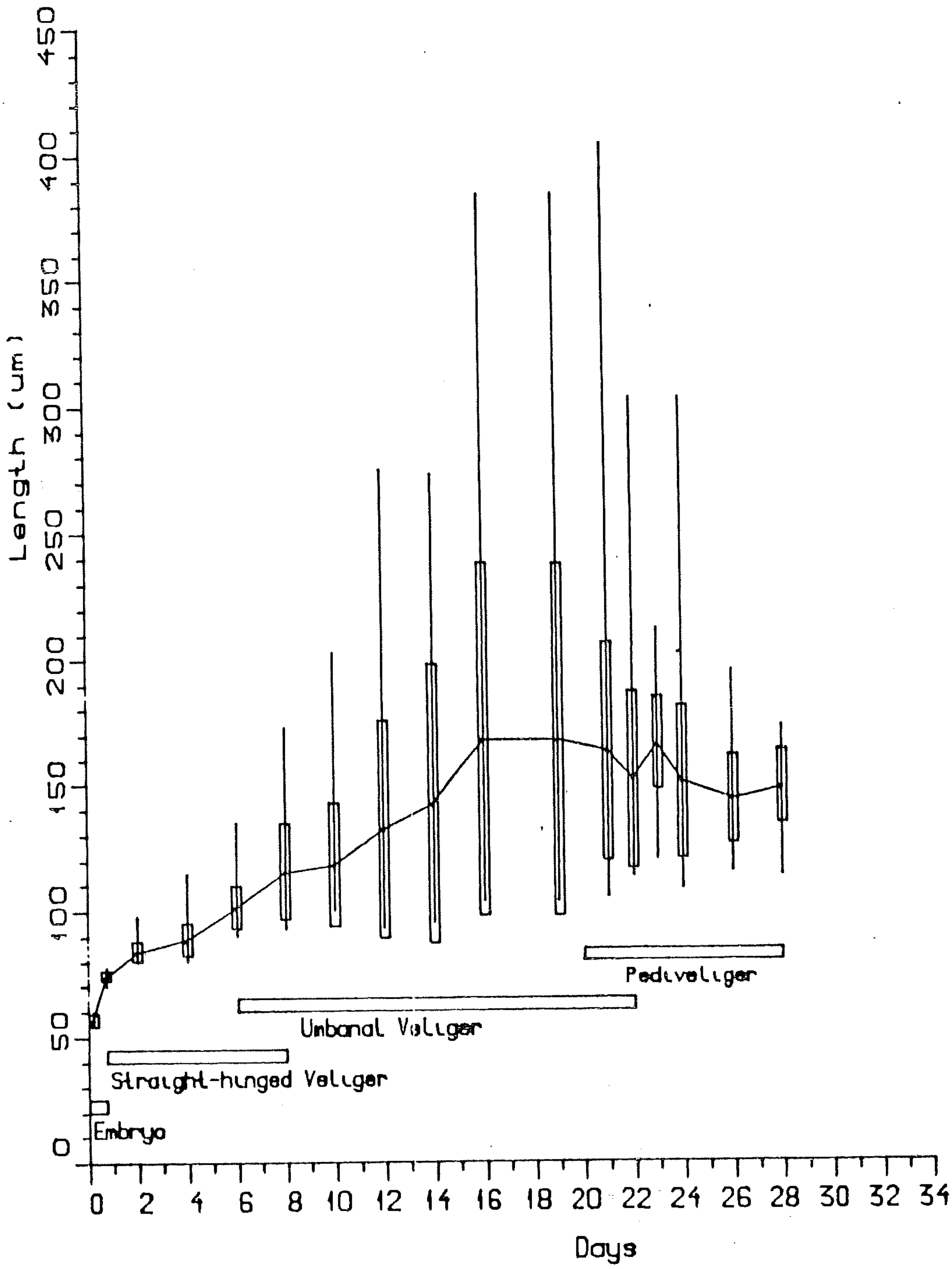
Rose, R.A., Campbell, G.R. and Sanders, S. The Planktotropic larval development and newly settled spat of the Saucer Scallop, Amusium balloti (Bernardi) (Mollusca:Pectinidae) (in prep.).

