# EFFECTS OF SALINITY ON THE REPRODUCTIVE CYCLE OF FEMALE FRESHWATER SHRIMP, *PALAEMONETES ARGENTINUS*

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## ABSTRACT

The South American shrimp *Palaemonetes argentinus* inhabits inland freshwater habitats such as lakes, rivers and streams, but also coastal brackish marshes and lagoons. Hence, this so-called "freshwater" shrimp is expected to be able to reproduce in a wide range of salinities. Here we examined effects of three salinities (1, 15, 25 ppt) on survival and reproductive cycle of females originating from two separate populations in Argentina: 1) Lake Chascomús (LC), a shallow inland lake with low and stable ionic concentrations; and 2) Vivoratá Creek (VC), a lotic stream draining into the adjacent brackish lagoon Mar Chiquita, where salinity is tide-dependent. After an observation period of four months, survival was generally high, without showing significant differences between salinities or populations. Ovarian maturation was measured on a scale ranging from "empty" ovaries (= females with newly laid eggs) to fully developed ovaries (= females ready to spawn). Most LC shrimps (> 80%) developed their ovaries again after an initial egg extrusion (beginning of the experiment) and produced new eggs at all tested salinities. In VC shrimps, ovarian maturation and egg deposition were successful in both the lowest and the highest salinity (1, 25 ppt), but developed ovaries were resorbed in most individuals at the intermediate salinity (15 ppt). Ovarian resorption was never observed at the highest salinity treatment, thus this phenomenon may have been provoked by unknown factors other than ionic concentration. At 15 and 25 ppt, the beginning of vitellogenesis started later and the total time to ovary maturation was longer than at 1 ppt, lengthening in both populations the time between consecutive spawning of eggs by nearly 10 days. Irrespective of the origin of a population, female *P. argentinus* survived and completed all reproductive events under a wide range of salinities, which supports the hypothesis that this species is in an early stage of evolutionary invasion of freshwater habitats.

KEY WORDS: freshwater invasion, ovarian cycles, *Palaemonetes argentinus*, population survival, reproductive plasticity

## INTRODUCTION

There is consensus that palaemonid shrimp (Decapoda: Caridea), a particularly diverse and ecologically important group (Bauer, 2004), evolved in the sea before invading also brackish coastal, estuarine, and eventually limnic habitats (Jalihal et al., 1993; Freire et al., 2003; Augusto et al., 2007). This taxon is therefore a good model for examining physiological and developmental changes associated with evolutionary transitions from marine to freshwater habitats. Freshwater-inhabiting palaemonids, especially species belonging to the genera *Macrobrachium*, *Palaemon*, and *Palaemonetes* show physiological characteristics that may indicate their recent evolutionary history in this habitat, in particular tolerance of high salinities, dependence on saline water for larval development, and reproductive migrations towards the sea (McNamara, 1987; Fidhiany et al., 1991; Freire et al., 2003; Bauer and Delahoussaye, 2008). Species living in estuaries or brackish water commonly show an extended and planktotrophic larval development, which is typical of ancestral marine palaemonids, while some species that have completely adapted to freshwater display an abbreviated larval development with only few non-feeding larval stages of short duration (Jalihal et al., 1993; Odinetz Collart and

Magalhães, 1994). All species belonging to the predominantly marine genus *Palaemon* exhibit, as far as this is known, an extended pattern of larval development, regardless of the salinity conditions prevailing in their habitats (Knowlton and Vargo, 2004). In the genus *Macrobrachium*, an abbreviated developmental mode has evolved in numerous (but not all) species that have successfully invaded freshwater habitats. This pattern is considered as a convergent trait, providing only little taxonomically useful information (Murphy and Austin, 2005).

Several species of *Palaemonetes* have been described from all continents with the exception of Antarctica (Collins, 1998). This widely distributed genus, ecologically important in estuarine and freshwater environments, has received less attention than *Macrobrachium* and *Palaemon* spp., likely because of their limited commercial value and overall similarity (Cheper, 1988; Collins, 1998; Reinsel et al., 2001). Most investigations have concentrated on salinity tolerance of adults (Pannikar, 1941; Sanz, 1984; Lowe and Provenzano, 1990) while effects of salinity on reproductive performance have hardly been investigated (Lowe and Provenzano, 1990). An abbreviated mode of larval development has been observed in some fully limnic

species (Guerao, 1993), whereas an extended pattern has been described for several others living in freshwater and brackish water, in particular in North America and Europe (Fincham, 1979; Knowlton and Kirby, 1984; Knowlton and Schoen, 1984; Knowlton and Vargo, 2004).

Among South American species, the so-called "freshwater" shrimp *Palaemonetes argentinus* Nobili, 1901 exhibits various characteristics that are intermediate between those of truly limnic and marine palaemonids. This widespread shrimp lives in rivers, streams and inland lakes in northern Argentina, Uruguay, and southern Brazil, but also in brackish stream habitats (Müller et al., 2004) and coastal lagoons such as the Laguna Mar Chiquita, Argentina (Spivak et al., 1994; Spivak, 1997). Its extended larval development ($\geq$ 9 zoeal instars) resembles the ancestral pattern (Menú-Marque, 1973). On the other hand, the first zoea is able to reach the following stage without feeding (facultative lecithotrophy), which is more common in limnic than in marine palaemonids (Anger, 2001). Its larval and embryonic development can be completed in a wide range of salinities (Anger et al., 1994; Spivak, 1997; Ituarte et al., 2005). Moreover, all developmental stages of a population from an inland lake are able to cope also with sudden increases in salinity (Ituarte et al., 2008). Despite the fact that the earlier process of development such as ovarian maturation has been previously described by histological studies (Goldstein and Lauria de Cidre, 1974), nothing is known about salinity effects on ovarian cycle in this shrimp species.

