IJST (2011) A2: 157-163
Iranian Journal of Science & Technology

http://www.shirazu.ac.ir/en

Freshwater green algae *Chlorella sp.* and *Scenedesmus obliquus* enriched with B group of vitamins can enhance fecundity of *Daphnia magna*

N. Mehdipour[1]*, M. Fallahi[2], G. Azari Takami[3], G. Vossoughi[1] and A. Mashinchian[1]

[1]*Department of Marine Biology, Faculty of Marine Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[2]*Internal Waters Aquaculture Research Institute, Anzali Port, Guilan, Iran*
[3]*Department of Aquatic Health and Diseases, Faculty of Veterinary Medicine, Tehran University, Tehran, Iran*
E-mail: neda.mehdipour@gmail.com; mahyarparvaneh2003@yahoo.com; takami85@hotmail.com; hvos40@yahoo.com; ali2m@yahoo.com

Abstract

One of the most important aims of aquatic hatcheries is availability to mass produce of *Daphnia magna* as a live food with high nutritional value in the shortest time. In the present study, *D. magna* fed with two freshwater green algae species, *Chlorella sp.* and *Scenedesmus obliquus,* enriched with different dosages of a mixture B group vitamins (0, 0.5, 1 and 2 $ml.l^{-1}$) were compared at two stages (at the beginning of the culture and at the end of logarithmic growth phase). The results showed that increases in the B group vitamin levels in both enriched algae performed, increasing the fecundity and population growth rate in *D. magna* at the two compared stages, but significantly only with the control group ($P<0.05$). The highest fecundity and population growth rate obtained in *D. magna* was when they were fed algae enriched with 2 $ml.l^{-1}$, but not significantly with 0.5 and 1 $ml.l^{-1}$ ($P>0.05$).

Keywords: Chlorella sp.; Scenedesmus obliquus; Daphnia magna reproductive performance

1. Introduction

Since *Daphnia magna* is one of the zooplanktons considered as live food for fish fry at the beginning of their active feeding, their mass production with high nutritional value is one the necessities of many hatcheries. *Daphnia* culture began 150 years ago, specially for feeding Sturgeon's larvae.

The color of *Daphnia* and their continuous crinkle-shape movements attract fish fry and because of their chemical compositions, they have a significant importance in aquaculture [1]. *Daphnia* is not a suitable prey organism for marine organisms, because it is a freshwater species and has a low content of essential fatty acids, particularly (n-3) HUFA [1-3].

Live prey organisms, *Daphnia*, rotifers and *Artemia*, can be bio-encapsulated with a variety of enrichment diets to manipulate their nutritional content including ω3 highly unsaturated fatty acids (FA) and vitamins. Nevertheless, the enrichment techniques are not applicable for all nutrients and prey organisms [4].

*Corresponding author
Received: 25 April 2010 / Accepted: 30 August 2010

Goulden *et al.* (1982) [5] proved that mass culture of *Daphnia* in the autotrophic system that equally contains green algae species will not be able to preserve *Daphnia* generation without adding an adequate mixture of B group vitamins. In this research, *D. magna* was fed on two freshwater green algae species, *Chlorella sp*. and *Scenedesmus obliquus*, enriched with a suitable mixture of B group vitamins to enhance its reproduction.

2. Material and methods

2.1. Mass culture of green algae Chlorella sp. and Scenedesmus obliquus

The Zander (Z-8+N) medium was used for massive and pure culture of the two green algae species. This is a general medium for culturing green and blue-green algae. Since green algae do not have heterocyst for nitrification, nitrogen should be added to their medium; therefore their medium is in the form of Z-8+N. The culture of algae was carried out in the laboratory by adding pure algal stock of the respected species to the medium. The amount of inseminated algae in samples was 1mg dried substance per liter of the

new medium. The algae were cultured at optimal conditions using mono white color (3500±350 Lux) with a photoperiod of 14L:10D, at 25±2ºC and pH=7.5-8 [6]. The algae were collected from the medium after 96 hours when they were at the end of their logarithmic growth phase and when they were at their maximum nutritional value and density. Three subsamples were used to determine the dry weight of the cultured algae while the rest were determined and before using as food for *Daphnia*. Algae at a density of 10 $mg.l^{-1}$ of *Daphnia* culture medium was used for the reproduction experiment [6].

