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Effects of increasing salinity on an *Artemia* population from Mono Lake, California

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Summary. Salinity increased from 48 to 93 g/l total dissolved solids (TDS) in Mono Lake, California between 1941 and 1982, and is expected to fluctuate between 169 and 248 g/l at equilibrium by the middle of the next century. In order to predict the consequences of this trend on the Mono Lake ecosystem, we determined effects of salinity on survival, growth, reproduction and hatching of *Artemia monica*, Mono Lake's only macrozooplankton species. Seven salinities ranging from 76 to 179 g/l were tested in a long-term experiment to determine both lethal and sublethal responses. The salt tolerance limit for subadult *A. monica* was between 159 and 179 g/l. Adult size, growth rates, and brood sizes decreased, and female mortality during reproduction increased, as salinity increased. Hatching of diapause eggs was delayed and total percent hatch decreased as salinity increased, and hatching failed at 159 g/l. The life-time reproductive potential of individual females decreased linearly over the seven salinities tested. Based on this study, we predict a decrease in the productivity of the *A. monica* population in Mono Lake and extinction of the species is probable before the lake reaches equilibrium.

Introduction

Salinity fluctuations caused by human activities are common in aquatic systems. In the American Great Basin of the western United States major transbasin water diversions and other alterations have caused salinity increases in Mono Lake, California (Mason 1967; Winkler 1977), Pyramid Lake, Nevada (Galat and Robinson 1983), Walker Lake, Nevada (Koch et al. 1977), Great Salt Lake, Utah (Whelan 1973) and Owens Lake, California. Reduction in species richness, (Bayly 1972; Koch et al. 1977; Por 1980; Rushford and Felix 1982), decreases in productivity and loss of higher trophic levels (Por 1980) have all been observed in lakes subjected to increasing salinity.

As is typical of hypersaline lakes, species diversity in Mono Lake is low (Mason 1967; Melack 1983). The phytoplankton is dominated year-round by a small coccoid green alga, and *Artemia monica* is the only macrozooplankton species. The *A. monica* population serves as the major food source for the nesting California Gull (*Larus californicus*) and many migratory waterfowl (Winkler 1977). The population dynamics of *A. monica* have been described by Lenz (1980, 1984). *Artemia* has two alternative reproductive modes. In ovoviviparous reproduction, females produce a thin shelled egg which completes development and hatches in the uterus. In the alternative mode, females produce a diapause cyst, which must undergo a dormant period before development resumes and hatching occurs (D'Agostino 1980). The success of the *Artemia* population in Mono Lake is dependent on the production and the hatching of overwintering eggs. These diapause eggs hatch in early spring, and in May the first adult *Artemia* are observed. For approximately one month *Artemia* females bear live young, and starting in June females switch to oviparous reproduction. The diapause eggs lie dormant on the bottom of the lake until the following spring.

Water exports from the Mono Lake basin have caused a decline in lake level and a salinity increase from ca. 48 to 93 g/l between 1941 and 1982. The lake will reach a dynamic equilibrium in the middle of the next century and salinities will fluctuate between 169 and 248 g/l, assuming diversions of 100,000 acre feet per year and a climate similar to 1937–1983 (Vorster 1985).

Currently there are public and governmental concerns over the productivity of *A. monica* as the lake salinity continues to increase. *Artemia* occupies a key position in Mono Lake's ecosystem (Mason 1967; Winkler 1977; Cooper et al. 1984; Lenz 1984), and reduced productivity or extinction of this species would have a major impact on other trophic levels. Laboratory studies can be predictive tools which provide insight into the potential responses of an organism to environmental changes. We investigated in a long-term experiment the effects of salinity on survival, growth, reproduction and hatching in *A. monica*.

Materials and methods

Mono Lake *Artemia* nauplii were raised in seven salinities of Mono Lake water (Table 1). Salinity treatments ranged from 76 g/l to 179 g/l, which was expected to produce from high to low survival and reproductive output. Salinities were chosen to include 88 g/l Mono Lake water (MLW) (1983 lake level) as the control and 159 g/l MLW which was expected to result in a 50% adult mortality (Herbst and Dana 1980). Total dissolved solids (TDS) were determined gravimetrically (APHA 1980) using 5 ml aliquots of water. Salinities given in g/l refer to the TDS of the water throughout this paper. PH of each salinity was measured

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Table 1. Total dissolved solids (TDS), % Mono Lake water (MLW) and pH of salinities used in bioassay

Treatment #	TDS	% MLW	pH
1	76 g/l	86%	9.8
2[a]	88	100	9.8
3	97	110	9.8
4	118	134	9.8
5	133	151	9.8
6	159	181	9.7
7	179	203	9.7

[a] 100% Mono Lake water collected from depth of 8 m in July 1983

using a Markson pH meter (model 95), with a high salinity probe and corrected for temperature.

All containers used in the bioassay were leached and acid washed prior to their use. The desired salinities were obtained by collecting water from Mono Lake from 8 m depth and evaporating it outdoors in shallow plastic pans under direct sunlight. After concentration water was filtered through a 0.45 μm Gelman minicapsule filter.

General culture technique

Cysts were provided by L. Drinkwater, who on September 19, 1982 collected females from Mono Lake and held them in the lab until their cysts dropped. These were collected, sieved and stored in Mono Lake water (1982 MLW, ca. 93 g/l) under nitrogen gas at 4° C to simulate the normal diapause period of three months (Dana 1981). Cysts were hatched at 16° C in 42 g/l MLW, which has been found to produce an 88% hatch in five days. Reduced salinity also synchronizes hatching such that a three-day incubation produces large numbers of nauplii in the same developmental stage (instar 1).

Newly hatched shrimp (instar 1) were transferred to four dram shell vials (21 mm × 70 mm) containing 5 ml of the test medium, three nauplii per vial, and cultured at 20° C. Brine shrimp nauplii can survive sudden shifts in salinity, and the first instar may be transferred from 5 g/l to 150 g/l salinity without any harm (D'Agostino and Provasoli 1968; Sorgeloos 1980). For each salinity there were three replicates of 20 vials each, totalling 60 shrimp per replicate. Vials in each replicate were placed together in small containers and covered to reduce evaporation.

