V

REVIEW ON PRODUCTION OF MUSSEL SEED

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Production of mussel seed requires priority attention for the expansion of mussel culture as an industry in India. Procurement of adequate seed from the wild beds even for the current experimental programmes is faced with problems. Two lines of approach have been suggested for enhancing seed production.

The first approach is to harness the production of seed in nature through three ways, namely exploitation of the mussel seed settling on the stone embankments and groynes laid as an anti-sea erosion measure, enhancement of spat fall on artificially laid collectors in the farms; and selecting areas where the current system and other environmental factors are conducive for larval transport and settlement of the pediveliger stage in the sites where breeding reserves of adult mussel are provided.

The second approach is through hatchery system. Although mussel hatcheries do not exist in Europe where world mussel production is concentrated in view of plentiful supply of natural seed, it is imperative for India to develop the hatchery technology as the natural seed availability is severely limited.

While discussing the above aspects of seed production, the paper reviews the larval history of mussel and the technologies available for induced spawning and larval rearing, with particular reference to larval food and diseases.

Introduction

Production of seed or young ones is one of the vital aspects of any farming activity either on land or in water. The ontogeny of marine organisms is such that the environment, which is dynamic and ever changing, plays the key role in the dispersal of the population and the success or failure of a brood in reaching its appropriate destination. This explains the uncertainty of availability of young ones for any mariculture operation and the need for human effort to ensure seed production through culture.

During the last few years technologies of mariculture have been developed in India for several economically important organisms but commercial culture can become truly successful only upon the availability of viable technologies for the production of seed. Culture of the green mussel *Perna viridis* and the brown mussel *P. indica* is one of the areas where considerable advances have been made on the techniques of farming but the basic seed material has always been obtained from the wild. Removal of seed mussel from the beds for farming comes in conflict with the interests of the natural fishery. The mussel beds in Europe—in Spain, the Netherlands, France and Italy—are extensive and spatfall has always been abundant. This is one of the reasons for the absence of mussel hatcheries in these countries. On the other hand, the mussel beds on the Indian coast are limited and scattered, and rocky surface area for spatfall is also restricted. The natural seed resource on the beds cannot support mussel culture industry of some magnitude. These factors make it imperative to develop techniques for seed production.

Larval History of Mussels

The reproductive 'strategy' of mussels is one of high fecundity, small eggs, external fertilisation and a pelagic larva that feeds on the phytoplankton (Bayne, 1976 *Marine mussels: their ecology and physiology* Camb. Univ. Press, 506, pp.[1]). The fecundity of the Indian species of mussels has not been worked out. However, some estimates are available for the European mussel *Mytilus edulis* which, though not accurate, may give an indication of the order of fecundity. Bayne (1975, in *physiological ecology of estuarine organisms* (Ed. Vernberg, Univ. Calif. press, 259-277[2]) estimated half

a million eggs per female of 4-5 cm in shell length. At the other extreme, Fretter and Graham (1964, *Reproduction in physiology of Mollusca*, Ed K. M. Wilbur and C. M. Yonge, Acad. press, N.Y., 127-164[3]), quoting Pelseneer gave an estimate of 10 million eggs.

The larval development of *Perna viridis* has been described by Rao *et al.* (1976, *Indian J. mar. Sci.*, **5** : 113-116[4]) and that of *P. indica* by Kuriakose (1980, Abs. 169, *Symp. Coastal Aquaculture*, Mar. Biol. Ass. India[5]). As reviewed by Bayne (1976[1]), development of mussels of other geographical regions has been studied in detail by several workers and the larva of *M. edulis* is one of the most frequently described of all lamellibranch larvae. In general, subsequent to external fertilisation, the embryo, through a process of cleavage and gastrulation, develops into the first stage of larva called trochopore, thence to a veliger. On the secretion of the larval shell, prodissoconch I, by the shell-gland of the veliger, the larva attains the straight-hinge or D-shaped stage. The mantle of the veliger then secretes the second larval shell, prodissoconch II, and the larva is called a veliconcha. As the larva approaches metamorphosis, a pedal organ develops and the larva with its functional foot is called the pediveliger. It is at this stage that the larva descends down from the plankton to sea bottom. Through a pattern of swimming and crawling behaviour it selects the substratum and attaches itself by the secretion of byssus threads after which metamorphoses, when a series of changes takes place in the organ systems towards adult organisation becomes possible. After metamorphosis, the adult shell dissoconch is secreted. The young post-larva with adult shell characteristics is described as a plantigrade (Bayne, 1976[1]). It derives the name spat and the process of settlement of a brood of spat is called spatfall.

