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The hatchery culture of bivalve mollusc larvae and juveniles

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Abbreviations used in this leaflet

cm	Centimetre (1/100 m)
°C	Degrees centigrade
g	Gram
h	Hour
l	Litre
M	Molar $\frac{\text{(molecular weight (g) of solute)}}{\text{litres of solution}}$
mg	Milligram (1/1000 g)
ml	Millilitre (1/1000 l)
mm	Millimetre (1/1000 m)
%	Percentage
PVC	Polyvinyl chloride
ppm	Parts per million (equivalent to mg/l)
psu	Practical salinity units [25 psu = 25‰ (parts per thousand), 30 psu = 30‰, 32 psu = 32‰ etc.]
µl	Microlitre (1/1000 ml)
µm	Micrometre (micron) (1/1000 cm)

1. INTRODUCTION

This leaflet is intended as a guide to the procedures used in the hatchery rearing of bivalve molluscs in the United Kingdom. These procedures have been used, by the Ministry of Agriculture, Fisheries and Food (MAFF) at the Fisheries Laboratory, Conwy, over several years.

Despite many years of research, there are still considerable problems in the reliable hatchery production of bivalve species. Successful hatchery production of larvae and spat is related more to the skill and experience of the staff than to the excellence of the facilities and equipment. A keen awareness of the importance of hygiene is essential. In general terms, greater success can be expected in the cooler months of the year when it is necessary to condition adults to spawn outside their normal breeding season. Higher mortalities of larvae, and on rarer occasions of spat, can be anticipated in the summer and are associated with higher temperatures which produce greater bacterial infection.

The species which are reared in commercial hatcheries in the UK (and northern Europe) include native and exotic (introduced or non-indigenous) species (Table 1). Importations of exotic species to the UK, with the exception of the American hard shell clam, were made via the hatchery and quarantine facilities at the Fisheries Laboratory, Conwy. The broodstock were held in conditions of strict quarantine and destroyed after spawning. The juvenile offspring were then reared in isolation with frequent histological checks being made on them by MAFF's Fish Diseases Laboratory, Weymouth, to ensure their freedom from non-indigenous parasites and organisms which cause disease. Small numbers of young adults were eventually supplied to British hatcheries for breeding purposes.

The general aspects of hatchery culture are outlined in Figure 1. This leaflet describes the husbandry of broodstock, larvae and juveniles within a hatchery. Broodstock conditioning, larvae culture and spat culture need to be carried out in separate areas to prevent mixing of sea water and the potential for spreading disease or bad hygiene problems.

2. CONDITIONING OF BROODSTOCK

Table 1. Bivalve species reared commercially in UK hatcheries

Species	Status	Year of introduction	Source
European (native) flat oyster = *Ostrea edulis* L.	Native	—	—
Carpet shell (palourde clam) = *Tapes decussatus*	"	—	—
Pacific oyster = *Crassostrea gigas* Thunberg	Introduced	1965 and 1972 1978	Canada USA
Manila clam = *Tapes philippinarum* Adams and Reeve	"	1980	USA
American hard shell (quahog) clam = *Mercenaria mercenaria*	" (accidentally?)	1910-1920?	USA?

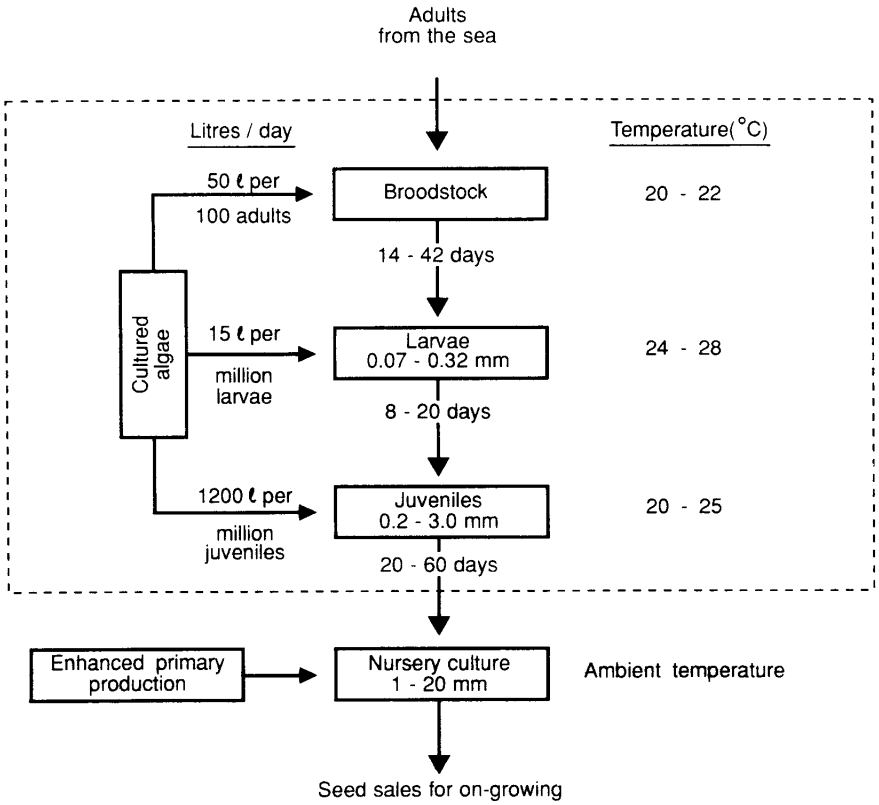

Figure 1. Outline of hatchery culture

For many bivalves in temperate coastal waters, sexual development starts in the sea when the water temperature exceeds 10-14°C. In temperate waters of the northern hemisphere, gametes develop during late May/June and mature during July/August, to be retained until spawning is stimulated by increased temperatures or by a series of thermal/handling shocks. In northern European waters, where sea water temperatures are rarely high enough to stimulate natural spawning of exotic species, mature gametes are retained into the winter and are then resorbed.

Maturity can be accelerated in the hatchery by keeping bivalves at elevated temperatures with a suitable food ration. It is possible to produce mature adults in the winter and early spring before adults in the sea start their sexual development. Thus, bivalve species in spawning condition can be made available for most of the year. To obtain spawnings in the autumn, it is possible to produce mature juveniles from early season spawnings by conditioning them at high temperatures and with high food rations.

2.1 Clams

Small clams, larger than 8 mm shell length (0.075 g live weight), can be used as breeding stock but, since egg production increases with increasing adult size, it is more usual to use mature adults of 35-45 mm shell length (10-20 g live weight). These will spawn 5-8 million eggs on average, depending on their condition and the time of year when they are brought into breeding condition. All of the clams mature first as males (protandric hermaphroditism) and then, as they become older, either change sex to female or remain as males. Populations of 2 and 3 year olds show a 50:50 sex ratio.

2.2 Oysters

Pacific oysters, like clams, mature first as males and then either change sex to female or remain as males. Flat oysters also mature first as males but, thereafter, change sex at regular intervals. On rare occasions, individuals remain either as males or females. At Conwy, mature adults of 30 g live weight (65 mm shell diameter for flat oysters, 70 mm shell length for Pacific oysters) are used as broodstock. Females of these sizes can be expected to release 1 million larvae (flat oyster) or approximately 50 million eggs (Pacific oyster) per individual.

2.3 Conditioning technique

Adult bivalves taken from the sea are brought into the hatchery and placed in a tray supported off the bottom in a tank similar to that shown in Figure 2. (The clams feed more efficiently if they are kept in a substrate of coarse sand or shell and gravel).

The sea water supply need not be filtered, since the diversity and abundance of natural food species, which may be present in the unfiltered sea water, can be beneficial to the conditioning of the broodstock. Salinity should exceed 25 practical salinity units (psu's) (UNESCO, 1981) and temperature should be 22 ± 2°C. Water flow rate through the tank should exceed 25 ml/min per adult and no more than 60 adults (20 g live weight) or 40 adults (30 g live weight) should be held in a tank of the dimensions shown. The water must run to waste and not be recirculated or used again.

