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RESEARCH ARTICLE

BIOMETRICAL CHARACTERIZATION OF *ARTEMIA FRANCISCANA* COLLECTED FROM TUTICORIN COAST, SOUTHEAST COAST OF INDIA

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ABSTRACT

In order to introduce *Artemia* cyst to larvicultural feeding of aquatic animals, biometrical characteristics of *Artemia* cysts and newly hatched nauplii collected from two different sites viz. natural and algae fed *Artemia* culture pond in Tuticorin, Tamil Nadu, India have been studied. The dry and decapsulated cysts diameters, cyst hydration, chorion thickness and total length of newly hatched nauplii collected from wild and algae fed pond were measured under microscope. The results showed that *Artemia franciscana* cyst size ranged from 216.66 to 223.33 µm size, the hydrated cyst size varied between 232 to 237.66 µm, Chorion thickness 4.6 to7.6 µm, and the naupliar length from 448.66 to 476.66 µm have been observed in the study environment. The smaller sized *Artemia* cyst and naupli was noticed in algae fed *Artemia* culture pond while compared to wild collection. The small size of *Artemia* is suitable for shrimp post larvae, ornamental and marine fish larval stage feeding and it could be a good alternate feed for replacing the imported expensive cysts.

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INTRODUCTION

The brine shrimp *Artemia* is the most popular live feed in aquaculture. Salt lakes and brine ponds with *Artemia* populations are found all over the world (Vanhaecke *et al*., 1987; Triantaphyllidis *et al.,* 1998) with exception of Antarctica (Claus *et al.,* 1977*). Artemia* has the survival ability to adapt, live and reproduce in hyper saline waters which are mainly located in very dry areas worldwide (Vanhaecke *et al*., 1987; Triantaphyllidis *et al.,* 1998). In order to sustain the fast growing aquaculture industry and meet the high demand for *Artemia* cysts (Dhont and Sorgeloos, 2002) natural resources other than Great Salt Lake in Utah (USA) should be exposed as alternative commercial sources (Triantaphyllidis *et al*., 1994; Lavens and Sorgeloos, 2000). The climate in the Tuticorin coast in the southeast coast of India is characterized by long dry summers and short rainy winters, which generally imply that the place will be suitable for sustainable aquaculture of *Artemia* and to study its cultural characteristics in this region. The fact on the presence of *Artemia franciscana* in Tuticorin salterns as per previous reports is great importance, areas of the world has provoked the complete replacement of the native *Artemia* sps. Characterization of various strains of brine shrimp is essential to enhance its potential in aquaculture (Abatzopoulos *et al.,* 1986). Biometrics of cysts has been reported to be one of the important characteristics to mark the different strains of *Artemia* (Tejeda., 1987). Variations in the cyst size and thickness of outer layer would correlate the strain characterization, those determined the influence of external factors on biomass, cyst production and morphometric of *Artemia franciscana* from Colombian Caribbean (Amargo *et al.,* 2003, 2004, 2005). Recently (Agh *et al*., 2009) and (Peykaran mana *et al*., 2011) have studied the morphometric, biometric and genetic characteristics of an *Artemia* from Iran. The decapsulated cysts of *Artemia* combine the physical properties of a dry artificial feed and the nutritional value of *Artemia* nauplii (Lim *et al.*, 2002). Subsequent studies demonstrated that decapsulated cysts will serve good feed similar to freshly hatched *Artemia* nauplii for the larvae of marine shrimps, freshwater prawns and lobsters (Bruggeman *et al*., 1980).

Artemia is the continuous filter feeder which selects suspended particles only on the basis of size (Gelabert and De la Cruz, 1990); although it's feeding behaviour may be affected by several factors that influence its filtration, ingestion or assimilation rates. The range of optimum particle

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concentrations for maximum ingestion is relatively wide, and is specific for each culture condition. In addition, it also various with age, because the feeding efficiency increases with the number of functional thoracopods (Lavens and Sogeloos, 1991). The quality of micro algae diets for *Artemia* has been the objects of several studies (Sick 1976; Johnson 1980; Fabregas *et al.* 1996, 1998) with different results, depending on the species of micro algae, on the culture conditions, and possible on the species of *Artemia* used for the feeding experiments. Our objective of the study was to differentiate and compare the wild *Artemia* populations in natural habitats of Tuticorin saltpans and from microalgae fed *Artemia* culture ponds.

