The growth, survival rate and reproductive characteristics of *Artemia urmiana* fed by *Dunaliella tertiolecta, Tetraselmis suecica, Nannochloropsis oculata, Chaetoceros* sp., *Chlorella* sp. and *Spirolina* sp. as feeding microalgae

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Abstract

This study was performed to compare the efficiency of six microalgae namely *Dunaliella tertiolecta, Tetraselmis suecica, Nannochloropsis oculata, Chaetoceros* sp., *Chlorella* sp. and *Spirolina* sp. on the growth, survival rate and reproduction efficacy in *Artemia urmiana* in laboratory conditions. *Artemia* cysts were harvested from Urmia Lake and hatched according to the standard method. Live microalgae were cultured using the f/2 culture medium. *Artemia* survival was determined in treatments on days 8, 11, 14, 17 and 20. A highly significant difference (p<0.01) were found among three microalgae in terms of length growth, survival rates and reproduction characteristics in *A. urmiana*. In spite of higher length growth of *A.urmiana* fed on *N. oculata* than *A. urmiana* fed by *T. suecica* but survival and reproduction in the latter was better than the first treatment. In general, *D. tertiolecta* was more efficient than other microalgae examined in the present study on *A. urmiana* concerning not only to growth and survival but also to reproduction mode. So, it is preferred to feed *A. urmiana*.

Keywords: *Artemia urmiana*, Microalgae, Length growth, Survival rate

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Introduction

Potentially, *Artemia* is an excellent feed for fish and crustaceans (Sorgeloos, 1980). The brine shrimp *Artemia* is probably the most popular live diet in aquaculture. *Artemia* is a non-selective filter feeder. It is able to use all nutrients that are smaller than its mouth. Various factors affect *Artemia*'s filtration rate, ingestion, digestion and feeding behavior. These factors include the quality and quantity of feed such as floatability, minimum solubility in water, digestibility and size and so on (Sorgeloos *et al.*, 1998).

Due to its particular biological characteristics, *Artemia* can be fed on different diets, from live microalgae to microcapsules and waste products from the food industry (Lavens and Sorgeloos, 1991). Microalgae strains are recognized as excellent sources of proteins, carbohydrates, lipids, and vitamins, and as food and feed additives. *Nannochloropsis* sp. is well known as a source of EPA, an important polyunsaturated fatty acid (Radhakrishnan *et al*., 2009). *Chlorella* sp. is also recognized as a source of EPA.

The bioencapsulation technique provides interesting opportunities for using *Artemia* biomass not only as food attractant, but also as carrier for administration of various products to the predator, such as essential nutrients, pigments, hormones, and prophylactic or therapeutic agents (Léger *et al.*, 1986, Majack *et al.*, 2000, Malpica Sanchez *et al.*, 2004). Suitable algal species for filter-feeding organisms such as *Artemia* are selected according to mass culture potential, cell size, digestibility and nutritional value (Hafezieh, 2004).

Diatoms are considered good sources of highly unsaturated fatty acids, especially of 20:5ω-3 (Lora-Vilchis and Voltolina, 2003). In contrast, chlorophytes are rich in C16 and C18 fatty acids (Dunstan *et al*., 1992; Brown *et al*., 1997;), and in particular *Chlorella* has also a high content of carotenoids and ascorbic acid (Czygan, 1968; Merchie *et al.*, 1995), which might be of importance for growth and especially long-term enhancement of the food quality of *Artemia*.

In natural habitats, microalgae form the main food source for *Artemia*. In Urmia Lake, for example, the microalga *Dunaliella* is the dominant species of the lake microalgal flora and composes more than 90% of algal density (Mohebbi *et al*., 2009; Mohebbi, 2010). Obviously, *Artemia* often feeds on *Dunaliella* in most of its natural habitats.

Considering the substantial growth of aquaculture activities, it is useful to study more about microalgae suitability for *Artemia* feeding. Besides, studies on native *Artemia* populations represent an alternative for the exploitation of natural resources, also favoring the development of the local aquaculture industry. While there are so many studies on the effect of different algae on various *Artemia* strains, there are

few studies related to *A. urmiana* fed on various microalgae. The purpose of this study was to investigate and compare the effects of various algae on the growth, survival rate and reproduction of *A. urmiana*, and to determine the most appropriate algal species for *A. urmiana* in laboratory conditions.

