EFFECT OF FOOD SHORTAGE ON GROWTH, ENERGETIC RESERVES MOBILIZATION, AND WATER QUALITY IN JUVENILES OF THE REDCLAW CRAYFISH, *CHERAX QUADRICARINATUS*, REARED IN GROUPS

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A B S T R A C T

The aim of this study is to analyze the impact of food shortage on growth performance, by means of energetic reserves (proteins, glycogen and lipids) mobilization and hepatopancreas cells analysis in *C. quadricarinatus* juveniles maintained in groups, as well as the effect on culture water quality. Two experiments were performed, each of them with two feeding regimes during 45 days. The Control feeding regime, in which crayfish were fed daily (once a day) throughout the experimental period (DF), and the Cyclic feeding regime, in which juveniles were fed for 2 or 4 days (once a day) followed by 2 or 4 days of food deprivation (2F/2D and 4F/4D, respectively) in repeated cycles. Cyclic feeding influenced growth, biochemical composition from hepatopancreas and muscle, and water quality. Juveniles cyclically fed were unable to maintain a normal growth trajectory during 45 days. Apparent feed conversion ratio, apparent protein efficiency ratio, hepatosomatic index and relative pleon mass were similar in cyclic and daily fed animals and no structural damage was found in the hepatopancreas of juveniles subjected to cyclic feeding. The novelty of this study was the significant accumulation of proteins in pleonal muscle in both cyclic feeding regimes (approx. 18%) suggesting that the storage of this constitutive material during food shortage may be an adaptation for a compensatory growth when food becomes abundant again. The cyclic feeding regimes had a positive effect on water quality decreasing inorganic nitrogen concentration. This was due to the reduction in the amount of animal excretes and feces in the group that received approx. 50% less feed. Additionally, water pH was higher in cyclic feeding tanks, as a result of lower organic matter decomposition and consequent release of CO_2. Accordingly, total ammonia in the water was significantly lower for the cyclic feeding regimes compared to their respective controls. This study suggests that the protocol of cyclic feeding could be applied at least 45 days in 1 g juveniles maintained in group conditions, without affecting the energetic reserves and hepatopancreas structure, emphasizing the high tolerance of this species to food restriction.

KEY WORDS: *Cherax quadricarinatus*, cyclic feeding, energetic reserves, hepatopancreas structure, water quality

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INTRODUCTION

The redclaw crayfish, *Cherax quadricarinatus* (von Martens, 1868), is a freshwater omnivorous species native to the north of Queensland and the southeast of Papua New Guinea. Saoud et al. (2013) and Zhu et al. (2013) recently reviewed the many physical, biological, and commercial attributes that make it suitable for commercial aquaculture. Recent studies focused on growth optimization of early juveniles of this species combined with the reduction in feeding costs (Ghanawi and Saoud, 2012). Enhancement in growth and feed efficiency, reduction in production costs and improvement of water quality can be attained by taking advantage of the growth response known as compensatory growth with consequent complete catch up (Turano et al., 2008; Cho and Heo, 2011; Sevgili et al., 2012). According to Jobling (2010), compensatory growth is a higher growth rate expressed by animals that had previous restricted growth when compared with the conspecifics, which have not experienced depression in growth. In turn, catch up is the convergence of growth trajectories of these animals that have different growth histories. Stumpf et al. (2010, 2011, 2012) when comparing daily and cyclically fed juveniles of redclaw crayfish *C. quadricarinatus* under individually rearing culture conditions found that newly independent juveniles (approx. 20 mg) when exposed to intermittent feeding for a short-term can catch up fully but failed when exposed for a moderate-term. However, when advanced juveniles (approx. 400 mg) were exposed to intermittent feeding for a long-term they can catch up fully. Several factors such as social behavior, water quality and feed quality may play a role in modifying compensatory and catch up growth (Cui et al., 2006).

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For example, gibel carp (*Carassius auratus* Linnaeus, 1758) and hybrid sunfish (*Lepomis cyanellus* × *L. macrochirus* Rafinesque, 1819) showed different growth responses when reared individually rather than in groups, a reaction attributed to the influence of social behavior (Hayward et al., 2000; Cui et al., 2006).

