A manual of techniques for culturing paua, *Haliotis iris*, through to the early juvenile stage

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Abstract

Tong, L. J., Moss, G. A., Redfearn, P., & Illingworth, J. 1992: A manual of techniques for culturing paua, *Haliotis iris*, through to the early juvenile stage. *N.Z. Fisheries Technical Report No. 31*. 21 p.

The prospective paua farmer is introduced to the biology of paua. The principles of paua rearing are discussed. These include broodstock management, spawning, fertilisation and larval rearing, settlement and early juvenile growth, and ongrowing to a marketable size. Cultivation methods and equipment are described.

A detailed recipe of equipment, timing, and techniques is given for spawning adult paua, rearing and settling larvae, and growing postlarvae through to 5 mm.

Introduction

Abalone populations are frequently affected by poor recruitment and overfishing (Mottet 1978, Sainsbury 1982, Murray & Ackroyd 1984, Shepherd *et al.* [1992]). Enhancement is an option that may supplement erratic recruitment, and outplanting of hatchery reared abalone has been carried out in several countries (Tegner & Butler 1989, Momma [1992], Schiel [1992]). Rearing techniques have also been applied to farming abalone (Hahn 1989a). In the last 20 y hatcheries and farms have been developed in Taiwan, Korea, the U.S., France, Mexico, Australia, and New Zealand (Hahn 1989a, Shepherd *et al.* [1992]).

The culture techniques used vary between countries and between institutions and farms (Hahn 1989a). At the MAF Fisheries Mahanga Bay hatchery in Wellington Harbour we have developed systems for producing larval and juvenile abalone, and results and work in progress have been described by Illingworth (1986) and Tong (1982, 1983, 1986). Techniques have been developed for holding broodstock, spawning, and larval rearing (Tong & Moss [1992]) and settlement (Moss & Tong 1992a, 1992b). Commercial scale settlement systems and larval and juvenile transport techniques have been developed (Tong & Moss [1992]). The techniques at Mahanga Bay were developed to minimise labour costs for application to commercial operations (Henriques *et al.* 1989).

There are three species of abalone in New Zealand waters: the black or rainbow paua, *Haliotis iris*; the yellowfoot or queen paua, *Haliotis australis*; and the whitefoot or virgin paua, *Haliotis virginea*. Only the black and yellowfoot species have potential for farming or reseeding. The whitefoot has a very slow growth rate (authors' unpublished data).

This manual has been written for prospective culturists, and it describes in detail the procedures to be followed for producing 5 mm paua. Techniques for ongrowing to a larger size are briefly outlined. Details of the technology used in other countries are given by Hahn (1989a) and Shepherd *et al.* [1992].

Biology of paua

Paua (*Haliotis iris*) are marine snails belonging to the class Gastropoda in the phylum Mollusca. The Haliotidae are universally called abalone. The shell is ear-shaped, with a large opening, and is typically perforated with a series of holes. The excretory products, as well as sperm and eggs, are carried out through the holes in the respiratory current, which enters at the front of the shell and flows over the gills (Figure 1).

Paua have a large muscular foot, which is attached to the shell by a large central muscle. The foot, which is the favoured part for eating, is used for creeping and attachment and can exert a very strong suction on to a rock surface. It is surrounded by the epipodium, a circle of sensory tentacles. Paua lack a blood clotting mechanism, and, if cut or damaged, may slowly bleed to death.

The gonad develops around a large digestive gland, which branches off the gut. When the foot and mantle are pulled back, the colour of the gonad and hence the sex of the animal can be determined. The ovary is green and the testis creamy white in mature animals.

Very small paua (less than 5 mm) will graze on microscopic plants or diatoms and ingest and utilise a range of bacteria and other microscopic organisms, as do other abalone (Garland *et al.* 1985, Hooker & Morse 1985, Norman-Boudreau *et al.* 1986). Larger juveniles and adults eat seaweeds and are generally opportunistic feeders (Poore 1972a) and will stay in one area catching and feeding on driftweed. Food is taken in at the mouth and broken down into small pieces by a ribbon of rasping teeth called the radula.

Paua are long lived and slow growing (Poore 1972b, Sainsbury 1982, Murray & Ackroyd 1984). An individual can take 6 y to reach the legal catch size of 125 mm, but in most areas the average time taken is about 10 y. Paua mature when they are 70–90 mm long (Poore 1973).

Spawning usually occurs in late summer to early autumn, when sea temperatures are falling (Poore 1973, Sainsbury 1982, Wilson 1988). Paua can spawn at other times of the year (authors' unpublished data), and in the south of New Zealand they may spawn earlier than in the north. Paua are broadcast spawners and release sperm and eggs directly into the surrounding sea water. Legal sized paua can spawn up to 11 million eggs in a season (Poore 1973). The eggs are about 230 µm in diameter, but the sperm are much smaller, with the head being about 4 µm and the flagellum 38 µm in length (McCardle 1984).

Fertilised eggs develop rapidly into free swimming trochophore larvae (Figure 2). Larvae change to become shelled veligers, which develop a foot, operculum, eye spots, mouth, and radula (Tong 1982). At the end of a free swimming period, larvae fall to the bottom and explore the rocky substrate. If the surface is suitable for settlement, larvae lose the ability to swim, and they crawl over the surface and start feeding. Paua preferentially settle on to the encrusting seaweed *Lithothamnium* (Tong *et al.* 1987, Moss & Tong 1992a), commonly referred to as "pink paint". The postlarval shell develops, and the first respiratory hole appears in the shell at about 2 mm in length.

Small juveniles live in the subtidal zone, but at about 10–15 mm they move into the intertidal zone and under small boulders and rocks (Poore 1972a). At maturity, paua move into deeper water.

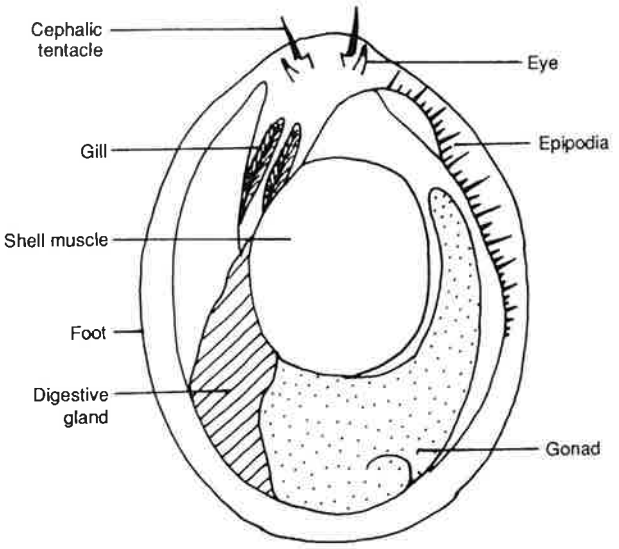

Figure 1: Diagram of a paua with the shell removed.

