Int. J. Aquat. Biol. (2017) 5(4): 246-251
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Original Article

Quality evaluation of the invader species, *Artemia franciscana* from Covelong salt works, Kelambakkam, South India

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Abstract: The quality of the invader brine shrimp, *Artemia franciscana*, colonized in Covelong salt works, Kelambakkam (South India) was evaluated based on biometrics, hatching characteristics and nutritional composition of cyst and freshly hatched nauplii. Hydrated cysts measured 238.31±10.42 µm in diameter, while the freshly hatched nauplii measured about 429.00±12.19 µm in length. A hatching of 76.80±5.68% was obtained with hydrated cysts. Time required to obtain 90% hatching was 22.31 hrs for this strain. The hatching synchronization time was of 4.41 hrs. The proximate composition of the decapsulated cysts and newly hatched nauplii showed high level of protein, energy content and low ash content. Fatty acids analysis of the freshly hatched nauplii indicated low level of EPA and absence (or undetectable) of DHA.

Article history:
Received 6 March 2017
Accepted 24 July 2017
Available online 25 August 2017

Keywords:
Artemia fransciscana
Biometrics
Hatching characteristics
Nutritional composition

Introduction

Providing nutritionally adequate diet is a major problem in finfish and shellfish industries, especially at hatchery level. The main thrust of basic fish and crustacean research, with regard to nutrition, is focused on ensuring efficient utilization of feed for their growth (Rao, 1994). In the natural environment, the young ones of marine fishes have less yolk reserves, compared to freshwater species, which are depleted rapidly. Also, the mouth of marine fish fry is relatively small (Lavens and Sorgeloos, 1996) and they can only feed on the pray of appropriate size. Many commercially important marine fish species have a reproductive strategy that results in larvae of altricial type. Altricial larvae require good amount of nutrients, the functional capacity and the develop-mental status of the larval digestive system is sufficient to support growth with live diets from first feeding onwards (Govoni et al., 1986). Altricial larvae having rudimentary digestive system (Conceicao et al., 2010; Wold et al., 2007) which makes them dependent on live food, such as *Artemia* and Rotifers, during their early stages of development (Rehberg-Hass et al., 2015). Because of essential growth factors present in natural prays, they promotes growth, high survival, conversion efficiency and enhanced immune response in predators in natural environment (Awaiss et al., 1992).

Although there are several live food organisms such as *Tubifex* sp., *Brachionus* sp., *Daphnia* sp., *Artemia* sp., and *Moina* sp. used in shellfish and finfish hatcheries, the brine shrimp, *Artemia* nauplius is universally accepted live feed in both finfish and shellfish hatcheries, because of their 'on demand' character, nutritional efficacy and mainly on the knowledge accumulated to improve the hatching quality of the harvested cysts (Sorgeloos et al., 1986). Due to increasing demand and various climatic phenomenons such as El-Nino, the global availability of *Artemia* cyst reduced drastically and the price rose exponentially (Lavens and Sorgeloos, 2000). This condition leads to the diversification of *Artemia* resources worldwide. However, the cyst quality in terms of hatching rate and nutritional values proved to

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DOI: https://doi.org/10.22034/ijab.v5i4.316

be highly variables, among, not only various strains and species of *Artemia* sp., but also different groups from the same location (Vanhaecke and Sorgeloos, 1982). Efforts were made for the mass production of *Artemia* cyst and biomass in man-made saltpans at Covelong salt works, Kelambakkam, Tamilnadu, India for hatchery operations. A total of 80 kg of cysts were harvested successfully in one hector of *Artemia* culture pond (Krishnakumar, 2012). The present study was undertaken to evaluate the quality of harvested cysts and nauplii in terms of its size, hatching characteristics and nutritional composition of *Artemia franciscana* (Krishnakumar, 2012, Sivagnanam et al, 2011), colonized in Covelong Salt works, Kelambakkam.

Materials and Methods

Sampling site and cyst collection: The brine shrimp cysts were collected from Covelong salt works, Kelambakkam, Southeast coast of India. The Covelong salt works, Kelambakkam is one of the major salt manufacturers in Tamilnadu. It covers an area of 1307 acre. This saltpan is divided in to series of salt ponds, such as reservoir, evaporation and crystallizers. This salt pan is sourced sea water from Bay of Bengal through backwater opened near Muttukadu. Salt is produced by evaporating the seawater using sunlight. The distribution of *Artemia* was restricted in evaporation only and no *Artemia* population was recorded in reservoir ponds (Krishnakumar, 2012).

