

Effects of temperature and salinity on prereproductive life span and reproductive traits of two species of *Artemia* (Branchiopoda, Anostraca) from Argentina: *Artemia franciscana* and *A. persimilis*

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Abstract The identification of the environmental conditions inducing different ecophysiological responses in the different strains and populations of the brine shrimp *Artemia* should improve the understanding of their biogeographic distribution. Nauplii from two Argentinean brine shrimp populations, *Artemia persimilis* from Salinas Grandes de Hidalgo (province of La Pampa) and *Artemia franciscana* from Laguna Mar Chiquita (province of Córdoba), were grown up until adulthood at different salinities (30, 60, 90, 120 gL⁻¹) and temperatures (12, 21, 28°C). The aim was to assess the effects of these different conditions on prereproductive life span and reproductive traits. Results evidenced that at 21 and 28°C, at any salinity, *A. franciscana* from Laguna Mar Chiquita attained higher survival and fecundity, after a shorter prereproductive period, than *A. persimilis* from Salinas Grandes de Hidalgo. These data support

that *A. franciscana*, considered a superspecies, exhibits higher phenotypic plasticity than *A. persimilis*, and that *A. persimilis* is better adapted to lower temperatures than *A. franciscana*. These differences in temperature and salinity tolerance could explain the present distribution of these two species in the South Cone in South America.

Keywords *Artemia persimilis* · *Artemia franciscana* · Argentina · Temperature · Salinity · Survival · Fecundity · Growth · Body length · Prereproductive period

Introduction

The brine shrimp *Artemia* (Crustacea, Anostraca) is distributed worldwide with the exception of Antarctica (Vanhaecke et al., 1987; Triantaphyllidis et al., 1998). In the New World, this genus is represented by three species: *Artemia franciscana*, Kellogg (1906), *Artemia persimilis*, Piccinelli & Prosdocimi (1968), and *Artemia monica*, Verrill (1869). It has been demonstrated that *A. franciscana*, endemic to the New World (North, Central and South America) is present in other continents as a consequence of its introduction for different purposes, aquaculture among them (Triantaphyllidis et al., 1998; Amat et al., 2005), while *A. persimilis* is found exclusively in hypersaline ecosystems of Argentina (Barigozzi

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1989; Triantaphyllidis et al., 1998; Rodriguez Gil et al., 1998; Cohen et al., 1999) and Chile (Gajardo and Beardmore 1993; Gajardo et al., 1998; De Los Rios and Zúñiga, 2000). This species was also reported in San Bartolomeo salterns, Sardinia (Italy) (Halfer-Cervini et al., 1968; Piccinelli and Prosdocimi, 1968). However, as this existence could not be confirmed (Barigozzi, 1989), the present distribution of *A. franciscana* and *A. persimilis* is not definitive (Gajardo et al., 1995; Triantaphyllidis et al., 1998).

It was demonstrated that *A. franciscana* replaces endemic brine shrimp species wherever it is inoculated (Triantaphyllidis et al., 1998; Amat et al., 2005). Different studies show *A. franciscana* as being the most euryhaline and eurythermal species in the genus (Wear and Haslett, 1986; Browne et al., 1988; Browne and Bowen, 1991), developing the highest levels of adaptation (Browne et al., 1988) or phenotypic plasticity. *A. franciscana* is the most fit among the bisexual species of *Artemia* due to the high reproductive performance of its females on the basis of largest offspring per brood, longest reproductive period and life-span (Browne et al., 1988). All this allows to consider *A. franciscana* as a species in evolutionary expansion (Gajardo et al., 2004) due to its colonist capacity (Amat et al., 2004).

The information cited above related to the fitness of *A. franciscana* has been obtained from the San Francisco Bay (California, USA) autochthonous population (Vanhaecke et al., 1984; Browne et al., 1988; Browne and Wanigasekera, 2000; Barata et al., 1996a, b). The variability of fitness for other populations of *A. franciscana* is not adequately known, and even less at the characteristic environmental conditions prevailing in the South Cone in South America. In this region, where *A. franciscana* populations were also found (Gajardo et al., 1995; Zúñiga et al., 1999; Papeschi et al., 2000; Amat et al., 2004), climatic and hydrological conditions are diverse, specifically in Argentina, a country that until recently was suspected to be inhabited only by *A. persimilis* populations.

Previous reports (Browne et al., 1984, 1988) have provided the evidence that American brine shrimp strains show a broad diversity in life-span characteristics, markedly different from other sexual or asexual Old World forms. It is inter-

esting to unveil the possible differences in prereproductive and reproductive characteristics for both American species, mainly from the point of view of their biogeographic distribution in Argentina.

The *Artemia* populations, that this work is dealing with, come from inland salt lakes or lagoons, permanent or temporary bodies of salt water with salinities $>30 \text{ gL}^{-1}$, lacking any present or recent connection with the marine environment (Williams, 2002). These ecosystems are usually confined to dry regions where evaporation normally exceeds precipitation. This pattern is only disturbed more or less periodically by the climatic phenomena known as El Niño Southern Oscillation (ENSO), especially in the mid-latitudes of the Western North and South America. Some of these lakes never dry out, although their water level may fluctuate considerably over long periods, with markedly different salinity, that can vary from <50 to 300 gL^{-1} without complete desiccation. Other water bodies are filled only episodically. They remain for years as simple dry salt pans or very shallow brines, and only fill up considerably if unpredictable and episodic rains fall on their surrounding catchment areas. Salinities are equally variable, because brines are seasonally produced by these rains that dissolve superficial salt beds, several cm thick (Angelelli et al., 1976), displaying high values immediately. Thus salinities show fluctuations following the annual cycles. Permanent salt lakes, during their moderate salinity periods, can harbour the halo-tolerant taxa from fresh waters, changing with increasing salinity to taxa found only in salt lakes. In temporary lakes or lagoons, with high salinity, biodiversity decreases favouring endemism and the restricted distribution of adapted species in a given genus that shows such cosmopolitanism. This is the particular case for the genus *Artemia*.

