A SIMPLIFIED HATCHERY TECHNIQUE FOR MASS PRODUCTION
OF PENAEID PRAWN SEED USING FORMULA FEED

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ABSTRACT

A technique for large-scale production of penaeid prawn seed using a dry
microparticulate formula feed, NPCL-17, is described. The details of preparing the
feed which is compounded from inexpensive, locally available raw materials such
as groundnut oil cake, fish meal, dried mantis shrimp, prawn waste and tapioca
and fortified with vitamins and minerals are given. The larvae are grown in outdoor
tanks filled with 2 m³ of seawater, filtered through 60-micron mesh nylon stocking
cloth. The daily ration of formula feed is offered in 4 equal instalments at 6
hourly intervals and the larval tanks are vigorously aerated. Apart from providing
nutrition for the larvae, the feed, under the influence of sunlight, helps to create
a natural ecosystem conducive for their survival. The easy-to-dispense dry feed
has greatly simplified the larval rearing procedures and has given an average sur-
vival rate of 66.7% from nauplius to postlarva. The rationale of the feeding sche-
dules and the particle size of the feed offered are discussed in the light of the
changes taking place in the functional morphology of the feeding appendages
during larval development.

INTRODUCTION

In the context of increasing global demand for prawns and the stagnation
of their production from the existing capture fisheries, most of the developing
countries in the tropical region have given high priority for promoting prawn
culture in impounded coastal waters and are engaged in setting up hatcheries to
provide the prawn culturists with adequate supply of prawn seed. Ever since
Hudinaga (1942) succeeded in rearing penaeid prawn larvae under laboratory
conditions, several workers have evolved more sophisticated procedures to in-
crease the survival of the larvae in culture systems (See Muthu 1982). It is well
recognised that the key factor in successful rearing of penaeid larvae is the
provision of appropriate feed for the larvae at the various stages of development
(Cook and Murphy 1966; 1969; Aquacop 1977; Mock et al 1980). The expen-
sive procedures involving high skill and technology needed for developing and
maintaining cultures of suitable live-feed organisms such as diatoms, brine
shrimp larvae, rotifers, cladocerans, nematodes, etc., have been the major con-
straints in large-scale production of prawn seed for aquaculture purpose.
Recently several substitute inert feed stuffs and feed formulations have been tried to overcome this difficulty. In small-scale rearing experiments Hirata et al (1975) used powdered soy cake to rear the larvae of *P. japonicus*, while Villegas and Kanazawa (1980) were successful with a particulate compounded diet. Shigueno (1975) reported that powdered formula feed could be used with fair results in rearing the larvae of *P. japonicus*. Jones et al (1979) reared the larvae of the same species to PL1 stage on microparticulate and microencapsulated diets. Hameed Ali (1980) and Alikunhi et al (1982) used bleached crustacean tissue suspensions prepared out of *Mesopodopsis* sp., *Metapenaeus dobsoni* and *Squilla* sp. to rear the penaeid larvae on a large scale.

Realising the importance of large-scale hatchery production of prawn seed for coastal aquaculture the Central Marine Fisheries Research Institute, Cochin, initiated work on different aspects of spawning and rearing of penaeid prawns under controlled conditions at their Narakkal Prawn Culture Laboratory (NPCL). Among the various research projects undertaken at this laboratory with the objective of simplifying the larval rearing procedures for mass production of prawn seeds, special attention was given to development of microparticulate larval feeds using locally available inexpensive raw materials. The results of this study concerning the larval rearing of the Indian white prawn *Penaeus indicus* H. Milne Edwards are reported here.

**Material and Methods**

The equipment and infrastructural facilities available at the NPCL were fully utilised for conducting these experiments. Besides standard laboratory equipment, the facilities included oil-free compressed air grid, filtered-seawater supply system, mass cultures of phytoplankton and other live feed organisms, portable plastic pools, brood-stock maintenance facility, growout ponds of varying sizes and glasshouse. The seawater used in the laboratory was pumped from the sea into large indoor tanks where it was allowed to settle for two days and then filtered through 60-micron nylon cloth. Even after filtration this seawater usually contained diatoms to the extent of 600-2000 cells/ml. The larvae used for these experiments were obtained from the routine prawn-breeding programme of NPCL using farm-grown spawners of *P. indicus*, induced to mature in the broodstock facility.

