Growth of tropical cladocerans *Ceriodaphnia cornuta* G.O. Sars, 1885 and *Moina micrura* Kurz, 1875 fed with different diets

N.L. Latib¹, F.Md. Yusoff^{1,2,*}, N. Nagao² and H. Nizar¹

¹Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

²International Institute of Aquaculture and Aquatic Sciences, Universiti Putra Malaysia, 71050 Port Dickson, Negeri Sembilan, Malaysia

³Laboratory of Marine Biotechnology, Institute of Biosciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

*Corresponding Author Email : fatimamy@upm.edu.my

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Abstract

Aim: To examine the effects of different feed types (live microalgae diets and processed diets) on the growth of tropical cladocerans, *Ceriodaphnia cornuta* G.O. Sars, 1885 and *Moina micrura* Kurz, 1875.

Methodology: Zooplankton samples were collected from a small pond in the Universiti Putra Malaysia (UPM). Live zooplankton samples were brought to the laboratory for identification, isolation, and mass culture. Experiments were accomplished by providing *ad libitum* feeding to zooplankton (approximately 1.0×10^6 cells mL^{-1} density for live algae diets and 0.01 mg mL^{-1} for processed diets) with three replicates for each diet. The two processed diets were the baker's yeast and *Spirulina* powder, and two live-algal diets were *Chlorella sorokiniana* Shihira and Krauss, 1965 and *Chlorella vulgaris* M. Beyerinck [Beijerinck], 1890. Zooplankton population density was counted every third day under a dissecting microscope by sampling 25 ml of well-mixed culture. The experiments were terminated when the population stabilized or began to decline.

Results: Cultures of *Ceriodaphnia cornuta* and *Moina micrura* showed the highest ($p < 0.05$) mean population density, specific growth rate and body size when fed with live algae diets compared to the processed diets. *Ceriodaphnia cornuta* produced the highest population density (1670.0 individual l^{-1}), specific growth rate ($\mu = 0.19$), and body size (393.0 μm) in *Chlorella sorokiniana* diet. While *M. micrura* generated the highest population density (3993.67 individual l^{-1}), specific growth rate ($\mu = 0.40$), and body size (783.97 μm) in the culture fed with *Chlorella vulgaris*. The lowest population density for *C. cornuta* was *Spirulina* powder culture and baker's yeast culture for *M. micrura*.

Interpretation: The results of the present study illustrated that both algae diets produced significantly higher ($p < 0.05$) population growth rate and body size than non-algal diets. Live algal diets are better food items for the growth of cladocerans, *Ceriodaphnia cornuta* and *Moina micrura* as compared to processed diets.

Key words: *Ceriodaphnia cornuta*, *Moina micrura*, Population density, Tropical cladocerans

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Introduction

Zooplankton forms one of the most important links in aquatic food chain and supports fishery resources in many different inland and marine ecosystems. Zooplankton commonly depends on algae, microbes, and particulate organic matter as its food sources in aquatic ecosystems (Alva-Martinez *et al.*, 2007; Long *et al.*, 2019; Tellioglu, 2013). In aquaculture industry, both phytoplankton and zooplankton function as live foods for commercially important fish and other aquatic organisms because they are easily digestible protein rich diets (Das *et al.*, 2012). The ability of live food species to move in water column make them regularly available to fish and shellfish larvae and projected to encourage larval feeding response (David, 2003). Most shellfish and fish larvae require phytoplankton and small zooplankton as their first food to achieve fast growth and high survival (Evjemo *et al.*, 2003). In fact, shellfish and fish larvae do not respond to artificial supplemented feed as well as they do to live feed.

Cladocerans are one of the important zooplankton groups, beside rotifers and copepods. Many species of cladocerans are filter-feeders and rely heavily on phytoplankton for their main feeding regime (Khatoon *et al.*, 2012), due to microalgae high nutritional content and suitable size (Choi *et al.*, 2014; Muñoz-Mejía and Martínez-Jerónimo, 2007). *Ceriodaphnia cornuta* and *Moina micrura* are freshwater zooplankton, commonly found in tropical lakes and ponds. *Moina micrura* is an important live food in aquaculture since it is rich in nutritional contents and capable of improving larval growth and development of many cultured species (Azuraiddi *et al.*, 2013). In general, cladocerans are ideal live foods as they have digestive enzymes, high nutritional quality, and suitable body sizes (0.2–6 mm) for many species of fish and invertebrate larvae (Dodson and Frey, 1991; Kumar *et al.*, 2005; Nandini and Sarma, 2003; Srivastava *et al.*, 2006).

