

Cyst Quality & Hatching in Parthenogenetic Brine Shrimp, *Artemia*

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Data on cyst size, naupliar size, biochemical constituents (carbohydrate, protein and lipid) and hatching characteristics (in relation to temperature, salinity, different glycerol concentrations and antibiotics) of *Artemia* collected from Balamba, Saurashtra coast are presented. Maximum hatching occurs at 30°C both at 5 and 35×10^{-3} salinities. Cyst viability can be increased using antibiotics which suppress associated bacterial growth. Low concentrations of glycerol (0.5 and 0.75 mol.l⁻¹) enhance the percentage of hatching of the cysts. On the basis of naupliar size, low ash content and high protein content it can be suggested that this strain is likely to be preferred by predators.

Brine shrimps have discontinuous distribution both in temperate and tropical coastal marine and inland saline lakes¹. Because of their geographical isolation, the brine shrimp population has diversified to more than 150 strains. As a result, each strain needs to be biologically characterised so that its suitability for use in aquaculture can be properly assessed.

Earlier, occurrence of *Artemia* from the solar salterns of Gujarat is reported². In the present paper, data on cyst size, naupliar size, biochemical constituents and hatching characteristics, viz. hatching in relation to temperature and salinity, hatching in different concentrations of glycerol, and effect of antibiotics on hatching of *Artemia* from the Saurashtra coast, India are presented.

Materials and Methods

Artemia cysts were obtained from the salterns at Balamba (lat. 23°42'N; long. 70°17'E).

Diameter of both hydrated (kept in ordinary seawater for 1 h), unhydrated and decapsulated (decapsulation by sodium hypochlorite treatment) cysts were determined by measuring 100 cysts individually. The mean number of cysts was determined by counting 10 replicates of 1 mg samples. Dry weight analysis was made on 15 replicates of 1 mg wet samples dried in an oven at 70°C for 24 h.

For biochemical measurements of nauplii, cysts were incubated in natural seawater (sal. 35×10^{-3} ; temp. $29 \pm 1^\circ\text{C}$). The first instar nauplii were fixed with Lugol's solution and length was determined using a microscope fitted with an ocular micrometer. Dry samples of both nauplii and cysts were incinerated at 600°C for 4 h in a muffle furnace and ash content determined. Carbohydrate, protein and lip-

id of the freshly hatched nauplii were estimated using standard procedures³⁻⁵. Hatching percentage, hatching output and hatching efficiency were calculated according to Vanhaecke and Sorgeloos⁶.

Experiments on hatching in relation to salinities (5, 15, 25, 35 and 50×10^{-3}) and temperature (25°, 30° and 35°C) were carried out following the methods of Sorgeloos and Persoone⁷.

To test the effect of antibiotics on hatching, streptomycin and penicillin were used. The hatching medium was 100 ml natural seawater filtered through 0.45 µm millipore filters. Two antibiotic treatment groups were used at 4 different cyst densities (2, 4, 6 and 8 mg each in 100 ml) with appropriate controls (in natural seawater). One group received 50 ppm of streptomycin and the other 50,000 units of penicillin (Sarabhai Chemicals, India). The antibiotics were added to the medium at 0 time and thereafter at 12 h intervals. At the end of 48 h, 1 ml samples (10) were drawn from each culture flask, nauplii killed by adding 1 or 2 drops of Lugol's solution and counted under a binocular microscope. Standard microbiological techniques were followed for estimating the total viable counts (TVC)⁸. Pour plate technique was followed employing nutrient agar (Himedia). The number of colony forming units appearing on the nutrient agar after 48 h are reported here.

Effect of different concentrations of glycerol on hatching was studied by hydrating the cysts in distilled water for 1 h. The settled cysts were collected and redried in an oven at 36°C for 2 d. Glycerol solution (mol. l⁻¹) ranging from 0.25 to 2 were prepared at 0.25 intervals. Cysts (5 mg) were placed in 10 ml beakers containing 5 ml of specified glycerol concentrations and incubated at room temperature ($29 \pm 1^\circ\text{C}$). Each concentration of glycerol was

tested in triplicate. Controls were also run in triplicate with similar concentrations of NaCl. Bacterial growth in these experimental containers was suppressed by adding penicillin at 1,50,000 units. l⁻¹. After 96 h all samples were fixed with Lugol's solution. The number of unhatched cysts, pre-nauplii and nauplii were counted and expressed as percentage of total number of cysts introduced.

