

Ontogeny of osmoregulation in the palaemonid shrimp *Palaemonetes argentinus* (Crustacea: Decapoda)

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ABSTRACT: Osmoregulation was studied in the zoeal stages I and VI, the first decapodid, the first juvenile, and in adults of the palaemonid shrimp *Palaemonetes argentinus*. The larvae hatch in freshwater creeks or in adjacent brackish coastal lagoons of the warm temperate southwestern coast of the Atlantic Ocean; larval development is possible in low salinities. To cope with these demanding environments, the capacity for osmoregulation is well developed at hatching, increasing only slightly throughout development. All the postembryonic developmental stages hyper-regulated at low salinity (1 to 10‰), hyper-osmoconformed at 17‰, and osmoconformed at higher salinities (26‰; up to 32‰ in adults). The type of osmoregulation did not change during development from larval hatching through the adult phase. The ecological implications and the evolutionary significance of osmoregulation in early life-history stages of *P. argentinus* and other aquatic crustaceans are discussed.

KEY WORDS: Osmoregulation · Ontogeny · Adaptation · Crustacea · Caridea · Palaemonidae · Larva *Palaemonetes*

INTRODUCTION

Salinity is one of the main environmental factors exerting a selection pressure on aquatic organisms. The ability of a species to adapt to environmental salinity and its fluctuations is thus a major adaptive process achieved through different behavioral and/or physiological mechanisms, among which osmoregulation is of major importance for some animal groups, including crustaceans. In adult crustaceans, osmoregulation has been studied in numerous species (reviews in Mantel & Farmer 1983, Péqueux 1995). More recently, several studies have stressed the adaptive importance of osmoregulation throughout the development of crustaceans, underlining the fact that the ability of each developmental stage to adapt to salinity and its variations is one of the factors permitting the successful

establishment of a species in a given habitat (review in Charmantier 1998). But the number of species in which the ontogeny of osmoregulation has been studied is still limited, and it was recently stated that future investigations should be conducted in species submitted to harsh environments (extreme and/or highly variable salinity) during their development (Charmantier 1998, Charmantier et al. 1998).

Among the candidate species in a series of studies in this field is the shrimp *Palaemonetes argentinus* (Nobili 1901) (Decapoda: Caridea: Palaemonidae). This species is found in an area covering the north and center of Argentina, Uruguay and southern Brazil (Spivak 1997). Although it is generally considered a freshwater species, it has also been found in brackish coastal lagoons extending along the coast of the warm temperate southwestern Atlantic Ocean (review in Spivak 1997), particularly in Mar Chiquita lagoon, Argentina, in which ample variations of salinity have been reported (Anger et al. 1994).

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The larval development of *Palaemonetes argentinus* was described from laboratory-reared shrimp (Menú-Marque 1973). It comprises a variable number of zoeal and decapodid stages. Under natural conditions, the biological cycle of *P. argentinus* can be accomplished in either freshwater or brackish water (review in Spivak 1997). The life history of a population living in the brackish lagoon of Mar Chiquita, Argentina, and in adjacent freshwater creeks has recently been described by Spivak (1997). All developmental stages of this species have been found together in this lagoon, indicating larval development within the parental habitat (Anger et al. 1994).

Since coastal lagoons like Mar Chiquita are characterized by highly variable salinity regimes, fluctuating between freshwater and seawater (Anger et al. 1994), this species is exposed to a wide salinity range throughout its life cycle. The objectives of the present study were thus (1) to determine the ability of selected postembryonic stages of development to osmoregulate, and (2) to relate their osmoregulatory capabilities with available data on the developmental ecology of this species.

MATERIALS AND METHODS

Specimens: origin and culture. Juvenile shrimp *Palaemonetes argentinus* were collected near the mouth of a freshwater creek, Arroyo Sotelo, flowing into the Mar Chiquita lagoon, Argentina (for more details of the habitat see Spivak 1997), and transported live in water from the capture site (1‰ salinity) to the marine biological station Helgoland, Germany. They were reared to adulthood at 1‰, 24°C and a 12 h light:12h dark cycle, with *Artemia* sp. nauplii or isopods (*Idotea* spp.; in late juvenile stages only) given as food.