2.2. Preparation of enriching solution

An appropriate vitamin mixture containing all B group vitamins applied for mono culture of *D. magna* with green algae (Table 1) was offered by Goulden *et al.* (1982) [5]. This vitamin mixture can be preserved in a dark, cold and dry place (-18 ºC) maximum for three weeks before use [5].

2.3. Mass culture of Daphnia magna

Two 20L aquariums (without any replication) containing 15L dichloride tap water, one for *Chlorella* sp. and the other for *Scenedesmus obliquus* culture as live food were used for mass production of *D. magna*. The density of *Daphnia* considered in the culture was 50 individuals per liter. Those cultured in 12:12h L:D light condition and 22±1 ºC were daily fed with green algae *Chlorella sp***.** and *Scenedesmus obliquus* [7].

2.4. Enrichment of algae with B group vitamins

Zero, 0.5, 1 and 2 ml of prepared B vitamin mixture were added to each liter of the algae culture medium at two periodic stages, one at the beginning of the culture (BCH) and one at the end of logarithmic growth phase (ELGP) for each treatment group separately, each with three replicates.

2.5. Study of Daphnia Fecundity and PGR

Forty eight 1.5L beakers containing 1L of dichloride tap water were used to investigate fecundity and population growth rate of *D. magna*. Ten individual *D. magna,* 3 days old were released in each of the containers. The water of the beakers was replaced daily with freshwater by adding 10mg enriched algae with different dosages during the period of the experiment [8]. After the maturation of *D. magna*, their neonates were isolated and counted everyday [9]. Fecundity and PGRP of *D. magna* were calculated based on the following equations [9]:

$F = Nn / Nm.T$ $r = (\ln Nm - \ln Nn)/t$

Where F=Fecundity of *Daphnia* and r= Population growth rate of Daphnia, Nn= Number of neonates, Nm=Number of maturated *Daphnia*=10, T= One day and t= ten days

3. Statistical analysis

Shapiro wilk test was used for examining the normality of data distribution. Because of the normality of the data distribution, Parameteric test was used for analyzing all of the measured factors. Two-way analysis of variance (ANOVA) was used for examining the existence of interactive effect by which some of the factors were simultaneously affected. In the analyses, the entire interactive effects were over 0.05 ($P>0.05$), so some of the factors were independently analysed and tested by One-Way ANOVA. The (multi domain) Duncan test was used for comparing the average of different replicates of each treatment.

Table 1. Vitamin mixture for monospecific culture of *Daphnia* on *Chlorella sp.* and *Scenedesmus obliquus* [5]

Nutrient	Concentration of stock solution ($\mu g.l^{-1}$)
Biotin	5
Thiamine	100
Pyridoxine	100
Pyridoxamine	3
Calcium Panthothenate	250
B12 (as mannitol)	100
Nicotinic acid	50
Nicotinomide	50
Folic acid	20
Riboflavin	30
Inositol	90

4. Results and discussion

According to the results (Fig.1) the average fecundity of *D. magna* (that means the number of newborns *Daphnia* per a mature *Daphnia* in one day) fed with *Chlorella sp.* enriched with different B group vitamins dosages at the BCH (0.5, 1 and 2 $ml.l^{-1}$) were respectively 1.557 ± 0.071, 1.543 ± 0.254 and 2.128 ± 0.375 number of neonates (day^{-1}), which showed an increase of 18%, 17% and 61% in proportion to the control group (1.314±0.404 number of neonates (day^{-1})). The utmost average of *D. magna* fecundity was obtained through being fed with enriched *Chlorella sp.* with a dosage of $2ml.l^{-1}$, but not significant differences with other dosages treatments (P>0.05) except the control (P<0.05). The average population growth rate of *D. magna* fed *Chlorella sp.* enriched with different dosages at the BCH (0.5, 1 and 2 $ml.l^{-1}$) were respectively 0.209 ± 0.003, 0.211 ± 0.006 and 0.247 ± 0.033 indiv./day (Fig.2) which showed an increase of 1%, 2%, 20% in proportion to the acquired amount from witness treatment 0.205 ± 0.024 indiv/day. Although the highest average of *D. magna* PGR was obtained in daphnia fed enriched algae with dosages of 2 $ml.l^{-1}$, there are no significant differences with other B group vitamins (0.5 and 1 ml. l^{-1}) (P>0.05) except the control group (P<0.05).

