A manual for the artificial propagation of the silverlip or goldlip pearl oyster, *Pinctada maxima,* (Jameson) from Western Australia

by

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Summary

The Western Australian Research Laboratories began investigating the feasibility of artificially propagating the silverlip or goldlip pearl oyster, *Pinctada maxima,* (Jameson) in 1982 and culminated its study by operating a pilot-scale, seasonal hatchery at the Broome Jetty, Broome, Western Australia from 1986 to 1989. This manual describes the hatchery facilities and husbandry techniques developed during this period for the spawning and culture of the larvae and newly settled spat.

key words: *Pinctada maxima*, site selection, artificial propagation, larvae, spat, hatchery and nursery facilities

Acknowledgements

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Mr B.K. Bowen, Director of Western Australian Fisheries, provided executive support for the project and Dr D.A. Hancock, former Chief Research Officer of the W.A. Marine Research Laboratories, was responsible for initiating this research.

Special thanks are also due to the W.A. Pearling Industry. This project would not have been possible without their assistance and cooperation in the collection of reproductive material and live pearl oysters for this study. The following companies, listed alphabetically, were particularly helpful: Arrow Pearling Company, Blue Seas Development, Broome Pearls Pty Ltd., Clipper Pearls, Cossack Pearls Pty Ltd, Cygnet Bay Pearls, Darella Holdings Pty Ltd, Maxima Pearling Company, Morgan and Company Pty Ltd, Paspaley Pearling Company Pty Ltd., Pearls Pty Ltd, Roebuck Pearl Producers Pty Ltd, and Willie Creek Pearling.

Mr C.R. Edwards, retired Senior Engineer of the Building Management Authority of Western Australia provided expertise in the planning, designing and construction of the Broome Research Hatchery.

Two previous maintenance engineers at the W.A. Marine Research Laboratories: Messrs E. Search and W. Gibson, were responsible for fabricating and maintaining much of the hatchery equipment. Mr. J. Gilbody helped during the construction phase of the Broome Research Hatchery. W.A. Fisheries Department biologists Messrs R. Dybdahl and M. Mannion and Mmes. S. Sanders and S. Harders, assisted throughout the course of this project. In particular, Mr. S. Baker made an outstanding contribution in helping to run the Broome hatchery.

A special thanks is extended to Dr T.G. Dix for his early involvement during the first FIRTA Project 82/25. During this time, he trained staff for this project and supervised construction of a temporary, warm water hatchery at the Tasmanian Fisheries Development Authority Research Laboratory, Taroona.

Finally, thank you to Ruth Leslie-Rose and the AppleCentre™ Perth, especially Carolyn Booth, for their assistance in the publication of this manual.

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1. Introduction

This manual provides practical information for the Western Australian Pearl Culture Industry on the husbandry or artificial propagation of the silverlip or goldlip pearl oyster, *Pinctada maxima,* (Jameson). The methods outlined should provide a useful guide for any future commercial scale production of hatchery seed. The most appropriate protocol adopted will depend on the particular production level and hatchery site. The equipment cited or described is not necessarily an endorsement of a particular product, material or brand.

This technical report is the first publicly available documentation on the successful propagation of *P. maxima* on a pilot-scale in Australia. Prior to 1986, the technology was available to only Japanese scientist and technicians. The manual contains a brief description of the Broome location and seawater quality at the hatchery, the hatchery facilities, the spawning methods, larval and post-larval culture, algal production, and early nursery production of spat.

The culture methods were derived from those previously published for other marine bivalves (e.g., edible oysters, clams, scallops and, in particular, the pearl oysters *Pinctada fucata* and *P. margaritifera*). These and other publications, related to selection of sites for aquaculture, algal culture and microbiology, are at the end of this manual.

2. Site Selection

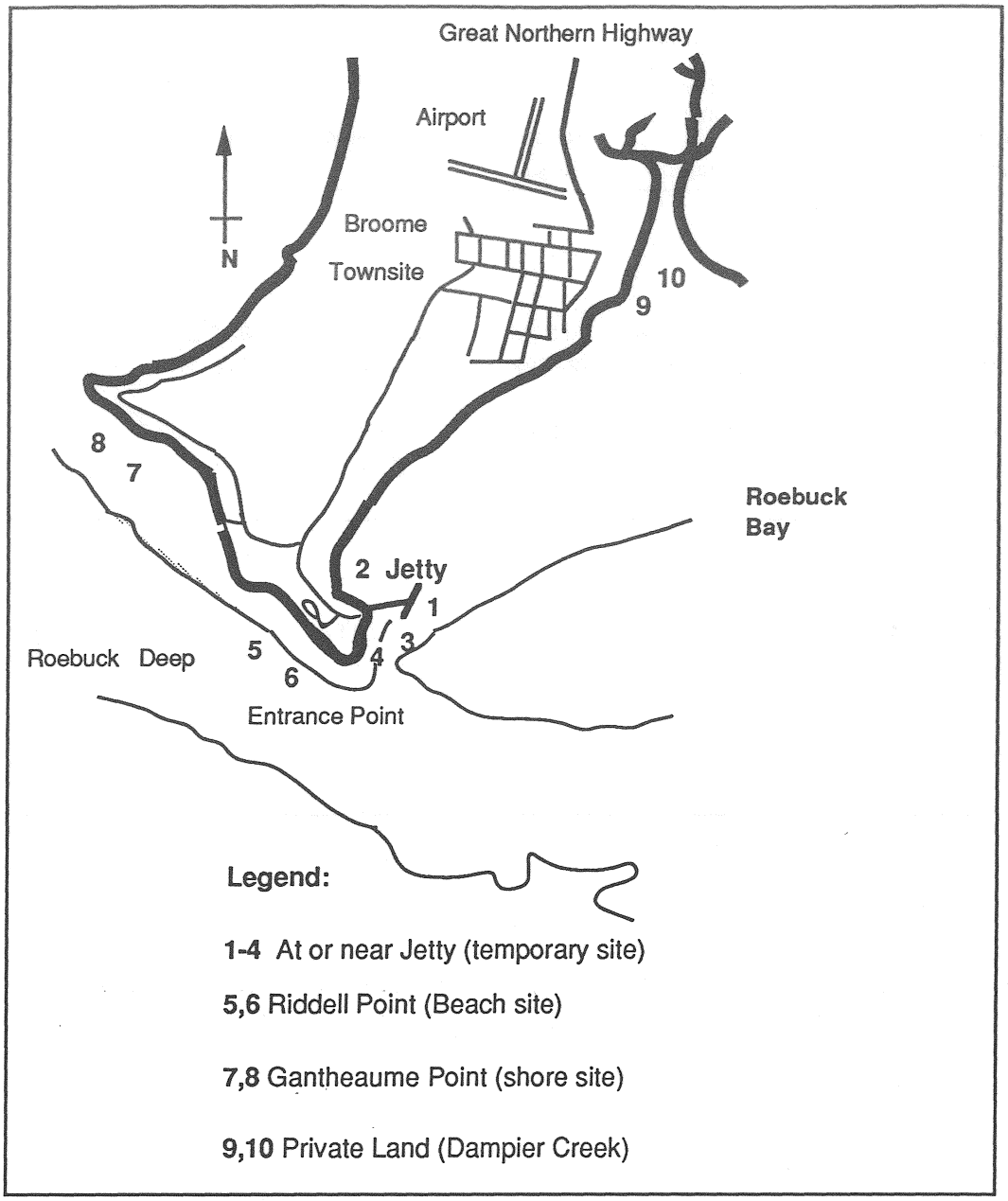
The location of a hatchery will require consultations with various governmental agencies, WA Pearl Producers Association and any private individuals or organizations with vested interest in the area selected. Information on the procedures for obtaining a licence and approval to develop an area for aquaculture can be obtained from the WA Fisheries Department's Interdepartmental Council (IDC) on aquaculture.

An on-site survey should consider road accessibility, availability of sea and freshwater resources, pollution sources (if they exist), and services and labour available to the site. According to New (1975), a one year survey should evaluate the following factors: 1) contour soundings and tidal range; 2) tidal stream velocity and direction; 3) seawater analysis during each season of the year at various depths for temperature, salinity, dissolved oxygen levels, chlorophyll content, particulate organics, dissolved organics, suspended solids, phosphates, silicates, nitrates, nitrites, and ammonia; 4) freshwater analysis (hardness, alkalinity, turbidity, nitrates, phosphates and minerals); 5) seawater beach extraction investigation; 6) plankton tows; 7) trawling and netting; 8) transects (physical and biological); and 9) calculation of the rate of oceanic water renewal at the site. If the hatchery is to be set up at an existing pearl culture farm, then information on most of these factors will already be available.

Prior to setting up the hatchery at the Broome Jetty, ten sites within the Broome area were evaluated: four locations at or near the Broome Jetty; two near Riddell Point (1km southwest of the Jetty abutment); two near Gantheaume Point Light (0.2km southeast of the Point); and two on private properties at Dampier Creek (Figure 1). The site at the end of the Broome Jetty was selected as the most suitable for the following reasons: the existence of a goods shed for housing the hatchery; the presence of freshwater, electrical power and telephone services; and, most importantly, access to a comparatively inexpensive supply of seawater.

The research hatchery was intended to be a temporary, pilot-scale facility. Therefore, some of the negative aspects of the site, which would seriously compromise a commercial operation in the longer term were over looked. These aspects were: lack of space, poor ventilation (resulting in water temperatures in header tanks reaching 34° C or greater), unusually high levels of aerosol contaminants (such as, unburnt hydrocarbons, bacterial and organic particulates), three-day long power shut-downs associated with off-loading of fuel from oil tankers and continuous vehicle and shipping activities. These factors were peculiar to a commercial shipping jetty.

Figure 1: Potential Broome Hatchery Sites



To establish the water quality of Roebuck Bay, surface and bottom water temperatures and various nutrient levels were measured for two years. The results are presented in Table 1 along with measurements taken from the fishing grounds off Eighty-Mile Beach. When the two locations are compared, seawater in Roebuck Bay averages 2° or 3°C warmer, has lower mean levels of total-phosphorous and total-nitrogen, and a higher mean level of ammonium nitrogen. In general, however, the nutrient levels at these two sites are typical of tropical, Indo-Pacific, coastal waters.

Table 1: Coastal water temperatures and nutrient levels for Roebuck Bay and the fishing grounds of Eighty Mile Beach (at similar depths).

Location	Parameter (mean ± S.D. and range)						
	Water Temperature (Celsius)	Ortho-phosphate Phosphorous (µg/L)	Total Phos-phorous (µg/L)	Ammonium Nitrogen (µg/L)	Nitrite plus Nitrate Nitrogen (µg/L)	Total Kjeldahl Nitrogen (µg/L)	Chlorophyll-a (µg/L)
Broome Jetty							
Surface	26.9 ± 3.8 (21.0 - 32.2)	6.2 ± 5.5 (1 - 24)	16.2 ± 6.8 (2 - 35)	10.3 ± 9.3 (2 - 52)	2.8 ± 1.4 (1 - 6)	181.3 ± 72.4 (48 - 314)	0.7 ± 0.4 (0.01 - 1.78)
Bottom	26.8 ± 3.6 (19.8 - 32.1)	5.4 ± 4.1 (1 - 19)	16.9 ± 7.9 (4 - 43)	10.2 ± 8.2 (1 - 33)	2.8 ± 1.4 (1 - 7)	160.1 ± 58.7 (37 - 258)	0.9 ± 0.5 (0.19 - 2.13)
Fishing Grounds							
Surface	24.0 ± 2.8 (20.0 - 26.8)	4.9 ± 1.3 (3 - 7)	30.4 ± 13.5 (17 - 62)	5.9 ± 4.8 (1 -13)	3.7 ± 0.9 (3 - 6)	315.1±211.6 (74 - 645)	0.3 ± 0.4 (0 - 1.35)
Bottom	23.8 ± 2.7 (20.0 - 26.7)	5.2 ± 0.8 (4 - 6)	26.9 ± 5.8 (19 - 35)	4.5 ±3.0 (1 - 9)	4.1 ± 1.1 (3 - 6)	331.9±179.7 (107 - 563)	0.3 ± 0.2 (0.1 - 0.6)

3. Infrastructure at the Broome Jetty

In cooperation with the WA Department of Marine and Harbours, the hatchery was located at the northeastern end of the 800m long Broome Jetty, and built inside a pre-existing, cyclone-rated, steel-framed, goods shed. The cement floor area allocated for the hatchery was 10m by 11m and partitioned off from the rest of the shed by a 2.5m high wire fence. A 6m transportable laboratory and two 3m high tank stands were placed within this area , along with an assortment of culture tanks, pumps, filters and piping. Two holes were drilled through the floor of the Jetty to allow for an intake pipe and drain. The hatchery was ventilated by a door and four windows. Two large, sliding doors located on each side of the shed and outside the hatchery area, also provided some extra ventilation.

Existing freshwater, electricity and telephone services were available at the shed, but due to Australian Maritime safety requirements, the electrical supply had to be shut-off when oil tankers were off-loading petroleum products. Generally, this occurred once every two months and lasted 1 to 3 days. During this time, master algal cultures and the best 5L and 20L bag cultures were transferred to a temporary laboratory.

4. Design and layout of Facilities

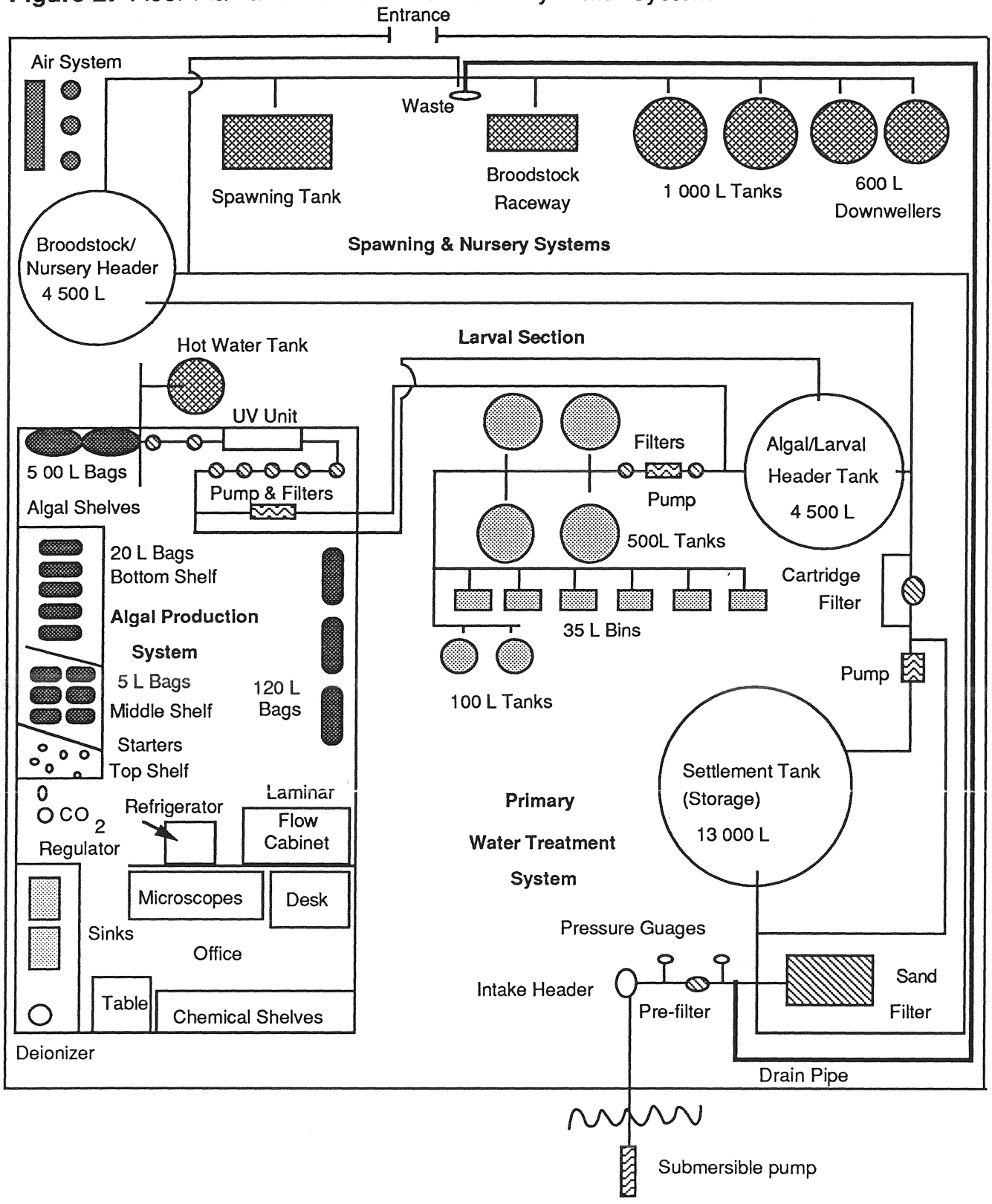
4.1 Overall Design

The four major components of any bivalve hatchery are: broodstock maintenance and spawning; larval culture; settling and post-larval (spat) culture; and algal production. The way these components are integrated into the hatchery's operations depends on the species being cultured and location of the hatchery. As an example of how they mesh together, the floor plan and pipe and instrument diagram of the Broome Jetty hatchery are illustrated in Figure 2. Pumps, filters, water pipes and tanks were interchangeable between the four subsystems to allow for greater flexibility in case of equipment failure or a shift in operational procedures.

