J. Natn. Sci. Coun. Sri Lanka 1995 **23**(4): 161-169

EFFECTS OF TEMPERATURE AND SALINITY ON SURVIVAL, GROWTH AND FECUNDITY OF THE BRINE SHRIMP *ARTEMIA PARTHENOGENETICA* FROM SRI LANKA

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(Received: 18 January 1995; accepted: 03 November 1995)

Abstract: Survival, growth and reproductive mode were studied in *Dunaliella* fed Sri Lankan *Artemia parthenogenetica* raised from hatching to reproductive maturity at different salinity and temperature combinations. Survival was high in 100 and 120ppt, both at 25° (94 and 72%, respectively) and 29°C (82 and 78%, respectively). Poor survival was recorded in higher salinities at 29°C and all salinities at 35°C. At 21°C survival decreased rapidly to near 10%, other than in 65ppt where survival was 48%. Growth rate at the tested salinities varied from 0.11mm day^{-1} to 0.16mm day^{-1} at 21°C and from 0.23mm day^{-1} to 0.34mm day^{-1} at 25°C. Similar growth (0.39mm day^{-1}) occurred in 100 and 120ppt at 29°C. No animals reached adult stage at 21°C whereas at 25° and 29°C, broodpouch development commenced by day 14 in 65, 80 and 100ppt. Reproduction occurred only at 29°C. Ovoviviparity occurred only in the 35 and 100ppt treatments on day 15 (at 68 and 56 nauplii day^{-1}, respectively). Subsequent gradual increase in salinity (4ppt day^{-1}) in the 35 and 100ppt treatments made the ovoviviparous groups to switch to oviparity at 132ppt (at 37 cysts adult^{-1}) and 136ppt (at 38 cysts adult^{-1}), respectively. Animals reared in 120ppt also reproduced, but without switching, showing oviparity (at 36 cysts adult^{-1}) with gradual salinity increase to 140ppt. Salinity-temperature combinations ranging from 100 to 120ppt and 25° to 30°C are, therefore, best for survival, growth and reproduction of Sri Lankan *Artemia*.

Key words: *Artemia*, cysts, *Dunaliella*, growth, nauplii, reproduction, salinity, survival, temperature.

INTRODUCTION

The brine shrimp *Artemia* is widely used as a larval feed in fish and shellfish hatcheries. Natural populations of *Artemia* inhabit geographically isolated biotopes having specific biotic and abiotic conditions.[1] In Sri Lanka, a parthenogenetic strain of *Artemia* inhabits solar salterns in Hambantota and Puttalam.[2,3] The abiotic conditions of *Artemia* biotopes show substantial differences in water temperature and salinity.[4] Such differences and the commercial importance of *Artemia* have prompted studies into many of its known species and strains with respect to their temperature and/or salinity effects on survival,[1] growth and mortality[5-7] and maturation and fecundity.[8] Results from these studies have illustrated the variation in temperature and salinity responses between *Artemia* strains. These findings have served to highlight the importance of selecting optimal ranges for known strains of *Artemia* for specific aquacultural applications.[1]

Salinity and temperature effects on the life cycle of Sri Lankan *Artemia parthenogenetica* are not documented. The promotion of a wider utilization and export of local *Artemia parthenogenetica* requires such data that are necessary

to determine optimum conditions for inoculating *Artemia* culture ponds. Field culture requires the introduction of the nauplius stage individuals directly into culture ponds.[9] Their subsequent survival, growth and reproduction under prevailing salinity and temperature conditions determine the size of the final cyst producing population. This paper reports on salinity and temperature effects on life cycle characteristics of the Sri Lankan *Artemia parthenogenetica*.

