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RESEARCH ARTICLE

Detection of Egg Diameters and Hatching Rates of California, China, Utah, and Vietnam Originated *Artemia sp*. in Different Temperatures and Salinity

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ABSTRACT

The aim of this study is to compared with amounts in per gram and the egg diameters, hatching rates in 25 ºC and 30 ºC temperatures and 25‰, 030‰, 035‰ salinity of *Artemia sp*. from four different origins (California, China, Utah and Vietnam). Egg count and diameter measurement was carried out with the use of binocular microscope. The most number of eggs in 1 gram was determined to be from Utah originated *Artemia sp*. while the least was determined to be from California originated ones. Hatching rates were detected to be the highest at 30ºC temperature, 25‰ salinity, at the 18th hour in Vietnam originated *Artemia sp*. In conclusion, it was detected that the hatching time for *Artemia sp*. varies depending on the temperature and salinity of the water.

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Introduction

Many fish and other aquatic creatures need to eat living organisms in the first stage of their development, i.e. when they hatch and the yolk sac retreats (Lavens et al. 1986). *Artemia sp*. is a zooplankton which grows in salt lakes, is used as live feed in aquaculture works (aquarium, fresh water, sea, etc.), and is one of the most preferred species due to its ease of production and high nutritional value. It is an essential live feed during various larva stages of especially sea fishes like bream, bass and sole fish and arthropods such as shrimp and lobster. *Artemia sp*. is frequently used live and frozen in the production of aquarium fishes because it has a high level of carotenoid pigment (Alpbaz et al. 1992). It is known that it was

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first used as live feed by Seale (1933) and Rollefsen (1939) in aquaculture (Sorgeloos, 1976). It is necessary for *Artemia nauplii* used in feeding of fish larvae as live feed to have the smallest size, to hatch at the same time and in a short period, and to be consumed at the stage it has the highest nutritional value (Alpbaz et al. 1992).

Artemia sp. forming durable cysts throughout its life cycle and these eggs being easily transportable and the possibility to keep the eggs in appropriate conditions for a long time are factors which popularized the use of this organism in aquaculture (Cirik and Gökpınar, 1993). Such that, *Artemia* eggs collected from salt lakes can be kept for many years and when needed, the eggs can be placed into salt water and larvae can be acquired. *Artemia sp*. of *Branchiopoda Crustacea* is an organism which is found in many parts of the world and which has a large salinity and temperature tolerance. Adult *Artemia* can resist salinity levels between 1‰ and 235% and temperatures between 6-37°C (Koray 1980, Alpbaz et al. 1992). Artificial incubation of *Artemia* eggs is carried out in prepared salt water solutions. The salinity of the used water needs to be 5-70‰ depending on the conditions (Çelikkale, 1978; Uçal, 1979; Sorgeloos, 1980). Other important environment parameters are pH, temperature, light, and oxygen concentration. The optimal temperature range for incubation of cysts and growth of nauplius is at 25-30°C temperature. Moreover, the temperature must remain stable during the incubation of eggs, otherwise, changing of temperature suddenly increase or decrease will be effect of hatching rate or biochemical composition of egg (Cirik and Gökpınar, 1993).

For this purpose; amount of eggs in one gram, egg diameters, and hatching rates in different salinity and temperatures of *Artemia sp*. from four different origins were compared in our study.

Material and Method

The China Mixed, Utah, and Vietnam originated *Artemia sp*. eggs used in the study were acquired from İzmir Rotifer Su Ürünleri A.Ş. and California originated *Artemia sp*. eggs were acquired from technical aquarium makers. The experiment system for the study was set up in Istanbul University Faculty of Aquaculture recirculating system and all egg hatching experiments were carried out here. Bain-Maria system was used for the opening of *Artemia sp*. eggs. An amount of tap water was put in two aquariums sized 50x50x100 cm and the experiment setup was formed by placing oval plastic bottles of 20 cm height and 5.5 cm diameter. Water temperatures were set in two different values as 25°C and 30°C with 100 Watt heaters. Salinity concentrations were set as 25‰, 30‰ and 35‰ by putting rock salt in the rested tap water and arranged in 3 repetitions for each experiment group. Additionally, air was connected to each experiment setup in order to provide the mixture for the eggs. This provided the settling of the eggs at the bottom and keeping the amount of oxygen dissolved in water at the required level. The eggs were weighed in sensitive digital scale in a way to be 0.20/0.25 gr for each group and the eggs were carefully placed in the readied cups which have the temperature and salinity levels set. First samples were taken 18 hours after the start of incubation of the eggs and hatching times were monitored. Samples of 0.5cc were taken from each experiment group with volumetric pipette at the end of 18 and 24 hours. Taken samples were immediately fixed with 4% formalin and their development was stopped. Count and measurement of the acquired samples were carried out under microscope. Count of all acquired samples in a binocular dissection microscope was carried out homogenously by pouring the 0.5 cc samples on 10x10 cm Petri dishes divided with 0.5x0.5 cm squares. The results of hatching rate, diameter and amount of eggs were subjected to variance analysis by the use of SPSS package program (SPSS 2004), and the differences between the averages found significant were determined by Tukey Multiple Comparison Test.