Due to space constraints and local tidal conditions, water supply to the hatchery was on a semi-continuous basis with some primary treatment before storage. The hatchery was designed to operate on a small volume, high turnover water system for seven or eight months of the year (September to March/April).

All pumps were made from inert materials, cast iron or marine-grade stainless steel. Materials, such as, copper, brass or bronze were avoided as they leach copper ions which can be toxic to bivalve larvae. Pipes, ball valves and hoses were PVC plastic and could be easily dismantled for cleaning or relocation. All electrical appliances were splash or water proof and all power point outlets were fitted with circuit breakers.

Figure 2: Floor Plan and Flow Chart of Hatchery Water System



4.2 Services

4.2.1 Seawater Supply and Treatment System

Good quality seawater is of prime importance for a hatchery to operate effectively and should be "clean", open coastal water with a salinity of around 34-35ppt (parts per thousand).

At the Broome hatchery, seawater was drawn from underneath the Jetty with a marine-grade stainless steel submersible pump attached to the end of a 14m, 100mm diameter, "heliflex", food grade, PVC delivery hose. Although the depth at which water was drawn varied from 3m to 12m due to a 9m tidal range, the pump's position always remained 6m above the sea bed and was held in position with four nylon stay lines. A block and tackle pulley system enabled both the hose and pump to be pulled up into the shed during a cyclone or to be stored during the off-season.

Seawater entering the hatchery was drawn at 200L/min and passed through a marine-grade stainless steel mesh filter to remove suspended particles larger than 100µm (1000µm = 1mm). The coarsely filtered or "raw" seawater was either pumped directly into a 4 500L fibreglass header tank, which supplied the broodstock/spawning and spat culture systems, or passed through a sand filter to remove particles greater than 30-50µm. This water was either used directly or stored in a plastic-lined, portable, 13 000L settlement tank. Prior to refilling the settlement tank, the sand filter was routinely backflushed with at least 1 000L of previously stored water from the settlement tank.

After a minimum of 12 hours storage, settlement tank water was used directly or pumped through a swimming pool, cartridge filter to remove particles greater than 10µm. This water was either used directly or stored in another 4 500L fibreglass header tank which supplied the larval and algal culture systems. Water from the header could be further filtered to the submicron level and/or irradiated with ultra-violet (UV) light depending on the application (Figure 2).

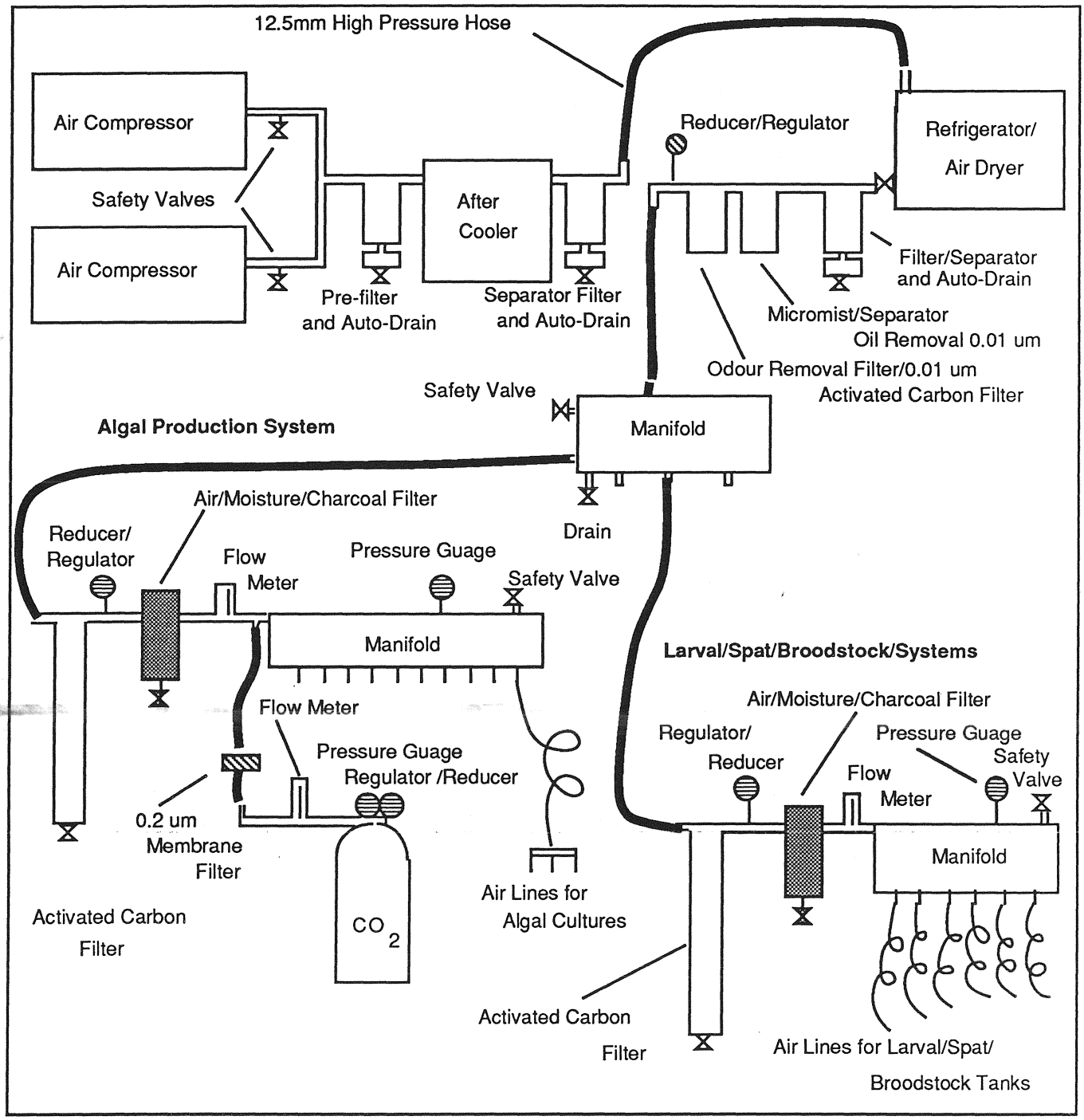
Both header tanks and settlement tank had plastic, conical lids to exclude dust and aerosol contaminants.

4.2.2 Air System

Air can be supplied by either an air compressor or blower depending on the requirements. Blowers produce large volumes of oil-free air at low pressure and are less prone to mechanical failure, whereas, compressors produce small volumes of high pressure air which are not necessarily oil-free and must be filtered.

At the hatchery the air requirements were low and adequately supplied by two 10 CFM piston compressors which were either operated together or separately depending on the demand. Air exiting the compressors was pre-filtered for water, dried, refrigerated to 20° C and filtered for submicron particles, moisture and oil, before entering a stainless steel manifold. Air from the manifold was delivered via flexible, high-pressure hoses to one of two regulators connected to an activated charcoal and submicron filter unit. One of these units supplied low pressure air to a PVC reservoir which distributed air to the broodstock/spawning, larval and post-larval culture systems, while the other supplied the algal production system (Figure 3). Air entering the reservoir for algal production was enriched with food grade carbon dioxide before use (Section 4.6).

Figure 3: Air Flow System



4.2.3 Heating System

Seawater used for spawning trials was heated with a single phase, thermostatically controlled, incoloy (steel alloy) filament, immersion heater which drew 2Kw.

4.2.4 Freshwater System

Freshwater was used mainly for cleaning equipment, pipes and washing down the hatchery floor. Water delivered to the algal production unit was either heated with an instantaneous hot water heater for washing-up glassware or deionized for the preparation of nutrients. Water consumption over three years averaged 57 kilolitres/mo or $53/mo.

4.2.5 Electrical System

The hatchery's main control panel supplied electricity to both single and three phase pumps, lights, heaters, compressors, ovens, power tools and the algal production unit. Within the algal production unit, a switch board distributed power to lights and timers, electronic balances and microscopes, a single phase pump, UV sterilizer, laminar flow cabinet, refrigerator, electric oven and stove, and three air conditioners. During normal operations, an average of 2 291 kw/hr/mo of power was consumed ($256/mo at $ 0.1117/kw/hr).

4.3 Broodstock/Spawning Unit

Two 650L cylindrical fibreglass tanks and one 400L asbestos raceway were used to maintain up to 150 broodstock oysters for gonad conditioning ("fattening"). A 600L fibreglass-coated, wooden, rectangular tank capable of holding 130 oysters was available for spawning trials. Seawater for spawnings could be cartridge-filtered from 10µm down to the submicron level and irradiated with UV light, using the same equipment for algal production. A 500L polycarbonate plastic tank was used to heat seawater, which could be either pumped directly into the spawning tank or mixed with ambient seawater to obtain the desired temperature. Fertilized eggs were collected with sieves and plastic buckets from the larval culture unit.

4.4 Larval Culture Unit

Four 500L polycarbonate tanks, two 1 000L fibreglass tanks, two 100L polyethylene plastic tubs and six 25L polycarbonate rectangular bins were available for rearing various batches of larvae. Each 500L and 1 000L tank had a plastic, conical lid to reduce the level of aerosol contaminants coming in contact with the cultures. Water used for rearing the larvae was pumped through either a 1µm or 10µm cartridge filter unit at a flow rate which could vary from 10L to 50L/min. PVC beverage hose (20mm diameter) was used for siphoning water during water changes. Various nylon mesh sieves (20, 25, 44, 60, 71, 80, 112, 120, 140, 150, 180, 200, 236, 450µm) were used to collect larvae during water changes.

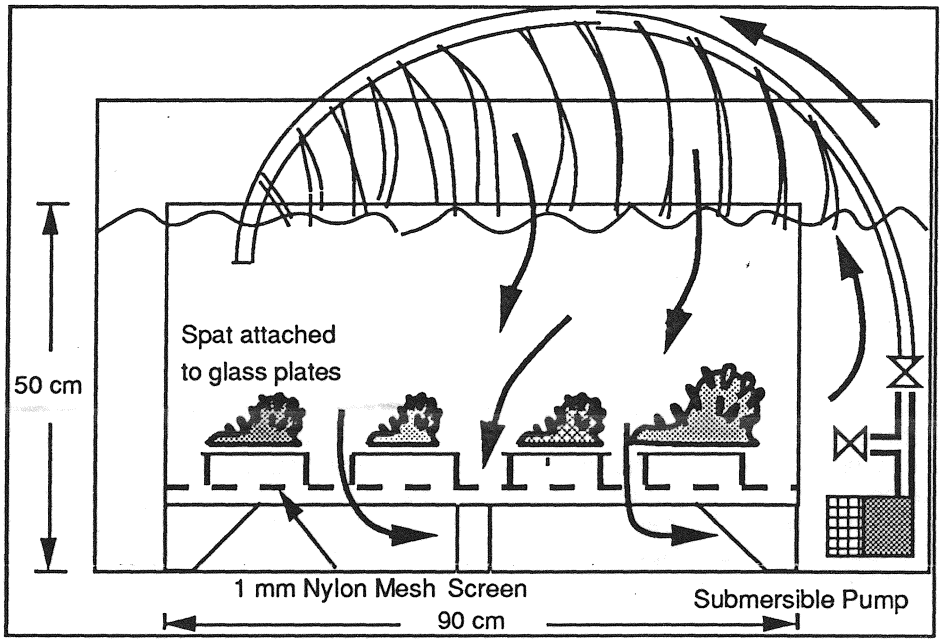
Sieves were constructed from PVC pipe (30cm dia.). Each sieve was 12cm or 20cm high and stood on legs which were 2cm high. Nylon mesh was held in place by two plastic rims cut from the same pipe. Each rim was shortened to match the inside circumference of the original pipe by cutting-out a thin section. This enabled them to be fitted tightly against the inside wall. The bottom rim had four sections cut out of it to form legs and was inserted into the sieve with the legs protruding out one end and glued or screwed into place. A nylon screen was placed over the other end of the sieve and the top rim was placed over the screen. Both of these were inserted into the sieve, tapped down to the bottom rim and held in place only by tension or silicone glue. If the screen was damaged, the glue and the top rim could be removed to replace the screen. Because of the way the legs were positioned, the sieves could be stacked by fitting the legs of one sieve into the top end of another.

4.5 Settling and Spat Unit

Settling of post-larvae was performed in the same tanks used for rearing the larvae. Dark glass or plastic plates, monofilament fishing line, and plastic mesh were placed in the culture tanks prior to settlement to collect the post-larvae (see Section 5.8 for details). Depending on the size of the post-larvae and subsequent spat, seawater was pumped through either 1 and 10 µm cartridge filters or through 20, 45 and 60µm nylon sieves.

Larger spat were either reared at sea under the Jetty in circular plastic-mesh trays or at the hatchery in rectangular or cylindrical tanks not in use for spawnings or larval culture. Spat were also reared in one of two downweller systems which consisted of a plastic cylinder (h = 0.5m, dia. = 0.9m) inserted into a 600L tank (Figure 4). Each cylinder stood upright on a set of legs and was open at the top and covered at the bottom with 1mm nylon mesh screen. A submersible pump situated underneath the screen was connected to a black polyethylene pipe which extended up and over the top of the downweller. The pump drew water from below the cylinder at 1 to 3L/min and sprayed it over the surface through perforations in the pipe.

Figure 4: Downweller Unit



4.6 Algal Production Unit

The algal unit, housed in a 6m by 3m by 2.4m cyclone-rated, transportable mounted on steel skids, could produce a total of 1 660L of algae at one time (for floor plan see Figure 5). Master or starter monocultures were grown in 250ml to 3 000ml conical flasks to seed 5L, 20L, 120L and 500L plastic bag cultures which were later harvested for food. Cultures 20L or less were maintained on a two-tiered shelf (Figure 6). The top shelf supported flask cultures while steel racks fitted below each shelf were used to hang twenty 5L bags from the top shelf and ten 20L bags from the bottom shelf. Fluorescent tubes positioned underneath each shelf provided lighting for the bag cultures while flask cultures were illuminated with incidental lighting within the transportable. Three 120L and two 500L bag cultures were held in wire mesh cages (Figure 7) and illuminated with fluorescent tubes vertically positioned behind each cage.

Algae were grown at 20° to 24° C by controlling the air temperature inside the transportable with two of three wall-mounted, 1 Hp (746W) air conditioners. Fluorescent lighting provided illumination on a 8-12hr dark/12-16 hr light cycle. Light intensities for various culture volumes ranged as follows: 12-39 $\mu E\ m^{-2}sec^{-1}$ (approx. 105 to 320 Lux) for flasks; 200-350 $\mu E\ m^{-2}sec^{-1}$ (1 450-2 850 Lux) for 5L bags; 180-400 $\mu E\ m^{-2}sec^{-1}$ (1 500-3 150 Lux) for 20L bags; 140-275 $\mu E\ m^{-2}sec^{-1}$ (1 100-2 350 Lux) for 120L bags; and 140-250 $\mu E\ m^{-2}sec^{-1}$ (1 100-2 060 Lux) for 500 bags.

Starter cultures less than 1 000ml were not aerated but stirred twice daily by hand to keep the algae in suspension. Cultures greater than 1 000ml were aerated with submicron filtered air which was intermittently enriched with filtered, food grade carbon dioxide stored in $63m^3$ pressurized cylinders. A flow meter regulated the amount of carbon dioxide enriching the air at 0.45% to 2% by volume (Figure 3).

To control contamination by micro-organisms, seawater used as a culture medium was either autoclaved or filtered, depending on the volume required.

Starter cultures or volumes less than 3L were sterilized in flasks placed in a pressure vessel or autoclave with a 39L capacity. The vessel, which was heated externally on a electric hot plate, was located outside the transportable. Seawater used for bag cultures was first filtered through a series of cartridge membranes ranging in pore size from 10µm, 5µm, 1µm, 0.6µm and irradiated with UV light before a final filtration through a set of 0.45µm and 0.2µm pharmaceutical grade membranes (Figure 8). A 3/4 Hp (560W) positive displacement pump was used to deliver the seawater.

Inoculations of new starter and 5L bag cultures were done aseptically inside a laminar flow cabinet using standard laboratory procedures. All bag cultures used air pressure to aseptically transfer algae from the donor to receiver via a temporary connection between their air-intake lines.

