JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 44, No. 1
February, 2013

Effects of Microalgae and Alfalfa Meal on Population Growth and Production of a Freshwater Rotifer, *Euchlanis dilatata* (Rotifera: Mongononta)

Omidvar Farhadian[1], Leila Daghighi, and Eisa Ebrahimi Dorche

Department of Natural Resources, Isfahan University of Technology, Isfahan 84156-83111, Iran

Abstract

Population growth and production of the freshwater rotifer, *Euchlanis dilatata*, were determined after being fed 1 of 12 diets: *Scenedesmus quadricauda* (S), *Chlorella vulgaris* (C), baker's yeast (*Saccharomyces cerevisiae*) (Y), alfalfa meal (*Medicago* spp.) (A), and eight combinations of S+C, S+Y, C+Y, S+C+Y, S+A, C+A, S+C+A, and Y+A (mixture in the same ratio by equal nitrogen weight). Results showed that both microalgal and alfalfa meal alone and in combination with Y had a significantly higher ($P < 0.05$) population growth and production compared with Y alone. The mean maximum *E. dilatata* population density fed on S, S+Y, C, C+Y, S+C, S+C+Y, Y, A, S+A, C+A, S+C+A, and Y+A diets were 65 ± 7.5 (mean $\pm$ SE), 124.6 ± 17.2, 115.8 ± 7.9, 41.5 ± 7.9, 131 ± 14.1, 93.5 ± 8.7, 14.5 ± 7.6, 129.5 ± 18.5, 255.0 ± 20.5, 180 ± 30.5, 124.5 ± 25.4, and 61.5 ± 24.5 individuals/mL, respectively. Correspondingly, the mean population growth rates (K) through the 16-d culture period were 0.29, 0.40, 0.45, 0.27, 0.34, 0.41, 0.26, 0.51, 0.58, 0.59, 0.39, and 0.45/d, respectively. Results indicated that alfalfa meal in combination with microalgae and yeast could be used as primary diets for *E. dilatata* culture. This may reduce the costs of rotifer production.

Microalgae such as green algae of *Chlorella vulgaris* and *Scenedesmus* spp. are generally used for culture of freshwater zooplankton, especially cladocerans and rotifers (Pena-Aguadoa et al. 2005; Abedian Kennari et al. 2008b; Espinosa-Rodriguez et al. 2011; Serrania-Soto et al. 2011). This is because these microalgae have sufficient nutrients to meet requirements in most zooplankton species (Ahlgren et al. 1992). Microalgae can be difficult to produce in mass scale and require large tank space; this can be a limiting factor in zooplankton mass cultures. Therefore, research to find partial replacement for microalgae is in progress. The use of baker's yeast, *Saccharomyces cerevisiae*, as food for mass production of rotifers has been used (Hirayama and Funamoto 1983; Lubzens et al. 2001; Pena-Aguadoa et al. 2005). Although easy and convenient mass cultivation of marine rotifers have been achieved by rearing them on baker's yeast (Watanabe et al. 1983; Hirayama 1987), their production is often unstable and they are nutritionally deficient for feeding to larval fish due to the lack of highly unsaturated fatty acids (HUFAs) (Hirayama and Watanabe 1973; Watanabe et al. 1978). Many early stages of cultured marine fish larvae are commonly fed rotifers in large amounts and are eventually switched to larger prey. The first-feeding fish larvae, especially marine fish larvae, are usually small in size, have poorly developed eyes, do not swim well, and require easily digested diets (Yoshimura et al. 1996; Sarma et al. 2003).

