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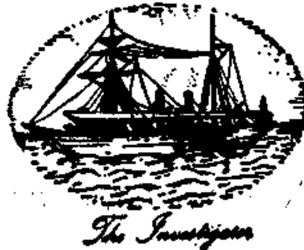

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EARLY LARVAL DEVELOPMENT OF EDIBLE OYSTER *CRASSOSTREA MADRASENSIS* (PERSTON)

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ABSTRACT

Mature male and female edible oyster *Crassostrea madrasensis* selected from the Tuticorin oyster farm were stripped and the eggs were artificially fertilized in glass containers. 91.5 to 92.2 percentage of eggs underwent successful fertilization and the larvae were reared upto umbo stage in one experiment. The size of the mature egg varied from 49 to 59 μ. A method of filtering and washing the eggs using centifugation was devised as the eggs could not be retained even by the finest sieve (Nylobolt bolting cloth 30 HD) available. The fertilized eggs were cultured in different vessels of size ranging from 1 litre to 80 litres containing filtered ultraviolet treated sea water. 85% of the fertilized eggs successfully reached the straight hinge-stage in 18 to 24½ hours. The umbo stage was reached in 11 days, provided *Chlorella salina* was supplied as food. Antibiotics were used to control other organisms developing in the culture and multivitamins as a source of nutritional supplement.

INTRODUCTION

THE DEVELOPMENT of a suitable hatchery for the artificial propagation of the edible oyster *Crassostrea madrasensis* has been necessitated in recent years. Loosanoff and Davis (1963) in mid 1940s modified Well's techniques and standardised a method to culture the American oyster larvae to metamorphosis and later developed methods for to a full fledged oyster hatchery including induced breeding. No successful work on the hatchery production of Indian edible oyster *C. madrasensis* has so far been done. The only available literature in this field is that of Devanesen (1955) who has made certain observations on the early developmental stages of *Ostrea madrasensis* (= *C. madrasensis*.) The present work is an attempt to induce the oyster *C. madrasensis* to breed in the laboratory and to rear the larvae.

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MATERIAL AND METHODS

Adult oysters were selected from the Tuticorin oyster farm, cleaned and placed in 3 litre glass troughs. Temperature and salinity manipulation to induce the oysters to breed in the laboratory proved ineffective. As an alternative stripping of oysters was tried with success. The right valve of the oyster was removed and the content of the gonad was taken out using a fine pipette. The gonadal content was examined under microscope to determine the sex as well as maturity. Round spherical eggs and actively moving sperms alone

were chosen. The gametes were separately diluted in filtered sea water and allowed to stand undisturbed for 20 minutes. By this time the eggs settled down and that helped to eliminate to a great extent the tissue fluids and tissue pieces which form a source of contamination in the culture. The sedimented eggs were separated and mixed with sperms in a petri dish of 15 cm diameter kept shaking to keep the eggs in suspension so as to give good percentage of fertilization and uniform development. Immediately after fertilization but before the commencement of the cleavage, the eggs were centrifuged twice at 1000 rpm, for one minute and the supernatant containing excess sperms and tissue fluids were eliminated.

Vessels of different sizes ranging from 1 litre conical flasks to large cylindrical plastic bins of 80 litres capacity were used for larval culture. For experimental work transparent wide-mouthed plastic bottles of 1 litre capacity were found to be the best suited. Sea water was filtered through cotton wool and subjected to ultraviolet treatment. The required quantities of antibiotics such as penicillin and streptomycin were added.

The fertilized eggs were transferred to the plastic bottles at a density of 1,80,000 eggs/500 ml of sea water. The first change of water was done 25 hours after fertilization and subsequently once in two days. Water was aerated each day for 10 minutes.

Unicellular algae were cultured in conical flasks of size ranging from ½ litre to 5 litres using Miquel's medium (modified by Ketchum and Redfield) and Erdscriber medium (Gross, 1937). The prevent contamination, the algal food was subjected to ultraviolet treatment prior to being supplied as food.

Narcotization and preservation of the larvae

To study the development of the zygote, cleavage pattern, larval movement and larval structure, samples were taken from the culture and observed under microscope continuously for the first 25 hours. For examination, the larvae were narcotized with menthol and made transparent with glycerol. Neutral red and Rose Bengal were used as stains. Whole mounts were used for photographing.

