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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).

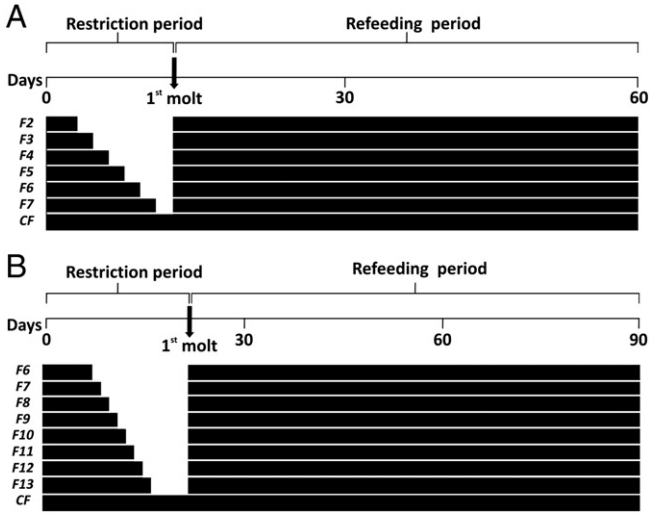

Fig. 1. General schedule of the treatments and protocols applied to determine the point-of-reserve-saturation of (A) stage III and (B) one-gram juveniles of *Cherax quadricarinatus*. The time when first molt occurred depended on each individual and this defined the end of restriction period and the beginning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

Five juveniles from each treatment group were sacrificed on day 30. Their hepatopancreas and pleon were removed and weighed, with the former being fixed and processed by a routine histological method (López Greco et al., 2007). Only the juveniles sacrificed in intermolt were taken into account. The hepatopancreatic tissues were dehydrated in alcohol series and embedded in paraffin. Seven-μm sections were stained with hematoxylin-eosin and observed under light microscopy. This histological protocol was followed after sacrificing animals at the end of the experiment (day 60).

2.2.2. J1 g experiment

A total of 180 1-g juveniles from 4 mothers were sampled, dried with paper towel and carefully weighed using an analytical balance (accuracy: 0.001 g). These juveniles (mean initial weight ± SD 0.95 ± 0.15 g) were randomly assigned to one of 8 feeding treatments and 1 control consisting of continuously fed (CF) animals (20 replicates per treatment, 5 replicates from each mother). This experiment, which lasted for 90 days, consisted of different treatments identified from F6 to F13, with an increasing number of feeding days (6 to 13 feeding days) beginning from the first day of the experiment and followed by continuous starvation until the next molt (restriction period). After the molt, each animal was fed daily until the end of the experiment on day 90 (refeeding period) (Fig. 1B). The treatments were selected on the basis of previous results. These data indicate that one-gram juveniles of *C. quadricarinatus* require more than 5 days of initial feeding to molt and that the proportion of molting juveniles which were fed for 15 days is similar to that of continuously fed juveniles (Calvo et al., 2009).

On feeding days, animals were offered a nutritionally balanced food (TETRA®) ad libitum once daily, and checked once daily for molts and deaths. After the first molt, weight and time to molt were recorded. Juveniles were also weighed on days 30, 60 and 90 of the experiment.

Mortality was recorded at the end of the restriction period and at the end of the experiment (day 90), when animals were sacrificed. The hepatopancreas and pleon were removed and weighed; the former was fixed and processed as described above.

2.3. Calculations and statistical analysis

Molting was calculated as the percentage of individuals in a given treatment that molted to the following stage. To estimate the PRS_{50}, data of molting were fit to a sigmoid curve using the equation $f = a/\{1 + \exp.-[(x-x0)/b]\}$ (Paschke et al., 2004; Stumpf et al., 2010). Growth was evaluated in terms of growth increment (GI), expressed as percentage and mean specific growth rate (SGR), expressed as percentage per day. These were calculated as follows: $GI = 100 \times ((W_t - W_0)/W_0)$ and $SGR = 100 \times (\ln W_t - \ln W_0)/t$, where W_t and W_0 are final and initial wet weights and t is the time, estimated as the number of days from the beginning of the experiment. GI was calculated after the first molt and at the end of both experiments, and SGR, in the middle and the end of both experiments.