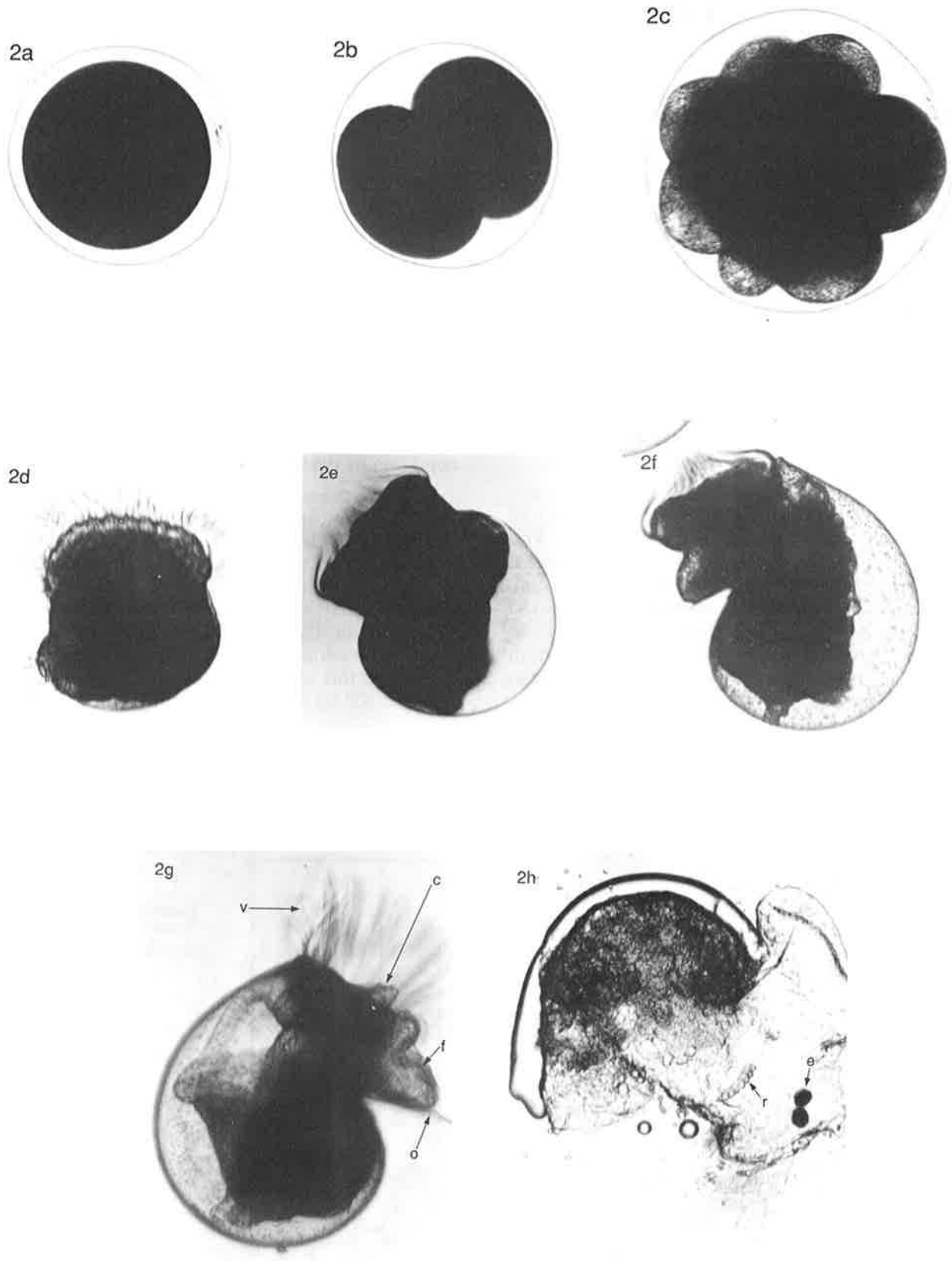

Figure 2: Egg and larval development of paua: a, fertilised egg; b, 2 cell stage — first division; c, 12 cell stage; d, trochophore larva; e, early veliger; f, mid veliger; g, late veliger (c, cephalic tentacle; f, foot; o, operculum; v, velum); h, veliger squash (e, eyespots; r, radula).

Principles of paua rearing

Broodstock management

Local populations of paua may vary in their ability to spawn from month to month and from year to year (Poore 1973, Sainsbury 1982). Thus, a hatchery cannot rely on collections of broodstock to provide regular supplies of larvae.

Our procedure is to collect broodstock in late summer to early autumn and hold them in the hatchery until required for spawning. In the system described below we had more spawnings and obtained larvae over a longer period than we could from wild caught paua (authors' unpublished data).

To hold broodstock animals you need the correct permits and documentation (Appendix 1).

At the Mahanga Bay hatchery broodstock are held at ambient temperatures (10–20 °C) on a 12/12 photoperiod in two 1000 l tanks (Figure 3). The tanks are shaded, so that light levels are maintained at less than 5 micro Einsteins per metre per second. Broodstock have been held at densities of 80–180 adult paua per tank, depending on the size of the animals. Paua ideally should be longer than 100 mm, at which length egg production per animal on average exceeds 100 000 (Poore 1973).

The tanks have a water-jet circulation system which delivers 11 l of water per min through each of eight 4 mm jets. The jets are set at an angle of 50–60° to the tangent, 55 mm from the centre of the tank. This provides water turbulence at the walls and exposes the paua to flowing, well-oxygenated water. The water is recirculated through a reservoir tank to which fresh sea water is added at a rate that changes the water in the system five times a day.

Once a week in winter and twice a week in summer the paua are fed locally abundant brown seaweeds such as *Macrocystis pyrifera*, *Lessonia variegata*, and *Durvillaea antarctica*. The seaweed is held close to the side of the tank by a sleeve of 70 mm plastic mesh, and it is maintained in good condition by the turbulent water flow.

In Japan the amount of seaweed eaten per day is monitored and is used to indicate the condition of the abalone (Uki & Kikuchi 1984, Hahn 1989b). This has not been tested in New Zealand with paua. Adult abalone can eat up to 10% of their body weight per day in summer; in winter they are likely to eat nearer 4% (Uki & Kikuchi 1984). We feed twice as much seaweed as will be eaten between feeds, and this equates to 12–16 kg of seaweed per feed per 100 legal size paua.