Shrimp were transferred to fresh media once per week. APHA (1980) guidelines for bioassays specify that changes in the toxicant should be no more than ±10%. The average increase in TDS between water changes for all treatments was 10.6% (range, 5.6 to 15.1%) with the highest values occurring in the lowest salinities. Better control of salinity change was obtained during the hatching experiments with an average salinity increase of 4.5% (range 0 to 10.5%) over the 14 day hatching period, with the greatest changes occurring in the lower salinities. Ammonium levels, measured on one occasion, were found to be variable (range 4.8 to 48.5 μM) with the lowest concentrations at the highest salinities.

Shrimp were fed daily on a food mixture developed by Aquafauna, Inc. specifically for culture of brine shrimp, which contained 79.7% rice brain, 13.7% whey and 6.6% *Spirulina*. From the nauplius to the juvenile stage, shrimp were fed 2×10^{-4} g food/shrimp/day; this was doubled for the adults.

Survival and growth

Survival was monitored daily for the first seven days, after which it was checked every two days until sexual maturity was reached. Dead shrimp were removed during each observation period. At the beginning of the experiment, 55 newly hatched 1st instar nauplii were randomly picked and measured. Subsequent length measurements on ten randomly chosen shrimp/replicate were made twice weekly. Measurements were made on animals lightly narcotized with chloroform, a technique used by Lochhead and Lochhead (1941) and Gilchrist (1960). Three drops of chloroform in 10 ml of media cause the animal to be motionless for a few minutes while the heart continues to beat and recovery is complete within 10 min (Gilchrist 1960). Once sexual maturity was attained, animals were photographed and measured from negatives, since the effect of chloroform on brine shrimp reproduction is unknown. Ten females and ten males per replicate were measured at seven to ten-day intervals.

Reproduction

Females and males were paired and cultured as described in the general culture section. There were 15 to 20 pairs/replicate and three replicates/treatment, except in salinity treatment 6 in which each replicate had six to ten pairs (due to high mortality prior to sexual maturity). Since no females survived to sexual maturity in treatment 7, reproductive studies could not be done at this salinity. Male shrimp which died during the experiment were replaced if another male from the same salinity was available. Otherwise, the female of that pair was removed from the experiment.

Individual cyst broods were tabulated and then pooled by brood number and replicate for each salinity. Throughout the duration of the reproductive phase the collected cysts were kept at 20° C in the test salinity and covered with Parafilm. Once all broods had been collected, nitrogen gas was bubbled into each vial for five minutes to purge oxygen. The vials were then capped and stored at 5° C for four months (116 days) to simulate the diapause period the cysts experience in Mono Lake (Dana 1981). A small amount of salt precipitation was noted in treatment 6 during the cold dormant period.

Hatching

Cysts were filtered onto Whatman #1 filters, rinsed in a distilled water bath for 30 seconds, then filtered onto a gridded Whatman #1 filter paper. The cysts and filter paper were placed on small glass beads in a 37 mm diameter plastic petri dish (with cover). The test salinity was pipetted into the petri dish to the top of the beads so as to just moisten the filter paper. Petri dishes were placed in 150 mm Nalgene desiccators which contained a reservoir of the test salinity thus minimizing evaporation and changes in salinity. This technique was developed by L. Drinkwater, Univ. California, Davis. All treatments were incubated at 15° C and consisted of three replicates with 150 to 200 cysts/replicate and were observed every three days for 14 days.

Experiments were run on cysts which were pooled from

the second and third broods from each salinity. Hatching success for different broods was tested on broods one through four for treatment 4. There was no significant difference among broods in hatching success (ANOVA, $P > 0.05$).

Two sets of hatching experiments were done. In the first, cysts were incubated in the salinity in which they were produced (source salinity). Secondly, cysts from each of the salinity treatments were incubated in a reduced salinity of 42 g/l to test for egg viability. We also compared hatching of the laboratory produced cysts to hatching of naturally produced cysts. In September 1983 cysts were gathered from females collected from Mono Lake. These naturally produced cysts were then subjected to the same diapause and hatching conditions as the treatment 2 (88 g/l) experimental cysts.

Second generation survival and growth

Cysts produced by the first generation were hatched in their respective salinities, transferred to vials and cultured as described in general culture techniques. Due to the low numbers of available cysts, only 30 nauplii per replicate were used. Second generation survival of treatments 6 and 7 could not be tested due to hatching failure in the former and high mortality in the latter. Naturally produced eggs from Mono Lake were hatched and cultured in 88 g/l MLW to compare to second generation survival in the test treatment of the same salinity. Survival was monitored every two days until day 21, at which time shrimp were preserved in 5% formalin and measured at a later date. Lengths were corrected for shrinkage due to formalin effects.

Statistical treatment

Variances were checked for homoscedasticity using Levene's test (Biomedical Computer Programs, BMDP), F-Max test, or F-test. Where variances were not significantly different, the following tests were used: ANOVA (BMDP), multiple regression (BMDP), and Student's *t*-test. Pairs of means were compared with the Bonferroni (BMDP) or *t*-test. Where homoscedasticity was not found, comparisons among treatments were conducted according to the procedure of Games and Howell or the G-test (Sokal and Rohlf 1981). Several data sets (length and brood size) were tested and found to be normal using probability graph paper, therefore normality was assumed for all remaining data sets.

The Kolmogorov-Smirnov test was employed to test differences between cumulative distribution curves (female age at first brood production). A G-test and a three-way contingency table were used to analyze frequencies in discrete categories. Except where noted, statistical tests were done according to Sokal and Rohlf (1981).

Proportions obtained in the hatching experiments were normalized with a logit transformation (Ashton 1972) prior to statistical analysis. The logit transformation was also used when analyzing proportions of first and second generation survival.