This study reports the differences in the prereproductive parameters of life-span, and in the reproductive characteristics between two populations of *A. persimilis* and *A. franciscana* from Argentina, where both species are found at present (Triantaphyllidis et al., 1998; Cohen et al., 1999; Pastorino et al., 2002). Populations from both species might have competed previously attaining their present biogeographic

distribution. Presently, they could be still competing. Consequently, this study was focused to know the responses of both *Artemia* populations to different conditions of temperature and salinity, in terms of nauplii survival until adulthood, growth rate, duration of the prereproductive period and fecundity.

Materials and methods

Following the experimental model suggested by Browne et al. (2000), this study aims to measure the survival, the growth rate, the fecundity and the duration of the prereproductive period in two populations from New World *A. franciscana* and *A. persimilis* species, using different combinations of culture salinities (30, 60, 90, 120 gL⁻¹) and temperatures (12, 21, 28°C). Higher salinity conditions were avoided in the experimental design due to the fact that salinities above 120 gL⁻¹ entail very long prereproductive periods so that the animals do not always reach sexual maturity and cultures usually crash (Abatzopoulos et al., 2003; Triantaphyllidis et al., 1995; Browne and Wanigasekera, 2000). Alternatively, a low salinity (30 gL⁻¹) condition was chosen to test the low tolerance limit for both species, useful to explain the performance of the brine shrimp populations in epicontinental ecosystems relying on precipitation to be filled up.

Both populations used are autochthonous from Argentina, i.e. *A. persimilis* from Salinas Grandes de Hidalgo (province of La Pampa; 37°13' S; 63°26' W) and *A. franciscana* from Laguna Mar Chiquita (province of Córdoba; 30°20' to 31°0' S; 62°40' to 63°10' W). They were chosen among the geographically closest Argentinean populations of both species. This allowed comparing their capabilities in terms useful to explain a competitive behaviour, likely to happen due to their neighbourhood and the putative expanding tendency of *A. franciscana*.

Original cysts collected from both places were processed in the laboratory according to standardized methods (Vanhaecke and Sorgeloos, 1980), and stored at 4°C in sealed plastic bags. Nauplii were obtained from cyst hatching after 24 h of hydration and incubation in diluted filtered sea water (18 gL⁻¹) at 28°C under continuous

gentle aeration and illumination. After hatching, samples of 100 instar-I nauplii were transferred to different 750 mL containers. The culture media for nauplii was *Tetraselmis suecica* mass culture kept at a salinity of 30 gL⁻¹ and *Dunaliella viridis* mass culture kept at a salinity of 90 and 120 gL⁻¹. A mixture of *T. suecica* (30 gL⁻¹) and *D. viridis* (90 gL⁻¹) cultures (1:1) provided the salinity of 60 gL⁻¹ culture medium.

Nauplii were directly transferred to containers with 30 or 60 gL⁻¹ media. The salinity of the containers of 90 and 120 gL⁻¹ was gradually increased to the desired level with microalgae culture mixtures in such a way that the animals were not stressed (120 gL⁻¹ was reached after 4 days). Three replicates were set up for each salinity at the three different temperatures. The nauplii culture containers were arranged in climatic chambers providing the three temperatures selected, under mild aeration and 12D:12L illumination regime.

Microalgal availability in culture media was controlled measuring the cell density in diluted samples with an haematocytometer, and their absorbance at 678 nm with a spectrophotometer Hitachi U-2001 (Marker et al., 1980; Lorenzen, 1967; Stein, 1973). These data were used to construct regression between absorbance and cell density values. Thus, the absorbance values allowed to assess the algae biomass present in the culture. The media were renewed every four days with microalgae culture. The quantity of food was increased up to a maximum density of 500,000 cells/mL, according to the *Artemia* population growth.

Initial length for 50 instar-I nauplii was measured in both populations, once fixed with 1% Lugol's solution, under a dissecting microscope to the nearest micrometer eyepiece mark (1mark = 20 µm). Survival, length and reproductive status were first monitored on the 8th day after hatching and every fourth day from then on until the moment when more than 50% of mature surviving females showed full ovisac (MT₅₀). In each control, dead and surviving animals were counted. Ten specimens (5 males and 5 females), randomly chosen from each sample, were anaesthetized with chloroform-saturated deionized water to measure their total length (Hontoria and

Amat, 1992) under the dissecting microscope. When females attained MT_{50} , the ovisacs of ten specimens, randomly chosen, were dissected, and the offspring counted and identified in order to assess their fecundity.

Statistics

Statistical analyses were performed with the SPSS 11.0 for Windows package (SPSS Inc., Chicago, IL, USA). The survival at MT_{50} (S_{50}), the total length at MT_{50} (TL_{50}), and the fecundity at MT_{50} (F_{50}) were compared for the two species and different conditions using three or two way ANOVA. The average growth rate at MT_{50} (AGR_{50}) was calculated dividing the total length mean of each replicate by the corresponding MT_{50} . The assumption of homogeneity of variances was contrasted using Levene's test (Sokal and Rohlf, 1995). When homogeneity of variances was accomplished, Tukey's test was employed to contrast the equality of means (Sokal and Rohlf,

1995). When heterocedasticity was significant ($P \leq 0.05$), Games–Howell test of mean comparison was employed to assess the treatment performance (Sokal and Rohlf, 1995).

The S_{50} and F_{50} data were treated with the arcsine square root and square root transformation, respectively ((Zar, 1974). The unusual result obtained from one of the replicates for *A. franciscana* survival at 21°C and 120 gL⁻¹ combination was eliminated according to Grubbs test for outliers (Grubbs, 1969; Stefansky, 1972). Moreover, S_{50} at 28°C and 30 gL⁻¹ for *A. persimilis* was 5.3% (sd 1.8). Due to this low survival, the evaluation of the other variables was not considered as representative.

