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Omidvar Farhadian
Isfahan University of Medical Sciences

Nasrollah M. Soofiani
Isfahan University of Technology

Eisa Ebrahimi dorcheh
Isfahan University of Technology

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Larval feeding behaviour of angel fish *Pterophyllum scalare* (Cichlidae) fed copepod *Eucyclops serrulatus* and cladoceran *Ceriodaphnia quadrangula*

Omidvar Farhadian, Rahman Kharamannia, Nasrollah Mahboobi Soofiani & Eisa Ebrahimi Dorche

Department of Natural Resources, Isfahan University of Technology, Isfahan 84156-8311, Iran

Correspondence: O Farhadian, Department of Natural Resources, Isfahan University of Technology, Isfahan 84156-8311, Iran. E-mails: omfarhad@cc.iut.ac.ir; farhadyo@yahoo.com

Abstract

Three different live diets, *Eucyclops serrulatus*, *Ceriodaphnia quadrangula* and equal combination of *E. serrulatus* copepodid and *C. quadrangula,* were offered to angelfish (*Pterophyllum scalare*) larvae viz 1-week, 2-week and 3-week old at prey densities of 2, 5 and 10 individuals mL^{-1}. Results showed that 1-week-old *P. scalare* larvae consumed *E. serrulatus* copepodid at a rate of 31.3–56.7 ind. h^{-1}, *C. quadrangula* at 8.0–12.0 ind. h^{-1}, and mixture of *E. serrulatus* and *C. quadrangula* at 20.7–40.7 ind. h^{-1}. For 2-week- and 3-week-old larvae, consumption rate increased accordingly. The electivity indices (E) of *P. scalare* (1-week-old larvae) for *E. serrulatus* copepodid were +0.18, +0.23 and +0.22 at prey densities of 2, 5 and 10 ind. mL^{-1} respectively. Tendency towards *E. serrulatus* copepodid consumption reduced by aging *P. scalare* as indicated by the E values for 2- and 3-week-old larvae. However, growth and survival of *P. scalare* larvae was greatest when fed on combination of copepod *E. serrulatus* and *C. quadrangula*.

Keywords: Ingestion rate, *Pterophyllum scalare*, *Eucyclops serrulatus*, *Ceriodaphnia quadrangula*

Introduction

Angel fish (*Pterophyllum scalare*) is one of the popular freshwater fish species in aquarium trade industry (Stoskopf 1993b; Garcia-Ulloa & Gomez-Romero 2005). This species is native of Amazon region of South America and belongs to cichlid fish family (Stoskopf 1993b). This species has high economic value due to its great world demand (Karayucel, Orhan & Karayucel 2006). One of the bottlenecks in aquaculture of this species is supplying appropriate food item during larval rearing. In general, poor nutrition is one of the biggest problems in the ornamental fish rearing. Formulated and manufactured feed has usually a short shelf life, hence, declining in quality through the breakdown of vitamins, proteins, fats and oils over the time (Landau 1992; NRC 1993; Garcia-Ulloa & Gomez-Romero 2005). Lower survival and growth rates of *P. scalare* are commonly obtained when such diets are used as the sole diet, for example, larvae fed by commercial extruder diet (Biomar).

One of the practical ways of overcoming these problems is the application of live zooplankton preys, such as ciliates, rotifers, cladocerans and copepods. Among them, cladocerans and copepods have been widely used as live food in aquaculture of fish (Chakrabarti & Jana 1990; Celino, Hilomen-Garcia & del Norte-Campos 2011) and shrimp larvae (Farhadian, Yusoff, Mohamed & Saad 2009; Khatoon, Banerjee, Yusoff & Shariff 2012). Moreover, cyclopoid copepod *Eucyclops serrulatus* and cladoceran *Ceriodaphnia quadrangula* are most abundant in fresh waters fish ponds. *Eucyclops serrulatus* is easily cultured under extreme environmental conditions, such as low oxygen conditions (Datry, Hervant, Malard, Vitry & Gibert 2003) and algal and animal diets (Monakov 2003; Nandini & Sarma 2007) with suitable survival rate. *Eucyclops serrulatus* has six naupliar (119- to 275-μm size) and five copepodid (305- to 530-μm size) stages before reaching adult stage. This wide spectrum of sizes makes it a more suitable prey for fish larvae. As for feeding habit, *E. serrulatus* consumes a wide range of cultured microalgae species, such as

Chlorella vulgaris and Scenedesmus spp. (Downing & Rigler 1984; Nandini & Sarma 2007), which makes it more convenient for mass culture. Cladocerans, on the other hand, are suitable zooplankton for fish larvae feeding due to their natural abundance, high nutritional quality, suitable sizes (0.2–6 mm), parthenogenetic reproduction strategies, richness in digestive enzymes and high caloric value (Nandini & Sarma 2003; Kumar, Srivastava & Chakrabarti 2005). Most early cultured aquatic larvae usually consume rotifers in large amount and then switch to larger prey such as *Moina* spp. and *Ceriodaphnia* spp. with increasing size (Khadka & Rao 1986; Domínguez-Domínguez, Nandini & Sarma 2002). For instance, larvae of *P. scalare* preferred cladoceran (Nandini & Sarma 2000), 5-week-old larvae of red eyed tetra *Moenkhausia sanctaefilomenae* consumed *Moina macrocopa* and *Ceriodaphnia dubia* intensively (Alanis, Sarma & Nandini 2009), and whiteleg shrimp, *Litopenaeus vannamei*, postlarvae consumed *Diaphanosoma celebensis* as a superior live food (Khatoon *et al.* 2012).

