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Short communication

Stocking density for the seahorse *Hippocampus reidi* in the pelagic phase and insights on the benthic phase in culture conditions

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1. Introduction

A trend in increasingly intensive fish farming systems has been observed that seeks to minimize production costs and maximize productivity without substantial growth reduction or mortality rise (Björnsson, 1994; El-Sayed, 2002). Usually, there is an inverse relationship between stocking density and growth rate (Holm et al., 1990; Lambert and Dutil, 2001; El-Sayed, 2002). Higher densities can also lead to potentially stressful social interactions, which usually raise mortality rates (Iguchi et al., 2003; Tapia-Paniagua et al., 2014).

Juvenile seahorses are commonly stocked at low densities in aquarium (1–2 juvenile/L) (Planas et al., 2017). At 15–30, exceptionally 10, days of age, *H. reidi* leaves the pelagic phase to take benthic habits (Hora and Joyeux, 2009), attaching to some holdfast with its prehensile tail. In Brazil, stocking density of *Hippocampus reidi* in commercial aquaculture varies with age and is habitually lowered from 6 to 0.6 individual/L between the pelagic and benthic phases. After settlement, extensive low-cost growth of this species has been achieved at very low stocking densities (0.04 ind./L) in net cages (Fonseca et al., 2015).

The objective of this study was to verify the best stocking density for *H. reidi* during the pelagic phase and the beginning of the transition process to the benthic phase.

2. Material and methods

The experiment was conducted at Laboratório de Ictiologia e Maricultura Ornamental (LabIMO), at Universidade Federal do Espírito Santo, Brazil (authorization from the Ethics Committee for the Use of Animals UFES, no. 037-2011), and lasted 15 days. Treatments were carried in triplicate using 1440 seahorse newborns (out of a total of 1454) released by one pregnant breeding male. Newborns were distributed immediately after birth into twelve 20-L aquaria at four stocking densities: 1 individual/L (20 ind./20 L-aquarium); 2 ind./L (40 ind./aquarium), 6 ind./L (120 ind./aquarium) and 15 ind./L (300 ind./aquarium).

The experiment was carried out in a recirculation system (described in Hora et al., 2017). Water flow to each aquarium was 40 L/h. Salinity, temperature, pH, ammonia, nitrite and dissolved oxygen were checked daily. Salinity was maintained at 20.4 ± 1.1 psu (mean ± standard deviation), according to the best conditions for growth and survival of *H. reidi* as described by Hora et al. (2016), and temperature was maintained at 24.9 ± 0.3 °C. Both parameters were measured with a YSI ec300 multi-parameter (precision of 0.1). The pH was measured with an electronic pHmeter of 0.1 precision (model ph-700, Instrutherm, USA) and was between 7.8 ± 0.6. Total ammonia and nitrite were measured with colorimetric kits and remained below 0.05 ppm. Oxygen was measured with an oximeter (MO-910, Instrutherm, USA) and remained at 6.9 ± 0.1 mg/L.

From 0 to 7 days of age, juveniles were fed wild zooplankton collected in the estuary of the Piraquê-Açu river (40° 09′ 14,41″W, 19° 57′ 01,13″S) with a net of 100 μm mesh size. The estuarine zooplankton community at Piraquê-Açú is widely dominated by copepods (*Acartia lilljeborgi*, *Temora turbinata*, *Parvocalanus crassirostris*, *Oithona oswaldocruzi*, *Euterpina acutifrons* and *Paracalanus parvus*) (Pereira J.B. and Nunes R.A. *personal communication*). Zooplankton was sorted (300 μm) to remove larger organisms and was offered to seahorses from two to five times a day until satiety, keeping prey density close to 3 ind./mL. Metanauplii of *Artemia* sp. enriched with Super-Selco® (INVE, USA) were offered from the fifth day after release (5 DAR) until the end of the experiment. Following the protocol described by Hora et al. (2017), feed replacement was gradual from 100% zooplankton on 4 DAR, to 75–25% zooplankton-*Artemia* sp. on 5 DAR, 50–50% on 6 DAR, 25–75% on 7 DAR and 100% *Artemia* sp. from 8 DAR onwards.