Results

First generation

Survival. *Artemia* survival for the first 26 days in treatments 1–7 is graphed in Fig. 1A. Mortality in treatments 1–5 was low and nearly constant over this time period. In treatments 6 and 7 we observed an initially high mortality, which leveled off after the sixth day. Only two males survived to sexual maturity in treatment 7 and adult female survival in treatment (as the proportion of females producing a third brood) was below 20% (see Table 3). Salinity effects on mortality were statistically significant for both juveniles (ANOVA, $P < 0.001$) and adult females (G-test, $P < 0.001$).

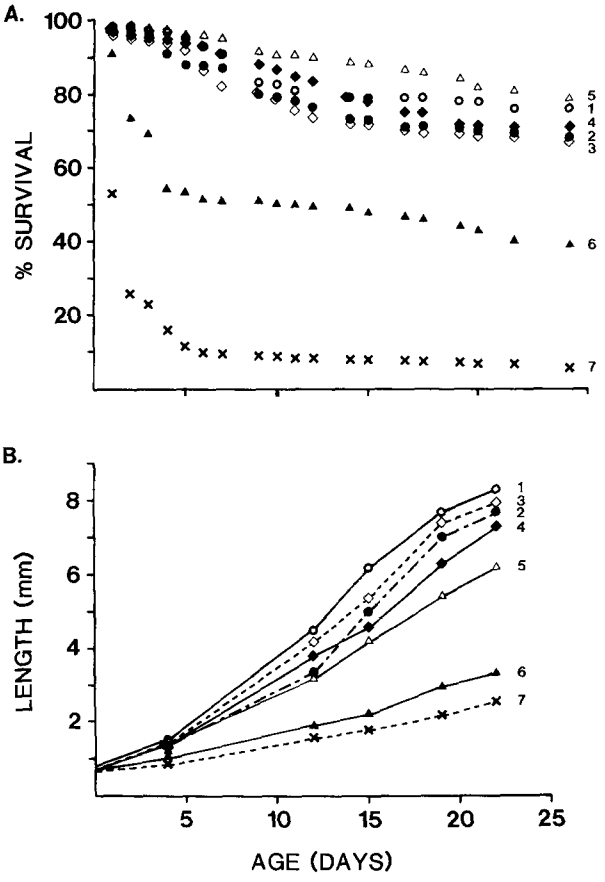

Fig. 1A, B. Mean *Artemia* survival (**A**) for the first 26 days and length (**B**) for the the first 22 days in salinity treatments 1 to 7. For length, sample size of 30 for treatments 1 to 6 on all dates. Sample size for treatment 7 for consecutive dates: 22, 16, 15, 10, 8

Growth. Growth rates from the naupliar to juvenile stage are presented in Fig. 1B. Between days 4 and 22 length increased linearly, and in general, growth rates decreased with increasing salinity. Salinity had a significant effect on growth rates over days 4–22 (ANOVA, $P < < 0.001$). Treatment 7 was not included in this analysis due to the small sample size. An ANOVA on day 22 (including treatment 7) revealed significant differences among mean lengths ($P < < 0.001$).

Differences in molting rate were also observed between treatments 6 and 7 and the lower salinities. Animals in treatments 1 through 5 had all reached the juvenile stages (instars 9–11) by day 22, whereas most animals in treatments 6 and 7 had only developed to instars 4 or 5 by this time.

The trend of decreased growth rates with increasing salinity established during the first 24 days continued during adult growth (Fig. 2). A two-way ANOVA on the slopes

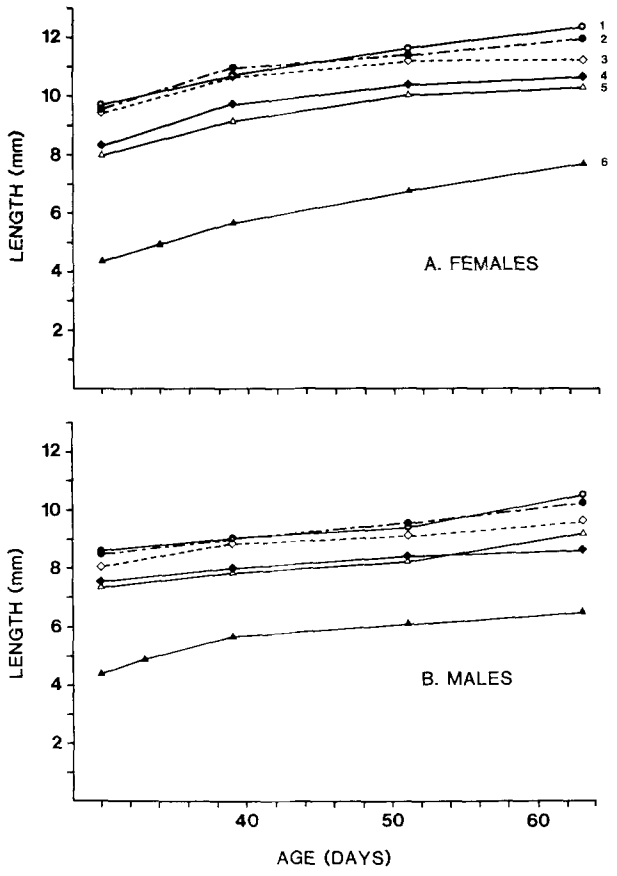

Fig. 2A, B. Mean adult female (**A**) and male (**B**) *Artemia* length for days 30 to 64 in treatments 1 to 6. Treatment 6 animals did not reach adult stage until after day 39. Treatment 7 animals not shown since only two adult males survived