Although studies on components of feeding behaviour of fish larvae (such as numbers of encounters, attacks, captures, ingestions, rejections and escapes) with regard to zooplankton preys have been extensively reported (Barbosa & Matsumura-Tundisi 1984; Nandini & Sarma 2000; Sarma, Lopez-Romulo & Nandini 2003; Graeb, Dettmers, Wahl & Caceres 2004), there are scarcity of reports on the measurement of consumption rate and prey selection especially by *P. scalare.* In general, prey selection in fish larvae is affected by a number of factors namely prey shape, mobility, density (Holmes & Gibson 1986; Knutsen 1992; Graeb *et al.* 2004), mouth size (Yasuda 1960; Cunha & Planas 1999), visual and spectral sensitivity (Neave 1984; Drost 1987).

The purpose of this study was to determine ingestion rates and prey selection by different size and age of larval *P. scalare* as a function of *E. serrulatus* and *C. quadrangula* densities.

Materials and methods

Prey preparation

Eucyclops serrulatus was isolated from a fish culture pond, in Karasgan fish propogation center, Isfahan, Iran. The gravid females were cultured at the rate of 25 individuals. L^{-1} in 5-L containers with filtered, autoclaved freshwater. *Eucyclops serrulatus* was cultured under 24.5°C temperature, 12 h:12 h light: dark, 60 μmol s^{-1} m^{-2} light intensity and the culture was continued for 4 weeks until copepod density reached 3 ind. mL^{-1}. The algae mixture of *Chlorella vulgaris* (5-μm length) and *Scenedesmus quadricauda* (11.5-μm length, excluding spines, and 5.9-μm width) in the same ratio (by equal nitrogen weight) was used to feed *E. serrulatus*. The protein content of *C. vulgaris* and *S. quadricauda* were 45.5% and 43.5% dry weight respectively (Farhadian, Daghighi & Ebrahimi Dorche 2012b). The available nitrogen content in each diet was estimated according to equation of N = per cent protein/6.25 as used by Farhadian *et al.* (2012b). The nitrogen content of of *C. vulgaris* and *S. quadricauda* was 7.28% and 6.96% dry weight, respectively, and ratio was calculated as 2.22:2.12 for algal diet mixture. The mixed algal diet used in this experiment was offered at a quantity of 10 mg dry weight L^{-1} day^{-1}.

Plankton nets of 40-μm and 100-μm mesh size were used to collect nauplii and copepodids of *E. serrulatus* respectively. However, the *E. serrulatus* used in this study was obtained from the populations grown in the laboratory using mixed algae. The copepodid of *E. serrulatus* ranged between 305 and 530 μm in length, 183–219 μm in width and 3.30 ± 0.10 μg in dry weight. Lengths of copepodids were measured from tip of the rostrum to the base of caudal rami using an ocular micrometer fixed on an inverted microscope. The dry weight of copepodids was determined by filtering and drying from known prey density according to the method described by Lavens and Sorgeloos (1996).

Ceriodaphnia quadrangula samples were collected from Hanna Dam Lake (Latitude = 31° 13‘–31° 14‘ N; Longitude = 52° 46‘–52° 47‘ E), a cold temperate eutrophic lake located in Eastern part of Isfahan province, central Iran. Resting eggs (ephippia) of *C. quadrangula* were collected in autumn (October–November 2010) from the water surface using a plankton net of 40-μm mesh size and kept in cool and dry place. Each resting egg contained one egg 290–520 μm in length and each gram of eggs contained 220–260 × 10^3 eggs. *Ceriodaphnia quadrangula* ephippia were identified and separated from other ephippia according Vandekerhkove, Declerck, Vanhove, Brendonck, Jeppesen, Conde and M. & Deester L. (2004). To prepare *C. quadrangula* stock, eggs were hatched in a 2-L beaker, stocked at a rate of 1 g L^{-1} of filtered and autoclaved EPA (96 mg NaHCO3, 60 mg CaSO4, 60 mg MgSO4 and 4 mg KCl in one litre of distilled water), at 25°C, pH = 7.5 and aerated vigorously. After 5 days, aeration was stopped, and hatched neonates were removed to a new container by siphoning. Four weeks before starting the main experiment, *C. quadrangula* were fed with mixed algal diet of *C. vulgaris* and *S. quadricauda* with the similar ratio and quantity explained for *E. serrulatus*. The adult of *C. quadrangula* ranged between 696.7 and 773.3 μm in length and 453.3 and 773.3 μm in width. The mean dry weight of *C. quadrangula* was 4.20 ± 0.15 μg. All the prey cultures were maintained at the Fishery Research Laboratory at Isfahan University of Technology (FRL-IUT), Iran. Each culture was examined daily, all exuvia and any dead individuals were removed and up to 20% of water was replaced daily. Some important nutritional value of *E. serrulatus* and *C. quadrangula* used in this experiment are present in Table 1.

Experimental procedure

Three sets of feeding experiments were designed in this study. In the first experiment, the ingestion rates of 1-week-old larvae fed with *E. serrulatus* copepodid, *C. quadrangula* and their combinations (1:1) were evaluated. The second and third experiments were designed to evaluate the ingestion rates of older larvae (2-week-old larvae and 3-week-old larvae) in the similar conditions to the first experiment. Angel fish larvae used at this study were obtained from propagation of existing brood stocks in our laboratory. Some important characteristics of experimental *P. scalare* larvae are present in Table 2. The experiments followed a 3 × 3 factorial design, 3 with prey types and 3 with prey densities with 3 replicates for each treatment in each set of experiments.