Chemicals used in nutrient solutions were weighed with an electronic balance and stored in a refrigerator. All freshwater used in preparation of nutrients was deionized. Washing-up and storage of glassware, microscopic examination of algal cultures and office work were performed inside the transportable. A compound microscope with a phase-contrast condenser and oil and phase objectives was used for inspecting bacteria, larvae and microalgae. A stereo microscope was used to inspect newly settled spat and young juveniles.

Figure 5: Floor Plan of Algal Transportable

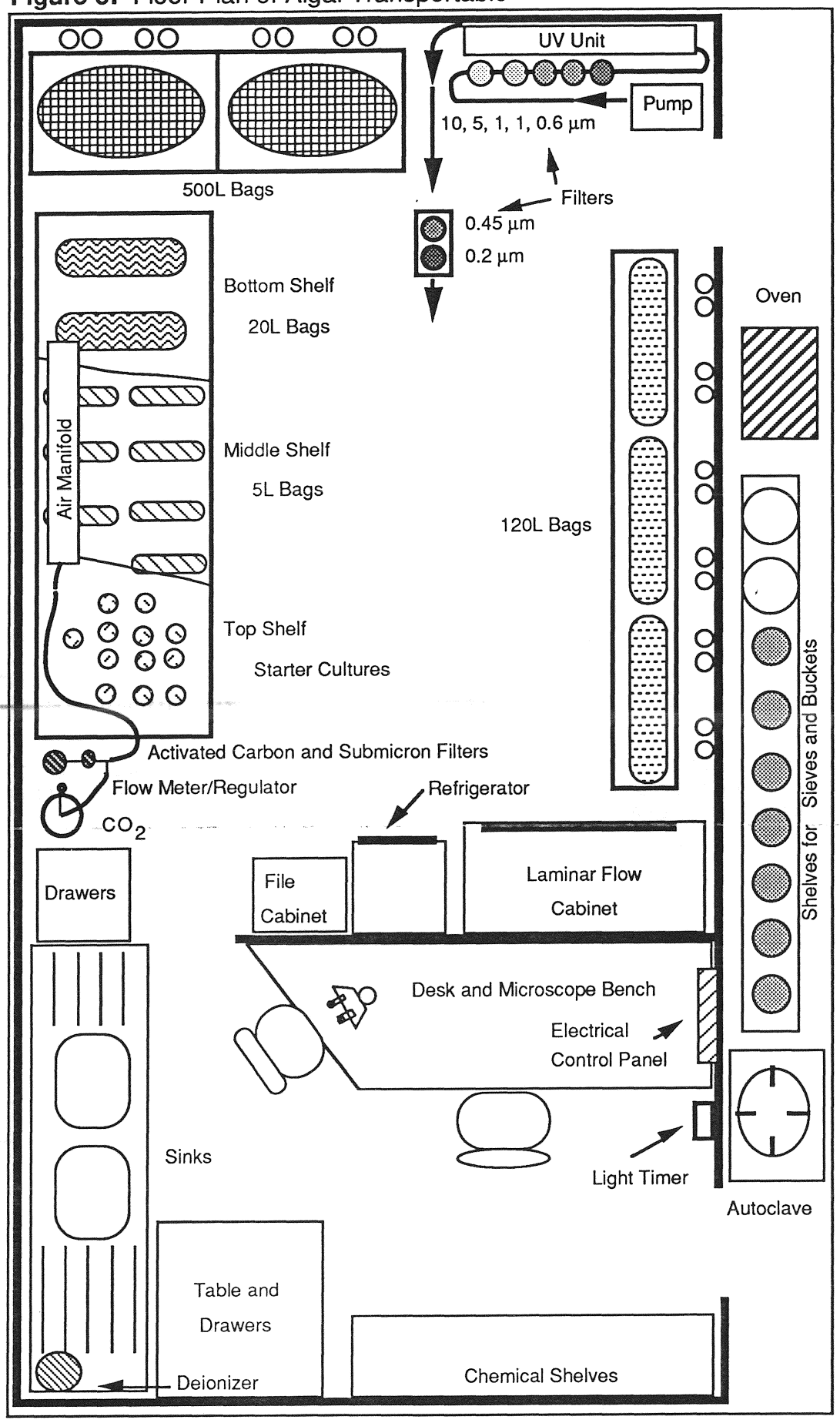


Figure 6: Algal Shelves and Bag Racks

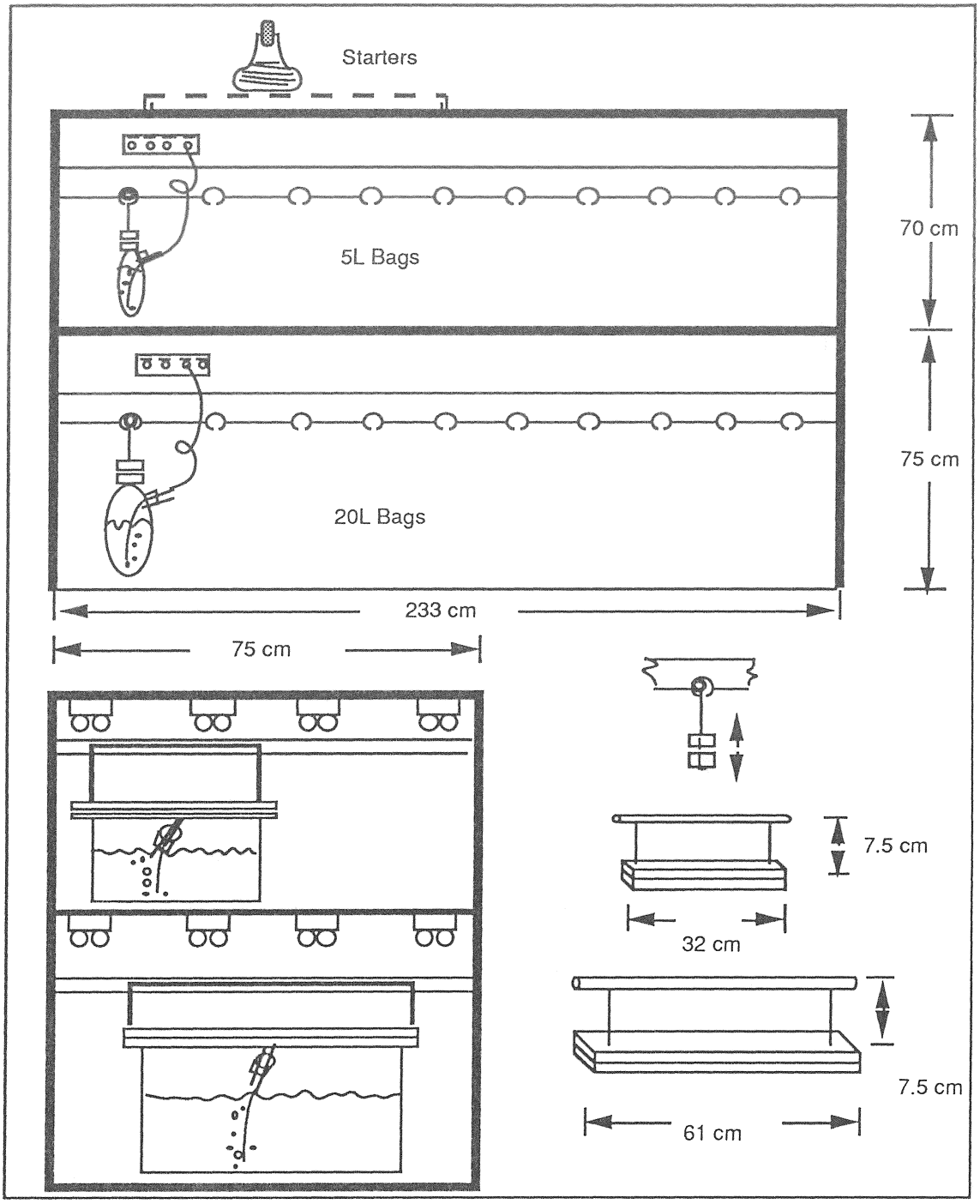


Figure 7: Wire Mesh Frames for 120 and 500L Bags

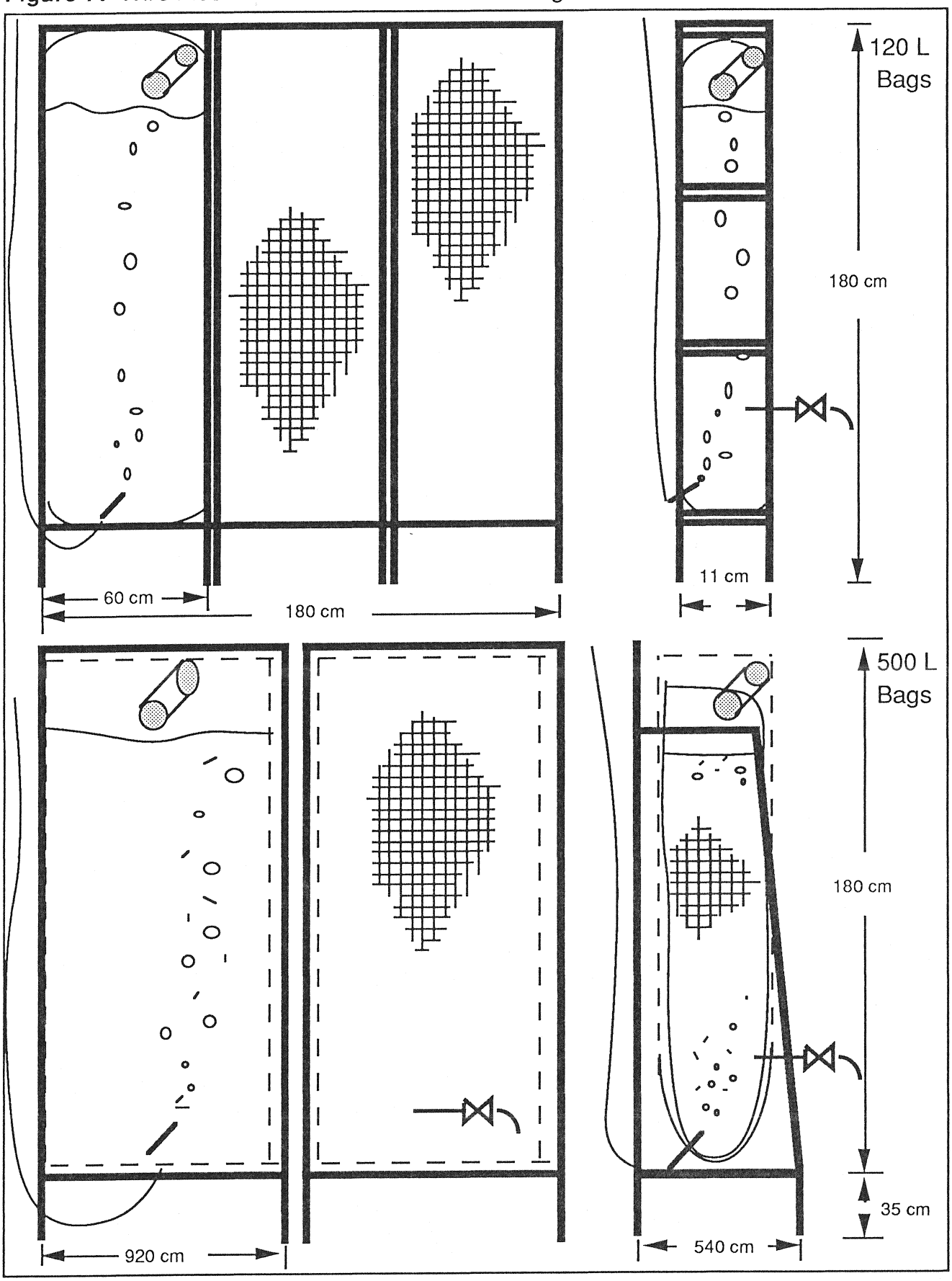
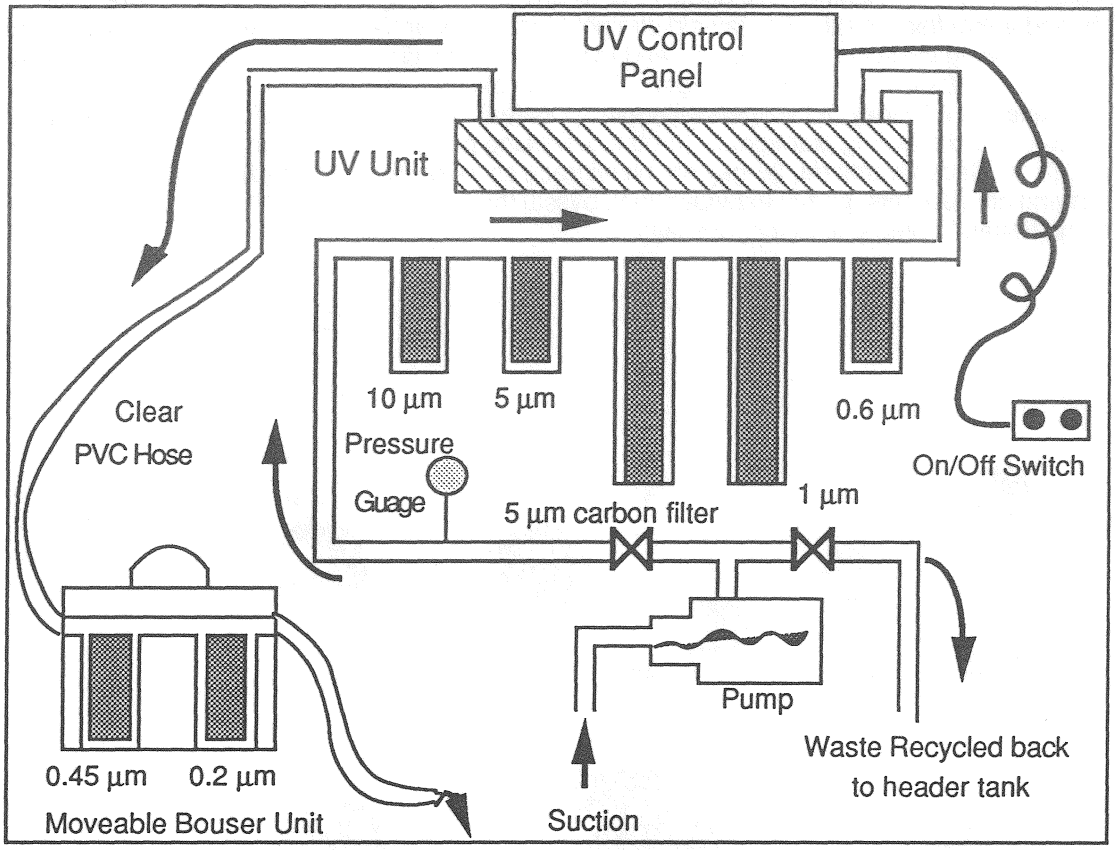


Figure 8: Algal Filtration and Ultraviolet Irradiation System



5. Husbandry

5.1 Life Cycle and Reproduction

Pinctada maxima is a protandric hermaphrodite (male before female) which is capable of changing its sex according to environmental conditions, such as, the availability of food. Generally individuals are one sex or the other during a complete reproductive cycle. *P. maxima* typically reaches maturity as male during the first year of its life or when the shell height (distance from the hinge to curved-growth margin) is 120mm or greater. As they grow (age), the incidence of female sexuality increases. In wild populations, the sex ratio is approximately 1:1 when the shell height of individuals is 200mm or greater. Female sexuality tends to be adversely influenced by poor growing conditions, causing oysters to remain as males or change from females to males.

The gonads are not discrete organs and develop between the tissues covering the stomach, liver and gut loop. Male gonads are white and female gonads yellow. Both sexes spawn several times during a season, releasing their spawn into the sea (Rose *et al.*, 1990). Fertilization and subsequent larval development occur in the plankton (Figure 9). Larvae grow from 6 µm to 12µm/day in shell length and after 16 to 35 days settle and metamorphose into spat (Figure 10). Newly settled spat and juveniles (Figure 11) grow at about 10 mm/mo (Figures 12). After 15 to 18 months they are usually greater than 120 mm in shell height and large enough for pearl cultivation.