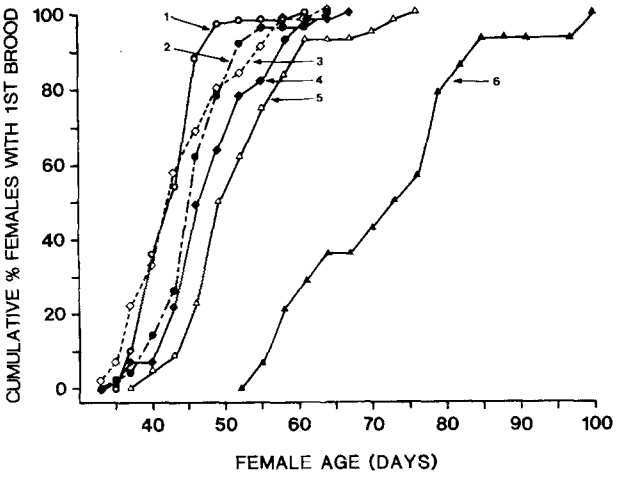

Fig. 3. Cumulative percent of females with a first brood as a function of their age for treatments 1 to 6

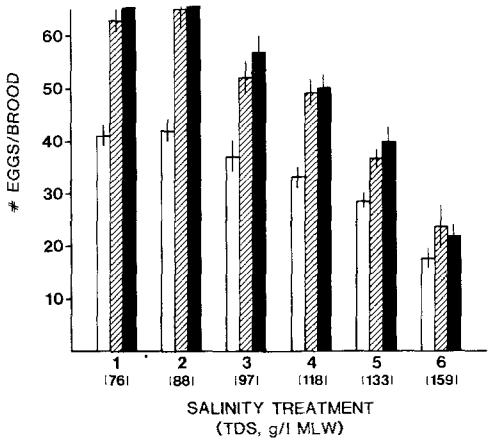

Fig. 4. Brood size in number of eggs per brood for treatments 1 to 6. Brood #1 (*open bars*), brood #2 (*cross hatched bars*) and brood #3 (*solid bars*) are shown separately for each salinity. *Vertical lines* indicate standard errors

of the growth curves in Fig. 2 (treatments 6 and 7 excluded, because of the small sample size due to high mortality), and one on mean lengths on day 63 (treatment 7 exlcuded), showed significant effects due to sex ($P << 0.001$) and salinity ($P < 0.01$) but not sex-salinity interactions. Females were always significantly larger than males (Bonferroni, $P < 0.05$), which is also true for natural populations of *Artemia* (Lenz 1984).

Reproduction. Three parameters of female reproductive output were monitored: age at first reproduction, brood size and reproductive mode. Female fertility was studied in treatments 1 through 6, since no females survived to sexual maturity in treatment 7. Salinity caused a delay in reproduction (Fig. 3). The cumulative distribution curve of treatment 6 was significantly different from all other treatments (Kolmogorov-Smirnov test, $P < 0.01$).

Brood size decreased with increasing salinity, a trend which was observed for all three broods (Fig. 4, ANOVA, $P << 0.001$). Except for treatment 6, brood #1 was always smaller than broods #2 and #3, which were of about equal size (ANOVA, $P < 0.01$). Brood size did not appear to differ between naupliar and cyst brood types.

Reproductive mode was dependent on both brood number and salinity. Three groupings emerged for the frequency of ovoviviparity among reproducing females (Table 2, G-test, $P < 0.05$): low for treatments 1–3 (<3%), moderate for treatments 4 and 5 (10.5 and 11.5%) and high for treatment 6 (30.8%). Incidence of ovoviviparity decreased with brood number for a given salinity and increased with salinity for a given brood number (three-way contingency table, $P < 0.01$, Table 2).

Hatching

Total hatch, cyst viability, and timing of hatch were affected by salinity. In Fig. 5 we present the percent hatch on day 14 for eggs incubated in their source salinity and in a reduced salinity (42 g/l). In the source salinity, cyst hatch decreased with increasing salinity. Mean percent hatch on day 14 was significantly affected by treatment (ANOVA, $P << 0.001$). Treatment 6 was not included in this analysis since no hatching was observed. Cyst viability was ascertained by reducing the incubation salinity to 42 g/l. Hatching in treatments 1–4 remained unaltered, however we observed a significant increase in the percent hatch in treatments 5 (*t*-test, $P < 0.01$) and 6 (Fig. 5).

The timing of hatching differed in the two incubation treatments. Cumulative hatching curves for treatments 1–5 were virtually identical for eggs incubated in the reduced salinity (Fig. 6A). In contrast, hatching curves of eggs incubated in their source salinity were delayed at higher salini-

Table 2. Reproductive Outcome, incidence of ovoviviparity

Treatment	Brood #	*N*	Percent	Percent pooled
1	1	56	1.8	
	2	53	3.8	2.7
	3	41	2.4	
2	1	47	8.5	
	2	48	0.0	2.9
	3	42	0.0	
3	1	43	4.6	
	2	37	2.7	2.7
	3	30	0.0	
4	1	41	26.8	
	2	33	3.0	11.5
	3	30	0.0	
5	1	41	22.0	
	2	38	2.6	10.5
	3	26	3.9	
6	1	13	38.5	
	2	9	11.1	30.8
	3	4	50.0	

G-test, run on pooled data (over broods 1, 2 and 3), indicates three groupings each significantly different from each other ($P<0.05$): treatments 1–3, treatments 4–5, and treatment 6. Within each group frequency of ovoviviparous broods not significantly different from each other

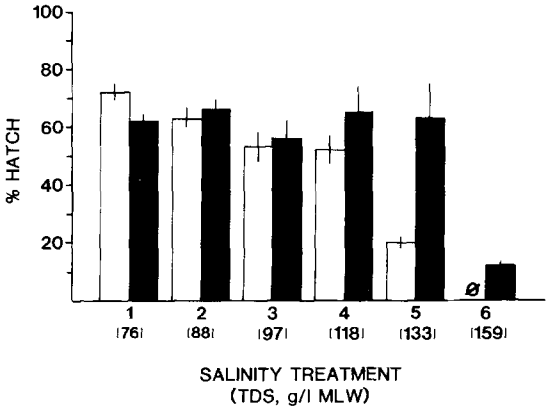

Fig. 5. Total percent hatch of cysts incubated for 14 days in source salinity (*open bars*) and in reduced salinity of 42 g/l (*solid bars*). *Vertical lines* indicate standard errors

ties (Fig. 6B). There was no effect of salinity on mean hatch on day three among treatments incubated in the reduced salinity. However, salinity had a significant effect on hatching for cysts incubated in the source salinity (*t*-test, $P<0.05$).

