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Mutual effect of light and turbidity on hatching of *Artemia franciscana* cysts

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Abstract
Artemia produces cyst in natural environments and at definite times of the year, but these cysts are inactive. However, they continue the trends in germinal growth by emergence of proper conditions, feeding from the environment. The effect of light and opacity together as the nonliving elements and the effective physical factors are investigated for the first time in this study. In order to find the best conditions for obtaining the highest efficiency and simultaneous hatching as well as the number of cysts of these aquatic animals, with regards to equal conditions in salinity and temperature. Light intensity was not significant on hatching (p=0.053), but it showed significant difference at light intensity of 1000lux (p<0.05). The lowest rate of hatching in 1500 lux light intensity was 17.75+8.62 and the highest rate of hatching was observed to be 25.94+8.3 in treatments with the light intensity of 1000 lux (p=0.032). The effect of turbidity was not significant on hatching (p=0.58).

Keywords: Effect, Light, Turbidity, Hatching, *Artemia franciscana*

Introduction
One of the most important matters in fishery industry is supplying suitable and valuable food for the fish, which usually allocate most part of consuming costs to itself (Lavens and Sorgeloos, 1996) [22]. Live food is considered as one of the most important and valuable nutritional elements in successful growth of fish and shrimps larva, being effective on the rate of growth and survival of most fish species during their larval stage (Sorgeloos *et al.*, 2001) [24]. With their vast diversity, live foods are different in their quality. *Artemia* nauplii is mostly used among different live foods (Lavens *et al.*, 1986) [23]. Using *artemia* started in 1939 for feeding aquatic animals, and it is extensively common today in that respect (Watanabe and Kiron, 1994) [31].

Artemia is of "Arthropoda" species in "Crustacae" division and is among "branchiopoda" group of animals, used as one of the most valuable aquatic animals in industrial, pharmaceutical, agricultural researches and especially in fishery studies and feeding aquatic animals (Triantaphyllidis *et al.*, 1998) [25]. *Artemia* produces cyst in natural environments and at definite times of the year, but these cysts are inactive. However, they continue the trends in germinal growth by emergence of proper conditions, feeding from the environment. Using scoured cysts, newly hatched nauplii and biomass artemia is important in all the different stages of growth for nutrition of larva and most of the growing aquatic animals during the growing period, causing faster growth, improving nutritional conditions, higher rate of lagging and increasing production (Agh, 1999; Agh *et al.* 1996) [9, 10]. Artemia newly hatched nauplii is used in growing aquatic animals more tthan any other stages. For more rate of using artemia cysts, it is better to have complete knowledge as much as possible, about the hatching characteristics of different strains of artemia (Bengtson *et al.*, 1991) [17]. Different factors are effective on hatching of artemia cyst, such as salinity, pH, temperature, oxygen, light and opacity (Alizadeh *el al.*, 2012; *Baqeri et al.*, 2005; Ghanbari *et al.,* 2011) [14-16]. Light is an important environmental factor in water environments and opacity is one of the important factors in water quality and artemia cysts hatching. Thus, determining the effects of non-biological elements could help in obtaining more appropriate results. The effects of different levels of light intensity on hatching parameters of Urumieh Lake cysts hatching and the rate of hatching of Urumiana artemia cysts in different light intensities are analyzed in this study (Qodratnama & Azari Takami, 2006) [13].

The effect of light and opacity together as the nonliving elements and the effective physical factors are investigated for the first time in this study. In order to find the best conditions for obtaining the highest efficiency and simultaneous hatching as well as the number of cysts of these aquatic animals, with regards to equal conditions in salinity and temperature.

Methods and Materials

The present study was done in autumn 2014, in the reproduction and growing workshop in natural resources faculty/ Gilan University. All the laboratory devices were disinfected by saline water before the test. The test began by placing some conical plastic bottles with approx. volume of 1.5lit.in an aquarium (with dimensions: 80x40x50cm). The bottles were filled with 800ml of distilled water and the bottles were labeled with numbers. For unique temperature conditions in the test, the bottles were placed in one aquarium and the aquarium was filled with water up to the height of 30cm. two 150w heaters (equipped with thermostat) were used in the aquarium for temperature requirements, and an air tube was placed beside each heater for homogeneity of the temperature in the system. The heaters were adjusted on 27 to 28 ˚C (Tayebi *et al.,* 2006) [13]. For the oxygen requirements of the cysts, the air tubes were placed in such a way that airing was done from the bottom of the bottles, in order to avoid the cysts sedimentation and deficiency of oxygen at the end of the bottles, and to provide similar proper conditions for the cysts. Water salinity was reached to 35ppm, by solving 29g of crushed salt stone from Urumieh Lake. The rate considered for *Artemia franciscana* in this experiment was 2g/lit. Fluorescent lamps were installed above the aquarium at different heights to provide the required light for the samples. The light intensity adjusted by lux meter for each group of samples on 500, 1000 and 1500 lux. Clay was used for the opaque condition. First, the required amount of clay was prepared from the soil laboratory in the faculty and the clay was properly grinded by a china pounder. Then the clay was mixed with water to separate the existing small quartz in the soil. After that, it was passed from a piece of cloth. The obtained solution was then boiled in a vessel and was used after drying for the test. Three types with different opacities of 0, 2 and 3g/lit. were prepared for the required analysis in the test.

