



CMFRI SPECIAL PUBLICATION

Number 60

SHRIMP FEED FORMULATION AND FEED MANAGEMENT

CMFRI
TRANSFER OF TECHNOLOGY SERIES

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

SHRIMP FEED FORMULATION AND FEED MANAGEMENT

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Front cover : *Some of the ingredients for shrimp feed and preparation of pellets.*

Back cover : *Sun drying of shrimp feed pellets.*

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P R E F A C E

Successful and sustainable aquaculture of finfish and crustaceans depends upon the provision of nutritionally adequate, environmental friendly and economically viable artificial feeds. Feeding increases the productivity of aquaculture farms and constitutes the highest single cost item of running expenditure. In view of this, it is imperative that artificial feeds besides being scientifically formulated and optimally processed, should be judiciously supplied considering the specific nutritional needs of the cultivated species and the intensity of the culture operations. In order to achieve maximum growth and best feed efficiency of a given feed from a culture system, the feeding strategies employed viz. feeding rate, frequency and methods of feeding are of primary importance.

The Central Marine Fisheries Research Institute has identified prawn nutrition as an important area of research and has been actively engaged in the formulation of suitable feeds for the commercially important species *i.e.* *Penaeus monodon* and *P. indicus*. These feeds are being tested under field conditions at South Chellanam, Ernakulam, where the dissemination of technology relating to on farm shrimp feed production has also been undertaken. This Publication on shrimp feed formulation and feed management, has been prepared as an easy to read guide for small scale prawn farmers, extension officers and others engaged in prawn culture. Besides, describing the feed requirements in brief, it deals with all the important aspects of feed management which are a must for getting the best feed performance about which most farmers are usually ignorant.

I wish to express my thanks to Dr. (Mrs.) Manpal Sanhotra, Scientist, P.N. P. Division for the efforts put in for the preparation of this Handbook based on her own work and experiences in the field and to Dr. (Mrs.) Krishna Srinath, Senior Scientist, S.E.E.T.T. Division for the help rendered in dissemination of technology. I thank Dr. M. Peer Mohamed, Head, Physiology, Nutrition and Pathology Division and Dr. N.N. Pillai Head, Crustacean Fisheries Division and Shri. D.B. S. Sehara, Head, S.E.E.T.T. Division who have gone through the text and gave their valuable comments. I also thank Dr. K. Rengarajan, Senior Scientist for editing this Handbook.

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INTRODUCTION

Shrimps are cultured in waters rich in nutrients and natural food derived both from plant and animal origin. Fertilization with either chemical or organic fertilizers increases production of food organisms resulting in higher shrimp production. This traditional culture of shrimp with only natural food gives around 200-700 kg/ha/season.

Supplementary feeds are source of nutrients which complement natural food to increase shrimp production. These low cost feeds are mainly processed pellets which primarily provide protein and energy to the culture animals. In extensive farming system, which is an improved traditional system, ponds are stocked with the desired species of shrimp and fed with locally available material as per requirement. This system of shrimp culture with supplementary feed yields 800 kg-1000 kg/ha/crop.

In the case of intensive shrimp farming systems in addition to selective stocking of desired species, feeds with high conversion ratios which provide all the nutrients essential for shrimp such as proteins, amino-acids, carbohydrates, lipids and fatty acids, vitamins and minerals are also required. Such systems with complete feed yield over 10,000 kg/ha/crop.

In the case of intensive system, ponds are stocked with seeds of fast growing shrimp at the rate of 1-3 lakh/ha, providing very good water management and using specially prepared feeds with high conversion rates. Thus the use of complete feed increases the carrying capacity of the pond by enabling higher stocking rates which in turn increases shrimp production and profit.

However, these feeds are expensive and take a considerable amount ranging from 50 to 70% of the total production cost. As the quality of feed and costs are directly related, improvements in feed quality, inevitably increase the production costs of feed. Therefore

quality feed and cost are critical and important factors in determining profitability in a shrimp farm. In the selection of an appropriate feed to match the shrimp farming, several factors have to be considered and these include expert farm management, farm infrastructure facilities such as pumps, paddle wheels, farm and pond size to have the desired production yield as all these factors are interrelated.

