CMFRI

WORKSHOP ON

MUSSEL FARMING

25 - 27 SEPTEMBER, 1980
MADRAS

CENTRE OF ADVANCED STUDIES IN MARICULTURE

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE

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TECHNICAL SESSION II	BIOLOGY, PHYSIOLOGY AND GENETICS OF MUSSELS	CMFRI-CAS/MF/80/BP-4

LIFE-HISTORY STUDIES ON INDIAN SEA MUSSELS

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Mussel culture operations are in good progress at the demonstration farms of the Central Marine Fisheries Research Institute at Vizhinjam and Calicut along the west coast and at Kovalam near Madras on the east coast. Experimental mussel farming is also undertaken by the National Institute of Oceanography at Dona Paula in Goa and at Ratnagiri by the Agricultural University of Maharashtra. Biological studies of an academic nature are being carried out at the University of Madras and Aurangabad. It has been shown that the yield rates are high enough to bring fair profits after covering the initial investment for building up farming facilities and recurring maintenance expenses. Some of the coastal fishermen have now learnt to supplement their income from regular fishing by taking to mussel culture under the technical guidance of the staff of the Central Marine Fisheries Research Institute.

Mussels are hardy to fluctuations in environmental conditions to a great extent and are fast growing. Being filter feeders, the mussels feed upon the phytoplankton, the first in the food chain, and build up highly proteinaceous meats which are directly utilizable by man for his food.

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Mussel culture augments food supplies to a great deal more efficiently than by farming most other food species. In the Indian waters two mussel species viz., the green mussel Perna viridis (L) and the brown mussel P. indica (Kuriakose) are known to occur. The biology and life history of the green mussel occurring along the Goa coast have been studied in some detail (Rao, et al, 1975 and 1976). Adequate information on these aspects is also available on Perna indica (Kuriakose, 1980). For mussel culture procurement of seed in adequate quantities is very essential. For farming at present the seed are being collected from the vicinities of the natural mussel beds. Mussel culture has come to stay, the interest being awakened by the initial success met with in the demonstration farms. Dependence on natural resources of seed with increase in demand in future is bound to be met with some difficulties. Further transport of the seed from distant collection grounds to farming sites will present problems apart from expense involved. The laboratory studies on green mussel have indicated the possibility of inducing both sexes to spawn, fertilizing the eggs and rearing the larvae to the seed or spat setting stage (Rao, 1976). These results are of much interest for establishing hatchery techniques of seed production under controlled conditions. The varied aspects of breeding biology, larval growth and habits and settlement of seed outlined here pertaining to P. viridis, are of fundamental importance in the scientific management of mussel farming.

Sexual Maturity of the Gonads and Spawning

The ripe gonad follicles of the mussels surround the visceral organs and enter the mantle wall. In female they are orange red and in male light cream yellow. In P. viridis

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sexual maturity is attained in all individuals at a length of 25 mm when they have completed 3 months of life after settlement as spat, but some with ripe gonads even at a much smaller size of 15 mm in length have been observed to spawn in the laboratory aquaria. Taking the species as a whole, there is breeding round the year with two spawning peaks. A difference has been noted in the spawning periodicity of the older mussels above the marketable size of about 60 mm in length and in the younger ones below that size. The spawning in the older ones commences from July and lasts till December with peak spawning in September-November which is followed by abundant spat setting in October-November. In the younger ones the spawning is from January to April with its peak in about February-March but this is not followed by an appreciable settlement of spat. During the breeding season mussels collected a few days before or after the full-moon days and kept in the laboratory aquaria spawned immediately but how far this is indicative of a lunar periodicity is to be ascertained by further observations. In the act of spawning the male discharges the milt through the exhalent siphon in a steady, visible, whitish streams which soon diffuses into the surrounding water turning it cloudy. In the case of the female, the eggs which are orange yellow and liberated in a streams, settle down at the bottom in the vicinity of the mussel discharging them.