Results

S_{50}

The evolution of survival until MT_{50} for both populations at all salinity–temperature combina-

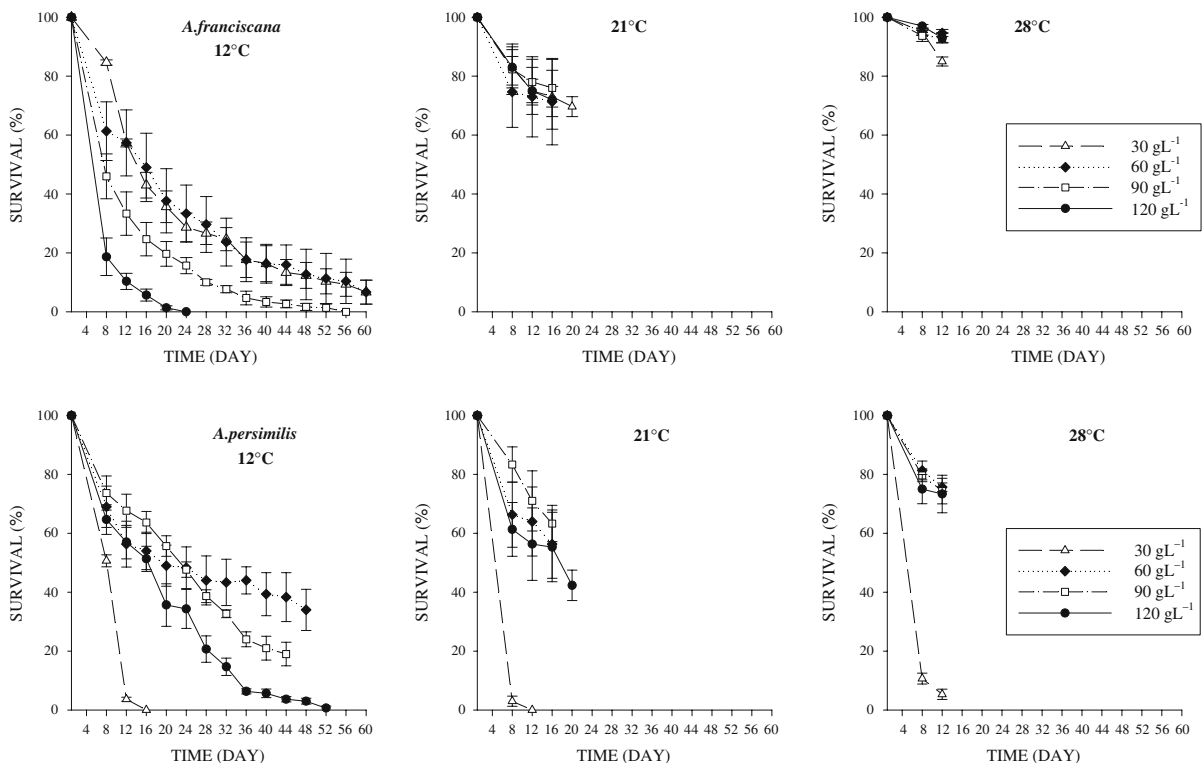


Fig. 1 Evolution of survival for *A. franciscana* and *A. persimilis* specimens until MT_{50} (time when 50% of the females reach sexual maturity) at different conditions of temperature and salinity

Table 1 Comparison of S_{50} , TL_{50} , MT_{50} , F_{50} and AGR_{50} for *A. persimilis* and *A. franciscana*. Mean (standard deviation). See text for the variable definitions

	S_{50} (%)		TL_{50} (mm)		MT_{50} (days)		F_{50} (offspring per female)		AGR_{50} (mm/day)	
	<i>A. franciscana</i>	<i>A. persimilis</i>	<i>A. franciscana</i>	<i>A. persimilis</i>	<i>A. franciscana</i>	<i>A. persimilis</i>	<i>A. franciscana</i>	<i>A. persimilis</i>	<i>A. franciscana</i>	<i>A. persimilis</i>
12°C	30	7.7 (4.1)*	0.0 (0.0)	—	60.0 (0.0)*	—	—	—	—	—
	60	6.7 (4.1)*	34.0 (7.0)	—	60.0 (0.0)*	48.0 (0.0)	—	85.3 (4.1)	—	0.19 (0.01)
	90	0.0 (0.0)	19.0 (4.0)	—	—	46.7 (1.0)	—	65.3 (5.2)	—	0.19 (0.02)
	120	0.0 (0.0)	0.7 (0.7)	—	—	—	—	—	—	—
21°C	30	69.7 (3.4)	0.0 (0.0)	—	20.0 (0.0)	—	108.6 (6.9)	—	0.45 (0.01)	—
	60	71.3 (14.7)	56.0 (11.4)	—	17.3 (1.3)	17.3 (1.3)	74.5 (5.2)	63.5 (4.1)	0.50 (0.02)	0.59 (0.03)
	90	76.0 (9.7)	65.0 (4.5)	10.3 (0.2)	17.3 (1.3)	17.3 (1.3)	97.1 (5.5)	53.1 (3.1)	0.50 (0.02)	0.56 (0.03)
	120	72.0 (10.0)	55.0 (7.5)	9.7 (0.2)	16.0 (0.0)	18.7 (1.3)	94.3 (2.7)	64.2 (10.2)	0.55 (0.01)	0.50 (0.03)
28°C	30	85.0 (1.5)	5.3 (1.8)	—	12.0 (0.0)	—	108.0 (6.8)	—	0.73 (0.01)	—
	60	94.7 (1.2)	75.7 (1.5)	11.0 (0.3)	12.0 (0.0)	12.0 (0.0)	143.3 (7.9)	107.9 (5.2)	0.81 (0.01)	0.91 (0.01)
	90	93.0 (1.5)	74.3 (4.3)	10.5 (0.2)	10.7 (1.3)	12.0 (0.0)	138.2 (6.1)	107.8 (5.9)	0.86 (0.03)	0.88 (0.01)
	120	93.0 (1.7)	73.3 (6.4)	9.6 (0.3)	12.0 (0.0)	12.0 (0.0)	177.6 (6.8)	120.6 (4.6)	0.83 (0.01)	0.80 (0.01)

(*) On the 60th day of the experiment, the 12°C climatic chamber was damaged and specimens from the 30 gL⁻¹ and 60 gL⁻¹ salinity replicates for *A. franciscana* died. The results were used to perform all statistical analyses

(—) These combinations did not allow achieving the mature female status

tions is shown in Fig. 1. S_{50} is presented in Table 1 and Fig. 2. On the 60th day of the experiment, the 12°C climatic chamber was damaged and the specimens at salinities of 30 and 60 gL⁻¹ for *A. franciscana* died. The results obtained from these combinations until that moment were used to perform statistical analyses, and they are indicated with an asterisk (*) in Table 1.