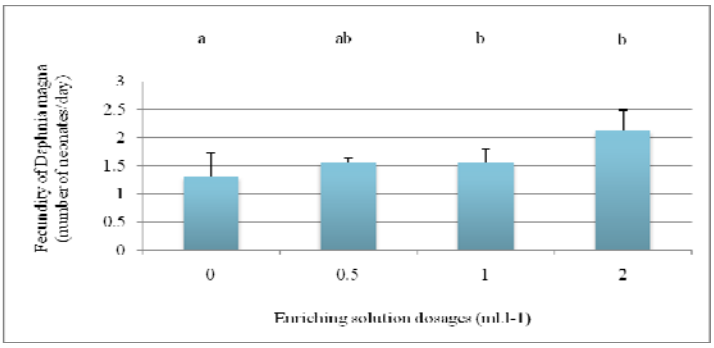

Fig.1. Fecundity of *Daphnia magna* fed with *Chlorella sp.* enriched with different dosages of B group vitamins mixture at BCH. Values are the mean ± SD (n=3). Different letters show significant differences (P<0.05).

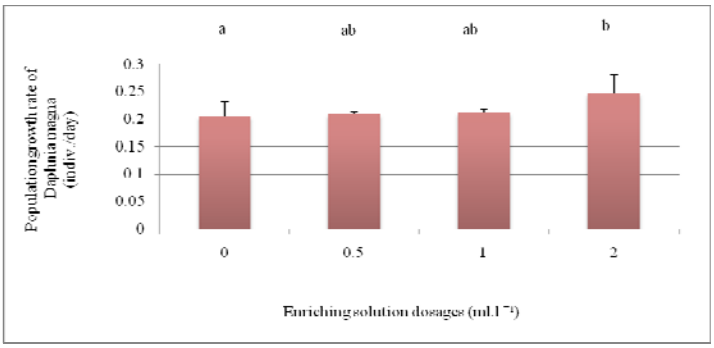

Fig. 2. Population growth rate of *Daphnia magna* fed with *Chlorella sp.* enriched with different dosages of a mixture of B group vitamins at BCH. Values are the mean ± SD (n=3). Different letters show significant differences (P<0.05)

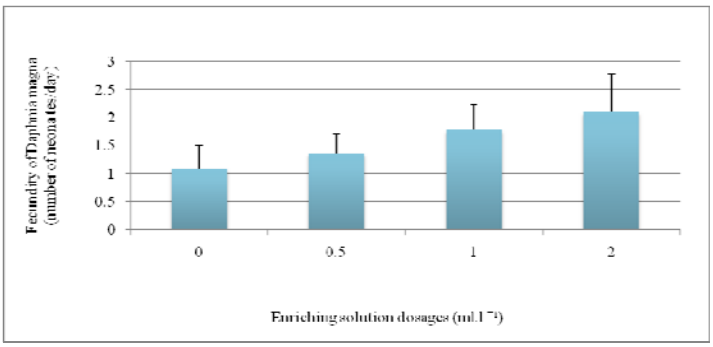

Fig. 3. Fecundity of *Daphnia magna* fed with *Chlorella sp.* enriched at ELGP of alga with different dosages of B group vitamins mixture. Values are the mean ± SD (n=3). No differences were found between treatments.

Figure 3 demonstrated that the average fecundity of *D. magna* fed with *Chlorella sp.* enriched with different vitamin B at the ELGP (0.5, 1 and 2 ml.l^{-1}) were 1.371 ± 0.354, 1.784 ± 0.448 and 2.1 ± 0.69 number of neonates (day $^{-1}$) respectively, which expressed a 25%, 62%, 91% increasing proportion to the control group 1.095 ± 0.42 number of neonates (day $^{-1}$). The utmost average fecundity was obtained in daphnia fed with enriched *Chlorella sp.* with 2 ml.l^{-1} vitamins B concentration, but there no significant differences between the treatments (P>0.05).

