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The Effects of *Dunaliella tertiolecta, Tetraselmis suecica* and *Nannochloropsis oculata* as Food on the Growth, Survival and Reproductive Characteristics of *Artemia urmiana*

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Abstract

Artemia is the most widespread live food used in the production of different stages of many aquaculture organisms. It is a non-selective filter feeding organism. Generally, microalgae are the most favorable feeds for *Artemia*, particularly when the algal species have suitable size, digestibility and nutrient values. This study was performed to compare the efficiency of three microalgae namely *Dunaliella tertiolecta*, *Tetraselmis suecica* and *Nannochloropsis oculata* for growth, survival and reproduction efficiency of *Artemia urmiana* in laboratory conditions. *Artemia* cysts were harvested from Urmia Lake and hatched according to standard methods. Live microalgae were cultured using the f/2 culture medium. *Artemia* survival was determined in treatments on days 8, 11, 14, 17 and 20. The results indicated a significant difference ($P<0.01$) among three microalgae in terms of growth, survival rates and reproduction characteristics in *A.urmiana*. In spite of higher length growth of *A.urmiana* fed on *N.oculata* than *T.suecica* but survival and reproduction in *A.urmiana* fed on *T.suecica* was better than the first treatment. In general, *D.tertiolecta* was more efficient than *T.suecica* and *N.oculata* on *A.urmiana*, hence, it is preferred for feeding *A.urmiana*.

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

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1. Introduction

Potentially, *Artemia* is an excellent food source, which could provide quality 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 the filtration rate, ingestion, digestion and feeding behavior of *Artemia*. 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 with 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, for food and feed additives. *Nannochloropsis* sp. is well known as a source of EPA, an important polyunsaturated fatty acid. *Chlorella* sp. is also recognized as 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 (Brown *et al.*, 1997; Dunstan *et al.*, 1992), 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 for long-term enhancement of 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). So, it is obvious that *Artemia* often feeds on *Dunaliella* in majority of its natural habitats. Considering the substantial growth of aquaculture activities, it is necessary to increase the studies 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. On the other hand, while there are so many studies on the effect of different algae on various *Artemia* strains, there are few studies on the *Artemia urmiana* feed 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* also to determine the most appropriate algal species for *A.urmiana* in the laboratory conditions.

2. Materials and methods

2.1.Microalgae culture

Stock culture of *Tetraselmis suecica* was provided from the Persian Gulf Ecology Research Institute in Bandar Abbas, Iran. *Nannochloropsis oculata* was sent from Aquaculture Research Institute of South in Ahvaz, Iran. Live microalgae were cultured using the f/2 culture medium (Guillard, 1975). An amount 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 cool enough, 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 the 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 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 became more concentrated through cooling in refrigerator. The concentrated alga was diluted up to a determined level (18×10^6 cells/mL) before use for *Artemia* feeding. To do this, the density of the alga was determined with a Neubar slide and a Nikon ECLIPSE 50i microscope.

2.2.Artemia culture

Artemia cysts were harvested from Urmia Lake and hatched according to Sorgeloos *et al.* (1987). *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) containing 1000 cc water with 80 ppt salinity. The vessels were placed in incubator with 25 ± 1º C temperature (Boone and Bass-Becking, 1931). Brine shrimp nauplii were experimentally kept under the following culture conditions: 25±2.5°C 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 algae *Dunaliella tertiolecta*, *Tetraselmis suecica* and *Nannochloropsis 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 of *Artemia* 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 the 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 the number of offspring, the number of reproduction in the study period, the day of first reproduction, the interval between two consecutive reproductions were calculated for each pair of *Artemia*. In this study, one way analysis of variance (ANOVA) and Duncan test were used to compare the average of properties. All diagrams were produced in Excel 2007.

3. Results

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

Survival indicated significant difference ($P<0.05$) between *Artemia* fed on *N.oculata* and those fed on *D. tertiolecta* and *T. suecica* so that *Artemia* fed on *N. oculata* showed lower survival percentages than the two latter treatments (Figure 2). Besides, the *Artemia* fed on *T.suecica* showed lower survival rates than those fed on *D. tertiolecta*, though this difference was not statistically significant (Figure 2). On the other hand, the survival rate among various days of experiment showed significant difference between days 8 and 11 and this difference was also observed 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 *Dunaliella tertiolecta* than *Artemia* fed on *Tetraselmis suecica* which in turn was higher than that of fed on *Nannochloropsis oculata* ($P<0.01$, Table 2). This pattern of survival was similarly observed on days 8, 11, 14, 17 and 20 of the experiment.

