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Effect of intermittent feeding on growth in early juveniles of the crayfish *Cherax quadricarinatus*

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ABSTRACT

Three experiments were conducted with juveniles of the crayfish *Cherax quadricarinatus* to investigate the effect of intermittent feeding regimes on growth and the ability to tolerate the shortage of food. In experiment 1, stage III juveniles were assigned to one of seven intermittent feeding groups (from FS1: 1 day fed/1 day non-fed to FS7: 7 days fed/7 days non-fed) and two control groups, continuously fed (CF) and continuously starved (CS) animals; this experiment comprised a short-term intermittent feeding period until the first molt, followed by a continuous feeding period. In the experiment 2, stage III juveniles were assigned to one of three intermittent feeding groups (FS2 to FS4) and one control group (CF); it consisted of a prolonged intermittent feeding period, until the end of the experiment. In the experiment 3, stage VI and VII juveniles were assigned to one of three intermittent feeding groups (FS2 to FS4) and one control (CF); it also consisted of a prolonged intermittent feeding period. The red claw crayfish juveniles were able to tolerate periods of intermittent feeding and underwent compensatory growth after continuous feed was re-established. The ability of crayfish to tolerate intermittent feeding was influenced by developmental stage and duration of the intermittent feeding period. Stage III juveniles survived, but decreased growth, when subjected to prolonged intermittent feeding. However, they showed full compensatory growth when the intermittent feeding period was short and followed by continuous feeding. On the other hand, stage VI–VII tolerated 60 days of prolonged intermittent feeding without any change in growth and survival. The hepatosomatic index (based on wet weight) values of the treatments and the control were similar, suggesting that intermittent feeding may not be considered a nutritional stress condition. The relative pleon weight (based on wet weight) values of the treatments and control were similar suggesting low use of nutrients from the muscle to increase the chance for survival. The juveniles of *C. quadricarinatus* can tolerate relatively long periods of low food availability and this is an important adaptation for their survival in changing/unpredictable environments and an attribute favorable for the production of the species.

1. Introduction

Variations in food availability are especially pronounced in freshwater environments, high latitudes and between groundwater and surface water ecosystems (Anger et al., 2004; Jalihal et al., 1993; Mezek et al., 2010). Food limitation may affect the survival of species and these can develop adaptations as a survival strategy (Mezek et al., 2010). The nutritional stress selects for an enhanced maternal energy investment into egg production, allowing a partially food-independent early larval development (Anger, 2001, 2006) and for abbreviated or direct developmental modes (Jalihal et al., 1993).

In addition, many animals have the capacity to compensate for weight loss, after a period of food deprivation (Li et al., 2005). Taking advantage of compensatory growth is one potential way to reduce costs and its use in aquaculture has been proposed as a tool to increase growth and improve food conversion efficiency (Foss et al., 2009; Hagen et al., 2009). The degree of compensatory growth is highly dependent not only on the species but also on the length and intensity of food deprivation (Hagen et al., 2009; Oh et al., 2008; Palma et al., 2010).

Decapod crustaceans also have the ability to tolerate starvation periods (Calado et al., 2007; Figueiredo et al., 2008; Gebauer et al., 2010; Wen et al., 2006) and to achieve compensatory growth after food restriction (Li et al., 2009; Singh and Balange, 2007; Wu et al., 2000, 2001; Wu and Dong, 2002a, b). *Fenneropenaeus chinensis* juveniles fed a low-protein diet showed compensatory growth after increasing dietary protein (Wu and Dong, 2002a). In addition, juveniles of this species produced a compensatory response when they were initially starved

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and then refed (Wu et al., 2000; Wu and Dong, 2002b) or exposed to different feeding levels and then refed ad libitum (Wu et al., 2001). *Macrobrachium nipponense* underwent compensatory growth after different periods of starvation followed by refeeding (Li et al., 2009). On the other hand, the effect of intermittent feeding on growth has been rarely evaluated (Mazlum et al., 2011; Wu and Dong, 2001; Zheng et al., 2008).

Feed cost represents a large portion (up to 80%) of total operating cost in intensive aquaculture (Thompson et al., 2010). For that reason, food restriction without effect on growth may be seen as an alternative strategy to reduce costs and increase profits of aquaculture operations (Bavčević et al., 2010). However, questions arise as to what extent should feeding rate be reduced without compromising the growth performance of the animals (Nunes et al., 2006).

Cherax quadricarinatus is an important species of freshwater crustacean for aquaculture. This species has been the subject of many studies concerning growth and nutrition (Campaña-Torres et al., 2008; Cortés-Jacinto et al., 2003, 2004a, b, 2005; Hernández-Vergara et al., 2003; Meade and Watts, 1995; Thompson et al., 2006; Villarreal-Colmenares, 2002). *C. quadricarinatus* has direct development and the first two lecithotrophic juvenile stages (JI and JII) stay with their mothers for 10 to 15 days (Levi et al., 1999). After molting to stage juvenile III (JIII) they become more independent from their mothers beginning the exogenous feeding (Levi et al., 1999). Following stages (III, IV and V) last approximately 10 days for each one and according to this, after 30 days juveniles are found in stage juvenile V or VI (Stumpf et al., 2010).

