CULTURE EXPERIMENTS WITH A FRESHWATER CLADOCERAN, *CERIODAPHNIA QUADRANGULA* (O. F. MÜLLER, 1785), AS SUITABLE LIVE FOOD FOR MAYAN CICHLID (*CICHLASOMA UROPHTHALMUS* GÜNTHER 1862) LARVAE

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ABSTRACT

Farhadian, O.; Khanjani, M. H.; Keivany, Y. & Dorche, E. Ebrahimi 2012. Culture experiments with a freshwater cladoceran, *Ceriodaphnia quadrangula* (O. F. Müller, 1785), as suitable live food for mayan cichlid (*Cichlasoma urophthalmus* Günther 1862) larvae. Braz. J. Aquat. Sci. Technol. 16(2): 1-11. eISSN 1983-9057. Effects of five different diets of green microalgae, *Scenedesmus quadricauda* (S), poultry manure (P), cattle manure (C), and two mixed diet of P+C (PC) and S+P+C (SPC); and interaction effects of water temperature (20, 25, 30°C) and photoperiod (24:0, 0:24, and 12:12, hours light: hours dark) on production and growth of *Ceriodaphnia quadrangula* were examined separately. Then, mass cultivation of *C. quadrangula* and its application for rearing of an ornamental fish larva, Mayan cichlid (*Cichlasoma urophthalmus*) were carried out. Results showed that mean population density and specific growth rate (*SGR*) of *C. quadrangula* obtained 0.15-3.70 ind. mL^{-1} (150-3700 ind. mL^{-1}) and 0.019-0.18 day^{-1}, respectively, maximum with S diet and minimum with C diet. The better mean population density and *SGR* for *C. quadrangula* determined at interactions 25 ºC with 24:0 and 12: 12, L:D, respectively, which was significantly higher than that at interactions 20 ºC and 30 ºC. Nutritional analyses showed that protein and lipid contents of *C. quadrangula* were 54% and 12.3% dry weight, respectively. The fatty acid contents of *C. quadrangula* were 27.3% and 63.7% of saturated and unsaturated fatty acids, respectively. Among PUFAs (Polyunsaturated Fatty Acids) content, 18:2n-6 and 18:3n-3 were in highest level. Early cichlid larvae (5-weeks-old, 15.8 mm length and 110 mg weight) and advanced larvae (10-weeks-old, 25.6 mm length and 240 mg weight) consumed *C. quadrangula* at range 220–584 ind. day^{-1} $larvae^{-1}$ and 528–1956 ind. day^{-1} $larvae^{-1}$, respectively, as well as suitable growth and survival rate. This study demonstrated that *C. quadrangula* could be used as live feed purposes for larval rearing in aquaculture.

Keywords: Freshwater zooplankton, diet, temperature and photoperiod, fatty acid, Mayan cichlid larvae, ornamental fish larvae, ingestion rate

INTRODUCTION

Cladocerans are small zooplanktonic crustaceans which almost exclusively live in freshwater. They are suitable live food sources used in aquaculture industry due to their abundance, tolerance to environmental conditions, high nutritional quality, ease of handling and sorting from other zooplanktons, suitable sizes (0.2-6 mm), parthenogenetic reproduction, short generation time, richness in digestive enzymes, and high caloric value (Nandini & Sarma, 2003; Kumar et al., 2005).

Most early fish larvae consume rotifers in large amount and they need larger prey such as *Moina* and *Ceriodaphnia* with increasing age and size of the fish larvae (Khadka & Rao 1986; Domínguez-Domínguez et al., 2002). For instance, larvae of angel fish preferred cladoceran (Nandini & Sarma, 2000) and 5-week-old larvae of red eyed tetra (*Moenkhausia sanctaefilomenae* Steindachner) hardly consumed *Moina macrocopa* Straus and *Ceriodaphnia dubia* Richard (Alanis et al*.,* 2009).

Determination of suitable culture requirements for cladocerans is very important and practical for its mass cultivation, such as food quantity and quality, water temperature, and photoperiod control growth and reproduction in cladoceran species (Boersma & Vijverberg 1996; Nandini & Sarma, 2000; Alva-Martinez et al., 2004). In this regard, sometimes light becomes a more important factor than temperature because it is the main stimulus for the periodic activity of many crustaceans (Jansson & Kallander 1984), inhibits growth, maturation and reproduction of aquatic invertebrates (Miliou, 1992), and increased feeding activities (Buikema, 1973). According to Segal (1970), reproductive activities are light dependent and appear to be mediated by photoneuroendocrine pathways. As the feeding rates increased, it enhanced the assimilation efficiency in zooplanktons, which in turn, could increase population growth rate.

Among the cladocerans, *Ceriodaphnia quadrangula* (O. F. Müller) is found in the freshwater ponds and lakes around the world and could be considered as a good replacement for *Artemia* and rotifer (Sharma & Chakrabarbarti 2000; Kumar et al., 2000). This species generally found in waters with temperature ranging from 15 to 25 °C (Rask et al., 1998).

