Combined effects of salinity and temperature on survival and reproduction of five species of *Artemia*

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Abstract

Survival and reproductive performance were measured at nine temperature–salinity (T–S) combinations (15°C, 24°C and 30°C/60 ppt, 120 ppt and 180 ppt) for four sexual (*Artemia franciscana*, *A. salina*, *A. sinica* and *A. persimilis*) and one parthenogenetic (*A. parthenogenetica*) species of brine shrimp. There was significant interaction between temperature and salinity for survival and reproductive traits. For most species there was disconcordance among seven performance estimators (LT_{50}, 21-day survival, r, and four female reproductive traits) for the optimal T–S combination. We propose that the best ecological estimator of reproductive success is cohort reproductive output because it incorporates both survival (l_x) and reproductive (m_x) functions. All species had maximum reproduction at 24°C; at 120 ppt for *A. parthenogenetica*, *A. sinica* and *A. franciscana*, and at 180 ppt for *A. salina* and *A. persimilis*. There was only one T–S combination (24°C/120 ppt) where all species completed their life cycle. While at least one *Artemia* species reproduced at eight of the nine possible T–S combinations, sustainable reproduction (where $R_o \geq 1$) occurred at only five T–S combinations. *A. parthenogenetica* had the narrowest tolerance range of T–S combinations, contradicting the general purpose genotype hypothesis advanced for obligately parthenogenetic species.   © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Temperature and salinity are among the most important physical factors in the life of marine and brackish water organisms. There is often a complex co-relationship between

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the two factors, where temperature can modify the effects of salinity, thereby changing the salinity tolerance range of an organism, and salinity can modify the effects of temperature accordingly (e.g. Kinne, 1963; Williams and Geddes, 1991).

The present study focuses on the combined effects of temperature and salinity on survival and reproduction of one parthenogenetic and four sexual *Artemia* species. The existence of sexual and obligately parthenogenetic species (the latter with diploid and polyploid populations), which can reproduce oviparously or viviparously, offers the opportunity for empirical investigations of lifespan and reproductive theory (Browne, 1980a,b, 1983, 1992; Zhang and Lefcort, 1991; Zhang and King, 1993; Barata et al., 1996a,b). *Artemia* (brine shrimp) inhabit hypersaline lakes and ponds, which differ in water chemistry, seasonality, species composition, and productivity (Lenz and Browne, 1991). In North and South America, Australia, and Oceania, only sexually reproducing populations have been found, but in the Old World, especially the Mediterranean Basin, both sexual and parthenogenetic monospecific populations occur, although a few populations are mixed (Browne and MacDonald, 1982; Barata et al., 1995). Seven species are currently recognized: *A. salina* (= *A. tunisisana*) (Europe and North Africa); *A. franciscana* (New World and Oceania); *A. monica* (Mono Lake, USA); *A. persimilis* (Argentina); *A. urmiana* (Lake Urmia, Iran); *A. sinica* (Asia); *A. parthenogenetica* (widely distributed throughout the Old World). Additional information on population locations and biogeography is available in Triantaphyllidis et al. (1998).

Artemia have been widely used as model organisms for biochemical, physiological, genetic and ecological studies with more than 5000 published papers (McCourt and Lavens, 1985). The differences in genetic constitution among *Artemia* species (Browne and Bowen, 1991; Pilla and Beardmore, 1994), as well as among populations within a species (Browne and Hoopes, 1990), enhance the probability for physiological variation among strains (Persoone and Sorgeloos, 1980; Vanhaecke et al., 1984).

Numerous studies (see Browne et al., 1988, 1991; Barata et al., 1996a and references therein) have been conducted on the effects of temperature on survival, life span and reproductive characteristics of *Artemia* populations. Studies of similar design (e.g. Triantaphyllidis et al., 1995) have estimated the effects of salinity on life-history traits. Such unifactorial experiments can estimate physiological tolerance of populations/ species and yield insight into competitive interactions among species (Browne and Halanych, 1989; Zhang and Lefcort, 1991; Barata et al., 1996b). Multifactorial designs usually more closely resemble the range of conditions encountered by organisms in natural habitats, but they often entail logistical and/or time constraints and the interpretation of the results can be more complicated.

