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Influence of algal diet on population density, egg production and hatching succession of the calanoid copepod, *Paracalanus parvus* (Claus, 1863)

N. Jeyaraj* and P. Santhanam
Department of Marine Science, School of Marine Sciences, Bharathidasan University, Tiruchirappalli-620 024, Tamil Nadu, India.
***Corresponding author: n_jeyaraj@yahoo.com**

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Keywords Microalgae, copepod *Paracalanus parvus*, Population density, Egg hatching rate

Abstract

Aquaculture is the most significant growth component of the Indian fisheries industry interest in marine fish culture has dramatically increased with the recent failures in the commercial fishery. The main bottleneck for fry production in the marine fish is associated with larval feeding. Many commercial marine finfish species that are raised through their entire life cycle are unable to grow if fed exclusively on formulated diets during their first developmental stages. It is well accepted that, many copepods are a valuable source of food for fish larval rearing although they are not often used in aquaculture industry. There has been no experimentation to measure the effect of algal diets for the aquaculture of copepods. Therefore, a series of experiments were conducted with *Paracalanus parvus* to effect of algal food type on population density, fecundity and egg hatching succession. We used six types of algal diets such as *Chlorella marina*, *Dunaliella* sp., *Isochrysis galbana*, *Nannochloropsis* sp., *Tetrasilmis* sp. and *Skeletonema costatum* for the present study and the experiment was lasted for 12 days at optimum temperature 25 ± 1°C and salinity 30 ± 1 ‰. The triplicates were made for each treatment. The result showed that the highest population density (1374 ± 76.33 ind./lit) was obtained in *P. parvus* which fed with *I. galbana* followed by *C. marina* (1013 ± 65.35 ind./lit), *Tetrasilmis* sp. (990.3 ± 86.55ind./lit), *Nannochloropsis* sp. (769 ± 52.50 ind./lit), *Dunaliella* sp. (431 ± 34.8 ind./lit) and *S. costatum* (258 ± 36.26). Further the algal diet also had a significant effect on egg production and hatching rate, though the highest egg hatching % (95.64 ± 2.72) was recorded in *P. parvus* fed with *I. galbana* diet. Whereas the lowest (26.60 ± 1.00) was recorded in *S. costatum*. The present study clearly indicated that, the diet *I. galbana* was the best feed for the successful culture of copepod, *P. parvus* in large scale. The short generation time and high reproductive potential make the use of this copepod as promising as live feed in aquaculture practices

Introduction

Many marine fishes produce small pelagic eggs. Larvae hatched from small eggs require a source of live food very soon after the onset of exogenous feeding (Schipp 2001). Most of the commercial species are reared using rotifers and *Artemia* nauplii since they can be cultured in large quantities at high densities. Unfortunately, using rotifers and *Artemia* during this early period in life history does not always promote optimal larval growth since these live preys may contain an inadequate fatty acid profile and, in some cases, be of an inappropriate size (Sargent *et al*. 1999; Olivotto *et al*. 2003). Because of this, there is a need for identification of alternative food sources. Several lines of reasoning suggest that copepods have potential as an alternative food resource in larval fish mariculture that might replace rotifers and *Artemia* (or) both (Cutts 2002). Copepods are an important food source for many developing larvae, post larvae and juvenile fish and crustaceans (Sun and Fleeger 1995). When provided as a first feed, copepod nauplii promote development and improve the survival rate of marine finfish larvae (Toledo *et al*. 1999). Additionally, the distinctive swimming behaviour of copepods is believed to

stimulate stronger foraging responses in some fish larvae, resulting in improved ingestion rates (Stottrup 2000). Mass culture of several marine copepods has been attempted to utilize them as live foods in marine finfish cultures (Støttrup *et al*. 1986; Sun and Fleeger 1995; Støttrup and Norsker 1997; Schipp *et al*. 1999; Payne and Rippingale 2001; Santhanam 2002 and Rajkumar 2006). Copepods for aquaculture purposes have been fed a variety of microalgae species. These microalgae have been fed alone or combined. Each microalga has a different nutritional content and cell size and little is known about the nutritional requirements of copepods. The effects of various dietary microalgae have on culture parameters have been evaluated with many copepods including *Acartia tonsa* (Støttrup and Jensen 1990; Støttrup *et al*. 1999; Kleppel and Burkart 1995), *Gladioferens imparipes* (Payne and Rippingale 2000), *Tisbe biminiensis* (Pinto *et al*. 2001), *Acartia omorii* (Shin *et al*. 2003), *Acartia sinjiensis* (Knuckey *et al*. 2005), *Pseudodiaptomous euryhalinus* (Cruz *et al*. 2009), *Temora stylifera* (Buttino *et al*. 2009) and *Pseudodiaptomus pelagicus* (Ohs *et al*. 2010).