Since preliminary experiments (Anger unpubl.) had shown that larval development requires brackish water, larvae were reared from hatching through metamorphosis at 5‰, using aerated beakers (1 l; other conditions as in juvenile rearing). Water and food (freshly hatched *Artemia* sp. nauplii) were changed daily. The developmental stages used in experiments were the first and the last zoeal stage (zoea I, VI), the first decapodid (postembryonic instar VII), the first juvenile stage (reached after a total number of 6 zoeal and 2 to 5 decapodid stages; for terminology of larval stages see Williamson 1982) and adults (males and females, total body length ranging from 2.4 to 3.6 cm).

The duration of successive larval stages was 2 to 3 d. Stages within each molting cycle (Drach 1939) were determined according to the time elapsed since the last preceding ecdysis, distinguishing between the initial postmolt stages (A and B), intermolt (C) in the middle,

and premolt (D) near the end of the molt cycle. Hemolymph samples were collected exclusively from stage C individuals. In zoeal, decapodid and first juvenile stages, this was defined as the middle of the instar period. The validity of this staging method was occasionally confirmed through microscopical observations taking the telson or uropods as reference body parts (Drach 1939, Drach & Tchernigovtzeff 1967, Anger 1983). In adult shrimps, stage C individuals were selected after microscopical observation of the uropods (Drach & Tchernigovtzeff 1967).

Preparation of media. Experimental media were prepared and stored in 5 l plastic containers for the entire duration of the experiments. Dilute media were prepared by adding desalinated freshwater to natural North Sea water. All experiments were conducted at a constant temperature of 24°C. Salinities were expressed as osmotic pressure (in mOsm kg⁻¹) and as salt content of the medium (in ‰); a value of 3.4‰ is equivalent to 100 mOsm kg⁻¹ (29.41 mOsm kg⁻¹ per 1‰). The osmotic pressure of the media was measured with a micro-osmometer Model 3 MO (Advanced Instruments, Needham Heights, MA, USA) requiring 20 µl sample⁻¹. Media with the following osmolalities (mOsm kg⁻¹) and corresponding salinities (‰) were prepared and used for all stages: 31 mOsm kg⁻¹ (1.1‰), 77 (2.6), 156 (5.3), 302 (10.3), 500 (17.0), and 755 (25.7). Adult shrimp were also exposed to seawater, 947 mOsm kg⁻¹ (32.2‰).

Osmoregulation. Zoeal and decapodid stages were exposed to the experimental media in covered petri dishes. In decapodids, a particular escape behavior (jumping) had to be prevented by placing additionally a sheet of nylon gauze (200 µm mesh size) on the water surface. Later stages (juvenile I, adults) were placed in glass vials (250 ml and 1 l capacity, respectively). These were covered with a convex glass lid, the bottom of which touched the surface of the media to prevent jumping; at the margins of the vials, a ring of air allowed for gas exchange.

Hemolymph osmolality was determined for each stage after a period of osmotic stabilization in each medium. Based upon results from previous studies on different species (Charmantier 1998), larvae and first juveniles were kept for 24 h in each medium before sampling; the acclimation time in adults was 48 h. Larvae and juveniles were superficially dried on filter paper, then quickly immersed in mineral oil to avoid evaporation and desiccation. The remaining adherent water was aspirated through a glass micropipette. Another micropipette was then inserted in the heart to sample hemolymph. In adult shrimp, hemolymph was collected from a cut fourth or fifth pereopod, after carefully drying it with filter paper.

For all developmental stages, hemolymph osmolality was measured with reference to the medium osmolality on a Kalber-Clifton nanoliter osmometer (Clifton

Technical Physics, Hartford, CT, USA) requiring about 30 nl. The results were expressed either as hemolymph osmolality or as osmoregulatory capacity (OC), defined as the difference between the osmolalities of hemolymph and the medium. Analysis of variance (ANOVA) and Student's *t*-tests were used for multiple and pairwise statistical comparisons of mean values, respectively, after appropriate checks for normal distribution and equality of variance (Sokal & Rohlf 1995).