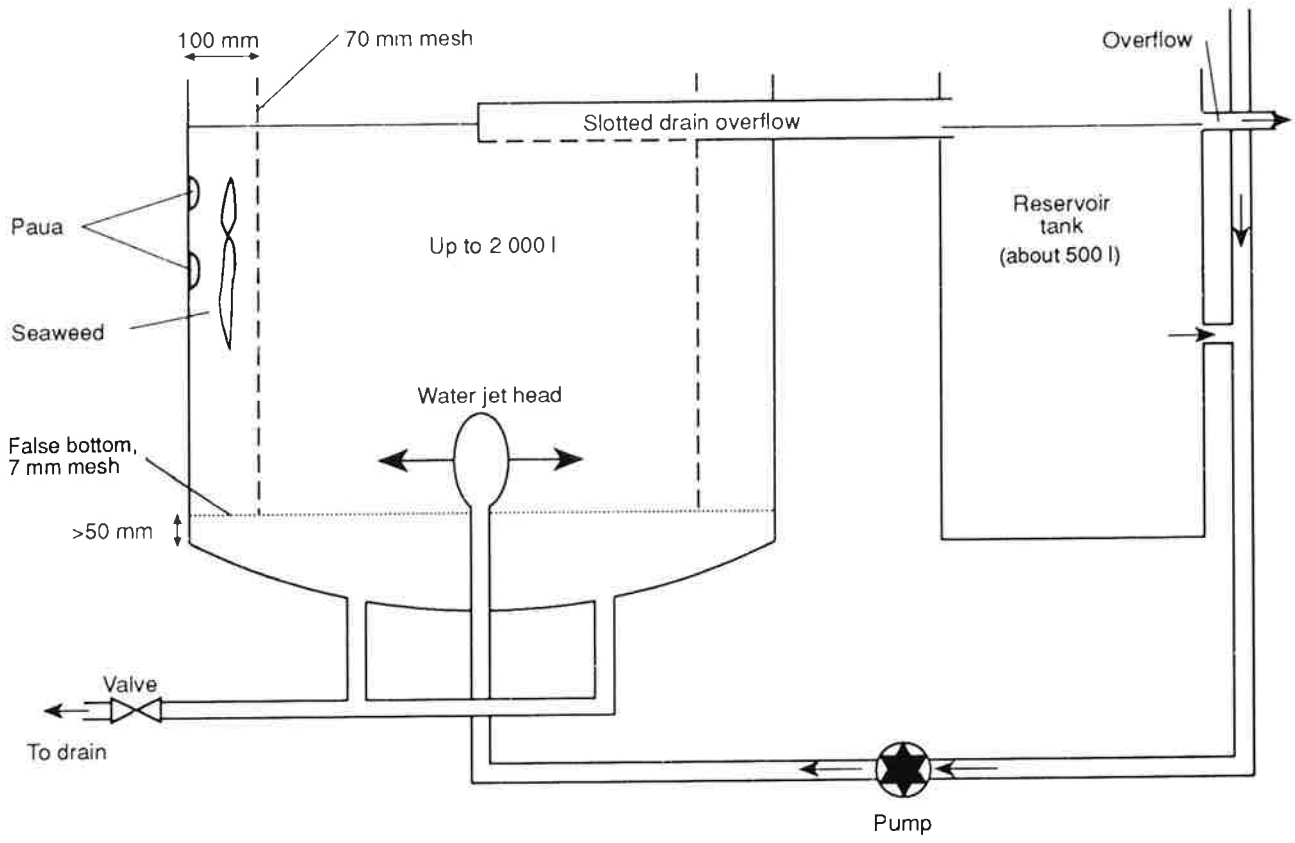

Figure 3: Diagram of a broodstock holding tank.

The broodstock tanks have a false bottom of 7 mm plastic mesh. The paua avoid the mesh by staying on the tank side. Faecal matter and fragmented seaweed fall through the mesh and are flushed out once a week.

Paua can be held in any bucket or tank, provided they can be fed with suitable quantities of seaweed, and are supplied with well-oxygenated water, and the system is cleaned regularly with minimal disturbance to the paua (Ebert & Houk 1984, Uki & Kikuchi 1984).

Spawning

Paua spawn readily if their gonads are very ripe, and they may spawn in the holding tanks if they are not handled carefully.

At the Mahanga Bay hatchery paua are induced to spawn in 12 l tanks (Figure 4) containing sea water at 15 ± 1 °C. Males and females are held in separate tanks, so that fertilisation can be controlled. We have spawned paua at temperatures of 12–18 °C.

A mixture of hydrogen peroxide and sodium hydroxide is used to stimulate spawning (Morse *et al.* 1978). We use this method because it is cheaper and more reliable than other techniques for our species (authors' unpublished data). Other methods that have been used to spawn abalone include temperature shock, air drying, and ultraviolet light irradiated water (Kikuchi & Uki 1974, Hahn 1989c).

Paua are spawning well if eggs and sperm are jetted out through the shell holes in a fine cloud (Figure 5) and are not clumped into sticky plugs of eggs or strings of sperm. A large female (150 mm) can release up to 5 million eggs at one spawning.

Fertilisation, hatching, and larval development

Paua eggs are fertilised in bucket sieves (Figure 6) with a minimum sperm density of 25 000 per ml. A higher density of sperm will not damage the eggs, provided the sperm are thoroughly washed out of the sieves within 30 min (Ebert & Houk 1984).

The fertilised eggs are placed into a hatching tank (Figure 7). Within 24 h free swimming trochophore larvae hatch, rise to the surface, and flow into the larval rearing tank. The egg membranes and poorly swimming aberrant larvae remain at the bottom of the hatching tank. In a further 24 h the larvae develop into the veliger stage.

Paua larvae have a large yolk supply, which is sufficient to feed them during the development period until they are ready to settle. No extra food is required during larval rearing, though there is evidence that the larvae absorb nutrients from the water (Jaeckle & Manahan 1989).

The rate of development of eggs and larvae depends on temperature (Table 1). At 15 °C the larvae hatch at about 20 h, and they are able to settle from about 7 days (Moss & Tong 1992a).

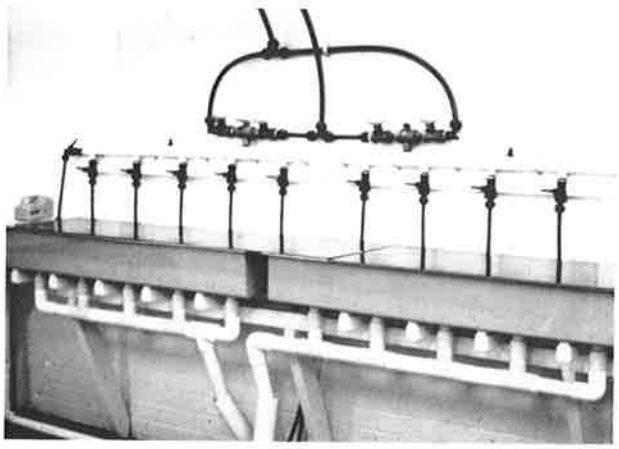

Figure 4: Spawning tanks.

Figure 5: Paua spawning: female (above), male (below).

Figure 6: Bucket sieve. We use a 30 cm long piece of 20 cm diameter pipe with 80 µm mesh on one end. It sits inside a 20 l bucket with holes cut in the side to regulate the water level. The volume of water in the sieve when placed in the full bucket is 10 l.

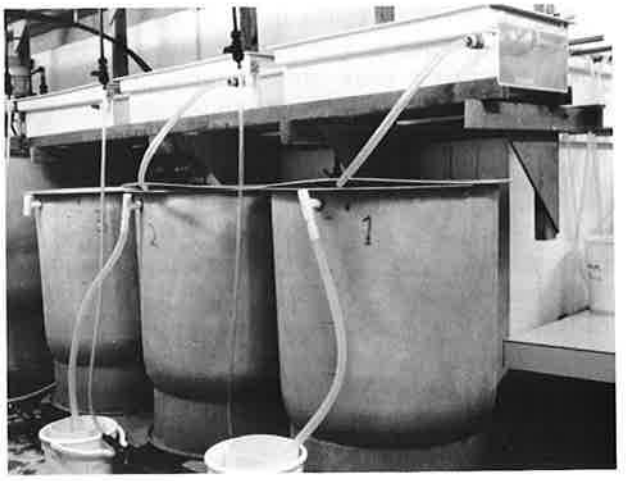

Figure 7: Hatching tanks and larval rearing tanks (above). Diagram of a hatching tank and a larval rearing tank with a banjo sieve (below).