MATERIAL AND METHODS

Description of sampling sites

Tuticorin is located in extreme southern parts of Tamil Nadu. This coastal city constitutes 70 percent of the total salt production of Tamil Nadu state and 30 percent of India. Tuticorin is the second largest producer of salt in India next to Gujarat. The total geographical area is about 4621 sq. km. and constituting about 3.5 percent and coastal line covered of 121km. Among the area coverage, 25,000 acres covered under saltpans production. Ground water holding 55 to 65 ppt of salinity and for helps in salt production. Veppalodai (Lat. 8° 53' 45.97"N ; Long.78° 09' 56.68"E) and Tharuvaikulam (Lat. 8° 44' 40.07"N ; Long.78° 07' 27.87"E) are the main areas for salt production which was chosen as sampling sites situated 10 km and 20 km north to Tuticorin city respectively. Samples of adult *Artemia* (Fig.1) and cyst were collected naturally from wild at Veppalodai (St. 1) and algae fed *Artemia* culture pond at Tharuvaikulam (St. 2) situated in Tuticorin coast. Adult brine shrimp samples were collected with 150 µm mesh plankton net and preserved with a neutralized 5% formalin solution before being studied in the laboratory. The collected cysts were hatched out for biometric analysis.

Biometrics of cysts

The cysts collected from two different sites were treated following the protocol described by Sorgeloos *et al.* (2008). The diameter of the hydrated and decapsulated cysts was measured under a microscope equipped with a calibrated micrometer; chorion thickness was measured according to Vanhaecke and Sorgeloos (2002).

Biometrics of nauplii

To analyze biometrical characteristic of *Artemia* naupli instar 1, cysts were hatched in natural seawater with salinity of 32 ppt, temperature and pH in the range of 28ºC and 8 under continuous illumination (2000 lux) and aeration. The length of nauplii instar 1 (Fig. 2) was measured under a microscope equipped with calibrated micrometer.

Hydrated cyst

When the cyst incubated in seawater, the biconcave cyst swells up and becomes spherical within 1 to 2 h. After 12 to 20 hrs of hydration, the cyst shell (including the outer cuticular membrane) bursts and the embryo surrounded by the hatching membrane becomes visible. Dry cysts are very hygroscopic and take up water at a fast rate, within the first hours the volume of the hydrated embryo increases to a maximum of water content; however, the active metabolism starts from 60% water content onwards, while environmental conditions are favourable.

Chorion thickness

The diameter of hydrated cysts was measured under a microscope provided with calibrated micrometer eye piece (Sorgeloos, 1997). For this purpose cysts were first hydrated during 2 hours in 10 g. L^{-1} sea water, until cysts were observed to be completely spherical, and then fixed in 1% Lugol solution at overnight. Concerning decapsulated cysts, 1 g of cysts from each sample was hydrated in 10 g. L^{-1} of sea water for 2 hours and then decapsulated with sodium hypochlorite (Sorgeloos *et al.*, 1986). The decapsulated cysts were fixed with 1% Lugol for 1 hour and left overnight in the dark (Sorgeloos, 1997). Decapsulated cysts diameter was measured as cited above and the chorion thickness was calculated by Sorgeloos (1997).

