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Effect of food restriction on female reproductive performance in the redclaw crayfish *Cherax quadricarinatus* (Parastacidae, Decapoda)

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Abstract

Aquaculture activity has grown remarkably in the last years, crustaceans being the most profitable products because of their high prices. Feeding costs represent a large portion of total operating costs in the aquaculture industry. In this context, the objective of the present study was to evaluate the effect of food restriction on the reproductive performance of redclaw crayfish *Cherax quadricarinatus*. Females at the onset of sexual maturity were stocked with males and were fed daily a formulated diet at 1.5% (control group) and 0.5% (restricted group) of their mean weight. The experimental period lasted 105 days. The percentage of ovigerous females and broods successfully hatched tended to be higher in restricted females compared with control females, but egg volume and weight did not differ between both experimental groups. However, the lipid concentration of rematuring ovaries was lower in restricted females than control females, suggesting that food restriction affects the amount of reserves transferred to the ovaries during vitellogenesis. The nutritional state of females was independent of the amount of food provided, as reflected by similar growth parameters and biochemical composition of the hepatopancreas in control and restricted females. Based on these results, we conclude that it is possible to reduce the feeding rate of *C. quadricarinatus* females to one-third of its original value without affecting their reproductive performance and somatic growth, at least for a 105-day period. This finding provides a useful tool for commercial producers to decrease production costs and improve water quality in culture systems.

Keywords: biochemical composition, *Cherax quadricarinatus*, food restriction, reproductive performance, somatic growth

Introduction

Global aquaculture production is growing in response to the increasing domestic consumption and export of fish food. The future of the aquaculture sector will be influenced by its capacity to address strategic challenges arising from population growth, urbanization and dietary diversification (FAO 2014). In recent years, the main purpose of aquaculture activity has been to maximize the production of high-quality organisms while reducing operating costs (Luchini & Panné Huidobro 2008; FAO 2014). The latter may be achieved by formulating cheaper diets or reducing the amount of food provided to the animals, as food can represent up to 50% of total production costs (Shiau 1998). These strategies may also improve water quality in culture systems. In this sense, Stumpf, Castillo Díaz, Viau, Valenti and López Greco (2014) found that when animals received 50% less food, there was a reduction in nitrogen concentration and organic matter decomposition due to the decrease in excretion products

and faeces. The improvement of water quality in aquaculture facilities may in turn reduce the negative impact of effluents on the environment.

The redclaw crayfish *Cherax quadricarinatus* (von Martens, 1898) is a decapod crustacean with several biological attributes that make it an excellent choice for aquaculture, such as high growth rate, multiple spawns *per* year and relatively non-aggressive behaviour (Meade & Watts 1995; Lawrence & Jones 2002). The species development is direct, with nine embryonic stages and two post-hatching stages, all of which feed on the yolk reserves accumulated in the eggs (embryo) or the cephalothorax (juveniles I and II). The successful reproduction of this crayfish is highly dependent on nutrition (Bromage 1995; Harrison 1997; García-Ulloa 2000), with diets of adequate nutrient composition being particularly important for gonad maturation and production of high-quality eggs (Rodríguez-González, Villarreal, Hernández-Llamas, García-Ulloa, Vázquez-Boucard & Serrano-Pinto 2011).

Ovarian maturation (i.e. vitellogenesis) involves the transference of reserves from the hepatopancreas to the gonad (Saoud & Ghanawi 2012; Rodríguez-González, Hernández-Llamas, García-Ulloa, Racotta, Montoya-Mejía & Villarreal 2014). Because of its role as digestion, nutrient absorption and reserve storage site, the hepatopancreas is used for monitoring the nutritional condition of cultured animals (Vogt, Storch, Quinitio & Pascual 1985; Evans, Fan, Finn, Dawson, Siva & Lee 1992; Icely & Nott 1992; McClain 1995; Johnston, Alexander & Yellowhees 1998; Jussila & Evans 1998; Sousa & Petriella 2000). Its structure is tubular, each tubule consisting of different cell types: E cells (embryonic), R cells (resorptive), F cells (fibrillar) and B cells (blisterlike). R cells are vacuolarized and store lipids and glycogen, F cells contribute to the secretion of digestive enzymes, and B cells are associated with nutrient absorption and synthesis of digestive enzymes (Gibson & Barker 1979; Al-Mohanna & Nott 1987; Caceci, Neck, Lewis & Sis 1988; Icely & Nott 1992; Franceschini-Vicentini, Ribeiro, Papa, Marques Junior, Vicentini & Moraes Valenti 2009).