Statistical analyses show differences between the two populations at the different conditions. Since the three-way interaction is not significant, it was eliminated from the model to homogenize the variances (Sokal and Rolf, 1995). The three-way ANOVA reports significant effects for the species ($P < 0.00001$), the temperature ($P < 0.00001$)

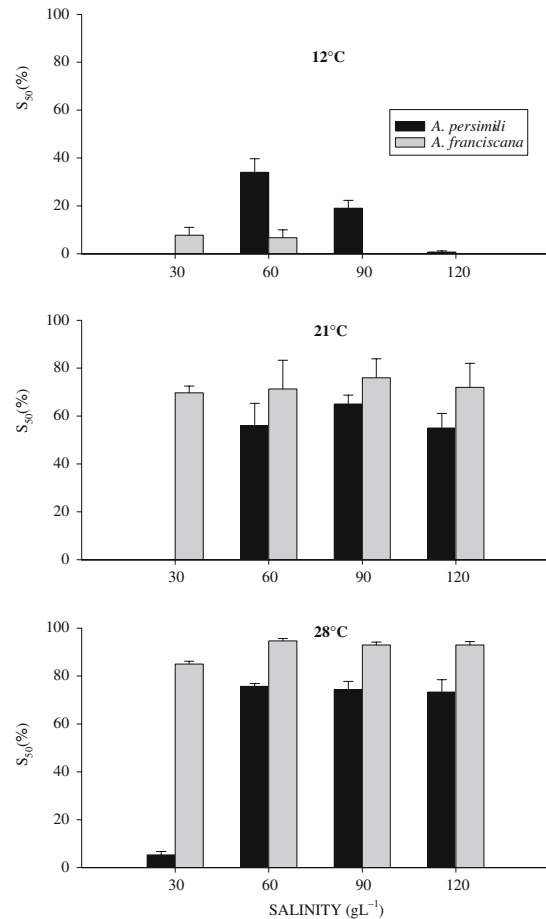


Fig. 2 Survival of *A. persimilis* and *A. franciscana* specimens at MT_{50} (time when 50% of the females reach sexual maturity) at 12 different combinations of temperature and salinity

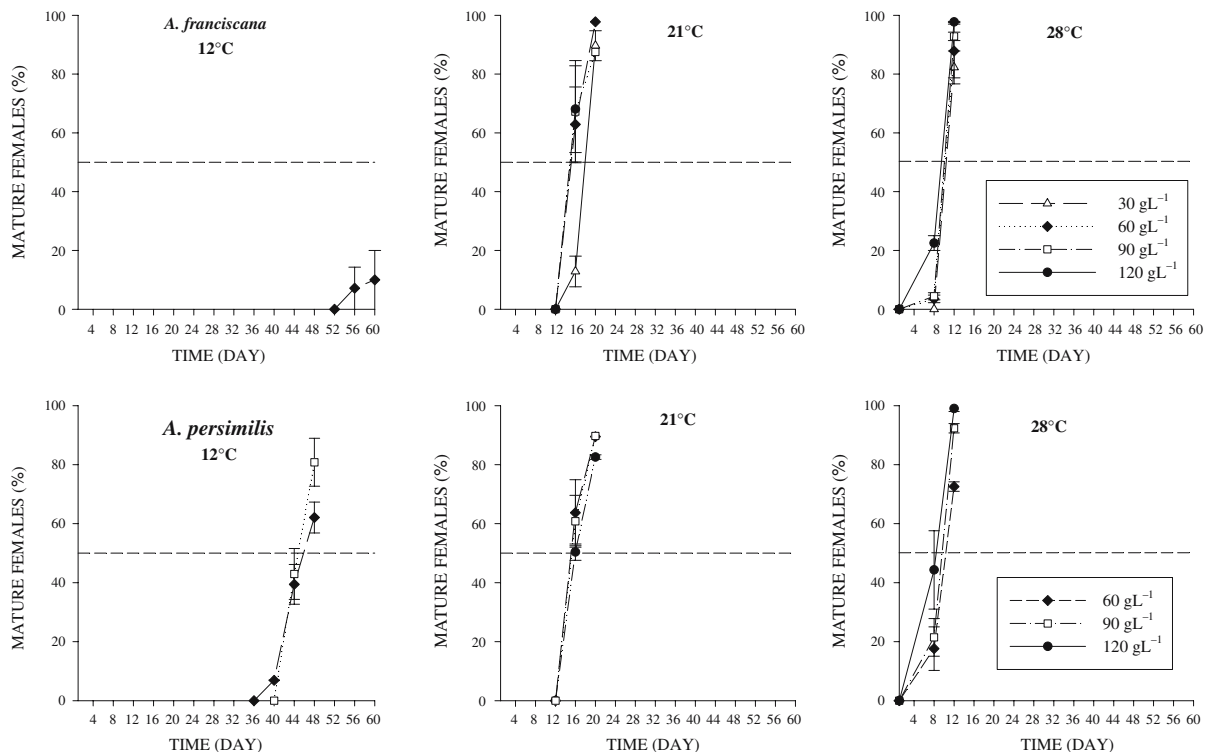


Fig. 3 Evolution of sexual maturity for *A. franciscana* and *A. persimilis* females until MT_{50} (time when 50% of the females reach sexual maturity) at different conditions of