Angel fish larvae (1-week-old larvae = 0.14 ± 0.01-μg dry weight) were given a short bath in filtered freshwater, and then transferred to beakers (2 L) with different prey densities. Beakers were covered by black plastic and fluorescent lighting was mounted over them. A total of 50 larvae were assigned to each beaker (25 larvae L^{-1}). Aeration and uniform distribution of food organisms was provided to each container. To determine the mean ingestion rate of larvae from 1-week-old larvae, each group of larvae were kept in the experimental beaker for about 3 days (65 ± 5 h). Similar protocols were followed for measuring the ingestion rate of 2-week-old larvae (dry weight = 2.3 ± 0.07 mg) and 3-week-old larvae (dry weight = 5.2 ± 0.12 mg).

To maintain water quality, constant prey density and appropriate size during the experiment, the larvae from the old container were transferred daily to a new container with new prey items of original densities. Three control beakers containing only prey item were used for each density. Prey reduction due to mortality within experimental containers was accounted by measuring prey death in control and old container after removal of larvae.

The preys counting were performed by taking five sub-samples daily from both well-mixed

Table 1 Mean (± SE) of protein, lipid, carbohydrate (% dry weight), highly unsaturated fatty acid (HUFAs) (mg/g dry weight) and n-3: n-6 ratio of *Eucyclops serrulatus* copepodids and *Ceriodaphnia quadrangula* used in this experiment.

Preys	Protein	Lipid	Carbohydrate	HUFAs	n-3:n-6
E. serrulatus	55.3 ± 4.5	20.4 ± 1.4	12.5 ± 0.4	25.9 ± 1.7	2.68:1
C. quadrangula	45.2 ± 3.4	12.3 ± 1.5	10.4 ± 0.9	10.7 ± 1.2	0.57:1

Table 2 Mean (± SE) of age, length and weight of different larvae of *Pterophyllum scalare* used in this experiment.

Larvae	Age(days after hatching)	Standard length (mm)	Body width (mm)	Wet weight (mg)	Dry weight (mg)
1-week-old-larvae	8.0 ± 2.0	4.5 ± 0.2	1.2 ± 0.1	0.9 ± 0.1	0.14 ± 0.01
2-week-old-larvae	16.0 ± 2.0	6.5 ± 0.8	2.2 ± 0.1	10.7 ± 1.2	2.30 ± 0.07
3-week-old-larvae	22.0 ± 2.0	8.5 ± 1.1	3.5 ± 0.4	25.5 ± 3.1	5.20 ± 0.12

control (without larvae) and experimental (with larvae) containers. Number of prey in each subsample was measured using Bogorov's plate chamber. At the end of the experiment, 10 larvae from each treatment were taken randomly to measure the dry weight (oven dried at 70°C for 10 h).

The survival rate and specific growth rate (SGR-based on dry weight) of larvae (Ricker 1979) were measured using the following formulas:Survival rate (%) = $(N/N_0) \times 100$,

where N_0 and N are the initial and final number of larvae;

SGR (% mg day^{-1}) = $(\text{Ln } W - \text{Ln } W_0) \times 100/D$,

where W_0 and W are the initial and final mean body weight (mg) of larvae, and D is duration of experiment.

Ingestion rate calculation

Ingestion rates (I_R) were calculated according to Paffenhofer (1971) based on the following formula:

$I_R = \{(C_o - C_{t)} - ((C_1 - C_2) \times C_o/C_1)\} \times \{V/n\ t\}$

Where

I_R = number of prey ingested larvae^{-1} h $^{-1}$ (Ingestion rate);

C_o = initial prey density in each experimental beaker;

C_t = final prey density in each experimental beaker;

C_1 = initial prey density in each control beaker;

C_2 = final prey density in each control beaker;

V = working volume of the beaker;

n = mean number of larvae at the beginning and the termination of experiment;

t = time

Weight-specific ingestion (WSI) was calculated using the following formula:

$$\text{WSI}(\%) = \frac{\#\text{of ingested prey} \times \text{prey dry-weight}(\mu g)}{\text{Larvae dry weight}(\mu g)} \times 100$$

Electivity of prey organisms by *P. scalare* in a 1:1 combination of *E. serrulatus* copepodid, and *C. quadrangula* was calculated using the following electivity (E) index from Ivlev (1961, cited by Yurochko 1976):

$$E = \frac{r - p}{r + p}$$

Where r is the per cent of one prey item in total ingested diet and p is the per cent of same prey item in the experimental beaker. The electivity ranges from +1 to −1, which indicate positive or negative selectivity.

Data analysis

All experiments were conducted using a completely randomized design with three replicates in each treatment. The collected data were analysed using two-way analysis of variance (ANOVA). Significant differences among treatments were determined using Duncan's Multiple Range Test at a 0.05 level probability. Specific growth rate and survival rate was arcsine-square root transformed to ensure a normal distribution prior to analysis (Zar 1984). All statistical analyses were carried out using SPSS (2002).

Results

Ingestion rates of *P. scalare* increased continuously with the increase in prey density being significantly different ($P < 0.05$) among different treatments (Fig. 1) in each set of experiment. The mean highest ingestion rates of *E. serrulatus* (56.7 ind. larvae^{-1} h^{-1}), *C. quadrangula* (12.0 ind. larvae^{-1} h^{-1}), and combination of *E. serrulatus* and *C. quadrangula* (40.7 ind. larvae^{-1} h^{-1}) for 1-week-old larvae were observed at the high prey density (10 ind. mL^{-1}) (Fig. 1a). These ingestion rates were 1.65, 2.94 and 1.98 times lower than 2-week-old larvae (Fig. 1b) and 1.62, 5.36 and 2.34 times lower than 3-week-old larvae (Fig. 1c) when fed at similar food densities.