The relationship among ovarian maturation, molting, spawning, embryo incubation and hatching has been described in caridean shrimps producing multiple broods per breeding season (see Bauer, 2004). Two main patterns were observed in *Palaemonetes* spp females: successive and alternate breeders. Successive breeders may produce a new brood very soon after hatching, i.e., ovary is mature and ready to spawn just when embryos hatch, after a parturial (spawning) molt (Bauer, 2004). *Palaemonetes pugio* Holthuis, 1949 is an example of such a pattern, although sometimes ovarian maturation is not completed until several days after hatching and the subsequent parturial molt is thus delayed (Bauer and Abdalla, 2000). Alternate breeders, the most common pattern of brood production in the genus *Palaemonetes* (Bauer, 2004), produce a new brood at every other molt, i.e., ovary is immature when embryos hatch, after hatching a non parturial molt occurs and ovarian maturation begins just after this molt.

The present study examines the hypothesis that *P. argentinus* is, on an evolutionary scale, in an early stage of invasion of freshwater environments. If this hypothesis is correct, then adults should survive and reproduce in a wide range of salinities, independent of their habitat of origin (brackish or freshwater). This prediction was tested under controlled experimental conditions analysing effects of three salinity treatments (1, 15 and 25 ppt) on female survival and ovarian maturation in shrimps originating from two geographically and ecologically separated populations living in habitats with different degrees of isolation from the sea. In addition, we present preliminary observations on the relationship of ovarian development, molting and spawning in this shrimp species at different salinity treatments.

## MATERIALS AND METHODS

### Study Sites

The study sites are located in the same geographic area, the Province of Buenos Aires, Argentina. Lake Chascomús (LC) is a shallow eutrophic lake with very low salinities (see Ituarte et al., 2007) varying between oligohaline (0.5-5 ppt) and hyposaline conditions (< 0.5 ppt), depending on rainfall, evaporation and winds (Maizels et al., 2003). It belongs to a system of shallow lakes ("Las Encadenadas de Chascomús") that are connected to the Salado River (Torremorell et al., 2009), which eventually drains into the southwestern part of the Río de la Plata estuary (Samborombón Bay, Fig. 1). The sampling site is nearly 70 km away from the Atlantic Ocean.

Vivoratá Creek (VC) is a lotic habitat with tide-dependent salinity variations ranging between mesohaline (5-18 ppt) and oligohaline conditions (0.5-5 ppt). The creek drains into the narrow southern part of the brackish coastal lagoon Mar Chiquita (Fig. 1), where strong seasonal, daily and local variations of salinity occur, depending on tides, direction and force of winds, and rainfall (Anger et al., 1994; Spivak et al., 1994). The sampling site is only about 3 km away from the Atlantic Ocean.

### Experimental Procedures

Females with fully developed ovaries and adult males were collected at the end of September 2005 in LC and at the beginning of November 2005 in VC, using a hand net (45 cm width, 30 cm deep, and 1 mm mesh size). Average salinity and temperature values, measured during the collections with a U-10 Horiba water quality checker, were $1 \pm 0$ ppt, $16.9 \pm 0.4°C$ in LC, and $2.4 \pm 0.8$ ppt and $23.5 \pm 0.4°C$ in VC (mean $\pm$ SD; $n = 5$ measurements).

In the laboratory, the shrimps were sorted in breeding pairs and kept in individual plastic containers (500 ml). The bottom of each container was covered with a layer (ca. 1 cm) of small stones, and an empty bivalve shell was offered as a refuge. A total of 90 breeding pairs were reared at three different salinities (1, 15, 25 ppt; 15 pairs per salinity treatment and population). Shrimps were transferred to each treatment in progressive acclimation steps of 2 h at 1, 5, 10 and 15 ppt (where applicable). All solutions were made by dilution of filtered seawater (Schleicher and Schuell filter paper 0859, pore size ca. 7-12 μm) with dechlorinated tap water. Aquaria were provided with constant aeration. The conditions of temperature ($21 \pm 2°C$) and light (14:10 h L:D cycle) simulated summer conditions. Every morning, a few drops of freshly hatched nauplii of *Artemia* spp and fish food (TetraMin Pro®; lipids 12%; proteins 46%) were given as food. Twice a week, the shrimps were fed with small pieces of fresh squid tentacles. Excess food was regularly removed from each container. Water was changed every 3-4 days. Breeding pairs were examined every morning, and events of death, molting, egg-laying, egg loss, or larval hatching, as well as the characteristics of the ovary (see below), were recorded daily. Dead shrimps (females and males) were replaced until day 6 of the experiments in order to maintain the same initial number of observed females. After this time, all dead shrimps were removed but not replaced.