To overcome these shortcomings and deficiencies that exist in yeast, the use of algal and non-algal diets in mixture with baker's yeast could support population growth and production better than mono-diets alone (Hirayama and Funamoto 1983; Hirayama and Satuito 1991). The nutritive quality of rotifers has been improved by feeding them microalgae or emulsified oil prior to feeding to fish larvae (Watanabe et al. 1983). In addition, microalgae mostly

[1] Corresponding author.

need vitamins (auxotrophic), which depend on their nutrient uptake, and could lead to a better understanding of rotifer growth and production (Scott 1981; Hirayama and Satuito 1991). Both nutrients and vitamins are consumed by rotifers via algal cells, algal culture medium, or a combination of both (Scott 1981). One of the suitable non-algal diets for zooplankton culture is organic fertilizers. Adequate populations of zooplankton and suitable pH may be achieved by using organic fertilizers (Anderson 1993b). In fact, the organic fertilizers differ in their physical properties, decomposability, available nutrients, and nutritional value of zooplankton (Barkoh and Rabeni 1990; Anderson 1993a). Alfalfa or *Medicago* spp. (Family Papilionaceae) is a common perennial crop species. Alfalfa meal as an organic fertilizer contains high protein and vitamins of A, B1, B6, C, E, and K as well as elements of Ca, K, Fe, and Zn (Radcliffe and Cochrane 1970; Briggs 1994; Zhu et al. 1996; Ehsanpour and Fatahian 2003). In addition, it is rich in essential enzymes of lipase, amylase, coagulase, emulsin, invertase, peroxidase, pectinase, and protase and may aid in digestion. Alfalfa meal has been used for culture of cladocerans, *Ceriodaphnia dubia* and *Daphnia magna* (Barkoh and Rabeni 1990). They concluded that both species survived better and produced more neonates in cultures with alfalfa meal and thus they recommended the use of alfalfa meal for culture of *Ceriodaphnia*. Alfalfa meal as non-algal diet, therefore, could be suitable as a food for freshwater rotifers, such as *Euchlanis dilatata*.

Euchlanis dilatata (Rotifera: Mongononta) is a freshwater littoral rotifer and is similar to many other rotifers because they are a cosmopolitan species and are distributed in both acidic and alkaline waters (Myers 1930; King 1967). This species is periphytic and its diet naturally includes diatoms, green and blue-green algae (King 1967; Gulati et al. 1987). Previous works indicated that *E. dilatata* have advantages compared to *Brachionus* spp., such as suitable lorica biovolume (120–150 μm^3 in *Brachionus* and 250–300 μm^3 in *E. dilatata*) (McCauley 1984; Abedian Kennari et al. 2008a), better consumption of planktonic algae, better competition, resistance to starvation, and ability to reproduce at lower food level (King 1967; Nandini and Sarma 2002). In our observation, we found that *E. dilatata* was frequently attacked and captured by ornamental fish larvae, such as angel fish (*Pterophyllum scalare*), guppy (*Poecila reticulata*), Mayan cichlid (*Cichlosoma urophthalmus*) and also larvae of sturgeon and cyprinids fishes at early larval stages (1-wk larvae). In contrast to literature available on rotifer *Brachionus* spp., research on the culture and production of the freshwater rotifer, *E. dilatata* is lacking. The objective of this study was to evaluate the effects of algal and alfalfa meal versus baker's yeast as diets on population density and growth rates on the freshwater rotifer, *E. dilatata*.

Materials and Methods

Diet Preparation

Green microalgae, *Scenedesmus quadricauda* (11.5 μm length, excluding spines, and 5.9 μm width) and *C. vulgaris* (about 5 μm), were cultured in Bold's basal medium (Nichols and Bold 1965), at a water temperature of 25.3 C, 12 h light : 12 h dark photoperiod, and a light intensity of 60 μmol photons/m^2/sec in 5-L carboys with mild continuous aeration for 7 d. The algal density was determined using a Neubauer hemocytometer (0.25 $mm^2 \times 0.1$ mm) under an inverted microscope (Ceti, Leuven, Belgium), both in live and fixed in Lugol's iodine solution (0.1 mL for 3 mL sample) (Martinez and Chakroff 1975). At the stationary growth phase, microalgal cells were harvested by centrifuging (Centurion Scientific Ltd, Stoughton, UK) at 503 *g* for 5 min. The harvest of microalgae occurred daily before starting to prepare the rotifer stock (Heasman et al. 2000) and was then chilled to 4 C. The dry weight of microalgal cells and yeast were determined by filtering and drying algae and yeast from aliquots of known concentration according to the method described by Lavens and Sorgeloos (1996). Protein contents of the diets were determined following the method described by Meyer and Walther