Most cladoceran species are capable of consuming unicellular algae with size ranging between 2 µm to 25 µm (Das *et al.*, 2012; Flores-Burgos *et al.*, 2003). However, large colonial, filamentous and spinous algal species are unfavorable for them (Fulton, 2006; Guo and Xie, 2006 and Lurling, 2003). Cladoceran species mainly depend on few groups of algae such as chlorophytes (Goldyn and Kowalczevska-Madura, 2012; Repka, 1997) cryptophytes (Li *et al.*, 1996), and autotrophic flagellates (Ahlgren *et al.*, 1990) for enhancement of their growth and reproduction. On the other hand, blue-green algae can reduce the growth of cladocerans (Urrutia-Cordero *et al.*, 2015) mainly due to cyanotoxin (Adibah *et al.*, 2020). Some studies have reported that cell morphology, biochemical composition, and nutritional content show difference in growth response of zooplankton towards microalgal species (Alva-Martinez *et al.*, 2004; Bec *et al.*, 2003; Elser *et al.*, 2001; Tönno *et al.*, 2016; Urabe *et al.*, 2003).

The quality and quantity of phytoplankton consumed are key factors that govern the growth of cladoceran populations (Choi *et al.*, 2014). This study evaluated the effects of using microalgae diets to improve the population growth rates of *Ceriodaphnia cornuta* and *Moina micrura*, which can be further used to enhance the live-food production for the aquaculture industry.

Materials and Methods

Isolation of species : *Ceriodaphnia cornuta* and *Moina micrura* samples were collected from pond 5 in Bukit Ekspo, Universiti Putra Malaysia, by passing water samples through a 60-µm sieve several times. Live *Ceriodaphnia cornuta* and *Moina micrura* samples were kept in one litre plastic bottles and carried to the laboratory for isolation and identification of species. The samples were then examined under dissecting microscope to confirm the species and then samples were cultured in one litre flasks containing filtered pond water using 25 µm net. One parthenogenetic female with eggs was transferred to a 50 ml beaker to ensure a pure mono-species stock. Many beakers, each containing one female were prepared as stock cultures. For stock culture, samples were maintained and fed with green water culture until the eggs were hatched and the neonates grew to adults. Each culture was examined daily during the stock maintenance. All dead individuals were noted and removed from the beaker. All wastes were also removed, and water in the beaker was changed daily up to 30%. Zooplankton from the third generation were selected for the study to ensure the parental effects and phenotypic tractability maintained from the stock culture according to Lynch and Ennis (1983).

Culture of algae as feed : *Chlorella vulgaris* and *Chlorella sorokiniana* were used in this experiment. Pure stock cultures of two algae were grown using BG-11 medium (Rippka *et al.*, 1979). The cultures were kept and maintained in General Research Laboratory, Department of Aquaculture, Faculty of Agriculture, UPM under the control temperature of $25 \pm 2^\circ\text{C}$ and light and dark cycle of 12 hr light:12 hr dark with mild continuous aeration. The algae concentrations were determined using an improved Neubauer hemocytometer (0.25 mm² x 0.1 mm) under a dissecting microscope after samples were fixed with Lugol's solution.

Experimental design : Experiments were performed to test the effects of different food types on population growth rate and density of *Ceriodaphnia cornuta* and *Moina micrura* using two live microalgae diets: *Chlorella vulgaris* and *Chlorella sorokiniana*, and two processed diets: baker's yeast, *Saccharomyces cerevisiae* and *Spirulina* powder, with three replicates for each diet. *Ceriodaphnia cornuta* and *Moina micrura* were fed with approximately 1.0×10^6 cells ml⁻¹ density for live algal diets and 0.01mg ml⁻¹ for processed diets every day. Water quality was regularly checked to avoid overfeeding, which was indicated by a

cloudy medium or left-over food precipitation (Matias-Peralta et al., 2011). Two hundred females were transferred to one litre conical flask containing filter-sterilized freshwater pond water (pH 6.9 - 7.5, temperature 25.0°C and dissolved oxygen 6.6 mg l⁻¹) for initial stocking density. Temperature, salinity and photoperiod were kept same as in the stock culture. The culture medium was changed 100% every third day. The flasks were manually shaken every day to maintain homogenous condition inside the flasks. When the population stabilized or began to decline the experiments were terminated. Every third day, population density for both species were determined by removing 25 ml of well-mixed culture and counted under a dissecting microscope (Azuraiddi et al., 2013). Population growth rate was calculated by the following formula:

$$\text{Population growth rate, } \mu = \frac{(\ln^{x_1} - \ln^{x_2})}{T}$$

Where, X_1 is the population density at the start of selected time interval; X_2 is the population density at the final of selected time interval and T is the time in days

