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ARTICLE

Fecundity and fertility of the longsnout seahorse, *Hippocampus reidi* (Teleostei: Syngnathidae), in tropical Brazil

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ABSTRACT: (Fecundity and fertility of the longsnout seahorse, *Hippocampus reidi* (Teleostei: Syngnathidae), in tropical Brazil). The longsnout seahorse, *Hippocampus reidi* Ginsburg, from the estuary of the Maracaípe River, in Ipojuca, PE, was studied in the field and laboratory between July/2001 and October/2002. Macroscopic analysis of its ovary showed that the structure is morphologically similar to the gonads of other marine teleosts, which are in the shape of a "Y." Histologically, the ovary exhibited a pattern of germinal ridges that had oogonia similar to those found in pipefish (relatives of *Hippocampus*). *Hippocampus reidi* exhibited partial spawning, telolecithal eggs, an average fecundity rate of 839.66 oocytes per lot, an average fertility rate of 691.4 offspring per brood pouch, oocytes that were 1.5–2.0 × 1.0 mm and newborns that were 5.0–7.0 mm tall. There was no correlation between the height or weight of a male and the number of embryos it incubated.
Key words: gonads, reproduction, telolecithal egg, brood pouch, mangrove.

RESUMO: (Fecundidade e fertilidade do cavalo-marinho do focinho longo *Hippocampus reidi* (Teleostei: Synnathidae) em regiões tropicais do Brasil). O cavalo-marinho do focinho longo *Hippocampus reidi* Ginsburg oriundo do estuário do rio Maracaípe, Ipojuca, PE foi estudado em campo e em laboratório entre julho/2001 e outubro/2002. A análise macroscópica do ovário mostrou ser este uma estrutura típica, morfologicamente semelhante às gônadas dos teleósteos marinhos, em forma de Y. Histologicamente, exibiu um padrão de cristas germinativas para as oogônias semelhante àquele encontrado em seus parentes peixes-cachimbo. O tipo de desova e o tipo de ovo exibido foi a parcial e o telolécito, respectivamente. A fecundidade média por lote foi de 839,66 ovócitos, enquanto que a fertilidade média foi de 691,4 alevinos por bolsa incubadora. O tamanho do ovócito variou entre 1,5 e 2,0 mm de comprimento x 1,0 mm da largura e o tamanho do recém-nascido variou de 5,0 -7,0 mm de altura. Não houve correlação entre a altura ou peso do macho e número de embriões incubados.
Palavras-chaves: gônadas, reprodução, ovo telolécito, bolsa incubadora, manguezal.

INTRODUCTION

Seahorses (*Hippocampus*) are fishes with peculiar characteristics, where the male is pregnant during a long part of its adult life (Martin-Smith *et al.* 2004, Silveira 2005). Worldwide, there are approximately 40 species of *Hippocampus* and knowledge about the fecundity and fertility of these species contributes to understanding their vulnerability. The fecundity and, consequently, the fertility of seahorses are low, and populations in certain regions are subject to intensive fishing and environmental degradation, making them difficult to find (Lourie *et al.* 1999).

The reproduction parameters of a species vary naturally because of diverse environmental conditions and, therefore, should be estimated using the measurable environmental characteristics of where they live. This is the case with many species of *Hippocampus* studied, which have characteristics in common (e.g., courtship rituals) but have fertility rates, fecundity rates and newborn size that vary (within the same species) based on the geographic region where individuals live. According to Lourie *et al.* (1999), *Hippocampus bargibanti* Whitley gives birth to one or two offspring while *Hippocampus kuda* Bleeker gives birth to up to 1,000 young each time. The size of newborns of several known species vary between 6.0 and 16.0 mm. Selman *et al.* (1991) described the histology of the ovary of *Hippocampus erectus* Perry, and suggested that the ovary of seahorses, which have two germinal ridges, evolved from the pipefish (another Syngnathidae) that have only one germinal ridge.

This study presents the first estimation of the fecundity and fertility rates of *Hippocampus reidi* Ginsburg, in a natural environment, in a tropical region of Brazil. This work also includes a morphological description of the ovary and ovocyte of this species, including histological comments, as well as data about incubation time and newborn height. The information gathered during this study, when included with data from past works, will contribute to creating guidelines for the proper management of this species.

