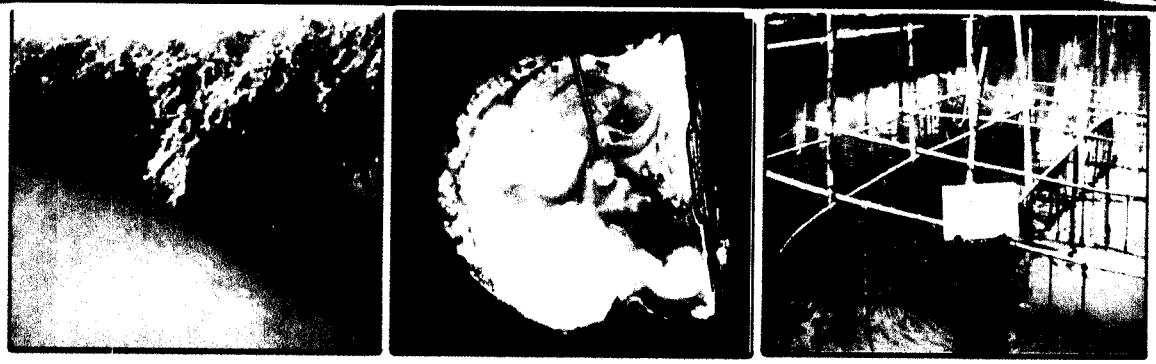
POSTGRADUATE PROGRAMME IN MARICULTURE

M.F.Sc. Mariculture

Practical Manual
Volume I



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INDIA

M.F.SC. MARICULTURE

PRACTICAL MANUAL

COURSE No. MC-501

CULTURE OF CRUSTACEA

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FACULTY MEMBERS : E.V. RADHAKRISHNAN
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JOSILEEN JOSE

Identification of culturable species of shrimps

Objective	:	To identify important culturable species of penaeid shrimps in India – *Penaeus indicus*, *P. monodon*, *P. merguiensis* and *P. semisulcatus*.
Principle	:	The key for identification provided gives important morphological characteristics for identifying the given specimens to species level.
Equipments/materials	:	Specimens of shrimp, dissection set, dissection board, tray and microscope.
Procedure	:	The steps given in the key for identification to be followed.
Observations	:	(Note down important characteristics of each species and draw figures)

Note: Important characteristics and diagram of each species to be given in the Record Book. The Record Book should be submitted in the following practical class.

Practical No. 2 Mary K. Manisseri

Identification of culturable species of shrimps (repeated)

Objective	:	To identify important culturable species of penaeid shrimps in India – *Metapenaeus dobsoni*, *M. monoceros*, *M. affinis* and *Parapenaeopsis stylifera*
Principle	:	The key for identification provided gives important morphological characteristics for identifying the given specimens to species level.
Equipment/materials	:	Specimens of shrimp, dissection set, dissection board, tray and microscope.
Procedure	:	The steps given in the key for identification to be followed.
Observations	:	(Note down important characteristics of each species and draw figures)

Note: Important characteristics and diagram of each species to be given in the Record Book. The Record Book should be submitted in the following practical class.

Practical No. 3

Mary K. Manisseri

Maturity stages of shrimp

Objective	:	To identify the different maturity stages of shrimp.
Principle	:	Maturity stages of female shrimp are determined by observing the stages of ovarian development. This helps in the selection of late-maturing or mature shrimp for brood-stock development.
Equipments/materials	:	Specimens of shrimp, dissection set, dissection board, tray and microscope.
Procedure	:	Observe the secondary sexual characteristics of male and female shrimp - classify the female specimens into immature, early maturing, late maturing, mature and spent-recovering stages.
Observations	:	(Note down important characteristics and draw different maturity stages).

Note: Draw maturity stages with notes on important characteristics in the Record Book.

Practical No. 4

Mary K. Manisseri

Female reproductive system of shrimp

Objective	:	To dissect and display the female reproductive system of (fully mature) shrimp.
Principle	:	Fully mature ovary is dark green in colour with well-developed anterior, middle and posterior lobes and clearly visible oviduct.
Equipments/materials	:	Specimens of shrimp, dissection set, dissection board, tray and microscope.
Procedure	:	Choose fully mature female specimens of shrimp – pin to the dissection board in a tray of water with dorsal side up - remove the exoskeleton on the dorsal side – trace anterior, middle and posterior lobes - trace oviduct from coxa of third peraeopod - display.
Observations	:	(Note down important characteristics and draw female reproductive system).

Note: Draw female reproductive system, label and submit Record Book in the following practical class.

Practical No. 5

Mary K. Manisseri

Examination of sperm and ova of shrimp.

Objective	:	To dissect out ovary and terminal ampoule of shrimp and examine ova and sperm.
Principle	:	Mature ovary contains ova belonging to the last stage of development before spawning. Fully developed permatophores can be dissected out from the terminal ampoule and examined under microscope.
Equipments/materials	:	Specimens of shrimp, dissection set, dissection board, tray and microscope.
Procedure	:	Repeat dissection and display of the female reproductive system – take small portions of ovary from different regions – examine eggs - dissect out terminal ampoule from coxa of fifth thoracic leg of male specimen – examine spermatophore under microscope.
Observations	:	(Note down important characteristics and make drawings).

Note: Record Book to be completed and submitted

Practical No. 6 E.V.Radhakrishnan

Identification of lobsters

Objective	:	To identify important culturable species of lobsters in India.
Principle	:	The key for identification provided gives important morphological characteristics for identifying the given specimens to species level.
Equipments/materials	:	Specimens of lobsters, dissection set, dissection board, tray and microscope.
Procedure	:	The steps given in the key for identification to be followed.
Observations	:	(Note down important characteristics of each species and draw figures)

Note: Important characteristics and diagram of each species to be given in the Record Book. The Record Book should be submitted in the following practical class.

Practical No. 7 A. Laxminarayana

Observation on Prawn filtration practices.

Objective: To study the prawn filtration practices.

Principle: The Shrimp/fish seed, entered during high tide are trapped in the pond and allowed to grow for 2 to 6 months. They are harvested during "thakkom" (8 days around full moon and new moon).

Equipments :- sluice gate, bamboo screen, close meshed conical net.

Procedure:- Allow the entry of shrimp/ fish seeds with tidal water into the paddy fields during high tide – place bamboo screen in the inner edge of sluice gate – harvest using close meshed conical net fitted to sluice gate during low tide.

Observations:- Observe catch. Note the total catch and species composition.

Inference:- Note the quality of catch.

Charts/ Tables/ Fig:- Prepare a chart of shrimp catch.

Practical No. 8 A. Laxminarayana.

Shrimp farming – operation and management.

Objective:- To operate the shrimp farm and manage successfully to achieve good production.

Principle:- To prepare the pond properly by eradicating the predators and fertilizing the pond

- stocking the pond with good quality seed after proper transportation and acclimation.
- to manage the water quality so as to ensure good production of shrimps.
- prevention and cure of shrimp disease to obtain good production.

Equipment/ reagents:- pH meter, lime, urea, superphosphate, cowdung, shrimp seed, refractometer, thermometer, sechhi disc, D.O. meter, feeds, feed check trays, cast net, electric net, microscope.

Procedure:- Dry the pond – sterilize – measure the pH – bring the soil pH to 7.5 by addition of lime – eradicate the predators using tea seed cake – fertilize pond with urea, superphosphate and cowdung/ chicken manure.

- select good quality shrimp seed – transport – acclimate – stock the pond – manage water quality
- feed with quality feed with good FCR – use feed check trays for feed management.
- check the stocked shrimps regularly to find out and manage diseases.

Observe:- Observe the preparation and fertilization of pond.

- selection of good quality seed, transportation and stocking.
- monitoring of various water quality parameters.
- feed and feed management.
- diseases of stocked shrimps.
- harvesting.

Inference:- Give your inferences on quality and quantity of shrimps.

Chart/ Tables etc:- Prepare a chart on the various water quality parameters.

- a table on feeding and quality & quantity of shrimps harvested.

Practical No. 9 A. Laxminarayana

Shrimp farming – assessing growth and production.

Objective: to assess the growth and production in shrimp farm.

Principle: to assess the growth and production in shrimp farm by regular sampling using cast net.

Equipments: cast net, scale, balance.

Procedure: Cast the net in 10 places representing the four corners and six middles. Count the number per haul and take the length and weight of the shrimp and calculate the standing crop directly in Kg/ ha, using the formula N/F x W x 10, where 'N' is the average number of shrimp per haul, 'F' is the factor for net used (5, 10, 20 respectively for 5, 7, & 9 cubit cast net) and 'W' is the average weight of the shrimp in grams. Measure the total length of the shrimps using a scale. Take the weight using a balance.

Observation: Assess the growth of stocked shrimps and estimate the total biomass in the pond.

Inference: Give your inferences on the growth and total production.

Charts/ Tables: Prepare a chart summarising the growth of shrimps. Also prepare a table indicating the total biomass during different periods of sampling.

Practical No.10 **Josileen Jose**

Collection, transportation and maintenance of wild shrimp spawners, mass production of *Chaetoceros*

Objectives:

i. To study different maturity stages in *Penaeus semisulcatus* by observing shrimp at the landing center.
ii. Selection of suitable spawners for hatchery seed production.
iii. Transportation the selected spawners to the hatchery in plastic jerry cans containing good quality sea water provided with aeration and maintenance of wild spawners in the hatchery tank under suitable environmental parameters.
iv. Mass production of mixed diatoms dominated with *Chaetoceros* spp.
v. To observe the spawning and behaviour of the spawner during mid night hours and examine the fertilized eggs under microscope and study its developmental stages.

Principle:

- A thorough knowledge of maturity stages is essential for the selection of ready spawners from wild for seed production. A fully mature shrimp with stage IV ovary ("diamond" shape in the 1st abdominal segment clearly visible through the exoskeleton against a light source) is dark olive green in colour with well defined anterior, middle and posterior lobes and oviduct.
- Spawning takes place invariably during night hours between 8pm – 2am. A fully mature shrimp will spawn the following night generally around 1030-1130pm. When the eggs are shed, the female simultaneously releases the sperms also from the thelycum. Fertilization takes place in the sea water and fertilized eggs sinks to the bottom.
- A good bloom of diatoms can be obtained within a period 4 days in good quality sea water provided with suitable chemicals, inoculam collected from sea and maintained with desired range of environmental parameters.
- Fertilized eggs are spherical with perivitelline space and cell division begins in the first hour itself. 2-cell stage can be seen with in an hour.

Equipments/ Materials:

Spawners, plastic jerry can (50 lit.), broodstock maintenance tank for ready spawners, FRP tanks for algal culture, refractometer, pH meter, spawning tank, cover for the spawning

tank, 100μ Filter, filter bags (1μ), scoop net, top pan balance, scale, microscope, chemicals- sodium orthophosphate, potassium nitrate, EDTA and sodium silicate.

Procedure:

- Classification of different maturity stages – immature, early maturing, late maturing, fully mature and spent/recovering stages -
- Selection and transportation of suitable spawners to the hatchery
- Stocking and rearing the spawners in hatchery tank under suitable environmental parameters.

Mass culture of diatom with, dominating

- Setting one ton fibre glass tank for diatom culture - inoculam collection from sea - filtration of 100 buckets of sea water through 50μ filter - addition of chemicals to the culture tank - vigorous mixing of the chemicals using plastic buckets - transfer the filtrate to the inoculam to the culture tank after removing the dirt and other unwanted materials trapped while filtering - proper aeration.

Setting spawning tank

- Clean the spawning tanks according to the number of spawners - fill the tank with known volume of sea water - addition of EDTA - note the details of the spawner like, total length, total weight, etc.- transfer of spawners to the spawning tanks.

Observations:

- Note down the important characteristics of the different stages of ovary
- Note the important water quality parameters of spawning tank-algal culture tank.
- Process of spawning* – behaviour of the animal – time of spawning- fertilized eggs under microscope to see the different stages of development.

Figures:

- Draw different stages of ovary – fertilized egg .

Practical No.11 **Josileen Jose**

Observation on egg development and larval stocking

Objectives:

i. To study the embryonic development.
ii. To estimate the total number of eggs spawned ie. fecundity.
iii. To estimate the total number of nauplii produced
iv. To estimate the rate of survival from egg to naupliar stage
v. To stock the nauplii in larval rearing tanks

Principle:

Rate of larval survival is calculated based on the egg count/nauplii count.

Equipments/materials:

Microscope, Glass beakers (100 ml-3 nos.), glass droppers, a small piece of black cloth.

Procedure:

Estimation of egg count

Take samples from the spawning tank - observe the development of nauplii under microscope - take three 100ml. samples from each spawning tank- (each student has to take the egg count) - count the total no. of eggs in each sample -take the average no. and estimate the total eggs spawned-removal of the unhatched eggs and other dirts from the spawning tank - collection of eggs using proper filters/egg collectors and wash the eggs in running water-clean the sides of the spawning tank-raise the water level and transfer back the washed eggs.

The total number of eggs is estimated using the following formula:

$$\text{Av.no.of eggs in sample} \times \frac{\text{Vol.of water in spawning tank (in litres)}}{0.1}$$

Nauplii count and Larval rearing

Nauplii count-total no. of nauplii – similar procedure followed for egg count - estimation-survival rate-egg to nauplii-setting larval rearing tanks based on the total no.of nauplii @ 80-100/litre - transfer of nauplii to larval rearing tanks - check the water quality parameters in each tank.

Water quality and other conditions for larval rearing:

Parameter	**Permissible range**
Salinity	29-34ppt
Temperature	26.0-32^{0} C
PH	8.0-8.4
Dissolved oxygen	3.0-8.0 ml/litre
Total ammonia	< 0.1 ppm
Nitrite	<0.05ppm
Light intensity During day time	20000-125000 lux

Observations :

Observe the development of phytoplankton in the previous day's culture.

Note the important water quality parameters of - algal culture tank- larval rearing tank.

Figures:

Draw different stages of embryonic development, nauplius stages I and II (N-1 & N-2).

Practical No.12 **Josileen Jose**

Observation on nauplus stage and estimation of phytoplankton cell concentration

Objectives:

i. To study the different stages of Nauplius (N-3 to N-5)
ii. To estimate the cell concentration of the phytoplankton culture.

Principle:

- The nauplius is pear shaped with 3 pairs of appendages and moults every 4-6 hrs. except the last sub stage. It passes through 6 nauplius sub stages before it metamorphoses into the protozoea. The does not have a mouth or alimentary canal and hence does not feed. It subsists on the yolk material present inside its body. Nauplius swims actively towards a weak source of light.
- The knowledge of cell concentration of algal culture is essential to determine the daily requirement of algal feed for feeding the shrimp larvae.

Equipments/materials:

Microscope, Glass beakers, glass droppers, phytoplankton counting tray (Rafter cell counter).

Procedure:

Nauplius observation

Observe the nauplius under microscope and record the developmental stage - check the water quality parameters.

Estimation of phytoplankton cell concentration

Clean the counting tray -take 1 ml. of phytoplankton sample -count the cells - estimate the total concentration in the mass culture tank.

Add *Chaetoceros* culture in each larval culture tank when the larvae reaches N-6 stage anticipating the next larval stage protozoea , which is a active filter feeder.

Collection and cleaning of materials for setting Rematuration System for shrimps

Collection of raw materials like sand, oyster shells, blue metal, coral stones for the filter bed - cleaning the materials with good quality water and drying - preparation of the net material for the filter bed - cutting required sizes of aeration tubes, air stones etc. for the air-lifting system for the recirculation.

Algal culture

Start new Mixed algal culture dominated with *Chaetoceros* spp. in one ton FRP tanks (2 no). Always check phytoplankton under microscope (for contamination, ciliates, etc) before feeding the larvae or using as inoculam. On sunny days the amount of inoculam should be reduced to avoid rapid decline of the culture.

Observations :

Observe the development of phytoplankton in the previous day's culture.

Note the important water quality parameters of - algal culture and larval rearing tanks.

Figures:

Draw nauplius stage VI, different phytoplankton species of the mixed algal culture.

Practical No.13 **Josileen Jose**

Observation on larval stages and setting a new rematuration system for spawners

Objectives:

i. To study the Protozoea –1 stage.
ii. To set a rematuration system (recirculating system with in-situ biological filter) for spawners.

Principle:

- Nauplius metamorphoses into protozoea stage after 36-48 hrs. It has 3 sub stages, each stage lasts one day. First protozoea stage (PZ-I) has a broad 'head' and a narrow 'tail' comprising segmented thorax and unsegmented abdomen with forked end. Eyes are sessile. The protozoea stage has an alimentary canal, mouth and feeding appendages and starts feeding algae present in the sea water. It has an efficient filtering mechanism to sieve the algal cells from the sea water. The protozoea swims actively in the water and is strongly attracted towards light.

- Ablated females are maintained in rematuration system provided with similar environmental condition of the sea. The range of water quality and other parameters to be maintained in the rematuration system are given below:

Parameter	**Permissible range**
Salinity	29-34ppt
Temperature	27.0-30^0 C
PH	8.0-8.2
Dissolved oxygen	4.0-6.0 ml/litre
Total ammonia	< 0.05 ppm
Nitrite	<0.005ppm
Light intensity During day time	< 500 lux

Under these conditions about 80% of the ablated females mature and spawn within a week's time after eyestalk ablation.

pH and ammonia are very crucial factors affecting the maturation process. The recirculation system with insitu biological filter keeps the system almost ammonia free and maintains a steady

pH. The first step in biological filtration is mineralisation of organic nitrogenous compounds, nitrification and denitrification by bacteria suspended in the water and attached to the different layers of biological filter. In the nitrification ammonia is oxidized to nitrite by *Nitrosomonas* spp. and nitrite to nitrate by *Nitrobacter* spp. During denitrification nitrate or nitrite reduced to either nitrous oxide or free nitrogen with help of both heterotrophic and autotrophic bacteria.