After 48 hours from starting the test for hatching *Artemia franciscana* cysts in three different light intensities, the content of the bottle was completely mixed and then three samples were taken from the surface, intermediate and end parts of the bottle, respectively, and a totally homogeneous sample was prepared from each bottle with the volume of 10ml. The samples were then poured in the sampling tubes, labeled and transferred into the laboratory. After that, every vessel with the sample was stabilized with formalin 4%. A few drops of the sample was poured inside a petri dish to determine the hatching rate and to measure a homogeneous sample, and the number of hatched nauplii and unhatched cysts were calculated in these drops, the number of nauplii in each drop were measured from the petri dish and the average rate of it (N) was calculated. Also, the number of nauplii in the umbrella stage in every sample and the average values (U) were estimated. The rate of hatching and the average value for the samples was calculated in this way, according to the following formula.

$$|H| = |\frac{N}{N+U}| \times 100$$

Rate of hatching

Data analysis was done by SPSS Ver. 16 software. After normalizing the obtained data for comparing the average values, the two-way ANOVA and LSD analyzing methods were used for significant differences with certain 8.3ty level of 95% in different treatments.

Results

The mutual effect of the two factors, namely light intensity and turbidity was not significant on the rate of hatching of different treatments ($p = 0.328$). Hence, the effect of each factor was considered separately.

Effect of Light Intensity

Light intensity was not significant on hatching ($p=0.053$), but it showed significant difference at light intensity of 1000lux ($p<0.05$). The lowest rate of hatching in 1500 lux light intensity was 17.75±8.62 and the highest rate of hatching was observed to be 25.94±8.3 in treatments with the light intensity of 1000 lux ($p=0.032$).

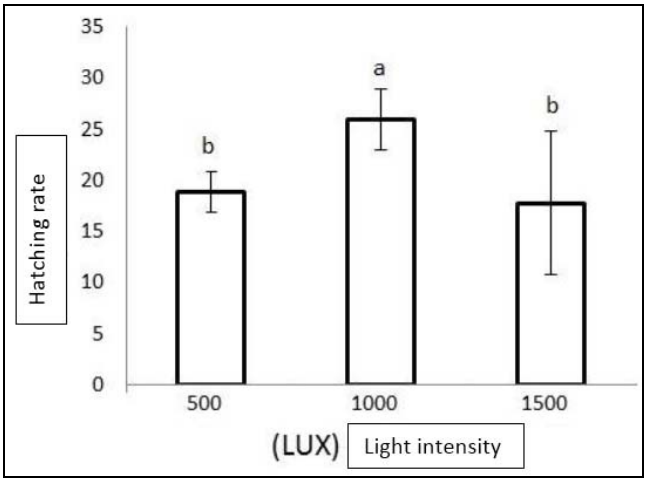

Fig 1: Rate of hatching (17.75 ± 8.62) of *Artemia franciscana* cysts by using different light intensities after 48 hours ($p<0.05$). Letter "b" indicates no significant difference ($p>0.05$), but the letter "a" (light intensity of 1000 lux) shows significant difference with "b" ($p<0.05$).

Effect of Turbidity

The effect of turbidity was not significant on hatching ($p=0.58$).