MATERIALS AND METHODS

Individuals of *Hippocampus reidi* were captured in the estuary of the Maracaípe River, in the city of Ipojuca, Pernambuco, in northeastern Brazil (8°32'14.9"S, 35°00'17.8"W). The seahorses were sampled during weekly dives between July/2001 and October/2002.

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In order to determine the fecundity rate of *H. reidi*, and to avoid killing the animals, the collecting method proposed by Vincent (1994b) was used. Sexually mature pairs were transported to a laboratory, measured, weighed and put into small aquariums so they could continue their courtship rituals as found in natural environments. Before coupling occurred, the male was removed from the aquarium causing the female to release its ovocytes, which fell to the bottom of the aquarium, allowing for an ovocyte count. Of the 11 females selected and observed, only three released ovocytes when the male was not present.

To evaluate the reproductive potential of the males, the males were captured when they were pregnant and kept in small aquariums until the offspring were born. At this point, the newborns inside the pouch of each male were counted (dead or alive). Of the 14 individuals selected and observed, 13 were pregnant and one was ready to receive eggs from a female. The height of the males and females was measured from the top of the head to tip of the stretched tail (Lourie *et al.* 1999), and the individuals were weighed on a semi-analytical digital scale, which was accurate to 0.001 g.

To observe the fertility rate and successive recoupling under laboratory conditions, a series of 13 60-L aquariums were employed to house the couples using a photoperiod of 10-hours of light per day (0.5 w fluorescent/liter). The physical and chemical parameters of the water were kept at 8.2 pH, salinity 30 ppm and ammonia and nitrite 0 mg/L, and were monitored with measurement kits for marine aquariums. The ambient water temperature was 28°-31°C. Food was given to the seahorses twice per day, which consisted of *Artemia salina* adults, the post-larva of the marine shrimp *Litopnaeus vanamei* and alevins of *Poecilia vivipara*. After coupling occurred, the incubation time of the male was monitored and, before giving birth, it was transferred to a small aquarium with an external filtering system (with no sand or shells on the bottom) so the offspring could be counted. On the same day as the birth of its offspring, the male was put back in the aquarium with its mate and observed.

Ovary and ovocyte morphology, and histology of the ovary

The ovary was removed from an individual of *H. reidi*, which was part of the Projeto Hippocampus collection (Maracaípe, PE), and it was morphologically analyzed and photographed using a binocular stereoscopic microscope coupled with a digital camera. For the histological analysis of the ovary, the organ was processed and embedded in paraffin, sectioned (5 µm thick) using a Leitz rotational microtome and stained with hematoxylin and eosin. The sections were analyzed using at Zeis Axiostar binocular microscope and photographed with a coupled digital camera. The ovocytes analyzed (n=30) were taken from live females (n=3, Maracaípe, PE) that spawned their eggs in the absence of a male. The ovocytes were fixed in a 10% buffered formol solution, analyzed under a binocular stereoscopic microscope and photographed with a coupled digital camera.

Table 1. Average fertility and fecundity of *H. reidi* in the Maracaípe mangroves, Ipojuca, PE.

Height (cm)	Nº of Alevins or Ovocytes	Weight (g)
	Males	
13.2	709	11.30
13.6	667	12.50
14.0	774	13.31
14.2	559	13.20
14.5	939	15.00
14.6	300	13.50
14.9	213	12.42
15.0	601	14.30
15.0	1355	7.42
15.2	1183	16.5
15.3	395	11.00
16.3	238	14.86
16.4	1055	22.53
	Males' Average	
14.8	691.4	13.68
	Females	
12.30	725	10.00
14.20	914	12.00
14.30	880	12.30
	Females' Average	
13.60	839.66	11.43

RESULTS

Fertility and Fecundity in natural environments

The individuals of *H. reidi* studied had between 213 and 1,355 alevins per pouch, were between 13.2 cm and 16.4 cm tall and weighed between 12.4 g and 17.42 g. The average fertility rate of the males (n=13) was estimated to be 691 alevins per pouch, and the average height of the males was 14.8 cm (Table 1). There was no correlation between the number of incubated embryos and height of the males studied (P=0.977; Fig. 1) or the weight of these males (P=0.720). Therefore, for any height or weight of a pregnant male, the number of incubated embryos is statistically similar and does not depend on the size of the male.