Although many studies were conducted on effects of different diets, temperature and photoperiod on different cladocerans, especially on *Daphnia* species (Gophen, 1976; Chinnery & Williams 2003), little information is available on growth and production of *C. quadrangula*. In addition, information about mass culture and nutritional values, especially fatty acid content, of *C. quadrangula* under suitable culture conditions and also its application as live food for freshwater fish larvae is not available on literature. Use of this species as live food is very suitable for aquarium or ornamental fish larvae, such as Mayan cichlid, *Cichlasoma urophthalmus*. This species belongs to the Cichlidae family and has interesting colors, with its eight black bands and its large ocellus on the caudal peduncle, which gives it its scientific name. It is native to the Atlantic slope of tropical Mesoamerica (Central America) and inhabits lakes, rivers, rocky shorelines, lagoons, estuaries, coastal islands and mangroves. It inhabits waters with temperatures from 18 to 34 ºC and salinity from 0 to 40 ppt. This species is a dietary generalist, consuming organisms from a variety of disparate taxa, especially crustacean zooplankton.

This paper aims to determine suitable diets and suitable interaction of temperature and photoperiod on growth and production of *C. quadrangula*; mass culture of *C. quadrangula* and its consumption by an ornamental fish, Mayan cichlid (*Cichlasoma urophthalmus*) larvae.

MATERIALS AND METHODS

Microalgal Preparation

Scenedesmus quadricauda, green algae was grown in Bold's basal medium (Nichols & Bold 1965), at 25.3 ºC, 12h:12h, light:dark cycle and 60 µmol photons m^{-2} s^{-1} light intensity in 10-litre carboys with mild continuous aeration for 7 days. The *S. quadricauda* concentration (density) was determined using an improved Neubauer hemocytometer (0.25 mm^2 x 0.1 mm) under an invert microscope (Ceti Belgium) according to Martinez & Chakroff (1975) after the samples were fixed in Lugol's iodine solution (0.1 ml for 3 ml sample). Algal cells were harvested by centrifugation (Centurion Scientific Ltd, Germany) at 3000 rpm for 10 minutes when the microalgae growth reached the stationary phase. The harvested microalgae were chilled to 4 °C for one week before starting the main experiment.

Ceriodaphnia quadrangula Stock Preparation

Ceriodaphnia quadrangula samples were collected from Hanna Dam Lake (31°13' - 31°14'N; 52° 46' - 52° 47' E; Altitude= 2300 m; Area=700 ha; Mean depth=10 m, Mean annual rainfall=380 mm), located in Eastern part of Isfahan province, central Iran. Resting eggs (ephippia) of *C. quadrangula* were easily collected in winter (Jan.-Mar.) from the water surface by sieve number 270 (mesh=0.05 mm) and kept in cool and dry place. Each resting egg contains one egg with 290-520 µm length and each gram of eggs contains 220-260 x 10^3 eggs. They were identified from other ephippia according Vandekerhkove et al. (2004). To prepare *C. quadrangula* stocks, eggs were hatched in a 2 L beaker, stocked at 1 g L^{-1} of filtered and autoclaved EPA (96 mg $NaHCO_3$, 60 mg $CaSO_4$, 60 mg $MgSO_4$, and 4 mg KCl in one liter of distilled water), at 25 °C and a pH of 7.5 and aerated vigorously. After 5 days, aeration was stopped and hatched nauplii were removed to a new container by siphoning out. Four weeks before starting the main experiment, they were fed with green microalgae, *Scenedesmus quadricauda* (11.5 µm length, excluding spines, and 5.9 µm width). The culture was maintained at the Fishery Research Laboratory at Isfahan University of Technology (FRL-IUT). During stocks maintenance, each culture was examined daily, all exuvia and any dead individuals were removed and up to 40 % of water was also changed daily.

Experiment 1- Effects of Different Diets on *C. quadrangula*

Ceriodaphnia quadrangula were fed with 5 diets including; *Scenedesmus quadricauda* (S), poultry manure (P), cattle manure (C), mixture of poultry and cattle manures (PC), and mixture of *S. quadricauda*, poultry and cattle manure (SPC) at low and high density (Table 1). This experiment was designed as factorial design of 5 (diet type) x 2 (diet density) with three replicates.

Experiment was run in 30 glass beakers (diameter=7.5 cm, volume=250 mL) each filled with 200 mL of filtered autoclaved of culture medium (EPA) and 5 individuals of *C. quadrangula* (from laboratory stock culture). The feeding frequency was twice per day (morning/evening). Aeration was provided at centre of each beaker using tubes with aperture of 5 mm and also was manually shaken twice a day to ensure homogenous condition inside the beakers. The culture medium (EPA) was changed every three days by passing the *C. quadrangula* culture through 40 µm plankton net which was small enough to retain the *C. quadrangula* but large enough to remove most of the detritus, other solid wastes and to prevent bacterial and algal growth on the walls and bottom. Each beaker was examined daily and any dead individuals and uneaten diet were removed. The culture characteristic were 25 ºC temperature, a 12 h:12 h light:dark cycle and 60 µmol photons m^{-2} s^{-1} light intensity. Population growth rate of *C. quadrangula* was studied for a 21-day

Table 1- Food type, food level and food density used in the experiment 1; *S. quadricauda* in cells mL^{-1}, Poultry manure (35% C, 15% N, 12% P) in mg day^{-1}, Cattle manure (25% C, 11% N, 3% N) in mg day^{-1}, mixed diet with same ratio in weight.

