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Effects of Salinity on the Hatching Efficiency of Artemia Cysts Decapsulation

Hnin Hnin Htun,
Department of Biotechnology, Mandalay Technological University, Mandalay, Myanmar

Htway Htway San,
Administative Department, Computer University (Taungoo), Taungoo, Myanmar

Zin Myo Swe
Department of Biotechnology
Mandalay Technological University,
Mandalay, Myanmar

Abstract: In this study, hypochlorite (liquid bleach, NaOCl) was used for *Artemia salina* cysts decapsulation as a decapsulation method which saves cost and labor because of increasing the hatching rate and speed. Cysts were removed from hypochlorite solution after 15 min. The stocking density in each hatching systems were 1gm cysts per 1L, replicated three times. The treatments were maintained at 25‰, 30‰, 35‰ and 40 ‰ saline solution and the pH between 7.5 - 8.5 respectively. A uniform temperature of 28ºC was maintained for all the treatments throughout the study period. However, among the treated cysts, the best hatching rate (83±1.7%) was achieved in cysts treated with (liquid bleach, NaOCl). Hatching efficiency of the cysts in different salinity levels was studied and described in this paper in salinity range of 25‰ to 40‰. Maximum hatching efficiency was recorded in the water having 30‰. The aim of this paper is to study the hatching efficiency of *Artemia* cysts in varying salinity decapsulation conditions.

Keywords: *Artemia salina*, Decapsulation, Hatching efficiency, Cysts, salinity

1. INTRODUCTION

Among the live diets used in the larviculture of fish and shellfish, nauplii of the brine shrimp *Artemia* constitute the most widely used food item. Annually, over 2000 metric tons of dry *Artemia* cysts are marketed worldwide for on-site hatching into 0.4 mm *nauplii*. Indeed, the unique property of the small branchiopod crustacean *Artemia* to form dormant embryos, so called 'cysts', may account to a great extent to the designation of a convenient, suitable, or excellent larval food source that it has been credited with. Those cysts are available year round in large quantities along the shorelines of hyper-saline lakes, coastal lagoons and solar salt works scattered over the five continents. After harvesting and processing, cysts are made available in cans as storable 'on demand' live feed. Upon some 24-hr incubation in seawater, these cysts release free-swimming *nauplii* that can directly be fed as a nutritious live food source to the larvae of a variety of marine as well as freshwater organisms, which makes them the most convenient, least labor-intensive live food available for aquaculture [1].

Live food is necessary for fish larvae, especially in marine species. Undeveloped digestive system of larvae, makes them completely dependent on live food. Live foods have other advantages over the formulated feed including appropriate size, palatability due to high water content, immersion in water column and stimulation of larval predatory behavior (Bengston, 2003). 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) [2].

Generally, *Artemia* cyst decapsulation depends on many factors like temperature, salinity, pH, cyst quality, aeration etc. Moreover, fixed temperature, cyst density and container's design also play important role in maximal production of *Artemia* cyst (Lavens and Sorgeloos 1996, Sharahi and Zarei 2016) [1] , [3].

Decapsulation is a process in which the chorion layer is dissolved and removed. Decapsulated cysts could be used as a food source for fish larvae, although its application is limited compared to the *nauplii*. Decapsulated cysts have been used to rear larvae of freshwater catfish (*Clariasgariepinus*), common carp (*Cyprinuscarpio*), and marine shrimp (*Penaeusindicus* and *Penaeusmonodon*) and milkfish (*Chanoschanos*) (Verreth et al., 1987; Vanhaecke et al., 1990; Stael et al., 1995; Ribeiro and Jones, 1998; Sui, 2000).

But, there was lack of research work of precise temperature and salinity or their combined effect on cyst decapsulation. That is why, an experiment was conducted to find out the influence of salinity and temperature on the decapsulation of cyst. This *Artemia* embryo was completely removed from the cyst by a short-term incubation in saline solution with aeration. This procedure is called decapsulation of cyst (Lavens and Sorgeloos 1996)[1].

Sorgeloos (1976) stated in his study that the highest hatching rate occurs when the water temperature is kept stable at 28ºC [8]. Similarly, in the study he conducted in 1980, he stated that quick and maximum amount of hatching of *Artemia* eggs occurs in 30 ºC temperature but at 33-40 ºC temperature the egg metabolism is delayed and deaths are observed (Sargeloos, 1980)[9]. Vanhaecke et al. (1984) have reported that the optimal salinity for egg hatching is 35‰ [10].