Hatching in naturally produced eggs was not significantly different from treatment 2. Both wild type and treatment 2 eggs were kept at 88 g/l, for both dormancy and incubation. Eggs produced in this experiment retained a similar hatching potential as naturally produced eggs.

Second generation

Survivorship and growth in the second generation was monitored for treatments 1 through 5. Treatments 6 and 7 were excluded because there was no second generation: in treatment 7 no first generation females reached maturity, and in treatment 6 cysts did not hatch in the source salinity.

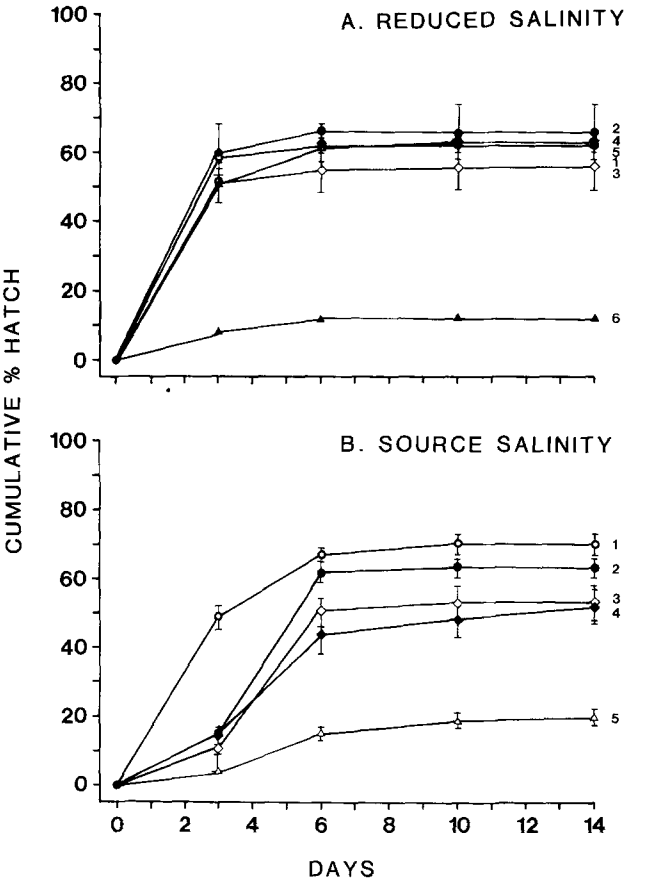

Fig. 6A, B. Cumulative percent hatch of cysts in reduced salinity (42 g/l, **A**) and in source salinity (**B**) during a 14 day incubation. *Vertical lines* indicate standard errors

A comparison of first and second generation survival and length is presented in Fig. 7A, B. Salinity had a significant effect on second generation survival (days 14 and 21, G-test, $P<0.001$). Survival of first and second generation treatment pairs (eg. first generation treatment 1 vs second generation treatment 1) were similar. Survival of wild type animals in 88 g/l was higher than in second generation animals raised in the same concentration (treatment 2, G-test, $P<0.001$), suggesting that we may have had some lab effects.

As in the first generation, growth rates decreased with increasing salinity. Salinity was found to have a significant effect on length (day 22, Games and Howell, $P<0.05$). Paired comparisons of lengths showed that first generation lengths were always significantly greater than second generation lengths (*t*-test, $P<0.01$, Fig. 7B). Mean length of animals from naturally produced eggs was greater than second generation animals raised in the same salinity, 88 g/l (*t*-test, $P<<0.001$).

Discussion

The impact of environmental stress on an organism depends on the frequency and extent of the disturbance and the compensatory abilities of the organism. Although compensatory mechanisms may allow the organism to survive, sublethal stress can still have detrimental consequences. The

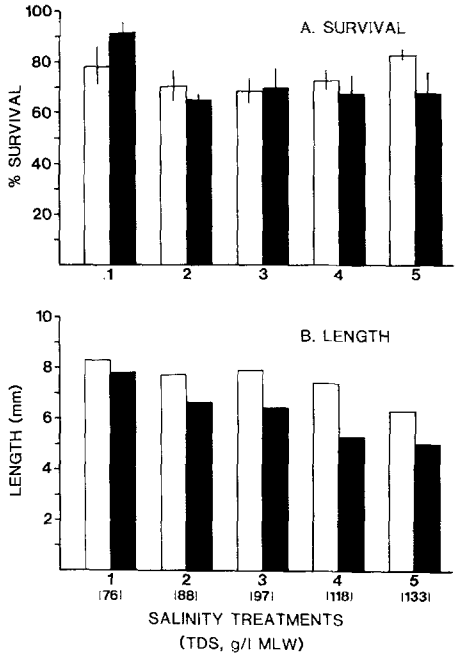

Fig. 7A, B. First generation (*open bars*) and second generation (*solid bars*) survival (**A**) and length (**B**) at 22 days. *Vertical lines* indicate standard errors. No error bars are shown for length, because the standard error was less than 0.1 mm

summation of sublethal responses results in reduced survival and reproductive potentials in the individual and consequently a change in population structure. In this experiment we observed sublethal and lethal salinity effects in *A. monica*. No second generation was produced in salinities above 133 g/l due to high mortality and hatching failure. Delays in reproduction and reduction in brood size occurred with increasing salinity.