Results and Discussion

Mean diameter of hydrated untreated and hydrated decapsulated cysts (Table 1) in the present study falls within the reported range⁹. The mean number of cysts. mg⁻¹ recorded in the present study is lower than that of Tuticorin¹⁰ and Sri Lankan¹¹ strains.

The hatching percentage was 36 ± 2.74 at 35×10^{-3} salinity and $29 \pm 1^\circ\text{C}$ temperature. The low percentage of hatchability may probably be due to frequent hydration and dehydration of the cysts in the field during rainy and summer seasons respectively and improper storage (cysts should be stored in vacuum sealed or nitrogen fluxed containers). Hatching efficiency and hatching output were 71.64×10^3 and 188.41 mg.g^{-1} respectively (Table 1). Compared with reported¹⁰ hatching output values of 12 different geographical strains, the present value is rather low.

The size of freshly hatched nauplii as an indicator of uptake potential by the predator will determine the food value of a given batch of *Artemia* cysts. Naupliar size and weight vary in different geographical strains, hence, the concept of hatching efficiency will be misleading. Therefore, it is necessary to consider hatching output.

The naupliar length recorded in the present study ($390 \pm 10 \mu\text{m}$) is smaller than the value given for the different geographical strains⁹. Thus, the smaller sized nauplii will be devoured more easily than the larger ones. The dry weight of nauplius recorded here ($2.63 \pm 0.11 \mu\text{g}$) falls within the recorded range of values ($1.61\text{--}3.33 \mu\text{g}$)¹².

In the present study, *Artemia* nauplii showed 5.9 ± 0.08 , 17.67 ± 1.25 , 18.58 ± 0.62 and $58 \pm 0.41\%$ respectively of ash, carbohydrate, lipid and protein contents. Considerable difference exists in the biochemical data reported by different authors^{11, 14-16}. It has been shown that the biochemical constituents are influenced by temperature-salinity¹⁴ and starvation¹⁷. Variations may also be due to differences between the strains (parthenogenetic and bisexual). Strict comparisons are not possible as the condition in which nauplii are hatched or the developmental stages of the larvae nor the methods of analyses are uniform. However, all the strains of *Artemia* have

Table 1—Cyst and Naupliar Characteristics of *Artemia*

Cyst diam. (μm)	
Untreated hydrated	253 ± 0.12
Hydrated-decapsulated	231 ± 0.51
Cysts (no. mg ⁻¹)	199 ± 6.29
Individual cyst dry wt (μg)	3.7 ± 0.36
Ash content of cyst (dry wt)	6.99 ± 0.6
Naupliar length (μm)	390 ± 10
Individual naupliar dry wt (μg)	2.63 ± 0.11
Hatching percentage*	36
Hatching efficiency†	71.64×10^3
Hatching output‡	188.41

$$* \frac{\text{Total no. of cysts hatched}}{\text{Total no. of cysts incubated}} \times 100$$

†No. of nauplii hatched.g⁻¹ cysts

‡Hatching efficiency $\times$ individual naupliar dry wt expressed in mg biomass. g⁻¹ cysts.

high percentage of protein which makes them nutritionally valuable as live feed.

Hatching percentage was high at salinities 5 and 35×10^{-3} (Table 2). Hatching was more at 30°C . This corroborates with the earlier findings^{11, 16, 18}. It has been shown that the different geographical strains of *Artemia* have different salinity and temperature optima for hatching.

The bacterial counts after 48 h in penicillin and streptomycin treated groups were much less than the control groups (Table 3) and penicillin was more effective than streptomycin. The percentages of nauplii hatched were more in the treatment groups than in the control (Table 3) indicating better hatchability due to suppression of bacteria by the antibiotics. A positive correlation between improved nauplii yield and bacterial suppression at high densities when treated with chloramphenicol and penicillin-streptomycin has been reported¹⁹.

It has been pointed out that many biochemical and physiological changes take place during the first 18-24 h of rehydration of *Artemia* cysts before they hatch²⁰. These may be critical in determining the hatching/survival of the nauplii. Low quality cysts contain foreign bodies with bacteria and fungi which may in turn cause mortality by mechanical blocking of pores in the egg membrane. The effect of bacterial exotoxins may also suppress hatching. Thus, suppression of bacterial growth during early stages of cyst rehydration is important for development and hatching of *Artemia* cysts.

Mean hatching percentage at different concentrations of NaCl was 86 ± 1.84 . Effect of glycerol on hatching of *Artemia* cysts after 96 h is shown in Fig. 1. The percentage nauplii decreased with increase in glycerol concentrations up to 1.5 mol.l^{-1} .

