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Substrate selection and effect of different substrates on survival and growth of juveniles of the freshwater crayfish *Cherax quadricarinatus* (von Martens 1868) (Decapoda, Parastacidae)

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Abstract Survival and growth of juveniles of the Australian freshwater crayfish *Cherax quadricarinatus* were evaluated in aquaria with four different substrates covering their bottom. The four substrates were plastic mesh, small stones, fine sand and bare glass without cover (control group). Two size classes of juveniles were evaluated: recently hatched or early ($\sim$0.02 g of body weight) and pre-fattening or advanced ($\sim$1 g of body weight). A group of twelve early or ten advanced juveniles were placed in each of the 24 aquaria. Each substrate had a total of six aquaria, three containing early juveniles and the other three containing advanced juveniles groups. Each aquarium had an excess small PVC pipes that served as shelters. After 12 weeks, early juveniles raised in small stones or fine sand had a statistically significant heavier body mass than the control group while the advanced juveniles did not show statistically significant differences in body weight. In a second experiment, ten early and ten advanced juveniles were individually placed in a circular glass aquarium divided into four sections each containing one of the same substrates used in the first experiment. Each individual was filmed for 24 h, and the time spent in each compartment was registered. Both sizes of juveniles showed a marked preference for the small stones. This experiment was repeated with animals used in the first experiment and observed the same selection pattern indicating that substrate selection is independent of body mass or previous acclimation to the different substrates.

Keywords *Cherax quadricarinatus* · Crayfish · Parastacidae · Growth · Substrate preference

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Introduction

The worldwide success of the freshwater crayfish aquaculture industry during recent decades has been based on the selection of species with adequate characteristics for both culturing and commercial purposes. There are more than 100 species of Australian crayfishes, but only three species of the genus *Cherax* are currently being farmed due to their high commercial potential. These are the "marron" *Cherax tenuimanus* (Smith), the "yabby" *Cherax destructor* (Clark) and the "red claw" *Cherax quadricarinatus* (von Martens), which is native to the rivers of northern Australia. Moreover, the red claw has been the first species of crayfish cultured in Australia (Jones 1990; Du Boulay et al. 1993). High growth rates and a high tolerance to wide variations in water quality make this crayfish species an excellent candidate for aquaculture (Masser and Rouse 1997). In fact, the red claw is being cultivated in several countries including Australia, China, Israel, United States, Cuba, Ecuador and Mexico (Garcia-Guerrero et al. 2003). In Argentina, there is a great interest in the commercial rearing of these crayfish, although its commercial exploitation is in the developing stage. Currently, several laboratory experiments are being conducted worldwide whose aim is to maximize the production of red claw crayfish. The methods of rearing, survival and the growth of the early stages of their life cycle are essential to obtain a high yield in their final commercial harvest. *C. quadricarinatus* exhibit high mortality during their first juvenile stages due to frequent molts, cannibalism and the morphological changes in their digestive system. Additionally, the juveniles of this crayfish are very aggressive and exhibit a strong competition for resources. They establish a hierarchy among neighboring individuals resulting in dominant individuals. Heterogeneity in size and body weight is commonly found in the cultivation of parastacids. Similar problems were reported in different species of crayfish in the first stages of life, and agonistic behavior and cannibalism are the main reasons for reducing yields in different culture systems (Mills and McCloud 1983; Mitchell and Collins 1989; Gydemo and Westin 1989; Matthews 1992; Geddes et al. 1993; Jones 1995b; Sáez-Royuela et al. 1995; Barki et al. 2006).

In order to improve the production of the first stages of life, it is necessary to optimize the conditions required for survival and growth in these vulnerable stages. The present study evaluated the effect of four different substrates on the growth and survival of juvenile *C. quadricarinatus* under laboratory conditions, as well as their substrate preference in early and advanced juveniles.

Materials and methods

Evaluation of the substrate type

Two sizes of *C. quadricarinatus* were used as early juveniles (0.02 g of body weight) those are kept at the hatchery in commercial operation and advanced juveniles (1 g of body weight) which are kept at the nursery and are at the pre-fattening stage. Early juveniles (15–20 days post-hatching) were randomly selected from a pool of juveniles composed from hatchings of several ovigerous females that had synchronized hatchings and then randomly assigned to each treatment. Advanced juveniles were selected at random from a pool of animals raised in indoor tanks in a farm. All the experiments were carried out in glass aquaria (900 cm^2 of bottom surface) filled with 8 l of dechlorinated tap water continuously aerated. Temperature was maintained in 25 ± 2°C, and photoperiod was held at 14:10 (light/dark). The water in all aquaria was changed twice a week. Every day, all animals were fed ad libitum with commercial pellets (Tetra Diskus®, 40% of protein), used in previous studies with *C. quadricarinatus* (Cahansky et al. 2008). Dissolved oxygen (Digital Oxygen Meter, precision of ±0.4 mg/l), pH (Jenco Model 6350, precision of ±0.01), ammonia (Wiener® kit), total hardness (as $CaCO_3$), alkalinity and nitrite levels (both measured with Acuanalítica® kit) were determined in each aquaria at the beginning and at the end of the experiment before changing water and feeding the animals.

