

# Estimating population parameters of longsnout seahorses, *Hippocampus reidi* (Teleostei: Syngnathidae) through mark-recapture

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Estimating population parameters is essential for understanding the ecology of species, which ultimately helps to assess their conservation status. The seahorse *Hippocampus reidi* is directly exposed to anthropogenic threats along the Brazilian coast, but the species still figures as Data Deficient (DD) at IUCN's Red List. To provide better information on the ecology of this species, we studied how population parameters vary over time in a natural subtropical environment. By combining mark-recapture models for open and closed populations, we estimated abundance, survival rate, emigration probability, and capture probability. We marked 111 individuals, which showed a 1:1 sex ratio, and an average size of 10.5 cm. The population showed high survival rate, low temporary emigration probability and variable capture probability and abundance. Our models considering relevant biological criteria illuminate the relatively poorly known population ecology and life history of seahorses. It is our hope that this study inspires the use of mark-recapture methods in other populations of *H. reidi* in a collective effort to properly assess their conservation status.

**Keywords:** Capture Probability, Emigration Probability, Population Ecology, Robust Design, Survival Rate.

Parâmetros populacionais são essenciais para compreender a ecologia das espécies, além de auxiliar a avaliação do seu status de conservação. Dentre as espécies de cavalo-marinho que ocorrem no Brasil, *Hippocampus reidi* é a mais abundante, o que expõe suas populações a frequentes ameaças antropogênicas. Entretanto, esta espécie ainda consta como Deficiente em Dados (DD) na lista vermelha da IUCN. Considerando esta falta de informações sobre populações de *H. reidi*, utilizamos métodos de marcação e recaptura para avaliar como parâmetros de uma população de ambiente subtropical variam ao longo do tempo. Baseado em históricos de captura individuais, combinamos modelos de populações abertas e fechadas para estimar abundância, taxa de sobrevivência, probabilidade de emigração e probabilidade de captura. Ao todo, marcamos 111 indivíduos, em proporção sexual de 1:1, e tamanho médio de 10,5 cm. