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Growth and metabolic parameters of common snook juveniles raised in freshwater with different water hardness

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ABSTRACT

The adaptation of common snook *Centropomus undecimalis* to low salinities and freshwater has being widely studied, but the definition of the best water hardness levels may facilitate the adaptation and growth of this species in freshwater. Consequently, the aim of this study was to evaluate survival, growth and metabolic parameters (blood glucose and osmolality and lactate in plasma and tissues) of common snooks raised in freshwater with different water hardness levels. In an acute experiment, juveniles were transferred from seawater 35 ppt to seawater 35 ppt (control) and freshwater at 20, 100, 250, 500 or 1000 mg $CaCO_3$ L^{-1}. Mortality after 96 h was 100, 79.17, 58.33, 4,17 and 50%, respectively. In another experiment juveniles (initial weight 10.9 ± 1.5 g) were maintained for 60 days in water hardness of 100, 500 or 1000 mg $CaCO_3$ L^{-1} or seawater (control). No mortality was observed in this experiment. The best weight gain, specific growth rate and feed intake in freshwater (values not significantly different from seawater) was observed in common snooks kept at 100 mg $CaCO_3$ L^{-1}. Blood and hepatic glucose did not differ significantly between treatments. Muscle glucose levels were significantly lower in fish kept at 100 and 1000 mg $CaCO_3$ L^{-1} than the other treatments. However, lactate levels were higher in the muscle of common snooks kept at 100 to 500 $CaCO_3$ mg L^{-1} and lactate in plasma was higher in those maintained at 1000 mg $CaCO_3$ L^{-1}. Plasma osmolality was not affected significantly by treatments. In conclusion, 100 mg $CaCO_3$ L^{-1} is the best water hardness to raise common snooks in freshwater.

1. Introduction

The common snook, *Centropomus undecimalis*, is a carnivorous teleost (Machado et al., 2013; Dutka-Gianelli, 2014) known for characteristics that qualify it for the practice of aquaculture, such as tolerance to low oxygen concentrations and wide variation of salinity, great rusticity, easy adaptation to inert diets and quality meat (Chapman et al., 1982; Tucker, 1987). Consequently, common snook may be an alternative species for marine culture in the tropical and subtropical regions of Americas, as well as in freshwater, in which it presents good growth rates (Alvarez-Lajonchère and Tsuzuki, 2008; Tucker, 1987; Tucker and Jory, 1991).

Euryhaline teleosts, such as the common snook, are able to maintain blood osmolarity in a tolerable range, independently of environmental salinity, due to an effective osmoregulation (Varsamos et al., 2005; Gracia-López et al., 2006). Exposure to a different salinity may alter the cost of ionoregulation and consequently the amount of energy available for fish growth (Gracia-López et al., 2006; Anni et al., 2016). Seawater-adapted fish transferred to low salinities need to reorganize their metabolism to face the ionoregulatory challenge (Boeuf and Payan, 2001; Morgan and Iwama, 1991). Several euryhaline teleosts present lower growth in lower salinities (Sampaio and Bianchini, 2002; Chen et al., 2009; Mylonas et al., 2009; Rhody et al., 2010) and in freshwater common snooks also showed lower plasma osmotic pressure (Gracia-López et al., 2006).

As waterborne Ca^{2+} reduces gill permeability (Wood, 2001; Baldisserotto, 2011), the increase of water hardness may be a good strategy to improve growth of common snook in freshwater. Tucker (1987) demonstrated that common snook presents good growth in freshwater at water hardness 269 and 705 mg $CaCO_3$ L^{-1}), but no

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study so far compared growth of this species in freshwater at different water hardness. Thus, the objective of the present study work was to evaluate the influence of different water hardness on growth, survival and physiological parameters (blood glucose and lactate in plasma and tissues, blood osmolality) of common snooks in freshwater.

2. Material and methods

2.1. Fish

The experiments were performed in the Marine Fish Culture Laboratory (LAPMAR) at the Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil. Juvenile common snook *Centropomus undecimalis* (initial weight 10.92 ± 1.52 g and total length 11.34 ± 0.68 cm) were obtained of a spawning induced in broodstocks as described by Passini et al. (2013) and maintained at the LAPMAR in salinity 35 ppt.

2.2. Acute experiment

Juvenile common snooks were transferred directly from a 500 L tank with seawater 35 ppt to continuously aerated 10 L aquaria containing seawater 35 ppt (control) or freshwater at 20, 100, 250, 500 or 1000 mg $CaCO_3\,L^{-1}$ (freshwater with the highest water hardness presented Ca^{2+} levels similar to seawater) (n = 8, three aquaria per treatment) and maintained for 96 h. The treatment with the highest water hardness level contains waterborne Ca^{2+} levels close to seawater. Water quality parameters were: 21.46 ± 0.65 °C, dissolved oxygen levels 6.25 ± 0.02 – freshwater and 4.98 ± 0.02 – seawater pH 7.34 ± 0.01 - freshwater and 7.78 ± 0.01 – seawater, alkalinity 17.24 ± 6.01 mg $CaCO_3\,L^{-1}$ - freshwater and 104.05 ± 33.31 mg $CaCO_3\,L^{-1}$ – seawater, water hardness 21 ± 3.57 mg $CaCO_3\,L^{-1}$; 103.54 ± 4.52 mg $CaCO_3\,L^{-1}$; 244.09 ± 8.89 mg $CaCO_3\,L^{-1}$; 543 ± 26.47 mg $CaCO_3\,L^{-1}$; 1077.08 ± 84.16 mg $CaCO_3\,L^{-1}$; seawater 5659 ± 196.35 mg $CaCO_3\,L^{-1}$, total ammonia nitrogen (TAN) 0.42 ± 0.21 mg L^{-1}. Fish were not fed through this experiment. Water renewal was 100% daily and dead fish were removed. Water of each treatment was previously adjusted to the experimental conditions and hardness was increased with $CaCl_2{\cdot}2H_2O$ if necessary.