The relative wet hepatopancreas weight (RHW = 100 * wet weight of hepatopancreas / body weight) and the relative wet pleon weight (RPW = 100 * wet weight of the pleon/body weight) were calculated for each sacrificed juvenile.

The parametric tests were applied when data met the appropriate assumptions; otherwise, equivalent non-parametric tests were used. One-way ANOVA or Kruskal–Wallis test (non-parametric) was used to test for differences in the time to molt, GI, SGR, RHW and RPW among treatments, followed by Tukey or Mann–Whitney (non-parametric) tests for multiple comparisons between treatments and CF (Zar, 1999). Weight data recorded throughout the experiment were analyzed by one-way repeated-measures ANOVA (Zar, 1999).

3. Results

3.1. JIII experiment

The results showed a significant relationship between percentage of molting and initial feeding period ($p < 0.001$), with F2 being the only treatment with a significantly lower value than that of the control ($p < 0.05$); the value of PRS_{50} obtained from the sigmoid curve was

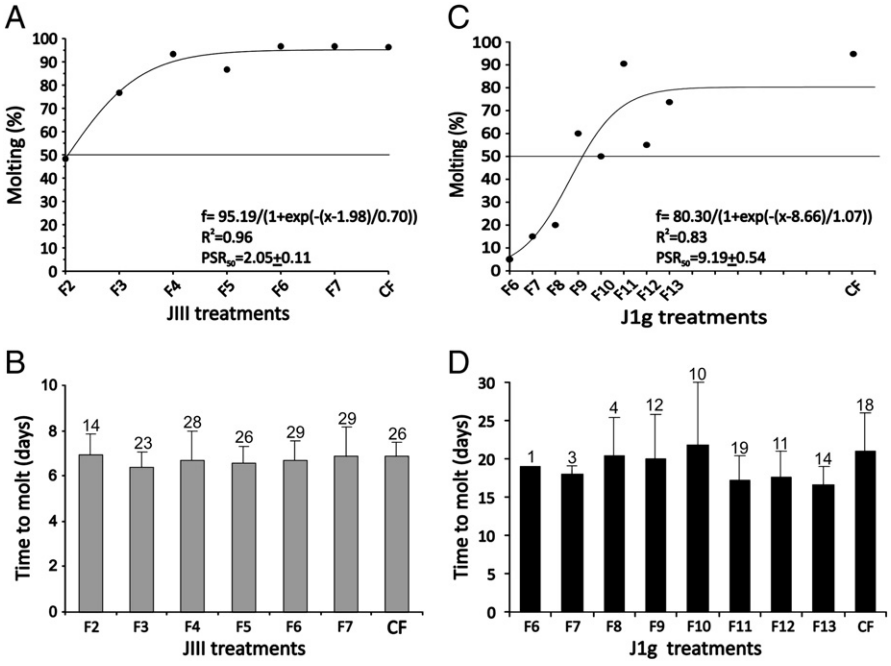

Fig. 2. Molting percentage and time to the next molt (mean ± SD) estimated for both experiments. (A) and (B): JIII experiment; (C) and (D): J1 g experiment. The numbers above the bars indicate the sample sizes.

2.05 ± 0.11 days (Fig. 2A). The time to molt for CF juveniles that molted to stage IV was 6.85 ± 0.61 days, with no significant differences between each treatment and CF ($p>0.05$; Fig. 2B). The GI obtained after molt was significantly higher for CF than for the treatments F2, F3 and F4 ($p<0.05$; Fig. 3A). The treatments had no significant effect on weight throughout the experiment ($p>0.05$; Fig. 4A).

During the restriction period, mortality tended to be higher in treatments with less feeding days (F2 and F3). However, these results could not be statistically analyzed due to violations of test assumptions. In contrast, at the end of the experiment, the mortality was independent of treatments ($p>0.05$; Fig. 5A). No significant differences in GI (Fig. 3A) or SGR (Table 1) were observed between treatments and CF ($p>0.05$). The RHW and RPW were similar among treatments at days 30 and 60 of the experiment ($p>0.05$; Table 2).