TABLE 1. *Mean (±SE, N = 3) protein and fatty acid composition (% total fatty acid) of* Scenedesmus quadricauda, Chlorella vulgaris *and baker's yeast used in this experiment.*

Fatty acids	*S. quadricauda*	*C. vulgaris*	Baker's yeast
Protein (% DW)	43.5 ± 2.23	45.5 ± 3.51	40.1 ± 2.33
C14:0	1.50 ± 0.20	0.63 ± 0.08	0.5 ± 0.01
C14:1n-5	–	0.28 ± 0.07	0.1 ± 0.00
C15:0	–	–	0.1 ± 0.00
C16:0	9.45 ± 1.30	24.93 ± 0.16	8.6 ± 0.02
C16:1n-7	0.75 ± 0.22	1.42 ± 0.02	15.5 ± 0.29
C17:0	–	–	–
C17:1n-7	–	–	–
C18:0	0.65 ± 0.15	8.26 ± 0.04	3.5 ± 0.05
C18:1n-9	3.20 ± 0.40	2.29 ± 0.01	20.1 ± 0.02
C18:1n-7	0.45 ± 0.05	30.03 ± 0.37	–
C18:2n-6	7.45 ± 1.25	6.58 ± 0.04	2.2 ± 0.00
C18:3n-3	25.61 ± 4.60	2.28 ± 0.01	0.8 ± 0.00
C20:0	–	0.76 ± 0.00	4.4 ± 0.06
C20:1n-9	0.35 ± 0.07	0.37 ± 0.01	–
C20:4n-6	–	–	–
C20:3n-3	–	–	–
C:20:4n-3	–	–	–
C20:5n-3	–	–	–
C22:0	–	0.83 ± 0.01	–
C22:1n-9	–	0.66 ± 0.00	–
C22:6n-3	–	–	–
C24:0	–	–	–
C24:1n-9	–	0.65 ± 0.01	–
Total SAFAs	11.60	27.43	17.1
Total MUFAs	4.75	35.7	35.7
Total PUFAs	33.06	8.87	3.0
Total n-3	25.61	2.28	–
Total n-6	7.45	6.58	–
n-3:n-6	3.44:1	0.35:1	–

– = not detectable. DW = dry weight; SAFAs = saturated fatty acids; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids.

(1988). In addition, the fatty acid composition of the microalgae and baker's yeast were prepared according to the direct methylation technique (Divakaran and Ostrowski 1989).

The microalgal density was controlled during *E. dilatata* culture to prevent any contamination as well as to keep the microalgal density constant during the experiment. The mixed microalgal diets were prepared with the same nitrogen weight ratio.

Baker's yeast was dissolved in distilled water in concentration of 1 mg/mL at laboratory conditions the same as microalgal cultures. The fatty acid composition of microalgae and yeast used are presented in Table 1.

Alfalfa meal used in this study was prepared from *Medicago sativa*, *Medicago rigidulla, Medicago polymorpha*, and *Medicago scutellata* harvested at an early blooming growth stage and then dried for 2 wk. Then, alfalfa meal was prepared with mixture of four alfalfa species in the same ratio in dry weight because the better rotifer performance was obtained in mixture compared to alone. The dry matter, organic matter, ash, crude protein, Ca, P, and K of alfalfa meal were determined according to AOAC (1990) for their mixture alfalfa meal which prepared in the same ratio in weight. The chemical compositions were 90.5 ± 2.2, 85.1 ± 1.5, 13.1 ± 0.6, 20.5 ± 3.2, 1.3 ± 0.1, 0.25 ± 0.02, and 1.5 ± 0.1% dry weight. The dried alfalfa meal was sieved (size <20 μm) and then spread on trays in very thin layer under

UV-irritation for 12 h before fed to rotifers to prevent possible microbial contamination.