Figure 4 showed the average PGR of *D. magna* fed with *Chlorella sp.* enriched with different B group vitamins concentrations at the ELGP (0.5, 1 and 2 ml.l^{-1}) were 0.209 ± 0.018, 0.223± 0.015 and 0.268 ± 0.085 indiv/day respectively, which revealed a 3%, 10% and 33% increase compared to the control group 0.201±0.049 indiv/day. Although there are no differences between dosage treatments (P>0.05) concerning the *Daphnia* fecundity, the highest average was obtained in those fed *Chlorella sp.* enrichment with 2ml.l^{-1} vitamins B.

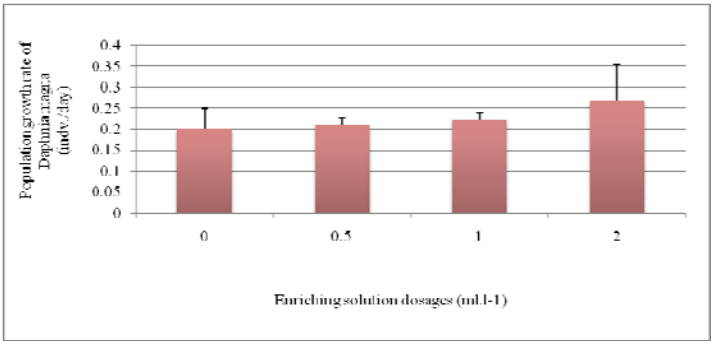

Fig. 4. Population growth rate of *Daphnia magna* fed with *Chlorella sp.* enriched at the end of logarithmic growth phase of alga with different dosages of a mixture of B group vitamins. Values are the mean ± SD (n=3).
There are not any differences between dosages treatments statistically.

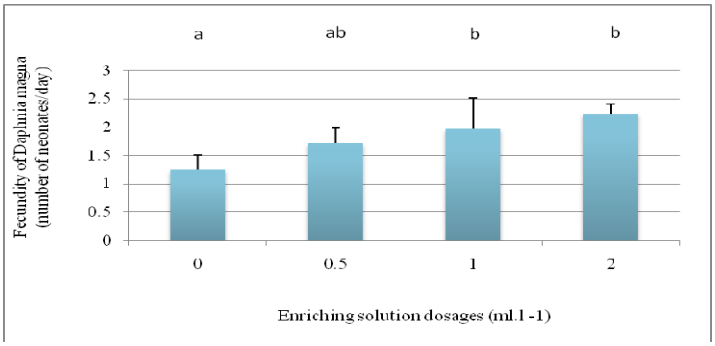

Fig. 5. Fecundity of *Daphnia magna* fed with *Scenedesmus obliquus* enriched with different dosages of a mixture of B group vitamins at BCH. Values are the mean ± SD (n=3). Different letters show significant differences (p<0.05).

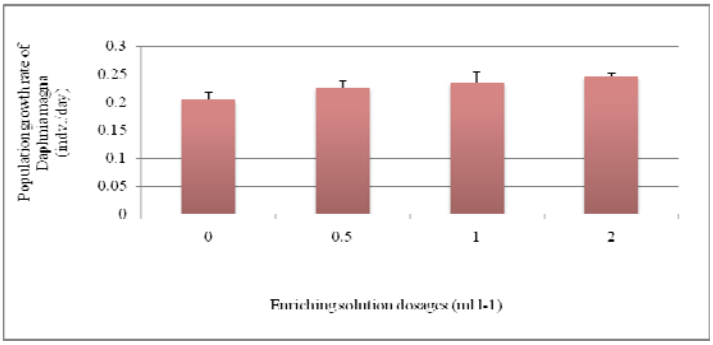

Fig. 6. Population growth rate of *Daphnia magna* fed with *Scenedesmus obliquus* enriched from the BCH with different dosages of a mixture of B group vitamins. Values are the mean ± SD (n=3).
Different letters show significant differences (p<0.05).