Twelve early juveniles and ten advanced juveniles were placed in each aquarium at a density of 133 and 111 individuals per m^2, respectively, similar to densities used previously by other authors (Jones 1995b; Masser and Rouse 1997). Each aquarium contained 1.9-cm-diameter PVC pipes in excess as refuge. For both age groups, four substrates were tested: bare glass (control), plastic mesh (a mesh similar to mosquito netting adhered to the bottom of the aquaria), stones (0.4 cm thick covering the bottom of the aquaria) and sand (0.4 cm thick covering the bottom of the aquaria). All substrates were tested in triplicate for a total of six aquaria for the two age groups. At the end of 120 days experiment, the mortality was recorded, removing dead animals from all aquaria daily, as well as the body weight. Prior to weighing in an electronic balance with a precision of ±0.1 mg, all animals were dried with a paper towel to remove excess water.

Growth data was analyzed using a one-way analysis of variance (ANOVA) and a post hoc multiple contrast (LSD). The homogeneity of variance was verified by a parametric Levene test. Survival data was analyzed with a one-tail Fischer test. In all tests, a significance level of $P < 0.05$ was used.

Substrate preference

In the substrate preference experiment, a circular glass aquarium (25 cm of diameter filled with 3 l of dechlorinated water) divided on the bottom into four equal sections each containing a different substrate was used. The substrates tested were the same substrates used in the first experiment: bare glass, plastic mesh, stones and sand. Ten early and ten advanced juveniles maintained as stock in the laboratory in glass aquaria containing refuges were tested individually. Additionally, ten individuals of 1 g from the first experiment were tested in the second round of this experiment. Each individual was recorded for 24 h and the time spent in each compartment was determined from this recording.

In the beginning of the video recording, each juvenile was placed in the center of the aquarium. The movement of each juvenile in the experimental arena was recorded with a web camera (Genius®) located directly above the center of the aquarium and connected to a PC (Fig. 1). A video surveillance program (Active Webcam) was used for recording at a rate of three frames/minute in all experiments. The experimental arena was placed in a closed room maintained at an ambient temperature of 25 ± 2°C and a 14:10 (light/dark) photoperiod. During the light phase, the light source was a white lamp located above the aquarium and connected to a timer. During the dark phase, the light source was infrared LEDs (IR, 940 nm wavelength) located around the webcam. The IR light was on during both the light and dark phases.

In the first part of the experiment, the animals used were from the laboratory stock. They were placed in the experimental arena 24 h prior to the beginning of the recording to allow the animals to acclimate to the arena. In the second part of the experiment, in which the animals maintained in a particular substrate for 120 days were used, no acclimation took place. Animals were not fed during the experiment. The time spend in each substrate

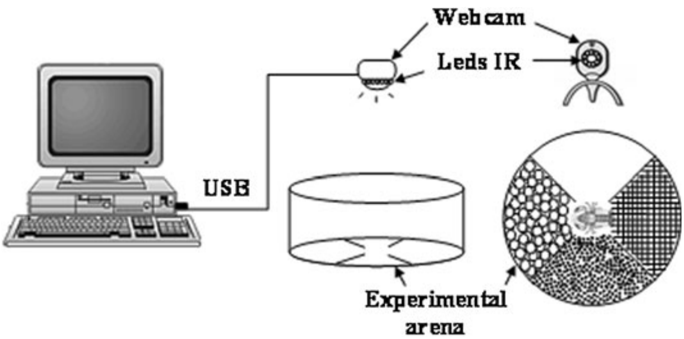

Fig. 1 Scheme of the experimental arena used for the substrate preference study. The recording was made with a webcam connected to a PC held by a tripod (not shown) above the experimental arena that was divided into four equal sections each containing a different substrate: stones, sand, plastic mesh and glass. IR LEDs were located around the webcam to allow recording during the night. Each juvenile was recorded during a continuous 24 h period

during the 24-h recording was calculated for each animal and was later expressed as the absolute frequency and analyzed using a X^2 test to evaluate substrate preference.

