The effect of salinity on the reproductive characteristics of parthenogenetic *Artemia* from South Africa

BF Williams and SA Mitchell*
Department of Zoology, University of the Orange Free State, Bloemfontein 9300, South Africa

Abstract

Separate cohorts of *Artemia* were reared in medium with salinities of 50, 75 and 100 g NaCl·ℓ^{-1} to determine the influence of salinity on reproductive strategies. *Artemia* grown at salinity of 50 g·ℓ^{-1} produced a mean of 356,1 (SD 73,17) progeny each, with 67,3% as cysts. The mean number of progeny produced at a salinity of 75 g·ℓ^{-1} was 957,6 (SD 583,3), of which 2% were cysts, and at 100 g·ℓ^{-1} it was 49,6 (SD 32) with 16% as cysts.

Introduction

The salinity tolerance of *Artemia* is well known. Persoone and Sorgeloos (1980) reported that it can survive at salinities ranging from almost zero to water supersaturated with NaCl. However, in practice the lowest salinity in which *Artemia* occurs naturally corresponds with the highest salinity tolerated by its predators. Bowen et al. (1985) showed that although various strains of *Artemia* tolerate a wide range of salinities, the dominant anion influenced survival, as organisms from a habitat where chloride was dominant were unable to survive in a medium high in carbonate, and vice versa.

There are a number of small, temporary salterns in the semi-arid regions of South Africa, some supporting small saltworks (Mitchell and Seaman, 1988). The dominant anion in the salterns in this area is chloride (Hugo, 1974). Brine for the saltworks is pumped from boreholes or pits in the salterns, many of which have no surface water for most of the year. While the salterns themselves offer an ephemeral habitat, the saltworks are both permanent and have generally stable salinities in the evaporating pans.

The present study investigated the life cycle and reproductive characteristics of the parthenogenetic strain of *Artemia* in the salterns of the Western Orange Free State and the Northern Cape Province of South Africa, in order that these parameters may be compared with those of *Artemia* from other localities as listed by Browne et al. (1984).

Materials and methods

Stock cultures of *Artemia* were held in aerated medium at a salinity of 50 g NaCl·ℓ^{-1} of salt obtained from a saltworks near Bloemfontein where *Artemia* occurs naturally. The dominant anion in this salt is chloride. For each experiment a number of females about to release nauplii were isolated and held overnight in medium of the required salinity. The following morning the mature females were replaced in the stock culture, and some of the released nauplii were used experimentally. Thus, the age of the organisms used in each experiment was known within 8 h.

Each cohort consisted of 25 organisms held individually in petri dishes (100 mm dia.) containing 50 mℓ of unaerated medium. The medium was replaced daily and contained sufficient *Chlorella* cells to feed the organisms for 24 h. Each organism was checked daily and the number of nauplii and cysts were recorded.

Reproductive characteristics were recorded for organisms surviving to maturity (Tables 1 to 3).

The organisms used in the experiment were fed on the *Chlorella* sp. cultured in the laboratory using dried cattle manure (1 g·ℓ^{-1}) supplemented by urea (100 mg·ℓ^{-1}) as the source of nutrients. The salinity of the algal medium was adjusted to the appropriate level before being added to the higher salinity treatments. Both the experimental animals and the stock cultures were held at 22°C ± 1°C.

The effect of three salinities (50, 75 and 100 g NaCl·ℓ^{-1}) on the life cycle and reproductive characteristics of *Artemia* was investigated. The differences in the means and standard deviations for each characteristic measured for the organisms in each of the three treatments were tested for significance by ANOVA. Where differences were found to be significant, the means of the individual treatments were tested against each other by the method of standard error between two means.

Results

Survival and life cycle characteristics

Approximately 60% of the organisms survived to sexual maturity in each of the three salinities tested, and the number surviving did not differ significantly between the treatments.

The longevity of *Artemia* during different phases of their life cycles in the three treatments (Table 1) was tested by ANOVA, and significant (at p0,05) differences were found for all but the post-reproductive phase (Table 2).

The *Artemia* grown at 75 g NaCl·ℓ^{-1} spent significantly longer in all phases of the life cycle except the post-reproductive period than those grown at either 50 or 100 g NaCl·ℓ^{-1}. The mean duration of the reproductive period for the organisms grown in the medium at 100 g·ℓ^{-1} was so short because 73% of the organisms in this treatment produced only one brood of offspring before dying, and none produced more than two broods. Organisms in this treatment died very soon after their final brood.

Reproductive characteristics

The total production of offspring, as well as the production of nauplii was highest at 75 g NaCl·ℓ^{-1} (Tables 1 and 3). The trend of

*To whom all correspondence should be addressed. Present address: Water Research Commission, PO Box 824, Pretoria 0001, South Africa.