Juveniles of *C. quadricarinatus* have strong starvation resistance because of their short point of reserve saturation (PRS) and their long time to reach the point of no return (PNR) (Stumpf et al., 2010; Calvo et al., 2011, 2012), leading to a low nutritional vulnerability index (NVI) according to Gebauer et al. (2010). A strong starvation resistance has been observed in larval decapods developing in freshwater, at high latitudes, where food availability may be unpredictable (Anger et al., 2004; Paschke et al., 2004; Calvo et al., 2012). A higher NVI has been observed in various marine species, e.g., in zoea II of *Hyas araneus* (Linnaeus, 1758), in some phyllosoma stages of *Thenus* sp. (Leach, 1815) and zoea I of *Petrolisthes laevigatus* (Guérin, 1835) (cf. Anger and Dawirs, 1981; Mikami et al., 1995; Gebauer et al., 2010). Consequently, food deprivation affects metabolic activity, and the essential processes are maintained at the expense of accumulated endogenous energy reserves, which results in a loss of mass (Wen et al., 2006). Calvo et al. (2013) demonstrated that *C. quadricarinatus* juveniles exposed to long starvation periods, showed significantly diminished lipids and proteins content from hepatopancreas and pleonal muscle, and a pronounced and quick replenishment when food was offered. The structure of the hepatopancreas, the main monitor organ for the nutritional health (Gibson and Barker, 1979; Vogt et al., 1985; Verri et al., 2001; Sánchez-Paz et al., 2007) also showed the impact of a long starvation period and an ability to recover after food was provided (Calvo et al., 2013).

The use of cyclic feeding regimes may also have an overall effect on pond water quality, particularly if feeding efficiency is increased, because it may help to improve overall nutrient retention and this would be beneficial for production methods (Turano et al., 2007, 2008). Besides, the reduction of food supplied improves the yields by decreasing costs.

This study was aimed to point out the impact of food shortage on growth performance, hepatopancreas cells, energetic reserves (proteins, glycogen and lipids) mobilization in *C. quadricarinatus* juveniles, as well as its effect on culture water quality. To our knowledge, it has never been evaluated in juveniles of *C. quadricarinatus* reared in groups.

MATERIALS AND METHODS

Experimental Procedures

Juveniles were obtained under laboratory conditions, from a brood stock supplied by Farm Pinzas Rojas, Tucumán, Argentina. Ovigerous females (mean wet body mass ± standard deviation: 59.78 ± 3.17 g) were placed in individual glass aquaria (60 × 40 × 30 cm) containing 30 l of dechlorinated tap water and continuous aeration. The pH varied from 7 to 8, hardness from 70 to 100 mg/l as $CaCO_3$ equivalents, and dissolved oxygen from 5-8 mg/l. Temperature was held at 27 ± 1°C by 100 W water heaters (ATMAN) and the photoperiod was 14:10 hours (light:dark). All these variables were in agreement with Jones (1997). Females were fed daily *ad libitum* with *Elodea* sp. and commercial balanced food (granules) for tropical fish Tetracolor (TETRA®), containing 47.5% protein, 6.5% lipid, 2.0% fiber, 6.0% moisture, 1.5% phosphorus and 0.1% ascorbic acid. This diet was

previously tested and showed to be adequate for rearing *C. quadricarinatus* under laboratory conditions (Sánchez De Bock and López Greco, 2010; Stumpf et al., 2010). Juveniles were separated from their mothers after reaching the free-living stage III (Levi et al., 1999) and maintained in glass aquaria (60 × 40 × 30 cm) under the same conditions described above, until reaching approx. 1 g. Then, they were transferred to the experimental units.

Two experiments were performed using feeding protocols, consisting of alternation of feeding days and food deprivation days. This will be referred as cyclical feeding from now on. These protocols were selected based on previous results, in which Stumpf et al. (2011) demonstrated that *C. quadricarinatus* juveniles fed for 2 or 4 days followed by 2 or 4 days of food deprivation had the same survival and mass gain as those fed daily when individually cultured. Juveniles were carefully dried with a towel paper, weighed (initial mass) using an analytical balance (0.1 mg precision) and randomly assigned to rectangular plastic tanks (33.5 × 25 × 19 cm) filled with 8 liters of fresh water (experimental units). Four juveniles were stocked in each tank, in a density of 50 crayfish/m², which match the stocking density used in grow-out ponds (Jones and Ruscoe, 2000). Each tank was provided with eight PVC tubes (10 cm long and 2 cm in diameter) and two pieces of synthetic net (30 × 23 cm) as shelters to reduce cannibalism. Photoperiod, temperature and water aeration were maintained as previously described for ovigerous females. The tank water was completely replaced once a week to remove uneaten food and accumulated excretions.