In India works on related to experimental biology of copepod very meager, James and Thompson (1986) have experimented the culture of brackishwater cyclopoids, (Santhanam 2002 and Rajkumar 2006) have successfully cultured the cyclopoid copepod, *Oithona rigida*, and calanoid copepods *Acartia clausi*, *A. erythraea*, to date, no published information is available as to the optimum microalgal diet conditions for the culture of *P.parvus*. Identification of an optimal microalgal diet, which is trouble-free to culture the copepod, is paramount to the successful culture of these organisms, especially as the scale of production increases. Through a series of laboratory experiments, the present study examined which microalgae species increased the survival, population density and fecundity of cultured populations of *P. parvus*.

Materials and methods

Stock cultures of microalgae

Algal species such as *Chlorella marina*, *Dunaliella* sp., *Isochrysis galbana*, *Nannochloropsis* sp., *Tetrasilmis* sp. and *Skeletonema costatum* stock cultures were obtained from Central Institute of Brackishwater Aquaculture (ICAR, Govt. of India, Chennai) and Central Marine Fisheries Research Institute (ICAR), Cochin. All the species of algae were grown at 25 °C, 30 ‰, and 14 L: 10 D light regimes and fertilized with f/2 medium (Guillard and Ryther 1962). The algae were harvested during the log phase (approx. 35,000 cells/mL) for feeding to the copepods.

Stock cultures of copepods

Copepod samples were collected from the coastal waters of Muthupet (Lat. 10^{o} 20'N and Long 79^{o} 35'E) during early in the morning using plankton net with 158µm mesh. The collected samples were provided with vigorous aeration by using battery aerators and the samples immediately transported to laboratory and thoroughly rinsed to reduce the contamination from other zooplankters. From the samples, *P. parvus* were identified under microscope using the standard key (Kasturirangan 1963). After the conformation, 200 numbers that including male and female *P. parvus* were isolated and stocked separately in an oval shaped, flat-bottomed fiberglass tank (0.54m dia, 0.81m length) filled with 100 litres of filtered (1µm) seawater and gentle aeration was given. The water quality parameters such as temperature, salinity, pH and dissolved oxygen were maintained in the ranges of 26 - 30 ^{o}C; 28 - 32 ‰; 7.5 - 8.5; 5.0 - 7.5 ml/l respectively (during rearing period) fed with a daily ration of mixed algae *viz*., *C. marina*, *Dunaliella* sp., *I. galbana*, *Nannochloropsis* sp., and *S. costatum* in the concentration of 20,000 cells/ml. The copepod cultures were harvested at every 12 (*P. parvus*) days by gentle siphoning. The generation time of *P. parvus* is about 10 - 12 days under optimal conditions at 26 - 30 ^{o}C and having 6 naupliar and 6 copepodite stages including the adult. Finally the adult male and female copepods were used to restart stock culture. Water quality parameters such as temperature, salinity, pH, Dissolved oxygen and the population density and survival of copepod were observed daily. For biological observations triplicate samples of 1ml was taken and counted for the different stages of copepods for average results.

