



CMFRI SPECIAL PUBLICATION
Number 57

**HATCHERY TECHNIQUES
AND CULTURE OF THE SEA-CUCUMBER
*HOLOTHURIA SCABRA***

A
CMFRI
TRANSFER OF TECHNOLOGY SERIES

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
Indian Council of Agricultural Research
Dr. Salim Ali Road, Post Box No. 1603, Tatapuram, P.O.
Ernakulam, Cochin 682 014, India

CMFRI SPECIAL PUBLICATIONS

1. Pearl culture training : Long-term and short-term courses. 1977, 39pp.
2. Mariculture research and developmental activities. 1978, 48pp.
3. Summer Institute in breeding and rearing of marine prawns. 1978, 129pp.
4. Economics of the indigenous fishing units at Cochin : A case study. 1978, 24pp.
5. Seminar on the role of small-scale fisheries and coastal aquaculture in integrated rural development, Madras, 6-9 December 1978. Abstracts, 1978, 44pp.
6. Proceedings of the first workshop on technology transfer in coastal aquaculture held at Cochin, 23-24 July, and Mandapam, 27-28 July 1979. 1979, 96pp.
7. Manual of research methods for crustacean biochemistry and physiology. 1981, 172pp.
8. Manual of research methods for fish and shellfish nutrition. 1982, 125pp.
9. Manual of research methods for marine invertebrate reproduction. 1982, 214pp.
10. Analysis of marine fish landings in India : A new approach. 1982, 42pp.
11. Approaches to finfish and shellfish pathology investigations. 1983, 43pp.
12. A code list of common marine living resources of the Indian seas. 1983. 150pp.
13. Application of genetics in aquaculture. 1983, 90pp.
14. Manual of research methods for invertebrate endocrinology. 1983, 114pp.
15. Production and use of *Artemia* in aquaculture. 1984, 74pp.
16. Manual on marine toxins in bivalve molluscs and general consideration of shellfish sanitation. 1984, 100pp.
17. Handbook of diagnosis and control of bacterial diseases in finfish and shellfish culture. 1984, 50pp.
18. Proceedings of the workshop on sea turtle conservation. 1984, 109pp.
19. Mariculture research: under the Centre of Advanced Studies in Mariculture. 1984, 136pp.
20. Manual on pearl culture techniques. 1984, 42pp.
21. A guide to prawn farming in Kerala. 1985, 96pp.
22. Water quality management in aquaculture. 1985, 96pp.
23. Hatchery production of penaeid prawn seed : *Penaeus indicus*. 1985, 41pp.
24. The present status of ribbonfish fishery in India. 1986, 49pp.
25. A practical manual for studies of environmental physiology and biochemistry of culturable marine organisms. 1986, 45pp.
26. Theorems in environmental adaptation. 1986, 50pp.
27. Bibliography of the publications by the staff of CMFRI 1948-85. 1986, 168pp.
28. The present status of our knowledge on the lesser sardines of Indian waters. 1986, 43pp.
29. Exploitation of marine fishery resources and its contribution to Indian economy. 1986, 32pp.
30. Seminar on potential marine fishery resources and its contribution to Indian economy. 1986, 32pp.

1 to 11 Out of Print

Contd. on back inside

**HATCHERY TECHNIQUES
AND CULTURE OF THE SEA-CUCUMBER
*HOLOTHURIA SCABRA***

TRANSFER OF TECHNOLOGY

D. B. JAMES

A. D. GANDHI

N. PALANISWAMY

J. X. RODRIGO

*Central Marine Fisheries Research Institute,
Cochin - 682 014*



**CMFRI SPECIAL PUBLICATION
Number 57**

**CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
Indian Council of Agricultural Research
Dr. Salim Ali Road, Post Box No. 1603, Tatapuram - P.O.
Ernakulam, Cochin 682 014, India**

LIMITED DISTRIBUTION

Published by : **DR. P. S. B. R. JAMES,**
Director,
Central Marine Fisheries Research Institute,
Cochin - 682 014.

Editing and layout by : **DR. K. RENGARAJAN,**
Senior Scientist,
Central Marine Fisheries Research Institute,
Cochin - 682 014.

Front cover : Seeds of *Holothuria scabra* produced at CMFRI hatchery at Tuticorin.

Back cover : Sea-cucumber hatchery of CMFRI at Tuticorin.

PRINTED IN INDIA
AT PAICO PRINTING PRESS, ERNAKULAM, COCHIN - 682 035

PREFACE

Hatchery practices in sea-cucumbers are followed in China and Japan and more recently in Korea and Russia also. At all these places only seed of *Stichopus japonicus* is produced. The seed, after retaining it for two or three months, is sea-ranched since it is expensive to maintain them in the hatcheries for a long periods.

In India the *Beche-de-mer* industry is very ancient one. Till recently the whole fishery was supported only by a single species namely *Holothuria scabra*. As a result of this the natural populations dwindled down alarmingly. In order to enrich the natural populations a Research Project was taken up by the **Central Marine Fisheries Research Institute** at Tuticorin Research Centre in 1987 on the hatchery and culture of sea-cucumbers. Break-through was achieved in 1988 in inducing *Holothuria scabra* to spawn in the laboratory for the first time by thermal stimulation and producing seeds. Since then several spawnings have taken place and seeds produced. In 1992 the Marine Products Export Development Authority, Cochin has sanctioned a Research Project for six lakhs rupees for three years on intensive seed production and sea-ranching of sea-cucumbers. This has given a further impetus to the work.

This Special Publication is a practical guide for seed production in the hatchery and culture of *Holothuria scabra* which is the most valuable species processed in India at present. It is well illustrated with colour photographs. I hope this special publication of the "Transfer of Technology Series" will be of interest to those who are involved in the *Bache-de-mer* industry.

The efforts of the team headed by Dr. D. B. James in the production of seeds in the hatchery and also in the preparation of this publication is highly appreciated and I congratulate them for this achievement. I also thank Dr. K. Rengarajan for editing and getting the publication printed in time.

P. S. B. R. JAMES
Director,
Central Marine Fisheries
Research Institute.

Cochin - 682 014,
24th January 1994.

CONTENTS

PREFACE	iii
INTRODUCTION	1
DISTRIBUTION AND BIOLOGY	2
HATCHERY SITE	5
HATCHERY FACILITIES	6
PERSONNEL REQUIREMENT	9
HATCHERY OPERATIONS	10
ENVIRONMENTAL FACTORS	17
NURSERY REARING	19
PREDATORS AND THEIR CONTROL	21
CULTURE PROSPECTS	22
GROW-OUT SYSTEMS	23
CULTURE OF ALGAL FOOD	25
ECONOMICS	27

INTRODUCTION

In India sea-cucumbers occur in the Gulf of Mannar, the Palk Bay, the Andaman and Nicobar Islands, the Lakshadweep Islands and also in the Gulf of Kutch. At present it is exploited only in the Gulf of Mannar and Palk Bay since there is no processing in the Andaman and Nicobar Islands and also at Lakshadweep. As a result of continuous exploitation over thousand years the natural populations have dwindled down considerably. Sea-cucumbers being defenceless animals do not offer any resistance at the time of capture and also being sluggish they do not make any attempts to escape from the collector. For these reasons and also due to over exploitation the natural populations have alarmingly decreased. This scarcity of sea-cucumbers has created a crisis for the *Beche-de-mer* industry due to shortage of raw material for processing.