Food Type	Food level (symbol)	Food density
S. quadricauda	Low (SL)	1×10^4
	High (SH)	50×10^4
Poultry manure	Low (PL)	5
	High (PH)	10
Cattle manure	Low (CL)	5
	High (CH)	10
Poultry manure + Cattle manure	Low (PCL)	2.5 + 2.5
	High (PCH)	5 + 5
S. quadricauda + Poultry manure + Cattle manure	Low (SPCL)	0.5×10^4 + 1.25 +1.25
	High (SPCH)	25×10^4 + 2.5 + 2.5

period. Measurements of C, N, and P in manures were determined according to APHA (1995).

Experiment 2 - Effects of Temperature and Photoperiod on *C. quadrangula*

To examine the effects of temperature (20, 25, and 30 °C), photoperiod (24:0, 12:12, 0:24, h light: h dark, L:D), five *C. quadrangula* females were reared in each beaker for a 21-day period. Experiment was run in 27 glass beakers (diameter=7.5 cm, volume=250 mL) containing 200 ml of filtered autoclaved culture medium (EPA). All the experiments were performed and maintained inside water baths equipped with fluorescent lamps. This experiment was designed as a factorial of 3 (temperature) x 3 (photoperiod) with three replicates.

Ceriodaphnia quadrangula population was counted under a dissecting microscope using Bogorov's plate chamber. At the end of the experiment 1 and 2, two 10-ml sub-samples from each beaker (6 for each treatment) were removed and all the individuals were counted and fixed with 5% formalin for length and width measurements (30 individuals for each treatment). The specific growth rate (*SGR*) of *C. quadrangula* population was calculated using the following formula (James & Al-Khars 1986):

$$SGR = \frac{\ln N_t - \ln N_o}{T}$$

Where, T is the culture days and N_o and N_t are the initial and final (highest) density of *C. quadrangula*, respectively. Doubling time (*Dt*) was calculated according to the following formula (James & Al-Khars 1986):

$$D_t = \frac{Ln2}{SGR}$$

Experiment 3 - Mass Production of *C. quadrangula* to Rear Mayan Cichlid Larvae

Ceriodaphnia quadrangula was cultured for two months in three plastic tanks (50 ± 3 L) containing EPA medium with initial density of 30 ind. L^{-1}. Green microalgae, *Scenedesmus quadricauda* was added at a concentration of 4-6 x 10^5 cells mL^{-1} every other day depending on *C. quadrangula* population size and growth of *S. quadricauda* in the cultures. Care was taken to avoid overfeeding, which was indicated by a cloudy medium or excess precipitation of food. This experiment was maintained at temperature of 25 ºC and continuous light. Aeration was provided at the centre and near the bottom of the tank, using glass tubes having an aperture of 10 mm. *C. quadrangula* were harvested after 30 days. At each harvest, cultures were passed through plankton nets (mesh size 40 µm) to obtain *C. quadrangula* individuals. The collected samples were randomly allocated to two sets. One set was washed with distilled water three times and kept in -40 ºC overnight and then freeze-dried to use for fatty acid analyses. An additional set of *C. quadrangula* was estimated by counting the total number of the population and were processed for feeding of Mayan cichlid larvae. After live harvesting of *C. quadrangula* individuals, ingestion rate of two groups of Mayan cichlid (different in age, size and weight, Table 2) were determined using different densities of *C. quadrangula*. The experiment was carried out with cichlid larvae which were obtained from aquarium shop located in Isfahan, Iran. The cichlid larvae stocks were acclimated to laboratory conditions of filtered autoclaved freshwater, 26 ± 1 ºC temperature and 12h:12h light : dark for 72 hours and fed on the artificial feed which was used in aquarium shop.

Cichlid larvae were carefully washed in filtered fresh water (Total water hardness=150 ±12 mg L^{-1}

Table 2 - Mean (± S.E, N=10) total length, width, wet weight, and dry weight of cichlid *Cichlasoma urophthalmus* larvae.

Larvae type	Length (mm)	Width (mm)	Wet-weight (mg)	Dry-Weight (mg)
Early larvae (5-weeks-old)	15.8 ± 1	3.2 ± 1	110 ± 10	44.1 ± 2
Advanced larvae (10-weeks-old)	25.6 ± 1	5.5 ± 1	240 ± 10	80.1 ± 5

as $CaCO_3$) and then transferred to beakers (2 L) with different *C. quadrangula* concentrations of 2, 5 and 10 ind. mL^{-1}. The cichlid larvae density in each beaker was 5 larvae L^{-1}. Each beaker was stocked with 10 larvae. Aeration and uniform distribution of *C. quadrangula* was provided. Each feeding treatment was replicated thrice. Three control beakers (without larvae) containing only *C. quadrangula* were used for each concentration. The fish larvae were transferred daily to new containers with the same volume of water and prey density. *C. quadrangula* counting and the required number for experiment were performed in a zooplankton chamber (Bogorov's plate chamber). The prey density in both control and feeding treatments (with larvae) was measured daily from at least 5 sub-samples. Ingestion rates (I_R) and weight specific ingestion (WSI) of Mayan cichlid larvae were calculated according to Farhadian et al. (2007).