Rose, R.A., Sanders, S.G. and Mannion, M. 1985. Phytoplankton production for mariculture. Aust. Microbiologist 6(2): 145.

TABLE 1. SUMMARY OF SUCCESSFUL SPAWNINGS OF *PINCTADA MAXIMA* CARRIED OUT AT W.A.M.R.L. INVOLVING MORE THAN 865 BROODSTOCK.

SPAWNING DATE	NUMBER OF STRAIGHT-HINGED VELIGERS PRODUCED ($x10^6$)	DEVELOPMENTAL STAGE AND MEAN SIZE REACHED (µm)	COMMENTS
APR. 1984	2.75	Straight-hinged veliger, 100	Non-viable gametes, low number of zygotes produced, high % abnormal
DEC. 1984	24.0	Early umbonal, veliger, 100	Mortality due to vibrio-contaminated larval food.
JAN. 1985	3.63	Pediveliger, 165	Poor spawning but 4% of the larvae reached 405 µm in shell length. Settlement was unsuccessful after 28 days.
MAR. 1985	0.27	Straight-hinged veliger, 84	As above for April 1984 spawning

FIGURE 1. GROWTH RATE OF *PINCTADA MAXIMA* EMBRYOS AND LARVAE SPAWNED IN JANUARY 1985 (TABLE 1) AND REARED AT 29-30°C. GREATEST DIAMETER OF EMBRYO AND SHELL LENGTH OF LARVA ARE EACH PLOTTED AS MEAN, STANDARD DEVIATION (RECTANGLES) AND RANGE. EACH POINT IS DERIVED FROM 30 TO 50 MEASUREMENTS.



Promising results from pearl oyster aquaculture

RESEARCHERS at the Western Australian Marine Research Laboratories recently overcame a major obstacle in their efforts to culture the tropical pearl oyster, *Pinctada maxima*.

Although the project began in July 1982, early efforts to successfully induce spawning and subsequent settlement of the larvae were hampered by insufficient knowledge of the species' reproductive biology.

However, last summer (1984-85) spawning techniques were developed and three spawnings were achieved using oysters collected from deepwater fishing grounds southwest of Broome. Latest report from the research team, consisting of Ms Serena Sanders, Mr Rand Dybdahl and Dr Bob Rose, was that the third batch of larvae reached the pediveliger stage (ranging from 165 to 405 µm in shell length).

Although settlement, which normally occurs three weeks after fertilisation, was not achieved on this occasion, the experience provided vital information on the feeding requirements of larvae which will be a valuable benefit for the ongoing program.

Early research

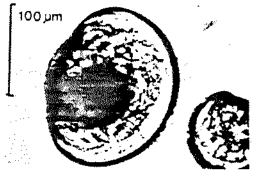
The study into the artificial propagation of the pearl oyster was undertaken by the Western Australian Department of Fisheries in response to predicted long-term requirements of the State's pearling industry, currently valued at about $24 million per annum. The predictions took into account dwindling stocks, high

by Cliff Young, assistant editor*

rates of mortality at oyster leases, and escalating costs of collecting natural oysters.

The Commonwealth Fishing Industry Research Trust Account had agreed to fund the project, and two main objectives were defined.

The immediate aim of the project was to investigate the feasibility of breeding pearl oysters artificially by modifying existing aquaculture techniques



Umbonal larva 10 days old.

developed for edible oysters and scallops.

The second, longer-term aim was to develop the technology required to supply commercial pearl leases with hatchery-produced oysters which were disease resistant.

The department considered that achieving these aims would help lessen the fishing pressure on natural populations and conserve the remaining stocks.

The project was subsequently divided into three phases, two of which have been completed. The first involved training personnel and developing hatchery facilities needed to culture tropical bivalves. To save on costs and time this phase was carried out at existing hatchery facilities in Taroona, Tasmania, under Dr Trevor Dix of the Tasmanian Fisheries Development Authority.