For example in intensive system the ponds are generally of the size of 0.1 to 0.5 ha and stocking rate varies from 3-10 lakh/ha. Very good water quality has to be maintained by providing even upto 300% exchange daily. In addition to this vigorous aeration is also provided by air blowers/paddle wheels/agitators. But in such systems high energy feed with good conversion ratios of 2 and below are a must and depend on the quality and convertability of the feed. These types of culture systems give a shrimp production of over 10 tonnes/ha/crop.

Quality of feed should therefore, be of an appropriate level as required by the farming system. The use of locally available ingredients such as mantis shrimp, *acetes*, squid waste and prawn head waste in the preparation of feeds can help a great deal in reducing the costs of feed. The price of these feeds ranges from Rs. 14 to 25/kg in comparison to Rs. 30 - 45/kg for the imported commercial feeds.

2

FEED REQUIREMENTS

Around 40 nutrients are believed to be essential for shrimp.

Proteins

These are the major organic material in the animal tissues constituting 65-75% of the total dry weight basis. As proteins are continuously used for growth and normal metabolism, a continuous and liberal supply is essential. Inadequate protein results in reduction or cessation of growth, loss of weight due to removal of proteins from tissues to sustain vital body functions. Recommended protein levels for commercial feeds are given in Table 1. If natural food is sufficiently available, these levels can be reduced.

Lipid

These are fat soluble compounds consisting of fats, phospholipids and sterols which are (i) concentrated and highly digestible sources of energy, (ii) components of tissues and (iii) carriers of fat-soluble vitamins. Lipids are essential for normal growth and functions of shrimp. Decreased growth and increased mortalities are associated with drastic fall in lipid levels exceeding 10%. Lipid levels recommended for commercial feed are given in Table 1.

TABLE 1. Recommended protein and lipid levels in percentage for shrimp feeds (as fed basis) in relation to body weight

Size of shrimp (g)	Protein level (%)	Lipid level (%)
0 - 0.5	45	7.5
0.5 - 3.0	40	6.7
3.0 - 15.0	38	6.3
15.0 - 40.0	36	6.0

Source: Akiyama, D.M. Warren G. Dominy and Addison L. Lawrence 1989. Penaeid shrimp nutrition for the commercial feed industry. *Soybeans, American Soybean Association*, 35 pp.

Fibre

This is the undigestible fraction in feeds. It is not digested at a significant level to be a factor in shrimp nutrition. High fibre feeds increase faecal production and consequently pollute the water. Fibre levels in commercial feeds should not exceed 4% and this limitation significantly increases the cost of feed formulation. Under less intensive systems of shrimp culture, fibre level can be increased, thus decreasing the cost of feed.

Minerals

Minerals are the inorganic (ash) component of feed. Approximately 20 minerals are required by shrimp and the essential functions of these are to act as constituents of the exoskeleton and as co-factors for normal metabolism and growth.

TABLE 2. Mineral requirements in shrimp diets

Calcium	2.8%	Iron	300 ppm
Phosphorus	1.8%	Copper	25 ppm
Magnesium	0.2%	Zinc	110 ppm
Sodium	0.6%	Manganese	20 ppm
Potassium	0.9%	Cobalt	10 ppm
		Selenium	1 ppm

Source: From various authors.

Shrimp like other aquatic animals, have the ability to absorb minerals from the surrounding water in addition to the ingested feed. Therefore, the dietary requirement for a particular element depends, to a large extent upon the concentration of it in water. Minerals are probably not so important in the diet since generally the water, especially brackish water, is rich in minerals.

Shrimps can absorb or excrete minerals directly from the aquatic environment *via* gills and body surfaces. As brackish water contains a high level of calcium, it is not considered essential. However, its level should be monitored to maintain a Ca : P ratio of 1:1 to 1.25:1 and should not exceed 2.8% in the feed. Phosphorus on the other hand needs to be supplemented and total phosphorus level in shrimp feed in approximately 1.5%. Ash levels should not exceed 15%. Mineral requirements in shrimp diets are given in Table 2.

