Seed Production in Sea Cucumbers

Dr D B James
Central Marine Fisheries Research Institute
Kochi - India

Introduction

ea cucumbers are one of the commercially important groups f animals of the sea. Processed sea cucumbers are a delicacy r the Chinese and form part of their life and tradition. In ade parlance, processed sea cucumbers are known as **eche-de-mar** or **Trepang**. It has no local market and the hole product is exported, chiefly to Singapore. An expensive em, quality **beche-de-mer** costs 25 dollars. India, at esent, exports **Beche-de-mer** worth of nearly Rs 1 crore. is procured in dry form, soaked in water, cleaned and oked in many delicious ways. It is rich in proteins and has low fat content. It has some medicinal value too.

ea cucumbers are sluggish animals with very little vements. They make no attempts to escape at the time of ture and also offer no resistance. For these reasons, they quickly exploited. There are over 650 species of sea umbers in various parts of the world. There are about species in the seas around India, of which 75 are from the llow waters within 20 metres depth. Of these, only a en are of commercial value.

to overexploitation of the resource, the **Beche-de-mer** stry is facing shortage of material for processing. The rnment of India imposed a ban in 1982 on the export of rial which is less than 3 inches (75 mm) in length. This ted a crisis for the industry. In order to overcome the tage of material, the Central Marine Fisheries Research tute (CMFRI) proposed a new research project on seed uction and sea ranching of sea cucumbers in 1987. success was achieved early in 1988 with the inducing most valuable sea cucumber, **Holothuria scabra**, to in the laboratory and produce seed. Since 1992, The he Products Export Development Authority (MPEDA) been partly funding this research project.

ed Production

a cucumber seed is produced in China and Japan and, in nt years, also in Korea and Russia. In India, a kthrough in inducing sea cucumber to spawn and uce seed was achieved in 1988. In other countries, the of **Stichopus Japonicus** alone is produced whereas in , the seed of the most valuable sea cucumber, **thuria scabra** is produced. Another commercially tant sea cucumber, **Holothuria atra** is also induced wn on several occasions and produce the larvae.

Brood Stock Material

Brood stock collection is an important aspect in any culture system. The brood stock material (Pe.I,A) is collected from the commercial catches meant for processing. Only large and healthy specimens are collected for this purpose. All injured specimens are discarded. They are stocked in a one-tonne tank brought from the natural beds. The sand is arranged in 100 mm thickness to enable the sea cucumbers to bury themselves.

The success of a hatchery depends on the condition of the brood stock and the health of the animals. The water in the tank is changed every day and the sand in the tank every fortnight. If the water is not changed every day, the sea cucumbers eviscerate throwing out all the internal parts including the gonads and the material does not serve any useful purpose for hatchery experiments. The sea cucumbers live on the organic matter present in the sand. If proper food

is not available, the animals become shrunken and the gonad is re-absorbed. It is desirable to stock 20 to 30 individuals per m3.

Collection timing is very important for the success of hatchery management. **Holothuria scabra** has two spawning peaks, one during March-April and the other during November-December. It is desirable to collect the brood stock material during the spawning peaks so that the chances of spawning are more as most of the specimens have ripe gonads ready for release.

Spawning

Spawning in sea cucumbers can be achieved in four ways natural spawning, stripping, thermal shock and stimulation through desiccation and flowing water.

i. Natural spawning

When the gonads are fully mature, the male and female breeders release their gametes without any inducement. At first, the male releases the sperm, which induces the female to release the egg after four hours. The male usually spawns at about 10 a.m.and the female release the egg at 2 p.m. The male releases sperm for more than an hour and the female releases the egg in about half-an hour's time. The female releases about a million eggs each time.

ii. Stripping

This method was used by the Japanese and the Chin during earlier days. The rate of fertilization is as low as per cent and the number of deformed individuals is large. this method, the back of the breeder is open with sess from the anus upwards. The ovary and the testis are tak out and dried in the shade. The ovary is then placed i container filled with sea water and torn lightly with tweez or scissors to release the eggs into sea water. The eggs then filtered off and set aside. The testis is placed in anot container with sea water and cut to pieces. The sperm swi out into the sea water. The sea water with eggs is th poured into the one with sperm for the eggs to be fertilis This method is suitable only for small-scale operations.

iii. Thermal shock

This method is often used to induce spawning in mar invertebrates, such as mollusca and echinoderms. This far the best method for inducing sea cucumbers to spaw the laboratory. The water temperature of filtered sea wa can be raised by exposure to intense sunshine, or with electric heating rod to raise the temperature by 3-5 degr than that of the filtered sea water. This thermal sh stimulates the breeders to discharge sperm or eggs.

iv Stimulation through desiccation and flow water

This method can be used after breeders have been conditioned for days. Stimulation for induc spawning is generally carried out dusk. First, the tank is emptied of the water the brood stock left to dr the shade for a period of time. The then subjected to high pressure water for several minutes. W applying water pressure, the should be scrubbed clean and later with filtered sea water. After breeders have been stimulated for 2 hours, they begin to move up the wall and move about frequently. F the male releases the sperm and three hours the female releases the By this method, 95-100 per fertilization can be achieved.

