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Closing the reproductive cycle: Growth of the seahorse *Hippocampus reidi* (Teleostei, Syngnathidae) from birth to adulthood under experimental conditions

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ABSTRACT

The seahorse *Hippocampus reidi* is one of two seahorse species occurring in Brazil, and is in demand for the ornamental aquarium and curio trades. Juvenile *H. reidi* siblings ($n = 615$) born in captivity were raised on a diet of wild zooplankton, enriched *Artemia* and wild mysid from birth to 109 days. Growth averaged 0.77 ± 0.01 mm day^{-1} for the whole period. However, after the onset of sexual maturation at about 60 days of age, growth decreased to 0.31 ± 0.10 and 0.53 ± 0.09 mm day^{-1} in males and females respectively. The first births of F3 juveniles were observed after 81 days of growth. All individuals were adult at the end of the study and sex ratio was 1M:1.2F. Total mortality was 11.7%, with mortality peaks apparently caused by changes in the offered diet; gradual dietary shifts may reduce the occurrence of such mortality peaks. This study indicates that *H. reidi* may be raised in captivity from birth to adulthood with relatively fast growth rate, low mortality and early maturity, thus, increasing the potential application of this seahorse species to commercial aquaculture and conservation initiatives.

1. Introduction

While destruction or degradation of estuarine and reef habitats threatens natural populations everywhere (Lourie et al., 1999), the commercial extraction of 25 million seahorses per year is probably one of the principal factors for the decline of natural populations of these fish in several parts of the world (Vincent, 1996a), including Brazil (Rosa et al., 2007). Seahorses are mainly sought for traditional Chinese medicine, but also for the ornamental and curio trades (see Gasparini et al., 2005). Overall, the commerce of seahorses involves many countries (at least 45, Lourie et al., 1999). Brazil, one of the ten biggest trading countries in the commercial trade of aquarium fish (Monteiro-Neto et al., 2003), due to an enormous volume of freshwater fishes being collected from the Amazonian region and elsewhere, was also the fourth major exporter of seahorses during the period 1998–2002 (Wabnitz et al., 2003). Two species of seahorse occur in Brazil: *Hippocampus erectus* Perry, 1810 (lined seahorse) and *Hippocampus reidi* Ginsburg, 1933 (longsnout seahorse). The latter, found from the Bahamas and United States to southeast Brazil, is more abundant (Figueiredo and Menezes, 1980; Lourie et al., 1999; Rosa et al., 2002). Among *Hippocampus* species, *H. reidi* is one of the most in demand for the international ornamental trade, including for use in public aquaria (Bull and Shedd, 2002).

All seahorse species are listed on the Appendix II of CITES (2008) and are cited as vulnerable on the Red List of Threatened Species (IUCN, 2008). In Brazil, both *H. erectus* and *H. reidi* are catalogued as "superexploited or threatened of superexploitation" (MMA, 2004), a categorization that has provided some measure of fishing protection and trade restriction, at least in the ornamental segment of seahorse commerce (e.g., Rosa et al., 2006). The aquaculture of ornamental organisms is an alternative to the capture of wild individuals, and may reduce the impacts upon natural populations (Vincent, 1996b). It is potentially a boon for the industry of ornamental fishes because various countries, such as Bahamas, Brazil and several states of the USA, have established importation quotas for wild-caught organisms (Tlusty, 2002). An interesting side effect of conducting research for the cultivation of seahorses is the possible furnishing of important life history information that may also be used in conservation and management of exploited species. There has been, however, little effort done (and success attained) to grow and breed *H. erectus* and *H. reidi* in Brazil and elsewhere (Olivotto et al., 2008). The goal of the present study was to determine the growth of *H. reidi* between birth and first reproduction and to provide information on growth rate, mortality, sex ratio and sexual maturation of young adults.