**Preparation of formula feed NPCL-17**

Locally available inexpensive raw materials such as prawn waste (a waste product of the prawn-processing industry consisting of prawn heads, appendages and peeled cuticle), mantis shrimp (a usually discarded by-catch of the trawlers), groundnut oil cake, fish meal and tapioca were used as main ingredients. The above materials were dried and powdered separately in a micropulveriser, fitted with 150-micron sieve, to form a fine powder. A 'feed base' was first prepared by thoroughly mixing these powders in the following composition.
Dried prawn waste .......... 25.0%
Dried, whole mantis shrimp .. 25.0%
Groundnut oil cake .......... 37.5%
Fish meal .................. 12.5%

Dried tapioca, powdered in the same micropulveriser, served as binder and carbohydrate source. For every 100 g of the feed base 20 g of tapioca powder was used. The feed was fortified with vitamins and minerals. To every 100 g of the feed base the following were added. Vitamin A (as acetate) USP 2000 I.U., thiamine mononitrate I.P. 5 mg, riboflavin I.P. 1 mg, nicotinamide I.P. 5 mg, pyridoxin hydrochloride I.P. 1.5 mg, calcium pantothenate USP 5 mg, cyanocobalamin I.P. 0.5 mg, ascorbic acid I.P. 25 mg, calciferol I.P. 200 I.U., DL-alpha tocopherol acetate 0.75 mg, biotin 0.025 mg, magnesium phosphate (dibasic) 24 mg, manganese hypophosphite 0.3 mg, dried ferrous sulphate 5.3 mg, calcium phosphate 0.104 g, calcium lactate 0.2 g, potassium dihydrogen phosphate 0.1 g.

The ingredients were mixed together in the proportion given above and blended with 40% by weight of water to obtain moist granules. They were kept in trays and steamed in a cooker for 10-15 minutes. After cooking, the cooked material was made into a dough, extruded through a pelletiser using a 3-mm die and dried in a hot-air oven at 65-70°C for 12 h. The dried pellets were powdered in micropulveriser using a 50-micron sieve and stored in air-tight polyethylene containers at room temperature.

The proximate composition of the formula feed NPCL-17 is as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>36.8%</td>
</tr>
<tr>
<td>Lipids</td>
<td>10.1%</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>29.8%</td>
</tr>
<tr>
<td>Ash</td>
<td>18.9%</td>
</tr>
<tr>
<td>Moisture</td>
<td>4.4%</td>
</tr>
</tbody>
</table>

Laboratory experiments in glass beakers

Initially, indoor laboratory experiments were carried out in glass beakers in order to determine whether P. indicus larvae could be reared through the various stages of development exclusively on a formulated diet. One litre of specially filtered (20 micron filter) seawater of 35 ppt salinity was taken in each beaker and 50 numbers of P. indicus obtained from the same brood were introduced. Continuous aeration was provided in each beaker through air stones and the experiment was carried out in ambient room temperature, which varied from 29.0 to 30.5°C. Three different feeding schedules were adopted in the beakers and the treatments were replicated thrice in a randomised block design. Feeding of the larvae was carried out once a day at the rates as follows:
Treatment Feed Feeding rate
1 No feed
2 Mixed diatom culture dominated by Chaetoceros affinis 80-100 x 10³ cells/ml 
3 Formula feed NPCL-17 0.16 mg/larva/day 

The daily ration of formula feed given in Treatment 3 was identical with the quantity of feed used in similar experiments with P. japonicus by Hirata et al (1975) and Villegas and Kanazawa (1980). The rearing medium was changed every day, when all the larvae were counted and staged under a binocular microscope. Since all the larvae did not metamorphose into the next stage at the same time, the stage of development of the larvae (S) in each beaker was expressed as:

\[ S = \frac{D}{\text{Total number of larvae in beaker}} \]

Where \( D = \sum \) Number of larvae in each stage times arbitrary value of that stage. For this an arbitrary value was assigned to each stage of larvae as follows:

<table>
<thead>
<tr>
<th>Stages</th>
<th>Arbitrary value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nauplius (N)</td>
<td>0</td>
</tr>
<tr>
<td>Protozoa-1 (P₁)</td>
<td>1</td>
</tr>
<tr>
<td>Protozoa-2 (P₂)</td>
<td>2</td>
</tr>
<tr>
<td>Protozoa-3 (P₃)</td>
<td>3</td>
</tr>
<tr>
<td>Mysis-1 (M₁)</td>
<td>4</td>
</tr>
<tr>
<td>Mysis-2 (M₂)</td>
<td>5</td>
</tr>
<tr>
<td>Mysis-3 (M₃)</td>
<td>6</td>
</tr>
<tr>
<td>Postlarva-1 (PL₁)</td>
<td>7</td>
</tr>
</tbody>
</table>

Larval rearing in outdoor pools using formula feed NPCL-17

Fifteen experiments were carried out in 2 m³ capacity circular plastic-lined pools kept inside a glass house in bright sunlight (50000 to 120000 lux). The pools were disinfected, cleaned and filled with seawater on the day the prawns were expected to spawn. The water in the pools was vigorously aerated. The hatched-out nauplii were stocked in the rearing pools at stocking densities varying from 6 to 12 N/l of seawater. Although the nauplius is a non-feeding stage 2-3 g/m³ of formula feed were added to the pool on the day of stocking itself. The dosage of formula feed was increased to 4 g/m³/day during the protozoal stage and to 5 g/m³/day in the mysis stage. The daily dose of feed was divided into four equal portions and added to the pool at 6 hourly intervals. By the time the larvae reached P₁ stage, the phytoplankton originally present in
the seawater medium, increased to 17000 to 42000 cells/ml. When the sunlight was intense the phytoplankton concentration often reached 80000 to 100000 cells/ml and under such circumstances the dosage of formula feed was reduced. It would appear that the unutilized formula feed in the medium was broken down by bacteria, thereby providing nutrients for development of phytoplankton under the influence of sunlight.

The larvae were examined under the microscope in the morning and evening every day to see if they were feeding well. From P1 stage onwards 1/3 volume of water from the pool was siphoned out through a filter box (300 micron mesh) and replaced with fresh seawater. Vigorous aeration was maintained throughout the period of rearing to promote aerobic decomposition of the sediments. The experiments were carried out in ambient temperatures varying from 25.4 to 34.6°C.

**RESULTS**

**Laboratory experiments in glass beakers**

The survival and development of the larvae in the beaker experiments are shown in Fig. 1 and Fig. 2 respectively. For each treatment the values plotted represent the averages of the three replicates. In the beakers where no feed was given all the larvae died after the second day in P1 stage indicating that the larvae, having exhausted the yolk reserves in nauplius stage, required energy from external sources of food at this stage.

![Survival of P. indicus larvae on different feed treatments in 1-L beakers.](image-url)
In phytoplankton-fed cultures (Treatment 2), however, the development of the larvae followed a normal sequence and the survival was as high as 90% up to M₃ stage, which they attained on the 7th day. It is obvious that up to this stage the feed provided was nutritionally adequate for their normal growth and survival. The survival rate, however, declined sharply to 48% between M₃ and PL₁, indicating that the phytoplankton alone was not sufficient beyond M₃ stage.

![Graph showing development of P. indicus larvae during different feed treatments in 1-L beakers.](image)

In Treatment 3, using formula feed, mortality at each larval stage was significant and the development was delayed, the PL₁ stage having been attained in 10 days, i.e., two days later than in Treatment 2. Besides, the survival rate of 12.5% at PL₁ stage was also relatively low indicating the formula feed by itself is a complete food for the larvae. Nevertheless, the fact that 12.5% larvae developed and survived up to the PL₁ stage on this diet alone showed that they were able to utilise this formula feed to a limited extent for their survival. The experiments in large outdoor pools were planned on the basis of evaluation of these results.