The second phase involved setting up a pilot scale hatchery, at the Department's Marine Research Laboratories at Waterman, to produce pearl oyster spat to be grown on pearl leases near Broome.

The third phase will be to optimise growth, survival and stocking densities of oyster spat placed on different pearl leases.

Hatchery

The tropical mollusc hatchery constructed at the Western Australian Marine Research Laboratories consists of three subsystems:

- a broodstock and spawning unit;
- a larval and spat unit; and
- an algal production unit.

The broodstock and spawning unit is capable of maintaining up to 160 pearl oyster broodstock at any one time, and of processing up to 80 broodstock during a single spawning session.

Larvae produced can be grown in either a single 4500-litre fibreglass tank or in several smaller fibreglass or PVC plastic tanks of between 200 and 500-litre capacity.

Spat can be reared in either a raceway or several different types of upwellers.

The algal unit has the capacity to grow up to 2300 l of unicellular microalgae. Maximum quantities of algae that can be harvested per day vary between 100 and 150 l, depending on the cell concentration required, and on whether the algae are cultured in batches or semi-continuously.

At present, seven species of unicellular microalgae (five flagellates and two diatoms) are grown as food for broodstock, larvae and spat.

Spawning techniques

To date about 40 spawning experiments involving more than 865 pearl oysters have been com-

Algal production unit showing 500-litre bag cultures (left) and starter (250-500 ml), 5 l and 20 l cultures in background.

The smaller, younger oyster (left) is used solely for cultivating pearls. The larger, older oyster (right) is used as broodstock. In the past, large oysters were exploited for their MOP (mother of pearl).

*This article was prepared from information supplied by Mr Rand Dybdahl, Dr Bob Rose and Ms Serena Sanders of the Western Australian Marine Research Laboratories, PO Box 20, North Beach, WA 6020.

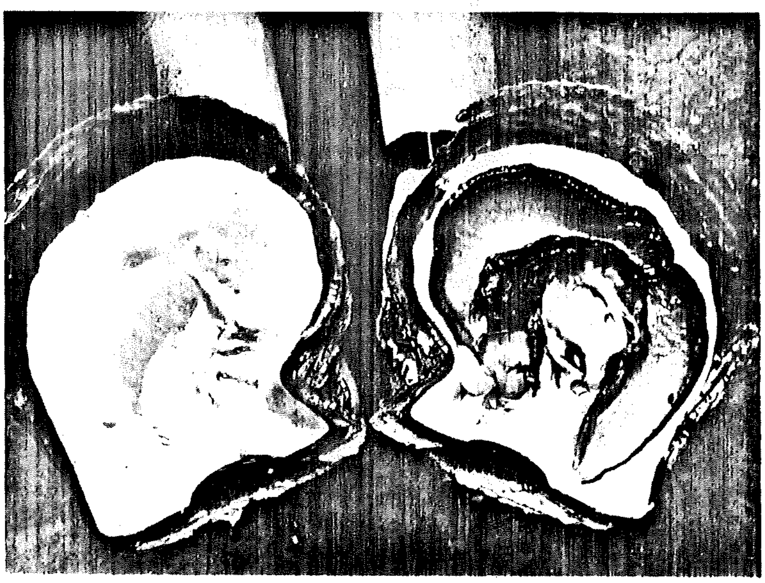
Pearl oyster broodstock which are being conditioned for spawning are held in baskets placed in a raceway.

pleted over the past two breeding seasons (1983-84 and 1984-85).

Latter experiments have utilised large amounts of heated, circulating water instead of still, aerated water as used in the past.

Attempts to induce spawning have involved manipulating water temperatures, using seawater irradiated with ultra-violet light, and the use of two chemicals known to have been used with success in past experiments with bivalves.

The chemicals are hydrogen peroxide buffered with TRIS (trishydroxymethylaminomethane), and serotonin, a neural transmitter. The former stimulates hormones responsible for expelling gametes from the gonads, and the latter is a vasoconstrictor, stimulating ciliary movement and the heart.



