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REPRODUCTIVE PERIOD, AVERAGE HEIGHT FOR THE DEVELOPMENT OF THE BROOD POUCH AND SEXUAL MATURATION OF THE SEAHORSE HIPPOCAMPUS REIDI (SYNGNATHIDAE) IN THE NORTHEAST OF BRAZIL

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Abstract

Seahorses (Syngnathidae: *Hippocampus*) are fish threatened with extinction worldwide, requiring management plans for their conservation. For this purpose, researchers seek to know the life history of each species, recording their occurrence and distribution in each habitat. The longsnout seahorse, *Hippocampus reidi*, presented a reproductive period throughout the year in the Maracaípe mangroves, PE, with peaks of the reproductive season occurring between May and November. The mean height at which the male develops the brood pouch occurred at 10.0 (± 1.1) cm and the height at the first gonad maturation (L_{50}) was 12.3 (± 0.48) cm. Population data obtained in this study aim to contribute to the elaboration of the management plan for the species in the Brazilian tropical regions.

Keywords: Fish, Reproduction, Biological Parameters, Reproductive Season, Reproductive Size

1. Introduction

The family Syngnathidae comprises seahorses and pipefishes, species circumglobally distributed, dwelling the waters of temperate and tropical regions of the world (Lourie & Randal, 2003). The vast majority of Syngnathidae are listed on the IUCN (2015) (International Union for Conservation of Nature) as "data deficient", revealing poor knowledge of biology of the species. Two Brazilian species, *H. reidi* and *H. erectus* are listed in the *IUCN* as "data deficient" or "vulnerable" species, respectively, since 1996. In Brazil, both, recently, were included in the official list of endangered species issued by MMA (Ministry of Environment), according to Ordinance MMA No. 445, dated December 17, (2014). *Hippocampus patagonicus*, the third Brazilian species, has not yet been evaluated under *IUCN* criteria, but its presence in Brazil is no longer questionable (Boehm *et al.*, 2013; Silveira *et al.*, 2014).

The need for data on basic biology and population dynamics for the creation of management plans for the species is an urgent task for Brazilian researchers, as such data are scarce. CITES (2003) (Convention on International Trade in Endangered Species of Wild Fauna and Flora), responsible for regulating fishing activities and protecting endangered fish, introduced Resolution No. 12:54 recommending a minimum universal height limit of 10 cm for all seahorse species in trade, including *H. reidi*. The minimum size/height for commercial fishing should be part of the management plan of each species. This requires knowledge of biological parameters, such as the size or age at first gonad maturation. In this paper, we emphasize the importance of determining the reproductive period, average height of formation of the brood pouch and average height at first gonad maturation.

The knowledge of the reproductive period with the presence of a peak or high breeding season allows for greater flexibility in conservation actions, since such information can provide subsidies for a closed season. Normally, the fish reproductive period is determined by a number of environmental factors acting on the physiology of the species, leading to gonad maturation and spawning, and its length can be measured by the number of consecutive spawns during this period. Many works on histological and macroscopic gonad analysis determine the extent of the reproductive period based on the sampled months, but the analyses are often performed on females and with the sacrifice of the animals (Adams, 1980; Isaac-Nahum & Vazzoler 1987; Vazzoler, 1996).

The reproduction of seahorses occurs through the transfer of hydrated female oocytes the into the male's pouch, when he releases his sperm for fertilization and incubation of eggs that are already rich in calf (the seahorse is ovoviviparous). After a certain period of

development (pregnancy), which varies according to the species and water temperature, fry, miniature replicas of the adult parents are born (Azzarello, 1991; Lourie *et al.*, 1999; Silveira, 2001).

In view of the peculiarities of the seahorse to become pregnant, we chose to analyze males and their incubation pouches (pregnant and nonpregnant) to determine the reproductive period, avoiding sacrifices. The physiological condition of the incubation pouch, usually denounced by its volume, is the main attribute for the classification of pregnant and non-pregnant males. Although the pregnant male exhibits his enlarged brood pouch, there is an intermediate situation that undermines the certainty of pregnancy, when it is not possible to observe the mating or the birth of offspring. This intermediate situation between pregnancy and non-pregnancy is called receptivity: a receptive male has a pouch like a pregnant pouch, meaning that it is ready to mate. This structure becomes bulky, due to the intense vascularization and modifications of the internal epithelium, which is preparing to receive the oocytes (Azzarello, 1991; Silveira, 2001).