The WSI significantly increased with increasing prey density ($P < 0.05$, Fig. 2). Relatively sharp increase in WSI was observed when larvae were fed *E. serrulatus*. The WSI values ranged from 103 to 187% for 1-week-old larvae (Fig. 2a), whereas for 2-week- and 3-week-old larvae, these values ranged from 177 to 309% and 221 to 303% for different prey densities respectively (Figs. 2b, 2c). The WSI data for *C. quadrangula* indicated that smaller larvae (1-week-old larvae) can ingest about 33–50% (Fig. 2a) while they were ingested at 82–148% and 161–270% by the larger larvae (2- and 3 week-old larvae respectively (Figs. 2b, 2c).

The ingestion rates of *P. scalare* fed on 1:1 combination revealed that individual ingestion rates of 3-week-old larvae were approximately 3.43, 2.66 and 2.34-fold higher than those of 1-week-old larvae and 1.57, 1.44 and 1.19-folds than

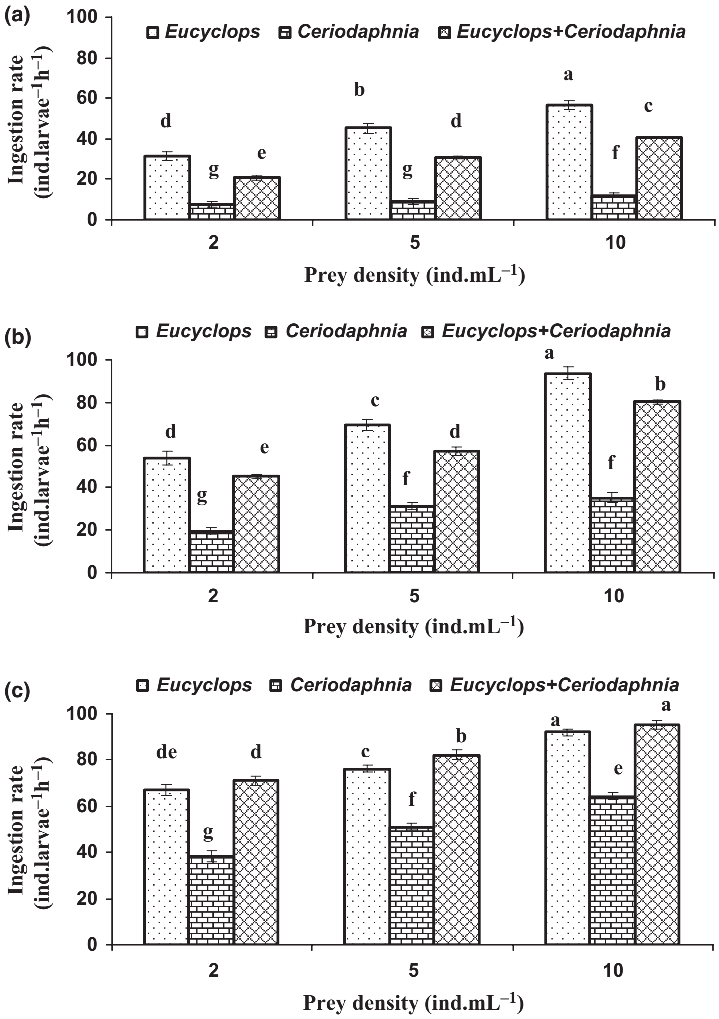

Figure 1 Ingestion rates (prey larvae^{-1} h^{-1}) of *Pterophyllum scalare* larvae (a = 1-week old, b = 2-week old, c = 3-week old) fed on different prey density (ind. mL^{-1}). Values are mean ± standard error from three replicates. Values with different letters are significantly different ($P < 0.05$).

2-week-old larvae for 2, 5 and 10 ind. mL^{-1} of initial prey density respectively (Fig. 3).

There were significant differences between ingestion rates of *E. serrulatus* and *C. quadrangula* at combined situation. The daily ratio of ingestion rates were about 68:32, 75:25, 74:26 for 1-week-old larvae (Fig. 4a), 71: 29%, 67: 33% and 68: 32% for 2-week-old larvae (Fig. 4b) and 48:52, 39:61 and 40:60 for 3-week-old larvae (Fig. 4c) at prey density of 2, 5 and 10 ind. mL^{-1} respectively.

The electivity indices (E) of different size groups of *P. scalare* larvae for *E. serrulatus* and *C. quadrangula* at three different prey densities are presented in Fig. 5. The electivity indices of 1-week-old larvae for *E. serrulatus* copepodids were +0.18, +0.23 and +0.22 at prey densities of 2, 5 and 10 ind. mL^{-1} respectively (Fig. 5A). For 2-week-old larvae, the E values for the same prey and density decreased, their values being +0.18, and +0.18, but not in the case of low density prey, but the E values for 3-week-old larvae were + 0.04, −0.05 and −0.04, indicating lower tendency towards *E. serrulatus* consumption. Tendency towards *E. serrulatus* copepodid consumption reduced by aging *P. scalare* as indicated by the E values for 2- and 3-week-old larvae (Figs. 5B, 5C). On the other hand, the E values of 1-week-old larvae for *C. quadrangula* were around −0.29, −0.41 and −0.40 at food density 2, 5 and 10 ind. mL^{-1} respectively. These E values for 3-week-old larvae were −0.04, +0.04 and +0.03 respectively. Thus, the E values for *C. quadrangula* increased positively at 3-week-old larvae as compared with the 2-week-old larvae and 1-week-old larvae.