Equipments/materials:

Microscope, Glass beakers, glass droppers, cavity slides, raw materials like cleaned sand, oyster shells, blue metal, coral stones for the filter bed, net material for the filter bed, aeration tubes, air stones, PVC pipes and elbows for air- lifting system for water recirculation.

Procedure:

Management of larval rearing tanks

Observe the protozoea-1 stage under microscope and check whether the larvae is fed well - give 30% water exchange –addition of algal feed to the larval culture tanks-

Setting a recirculating Rematuration System for spawners

Setting the RMT in one ton/ five ton tank - cleaning the tank with good fresh water and final rinsing with sea water - lay down the PVC pipes and fix the arising arms of PVC - spread the coral stones & oyster shells and fill the gaps with ½" & ¼" blue metal - spread the charcoal uniformly -above that spread the filter cloth - spread a 20cm layer of cleaned sand over it - fill the tank with filtered sea water (80%) - give aeration and check the aeration points and adjust the water circulation in such a way that 8-10 cycles per day - check the pH and maintain the pH between 8-8.2. if necessary adjust the pH by adding sodium bicarbonate -transfer the spawned animals for rematuration to the RMT if the water clarity is good or the following day.

Observations :

Study the protozoea-1 stage under microscope with its morphological changes from the previous stage ie. N-VI.

Observe the development of phytoplankton in the previous day's culture.

Note the important water quality parameters of - algal culture, larval rearing tanks and RMT.

Figures:

Draw Protozoea-I stage and Rematuration tank (RMT) and cross section of RMT.

Practical No. 14 **Josileen Jose**

Larval observation - Protozoea II, chlorella/Nannochloropsis mass culture, eyestalk ablation

Objectives:

i. To study the Protozoea –II stage.
ii. Mass production of Chlorella/ Nannochloropsis.
iii. Eyestalk ablation for induce maturation in female shrimps.

Principle:

- Protozoea - I lasts for a day and metamorphose to protozoea - II stage; in this stage eyes become stalked, rostrum develops supra-orbital spine appears and abdomen become segmented.

- Chlorella/ Nannochloropsis mass production – essential for the rotifer culture – a live feed used for shrimp / crab larval development. It can be produced by either by GNOC (Groundnut oil cake) method or Chemical method.

- During certain months, it may be difficult to get wild spawners from the sea. Then, female shrimps with ovaries in the early stages of development can be induced to develop their ovaries and spawn under controlled conditions. The unilateral eyestalk ablation technique is used for inducing the shrimps to mature in captivity. In the normal shrimp, the development of the ovary is controlled by a hormone secreted by the endocrine gland situated in the eyestalk. The optic ganglia and the related neurosecretory centers which produce an ovary inhibiting hormone are removed by eyestalk ablation process. This initiates the development of the ovaries and within 5-10 days spawning takes place, which may vary depending on the stage of the ovary, feed used and conditions of the maturation pool. A small portable electrocautery apparatus is used for eye-stalk ablation. Morality due to cauterization is rare.

Equipments/materials:

Microscope, Glass beakers, glass droppers, cavity slides, Electro-cautery apparatus, Shrimps for eye-stalk ablation.

Procedure:

Management of larval rearing tanks

Observe the protozoea-II stage under microscope and check whether the larvae is fed well - give 50% water exchange – addition of algal feed to the larval culture tanks.

Chlorella Mass culture

Clean a 1t fibre glass tank for chlorella culture - fill the tank with filtered sea water. give aeration- add chemicals to the sea water if 'chemical method' is following or extract of "ground nut oil cake + chemicals for "GNOC" method- add innoculam------observe the developments in the following days.

Eyestalk ablation

Select the healthy spawner and hold the animal gently but firmly without jumping out of the hand. One of the eyes is cut by passing the red hot loop of the cautery through the middle of the eyestalk. Cauterisation seals the cut end and prevents bleeding. Release the animal gently back to the maturation pool.

Observations :

Study the protozoea-II stage under microscope with its morphological changes from the previous stage ie. PZ- I

Observe the development of phytoplankton in the previous day's culture.

Note the important water quality parameters of - algal culture, larval rearing tanks and RMT.

Note the changes of eyestalks animals daily. This can be easily done during night hours without disturbing the animals with the help of a underwater torch.

Figures:

Draw Protozoea-II stage .

Practical No.15 **Josileen Jose**

Larval observation - Protozoea III

Objectives:

To study the Protozoea –III stage.

Principle:

- Protozoea – II lasts for a day and metamorphose to protozoea - III stage; uropods develop, telson demarcated from the last abdominal segment, first abdominal segments with dorsal spine.

Equipments/materials:

Microscope, Glass beakers, glass droppers, cavity slides, under water torch.

Procedure:

Management of larval rearing tanks

Observe the protozoea-III stage under microscope and check whether the larvae is fed well - give 50% water exchange – addition of algal feed to the larval culture tanks.

Observations :

Study the protozoea-III stage under microscope and compare the morphological changes from the previous stage ie. PZ- II

Observe the development of phytoplankton in the previous day's culture.

Note the important water quality parameters of - algal culture, larval rearing tanks and RMT.

Note the changes of eyestalk ablated animals daily. This can be easily done during night hours without disturbing the animals with the help of a underwater torch.

Figures:

Draw Protozoea - III stage .

Practical No.16

Josileen Jose

Larval observation – Mysis I, Prawn-egg custard preparation and Chlorella-rotifer culture

Objectives:

i. To study the Mysis –1 stage.
ii. Mass production of rotifers feeding with Chorella/Nannochloropsis
iii. Prawn - egg custard preparation for the post larvae.

Principle:

- The protozoeal stage is followed by the mysis phase in which the carapace covers the thorax, 3rd maxillipeds and the 5 pereopods are functional with well developed exopods, the first 3 pereiopods have rudimentary chelae, pleopods if present rudimentary without setae. Mysis looks like a miniature shrimp in its appearance. Unlike the protozoeal sub stages, which are distinguished by clear-cut morphological changes, the mysis sub-stages are separated only by small increase in the size of the larvae and the length of the pleopods. In the mysis stage also the larvae retain the filtering mechanism for feeding on algal cells. The mysis do not swim actively like the protozoea and are also less responsive to light. They hover in the water column with head pointing obliquely downwards. The mysis lasts for 3-4 days before metamorphosing to the 1st postlarval stage.

- Rotifers raised from Chorella / Nannochloropsis are nutritionally high than rotifers developed from other sources and give good results in larval development.

- Proved to be a good feed for the post larvae.

Equipments/materials:

Microscope, Glass beakers, glass droppers, cavity slides, under water torch, rotifers for inoculation, hen's eggs-2-3 no., small prawns –1 Kg.

Procedure:

Management of larval rearing tanks

Observe the Mysis –I stage under microscope and check whether the larvae is fed well - give 50% water exchange –addition of algal feed to the larval culture tanks.

Prawn egg custard preparation

Peel and devein 1 Kg small prawns -take the total weight of cleaned small prawns - add chicken egg to the prawn meat (one egg for 200g of meat) - finely blend it in a mixer grinder-transfer the contents in to an aluminium container - cook it in a pressure cooker for 20 minutes without 'weight'- cut the prawn-egg custard into small pieces of required sizes for daily use and allow it to cool - keep it in a freezer.

Chlorella-rotifer culture

Estimate the total conc. of the *chlorella*/ Nannochloropsis culture - innoculate the chlorella/ Nannochloropsis culture with rotifer -daily record the rotifer development -take the count and estimate the density.

Observations :

Study the Mysis-I stage under microscope and compare the morphological changes from the previous stage ie. PZ- III

Observe the development of phytoplankton in the previous day's culture.

Note the important water quality parameters of - algal culture, larval rearing tanks and RMT.

Note the changes of eyestalk ablated animals daily. This can be easily done during night hours without disturbing the animals with the help of a underwater torch.

Figures:

Draw Mysis - I stage and rotifer .

Practical No.17 **Josileen Jose**

Larval observation - Mysis II

Objectives:

To study the Mysis-II stage.

Principle:

The second mysis stage has pleopod buds.

Equipments/materials:

Microscope, Glass beakers, glass droppers, cavity slides, under water torch.

Procedure:

Management of larval rearing tanks

Observe the Mysis-II stage under microscope and check whether the larvae is fed well - give 50% water exchange –addition of algal feed to the larval culture tanks.

Observations :

Study the Mysis-II stage under microscope and compare the morphological changes from the previous stage ie. Mysis-I.

Observe the development of phytoplankton in the previous day's culture.

Note the important water quality parameters of - algal culture, larval rearing tanks and RMT.

Note the changes of eyestalk ablated animals daily. This can be easily done during night hours without disturbing the animals with the help of a underwater torch.

Figures:

Draw Mysis-II stage .

Practical No.18 **Josileen Jose**

Larval observation - Mysis III

Objectives:

To study the Mysis-III stage.

Principle:

The third mysis stage has two segmented pleopod buds.

Equipments/materials:

Microscope, Glass beakers, glass droppers, cavity slides, under water torch.

Procedure:

Management of larval rearing tanks

Observe the Mysis-III stage under microscope and check whether the larvae is fed well - give 50% water exchange –addition of algal feed to the larval culture tanks.

Observations :

Study the Mysis-III stage under microscope and compare the morphological changes from the previous stage ie. Mysis-II.

Observe the development of phytoplankton in the previous day's culture.

Note the important water quality parameters of - algal culture, larval rearing tanks and RMT.

Note the changes of eyestalk ablated animals daily. This can be easily done during night hours without disturbing the animals with the help of a underwater torch.

Figures:

Draw Mysis - III stage .

Practical No.19 **Josileen Jose**

Larval observation – Postlarva-I and searanching of the post larvae

Objectives:

i. To study the Postlarval stage.
ii. To estimate the total number of PL produced and its survival rate.

Principle:

The first post larvae (PL-1) superficially resembles the 3rd mysis except for the development of setae on the pleopods. But there is a drastic change change in the feeding appendages. The filter feeding mechanism is lost and the mandibles lose serrations and acquire sharp cutting edge. The first three walking legs lose the swimming setae and their claws become functional, capable of picking larger particulate matter. The hatchery phase (from nauplius to PL-5) takes 13-14 days. The nursery phase lasta for about 15-20 days after the PL-5 stage, till the post larvae reach the stockable size of 20-25mm.

Equipments/materials:

Microscope, Glass beakers, glass droppers, cavity slides, under water torch.

Procedure:

Management of larval rearing tanks

Observe the post larva-1 under microscope and record the details of the stage - check the water quality parameters - give 50% water exchange - provide sufficient phytoplankton - addition of prawn-egg custard to the culture tank

Observations :

Study the postlarva-I stage under microscope and compare the morphological changes from the previous stage ie. Mysis-III.

Observe the development of phytoplankton in the previous day's culture.

Note the important water quality parameters of - algal culture, larval rearing tanks and RMT.

Note the changes of eyestalk ablated animals daily. This can be easily done during night hours without disturbing the animals with the help of a underwater torch. When the reaches stage IV separate it for spawning.

Figures:

1. Draw postlarva-I stage .
2. Prepare a chart of entire hatchery operations from collection of spawners to searaching of postlarvae (with date)
3. Write a detail report of training on hatchery seed production of shrimp.

Practical No.20 **Josileen Jose**

Embryonic development and Larval stages of portunid crab

Objectives:

To study the different larval stages of the portunid crabs- *Scylla* spp and *Portunus pelagicus*.

Principle:

In portunid crabs after spawning eggs are carried by the females in their abdominal chamber, attached to their pleopodal endopodites. This forms a compact berry and incubation period is around 10 days in tropical waters. Newly spawned eggs are bright yellow in colour and as the incubation progress the colour changes to dull yellow to deep grey. In advanced stages of incubation twitching of the developing zoea can be seen clearly through the egg membrane. Egg hatches out as zoea and passes through different zoeal stages and metamorphose to megalopa and then to crab stage. Thorough knowledge of embryonic development and larval stages is essential for the production of crab seeds.

Equipments/materials:

Microscope, Glass beakers, glass droppers, cavity slides, larval stages of the crab.

Procedure:

Zoeae are with a long rostral and dorsal spines and a short lateral spine on the carapace. In first zoea, eyes are sessile, abdomen is 5 segmented and a forked tail. Inner margin of each fork bears three long serrated setae. In the second zoea eyes become stalked and inner median margin of caudal furca develops a pair of short plumose setae. In the third zoea abdomen become six segmented. In the fourth zoea abdomen develops paired pleopod buds at the ventral posterior end of the second to fifth segments. In the fifth zoea, pleopod buds are biramous, elongated and well developed. Zoeal stages are attracted towards light and they often aggregate into groups along the sides of the rearing tanks. Megalopa is quadrangular in shape and with 6 segmented abdomen with dorso ventrally flattened tail. It usually clings to a substratum and with a peculiar 'whirling' movement. Megalopa stage often restricted to the sides and bottom of the rearing tank.

Megalopa transforms to crab stage which resembles an adult crab. The margins are serrated with 9 anterolateral spines and periopods are well developed.

Observations :

Note the important identification characters of different zoeal stages, megalopa and crab stage. Study thoroughly the different stages and compare between them.

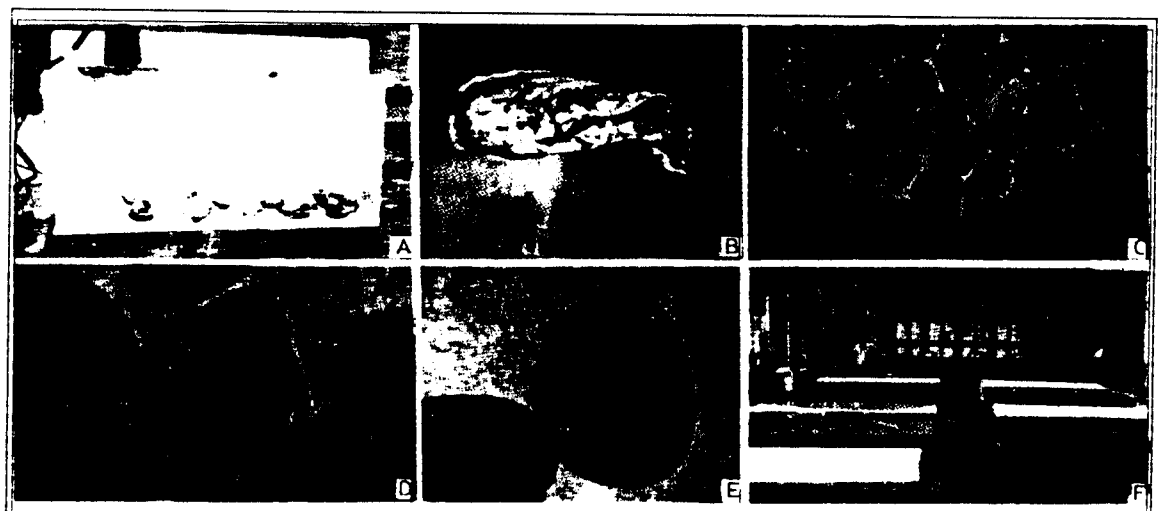
Figures:

Draw the diagrams of eggs, all zoeal stages, megalopa and crab stage.

Culture of Mollusca

(MC-502)

Post Graduate Programme in Mariculture
Central Marine Fisheries Research Institute
Cochin- 682014



Practical Outline

Contents

Title: Pearl Oyster Surgery

Practical 1

Objective: Identification of surgical Instruments and its uses in pearl surgery

Principle: Surgical instruments used in pearl surgery are specific for different steps involved in nucleus implantation

Equipment: Pearl surgery equipment

Procedure: Identify the surgical instruments. Draw the item and mention its use

Observations: Identify the tools and observe the design of each tool

Inferences: Each tool in pearl oyster surgery is designed for a specific purpose.

Faculty : Shri.I.Jagadis

Title: Pearl Oyster Surgery

Practical No. 2

Objective: Technique of nucleus implantation *in Pinctada fucata*

Principle : The manle of donor pearl oysters are used as graft in pearl surgery.

Equipment : Surgical equipment, pearl oysters, eosin, troughs

Procedure:

1. Select healthy oysters, place the oysters with their hinge down and narcotizate with menthol.
2. Select healthy oysters for graft preparation
3. Cut the mantle into small uniform size pieces.
4. Treat the grafts with eosin

Observations: Observe the different parts of mantle epithelium.

Inference :

Faculty : Shri.I.Jagadis

Title: Pearl Oyster Surgery

Practical No. 3

Objective: Nucleus implantation *in Pinctada fucata*

Principle: Pearl can be produced by insertion of graft tissue and nucleus near the gonad region of healthy pearl oysters

Equipment: Pearl surgery equipment, pearl oysters, graft tissue, nucleus, plastic troughs, menthol

Procedure:

1. Sterilise the nuclei
2. Peg the recipient oysters
3. Clamp the oysters
4. Make the incision for graft insertion, insert the graft
5. Insert the nucleus
6. Place the oysters for convalescence

Observations: Observe the site of incision and the method of placing the graft and nucleus in the gonad of recipient oysters

Inference: Pearls can be produced by implantation of graft and nuclei in pearl oysters.