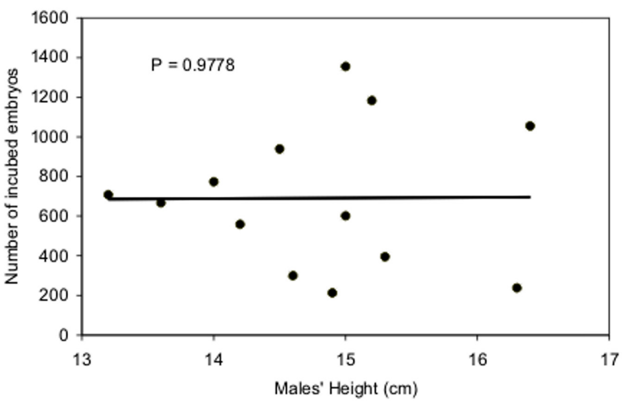

Figure 1. Diagram of the dispersal of the number of incubated embryos in relation to the height of male *Hippocampus reidi* individuals in the Maracaípe mangrove, in Ipojuca, PE.

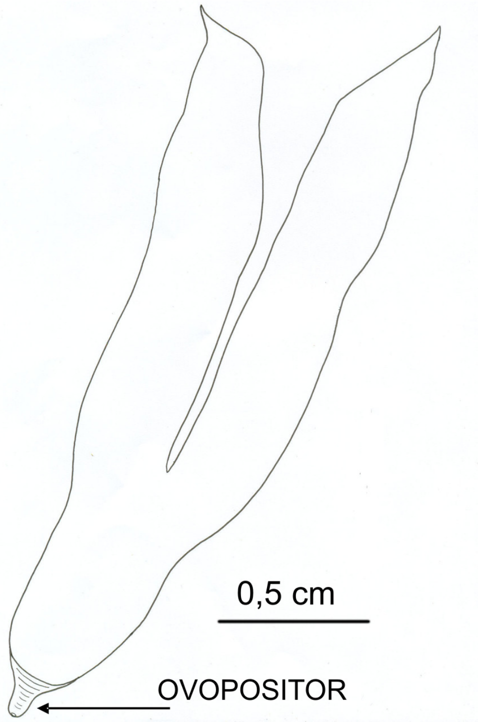

Figure 2. Ovary of *Hippocampus reidi* showing the ovipositor.

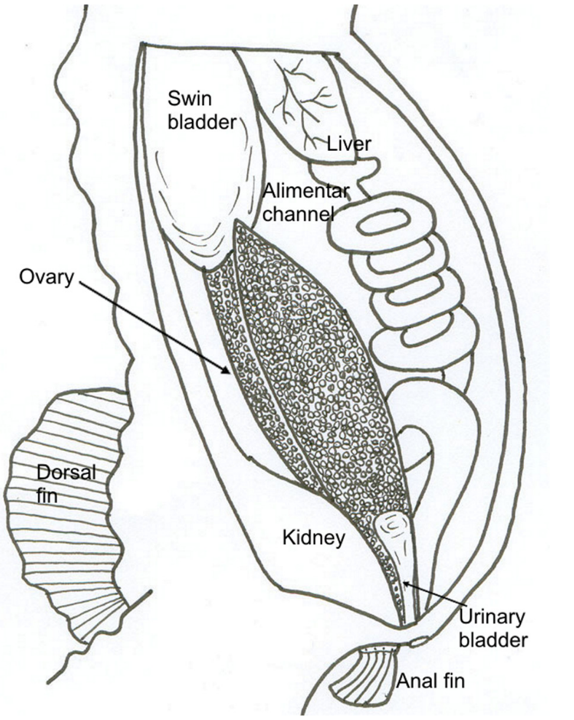

Figure 3. Diagram showing the position of the ovary in *Hippocampus reidi* within the body cavity.

The fecundity of *H. reidi*, derived from natural environments (n=3), was 725–914 ovocytes, with an average of 839.66 ovocytes per lot spawned. The females used in this study had a height between 12.3 cm and 14.3 cm and weighed between 10.0g and 12.3g, respectively. However, the fact that one male had 1,355 alevins in its pouch suggests that the ovocyte limit for this species is probably higher.

Ovary structure and egg type

The ovary of *H. reidi* is an organ composed of two cylindrical tubes that are basally fused creating a "Y" (Fig. 2). The lower part that is fused is contiguous to the ovipositor, a retractile structure exposed during coupling through the urogenital opening. A supporting mesentery drives nerves, blood and lymphatic vessels that feed the organ, which is situated between the kidneys and the urinary bladder, beneath the swim bladder (Fig. 3).

