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Original Article

Investigation of different levels of glycerol on cyst hatching percentage, total length and survival of *Phallocryptus spinosa* and *Artemia franciscana*

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Abstract: *Artemia* and fairy shrimps due to propitious size and wide distribution throughout the world, purvey ample feed resources with relatively favorable nutritional value to profitable aquatic species. Ambient water physical-chemical properties affect the biological function of zooplanctonic cysts. Glycerol is an alcoholic compound which is soluble in water and it has three hydroxyl groups that are responsible for solubility in water. There is a relationship between the presence of free glycerol in water and cysts metabolic rate and dormancy duration. The present study aimed to investigate the effects of different levels of free glycerol in the hatchery water on hatching percentage, total length of nauplius and mortality rate of *Phallocryptus spinosa* and *Artemia franciscana* cysts. In this experiment, four triplicate treatments including 0% (control), 0.1%, 1% and 10% of glycerol were used on *A. franciscana* and *P. spinosa* hatchery water for 48 hours and 72 hours, respectively. The results revealed that 0.1% glycerol was the most efficient level for hatching percentage of *P. spinosa* cysts with 28.86±1.6%. Also, the size of total length of newly hatched nauplii in this treatment was 0.75±0.08 mm that was significantly greater than the other treatments ($P \leq 0.05$); there was no significant difference in the mortality percentage between this treatment and the control treatment. In *A. franciscana*, the highest hatching rate (68.33±4.71%) and nauplius length (0.90±0.08 mm) were recorded in the 1% glycerol treatment. According to the results, glycerol at 0.1% level for *P. spinosa* and 1% level for *A. franciscana* are suitable in the cysts hatching media to increase hatching rate and nauplii performance. Our work could contribute to a better understanding of the hatching biology of dormant life stages in zooplanctonic crustaceans.

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Introduction

Along with the development of aquaculture in recent decades, usages of another class of freshwater crustaceans belong to Anostraca such as *Artemia* and fairy shrimp have been of great interest. These organisms with their unique biological characteristics can live in a wide range of environmental conditions and have a high potential to produce high biomass, rapid growth and cysts production. Anostracas have a wide distribution in the world and since the beginning of 1910; nine families, 27 genera and 266 species of them have been recorded (Brtek and Mura, 2000). There is still no artificial feed formulation substitute for *Artemia*. *Artemia* is the most widely used live feed in larviculture due to its high nutritional quality (Sorgeloos et al., 1986). In fact, *Artemia* remains essential food item in most marine finfish and shellfish hatchery operations especially during the earliest life stages (Kolkovski et al., 2004).

Several studies examined temperature, salinity, soluble oxygen content and presence of few substances as influential factors on growth and reproduction of different *Artemia* and fairy shrimps species in hatching condition (Triantaphyllidis et al., 1995; Brown and Wanigasekera, 2000; Lotfi et al., 2003; Abatzopoulos et al., 2003; Hafezieh and Hosseinpour, 2009; Agh et al., 2008). Salinity, water temperature and nutrition are the most important factors affecting the growth and reproductive performance of *Artemia* and fairy shrimp in hatching conditions or natural ecosystems or artificial environment (Wear and hustler, 1987;

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Triantaphyllidis et al.,1995; Zmora and Shpigel, 2006; Atashbar et al., 2014).

The dehydrated encysted embryos resume development upon rehydration and aerobic incubation with an adequate salinity, coinciding with a decrease in the trehalose level of the cysts accompanied by a corresponding increase in the contents of glycogen and glycerol (Nambu et al., 1997). When the nauplius is fully developed, the shell splits, which is the result of free glycerol production and causes a considerable uptake of water (Clegg, 1964). Glycerol or glycerin is an alcoholic compound which is soluble in water and it has three hydroxyl groups that are responsible for solubility in water. Since, glycerol forms fats via its combination with fatty acids and glycolysis pathway in the studied organisms cysts, they absorb the glycerol as a precursor to provide energy for cellular metabolism. Study of the glycerol content in extracts of *Artemia salina* cysts revealed an absolute influence of glycerol on the *Artemia* cysts metabolism (James and Clegg, 1962). The main objective of the present study was to investigate on the effects of different free glycerol contents on hatching rate and quality of the larvae of *Phallocryptus spinosa* and *Artemia franciscana* as two important zooplanctonic crustaceans as live feed.