At this juncture the Central Marine Fisheries Research Institute has taken up a Project on the hatchery production of seeds and culture of sea-cucumbers. The CMFRI, in its attempt, succeeded in inducing *Holothura scabra* to spawn for the first time in the laboratory by thermal stimulation. Since then several spawning have been obtained and the seeds produced are now grown in the sea under natural conditions. It is proposed to scale up seed production for sea-ranching programme to enrich the natural populations. The present publication on hatchery production of seeds and culture of sea-cucumbers will be useful to the entrepreneurs. The technology presented here is the result of continued research on various aspects of the hatchery system. The technology is simple for adoption and low-cost one in terms of equipment and expenditure. Besides basic information on the hatchery and culture of sea-cucumbers, some information on the biology and distribution of Indian sea-cucumbers along with production of feed for larvae are given.

2

DISTRIBUTION AND BIOLOGY

Distribution

Sea-cucumbers belonging to the families Holothuridae and Stichopodidae are used in processing for *Bache-de-mer*. They enjoy a world wide distribution and found in large numbers in the Indo-west Pacific region comprising of the Islands of the Western Indian Ocean, Mascarene Islands, East Africa and Madagascar, Red Sea, Southeast Arabia, the Persian Gulf, west coast of India and Pakistan, Maldives and the Lakshadweep, Sri Lanka, Bay of Bengal including the Andaman and Nicobar Islands, the East Indies, North Australia, the Philippines, China and Southern Japan, South Pacific Islands and the Hawaiian Islands. In the Gulf of Mannar and Palk Bay, the commercially important species are :

<i>Holothuria scabra</i>	<i>Actinopyga echinites</i>
<i>H. spinifera</i>	<i>A. miliaris</i>
<i>H. atra</i>	<i>Stichopus variegatus</i>
<i>Bohadschia marmorata</i>	<i>S. chloronotus</i>

In the Andaman and Nicobar Islands the following species are of economic importance :

<i>Actinopyga echinites</i>	<i>A. miliaris</i>
<i>A. lecanora</i>	<i>A. serratidens</i>
<i>A. mauritiana</i>	<i>Bohadschia marmorata</i>

<i>B. graeffei</i>	<i>Stichopus chloronotus</i>
<i>B. vitiensis</i>	<i>S. variegatus</i>
<i>Holothuria scabra</i>	

In the Lakshadweep, important species are :

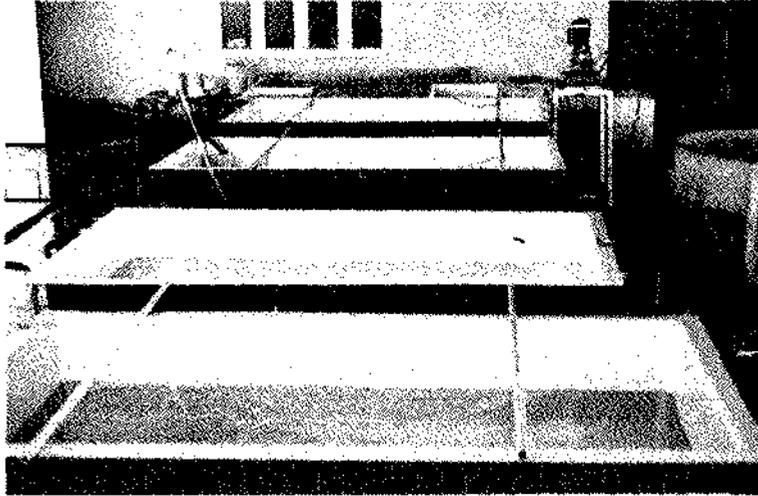
<i>Actinopyga echinites</i>	<i>B. graeffei</i>
<i>A. mauritiana</i>	<i>B. marmorata</i>
<i>A. miliaris</i>	<i>Stichopus chloronotus</i>
<i>A. serratidens</i>	<i>S. variegatus</i>
<i>Bohadschia argus</i>	<i>Thelenota ananas</i>

The commercially most important and valuable species *Holothuria scabra* which is common in the Gulf of Mannar and the Palk Bay and the Andaman and Nicobar Islands, is not distributed in the Lakshadweep. Even though several valuable species occur in the Andaman and Nicobar Islands and also in the Lakshadweep, at present there is no exploitation of the species. At present *H. scabra* is also reported from Malvan on the west coast of India. Its occurrence from the Gulf of Kutch is a distinct possibility. All the commercially important species of sea-cucumbers are coral loving and they occur right from the intertidal zone to a depth of 20 metres.

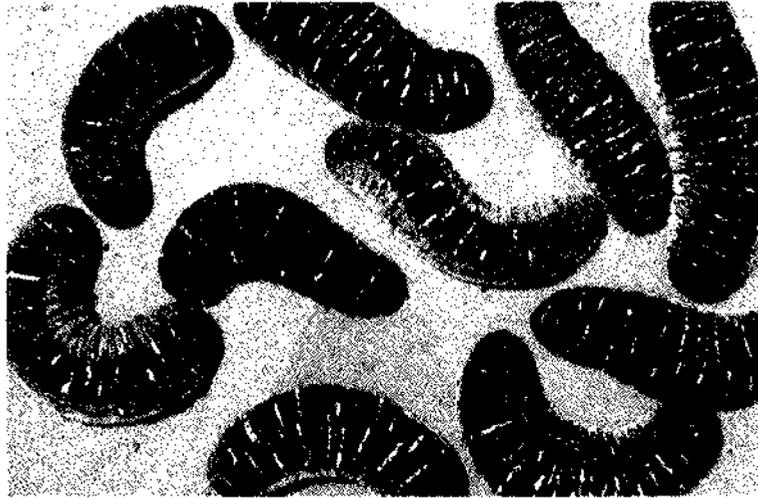
Biology

Though the sea-cucumbers are economically important, not much attention is paid to the biology of the animals. For the first time some information is collected on the biology of the sea-cucumber *Holothuria scabra*. *Holothuria scabra* is known to reach a maximum length of 400 mm and the weight in live condition is 500 g. It prefers sandy-muddy substratum. It is often found buried with the posterior end always above the surface of mud. It is known to prefer slightly less saline areas. Usually smaller forms are found near the shore. As they grow, they migrate to deeper waters for breeding. It

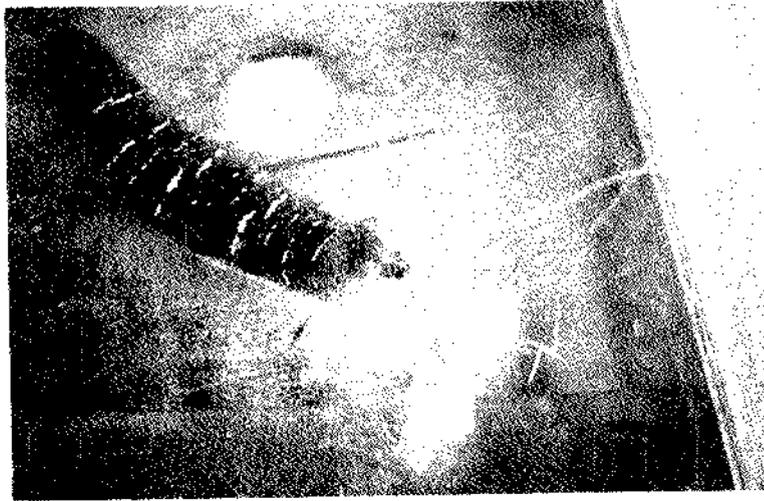
breeds twice in an year in the Gulf of Mannar. The first spawning season is from March to May and the second one is during October-December. In 18 months this species reaches sexual maturity. Size at first maturity for females is estimated at 213 mm and for males at 210 mm. The fecundity is estimated at ten lakhs. The longevity is estimated as ten years. At the end of first, second, third, fourth and fifth years it reaches a length of 136 mm, 225 mm, 284 mm, 322 mm and 348 mm respectively. They feed on sand and mud, and subsist on the organic matter present in it.