temperature and salinity. Dash line represents the 50% of females with full ovisac when MT_{50} is calculated

and the salinity ($P < 0.00001$) factors, and also for species–temperature ($P < 0.00001$), the species–salinity ($P < 0.00001$) and the temperature–salinity ($P < 0.0001$) interactions. The factor with highest sum of squares and, consequently, with highest effect on survival is the temperature. In this way, Tukey's test finds differences ($P < 0.00001$) between 12, 21 and 28°C. *A. franciscana* presents higher survival than *A. persimilis* at 21 and 28°C, while at 12°C *A. persimilis* attains better survival than *A. franciscana*. Not surprisingly, survival rates improve with increasing temperature for both species.

In terms of salinity, the most important differences are found at 30 gL⁻¹, at which *A. persimilis* cannot survive properly whatever the temperature (12, 21 and 28°C) assayed. A two-way ANOVA for *A. persimilis* was used to look for differences to explain these results. Thus, salinity produces the greatest effect on *A. persimilis* survival ($P < 0.00001$). A Games-Howell

post hoc mean comparison analysis shows that salinity segregates two different survival groups of means: 30 gL⁻¹ and 60–90–120 gL⁻¹. On the other hand, two different groups of means are segregated according to the increase of temperature and their effect on survival: 12 and 21–28°C.

A two-way ANOVA for the *A. franciscana* data shows that the temperature is the only factor producing significant differences on the survival of this species ($P < 0.00001$). A Games-Howell *post hoc* analysis reveals that the three temperatures produce significantly different effects on *A. franciscana* survival.

MT_{50}

Table 1 shows the MT_{50} results obtained at the 12 temperature-salinity combinations for both species. Those combinations that did not attain final results because of the 12°C climatic chamber

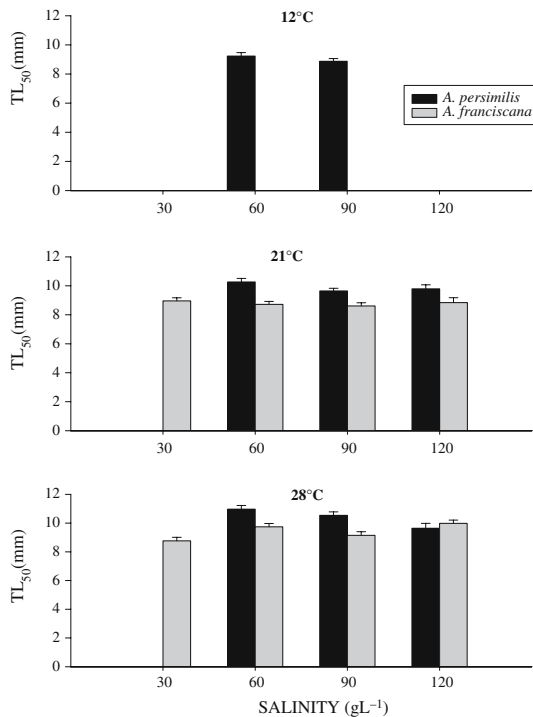


Fig. 4 Total length of *A. persimilis* and *A. franciscana* specimens at MT₅₀ (time when 50% of the females reach sexual maturity) at 12 different combinations of temperature and salinity

failure are referred as survival with an asterisk in Table 1. Obviously, sexual maturity occurs sooner the higher the temperature, producing a decreasing tendency in MT₅₀ results (12 > 21 > 28°C) for both species. **Figure 3 shows the evolution of sexual maturity status for *A. franciscana* and *A. persimilis* females at all the experimental conditions.** The biggest divergence in MT₅₀ between both species occurs at 12°C (Fig. 3, Table 1). At this temperature, *A. persimilis* from Salinas Grandes de Hidalgo reached its MT₅₀ sooner than *A. franciscana* (Fig. 3, Table 1). According to the results, temperature is the most important factor on the MT₅₀ variable, while salinity seems not to affect the time needed to reach maturation.

TL₅₀ and AGR₅₀

The results obtained for TL₅₀ are presented in Table 1 and Fig. 4. Females from the *A. persimilis* population are longer than those from *A. fran-*

ciscana at MT₅₀ at all combinations of temperature and salinity. The only exception to this fact is found at 28°C and 120 gL⁻¹ combination (Table 1). However, the species factor is not significant in the statistic comparisons. Consequently, average growth rate (AGR₅₀) tends to be higher for *A. persimilis* at all conditions with the exception of the combinations of 28°C at 120 gL⁻¹, and 21°C at 120 gL⁻¹ (Table 1). On the other hand, the ANOVA analysis reflects the differences in temperature in a significant way ($P < 0.00001$), showing a relationship between the temperature increase and a better growth (AGR₅₀) (Figs. 5 & 6). This is more evident from 12 to 21°C than from 21 to 28°C.