The spawned egg of *M. edulis* is 68-70 μm in diameter and during the veliconcha stage there is considerable growth in size (from 110-250 μm in shell length); at 220-260 μm the larva acquires a pair of pigmented spots and subsequently, with the development of a foot, becomes a pediveliger (Bayne, 1976[1]).

Kuriakose (1980[5]) has observed that the ripe ovum of the brown mussel *Perna indica* measures 55 μm and at the early pediveliger stage the larva is 300 $\times$ 260 μm. When the larva is about 10-12 days after fertilisation, the pediveliger (325 $\times$ 265 μm) settles down on a substrate. The spawned eggs of the green mussel *P. viridis*, as observed by Rao *et al.* (1976[4]), measures 45-50 μm in diameter and the pediveliger is about 300 μm in shell length, the stage being reached after the 16th day from fertilisation.

The time required for development to various stages depends on the species and the environmental factors, of which temperature is the most significant. The mussel larvae (in temperate waters) require between 15 and 35 days to grow from fertilisation to the pediveliger stage when settlement and metamorphosis become possible, and a larval period of 3 weeks is a reasonable approximation (Bayne, 1976[1]).

Considering the fecundity estimates available for mussels, the survival rate from egg to adult stage is very low. In the case of marine bottom invertebrates, Thorson (1946, 1950) and Mileikovsky (1971), as cited by Bayne (1976[1]), have estimated that mortality during the free-swimming larval period is considerable, possibly approaching 99%. The main mortality factors have been identified as predation, excessive dispersal to areas where suitable sites for post-larval survival do not exist and death due to extreme physical factors. Excessive larval dispersal may cause the fluctuations and uncertainties of spatfall in the natural beds.

Utilisation of Wild Seed in Mussel Culture

Given the reproductive strategy of mussel, as indicated earlier, the pelagic larvae in different stages of development can be expected to be present in abundance in the coastal waters during the spawning season. Since the natural mussel beds are restricted to a few rocky patches it can be presumed that only a fraction of the larval population gets a chance to settle on these beds and the rest suffers mortality due to various reasons. Spatfall on the beds is more or less abundant and Jones (1950, *J, Bombay nat. Hist. Soc.*, **49** (3) : 519-528[6]) observed that 'there is absolutely no dearth for mussel spat'. Appukuttan (pers. comm.) has estimated a density of 10-15 kg of mussel seed (shell length 20-35 mm) per square metre of rocky surface in good collection grounds near Vizhinjam. The current experimental and demonstration mussel farms in India largely use the seed collected from the natural grounds (Calicut-Tellicherry, Vizhinjam-Neendakara ; Ennore-Cuddalore). However, seed supply from these grounds cannot meet requirements for expansion of mussel culture as an industry. The experimental programme on the east coast near Madras already faces the problem of seed inadequacy. It has also been stated earlier that collection of seed from the exploited natural beds comes in conflict with the interests of the existing sustenance fishery. Therefore it is necessary to organise a system of seed production for commercial use.