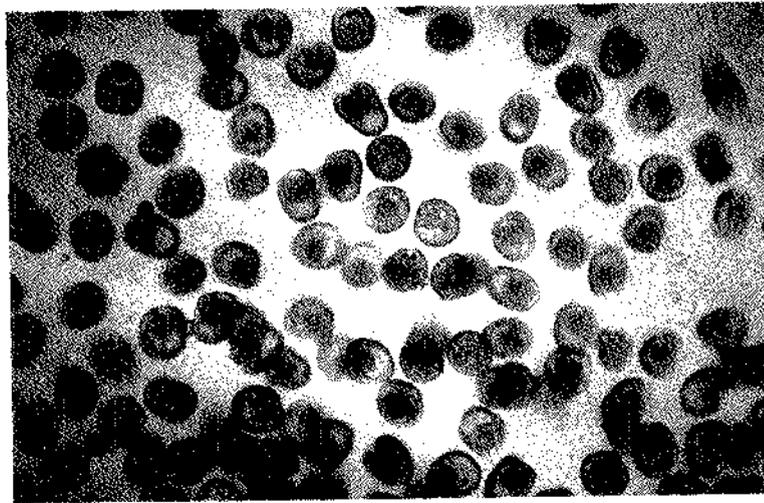
I. Sea-cucumber hatchery at Tuticorin Research Centre of CMFRI.



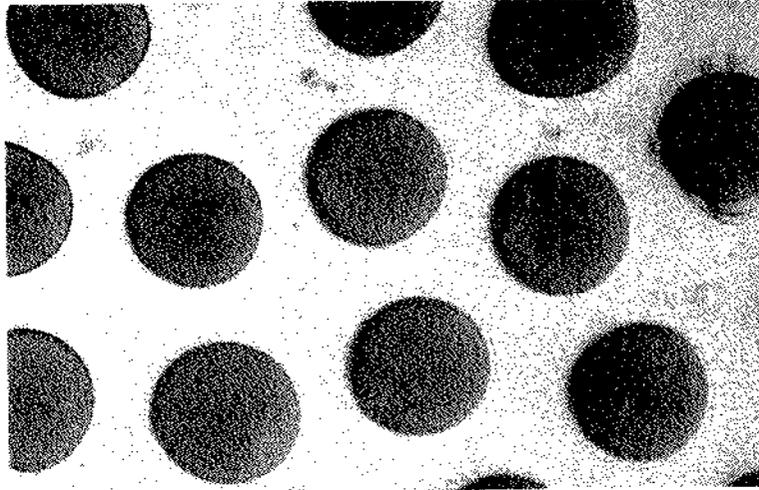
II. Brood-stock of *Holothuria scabra*.



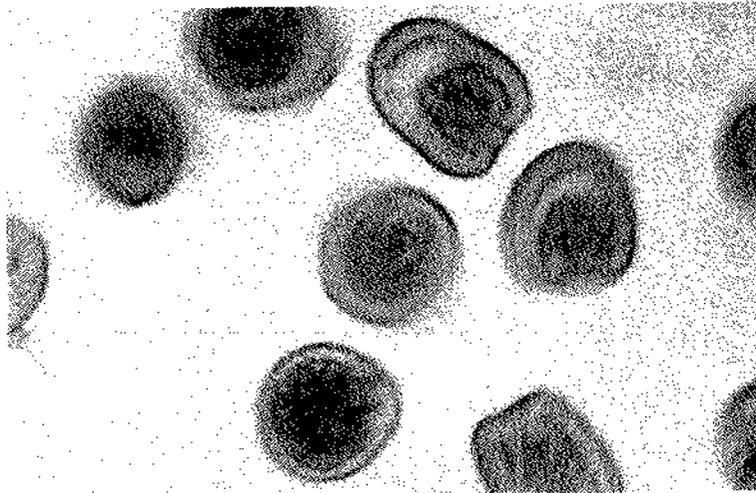
III. Male *Holothuria scabra* releasing the sperms.



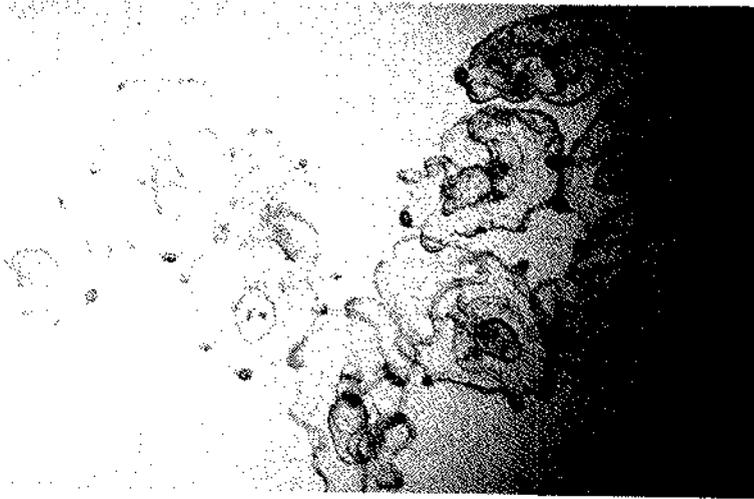
IV. Group of eggs.



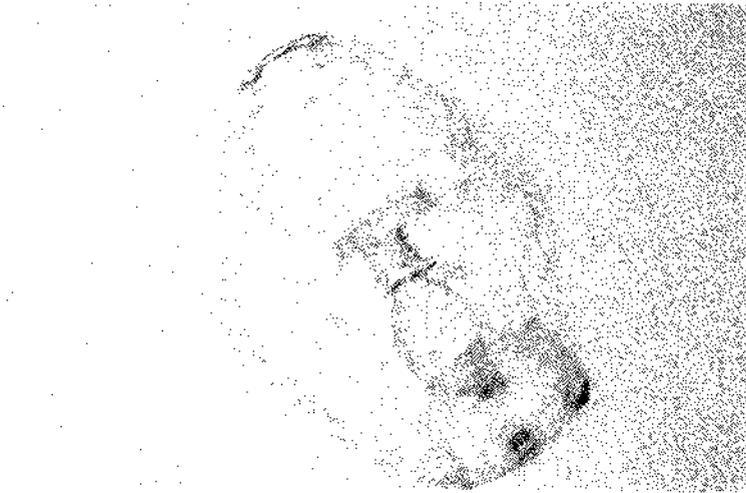
V. Fertilized eggs (enlarged view).



VI. Gastrula (at the top of the photograph).



VII. A collection of early auricularia.



VIII. Early auricularia.

3

HATCHERY SITE

Suitable site selection is an important basic requirement for establishing a viable hatchery. The success of hatchery operations mainly depend on several factors concerned with the site.

The following are the primary requirements to be considered, while selecting a site for the hatchery.

1. The sea-cucumber hatchery must be located near to the shore with adequate supply of seawater free from pollutants, suspended particles, silt, etc.
2. The salinity of the seawater must be between 30 and 40 ppt throughout the year.
3. Hatchery should be away from industrial and domestic sewages and from river mouth to avoid dilution of seawater during monsoon.
4. The sea bottom must be rocky or coralline so as to get clean seawater throughout.
5. Supply of freshwater must be ensured at the hatchery site.
6. Approachable road must be available for easy transport.
7. Hatchery area should not be affected by cyclones and other natural calamities like sea or soil erosion.
8. Hatchery site must be provided with electricity.
9. The area from hatchery site to farm should be free from fishing operations.

4

HATCHERY FACILITIES

The basic facilities required for the production of sea-cucumber seeds are described below.

Building

The hatchery is 24 x 12 m in area with light roofing (Pl. I). Cuddapha stone flooring with proper slope should be given and a straight closed gutter runs in the middle along the entire length of the hatchery to collect the water spilled to keep the floor dry and clean. Proper ventilation should be provided at the sides of the hatchery building.

