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Tolerance and growth of the longsnout seahorse *Hippocampus reidi* at different salinities

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ABSTRACT

Hippocampus reidi is one of the most popular seahorse species in the aquarium trade. The commercial breeding of this species is an alternative to reducing the fishing pressure on natural populations. Two experiments with newly born juveniles were conducted in this study to assess salinity tolerance (Lethal Time for 50% of the population-LT_{50}), survival and growth during the first 10 days of life. A third experiment determined the isosmotic point of adults. The highest LT_{50} was observed at 10 psu, followed by 15, 20, 25, 30, and 35 psu, and the lowest LT_{50} was recorded at 5 psu. A negative relationship between salinity and final weight and between salinity and final height was observed through the regressions performed, such that a decrease in salinity (until the lowest level analysed; 10 psu) implied a higher growth in height and weight. The highest survival rates were observed between the salinities 10 and 25 psu. The isosmotic point of the species was determined at 11.68 psu (303.38 mOsm/kg), which is in agreement with the salinity where the best salinity results were observed in the first two experiments. Using an intermediate salinity to produce *H. reidi* on a commercial scale would be advantageous because of survival and growth improvement in addition to requiring a lower seawater uptake and less cost for purchase artificial salt.

1. Introduction

The longsnout seahorse *Hippocampus reidi* (Ginsburg, 1933) is found from North Carolina (USA), Bermuda and the Bahamas to Rio Grande do Sul (Brazil) (Froese and Pauly, 2013) and is one of the most popular species of the genus in the ornamental trade market. Since 2004, all seahorse species have been listed in Appendix II of CITES (2014) in view of the degradation of their natural habitats and, mainly, the high fishing pressure to supply the traditional Chinese medicine and the aquarium and craft trades. In Brazil, export quotas were established in 2004 for *H. reidi* and *Hippocampus erectus* (Silveira et al., 2014), aiming to control and reduce capture for international trade (*Hippocampus patagonicus* was recently recorded in Brazil, and collection is not permitted). Each company has the right to export 250 individuals of each species per year but there is no restriction on the domestic market.

Another tool to reduce the fishing pressure on natural populations of *H. reidi* is commercial breeding, which is already performed in Brazil on a small scale (Hora and Joyeux, 2009; Koldewey and Martin-Smith, 2010). The life cycle of *H. reidi* in captivity is already known (Hora and Joyeux, 2009). However, there are few studies on the production of this species (Olivotto et al., 2008; Pham and Lin, 2013; Willadino et al., 2012; Melo-Valencia et al., 2013; Souza-Santos et al., 2013). This lack of studies causes many problems that generate high mortality rates, especially during the first days of life when the animals are more fragile and susceptible to disease (Koldewey and Martin-Smith, 2010).

H. reidi inhabits coastal and estuarine regions (Foster and Vincent, 2004) in salinity between 5 and 43 psu (e.g., Silveira, 2005; Mai and Velasco, 2012). Salinity is known to be one of the most important abiotic water parameters able to influence fish development at different life stages. Salinity may affect fertilization and egg incubation (Griffin et al., 1998; Zhang et al., 2010), larval growth (Ostrowski et al., 2011; Tsuzuki et al., 2008), and even the development of juveniles and adults (Lin et al., 2009; Resley et al., 2006). Marine and estuarine fish spend more energy for osmoregulation when in an environment with a salinity level different from their body concentration. This energy expenditure can vary from less than 10% up to 50% of their standard metabolism (Boeuf and Payan, 2001) and can negatively affect animal growth and survival. Thus, while *Hippocampus kuda* at the age of nine

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weeks show extreme salinity tolerance, growth and survival were better at intermediate salinities (Hilomen-Garcia et al., 2003). Nonetheless, growth and survival at different salinity levels were not tested in juveniles or other seahorse species (Lin et al., 2009), including *H. reidi* (Melo-Valencia et al., 2013).

The objective of this study was to assess the tolerance to salinity and the growth of *H. reidi* juveniles during their first days of life and to determine the isosmotic point of the species. Such studies will contribute to improvements in seashore culture, there by meeting the needs of the *H. reidi* breeding industry and helping to reduce capture of these animals in the natural environment.

2. Methodology

Three experiments were conducted: 1) tolerance to salinity in *H. reidi* juveniles during the first days of life, 2) growth and survival at different salinity levels, and 3) determination of the isosmotic point of adult animals.

2.1. General maintenance conditions

The experiments were performed at the Laboratory of Ichthyology and Ornamental Mariculture (Laboratório de Ictiologia e Maricultura Ornamental), located at the Oceanographic Base of the Federal University of Espírito Santo (Universidade Federal do Espírito Santo - UFES) (Aracruz, state of Espírito Santo- ES, Brazil) (authorization Ethic Committee for the Use of Animals UFES no.037-2011). Fifty-one *H. reidi* adults were captured in Espírito Santo Bay (Vitoria-ES, Brazil, 20°19′ S, 40°20′ W) (authorization ICMBio SISBIO no. 23924-1), including females and males in advanced pregnancy. The salinity was 28.0 ± 3.2 psu in the capture site.