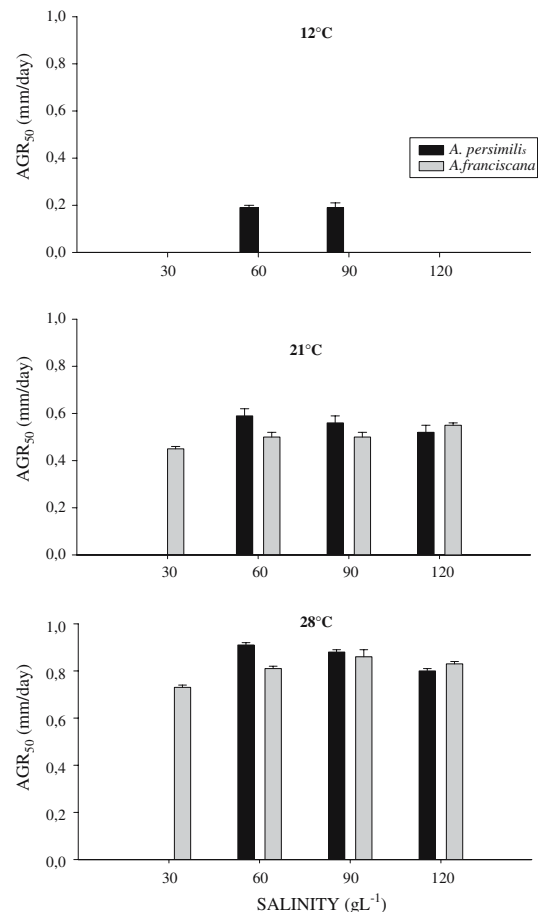


Fig. 5 Average Growth Rate (AGR₅₀) of *A. persimilis* and *A. franciscana* specimens at MT₅₀ (time when 50% of the females reach sexual maturity) at 12 different combinations of temperature and salinity

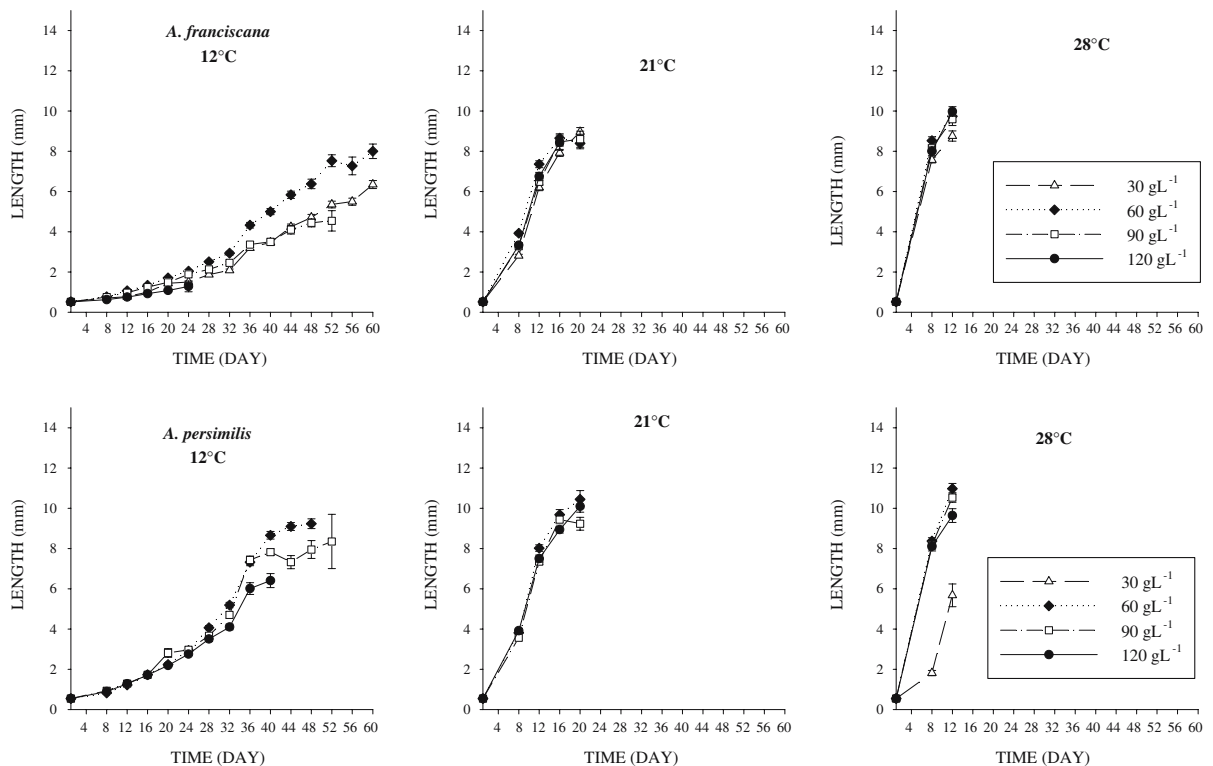


Fig. 6 Evolution of body length of *A. franciscana* and *A. persimilis* specimens until MT_{50} (time when 50% of the females reach sexual maturity) at different conditions of temperature and salinity

Salinity especially affected the growth of the *A. persimilis* population from Salinas Grandes de Hidalgo. For this population, the Games and Howell mean comparison *post hoc* test finds differences between two groups of means: 30 gL^{-1} and $60\text{--}90\text{--}120 \text{ gL}^{-1}$ ($P < 0.00008$). These results are also confirmed by the significant ($P < 0.00001$) interaction showed by the species–salinity factors. This fact evidences the negative influence of low salinity on the *A. persimilis* growth, while *A. franciscana* seems to be unaffected.

F_{50}

Table 1 and Fig. 7 show the results of fecundity at the different conditions for both species. When the experimental conditions allowed survival and the achievement of maturity, females from the Argentinean *A. franciscana* population from Laguna Mar Chiquita showed a higher fecundity than *A. persimilis* females from Salinas Grandes de Hidalgo, i.e., at 21 and 28°C at any salinity

combination (Fig. 7). A three-way ANOVA analysis was performed including *A. persimilis* fecundity at 12°C, but without those for *A. franciscana*. The reason for this is none of the *A. franciscana* individuals were fertilized at this temperature as already stated. The ANOVA results show significant differences for the species ($P < 0.00001$), the temperature ($P < 0.00001$) and the salinity ($P < 0.00005$) factors. Tukey's *post hoc* mean comparison test for fecundity separates the results in three groups of means for salinities: 30, 60–90 and 120 gL^{-1} . Indeed, at 120 gL^{-1} , *A. persimilis* shows higher offspring production at 21 or 28°C, and *A. franciscana* the most productive combination at 28°C. Tukey's analysis finds differences between 21°C and 28°C ($P < 0.00001$) in the pooled results for both species, while differences found between 12°C and 28°C ($P < 0.00001$) only reflect the *A. persimilis* differences.