Deepwater pearl oyster (approximately 2.5 kg wt) with valves separated, showing viscera attached to right valve.

Unfortunately, both chemicals have proved inconclusive in inducing spawning in the pearl oyster, although part of the problem could be related to the broodstock being insufficiently ripe.

Obtaining ripe broodstock has been the major problem encountered by the research team. This, coupled with the long distances over which the oysters have to be flown (from the fishing grounds off Broome to the research laboratories in Perth is 2274 km), has made the team consider setting up a pilot hatchery at Broome.

The Fisheries Department is planning to build a field station at Broome before the end of this year.

Tidal-powered upwellers

One of the possible nursery-culture systems under consideration for use at Broome is a modified version of a tidal-powered floating upweller system, or 'flupsy', used for bivalves in England and Ireland.

By utilising the tides at Broome, a flupsy would eliminate the need for costly pump-driven circulation systems and would also save on maintenance.

Two modified flupsys have already been built at the Western Australian Marine Research Laboratories and these will be tested at Broome in the near future.

If successful, a larger aluminium upweller raft has been designed as a more permanent fixture.

The larger version would be 2.4 metres long and 1.8 metres wide and could be walked on to facilitate maintenance and tray-changing operations.

Pilot experiments using wild spat for ongrowing have already been carried out off Broome using more conventional oyster trays.

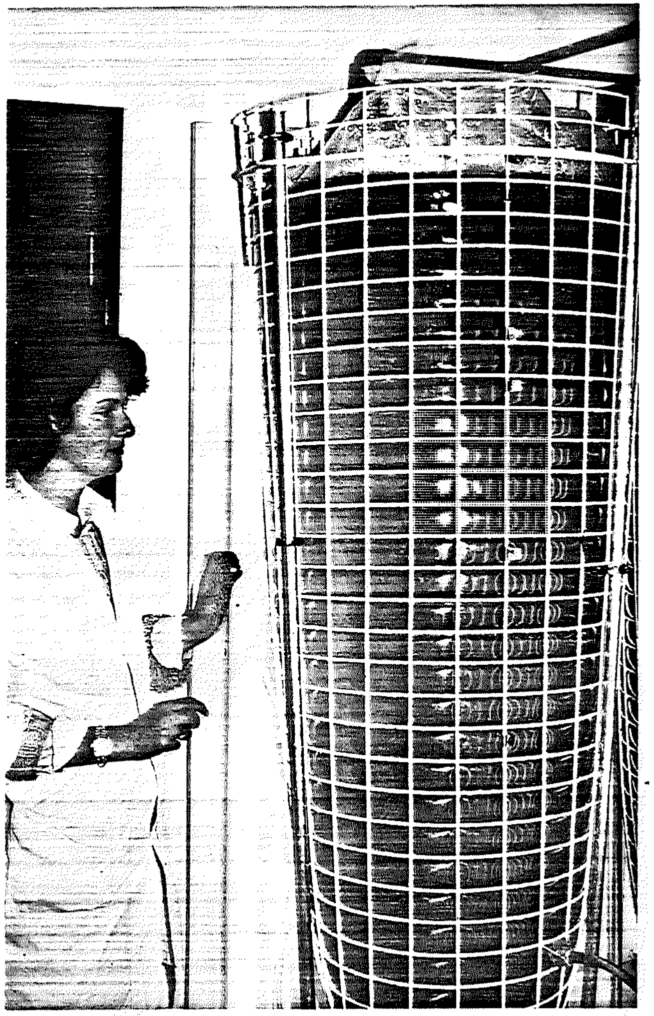
These experiments show that major problems to overcome will be excess silting, intense predation, fouling and damage from severe weather conditions, such as cyclones.

Department Develops Oyster Culture Techniques

Broome's high tidal range has proved to be a bonus for the Department of Fisheries and Wildlife's pearl oyster programme. A modified version of an Irish-designed tide-powered floating upwelling system ("Flupsy") will be used as a nursery to rear pearl oyster spat to their juvenile size stage. The following story was written from material and background information supplied by the Research Officer in Charge of the pearl oyster programme, Mr. Rand Dybdahl.