2. Material and methods

2.1. The rearing system

The facilities for rearing *H. reidi* are that of the marine ornamental fish exporter Juan Pablo de Marco e Irmão Ltda, in the state of Espírito Santo, Brazil. The closed seawater system, of about 10 000 L total, is built around the following set of equipment to maintain water quality: a biological filter composed of 1000 kg coralline algae skeletons, a 12 000 L h^{-1} skimmer assemblage, a 90 W UV sterilizer, a cooling system maintaining temperature below 28 °C and a 14 000 L h^{-1} pump. [A fluidized sand-bed filter was later installed to lower nitrite

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concentration.] The seahorses are held, bred and raised in a series of 50, 120 and 200-L tanks annexed to the system. The glass tanks are either hexagonal (50 L) or rectangular (the others) and are painted blue on rear and lateral sides. Seawater flow was set at one tank volume per hour with inflow at the center (hexagonal) or side (rectangular) of the tank and whole-column outflow on the opposite side. Tanks are not aerated (no air stones) and are furnished with artificial plants to provide holdfasts. Seahorses have free access to the water surface. Physico-chemical parameters of water (temperature, salinity and pH; all at precision 0.1) were measured two or three times a week. During the experiment, temperature varied between 22.0–26.6 °C, pH 8.2–8.4 and salinity 26.5–29.0. Photoperiod was 12L:12D with light provided by 40 W fluorescent tubes located 50 cm above the water. Tanks were siphoned 5 times a day to remove detritus from the bottom.

The broodstock used in this study had been held for one year or more in a 200 L-tank at the facilities. Reproduction, evidenced by the liberation of juveniles by males, had been ongoing for years, but with extremely low survival. In this study, conducted between April and June 2007, 615 *H. reidi* juveniles from a 1089-strong brood were used. The juveniles were the progeny of a single male from amongst the few tank-born and raised survivors and, as others in the broodstock, had lost most of its tail to tail-rot disease. The F2 juveniles were distributed equally (3×205 individuals) between three 50 L tanks. When 14 and 45-days old, they were counted and transferred first to three 120 L tanks and later to three 200 L tanks where they remained to the end of the study. A final count was done on the 109th day of life and last day of the experiment.

2.2. Culture diet

Specialized food for seahorses does not exist on the Brazilian market. Thus, live zooplankton, mainly copepodites and copepods, were captured with a 100 µm-mesh conical plankton net from a nearby estuary (Canal da Passagem, in Vitória; 20°19′ S, 40°20′ W). Zooplankton was further sieved (200 µm) to remove larger organisms. Overall, estuarine copepod communities in that estuary are strongly dominated by *Acartia lilljeborgi*, *A. tonsa*, *Paracalanus parvus*, *P. quasimodo*, *Parvocalanus crassirostris*, *Temora turbinata*, *Oithona hebes*, *O. oculata* and *Euterpina acutifrons* (Sterza and Loureiro Fernandes, 2006). In addition, two lots of brine shrimp (*Artemia salina*, Linnaeus, 1758; Brand INVE) were decapsulated three days apart and set to hatch in two 500 L-tanks. In these, they were fed brewer yeast until they were about 15-days old. In the 24 h before being given to *H. reidi*, brine shrimp were fed fish-oil enriched-food (Super Selco®) at the recommended inclusion rate. Finally, the mysid *Mysidium gracile* Dana, 1852 (Crustacea: Mysidacea) was captured manually with a small hand-net of 500 µm mesh at a nearby beach (Praia do Canto, Vitória). All wild food was rinsed with system water before being briefly held in tanks, given as food or frozen.

Original broodstock were routinely fed *ad libitum* five times a day with live or frozen *M. gracile*. During the study, food was offered 5 times a day to F2 juveniles, with diet changes spread over three days. Live zooplankton was offered from birth (day 0; *Age* = 0) to 6 days of age (day 6) at concentration 5 mL^{-1}. One-to-fourteen-days-old brine shrimp was given from day 3 to day 25 at the same concentration (5 mL^{-1}). Live or frozen *M. gracile* was offered *at libitum* from day 22 to the end of the study.