Figure: Draw the location of the nucleus implantation in pearl oysters

Faculty : Shri.I.Jagadis

Title: Pearl Oyster Farming

Practical No. 4

Objective: Fabrication of pearl oyster holding cages

Principle: Closed containers can be used for rearing pearl oysters

Equipment: Iron / aluminium frames, nylon net, old fish net, nylon thread, scissors, knives

Procedure: Cover the iron frames/base with nylon net. Prepare square- cages lantern cages, book type cages and traditional box type cages. Also stitch/ attach the velon pouch on the base for collecting the rejected nucleus

Observations: Draw the cages and the write down the procedure for tying different knots

Inferences : Different types of cages can be used fro studying the individual growth rate or for rearing oysters. Pouches can be used to collect rejected nucleus /pearls

Faculty : Shri.I.Jagadis

Title: Pearl Oyster Farming

Practical No.5

Objective: Fabrication of rafts for bivalve farming

Principle : Wooden rafts can be used for bivalve culture. The rafts can be suspended by floats and anchored to the seabed by different anchoring devices.

Equipment: Wooden poles, barrels, anchors, nylon/coir ropes, knives. scissors.

Procedure:

1. Select the bamboo poles/ casuarinas poles
2. Construct a 5X5 m raft by tying the poles
3. Place the barrels and tie them in the four corners and center for floatation.

Observation: Observe the quality of the wood/ bamboo used, the different methods of fixing the floats and anchors. Note the length of anchor chain/rope.

Inference: Draw the raft and flotation/ anchoring devices

Faculty : Shri.I.Jagadis

Title: Pearl Oyster Farming

Practical No. 6

Objective: Pearl oyster farm management

Principle: Sedentary bivalves are attacked by foulers and borers which have to be cleaned periodically to avoid mortality of farmed organisms.

Equipment: Knives, 5% formalin, plastic bottles

Procedure: Visit the pearl farm; observe the fouling on cages and pearl oysters. Identify the foulers and other organisms associated with pearl farms. Clean the cages observe the pearl oyster and restock the cages.

Observations: Draw the foulers and the common borers and write a note on its significance

Inferences:

Faculty : Shri.I.Jagadis

Title: Pearl Culture

Practical No. 7

Objective: Grading of pearls

Principle : The quality of pearls produced by pearl oysters is dependent on the the uniformity of nacre coating, no.of projections formed and luster of the coating.

Equipments : Different types of cultured pearls, hand lens

Procedure: Sort and grade the pearls into A, B and C based on the nacre coating and luster

Observations : Write down the main points upon which pearls are graded.

Inferences: The quality of pearls can be graded based on the nacre coating and number of projections.

Faculty : Shri.I.Jagadis

Title: Half pearl in Abalone

Practical No. 8

Objective: Half pearl production in Abalone

Principle: Pearls can be produced by insertion of nucleus in the abalone, *Haliotis varia*

Equipment: Abalones, surgical tools, troughs, shell beads

Procedure: Demonstration of nucleus implantation in abalone

Observation: Observe the procedure followed for pearl production in abalones

Inference: Pearls can be produced from abalones.

Faculty : Shri. Boby Ignatius

Title: Bivalve Hatchery

Practical 9

Objective: Induced spawning of bivalves

Principle: Bivalves can be induced to spawn

Equipment: Jumo thermometer, silica immersion heater, NaOH, 0.1N solution of Ammonium hydroxide, TRIS buffer, dissection set

Procedure:

1. Select of ripe oysters
2. Thermal Stimulation – set up the jumothermometer, silica immersion heater, aerator, Perspex tank.
3. Chemical stimulation – prepare different pH using NaOH, TRIS buffer – preparation of different concentration of Hydrogen peroxide – injection of 0.1N solution of Ammonium hydroxide.
4. Biological stimulation – add sperm suspension

Observation: Write down the different methods and the response to each treatment

Inference: Bivalves can be induced to spawn by thermal, chemical and biological

Faculty : Dr.S.Dharmaraj and Dr.P.Muthiah

Title: Bivalve Hatchery

Practical 10

Objective: Embryonic development of bivalve larvae

Principle: The fertilized egg of bivalve passes through different developmental stages and shows phototropism

Equipment: Microscope, slides, fertilized bivalve eggs, light source

Procedure: Separate the healthy fertilized eggs from the spawning container and observe them under the microscope

Observations: Study the embryonic larval development of bivalves by Observing the cell division – formation of morula, blastula, gastrula, trochophore and veliger larvae – method of reorientation of cells – formation of dermal layers, archenterons etc. time sequence of development – larval behaviour – phototropism.

Inference: Draw the different stages of development, mark the main parts and measure the different stages

Faculty : Dr.S.Dharmaraj and Dr.P.Muthiah

Title: Bivalve Hatchery

Practical 11

Objective: Larval rearing and the development of bivalve larvae from fertilization

Principle: The developing larvae of bivalves have to be checked and must be provided with required feed.

Procedure: The larval development and rearing methods to be familiarized by the following steps

1. Estimation of larvae, stocking and feeding schedules
2. Standardisation of techniques on larval density, larval feeding.
3. Observation of different stages of larval development.
4. Estimation of spat.

Observations: Draw the different stages of development, mark the main parts and measure the different stages

Inferences: The quantity of feed required for each larval stage is different. The different stages of bivalve larvae have anatomical adaptions to suite its mode of life

Faculty : Dr.S.Dharmaraj and Dr.P.Muthiah

Title: Bivalve Hatchery

Practical 12

Objective: Phytoplanters are used as feed in bivalve hatchery and calculate the feed to be given at each stage

Principle :

Equipment :

Procedure: Identification of *Isocrysis galbana*, *Chaetoceros* sp, *Nannochloropsis* sp .Calculate the feed for each tank

Remarks: Draw the different phytoplankton and write a note on its significance in bivalve hatchery

Faculty : Dr.S.Dharmaraj and Dr.P.Muthiah

Title: Bivalve Hatchery

Practical 13

Objective: Spat collection on cultch and production of culthless spat

Principle: Polythene sheets can be used for production of cultchless spat

Equipment: Oyster shells, setting tanks with larvae, polythene sheet

Procedure:

Clean oyster shells
Place them in the setting tanks,
Placing polythene sheet for cultchless spat

Observations: Observe the difference in pearl oyster and edible oyster spat setting tanks and note it down

Inference: Spat can be collected by placing suitable cultch material and production of cutchless spat is possible by placing polythene sheets

Faculty : Dr.S.Dharmaraj and Dr.P.Muthiah

Title: Oyster culture

Practical 14

Objective: Estimation of condition index in edible oyster

Principle: Condition index of oysters is related to the reproductive stage of the oyster

Equipment : Measuring jar with a side tube, oysters, microscope

Procedure: Calculate the condition index (CI) of oysters by the formula

$$CI = \frac{\text{dry soft tissue wt (g)} \times 1000}{\text{internal shell cavity volume (ml)}}$$

Note the reproductive stage of the oyster
Find the difference in CI between different stages

Observations: Observe the variation in condition index of oysters relative to its maturity stage.

Inference: The condition index of oysters is high when the gonad is fully mature

Dr.P.Muthiah

Title: Oyster culture

Practical 15

Objective: Preparation of oyster rens

Principle : Spat of oysters can be collected by placing cultch in the natural beds or farm area

Equipment : Modified hammer, empty shells, brushes, nylon ropes

Procedure: Select, clean and treat empty oyster shells . Drill a hole and string the shells

Observation: Count the number of spat on an cultch which has been placed Earlier

Inference:

Faculty : Dr.P.Muthiah or Dr.V.Kripa

Title: Oyster culture

Practical 16

Objective: Depuration of bivalves

Principle: Depuration of bivalves improves the quality of the meat

Equipment: Not required

Procedure: Visit the oyster farm and observe the different water treatment and oyster holding facilities for depuration

Observation: Write down the procedure and give a diagrammatic sketch of the depuration protocol.

Inference:

Faculty : Dr.P.Muthiah or Dr.V.Kripa

Title: Oyster culture

Practical 17

Objective: Oyster harvest and heat shucking

Principle: Oysters are harvested when the oyster meat fills the cavity and by steaming, shucking of meat is easier

Equipment: Not required

Procedure: Visit the oyster farm, harvest the rens, jet wash and heat shuck the rens.

Observation: Note the weight of rens, find out the production per ren and also the difference in meat weight after heat shucking

Inference:

Faculty : Dr.P.Muthiah or Dr.V.Kripa

Title: Mussel culture

Practical 18

Objective: Mussel harvest and heat shucking

Principle: Mussels are harvested when the meat fills the cavity and by steaming, shucking of meat is easier

Equipment: Not required

Procedure: Visit the farm, harvest the mussel ropes, jet wash and heat shuck

Observation: Note the weight of mussel ropes, find out the production per rope and also the difference in meat weight after heat shucking

Inference:

Faculty : Dr.K.S.Mohamed or Dr.V.Kripa

Title: Mussel culture

Practical 19

Objective: Estimation of growth and production

Principle: Production /Yield from a farm can be estimated based on sub sample analysis

Equipment: Vernier calipers, field balance

Procedure: Visit the mussel farm, harvest the mussel ropes, and take the length and weight measurements and calculate the production per farm

Observation: Note the different size groups of mussels, the average weight per mussel rope and condition of mussel meat

Inference:

Faculty : Dr.K.S.Mohamed or Dr.V.Kripa

Title: Mussel culture

Practical 20

Objective: Mussel seeding

Principle: Mussel seed can form new byssus threads and attach to ropes.

Equipment : Mussel seed, nylon rope, biodegradable cloth, needle, troughs

Procedure: Visit the coastal intertidal regions during low tide, collect the mussel seed, clean it and seed it on the nylon rope by the traditional method and by using the new device .

Observation: Write down the approximate seed used per rope. Estimate the seed required for seeding 100 ropes of 1.5 m length.

Inference:

Faculty : Dr.K.S.Mohamed or Dr.V.Kripa

Title: Gastropod culture

Practical 21

Objective: Hatchery rearing techniques of commercially important whelks

Principle : The whelk larvae can be reared in hatcheries

Equipment: Microscope, slides, droppers

Procedure:

Observation: Observe the brood stock and juvenile rearing systems and the feeding protocol . Draw the different larval stages of whelks and label the parts.

Inference: The larvae of whelks have different feed preferences. With anatomical changes larvae metamorphoses to juveniles.

Faculty : Dr.V.Kripa

M.F.SC. MARICULTURE

PRACTICAL MANUAL

COURSE No. 503

CULTURE OF FINFISH AND SEA CUCUMBER

Course Teacher : Dr. L. Krishnan
Faculty Members : Dr. G. Gopakumar
Smt. Grace Mathew
Shri. A. Raju
Dr. K. K. Joshi
Smt. P.S. Asha

Practical No.1 Teacher K.K.Joshi

Title: Identification Cultivable Marine FinFishes

Objective: To learn the methodology of identification fish samples

Principle: Identification of important cultivable marine finfishes using the morphometric and meristic characters and taxonomic monographs.

Requirements: Groupers sample
Scale
Needles
Lens
Microscope

Procedure: Take the linear measurements and meristic counts of the given fish. Observe the characters and compare with the monograph. Identify the fish up to species level by studying the distinguishing characters. Write down important characters of family, genera and species.

Observation: Draw neat - labelled sketches

Teacher: Grace Mathew, P.S

Practical No.1

Title: Determination of maturity stages in finfishes(Groupers)

Time: 2 hours

Objectives : To determine of maturity stages in finfishes(Groupers)

Principle : A thorough knowledge of maturity stages of broodstock fishes is essential in the success of hatchery operations.

Equipments/Reagents : Microscopes, glass slides , glass beakers, cannulae, needles, formalin etc.

Procedure : The broodfishes are shown and the features explained. The gadgets used and the methodology are also explained in detail. A sample netted out. Proper ways of handling the animals shown. Insertion of cannulae, aspirating and extraction of a portion of the gonad etc. explained and shown. The tissues extracted taken into watch glass or cavity block and observed under microscope using glass slide. Oocytes observed, size of oocytes measured using micrometer. Examination of male fishes were also shown. The male fish was gently pressed along the sides of the abdomen when the whitish coloured milt oozes out.

Observations: The maturity stages of broodstock fishes are observed. Size of ova and the quantum of milt observed.

Inferences: Fish is mature with oocytes in the ripe stage of spawning /male fish ready for spawning.

Teacher : A. Raju

PRACTICAL No.1

TITLE : IDENTIFICATION OF CULTIVABLE MARINE AND BRACKISHWATER FINFISHES

Objective:

Raising broodstock; accelerating maturation, spawning and seed production by hypophysiation.

Principle :

Captive broodstock of cultivable finfishes would enable to undertake induced breeding and pave way for production of seeds.

Equipments/Reagents:

Scale, divider and dissection set

Procedure:

Fish specimens are collected from the fish landing centers and brought to the laboratory. They are segregated into groupwise. The morphometric and meristic characters are taken. They are identified using identification key. Then they are preserved in 7% formalin and kept with labels for future reference.

Obsaervation:

Colour of fish and pigments on the body are noted. This may help for easy identification.

Inference:

The given specimen is identified as ----------.

Charts, tables, figs., etc.

Draw the figure of the identified fish specimen.

Practical No.1

Teacher: **G.Gopakumar.**

Title: **Identification of major groups of marine ornamental fishes**

1. **Objective:** To identify major groups/species of marine ornamental fishes belonging to clownfish, damselfish, Moorish idol, banner fish, lion fish, butterfly fish, box fish, cardinal fish, parrot fish, wrasses, trigger fish, puffer fish, rabbit fish, squirrel fish and eels.

2. **Principle:** Separation of different groups/species by colour pattern, morphometric studies, meristic counts and other external and internal features.

3. **Equipments/reagents:** Measuring scale, divider, forceps, dissection microscope, formalin, glass bottles, glass jars, tray, needles, glouse, (reference books / identification sheets)

4. **Procedure:** Wash the fish thoroughly in running water, note the colour, shape, determine the probable family, take the standard morphometric measurements & meristic counts as applicable to the family, after confirming the family, proceed to find out the genus and species by examination of the required external and internal features.

5. **Observations:** The colour and length of the fish is noted. The morphometric and meristic counts as required are recorded. Record the exclusive features that separate the species from others.

6. **Inferences:** Based on the observations recorded, the fish is identified as belonging to a particular species.

7. **Charts, tables & figures:** The figures of the species identified and their exclusive features are drawn.

Practical No: 1 **Teacher : P.S. Asha**

Title: Spicule preparation and identification of cultivable sea cucumber species.

Objective : To prepare the spicules from cultivable sea cucumber species

Principle : Spicules from various parts of the body (body wall, tentacles, podia etc.,) are separated by treating thin sections with caustic soda solution or bleach or sodium hydroxide, to dissolve the organic material

Equipment s/ Reagents : Hypochlorite solution, D.P.X. mountant, distilled water, microscope, forceps, slide, cover-slip, wash bottle, blade, needle etc.,

Procedure :

Thin sections of tissue are removed from bivium, trivium, oral tentacles and podia of sea cucumber and macerated in sodium hypochlorite (bleach), or sodium hydroxide, or caustic soda solution, in order to dissolve the organic material.

After washing in distilled water, the spicules are rinsed in alcohol and can be processed with a drop of a mountant (D.P.X. mountant).

After processing, the spicules can be observed either on permanent slides with a light microscope, or prepared for scanning electron microscope and take the measurements.

Observation: Spicules of various size and shapes

Inferences : Based on the spicules characteristics species identification can be conducted.

Practical No.2 Teacher K.K.Joshi

Title: Identification Cultivable Brackish Water Fishes

Objective : To learn the methodology of identification fish samples

Principle: Identification of important cultivable marine finfishes using the morphometric and meristic characters and taxonomic monographs.

Requirements: Mullets, Erotplus, Milk fish
Scale
Needles
Lens
Microscope

Procedure: Take the linear measurements and meristic counts of the given fish. Observe the characters and compare with the monograph. Identify the fish up to species level by studying the distinguishing characters. Write down important characters of family, genera and species. Prepare note on the culture importance

Observation: Draw neat - labelled sketches

Teacher : A. Raju

PRACTICAL No.2

TITLE : IDENTIFICATION OF CULTIVABLE MARINE AND BRACKISHWATER FINFISH SEED

Objective :

To conduct culture experiments on fast growing finfish species and assess the production. To develop grow-out technology for farming cultivable finfish species in ponds, pens and cages.

Principle

Farming of cultivable finfishes by stocking the seeds under different culture systems would help to increase the fish production and compensate the demand in the export market.

Equipments/Reagents:

Seed collection net, collection bottles and formalin.

Procedure:

Operate a net in the seed potential grounds and collect the finfish seed. Preserve in formalin and bring them to the laboratory. Segregate them into different groups and identify them into specieswise. Keep them in the specimen bottles with 7% formalin and label for future reference.

Observation:

Colour, pigments and other characters of the seed are observed. The place and method of collection are noted.

Inference:

The collected finfish seed identified as ----------.

Charts, tables, figs., etc. :

Draw the sketch of the finfish seed.

Practical No: 2 **Teacher : P.S. Asha**

Title: Practicals : Observation on larval rearing techniques of holothurians.

Objective : To study the techniques of induced spawning , rearing and feeding patterns of various larval stages of holothurians .

Principle : Adults sea cucumbers are induced to spawn by suitable stimuli, the auricularia larvae are reared with micro alga *Isochrysis galbana* to get maximum settlement and the juveniles are reared with Sargassum extract up to 60mm size.