Studies of the artificial propagation of pearl oyster seed began in July 1982 to examine the feasibility of artificially propagating the tropical pearl oysters *(Pinctada maxima)* on a commercial scale by modifying existing techniques applied to edible oysters and scallops. The research was undertaken in response to predicted long-term requirements of the Western Australian pearling industry resulting from dwindling stocks, high mortality rates at leases and escalating costs of collecting natural oysters.

Construction of an experimental hatchery for both tropical and temperate molluscs at the Western Australian Marine Research Laboratories, Waterman, was completed towards the end of October 1983. The hatchery consists of 3 sub-systems: an algal production unit, a broodstock and spawning unit and a larval and spat unit. The algal unit has the capacity to grow up to 2300L of unicellular micro-algae. Currently 7 special of micro-algae (2 diatoms and 5 flagellates) are grown as a food source for broodstock larvae and spat. The broodstock and spawning unit is capable of maintaining up to 100 pearl oyster broodstock at any one time and of processing up to 80 broodstock during a single spawning session. Larvae produced can be grown in either a single 4500 L fibreglass tank or several small fibreglass or PVC plastic tanks (200, 400 and 500 L).

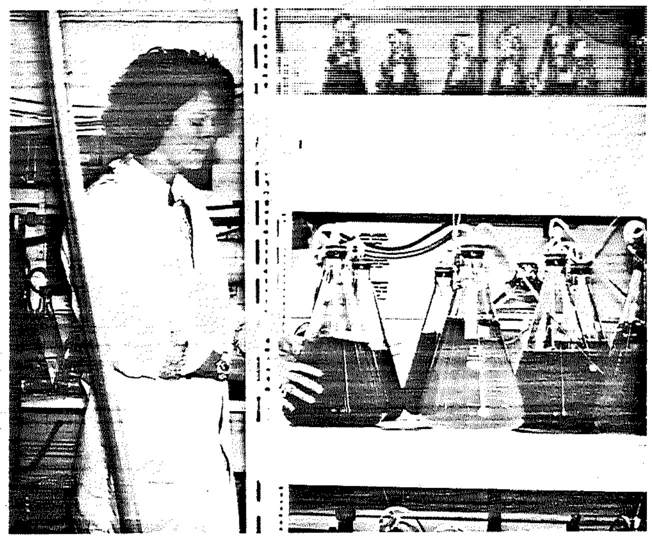


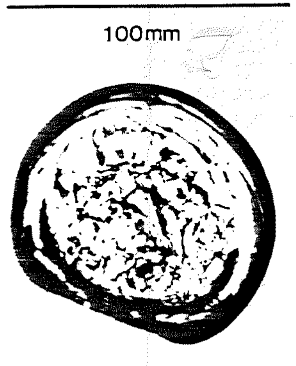
▲ Giant plastic bags are used to culture algae at Waterman to feed the oyster spat. (Photo P. Cogan)

Spat can be reared in either a raceway or several different types of upwellers.

The hatchery project was funded by the Commonwealth Fishing Industry Research Trust Account and the hatchery was developed by Dr. Bob Rose and operated by Mr. Dybdahl, and Miss Serena Sanders.

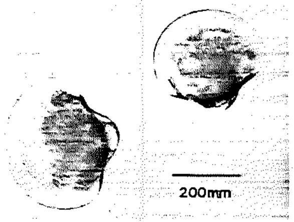
Indoor upwelling systems are used in Great Britain and elsewhere to grow newly settled spat (0.3 mm) of edible oysters in deep beds, through which water circulates, so they can be transferred to the sea. British hatcheries are currently producing millions of juvenile oysters a year which has revived interest in growing



