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HATCHABILITY OF THE FIRST CYSTS OF *ARTEMIA* PRODUCED IN SALT-PANS IN BANGLADESH

M. Paranthaman, E. Naylor and N. Mahmood*

School of Ocean Sciences, University of Wales-Bangor, Menai Bridge, Anglesey, Gwynedd, U.K.

* *Institute of Marine Sciences, University of Chittagong, Chittagong, Bangladesh*

Abstract

Hatchability was investigated in Bangladesh-produced cysts of *Artemia*, the first such cysts to be produced in salt-pans in that country, using imported stock from Great Salt Lake (USA). Optimal hatching conditions of 25°C temperature, 25 ppt salinity and 0.14×10^{16} quanta/sec/cm^2 light intensity were defined. The maximum hatch achieved in the Bangladesh material by this means was 39%, compared with 70% in control cysts imported directly from the USA.

Since the Bangladesh material, unlike the USA cysts, may not have experienced low environmental temperatures during diapause, further experiments involving chilling were carried out. The USA cysts hatching rate was not increased by this means, but the Bangladesh material hatching rate was increased to nearly 60%. The production in preliminary trials of *Artemia* cysts with a potential hatching rate not much lower than that of the parent stock, gives encouragement for further development of *Artemia* culture in Bangladesh.

Introduction

In Recent years, increased demand has resulted in world-wide efforts to extend the geographical range of the culture of brine shrimp *Artemia salina* L. in salt-pans and saline lakes, sometimes in developing countries (Sorgeloos, 1980). This necessitates the optimization of hatchability rates of harvested cysts in each new source. One such potential new source locality is in Bangladesh where the first cysts from field trials have been produced using imported USA cysts from Great Salt Lake, Utah (Mahmood, 1990). The present study sets out to determine the suite of conditions which would provide optimal hatching in the Bangladesh-produced material, particularly bearing in mind the different climate regime and lower environmental temperature which the Utah parent stock would experience.

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Materials and Methods

The source material for the present study was produced in two experimental ponds, each 45 m^2 in area, at Chanua, Banskhalii Chittagong, Bangladesh (Mahmood, 1990), *Artemia* nauplii hatched in seawater (35 ppt) from 30 g of Great Salt Lake cysts were introduced into the ponds in mid-January, 1989. Each pond was initially fertilized with

180 g of urea and 3.6 kg of dry chicken manure, and subsequently with additions of these fertilizers at 45 g and 900 $g.wk^{-1}$ respectively. The trials lasted for 3 months, during which time the salinity of the ponds rose from 60 to 120 ppt and noon temperature from 22 to 34°C. At the end of March 1989, a total of 517 g dry weight of cysts was harvested, p epared by washing and sun-drying, from which representative material was studied for hatchability at the Marine Science Laboratories, Menai Bridge, U.K.

A small quantity of dry cysts of *Artemia* was placed in filtered sea water (32 ppt.) in solid watch glass and mixed by micropipette. Immediately, with the micropipette, random samples, each of 10 cysts, were then transferred one by one from the watch glass to multi-chambered petridishes each containing 5 ml of filtered sea water. Each petridish contained 25 chambers and two such petridishes were used to carry out 50 replicates, for each combination of environmental factors tested. Incubations were then carried out for 50 hours in various factor combinations, *viz.* 15, 20, 25, 30 and 35°C (at 32 ppt and 0.14×10^{16} quanta/sec/cm^2 ; 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 ppt (at 25°C and 0.14×10^{16} quanta/sec/cm^2; dark, 0.14×10^{16} and 0.60×10^{16} quanta/sec/cm^2 (at 25°C and 32 ppt); and cooling to 2.5°C and — 22°C for different time periods with subsequent incubation at 25°C, 32 ppt and 0.14×10^{16} quanta/sec/cm^2.

The cumulative numbers of nauplii emerging were counted under a binocular microscope at five hour intervals. Cumulative hatch numbers at each temperature, salinity, light and chilling treatment were then tested by analysis of variance, significant differences being determined by the Tukey or Bonferroni test. In each case the results with Bangladesh-produced cysts of *Artemia* were compared with results from control experiments using csysts obtained direct from the parent stock in Great Salt Lake (USA).