The development of the brood pouch in seahorses enables sexual differentiation, since only males possess them, but the formation of this structure occurs within a size/height range. The calculation of an average height for the development of the pouch is of utmost importance, as it allows the classifications of gender (male or female), and juvenile, which is the sexually undifferentiated individual, thus enabling the determination of the population structure closer to reality (Silveira, 2005). The lack of these data leads to a deviation in sex ratio, producing a biased sample towards one sex over the other: if we use the shortest male with brood pouch to consider the height for the development of the pouch, the population will tend towards females (Baum *et al.*, 2003; Silveira, 2005), whereas, if we use the highest male with the pouch still developing, the sample will possibly tend to have more juveniles/undifferentiated and less females.

The size at the first gonad maturation (L_{50}) in fish is understood as the average length with which 50% of the population of a given species starts their reproductive activities (Vazzoler, 1996). Its establishment is essential for the control of natural stocks, since it provides elements for the determination of a minimum size for commercial captures. The L_{50} represents a fraction of the average maximum length (Linf) of the population, and can be estimated from this parameter. According Vazzoler (1996), the methodological pattern of the logistic curve for the calculation of the average size at first gonad maturation has asymptote at 1, but this presupposes that all individuals find themselves in reproductive status, which does not occur. For this reason, an alternative method that utilizes a variable

asymptote related to the frequency of mature individuals is used for obtaining more accurate results (Fontoura *et al.*, 2009). Thus, this study estimated the reproductive period, the average height for the development of the pouch and the average height at first gonad maturation of *H. reidi*, aiming to contribute data to the national plan for the management of seahorses proposed by the Brazilian Ministry of Environment.

2. Material and Methods

2.1 Area and data collection in the mangrove

Field studies were conducted in the estuary of the Maracaípe River (8 º 32'14, 9" S and 35 º 00'17, 8" W), municipality of Ipojuca, State of Pernambuco, northeastern Brazil. Data were collected along 25 points, including the entire estuarine area shown in Fig. 1. The samplings were made weekly via *snorkel* at low tide, between June 2001 and July 2003.

When found, the animals were caught by hand and placed in plastic containers with water from the site for transportation to the laboratory. All observed seahorses were collected for analysis, had their heights measured (linear measurement from the top of the head to tip of the tail held straight, as proposed by Lourie *et al.*, (1999) and records on the pregnant, nonpregnant and receptive state of males were taken, as well as observations on the formation of the juvenile pouches.