The combined effects of temperature and salinity on tolerance, survival and reproduction have been investigated in *Artemia* by several researchers, albeit with significant limitations. Vanhaecke et al. (1984) examined the combined effects of temperature and salinity on the survival of 13 geographical strains encompassing four species. They noted that although salinity resistance is high, a tolerance range from 3 ppt to 300 ppt, as generalized for the genus *Artemia*, is not valid for all *Artemia* species, particularly at lower salinities. Since the experimental period was limited to nine days, their data are limited to sub-adult survival. Wear and Haslett (1986) and Wear et al. (1986) studied the effects of temperature and salinity on *A. franciscana* from Lake

Grassmere, New Zealand, and reported that more than 90% of the nauplii survived to maturity within the range 20–28°C/100–170 ppt, and that adults were more tolerant than juveniles. However, since no other species except *A. franciscana* from Lake Grassmere were tested, no direct intraspecific or interspecific comparison can be made. Vanhaecke and Sorgeloos (1989) examined *Artemia* populations to determine the effects of temperature and salinity on hatchability of cysts, resistance of larvae, and biomass production. Using two salinities (35 and 120 ppt) they found that for some populations temperature tolerance (at 25°C, 30°C, 34°C and 37°C) was clearly affected by salinity. *A. salina* and *A. persimilis* strains were the least tolerant to high temperatures, with *A. franciscana* having the highest temperature tolerance and *A. parthenogenetica* strains intermediate. However, the experimental period was again limited to nine days, well before reproductive maturity is reached in *Artemia*. Thus, previous studies of *Artemia* on the effects of temperature and/or salinity incorporated one variable, examined a single species, or were limited to prereproductive effects. The present study is the first three factorial (temperature, salinity, species) approach to life span and reproductive trait analysis. The aims of this study are: (1) to assess the combined effects of temperature and salinity on *Artemia* survival and reproduction; (2) to determine the level of concordance among measures of survival, lifespan and reproductive performance; (3) to determine which estimator is most appropriate for determining ecological success; (4) to compare laboratory performance with field data and geographical distribution.

2. Materials and methods

2.1. Artemia populations

The origins of the *Artemia* cysts used in the experiment are as follows: (1) *Artemia parthenogenetica* (diploid and tetraploid parthenogenetic) from Margherita di Savoia, Italy; (2) *A. salina* (= *A. tunisiana*) (diploid sexual) from Larnaca, Cyprus; (3) *A. sinica* (diploid sexual) from Yuncheng, Shanxi China; (4) *A. franciscana* (diploid sexual) from San Francisco Bay, USA; (5) *A. persimilis* (diploid sexual) from Hidalgo, Argentina. Thus, populations from each of the five principal *Artemia* species were tested. *A. monica* was not used in the study since it requires a specialized ionic medium (containing boron) toxic to most *Artemia* species (Lenz, 1987; Bowen et al., 1988). It and *A. urmiana* have each been reported from a single location (Triantaphyllidis et al. 1998).

2.2. Culture conditions

Solutions of different salinities (60 ppt, 120 ppt and 180 ppt) were prepared using synthetic sea water (Instant Ocean) and NaCl. Salinities were measured using hydrometers (temperature corrected). Two of the three experimental temperatures (15°C and 30°C) were conducted using constant temperature chambers. The 24°C experiment was conducted in a thermostatically controlled laboratory room. For all areas, temperature was maintained within±1°C. Jars within each treatment were randomly moved ever three days in order to minimize gradient effects.

2.3. Survival experiments

Artemia were mass-hatched from cysts in synthetic sea water and individuals were immediately transferred into jars containing 100 ml of brine at 60 ppt, 120 ppt and 180 ppt. The jars were then placed randomly at appropriate temperatures under constant fluorescent lighting. No artificial aeration was used. For each of the five species, ten jars, each containing five individuals ($n = 50$), were established for nine salinity–temperature combinations: 60 ppt, 120 ppt and 180 ppt at 15°C, 24°C and 30°C. Thus, the experimental design incorporated three variables (species, salinity and temperature) for a total of 45 treatment conditions ($5 \times 3 \times 3$), and a total n of 2250 individuals (45×50). *Artemia* were fed yeast and *Dunaliella* (a unicellular algae) alternately every other day (the maximum amount which could be filtered during the two day period).

Survival census was initiated 24 h after the hatching of the cysts. Salinity was measured every third day and corrected by adding fresh water. The number of dead and living individuals were counted on days 1, 2, 3, 5, 7, 14 and 21 in every jar. (Day intervals were selected based on a pilot study, which indicated that the highest mortality occurred in the first five days). LT_{50} was recorded for each cohort ($n = 50$) over the entire life cycle.

2.4. Lifespan and reproductive experiments

At reproductive maturity, surviving individuals used in the 21-day survival experiment were transferred to glass jars containing 250 ml of brine and algal culture. For the parthenogenetic species, one female was placed in each jar, whereas for each sexual species, a male–female pair was placed in each jar. Upon the death of a partner, males and females were again paired with a survivor from another pair raised at the same T–S combination. Approximately 20% of the culture water was removed every seven days and replaced by fresh brine. Individuals were monitored throughout their life cycle and eleven life-history traits recorded for each species: (1) Number of reproductive females; (2) Female pre-reproductive period (days); (3) Female reproductive period (days); (4) Lifespan for reproductive females (days); (5) Offspring per brood; (6) Broods per female; (7) Offspring per female; (8) Offspring per day per female; (9) Interbrood interval (days); (10) Percent of offspring encysted; (11) Male life span (days).