That like most other bivalves mussels can be induced to spawn is known from the observations of Field (1922), Iwata (1950, 195[illegible]), Loosanoff and Davis (1963) and Ino (1973). When mussels are vigorously shaken up in water and allowed to remain undisturbed for a time, they would liberate spawn; if an electric shock stimulation at 20 volts for 5 seconds is administered the mussels would react in the same manner;

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and thermal shock given by keeping the mussels at a higher water temperature than that of the environment from which they are taken mussels would respond in releasing the eggs. Some chemical solutions as NH_4 OH, KCl etc. in weak dilutions would also produce the same result when they are injected in minute quantities into the tissues of the mussels. In the European mussel Mytilus edulis pricking the posterior adductor muscle initiates spawning. The centre of nervous control for spawning reaction is considered to be located in the posterior adductor muscle. It is also known that when ripe reproductive elements of a mussel of one sex are placed in water, spawning reaction is initiated in mussels of the opposite sex, particularly when this is done along with the thermal shock treatment. With the exception of electrical stimulation the above cited methods have been tried by the present writer in inducing P. viridis to spawn. No particular method gave unfailing results in all the experiments. However, the thermal shock treatment of increasing the water temperature in which the mussels were kept to about 5°C above the prevailing water temperature (not exceeding 35°C in the experiments) gave satisfactory results during the breeding season. The males reacted very favourably in about 30 minutes in most cases, (75 % cases) but in only about 20% of the experiments the females responded. The eggs discharged were in very good condition and they were readily fertilized with sperm liberated by the males in the same experiments.

Structure of the Egg, Fertilization, Early Development upto Trochophore

The spawned out egg, which is orange red in colouration and enclosed in a thin vitelline membrane has granular yolk in its cytoplasm surrounding a centrally placed nucleus and measures about 50 um in diameter. /

/Fertilization is

external when ova and spermatazoa are shed into the waters in the natural environment. For tracing developmental stages the spawned out eggs were mixed with milt in the finger bowls and the developmental processes were followed by microscopic examination. A large number of spermatazoa swarm round the egg and one succeeds in fertilizing the egg. The first polar body is given off 20 minutes after fertilization followed by the second polar body about 10 minutes after. Simultaneously with the formation of the second polar body at the vegetative pole of the egg a hyalene lobe like protrusion the 1st polar lobe appears. It may be noted that the formation of the polar lobes is a characteristic feature in the segmentation of the eggs of Mytilus and a few other bivalve species (Reverberi, 1971). The egg undergoes cleavage dividing into 2 cells viz., the AB and CD cells. Along with the polar lobe at this stage a three lobed (trefoil) appearance is presented. The first polar lobe is now drawn into the CD cell with the result that this cell is larger than the AB cell. Before the second cleavage starts, the second polar lobe appears at the vegetative pole of the CD cell. The second cleavage results in four cells A, B, C and D soon after which the second polar lobe gets absorbed into the D cell. The third cleavage is 'spiral' and 'dexiotropic' which divides A, B and C cells equally into two halves each and the D cell into one small cell and one large cell. Further cleavages follow soon, the smaller cells spreading over the larger cells, giving rise to blastula stage which develops cilia and begins to rotate. Gastrulation takes place by epiboly the blastopore appearing first at the vegetative pole but subsequently shifting ventral wards. The embryo now undergoes a little elongation, broad at the apex and somewhat narrow posteriorly, passing thus to the next stage the trochophore which is reached in 6 to 8 hours after the fertilization of the egg and measures about 58 um on its long axis.

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It has an apical tuft of a few larger cilia, an archenteric space within and a stomodeal pit where the blastophore has closed i.e. ventralwards. Mouth and anus are absent and the late trochophore stage has dorsally a shell gland developed (Figs. 1-9).

Early and Late Veliger Stages

The velum with larger and powerfully vibratile cilia and the first larval shell or prodissoconch I are simultaneously formed after the trochophore stage. The larval shell is D-shaped and the two valves of the shell are united at the hinge which is straight. Hence the larva is said to be at the straight-hinge stage which is reached in about 18 hours after fertilization. It has been observed to measure 62 um at hinge. The well developed velum which is the only organ for locomotion has in its middle one or two slender long cilia, of the apical tuft of cilia of the trochophore. It has no mouth, oesophagus or intestine and anus. The archenteric space of the trochophore persists. This first stage veliger is apparently incapable of ingesting food (Fig. 10). In the next stage the larval shell grows a little bigger and the retractor muscles of the velum are clearly seen (Fig. 11).