Materials and methods

Microalgae culture

Stock culture of *T. suecica* was provided from the Persian Gulf Ecology Research Institute in Bandar Abbas (Iran). *N. oculata* was sent from Aquaculture Research Institute of South (Ahvaz, Iran).

Live microalgae were cultured using the f/2 culture medium (Guillard, 1975). A volume of 20 mL sea water (20-24 ppt) was poured into twenty five 75-mL test tubes and 40 µL of f/2 medium was added to each tube. When the tubes were cooled, 1-2 drops of vitamin solution was added to each tube. A little of alga was removed from stock culture by forceps and transferred into test tubes. The tubes were placed in suitable condition and stirred several times daily. After a few days, the tubes went green. Then the alga of each tube was transferred into a 250-mL or 500-mL flasks which contained the f/2 medium and vitamin. Similarly, this cycle was repeated until the algae were finally transferred into 30-L plastic bags and 100-L tanks. When the algal density reached a maximum level, aeration was interrupted. Then, the algal solution was concentrated more by cooling in the refrigerator. The concentrated alga was diluted up to a determined level (18×10^6 cells/mL) before use for *Artemia* feeding. The density of the alga was determined using a Neubar slide and a Nikon ECLIPSE 50i microscope.

Artemia culture

Artemia cysts were harvested from Urmia Lake and hatched according to Sorgeloos *et al*. (1986). *Artemia* were starved during the first 24 hr in order to allow yolk resorption (Teresita and Leticia, 2005). Newly hatched larvae were enumerated and 500 larvae were placed in one conical vessel (4 repeats from each treatment) that contained 1000 cc of water with 80 ppt salinity. The vessels were placed in the incubator with 25 ± 1º C temperature (Boone and Bass-Becking, 1931).

Brine shrimp nauplii were experimentally kept under the following culture conditions: 25±2.5ºC water temperature, 30±1.3 ppt salinity, 8.0±0.4 pH and >5 mg L^{-1} dissolved oxygen. Feeding the larvae was started according to Coutteau *et al*. (1992) 24 hr after hatching of the cysts. The used food composed of the algae *D. tertiolecta*, *T. suecica* and *N. oculata*. At the beginning, *Artemia* density was one larva per 2 mL of water which was reduced to one *Artemia* per 3 mL and one *Artemia* per 4 mL on days 8 and 14, respectively (Boone and Bass-Becking, 1931).

On days 8, 11, 14, 17 and 20, ten animals were taken out from each container (30 per treatment) and

measured (from the naupliar eye to the telson; (Amat, 1980) using Motic Images plus 2.0 software.

Artemia survival percentages were determined in three treatments on days 8, 11, 14, 17 and 20 (Cruz *et al.*, 1993).

When the *Artemia* were grown as adults, 30 females and 30 males were randomly selected and transferred into cylindrical bottom-conical small vessels named falkons (one female and one male *Artemia* in each falkon). In order to control the falkons' temperature, they were placed in special boxes (Racks) which in turn were put in aquariums with 25°C temperature (Boone and Bass-Becking, 1931). For each *Artemia* one drop of the enumerated algae (18×10^6 cells/mL) was daily added into the falkons. The water content of the falkons was changed daily. At the same time, the probable produced cysts or larvae were counted using a WILD M3C model stereomicroscope (Mohammadyari, 2002). The type and number of offspring, the reproduction rate in the study period, the day of first reproduction, the interval between the two consecutive reproductions were calculated for each pair of *Artemia*.

One way analysis of variance (ANOVA) and Duncan test were used to compare the average of properties. All diagrams were produced in Excell 2007.

Results

A significant difference ($p<0.01$) was observed between length growths of *A. urmiana* fed on three different microalgae so that, the *A. urmiana* fed on *D. tertiolecta* and *T. suecica* showed the highest and the lowest length growth, respectively (Fig. 1). In the study period (20 days) the mean of length growths were 5.171 mm, 4.555 mm and 3.131 mm 4943.44 mm and 4820.024 mm in *A.urmiana* fed on microalgae *Dunaliella tertiolecta*, *N. oculata* and *T. suecica*, *Chaetoceros* sp., *Chlorella* sp. and *Spirolina* sp. respectively (Table 1).