Based on current knowledge on natural seed production, three approaches seem possible. The first one is the profuse spatfall that takes place on granite embankments and groynes laid along the coast of central Kerala for prevention of sea erosion. Jones and Alagarswami (1973, *Proc. Symp. Liv. Res. Seas around India*, 641-647[7]) have drawn attention to the remarkable feature of carpet-like settlement of young mussels of *P. viridis* on these rocks. Later, Nair *et al.* (1975, *Indian J. Fish.*, **22** (182): 236-242[8]) surveyed the area between Shertalai and Cochin and found the density of spat to be 220-248 per 100 cm^2 on the rocks submerged most of the time and 112-170 spat per 100 cm^2 in less favourable, surroundings. Natural beds of mussel in this region are not known and the settlement must be from the pelagic phase larvae dispersed in the coastal waters. Since the peak mussel spawning along Kerala coast is during the south-west monsoon (July-August) it is not easy to collect the spat by other means such as laying spat collectors. The stone embankments can form one source of supply of seed for mussel culture.

Secondly, spatfall occurs in the mussel culture farm itself at Vizhinjam and Calicut and it is collected on the frills of split nylon ropes and other cultch materials (Appukuttan, 1980, *Mar. fish. Infor. Serv. T & E. Ser.*, **16** : 13-16[9]). At Vizhinjam the spat collected in the farm is also used for culture but the quantity is inadequate to meet the seed requirements of the farm. There is need for improvement of the techniques of spat collection towards increasing seed production by this method.

The third possibility is production of seed in the farm by keeping a breeding stock of mussels as has been demonstrated in the farm near Madras (Rangarajan, 1980, Abst. 164, *Symp. Coastal Aquaculture.*, Mar. Biol. Ass. India[10]). Dense spatfall on tiles has been obtained by this method. It is probable that the coastal current system at the site of farm (Kovalam) is favourable so that the pediveliger larvae in the pelagic phase reach the farm area for settlement. It is also worthwhile examining the principle of artificial biocoenosis by the application of which animals with gregarious habits can be concentrated (Achari, 1980, Abst. 29, *Symp. Coastal Aquaculture.*, Mar. Biol. Ass. India.[11]).

These three possibilities need further consideration and intensive experimental work with a view to establishing mussel seed farms, even if seasonal, for supply of seed to culture farms. Economic considerations will weigh upon such attempts, although technical feasibility may be established.

Artificial Production of Seed

Artificial seed production has the advantage of assured seed supply and stock improvement through genetic manipulation. During the last three decades there has been a growing interest in the world for the production of seed of cultivable molluscs through hatchery system. Loosanoff and Davis (1963, *Adv. mar. biol.*, **1** : 136[12]) and Imai (1977, *Aquaculture in shallow Seas;* Oxford & IBH Publishing House, New Delhi, 615 pp[13]) have produced comprehensive treatises on the subject. Although techniques have been developed for several species, commercial hatcheries exist only for a selected few species such as the edible oysters, clams and abalones (Davis, 1969, *Trans. American Fish. Soc.*, **98** (4): 743-750[14]). Ukeles (1975, *Proc. First Internat. Conf. Aquacult. Nutrition*, 127-162[15]) mentions that there are about 35 pilot plants and commercial shellfish hatcheries in the United States and a considerable number abroad. Mason (1976, *Marine mussels: their ecology and Physiology* (Ed. B. L. Bayne), Camb. Univ. Press ; 385-410[16]) considers that the cost of rearing mussel larvae could not be supported owing to the lower price fetched by mussels than oysters and that for this reason mussel cultivation is based on the collection and raising of naturally settled spat. Seed availability had not been a restrictive factor for mussel culture in Europe. According to Korringa (1976, '*Farming of marine organisms low in the food chain;* Elsevier Sci' Publ. Amsterdam, 264 pp.[17]) 60 to 70% of the seed used for mussel farming in Galicia, Spain, comes from the settlement of young mussels on the rocks in the intertidal zone and the rest from spat collection with the aid of ropes hung from the floating parks. But inadequacy of natural seed is a constraint in India in view of the nature of distribution of the mussel beds. It would, therefore, be necessary to develop capability for hatchery production of seed. Korringa (1976)[17] states that though it is still uncertain whether hatcheries will ever play an important role in providing shellfish farms with the seed stock they need on a truly commercial scale, it should be realised that the development of these techniques may prove to be of the greatest importance for the future of these industries. The process of artificial production of seed is generally common for many species of bivalves as is seen from the fact that, in U.S.A., an oyster hatchery switches over to clam seed production easily. The techniques of seed production of mussels and constraints thereof are briefly outlined below.