Figure 9 Embryonic, larval and early spat development of *Pinctada maxima*. (A) Early cell division one hour after fertilization: ovum (o); early first cleavage (1C); second cleavage (2C) with embryo displaying three out of four cells and a polar lobe (pl). B) Gastrula five hours after fertilization. C) Eight hour old trochophore showing shell formation (sh) and apical flagella (af). D) Day-old straight-hinged veliger (D-shaped larva). E) Eight-day-old, early, umbonal veliger showing indistinct umbo (u), extended velum (v) and digestive diverticulum (dd) as a dark patch covering viscerum. F) 10-day-old umbonal veliger. G) 14-day old umbonal veligers, including a larger, rapidly developing "streaker". H) 21-day old veligers with eye spots (es). I) 24-day old pediveliger with foot (f) slightly extended. J) 25-day old crawling plantigrade (metamorphosing pediveliger) showing extended foot, rudimentary gill filaments (gf). K) Recently settled, 28-day old plantigrade showing dissoconch (d) and gill filaments. L) 35-day old spat displaying gill filaments, partial extended foot, byssal threads (bt) and mantle (m), with dark pigment spots. (From Rose and Baker, in preparation for publication)

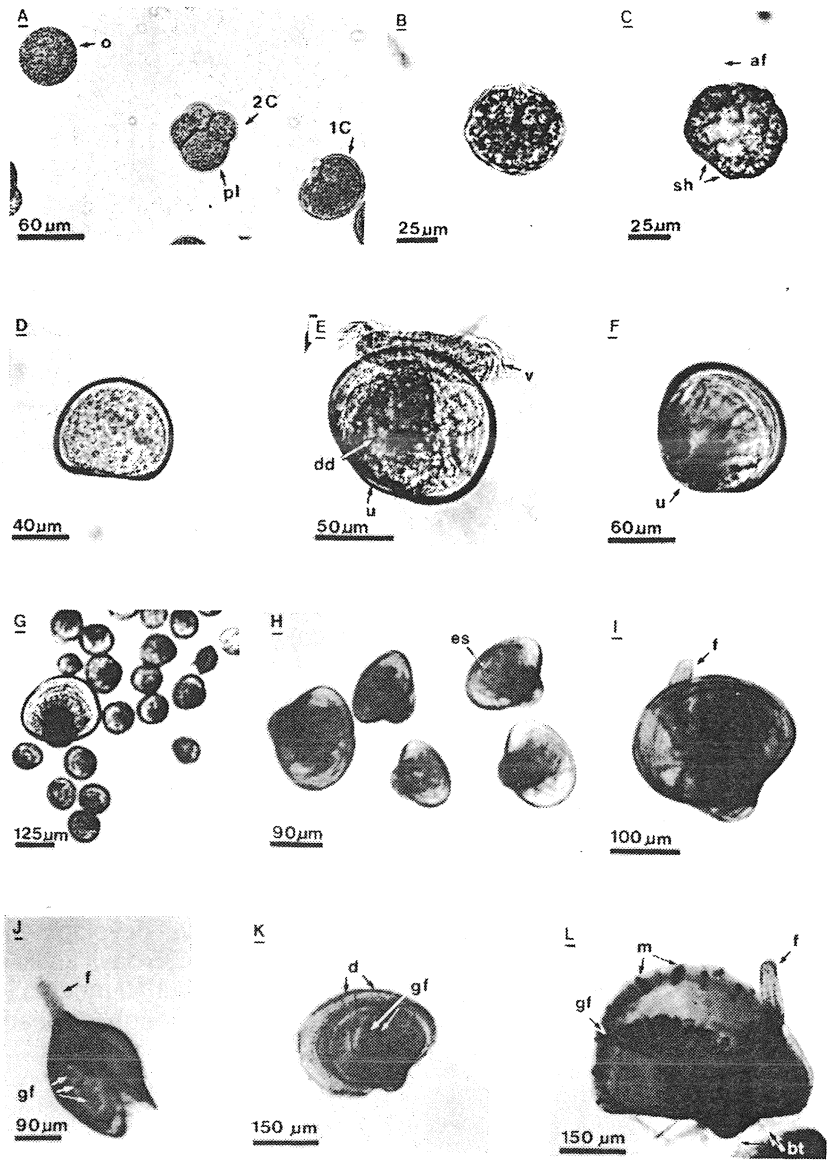


Figure 10 Growth rate of *Pinctada maxima* larvae and post-larvae from several batches reared at temperatures averaging 27-29°C.

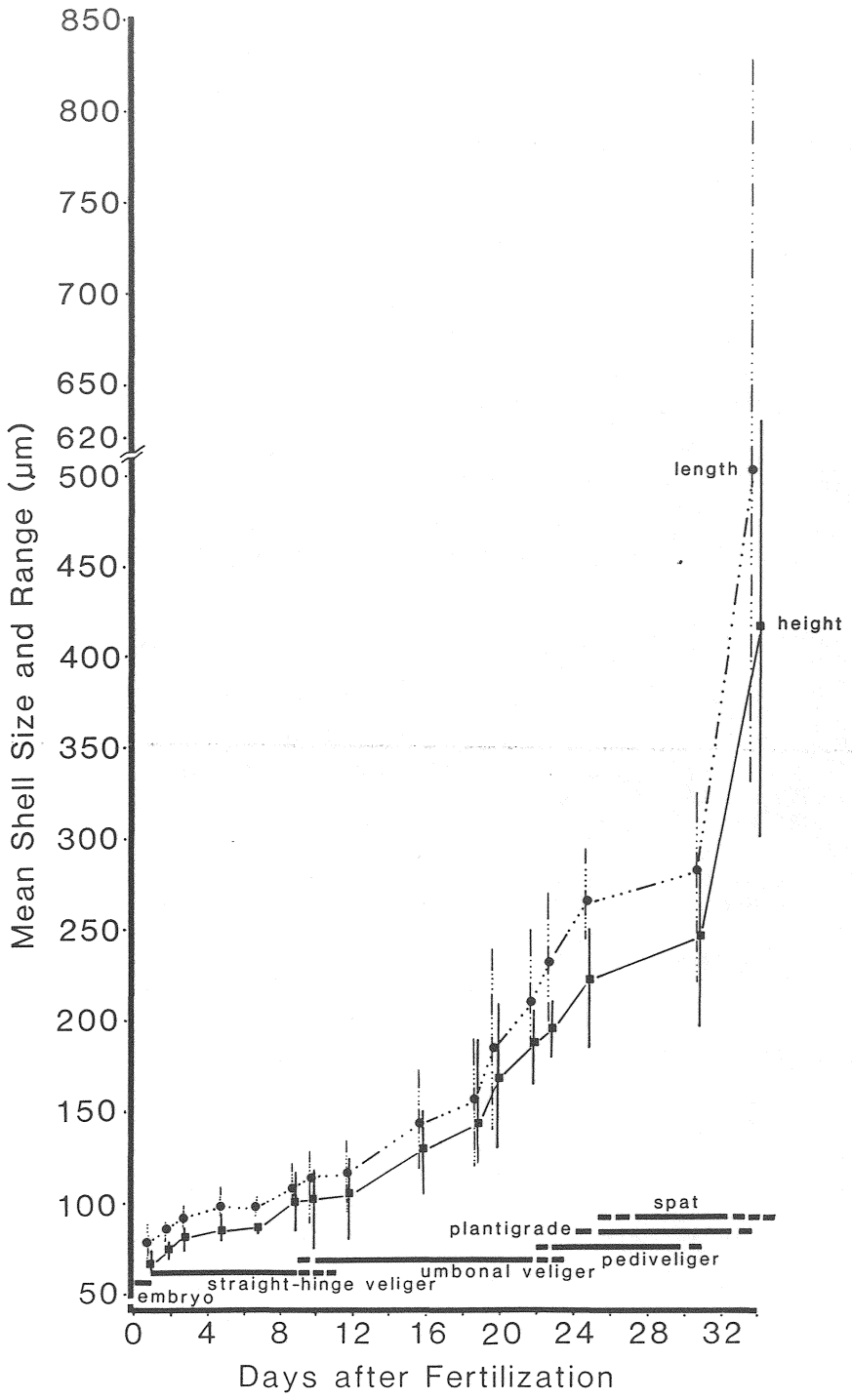


Figure 11 Juvenile *Pinctada maxima*. (A) Outside view of the left valves of five juveniles at different ages, from left to right: 2.5, 3, 4, 4.3 and 5 months after fertilization, respectively. Note growth processes (gp). (B) Inside view of paired valves of juveniles five months after fertilization (four months after setting), illustrating different growth rates of individuals from same spawning. Note nacreous (n) and periostracal(p) layers of shell. Scale in millimetres (from Rose and Baker, in preparation).

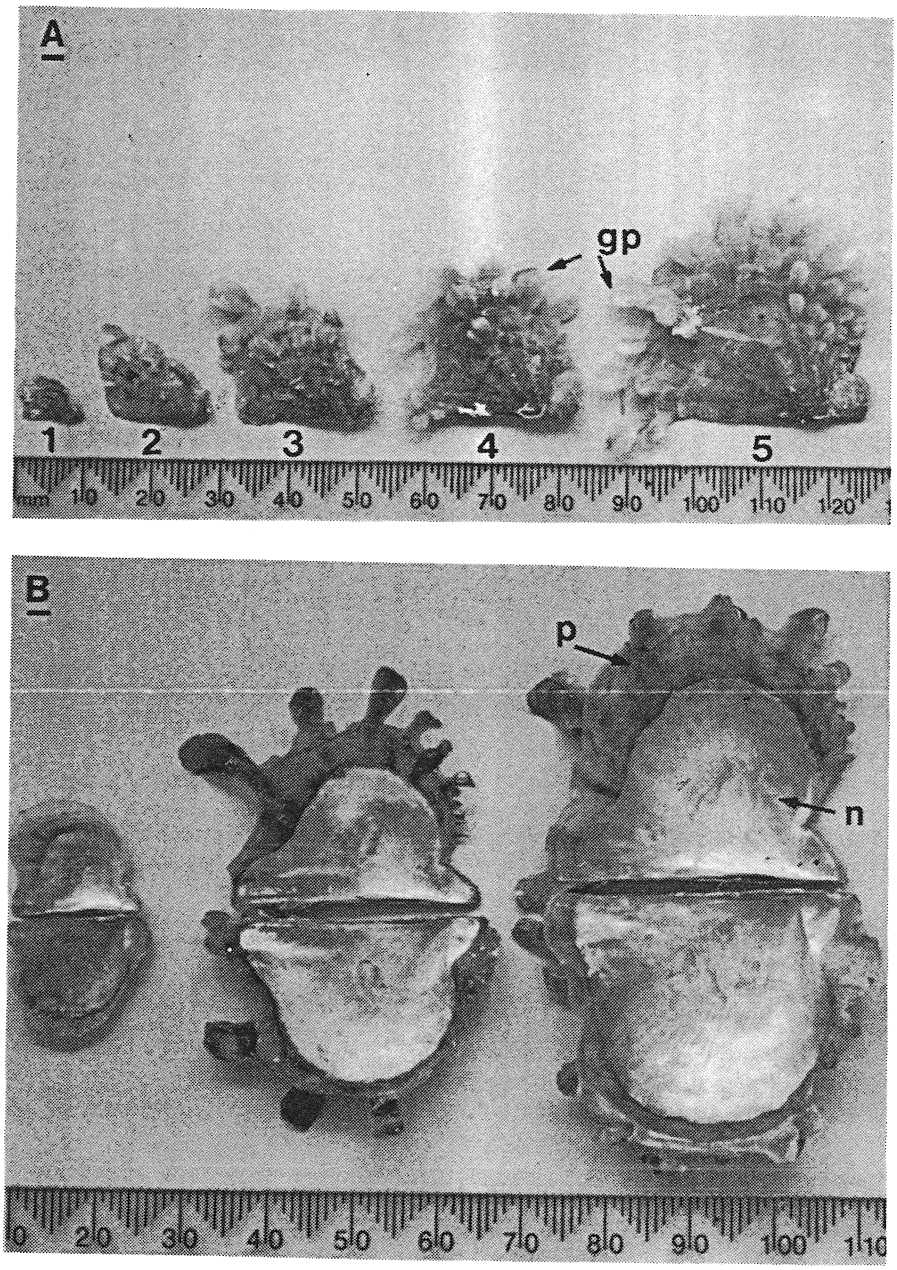
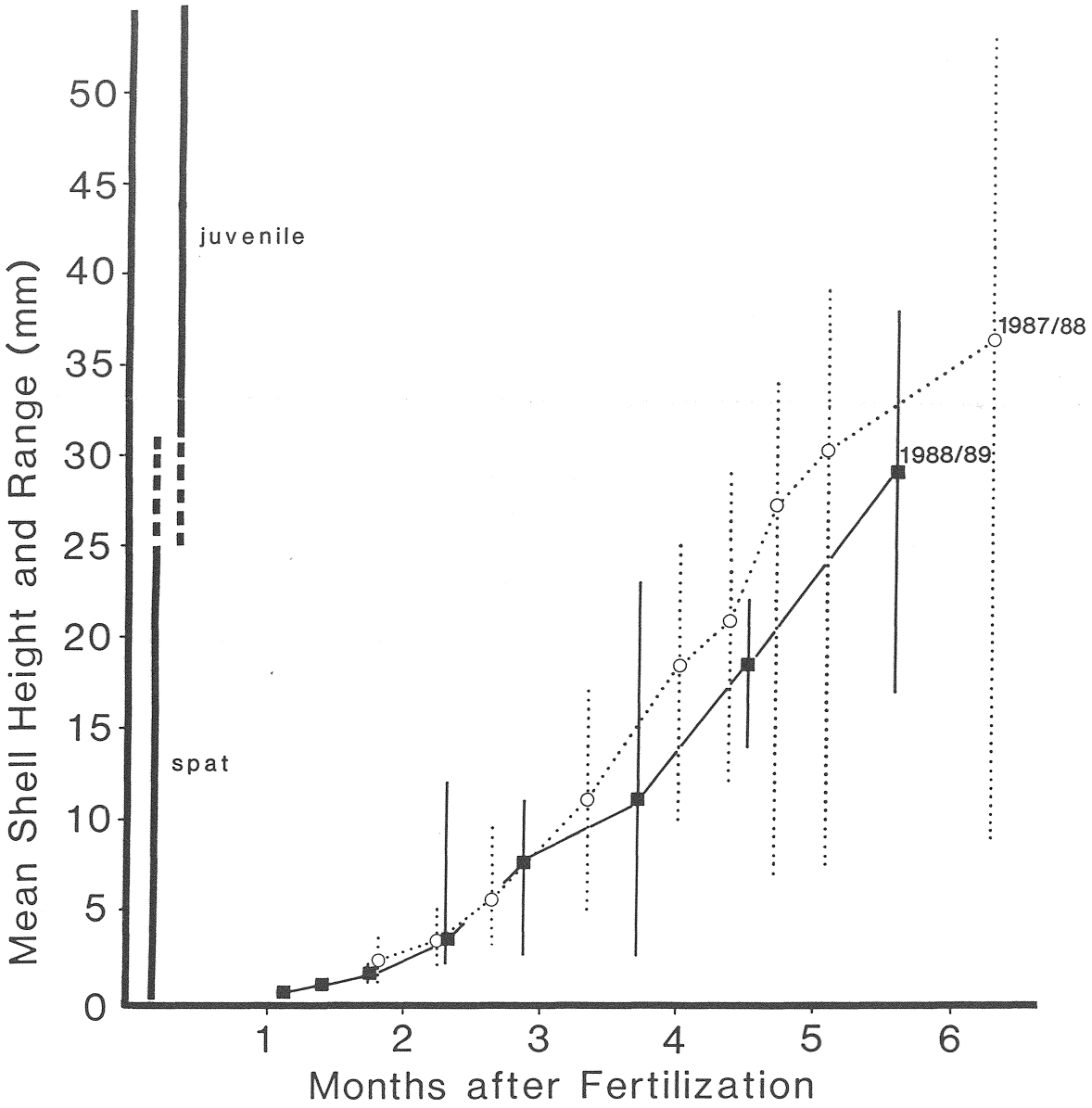


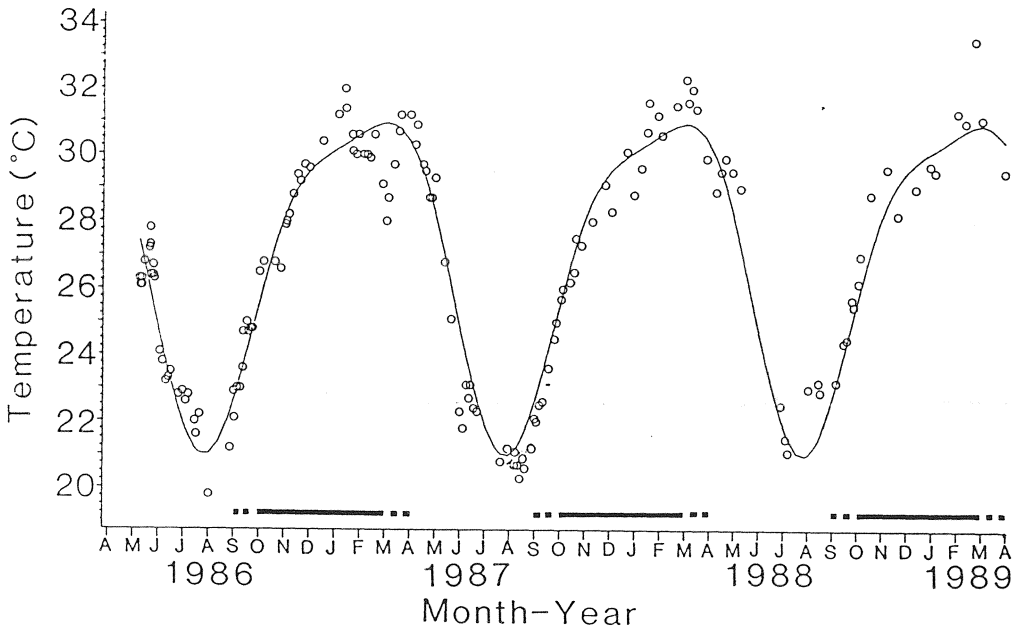
Figure 12 Growth rate of *Pinctada maxima* spat and juveniles reared in downwellers in the nursery for approximately six months at temperatures averaging 28-31°C.