Cyst decapsulation

The live naupli of the brine shrimp *Artemia* are excellent food for most fish and crustacean larvae. The non-hatched cysts or cyst shells which are ingested by a predator cannot be digested and may cause blockage of the gut or have other harmful effects (Herald and Rackowicz, 1951; Morris 1956; Rosenthal 1969; J.E. Shelbuorne cited by Provasoli (1969) and Stults (1974). Moreover as external surfaces of cyst shells carry spores of bacteria, plant and even animal species (Gilmour *et al*., 1975), serious infections can occur in fish or crustacean cultures after the addition of mixed suspensions of nauplii and cysts (Shelbourne, 1964; Mac Farlane, 1969). For these reasons, when *Artemia* nauplii are used as a live food source in aquaculture, the nauplii are usually separated from the hatching debris. However, the separation techniques are in many cases not very efficient or require the use of special separator boxes (Sorgeloos and Persoone, 1975). The dry cysts are hydrated in a funnel shaped container with seawater or tap water and kept in continuous suspension by aeration from the bottom. After 1h, the suspension is diluted with an equal volume of commercial hypochlorite to obtain a final concentration of active ingredients (most commercial brands contain 5.25% active chlorine). The oxidation process starts immediately and, as the chorions dissolve, a gradual colour change is observed in the cysts from dark brown via. white to orange. Within 7-10 minutes, the chorions disappear completely and the decapsulated cysts should be filtered immediately and thoroughly washed with seawater or tap water in order to remove all traces of hypochlorite. The treated cysts are now either incubated directly for hatching or, after immediate dehydration in a brine solution or stored for later use.

RESULTS

The diameter of hydrated, non-decapsulated and decapsulated cysts, chorion thickness and the instar-1 naupliar length of *Artemia* population collected from two different sites were summarized below.