Table 2—Hatching Percentage at Various Salinities and Temperatures

Salinity ($\times 10^{-3}$)	Temperature ($^{\circ}\text{C}$)*	
	25	30
5	20 $\pm$ 0.71	31 $\pm$ 3.49
15	15 $\pm$ 1.0	20 $\pm$ 0.84
25	18 $\pm$ 0.84	25 $\pm$ 1.14
35	23 $\pm$ 1.3	36 $\pm$ 2.74
50	10 $\pm$ 0.89	16 $\pm$ 0.84

*At 35 $^{\circ}\text{C}$ no hatching at all salinities tested.

Table 3—Effect of Antibiotic Treatment on Growth of Bacteria and Hatching of *Artemia*

Density (mg cysts $\times$ 100 ml $^{-1}$)	Control ($\times 10^3$)	Strepto- mycin ($\times 10^3$)	Penicillin ($\times 10^3$)
Colony forming units of bacteria			
2	500	2	10
4	540	310	2
6	440	264	2
8	490	44	8
Percentage nauplii hatched at 48 h			
2	75.4	78.0	85.4
4	63.0	88.0	94.2
6	67.0	84.0	95.6
8	63.0	75.4	84.2

At higher concentrations, lowest percentage of pre-nauplii were observed. Between 0.5 and 0.75 mol.l $^{-1}$, maximum pre-nauplii were recorded, thereafter with increase in glycerol concentrations, there was decrease in percent pre-nauplii up to 1.5 mol.l $^{-1}$. Above this concentration there was no pre-nauplius. The non-hatched cysts in glycerol media amounted to not more than 25-40% up to 1 mol.l $^{-1}$ solutions. At higher concentrations, the percentage of non-hatched cysts increased resulting in decrease in number of pre-nauplii and nauplii in the media. Nauplii found in 0.25 to 1.25 mol.l $^{-1}$ glycerol concentrations were non-motile.

Geldiay *et al.*²¹ have recorded free swimming nauplii in NaCl concentrations from 0.2 to 1.8 mol.l $^{-1}$ with 68-70% hatching and nauplii from 0.2 to 1.4 mol.l $^{-1}$ glycerol solutions. They also reported that after the rupture of chitinous membrane, there was a rise in external sodium with a consequent decrease in internal sodium levels. Non-motile nauplii in lower concentrations of glycerol and absence of pre-nauplii at higher concentrations observed in the present study may possibly be due to the loss of sodium ions as suggested by Geldiay *et al.*²¹ This study further confirms their findings that although glycerol is interchangeable with water, it can prevent post em-

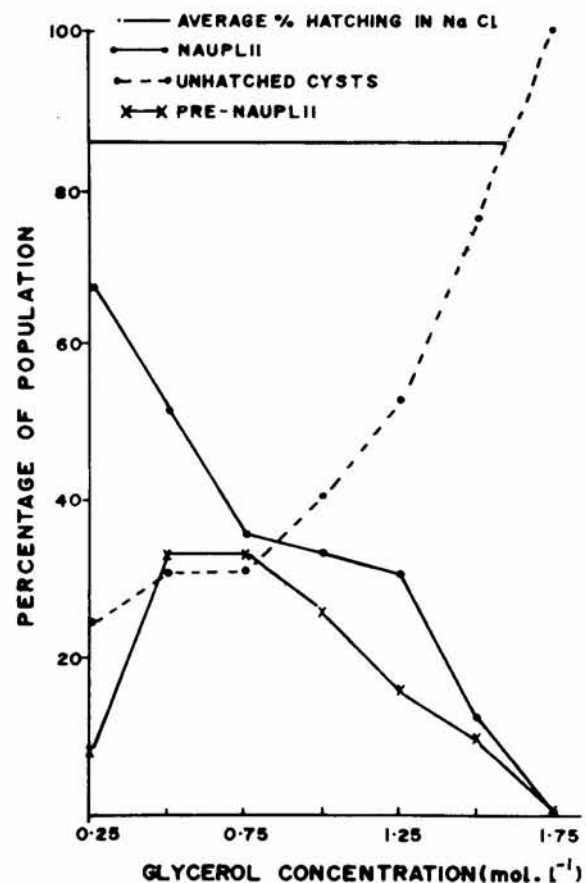


Fig. 1—Effects of increasing concentrations of glycerol on the hatching of *Artemia* after 96 h incubation period

bryonic development at higher concentrations. Glycerol dependent hyperosmotic regulatory system dominant during hatching appears to be replaced by the sodium hypoosmotic regulatory system which plays an important role in maintaining larval water balance²²⁻²⁴. Therefore, as soon as the chitinous membrane ruptures during the normal incubation period, the external medium must be seawater.