METHODS AND MATERIALS

Artemia parthenogenetica cysts produced in Mahalewaya saltern, Hambantota, were used for studying survival, growth and fecundity at different salinities and temperatures. *Artemia* cysts were hatched in sea water of 35ppt salinity at a room temperature of 29°C under continuous illumination and aeration. Hatched instar I nauplii were introduced at a density of 50 nauplii/250 ml into glass conical flasks containing salt solutions of 35, 65, 80, 100 and 120ppt. The solutions at each of the salinities were maintained at temperatures of 21, 25 and 29°C with continuous aeration. Additionally, 140 and 180ppt salinities were also used at 29°C and at 35°C. The higher salinities were used only at the higher temperatures because, under field conditions in *Artemia* ponds, the high salinity levels occur only under increased evaporation rates associated with high temperatures. All experiments were conducted in triplicate. The solutions of different salinities were prepared by dissolving commercial salt in sea water. Experiments at 21°C and 25°C temperatures were carried out in a temperature controlled room in thermostatically controlled water baths. Experiments at 35°C were carried out in a constant temperature water bath (Kottermann).

The feeding schedule[1] was as follows: *Artemia* nauplii were fed daily with the alga *Dunaliella* sp. commencing at a density of 6.0×10^6 and 8.4×10^6 cells per day at 21°C and 25°C, respectively, and at 10.8×10^6 cells per day at 29°C and 35°C, doubling the algal densities every two days. Algal cultures were counted using a haemocytometer and the required concentrations for feeding were prepared after centrifugation. Faecal matter was siphoned out and 25% of the medium was renewed every 2d. Survival was checked every 3d up to 14d. Body lengths were determined every 3 d for a random sample of 20 animals per flask. For body length measurements, each animal was transferred on to a cavity slide and the water was gently drawn out using a paper towel. The total length was measured from the anterior margin of the head in front of the ocellus to the base of the caudal furca[5] using a binocular microscope fitted with a calibrated eye-piece micrometer.

Ten adult animals were placed individually in glass vials of 50 ml capacity at 29°C in the salinities of 35, 100 and 120ppt which were the salinities in which animals attained adult stage and feeding was continued. The salinity was gradually increased at 4ppt day^{-1} by addition of a concentrated salt solution. The vials were examined daily for the presence of nauplii or cysts and counts were made of any nauplii or cysts that were produced.

RESULTS

Survival: Percentage survival at different temperatures and salinities is illustrated in Figure 1 a-d. A rapid reduction in survival was seen at 21°C in all salinity levels except in 65ppt in which survival had stabilized at 48% by day 14. At 25°C survival was high in 100ppt and 120ppt salinities.