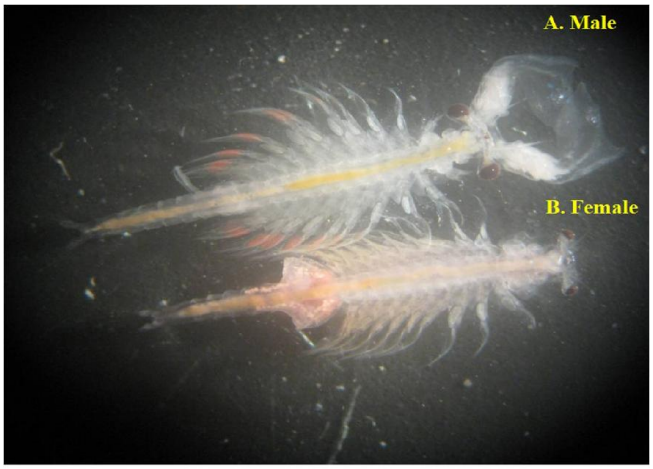

Fig. 1. Male and female *Artemia* adult under microscopic view

Fig. 2. Microscopic view of Artemia nauplii

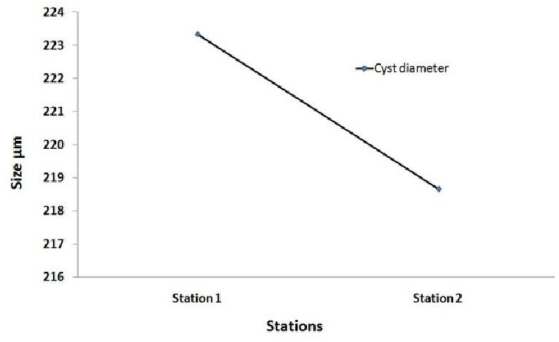

Fig.3. Diameter of *Artemia* cyst

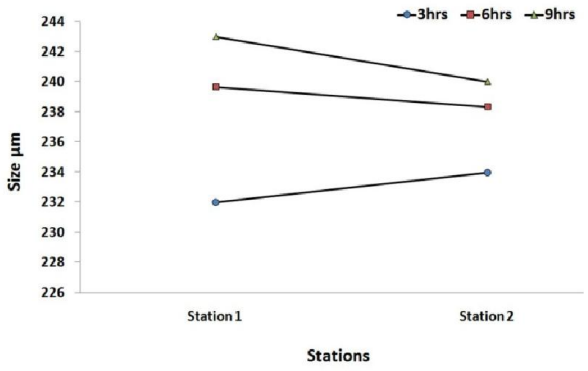

Fig.4. Hydration of *Artemia* cyst

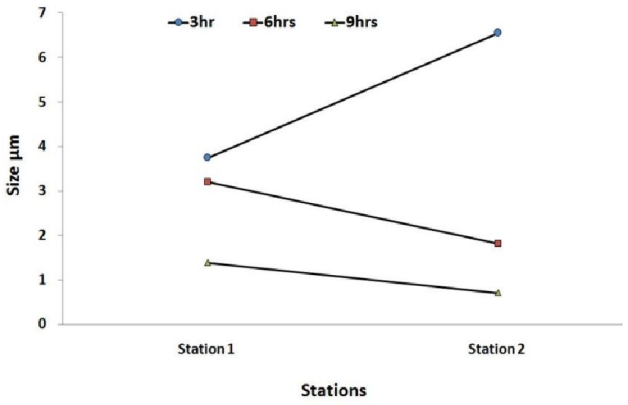

Fig.5. Hygroscopic nature of *Artemia* cyst

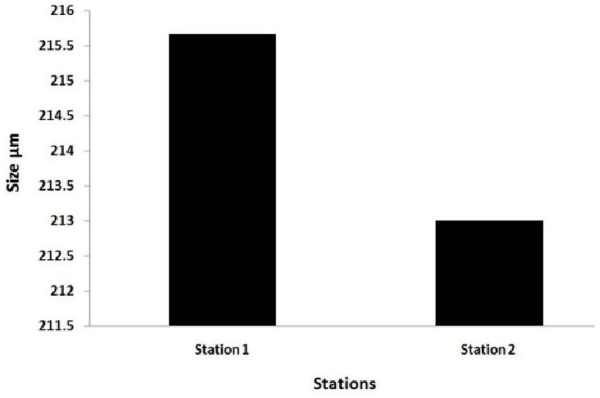

Fig.6. Decapsulated size of *Artemia* cyst

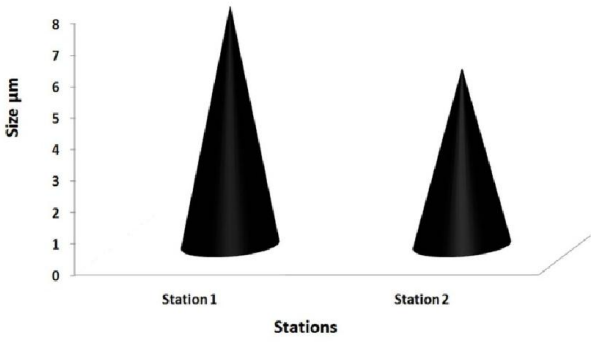

Fig.7. Chorion thickness of *Artemia* cyst

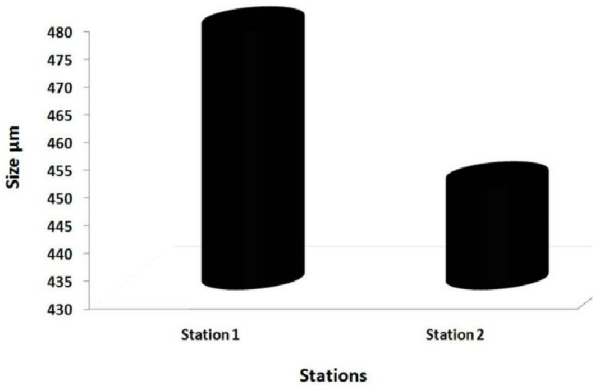

Fig.8. Size of *Artemia* Instar-1 nauplii

Biometrics of cyst

The cyst diameters varied between the two stations and the maximum size of cyst were recorded in St.1 (223.33µm) and minimum observed in St.2 (218.66µm), detailed results were showed in Fig.3.

Hydrated cyst

Hygroscopic nature was observed in the *Artemia* dried cyst collected from two different sites. Cyst hatched out during hydration process. Three different time duration for hydration interval viz. 3, 6 and 9 hours were analysed. The uptake of water by cyst was more in the first 3 hours when compare to next 6^{th} and 9^{th} hours. The maximum water uptake in first 3 hrs were noted in St.2 (234µm) and minimum in St.1 (232 µm), and the variations were described in Fig.4 and percentage level of hygroscopic difference showed in Fig.5.

Decapsulated cyst

Comparison between the decapsulated cysts collected from two different stations showed a significant difference. However, it was demonstrated that the cyst collected from St.2 (213µm) were smaller than those harvested from other site St.1 (215.66µm) and results were presented in Fig. 6.