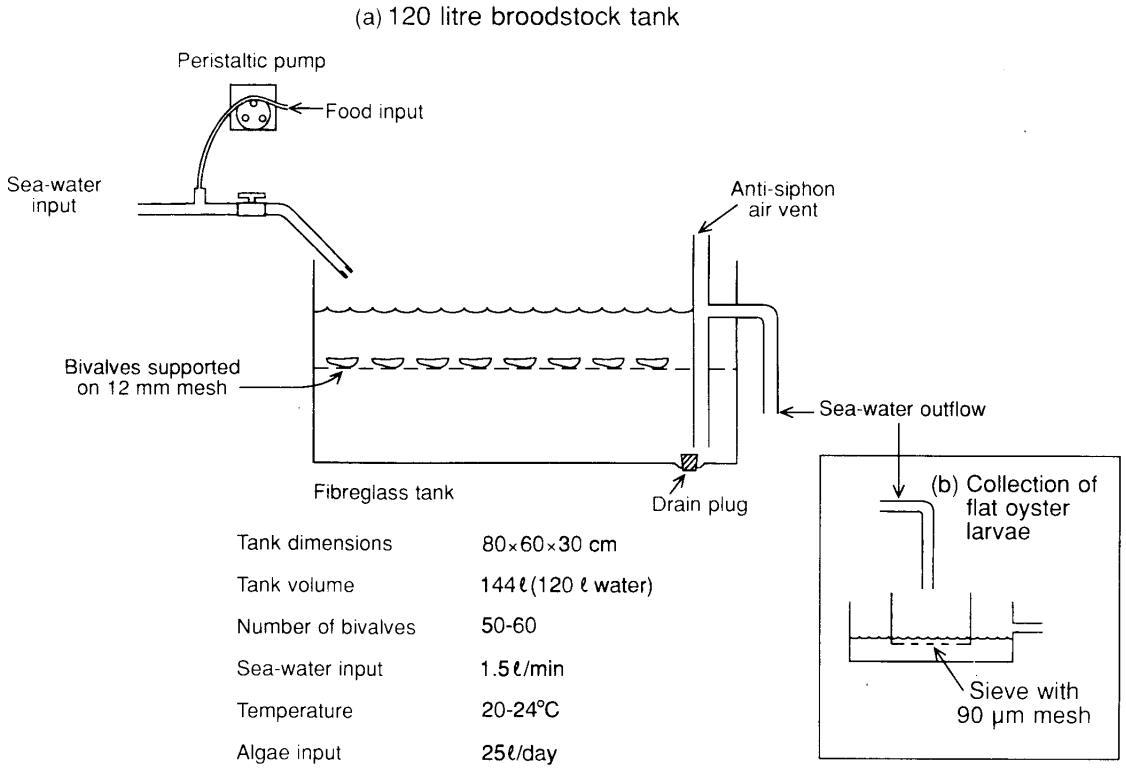

Figure 2. The broodstock conditioning system

A hatchery which is situated in an area free from pests and diseases (as designated under the Molluscan Shellfish (Control of Deposit) Variation Order 1983) (Great Britain - Parliament, 1983)* may be allowed under licence, to deposit broodstock originating from outside the designated area. Conditions of the licence may require strict quarantine for the broodstock with sterilization of effluent water and broodstock residues (e.g. by chlorination with a strong sodium hypochlorite solution), and final safe disposal of broodstock to ensure that no pests or diseases reach the local waters.

Cultured species of marine algae are used as the principal food supply during conditioning. *Tetraselmis suecica*, *Thalassiosira pseudonana* and *Skeletonema costatum* are useful species which can be cultured on a large scale. Each adult requires about 200 million cells of *Tetraselmis*, 2000 million cells of *Thalassiosira* or 1000 million cells of *Skeletonema* per day.
A mixture of these species, on a proportional basis, is better than a single-species diet. A food ration, equivalent to 6% of the initial dry meat weight of the broodstock in dry weight of algae (see Sub-section 10.2) per day, is recommended.

Broodstock take 6-8 weeks to reach spawning condition in winter and early spring and a progressively shorter period as the natural breeding season approaches.

The time taken to reach spawning condition can be calculated in terms of 'day degrees' (D°):

$$D° = d (t - t_o) \qquad \text{..... Equation 1}$$

where D° = the number of corrected day degrees (i.e. above the threshold temperature),
d = the number of days,
t = the ambient temperature, and
t_o = the threshold temperature for the start of gonadal development. In temperate climates where t_o for many bivalves is between 10°C and 14°C, D° is between 300 and 500.

3. SPAWNING

3.1 Clams

Spawning is the procedure by which mature clams are induced to liberate their gametes in response to applied stimuli. Unlike other bivalves reared in hatcheries, viable larvae cannot be obtained from artificially stripped gametes — eggs need to undergo a maturation process during passage down the oviducts. Various stimuli can be applied to induce spawning; the most successful are those that are natural and minimize stress to the animals. The description which follows is of a technique called thermal cycling and is the method used at the Fisheries Laboratory, Conwy.

Mature clams, taken from broodstock conditioning tanks, are cleaned externally to remove any adhering debris and are placed in a spawning tank. The type of tank preferred at Conwy is a shallow fibreglass trough of approximately 150 x 50 x 15 cm depth. It is fitted with a stand-pipe drain and two filtered sea-water supplies, one heated to 18-20°C and the other to 28-30°C. The base of the trough is covered by black plastic sheeting to provide a dark background against which gametes being liberated can be readily seen (Figure 3).

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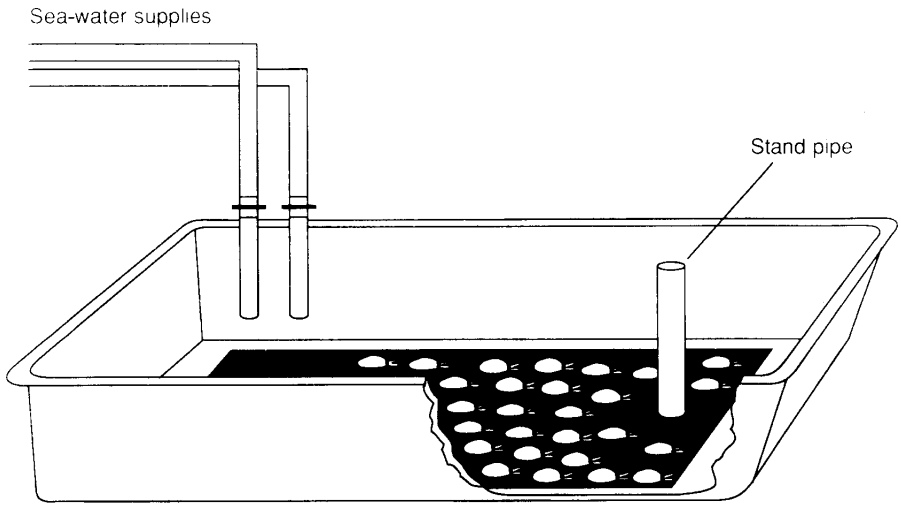


Figure 3. The spawning trough

The trough is part-filled with the cooler water to a depth of about 10 cm and a small amount of cultured algae is added to stimulate the clams to extend their siphons and start pumping activity. After 15-30 minutes, the water is drained and replaced with water at 28-30°C, again with a small addition of algae. This water is drained after a similar time period and replaced with cooler water and the procedure is repeated. The number of cycles which are necessary to induce spawning depends on the readiness of the clams to spawn. In summer, clams may spawn within an hour but, earlier in the season, it may take 4-6 hours of cycling before the first animal spawns. Generally, if the clams do not respond within a 6-hour period they are returned to the conditioning tanks for a further week. Clams may start spawning on the cool or warm part of the cycle. Often, males will spawn first but this is not always so.

Additional stimulus can be provided by the addition of stripped eggs or sperm. In Manila clams, the gonad is located at the base of the foot and can be seen when the mantle and gill tissues are lifted. If the gonad is carefully punctured with a Pasteur pipette, and suction applied, quantities of gametes can be withdrawn which can then be placed in a small volume of filtered sea water. This gamete suspension can then be dispensed towards the inhalent siphons of actively pumping clams with a Pasteur pipette, so that they are drawn into the mantle cavities by the pumping actions of the animals. (The inhalent siphon is the siphon furthest away from the hinge line and has the largest diameter of aperture). This additional stimulus, usually applied during the second warm cycle, almost always produces a spawning response within 0-4 hours of adding the gamete suspension. When spawning occurs, eggs and sperm are expelled through the exhalent siphon.

When the first clams to spawn are males, it is good practice to remove them from the trough and leave them out of water until sufficient eggs have been collected from spawning females. The reason for this procedure is that sperm ages rapidly and, if it is more than 1-hour old at the time of fertilization, a low fertilization rate will be obtained.

As each female clam begins to spawn, it is necessary to transfer it from the spawning trough to an individual spawning dish or beaker of about 1 l capacity containing about 400 ml of filtered sea water at 24-26°C (Figure 4). The same procedure applies to spawning males which can be identified as such by the milky appearance of the continuous stream of sperm liberated from the exhalent siphon, as against the granular clumps of eggs shed by a female.

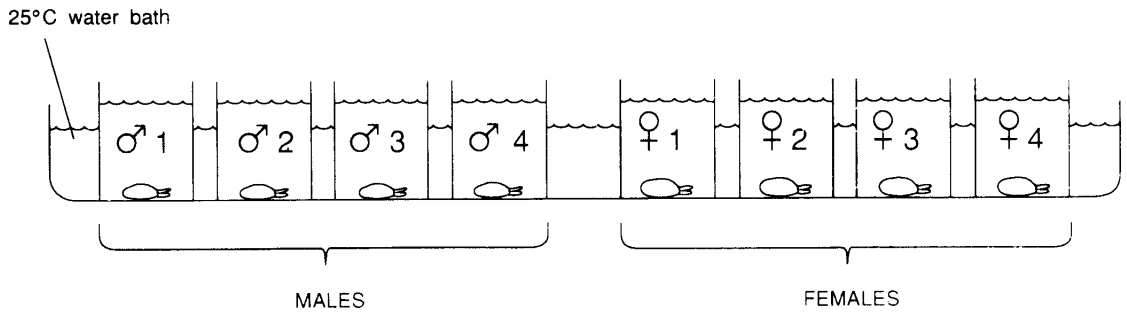


Figure 4. Collection of clam eggs and sperm

The time for completion of spawning for an individual clam is variable but gamete liberation rarely lasts for more than 15 minutes. It may, however, be necessary to remove a spawning female from its container and place it in a fresh one if large numbers of eggs have been liberated. The presence of dense concentrations of eggs in the water inhibits pumping activity and hence the expulsion of eggs. In addition, the female may start to filter eggs out of suspension.

Eggs quickly settle to the base of the dish. When spawning has been completed, any clumps of eggs can be separated, by carefully pouring the contents of the dish through a 60 μm nylon mesh sieve, retaining the separated eggs on a 15 or 20 μm mesh sieve. The eggs are then gently washed into a clean glass or plastic container with filtered sea water at 25°C. When first spawned the eggs are pear-shaped, but they rapidly assume a spherical shape once in contact with sea water. Eggs from the different females are collected separately to provide the opportunity to visually assess quality using a microscope at about x 100 magnification. Batches of eggs which do not round-off after about 10 minutes in sea water, or which are not of a uniformly dense, granular appearance, should be discarded. Reproductive development in the clams is not completely synchronous so that, at any point in time, eggs spawned by different females will be at different stages of maturation.