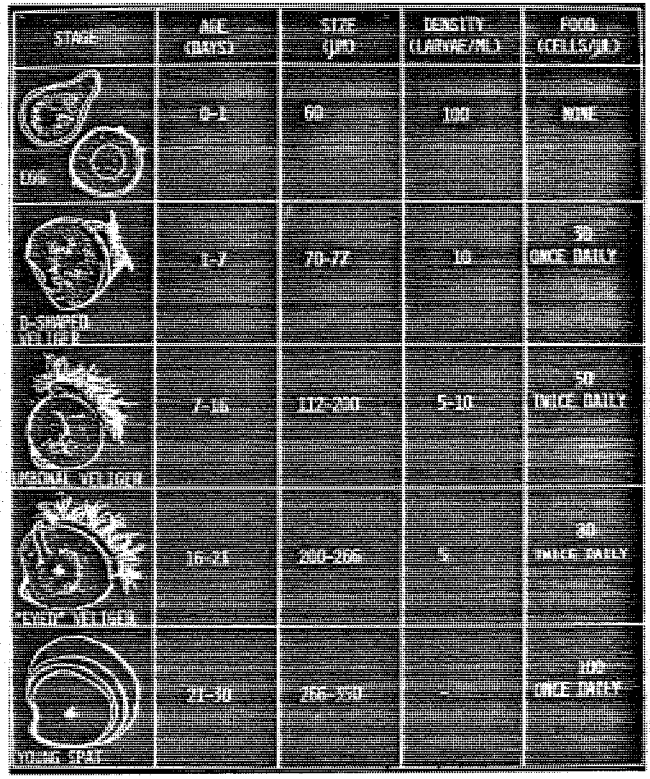


▲ 11 day old D-shaped veliger at early umbonal stage. (Photo B. Rose)

◄ Various preparations are made up to compare algae production rates. (Photo P. Cogan)



▲ Veligers of *Pinctada* sp. at late umbonal stage collected from plankton off Eighty Mile Beach (Photo B. Rose)



STAGE	AGE (DAYS)	SIZE (μm)	DENSITY (LARVAE/ML)	FOOD (CELLS/μL)
EGG	0-1	60	100	NONE
D-SHAPED VELIGER	1-7	70-72	10	30 ONCE DAILY
UMBONAL VELIGER	7-16	112-200	5-10	50 TWICE DAILY
EYED VELIGER	16-21	200-266	5	80 TWICE DAILY
YOUNG SPAT	21-30	266-350	-	100 ONCE DAILY

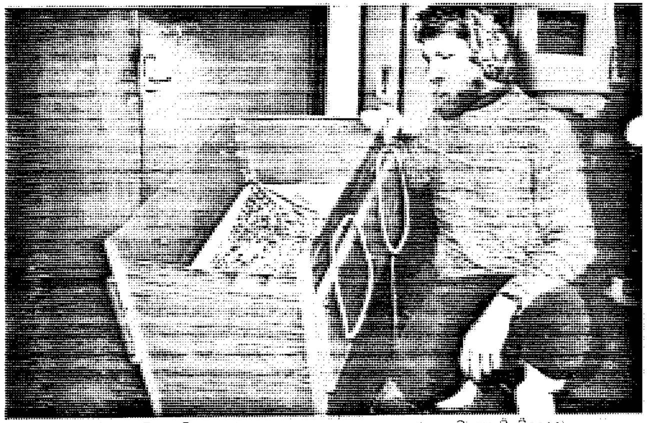
◄ Summary of Pearl larval stages, culture densities and feeding regimes. (Photo B. Rose) oysters. Mr Dybdahl read about the "Flupsy" which he believed could be used at Broome, where there is a very high tidal range. He obtained a plan and details from the Irish Aquaculture Association and one was built by workshop staff at Waterman so the system could be tested.

The tide-powered "Flupsy" was developed at Conwy in Wales for holding small hatchery reared oysters from 10 mg live weight, at any site where there is water deep enough to moor the system and a reasonable current flow of 50-100 cm/sec. The bivalves are kept near the water surface by flotation and the system is secured to the seabed by a single point mooring which allows it to swing into the tide. Water containing phytoplankton is thus forced up through the concentrated bivalve spat inside the upweller so they get an adequate food supply. A tide powered upweller eliminates the need for costly electric pumps to circulate the water and cuts down on maintenance.