In *A. franciscana*, a relationship is evident between the increase of temperature and a higher

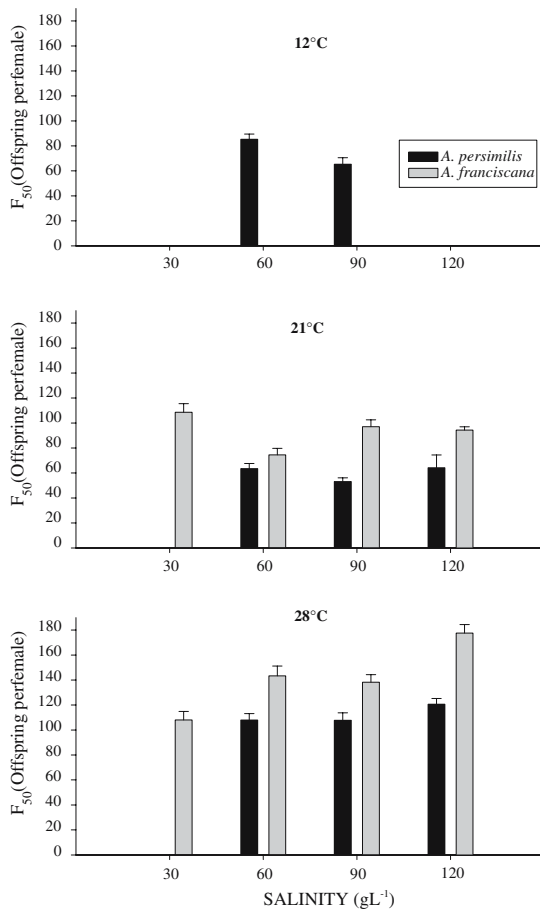


Fig. 7 Fecundity of *A. franciscana* and *A. persimilis* females at MT_{50} (time when 50% of the females reach sexual maturity) at 12 different conditions of temperature and salinity

fecundity (Table 1, Fig. 7). This relationship was not apparent for *A. persimilis* females between 12 and 21°C, although at 28°C reproductive output was the highest for this species.

The ANOVA results are significant for the species–salinity ($P < 0.005$) and the salinity–temperature ($P < 0.00001$) factor interactions. The significant interaction for the factors species–salinity indicates a different trend in both species for the fecundity as salinity changes. *A. franciscana* increases its fecundity along the salinity range, whereas *A. persimilis* presents similar results at different salinities. On the other hand, the salinity–temperature interaction reflects clear differences in fecundity between 28 and 21°C with increasing salinity, namely in *A. franciscana*.

Discussion

The *Artemia* species autochthonous from Argentina are expected to display a distribution primarily ruled by their adaptation to the prevailing environmental conditions in the diverse geographical Argentinean regions. The populations here studied are found in a large (2,000–6,000 km²) permanent salt lake as Laguna Mar Chiquita (province of Cordoba) in the case of *A. franciscana*, and in a fluctuating salt lagoon (3,000 ha) as Salinas Grandes de Hidalgo (province of La Pampa) for the *A. persimilis* population.

According to Reati et al. (1997), Angelelli et al. (1976), and our own data not presented here, the ionic composition of the brines from both water bodies studied shows an average ionic dominance $Na^+ - Cl^- - SO_4^{2+}$. This is also true for most of the other saline ecosystems found in this geographic region. When these salt lakes were prospected in search of *Artemia* populations, the average sodium and chloride proportions in percentage of total ions was 32 and 44%, respectively for Mar Chiquita (1997), and 38 and 59% for Hidalgo Saltworks (1992). Average proportions of SO_4^{2-} were more variable. They attained 17 and 2% for Mar Chiquita and Hidalgo, respectively, in concordance with the salinity changes. Thus, the high levels in Mar Chiquita were due to the low salinity recorded in this salt lake during the last two decades (around 40–50 g/L, although during the last century in Mar Chiquita the salinity fluctuated between 25 and 360 g/L (Reati et al., 1997).

Laguna Mar Chiquita is located into the temperate climatic region named Chaco-Pampa Plain (18–20°C annual average temperature), according to the overview on Argentinean lakes by Quiros and Drago (1999). In addition, it undergoes long periods of flooding waters of low salinity due to important fresh water inputs from three influent rivers (Reati et al., 1997). Salinas Grandes de Hidalgo lagoon is located in La Pampa region, at 700 km South of Laguna Mar Chiquita. It has a colder climate (14–16°C annual average temperature), and usually develops high salinities as soon as rain and ground water fill it up after dry periods. Salinas Grandes de Hidalgo is an endorheic saline basin lying in the Southern corner

of the Pampa Plain, very close to the Patagonia Plateau border (12–14°C annual average temperature).

Other evidence for the relationship of *A. persimilis* with colder ecosystems comes from previous prospective research on the distribution of the brine shrimp populations in Argentina, conducted between 1992 and 2003. *A. persimilis* populations were only found in the hypersaline ecosystems scattered in the South of Buenos Aires, Rio Negro, Chubut and Santa Cruz provinces. The population found in San Julián (Santa Cruz province, 49°17' S, 67°46' W) is the southernmost *A. persimilis* population prospected in Argentina. In this region, there is an average annual temperature of 8–9°C (minimum mean –3°C in winter, maximum mean 19°C in summer, Servicio Meteorológico Nacional. Fuerzas Aéreas Argentinas—www.meteonet.com.ar).

Differences in environmental conditions produce genetic adaptations and, consequently, different physiological responses (Kinne, 1970). However, the impact of the environmental stress on an organism depends on the frequency and extent of the disturbance and the compensatory abilities of the organism (Dana and Lenz, 1986). In this way, temperature and salinity are two important factors affecting the life span characteristics of different strains of the genus *Artemia* (Barata et al., 1996a, 1996b; Browne et al., 2000).