The effect of using diets with different proximate compositions has been extensively studied in *C. quadricarinatus* (Saoud, Garza De Yta & Ghanawi 2012). However, to our knowledge, no study has ever addressed the effect of food restriction on the reproductive performance of this or any other decapod species. This kind of research may allow producers to determine the amount of food that minimizes production costs and improve water quality without affecting animal performance.

Based on all the above-mentioned considerations, the objective of this study was to evaluate the effect of food restriction on the reproductive performance of *C. quadricarinatus* female spawners.

Materials and methods

Animals

The reproductive stock used in this study (36 females and 12 males) was obtained under laboratory conditions. Juveniles were placed in glass aquaria measuring 60 × 40 × 30 cm at a density of 62.5 animals m^{-2}. The aquaria were filled with 30 L of dechlorinated tap water (pH 7.5, hardness 80 mg L^{-1}, as $CaCO_3$ equivalents) under continuous aeration and at a constant temperature of 27 ± 1°C (the minimum and maximum temperatures were 26 and 28°C respectively). Water was exchanged completely once a week to remove faeces and uneaten food. PVC tubes (10 cm in diameter and 25 cm long) were provided for shelter (Jones 1997), and no bottom substrate was used. The photoperiod was 14:10 h (light:dark). Juveniles were fed daily *ad libitum* an experimental food formulated for *C. quadricarinatus*, containing equal quantities of animal and vegetable proteins (Gutiérrez & Rodríguez 2010). Its proximate composition was 38% minimum crude protein, 6% minimum crude fat, 16% ash and 4% moisture. The experiment was initiated when animals reached approximately 20 g, which is the size at onset of functional sexual maturity (Vazquez, Tropea & López Greco 2008).

Experimental design

Mature crayfish with an average weight of 24.9 g for males and 17.2 g for females were randomly distributed in 12 aquaria measuring 60 × 40 × 30 cm, in a ratio of three females to one male (16.7 animals m^{-2}). The reproductive condition of these crayfish was similar, because they had never mated before the experiment. Hence, the spawns analysed corresponded to the first spawns of all the experimental females. This allowed discarding a possible effect of maternal

senescence, which may be produced by successive spawning throughout life, on present results.

Each aquarium was a replicate and was randomly assigned to one of the following treatments (six replicates *per* treatment):

- *Control*: individuals fed daily at 1.5% of the mean weight of all the animals in the aquarium.
- *Restricted*: individuals fed daily at 0.5% of the mean weight of all the animals in the aquarium.

Crayfish were fed for 5 days followed by 2 days of food deprivation in repeated cycles, and were maintained under the same conditions of water quality, temperature and photoperiod as described above. There were no others sources of food in the system besides the formulated diet provided by the researchers. The animals were weighed at days 14, 40 and 90 of the experimental period, which lasted 105 days, and a mean weight value was calculated for each replicate. This value was used to estimate the amount of food to be provided until the following weighing event.

The aquaria were visually inspected once daily for the presence of moults and deaths. Dead crayfish were removed from the aquarium, and the remaining animals were weighted again for the recalculation of food to be provided. Cannibalism was avoided during the entire experimental period by placing recently moulted crayfish in individual containers until hardening of the exoskeleton. Although the aggressive behaviour of redclaw crayfish is lower than that of other commercially important species, some competition for food was detected among individuals when feeding them. Hence, it might be possible that not all the females from the same aquarium fed similarly, and for this reason, we treated them as pseudoreplicates in the statistical analysis of the data.