The paired ovaries of *P. argentinus* are situated in the cephalothorax, dorsally to the cardiac stomach and the hepatopancreas. They are visible through the thin transparent exoskeleton, as described also for other caridean shrimps (Bauer, 2004). The ovarian cycle can be described for live animals on the basis of changes in size and color of the gonad, and four stages of ovary maturation are usually defined (Bauer and Abdalla, 2000). The characteristics that define each stage in this study were the following: stage 1 or "empty" ovaries, corresponding to females with newly laid eggs. No evidence of ovarian development but, occasionally, translucent oöcytes were observed under a stereomicroscope in the "ovarian zone," i.e., between the cardiac stomach and the heart. Stage 2, ovaries pale gray, filled with oöcytes; cardiac stomach and hepatopancreas visible; stage 3, ovaries brown or green, filled with oöcytes; ovarian filaments larger, occupying the space between the anterior and the posterior part of the cephalotorax (near the heart); stage 4, fully developed ovaries, filling most of the cephalothorax and the first pleomere; cardiac stomach and hepatopancreas no longer visible; females ready to lay eggs.
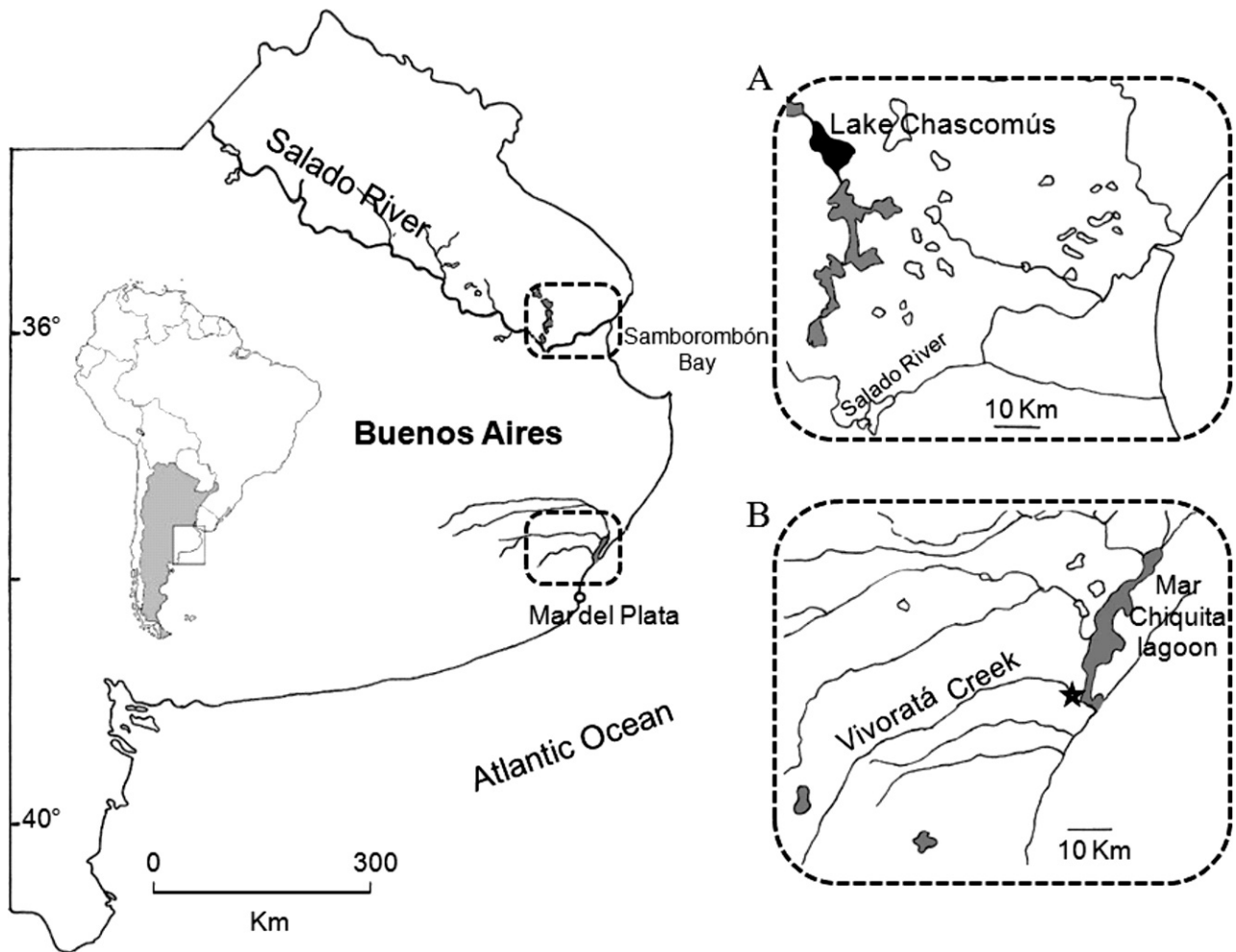
Fig. 1.  Location of the *Palaemonetes argentinus* populations studied in the Province of Buenos Aires, Argentina. A, System of shallow lakes ''Las Encadenadas de Chascomús,'' Lake Chascomús in black; B, the star shows the sample site in Vivoratá Creek.

Although ovarian maturation process is a continuum, the start of maturation could be unequivocally defined as the day when the ovary was seen colored (pale gray) for the first time.