The conditioning room (4.0 x 2.5 m) with asbestos sheets and false ceiling with thermocol, is used for conditioning the sea-cucumber prior to inducement for spawning. The room temperature of the conditioning room is kept between 18 - 25° C by using air-conditioners.

Seawater supply

The sea water filter system consists of a draw well, sedimentation tank, filter bed, storage sump, overhead tank and PVC delivery lines to the hatchery. Sea water is drawn into the well through a 15 cm dia PVC pipe by gravitation. The water is pumped to the sedimentation tanks using 1 HP electric pump sets to allow the larger particles to settle at the bottom. The clear supernatant seawater is passed through filter bed. The filter bed consists of fine river sand at the top, charcoal, pebbles and granite stones at the bottom. The filtered sea water is pumped to the storage sea water sump (20,000 l cap.) and the same is pumped to the overhead tank (10,000 l cap.) using 1 and 7.5 HP electric pumpsets respectively.

Air-supply system

The air supply system consists of air compressors, filters, PVC air grid, polythene tubes, diffuser stones and air regulators. Air compressor with a 1 HP electric motor gives a high output at low pressure. It has got a storage tank which is automatically cut-off when the tank is full allowing the compressor sufficient rest. The air flow is regulated passing through series of filter by removing oil and moisture content in the air. The air pipe of 25 mm dia run along the entire length of the hatchery and the air is drawn at the required places from the pipelines through polythene tubes and diffuser stones and adjusted with the help of a gate valve connected to the polythene tubes. By keeping a standby compressor the air supply system can be well managed.

Generator

Three phase generator of 10 KVA capacity operated by a 16 HP diesel motor is to be used in case of electricity failure.

Conditioning room

The conditioning room is 6 x 8 m in size with thermocol ceiling. Two air-conditioners (1.5 tonne cap.) are installed in the room for controlling the temperature to a desired level. The matured sea-cucumbers can retain sexually ripe gonads at the temperature. The sea-cucumbers are kept in the 100 l FRP rectangular tank provided with sand at the bottom of the tank. To this small quantities of algal powder is added. This forms excellent food for the brood stock.

Spawning tank

A perspex rectangular tank of 100 l capacity is arranged for spawning of sea-cucumbers. Jumo thermometer, silica cased immersion heater and an aerator are fitted to the tank during thermal stimulation and these are connected to an automatic ECE controller.

Larval rearing tanks

One tonne capacity FRP rectangular (200 x 100 x 50 cm) tanks are used for rearing the larvae of sea-cucumber. Five to ten tanks are

required for the hatchery. These tanks must have smooth inner surface with white colour which will help in observing the larvae easily.

Juvenile rearing tanks

The settled juveniles are reared in one tonne FRP tanks similar to larval rearing tanks. Five to ten such juvenile rearing tanks are required for the hatchery. These tanks should have smooth surface and provided with fine sand at the bottom for rearing the juveniles.

Algal culture tanks

The tanks used for algal culture are similar to the earlier two types. These tanks must have smooth white inner surface required for mixed algal culture. The seawater in the algal culture tank is agitated with a pair of diffuser stones. The mixed algae cultured in these tanks are suitable for feeding the juveniles of sea-cucumber. *Isochrysis galbana* given to larvae as feed is cultured under controlled temperature conditions.

Other equipments

Different mesh size (40, 80, 200 μm) sieves, a good microscope to observe the condition and to measure the larvae, a haemocytometer to count algal cell concentration, a plankton counting chamber to estimate the larval density are the other requirements for a hatchery. The pH meter, thermometer, refractometer, oxygen analysing unit, are the equipments used for monitoring the water quality. Glassware such as trays, beakers, conical flasks, embryo cups, petri-dishes, pipettes, microslides, cover slips, plastic containers such as buckets, basins, mugs and perspex tanks are the requirements.

For facility and equipment requirements, Annexure I on page 30 may be referred.

PERSONNEL REQUIREMENT

One supervisor with thorough knowledge on hatchery management, one technical assistant with experience in larval estimation, stocking, measurement and algal cell counting and two skilled helpers to change water for the larvae/juveniles and feed them daily will be enough to produce mass production of sea-cucumber in the hatchery and to rear them in the open sea. Providing running sea water system both for the larvae and juveniles would minimise labour in the hatchery. Hence man power may be utilised for effective management of juveniles in the culture area.

6

HATCHERY OPERATIONS

Collection of brood stock material

Brood stock material is collected from the commercial catches meant for processing. Only large and healthy specimens (Pl. II) alone were selected for this purpose. Specimens which were injured during capture and those which have been eviscerated were rejected. The specimens are stocked in one tonne capacity tanks with sand brought from the natural beds. The sand is arranged in six inch thickness to enable the sea-cucumbers to bury in the sand.

Maintenance of brood stock

The success of the hatchery depends on the healthy condition of the brood material. The water in the tanks is changed every day and the sand is changed every fortnight. If the water becomes stale the holothurians eviscerate rendering themselves useless for breeding purpose. Fresh algae is brought from the sea and ground to a fine paste in a mixer and small quantities of this is put in the broodstock tanks weekly once. Care should be taken not to put too much of the same since it will make the water foul. The sea-cucumbers live on the organic matter present in the sand. The algal paste settles down to the bottom and this is consumed by the holothurians along with the sand. If proper food is not provided the animals become shrunken and the gonad is re-absorbed rendering the material unfit for spawning purposes. It is desirable to keep 20 - 30 adults in one tonne tank.

Collection time

Collection time is very important for the success of hatchery management. *H. scabra* is known to have two spawning peaks, one

in March-May and the other in October-December. It is essential to collect the brood stock material during the spawning season since the chances of induced breeding are more, since most of the specimens will be ripe and ready to release the eggs. A small rise in temperature is enough to induce them to spawn. At present there is no known method to hasten the maturation process. Therefore it is desirable to collect the material during the breeding peaks and induce them to spawn.

Spawning

Spawning can be achieved in four ways. They are described below briefly. The specimens are induced to spawn in the laboratory by thermal stimulation for seed production.

i. Natural spawning

When the gonads are fully mature, the male and female breeders release the sperms and eggs without any stimulation. At first the males release the sperms usually at noon and this is followed by females which release the eggs after an interval of one to two hours.

ii. Stripping

This method is used only in small scale experiments since the fertilization rate is low and also the number of deformed larvae are more. In this method ripe specimens are selected during the breeding peaks. The specimens are cut on the dorsal side from the cloaca to mouth. The fully mature ovary which is always translucent is taken out with a forceps and dried in the shade for sometime. The ovary is then placed in clean sea water in a large petridish and lightly punctured with a scissors to release the eggs into sea water. Likewise the ripe testis is taken out and cut into pieces. When the sperms swim about, this water is poured into a ten litre beaker having the eggs with slight aeration to mix the eggs and sperms to achieve higher rate of fertilization.

iii. Thermal stimulation

This is by far the best and most reliable method available at present, to induce the holothurians to spawn. First the temperature

in the brood-stock tanks is noted. If the temperature is 28° C the specimens are introduced into water having a temperature of 32° C. Sea water is heated with an immersion rod and this hot water is carefully mixed with normal sea water to get the desired temperature. Usually a rise of 3-5° C is enough to induce them to spawn. This method is widely used.

iv. Stimulation through drying and powerful jet of water

This method can be used after the breeder has been conditioned for more than one week in the hatchery. First all the water in the brood-stock tank is removed and the specimens are dried in the shade for about half an hour. Then the specimens are subjected to powerful jet of sea water for a few minutes. After this the specimens are put back into the tank with sea water. After 1-2 hours, the specimens begin to move up the tank wall and begin to show swaying movements. First the male releases the sperms and then after one hour the female releases the eggs.