Equipment s/ Reagents : Brood stock, Larval and juvenile rearing tanks, microscope, Micro algal inoculums (*Isochrysis galbana, Chaetoceros calcitrans*), Walnes medium , transparent buckets, basins, dropper, slide, cover-slip, embryo cup, dropper, wash bottle, distilled water, aeration tubes, stones, control, Powdered feed (prepared from rice bran, Soya bean, and Sargassum), Algamac, Sargassum extract , Sargassum powder, fine sand etc.,

Procedure :

Brood stocks of sea cucumbers, collected from wild are induced to spawn by addition of powdered feed (50g/500l). The early auricularia are staked at a rate of 0.5/ml in clean filtered sea water and are fed with micro algal food (*Isochrysis galbana* at a rate of 20,000 cells /ml in the early stages, mixture of *Isochrysis galbana* and *Chaetoceros calcitrans* at 40,000 cells/ml in the later stages). Growth and survival rate of the larvae are assessed by taking the measurements and average of three one ml sample on alternate days. The Doliolariae larvae obtained after 10^{th} day are induced to settle by the addition of

Algamac (0.5g/500l). The Pentactulae obtained after 15 days are reared with Sargassum extract (20ml) up to a size of 20mm and later with a mixture of Sargassum powder and fine sand up to 60mm.

Observation:

Various larval stages (Auricularia, Doliolaria, Pentactula) are observed under microscope for growth, survival and feed in take.

Inferences : Holthurians can be better induced to spawn by adding powdered feed (50g/500l). The presence of lipid sphere in the late auricularia stage indicates the larval competency and it's readiness to metamorphose in the congenial environmental condition (water temperature 29-32°C).

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Teacher: Grace Mathew, P.S

Practical no.2

Title: Observations of techniques of induced breeding and larval rearing of cultivable fishes(groupers) Time : 2 hours

Objective: To observe the techniques of induced breeding and larval rearing of cultivable fishes(groupers)

Principle: Induced breeding techniques and also larval rearing techniques of marine finfishes.

Equipments /Reagents : Nets, sieves, microscopes, glass beakers, syringes etc.

Procedure : Hormonal as well as environmental inducement can be given to the fishes. Hormone inducement mainly for sex inversion. Environmental manipulation mainly by temperature variation as well as water level variation.

When spawning has taken place, different egg collection methods, counting of eggs in aliquot samples, examination of eggs under microscope, development of embryo shown, methods of incubation and hatching, preparation of larval rearing tanks, flow-through method. development of a green water system and providing larval feed, screening the right size of feed, cleaning the larval rearing tanks.

Observations : Induced breeding techniques and larval rearing methods are observed.

Practical No.2

Teacher: **G.Gopakumar.**

Title: **Reproductive biology of clownfish.**

1. Objective: To study the reproductive biology of clownfish.

2. Principle: Sex reversal, protandrous hermaphroditism and monogamy in the pair formation and breeding of clownfishes.

3. Equipments/Reagents: Stereozoom research microscope, ocular micrometer, microneedles, forceps, formalin.

4. Procedure: Identification of clownfish pair, microscopic examination of different stages of development of clownfish eggs, taking morphometric measurements of clownfish eggs, examination of clownfish larval stages, taking morphometric measurements of larval stages, examination of larval gut and observation on food and feeding, examination of live feeds for feeding clownfish larvae.

5. Observations: Recording the morphological differences between clownfish male and female, describing the different developmental stages of clownfish eggs and larvae, recording the morphometric measurements of eggs and larvae of clownfish, observations on the gut analysis of clownfish larvae, observations on different types of live feeds for feeding clownfish larvae.

6. Inference: The pairs of clownfishes are distinguished. The shape, size and developmental stages of clownfish eggs and larvae are inferred. The food and feeding and live feeds employed for clownfish larval rearing are studied.

7. Charts, tables & figures: The morphometric measurements of clownfish eggs and larvae are tabulated in a table. The different developmental stages of eggs and larvae are drawn.

Teacher : A. Raju

PRACTICAL No.3

TITLE : DETERMINATION OF MATURITY STAGES IN FINFISH

Objective :

To determine the different stages of maturity in cultivable finfish species.

Principle:

The determination of maturity stages would enable to know the condition of gonad of both sexes. The egg size would help to calculate the hormone dose for induced breeding in fish.

Equipments/Reagents:

Monocular microscope, ocular meter scale, stage micrometer, glass slides, canula tube, electrical balance, gilson fluid or ethyl alcohol/acetone/1% formalin.

Procedure:

Cut open the fish (if dead) and remove the ovary. Measure and weigh the ovary. Take subsamples from the ovary and separate the eggs. Keep them in the slide and take ocular division of atleast 50 eggs. Calculate the average. Then find the diameter of the egg by caliberating the ocular scale with stage micrometer reading. Repeat it. In live specimens, the eggs are drawn by cathedarisation and measured as per the above method.

Observation

The nature of the eggs (opaque, transparent or semi transparent) will be noted.

Inference

Based on the egg size, the fish is in ---------- stage of maturity.

Charts, tables, figs., etc.

Draw sketch of the egg.

Practical No.3 Teacher K.K.Joshi

Title : Determination of Maturity Stages in Fish

Objective: To determine the maturity stages of fish

Principle: Classification of maturity stages is a common method for the determination of the cycle of maturity of gonads. The species which have prolonged breeding season are those inn which the ovaries include several batches of eggs destine to become mature and shed periodically. The population consists of fishes of variable stages of maturity. There are five stages of maturity. They are stage I: Immature, stage II: Maturing; stage III: Mature, stage IV: Ripe, stage V spent.

Equipments/Reagents: Fresh fish
Dissection tools
Measuring scale
Weighing balance
Microscope

Procedure: Measure the total length and weight of the specimens. Cut open and note the sex, colour and appearance of the gonads. Study the eggs under microscope. Classify each ovary into stage I, II, III, IV and V.

Observation: Identify the stage and draw neat sketch

Inference: Tabulate the results and draw a bar graph.

Practical No.3

Teacher: **G.Gopakumar.**

Title: **Reproductive biology of damselfish.**

1. **Objective:** To study the reproductive biology of damselfish.

2. **Principle:** Sex reversal, protogynous hermaphroditism and polygamy in the pair formation and breeding of damselfishes.

3. **Equipments/Reagents:** Stereozoom research microscope, ocular micrometer, microneedles, forceps, formalin.

4. **Procedure:** Identification of damselfish pair, microscopic examination of different stages of development of damselfish eggs, taking morphometric measurements of damselfish eggs, examination of damselfish larval stages, taking morphometric measurements of larval stages, examination of larval gut and observations on food and feeding, examination of live feeds for feeding damselfish larvae.

5. **Observations:** Recording the morphological differences between damselfish male and female, describing the different developmental stages of damselfish eggs and larvae, recording the morphometric measurements of eggs and larvae of damselfish, observations on the gut analysis of damselfish larvae, observations on different types of live feeds for feeding damselfish larvae.

6. **Inference:** The pairs of damselfishes are distinguished. The shape, size and developmental stages of damselfish eggs and larvae are inferred. The food and feeding and live feeds employed for damselfish larval rearing are studied.

7. **Charts, tables & figures:** The morphometric measurements of damselfish eggs and larvae are tabulated in a table. The different developmental stages of eggs and larvae are drawn.

COURSE MC 504

SEAWEED CULTURE AND UTILIZATION

PRACTICAL MANUAL

MC – 504

Practical -1

Dr. N. Kaliaperumal

Date:

Time –3h

Topic - Collection and preservation of seaweeds, Methods of biomass estimation.

Objective: To estimate the seaweed biomass per unit area .

Principle: Estimation of seaweed biomass by quadrate sampling and line transect method and evaluation of natural stock species diversity per unit area

Collection and preservation of seaweeds:

- Collection of seaweeds from the intertidal and subtidal regions from the seaweed growing localities such as Krusadai Island, Pudumadam, Thonithurai and Rameswaram
- Preservation of collected seaweeds in 5% formalin after specieswise sorting and cleaning them in seawater for the morphological and anatomical studies.

Methods of biomass estimation

- There are two methods for estimation of seaweed biomass
- quadrate sampling method
- line transect method.

Quadrate sampling method

- In this method seaweeds are collected randomly from 10 to 15 quadrates of 1.0 m^2 or 0.5 m^2 areas in the intertidal and subtidal regions using metal quadrates once or twice in a month.
- Species wise fresh weights are recorded after cleaning and washing the seaweeds. These algae are then dried in the sun and later in an oven at 60° C to a constant weight and the dry weights are noted.
- The fresh and dry weights of the seaweeds per meter square are calculated to assess the seasonal changes in the biomass / standing crop of seaweeds in a particular area during one year.

- In this method several or required equidistant transects are fixed covering the intertidal and subtidal regions.
- Seaweeds are collected from one square metre area using 1.0 m^2 metal quadrates along the transects and the biomass (wet weight) for each species is taken.
- The quantification of biomass is done using the following formula

$$\text{Estimated biomass from a zone} = \frac{\text{Total weight of seaweed collected from the sampled area}}{\text{Area studied in the Zone}} \times \text{Total area of zone}$$

Observation:

Inference:

Figures:

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Practical : 2,3,4 Morphological and anatomical characterization for identification of economically important seaweeds

Types of seaweeds

Chlorophyceae (Green algae)

1. *Enteromorpha compressa*
2. *Ulva reticulata*
3. *U.lactuca*
4. *Chaetomorpha antennina*
5. *Cladophora sp.*
6. *Caulerpa racemosa*
7. *C.sertulariodes*
8. *Codium tomentosum*

Phaeophyceae (Brown algae)

9. *Dictyota dichotoma*
10. *Padina boergesenii*
11. *Spatoglossum asperum*
12. *Stoechospermum marginatum*
13. *Hydroclathrus clathratus*
14. *Cystoseira trinodis*
15. *Hormophysa triquetra*
16. *Sargassum wightii*
17. *Turbinaria conoides*

Rhodophyceae (Red algae)

18. *Porphyra vietnamensis*
19. *Pterocladia heteroplatos*
20. *Gelidium pusillum*
21. *Gelidiella acerosa*
22. *Gracilaria edulis*
23. *G corticata var corticata*
24. *G corticata var cylindrica*

25. *G. foliifera*

26. *G. crassa*

27. *G.verrucosa*

28. *Sarconema furcellatum*

29. *Hypnea valentiae*

30. *Acnathophora spicifera*

31. *Laurencia papillosa*

32. *Asparagopsis taxiformis*

Cyanophyceae (Blue-green algae)

33. *Lyngbya majuscula*

Objective : To identify the economically important seaweeds by morphological and anatomical features.

Principle : The morphological and anatomical features of the economically important seaweeds and other seaweeds will be worked by critically examining their morphological characters using binocular microscope and anatomical characters by taking transverse sections of the thallus and observing under the microscope, drawing diagrams and labelling different parts.

Requirements : Seaweed (fresh), blade, pith, microscope, eosin, glycerin brush, needle, watch glass, slides, coverslips

Procedure :

Morphology	**Anatomy**
Habit and Habitat	Transverse section
Thallus structure	Observation
Branching pattern	Cell type
Colour of the thallus	Cell size
Apical tip shape	Cell numbers

Identification of reproductive bodies in seaweeds

1. Unilocular sporangium	-	*Ectocarpus* spp
2. Tetrasporangia	-	*Dictyota dichotama*, *Padina*, *Gelidium*, *Pterocladia*, *Gracilaria*, *Hypnea* and *Acanthophora* spp.
3. Stichidium	-	*Gelidiella acerosa*, *Gelidiopsis variabilis*
4. Plurilocular sporangia	-	*Ectocarpus* spp.
5. Gametangia	-	*Acetabularia* spp.
6. Antheridial sori	-	*D.dichotama*
7. Oogonial sori	-	*D.dichotata*, *Sargassum wightii*, *S.ilicifolium*, *S.myriocystum*, *Turbinaria conoides*
8. Raceptacles	-	*Sargassum* spp, *Turbinaria* spp.
9. Cystocarp	-	*Gelidium pusillum*, *Pterocladia heteroplatos*, *Gracilaria spp*, *Hypnea* spp

Observations: 50 pages need to be added

MC – 504

N.Kaliaperumal

Practical: 5

Title: Seaweed Cultivation by Vegetative Propagation

Objectives : To study the method of seaweed cultivation by vegetative propagation

Principle : To study the selection of culture sites, method for vegetative propagation of seaweed, their Growth, harvest and processing

Requirements : Coir Rope, Casuarina poles, Seed material etc

Procedure :

- Selection of site
- Fabrication of coir rope and HDP rope nets:
- 5 x 2 m size nets, 2.5 cm thick coir rope is necessary. Four wooden poles or G.I. pipes are to be erected on the ground at a rectangular position of 5 x 2 m area.
- Fabrication of HDP rope nets is similar to that of coir rope nets but at the mesh intersections knots are to be put instead of inserting the ropes.
- For the border of the net 4 mm HDP rope has to be used and for the netting 3 mm thick HDP rope has to be used. The mesh size could be 10 cm.
- Collection and Transportation of Seed materials:
- Collection can be made by handpicking. After the collected materials are brought to the shore, the plants have to be transferred to plastic bins or fiberglass tanks containing fresh seawater.
- During long distance transport, frequent change of seawater on the way is necessary.
- Introduction of seed materials
- In the culture of *Gelidiella acerosa* by coral stones method iron nails are to be hammered on the coral stones
- Management of seaweed farms

- Observations on the growth of seaweeds cultured should be made at frequent regular intervals by taking random measurement of their length and biomass (weight).
- In the field cultivation, the following hydrological and environmental parameters should be monitored regularly.
  - Water movement
  - Water clarity and turbidity
  - Light intensity
  - Seawater temperature
  - pH, dissolved oxygen and salinity of seawater in the culture sites
  - Nutrients – Phosphate, Silicate, Nitrite and Nitrate of seawater
  - Silting or Sedimentation
  - Fouling organisms – Epiphytes and epifauna
  - Predators
  - Promoting the growth by pretreating the seed materials with hormones before introduction in the sea or by fertilizing the culture areas.

Harvesting

The harvesting of cultured algae should be made after 1-2 months when the seedlings reach harvestable size. Harvesting can be done by handpicking or using scissors / knives. If the conditions are favourable in the field for further cultivation, the remnants of the first harvest can be allowed for subsequent growth and harvest. A part of harvested crop can also be used as seed material for further cultivation.

Processing

- The harvested seaweeds should be thoroughly washed in seawater to remove the sand and other foreign matter and then sun dried. Species of *Sargassum* and *Turbinaria* which are to be stocked for longer period, should be treated with formalin in wet condition and then dried.
- In formalin treatment, the fresh seaweeds are soaked in 2% formalin in cement or wooden tanks for 2 hours and then dried in the sun. The formalin treatment is also done by spray technique. In this method the fresh seaweed is spread in a layer and formalin sprayed over it and layers

of seaweed are spread above it and formalin sprayed. This process is continued till all fresh seaweed received formalin treatment.

After 2 hours the plants are spread and dried in the sun. In the industries the seaweeds are cleaned to remove all the mixed algae and then soaked and washed in fresh water to remove debris and sand particles. The materials are then used for phycocolloid extraction.

Observation :

MC – 504

N. Kaliaperumal

Practical: 6 Title : Extraction of agar and estimation of mannitol by laboratory method- Part-I

Objectives : Extraction of Agar from Indian agarophytes

Principle : To estimate the total agar content of *Gracilaria* and *Gelidiella* species collected from Gulf of Mannar

Requirements: Dried and bleached seaweeds, Pressure cooker Organdy cloth, Velon screen

Procedure :

Take a 10 g of bleached sundried seaweed sample, cut into small pieces, wash twice and put in a 2 l conical flask. Add 300 ml of distilled water and cover with a cotton plug. Keep the conical flask in an autoclave and cook for 1-2 hours at 20 lb pressure. The extract is filtered in trays using organdy cloth and cooled at room temperature. The gel is then cut into strips and frozen in a refrigerator for one day. The frozen gel is thawed and dried in the sun using velon screen frames.

Part-II

Objective : Extraction of Mannitol from marine algae

Principle : To estimate the total mannitol content of *Sargassum* and *Turbinaria* species collected from Gulf of Mannar

Requirements : Dried and bleached seaweeds, Conical Flask, 0.1N H_2So_4 , 0.1N periodic acid, potassium iodide , 4N H_2So_4, starch, 0.1N $Na_2 S_2 O_3$

Procedure :

Weigh 200 mg of brown algal powder and put it in a conical flask. Add 5 ml of 0.1N H_2So_4 and leave it to digest for sometime, then add 5 ml of 0.1N periodic acid and note the time. Exactly after 1 minute, add 2 gms of potassium iodide crystals and immediately add 20 ml of 4N H_2So_4 followed by 5 ml of 1% starch

solution. Then titrate it against 0.1N $Na_2 S_2 O_3$. Disappearance of blue colour is the end point. Note the burette reading. For each material 4 samples may be titrated.

In the same manner blank titration without seaweed powder may be done and note the reading. Find out the difference between BTV (blank titration value) and titration values with seaweed powder. Convert the difference for 1 gm powder.

Observation

The percentage content of mannitol may be calculated as follows:
1cc of N/10 $Na_2S_2O_3$ = 0.0018217 gm. So percentage of mannitol = 0.0018217 x X x 100 x 100 where 'X' is the volume of $Na_2S_2O_3$ (0.1 N consumed for 1 g of seaweed powder).