Statistical analysis : All the data recorded were analyzed by one-way analysis of variance (ANOVA) with the Statistical Package for Social Science, SPSS (version 20). Duncan's Multiple Range Test was used to test the mean differences. All data were tested for homogeneity of variance earlier to satisfy the condition of normal distribution.

Results and Discussion

In this study, the population densities and growth rates of *C. cornuta* and *M. moina* fed with algal diets were significantly higher ($p < 0.05$) as compared to those fed with baker's yeast and *Spirulina* powder (Figs. 1, 2, 3 and 4). *Ceriodaphnia cornuta* fed with *Chlorella sorokiniana* showed the highest ($p < 0.05$) mean population density (1670.00 individual l⁻¹) as compared to other diets (Fig.1). However, the population density fed with *C. sorokiniana* started declining on day 18 due to a highly crowded condition (Fig. 1). Meanwhile, there were no significant difference ($p > 0.05$) between cladocerans fed with *Chlorella vulgaris*, baker's yeast and *Spirulina* powder as *C. cornuta* showed poor growth in all these cultures. *Spirulina* powder failed to produce sustainable population density because all the individuals started to decline and die during first three days of culture (Fig. 1).

Only *C. cornuta* fed with *C. sorokiniana* showed significantly higher ($p < 0.05$) specific growth rate when compared with other diets. The highest specific growth rate was recorded in the cultures fed with *C. sorokiniana* (0.19) followed by cultures fed with *C. vulgaris* and baker's yeast (Fig. 2). Negative growth rate was recorded for *Spirulina* powder because of the inability of *C. cornuta* to survive when fed with *Spirulina* powder (Fig. 1).

Fig. 1 : Mean population densities of *Ceriodaphnia cornuta* fed with different diets ($n = 3$). All *Ceriodaphnia cornuta* fed with *Spirulina* powder died after three days. Vertical bars indicate standard error of means.

Fig. 2 : Mean specific growth rates (μ) of *Ceriodaphnia cornuta* fed with different diets. Bars with different letters indicate that the means are significantly different at $p < 0.05$.

Fig. 3 : Mean population densities of *Moina micrura* fed with different types of diet.

Different from *C. cornuta*, *M. micrura* showed significantly higher densities and growth rates when fed with both *Chlorella* species as compared to baker's yeast and *Spirulina* powder (Fig. 3 and 4), probably due to latter's bigger size that allowed them to filter larger food particles. *Moina micrura* cultured with *Spirulina*

powder showed slow growth with a long lag phase and delayed peak density as compared to *C. cornuta*, which did not show any significant growth (Fig. 1 and 3). Even though *Spirulina* powder had the longest culture period up to 30 days for *M. micrura*, however, the population density was significantly lower ($p < 0.05$) as compared to live microalgae diets. The other three diets showed almost similar pattern of population growth curve when they reached maximum density on 12th day for those fed with *C. vulgaris* and *C. sorokiniana*, and 15th day for baker's yeast.

In general, the mean body size for *C. cornuta* was less than 400 μm . Individuals of *C. cornuta* fed with *C. sorokiniana* showed the biggest ($p < 0.05$) mean size (392.99 μm), followed by those fed with *C. vulgaris* and baker's yeast (Fig 5). No significant difference ($p > 0.05$) was detected between those fed with *C. vulgaris* and baker's yeast. *Ceriodaphnia cornuta* fed with *Spirulina* powder died after 9th day of culture.

The mean size of *M. micrura* fed with *C. vulgaris* were significantly different ($p < 0.05$) as compared to baker's yeast but had no significant difference ($p > 0.05$) with *C. sorokiniana* and *Spirulina* powder. The biggest individuals of *M. micrura* were found in culture with *C. vulgaris* (783.97 μm) followed by *C. sorokiniana* and *Spirulina* powder (Fig. 6). The smallest individual *M. micrura* was observed in the culture of baker's yeast.