Cyclic Feeding Experiments

Experiment 1 follows a completely randomized design with two treatments (feeding regimes) and five replicates (tanks). Forty juveniles (4 juveniles in each tank × 5 tanks × 2 treatments) weighing 1.03 ± 0.04 g were randomly selected from a pool of animals hatched from 4 females and subjected to two feeding regimes for 45 days. They were: (a) Control, in which crayfish were daily fed (once a day) throughout the experimental period (DF) and (b) Cyclic Feeding, in which juveniles were fed for 2 days (once a day) followed by 2 days of food deprivation (2F/2D) in repeated cycles. The animals were fed with Tetracolor (TETRA®) at 4% of their body mass (an amount of food in excess according to previous data).

Experiment 2 was designed and conducted as Experiment 1, except for the Cyclic Feeding treatment, in which juveniles (1.03 ± 0.04 g) were fed for 4 days (once a day) followed by 4 days of food deprivation (4F/4D) in repeated cycles. Juveniles from both experiments were weighed on days 15, 30 and 45. At the end of the experiments, juveniles were anesthetized with ice for 5 minutes, killed and their hepatopancreas and pleon were removed, weighed and subjected to histological and biochemical analysis.

Histological Analysis

Two hepatopancreas per replica ($n = 10$ for each feeding regime in both experiments) were fixed, dehydrated in alcohol series and embedded in paraffin. Sections (7 μm thick) were stained with hematoxylin-eosin (López Greco et al., 2007).

Biochemical Analysis

For biochemical analysis, hepatopancreas and pleon of juveniles were stored at −70°C. Two hepatopancreas from each replicate ($n = 10$ for each feeding regime in both experiments) and 2-3 pleonal muscles from each replicate ($n = 10$-15 for each experiment) were used for determination of total protein, total lipids and glycogen. Total protein was determined with Folin phenol reagent, using serum bovine albumin as the standard, according to Lowry et al. (1951), and measured with a spectrophotometer at 650 nm. Values were expressed as mg of total protein/g of tissue. Total lipids were determined by the sulfophosphovanillin method according to Folch et al. (1957) modified by Frings and Dunn (1970), and measured with a spectrophotometer at 530 nm. Values were expressed as mg of total lipids/g of tissue. Glycogen was determined following the method described by van Handel (1965), and glycogen levels were determined as glucose equivalent (glucose-oxidase method), after acidic hydrolysis according to the method described by Geary et al. (1981). Glucose was quantified using glycemia commercial kit (Wiener-Lab AA) and measured with a spectrophotometer at 505 nm. Values were expressed as mg of glycogen/g of tissue.

Water Quality Analysis

To determine the effects of cyclic feeding on water quality, the following parameters were measured once a week before complete replacement of the tank water: dissolved oxygen (DO) using a Tracer model oxygen meter

(0.02 mg/l precision; LaMotte), pH using a Tracer model pH meter (0.01 precision; LaMotte), and total ammonia ($NH_3 + NH_4^+$) and total hardness by colorimetric methods using ACUANALITICA® commercial kits.

Data Analysis

For both experiments, juvenile growth performance was calculated on a wet mass basis. The variables analyzed were: Survival (S (%) = (final number of crayfish/initial number of crayfish) × 100); specific growth rate (SGR (%/day) = ((ln final body mass − ln initial body mass)/(final time − initial time)) × 100); coefficient of variation (CV (%) = (standard deviation/mean) × 100); apparent feed conversion ratio (AFCR = (total food supplied/(final body mass − initial body mass))) (Hernández-Vergara et al., 2003; Thompson et al., 2004; Mazlum et al., 2011); apparent protein efficiency ratio (APER = (mass gain/protein provided)); hepatosomatic index (HSI (%) = (hepatopancreas mass/final body mass) × 100) and relative pleon mass (RPM (%) = (pleon mass/final body mass) × 100).