5.2 Breeding Season

The breeding season of wild oysters begins in September/October during the annual, rapid rise in water temperature and extends over the summer before finishing in March/April when the temperature drops (Figure 13). Records of the number and intensity of viable gametes per spawning indicate that there are generally two spawning peaks, a primary one during November/December and a secondary during February/March. On the basis of successful hatchery spawnings, the months of the breeding season can be ranked in decreasing order as follows: November; December; October = March; February; January; and April (Rose *et al.*, 1990).

Figure 13 Annual breeding season of *Pinctada maxima* derived from six years of recorded field and hatchery spawnings (horizontal bars: solid portion signifies major spawning period). The surface seawater temperatures (°C) (o—o) at the Broome Jetty from April 1986 to April 1989, are also plotted. Cyclical temperature curve plotted was determined by the following equation: $°C = 26.95 + 2.46 \sin Ø + 4.09 \cos Ø - 0.78 \sin (2Ø) - 0.96 + \cos (2Ø)$, when $Ø = 2\pi$ (day of year)/365, $r^2 = 0.93$ and n = 150 (from Rose *et al.*, 1990).



5.3 Broodstock

5.3.1 Size Requirements

Successful propagation begins with healthy parent stock. Broodstock should be at least 140mm or more in shell height and show signs of actively growing finger processes which are indicative of a healthy individual. Oysters used for half-pearl culture are an ideal size for broodstock. Their shells should not show signs of heavy infestation with boring worms (*Polydora*) or sponge (*Cliona*) since these parasites could affect gonad development or contaminate larval cultures. Stunted or dwarfed oysters ("clunkers") should be avoided as they may be diseased or slow growers; thus, affecting the robustness and growth rate of their progeny. Old, larger oysters are more difficult to spawn in the hatchery than younger, smaller oysters and are not recommended as broodstock. Oysters seeded for pearls can be used as broodstock since this operation does not adversely affect sexual development.

5.3.2 Collection, Transportation and Holding of Broodstock

Deep water oysters collected periodically from the fishing grounds throughout the breeding season are the most reliable stock of ripe oysters for induced spawnings. To avoid spawnings during transportation, males and females should be separated and placed in separate tanks with running water. Males with extremely ripe looking gonads should not be placed in tanks but covered with Hessian bags and kept cool and damp with running water from the boat's deck hose. They should not be exposed to wind which could cause desiccation (drying out) of their mantle and gill tissues.

Broodstock can be held on surface longlines or on the bottom in the same manner as oysters used for pearl culture but they should be spread-out to avoid overcrowding as this can adversely affect sexual development. Those left on surface longlines take at least five weeks to mature from the resting and early gonad development stages to the spawning-ripe stage. Broodstock should be cleaned occasionally so as to avoid heavy fouling on both their shells and nets.

5.3.3 Monitoring of Gonad Development and Broodstock Conditioning

Oysters which have been collected should be visually sexed using shell openers and a long spatula. Open the oyster carefully so that the mantle is not damaged and the gap between the valves is less than the width of your thumb or 2.5cm. For a right-handed person, the oyster should be oriented so that the right or convex valve is held in the palm of the left hand, with the growth margin facing towards the left shoulder and the shell hinge resting against the fingers. In this position, the left or flat valve should be on top. On the side where the foot and byssal threads appear, push to one side the mantle to find the stomach and gut loop. There is enough space here to see the gonads developing over the surface of these tissues and to determine their sex and stage of development. This area is also where the nucleus is implanted for pearl production.

Broodstock should be visually monitored for gonad development using the following scheme:

Stage 0: gonad tissue flaccid or invisible, sex indeterminate;

Stage 1: gonad visible but proliferation to gut loop is slight and proximal, gonad appears granular and difficult to sex by colour (male-white and female-yellow);

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

Stage 3: gonad ripe and bulging, gonad tissue extends over the surface of stomach, gut loop and liver; gonad appears confluent and when pierced gametes run freely and profusely.

Conditioning oysters to spawning ripeness (stage 3) in the hatchery was not adequately demonstrated for both sexes during this study and requires further investigation. On a few occasions, however, groups of 10 or 11 near-ripe males held in tanks for one to two weeks released viable spawn just after a water change. Prior to these spawnings broodstock had been kept in the dark in aerated, unfiltered seawater. Every day these tanks were siphoned for faeces and debris and refilled with new water and enough algae to turn the water opaque.

5.4 Spawning

To obtain the best quality sperm and eggs, only oysters with gonad development at stage 3 should be used. Females induced to spawn at the hatchery were estimated to produce between 0.5×10^6 and 12×10^6 eggs/individual, with each female typically producing 2×10^6 to 3×10^6 eggs. To compensate for the variability in number of eggs produced per female and to reduce the incidence of polyspermy (multiple fertilization of a single egg), the sex ratio should be approximately one male to four or six females.

5.4.1 Hatchery Spawnings

5.4.1.1 Temperature Induced

Oysters selected for spawning should be brushed clean and placed directly into a tank with running seawater filtered to at least 10µm or with ultra-violet irradiated seawater filtered to 1µm. Alternatively, after cleaning the oysters, they may be exposed to air for 1 to 2 hours before being placed into the spawning tank.

After 20-30 minutes in the tank, when the oysters have all opened their shells and are actively pumping, the water flow should be stopped and the temperature gradually raised 2° to 5°C above ambient. The final temperature should not exceed 33°-34° C since at these temperatures oysters can become stressed and the incidence of abnormal fertilization and subsequent cell division increases.

After another 20 to 30 minutes, the warm water should be drained and a new batch of ambient, flowing, filtered water introduced to the tank and the temperature raised as described above. If oysters have not spawned after two or three water changes, then a sperm suspension obtained from the gonad of a sacrificed male should be introduced into the tank at the beginning of the next cycle.

The suspension should be prepared by gently cutting the tissue covering the gonad, taking care not to cut the digestive gland. Place a 20 or 25µm, nylon mesh gauze over the incision (to reduce gonadal debris) and gently squeeze and wash the wound with filtered seawater. Collect the sperm oozing from the wound with a pipette or allow it to drip into a container filled with filtered seawater. After mixing the sperm, pour the contents directly into the tank or slowly introduced small amounts to individuals with a pipette. Place each drop over the inhalant aperture, which is formed by the mantle skirt and located antero-laterally to the gills or near the byssal threads.

Extremely ripe males do not need to be stimulated by a sperm suspension and will spawn almost immediately or within 20 minutes after exposure to running, filtered seawater. Generally, however, males will spawn 15-20 minutes to 3 hours after coming into contact with warm water and/or a sperm suspension. Females will spawn 10 to 20 minutes after the release of actively motile sperm.

Spawn is discharged from an individual's exhalent aperture, which is formed by the mantle and located postero-laterally or furthest from the foot and byssal threads. Sperm first appears as a white cloud, whereas, eggs appear as a cloud or tiny, granular, yellow-orange specks. Spawn which clump together and sink to the bottom of the tank, are poor quality gametes and should be discarded.

After a tank becomes cloudy with spawn wait at least 5 minutes, then drain the tank to collect the eggs (see section 5.4.3 and 5.4.4 on egg collection and counting). Refill the tank with filtered seawater and begin a new cycle until all spawning has ceased. Save some of the spawn water siphoned from the tank and add it to the tank if oysters do not begin spawning.

If a spawning has not occurred after 5 or 6 hours, then the oysters are not sufficiently ripe and should be returned to their conditioning tanks or placed back in the sea.

5.4.1.2 Chemically Induced

The procedures described here do not always work and often result in the release of suboptimal gametes. However, they may be useful for spawning near-ripe oysters at the beginning and end of the breeding season, or when oysters fail to respond to warm water and sperm-suspension stimuli. Experiments performed at the Broome hatchery have indicated that broodstock will sometimes spawn when exposed to a weak solution of hydrogen peroxide (H_2O_2) after adjusting the hydrogen ion concentration of the seawater to pH 9.0 with an alkaline. Furthermore, these alkaline compounds (e.g., ammonium hydroxide (NH_4OH), sodium hydroxide (NaOH) or TRIS buffer) by themselves, were sometimes found to induce spawnings after raising the pH of seawater to 9.0-9.5.

To reduce the need for large amounts of expensive chemicals, place approximately 20 broodstock into a tank filled with 100L of seawater. After the oysters have opened up and are actively pumping, adjust the seawater to a pH of 9.0 with one of the alkaline compounds above. Use either pH paper or a pH meter to determine the alkalinity of the seawater. Then add either 6% or 30% H_2O_2 (Unilab, AR grade) to the seawater to obtain a final concentration of 0.006%. For example:

ml required of H_2O_2 = (final conc.)(vol. of tank)/(strength of H_2O_2)
= (0.006/100)(100,000 ml)/(30/100)
= 20 ml

where : 0.006% = 0.006/100
30% = 30/100
100 L = 100,000 ml

The introduction of the alkaline compound separately or in combination with 0.006% H_2O_2 can be done in conjunction with the thermal-sperm stimuli described in section 5.4.1.1. After raising the water temperature add the chemical(s) and wait a few minutes before adding the sperm suspension. Wait 30 minutes then drain the tank and begin again. If a spawning has not occurred after 3 or 4 cycles, then neither sex is sufficiently ripe to spawn. If a spawning occurs wait at least 5 minutes before draining the tank to collect the eggs.

Serotonin, a naturally occurring neurotransmitter which stimulates heart and ciliary beating in molluscs, has been found to induce spawning in a number of clam, mussel and scallop species. However, at the Broome hatchery, experiments in which various concentrations of serotonin were injected into the gonad of *P. maxima* proved to be ineffective.

5.4.1.3 Stripping gametes for chemically induced fertilization

If access to spawning-ripe broodstock is not possible then, as a last resort, gametes of both sex can be stripped from the gonads of sacrificed oysters as described in section 5.4.1.1. Select the ripest males and females and collect the gametes of each sex separately in 1L of ultra-violet irradiated seawater filtered to at least 1µm. Add to the egg suspension enough decinormal ammonium hydroxide (0.1N NH_4OH) to make a 0.5% solution (final concentration 0.0005N NH_4OH). For example:

ml of required 0.1N NH_4OH = (0.5/100)(1000ml)
= 5ml
where: 0.5% = 0.5/100
1L = 1000ml

(Note: Concentrated NH_4OH (also called "880 ammonia") contains 28% by weight of ammonia and is 14 Normal, and must be diluted accordingly (add 1 part to 139 parts water) to obtain the decinormal working solution described above.)

Stir thoroughly and leave for approximately 40-50 minutes, checking the eggs under the microscope every 10 minutes. When the germinal vesicle (or nucleus) in 70% to 90% of the eggs is no longer visible and the eggs are round and not pear-shaped, add 1-4 ml of sperm to the 1L egg container. Mix thoroughly and leave for only 1-2 minutes before washing the eggs and re-suspending them in a 20L container of seawater filtered to 1µm. Two 1ml samples should be taken from the container to determine the average proportion of fertilized eggs (those with protruding polar bodies or those beginning cell division) from the total number of eggs counted.

Eggs should be washed quickly after insemination to reduce the destructive effects of NH_4OH on cell division. The harmful effects of the ammonium ions are not noticeable up to the 4-cell stage, but beyond this stage development can become abnormal and retarded. To avoid polyspermy, do not add more than 4ml of sperm as this may adversely affect normal development.

5.4.2 Field Spawnings

Field spawnings are a good method of obtaining large numbers of fertilized eggs from several thousand oysters. This method, however, is only practical if the hatchery operations can be synchronised with the transportation of culture oysters during the peak breeding season (October/November/early December).

During transportation the carrying tanks must be continuously monitored for signs of spawning. When this happens, wait 2 to 3 minutes before turning off the tank's water pump. Leave the oysters undisturbed for another 15 or 20 minutes or until they have finished spawning. Collect a sample of spawn with a glass container and check it for the presence of tiny yellow-orange specks (eggs). If they are present begin siphoning the tank water through two sieves to collect the eggs in the same manner described in section 5.4.3. After rinsing and pouring the eggs into a 20L bucket partially filled with 1µm filtered seawater, fill the bucket up to 20L and place a tight-fitting lid over the top. Continue this process until a sufficient quantity of eggs has been collected. Avoid putting too many eggs in one bucket, just enough so as to give the water column an evenly dispersed granular appearance (no more than 100-150 eggs per ml).

The buckets should be transported back to the hatchery within 12 to 24 hours. Upon arrival at the hatchery, the eggs should be counted as described in section 5.4.4.

5.4.3 Collection of eggs

Eggs produced during a spawning should be collected by siphoning or draining the water from the spawning tank and passing it through a 100µm and 20 or 25µm mesh sieve. The larger mesh sieve, which will let eggs and sperm through, should be placed on top of the smaller one to collect most of the mucus, faeces and gonadal debris. The smaller sieve, which will retain only eggs, should sit in a water bath of continuously running filtered seawater.

Suspending the eggs in the water bath will help to keep the eggs clean and minimize contact with the mesh screen. When the sieve becomes clogged with eggs and mucus, or becomes too full, replace it with a new one or stop the siphoning. The clogged sieve should be gently rinsed with at least 1µm filtered seawater. Before

pouring the eggs into a partially filled calibrated container, pass them through a 100 µm mesh sieve to collect any additional debris.

Another batch of eggs may be added to the container provided the density does not become too great. As discussed in section 5.4.2, the egg concentration within a 20L container, for example, should not be greater than 100 to 150 eggs per ml. Once a container has been filled to a predetermined volume, place a tight lid over the top and set it aside. Eggs should be counted and placed in their incubation tanks within 1 hour of collection, even if broodstock have not finished spawning.

If the spawning has been strong, producing large quantities of eggs, keep only eggs which sink to the bottom. Separate these from the floating eggs by gently siphoning only the upper water column, leaving the bottom untouched. Refill the container, let the eggs sink and repeat this procedure until the container holds only eggs which sink to the bottom.

5.4.4 Egg counts

To count eggs within a container gently, but thoroughly, mix the egg-water suspension with a plunger (a plastic 500ml graduated cylinder with a base stand will work). Move the plunger up and down with one hand while at the same time inserting a 1ml pipette into the container to take a sample. The pipette should have the tip removed and recalibrated to measure 1ml. To draw the pipette out of the water without losing it's contents, press the index finger over the top end and lift the pipette out at a slightly horizontal angle. If the sample is greater than 1ml slowly release the finger to allow some of the contents to escape. Once the exact amount is obtained transfer the sample to a Sedgwick-Rafter counting chamber and slide the glass cover over the top. At least two samples should be taken per container to obtain an average count.

Place the chamber under the compound microscope at low magnification of 40x to 100x (4x or 10x objective with a 10x ocular eyepiece). Count the number of eggs per ml sample and take the average. Multiply this by the number of mls in the container to get the total number of eggs.

For example:

sample 1 = 95 eggs per ml
sample 2 = 105 eggs per ml
average = 100 eggs per ml
container size = 20 L
if 1000ml = 1 L,
then 20 L x 1000ml/L = 20,000ml

therefore: total number eggs per 20,000ml container = 20,000ml x 100 eggs/ml = 2,000,000 eggs

Keep the egg count per 1ml sample less than 200 as counts become inaccurate at high numbers. If the count per ml is greater than 200, dilute the egg water by increasing the volume in the container (or subsample) as this will make counting easier. To assist with counting use a mechanical counter.

5.5 Larval Rearing

At the Broome hatchery, eggs developed into a trochophore within 7 hours and D-shaped larvae within 18 hours after fertilization at temperatures ranging from 27-30°C. By the end of 24 hours, they had a fully developed digestive tract and their shells were approximated 80µm in length, along the greatest distance parallel to the hinge line, and 70µm in height, along the greatest distance perpendicular to the hinge line (Figure 9D). The shell shape for at least 80% of the larvae did not change until day 10, when they were approximately 115µm in length and 105µm in height. At this time an umbo (knob or bump) formed on both valves near the hinge line (Figure 9F). By day 18, larvae which measured 210 x 190µm or greater had developed red eye spots and by day 22, those which were 240 x 220µm had a foot (the pediveliger stage) (Figure 9H and I). Settlement and metamorphosis for the majority of larvae began any time after day 24 or when the larvae averaged 250 x 230µm.

