TRAINING IN PRAWN HATCHERY TECHNOLOGY

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LIFE CYCLE AND REPRODUCTIVE BIOLOGY OF PENAEID PRAWNS

By

A. Laxminarayana

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a. Life Cycle

Almost all the cultivable species of marine prawns belonging to the genera Penaeus and Metapenaeus mature and spawn in the sea where the larvae also develop and after a series of moults, metamorphose into postlarvae. These postlarvae are carried by the tides into the estuaries and backwaters where they settle down and grow rapidly into juveniles. After attaining a particular size, the juveniles migrate back to sea for gonadial maturation and spawning.

The above pattern is typical of Penaeus indicus, P. monodon and other species, while in some such as Parapenaeopsis stylifera the entire life history is spent in the marine environment.

b. Reproductive Biology

In nature penaeid prawns breed only in the sea. They are sexually dimorphic, the male being generally smaller than the females. The males may become mature even in brackishwater ponds but the females never 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 petasma to the thelycum

of female. In penaeids with a closed thelycum (e.g. Penaeus indicus, P.monodon etc.) mating takes place between a newly moulted "soft" female with immature ovaries and a mature male in the intermoult phase; the spermatophores are tucked safely inside the seminal recepticles, close to the thelycum where they are retained until the prawn moults again; there is a time lag between mating and spawning. In penaeids with open thelycum (e.g. P.stylifera, 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 the sea water.

Egg production in prawns, as in other crustaceans is a cyclic process under the hormonal control of the neuro-secretory centres. Among these centres, the X-organ sinus gland complex in the eyestalk produces the gonad inhibiting hormone (GIH) which inhibits vitellogenesis, while the centres in the brain and thorasic ganglia produce the gonad stimulating hormones (GSH) which promote vitellogenesis. During the quiescent phase 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 take place under the influence of GSH. On the basis of this hypothesis, the technique of unilateral eyestalk ablation has been evolved for inducing the penaeid prawns to mature in captivity. By removal of one eyestalk, the titre of GIH is arbitrarily reduced and this leads to ovarian development.

Soon after ablation the prawns start feeding voraciously. Females weighing 90-120 g in the case of P.monodon and 15-25 g in P.indicus respond well to unilateral ablation of eye-stalk, when kept in flow-through system under subdued light (360-500 lux). Complete maturation is observed within 3 days to 15 days in different species.

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BROODSTOCK MANAGEMENT AND SPAWNING OF PENAEID PRAWNS

By

A. Laxminarayana

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

1. Size of prawns

The size of the prawns 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 prawns 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 only on females and not 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 off the ye-stalk with a pair of scissors or making a slit in the cornea and squeesing out the contents.

3. Management of maturation pools:

The ablated females are introduced into the maturation pool 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 pool it is advisable to keep a few males in the maturation pool. In a 10,000 litre pool 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	500 - 3600 lux

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

The pH of the sea water in the pool is maintained between 8.0 and 8.2 by addition of sodium carbonate (Ca 25 g/m^3 of water every day).or slaked lime (Ca.25 g/m^3 of water every alternate day). The prawns are fed with clam or mussel meat @ 12.5 - 15%

of prawn 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 sea water are measured in the morning and evening. The salinity of the water may be checked once in two days. If the salinity 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 prawns 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 sea water in the maturation pool is also totally replaced before introducing a fresh batch of ablated females into the pool. If it is difficult to get large sized prawns for eye ablation, the spent females can be reintroduced into the pool for rematuration.

4. Spawning

The females with fully mature ovary are removed with a dipnet from the maturation pool 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, alinity 30-34 ppt and the pH 8.0-8.2. Mild aeration is given. Disodium salt of EDTA is added to the water @ 0.1 gm per 100 litres of

water. The tank is covered with ablack 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 female is removed only in the morning and returned to the maturation pool, if it is in good condition for rematuration.

5. Counting of eggs and nauplii

For estimating the number of eggs produced, the eggs are disperesed in the water by thorough mixing and three 100 ml samples are taken with a beaker. The number of eggs in each sample is counted and the averagenumber in 100 ml calculated. The total number of eggs is estimated thus:-

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

The nauplii hatch out by afternoon and the number of nauplii in the tank is estimated following the procedure adopted for counting the ggs.

To separate the nauplii from the dead eggs, the aeration is stopped and a beam of light from a torch or other source is directed on the water surface. The nauplii attracted by the light, congregate at the surface while the dead eggs sink to the bottom from where they are siphoned out along with the sediments. The light is turned off and the counted nauplii in the spawning tank are allowed to flow into the rearing tank through a flexible PVC hose attached to the ball valve at the bottom of the tank.

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LARVAL STAGES OF PENAEID PRAWNS
- MORPHOLOGY AND BEHAVIOUR

By

A. Laxminarayana

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1. Eggs

The eggs of penaeid prawns are about 0.3 to 0.45 mm in diameter with a perivitelline space, the width of which determines the buoyancy of the eggs. A narrow perivitelline space (15 microns) which is characteristic of the genus Penaeus makes this eggs less buoyant than the eggs of Metapenaeus dobsoni and Parapenaeopsis stylifera which have a wide perivitelline space (85 micron and 60 microns respectively). The small size of the eggs with the limited store of yolk results in a short embryonic life. The eggs hatch out 14-17 hours after spawning. The rate of development of the eggs depend solely on the ambient temperature, the higher the temperature, the shorter the duration of embryonic stage. Apart from the perivitelline membrane or the egg membrane, the developing nauplius is ensheathed by a thinner membrane which is the vitelline membrane. At the time of hatching, the nauplii gets rid of both the membranes.

2. Nauplius

The body is pear shaped with 3 pairs of appendages, the uniramous antennules and the biramous antennae and mandibles. There are no spines or processes on the mandible for feeding purposes; It is purely a swimming organ. The mouth and

alimentary canal are not formed and the nauplius does not feed, depending entirely on the internal yolk for development. There are usually 6 naupliar sub-stages identifiable by the increase in the number of setae in the caudal lobes and the antennal exopods. While the duration of each sub-stage is only 4-6 hours, the 6th sub-stage is extended for about 12 hours. The nauplius phase lasts for 36-48 hours. Being a non-feeding stage, the rate of development of the nauplius is dependant only on the ambient temperature.

3. Protozoea:-

The nauplius metamorphoses into the protozoea which is characterised by 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 that is bifurcate posterially with at least 7 setae on each furca, well-developed biramous 1st and 2nd maxilipeds and 3rd maxillipedes that are absent or rudientary. The protozoea has functional alimentary canal and feeding appendages (mandible, maxillulae and maxilla). It is a voracious filter feeder, feeding on phytoplankton. Under good feeding conditions this stage lasts for 3-4 days when the temperature is 27-29°C. The duration of the protozoeal phase is more dependant on availability of suitable feed than on temperature; if nutritionally adequate feed is not available in sufficient quantity the protozoeal stage is prolonged. The larvae become weak and die.

The protozoeal phase is clearly divided into 3 distinct sub-stages in all the penaeids. They can be distinguished as follows:-

Protozoea I : Eyes sessile; rostrum or supra-orbital spines absent; peraeopods absent; abdomen unsegmented;

Protozoea II : Eyes stalked; rostrum and supra-orbital spines (if any) appear; first 5 abdominal segments demarcated, telson not separable from last abdominal segment; uropods absent;

Protozoea III : Uropods present, telson separated from last abdominal segment; first 5 abdominal segments with dorsal spines;

4. Mysis:-

The protozoeal phase is followed by the mysis phase in which the carapace covers the thorax, the 3rd maxillipeds and the 5 pereopeds 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.

Unlike the protozoeal sub-stages, which are distinguished by clear-cut morphological changes, the mysis sub-stages are separated only by small increase in (a) the size of the larvae, (b) the length of the pleopods and (c) the number of setae on

into 4-5 sub-segments in protozoea I and II. As the mysis stage 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 serves as a sensory organ. Similarly the natatory antenna of the protozea has a fully segmented exopod for greater flexibility; in the 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 the main swimming organs during the mysis stage. The endopods of the periopods which bear long terminal setae during the mysis stage are also natatory in function. The uropods which are fully developed in the mysis stage form, along with the telson, the tail-fan which, when flicked by the flexure of the abdomen, suddenly jerks the larva backwards and enables it to escape from predators. The appearance of setae on the pleopods during the postlarval phase coincides with the disappearance of the thoracic exopods and the natatory function is taken over by the pleopods.

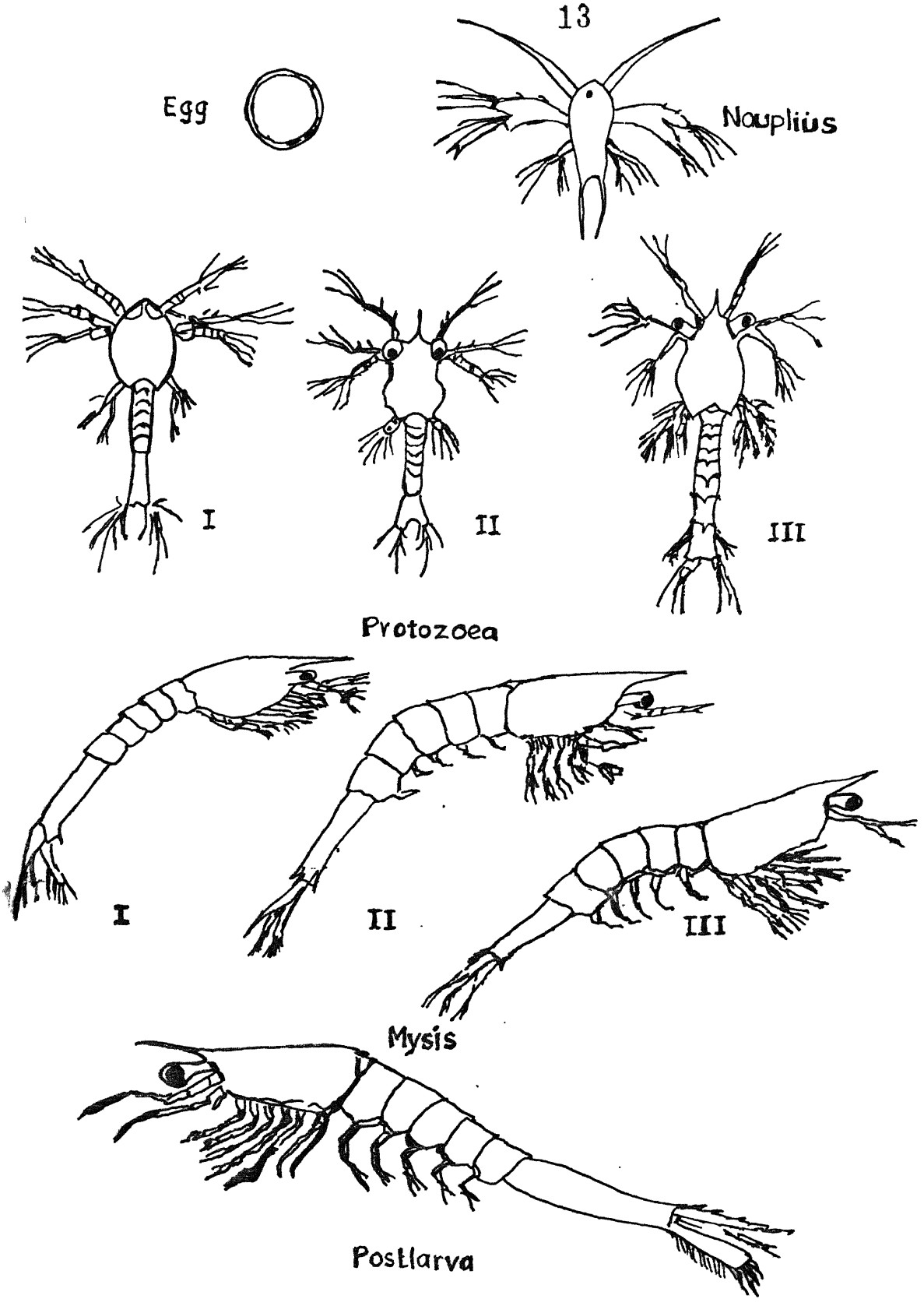
Feeding appendages:-

The mouth parts of the protozoea and mysis stages of penaeids are adopted for filter feeding. They have a well-developed maxillary filter with numerous close-set filtering setae on the endites of the protopods; among the filtering setae which have fine setules pinnately arranged on either side, a few stronger setae with stiff bristles are interspersed,

perhaps providing reinforcement to the delicate filter. The space between the setules of the filtering setae are small enough to retain the phytoplankton on which they feed. The brush-like setae on the maxillulary endites sweep the phyto-plankton collected on the maxillary filter into the mouth. The maxillary filter is supplemented by a coarser filter made up of numerous barbed setae on the protopod of the first maxilliped. The mandible is weak with serrated teeth in between the incisor and molar processes. The endopods of the thoracic legs are tipped with long weak setae and are unsuitable for holding the prey.

