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Growth performance and nutritional profile of a cyclopoid copepod *Oithona similis* isolated from Kochi, south west coast of India

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Original Article

Abstract

A series of experiments of each 25 days were conducted to evaluate the suitability of four microalgal diets for the culture of the tropical cyclopoid copepod *Oithona similis*. The mono-algal diets were *Chaetoceros calcitrans, Isochrysis galbana, Chlorella marina* and *Nannochloropsis oculata*. Present work was carried out in a Completely Randomized Design (CRD) with four treatments and three replicates. After feeding *O. similis* with the 4 algal diets for 25 days, population density of adults, copepodites, nauplii and egg bearers were determined. Density and population growth rate of all stages were the maximum when fed with *C. calcitrans* and it was confirmed as an excellent diet for *O. similis*. Growth performance as indicated by population density and growth rate was significantly ($P<0.05$) higher for all stages when fed with *C. calcitrans* compared to the rest of the diets. The biochemical profile of *O. similis* showed superiority in protein (55.6%), and lipid (33.4%) contents on feeding with *C. calcitrans.* Since the strain is cultivable with good nutritional profile and high survival rate, it gives an immense scope of high value larval feed for use in marine hatcheries. Based on the current results, it is suggested that among the diets tested, the diatom *C.calcitrans* was the best for enhanced production of all stages of *O. similis* in controlled conditions followed by *I. galbana*.

Keywords: *Tropical copepods, Oithona similis, growth rate, density, nutritional profile*

Introduction

The superiority of copepods as live feeds in marine fish larviculture has increased the interest towards controlled culture of copepods (Stottrup, 2003; Lee *et al.*, 2006). Copepods are superior among live feeds due to its acceptance, digestibility and movement in the water column and a very ideal biochemical profile that meets the requirement of the most marine fish larvae (Evjemo *et al.*, 2003; Van der Meeran *et al.*, 2008). Nutritional quality of copepods in larviculture is determined by larval growth rate, survival, pigmentation and successful metamorphosis (Holmefijord *et al.*, 1989; Naess *et al.*, 1995; Naess and Lie, 1998; Shields *et al.*, 1999). Use of copepods for marine fish larvae has reduced frequencies of skeletal deformities, improved larval pigmentation, survival and growth rate during larval and early juvenile stages (Shields *et al.*, 1999; Payne and Rippingale, 2000; Stottrup, 2000).

For marine fish larviculture, the cyclopoid copepod *Oithona* sp. can be used as live feed due to its abundance, ease of

culture and nutritional quality. The polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and the calcium content in *Oithona* sp has been reported as higher than that in *Artemia* and rotifer (Chilmawatia and Sumintoa, 2016). Using *Oithona* as feed in larvae have shown increased level of EPA and DHA composition in *Cromileptes altivelis* (Valenciennes 1828) (Aliah *et al*., 2010), growth and survival rate of *Hippocampus kuda* (Chilmawatia and Sumintoa, 2016), *Chanos chanos* (Forsskål, 1775) (Raj *et al*., 2003) and *Lates calcarifer* (Santhanam and Perumal, 2012). *O. similis* is one of the most important copepod species in the world (Gallienne and Robins, 2001) and it is reflected in its abundance, biomass and trophic role within the aquatic ecosystem (Fransz and González, 1995; Metz, 1996; Atkinson and Sinclair, 2000). During its whole life span, *Oithona similis* Clauss, 1866 is an important predator as well as an important prey organism. In contrast to the nauplii of many other copepod species, the ones of *Oithona* spp. start to feed immediately after hatching (Uchima and Hirano, 1986; Hirst and Ward, 2008). There is still only very little information on mass culture of *Oithona* sp. as live feeds in marine larviculture. It has been reported that there are still no ideal phytoplankton diets to culture *Oithona* sp. in mass condition (Santhanam and Perumal, 2012; Vasudevan *et al*., 2013).