The experiments began when field-caught females with fully developed ovaries (stage 4) spawned. They were terminated when either a subsequent brood was laid in the laboratory, or if the ovaries had not matured by day 110. The following variables were recorded:

1) percentage of *i)* survival (initial $n = 15$ females); *ii)* females that failed in complete egg incubation; and *iii)* females that produced a second clutch;
2) days elapsed between *i)* first spawning and the start of ovarian maturation (denoted as ''time to start of maturation''); *ii)* first spawning and egg loss (denoted as ''time to egg loss''); *iii)* first spawning and hatching (embryonic development); *iv)* the start of ovarian development and second spawning (denoted as ''time to egg-laying''); *v)* hatching and parturial molt; *vi)* hatching or egg loss and first non parturial molt; *vii)* first (or second or third) non parturial molt and second spawning; *viii)* first non parturial molt and second non parturial molt; *ix)* second non parturial molt and third non-parturial molt; and *x)* consecutive spawnings (denoted ''time between clutches'').

### Statistical Analysis

Survival curves were compared between salinities and populations using a nonparametric LogRank test, a specific test of the Kaplan-Meier survival analysis (statistics software: SigmaStat® for Windows, Version 3.5). It provides a chi-square statistic, assuming that all survival time data are equally accurate and all data will be equally weighted in the analysis. Differences in periods of time (''to egg loss,'' ''to start of maturation,'' ''to egg-laying,'' ''between clutches'') were evaluated by two-way ANOVA with unequal replication, with salinities and populations as factors (Zar, 1996). All ANOVAs were performed after checks for normal distribution and equality of variance (Kolmogorov-Smirnov and Cochran tests, respectively; Underwood, 1997). Data were transformed when necessary. When ANOVA indicated significant differences between treatments, they were tested *a posteriori* with the Honestly Significant Difference (HSD) test for different sampling sizes (Zar, 1996).

### RESULTS

#### Survival

At least 86% of the females survived at the lowest and intermediate salinities, but mortality rates were high at 25 ppt, especially during the first two weeks of the experiment (Fig. 2). Despite seemingly higher mortality at 25 ppt, these survival curves did not differ significantly among salinities (LC: $\chi^2 = 3.04$, $d.f. = 2$, $P = 0.22$; VC: $\chi^2 = 5.55$, $d.f. = 2$, $P = 0.06$; Fig. 2). Likewise, no significant differences in mortality were detected between populations ($\chi^2 = 2.4$, $d.f. = 1$, $P = 0.12$).
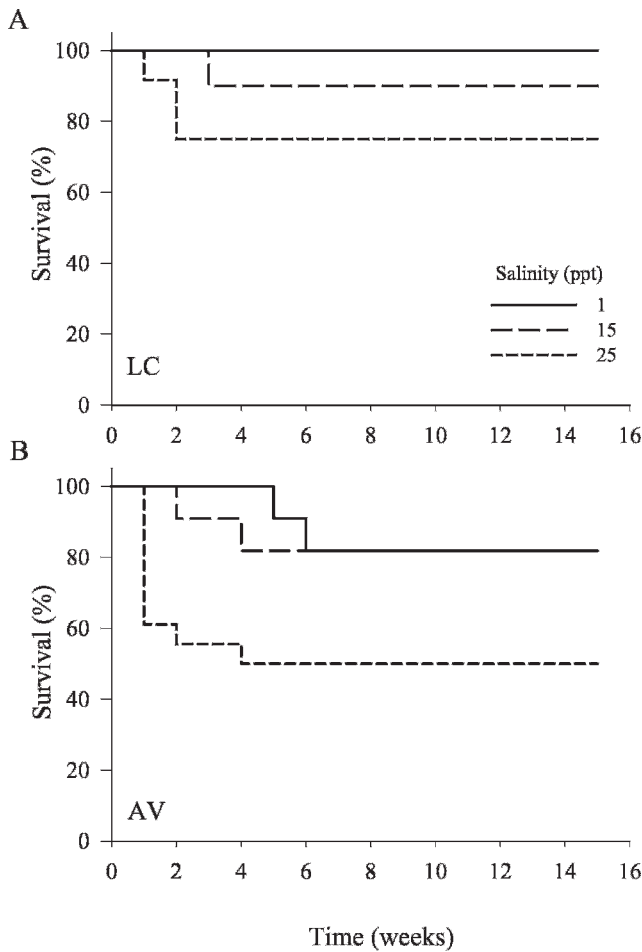
Fig. 2. LogRank survival graph showing the percentage of females of *Palaemonetes argentinus* from (A) Lake Chascomús (LC) and (B) Vivoratá Creek (VC) that survived at three salinities (1, 15, 25 ppt) during the study period.



Fig. 3. Egg loss of *Palaemonetes argentinus* in Lake Chascomús (LC) and Vivoratá Creek (VC) at three salinity treatments (1, 15, 25 ppt). A, Percentage of females incapable of keeping their first clutch of eggs during the first week of experiment. Number of females alive in each treatment is indicated above bars. B, Days (mean ± SD) elapsed between first spawning and egg loss, above bars the number of shrimps.