A sudden transformation in the mouth parts takes place when the mysis metamorphoses into the postlarvae. The mandibles lose the serrated teeth and develop a sharp cutting edge in their place; the maxilla loses the filtering setae and the endites become highly reduced; the protopod of the first maxilliped becomes broad and acquires stiff bristles; and the chelae on the periopods become functional. The appendages are now capable of dealing with prey animals and the postlarvae become carnivorous.

The structure of the appendages clearly shows that the protozoea and mysis are adapted for filtering the phytoplankton suspended in the water and that they are incapable of deliberately pursuing and capturing any moving animal prey. At the Narakal Research Centre of CIBA., Penaeus and Metapenaeus have been successfully reared from protozoea to postlarva on a diet of phytoplankton alone, without giving them any animal feed. However all over the world rotifers or freshly hatched Artemia nauplii are fed to the mysis stages. In view of the fact that


Egg
Nauplius
I
II
III
Protozoea
I
II
III
Mysis
Postlarva

the appendages are unsuitable for capturing moving prey it is certain that a lot of this food is wasted. However, it is possible that the rotifers or Artemia nauplii which accidentally come into contact with the oral region of the larvae may be held by the coarse setae of the first maxillipeds and perhaps also by the stiff barbed setae on the endites of the maxillule and eaten.

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 post-larvae 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 protozoea of Parapeneopsis with their longer antennular peduncle and very long apical setae swim at a slower pace than the protozoea of Metapenaeus and Penaeus which have shorter antennuler peduncles and apical setae.

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. If they become listless and are not attracted towards a beam of weak light it is a sure sign that all is not well with them. When the aeration is stopped the healthy nauplii and protozoea form swirling swarms at the surface reminiscent of a swarm of bees. There seems to be some attraction between the members of a swarm.

The moult to the first mysis transforms a very active protozoea of Penaeus into a relatively sluggish animal which hangs with the anterior end pointing obliquely downwards. It hovers around like a helicopter and jumps back suddenly by flexing the abdomen. The mysis of Metapenaeus on the other hand is oriented horizontally and is more active than the mysis of Penaeus. The late stage mysis of Parapenaeopsis are strong swimmers because the exopods of pereopods acquire additional pairs of setae with each moult of the mysis. In the other two genera the uropod setae are fewer in number and remain constant in all the mysis sub-stages.

With the acquisition of plumose setae on the pleopods the postlarvae become horizontally oriented while swimming. The first postlarvae of Metapenaeus and Parapenaeopsis promptly settle to the bottom of the container but the early postlarvae of Penaeus continue to be pelagic for 4-5 days. The postlarvae of P. indicus are the most active while those of P. monodon and P. semisulcatus tend to rest on the sides of the container in a vertical position.

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LARVAL REARING TECHNOLOGY FOR PENAEID PRAWNS

By

A. Laxminarayana

The nauplii are counted and transferred to the larval rearing tanks at a stocking density of 75,000 nauplii/m^3 (1,50,000 per 2 tonne tank). The sea water supplied to the rearing tank is filtered through a 50 micron mesh bolting cloth bag. Fabric filters of 10 and 5 are very effective in removing all particulate content upto that size.

Management of larval rearing pool:-

The pools are managed as shown below:-

Day	Stage	Seawater removed (litres)	Algal culture added (litres)	Particulate feed (g)	Seawater addition (litres)	Total vol. of water made upto(litres)
1	N 2	-	-	-	1000	1000
2	N 5	-	100	-	-	1100
3	PZ 1	-	150-200	-	700-750	2000
4	PZ 2	500	150-250	-	250-350	2000
5	PZ 3	500	150-250	-	250-350	2000
6	M 1	500	150-250	-	250-350	2000
7	M 2	500	150-250	-	250-350	2000
8	M 3	500	150-250	10-15	250-350	2000
9	PL 1	750	100-150	12-15	600-650	2000
10	PL 2	750	100-150	12-25	600-650	2000
11	PL 3	750	100-150	12-25	600-650	2000
12	PL 4	750	100-150	12-25	600-650	2000
13	PL 5	750	100-150	12-25	600-650	2000

Water quality and other conditions condusive for larval rearing are given below:-

Parameters	Permissible range
Salinity	29-34 ppt
Temperature	26.0 - 32.5°C
pH	8.0 - 8.5
Dissolved oxygen	3.0 - 8.0 ml/Litre
Light intensity during day time	20,000 - 1,25,000 lux
Total ammonia	0.1 ppm
Nitrite	0.05 ppm

The first four parameters can be monitored daily. Ammonia and nitrite levels may be monitored 2-3 times a week. The water from the larval rearing tanks is removed by keeping a siphon inside an open filter box (100 microns mesh size) to prevent the loss of larvae. The algal 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. Algal cells counts are made using a rafter cell or a haemocytometer. After some experience in larval rearing it is possible to judge whether the algal concentration in the larval tanks is adequate by observing the feeding condition of the larvae and the larvae. The larvae are examined under the microscope every two hours to see if the gut is full and also to note their general condition. The sediments are not removed usually. But if turbidity is too high the sediments have to be

siphoned out after stopping the aeration for about 10 minutes. Good aeration prevents water spoilage. The presence of algal cells in the medium stimulates the natural growth of copepod and rotifer populations which also serve as food for the prawn larvae in the postlarval stages.

When the larvae are in h althy condition they swarm at the surface during the nauplius and protozoea stages, if the aeration is stopped for about 10 minutes; during the mysis stage they are more dispersed in the water column. When the feeding conditions are good the protozoea can be seen with long faecal string trailing from their posterior end.

Larval counts and harvesting:

In the rearing tanks larval counts are taken when the developing larvae reach the PZ 1, M1-M3 and PL 1 stages. After vigorous aeration and gentle mixing, four samples are taken from 4 different places in the larval tank in 1 litre beakers and the larvae counted. The average number of larvae per litre of the 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 PL 5. 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 PL 5 before they are stocked in the nursery.

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NURSERY REARING TECHNIQUES

By

S.M. Pillai

The hatchery reared seed prawns from PL 5 stage are to be reared upto PL 20 stage which is the early juvenile stage for stocking in grow-out ponds in seperate place called nurseries. The nurseries act as link between hatchery and grow-out. Nursery rearing of hatchery produced seed is a must since the seed of PL 5 stage are small in size, can not withstand fluctuations in environmental factors and also unable to compete for food and space. Hence they should be moved to larger space for both growth and survival.

Type of nurseries:

Nurseries can be classified broadly into two categories:-

1. Hatchery associated nurseries.
2. Farm associated nurseries.

Cement cisterns, plastic pools, raceways, cages, pens, FRP tanks and earthern ponds of different dimentsions are used as nursery systems.

Hatchery associated nurseries:

Cement cisterns, plastic pools, raceways and FRP tanks are used for nursery re-ring of prawn larvae. The size and number of the re-ring units can be chosen as per the requirements and targeted production. The size of the nursery tank should be at least 5 times larger than the hatchery tank

The procedure followed is as follows:-

1. The rearing units are disinfected, cleaned and dried.

2. The tanks are kept dry for two days.
3. Filtered sea water is taken upto 60 cm level.
4. Salinity is adjusted and maintained to the level at which the PL 5 is reared.
5. Stocking is to be done at early morning or late evening hours to reduce the stress to the larvae.
6. Stocking density can be between 6,000 to 8,000 per m^3 (= per ton).
7. Monitor temperature, salinity, pH and DO regularly and corrective measures taken immediately to maintain these factors at their desired levels.
8. Aeration should be provided @ one air stone per sq. meter. A flow-through system to ensure 300% daily exchange of water will be ideal.
9. Supplementary feeding is to be done with particulate diet as per the rate shown below:-

   PL 5 to PL 10 = 500 microns feed particles fed @ 1 g/day/1000 larvae.

   PL 10 to PL 20 = 1000 microns feed particles fed @ 5 g/day/1000 larvae.

   Above PL 20 = 3 mm pellets.

The feed ration for a day should be given in equal instalments, at least 4 to 6 times in a day so that it will be better utilised and the wastage minimised.

10. If algal bloom is noticed, the accumulated sediment may be removed and the water replenished.

11. The salinity may be reduced to 10-20 ppt by the addition of required quantities of brackish/fresh water when the postlarvae attain PL 15 stage.

12. The stock should be sampled periodically to assess growth.

13. Biofilters may be installed in the nursery tanks for maintenance of better water quality.

14. Addition of subsrata may be provided for the movement of larvae and more grazing area.

15. At PL 20 stage the larvae should be harvested.

16. In raceways air lifts are provided for better water circulation and oxygenation and as such high density larval rearing can be done in such systems.

Farm associated nurseries:-

The farm associated nurseries are earthen ponds or pens. A portion of the farm area may be reserved for nursery pond. Generally, 10% of the total farm area can be the nursery area. Alternatively a portion of the grow-out pond itself can be converted into temporary nursery by constructing a temporary bund or barricading with bamboo and velon net screen to form a pen. The procedure followed in farm related nurseries is listed below:-

1. The nursery area should be cleared off predators and other unwanted organisms by application of ammonia or mohua oil cake.

2. The pond may be dewatered and exposed to sunlight which eliminates predators and accelerates decomposition of organic matter by oxidation.

3. Water should be taken through proper screens, initially upto 30 cm.

4. Fertilization should be done with inorganic fertilizers such as urea and super phosphate @ 200 kg/ha. each.

5. After two days, water is again taken and the level raised to 80 cm.

6. Stocking is then done with the hatchery reared PL 5 after proper acclimatization.

7. The hydrological factors like temperature, salinity, pH and DO are to be monitored regularly.

8. Periodic manuring and/or fertilizing is to be followed to augment the production of natural food organisms.

9. Water exchange may be done carefully through proper screens to check the entry of predators and unwanted organisms.

10. Addition of substratum may be given to increase the grazing area for the larvae and for protection especially to the newly moulted ones.

11. Supplementary feeding may be followed @ 5% of the body weight with 3 mm feed pellets.

12. Once the prawns reach PL 25 stage, they may be harvested and stocked in grow-out ponds.

13. If the nursery is within the grow-out the bund or the protective barricade may be removed and the prawns are allowed to grow in stock ponds.

In hatchery associated nurseries the survival recorded ranged from 70-90% when the larvae are held for 15 days (from PL 5 to PL 20). However, in earthern nursery ponds the survival varies from 60-80%.

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CULTURE OF LIVE FOOD ORGANISMS FOR PRAWN LARVAE AND FEEDING STRATEGIES

By

S.M. Pillai

Successful hatchery production of prawn seed is dependant on the provision of adequate amount of larval feeds specific to the various developmental stages of the penaeid prawn larvae. In natural environment the larvae feed on the food organisms available in the sea water. But in a hatchery set up, each larval stage should be fed with the specific type of feed. Prawn larvae exhibit wide range of feeding habits. The nauplii do not feed at all, but subsist on the yolk. The protozoea feeds on minute food organisms, namely phytoplankton. The mysis stage of the larvae feeds on small animal food organisms. At postlarval stages, they feed on a variety of animals and plant matter.

Types of live food organisms for prawn larvae:-

In the controlled hatchery system, successful rearing of prawn larvae is related to the supply of a variety of food organisms. Although attempts are being made to rear larvae on artificial diets, phytoplankton serves as the principal food item of larvae. The desired larval food organisms are listed below:-

I Phytoplankton

- Diatoms

1. Chaetoceros sp.
2. Skeletonema costatum
3. Thalassiosira sp.
4. Nitzchia sp.
5. Cyclotella sp.

- Flagellates
  1. Tetraselmis sp.
  2. Isochrysis sp.
  3. Monochrysis sp.
- Green algae
  1. Chlorella sp.

II Zooplankton

- Rotifer
  1. Brachionus plicatilis
  2. Cladocera
     1. Moina
     2. Daphnia

Brine Shrimp

1. Artemia

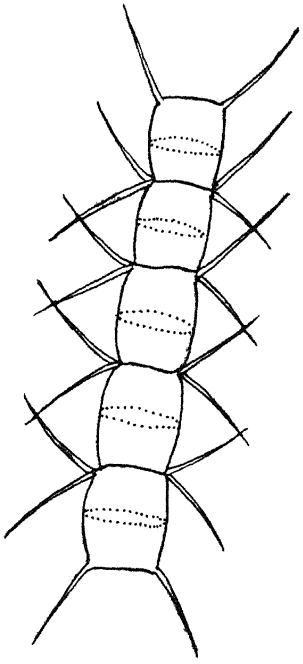
Culture of Phytoplankton:-

Culture of algae or phytoplankton is done in two set-ups.