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)[11].

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*a nd 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 [12].

Ahmed *et al.* (1997) performed an experiment on the effect of decapsulation on viability and hatching of *Artemia* cysts at different salinities. They found highest hatching rate (75.7 ± 2.5 %) at 30 ‰ and lowest (70.0 ± 1.7 %) at 25 ‰ after 24 hrs of incubation [13].

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) [14].For this purpose hatching efficiency and hatching rates in different salinity of *Artemia* sp. compared in our study.

2. MATERIALS AND METHODS

2.1 Ultrastructure of *Artemia* Cyst

The hard, dark brown, external layer of a cyst, the chorion (Figure. l), which can be removed in a hypochlorite solution, is lipoproteinaceous and is impregnated with haematine, a derivate of haemoglobin (Dutrieu, 1960; Linder, 1960; Anderson et al., 1970) [15]. It has numerous interconnected canals which are filled with air and are in contact with the surface of the cortical layer. According to Mathias (1937) this alveolar layer contributes to the buoyancy of the cyst [16].

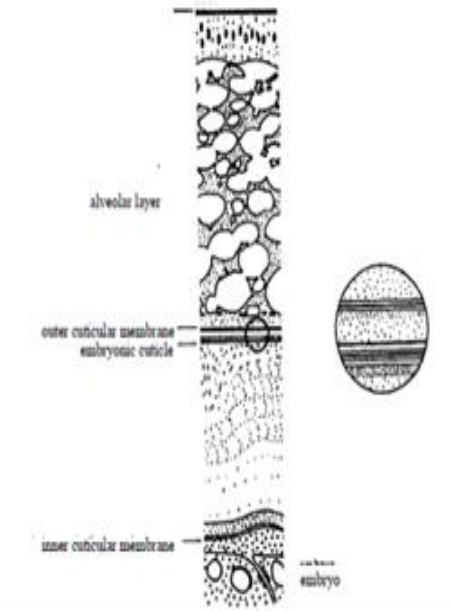

Figure 1. Schematic Diagram of the Ultrastructure of an *Artemia* Cyst. (Modified from Morris and Afzelius, 1967)[17]

2.2 *Artemia* Decapsulation Procedure

1. Cyst hydration: Hydrate 1gm of dried cyst with 100 µm mesh bag in 1 L of tap water in a cup for 15 minutes at room temperature. Examine the cyst under a microscope with top lighting before proceeding. In the figure 2, dry cysts are dimpled, resembling a deflated basketball, whereas fully hydrated cysts are completely spherical in shape. The cysts must be fully hydrated prior to the decapsulation step.

2. Decapsulation: Add the chilled bleach (NaOCl) to the cup and continue with pushing for 15 minutes. When the cysts are 90% orange, stop the reaction and rinsing well with cool tap water.

3. Neutralization of residual chlorine: To neutralize any residual chlorine transfer the mesh bag to a clean 2 L cup and pour the 1.0% sodium thiosulfate (1L) into the bag. Soak the cysts in the sodium thiosulfate solution for ~1 min, then rinse the cysts with dechlorinated tap water. And then, 5mL of the 100 µm mesh bag water sample tested with one drop of otholidine solution. When the sample water appeared light yellow color, it was indicated for presence of chlorine residues and the colorless of water sample was free of chlorine residues.

4. Dehydration for long-term storage: Transfer the cysts back to the cup with 2 L of saturated brine and aerate for 24 hours (save back a filled squirt bottle of saturated brine solution to help transfer cysts to pail). Add granular NaCl (30gm/1L) 30‰ as needed to keep the solution saturated during the dehydration process. Transfer dehydrated cyst 5 L pail and fill with fresh saturated brine 30‰.

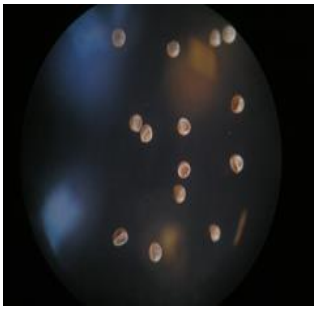

Figure 2. Dry *Artemia* Cysts

3. RESULTS AND DISCUSSIONS

The chorion of the brime shrimp cysts is removed without affecting the viability of the embryos by a process known as decapsulation. The decapsulated cysts can be supplied as feed for shrimp larvae. The process of decapsulation involves hydration of the cysts for 15 minutes in clean water.

