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Effect of long-term exposure to high temperature on survival, growth and reproductive parameters of the "redclaw" crayfish *Cherax quadricarinatus*

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ABSTRACT

The effect of temperature on survival, growth and reproductive parameters of *Cherax quadricarinatus* from a stage of early sexual differentiation to a stage of sexual maturity was investigated. Growth performance was expressed as specific growth rate (SGR) and growth increment (GI), while reproductive performance was evaluated in terms of the gonadosomatic index (GSI), mean oocyte diameter (MOD), proportion of mature vs. immature testicular lobes and structure of the vas deferens. The experiment consisted of males and females exposed separately to two treatments (water temperature at 27 and 30 °C) during a 360-day period. Survival was similar between treatments for each sex and between sexes for each treatment. Female growth, GSI and MOD did not differ between treatments. High temperature induced spawning in at least 20% of females. This treatment negatively affected growth of males, but they showed higher GSI and a greater proportion of mature testicular lobes, thus indicating an accelerated spermatogenesis. At 27 °C, males grew more than females from an approximate size of 15 g, confirming previous results about the differential growth of both sexes. We conclude that the long-term exposure to 30 °C is favorable for ovary maturation and spawning in females, but it clearly affects male somatic growth and consequently, the yields of culture. Both results are especially useful for the species culture in tropical countries. The effect of this water temperature on male reproductive performance deserves further research.

1. Introduction

Decapod crustaceans have a great potential for aquaculture, with penaeid shrimp species being one of the most important groups from a commercial point of view (FAO, 2006). In relatively recent times, the culture of crayfish of the genus *Cherax* has begun to develop, not only in Australia, their native country, where they inhabit freshwater bodies such as rivers and lakes, but also in many tropical and subtropical countries, such as China, Israel, Ecuador, Mexico, United States of America and Argentina (Medley et al., 1994; Rouse, 1995; Rodgers et al., 2006). This genus includes three commercially important species, the "redclaw" *Cherax quadricarinatus*, the "marron" *Cherax tenuimanus* and the "yabbie" *Cherax destructor*.

C. quadricarinatus is advantaged with respect to the other two species by several biological and commercial attributes that make it an excellent choice for aquaculture. Although it is native of a tropical region, it grows well at temperatures between 24 and 30 °C, under which it reaches the commercial size (50–100 g) in an approximate 7 months-period. On the other hand, it can tolerate a broad range of water quality conditions, including low oxygen concentrations (>1 ppm); hardness and alkalinity (20 to 300 ppm) and pH (6.5 to 9) (Masser and Rouse, 1997; Luchini, 2004; Panné Huidobro et al., 2004).

C. quadricarinatus is a gonochoristic species, the male reproductive system consisting of a pair of testes connected in their middle portion to the vasa deferentia, which open in the *appendices masculinae* at the bases of the fifth pair of walking legs (Hobbs et al., 2007; López Greco et al., 2007). The female reproductive system consists of a pair of ovaries, from which the oviducts extends laterally to connect with the genital openings located at the bases of the third pair of walking legs (Vazquez et al., 2008). Sexual maturity is reached in both sexes from an approximate weight of 18 g. Females have a relatively high fecundity (100–1000 eggs *per* spawning event, depending on size), and they can spawn multiple times (3 to 5 times in a year) throughout a period of about 6 months and under temperatures above 22 °C. Development of this species is direct, meaning that the adult morphology is achieved without progression through a morphologically distinct, free-living larval phase. This greatly simplifies breeding under cultured conditions (Masser and Rouse, 1997; Luchini, 2004; Panné Huidobro et al., 2004).

Many studies have analyzed the effect of different physical variables, such as temperature, salinity and water calcium concentration on

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growth and survival of freshwater crayfish (King, 1994; Jones, 1997; Verhoef and Austin, 1999; Zhao et al., 2000; García-Guerrero et al., 2003; Carmona-Osalde et al., 2004; Hammond et al., 2006; Harlioglu, 2009), and penaeid shrimps (Bartlett et al., 1990; Lester and Pante, 1991; Bray et al., 1985; Wyban et al., 1995; Ponce-Palafox et al., 1997). Temperature is considered to be one of the most important physical factors influencing metabolic activity (García-Guerrero et al., 2003), food intake (Whitmore, 1997) and growth (Ponce-Palafox et al., 1997). In order to improve yields in production systems, many of the studies mentioned above focused on determining the upper and lower limits of the optimal temperature range that includes the water temperature at which growth parameters and survival are optimum (King, 1994; Jones, 1997; Ponce-Palafox et al., 1997; Zhao et al., 2000; García-Guerrero et al., 2003; Carmona-Osalde et al., 2004; Hammond et al., 2006). However, there is a general lack of information on the possible effect of temperature on reproductive parameters; in particular no analysis has ever been made of the effect of temperature on both female and male reproductive systems from a histological point of view.

Temperature was found to affect sperm quality in penaeid shrimps (Bray et al., 1985; Pascual et al., 1998; Perez Velazquez et al., 2001), and to significantly reduce sperm production in *C. quadricarinatus* males (Bugnot and López Greco, 2009). On the other hand, ovarian development in *Procambarus llamasi* and *C. quadricarinatus* seems to be positively influenced by increasing temperature (Carmona-Osalde et al., 2004; Sánchez de Bock and López Greco, 2009, respectively), a tendency also seen in the spawning rate of *C. quadricarinatus* females (Yeh and Rouse, 1995).