Inference:

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Practical: 7 Title : **Qualitative and quantitative estimation of agar**

Objectives : To make qualitative and quantitative estimation of agar

Principle : To find out the quality of agar

Requirements : 100 ml beaker, agar powder, heater, spectrophotometer

Qualitative estimation of agar

Gel clarity

Agar or carrageenan gel of 1.0% is prepared in absorption cell. The optical density is determined at 500 nm in a spectrophotometer or colorimeter using distilled water as a standard.

Gel strength

Prepare 1.5% agar or carrageenan solution. Take 100 ml of it in 100 ml beaker. Allow the gel to cool at room temperature. Then keep the beaker containing the gel over night in a refrigerator at 25° C. Remove the beaker with the gel from the refrigerator and place the gelometer gently on the surface of the gel so that bottom of the gelometer rests on the surface gently. Add weights gradually on the pan of the gelometer until it breaks the gel. Note the weight required to break the gel. The weights used to break the gel and the weight of the gelometer is added to know the gel strength of the agar which is expressed in g/cm^2.

Gelling temperature

Pour 100 ml of 1.5% solution of agar into 100 ml beaker. Keep the beaker in a water bath with running cold water. Insert a thermometer into the solution using a stand. Allow the gel to cool slowly at room temperature. Drop 3 mm stainless steel balls or glass beads one by one at frequent intervals on the surface of the solution at near 40° C. The temperature at which the beads stay on the surface of the gel is the gelling temperature and the value is expressed in Celsius.

Melting temperature

Keep 100 ml beaker containing 1.5% agar in a water bath. Insert a thermometer in the gel in such a way that the bulb of the thermometer is at the centre of the gel mass and then heat the water bath. Wait till the mercuric column of the thermometer shows 50° C. Thereafter drop the glass or stainless steel beads on the surface of the gel. The temperature at which the beads reach the bottom of the beaker is the melting temperature which is expressed in Celsius.

Quantitative estimation of agar

The quantity / yield of agar is estimated by the following formula

$$\text{Yield of Agar (\%)} = \frac{\text{quantity of agar obtained}}{\text{quantity of seaweed used}} \times 100$$

Observation :

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Practical: 8 Title : Extraction of algin, its properties

Objective: To extract alginic acid from *sargassum* and *Turbinaria*

Principle: To extract and estimate qualitatively the alginic acid from the supplied brown algae

Extraction of Algin

Take 10 g brown algal powder and 3 g of sodium carbonate. Put them in 500 ml beaker and add 400 ml of distilled water. Boil it in a water bath for 2-3 hours and stir frequently. Remove the beaker containing the macerated material and cool it at room temperature. Filter the macerated material to a 1000 ml beaker through bolting silk cloth and discard the residue. Add 1:2 hydrochloric acid to the filterate till complete precipitation of alginic acid. Collect the precipitated alginic acid using bolting silk cloth. Wash the alginic acid in warm water to remove acidity. Dehydrate the alginic acid by washing it in rectified or denatured spirit. Collect the alginic acid in a petridish and dry it in an oven at 60° C for 24 hours.

Viscosity of algin

Dissolve thoroughly 2 g of algin in 200 ml water in a 250 ml beaker. The viscosity of the solution is measured using a Brookfield viscometer. Insert a suitable spindle such as No. 2 or 3 into the solution and run the spindle at the rate of 20 rpm at 25° C to read a stable value indicated and multiply with the corresponding; spindle coefficient. The product is absolute viscosity in cp (milli Pascal-second). For instance, at 20 rpm 25° C and use No.3 spindle, the value indicated is 36.1, then 36.1 x 50 (spindle coefficient) = 1800 m.Pa.s (centipoises).

Observation:

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Practical: 9 Title : Methods of preparation of seaweed food products and recipes

Objective: To study the different recipes prepared from seaweeds

Principle: To prepare different recipes from seaweed

Procedure:

Salad

The following seaweeds are used for making salads either singly or in combination of two or three seaweeds. *Caulerpa racemosa*, *C.sertularioides*, *Codium* spp, *Gracilaria verrucosa*, *G.eucheumoides*, *Hydroclathrus clathratus*, *Laurencia papillosa* and *Porphyra* spp.

Fresh seaweeds are cleaned of sand, debris, attached stones etc. and then washed in freshwater. Chopped tomatos, carrot, onion, chilly and ginger are added and mixed. Salt is added to taste.

Seaweed masala

Cut onion and green seaweed (*Ulva lactuca*) into pieces and garnish them in low fire with oil, mustard and curry leaves. When about to turn grey, add chilly powder, coriandrum powder, turmeric powder, salt, ginger and tomato pieces and mix well. It can be eaten with rice and chappatis.

Seaweed pickle

Take cleaned fresh seaweed *(Gracilaria edulis)* and remove moisture with cloth. Cut into small pieces. Soak in vinegar for 2 days. Remove from vinegar, add gingelly oil, chilli powder, mustard and fenugreek powder. Season with asafoetida. Add peeled garlic. Mix thoroughly and bottle.

Seaweed wafer

Boil cleaned dried seaweed (*Gracilaria edulis*) in water. Filter through organdy cloth. Add rice paste, chilly paste and asafoetida powder. Add gingelly seed and cumin

seed and mix well. Cook together Dry the paste in open sun in small lumps on cloth. Store in air tight jar before serving fried in oil.

Seaweed porridge

Boil dried cleaned seaweed (*Gracilaria edulis*) in water for 20 minutes. Grind it into a fine paste. Boil the paste in water. Add sugar and milk and mix thoroughly. Add cashew nut, raisins and cardomon. Serve hot.

Seaweed jelly

Boil cleaned dried seaweed (*Gracilaria edulis*) in water for 45 minutes. Stir frequently. Filter through organdy cloth into a vessel. Add sugar, lemon juice, food essence and food colour to taste in hot condition. Mix thoroughly. Pour in an enamel or stainless steel tray. Allow to set. Refrigerate for minimum 30 minutes. Cut into pieces and serve.

Seaweed Jam

Prepare sugar syrup. Add seaweed powder (*Ulva lactuca*) and boil for 15 minutes with constant stirring. Add edible colour and essence. Ready to serve.

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Practical: 10 Topic : Distribution and collection and morphological characterization of seagrasses

Objective : To identify different seagrasses of Indian coast by morphological characterization.

Principle : To study the morphological and anatomical variation in different species of seagrasses.

Procedure :

The following seagrasses species will be collected from the intertidal and subtidal regions and their morphological characters will be worked by critically examining their external features, drawing diagrams and labeling the parts.

Seagrasses

Enhalus acoroides
Halophila ovalis
H.ovata
Thalassia hemprichii
Cymodocea rotundata
C.serrulata
Syringodium isoetifolium
Halodule uninervis

Observation :

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Practical: 11 Topic: Extraction and estimation of photosynthetic pigments from seaweeds and tracing the absorption spectrum of chlorophyll.

Objectives : Extraction of photosynthetic pigments from red and green macroalgae

Principle : Estimation of photosynthetic pigments such as chlorophyll a, b, total chlorophyll, phycobiloprotein, and carotenoids from seaweeds and to study the absorption spectrum of the chlorophyll.

Requirements : Seaweed –*Gracilaria* sp & *Ulva* sp
Acetone - 90%, distilled water
0:5 M Phosphate buffer pH (6.8)
Mortar cum pestle
Test tubes, Centrifuge tubes, beaker etc

Equipments required: Refrigerated centrifuge
UV-Vis spectrophotometer
Electronic balance

Chemical preparation:

90% acetone: 90 ml of pure acetone +10 ml of distilled water

0.5 M Phosphate buffer pH (6.8):

1.0 M Sodium hydroxide –100 ml

sod.dihydrogen phosphate –100ml

Add x ml of !.0 M sodium hydroxide to 50 ml of 1.0 M sodium dihydrogen phosphate till the pH is 6.8. Dilute the solution to 100 ml.

Procedure :

- Grind a known quantity of seaweed (appx 100mg) in a mortar cum pastle with 90% acetone.
- Make up the volume to 5 or 10 ml
- Centrifuge at 10,000g for 10 min by Hitachi refrigerated centrifuge and collect the clear supernatant

- Take optical density in 630,663 and 450 nm with reference to 90% acetone
- Take again fresh known quantity of seaweed (100 mg from red algae)
- Grind it with 0.5 M Phosphate buffer pH 6.8
- Centrifuge it at 10,000g for 10 min by Hitachi refrigerated centrifuge and collect the clear supernatant
- Take OD at 498, 614 and 651 nm with reference to phosphate buffer

Calculation :

Chlorophyll	-	11.47 (OD663) - 0.4 (OD 630)
Phycoerythrin	-	155.8 OD498-40.4 OD614-10.5 OD651
Phycocyanin	-	151.1 OD614-99.1OD651
Allophycocyanin	-	181.3 OD651-22.3 OD614
Carotenoids	-	$\frac{OD450 \times 10}{2.5}$

Absorption spectrum

-Arrange the equipment for baseline correction from 400-700 nm with 90% acetone

-Take the clear extracted solution of chlorophyll

-Scan the same from 400-700 nm

-Take a print out and observe the peak and crest

- Compare with the absorption maxima of the chlorophyll sample

Observations:

Inference:

Figure:

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Practical: 12 & 13 Title: Reproductive structure, spore out put and observation on their survival and growth in laboratory condition.

Objectives : To observe the reproductive structure of *Gracilaria* sp. by making transverse section and observe on spore output their survival and growth.

Principle : To know about the structure of cystocarps, carpospore liberation , their nursery rearing and growth in laboratory condition

Requirements : Live Cystocarpic plants of *Gracilaria* sp. Watchglass, slides, coverslip, Beaker, Test tubes (100 ml capacity) Blade, pith, Eosin, Glycerin, microscope and growth Chamber, Enriched Pravosali's medium, seawater

Chemical preparation: As per the standard procedure prepared the stock solution of Pravosali's medium.

Procedure :

Part-I

- Take few live cystocarpic plants of any *Gracilaria* sp.
- Make few transverse section with the help of a new blade
- Select the best section, stain it with eosin and mount with glycerin
- Observe under microscope
- Draw the diagram label the necessary parts of the cystocarps

Observation :

Part-II (Group activity)

- Take a beaker of 1 l capacity filled with filter and sterilized seawater
- Place few glass slides and cover slips inside the beaker
- Place a nylon piece on the top of the beaker so that the nylon is just dipped in water
- Place a few healthy live cystocarpic plants of *Gracilaria* on the net piece. Care should be taken that the cystocarpic plant is not exposed to dessication.
- Keep it overnight
- Next day observe the glass slides under the microscope and observe the spores attached to the slides or the coverslips
- Note down the number of spores per unit area and their size by an ocular micrometer.Note down the size after calibrating the ocular by a stage micrometer.
- Place the glass slides and the coverslips in a test tube filled with enriched seawater and kept under constant light, temperature and photoperiod in a growth chamber.
- Take regular observations till the spores changed to germling stage.
- Find out the Crop growth rate of the spores and draw a growth curve.

Observation :

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Practical: 14 Title.: Estimation of total protein content in seaweed.

Objectives : To estimate the protein content of the seaweed supplied to the student.

Principle : To find out the total protein content in marine algae

Method used : Lowry's method

Requirements : Dried and powdered seaweed ,Test tubes, Beaker, Glass rod. Reagent bottle, Sodium hydroxide, Sodium carbonate, coppersulphate, Sodium potassium tartarate, Folin's reagent, Bovine serum albumin. conc sulphuric acid

Chemical preparation:

1 N NaOH – 4g of NaOH in 100 ml of dis.water

2% copper sulpahate - 2 g $CuSO_4$ in 100 ml dis.water **(A)**

4% Na-K tartarate - 4 g Na-K tartarate in 100 ml dis.water**(B)**

3% Na_2CO_3 in 0.1 N NaOH- 3 g Na_2CO_3 in 0.1 N NaOH **(c)**

Folin Phenol reagent - Diluted with Dist water (1:1)

Bovine serum albumin - 25 mg in 50 ml of dist.water

Preparation of standard curve -

Make different concentration of standard bovive solution as follows.

Standard Bovine solution(ml)	1 N NaOH (ml)	Resulting protein (mg)
0.5	0.0	0.25
0.4	0.1	0.20
0.3	0.2	0.15
0.2	0.3	0.10
0.1	0.4	0.05
0.0	0.5	0.00 (Blank)

Procedure :

- Prepare the alkaline copper tartarate solution in the following method
- ml of A + 2 ml of B + 96 ml of C (Prepared afresh)
- Add 5 ml of the reagent to each sample tube containing BSA
- Add 0.5 ml of diluted Folin's reagent
- Add 5 ml of concentration sulphuric acid
- Allow to stand for 20 min,Take OD at 660 nm
- Draw the standard curve

Procedure for the seaweed sample:

- Weigh 10 mg of dried powdered sample to a 10 ml cap test tube
- Add 5 ml of 1 N NaOH solution, capped it with marble and allow to
- stand for 24 h in room temperature
- Pipette out 0.5 ml of extract to another test tube
- Add 5 ml of alkaline copper tartarate and 0.5 ml of folin's reagent
- Add 5 ml of concentrated sulphuric acid. Allow to stand for 20 min
- Take OD at 660 nm by a Hitachi spectrophotometer
- Estimate the quantity of protein from the standard curve

Calculation :

Percentage of protein = $\frac{\text{mg of protein X 10}}{\text{mg of tissue}} \times 100$

10 is the dilution factor

Observations :

Inference:

Graph:

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Practical: 15 Title : Estimation of total carbohydrate content in seaweed.

Objectives : To estimate the carbohydrate content of the seaweed supplied to the student.

Principle : To find out the total total carbohydrate content in marine algae

Method used : Phenol sulphuric method (Duboi's 1956)

Requirements : Dried and powdered seaweed ,Test tubes, Beaker, Glass rod, Reagent bottle, Trichloroacetic acid, Phenol, conc sulphuric acid

Chemical preparation:

5% TCA	–	5g of TCA in 100 ml of dis.water
5% Phenol	-	5 g Phenol in 100 ml dis.water
Glycogen	-	25 mg glycogen in 50 ml of 5% TCA

Preparation of standard curve -

Make different concentration of standard Glycogen as follows.

Standard Glycogen (ml)	5% TCA (ml)	Resulting carbohydrate (mg)
0.20	0.00	0.100
0.15	0.05	0.075
0.10	0.10	0.050
0.05	0.15	0.025
0.00	0.20	0.000(Blank)

Procedure :

- Add 1 ml of 5% phenol to each sample tube containing Glycogen
- Add 5 ml of concentration sulphuric acid, mix by tapping
- Allow to stand for 30 min.Take OD at 490 nm
- Draw the standard curve

Procedure for the seaweed sample:

- Weigh 5 mg of dried powdered sample to a 15 ml cap test tube
- Add 10 ml of 5% TCA solution, mark it and allow to heat in a water bath for 3 h
- Remove the tube, cool and return to the same volume by adding dis.water
- Pipette out 0.2 ml of extract to another test tube
- Add 1 ml of 5% phenol
- Add 5 ml of concentrated sulphuric acid. Allow to stand for 30 min
- Take OD at 490 nm by a Hitachi spectrophotometer
- Estimate the quantity of carbohydrate from the standard curve

Calculation :

Percentage of carbohydrate = $\frac{\text{mg of carbohydrate X 10}}{\text{mg of tissue}} \times 100$

10 is the dilution factor

Observations :

Inference:

Graph:

MC – 504 Reeta Jayasankar

Practical: 16 Title: **Estimation of total lipid content in seaweed.**

Objectives : To estimate the lipid content of the seaweed supplied to the student.

Principle : To find out the total total lipid content in marine algae

Method used : Folch's method followed by Phospho Vanillin method

Requirements : Dried and powdered seaweed, Test tubes, Beaker, Glass rod, Reagent bottle, Sodium Chloride, orthophosphoric acid, Vanillin,reagent, Cholesterol, Chloroform and methanol.

Chemical preparation:

4% orthophosphoric acid– 800ml OPA in 200 ml of dis.water (A)

2% Vanillin in solution A - 2g Vanillin in 200 ml of (A)

Chloroform:Methanol - 2:1 ratio (B)

Cholesterol - 8 mg glycogen in 4 ml of (B)

0.9% Sodium chloride - 900 mg NaCl in 100 ml of Dist.water

Procedure :

Extraction of lipid

- Take 100 mg dried algal powder, Put in a screw cap vial with 25 ml of 2:1 chloroform and methanol mixture
- Loosely cap the vial and heat at 60^{0}C for 15 min.
- Cool and make up the volume. Shake well and filter in Whatman 541 filter paper
- Collect the filtrate in 30 ml screw cap tube marked in 20 ml.
- Add 4 ml of distilled water. shake well, centrifuged at 1500 rpm
- Decant the aqueous phase. Evaporate the sample at 60°C under steam of air.

- Dissolve the residue in 1 ml of methanol and again 2 ml of chloroform. Further 2ml of chloroform methanol mixture, mix well
- Add 0.2 ml of 0.9% NaCl. Pour to a separating funnel, Mix well and allow to stand for few hours.
- Remove the lower phase into a clean tube and make up the volume to original.
- Take 0.5 ml of extract to a clean tube.Allow to dry in a vaccum dessicator over silica gel.
- Add 0.5 ml of concentration sulphuric acid, mix by tapping, Plug the tube with non-absorbant cotton. Place in boiling water bath for 10 min. cool the tube

Estimation of lipid

- Take 0.2 ml of this acid digested extract in a separate tube .
- Add 5 ml of Vanillin reagent, Mix well and allow to stand for 30 min.
- Take OD at 520 nm
- Take 0.2 ml of standard cholesterol and read the OD after adding Vanillin reagent and compare with the sample

Calculation :

Percentage of lipid = $\frac{\mu g \text{ of unknown} \times \mu g \text{ standard} \times \text{dilution factor}}{\mu g \text{ of known}}$

It is expressed in µg lipid/ml or % of lipid

Observations :

Inference:

Graph:

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Practical: 17 Title : Morphological anatomic characterization of seagrasses

Objectives : To identify the morphological and anatomical features of two species of seagrasses and to identify the name of the given species.