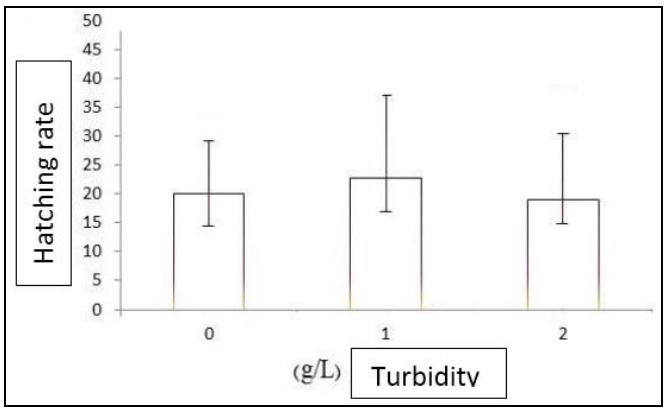

Fig 2: Rate of hatching of *Artemia franciscana* cyst using different rates of turbidity after 48 hours

Discussion and Conclusion

The effects of light intensity and opacity was analyzed in the study on artemia cysts hatching. According to the results, different opacities and used homogeneous salinity within this test had no significant difference in the rate of artemia hatching, but the differences of light intensity were effective on hatching of artemia. Due to the nutritional and economic values of artemia cysts, it would be better to be informed about the characteristics of hatching different strains of artemia for more appropriate use of these cysts. Identifying the characteristics of hatching is important since these characteristics are different in each strain or even in every package of artemia cysts. Usually, naupolii is used for feeding of growing aquatic animals larva after maximum hatching of the cysts. However, it should be noted that the nutritional value of the artemia that is to be used as a nutritional material for growing specific aquatic animals is rather more important than the characteristics of its hatching (Bengtson *et al.*, 1991; Browne and Wanigasekera, 2000) [17, 19].

According to various studies, hatching of artemia cysts is affected by lighting conditions (Lavens and Sorgeloos, 1987; Lavens *et al.*, 1986) [21, 23]. Light intensity, duration of lighting and light wavelength are among the different light parameters that affect hatching (Browne and Wanigasekera, 2000) [19]. By placing in aerobic conditions, the cysts are immediately becoming sensitive to light after being hydrated. After absorbing the light by optical receivers, respiration and metabolism of carbohydrates start in the cysts, leading to increasing pH, and by activation of trihalase enzyme, trihalose is dissolved (Vanhaecke *et al*., 1981; Vanhaecke and Sorgeloos, 1980) [29. 30]. On the other hand, by dissolving trehalose to glycogen and glycerol, the cysts shells are torn apart and hatching will occur (Van Stappen, 1996) [28]. According to Vanhaecke *et al.* (1981) [29] in different geographic strains of artemia, the cysts' stimulation thresholds by light are different that is due to the difference in chorion layer characteristics of the cysts, such as thickness of the layer and hematinic compaction or the pigments responsible for absorbing the light (Blust *et al*., 1992) [18]. According to the mentioned studies, there is a direct relation between the cyst metabolism and the light intensity. The direct relation significance between the rate of light intensity and hatching was obtained according to the mentioned studies (García-Ortega *et al*., 2001) [20]. However, it is to note that by increasing light intensity, the rate of hatching will increase, too (Van der Linden *et al*., 1985; Van der Linden *et al*., 1991) [26, 27]. Furthermore, Vanhaecke *et al.* studied the effect of different light intensities of 200-2000 lux on hatching. According to the obtained results, the rate of hatching shows an ascending trend by increasing the light intensity. By increasing the light intensity up to 1000 lux, a significant increase was observed the present study, in the rate of hatching.

On the other hand, the temperature range (about 27-29 ˚C) was considered for high rate of hatching. It was concluded in these studies that the cysts have better hatching rate in this temperature. But, the results of other investigations showed that the temperature range (23-40 ˚C) prevents metabolism in the cysts in the hatching stage. Lack of significant difference in hatching of artemia cysts in different tested opacities shown in this study is probably because the created opacity in the hatching system is not in such a range to have significance in hatching mechanism. Different light intensities in this experiment causes difference in hatching of artemia cysts that indicates the effect and role of lighting factors in hatching of *Artemia franciscana* cysts. The reason for reducing the hatching rate of artemia cysts compared to the expected range could be due to various reasons including lack of light homogeneity in the hatching system of the cysts and temperature range changes over the tolerable threshold of the cysts for appropriate hatching. But, the opacity up to this rate of turbidity has no effects on hatching. Using the light intensity of 1000 lux that is almost equivalent to natural light is however recommended for hatching of artemia cysts.

Appreciation

This research was done in the reproduction and growing workshop in natural resources faculty/ Gilan University. We hereby appreciate and gratify the relevant authorities of the workshop, Messrs. Mohammadi and Faridi, as well as the head of pedology library of jungle group, Mr. Nabavi for providing the required facilities for this research.

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