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Live feed research for larviculture of marine finfish and shellfish

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Introduction

The major expansion of marine finfish and crustacean aquaculture since 1980s around the world can be attributed to the development of standard techniques for mass production of live feed. Even though most farmed marine animals are either carnivorous or omnivorous from their post-larval stage, microalgae are required for larval nutrition during a brief period either for direct consumption or indirectly as food for live prey fed to small larvae. The hatchery production of penaeid shrimp post-larvae depends on the use of live diatoms for the early stages and *Artemia* for later stages. Globally the hatchery production of juveniles of marine finfish is achieved by the use of rotifers and *Artemia.* Microalgae are also routinely used in the 'green water technique' employed for marine finfish larviculture. Most marine finfishes have altricial larvae and when yolk sac is exhausted, they remain in an undeveloped state. The digestive system is rudimentary, lacking a stomach and much of the protein digestion takes place in the hindgut epithelial cells. Altricial larvae cannot digest formulated feed and hence live feed is vital for their survival. Live feed organisms are able to swim in the water column and are thus constantly available to the larvae. The movement of live feed in water stimulates larval feeding responses. Live feed organisms with a thin exoskeleton and high water content may be more palatable to the larvae when compared to the hard formulated diets.

The development of culture methods for copepods as live feed, studies on green water techniques with different microalgae in larviculture, nutritional enrichment of rotifers for use as live feed in larviculture, isolation of new strains of microalgae, experiments on microalgal mass cultures, use of macro live feed in lobster larviculture, use of copepods in finfish larviculture and fatty acid profiling of enriched rotifers were the major aspects of research carried out by CMFRI under the X plan project on live feed.

Copepods as live feed

It is well understood that small-sized live feed with sufficient DHA, EPA and ARA is the key factor for the success of larval rearing of marine fishes with altricial larvae including groupers and many ornamental fishes. Improved growth, survival and

normal pigmentation have been documented for several marine finfishes reared with copepods. One of the major advantages of copepods is the wide range of body sizes both within and between species. The larvae that hatch from copepod eggs, are nauplii (NI) which develop through 5 or 6 moults before passing into the copepodite stages. The early stage nauplii and copepodites are extremely useful as initial prey for species with larvae having small mouth gape.

Two species of copepods *viz.,* the harpacticoid *Euterpina acutifrons* and the calanoid *Pseudodiaptomus serricaudatus* were selected based on their small size for the study. The size details were as follows:

Pseudodiaptomus serricaudatus

Adults	:	643 – 728 µm
NI	:	50 - 65 µm
Last stage nauplius	:	185 - 190 µm
Copepodites	:	200 -514 µm

Euterpina acutifrons

Adults	:	485 - 571 µm
NI	:	41 - 52 µm
Copepodites	:	285 - 571 µm

Culture of the calanoid copepod *Pseudodiaptomus serricaudatus*

Experiments on culture of *P. serricaudatus* were conducted in two-tonne capacity tanks. Initially green water was developed in the tank by adding sufficient quantity of *Nannochloropsis* culture cell counts ranging from 3 x 10^5 cells / ml to 5 x 10^5 cells / ml. Adult copepods were introduced into the green water.

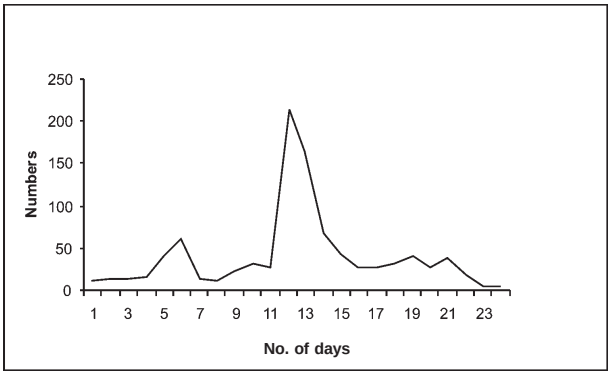

Fig. 1. Daily counts of non-egg bearing copepods

Green water was replenished daily so as to maintain the cell count throughout the period of experiment. Daily counts were taken on the number of non-egg bearing copepods, egg bearing copepods, nauplii and copepodites (Fig. 1-4).