Settlement lasted over eight days for some batches (day 24 to 32) with the plantigrade or post-larvae ranging from 256 x 226µm to 350 x 290µm (Figure 9K). By day 35, one week old spat displayed a well-developed mantle, gill fillaments, foot and byssal threads. They averaged 575 x 475µm and ranged from 430 x 330µm to 1 000 x 750µm (Figure 9L).

Approximately 10% of the larvae failed to develop umbones until day 16. In contrast the other 10% grew so rapidly that by days 12 to 14 they were pediveligers and averaged 253 x 218µm.

5.5.1 Egg and Embryo Incubation

Depending on the scale of operation, eggs which have been counted can be placed directly into large larval rearing tanks (greater than 1 000L) or placed in smaller volume hatching bins and transferred to rearing tanks when they have developed into swimming D-shaped larvae.

If eggs are to be placed in hatching bins, then they should be incubated at 27-28°C. After 18 hours, unfertilized eggs and deformed embryos will have settled onto the bottom of the bin and healthy larvae will be seen swimming in the water column. At this time the larvae can be siphoned from the water column, leaving the last 10 cm of water

above the bottom undisturbed so as to avoid the collection of unhealthy or dead individuals. Collect the larvae onto a sieve sitting in a water bath, rinse and concentrate them into a container of known volume and calculate the total number using the same procedure described in section 5.4.4.

It is possible to siphon the bins after 7-8 hours when the eggs have developed into trochophores, however, care should be taken since they do not have a shell and could be damaged if collected onto a sieve when concentrating them for a count.

Initial egg density of each incubation bin should not be more than 30 eggs/ml. To calculate the volume required, first determine the total amount of eggs spawned (see section 5.4.4) then divide this by the number of eggs/ml. For example:

required density	= 30 eggs/ml
total number of eggs	= 12×10^6
volume required	= $(12 \times 10^6$ eggs)/(30 eggs/ml)
	= 400,000ml
	= 400L

5.5.2 Tank Culture

After counting the larvae, they should be poured into a culture tank already filled with seawater filtered to 1µm and algal food. The size of the vessel will be dictated by the production scale of the hatchery and number of larvae. The procedures described here relate particularly to volumes of 500L and 1 000L. Tables 2 and 3 show stocking densities, number of water changes, culling periods, food concentrations per ml, and percentage composition of microalgal species in diet. They are provided as an example of the husbandry techniques used at the Broome hatchery.

The larvae should be reared at a constant temperature between 27° and 30°C. The actual temperature selected within this range is not important and will depend mainly on the ambient water temperature for that time of year. The larvae may be kept in a dark or dimly lit room. However, this is not critical except that it helps to maintain a constant culture temperature, inhibit unnecessary phytoplankton growth and facilitate observations on larval behaviour with a portable light source.

Tanks selected for larval culture should be first washed and disinfected. Although the type of disinfectant is not important, it should be used sparingly and only as directed by the manufacturer. The most common disinfectant used in hatchery operations is chlorine (sodium hypochlorite) at 10 parts per million (ppm). A commercial bleach (Clorox) or a disinfectant (Clorofos) used by the dairy and food industries is also suitable.

5.5.3 Stocking Densities

The initial density should be no more than 5 larvae/ml, and should be reduced to 2 larvae/ml by day 10 and 1 larva/ml by day 14. The density should be maintained at 1 larva/ml or 1 larva/2ml by day 18 or at the time of settlement. Densities should be adjusted during water changes or when larvae are being selected or culled on the basis of shell length (Table 2).

5.5.4 Water Changes and Aeration

Water changes may be flexible and dictated by the health and growth rate of the larvae or quality of the water (see section 6.2). They should be done by gently siphoning the culture water through a sieve placed in a water bath as described in section 5.4.3. Before returning the larvae back to their tanks, pour them into a precalibrated container. Take two 1ml samples and multiply the average count per ml by the total number of mls within the container to determine the total number of larvae present (see section 5.5.1). Adjust the stocking density according to Table 2 with the volume of the culture tank.

For culture volumes of 500L or less, it may be necessary to change half of the water on days 6 and 10, and all of the water on days 14 and 18 (Table 2). Slow growers may need the water changed on day 22 or just before settlement. For larger volume tanks of 1 000L or more, a complete water change can be made on days 5 and 15 or on days 10 and 18.

There may be no need to change the water in larger tanks except when reducing the stocking density or culling the fast from the slow developers. This technique is possible only if there are no signs of overfeeding (excess algae covering the floor of the tank) or deterioration in water quality. At the Broome hatchery, some larval batches reared in both 500L and 1 000L vessels were left until day 10 before a complete water change and then again until after settlement on day 28 or 33.

During the half water changes do not clean the tanks but siphon any debris which may have settled on the bottom (dregs). Make sure this water is kept separate from the larvae and discard it. During the half water change on day 10, transfer half of the culture's volume to a new tank to reduce the density to 2 larvae/ml. During full water changes wash the tanks with a brush after collecting the larvae.

All sieves should be washed in freshwater and disinfectant after they have been used or before they are used for another batch. When storing sieves make sure they stay dry and free of dust. These procedures will help prevent cross contamination between different batches and reduce the spread of disease.

Aeration of the tanks is not necessary until just before the settlement period from day 18 onwards (Table 2). This ensures that the food, larvae and metamorphosing post-larvae are evenly distributed throughout the water column after the introduction of settlement collectors. Air bubbles from the aerator should not be too fine as their only purpose is to gently stir the water to keep the larvae and algal food in suspension rather than to provide oxygen to larvae.

5.5.5 Selection of Larvae (Culling)

Culling is necessary to remove the slow growing larvae which compete for food with larger larvae and to prevent overcrowding during the settlement. Differences in individual growth may become great enough after day 10 or 12 so that fast growers can be easily isolated from slow growers during the third water change on day 14 (Table 2). The second culling should occur on day 18 or when the fast growing larvae have reached the eye-spot or pediveliger stage (Table 2). At the Broome hatchery this occurred on days 21 to 24 for some larval batches. A third culling may be required on day 22.

Culling should be done in conjunction with a water change. First, collect the larvae onto a sieve sitting in a water bath. The mesh should be fine enough to collect the smallest size range of larvae. Rinse and pour the larvae into a container before sorting them into different size classes. Next, slowly pour the larvae from the container onto a second, larger mesh sieve which has been placed in a water bath. Larvae which pass through are then placed into a separate container. Those which were collected on the second sieve are placed into a different container or, if necessary, poured onto a third, even larger mesh sieve. Each size class should then be counted and put into their appropriate culture vessels, which have already been cleaned and filled with freshly filtered, 1µm seawater.

When culling larvae for the first time, use two sieves with mesh sizes of 60 and 120µm, if only a few million larvae are being cultured. If tens of millions are being cultured, use three mesh sizes (60, 80 and 120µm) and, in both cases, discard larvae collected on the 60µm mesh.

During the second culling, use two (100 and 140µm) or three (100, 120 and 140µm) mesh sizes according to the number of larvae present. Discard those larvae collected on the 100µm mesh sieve if large numbers are involved. Larvae collected on the 140µm sieve should be placed into a settlement tank. If a third culling is required use 120 and 140µm mesh.

5.5.6 Feeding

Algae harvested as larval food should be young, clean and healthy. Before feeding the larvae, filter the algae through a 20-25µm mesh sieve to collect any debris or to reduce the clumping of cells. Also allow it to acclimate to the same temperature as the larval culture since a difference of 4° to 7° C may exist between the two culture media. Such a temperature "shock" could affect the behaviour and physical state of the algae and thus reduce its food value.

Microalgal species suitable as food for *P. maxima* larvae are: Tahitian strain of *Isochrysis galbana* (T. Iso); *Chaetoceros calcitrans* (Chaeto C.); *Chaetoceros gracilis* (Chaeto G.); *Nannochloropsis oculata* (Nanno.); and *Tetraselmis chuii* (Tetra C.). The golden-brown flagellate, T. Iso (6µm in long diameter) and the two diatoms, Chaeto C. and G. (3-4µm in long diameter) are the most nutritious foods. Larvae can be reared separately on either T. Iso, Chaeto C. or Chaeto. G. but it is better to provide them with a mixed diet. The two "green" species (Nanno. and Tetra C.) are less important. Nanno.(which previously was named *Chlorella* sp.) is the smallest of all these species (2-3µm in diameter) and is suitable food for young, pre-umbonal larvae (< 114µm). Tetra C. is larger (10-13µm longest diameter) and suitable for older larvae and spat.

The percentage composition of species used as food does not appear to be critical as long as T. Iso or either of the two Chaeto species make up at least 60-70% of the diet. An example of the amount and diet composition of algae fed to larvae, post-larvae and spat per day at the Broome hatchery is presented in Table 4. After day 10, the amount of food fed to the larvae may need to be varied according to how the larvae are growing and swimming. Pediveligers should not be overfed and feeding rates adjusted to their clearance rate. Excess algae should not be allowed to settle on the bottom of the culture vessel.

If the algal unit has a small production capacity, it may be more practical to rear larvae at lower temperatures (25-27° C) since their metabolism is slower at these temperatures and consequently they eat less.

5.5.7 Labelling and Measurements

All batches should be labelled or identified with the following information:
date of spawning; batch code; number of larvae. In addition, a written record should also be kept of this information, as well as, details of the spawning trial and total number of eggs spawned.

Approximately 50 larvae should be measured at least every four days or during a water change and culling period. Microscopic examination of the larvae provides information on their growth rate and general condition which will help with improving the feeding regime or to identify the presence of disease at an early stage before it spreads.

Table 2: Larval Husbandry Schedule

Day	Larval Density			Selection	Water		Clean	Change	Aeration
	Larval tank		Setting Tank	(mesh µm)	Change		Tank	Tank	Setting
	No. 1	No. 2	No. 1		half	full			Tank
1	4/ml								
2									
3									
4									
5									
6	2/ml				x				
7									
8									
9									
10					x				
11									
12									
13									
14	2/ml	1/ml		60, 80, 120		x	x	x	
15	(<170 um)	(>170 um)							
16									
17									
18	1/ml		1/ml	100, 120, 140		x	x	x	x
19	(<170 um)		(>200 um)						
20									
21									
22			1/ml	120, 140	x	x	x	x	x
23			(>200 um)		Setting	Larval			
24					Tank	Tank			
25									
26									
27									
28									
29									
30									

Table 3: Larval and Post-Larval Feeding Schedule

Day	Shell Length (µm)	Algal Density	% algal species		
	Mean (range)	cells/ml	T. Iso	Chaeto	Nanno
1	80 (65-85)	5 000	100	0	0
2		6 000	70	15	15
3		7 000	70	20	10
4		8 000	70	25	5
5	98 (90-105)	10 000	70	25	5
6		12 000	65	35	5
7		14 000	60	35	5
8		18 000	60	35	5
9		20 000	60	35	5
10	115 (90-130)	25 000	55	40	5
11		28 000	55	40	5
13		30 000	55	40	5
14		32 000	55	40	5
15	144 (115-250)	34 000	50	45	5
16		38 000	50	45	5
17		40 000	50	45	5
18	165 (120-280)	42 000	45	50	5
19		44 000	45	50	5
20	185 (140-300)	48 000	45	50	5
21		50 000	40	55	5
22		"	40	55	5
23		"	40	55	5
24		"	35	60	5
25	237 (150-315)	"	35	60	5
26		"	35	60	5
27		"	30	65	5
28		"	30	65	5
29		"	25	70	5
30	281 (220-325)	"	25	70	5

5.6 Settlement

When larvae are greater than 220µm in shell length, they develop two red eye spots (one on each valve). Two days later, or when they are 240µm in length, they develop a ciliated foot which can be used for swimming, probing and crawling (the pediveliger stage). At this time they are approximately four days from settlement and metamorphosis into post-larvae or plantigrades. The pediveliger becomes geopositive or photonegative during this period and begins to search for a place to settle. Larvae collected on 140µm mesh sieves (diagonal = 198µm) will be approximately 200µm or greater at this time and should be transferred to a settlement tank.

The settlement period can last for approximately one week. To determine when settlement has begun, use PVC pipe, grey plastic strips or dark glass plates placed into the culture or settlement tanks to attract the early settlers. Post-larvae or newly settled spat which are 250µm or more in shell length will attach to these materials and will be easy to distinguish. If necessary, they can be dislodged from the collector since attachment is not permanent at this time.

5.6.1 Settlement Techniques

There are two methods of settling larvae:

First, pediveligers are allowed to settle and metamorphose into post-larvae on the floor and walls of the settlement tank without collectors. With this method, the water must not be changed. Consequently, it is important not to overfeed the pediveligers or post-larvae since the uneaten algae will accumulate on the floor of the tank and lead to high bacterial levels which are not conducive to good larval health.

The number of larvae settling should be controlled by providing them enough bottom space. The best density is 0.5×10^6 to 1×10^6 pediveligers/m^3 (1 000L) of seawater.

By day 30, or when there are no pediveligers left in the water column, drain the settlement tank and wash the bottom with a fine spray (atomizer) to remove dead and unhealthy larvae. Count the number of settled spat by estimating the number of spat occurring on $10cm^2$ of the floor of the tank.

Collect the attached spat by gently brushing them off the tank's bottom with a soft paint brush and pour them into a small container holding five or six collectors made from synthetic fibres (e.g., plastic shade cloth), nylon netting or plates of grey plastic or dark glass. The spat should attach to the collectors within 30 minutes to 1 hour. When they have attached, transfer them to the nursery.

The advantage of this method is that mortality of spat after settlement is reduced because the stocking density or number or spat attached to each collector can be controlled. The disadvantage is that if the pediveligers and newly settling post-larvae are overfed, the tank bottom will become dirty and this will adversely affect the success of settlement.

Second, post-larvae are allowed to settle on collectors placed into the settlement tanks. The type of collector used can be similar to the above or a typical Japanese pearl net with the pocket or inside panel filled with unravelled nylon rope. Moderately vigorous aeration is required to keep the tank water constantly stirred since post-larvae lose their swimming ability. Larvae will attach to the collectors with the aid of mucus threads.

The advantage of this method is that it is easier because there is less handling of the post-larvae and newly settled spat. The disadvantage is that it is difficult to count the number of spat attached to the collectors.

5.7 Post-larval Culture

Techniques for rearing post-larvae or newly settled spat are similar to those used for larvae. Culture water should be changed every three days and at the same time the tank should be cleaned. The level of filtration of the seawater should be at least 10µm and aeration should be vigorous enough to ensure that there is adequate circulation through the collectors. The stocking density per tank should be about 0.5 post-larva/ml or less.

During settlement and the first two weeks afterwards, recently metamorphosed spat have low energy reserves and should not be exposed to undue stress. At this critical stage of development it is important to feed them according to their clearance rate and not to overfeed them. The food concentrations should be similar to those shown in Table 3 for larvae after day 21 (i.e , 50 000 cells/ml every 24 hours).

Daily concentrations may need to be increased up to 65 000 cells/ml and the total ration may need to be spread over two or three feeding periods. Experiments at the Broome hatchery have shown that if large amounts of food are poured in all at once much of the algae will settle out of the water column before the spat can eat it, or the spat will ingest but not digest the algae. In other words, they filter the algae from the water but do not utilize it as food. At the Broome hatchery, post-larvae were fed twice daily: early morning and late afternoon.

5.8 Spat Culture (nursery)

To maximize growth and survival of young spat or juveniles entering grow-out, bivalves are typically reared in a nursery system. The appropriate husbandry techniques adopted will depend on how different stocking densities and equipment affect growth and survival.

At Broome, spat were reared either at the hatchery in tanks (with or without downwellers) or at sea in circular, plastic mesh trays (see section 4.5). There was no noticeable difference in the growth or survival of spat reared in either tanks vigorously aerated or downwellers at the same density. However, the larger, downweller systems were more appropriate for culturing large numbers of spat (5 000 to 20 000).

Spat cultured in downwellers or tanks at a density of 4 individuals/100cm^2 grew an average of 9.6mm/mo in shell height (distance perpendicular from the hinge line to edge of the growth margin) while those stock at 25 individuals/100cm^2 averaged 6mm/mo. Spat cultured in circular, plastic trays at a density of 3 individuals/100cm^2 grew an average of 9.2mm/mo in shell height while those stocked at 7 individuals/100cm^2 averaged 7.3mm/mo. Faster growing spat cultured in the hatchery or sea nursery system increased in wet weight from 0.1 gram to 9.0 grams in a little over three months. Mortality of both spat and juveniles six months after settlement was 1 to 2% for those reared in the hatchery and 9 to 12% for those reared at sea.

The main advantage of the hatchery nursery is that mortality is reduced and juveniles can be grown in a controlled environment to a size large enough to fit into standardized nets used by the pearl culture industry. This approach does away with having to change the size of plastic mesh nets or trays as the juveniles grow or with having to routinely cull and clean these containers during grow-out. The disadvantage of this approach is that it may not be cost-effective on a large scale since it will strain the hatchery's algal production and water supply system. The sea nursery system is labour intensive and juvenile mortality is higher, but it may be more cost-effective as there are no expenses associated with feeding or supplying water.