3.2. J1 g experiment

There was a significant relationship between percentage of molting and initial feeding period ($p<0.001$), with juveniles fed less than nine days (F8, F7, F6) showing a significantly lower value than that of the control ($p<0.05$); the value of PRS_{50} obtained from the sigmoid curve was 9.19 ± 0.54 days (Fig. 2C).The time to molt for CF juveniles was 21.08 ± 4.91 days and no significant differences were found between each treatment and CF ($p>0.05$) (Fig. 2D). The GI obtained after molt for CF was similar to that obtained for the treatments ($p>0.05$) (Fig. 3B). The treatments had no significant effect on weight throughout the experiment ($p>0.05$) (Fig. 4B). The treatments F6, F7 and F8 were not included in the analyses of the time to molt, GI and weight obtained throughout the experiment because of their low percentage of molting ($p<0.001$).

During the restriction period, mortality was higher for F6, F7 and F8 than for CF ($p<0.001$), while at the end of the experiment, mortality was higher for F9, F8, F7 and F6 than for CF ($p<0.05$) (Fig. 5B). No significant differences in GI (Fig. 3B) or SGR (Table 1) were observed between treatments and CF ($p>0.05$). The RHW and RPW were similar among treatments at day 90 of the experiment ($p>0.05$) (Table 2).

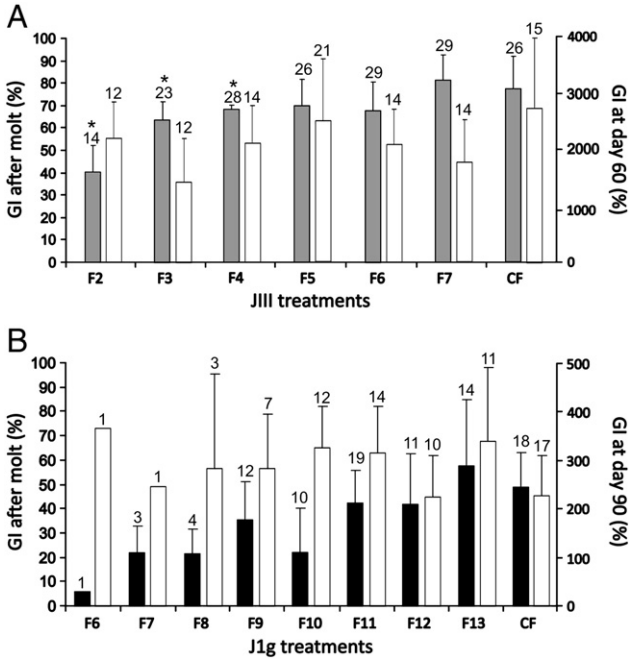

Fig. 3. Growth increment (GI; mean ± SD) estimated for both experiments. (A): After molt (gray bars) and at day 60 (white bars) of the JIII experiment; (B): after molt (black bars) and at day 90 (white bars) of the J1 g experiment. Each treatment was compared with its corresponding control. The numbers above the bars indicate the sample sizes. Asterisk (*) indicates significant difference ($p<0.05$).