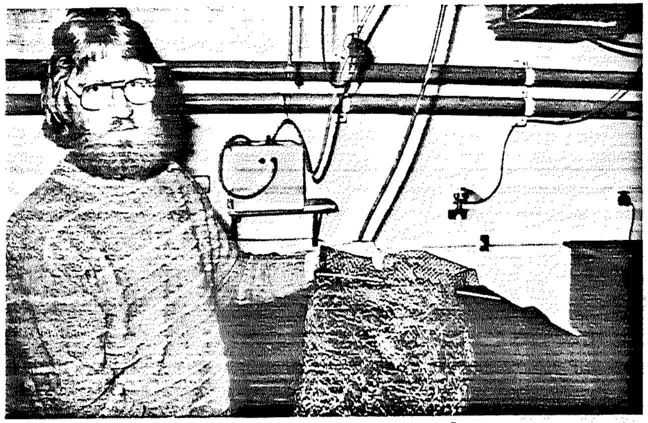
The system is accessible at all stages of the tide and experiments have shown that at low stocking densities it gives similar growth to that in shore trays nearby.

During tests in Wales, edible oysters of 6-20 mg live weight (3-5 mm shell diameter) were grown to 200-1000 mg live weight (10-20 mm shell diameter) depending on initial stocking density, after 3 to 4.5 months in the sea. Maximum stocking capacities of 9 kg to 16 kg (up to 6 g/cm^2 of water intake area) have been reached in the systems tested. Growth was better when the oysters were held in a stock of trays in the "Flupsy" rather than in a thick layer in a deep box but the later proved easier to manage.

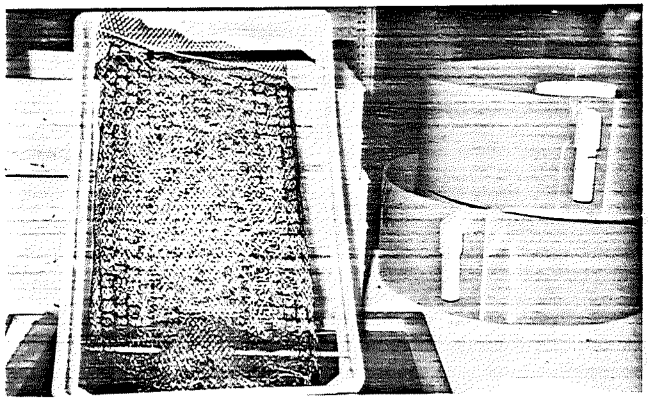
Plywood was used to build the first "Flupsys" in Wales in 1978 and 1979 and the trays and tops and bottoms were covered with either 1.5 mm mesh coated fibreglass or 6 mm mesh polyethylene netting, depending on the size of the oysters. The netting



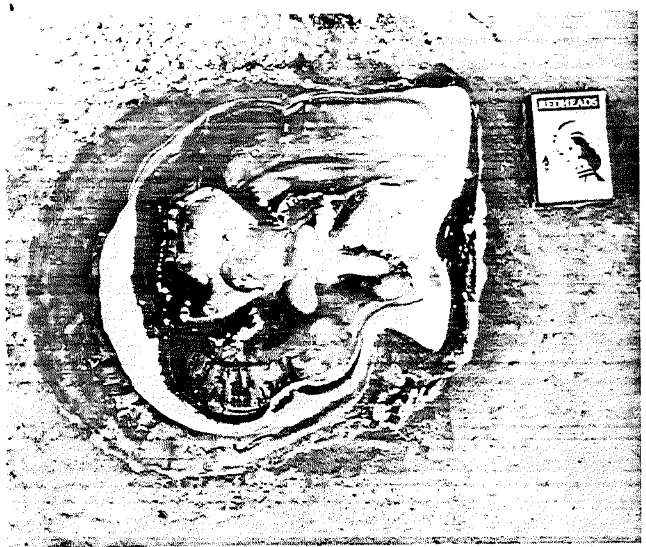
Research Officer Rand Dybdahl opens up one of the upwellers. (Photo P. Cogan)