Nutritional Analyses

For the nutritional analyses, collected samples of *C. quadrangula* and also *S. quadricauda* (collected by centrifugation at 4000 rpm for 5 minutes) were freeze-dried and then protein and lipid contents were determined following the method described by Meyer & Walther (1988). Fatty acid contents of samples were prepared according to the direct methylation techniques (Divakaran & Ostrowski 1989).

Statistical Analysis

Data were analyzed using two-way analysis of variance (ANOVA). Differences in treatment means were compared by Duncan's multiple range tests. The maximum population specific growth rates (*SGR*) were transformed to Arcsine square root to ensure a normal distribution (Zar, 1984) and tested for statistical significance by two-way ANOVA. All statistical analyses were carried out using statistical package of SPSS (SPSS, version 2002).

RESULTS

Experiment 1

The highest mean population density and specific growth rate of *Ceriodaphnia quadrangula* were obtained with *S. quadricauda* (3.7 ind. mL^{-1}, 0.18 day^{-1}) and its mixture with poultry and cattle manure (1.57 ind. mL^{-1}, 0.138 day^{-1}) at high food level followed by SL and SPCL diet. The poultry manure and its mixture with cattle manure gave a density of 0.66-0.32 ind. mL^{-1} and growth rate of 0.095-0.058 day^{-1}, maximum with low food density and minimum with high. Cattle manure solely was not able to support population and growth at both food densities (Fig. 1). Results showed that mean length of *C. quadrangula* varied from 800 to 521 µm depend to diet, larger fed on CL and smaller fed on SH diet (Fig. 2).

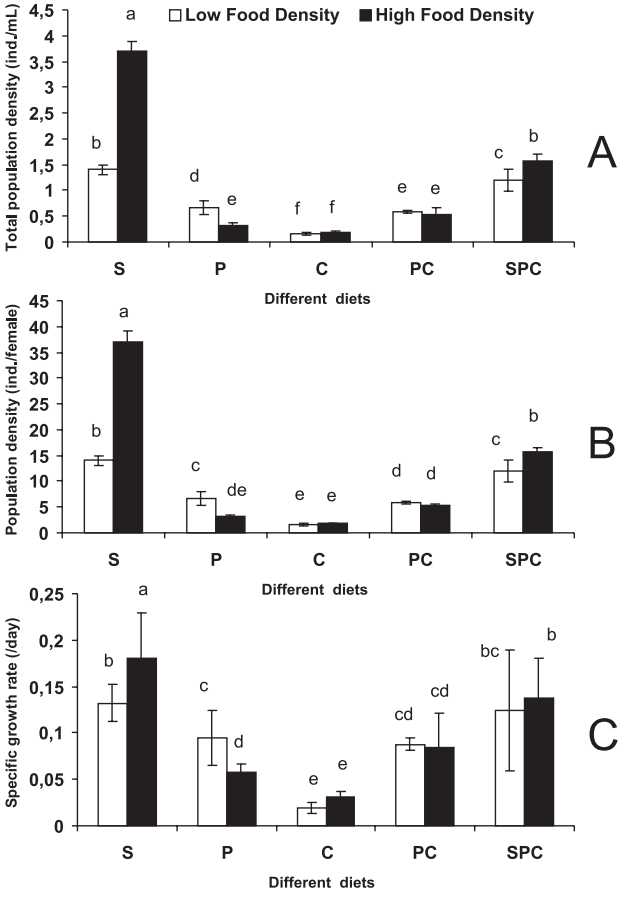

Figure 1 - Mean (±SE, N=6) population density (ind. mL^{-1}) (A), density (ind./female) (B), and population specific growth rate (SGR) (C) of *Ceriodaphnia quadrangula* in different diets. Bars in the same letters are not significantly different (P>0.01).

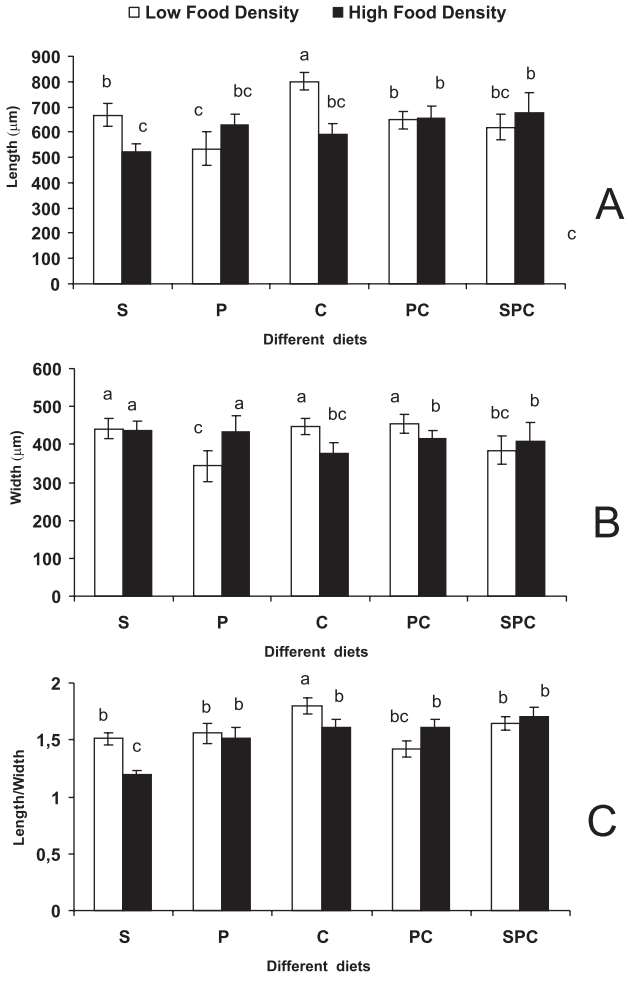

Figure 2 - Mean (± SE, N=30) body length (A), body width (B), and length / width (C) of *Ceriodaphnia quadrangula* in different diets. Bars in the same letters are not significantly different (P>0.01).