RESULTS

Table 1 shows the effects of temperature, salinity and light on the hatching percentage of Bangladesh cysts of *Artemia* compared with Utah controls. The optimal conditions for the experimental groups and the control groups were 25°C, 25 ppt and 0.60×10^{16} quanta/sec/cm^2 light intensity, and 25°C, 10 ppt and 0.14×10^{16} light intensity respectively.

TABLE 1. *Maximum cumulative hatch percentages of Bangladesh and USA cysts of* Artemia *at various temperatures (at 32 ppt and 0.14×10^{16} light intensity), various salinities (at 25°C and 0.14×10^{16} light intensity) and at various light intensities (at 32 ppt and 25°C). ± Standard deviation. Asterisked values are significantly lower ($P < .05$) than the underlined maximum values, as determined by the Tukey test*

		Bangladesh	USA
Temperature (°C)			
15	..	19.8 ± 9.4*	31.0 ± 12.2*
20	..	23.0 ± 11.8	47.6 ± 13.9*
25	..	28.0 ± 16.7	68.6 ± 12.0
30	..	20.2 ± 12.2*	64.4 ± 8.4
35	..	12.6 ± 7.5*	51.2 ± 12.6*
Salinity (ppt)			
5		27.4 ± 15.9*	59.4 ± 13.9
10	..	30.0 ± 12.3*	67.6 ± 11.9
15	..	31.8 ± 16.5	67.4 ± 11.7
20	..	31.6 ± 10.3	63.0 ± 9.7
25	..	38.8 ± 17.7	56.8 ± 14.6*
30	..	35.6 ± 11.3	62.4 ± 12.7
35	..	33.6 ± 13.1	55.4 ± 15.7*
40	..	31.6 ± 8.7*	56.2 ± 15.9*
45	..	28.8 ± 10.0*	60.2 ± 12.7
50	..	22.8 ± 7.8*	62.6 ± 15.6
Light incubation (quanta/sec/cm^2)			
0.00 (dark)	..	17.4 ± 8.8*	55.0 ± 9.5*
0.14×101^{16}	..	28.0 ± 16.9	68.6 ± 12.0
0.60×101^{16}	..	29.8 ± 10.4	66.8 ± 10.6

The effects of chilling cysts at 2.5°C for various lengths of time are given in Table 2. These show for Bangladesh cysts a markedly increased cumulative hatch following chilling at 2.5°C for 40 hours, but with no statistically significant effect of chilling on the cumulative hatch of parent USA material. Table 3 plots the maximum cumulative hatch values after 1, 5 and 10 days chilling at -22°C, with subsequent culture in optimal conditions derived from the initial experiments, which were the highest of all hatching values obtained for Bangladesh material. The values after chilling for 5 and 10 days were not significantly different from each other ($P < 0.05$) and were only just over 10% lower than the control hatching rates using Utah material.

The temporal pattern of hatching obtained in a range of conditions for Bangladesh and Utah cysts is illustrated in Fig. 1 and 2. These confirm the additive improvement of percentage hatching in the Bangladesh material by combining optimum conditions of salinity, temperature, light and chilling. Not only was the maximum hatching percentage obtained only about 2% less than that in controls, but the initial rate of hatching was indeed slightly better than in controls.

Discussion

The important role of *Artemia* in aquaculture demands a continuous effort to improve hatching and culturing conditions and present results sought to define optimum conditions for the hatchability of the first Bangladesh produced cysts of *Artemia*.

TABLE 2. *Maximum cumulative hatching percentages* (± *s.d.*) *of Bangladesh and USA cysts of* Artemia *after different chilling times and kept subsequently at* 32 *ppt*, 25°C *and* 0.14×10^{14} *light intensity* (*quanta/sec/cm*^{2}). *Asterisked values are significantly higher than non-chilled controls, as determined by the Bonferroni test* ($P < .05$.)