When separation and examination of the eggs are complete, batches of eggs which appear to be in good condition can be pooled. Sperm from the various males which spawn are similarly pooled. It is good practice to use eggs from at least six females and sperm from a similar number of males for a production run. This ensures a good genetic mix in the offspring depending on the degree of heterozygosity of the parents.

It is desirable to estimate egg numbers before fertilization, since development will be impaired if the density of embryos per unit volume beyond early cleavage exceeds certain limits. This density and the method used to determine egg numbers are described later in Section 6.

3.2 Pacific oyster

Thermal cycling can also be used to induce Pacific oyster broodstock to spawn. A faster method, however, provided unlimited numbers of broodstock are available, is to artificially remove gametes from the adults (Helm and Millican, 1977). If the flat shell valve is removed, the gonad can be punctured with a Pasteur pipette and the gametes withdrawn. Eggs or sperm are dispensed into 400 ml of filtered sea water at 24-26°C in a 1 l beaker, using a separate beaker for each oyster.

Eggs are poured carefully through a 60 μm nylon mesh sieve and collected on a 15 or 20 μm mesh sieve. Treatment of eggs and sperm is then the same as that for clams.

3.3 Native flat oyster

This species is larviparous (i.e. egg fertilization and early development of larvae take place within the gill cavity of the parent). The larvae are shed at approximately 170 μm shell length, into the surrounding water. They may be collected from the broodstock conditioning tank by placing a 90 μm nylon mesh sieve under the sea-water outflow pipe (see Figure 2(b)). The sieve should be kept clean and checked regularly during the day for the presence of larvae. Treatment and estimation of numbers of larvae are described in Section 6.

4. FERTILIZATION : CLAMS AND PACIFIC OYSTER

After pooling the eggs and sperm in separate containers, fertilization is carried out by adding 2 ml of a dense sperm suspension to each litre of the egg suspension. The sperm should be less than 30 minutes old from the time of spawning and the eggs less than 60 minutes old. After addition of the sperm, the contents of the container should be gently agitated and then allowed to stand for 60-90 minutes. Within this period at 25°C the fertilized eggs divide, first into two cells of equal size and then into four cells composed of one large cell capped by three much smaller cells (Figure 5). Assessments of the percentage of eggs developing normally can be made using a relatively low-power microscope (x 20 magnification). Fertilization rates almost invariably exceed 90%.

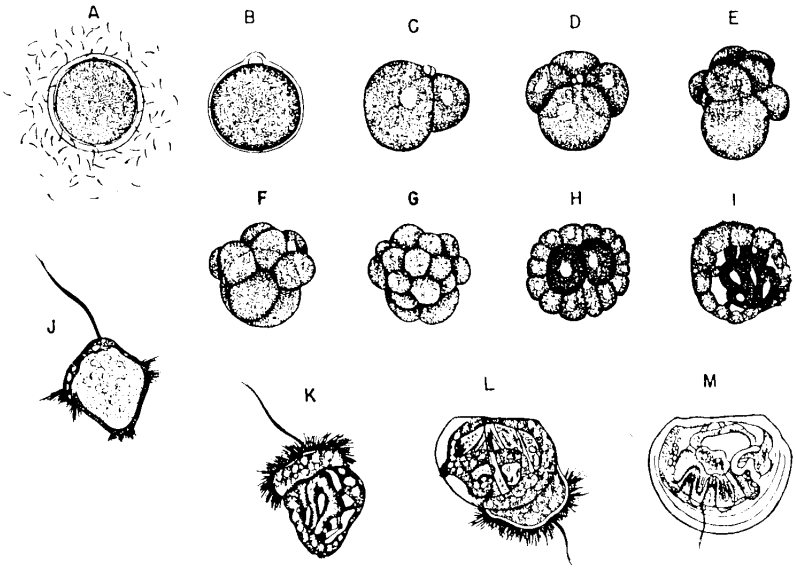


Figure 5. Development of the Manila clam embryo

5. EMBRYONIC DEVELOPMENT : CLAMS AND PACIFIC OYSTER

Fertilized eggs are left to develop to the fully-shelled, D-veliger stage in flat-bottomed rearing vessels (Figure 6). This early veliger stage is known as the D-larva stage because of the characteristic "capital D" shape of the shell.

Vessels of 100-250 l are ideal, preferably made from pigment-free, translucent polyethylene. New vessels must be filled with sea water and allowed to soak, with weekly changes of water, for three

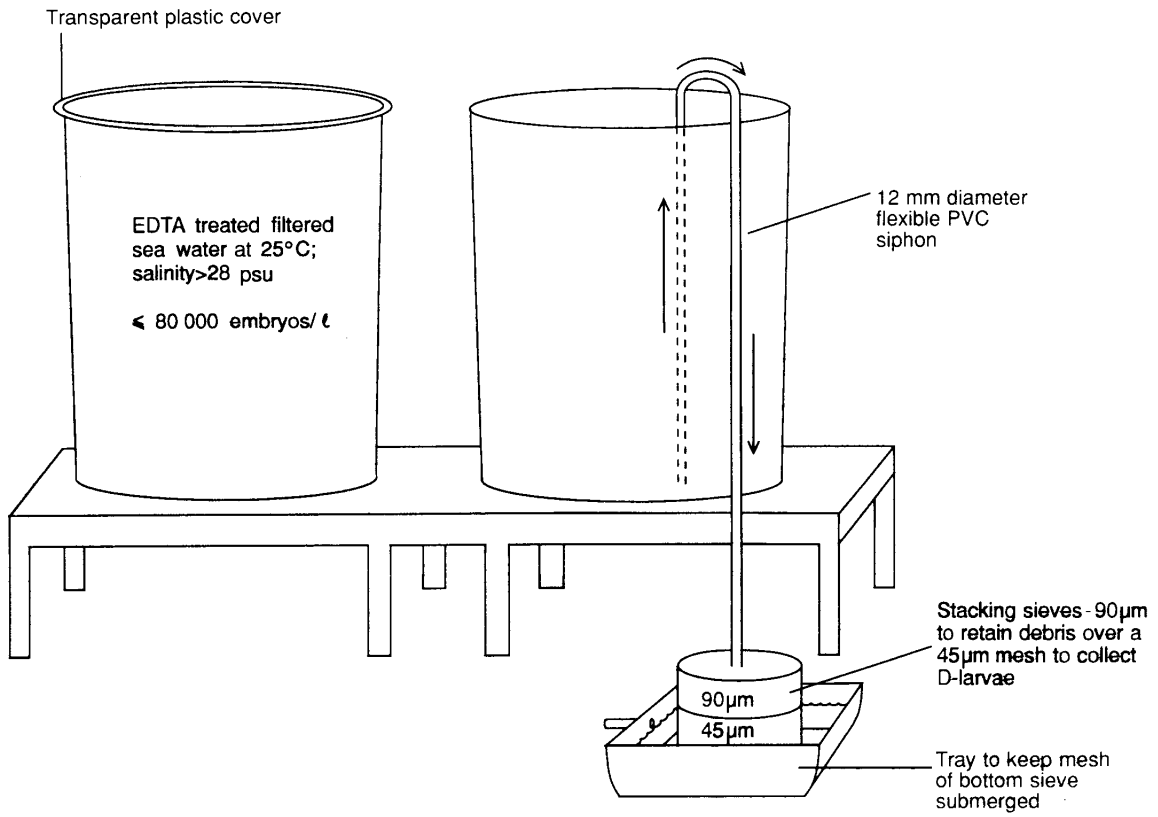


Figure 6. Rearing vessels for embryos and larvae: the method for recovering D-larvae is shown

months before use. This allows toxic substances (which may be harmful to larvae) to leach from the surface of new plastics. Vessels are filled with sea water, filtered by either a rapid sand filter, a beach well, a diatomite filter or similar device, which will retain particles greater than 2 µm in diameter. The water is heated to 25°C and salinity should be 25 psu for Pacific oysters and above 25 psu for clams. It is often beneficial to filter the water and to fill the vessels 24 hours before they are needed. The water is then treated with 1 mg of EDTA (disodium salt — as used in the preparation of algae culture medium) per litre (Utting and Helm, 1985) and is vigorously aerated for 24 hours. The air-flow is switched-off during embryonic development.

Embryos are stocked in the vessels about two hours after fertilization at a maximum density of 80 000/l and fully developed D-larvae are recovered, by the method illustrated in Figure 6, 24 hours later.

In cultures of up to 200 l capacity, 30-50% of the initial number of embryos may be recovered as perfectly formed D-larvae. Imperfectly formed D-larvae (i.e. those with misshapen shells) are unlikely to develop further. The normal D-larvae should have a mean shell length of about 90-95 µm (clams) and 70-75 µm (Pacific oysters) (measured by monocular microscope, x 100 magnification fitted with an eye-piece graticule calibrated against a micrometer slide).

Normal D-larvae are retained by a 35 µm sieve and the number recovered is estimated as described in the following section.

6. ESTIMATING NUMBERS OF EGGS AND LARVAE

Care is needed when handling eggs and larvae. During transfer to other containers, it is important to ensure that the mesh of the sieve is always submerged (Figure 7(a)).

Much of the equipment used in the hatchery needs to be specially made. For example, sieves are prepared from PVC tubes or high-impact, rigid polystyrene horticultural box containers. The bases are removed from the latter and nylon monofilament meshes fixed with PVC solvent cement. Perforated plunger agitators and counting slides are made in the workshop from acrylic or PVC sheet and rod.

Specific equipment, which can be purchased, includes adjustable volume automatic pipettes (0.1-1.0 ml and 1.0-5.0 ml ranges are useful) and various measuring cylinders from 25 ml to 2000 ml in volume.