Results

Evaluation of the substrate type

The mean body weight of early juveniles in the stone and sand substrates was significantly statistically greater ($P < 0.05$) than those in the glass (control) substrate, while the plastic net caused an intermediate effect. The mean body weight of the advanced juveniles was not statistically different among the substrates ($P > 0.05$) although a similar trend observed as in the early stage, i.e., a heavier body weight of late stage juveniles maintained in stones or sand (Table 1).

There were no significant differences ($P > 0.05$) in survival rates among the treatments. However, advanced juveniles exhibited a greater survival rate at the end of the experiment, independent of treatment. Early juveniles showed high mortality rates in the first few weeks of the experiment resulting in a low survival rate at the end of the experiment (Table 1).

Table 1 Mean body weight and survival (±standard errors) versus substrate type for early (0.02 g) and advanced (1 g) juveniles after 120 days

Substrate type	Early juveniles		Advanced juveniles	
	Mean body weight (g)	Survival (%)	Mean body weight (g)	Survival (%)
Bare glass	0.94 ± 0.21^{a}	50 ± 1.0	3.10 ± 0.31	80 ± 1.0
Plastic mesh	$1.48 \pm 0.22^{a,b}$	27.78 ± 0.41	3.41 ± 0.36	65 ± 0.5
Sand	1.69 ± 0.11^{b}	33.33 ± 0.0	3.82 ± 0.41	85 ± 0.5
Stones	1.71 ± 0.25^{b}	37.50 ± 0.5	3.84 ± 0.24	85 ± 0.5

Different letters (a, b) represent statistically significant differences ($P < 0.05$) for the same group of juveniles

The water quality parameters did not change significantly ($P > 0.05$) among treatments. The means values of pH was in the range 7–8, dissolved oxygen between 5 and 6 mg/l, ammonia values were below of 0.04 mg/l, alkalinity show a range between 60 and 75 mg/l, hardness between 84 and 90 mg/l and nitrite values registered were in a range 0–0.02 mg/l. These results were within acceptable limits for culturing of red claw crayfish (Jones 1990; Hutchings and Villareal 1996; Masser and Rouse 1997).

Substrate preference

All the stock juveniles exhibited a significant ($P < 0.01$) marked preference for stones when compared to the three other substrates (Fig. 2). Similarly, the juveniles that participated in the first experiment, in which they were acclimated for 120 days to one of these four substrates, also exhibited strong significant ($P < 0.01$) preference for stones (Fig. 3).

Discussion

The results of the current experiment indicate that the type of substrate used for early juveniles is an important factor since it significantly affects their growth. In contrast, the substrate type does not seem to affect the growth of advanced juveniles. However, animals kept in stones attained higher body weights. There were no significant differences among the substrates in survival in both groups of juveniles; nevertheless, we observed a trend of higher mortality in stones when compared to control (glass) for early juveniles. Although the number and frequency of molts could not be adequately monitored in both the stone and sand because small molted exoskeletons mixed with those substrates, the growth rate of juveniles kept in either stones or sand was faster than that of control. Jones (1995b) stated that under good rearing conditions, juvenile crayfish of *C. quadricarinatus* molt and grow rapidly. Therefore, the faster growth rate observed in stones and sand may be attributed to a higher frequency of ecdysis when compared to control.

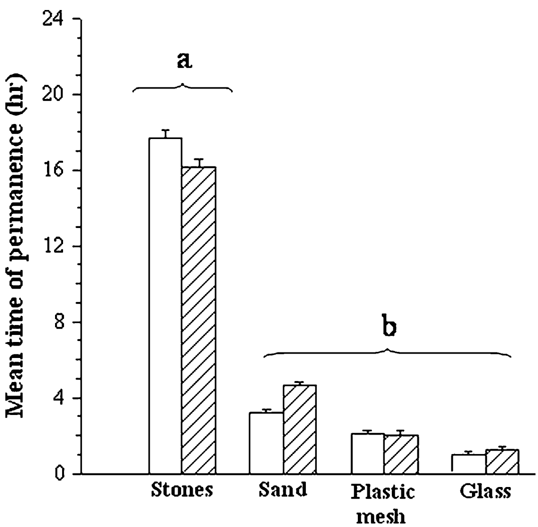

Fig. 2 Substrate preference for early (0.2 g, *empty column*) and advanced (1 g, *dashed column*) juveniles previously expose to the substrates of the experimental arena (represented in the *x* axis) during 1 day. The *columns* represent the average time, in hours, spent during the 24-h experiment in each substrate (glass, plastic mesh, stones and sand), and the *bars* show standard errors (SE). *Different letters* (*a*, *b*) represent statistically significant differences ($P < 0.01$) between substrates for both groups of juveniles