In *H. reidi*, each ovarian cylinder has two dorsal germinal ridges, which develop the follicles with the stem cells. The oogonia are restricted to the ridges, and in the ovary lumen there are only mature ovocytes (Fig. 4). When the ovocytes of *H. reidi* are ready to be fertilized they are hydrated, and are bright yellow, orange or sometimes reddish in color because they contain a large amount of vitellus. Each ovocyte is pear-shaped and the vitellus is concentrated in the widest region (the vegetable pole), which is opposite the animal pole where the embryo develops. The ovocyte dimensions (fixed ovocytes, n=30) were 1.5–2.0 × 1.0 mm, with an average of 1.81 × 1.0 mm, and the vitelline occupied an average of 68.23% of this space.

Observed incubation time in the laboratory and newborn height

After the transfer of ovocytes into the pouch of each male, and subsequent fertilization, there was an incubation or pregnancy period that ranged from 12 to 20 days; 12 days was the most common (personal observation). The water temperature ranged between 28º C and 31° C; the highest temperatures were associated with the shortest incubation times, but the frequency of this was not quantified. From the 13 couples studied, 58 reproductive events were observed, and nine couples reproduced at least two consecutive times during a period of one to 15.5 months (Table 2). The sum of the reproductive events within the

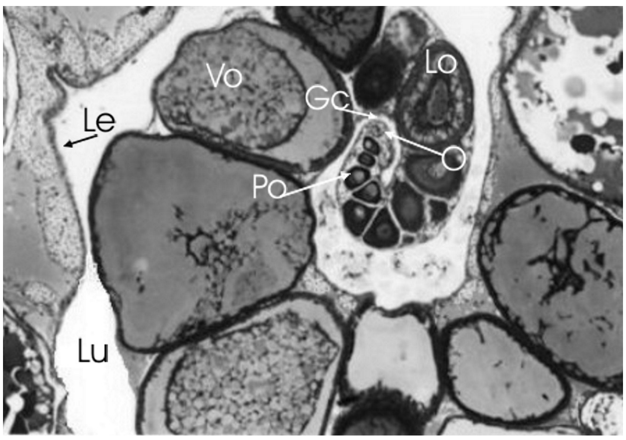

Figure 4. Histological section of the ovary of *Hippocampus* reidi showing the phases of the oocyte and the germinal ridge (Gc); Oogony (O) Oocyte primary (Po); Vitellogenic oocyte (Vo); Lipid oocyte (Lo); Lumen (Lu); Luminal epithelium (Le). X 250.

Table 2. Number of consecutive reproductive events per *H. reidi* couple and average individual fertility during the sample period (in the laboratory).

N	Nº of reproductive events/couple	Average fertility/ individual	Sample period (months)
1	11	451	15.5
2	11	354	13.0
3	12	600	13.0
4	04	310	12.0
5	03	337	5.0
6	06	365	5.0
7	02	298	4.1
8	01	348	1.0
9	03	238	1.1
10	02	130	5.0
11	01	495	2.0
12	01	403	1.0
13	01	774	4.0

laboratory (58) produced 24,371 alevins, with an average of 392.54 newborns per pouch for each pregnancy. When expelled through the opening of the pouch of the males, after the vitelline sac was fully consumed, the alevins (which were equally sampled from three males captured in the Maracaípe mangroves) had a height ranging between 0.5 cm and 0.7 cm, with an average height of 0.6 cm (n=30, Fig. 5). Larvae, which had not consumed the vitelline sac, were rarely expelled with the alevins, but the frequency of this event was not quantified.

Figure 5. Newborn (6 mm height) of *Hippocampus reidi*.