TABLE 3. Vitamin requirements of cultured shrimp

Vitamin	Quantity required per kg of feed	Vital functions	Deficiency symptoms
Water Soluble			
Thiamine (B ₁)	150 mg	Controls carbohydrate metabolism.	Anorexia, poor growth, pigmentation, mortality.
Riboflavin (B ₂)	100 mg	Helps in fatty acid, amino acid and carbohydrate metabolism.	Anorexia, poor growth abnormal swimming behaviour, mortality.
Pyridoxine (B ₆)	50 mg	Helps in many enzymatic reactions and in amino acid metabolism.	Poor growth, mortality, hyperirritability.
Pantothenic acid	100 mg	Cholesterol synthesis enzyme reactions.	Abnormal gill features, anorexia.
Niacin	300 mg	Helps tissue oxidation energy metabolism synthesis of fatty acids.	Poor growth, anorexia, lethargy and mortality.
Biotin	1 mg	Helps in carboxylation and transcarboxylation.	Anorexia, slow growth, depigmentation.
Inositol	300 mg	Tissue formation.	Slow growth, anorexia.
Choline	400 mg	Transmethylation, lipid transport and nerve impulse.	Poor growth, fatty tissue
Folic acid	20 mg	Metabolism of amino acids, biosynthesis of purines and amino acids.	Poor growth and lethargy.
Vit. B ₁₂	0.1 mg	Nucleic acid synthesis, carbohydrate and lipid metabolism.	Poor growth, anorexia.
Ascorbic Acid	200 mg	Formation of collagen and steroid hormones.	Black death, light colour hepatopancreas, reduced growth.
Fat Soluble			
Vit. A	1500 IU	Calcium transport and reproduction, embryonic development.	Depigmentation, soft exoskeleton.
Vit. D	7500 IU	Calcium and phosphorus metabolism.	Poor growth soft exoskeleton.
Vit. E	400 mg	Antioxidant.	Reduced growth and depigmentation.
Vit. K	200 mg	Blood coagulation, electron transfer.	Hemorrhaging of tissues.

Source : from various authors

Vitamins

Vitamins are complex organic compounds required in trace amounts for normal growth, reproduction, health and general metabolism. In cultured shrimp, supplementation of vitamin premixes has been shown to improve health, especially if combined with mineral supplementation. Vitamin requirements are greatly affected by various factors such as availability of natural food, size and growth rate of shrimp and methods of feed production. A few of the essential vitamin requirements of cultured shrimp along with their vital functions and deficiency symptoms are given in Table 3.

3

ATTRACTABILITY AND PALATABILITY

When shrimps are fed, 'attractants' leach from the feed pellets into aquatic medium and these are detected by shrimps with chemoreceptors distributed throughout their body. The shrimps detect and feed by smell and not by sight. Therefore attractability and palatability of a feed are also critically considered, as a nutritionally balanced feed is of little value if not consumed by shrimp. Feed with good attractable smell and good palatability, will bring shrimps to feed voraciously which results in high production.

A misconception among farmers is that when they smell the feed they can determine the feeds attractability and palatability to shrimp. A feed may be palatable to shrimp and yet have no smell which can be detected by a farmer as the mechanism of smelling is different in man and shrimp.

A method for determining a feed's attractability and palatability, is to watch a shrimp being fed in a bucket. Within two minutes of its being given the feed, the shrimp will become active and search for the feed. If it does not respond to the feed, the feed is not attractable and should not be used. Within 30 minutes, the shrimp intestine should be filled with feed, which confirms the consumption of the feed. If shrimps fondle the pellets and then drop them without eating, the feed is not palatable though attractable and should not be used.

4

PROPER PREPARATION AND APPEARANCE OF FEED

As shrimps feed only by smell, colour is irrelevant. However, feeds should be uniform in colour. Colour variation in pellet indicates inadequate mixing of ingredients and/or a variation in cooking of the feed. Inadequate mixing results in non-homogeneous distribution of the nutrients. Overcooking can destroy essential nutrients such as vitamins, aminoacids and render them unavailable, while undercooking can result in poor water stability. As shrimps can sort large particles of ingredients, feeds should be properly powdered so as to be homogeneously balanced. Uneven particle size is also an indicator of poor feed processing.

Feeds should not contain cracks or fractures, but should be uniform in texture. When cracks are there water will seep into the pellet and reduce the water stability. The feed should also not stick together. Sticking or clumping indicates inadequate drying before packing. The nutritional quality of wet feeds deteriorates rapidly. Shrimp feed should contain a maximum of 2% fines or dust, as excessive fines are a result of poor processing and result in feed wastage as it will not be consumed by the shrimp and will only result in water pollution.