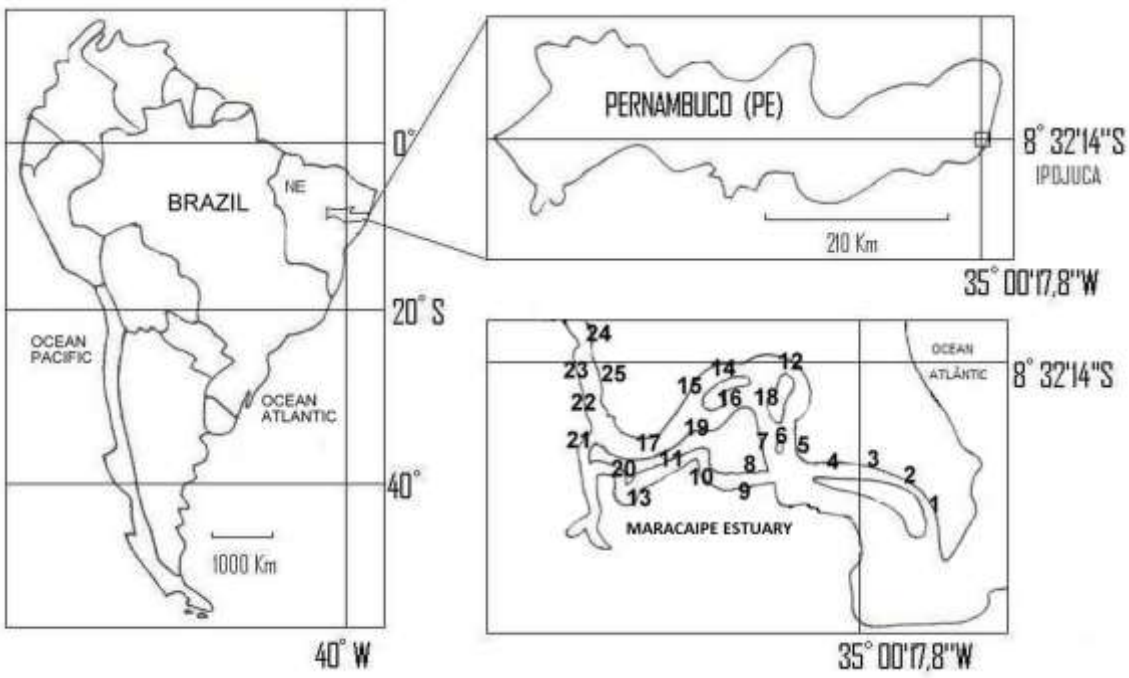

Fig. 1 Location Maracaípe River estuary in South America: Northeast of Brazil, Pernambuco state, Ipojuca.

2.1 Reproductive period and height of the first gonad maturation

The reproductive period was established through the relative frequency of pregnant males during the months of sampling. Seahorses with pouch, which caused doubts about their pregnancy, were classified as receptive (meaning being able to reproduce).

The pregnant were classified by visual analysis and external examination of the pouch, and some were kept in the laboratory for confirmation of pregnancy at birth and offspring count. Both the pregnant and the receptive males were used to establish the reproductive period. To measure the difference between males from the high and low reproductive season, the Mann-Whitney test was accomplished (two independent samples).

The determination of the size at first maturation was effected through the relative frequency of receptive and pregnant males according to the length class, with adjustment in the following logistic function

$$F = \frac{A}{1 + e^{-r(L_t - L_M)}},$$

where: F is the frequency of pregnant individuals by height class; A is the asymptote of the logistic curve related to the average frequency of pregnant males; L_t is the height of the body and L_m is the estimated size at first maturity.

The adjustment was done by nonlinear regression routine using SPSS 11.0 and fit through the Levenberg-Marquardt algorithm.

2.3 Average Height for the Development of the Incubation Pouch

The animals used for the calculation of average height of the brood pouch formation were juveniles from the smallest seahorse with fully formed pouch to the greatest with the pouch still in formation. Juveniles with small pouch in formation (values below the lowest male with fully formed pouch), were not considered for calculus just because we seek the average height of the seahorse with a fully formed pouch. The average height of the brood pouch formation was estimated by linear regression, using the various classes in height, and the variable indicating the brood pouch formation assumed two values, "1" for pouch formed and "0" for pouch in formation.

3. Results

3.1 Reproductive Period

The reproductive period of *H. reidi* in the mangrove of Maracaípe extends year-round with the presence of pregnant males in different height classes (Fig. 2). Although having a long reproductive period (Fig. 3), there appears to be a cyclical variation in reproductive intensity, with a higher frequency of pregnant males between the months of May and November (high reproductive season), comprising the winter (rainy season) and part of the spring. In the remaining months, *H. reidi* presented a moderate reproductive activity, reaching the lowest frequency in the dry season, between the months of February and April. Of the 435 sampled seahorses, 45% were male (8 individuals), and the remaining 55% were distributed between females and juveniles. Of the 198 assessed males, 102 individuals were pregnant. It was also observed that a greater number of seahorses (60 individuals) were not pregnant during the low season against only 30 individuals, which were not pregnant during the peak reproductive season (binomial test for comparing two proportions: $Z = 5.2574$, $p < 0.0001$).