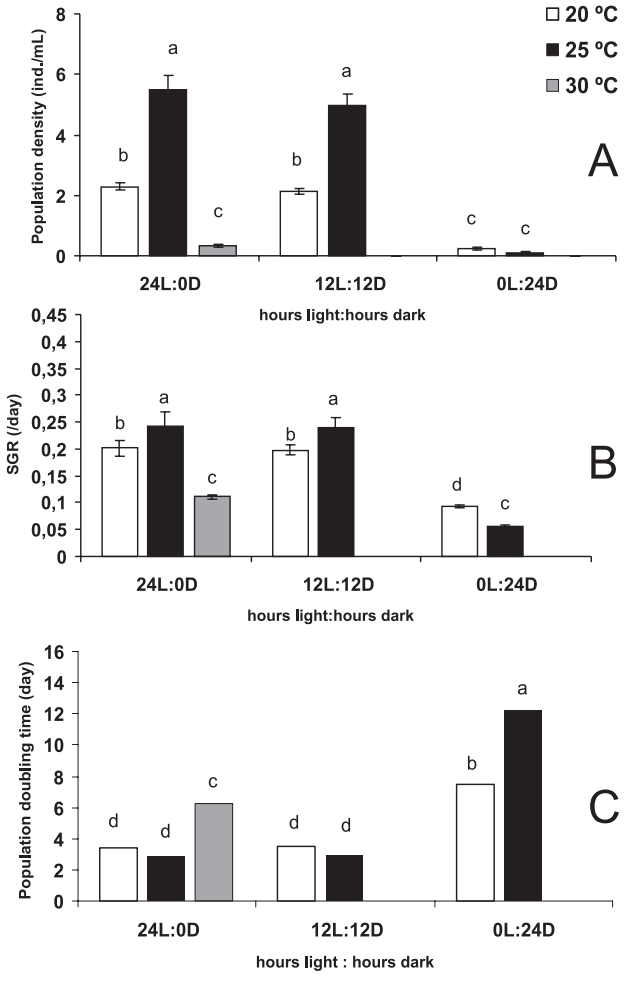

Figure 3 - Mean (±SE, N=6) population density (A), population specific growth rate (SGR) (B), and population doubling time (C) of *Ceriodaphnia quadrangula* in different temperature and different photoperiod. Bars in the same letters are not significantly different (P>0.01).

Experiment 2

The average population density of *C. quadrangula* ranged from 5.51 to 0.11 ind. mL^{-1} on the 21st day of culture, the highest at photoperiod of 24: 0 and water temperature of 25 ºC, and the lowest at photoperiod of 0:24 (Fig. 3A). The *SGR* and *Dt* for *C. quadrangula* in different photoperiod and temperature levels are shown in Figures 3B and 3C. *SGR* ranged from -0.03 to 0.24 (day^{-1}), the highest at photoperiod of 24:0 and a temperature of 25 ºC, and the lowest at 0:24, and 30 ºC.

Female adults of *C. quadrangula* significantly varied in length, width and length to width ratio at treatment interactions (Fig. 4). Results showed that maximum length (731µm) and width (491µm) were obtained at 20 ºC under photoperiods of 12:12 and 0:24 LD. In contrast, length to width ratio was highest under photoperiod of 24:0 and temperatures of 20 and 30ºC.

Experiment 3

Mass production of *C. quadrangula* in plastic tanks (50 L) had a density of 2000 ± 235 ind. L^{-1} after 20 day culture fed on *S. quadricauda*. The protein, lipid and fatty acid profiles of *C. quadrangula* grown on *S. quadricauda* are presented in Table 3. The protein and lipid contents of *C. quadrangula* were 54% and 12.3% dry weight, respectively. Monounsaturated fatty acids (MUFAs) constituted the major part of the fatty acids (36.91 %) followed by saturated fatty acids (SFAs) (27.03 %) and polyunsaturated fatty acids (PUFAs) (26.74 %). The major SFAs, MUFAs, and PUFAs for *C. quadrangula* were C16:0 (14.5 %), C16:1n-7 plus C18:1n-9 (12.2 and 12.8 %) and C18: 2n-6 (16.4 %), respectively. In general, the suitable n-3/n-6 of 0.57:1 for *C. quadrangula* makes it as more suitable and nutritious prey for fish larvae.

Ingestion rates increased continuously with the increase in prey density and cichlid larval size.

Table 3 - Mean (±SE, N=3) protein, lipid and fatty acid composition (% total fatty acid) of *Scenedesmus quadricauda* and *Ceriodaphnia quadrangula*.