Fertilization

It is important to ensure a high ra the artificial breeding by obtaining quality eggs. Therefore, it is neces to handle the eggs carefully as soo they are released. The fertilizat external and takes place in the w After the eggs and sperm are rele

the breeders are removed from the tank. The eggs are washed several times in order to remove the excess sperm which might pollute the water in the tank resulting in reduced fertilization and a large number of deformed embryos.

Early Development

The female usually releases about one million eggs. About 0.75 million eggs can be stocked in 750 litres of water. After fertilization, the eggs undergo cleavage (pl,I,B) transformed into blastnla stage (pl.I,C) next day. The eggs are spherical, white and visible to the naked eye and are found floating. The diameter of the eggs varies from 180 to 200 u. On the third day, auricularia larva (pl.De pl.II, A) was formed. The auricularias are fed on the microalga **Isochrysis galbana** and a mixed culture dominated by the species of diastoma **chaetoceros sp**. and **Skeletonema sp**. The auricularia actively feed on **Isochrysis galbana**. The mouth region exhibited constant pulsating movements and the yellowish-green concentration of the feed in the stomach is seen in circular movement. As days pass on, the auricularia become more and more transparent and the lateral projections become more prominent. On each side of the larva there are four lateral projections and at the tip of each projection there is a round structure. The pre-oral and anal bands have a number of pigment spots. The length of auricularia at this stage varies from 660 to 1050 u. Some of the auricularia are smaller in size. A few of the auricularia are transformed into doliolaria stage on (pl.II,B) on the tenth day. Th doliolaria are barrel-shaped with five bands and with two tentacles projecting out. the posterior portion is slightly tapering. On each side there are five round structures with ossicle distinct at the posterior end. The doliolaria move fast in the forward direction. Their length varies from 420 to 570 u.

On the thirteenth day, some of the doliolaria transform into pentactula stage (pl.Ii,c). The body of pentactula is tubular with five tentacles at the anterior end and with one short stumpy tube-foot at the posterior end. The cloacal opening is distinct. The colour is greenish brown. The length varies from 330 to 750 u. By 18th day, the tubefeet and tentacles become more distinct and a number of tables are seen projecting from the skin. The tentacles have a web in between them. At the posterior end, two long tubefeet are seen. The spires of the tables are projecting from the skin. The tentacles and tubefeet also have tables. The length of the specimens varies from 550 to 720 u. The pentactula have the habit of moving to the edge of the tank and remain just below the surface of water. Soon they settle down at the bottom of the tank. The seed (pl. II,D) produced is transferred after two months.

Rearing of the postlarvae

Preparation of the rearing tanks

Rearing tanks and other tanks used in breeding, especially the new tanks, must be scrubbed clean and filled with water for 20 days, during which period the water is changed repeatedly in order to lower the pH to less than 8.5. Bef the tanks are used, they are scrubbed and filled with wa containing 40 pp, bleaching powder and then washed cl with filtered sea water before the larvae are introduced

The early development of fertilized egg took place on surface and column of water. From auricularia stage larvae started feeding on pentactula and settle down to bottom of the tank. The larvae are reared in one-tonne t in filtered and aerated sea water of salinity 32-34 per and temperature 27-29oc. The water is changed every The temperature, pH and salinity are regularly monito

Rearing density: Strict control of rearing density of lar i.e. the number of larvae per ml of sea water is first calcul At present, there are two methods to rear larvae still w rearing and flowing water rearing. Auricularia during early and middle stages, concentrate at the surface of wa If the density of the larvae is more they will form as a ball sink resulting in death. Therefore, the rearing den should be controlled to ensure better survival rate. optimum density of postlarvae is 300-700 per litre. In a tonne tank with 750 litres of water, 3,75,000 auricul can be stored.

Selection and counting of Larvae: After fertilized are moved to rearing tanks, they develop into early auricu stage in about 50 hours. The bottom of the rearing t should be cleaned thoroughly. Healthy larvae occupy surface layer of water, while deformed larvae and dead generally stay in the lower layer of water coloum or a bottom of the tank. All the dead individuals, defo larvae and sediment should be siphoned out in order to the tanks. After the tanks are cleaned, the water in the should be gently stirred so that the larvae can be unifo distributed. A sample is then taken for counting the la Samples are takne separately from two ends and the m of the tank in a 250 ml beaker. This sample is unifo stirred and one ml sample is taken in a pipette and pu plankton counting chamber. The number of larvae is co in each ml. Like this, two more samples have to be take the average of three counts is taken as an indication o density of larvae. The result of the count would whether the density is desirable or not.