Materials and Methods

The *P. spinosa* cysts were collected from the Eastern Azarbaijan Province water recources body and and *A. franciscana* cysts were obtained frim ornamental fish center market. The experiment was conducted in the Laboratory of Fisheries Department in the University of Tehran. Experiment procedure was designed by triplicate in four treatments including 0% (control), 0.1%, 1% and 10% of glycerol. Eech treatment had 200 cysts of to include *P. spinosa* and *A. franciscana* in 100-ml cylindrical containers kept under 2000-lux light in incubators. The treatments varies solely in temperature of aeration being 20°C and 30°C for the *P. spinosa* and *A. franciscana* treatments respectively. Also water salinity for *A. franciscana* and *P. spinosa* treatments were adjusted in 30 ppt and 1 ppt, respectivly. Water salinity was made by $CaCl_2$ that prepared from the Merck company. Also, pH in the treatments were 7.1±0.2 during the research period.

In order to remove diapause, the cyst samples of both species were prepared according to Levanse and Sorgeloos (1996) chilling method. Thus, the cysts were stored at -20°C during a month in the freezer and then exposed to room temperature throughout a week leading to hatch stage (Levanse and Sorgeloos, 1996).

Artemia franciscana and *P. spinosa* cystes were shaken 48 and 72 hours after incubation, respectively to provide homogenized fairy shrimp and Artemia naupili. The samples (10 ml) were fixed in 4% formalin and the hatching percentage was calculated by the below formula (Gholamzadeh et al., 2018).

$$\text{Hatching percentage} = \frac{number\ of\ hatched\ cycts}{number\ of\ cycts + number\ of\ nauplii} \times 100$$

To calculate the percentage of mortality, the number of dead nauplii in the 10 ml samples were divided into number of hatched nauply as below formula.

$$\text{Mortality percentage} = \frac{number\ of\ dead\ nauplii}{number\ of\ hatched\ nauply} \times 100$$

To achieve the desired amount of glycerol ratio in the treatments, the ratio of 0% (control), 0.1%, 1% and 10% liquid glycerol was diluted to the incubation containers. For obsevation of hatching fairy shrimp and artemia cysts procces and nummerating their mortality, we took samples from the treatments and studied them under the steromicroscop and to measure their length and then were moved on the lam under optical microscopy. Also we used from image J software to analysis of the images (Drewes, 2006).

Before analyzing the variance, the data normal distribution was confirmed by the Shapiro-Wilk test then the quality parameters were analyzed by one-way ANOVA and significant differences among the means were found (P<0.05) by Duncan's test in SPSS software version 21 (IBM Co. NY, USA).

Results

Various glycerol concentrations in hatching media caused different records of mortality and hatching percentage in the treatments. The results showed that the highest hatching rate were recorded in *P. spinosa*

Table 1. Hatching percentage of *Phallocryptus spinosa* and *Artemia franciscana* exposed to different glycerol level (Values are represented as means±SD (n = 3). Means in the same row with different superscript show significant differences (P<0.05)).

treatments / species	Control	0.1% glycerol	1% glycerol	10% glycerol
P. spinosa	14.4±1.5^{a}	28.86±1.6^{b}	25.5±20^{ab}	0±0^{c}
A. franciscana	55±4.08^{a}	63.33±2.35^{ab}	68.33±4.71^{b}	0±0^{c}

Table 2. Total length (mm) of *Phallocryptus spinosa* and *Artemia franciscana* nauplii in different glycerol level (Values are represented as means±SD (n = 3). Means in the same row with different superscript show significant differences ((P<0.05)).

treatments / species	Control	0.1% glycerol	1% glycerol	10% glycerol
P. spinosa	0.61±0.04^{a}	0.75±0.08^{b}	0.65±0.02^{ab}	-
A. franciscana	0.87±0.01^{a}	0.89±0.02^{a}	0.90±0.08^{a}	-

Table 3. Mortality percentage of *Phallocryptus spinosa* and *Artemia franciscana* nauplii in Different glycerol level (Values are represented as means±SD (n = 3). Means in the same row with different superscript show significant differences ((P<0.05)).

treatments / species	Control	0.1% glycerol	1% glycerol	10% glycerol
P. spinosa	15.49±10.8^{a}	22.33±15.92^{ab}	27.66±4.49^{b}	-
A. franciscana	17.33±2.05^{a}	24±1.41^{b}	20±1.63^{ab}	-

(28.86±1.6%), *A. franciscana* uppermost hatching percentage (68.33±4.71%) in the 0.1% and 1% glycerol treatments, respectively. Also, the results showed no hatching in the cysts of the both *P. spinosa* and *A. franciscana* in the 10% glycerol treatment (Table 1).