E. dilatata Stock Preparation

Euchlanis dilatata samples were collected from Hanna Dam Lake (Latitude = 31°13′–31°14′N; Longitude = 52°46′–52°47′E), located in Eastern part of Isfahan province in central Iran. To prepare *E. dilatata* stock, an asexually reproducing rotifer isolated in laboratory was used throughout the experiment. The rotifers were fed for several generations in a 50-mL flask. The autoclaved medium of EPA (96 mg NaHCO3, 60 mg CaSO4, 60 mg MgSO4, and 4 mg KCl in 1 L of distilled water according to Flores-Burgos et al. 2003) was used. The stock was maintained at water temperature of 25 C, an initial pH of 7.5 and aerated vigorously using mixed diets of S+C+Y+A (mixture in the same ratio by equal nitrogen weight) to counterbalance possible effects of diet, which they had previously been fed. The stock culture was maintained at the Fishery Research Laboratory at Isfahan University of Technology (FRL-IUT) under 12 h light : 12 h dark photoperiod and light intensity of 60 µmol photons/m^2/sec. During daily maintenance of stock cultures, dead individuals were removed and quantified and 20% of the medium was exchanged every other day.

Experimental Procedure

We tested the effects of feeding 12 diets of monoalgae, non-algae, and their mixture on population growth rates and production of *E. dilatata*. The diets were *S. quadricauda* (S), *C. vulgaris* (C), baker's yeast (*Saccharomyces cerevisiae*) (Y), alfalfa meal (*Medicago* spp.) (A), and eight combinations of S+C, S+Y, C+Y, S+C+Y, S+A, C+A, S+C+A, and Y+A (mixture in the same ratio by equal nitrogen weight) (Barkoh et al. 2005). The protein content of S, C, Y, and A was 43.5, 45.5, 40.1, and 20.5 % dry weight, respectively. The available nitrogen content in each diet was estimated according to equation of N = Percent protein/6.25 as used by Anderson (1993b) and Barkoh et al. (2005). The nitrogen content of S, C, Y, and A was 6.96, 7.28, 6.41 and 3.28% dry weight, respectively, and ratio was calculated as 2.12:2.22:1.95:1 for S:C:Y:A. The diets used in this experiment were offered at a quantity of 10 mg dry weight/L/d.

The experiment consisted of a completely randomized design with 12 treatments (12 diets) with 3 replicates each treatment. It was run in 36 glass 250-mL flasks each filled with 200 mL of filtered autoclaved of culture medium (EPA) and 200 individuals of *E. dilatata* (from laboratory stock culture). The feeding frequency was twice daily (morning and evening). Flasks were shaken manually four times per day to ensure proper mixing inside the flasks. The culture medium was entirely exchanged every 3 d by pouring the *E. dilatata* culture through a 40-µm plankton net which was small enough to retain the *E. dilatata* but large enough to remove most of the detritus, other solid wastes. This also allowed bacterial and algal growth on the flasks to be cleaned. Each flask was examined daily and any dead individuals and uneaten diet were removed as much as possible. The rotifer culture conditions were 25 C temperature, a photoperiod of 12 h light : 12 h dark cycle and 60 µmol photons/m^2/sec light intensity. Population growth rate of *E. dilatata* was studied for a 16-d period.

The *E. dilatata* population was quantified every other day by removing 5-mL subsamples from each flask (three for each diet) and placing under a dissecting microscope using a Bogorov's plate chamber. All rotifers were counted alive. At end of the experiment (Day 16), rotifers were fixed with a NaCl solution of 5 g/L for length and width measurements (30 individual rotifers for each replicate). The population growth rates (K) were calculated using the formula: $K = \ln N_t - \ln N_o/t$ where N_o and N_t are the initial and final (highest) densities of *E. dilatata*, respectively, and t is the culture days (James and Al-Khars 1986). Doubling time (Dt) was calculated using the formula: $Dt = 0.693/K$ (James and Al-Khars 1986). The length and width of

lorica were measured using an ocular micrometer under microscope using magnification of ×40.