Fatty acids	*S. quadricauda*	*C. quadrangula*
Protein (% DW)	43.5 ± 2.23	54 ± 3.27
Lipid (% DW)	9.23 ± 1.10	12.30 ± 1.10
C14:0	1.50 ± 0.20	3.21 ± 0.12
C14:1n-5	-	0.52 ± 0.15
C16:0	9.45 ± 1.30	14.5 ± 1.50
C16:1n-7	0.75 ± 0.22	12.20 ± 1.32
C17:0	-	2.05 ± 0.05
C17:1n-7	-	1.62 ± 0.05
C18:0	0.65 ± 0.15	5.52 ± 0.30
C18:1n-9	3.20 ± 0.40	12.85 ± 0.65
C18:1n-7	0.45 ± 0.05	9.52 ± 0.40
C18:2n-6	7.45 ± 1.25	16.41 ± 0.56
C18:3n-3	25.61 ± 4.60	4.21 ± 0.05
C20:0	-	0.80 ± 0.07
C20:1n-9	0.35 ± 0.07	0.20 ± 0.03
C20:4n-6	-	0.65 ± 0.01
C:20:3n-3	-	0.51 ± 0.01
C:20:4n-3	-	0.45 ± 0.03
C20:5n-3	-	3.23 ± 0.35
C22:0	-	0.53 ± 0.05
C22:6n-3	-	1.28 ± 0.19
C24:0	-	0.42 ± 0.03
Total SFAs	11.60	27.03
Total MUFAs	4.75	36.91
Total PUFAs	33.06	26.74
Total n-3	25.61	9.68
Total n-6	7.45	17.06
n-3 : n-6	3.44 : 1	0.57 : 1

Results showed that early cichlid fish larvae consumed *C. quadrangula* at 220–584 ind. day^{-1}. Similarly, advance cichlid fish larvae ingested *C. quadrangula* at 528–1956 ind. day^{-1}. This study indicated that cichlid larvae, at early and advanced stages, can ingest *C. quadrangula*. The WSI significantly increased with increasing prey density ($p<0.05$, Fig. 5). The WSI values ranged from 2.99 to 7.95 % for while for early fish larvae, these values ranged from 3.95 to 14.62 % for advance larvae per day at different food concentrations (Fig. 5). The larvae dry weights in each larval group were significantly higher at 10 ind. mL^{-1} than 2 and 5 ind. mL^{-1} (Table 4). The maximum survival rate and dry weight were observed at 10-week larvae at prey density of 10 ind. mL^{-1}.

DISCUSSION

Suitable Diets for *C. quadrangula*

Ceriodaphnia quadrangula population grew significantly ($p<0.05$) higher in S and SPC than in P, C and PC (Fig.1). Comparison between different manure diets (P, C and PC) showed that P diet gave better performance in terms of population and growth on *C. quadrangula*, especially at low manure density. In this study, mean population density at different examined diets ranged between 0.15 and 3.70 ind. mL^{-1} (150-3,700 ind. L^{-1}). Our results could support the findings of previous studies on *Ceriodaphnia cornuta* Sars (733-1,930 ind. L^{-1}) using organic manures (Srivastava et al., 2006), on *C. cornuta* (50-10,232 ind. L^{-1}) using chicken manure (Altaff & War, 2010), on *Moina mongolica* Daday (540-3,500 ind. L^{-1}) using green algae *Nananochloropsis oculata* Droop (He et al., 2001).

In fact, the numerical differences could be attributed to inoculum and residual densities of cultured organisms (Malhotra & Langer, 2010) because the optimum space requirement for each species may be different. The increasing population density of *C. quadrangula* with algal concentration is common in laboratory experiments (Nandini & Rao 1998). Nandini & Sarma (2003) stated density of cladocerans under the highest algal food level varied from 2,300 to 71,000 ind. L^{-1}. They reported that *Chlorella* at a density of $0.8x10^6$ cells mL^{-1} gave better growth for cladoceran species. The lower density of *C. quadrangula* fed on *S. quadricauda* could be described by spine cells in *S. quadricauda* compared to spineless cells such as *Chlorella* (Fileto et al., 2004). Generally, size, shape, spines, colonies, filaments, hard cell walls and gelatinous sheaths, and movements of microalgae affect on feeding activities in cladocerans (Fileto et al., 2004). Although most of cladocerans filter organic manures, the size of particle and filtering efficiency are depend on their setae appendage morphology (He et al., 2001). In this study, lower performance of manure diets, especially at high concentration, may be due to low quality, lower filtration rate and aggregation of manure particles. Therefore, better growth and production in low concentration of manures could be attributed to a good adaptation of *C. quadrangula* as reported by Stemberger & Gilbert (1985). Therefore, poultry manure can be used as diet for mass culture of cladoceran, *C. quadrangula* as well as a suitable replacement for microalgae such as *S. quadricauda*.