Results (Fig.5) revealed that the average fecundity of *D. magna* fed with *S. obliquus* enriched with different dosages of B group vitamins at the BCH (0.5, 1 and 2 ml.l^{-1}) were respectively 1.721 ± 0.265, 1.969 ± 0.539 and 2.233 ± 0.175 number of neonates (day $^{-1}$) which showed an increase of 36%, 56%, 77% in proportion to the acquired amount from witness treatment 1.257 ± 0.248 number of neonates (day $^{-1}$). The utmost average of *D. magna* fecundity was obtained through being fed with enriched algae with dosages of 2 ml.l^{-1}, which has a significant difference only with the control group (P<0.05).

The averages of PGR in *Daphnia* fed *S. obliquus* enrichment with different dosages of B group vitamins (0.5, 1 and 2 ml.l^{-1}) were 0.226 ± 0.011, 0.234 ± 0.018 and 0.245 ± 0.005 indiv/day respectively (Fig. 6). This shows an increase of 11٪, 15٪, 20٪ compared to the witness vitamin treatment 0.203 ± 0.015 indiv/day. Although the utmost average of fecundity was obtained in *D. magna* enriched with 2 ml.l^{-1} vitamin B mixture, it has no differences with 0.5 and 1 ml.l^{-1} vitamin dosages statistically (P>0.05). The PGR of the control group was the least value to have significant differences with others (P<0.05).

Figure 7 evidenced that the average fecundity of *D. magna* fed with *S. obliquus* enriched with different dosages at the ELGP (0.5, 1 and 2 ml.l^{-1}) were 2.029 ± 0.311, 2.253 ± 0.668 and 3.106 ± 0.857 number of neonates (day $^{-1}$) respectively, which showed an increase of 47٪, 64٪, 83 ٪in proportion to the acquired amount from the control group 1.373 ± 0.534 number of neonates (day $^{-1}$). The utmost average of *D. magna* fecundity was obtained through being fed with enriched algae with dosages of 2 ml.l^{-1}, but there are no differences between treatments (P>0.05).

Results (Fig.8) showed that the average population growth rate of *D. magna* fed with *S. obliquus* enriched with different dosages at the ELGP (0.5, 1 and 2 ml.l^{-1}) were respectively 0.227 ± 0.007, 0.231 ± 0.022 and 0.248 ± 0.013 indiv/day which showed an increase of 15%, 17%, 26% in proportion to the acquired amount from the control group 0.196 ± 0.015 indiv/day. There are no differences between treatments (P>0.05), nonetheless, the utmost average of *D. magna* fecundity was obtained through being fed with enriched algae with dosages of 2ml.l^{-1}.

During recent decades, several techniques have been used to increase the nutritional value of Daphnia or their food resources.

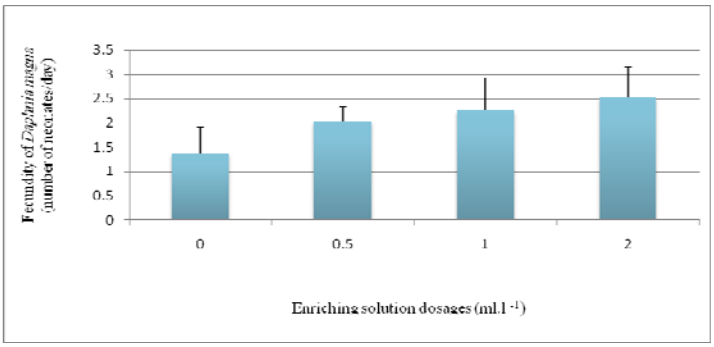

Fig. 7. Fecundity of *Daphnia magna* fed with *Scenedesmus obliquus* enriched at the end of logarithmic growth phase with different dosages of a mixture of B group vitamins. Values are the mean ± SD (n=3). No differences were found between groups