Statistical Analysis

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

Results

Nutritional values of the microalgae used for culturing *E. dilatata* presented in Table 1. The composition of saturated fatty acids (SAFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) were significantly different ($P < 0.05$) among the algal foods. Amount of PUFAs in *S. quadricauda* (33.1%) was higher significantly compared to *C. vulgaris* (8.9%). On the contrary, the amounts of SAFAs and MUFAs in *C. vulgaris* food, 27.4 and 35.7%, respectively, were higher significantly ($P < 0.05$) compared to *S. quadricauda* (11.6 and 4.8%). The dry weights were 0.50×10^{-5} µg/cell and 1.55×10^{-5} µg/cell for the algae, *S. quadricauda* and *C. vulgaris*, respectively.

Population dynamics of *E. dilatata* fed different diets (Fig. 1) showed that diets mixed with yeast (Y) (dry weight $= 2.3 \times 10^{-5}$ µg/cell) decreased population growth, while diets mixed with algal (S and C) and alfalfa (A) significantly ($P < 0.05$) increased the population. *E. dilatata* reached the peak population density at different rates with each diet, diets containing Y took the shortest time to reach the peak.

The daily population increase rate (K) and doubling time (Dt) of *E. dilatata* fed different diets (Fig. 2) showed that maximum K and minimum Dt mostly obtained at second culture day. The range of K was 0.05–1.60/d, lowest with Y and highest with C+A, while Dt

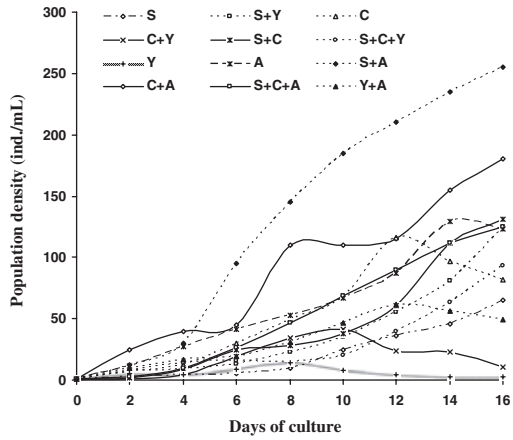

FIGURE 1. *Mean of population density (individuals/mL)* Euchlanis dilatata *fed on different algal and non-algal diets during culture days.* Scenedesmus quadricauda *(S),* Chlorella vulgaris *(C), baker's yeast (*Saccharomyces cerevisiae*) (Y), and alfalfa meal (*Medicago *spp.) (A).*

ranged 0.43–12.10 d, lowest with C+A and highest with Y. Generally, the increased rate of population growth reduced with the number of culture days of rotifer *E. dilatata*.

To compare the effects of each diet, mean production (based on individuals/mL/d), mean population increase and mean doubling time of the population of *E. dilatata* were also calculated (Fig. 3). Results showed that diets of A, S+A, and C+A were faster than Y, S+Y, and C+Y. The mean production and mean daily population increase (K) of *E. dilatata* through experiment ranged from 5.7 to 129.9 individuals/mL/d and 0.26 to 0.59/d, respectively, maximum with S+A and C+A; and minimum with Y diet (Fig. 3).

The peak population time of *E. dilatata* fed on C, C+Y, Y, and Y+A were obtained in Days of 12, 10, 8, and 12, respectively, which were significantly ($P < 0.05$) shorter than that other diets (Fig. 4). The highest population densities attained were 255 individuals/mL (at Day 16) and 180 individuals/mL (at Day 16) for S+A and C+A, respectively (Fig. 4).

Means of lorica length and width of *E. dilatata* varied according to the examined diets. The measured lorica length ranged from 195 to 302 µm, those fed Y were larger than