Spawning behaviour

In sea-cucumbers the sexes are separate and it is not possible to distinguish the sexes externally. Only microscopical examination of the gonads will indicate whether they are males or females. Ripe ovary is translucent and ripe testis is milky white in colour. At the time of spawning also it is possible to differentiate the sexes since the spawning behaviour of males and females is different. In all cases only the males spawn first and this is followed by the females. In the case of *H. scabra* the males first lift the anterior end and exhibit swaying movements. After exhibiting such movements for sometime the males start releasing the sperms (Pl. III) from the gonopore situated on the dorsal side near the oral region. The males when they start releasing the sperms keep on doing so for one to two hours depending on the size of the testis inside. A distinct papilla is discernible in the case of males at the time of spawning. In the meanwhile if there are ripe females they start reacting to the sperms, released into the water. The anterior region of the female gets bulged due to the pressure built in inside.

The eggs are released in a continuous and powerful jets intermittently whereas the sperms are released more or less continuously without force. The egg mass released is light yellow muscus-like in appearance. The powerful jet of eggs helps in the dispersal over a wide area.

Fertilization

It is important to ensure a high rate of survival in artificial breeding by obtaining high quality eggs. Therefore it is necessary to handle the eggs carefully as soon as they are released. The fertilization takes place outside in the water. After the eggs and sperms are released, the breeders are removed from the tank. The eggs are washed several times in order to remove the excess sperms which might pollute the water in the tank resulting in reduced fertilization and large number of deformed embryos.

Early development

The female usually releases about one million eggs. The eggs (Pl. IV & V) are spherical, white and viable to the naked eye. The diameter of the eggs varied from 180 to 200 μ . About 0.75 million eggs can be stored in 750 litres of water. After fertilization the first polar body appears within 20 - 30 minutes. The first cleavage takes place after 15 minutes. Four celled stage is formed after 20 minutes. Early blastula is reached after 40 minutes. In three hours the blastula is fully formed. After 24 hours the gastulla (Pl. VI) is fully developed. It is oval in shape and motile. After 48 hours early auricularia appears. On the tenth day doliolaria is found and after three days pentactula stage is reached. The various types of larvae are briefly described below.

Auricularia larva

Early auricularia (Pl. VII & VIII) is formed after 48 hours. After five or six days, late auricularia (Pl. IX) is formed. It is slipper-shaped, transparent and pelagic in habit. It has a preoral loop anteriorly and anal loop posteriorly. These bands help in locomotion. The digestive tract consists of mouth, an elongated pharynx and sacciform

stomach. The early auricularia larva measures on an average 563 μ . The late auricularia has an average length of 1.1 mm. On the tenth day the auricularia larvae metamorphose to doliolaria larva.

Doliolaria larva

The doliolaria (Pl. X) is barrel-shaped with five bands around the body. These larvae measure 460-620 μ . Rapid changes occur inside the body and all adult features of the holothurian set in. This stage is short and lasts only for two or three days and subsequently transform into a creeping stage known as pentactula.

Pentactula larva

The pentactula (Pl. XI) is tubular with five tentacles at the anterior end and with a single tubefoot at the posterior end which helps in the locomotion of the larva. The pentactula creeps over the sides and bottom of the tank. They actively feed on benthic algae and other detritus matter. The pentactula measures 600-700 μ . If they are fed on algal extract some of them reach a length of 10 mm in one month's time and transform into juveniles (Pl. XII).

Rearing of larvae

Rearing tanks and other tanks used in the hatchery, especially the new ones must be scrubbed clean and filled with water for 10 days, during which period the water is changed daily in order to lower the pH below 8.5. Again the tanks are scrubbed and filled with water containing 40 ppm bleaching powder and then washed clean with filtered sea water before the larvae are released.

Rearing density

Strict control of rearing density of the larvae is to be observed. The larvae can be reared in still waters or running waters. If the density of the larvae is more, they will form a ball and sink resulting in death. Therefore the rearing density should be controlled to ensure better survival rate. The desirable density of larvae is 300-700 per litre. In one tonne tank with 750 litres of water, 3,75,000 larvae can be stocked.

Selection and counting of the larvae

After fertilization the eggs are removed to rearing tanks, they develop into early auricularia larvae in 48 hours. The bottom of the rearing tank should be cleaned thoroughly. Healthy larvae occupy the surface layer of water, while deformed ones and dead larvae generally stay and settle at the lower layer of the water column or at the bottom of the tank. All the dead individuals, deformed larvae and sediment should be siphoned out in order to clean the tanks. All the larvae are taken in a sieve and put into a beaker of 10 l capacity. Mild aeration is given to the larvae. The water is uniformly stirred and one ml sample is taken in a pipette and put in a plankton counting chamber. The number of larvae are counted in each ml. Like this three samples have to be taken and the average of three counts is taken as an indication of the density of the larvae. The result of the count would show whether the density is desirable or not. The period of auricularia larval development can be divided into three stages viz. early, middle and late stages. As they develop from one stage to the next, the bottom of the tanks must be cleaned completely once, or the larvae are shifted to another tank. Normally the larvae are taken out once in three days so that the tanks can be cleaned thoroughly to avoid infestation of other organisms. On other days the water level is reduced to more than half by keeping the sieve inside the tank. The sediment must be removed to keep the water fresh. An upto date information on the survival rate at each 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 the course of time. These and the left over food produce harmful substances like hydrogen sulphide and nitrogen wastes. With the rise of temperature, bacteria also develop. Poor water quality directly affects the normal development of larvae. Therefore proper water management and sanitation is essential. While water is changed by keeping the sieve inside the tank, the mesh size of the sieve must be smaller than the larvae. Normally 80 μ sieve is used since the

larvae and even the eggs are larger in size than the mesh in the seive. While the water is being changed someone should constantly stir the water lightly all round the tank. This will prevent the loss of larvae during the water change, since siphoning would normally force the larvae to stick to the seive causing injury to the larvae. The sediments at the bottom of the tank should be siphoned out completely every three or four days.

Larval feeding and feeding rates

Suitable and high quality microalgae and correct feeding rates are important to the success of rearing. As the larvae progress in development the alimentary canal is well formed and the larvae must be given diet immediately. The feeding mechanism of the larvae consists of conveying the suspended bits of organisms and unicellular algae into the alimentary canal through the mouth parts by swaying of the hair-like structures round the mouth. The effectiveness of various microalage were tried. Better growth rate was obtained when fed on microalga *Isochrysis galbana*. With this microalga the mortality rate was also found less. After four or five days the larvae are also fed with mixed culture chiefly consisted of phytoplankton *Chaetoceros* sp.

The larva requires different quantities of diet during different developmental stages. Unicellular algae are fed twice in a day, but the quantity given each time depends on the particular stage of larvae. In general 20,000 to 30,000 per ml in the rearing tank water is maintained. The microalga *Isochrysis galbana* cultured usually has a concentration of 80,000 cells per ml. When the bloom is good it reaches one million mark. The quantity of diet given should be increased or decreased depending on the quantity of food in the stomach of the larvae. This can be visually checked every day before feeding them. Unicellular algae during the peak period of their reproduction are the most preferred diet for the larvae.

ENVIRONMENTAL FACTORS

Monitoring of the environmental factors is of paramount importance since the larvae and seeds are sensitive to the environmental changes and easily succumb when conditions are adverse.

Temperature

The ideal temperature for rearing of the larvae was found to be 27 - 29° C. The temperature of the water should be noted twice in a day, both in the morning and also during the afternoon.