The present study has evaluated the egg, nauplii and copepodites production and adult population growth of the cyclopoid copepod *Oithona similis* fed with monocultures of micro algae namely *Isochrysis galbana*, *Cheatocerous calcitrans*, *Nannochloropsis oculata* and *Chlorella marina*.

Material and methods

The experiment was conducted at the experimental marine hatchery of Central Marine Fisheries Research Institute (CMFRI), Kochi, Kerala, India following a Completely Random Design (CRD) with four treatments spanning 25 days for each treatment. The treatments were culture of *Oithona similis* fed with phytoplankters (i) *I. galbana*; (ii) *C. calcitrans* (iii) *N. oculata* and (iv) *C. marina*.

Phytoplankton culture

The phytoplankton *C. calcitrans* (Ehrenberg, 1844), *N. oculata* (Droop) (Hibberd, 1981), *C. marina* and *I. galbana* (Parker, 1949) chosen to feed *O.similis* were sourced from the Microalgal Culture collection at Central Marine Fisheries Research Institute (CMFRI), Kochi, Kerala, India. The algal species were cultured at 25±1ºC in 1-20L glass containers using f/2 medium (Guillard and Ryther, 1962) in seawater of salinity 30 ppt. and pH 8.2. Silicate was supplemented for the culture of the diatom *C. calcitrans*. The photoperiod was set at light: dark cycle of 12 h: 12 h. Microalgae in the exponential growth phase was used to feed *O. similis* because of the high nutrient content in that stage (Creswell, 2010). The density of microalgae (Nos. mL^{-1}) was calculated each day by taking a sample and counting under a microscope using haemocytometer (Improved Neaubouer volume 0.0025 mm^3).

Experimental setup

Four separate experiments were carried out to assess the influence of the four microalgae on *O. similis* culture productivity, *i.e.* the population density of adult and egg bearers, production of egg, nauplii, and copepodites over a 25 day culture period. Three replicates were maintained for each treatment.

Oithona similis culture

O. similis used for the present study was isolated from the wild and stock cultures were maintained in 1000 ml glass containers in CMFRI laboratory. Culture of *O. similis* for experiments was done for 25 days in 3 L glass containers filled with sterilized seawater of salinity 30 ppt and pH 8.5. Initial density was 1 individual mL^{-1} and consisted of all stages of *O. similis*. The feeding was done once in a day using 1-2 $\times$ 10^6 cells mL^{-1} of phytoplankton. Every 5th day, the culture water was fully changed by filtering the culture through a series of sieves of mesh size 500, 200 and 60 μm. The sieves of varying mesh size were used to collect the different stages of the copepod. The stages were examined and counted under light microscope (10X) using Sedgwick -Rafter zooplankton counting chamber.

The population density of adult and egg bearing copepods was determined by following Camus and Zeng (2008 & 2009). *O. similis* were acclimatized to all four diets for 3 days in 3 L flasks before being used for the experiment. After acclimatization period, fifty *O. similis* adults were introduced into each 1 L flask, for recording the population growth. Three replicates were kept for each treatment. Next 25 days, *O. similis* were fed daily with the designated diets (*I. galbana*; *C. calcitrans*, *N. oculata,* and *C.marina*) at a cell density of 1$\times$ 10^5 cells mL^{-1} and approximately 50% of the culture water was exchanged daily by gently siphoning through a sieve. Population growth was determined on day 5, day 10, day 15, day 20 and day 25.

For estimating nauplii and copepodite production, 50 adults of *O. similis* were randomly selected from the 3 L cultures and transferred into 12 one litre flasks and fed with the 4 experimental diets (3 replicates/ treatment). Nauplii and copepodites numbers were estimated for each microalgal diet by counting using a Sedgwick rafter cell counter and compound microscope at every 5 days intervals for 25 days. Average value of three subsamples (5 mL to 10 mL) taken from the each treatment was used to calculate the population growth rate (r) and density of various developmental stages of *O. similis*.