Cohort reproductive output was calculated for each species for each T–S combination by multiplying reproductive output per female by the number of females (from the initial cohort of 50) which survived to reproduce. Since only half of the cohort was female for the sexual species, the numbers for *A. parthenogenetica* populations were halved for comparative purposes.

Two additional ecological performance indicators were calculated. Net reproductive rate (R_o) was estimated by multiplying cohort reproductive output by the proportion of females which survived to reproduce and dividing by the initial number of females in the cohort. The resulting value is the product of survivorship (l_x) and fertility (m_x) functions. However, R_o cannot validly be compared across populations due to differences in generation times (Stearns, 1992). The intrinsic rate of increase (r) is preferred for

comparing ecological performance. It can be estimated by calculating $r = \ln R_o / T$, where $T = 1.5$ prereproductive periods (Stearns, 1992, p. 26).

2.5. Statistical analyses

Survival from 0 to 21 days for each individual was analyzed using ANOVA via SYSTAT. Data were arcsine transformed, and the assumption of homogeneity of variances was satisfied using F-max tests (Sokal and Rohlf, 1995). LT_{50} was recorded for each cohort ($n = 50$) over the entire life cycle. Since LT_{50} values are non-parametric, significance among populations was estimated via goodness of fit (Sokal and Rohlf, 1995, pp. 689–695). Interpopulation comparisons of lifespan, lifespan components and female reproductive traits (Table 2, columns 4,6–14) were determined by single-factor ANOVA if the data were normal and homoscedastic (determined by F-max tests). If the data were significantly heteroscedastic, Games-Howell approximate tests of equality of means when variances are heterogeneous were conducted. This method performs unplanned comparisons between pairs of means using a studentized range with specifically weighed average degrees of freedom and a standard error based on the averages of the means (Sokal and Rohlf, 1995, pp. 402–403). For each T–S combination, male and female lifespans were compared by Wilcoxon test. The number of reproductive females among species at 24°C/180 ppt (the only T–S combination where all species reproduced) was tested by the goodness of fit G-test, where the expected value was the average number of reproducing females for the five species.

3. Results

3.1. 21-Day survival

Table 1 lists percent mortality by day 21 at nine T–S combinations for five species of *Artemia*. ANOVA revealed that there are significant effects in survival by species, salinity and temperature, as well as significant interaction among all three variables ($p < 0.001$) for all tests (Table 2). Of the 45 species–temperature–salinity combinations tested, 100% mortality by day 21 occurred in 13 combinations. Survivorship was retested for these 13 S–T combinations and again 100% mortality occurred by day 21 for these S–T combinations. Mortality at 21 days appeared to increase with temperature, with 100% mortality occurring in 0% of the 15°C cultures, 27% of 24°C cultures and 60% of those at 30°C. However, there is no apparent linear relationship between mortality and increasing salinity, with 100% mortality occurring in 47%, 14% and 27% of the cultures at 60 ppt, 120 ppt and 180 ppt respectively. Although there was significant interaction between salinity and temperature, the data indicate that temperature has a greater effect on mortality than does salinity (over the ranges tested). The 21-day survival data indicate that there is no one optimal T–S combination for all species, with each species having a different optimum.

Table 1
Survival of five species of *Artemia*, measured as 21-day survival and LT_{50} values. Each of the nine temperature–salinity combinations had an initial cohort of 50 individuals

Temp (°C)	15			24			30		
Salinity (ppt)	60	120	180	60	120	180	60	120	180
21-Day Survival (%)									
A. parthenogenetica	68	72	54	15	24	82	0	0	0
A. salina	10	46	76	0	51	79	0	0	0
A. sinica	72	32	37	0	50	30	0	57	8
A. franciscana	80	58	74	24	73	0	15	5	0
A. persimilis	50	24	26	0	74	65	0	28	43
LT_{50} (d)									
A. partehnogenetica	29	32	25	18	14	47	4	4	3
A. salina	4	18	37	4	22	48	2	4	3
A. sinica	37	18	18	4	18	11	2	31	1
A. franciscana	25	23	39	11	26	1	11	4	1
A. persimilis	18	14	18	5	40	47	3	14	14

3.2. LT_{50} values

Another method of summarizing survivorship is via LT_{50} values calculated over the entire lifespan, rather than the 'snapshot' produced by 21-day mortality. LT_{50} values (Table 1) were found to be significantly different among the five species at nine T–S combinations ($p < 0.01$) by goodness of fit tests. When averaged for all species–salinity combinations, LT_{50} values (mean±SD) decreased from 23.7±9.8 to 21.1±16.8 to 6.7±8.0 days for 15°C, 24°C and 30°C, respectively. Thus, LT_{50} values reinforce the previous conclusion obtained from 21-day mortality that mortality increases with temperature. Optimal T–S combinations for LT_{50} and 21-day survival are concordant only for two of the five species, indicating that for most species one is not a good predictor of the other.