## Salinity Effects on the Female Reproductive Cycle

All females with pre-spawning ovaries (ovarian stage 4) collected from the field and individually paired with males went through a parturial (spawning) molt and subsequently became ovigerous; however, some lost their eggs. The highest and lowest percentage of shrimps that failed to retain their first clutch in the laboratory was observed in LC at 25 ppt and in VC at 1 ppt (53 and 6%, respectively; Fig. 3A). The time from spawning to egg loss was similar between treatments ($F_{1, 15} = 0.74, P = 0.4$) and populations ($F_{1, 15} = 0.22, P = 0.6$), and there was no significant interaction between these factors ($F_{1, 15} = 2.7, P = 0.12$; Fig. 3B). It averaged 4.04 ± 1.71 days.

Alternate breeding was the most common pattern of brood production in both populations, mainly at the lowest salinity treatment (Table 1). A non-parturial molt (without spawning) occurred within 1-3 days after the hatching of embryos or within 1-28 days after egg loss, and after this molt ovarian maturation occurred, leading to the next spawn (Table 1). However, one shrimp from LC (1 ppt) had the ovary mature at hatching, and had a parturial (spawing) molt only 3 days after hatching, i.e., fast successive parturial (Table 1). Two other patterns of brood
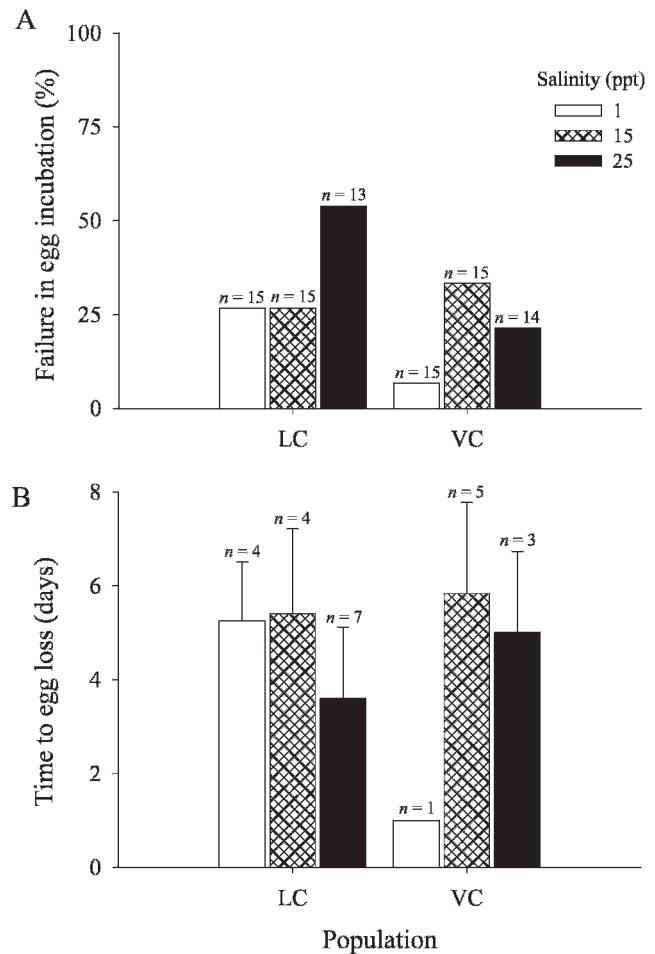
production were observed in both populations mainly at the intermediate and highest salinity treatments: ovary maturation took place after two or even three non-parturial molts prior to the next spawn (Table 1).

Shrimps from both populations were able to complete ovarian maturation and produce a second clutch of eggs under all experimental conditions (Fig. 4A, Table 1), including females that were incapable of incubating their first brood. In nine of the twelve shrimps from VC kept at 15 ppt ovaries were resorbed after they had reached stages 2 or 3 of maturation, whereas just one individual was unable to continue ovarian cycle and it remained in stage 1 (NOM in Table 1). Ovarian resorption was never observed in shrimps kept in other treatments, whereas three females at different treatments remained with ovaries in stage 1 until the experiments were finished (Table 1).

The periods of time "to start of maturation," "to egg-laying," and "between clutches" were significantly affected by salinity, but there were no significant differences between populations or interactions between these factors (Table 1; Fig. 5). All these periods were significantly shorter at 1 ppt than at higher salinities, while no significant differences

Table 1. Patterns of brood production and timing of reproductive events observed in *Palaemonetes argentinus* females from Lake Chascomús (LC) and Vivoratá Creek (VC) at three salinity treatments (1, 15, 25 ppt). Three patterns of brood production (see Bauer and Abdalla, 2000) were observed in *P. argentinus*: A, alternate breeders, after either hatching or egg loss a single non-parturial molt (first NP molt) occurred, then ovarian maturation begun, leading to the production of the second spawning; FS, fast successive breeders: a parturial (spawning) molt occurred just after the first brood hatched; O, other breeders: after either hatching or egg loss two or three non-parturial molts occurred, then ovarian maturation begun, and a second spawning was produced. The ovaries of some females, denoted as NOM, did not maturate during the study period. *n*: number of observed shrimps; NP: non-parturial molts; P: parturial (spawning) molt; Re: ovary resorption. Data are mean days ± SD.