Data were tested for normality by Shapiro-Wilk's W-test and homocedasticity by Levene's test before analysis. Survival (following arcsine transformation), body mass, SGR, CV, AFCR, APER, total food supplied, HSI, RPM, energetic reserves and water quality parameters were subjected to Student's t-test. When these data did not meet the appropriate assumptions the Mann-Whitney U-test was used. Significance level was set at $P < 0.05$ (Zar, 1999). All statistical analyses were performed using STATISTICA, version 8.0.

RESULTS

Growth and Survival Performance

Juvenile survival at day 45 was 100% (DF) and 90% (2F/2D) in experiment 1, and 95% (both feeding regimes) in experiment 2 showing no difference between treatments (Mann-Whitney U-test; $P > 0.05$ and Student's t-test; $P > 0.05$, respectively). Total food supplied was 46% and 49% lower (Student's t-test; $P < 0.05$) in 2F/2D and 4F/4D treatments, respectively, than in their controls (Table 1). Nevertheless, the final mean body mass was 13% and 25% lower in the 2F/2D and 4F/4D treatments, respectively (Student's t-test; $P < 0.05$) (Table 1). SGR was lower (Student's t-test; $P < 0.05$) in the 2F/2D and 4F/4D treatments, respectively, than in the controls (Table 1). Variation in body mass between individuals (estimated by the CV) did not differ significantly between feeding regimes in both experiments (Student's t-test; $P > 0.05$) (Table 1).

Juveniles from the 2F/2D and 4F/4D treatments had similar (Student's t-test; $P > 0.05$) apparent feed conversion ratio and apparent protein efficiency ratio than the controls at the end of the experiment (Table 1). The HSI and RPW, were similar (Student's t-test; $P > 0.05$) in both feeding regimes for each experiment (Fig. 1).
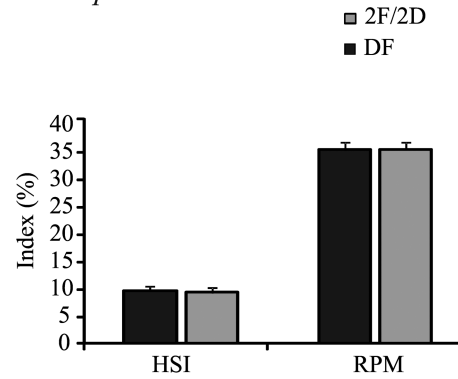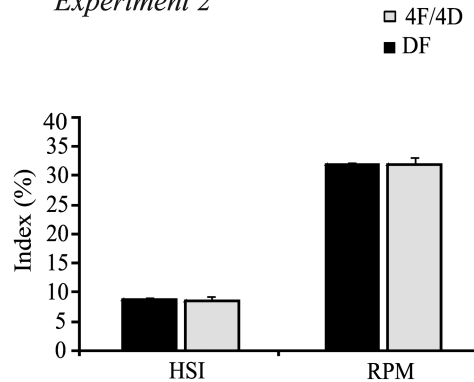

Fig. 1. Wet hepatosomatic index (HSI) and relative pleon mass (RPM) of *C. quadricarinatus* juveniles at day 45. DF = juveniles daily fed throughout the experimental period; 2F/2D and 4F/4D = juveniles fed for 2/4 days followed by 2/4 days of food deprivation in repeated cycles during the experimental period. Values are expressed as means ± standard error of five replicates per treatment.

Histology of the Hepatopancreas

The hepatopancreas was characterized based on recent descriptions of this digestive gland for this species (Calvo et al., 2011, 2012). The structure of the hepatopancreas of *C. quadricarinatus* resembles that of other decapod crustaceans and it is composed of numerous blinded tubules with four main cell types, i.e., E-, F-, B- and R-cells (Calvo et al., 2011). E-cells have a prominent nucleus occupying most of the basophilic cytoplasm and they are located at the distal

Table 1. Growth performance and production variables of *C. quadricarinatus* juveniles in both experiments, over a 45-day period. Values are expressed as means ± standard error of five replicates per treatment. SGR = specific growth rate; CV = coefficient of variation; AFCR = apparent feed conversion ratio; APER = apparent protein efficiency ratio; DF = juveniles daily fed throughout the experimental period; 2F/2D and 4F/4D = juveniles fed for 2/4 days followed by 2/4 days of food deprivation in repeated cycles during the experimental period. An asterisk (*) indicates significant differences ($P < 0.05$) between cyclic feeding and control for each experiment (Student's t-test).

