SHORT-TERM TRAINING COURSE IN BACKYARD HATCHERY TECHNOLOGY FOR WHITE PRAWN

PENAEUS INDICUS

COURSE CONTENT

CENTRAL INSTITUTE OF BRACKISHWATER AQUACULTURE

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INFRASTRUCTURE FACILITIES NEEDED FOR THE BACKYARD HATCHERY AND THE BACKYARD HATCHERY TECHNOLOGY

A. Laxminarayana

a) Infrastructure facilities:

For the successful operation of the backyard hatchery, the following facilities are needed:-

1. 5 H.P Pumpset for pumping seawater
2. 1 H.P Pumpset for water exchange
3. Overhead tank for water storage
4. 2 H.P Air Blower (three phase)
5. 1 H.P Air Blower (single phase)
6. 7.5 H.P Generator
7. 2 t FRP tanks for larval rearing
8. 1 t FRP tank for the culture of phytoplankton
9. 200 litre tanks for spawning
10. Chemicals for phytoplankton culture
11. Squilla powder for feeding the postlarvae
12. Domestic mixie
13. Standard sieves (100, 200, 500 and 1000 microns)
14. Aeration grid and aeration system
15. Nylobolt silk cloth for filtering seawater (50 micron)
16. Glasswares
17. Plasticwares
18. Closed meshed velon netting
19. Seed transportation bags (8 litre capacity)
20. Oxygen cylinder (75 kg/cm^2)
21. pH meter
22. Refractometer
23. Freshwater supply system

b) The Backyard Hatchery Technology:-

The backyard hatchery system for the production of *Penaeus indicus* seed involves minimum exchange of seawater. This system will be specially useful in places where the availability of seawater is a problem. The feeds used here are mixed culture of phytoplankton and mantis shrimp powder.

The nauplii are stocked in 2 tonne tank at a stocking density of 100 nauplii/litre in 300 litres of filtered seawater of salinity 32$\pm$1 ppt. When the larvae reach the protozoea I stage about 100 litres of seawater and separately cultured mixed culture of phytoplankton dominated by *Chaetoceros* sp. is added to the larval rearing tank. Everyday, till the larvae transform to the postlarval state, seawater and mixed culture of phytoplankton is added to the larval tanks depending on the concentration of phytoplankton as indicated by the intensity of colour.

From postlarval stage I onwards, squilla powder is given as feed in three divided doses. From PL3 to PL5, 1/3 of the water in the tank is changed on alternate days. From PL3 onwards the salinity of the medium is gradually brought down. From PL7 onwards 50% of the water in the larval tank is changed on alternate days, using brackishwater. By the time, the larvae attain the PL20 stage the salinity of the tank will be 15 to 16 ppt. This seed (PL20) can be stocked directly in the grow-out ponds without acclimation.

The particle size of the squilla powder supplied to the postlarvae is as follows:-

PL1 to PL5	- 200 micron
PL5 to PL15	- 500 "
PL15 to PL20	- 1000 "

Throughout the rearing period, aeration is provided to the rearing tank from a 2 H.P air blower.

The method described above gives only guideline procedures. By paying careful attention to water quality and condition of the larvae, the volume of water exchanged and the amount of feed given should be judiciously varied to meet the exigencies of the situation.

The average survival rate from nauplius 1 to PL20 will be 35% in the case of Penaeus indicus.

This system of hatchery production of shrimp seed can be used to establish a chain of backyard hatcheries in the country.

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BROODSTOCK DEVELOPMENT AND SPAWNING OF PENAEID SHRIMPS

A. Laxminarayana

At the outset, some aspects of penaeid reproduction relevant to broodstock development and amanagement are summarised below:-

I a) Reproductive Biology

In nature, penaeid shrimps breed only in the sea. They are sexually dimorphic, the male being generally smaller than the females. The males mature even in the brackishwater ponds but the females normally do not attain full ovarian development in such ponds. The males produce non-motile sperms which are packed inside spermatophores. At the time of mating the male transfers the spermatophores with the help of its petesma to the thelycum of female. In penaeids with a closed thelycum (e.g. *Penaeus indicus*, *P. monodon* etc.).mating takes place between a freshly moulted "soft" female with immature ovaries and the spermatophores are tucked safely inside the seminal receptacles close to the thelycum where they are retained until the shrimp moults again; there is a time lag between mating and spawning. In penaeids with open thelycum (e.g. *P. styliferus*, *P. vannamei*, *P. stylirostris* etc.) mating takes place between a "Hard" intermoult female with ripe ovaries and a hard mature male in the intermoult phase; the spermatophores are attached superficially on the surface of the thelycum and can easily be dislodged; spawning takes place soon after mating. In both types of penaeids, at the time of spawning the male is not present; the female simultaneously releases the eggs from the oviduct and the sperms from the spermatophores and fertilization takes place in seawater.

Egg production in penaeid shrimps, as in other crustaceans, is a cyclic process under the hormonal control of the neurosecretory centres. Among these centres, the X-organ sinus gland complex in the eyestalk produces the gonad inhibiting

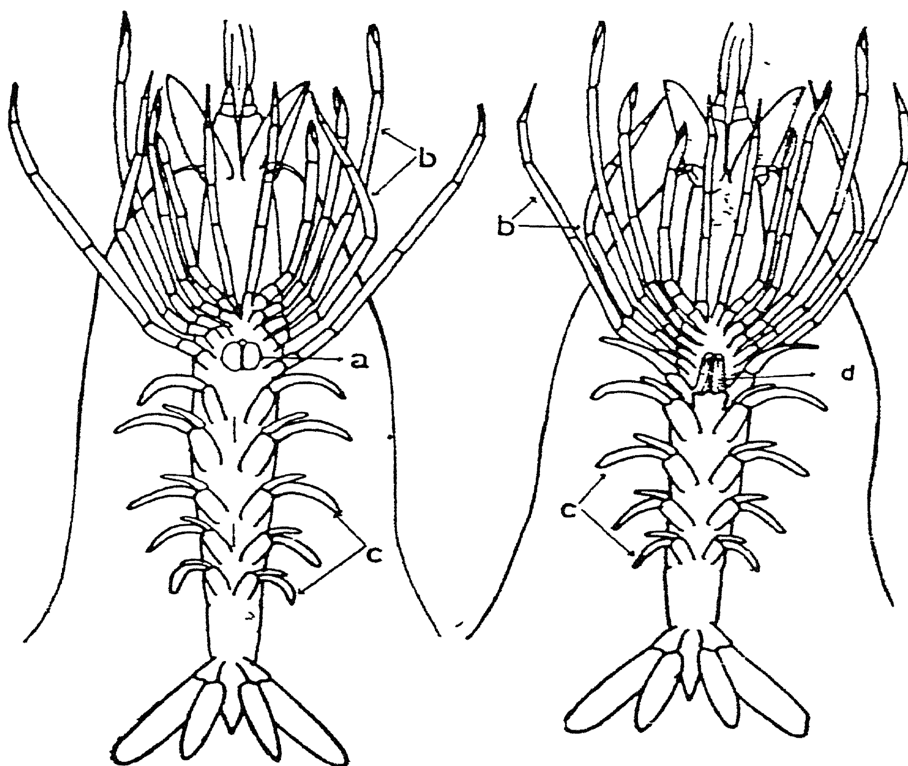

Fig. 1. *Penaeus indicus*: Male (right) and female (left). a. thelycum, b. walking legs, c. swimming legs, and d. petasma.

(Silas *et al.*, 1985)

hormone (GIH) which inhibits vitellogenesis, while the centres in the brain and thoracic ganglia produce the gonad stimulating hormones (GSH) which promote vitellogenesis. During the quiescent phse of the ovary, the X-organ seems to produce a high titre of GIH which restrain vitellogenesis either directly or through its action on the neurosecretory centres which produce the GSH. In nature, when the physiological and environmental conditions are conducive to reproduction, the titre of the GIH secreted by the X-organ complex is probably reduced, thereby allowing vitellogenesis to proceed under the influence of GSH. On the basis of this hypothesis, the technique of unilateral eyestalk ablation has been evolved for inducing the penaeid shrimps to mature in captivity. By removal of one eyestalk, the titre of GIH is arbitrarily reduced and this leads to ovarian development.

II Broodstock management and spawning:

The unilateral eyestalk ablation technique is employed for inducing the shrimps to mature in captivity.

1. Size of prawns:- The size of the shrimps used is critical. In the case of *P. indicus* the females should be larger than 145 mm in total length (20 g) and the males larger than 140 mm total length (17 g). The shrimps may be collected either from the sea or from the culture ponds. The females of this size collected from sea or ponds are usually impregnated, but have immature ovaries and the males are fully mature with the white spermatophores visible at the base of the 5th walking legs. Eyestalk ablation is done on females and not normally on males.

2. Eyestalk ablation:- A small portable electrocautery apparatus is used for eyestalk ablation. One of the eyes is cut by passing the red hot loop of the cautery through the middle of the eyestalk. The optic ganglia and the related neurosecretory centres which produce an ovary inhibiting hormone are removed by this process. Cauterisation seals the cut end and prevents bleeding. Mortality due to cauterisation is nil. Other forms of ablation involves cutting of the ye-stalk with a pair of

increases above 34 ppt due to evaporation it may be brought down by adding freshwater to maintain the salinity between 29 and 34 ppt.

In a commercial hatchery, although everyday monitoring of ammonia and nitrite levels is not required, base-line information as well as occasional checks on these parameters are essential. In case of acute water quality management problems, expert advice may be needed.

If shrimps suitable for ablation are readily available it is advisable to discard the spent females and use a fresh batch of ablated females for every hatchery run. The seawater in the maturation tank is also totally replaced before introducing a fresh batch of ablated females into the tank. If it is difficult to get large sized shrimps for eye ablation, the spent females can be reintroduced into the tank for rematuration.

4. Spawning:- The females with fully mature ovary are removed with a dipnet from the maturation tank and transferred in the evening to the spawning tanks each containing 200 litres of the water filtered through a 50 micron mesh bolting cloth. Only one spawner is kept in each tank. The temperature of water is 27°C-29°C, salinity 30-34 ppt and the pH 8.0-8.2. Mild aeration is given. Disodium salt of EDTA (chelating agent) is added to the water @ 0.1 gm per 100 litres of water. The tank is covered with a black lid to protect the female from strong light and to prevent it from jumping out of the tank. The lights are switched off during night. Spawning usually takes place between 8 p.m and 2 a.m. The females is removed only in the morning and returned to the maturation tank, if it is in good condition for rematuration.

5. Counting of eggs and nauplii:- For estimating the number of eggs produced, the eggs are dispersed in the water by thorough mixing and then 100 ml samples are taken with a beaker. The number of eggs in each sample are counted and the average number in 100 ml calculated. The total number of eggs is estimated thus.

scissors or making a slit in the cornea and squeezing out the contents.

3. Management of maturation tanks:

The ablated females are introduced into the maturation tank along with a few males. The male to female ratio need not exceed 1:4.

If the females are all impregnated there is no need for males. However, to impregnate females that may moult in the maturation tank it is advisable to keep a few males in the maturation tank. In a 10,000 litre tank where the biological filter is functioning well, 50 P. indicus (40 females and 10 males) can easily be maintained.

Water quality and other conditions conducive for maturation:-

Parameters	Permissible range
Salinity	29-34 ppt
Temperature	27-29°C
pH	8.0-8.2
Dissolved oxygen	4.0-5.5 ml/litre
Total ammonia	0.02-0.07 ppm
Nitrite	0.003-0.02 ppm
Light intensity during day time in the shed	60-100 lux

Under these conditions about 70% of the ablated females mature and spawn within 4-5 days after eyestalk ablation.

The pH of the seawater in the pool is maintained between 8.0 and 8.2 by addition of sodium carbonate (approx 25 g/m^3 of water every day) or slaked lime (approx 25 g/m^3 of water every alternate day). The shrimps are fed with clam or mussel meat @ 12.5-15% of shrimp biomass per day in the evening. The unused food and faecal pellets are siphoned out in the morning.

The temperature, pH and dissolved oxygen content of the seawater are measured in the morning and evening. The salinity of the water may be checked once in two days. If the salinity

Av. No of eggs in sample x $\frac{\text{volume of water in tank (litr.)}}{0.1}$

The fertilization rate is also calculated by taking a sample of eggs (fertilised and unfertilised) and counts taken under the microscope.

One of the problem faced in the hatchery is the release of non-viable eggs due to the absence of sperms in the thelycum. It is difficult to get the once spawned females to achieve impregnation in the maturation facilities. Such females very often spawn unfertilized eggs when their thelycum is devoid of spermatophores and the eggs thus spawned are non-viable. To solve the problem of lack of mating in broodstock shrimps two techniques may be used namely artificial insemination or artificial spermatophore transfer and *in vitro* fertilization.

a) Artificial insemination (Artificial spermatophore transfer)

The spermatophores from the fully mature males (130-140 mm in length) were removed by giving a mild electric shock (6 volts) to the base of the fifth pair of walking legs. This process is known as Electroejaculation of spermatophores. These spermatophores are implanted into the thelycum of the freshly moulted ("soft") eye ablated females. For insemination, the moulted female is caught from the tank with a soft hand net and held with the left hand, ventral side up and the pair of spermatophores quickly inserted into the thelycum of the female. The female is allowed to recover in the trough of seawater which is continuously aerated. Later on, the female is introduced into a 250 litre tank filled with seawater (salinity 32-33 ppt).