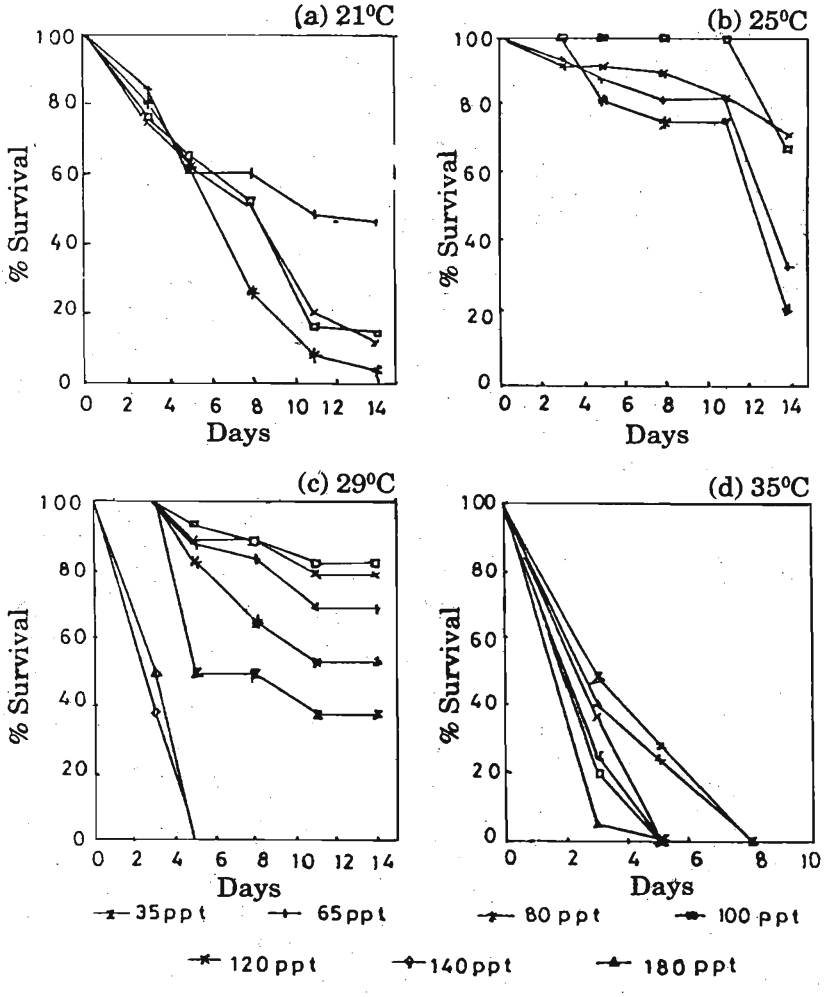

Figure 1 a-d: Percentage survival in *Artemia parthenogenetica* at varying temperatures and salinities.

At room temperature of 29°C, 100% mortality was observed by day 5 in 140ppt and 180ppt salinities. Until day 3 there was 100% survival in the salinity levels ranging from 35ppt to 120ppt. Survival decreased rapidly from 100% at day 3 to 48% at day 5 at 35ppt salinity. There was 68% and 52% survival at salinities of 65ppt and 80ppt at day 14. The highest survival (82%) at day 14 was attained in 100ppt salinity followed by 78% survival in 120ppt salinity. The lowest survival (37%) at room temperature was in 35ppt salinity at day 14.

At 35°C there was a rapid decrease in survival within two days of the experiment resulting in complete mortality by day 5 at all salinity levels except at the low salinity levels of 35 and 65ppt. Even at these 35 and 65ppt salinities, 100% mortality occurred by day 8.

Growth: The mean length of instar I nauplius of Sri Lankan *Artemia* was 475.4µm. The mean lengths of *Artemia* at different temperatures and salinities are illustrated in Figure 2 a-c. Length measurements were discontinued after 25 days or when there was more than 75% mortality.

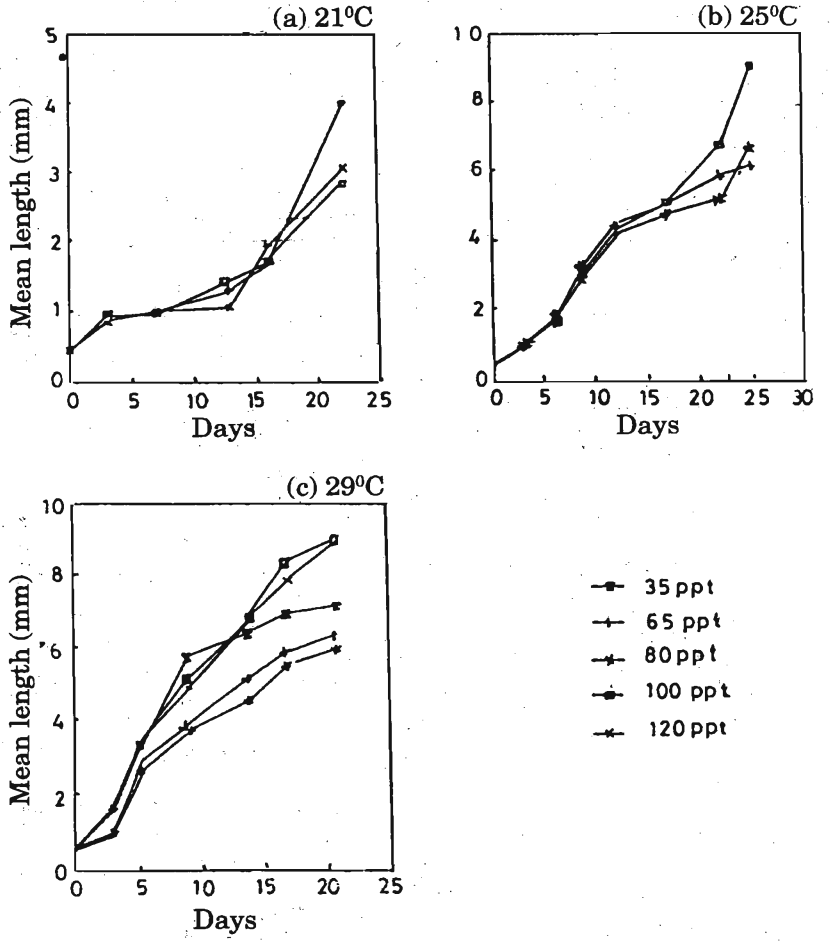

Figure 2 a-c: Variation in mean body length (mm) in *Artemia parthenogenetica* at varying temperatures and salinities.