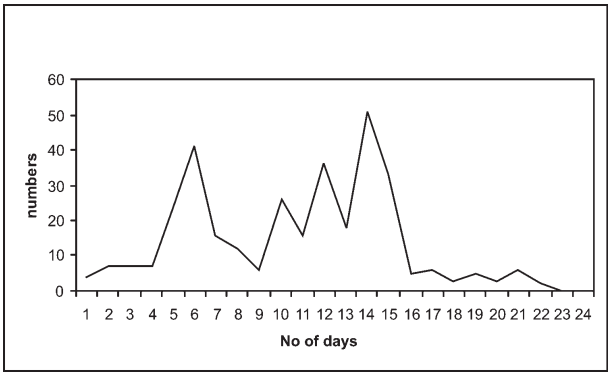

Fig. 2. Daily counts of egg bearing copepods

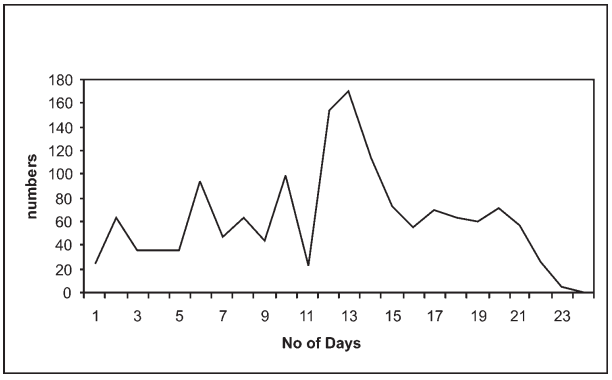

Fig. 3. Daily counts of nauplii

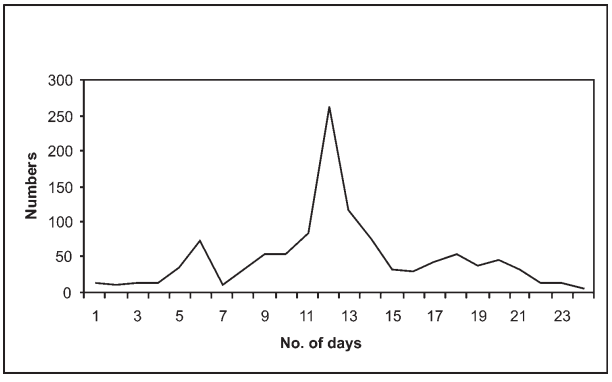

Fig. 4. Daily counts of copepodites

Mixed culture of *P. serricaudatus* and *E. acutifrons*

Since larviculture by employing mixed culture of copepods was found to be more successful as experiments were conducted on mixed culture of the