The implanted female fed with clam meat *ad libitum*. The water in the tank is changed daily. The eye ablated femal mature 4 to 7 days after implantation. When the fully mature, the female is kept for spawning. The spawning takes place at night.

b) In vitro fertilization

The fully mature female is kept for spawning in a transparent perspex tank. Mean while, the spermatophores from mature males are removed by electroejaculation and a sperm suspension was made by mascerating the spermatophores in filtered seawater (salinity 33 ppt and pH 8.2). The sperm suspension was taken in a 50 ml beaker.

The female starts spawning at night. At the time of spawning, the female was caught and the spawning was stopped by lifting the shrimp out of water. The ovary was cut with scissors and the eggs are mixed with the sperm suspension taken in the 50 ml beaker. Later, the egg sperm suspension is transferred to a 250 ml conical flask containing 100 ml of filtered seawater. The flask is moved rapidly to get a whirlpool movement for nearly 5 minutes. Fertilization is observed 10 minutes after mixing the sperms with eggs. The fertilized and unfertilized eggs are transferred into FRP tank containing 50 litres of filtered seawater. Larvae start hatching within 12-14 hours after fertilization.

The techniques of artificial insemination and in vitro fertilization are useful in solving the problem of non-mating in broodstock shrimps. These techniques are also useful in selective breeding and hybridization of penaeid shrimps.

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b) Nauplius:

The body of the nauplius is pear shaped with 3 pairs of appendages, the uniramous antennules and the biramous antennae and mandibles. The mandibles are used as swimming organs and they are devoid of spines or processes used for feeding. Since the nauplii subsist on the yolk present for development and since they do not feed, the mouth and alimentary canal are not developed at this stage.

Usually 6 naupliar sub-stages are identified by the increase in number of setae on caudal lobes and the antennal exopods. The duration of each sub-stage lasts for 4-6 hours, except the 6th sub-stage which is completed in 12 hours. The duration of nauplius phase is 36-48 hours.

c) Protozoea:

The nauplius metamorphose into protozoea. The protozoea is characterised by the presence of a large carapace that does not cover the thorax completely, a slender thorax and abdomen, uniramous antennules and biramous antennae with fully segmented exopods, abdomen bifurcated posteriorly with at least 7 setae on each furca, well developed biramous 1st and 2nd maxillipeds and the 3rd maxilliped is rudimentary or absent. The alimentary canal is functional and the feeding appendages like mandibles, maxillules and maxillae.

The protozoea feed voraciously on phytoplankton. At a temperature range of 27-29°C and good feeding conditions this stage lasts 3-4 days. The availability of adequate quantity of feed determines the duration of this stage. If feed is not provided properly the protozoeal stage is delayed and the larvae become weak and die.

There are 3 clear sub-stages in protozoea phase as detailed below:

At protozoea I, the eyes are sessile, rostrum or supra-orbital spine absent, pereopods absent and abdomen unsegmented.

MORPHOLOGY AND BEHAVIOUR OF PENAEID PRAWN LARVAE, THEIR REARING AND TRANSPORTATION

S.M. PILLAI

The penaeid prawn larvae vary in shape and size and exhibit characteristic behaviour specific to the species concerned. Although naupliar, protozoeal, mysis and post-larval stages are common to all the penaeid prawns, there are clear variations in each larval stage among the different species of prawns. Various types of larval rearing techniques are practised in hatcheries in different regions of the world. Production of quality seed is the ultimate aim of the hatchery which involves hatchery and nursery rearing. The end product of seed production of prawns is postlarvae (PL 20) which are to be supplied to the farmers for stocking in grow-out ponds. The details of postlarval production from eggs to PL 20 and their transportation are described below.

1. Morphology and behaviour of prawn larvae

a) Eggs:

The diameter of penaeid prawn eggs vary from 0.25 to 0.41 mm with a perivitelline space which determines the buoyancy of the eggs. The eggs of *Penaeus* group have narrow perivitelline space (15 microns) and thus they are less buoyant compared to the eggs of *Metapenaeus dobsoni* and *Parapenaeopsis stylifera* which have 85 micron and 60 micron perivitelline space, respectively and thereby they are more buoyant. The embryonic life is short due to the small size of the eggs and the limited yolk stored in the eggs. The eggs hatch out 9-17 hours after spawning. The rate of development of the eggs is related to temperature, higher the temperature shorter the duration of embryonic stage. In addition to the perivitelline or egg membrane, the developing nauplius is covered by a thinner vitelline membrane. The nauplii hatch out by rupturing these two membranes.

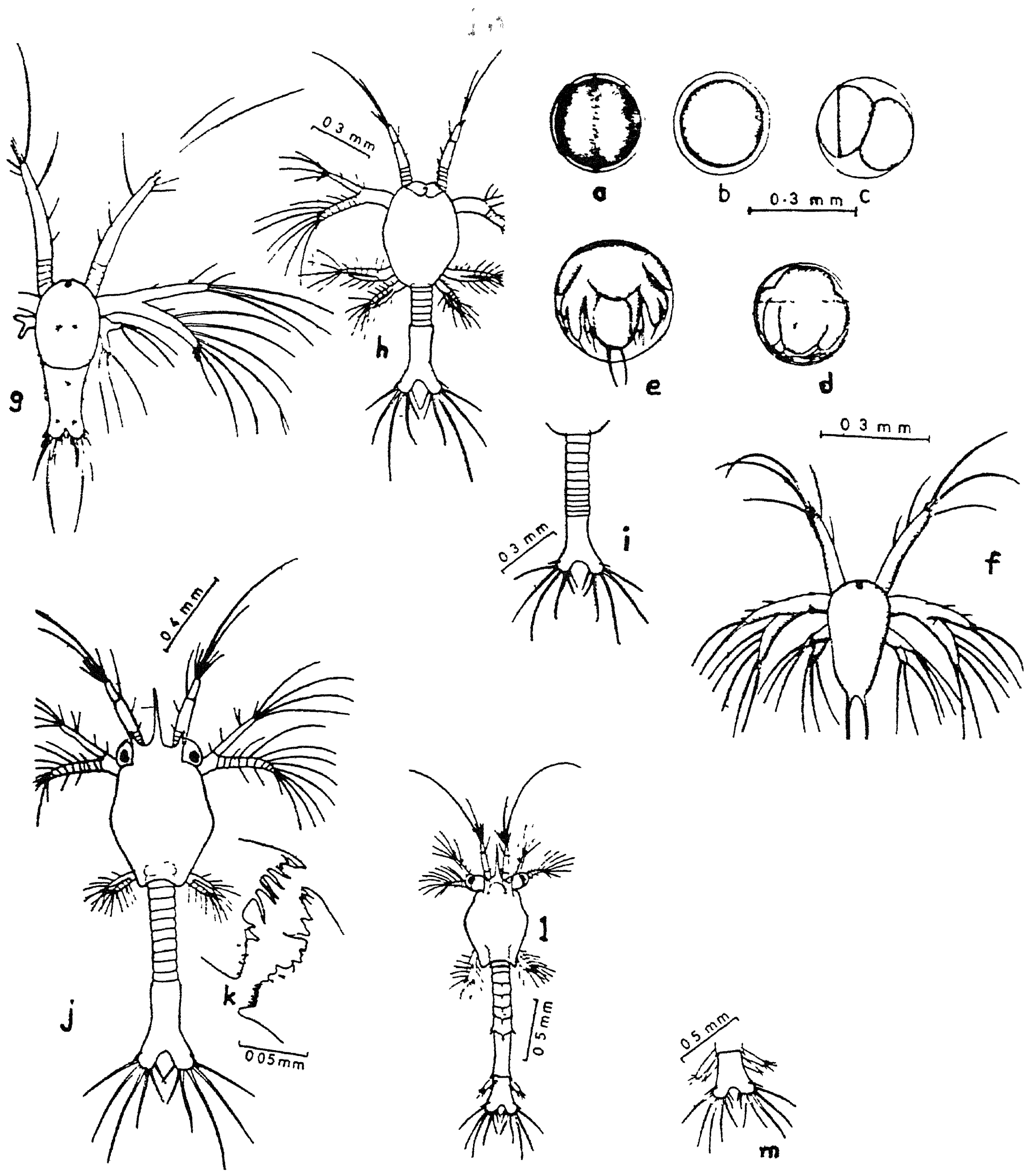

Fig.1-Larval development of Penaeus indicus H.M. Edw. (after Muthu et al., 1978)
a,b,c-eggs at different stages of development;
d and e-developing nauplius inside the egg;
f-Nauplius-1;
g-dorsal view of nauplius-VI;
h-dorsal view of protozoea-1;
i-abdomen of protozea-1 in advanced stage;
j-dorsal view of protozoea-II;
k-mandibles of prtozoea-II;
l-dorsal view of protozoea-III;
m-Uropod and telson of protozoea-III

In protozoea II, the eyes are stalked, rostrum and supra-orbital spine appear, first 5 abdominal segments demarcated, telson not separated from the last abdominal segment and uropods absent.

Protozoea III is characterised by the presence of uropods, telson separated from the last abdominal segment and the presence of dorsal spines on the first 5 abdominal segments.

d) Mysis:

The protozoeal phase is followed by the mysis phase. At mysis stage the carapace covers the thorax, the 3rd maxillipeds and the 5 pereopods are functional with well developed exopods, the first 3 pereopods have rudimentary chelae, pleopods, if present, are rudimentary without setae, antennal exopod is unsegmented and scale-like and the telson is narrow and notched medianly.

As in protozoeal phase, three distinct, sub-stages are recognised in mysis stage also. These sub-stages are distinguished by the little increase in the size of the larvae, the length of the pleopods, the number of setae on the antennal scale, maxillary exopod and theuropod rami. As the mysis stag is approached the antennule loses its mobility when these sub-segments fuse into a single basal segment in protozoea III. In the mysis stage the antennule altogether loses its natatory function and becomes a sensory organ. Likewise, the natatory antennae of the protozoea has a fully segmented exopod for greater flexibility; in mysis stage the exopod loses its segmentation and is transformed into a scale which perhaps serves as a stabilizing organ. The setose exopods of the thoracic appendages are main swimming organs during mysis stage. The endopods of the pereopods with their long terminal setae are also natatory in function. The fully developed uropods along with the telson form the tail fan which when flicked by the flexure of abdomen suddenly jerks the larvae backwards and enables it to escape from predators. The mysis stage lasts 3-4 days.

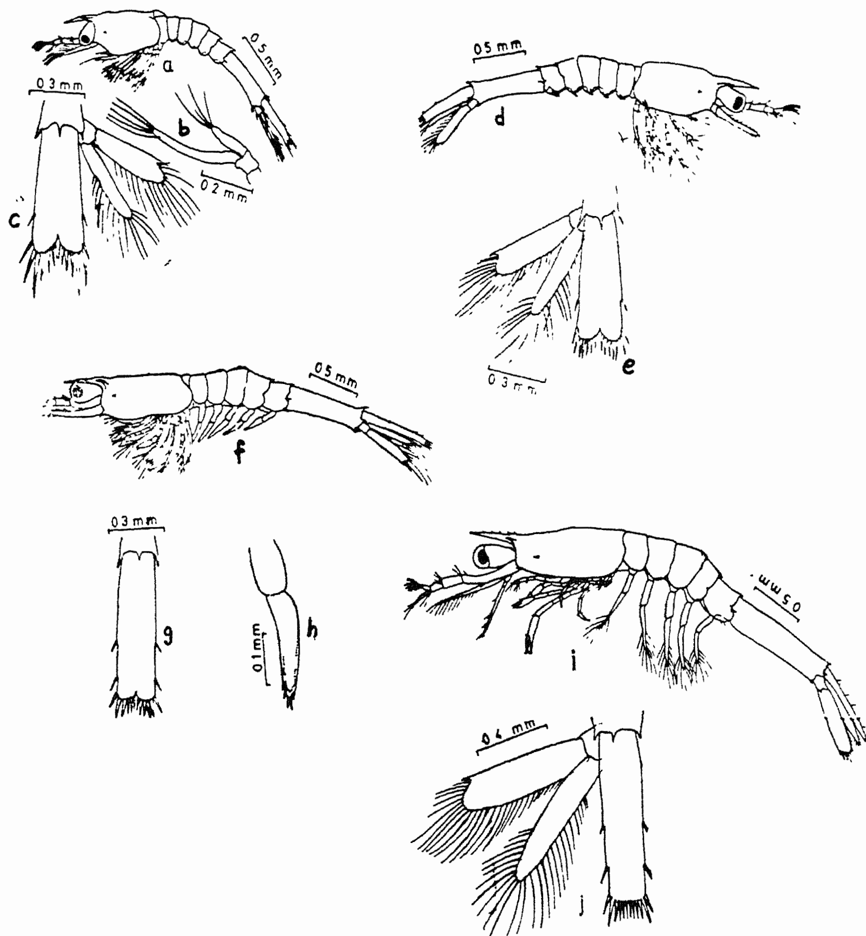

Fig.2 Larval development of *Penaeus indicus* H.M.Edw. (after Muthu *et al*, 1978
a-Lateral view of mysis-i
b-Fifth pereopod of mysis-i
c-Uropod and telson of mysis-1
d-Lateral view of mysis-ii
e-Uropod and telson of mysis-ii
f-Lateral view of mysis-iii
g-Telson of mysis-iii
h-Pleopod bud of mysis-iii
i-Lateral view of postlarva-i
j-Uropod and telson of postlarva-1

Although 3 sub-stages were distinguished in mysis phase for *Penaeus* group, the *Metapenaeus* exhibit 4 to 6 sub-stages. However, in *Parapenaeopsis*, *Sicyonia* and *Parapenaeus* the respective sub-stages recognised were 7, 4 and 14.

e) Postlarva:

The mysis sub-stage III transforms into the postlarvae characterised by development of swimming setae on the pleopods, loss of exopods on the pereopods and the 2nd and 3rd maxillipeds and assumption of the prawn-like body shape. The postlarvae lose the filter feeding habit and became capable of handling small prey animals.