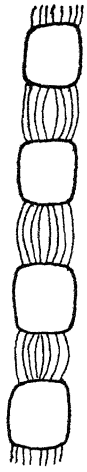
1. Indoor culture or maintenance culture
2. Outdoor mass culture

Culture of algae involves a number of stages before mass culture and these are done in indoor facilities. They are:-

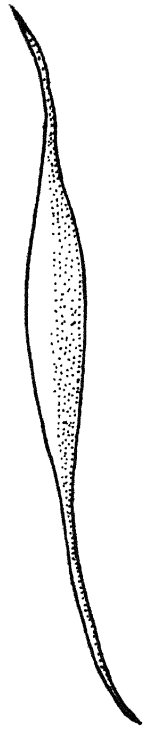
1. Selection of suitable containers
2. Preparation of suitable culture media.
3. Isolation of the desired species.
4. Management of the cultures under controlled temperature, light and aeration.
5. Harvesting and preservation.



CHAETOCEROS Sp.



SKELETONEMA Sp.



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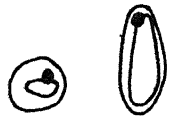

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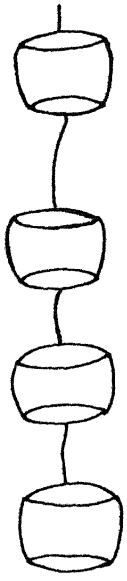

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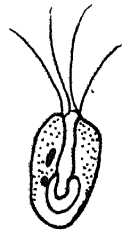


TETRASELMIS Sp.

ISOCHRYSIS Sp.

1. Culture containers

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

2. Isolation of desirable species of algae for culture

The presence of desirable species of algae in the seawater is first to be ascertained. This can be done by sieving seawater through plankton net made of silk bolting cloth (180 meshes/Sq.inch). The sample is examined under microscope for the presence of the algae after sedimenting or centrifuging. Then the desired species can be isolated using any one of the following methods:-

1. Pipette method:

   Large organisms can be pipetted out using a micropipette and transferred to culture tubes.

2. Centrifuge or washing method:

   By repeated centrifuging of the sample and inoculating the deposit.

3. By exploiting the phototactic movements:

   By this method most of the phytoflagellates can be isolated.

4. Agar plating method:

   The required species to be isolated can be picked up by a platinum needle or loop and streaked on agar plates.

5. By dilution method:

By serial dilution technique, the desired species can be isolated.

3. Culture media

The nutrients, nitrates and phosphates are required in the ratio of 16:1 (N:P) for the growth and reproduction of phytoplankton. So the culture media should contain these nutrients besides other nutrients, trace metals, vitamins and amino acids. For diatom culture, the media should contain silicates. The absence of any one of these growth promoting substances, reflect on the growth of the algae or flagellates. A few media used for culture of diatoms and flagellates are given below:-

a. Schreiber medium

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

b. Miquel's solution

A	B	
Potassium nitrate 20.2 g in 100 ml distilled water	Sodium phosphate	4 g
	calcium chloride	4 g
	Ferric chloride	2 g
	Conc. HCl	2 ml
	Dissolve in 100 ml distilled water.	

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

c. Walne's Medium

$NaNO_3$ - 100.00 mg

Na_2 EDTA - 45.00 mg

H_2BO_3 - 33.60 mg

$NaH_2PO_4 \cdot 2H_2$) - 20.00 mg

$FeCl_3 \cdot 4H_2O$ - 0.36 mg

Vitamins

B1 - 0.1 mg

B12 - 0.005 mg

Trace Metals

$ZnCl_2$ - 0.021 mg

$CoCl_2$ 6 M_2O - 0.020 mg

$(NH_4)_6 Ho_7 O_{24} \cdot 4H_2O$ - 0.009 mg

$CuSO_4 \cdot 5H_2O$ - 0.02) mg

Sea water to 1 litre

d. TMRL medium

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 separate bottles. Add 1 ml each to 1 litre filtered sea water.

e. For mixed culture of phytoplankton:

Potassium nitrate	1.2 g
Sodium phosphate	0.2 g
Sodium silicate	0.6 g
EDTA	0.6 g

Dissolve the chemicals in distilled water and add to 100 litre of unfiltered seawater.

4. Management of the culture:

Stock cultures of all the micro-algae are maintained in a special room in phyco lab having uniform temperature and light.

Since light is essential for photosynthesis of algae, in algal culture room light intensity of 1000 to 3000 lux is provided with flourescent lamps.

For maintenance of flagellates the temperature of the stock culture room should be maintained between 23-25°C during day and 20-23°C during night.

Aeration must be provided to all culture containers because besides providing oxygen it helps to keep the cells in suspension.

distributes nutrient salts uniformly in the medium and provides CO_2 for photosynthesis.

Growth phases of the algal culture

The increase in cell numbers in a culture follows characteristic pattern and the following four phases have been recognised. The typical growth curve of an algal population is shown in Figure.

a. Lag or induction phase:-

The cells taken from the stock culture and inoculated to the new flask have to acclimatise to the new medium. So there is no cell division for a few hours and this phase is known as lag or induction phase.

b. Exponential or growing phase:-

The cells divide steadily at a constant rate and the growth continues till the culture reaches its maximum concentration.

c. Stationery phase:-

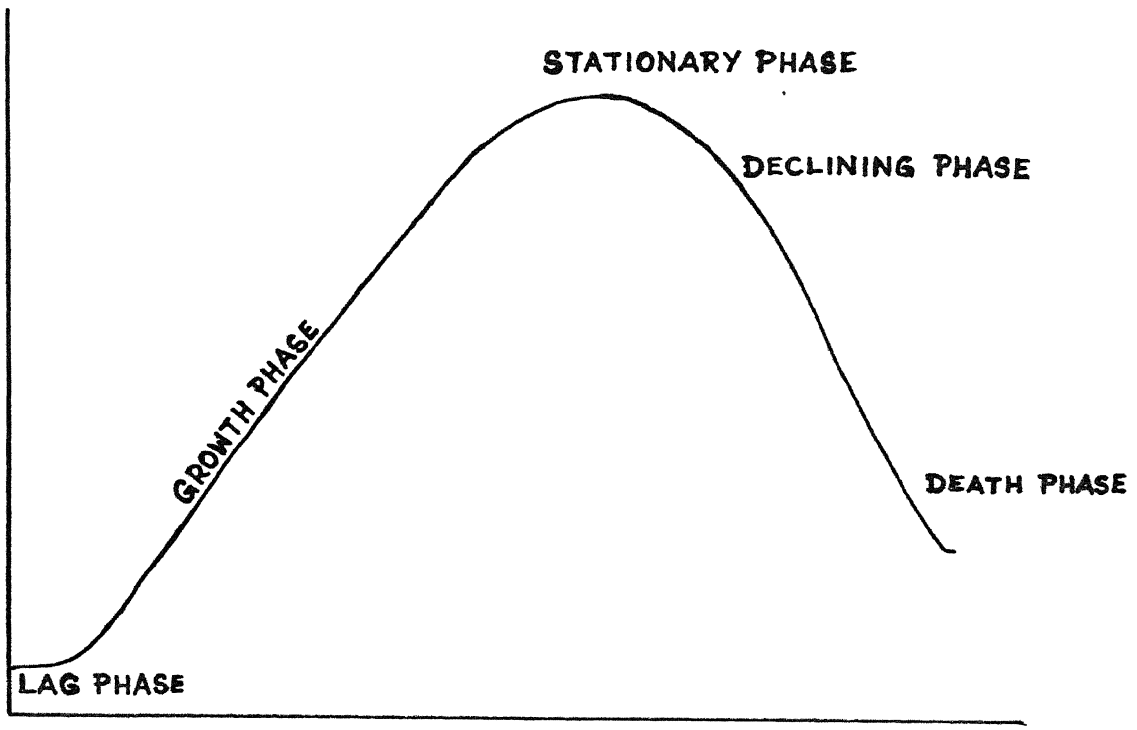
Once the cells reach maximum concentration, growth is arrested and the culture is stationery. The population more or less remains constant.

d. Death or declining phase:

After a long period in stationery phase, the cell may lose its vitality and start to die and the population declines.

Determination of cell densities:

Since most of the micro-algae are less than 10 μ in size, counting is done with haemocytometer.


STATIONARY PHASE
DECLINING PHASE
GROWTH PHASE
DEATH PHASE
LAG PHASE

Haemocytometer has 9 chambers, 4 sides having 16 divisions and 5 chambers with 25 divisions in the central block. At least counting should be done in 3 chambers. The cell density is calculated using the formula.

Cell Density (cells/ml) = Average counts per chamber x 10^4.

5. Harvest and preservation:

Harvesting algal cultures is a simple process wherein the 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 is high and deplete the culture early in the stationery phase.

Sometimes especially during adverse weather conditions it is a problem to maintain the culture and assure constant supply to meet the hatchery requirements.

For concentration and preservation of the algae, freeze drying or sun drying can be adopted. Freezing can be done by flocculation of the algae by adding alum or lime or adjusting the pH using Sodium hydroxide. Known quantity of algae could be preserved by this method so that it can be used for feeding the larvae later during adverse weather conditions when the culture fails or takes longer time to reach the required cell density.

During flocculation the pH of the algal culture is increased by the addition of Sodium hydroxide under vigorous stirring. The culture is then left for one hour undisturbed. The algal mass deposits on the bottom. The clear water is decanted and sediment collected. Then the pH is brought down to the original value by adding dilute HCl. The algae is now ready for freezing or drying.

Drying can be done by pouring the algae in enamel trays and keeping in sun light. Once dry the algae can be scrapped from the trays and kept in air tight glass bottles.

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

Mass culture of phytoplankton at Narakal

At Narakal Research Centre of the Central Institute of Brackishwater Aquaculture, mixed culture of phytoplankton predominated by Chaetoceros affinis is raised for feeding prawn larvae.

The culture is done in 1000 litre capacity, white fibreglass tanks placed under the glass-roofed shed. Fresh seawater (30-34 ppt salinity) filtered through 50 micron mesh bolting cloth is taken into the tank and it 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 intensity of the light in the shed varies from 20,000 to 1,20,000 lux during day time and the temperature of the seawater from 28°C-35°C. Under these conditions, the diatoms multiply rapidly and golden-brown bloom is obtained within 24-48 hours. The culture is dominated by Chaetoceros forming 75-90%.

The other diatoms account for 10-25% and represented by species of Thalassiosira, Skeletonema and Nitzchia. The concentration of diatoms will be 3-4 lakh cells/ml of culture. This culture is used for feeding larvae and also as inoculum for fresh batch cultures. The algal cultures will be ready for use 16-20 hrs after inoculation.

Culture of Rotifer, Brachionus plicatilis

The rotifer, Brachionus plicatilis is used as larval feed for mysis and postlarval stages. It can be cultured by two methods. In the first method it is cultured by adding the feed into the tank. In the second method, the rotifer and feed are cultured together in one tank.

The procedure for culture of rotifer followed at Narakal Research Centre of CIBA is given below:-

Seawater is first filtered through 50 micron net and taken into 2 ton, 10 ton or 40 ton tanks. It is then fertilized with groundnut oil cake (juice), urea and superphosphate at the rate of 200 g, 2 g and 2 g respectively per ton of water. The tanks are well aerated and inoculated with starter culture of Chlorella. After a day or two when the pH increases above 7, a starter culture of B. plicatilis is added to the tanks. Within 2-3 days a bloom of Chlorella develops and the rotifers multiply rapidly. A population density of 250 nos/ml within 4-6 days is obtained. Harvesting is done in the morning every day with a plankton net (40 micron mesh nylobolt).

The harvested rotifers are washed in clean seawater filtered through 40 micron nylobolt and frozen into blocks in a deep freezer using 10% glycerine as cryoprotectant.

Artemia nauplii

Though Artemia nauplii are not being used at CIBA, all commercial hatcheries use Artemia nauplii which are given from M_3 to PL 5. San Franscisco strain of Artemia cysts have a high percentage of hatching. These cysts are placed in normal seawater. Within 24 hours the nauplii hatch out. For production of one million postlarvae the requirement will be 8-10 kg cysts.

Feeding strategies

The larval stages of prawns should be fed @ 50,000 to 1,00,000 cells/ml of diatoms. Feeding should be done periodically so that at any given time the algal cell count should not come down below 50,000 cells/ml in the larval rearing tanks. Rotifers are fed from mysis stage onwards @ 100 Nos/larvae per day.

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PREPARATION OF MICRO PARTICULATE FEED

By

S.A. Ali

Micro particulate compounded diet is used for rearing the postlarvae (postlarvae 1 onwards) in hatchery and nursery. It is prepared using low cost ingredients available locally. The procedure for preparing the micro particulate diet is outlined below:-

Composition of the diet

Prawn waste	20 parts
Mantis shrimp	20 "
Fish meal	10 "
Ground nut cake	30 "
Tapioca	20 "
Vitamin mixture(*)	1 part
Mineral mixture (**)	5 parts

(*) Vitamin mixture 2 tablets of Becadex multivitamin tablets per Kg of feed.

(**) Mineral mixture 10 g of calcium carbonate and 40 g of potassium dihydrogen orthophosphate per kg of feed.