For decapsulation of 1gm of cysts, a solution is prepared by dissolving 0.5 g of bleaching powder in water and cysts were removed from hypochlorite solution after 15 min. Thereafter, the cysts were washed with clean water 5 times prior to incubation. Continuous aeration was provided to ensure dissolved oxygen up to 5 ppm and suitable turbulence for cysts.

Four types of decapsulation salinity solution were prepared (e.g. 25‰, 30‰, 35‰ and 40‰). Three replicates were maintained for each treatment. Decapsulation was performed at 28°C. Temperature and salinity of water were checked using a refractometer, and thermometer.

After keeping the solution overnight with aerator (SOBO-348A air pump) at (pH 8.0-8.5), the supernatant was taken for decapsulating the cysts. Hatching rate was recorded at 24hr after incubation and determined by counting and averaging the *nauplii* in three samples (0.1 ml).

Table 1 shows the hatching rates of *Artemia* eggs at 24th hours in 28°C and 25‰, 30‰,35‰ and 40‰ salinity ratios. When the table is examined, it was observed that the hatching rate at the salinity 40‰ was generally lower compared to

other groups. It was detected that the highest hatching rate was at the 24hr, 30‰ salinity, in *Artemia sp.* OUXE(Great Salt Late –Uta USA).

Table 1: Hatching Rates of *Artemia* Eggs at 24 hr in 28°C with 25‰, 30‰, 35‰ and 40‰

Artemia Types	24hr(Hatching Time			
	25‰	30‰	35‰	40‰
OUXE(Great Salt Late –Uta USA)	73±2.6	83±1.7	71±2.6	63±2

There was general increase in the hatching rate of *Artemia* cysts in different salinity levels. In this study, temperature was uniform (28ºC) for all the three salinity levels tested and pH of the rearing medium was (7.7-8.5) for (25‰, 30‰, 35‰ and 40‰) saline solutions respectively.

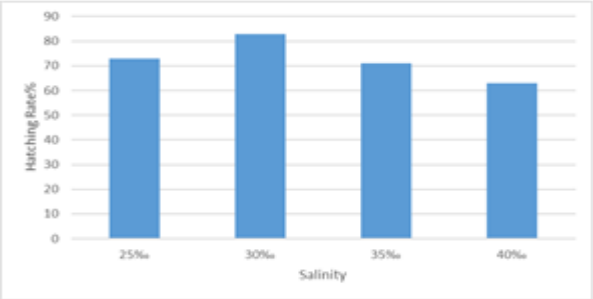

Figure 3. Effect of Salinity on Decapsulation of *Artemia* Cyst at 28°C

Water temperature, and pH is probably the most important environmental variables in *Artemia salina* cultures, because it directly affects metabolism, oxygen consumption, growth, moulting and survival (Herbst, 2001)[18].

The effect of salinity on decapsulation of *Artemia* cyst at 28°C is presented (Figure 3). Highest hatching rate (83±1.7) % was observed in 28ºC temperature at 30‰ salinity. Lowest hatching rate (63±2) % was found in 30°C at 40‰. Data are recorded as mean ±SEM. Bar with different letter show significantly difference ($p < 0.05$, analyzed by one way ANOVA).This experiment was conducted to find out the influence of salinity and temperature on the decapsulation of cyst.

4. CONCLUSIONS

The variation of *Artemia* decapsulation rate could be the effect of different above mentioned factors. It can be concluded from the above discussion and the findings of this study that the decapsulation rate of *Artemia* cyst greatly depends on salinity and temperature.

This result, 30‰ salinity and 28°C could be the best combination of higher rate of decapsulation of *Artemia* cyst. Moreover, decapsulation rate decreases above 35‰ and 28°C. The best survival rate is most probably achieved at 30‰.Therefore, we may conclude that 30‰ salinity at 28°C could enhance survival of *Artemia*.

This paper deals with the study of hatching efficiency of *Artemia* cysts in decapsulation condition. *Artemia* cysts found a direct correlation with the environmental factors, which is supported by the present findings.

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6. REFERENCES

[1] Lavens, P. and Sorgeloos, P. 1996. Manual on the production and use of live food for aquaculture FAO Fisheries Technical Paper. No. 361. Rome, FAO. 295 pp.