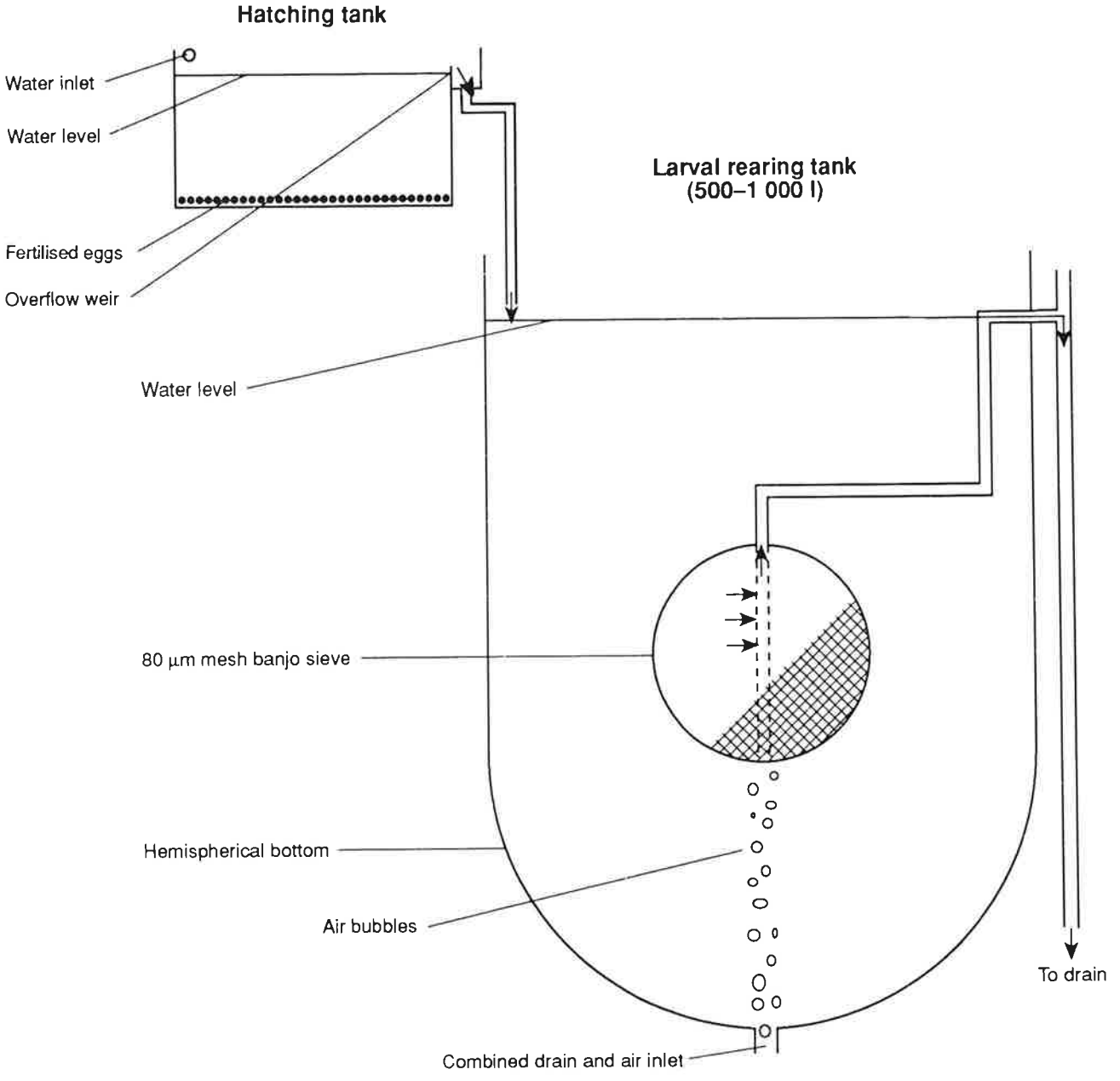

Table 1: Rate of egg development and time to formation of 5–6 rows of chitinised teeth on the radula, by temperature

Temperature (°C)	Time to first division (min)	Time to hatching (h)	Time to 5–6 teeth (days)
13.0	110	26.0	9
13.5	107	22.0	8
14.5	104	21.5	8
15.0	90	20.0	7
15.5	85	20.0	7
16.0	85	18.0	6
16.5	80	17.0	6
17.0	77	17.0	5
17.5	75	16.0	5

The larval culture system

The water in which the larvae are cultured should be filtered to a nominal 1 μm level. High levels of bacteria (2 x 10^6 bacteria per ml) can be lethal to mollusc larvae (Lewis *et al.* 1988). In a tank of static, warm sea water bacterial numbers can multiply 10 times in 24 h (MAF Fisheries unpublished data). A flow-through system and lids on the tanks will reduce bacterial contamination.

Paua larvae are reared in 500 or 1000 l tanks (*see* Figure 7) in a flow-through system at a density of up to six larvae per ml. Larvae are retained in each tank by a "banjo" sieve on the outlet. To prevent larvae from being sucked on to the mesh, the maximum flow rate should not exceed 4 l per min for a filter surface area of 0.25 m^2. In addition, the water in the tanks should be gently aerated.

The water temperature should not vary by more than a few degrees, and any change in temperature should be gradual. Larvae can be reared in water over a range of salinities (Hahn 1989d), but a sudden drop in salinity of about 5 milli-Siemens can cause high mortalities (authors' unpublished data).

Settlement and early juvenile growth

The criteria used to determine larval readiness to settle are 4 buds on the cephalic (head) tentacles (Seki & Kan-no 1977, 1981a), an ability to crawl on a surface (Ebert & Houk 1984), and 5–6 rows of teeth on the radula (Tong 1982, Tong & Moss [1992]). Settlement is slow (Morse *et al.* 1979a), and it can take up to 48 h on an artificial surface (Moss & Tong 1992b).

Settlement can be delayed for several days by not providing the larvae with a suitable settlement surface. The larvae continue to develop during this time, and the only quantitative measure of further development is the number of rows of teeth on the radula. Postlarval survival is improved if the larvae are settled when they have 10–12 rows of teeth on the radula (Moss & Tong 1992a). At 15 °C these larvae would be 10 days old.

A method to prepare larvae, so that the rows of teeth on the radula can be counted, is described in Appendix 2.

Benthic diatoms suitable for food at settlement can be isolated from sea water and cultured in the hatchery. An alternative management programme can be used to allow natural diatoms to establish and grow in the tanks.

At Mahanga Bay we culture benthic diatoms in plastic bags under artificial light. In California white polythene plastic containers are used for diatom settlement and culture substrates (Ebert & Houk 1984). The Japanese fertilise tanks with inorganic nutrients to encourage natural diatom blooms (Hahn 1989e).

At settlement the layer of diatoms should appear as a faint yellow-brown layer which becomes obvious when a finger is run along the side of the tank or a plate to expose the settlement surface.

At Mahanga Bay settlement takes place in a V-shape tank system (Illingworth 1986), which provides a high surface area to volume ratio (Figure 8). Suitable surface texture and adequate preparation of the surface with bacteria and diatoms are essential for ensuring high settlement rates and postsettlement survival (Tong & Moss [1992], Moss & Tong 1992b).