Dissolved oxygen

Dissolved oxygen level varies with water temperature. The higher the temperature, the lower the dissolved oxygen level. The normal range for dissolved oxygen is 5-6 ml/l. Always aeration is given to the larval tanks throughout the day to see that the oxygen level does not go down much. For one tonne tank generally two aerators are provided one at either ends.

pH

Under normal conditions, the rearing sea water is generally alkaline with pH of 7.5-8.6. Tests have shown that the larvae of *H. scabra* adopt to a fairly wide range of pH. However when pH rises over 9.0 and drops below 6.0 the moving ability of the larvae weakens and growth stops. Therefore the pH of the water must be between 6.0 and 9.0.

Salinity

Salinity of normal sea water is 35‰. If the salinity is low all the larvae will die. The lethal critical salinity is 12.9‰. The optimum

salinity for larval development ranges from 26.2 to 32.7‰. In this range the higher the salinity, the quicker is the development. Too high or too low salinity adversely affects the normal development of the larvae, resulting in large number of deformed larvae causing death. Salinity estimation is, therefore an important routine work throughout. If the specific gravity of the water is found out the measured value can be converted into salinity value.

Ammonical nitrogen

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

NURSERY REARING

The doliolaria (13-15th day) will settle on hard surfaces when food is sufficient and also when proper substratum is available for them to settle. If these two conditions are not satisfied they continue to swim in the tanks for a long time. Therefore correct feed is given to the larvae and often "settlers" (settling bases) are provided for them to settle. It has been observed in case of *H. scabra*, the doliolaria larvae settle when once algal extract is given without keeping any "settlers".

Types of settling bases

Two types of settling bases are tried for the larvae. In the first type polythene sheets are taken and kept in a tank outside the hatchery where there is good sunlight. Into these tanks filtered seawater is circulated continuously for four or five days. Benthic diatoms and other algae settle on the plates. These plates are taken inside the hatchery and suspended in the tanks which have doliolaria about to settle down. The hard surface and the food induces them to settle on the plates. One disadvantage with "settlers" of this type is that the benthic algae which settles on the plates comes off completely after four or five days. In the other type of "settler" the polythene sheets are kept in a tank having sea water. To this some algal extract filtered through 50 micron sieve is added. Usually species of *Sargassum* are used to make the extract and this is put in the tanks with small quantities of sea water. The algal extract will stick to the plates. Fresh extract is daily put and the water is also daily changed. After four or five days the polythene sheet is covered with fine coat of algal extract and this serves as a good settling base for the larvae. If food is not provided on the "settlers" the larvae

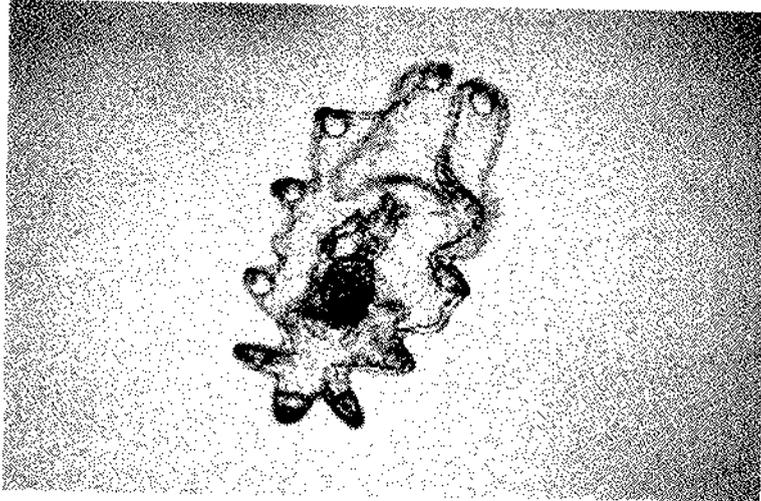
will die. The settling bases should not have any toxicity and should be easily available and also should be inexpensive.

Diet for the juveniles

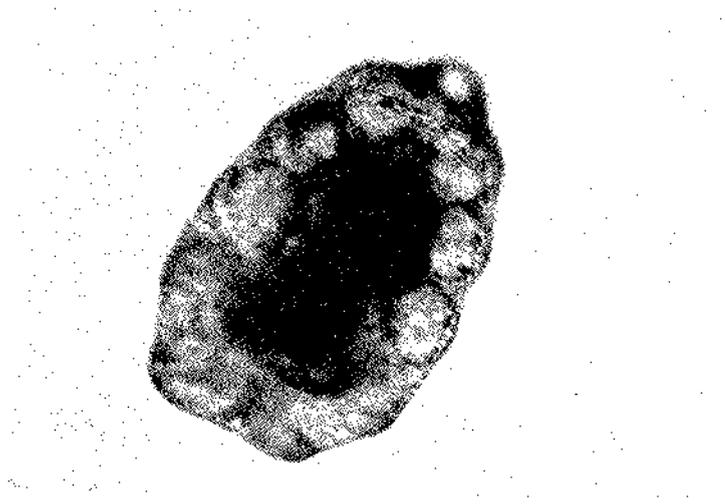
After settling down, the juveniles have only weak moving ability. First algal extract should be given by filtering through 40 microns seive and after one month 80 micron seive can be used. This filtered extract is daily given to the juveniles both in the morning and also in the evening. The seed is found to feed actively on the algal extract and grow well due to the high protein content. After one month larger ones (15-20 mm) are separated and put in a tank with very fine sand. To this daily algal extract is given. After two months fine algal powder is also added.

Density for the juveniles

When the larvae develop into juveniles, they begin to crawl. Most of them stay on the settling bases. After 15 days of settlement, they can be seen with naked eye. At this stage the number of juveniles should be estimated. A random sampling is made with a frame of 400 sq.cm. The estimates should be made from either end and also from the mid point. To get realistic estimates the sampling area should be over 5% of the tank area. In order to achieve increased survival rate, it is necessary to control the density of the juveniles on the settling bases at the optimum level. Too many juveniles in a limited area and insufficient diet will adversely affect the growth and survival. Therefore after they are counted, their density should be adjusted to an optimum of 200-500 individuals for one sq.m.



IX. Late auricularia.



X. Doliolaria.



XI. *Pentactula* (posterior tube-foot hidden from view).



XII. Just metamorphosed juveniles.

PREDATORS AND THEIR CONTROL

Predation

Copepods and ciliates are the main predators on the auricularia larvae since their movements are sluggish. They attack the larvae at the sides and injure the bodies. Finally the larvae die due to the injuries caused. They also harm the juveniles by reproducing fast in the rearing tanks and compete for food with the juveniles. Algal extracts given for seeds is found in the alimentary canals of copepods also. Infested juveniles assume ball shape and they die gradually. The auricularia larva is the most vulnerable for the attack of the predators because of their extended life for several days.

Predator control

Control trials on copepods with different chemicals at different concentrations have been experimented. Chemicals containing organo-phosphorus can be tried. Copepods can be killed with 2 ppm dipterx in two hours with no harmful effects on the seed. However it is necessary to be careful and give attention to the preparation of dipterx solution of appropriate concentration. The solution should be evenly sprinkled into the tanks and the water of the tank must be changed completely after two hours. This is very important otherwise it will also affect the juveniles.

10

CULTURE PROSPECTS

The countries like China and Japan which produce seeds of *Stichopus japonicus*, do not culture them to harvestable size. After the seeds reach a length of 20-30 mm, they are sea-ranched in suitable areas for further growth and for enriching the natural populations. They find it expensive to maintain the juveniles in the laboratory over long periods to reach harvestable size. Ofcourse the growth rate in India for *H. scabra* is found to be faster when compared to the growth rate in *S. japonicus* in China and Japan.