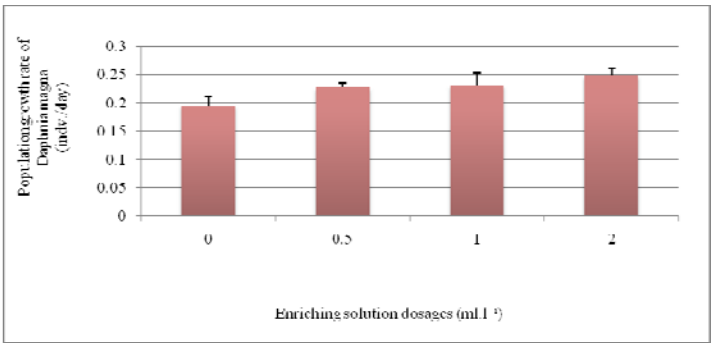

Fig. 8. Population growth rate of *Daphnia magna* fed with *Scenedesmus obliquus* enriched at the ELGP with different concentrations of a mixture of B group vitamins. Values are the mean ± SD (n=3). No differences were observed statistically between groups.

Some of them are as follows: Enrichment of green algae fed by *Daphnia* with vitamins B12 and B1 [10]. Vitamins B12, B7 and B1 [11], an appropriate mixture of B group vitamins [5]. Enrichment of cultured zooplankton with vitamins A, E, C and highly unsaturetad fatty acids [4, 12 and 13], Lipid emulsions [12-16] and essential fatty acids and vitamin C [17, 18]. Studies by Lwoff and Dusi [19] discovered that some members of the Chlorophyta require B group vitamins as growth factors in their culture. Although vitamins have no effective role in producing energy, they have such an importance in metabolic reactions and the natural growth of body cells that the lack of any of them will cause serious disruptions in animals. Since B group vitamins and their derivatives function as coenzymes (CoA, StylCoA, NAD^{+}, $NADP^{++}$, FMN, FAD, B6PO4, THF, COBAMID) in the catalysis of metabolic reactions and because animal cells are not able to synthesize these coenzyme groups, they should be taken through nutrients [20]. Although most of the B group vitamins are synthesized by unicellular and herbal cells in nature, the results of different researches indicate that when the objective of mass culture of an algae species is using them as live food in aquaculture, their enrichment with vitamins and other nutrients before being used is necessary. Some of the B group vitamins such as vitamins B12, B7, B1 are essential for the growth of many algae including green algae, and they should be taken in from their culture environment. It has been estimated that about 70 percent of all plankton algae need vitamin B12 as a growth factor in their culture [21]. The uptake of vitamin B12 by different strains of freshwater *Chlorella* (*Chlorella vulgaris*) was measured by Maruyama *et al.* [22]. The results showed that various strains of *Chlorella* are able to uptake B12 from their medium, but they differ in their amount of uptake. Some of the Chlorella strains are able to absorb more than 81 percent of the vitamin from the medium, without any requirement for this amount of the vitamin. The effect of algal food quality on the *Daphnia* reproduction was studied in several cases [10, 5, 23, 24, 1, 25, 26 and 27]. D'Agostino and Provasoli (1970) [10] concluded that *D. magna* fed with green algae enriched with vitamins B1, B7 and B12 grow at least to 200 generations. The results of Lewis [28], and Murphy [26] indicated that the addition of vitamins to the medium in which the algae food is grown with crustacean, may allow a continuous culture of herbivorous crustacean which are considered difficult to grow. Goulden *et al*. [5] proved that mass culture of *Daphnia* in the autotrophic system that equally includes two green algae species will not be able to preserve *Daphnia* generation without adding an adequate mixture of B group vitamins. The influence of vitamin B12 deficiency on the reproduction of *Daphnia plux* was also studied by Keating [29]. According to these results, the fecundity of *Daphnia* increased with the concentration of B12 in the media. The nutritional effect of freshwater *Chlorella* (*Chlorella vulgaris*, k-24) containing vitamin B12 in its cells on the growth of marine Rotifer *Brachionus plicatilis* was studied by Hirayama *et al*. [24]. According to this research, freshwater *Chlorella* which is produced by traditional culture cannot support rotifer growth under bacteria-free conditions, therefore, rotifers fed with *C. vulgaris* show very suppressed growth. However, *Chlorella* enriched with vitamin B12 (by adding the vitamin solution in to the suspension or by culturing the *Chlorella* in a medium containing vitamin B12) can support rotifer growth, because the nutritional value of *Chlorella* following enrichment is greatly improved and almost at the same level as that of marine *Chlorella*. In this study the highest rotifer yield was obtained from the group cultured with *Chlorella* containing more vitamin B12 in their cells [30, 31]. The effect of vitamin B12 enriched *Thraustochytrids* on the population growth of rotifers, was studied by Hayashi *et al.* [23]. The feeding of B12 enriched thraustochytrids to rotifers greatly improved the population growth of rotifers, In that study, it was found that B12 is taken up into thraustochytrids cells, and that the addition of B12 to the medium decrease the cellular contents of odd numbered fatty acids [23]. It is necessary to mention that *Daphnia magna* fed with vitamin-enriched algae are less sensitive to a chronic copper stress than *D. magna* fed a trout-granule diet, this conclusion is based on the responses of cohorts as measured by survival, mean brood size and the instantaneous population growth rate [27]. In the present study it was found that enrichment of green algae *Chlorella sp. & Scenedesmus obliquus* with an appropriate vitamin mixture which consists of all B group vitamins, from the beginning of culture, can increase *Daphnia magna* fecundity and population growth rate, and feeding *D. magna* with algae enriched with increased dosages of enriching solution causes its fecundity to increase.