Other techniques to promote settlement have been used overseas. Gamma amino butyric acid (GABA) is used in the U.S. (Morse *et al.* 1979a, Morse [1992]) and Mexico (Searcy-Bernal *et al.* [1992]). It is a commercially available chemical, which occurs naturally on the surface of *Lithothamnion* (Morse *et al.* 1979a). We have found that GABA will increase the speed of settlement, but not the postsettlement survival (Moss & Tong 1992b), and we do not consider it to be essential. The Japanese do not use GABA, but they have found the mucous trails of actively feeding abalone can be used to induce settlement (Grant 1981, Seki & Kan-no 1981b). We have not tested the Japanese technique.

The major problem after settlement is regulating the supply of food. We have no measure of food densities, which can be judged only by eye and experience (Ebert & Houk 1984, McMullen & Thompson 1989). If the diatom layer becomes too thick, it becomes difficult to see the postlarvae, and bacteria and protozoa increase and may cause high mortalities (Morse *et al.* 1979b). Rapid algal growth can be controlled by shading the tank.

Where surface food is insufficient, the postlarvae will graze off all the algae and the surface is easily seen. In this situation, depleted diatom growth can be encouraged by increasing the illumination or adding nutrients. Alternatives are to add extra cultured microalgae or extra plates with a diatom coating, or transfer some paua to another tank by moving the plates.

When the juveniles reach 5 mm they can be weaned from the microalgal diet to a macroalgal diet. An estuarine seaweed, *Gracilaria chilensis*, is appropriate. *Gracilaria* is not a natural food of

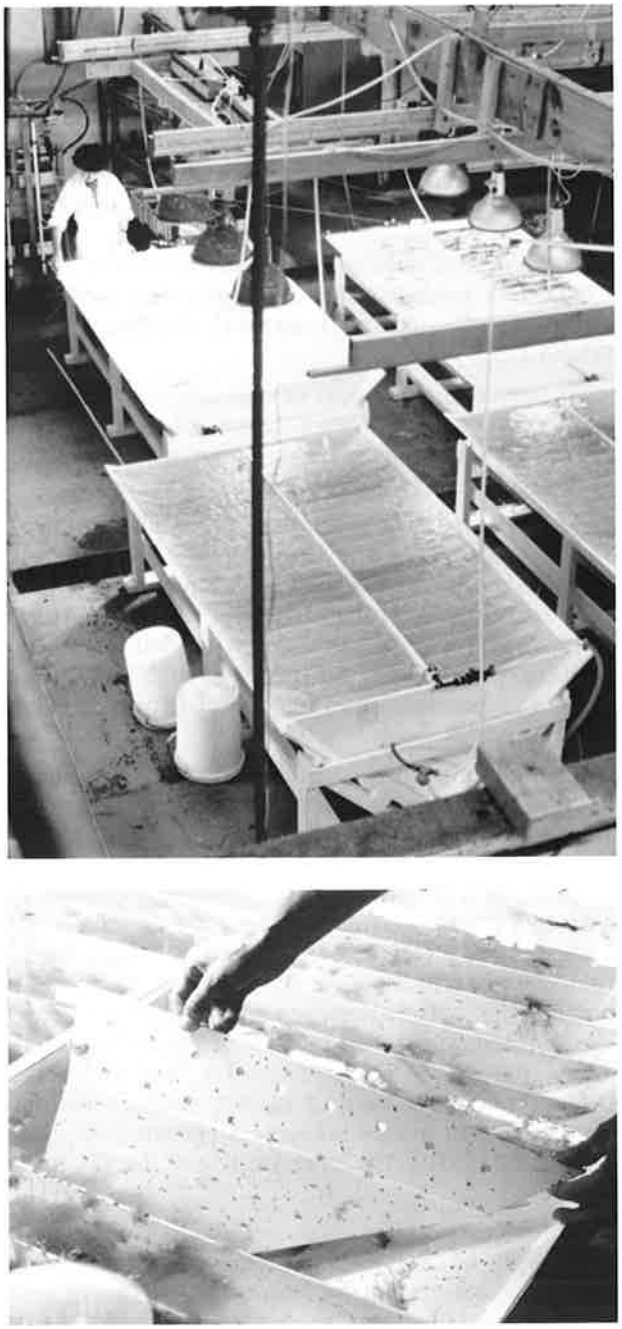

Figure 8: V-shaped settlement tanks and plates (above). Close-up of the settlement plates showing settled paua (below).

paua, but it promotes rapid growth (Tong 1983, Pickering 1986, 1990), and it can be harvested or cultured (Terzaghi *et al.* 1987). In a V-shape system with plates spaced every 100 mm we add about 50 g of *Gracilaria* in each space every 4–5 days.

The numbers of paua that settle and survive through to the early juvenile stage can vary greatly, but there is no accurate method to calculate numbers, except when the tank is emptied. Subsampling, by counting paua on randomly selected plates, will not give a true estimate because the distribution in a tank is usually very uneven. As a guide, if there are too many paua, they will pile on top of each other at the bottom of the tank, eat all the seaweed within 1 or 2 days, and block air holes. In extreme cases anoxic conditions can occur at the bottom of the tank and kill the juveniles. Numbers can be reduced either by transferring paua on the plates to another tank or removing the paua after they have been anaesthetised (*see* Appendix 3).

Ongrowing to a marketable size

Abalone are marketable at lengths of 50–75 mm (McMullen & Thompson 1989, R. Brown, Crystal Park paua farm, pers. comm.), which correspond to meat weights of 10–30 g (Table 2). In the wild paua can reach 50 mm in 2–3 y (Poore 1972b, Sainsbury 1982). Growth is normally fastest up to about 70 mm, at which size most animals are sexually mature.

Abalone within a batch will grow at markedly different rates (Henriques *et al.* 1989). To maintain similar size groups in a tank, commercial growers normally sort their abalone by size (Hahn 1989f, McMullen & Thompson 1989). Growth rates of paua should be monitored, and ideally the lengths of at least 200 randomly selected animals from each tank should be measured every 2 months. Vernier calipers are normally used to measure the longest length of the shells to the nearest millimetre, and a mean and range of sizes should be calculated. In a commercial operation this practice is difficult and laborious, and paua are usually sorted by eye (R. Ewing, Taranaki Aquagardens, pers. comm.).

Table 2: Length-weight relationship for juvenile paua grown at the Mahanga Bay hatchery

Length (mm)	Total weight (g)	Meat weight (g)
10	0.1	0.1
15	0.4	0.3
20	1.0	0.6
25	1.9	1.3
30	3.3	2.2
35	5.1	3.4
40	7.6	5.1
45	10.7	7.2
50	14.6	9.8
55	19.4	13.0
60	25.1	16.9
65	31.7	21.4
70	39.5	26.7
75	48.4	32.7

Food requirements

Paua larger than 5 mm will eat a wide range of seaweeds, but generally prefer and grow best on red seaweeds (Poore 1972a). Other studies have shown a preference for *Gracilaria* (Dutton & Tong 1981) and *Undaria* (Middlemass 1990).

There is some evidence that mixed diets give better and more sustained growth in abalone (Day & Fleming [1992]). Seaweeds grown under different

conditions will have different chemical compositions, and the food values of seaweeds harvested from the wild will vary seasonally and geographically (Uki 1981).