2. Larval behaviour:

The penaeid nauplii are attracted towards a weak source of light but bright sunlight is harmful to them. The protozoea stages are also positively phototactic to weak light. Attraction to low light intensities becomes less pronounced in the mysis stage; the third mysis and postlarvae are not attracted by low light intensities. The nauplii at rest remain suspended with the ventral side up in the water. They swim in short spurts. The protozoea are very active and swim swiftly in a horizontal position with the dorsal side up. The rate of movement doubles with each moult. The protozoea swim ceaselessly and can be seen trailing a long "tail" of faecal matter if they are well fed and healthy. When the aeration is stopped, the healthy nauplii and protozoea form swirling swarms at the surface like a swarm of bees. There seems to be some attraction among the members of a swarm.

The active protozoea transforms into a relatively sluggish animal following moult into mysis stage and they hang with the anterior end pointing obliquely downwards. It hovers around like a helicopter and jumps back suddenly by flexing the abdomen.

With the appearance of plumose setae on the pleopods the postlarvae become horizontally oriented while swimming. The early postlarval stages of Penaeus are pelagic for 4-5 days; the postlarvae of P. indicus are the most active compared to other species. Till mysis stage the larvae move backwards and at postlarvae they exhibit forward movements.

3. Larval rearing techniques

After spawning, the eggs are counted to estimate the spawning rate. The eggs are examined under the microscope to ascertain the fertilization rate by taking adequate samples and examining at least 50 eggs. The fertilized eggs are transparent with distinct perivitelline space. The unfertilized eggs are opaque. The hatched-out nauplii are counted to estimate the hatching rate again by taking 2-3 aliquot samples.

The nauplii are then transferred into rearing units by siphoning with great care to avoid handling stress. Taking advantage of their phototactic behaviour, the nauplii concentrated in one corner of the tank is siphoned out. Tanks of 2-10 tonne capacity are used to rear larvae. The stocking density of nauplii is done @ 1,00,000 nauplii/tonne or 100 nauplii/l. One 2 tonne tank can hold, 2,00,000 nauplii which will be the ideal tank size to manage the rearing in backyard set-up. Sea water filtered through a 50 micron mesh bolting cloth bag is used in the larval tanks. Fabric filters of 10 micron and 5 micron are very effective in removing all particulate contents upto that size. The tanks are aerated continuously with air diffuser stones.

Management of larval tanks

The larval rearing tanks are cleaned and disinfected before the start of the next larval run. To clean, the inner side of the tanks are smeared with bleaching powder and left for 10-12 hours before scrubbing and cleaning. Cleaning is repeatedly done with water till the smell of chlorine vanishes. Then the tanks are kept dry for 24 hours before use.

The management of the larval tanks is done as per the details given below:-

Day	Stage	Seawater removed (lit)	Algal culture added (lit)	Particulate feed (g)	Seawater added (lit)	Total vol. of water made upto (lit)
1	N2	-	-	-	1000	1000
2	N5	-	100	-	-	1100
3	PZ1	-	150-200	-	700-750	2000
4	PZ2	500	150-250	-	250-350	2000
5	PZ3	500	150-250	-	250-350	2000
6	M1	500	150-250	-	250-350	2000
7	M2	500	150-250	-	250-350	2000
8	M3	500	150-250	10-15	250-350	2000
9	PL1	750	100-150	12-15	600-650	2000
10	PL2	750	100-150	12-15	600-650	2000
11	PL3	750	100-150	12-15	600-650	2000
12	PL4	750	100-150	12-15	600-650	2000
13	PL5	750	100-150	12-15	600-650	2000

However in backyard hatcheries, the water volume in larval tanks is gradually increased by adding algal water upto PL1 stage. From PL1 to PL5 stage daily 1/3 quantity of water is exchanged and this is increased to 50% during PL5 to PL20 stages on alternate days.

The water quality and other conditions conducive to larval rearing are as follows:-

Parameters	Permissible range
Salinity	27-34 ppt
Temperature	26.0-32.5°C
pH	8.0-8.5
Dissolved oxygen	3.0-8.0 ml/1
Light intensity during day time in tanks kept in the open	20,000-1,25,000 lux
Total ammonia	0.1 ppm
Nitrite	0.05 ppm

The first four parameters should be monitored daily. Ammonia and nitrite levels may be monitored 2-3 times a week. From the larval rearing tanks water is removed by keeping a siphon inside an open filter box to prevent the loss of larvae. 50 micron mesh is used till protozoea 3, 100 micron mesh upto mysis 3 and 250 micron mesh from postlarvae 1 onwards. The diatom culture is allowed to flow from the culture tank directly into the larval rearing tanks through flexible PVC hose for feeding the larvae. The concentration of algal cells in the larval tanks is usually 30,000-40,000 cells/ml. Diatom cell density is estimated using a Haemocytometer. By experience it will be possible to ascertain the adequate quantity of diatoms in the tanks by observing the feeding condition of the larvae. The larvae should be examined once in two hours to make sure the fullness of the gut of the larvae and also to know their general condition.

Normally the sediments are not removed. However, if turbidity is too high the sediments have to be siphoned out after suspending aeration for ten minutes. By efficient aeration water quality of the tanks can be maintained. Since the larval tanks contain algal cells it stimulates the natural growth of copepods and rotifers which also form the food component of the postlarvae.

Larval counts and harvesting:

Larval counts were made when the developing larvae reach PZ1, M1, M2, M3, PL1 and PL5 stages. Four samples were taken after vigorous aeration and gentle mixing, from four places in the tank. The larvae are then individually counted. The average number of larvae per litre of sample is raised to the total volume of water in the tank to get an estimate of the total number of larvae. The postlarvae are harvested at PL5. After reducing the water level by siphoning, the ball valve is opened and the postlarvae are collected in buckets. Sample counts are made to get an estimate of PL5 before they are stocked in the nursery.

4. Nursery rearing technique:

The nursery acts as a link between the hatchery and grow-out. Since the hatchery reared PL5 are smaller in size and unable to withstand fluctuations in environmental factors and can not fully stand on the interspecific competition for food and space, they are to be nursed in special areas called nurseries. Cement cisterns, plastic pools, raceways, cages, pens, FRP tanks and plastic lined earthern ponds of different dimensions are used as nursery system. The size and number rearing units can be chosen as per the targeted production. Normally the size of the nursery tank should be at least 2-3 times more than the rearing tank.

The nursery tanks are disinfected, cleaned and dried before filling with filtered seawater. The salinity is maintained to that level at which PL5 are produced. Stocking of PL5 is done @ 6,000 to 8,000/m^3 either during early morning or late evening hours. The tanks are to be aerated with air stones @ one stone/m^2. The following feeding regime are suggested:-

PL5 to PL10 = 500 microns feed particles
fed @ 1 g/day/1000 larvae

PL10 to PL20 = 1000 microns feed particles
fed @ 5 g/day/1000 larvae

Above PL20 = Crumbles

The feed ration for the day is provided in 4-6 equal instalments. Regular monitoring of temperature, salinity, pH and DO are to be done to avoid problems and to take corrective measures.

However in backyard hatcheries upto M3 the larvae are fed with diatoms alone. From PL1 to PL5 the larvae are fed with 200 micron squilla powder. While PL5 to PL15 stages are reared with 500 micron squilla powder, the particle size is increased to 1000 micron from PL15 to PL20 stages.

When the postlarvae reach PL15 stage the salinity is gradually brought down to 10-20 ppt by the addition of required quantities of brackish/freshwater. In backyard hatcheries reduction of salinity is done from PL3 stage and slowly brought down to 20 ppt at PL20 stage.

5. Seed Transportation

The transport of nursery reared prawn seed from the hatchery to the form is the last step in hatchery production of seed prawns. Prawn seed can be transported either in open or closed containers. Open containers can be used to transport seed over short distances involving maximum of 2 hours. For long distance transport closed containers like polythene bags are the ideal packing units which can be enclosed in paper cartons.

The polythene bag is first filled with 6 litres water and the remaining part is filled with oxygen and sealed tightly. Seed of *P. indicus* can be transported @ 50 to 500 nos/litre by different mode of transportation and different durations. Shorter the duration of transport more the numbers of larvae transported. Packing of the seed should be done during morning or evening hours and night will be ideal period for transport since the metabolic activity of the postlarvae will be lower at lower temperatures of night. To minimise the metabolic activity of the larvae the temperature of the water can be lowered by placing ice blocks packed in polythene sachets in the bag or saw dust in the cartons and thermal fluctuations can be checked by placing theremocole sheets.

During long distance transport, *Artemia* nauplii are introduced in the polythene bags to avoid cannibalism among the prawns.

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CULTURE OF PHYTOPLANKTON

S.M. PILLAI

Availability of sufficient quantity of feeds specific to the various larval stages of penaeid prawns is one of the prerequisite for successful hatchery production of prawn seed. In nature, the prawn larvae feed on a variety of food organisms. But in hatchery, each larval stage should be fed with specific type of feed. The feeding habits of each larval stage is different. While the nauplii do not feed at all, but subsist on the yolk, the protozoea feeds on phytoplankton. At mysis stage they feed on small animal food organisms in addition to phytoplankton. A variety of animal and plant matter form the food spectrum of postlarval stages of prawns.

Types of live food organisms for prawn larvae

In hatchery although attempts are on to rear larvae with artificial diets, still phytoplankton forms the essential food component of the early larval stages. The common species of phytoplankton (Fig.1) used as larval diets are as follows:-

a) Diatoms
1) *Chaetoceros* sp.
2) *Skeletonema* sp.
3) *Thalassiosira* sp.
4) *Nitzschia* sp.
5) *Cyclotella* sp.

b) Flagellates
1) *Tetraselmis* sp.
2) *Isochrysis* sp.
3) *Monochrysis* sp.

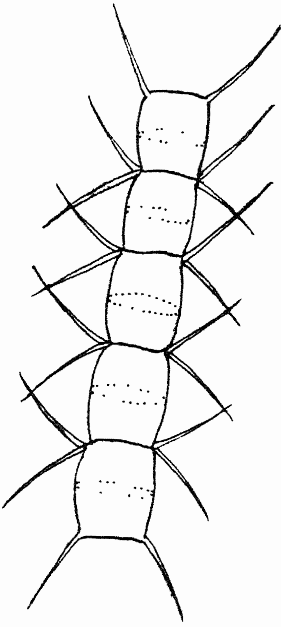
c) Green algae
Chlorella sp.

Some common forms of diatoms and algae used to feed the prawn larvae at Narakkal Research Centre of Central Institute of Brackishwater Aquaculture and their sizes are as follows:-

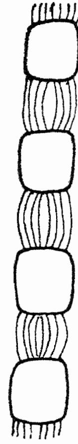
Chaetoceros sp.	12 - 15 micron
Skeletonema sp.	12 - 15 "
Thalassiosira sp.	15 - 18 "
Nitzschia sp.	30 "
Chlorella sp.	3 "
Tetraselmis sp.	22 "

Culture of phytoplankton

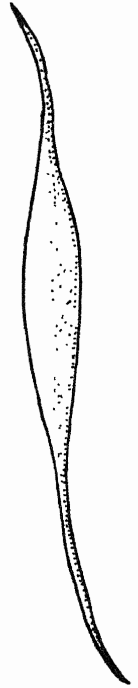
Culture of phytoplankton is done in two set-ups; the indoor culture or maitenance culture and the outdoor mass



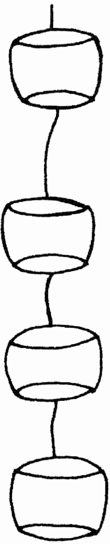
CHAETOCEROS Sp.



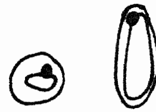
SKELETONEMA Sp.



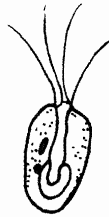
NITZSCHIA Sp



THALASSIOSIRA Sp.



CHLORELLA Sp.



TETRASELMIS Sp.