Experimental design and setup

For the experiment on effect of algal type on copepod survival, ten individuals were maintained separately in each bowl for each feed. They were transferred daily to a new bowl with freshly filtered seawater and feed. The daily mortality was recorded carefully. The experimental sets were maintained at 28 ± 2 ^{o} C and 30 ± 1 ‰ till the death of all animals. The separate experiment was maintained to examine the effect of algal type on population density, egg production and hatching success of *P. parvus*. All experiments were carried out using 1000 mL glass bowl that were placed under similar culture conditions as described for the stock cultures. For both experiments, there were six diet treatments with triplicates for each treatment was followed. The six algal diet *viz*., *C. marina*, *Dunaliella* sp., *I. galbana*, *Nannochloropsis* sp., *Tetrasilmis* sp. And *S. costatum* concentrations were determined daily using a haemocytometer to count the number of cells per ml under a microscope. For all treatments and throughout the experiments, feeding ration of all algae in cells/ml were based on their equivalent to optimal feeding levels of ash free dry weight (AFDW) ml - 1 as recommended by (Knuckey *et al*. 2005). The feeding rations of the algal diets used in this experiment were 20,000 cells/ml. At the beginning of the population density experiment,

10 adult *P. parvus* were stocked into each triplicate culture glass bowl. Adult copepods were separated from the stock culture by draining culture water through a 180 µm sieve. The copepods caught on the mesh were then immediately placed in a petri dish with a small amount of seawater. Individuals were randomly captured using a fine-tipped pipette and transferred to the

experimental bottles. For entire experimental period (12 days), the copepods were fed the designated diets daily, and approximately 50 % culture water was exchanged daily using a siphon with a 38 µm mesh attached to the end to prevent removal of the copepods. After 12 days, all the contents of each replicate bottle were drained through a 38 µm sieve and the total number of nauplii, copepodites and adults of *P. parvus* retained were fixed in 5 % formalin solution. The counting of *P. parvus* nauplii, copepodites and adults were made using a Sedgewick Rafter counter and a high-powered microscope.

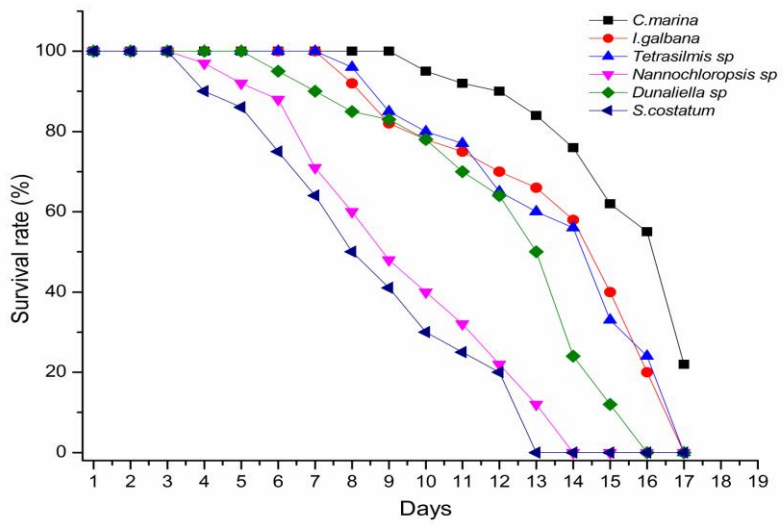
For the egg production and hatching experiment, the male and females of copepods were isolated and kept in beakers containing filtered seawater and incubated for 24 hours. After that egg were drained onto a 38 µm sieve, which were then rinsed and placed in fresh water for 2.5 min to kill all post-egg-stage copepods. The eggs were then returned to seawater and the number of eggs per replicate was counted under a high powered microscope before being placed in 2 ml of seawater in sealed specimen containers for incubation under identical conditions (12 h:12 h light:dark cycle, 28 ± 1 °C and salinity 34 ± 1 ppt). Based upon pilot trial, a incubation period of 48 h was selected. After 48 hr, the number of unhatched eggs per replicate was counted and the hatching rate (%) was subsequently.

Results

Survival rate of *P.parvus* in different algal diet

The survival of *P.parvus* recorded in the present study was not significantly different among the diets treatments. The 100% survival was noted for about 3-9 days at *C.marina*. In *C. marina*, 100% survival occurred up to 9th day, 62% survival observed on 15th day and total mortality occurred on 17th day onwards. wereas *Nannochloropsis* sp. and *S.costatum* resulted the poor survival than the rest of the feed types, where 100% survival was observed up to 3nd day only, after that survival decline to 41% and 48% on 9th day respectively and complete mortality observed on 13th and 14th day onwards (Fig.1).