The larval survival and specific growth rate (based dry weight) in different larval size groups

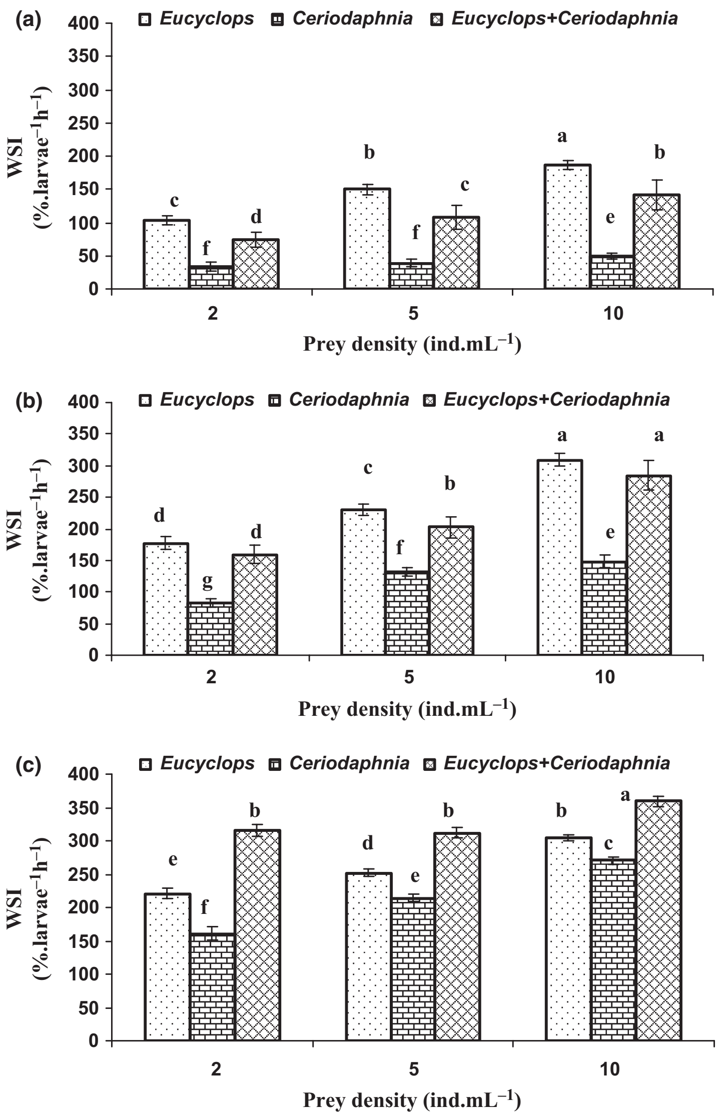

Figure 2 Weight-specific ingestion (WSI) (% larvae^{-1} h^{-1}) of *Pterophyllum scalare* larvae (a = 1-week old, b = 2-week old, c = 3-week old) fed on different prey density (ind. mL^{-1}). Values with different letters are significantly different ($P < 0.05$).

that were fed on *E. serrulatus* alone and its combination with *C. quadrangula* were higher than those fed on only *C. quadrangula* (Table 3). The survival rates were 86.0%, 81.5% and 91.5% at 1-week-old larvae when fed with *E. serrulatus, C. quadrangula* and *E. serrulatus* + *C. quadrangula* treatments at maximum prey density. Correspondingly, survival rates for 2-week- and 3-week-old larvae fed the same prey and density ranged between 86% and 93%, and 91.5% and 100% respectively (Table 3).

Discussion

The copepod and cladoceran are natural nutritive prey for freshwater fish larvae (Nandini & Sarma 2000, 2007). They have suitable size and nutritional value that make them a suitable live food for aquaculture industry (Nandini & Sarma 2000; Farhadian, Khanjani, Keivany & Ebrahimi Dorche 2012a). In this study, *E. serrulatus* and *C. quadrangula* successfully were cultured on mixed green microalgae of *C. vulgaris* and *S. quadricauda*. Our findings showed that their nutritional value of mentioned prey could be met nutritional requirements of some of cultured fish larvae (Farhadian *et al.* 2012a). Similar to most fish larvae, feeding of *P. scalare* larvae is also based on consumption of zooplankton (Garcia-Ulloa & Gomez-Romero 2005).

This study demonstrates that type of zooplankton and its density influence ingestion rate and