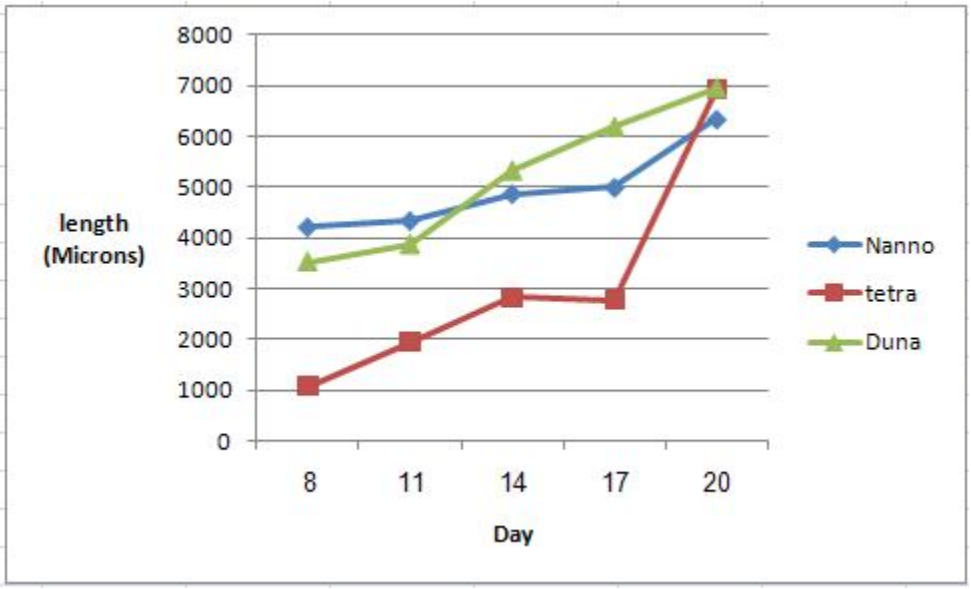

Figure 1. Length growth of *A.urmiana* treated by three microalgae feeds

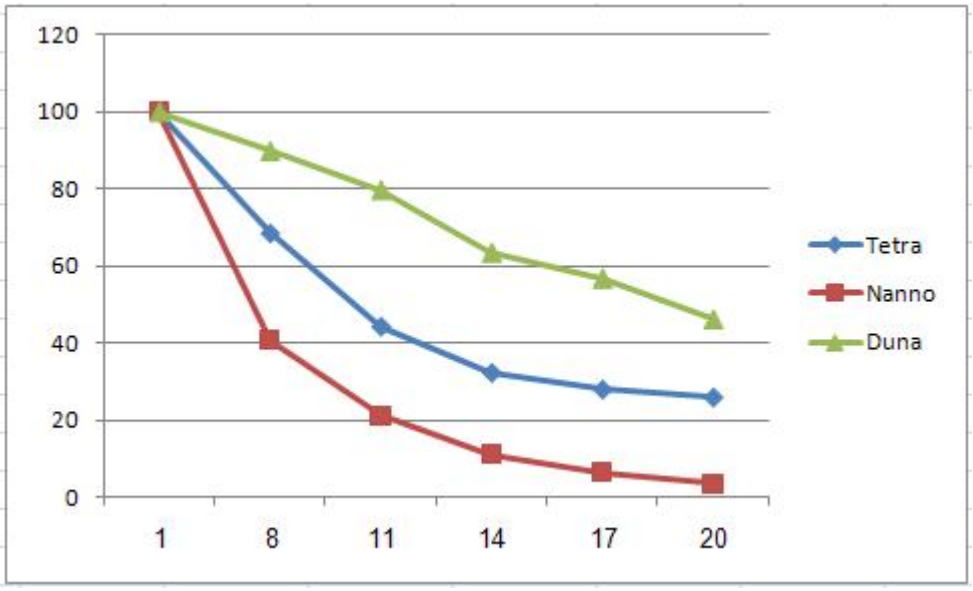

Figure 2. Survival percentages for three different microalgae fed to *Artemia urmiana*