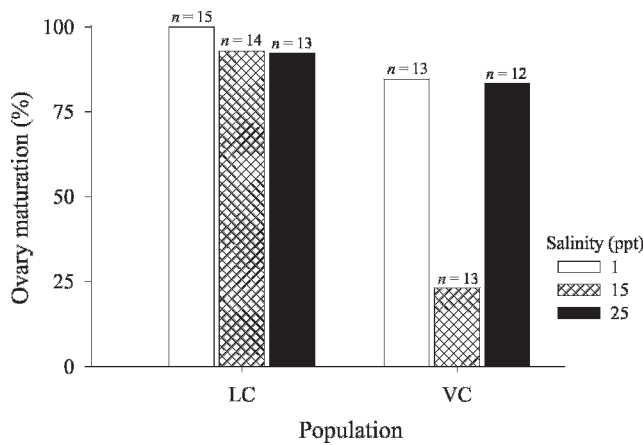| Population | Salinity (ppt) | Pattern of brood production | n | Time between events (days ± SD) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1st spawning - hatching | 1st spawning - egg loss | Hatching - P molt | Hatching/egg loss - 1st NP molt | 1st NP molt - 2nd spawning | 1st NP molt - 2nd NP molt | 2nd NP molt - 2nd spawning | 2nd NP molt - 3rd NP molt | 3rd NP molt - 2nd spawning | Total (1st spawning - 2nd spawning) |
| LC | 1 | A | 10 | 24.9 ± 0.7 | - | - | 1.4 ± 0.8 | 15.5 ± 1.3 | - | - | - | - | 42.8 ± 1.9 |
| | 1 | A | 4 | - | 4.7 ± 1.5 | - | 21.7 ± 1.5 | 15 ± 0.8 | - | - | - | - | 42.2 ± 1.2 |
| | 1 | FS | 1 | 27 | - | 3 | - | - | - | - | - | - | 30 |
| | 15 | A | 7 | 25.8 ± 1.1 | - | - | 1.8 ± 0.7 | 23.6 ± 9.1 | - | - | - | - | 52 ± 9.1 |
| | 15 | A | 2 | - | 5.5 ± 2.1 | - | 21 ± 4.2 | 17 ± 1.4 | - | - | - | - | 45 ± 1.4 |
| | 15 | O | 2 | 26 ± 0 | - | - | 2 ± 1.4 | - | 19 ± 4.2 | 19.5 ± 2.1 | - | - | 74 ± 8.5 |
| | 15 | O | 2 | - | 4.5 ± 0.7 | - | 23.5 ± 0.7 | - | 19.5 ± 0.7 | 17.5 ± 0.7 | - | - | 66 ± 0 |
| | 15 | NOM | 1 | 26 | - | - | 3 | - | 18 | - | - | - | - |
| | 25 | A | 3 | 26.3 ± 0.6 | - | - | 2 ± 0 | 20.6 ± 4.5 | - | - | - | - | 50 ± 4 |
| | 25 | A | 6 | - | 2.2 ± 1.5 | - | 19 ± 11.1 | 24 ± 7.1 | - | - | - | - | 46.5 ± 10.3 |
| | 25 | O | 1 | - | 3 | - | 20 | - | 18 | 26 | - | - | 59 |
| AV | 1 | A | 11 | 22.2 ± 0.9 | - | - | 1.7 ± 1.2 | 18.3 ± 5.7 | - | - | - | - | 42 ± 0.6 |
| | 1 | O | 1 | 22 | - | - | 5 | - | 16 | 18 | - | - | 62 |
| | 1 | NOM | 1 | 24 | - | - | 3 | - | 18 | - | - | - | - |
| | 15 | A | 1 | 24 | - | - | 2 | 36 | - | - | - | - | 62 |
| | 15 | A | 2 | - | 5 ± 1.4 | - | 15.5 ± 4.9 | 20.5 ± 2.1 | - | - | - | - | 46 ± 1.4 |
| | 15 | Re | 3 | 23.7 ± 1.5 | - | - | 2 ± 1 | - | 18.7 ± 1.1 | - | 21.3 ± 3 | 15 | - |
| | 15 | Re | 3 | 22.7 ± 2.5 | - | - | 11.3 ± 14.7 | - | 17.7 ± 3.8 | - | - | - | - |
| | 15 | Re | 2 | - | 2 ± 1.4 | - | 23 ± 1.4 | - | 29.5 ± 17.7 | - | - | - | - |
| | 15 | Re | 1 | - | 6 | - | 23 | - | 23 | - | 23 | 23 | - |
| | 15 | NOM | 1 | 20 | - | - | 6 | - | 16 | - | 23 | 15 | - |
| | 25 | A | 4 | 24.7 ± 3.8 | - | - | 1 ± 1 | 20.5 ± 5.7 | - | - | - | - | 46.2 ± 6.4 |
| | 25 | A | 2 | - | 5.5 ± 3.5 | - | 13.5 ± 6.4 | 18.5 ± 0.7 | - | - | - | - | 53 ± 12.7 |
| | 25 | O | 2 | 20.5 ± 6.4 | - | - | 0.5 ± 0.7 | - | 11.5 ± 3.5 | 24 ± 12.7 | - | - | 56.5 ± 2.1 |
| | 25 | O | 1 | 22 | - | - | 1 | - | 6 | - | 15 | 5 | 49 |
| | 25 | O | 1 | - | 3 | - | 23 | - | 15 | 16 | - | - | 57 |
| | 25 | NOM | 1 | 18 | - | - | 5 | - | - | - | - | - | - |