Result and Discussion

Artemia sp. exists in many parts of the world, has a large salinity and temperature tolerance and it is reported that its adult members can adapt to salinity values between 1‰ and 235% and temperatures between 6-37°C (Alpbaz et al., 1992). Artificial incubation of *Artemia* eggs are carried out in prepared salt water solutions. Thus, incubation experiments in different temperature, salinity, and times of *Artemia sp*. eggs from 4 different origins were carried out in this study. The results from each *Artemia sp*. from 4 different origins in two temperature and three salinity values were evaluated separately according to the results of the conducted experiment.

The number of eggs in 1 gram and egg diameters of California, Utah, China, and Vietnam *Artemia sp*. are presented in Table 1. It was detected that the number of eggs in one gram was the most in Utah originated Artemia sp. and the least in California originated Artemia sp. ($P<0.05$). It was detected that the egg diameter of California originated Artemia sp. was bigger than the other groups ($P < 0.05$). It was observed that the egg diameters of Utah and China originated Artemia sp. were similar ($P > 0.05$).

Table 1: Number of *Artemia* dry eggs in 1 gr

Artemia origin	Number of Eggs in a Gram	Egg Diameter (µ)
California	225.000 ± 7.01^{d}	275 ± 2.01^{a} µ
Utah	275.000 ± 7.71^{a}	265 ± 1.93^{b} µ
China	245.000 ± 7.00^{c}	265 ± 3.02^{b} µ
Vietnam	255.000 ± 7.78^{b}	235 ± 4.08^{c} µ

The values are presented as mean and standard deviation (Mean ± SD). The statistical difference between the values in the same column shown with

separate upper letters was found significant at 95% confidence level according to the Tukey multiple comparison test.

Artemia sp. is an organism found in many regions of the world and can live in a large salinity and temperature range (Alpbaz et al., 1992). For this purpose, hatching rates of *Artemia sp*. detected at 18th and 24th ours in 25ºC and 25‰, 30‰, and 35‰ salinity are presented in Table 2. When the hatching rates were compared and 18th and 24th hours were evaluated, it was observed that Utah originated *Artemia sp*. eggs in 30% salinity had a higher hatching rate compared to other groups ($P < 0.05$). Moreover, it was observed that there was a decrease in hatching rates in the 25‰ salinity group in 24th hour compared to 18th hour and the lowest hatching rate was in the China originated *Artemia* group at the 24th hour, and 25‰ salinity ($P < 0.05$).

Table 2: Hatching Rates of *Artemia* Eggs at 18th and 24th hours and 25ºC in 25‰, 30‰ and 35‰ Salinity

Artemia Types	Number of Eggs"(gL^{-1})					
	18th hour			24th hour		
	25‰	30‰	3‰	25‰	30‰	35‰
California	60±2^{a}	40±2^{b}	60±2^{b}	40±2^{a}	6± 2^{b}	60±3^{a}
Utah	40±1^{b}	75±2^{a}	68±2^{a}	30±2^{b}	68±3^{a}	55±2^{ab}
China	25±1^{c}	27±2^{c}	50±2^{c}	28±2^{b}	50±2^{c}	38± 2^{b}
Vietnam	10±2^{d}	10±1^{d}	11±1^{d}	5 ± 1^{c}	11±1^{d}	16± 1^{c}

The values are presented as mean and standard deviation (Mean ± SD). The statistical difference between the values in the same column shown with separate upper letters was found significant at 95% confidence level according to the Tukey and Duncan multiple comparison test.