Egg characterization

The aquaria were visually inspected once daily for the presence of ovigerous females. When detected, they were placed individually in plastic aquaria measuring 33.5 × 25 × 19 cm, under the same experimental conditions as described above. At day 16 of the incubation period, 5–10 eggs were gently removed from the first left pleopod of each female to evaluate egg quality. The samples were weighed (wet weight; precision: 0.1 mg), and the individual egg weight was calculated by dividing the sample weight by the number of eggs. Egg volume was determined using the formula for an ellipsoid $4/3 \times \pi \times r1 \times r2 \times r3$, where r1, r2 and r3 are the radii of the dorsal–ventral, anterior–posterior and right–left axes respectively. These axes were measured using a stereomicroscope. Finally, the dry weight of egg samples was determined (precision: 0.1 mg) following oven drying of the eggs at 55–60°C during 48 h (Hines 1982).

Female characterization

At the end of the experiment, all females were weighed (final body weight; precision: 0.1 mg) and their postorbital cephalothorax lengths were measured from behind the eye to the posterior end of the cephalothorax (precision: 0.01 mm). Females were then sacrificed after being cold-anaesthetized at −20°C for 15 min. Their carapace was removed, and the hepatopancreas and ovaries were rapidly dissected and weighed (precision: 0.1 mg). The ovaries were stored at −70°C for biochemical analysis. A portion of each hepatopancreas was fixed in Bouin's solution for 4 h at room temperature for histological examination. The tissues were then dehydrated in an alcohol series and embedded in paraffin. Sections (7 μm thick) were stained with haematoxylin–eosin (López Greco, Vazquez & Rodríguez 2007) and characterized based on recent descriptions of the hepatopancreas structure for *C. quadricarinatus* (Calvo, Stumpf, Pietrokovsky & López Greco 2011; Calvo, Tropea, Anger & López Greco 2012). The remaining portion of each hepatopancreas was stored at −70°C for biochemical analysis.

Biochemical analysis

Protein concentrations of the hepatopancreas and ovaries were determined spectrophotometrically, according to the method described by Bradford (1976). Samples ($n = 3$) weighing 60 mg were homogenized in 4:1 volume:weight of 50 mM L^{-1} Tris-HCl buffer, pH 7.5, and centrifuged at 10 000 × g for 30 min in a refrigerated centrifuge (4°C). Total proteins were estimated in the supernatant using the Coomassie blue dye method, with bovine serum albumin as standard. Absorbance was read at 595 nm.

Lipid concentrations of the hepatopancreas and ovaries were determined using the sulfophosphovanillin method described by Folch, Lees and Sloane-Stanley (1957) and modified by Frings and Dunn (1970). Samples ($n = 3$) weighing 50 mg were homogenized in 4 mL of a mixture of chloroform and methanol (2:1, v v^{-1}), then mixed with 0.9% NaCl and centrifuged to separate the lipid fraction. Extra virgin olive oil diluted with absolute ethanol was used as standard. Absorbance was read at 530 nm.

Glycogen concentration of the hepatopancreas was determined following the method described by Van Handel (1965). Samples ($n = 3$) weighing 80 mg were boiled with 4:1 volume:weight of KOH 30% for 1 h. After cooling, glycogen was precipitated with the addition of 75 μL of saturated Na_2SO_4 and 1875 μL of absolute ethanol, and centrifuged at 2000 × *g* for 10 min. The precipitate was then dissolved in 250 μl of distilled water, and glycogen was measured using the anthrone reagent method. Rabbit liver (Fluka; Sigma-Aldrich, St. Louis, MO, USA) was used as standard, and absorbance was read at 620 nm.

Calculations were performed on a wet weight basis, and values were expressed as mg g^{-1} in all cases.

Statistical analysis

The following variables were calculated at the end of the experiment:

Percentage of ovigerous females = 100 × (number of ovigerous females/total number of females);

Percentage of broods successfully hatched = 100 × (number of broods successfully hatched/total number of ovigerous females);

Gonadosomatic index = 100 × (reproductive system weight/female final body weight);

Hepatosomatic index = 100 × (hepatopancreas weight/female final body weight);

Specific growth rate = 100 × (($\log_e$ final body weight − $\log_e$ initial body weight)/time), where time was expressed in days (Evans & Jussila 1997).