5

SIZE OF FEED PELLETS

The size of feed pellets is not related to the mouth size of shrimp. As shrimps carry the feed particles to their mouth as they feed and often swim with the pellets, the size should be small enough to be carried to the mouth and also enable the shrimps to carry the pellet while swimming. Recommended feed pellet sizes for shrimp are given in Table 4.

TABLE 4. Recommended pellet sizes with regard to shrimp body weight

Shrimp weight (g)	Pellet size (mm)
0-3	1 (crumble)
3-15	2 x 4
15-40	2.5 x 5

Source : Akiyama, D.M., Warren G. Dominy and Addison L. Lawrence 1989. Penaeid shrimp nutrition for the commercial feed industry. *Soybeans, American Soybean Association*, 35 pp.

Shrimps do not require more than 3 sizes of feed (Fig. 2). However, the smaller the feed size, the more the feed particles per unit weight would be available, thereby feeding a greater number of shrimps.

6

STABILITY OF FEED IN WATER

Shrimps are slow continuous feeders and the feeds should be water stable, so that the full feed is consumed without waste. Feeds with poor water stability, disintegrate rapidly and cause



Fig. 1. Stability of shrimp feeds in Water : A. feed with good stability in water swells, but remains intact till consumption and B. feed with poor stability in water disintegrates immediately leading to water pollution.

feed waste, water pollution and give poor feed conversion ratios (FCR).

A water stability of 2.5 hours is quite satisfactory. Within this time all the attractants would have leached out and the feed will not be consumed. However, optimum water stability is dependent on feed management. If shrimps are fed 4 - 5 times per day and all the feed is consumed within 30 minutes, the required stability will be only one hour (Fig. 1).

7

FEED MANAGEMENT

This is one of the most important factors in determining the profitability of a shrimp farm. Because the high input costs is related to feeding shrimp and the effect of the feed on the culture conditions, water and substrate quality. Shrimps are nocturnal and continuous intermittent feeders and this behaviour dictates the feed management strategy. They should be fed several times rather than once and the major portion of the ration should be given at night when they are most active. Recommended feeding rates, feeding times and quantity of feed to be given are dealt below.

Feeding methods for shrimps

a. *Feeding area*

- Pond < 1 ha, feed around the ponds.
- Pond > 1 ha, feed along the sides and centre (Using boat or mechanical feeder).

b. *Feeding monitoring*

- Number of Feeding Trays : 4 - 6/1 ha pond.
- Dimension of Feeding Trays : 1.0 x 1.0 m or 0.7 x 0.7 m.

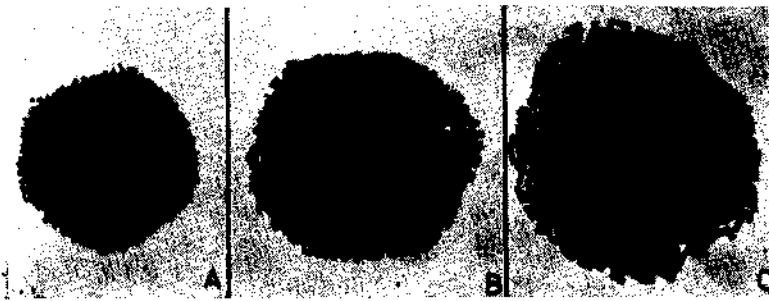


Fig. 2. The three recommended shrimp feed types : A. Starter, B. Grower and C. Finisher.