Larval rearing in outdoor pools using formula feed NPCL-17

These experiments were conducted with the objective of utilising the formula feed in combination with the phytoplankton production naturally taking
place in seawater in the presence of sunlight. The 15 sets of experiments making use of 921000 N\textsubscript{1} to produce 614000 PL\textsubscript{1} of *P. indicus* gave an average survival rate of 66.7\%. The survival rate in individual experiments, however, ranged from 32.2\% to 96.8\% (Table 1). The development period from N\textsubscript{1} to PL\textsubscript{1} ranged from 7 to 8 days in the warmer months and 9 to 10 days in cooler months.

When the recorded survival rates are plotted against stocking densities (Fig. 3) there seems to be no correlation between them. At the highest stocking density tried during the present experiment (52 N\textsubscript{1}/l) the survival rate was 92.3\% whereas at the lowest stocking density (6 N\textsubscript{1}/l) the survival rate was 66.7\%. The lowest survival rate of 30.2\% was obtained in the experiment in which stocking density was 26.5 N\textsubscript{1}/l. The usual inverse relationship between stocking density and survival rate was not evident at the stocking densities tried. This may become relevant at higher stocking densities. In other words the system can sustain stocking densities higher than 52 N\textsubscript{1}/l.

It is interesting to note that the quantity of formula feed added to the pools, when calculated in terms of the amount of feed per larva per day, did not show any relationship to the survival rate of the larvae at the level of population densities employed (Table 1). Therefore, it was obvious that the larvae had also made use of other food from the medium (i.e., the phytoplankton which developed naturally in the pools) in addition to the formula feed provided. Periodic microscopic examination of the larvae showed that their gut contents consisted chiefly of greenish substance particularly in the early protozoal stages and, in addition, the formula feed was also seen in gut of the larvae from the P\textsubscript{2} stage onwards. That the larvae have been ingesting the formula feed was evident from the long fecal trails they developed 10 minutes after introduction of the feed and the good survival of the larvae was clear indication of its assimilation by the larvae. Since the pools were set up outdoors exposed to sunlight.
and since the medium was not sterilized the normal primary and secondary production process continued to take place in the medium, thereby providing a natural ecosystem for the prawn larvae.

**DISCUSSION**

The experiments conducted during this study have given an insight into the role played by microparticulate feeds in larval rearing of penaeid prawns. The exclusive use of formula feed NPCL-17 in the beaker experiments using specially filtered seawater resulted in poor survival and delayed development of the larvae, indicating that the feed by itself is not adequate for the larvae. But the same feed when used in the large pools exposed to sunlight gave high survival rates (average 66.7%) and the duration of larval development was normal. In the latter case, the phytoplankton which was observed to develop in the larval rearing pools under the influence of sunlight has been an additional source of nourishment to the larvae. It was possible that the unutilised particles of the formula feed might have also acted as organic fertilizer stimulating the growth of the phytoplankton. It is interesting to note that Hirata et al. (1975), Hameed Ali (1980) and Alikunhi et al. (1982), who reared penaeid larvae on particulate diets in outdoor tanks exposed to sunlight, have reported the presence of good diatom concentrations in their larval rearing containers. The success of their rearing methods is possibly due to the additional nutrition provided to the larvae by the diatoms growing in the medium. The point to be emphasised here is that the particulate diets and tissue suspensions may not be effective as larval feed when they are grown indoors under poor light conditions.

Rearing of penaeid larvae in outdoor tanks in which natural growth of phytoplankton is stimulated by adding chemical nutrients is, no doubt, closely similar to the technique of rearing them in outdoor tanks with particulate feed. But the former method is however impracticable for large-scale commercial rearing in hatcheries due to the uncertainty in development of phytoplankton in the rearing media. Regulating the growth of phytoplankton consisting of desired species at optimum concentrations in larval culture media has been found to be very difficult under conditions obtaining in hatcheries. It is in this context that the success of larval rearing using formulated dry particulate feeds assumes importance.