Table 2
Results of the three factorial Analysis of Variance performed for 21-day survival values for five *Artemia* species at nine temperature–salinity combinations

Source	*F*-ratio	Probability
Species	11.20	< 0.001
Salinity	95.69	< 0.001
Temperature	523.76	< 0.001
Species × Salinity	34.27	< 0.001
Species × Temperature	26.15	< 0.001
Salinity × Temperature	34.34	< 0.001
Species × Temperature × Salinity	21.85	< 0.001

Table 3
Comparison of lifespan components and reproductive values for five species of *Artemia* (mean±SD)

Species	Temp [°C]	Sal [ppt]	No. of rep.♀♀	Pre-Rep.♀ period (d)	Rep.♀ period (d)	♂ Lifespan (d)	Rep.♀ lifespan (d)	Offspring/brood	Broods/♀	Offspring/♀	Offspring/♀ day	Interbroodal interval	% Cysts	Cohort reprod. output	Net reprod rate (R_o)	Intrinsic rate of increase (r)
A. parthenogenetica	24	120	4/50	40.0±5.6	66.5±21.3	–	111±21.9	57.3±11.7	10.8±2.1	603±102.2	9.8±3.5	6.2±1.2	17.1±14.9	2412	3.86	0.023
	24	180	23/50	36.4 + 6.7	71.2 + 44.5	–	113 + 46.3	42.7 + 11.8	11.7 + 6.8	541.7 + 395.3	7.6 + 2.2	5.8 + 1.2	81.7 + 35.6	12459	114.62	0.087
A. salina	15	120	6/25	43.7±8.5	10.7±11.7	75.6±13.4	64.5±11.3	41.7±28.0	2.3±1.5	88.8±57.9	24.7±34.9	3.5±2.6	100±0.0	533	5.12	0.025
	15	180	2/25	60.5±3.5	1.0±0.0	69.5±9.0	66.5±5.0	23.0±7.1	1.0±0.0	23.0±7.1	23.0±7.1	1.0±0.0	100±0.0	46	0.14	− 0.022
	24	120	6/25	20.3±3.5	17.2±9.3	99.3±29.7	38.7±10.2	35.6±12.2	3.8±2.0	130.7±77.3	8.5±3.5	4.5±1.5	100±0.0	784	7.53	0.066
	24	180	14/25	29.8 + 4.6	37.6 + 20.2	132.1 + 57.0	71.1 + 22.7	30.7 + 7.2	8.5 + 3.5	259.6 + 113.8	7.6 + 2.7	4.3 + 0.7	100 + 0.0	3634	81.40	0.098
A. sinica	24	120	5/25	22.8±4.1	22.0±28.4	88.3±29.1	51.8±28.7	47.0±50.4	4.0±3.2	287.4±394.3	10.4±3.1	3.9±3.4	28.5±44.0	1437	11.50	0.071
	24	180	4/25	26.0±1.2	9.8±7.6	87.8±24.0	44.8±15.6	40.9±18.9	2.8±1.7	121.0±100.9	18.6±16.9	3.1±1.4	80.3±39.3	484	3.10	0.029
	30	120	8/25	20.4 + 4.4	15.5 + 5.5	47.6 + 6.3	40.9 + 4.4	44.7 + 18.2	3.9 + 1.2	182.3 + 99.7	12.4 + 7.5	4.2 + 1.6	22.9 + 42.5	1459	18.66	0.096
A. franciscana	15	60	2/25	56.0±9.9	15.0±8.5	71.6±10.3	87.5±5.0	21.8±8.3	2.5±0.7	57.5±36.1	3.8±0.3	5.8±1.8	0.0±0.0	115	0.37	− 0.039
	24	60	2/25	35.0±9.9	5.0±5.7	73.5±12.0	49.0±2.8	44.8±3.2	1.5±0.7	66.0±26.9	28.2±26.6	2.8±2.5	0.0±0.0	132	0.42	− 0.017
	24	120	13/25	24.2±5.6	28.2±24.7	88.9±35.2	58.2±24.2	74.6±34.9	5.4±3.8	470.5±438.5	27.5±20.5	3.9 ±2.2	22.1 ±34.1	6116	127.21	0.133
	30	120	3/25	13.3 + 2.5	2.7 + 2.9	25.3 + 4.0	18.7 + 4.6	47.1 + 27.0	1.7 + 1.2	78.7 + 61.1	39.2 + 30.2	1.3 + 0.6	0.0 + 0.0	236	1.13	0.066
A. persimilis	15	60	2/25	25.0±1.4	1.0±0.0	46.8±6.5	29.0±2.8	16.0±1.4	1.0±0.0	16.0±1.4	16.0 ±1.4	1.0±0.0	0.0±0.0	32	0.10	− 0.061
	15	180	2/25	34.0±5.7	6.0±5.7	62.8±11.5	43.0±1.4	28.1±3.4	2.5±0.7	69.0±11.3	19.1±16.1	2.2±1.7	0.0±0.0	138	0.44	0.016
	24	120	12/25	19.4±2.7	31.5±23.6	59.3±21.2	61.9±27.3	61.6 ±21.8	6.0±4.3	426.5±350.2	17.5±12.1	5.7±6.4	91.9±14.9	5118	98.27	0.157
	24	180	10/25	26.4±15.4	57.0±26.3	101.9±21.3	95.6±21.9	48.3±11.8	9.6±3.4	481.8±216.9	8.8±3.7	6.0±1.6	93.0±12.6	4818	77.09	0.110
	30	120	5/25	18.4±7.3	10.0±5.3	43.5±7.4	35.0±7.0	36.0±9.7	3.6±1.3	138.8±85.3	13.8±2.7	2.6±0.6	48.0±40.3	694	5.56	0.062
	30	180	11/25	9.7±1.0	8.3±5.3	32.6±6.6	27.2±9.3	41.9±11.1	3.3±1.6	138.8±89.3	21.6±15.0	2.3±0.7	45.7±43.9	1527	26.88	0.226