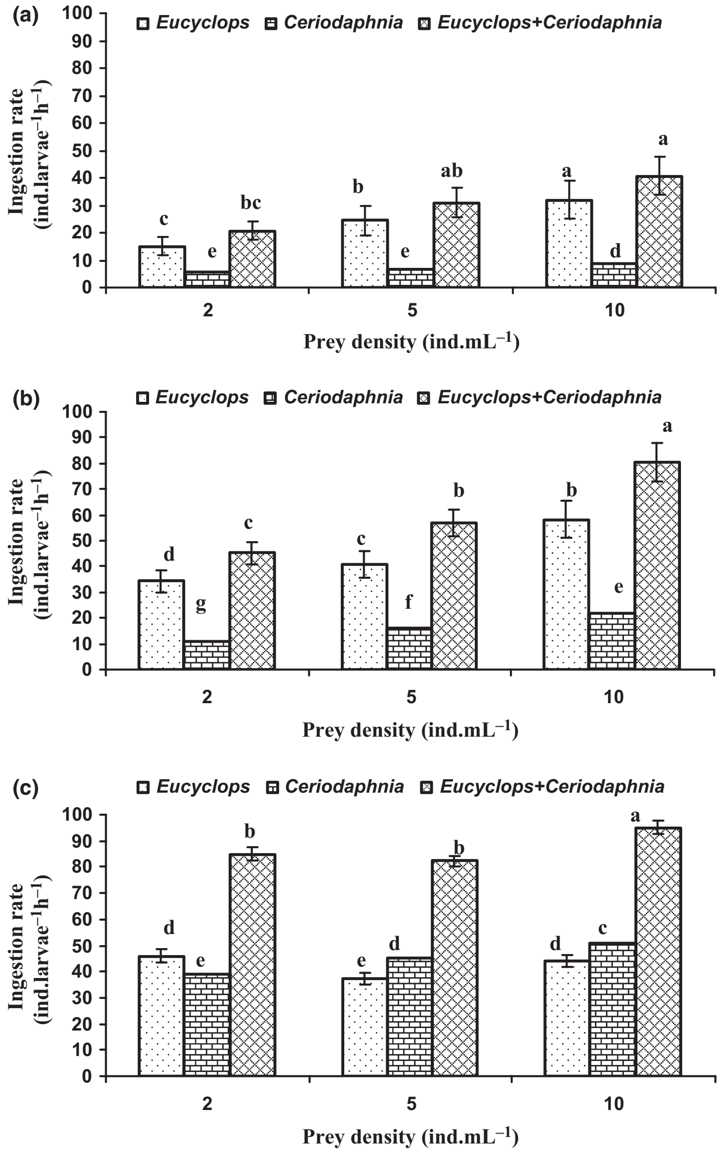

Figure 3 Ingestion rates (prey larvae^{-1} h^{-1}) of *Eucyclops serrulatus* copepodids and *Ceriodaphnia quadrangula* when offered in 1:1 combination at different prey density (ind. mL^{-1}) to *Pterophyllum scalare* larvae (a = 1-wk-old, b = 2-wk-old, c = 3-wk-old). Values with different letters are significantly different ($P < 0.05$).

prey selection by different size classes of *P. scalare* larvae. Generally in all fish species, there is an age-dependent increase in body size that cause different responses to various aspects of feeding behaviour such as encounter, attack, capture and ingestion (Nandini & Sarma 2000; Sarma *et al.* 2003; Graeb *et al.* 2004). Accordingly, *P. scalare* larvae showed similar behavioural response to different preys during our study period, as indicated by changes in ingestion rate and selectivity of prey.

In general, ingestion rate increased with increasing density of prey items, which could be attributed to the higher chance of encountering larvae with prey items. Previous researchers also reported an increased ingestion rate with increasing food or prey density in fish and crustaceans (Chakrabarti & Jana 1990 on common carp, *Cyprinus carpio*; Celino *et al.* 2011 on common seahorse, *Hippocampus kuda*; Graeb *et al.* 2004 on yellow perch, *Perca flavescens*; Yufera, Rodriguez & Lubian 1984 on grooved shrimp, *Penaeus kerathurus*; Chu & Shing 1986 on greasyback shrimp, *Metapenaeus ensis*; Alam 1992 on giant freshwater prawn, *Macrobrachium rosenbergii*; Farhadian, Yusoff & Arshad 2007 on giant tiger prawn, *Penaeus monodon*; Farhadian *et al.* 2012a on Mayan cichlid, *Cichlasoma urophthalmus*). It is also well established that increased chance of