References

[1] Lavens, P. & Sorgeloos, P. (1996). *Manual on the production and use of live food for aquaculture.* FAO Fisheries Technical Paper. Laboratory of Aquaculture and Artemia Reference Center. University of Ghent, Ghent. 284.

[2] Von Elert, E. & Wolfform, T. (2001). Supplementation of Cyanobacterial food with polyunsaturated fatty acids does not improve growth of *Daphnia*. *Limnology, Oceanography*, *46*, 1552-1558.

[3] Von Elert, E., (2002). Determination of limiting polyunsaturate fatty acid in *Daphnia galeata* using a new method enriches food algae with single fatty acid. Limnology, *Oceaography*, *46*, 1764-1773.

[4] Coutteau, P. & Sorgeloos, P. (1997). Manipulation of dietary lipids, fatty acids and vitamins in zooplankton cultures, *Freshwater Biology*, *38*, 501-512.

[5] Goulden, C. E., Comotto, R. M., Hendrickson, J. A., Horring, L. L. & Johnson, K. L. (1982). Procedures and recommendations for the culture and use of *Daphnia* in bioassay studies. Aquatic Toxicology and Hazard Assessment. *American Society for Testing and Materials*, 139-160**.**

[6] Ordog, V. (1981). Apparatus for Laboratory algal bioassays. *Hydrobiologia, 16*, 127-136.

[7] Fallahi, M., Pirri, H., Ramezani, R., Mohammadi, V. & Salavatian, S. M. (2005). Final report of algae culturing. *Iranian fisheries research organization*. Tehran.

[8] Piri, M. (1998). Effects of some herbicides commonly used in Iran on *Selenastrum capricornatum* and *Daphnia magna*. Ph.D. Dissertation. University of Hungary. Hungary.

[9] Downing, J. A. & Rigler, F. H. (1984). *A manual on methods for the secondary production in freshwater*. IBP. Handbook. No. 17, 2nd ed. Blackwell Scientific Publications, Oxford.

[10] D, Agostino, A. & Provasoli, L. (1970). Dixenic culture of *Daphnia magna*, Straus. *Biol. Bull*, *139*, 485-494.

[11] Provasoli, L. & Carlucci, A. F. (1974). *Vitamins and growth regulators. Algal physiology and biochemistry.* Blackwell Scientific Publication, Oxford.

[12] Hafezieh, M., Mohd Saleh Kamarudin, S., Bin Saad, C. R., Abd Sattar, M, K., Agh, N., Valinassab, T., Sharifian, M. & Hosseinpour, H. (2010) Effects of enriched *Artemia urmiana* with HUFA on growth, survival, and fatty acids composition of the Persian sturgeon larvae (*Acipenser persicus). Iranian Journal of Fisheries Sciences, 9*(1), 61-73.