Chilling at 2.5°C Time (Hrs.)		Bangladesh Non-chilled controls	Bangladesh Chilled	USA Non-chilled controls	USA Chilled
10	..	2.46 ± 19.8	24.2 ± 19.6	68.6 ± 12.0	69.2 ± 10.5
20	..	22.4 ± 17.0	22.2 ± 15.3	65.2 ± 12.0	62.0 ± 13.6
40	..	26.4 ± 17.4	28.0 ± 22.7*	65.6 ± 15.1	64.0 ± 10.9
60	..	28.2 ± 18.1	38.2 ± 22.4*	61.6 ± 12.5	62.2 ± 15.3

TABLE 3. *Maximum cumulative hatching percentages* (± *s.d.*) *of Bangladesh and USA cysts of* Artemia *after different chilling times and kept subsequently at* 25 *ppt.*, 25°C *and* 0.14×10^{14} *light intensity* (*quanta/sec/cm*^{2})

Chilling at -22°C Time (days)		Bangladesh	USA
1	..	42.8 ± 13.0	67.4 ± 10.9
5	..	58.8 ± 15.7	68.6 ± 10.6
10	..	60.4 ± 15.0	68.0 ± 9.7

The lowest temperatures at which *Artemia* appears to survive, except as cysts, is 6°C and the maximum temperature that *Artemia* populations tolerate has been reported to be close to 35°C (Persoone and Sorgeloos, 1980). Optimal temperatures for early larvae of *Artemia* appear to be close to the reported optimum hatching temperature of approximately 30°C (Von Hentig, 1971), though in this there is some variation from race to race (Sorgeloos, 1975). Present results confirm that temperature is a significant influencing factor on the hatchability of Bangladesh-produced cysts of *Artemia*, the maximum cumulative hatch occurring at 25°C. Qualitatively, but not quantitatively, the hatching pattern of Bangladesh produced cysts was similar to that of Great Salt Lake material used as controls the lower hatching rate of the Bangladesh material requiring further explanation.

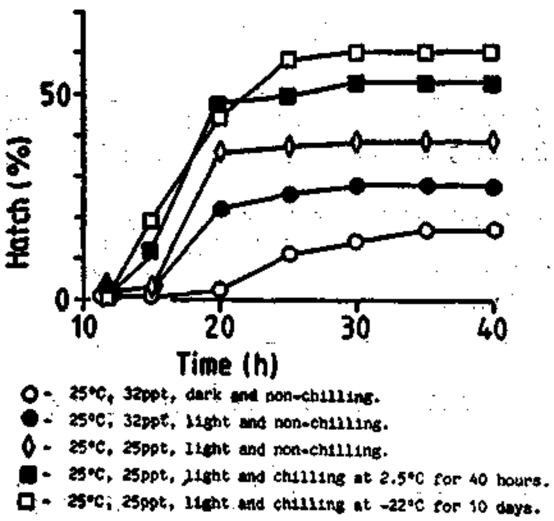

FIG. 1. Cumulative hatching percentage of Bangladesh produced cysts of *Artemia* in different hatching conditions.

For reasons of practical convenience natural sea water is often used to hatch *Artemia*. This presumably arises, because it is generally accepted that *Artemia* is a euryhaline organism, with a wide salinity tolerance variously reported as 3 - 300 ppt (Bayly, 1972) and 35-110 ppt (Vanhaecke *et al.*, 1984), with some geographical variation. However, most authors now agree that hatching rates are greatest at salinities of less than that of sea water (Sorgeloos, 1980; Vanhaecke *et al.*, 1980; Bruggeman *et al.*, 1980; Vanhaecke and Sorgeloos, 1983; Thun and Starrett, 1987). This was confirmed in the present study in which it was shown that 25 ppt is the optimum salinity and 15-35 ppt is the optimum range for the maximum hatchability of Bangladesh-produced cysts, of *Artemia*. In the control experiments, Great Salt Lake stock showed little variation in hatching rate over the range 5-50 ppt salinity, but highest rates were again in low salinities at 10-15 ppt. Present results therefore confirm the suggestion that use of a low salinity medium assures increased hatching outputs (Vanhaecke and Sorgeloos, 1983). This and demonstration of higher energy content nauplii hatched in low salinities (Vanhaecke *et al.*, 1980, following Clegg, 1964 and Conte *et al.*, 1977) argue strongly for the use of low salinities for the hatching of *Artemia* in commercial hatcheries.