When the auriculariae are in the early stage, they are at the density level of about 500 per litre. The pe auricularia development can be divided into three st viz., early middle and late stages. As they develop fro stage to the next, the bottom of the tanks must be cl completely once, or the larvae moved to another Normally the larvae are taken out after every three d that the tanks can be cleaned thoroughly to avoid infes of ciliates and also copepods. On other days, the wate is reduced to more than half by keeping the sieve ins tank. Any sediment must be removed to keep the fresh. An uptodate information on survival rate a developing stage is necessary.

Water management

In the course of rearing, the larvae eject faeces and consume dissolved oxygen constantly. Some of the larvae die in due course of time. These and the leftover food produce harmful substances such as H2S and NH3. In addition, bacteria reproduce rapidly with rise of temperature. Poor water quality directly affects the normal development of larvae. Therefore, proper water management and sanitation are essential. Regular cleaning of tanks and chaning of water is essential. The dirt and deformed larvae at the bottom of the tank are siphoned out every day. The mesh size of sieve must be smaller than the larvae. Normally 80 u sieve is used since the auriculria larvae and even the eggs are bigger in size than the sieve. While the water is being changed, the sieve is kept inside the tank. Someone should constantly stir the water lightly all round while the water is being changed. This will prevent loss of larvae during water change, since siphoning would normally force the larvae to stick to the sieve causing mechanical injury to the larvae. The sediment at the bottom of the tanks should be siphoned out completely every three or four days.

Larval feeding and feeding rates

Suitable and high quality microalgae and correct feeding densities are the key to successful rearing. As the larvae of **Holothuria scabra** and **H.atra** develop into early auriculariae larvae, its alimentary canal is well formed and the larvae must be given diet immediately. The feeding mechanism of the larvae consists of conveying the suspended food organisms and unicellular algae into alimentary canal through the mouth parts by the swaying of the peristomial cilia. The effectivenes of **Isochrysis galbana, Duniella salina, Dicrateria** sp, and mixed feed consisting of all the above mentioned microalgae were tried. The results showed that the growth rate is better when fed with **Isochrysis galbana** and also the mortality rate is low. After four or five days the auriculara are fed with mixed culture. This chiefly consists of the phytoplankton **Chaetoceros sp.**

The larvae require different quantities of diet during different developmental stages. Unicellular algae are fed twice a day, the quanity given each time depends on the particular stage of the larvae.

In general, 20,000 to 30,000 cells per ml in the rearing tank should be maintained. The microalga isochrysis galbana culture initially has a concentration of 80,000 cells per m. When the culture is good it reaches one-million mark. The quantity of feed should be increased or decreased depending on the quantity of food in the stomach of the larvae. This can be periodically checked every day before feeding them. Unicellular algae during the peak period of reproduction are the most preferred diet for larvae.

Environmental factors

Monitoring of the environmental parameters is of paramount importance in the culture operations since the larvae and juveniles are very sensitive to environmental changes.

Temperature

At Tuticorin, the temperature of sea water ranges from 26-30oc. The ideal temperature for rearing larvae is found to be 27-29oc. The water temperature should be noted twice in a day, in the morning and the afternoon.

Dissolved oxygen

Dissolved oxygen level varies with water temperature. The higher the temperature, the lower the Do level. At Tuticorin, the normal range for dissolved oxygen is 5-6 ml/litre. Always, areation is given to the larval tanks throughout the day to see that the oxygen level does not go down much. For a one-tonne tank usually aeraters are provided, one at either end.

pH

Under normal conditions, rearing sea water is generally alkaline with pH at 7.5 to 8.6. Tests have shown that the larvae of **Holothuria scabra** and **H. atra** larvae and juveniles adapt to a fairly wide range of pH. However, when pH rises over 9 or drops below 6.0, the moving ability of the larvae weaknes and growth stops. Therefore, the pH value of the water must be maintained between 6 and 9.

Salinity

Salinity of normal sea water at Tuticorin ranges from 31 to 34 per cent. If the salinity is low, all the larvae will die. The lethal critical salinity is 12.9 per cent. The optimum salinity for larval development ranges from 26.2-32.7 per cent. In This range, the higher the salinity, the quicker is their development. Too high and too low salinity adversely affects the normal development of the embryo and the larvae, resulting in the death of a large number of deformed larvae. Salinity estimation is, therefore, an important routine work throughout the entire rearing period. A salinity refractometer is now commonly used for quick salinity estimation. If a specific gravity mater is used, the measured value can be converted into salinity value.

Ammonical Nitrogen

The ammonical nitrogen content of sea water is very low. The sources in the breeding tanks are mainly the metabolites of larvae, the unconsumed diet and decomposing organisms. Too much accumulation of NH3 can be harmful to the larvae. The larvae can develop normally with an ammonical nitrogen content of 70-430 mg per m3 water. When its content is over 500 mg per m3, it will have a harmful effect on the development and growth of the larvae.