5.8.1 Hatchery Nursery

Approximately 42 to 44 days after fertilization (two-three weeks after settlement), when the spat are 1.2mm or more in shell length, they should be transferred to the nursery. Tanks or downwellers should be cleaned every 3 to 6 days and the water changed at least every 2 to 3 days. The spat should be lightly sprayed with seawater to clean away faeces and pseudo-faeces (debris filtered out of the water by the gills but rejected as food).

The level of water filtration should be gradually reduced from 10µm to 20 or 30µm over the next two or three months so that spat are exposed to naturally occurring phytoplankton and silt. Completely unfiltered "raw" seawater is undesirable as it allows organisms to enter into the system which compete with the spat for food. Moreover, these organisms create a fouling problem (e.g., barnacles, tube worms, tunicates, other bivalves). However, before spat are transferred to the sea nursery or grow-out, they should be exposed for several days to unfiltered seawater. This will reduce any stress which may result from a change in water quality between the hatchery and natural environment.

The food concentration should be increased during this period. This increase in demand for food may place a strain on algal production but it can be ameliorated to some extent by increasing the amount of Nanno. and Tetra C. fed to the spat. In addition, as the spat become larger the bacterial load of the algal food becomes less important. Food not suitable for larvae because it has a higher level of bacteria or is too old can be used for spat culture instead of discarded. At a density of 25 individuals/100 cm^2, spat can be fed according to the schedule presented in Table 4.

Feeding times should correspond with clearance rates. Like post-larvae culture, feeding may be necessary three times a day or a drip feed system may have to be considered. During this culture phase spat require large amounts of food and will filter the water rapidly.

Spat should be culled from their culture tanks periodically as they grow to reduce the competition for food and space. When culling, spat will have to have their byssal threads cut with a razor if they are attached to a flat surface. If they are attached to nylon filament or plastic mesh it may be easier to transfer the entire bundle initially and separate the juveniles from the mesh later when they are approximately 50mm x 30mm or greater in shell dimension. The actual times to cull and transfer spat to sea nursery or grow-out will depend on the growth rate of the spat and production strategy.

Table 4: Feeding Schedule for Post-Larval and Newly-Settled Spat

Day	Shell Length (mm) Mean (range)	Algal Density (cells/ml) AM	PM	% Algal Species T. Iso	Chaeto	Tetra C.
43		26 000	34 000	30	60	10
:						
48	1.60 (1.00-2.48)	30 000	35 000	30	60	10
:						
53		30 000	40 000	30	60	10
:						
58		30 000	50 000	35	60	5
:						
63		35 000	50 000	35	60	5
:						
68	5.10 (3.00-14.00)	40 000	50 000	35	60	5
:						
73		45 000	50 000	40	55	5
:						
78		45 000	55 000	40	55	5
:						
83		45 000	50 000	40	55	5
:						
88		45 000	55 000	45	50	5
:						
93		50 000	50 000	45	50	5
:						
98	14.80 (3.50-20.00)	52 000	53 000	45	50	5
:						
103		53 000	55 000	50	45	5
:						
108		55 000	55 000	50	45	5
:						
113		55 000	55 000	50	45	5
:						
118		55 000	60 000	55	40	5
:						
123		55 000	60 000	60	35	5
:						
128		60 000	60 000	65	30	5
:						
135	41.70 (7.00-50.00)	60 000	60 000	70	25	5
:						
138		60 000	60 000	75	20	5

5.8.2 Sea Nursery

Spat can be transferred to the sea almost any time after post-larval culture (approximately one month after settlement). However, they must be well protected from small predators such as fish, worms and crabs. This will require small mesh enclosures which must be monitored routinely for fouling and damage. At Broome it was more practical to transfer spat two months after settlement when they were 15mm x 10mm. This avoided using small mesh enclosures which fouled and damaged quickly. When selecting a mesh size it is important that the spat can not slip through diagonally. Trays or cages should be cleaned once weekly or when necessary. A fine high pressure spray can be used, provided it is not too strong.

5.8.3 Counting Spat

Post-larvae and newly settled spat are difficult to count accurately unless several samples are taken to get an average. Small young spat (0.05mm to 1.0mm) which are dislodged from their collectors are first collected on a sieve and graded. The sieve fraction to be counted is then rinsed into a container and filled to a precalibrated volume. As with larvae, use a plunger to keep the spat in suspension and take a 2ml sample. A 2 or 3ml pipette should be wide enough to collect the spat provided the tip has been removed to prevent clogging. The sample must be taken quickly so that the spat do not have a chance to settle out of the water column or adhere to the inside wall of the pipette. Pour the 2ml sample into a petri dish with a grid etched on the bottom and count the spat using a mechanical counter. After several samples, take the mean and divide this by the sample size to obtain the mean per ml. Multiply this number with the volume of the container in mls to calculate the total number of spat. For example:

mean count	= 75 spat/2ml = 37.5 spat/ml
volume of 10L container	= 10L x 1 000ml/1L = 10 000 ml
total number of spat	= 37.5 spat/ml x 10 000ml = 375 000 spat

Larger spat can be counted indirectly using the weighing method. After they have been removed from their collectors during a culling, place them on a sieve and allow the water to drain for several minutes to remove excess water. Take a small sample of about 150-200 spat and weigh them to the nearest 0.01g. Next spread the spat out and count the actual number within the sample. Repeat this procedure several times to get the average weight of each spat. Finally, weigh all the spat on the sieve and calculate the total number of spat present by dividing the total weight by the average weight of an individual spat.

5.9 Algal Production

Algal culture is perhaps the most crucial operation of the hatchery since, without food, larval and spat culture would not be possible. Algae must be grown under hygienic conditions and harvested during their active growth phase to ensure that they are nutritious and not heavily contaminated with bacteria. Generally, "young, clean" algae are reserved for larvae and newly settled spat. Older cultures can be used for larger spat and for broodstock conditioning.

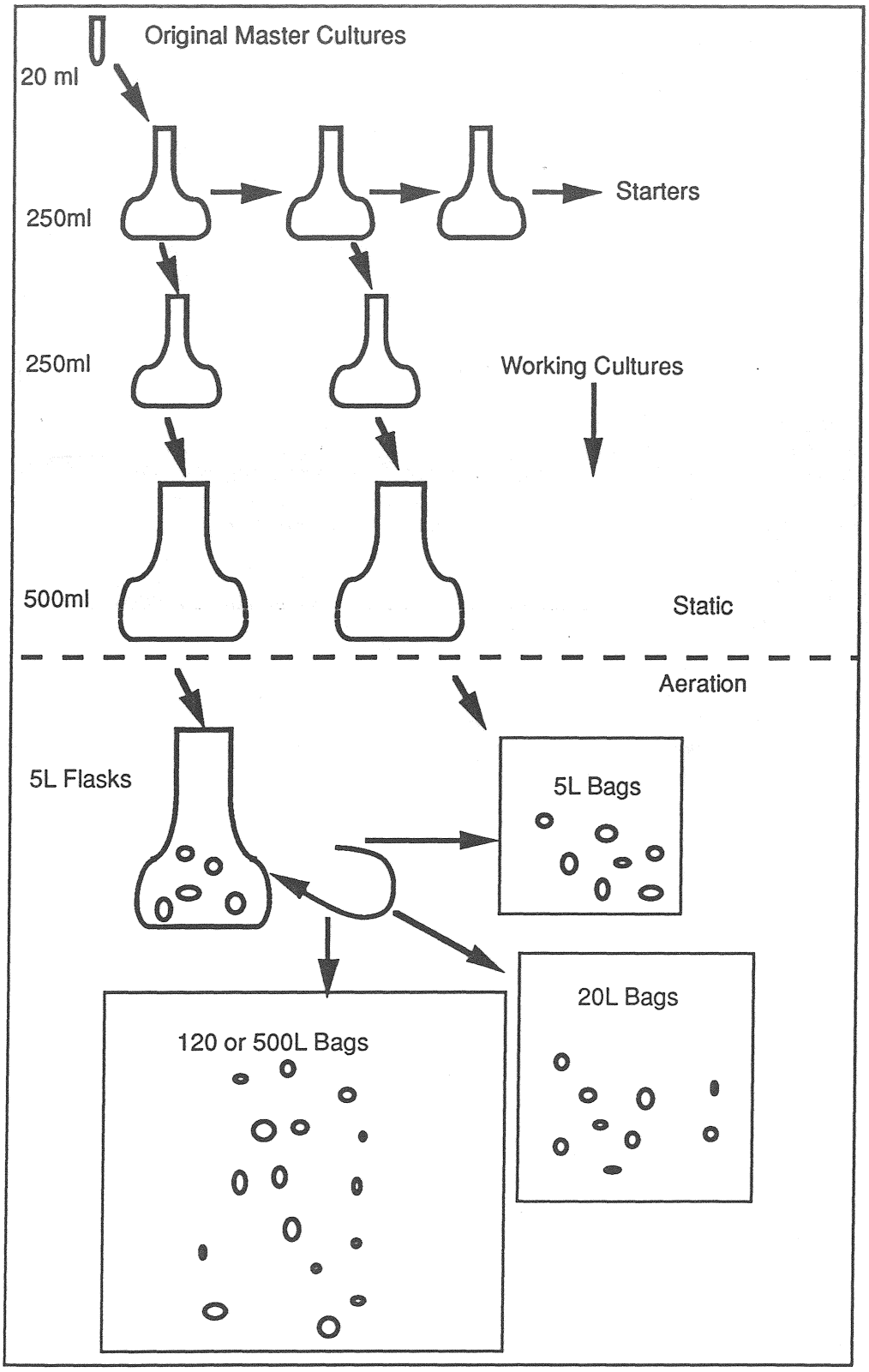
The preparation, production, maintenance and hygiene of microalgae at Broome will not be described in detail here since they are similar to those already published elsewhere (e.g., Stein (1973), Guillard (1983), Lewis *et al.* (1986)). The facilities at Broome (section 4.6) and techniques employed which are relevant to pearl oyster husbandry will be discussed only.

The algal species mentioned in section 5.5.6 can be obtained from most state universities or from C.S.I.R.O. Marine Laboratories, G.P.O. Box 1538, Hobart, Tasmania, Australia 7001. Monocultures obtained from these institutions should be axenic (free of foreign matter) and maintained in that condition. These "master or starter" cultures may collapse or become contaminated from time to time so it is best to use a local supplier who can provide replacements rapidly.

5.9.1 Production Strategy

Algal monocultures are grown either in sequential batches of increasing size or semi-continuously using 200L and 500L cultures (Figure 14). Starters (less than 500ml) are used to inoculate 5L flasks and these, in turn, are used to inoculate new 5L cultures or 20L, 200L or 500L bags. If 5L bags are used, they should not be used to reinoculate new 5L bags and should be either harvested or used to inoculate larger bag cultures, providing their bacterial levels are low. Never inoculate a smaller culture with a larger one. All cultures in glass flasks which are 5L or less in volume should be axenic. Always keep records of the batches (inoculation dates, cell densities, nutrients used, bacteria levels, etcetera).

Figure 14: Inoculation Scheme (all flask cultures should be axenic)



5.9.2 Preparation of culture medium

Seawater used for starter cultures and 5L glass flasks should be submicron filtered and autoclaved at 121^{0}C and 103.4 Kpa (15 psi or 1 atmosphere) to keep monocultures free of bacteria. Volumes of less than 500ml will be sterilized in 15 minutes at these conditions while volumes of 1L and 4.5L will require 20 minutes and 1 hour, respectively.

Seawater for bag cultures can not be autoclaved but should be UV treated and filtered.to the submicon level. Although this procedure does not reliably eliminate bacteria, it does reduce their numbers to such low levels that the algae produced are generally suitable food for spat and broodstock. If bacterial levels are excessively high, however, it may be necessary to disinfect the water for 12 hours with 1 ml sodium hypochlorite per 10L. Then aerate and dechlorinate the water with 0.5ml of 1N sodium thiosulphate per 10L two hours before inoculating the bag.

Disinfecting the seawater with chlorine should be used with discretion as it will not routinely kill all the bacteria. The few bacteria that do survive, will be chlorine resistant and flourish without any competition for limited resources. Algal cultures with seawater prepared this way invariably become contaminated with high levels of bacteria. Therefore, it may be more practical to culture algae in seawater already contaminated with a mixed bacterial flora which are less likely to reach dangerously high levels because of competition (Lewis *et al*, 1986). Furthermore, if algae from 5L bags, which are already contaminated, are used to inoculate 200 and 500L bags , then it would be a waste of time and chlorine to disinfect the seawater.

Before inoculation, recently filtered and/or autoclaved seawater must be enriched with nutrients to enhance rapid algal growth. Although there are a variety of nutrient recipes, those used at Broome were based on Guillard's f/2 medium and modified (mod) F (see Tables 5 and 6). All stock solutions for these recipes are bottled and stored in the refrigerator.

To enrich seawater for 20L cultures or less use Guillard's f/2 medium. Add 1 ml of each f/2 stock solution listed in Table 5 (1 to 4) to each litre of seawater, except for the vitamin stock (5) which should be added at 0.5ml per litre of seawater. For starter cultures the nutrients are added directly to the seawater before it is autoclaved. Phosphate, however, can be autoclaved separately from the seawater to prevent precipitation. Add 1ml of phosphate to 3ml of distilled or deionized water, autoclave for 15 minutes and add aseptically to seawater later. For 5L and 20L cultures, the stock solutions can be mixed together, autoclaved for 15 minutes and added to the seawater aseptically just prior to inoculation. The phosphate can be kept separate from the other stock solutions.

If the seawater is being prepared for flagellates, metasilicate (stock solution 4, Table 5) is omitted. Unlike flagellates, diatoms (Chaeto C. and G.) are given metasilicate and sometimes ammonium chloride instead of sodium nitrate. Otherwise the nutrient media for diatoms and flagellates are the same. Vitamins (stock solution 5) should be acidified (pH 4.5-5.0) with HCl to keep them stable during autoclaving.

For 200L and 500L cultures mod F is used to enrich the seawater at 0.5ml of stock solution per litre of seawater (Table 6). Trace metals are not used in mod F as they are assumed to be added with the tap water. It may be necessary to add TRIS buffer to the stock solution to adjust the pH to 4.5-5.0. Autoclave the amount of mod F required for 15 minutes before adding it to the seawater culture. For diatoms a stock solution of metasilicate (stock solution 4, Table 5) is also added at 1ml/L seawater.

Table 5: f/2 Nutrient Medium (to each litre of seawater add 1ml stock solution, except for vitamin stock add 0.5ml/L)

STOCK SOLUTIONS	CONCENTRATION (g/L distilled H_2O)
1. NITRATE ($NaNO_3$) *	75
2. PHOSPHATE ($NaH_2PO_4H_2O$)	5
3. TRACE METALS	
METAL CHELATE (Na_2EDTA)	4.36
FERRIC CHLORIDE ($FeCl_36H_2O$)	3.15
COPPER ($CuSO_45H_2O$)	0.01
ZINC ($ZnSO_47H_2O$)	0.022
COBALT ($CoCl_26H_2O$)	0.01
MANGANESE ($MnCl_24H_2O$)	0.18
MOLYBDATE ($Na_2MoO_42H_2O$)	0.0063
4. METASILICATE ($Na_2SiO_39H_2O$)**	15-30
5. VITAMINS	
CYANOCOBALAMIN (B12)	0.001
BIOTIN	0.001
THIAMINE HCl (B1)	0.2

* For some diatoms 106 g/L NH_4Cl is used as the nitrogen source

** Add for diatoms only

Table 6: Modified F Nutrient Medium (to each litre of seawater add 0.5ml of stock solution and for diatoms also add 1 ml of metasilicate)

STOCK SOLUTION	CONCENTRATION (g/L Tap H2O)
NITRATE	300
CALCIUM GLYCEROPHOSPHATE	10
METALCHELATE	7.5
FERRIC CHLORIDE	10.5
VITAMINS	
CYANOCOBALAMIN	0.001
BIOTIN	0.001
THIAMINE HCl	0.2

5.9.3 Aeration

Starter cultures or those less than 1L are kept static. Cultures of 5L or greater are aerated with oil-free, submicron filtered air enriched with CO_2. The air is only enriched with CO_2 (0.45 -2% by volume) during the light period or photosynthetic cycle and not during the dark period or respiration cycle. Carbon dioxide is considered a nutrient but can be toxic at high levels.

5.9.4 Inoculations and harvesting

Small 200-500ml volumes of monocultures are maintained by regularly inoculating new sterile media from 2-3 week old starters. Transfers are made aseptically inside a laminar flow cabinet by first flaming the neck and mouth of the donor and receiver vessels before rapidly and neatly pouring 50ml to 100ml of inoculum into the receiver. When finished quickly replace the cotton bung and alfoil cover. Any hospital, technical college or university will be able to provide details on aseptic laboratory techniques.(also see literature cited).