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TABLE 1
REPRODUCTIVE CHARACTERISTICS OF THE PARTHENOGENETIC *ARTEMIA* FROM THE ORANGE FREE STATE WHEN REARED IN MEDIA WITH DIFFERENT SALINITIES (g NaCl·ℓ^{-1}), PRESENTED AS MEAN (STANDARD DEVIATION)

Characteristic	Salinity 50	75	100
Life cycle characteristics			
Pre-reproductive period (days)	20,23 (1,64)	29,00 (2,12)	21,54 (0,78)
Reproductive period (days)	20,92 (3,01)	29,40 (10,50)	1,71 (0,95)
Post-reproductive period (days)	3,77 (2,92)	5,20 (5,68)	2,15 (1,63)
Total life-span (days)	44,23 (5,02)	62,60 (8,56)	25,00 (1,47)
Reproductive characteristics			
Offspring/brood	70,69 (43,61)	140,97 (62,76)	34,41 (18,14)
Broods/♀	5,08 (0,86)	6,60 (2,07)	1,38 (0,51)
Offspring/♀·d^{-1} (Reproductive phase)	16,83 (4,05)	31,33 (11,15)	28,40 (13,61)
Interval between broods (days)	3,65 (2,22)	4,25 (1,42)	2,50 (1,18)
% offspring encysted	67,27 (21,90)	2,01 (8,00)	16,0 (32,00)
% mixed broods (nauplii and cysts)	6,35	0	0
Total offspring/♀	356,08 (73,17)	957,60 (583,30)	49,62 (22,49)

TABLE 2
THE SIGNIFICANCE (AT p0,05) OF DIFFERENCES OBSERVED FOR THE DURATION OF THE PHASES OF THE LIFE CYCLE OF THE PARTHENOGENITIC ARTEMIA IN DIFFERENT SALINITIES.50 = 50 g·ℓ^{-1}; 75 = 75 g·ℓ^{-1}; 100 = 100 g·ℓ^{-1}

Pre-reproductive	50 < 100 < 75
Reproductive	100 < 50 < 75
Post-reproductive	Not significant
Total life-span	100 < 50 < 75

TABLE 3
THE SIGNIFICANCE (AT p0,05) OF DIFFERENCES OBSERVED FOR THE REPRODUCTIVE CHARACTERISTICS OF THE PARTHENOGENETIC ARTEMIA. 50 = 50 g·ℓ^{-1}; 75 = 75 g·ℓ^{-1}; 100 = 100 g·ℓ^{-1}

Offspring/brood	100 < 50 < 75
Broods/♀	100 < 50 < 75
Offspring/♀·d^{-1}	50 < 75, 100
Interval between broods	Not significant
% offspring encysted	75, 100 < 50
Total offspring/♀	100 < 50 < 75

offspring production was similar at the three salinities tested although less distinct at 100 g NaCl·ℓ^{-1}, in that mean fecundity increased with age to a maximum, then decreased (Fig. 1). In addition to the much higher fecundity shown by the organisms grown in the medium with 75 g NaCl·ℓ^{-1}, the organisms were a mean of 8 d older than those grown in the other two salinities tested before they reached sexual maturity and the reproductive period lasted longer (Fig. 1, Table 1).

Very few of the offspring from the organisms grown in the medium with 75 g NaCl·ℓ^{-1} were encysted. Although the mean proportion of encysted offspring was substantially higher amongst the organisms grown at 100 g NaCl·ℓ^{-1}, the difference is not significant because the variation between individuals in the latter treatment was so great (Table 3). The only reproductive characteristic measured which did not differ significantly between the treatments was the interval between broods (Table 3).

Discussion

The results indicate that variation in the salinity of the medium has a marked effect on the life cycle characteristics of the parthenogenetic *Artemia* from the inland salterns of South Africa. It is also apparent that both the fecundity and the longevity of the organisms were severely reduced at the highest salinity tested in this study (100 g NaCl·ℓ^{-1}). Individual organisms were not limited to either nauplius or cyst production, but were able to produce both at all of the salinities tested. However, only in the medium containing 50 g·ℓ^{-1} did the *Artemia* produce significantly large numbers of both nauplii and cysts.

Optimal reproduction (in terms of both numbers and nauplii) was obtained at a salinity of 75 g NaCl·ℓ^{-1}, i.e. a salinity considered near optimal by both Browne et al. (1984, 90 g·ℓ^{-1} TDS) and Bowen et al. (1985, 80 to 85 g·ℓ^{-1} TDS). However, considering the severe decline in fecundity shown by the organisms in the present study between the salinities of 75 and 100 g·ℓ^{-1} TDS, the optimum for this population is probably lower than that of the populations studied by Browne et al. (1984) and Bowen et al. (1985).

