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Early and late effects of feed restriction on survival, growth and hepatopancreas structure in juveniles of the red claw crayfish *Cherax quadricarinatus*

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ABSTRACT

The objective of this study is to estimate the point-of-reserve-saturation 50 (PRS_{50}) of stage III (JIII) and 1-gram (J1 g) juveniles of *Cherax quadricarinatus* and to evaluate the early and late effects of feeding restriction on survival, growth and hepatopancreas structure. The experiments consisted of different feeding treatments followed by continuous starvation until molting to the following stage (restriction period). After molting, juveniles were fed daily until the end of the experiment (refeeding period). The PRS_{50} estimated for JIII was 2.05 ± 0.11 days, according to which 2 feeding days were required for 50% of the JIII to molt to JIV. However, the value of growth increment and the presence of hepatopancreatic abnormalities showed that these molted juveniles were not in optimal conditions. Their hepatopancreas showed a significant recovery during the refeeding period. This suggests that mortality in JIII exposed to a feeding restriction period close to the PRS_{50} occurs earlier than in the following stages and that the survivors recover after a refeeding period. The PRS_{50} of JIII could be used to test offspring quality, with the immediate advantage of reducing maintenance costs of poor-quality juveniles. The PRS_{50} estimated for J1 g was 9.19 ± 0.54 days; those fed for less than 9 days exhibited higher mortality during the restriction period, and those of F8 and F9 had histological abnormalities after the refeeding period. The mortality in J1g of F9 increased at the end of the experiment, suggesting that although they would be able to molt in a proportion similar to the control, they die later as a consequence of the restriction period. In this study, the relative wet hepatopancreas weight (RHW) was similar among treatments and between both experiments even when histological examination showed nutritional stress, implying that the RHW estimated with wet weight is a poor indicator of nutritional status. An adequate management in terms of reducing the amount of food and the use of proper tools for monitoring the health of cultured animals are essential for improving profits. In this context, the values of PRS_{50} and the information obtained from the present study are useful to establish a feeding schedule for the production of *C. quadricarinatus*.

1. Introduction

The growth in aquaculture production of major species groups increased considerably between 2000 and 2008, with crustacean production rising at an average annual rate of about 15% (FAO, 2010). The red claw crayfish *Cherax quadricarinatus* is a freshwater, omnivorous species native to the North of Queensland (Australia) and the Southeast of Papua New Guinea. It has great aquaculture potential because of its high growth rate, easy management and high productivity (Cortés-Jacinto et al., 2003; Jones, 1997). Moreover, the red claw crayfish is well valued in the market and used worldwide for human consumption and ornamental purposes (Luchini and Panné Huidobro, 2008). Currently, the species is cultured intensively and semi-intensively in many countries including Australia, United States, China, Ecuador, Mexico and Argentina (Luchini, 2004; Rodgers et al., 2006). Therefore, studies of growth improvement and feeding efficiency aimed at increasing yields are worthy of particular attention (Rodgers et al., 2006).

Most research addressing the nutritional requirements of *C. quadricarinatus* has involved preadults and adults (Campaña-Torres et al., 2008; Cortés-Jacinto et al., 2005; Saoud et al., 2008; Villarreal-Colmenares, 2002), while only a few used earlier developmental stages (Gu et al., 1996; Stumpf et al., 2010). The mortality in nursery juveniles of the red claw crayfish was reported to range between 50 and 85% (Jones, 1995; Masser and Rouse, 1997). During this period, stage III juveniles switch from an endogenous food source to

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exogenous feeding (Levi et al., 1999) and therefore qualitative and quantitative deficiencies in the diet have adverse effects on early survival (García-Guerrero et al., 2003).

The "nutritional vulnerability" (Sulkin, 1978) or "nutritional flexibility" (Sulkin and Van Heukelem, 1980) at early developmental stages of crustaceans has been studied in crabs (Anger, 1995; Figueiredo et al., 2008; Gebauer et al., 2010; Harris and Sulkin, 2005), shrimps (Paschke et al., 2004; Thessalou-Legaki et al., 1999; Zhang et al., 2009) and in the lobster *Panulirus cygnus* (Liddy et al., 2003). Most often, the nutritional vulnerability of larvae has been quantified by means of the point-of-no-return and the point-of-reserve-saturation (PRS). PRS_{50} is the time (in days) when 50% of the individuals at a given stage of development are capable of molting to the following stage (Anger and Dawirs, 1981; Gebauer et al., 2010; Paschke et al., 2004). Recently, Stumpf et al. (2010) estimated the PRS_{50} value for stage III of *C. quadricarinatus*, but the long-term effects of feeding restriction are unknown.

In crustaceans, the digestive gland or hepatopancreas (Van Weel, 1974) is used for monitoring culture health (Vogt et al., 1985) because it is the site of digestion, nutrient absorption, reserve storage and synthesis and secretion of digestive enzymes (Icely and Nott, 1992; Johnston et al., 1998; Sousa and Petriella, 2000). The organ is compact, bilobulated and fills most part of the cephalotorax. Histologically, it has tubular structure (Cuartas et al., 2002; Sousa and Petriella, 2006), with each tubule consisting of different cell types, i.e. E-cell (embryonic), R-cell (resorptive), F-cell (fibrillar), and B-cell (blisterlike) (Al-Mohanna and Nott, 1987, 1989; Caceci et al., 1988; Franceschini-Vicentini et al., 2009; Gibson and Barker, 1979; Icely and Nott, 1992). Starvation, salinity changes and dietary components have been reported to cause alterations in hepatopancreas structure (Anger and Hayd, 2009; Díaz et al., 2010; Jones and Obst, 2000; Li et al., 2008). However, histological changes in this organ have never been examined in previous studies on *C. quadricarinatus*. Therefore, the objective of this study is to estimate the point-of-reserve-saturation of stage III and 1-gram juveniles of *C. quadricarinatus* and to evaluate the early and late effects of feeding restriction on survival, growth and hepatopancreas structure.