The results of nauplius' total length have been presented in the Table 2. The results confirmed that in the *P. spinosa* nauplius, the highest total length was recorded in the 0.1% glycerol treatment and this treayment was signifilantly different with the control treatment. Total length of the *A. franciscana* nauplius among the treatments were similar and there were no significant differences among the glycerol treatments (Table 2).

The results showed that the highest mortality percentage of *P. spinosa* nauplius (27.66±4.49%) was recorded in the 1% tretament but in the *A. franciscana* was recorded (24±1.41%) in the 0.1% percentage. In the *P. spinosa*, there was sinificantly different between the 0.1% and the control treatment but in the *A. franciscana* these treatments had no significant different (Table 3).

In general, results of the present study about *P. spinosa* implies that the best hatching percentage, total length, and the mortality rate occurs in the 0.1% glycerol treatment. Although, the mortality rate of this treatment was greater than the control, this difference was not statistically significant. Also, the effects of various glycerol levels on the *A. franciscana* larval quality suggested that 1% glycyrol level is the best experimental treatment. Nevertheless, increasing the amount of glycerol did not induce significant differences in total length of *A. franciscana* and treatments with 0.1% glycerol significantly increased the mortality rate in comparison to the control treatment while 1% level of glycerol did not show any significant difference.

Discussion

The effects of external osmotic pressure on growth and hatching, respiration with changes in glycogen conctent, glycerol and trehaloses in different *Artemia* species cysts have been investigated. The column at the right attempts to account for the decrease in trehalose by increases in glycogen, glycerol, and glucose equivalents of the amount of oxygen consumed (Clegg, 1964). The severity of this action is directly related to external osmotic pressure factors. Glycerol exists in two parts of the cyst, the space

filling between embryonic layer and the shell from where glycerol is emitted to the environment as the shell is discharged and embryo produces embryonic glycerol immediately after hatching (Jmaes and Clegg, 1964). If free glycerol in *Artrmia* cyst is functioning osmotically during the lag period, and thereby enabling the embryo to emerge, then the osmotic pressure exerted by this glycerol can be expected to bear some relation to the external osmotic pressure (Clegg, 1964). The present study investigated how different levels of glycerol can affect on the hatching percentage of two zooplanctonic crustacean species i.e. *P. spinosa* and *A. franciscana* and to what extent help to improve the larval quality of the larva. The level of growth and survival of *Artemia* in nature and cultivation conditions mainly depends on temperature, salinity, quantity, and quality of food (Sorgeloos, 1986; Saravanakumar and Soundarapandian, 2009). According to the present study, glycerol factor also can affect the quality of *P. spinosa* and *A. franciscana* nauplii. The results indicated that the presence of specific amounts of glycerol in the incubation system of *P. spinosa* and *A. franciscana* cysts increased hatching percentage. Glycerol, an osmotically active metabolite produced by the embryo, is responsible for mechanically breaking the tertiary shell in Artemia cysts (Murugan and Dumont, 1995). It seems that the presence of glycerol in the cysts incubation system caused a change in the osmotic pressures on both sides of the cyst shell brought about changes in the time and hatching percentage in the studied species. The differences between the best treatment of glycerol level in *P. spinosa* and *A. franciscana* hatching percentage could be due to the presence of different glycerol amounts and variation in the pressure between the hatching media and the cysts internal condition. It seemed possible that glycerol might be involved in shell-rupture because of its presence in high concentration, its relatively low molecular weight, and its well-known hygroscopic properties (Clegg, 1964).