3.3. Reproduction and lifespan

Lifespan, lifespan components and female reproductive values for the five *Artemia* species are listed in Table 3 (columns 4–16). A three-fold difference among species were found for the ability to survive and reproduce at different T–S combinations; *A. parthenogenetica* (as represented by the Margherita di Savoia population) appears to be a niche specialist, reproducing at only two T–S combinations while *A. persimilis* is relatively euryhaline and eurythermal, reproducing at six T–S combinations. Intermediate 'niche' breadth was found for *A. franciscana*, *A. salina* (four T–S combinations for each) and *A. sinica* (three T–S combinations).

Table 4 lists probability values determined by single factor ANOVA for each trait regarding comparisons among T–S combinations within each species. The power of many tests is low due to small sample size caused by high mortality within a cohort prior to reproductive maturity. However, where data could be collected for a relatively large number of reproductive females (i.e. *A. persimilis* and *A. salina*), significant differences were found for most lifespan and reproductive traits.

For the four sexual species, males lived on average 42% longer than females. Male lifespan was longer than female lifespan in 15 of the 17 T–S combinations where reproduction occurred ($p < 0.01$ by goodness of fit). With regard to individual T–S combinations, sample numbers were not large enough for some T–S combinations for statistical testing but significant differences were found for *A. salina* at $24°/120$ and $24°/180t$, for *A. sinica* at $30°/120$, and for *A. franciscana* at $24°/120$ ($P < 0.05$, Wilcoxon test).

3.4. Interspecific comparisons of life-history traits

The number of reproductive females differs significantly among species at $24°C/180$ ppt (Table 3, column 4, $p < 0.01$ by goodness of fit test), the only T–S combination where all species reproduced. For interspecific comparison, the total number of females for *A. parthenogenetica* should be halved since all 50 nauplii were females, whereas for each sexual species only 25 nauplii were females (presuming a male:female ratio of

Table 4
P values for differences among temperature–salinity combinations within each species

Species	A. parthenogenetica	A. salina	A. sinica	A. franciscana	A. persimilis
# of Rep. ♀♀	< -0.001	< 0.05	ns	< 0.01	< 0.01
LT$_{50}$ (Days)	< 0.001	< 0.001	< 0.01	< 0.001	< 0.001
♀ Pre-Rep. Period	ns	ns	ns	< 0.01	< 0.01
♀ Rep. Period	ns	< 0.01	ns	ns	< 0.01
Rep. ♀ Lifespan	ns	< 0.01	ns	< 0.05	< 0.01
Offspring/Brood	ns	ns	ns	ns	< 0.01
Broods/♀	ns	< 0.01	ns	ns	< 0.01
Tot. Offspring/♀	ns	< 0.01	ns	ns	< 0.01
Offspring/Day/♀	ns	ns	ns	ns	< 0.01
Interbroodal Int.	ns	ns	ns	ns	< 0.01

1:1). At other T–S combinations the numbers of reproductive females were not statistically significant. All species except *A. sinica* had the highest number of reproductive females at 24°C, with salinity of 120 ppt or 180 ppt.