ISOCHRYSIS Sp.

culture. Outdoor mass culture is taken up after indoor cultur which involves a series of steps as follows:-

1. Selection of suitable containers
2. Preparation of culture media
3. Sterilisation of glass containers
4. Isolation of the desired species
5. Management of the culture under controlled light, temperature and aeration.
6. Harvesting and preservation

1. Culture containers

For indoor phytoplankton culture test tubes, Erlenmeyer flasks, Haffkine flasks, polythene bags, glass carbuoys and perspex tanks can be used. For outdoor culture large glass aquaria, FRP tanks or cement tanks of different sizes can be used.

2. Culture media

For proper growth and reproduction of the phytoplankton the nutrients, nitrates, silicates and phosphates should be available. The culture media should also contain nutrients like trace metals, vitamins and amino acids. The absence of any one of these growth promoting substances will affect the growth of the phytoplankton.

The following are some media used for culture of algae or flagellates:

(i) Schreiber medium

Sodium nitrate	0.1 g
Sodium acid phosphate	0.02 g
Soil extract	50 cc
Filtered water	1 litre

(ii) Miquel's solution

A	B
Potassium nitrate - 20.2 g	Sodium phosphate - 4 g
in 100 ml distilled water	Calcium chloride - 4 g

Ferric chloride - 2 g
Conc. HCl - 2 ml
Dissolved in 100 ml distilled water.

Add 0.55 ml of A and 0.5 ml of B to each litre of filtered sea water.

(iii) <u>Walne's medium</u>

Na NO_3	- 100.00 mg
Disodium salt of EDTA	- 45.00 mg
$H_2 BO_3$	- 33.60 mg
Na $H_2 PO_4 . 2 H_2O$	- 20.00 mg
Fe Cl_3. [illegible]H_2O	- 0.36 mg
Vitamins	
B1	- 0.10 mg
B12	- 0.005 mg
Trace metals	
Zn Cl_2	- 0.021 mg
Co $Cl_2 . 6H_2O$	- 0.02 mg
$(NH_4)_6 Mo_7 O_{24} . 4H_2O$	- 0.009 mg
Cu $SO_4 . 5 H_2O$	- 0.[illegible]2[illegible] mg
seawater	- 1 litre

(iv) <u>TMRL medium</u>

Potassium nitrate	- 10 g
Sodium phosphate	- 1 g
Ferric chloride	- 0.3 g
Sodium silicate	- 0.2 g

Prepare each chemical in 100 ml distilled water in seperate bottles. Add 1 ml each to [illegible] 1 litre filtered seawater.

(v) <u>For mixed culture of phytoplankton</u>

Potassium nitrate	- 1.2 g
Sodium/Potassium phosphate	- 0.3 g
Sodium silicate	- 0.6 g
EDTA	- 0.6 g

The above nutrients are dissolved in 100 litres of sea water filtered through 50 micron mesh nylobolt cloth.

3. Sterilisation of glass containers

All the containers used for culture of phytoplankton are to be cleaned and dried before start of the culture. The containers should be sterilised in an autoclave. Sterilisation is very important since even a slight contamination will lead to the loss of the entire stock cultures.

4. Isolation of desirable species

The seawater is first sieved through a plankton net (50 micron mesh size). The sample is then examined under microscope for the presence of desirable species of algae after sedimenting or centrifuging. Then the species is isolated using any one of the following methods

i) Pipette method:
Using a micropipette large organisms can be pipetted out to culture tubes.

ii) Centrifuge or washing method:
By repeated centrifuging at 500-1000 rpm of the sample and inoculating the deposit.

iii) By exploiting the phototactic movements:
By this method most of the phytoflagellates can be isolated.

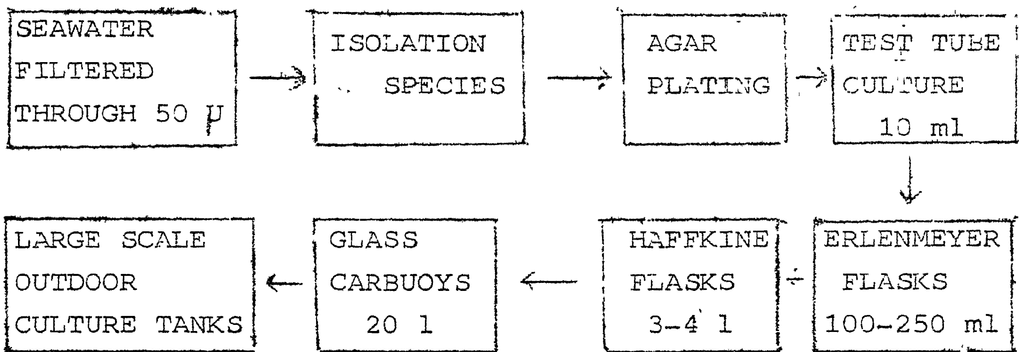
iv) Agar plating method:
The required species is picked up by a platinum needle or loop and streaked on agar plates.

5. Management of the culture

Stock cultures of micro-algae are to be maintained in a special room under uniform temperature and light. Since light is essential for photosynthesis of algae, light intensity of algal culture room is maintained between 1000 to 3000 lux with flourescent lamps. Temperature of the stock culture room should be maintained between 23-25°C during day and 20-[illegible]°C

during night. Aeration has to be given continuously to the culture containers since besides providing oxygen it helps t keep the cells in suspension, distributes nutrients uniforml in the medium and provides carbon-di-oxide for photosynthesi

The scaling up of phytoplankton culture is indicated in the following flow diagram:-



Growth phases of the algae

The multiplication of algal cells in culture follows characteristic pattern with four distinct phases of growth. The algae exhibit "Sigmoid" growth curve as shown in Fig.

a) Lag or induction phase:

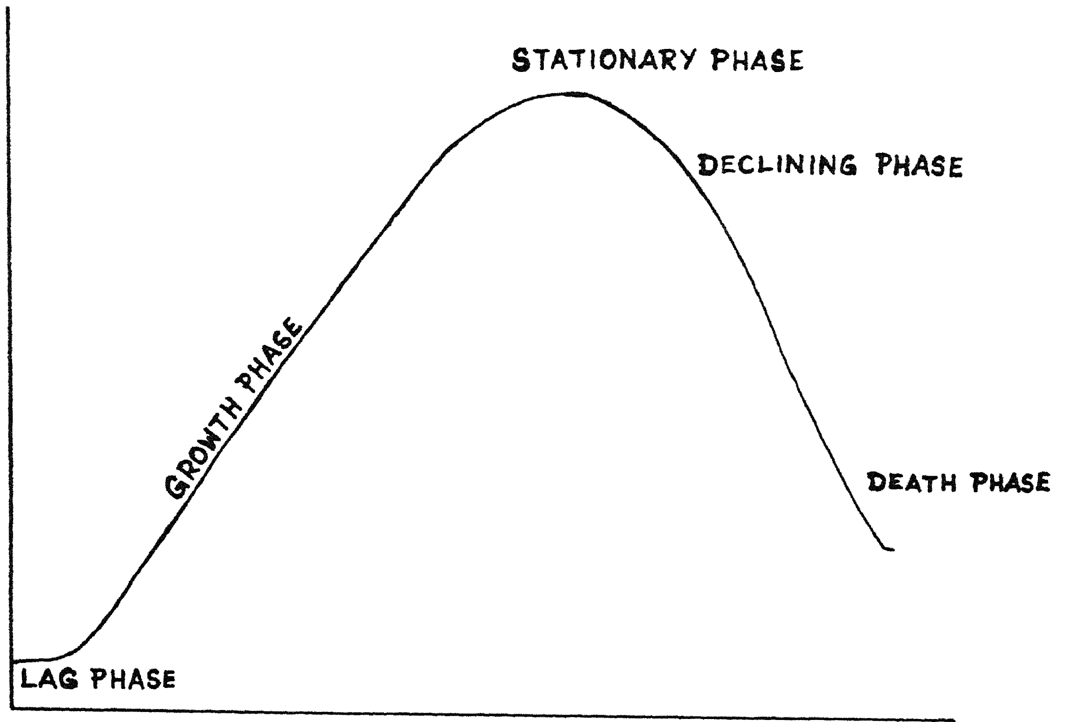
When a new algal culture flask is inoculated with stock culture, there will not be any cell division for a few hours since the inoculated cells [illegible] a short time to acclimatise to the new medium. This period is called lag or induction phase.

b) Exponential or growing phase:

During this phase, the cells divide steadily at a constant rate and it continues till the culture attains maximum concentration.

c) Stationery phase:

Once the cells reach maximum concentration, growth is arrested and the culture becomes stationery. The cell density remains more or less constant.


STATIONARY PHASE
DECLINING PHASE
GROWTH PHASE
DEATH PHASE
LAG PHASE

d) Death or declining phase:

After a long stationery phase, the cell may loose its vitality and start to die, resulting in a decline of the population.

Determination of cell densities

Majority of the cultured micro-algae are less than 10 µ in size. They can be counted with a Haemocytometer.

The Haemocytometer has 9 chambers, 4 sides having 16 divisions and 5 chambers with 25 divisions in the central block. Counting of the cells should at least be made from 3 chambers. The cell density can be calculated using the formula s.

Cell density (cells/ml) = Average counts per chamberx10^4.

6. Harvest and preservation

Harvesting of algal culture is a simple process wherein known quantity of algal cells are poured into the larval rearing units. Harvest should be done at the late phase of logarithmic growth when the density of cells will be high and utilise the culture early in the stationery phase.

During adverse weather conditions it will be difficult to maintain the culture and thereby to ensure constant supply to the hatchery.

Preservation and concentration of algae can be accomplished by freeze drying or sun drying. Freezing can be done by flocculation of the algae by adding alum or lime or adjusting the pH using sodium hydroxide. By this method known quantity of algae could be preserved so that it can be used during adverse weather conditions when the culture fails or takes longer time to attain the required cell density.

During flocculation the pH of the algal culture is increased to 9.4 by adding sodium hydroxide under vigorous stirring. The culture is then left undisturbed for one hour. The algal mass gets deposited on the bottom of the container.

The clear water is decanted and sediment collected. The pH is then brought down to the original value of 8.2 by adding dilute HCl. The algae is now ready for freezing or drying.

Drying is done under sunlight by pouring the algae into enamel trays. Dried algae can be scrapped and stored in air tight glass bottles.

Before freezing the algae have to be treated with few drops of cryoprotectant like dimethyl sulphoxide or glycerol. The frozen algae can be stored for 3 months.

Mass culture of phytoplankton

Mixed culture of phytoplankton, dominated by *Chaetoceros affinis* is raised for feeding prawn larvae at the Narakal Research Centre of the Central Institute of Brackishwater Aquaculture.

Mixed culture of phytoplankton is done in 1000 litre capacity white FRP tanks. The tanks are placed under the glass roofed hatchery shed. Fresh seawater (30-34 ppt) filtered through 50 micron mesh bolting cloth is taken into the tank and is fertilized with the following nutrients.

Sodium nitrate	-	12 ppm
Potassium orthophosphate	-	3 ppm
Sodium silicate	-	6 ppm
EDTA	-	6 ppm

The tank is aerated with two air stones. The light intensity in the glass shed varies from 20,000 to 1,20,000 lux during day and the temperature 28-35°C. The diatoms multiply rapidly and the characteristic golden brown bloom is achieved within 24-48 hrs. This culture is dominated upto 75-90% by *Chaetoceros*. The other forms like *Thalassiosira*, *Skeletonema* and *Nitzschia* constitute 10-25%. The cell density will be 3-4 lakh cells/ml. This culture is used for feeding prawn larvae and also as inoculum for fresh batch cultures. Generally the algal culture will be ready for use 16-20 hrs after inoculum.

Feeding Schedules:

The larval stages of prawns should be fed @ 30,000 to 40,000 cells of diatom/ml of water. Feeding has to be done 4 to 5 times in a day so that at any given time, the algal count in the larval rearing tanks should not be less than 30,000 cells/ml. In the Japanese system of seed production the larval and diatom cultures are done together in the same container so that the larvae feed continously. The feeding activity of the prawn larvae could be assessed by the green colour of the tubular alimentary tract characteristic of the intense ingestion of algal cells and the presence of long and continuous strands of faecal matter appearing as "tail" of the larvae.

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NUTRITIONAL REQUIREMENTS AND COMPOUNDED FEED FORMULATION FOR PENAEID PRAWNS

S. AHAMAD ALI

Like any other animals, prawns have their specific nutritional requirements. For healthy and faster growth, the prawn diet should have adequate levels of energy, vitamins and minerals. The main sources of energy in the diet are protein, fat and carbohydrate. For preparing a nutritionally balanced feed, the requirements of these nutrients are determined for candidate species. Detailed information on the requirements of penaeid prawns which has accumulated in the literature is briefly discussed below.

Energy requirements:

Penaeid prawns require about 3500 to 4000 Kcal of digestible energy per kilogram in their diet. One gram of protein provides 5.6 Kcal of energy whereas a gram of lipid is equal to 9.45 Kcal of energy. The energy equivalent of carbohydrate is 4.1 Kcal/g.