c. *Feed monitoring schedule*

Day of culture	Average body weight (g)	Amt. of feed % of biomass	% of feed put per feeding tray*	Feed tray monitoring time (hr)
1 - 7	0.02 - 0.4	20.0 - 17.0	0.6	2.5
8 - 14	0.5 - 1.3	16.8 - 14.2	0.6	2.5
15 - 21	1.4 - 1.9	14.02 - 11.8	0.8	2.0
22 - 28	2.0 - 2.7	11.6 - 9.9	0.8	2.0
29 - 35	2.8 - 3.4	9.7 - 8.4	0.8	2.0
36 - 42	3.5 - 4.2	8.2 - 7.1	1.0	1.5
43 - 49	4.3 - 5.1	6.9 - 6.1	1.0	1.5
50 - 56	5.2 - 6.0	6.0 - 5.3	1.0	1.5
57 - 63	6.2 - 7.0	5.2 - 4.6	1.0	1.5
64 - 70	7.2 - 8.1	4.5 - 3.9	1.0	1.5
71 - 79	8.3 - 9.3	3.8 - 3.4	1.2	1
78 - 84	9.5 - 10.8	3.3 - 3.0	1.2	1
85 - 91	11.0 - 12.5	2.9 - 2.7	1.2	1
92 - 98	12.7 - 14.1	2.6 - 2.55	1.2	1
99 - 105	14.4 - 15.9	2.5	1.2	1
106 - 112	16.2 - 17.8	2.5	1.2	1
112 - 119	18.1 - 19.8	2.5	1.2	1

* This is workable for 4-6 feeding trays/1 ha pond.

Note : The above Table is a guideline only, further adjustment has to be done through feed tray monitoring.

d. *Feeding times and % of daily ration at each feeding*

Time	% of daily ration
0600	20
1000	10
1400	10
1800	30
2200	30

e. *Point of feeding adjustment*

Feed consumption	Point/Feeding tray
Fully consumed	3
< 10% Excess	1
> 10% Excess	0

Feeding monitoring (Points) Feed rate adjustment

15 - 19	+ 10%
11 - 14	+ 5%
8 - 10	Maintain
4 - 7	- 5%
0 - 3	- 10%

Determination of feeding rates

a. *Average body weight (ABW)*

- to be done weekly
- Weight samples (at least 100 shrimps to obtain ABW).

b. *Estimated survival*

- by net-casting and also depending on the experiences of the farmer.

c. *Feeding rate as % of body weight/day*

Shrimp size (g)	Supplementary feeding (%)	Complete feeding (%)
0 - 3	10.0 - 4	15 - 8
3 - 15	4.0 - 2.5	8 - 4
15 - 40	2.5 - 2	4 - 2

d. *Feeding guide*

Size/weight (g) of prawn	% of feed to body weight to be fed/day	No. of feedings/day
<1	20 - 15	3
1	15	4
2	12	4
3	9	4
4	7	4
4 to 6	7 - 5	4-5
6 to 8	5 - 4	4-5
8 to 10	4 - 3	4-5
10 to 12	3 - 2.8	4-5
12 to 15	2.8- 2.5	4-5
15 to 17	2.5	4-5
17 to 20	2.5	4-5
>20	2.5	5

e. *Formulations to determine feeding rates*

- i. Stocking no. X survival rate X Av. Body weight = Biomass.
- ii. Biomass X % Body weight feed per day = Amount feed per day.

Feed management should be regulated by feed consumption. Shrimp appetite will vary according to environmental conditions *i.e.* water quality, sunny/overcast days and physiological conditions such as disease and moulting. Feed consumption may be monitored by the use of feeding trays (Fig. 3), the number of which varies with the farmer's ability. Location of trays is important and areas near or before aerators should be avoided. As feed consumption may vary within a day due to environmental factors or condition of shrimps, feed consumption should be monitored daily.

Properly managed feeds will improve shrimp production and increase profitability. Overfeeding reduces profits and increases mortalities as the uneaten feed pollutes water and substrate quality, and increases the food conversion ratio.

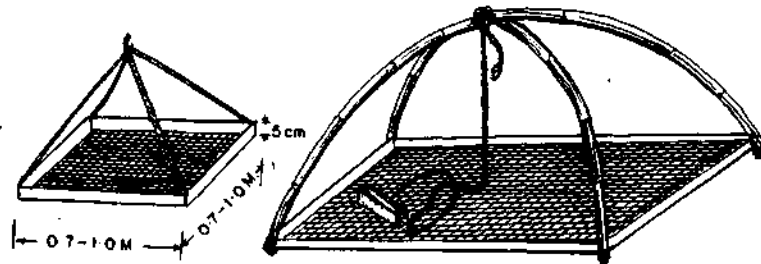


Fig. 3. Feeding trays used for feeding shrimps.