Juvenile paua can eat up to 20% of their body weight per day of seaweed (authors' unpublished data), and other species of abalone may eat as much as 30% (Hahn 1989g). Thus, it is particularly important to provide sufficient good quality food for an abalone farm. A farm producing 1 million paua per year will require about 1400 t of seaweed (P. Redfearn pers. comm.). An artificial diet may be an alternative. The Japanese company Nikon Nasan Kogyo K.K. produces a pelletised food, and companies in Australia (Anon. 1991) and New Zealand are developing and testing artificial foods.

Environmental requirements

The effects of oxygen levels, water temperature, salinity, organic acids, and heavy metals on the survival and growth of abalone were discussed by Hahn (1989d). However, there is little information on the environmental requirements of paua. They grow faster in warmer water (Tong 1980, 1982), but temperatures over 20 °C can cause mortalities. Shortterm (up to 48 h) falls in salinity of 10 milli-Siemens have been tolerated at the Mahanga Bay hatchery (MAF Fisheries unpublished data), but we do not have growth data for paua reared at lower salinities.

Sand and silt kill paua (Sainsbury 1982), and they should be avoided in culture systems, where even small amounts will stress juveniles (R. Brown, Crystal Park paua farm, pers. comm.).

Abalone should be cultured in systems which have a good flow of clean, well-oxygenated water with minimal fluctuations in temperature and salinity (Hahn 1989d).

Ongrowing systems

Systems for ongrowing abalone include land-based raceways, shore-based containment systems, and suspended cages or barrels (Hahn 1989f).

Raceways have the advantage that servicing is not weather dependent, but the disadvantage that they must have a supply of pumped water and aeration (Svenson 1988).

Shore-based containment systems are built where part of the system is submerged at all times and where there is a good water exchange. In exposed areas containment systems must be strong enough to withstand storm damage.

Suspended systems consist of cages, or of containers with meshes to allow water movement, but which retain the abalone and their food (Hahn 1989f, McMullen & Thompson 1989, Yoo 1989). The containers are suspended in the water column in situations which allow good water exchange.

The maintenance of both shore-based systems and suspended systems can be weather dependent.

A recipe for breeding and rearing paua

Facilities

Tanks

broodstock holding tanks (*see* Figure 3);
12 l spawning tanks (*see* Figure 4) with covers;
washing unit (Figure 9);
hatch tank (*see* Figure 7);
larval rearing tank (*see* Figure 7);
V-shape settlement tanks (*see* Figure 8).

Laboratory equipment

chemicals to induce spawning (Appendix 4);
measuring cylinders and beakers;
80 µm mesh bucket sieves (*see* Figure 6);
400 µm sieve;
20 l white buckets;
nontoxic PVC hose for siphoning eggs and sperm from tanks;
compound and dissection microscopes;
microscope slides;
haemocytometer;
Stempel pipette and counting chamber;
80 µm mesh banjo sieves (*see* Figure 7) — 500 and 150 mm diameter;
1 µm cartridge filters and housing.

Other

supplies of adult paua (*see* Appendix 1);
benthic diatom cultures.

NB: Unless otherwise stated, all sea water should be filtered to about 2 µm.

Buckets and sieves used for handling the eggs and sperm should be checked for damage and cleaned thoroughly before spawning starts. After the paua spawn, work carefully and quickly, because delays may result in aberrant larval development.

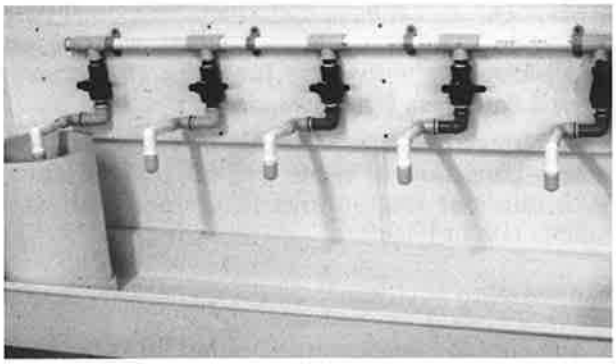

Figure 9: Washing unit with sieve.

Broodstock

From late summer onwards 20–30 adult paua should be collected every 2–3 weeks and tested to see if they are ready to spawn (use the spawning procedure described below). When 2 or 3 males and females spawn readily, a further 100–200 paua should be collected as broodstock for the year. These animals should be held in the broodstock tanks and should be well fed. Care must be taken to avoid damaging paua when they are collected, because they lack a blood clotting mechanism, and, if cut or damaged, may slowly bleed to death.

Spawning and rearing

Day 1

(Time)

0800 Select 8–12 females with bulging ovaries and 6–8 males with large creamy-white testes from the broodstock. A large gonad size suggests a paua will spawn, but it is not a guarantee. Place two paua in each 12 l tank of flowing sea water and keep the males and females separate.

0845 Prepare a 6% hydrogen peroxide solution by mixing one measure of 30% hydrogen peroxide with four measures of distilled water (*see* Appendix 4).

0900 Turn off the water flow to the 12 l tanks. Add 40 ml of the 6% hydrogen peroxide solution and 13 ml of a 1 M sodium hydroxide solution (*see* Appendix 4) to the water in the tanks, mix, and leave for 3 h.

1200 Empty the sea water and chemicals from the tanks and flush through with sea water. Continue flushing the tanks for 15 min, or for at least three complete changes of water.

1215 Turn the water off and cover the tanks.

1330 Male paua will start spawning about 1.5 h after flushing and females about 2 h. The time period can be longer (up to 4 h), depending on the water temperature.

1430 Gently siphon the eggs through a 400 µm mesh sieve (to remove faecal matter) into a bucket sieve (*see* Figure 6). A suitable density is 300 000 eggs per sieve (or a density of about 1000 eggs per cm^2 of sieve). Higher densities can block the meshes and stop the water flow through the sieve. Egg counts should be made to ascertain numbers in the sieves (Appendix 5), but with experience, egg numbers can be estimated.

1435 Transfer the sieve to the washing unit (*see* Figure 9), and thoroughly rinse the eggs for 15–30 min to remove detritus and maintain ambient oxygen levels. Agitate the sieve every few minutes to prevent clogging.

1440 Siphon the sperm from several males into a bucket, and check under a microscope to see if they are moving vigorously. If they are not, collect freshly spawned sperm. The sperm density should be calculated by use of a haemocytometer (*see* Appendix 5); with experience, the density can be estimated visually on the basis of the degree of opacity or milkiness.

1450 Examine the eggs under a microscope to ensure that they have a well-rounded shape and a uniformly dense, rather than granular, yolk. Just after the eggs are released they may be irregularly shaped, but will quickly become round.

1500 Always recheck sperm for motility just before fertilisation. If the sperm are no longer moving vigorously, collect freshly spawned sperm. Remove the sieve containing the eggs from the washing unit and replace it in its bucket. To fertilise the eggs add sperm to the bucket sieve to achieve a density of at least 25 000 sperm per ml (*see* Appendix 5). Occasionally agitate the mixture of sperm and eggs. Eggs and sperm should not be left together for more than 15 min, because sperm can break down the egg membrane.

1515 Transfer the sieve to the washing unit (*see* Figure 9), and rinse the eggs for 10–15 min to remove the excess sperm.