Explanation to Plates/Pictures/Photes

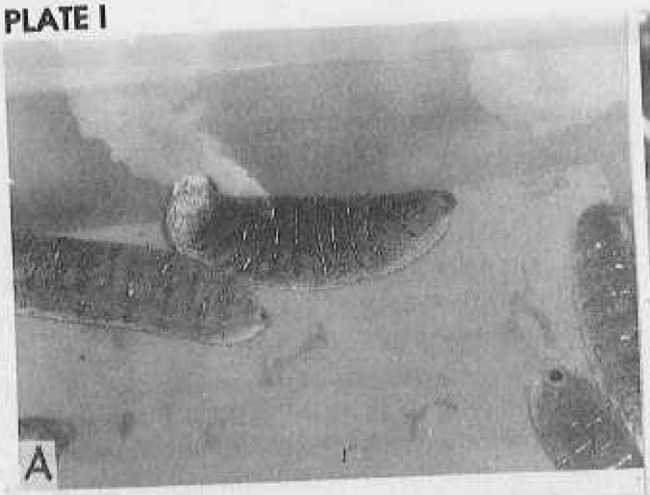
PLATE I

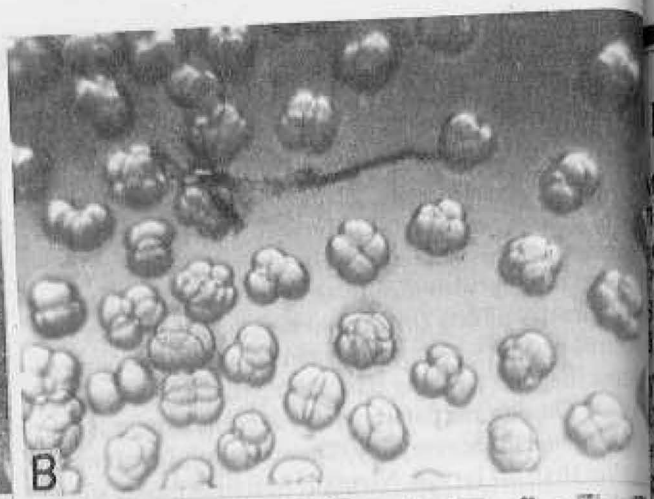
A. Brood stock material
B. Four - celled and eight - celled stages
C. Blastula
D. A group of Auricularia

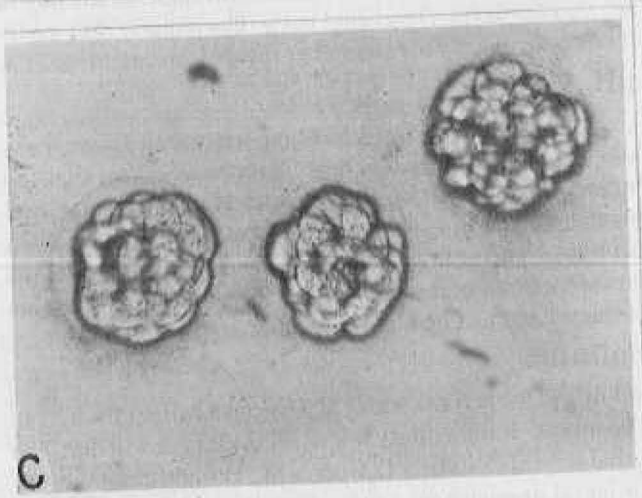
PLATE II

A. A single late Auricularia
B. Doliolaria
C. Pentactula
D. Seed of Holothuria scabra produced in the hatchery

PLATE I







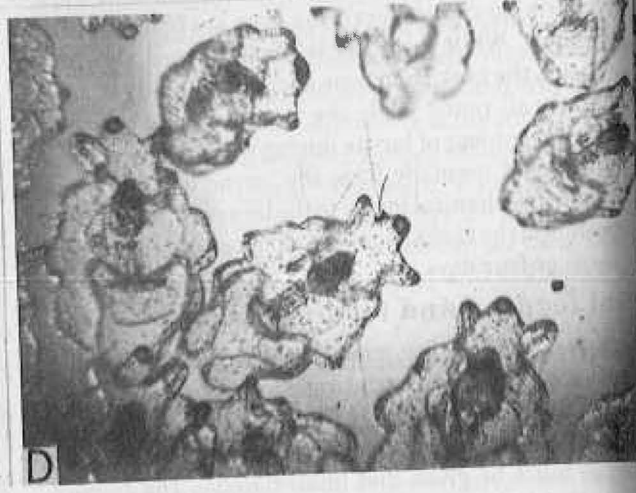


PLATE II