Alpbaz et al. (1992), in a study they conducted, stated that *Artemia* eggs reach maximum hatching between 18-24 hours in a 20 liters or 75 litersconic ventilated medium at 25-30ºC and their hatching continues after this time as well. Güven and Erençin (1992) have examined the hatching in different temperature and salinity parameters at 24th and 48th hours and stated that better hatching occurs at the 48th hour. Koray (1988) stated that the hatching of *Artemia sp*. eggs occurs between 36-38 hours depending on the medium temperature (25-30ºC) and pH (8-8,2).

Table 3 shows the hatching rates of Artemia eggs at 18th and 24th hours in 30ºC and 25‰, 30‰ and 35‰ salinity ratios. When the table is examined, it was observed that the hatching amount of Vietnam originated *Artemia sp*. was generally lower compared to other groups ($P < 0.05$). It was determined that the hatching amounts of California and Utah originated *Artemia sp.*, which are prevalently used in fish and other aquatic farming, were higher compared to other groups ($P < 0.05$). It was detected that the highest hatching rate was at the 18th hour, 25‰ salinity, in *Artemia sp*. originated in Utah.

Table 3: Hatching Rates of *Artemia* Eggs at 18th and 24th hours and 30ºC in 25‰, 30‰ and 35‰ Salinity

Artemia Types	Number of Eggs(gL^{-1})					
	18th hour			24th hour		
	25‰	30‰	35‰	25‰	30‰	35‰
California	75±2^{ab}	60±2^{a}	54± 2^{b}	60±2^{b}	70±2^{a}	48±^{3ab}
Utah	80± 1^{a}	32±2^{b}	63± 2^{a}	75±2^{a}	35±3^{c}	54±2^{a}
China	30± 1^{c}	29±2^{b}	46± 2^{c}	15±2^{c}	45±2^{b}	40±2^{b}
Vietnam	5 ± 2^{d}	12±1^{c}	56± 1^{b}	3 ± 1^{d}	45±1^{b}	11±1^{c}

The values are presented as mean and standard deviation (Mean ± SD). The statistical difference between the values in the same column shown with separate upper letters was found significant at 95% confidence level according to the Tukey and Duncan multiple comparison test.

Sorgeloos (1976) stated in his study that the highest hatching rate occurs when the water temperature is kept stable at 28ºC. Similarly, in the study he conducted in 1980, he stated that quick and maximum amount of hatching of *Artemia* eggs occurs in 30ºC temperature but at 33-40ºC temperature the egg metabolism is delayed and deaths are observed (Sargeloos, 1980).

Vanhaecke et al. (1984) have reported that the optimal salinity for egg hatching is 35‰. It was determined that larva production from *Artemia* eggs can be carried out in artificially prepared salty waters and the salinity can change between 5-70‰ (Çelikkale, 1978). Sorgeloos and Persoone (1980) detected that *Artemia sp*. spends a lot of energy for osmoregulation at high salinity and salinity has an effect on physiochemical factors affecting the egg metabolism of *Artemia* and that salinity needs to be under a certain threshold in order for cyst development to begin. In a study they conducted, Güven and Erçetin (1992) have examined the hatching of *Artemia* eggs in different salinities (25‰, 30‰, 32‰ and 35‰) and stated that the difference salinity levels have not found a significant effect on hatching rates.

Jorge et al. (2017) carried out a production study with *Artemia franciscana* species they acquired from different regions of the internal waters of Mexico at 60, 80, 100, and 120 gL^{-1} salinity rates. They reported that the best egg hatching ratio is at 25ºC temperature and 120 gL^{-1} salinity and as the salinity ratio increases, the hatching rate increases as well.

According to the results I acquired in the study, it was detected that when the number of eggs in a gram and hatching rates are evaluated, the best result was with Utah originated *Artemia* group which is prevalently used in farming as live feed. Thus, it was once more detected that temperature has an effect on hatching of *Artemia sp*. eggs. Moreover, this was parallel to the values detected in the previously conducted studies.

Conclusion

Artemia sp. has an important place as a live feed source in farming of fish and other aquatic organisms.

Incubation experiments of *Artemia sp*. eggs from 4 different origins at different temperatures, salinity, and times were carried out in this study. According to the experiment results, although good results were acquired from *Artemia sp*. from 4 different origins in both temperatures and three different salinity values separately, best hatching was observed in Utah originated *Artemia sp*. at the 18th hour, 30^{o}C temperature and 25‰ salinity values. Although the importance of the sizes of *Artemia*, which have a commercial importance, is known in use as live feed in fish and other aquatic larvae, prevalent selection of Utah and California originated ones being a better choice, since the hatching rate is important in terms of efficiency, was evaluated with this study.

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