6.1 Procedural steps

(i) Wash and sieve the eggs or larvae and transfer them to a 1 or 2 l measuring cylinder.

(ii) Make the volume up to the graduation mark with filtered sea water.

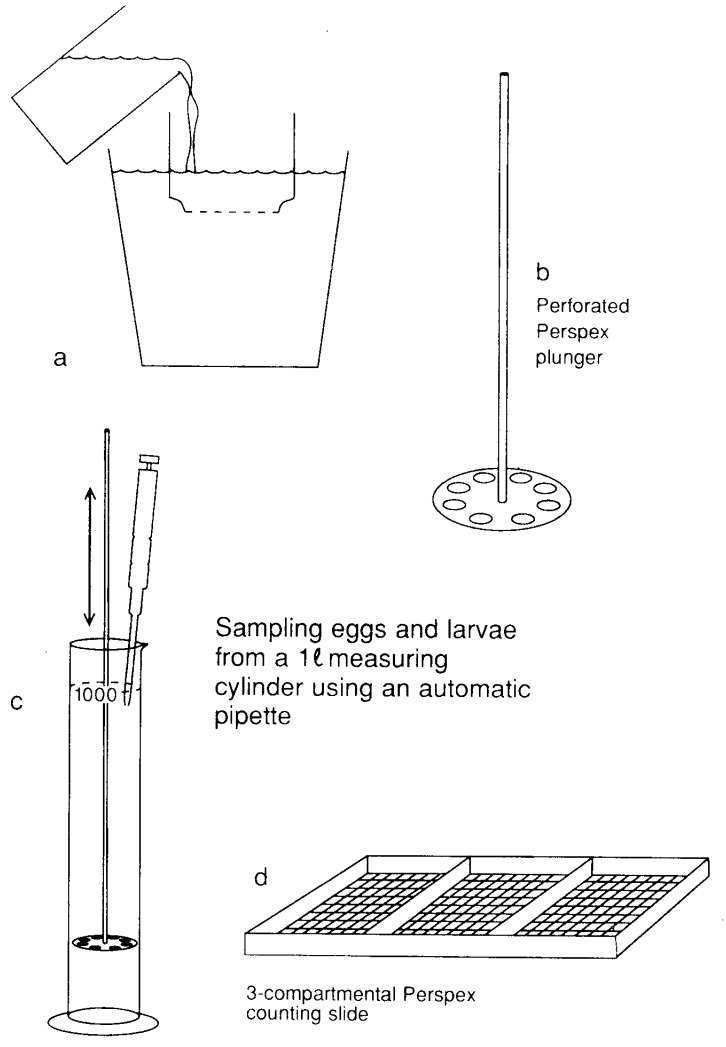


Figure 7. Equipment used for estimating eggs and larvae

(iii) Set an automatic pipette at 0.5 ml and, while agitating the contents with a perforated plunger, take three replicate samples from the cylinder. Make sure that the eggs or larvae are evenly distributed in the water column during sampling (see Figure 7(c)).

(iv) Transfer the samples to the compartments of the counting slide. Each compartment should be etched with a suitable grid to facilitate counting (Figure 7(d)).

(v) Count the eggs or larvae in each sample using a binocular microscope.

(vi) Calculate the total number of eggs or larvae according to the following example:

counts of eggs or larvae in samples = 236, 254, 239 (mean = 243);

volume of sample = 0.5 ml;

total volume of cylinder = 1000 ml;

therefore, the total number of eggs or larvae = 1000/0.5 x 243 = 486 000.

With very high numbers, smaller volume samples, a larger volume measuring cylinder, or a combination of the two, may be used. In the case of eggs, which are very delicate, it may be easier to transfer the egg suspension to a large container, (for example, a 10 l polyethylene bucket). In this case, make up the volume to the calibration line and withdraw samples while gently agitating the contents of the bucket with a large diameter, perforated, plastic plunger.

An electronic particle counter, such as one of the range of models supplied by Coulter Counter, though expensive, is an invaluable aid in determining numbers of eggs and larvae and is also of value in determining cell densities in cultures of unicellular algae.

7. REARING OF LARVAE

7.1 Basic conditions

The larvae can be grown equally well in flat-bottomed vessels (see Figure 6) or in conically-based, fibreglass tanks fitted with bottom drains (Figure 8).

Clam and Pacific oyster D-larvae can be grown at densities of up to 15 000-20 000/l but growth and survival are improved considerably at densities below 10 000/l.

Growth is improved by ensuring that the water is sufficiently turbulent to keep the larvae in suspension. Vessels may be aerated by a single central aerator at flow rates of up to 200 l/h, depending on the size of the container. Excessive aeration may depress the growth of larvae. Compressors which produce carbon or oil in their air supplies are unsuitable for the hatchery. The air is filtered to 0.45 μm particle size by a series of cartridge filters of decreasing porosity. This is needed to reduce air-borne contaminants which may include harmful micro-organisms.

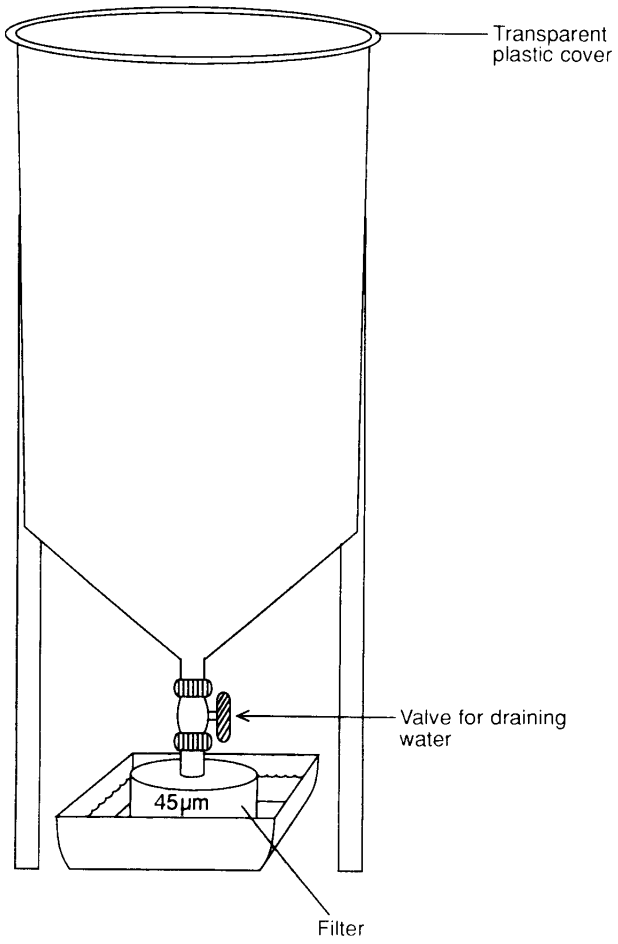


Figure 8. Conical larvae rearing tank

Sea water for the larvae is filtered to about 2 µm particle size and is heated to 24-26°C. Salinity should be 25 psu for Pacific oyster larvae, above 25 psu for clam larvae and above 30 psu for flat oyster larvae. Following filtration, sterilization of the water by ultraviolet light to reduce bacterial contaminants is optional, but advisable.

Clam and Pacific oyster D-larvae, and newly released flat oyster larvae are at the stage where they need feeding with unicellular algae. Suitable food species include:

Chaetoceros calcitrans;
Thalassiosira pseudonana (3H);
Isochrysis galbana (or 'Tahiti Iso'); and
Tetraselmis suecica (for larvae >120 µm in length).

Methods for the mass production of these algae are described elsewhere (Helm *et al.*, 1979; Laing, 1979).

Diets of mixed algae are beneficial. A suitable diet for the D-shelled larvae is a mixture of *Chaetoceros* and *Isochrysis*. Food rations are usually quoted as the number of algal cells per microlitre (cells/µl). When a mixture of *Chaetoceros* and *Isochrysis* is fed, the most suitable cell densities are 125 cells/µl

and 50 cells/µl respectively. *Isochrysis* and *Chaetoceros* can be fed alone at 50 cells/µl and 250 cells/µl respectively. With a two-species diet, each alga is added at 50% of the cell density at which it is fed as a single species diet. Larvae greater than 120 µm mean shell length grow best on a three-species mixture comprised of the following proportions:

33 cells/µl *Isochrysis* + 83 cells/µl *Chaetoceros* + 3.3 cells/µl *Tetraselmis*.

Volumes of the algal species needed to achieve the required cell densities are calculated from the following formula:

$$\text{Volume (ml)} = \frac{\text{Required cell density (cells/µl)} \times V \times 1000}{\text{Cell density of harvested algae (cells/µl)}} \qquad \text{.....Equation 2}$$

where V = the volume of the rearing vessel in litres.

Over-feeding is equally as damaging to the performance of larvae as under-feeding. With high densities of larvae, it is necessary to add the total daily ration in two or more feeding sessions to avoid grossly exceeding the optimum cell density.

The number of algal cells eaten per day, by larvae of different shell lengths grown at 24°C, is shown in Figure 9. One cell of *Tetraselmis* is calculated as being equivalent in cell volume to 10 cells of *Isochrysis* or 25 cells of *Chaetoceros*, and values shown are in *Isochrysis* cell equivalents. Numbers of cells eaten per larva per day increase as the larvae grow (Figure 9) but with clams decrease once a size of 190 µm is reached (see Sub-section 8.1).