The larval shell viz. the prodissoconch I grows as a result of deposition fresh shell material secreted by the mantle. This fresh deposition shows clear concentric lines of growth. The growing shell is now in prodissoconch II stage and the larva is often termed veliconcha (Bayne, 1976).

Between the third and the nineth day of development most of the larval structures gradually make their appearance. At first the alimentary tract with the mouth,

oesophagus, stomach with the associated digestive gland, a coiled or looped intestine followed by rectum and anus are formed; the anterior adductor muscle precedes the posterior adductor muscle; the statocyst and the rudiments of labial palps, foot and gills are also recognisable. (Figs. 12 and 13). The hinge is still straight.

By fourteenth to sixteenth day after fertilization the larva has grown to 225 - 278 um in length, developing a distinct umbo on the hinge. Hence the larva is said to be in the umbonal stage. In addition to the structures already mentioned a dark pigmented eye spot has appeared. Labial palps, the gill filaments and the foot have grown larger than in the earlier stages. The shell turns from yellowish to deep brown hue at the hinge. The velum continues to be the chief locomotor organ.

The Pediveliger and Spat

By eighteenth day some of the larvae have grown to 300 um in length; the shell is now a little oblique and the velum is still large and used for swimming most of the time near to the bottom of the culture bowl. The finger-shaped foot is often protruded and used for creeping. The pediveliger as it is now called fixes itself to substratum by byssus formation from the secretion of the pedal glands of the foot and under goes metamorphosis.

The changes that take place in metamorphosis are absorption of the velum, disappearance of the eye spot by cytolysis, elaboration of the labial palps and gills, a further enlargement of the posterior adductor muscle, and a gradual reduction of the anterior adductor muscle.

There is a marked oblique growth of the shell after the setting of the spat with the result that the umbo is seen at the anterior extremity of the shell.

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It is of some interest to note that there is no uniform development of the larvae resulting from the same batch of eggs spawned by a female. While some have far advanced in development upto eyed veliger stage others have been noted to be still in the late straight hinge stage. This disparity in growth was not due to lack of or insufficiency of food as the larvae were fed on a plentiful supply of algal cultures.

Duration of Larval Life

In the laboratory culture of the larvae, the pediveliger stage was first observed on the 16th day and spat settlement on the 19th day after fertilization. In the natural environment the duration of larval life may be shorter by a few days.

It has also been observed that after the pediveliger stage is reached settlement of spat has been delayed for prolonged periods. In the laboratory cultures most of the pediveligers continued to remain active without settlement for a maximum period of 56 days (Rao et al, 1976). The ability to delay settlement perhaps helps seeking favourable substrata, especially when the larvae are drifted by currents to distant environments.

Larval Nutrition

In rearing the bivalve larvae, algal cultures of a large number of species are widely used (Loosanoff and Davis, 1963). The algal species selected for the purpose should be small enough to be ingested, nutritious and readily acceptable to the larvae. Motile or floating species are generally preferred to non motile ones as the latter sink to the bottom of the culture vessels and not easily available to the free swimming larvae. In rearing the

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larvae of P. viridis cultures of Chlorella, Tetraselmis gracilis, T. chui and Synechocystis were used. The three latter species grew fast in the subcultures. The mussel larvae commenced feeding only from the third day after the fertilization of the egg by which time the alimentary tract was clearly formed. When Synechocystis alone was used, the larvae remained stunted. When a mixture of Synechocystis, Tetraselmis gracilis, T. chui and Chlorella were used, growth rate was favourable. Species like Isochrysis galbana and Monochrysis lutheri which are known to promote very good growth in bivalve /in general were /larvae not available for experiments.

Growth, Life Span and Maximum Size

Paul (1942), Ranade et al (1973) and Rao et al (1975) have furnished information on the growth rates of P. viridis. Paul's observations show that a maximum size of 14.5, 19.0, 55.5 and 93.0 mm in length was attained by the green mussel at Madras harbour in 30,84, 167 and 321 days. Ranade et al observed an average growth of 7.5 mm a month during October to May in spat set on Ratnagiri coast. A study by Rao et al (loc. cit) by length frequency distribution of mussels along the Goa coast shows modal size of 96 mm at the end of 1st year, 132 mm at the end of /and 2nd year/ 156 mm in the 3rd year, the average annual rates of increase being 96 mm, 36 mm and 24 mm at average monthly rates of 8, 3 and 2 mm respectively. It was also observed that in seed set fresh on floating buoys at Vengurla Bay the modal shifts indicated an average monthly growth of 8-8.8 mm during January to April in 1973 and a little less during October to March (1973-1974) being 6.0 mm. The maximum size observed in each month was much higher as

