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Culture of the Calanoid Copepod, *Acartia erythraea* and Cyclopoid Copepod, *Oithona brevicornis* with Various Microalgal Diets

(Kultur Kopepod Kalanoid, *Acartia erythraea* dan Kopepod Siklopoid, *Oithona brevicornis* dengan Pelbagai Diet Mikroalga)

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ABSTRACT

Two experiments were conducted to develop Acartia erythraea *and* Oithona brevicornis *cultures: The performance of five microalgal diets to produce nauplii, copepodites and adults of* A. erythraea*; and the performance of the same diets to produce nauplii, copepodites and adults of* O. brevicornis*. The five different microalgal diets were* Isochrysis galbana *(IG),* Chaetoceros affinis *(CA),* Chlorella marina *(CM),* Nannochloropsis oculata *(NO) and mixed algae (mixture of IG, CA, CM and NO at an equal abundance to provide the exact cell density). The results indicated that the abundance of both* A. erythraea *and* O. brevicornis *was higher in tanks supplied with IG and mixed algae than the tanks supplied with CA, CM and NO. IG and mixed algal diets were statistically similar on the mean abundance for both* A. erythraea *and* O. brevicornis*. The maximum production of* A. erythraea *nauplii was observed on day 12 of culture period and the nauplii production decreased from day 13 onwards. The mean abundance of* A. erythraea *copepodites and adults increased along with time up to the end of the culture period. In the case of* O. brevicornis *nauplii, the maximum abundance was observed on day 9 day of culture period and the nauplii production decreased from day 10 onwards. The mean abundance of* O. brevicornis *copepodites and adults increased gradually from the beginning to the end of the culture period. Under the experimental conditions of this study, both IG and mixed algal diets can be recommended for the best growth performance of A. erythraea and* O. brevicornis.

Keywords: Acartia erythraea*; calanoid copepod; cyclopoid copepod; microalgal diet;* Oithona brevicornis

ABSTRAK

Dua eksperimen telah dijalankan untuk menghasilkan kultur Acartia erythraea *dan* Oithona brevicornis*: Prestasi lima diet mikroalga untuk menghasilkan nauplii, kopepodites dan dewasa* A. erythraea*; dan prestasi diet yang sama untuk menghasilkan nauplii, kopepodites dan dewasa* O. brevicornis*. Lima diet mikroalga tersebut adalah* Isochrysis galbana *(IG),* Chaetoceros affinis *(CA),* Chlorella marina *(CM),* Nannochloropsis oculata *(NO) dan alga campuran (campuran IG, CA, CM dan NO pada jumlah yang sama banyak untuk mendapatkan kepadatan sel yang tepat). Keputusan menunjukkan bahawa bilangan kedua-dua* A. erythraea *dan* O. brevicornis *adalah lebih tinggi di dalam tangki yang dibekalkan dengan IG dan alga campuran daripada tangki yang dibekalkan dengan CA, CM dan NO. Diet IG dan alga campuran juga menunjukkan statistik yang sama dalam purata kelimpahan kedua-dua* A. erythraea *dan* O. brevicornis*. Bilangan maksimum naupli* A. erythraea *diperhatikan pada hari ke-12 tempoh kultur dan pengeluaran nauplii menurun bermula dari hari ke-13 dan seterusnya. Purata kopepodites dan* A. erythraea *dewasa meningkat sejajar dengan masa sehingga akhir tempoh kultur. Dalam kes naupli* O. brevicornis*, kepadatan maksimum diperhatikan pada hari ke-9 tempoh kultur dan pengeluaran nauplii menurun pada hari ke-10 dan seterusnya. Purata kopepodites dan* O. brevicornis *dewasa meningkat secara beransur-ansur dari awal hingga akhir eksperimen. Di bawah keadaan eksperimen dalam kajian ini, kedua-dua diet IG dan alga campuran boleh disyorkan untuk prestasi pertumbuhan terbaik bagi* A. erythraea *dan* O. brevicornis.