One-way analysis of variance (ANOVA) was used to compare egg volume and weight (wet and dry), gonadosomatic index, biochemical composition of the ovaries, hepatosomatic index, female final body weight, specific growth rate, and postorbital cephalothorax length between control and restricted groups. The biochemical composition of the hepatopancreas was analysed using multivariate analysis of variance (MANOVA). The percentage of ovigerous females and percentage of broods successfully hatched were compared between treatments with the Fisher's exact test. Results *per* treatment are presented as means ± SE. All tests were carried out at the 95% significance level (Sokal & Rohlf 1995).

Results

Females spawned only once during the experimental period, which corresponded to their first spawn. No significant differences were found between treatments in the percentage of ovigerous females and percentage of broods successfully hatched ($P > 0.05$; Table 1), although both variables tended to be higher in the restricted group. The amount of food provided to the females had no effect on egg production, as reflected by similar egg volume and weight (wet and dry) in the control and restricted treatments ($P > 0.05$; Table 1). Mean values were 6.2 mm^3, 4.7 and 2.1 mg for the volume, wet weight and dry weight of eggs respectively.

Table 1 Effect of food restriction on reproduction and growth of *Cherax quadricarinatus* females over a 105-day period*,†,‡

Variable	Control	Restricted
Ovigerous females (%)	20.83[a]	33.33[a]
Broods successfully hatched (%)	40[a]	75[a]
Egg volume (mm^3)	6.07 ± 0.44[a] (5)	6.37 ± 0.89[a] (6)
Egg wet weight (mg)	4.60 ± 0.28[a] (5)	4.77 ± 0.51[a] (6)
Egg dry weight (mg)	1.99 ± 0.16[a] (5)	2.14 ± 0.19[a] (6)
Final body weight (g)	20.87 ± 4.18[a] (3)	20.03 ± 4.07[a] (3)
Specific growth rate (%/day)	0.37 ± 0.24[a] (3)	0.32 ± 0.19[a] (3)
Postorbital cephalotorax length (mm)	33.42 ± 2.22[a] (3)	33.63 ± 3.93[a] (3)

*The initial mean body weight of females was 17.19 g (13.45–23.42 g). The final body weight and postorbital cephalothorax length were recorded at day 105 of the experimental period.
†Values in parentheses are the number of replicates (i.e. aquaria) used to calculate each variable.
‡Within a row means followed by the same superscript are not significantly different ($P > 0.05$).

The gonadosomatic index tended to be lower in restricted females at the end of the experiment, yet this difference was not statistically significant ($P > 0.05$; Table 2). The biochemical composition of ovaries differed between treatments, with lipid concentration being significantly lower in restricted females ($P < 0.05$). However, protein concentration did not depend on the amount of food provided, reaching a mean value of 39.2 mg g^{-1} ($P > 0.05$; Table 2).

The hepatosomatic index and biochemical composition of the hepatopancreas were likewise similar in the control and restricted treatments; the concentrations of lipids, proteins and glycogen averaged 58.9, 17.5 and 2.4 mg g^{-1} respectively ($P > 0.05$; Table 2). The general structure of the hepatopancreas was unaffected by food restriction (Fig. 1a,b). Nevertheless, some minor alterations were observed in the hepatopancreas of restricted females, comprising enlarged and abnormally shaped tubular lumen (Fig. 1c), widening of the intertubular space (Fig. 1d), hypertrophy and coalescence of B cells (Fig. 1e), and disorganization of the tubular epithelium (Fig. 1f).

Regarding female growth, no differences were observed between treatments in final body weight, specific growth rate and postorbital cephalothorax length ($P > 0.05$; Table 1).