Suitable Interactions of Temperature and Photoperiod for *C. quadrangula*

Ceriodaphnia quadrangula grew at interactions of 20 ºC and 25 ºC with photoperiod of 24:0 (hL:hD) and 12:12. Contrarily, this species did not grow well at interactions of 0:24 with three levels of temperatures. As indicated by other researchers, water temperature strongly affects growth and reproduction in cladoceran cultures (Benider et al*.,* 2002; Ovie & Edborge, 2002; Rose et al., 2002). The possible reason for the lower growth and production levels at higher temperatures (e.g., 30 °C in this study) could be related to higher energy requirements resulting from increasing respira-

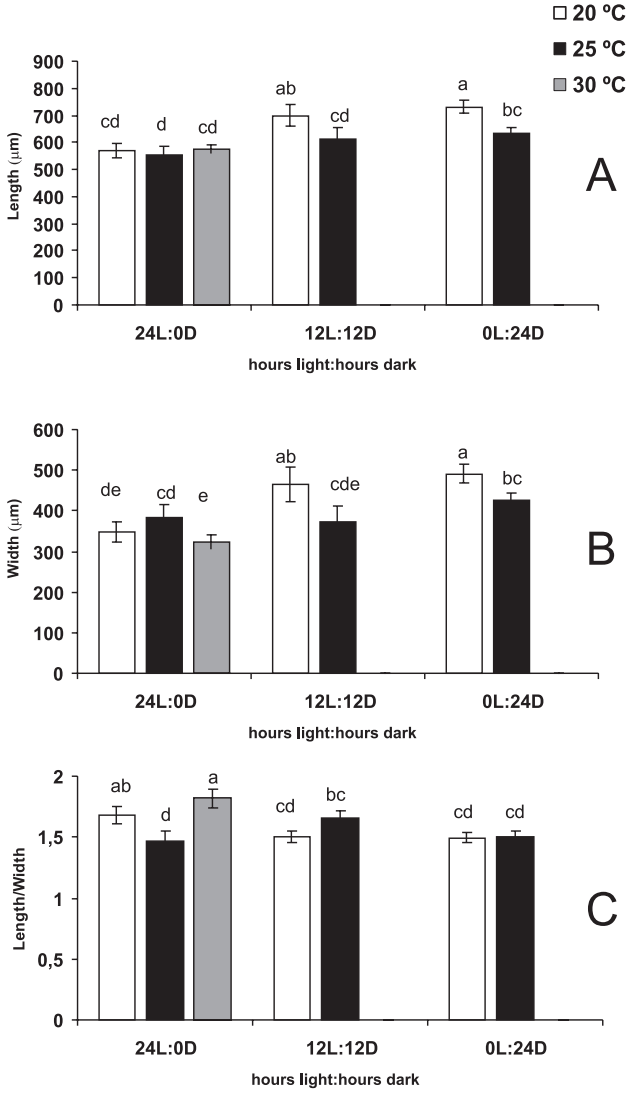

Figure 4 - Mean (± SE, N=30) body length (A), body width (B), and length / width (C) of Ceriodaphnia quadrangula in different temperatures and different photoperiods. Bars in the same letters are not significantly different (P>0.01).

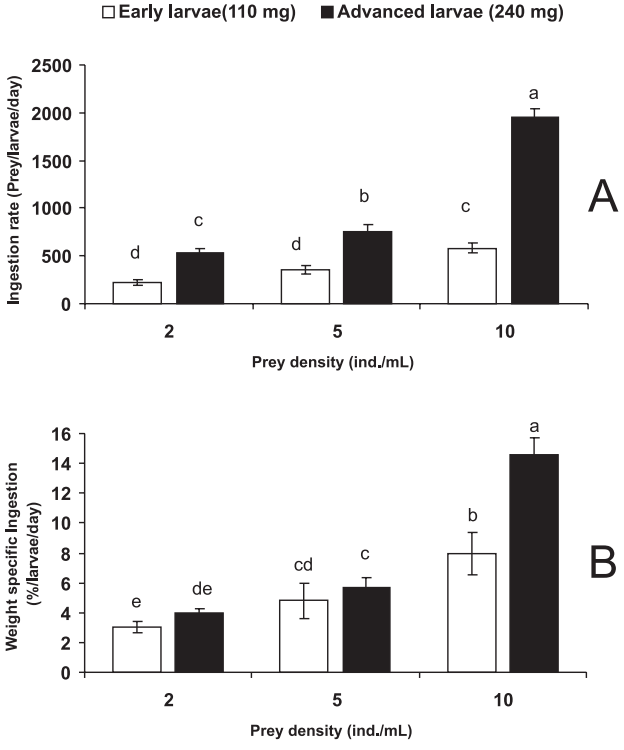

Figure 5 - Mean (±SE, N=3) ingestion rates (A) and weight specific ingestion (WSI) (B) of Mayan cichlid larvae fed on different Ceriodaphnia quadrangula density. Bars in the same letters are not significantly different (P>0.01).

Table 4 - Dry weight (mg larvae^{-1}) and survival rate (%) of cichlid *Cichlasoma urophthalmus* larvae fed on *Ceriodaphnia quadrangula* in 10 days period at three prey densities (ind. mL-1). Values are mean ± standard error from three replications. Values within each row do not share the same superscript are significantly different (P<0.05).

