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EFFECT OF NATURAL, ARTIFICIAL AND GROUND SALINE WATERS ON HATCHING OF *ARTEMIA FRANCISCANA* CYSTS

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Abstract: *Artemia* is a branchiopod crustacean of hypersaline environments. Its cysts form the source of most well known live food in global aquaculture for growing larval stages of prawns and fishes. A comparative study was made on hatching percentage of cysts of *Artemia franciscana* in natural sea water, ground saline water and artificial sea water. The highest value of 75 was recorded in artificial sea water followed by 60 % in natural sea water and 59 % in ground saline water on 24 hours of cyst incubation. Freshly hatched nauplii measured 360-370 µm. Important water quality parameters were also analysed. Dissolved oxygen varied from 4.8 to 5.2 mg/l while dissolved free carbon dioxide was nil in all the trials. The level of phosphorus in natural and ground saline water was 3.83 mg/l and 0.7 mg/l, respectively, which was much higher than the favourable range (0.05-0.5 mg/l). On the other hand, phosphorus value of 0.09 mg/l was very close to normal range in case of artificial sea water. The hatching salinity and temperature were maintained at 30 ppt and 26±2 °C, respectively.

Key words: Live food, *Artemia franciscana*, *Artemia* cyst hatching

INTRODUCTION

Suitably selected live food organisms lead to the success of aquaculture hatcheries involved in producing larvae of finfish and shellfish species for their aquaculture. Despite encouraging results with formulated feeds (Fernández-Díaz and Yufera, 1997; Cahu and Infante, 2001), most commercial finfish species do not grow well when fed exclusively on these feeds during their early developmental stages (Bonaldo *et al*., 2011). Microalgae, certain ciliates including *Fabrea salina* (Pandey *et al*., 2008), a variety of rotifers, copepods and cladocerans are common examples of live foods. However, well known for its good nutritional composition and ready supply in encysted form anywhere in the world throughout the year, *Artemia* is the most widely used life food organism for hatchlings and juveniles of marine and freshwater species. This food organism is an important inhabitant of hypersaline water bodies such as solar salt pans and salt lakes where it filter-feeds on microalgae, bacteria and detritus. It is never found in seas. Based on various factors that impact aquaculture market, the global demand of *Artemia* cysts, 2500 – 3000 tonnes/year, had been expected to increase further (FAO, 2011). This branchiopod crustacean grows through 15 successive molts to become an adult of 1.0-2.0 cm in length. By virtue of its role as the harbinger of better salt production, *Artemia* is popularly called as brine shrimp or sea monkey. Similarly, aquarium dealers in the US call it 'sea monkey' to promote sale of its cysts to aquarium lovers. In India, many workers have reported its occurrence in various salt pans (Ansari, 1987 and Royan, 1979) and Salt Lake in Rajasthan (Baid, 1958). However, due to poor processing methods employed and consequently lower hatching percentage, the *Artemia* cysts derived from Indian strain are relatively less in demand by most hatchery owners of the country.

Both parthenogenetic and zygogenetic populations of *Artemia* exist in the natural environment. In order to investigate the usability of *Artemia* cysts in inland regions in India and elsewhere, the present comparative study was performed on their

hatchability in various saline media namely natural sea water, artificial sea water and ground saline water. The cysts used were of *Artemia franciscana* obtained from the Great Salt Lake, Utah, United States which has been the major site of their production and global supply. Their size was 160-200 µm. Findings of this study have potential to rely upon tested alternatives of natural sea water for hatching *Artemia* cysts.