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shown in the table below:

Month	Age (months)	Modal size (monthly average) mm	Maximum (Monthly average) mm
January	3.5	30 (8.56)	34.5 (9.84)
February	4.5	39 (8.66)	42.0 (9.2)
March	5.5	45 (8.66)	56.5 (10.2)
April	6.25	54 (8.8)	63.0 (10.8)

It is seen from the table that a certain proportion of individuals attain a maximum size of 60 mm in about 6 months after initial settling.

The maximum size of the mussel along the Goa coast observed was 145 mm in length weighing 72 gm (after preservation in 5% formalin). The maximum size of specimen kept in Singapore National Museum is reported to be 170 mm in length (Kow et al, 1973). Deduced from the size frequency studies carried out here, this length is expected to be attained in the fourth year of its life. The life span does not seem to be beyond 4 years.

Some ecological considerations and conclusion

Mussels are littoral in their distribution, densely set over rocky coasts. The setting of spat is immense after the monsoon months commencing from about November and extending to April along the Goan coast. The regions where the spat set are from about 1 m high at LWST to varying depths depending on the availability of hard substrata. In the shallow waters mussels become scarce by April as they are fished and removed by local people rather indiscriminately. In the deeper waters they thrive all round the year as their removal is met with some

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difficulty as only a few fishers who know diving can approach the beds. The deeper waters in the intertidal region seem to favour growth better than in the very shallow waters where partially or wholly the mussels are exposed at low tides. The main source of collection of spat for culture purposes are vicinities of the natural beds and the pattern of depthwise setting of the spat needs an intensive study of the production areas.

In the laboratory rearing of mussel larvae it has been found that although pediveliger stage is reached in large number of larvae, the setting of spat has been observed to be poor as it is not known the kind of culch that is favoured most for settlement. The elucidation of this helps hatchery techniques.

The adult mussels appear to tolerate variations in water salinities of the environment a great deal more than the larvae as shown by Kow et. al. (1973)(10 to 35‰ by the adults and 26 to 29‰ by the larvae). Observations along the Goan coasts have shown total absence of larvae in July - August at stations where salinities were low (6.8 to 13.99‰) although these months were within the breeding period. The occurrence of greater densities of larvae have been found to coincide with periods when the salinities were high. The choice of culture sites and collection sites of spat should be in such localities where fairly high salinities prevail for most part of the year. Information on the nutritional requirements of the growing mussels and fouling organisms associated with mussel settlements is still inadequate and these aspects need intensive studies.