The proximate composition of the feed should be:-

Moisture not more than 6%

Crude protein not less than 38%

Lipid not less than 6%

Carbohydrate not less than 24%

Crude Fibre not more than 6%

Ash not more than 20%

Ingredients

All the ingredients should be obtained in dry form. Generally, fishmeal, ground nut cake and tapioca powder are available as dry materials. Prawn waste and mantis shrimp some times have to be collected in fresh condition and dried in the oven at 60°C. Where there is no possibility of electrical drying, the materials may be dried in the sun. But as far as possible sun drying may be avoided.

Powdering:

The ingredients may be powdered either individually or after mixing them in an electrical grinder and the powder should pass through 0.5 mm sieve.

Feed compounding:

The feed should be compounded by mixing the ingredients in the proportions as per the formula. It should be mixed properly and homogenised. Water (400 to 600 ml per kg of dry feed) should be added and made into a dough. The feed should be steamed in cooker (without weight) for 10 minutes. It should be pelletised in 3 mm diameter die and dried at 60°C for 12 hours. The dry feed should be stored in polythene bags kept in good containers.

Preparation of micro-particles:

The dry pellets of the feed is prepared into micro-particles of required size. The pellets are ground in an electrical grinder with controlled speed. Then the powdered material is sieved through the required size sieve. For postlarvae 1 upto PL 10 the particle size of diet can be between 200-500 microns. From PL 10 upto PL 20, the particle size of

diet can be 1000 microns. After that 3 mm pellets can be used for feeding the juveniles.

The details of particle size, feeding rate and frequency feeding of the larvae in hatchery and nursery are summarised below:-

Stage of larvae	particle size of feed	quantity of feed per day per 1000 larvae	scheduled time of feeding
Mysis III upto post-larvae PL 5	200 microns	200 mg	Divide feed in three equal parts and feed in the morning, afternoon and evening
PL4-PL10	500 microns	1000 mg	-do-
PL11-PL25	1000 microns	2-3 g	-do-
PL25 and above	3 mm pellets	10% body weight	-do-

Rearing of larvae using micro-particle diet:

The early larvae (from protozoea I onwards) can also be reared using the same micro-particulate compounded diet 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 also phytoplankton cells which will grow in the culture tanks. Thus the growing larvae receive a mixed diet which is very conducive for their healthy growth. The advantage of this method is that separate culture of algae can be avoided and the same compounded feed can be used until the larvae attain stockable size, simplifying considerably, the rearing technique. Using this method

a survival rate as high as 80% could be achieved. The rate of feeding of 50 micron size micro-particulate feed is 5 grams per day in three divided doses, in one ton capacity tank when 1,00,000 (one lakh) nauplii are stocked.

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DISEASES OF PRAWN LARVAE

By

S.C. Mukherji

INTRODUCTION

Crustacea are attracting the most attention today in our country as mariculture species like shrimps, lobsters and some of the crabs are gaining importance for its export potentials. However, commercial scale production of these species have not touched the peak due to some short-comings in available technology. Culture of other species is still in the experimental, developmental or pilot plant stages with much still to be achieved under the heads of inexpensive defined diets, maintenance of water quality, larval survival and disease control.

Almost without exception, the infectious disease problems that have surfaced in crustacean culture are microbial in origin ie. bacteria, fungi and protozoea all seem to be of significance, with bacteria well in the lead. Among the bacteria, the shell-destroying forms and the holophilic vibrios are note-worthy. Some of the most serious diseases of lobsters like gaffkaemia and shell disease cause mortalities in concentrated populations. Shell disease, ciliate disease and gray crab disease manifest themselves in shedding tanks. Much can be learned about the role of pathogens in mariculture populations through studies of these artificially held animals. Some of the important diseases that are now or may become problems for successful shrimp culture has been summarized below. It should be pointed out that many other parasites of shrimps from natural waters have been identified,

particularly protozoa and worms, but these have not yet been demonstrated to be of significance to maricultue populations. It should also be pointed out that some of the organisms responsible for so called "diseases" are facultative pathogens and are able to prosper when culture conditions are less than optimal.

Virbiosis of shrimps (V. parahemolyticus)

Species affected : Pink shrimp, P. duorarum
Brown shrimp, P. aztecus
White shrimp, P. setiferus

Gross signs: Animals become uneasy and jumps hitting the cover of the aquarium. They drop down to the bottom lying on their sides and then jump again. Die within 3 hours often in the upright position

Haemolymph clots slowly and become turbid, body muscle may become milky, hemocyte numbers may be reduced.

Cause: Exotoxin liberated by the bacteria

Method of Diagnosis: Isolation of causative agent on blood agar or brain heart infusion agar.

Effect on host: Behavioral abnormalities and rapid death.

Treatment: Nothing reported, but oxytetracycline in water may be tried.

Preventive measures: Water sterilization and filtration. Avoid use of contaminated natural food and avoid excessive handling.

FILAMENTOUS BACTERIAL DISEASE

Common name : Leucothrix disease

Species affected : Brown shrimp

White shrimp

Californian brown shrimp

Cause: Filamentous bacteria of genus Leucothrix

Gross signs: Filamentous growth often on appendages on Post-larvae.

Diagnosis: Direct microscopic examination of fresh mounts.

Growth on non-selective media.

Effect on host: Can produce mortalities of post.larvae with heavy infestations. Adult shrimp can be killed by inoculation of cultured bacteria. Mortality of shrimps with heavy infestations of gills usually occurs during or immediately following moulting.

Treatment: Potassium permanganate (5 to 10 ppm in 1 hr. static treatment) effective for 10 days.

Preventive measure: Maintenance of good water quality.

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LARVAL MYCOSIS OF SHRIMPS

Common Name : Larval mycosis.

Species affected : White shrimp P. setiferus

Brown shrimp: P. aztecus

Gross signs: Systemic infection of larvae with extensive highly branched fungal mycelium throughout body. The animal become yellowish green in colour with numerous oil droplets.

Cause: Fungus Lagenidium Sp.

Method of diagnosis: Gross signs and isolation of fungus in Sabouraud agar or broth. Sporulation is induced by transfer of cultured mycelium to sterile seawater.

Effect on host: Mortalities are rpoduced rapidly in hatchery tanks among larvae upto first mysis stage only. Mortalities may reach 100% within 2 days.

Treatment: Malachite green is reported as effective measure at .001 to 006 ppm 'Traf lan' @ 0.01 ppm.

Prevention measures: Chlorination and filtration of water is an effective measure.

FUSARIUM DISEASE

Cause: Fungus Fusarium Sp.

Possibly several species and other fungi may be involved as well.

Gross signs: Black coloured gills. The disease comes in form of epizootic and cause about 90% mortalities. The fungus typically affect the gills, basal segments of walking legs and body wall behind the gills.

Treatment: Malachite green (0.5 to 01 ppm) for 24 hours effective against exposed spores and hyphae. But internal hyphae and spores are not affected.

COTTON DISEASE OF SHRIMPS

Cause: Several microsporidiam protozoans. eg. Nosema nelsoni. Pleistophera sp and Thelohania duprara.

Gross signs: Opaque white areas in abdominal muscles which

is often extensive. Sometimes there is blue-black colour on back and sides of the shrimp. It also invades the digestive tract and heart.

Method of diagnosis: Gross signs provide a good clue. Blue-black pigmentation especially found with Pleistophora sp. infections. Microscopic examination of fresh squashes from infected muscles reveal microsporidian spores. Stained spores give clue for the diagnosis.

Effect on the host: Transmission is probably by ingestion of spores or of intermediate hosts which is fed on the spores. Multiple infections can occur. Infected individuals can be weakened or killed especially if other environmental stresses exist.

Treatment: Non reported.

FUNGUS DISEASE OF FRESHWATER SHRIMP

Cause: Considered to be caused by an unknown fungus.

Species affected: Macrobrahium rosenbergii

Gross signs: Usually the shrimp larvae are affected. Small opaque whitish patches occur first at the base of appendages and in tail of larvae then spread throughout the entire body.

Effect of host: Produce sporadic heavy larval mortalities.

Preventive measures: Infected larvae should be removed. Troughs and tanks should be cleaned and disinfected.

Known geographic range:

Ubiquitous in marine water. This can also cause enteric isturbances in humans. Another vibrio, V. panubirus has been reported from pond culture shrimp (P. japonicus) in Japan where it causes blackening of gills.

2. Vibrio alginolyticus

Gross signs:

Shrimp become lethargic and disoriented. Abdominal muscle become opaque white with red discolouration of pleopods and periopods. Infected animals die in upright position.

Diagnosis:

Isolation of bacteria from haemolymph of maribund animals on trypticasesoy agar with 2% salt.

Effect on host:

Mass mortalities occur in the aquarium held larvae. Major losses are reported in tank held juveniles and adults. In some instances mortalities upto 100% have been reported in tank-held population.

Brown Spot Disease

Species affected : Pink Shrimp
White Shrimp
Brown Shrimp

Cause: Several species of chitin-destroying bacteria especially V. anguillarum and Pseudomonas have been isolated.

Gross signs: Brownish eroded areas appear on the exoskeleton as small circular spots.

Diagnosis: Brownish spots often with white margins and depressed centres on exoskeleton. Sometimes necrosis appear in the underlying tissues, bacterial isolates include chitin-destroying organisms.

Effect on the host : In some instances tank-held populations may be infected rapidly producing 100% infection with mortalities due to gill destruction. There may be progressive destruction of the exoskeleton depending on the route of entry of the secondary pathogens.

Treatment : Mixtures of malachite green and formalin at 0.05 to 1 ppm and 20 to 75 ppm respectively is effective in reducing the losses.

Terramycin (20 g/45 g ration fed for 14 days) is also effective.

Preventive measures: Adequate water filtration and sterilization. Remove infected and dead individuals, Prevent injuries which probably serve as primary portals of entry.

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TRANSPORT OF PRAWN SEED

By

S.M. Pillai

For scientific prawn farming, the availability of required numbers of seed at the proper time is the most important aspect. This becomes more significant when high density intensive farming is practised. Transport of prawn seed from the hatchery after nursery rearing to the farm is the last step in hatchery production of seed prawns.

Types of Seed Transport:-

Seed prawns can be transported by two methods:-

1. Transportation in open containers.
2. Transportation in closed containers.

1. Transportation in open containers:-

For short distance transport of seed this method is employed. The duration of the transport can be upto 2 hours with at least one or two water exchanges.

2. Transportation in closed containers:-

For long distance transport, the seed should be packed under oxygen in proper containers.

Steps involved in seed transport:-

The entire operation of transport of prawn seed from the hatchery to the farm may be classified into the following steps:-

1. Selection of containers

The selection of containers depends upon the mode and duration of transport. For short duration transport

FRP tanks, GI tanks, Jerry cans, Plastic bins, Drums and Buckets etc. are used. For long distance transport polythene bags are the ideal for packing the seed and then they can be enclosed in paper cartons.

2. Oxygen packing:-

Oxygen packing of the seed is a must if the distance and duration of transport is longer and also when the quantity of seed is more. Polythene bags are the ideal containers for oxygen packing. DO content of 2.5 ml/litre is found optimal for seed transport. 1/3rd of the bag is filled with water and the remaining part is filled with oxygen and sealed tightly in this transport.

3. Stocking density:-

The density of the seed to be stocked depends upon the species; its size and behaviour. P. monodon seed in densities of 20 to 6000 Nos/litre was transported depending upon the duration of transport. For a duration of 36 hours, the ideal density would be 500 nos/litre under oxygen packing. The seed of P. indicus was transported @ 50 to 500 nos/litre by different modes of transportation and different durations. A density of 250 seed/litre for the journey involving 24 hours and 100 seed/litre for a longer period is recommended for P. indicus.

4. Mode of transport.-

Seed is transported by road, rail or air depending upon the distance to be transported. To nearby places from the seed production centres, seed could be transported manually in open containers.

Duration of transport.-

The duration of transport again varies depending on the distance.

6. Time of transport -

Transport of prawn seed should be done either during morning or evening hours. This is more so important for open container transport so that the day time high temperature can be avoided. Transport of seed at night hours is ideal since the metabolic activity of the larvae will be lower at lower temperatures at night.

Precautions during seed transport:-

1. The containers selected should not be toxic to the seed.
2. The polythene bags used for oxygen packing should be tested for leaking before and after packing.
3. For shorter duration transport in open containers twigs of grass may be provided for seed to rest and to minimise their locomotion.
4. During open transport periodic checking of the prawns should be done for their general healthiness and at signs of stress the water may be exchanged.

5. During long distance transport, nauplii of live food organisms such as Artemia or Moina may be also introduced in the polythene bags to avoid cannibalism among the prawns.

6. The level of oxygen should not fall below 0.2 ml/litre.

7. The pH should not fall below 7.

8. The level of ammonia should not be more than 80 ppm.

9. The metabolic activity of the larvae can be minimised by lowering the temperature of the transport medium by placing ice or sand dust in the cartons.

10. Packing stress should be minimal.

11. Packing and transport of the seed should be done during evening.

12. Thermocole sheets placed at the sides, bottom and top in the card-board carton will insulate the consignment against thermal fluctuation.