Fecundity: Reproductive characteristics of *Artemia* are summarized in Table 1. *Artemia* did not attain reproductive maturity at any of the tested salinities at 21°C. A slight appearance of broodpouches was observed at 65, 80 and 100ppt salinities at 25°C. They grew only to pre-adult stage at 25°C. At 29°C in both 100 and 120 ppt salinity, *Artemia* attained reproductive maturity in 14 to 15 days within a length range of 6.9 to 7.0 mm.

Table 1: Reproduction and fecundity of *Artemia parthenogenetica* in laboratory experiments at different temperature and salinity combinations.

Experimental conditions			Reproduction and Fecundity					
			Naupliar production			Cyst production		
T(°C)	S(ppt)	Age at maturity (days)	nos/parent	Age (days)	Salinity (ppt)	nos/parent	Age (days)	Salinity (ppt)
21	65	n.a.		n.p.			n.p.	
	80	n.a.		n.p.			n.p.	
	100	n.a.		n.p.			n.p.	
	120	n.a.		n.p.			n.p.	
25	65	n.a.		n.p.			n.p.	
	80	n.a.		n.p.			n.p.	
	100	n.a.		n.p.			n.p.	
	120	n.a.		n.p.			n.p.	
29	35	14	68	15	35	37	46	132
	65	n.a.		n.p.			n.p.	
	80	n.a.		n.p.			n.p.	
	100	14	56	15	100	38	25	136
	120	15		n.p.		36	26	140
	140	n.a.		n.p.			n.p.	
	180	n.a.		n.p.			n.p.	
35	35	n.a.		n.p.			n.p.	
	65	n.a.		n.p.			n.p.	
	80	n.a.		n.p.			n.p.	
	100	n.a.		n.p.			n.p.	
	120	n.a.		n.p.			n.p.	
	140	n.a.		n.p.			n.p.	
	180	n.a.		n.p.			n.p.	

n.a. = not attained T = temperature in °C d = days
n.p. = not produced S = salinity in ppt

At 100 ppt salinity at 29 °C adults with broodpouches were observed on day 14, and on day 15 five adults released nauplii at 56 numbers adult^{-1} (Table 1). With gradual increase in salinity, nauplii were released at 62 numbers adult^{-1} at 108ppt after an interval of 4 days from the initial release of nauplii. There was no release of nauplii by *Artemia* from 108 to 136ppt salinity. Two animals released cysts at 38 numbers adult^{-1} and 36 numbers adult^{-1} at 136ppt on day 25 and 140ppt on day 26, respectively. There was 100% mortality in *Artemia* two days after the salinity was increased to 140ppt.

In the treatments maintained at 29°C and 120ppt, adults with broodpouches were observed on day 15. As salinity was gradually increased at a rate of 4ppt per day cysts were released at 140ppt at 36 cysts adult^{-1} initially at day 24, and 40 cysts adult^{-1} 4 days thereafter at the same salinity. Ovoviviparity was not observed in these adults.

Artemia initially reproduced ovoviviparously in 35ppt at 68 nauplii per female and as salinity was increased upto 100ppt, ovoviviparity re-occurred at

3 to 4 day intervals. Oviparity occurred at 132ppt and continued upto 148ppt salinity after which there was 100% mortality. *Artemia* did not reproduce from 100 ppt to 132ppt salinity. The highest number of nauplii (160/female) were produced at 60ppt followed by 135 nauplii/female at 40ppt salinity. With regard to cyst production, fecundity ranged from 37 to 42 cysts/female.