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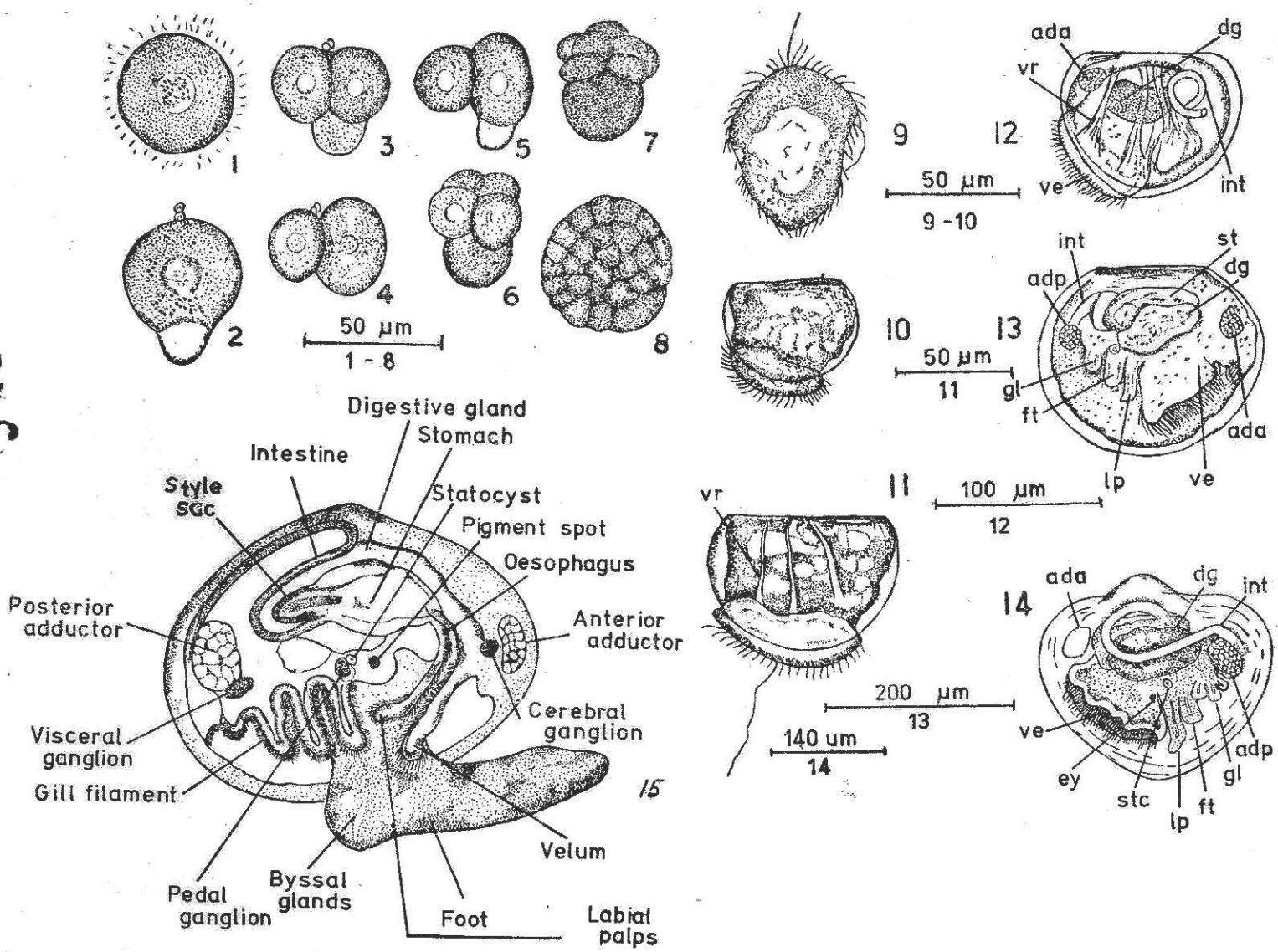

Life-History stages of *Perna viridis* (L): 1. Spawned out egg being surrounded by spermatozoa, 2. Fertilized egg with the 1st and 2nd polar bodies extruded out, 3. Trefoil stage after 1st cleavage with AB and CD cells and the 1st polar lobe, 4. Absorption of the 1st polar lobe into CD cell, 5. Appearance of 2nd polar lobe from CD cell before the 2nd cleavage appears, 6. AB and CD cells formed as a result of 2nd cleavage, also 2nd polar lobe is prominently seen, 7. Third cleavage divides the A, B, and C cells equally into D cell 2nd polar lobe enters and therefore it divides, 8. Smaller cells spread over larger cells forming blastula, 9. The trochophore stage, 10. Straight-hinge stage, D–larva or prodissoconch - 1st stage veliger, 11. Straight-hinge stage veliger developing velar retractors, 12. Prodissoconch – 2nd stage veliconch early stage with digestive gland, stomach and other parts of alimentary tract-Anterior adductor formed, 13. Prodissoconch stage further advanced in development, posterior adductor, statocyst, rudiments of labial palps, foot and gill seen, 14. Fully formed eyed veliger larva, and 15. Diagramatic representation of plantigrade larva of *Mytilus*.

Source : Rao *et al.*, 1976.
Figs. 1 - 14. *Indian J. Marine Sci.*, **5** : 113-116.
Fig. 15. Bayne, 1976.

Abbrevations : *ada* - Anterior adductor muscle; *adp* - Posterior adductor muscle; *dg* - digestive gland; *ey*- Eye-spot; *ft* - foot; *gl* - gill; *int* - intestine; *lp* - labial palp; *st* - stomach; *stc* - statocyst; *ve* - velum; and *vr* - velar retractors.