According to Ger et al. (2016), cyanobacteria is regarded as a poor quality food mainly due their toxicity and morphology. For example, *Spirulina* is a filamentous, long, and appears as blue-green spiral threads. Previous studies have shown that most cladoceran species do not easily ingest large colonial, filamentous and spinous microalgal species (Alva-Martínez et al., 2007; Hartmann and Kunkel, 1991; Lurling, 2003). Most zooplankton species that feed on cyanobacteria alone tend to grow slower, delayed reproduction, and generally increased mortality (Munirasu et al., 2016; Tillmanns et al., 2008; Webster and Peters, 1978). The low population growth observed in cultures with *Spirulina* powder diet might have been due to the relatively large size of algal cells and its filamentous composition that makes them less acceptable for both cladocerans.

Gulati and DeMott (1997) reported that zooplankton fed with only baker's yeast proved to be a poor dietary source. However, mixed baker's yeast and other food sources such as microalgae could increase the survival level of zooplankton (LaRocca et al., 1994; Gomez et al., 2012; Khatun et al., 2014). Zooplankton species including *C. dubia* that were given diet containing baker's yeast reached low growth density (Pena-Aguado et al., 2005). In addition, population growth of *Apocyclops dengizicus* fed on baker's yeast also resulted in poor population growth density (Farhadian et al., 2008).

Cladocerans are able to eat unicellular algae of dimension 2 μm to 25 μm (Flores-Burgos et al., 2003). Previous

Fig. 4 : Mean specific growth rates (μ) of *Moina micrura* fed with different types of diets. Bars with different letters indicate that means are significantly different at $p < 0.05$.

Fig. 5 : Mean body size of individual of *Ceriodaphnia cornuta* fed with different types of diets. Different letters indicate significant difference ($p < 0.05$). All *C. cornuta* fed with *Spirulina* powder died on 3rd day of culture.

Fig. 6 : Mean body size of *Moina micrura* fed with different types of diets. Different letters indicate significant difference ($p < 0.05$). Vertical bars are standard errors of the means.

studies suggested that maximum food particles that could be ingested depend on the body size (Bogdan and Gilbert, 1984; Burns, 1968). Vijverberg (1989) reported that green algae were good quality food sources for zooplankton. This evidence was

supported by Alva-Martinez *et al.* (2007) when *C. dubia* had a longer culture period when fed with *C. vulgaris* than cyanobacterial species. *Moina macrocopa* generally has higher growth rate when fed with *C. vulgaris* than many species of cladocerans, including *C. dubia* (Mangas-Ramirez *et al.*, 2002; Pena-Aguado *et al.*, 2005).

In this study, *C. cornuta* was able to grow well on *C. sorokiniana* but showed poor growth on *C. vulgaris*, probably due to the fact that *C. sorokiniana* was smaller (mean size < 3 µm) than *C. vulgaris* (mean size > 5 µm). Umi *et al.* (2020) reported that *C. cornuta* was dominant in eutrophic lake as they can feed on small bacterial cells from the decomposed algal mass. Thus, lower growth rates of small *C. cornuta* when fed with *C. vulgaris* could be due to larger cell size of *C. vulgaris* as compared to *C. sorokiniana*. In addition, the body size of *C. cornuta* was approximately two times smaller than *M. micrura* (Fig. 5, 6). In this study, the body size of *C. cornuta* was less than 400 µm, indicating that they have small body size compared to previous reports (Freitas *et al.*, 2007; Srivastava *et al.*, 2006). Muñoz-Mejía and Martínez-Jerónimo (2007) reported that cladocerans are able to eat and adapt to any food item within the size range of their filtering capacity. When they have a small body size, distances between the setulae on moving appendages, which act as filters the feed might also small in size. This would restrict their diets and cause difficulty for small-sized cladocerans to eat big food particles size (He *et al.*, 2001).

In conclusion, live microalgae diets such as *Chlorella* spp showed better effects on the growth of cladoceran zooplankton populations (*C. cornuta* and *M. micrura*) compared to bakers' yeast and *Spirulina* powder, which could be due to better nutritional quality of the fresh live foods than processed ones. This study showed the correct matching between the zooplankton body size and the size of food particles is important in determining the effectiveness of food type on zooplankton growth rate.

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