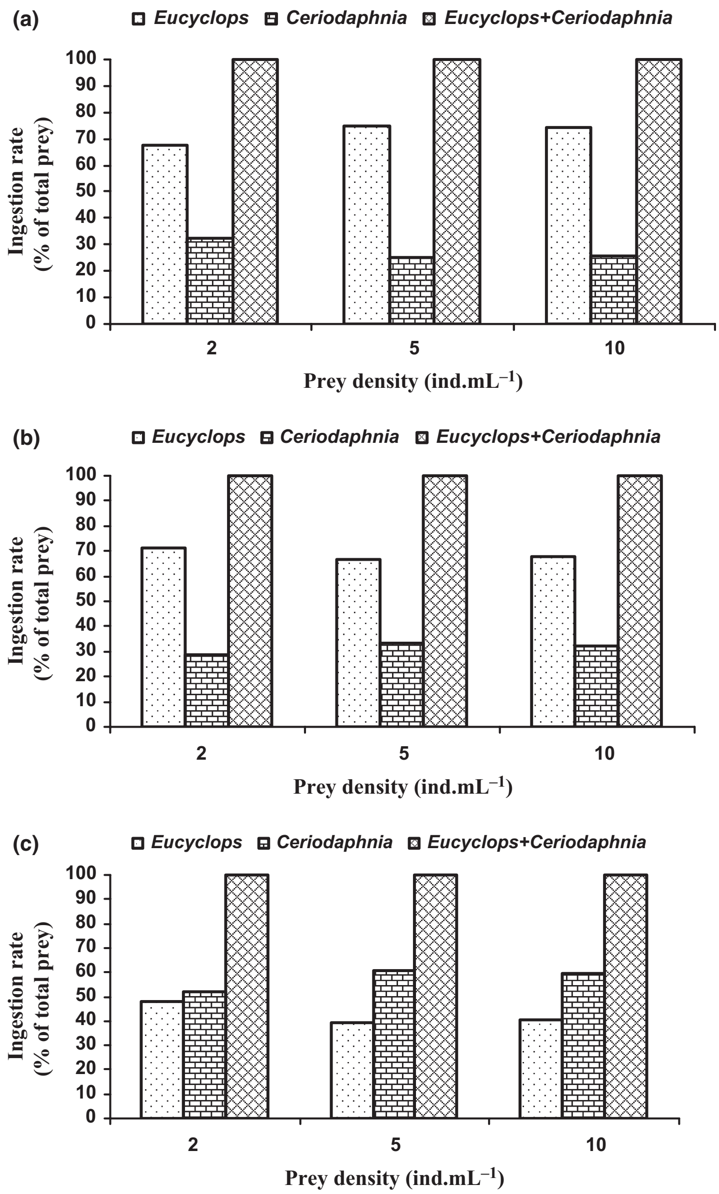

Figure 4 Ingestion rates (% of total prey) of *Eucyclops serrulatus* copepodids and *Ceriodaphnia quadrangula* when offered in 1:1 combination at different prey density (ind. mL^{-1}) to *Pterophyllum scalare* larvae (a = 1-week old, b = 2-week old, c = 3-week old).

encounter is very important for capture success by fish larvae, especially at early larval stage (Nandini & Sarma 2000; Sarma *et al.* 2003; Graeb *et al.* 2004).

In terms of maximum ingestion and maximum survivorship of 1-week-old larvae, optimal feeding rates were estimated at 56.7 ind. h^{-1} for *E. serrulatus*, 12.0 ind. h^{-1} for *C. quadrangula* and 40.7 ind. h^{-1} for *E. serrulatus* + *C. quadrangula*. Correspondingly, for 2-week-old and 3-week-old larvae, these values were 93.7 and 92.0 ind. h^{-1} for *E. serrulatus*, 35.3 and 64.3 ind. h^{-1} for *C. quadrangula* and 80.3 and 95.3 ind. h^{-1} for *E. serrulatus* + *C. quadrangula* respectively.

The ingestions rate of *E. serrulatus* by 1-week- and 2-week-old larvae when offered together with *C. quadrangula* differed from the time that similar combination presented to 3-week-old larvae (Fig. 4). Therefore, it can be concluded that younger larvae have a higher preference towards *E. serrulatus,* whereas the older larvae have a higher tendency towards *C. quadrangula* consumption. On the other hand, the ingestion rates of *E. serrulatus* nauplii by 1-week-, 2-week- and 3-week-old larvae

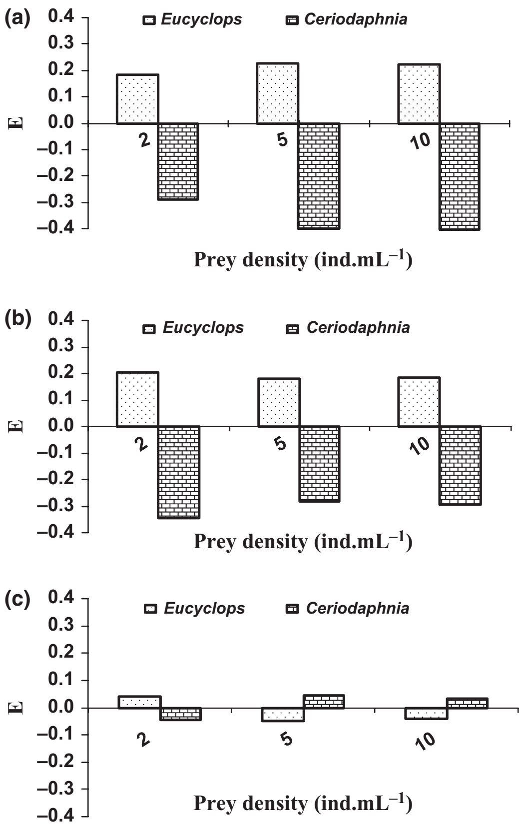

Figure 5 Electivity index (E) of *Eucyclops serrulatus* copepodids, *Ceriodaphnia quadrangula* (1:1 combination) by *Pterophyllum scalare* larvae (a = 1-week old, b = 2-week old, c = 3-week old) fed on different prey density (ind. mL^{-1}).

were very low due to small size and weight, and perhaps low capturing ability of larvae,. That is why *E. serrulatus* copepodids were used instead of nauplii as a diet.

The larger larvae of *P. scalare* (2-week and 3-week old) showed a better growth and survival rate when feeding on *C. quadrangula* than on *E. serrulatus* copepodids. An increase in the age of larvae not only increase gape size but also enhanced probability of capturing, attacking and encountering that result in a higher ingestion rate in larger larvae (3-week old vs. 1- and 2-week old). In general, larvae of most fish cannot consume prey larger than 500-μm diameter in the first weeks (Khadka & Rao 1986; Mookerji & Rao 1994). Actually, the gape size is a good indicator of prey size that fish larvae could handle (Yasuda 1960; Cunha & Planas 1999). Larvae of *P. scalare* have slightly larger mouth sizes at first week that make it suitable for a better ingestion of *E. serrulatus* copepodids than *C. quadrangula*. Cladocerans were also less important than copepodid in 1-week-old larvae, perhaps, because of inappropriate size. Furthermore, larvae of *P. scalare* may become more effective predators as soon as grow up and become more capable for improving prey capturing. This finding could possibly be explained by the size-efficiency hypothesis (Brooks & Dodson 1965). Differences in size between *E. serrulatus* and *C. quadrangula* may effect on feeding behaviour of *P. scalare* larvae.