(*a*) *Induced spawning*

Field (1922, *Bull. Bur. Fish. Wash*, **38** : 127-260[35]) who is one of the earliest workers on the biology of

mussel, found that rough handling of *M. edulis*, such as shaking them in a dish of sea water, would induce spawning. Iwata (1949, *Bull. Jap. Soc. sci. Fish.*, **15** : 439-442[18], **15** : 443-446[19]), (1951 **17** (1) : 15-18[20]), (**17** (3) : 91-93[21]), (**17** (3) : 94-95[22])' (67 : 96-97[23]), (1952 **17** (6) : 157-160[25]) *b Biol. J. Okayama Univ.*,) **1**(1-2) : 1-11[25]) has done considerable work on the spawning of *Mytilus edulis*. He (1949 [19]) reported that spawning could be induced by stimulation. A stimulation by 20 volts of 5 seconds duration was sufficient to induce the discharge of gametes from every ripe mussel.

Iwata (1951[22], 1952[25]) conducted experiments on chemical stimulation of spawning by dipping the mantle piece of *M. edulis* for 10 minutes in M/2 aqueous solution of NH_4Cl and M/3 solution of $BaCl_2$ and found them effective. Morse *et al.* (1978, *Proc. World. Maricult. Soc.*, **9** : 543-547[24]) used hydrogen peroxide in an alkaline medium to induce spawning in *M. edulis* and *M. californianus*.

Thermal stimulation has also been employed by some workers on mussels. Iwata (1951[25]) found that spawning could be induced in *M. edulis* by a sudden rise of temperature from 7° to 15°C. Rao *et al.* (1976[4]) obtained spawning in *Perna viridis* by raising the temperature from 26.5°-28.0°C to 32°-35°C. Bayne (1965 *Ophelia*, **2**: 1-47[25]) observed adult *M. edulis* in a condition responsive to spawning by raising the temperature from 7° to 13°C over 25-30 days. Hrs-Brenko (1973, *Aquaculture* **2**: 173-178[26]) was successful in conditioning mussel for spawning by raising the temperature from 1° to 18°C within 13 days.

Loosanoff and Davis (1963[12]) failed with the usual methods of spawning induction on *M. edulis*. They found that a simple method of gently touching the adductor muscle several times with the point of a needle or inserting a small wooden wedge between the shells to stretch the adductor was very effective to induce spawning in the species.

(b) Rearing of Larvae

Compared to the work on the culture of larvae of oysters and clams, rearing of mussel larvae has received very little attention. Loosanoff and Davis (1963[12]) have spawned *M. edulis* in the laboratory and reared the larvae to metamorphosis and settling. They fed the larvae with an algal mixture consisting chiefly of *Chlorella* and other green forms and the larvae grew remarkably well. They found that there were large variations in size even among larvae that originated from the same parents and that the size at which the larvae metamorphosed varied almost by 90 μm. Hirano and Oshima (1962), as cited by Imai (1977[13]), used *Chlamydomonas* sp. as food for *M. edulis* larvae. Rao *et al.* (1976[4]) reared the larvae of *Perna viridis* with cultures of *Chlorella, Tetraselmis gracilis, T. chui* and *Synechocystis* sp. and found that growth was good when the larvae were fed on a mixture of *T. gracilis* and *Synechocystis*. The larval rearing was carried out in finger bowls and no larval settlement had occurred although the larvae were reared up to 56 days (Rao *et al.*, 1976[4]). Kuriakose (1980[5]) succeeded in getting the larvae of *Perna indica* metamorphose and settle on ground glass and fragments of filamentous algae without artificial feeding. The work so far carried out at CMFRI (1978 *Anuual Report*, 78., CMFRI., 115 pp.[27]) on the rearing of larvae of *P. indica* at Vizhinjam and *P. viridis* at Madras have given only partial success up to the straight-hinge veliger stage.