OBSERVATIONS

Mature female oysters are characterised by the presence of round spherical eggs whereas the immature ones by oval or flask shaped eggs (Pl. I A). The ripe spherical eggs are characterised by a round nuclear region (29 to 33 μ diameter) in the centre surrounded by granular cytoplasm (Pl. I B). The size of the mature eggs range from 49 to 59 μ in diameter; the average being 54 μ. The average dimension of the immature eggs is 43 μ broad and 79 μ long. The fertilized egg contracted and assumed a globular shape if it was not round before. The lighter nuclear region disappeared (Pl. I C).

In 30 minutes after fertilization (Table 1) the polar body is formed (Pl. I D). Then the first cleavage divides the zygote meridianally into two unequal cells representing the anterior and posterior ends of the embryo (Pl. II A). The plane of the second division is at right angles to the first. Both the blastomeres divide synchronously and separate into four quadrates (Pl. II B, C). Further ensuring cleavages result in the multicellular stage (Pl. II D). The embryo later reaches the blastula stage and starts rotating and swimming by means of cilia developed on its surface. In 5½ to 6½ hours after fertilization the gastrula stage is reached. This is followed by the trochophore stage. The shell gland begins to secrete the shell and by 18 to 24½ hours 90% of the fertilized eggs reach the straight hinge or the D-type larvae (Fig. 1 a, b). The average dimension of the larvae is 63 × 49 μ. The colour of the larvae in the area of the digestive

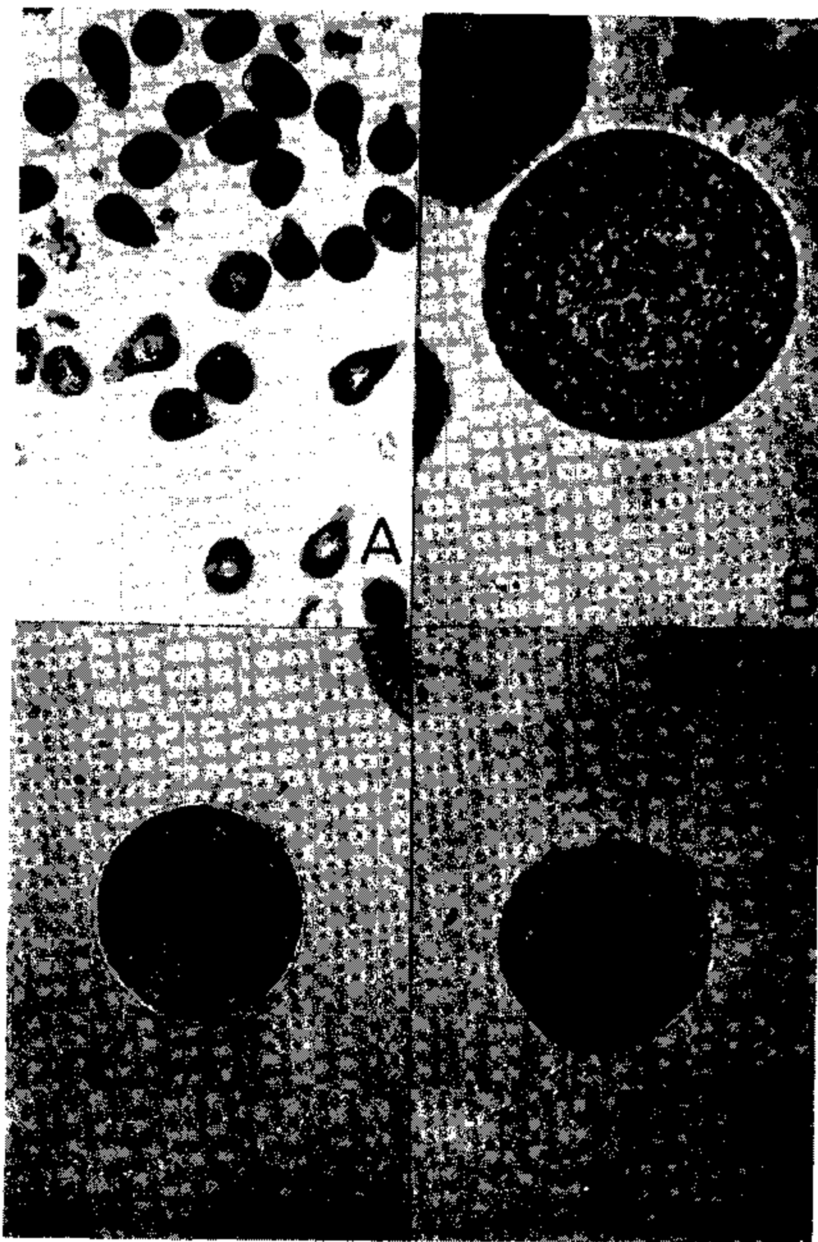

PLATE I. A. Mixture of mature and immature eggs stripped from a female oyster, B. Mature unfertilized egg enlarged, C. Egg immediately after fertilization and D. Formation of polar body.