MATERIAL AND METHODS

The natural sea water having a salinity of 35 ppt was collected from the Andheri area of Mumbai coast during low-tide and it was left undisturbed for 2 days for settling particulate materials. The ground saline water of 0.5 ppt was pumped out from a borewell of the Central Institute of Fisheries Education, Mumbai. The artificial sea water with 35 ppt salinity was prepared by adding its different constituents in appropriate proportion (Harvey, 1960). In fact a salinity of 30 ppt in all these three water types was adjusted by adding freshwater or sea salt as required i.e. the salinity of natural sea water and artificial sea water was lowered from 35 ppt to 30 ppt by adding freshwater and the salinity of ground saline water was raised from 0.5 ppt to 30 ppt by adding sea salt (salt). In this way, twelve litres each of natural sea water, ground saline water and artificial sea water were made ready for the experiment. In triplicate, four litres of these three water types were taken in serially arranged glass jars for hatching 2 g of cysts of *Artemia franciscana* in each jar. Before immersing cysts in jars, these were treated with sodium hypochlorite solution @15 ml/g cyst taken in a beaker for 10 minutes for decapsulation. Since the reaction of cyst shell (chorion) with sodium hypochlorite is exothermic and rising temperature may damage the embryo, the beaker having these was placed in a small tray containing ordinary water and ice cubes to keep the cysts' temperature below 40ºC. During this period, cysts were constantly stirred using a glass rod to facilitate uniform cooling. The whole content was filtered through a plankton net of 100 µm mesh size. The decapsulated cysts were washed thoroughly for few minutes by freshwater to remove traces of toxic chlorine. To ensure complete removal of chlorine, decapsulated cysts were dipped in 0.1 % sodium thiosulphate solution for less than a minute and then rinsed again with water. Now these cysts were introduced in jars which were supplied with vigorous aeration through 4 mm diameter PVC tubes. Instead of using perforated stone usually fixed at the end of aeration tube, a small piece of intact stone of 25 g was tied behind the tip of each tube. This manipulation is necessary as it helps not only in aeration but also promotes circulation of cysts throughout the water column required for proper hatching. Water quality parameters (Table 1) such as dissolved oxygen, dissolved carbon dioxide, salinity, temperature, pH, alkalinity, chemical oxygen demand, nitrite nitrogen, nitrate nitrogen, ammonium ion and phosphorus concentrations were analysed as per Standard Methods for the Examination of Water and Wastewater (APHA, 2005). Though continuous illumination of about 2000 lux is suggested for best hatching results, hatching sets in the present experiment were arranged in an open area in moderate sunshine. The duration of light and dark hours was 14L:10D. After 24 hours of incubation, the aeration was stopped. The unhatched cysts settled down on to the bottom of jars within few minutes while nauplii remained swimming homogenously. A single sample of 5 ml was taken randomly from the bottom of each jar by simple pipette and placed in petri dishes for counting the number of unhatched cysts and freshly hatched nauplii carefully with naked eyes. The counting of nauplii was made easier by pouring 5-6 drops of 5 % formalin in petri dishes to make motionless or kill fast-swimming nauplii. The nauplii were removed with forceps one by one from the dish leaving behind unhatched cysts. The hatching percentage was calculated as- No. of nauplii / (No. of unhatched cysts + No. of nauplii) x 100

RESULTS AND DISCUSSION

The results showed that the highest hatching percentage of 75 was found in artificial saline water whereas it was 60 % and 59 % in natural saline and ground saline water, respectively. Immediately after hatching, nauplii measured 360-370 µm in length in all cases. Contrary to expected higher hatching percentage in natural sea water, its highest value in artificial sea water is most probably due to lack of pollution in artificial sea water. Moreover artificial

sea water contained the required nutrients in favourable range. The level of phosphorus in natural and ground saline water was 3.83 mg/l and 0.7 mg/l, respectively, which was much higher than the favourable range (0.05-0.5 mg/l). On the other hand, phosphorus value of 0.09 mg/l was very close to normal range in case of artificial sea water. Highest (63 %) and lowest (25 %) hatching percentage have been obtained at 27°C and 35°C, respectively (Singh and Khandagale, 2006). Similar to pH values in present study, optimal hatching occurs in the pH range of 8-8.5 (Rajkumar and Babu, 2015).