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Selection of site for prawn hatchery and its facilities

By

K.V. George

Selection of site for constructing the hatchery for prawn seed production is a crucial factor. It has a major influence on the successful technical operations and economic viability of the hatchery.

The following are important criteria to be taken into consideration for choosing site for the hatchery:-

1. The success of a hatchery is mainly depending upon the availability of clear sea water. It should be of good quality and have a salinity of 28 - 34 ppt throughout the year. For this the site should be away from river mouths so that sea water is not diluted by the freshwater discharge from the rivers. Turbid sea water is not recommended as this would bring in lot of silt and detritus.

2. The sea bottom near the site should be sandy or rocky and not muddy. The seashore should be flat or gently sloping so that fixing of foot-valve becomes easy for sea water pumping.

3. The site should be situated in the neighbourhood of prawn culture systems.

4. The proximity to a fishing harbour would be advantageous so that collection of gravid females and adult prawns for brood stock will be easier.

5. The area should be easily approachable by good roads to facilitate free transportation of materials.

6. It should not be located near sources of thermal, sewage or industrial pollution.

7. Fresh water facility should be available at the site.

8. Slushy and marshy low lying areas are to be avoided. Sandy and hard elevated areas are recommended for easy construction of hatchery and to avoid water logging.

9. Areas subject to sea erosion or soil erosion should be avoided. The area should not be affected by cyclones and flood.

10. Power supply should be available in the vicinity so that expenditure on running operations can be reduced considerably.

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ENGINEERING INPUTS IN A SHRIMP HATCHERY*

By

I.C. BEHERA

AQUACULTURE ENGINEER, MPEDA

1. INTRODUCTION

Expansion of shrimp farming activities in the recent past and scientific culture practice with higher stocking density of farm ponds have raised a rapidly increasing demand for selective species of seed in coastal aquaculture. The erratic and unreliable availability of natural seed stock has compelled the farmers to look for hatchery supply of shrimp seeds. Inspite of a few commercial hatcheries established in the country, a wide scope exists for setting up further hatcheries to bridge the gap progressively increasing between the demand and the supply.

Hatchery technology is not new in India. More than a decade has elapsed since the success has been achieved in the induced breeding of marine shrimp and larval rearing under controlled condition. Yet application of engineering skill in establishing a hatchery is still at the nascent stage. It would not be over emphatic to say that engineering input is a must at every stage of hatchery operation.

Site appraisal, design, construction and operation of the systems to maintain a highly conducive aquatic environment need the expertise of one branch of engineering or the other.

Paper presented at the "National Workshop on Aquacultural Engineering Education in India": 16-17 March 1988, organised by the Indian Institute of Technology, Kharagpur.

However, an engineer must understand the basic principles of hatchery operations and tune his skill, knowledge and experience to the complex requirements of various hatchery components. A schematic diagram of a complete hatchery production cycle is presented at Fig.1 to acquaint with its components.

2. SITE APPRAISAL

Success of a Shrimp Hatchery Project rests with the critical assessment of physical conditions of the surrounding environment as regards to quality and quantity of available process seawater and freshwater, soil conditions, climatic factors and level of infrastructure. For a gross site appraisal, a checklist is presented at Table - 1 as ready reckoner registering all known physical parameters. It is a precursor to ascertain the quality of seawater by the given indicative parameters as:

Salinity	: 30 to 34 ppt	; Dissolved oxygen	: 90%
pH	: 8.0 to 8.4	; Turbidity	: Clear, nominal particle filtration to 10 microns
Temperature	: 28°C to 31°C		

3. THE DESIGN

A hatchery complex is to be designed based on the site evaluation, appropriate technology intended to adopt, functioning of various components and interactions thereof. The design consists of finding out specifications and capacities of culture tanks for each component, water supply and distribution system, aeration and finally an effective and compact layout.

3.1 Components

Production of postlarvae - 20 stage (PL - 20) being the target for a shrimp hatchery complex, its design is done, from the post larval rearing facility to the maturation including live feed culture tanks (Fig. 1). Growout ponds could form part of the hatchery complex to develop potential breeders; however it is only optional and so its design is felt beyond the scope of this paper.

(a) Common requirement of tanks

(i) Smaller tanks of circular/oval/rectangular shape with rounded corners are preferable for easy cleaning, seterilisation and prevention of cross-contamination of disease; (ii) construction materials may be of FRP, brickmasonry, RCC or ferrocement, (iii) facility for water supply and continuous aeration for each tank: (iv) easy and complete draining by PVC standpipes or suitable valves, (v) sufficient clearance between tank - drainout and floor; (vi) all interior surfaces be smooth - F R P tank to have proper resin covering over fibreglass mattings and masonry tanks coated with food grade epoxy (with minimum 3 mm thickness); (vii) effective water circulation to prevent settlement of algal cells, food particles and to keep cultured organisms evenly distributed; (viii) catwalk 1 for easy operation and maintenance; and (ix) calibrations to measure the depth and volume of water.

(b) Specific requirements of tanks

Specific requirements of tanks for different hatchery components are given in Table 2.

3.2 Water supply and distribution

Water being the "life blood" of a hatchery, its supply and distribution must be carefully designed, by finding out the daily water requirement, designing water supply elements and effectively controlling and monitoring water quality.

(a) Water requirement

Water requirement for a hatchery being a function of the total volume of tanks and rate of exchange; it may be expressed in terms of Average Daily Flow (ADF) based on peak demand. Indicative rates of water exchange for different components are given in Table-2.

The average daily flow requirement could be reduced by more than 50% through introduction of recirculation system either in individual components or in the hatchery as a whole. This helps to maintain a stable water condition and conserve water and energy. Typical water recirculation systems through a pair of maturation tanks and for the hatchery as a whole are presented in Fig.2 and Fig.3.

Apart from seawater, a shrimp hatchery needs freshwater for vital operations like, (i) inducing molting in the broodstock by reducing salinity by 4-5 ppt for two days and then increasing to normal seawater salinity so as to enable mating, (ii) reducing salinity of PL nursery tanks gradually everyday to match the salinity of growout ponds, and (iii) for rotifer culture which needs 75% seawater and 25% freshwater.

(b) Water supply elements

On finding out the capacities of tanks and the ADF, a schematic flow diagram (Fig.4-a) is drawn to represent the water supply and distribution scheme for the hatchery, followed by the design of various elements in the scheme.

(i) Pumps

A series of pumps as shown in Fig.2(a) is required for the water supply scheme. Each of these pumps should have a standby with individual suctions and a common delivery with necessary non-return valves (Fig.2-b). Construction material for the pump impeller could be of bronze or 316 SS; shaft made of 316 SS and foot valve made of bronze or polypropylene. Non-metalic pumps could also be preferred (Frazer, 1986).

(ii) Filters

Plain sedimentation (optional) could precede filtration (Fig.4-a) to remove gross turbidity and provide additional storage against abrupt fluctuations in the quality of water from main source.

Sand filters are common choice for filtration of seawater and freshwater. Fig.4-b presents a slow-sand filter with backwash and pumping of filtered water to overhead tanks. However, due to bulk construction, frequent backwashing and slow rate of recovery in slow-sand filters, in recent years rapid-sand and pressure-sand filters are being commonly used. Pressure-sand filters are compact in design and easy to install. Minimum two pressure-sand filters are to be installed for continuous operation. Further a number of on-line catridge, UV filters could be used for optimum filtration efficiency.

(iii) Overhead water tanks (Fig.2-b)

Basic requirements are - (i) Capacity for seawater tanks at least 50% of the daily hatchery demand that freshwater 1/3rd of seawater; (ii) multiple tanks with interconnections to isolate each other for maintenance of tanks and disinfection of water without interrupting the supply; (iii) outlets for complete drainage of each tank; (iv) wider tanks than deeper ones to minimise pressure variation due to water level fluctuations and easy cleaning and sterilisation; (v) water temperature at the same level of hatchery tanks; (vi) maintaining a level indicator at the tank-outside to estimate volume of water in the tank for water treatment; (vii) continuous aeration of entire water column to avoid stratification and oxygen depletion due to water treatment; (viii) air piping to the tanks at higher level than the over flow outlet, (ix) level sensing (optional) to switch on/off the pump PS-1 at preselected low and high water levels, which could help in preventing overflow of tanks, pumps running dry and regular attendance for operation; (x) common overhead platform for both seawater and freshwater; (xi) lids to prevent sunlight penetration and contamination by birds, insects etc. and (xii) construction materials my be of RCC/Ferrocement (with inside epoxy coating), pressed steel (with inside FRP lining) or readymade HDPE tanks.

(iv) Pipe line & fittings

Basic requirements of pipe lines and fittings are as floows: (i) All pipes and pipe fittings should be of standard

size. (ii) Inside the hatchery building, distrbutor pipes must be laid on walls along with air pipes. (iii) Uniform pressure should be ensured at all pipe outlets with independent control valves for each tank at easily accessible heights. (iv) Quick Release Couplings may be used for intermittent supplies to avoid complex plumbings, eg. for supply of premixed seawater and fresh-water from premix tank No.4 (Fig.4-a) to Maturation, Algal Culture and PL rearing. (v) Pipe materials could be of PVC or HDPE and valves of bronze, pp or 316 SS.

(c) Water quality control & Monitoring

Design of facilities for optimum control of water quality needs acquaintance with the process of water treatment. All control measures shall be effective only when a high degree of hygiene is maintained simultaneously.

(i) Water treatment

Eventhough most of the organic impurities are removed by mechanical filtration, some bacteria, water molds, viruses etc. escaping the filters may contaminate water which might be lethal to culture organisms. After physical impurities are removed, water should be disinfected before let out to hatchery components. 30 ml of 10% or 60 ml. of 0.5% Sodium Hypochlorite (NaOcl) is added per cu.m of stored water. After sometime (at least 15 minutes) chlorine is neutralised by adding sodium thiosulphate to the storage tanks at the rate of 50 gms per cu.m of water (Cook, 1977). As the chemical reaction reduces dissolved oxygen, water should be aerated well before use. Water could further be exposed to U.V. radiation for critical hatchery components like larval rearing and Algal Culture.

(ii) Maintaining Hygiene

Sterilisation and disinfection of all containers, pipelines etc, should be carried out as and when the operation permits.

Primary Water Intakes

Sodium Hypochlorite (NaOCl) could be allowed to drip through a flexible tubing laid along with pump suction line to the point of water inlet. (Fig.4-c); This almost kills everything in the pipe line. 4 litres of NaOCl dripped for 15 minutes is enough for a 2" pipe line. The treated water is wasted. To prevent air getting sucked through the intake, the flexible tubing should be closed after each use. (Cook, 1977).

Containers

All culture tanks are cleaned thoroughly before and after use. Cleaning is done by scrubbing with detergent solutions rinsed with clean freshwater and disinfected by hypochlorite solutions (8-25 mg/l for a contact time of 8 to 10 hour respectively). High pressure hydrants could be used for rinsing tanks. Portable F R P tanks are exposed to direct sunlight and immobile tanks inside hatchery to U V radiation to kill potential disease organisms.

Similarly the overhead water tanks and sedimentation tanks (if used) must be cleaned, rinsed and exposed to sunlight for total disinfection at regular intervals.

Sand Filters

Filter materials and filter chambers should be cleaned and disinfected at least once a month. Filter materials are removed and digested in freshwater repeatedly till clean, sun dried and

put back to cleaned filter chambers. Collecting underdrains (PVC manifold), sumps etc. should also be cleaned.

Floors/Drains

Floors and drains of the hatchery complex should also cleaned, disinfected and flushed with high pressure hydrants. Floors and drains should not have any pockets or crevices etc. to trap water. As huge quantity of waste water is released from the hatchery complex, it should be discharged at a point where it may not re-enter the intake system. However, the waste waster could also be treated (Fig.3) to protect the water environment.

Monitoring Water Quality

Primary parameters such as salinity, temperature, DO, pH etc, should be monitored at least twice daily. Micro processor technology could be adopted to monitor water temperature, DO etc. and to control feeding programmes. The monitored data should be recorded for analysis and further programming.

3.3. Aeration

Continuous aeration is essential for entire hatchery operations to maintain desired level of dissolved oxygen, ensure even water temperature and help in the reduction of ammonia content in the tank water. The basic design considerations are, (i) volume of air and (ii) total head of water column against which air is to be delivered. It is estimated that 0.004-0.005 Cu.m of air per min. delivered per Cu.m. of water is sufficient to oxidise dissolved organic matter in the tank (NACA, 1986). However, minimum rate of air flow is 0.0017 Cu.m. per min (0.06 CFM) required by an air stone. Depending on the components,

the air flow may vary btween 0.004 and 0.03 Cu.m. per min. per Cu.m of water. For aeration, the total head is the maximum depth to which air needs to be delivered at any point of distribution. This is not more than 2 m for a hatchery system, which worksout to 0.2 kg, per sq. m (2,857 psi).