Animals allocated to experiments 1 and 2 were kept in 60 and 140 L tanks that were part of a saltwater recirculation system of approximately 4000 L equipped with a skimmer, a biological sand filter, and calcareous algae. Physical and chemical parameters were monitored daily. Temperature and salinity were measured with a multi-parameter meter (model EC300, YSI, USA). The temperature ranged from 25.8 °C to 26.5 °C, and salinity was maintained at 30 psu. The pH was measured with a pH meter (model pH-700, Instrutherm, USA) and ranged from 8.3 to 8.4. Nitrite and total ammonia were measured by photocolorimetric method, and they remained below 0.05 mg/L. The concentration of dissolved oxygen, measured by an ox meter (model MO-910, Instrutherm, USA), was 6.6 ± 0.3 (mean ± standard deviation) mg/L. The water quality parameters were monitored with the same equipment in all experiments.

The experiments were conducted under a 12-hour photoperiod (12 h light and 12 h dark). The light originated from 15-W fluorescent lamps that were located 30 cm above the tanks.

The animals were fed four times a day, twice in the morning and twice in the afternoon until satiation. The food consisted of live marine caridean shrimp collected in the natural environment and marine mysids (*Mysidium gracile*) collected and frozen. The tanks were siphoned three times daily in the afternoon before supplying food and 1 h after the fourth feeding to remove food debris and faeces from the bottom.

2.2. Experiment 1 - salinity tolerance of juveniles

In this species, newborn juveniles are large-sized (6 to 8.5 mm in height; Hora and Joyeux, 2009; Willadino et al., 2012; Souza-Santos et al., 2013). Immediately after birth, 720 juveniles were randomly captured among more than 1000 siblings from a single brood of a single male and were directly transferred to the experimental units to test their resistance to abrupt salinity change. The experimental units consisted of 24 3-L tanks with moderate aeration, each tank holding 30 individuals (10 individuals/L).

Eight salinity treatments were tested (0, 5, 10, 15, 20, 25, 30, and 35 psu) with three replicates per treatment. Salinity 0 was included in the experiment because it may be a common occurrence in some localities where this coastal and estuarine seahorse is found. It also is a salinity remarkably close to the lowest salinity reported in the wild for the species. Deionised water was added to reduce salinity. Conversely, to increase salinity, the water was heated to accelerate the evaporation process until reaching the desired salt level (Sampaio and Bianchini, 2002). The animals were not fed throughout the experimental period in order not to introduce any bias caused by zooplankton tolerance to salinity (newborns only accept live food). Thirty percent of the water was renewed daily, the temperature was maintained at 25.0 ± 0.4 °C, the pH was 8.1 ± 0.3 and nitrite and ammonia levels were below 0.05 mg/L. Dead individuals were counted and removed every 6 h during the first 72 h, and then every 12 h. The experiment was ended when all individuals had died. Death was defined as the absence of opercular movement or the absence of a reaction to mechanical stimulation.

2.3. Experiment 2 - survival and growth of juveniles at different salinities

2.3.1. Experimental design

The survival and growth of the *H. reidi* juveniles were evaluated during the first 10 days of life at seven salinities: 5, 10, 15, 20, 25, 30, and 35 psu. This period of initial life was chosen because the newly born juveniles are planktonic (Hora and Joyeux, 2009) and because it is when the highest mortality under culture conditions occurs, therefore considered the most critical period in the production of this species (Olivotto et al., 2011). Treatments were performed in triplicate using newborns from ten males (breeders). Each male provided two groups from a single brood (except for a brood from which three groups were separated). Each group comprised 60 randomly selected offspring. These two (or three) groups produced by a single male were placed in replicates of different salinities.

The experiment was conducted in 40-L tanks with moderate aeration (1.5 seahorse/L). All juveniles were transferred in the same water where they were born (salinity of 30 psu) to a tank, and the salinity was gradually decreased by dripping freshwater at a rate of 2.5 psu per hour, for acclimation at each salinity. Once the water reached the desired salinity level, the juveniles were allocated to their corresponding treatment. The same method of experiment 1 was used to increase the salinity, also adopting the same rate. The zero salinity was not analysed because all animals subjected to this treatment rapidly died during the tolerance test (see results: Experiment 1).

The temperature in the tanks during the experiment was 25.3 ± 0.5 °C. The pH was 7.6 ± 0.6, and the nitrite and ammonia levels remained below 0.05 mg/L. Fifty percent of the water was renewed daily.

2.3.2. Feeding

Juveniles were fed exclusively on live wild zooplankton from the first to fifth day of age. The zooplankton was sampled at the Piraquê-Açu River estuary (Aracruz - ES, Brazil 40°09′14.41″ W, 19°57′01.13″ S) with a conical plankton net with a 100-μm mesh size. After sampling, the zooplankton was sorted (300 μm) to remove larger organisms. The estuarine zooplankton community at Piraquê-Açu is widely dominated by copepods (*Acartia lilljeborgi*, *Temora turbinata*, *Parvocalanus crassirostris*, *Oithona oswaldocruzi*, *Oithona oculata*, *Euterpina acutifrons* and *Paracalanus parvus*) (Pereira J.B. and Nunes R.A. *personal communication*). Barroso (2004) reports that the salinity at the estuary mouth (near the zooplankton sampling site) ranges from 11.60 to 34.05 psu, with an average of 30.45 (±4.41 psu). The collected zooplankton was acclimated to each tested salinity and directly offered to fish (non- enriched). The feed was offered two to four times daily until satiation.

Metanauplii of *Artemia* sp. Enriched with SuperSelco® (INVE, USA) were offered from day 5 until the end of the experiment, following the