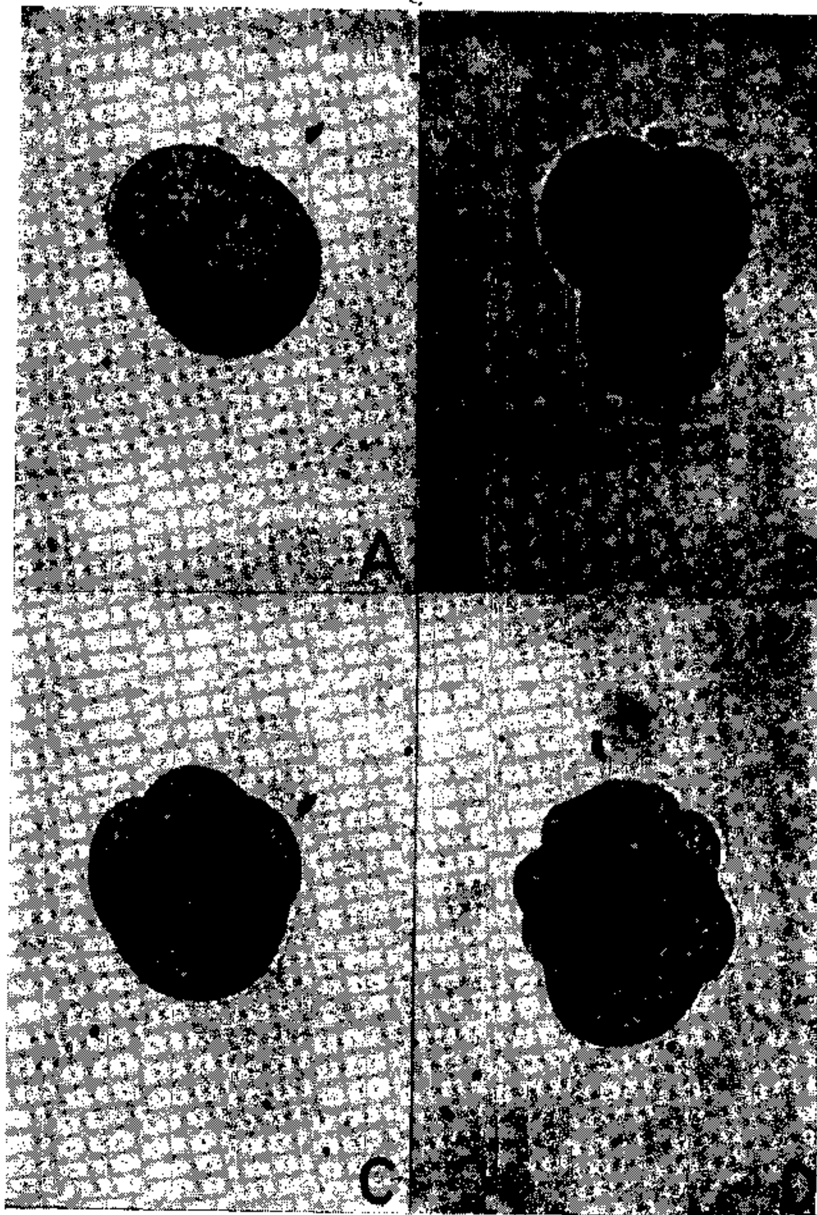

PLATE II. Different cleavage stages of the oyster egg : A. 1st cleavage, B. Commencement of 2nd cleavage, C. 2nd cleavage completed and D. multicelled stage.

TABLE 1. *Course of development and the time required for the artificially fertilized eggs of* C. madrasensis *to reach the different developmental stages*

Course of development	Time after fertilization
Release of polar body ..	30 m
Ist cleavage ..	40 ,,
2nd cleavage ..	50 ,,
3rd cleavage ..	60 ,,
Multicelled stage ..	1 h 10 m to 2 h
Blastula stage ..	1 ,, 50 ,, to 3 ,,
Rotating & Swimming stage ..	3 ,, 50 ,, to 5 ,,
Gastrula ..	5 ,, 50 ,, to 6 ,, 30 m
Trochophore ..	6 ,, 30 ,, to 13 ,, 50 ,,
Straight hinge stage ..	18 ,, 00 ,, to 24 ,, 30 ,,
Umbo stage ..	11 days

FIG. 1. a. Straight-hinge stage (the D-type larvae) with the velum expanded and b. Straight hinge larvae as seen at the upper layers of the culture medium.

diverticulum is golden brown. But the colour as a whole is usually pinkish orange.