An evaporation pond was chosen for mass production of *Artemia* biomass and cyst. The pond was modified and care was taken to prevent the entry of predators in to the pond. The pond was filled with 80 ppt of salinity, rich with algae. Locally available cysts were used for *Artemia* inoculation in the culture pond. Once the biomass increased in the culture pond, salinity was increased by pumping high salinity water (>180 ppt) in to the culture to induce cyst production. Immediate next cysts were found floating near the shore.

The floating cysts were harvested using 200 µm pore sized scoop net from the culture pond. The collected cyst were cleaned free from other debris by sieving them using different pore sized meshes (300, 250 and 200 µm) and soaked in brine solution (of >180 ppt of salinity for about 1 hr) and in freshwater (0 ppt salinity), and then treated as described by Sorgeloos et al. (1986). The cysts were layered and dried in a thermostatically controlled oven at around 35°C.

Biometry and cyst hatching: The diameter of one hundred fully hydrated (soaked in seawater for 1 hr) and decapsulated cysts were measured using an ocular micrometer (ERMA) calibrated with stage micrometer fitted in compound microscopes. The hydrated cysts were decapsulated according to Sorgeloos and Kulasekarapandian (1984). For hatching, the cysts were incubated in filtered seawater of 35 ppt salinity at 30°C under continuous illumination (~1000 lux). Hatching characteristics such as hatching percentage, hatching efficiency and hatching rate were determined as described by Sorgeloos and Kulasekarapandian (1984) and Vanhaecke and Sorgeloos (1982). Hatching was performed in 100 ml conical glass tubes, with three replicates.

Proximate analysis: Proximate analysis of decapsulated cyst and nauplii was carried out following the standard methods of AOAC (2000). Nitrogen content of the sample was analysed using Kjeltec system (2100 Kjeltec Auto distillation, Foss Tecator, Sweden) and crude protein (CP) was calculated by multiplying nitrogen percentage by 6.25. Ether extract (EE) was determined using Soxtec system (1045 Soxtec Extraction Unit, Foss Tecator, HÖganäs, Sweden) using diethyl ether (boiling point, 92°C for 90 min) as a solvent and ash content was estimated by incinerating the sample in a muffle furnace at 600°C for 12 hrs. Total carbohydrate (TC) was calculated by differences as TC=100-(CP+EE +ash).

Fatty acid profile was determined as fatty acid methyl esters (FAMEs). The FAMEs were prepared according to the method of AOAC (2000). The prepared methyl ester was injected to the gas chromatography (GC-MS, GC: Agilent technologies; Model 6890N; Germany and MS: Agilent technologies; Model l 5973; USA) equipped with the flame

Table 1. Biometry of cyst and freshly hatched nauplii.

Measured parameters	Diameter/Length
Hydrated cysts (μm)	238.31±10.42
Decapsulated cysts (μm)	224.07±12.77
Nauplii (μm)	429.00±12.19

(* mean±SD of 100 observations)

ionisation detector (FID) at a split ratio of 1:20. A fused silica capillary column (30.0 m X 250 μm X 0.25 μm), coated with bonded polyglycol liquid phase, was used. The analytical conditions were: injection port temperature of 250°C and detector temperature of 250°C. The oven was programmed from 170°C to 225°C at a rate of 1°C/min. Retention times of FAME standards were used to identify chromatographic peaks of the samples. Fatty acid content was calculated, based on the peak area ratio and expressed as mg fatty acid/ g oil.

Statistical analysis: All the data were subjected to statistical analysis (One-way ANOVA and Duncan) using SPSS statistical software (version 11.5 for windows, SPSS, Chicago, IL, USA). Limits of significance for all critical ranges were set at $P<0.05$.

Results

Mean diameter of hydrated and decapsulated cysts were measured 238.31±10.42 μm and 224.07±12.77 μm, respectively, while the nauplii had a mean length of 429.00±12.19 μm (Table 1). As much as 76.80% of hatching was obtained prior to decapsulation and the percentage of hatching increased significantly, after decapsulation, up to 88.96% with the difference of 5.16%. The best hatching efficiency obtained was 221078.60±12829.33 nauplii g^{-1}. Hatching rate revealed that the first appearance of nauplii (T_0) was observed on 15.35 hrs of incubation and 90% of hatching was obtained (T_{90}) after 21.31 hrs after incubation under normal conditions (30 ppt and 30°C) (Table 2).