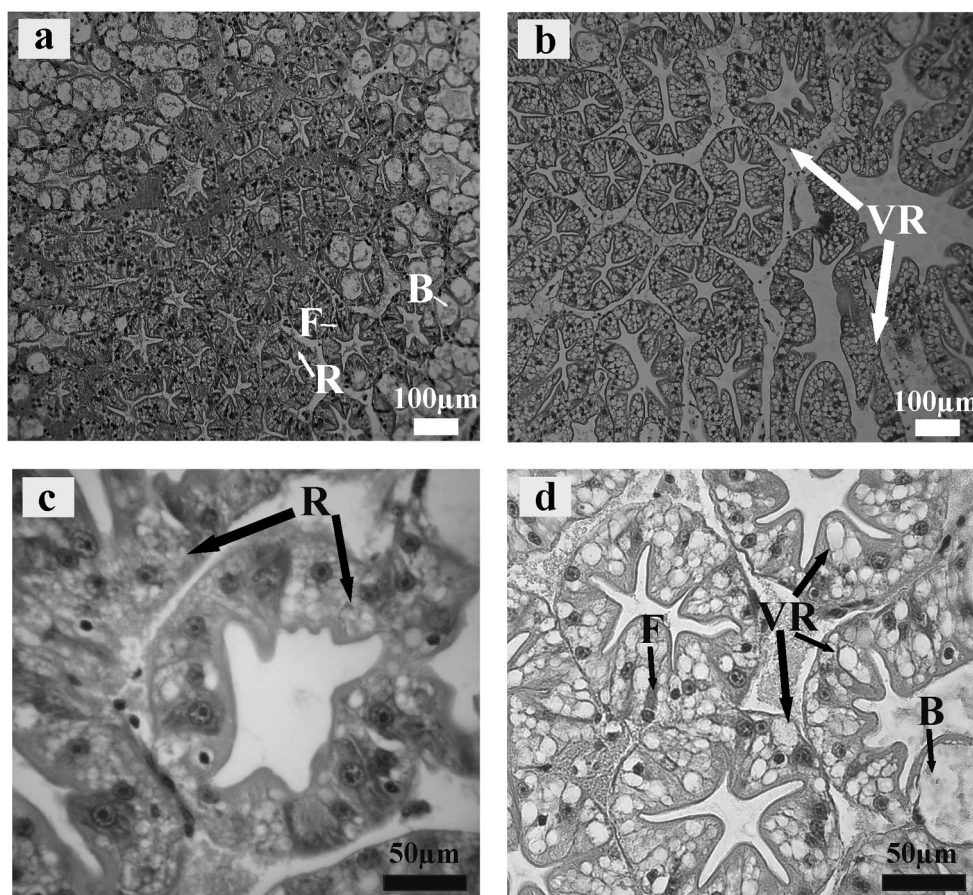|	Experiment 1		Experiment 2	
	Cyclic feeding (2F/2D)	Control (DF)	Cyclic feeding (4F/4D)	Control (DF)
Body mass (g)	2.65 ± 0.23*	3.29 ± 0.12	2.22 ± 0.22*	3.44 ± 0.36
SGR (%/day)	2.04 ± 0.24*	2.60 ± 0.19	1.52 ± 0.09*	2.14 ± 0.25
CV (%)	31.09 ± 6.58	23.98 ± 5.22	30.30 ± 2.77	30.40 ± 8.62
AFCR	4.69 ± 0.94	5.52 ± 0.30	5.68 ± 0.35	7.33 ± 1.20
APER	0.50 ± 0.07	0.39 ± 0.02	0.38 ± 0.02	0.32 ± 0.05
Total food (g)	6.65 ± 0.24*	12.36 ± 0.15	5.83 ± 0.16*	11.44 ± 0.47

Fig. 2. Histological sections of the hepatopancreas of *C. quadricarinatus* juveniles from Experiment 2, stained with hematoxilin-eosin: (a) and (c) DF = juveniles daily fed throughout the experimental period, (b) and (d) 4F/4D = juveniles fed for 4 days followed by 4 days of food deprivation in repeated cycles. R: R-cell; VR: enlarged vacuoles from R-cells; B: B-cell; F: F-cell. Magnification: (a, b) ×10; (c, d) ×40. Scale bar: (a, b) 100 μm; (c, d) 50 μm.