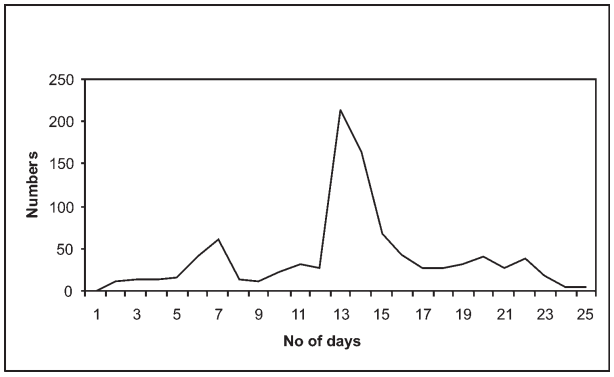

Fig. 5. Daily counts of non-egg bearing copepods

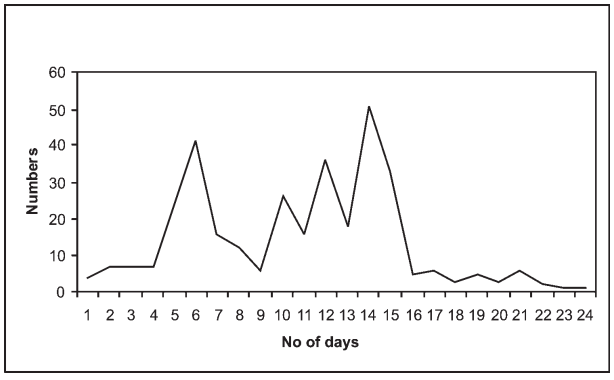

Fig. 6. Daily counts of egg bearing copepods

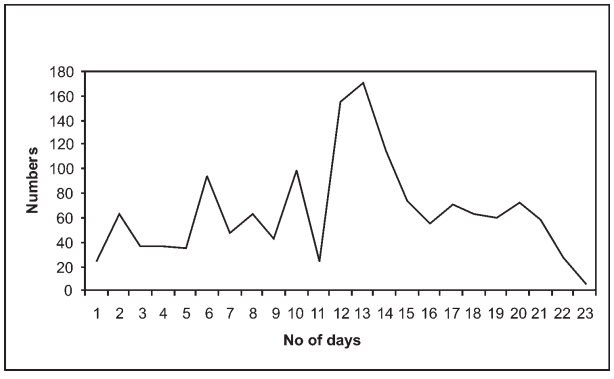

Fig. 7. Daily counts of nauplii

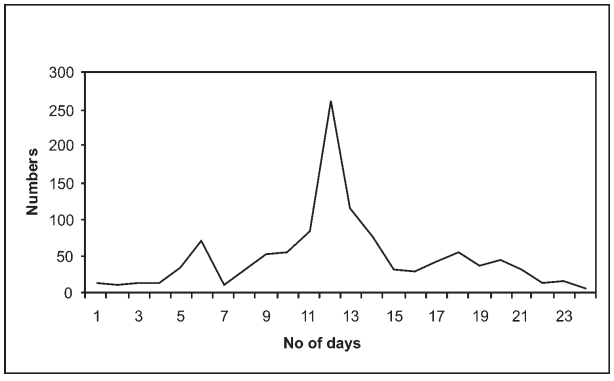

Fig. 8. Daily counts of copepodites

two species selected *viz., P. serricaudatus* and *E. acutifrons*. The methodology was same as that employed for the culture of *P. serricaudatus.* Daily counts of different stages recorded during the experiment are given in Fig. 5-8.

Larviculture experiments using copepods as live feed

It was found that co-culturing of copepods in green water in the larviculture tank is the most effective method of larval rearing of marine finfishes. Initially green water was developed in larval rearing tanks (5 tonne capacity FRP tanks) by adding sufficient quantity of *Nannochloropsis* culture so as to get a cell count ranging from 3×10^5 cells / ml to 5×10^5 cells/ml. Adults of *E. acutifrons* and *P. serricaudatus* were introduced into the tanks. When the copepods have started their growth phase as can be noted by counting the number of egg-bearing copepods, nauplii and copepodites per 50 ml, newly hatched larvae were introduced into these tanks.

Experiments on the larviculture of five species of ornamental fishes *viz., Dascyllus trimaculatus, D. aruanus, Pomacentrus caeruleus, Neopomacentrus nemurus* and *Chromis viridis* were conducted by this method. The number of adult copepods, egg bearing copepods, nauplii and copepodites per 50 ml of the larviculture tanks during the initial phase of successful larval rearing experiments for the above five species were recorded.