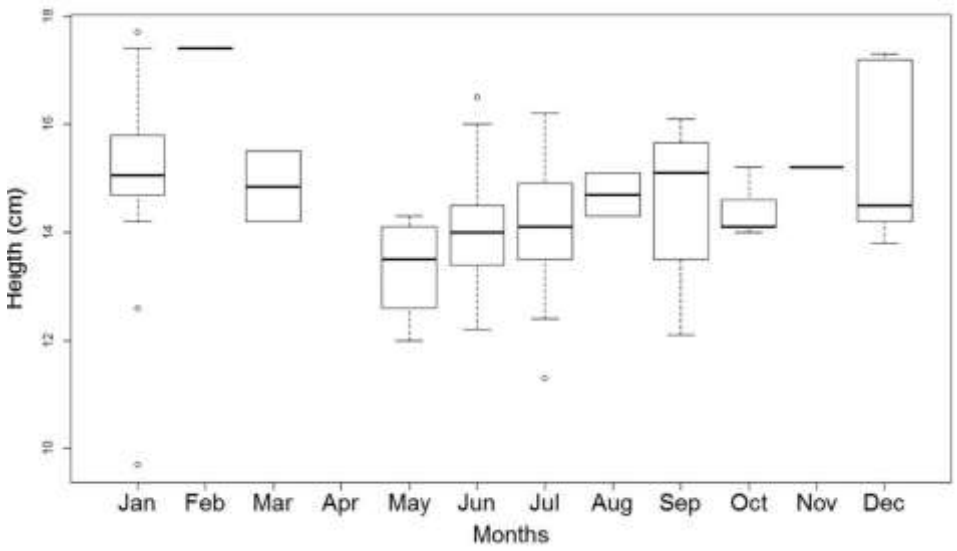

Fig. 2 Pregnant males of species *Hippocampus reidi* distributed by height classes in the estuary of the River Maracaípe Ipojuca, PE between 2001 and 2003.

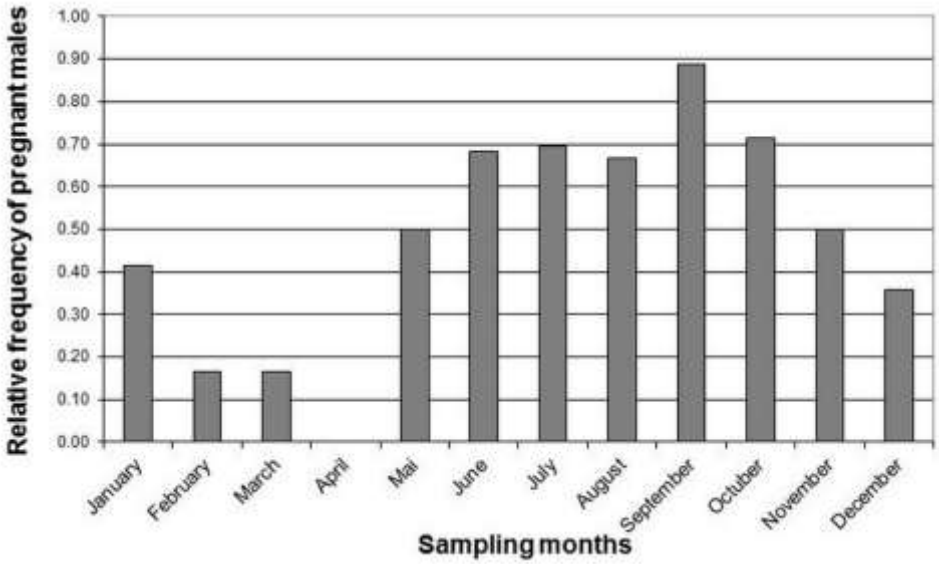

Fig. 3 Breeding season of *Hippocampus reidi* in mangrove Maracaípe Ipojuca, PE, Brazil.

3.2 Average Height for the development of the Pouch Incubator and at First Gonad Maturation

The identification of the development process of the brood pouch in *H. reidi* occurred in individuals with heights between 6.7 cm and 11.1 cm, with the average at 10.0 ±1.1cm (CI = (7.844, 12.156)) (n = 23). The estimated average height at the first gonad maturation (L_{50}) in *H. reidi* (n = 102) was 12.3 cm (r^2 = 0.84, Table 1, Fig. 4). Considering the confidence interval, we can say, with 95% confidence, that males of *H. reidi* initiate the reproductive activity with heights between 11.1 and 13.5 cm. The shortest receptive male measured 9.7 cm, whereas the longest pregnant measured 17.7 cm.

Table 1 Setting using nonlinear regression. SPSS 11.0 - Levemberg-Marquard algorithm. r^2 = 0.83621. *A*, asymptote of the logistic curve; L_{50}, average height at the first gonad maturation.