part of the hepatopancreatic tubules. F-cells are cylindrical, with a central nucleus and basophilic cytoplasm and they are found in the medial and proximal parts of the tubules. B-cells have a large vacuole which displaces the nucleus basally and they are more abundant in the medial and distal parts of the tubules. R-cells are cylindrical and 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 (Calvo et al., 2011) (Fig. 2). In our study, after 45 days of cyclic feeding there were no structural differences in hepatopancreas between cyclic feeding and control for both experiments. The only abnormality found in the hepatopancreas of juveniles subjected to 4F/4D cyclic feeding was the presence of R-cells with larger vacuoles than those from control juveniles (Fig. 2).

Energetic Reserves in the Hepatopancreas and Pleonal Muscle

At the end of Experiment 1, juveniles from the 2F/2D regime had hepatopancreas glycogen content significantly lower and pleonal muscle protein content significantly higher than juveniles from the DF regime (Student's t-test; $P < 0.05$) (Table 2). No significant differences (Student's t-test; $P > 0.05$) were found in the lipid content of both tissues between juveniles subjected to cyclic feeding and control (Table 2). In

Experiment 2, juveniles from the 4F/4D feeding regime had significant higher pleonal muscle protein content (Table 2) than juveniles from the control (Student's t-test; $P < 0.05$). The glycogen and lipid contents of the hepatopancreas and pleonal muscle were statistically similar for cyclic feeding and control (Student's t-test; $P < 0.05$).

Water Quality

Water temperature, dissolved oxygen and total hardness were similar between cyclic feeding and control, whereas pH were significantly higher (Student's t-test; $P < 0.05$) in cyclic feeding in both experiments (Table 3). In both experiments, total ammonia was significantly lower in cyclic feeding (Student's t-test; $P < 0.05$).

DISCUSSION

In the present study juveniles (1 g) maintained in groups and exposed to cyclic feeding regimes for 45 days were unable to maintain a normal growth trajectory. Although 2F/2D and 4F/4D regimes did not lead to compensatory responses, our results in terms of survival and the effects on the accumulation and mobilization of energy reserves were very promising.

The application of cyclic or intermittent feeding regimes has been used few times in decapod crustaceans. Mazlum

Table 2. Energy reserves (mg/g) in the hepatopancreas and pleonal muscle of *C. quadricarinatus* juveniles in both experiments, over a 45-day period. Values are expressed as means ± standard error of five replicates per treatment. DF = juveniles daily fed throughout the experimental period; 2F/2D and 4F/4D = juveniles fed for 2/4 days followed by 2/4 days of food deprivation in repeated cycles during the experimental period. An asterisk (*) indicates significant differences ($P < 0.05$) between cyclic feeding and control for each experiment (Student's *t*-test).

	Experiment 1		Experiment 2	
	Cyclic feeding (2F/2D)	Control (DF)	Cyclic feeding (4F/4D)	Control (DF)
Wet hepatopancreas (day 45)				
Total protein	27.3 ± 1.3	29.4 ± 0.9	17.2 ± 0.6	16.2 ± 0.7
Total lipids	72.6 ± 12.5	85.7 ± 9.5	133.7 ± 12.7	100.4 ± 11.6
Glycogen	5.2 ± 0.9*	26.7 ± 6.6	40.1 ± 9.5	22.3 ± 6.4
Wet abdominal muscle (day 45)				
Total protein	53.3 ± 2.1*	45.2 ± 1.6	35.3 ± 1.7*	30.0 ± 1.0
Total lipids	3.0 ± 0.8	3.0 ± 1.0	16.4 ± 3.9	13.6 ± 2.3
Glycogen	54.3 ± 21.6	30.3 ± 11.2	72.0 ± 25.3	90.4 ± 18.0

et al. (2011) when comparing the daily and cyclically fed juveniles of narrow-clawed crayfish *Astacus leptodactylus* (Eschscholtz, 1823) found that providing food every 2 days during 90 days and in group conditions, a similar growth was achieved when compared to those fed daily. In turn, Wu and Dong (2002) when comparing the daily fed and cyclically fed juveniles of the Chinese shrimp *Fenneropenaues chinensis* (Osbeck, 1765) found that juveniles failed to catch up the control in body mass when exposed intermittently to 8 days of starvation and 32 days of feeding, during 40 days in group conditions. According to our previous studies mentioned above in the section of introduction (Sumpf et al., 2010, 2011, 2012) and the present results, it seems that catch up growth may depend on individual or group conditions, and the compensatory growth may depend of the restitution of food the feeding cycle.