Artemia has been the subject of numerous salinity studies, almost all of them short-term and partial life cycle (eg: Croghan 1958a, b; D'Agostino and Provosoli 1968; Engel and Angelovic 1968). Most of these studies were limited to the San Francisco or Great Salt Lake brine shrimp populations (*A. franciscana*) and to salinities lower than those tested here. Recent work by Bowen et al. (1985) has shown that the many *Artemia* populations have different physiological tolerances to specific ions and ionic ratios. Therefore, comparisons between *A. monica* and other *Artemia* populations must be made cautiously because response to increasing salinity may depend on the individual population and ionic constituents of the salinity tested. Mono Lake, a triple water lake, is rich in chloride, carbonate and sulfate, which is unusual for *Artemia* habitats (Cole and Brown 1967).

Artemia has been observed in crystallizing brines in natural environments (Mitchell and Geddes 1977, Dana 1981), however, these shrimp usually represent senescent populations. In laboratory bioassays Croghan (1958a) found a salt tolerance limit of 300 g/l for adult brine shrimp from the Great Salt Lake, Utah. Nauplii from this same population could only tolerate 2.5–3.0 M NaCl (146–175 g/l) (Conte et al. 1972, 1973). The lethal salt tolerance limit found here (159–179 g/l) for subadult stages is similar to Conte's values for nauplii, but lower than both Croghan's values and earlier reports for adult *A. monica* (184 g/l, Herbst and Dana 1980). Subadults and nauplii appear to be less euryhaline than adults. Vanhaecke et al. (1984) found decreased survival of subadults between salinities of $70^0/_{00}$ and $120^0/_{00}$ for several *Artemia* strains, although survival at a given salinity was dependent on the temperature.

While *Artemia* can maintain osmotic homeostasis in elevated salinities, increased energy requirements may affect other functions such as growth and reproduction. Adult size and growth rates of *Artemia* in our study were inversely proportional to salinity, in accord with other studies (Bond 1933, Gilchrist 1960). Reeve (1963) found a maximum in growth rate of young Great Salt Lake *Artemia* at seawater concentrations (35 g/l). This agrees well with our findings of increased growth rates with lowered salinities and it suggests that *A. monica* may already be growing non-optimally at the lowest salinity tested (76 g/l) in our experiments.

The significance of a slower growth rate is seen in the subsequent delays in reproduction (Fig. 3). These delays would probably be even more pronounced at spring temperatures of 5° to 10° C when growth rates in Mono Lake are very low (Dana and Lenz 1982). Timing of *Artemia* reproduction in the Mono Lake ecosystem may be an important determinant not only for its own seasonal population dynamics, but also for bird populations which utilize the shrimp as a food source during breeding and migration (Winkler 1977).

Other reproductive characteristics affected by salinity included brood size, mode of reproduction, and female mortality during reproduction. Brood size was inversely related to salinity (Fig. 4) and mortality of adult females during reproduction increased with salinity (Table 3). Further evidence of salinity related stress was the significantly greater proportion of females producing nauplii in the higher salinities (Table 2). Browne (1980) surmises that cyst production is more costly than nauplius production based on his demonstration that cyst broods were smaller than naupliar broods, and on the work of Clegg (1962) and Von Hentig (1971) which showed that 22% of the dry mass of the cyst is utilized for encapsulation. The increased proportion of naupliar broods and smaller brood size produced at higher salinities may then reflect a reduction in the amount of energy available for reproduction, to compensate for the increased osmotic work. As the salinity increases in Mono Lake one would predict that, other factors being equal, proportionately fewer cysts will be produced, resulting in a reduced cyst pool for future years.

Salinity had a large effect on hatching of dormant cysts, with low hatching observed in 133 g/l MLW and no hatching at 159 g/l MLW (Fig. 5). These limits are higher than those found for other *Artemia* populations where salinities above 75 ppt. seawater caused decreases and delays in hatching (Royan 1975, Ivanovskii et al. 1980). Before cysts can initiate metabolic activity for emergence, they have to attain a critical level of intracellular hydration (Clegg 1974, 1976). The critical hydration level found by Clegg for San Francisco cysts was 1.25 M NaCl solution (ca. 73 g/l). Below this hydration level metabolism and emergence did not occur. A range of salinity hatching thresholds have been reported by Collins (1977), from 0.45 M NaCl for the Jesse Lake, Nebraska population up to 1.7 M NaCl for the Great Salt Lake population. Although the critical hydration level appears to be population dependent, salinity thresholds for most populations are less than 1.25 M NaCl.

A. monica may utilize different physiological and biochemical mechanisms for the hatching process than other

Table 3. Parameters used to determine reproductive potential of first generation females. Reproductive potential calculated over three broods before (RP) and after (RPH) hatching factored in

Treatment	TDS (g/l)	Brood #	Reproductive Parameters				Reproductive RP(5)	Potential RPH(6)
			(1)	(2)	(3)	(4)		
1	76	1		0.983	41.9			
		2	0.761	0.947	63.2	0.702	114.0	80.0
		3		0.714	68.2			
2	88	1		0.870	41.7			
		2	0.678	0.879	64.8	0.627	97.8	61.3
		3		0.778	65.6			
3	97	1		0.933	37.0			
		2	0.667	0.837	52.8	0.532	81.6	43.4
		3		0.769	56.7			
4	118	1		0.688	33.2			
		2	0.706	0.586	49.4	0.518	54.6	28.4
		3		0.508	50.3			
5	133	1		0.723	28.5			
		2	0.794	0.673	36.5	0.202	52.0	10.5
		3		0.509	39.8			
6	159	1		0.625	19.4			
		2	0.294	0.550	24.1	0	8.7	0
		3		0.176	24.0			
7	179	1		0	0			
		2	0.017	0	0	0	0	0
		3		0	0			

1: proportion surviving to juvenile stage
2: proportion females producing cyst brood
3: mean #cysts/brood
4: mean proportion of cysts hatched
5: RP = mean # offspring produced/female, over three broods; before hatching
$= \#1 \times ((\#2 \times \#3) + (\#2 \times \#3) + (\#2 \times \#3))$
brood 1 brood 2 brood 3
6: RPH = mean # offspring produced/female, over three broods; after hatching
$= RP \times \#4$

Artemia populations, since they have aberrant dormancy characteristics: instead of the usual dehydration-rehydration requirements of most *Artemia* populations, *A. monica* produces cysts which sink and undergo an obligate cold dormancy in the hydrated state (Dana 1981).