Population growth rate (r) was calculated using the data obtained for each treatment by following Krebs (1985) formula as reported by Cheng *et al.* (2011)

$$r = \frac{\ln Nt - \ln N_0}{t}$$

Where, t is the total duration of culture in days, N_0 and Nt are initial and the final density of *O.similis* (in d· mL^{-1}).

Egg production was calculated by using the formula from Zamora-Terol *et al.* (2014) as given below:

$$\frac{\sum s \times e}{\sum n}$$

Where, s is egg sac; e is average number of egg in one sac; and n is number of ovigerous female (ind).

Biochemical analyses

Mass culture of *O. similis* was carried out for biochemical analyses using the microalgal diets which gave better growth in previous experiments. The diets used were *C. calcitrans, I. galbana and N. occulata.* Cultures of *O. similis* in triplicate was maintained in 500 L FRP tanks provided with continuous aeration for 25 days. The copepod was fed with the algal diets at the rate of 1 x 10^5 cells ml^{-1}. For biochemical analyses, the cultures which contained all the stages of *O. similis* were filtered and excess moisture was removed using absorbent paper and was dried in a hot air oven at a temperature of 60° C to obtain constant weight. The dried triplicate samples were pooled together for analyses. Protein content was determined by following Lowry's method with bovine serum albumin (BSA) as standard (Lowry *et al.*, 1951). The phenol- sulphuric acid method of Dubois (Dubois *et al.*, 1956) was followed to estimate the carbohydrate content in the copepod sample and Bligh and dyer method (Bligh and Dyer., 1959) for estimation of total lipid.

Statistical analyses

Data were analysed using one-way ANOVA and whenever significant differences ($p<0.05$) were recorded, Tukey's multiple comparisons test was used to determine specific differences among treatments ($p<0.05$). All statistical analyses were conducted using SPSS program Ver. 16. Data are presented as mean±standard deviation (SD).

Results and discussion

The copepod *Oithona similis* fed with four mono algal diets for a period of 25 days was examined and growth in number

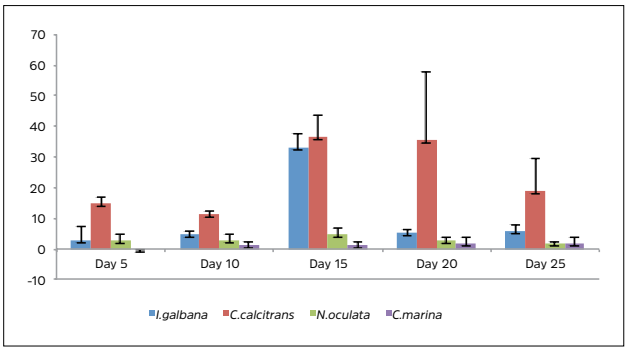

Fig. 1. Density of nauplii (ind.mL^{-1}) in *Oithona similis* culture fed with different algal diets

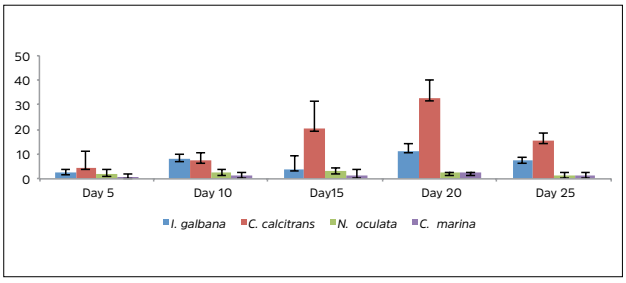

Fig. 2. Density of copepodites (ind.mL^{-1}) in *Oithona similis* culture fed with different algal diets