Aquaria were siphoned three times a day, and 20–30% of the whole system water was renewed at the end of the first week. Photoperiod was kept at 12 h of light and 12 h dark (Hora et al., 2017) and light was provided by 9 W fluorescent lamps ('daylight', i.e. 6500 K) installed 25 cm from the water surface, supplying 2500 ± 400 lx to the aquaria.

Growth, as total height (H) and wet weight (W), was estimated as the average of six individuals randomly sampled on the day of release (day zero; Age = 0), 5, 10, and 15 DAR (day after release) of each experimental unit (n = 276). The animals were anesthetized with benzocaine (30 mg/L), photographed and weighed (W, in mg) on a

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0.0001 g precision balance using a 50 mL beaker filled with water and returned to their original aquarium. No behavior change was detected after biometry and the fish were able to feed immediately. Graduated photos of each individual were used for measurement in the SigmaScan Pro 5® program. Height (*H*, in mm) was defined as the sum of crown height, trunk length and tail length (Lourie et al., 1999).

The following variables were calculated from the biometric data:

Daily growth rate (DGR, $mm\cdot day^{-1}$) is the slope b of the linear regression

$$: H = a + b\cdot \mathrm{Age}$$

Specific growth rate in weight (SGR,$\%\cdot day^{-1}$)

$$: \mathrm{SGR} = 100\,[ln(final_W) - ln(initial_W)]/\mathrm{Age}$$

Height and weight were tested by repeated-measures ANOVAs using 5, 10 and 15 DAR data with full factorial model and type-III sum of squares. Data normality, homoscedasticity and sphericity were tested by Levene's and Mauchly's tests, respectively, prior to analysis and found adequate (i.e., $P > 0.05$). One-way analyses of variance (ANOVA) were applied to test for difference in DGR, SGR and survival among density treatments at the end of the experiment (15 DAR). Survival data were neither normal nor homoscedastic (Levene's test) and were sine-arc transformed prior to analysis. All analyzes were performed using SPSS 16.0 assuming a significance level $\alpha = 0.05$. Results are presented as mean ± standard error of the three replicates.

3. Results

Weight and height increased with age but did not vary significantly among the stocking densities tested (Fig. 1 and Table 1). While the Age*Density interaction was, marginally, significant for weight (but not for height; Table 1), no obvious pattern was detectable.

Also, no differences were detected in daily growth rate, specific growth rate or survival among treatments (Table 2) (ANOVAs; $P > 0.05$).

4. Discussion

At 15 DAR stocking density in the range 1–15 ind./L had no significant effect on survival and growth of *H. reidi* juveniles. Similar results were reported for *Hippocampus abdominalis* (Martinez-Cardenas and Purser, 2012) and *Hippocampus whitei* (Wong and Benzie, 2003) in experiments of 42 and 107 days of duration, respectively. In these experiments, which did not differentiate the planktonic from the benthic phase, the density ranged 1–15 ind./L for *H. abdominalis* and 0.5–1 ind./L for *H. whitei*. Densities of 2–5 ind./L were used in studies of *H. reidi* cultivation in the planktonic phase (Olivotto et al., 2008; Hora and Joyeux, 2009; Willadino et al., 2012; Pham and Lin, 2013). The commercial seahorse aquaculture in Brazil normally used 3–6 ind./L in pelagic phase (J.P. Demarco, personal communication).

The densities used for seahorses are considered low compared to those reported for other species of marine fish (Martinez-Cardenas and Purser, 2012). These comparisons are, however, skewed since syngnathids present precocious development and are released as larvae in an advanced stage of development (Novelli et al., 2015), larger and heavier than altricial larvae. While many larvae used in marine aquaculture are born with approximately 2 mm, *H. reidi* is born with 8 mm, about 64 times heavier if considering a cubic weight-length relationship (King, 1995; Froese, 2006). Thus, if *Gadus morhua* larvae can be maintained at densities of 50 to 300 larvae/L for 36 days without compromising their performance (Baskerville-Bridges and Kling, 2000), the highest densities used in the present experiment (15 ind./L) actually correspond in weight to 960 altricial larvae/L.