In *Dascyllus aruanus,* the number of non-egg bearing copepods, egg bearing copepods, nauplii and copepodites per 50 ml in the first phase of larviculture ranged from 7–152, 1-109, 3-273 and 12–173 respectively. In *D. trimaculatus,* the number of non-egg bearing copepods, egg bearing copepods, nauplii and copepodites per 50 ml in first phase of larviculture ranged from 22-109, 7-97, 35-203 and 37-163 respectively. In *Pomacentrus caeruleus,* the number of non-egg bearing copepods, egg bearing copepods, nauplii and copepodites per 50 ml in first phase of larviculture ranged from 21-263, 7-41, 23-132 and 17-73 respectively. In *Neopomacentrus nemurus,* the number of non-egg bearing copepods, egg bearing copepods, nauplii and copepodites per 50 ml in first phase of larviculture ranged from 11 – 230, 3-41, 24 – 171 and 2-262 respectively. In *Chromis viridis,*

the number of non-egg bearing copepods, egg bearing copepods, nauplii and copepodites per 50 ml in the first phase of larviculture ranged from 5 – 61, 1-62, 21-116 and 6 - 41 respectively.

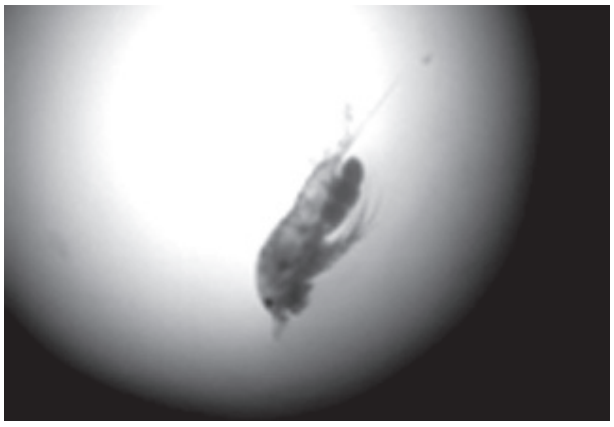

Fig. 9. Adult - *Euterpina acutifrons*

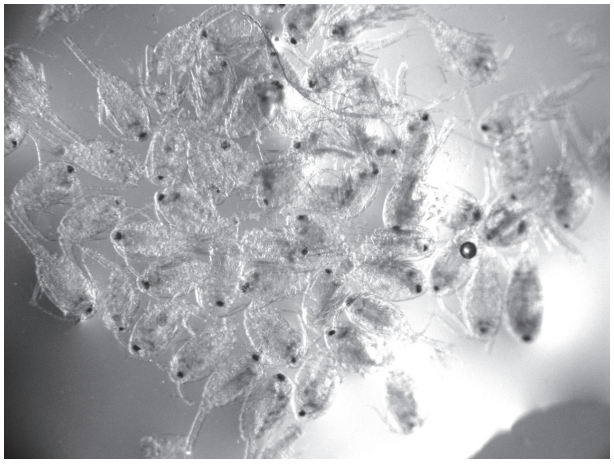

Fig. 10. *P. serricaudatus* in their mass culture

Fig. 11. Nauplius of *P. serricaudatus*

Larviculture of honey comb grouper, *Epinephelus merra*

Successful larviculture of honey-comb grouper was achieved by the green water technique and pseudo green water techniques and by first feeding with the nauplii of *P. serricaudatus* and *E. acutifrons* during the first fifteen days of larval culture. The nauplius concentration maintained in the larval rearing tanks ranged from 4 – 13/ 50 ml and the copepodite concentration ranged from 2 to 11/50 ml during the first fifteen days.

In all the larviculture experiments, it was found that the survival of larvae during the first fifteen days was directly proportional to the density of nauplius / copepodite stages in the rearing tanks.

Introduction of rotifers along with copepods in the larviculture tanks

One experiment each on the larviculture of *D. trimaculatus* and *D. aruanus* was conducted by supplementing rotifers along with copepods from the end of first week of larviculture. It was noted that within 2-3 days, the rotifers bloomed in the rearing tanks resulting in the depletion of copepods. In both the experiments, complete mortality of larvae was noted.