Kata kunci: Acartia erythraea*; diet mikroalga; kopepod kalanoid; kopepod siklopoid;* Oithona brevicornis

INTRODUCTION

The larvae of many fish species across all ecosystems rely principally on live animals for their nutrition. Therefore, identifying and evaluating suitable live feeds are fundamental for maximizing survival and growth of fish larvae for aquaculture. Among various live feeds, rotifers have been used as a first feed for most fish larvae for a long time as they can generally be cultured in large quantity and high density. However, many studies have suggested some live feeds such as rotifers or *Artemia* are not suitable as a first diet for the early larval stage of many fish (Drillet et al. 2008; Lee et al. 2005; Rajkumar & Kumaraguru Vasagam 2006). There are some evidences that some species of calanoid and cyclopoid copepods are more suitable than rotifers or *Artemia* (Drillet et al. 2011; Stottrup 2006). Drillet et al. (2011) and Puello-Cruz et al. (2009) observed that the nauplii of *Acartia* spp. (calanoid copepods) are more suitable than rotifers or *Artemia* for many marine fish larvae (red snapper, groupers, halibut, cod, flounder and barramundi) as nauplii of *Acartia* spp. are very small, easily digestible and nutritionally rich. *Acartia* spp. are very rich in HUFA, especially the EPA and DHA and ARA (Schipp et al. 1999). Moreover, calanoid copepods are suitable because of their size range (length 80 to >900 µm and weight 3-5 µg in dry matter basis), pelagic life cycle and producing resting eggs (Rajkumar & Kumaraguru Vasagam 2006; Ribeiro & Souza-Santos 2011).

Like the calanoid copepods, some cyclopoid copepods are also more suitable than rotifers or *Artemia* for many marine fish larvae (Stottrup 2006). The cyclopoid copepods of the genus *Oithona* have short generation period and worldwide geographic distribution, abundance, high rate of reproduction, small size, and high nutritive value. They are considered nutritionally superior than the *Artemia* nauplii especially during metamorphosis of larvae (Hernandez Molejon & Alvarez-Lajonchere 2003; Stottrup 2006). Some cyclopoid copepods (*Oithona* spp.) are considered to be the most suitable species for mass culture among all copepods as they are relatively easy to culture (Lipman et al. 2001). According to Stottrup (2006), they are ideal supplement to the traditional live feed during the larval stages for many fishes. Unfortunately, the culture technology of *Acartia* spp. (calanoid copepod) and *Oithona* spp. (cyclopoid copepod) is not well developed, though both are likely very good candidates for studies on the development of culture technology.

Many copepods are filter-feeders, and they feed primarily on phytoplankton. However, the information regarding their food preference is insufficient for batch culture production. Suitable microalgal diets may improve their growth, survival and production (Puello-Cruz et al. 2009; Rahman et al. 2010, 2008a, 2008b). Many scientists have attempted to culture different species of copepods by providing various phytoplanktons for food (Buttino et al. 2009). However, the food preference for microalgal species by different copepods is often species specific and can also vary with their developmental stage and availability of preferred natural food. Optimising copepod diets to meet their preferences can influence their growth, egg production and success of egg hatching (Milione & Zeng 2008; Rahman & Meyer 2009; Rahman & Verdegem 2010). The present paper describes a study of culturing *A. erythraea* and *O. brevicornis* using various microalgal diets namely *Isochrysis galbana* (IG), *Nannochlorpsis oculata* (NO), *Chlorella marina* (CM) and *Chaetoceros affinis* (CA) and then one with all the micro-alagal species combined under laboratory condition. The objective of this study was to assess effects of these different microalgal diets on the culture performance of *Acartia erythraea* and *Oithona brevicornis*.

Materials and Methods

The samples of microalgal species IG, NO, CA and CM were collected from the Central Institute of Brackishwater Aquaculture, Chennai and Central Marine Fisheries Research Institute, Cochin, India for microalgal culture. All the microalgal species were cultured with f/2 medium at 28°C. The salinity and light regimes were maintained 30‰ and 14 l: 10 D, respectively. The algae were harvested during cell abundance of 30,000 cells/mL log phase) to feed the copepods.