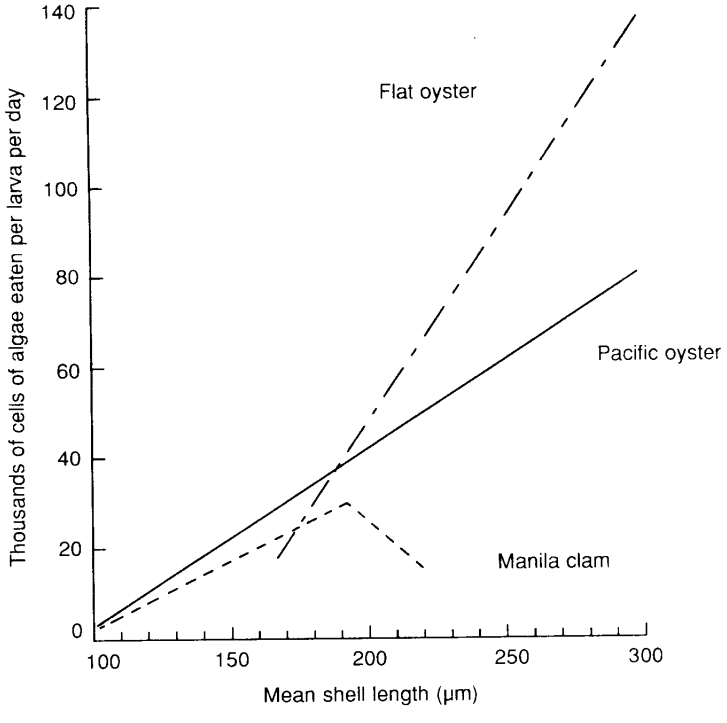


Figure 9. Feeding of larvae: number of algal cells (equivalent in size to Isochrysis) eaten per day at 24°C

7.2 Starting a larvae culture

(i) Fill a clean larvae rearing vessel with diatomite or sand-filtered sea water at about 25°C and at the appropriate salinity.

(ii) Abnormally high mortalities of larvae may be caused by bacteria. Thus, it is advisable to treat the water with ultraviolet light before filling the vessels. If mortalities persist, a broad-spectrum antibiotic may be used under veterinary prescription.

(iii) Add clam and Pacific oyster D-larvae to the vessel at a density of 10 000 larvae/l and add newly-liberated flat oyster larvae at a density of 2000 larvae/l.

(iv) Calculate the volumes of algae needed (see Sub-section 7.1) and add to the vessels to provide the required food ration.

(v) Aerate the water gently for D-larvae but more vigorously for larger larvae (>140 μm). Flow rates of air for optimum growth and spat production vary with size and shape of container. As a guide, an aeration rate of 200 l/h and 350 l/h are required for conical containers with 75 l and 350 l capacities (Helm and Spencer, 1972).

(vi) The culture is now left for 24 hours before further husbandry is necessary.

7.3 Husbandry of larvae cultures

Since larvae cultures are operated as static water systems (i.e. without a continuous exchange of water) they require daily attention. The daily addition of algae and the accumulation of waste products during the larvae's normal feeding, respiration and excretion can lead to harmful water quality in the vessel. It is normal to change the water in the culture vessels three times per week at approximately two-day intervals. The standard practice at Conwy is to change the water on Monday, Wednesday and Friday of each week.

7.3.1 Husbandry at water changes

The procedure is similar to that for embryos (see Section 5).

(i) Water is syphoned from the vessel into a sieve with a mesh sufficiently large enough to allow larvae to pass through but which retains debris. Initially, a 200 μm sieve is ideal. The larvae are retained on the 45 μm aperture mesh of the lower sieve.

(ii) Wash any remaining larvae from the vessel into the sieve.

(iii) Wash the vessel with a sponge and hot detergent solution and rinse well.

(iv) Re-fill the vessel with appropriately treated sea water.

(v) Grade the larvae by washing them through a stack of sieves of descending apertures with filtered sea water. Suitable mesh sizes are shown in Figure 10.

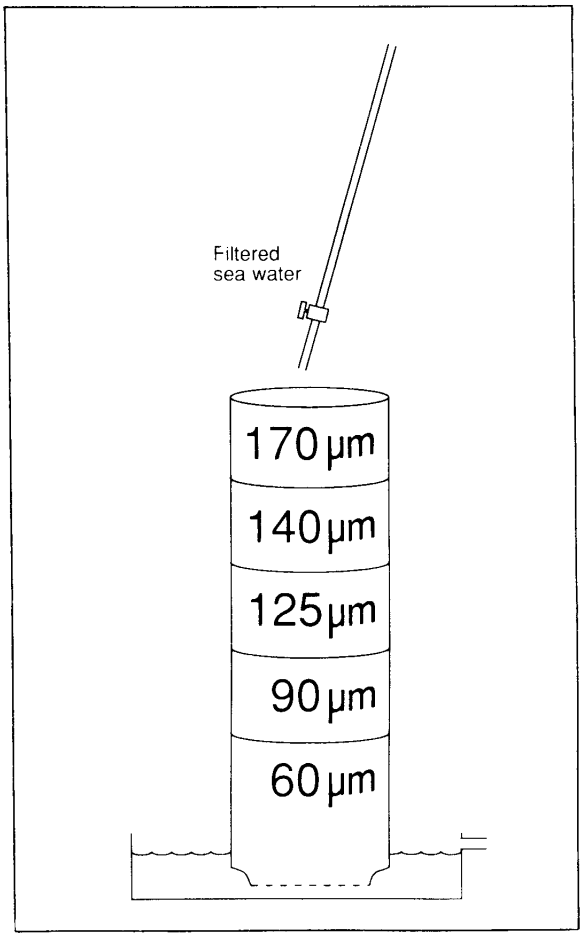


Figure 10. Stack of sieves showing meshes which are suitable for Manila clams. Oysters near to settlement require meshes of 210 µm and 245 µm and Pacific oyster D-larvae require meshes of 35 µm and 45 µm in addition to those shown

(vi) Take small samples of retained larvae from each sieve and observe their appearance and activity with a microscope. Discard any sieved fraction containing predominantly dead larvae (i.e. empty shells and those containing decomposing tissues). Healthy larvae have yellow-brown colouration with a dark digestive gland.

(vii) Wash the healthy larvae into a measuring cylinder. Sample (see Section 6) to determine the total number surviving. Measure a sample of 50-100 larvae and calculate the mean shell length. The addition of a few drops of formalin will immobilise the larvae.

(viii) Return the larvae from the cylinder to the rearing vessel and adjust the aeration to the required level.

(ix) Feed.

(x) Repeat this procedure at the next water change.

7.3.2 Husbandry between water changes

This consists of replacing the algae grazed down in the previous 24 hours. A sample of water is taken from the vessel and the residual algal cells are counted, either microscopically using a haemocytometer, or with a Coulter Counter.

BIVALVE LARVAE

Species : Manila clam — **Daily record**

Date	Day	Vessel vol (l)	Expt.No.	T(°C) 26.1
	12	125	9	S(psu) 30

Mean shell length (µm) 211.4 **% eyed**

Class	Frequency	No.=	Diff
160			-31
170	I	1	-4
180	III	3	-9
190	IIII	4	-8
200	卌 卌	10	-10
210	卌 卌 卌 卌 IIII	24	
220	IIII	4	+4
230	III	3	+6
240	I	1	+3
250			+13
		n=50	=-1.8 x 2
			=-3.6

Water treatment

Answer ✓ or x

1.Water change? ☑

If ✓

2.Water filtered? ☑

3.UV treated? ☑

4.EDTA added? ☑

5.Antibiotics? ☒

If ✓ specify:-

.....

6.Other treatment? Specify:

.....

Larvae observations

Colour: Very good

Activity: Very good

Sub-sample vol 0.5 ml/ 2 l

Counts: 183,177,180

Total number: 720,000

Feeding

Residual algae		Algae added			
Species	Cells/µl	Species	Cells fed	ml added	Cells/ µl
ISO	15.6	T.ISO	100	954	13100
CHAET		CHAET	100	236	53000
TET	1.2	TET	10	521	2400

Spat counts (oysters)

Sub-sample vol..... ml/..... l

Counts:

Total number:

Grand total:

Larvae grading	35	45	61	90	124	140	170	210	236	265	µm sieve	
Approx.%				5 poor	10	45	40	few				
Retain ✓ Discard x				x	✓	✓	✓	✓				

Notes: Large number of pediveligers

Figure 11. Specimen of daily record sheet

Daily records are kept of culture temperatures, residual algae and any additions of food needed to restore optimum food cell concentrations (see specimen copy of record sheet, Figure 11). Volumes of additional algae are calculated from the formula given previously.

8. GROWTH OF LARVAE

Information on the growth and development of larvae is shown in Figures 12 and 13.

8.1 Clams

Growth in the first eight days, when the larvae are actively swimming, is rapid. Growth rates decrease as larvae reach the pediveliger stage (ca 200 μm) when they gradually change to a more sedentary habit, crawl by means of a foot, and undergo metamorphosis (ca 230 μm) to the juvenile or spat stage. Feeding rate decreases during this transition (see Figure 9). Young spat tend to crawl up vertical surfaces and cluster together using their byssal threads.