The similarity of the variation coefficient between treatments in both experiments showed that some juveniles exposed to cyclic feeding did not monopolize the food supply and did not generate variation in size. Apparent feed conversion ratio, apparent protein efficiency ratio, hepatosomatic index and relative pleon mass were also similar between animals fed cyclically and daily, and no structural damage was found in the hepatopancreas suggesting that the cyclic feeding does not change the ability of the crayfish to digest food and obtain nutrients for growth. In addition, substantial protein reserves were accumulated in the muscles of the pleon. These results suggest that 1 g juveniles *C. quadricarinatus* subjected to cyclic feeding may show a compensatory re-

sponse after a restoration of feeding, but further studies need to be done.

Crayfish showed an important capability to deal with food shortage (significant reduction of food offered, approximately half in relation to the controls) demonstrating a high survival. Animals show behavioral, physiological and biochemical responses to reduce maintenance metabolism to prolong survival, and energy reserves can cover metabolic needs to face food deprivation periods (Wang et al., 2006). Anger (2001) suggests that the sequence in using energy reserves in crustaceans under starvation conditions are lipids followed by protein. Lipids are mobilized for energy production in a short period of lack of food, while the pool of proteins remains initially stable reflecting their importance as structural components. If the stress continues, proteins are sacrificed for energy production. In a recent study Calvo et al. (2013) observed in juvenile *C. quadricarinatus* (1 g) exposed to 50 days of severe starvation (close to the point of no return) that the lipids of hepatopancreas and protein of pleonal muscle were consumed to ensure survival and thus the animal stopped growth. In the current study, other responses were observed in the energetic reserves considering the cyclic feeding as a moderate food deprivation which allowed the growth of these juveniles. There was only a significant utilization of hepatopancreatic glycogen in juveniles of 2F/2D. These juveniles showed a decrease of approx. 80% of this carbohydrate when compared to DF. Glycogen stores can be mobilized when needed to serve as precursors of metabolic intermediates in energy production, for synthe-

Table 3. Overall tank water quality variables over a 45-day period in *C. quadricarinatus* juveniles in both experiments. Values are expressed as means ± standard error of five replicates per treatment. T = water temperature; DO = dissolved oxygen; $NH_3 + NH_4^+$ = total ammonia; DF = juveniles daily fed throughout the experimental period; 2F/2D and 4F/4D = juveniles fed for 2/4 days followed by 2/4 days of food deprivation in repeated cycles during the experimental period. An asterisk (*) indicates significant differences ($P < 0.05$) between cyclic feeding and control for each experiment (Student's *t*-test).

	Experiment 1		Experiment 2	
	Cyclic feeding (2F/2D)	Control (DF)	Cyclic feeding (4F/4D)	Control (DF)
T (°C)	26.34 ± 0.13	26.23 ± 0.06	26.42 ± 0.14	26.59 ± 0.17
DO	7.00 ± 0.13	7.05 ± 0.18	7.22 ± 0.08	7.13 ± 0.16
pH	7.35 ± 0.12*	6.98 ± 0.10	7.62 ± 0.04*	7.34 ± 0.05
$NH_3 + NH_4^+$ (mg/l)	0.41 ± 0.03*	1.07 ± 0.11	0.20 ± 0.02*	0.32 ± 0.03
Total hardness (mg/l)	81.00 ± 1.40	84.16 ± 2.25	122.50 ± 1.84	125.83 ± 1.81

sis of nonessential amino acids, nucleic acids and cuticular chitin (Anger, 2001). One possible explanation for finding glycogen mobilization in hepatopancreas of 2F/2D and not in 4F/4D is that at day 45 the former had a single day of re-feeding compared to 4 days of the second regime, suggesting that the first days of re-feeding are the key for the recovery of glycogen.