Our findings showed that availability (prey density) of copepod and small cladocerans is also important factor in growth and survival rates of *P. scalare*. However, the rates of growth and survival were different in response to the food items. The differences could be attributed to morphological differences in both *P. scalare* larvae and the zooplankton that might affect components of feeding behaviour (Nandini & Sarma 2000; Sarma *et al.* 2003; Graeb *et al.* 2004). The copepodid of copepods are cylindrical with uniform body width and relatively faster swimming (Williamson & Reid 2001). Contrary, cladocerans are spherical shaped and move by small jumps through the water column. It is noted that capture success is higher for 2-week- and 3-week-old larvae when feeding on

Table 3 Mean (± SE) of survival rate (%) and specific growth rate (% mg day^{-1}) of *Pterophyllum scalare* larvae fed on *Eucyclops serrulatus* copepodids, *Ceriodaphnia quadrangula* and their 1:1 mixture at maximum prey densities. Values in each column with different superscript letters are significantly different ($P < 0.05$).

	Survival rate			Specific growth rate		
Preys	1 week	2 weeks	3 weeks	1 week	2 weeks	3 weeks
Eucyclops	$86.0^{b} \pm 3.0$	$89.0^{b} \pm 1.5$	$93.0^{b} \pm 3.0$	$19.2^{b} \pm 1.7$	$15.1^{b} \pm 1.2$	$12.1^{b} \pm 0.1$
Ceriodaphnia	$81.5^{c} \pm 6.0$	$86^{c} \pm 1.5$	$91.5^{b} \pm 1.5$	$18.8^{b} \pm 2.5$	$14.5^{b} \pm 2.1$	$11.8^{b} \pm 1.7$
Eucyclops + *Ceriodaphnia*	$91.5^{a} \pm 3.0$	$96^{a} \pm 1.5$	$100^{a} \pm 0.0$	$23.2^{a} \pm 1.8$	$16.5^{a} \pm 1.2$	$12.3^{a} \pm 1.7$

cladocerans, but a higher tendency towards copepodid was noticed in the case of 1-week-old larvae. Similar findings have been reported for the other ornamental fish larvae. For example, black crappie *Pomoxis nigromaculatus*, freshwater drum *Aplodinoyus grunniens* and bluegills *Lepomis macrochirus* feeding on copepod nauplii at small sizes, and cladocearn and adult copepods with increasing length/age and gape width (Barbosa & Matsumura-Tundisi 1984; Nandini & Sarma 2000; Sarma *et al.* 2003; Graeb *et al.* 2004).

There is virtually little information concerning ingestion and survival rates of *P. scalare* larvae fed on copepod species for comparison with our results. However, Brick (1974) and Bryan and Madraisau (1977) reported a better survival rate for larvae of *Scylla serrata* and *Siganus lineatus*, respectively, when fed on copepod compared with *Artemia*. Our present study showed that the highest ingestion rates were 41.5 ind. h^{-1} for 1-week-old larvae and 101.6 ind. h^{-1} for 2-week-old larvae when fed *E. serrulatus* and *C. quadrangula* respectively. This study indicated that *P. scalare* larvae can generally ingest *E. serrulatus* selectively much better than *C. quadrangula* at 1:1 combination, hence suggesting a better prey for larvae culture of *P. scalare*. Growth rate and dry weight of our larvae also improved significantly when offered *E. serrulatus* as prey solely or in combination with *C. quadrangula*. According to Ryer and Boehlert (1983), the total energy gained is increased by the greater number of prey items; therefore, the increasing ingestion rate of *P. scalare* larvae on mixed diets may have led to faster growth and better survival rate. In addition, the *P. scalare* may discrepate between *E. serrulatus* and *C. quadrangula*, due to differences in their nutritional quality including levels of highly unsaturated fatty acids (HUFAs). Studies conducted by other workers on yellowtail clownfish, *Amphiprion clarkia* (Olivotto, Buttino, Borroni, Piccinetti, Malzone & Carnevali 2008) and on seahorse, *Hippocampus kuda* (Celino *et al.* 2011) also strongly suggested the use of copepod as live food.

Our findings demonstrate that *E. serrulatus* and *C. quadrangula* on its own or in combination influence the survival and growth of *P. scalare* larvae. We speculate that the higher observed survival and growth rates of larvae fed on *E. serrulatus* and *E. serrulatus* + *C. quadrangula* could possibly resulted from the higher capturing and ingestion rate, lower time consuming and higher digestibility (Confer & Lake 1987), higher energetic gains (Graeb *et al.* 2004) and a better biochemical composition (Delbare, Dhert & Lavens 1996; Sargent, McEvoy, Estevez, Bell, Bell, Henderson & Tocher 1999; Olivotto *et al.* 2008) of copepod prey. Overall, survival and growth of *P. scalare* was improved by application of diets containing *E. serrulatus* copepodid, as larval age increased.

Conclusion

Our research on quantification of prey ingestion rate, prey selection, survival and growth rate of different size *P. scalare* larvae showed that application of copepod as live food results in high performance, especially in 1-week-old larvae. Daily rations (live feed) for *P. scalare* larvae were found to be size, type and density dependent. Based on present results, optimum *P. scalare* larvae rearing practice could be attained at high prey density of 5–10 ind. mL^{-1} *E. serrulatus* or *E. serrulatus* + *C. quadrangula*; however, more experimentation is necessary to assay the effect of higher densities of prey on *P. scalare* performance.

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