The feed conversion ratio depends on both feed management and feed quality. If properly managed, the FCR will be low (better) even with inferior quality feed.

8

FEED STORAGE

Proper feed storage is essential to maintain feed quality as well as to avoid any loss due to insect and rodent infestation. Some guidelines to maintain feed quality are as follows :

1. Store in dry, cool and well-ventilated area.
2. Do not store directly on concrete floors or in contact with concrete walls. Stacking of bags on wooden pallets as given in Fig. 4.
3. Keep away from direct sunlight.
4. Feeds should not be stored for more than 3 months.
5. Ensure First-in First- out management.
6. Spoiled or old feed should not be used, as the economic loss of feeding shrimp with spoiled or old feed may be greater than discarding the feed.

c. *Feeding rate as % of body weight/day*

Shrimp size (g)	Supplementary feeding (%)	Complete feeding (%)
0 - 3	10.0 - 4	15 - 8
3 - 15	4.0 - 2.5	8 - 4
15 - 40	2.5 - 2	4 - 2

d. *Feeding guide*

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<1	20 - 15	3
1	15	4
2	12	4
3	9	4
4	7	4
4 to 6	7 - 5	4-5
6 to 8	5 - 4	4-5
8 to 10	4 - 3	4-5
10 to 12	3 - 2.8	4-5
12 to 15	2.8- 2.5	4-5
15 to 17	2.5	4-5
17 to 20	2.5	4-5
>20	2.5	5

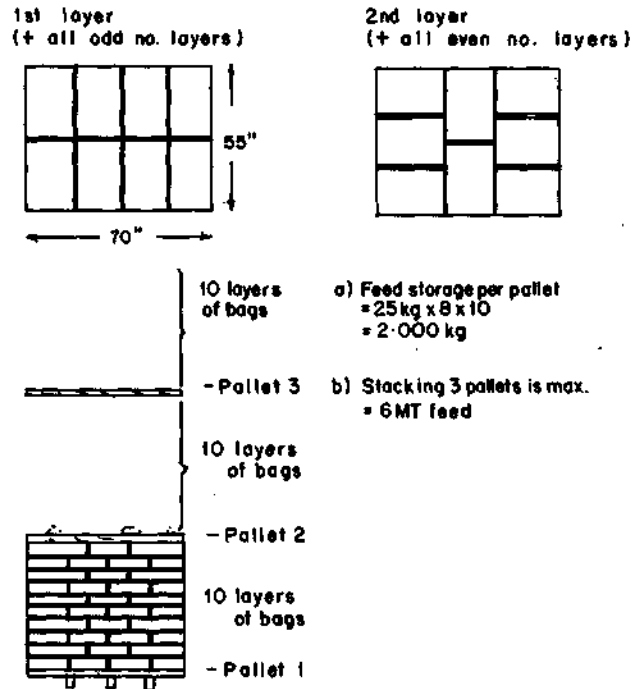
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FEEDING PRINCIPLES

1. Give less quantity of feed, but in higher frequency *i.e.* more number of times/day.



Pallets to be placed in pairs with a 2 ft ventilation space between pairs.

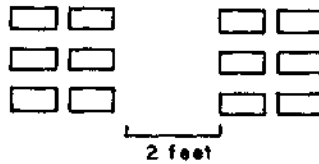


Fig. 4. Scheme of stacking feed bags on wooden pallets.

2. Feed less when the pond water is more fertile. Feed more in reverse.
3. Feed less per meal by increasing feeding frequency when the temperature of water exceeds 32° C. Feed more in good weather.
4. Feed less or temporarily stop feeding during heavy rain.
5. Feed less on the day when mass moulting occurs and increase moderately two days after moulting.
6. Difference in size of shrimps is because of lack of feed for long periods. Increase feed quantity when water quality is good.
7. When water quality is poor, improvement of water quality should be done in priority and at the same time feeding quantity decreased or stopped temporarily if mortality takes place.

10

SELECTION OF HEALTHY POST-LARVAE

Tips to identify healthy Post-larvae

1. Activeness : Place some PL (about 100) in a basin, stir the water and check. Healthy PL will swim against the current. Weak PL will gather at the centre.
2. Even size : Healthy prawns in uniform sizes are the results of good management. Uneven size may be due to :
 - a. Different stages
 - b. Under feeding
 - c. Disease, and
 - d. Poor water quality.