Cysts and nauplius production were only observed in *A.urmiana* fed on *D. tertiolecta* and *T. suecica*. In other words, *A.urmiana* fed on *N. oculata* 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 *Tetraselmis suecica* (Table 3). Mean cysts production in *A.urmiana* treated with *Dunaliella tertiolecta* and *Tetraselmis suecica* were 12.87 and 2.47 cysts in the experiment period respectively. On the other hand, *A.urmiana* fed on *Dunaliella tertiolecta* and *Tetraselmis suecica* produced 8.36 and 2.60 naplius in the experiment period respectively. Also, significant differences was 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 regards to the interval between the two consecutive reproductions.

There was a significant difference (P<0.01) 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 naplious production.

Table 1. Mean length growth between *A.urmiana* fed on three different microalgae (P<0.01)

Microalga	N	Mean length growth (mm) ±Std. Deviation
Tetraselmis suecica	152	3131.14±1447.033
Nannochloropsis oculata	66	4555.47± 719.085
Dunaliella tertiolecta	150	5171.29±2432.937

Table 2. Mean survival rates for *A.urmiana* fed on three 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

Table 3. Cysts and nauplius production in *A.urmiana* fed on three 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

4. 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. It can be used in different forms in hatcheries and nurseries, e.g. decapsulated cysts, nauplii, metanauplii, juvenile and adult stages, 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, on their culture conditions, and possibly on 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 *Tetraselmis 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 our study result in which we obtained a mean length of 3.01mm for *A.urmiana* fed on *Tetraselmis suecica* on day 14. These differences may be attributed either to *Artemia* species or to Mexican tropical climate which were highly dissimilar.

The results of the present study confirmed the results obtained by Voojodzadeh *et al.*, (2007) who found that *A.urmiana* fed with *Nannochloropsis 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 *Tetraselmis suecica* had the lowest length growth among treatments which was not in agreement with their work.

On the other hand, Fabregas *et al.*, (1996) evaluated *Tetraselmis suecica* nutritional value on *Artemia* total growth, survival and reproduction characteristics in different culture concentrations. They obtained the best results when *Artemia* fed on *Tetraselmis suecica* grew at a nutrient concentration of 8 mg atom N 1-1. This concentration was relatively higher than the *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.

In spite of the fact that *Tetraselmis suecica* induced lower growth (mean length= 3131.14 µm) in *A. urmiana* than *Nannochloropsis oculata* (mean length= 4555.47 µm) in our study, but reproduction outcome was better than *A. urmiana* fed on *Nannochloropsis oculata* (Table 3). This suggested that *Tetraselmis suecica* had more efficiency to differentiate sexual capabilities in *A.urmiana* than *Nannochloropsis oculata*. As is shown in Fig.1 *A.urmiana* fed on *T.suecica* indicated a lower growth rate 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 (Figure 1). This suggested that *A.urmiana* fed on *T.suecica* grew to adults at the end of trial (day 20), but *A.urmiana* fed on *N.oculata* did not reach the length or differentiation capable of producing cysts or naplious. On the other hand, the comparison of reproduction characteristics between *A.urmiana* fed on *D.tertiolecta* and *T.suecica* showed that *D.tertiolecta* had better reproduction outcomes than using *T.suecica* for *A.urmiana.*

4. Conclusion

In general, the results of the present study indicated that *Dunaliella 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 a preferable food for *A.urmiana* than the two other microalgae. Hannah *et al.,* (2013) evaluated the nutritional value of four microalgae namely *Chaetoceros calcitrans, Skeletonema coastaum, Dunaliella salina & 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. nauplii. Their finding was in agreemtn with our study in which it was suggested that another species of *Dunaliella* (i.e. *D.tertiolecta*) was preferable food source for Artemia. This was the case in *A.urmiana*'s natural habitat (i.e. Urmia Lake) in which *Dunaliella*.spp composed more than 90% of the total algal density (Mohebbi, 2010).

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