

## Variations in hatching characteristics of Artemia cysts of Indian saline ponds in relation to salinity

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The hatching quality of Artemia cysts vary to a large extent under the influence of hydrological factors. However, several persons are of opinion that the cysts processing and incubation conditions affect the hatching quality of Artemia cysts. The present paper reports on the fluctuations in hatching quality of Artemia cysts in relation to salinity. Artemia cysts were collected from the saline ponds. Hatching percentage, hatching efficiency, and hatching output of cysts produced, in higher salinities were, found to be better than those collected from lower salinities. An increase in hatching percentage, hatching efficiency and hatching output were observed with an increase in salinity. The study has provided a fund of knowledge in ensuring a better management and control over the natural population for profitable harvest of Artemia cysts, thus obviating the need to depend on the unpredictable supply of Artemia cysts from wild populations.

### 1. INTRODUCTION

The studies on ecology and distribution of Artemia have been an area of scientific interest for many years. However, very little emphasis has been given to the impact of environmental factors on the cyst quality and hatching characteristics of Artemia.

The nutritional and hatching quality of Artemia cysts vary to a large extent under the influence of hydrological factor. Many laboratory experiments have been performed to study the hatching quality of bring shrimp. The egg size and hatchability are greatly influenced by ecological factors (Collins, 1977), Covens et al. 1986 a). Several authors reported that the cyst processing and incubation conditions, affects the hatching quality of Artemia (Venhaecke and Sorgeloos, 1983; Niniura, 1988). Numerous studies on the biochemical change of adult Artemia, *nutritional* differences and hatching quality of cyst produces under different conditions have been conducted (Rahaman and Rathinasamy, 1997). But only very little attention had been paid on the variations in hatching characteristics of natural population of Artemia in relation to salinity. Hence the objectives of the present study was to collect sufficient information on the fluctuations in the hatching quality of Artemia cysts for a proper understanding of critical parameters for the production of good quality cysts.

### 2. STUDY AREA

Investigations were made at two selected salt pans located at Vedaranyam, (Station I) in Nagapattinam district (10, 01' N, 79 59' E) and Kelanibakkam (Station II) (12, 08' N, 80, 02' E) m Chengleput district of Tamilnadu, India, where natural Artemia populations existed (Rahaman et al 1993).

The dry climatic conditions prevailing in these areas are ideally suited for the production of salt by solar evaporation of sea water. The average air temperature during summer months is around 32 to 33 °C and average wind velocity and evaporation rates are 3.4 m/s and 1.77 cm/day respectively. The salt operations are seasonal; the production period is from January to September. Due to the north east monsoon the salt; operations are suspended from October to December.

The Vedaranyam saltworks, under investigation has a total area of about 93 hectare of which 81 hectares are used for the production of edible salt and remaining area for industrial salt production. The total annual production from the saltwork is around 4570 tonnes NaCl (= 49 tonnes/hectare) operating with a work force of 1000 to 1500 people, producing 610 tonnes industrial salt and 3960 tonnes edible salt. The Kelambakkam Balloons having a total area of 525 hectare have a total production of approximately 18,000 tons/year (12,000 tonnes edible salt + 6,000 tonnes industrial salt).

The seawater, which enters into the feeder canal by tidal action, is pumped into a series of evaporators; each one acre in area and a depth of 75 to 90 cm. The brine is then stored in reservoirs from where it is charged into consolidated crystallizers for the solar evaporation. When the salt is separated from the brine and settled in the pans they are scrapped out using wooden scrapers.

From both saltworks samples were taken from a selected stations. Station I was evaporator where ovoviviparous mode of reproduction was observed and Station II was the evaporator where *Artemia* reproduced viviparously.

### 3. MATERIALS AND METHODS

The materials for the present study were collected at fortnightly intervals for a period of one year extending from July 1990 to July 1991 from the selected stations between 7.00 to 12.00 hours. Water and cyst samples were collected simultaneously to study the cyst hatching quality characteristics. Water salinity was recorded with a refractometer (Biomarine, USA).

The cysts were collected using a hand net having mesh size of 50  $\mu\text{m}$ . The techniques outlined by Sorgeloos et al., (1986) was followed for cyst harvesting and processing. Cysts after collection were sieved in double-screened net having two

different mesh sizes (600  $\mu\text{m}$  and 150  $\mu\text{m}$ ) to remove the dead *Artemia* and other larger impurities. The cleaned cysts were preserved in common salt and were taken to the laboratory. In the laboratory cysts were processed by density separation with brine and fresh water and dried under shade in sunlight; weighed and stored in deep freezer.