Delays in hatching observed in higher salinities (Fig. 6B) may also be related to the hydration level of the cyst. Clegg (1964) demonstrated that the delays at higher osmotic pressures were due to a decrease in the effective concentration of water in the environment. Conte et al. (1977) confirmed Clegg's work and concluded that development of the cyst is dependent upon the state and rate of hydration, and can be manipulated by the external salinity.

While compensatory mechanisms may allow an organism to counteract a given stress at one stage of its development, later ontogenetic stages may suffer a decrease in productivity or even death. As Rosenthal and Alderice (1976) state, "Thus, the organism trades certainty of death at one stage for a lower probability of survival at a later stage". In some cases, the response to stress in our experiment is clear: low survival, delayed and reduced reproduction and hatching failure above 133 g/l MLW. The more subtle responses of stress are not always readily visible and the compounding effects throughout the life cycle must be taken into account. Female reproductive potential (over three broods) was calculated for each salinity level based on measured rate of juvenile survival, female mortality during each brood interval, brood size, and hatching success. Reproductive potential decreases linearly as salinity increases (Fig. 8, Table 3). The lack of a "plateau" at the lower salinities suggests that even the lowest salinities tested in this bioassay are not optimal and represent some stress. In higher salinities the compounding effects are substantial, resulting in low reproductive potential and hence reduced survival potential of the population. These calculations, of course, do not take into account additional factors that may be present in the natural environment, but they indicate how salinity stress is translated through the life cycle.

Water diversions at Mono Lake have occurred since 1941 and if continued, will bring the lake to a dynamic equilibrium with salinity fluctuating between 169 and 248 g/l (Vorster 1985). Our salinity experiments can be used cautiously to predict the response of *A. monica* at increased salinities. This study does not account for possible long-term adaptations, which might increase salinity tolerance. Neither does it consider other factors which are likely to change concurrently as the lake's salinity increases.

Nevertheless, it is clear that the continued existence of *A. monica* is dependent on the most salinity sensitive stages in its life cycle: dormancy and hatching. The constraints of hydration levels in the cysts at elevated salinities discussed earlier will inevitably cause a decrease and eventually

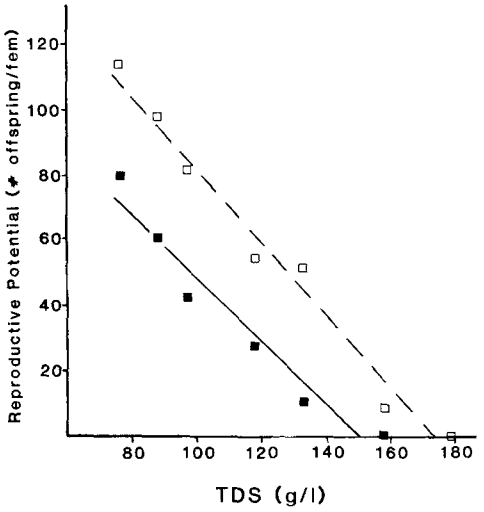

Fig. 8. Reproductive potential of first generation females over three broods before hatching success (RP *open squares*) and after hatching success (RPH *closed squares*) taken into account. See Table 3 for calculations of RP and RPH. Lines determined from linear regression

a total loss of viability of the cysts. Hatching from the encysted egg is a critical stage in the life cycle because it is the seed for the *Artemia* population for the entire year. Our experiments predict a loss of hatching of the cysts at a salinity between 130 and 160 g/l. Based on these salinity considerations alone extinction of the species is highly probable above 133 g/l, well below the salinities projected for Mono Lake when it reaches equilibrium.

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References

American Public Health Association (1980) Standard methods for the examination of water and wastewater. 2nd edition. Washington D.C.

Ashton WD (1972) The logit transformation. Hafner Publishing CO., New York

Bayly IAE (1972) Salinity tolerance and osmotic behavior of animals in athalassic saline and marine hypersaline waters. Ann Rev Ecol Syst 3:233–269

Bond RM (1933) Observations on *Artemia franciscana* Kellogg especially on the relation of environment to morphology. Int Rev Hydrobiol 28:117–125

Bowen ST, Fogarino EA, Hitchner KN, Dana GL, Chow VHS, Buoncristiani MR, Carl JR (1985) Ecological isolation in *Artemia:* population differences in tolerance of anion concentrations. J Crustacean Biol 5:106–129

Browne RA (1980) Reproductive pattern and mode in the brine shrimp. Ecology 61:466–470

Clegg JS (1962) Free glycerol in dormant cysts of the brine shrimp *Artemia salina*, and its disappearance during development. Biol Bull 123:295–301

Clegg JS (1964) The control of emergence and metabolism by external osmotic pressure and the role of free glycerol in developing cysts of *Artemia salina.* J Exp Biol 41:879–892

Clegg JS (1974) Interrelationships between water and metabolism in *Artemia salina* cysts: hydration-dehydration from the liquid and vapour phases. J Exp Biol 61:291–308

Clegg JS (1976) Interrelationships between water and metabolism in *Artemia* cysts II. Carbohydrates. Comp Biochem Physiol 53A:83–87

Cole GA, Brown RJ (1967) The chemistry of *Artemia* habitats. Ecology 48:858–861

Collins NC (1977) Ecological studies of terminal lakes: Their relevance to problems in limnology and population biology. In: Greer DC (ed) Desertic terminal lakes, Proceedings. Utah Water Research Laboatory, Utah, pp 411–420

Conte FP, Droukas PC, Ewing RD (1977) Development of sodium regulation and de novo synthesis of Na+K-activated ATPase in larval brine shrimp, *Artemia salina.* J Exp Zool 202:339–362