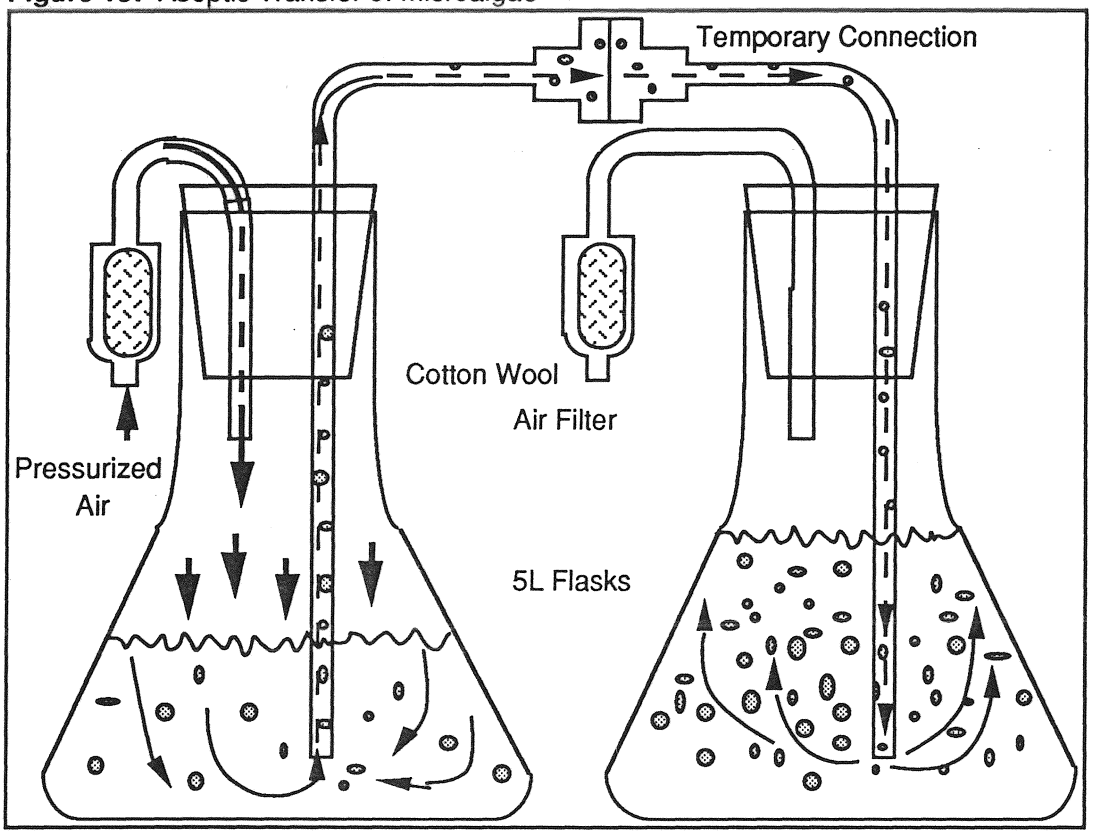
New cultures of 5L or greater are inoculated every 3 or 4 days by using air pressure to aseptically transfer algae from the donor to receiver via a temporary connection made between their air-intake lines (Figure 15).

Algae from 5L flasks or bags are harvested within 3 to 5 days after inoculation. The cell density per ml is routinely counted to determine the volume of algae required per feeding. This is achieved by taking a dilute sample of algae and using a haemocytometer to count the cells under a microscope at 100x, 200x or 400x magnification (see Dupuy *et. al.*,1977, or Guillard, 1978, for details).

In addition to counting, algal cells should be microscopically checked for microbial contamination and changes in cell morphology. Any cultures which do not appear physically normal, or which smell, should be discarded immediately.

At the Broome hatchery, cell densities in 5L bags typically ranged from 2.5 x 10^6 to 4.5 x 10^6 cells/ml for T. Iso, 10 x 10^6 to 20 x 10^6 cells/ml for Nanno. and 0.4 x 10^6 to 1.5 x 10^6 cells /ml for Tetra C. by the third day after inoculation. Densities for both Chaeto C. and G. ranged from 3 x 10^6 to 13 x 10^6 cells/ml by the third day.

Figure 15: Aseptic Transfer of Microalgae



5.9.5 Cleaning Equipment

All glassware and paraphernalia associated with algal culture must be washed with biodegradable detergent and hot water, rinsed in hot and cold water, a weak acid solution (20-30%) and finally deionized water before being dried. Store all equipment in dust-proof shelves. All containers must be sealed with tops, alfoil and bottled nutrients should be sterilized before storing.

The housing units for filter and pipe work and air lines should be washed and disinfected with freshwater and chlorine (10ppm) routinely. Filters should be flushed in freshwater and then left to dry in an oven set at 50-60°C.

6. Hygiene (Bacterial Monitoring)

Strict hygienic practices are necessary to keep bacterial levels in the algal, larval and spat cultures low and to ensure that they are *Vibrio* free. Routine monitoring of these cultures should be performed using standard microbiological techniques (Stein, 1973 and Lewis *et al.*, 1986). Surfaces of equipment should be kept clean either mechanically or chemically with disinfectants (e.g., 75 - 95% alcohol (v/v), hot (70°C) soapy water, chlorine, acid).

Marine agar (MAV), and seawater agar with vitamins (SWAV) are used as general growth media to qualitatively and quantitatively test for the presence of marine bacteria. Presumptive marine *Vibrio* species are identified using T.C.B.S. agar (thiosulphate-citrate bile salt-sucrose) made with additional sodium chloride to bring the salinity to that of seawater. Preparation of these media, the equipment required and procedures for testing are described by Lewis *et al* (1986).

To qualitatively test for bacteria, a sterilized swab (cotton bud) is used to sample a culture or surface and inoculate an agar plate. To quantify the presence of bacteria, dilute samples are collected onto membrane filters (pore size 0.45µm and 0.2µm) and placed onto the surface of the agar plate. Alternately, a dilute sample is placed directly onto the surface of the agar and spread with a sterilized L-shaped glass rod. Inoculated plates are then labelled with details of the sample and dilution. After one or two days the colonies are scored or counted to estimate the bacterial cells per ml. Inoculations should be done aseptically.

Starter and 5L flask cultures should be free of bacteria. At Broome, the level of bacteria present in 5L and 20L bags harvested for larvae and spat ranged from 10^1-10^4 and 10^1-7 x 10^4 bacteria per ml, respectively. Algae from 120L and 500L bags used for spat and broodstock with levels which ranged between 10^5 and 10^6 should be discarded. As a general rule of thumb, cultures with 10^6 bacteria per ml or more are harmful to bivalve larvae and juveniles and should be discarded.

The level of bacteria for the settlement tank, and algal delivery pipe at Broome typically ranged from, 2 x 10^3 -4.66 x 10^3 and 2 x 10^2-2.98 x 10^3 bacteria per ml, respectively. Compared to temperate waters these levels were up to two orders of magnitude higher. The bowser connected to the UV unit and submicron filters (Figure 8) which provide seawater for algal cultures had a range of 0-20 bacteria/100ml.

7. Husbandry Problems

7.1 Antibiotics

To control an outbreak of disease in larval cultures or to reduce the bacterial level in algal cultures, antibiotics can be used with discretion. They should not be used indiscriminately or as a method of preventing disease, as there is the possibility of selecting for an antibiotic-resistant bacterium. Eggs and embryos should not be exposed to antibiotics as they can be adversely affected.

If antibiotics are used then the most common treatment is a solution of penicillin G and streptomycin sulphate at 10 000 to 15 000 units of penicillin and 13 to 20mg (1 000 mg = 1g) of streptomycin per litre of seawater. Less commonly used antibiotics are neomycin at a concentration of 100mg per litre or a wide spectrum antibiotic such as chloramphenicol sodium succinate (chloromycetin) at 8 to 10mg per litre.

Since it is the volume of water which is important and not the larval density, larvae can be collected into a small container before exposing them to the antibiotic(s). After 1 to 2 hours, rinse the larvae with UV treated seawater before returning them to a clean tank. Algal cultures should be exposed to the antibiotics for 2 to 3 hours before feeding it to the larvae or spat.

Larvae are sensitive to fungicides and if infected with fungus should be treated by exposure to warm temperatures of up to 34° C for 12 to 24 hours. Fungal infections in algal cultures are treated with cycloheximide (final concentration 75mg per litre). Do not let your skin come in contact with this fungicide. If this occurs, wash immediately with soap and water.

7.2 Broodstock Management

This aspect of artificial propagation is often the most difficult and neglected. It is, however, the most important in terms of determining the viability of progeny reared in the hatchery.

7.2.1 Sick or Moribund Broodstock

Outbreaks of disease or moribund (dead or dying) oysters are unusual amongst broodstock kept at low densities at sea or in the hatchery. If signs of sick or unhealthy oysters are discovered, isolate them from the others immediately using the same practices adopted by culture farms. If in the hatchery, close down the conditioning or holding tank and wash out the entire broodstock unit with Clorofos or chlorine (see section 5.5.2). The cause of death may be due to disease but more than likely it is the result of poor environmental conditions which cause stress (i.e., high temperatures, widely fluctuating salinities, desiccation, poor water circulation, and poor quality water and food). Healthy oysters should show signs of shell growth, feed and defecate regularly, close their shells rapidly when disturbed and display fully extended, thick mantles when feeding.

7.2.2 No Spawnings or Only One Sex Participating

Oysters which do not spawn should be sexed and scored for gonadal development and then closely monitored for an improvement in ripeness. Occasionally, when ripe oysters do not release any gametes they can be returned to their conditioning tanks or to sea and tried again the following day. If only one sex spawns it is usually the male. Check the gametes under the microscope for motility and shape. Slow moving sperm is a sign that the males are not fully ripe and in this condition the sperm will not trigger females to release eggs. Also check female oysters as they may not be sufficiently ripe.

7.2.3 Release of Poor Quality Gametes

Poor quality spawn is often a sign that the oysters have been stressed. Check the husbandry protocol and history of when, where and how the broodstock were collected. These oysters should probably be left for a few weeks and closely monitored for improved gonadal development before attempting to spawn them again. The most reliable method for producing strong spawnings and viable gametes is to collect fresh broodstock from the wild at the beginning to the spawning season.

7.3 Larval Culture

7.3.1 Diagnosis of Healthy and Diseased Larvae

In addition to measuring the shell growth of larvae, they should be inspected microscopically at least once every two days to monitor their health and development. Healthy larvae swim actively and filter algae from suspension quickly. They grow rapidly, have a well developed velum, clean and smooth shells and are brown-gold in colour.

Sick larvae will grow slowly, appear pale in colour, and often have debris attached to the surface of their velum and shell. Protozoans will be found swimming or crawling over the outside of their shells. The presence of protozoans are good indicators of bacterial infestations since they feed on bacteria. Moribund larvae and empty shells will be swarming with bacteria and protozoans.

Occasionally larvae will become infected with fungi. When this occurs thin thread-like mycelia can be observed protruding from the shell or ramifying throughout the viscerum.

Outbreaks of disease will cause a dramatic loss of larvae with only a few survivors remaining after 1 or 2 days. At Broome, disease outbreaks were rare and usually related to overcrowding and foods contaminated with bacteria. Any infected larval batches should be disinfected and discarded. Tanks, bins, sieves, aerators, piping and pumps should be thoroughly washed, disinfected and l eft to dry for several days before using them again.

7.3.2 Crashed Cultures

For some unknown reason larval batches will crash. Why this occurs may be related to subtle changes in weather or to early spring (pre-cyclonic) phytoplankton blooms.which affect the water quality. Generally conditions which cause crashes come and go quickly.

7.3.3 Slow and Fast Growth

Several factors can be responsible for variations in growth rate. Some of the obvious ones are temperature, feeding rates and quality of the food (age, type, bacterial load). Less obvious factors may be related to viability of the eggs due to broodstock health.

Algal cultures should be checked to make sure they are not contaminated with foreign algae (blue-green or brown-green species) and bacteria. Algal food contaminated with a blue-green algae can be detected when the larval culture tank develops light green scum on the walls of the tank at the water-air interface. Food contaminated with brown-green algae develops tiny green-brown globules near the surface. Improved water filtration and the use of pure algal cultures will eliminate this problem.

Seawater for larval cultures should contain only larvae and algal food. If large blooms of protozoans are present, then competition for space and food will occur which will slow larval growth. To avoid this problem seawater should be filtered to the 1µm level.

Natural variations in individual growth of larvae should be expected but slow growth rates can be avoided by separating the fast growers from the slow growers or by increasing the food ration to lessen competition. The food ration should be set so larvae grow between 6 and 10µm per day.

7.3.4 High Levels of Mortalities and Abnormalities

Mortalities which are not preceded by periods of slow growth indicate poisoning or disease. Mortalities following periods of prolonged slow growth are due to poor husbandry. High levels of abnormality are usually related to poor quality gametes.

7.3.5 Larvae on Bottom of Tank

Larvae which are inadequately fed will tend to settle out of the water column. When properly fed they should rise from the bottom and return to swimming. If this does not happen then the culture has crashed.

Do not disturb settled-out larvae as this will stir-up the bottom sediment and stress the larvae. If piles of larvae are found on the tank bottom after feeding, collect some and observe them microscopically. Take a dilute sample of the larvae and sediment to inoculate a marine agar and T.C.B.S. plate. This will quantitatively test for the general bacterial level and for *Vibrio*. Sick or moribund larvae should be siphoned from the bottom and discarded. Larvae remaining in the water column should be collected on a sieve, treated with antibiotics if necessary and transferred to a clean tank of seawater.

7.3.6 Sediment on Bottom of Tank

When noticeable amounts of sediment accumulate on the bottom do not disturb it but dispose of it during the next water change. The presence of sediment could mean the larvae are being overfed or that the algae fed to the larvae are unused and dropping out of the water column. To prevent or reduce the amount of drop-out, acclimate the algae to the temperature of the larval culture before pouring it in.

7.4 Post-larval/Spat Culture

Young healthy post-larvae and spat grow rapidly. Post-larvae and newly settled spat will display a smooth, clean dissoconch (adult shell) while spat will have an abundance of large finger projections on the shell surface. Their viscerum and mantle should be visible through the shell.

7.4.1 Cleaning/Fouling

Fouling organisms can be a problem if they become too numerous because they will tend to crowd and smother spat as well as compete for their food. The net result will be to stress the spat, possibly causing death or extra maintenance work. To kill small fouling organisms attached to the surface of spat shells, expose the spat to freshwater for 10 minutes. In the hatchery, large fouling organisms can be avoided by filtering the water for the nursery system to the 50µm level. Routine cleaning of spat cages used for sea nursery culture will be required to avoid fouling.

8. General Operation Schedule

To summarize the general operations at Broome, a time schedule of the procedures for rearing a single batch of larvae to settlement are outlined below. The actual schedule implemented at a commercial hatchery will vary slightly due to differences in the site, scale of operation and experience of the staff. Any effort at streamlining this schedule (and thus optimizing productivity) will depend on management's ability to keep accurate records, and identify and eliminate problem areas quickly.

Day before: Clean and fill larval tanks and hot water tanks for spawning the next day; select broodstock.

Day 0: Clean and spawn oysters; count and place embryos into hatching bins or larval tanks.

Day 1: Count and distribute D-shape larvae to tanks; feed larvae.

Day 2-5: Inspect and feed larvae daily according to feeding regime.

Day 5-10: Half water change of larval tank; measure and reduce stocking density; feed larvae according to schedule or clearance rate.

Day 11-15: Full water change, clean and change tanks; select larvae greater than 170µm, count and place in separate tank; feed larvae.

Day 16-20: Full water change, clean and change tanks; prepare collectors, select for larvae greater than 200µm, count and place in aerated settlement tank; feed larvae twice daily

Day 21-25: Full water change, clean and change tanks: prepare more collectors if necessary, select for larvae greater than 200µm, count and place in aerated settlement tank; return slow growers (< 200µm) to larval tank; feed larvae and post-larvae twice daily according to schedule or clearance rate.

Day 26-30: Change settlement tank water every third day; feed post-larvae twice daily.

Day 30-41: Change and clean settlement tanks; transfer post-larvae to nursery

To gain an idea of routine duties and tasks involved in operating a pilot-scale pearl oyster hatchery on a seasonal basis, a list of some of the obvious chores and man hours/week to attend to each chore is presented below. The times presented are based on a two-man hatchery operation at Broome using manual or semi-automatic facilities. The staff operated for a minimum of 100 man hours/week.

Chore	*Man hours/week*
Maintaining broodstock	10
Maintaining algal cultures	24
Preventive and corrective maintenance	8
Spawning	10
Feeding larvae and spat	12
Handling spat	4
Changing culture water	12
Cleaning tanks	4
Cleaning collectors, downwellers, trays	4
Calculating algal and larval densities, record keeping	6
Miscellaneous	6

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