Hatching characteristics such as chorion thickness, hatching percentage, hatching efficiency, hatching rate, individual naupliar dry weight and hatching output were determined. Collation thickness was calculated by measuring the diameter of the hydrated and capsulated cysts using ocular micrometer. Newly hatched nauplii were dried in hot air oven at 60  $^{\circ}\text{C}$ , dry weight was determined and the average individual dry weight was calculated. For the estimation of hatching percentage, efficiency and output the cysts were incubated at room temperature under continuous aeration and illumination of 1000 lux in 35 ppt seawater for 48 hours (Sorgeloos et al., 1986) and calculated using the formula given below.

Hatching percentage (H %) = number of nauplii which can be produced from 100 full cysts containing an embryo; this criterion does not take into account cyst impurities, e.g. cracked shells, sand, salt etc.

Table - 1 - HATCHING CHARACTERISTICS OF VEDARANYAM CYSTS

| Month  | Chorion Thickness ( $\mu\text{m}$ ) | Naupliar Individual Dry wt. ( $\mu\text{g}$ ) | Hatching efficiency (No/g) | Hatching Rate |          |          |       | Hatching output (mg/g) |
|--------|-------------------------------------|---|----------------------------|---------------|----------|----------|-------|------------------------|
|        |                                     |   |                            | $T_0$         | $T_{10}$ | $T_{90}$ | $T_s$ |                        |
| Jul'90 | 9.84                                | 2.38  | 1,60,000                   | 23.0          | 26.5     | 32.4     | 5.9   | 380.8                  |
| Aug.   | 10.17                               | 2.24  | 1,82,000                   | 24.5          | 26.0     | 31.5     | 5.5   | 407.7                  |
| Sep.   | 9.58                                | 2.41  | 1,35,000                   | 22.0          | 24.0     | 30.7     | 6.7   | 325.4                  |
| Oct.   | 9.61                                | 2.44  | 1,15,000                   | 22.3          | 26.0     | 33.2     | 7.2   | 280.6                  |
| Nov.   | 9.65                                | 2.42  | 1,22,000                   | 23.1          | 24.0     | 30.9     | 6.9   | 295.2                  |
| Dec.   | 8.17                                | 2.51  | 88,000                     | 19.5          | 23.4     | 31.5     | 8.1   | 220.9                  |
| Jan'91 | 8.13                                | 2.51  | 88,000                     | 19.0          | 23.2     | 31.1     | 7.9   | 220.9                  |
| Feb.   | 8.42                                | 2.49  | 1,04,000                   | 20.1          | 24.3     | 32.3     | 8.0   | 259.0                  |
| Mar.   | 9.08                                | 2.45  | 1,15,000                   | 21.5          | 25.6     | 33.1     | 7.5   | 282.9                  |
| Apr.   | 8.16                                | 2.54  | 64,000                     | 18.5          | 24.5     | 32.5     | 8.0   | 162.6                  |
| May.   | 9.92                                | 2.38  | 175,000                    | 23.0          | 26.2     | 32.0     | 5.8   | 416.5                  |
| Jun.   | 9.54                                | 2.44  | 1,36,000                   | 22.0          | 24.0     | 31.7     | 7.7   | 331.8                  |
| Jul.   | 9.51                                | 2.45  | 1,32,000                   | 21.7          | 23.9     | 31.5     | 7.6   | 323.4                  |