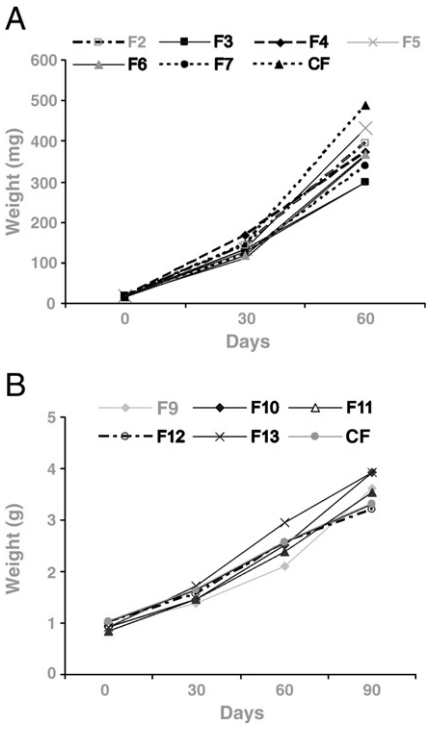

Fig. 4. Mean weight of juveniles during both experiments: (A) JIII experiment; (B) J1 g experiment. For the JIII experiment the final sample size was: 6(F2), 8(F3), 7(F4), 16 (F5), 8(F6), 9(F7), 10(FC). For the J1 g experiment the final sample size was: 7(F9), 8 (F10), 13(F11), 10(F12), 11(F13),17(FC).

3.3. Histological study of the hepatopancreas

The structure of the hepatopancreas of *C. quadricarinatus* resembles that of other decapod crustaceans. It is composed of numerous blind-end tubules with four main cell types, namely, E-, F-, B- and R-cells. E-cells are cuboidal and have a prominent nucleus occupying most of the basophilic cytoplasm; they are located at the distal part of the hepatopancreatic tubules. F-cells are cylindrical, with a central nucleus and basophilic cytoplasm; they are found in the medial and proximal parts of the tubules. B-cells are the largest cell type, with a large vacuole which displaces the nucleus basally; they are more abundant in the medial and distal parts of the tubules. R-cells are the most numerous cell type in the hepatopancreas; they are cylindrical, contain many small vacuoles and the nucleus is located centrally or basally; they are more abundant in the medial and proximal parts of the tubules (Fig. 6A,B).

After the restriction period the hepatopancreas showed several abnormalities: 1) structural disorganization of the tubules (Fig. 6D), characterized by the discontinuity of the epithelium; 2) an enlarged tubular lumen (Fig. 6C) resulting from thinned epithelium; occasionally, this made cell identification difficult; 3) hypertrophy of B-cells (Fig. 6F), larger vacuoles, tending to coalesce into larger ones; and 4) R-cells showing larger vacuoles than those in the control (Fig. 6E).

All these abnormalities were found in JIII who were fed for less than 5 days (F4, F3 and F2 treatments), with F2 showing the most pronounced alterations. At the end of the JIII experiment (day 60), abnormalities were observed in some of the treatments, particularly those involving less than 4 feeding days (F3 and F2 treatments); these consisted in structural disorganization of the tubules, hypertrophy of B-cells and presence of large vacuoles in R-cells.

In the J1 g experiment, the histological examination of the hepatopancreas showed the same abnormalities as in the JIII experiment (Figs. 6 C,D,E,F). These were exclusively present in

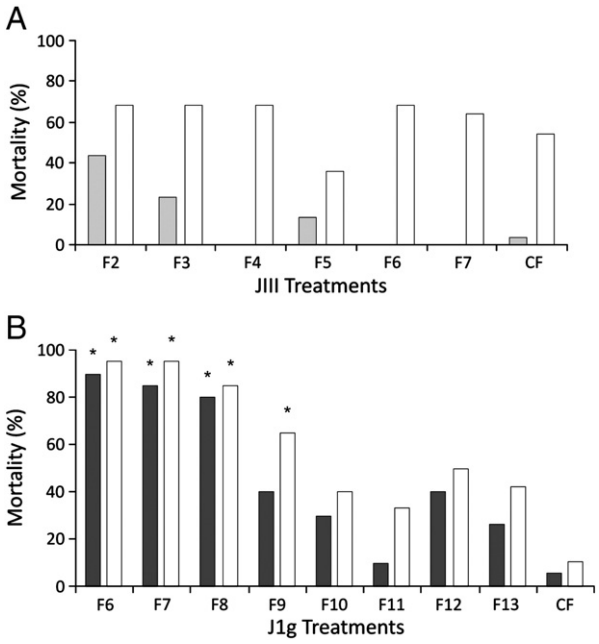

Fig. 5. Mortality estimated for both experiments. (A): During the restriction period (gray bars) and at day 60 (white bars) of the JIII experiment; (B): during the restriction period (black bars) and at day 90 (white bars) of the J1 g experiment. The comparisons were done between each treatment and its corresponding control. Asterisk (*) indicates significant difference ($p<0.05$).

animals fed for less than 10 days (F9, F8, F7 and F6), the most pronounced being found in the only survivors of F6 and F7. The hepatopancreas of J1 g fed for more than 9 days were similar to those of the control.