Discussion

This study is, to our knowledge, the first attempt to analyse the effect of food restriction on the reproductive performance of a decapod crustacean. The percentage of ovigerous females and percentage of broods successfully hatched tended to be higher in restricted females compared to control females, but no differences were observed in the volume and weight of eggs produced by both experimental groups. These variables are generally considered as good indicators of egg quality because they reflect the amount of nutrients stored in the yolk (Attard & Hudon 1987; Clarke 1993). Besides, they are easy and quick to assess (García-Ulloa, Rodríguez & Ogura 2004). Egg quality is generally dependent on the diet provided to crustacean spawners, as substantial quantities of proteins and lipids synthesized from ingested food are required during ovarian maturation (Bromage 1995; Harrison 1997; García-Ulloa 2000). Rodríguez-González, García-Ulloa, Hernández-Llamas and Villarreal (2006a) found that the eggs of *C. quadricarinatus* were larger and heavier when females were fed diets containing 32–37% protein compared to those with 22–27% protein. The diet used in the present study had 38% protein, which may explain the similar values of egg volume and weight between that study and ours. Tropea, Arias, Calvo and López Greco (2012) also reported similar values for both variables, but they fed broodstock a commercial diet (Tetracolor, Tetra GmbH, Melle, Germany) with higher protein content (47.5%). It seems from our results, and those reported by Tropea *et al.* (2012) that feeding females with the formulated diet and Tetracolor, respectively, lead to the production of similar-sized eggs, which may reflect similar yolk contents (Clarke 1993). Hence, from the comparison of both studies we conclude that the commercial and formulated diets may meet equally well the nutritional requirements for vitellogenesis.

More importantly, present results suggest that food restriction had no effect on the amount of metabolic reserves that females transferred to the

Table 2 Effect of food restriction on biochemical composition of the hepatopancreas and ovaries of *Cherax quadricarinatus* females over a 105-day period*,†

Variable	Control	Restricted
Gonadosomatic index (%)	3.98 ± 1.97^{a} (3)	1.12 ± 0.55^{a} (3)
Ovary lipids (mg g^{-1})	47.70 ± 5.20^{a} (3)	23.00 ± 11.08^{b} (3)
Ovary proteins (mg g^{-1})	33.90 ± 24.26^{a} (3)	44.40 ± 21.98^{a} (3)
Hepatosomatic Index (%)	7.01 ± 1.21^{a} (3)	7.53 ± 0.53^{a} (3)
Hepatopancreas lipids (mg g^{-1})	63.80 ± 25.89^{a} (3)	53.90 ± 11.82^{a} (3)
Hepatopancreas proteins (mg g^{-1})	24.20 ± 5.59^{a} (3)	10.80 ± 11.13^{a} (3)
Hepatopancreas glycogen (mg g^{-1})	2.00 ± 0.45^{a} (3)	2.80 ± 0.65^{a} (3)

*Values in parentheses are the number of replicates (i.e. aquaria) used to calculate each variable.

†Within a row means followed by the same superscript are not significantly different ($P > 0.05$).