Nowadays, the use of materials, compounds and resources improving the probability of growth, survival rate and reproductive performance of live feeds in breeding conditions are of particular importance regarding the mass production. When the nauplius is fully developed, the shell splits, which is the result of free glycerol production and causes a considerable uptake of water (Wheeler et al., 1979). Researches has examined the important factors such as temperature, salinity, oxygen concentration and alkalinity on growth conditions and reproductive performance of *Artemia* and fairy shrimp (Triantaphyllidis et al., 1995; Brawn and Wanigasekera, 2000; Lotfi et al., 1382; Abatzopoulos et al., 2003; Hafezieh and Hosseinpour, 2009; Agh et al., 2008). In addition, results of current study suggested that raising the amount of glycerol to certain levels boosts the hatching percentage of both spiecies, *A. franciscana* and *P. spinosa*. Low concentration of glycerol (0.5 and 0.75 mol/l) enhance hatching percentage of the *A. parthenogenetica* cysts (Royan et al., 1987). In contrary to a previous study, advocating the downregulating effects of glycerol content increase on *A. parthenogenetica* hatching percentage (Royan et al., 1987). The results of current study corroborate a concordance between increment of glycerol and augmented hatching rate and larva quality for both tested spiecies when other influencial factors except for glycerol level remains unwavering. A lowered hatching at higher concentrations of glycerol, conversely, might be due to an increase in the viscosity of the medium and perhaps to the rapid development of fungi and baeteria (Murugan and Dumont, 1995). Another study showed that hatchability was significantly increased (23%) in 0.0125% glycerol and concentration above 0.025% glycerol had no or a negative influence on hatching of the *Thamnocephalus platyurus* (Murugan and Dumont, 1995). Carbohydrate metabolism, which involves the conversion of trehalose to glycerol and is required for hatching, responds to increasing salinity as reported in other Artemia species: increasing amounts of glycerol must be synthesized as salinity is raised (Drinkwater and Crowe, 1991).

Despite the rising trend of hatching percentage as hatching medium was enriched by more glycerol,

hatching was impeded in treatments containing highest glycerol amount (10%). This observation might be induced by several reasons. For instance, osmotic pressure could be the reason due to the fact that adding superfluous amounts of glycerol is likely to alter the osmotic pressure between cyst internal condition and hatching environment dramatically resulting in reversed or ceased water flow. The accumulating glycerol in the egg becomes the osmotic gradient and eventually the inelastic alveolar layer is fractured, rupturing the shell and propelling the instar from the shell (Trotman 1991). From other point of view, adding excess amounts of glycerol afflicts the water quality and hatching medium ending up without any hatching for both examined species. The influence of different factors such as the level of water hardness and various ratios of water hardness factors such as calcium and magnesium on the hatching percentage and quality of larvae *P. spinosa* and *A. franciscana* were investigated. The results indicated that increasing the water hardness up to 500 mg/L can affect the amount of hatching rate of *P. spinosa* and *A. franciscana* and improve the nauplii quality (Gholamzadeh et al., 2018). Though glycerol, accumulated inside the cysts, aids the embryo to rupture the shell, its role in hatching when added externally is indeed not known (Murugan and Dumont, 1995). The stimulation of net glycerol synthesis by increased osmotic pressure indicated that free glycerol might play a role in overcoming the osmotic pressure difference between the interior of the Artemia cyst and its environment (Clegg, 1964).

Several researches studied the combined effects of photoperiod, temperature, and salinity, each with three levels, on the hatching percentage of *Artemia* cysts. Photoperiod and temperature, as well as temperature-salinity interaction, were found to significantly affect the hatching percentage of *Artemia* (Arun et al, 2017). Increasing the amount of glycerol in the hatching environment decreases the *A. parthenogenetica* hatching percentage (Royan et al., 1987). Bacteria are commonly found associated with the shell surface of most cysts. The degree of contamination varies considerably, but often bacteria are found completely coating the outer shell layer (Wheeler et al., 1979). *Vibrio* spp. has became dominant after 24 hours, probably because during hatching, *Artemia* cysts are broken and a reserve organic substance, glycerol, is excreted to hatching water (Sorgeloos et al., 1986). Glycerol is an organic substrate that is utilized efficiently by *Vibrio* spp. A very low inoculum of this population could became dominant, utilizing glycerol rather than the Gram-positive population (López-Torres and Lizárraga-Partida, 2001). Bioflocs grown on glycerol as carbon source inhibit quorum sensing-regulated bioluminescence in *V. harveyi* and protect brine shrimp larvae from vibriosis (Crab et al., 2010).

The results of this experiment proved that under constant conditions for other factors influencing in hatching rate, increasing the amount of glycerol can increase the hatching and larval quality of these two species. The present study confirmed that the presence of glycerol in the incubation media of the *P. spinosa* and *A. franciscana* increase the hatching percentage in both species. However, in neither of these species, in the treatment of 10%, glycerol content had not any hatching and this issue can be indicative of several factors. By increasing the concentration of glycerol in the hatching media, the amount of exogenous pressure in the environment, outside and inside the cyst is equal and in cases of high levels of glycerol, by increasing the concentration of glycerol, the incubation medium increases the pressure in this environment and causes inhibition of water absorption by the cysts. On the other hand, it can be concluded that increasing the amount of glycerol to 10%, reduces the water quality factors for the species, which caused the hatching percentage in *P. spinosa* and *A. franciscana* to be zero.