Proximate composition of decapsulated cysts showed high level of protein, lipid, carbohydrate, energy content and low ash content compared to freshly hatched nauplii. The protein and carbohydrate amounted to 54.13 and 18.85% respectively in cysts

Table 2. Hatching characteristics of *Artemia* cysts from Covelong Salt works.

Characters		KBM strain of *Artemia**
Hatching efficiency (no. nauplii/g)		221078.60±12829.33
Hatching rate (hrs)		
	T_0	15.35±0.07
	T_{10}	17.91±0.51
	T_{90}	22.31±0.19
	T_S	4.41±0.44
Hatching percentage (%)		76.80±5.68

T0: time until appearance of the first nauplii; T10: time until 10% hatching is attained; T90: time until 90% hatching is attained; TS (T90-T10): determination of hatching synchrony (* mean±SD of three observations)

and 52.86 and 11.01% respectively in nauplii. Lipid content registered in nauplii was high (15.46%) compared to cyst (14.23%). High level of carbohydrate and energy was recorded in cyst as 18.85±0.25 and 351.80±1.91 kcal $100g^{-1}$, respectively (Table 3). A total of 18 fatty acids were recorded in freshly hatched *Artemia* nauplii, 8 saturated and 10 unsaturated fatty acids. Among the saturated fatty acids, palmitic acid and stearic acid were recorded high of 14.31 and 5.66 $mg.g^{-1}$, respectively. Lignoceric acid and lauric acid registered with minimum content of 0.07 and 0.11 $mg.g^{-1}$, respectively. Among the 10 unsaturated fatty acids, α-linolenic acid and oleic acid amounted to be 33.36 and 31.01 mg.g-1, respectively and erucic acid was recorded with low amount of 0.05 $mg.g^{-1}$ (Table 4).

Discussion

The brine shrimp *Artemia* is a micro-crustacean, with a wide distribution over all continents except Antarctica well-adapted to the harsh conditions that severely hypersaline environments impose on survival and reproduction (Vanhaecke and Sorgeloos, 1982). *Artemia* is well-known as an invaluable live food for aquaculture (Sorgeloos et al., 2001; John et al., 2004; Ben Naceur et al., 2012). Due to the increasing aquaculture practices worldwide, demand for quality *Artemia* cyst has been increased and price rose exponentially (Lavens and Sorgeloos, 2000). During 1980's, parthenogenetic species of *Artemia* was dominated in Kelambakkam salt pans and the size of

Table 3. Proximate composition of the cyst and nauplii of KBM strain.

Proximate composition	Cyst	Nauplii
Total Protein (dry wt. %)	54.13±3.16	52.86±2.56
Total Lipid (dry wt. %)	14.23±0.21	15.46±0.36
Total Carbohydrate (dry wt. %)	18.85±0.25	11.01±0.75
Ash (dry wt. %)	5.98±0.14	9.79±0.43
Energy (kcal/100g)	351.80±1.91	330.34±1.50

(mean±SD of five observations)

Table 4. : Fatty acid content in freshly hatched *Artemia* nauplii.

Fatty acids	Mg.g^{-1}
C12:0	0.11
C14:0	0.98
C15:0	0.23
C16:0	14.31
C17:0	0.92
C18:0	5.66
C22:0	0.18
C22:0	0
C23:0	0
C24:0	0.07
C16:1n-7	3.16
C18:1n-9	31.09
C22:1n-9	0.05
C18:2n-6	7.71
C18:3n-3	33.36
C20:2	0.27
C20:3n-6	0.09
C20:3n-3	0.87
C20:4n-6	0.37
C20:5n-3	1.46

the nauplii was fairly larger to feed the developing shrimps larvae at hatchery level. But at present bisexual strain of *Artemia* was a colonized in Kelambakkam saltpans (Sivagnanam et al., 2011).