2. Materials and methods

2.1. Conditions for broodstock maintenance and selection of juveniles

Stage III (JIII) and 1-gram juveniles (J1 g) were obtained under laboratory conditions from reproductive stocks supplied by Farm Las Golondrinas, Entre Ríos, Argentina. Ovigerous females (mean wet body weight ± SD 59.78 ± 3.17 g) were placed individually into 30-l glass aquaria (60 × 40 × 30 cm) containing dechlorinated water (pH 7–8, hardness 70–100 mg/l as $CaCO_3$ equivalents) under continuous aeration to maintain a dissolved oxygen concentration of 5–8 mg/l, and a photoperiod of 14 l:10D (Jones, 1997).

Temperature was held constant at 27 ± 1 °C by ATMAN water heaters (100 W). The females were fed daily ad libitum with *Elodea* sp. and commercial balanced food for tropical fish Tetracolor, TETRA®, containing 475 g/kg crude protein, 65 g/kg crude fat, 20 g/kg crude fiber, 60 g/kg moisture, 15 g/kg phosphorus and 100 mg/kg ascorbic acid. This diet was previously found to be adequate for the studied species (Sánchez de Bock and López Greco, 2010; Stumpf et al., 2010). After reaching the free-living stage III (Levi et al., 1999), juveniles were separated from their mothers and maintained under the laboratory conditions described above.

One-gram juveniles were obtained from the same broodstocks as JIII. The former were maintained in 30-l glass aquaria (60 × 40 × 30 cm) until reaching about 0.5 g and then stocked individually. They were weighed after every molt and those of 1 ± 0.2 g were randomly assigned to the feeding treatments described below.

2.2. Experimental conditions

The experiments consisted of different feeding treatments followed by continuous starvation until molting to the following stage (restriction period). After molting they were fed daily until the end of the experiment (refeeding period) (Fig. 1).

During the experiment, juveniles were placed in individual plastic containers (500 cm^3) with a piece of synthetic net as shelter (3 × 3 cm) and 350 ml of dechlorinated water under continuous aeration. These containers were placed in aquaria of 53 × 40 × 12 cm with water maintained at 27 ± 1 °C by ATMAN water heaters according to previous studies (Stumpf et al., 2010). For both experiments, water quality was monitored weekly and the physico-chemical parameters (i.e. dissolved oxygen 5.6–7.74 mg/l, pH 7.61–7.92, hardness 65–95 mg/l as $CaCO_3$ equivalents and nitrites <0.05 mg/l) were maintained within the optimal ranges recommended for *C. quadricarinatus* (Jones, 1997).

2.2.1. JIII experiment

A total of 210 stage III juveniles from 5 mothers were sampled, dried with paper towel and carefully weighed using an analytical balance (accuracy: 0.001 g). These juveniles (mean initial weight ± SD 16.80 ± 1.13 mg) were randomly assigned to one of six feeding treatments and one control consisting of continuously fed (CF) animals (30 replicates per treatment, 6 replicates from each mother). This experiment, which lasted for 60 days, consisted of different treatments identified from F2 to F7, with an increasing number of feeding days (2 to 7 feeding days) beginning from the first day of the experiment and followed by continuous starvation until molting to stage IV (restriction period). After this molt, each animal was fed daily until the end of the experiment on day 60 (refeeding period) (Fig. 1A). The treatments were selected on the basis of previous results. These data demonstrate that stage III juveniles of *C. quadricarinatus* are unable to molt if unfed or fed for one day, requiring at least 2 days of initial feeding (Stumpf et al., 2010).

On feeding days, animals were offered a nutritionally balanced food (TETRA®) ad libitum once daily, and checked twice daily (morning and afternoon) for molts and deaths. After molting to stage IV, weight and time to molt were recorded. Juveniles were also weighed on days 30 and 60 of the experiment. The mortality of the experimental groups was recorded at the end of the restriction period and at the end of the experiment (day 60).

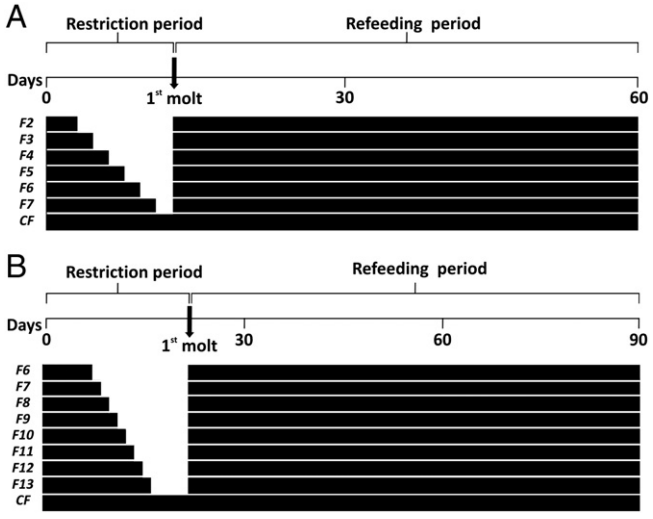

Fig. 1. General schedule of the treatments and protocols applied to determine the point-of-reserve-saturation of (A) stage III and (B) one-gram juveniles of *Cherax quadricarinatus*. The time when first molt occurred depended on each individual and this defined the end of restriction period and the beginning of the refeeding period. ■Fed □Unfed.