Biometrical Study Of Brine Shrimp (*Artemia franciscana*) With Special Emphasis On Hatching Efficiency And Hatching Percentage In Tropical Condition At Bangladesh

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Abstract

The present study was carried out to investigate the hatching efficiency and hatching percentage of untreated and decapsulated cysts of *Artemia franciscana* at three salinities; 30 ppt, 35 ppt and 40 ppt. This study revealed that the hatching efficiency and hatching percentage were higher in case of decapsulated cysts. First hatching of nauplius from cysts were observed after 14 hours and 13 hours of incubation for untreated and decapsulated cysts, respectively. The values of hatching efficiency and hatching percentage were also higher at 35 ppt in untreated and decapsulated cysts. At 35 ppt, the hatching efficiency after 48 hours of incubation was 1.42×10^5 for decapsulated cysts and the value for untreated cysts was 8.96×10^4 nauplii per gram cysts. After 96 hours of incubation, the hatching percentage for decapsulated cysts were 75%, 89% and 90% at 30 ppt, 35 ppt and 40 ppt, respectively; and the values were 55%, 62.5% and 60% for untreated cysts at respected salinities, 30 ppt, 35 ppt and 40 ppt. The mean diameter for untreated and chorionic cysts were 242.2±1.90 µm and 213.5±3.167 µm respectively, with a chorionic thickness of 14.35 µm. This study would help the hatchery managers to understand the efficiency of larval live feed, since adequate data are lacking on the hatching performances of *Artemia* cysts in Bangladesh.

Keywords: Hatching efficiency, Hatching percentage, Decapsulation, Chorionic cysts

Introduction

The brine shrimp, *Artemia*, being the most reliable and obligate live feed item for fishes, have widely been used by the aquaculturists since long. It is a typical inhabitant of hypersaline environments, where other few animals can survive. Their unusual mode of reproduction in which females produce either free-swimming nauplii or dormant cysts, have probably enabled them to survive in such conditions (MacRae 2003).

Considering their nutritional value, easy hatching process from cysts and year round availability (Sorgeloos *et al*. 1978 and Bengtson *et al*. 1991), it has become popular by the hatchery managers to use as a convenient substitute for the natural plankton diet of fish and shrimp larvae. From the point of nutritional view, the young nauplii have a protein reserve of 60%, where adults have 58% of protein content (Ahmed and Awal 1994). The energetic content of the newly hatched nauplii can be increased by the decapsulation process, which causes the nauplii to spend less energy to emerge from the cysts (Treece, 2000). However, the dried decapsulated cysts have also proven to be used as an appropriate feed in larval rearing of fish (Van Stappen 1996, Lim *et al*. 2003). This present study aims to evaluate the potential process of hatching of *Artemia* for producing more nauplii from one gram of cysts. In Bangladesh, since the fundamental works for the development of *Artemia* are lacking (Sultana *et al*. 2009), this study will help to be used effectively in the commercial production of *Artemia* larvae in our country.

Materials and Methods

Culture media:

Unpurified brine water was collected from the saltpans of Teknaf area of Cox's Bazar used as culture media for hatching of nauplii from *Artemia franciscana* cysts.

Hatching efficiency (HE) and hatching percentage (HP %):

For determining the HE and HP%, simplified method was used as proposed by Sorgeloos and Kulasekarapandian (1984), in which 80ml of salt water was taken in 250ml measuring conical flask. The media was then aerated and 250 mg cysts were added. After one hour, the volume was made 100ml by adding salt water. Continuous aeration and light illumination were provided.

For hatching efficiency, five sub-samples of 0.25 ml were taken after 48 hours in five separate petri dishes and the volume was made 1 ml by

adding fresh salt water. Then the number of larvae per petri dish were counted by using magnifying glass and an average (N) was taken. Then the HE was determined by using following formula (Sorgeloos and Kulasekarapandian 1984):

$$\text{HE} = \frac{No.of\ nauplii}{One\ gram\ of\ product}$$

$$= \frac{N \times 4 \times 100 \times 4}{1}$$

For hatching percentage, five sub-samples of 0.25 ml were taken and then made 1 ml in the same way stated above. Samples were collected after 12 hours of incubation at different time intervals. The number of nauplii and unhatched cysts were then counted using SR cell counter, under microscope (at 10X magnification). The HP (%) was determined by using the following formula (Sorgeloos and Kulasekarapandian 1984):

$$\text{HP}(\%) = \frac{No.of\ nauplii \times 100}{No.of\ nauplii + No.of\ unhatched\ cysts}$$

Decapsulation

Cysts were decapsulated following the process proposed by Sorgeloos and Kulasekarapandian (1984). For decapsulation, 0.1 gm of dry cysts was taken in a conical, transparent plastic container and incubated in salt water for 1 to 2 hours to ensure complete hydration. After 2 hours, 0.5 gm of bleaching powder was added to the salt water and the media was aerated. Ice was added to the media to maintain the temperature beyond 40℃. The color of the cysts was observed. When the color of the cysts changed from dark brown to orange, the cysts were filtered from the decapsulation solution and rinsed with cool tap water until no chlorine smell persisted. The sample of decapsulated cysts was then incubated for determining hatching efficiency and hatching percentage. The samples were also collected to measure the cyst diameter.