GROW-OUT SYSTEMS

After the juveniles are grown for a month or two in the nursery, they have to be transferred to the sea for further growth. By the end of two months most of the seeds will reach 40 mm in length. There is vast difference in the growth rate of the juveniles belonging to the same brood. Therefore some culling is necessary. Fast growing seeds are separated and transferred to grow-out systems. The juveniles (Pl. XIII) can be grown under three different conditions depending on the number of juveniles on hand.

In the first method, rectangular iron cages of the size 1.0 x 0.6 m are taken and they are closely woven with 2 mm nylon rope into 900 sq. mm net work. Fine velon screen is taken and stitched as a lining for the rectangular box type cages. The mesh for the velon screen should be very fine otherwise the sand placed in the box will escape. Along with fine sand algal powder is also kept as food for the seed. Four casurina poles are driven into the sea bottom at a depth of one metre and the boxes are securely tied to the poles and kept at the bottom. After introducing the juveniles into the box the lid is properly closed and stitched to prevent the juveniles from escaping into the sea. These boxes are removed every month to clean the cages (Pl. XIV) from fouling organisms and also to note the length and weight of individual sea-cucumbers. All the sides of the rectangular box cages are thoroughly scrubbed with brush to remove the fouling organisms. This operation not only helps to remove the fouling organisms, but also allows free flow of water into the box. Since the space is limited large number of juveniles cannot be grown by this method. Due to limited area the growth is also found to be somewhat slow.

In the second method an old one tonne tank is fixed at the bottom of the sea at 1.5 m depth. A rectangular frame of slightly larger the size of the tank, made of casurina poles is fixed at the sea bottom and the tank is fixed/slided into it. Before fixing the tank at the bottom of the sea it is first filled with fine sand to one fourth from natural habitat. The sand should be free from predators such as crabs and other unwanted organisms. Fresh algae from the sea is collected and dried. Then the dried algae is made into powder and powder is mixed with fine sand and transferred to the tank. The algal powder helps in better growth of the seeds. The tank is covered by velon screen and securely tied to prevent the entry of unwanted organisms particularly predators. The specimens are removed every month and examined for their growth. Growth of the juveniles (Pl. XV) reared in tank was found to be better and also faster due to more space and good circulation of water. The mortality is also found to be less.

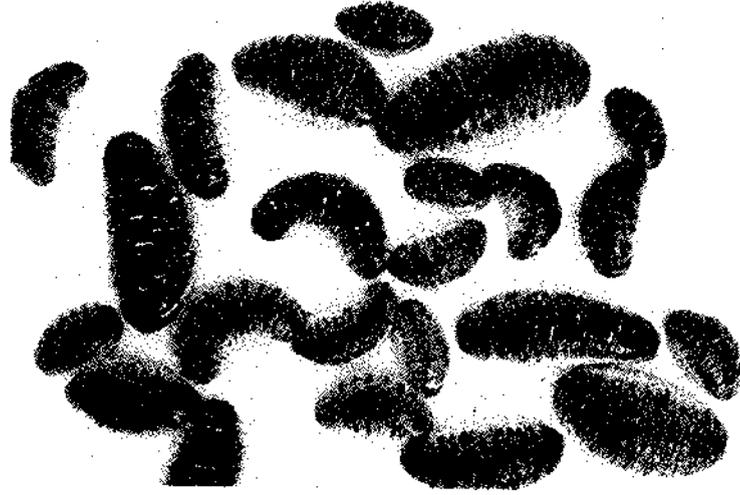
In the third method, pens (Pl. XVI) are erected in shallow water in sheltered bays. The pens can be made of bamboo screens or palmirah rafters. Also velon screen pens can be constructed and fixed in the sea. The mesh for the velon screen should be about 4 mm to allow free flow of water and it is desirable to have the bottom also covered by velon screen for better retrieval, since *H. scabra* is known to burrow and escape. Pens with the above material can be constructed to an area of 25 sq.m. The pens have to be periodically examined to see that they are not damaged by crabs and other boring organisms. *H. scabra* is known to reach harvestable size in 18 months in the natural environment. In the culture systems the growth is expected to be faster. Though the culture practices in holothurians are in infancy, the prospects seem to be excellent since at the end even if 1000 holothurians are harvested one can get Rs. 30,000/- to Rs. 50,000/-.



XIII. Seeds produced in the hatchery.



XIV. Cleaning of rectangular cages for sea-encumber culture.



XV. Juveniles grown in one tonne tank in the sea.



XVI. Nylon screen pen erected in sea.

CULTURE OF ALGAL FOOD

Phytoplankters are mostly unicellular algae and efficient in utilising the solar energy to convert the inorganic substances into organic compounds suitable for primary consumers in the aquatic environment. As the whole body is nutritious, they have a high protein content that is easily digestible. Because of these qualities the nature presents the phytoplankton as primary producers forming the base of the aquatic food chain. Such qualities have created a high demand for these micro-organisms in the hatchery operations as essential feed.

Feeding the holothurian larvae with algae

The success of the hatchery operations depends upon the continuous production and supply of high quality feed. In order to assure a speedy growth of the larvae the feed selected should have high protein content and must be able to be cultured in mass scale and supplied to the hatchery. The species produced as feed should be acceptable to the larvae of the cultured organisms. During the initial stages of hatchery development, the holothurian larvae were found to thrive well with a sufficient supply of phytoplankton. Quick development and high survival rate could be achieved with the supply of easily digestible flagellates belonging to the algal classes Chrysophyceae and Haptophyceae. Quality of feed differs with different development stages. Combination of selected species of algae was found to yield good results. The larvae of *H. scabra* were fed on *Isochrysis galbana*, *Dicrateria* sp. and *Dunaliella* sp. The growth and survival rate was found to be more when fed on *Isochrysis galbana*. In addition a mixed culture of diatoms dominated by *Chaetoceros* sp. also was found suitable in enhancing the growth rate of the holothurian larvae.

The details and methods of microalgal culture; isolation techniques of required species especially serial dilution culture technique, culture media such as 'Conway' or Walne's medium, Miquel's medium, TMRL (Tung Kang Marine Research Lab.) medium, etc.; outdoor culture of mixed algae, stock culture maintenance, mass culture of flagellates; different phases in mass culture of algae such as Lag or induction phase, exponential phase or growing phase, declining phase, stationery phase, death phase, etc.; determination of cell concentration, different factors effecting microalgal culture, harvesting of cultured algae and problems and constraints faced in microalgal culture, have been elaborately given and discussed in different Special Publications and Brochures particularly in the 'Transfer of Technology Series' of CMFRI and recently by Gopinathan (1993, Microalgal culture. *In : Live Feed. Handbook on Aquafarming*, MPEDA, pp. 1-15).

ECONOMICS

A tentative expenditure both capital and recurring, investments and economics of sea-cucumber culture is given below :

Hatchery seed production

Total number of tanks required	:	6 (1 tonne capacity)
Stocking rate of auricularia larvae	:	3.75 lakhs/1 tonne tank
Total auricularia stocked in one run	:	1 million
Expected percentage production of juveniles (10%)	:	1 lakh
Survival of juvenile at the end of 18 months in the sea	:	40%
Net production of harvestable sea-cucumbers	:	40,000

CAPITAL EXPENDITURE

	Rs.
A. <i>Building and tanks</i>	
Hatchery shed with light roofing (30 m x 10 m)	1,00,000
Room for Generator/Compressor (27 sq.m. @ Rs. 500/- per sq. m.)	13,500
Seawater sump 14 sq.m. @ Rs. 750/- per sq.m.	10,500
Sedimentation tank 8.4 sq.m. @ Rs. 750/- per sq.m.	6,300
Filter bed 4.5 sq.m. @ Rs. 750/- per sq.m.	3,375