[13] Hafezieh, M., Kamarudin, M. S. & Agh, N. (2008). Nutritional enhancement of total lipid, n-3 and n-6 fatty acids in *Artemia urmiana* nauplii by enriching with ICES/30/4. *Pakistan Journal of Biological Sciences, 11*(17), 2167-2170**.**

[14] Park, S., Brett, M. T., Muller-Navarra, D. C. & Goldman, C. R. (2002). Essential fatty acid content and the phosphorus to carbon ratio in cultured algae as indicators of food quality for *Diphnia*. *Freshwater Biology, 47*, 1377-1390.

[15] Plath, K. & Boersma, M. (2001). Mineral limitation of zooplankton: Stoichiometric constraints and Optimal foraging. *Ecology*, *82*, 1260-1269.

[16] Sunbom, M. & Vrede, T. (1997). Effect of fatty acid and phosphorus content of food on growth, survival and reproduction of *Daphnia*. *Freshwater Biology*, *38*, 665-674.

[17] Abedian Kenari, A. M., Ovissi pour, M. R. & Nazari, R. M. (2007). Effect of N3-HUFA enriched *Daphnia magna* on growth, survival, stress resistance and fatty acid composition of larvae of Persian sturgeon (*Acipencer percicus*). *Iranian Journal of Fisheries Sciences, 7*, 1-14.

[18] Ovissi pour, M. R., (2006). *Daphnia magna* enrichment with fish oil & vitamin C and its effect on growth, survival and body compositions of Persian sturgeon (*Acipencer percicus*) larvae. Ms.c. Dissertation, Tarbiat Modares University.

[19] Croft, M. T., Warren, M. J. & Smith, A. G. (2006). Algae need their vitamins. *American Society for Microbiology, Eukaryotic cells, 5*, 1175-1183.

[20] Shahbazi, P. & Malekniya, N. (2003). *General Biochemistry*, Vol .1, 20th Ed. Tehran, Tehran University Press.

[21] Azari Takami, G. & Amini Charmehini, M. (2009). *Plankton culture manual*. Tehran, Tehran University Press.

[22] Maruyama, I., Ando, Y., Tadahiko, M. & Hirayama, K. (1989). Uptake of vitamin B12 by various strains of unicelluar algae *Chlorella*. *Nippon Suisan Gakkaishi*, *55*, 1785-1790.

[23] Hayashi, M., Yukino, T., Watanabe, F., Miyamoto, E. & Yoshihisa, N. (2007). Effect of vitamin B12-enriched Thraustochytrids on the population growth of rotifers. *Biosci. Biotechnol. Biochem*, *71*, 222-225.

[24] Hirayama, K., Maruyama, I. & Maedo, T. (1989). Nutritional effect of freshwater *Chlorella* on growth of the rotifer *Brachionus Plicatilis*. *Hydrobiologia*, *186/187*, 39-42.

[25] Kilham, S., Kreeger, D., Goulden, C. & Lynn, S. (1997). Effects of food quality on fecundity and population growth rates of *Daphnia*. *Freshwater Biology*, *38*, 639-647.

[26] Murphy, J. S. (1970). A general method for culturing of water flea, *Moina macrocopa Straus* in artificial culture medium. *Tohoku J. Agric. Res*, *5,* 27-36.

[27] Winner, R. W., Keeling, T., Yeager, R. & Farrell, M. P. (1977). Effect of food type on the acute and chronic toxicity of copper to *Daphnia magna*. *Freshwater Biology, 7*, 343-349.

[28] Lewis, A. G. (1967). An enrichment solution for culturing the early developmental stages of the planktonikc marine copepods *Euchaeta japonica* Marukawa. *Limnol. Oceanog, 12*, 147-148.

[29] Keating, K. I. (1985). The Influence of vitamin B12 deficiency on the reproduction of *Daphnia plux* leyding (Cladocera). *Journal of Crustacean Biology*, *5*, 130-136.

[30] Maruyama, I. & Hirayama, K. (1989). The culture of the rotifer *Brachionus plicatilis* with *Chlorella vulgaris* containing vitamin B_{12} in its cells. *Journal of the World Aquaculture Society, 24*, 194-198.

[31] Maruyama, I., Nakao, T., Shigeno, I., Ando, Y. & Hirayama, K. (1997). Application of unicellular algae *Chlorella vulgaris* for the mass-culture of marine rotifer *Brachionus*. *Hydrobiologia*, *358*, 133-138.