Parameter	Estimate	Standard Deviation	Confidence Limit (95%)	
			Lower	Upper
A	0.741	0.0845	0.5330	0.9490
r	1.412	0.8253	-0.6073	3.4310
L_{50}	12.300	0.4828	11.1200	13.4800

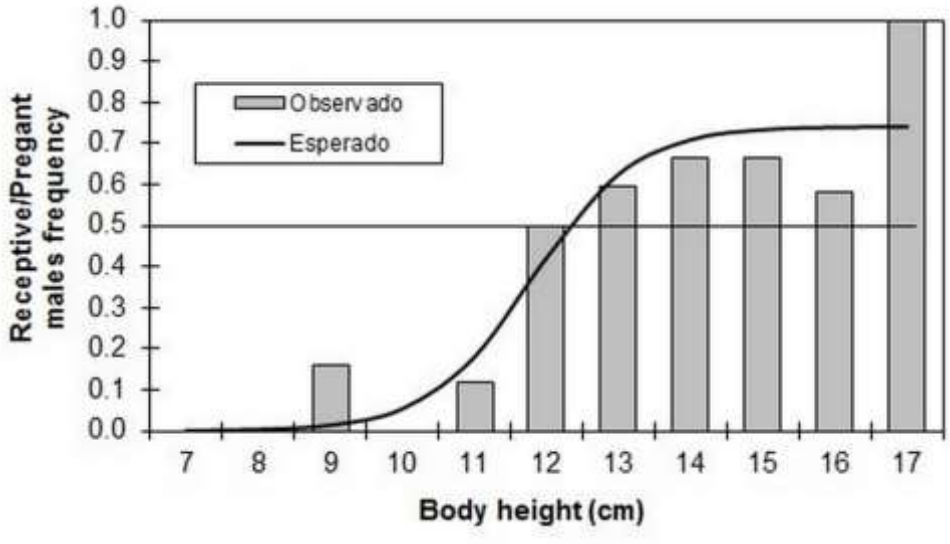

Fig. 4 Height first maturation in *Hippocampus reidi* sediments from Maracaípe Ipojuca, PE, Brazil (nonlinear regression with adjustment through the Levemberg-Marquardt algorithm).

Given that the sampling effort was evenly distributed throughout the year, the variable asymptote of the logistic curve (A = 0.741) permits us to infer that, as an annual average, adult males are pregnant approximately 74.1% of the time.

4. Discussion

According to Hatcher *et al.*, (1989), the reproductive season for fish in the tropical regions is usually more extensive than for fish of temperate regions, and is often determined by rainfall regimes and water current patterns. According to Taylor (1990), estuarine species can spawn repeatedly during a long reproductive season, and the duration of this season also varies according to the species and with latitude. Also, the depletion of energy reserves, high temperatures and reduced food availability may disrupt spawning during the annual cycle. According to Lourie *et al.*, (1999), the *Hippocampus* reproductive period varies from two to nine months, depending on the species and geographical distribution. Even though this author does not present data on *H. reidi,* they state that *Hippocampus kuda*, which also inhabits estuaries in Indonesia, maintains its reproductive period during the rainy season. According to Foster & Vincent (2004), *Hippocampus trimaculatus* in China, has a reproductive peak between March and May, but remain reproductive throughout the year at constant temperatures. *Hippocampus comes* in the Philippines, has a reproductive peak in the rainy season (July to December), maintaining its reproductive activity throughout the year (Perante *et al.*, 2002). In this work, as in Silveira (2005), *H. reidi* maintains its reproductive function throughout the year and their reproductive peak or high reproductive season occurs

between the months of May and November, including the winter (rainy season) and part of the spring. Studying *H. reidi* in an estuary in the state of Piauí (Brazil), Mai & Velasco (2011) found the same period and reproductive peak described in this work. Rosa *et al.*, (2007) found different reproductive peaks for the same species in other places in Brazil. This reproductive intensity related to the rainy season may be associated with a higher food intake by juveniles, with the intensification of the secondary production (Day *et al.*, 1989), as many species of either estuarine-dependent or resident fish maintain this reproduction pattern, in order to ensure that their offspring has the food resources at birth, necessary for the early life stages.