In another hand, the *Artemia* was tested to study the combined effects of photoperiod, temperature, and salinity, each with three levels, on the hatching percentage of their cysts. Photoperiod and temperature, and temperature-salinity interaction, were found to significantly affect the hatching percentage of *Artemia* (Arun et al, 2017). According to the outcomes of the current study, addition of glycerol in the incubation water enhances the hatching percentage and nauplii quality of *P. spinosa* and

A. franciscana cysts. In order to produce fine and qualified aquatic species, making a significant contribution to aquatic animal and living food production, it is suggested to benefit from the factors influencing the production and quality of *P. spinosa* and *A. franciscana* larvae. Glycerol is a crucial material in hatching environment serving capabilities to obstruct or promote hatching rate. The results in this study presented either hatching stoppage and enhancement when hatching medium of *P. spinosa* and *A. franciscana* included high glycerol content and certain glycerol amount respectively.

In conclusion, quality parameters of the *P. spinosa* and *A. franciscana* cysts were investigated in responses to variation of hatching medium glycerol level. *P. spinosa* responsed to the different levels of glycerol treatments and recorded the highest mortality percentage in the 1% glycerol treatment while in the *A. franciscana* irregular response recorded a significant mortality at 0.1% level of glycerol and no statistically different in mortality rate among the fifferent glycerol concentrations. In terms of hatching percentage, *P. spinosa* and *A. franciscana* cysts revealed positive trend of improvement as glycerol level increased, but reached the optimum amount at 0.1% glycerol level for *P. spinosa* and 1% for *A. franciscana* cysts. Although, hatching rate and mortality percentage was affected by glycerol level variation, total length response of *A. franciscana* cysts remained unchanging as glycerol level varied. Overall, according to the results, glycerol at 0.1% level for *P. spinosa* and 1% level for *A. franciscana* are suitable in the cysts hatching media to increase hatching rate and nauplii growth.

References

Abatzopoulos T.J., El-Bermawi N., Vasdekis C., Baxevanis A.D., Sorgeloos P. (2003). Effects of salinity and temperature on reproductive and life span characteristics of clonal Artemia. (International Study on Artemia. LXVI). Hydrobiologia, 492: 191-199.

Agh N., Van Stappen G., Bossier P., Sepehri H., Lotfi V., Razavi Rouhani S.M., Sorgeloos P. (2008). Effects of salinity on survival, growth, reproduction and life span characteristics of Artemia population from Urmia Lake and neighboring lagoons. Pakistan Journal of Biological Sciences, 11(2): 164-172.

Arun V., saharan N., Ramasubaranian V., Babitha rani A., Salin K., Ravindra S., Harsha H., Deepak G.P. (2017). Multi-response optimization of *Artemia* hatching process using split-split-plot design based response surface methodology. Scientific Reports, 7: 40394.

Atashbar B., Agh N., Stappen G., Mertens J., Beladjal L. (2014). The combined effect of temperature and salinity on hatching characteristics of three fairy shrimp species (Crustacea: Anostraca). Journal of limnology, 73(3): 574-583.

Brown R.A., Wanigasekera G. (2000). Combined effects of salinity and temperature on survival and reproduction of five species of Artemia. Journal of Experimental Marine Biology and Ecology, 244: 29-44.

Brtek J., Mura G. (2000). A revised key to families and genera of the Anostraca with notes on their geographical distribution. Crustaceana, 73: 1037-1088.

Clegg J.S. (1964). The Control of emergence and metabolism by external osmotic pressure and the role of free glycerol in developing cysts of *Artemia salina*. Journal of Experimental Biology, 41: 879-892.

Crab R., Lambert A., Defoirdt T., Bossier P., Verstraete W. (2010). The application of bioflocs technology to protect brine shrimp (*Artemia franciscana*) from pathogenic *Vibrio harveyi*. Journal of Applied Microbiology, 109: 1643-1649.

Drewes C. (2006). Quantitative investigations of hatching in brine shrimp cysts., in Tested Studies for Laboratory Teaching, Volume. Proceedings of the 27th Workshop/Conference of the Association for Biology Laboratory Education (ABLE). pp: 299-312

Drinkwater L.E., Crowe J.H. (1991). Hydration State, metabolism, and hatching of Mono Lake artemia cysts. The Biological Bulletin, 180: 432-439.