	5-weeks-old larvae			10-weeks-old larvae		
Prey density	2	5	10	2	5	10
Dry-weight	48.3 ^{f} ± 0.5	52.6 ^{e} ± 1.3	57.6 ^{d} ± 2.1	87.8 ^{c} ± 3.2	90.2 ^{b} ± 2.5	97.1 ^{a} ± 4.2
Survival rate	80.3 ^{e} ± 3.3	90.3 ^{c} ± 3.3	96.6 ^{b} ± 3.3	86.6 ^{d} ± 6.6	100 ^{a}	100 ^{a}

tion and feeding rates at higher temperatures (Gophen, 1976).

In this study, the maximum mean specific growth rate (*SGR*) for *C. quadrangula* at 25 ºC was significantly higher than that at 20 ºC and 30 ºC. According to Nandini & Sarma (2003), cladocerans have *SGR* values ranging from 0.01 to 1.50 day^{-1} depending on the species, food type and density, and temperature (Nandini & Sarma, 2000; 2002). Higher *SGR* values in the current study, compared to other cladocerans such as *Simocephalus* and *Daphnia,* could be attributed to their shorter lifespan and higher fecundity (Stearns, 1976), shorter maturation times, ripening of gametes and their release, and continuous breeding of females in these conditions.

Findings of this study showed that the mean total production of *C. quadrangula* at interaction 20 ºC, 25 ºC and 30 ºC with photoperiod of 24:0 were 2.28, 5.51, and 0.34 ind. mL^{-1}, respectively. These production levels were slightly lower compared to levels obtained for other species of freshwater cladocerans as recorded by Nandini & Sarma (2003) at interaction 25 ºC and 12:12 LD. The differences could mostly be attributed to species, food type and density, initial culture density and culture volume.

In this study we found smaller sizes in *C. quadrangula* reared under 24:0 LD in all three levels of temperature. This could be due to higher molting rate and higher reproduction. Furthermore, smaller adult sizes of *C. quadrangula* may be related to size of the ingested microalgae. Since *C. quadrangula* ingested smaller sizes of *S. quadicauda* under continuous light, thus they had smaller sizes compared to other treatments (DeMott, 1985; Kobayashi, 1991).

Nutritional Values of *C. quadrangula*

In this study, the protein and lipid contents of *C. quadrangula* were 54% and 12.3% dry weight, respectively. These amounts meet a major part of nutritional requirements of fish larvae. The differences in protein and lipid content are varied depend on species, age of individuals, physiological conditions, food and temperature.

As shown in Table 3, in the fatty acid profile of *C. quadrangula*, 27.3 and 63.7 % of saturated and unsaturated fatty acids were determined, respectively. *C. quadrangula* grown on *S. quadricauda* contained higher amounts of 18-carbon unsaturated fatty acids such as linoleic acid (18:2n-6), linolenic acid (18:3n-3), oleic acid (18:1n-9), vaccenic acid (18:1n-7) than 20-carbon unsaturated fatty acids which are useful for freshwater fish larvae and fry (Oka et al., 1982). Palmetic acid (16:0), palmitoleic acid (16:1n-7), myristic acid (14:0) and stearic acid (18:0) were higher than other saturated fatty acids.

Watanabe (1987) stated that freshwater species required mainly 18:2n-6 or 18:3n-3 or both as essential fatty acids and increase growth and survival in fish larvae. Lower content of HUFAs detected in *C. quadrangula* may be attributed to low efficiency of delta-5 and delta-6 desaturaes as reported by Bec et al. (2003) for Daphnidae *Simocephalus vetulus*. Another reason for lower fatty acid content of *C. quadrangula* can be associated with lower filtration and assimilation of *S. quadicauda* due to its unsuitable morphological (such as presence of horns in the apical cells) as well as unbalance biochemical properties. For example, Brett et al. (2006) reported that the differences in n-3 to n-6 fatty acids in *Daphnia* were strongly dependent on diet. In general, *C. quadrangula* has suitable fatty acid content, which made it as good live prey for fish larvae. In general, aquatic animals can synthesize saturated fatty acids and lower unsaturated fatty acids to PUFAs (Halver, 1980). The 20-carbon chained PUFAs are essential for proper development of fish larvae and fry (Oka et al., 1982), and these were accumulated in *C. quadrangula* (Table 3).

Rearing of Mayan Cichlid (*Cichlasoma urophthalmus*) Larvae

In this study, cichlid larvae ingested *C. quadrangula* in both larval sizes (Table 2, Fig. 5) due to its suitable body size and absence of spine in the caudal parts. Some species of zooplankton are not consumed by fish larvae due to spines in body (Zaret, 1980). Comparing results between 5-weeks-old and

10-weeks-old larvae showed that ingestion rate forthe latter were increased. These differences could be related to improvement of prey capture and handling skills of 10-weeks-old cichlid larvae, therefore they consume higher numbers with increasing prey density (Alanis et al*.,* 2009).

CONCLUSION

As conclusion**,** *C. quadrangula* had better growth and reproduction on S and P diets, and interactions of 20 ºC and 25 ºC with 24:0 and 12:12, L:D, its suitable body size (521-800 µm in length), its fatty acid content (PUFAs= 26.74% total fatty acid, n-3: n-6 = 0.57:1), its consumption by cichlid larvae (220-1956 ind. day^{-1} larvae^{-1}), suitable larval growth and survival rate make it as a live food to use in aquaculture industry.

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