The rate of fertilization was 91.5 to 95.2% and 85% ultimately reached the straight hinge state. *Tetraselmis gracilis* and *Synechocystis salina* proved to be too big for the larvae to feed. In one experiment when *Chlorella* was supplied as food the larvae developed to umbo stage in 11 days.

During the initial experiments the larvae did not survive beyond 24 hours. This was due to multiplication of protozoans. Series of experiments conducted to ascertain the optimum concentration of antibiotics required to effectively control the unwanted organisms revealed that 37.5 ppm of penicillin or 50 ppm of streptomycin or 75 ppm of chloromphenicol is effective. Of these the use of chloromphenicol and streptomycin separately or in combination gave better result than the use of penicillin. However, the larvae did not develop beyond straight hinge stage in most of the experiments.

Discussion

The size of the ripe eggs of *Ostrea madrasensis* (= *Crassostrea madrasensis*) as reported by Devanesen (1955) ranges from 51 to 85 μ in diameter. But the present study reveals that the size of the ripe eggs of *C. madrasensis* ranges from 49 to 59 μ and that of unripe eggs from 43 to 79 μ. Similarity egg size is reported in American oyster - 50 to 55 μ (Loosanoff and Davis, 1963) and in Portuguese oysters - 50 to 58 μ (Imai, 1971).

The eggs of *Crassostrea madrasensis* are too small to be retained even by the finest sieve available — the nylobolt bolting cloth 30 HD. Loosanoff and Davis (1963) experienced the same difficulty with the eggs of *Crassostrea virginica*. They devised the method of 'sedimentation' to partially free the eggs from body fluids, sperms and other contamination. In the present work it is found that centrifuging the eggs is the best alternative to wash and filter the eggs in the absence of a suitable sieve.

Larval development of *Crassostrea madrasensis* follows the usual pattern observed in other oysters. The larvae reached the shelled stage as early as 18 to 24½ hours with a shell length of 63 μ. This more or less agrees with Loosanoff and Davis (1963) who reported that the larvae of *Crassostrea virginica* reached the shelled stage in 24 hours with a shell length ranging from 68 to 75 μ. Imai (1971) record that in portuguese oysters the larvae reach the shelled stage with a shell length of 75 μ in 24 hours

The acceptance of the algae as food depends mainly on their cell size. The larvae of *Crassostrea madrasensis* do not accept *Tetraselmis salina* (8-12 × 6.5-8 × 4.5 microns in size) and *Synechocystis salina*. But it accepts *Chlorella salina* whose size is smaller and ranges from 5-7 microns. This agrees with the finding of Walne (1974) who reports that *Ostrea edulis* fails to ingest anything measuring more than 10 microns in diameter. But he holds the view that *Chlorella* is not a good food, for the digestive enzymes of the larvae of *Ostrea edulis* cannot penetrate the cell wall. It is seen that the larvae of *Crassostrea madrasensis* accept *Chlorella* only in the absence of other suitable food. If the unicellular algae acceptable for the larvae are identified, segregated and cultured, the rearing of the larvae of *Crassostrea madrasensis* will become easy and successful.

References

Devanesen, D. W. 1955. Report No. 1 on the Madras edible oyster (*Ostrea madrasensis*). Govt. Press, Madras, **2** : 60 pp.

Gross, F. 1937. Notes on the culture of some marine plankton organisms *J. Mar. Biol. Ass. U.K.*, **21** (2): 753-768.

Imai, T. 1971. The rearing of larvae and seedlings of bivalves. *Aquaculture in shallow seas, progress in shallow sea culture.* Amerind publishing Co. Pvt. Ltd., New Delhi. pp. 579-600.

Loosanoff, V. L. and H. C. Davis 1963. Rearing of Bivalve Mollusks. *Advances in Marine Biology*, **42** (4) : 607-624.

Walne, P. R. 1974. *Culture of Bivalve Molluscs 50 years experience at Conwy.* White friars press, London. pp. 173.