The size of the food particles offered to the larvae seems to have profound influence on the success of rearing penaeid larvae. NPCL-17 was dispensed in the form of micropulverised powder obtained after passing through a sieve of 50-micron mesh. The particle size of the conventional species of diatoms offered as natural feed of penaeid larvae fall within 5 to 20 microns. Chaetoceros affinis which was the dominant diatom in the seawater at Narakkal was 5-8 microns in size. Jones et al. (1979) found that the average size of particles ingested by the larvae of *P. japonicus* increased from about 10 microns in *P.* to
**Table 1. Mass rearing of *P. indicus* larva in outdoor pools using formula feed.**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Dates</th>
<th>Duration in days</th>
<th>Temperature range ºC</th>
<th>Stocking density N/1</th>
<th>Population No. x 10³</th>
<th>Quantity of feed added mg/larva/day</th>
<th>Percent survival rate N₁ to PL₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4-3-82 to 12-3-82</td>
<td>8</td>
<td>30.0-32.5</td>
<td>35.5</td>
<td>71</td>
<td>66</td>
<td>64</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>8</td>
<td>30.0-32.5</td>
<td>42.5</td>
<td>85</td>
<td>73</td>
<td>52</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>8</td>
<td>29.5-32.1</td>
<td>22.5</td>
<td>45</td>
<td>35</td>
<td>27</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>8</td>
<td>29.0-32.0</td>
<td>32.0</td>
<td>64</td>
<td>49</td>
<td>47</td>
</tr>
<tr>
<td>5</td>
<td>21-3-82 to 29-3-82</td>
<td>8</td>
<td>31.0-33.0</td>
<td>6.0</td>
<td>12</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>26-3-82 to 3-4-82</td>
<td>8</td>
<td>31.0-34.0</td>
<td>20.0</td>
<td>40</td>
<td>39</td>
<td>30</td>
</tr>
<tr>
<td>7</td>
<td>29-4-82 to 6-5-82</td>
<td>8</td>
<td>32.0-34.6</td>
<td>25.0</td>
<td>50</td>
<td>41</td>
<td>35</td>
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<tr>
<td>8</td>
<td>27-11-82 to 5-12-82</td>
<td>8</td>
<td>28.0-31.5</td>
<td>26.5</td>
<td>53</td>
<td>38</td>
<td>28</td>
</tr>
<tr>
<td>9</td>
<td>1-12-82 to 10-12-82</td>
<td>9</td>
<td>27.3-29.8</td>
<td>36.0</td>
<td>72</td>
<td>63</td>
<td>58</td>
</tr>
<tr>
<td>10</td>
<td>4-12-82 to 13-12-82</td>
<td>9</td>
<td>27.4-31.4</td>
<td>45.0</td>
<td>90</td>
<td>53</td>
<td>41</td>
</tr>
<tr>
<td>Date</td>
<td>Container</td>
<td>Temperature (°C)</td>
<td>Salinity (ppt)</td>
<td>pH</td>
<td>Calcium (mg/L)</td>
<td>Magnesium (mg/L)</td>
<td></td>
</tr>
<tr>
<td>----------</td>
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<td>------------------</td>
<td>----------------</td>
<td>----</td>
<td>----------------</td>
<td>------------------</td>
<td></td>
</tr>
<tr>
<td>5-12-82</td>
<td>9</td>
<td>27.2-29.9</td>
<td>15.5</td>
<td>31</td>
<td>30</td>
<td>30</td>
<td>0.19-0.26</td>
</tr>
<tr>
<td>10-12-82</td>
<td>9</td>
<td>26.7-31.4</td>
<td>25.0</td>
<td>50</td>
<td>43</td>
<td>31</td>
<td>28</td>
</tr>
<tr>
<td>5-1-83</td>
<td>9</td>
<td>25.4-28.9</td>
<td>25.0</td>
<td>50</td>
<td>41</td>
<td>38</td>
<td>32</td>
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<tr>
<td>21-1-83</td>
<td>10</td>
<td>26.0-29.1</td>
<td>52.0</td>
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<td>101</td>
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<td>96</td>
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<tr>
<td>21-1-83</td>
<td>10</td>
<td>26.0-29.1</td>
<td>52.0</td>
<td>104</td>
<td>100</td>
<td>95</td>
<td>91</td>
</tr>
</tbody>
</table>