(a) Aerating machinery

Considering large volume of low pressure air required, either Twin Lobe or Turbine type multistage blowers are preferred, as their performance are (i) continuous duty, (ii) air delivery free fom oil vapour, moisture or other contamination and completely uniform and free from pulsation, (iii) need less maintenance and (iv) availability in wide range of capacities (upto 1,00,000 Cu.m. per hr.). However, air blowers should be installed on anti-vibration mountings and in duplicates and should have inlet and outlet air filters.

(b) Air pipelines

Basic requirements are (i) initial length made of aluminium to absorb heat from the blower and rest PVC pipes (ii) condenser unit in air line inside air conditioned rooms to remove condensed water vapur, (iii) air release to tank bottoms through air stones fixed to flexible PVC or polyethylene tubings and (iv) in each section an additional air outlet with quick release coupling for use outside culture tanks.

3.4 Emergency power supply

Diesel generating set with capacity to operate air blowers, artificial lights, air conditioners and pumps is a mandatory provision in case of mains failure. The basic requirements are

(i) an automatic mains failure (ANF) controlled panel synchronised to the mains supply as larval rearing, Artemia and rotifer tanks may be affected within minutes of power failure, (ii) sequential start for essential units in decending order of priority, say, blowers, lights and so on, (iii) D.G. set and connected installations confirming to relevant sections of Indian Electricity Act and (iv) an additional set as stand by to the original set to operate at least the air blowers.

3.5. Hatchery layout/building

The optimal juxta-position of the hatchery components is determined on the basis of their interrelationship (Fig.1), corresponding layout of hydraulic and pneumatic plumbings, drainage system and most importantly, the operating needs. The specific requirements of the hatchery buildings are, therefore (i) layout of components consistant with operational sequence, (ii) no thorough-fare through any components - approach through corridors only, (iii) machine rooms (for air blowers, D G set etc.) and repair workshop isolated from the main building as the hatchery operations are not compatible with noise, vibration and exhaust fumes, (iv) anti insect nettings on wall openings, (v) large doors on outside wells to remove tanks for maintenance (vi) drains with adequate capacity and generating self cleaning velocity and (vii) provision of roof extractors and exhaust fans to remove vitiated air caused by culture process, air borne contaminants and to maintain satisfactory thermal environments.

4. Construction

As evident, a shrimp hatchery complex involves civil, mechanical, electrical and some other engineering skills. As a result, the network analysis indicating the events and activities in the lines of CPM/PERT is necessary for an effective planning and execution of the project. Prior to the construction and installations, the interdependent and interrelated features of the work must be set down. Pipes through RCC tanks, pipe supports welded to the roof truss work, specifying D.C. Set room as per electrical inspectorates requirements etc. are a few examples.

5. Operation and maintenance

Apart from the design, construction and ensuring hygiene, operation of the process facilities with preventive maintenance need due attention of engineers. A maintenance schedule for civil and mechanical structures against adverse coastal conditions and for all machineries as per manufactureres recommendations with minimum possible repairs are required for continuous operation lest major breakdowns call for a total shut down.

6. Conclusions

From the foregoing discussions, it is seen that the construction and successful commissioning of a shrimp hatchery is the culmination of various engineering inputs synchronising the site and available materials with the biological needs typical to penaeid shrimp. The paramount importance with successful operation of a shrimp hatchery lies with a site having adequate quantity and quality of seawater, which cannot be compensated at a later stage. On the otherhand, the engineer should not only

evaluate the available technology and operation procedures but also further his understanding of the biological requirements to exercise some flexibility in designing a shrimp hatchery.

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TABLE - 1

CHECKLIST FOR SITE APPRAISAL OF A SHRIMP HATCHERY

Items	Total points	Evaluate out of total points	Remarks
1. SEA WATER (150)			
Period of availability possibility of abrupt reduction in salinity due to river discharge	60		Decision: Reject the site if TE is less than 75%. Site acceptable for limited production if TE is between 75% and 85%. Site favourable for commercial production if TE is 85% and above.
	40		
pollution level	30		
Distance of seawater source	20		
2. TOPOGRAPHY 950)			
Land undulations and natural barriers	30		
Waste water disposal	20		
3. SOIL 930)			
Land bearing	20		
Sub soil condition	10		
4. CLIMATE (20)			
Wind pressure	10		
Sunlight hours	10		
5. INFRASTRUCTURE (150)			
Construction materials	20		
Freshwater	30		
Electricity	30		
Machinery and equipments, after sales service	20		
Skilled labour	10		
Market for hatchery produce	10		
Accessibility to site	20		
Possibility of improvement	10		
TOTAL EVALUATION (TE)	400		

TABLE - 2

SPECIFIC REQUIREMENTS OF TANKS

(A) : Standard sizes adopted (B) : Stocking capacity (C) Rate of water exchange (indicative)
(D) : Light conditions (E) Construction materials
(F) : Other requirements

Maturation

(A) 10 - 12 T circular (dia.4m)
(B) 7 adult shrimp per sq.m.
(C) 200% for flow-through or 50% for recirculation
(D) Reduced light (100 Lux at tank water surface, Photo-period control of 14 hrs. light to 10 hrs. dark
(E) : FRP opaque or RCC/Ferrocemer with dark green expoxy coating of inside surface
(F) : Sand substratum, if necessary; underwater torch to observe ovary development.

Spawning/Hatching

(A) : 259 - 500 Litres cylindro-conical (dia.0.75 - 1m)
(B) : One spawner per tank
(C) : 400%
(D) : Dark room
(E) : RFP opaque
(F) : Lid for tank

Larval rearing

(A) : 2T cylindroconial (Dia.1.5m) or 10 T oval/rectangular
(B) : 100 nos. nauplii to 50 nos. PL5/lt.
(C) : 200%
(D) : Moderate light
(E) : FRP translucent or RCC Ferrocement with white epoxy coatingof the inside surface

Algal Culture

(A) : 250 litre - 2 T cylindro-conical
(B) : 100000 cells per ml
(C) : 50%
(D) : Day light, fluorescent lamps around tanks, 5000 lux at pure culture shelves with 18 hrs. light to 6 hrs. dark
(E) : FRP translucent
(F) : FRP translucent roofing for scaling up: barrel pump for algae transfer to larval rearing tank

Artemia

(A) : 500 litre - 1 T cylindro-conical
(B) : 200000 artemia nauplii per litre
(C) : 50%
(D) : Reduced light
(E) : FRP opaque with translucent window at bottom with PP Ball valve for harvest
(F) : Lid for each tank

PL Rearing (Nursery)

(A) : 10 T or bigger tanks with oval or rectangular shape
(B) : 25 nos. of PL5 to 10 Nos PL20;t
(C) : 100%
(D) : Day light
(E) : Same as larval rearing
(F) : Installed outside hatchery building.

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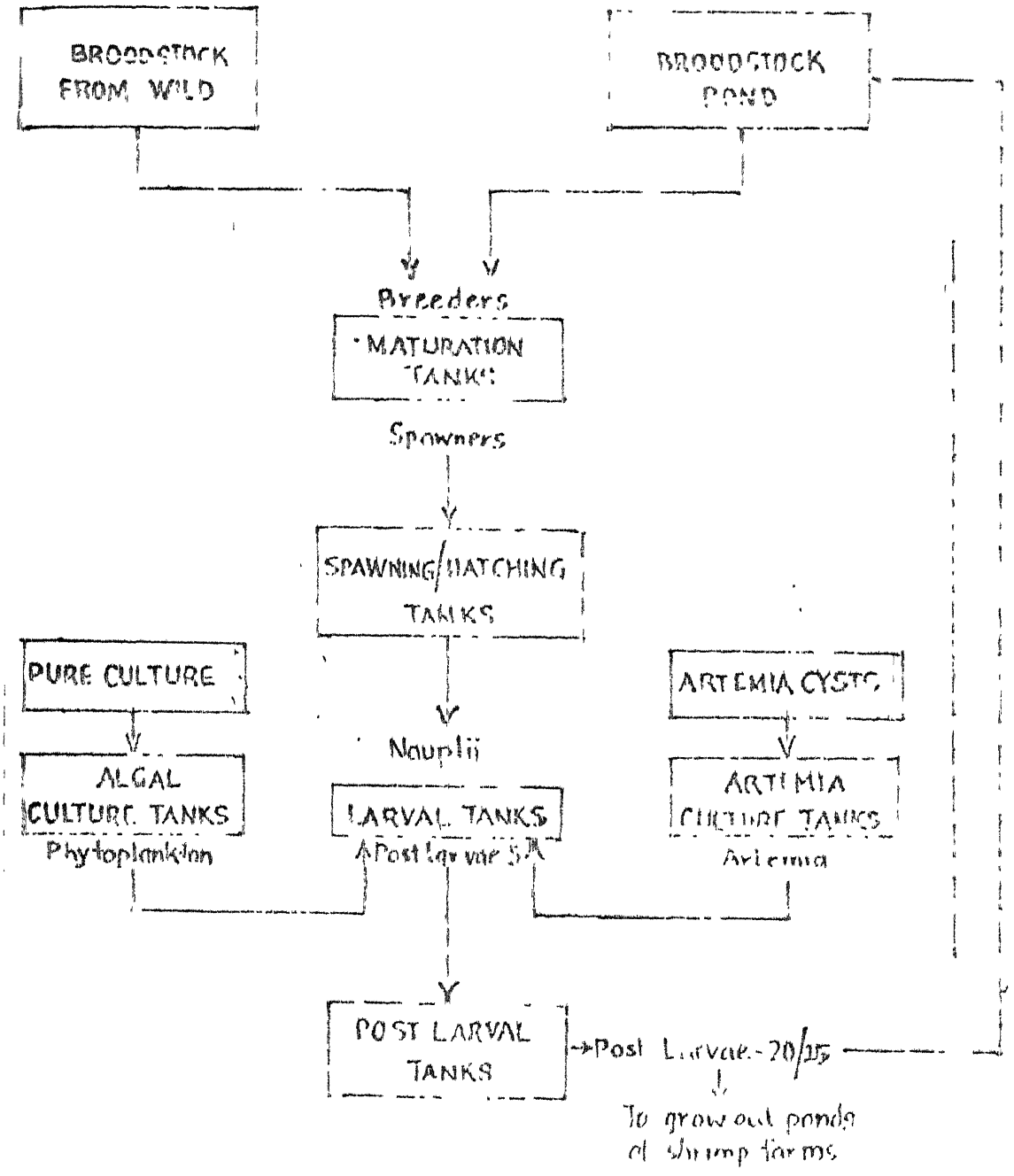
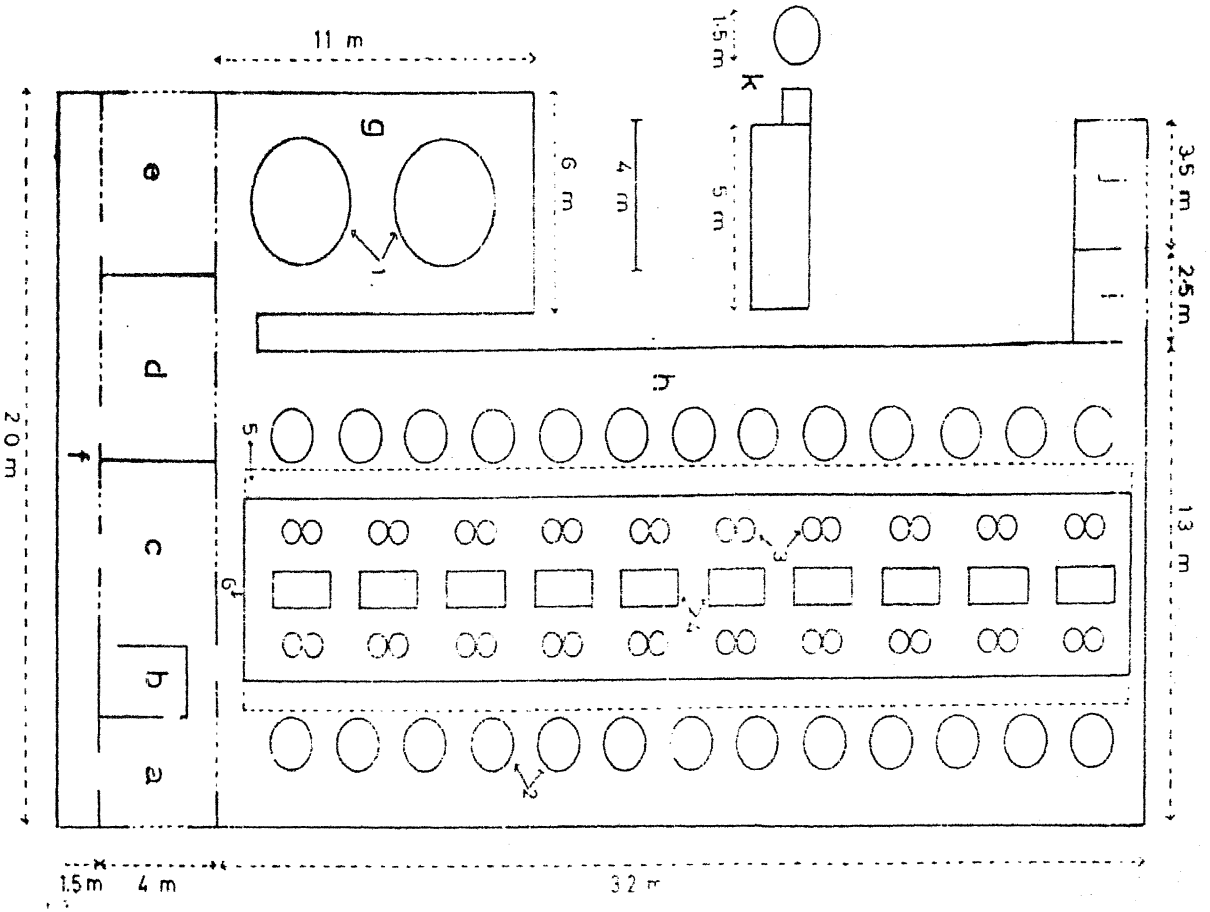


Fig.1. SCHEMATIC DIAGRAM OF OPERATIONS THROUGH A HATCHERY COMPLEX



Lay-out of hatchery complex: a. duty room, b. toilet, c. laboratory, d. feed preparing room, e. store, f, verandah, g. maturation shed, h. larval rearing shed, i. air blower room, j. generator room and k. freshwater sump and overhead tank. 1. maturation tanks, 2. larval rearing tanks, 3. spawning tanks, 4. algal culture tanks, 5. cat-walk and 6. raised platform.