Not surprisingly, female pre-reproductive period (Table 3, column 5) decreased with increasing temperature for all species. However, the length of female pre-reproductive period does not appear to be a linear function of salinity. Two observations with regard to individual species are warranted. Firstly, *A. parthenogenetica* reproduced at only two T–S combinations and at both combinations took significantly longer to mature than sexual species. Secondly, *A. persimilis* was the earliest to mature at every T–S combination, except at 24°C/180 ppt where *A. sinica* had a slightly shorter period.

All species had maximum female reproductive periods at 24°C at 120 or 180 ppt (Table 3, column 6). Old World species appear to have relatively limited temperature ranges at which reproduction occurred. For *A. salina*, no reproduction occurred at 30°C, whereas for *A. sinica*, no reproduction occurred at 15°C, while *A. parthenogenetica* had no reproduction at either 15° or 30°. The two New World sexual species had the broadest temperature and salinity ranges where reproduction occurred, with *A. persimilis* reproducing at six and *A. franciscana* at four T–S combinations.

The components of female lifespan (pre-reproductive, reproductive and post-reproductive period) are listed in Table 3 (columns 5,6,8). At 15°C, the reproductive periods were small with long prereproductive periods. Reproductive periods were also small at 30°C. Only at 24°C was the majority of a female's life spent in the reproductive phase. The only T–S combination where all species reproduced and where components of female lifespan can be compared is 24°C/120 ppt. The four sexual species showed relatively little variation in the length of the pre-reproductive period, whereas that value for the parthenogenetic species is approximately twice that of any of the sexual species. However, the reproductive period of the parthenogenetic species is also more than twice that of any sexual species.

3.5. Reproductive traits

All species had peak production of offspring at 24°C, at either 120 to 180 ppt (Table 3, column 11). For any one T–S combination, *A. parthenogenetica* produced the highest number of offspring, followed by *A. franciscana* and *A. persimilis*. The two Old World sexual species, *A. salina* and *A. sinica*, had the least number of offspring.

We propose that cohort reproductive output (Table 3, column 15) is the best estimator of reproductive success since it represents the combined effects of survival and fecundity. All species except *A. sinica* had maximum cohort reproduction at 24°C/120 ppt or 180 ppt. For a starting cohort of 25 females, the reproductive output of *A. salina* at its optimal S–T combination was < 25% that of *A. parthenogenetica* or *A. franciscana*. For some species, comparisons between cohort reproductive output and previous estimators of survival and/or reproduction are divergent (Table 5). For example, the largest value for reproductive output per female for *A. parthenogenetica* is at 24°C/120 ppt, but the cohort reproductive output at that T–S combination is < one-sixth that at 24°C/180 ppt due to the small number of reproductive females at 24°C/120 ppt.

Table 5
Temperature–salinity combinations for five *Artemia* species where optimum performance occurred for seven survival and reproductive traits

Species	LT$_{50}$	21-Day survival	Number of rep. females	Number of rep. days	Number of offspring per female	Cohort reproductive output	Intrinsic rate of increase (r)
A. parthenogenetica	24/180	24/180 or 15/120	24/180	24/180	24/120	24/180	24/180
A. salina	24/180	24/180	24/180	24/180	24/180	24/180	24/180
A. sinica	15/60	30/120	30/120	24/120	24/120	30/120	30/120
A. franciscana	15/180	15/60	24/120	24/120	24/120	24/120	24/120
A. persimilis	24/180	24/120	24/120	24/180	24/180	24/120	30/180

4. Discussion

4.1. Survival and reproduction

The lengths of the pre-reproductive and reproductive periods have a pronounced effect on fitness in *Artemia* (Browne et al., 1988) and almost all organisms (Stearns, 1992). At their optimal T–S combinations, the females of all five species spent the majority of their lifespan in the reproductive phase, but when deviations from the optimal environment occur, the reproductive component declines both on an absolute (number of days) and relative (proportion of lifespan) basis.