Recently, Stumpf et al. (2010) demonstrated that juveniles JIII can tolerate and compensate for a short period of intermittent feeding. Thus, the objective of this work was to evaluate the effect of short and long-term intermittent feeding on growth and survival at different stages of development of juveniles of the red claw crayfish, *C. quadricarinatus.*

2. Materials and methods

2.1. Animals

Juveniles of the crayfish *C. quadricarinatus* were obtained under laboratory conditions from reproductive stocks supplied by the farm Las Golondrinas (Entre Ríos) in Argentina. Ovigerous females weighing between 57 and 64 g were placed individually into 30-l glass aquaria (37×27×18 cm) containing a PVC tube (10 cm in diameter and 25 cm long) as shelter (Vazquez and López Greco, 2007), and dechlorinated water (pH 7–8, hardness 50–100 mg/l as $CaCO_3$ equivalents) under continuous aeration to maintain a dissolved oxygen concentration (DO) higher than 5 mg/l. The photoperiod was 14L:10D (Jones, 1997) and temperature was held constant at 27±1 °C by ATMAN water heaters (100 W) (Jones, 1997). These females were fed daily ad libitum with *Elodea* sp. and commercial balanced food for tropical fish Tetracolor, TETRA® (Sánchez de Bock and López Greco, 2010; Stumpf et al., 2010; Vazquez et al., 2008). Juveniles were separated from their mothers after reaching the free-living stage III (Levi et al., 1999). Until the beginning of the experiments, they were fed a protein-rich diet and maintained under the conditions of water quality, temperature and photoperiod described above.

2.2. Experimental procedures

Three experiments were conducted. They comprised an intermittent feeding period followed or not by a continuous feeding period. The intermittent feeding period, consisted of alternating days of feeding and non-feeding.

In all the assays, each juvenile was carefully dried with a paper towel, weighed (initial weight) with an analytical balance (precision 0.1 mg) and randomly assigned to a single plastic container with water. The containers used for juveniles had a capacity of 500 cm^3 each, were filled with 300 ml of dechlorinated water and were provided with a 5×5 cm-synthetic net as shelter according to previous studies (Stumpf et al., 2010). During these experiments, the plastic containers were cleaned by siphoning and the water was totally renewed every day, in order to remove any food remaining. These water changes maintain levels of ammonia and nitrites undetectable, ensuring high water quality (Meade and Watts, 1995).

The water quality parameters were measured once a week in the water supply tank (200 L) for the experiments. These parameters, i.e. dissolved oxygen (5.4–8.0 mg l^{-1}), pH (7.3–8.4), hardness (60–100 mg l^{-1} as $CaCO_3$ equivalents) and temperature (26–29 °C) were within the ranges recommended for aquaculture (Boyd and Tucker, 1998; Jones, 1997). The photoperiod was 14L:10D (Jones, 1997).

On feeding days, animals were fed ad libitum once a day with Fundus® (Fideos Don Antonio SA), with a composition of crude protein 50.40%, crude fat 20.49%, 19.26% carbohydrates, 2.40% fiber and 6.82% moisture (Centro de Investigaciones Biológicas del Noreste, S.C. — Laboratorio de Analisis Químicos Proximales — México). This formulated food is a commercial trout diet and currently manufactured in Argentina and used in the crayfish culture.

2.2.1. Experiment 1: effects of a short-term intermittent feeding period followed by continuous feeding period on stage III juveniles

A total of 216 stage III juveniles with an average initial body weight of 17.01±1.24 mg were randomly assigned to one of seven treatments and to one of two control groups (24 replicates). In this experiment, which lasted for 45 days, the intermittent feeding period was applied until molting to stage IV follows of the continuous feeding period until the end of experimental period, at day 45. The following treatments were performed: FS1 (1 day fed/1 day non-fed), FS2 (2 days fed/2 days non-fed), FS3 (3 days fed/3 days non-fed), FS4 (4 days fed/4 days non-fed), FS5 (5 days fed/5 days non-fed), FS6 (6 days fed/6 days non-fed) and FS7 (7 days fed/7 days non-fed). The controls consisted of CF (continuously fed) and CS (continuously starved) animals. These treatments were selected on the basis of previous results showing the best outcome for survival and growth (Stumpf et al., 2010).

2.2.2. Experiment 2: effects of a long-term intermittent feeding period on stage III juveniles

A total of 160 stage III juveniles with an average initial body weight of 16.78±1.16 mg were randomly assigned to one of three treatments and to a control (40 replicates). The treated juveniles were subjected to intermittent feeding period until the end of the experiment at day 60. The following treatments were performed: FS2 (2 days fed/2 days non-fed), FS3 (3 days fed/3 days non-fed), and FS4 (4 days fed/4 days non-fed). The control was CF. These treatments were selected on the basis of the experiment 1 results.

2.2.3. Experiment 3: effects of a long-term intermittent feeding period on juveniles at stages VI and VII

A total of 96 juveniles with an average initial body weight of 0.36±0.05 g (stages VI and VII) were randomly assigned to one of three treatments and to a control (24 replicates). The treated juveniles were subjected to intermittent feeding period until the end of the experiment at day 60. The following treatments were performed: FS2 (2 days fed/2 days non-fed), FS3 (3 days fed/3 days non-fed), and FS4 (4 days fed/4 days non-fed). The control was CF.

2.3. Calculations and data analysis

Molts and deaths were checked twice a day (morning and afternoon). Juveniles were weighed after molting to stage IV and at days 15, 30 and 45 (experiment 1). In the experiments 2 and 3 juveniles were weighed at days 15, 30, 45 and 60. The duration of stage III and