Principle : To differentiate between sea grasses and sea weeds with their morphology and anatomical features.

Procedure : Observes the morphological features of the species, draws labeled sketches, takes CS of the stem, understands the anatomical characteristics.

Observations :

Morphology

Habit-

Leaf : Length
Breadth
Venation
Margin
Sheath
Ligules

Stem : Nodes
Internode - length
Thickness

Root : Nature of root system
Lateral roots
Root caps

Anatomy

stem C.S.
epidermis
cortex
medullary cells
vascular elements

Inference : The given specimen is identified as .....

Practical: 19 Title: Preparation of chemicals and glasswares for tissue culture of seaweed

Objective : To prepare medium and necessary glass wares for the Tissue culture of seaweeds.

Principle : To understand the essential requirement of seaweed tissue culture.

Requirements :

Conical flasks	-	500 ml
Beaker	-	500 ml
Reagent bottles	-	1 lit - 2
		100 ml - 2

Balance (top pan)
Distilled water
Filtered seawater.

PROCEDURE

-Clean the glasswares with detergent and rinse them with tap water.

-Sterilize them in an autoclave for 30 min. at 15 lbs.

Preparation of culture medium

Walne"s medium consists of 1 ml of stock A solution, 0.5 ml of stock B solution and 0.1 ml each of Vitamin B1 and B 12 in a final volume of 1 lit. of filtered sea water of 30 – 32 ppt salinity.

Stock A - Weigh the following chemicals and make up the volume to 1 lit. and store in a clean reagent bottle.

Potassium nitrate	-	100 g
Sodium orthophosphate	-	20 g
EDTA	-	45 g
Boric acid	-	33.4 g
Ferric chloride	-	1.3 g
Manganous chloride	-	0.36 g

STOCK B Similarly prepare stock B in one lit. of distilled water with the

following salts

Zinc chloride	-	4.2 gm
Cobalt chloride	-	4.0 gm
Copper sulphate	-	4.0 gm
Ammonium molybdate	-	1.8 gm

VITAMINS

Vitamin B1	-	200 mg in 100 ml distilled water.
Vitamin B12	-	10 mg in 100 ml distilled water.

Refrigerate the stock solutions and vitamins when not in use.

-Autoclave the medium and both stock solutions (A & B) in sea water at 15 lbs for 30 minutes.

-Add the vitamins using a syringe fitted with filter (2 μ)

Observation:

Inference :

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Practical: 20 Title: Tissue culture of seaweed (Group studies)

Objective : To culture the thallus of seaweed in liquid culture medium

Principle : To find out growth rate of seaweed cultured in laboratory conditions and to observe the changes while maintaining seaweed in laboratory conditions.

Procedure

- Clean the thallus of seaweeds in detergent solution and with excess seawater.
- Note the initial weight of thallus bits to be (W_o).
- Cut them into small bits (2 cm to 3 cm size) for the laminar flow hood and transfer them to the conical flask containing sterilized medium.
- Plug them with cotton and label them the details such as name of the species, date of incubation etc.
- Incubate them under light. Observe the changes every alternate days.
- Note the weight gained or lost to the thallus bits after 10 days (W_1).
- Calculate the growth rate using the following formula.

  Daily growth rate (DGR) = (W1 – W0) / T

  Where (W_1) - Final weight of thallus

  (W_0)- Initial weight of thallus

  T - Total days of incubation.

Observation:

Initial weight of the thallus (W_0).

Final weight of the thallus (W_1).

Time period for incubation (T)

Calculation:

DGR =

Graph:

Practical: 21 Title: Extraction of Carrageenan from *Hypnea* and *Kappaphycus*

Objective : To extract Carrageenan from seaweed samples and to estimate the yield.

Principle : Hypnea species contains λ carrageenan where as kappaphycus species contains k carrageenan.

Requirements : Bleached and dried seaweeds – pulverised.
Balance
Distilled water
NaOH, Na_2CO_3
Erlenmeyer flask, Beaker (500 ml)
Autoclave / Pressure cooker
Isopropyl alcohol.

Procedure

- Soak 5 gm of dried seaweed powder in about 100 ml of distilled water- make alkaline with 1N NaOH (pH – 8) or sodium bicarbonate.
- Use 500 ml flask. Autoclave for 1½ h by covering the flask with aluminium foil.
- Filter the viscous solution while it is hot into a 500 ml beaker.
- Cool the extract to room temperature.
- If the extract gels immediately on cooling it indicates presence of kappa or iota carrageenan,
- If the extract does not gel on cooling but remains highly viscous the carrageenan can be precipitated by adding 2.5 times the volume of isopropyl alcohol.
- Remove carrageenan by filtration and dry in an oven at 60 ° C over night.
- Place the gel in a freezer.
- On the following day frozen gel can be and then dried, Weigh the dry carrageenan obtained and calculate the yield of carrageenan from dry weight of seaweed used.

Observation

1. The carrageenan obtained from the seaweed appears to be ....carrageenan.

2. The yield of carrageenan from the given sample is .....% /g dry wt.

Inference:

M.F.SC. MARICULTURE

LECTURE OUTLINE

COURSE No. 505

ECOLOGY OF CULTURE SYSTEMS

Course Teacher : D. PREMA
Faculty Members : C.P. GOPINATHAN
G.S.D. SELVARAJ
T.S.NAOMI
REETA JAYASANKAR
MOLLY VERGHESE
IMELDA JOSEPH
A. NANDAKUMAR
GEETHA ANTONY

CONTENTS

Water quality parameters in mariculture systems (Part I)

Water quality – definition – water quality monitoring - importance - water quality parameters related to maricultre – physical – chemical

Physical factors : Temperature – absorption of solar energy at sea surface – factors affecting - vertical temperature profiles - formation of thermal stratification – typical profile – thermocline – definition - stability of the stratification

Measurement of temperature at surface, sub surface depths – instrument used – protected reversing thermometer – mechanism of recording temperature – accuracy of measurements

Factors affected by changes in temperature – physical – density, viscosity of water, solubility of gases, chemical – biological processes in the sea

Light – importance of the parameter – penetration of light (IR and Visible portion of the spectrum) to different depths – basis of degree of penetration – factors affecting – euphotic zone

Instruments for measuring light penetration – Secchi disc, transparency meter

Water quality parameters in mariculture systems (Part II)

Salinity – definition –unit – ratio of constant composition of in sea water – average salinity of sea water – factors causing variations in salinity – evaporation, precipitation, fresh water discharge from rivers – salinity tolerance – stenohaline and eurihaline forms

Salinity measurements – determination of chlorinity – titration method – Knudsen's equation – use of "standard sea water"
Determination of electrical conductivity of sea water – application of the relationship between conductivity and salinity – use of Salinometers – Refractometers – principle

pH – definition – principle system regulating pH in water – factors affecting variations in pH of sea water – environmental, biological – pH range of sea water – factors affecting marine organisms

pH measurements – use of pH meters – calibration - procedure

Turbidity – causes of turbidity – plankton blooms, concentration of humic substances, suspended particles of clay – problems due to turbidity

Turbidity measurements – estimation of total suspended solids (TSS) in sea water - unit - use of Nephelometer – unit of turbidity (ntu)

Water quality parameters in mariculture systems (Part III)

Chemical factors : Alkalinity - What is alkalinity - sources – unit of alkalinity - predominant basis contributing to alkalinity – effects due to high alkalinity

Determination of alkalinity – titration method – steps involved – phenolphthalein alkalinity - total alkalinity

Total hardness – what is total hardness – unit of expression – causes of hardness in water – polyvalent metallic ions – natural hardness – limestone – effect of hardness in water

Estimation of total hardness – titration method – principle

Dissolved oxygen – importance of the parameter – solubility of oxygen in water – factors influencing solubility - oxygen exchange across the sea surface – photosynthetic production of oxygen – dissolved oxygen stratification in culture systems

Estimation of dissolved oxygen in water – titrimetric method - Winkler method – sampling and storage – procedure

Water quality parameters in mariculture systems (Part IV)

Inorganic salts - major dissolved nutrients in sea water – phosphorus, nitrogen and silicon - their role in aquatic productivity – availability of nutrients at different layers – factors influencing concentration – light, temperature, water movement, river influx, extend of productivity - nutrient utilisation - process of photosynthesis – nutrient regeneration process - replenishment of surface layers – factors influencing – physical

Methods of estimation – orthophophosphate, nitrate, ntrite, silicate – principles - instruments involved

Bio-chemical oxygen demand (BOD) – what is meant by BOD - factors affecting BOD - purpose of BOD tests – water pollution investigation

Determination of BOD in samples – direct method , dilution method - preparation of dilution water - dilution scale – basis for dilution

Chemical Oxygen Demand (COD) - usefulness of COD tests - principle involved

Estimation of COD by different methods – digestion method – heat dilution procedure for brackish waters

Circulation and mixing pattern in coastal water bodies

Coastal Water bodies - regions included– their characteristic features - factors affecting – discharge of continental materials – biological productivity

Major circulation processes in the region – causative factors inducing circulation and mixing - thermohaline circulation – wind action at surface – effects of tidal currents

Thermohaline circulation : factors causing – evaporation, surface cooling - convective flow – effect on density of water masses – sinking process

Wind driven circulation : water movement – effect on coastal waters – upwelling - sinking

Tidal currents : factors causing formation of tides – oscillation and period – spring tide - neap tide

Factors affecting circulation and mixing – effect on thermal barriers - oxygenation of deeper layers – nutrient replenishment in surface layers - primary productivity of the euphotic zone – dilution of coastal pollution – influence on living organisms

Monsoon and its effect on coastal water bodies

Monsoon – origin of terminology – monsoons over India – causes of monsoon – wind patterns along the shores of the Indian Ocean - components determining the origin and development of monsoons - seasonal variations in sea surface temperature (SST) – variations in air-sea interaction

Phases of monsoon – wet phase - duration – average rainfall – dry phase– what is monsoon failure – monsoon forecasting – different ranges of forecast - effect of monsoon on physical and chemical properties of coastal water bodies

Upwelling – factors causing the phenomenon – upwelling process – upwelling areas – upwelling along the West Coast of India – causative factors – wind and current pattern during upwelling – period, intensity and duration of the process – effect on water properties – influence on coastal productivity

Mud banks – characteristic features – formation of mud banks – major locations of formation along the south west coast of India – reasons for tranquillity – properties of mud banks – physical - chemical

Coastal Ecosystem Analysis: Part-I. An overview

Introduction - Ecology - Ecosystem

The Environment - Hydrosphere - Atmosphere - Lithosphere - Biosphere

Coastal Zone - Continental shelf (classification) –Inshore - offshore waters - Neritic - Littoral zones

Inshore waters - Extent (upto 50 m depth contour)

Indian coast (area) - 0.193 million km^2 (42% of Continental shelf)
- East coast: 78,500 km^2 (41%)
- West coast: 1,14,500 km^2 (59%)

Estuary-13% of global coastline - Factors governing formation and life-span of estuary

Estuarine ecosystems - Mangroves, salt marshes, backwaters and lagoons

Tides - Spring tide - Neap tide - Full moon tide - New moon tide -Tidal amplitude - Role of tides

Diurnal studies - High and Low tide data

Types of Estuaries - Classification
- Prichard (1967)
- Mc Hugh (1966)
- Hansen and Ratray (1966)
- Nair and Thampi (1982)

Hydrodynamics of estuaries - Formation of mudflats and mangroves

Station fixing and sampling techniques

Coastal Ecosystem Analysis : Part-II. Structure and Functions

Boundaries - Physical and Ecological

Coastal Ecosystems: Marine zone –Estuarine zone - Mangroves - Salt marshes - Lagoons and Atolls - characteristics

Organic cycle: Within the phytomass - Within the Ecosystem - Outside the Ecosystem

Ecological Reservoirs (storage of organic matter): Living tissue - Detritus - Dissolved form (solution)

Organic carbon compounds in nature - (0.05%) - Storage: Fossil – detritus - biomass

Biomass: Primary - secondary - tertiary levels

Food-chain and Food-web – description - Trophic cycle (Food-chain) in the sea - Detrital Food-web in the Mangroves / Estuarine system

Organic cycling in the Mangrove ecosystem - Factors governing organic cycling

Different Types of Culture Systems

Introduction – characteristics - Freshwater – Estuary - Open sea - Salt pans

Pond culture – types - Freshwater - Perennial ponds - temporary ponds - Holding pond - Rearing pond - Growing pond - Excavated ponds - Levee ponds - Factors considered before building a pond - Preparation and management of fish culture ponds - Ecology of fish pond – factors affecting ecology – physical - chemical - biological

Culture in Brackish water ponds - Traditional culture practices - in impoundments - in paddy fields - in small shallow coastal lagoons

Other culture systems – features - Raft culture - Rack culture - Cage and Pen culture systems – advantages - disadvantages - Raceway and Tank culture – method – open system – closed system – advantages - disadvantages - Culture in salt pans – features - Sewage fed fisheries – features - Monoculture - Polyculture - Extensive and Intensive culture practices

Lecture 10

Prepared by Shri G.S.D. Selvaraj

Dissolved Oxygen and pH in Culture Systems

Dissolved Oxygen - The most critical factor - Sources of addition (Physical and biological) - Sources of removal (Physical, chemical and biological)

Solubility - Role of Temperature and Salinity - Supersaturation

Factors influencing dissolved Oxygen and pH

Diurnal fluctuation - Culture systems

Seasonal fluctuations - Coastal waters (winter-summer-monsoon)

How to check dissolved Oxygen - Sampling techniques

Coastal waters - At lowest and highest tides
- At bihourly intervals
(Diurnal and Tidal cycle studies)

Culture ponds - Early morning and late afternoon
- BOD / COD Tests

Determination of Compensation depth

pH & Dissolved Oxygen range and optimum in Culture Systems

Main causes for Oxygen depletion in Culture ponds

- Respiratory loss
- Phytoplankton depletion
- Eutrophication (aftereffect)
- Bacterial oxidation processes

Factors governing dissolved Oxygen in the Culture ponds - Primary Production - Feed - Seed density - Illustrations and Precautions

Biological Oxygen Demand (Net BOD) in Coastal Waters

BOD = Biological/Biochemical Oxygen Demand

- A derivative of Oxygen measurement
- Role of dissolved oxygen in coastal waters
- To determine water quality and healthy survival of organisms

Oxygen user levels : Primary, Secondary & Tertiary

Primary level : Phytoplankton and Bacteria

Oxygen requirement for Tertiary / culture organisms

Drawbacks of existing methods to assess O_2 level:

- BOD estimation (drawbacks)
- COD estimation (drawbacks)

Net BOD estimation at primary-Secondary production level:

(Modified Light and Dark bottle Oxygen Technique)

L-I value (12 hrs) = 12 light hours of the day

D-I value (12 hrs) = 12 night hours of the day

[(L-I) + (D-I)] 12 hrs = Net BOD value per 24 hrs

Water quality Test : + value = good; - value = Not good

Result: More realistic to the environment

Net BOD - An index to water quality

Carbon dioxide - Bicarbonate-Carbonate Buffer System in the Sea

Inorganic Carbon in the sea

CO_2 = 1%; HCO_3^- = 90%; CO_3^{2-} = 9%

Organic Carbon compounds on Earth = 0.05%

Fossil = 64%; Detritus = 32%; Living mass = 4%

Carbon dioxide - Most oxidised state of Carbon

Link between organic and inorganic forms of Carbon

Sources of CO_2 in sea water

Role of CO_2 (Photosynthesis & Respiration)

Carbon dioxide Cycle: - Within phytomass
- In the ecosystem

Factors influencing Bicarbonate - Carbonate buffer system

- Diurnal variation
- Photosynthesis and respiration
- Excess photosynthesis depletes HCO^-_3 pool
- Hydrolysis of carbonate ions and release of OH^- ions - pH rise
- Phytoblooms - pH rise (afternoon)
- Respiration - pH fall (at night)
- Reversible buffer action - At night

Precaution : Excess bloom – pH rise - stress to organisms

Regulate photosynthesis = Equilibrium

Nutrient Cycles in Coastal Ecosystems: Part- I. Nitrogen Cycle

Nitrogen store house - Living tissue
- Detritus/sediment
- Dissolved form

Cycle of Nitrogen - Within the phytomass (Part)
- Within the Environment (Rest)

Phytomass : Uptake of inorganic form and release of organic form

Environment: Recycling of organics to inorganics

Source: Inputs from neighbouring environments (River and Sea to Estuary)

Nitrogen cycle: Involves four processes - Parabiosis

(i) Nitrogen Fixation: (Incorporation in the cell) (uptake, assimilation, deposition, Utilisation) (eg) Mangrove vegetation

Nitrogen fixing bacteria
- *Acetobactor* (Aerobic)
- *Clostridium* (Anaerobic)
- *Rhizobium* (Symbiotic)

(ii) Ammonification: (Reduction process) : Breaking down of organic N. compounds to simpler forms of Ammonia

(iii) Nitrification: (Oxidation process): Ammonia to Nitrite:
- *Nitrocystis*
- *Nitrococcus*
- *Nitrosomonas*

Nitrite to Nitrate: *Nitrobacter*

(iv) Denitrification: (Reduction process) - (in oxygen deficit environment)

Nitrate to Nitrite (Heterotrophic bacteria)

Nutrient Cycles in Coastal Ecosystems: Part-II. Phosphorus Cycle

Sources - Input from land drainage/rivers

- Input from the Sea during high tide
- *In situ* production by recycling process

Transport - Through Flood flow

- Through tides
- Through migration of organisms

(Removal - Biological and non- biological processes)

Storage - Living tissue, Detritus, dissolved form

- Soluble and insoluble forms
- Organic and inorganic forms

Inorganic phosphorus :	(i) Orthophosphate	- 1%
	(ii) Hypophosphate	- 87%
	(iii) Phosphate	- 12%

(96% of phosphate and 44% of Hypophosphate are present as ions (with Calcium & Magnesium)

Phosphate recycling: Faster than Nitrogen cycle

- partly within the phytomass

(uptake of inorganic P. and release of organic P by excretion and death)

- rest within the environment by microbial action

(In shallow waters/culture systems, recycling occurs mostly in sediments)

- Recycling faster in summer months

By decomposition, zooplankton releases phosphate faster than phytoplankton cells - Role of Protozoans in Phosphorus regeneration.