The findings of Browne et al. (1988) that 15°C is near the lower temperature limit for successful reproduction in *Artemia* is supported by the present study. *Artemia salina* appear to be adapted to cold temperature (i.e. 15°C) relative to other species, although *A. persimilis* and *A. franciscana* can also reproduce at 15°C. This is reflected in a study of a New Zealand population of *A. franciscana* which was found to initiate rapid population increase in the field at 15°C (Wear and Haslett, 1986; Wear et al., 1986). Apparently, the relatively high survival rates found for some species at 15°C (e.g. *A. parthenogenetica*) do not translate into high reproductive output at this temperature. Our results also indicate that at 30°C *Artemia* approach their upper temperature limit for successful reproduction. However, salinity tolerance for the genus is higher than the 180 ppt upper limit used in this study because four of five species produced relatively high numbers of offspring at the highest salinity tested (180 ppt).

Our results show that species which has a higher reproductive output compared to other *Artemia* species at one T–S combination does not necessarily have a higher reproductive output at a different T–S combination, indicating that comparative analysis of different species based on just one T–S combination may be misleading. Unless a multi-factorial approach is employed it is difficult to compare species' survival, reproduction and competitive performances. This study demonstrates not only that there are significant interactions between temperature and salinity, but that the extent of interaction varies significantly among species.

While the results of this study demonstrate that temperature and salinity significantly interact, additional factors such food quality and quantity, and oxygen levels, may interact with various T–S combinations or with each other. All of the *Artemia* species in this study were raised in identical conditions (except for variation in temperature and salinity) which we believe approximate field conditions and hence are useful for comparative purposes. Different culturing conditions are likely to produce varying results. Triantaphyllidis et al. (1995) measured the effects of 35 ppt, 60 ppt, 100 ppt, 140 ppt and 180 ppt salinity (all at 25°C) on lifespan and reproductive performance of an *A. parthenogenetica* population from Tanggu, China, and a population of *A. franciscana* from San Francisco Bay, USA. Culture conditions were a diet of *Dunaliella terticola* and a yeast based feed (LANSY), and constant aeration. These conditions are probably optimal for rearing *Artemia* in the laboratory but are less reflective of field conditions, regardless of whether food is a limiting factor. Constant aeration may be a critically artificial factor since oxygen level is negatively correlated with salinity and low oxygen level has numerous effects, many of which are adverse on *Artemia*, and has been shown

to affect intra- and inter-specific competitive abilities (Varo et al., 1993; Barata et al., 1996a,b). Triantaphyllidis et al. (1995) reported optimal salinity for *A. parthenogenetica* from Tanggu to be between 60 and 100 ppt while SFB was euryhaline, generally reflecting our results. The results for SFB were also comparable for our 24°C/120 and their midrange salinities (25°C/100–140 ppt) for most reproductive and lifespan traits, suggesting that differences in culture conditions between the two groups had little effect at optimal salinity levels. However, at 25°C/60 they reported 70%–80% survival for SFB while our results were 16% survival and a longer reproductive period. Vanhaecke et al. (1984) using similar culture conditions with constant aeration also reported high survivorship for *A. sinica* and *A. salina* at 25°C/60 ppt, whereas survival was zero at 24°C/60 ppt for these species in our study (although it should also be noted that geographically different populations of each species were used for the two studies).

A. *parthenogenetica* reproduced at only two T–S combinations, indicating low phenotypic plasticity compared to sexual species of *Artemia*. This is the opposite expectation for an obligately parthenogenetic species compared to its related sexual species as advanced by the general purpose genotype hypothesis (Lynch, 1984). However, the performance of *A. parthenogenetica* from Margherita de Savoia may not be representative of other *A. parthenogenetica* populations (Browne, 1992). Basil et al. (1987) reported the occurrence of *A. parthenogenetica* in India at temperatures ranging from 34 to 36°C. at salinities of 155–204 ppt, although it is not known whether the occurrence represents a transitional or permanent population. The Tanggu (China) *A. parthenogenetica* population has been shown to successfully reproduce from 35 to 180 ppt at 25°C. In addition, the Margherita de Savoia population contains a relatively low number of genotypic clones compared to some parthenogenetic diploid *Artemia* populations (Abreu-Grobois and Beardmore, 1982; Browne and Hoopes, 1990), which may limit its physiological tolerance range.

While the ability to reproduce in a particular T–S combination is a useful estimate of fitness, for reproduction to be sustainable, the net reproductive rate $(R_o) \geq 1$ and the intrinsic rate of increase $(r) \geq 0$. Values of r are < 0 for five T–S combinations where reproduction occurs (Table 3, column 17), indicating that the populations are not sustainable at these T–S combinations. When sustainability is taken into account, the number of T–S combinations decreases from four to three for *A. salina*, from four to two for *A. franciscana*, and from six to four for *A. persimilis*. However we would emphasize that only one population of each species was examined and that as more populations are tested the number of T–S combinations suitable for sustained reproduction will likely increase for some *Artemia* species.