Detailed techniques are available for the harvesting, processing and storage of *Artemia* cysts (Sorgeloos *et al*., 1986; Stappen, 1996). This unique animal remains alive in diapause state within the cyst as long as anhydrous and anaerobic conditions are maintained. In fact, *Artemia* starts producing brown coloured cysts (eggs) instead of nauplii during unfavourable conditions of higher salinity (above 100 ppt) and/or low dissolved oxygen levels. It is known that *Artemia* can withstand sudden increase in salinity up to 150 ppt without any mortality up to 6 hour and with low mortality up to 24 hour of incubation (Vikas *et al*., 2016). The uterus of a single female can contain up to 200 cysts. These cysts are collected, washed, dried, sieved, packed in air-tight tins and sold in the market under different brand names. When required, cysts are immersed in saline water to break their cryptobiotic state. The metabolic activity of the embryo is activated. Consequently the embryo emerges from the cyst in the form of a nauplius which is the most common stage of *Artemia* for its use as food in larviculture.

Innumerable studies have been made to the elucidation of the biological, biochemical, ecological and other aspects of *Artemia* cysts hatching. However, hatching percentage is not strain specific as it is influenced by a wide array of factors like harvesting, processing, storage and hatching techniques as well as production conditions that affect the parent generation. Nevertheless, all these efforts have hitherto not resulted in a complete understanding of all processes involved. Genotypic differences may be the basis of varying hatching characteristics among brine shrimp populations (Vanhaecke and Sorgeloos, 1982). However, comparative experiments on reproductive and life-span characteristics in 12 *Artemia* strains showed that hatching is strongly correlated with the environment and not with genotype (Browne *et al*., 1984). Hatching percentage varies from about 20 to 90 % of the total cysts (Vanhaecke and Sorgeloos, 1983). This quality criterion accounts for the price differences among *Artemia* batches. The number of nauplii hatching per gram of cysts can vary from <1,00,000 to >3,00,000. The removal of hard shell or cyst decapsulation has many advantages. As embryo becomes disinfected, it prevents entry of bacteria associated with shell into culture tanks (Sorgeloos *et al*., 1977). It also prevents gut obstruction by empty shells in larval fish (Bruggeman *et al*., 1980). In fact, the cysts or cyst shells which are ingested by the predator animal can not be digested and may cause many deleterious effects (Herald and Rackowicz, 1951). When decapsulated cysts are subjected to hatching, their hatchability is improved, again because no shell breakout is needed (Bruggeman *et al*., 1980).

Though artificial saline water has shown better result for hatching *Artemia* cysts, its preparation is costly and thus should be used only when cost-benefit ratio is favourable to hatchery owners. Similarly ground saline water with added crude salt can be used successfully in absence of natural saline water. India has about 7 million hectares of saline soils where the ground water is highly saline. The salinisation is extensive in the Indus-Ganga plains of north-western India and in the states of Punjab, Haryana, Gujarat and Rajasthan with a salinity of up to 15 ppt. Smaller areas of ground saline water are also found in southern India. All these areas of the country are entirely unfit for agricultural activities. These can

Table 1. Water-quality parameters of different saline media with hatching percentage

Water type	DO (mg/l)	DCO_2 (mg/l)	Salinity (ppt)	Temp. (°C)	pH	Alkalinity (mg//l)	COD (mg/l)	NO_2-N (mg/l)	NO_3-N (mg/l)	NH_4^+ (mg/l)	Phosphorus (mg/l)	Hatching%
Natural sea water	5.2	Nil	30	26±2	9	160	36	0.215	1	0.33	3.83	60
Ground saline water	4.8	Nil	30	26±2	8.7	102	36	0.379	<1	2.7	0.7	59
Artificial sea water	4.8	Nil	30	26±2	9	164	36	0.257	3.1	0.49	0.09	75

be developed for culture of *Artemia*, marine fish and prawn species in ground saline water ponds. The present findings suggest that hatching of *Artemia* cysts in ground saline water is quite comparable to that of natural sea water.

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REFERENCES

Ansari, S.K.R. 1987. *The brine shrimp Artemia- Ecology, cyst hatching, development and mass culture*, M.Sc. Thesis, University of Bombay, India.

APHA 2005. *Standard Methods for the Examination of Water and Wastewater*, 21st Edition, APHA, AWWA and WEF.