Diameter of untreated cysts:

The diameter of untreated cysts were measured under microscope (at 10X magnification) using an ocular micrometer. The cysts were collected after 2 hours of incubation.

Chorionic thickness:

Chorionic thickness of cysts were measured by using the following formula (Vanhaecke and Sorgeloos, 1980):

$$\text{Chorionic thickness} = \frac{\textit{mean diameter of untreated cysts} - \textit{mean diameter of decapsulated cysts}}{2}$$

Results and Discussion

The mean values for untreated and decapsulated cysts were 242.2 ± 1.90 µm and 213.5 ± 3.167 µm respectively. The chorionic thickness was measured from the mean diameter of chorionic hydrated and decapsulated cysts and the value is 14.35 µm (Table 1).

Table 1. Determination of Chorionic cysts

Diameter of untreated cysts (µm)	Diameter of decapsulated cysts (µm)	Chorionic thickness
Mean ± SE	Mean ± SE	
242.2 ± 1.90	213.5 ± 3.167	14.35

The highest HE for untreated cysts was obtained at 35 ppt (89,600 N/gm cysts), followed by 40 ppt (56,000 N/gm cysts) and 30 ppt (27,200 N/gm cysts). The highest hatching efficiency for decapsulated cysts was also found at 35 ppt, the value of which is 1,42,720 N/gm cysts, follwed by 40 ppt (1,41,120 N/gm cysts) and 30 ppt (1,25,760 N/gm cysts) (Table 2).

Table 2. Hatching efficiency of untreated and decapsulated cysts at different salinities

Salinity (ppt)	HE (N/gm cysts) of untreated cysts	HE (N/gm cysts) of decapsulated cysts
30	27,200	125,760
35	89,600	142,720
40	56,000	141,120

At each salinity, hatching efficiency of decapsulted cysts are higher than the hatching efficiency of untreated cysts (Figure 1).

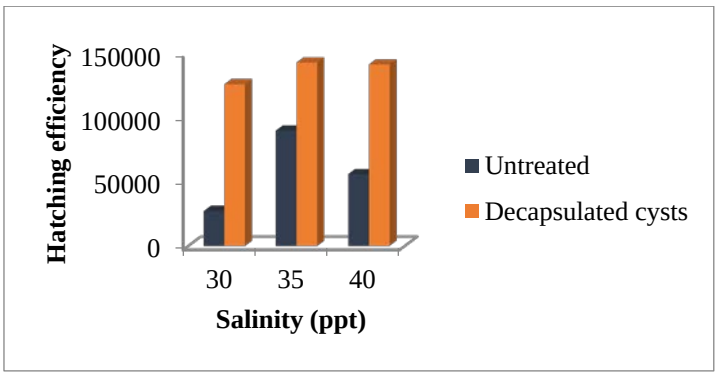

Figure 1. Comparison of hatching efficiency of untreated and decapsulated cysts

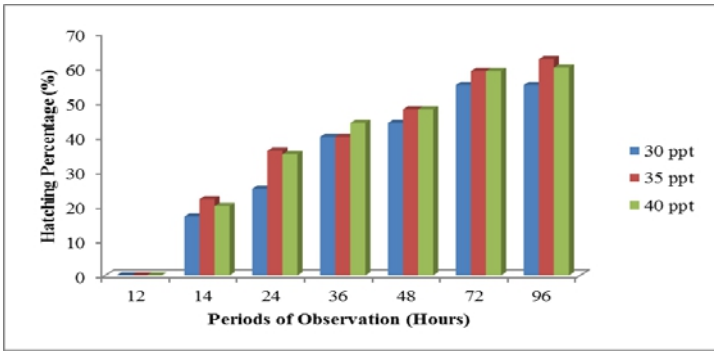

Figure 2. Hatching percentage of untreated cysts at different level of salinities

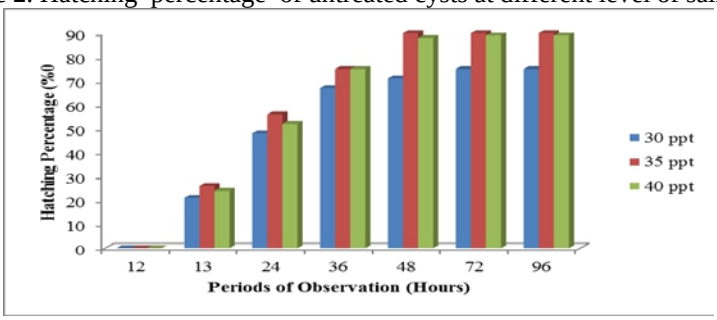

Figure 3. Hatching percentage of decapsulated cysts at different level of salinities