Protein requirement:

Protein is the most important and expensive component in prawn diet. Penaeid prawns require 30 to 40% of high quality protein in their diet. Proteins, rich in essential amino acids such as arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine, should be provided in the diet. Animal proteins derived from marine origin are rich in these amino acids. Plant protein materials can be used to balance the deficient amino acids.

Lipid requirements:

The lipid requirement in the diet of prawn is between 8 and 10%. But the lipid supplied in the diet should be rich in polyunsaturated fatty acids (PUFA) such as linoleic acid (18:2w6), linolenic acid (18:3w3), ecosapentaenoic acid

(20:5w3), docosahexaenoic acid (22:6w3). These fatty acids are found to be essential for prawns and should be supplied through the diet. In addition to the simple fats, the lipid includes phospholipids, steroids, carotenoids, vitamins D, E and K. Phospholipids such as lecithin and cephalin are important and essential for growth and survival of prawn larvae and juveniles. These should be provided at 1-2% level in the diet. Similarly prawns require about 0.5% of cholesterol in their diet. Eventhough the quantitative requirements of vitamins D, E and K in the diet of prawn are not fully defined these are included in the diet.

Lipids derived from marine animals are found to be rich in PUFA. Plant oils on the other hand are good sources of phospholipids and other fatty acids.

Carbohydrate requirement:

Carbohydrates are classified as monosaccharides (glucose), disaccharides (sucrose) and polysaccharides (starch). Among them prawns are found to utilize, disaccharides and polysaccharides better. In practical feeds starch is generally used as sources of carbohydrate. According to the requirement of energy, carbohydrate level in the diet of prawn can be between 20 and 40%. Apart from this the diet should also contain the polysaccharide cellulose which should not be more than 6%. This is found to be necessary for healthy growth of prawns and better conversion efficiency of feed.

Mineral requirements:

Inorganic elements like calcium, phosphorous, sodium, potassium, magnesium, iron, manganese, copper, chlorine, iodine, cobalt, zinc, etc. are generally known as mineral elements. These are required in small quantities in the diet and therefore are categorised as minor nutrients. The mineral nutrients are important and essential in the diet of prawns. Their deficiency in the diet often causes disease.

Most of the studies conducted on the calcium requirements for penaeid prawns recommended a calcium requirement of 1% in the diet, eventhough there are evidences that prawns are capable of absorbing calcium from water. The requirement of phosphorous in the diet of prawn is shown to be between 1 to 2%. The ratio of calcium and phosphorous seems to be significant in the diet of prawns and it should be at least 1:1. The requirement of other minerals may be as follows. Magnesium 0.3%, potassium 0.9%, copper 0.06 to 0.6% and zinc 0.07%. In addition to these, cobalt, iodine, iron, and manganese may be required in trace quantities which are not yet defined.

Suitable salts of these lements can be used for preparing mineral mixture and should be used at appropriate levels in the diet.

Vitamin requirements:

Vitamins are essential in the diet of prawn and their absence in diet leads to deficiency diseases. Prawns require most of the vitamins of B-group, vitamins C and E. The quantitative requirement of thiamine is 6-12 mg and that of pyridoxine is 12 mg per 100 g of diet. The diet of prawn requires 60 mg of choline, and 200-400 mg of inositol. Vitamin C (ascorbic acid) is required in higher amounts which is about 0.5 to 1.0%. Its deficiency in the diet is found to cause a disease syndrome called 'black death' with characteristic blackening of esophagus wall, cuticle, gastric wall, hind gut and gills in penaeid prawns like *Penaeus californiensis* and *P. stylirostris*. β-carotene should be supplied in the diet and the prawns are capable of converting it into vitamin A. The requirement of vitamins D and K for prawns is not established.

Formulation of compounded feeds

After understanding the nutritional requirements of candidate species of prawns, compounded feeds are formulated by balancing the requirements with practical feed materials available in the region. The ingredient composition of a feed

is made through balancing the major nutrients protein, lipid and carbohydrate. The feed formula is completed by incorporating adequate levels of mineral and vitamin mixtures. For formulating good quality feeds, the raw materials available in the region should be identified and selected and tested for their nutritional quality.

Selection of raw materials:

The feed materials required for formulating prawn feeds are protein sources (animal and plant origin), lipid sources and carbohydrate sources. The criteria for selection of a raw material is that the selected material should have good quality and should be available in large quantities whenever they are required at a reasonable price.

Protein sources:

The typical animal protein sources are fish meal, squid meal, prawn head meal, squilla meal, cuttlefish meal, clam meal and crab meal. In addition to these, materials like meat meal, blood meal and feather meal may also be used wherever available, after testing their suitability for prawns.

The important plant protein sources are soybean meal, ground nut cake, gingelly cake, mustand cake, coconut cake and any other residues of oil seeds after extraction of oil. Care should be taken in using the plant protein sources since some of them contain toxins or growth inhibiting factors. For example soybean cake contains a trypsin inhibiting factor and mustard cake contains thiocyanates which are toxic to animals. Suitable methods should be employed either to destroy them or remove before they are used in feed preparation.

There are also some single cell protein sources such as *Spirulina* and yeast which can be used in the feed formulas

Lipid sources:

Some of the important lipid sources which can be used in prawn feeds are cod liver oil, shark liver oil, sardine oil, soybean oil and sobyean lecithin. Most of these lipids are

rich in PUFA and should be preserved from oxidation. This [illegible] done by adding antioxidants such as butylated [illegible] anisol [illegible] ethoxyquin at 100 ppm. The steroid cholesterol [illegible] [illegible] supplementing its requirement in the feed.

Carbohydrate sources:

The important carbohydrate sources are wheat flour, [illegible] flour, maize flour, jowar and tapioca powder. All these materials are good sources of starch in the feed [illegible] can also [illegible] as binders if appropriate method of preparation is adopted.

The proximate composition of some selected feed materials is given in Appendix-1.

Feed formula:

After selection of the raw materials, a feed formula can be evolved by balancing the nutrients in the feed at required levels. One of the simplest methods by which a feed formula [illegible] be evolved is the 'Square' method. In this method either the protein or the energy of the feed can be balanced. The method is illustrated by the following example.

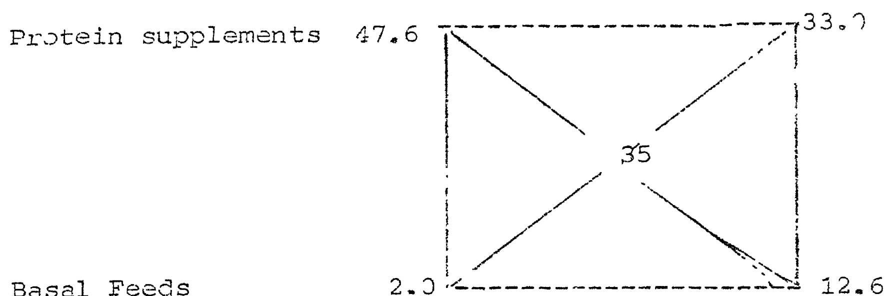
Example:

To prepare a feed with 35% protein using four ingredients fish meal (protein 60%), prawn head meal (protein 35%), soybean cake (protein 48%) and tapioca (protein 2%). In this case the ingredients are grouped into protein supplements (having more than 20% protein) and basal feeds (having less than 20% protein). The protein content in each group is averaged as follows:-

Protein supplements -	Fish meal	= 60.0
	Prawn head meal	= 35.0
	Soybean cake	= 48.0
		143.0
	Average $\frac{143}{3}$	= 47.6

Basal Feeds : Tapioca = 2.0%

Now a square is constructed and the names of the feed materials are written on the two left corners along with the protein content. The required protein level is written in the middle of the square. Next, the protein level of the feed is subtracted from that of the ingredients and the answer is placed in the corner opposite to the corresponding feed stuff ignoring the positive or negative sign as given below:-



Add the figures on the right hand side of the square 33.0+12.6 = 45.6. Now to make the feed with 35% protein we must mix

Protein supplements $\frac{33.0}{45.6} \times 100 = 72.37$

Basal Feeds $\frac{12.6}{45.6} \times 100 = 27.63$

The protein supplements are 72.37% in the feed and the basal feed is 27.63%.

The feed formula using these materials can be written as follows:-

Fish meal	$\frac{72.37}{3}$	= 24.1%
Prawn head meal	$\frac{72.37}{3}$	= 24.1%
Soybean cake	$\frac{72.37}{3}$	= 24.2%
Tapioca powder		= 27.6%
	Total	100.0

For adding vitamin and mineral mixture, appropriate amount of tapioca may be replaced. The feed formulated above contains 35% of crude protein.

Feed Binders:

Prawn feeds, when put in water should not dissolve and disintegrate. The feed should be stable in water for at least three to four hours. For making the feed waterstable, suitable binding material should be used, which keeps the feed ingredients together. Many chemical substances can be used as binders. Some examples of binding materials are agar agar, carboxy methyl cellulose (CMC), gelatin, gum, polyvinyl alcohol (PVA), sodium alginate and starch. Any natural source of starch can be used as binder. In such a case the feed should be cooked at 100°C for 5-10 minutes in order to gelatinize the starch. In practical feeds starch is a good and economical binder.

Preparation of Feed:

The composition of the feed used for feeding the postlarvae of *Penaeus indicus* in CIBA Hatchery at Narakal is given below:-

Composition	
Fish meal	20.0%
Prawn head meal	26.0%
Ground nut cake	20.0%
Tapioca	25.5%
Lecithin (soybean)	2.0%
Vitamin mix1	1.4%
Mineral mix2	5.1%
Total	100.0

1. Vitamin mix: The vitamin mixture consists of ascorbic acid 0.5 g, choline chloride 0.7 g, niacin 0.05 g, pantothenic acid 0.02 g, pyridoxine 0.1 g and thiamine 0.01 g.

2. Mineral mix: The mineral mixture consists of calcium carbonate 1.0 g, potassium dihydrogen orthophosphate 4.0 g, copper sulphate 0.06 g and zinc sulphate 0.07 g.

The proximate composition of the feed is as follows:-

Moisture	5.2%
Crude protein	38.5%
Lipid	8.0%
Carbohydrate	27.3%
Crude fibre	6.7%
Ash	14.3%

Processing of ingredients:

All the ingredients should be obtained in dry form. Fish meal, groundnut cake and tapioca powder are available as dry materials. Prawn head waste may be collected in fresh condition and dried in an electrical oven at 70°C. The dry ingredients are powdered individually in an electrical grinder and should pass through 0.5 mm sieve.

Preparation of stock feed:

The ingredients are mixed according to the formula. The feed should be thoroughly homogenised with 600 ml of water per kg of dry feed. The dough so obtained is steamed in a cooker (without weight) for 10 minutes. The feed is pelletized using a 3 mm diameter die and dried in an electrical oven at 70°C for 24 hours. The dry feed should be stored in polythene bags kept in good containers. This is the stock feed.

Preparation of micro-particulate feed:

For preparing micro-particulate feed, the pellets from the stock feed are taken and powdered in an electrical grinder with controlled speed. The powdered mash is sieved through 100 and 200 micron sieves. The particles passed through 200 micron sieve but retained by 100 micron sieve are taken as 200 micron-particles. Similarly the particles of 500 and 1000 microns are prepared by passing through these two respective sieves.

The micro-particulate feed is introduced to mysis III or postlarvae I in the hatchery and continued upto PL20 and beyond in the nursery. The details of particle size, feeding rate and

frequency of feeding of the larvae in hatchery and nursery are summarised below.

Stage of larvae	Particle size of feed (microns)	Quantity of feed per day per 1000 larvae	Schedule and time of feeding
Mysis III upto postlarvae PL5	200	200 mg	Divide the feed in three equal parts and broadcast in the larval tank in the morning, afternoon and evening
PL5 to PL10	500	1 00 mg	-do-
PL11 to PL25	1000	2-3 g	-do-
PL25 and above	3 mm pellets	10% of body weight	-do-

Rearing of larvae using micro-particulate feed:

The early larvae (from protozoea I onwards) can also be reared using the same micro-particulate feed with a particle size of 50 microns, in open culture systems. In this method the rearing tanks are exposed to sun light under a glass house. The larvae feed on a mixture of feed particles and algal cells that grow in the culture tanks. This mixed diet system is conducive for healthy growth of prawn larvae. The advantage of this method is that separate culture of algae is not necessary and the same particulate feed can be used until the postlarvae attain stockable size. This simplifies the larval rearing technique considerably. Using this method a survival rate as high as 80% at PL5 stage, could be realised in P. indicus. The rate of feeding of 50 micron particle feed is 5 grams per day in three divided doses in one tonne capacity tank when 1,00,000 nauplii are stocked.

Feeding prawn larvae in Backyard Hatchery:

In the backyard hatchery technique, the prawn larvae are reared from protozoea I stage upto postlarvae I exclusively with mixed phytoplankton. The postlarvae are fed with Squilla

powder. For this purpose fresh squilla is obtained and sundried. It is powdered in a grinder and prepared into particles of 200, 500 and 1000 microns by sieving through the respective size sieves. The postlarvae I are fed with 200 micron particles until they reach PL5. The postlarvae PL6 are fed with 500 microns and postlarvae PL15 are fed with 1000 micron particles. The rate of feeding is 9 g per day in three divided doses at PL1 stage in 2 tonne tank having 2 lakh nauplii stocked. At PL10 stage the rate of feeding is 12 g and at PL20 it is 15 g a day in three divided doses.