In conclusion it may be stated that maximal hatching is attained at 30 $^{\circ}\text{C}$ both at 5 and 35 $\times 10^{-3}$ salinities. It has been demonstrated that at lower salinity, the hatching rate increases and the nauplii have a higher energy content and high hatching efficiency²⁵. Hence, it is advisable to hatch the cysts in 5 $\times 10^{-3}$ saline medium. Harvesting of cysts preferably soon after the release and subsequent storage in super saturated brine solution would enhance hatching efficiency. Viable cysts when suspended in seawater tend to settle and the collection of such cyst with proper storage will guarantee quality cysts. Cyst viability can be increased using antibiotics which suppress associated bacterial growth. Based on naupliar size, low ash content and high protein content, this strain is likely to be preferred by predators.

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References

- 1 Stella E, *Z Ind abst Vererbungslehre*, **65** (1963) 412.
- 2 Royan J P, *Mahasagar-Bull Natn Inst Oceanogr*, **12** (1979) 271.
- 3 Dubois M, Gillas K A, Hamilton J K, Rebus R A & Smith F, *Anal Chem*, **28** (1956) 350.
- 4 Folch J M Lees & Stanley G H S, *J Biol Chem*, **226** (1956) 497.
- 5 Raymont J E G, Austin J & Linford E, *J Cons Perm Int Explor Mar*, **28** (1954) 354.
- 6 Vanhaecke P & Sorgeloos P, Paper presented at *World Mariculture Society—Technical Sessions*, Seattle (WA-USA), March 8-10, 1981.
- 7 Sorgeloos P & Persoone G, *Aquaculture*, **6** (1975) 303.
- 8 Society of American Bacteriologists, *Manual of Microbiological Methods* (McGraw-Hill, New York) 1957, 357.
- 9 Vanhaecke P & Sorgeloos P, in *The brine shrimp Artemia*, Vol 3, edited by G Persoone, P Sorgeloos, D Roels and E Jaspers (Universa Press, Wetteren, Belgium) 1980, 393.
- 10 Royan J P, *Indian J Mar Sci*, **7** (1978) 116.
- 11 Manjula V N, *A Study on the Sri Lankan Strain of brine shrimp Artemia*, B.Sc. dissertation, University of Colombo, Sri Lanka, 1984.
- 12 Vanhaecke P & Sorgeloos P, in *Book of Abstracts. Twelfth Annual meeting of the World Mariculture Society*, 1982.
- 13 Dutrieu J, *Arch Zool Exp Gen*, **99** (1960) 1.
- 14 Von Hentig R, *Mar Biol*, **9** (1971) 145.
- 15 Benijits F, Van voordens E & Sorgeloos P, in *Proceedings of 10th European Symposium on Marine Biology*, Vol. 1, edited by G Persoone and E Jaspers (Universa Press, Wetteren, Belgium) 1975, 620.
- 16 Royan J P, in *The brine shrimp Artemia*, Vol 3, edited by G Persoone, P Sorgeloos, O Roels and E Jaspers (Universa Press, Wetteren, Belgium) 1980, 223.
- 17 Paffenhofer G A, *Helgolander Wiss Meeresunters*, **16** (1967) 130.
- 18 Bhargava S C, Jakhar G R, Saxena M M & Sinha R K, in *Second International Symposium on the brine shrimp Artemia*, 1-5, 1985 Antwerpen, Belgium, Abstract No. 17.
- 19 Coleman D E, Nakagawa L K, Nakamura R M & Chang E, in *The brine shrimp Artemia*, vol. 3, edited by G Persoone, P Sorgeloos, O Roels and E Jaspers (Universa Press, Wetteren, Belgium) 1980, 153.
- 20 Clegg J S, *Trans Am Microsc Soc*, **93** (1974) 481.
- 21 Geldiay R, Koray T & Buynkisik B, in *The brine shrimp Artemia*, vol. 3, edited by G Persoone, P Sorgeloos, O Roels and E Jaspers (Universa Press, Wetteren, Belgium) 1980, 147.
- 22 Conte F P, Hootman S R & Harris P J, *J Comp Physiol*, **80** (1972) 239.
- 23 Conte F P, Dronkas P C & Ewing R D, *J Exp Zool*, **202** (1977) 339.
- 24 Ewing R D, Peterson G L & Conte F P, *J Comp Physiol*, **80** (1972) 247.
- 25 Vanhaecke P, Cooreman A & Lavens P, *Jaarverslag F.K.F.O.*, 20010.78 (1980) 42.