The experiment of AQUACOP (1980 Abstr. 95, *Symp. Coastal Aquaculture*, Mar. Biol. Ass. India[28]) on mass production of green mussel *Perna viridis* in Tahiti (French Polynesia) is of great interest for tropical mussel culture, particularly to India where the same species is of considerable importance. Feeding the mussel larvae with *Chaetoceros gracilis* and *Isochrysis* sp. AQUACOP (1980[28]) had achieved from 30 to 60 % spat settlement on nylon meshes between the 15th and 20th day. This is one of the very few works on hatchery development for seed production of molluscs in the tropical waters.

(c) Problems of hatchery production

The major technical components of any shellfish hatchery programme include spawning of adults and handling the young up to a stage when they can be transplanted to the natural beds or culture farms. Several methods for inducing the mussel to spawn are available as mentioned earlier and a concerted effort on use of these techniques on the Indian species can quickly determine an effective and cheap method. It is in the second area of handling the larvae through development to settlement that success would depend on several factors. These are water quality, larval food and disease control. These problems have been reviewed in detail by several workers (Loosanoff and Davis, 1963[12] ; Davis, 1969[14] ; Loosanoff, 1971 *Proc. Conf. Artificial Propagation of Commercially valuable shelfish-oysters*, College of Marine Studies Univ. Delaware ;[29] Ukeles 1971, 1975 *Proc. Conf. artificial propagation of Commercially valuable shelfish oysters ;* College of Marine. Studies, Univ. of Delaware[30]). The sea water used for larval rearing has to be assessed

for its freedom from suspended matter and pollutants, temperature, salinity and pH range and nutritional value as each of these factors may play a critical role. Ukeles (1975[15]) states that observations in laboratories, pilot plants and commercial hatcheries emphasize the importance of sea water quality to the successful culture of bivalves.

Feeding the bivalve larvae is a major constraint in the hatcheries. On top of the list of problems in the bivalve larval rearing laboratories and commercial aquaculture plants is the one of providing an optimal nutritional support that is efficient and economical for culturing animals under controlled conditions (Ukeles, 1975[15]). The naked flagellates *Isochrysis galbana* and *Monochrysis lutheri* have almost become the universal food for the early larval stages of most of the bivalves in rearing. Bayne (1965[25]) reared *M. edulis* larvae on the diet of *I. galbana*. Mussel larvae have also been successfully reared using *Chlorella* (Loosanoff and Davis, 1963) or *Chaetoceros* (AQUACOP, 1980[28]). In the field of larval nutrition considerable effort is required in India to identify suitable food organisms for the different stages of larva and culture them on a mass scale.

Disease of larvae, although little understood, can cause heavy mortalities. Davis *et al.* (1954, *Science*, **120:** 36-38[31]) have described how a fungus can occasionally acquire epidemic proportions in some larval cultures, killing most of the larvae in 2-4 days. Vishniac (1955, *Mycologia*, **47:** 633-645[32]) found that the fungus *Sirolpidium zoophthorum* was responsible for the above mortality among clam larvae. Guillard (1959, *Biol. Bull.*, **117:** 258-266[33]) isolated two virulent bacterial clones, one of which appeared to be a species of *Vibrio* and the other of *Pseudomonas*. Tubiash *et al.* (1965 *J. Bacteriol*, **90:** 1036-1044[34]) found bacillary necrosis among larval and juvenile molluscs. Ciliates are commonly found among dead and moribund larvae of bivalves in the culture vessels.

Loosanoff and Davis (1963[12]) found that precautionary measures consisting of cleanliness of the rearing tanks and ultraviolet treatment of the seawater can to a large extent control fungus infections. They observed that antibiotics at low concentrations such as streptomycin, aureomycin, Combistrep (a mixture of dihydrostreptomycin and streptomycin sulphate) and Sulment (sulphamerazine) increased the rate of growth of clam larvae. Loosanoff and Davis (1963[12]) have also suggested control measures against fouling and competing organisms. The above problems are common to larval rearing of any bivalve and, with regard to mussel, detailed work will become necessary as progress is made in larval culture technology.