Phosphorus cycle - Not fully understood. (More research envisaged)

Lecture 15 Prepared by Shri G.S.D. Selvaraj and Smt. Molly Varghese

Organic Production in Coastal Ecosystems

Organic production - Primary, Secondary and Tertiary production

Store house - Living tissues, detritus, dissolved form

- Primary production - (Basic source)
- Methods for estimation of primary production

Sources : River, Sea and *in situ* production

POM and DOM: POM: > 0.45 µm (SPM and Detritus)

- SPM = Living and Non living-Seston (Dry wt in mg / l)
- Primary production (g.C/m^3/day) - Conversion factor
- Secondary production (g.C/m^3/day)-Conversion factor
- Factors influencing primary & secondary production
- DOM : < 0.45 µm (Dissolved organic matter)

Role of Mangroves and other macrophytes

Mangrove ecosystem - Highly productive

Pitchavaram Mangroves : organic production

SOM = 340 million tonnes/year

DOM = 0.1 million ton/year

Factors influencing sedimentation : (physical, chemical and biological)

Estuarine systems : *In situ* production = > 80%

Sedimentation increases organic load

Humus - Fat of the soil

Estimation: Organic Carbon in sediment (%)

DOM estimation: (i) Dry combustion method

(ii) Wet oxidation method

Removal of DOM: Biological and Non biological

Functions of DOM: Growth, behaviour, mating etc.

Harmful effect: (eg) pollutants

Primary Production

Primary production – definition – significance in fisheries – methods of estimating primary production – light and dark bottle oxygen technique – advantages - ^{14}C technique – principle - Chlorophyll *a*, *b*, and *c* estimation – principle and procedure - Determination of total cell counts - principle - rates of primary production in various ecosystems – estimation of potential resources based on primary production – significance of primary production in mariculture.

Factors influencing Primary Production

Physical - chemical - biological factors - effect of monsoon – eutrophication – definition of the problem – natural – artificial - Red tide - toxic algal blooms and harmful effects - Causative organisms - Factors influencing - Harmful effects - Water quality management in algal culture systems - benthic production - Methods of estimation and assessment of potential resources

Primary (Photosynthetic) Production in Aquatic systems
Part-I. Gross Primary Production (L&D O_2 Technique)

Introduction - Methods to determine phytoplankton production
- Cell counts
- Chlorophyll estimation
- C^{14} Technique
- L & D bottle Oxygen Technique

Merits and demerits of different methods

Advantage of Oxygen Technique - Simple and widely used - Provides rate of primary production - Provides gross and net values

Gross primary production = L-D per 12 hrs.

Net primary production = L-I per 12 hrs.

Photosynthetic quotient and conversion factor

Derivation: mg. C. / l = g. C. / m^3

Optimum incubation time - Sea (3 hrs)
- Estuary (2.5 hrs)

Respiratory loss = I-D value

Column production estimation in the sea

Estimation of primary production in Culture ponds

Validity of L-D for G.P.P (L and D bottle components after incubation)

Primary (Photosynthetic) Production in Water
Part-II. Net Primary Production (L&D O_2 Technique)

Introduction - Parameter to assess water quality and potential fishery resources

Light and Dark bottle Oxygen Technique

N.P.P. = G.P.P. minus Respiratory loss by phytoplankton

i.e. (L-I) = [(L-D) - (I-D)]

Factors influencing I-D value: - Respiration by phytoplankton
- Respiration by micro zooplankton
- Bacterial reactions (oxidation - reduction processes)

Validity of L-I values for NPP in primary production experiments

Light bottle components (after incubation)

Factors influencing Light bottle samples (coastal waters)

Result: N.P.P. value is masked (by bacterial interference)

Rectification : Improvement of Formula for N.P.P

Determination of Respiratory loss by phytoplankton during photosynthesis

N.P.P. = 80% of G.P.P = 0.8 (L-D) 12 hrs

Water quality Test = % of L-I in L-D value

> 75%	= Best quality
50-75%	= Better (Replenishable)
25-50%	= BOD more (Water exchange advised)
<25%	= Not good

Methods of estimation of primary production of macrophytes.

Estimation of biomass by quadrate method and analysis of frequency, Density, abundance and percentage of cover in a particular distribution - Estimation of photosynthetic pigment during different period of growth – Chlorophyll-carotenoid-phycoerythrin-phycocyanin-allophycocyanin- their structure-qualitative characterization-Rate of oxygen evolution - Using standard oxygen electrode - Light and dark bottle method – DO of the benthic macrophytes-Polarographic method –Estimation of DO of seaweed and microalgae-Rate of respiration-Need for dark adaptation of the algae- Infra red gas analyzer (IRGA)-insitu photosynthetic activity- Rate of CO_2 utilization – use of stable isotope-Measurement of production through $^{14}CO_2$ techniques using enzyme RUBP carboxylase -Short term exposure - Long term exposure - Use of GM counter - Liquid Scintillation counter –identification of the intermediate compound-Specificity of PSI and PSII photosystem by mild detergent treatment- Efficacy of both the system- methyl viologen test and DCPIP reaction-Use of artificial electron donor and electron acceptor - Using inhibitor like KCN, Sodium azide and herbicide in the electron transport chain - Fluorescent kinetics and quantum yield- Use of Oscilloscope – slow kinetics and fast kinetics

Classification of major phytoplankton organisms

Diatoms – taxonomy – identification – features – cell characteristics – structure – reproduction – significance as feed

Dinoflagellates – taxonomy - identification – features - structure – cell characteristics – reproduction – significance - Red tide - Harmful effects to the aquatic organisms

Silicoflagellates – taxonomy - identification – features - structure – cell characteristics – reproduction - significance

Coccolithophores - taxonomy - identification – features - structure reproduction - significance

Blue Green Algae - Role of blue – green algae – structure – reproduction - Red tide - Harmful effects to the fauna

Nannoplankton - Organisms – Importance in hatcheries – Role of phytoplankton in food chain

Classification of macrophytes in coastal water bodies

Coastal vegetations - environmental influence on coastal vegetation, exposure to seawater - tidal amplitude - distance from the sea - topography - soil condition-Marine algal vegetation in littoral and sub littoral area – Chlorophyta- Phaeophyta- Rhodophyta- important characteristics for classification- branching pattern- morphology of the thallus- reproductive structure- pigment constituents- apical structure- dichotomy- distribution of marine algae in different depth- light an important factor influencing vertical distribution- Algal vegetation of salt and brackish water –salt tolerant variety- estuarine variety- aquatic weed – floating- submerged- partially submerged- Vegetation in sand dunes – Xerophytes distribution-characteristic features -Specialized vegetation associated with drift-line - Vegetation in shingle beaches - Vegetation in coastal cliffs – Mangrove

Classification of Zooplankton

Introduction - Marine Habitat - Drifting life of the environment - Classification – Phylum–Class– Order – Family. Characteristic features of common zooplankters. **Phylum Protozoa-**S.phylum Sarcomastigophora - Class Chrysomonadina Order Dinoflagellida - *Noctiluca, Ceratium, Peridinium* - Class Rhizopoda- Order Foraminiferida -*Globigerina*- **Phylum Coelenterata (Cnidaria)** -Class Hydrozoa - Order Hydroida - *Obelia, Liriope* Order Siphonophora, *Physalia, Porpita.* Class Scyphozoa –*Aurelia*-**Phylum Ctenophora**–Class Tentaculata– Order Cydippida– *Pleurobrachia*- **Phylum Bryozoa** – Cyphonautes **Phylum Brachiopoda** – Lingula&Pelagodiscus **Phylum Phoronida** – Actinotrocha **Phylum Chaetognatha** – *Sagitta enflata*- **Phylum Annelida** – Class Polychaeta (Bristle worms) – *Tomopteris*- **Phylum Arthropoda** - Class Insecta- Class Crustacea – S.class Cirripedia- Order Thoracica- Families Balanidae & Lepadidae- S.class Ostracoda- S.class Branchiopoda- Order Diplostraca- S.order Cladocera- *Evadne, Penilia* - S.class Entomostraca- Order Copepoda -S.orders Calanoida, Cyclopoida and Harpacticoida- S.class Malacostraca- Super Order Peracarida- Order Mysidacea- Order Amphipoda- Order Isopoda- Order Tanaidacea- Family Tanaidae- Super Order- Eucarida- Order Euphausiacea -Order Decapoda – S.order Natantia- Tribe Penaeidea- Family Penaeidae – *Penaeus, Metapenaeus* Family Sergestidae – *Lucifer, Acetes*- Tribe Caridea – S.order Reptantia – Families Palinuridae & Scyllaridae (lobsters) S.order Anomura–S.order Brachyura –S. order Hoplocarida –Stomatopods - **Phylum** Mollusca –Class Gastropoda- Order Heteropoda – Order Pteropoda –Class Lamellibranchiata **Phylum Echinodermata** – Larval forms - Class Asteroidea– bipinnaria-brachiolaria- Class- Echinoidea- echinoplutei - Class Ophiuroidea–ophioplutei Class Holothuroidea –auricularia-doliolaria - pentacularia

Phylum Chordata – S.phylum Urochordata (Tunicata) Class Thaliacea, Order Doliolida –*D. gegenbauri*- Order Salpida –*S. democratica*- Order Pyrosomida, *P.atlanticum.* Class Copelata (=Larvacea=Appendicularia) *Oikopleura, Frittilaria*

Factors influencing zooplankton abundance & distribution

Introduction - Lower trophic level consumers - principal herbivorous component - spatial & temporal changes - biological & physico-chemical conditions of the marine environment

Factors affecting zooplankton abundance – Physico chemical - Temperature – thermocline – moulting – spawning - Light – vertical migration - Salinity – tolerance - Dissolved oxygen level – optimum - Water movement - Tidal currents - Upwelling - Turbidity – Biological - Food variability – scarcity – predation - competition - Environmental preferences – Multiple strategies

Marine ecosystems – Life cycle – Holoplankton - meroplankton - tychoplankton - larval behaviour and abundance - faunal assemblages in relation to the habitat

Estuarine ecosystems - Circulation pattern – grazing - patchy distribution - tolerance - riverine discharge - environmental stress – biotic & abiotic factors of importance - prey–predator interactions - seasonal changes in community structure

Assessment of Secondary production (Part I)

Introduction - The linear food chain concept - trophic levels - primary – secondary - tertiary consumers - production - secondary production definitions

Basis for production measurements - Two main approaches : a) Observational (field data) b) Experimental

a) **Observational:** recruitment time method - definition - generation time method - definition - growth increment method – definition - using identifiable life stages – copepods

1. Growth increment method

B = N x w, where **B** - biomass, **N** - number of individuals in a population, **w** - mean weight of an individual. Production (P_t) during a time interval t_1 to t_2 ,

$P_t = (N_1 - N_2) \frac{w_1 + w_2}{2} + B_2 - B_1$, $B_2 - B_1$ - increase in biomass

during the time interval, $t_1 - t_2$ and the subscripts **1, 2** pertain to t_1 (initial) & t_2 (final) - concept of production of zooplankton population - definition.

P_t (annual production) - $P_t = P_{t1} + P_{t2} + P_{t3} ------ P_{ti}$

Crustaceans – determinate growth pattern - each stage limited by size of exoskeleton

2. Using primary production, predict secondary production & yields of fish

E, Ecological Efficiency (Definition) – Approximated from - **Transfer Efficiency (E_T)**

$E_T = \frac{P_t}{P_{t-1}}$ Annual production at trophic level **t**

P_{t-1} Annual production in the preceding trophic level $_{t-1}$

$P = BE^n$, where, **B** - annual primary production, **E** - the ecological efficiency and **n** - the number of trophic levels

Prediction – Wet wt of fish & dry wt of planktivorous fish – method

3.Production to Biomass (P/B) ratio :

b) experimental :
(i) Laboratory-scale experiments
(ii) Enclosed ecosystem experiments
(iii) Computer model simulations

Assessment of Secondary production (Part II)

Theoretical concepts and applications - Secondary production – regularity & irregularity in the production cycles - Higher latitudes - Mid latitudes

Tropics - principal pathways - open ocean **a)** Microbial loop – DOM - bacteroplankton (0.2 – 2.0µm) – heterotrophic protists-metazoal zooplankton – fish **b)** Picoplankton (prokaryotic & eukaryotic cells, 0.5-2.0 µm) –bacteroplankton - metazoal zooplankton - fish **c)**Traditional food chain – phytoplankton – zooplankton – fish - In estuaries – In coral reefs - In atolls –In mangroves

Volume of water in m^3 filtered by the zooplankton net fitted with a digital flow meter

$V = \frac{A \times R}{K}$ Where, **A** - the area of the mouth of net ring in m^2 (Πr^2)
R - revolutions of flowmeter during operation of the net.

$K = \frac{R}{L}$ $\frac{\text{(Revolutions)}}{\text{(Length of tow)}}$

Calibration factor of flowmeter - **(m/rev)**

Average displacement volume of the zooplankton in ml/m^3 converted to ml/m^2 for a particular depth using the formula, $N \times \frac{D}{V}$ where

N = Nos. or Dis.Vol. of zooplankton

D = Depth in m

V = Volume of water in m^3

Assuming zooplankton standing stock in steady state - secondary production estimated as 1 ml = 0.025 g cm^{-2} - modified method - The annual secondary production - the average generation time of tropical copepods (18 days) - multiplying with 365/n, where n - the generation time of copepods.

Ichthyoplankton of inshore waters (Part - I)

Introduction - Ichthyofauna spawning ground – spawning season Ichthyoplankton survey - Methods used in identification of fish eggs and larvae - series method - hatching method.

Sampling - Sampling design - quantitative – qualitative - standard sampling. Sampling methods – tows - sampling with plankton nets – Bongo net – gauze – cod end bucket – filtration efficiency – flow meter - closing net – tripping mechanism – messenger

Quantitative analyses - Zooplankton volume determination - fractioning sample – Folsom splitter.

Qualitative analyses - Sorting fish eggs and larvae - counting – counting chamber

$$\text{Standardised number of spawn products (No. / } m^3) = \frac{N \times D}{m^3}$$

Where, N = Number of eggs/larvae

D = Depth of collection (in m)

m^3 = Volume of water filtered

Ichthyoplankton of inshore waters of the sea (Part II)

Structure of fish egg and larva – terminologies - definitions

Guidelines - identification - **fish eggs-** Egg size – shape - extent of perivitelline space - nature of egg membranes – Yolk - oil globule – number-size & position - embryonic characters

Guidelines - identification **- fish larvae -** Morphometrics - measurement of body parts - fin positions - Meristics - number of myotomes / vertebrae – spines / rays - Pigment pattern - type – nature – position - changes during development - Specialised characters peculiar shape of body / eye - elongation of spines / rays

Common coastal **Ichthyoplankton** families – salient **diagnostic** characters -

Order Anguilliformes – Family Ophichthidae (snake eels)

Order Clupeiformes – Family Clupeidae (sardines) - *Sardinella longiceps* myotome 48 - Family Engraulidae (anchovies) - *Stolephorus punctifer* myotome 42.

Order Cyprinodontiformes – Family Hemiramphidae (half beaks) - Family Belonidae (needle fishes) - Family Oryzidae (rice fishes)

Order Myctophiformes – Family synodontidae (lizard fishes)

Order Scorpaeniformes – Family Platycephalidae (flat heads)

Order Perciformes – Family Ambassidae (glass fishes) - Family Carangidae (trevally/scad) - Family Leiognathidae (silver bellies) - Family Lutjanidae (snappers) - Family Sciaenidae (croakers) - Family Scombridae (mackerel, tuna) mackerel 31, tuna 39-42.