Conte FP, Hootman SR, Harris PJ (1972) Neck organ of *Artemia salina* nauplii. A larval salt gland. J Comp Physiol 80:239–246

Conte FP, Peterson GL, Ewing RD (1973) Larval salt gland of *Artemia salina* nauplii. Regulation of protein synthesis by environmental salinity. J Comp Physiol 82:277–289

Cooper SD, Winkler DW, Lenz PH (1984) The effect of grebe predation on a brine shrimp population. J Anim Ecol 53:51–64

Croghan PC (1958a) The survival of *Artemia salina* (L.) in various media. J Exp Biol 35:213–218

Croghan PC (1958b) The osmotic and ionic regulation of *Artemia salina* (L.). J Exp Biol 35:219–233

D'Agostino A (1980) The vital requirements of *Artemia:* physiology and nutrition. In: Persoone G, Sorgeloos P, Roels O, Jaspers E (editors) The brine shrimp *Artemia*, vol 2. Physiology, biochemistry, molecular biology. Universa Press, Wetteren, Belgium, pp 55–82

D'Agostino AS, Provasoli L (1968) Effects of salinity and nutrients on mono- and diaxenic cultures of two strains of *Artemia salina.* Biol Bull 134:1–14

Dana GL (1981) Comparative population ecology of the brine shrimp *Artemia.* Masters thesis, San Francisco State University

Dana GL, Lenz PH (1982) Spring hatching dynamics of *Artemia* in Mono Lake, California. Unpublished report

Engel DW, Angelovic JW (1968) The influence of salinity and temperature upon the respiration of brine shrimp nauplii. Comp Biochem Physiol 26:749–752

Galat DL, Robinson R (1983) Predicted effects of increasing salinity on the crustacean zooplankton community of Pyramid Lake, Nevada. Hydrobiol 105:115–131

Gilchrist BM (1960) Growth and form of the brine shrimp *Artemia* (L.). Proc Zool Soc London 134:221–235

Hentig R von (1971) Einfluß von Salzgehalt und Temperatur auf Entwicklung, Wachstum, Fortpflanzung und Energiebilanz von *Artemia salina.* Mar Biol 9:145–182

Herbst DB, Dana GL (1980) Environmental physiology of salt tolerance in an alkaline salt lake population of *Artemia* from Mono Lake, California, U.S.A. In: Persoone G, Sorgeloos P, Roels O, Jaspers E (editors) The brine shrimp *Artemia* , vol 2. Physiology, biochemistry, molecular biology. Universa Press, Wetteren, Belgium, pp 157–167

Ivanovskii YA, Mitrofanov YA, Chaga IL (1980) Hatching of *Artemia salina* at different salinity and temperature regimes. Sov J Mar Biol 6:202–207

Koch DL, Mahoney JL, Spencer JJ, Jacobson RL (1977) Limnology of Walker Lake. In: Greer DC (ed) Desertic terminal lakes, Proceedings. Utah Water Research Laboratory, Utah, pp 355–369

Lenz PH (1980) Ecology of an alkali-adapted variety of *Artemia* from Mono Lake, California, U.S.A. In: Persoone G, Sorgeloos P, Roels O, Jaspers E (editors) The brine shrimp *Artemia,* vol 3. Ecology, culturing, use in aquaculture. Universa, Press, Wettern, Belgium, pp 79–96

Lenz PH (1984) Life-history analysis of an *Artemia* population in a changing environment. J Plankton Research 6:967–983

Lochhead JH, Lochhead MS (1941) Studies on the blood and related tissues in *Artemia* (Crustacea Anostraca). J Morphol 68:593–632

Mason DT (1967) Limnology of Mono Lake, California. Univer Calif Publ Zool 83

Melack JM (1983) Large, deep salt lakes: a comparative limnological analysis. Hydrobiologia 105:223–230

Mitchell BD, Geddes MC (1977) Distribution of the brine shrimps *Parartemia zietziana* Sayce and *Artemia salina* (L.) along a salinity and oxygen gradient in a South Australian saltfield. Freshwater Biol 7:461–467

Por FD (1980) A classification of hypersaline waters based on trophic criteria. Mar Ecol 1:121–131

Reeve MR (1963) Growth efficiency in *Artemia* under laboratory conditions. Biol Bull 125:133–145

Rosenthal H, Alderice DF (1976) Sublethal effects of environmental stressors, natural and pollutional on marine fish eggs and larvae. J Fish Res Bd Can 33:2047–2065

Royan JP (1975) Influence of salinity and temperature on the hatching of brine shrimp, *Artemia salina* cysts. Mahasagar 8:183–185

Rushford SR, Felix EA (1982) Biotic adjustments to changing salinities in the Great Salt Lake, Utah, USA. Microbiol Ecol 8:157–161

Sokal RR, Rohlf RJ (1981) Biometry. 2nd edition. WH Freeman and CO., San Francisco

Sorgeloos P (1980) The use of the brine shrimp *Artemia* in aquaculture. In: Persoone G, Sorgeloos P, Roels O, Jaspers E (editors) The brine shrimp *Artemia*, vol 3. Ecology, culturing, use in aquaculture. Universa Press, Wetteren, pp 25–46

Vanhaecke P, Siddall SE, Sorgeloos P (1984) International study on *Artemia*. XXXII. Combined effects of temperature and salinity on the survival of *Artemia* of various geographical origin. J Exp Mar Biol Ecol 80:259–275

Vorster PT (1985) A water balance forecast model for Mono Lake, California. Masters thesis, California State University, Hayward, California, USA

Whelan JA (1973) Great Salt Lake, Utah: chemical and physical variations of the brine, 1966–1972. Utah Geologic and Mineral Survey. Water Resources Bull 17

Winkler DW (1977) (ed) An ecological study of Mono Lake, California. Institute of Ecology, University of California, Davis, Publ No 12

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