Micro-encapsulated feeds:

Micro-encapsulated feeds are prepared by encapsulating a feed material with a suitable coating wall. The size of capsules range from 10 microns to 500 microns. Micro-encapsulated feeds are primarily meant for feeding prawn larvae. The central feed mixture is called the core and capsulating material is called the outer wall. Micro-encapsulation is achieved by different methods. These are nylon-protein encapsulation, gelatin-gum, sodium alginate-calcium chloride, zein and carrageanan encapsulation techniques. Both liquid and solid materials can be encapsulated. The greatest advantage of micro-encapsulation is that leaching of the nutrients from the diet into the water is prevented. When these capsules are ingested by the larvae, the capsule wall is ruptured by the digestive juices and the core material is released into the stomach. A typical example of micro-encapsulation of a diet is described below. The principle involved in this method is that a chemical diamine reacts with another chemical sebacoyl chloride in solvent medium and forms nylon-protein wall to encapsulate a diet mixture. The materials required are:-

1. Any diet mixture (liquids are preferable).
2. Diamino hexane solution: Prepare by dissolving 0.92 g of 1, 6-diaminohexane + 20 ml of 0.45 M sodium bicarbonate-sodium carbonate buffer (pH 9.8).
3. Mixed solvent : Mix chloroform and cyclohexane in 1:4 ratio.

4. Sebacoyl chloride.
5. Sucrose mono-laurate.
6. Span 85 (a detergent).

Procedure:

Take 25 ml of mixed solvent and 0.5 ml of span 85. To this add 0.5 ml of diamine solution and the diet mixture, homogenise for 3 minutes. Take another 10 ml of mixed solvent and 0.2 ml of sebacoyl chloride. Mix both the mixtures, stir well and add another 30 ml of mixed solvent. The micro-encapsulated diet is precipitated. The particle size of capsules depends upon of speed of stirring. Wash the capsules so obtained with 100 ml of mixed solvent 2 or 3 times. To the capsules add 7 ml of sucrose monolaurate and stir for 10 minutes. Filter and take the capsules and keep them in 2 litres of distilled water for 24 hours. Wash the capsules with water twice. The micro-capsules so obtained should be stored in 1 molar sodium chloride solution.

Micro-encapsulated diets can also be prepared by spray drying technique.

Quality control and storage of feeds:

Checking the quality of the raw materials and the final product is very essential. The raw materials and feeds should conform to the nutritional composition required. Analysis of proximate composition of feeds and feed materials reveals their quality. The proximate composition consists of estimating moisture, crude protein, lipid, carbohydrate, crude fibre and ash. These can be determined by standard AOAC methods.

Proper storage of feeds and feed materials is very important. Improper storage of feeds leads to deterioration of quality as a result of which desired results cannot be achieved. It may often lead to large scale mortalities of cultured animals. During storage feeds are subjected to attack by insects, moulds and rodents. The insect and rodent attacks

can be effectively controlled by storing in [illegible] containers.

Moisture content in feeds plays an important [illegible] moisture in feeds should not be more than 5 to 6%. If the moisture content is more than 10% mould growth may occur. [illegible] can lead to the production of aflatoxin in feeds and feed materials, which is very toxic to prawn larvae and juveniles. Oil cakes such as groundnut cake, coconut cake, soybean cake etc. are more prone to aflatoxin contamination. Mould growth can be prevented by controlling the moisture in the feed and [illegible] by using preservatives such as sodium benzoate or salts of propionic acid at about 100 ppm.

Since prawn feeds contain lipids with polyunsaturated fatty acids, these are easily oxidised when exposed to atmospheric air. This reduces the nutritional quality of the feed. It can be controlled by storing the feeds in air tight containers and using some anti-oxidants such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) and ethoxyquin at 100 ppm.

Suggested Further Reading

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Appendix-1

Some raw materials used for compounding feeds and their composition

Name of raw material	percent on dry basis				
	Crude protein	Fat/ lipid	Carbohydrate	Crude fibre	Ash
1	2	3	4	5	6
I. Energy Feeds:					
Barley	9.9-20.29	2.17	16.34	5.81	2.36
Corn (Maize)	6.2-9.6	4.3-5.5	69.6-70.7	1.4	1.4
Oats	12.0	2.4	63.7	5.0	-
Rice (whole)	8.4	2.1	76.7	0.7	0.8
Rice (broken)	7.5	0.5	79.9	0.3	0.5
Rice bran	13.24-15.80	18.2	47.43	9.0	11.8
Rye	11.6	1.7	69.8	1.9	2.0
Sorghum (Milo)	11.0	2.8	71.6	2.0	-
Tapioca	2.0	0.54	68.50	-	1.[illegible]5
Wheat grain	13.07	1.96	63.61	3.91	3.85
Wheat flour	10.80	1.10	74.60	0.20	0.5
Wheat bran	13.90	4.20	55.60	10.50	5.3
II. Protein supplements:					
(a) Plant materials:					
Brewer's grains	26.0	6.0	41.8	15.0	-
Coconut cake	25.96	11.2	22.19	-	8.88
Cottonseed cake	42.0	2.0	30.0	11.0	-
Distiller's grains	27.0	-	41.0	12.0	-
Gingelly cake	34.03	10.8	24.76	-	12.52
Gluten (wheat)	25.0	2.0	48.0	8.0	-
Groundnut cake	48.42	7.56	28.18	-	6.03
Linseed	35.0	2.0	39.0	9.0	-
Malt sprouts	26.0	1.0	44.0	14.0	-
Rapeseed cake	46.0	1.0	31.0	5.0	-
Sunflower cake	47.0	3.0	24.0	11.0	-

1	2	3	4	5	6
(b) Animal materials:					
Blood meal	80.0	2.0	-	-	0.52
Clam meat (Sunneta scripta)	48.10	13.55	16.69	-	7.62
Crab meal	30.0	1.7	-	-	-
Fish meal (Brownfish meal low protein: whitefish meal high protein)	52-74	1.0-10-0	-	-	14.0-31.
Mantis shrimp (Squilla)	44.06	7.55	1.27	-	23.63
Meat meal	53.0	10.0	-	-	12.03
Meat and bone meal	51.0	10.0	-	-	16.07
Mysid meal	76.05	2.72	5.57	-	15.66
Prawn waste meal	35.20	6.60	0.97	-	23.95
Silkworm pupae					
(1) whole	55.91-57.5	24.5-29.7	5.58	-	2.98
(2) defatted	75.36	1.75	8.40	-	5.59
Shrimp meal	36.0-48.0	3.0	-	-	-
Squid meal	81.38	9.63	5.33	-	3.66
III Non-conventional food ingredients:					
House fly larvae	45.0	15.0	-	-	8.0
Poultry feather meal (hydrolysed)	80-85	2.5	-	1.5	3.0
Single cell protein					
(i) Krill	55.0	10-15	-	-	15.2
(ii) Marine yeast	25.63	2.69	63.50	4.27	3.91
(iii) Petroleum yeast	61.22	2.10	26.24	3.9	6.54
(iv) Sludge	43.0	0.43	15.0	28.0	3.0
(v) Spirulina	60.89	9.0	6.63	-	13.0

METHODS OF WATER ANALYSIS

S. AHAMADALI

Management of water quality in the larval rearing tanks in the hatchery and also in the broodstock tanks is very essential. Water quality management includes prevention and control of disease spreading organisms and the control of chemical constituents in the water. While the management of diseases is dealt separately elsewhere, the management of chemical quality of the water is presented here.

The important chemical and physical parameters that should be monitored regularly are salinity of the water, temperature, pH, dissolved oxygen, total ammonia, nitrate, nitrite and light intensity during day time. The permissible range of these parameters in hatchery and broodstock tanks are as follows:-

Parameters	in Hatchery tanks	in broodstock tanks
Salinity	29-34 ppt	29-34 ppt
Temperature	26-32.5°C	27-29°C
pH	8.0-8.5	8.0-8.2
Dissolved oxygen	3.0-8.0 ml/l	4-5.5 ml/l
Total ammonia	0.1 ppm	0.02-0.07 ppm
Nitrite	0.05 ppm	0.0003-0.02 ppm
Light intensity during day time	20,000-1,25,000 lux*	< 100 lux

*Direct incident sunlight on larval tanks is not desirable.

Water sampling for analysis:

Taking a representative sample of water for analysis is essential to ensure that the results truly represent the entire lot. This is easy if the quantity of the water to be tested is small. It can be properly mixed and the sample taken. But if the water mass is huge proper sampling method should be adopted. Water sampling bottles are available for this purpose which can be purchased and used.

Temperature:

The temperature of the water can be measured using a thermometer graduated in degrees centigrade.

pH:

pH of a medium is the measure of hydrogen ion concentration in it. If the pH of water is 7, it is neutral and if below 7, it is acidic and above 7, it is alkaline (basic). Any pH meter can be used for recording the pH of the medium. Before using the pH meter, its electrode should be in order with proper filling of saturated solution of potassium chloride (KCl). Most of the pH meters need calibration before use. This can be done by dipping the electrode in a buffer solution of known pH and adjusting the pH to that value. Buffer tablets of pH ranging 4 to 9 are available which can be used for this purpose.

Light intensity:

Light intensity can be measured with the help of a 'Lux' meter.

SALINITY:

Reagents:

1. Silver Nitrate (24.5 gm/litre)
2. Potassium Chromate (10%) 10 gms in 100 ml. of water.
3. Standard Seawater

Procedure:

Pipette 10 ml of standard seawater into a 250 ml conical flask. Add 4 drops of potassium chromate solution and titrate against silver nitrate solution till the colour changes to red. Pipette out 10 ml of the seawater sample into the conical flask and proceed as above.

Calculation:

Let volume of Silver nitrate for 10 ml of standard seawater = V_1 ml

Volume of silver nitrate for 10 ml of sample = V_2 ml

Salinity of standard seawater = S ‰

Salinity of sample = $\frac{V_2 \times S}{V_1}$ ‰

Salinity refractometers available in the market can give salinity reading instantaneously upto an accuracy of ± 1 ppt. It is necessary to see that the instrument shows 0 value when distilled water is used. If the value is not zero, it can be adjusted to zero by adjusting a small screw.

DISSOLVED OXYGEN:

Reagents

1. Sodium thiosulphate solution (1.25 gms in 1 litre)
2. Starch solution - 1 gm starch made into a paste with distilled water and diluted to 100 ml, boil and cool.
3. Winkler solution A (20 gms of Manganese chloride in 100 ml of water)
4. Winkler solution B (41 gm of sodium hydroxide + 25 g of potassium iodide in 100 ml water)
5. Concentrated Hydrochloric Acid.
6. Standard potassium iodate (Accurately weigh out 0.1784 g of potassium iodate into a 1 litre volume tric flask and dissolve and make up to the volume : This is 0.005N solution).
7. Potassium iodide (solid).

Procedure:

Collect the water sample in a 125 ml glass stoppered bottle without entangling any air bubbles. Take out the stopper and add 1 ml each of winkler A and winkler B solution. Close the bottle. Shake the bottle gently till the precipitate formed is evenly distributed. Allow to settle. Then add 2 ml conc. Hydrochloric acid, close the bottle and gently shake till the precipitate is completely dissolved.

Pipette 10 ml of potassium iodate solution into a conical flask. Add 1 gm of potassium iodide and 2 ml of Conc. Hydrochloric acid. Dilute to 100 ml and titrate against sodium

thiosulphate solution till the colour becomes pale yellow. Add 1 ml of starch solution, shake well and continue the titration till the blue colour disappears. Repeat until concurrent titre values are obtained.

Pipette out 100 ml of the preserved sample and titrate against sodium thiosulphate as above.

Calculation

Calculate the normality of potassium iodates as

$$= \frac{\text{Weight in litre}}{35.67} = N_1$$

Calculate normality of thiosulphate as

$$= \frac{N_1 \times 10}{\text{Titre value of thiosulphate for 10 ml of potassium iodate}} = N_2$$

Amount of dissolved oxygen in ml/litre

$$= \frac{\text{ml. thio.} \times N_2 \times 8 \times 1000 \times R}{100 \times 1.429}$$

(where 1.429 being weight of 1 ml of oxygen in milligrams. R is shown as the correction factor and which is roughly equal to 1.01 in majority of the cases).

REACTIVE PHOSPHOROUS:

Water samples may be collected in polythene bottles of roughly 150 ml capacity and analysis should be carried out within an hour of collection. If the analysis is to be delayed, the samples must be stored in refrigerator.

Reagents

1. Ammonium molybdate solution

Dissolve 15 gms of A.R. quality ammonium molybdate in 500 ml distilled water. Store in plastic bottle and keep away from sunlight.

2. Sulphuric acid solution

Add 140 ml of A.R. quality sulphuric acid to 900 ml distilled water and mix well.

3. Ascorbic Acid solution

Dissolve 27 gm of ascorbic acid (A.R. quality) in 500 ml distilled water. Store the solution in refrigerator after use.