1525 Rinse an egg hatching tank (*see* Figure 7) with fresh water, and then sea water, and fill with 15 °C sea water.

1530 Pour the eggs into the hatching tank, distribute them evenly, and allow them to settle, so that a single layer of eggs covers the bottom. Use a density of about 3 million eggs per 0.5 m^2 of tank bottom.

1600 Turn on the water, and adjust the flow rate to ensure that the eggs are not disturbed. The water flow reduces bacterial buildup in the hatching tank and maintains oxygen levels around the eggs.

Day 2

0800 Check the water temperature in the egg hatching tank; this will give an idea of hatching time (*see* Table 1). When the first larvae hatch, turn off the water flowing through the tank until the rearing tank is ready. Healthy trochophore larvae swim to the surface of the water where they can be seen as small green-white dots and often form dense shoals. (*See* Figure 2 for the egg and larval stages through to settlement.)

0830 Clean a 500 l larval rearing tank (*see* Figure 7) with a chlorine solution of about 10 µg per g (or about 50 ml of commercial chlorine bleach in 5 l of fresh water). Leave the solution in the tank for 5–10 min, and then rinse with fresh water, followed by 1 µm filtered sea water.

0845 Fill the rearing tank with 1 µm filtered sea water and connect a 500 mm diameter, 80 µm mesh banjo sieve to the outflow pipe.

0900 Place the outlet hose from the hatch tank into the rearing tank. Turn on the sea water to the hatch tank at a flow not exceeding 4 l per min, so that the larvae flow gently into the rearing tank. Gently aerate the rearing tank. All the larvae should hatch and be washed into the rearing tank within 7 h.

1600 Remove the hatching tank outlet hose from the rearing tank. Turn on the supply of 1 µm filtered sea water to the rearing tank at a rate of 2–4 l per min. Up to 3 million larvae can be reared in a 500 l tank.

Day 3

0800 Record the water temperature in the 500 l larval rearing tank. Turn off the water flow and the aeration, and let the water stand for 30 min.

0810 Remove the 1 µm filter from the housing and replace it with a clean filter. The used filter should be flushed through with fresh water and then left to air dry. Once dry the filter can be reused.

0830 Siphon out and discard the dead, weak, and aberrant larvae, which will have sunk to the bottom of the tank.

0845 Remove the banjo sieve and wash it in fresh water.

0900 Rinse the clean banjo sieve in 1 µm filtered sea water and replace it in the tank. Reset the water and air flows.

1600 Check the water temperature, flow rate, and aeration.

Day 4

0800 Record the temperature and turn off the water and air.

0810 Remove the 1 µm filter from the housing and replace it with a clean filter. Clean the used filter, as on day 3.

0830 Siphon the dead and aberrant larvae from the bottom of the tank.

0845 Gently siphon or drain the healthy larvae out of the tank and into several bucket sieves. Be careful not to siphon too many into a sieve, because the larvae can clog the meshes and cause the sieve to overflow. We collect up to 0.5 million larvae on each sieve.

0945 Leave the larvae standing in the sieves with a gentle flow (about 1 l per min) of 1 µm filtered sea water). Clean the now empty 500 l tank, as on day 2, and refill it with 15 °C, 1 µm filtered sea water. Clean the banjo sieve, as on day 3.

1045 Replace the larvae in the clean tank. Replace the banjo sieve, and reset the water and air flows through the tank.

1600 Check the water temperature, flow rate, and aeration.

Day 5

0800 Repeat the day 3 procedure. Patches of dead larvae on the bottom of the tank usually indicate a bacterial problem. If this is so, repeat the day 4 procedure. Wash the larvae well, with 1 µm filtered sea water, while they are in the bucket sieves.

Day 6

0800 Repeat the day 3 or 4 procedure, as necessary.

Day 7

0800 Repeat the day 3 or 4 procedure, as necessary. If the temperature has been 15–16 °C through the rearing period, the larvae should have 5–6 rows of radula teeth and could be settled. At this temperature we would recommend that the larvae be held for at least another day before settlement.

Settlement

These procedures for settlement run concurrently with the spawning and rearing procedures.

Day 1 Day of spawning.
Thoroughly clean the settlement plates and V-shape tank (*see* Figure 8). Fill the tank with fresh water, and leave it to soak for 24 h.

Day 2–3 Empty the V-shape tank, and refill it with 2 µm filtered sea water. Maintain the water flow through the tank to allow a bacterial film to develop on the surfaces.

Day 4 Turn off the water, and place the plates in the tanks on an angle to facilitate settlement on the upper surface. Add 10 l of a straw coloured mixture of cultured benthic diatoms to the tank (about 8 x 10^6 cells per ml). Aerate the tank for 5 min. Turn off the air and water for 12–24 h, to give the diatoms time to settle and establish.

Day 5 Turn over the plates in the tank, and add another 10 l of a straw coloured mixture of diatoms. Aerate for 5 min, and then leave the air and water off for a further 12–24 h.

Day 6–8 Turn on the air and water. Check the buildup of diatoms by running a finger over the side of the tank. A light yellow-brown film should be visible. If there is a heavy brown layer of diatoms, cover the tank with shade cloth and leave.

Day 9–10 When the larvae have about 10 rows of teeth on the radula, attach two banjo sieves (150 mm diameter, 80 µm mesh) to the outflows of the V-shape tank. Drain or siphon the larvae out of the larval tanks into the bucket sieves. Calculate the number of larvae (*see* Appendix 5). Add about 200 000 to each 2 m length of V-shape tank, distribute evenly, and maintain this distribution with gentle aeration. Best settlement on the plates occurs if they are left on an angle.

NB: Larvae can be settled on day 7 (with 5–6 rows of teeth on the radula), but we recommend that they be held longer in the larval culture to increase their postsettlement survival (Moss & Tong 1992a).

Day 10–11 One day after larval introduction.
With the water and air off, check the water surface in the tank for swimming larvae. Redistribute any concentrations of larvae by ladling with a beaker. Remove and clean the banjo sieves, being careful not to allow any of the larvae to drain out of the tank. Replace the banjo sieves, and turn the air and water back on.

Day 11–12 Two days after larval introduction.
Repeat the day 10–11 procedure unless all the larvae have settled; if so, follow the procedure for day 12–13.

Day 12–13 Three days after larval introduction.
Check that there are no swimming larvae, then remove the banjo sieves. Check the layer of diatoms on the sides of the tank; if the layer is dark brown, cover the tank with shade cloth until small grazing trails appear.

For the next 6–8 weeks it is important to control the growth of diatoms. Shade the tanks when algal growth is rapid, which usually happens during the first 2 weeks. Remove the shading once the paua and their grazing trails become visible. Maintain food levels by settling extra diatoms or adding more diatom covered plates. The degree of filtration of the sea water may be changed from 5 to 70 µm to allow more food to enter the tank, but remember that fine silt, which can kill small paua, may also enter.

At 8–10 weeks the paua should have reached 2–3 mm and have developed their first respiratory hole.

When the paua reach 5 mm, at about 4–6 months, they will be eating the diatoms faster than they can be added to the tanks. At this stage small quantities of *Gracilaria* or other suitable seaweed should be added to the tanks to wean the paua off the diatoms.