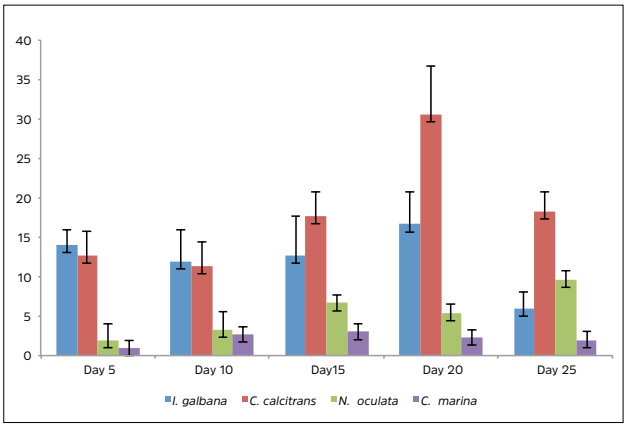

Fig. 3. Density of adults (ind. mL^{-1}) in *Oithona similis* culture fed with different algal diets

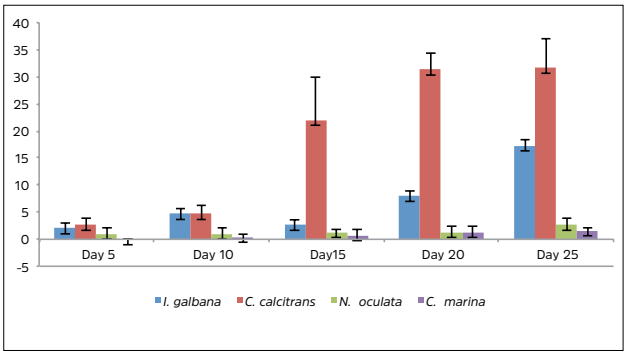

Fig. 4. Density of egg bearers (ind.mL^{-1}) in *Oithona similis* culture fed with different algal diets

Table 1. Population growth rate of different stages of *O. similis* in 25 days culture fed on different phytoplankton

Sl. No	Diets	Adult (d^{-1})	Egg producers (d^{-1})	Nauplii (d^{-1})	Copepodite (d^{-1})
1	*I. galbana*	0.0916±0.01	0.1141±0.03	0.117±0.01	0.0796±0.03
2	*C. calcitrans*	0.1163±0.03	0.1382±0.02	0.118±0.04	0.1091±0.01
3	*N. oculata*	0.0647±0.01	0.1013±0.03	0.088±0.01	0.0114±0.01
4	*C. marina*	0.0602±0.01	0.0207±0.01	0.088±0.03	0.0114±0.03

Table 2. Density of *Oithona similis* in 25 d culture by feeding with different algal diets

Sl. No	Diets	Adult (ind · mL^{-1})	Egg producers (ind · mL^{-1})	Nauplii (ind · mL^{-1})	Copepodite (ind · mL^{-1})
1	*I. galbana*	6 ± 2b	17.33 ± 6.11a	6 ± 2a	7.33 ± 1.15a
2	*C. calcitrans*	18.33 ± 2.51a	31.66 ± 5.50a	19 ± 10.53a	15.33 ± 3.05a
3	*N. oculata*	9.66 ± 1.15a	2.66 ± 1.15b	2 ± 0b	1.33 ± 1.15b
4	*C. marina*	2 ± 0b	1.66 ± 0.57b	2 ± 2b	1.33 ± 1.15b

Values (Mean ± S.D) within a column with different superscript letters were highly significant ($P < 0.05$).

was analyzed. Result of the study indicated that feed play a significant role in the population growth of cultured copepods. Growth was analyzed on day 5, day 10, day 15, day 20 and day 25 and is presented in Figures 1, 2, 3 and 4.