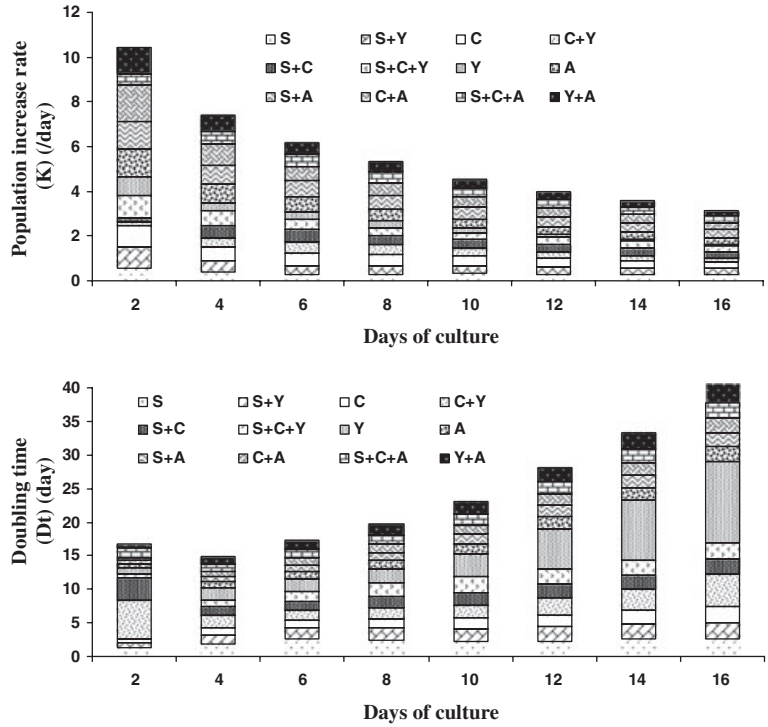

FIGURE 2. *Mean of population increase rate (*K*) (/d) and doubling time (d) (Dt) densities* Euchlanis dilatata *fed on different algal and non-algal diets during culture days.* Scenedesmus quadricauda *(S),* Chlorella vulgaris *(C), baker's yeast (*Saccharomyces cerevisiae*) (Y), and alfalfa meal (*Medicago *spp.) (A).*

those fed C+A, while the lorica width ranged from 108 to 257 μm, those fed with Y being larger than those fed C (Fig. 5).

Discussion

The results of this study indicate that the diets containing Y compare to diets of algal and alfalfa meal had reduced population growth and production of freshwater rotifer, *E. dilatata*. Generally, baker's yeast can be used as an algal substitute in live food production. There are obvious benefits to this practice, such as the reduction of algal production facilities. However, baker's yeast contains mostly palmitoleate (C16:1n-7) and oleic (C18:1n-9) fatty acids, it is completely devoid of PUFAs (Farhadian et al. 2008). Population growth of *E. dilatata* fed on Y alone was poor, probably due to their lack of PUFAs content and vitamins, lower filtration rate and aggregation of Y. On the contrary, the results obtained in this study stated that the diets containing microalgal diets (S, C, and S+C) and non-algal diets of A and their combination have a positive supplementary effect on the nutritive deficiency of Y for population growth and production of rotifer *E. dilatata*. Diets containing algal and alfalfa meal experienced increased production, which may be due to the PUFA content of microalgae and the chemical composition and nutritive value of the alfalfa meal.

The mean population growth rate (K) in *E. dilatata* ranged from 0.26 to 0.59/d. These results could be comparable with the findings of previous studies on freshwater rotifer species of *Brachionus patulus* (0.002–0.578/d) and *E. dilatata* (0.006–0.447/d) using *C. vulgaris* (Nandini and Sarma 2002) and Lecanidae (in general, <0.5/d) (Sarma et al. 2010; Serrania-Soto et al. 2011).

The highest population density of *E. dilatata* ranged from 14.5 to 255 individuals/mL,