Six survival and reproductive traits which could be used as performance estimators are listed in Table 5. Optimum T–S combinations are consistent for only one species (*A. salina*), and are discordant for the remaining four species. Hence, for most *Artemia* species, optimal environments determined by one estimator cannot be extrapolated to other estimators. A population which has high 21-day survival or high LT_{50} value at one T–S combination may have relatively low reproductive output at that same T–S combination. For example, no *A. franciscana* females reproduced at 15°C/180 where the maximum LT_{50} for this species occurred.

4.2. Comparison of field and laboratory data

Hammer and Hurlbert (1992), Wurtsbaugh and Berry (1990) and Dana and Lenz (1986) have reported natural populations of *Artemia* at 33 ppt, 50 ppt and 48 ppt, respectively. However, it is not known whether natural populations of *Artemia* can successfully complete their life cycle at these salinities and/or the observed individuals were migrants from higher salinity environments. A lower limit of 60 ppt for permanent field habitats has been attributed to predation by fish and aquatic invertebrates (Carpelan, 1957; Kristensen and Hulscher-Emeis, 1972). For example, at Salin de Giraud France, *A. parthenogenetica* are found at low population densities when salinities are < 80 ppt (MacDonald and Browne, 1989). The results from this study suggest that, in addition to predation, physiological limits prevent *Artemia* from permanently inhabiting waters where salinity is < 50 ppt, although relatively low levels of reproduction in the laboratory have been reported at < 50 ppt (Triantaphyllidis et al., 1995).

Of the five species, *A. persimilis* had the widest temperature and salinity range, the highest mean reproductive output and the shortest pre-reproductive period. This is surprising given the compact geographical range of *A. persimilis*, which has been reported from only a few saline lakes on the Grand Pampas (Hildago) area of Argentina (Triantaphyllidis et al., 1998). It may be that seasons and/or diurnal temperature/salinity fluctuations are large in these lakes but no information about the natural habitats of *A. persimilis* is currently available.

Our results indicate that *A. franciscana* is both euryhaline and eurythermal since it reproduces at four S–T combinations. *A. franciscana* is a 'superspecies' distributed throughout the Americas and Oceana, and exhibits high levels of phenotypic plasticity. It has also been successfully introduced into Asia, Africa and Europe, sometimes eliminating native *Artemia*. Previous data (Wear and Haslett, 1986; Browne, 1988; Browne et al., 1991) have suggested that it has the greatest temperature tolerance range of any *Artemia* species.

A. salina appears to be cold-adapted and intolerant to low salinity (60 ppt) at any temperature. Survival and reproductive performance estimators consistently identified 24°C/180 ppt as its optimal niche. Our data, combined with the high population growth and encystment rates at cold temperatures for this species (Barata et al., 1996b), indicate that *A. salina* is adapted to conditions found in ephemeral inland lakes. In the Mediterranean region, where *A. salina* occurs, these are often filled with water only during the winter months (Browne and MacDonald, 1982).

In the Mediterranean basin, polyploid *A. parthenogenetica* are found at higher latitudes than diploid sexual *A. salina*, with diploid parthenogenetic populations usually occurring in inland salinas and salt lakes whose ionic proportions are often considerably different than those of sea water (Amat, 1980; Browne and MacDonald, 1982; Zhang and Lefcort, 1991; Barata et al.,1995,1996b). Most authors attribute this distribution to the higher survival and fecundity of the polyploid parthenogens at extreme temperatures (Zhang and Lefcort, 1991; Browne, 1992,1993; Zhang and King, 1993). Recent data (Barata et al., 1996b) demonstrate that polyploid parthenogenetic *Artemia* from Spain have broader temperature tolerance ranges than diploid sexual or parthenogenetic

Spanish populations. Our results, however, show that *A. parthenogenetica* from Margherita di Savoia can reproduce at only two T–S combinations. This apparent contradiction may be due to the wide variation among *A. parthenogenetica* populations in physiological tolerance and life history traits, which often are as large as the differences found among separate *Artemia* species (Browne, 1992).

This study expands our understanding of multifactorial effects of the physical environment on sexual and parthenogenetic organisms, using *Artemia* as a model. To more completely understand these effects, numerous populations of each species need to be compared before the limits of even such relatively easily measured parameters as temperature and salinity can be defined for each species. A further complication is the choice of culture conditions, including whether optimal conditions or those more reflective of field conditions should be used. However, multifactorial experiments quickly encounter logistical and time constraints, as evidence by this study where even a simple three factorial design resulted in 45 species/temperature/salinity combinations. Nevertheless, if we wish to proceed beyond a rudimentary understanding of physiological constraints on species' biogeographic distributions, population ecologies and competitive interactions, increasingly complex multifactorial approaches must be utilized.

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