Figure 1. The survival rate (%) of *Paracalanus parvus* fed at six different microalgae diets. under identical condition of 25 ± 1 °C, 30 ± 1 ppt and photoperiod 12 L : 12 D.



Egg production and egg hatching rates

A significant difference in egg production was detected within each diet treatment. The maximum egg production of 37.33 ± 2.51 eggs/female/day was achieved at *I. galbana* feed which was more than six times higher than that of the *S. costatum* (5.0 ± 1.0 eggs/female/day) the mean percentage of egg production was significantly different among the diets (pb 0.3341) among treatments The highest mean hatching rate (35.66 ± 1.50) was recorded in *I. galbana* fed copepod and the lowest hatching rate (1.33 ± 1.15) was noted at *S. costatum* fed copepod. The maximum percentage of (95.64 ± 2.72) hatching was obtained in copepod fed with *I. galbana* whereas minimum percentage (26.60 ± 1.00) was noticed in *S. costatum* fed copepod (Table. 1).

Table 1. Mean±S.D. egg production, egg hatching rate and percentage of egg hatching for *P. parvus* cultured in fed the six treatment dietary microalgae

Algal diet	Egg production rate Eggs/female/day	Hatching rate	Hatching (%)
C. marina	26.33 ± 1.52	23.30 ± 1.52	88.58 ± 0.64
I. galbana	37.33 ± 2.51	35.66 ± 1.50	95.64 ± 2.72
Tetrasilmis sp.	19.66 ± 0.57	14.00 ± 1.00	71.14 ± 3.43
Nannochloropsis sp.	12.33 ± 1.15	7.33 ± 0.57	59.60 ± 5.15
Dunaliella sp.	8.33 ± 0.57	4.00 ± 1.00	47.68 ± 9.24
S. costatum	5.00 ± 1.00	1.33 ± 1.15	26.60 ± 1.00

Population density

Twelve days culture on copepod fed at different algal diets showed that the diet had a significant effect on the population density of *P. parvus*. Mean population density of *P. parvus* after being fed a designated diet was highest for the *I. galbana* with a final population density of 1374 ± 76.30 ind./l with nauplii (618), copepodites (534), adults (221) followed by *C. marina* (1013 ± 65.35ind./lit), *Tetrasilmis* sp. (990.3 ± 86.55ind./lit), *Nannochloropsis* sp. (769 ± 52.50 ind./lit), *Dunaliella* sp. (431 ± 34.8 ind./lit) and *S. costatum (*258 ± 36.26) total number of copepods (or) total population was significantly (pb0.005) different among the treatments (Fig. 2). Population density at *I. galbana* treatment was significantly higher than other algal diet treatments. The *S. costatum* diets produced the lowest population number at the end of the experiment. Fig. 3 showed a breakdown of different life-cycle stages of the *P. parvus* population fed at various algal diets for 12 days. It showed that there were substantial differences in the numbers of the three life-stage categories, *ie*, nauplii, copepodites and adults in different diet treatments. Most notably, the number of nauplii and copepodites was markedly higher for the *I. galbana* subsequent *C. marina* diet treatment whereas the nauplii production was very low for the *S. costatum* (Fig. 3).

Figure 2. Mean total population (including nauplii, copepodites and adults) (± SE) of *Paracalanus parvus* cultured on six different algal diets for12 days at temperature 25 ± 1 °C, salinity 30 ± 1 ppt and photoperiod 12 L:12 D