4. Potassium antimony tartrate solution

Dissolve 0.34 gm of pure reagent grade potassium antimony tartrate in 250 ml distilled water.

5. Mixed Reagent

Mix together 100 ml of ammonium molybdate, 250 ml of sulphuric acid, 100 ml of ascorbic acid and 50 ml of antimony tartrate solution and mix well. This solution can be kept for 6 hours, and the above quantity is sufficient for about 50 samples.

This reagent should be prepared as and when required.

Procedure

Take 100 ml of sample and add 10 ml of mixed reagent. After 5 minutes measure the absorbance at 885 $m\mu$ in a spectrophotometer or Colourimeter.

Warm another portion of the sample to laboratory temperature in a thermostated water bath and measure the value to obtain a turbidity correction. Correct the measured value of the sample by subtracting both the turbidity and reagent blank. Calculate the phosphate concentration in microgram atoms of phosphate phosphorus per litre as equal to correct absorbance x F.

In the present case F is equal to 5 (changes with instrument).

Alternate method:

Dissolve accurately 0.186 gm of anhydrous potassium dihydrogen phosphate in 1000 ml of distilled water. Store in a dark bottle with 1 ml of chloroform. 1 ml of the solution is made upto 100 ml. From this, 5 ml is taken and diluted to 100 ml. 100 ml sample is taken in a conical flask, and 10 ml of mixed reagent is added to the standard and sample. After

10 minutes the colour comparison of these 2 solutions is made using Nessler Cylinders.

The strength of the colour developed being proportional to amount of phosphate, calculate the phosphate concentration in sample using the strength of the standard potassium phosphate solution.

NITRATE

Reagents

1. Phenol solution

Dissolve 46 gm of dry A.R. quality phenol in 1000 ml of distilled water. Store in a glass bottle tightly stoppered.

2. Sodium hydroxide

Dissolve 29 gms of A.R. quality Sodium hydroxide in distilled water. Cool and dilute to 2000 ml.

3. Buffer Reagent

Pipette out 25 ml of phenol solution into a dry beaker and add 25 ml of sodium hydroxide solution. The solution is stable for one hour.

4. Copper sulphate solution

Dissolve 0.1 g of A.R. Copper sulphate in 1000 ml of distilled water.

5. Hydrazine sulphate solution

Dissolve 14.5 gm of A.R. quality Hydrazine sulphate in 2000 ml of distilled water. Store in a dark glass bottle. The solution is stable for one month.

6. Reducing agent

Mix 25 ml of copper sulphate solution and 25 ml of Hydrazine sulphate solution in 50 ml measuring cylinder. The solution is stable for one hour.

7. Acetone

8. Sulphanilamide solution

Dissolve 5 gm of sulphanilamide in a mixture of 50 ml conc. hydrochloric acid and about 300 ml of distilled water diluted to 500 ml with water. It is stable for many months.

9. N-Naphthyl Ethylene Diamine Di-hydrochloride solution (N.N.E.D.)

Dissolve 0.5 gm of N.N.E.D. in 500 ml of distilled water. Store the solution in a dark bottle.

10. Standard Nitrate solution

Dissolve 1.53 gm of analytical grade potassium nitrate in 1000 ml; 1 ml of this solution = 15.0/ ug of nitrogen. Dilute 5 ml of this solution to 250 ml with water. Store in dark bottle.

Procedure:

Measure out 50 ml of the seawater sample with a 50 ml measuring cylinder into a 250 ml conical flask (sample should acquire room temperature). Add 2 ml of buffer reagent and mix. After the buffer has been added to all the samples, add with rapid mixing, 1.0 ml of reducing agent and keep the flasks away from sunlight in a dark place for about 20 minutes. Add 2 ml of acetone, and after 2 minutes add 1 ml of sulphanilamide solution. After 2 minutes, but not later than 8 minutes, add 1.0 ml of N.N.E.D. solution and mix. Measure absorbance at 545/ in a colourimeter. Calculate the nitrate present in the sample using the formula

$$\text{Nitrate in 50 ml of seawater} = \frac{\text{absorbance of sample}}{\text{absorbance standard}} \times \text{amount of nitrate in standard}$$

NITRITE

Reagents

1. Sulphanilamide solution (as in Nitrate method)

2. N-Naphthyl Ethylene Diamine Dihydrochloride (N.N.E.D.)

(as in Nitrate method)

3. Standard Nitrite solution

Dissolve 0.345 gm of A.R. quality sodium Nitrite in 1000 ml of distilled water. Store in a dark bottle with 1 ml of chloroform. 1 ml of this solution = 5 ug of nitrite nitrogen. Dilue 10 ml of the solution to 1000 ml with distilled water and use for analysis.

Procedure

Measure out 50 ml of seawater sample in the conical flask. Add 1 ml of sulphanilamide solution to each sample. After 2 minutes but not later than 8 minutes, add 1 ml of N.N.E.D. solution to each and mix immediately. Carry out the procedure with standard nitrite solution also.

Measure the absorbance in a calourimeter at 545 mμm.

$$\text{Amount of nitrite present in water sample} = \frac{\text{absorbance of sample} \times \text{amount of nitrite present in standard}}{\text{absorbance of standard}}$$

AMMONIA

Reagents

1. Phenol-alcohol solution. Dissolve 10 g of reagent grade phenol in 100 ml of 95% (V/V) ethyl alcohol.
2. Sodium nitroprusside (0.5%) - Dissolve 1 g of sodium nitroprusside in 200 ml of water.
3. Alkaline solution: Dissolve 100 g of trisodium citrate and 5 g sodium hydroxide in 500 ml of water.
4. Sodium hypochlorite solution: Use a solution of commercial hypochlorite which should be at least 1.5 N.
5. Oxidising solution: Mix 100 ml of sodium citrate solution and 25 ml of hypochlorite solution and use the same day. Prepare this solution freshly for every day use.
6. Standard ammonia solution: Accurately weigh 0.1 g of ammonium sulphate (Analar grade) in 1 litre of distilled water. 1 ml of this solution is equal to 1.5 micrograms of ammonia nitrogen (N).

Procedure:

Preparation of standard graph

Take five 50 ml volumetric flasks and pipette 1 ml, 2 ml, 3 ml, 4 ml and 6 ml of standard ammonia solution. To each flask add 2 ml of phenol solution, 2 ml of sodium nitroprusside solution and 6 ml of oxidising reagent. Mix well and allow it to stand for 1 hour. Make upto the mark with distilled water and measure the absorbance in colourimeter or spectrophotometer at 640 μnm. Draw a graph between ammonia concentration and absorbance.

Take the water to be tested in 50 ml volumetric flask upto half-level. Add 2 ml of phenol solution, 2 ml sodium nitroprusside solution and 6 ml of oxidising agent. Make up to the mark with the same water sample to be tested. Allow it to stand for 1 hour and measure the absorbance as in the case of standard. Calculate amount of ammonia nitrogen present in the water sample from the standard graph. This value will give the ammonia nitrogen present in 40 ml of water sample. If the value is multiplied by 25 gives the ammonia N present in 1 litre.

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DISEASES OF PENAEID SHRIMP LARVAE

A. Laxminarayana

Successful rearing of penaeid larvae can at times be threatened by devastating effect of disease organisms, especially when they are reared at high densities. It should be remembered that prevention is better than cure because in intensive continuous culture systems, potential pathogens are always ready to proliferate. The routine techniques of penaeid larval rearing include the use of chlorination of the rearing systems and preventive antibiotic and antifungal treatments.

The penaeid larval diseases can be classified under (1) larval diseases (2) Bacterial diseases (3) Fungal diseases (4) Larval fouling by ciliates, protogens and other epibiontes and (5) Nutritional diseases.

1. Viral disease:

Viral infections appear mainly during postlarval and juvenile stages. Two groups of viruses have been implicated Baculovirus group of viruses (e.t. MBV) or Parvovirus or a Picornavirus group (e.g. IHHNV). The baculovirus group affects the midgut and hepatopancreas causing remarkable cytopathological changes that includes the presence of polyhedral inclusion bodies (PIB's) in nucleus, nuclear hypertrophy, chromatic diminution and nucleolar degeneration in the epithelial cells of the hepatopancreas. Epizootics may occur in protozoeal and mysis larval stages. The infections hypodermal and hematrpoetic necrosis Virus (HHNV) do not normally affect the midgut and hepatopancreas. It's target organs include ectodermally derived (like cuticular hypodermis or epidermis, cuticular epithelium and the nerve cords) and mesodermally derived tissues (striated muscle, connective tissues, hemocytes and hematopoctic tissues, mandibular organ, heart and the gonads). Certain patterns of necrosis and cellular inflammatory response are suggestive of IHHN infection.

In nature transmission is achieved through cannibalism of infected shrimp by uninfected shrimp.

Preventive measures:

Eradication of known IHHNV/BV contaminated stocks is recommended as the perferred means of control for this disease. All the breeders must be checked before taking them to the maturation facility. Stresses due to handling seem to encourage the establishment of viral disease. Therefore, the water used in the hatchery should be free of toxicants and pollutants.

2. Bacterial diseases:

(a) External necrosis:

This occurs in larval and postlarval stages of all penaeids. Under the microscope, the first trouble can be seen in protozoea [illegible] thereafter starts with a liquefaction of the gut contents. Algae are incompletely digested and a green liquid fills the gut instead of shaped faeces as in healthy larvae. Necrosis often starts on an antennae or a newly formed appendage like the uropods in protozoea III or the pleopods in mysis. Necrosis starts at the tip of the appendages and progresses towards the base and the necrosed area gets melanized. Weakened larvae have broken setae, opaque and more or less eroded appendages. Dying larvae are often ready to moult or failed to moult, the old carapace being held back by the necrosed appendages. The attack is more serious if it starts early in larval development. Postlarvae are rather resistant to disease.

(b) Internal necrosis:

Normally this follows external necrosis and is characterised by the necrosis at the region of the moults consequent to which the larvae cease to eat. Hepatopancreas and stomach become greyish. This disease is therfore known as 'grey stomach disease'. Protozoeal stage is the most prone to this disease and is noted usually after a long period of hatchery operation.

Prevention and therapy:

Disinfection of tanks and chlorination and filtration of the seawater supply is helpful as a preventive measure. The use of treated water ensures larval rearing to begin in optimal condition, but bacterial proliferation in an intensive larval system cannot be avoided. Even with a total daily water exchange larvae keep the bacteria inside the gut or fixed on the shell; this flora can be harmful only if its level is too high. The action of the preventive routine antibiotic treatment would then lead to a partial destruction of this flora.

Preventive antibiotics include streptomycin-bipenicillin (2 ppm), erythromycin phosphate (1 ppm AP), Tetracyclin chlorohydrate (2 micro litre/ml), sulphamethazin (3 ppm AP), furacin (0.1 ppm), chloramphenicol and furazolidone. In some of the hatcheries, the routine treatment for the larval rearing use chloramphenicol and furazolidine. The preventive dose is administered routinely every alternate day till the postlarval (PL5 stage). Curative dose is applied in case of apperance of necrosis.

3. Fungal disease:

The mycotic agents associated with fungal attacks are Lagenidium sp. Sirlpidium sp and Haliphthoros sp. The sign of contamination is the infestation of dead larvae. Highly branched fungal mycolium can be seen throughout the body, when observed under the microscope. Evolution of the disease is fast (24-48 hrs) especially in early larval stages and the thalli soon replace all the tissues of the larvae which form spore reservoirs. In Lagenidium sp. the reproductive form is an ex external tube which gives birth to a sporangium but in Sirolpidium sp. spores are formed in the mycelium inside the larvae. Infected individuals become immobile and settle to the bottom.

Prevention and treatment:

Preventive measures include chlorination and filtration of the seawater supply. Malachite green (0.009 mg/l) added to the spawning tanks containing the gravid female is effecive in inhibiting infection. As the spores settle preferentially on dead eggs or dead bodies, it is necessary to remove such decaying matter from the rearing tanks. Treflan-R (0.1 mg/l) is effective in destroying the zoopores. Trifluralin is the active incredient of the commercial herbicide TREFLAN-R.

Tuflan doses:

Nauplius	10 cc/m^3
Protozoea	20 cc/m^3
Mysis	30 cc/m^3
Postlarvae	40 cc/m^3

4. Larval fouling by ciliates, proteozeans and other epibiotes:

All the stages of the larvae can be affected. They are often found swimming around the larvae, upon the gills, between small setae or fixed on the shell. Ciliate infection is characterised by a matlike apperance on the gills and appendages due to the attachment of stalked colonies. These fouling organisms are present most of the time in the rearing medium and are not directly pathogenic. But their accumation on the larvae especially on the gills interfere with gas exchange. Heavy infestation can also affect feeding.

Preventive measures: Always maintain hygienic conditions and filter the incoming seawater properly. Accumulation of organic matter should not be allowed.

Nutritional diseases:

The black stomach disease is nutritional in origin and is associated with the use of Chaetoceros. The condition is characterised by the presence of black plug in the stomach obstructing the digestive tract. This is caused due to incomplete digestion of algae and normally appears when old Chaetoceros culture is used to feed the larvae.

Remedial measures: The remedial measures include monitoring the algal quality and evolution of the algal bloom in the larval rearing tanks. Change water and add fresh algae.