The hatching percentage of untreated and decapsulated cysts were obtained at different hours of incubation at 30, 35 and 40 ppt. The highest hatching percentage of untreated cysts at 30 ppt was 55% and observed after 72 hours of incubation. Similarly, the highest hatching percentages at 35 and 40 ppt were 62.5% and 60% respectively and observed after 96 hours of incubation (Figure 2). Both Figures (3 and 4) show that until 12 hours of incubation there were no hatching of nauplii from cysts. The values of hatching percentage of decapsulated cysts ranged from 75% to 90%, which are much higher than the hatching percentages of chorionic cysts, which ranged from 55% to 62.5% (Table 4 and 3).

Table 3. Determination of hatching percentage (%) of untreated cysts at different period of time

Salinity	Hatching percentage (%) of untreated cysts at different period of observation						
	12 h	14 h	24 h	36 h	48 h	72 h	96 h
30 ppt	No	17	25	40	44	55	55
35 ppt	No	22	36	40	48	59	62.5
40 ppt	No	20	35	44	48	59	60

Table 4. Determination of hatching percentage (%) of decapsulated cysts at different period of time

Salinity	Hatching percentage (%) of decapsulated cysts at different period of observation						
	12 h	13 h	24 h	36 h	48 h	72 h	96 h
30 ppt	No	21	48	67	71	75	75
35 ppt	No	26	56	75	90	90	90
40 ppt	No	24	52	75	88	89	89

Asem *et al*. (2007) found the mean diameter for untreated cysts ranging from 247.63±11.47 to 259.34±11.36 µm; and the values for decapsulated cysts were 231.29±10.43 to 251.6±11.24 µm, with the chorionic thickness ranged from 1.31 to 9.37 µm. Naceur *et. al*. (2012) studied 17 populations and showed that the mean diameter for untreated cysts ranged from 221.0 to 284.9 µm, and the values for decapsulated cysts ranged from 208.2 to 258.8 µm, with the chorionic thickness ranged from 4.7 to 13.3 µm. Abatzopoulos *et. al*. (2006a) found the mean diameter for untreated cysts ranged from 262.7 to 286.6 µm, decapsulated cysts from 258.6 to 274.4 µm, with the chorionic thickness ranged from 1.2 to 9.3 µm. In the other study of the same population, the mean diameter of untreated and decapsulated cysts ranged from 249.8 to 280.7 µm and 218.4 to 259.8 µm, respectively. The chorionic thickness ranged from 2.7 to 15.6 µm Abatzopoulos *et. al*. (2006). The variation between the mean diameter of untreated and decapsulated cysts, as well as, in chorionic thickness may be due to the cause of seasonal fluctuation in physico-chemical parameters and food availability (Abatzopoulos *et al*. 2006). These differences may be found in a site at different periods of time (Asem *et al*. 2007).

According to Vanhaecke and Sorgeloos (1980), in case of decapsulated cysts the hatching output increasing than untreated cysts. The nauplii also contain less energy since the embryos spend less energy to hatch out from decapsulated cysts. 1.06×10^5 N/gm cysts in 1977 and 1.92×10^5 N/gm cysts in 1979 was reported to be hatched from Great Salt Lake (Vanhaecke and Sorgeloos, 1983). Hudaidah (2011) could produce 2.76×10^5 to 2.90×10^5 N/gm in untreated cysts. According to Sato (1967), cyst hatching efficiency is greatly related to pH, which usually decreases at low pH, below 8.0. However, cysts having larger diameter produce less nauplii from one gram of dry cysts (Camargo *et al*. 2005).

Abatzopoulos *et al*. (2006) studied the hatching percentages of *Artemia* cysts from seven sites in Urmia lake, Iran. Only two samples showed satisfactory results after 48 hours of incubation and the values were 72.9 and 97.8%. Hudaidah (2011) found significant differences in hatching

percentages among batches from the same strain. The values ranged from 74 to 88% and 79 to 91% after 24 hours and 48 hours of incubation, respectively. In the present study, the hatching percentages were found to be influenced by salinity in case of both untreated and decapsulated cysts. The optimum salinity was 35 ppt. The hatching percentages were also higher for decapsulated cysts.

Conclusion

Artemia is being used in large scale in the hatcheries of Bangladesh. This study would help the hatchery managers and aquaculturists who want to feed their fish larvae with the most reliable live feed items, since adequate data are lacking on hatching performances of *Artemia* cysts.

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