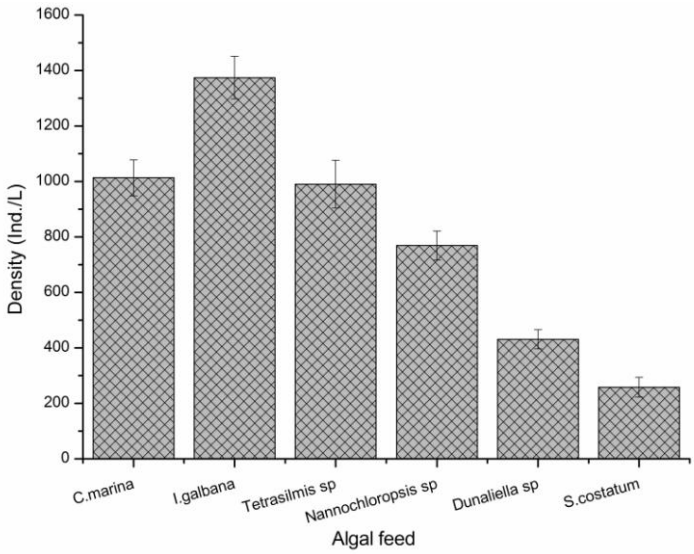


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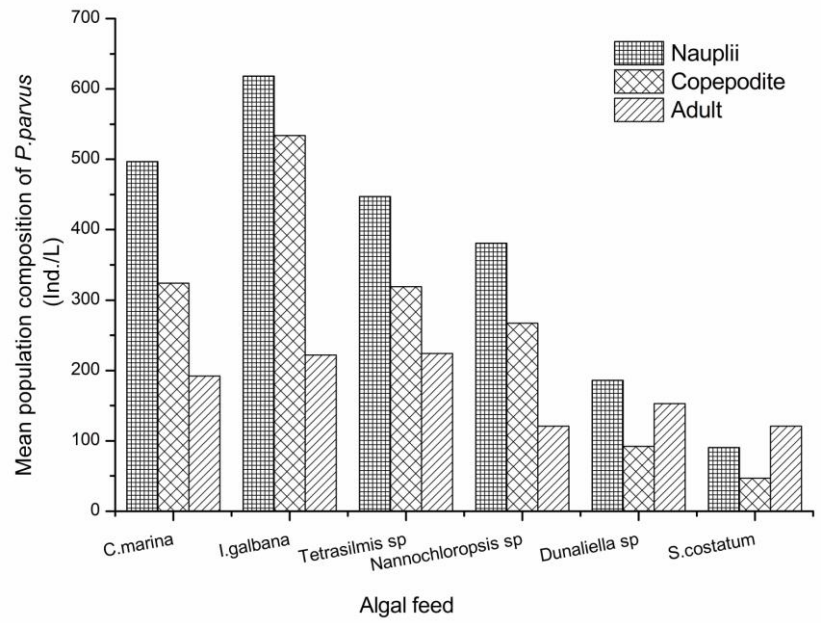
Figure 3. Mean number of life-stages (nauplii, copepodites and adults) within the population of *Paracalanus parvus* cultured for 12 days on six different algal diets at temperature 25 ± 1 °C, salinity 30 ± 1 ppt and photoperiod 12 L : 12 D.



Discussion

On the basis of different type of micro algae unaccompanied, all the algae used in the present study ought to be available for uptake by copepods, though the highest survival rate observed in *C.marina* fed copepod *P. parvus* and egg production rates and population density were observed in *I. galbana* fed *P. parvus* respectively. The efficacy of each algal diet differed in copepod, *P. parvus.* The aquaculture industry develops the need for higher density cultures of small prey items suitable for high-value finfish will increase. In these circumstances, the culture of small calanoid copepods such as the *P. parvus* of the present study may be enviable. The present result showed that the first-rate survival was observed in copepod fed with the diet of *C. marina* may be due to the favorable size of the prey (Shrivastava *et al*. 1999). *C. marina* is comparatively smaller in size than other type of feeds used in the present study (Fig.1). copepods had a less survival in *S. costatum* feed type may be due to the less consuming capability of copepods on chain forming diatoms and also the mouth parts of copepods are not facilitating the capture of larger food organisms and therefore presently low survival was found in *S. costatum* which is similar to earlier reports of (Perumal *et al*. 2000 and Ganf and Russell 1985). McKinnon *et al*. 2003) reported that larger algal species such as *Rhizosolenia alata* (16–23 Am wide by 150–260 Am long) were only available to CIV or later stages. For paracalanid copepod, the preferred prey size was >10 Am, though Paracalanus was able to take a 4.5-Am of *Isochrysis* throughout its life history (Paffenhofer 1984)

The findings of these studies have shown clear effects of diets on those parameters related to the productivity of copepods. Egg production is one of the principal factors determining population density in copepods, and it has been linked to the quality of diet (Uye 1981), in particular the EPA (eicosapentaenoic acid) to DHA (docosahexaenoic acid) ratio of the diet (Stottrup and Jensen 1990). EPA and DHA are highly unsaturated fatty acids (HUFAs), which are essential nutrients for most cultured fish and crustaceans as they are required for normal growth, development of the nervous system, and cell membrane functions (Anderson and De Silva 2003). Analysis of population density is probably more relevant to the ultimate goal of improving productivity of copepod culture for hatcheries because it provides a summary of the dietary effects on a range of inter-related parameters, including egg production, egg hatching rates, nauplii and copepodite development time and survival.