Table - 2 - HATCHING CHARACTERISTICS OF KELAMBAKKAM CYSTS

| Month   | Chorion Thickness ( $\mu\text{m}$ ) | Naupliar individual Dry wt ( $\mu\text{g}$ ) | Hatching efficiency | Hatching Rate |          |          |       | Hatching output (mg/g) |
|---------|-------------------------------------|--|---------------------|---------------|----------|----------|-------|------------------------|
|         |                                     |  |                     | $T_0$         | $T_{10}$ | $T_{90}$ | $T_s$ |                        |
| Jul '90 | 11.28                               | 2.31   | 1,85,700            | 27.0          | 28.9     | 34.6     | 5.7   | 429.0                  |
| Aug.    | 11.14                               | 2.37   | 1,73,400            | 26.5          | 28.6     | 34.6     | 6.0   | 411.0                  |
| So      | 11.15                               | 2.39   | 1,69,000            | 26.0          | 28.7     | 35.0     | 6.3   | 403.9                  |
| Oct.    | 10.09                               | 2.49   | 84,000              | 25.4          | 27.4     | 33.9     | 6.5   | 209.2                  |
| Nov.    | 10.24                               | 2.41   | 98,400              | 25.6          | 27.3     | 33.6     | 6.3   | 237.1                  |
| Dec.    | 10.75                               | 2.40   | 1,14,000            | 25.9          | 27.8     | 33.8     | 6.0   | 273.6                  |
| Jan'91  | 11.14                               | 2.35   | 1,79,000            | 26.2          | 28.4     | 34.0     | 5.6   | 420.7                  |
| Feb.    | 11.18                               | 2.39   | 1,76,000            | 26.0          | 28.5     | 34.4     | 5.9   | 420.6                  |
| Mar.    | 11.25                               | 2.30   | 1,83,900            | 26.8          | 28.9     | 34.6     | 5.7   | 423.0                  |
| Apr.    | 11.78                               | 2.17   | 1,97,000            | 27.3          | 29.0     | 34.2     | 5.2   | 427.5                  |
| May.    | 11.45                               | 2.27   | 1,84,500            | 26.8          | 28.7     | 34.3     | 5.6   | 418.8                  |
| Jun.    | 11.46                               | 2.24   | 1,89,000            | 27.0          | 28.9     | 33.4     | 5.5   | 423.4                  |
| Jul.    | 11.34                               | 2.25   | 1,86,000            | 26.8          | 28.6     | 34.4     | 5.6   | 418.5                  |

Hatching Rate (HR) = This criterion refers to the time period from incubation start (Hydration of the cysts) till nauplii release (hatching). The following time intervals are considered.

$T_0$  = incubation time till appearance of first free swimming nauplii

$T_{10}$  = incubation time till appearance of 10% of total hatching nauplii

$T_{90}$  = incubation time till appearance of 90% of total hatching nauplii

$T_s = T_{90} - T_{10}$  this value gives an indication of the hatching synchrony.

Hatching efficiency (HE) = number of nauplii which can be produced out of 1 gram dry cyst product when incubated for 48 hours under standard hatching conditions (No of nauplii/g of cyst).  
Hatching output (H0) = dry weight biomass of nauplii which can be produced out of 1 gram dry cyst product when incubated under standard hatching conditions (mg nauplii/g of cyst).

Statistical analyses were carried out to determine the correlation between salinity and hatching characteristics (Snedecor & Cochran, 1968).

#### 4. RESULTS

The hatching characteristics of *Artemia* are summarised in Table 1 and 2.

The hatching quality of cysts produced in higher salinities was found to be better than those collected from lower salinities. The quality of Kelambakkam cysts was superior to Vedaranyam cysts. Hatching percentage, efficiently and output of Kelambakkam cysts were higher. The chorion thickness of Vedaranyam cysts were smaller than Kelambakkam. An increasing trend was observed in shorten thickness with salinity. The values varied from 8.13 to 10.17  $\mu\text{m}$  in Vedaranyam cysts and 10.09 to 11.78  $\mu\text{m}$  in Kelambakkam cysts. The individual naupliar length ranged from 439.5 to 469.2  $\mu\text{m}$  in Kelambakkam and 424.04 to 483.6  $\mu\text{m}$  in Vedaranyam. An increase in hatching percentage; hatching efficiency and hatching output was observed with increase in salinity. The hatching % of Vedaranyam cysts increased from 42.3 (54 ppt) to 80.3% (243 ppt) with an increase in salinity. Similarly at Kelambakkam a maximum of 82.5% hatching was obtained in the cyst collected from 234-ppt water and minimum of 48% in cyst collected from 42.8 opt. Similar trend was also noticed 'in hatching efficiency and hatching output. The efficiency' values increased from 64,000 to 1,82,000 nauplii/g and 84,000 to 1,97,000 nauplii/g with increasing, salinity in Vedaranyam and Kelambakkam cysts respectively. The hatching output ranged from 162.6 to 416.5 mg/g at Vedaranyam and 209.2 and 429 mg/g at



Kelambakkam. Pronounced variations were also observed in the hatching rates. Hatching synchrony values were higher for the cysts collected at lower salinity. The values increased from 5.5 to 8.1 hours and 5.2 to 6.5 hours respectively in Vedaranyam and Kelambakkam cysts collected during the study period. A positive significant correlation was observed between salinity and chorion thickness, hatching %, hatching efficiency and hatching output, whereas, a negative relationship between naupliar length, weight and hatching synchrony (Table 3).