4. Discussion

The two size groups of juvenile *C. quadricarinatus* were affected differently by feeding restriction and refeeding. Stage III juveniles were affected in growth and survival during the restriction period, while 1-gram juveniles were affected in mortality during both periods.

The PRS_{50} estimated for JIII (2.05 ± 0.11 days) represented about 30% of the stage duration (6.85 ± 0.61 days). This result is in agreement with that of Stumpf et al. (2010) for the same species and with those obtained for brachyuran larvae (Giménez, 2002; Mikami et al., 1995; Staton and Sulkin, 1991) and shrimp larvae (Paschke et al., 2004).

According to PRS_{50} 2 feeding days would be enough for 50% of the JIII to successfully molt to the following stage. This may account for the similar time to molt among different treatments, suggesting that it is unnecessary to feed JIII juveniles for more than 2 days. However, the percentage of molting, the value of GI and the presence of hepatopancreatic abnormalities showed that molted F2 juveniles were not in optimal conditions. Nonetheless, the analysis of the weight revealed that the differences in growth found after the restriction period were compensated at day 30. Likewise, the histological analysis of individuals from F2 revealed abnormalities at day 30 which improved at day 60, thereby suggesting a substantial recovery. At the end of the experiment, all the treatments showed values similar to the control for all the variables, and, particularly, the mortality obtained for F2 was reached by the other treatments. 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. In shrimp aquaculture, stress tests are commonly applied to early larval stages to evaluate offspring quality. These tests are based on the exposure of shrimps to environmentally adverse conditions like starvation and low levels of salinity, dissolved oxygen or temperature (Palacios et al., 1999; Racotta et al., 2003). Likewise, PRS_{50} could be used to test offspring quality, with the immediate advantage of reducing maintenance costs of poor-quality juveniles.

Table 1
Specific growth rate (SGR; mean ± SD) of *Cherax quadricarinatus* juveniles calculated at the end of both experiments.

JIII experiment (60 days)		J1 g experiment (90 days)	
Treatments	SGR (% $days^{-1}$)	Treatments	SGR (% $days^{-1}$)
F2	7.8 ± 2.0	F6	1.7 ± 0
F3	7.1 ± 1.6	F7	1.4 ± 0
F4	7.4 ± 2.4	F8	1.4 ± 0.6
F5	7.6 ± 3.8	F9	1.5 ± 0.3
F6	7.6 ± 1.6	F10	1.6 ± 0.2
F7	4.7 ± 1.5	F11	1.6 ± 0.3
CF	6.7 ± 2.4	F12	1.3 ± 3.0
–	–	F13	1.6 ± 0.4
–	–	CF	1.3 ± 0.3

The PRS_{50} estimated for J1 g (9.19 ± 0.54 days) represented about 45% of the stage duration (21.08 ± 4.91 days), indicating that J1 g need more food to accumulate sufficient reserves for molting to the next stage than JIII. The PRS_{50} of J1 g is similar to that estimated for juvenile *Fenneropenaeus chinensis* (11.55 days) of about 0.7 g (Zhang et al., 2009). The majority of the PRS values reported in the literature correspond to crustacean larvae (Anger and Darwis, 1981; Bas et al., 2008; Gebauer et al., 2010; Giménez, 2002; Liddy et al., 2003; Paschke et al., 2004), possibly because the effect of temporary starvation is one important aspect of larval nutritional ecology (Zheng et al., 2005). However, information on PRS not only enhances the knowledge of starvation resistance but also is useful in the design of feeding regimes in aquaculture (Zhang et al., 2009).