Survival indicated significant difference ($p<0.05$) between *Artemia* fed on *N.oculata* than those fed on *D. tertiolecta* and *T. suecica* so that *Artemia* fed on *N. oculata* showed lower survival percentages than the two latter treatments (Fig. 2). Besides, the *Artemia* fed on *T. suecica* contained lower survival rates than those fed on *D. tertiolecta*, though this difference was not statistically significant (Fig.2). The survival rate in various days of the experiment showed significant difference between days 8 and 11 and between days 11 and days 14, 17 and 20 ($p<0.05$).

There was no significant difference in survival percentages among repeats in three different microalgae. However, survival percentages among various days of the experiment suggested that it was higher in *Artemia* fed on *D. tertiolecta* than *Artemia* fed on *T. suecica* which in turn was higher than those fed on *Nannochloropsis oculata* ($p<0.01$, Table 2). On the other hand, *Spirolina* sp. induced the highest mortality in *A. urmiana* (Fig 2). Also, *A. urmiana* fed by *Chaetoceos* sp. and *Chlorella* sp. indicated relatively similar survival patterns (Fig. 2). This pattern of survival was similarly observed on days 8, 11, 14, 17 and 20 of the experiment.

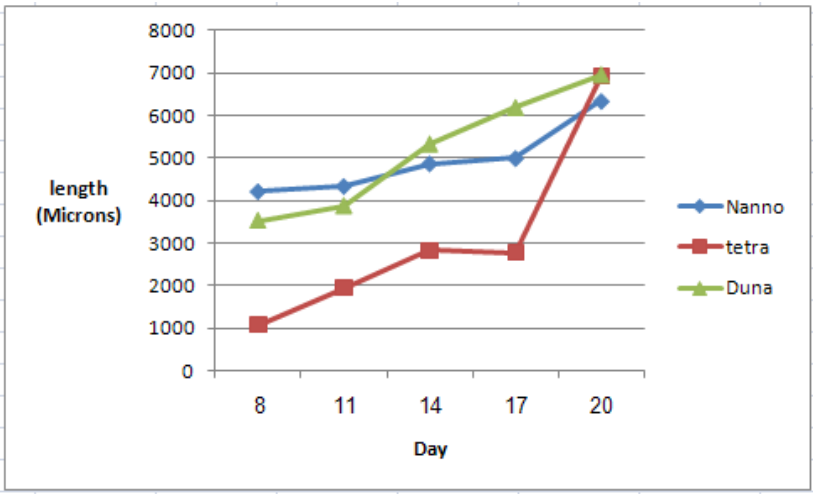

Figure 1: Length growth of *Artemia urmiana* treated by three microalgae feeds.

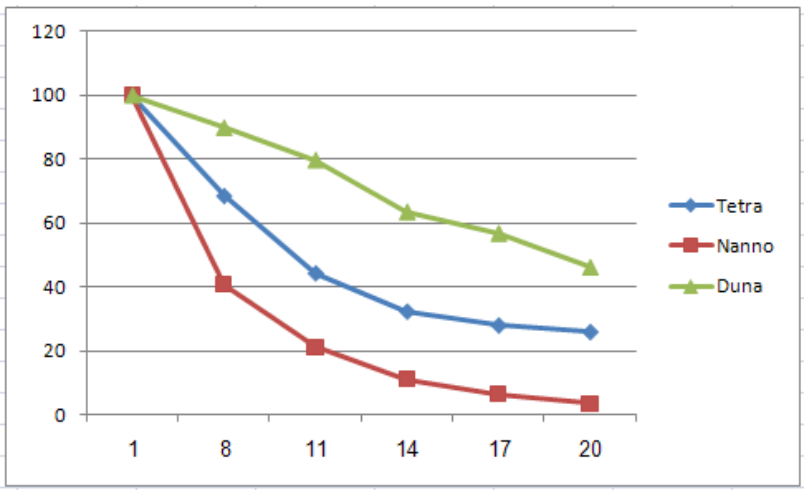

Figure 2: Survival percentages for three different microalgae fed to *Artemia urmiana.*

Table 1: Means of length growth between *Artemia urmiana* fed on different microalgae (p<0.01).