Baid, I. C. 1958. Occurrence of *Artemia salina* in Sambhar lake, Rajasthan. *Current Science*, 27(2): 58-59.

Bonaldo, A., Parma, l., Mandrioli, l. Sirri, R., Fontanillas, R., Baldiani, A. and Gutta, P. P. 2011. Increasing dietary plant proteins affects growth performance and ammonia excretion but not digestibility and gut histology in turbot (*Psetta maxima*) juveniles. *Aquaculture*, 318: 101.

Browne, R. A., Sallee, S. E., Grosch, D. S., Segreti, W. O. and Purser, S. M. 1984. Partitioning genetic and environmental components of reproduction and lifespan in *Artemia*. *Ecology*, 65: 949-960.

Bruggeman, E., Sorgeloos, P. and Vanhaecke, P. 1980. In *The Brine Shrimp Artemia* (eds. Persoone, G., Sorgeloos, P, Roels, O. and Jaspers), Universa Press, Wetteren, Belgium, 3: 260-269.

Cahu, C. and Infante, J.Z. 2001. Substitution of live food by formulated diets in marine fish larvae. *Aquaculture*, 200: 161.

FAO. 2011. Cultured Aquatic Species Information Programme. *Artemia* spp. Text by Van Stappen.

Fernández-Díaz, C. and Yufera, M. 1997. Detecting growth in gilthead seabream, *Sparus aurata* L., larvae fed microcapsules. *Aquaculture*, 153: 93.

Harvey, H. W. 1960. *The chemistry and fertility of seawaters*, University Press, Cambridge, 240 p.

Herald, E. S. and Rackowicz, M. 1951. Stable requirements for raising seahorses. *Aquar. Jr.* 22:234-242.

Pandey, B.D., Yeragi, S. G., Reddy, A. K. and Hagiwara, A. 2008. Life strategies and aquacultural usability of a hypersaline ciliate, *Fabrea salina*. *Current Science*, 94(3): 307-309.

Rajkumar, G. and Babu, D. E. 2015. Effect of light, temperature and salinity on the growth of *Artemia*. *International Journal of Engineering science Invention*, 4(12):7-14.

Royan, J.P. 1979. Occurrence of *Artemia* sp. in the Gulf of Kutch. *Mahasagar- Bull Nat Inst Oceanogr*, 12(4): 271-272.

Singh, R.K. and Khandagale, P. 2006. Optimal water characteristics for commercial production of cysts of the brine shrimp, *Artemia* in salt ponds. *Crustaceana*, 79(8):913-923.

Sorgeloos, P., Bossuyt, E., Lavina, E., Baeza-Mesa, M. and Persoone, G. 1977. Decapsulation of *Artemia* cysts: a simple technique for the improvement of the use of brine shrimp in aquaculture. *Aquaculture*, 12: 311-315.

Sorgeloos, P., Lavens, P., Léger, P., Tackaert, W. and Versichele, D. 1986. Eds. *Manual for the culture and use of brine shrimp Artemia in Aquaculture*, *Artemia* Reference Center, pp. 319.

Stappen, G..V. 1996. *Manual on the production and use of live food for aquaculture*, Lavens, P. and Sorgeloos, P., Eds. (FAO, Rome, Italy), 361: 79-136.

Vanhaecke, P. and Sorgeloos, P. 1982. International study on Artemia: XVIII. The hatching rate of *Artemia* cysts- a comparative study. *Aquacult. Eng.*, 1(4): 263-273.

Vanhaecke, P. and Sorgeloos, P. 1983. International study on *Artemia*: XIX. Hatching data for ten commercial sources of brine shrimp cysts and re-evaluation of the "hatching efficiency" concept. *Aquaculture*, 30: 43-52.

Vikas, P.A., Thomas, P.C., Sajeshkumar, N.K., Chakraborty, K, Sanil, N.K. and Vijayan, K.K. 2016. Effect of salinity stress on biochemical constituents and ArHsp22 gene in *Artemia franciscana*. *Indian J. Fish.*, 63(3): 150-156.