Container 2 ton plastic pool; Salinity range 33.00-34.95 ppt; pH range 7.82-8.57
about 28 microns in M3 stage. While rearing the larvae with particulate diets
the frequency of feeding is to be given due consideration, as excess of left-over
food in the medium causes pollution of water resulting in mortality. Delayed
feeding also produces disastrous effects. These problems can be overcome by
continuous aeration of the medium and by adding the daily ration in installments
at periodic intervals. Accordingly, in the present experiments, the daily ration
was divided into 4 equal parts and dispensed at 6-hourly intervals. Hirata et al
(1975), Jones et al (1979 and Villegas and Kanazawa (1980) have given the
daily ration of particulate feed in a single dose. Hameed Ali (1980) and Alikunhi
et al (1982) have dispensed the daily ration of wet tissue suspension in 5-h
intervals.

Very high survival rates up to M3 stage have been recorded in the indoor
experiments conducted in 1-m³ capacity pools on an exclusive diet of mixed
phytoplankton without using any zooplanktonic elements. This confirms the obser­
vations of Muthu (1982), who showed that the feeding appendages of protozoea
and mysis of penaeids are adapted only for filter feeding. In these stages the
numerous long setae and the close-set setules of the second maxilla form an
effective filtering device for seiving out the phytoplankton cells from the sea
water. The mandibles are weak and the first three pereopods have only rudimen­
tary chelae which are incapable of grasping moving prey. When Mt meta­
morphoses into PLi, the maxillary filter is abruptly lost, the serrated teeth on
the mandible are replaced by a sharp cutting edge and the chelae on the first
three pereopods become functional. The postlarva ceases to be a filter feeder
and becomes capable of capturing and dealing with larger food organisms thereby
transforming into an effective carnivore. That there is a sudden change in the
feeding habits when M3 metamorphoses into PLi is also confirmed by the
experiments of Jones et al (1979), who demonstrated through accumulative
particle size analysis of microcapsules in the medium, that the mean particle
diameter of the microcapsules increased after the larvae had fed on them during
the protozoea and mysis stages, while it abruptly declined during the postlarva
stage. The sudden decline in survival rate from Mt to PLi observed in our
beaker experiments in which the larvae were fed only with mixed phytoplankton
cultures (Fig. 1) also support the view that the feeding habits change abruptly
at the time of metamorphosis. Emmerson (1980), using pure cultures of Thala­
ssiosira weissflogii as feed, has shown that the filtration rate of the larvae of P.
indicus increased steadily from 2500 cells/larva/h in Pl stage to 12,000 cells/
larva/h in Mt stage and then suddenly declined to 6,000 cells/larva/h in early
PLi stage. He also attributed the reduced filtration rate of the early PLi stage
to the reduction in the size of the filter-feeding appendages at this stage. In the
light of these observations the conventional practice of feeding zooplanktonic
organisms to the prawn larvae during the mysis stage should be critically re­
examined. Since the larvae at this stage are not physically equipped to capture
moving prey organisms, large quantities of the costly Artemia nauplii that are
given as feed for the early mysis stage larvae may be wasted, although those accidentally coming into contact with the larvae may be retained by the coarser setae on the maxillipedes and eaten. It is therefore suggested that the introduction of zooplanktonic feed in larval tanks can be delayed till the M stage is about to metamorphose into PLI. At the NPCL, however, the postlarvae are fed on coarser particles of the formula feed passed through a 300-micron mesh sieve.

In comparison with the methods of rearing penaeid larvae extant in various countries the new method using dry formulated feed has the following advantages:

1. Elaborate arrangements for developing and maintaining liv-feed cultures are not required. This will result in considerable savings in space, labour and equipment, thereby reducing the cost of production of prawn seed.

2. Rearing procedures are greatly simplified so that even unskilled labourers with a little training can manage the hatchery operations.

3. Formula feed is prepared out of inexpensive raw materials through simple processes requiring no specialised equipment.

4. The feed can be prepared in large quantities in advance and stored in airtight containers to be used off the shelf whenever necessary, whereas the commonly used live feeds and wet tissue suspensions will have to be freshly cultured or processed each time.

5. The dry particulate feed can also be dispensed through automated feeding devices in sophisticated hatchery systems.

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REFERENCES