J1 g fed for less than 9 days exhibited higher mortality than the control during the restriction period, to the extent that only one survivor was recorded in treatments F6 and F7 at the end of the experiment. There was an increase in the mortality of treatment F9 during the refeeding period, with significant difference from the control occurring at the end of the experiment. In addition, the J1 g of F8 and F9 presented histological abnormalities. These results suggest that although J1 g fed for 9 days would be able to molt in a proportion similar to the control, they die later as a consequence of the restriction period.

The histological structure of the hepatopancreas of *C. quadricarinatus* was similar to that of other studied species (Al-Mohanna and Nott, 1987, 1989; Caceci et al., 1988; Cuartas et al., 2002; Franceschini-Vicentini et al., 2009; Gibson and Barker, 1979; Icely and Nott, 1992; Johnston et al., 1998; Sousa and Petriella, 2000, 2006). This is the first study of the effect of partial food restriction on the hepatopancreas structure of this species. The identified abnormalities could be useful for assessing nutritional status in aquaculture although it needs further research.

The RHW is considered a good indicator of nutritional status. However, Jones and Obst (2000) found that RHW calculated with dry hepatopancreas weight correlated with moisture, lipid, protein and energy in the organ, while a poor correlation was obtained using RHW from wet hepatopancreas. In this study, the RHW was similar among treatments and between both experiments even when histological examination showed nutritional stress. The values were also similar to those obtained for the same species by other authors (Evans et al., 1992; Jussila and Evans, 1998; Loya-Javellana et al., 1995), implying that RHW estimated on wet weight is not a good indicator of nutritional status under the assayed experimental conditions (e.g. number of starvation days).

Table 2
Relative hepatopancreas weight (RHW; mean ± SD) and relative pleon weight (RPW; mean ± SD) of *Cherax quadricarinatus* juveniles estimated for both experiments.

JIII Experiment					J1g Experiment		
Treatments	30 days		60 days		Treatments	90 days	
	RHW (%)	RPW (%)	RHW (%)	RPW (%)		RHW (%)	RPW (%)
F2	8.2 ± 1.1	28.6 ± 1.2	7.0 ± 1,1	30.8 ± 2.1	F6	7.6 ± 0	30.7 ± 0
F3	6.6 ± 1.3	28.1 ± 1.3	8.1 ± 1,0	29.0 ± 2.9	F7	7.8 ± 0	32.0 ± 0
F4	6.5 ± 4.9	26.9 ± 4.9	7.4 ± 1.5	30.2 ± 1.9	F8	7.8 ± 0.6	31.3 ± 1.3
F5	6.9 ± 2.0	28.3 ± 2.0	7.4 ± 1.2	31.9 ± 9.3	F9	6.3 ± 1.3	30.5 ± 1.0
F6	6.8 ± 2.4	26.7 ± 2.4	7.5 ± 1.0	29.8 ± 1.2	F10	7.0 ± 1.2	30.9 ± 1.2
F7	6.7 ± 3.7	25.9 ± 3.7	7.7 ± 0.5	31.0 ± 1.9	F11	7.5 ± 1.1	30.2 ± 2.5
CF	6.6 ± 3.7	28.8 ± 1.4	6.8 ± 1.5	32.8 ± 3.4	F12	7.1 ± 0.7	31.5 ± 1.8
–	–	–	–	–	F13	7.1 ± 0.7	31.4 ± 1.1
–	–	–	–	–	CF	7.3 ± 1.0	30.3 ± 2.1

Taking into account that food represents between 50% and 70% of operating costs in aquaculture production (Cortés-Jacinto et al., 2005; Thompson et al., 2005), an adequate management in terms of reducing the amount of food and the use of proper tools for monitoring the health of cultured animals is essential for improving profits. In this context, the values of PRS_{50} and the information