Nutritional enrichment of live feed

Enriched rotifer for larviculture of the clownfish *Amphiprion ocellaris*

(i) Rotifer (100-150 nos./ml) enriched with *Chlorella salina* (60-70 x 10^6) showed 45% of larval survival from 0 to 15 day of post-hatch.

(ii) Rotifer (100-150 nos./ml) enriched with *Nanochloropsis occulata* (60-70 x 10^6) showed 68% of larval survival from 0 to 15 days post-hatch.

(iii) Feeding the larvae of clown fish with rotifer (100-150 nos./ml) enriched with *C. salina*

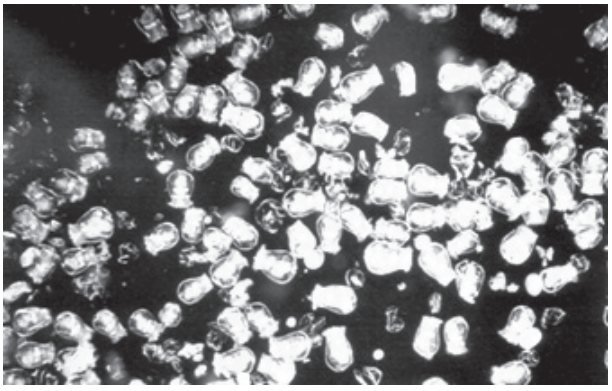

Fig. 12. Rotifer enriched with cod liver oil

(60-70 x 10^6) and *N. oculata* (60-70 x 10^6) in 1:1 proportion showed 80 to 85% larval survival from 0 to 15 days post-hatch.

(iv) Larvae fed with Rotifer (100-150 nos./ml) enriched with cod liver oil alone did not support larval survival. However larvae fed with oil enriched rotifer along with green algae *N. oculata* (60-70 x 10^6) and *C. salina* (60-70 x 10^6) showed 35 to 40 % larval survival.

Influence of different greenwater systems on larval rearing of the clownfish *A. percula*

Experiments on influence of different green water systems on larval rearing of *A. percula* using *Chlorella* sp., *Nanochloropsis* sp. and their combination in 1:1 proportion showed that

(i) use of *Chlorella salina* (60 x 10^6) as green water immediately after hatching to 3rd day of post-hatch gave 50 to 60% larval survival.

(ii) use of *Nanochloropsis* (60 x 10^6) as green water immediately after hatching to 3rd day of post-hatch gave 60 to 70% larval survival.

(iii) use of *Nanochloropsis* (60 x 10^6) and *Chlorella salina* (60 x 10^6) in 1:1 proportion as green water immediately after hatching to 3rd day of post-hatch gave 80 to 90% larval survival.

Influence of green water on larval rearing of blue damsel

Effect of microalgae on the feeding of blue damsel larvae was studied. The feeding intensity was calculated by observing number of rotifers in the gut of larvae reared in various algae. It was observed that larvae reared in *Nanochloropsis* showed slight increase in the feeding intensity when compared to other treatments.

Rotifer enrichment for crab larviculture

Rotifers were enriched with 'algamac' (commercial product) @ 300 mg/million of rotifers for 6-12 h. These enriched rotifers were fed to crab larvae. No noticeable improvement in the zoeal development of marine crab (*Portunus sanguinolentus*) was noted as compared to the non-enriched rotifers.

***Artemia* enrichment for lobster larviculture**

Artemia cysts (OSI-Brand) were hatched out and subjected to enrichment over a period of 12 – 24 h using different emulsions –

a) Raw sardine oil suspension extracted from boiled and pressed sardine meat was used as enrichment medium @ 1 ml / 100 ml, 2 ml / 100 ml and 3 ml / 100 ml.

b) A combination of fish meal powder and fine rice bran (1 : 9) was fermented over a period of 24 – 48 h. The liquid, filtered through 20 µ mesh cloth was used.

c) OTC (20 ppm) – treated and fed for ciliate infection

d) Spirulina mixed in water

e) A combination of spirulina and cod liver oil.

f) Cod liver oil suspension @ 1 ml in 100 ml seawater.

g) Clam meat suspension in *Nanochloropsis* medium.