## 5. DISCUSSION

Cyst quality is a paramount concern where marketing is involved. If not, production will not find a suitable niche in export market to get attractive prices. Environmental factors effect the quality of *Artemia* cysts to a larger extent. As stated earlier two types of cyst were encountered in the present study. One with thin chorion (less than 11  $\mu\text{m}$ ) and lighter in colour and which hatch out immediately and the other type with thick chorion (grater than 11  $\mu\text{m}$  and darker in colour and which remains in diapause for a long time. The appearance of two types of *Artemia* cysts, darker one with average diameter of 223  $\mu\text{m}$  and lighter one with average diameter of 266  $\mu\text{m}$  have been reported from the salt ponds of Elat, Israel (Zmora and Popper, 1985). The hatchability of darker cysts was better than lighter cysts. Layers et al., (1986) revealed an interaction between environmental conditions and hatching characteristics.

The diameter of light colour (282.4) cysts was bigger than dark colour (279.2) cysts. Contrary to this Sorgeloos et al., (1986) opined that the place and conditions under which they were produced do

not affect the diameter of cysts. Increase in the size of light colour cysts can be due to the hydration of the cyst in lower salinity (because of thin chorion). Similarly the smaller size of dark colour cysts can be attributed, to the dehydration in higher salinity. The thin cuticle of the light colour cyst was due mainly to the low haematin content. On the other hand the chorion of cyst produced in higher salinity was comparatively thicker than those produced in low saline period. Low oxygen levels (noticed during high salinity) are known to induce synthesis of haemoglobin which on reduction is converted into haematin. Under extreme conditions a thick layer of haematin may be secreted by the shell glands in order to withstand the stresses. This explains the thick chorion produced during low dissolved oxygen levels. Cysts with low content of haematin in the chorion are not well protected against the UV radiation. The thick chorion protects the embryo from damages. This accounts for the increase in hatchability in higher salinity. Moreover as the cyst collected during the hypersaline conditions were smaller and lighter in weight. One gram of cyst contained more number of eggs than those produced in low saline periods. Cysts with a thick chorion would be utilising more energy for the breaking of the shell than those with a thin chorion. Hence the length and weight of the freshly hatched nauplius hatched out of the cyst produced in lower salinity is slightly bigger. The diameter of Kelambakkam and Vedaranyam cysts were almost same as that of Tuticorin strain (283.8  $\mu\text{m}$ ; Royan et al., (1987) but bigger than that of the parthenogenetic strain reported from Bombay (267.94  $\mu\text{m}$ ) (Ansari, 1987). The differences in hatching quality of Kelambakkam and Vedaranyam strains can be attributed to the variations in ecological factors.

TABLE - 3 CORRELATION BETWEEN HATCHING CHARACTERISTICS AND SALINITY

| Artemia Strain | Chorion Thickness | H %   | HE    | Hatching Rate  |                 |                 |                | Naupliar Length | Individual dry weight | H 0   |
|----------------|-------------------|-------|-------|----------------|-----------------|-----------------|----------------|-----------------|-----------------------|-------|
|                |                   |       |       | T <sub>0</sub> | T <sub>10</sub> | T <sub>90</sub> | T <sub>s</sub> |                 |                       |       |
| Vedaranyam     | **                | **    | **    | **             |                 |                 | **             |                 | **                    | **    |
|                |                   | 0.931 | 0.911 | 0.953          | -0.134          | -0.134          | -0.944         | -0.272          | -0.969                | 0.894 |
| Kelambakkam    | **                | *     | **    | **             | **              | *               | **             | **              | **                    | **    |
|                | 0.99              | 0.60  | 0.98  | 0.90           | 0.95            | 0.58            | -0.867         | -0.934          | -0.867                | 0.949 |

\* - Significant (%5); \*\* - Highly Significant (1 %).

These types of ecological, studies are essential for the future management and optimal exploitation of saline biotopes for the harvesting of quality *Artemia* cysts for aquaculture purposes in order to save on importation of very expensive cysts. Considering the several thousand of hectares of salt pans along the east and west coast of India, the proper management of solar saltworks could yield sufficient quantities of good quality cysts.

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