Gholamzadeh P. (2018). Investigation of effects of different levels of glycerol and water total hardness and various Ca: Mg ratios on larval quality of the fairy shrimp *Phallocryptus spinosa* and *Artemia franciscana*. Master's Thesis. The University of Tehran.

Gholamzadeh P., Rezaei Tavabe K., Rafee G., Seidgar M. (2018). The study of different levels of hardness and different rations of Ca and Mg water hardness on cyst hatching and larval quality of fairy shrimp: *Phallocryptus spinosa*. Journal of the Animal Environment, (Accpeted paper).

Hafezieh M., Hosseinpour H. (2009). Effect of salinity on

the reproduction model of Artemia in Urmia Lake. Journal of Biology, 1(2): 21-28.

Jmaes S., Clegg B. (1964). The Control of emergence and metabolism by external osmotic pressure and the role of free glycerol in developing cysts of *Artemia salina*. Journal of Experimental Biology, 41: 879-892.

Jmaes S., Clegg B. (1962). Free glycerol in dormant cystes of the brine shrimp *Artemia salina*, and its disappearance during development. Biological Bulletin, 123(2): 295-301.

Kolkovski S., Curnow J., King J. (2004). Intensive rearing system for fish larvae research II Artemia hatching and enriching system. Aquacultural Engineering, 31: 309-317.

Lavens P., Sorgeloos P. (1996). Manual on the production and use of live food for aquaculture. FAO Technical Paper. 305 p.

Lotfi G.G.V., Agh N., Sepehri H. (2003). Effects of different salinities on growth, survival, life history and reproductive charachteristics of three populations of Artemia from Iran. Journal of Science, 2: 305-16.

López-Torres M.A., Lizárraga-Partida M.L. (2001). Bacteria isolated on TCBS media associated with hatched *Artemia* cysts of commercial brands. Aquaculture, 194: 11-20.

Murugan G., Dumont H. (1995). Influence of light, DMSO and glycerol on the hatchability of *Thamnocephalus platyurus* Packard cysts. Hydrobiologia, 298: 175-178.

Nambu Z., Nambu F., Tanaka S. (1997). Purification and Characterization of Trehalase from Artemia Embryos and Larvae. Zoological Science, 14: 419-427.

Rezaei Tavabe K., Rafiee G., Shoeiry M.M., Houshmandi S. (2015). Effects of water hardness and Calcium: Magnesium ratios on reproductive performance and offspring quality of *Macrobrachium rosenbergii*. Journal of the world Aquaculture Society, 46(5): 519-530.

Royan J.P., Sumitra K., Ramaiah N. (1987). Cyst quality and hatching in parthenogenetic brine shrimp, *Artemia.* Indian Journal of Marine Sciences, 16: 249-252

Saravanakumar P., Soundarapandian G. (2009). Effect of different salinities on the survival and growth of *Artemina* Spp. Current Research Journal of Biological Sciences, 1(2): 20-22.

Sorgeloos P., Lavens P., Léger P., Tackaert W., Versichele D. (1986). Manual for the culture and use of brine shrimp Artemia in Aquaculture. Belgium: *Artemia* Reference Center, Faculty of Agriculture, State University of Ghent, 10: 91-95.

Triantaphyllidis G., Poulopoulou V., Abatzopoulos T.I., Perez A.A.P., Sorgeloos P. (1995). International study on Artemia XLIX. Salinity effects on survival, maturity, growth, biometrics, reproductive and lifespan characteristic of a bisexual and a parthenogenitc population of Artemia. Hydrobiologia, 302: 215-227.

Trotman C.N.A. (1991). Normality and abnormality in early development. In: R.A. Browne, P. Sorgeloos, C.N.A. Trotman, (eds.). Artemia Biology. CRC Press, Boca, Raton, Florida. pp: 75-92.

Wear R.G., Huslett S.J. (1987). Studies on the biology and ecology of *Artemia* from Lake Grassmere, New Zealand. In: P. Sorgeloos, D.A. Bengtson, W. Decleir, E. Jaspers (eds.). *Artemia* Research and its Applications. Ecology, Culturing, Use in Aquaculture, Universa Press, Wetteren, Belgium. pp: 101-126.

Wheeler R., Yudin A.I., Clark W.H. (1979). Hatching events in the cysts of *Artemia salina*. Aquaculture, 18, 59-67.

Zmora O., Shpigel M. (2006). Intensive mass production of Artemia in a recirculated system. Aqauculture, 255: 488-494.