Fig. 4. Ovarian maturation of *Palaemonetes argentinus* in Lake Chascomús (LC) and Vivoratá Creek (VC) at three salinity treatments (1, 15, 25 ppt). Bars show the percentage of shrimps successful in completing ovary maturation and producing a second clutch, number of females alive in each treatment is indicated above bars.

were detected between 15 and 25 ppt (Fig. 5). The interval between egg-laying and the start of ovary maturation was $26.4 \pm 3.5$ days at 1 ppt vs. $33.8 \pm 8.1$ (average time for 15 and 25 ppt, HSD, $P < 0.01$; Fig. 5A). The time elapsed between the start of ovary maturation and egg-laying lasted $16.3 \pm 4.0$ days at 1 ppt vs. $19.4 \pm 4.0$ days on average for higher salinities (HSD, $P < 0.05$; Fig. 5B). Finally, the period between consecutive egg-laying events was $42.8 \pm 5.7$ at 1 ppt vs. $52.3 \pm 8.9$ days (average time for 15 and 25 ppt, HSD, $P < 0.05$; Fig. 5C).

## DISCUSSION

Our experimental data demonstrate that the "freshwater" shrimp *P. argentinus* survives for extended periods in a wide range of salinities (1-25 ppt), and that it is able to reproduce successfully under these conditions. Survival rate did not differ significantly among salinities in both populations, probably due to the low number of individuals, but a clear trend was observed: VC shrimps seemed to be more

Table 2. Two-way ANOVAs for timing of the ovarian cycle events observed in *Palaemonetes argentinus* from Lake Chascomús (LC) and Vivoratá Creek (VC) at three salinity treatments (1, 15, 25 ppt). A, Days elapsed between first spawning and the start of ovarian maturation; B, days elapsed between the start of ovarian development and second spawning, and C, days elapsed between consecutive spawnings. MS: mean squares.

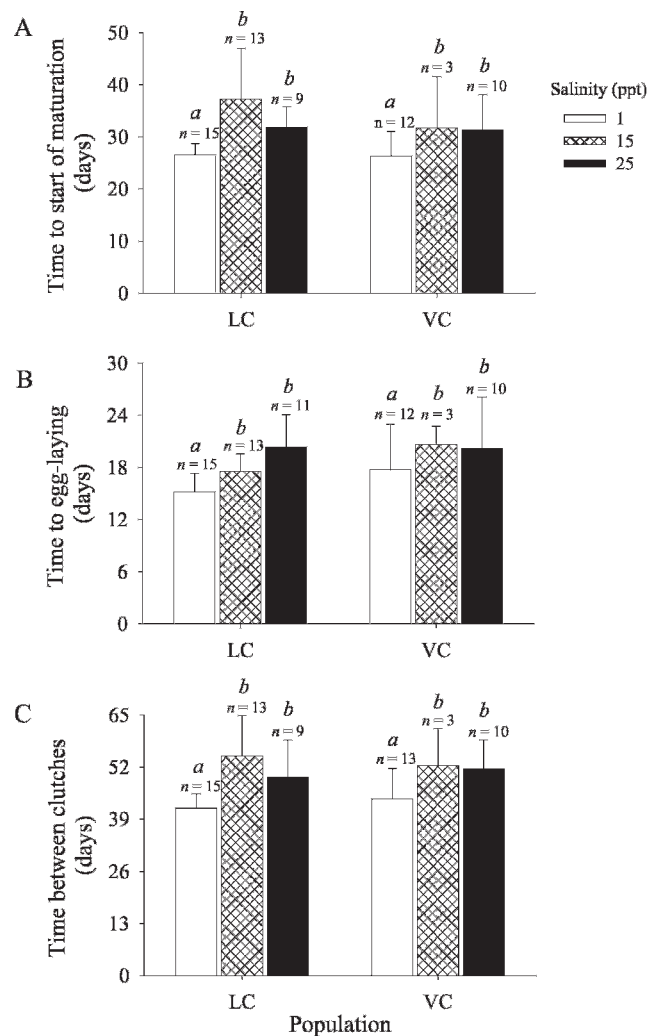|   | Source of variation | d.f. | MS | F | P |
|---|---|---|---|---|---|
| A | Salinity | 2 | 0.00028 | 8.95 | < 0.001 |
|   | Population | 1 | 0.000063 | 2.01 | 0.16 |
|   | Salinity * population | 2 | 0.000015 | 0.49 | 0.62 |
|   | Error | 54 | 0.000031 | | |
| B | Salinity | 2 | 0.00094 | 8.97 | < 0.001 |
|   | Population | 1 | 0.00029 | 2.74 | 0.1 |
|   | Salinity * population | 2 | 0.00016 | 1.58 | 0.21 |
|   | Error | 58 | 0.0001 | | |
| C | Salinity | 2 | 0.000099 | 8.22 | < 0.001 |
|   | Population | 1 | 0.000003 | 0.29 | 0.59 |
|   | Salinity * population | 2 | 0.000003 | 0.28 | 0.76 |
|   | Error | 56 | 0.000012 | | |



Fig. 5. Timing of the ovarian cycle events observed in *Palaemonetes argentinus* from Lake Chascomús (LC) and Vivoratá Creek (VC) at three salinity treatments (1, 15, 25 ppt). A, Days elapsed between first spawning and the start of ovarian maturation; B, days elapsed between the start of ovarian development and second spawning, and C, days elapsed between consecutive spawnings. Different letters indicate significant differences among salinities; number of shrimps in each treatment is shown above bars.