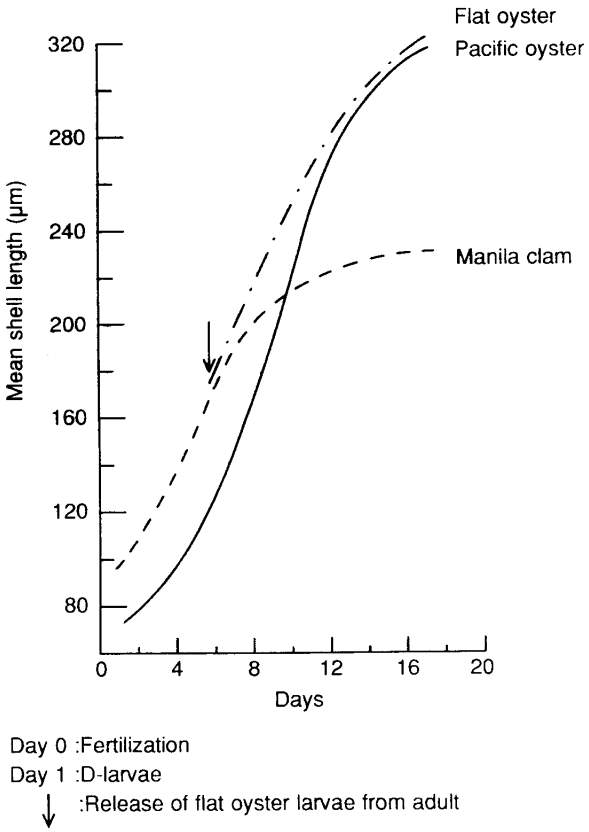


Figure 12. Growth of bivalve larvae at 25°C

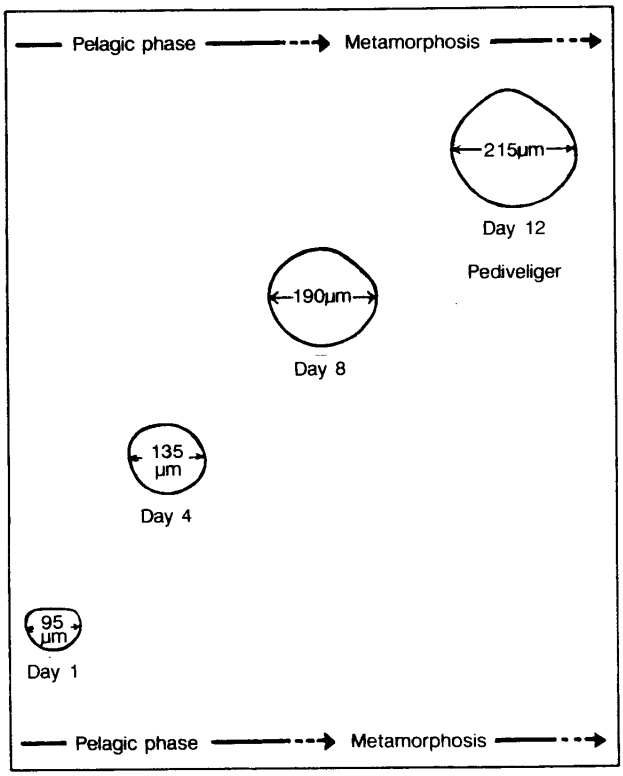


Figure 13. The appearance of Manila clam larvae reared at 25°C

8.2 Oysters

Pacific oyster larvae grow rapidly to metamorphosis during their first twelve days in culture. Flat oyster larvae, however, are quite large when liberated from the parent (approximately 170 μm), and hence their free-swimming stage is reduced to about seven days at 25°C.

Before metamorphosis (at 280-300 μm), oyster larvae cement themselves permanently to a suitable substrate. This phase is called "settlement". The settlement substrate used in the hatchery at Conwy is a matt-surfaced, black, PVC disc placed flat on the bottom of the rearing vessel. (An alternative technique used in some hatcheries is to add, to the rearing vessel, scallop shell ground into fragments of approximately 180 μm in diameter. These fragments provide settlement surfaces for individual larvae. The chemicals epinephrine and nor-epinephrine added at concentrations of 10^{-4}—10^{-5} M can also be used to induce larvae to settle and metamorphose without the provision of a settlement surface (Coon *et al.*, 1986)).

Larvae which are ready to settle can be recognised under the microscope by the presence of a dark, pigmented eyespot on the tissues inside the shell. When approximately 70-80% of larvae have reached the "eyed" stage, the PVC disc is added to the rearing vessel and the vessel is illuminated. Larvae swim away from the light and attach to the disc. "Settled" oysters are removed daily from the disc with a razor blade and the numbers are estimated (see Section 6).

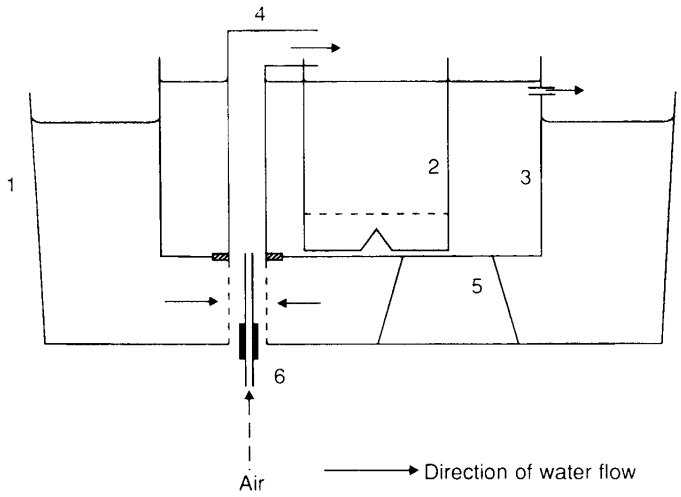
9. HUSBANDRY OF CLAM PEDIVELIGERS

Clam pediveligers are usually left in the rearing vessels until day 16 and, thereafter, those retained on a 170 μm sieve are transferred to recirculating sea-water systems of 100-200 l capacities operated by air-lift (Figure 14). Smaller larvae are retained in the larvae vessel until they are large enough to transfer.

Survival from the D-larva to the pediveliger is variable but should be greater than 40%. Optimum survival occurs early in the year, with the poorest survival usually in the summer, again associated with higher temperatures and bacterial infection.

An air-lift downwelling recirculation system, suitable for holding clam pediveligers during metamorphosis, is shown in Figure 14. The husbandry needed for this type of system is similar to that for the larvae vessels (see Sub-section 7.3) and the clams are fed with the same diet of algae. As pediveligers complete metamorphosis and start to grow rapidly, they require additional food above the daily basic ration. A 50% supplement may be added initially and this can be increased further with evidence of higher grazing rates.

By day 35, many of the spat should be retained on a 440 μm mesh. These spat may be transferred to an upwelling system (Figure 15). It is sufficient to grade pediveligers and early spat once each week. Meshes of 210 μm, 265 μm, 350 μm, 440 μm, 660 μm, 850 μm, 1000 μm and 1500 μm are useful



1 Reservoir tank
2 Downwelling cylinder, >20 cm in diameter fitted with 140 μm mesh at the base
3 Constant head tank
4 Air-lift pipe
5 Support
6 Air inlet

Figure 14. An air-lift downwelling, re-circulation system suitable for clams during metamorphosis

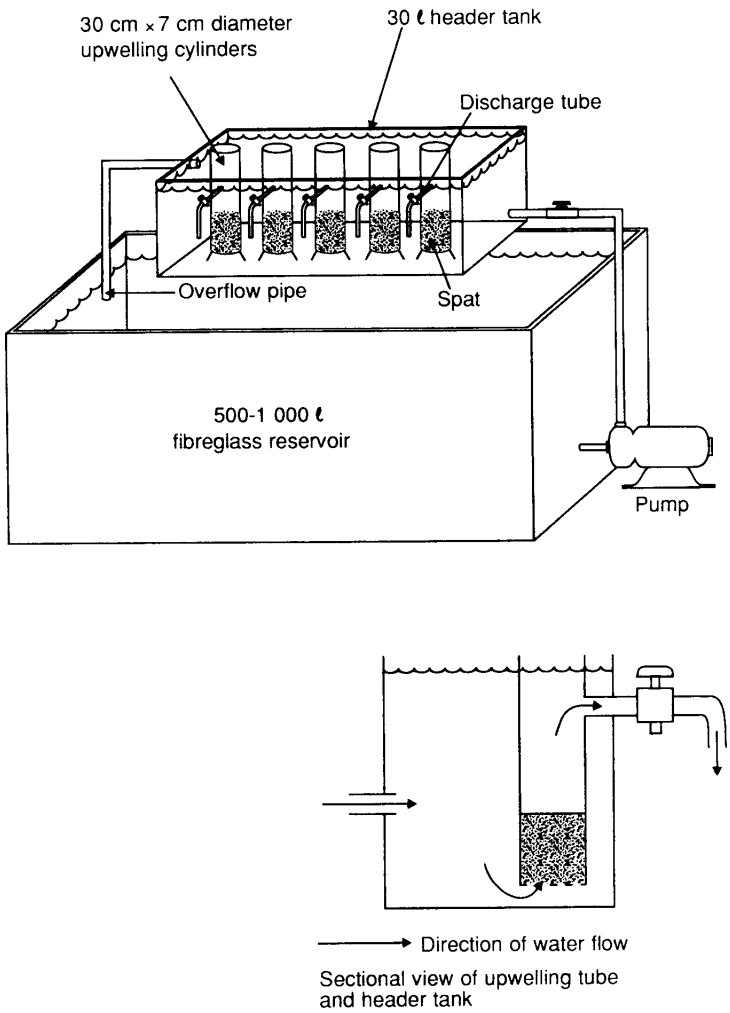


Figure 15. A nursery upwelling recirculation system as used at the Fisheries Laboratory at Conwy

for grading. The tanks need washing with detergent and hot water at each water change. Periodic cleansing of valves and pipework, by internal brushing, is necessary to clear accumulated algal debris. The spat may be sprayed with fresh or sea water with sufficient pressure to remove faeces, pseudofaeces and uneaten food. A 100 l downwelling system is suitable for growing 0.5 million small spat to a size which will be retained on a
440 μm mesh. Spat are particularly vulnerable during this stage and although 70% survival may be achieved in good conditions, survival is likely to vary widely.

10. HUSBANDRY OF SPAT

10.1 Nursery upwelling systems

Upwelling systems are suitable for oysters immediately after 'settlement' and for clams after completing metamorphosis in the downwelling system (see Section 9). A 210 μm mesh, fitted to the base of the upwelling cylinder, is appropriate for newly-settled oyster spat. Once clam spat are retained on 440 μm mesh, they are fairly robust and easy to handle. They are no longer able to swim but are extremely mobile and can crawl up vertical surfaces. As a consequence, many will climb above the water/air interface where they become stranded and desiccate, and eventually die. Care must be taken to dislodge these stray spat every day.