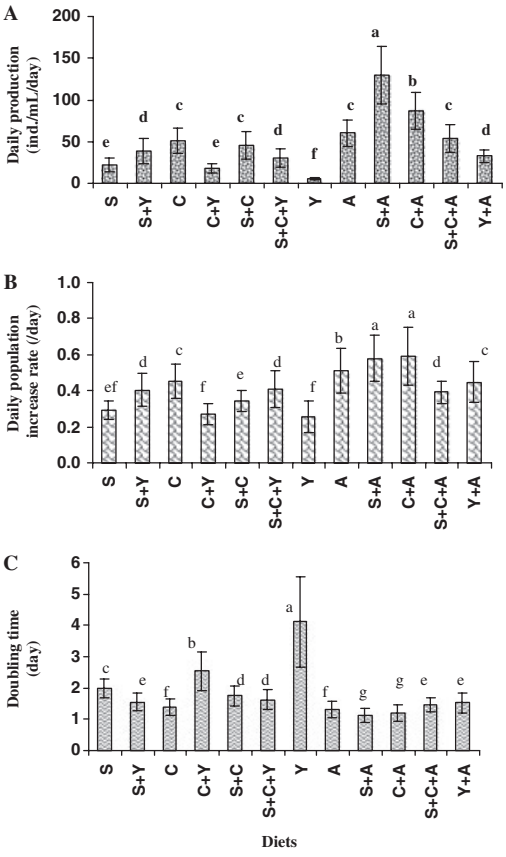

FIGURE 3. *Mean (±SE) of daily production (individuals/mL/d) (A), daily population increase rate (/d) (B), and doubling time (d) (C) of* Euchlanis dilatata *fed on different algal and non-algal diets. Bars with the same letters are not significantly different (*$P > 0.05$*).* Scenedesmus quadricauda *(S),* Chlorella vulgaris *(C), baker's yeast (*Saccharomyces cerevisiae*) (Y), and alfalfa meal (*Medicago *spp.) (A).*

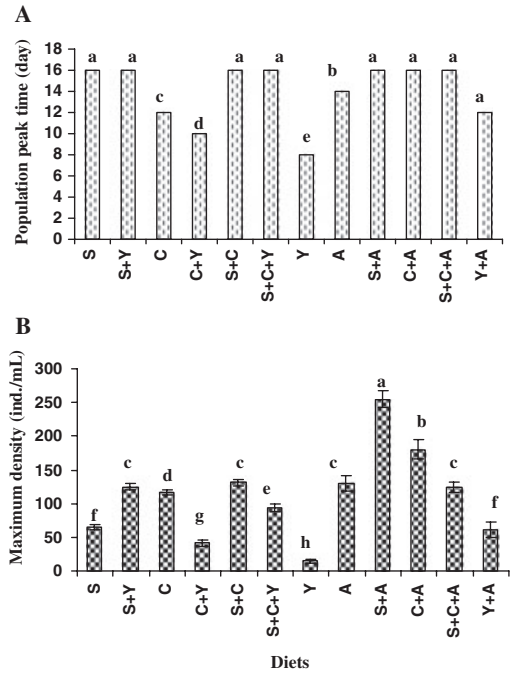

FIGURE 4. *Mean (±SE) of population peak time (d) (A) and maximum density of population (individuals/mL) (B) of* Euchlanis dilatata *fed on different algal and non-algal diets. Bars with the same letters are not significantly different (*$P > 0.05$*).* Scenedesmus quadricauda *(S),* Chlorella vulgaris *(C), baker's yeast (*Saccharomyces cerevisiae*) (Y), and alfalfa meal (*Medicago *spp.) (A).*

depending on diets. The similar density of 290 individuals/mL was reported by Nandini and Sarma (2002) for this species. Pena-Aguadoa et al. (2005) reported that *Brachionus rubens* reached higher densities on a mixture of *Chlorella* and yeast than on *Chlorella* alone. *Chlorella* and *Scenedesmus* have similar PUFAs, but *Scenedesmus* is richer in lipids, protein, nitrogen, and phosphorus levels (Ahlgren et al. 1992). Most literature showed that fatty acid content of diets plays a major role in determining levels of zooplankton production. For instance, Gulati et al. (1987) stated that *E. dilatata* was well adapted to consume planktonic algae such as *C. vulgaris* as well as for most rotifers (Rothhaupt 1990; Nandini and Rao 1998).