DISCUSSION

Ovary structure, egg type, fecundity and type of spawning

The ovary of *H. reidi* is morphologically similar to that of other teleosts, which have two cylindrical tubes that are basally united and form a "Y"; however, it differs in the oviduct that extends itself into an ovipositor, a retractile structure that helps transfer the ovocytes into the male's pouch, preventing the ovocytes from contacting the external environment (Selman *et al.* 1991, Silveira 2001). A peculiar characteristic of the ovary in *H. erectus* is the formation of two dorsal germinal ridges (from where the follicles emerge and the oogonia develop), and the mature ovocytes are found only in the ovary lumen. In *H. reidi* the structures are arranged in the same way. In a study by Selman *et al.* (1991), the size of a mature ovocyte in *H. erectus* was 2.4–2.6 × 0.4–0.75 mm. The average length of the ovocyte of *H. fuscus* Ruppell was 2.27 mm in a study by Vincent (1994) while that of *H. reidi* (based on our study) was 1.5–2.0 × 1.0 mm, whose vitelline space occupied approximately 70% of the ovocyte, characterizing it as a telolecithal "egg," which is in accordance with Azzarello (1990) and Silveira (2000a, 2001). The size of an ovocyte is related to reproductive behavior, in that species with internal fertilization generally have a higher number of ovocytes than species with external fertilization that do not care for their offspring (Vazzoler 1996). According to Vincent (1994b) ovocyte size is related to the advantages of high fecundity and the provisions provided to each newborn (which accounts for 96.2% of the variation found in the size of newborns), and to the latitude where a species lives because species from lower latitudes have been shown to have smaller ovocytes. Among the ovocytes of the *Hippocampus* species mentioned earlier, those of *H. reidi* (observed in our study) were the smallest, which is in agreement with Vincent (1994b) who observed small ovocytes (1.2 mm) in this species and a higher number of spawnings in relation to the others, suggesting a higher fecundity rate.

Fecundity depends on the volume of the celomatic cavity that shelters the mature ovary and also on the size of ovocytes produced, which varies based on the size of the female, is greater as the female grows larger, and is more related to the length of the individual rather than to its age (Vazzoler 1996). Although the sample number for spawning *H. reidi*, in the absence of a male, was very small (n=3), the data suggest that fecundity increases as the size of the female increases. In addition, the data were obtained in August and September, which according to Silveira (2005) is during the peak of the reproductive season (May to November). Studies have shown that egg size and fecundity are related to the latitude where a species occurs, which can vary (Vazzoler 1996, Vincent 1990).

The type of spawning found in fishes is characterized by how the ovocyte develops, the spawning frequency during the reproductive period and the number spawning periods during the lifetime of an individual (Vazzoler 1996). According to Vincent (1994a), seahorses spawn multiple times during each reproductive period, maturing, ovulating and spawning part of their post-vitellogenic ovocytes during each coupling. However, Teixeira and Musick (2001) observed, in the ovary of *H. erectus*, the development of similar-sized ovocytes and concluded that this species spawns all of its eggs at one time per reproductive period. This behavior, called total spawning, is different from other seahorses that spawn several times during a reproductive period (Vincent 1994a*,* Silveira 2001, Silveira 2005, Mai and Loebmann 2009). Laboratory experiments (personal observation) have shown that seahorses spawn multiple times and during a reproductive period form couples that are extremely synchronized (during the final maturation period of the ovocytes) to maximize the reproductive potential of both sexes. Even during reproduction under laboratory conditions, it is difficult to visually determine if a female is sexually mature. Therefore, it is necessary to verify this by observing the coupling event and the offspring that are produced, or to observe the response to a spawning stimulus. In field studies, it is even more difficult (and not recommended) to visually determine if a female is sexually mature and, according to Baum *et al.* (2003), data collected in this way should not be considered accurate.

Incubation time, fertility, newborn height and reproductive success

Fertility in seahorses varies among the species and within species and, in the second instance, is limited by the physical capacity of the pouch. Lourie *et al.* (1999) gathered known data about 32 species of seahorses, among which 14 had records relating to the formation of offspring.