Microalga	N	Mean of length growth (mm) ±Std.Deviation
Tetraselmis suecica	152	3131.14±1447.033
Nannochloropsis oculata	66	4555.47± 719.085
Chaetoceros sp.	81	4943.44±542.92
Chlorella sp.	75	4820.024±552.82

Cysts and nauplius production were only observed in *A. urmiana* fed on *D. tertiolecta* and *T. suecica. Chaetoceos* sp. and *Chlorella* sp. . In other words, *A.urmiana* fed on *N. oculata* and *Spirolina* sp.did not mature to produce cysts or naplius. The comparison of cysts and nauplius production between *A. urmiana* fed on *D. tertiolecta* and *T. suecica* indicated a significant difference (p<0.01). *A. urmiana* fed on *D. tertiolecta* produced much more cysts and naplius than the *A. urmiana* fed on *T. suecica* (Table 3). The mean cysts production in *A. urmiana* treated with *D. tertiolecta* and *T. suecica*

Chaetocerus sp. and *Chlorella* sp. were 12.87, 2.47, 1.2 and 1.6 cysts over the experiment period, respectively. Also, *A. urmiana* fed on *D. tertiolecta* and *T. suecica Chaetocerus* sp. and *Chlorella* sp. produced 8.36, 2.60, 1.19 and 1.50nauplius in the experiment period respectively. Significant differences were observed between *A. urmiana* fed on *D. tertiolecta* and *T.suecica* in terms of the number of reproductions in the study period and the day of first reproduction ($p< 0.01$), but these two treatments did not indicate any significant differences with regard to the interval between two consecutive reproductions.

There was a significant difference ($p<0.01$) only between repeats 1 and 3 in *A. urmiana* fed on *D. tertiolecta*. Other repeats did not indicate any significant differences in terms of cysts and nauplius production.

Table 2: Means of survival rates for *Artemia urmiana* fed on different microalgae.

Microalga	Days	mean±Std.Deviation
Dunaliella tertiolecta	8	482.75± 0.00
	11	264.25± 0.00
	14	132.25 ± 0.00
	17	107.50 ± 0.00
	20	91.75 ± 0.00
Tetraselmis suecica	8	342.25± 30.66
	11	221.50 ± 4.79
	14	124.25 ± 76.63
	17	106.50 ± 68.07
	20	98.50 ± 63.84
Nannochloropsis oculata	8	204.00 ± 26.14
	11	106.25 ± 42.94
	14	55.75 ± 16.52
	17	32.00 ± 6.27
	20	17.75 ± 3.30
***Chaetocerus* sp.**	8	75.1±7.3
	11	54.3 ± 5.2
	14	42.9±7.1
	17	32.5±6.9
	20	23.9±4.6
***Chlorella* sp**.	8	73.1±4.1
	11	52.1±6.4
	14	42.5±7.5
	17	33±6.3
	20	23.5±5.7
***Spirolina* sp.**	8	7.1±2.1
	11	1.45±1.1
	14	0.4±0.6
	17	0.0±0.0
	20	0.0±0.0

Table 3: Cysts and nauplius production in *Artemia urmiana* fed by different microalgae.

Microalga	repeat	Cysts (mean±Std.Deviation)	Nauplius (mean±Std.Deviation)
Dunaliella tertiolecta	1	12.975± 12.057	9.077± 9.076
	2	13.110± 5.030	11.306± 7.235
	3	10.183± 6.357	14.816± 7.504
Tetraselmis suecica	1	3.304± 2.944	2.819± 4.389
	2	2.829± 2.251	2.799± 2.764
	3	1.355± 1.247	2.235± 2.586
***Chaetocerus* sp.**	1	1.255±1.235	1.191±1.242
	2	0.812±0.682	1.352±1.110
	3	0.954±0.825	1.542±1.365
***Chlorella* sp.**	1	1.626±1.411	1.547±1.324
	2	1.360±1.032	1.881±1.547
	3	1.502±1.361	1.425±1.256

Discussion

It is well accepted that *Artemia* is the most widespread live food item used in the production of shrimp, prawn and fish larval stages. The organism can be used in different forms in hatcheries and nurseries, e.g. decapsulated cysts, nauplii, metanauplii, juvenile and adult stages, and frozen and freeze-dried *Artemia* biomass. *Artemia* biomass is nowadays more frequently used for specific stages of aquatic species as it enhances production characteristics and overall stress resistance and/or decreases cannibalism in dolphin fish and lobster larviculture (Lavens and Sorgeloos, 1991).

The quality of microalgae diets for *Artemia* has been the object of several studies (e.g. Sick, 1976; Johnson, 1980; Fábregas *et al*., 1996, 1998) with different results, depending on the species of microalgae, culture conditions, and possibly the species of *Artemia* used for the feeding experiments.