For copepod culture, *A. erythraea* and *O. brevicornis* samples were obtained using a plankton net (mesh size 158 µm; opening diameter 0.35 m) from the Coleroon waters during early morning of the full moon phase. After collection, the adult copepods and later-stage copepodites of *A. erythraea* and *O. brevicornis* were isolated by screening. Nauplii were isolated by 190 µm mesh screen and copepodites were isolated by a 500 µm mesh screen (Schipp et al. 1999). The remaining were adult copepods. The different copepod stages were confirmed under a microscope. The counting was performed in a S-R (Sedgewick Rafter) cell under a microscope. The large copepodites and adults of copepods were used for the stock culture, which was maintained in two separate flat-bottomed rectangular fiberglass tanks filled with vigorously aerated seawater that was filtered with UV at 200 L per min. The diameter and height of each tank were 550 and 850 mm, respectively. Similar tanks were also used for the experiments. Two experiments were conducted: The effects of five microalgal diets on nauplii, copepodites and adults of *A. erythraea*; and the effects of the same diets to produce of nauplii, copepodites and adults of *O. brevicornis*. The abundance of algae in each microalgal diet was 30,000 cells mL^{-1}.

A total of 15 fiber glass tanks (five microalgal diets with three replications) were used in each experiment. A membrane filter (pore size more than 1 µm) was used. The adult stocking abundance of *A. erythraea* and *O. brevicornis* in the experimental tanks were 307 and 352, respectively. During the rearing period, salinity, temperature, dissolved oxygen (DO), and pH were maintained at between 30 and 34‰, 28 and 32°C, 5 and 6.8 mL L^{-1} and 7 and 8.5, respectively. The culture periods of *A. erythraea* and *O. brevicornis* were 14 days and 10 days, respectively. At the end of the culture period, *A. erythraea* and *O. brevicornis* were harvested by a gentle siphoning.

All the data were statistically analyzed through the one-way ANOVA (at p=0.05 level of significance) after checking for normal distribution and homogeneity of variance. Statistical package SPSS (version 17) was used to analyze all the data. If there was any significant effect ($p<0.05$), the mean differences were analyzed through Tukey test.

Results

Effects of Microalgal Diets on *A. erythraea* Culture

Microalgal diets significantly affected the mean abundance of nauplii, copepodites and adults of *A. erythraea* ($p<0.05$).

Over a 14-day operation, the highest mean abundance of nauplii, copepodites and adults of *A. erythraea* was observed in tanks supplied with IG and mixed algae and followed by the tanks supplied with CM and CA (Table 1). The lowest average abundance of nauplii, copepodites and adults of *A. erythraea* was observed in tanks supplied with NO. Mean abundance of nauplii, copepodites and adults of *A. erythraea* was changed significantly (p<0.05) over time, though the changing trends were significantly different in different microalgal feed tanks (Table 1; Figure 1). The maximum production of nauplii was attained on day 12 of culture and the nauplii production decreased from day 13 onwards. The mean abundance of copepodites and adults of *A. erythraea* increased with increasing time. The maximum mean abundance of copepodites and adults of *A. erythraea* was observed on day 14 of culture (Figure 1).

EFFECTS OF MICROALGAL DIETS ON *O. BREVICORNIS* CULTURE

The mean abundance of *O. brevicornis* nauplii was statistically similar (p>0.05) in all microalgal feed tanks, while mean abundance of copepodites and adults of *O. brevicornis* was statistically different (p<0.05) in different microalgal feed tanks (Table 1). Over a 10-day culture period, the overall highest mean abundance of copepodites and adults of *O. brevicornis* was observed in tanks supplied with IG and mixed algae. The mean abundance of copepodites and adults of *O. brevicornis* was statistically similar in tanks supplied with CM, CA and NO. Mean abundance of nauplii, copepodites and adults of *O. brevicornis* changed significantly (p<0.05) over time, but the changing trends were statistically similar (p>0.05) in all the microalgal feed tanks (Table 1). The maximum abundance of nauplii was attained on day 9 of culture, and it was noticed that the nauplii production decreased from day 10 onwards (Figure 2). The mean abundance of copepodites and adults of *O. brevicornis* increased gradually from the beginning to the end of the culture period.

DISCUSSION

Acartia erythraea and *O. brevicornis* are the most commonly occurring copepod species in Coleroon coastal waters. These copepods have the capacity to grow fast and breed continuously with high reproductive capacity (Rajkumar 2006). Growth of any organism is dependent on its food in the culture system (Amira et al. 2016; Antony et al. 2014; Khatune-Jannat et al. 2012; Rahman et al. 2009). Similarly, growth and abundance of copepods are totally dependent on the available food in the culture system. The results of this study showed that the various species of microalgal diets affected the production of nauplii, copepodites and adults significantly for both *A. erythraea* and *O. brevicornis*. However, the highest production of nauplii, copepodites and adults of *A. erythraea* and *O. brevicornis* was observed with the IG diet compared to the other three mono-algal diets. This indicates that the *I. galbana* (IG) is an excellent mono-algal diet for both *A. erythraea* and *O. brevicornis*. This is in agreement with Puello-Cruz et al. (2009), who also considered IG as one of the best food sources for calanoid copepods.