susceptible to the highest salinity treatment than LC shrimps. It is interesting to note that in their natural habitat the populations studied here are not normally exposed to strong variations in salinity. The highest average salinity reported before for Lake Chascomús (LC) was 1.5 ppt (Maizels et al., 2003), and the highest single value registered during the present study was 3 ppt. Vivoratá Creek (VC) is directly connected with Mar Chiquita Lagoon, where strong salinity variations occur (Anger et al., 1994; Spivak et al., 1994). During a four-year sampling period (Ituarte, 2008), we occasionally registered salinity values up to 15 ppt. Therefore, salinities we measured at both sites are much lower than the two higher salinities assayed in our experiments. Accordingly, the strong salinity tolerance observed in both populations is likely associated with their evolutionary history rather than the present salinity conditions in their respective habitats.

In most decapods, successful reproduction usually takes place in a narrower range of salinities than required for

survival (Sastry, 1983). Shrimps from the VC population kept at 25 ppt showed a clear increase in mortality at the beginning of our experiment, but more than 83% of the individuals developed their ovaries and produced eggs in this treatment (Figs. 2, 4). Moreover, if the intermediate salinity (15 ppt) would have caused the observed ovarian resorption in VC shrimps, then we should expect an even stronger salinity effect on ovarian maturation at 25 ppt. Therefore, unknown factors other than high salinity may explain the initial mortality at 25 ppt and the ovarian resorption at 15 ppt in shrimps from the VC population. For example, it has been shown that parasitic isopods may reduce salinity tolerance and inhibit ovary maturation in caridean shrimps (Moles and Pella, 1984; Calado et al., 2005). Bopyrid-parasitized shrimps were found at VC, but never in the LC population. Although we used unparasitized shrimps in our experiments, juvenile isopods or other parasites (see Martorelli and Schuldt, 1990; Anderson, 1990) could have remained undetected. Future studies should thus evaluate the effects of prevalence and density of parasite larvae on the salinity tolerance of *P. argentinus* (Pung et al., 2002, 2006). In addition, the resorption of developing oöcytes (= oösorption) in the ovary may occur under stressful conditions (Hornung and Warburg, 1994); oösorption in response to physiological conditions, unfavorable environmental factors, or both, is a well documented phenomenon in insects and crustaceans (Kotaki, 2005; Hinsch, 1992, respectively). Despite the fact that no single clear explanation is possible for the ovarian resorption of *P. argentinus* observed in only one population and in a particular salinity treatment, we think that this phenomenon could be accounted for by variation in the internal condition of females rather than ionic concentration.

Salinity changes during oöcyte maturation may modify egg characteristics affecting the quality of the larvae, and eventually, population success. In *P. argentinus*, the delay of the beginning of ovary maturation and the lengthened ovarian maturation at high salinities (15, 25 ppt) suggest physiological costs for both the start of vitellogenesis and the process of yolk production in growing oöcytes. Moreover, there was a longer interval between spawnings of consecutive clutches at 15 and 25 ppt, which was caused by both a delay in the beginning of a new ovarian cycle and a longer process of ovarian maturation (Fig. 5). These results indicate that populations of *P. argentinus* living in environments with low and stable ion concentrations can produce more egg clutches in a single reproductive season. In this sense, the fitness of females is enhanced at 1 ppt, suggesting that freshwater is a better condition for reproduction in this species. On the other hand, ionic composition varies in salts from marine and freshwater origin, which may affect physiological mechanisms involved in salinity tolerance of freshwater invertebrates (see Zalizniak et al., 2006). The extreme euryhalinity of *P. argentinus* makes it a suitable model for investigations of the effects of different proportions of ions, especially of key ions such as $Ca^{+2}$ and $Mg^{+2}$, on the structure and function of yolk lipoproteins in growing oöcytes, as well as carry-over effects on embryos and larvae.

Reproduction of *P. argentinus* occurred in a wider range of salinities than reported for a freshwater shrimp from the United States, *Palaemonetes paludosus* (Gibbes, 1850), which is able to lay eggs at salinities up to 20 ppt but not at 25 ppt. In addition, the percentage of ovigerous females dropped in *P. paludosus* drastically at 10 ppt (Lowe and Provenzano, 1990). This intrageneric difference may be related to the fact that *P. paludosus* has an abbreviated larval development, whereas *P. argentinus* has an extended mode, which is typical of marine or brackish-water inhabiting rather than limnic species (Menú-Marque, 1973; Anger, 2001).

In summary, reproductive events in *P. argentinus* are affected by high salinities, even though reproduction was successful irrespective of population origin. The response of ovary maturation to salinity treatments in terms of start and time to development was highly flexible indicating that the process of vitellogenesis is in this species a plastic trait, and salinity does not seem to be a limiting factor for the occurrence of stable populations in both freshwater and brackish water environments. Future studies should explore mechanisms that allow this species to reproduce successfully in a wide salinity range. Regardless of population origin, the ability of this "freshwater" shrimp to survive and reproduce also at relatively high salt concentrations (25 ppt) supports the view that this species is still in an early evolutionary stage of its invasion of freshwater environments (Menú-Marque 1973; Anger 2001).

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