The present study showed that the, *I. galpana* fed copepod *P. parvus* produced the maximum mean population density it may be *Isochrysis* sp having high concentration of HUFA content. Watanabe (1991) reported that the haptophyte, *Isochrysis* sp. is a suitable food for all naupliar stages and small copepodid stages, which is rich in the HUFA content that will gave the best growth response in copepods. Similarly, reported that *Isohcrysis* have a high concentration of DHA and medium

to high amounts of n-3 fatty acid (Navarro *et al*. 1999). Meanwhile, the highest egg hatching rate (95.52%) was obtained from the diet of *I.galbana*. Of the many algal species that have been used in aquaculture, *Chlorella* and *Isochrysis* have been clearly successful as a food for rearing copepod species (Anraku and Omori 1963; Stottrup *et al*., 1986; Rippingale and Payne 2001; Cruz *et al*. 2009). The algal species has been successfully used as a single diet (Mc Kinnon *et al*. 2003) found that *Rhodomonas* supported high egg production rates of *A. sinjiensis* (up to 33 eggs/female/ day) while (Knuckey *et al*. 2005) found *Rhodomonas* supported higher development rates of *A. sinjiensis* nauplii than a binary diet of *Tetraselmis* and *Isochrysis.* Recently Ananth and Santhanam (2011) confirmed that *C.marina* supported higher development rates and population density of harpacticoid copepod, *Macrosetella gracilis*. In our study *C. marina* gave second highest population density than other diets. Similarly Lee *et al*. (2006) suggested that the *Tetraselmis* sp. and *Isochrysis* sp. diets could be the most favorable diet for mass culture of *P. nana.* A very recently Cruz *et al*. (2009) reported that *Chaetoceros muelleri* and *Isochrysis galbana* enhanced population in *Pseudodiaptomus euryhalinus*. Similarly, highest mean egg production while *I. galbana* as a feed in *A. tonsa* (Teixeira *et al*. 2010).

The present study indicated that diatoms *S. costatum* gave low density and hatching rate this may be due to chain forming phytoplankton and nutritional deficiency of the diet. McKinnon *et al*. (2003) reported that larger algal species may not prepare copepod it may be due to the less consuming capability. A number of studies have confirmed that copepods fed binary algal diets often exhibit higher fecundity compared to those fed monoalgal diets (Stottrup 1990; Rippingale and Payne 2001; Morehead *et al*. 2005; Lee *et al*. 2006). Payne and Rippingale (2000) suggested that a diet of *Isochrysis* sp. is likely to compensate on their essential fatty acid profile to achieve a balanced EPA to DHA ratio and thereby boost *G. imparipes* productivity. Although *I. galpana* has been shown to be the best among algal diets tested for egg production (Milione and Zeng 2007) in *Acartia sinjiensis*. Støttrup and Jensen (1990), who found that *A. tonsa* stopped producing eggs when fed with this microalga apparently because *Dunaliella* sp. is deficient in HUFAs the present study agreed with this report less amount of egg production were observed in *P. parvus* fed *Dunaliella* sp. Payne and Rippingale (2000) found that *Nannochloropsis oculata* supported very low nauplii development for *G. imparipes* as compared to other algal diets. Morehead *et al*. (2005) also found that *N. oculata* did not support population growth in *A.tranteri*. The futility of *N. oculata* as a monoalgal diet may relate to its lack of DHA and other HUFAs.

Conclusion

Finally, it is worth noting that, this study was conducted at laboratory scale over relatively short period of time. However, it clearly served the purpose of identifying the optimal microalgal diets for culturing copepod *P. parvus* which are likely to be applicable in larger-scale cultures. Based on the findings of this study, to achieve maximum productivity of *P. parvus* culture for aquaculture purposes, the species can be cultured using the *Isochrysis galbana* as feed.

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