	Rs.
Pump house 14.6 sq.m. @ Rs. 750/- per sq.m.	10,950
Overhead tank - 10,000 l capacity	50,000
Total	1,94,625
 <i>B. Fibreglass tanks</i>	
1 tonne capacity Broodstck/ larval/juvenile FRP tank - 6 no @ R. 5000/-	30,000
100 l capacity spawning FRP tank - 4 no. @ Rs. 500/-	2,000
200 l capacity mixed culture FRP tank - 1 no @ Rs. 1000/-	1,000
Total	33,000
 <i>C. Major equipments</i>	
10 KVA Generator - 1 No	50,000
Air Compressor - 1 No	10,000
7.5 HP Electric pump - 1 No	15,000
1.0 HP Electric pump - 2 Nos	7,000
Microscope, pH meter, Salinometer	15,000
Chemical balance	5,000
Furniture	25,000
ECE Controller, Silica cased immersion heater, jumo thermometer	4,000
Air conditioner - 2 Nos	40,000
Total	1,71,000
Total capital cost (A + B + C)	<u>3,98,625</u>

RECURRING EXPENDITURE

	Rs.
<i>A. Interest</i>	
On Rs 3,98,625 @ 15%	60,000
<i>B. Depreciation</i>	
On building and fibreglass tanks @ 5%	7,000
On equipment @ 10%	17,000
<i>C. Salaries</i>	
One Technician @ Rs. 2000/- per month for 18 months	36,000
Two helpers @ Rs. 500/- per month for 18 months	18,000
<i>D. Contingencies</i>	
Plastic ware, flexible PVC hoses, glassware, bolting silk, etc.	5,000
Energy cost (Electricity and Diesel)	15,000
Chemicals	2,000
Other contingencies	5,000
<i>E. Maintenance</i>	5,000
<i>F. Annual lease for land</i>	3,000
Total recurring expenditure (A to F)	<u>1,73,000</u>

REALISATION AND PROFITABILITY OF SEA-CUCUMBER PRODUCED

Total Sea-cucumber production	: 40,000 Nos.	
Cost of each sea-cucumber	: Rs. 20/-	
Total amount realised (40,000 x 20)	: Rs. 8,00,000	
Less : Non-recurring and Recurring Expenditure (Rs. 3,98,625 + Rs. 1,73,000)	: Rs. 5,71,625	
Net Profit	: <u>Rs. 2,28,375</u>	

ANNEXURE I

LIST OF EQUIPMENTS REQUIRED

<i>Equipment/facility</i>	<i>Quantity required</i>
Generator (10 KVA)	1
Air compressor 200 ~ 220 1420 RPM 50 HZ S1 Rating output 0.75 KW AMP 73	1
Fibreglass rectangular Broodstock/larval/ Juvenile rearing tanks (1000 l capacity)	6
Fibreglass rectangular (100 l capacity) spawning tank	4
Fibreglass tank rectangular (200 l capacity) Mixed culture tank	2
Binocular microscope	1
Mixie	1
ECE controller	1
Jumo thermometer (0-50°C) (For thermal stimulation)	1
Silica cased immersion heater	1
Air - conditioner 2 tonne capacity	2
pH meter	1
Salino-refractometer (Temperature compensated) (0-50°C)	1
Chemical balance	1
Thermometer (0-50°C)	3

<i>Equipment/facility</i>	<i>Quantity required</i>
<i>Laboratory glassware</i>	
Beaker 10,000 ml	6
Beaker 5,000 ml	6
Beaker 3,000 ml	4
Beaker 1,000 ml	6
Beaker 500 ml	6
Beaker 250 ml	6
Conical flasks 250 ml	5
Oxygen bottles 125 ml	10
Volumetric pipettes (assorted sizes)	10
Burettes (10 ml)	2
Burettes (50 ml)	2
Petridishes (150 mm dia)	2
Embryo cups (50 x 50 mm)	6
Micro slides with cavity	2
Micro slides (in box)	1
Cover slips (in box)	2
Plankton counting chamber (1 ml capacity)	2
<i>Plasticware</i>	
Plastic buckets (15 l)	6
Plastic buckets (5 l)	6
Plastic buckets (3 l)	6
Basins (20 l) capacity	6
Polyethelene flexible hoses (20 mm dia)	10 m

<i>Equipment/facility</i>	<i>Quantity required</i>
PVC pipes (150 mm dia for sieves)	6
Polythene sheets (for mixed algal culture)	10 m
Bolting silk cloth	
40 microns	1 m
80 microns	1 m
140 microns	1 m
180 microns	1 m
200 microns	1 m
Velon screen	
1 mm mesh	30 m
4 mm mesh	30 m
Tank cover cloth (Black)	30 m
Nylon rope (2 mm)	10 kg
Nylon rope (5 mm)	10 kg
Casuarina pole (3 m length)	50 Nos
Seawater drawing distribution grid made of 50 mm and 25 mm grid PVC pipelines and valves.	as required
Aeration grid made of 25 mm rigid PVC pipelines with copper nozzles, 5 mm polythene tubes, plastic 'T' joints and regulators and diffuser stones.	as required.

31. An appraisal of the marine fisheries of West Bengal. 1987, 32pp.
32. An appraisal of the marine fisheries of Orissa. 1987, 36pp.
33. An appraisal of the marine fisheries of Andhra Pradesh. 1987, 52pp.
34. An appraisal of the marine fisheries of Tamil Nadu and Pondicherry. 1987, 63pp.
35. An appraisal of the marine fisheries of Kerala. 1987, 42pp.
36. An appraisal of the marine fisheries of Karnataka & Goa. 1987, 104pp.
37. An appraisal of the marine fisheries of Maharashtra. 1987, 46pp.
38. An appraisal of the marine fisheries of Gujarat. 1987, 51pp.
39. An appraisal of the marine fisheries of Lakshadweep and Andaman & Nicobar Islands. 1987, 18pp.
40. National symposium on research and development in marine fisheries, Mandapam Camp 16 - 18 September 1987 (Abstracts). 1987, 112pp.
41. A manual for hormone isolation and assay. 1987, 46pp.
42. Manual of techniques for estimating bacterial growth rates, productivity and numbers in aquaculture ponds. 1987, 28pp.
43. Nutritional quality of live food organisms and their enrichment. 1987, 28pp.
44. An evaluation of fishermen economy in Maharashtra and Gujarat - A case study. 1988, 80pp.
45. Motorization of country craft in Kerala - an impact study. 1989, 74pp.
46. Atlas of clam resources of Karnataka. 1989, 56pp.
47. Annotated bibliography of commercially important prawns and prawn fisheries of India. 1989, 326pp.
48. The Indian Oil sardine *Sardinella longiceps* Valenciennes - An annotated bibliography. 1990, 80pp.
49. Hatchery production of Pearl oyster spat : *Pinctada fucata*. 1991, 36pp.
50. Annotated Bibliography of the Silverbellies (Pisces : Leiognathidae). 1992, 220pp.
51. Bibliography (Part 2) - The Publications by the staff of Central Marine Fisheries Research Institute 1986-1990. 1992, 112pp.
52. The Indian mackerel *Restrelliger kanagurta* (Cuvier) - An annotated bibliography. 1992, 126pp.
53. Mariculture research under the Postgraduate Programme in Mariculture - Part 2. 1993, 176 pp.
54. Mariculture research under the Postgraduate Programme in Mariculture - Part 3. 1993, 155pp.
55. Mariculture research under the Postgraduate Programme in Mariculture - Part 4. 1993, 134pp.
56. Mariculture research under the Postgraduate Programme in Mariculture - Part 5. 1993, 154pp.
57. Hatchery techniques and culture of the Sea-cucumber *Holothuria scabra*. 1994, 40 pp.
58. An annotated bibliography on Sea-cucumbers. 1994, 92 pp.
59. A Hand-book on India Sea-cucumbers. 1994, 48 pp.