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Appendix 1: Rules, Regulations, and Permits required

The rules and regulations for farming paua and collecting seaweeds are being revised. Contact your local MAF Fisheries office and discuss your plans at the earliest opportunity. They will tell you what permits are required.

To collect adult paua from the wild for your broodstock, you will either require quota or will need to purchase them from a quota holder.

Appendix 2: A method to prepare larvae for counting the rows of chitinised teeth on the radula

1. Collect 30–40 larvae in a small sieve. (A 25 mm long piece of 15 mm diameter PVC water pipe with 80 μm mesh glued on to one end works well.)
2. Place the sieve containing the larvae into 3–4 ml of sea water. Add 4 drops of concentrated hydrochloric acid (HCl). Agitate, leave for 4 min, and then remove the sieve from the acid bath. The acid dissolves the larval shell, but leaves the body (and radula) intact.
3. Dehydrate the larvae by transferring the sieve containing them through a series of alcohol baths. Start with a 70% ethanol or methanol solution, and progress through 90% and 95% to a 100% solution. The larvae should be left in each solution for 2–3 min.
4. Pipette 10–15 larvae from the sieve, and place them on a clean microscope slide. Squash the larvae on the slide with a coverslip.
5. View the slide under a compound microscope. The radula can be seen close to the eye spots (*see* Figure 2 h).

A clearer preparation can be achieved as follows, from step 3 above:

Pipette 10–15 larvae from the sieve and place them on a clean microscope slide, and let the 100% alcohol evaporate. Add 2 drops of a 1 M potassium hydroxide (KOH) solution to clear the tissues and squash the larvae with the coverslip. (To make the 1 M KOH solution, dissolve 5.6 g of KOH pellets in 100 ml of distilled water. This solution can be stored for future use.)

NB: It is often difficult, even for experienced observers, to find the radula when there are only one or two rows of teeth. The radula in ready-to-settle larvae with five or more rows of teeth can be seen easily by use of the above technique.

WARNING: Both potassium hydroxide and hydrochloric acid can be dangerous, and the following procedures should be followed if contamination occurs.

1. Potassium hydroxide (KOH)

Skin **Drench the skin with water, then 1% acetic acid. Blisters or burns must receive medical attention. Remove and wash contaminated clothing before reuse.**

Mouth **Wash thoroughly with water. Give plenty of water to drink, followed by vinegar, or 1% acetic acid, or plenty of lemon juice.**

IF NECESSARY, SEEK MEDICAL ADVICE

2. Hydrochloric acid (HCl)

Skin **Drench skin with water, then apply magnesia/glycerol paste. Blisters or burns must receive medical attention. Remove and wash contaminated clothing before reuse.**

Mouth **Wash mouth out thoroughly with water, and give water to drink, followed by milk of magnesia.**

IF NECESSARY, SEEK MEDICAL ADVICE

Appendix 3: Use of benzocaine for anaesthetising abalone

1. Dissolve benzocaine (ethyl p-aminobenzoic acid) in commercial grade 95% ethanol to make a final concentration of 100 ppm (*see* table below). Mix with sea water. Place the plates with paua on into the sea water and leave for 5–8 min. Do not leave the paua in the benzocaine for more than 10 min. If benzocaine is used on a tank of animals, make sure the tank can be drained quickly.

   Quantity of benzocaine dissolved in commercial grade 95% ethanol to make a final concentration of 100 ppm, per tank size:

Tank size (l)	Ethanol (ml)	Benzocaine (g)
70	52	2.77
100	74	3.96
500	371	19.79
1 000	742	39.58
2 500	1 855	98.95

2. Gently hose paua off the plates into a mesh bag or sieve. Revive the paua in running sea water. While anaesthetised, the paua can be sorted into sizes by use of large sieves.

   Benzocaine works best at temperatures above 15 °C, or with water raised 3–4 °C above ambient.

WARNING: Avoid prolonged exposure to benzocaine.

Appendix 4: Chemicals required to induce spawning

1. Hydrogen peroxide (H_2O_2)

   A stock solution of hydrogen peroxide is usually 100 volumes or 30%. It should be kept in a fridge and in the dark. The stock solution deteriorates with time, so it is best to replace it every 3–4 months. This solution is diluted with distilled water immediately before use.

2. Sodium hydroxide (NaOH)

   A 1 M solution is made by dissolving 40.0 g of sodium hydroxide pellets in distilled water and making the solution up to 1 l. Do not handle the pellets; use a spatula to spoon them out for weighing. This solution can be kept for later use.

WARNING: Both these chemicals can be dangerous, and the following procedures should be followed if contamination occurs.

1. Hydrogen peroxide (H_2O_2)

Skin **Drench the skin with water. Wash clothing before reuse.**

Mouth **Wash out the mouth with water, and give water to drink.**

IF NECESSARY, SEEK MEDICAL ADVICE

2. Sodium hydroxide (NaOH)

Skin **Drench the skin with water, then 1% acetic acid. Blisters or burns must receive medical attention. Remove and wash contaminated clothing before reuse.**

Mouth **Wash thoroughly with water. Give plenty of water to drink, followed by vinegar, or 1% acetic acid, or plenty of lemon juice.**

IF NECESSARY, SEEK MEDICAL ADVICE

Appendix 5: Counting techniques

1. Eggs and larvae

   Concentrate the eggs or larvae into 20 l of filtered sea water. Agitate to distribute evenly, and take a minimum of four 5 ml samples by using a Stempel pipette. Count the eggs or larvae in the samples by using a microscope. Calculate the total number of eggs or larvae by multiplying up.

2. Sperm

   Sperm are counted by use of a haemocytometer. Siphon the sperm into a bucket, agitate, and take a 5 ml sample. Place the sample in a watch glass, and mix with 0.5 ml of Lugol's iodine. (NB: Lugol's iodine will kill eggs and sperm, so it should be kept away from the spawning area.)

   Transfer a few drops of the mixture to the haemocytometer, and allow it to flow by suction under the coverslip. Count the sperm in 10 of the small squares on the haemocytometer, and calculate the average number per square. Multiply the average by 4.4 x 10^6. This will give the density of sperm per ml in the bucket.

Calculate the volume of sperm required by using the following formula:

$$V = \frac{SD_2 \times V_2}{SD_1}$$

where V = volume of sperm suspension required;
V_2 = volume of egg suspension;
SD_1 = calculated sperm density;
SD_2 = sperm density required.

For quick reference, use the following table, which gives quantities of sperm suspension required for final densities of 25 000 and 100 000 sperm per ml in a 10 l container:

Haemocytometer count (sperm per square)	Sperm density (sperm per ml)	25 000 per ml	100 000 per ml
0.1	4.4 x 10^5	570	2 280
0.2	8.8 x 10^5	285	1 140
0.3	1.3 x 10^6	190	760
0.4	1.8 x 10^6	145	580
0.5	2.2 x 10^6	115	460
0.6	2.6 x 10^6	95	380
0.7	3.1 x 10^6	85	340
0.8	3.5 x 10^6	75	300
0.9	4.0 x 10^6	65	260
1.0	4.4 x 10^6	60	240
2.0	8.8 x 10^6	30	120
3.0	1.3 x 10^7	20	80
4.0	1.8 x 10^7	15	60
5.0	2.2 x 10^7	15	60

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