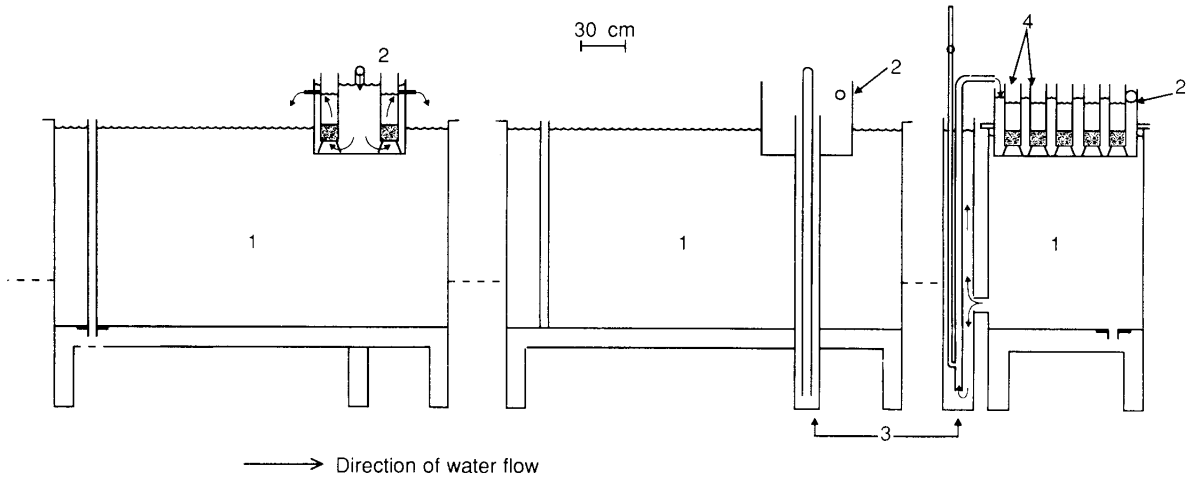


Figure 16. Spat upwelling recirculation systems. Optimum biological loading = 600 g live weight of spat divided between 10 tubes (60 g biomass/tube). Optimum flow rate of sea water = 25-30 ml/min per gram live weight biomass = 1.5-1.8 l/min (per tube). The airlift system must raise about 18 l/min to the header tank. Optimum daily food supply for 600 g biomass = 171 l of Tetraselmis equivalents at 1 million cells/ml (= 3 feeds of 57 l/day) = 34 g/day of spray-dried algae. Operating temperature is 24°C for most species. The frequency of water change = 3 times per week at recommended loading. Note: With minor modification, these systems can also be operated as downwellers, 'splash-jet' tray systems, or as partial flow-through systems

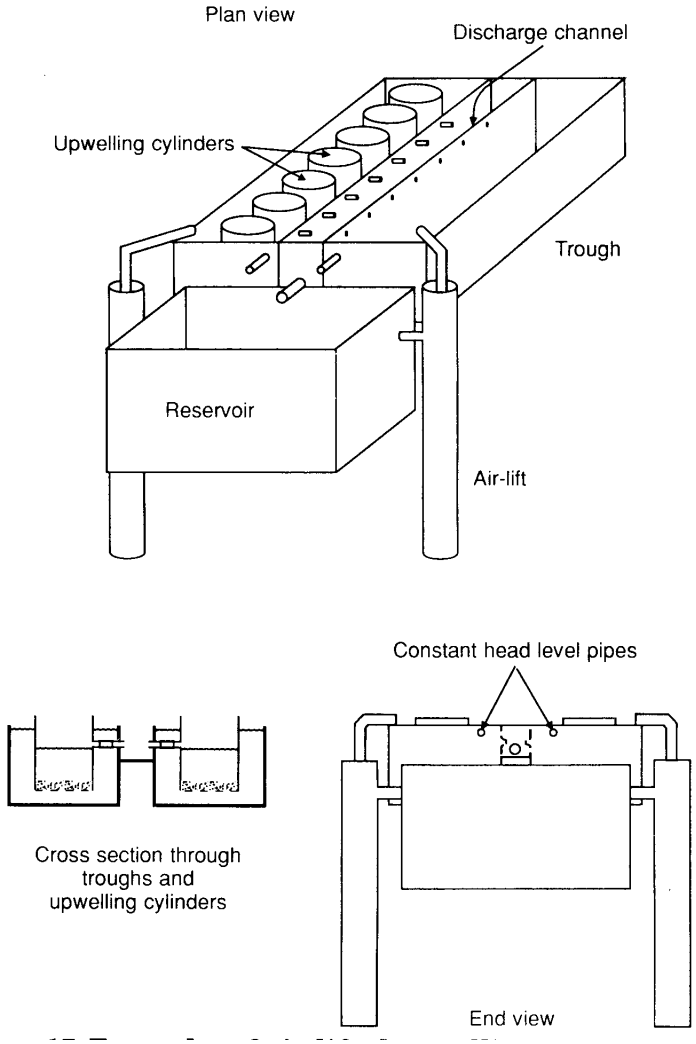


Figure 17. Examples of air-lifted upwelling nursery systems

Three types of upwelling systems are shown (see Figure 15 - Section 9; and Figures 16 and 17) in which water flow is induced through the cylinders, by creating a difference in the head of water. The water may be recycled with an electrically-driven pump (see Figure 15), as is the case at Conwy, or by air-lift (Figures 16 and 17). Valves may be fitted to the overflows of the upwelling cylinders, since spat growth is strongly influenced by water flow rate. Flow rates of 20-50 ml/min per gram should be used.

Sea water, coarsely filtered through a 45 μm mesh, may be used so that spat can benefit from the presence of naturally occurring algae in addition to those offered as food. The water is kept at 22-25°C and is changed three times each week when the biomass of spat is about 200 mg/l. The systems are thoroughly cleaned at each water change and the spat washed (see Section 9). Spat grading is generally carried out once per week where the objective is to group spat of a similar size together in an upwelling container. Individuals within a particular batch of spat show widely differing growth rates.

Nursery upwelling systems need to be of relatively large volume, since only small biomasses of spat can be grown successfully per unit volume of water. The consequences of over-stocking of clams, and competition for food, on growth rate are illustrated in Figure 18. It is good practice to regulate the biomass to a maximum of 200 mg/l of the system volume. For example, a 2000 l system can be used to grow a maximum of 400 g live weight of spat, irrespective of the mean live weight of the spat. To help with calculating the numbers of spat which can be held at different mean live weights, a length to weight conversion graph is given (Figure 19). Four hundred grams live weight can be made up of 400 000 x 1 mg (live weight) spat of 1.5 mm mean shell length or 40 000 x 10 mg (live weight) spat of 4.4 mm mean shell length.

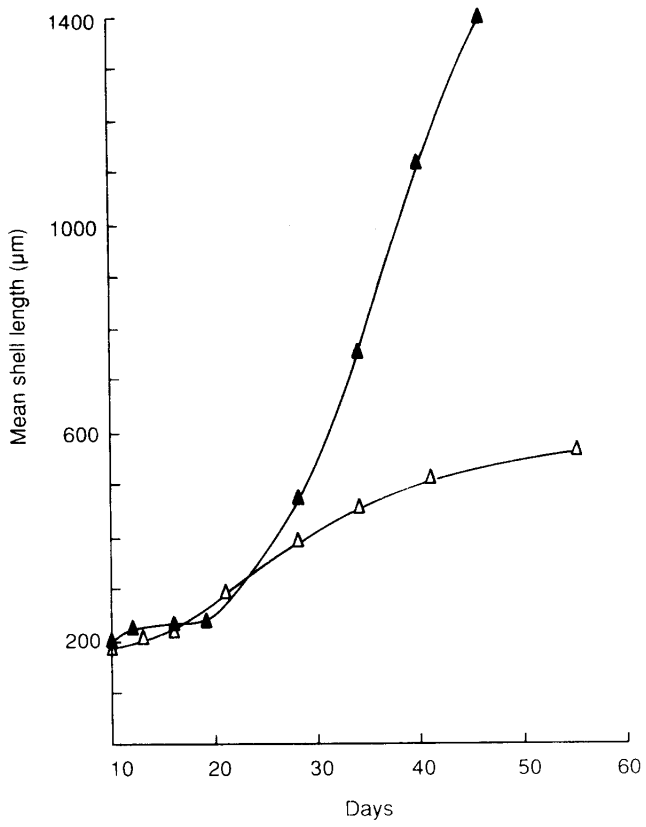


Figure 18. Growth of Manila clam spat at initial stocking densities of 400 mg/l (Δ) and 50 mg/l (▲)