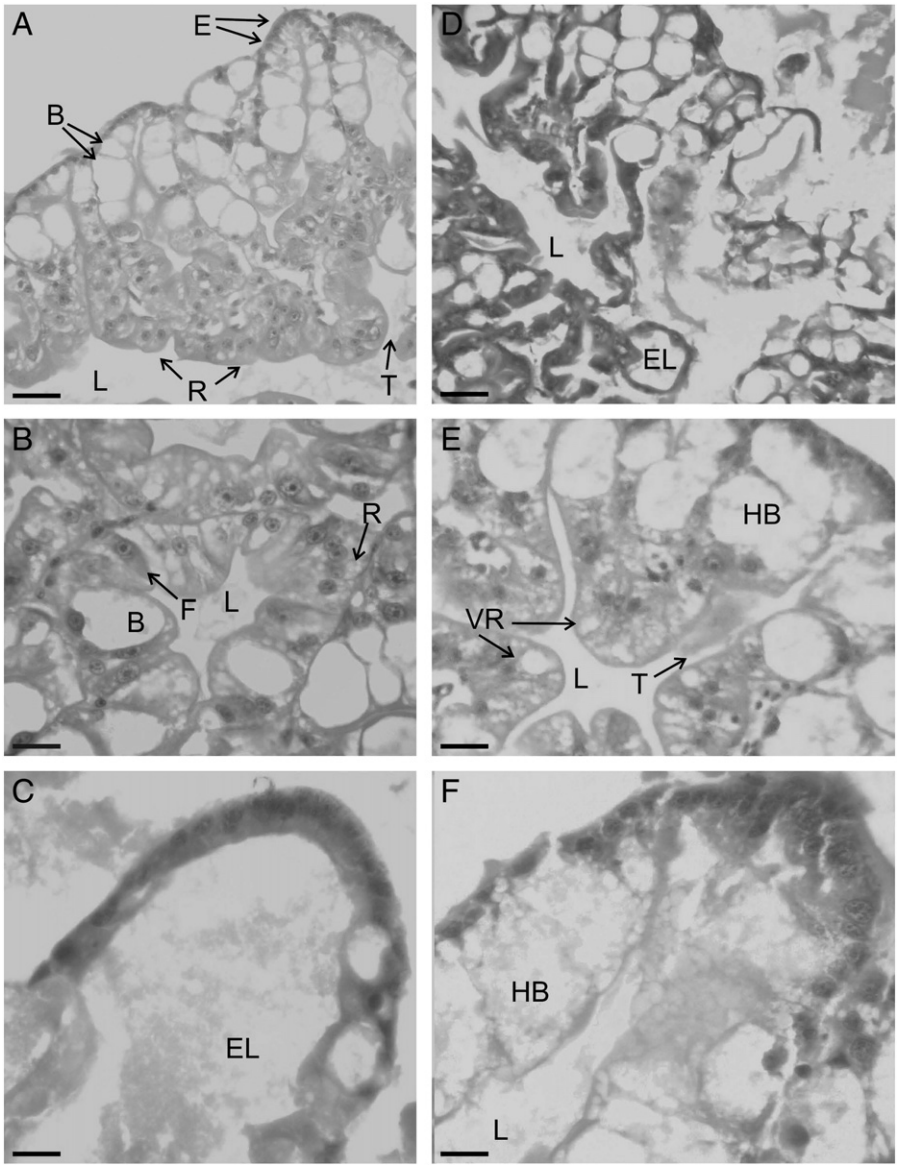

Fig. 6. Histological sections of the hepatopancreas from juveniles of *Cherax quadricarinatus* continuously fed (A,B) and abnormalities after feeding restriction (C,D,E,F). (A): Longitudinal section of the apical portion of tubules; (B): cross section of a tubule; (C): enlarged tubular lumen; (D): longitudinal section of disorganized tubular structure; (E): R-cells with enlarged vacuoles; (F): hypertrophy of B-cells tending to coalesce into larger one. Scale bars: (A) = 50 μm; (B),(C) = 20 μm; (D) = 60 μm; (E) = 30 μm; (F) = 10 μm. B: B-cell; E: E-cell; EL: enlarged tubular lumen; F: F-cell; HB: hypertrophy of B-cells; L: lumen of the tubule; R: R-cell; T: hepatopancreatic tubule; VR: enlarged vacuoles from R-cells.

obtained from the present study are useful to establish a feeding schedule for the production of *C. quadricarinatus*.

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