Browne et al. (1984) and Lenz and Browne (1991) argue that both a higher level of progeny encystment and a shorter life-span may be expected from *Artemia* populations which occupy unstable habitats. In the case of the study population, progeny encystment was highest (50%) at the relatively low salinity of 50 g NaCl·ℓ^{-1}, and even at a salinity that severely shortened the reproductive life cycle, progeny encystment remained low (16%). At the same time, the life-span was longest at 75 g NaCl·ℓ^{-1}, indicating that the life-span is not directly correlated with salinity. Thus, this strain of *Artemia* does not respond to increasing salinity by a swing from naupliar to cyst production.

Lenz and Browne (1991) cite one instance where a Central American population of *Artemia* reproduced oviparously at low salinities, but ovoviviparously at higher (>90g NaCl·ℓ^{-1}) salinities. This pattern is similar to the pattern observed during this study where the highest proportion of encystment of progeny occurred at the lowest salinity tested (50 g NaCl·ℓ^{-1}). However, Lenz and Browne (1991) also report a substantial decrease in offspring at the higher salinity and say that it is not clear whether this was due to food availability. This study, where the food was in excess for all treatments, showed that the *Artemia* produced double the number of nauplii at the salinity at which the lowest proportion of encystment occurres.

While the population studied here occurs mainly in the saltworks which offer a very stable habitat, these saltworks are a recent artefact of human activity in the area. The organisms would have originally occupied the natural salterns which are not stable, and may have inundations which last for short periods interspersed with dry periods which may occasionally last for longer than a year. According to Browne et al. (1984) and Lenz and Browne (1991), the population studied here should have a short life cycle and a high proportion of progeny encystment. However, the highest proportion of progeny encystment occurred at the lowest salinity tested (50 g·ℓ^{-1}), and at the optimum salinity tested ≈ 98% of the progeny were nauplii. This may indicate that this population of *Artemia* is not endemic to this habitat, bearing out the hypothesis of Seaman et al. (1991) that this strain was introduced to South Africa.

Where *Artemia* are being cultured for nauplii or cyst production, productivity would be increased by the use of a parthenogenetic strain where all of the adult organisms present in the culture are producing progeny. However, the large influence of salinity on both the fecundity and the proportion of encysted offspring shown by this strain of *Artemia* would mean that if this

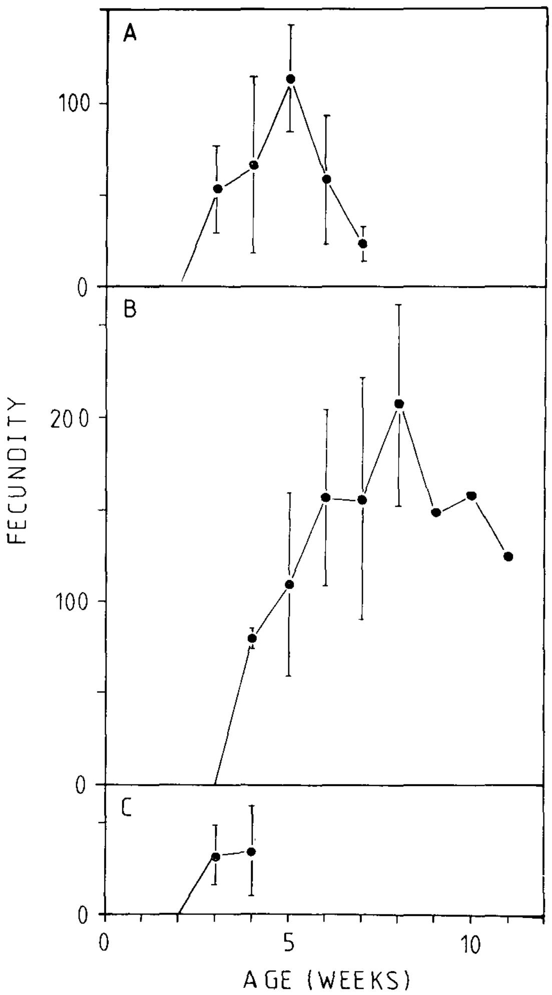

Figure 1
The mean (± 95% confidence limits) weekly fecundity of Artemia *reared at different salinities*
A - 50 g NaCl·ℓ^{-1}
B - 75 g NaCl·ℓ^{-1}
C - 100 g NaCl·ℓ^{-1}

organism is to be used in aquaculture the salinity would have to be carefully controlled to stimulate the type of production required. However, the high proportion of nauplii (≈98%) produced by females held in a medium of 75 g $NaCl \cdot \ell^{-1}$ lindicate that this would be a strain worth considering where naupliar production is required.

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