3. Disease free :
- | <i>Action</i> | <i>Rationale</i> |
|---|--|
| a. Check if larvae are in red colour. | Caused by expansion of chromatophores it indicates stress in the larvae. |
| b. Any chronic or acute mortality. | Indicative of advanced disease stress. |
| c. Any limb or appendage necrosis. | Indicative of bacterial infection. |
| d. Check for fouling organism, e.g. Zoothamnium | Indicative of poor water quality overfeeding. |
| e. Check hepatopancreas. | Brownish - normal; whitish - disease problem. |
4. Gut : Full gut indicates good health, empty gut indicates possible stress problem.
5. Moulting : Moulting indicative of nutritional disorders related to sterol, phospholipid or calcium/phosphorous.
6. Pigmentation :
- | | | |
|-----------|------------------|-------------|
| a. Shape | radiating strong | |
| | finger like | - healthy |
| | Round shape | - unhealthy |
| b. Colour | | |
| | Red | - healthy |
| | Yellow | - average |
| | Blue | - unhealthy |
7. Muscle development : The ratio of width of gut and body depth below the gut at the 6th segment - healthy PL should be 1 : 4 or > 4 (Fig. 5)

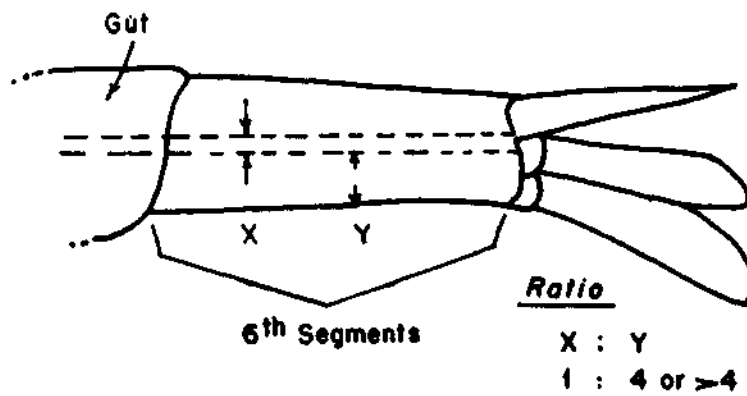


Fig. 5. The ratio of width of gut and body depth in shrimp.

8. Use of Antibiotic results in:
 - a. With - PL shorter, fatter and darker in colour.
 - b. Without - PL longer, thinner and lighter in colour.
9. Stress test : Lower temperature to 20° C in short period (< 10 minutes). Strong healthy PL will survive.
10. External features : For a healthy PL, the antennal scales of head portion are close together whereas the tail fan is widely opened.

CMFRI SPECIAL PUBLICATIONS AND BULLETINS

I. SPECIAL PUBLICATIONS

Spl. Pub. No.	Title	Year	Price	
			Indian Rs.	US \$
1	2	3	4	5
1	Pearl culture training : Long-term and short-term course.	1977	5	2
2*	Mariculture research and developmental activities.	1978	26	10
3	Summer Institute in breeding and rearing of marine prawns.	1978	20	5
4	Economics of the indigenous fishing units at Cochin : A case study.	1978	5	2
5	Seminar on the role of small-scale fisheries and coastal aquaculture in integrated rural development. Madras, 6 - 9 December 1978. Abstracts.	1978	10	5
6	Proceedings of the first workshop on Technology Transfer in coastal aquaculture held at Cochin, 23 & 24 July and Mandapam, 27 & 28 July 1979.	1979	15	5
7*	Manual of research methods for crustacean biochemistry and physiology.	1981	70	25
8*	Manual of research methods for finfish and shellfish nutrition.	1982	131	45
9	Manual of research methods for marine invertebrate reproduction.	1982	40	15
10	Analysis of marine fish landings in India : A new approach.	1982	10	5
11*	Approaches to finfish and shellfish pathology investigations.	1983	54	20
12*	A code list of common marine living resources of the Indian seas.	1983	80	25
13*	Application of genetics in aquaculture.	1983	50	15
14*	Manual of research methods for invertebrate endocrinology.	1983	63	20
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