Chorion thickness and naupliar length of the cyst

Chorion thickness values were maximum in St.1 (7.6 µm) and minimum in St.2. (5.6 µm) showed in Fig.5. The instar-1 naupliar length of *Artemia* population harvested from two different stations is summarized in Fig.7. The largest size of instar-1 recorded in St.1. (476.66 µm) and the smaller in St.2 (448.666 µm) showed in Fig.8.

DISCUSSION

Although the structure of *Artemia* cyst is same in all the strains but they have quantitative differences that have a great impact on their use in aquaculture. Vanhaecke & Sorgeloos (1980) studied the whole cyst, decapsulated cyst and chorion thickness diameter varies in 24 geographical regions. The results showed significant differences of mentioned parameters in various populations. Also, they suggested that the cyst diameter was related to genetic characteristics. They concluded that the largest cyst diameter was due to parthenogenesis population of Margarita Di Savia Italy (284.9+14.6 µm) whereas *Artemia franciscana* from San Francisco Bay had the smallest cyst diameter (223.9±11.7 µm). The largest and smallest decapsulated cyst diameters were recorded from *Artemia* sp. from Tuticorin, India (262.7±11.5 µm) and *A. franciscana* from San Francisco Bay (207.7±11.1 µm), respectively Vanhaecke & Sorgeloos, (1980). Pilla and Beardmore (1994) reported that the whole cyst diameter for *A. sinica, A. urmiana* and *Artemia* sp. was 232.75±11.22 µm, 265.85±15.85 µm and 232.75±11.22 µm, respectively; which shows a significant difference among specimens. Mayer (2002) showed that *Artemia* sp. populations from Portorico & Dominican have a significant difference on whole cyst diameters. Asem *et al.,* (2007) made a survey in Urmia Lake and carried out sampling from 26 stations and consequently found out that cyst & decapsulated cyst diameters and chorion thickness of collected cysts were different; namely the largest cyst diameter was (259.34±11.36 µm) in N (3-1) inhabitant (Asem *et al.,* 2007; Peikaran Mana, 2007). In this study, the *Artemia* cyst & decapsulated cyst diameters and chorion thickness parameters were measured from 2 different regions of Tuticorin. The results indicate a high variation of maximum 223.33 µm, minimum of 218.66 µm for cysts, the decapsulated cysts recorded of maximum 215.66 µm, minimum of 213 µm and 7.6, 5.6 µm of chorion thickness respectively. *Artemia* cysts diameters of Urmia Lake (265.85 ± 15.85 µm) have been reported by Pilla and Beardmore (1994) whereas Asem *et al*., (2007) obtained different results. The reason may be due to the salinity changes, food availability, environmental changes especially nutritional and other physico-chemical factors, precision of measuring instruments. Triantaphyllidis *et al*., (1996) showed that the diameters of untreated cysts from Namibia and Madagascar were 247.7 ±11 µm and 285.9± 11.6 µm; also for decapsulated cysts were 233.1±9.8 µm and 246.2 ±11.7 µm, respectively. Their study indicated that the cysts from Namibia were smaller than Madagascar ones. Abatzopoulos *et al.,* (1998) reported that *A. tibetiana* is the biggest recorded in size for *Artemia* species (323± 11.2 µm and 230±14.6 µm). Cohen *et al*., (1999) found diameter ranges between 246.1± 21 µm and 230.3 ± 1 µm for *Artemia* populations from Argentina.

Comparing the results of this survey with other researches on other species of *Artemia*, it can be concluded that in spite of the existing variety in *Artemia* cyst diameter in 2 different regions of Tuticorin, their size are in the range of other species or strains of *Artemia franciscana* cysts or a little larger. The world average of nauplii size studied in the 2 regions were showed a little difference, therefore they have more advantages comparing other nauplii achievement from other parts of the world**.** It is concluded that cysts from algae fed *Artemia* culture ponds have smaller size and greater cyst numbers per gram than the others. On the other hand, nauplii from cysts of algae fed *Artemia* culture ponds regarding to their suitable size may have a great potential for use in larviculture of various aquatic animals especially for shrimp, and have a great potential to compete with cysts from other parts of the world, especially after processing, drying and packaging.

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