[2] Bengston D. A. (2003). Status of marine aquaculture in relation to live prey: Past, present and future. In: J. G. Støttrup, and L. A. McEvoy (Eds.) Live Feeds in Marine Aquaculture, Wiley-Blackwell. pp: 1-16.

[3] Lavens, P., Leger, P., Sorgeloos, P. (1986). Production, Utilization and Manipulation of Artemia as Food Source for Shrimp and Fish Larvae, Actualities de Biochemie Marine, Oceanis, 9: in pres 1-16.

[4] SharahI, A.R. and Zarei, S. 2016. Mutual effect of light and turbidity on hatching of Artemiafranciscana cysts. International Journal of Fauna and Biological Studies 3(2): 03-06.

[5] Vanhaecke P., De Vrieze L., Tackaert W., Sorgeloos P. (1990). The use of decapsulated cysts of the brine shrimp Artemia as direct food for carp C y p r i n u s c a r p i o L. larvae. Journal of World Aquaculture Society, 21: 257–262.

[6] Verreth J., Segner H., Storch V. (1987). A comparative study on the nutritional quality of decap-sulatedArtemia cysts, micro-encapsulated egg diets and enriched dry feeds for Clariasgariepinus (Burchell) larvae. Aquaculture, 63: 269–282.

[7] Ribeiro F.A., Jones D.A. (1998). The potential of dried, low-hatch, decapsulatedArtemia cysts for feeding prawn post-larvae. Aquaculture International, 6: 421-440.

[8] Sorgeloos, P. (1976). The Brine Shrimps Artemia salina; A Bottleneck in Mariculture Pillay T.V.R., Dill. Wm. AAdvens in Aquaculture, Fishing News Boks Ltd. Farnhom, 321-324.

[9] Sorgeloos, P. (1980). Life History Of The Brine Shrimp Artemia, Proceeding of the International Symposium on the Brine Shrimp Artemiasalina, Corpus Christi, Texas, USA, Univesal Pres, Wetteren, Belgium, 19-23.

[10] Vanhaecke, P., Siddall, E.S., Sorgeloos, P. (1984). International Study on Artemia XXXII. Cambined Effects of Temperature and Salinity on the Survival of Artemia of Various Geographical Origin, J. Exp. Mar. Biol. Ecol., Vol: 80, pp: 259-275.

[11] Çelikkale, M.S. (1978). Artemia salina nauplii Yumurtalarının Tuzlu Suda Açılımve Normal SudaYaşama Süreleri Üzerine BirAraştırma, Ankara Üniversitesi ZiraatFakültesiYıllığı, Cilt:28 Sayı:2 Sayfa:447-456.

[12] Güven, E., Erençin, Z. (1992). Determination of Hatching Rates of the Brine Shrimp, Artemia sp., Depending on the Temperature and Salinity in

Laboratory Conditions, İ.Ü. Su Ürünleri Dergisi, Cilt:6, Sayı 2, Sayfa:61-70

[13] Ahmed, S.U., Rahman, M.A., Islam, M.N. and Kamal, M. 1997. Effect of decapsulation on viability and hatching performance of Artemiacysts at different salinity levels. BangladeshFish. Res. 1(2): 67-7 4.

[14] Alpbaz, A., Temelli, B., Özden, O., Korkut, A.K., Güner, Y.(1992).Farklı Orjinli Artemia Yumurtalarının Açılım Oranları Üzerinde Araştırmalar, E.Ü. Su Ürünleri Dergisi, Sayı: 31-32, Cilt: 8 Sayfa: 116-123.

[15] Dutrieu, J., 1960. Observations biochimiques et physiologiques sur le développement d'Artemiasalina Leach. Arch. Zool. Exp. Gén., 99: 1—134.

[16] Mathias, P., 1937. Biologie des Crustacés phyllopodes. ActaSei. Ind., 447: 1—107.

[17] Morris, J.E. and Afzelius, B. A., 1967. The structure of the shell and outer membranes in encysted Artemiasalina embryos during cryptobiosis and development. J. Ultrastruct. Res., 20: 244—259.

[18] Herbst D.B., (2001). Gradient of salinity stress, environmental stability and water chemistry as a template for defining habitat types and physiological strategies in inland salt waters. Hydrobiologia 466: 209-219.