2.3. Data collection

Behavior and morphological variables were observed routinely at each feeding as part of the rearing process. In particular, the times at which individuals settle, change color, court and give birth were recorded.

Dead seahorses were collected and counted daily while siphoning tank waste. Mortality estimates were checked against the enumeration of survivors on days 14, 45 and 109; no discrepancy was detected. Daily mortality (*M*, in percentage) was computed as the ratio between the number of daily deaths and the initial number of fish (615). Six individuals (two haphazardly sampled from each tank) were regularly sacrificed and preserved in ethyl alcohol 70%. Removal occurred every 2 days to day 16, every 4 days to day 32, and every 7 days to day 109 at which the experiment was considered completed because 100% of animals collected were sexually mature. Fish on day 67 were not collected and, thus, $n = 132$. On day 0 (birth), 6 of the 574 non-used individuals were collected and preserved as noted above (i.e., $n_{total} = 132 + 6$). On all preserved animals the height (*H*, in mm), as the sum of crown height, trunk length and caudal length (Lourie et al., 1999), was estimated using an ocular micrometer mounted on a dissecting scope. Individuals were weighed (wet weight, *W*, in mg) on an analytical balance of 0,1 mg precision. All fish were deposited at the fish collection of Universidade Federal do Espírito Santo (CI-UFES 1027).

From the 60th day of life onward, reproductive development was studied systematically. External identification of sex is possible in maturing fish, with males identified by the development of the brood pouch and the females by the presence of a salient sexual pore (Rosa et al., 2005). The onset of sexual maturation was estimated to be the day in which sacrificed animals presented either maturing ovaries or developing pouch. Females were dissected and ovaries examined macroscopically for size, volume used of the visceral cavity, coloration and external visualization of large oocytes through the muscular wall of the organ to be classified into three broad stages of gonad development. Thus immature females (IM) have small, translucent ovaries occupying less than about 5% of cavity and without visible oocytes; maturing females (MF) show small to medium-sized oocytes visible under the wall of pale-orange gonads filling up to 30% of cavity; mature females (M) have large (using 40–80% of cavity) orange ovaries where large prespawning oocytes are clearly distinguishable. Ovaries were weighed (wet weight, 0.1 mg precision) and the gonadosomatic index (*GSI*, in percentage) computed as gonad weight divided by total weight (gonads below the detection limit were assigned a nominal weight of 0.1 mg). Oocytes from MF and M gonads were enumerated and their average length and width were estimated through the measurement of 10 oocytes with an ocular micrometer. Males were classified into three stages based on the development of the brood pouch: initiating (I) with the presence of a small cleft in the abdominal region, below the anal fin; intermediary (IT) on which a small developing pouch already is visible, and developed (D) where the pouch is completely formed. Testes were not extracted.

2.4. Statistical analysis

Differences in height and *GSI* between developmental stages were tested through ANOVA type III with *post-hoc* Tamhane tests for unequal variances. Height-at-age data were fitted by linear regression ($H = a + b$ Age), with the slope *b* corresponding to the growth rate (in mm day^{-1}). Welch's approximate *t* and number of degrees of freedom (Zar, 1999; see Joyeux et al., 2008 for an application of the method) was used to compare growth rates. The height–weight relationship were considered to follow a growth model of the type $W = a\,H^{b}$ where b is the allometric coefficient. Parameter estimation was conducted by least squares linear regression on Naperian log–log transformed data, i.e. $\ln(W) = \ln(a) + b \ln(H)$. The allometric coefficient (b) was tested with the 2-tailed Student's *t*-test to detected differences from the isometric value ($b = 3$) with *n*-2 degrees of freedom (Zar, 1999). All analyses used $\alpha = 0.05$.

3. Results

3.1. Behavior

During the two first weeks of life, juvenile *H. reidi* displayed pelagic behavior. On day 15, numerous juveniles already were observed using artificial plants as holdfast. One-month-old juveniles were almost all benthic and many were starting to change coloration from blackish to