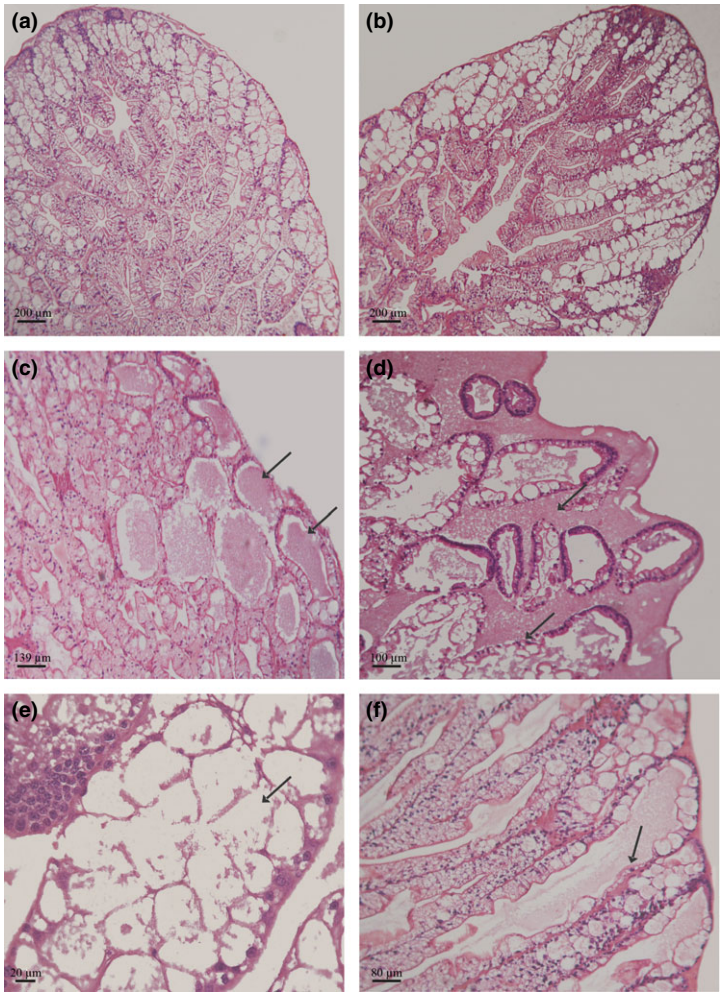

Figure 1 Histological sections of the hepatopancreas of *Cherax quadricarinatus* females fed at 1.5% (a) or 0.5% (b-f) of their mean weight, stained with haematoxylin–eosin. (a) General view showing the normal hepatopancreas structure; (b) general view showing some abnormalities in the hepatopancreas structure; (c) enlarged tubular lumen that lost the typical star-shape; (d) widened intertubular space and thinner tubule epithelium; (e) hypertrophy and coalescence of B cells; (f): cellular disorganization of the tubular epithelium. [Colour figure can be viewed at wileyonlinelibrary.com]

eggs. However, it may alter vitellogenesis during ovarian rematuration, as reflected by a lower gonadosomatic index in restricted females at the end of the experimental period. This index represents an easy way to measure size and weight changes of the gonad relative to the whole organism, but provides no information about the internal changes in the gonad (Grant & Tyler 1983; McRae & Mitchell 1995). Such changes may be detected by determining its biochemical composition, which was partly affected by food restriction. In particular, the lipid concentration of maturing ovaries was lower in restricted females than control females. Considering that the required lipids for gonad maturation come from the diet (Rodríguez-González, Hernández-Llamas, Villarreal, Saucedo, García-Ulloa & Rodríguez-Jaramillo 2006b), it seems that food reduction was too great for adequate ovarian rematuration. Lipids are essential as structural components of cell membranes and as energy source for the developing embryo (Coutteau, Geurden, Camara, Bergot & Sorgeloos 1997). The presence of lipid reserves in the eggs is particularly important in decapods with direct development, such as *C. quadricarinatus*, because the embryogenesis is lengthy in this group and, consequently, the embryos hatch at a more advanced stage of development (Herring 1974). For this reason, future studies may be necessary to evaluate a potential negative effect of food restriction on egg quality over successive periods of ovarian rematuration (i.e. consecutive spawns).

The ovarian protein content was highly variable among females from both treatments, as previously reported by Rodríguez-González *et al.* (2006b). They showed that this parameter varies in a wide range for each gonadosomatic index of

C. quadricarinatus females at first maturation, increasing during the ovarian maturation process. Hence, both the intrinsic variability of this parameter and the fact that females had ovaries at different maturation stages when the experiment was ended (as reflected by the variable gonadosomatic index) may explain the high variation observed in ovary proteins.