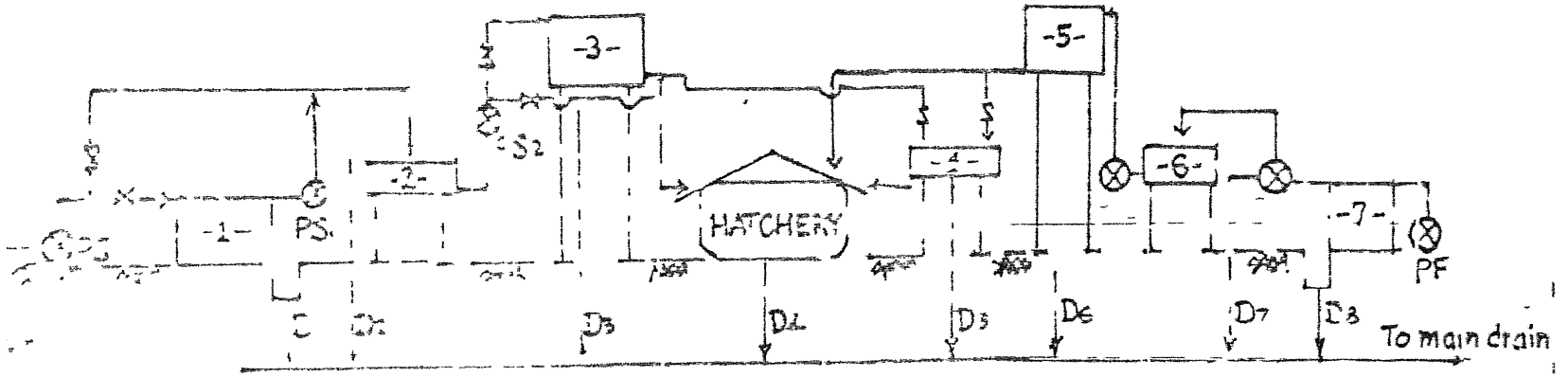


FIG.2a FLOW DIAGRAM OF SEAWATER/FRESH WATER TO A SHRIMP HATCHERY

EXPLANATION TO FLOW DIAGRAM

-1-2- Raw water pumps for seawater/fresh water
-3-7- GR Storage cum sedimentation tanks for....
PS1/PF1: Pumps to sand filters for---
2-6- Sand filters for [illegible] sand filter as alternative)
PS2/PF2: Pumps from sand filter to overheads for---
-3-5 : Overhead tank for----
-4- : Premixing tank for---
D1-D8: Drain outs

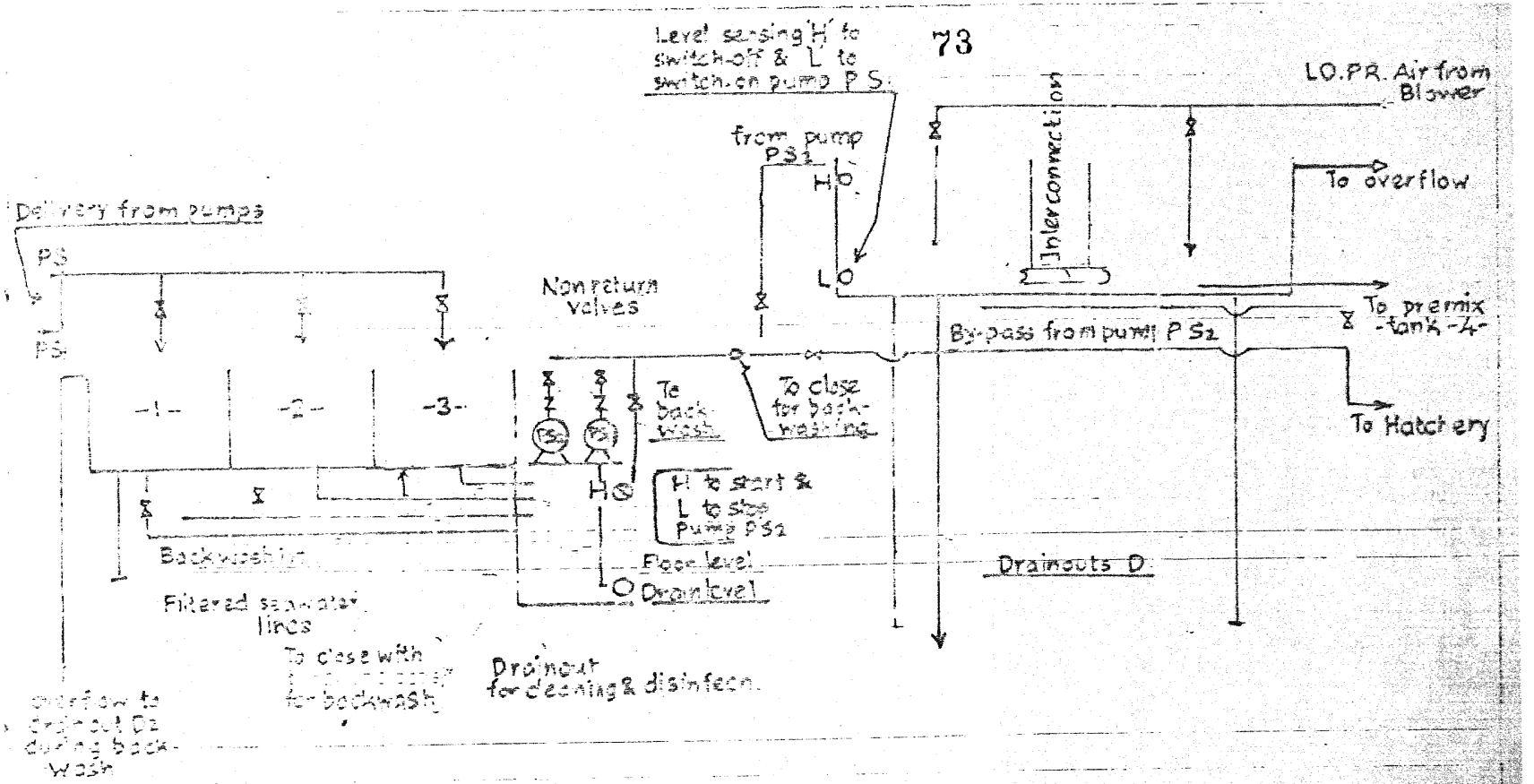


Fig. 2b DETAILS OF SAND FILTER & FILTERED WATER OVERHEAD TANKS

NOTE: Pump PS2 in duplicate
1,2,3: Sand filters
Filtered water collection sump

Tanks in duplicate with interconnection and independent drainouts

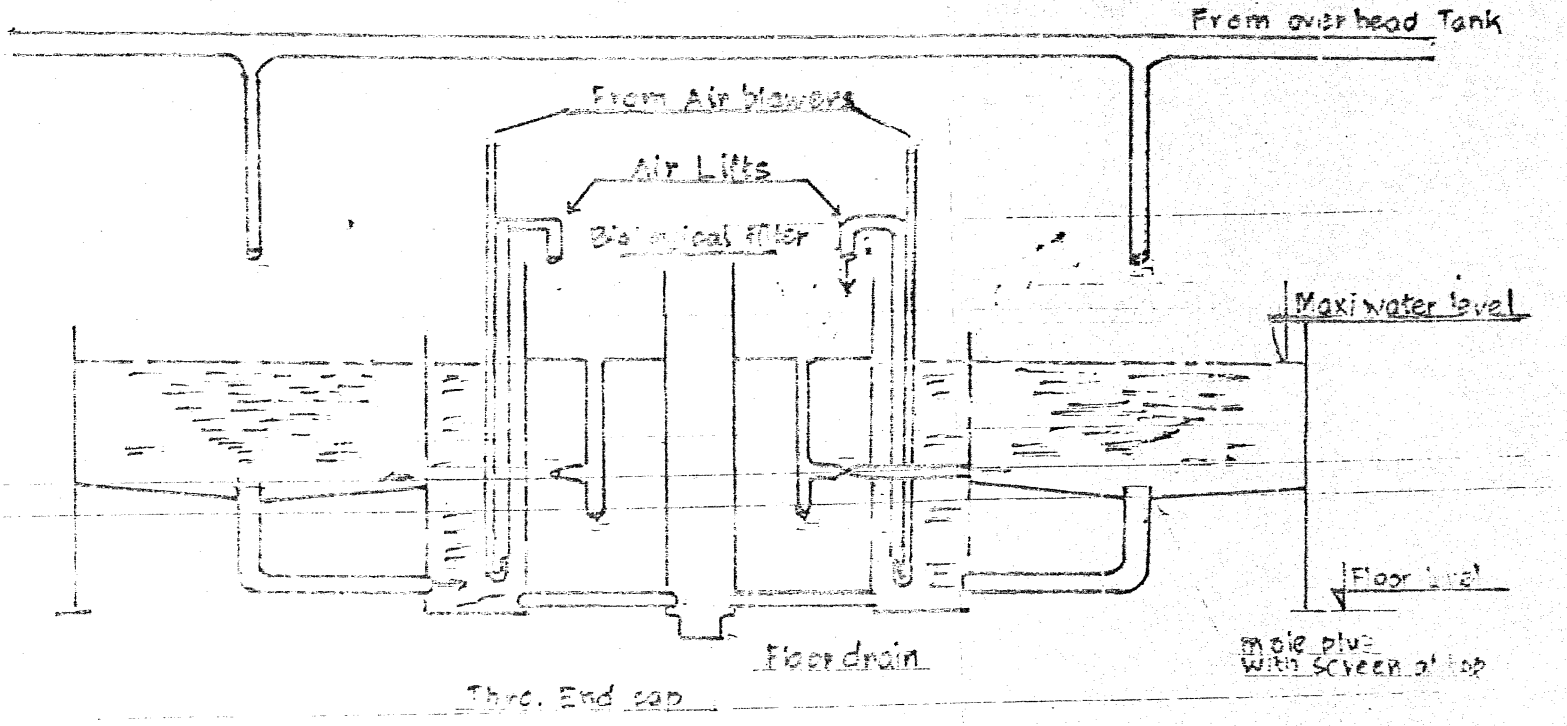
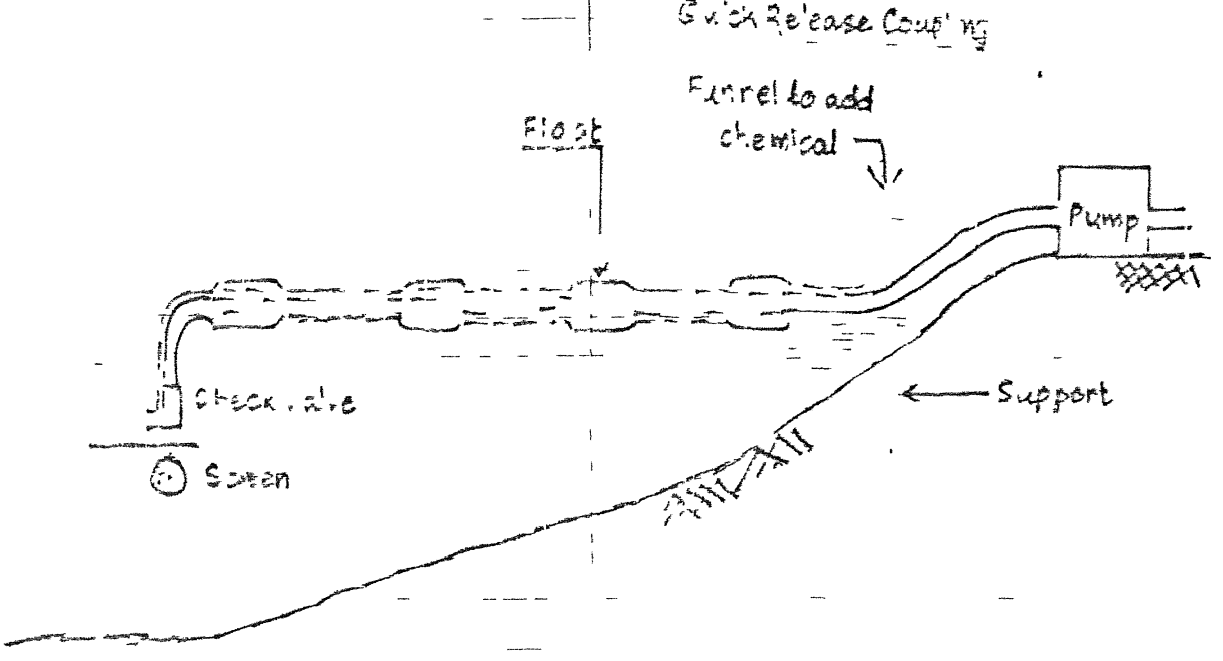
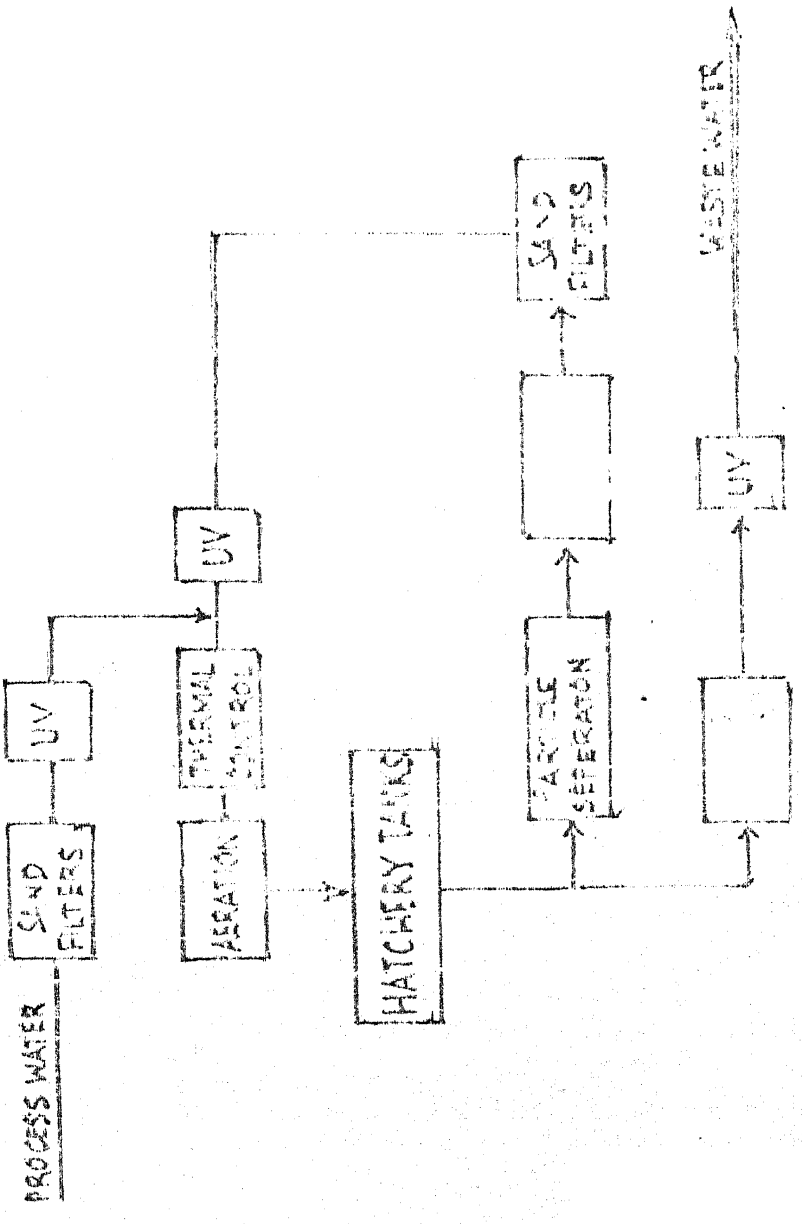