Browne et al. (1988, 2000) found that 15°C is near the lower temperature limit for successful reproduction in the genus *Artemia*. This is in agreement with the results reported here. *A. persimilis* from Salinas Grandes de Hidalgo and *A. franciscana* from Mar Chiquita show the lowest survival and offspring production, and shortest body length at 12°C. Although, *A. persimilis* appears to be better adapted to lower temperatures than *A. franciscana*, which is, in turn, more thermo-tolerant at the moderate and high temperatures assayed (see Figs. 1, 3 and 6).

Some mortalities happened at the beginning of the experiment during the first instar stages of development, when, according to Clegg et al. (2000), the capacity of acclimatizing is probably more critical. Provasoli and Shiraishi (1959) too suggested that the 5th instar to juvenile step is a particularly critical period in the life of *Artemia*.

Prereproductive periods contribute markedly to the fitness in almost all organisms (Stearns, 1992), including the *Artemia* genus (Browne et al., 1988). Thus, an inverse relationship between development time and temperature is well documented for many zooplankters, such as copepods (Botrell et al., 1976; Hart, 1990). Results for 33 species of copepods from 181 published studies (Huntley and Lopez, 1992) show that temperature accounted for 91 % of the variation in copepod growth rates. This is also found in the *Artemia* genus, where an increase in environmental temperature leads to a decrease in the prereproductive period (Browne et al., 1988, 2000; Abatzopoulos et al., 2003).

Besides, the temperature affects fecundity in the *Artemia* populations studied more than salinity. The best argument supporting this assertion could be that *Artemia* displays an r-strategy of reproduction. An increase in temperature implies a reduction of prereproductive and reproductive periods, and life span, that requires the production of a great number of offspring within the shortest time (Abatzopoulos et al., 2003; Triantaphyllidis et al., 1995). This is observed in our experimental results where increasing temperatures produced higher fecundities, especially in *A. franciscana*. There are significant differences in fecundity between *A. persimilis* and *A. franciscana*, the latter being more productive in all temperature–salinity combinations, except at 12°C. It is well documented that *A. franciscana*, as an r-strategist, shows high fecundity, viable and good quality offspring per brood, per day and per female (Browne and Halanych, 1989). This fact grants important colonising abilities.

Low salinity (30 gL⁻¹) produced important mortalities in *A. persimilis* nauplii. These results are in disagreement with Vanhaecke et al. (1984) and Browne et al. (2000) where *A. persimilis* is reported to stand the widest temperature and salinity ranges. Our data suggest that this particular *A. persimilis* population stands the lower temperature extreme, but it is the *A. franciscana* population studied here which shows the better performance in the salinity range assayed.

Life span characteristics, like prereproductive period or growth rate, are also affected by salin-

ity, at least a development delay is caused at higher salinities as reported by Abatzopoulos et al. (2003) and Baxevanis et al. (2004). However, the output from the present study allows to state that salinity was not directly involved in differences in prereproductive periods between the species studied, especially at moderate and high temperatures. Perhaps the salinity range used did not promote more differences, since 120 gL^{-1} is still into the salinity range that usually can be considered as optimal (Dana and Lenz, 1986). High salinity would not become in this case a limiting factor that could induce abnormal development results. Nevertheless, it is not easy to ascertain this point since salinities above 120 gL^{-1} entail very long prereproductive periods so that the animals cannot reach sexual maturity before cultures crash (Abatzopoulos et al., 2003; Triantaphyllidis et al., 1995; Browne and Wanigasekera, 2000) in laboratory conditions.

The effects of salinity on fecundity are documented by Dana and Lenz (1986) in terms of a decrease in reproductive potential (fecundity) as salinity increases. This is not confirmed by our results, even though fecundity is significantly affected by salinity. This behaviour is more apparent for *A. franciscana* (Fig. 7) than for *A. persimilis*.

The two populations studied exhibit significant differences in their survival, fecundity, growth, and duration of prereproductive period at different salinity and temperature conditions. The effect of temperature is more pronounced. These differences could be the explanation of their biogeographic distribution, since presently *A. persimilis* is found only in the Southern regions of Argentina and Chile (Triantaphyllidis et al., 1998; Amat et al., 2004; Gajardo et al., 2004). In fact, the population differences in performance found in this study can be linked to the climatic characteristics of the ecosystems inhabited by the two *Artemia* populations. The regional features, namely, the decrease of temperature from North to South, and the neotectonic patterns, determine the ecological complexity of the lakes in this region, especially in the Southern part, near Patagonia.

While Laguna Mar Chiquita is a permanent salt lagoon with constant incorporation of water (Quirós and Drago, 1999; Reati et al., 1997), but

with periods of fluctuating salinities, Salinas Grandes de Hidalgo is located in a region with plentiful natural depressions keeping important evaporitic deposits. These halites allow the formation of scattered brine water bodies filled up by precipitation and groundwater inflow. Probably, *A. franciscana* from Laguna Mar Chiquita has developed mechanisms of adaptation to wide salinity fluctuations through an enhanced osmoregulatory capacity. However, it has not developed any special adaptation to low temperatures, since its niche is situated in a temperate climatic zone. On the other hand, *A. persimilis* from Salinas Grandes de Hidalgo stands colder temperatures and dry periods along the year. This population must be adapted to low temperatures and to cope with sharp salinity changes.

Conclusions

The *A. franciscana* population from Laguna Mar Chiquita (province of Cordoba, Argentina) exhibits a higher phenotypic plasticity than the *A. persimilis* population from Salinas Grandes de Hidalgo (province of La Pampa, Argentina). The *A. persimilis* population seems to be better adapted to low temperatures than *A. franciscana*, but does not tolerate low salinities. These different tolerances to temperature and salinity ranges could explain their present distribution in Argentina where *A. persimilis* is localized from Buenos Aires and La Pampa provinces towards the South of the country, while *A. franciscana* is likely restricted to the Northern provinces, despite its demonstrated colonizing ability and the suspected status of species in evolutionary expansion.

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