RESULTS

Osmoregulation by the developmental stages was evaluated over a wide range of salinities. The results are given as variations in the hemolymph osmolality and as OC in relation to the osmolality and salinity of the medium (Figs. 1 & 2).

The pattern of osmoregulation did not change during development. All tested stages hyper-regulated in the dilute media (<17‰), hyper-osmoconformed at 17.0‰ and isoconformed at 25.7‰; the adult shrimp isoconformed also in seawater (32.2‰). Thus, the pattern of hyper-iso-osmoregulation was present already in the zoea I, and it persisted in the decapodid, juvenile and adult stages of the life cycle (Fig. 1).

The ability to hyper-osmoregulate at low salinity tended to increase slightly during early development, as demonstrated by variations in hemolymph osmolality (Fig. 1) or in OC (Fig. 2). At very low salinities (1.1 and 2.6‰), the hyper-OC did not change between zoeal stages I and VI, then it increased slightly in the deca-

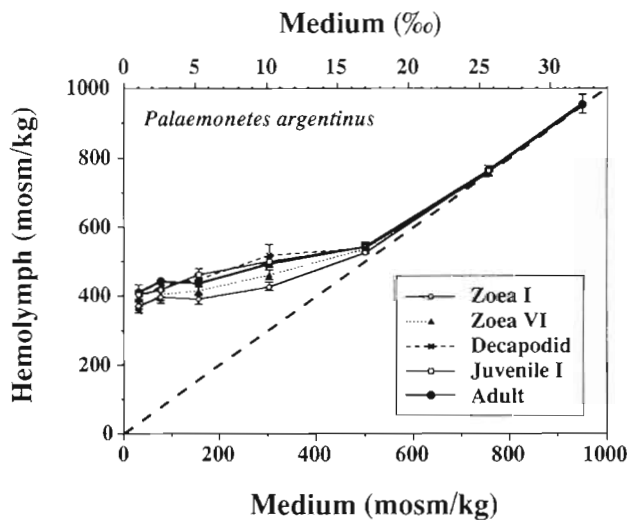


Fig. 1. *Palaemonetes argentinus*. Variations of the hemolymph osmolality in different stages of postembryonic development in relation to the osmolality and salinity of the medium at 24°C; error bars: $\bar{x} \pm SD$; n = 4 to 10 (zoeae I and VI) and 7 to 12 (later stages) individuals; dashed line: isoconcentration

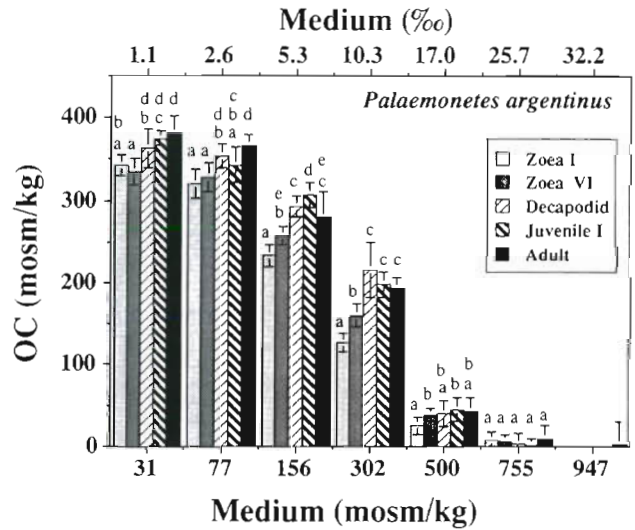


Fig. 2. *Palaemonetes argentinus*. Variations of the osmoregulatory capacity (OC) in different stages of postembryonic development in relation to the osmolality and salinity of the medium at 24°C; error bars: $\bar{x} \pm SD$; n = 4 to 10 (zoeae I and VI) and 7 to 12 (later stages) individuals; different letters above error bars indicate significant differences between stages (p < 0.05)

podid stage. No important variation in OC was noted in later developmental stages, i.e. in the juvenile I and adults. At moderately low salinities (5.3 and 10.3‰), the hyper-OC increased significantly from zoea I to zoea VI and the decapodid stage. In subsequent stages, it remained almost unchanged, with a slight tendency to decrease in adults at 5.3‰. At 17.0 and 25.7‰, there was little ontogenetic variation in OC. At 25.7‰, all stages osmoconformed, as did the adults at 32.2‰.