Present study reveals that the diameter of cyst and the nauplii length are smaller and comparable to the San Francisco Bay strain. The cysts hatching characters such as hatching percentage, hatching rate were seemed to be depends on salinity, temperature and pH (Krishnakumar, 2012). Earlier experiments on the hatching characteristics revealed that maximum hatching percentage was obtained with 30 ppt salinity (Data not shown here). The hatching efficacy of Kelambakkam *Artemia* population yielded about 221078 nauplii/g of cyst; which means that 4.52 g of cysts needed to get 1 million of *Artemia* nauplii. Kara et al. (2004) observed obtained 1 million nauplii from 5.5 g of cysts Chott Marouane (Northeast Algeria) strain of *Artemia*. *Artemia* cysts of Kelambakkam begin to hatch after 15 hrs of incubation whereas Sorgeloos et al. (1986) reported the time limits from 17.91 to 22.31 hrs. The hatching synchronization time was relatively small (4.41 hrs) and is still within the limits of 4.4–17.3 h as reported by Sorgeloos et al. (1986). This synchronized condition; it is possible to collect most of the nauplli in early stage (i.e. first instar stage) before stage II conversion. If the size of nauplii size and mobility increased, they lose 27% of their energetic value (Lavens and Sorgeloos, 1996), and become less accessible to predators.

The proximate composition of *Artemia* differed species to species and even location to location in the same area. Protein content averaged 54.13% in Kelambakkam population and was higher compared to 53.25% in San Francisco Bay strain of *Artemia* (Barrata et al., 1996), 58.4% in *Artemia* from Texococo Lake in Mexico (Barrara et al., 1994), 52.9-74.04% in *Artemia* from natural habitats in Spain (Gonzalbo et al., 1987), and in *Artemia* from the Great Salt Lake (Utah) had 58.39% (Gonzalbo et al., 1987). Rich proximate composition was observed in *Artemia* nauplii may because of the naturally existing algae of rich protein sources such as fresh *Spirulina* (Castro, 1993; Leger et al., 1986). Although wild *Artemia* lacks an external protein source, their natural protein content might be originated from feed sources that thrive in their natural environment. The average lipid content of 14.23% was recorded with Kelambakkam strain which was higher compared to Pichilingue strain (7.78%) (Naegel et al., 2002) and the lipid content varied from 6.45 to 14% for various populations of *Artemia* determined (Gonzalbo and

Amat, 1988). Barrera et al. (1994) obtained lipid contents of 7.29 and 3.37%, respectively, from Texcoco Lake *Artemia* and *A. franciscana* from San Francisco. The ash content of *Artemia* cyst and nauplii amounted to 5.98 and 9.79%, respectively. In case of ash content due to the feeding on organic matter that cause ash accumulation in the telopodites and the digestive tube, thus increasing the ash proportion and lowering that of other nutritional elements (Gonzalbo et al., 1987). Nevertheless, the ash content of the natural populations of the *Artemia* nauplii and decapsulated cyst in this study was not substantially different from that reported for other *Artemia* grown also under controlled conditions with different feed supplements (Barrera et al., 1994; Leger et al., 1986; Rosinvalli et al., 1987).

Sakamoto et al. (1982) have reasoned that the biochemical composition of the *Artemia* reflects the diet administered. Eicosapentaenoic (EPA) and decosahexanoic (DHA) fatty acids are considered essential components of the diet of marine organisms (Kanazawa et al., 1979; Watanabe, 1993). Eicosanoid production from arachidonic acid (n-6 fatty acid) is modulated by EPA, and failure to supply these two essential fatty acid in the appropriate balance may result in adverse biochemical responses when fed to the predator organisms (Sorgeloos and Kulasekara-pandian, 1984). In the present study, EPA reached a value of only 1.46 $mg.g^{-1}$ and no DHA was detected. Lavens and Sorgeloos (1991) found that when *Artemia* was cultivated with agricultural sub-products, the harvest contained small amounts of EPA and DHA acid. Therefore, it is reasonably to assume that the low amounts of EPA and DHA found in the present study is likely to be due to the mixing of agricultural products in the diet.

Compilation of results on the hatching characteristics and nutritional composition of *A. franciscana*, confined to Kelambakkam is very much suitable for shellfish and finfish aquaculture industries at hatchery level. Hence, mass propagation of the brine shrimp *Artemia* in Kelambakkam saltpan is proposed to reduce the feed cost at hatchery level and production of more *Artemia* cyst using the species available locally.

Acknowledgments

Financial assistance from the Ministry of Earth Sciences (MoES/11-MRDF/1/8/P/07), Government of India and thanks are due to Salt Commissioner, Jaipur, Deputy Salt Commissioner, Chennai and saltpan lessee of Cove long Salt Works (SKMS Salt Works), Kelambakkam are gratefully acknowledged for their kind permission for salt pans.

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