There is no previous study comparing the effects of IG diet on the production of *A. erythraea* and *O. brevicornis*, but Payne and Rippingale (2001, 2000) reported greater survival and nauplii production of *Gladioferens imparipes* copepod in IG diet compared to *Chaetoceros, Dunaliella* and *Nannochloropsis* mono-algal diets. Similar effects of IG diet were also observed by Stottrup and Jensen (1990) on the growth efficiency of *A. tonsa* fed five different mono-algal diets (IG, *Dunaliella tertiolecta, Rhodomonas baltica, Ditylum brightwellii* and *Thalassiosira weissflogii*). The most plausible reason for better performance of IG diet on nauplii, copepodites and adults of copepod production might be nutritional quality of IG, which is very rich in micronutrients including HUFAs and DHA (Payne & Rippingale 2000). According to Brown et al. (1989) and Puello-Cruz et al. (2009), IG is considered one of the very good foods for copepods as they contain very high quantity of essential fatty acids specially 22:6n-3 and 20:5n-3, which have positive influence on the growth performance

TABLE 1. Effects of different microalgal diets on the mean abundance (individual L^{-1}) of adults, copepodites and nauplii of *A. erythraea* and *O. brevicornis* in tanks based on repeated measure one-way ANOVA

Stage	Treatment	Time	Treatment ×Time	Treatment mean ± SE				
				IG	CM	CA	NO	Mixed algae
Acartia erythraea								
Nauplii	**	**	**	1741 ± 245[a]	1637 ± 215[b]	1641 ± 237[b]	1467 ± 209[c]	1749 ± 245[a]
Copepodites	**	**	**	591 ± 106[a]	545 ± 99[b]	547 ± 95[b]	434 ± 82[c]	597 ± 107[a]
Adults	**	**	**	420 ± 61[a]	361 ± 53[b]	357 ± 55[b]	332 ± 52[c]	435 ± 62[a]
Oithona brevicornis								
Nauplii	ns	**	ns	1688 ± 244	1679 ± 243	1709 ± 243	1667 ± 240	1716 ± 251
Copepodites	**	**	ns	395 ± 78[a]	343 ± 72[b]	389 ± 76[ab]	386 ± 76[ab]	403 ± 79[a]
Adults	*	**	ns	241 ± 41[a]	237 ± 40[ab]	230 ± 38[b]	238 ± 40[ab]	243 ± 41[a]

Value are expressed as mean ± SE of three replicates in each group. ANOVA was followed by Tukey test if any effect is significant Mean values in the same row with uncommon superscript are significantly different (p<0.05). *, ** and ns indicate p<0.05, p<0.005 and not significantly different, respectively