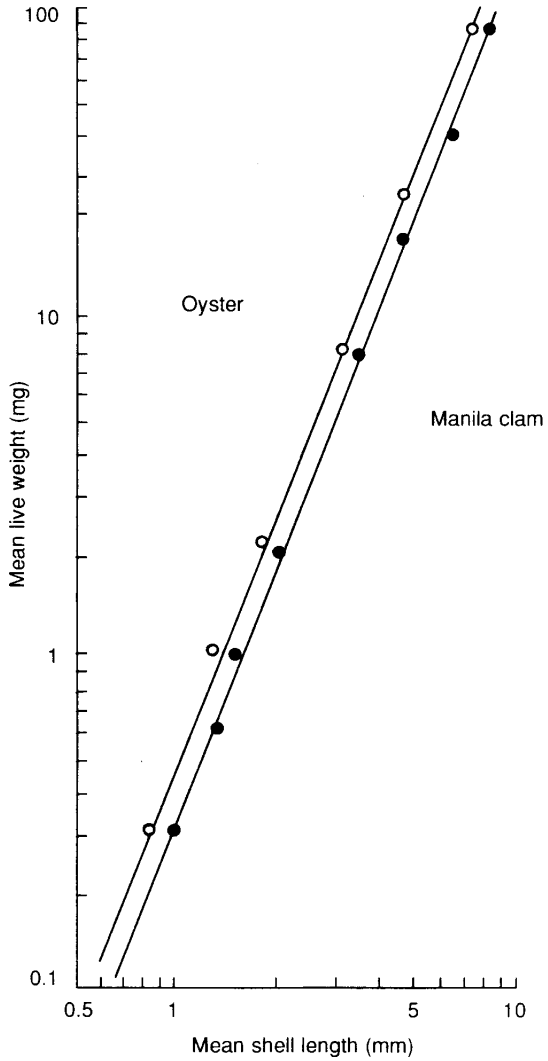


Figure 19. Length/weight relationship of clam and oyster spat

10.2 Food Requirements

Spat growth is largely influenced by the amount of food available for feeding. The ration is calculated as dry weight of algae (mg) fed over a week, per milligram of live weight of spat at the beginning of the week. The amount of growth which may be expected with Pacific oysters fed different rations of *Tetraselmis* is shown in Figure 20. One million *Tetraselmis* cells are equivalent to 0.2 mg dry weight (2.5 million cells to 0.5 mg, etc).

Since feeding is based on the weight of spat in the system, their biomass must be determined each week. Feeding a ration of 0.4 mg dried algae per milligram (live weight) of spat per week provides good spat growth without excessive cleaning problems associated with higher levels of feeding. The amount of food required daily can be calculated from the following formula:

$$F = (S \times 0.4) / 7 \qquad \text{..... Equation 3}$$

where F = the dry weight of algae required per day (mg),
and S = the live weight of spat (mg) at the beginning of each week.

For example, 200 g of spat (200 000 mg) require 200 000 x 0.4 / 7 = 11 429 mg (11.43 g) dry weight of algae per day. To determine the number (in millions) of algal cells equivalent to this weight of food, divide by the weight (in mg) of one million cells. Table 2 gives the weights of cells for commonly used species of algae.

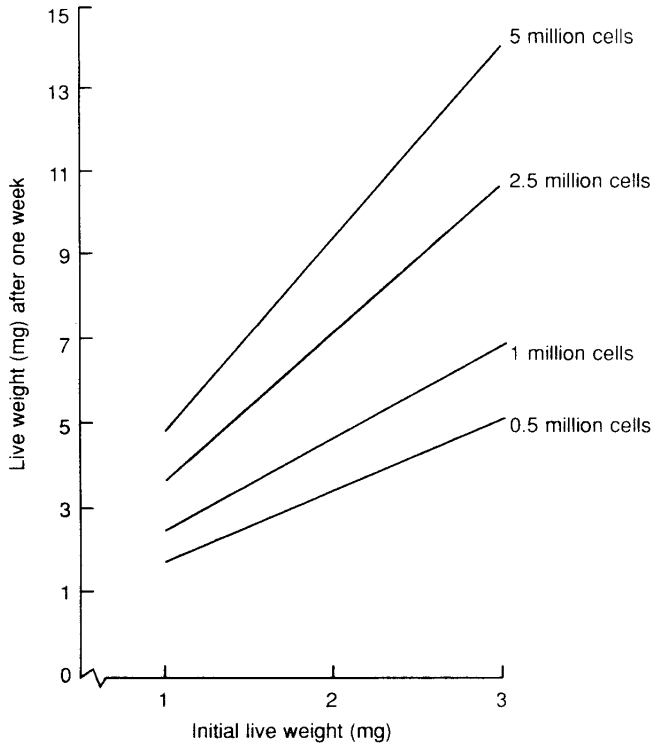


Figure 20. Growth of Pacific oysters fed different rations of Tetraselmis (millions of cells/mg of spat per week). Data are calculated from an equation (unpublished data) derived from experiments in which the spat were kept in trays supplied with water entering as 'splash jets' into their tops. The water temperature was 23-25°C and the salinity was 25 psu

Table 2. Dry weight (mg) per million cells - for common species of algae

Species of algae	Weight
T-ISO	0.02
Skeletonema costatum	0.032
Chaetoceros calcitrans	0.007
Chroomonas salina	0.13
3H (*T. psuedonana*)	0.02
Tetraselmis suecica	0.20

Therefore, to feed 11 429 mg of *Skeletonema* daily, 11 429/0.032 = 357 156 million cells would be required. To convert this number of cells into the volume of algae culture (in litres) required, divide by the concentration of cells in the culture (cells per µl). Continuing with the above example it can be seen that, if the *Skeletonema* were harvested at 7000 cells/µl, then a volume of 357 156/7000 = 51.02 l of this *Skeletonema* culture would be needed for feeding each day. The daily food requirement of spat in upwelling systems can be calculated from one equation, thus:

$$V = (S \times 0.4)/(7 \times W \times C) \quad \text{..... Equation 4}$$

where S = the live weight of spat (mg) at the beginning of the week,
W = the weight (mg) of one million algal cells (see Table 2 above),
and C = the cell concentration (cells/µl) of the culture.

It is preferable to feed a mixture of two or more species of algae, with one of the best combinations being a mixture of 50% *Skeletonema* and 50% *Tetraselmis*. To determine the amount of each species to feed, Equation 4 is used to calculate the total volume (V) of each type of alga which would be required if it were the only food, and the result is divided by 2.

It is possible to replace the 50% live *Tetraselmis* in the mixture with the equivalent amount of commercially available spray-dried *Tetraselmis* (Celsys, Cambridge, UK). This will give a growth identical to that of spat fed on the same mixture of an "all live" algae diet (Laing, 1990). The weight of dried food required is calculated as F (see Equation 3) divided by 2. For example, 200 g live weight of spat will require 200 000 x 0.4 / 7 = F = 11 429 / 2 = 5714 mg (5.714 g) per day. To give the best results, this amount of dried food is mixed with about
800 ml of sea water in a domestic blender for 5 - 10 seconds before feeding.

10.3 Growth and survival

Examples of growth in Manila clam and Pacific oyster spat are shown in Figure 21. Pacific oysters grow faster than Manila clams but both species usually reach 5 mg (2-3 mm) within 6 weeks in indoor nurseries. Survival rates are variable but 50-90% may be expected providing spat quality at "settlement" is satisfactory and adequate feeding and husbandry are maintained.

It is usual for hatcheries to sell clam and oyster spat, or put them into outdoor nurseries, at a mean shell length of 2-3 mm (5 mg), because of the considerable expense of growing them further under closely controlled conditions indoors. If there is a big difference between the temperature of hatchery rearing systems and the ambient temperature of the sea, subsequent spat survival will be greater if they are acclimated gradually before transfer by decreasing the temperature in the spat system by 2°C per day.

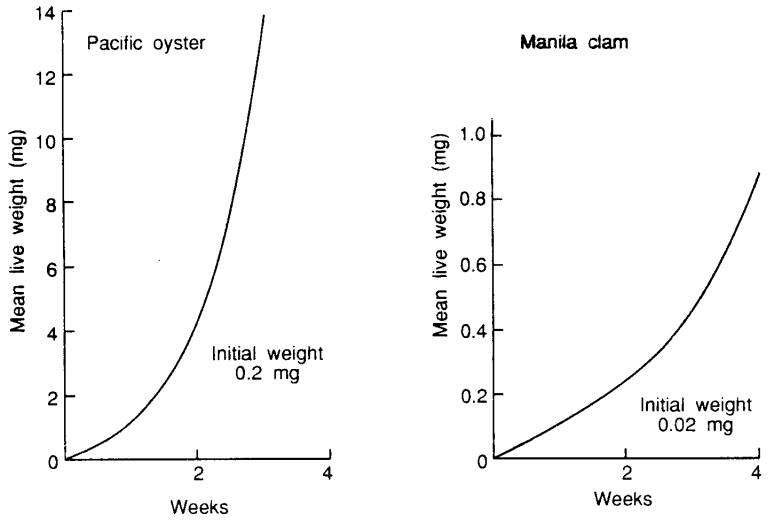


Figure 21. Examples of growth of Manila clam and Pacific oyster spat in the same upwelling system fed with Tetraselmis at a rate of 0.28 million cells/day per milligram of live weight of spat. Water temperature for the period averaged 22°C, salinity 30 psu and flow rate 30 ml/min per gram of spat through each upweller

11. CONCLUSIONS

This leaflet provides a brief review of the techniques used for the reliable hatchery culture of bivalve species most commonly reared in the United Kingdom. The techniques are appropriate for the rearing of native flat oysters (*Ostrea edulis*), Pacific oysters (*Crassostrea gigas*), Manila clams (*Tapes philippinarum*), native palourdes (*Tapes decussatus*) and American hard shell clams (*Mercenaria mercenaria*). With modifications, the techniques can also be used for the culture of other bivalve species.

The initial selection of a suitable site on which to build the hatchery is of utmost importance to its subsequent success for production of bivalve seed . A supply of high-quality sea water, free from pollution and organisms causing disease, is essential. The design of the hatchery is critical, to ensure that aspects of hygiene and safety are of the highest standards.

As this leaflet does not include all of the details relevant to hatchery culture, a list of further reading is given in Appendix 1.

ACKNOWLEDGEMENT

We would like to thank our colleague Mike Helm, (now in Blandford, Nova Scotia), for his help in the preparation of this leaflet.

The reference to proprietary products in this leaflet should not be construed as an official endorsement of these products, nor is any criticism implied of similar products which have not been mentioned.

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