The biology of *C. quadricarinatus* is modulated by temperature in many other aspects. King (1994) and Jones (1997) studied the effect of different water temperatures on the growth and survival of hatchlings and juveniles, respectively. Growth of juveniles was best between 24 and 28 °C and strongly reduced below 20 °C and above 34 °C, while their survival was significantly lower at 34 °C (Jones, 1997). Hatchlings reached a maximum growth rate at about 30 °C, while 15 °C was lethal (King, 1994). The effect of temperature on survival and duration of development from egg extrusion to juvenile stage was also investigated, with both parameters decreasing with increasing temperature (García-Guerrero et al., 2003). All these studies analyzed the effect of temperature on certain stages of the species life cycle.

Based on the considerations mentioned above the objective of the present research was to investigate the effect of long-term exposure (one year) to a temperature higher than the one commonly used in culture, on survival, growth and gonad development of *C. quadricarinatus*. This was investigated along male and female ontogeny, namely, from a stage of early sexual differentiation to the sexual maturity stage.

2. Materials and methods

2.1. Animals

Juveniles were obtained under laboratory conditions, from a reproductive stock supplied by the Farm Las Golondrinas, Entre Ríos, Argentina. Each ovigerous female was maintained in an individual glass aquarium of 60×40×30 cm containing 20 L of dechlorinated tap water and under continuous aeration. The total number of aquaria used for these females was 6. The temperature was held constant at 26–27 °C by ALTMAN water heaters (100 W, precision 1 °C), and the photoperiod was 14:10 (L:D). Each aquarium was provided with a PVC tube (10 cm in diameter and 25 cm long) as shelter (Jones, 1995a). Females were fed daily *ad libitum* with *Elodea* sp. and commercial Tetradiskus granules (approximate composition: min. crude protein 47.5%, min. crude fat 6.5%, max. crude fiber 2.0%, max. moisture 6.0%, min. phosphorus 1.5% and min. ascorbic acid 100 mg Kg^{-1}). When juveniles became independent at stage 3 (Levi et al., 1999), they were separated from their mothers. After reaching approximately 0.200 g, they were sexed by manual examination for the presence of genital openings at the bases of the third (females), fifth (males) or both (intersex specimens) pairs of walking legs.

Intersex specimens were discarded, while 72 males and 72 females were selected for the experiment. There was no significant difference ($p>0.05$) in the average initial weight between male (0.211 ± 0.018 g) and female (0.216 ± 0.007 g) juveniles.

2.2. Experimental design

Male and female juveniles were treated in separate aquaria and randomly assigned to each of the following treatments:

- Normal culture temperature (C): water temperature maintained at 27 ± 1 °C
- High temperature (HT): water temperature maintained at 30 ± 1 °C

Each experimental group was run in triplicate (3 aquaria *per* treatment and *per* sex), with a total of 12 crayfish *per* aquarium (50 animals/m^2) and a total number of 12 aquaria. Organisms were maintained in a 60×40×30 cm glass aquarium filled with 20 L of dechlorinated tap water with continuous aeration. PVC tubes (10 cm in diameter and 25 cm long) and onion bag mesh were used as shelter. The experiment was performed under constant conditions of photoperiod (14:10 (L:D)) and temperature (100 W ALTMAN aquarium heater, precision 1 °C). The temperature was monitored daily to ensure a maximum oscillation of 1 °C around the treatment values. The juveniles were fed daily *ad libitum* with commercial Tetradiskus granules and *Elodea* sp. All aquaria were cleaned and water was completely replaced once a week. The experimental period comprised 360 days, during which animals were sexed, weighed (precision 0.01 mg) and their mortality recorded every 2 weeks. The presence of ovigerous females and the differentiation of the "red patch" in males were also recorded.

2.3. Morphological and histological examination

At the end of the experiment, all animals were weighed (precision: 0.1 mg) and the following morphometric variables were measured (precision: 0.01 mm): cephalothorax length (CL), from the tip of the rostrum to the end of the cephalothorax; post-orbital cephalothorax length (POL), from behind the eye to the end of the cephalothorax; pleon width (PWi), measured in the second abdominal segment; length, width and height of the chelae (LC, WC and HC, respectively); and length of the red patch (LRP), when present. The weight of the chelae (CWe) and pleon (PWe) of each animal were also recorded.

After being cold-anaesthetized at −20 °C for 15 min the carapace was removed and the gonads were inspected to determine their relative size, form and color. In females, the stage of ovarian development was assessed using a four-stage ovary color pattern as follows: transparent (stage I), cream to pale orange (stage II), orange with some olive green oocytes (stage III), and fully olive green (stage IV) (Vazquez et al., 2008). The ovaries, testes and vasa deferentia were quickly dissected, weighed, and fixed in Bouin's solution for 4 h at room temperature. The tissues were then dehydrated, embedded in Glycol Methacrylate (Leica Historesin, Leica Microsystems), sectioned at 4 μm thick, and stained with Toluidine blue. At least three slides from each crayfish were inspected under light microscope. The oocyte diameters in each female were measured with an 8X Zeiss microscopic ocular lens, calibrated against a Leitz Wetzlar plate with 10-μm spacing on a representative section of each ovary. Only those oocytes with visible nuclei were measured. In males, the number of mature (containing spermatozoa) and immature testicular lobes were determined under light microscope. The structure of the vas deferens (VD) epithelium, the relative amount of secretion and the components of the primary and secondary layers of the spermatophore, including