Polyethylene mesh is used for oyster spat settlement. (Photo P. Cogan)

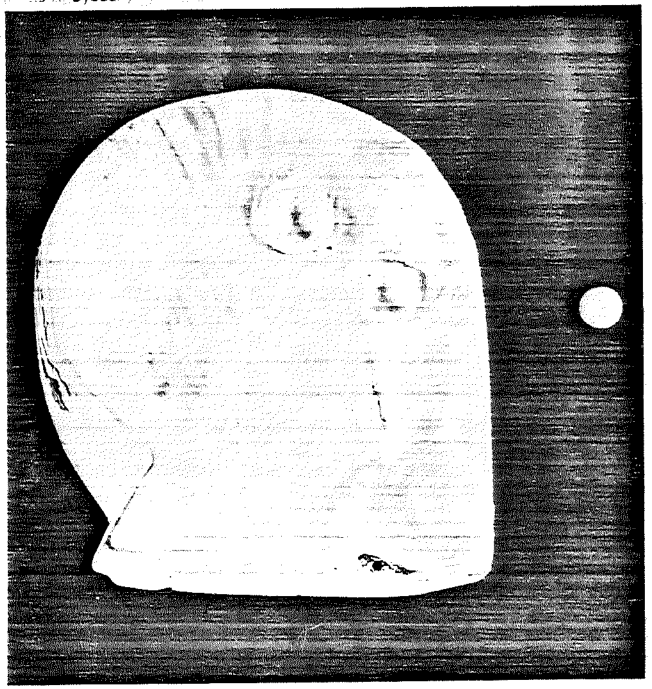


The mesh fits on trays inside the upwellers. (Photo P. Cogan)



A freshly opened Pearl Oyster. Note large size compared to the matchbox (Photo B. Rose)

Cultured half pearls have to be cut from the shell. Separate round pearls are much more valuable. (Photo R. Dybdahl)



was fixed to the frame with wooden battens and access to the interior of the trays was made by removing the top battens and mesh. The stack of "Flupsy" trays was secured within the compartment by rubber bands stretched across the top and attached by metal hooks to an eye bolt.

In 1980 the "Flupsy" was redesigned and made of stronger and lighter materials. The deflector plates were incorporated into a fibreglass base which was bolted on to an upper frame made from 2.5 cm angle iron. Flotation was provided by two expanded polystyrene filled 15 cm plastic pipes. They were each 1 m long. But they proved inadequate to keep it at the water surface at current speeds greater than 50 cm/sec and extra flotation was provided by attaching a 50 kg buoy.

When the plan and details of "Flupsy" arrived in W.A. they were adapted in the workshops at WAMRL to suit local conditions. The first one was moored near Waterman but it disappeared before any results could be obtained. Another was constructed and it will be field tested at Broome. A larger aluminium upweller has been designed and it will be big enough (2.4 m long and 1.8 m wide) to walk on to carry out maintenance and make tray changing easier.

The "Flupsy" is small enough to be transported easily to Broome, where spat, which are grown on to nylon mesh, will be placed in the "Flupsy" trays so they can be reared from 5 mm to about 30 mm. Spat taken from the hatchery cannot be placed in normal farm cages because of the danger of being eaten by a variety of predators.

F 82/249

extract from FIRC 27 minutes

FIRTA 82/25 <u>Studies on the Artificial Propagation of Pearl Oyster Seed</u>

FIRC supported continued funding of this project at $103,818 for 1984/85. The grantees comprised the only professional group in Australia with expertise in pearl oysters.

The progress report on this project was contained in the continuing application. FIRC accepted the report and noted that further work was needed. The report of the "Workshops on the Cause of Mortality and the Artificial Propagation of the Cultured Pearl Oyster" attached to the application was also noted.