Koldewey and Martin-Smith (2010), based on (Woods, 2003), conclude that adult of *Hippocampus abdominalis* does not respond well to high densities due to increased stress, leading to reduced growth and survival and higher susceptibility to disease. However, seahorses do not show marked dominance behavior and there is no record of cannibalism, a behavior that is typically exacerbated at high density (Hecht and Pienaar, 1993). During the pelagic phase, ethological and behavioral consequences of crowding and associated stress should be reduced in relation to what is expected during the benthic phase since both food and seahorses are well distributed in the water column and feeding competition is likely to be low.

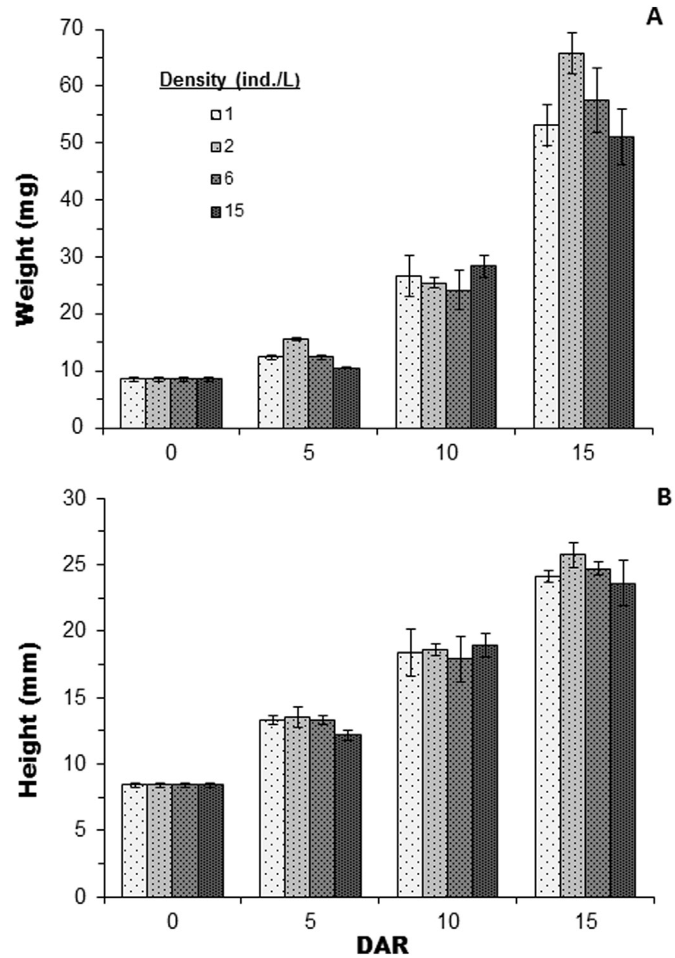

Fig. 1. Weight and height change in *Hippocampus reidi* from day of release to 5, 10 and 15 DAR in tanks of different stocking densities: (A) wet Weight and (B) total Height (mean ± standard error). No significant difference among stocking density treatments (1, 2 6 and 15 ind./L) was detected (repeated-measures ANOVAs; $P > 0.05$) for either weight or height.

Table 1
Summary statistics for the repeated-measures ANOVAs testing for differences in height and weight of pelagic phase seahorses *Hippocampus reidi* among stocking density treatments (1, 2, 6 and 15 ind./L) between release by male and the age of 15 DAR (days after release) df: degrees of freedom; *P*: significance.

Source	Height			Weight		
	df	Mean square	*P*	df	Mean square	*P*
Within-subjects effects						
Age (DAR)	2	394	< 0.001	2	6.159	< 0.001
Age * Density	6	1.27	0.22	6	46.4	0.05
Between-subjects effects						
Density	3	1.77	0.51	3	57.7	0.44