Like all ectothermic species, embryonic development (which occurs inside the pouch of a seahorse) varies based on water temperature and the species. Seahorses are ovoviviparous and the eggs burst inside the pouch releasing fully developed individuals (called alevins) that are able to swim and eat without parental care (Boisseau 1967*,* Azzarello 1991*,* Mi 1992*,* Silveira 2001). *Hippocampus abdominalis* Lesson, the largest species of seahorse (32.2 cm), has a pregnancy period of 28 days and gives birth to 300–700 offspring that are approximately 13.0 mm tall when born (Filleul, unpublished data). In a study by Woods (2000) the same species had an average of 269 alevins per pregnancy (53–721), which had an average height of 15.65 mm at birth. *Hippocampus zosterae* Jordan and Gilbert, which reaches 3.8 cm as an adult, is pregnant for a period of 10 days and gives birth to 3–25 offspring (Lourie *et al.* 1999) that are, on average, 8.0 mm tall (Strawn 1958). Medium-sized species, like *H. capensis* Boulenger (11.2 cm) incubate their embryos for 2 to 3 weeks and release 7–95 offspring that are 8.0–16.0 mm tall; *H. comes* Cantor (14.6 cm) is pregnant for 2 to 3 weeks, and releases 200–350 offspring that are approximately 10.0 mm tall. *Hippocampus fuscus* (12.0 cm) incubates its eggs 14 days and releases 20–110 offspring that are 10.0 mm tall; *H. hippocampus* Linneaus (13.0 cm) incubates its eggs for a period of 14 to 15 days and releases 400 offspring that are 10.0 mm tall; *H. whitei* Bleeker (13.0 cm) incubates its eggs for 21–22 days and releases between 100–250 offspring that are approximately 8.5 mm tall; and *H. trimaculatus* Leach (15.0 cm) gives birth to 400-1,000 offspring (Lourie *et al.* 1999). According to Lourie at al. (1999), *H. reidi* reaches 17.5 cm tall, incubates its eggs for 14 days, and releases 200–1,572 offspring that, on average, are 7.0 mm tall at birth. In a study by Mai and Loebmann (2009), which monitored *H. reidi*, males ranged in height from 15.1 cm to 16.5 cm (mean 15.6 cm), newborns ranged from 0.44 cm to 0.66 cm (mean 0.54 ± 0.051cm; n= 30) and the number of offspring ranged from 202 to 652 (mean 375 ± 242.4; n= 3). In this work, where individuals of this species were captured in the estuary of the Maracaípe River, the largest non-pregnant specimen of *H. reidi* was 17.7 cm tall and the largest pregnant specimen was 16.4 cm tall (the largest known specimen of *H. reidi* captured in the state of Pernambuco was 19.0 cm tall, personal observation). Males were usually pregnant for a period of 12 days and the average number of offspring was 691.4 (213–1,355), which had an average height of 6.0 mm at birth. There was no correlation between the height or weight of a male and the number of embryos it incubated, which suggests that offspring size, besides being influenced by the physical space in the pouch, depends on the fecundity of the female. According to Vincent and Giles (2003), offspring size showed a positive linear relationship with the weight and height of the female, showing no expressive correlation with the size of the male, which was a result of larger females producing more ovocytes.

According to Tresher (1983), the size of the ovocytes and offspring varies based on the latitude where a seahorse lives and is not related to the size of the male. The cause of this is thought to be environmental variables, such as photoperiod and water temperature, where at higher latitudes there is an increase in the size of the eggs produced by the female and an increase in the size of the offspring at birth.

According to Foster and Vincent (2004), reproductive success is estimated by the total number of offspring, number of couplings per reproductive season, number of reproductive seasons per year and the survival rate of offspring. In addition, these authors suggest that indicators of reproductive success, which are easiest to measure, are the number of offspring and their size at birth. These authors also note that, at birth, seahorses are larger than other marine teleosts because they are born as alevins, while other teleosts are born as larvae. The larger body size at birth suggests there would be an increase in survival rate; however, the low fecundity and fertility of seahorses compared to other marine teleosts, such as *Sardinella brasiliensis* Steindachner (which has a fecundity estimated at 38,000 ovocytes per lot; Vazzoler 1996), suggests that this is not true. Based on the data in this study, which showed that this species usually has a 12-day pregnancy period and recouples two days after birth (only for mating pairs), a female could potentially produce 1,679.32 ovocytes per month (two lots) and 11,752.24 ovocytes during each reproductive season (14 lots from May to November). Taking the fertility data into consideration, which had a higher sample number, 6,914 alevins would be produced during each reproductive season.

Observations *in situ*, in this work, showed the immediate predation of the alevins of *H. reidi* released in the Maracaípe mangroves, after they were born in the laboratory. Small carnivorous fishes quickly devoured the offspring, which were between 0.5 and 0.7 cm, although they had totally formed external skeletons (Silveira 2000b). Many of the alevins were taken by the watercourse to the sea or into the estuary, depending on the tide. Actually, seahorses born in the mangroves might have a higher survival rate than the alevins released in this study because they are usually born at night, which reduces the risks of immediate predation. It is also possible that the males give birth in sites that are safer compared to where our study released the offspring.

Methodologies to estimate the survival rate of seahorses, after they disperse at birth, have not been developed. The only belief is that in the not-so-distant past, seahorses were abundant in several regions along the Brazilian coast (Silveira 2001, unpublished data) and, certainly, used the same reproductive strategy, suggesting that their current reproductive success is directly tied to their overexploitation and changes in their environment.

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