The formation of the brood pouch in the male is a phase of great importance in the development of the sea horse, as it enables the secondary sex differentiation: only males have this structure. For this reason, this is the morphological feature that allows for the determination of a population structure in a safer manner. The determination of the average height for the development of the pouch enables the observation of the population structure, minimizing errors. According to Silveira (2005, 2009) and this work, fish up to 10.0 cm in total height without the presence of this structure are considered juveniles (sexually undifferentiated); from 10,1 cm and higher, without the presence of the brood pouch, are considered females; and with the pouch at any time, are males. Baum et al (2003) acknowledge that the lack of determination of this parameter hinders the analysis of population structure, overestimating the number of females. The authors considered the smallest observed male with a developed pouch to determine the size for the formation of this structure. However many males that developed their pouches after this size were quantified as females, diverting the sexual population rate. Before Silveira (2005), there were no criteria for studying the population structure of seahorses based on this parameter. The determination of an average height for the development of the pouch is necessary to classify the data. Although we know that the formation of the incubator in males can still occur, even after the height of 10.0 cm, the error to which we are exposed is much smaller. In our observations (n = 23), we detected two cases of brood pouch in development after the height of 10.0 cm (10.3 to 11.1 cm). If we were to confront the height of the shortest observed male with the brood pouch in development with the height of formation of this structure, the lowest value would be 6.7 cm and the value for fully formed individuals, 8.7 cm, determining the classification of all samples above this value (some future males) as females, diverting the sexual rate.

Previous experience in cultivation and maintenance of seahorses in laboratory (Silveira, 2000) showed that inadequate conditions, such as insufficient supply, lead to slow

growth, delaying the development of structures, such as the pouch. Species of seahorses cultivated (*ex situ*) or developed in the natural environment (*in situ*), showed formation of the brood pouch between 4.0 and 13.0 cm height, corresponding to the ages between four months and one year (Wilson & Vincent, 1998; Lourie *et al.*, 1999). This is a wide range of height for the development of the pouch , which, besides the life history of each species, could be explained by the origin of the data (*ex situ*, *in situ*), taking into account its geographical distribution and its respective climate (tropical or temperate), an important determinant in the dynamics of each species.

According to Vazzoler (1996), with the proximity of the first gonad maturation age, environmental factors act on individuals, determining where and when each species will reproduce, so as to maximize the availability of adequate food and minimize the risk of predation on the offspring. In the early stages of development, juveniles use the energy from food almost entirely for somatic growth, until it reaches the minimum critical size, when the process of gonad development begins. Thereafter, part of the energy is allocated to the gonad development and reproductive events that are established with the L_{50}, here referred to as the average height of first gonad maturation. According to Foster & Vincent (2004), the height at first gonad maturation in seahorses is usually underestimated, as it has been considered as the smallest size achieved by the male before presenting a fully developed brood pouch. (Wilson & Vincent, 1998; Baum *et al.*, 2003).

Also according to Foster & Vincent (2004), the development of the pouch may not be an indication of physiological maturity, as observed in *H. trimaculatus*, which develops the pouch when it reaches heights of 8.0 to 9.0 cm, but becomes sexually mature only when it reaches the height of 12.0 cm, confirmed by the histological analysis of the testes. For Lovett (1969), *Hippocampus abdominalis* (maximum height of 32.2 cm) was considered mature after achieving the height of 14.0 cm and the weight of 10.0 g, completing 12 months of age in this developmental stage, whereas Forteath (1997) states that this same species achieves sexual maturity at 8 months of age. *Hippocampus japonicus* (8.0 cm) achieves maturity between the ages of 3-8 months, with a height of 4.5-5.5 cm (FAO, 1990); the reproductive life of *H. kuda* begins at 9-12 months of age (FAO, 1990) and, according to Lourie et al (1999), at 7-8 months. According to those authors, many species of *Hippocampus* mature during the first reproductive season after birth, with ages between 6 and 20 months. Only small specimens, such as *Hippocampus zosterae* (maximum height of 3.8 cm) mature at 3 months of age (Strawn, 1958). However, the vast majority of seahorse species lack studies on their biology. In the state of Pernambuco, the average height of *H. reidi* at first maturity