We also observed a tendency to mobilize more lipids of the hepatopancreas in juveniles of 2F/2D regime (approx. 15% lower with respect to DF) and a tendency to accumulate more glycogen and lipids in hepatopancreas of juveniles in 4F/4D regime, which were approx. 79% and approx. 33% higher with respect to the control. It should be noted that the higher variation found among individuals within treatments may have masked any feeding regime effect on the studied reserves. The novelty of this study was the significant accumulation of proteins in pleonal muscle in both cyclic feeding regimes (approx. 18%). Pleonal muscles are considered the main local storage of protein in crustaceans and is only used when deprivation is prolonged, contributing to the maintenance of animal metabolism (Barclay et al., 1983; Dall and Smith, 1986; Anger, 2001; Silva-Castiglioni et al., 2007). The storage of constitutive material in pleonal muscle during food shortage is a conceivable adaptation for compensatory growth when food becomes abundant again. It may be possible because the high protein diet content compensates the reduced amount supplied.

This is the first study of the effect of cyclic feeding protocol on the hepatopancreas structure of this species showing that in general it was not negatively affected. Four major cell types are found in this gland (E, F, B and R), with F-cells being responsible for the release of digestive enzymes into the lumen of the digestive gland which are secreted into the foregut for extracellular digestion and B-cells playing a role in the intracellular digestive phase and the elimination of residual undigested substances (Brunet et al., 1994). R-cells are the most abundant, fulfilling the function of intracellular absorption and storage of nutrients (lipids and glycogen) in their vacuoles, although F and B-cells also take up some nutrients through pinocytosis (Sousa and Petriella, 2000; Anger, 2001; Berillis et al., 2012). As an antecedent on effects of food restriction on the structure of the juveniles of *C. quadricarinatus*, Calvo et al. (2011) detected many structural changes in the hepatopancreas due to partial food privation (juveniles fed for less than 9 days) including structural disorganization of the tubules and enlarged tubular lumen and juveniles that exhibited these characteristics also showed higher mortality during this restriction period. The only alteration that was observed in our study, i.e. the larger vacuoles in the R-cells in juveniles subjected to the 4F/4D regime, was probably due to the storage of a larger amount of lipids and glycogen. The same structural change was previously found in the hepatopancreas of juveniles fed a diet rich in lipids (Sacristán et al., 2011). Berillis et al. (2012) when comparing fed and starved groups of spiny lobsters with a novel image processing method to determine the nutritional condition found that the R-cell area is directly related to the amount of stored nutrients as lipid vacuoles and/or glycogen granules. In addition, F-cell numbers and enzyme production can

increase to replenish gastric fluid in preparation for the next meal in cultured juvenile spiny lobster, *Jasus edwardsii* (Hutton, 1875) under short-term food deprivation (Simon, 2009). However, Stumpf et al. (2014) when comparing daily fed and cyclic fed groups of red claw crayfish, found similar activity in digestive enzymes after long-term of food deprivation (45 days) suggesting that the number of cells F would not be affected. Thus, 45 days of cyclic feeding (long-term of food deprivation) may not be sufficient time to address drastic changes in the structure of the digestive gland.

The cyclic feeding regimes had a positive effect on water quality maintaining all the values recorded within the recommended range for *C. quadricarinatus* (Masser and Rouse, 1997). A decreased concentration of inorganic nitrogen was also detected due to the reduction in excretes and feces production by animals which received approx. 50% less feed. In addition, the uneaten food wastes should be lower in cultures subjected to cyclic feeding. In the present study, water pH was higher in cyclic feeding tanks, which may result from lower organic matter decomposition and consequent release of CO_2. Accordingly, total ammonia in the water was significantly lower for the cyclic feeding regimes compared to their respective controls. The decomposition of feces and leftover diet released nutrients in water column, mainly ammonia and phosphorus (Boyd, 1995). These nutrients are essential for phytoplankton proliferation, which may lead to eutrophication. On this basis, the implementation of cyclic feeding periods in commercial production ponds may lead to better water quality and consequently a reduction of the negative impact of effluents on the environment.

In summary, the results obtained in the present study infer that the protocol of cyclic feeding can be applied at least 45 days in 1 g juveniles maintained under group conditions without affecting the energetic reserves and hepatopancreas cells, emphasizing the high tolerance of this species to food restriction. These responses also suggest that when food availability returns to normal values, the reserves could be used to improve growth rate and thus compensate the previous lower growth. Further research is needed to test this hypothesis. Finally, present results suggest that the implementation of cyclic feeding regimes is a potentially effective strategy to reduce inorganic nitrogen accumulation in culture ponds of *C. quadricarinatus*.

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