Since 32.2‰ is an unusually high salinity for *Palaemonetes argentinus* under natural conditions, a group of 13 adult shrimps was exposed for several days to this medium. The water was aerated and changed regularly, and the shrimps were fed. The median lethal time (LT 50) for 7 of them was 11 d. No mortality was noted in a control group of shrimp maintained for the same time at 1‰.

DISCUSSION

Among the decapod species which have been studied for their osmoregulation, most marine and brackish-water caridean shrimps hyper-hypo-osmoregulate as adults. In particular, they display an efficient hypo-regulation in seawater (925 to 1000 mOsm kg⁻¹, ca 31.5 to 34.0‰; review in Mantel & Farmer 1983). In the genus *Palaemonetes*, reported hypo-osmoregulatory capacities in seawater were -320 mOsm kg⁻¹ in *P. varians* (Potts & Parry 1964), -380 mOsm kg⁻¹ in *P. inter-*

medius (Dobkin & Manning 1964), and $-275 \text{ mOsm kg}^{-1}$ in *P. pugio* (Roesijadi et al. 1976). This is in contrast to adult *P. argentinus*, which hyper-regulate at low salinities and hyper-osmoconform or isoregulate at higher salinities up to 32.2‰.

Many freshwater caridean shrimps are actually euryhaline and can thus adapt to relatively high salinities in the range of 25 to 33‰. Some of these have retained a capacity to hypo-regulate in seawater, e.g. *Macrobrachium rosenbergii* (Sandifer et al. 1975) and *M. petersi* (Read 1984), while others osmoconform at 700 mOsm kg^{-1} (ca 23.8‰) and above, e.g. *Palaemonetes paludosus* (Dobkin & Manning 1964). *P. argentinus* clearly belongs to this latter group. It is a strict osmoconformer in salinities of about 20 to 32.2‰. Survival under these conditions is limited to a few days, and, in brackish-water habitats such as Mar Chiquita lagoon, the shrimps tend to avoid high-salinity areas (Anger et al. 1994).

At the other end of the salinity spectrum, *Palaemonetes argentinus* is well adapted to low and very low salinities through efficient hyper-osmoregulation. This supports the general view of this shrimp as a freshwater species (Spivak 1997). At 31 mOsm kg^{-1} (1.1‰; i.e. at conditions close to freshwater), adult individuals maintain hemolymph osmolality at about 410 mOsm kg^{-1} . This average value is within the 350 to 550 (most frequently 400 to 500) mOsm kg^{-1} range reported for hemolymph osmolality in freshwater decapods, including caridean shrimps (e.g. *P. antennarius*: 400 mOsm kg^{-1} , Parry 1957; *P. paludosus*: 470 mOsm kg^{-1} , Dobkin & Manning 1964), crayfishes, and some potamid crabs (review in Mantel & Farmer 1983). The strong ability of *P. argentinus* to hyper-regulate in media close to freshwater most probably depends, at least in part, on active ion pumping by specialized osmoregulatory tissues in the branchial chamber. Their location would be worth studying. In addition, it has been demonstrated that some freshwater caridean shrimps can, as do crayfishes, produce a hypo-osmotic urine to prevent ion loss (reviews in Mantel & Farmer 1983, Péqueux 1995). Very few palaemonid shrimps have been studied with respect to urine concentration, and since several of them, including *P. argentinus*, are known to penetrate into freshwater, the contribution of the excretory organs to hyper-osmotic regulation in this species would be another interesting field of research.