was 12.3 cm at around seven months of life (Silveira, 2005), which is similar to the size of first reproduction found by Mai & Velasco (2011) that was 12.4 cm for males. The height extremes found for receptive/pregnant males were 9.7 cm and 17.7 cm, respectively. May & Velasco (2011) observed the smallest male of *H. reidi* , which exhibited a developed pouch at the height of 9.0 cm and became pregnant at the height of 9.5 cm. As reported by Curtis & Vincent (2006), the standard length (SL) of *Hippocampus guttulatus* Cuvier 1829 found in an estuary in Portugal was 10.94 cm at first sexual maturity. Silveira (2005) observed that not all males apt to reproduction were pregnant, and that a logistic curve with the asymptote at 1 (Vazzoler, 1996) overestimated the size at first maturity, and, for that reason opted for the calculation with a variable asymptote in the current work, as described in Fontoura *et al.*, (2009). In samples uniformly distributed throughout the year, the value of the variable asymptote is related to the average frequency of males in reproductive activity, indicating that they remain pregnant almost constantly, i.e., 74.1% of the time on average. These results are in agreement with those observed by Martin-Smith *et al.*, (2004), who stated that the ban on fishing of pregnant males is a problem for trade, as they are in pregnancy state most of the time.

Wilson & Vincent (1998) substantiated that *Hippocampus barbouri*, *Hippocampus hippocampus*, *Hippocampus fuscus* and *Hippocampus ingens* reach maturity at 4-5 months, but age is not considered the best indicator for maturity. The height of the individual is the best indicator, because it was observed that populations of *H. trimaculatus* from North and South China reached maturity at the same size, but with a gap of two months between the populations.

Although there are records of pregnant males of *H. reidi* with only 8.0 cm (collection LABAQUAC / PH 105r) in the South of Brazil, the difference in size at first maturity along a latitudinal gradient is not necessarily presumed. Albeit L_{50} was estimated at 12.3 cm in Maracaípe, a receptive male with 9.7 cm (Figure 3) was also captured in this locality. Thence, it is not possible to determine whether the isolated data recorded in southern Brazil are characterized as general trend or apply only to an animal captured at the lower end of the maturity curve. Rosa *et al.*, (2007) obtained an average height of 15.58 cm for pregnant males of *H. reidi*, with a variation range between 6.5 and 20.0 cm in several Brazilian regions (NE, SE and S).

The height at the first gonad maturation is a very important factor to determine the minimum size/height for commercial fishing. CITES (2003) determined a universal size of 10

cm as a minimum height limit for the capture of *H. reidi* and *H. erectus*, among other species of seahorses, without harming their reproduction and maintenance in nature.

According to our results, *H. reidi* does not fit the CITES decision, and the adoption of this resolution in our territory, especially in tropical regions, can lead to an even more severe depletion of these overexploited populations.

In Brazil, seahorses are still not fully covered by specific protection laws. There are only export quotas for other ornamental fish (MMA IN No 5/2004), which are not effectively enforced (Nottingham *et al.*, 2005). Such specific protection laws or management plans for the species, currently required by MMA, have become indispensable and urgent, in view of the increasing depletion of natural stocks in our country (Silveira, 2001, 2005). The creation of management plans for seahorses requires, among others, knowledge of the reproductive period of the species; information on the average height for the development of the brood pouch; and data on the average height at first gonad maturation established for their region, mainly taking into account the thermal gradient.

5. Conclusion

Although currently seahorses integrate the list of threatened species in Brazil (MMA Ordinance No. 445), it has not been a consistent structured management plan, or species recovery, and there is still no supervisory support. Soon (2016), the MMA will review the manager plan for the use of seahorses in Brazil (Marilia Marques Guimaraes Marini, Coordinator of National Action Plans of Endangered Species, personal communication), and thus, this study reinforces important points obtained in Silveira (2005) and which are suggested for tropical regions:

1 – Our studies allow us to suggest greater concern for *H. reidi* during either part or throughout the whole high reproductive season (May to November), with the possibility of establishing a closed season in Brazilian tropical regions. The social implications of this measure should be discussed together with the species management plan developed by researchers and by the Brazilian Environmental Ministry.

2 – We propose the inclusion of data collection, such as average height for the development of the pouch, in the seahorse study routines, to determine the population structure with the least possible error, with the intention of improving the method.

3 – Finally, we also suggested that the minimum size/height for commercial fishing of *H. reidi* in the tropical Brazilian territory be based on the average height of 12.3 cm at first gonad maturation of the species. It is our understanding that the minimum height of

commercial fishing of *H. reidi* should be 13.0 cm, as this will allow the fish to reproduce at least once before they are captured.

Despite all efforts and studies conducted to date, the acquired knowledge of seahorses is still small. Apart from ongoing studies, new and repeated observations are necessary to attain consistency and certainty about our statements.

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