The mean production of *E. dilatata* fed on S+C+A and Y+A diet were 53.5 and 32.5 individuals/mL/d, respectively, which were significantly ($P < 0.05$) higher than S and C+Y diets, likely because A was part of the diet combination. Another possible reason for the lower production with the S diet could be a lower ingestion rate of the S diet, especially when compared with diets containing C. The lower density of *E. dilatata* fed on S diet compared to C could be described by spine cells in S compared to spineless cells such as C diet (Filito et al. 2004; Mayeli et al. 2004; Ajah 2008), which decreased ingestion of S diet. Generally, size, shape, spines, colonies, filaments, hard

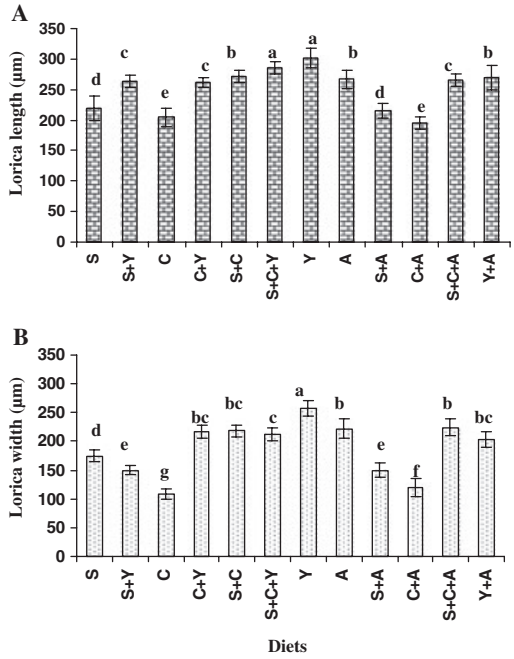

FIGURE 5. *Mean (±SE) of lorica length (µm) (A) and width (µm) (B) of* Euchlanis dilatata *fed on different algal and non-algal diets. Bars with the same letters are not significantly different (*$P > 0.05$*).* Scenedesmus quadricauda *(S),* Chlorella vulgaris *(C), baker's yeast (* Saccharomyces cerevisiae*) (Y), and alfalfa meal (* Medicago *spp.) (A).*

cell walls and gelatinous sheaths, and movements of microalgae affect on feeding activities in rotifers *Asplanchna priodonta*, *Brachionus calyciflorus*, and *B. patulus* and also small size cladocerans (Filito et al. 2004; Mayeli et al. 2004; Ajah 2008). In addition, the numerical differences between this study with previous could be attributed to inoculum and residual densities of cultured organisms because the optimum space requirement for each species may differ. Another reason related to higher growth rates of *E. dilatata* in diets containing algal and alfalfa could be a result of shorter maturation times, ripening of gametes and their release, and continuous breeding of females in these conditions.

The better survival and reproduction of *E. dilatata* fed diets containing alfalfa meal and its combination could be attributed to its nutritional values, better and efficient biodegradability of alfalfa meal. In addition, the better performance of alfalfa meal as dietary source may related to its suitable suspension in culture medium provide a greater probability of consumption directly by *E. dilatata* as reported by Anderson (1993a).

The effects of organic fertilizers such as alfalfa could be related to pH as previous reported by Barkoh et al. (2005) for some organic fertilizers. In this study, the pH ranged from 6.5 to 7.5 throughout the experiment. Attempts were made to maintain water quality constant in all treatments; therefore, we believe that the differences observed in population did not result from pH or other water quality parameters. Although the pH of cultures varied from 6.5 to 7.5, it was within the optimal pH range of 6.8–7.8 reported for culture of zooplankton (Ivleva 1973). In conclusion, organic fertilizers such as alfalfa meal not only can be directly used as food source for freshwater zooplankton production but also enhances secondary productivity and ultimately fish production as long as pH is maintained in the proper ranges (Mims et al. 1993). Therefore, we believe that use of alfalfa meal during green water practices in earthen ponds could increase pond biota such as live food production, especially rotifers such as *E. dilatata*. In addition, our results showed that alfalfa meal could effectively supplement algal and yeast diets, and therefore could reduce the costs of production of rotifers.

Acknowledgments

Support for this research was provided by Isfahan University of Technology (IUT), Isfahan, Iran. The authors wish to recognize the technical assistance of Safiallah Heidari and Delaram Tagavi.

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