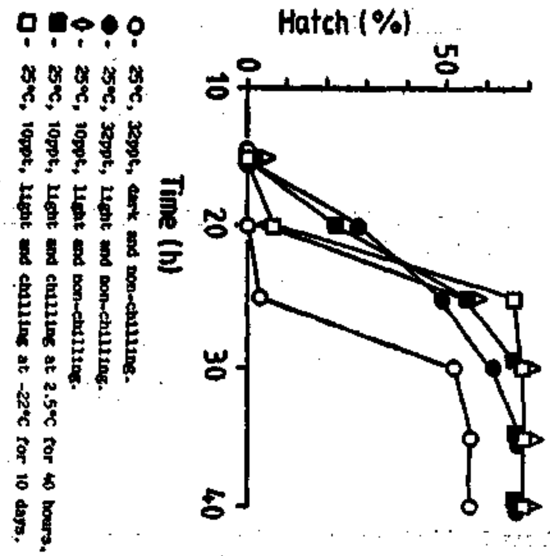

FIG. 2. Cumulative hatching percentages of Great Salt Lake (USA) cysts of *Artemia* in different hatching conditions.

Light conditions also influence the hatching efficiency of *Artemia* cysts (Sorg loos, 1980), an increase in hatching rate occurring in light, as compared to controls incubated in darkness (Sorgeloos, 1973). This is confirmed in the present study, cultures under illumination showing statistically increased hatching efficiency in both Bangladesh and control USA

Artemia cysts. The mechanism of light enhancement of hatching is not yet fully understood, but Sorgeloos and Persoone (1975) have reported that a minimal dose of light energy is needed to trigger onset of metabolism in the encysted embryo. Light absorption by the cyst chorion has been studied in *Artemia* by Iwasaki *et al.* (1980) and important differences in this characteristic between strains have been reported (Persoone and Sorgeloos 1980; Vanhaecke and Sorgeloos, 1980; Bruggeman *et al.*, 1980; Vanhaecke *et al.*, 1981) suggesting that there may be strain differences in light requirements. Hatching rate may also vary with exposure times and wave length as well as with light intensity (Van Der Linden *et al.*, 1985).

The results discussed so far clearly demonstrate that, even when optimum conditions of temperature, salinity and light are used for the hatching of Bangladesh *Artemia*, that the hatching rates of 30-40 % are significantly below the hatch rates of 70 % in control stocks obtained directly from Great Salt Lake. This led to consideration of the possibility that the Bangladesh produced cysts may not have experienced such low temperatures during diapause as the parent stock in N. America might experience in winter. Experiments involving chilling of the cysts confirmed that this appeared to be so. Climatic adaptations resulting in strain-specific temperature responses among *Artemia* would not be surprising since similar temperature related adaptations occur in geographically separated populations of other invertebrates (Lavens and Sorgeloos, 1987).

The exact biochemical mechanisms involved in the diapause process are not yet fully understood (Clegg and Conte, 1980). However, pre-incubation in low temperature is probably an effective factor in terminating dormancy in *Artemia* from temperate latitudes (Lavens *et al.*, 1986). From the present study this treatment appears to be necessary for such a strain even after a complete generation at subtropical latitudes. Several other diapause inhibition methods reported as effective for *Artemia*, including exposure to repeated dehydration and hydration, U.V. irradiation, cosmic radiation, magnetic fields, organic solvents, peroxide treatment and manipulation of internal pH (Lavens *et al.*, 1986), were no considered in the present study.

The maximum hatching achieved in the present study using combined optimal hatching conditions after exposure to chilling for some hours or days is only about 12 % less than the maximum hatching achieved using USA cysts as controls. This seems not unreasonable for the first batch of such cysts produced in Bangladesh from material imported from the USA. It offers promise for further improvements with increased experience of this culture technique in Bangladesh and offers considerable scope for commercial development in the technique in the country.

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