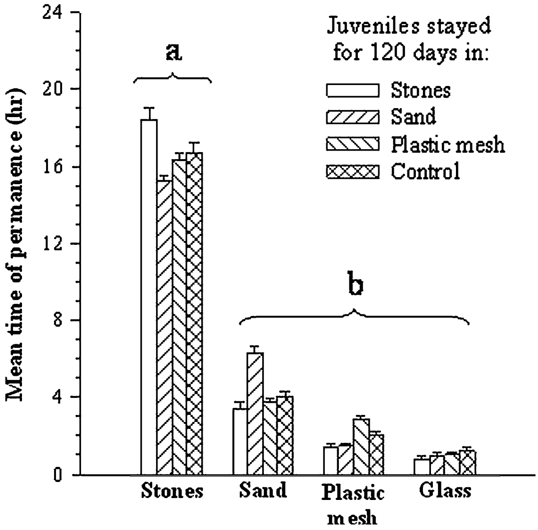

Fig. 3 Substrate preference of advanced juveniles (1 g) previously expose to one of the four substrates during 120 days. The *columns* represent the average time, in hours, spent during the 24-h experiment in each substrate of the experimental arena: glass, plastic mesh, stones and sand (represented in the *x* axis). *Bars* show standard errors (SE). *Different letters* (*a*, *b*) represent statistically significant differences ($P < 0.01$) between substrates

Higher frequency of molts indicates a faster rate of growth, which on one hand is beneficial; however, it also leads to a critical event in these animals' lives by making them vulnerable to attacks by conspecifics that are often their predators. Studies carried out in other Australian parastacids showed that individual rearing systems had a higher survival rate than communal rearing (Geddes et al. 1988; Du Boulay et al. 1993; Morrissy et al. 1995). On the other hand, the higher risk of mortality due to cannibalism at the early stages of life than in the later stages may partially explain the higher survival rate of advanced juveniles compared to that of early juveniles. Therefore, cannibalism is likely to have a several impact during this early life phase. This result corresponds within the normal range reported in farms (Jones et al. 1996).

In the stones and sand, a marked heterogeneity in final body weight was observed in early juveniles. This was expected because previous studies showed a differentiation into large dominant individuals and smaller subordinates during this growth phase (Jones 1990, 1995a, b). This disparity in size resulted in unequal competition for resources among individuals during the course of the experiment. In fact, Barki et al. (2006) observed that *C. quadricarinatus* small juveniles have a reduced growth rate when they are placed together with larger individuals than when they are placed with individuals of the same size.

A study carried out in early juveniles in white claw crayfish (*Austropotamobius pallipes*) showed that the difference in size among individuals as well as the competition for resources increased during the length of study (Sáez-Royuela et al. 2001). These authors also observed marked aggressive behavior as well as cannibalism that resulted in a low survival and an elevated growth rate of the survivors. Studies made on the crayfish species *Procambarus clarkii* (Figler et al. 1999) and *Pacifastacus leniusculus* (Ranta and Lindström 1992) reported that competition for resources and predatory interactions among juvenile crayfish increased the heterogeneity of size during cultivation.

In nature, *C. quadricarinatus* and other Australian crayfish are found in either rocky or sandy areas, as well as in areas with aquatic vegetation or fallen timber in the water. Juveniles prefer the shores of rivers and use stones as refuge to protect themselves from predation or attacks by conspecifics (Jones and Ruscoe 2001; Molony and Bird 2005). It has been suggested that these habitat may also play an important role in providing refuge during ecdysis, when vulnerability to predation is heightened (Lowery 1988; Fielder and Thorne 1990; Smallridge 1994).

Previous studies with *C. quadricarinatus* have clearly stated the relevance of providing suitable habitats for early juveniles during their culturing, also showing the ability of this species for displaying clear preferences for some of the types of habitats offered (Du Boulay et al. 1993; Jones 1995a, b; Karplus et al. 1995). In this study, juveniles showed marked preference for stones over the other substrates independently of body size or acclimation to a particular substrate. It is feasible that a nutritional or behavioral response to a substrate similar to the one found in nature (among all substrates tested in this study stones were the most natural substrate for this species) increased the growth and molt rate of these juveniles.

Therefore, with the aim of improving the growth of juvenile *C. quadricarinatus* in cultivation systems, it is recommended the use of stones as substrate especially for early juveniles kept in hatcheries. Nevertheless, future studies should consider the presence of adequate shelters that allow the development of a more complex environment. Such an environment would decrease aggressive interactions and cannibalism, improving survival rate of juveniles especially in early stages of their lives when mortality is higher than advanced juveniles.

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