Feeding experiments showed that raw sardine oil suspension produced good results when fed to *P. rugosus* phyllosoma, while feed reception was poor with cod liver oil. A combination of spirulina and cod liver oil proved to be good for spiny lobster.

P. homarus phyllosoma (stages I and II) also showed good feed reception when fed with clam meat suspension in *Nanochloropsis* medium, but there was an increase in the incidence of ciliate infections. Feed reception of scyllarid and spiny lobster larvae to OTC enriched *Artemia* was poor.

Fatty acid profile of rotifer enriched with *Chlorella* sp.

Samples of rotifers grown on *Chlorella* was harvested and profiled for fatty acids. As reported by earlier workers the polyunsaturated fatty acid (PUFA) content of rotifers grown exclusively on phytoplankton is low.

Microalgae

Isolation of new strains

Few new strains of microalgae were isolated and maintained in the stock culture room at Cochin. They

included *Chlorococcus,* marine *Spirulina*, *Chaetoceros, Nannochloropsis, Synecococcus* and *Anabaena* from Andaman Nicobar Island, *Nannochloropsis* from Cochin, *Chaetoceros* from Cochin, one species belonging to Chrysophyte group and *Chlorococcus* isolated from the backwaters of Cochin and maintained in marine enriched medium in pure form.

Experiments on mass culture of microalgae and their use in larviculture

Two sets of experiments were conducted each to increase the cell concentrations in the mass culture systems and also to increase the culture duration. Instead of giving the nutrients in a single dose, partial supply of nutrients were tried and found that the culture period can be extended by this method but the cell concentrations were found reduced.

Apart from these, different types of modified harvest methods were studied to lengthen the growth phase, especially the steady phase of the cultures with maximum cell concentrations. Partial harvest followed by transfer of the entire culture to freshly prepared tanks was found the best among the different systems studied.

Macro live feed

Ctenophore culture and enrichment for lobster larviculture

Ctenophores collected from the wild were maintained and reared in glass aquaria. The ctenophores were weaned to a diet of chopped clam/ shrimp/fish meat, which was found to be accepted readily. Consumption rates and food conversion were found to be good with clam meat. Assimilation of fatty tissue into radial canals was found to take place very fast. The enriched ctenophores were chopped before feeding the lobster larvae. Initial trials were done in *Petrarchus rugosus* larvae, which however, did not show good reception. The same when fed to PIII larvae of *Thenus orientalis*, were found to be accepted readily.

Under high saline conditions ctenophores can grow and multiply very fast when grown in large cement tanks with no direct sunlight, good zooplankton density and water column height (>2 m). However, the foraging plankters have to be introduced in large quantities. The results obtained in cement tanks of 4 x 2 x 12 m dimensions were encouraging. Scyllarid phyllosoma in advanced stages of development, when released into these tanks, were found to cling on to the medusae and swim around, thus confirming theories regarding the dispersal and feeding strategies employed by the phyllosoma.

Fig. 13. Comb jelly reared in large cement tanks

Conclusion

It is quite obvious that live feed is indispensable in marine larviculture and hence live feed research is an integral part of finfish and shellfish seed production. Research should be intensified on technologies for high density and stable production of suitable live feed. The identification of causes for sudden crashes in culture and remedial measures for the same requires priority attention. Genetic manipulations for the production of super small strains of rotifers and the commercial production of rotifer cysts are also major areas for intensified research. The production of high density microalgae in photobioreactors and by fermentation techniques is a potential area for future development. The copepod culture techniques, which is the key to the success of rearing of altricial larvae of marine finfish still remains to be standardized. Hence this should receive more focused research than the better studied traditional live feeds. Advances in live feed research can pave the way for the commercialization of seed production technologies for many species of marine finfish and shellfish in future years.