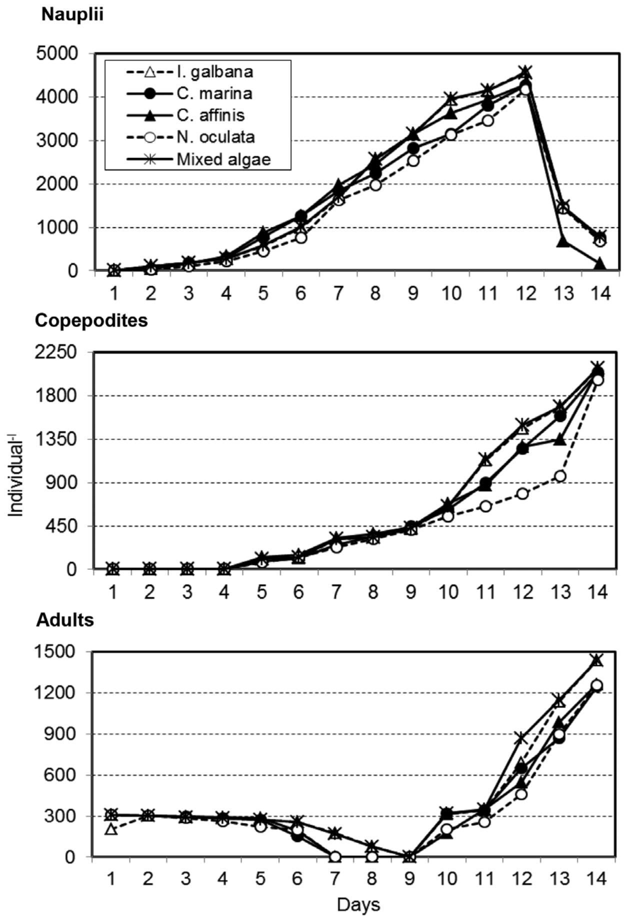

FIGURE 1. Interaction effects of treatment (microalgal diets) and time (days) on the mean production of adults, copepodites and nauplii of *A. erythraea*

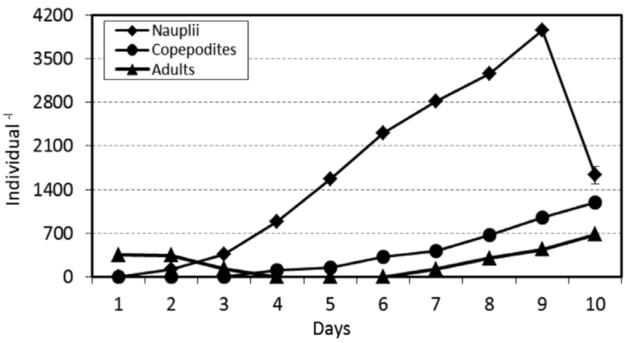

FIGURE 2. Temporal mean production of adults, copepodites and nauplii of *O. brevicornis*

of copepods. The performance of a diet also depends on its digestibility and composition (Rajkumar et al. 2013; Siddik et al. 2015; Wu et al. 2015). Thus, the low performance of CA, CM and NO mono-algal diets might be explained by the toughness and poor digestibility of their cell wall (Payne & Rippingale 2000).

In this study, the effects of the mono-algal IG diet were similar to the effects of the mixed algal diet on nauplii, copepodites and adults of copepod production. Our result of the effects of mixed algal diet on nauplii, copepodites and adults of copepod production are in agreement with the results of Kleppel and Burkart (1995) and Puello-Cruz et al. (2009). They concluded that mixed algal feed is better than mono-algal feed for copepods, because a mono-algal feed may not fulfill all of its nutritional requirements. Apart from the IG diet, it is also likely that the three other mono-algal feeds did not meet the nutritional requirement of the copepods because of their inadequate quantity of essential nutrients. However, the mixed diet likely fulfilled their nutritional requirements (Rahman & Mayer 2009; Rahman

2015a, 2015b; Wu et al. 2015). This was also confirmed by Milione and Zeng (2007), who observed that the production *A. sinjiensis* was higher with mixed algal diet than with mono-algal diets.

In the present study, we recorded the maximum density of *A. erythraea* with 4583 nauplii, 2097 copepodites and 1429 adults per litre while the maximum density of *O. brevicornis* was 4211 nauplii, 1249 copepodites and 710 adults per litre. The recorded maximum density of *A. erythraea* and *O. brevicornis* in the present study exceeded the maximum density of a temperate water species *Acartia* sp. (Ohno & Okamura 1988). Our findings concur with Rajkumar et al. (2004), who recorded a maximum 4276 nauplii, 2063 copepodites and 1420 adults of *A. clausi* per litre of water supplying a mix microalgal diet containing CM, *N. salina* and IG at an equal ratio.

The growth of copepods greatly depends on temperature and microalgal diet (Drillet et al. 2011; Milione & Zeng 2008). In the present study, we observed the highest nauplii abundance at day 12 for *A. erythraea* and day 9 for *O. brevicornis* by maintaining temperature between 28 and 32°C. However, the effects of temperature on the *A. erythraea* and *O. brevicornis* growth are not well understood. Therefore, more research is needed to elucidate the optimum temperature for the best growth of this two copepod species. In conclusion, both the IG and mixed algal diets had similar effects on the growth of *A. erythraea* and *O. brevicornis*. Under the experimental conditions of this study, both the IG and mixed algal diets can be recommended for the best growth performance of *A. erythraea* and *O. brevicornis*.

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