On the other hand, food restriction had no effect on the hepatosomatic index and biochemical composition, with index values resembling those obtained by Rodríguez-González *et al.* (2006b) for females of the same species. These results suggest that the food restriction protocol applied (i.e. crayfish fed daily at 0.5% of their weight, for five days followed by two days of food deprivation in repeated cycles, during a 105-day period) was not severe enough to deteriorate the nutritional state of crayfish. However, we found minor alterations in the histological structure of the hepatopancreas of restricted females that were similar to those reported by Calvo *et al.* (2011) and Sacristán, Ansaldo, Franco Tadic, Fernández Giménez and López Greco (2016) for *C. quadricarinatus* juveniles exposed to severe starvation. Alterations in the hepatopancreas structure may indicate nutritional stress, because changes in its cell types are related to alterations in nutrient absorption or storage and protein synthesis mechanisms (Berillis, Simon, Mente, Sofos & Karapanagiotidis 2013). Present results may not reflect nutritional stress of females, because the histological alterations of their hepatopancreas were minor, and growth variables were similar to those of control females. Instead, they may reflect a higher effort or capacity of restricted females to digest nutrients in order to compensate for lower food availability. In particular, the hypertrophy of B cells may be due to an increased synthesis of digestive enzymes and consequently higher intracellular digestion rates. On the other hand, the disorganization and lower height of the tubular epithelium may be related to the lipid content in the hepatopancreas (Simon 2009), which tended to decrease in restricted females. This may in turn affect the transfer of nutrients to the ovary for vitellogenesis and explain the lower lipid content found in the ovaries of restricted females at the end of the experimental period. It cannot be discarded that the histological alterations observed in the hepatopancreas as a consequence of food restriction were the prelude of more severe biochemical changes. If that were the case, these minor alterations may indicate the maximum period during which food restriction can be applied with no strong negative effects. Future studies may confirm if food restriction continuing beyond 105 days affects more severely the hepatopancreas structure and function in adult females as a result of nutritional stress, and its consequence on nutrient transference to the ovary.

Several authors have reported a negative correlation between the hepatosomatic and gonadosomatic indices in *C. quadricarinatus* and other decapods, reflecting the transference of reserves from the hepatopancreas to the gonad during vitellogenesis (Galois 1984; Castille & Lawrence 1989; Mourente, Medina, González & Rodríguez 1994; Rodríguez-González *et al.* 2006b). Interestingly, lipid concentration of the ovaries, but not the hepatopancreas, was reduced by food restriction, which suggests that restricted females would rather store reserves in the hepatopancreas (probably to maintain their growth rate) than use them for vitellogenesis. The differential allocation of lipids may be an adaptive response of crayfish to cope with lower food availability, 'getting prepared' for potential compensation when favourable conditions are re-established (Stumpf *et al.* 2014). In this sense, female investment in maintenance of growth and survival during food restriction might lead to better reproductive conditions in the future. However, only the re-establishment of normal feeding conditions (i.e. control conditions) may allow determining whether compensation is actually directed towards reproduction following the restriction period.

As already mentioned, no differences were observed in growth parameters between control and restricted females at the end of the experiment. Most of the studies that evaluated *C. quadricarinatus* growth were performed on juveniles weighing 1–6 g (e.g. García-Ulloa, López-Chavarín, Rodríguez-González & Villarreal-Colmenares 2003; Hernández-Vergara, Rouse, Olvera-Novoa & Davis 2003; Thompson, Muzinic, Engler & Webster 2005). The fact that we analysed growth in sexually mature females (initial wet weight: 17.2 g) that reproduced during the experimental period may explain the lower specific growth rates observed with respect to the ones shown in those studies. Accordingly, we found similarities with other studies performed on pre-adult crayfish (initial weights between 13 and 17 g), which reported

specific growth rates ranging from 0.3 to 0.6%/day (Jones & Ruscoe 2000, 2001; Barki, Karplus, Manor, Parnes, Aflalo & Sagi 2006; Pavasovic, Anderson, Mather & Richardson 2007). This may indicate that females were in good nutritional condition after being fed with our formulated diet. The lack of effect of food restriction on female growth performance agrees with previous results reported by Stumpf, Calvo, Díaz, Valenti and López Greco (2011), who exposed *C. quadricarinatus* juveniles to food restriction for up to 60 days with no changes in growth and survival, showing great resistance to starvation. Such resistance may be a result of the highly variable biotic and abiotic conditions in the freshwater environments inhabited by the species (Masser & Rouse 1997), which may consequently experience transitory periods of shortage in food availability (Jones & Obst 2000).

In conclusion, present results show that it is possible to reduce the feeding rate of *C. quadricarinatus* females to one-third of its original value without affecting their reproductive performance and somatic growth, at least for a 105-day period. This finding provides a useful tool for commercial producers to lower production costs and improve water quality in culture systems.

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