The pattern of osmoregulation does not change during the postembryonic development of *Palaemonetes argentinus*. The zoea I and VI and, most probably, all other zoeal stages are hyper-isoregulators, with a slight ontogenetic increase in the ability to hyper-regulate at 5 and 10‰. In decapodids, which are morphologically and physiologically transitional between zoeae and juveniles, the ability to hyper-regulate at

low salinities increases, but the pattern of hyper-isoregulation remains the same. This also applies, without important changes in regulation efficiency, to the first juvenile and the adult stages.

This ontogenetic pattern in the capability for osmoregulation can be assigned to 1 of 3 principal types described by Charmantier (1998). *Palaemonetes argentinus* belongs to the second category, in which the adult type of efficient osmoregulation is established as early as in the first postembryonic stage. Among the other crustaceans belonging to this category there are several branchiopod cladocerans (Aladin & Potts 1995), *Artemia salina* (Conte 1984), the amphipod *Gammarus duebeni* (Morritt & Spicer 1995), the isopods *Cyathura polita* (Kelley & Burbanck 1972) and *Sphaeroma serratum* (Charmantier & Charmantier-Daures 1994), and, among the decapods, the palaemonid shrimp *Macrobrachium petersi* (Read 1984) and the crayfish *Astacus leptodactylus* (Charmantier-Daures & Charmantier 1997). A feature common to these species is that they live in osmotically harsh habitats. Since they are exposed to high osmotic stress upon hatching, these species have adapted through the appearance of efficient osmoregulatory processes in the first (and subsequent) postembryonic stages. In freshwater species (including *P. argentinus*) this translates into high values of hemolymph osmolality at very low salinity. As the ability to osmoregulate depends on specialized tissues and organs (review in Charmantier 1998) their structural and functional ontogeny will be an interesting field for future research throughout the development of *P. argentinus*. Since, in this species, the pattern of osmoregulation is basically the same from early zoeae to adults, additionally the question arises whether the same or different osmoregulatory tissues and organs are involved throughout ontogeny.

There is mounting evidence that the ability of young developmental stages to osmoregulate is an important adaptive process. The possession of high osmoregulatory capabilities at hatching is one of the adaptive factors permitting the development of *Palaemonetes argentinus* in freshwater or in coastal areas such as Mar Chiquita lagoon. This shallow lagoon covering 46 km^2 is constantly fed with freshwater (salinity ca 1‰), and seawater (ca 33.5‰) can intermittently enter and leave according to different factors associated with the topography of the mouth of the lagoon, the lunar cycle, tidal amplitude, and the direction and strength of winds. The resulting salinity in the habitat of *P. argentinus* can be low (1 to 5‰) for extended periods of several days, or it can vary between 1 and 30‰ within a few hours (Anger et al. 1994). Given the important osmoregulatory abilities of this species at all postembryonic stages in media ranging from freshwater to seawater, the palaemonid shrimp *P. argenti-*

nus has been able to invade freshwater habitats, while retaining the ability of adapting to brackish habitats with variable salinity.

Similar ecophysiological correlations have been established in species living in habitats with demanding salinity features, for instance under variable conditions in shallow coastal waters or lagoons (*Sphaeroma serratum*: Charmantier & Charmantier-Daures 1994; *Gammarus duebeni*: Morrith & Spicer 1995), temporary rainfall puddles (*Uca subcylindrica*: Rabalais & Cameron 1985) and supratidal rockpools (*Armases miersii*: Charmantier et al. 1998), in extremely high salinities (*Artemia salina*: Conte 1984), in freshwater (*Astacus leptodactylus*: Charmantier-Daures & Charmantier 1997), and in species that accomplish ontogenetic migrations between habitats with different salinity regimes (*Macrobrachium petersi*: Read 1984; *Penaeus japonicus*: Charmantier et al. 1988). An improved understanding of the ecological implications and of the adaptive and evolutionary significance of the ontogeny of osmoregulation should come from further studies conducted on other species living in diverse habitats, and from investigations on the physiological basis of the ontogeny of osmoregulation.

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