Fig: 3 A PAIR OF MATURATION TANKS WITH CONTINUOUS WATER CIRCULATION


Quick Release Coupling
Funnel to add chemical
Float
Pump
Check valve
Support
Screen


PROCESS WATER
SAND FILTERS
UV
AERATION
THERMAL
UV
HATCHERY TANKS
PARTICLE SEPERATION
SAND FILTERS
UV
WASTE WATER

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The views expressed in this paper need not necessarily be that of the organisation.

Hatchery Management

By

K.V. GEORGE

The successful functioning of a prawn hatchery is based on proper management in the various phases of its construction and operation. Adequate technical know-how and expertise are essential for this. The following are the major areas which need proper management.

1. Selection of site.
2. Design, lay-out and construction of hatchery and other infrastructure facilities.
3. Sea water pumping system an storage facility for steady supply of good quality sea water.
4. Collection of spawners from the wild.
5. Broodstock management.
6. Selection of spawners.
7. Facilities for the spawning and rearing of larvae.
8. Water management - most important.
9. Nursery management.
10. Feed and feeding schedule.
11. Uninterrupted aeration facility.
12. Assured supply of electricity.
13. Availability of fresh water.
14. Appropriate stocking densities in the rearing and nursery tanks.
15. Seed collection, packing and transportation.
16. Proper maintenance of hatchery equipments.

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ADAPTATION OF TECHNOLOGY TO OTHER SPECIES OF PRAWNS

By

A.V.P. Rao

The maturation and larval rearing technologies evolved for Penaeus indicus can easily be adapted to other penaeid prawns since the reproductive biology, larval duration and postlarval requirements are identical for most of the species. In fact most of the marine prawns occurring along the Indian coasts were successfully bred and their postlarvae produced at the CMFRI and at CIBA the seed of Penaeus indicus, P. merguiensis, P. monodon, P. semisulcatus and P. japonicus was produced.

Penaeus monodon:-

This species, as compared to P. indicus has higher fecundity. The number of fertilized eggs spawned by adult females with a carapace length of 53.1 mm to 81.3 mm varies from 2.48 lakhs to 8.11 lakhs, with higher fecundity recorded from larger females. Hence the resultant nauplii due to their large numbers require more space for larval as well as postlarval rearing. For the larval rearing of P. monodon the same feeds viz., Chaetoceros sp, Thalassiosira sp, Tetraselmis sp, Cyclotella sp, Brachionus plicatilis, tissue suspension of Oratosquilla nepa, Perna viridis or egg custard is used. However, though this species is hardy as adult, the larval rearing is more difficult than that of P. indicus and the average survival between nauplius and postlarva (PL-2) is around 50%.

The capacity of algal culture tanks is 15% of the larval tank capacity.

The space for postlarval rearing is about five times more than the larval tank capacity. Bottom aeration of the postlarval tank through a perforated rigid PVC pipe along the longitudinal axis of the tank provides sufficient aeration. A postlarval tank with 0.5% gradient at the bottom and provided with a flow-through system can be very ideal. The postlarval tanks can be constructed in RCC and kept in the yard with individual covers to protect them from strong sunshine and rainfall.

P. japonicus:- This species is a burrowing form in the juvenile and adult stages and hence a part of the holding tanks for them should have sandy bustratum to provide for their burrowing habits. Methods of rearing the larvae and postlarvae are the same as employed for P. indicus. Since the larvae and early postlarvae are more sensitive to nitrite the quality of water should be of a very high order.

P. semisulcatus:- This species prefers higher salinities and has fecundity more or less the same as that of P. monodon. Hence the requirement of space for larval and postlarval rearing will also be identical. The conditioning to low salinities carried out in the case of P. monodon is not required, since they are normally stocked in ponds with a salinity range of 33 to 37 ppt. However, conditioning the postlarvae to higher salinities is essential.

P. merguiensis:- This species in all the aspects of its maturation, spawning, larval and postlarval rearing resembles P. indicus. Hence the technology for P. indicus seed production as such can be adapted.

Metapenaeus spp:- The eggs of Metapenaeus spp are generally smaller than the penaeid eggs. Hence while washing the eggs before transferring them to hatching tanks a smaller mesh than what is used for penaeid eggs is required. The postlarvae are much smaller than the penaeid postlarvae. Hence smaller meshed nets have to be used.

Though seed production of commercially important species of penaeid prawns was achieved repeatedly without using Artemia nauplii it has been now established that for sustained and commercial production of seed with robust health and resistant to diseases, Artemia nauplii are required. Since the nauplii that hatch out of Indian cysts are larger than those from the cysts of San Francisco strain, the latter are preferred. Utilising the Artemia nauplii once a day as feed for the stages between mysis-3 and postlarva-5 results in vast improvement in survival rate, especially in the subsequent stages.

Macrobrachium rosenbergii:- Wherever the salinity is lower during a part of the year such as in Kerala, the hatchery can become versatile by switching over to the production of seed of M. rosenbergii or M. malcolmsonii as per the availability of the brood stock. The required range of salinity is 12 - 16 ppt. Stored brine can also be used for making up the salinity of the above range.

Slate-grey coloured egg masses carried by females are ready for hatching in 24 - 36 hours. The larvae known as zoeae pass through eleven stages followed by the postlarval stage.

The entire metamorphosis to first postlarva requires on an average 30 days. When the temperature of the water is either too low (below 25°C) or too high (above 32°C) the larval metamorphosis takes a longer period - sometimes as long as 54 days. Just as in penaeid prawns the particle size of the food is gradually increased from 60 microns to 300 microns. A vareity of foods are used, both natural as well as compounded such as pond snail (Pila globosa), blood clam (Anadara granosa), green mussel (Perna viridis), tubificid worms, egg custard, tuna meat, trash fish and prepared feeds in dough form. Daily cleaning of the bottom of the tank to remove the unused feed and maintaining the water quality are very essential. The postlarvae require slow acclimatisation to freshwater conditions over a period of 24 hours and reared for two more weeks in nursery tanks before they are stocked in the grow-out ponds along with the Indian and exotic carps. Installation of air-lift pumps in the larval and postlarval tanks helps in maintaining water quality.

Thus one hatchery constructed for producing the seed of any one species of prawns can be used for other species as well with minor modifications, so that the facility can be used for a longer period during the year and becomes more remunerative.

METHODS OF WATER ANALYSIS

By

S.A. Ali

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 *	500-3,600 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. Bu fer 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.
3. Standard Sea water

Procedure:

Pipette 10 ml of standard sea water 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 sea water sample into the conical flask and proceed as above.

Calculation:

Salinity is calculated as follows:-

Let Volume of Silver nitrate for 10 ml of standard sea water = V_1 ml

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

Salinity of standard sea water = S °/₀₀

Salinity of sample = $\frac{V_2 \times S}{V_1}$ °/₀₀

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 a 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 volumetric 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 solidum thiosulphate as above.

Calculation

Calculate the normality of potassium iodate 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 Phosphorus

Samples to be collected in Polythene bottles of roughly 150 ml capacity and analysis is to 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

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

2. Sulphuric acid solution

140 ml of A.R. quality sulphuric acid added to 900 ml distilled water.

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 good quality of 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 tartarate solution. 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 μ in a spectrophotometer or Calourimeter.

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 standard 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 gm 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 distilled water. Diluted to 500 ml with water. It is stable for many months.

9. N1-Naphthyl Ethylene Diamine Di-hydrochloride solution(N.N.E.D.)

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

10. Standard Nitrate solution

Dissolve 1.53 gm of analytical reagent quality potassium Nitrate in 1000 ml; 1 ml = 15.0/ µg of Nitrogen. Dilute 5 ml of this solution to 250 ml with water. Store in dark bottle.

Procedure

Measure out 50 ml of the sea water sample with a 50 ml measuring cylinder into a 250 ml conical flask (sample should acquire room temperature). Add 2ml of buffer Reagent and mix. After the bufferhas 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 calourimeter.

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

NITRITE

Reagents

1. Sulphanilamide solution (as in Nitrate method)
2. N1 Napthyl Ethylene Diamine Dihydrochloride (NNED)

   (as in Nitrate method)
3. Standard Nitrite solution

Dissolve 0.345 gm of A.R. sodium, Nitrite in 1000 ml of distilled water. Store in a dark bottle with 1 ml of chloroform. 1 ml = 5 µg. Dilute 10 ml of the solution to 1000 ml with distilled water and use for analysis.

Procedure

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

Measure the absorbance in a calourimeter at 545 µm.

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

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 calourimeter or spectrophotometer at 640 um. 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 in the water sample from the standard graph. This value will give the ammonia a 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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