The different phytoplankton diets gave a different effect on growth performance of *O. similis*. Population growth rate of all four stages viz. nauplii, copepodite, adult and egg bearers of *O. similis* in 25 days fed on different phytoplankton is given in Table 1. Among treatments, *O. similis* fed with *C. calcitrans* recorded with the maximum population growth rate for all stages followed by *I. galbana* and *N. oculata*. *O.similis* fed with *C. marina* had been recorded with the lowest density with respect to number of adult copepods and growth (Table 2). In the density analysis of *O. similis* the highest average composition for all stages was observed in *O. similis* fed with *C. calcitrans,* followed by *I. galbana*, *N. oculata* and *C. marina* respectively. The maximum density of nauplii and copepodites were recorded in treatment using *C. calcitrans* as feed and the minimum was in *C. marina*. The maximum density of egg bearers was also obtained in cultures on feeding *C. calcitrans* (Table 2; Fig. 1, 2, 3 & 4). The details of egg production of *Oithona similis* in 20 d by feeding on different algal diets are given in Table 3. Similar the case with density and growth, the maximum egg production was recorded on feeding with *I. galbana* and *C. calcitrans*.

The favorable result on population density, growth and egg production of *Oithona similis* was obtained in *C. calcitrans* at a temperature range of 26-30°C, salinity 30 ppt and food concentration of $1x10^5$ cells ml^{-1}. Maximum density was found by using *C. calcitrans* as feed and it was significantly higher ($p < 0.05$) from other treatments which showed lower density (Table 2).

The biochemical analyses of *O. simils* fed on different micro algal diets in mass culture was carried out and was calculated in dry matter percentage. With all diets the protein composition was > 50%, lipid > 30% and carbohydrate < 10%. For *O. similis* fed with *C. calcitrans* the biochemical composition estimated was protein 55.6%, lipid 33.4% and carbohydrate 8.15%. For *O. similis* fed with *I. galbana* the protein content 51.6%, total lipid 32% and carbohydrate content 7.3%. While, feeding with *N. oculata,* the protein content was 45.74%, total lipid 29%, and carbohydrate 7.3%. Significant variation in nutrient composition while using *C. calcitrans* as feed revealed the fact that among the different algal diets provided, *C. calcitrans* and *I. galbana* as possible diets for *O. similis* in mass culture.

In the present study we have used four algal strains at the rate of $1x10^5$ cells mL^{-1} for culturing *O. similis* under controlled conditions to identify the best diet for the growth and production. The best growth performance for *O.similis* was obtained in cultures using *C. calcitrans* as feed followed by *I. galbana*. It has been reported that *C. calcitrans* has a rich calcium content of 0.59 % and phosphate content of 0.57 % in the cells (Lee *et al.*, 2006; Puelo-Cruz *et al.*, 2009). Mineral content in the diet is important for growth and reproduction of all copepods including *O. similis* and diatoms have always been considered as a suitable source of nutrients for zooplankton to sustain secondary production in terms of reproduction (Payne and Rippingale, 2000). *N. oculata* has not been reported as feed for production of *O. similis*. Most of the studies were focused on feeding *Oithona* sp with *Chaetoceros* or *I. galbana.* Iwasaki

Table 3. Egg production of *Oithona similis* in 20 d culture by feeding on different algal diets

Days	1	5	10	15	20
I. galbana	14	14	18	26	26
C. calcitrans	14	14	18	26	26
N. oculata	14	10	10	18	18
C. marina	14	10	10	18	18

et al. (1977) have been estimated optimum concentrations of micro algae for *Acartia clausi* in a 1:1 combination of *I. galbana* and *Monochrysis lutheri* as $1x10^6$ cells ml^{-1} at 15°C and $1.5x10^6$ cells ml^{-1} at 20°C. While feeding with *Chaetoceros muelleri*, the copepod *Pseudodiaptomus euryhalinus* had shown the best production as reported by Lee *et al.* (2006). Payne and Rippingale (2000) have also been reported that copepods fed by *C. calcitrans* tended to have a shorter time to maturity and produce more nauplii than those fed by *Dunaliella*. The present study also is in conformity with the earlier studies showing that *C. calcitrans* as the preferred diet for *Oithona sp.* (Chilmawatia and Sumintoa 2016). Based on the results obtained, it has been concluded that among the four different microalgal diets *C. calcitrans*, *I. galbana* or *N.oculata* can be used for mass culture of *O.similis*. However, it has been concluded that *C. calcitrans* is the most preferred diet of *O. similis.*

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