Lethal concentrations (LC_{50}) were calculated from mortality data every 24 h with the software “Trimmed Spearman Karber Method”.

2.3. Growth experiment

Common snooks (n = 204) were kept in a continuously aerated 10,000 L tank, salinity 32 ppt and 28 °C for five months. After, fish were transferred to a 500 L tank where water was adjusted gradually through four days to freshwater with water hardness 500 mg $CaCO_3\,L^{-1}$ (treatment with best survival in the acute experiment of transfer to freshwater) and temperature to 32 °C, with 1 °C increase day^{-1}, as suggested by Silva (2016). Fish were then randomly distributed into four groups (freshwater, water hardness 100, 500 or 1000 mg $CaCO_3\,L^{-1}$ (treatment of 20 mg $CaCO_3\,L^{-1}$ was not used in this experiment because provoked total mortality in the acute experiment) or seawater (control), each group in an independent recirculated system consisting of three 150 L tanks (n = 17 fish each tank), 50 μm filter, biofilter, skimmer and UV sterilizer (60 W) and temperature control. Fish were kept in a 12 h light: 12 h dark cycle and were fed four times a day until apparent satiation with commercial feed (Nutripiscis AL 45, 2.6 mm diameter, 45% crude protein - Paulínia - São Paulo, Brazil) for 60 days. Water quality parameters were: 31.52 ± 0.24 °C, dissolved oxygen levels 5.64 ± 0.02 – freshwater and 4.58 ± 0.03 – seawater, pH 7.34 ± 0.01 - freshwater and 7.78 ± 0.01 – seawater, 20.35 ± 0.47 mg $CaCO_3\,L^{-1}$ - freshwater and 95.19 ± 1.91 mg $CaCO_3\,L^{-1}$ – seawater, water hardness 103 ± 1 mg $CaCO_3\,L^{-1}$; 510 ± 4 mg $CaCO_3\,L^{-1}$; 1000 ± 8 mg $CaCO_3\,L^{-1}$; seawater 5789 ± 76 mg $CaCO_3\,L^{-1}$, TAN 0.37 ± 0.09 mg L^{-1} and nitrite 0.16 ± 0.04 mg L^{-1}.

Every 20 days of experiment all common snooks were anesthetized with 50 mg L^{-1} benzocaine, measured (total length) and weighed (body weight). Survival (S) was calculated using the following formula: $S = (Nf / Ni) \times 100$; where Nf and Ni are the number of fish at the end and the beginning of the experimental period, respectively. Weight gain (Wg), specific growth rate (SGR) and feed conversion (FC) were calculated as follows:

$Wg = Wf - Wi$, where Wf is the final body weight (g) and Wi is the initial body weight (g);

$SGR = [(\ln Wf - \ln Wi) / t) \times 100]$, where t is the time of experiment (days); and

$FC = FI / Wg$, where FI is feed intake (the total amount of feed consumed by the fish) (g).

The protocol was approved by the Ethics and Animal Use Committee of the Universidade Federal de Santa Catarina (UFSC), number PP00861/CEUA/PROPESQ/2013.

2.4. Water quality

Daily measurements of temperature and dissolved oxygen levels were made with oxygen meter Pro 20 (YSI, USA), pH with pH meter EcoSense pH 10 (YSI, USA), alkalinity following Apha (2005) and water hardness according to Eaton et al. (2005). Water hardness was adjusted with $CaCl_2{\cdot}2H_2O$, alkalinity with $NaHCO_3$ and pH was raised with NaOH 2N when below 7.0. Every week TAN (every two days in the acute experiment) and nitrite were determined according to Parsons et al. (1984). Water samples were collected every 15 days to determine Ca^{2+} levels with a colorimetric kit (Doles) and osmolality with a vapor osmometer (Vapro 5520, Wescor Inc., Utah, EUA).

2.5. Sampling and biochemical analysis

Blood was collected from the caudal vein of fish anesthetized with benzocaine with heparinized needle and syringe. A portion was used to measure glucose with a portable glucose meter (Ultra 2 System Kit Glico Apar, Onetouch, Brazil). The remaining sample was centrifuged for 10 min, 2000 × g, 4 °C and resulting plasma stored at − 20 °C for posterior analysis of lactate. Fish were then euthanized with 300 mg L^{-1} benzocaine and muscle and liver were collected.

Tissues were weighed and homogenized with 1 mL of TCA (trichloroacetic acid) 10% to obtain an acid deproteinized extract using the homogenizer, disintegrator and emulsifier TURRAX type (Marconi Equipment for Laboratory Industry Ltda, São Paulo, Brazil. The tissue homogenates were centrifuged at 917 × g for 5 min, 4 °C. Glucose and lactate were measured in the plasma and tissues according to Dubois et al. (1956) and Harrower and Brown (1972) methods. Plasma osmolality was determined using the same methodology described for water samples.

2.6. Statistical analysis

The data were submitted to Levene's and Kolmogorov-Smirnov's tests to determine homogeneity of variances and normality. Survival in the acute experiment was compared by the Chi-square test. Data of weight and length were compared between groups by two-way ANOVA (time X treatment), while one-way ANOVA was used for the remaining variables, and where appropriate, followed by Tukey's post hoc test. Statistica software (version 11.0) was used and the minimum significance level for all analyses was 95% ($P < 0.05$). Data are reported as mean ± standard deviation (SD).