DISCUSSION

The best conditions for survival of Sri Lankan *Artemia* from hatching to maturity were at 100ppt and 25°C with 100 and 120ppt salinities at 25° and 29°C also supporting high survival rates. These conditions should therefore be maintained in ponds for favourable survival during the growth phase of Sri Lankan *Artemia*. The higher salinities (140 and 180ppt) at 29°C and the range of salinities from 65 to 140ppt at 35°C are very unfavourable to the younger stages and should be avoided at the inoculation stage of pond culture. High mortalities at higher temperatures that were recorded in the present study were also evident under field culture conditions where total mortality of Sri Lankan *Artemia* populations occurred when pond waters at Palavi reached 33°C.[10] Such detrimental effects of high temperature on survival dictate that culture conditions should be manipulated so as to avoid pond waters reaching high temperature values, which tends to occur particularly during the later periods of the dry season.

Survival studies on a variety of other *Artemia* strains have shown increased survival to occur over a wider range of temperatures than reported by us, with survival in excess of 90% being reported over a 20 to 29°C temperature range in the Indian Tuticorin *Artemia* and survival decreasing to 50% with increase in temperature to 32 to 33°C.[1] *Artemia* from Iraq also displayed higher survival at lower temperatures of 15 to 25°C but became motionless at 35°C and further temperature increases (to 42 to 44°C) resulted in total mortality.[11] For the Indian Tuticorin strain, the preferred temperature was at 27°C and optimal survival occurred at 30 to 40ppt salinity range.[12]

Reports indicate a wide range of variability in the survival and growth of *Artemia* at different salinities and temperatures, e.g. *A. franciscana* from Lake Grassmere, New Zealand, achieved over 90% survival and fastest growth at 20 to 28°C in 100 to 170ppt salinity range[8]; 50 to 100ppt salinity range for increased growth in Tuticorin *Artemia*[13]; a higher growth rate in *Artemia* from Sambhar Lake, India reared at 12.5% (corresponding to 125ppt) salinity than at a low salinity of 6.5%[6] (corresponding to 65ppt). These reports indicate that the extent to which growth is influenced by the salinity of the external medium varies with the stock and sex of *Artemia*.[5]

Even though it had been found that reproductive maturity in parthenogenetic females from Sri Lanka was attained in 15 to 17 days when reared at 35ppt and at 140ppt at 25°C,[5] in the present study only a slight development of broodpouches was observed in Sri Lanka *Artemia* at 25°C at the low salinities tested. *Artemia* from Sambhar Lake, India, became reproductively mature in 15 to 17 days when

reared at 125ppt salinity while those reared at a low salinity of 6.5% became reproductively mature in 20 to 25 days.[6] The tolerance threshold of *Artemia* is strain-dependant and as far as optimum temperature is concerned it has been observed that there are probably as many temperature optima as there are *Artemia* habitats.[4]

In the present study *Artemia* reproduced ovoviviparously at 100ppt and oviparous reproduction occurred later when salinity was increased gradually to 132ppt. Similarly it had been found that the first brood of offspring in *Artemia* was ovoviviparous both in the wild and in the laboratory.[14] Ovoviviparous reproduction, however, was not observed in Sri Lankan *Artemia* cultured in 120ppt salinity or at increase of salinity from 120 to 140ppt since these animals produced cysts at 136ppt without prior nauplii production. Under field conditions at Mahalewaya saltern *Artemia* commenced cyst production at a similar salinity of 132ppt.[3] Observations on cyst production reported here point to the possibility of manipulating salinity values in the salterns for triggering cyst production. Reports have indicated that in parthenogenetic populations, the percent of offspring born viviparously was higher than encysted offspring.[15] Fecundity observations from the present study lend support to this viewpoint. The salinity range of 100 to 120ppt observed in the Mahalewaya salterns as carrying the highest populations of Sri Lankan *Artemia* relate favourably to the observations made under laboratory conditions.[10] A salinity range of 100 to 120ppt in combination with a temperature range of 25 to 29°C are recommended as optimal ranges for these two parameters for survival, growth and reproduction of *Artemia* from Sri Lanka.

Acknowledgments

This work was funded by the Natural Resources, Energy and Science Authority (RG/87/B/3). Laboratory facilities were provided by the Department of Zoology, University of Colombo and the National Aquatic Resources Agency.

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