Order Mugiloidei - Family Mugilidae (mullets)

Order Gobioidei – Family Gobidae (Gobies)

Order Pleuronectiformes – Family Bothidae (left-eye flounders) - Family Pleuronectidae (right-eye flounders) - Family Cynoglossidae (tongue fishes) - Family Soleidae (Soles)

Soil / Sediment – An Overview and Physical properties of soil

Soil – definition – submerged soil – sediment - surface soil – subsoil - soil profile –soil horizon –mineral soil - organic soil – Pedology - definition – Edaphology – definition –soil components - soil solids - inorganic – organic – soil water - a dynamic solution – soil air – a changeable constituent – soil as a biological laboratory – factors affecting nutrient availability in soil – soil as a colloid – definition of colloid – clay colloids – silicate clays and hydrous oxide clays – sources of negative charge in clay colloids – amorphous colloids in soil – organic colloids in soil – humus – composition – properties

Physical properties of soil: Soil depth – Soil texture – definition – the USDA classification of soil into textural classes - textural triangle diagram – Particle density of soil – factors affecting - unit of expression – Bulk density – a measure of compactness of soil – unit of expression – Pore space in soil – formula for calculation of pore space – Structure of soil – definition – types of soil structure – aggregation - role of binding agents – Soil consistence – definition – plasticity indices – soil cohesion – factors responsible for cohesion – soil cementation – cementing agents – levels of cementation of soil –swelling and shrinkage of soil – soil colour

Sedimentation process: What is sedimentation - Mechanism of sedimentation - Small scale process - Gravitational process – Entrainment process - Large scale process - Factors influencing sedimentation - Physical – deforestation – tidal / fresh water currents - semidiurnal tides – topography - upwelling currents - density currents - depth of estuary - Chemical - flocculation - chemical interaction - pollutant reactions - Biological - microbial organisms - mangrove roots- benthic algae and invertebrate feeders - uses of sediments - disadvantage of over sedimentation

Chemical properties of soil / sediment

Ion exchange - definition – cation exchange – anion exchange – factors affecting ion exchange – unit of expression – effect on nutrient availability

Soil reaction - definition – pH – active acidity- exchange acidity - reclamation of acidic and alkaline soils – nutrient availability in relation to pH – pH kinetics in aerobic soil and submerged soil – pH of different kinds of sediments

Specific conductance – definition – measurement – properties of salt affected soils - kinetics of specific conductance in aerobic and submerged soil

Oxidation - reduction potential – definition – differentiation in aerobic and submerged soil – measurement – factors affecting Eh – effect on marine environment – sequential reduction in lakes

Organic carbon – origin in soil – effect on soil fertility – decomposition of organic matter – mineralization and immobilization in aerobic and submerged soil – C/N ratio – factors affecting mineralization

Nitrogen – forms of occurrence in sediment – the nitrogen cycle in sediment – aminisation – ammonification – nitrification – nitrogen fixation – denitrification – factors affecting nitrogen availability

Phosphorus – forms present in sediment – factors affecting phosphorus availability – phosphorus fixation by sediment in acidic and alkaline conditions

Potassium – the potassium cycle in sediment – factors affecting potassium availability

Sulfur – forms of sulfur present in sediment – the sulfur cycle – factors affecting sulfur availability

Iron – forms of iron present in sediment – factors affecting iron availability - **Manganese** – forms of manganese present in sediment – factors affecting manganese availability - **Silicon** – forms of silicon present in sediment – factors affecting silicon availability

Sediment - Water Interactions

Dynamics of dissolved oxygen in water and sediment – stratification in lakes – Adsorption of chemical substances by mud and release into the water based on redox potential – changes in the nitrogen economy of bottom muds - Phosphorus sorption by mud and release into water based on change in pH – Sorption and release of heavy metals by bottom mud based on changes in salinity

Minerals and trace elements in aquatic ecosystem: Eutrophication – definition - nutrient load - Primary nutrients – Nitrogen, Phosphorus, Potassium – forms of occurrence – source – functions – favourable range in brackish and marine environments - Secondary nutrients – Calcium, Magnesium, Sulfur and silica - functions – favourable range in brackish and marine environments - Micronutrients – Chlorine – Manganese – Iron – Copper – Zinc - Cobalt - functions – favourable range in brackish and marine environments

Decomposition of organic matter in bottom sediment: Types of decomposition of soil organic matter – aerobic – anaerobic – factors affecting aerobic decomposition - factors affecting anaerobic decomposition – differentiation of end products in aerobic and anaerobic decomposition - influence of microbes – effect of type of carbonaceous material present in the environment - Effect of C/N ratio on decomposition of organic matter – influence of organic matter decomposition on nitrogen cycle - Effect of C/P ratio in organic matter decomposition and phosphorus cycle - Effect of C/S ratio in organic matter decomposition and sulphur cycle - Functions of soil organic matter

Occurrence and distribution of microbes in seawater (Part I)

General Marine environment – importance of marine microbiology- microbial standing crop- microbial elements- bacteria- fungi- yeast- flagellates- ciliates- unicellular/ multicellular microalgae-Factors influencing the distribution of bacteria in the sea - fluctuations in numbers of microorganisms - distance from land - effect of tides - diurnal fluctuations in the bacterial population - vertical distribution of marine bacteria - effect of solar radiations - temperature as an ecological factor - seasonal distribution of marine bacteria - effect of other organisms - the antagonistic effects of microorganisms - bacteriophage in seawater - effect of solid surfaces - effect of sedimentation - effect of organic matter;

Physico-chemical factors influencing bacterial distribution – light - turbidity – pH - redox potential – salinity - inorganic and organic substances - dissolved gases - Biological factors influencing bacterial distribution - competition for nutrients - bacteria and fungi as food for other organisms – photosynthetic bacteria- bacteria and fungi parasitized by other microorganisms.

Habitats for microorganisms – Neuston / pleuston – Nekton - Epibiotic habitat - Endobiotic habitat; Water borne microbial habitats in sea – Epipelagic – Mesopelagic –Bathypelagic – Abyssopelagic - Benthic habitats.

Occurrence and distribution of microbes in seawater (Part II)

Marine sediment- redox potential- bottom rich in organic compounds- negative Eh in clayey sediment- reducing bacteria increase – Eh decrease and depth- coarse sediment – less reducing- pH- increase and depth- negative Eh correlates with hydrogen sulphide *(Desulphovibrio)*- heterotrophic count reduce with depth- bacterial population in different types of sediment- sand- silt- clay- colloid- microbial population of sediments – their functions- total aerobes- total anaerobes- ammonification- urea fermentation- gelatin liquefaction- denitrification (to nitrogen)- nitrate reduction (to nitrite)- starch hydrolysis- glucose fermentation- xylose fermentation- cellulose decomposition- fat hydrolysis- chitin digestion- red clays in bottom - vertical distribution of bacteria in mud - factors influencing abundance of bacteria in mud - microbial oozes- siliceous and calcareous oozes- calcium carbonate precipitation- zooxanthellae- coral reef formation- microbes in estuarine sediments- organic- high sulphide- organic matter- purple bacteria- bloom of cyanobacteria- later diatom bloom- bacteria are heterotrophic- more chitin and lignin degrading microbes (*Actinomycetes*)- proteolytic bacteria maximum- chitin digestion- gram negative rods dominate surface -Culture Systems - Microbial habitats - Water- Sediment - Cultured organisms- autotrophs- chemoautotrophs- heterotrophs- Sampling methods - surface sampling systems - water column samplers - net samplers - water samplers - bottom sampling systems - grab samplers;

Lecture 34

Prepared by Dr.Imelda Joseph

Methods for estimation of bacterial population

Determination of biomass - direct methods –sample preparation -Microscopic examination – fresh samples / preserved samples - direct counts- aliquot sample on slide-haemocytometer- count organisms in the field of microscope- epifluorescence microscopy - phase contrast and light microscopy - Direct counting- slide count- viable counts- absolute concentration- viable organisms- principle- microbial population – diluted- sample preparation – homogenized sample- dilution procedure- serial dilution- 1 ml to 10 ml- choice of dilution- pour plate method- solid media- liquid culture- 200 to 300 viable colonies ideal- less error; Isolation and purification of major groups of microbes from culture systems – classification prokaryotes- bacteria- cyanobacteria- archaeobacteria- classification of bacteria- autotrophs- anaerobic- sulphur bacteria- *Chlorobium; Chromatium, Rhodospirillum*- Heterotrophs- aerobic; *Vibrio* –32% total bacterial population- Pseudomonas- Alcaligenes- flavobacterium- *Bacillus, Micrococcus- Cytophagae- Enterobacteriaceae*- contaminants- common shapes of bacteria- spherical coccus- rod shaped bacilli/ mostly single cell- spiral spirillum/ mostly single cell- common arrangement of bacterial cells- pairs/ diplo; parallel chains/strepto; irregular clusters/ staphylo; regular packets-perpendicular 4 cells each/tetrad/ 8 cells each Sarcinae- basic techniques - sterilization of media and equipments - culture vessels - enrichment cultures - enrichment by direct plating - membrane filtration-characteristics of membrane filters- cellulose esters- pore size- application in microbiology- Isolation (Pure cultures) - streak plate method - pour plating - dilution in a liquid medium - preservation of cultures – Methods- Identification - diagnostic tables.

Faecal pollution through sewage

Survival of indigenous and non-indigenous organisms in the aquatic environment - Microflora and sewage – domestic- waste water- remains of food- Faecal contaminants –process- removing pollutants continuously- physical- sedimentation- chemical- oxidation- biological- fish- microbes- decisive role- bacteria- fungi- organic compound breakdown- solids and liquids to carbon dioxide- water- inorganic salts- remineralization- change in microbial population- domestic sewage- proteolytic to cellulose degrading- human pathogens in sea water – *Salmonella* – *Shigella* – *Leptospira* - *E.coli* -*Vibrio cholerae* - *Mycobacterium tuberculosis*.

The role of microorganisms in the self-purification of waters - Pollution by refuse and sewage - natural self-purification of polluted systems – putrefying bacteria- groups breaking down sugar- starch- fat- urea- cellulose- coliform bacteria- indicator- faecal matter- *Escherichia coli*- *Streptococcus faecalis*- bacteriophages- anoxic conditions- H2S production- sulphur oxidizing bacteria- denitrifiers- iron bacteria- methane producers- how and extent of self-purification - factors affecting self-purification - change in population of micro organisms during different stages of self-purification – bioremediation in culture systems of pollutants;

Hydrogen sulphide production in mariculture systems

Suphur- occurs in different forms- marine environment- hydrogen sulphide gas- sewage/ rotten egg smell- detection level- 0.002 mg/l- human beings- Heterotrophs precipitating metals as sulphides - hydrogen sulphide formation - during the decomposition of proteins - from reduction of sulphates - fixation in sediments as metallic sulphides – escape into the overlying water - depletion of oxygen – role of anaerobic conditions - Sources of hydrogen sulphide prduction – Causes for production of hydrogen sulphide - Microbes involved in hydrogen sulphide production – microbes involved in oxidation of sulphide - role of oxygen – role of environmental conditions- aerobic decomposition- organic matter- anaerobic decomposition- reducing bacteria-

Microbial Sulphur cycle - liberation of sulphur from organic compounds - sulphate reduction - bacteria involved in the process - oxidation of sulphur compounds – role of achromic sulphur bacteria – role of purple sulphur bacteria.

Role of microbes on the regeneration of nutrients

Production of organic matter - chemo-autotrophic bacteria occurring in the aquatic habitat - breakdown of organic matter – proteolysis – lipoclastic / lipolytic bacteria - decomposition of carbohydrates - lignin decomposition - chitin decomposition - bacterial oxidation of hydrocarbons - methane fermentation by methanogens - marine humus – formation

Nutrient cycles in marine and estuarine systems – CHNOPS- occur in different forms- change in chemical form- aerobic- anaerobic- activities- soil microbes- cycling of carbon- nitrogen- sulphur- material interconversion- carbon cycle- carbon dioxide fixation- photosynthesis- chemosynthesis- forms of carbon- plant derived compounds- aerobic- anaerobic cycling- fermentation- respiration;

Nitrogen cycle - ammonia production - bacterial oxidation of ammonia - autotrophic bacteria involved in oxidation of ammonia - oxidation of nitrite to nitrate – bacterial genera involved in the process - reduction of nitrate to nitrite - bacterial genera involved in the process - nitrogen fixation - bacteria involved in the process.

Phosphorus cycle - assimilation of phosphate by microorganisms - regeneration of phosphate from phospholipids and nucleioproteins – microorganisms involved in the process - effect of bacteria on solubility of phosphate.

Lecture 38

Prepared by Dr.Reeta Jayasankar

Role of aquatic weeds in estuarine culture system and weed control

Classification of aquatic weed

Floating type : *Lemna minor, Nymphaea alba,Eichhornia, Salvinia, Pistia* - Submerged type : *Elodea, Myriophyllum, Ceratophyllum* - Emerged type : *Phragmites, Sagittaria, Typha, Nymphaea, Nelumbo* - Algae: *Enteromorpha*

Control of Aquatic weed

Mechanical control - Manual method: - Emergent weed: cutting with suitable tools - Free floating weed:Floating bamboo rafts, nets to encircle the weed and pulling ashore - Submerged weed: Pulled by hand at regular interval - Power operated machines - Motorised hand tool - - Non motorized method for free floating type - Fully powered weed cutter - General consideration - Involvement of local people and resource - Pilling these weeds and burning - Choosing best time for harvest eg. Typha at flowering and post flowering stage delays re growth - Chemical method - Using herbicides - Free floating - Water hyacinth and Eichhornia : 2,4 D, 2,4D sodium salt, 2,4D urea - Salvinia:Paraquat - Pistia straitiota:2,4D ester and 24D butunyl ethanol - Floating rooted - Nymphaea : 2,4 D BEE - Nelumbo : 2,4D salt - Rooted submerged - Phargmites, Typha: Delapan - Ipomea, Typha, Cyperaceae:Amitrole - Biological control - Use of competitive organism: Use of Flea beetle (*Agasicles hygrophila*) in south eastern U.S to suppress alligator weed *Alternanthera philoxeroids*

Prepared by Dr. Geetha Antony

Potential Live Feeds of Coastal Waters

Introduction - Importance of live food organisms in culture of fin / shell fishes - Live feed and target organisms – size - mobility - nutritional value

Live feed organisms - **Infusoria** - Taxonomic position – Morphology - Food and feeding habits - nutritional requirements – Reproduction - Evaluation and improvement of nutritional value - probiotics – **Rotifers** - Taxonomic position – Morphology - Food and feeding habits - nutritional requirements – Reproduction - Evaluation and improvement of nutritional value - probiotics – **Cladocerans** - Taxonomic position – Morphology - Food and feeding habits - nutritional requirements – Reproduction - Evaluation and improvement of nutritional value - probiotics – **Artemia** - Taxonomic position – Morphology - Food and feeding habits - nutritional requirements – Reproduction - Evaluation and improvement of nutritional value - probiotics – **Copepods** - Taxonomic position – Morphology - Food and feeding habits - nutritional requirements – Reproduction - Evaluation and improvement of nutritional value - probiotics – **Mysids** - Taxonomic position – Morphology - Food and feeding habits - nutritional requirements – Reproduction - Evaluation and improvement of nutritional value - probiotics

Lecture 40 Prepared by Dr. Geetha Antony

Water Quality Issues in the Maintenance of Live feeds in Laboratories

Introduction – Isolation - pure culture - stock culture - mass culture

Culture of live feeds - Infusoria – Culture Methods - Rotifers – Culture Methods - Cladocerans – Culture Methods - Artemia - Culture Methods – Copepod - Culture Methods

Maintenance of live feed in laboratory – factors affecting live feed culture - Physical - Light - Temperature – Chemical - Dissolved oxygen - Salinity - pH - Ammonia - Nitrite - Biological - Feeding strategies - Bloom / swarm - Infestation - Effect of factors on - Survival - growth - reproduction - production

Lecture 41 Prepared by Dr. D. Prema

Toxicities and Optimum ecological conditions in ponds

CO_2 toxicity – effect of CO_2 toxicity on fish – conditions leading to CO_2 toxicity - effect of normal levels of CO_2 in pond environment - Toxic forms of nitrogen – ammonia and nitrite toxicity - Source of ammonia – factors affecting ammonia toxicity – effect of ammonia on fish – lethal concentration and sublethal effects on fish - Nitrite toxicity – effect on fish - methaemoglobinemia – sublethal effects - source of nitrite – factors affecting nitrite toxicity - effect of chloride on nitrite toxicity - H_2S toxicity – formation – factors affecting H_2S toxicity – effect of H_2S toxicity on fish - Correction of different toxicities - methods

Optimum and adverse levels of various water quality parameters and their effect on fish - DO – pH - CO_2 - BOD - COD - ammonia N - nitrite N - nitrate N - H_2S - CH_4 - chlorine - heavy metals - pesticides - Favourable range of soil parameters for mariculture - soil nature - soil colour – pH - water retention capacity - sand - silt - clay – nitrogen – phosphorus - potassium - organic carbon - electrical conductivity - Classification of muds based on their fish / shrimp production potential with respect to different soil parameters

Lecture 42 Prepared by Smt. Molly Varghese

Water Quality Management in Culture and Hatchery Systems

Introduction - Water quality problems – causes - natural quality of water - concerns with events develop as a result of culture activities - concerns with pollutants which may enter from surroundings

Improvement of water quality – methods - Liming – when needed - advantages - liming material - liming rate - method of application Fertilization – when required – advantages - chemical fertilizers - organic fertilizers or manures – rate, frequency and method of application – Aeration - Circulation - Water exchange - Biofilters in marine hatchery - advantages

Phytoplankton control – use of algicides use of plankton feeding fish – use of macrophytes - water change - nutrient manipulation - Other treatments - application of $KMnO_4$ - treatment with chlorine – application of piscicides - application of ammonia – application of mahua oil cake – application of chlorinated hydrocarbon – application of insecticides – application of tea seed cake - turbidity and sedimentation – treatment with alum

Biological factors affecting water quality – Phytoplankton - other aquatic plants - zooplankton