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Practicals under Broodstock development and spawning of penaeid shrimps

A. Laxminarayana

1. Sexual dimorphism

Female

a) Relatively larger than males

b) The sternites of the last three thorasic somites are modified as 'thelycum'.

c) The external genital aperture is situated in the proximal podomere of the third leg

Male

a) Relatively smaller than females

b) The endopodes of the first pair of pleopods are modified into a copulatory organ known as 'petasma'.

c) The internal genital aperture is situated in the proximal podomere of the fifth leg

II Maturity stages

a) Immature stage: The ovaries thin, trnsluscent, unpigmented and confined to the abdomen.

b) Early maturing stage: The ovary increasing in size and the anterior and middle lobes developing. The dorsal surface light yellow to yellowish green

c) Late maturing stage: The ovary is light green and is visible through exoskeleton. The anterior and middle lobes fully developed.

d) Mature stage: The ovary dark green and clearly visible through exoskeleton.

a) stage 1: Testes lobes not developed. Spermatozoa not formed.

b) Stage 2: Testes lobes fairly developed.

c) Stage 3: Testes lobes well developed

d) Stage 4: Testes lobes well developed. Spermatozoa present in the terminal ampoule. This is the fully mature stage.

e) Stage 5: Testes lobes contain only spermatozoea but not other stages of spermatogenesis.

e) Spent recovering: Distinguishable from immature virgin females only from the size of shrimp. Examine the eggs collected from the ovary under microscope.

III Eyestalk ablation:

1. Select impregnated females in the size range of 145-160 mm in total length.

2. Carry out unilateral eyestalk ablation of the females by passing the red hot loop of the Electrocautery through the middle of the eyestalk.

IV Electro ejaculation of spermatophores and preparation of sperm suspension

1. Give a mild electric shock (6 volts) using electro-cautery apparatus to the base of the 5th pair of walking legs when the spermatophores are immediately ejaculated.

2. Take out the spermatophores with the help of a forceps and introduce the spermatophores into an embryo cup containing seawater (salinity 33 ppt, pH 8.2).

3. The spermatophores are mascerated to release the sperm.

4. This sperm suspension is later transferred into a 50 ml beaker containing seawater.

V. Spawning:

1. Select femaleswith fully mature (diamond shaped) ovary.

2. Fill the spawning tanks with seawater, filtered through a 50 micron mesh bolting cloth. Add disodium saltof EDTA to the spawning media @ 0.1 g per 100 litres of seawater. Provide aeration.

3. Keep the sperms in the spawning tank. Only one spawner in each tank.

4. Cover spawning tank with a lid.

5. Switch off lights during night.

6. Spawning takes place between 8.00 am and 2 am.

7. Remove spent female in the morning; Return the same to maturation tank. Record its length measurements.

8. Find out the fertilization rate under microscope.

$$\frac{\text{No of fertilized eggs x 100}}{\text{Total No of eggs}} = \%$$

9. Egg start hatching within 12 to 17 hours depending upon the ambient temperature.

10. Count number of nauplii by sampling. The larvae are dispersed in water by thorough mixing and three 100 ml samples are taken with the beaker. The number of eggs in each sample is counted and the average number in 100 ml calculated.

11. Count number of unhatched eggs by sampling

12. Calculate the hatching rate as follows:-

$$\text{Hatching rate} = \frac{\text{No of nauplii hatched x 100}}{\text{No of eggs spawned}} = \%$$

VI <u>Artificial insemination</u>:

1. Ablate females

2. Keep ablated females individually in 200 l tanks filled with seawater and provided with aeration.

3. Feed the ablated females with clam meat.

4. Change entire water daily in the tanks after removing uneaten food and feacal pellets etc.

5. Few unablated mature males also kept separately in 200 l tank and fed with clam meat. Change water daily.

6. Observe the moulting daily.

7. After moulting catch soft females with a handnet and hold with left hand, ventral side up.

8. Implant the electroejaculated spermatophores into the thelycum.
9. Allow the female to recover in the trough of seawater which is continuously aerated.
10. Release the implanted female into a 250 litre tank filled with seawater.
11. Feed the implanted female daily with clam meat.
12. Change water daily, remove uneaten food and feacal matter etc.
13. Observe the full development of ovary within 4 to 7 days after implantation.
14. When fully mature, keep the female for spawning individually in spawning tanks.
15. Spawning takes place at night.
16. Remove the spent female the next day and return to the same tank from where it was taken.
17. Count the number of nauplii and unhatched eggs the next day.
18. Calculate the hatching rate.

VII <u>In vitro fertilization</u>:

1. Female shrimps measuring 140 mm and above are kept individually for moulting in 300 litre tanks containing seawater.
2. Few mature males are also kept in another tank.
3. The shrimps are fed *ad libitum* on clam meat.
4. Water in these tanks was changed daily.
5. Note if there is any moulting in the tanks.
6. Two days after moulting, unilateral eyestalk ablation of the moulted females was carried out.
7. The females mature 3 to 5 days after ablation.
8. The fully mature females kept for spawning.

9. At the time of spawning, females were caught and spawning was stopped by lifting the shrimp out of water.
10. The ovary is cut and the eggs are mixed with the sperm suspension taken in a 50 ml beaker.
11. The egg-sperm suspension is transferred to 250 ml beaker containing 100 ml of filtered seawater.
12. The flask is moved rapidly to get whirlpool movement for nearly 5 minutes.
13. Fertilization observed 10 minutes after mixing eggs with sperms.
14. The fertilized and unfertilized eggs are transferred into FRP tank containing 50 litres of seawater.
15. Larvae start hatching within 12-17 hours after fertilization.

Larval rearing of *P. indicus*

S.M. Pillai

- Containers

  FRP tanks of 2 tonne capacity.
  Disinfect with bleaching powder or calcium hypochlorite
  Clean and dry the tanks.

- Water filtration

  Filter sewater with 50 micron nylobolt.
  Fill tanks with filtered seawater.

- Aeration - with diffuser air stones.

- Hatching rate

  Estimate hatching rate by taking 3 representative aliquot samples (100 ml).
  Examine the general health of the larvae.

- Stocking

  Transfer nauplii into rearing tanks by siphoning after concentrating them by focusing light. Stock @ 100 nauplii/litre of water.

- Water quality

  Maintain the water quality as follows:-
  Salinity - 27 to 34 ppt, Temperature 26 to 32.5°C, pH 8 to 8.5, DO 3-8 ml/l, Total ammonia 0.1 ppm, Nitrite 0.05 ppm.

- Larval stages

  Nauplius - 6 sub stages (N1 through N6)

Stage:	N1	N2	N3	N4	N5	N6
Furcal setae	1+1	1+1	3+3	4+4	6+6	7+7
Duration in hours	4	3-4	6-8	3-4	10-12	15-24
Size (mm)	0.28-0.31	0.29-0.32	0.29-0.32	0.34-0.38	0.35-0.41	0.43-0.54

Protozoea - 3 substages called PZ1, PZ2 and PZ3.

PZ1 : Sessile eyes, rostrum and pereopods absent and abdomen unsegmented. Duration 24 to 48 hou. Size 0.88-0.91 mm

PZ2 : Eyes stalked, rostrum appear, first 5 abdominal segments demarcated, uropod absent. Duration 48 to 72 hours. Size 1.40-1.55 mm.

PZ3 : Uropod present, telson seperated from last abdominal segment, dorsal spines present on the first 5 abdominal segments. Duration 24 to 36 hours. Size 2.41-2.73 mm.

Mysis - 3 substages. They are M1, M2 and M3.

M1 : Larvae assume shrimp like shape, rostrum long and curved, minute abdominal buds on the first five abdominal segments. Duration 48 to 72 hours. Size 3.07-3.65 mm.

M2 : Appearance of unsegmented pleopod buds. Duration 24 to 48 hours. Size 3.39-3.58 mm.

M3 : Development of 2 segmented pleopod buds. Duration 24 to 48 hours. Size 3.43-4.17 mm.

Postlarva 1 - Rostrum with 1 or 2 dorsal spines, pleopods well developed and setose. Duration 24 to 30 hours. Size: 4.55-5.26 mm.

- Feeding

At N6 stage algal water is introduced into the tank.
Feeding starts at protozoea 1 stage.
PZ1 to M3 stage - mixed diatoms @ 30,000 to 40,000 cells/ml.
Feed 4-5 times.
PL1 to PL5 - 200 micron squilla powder
PL5 to PL15 - 500 " " "
PL15 to PL20 - 1000 " " "
Feeding done 3 times per day.

- Water management

Start with 100 and gradually increase water level.
Upto postlarva 1 there is no water exchange, except addition of algal water.

From PL1 to PL5 daily 1/3 quantity of water is exchanged. From PL5 to PL20, on alternate days 50% water is exchanged. Use 250 micron mesh filter cloth.

- Larval counts

  Estimate survival of the larvae at PZ1, M1, M2, M3, PL1 to PL20 by counting 3 aliquot samples.

- Management

  Observe larvae for their general behaviour, feeding activity, health and disease incidence, if any. Maintain hygienic conditions in the hatchery. Ensure proper aeration.

  Maintain water quality. Bring down the salinity gradually from PL3 to 15-16 ppt at PL20.

- Harvest

  Harvest by reduction of water and scooping larvae with standard measure strainer. Sample minimum four measures to estimate PL20 production.

- Packing and transport

  Pack in polythene bags. Pour 6 litre water and fill it with O_2. Stock PL20 @ 3,000 nos/litre. During long distance transport introduce Artemia nauplii into the bag to prevent cannibalism. Reduce water temperature by placing blocks of ice in sachets into the bag.

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Mixed culture of phytoplankton

S.M. Pillai

For feeding prawn larvae, large scale cultures of phytoplankton is done in the hatchery. The various steps involved are:-

- Containers

  One tonne capacity white FRP tanks.

  Disinfect, clean and dry the containers.

- Water filtration

  Filter seawater with 60 micron nylobolt.

  Fill the tank with filtered water.

- Culture medium (for one tonne water)

Sodium nitrate	-	12 ppm
Potassium orthophosphate	-	3 ppm
Sodium silicate	-	6 ppm
EDTA	-	6 ppm

  Add the nutrients into the FRP tanks.

- Aeration

  Two air stones in one tank

- Maintain salinity 30-34 ppt and temperature at ambient condition. Light intensity at 20,000 - 1,20,000 lux during day time in open shed.

- Cell count

  By Haemocytometer. Place 1 ml algal water and count the cells in 3 chambers. Cell density (cells/ml) = Average count per chamber x 10^4.

- Cell density of 3-4 lakhs/ml in 24-48 hours. Appearance of golden brown colour indicates maximum cell density.

- Harvest

By siphoning and flocculation. For flocculation dissolve 100 g Na OH in 100 ml distilled water. Add sodium hydroxide solution drop by drop to the algal water and increase the pH to 9.4. Remove the settled algal mass. Bring down the pH to 8.2 by adding dil. HCl drop by drop.

Preservation and storage

Sun drying or freeze drying. Add few drops of cryoprotectants like glycerol or dimethyl sulphoxide before freeze drying the algae. Frozen algae can be stored for 3 months.

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PRACTICAL

S. AHAMAD ALI

Preparation of Micro-particulate feed for prawn larvae

A. Equipments :
1. Grinder (Pulverizer).
2. Pressure cooker
3. Pelletizer.
4. Dryer (Hot Air Oven)
5. Sieves (100, 200, 500 and 1000 microns)
6. Plastic basins (50 l capacity)
7. Containers for storage
8. Aluminium tray for drying the feed (Size of trays suitable to the dryer)
9. Balance to weigh 10 kg
10. Sieve shaker

B. Procedure (Final action):

Grind the ingredients prawn waste, groundnut cake, fish meal, tapioca in the pulverizer passing through 0.5 mm sieve. Weigh the powdered ingredients and mix them according to the formula for a batch of 5 kg, and thoroughly homogenise in a plastic basin. Add water (600 ml per kg of dry feed) and make it into a dough. Take the dough into cooker containers and steam it for 10 minutes without weight on the cooker. Pelletize the feed using a 2 mm or 3 mm diameter die and collect the pellets in trays. Dry the pellets in the dryer at 70°C for 24 hours. Store the dry pellets in good containers. This is stock feed.

C. Preparation of Micro-particles of feed:

Take aliquots of the stock feed according to the quantity required and grind it in the pulverizer without sieve. This can also be done in a mixie. Sieve the mash so obtained through these sieves in the sieve shaker and collect the particles passing through each sieve separately. Store the feeds properly in polythene bags kept in air tight containers.

D. Feeding prawn larvae with micro-particulate feed:

The prawn larvae are fed in the hatchery and nursery with the micro-particulate feed according to the schedule below:-

stage of larvae	Particle size of feed (in microns)	Quantity of feed to be fed for 1000 larvae/day	Schedule and time of feeding
Mysis III upto postlarvae PL5	200	200 mg	Divide the feed in three equal parts and broadcast in the larval tank in morning, afternoon and evening
PL5 to PL10	500	1000 mg	-do-
PL11 to PL25 and beyond	1000	2-3 g	-do-