Maldonado-Montiel and Rodríguez-Canché (2005) reared a Mexican local *Artemia* with rice bran (days 1- 6) and microalga *T. suecica* (days 7-15). They reported 79% survival rate at the end of trial which was higher than the value observed on day 14 in our study. They also measured a mean length of 5.34mm for *Artemia* at the end of their experiment (day 15). This value was higher than that in our study for which we obtaineda mean length of 3.01mm for *A. urmiana* fed on *T. suecica* on day 14. These differences may be attributed either to *Artemia* species or to Mexican tropical climate, sharply different than ours.

The results of the present study confirmed those obtained by Voojodzadeh *et al*. (2007) who found that *A. urmiana* fed with *N. oculata* did not produce any cysts or larvae even though they were reared until day 30. However, our study indicated that *A. urmiana* fed on *T. suecica* had the lowest length growth among treatments

which was not consistent with the work of Voojodzadeh *et al*. (2007).

A.urmiana fed with *Spirolina* sp. had the lowest (18.5%) survival rate and indicated statistically significant difference with other algae examined in this study (P< 0.00). this was due to large size of this alga which was unsuitable for Artemia. In fact, *Spirolina* sp. should be powdered before was fed to Artemia (Garcia-Ulloa and Garcia-Olea, 2004).

On the other hand, Fabregas *et al*. (1996) evaluated *T. suecica* nutritional value on *Artemia*'s total growth, survival and reproduction characteristics in different culture concentrations. They obtained the best results when *Artemia* were fed on *T. suecica* grown at a nutrient concentration of 8 mg atom N 1-1. This concentration was relatively higher than that of *T. suecica* concentration we used in our study. Therefore, we may attribute the lower length growth of *A.urmiana* fed by *T. suecica* to lower concentration of this microalga.

Study conducted by Hafezieh (2004) indicated that the application of *Chaetoceros* sp. as live food for *A.urmiana* had significantly different effect on body length than *Chlorella* sp. which confirms our study. However, in our study *Chaetoceros* sp. had higher effect on Artemia body length than *Chlorella* sp. that was reverse to Hafezieh (2004).

In spite of the fact that *T. suecica* induced lower growth (mean length = 3131.14 µm) in *A. urmiana* than *N. oculata* (mean length = 4555.47 µm) in our study, but reproduction outcome was better than *A. urmiana* fed on *N. oculata* (Table 3). This suggested that *T. suecica* had higher effects in differentiating sexual capabilities in *A. urmiana* than *N. oculata*. As shown in Fig.1, *A. urmiana* fed on *T. suecica* indicated a lower growth rates than *A. urmiana* fed on *N. oculata* on days 8, 11, 14 and 17. However, the growth rate of *A. urmiana* fed on *T. suecica* was higher than *A. urmiana* fed on *N. oculata* from day 17 to 20 (Fig. 1). This suggested that *A. urmiana* fed on *T. suecica* grew to adults at the end of the trial period (day 20), but *A. urmiana* fed on *N.oculata* did not reach the length or differentiation that could produce cysts or nauplius. The comparison of reproduction characteristics between *A. urmiana* fed on *D. tertiolecta* and *T. suecica* showed that *D. tertiolecta* had better reproduction outcomes for *A.urmiana* than *T.suecica* .

We can conclude that *D. tertiolecta* has higher potential in creating better reproductive characteristics in A. urmiana than other algae. In this respect, *T. suecica* is located after *D. tertiolecta* and before *Chlorella* sp. and *Chaetoceros* sp. is at the end of this list. In general, the results of the present study indicated that *D. tertiolecta* had higher efficiency than the two other microalgae on *A. urmiana* in terms of length growth, survival rates and reproduction outcomes. Therefore, *D. tertiolecta* is suggested as a preferable food for *A. urmiana*. Hannah *et al*.

(2013) evaluated the nutritional value of four microalgae namely *Chaetoceros calcitrans, Skeletonema coastaum, Dunaliella salina* and *D. bardawil* for Artemia sp. nauplii. They concluded that among the four microalgae tested, *D. salina* could be used as a potential live feed to improve the nutritional status of *Artemia* sp. as nauplii. Their finding was notconsistent with our results and they suggested that another species of *Dunaliella* (i.e. *D. tertiolecta*) was preferable food source for *Artemia*.

In the natural habitat of *A.urmiana* (i.e. Urmia Lake) *Dunaliella* spp compose more than 90% of the total algal density (Mohebbi, 2010).

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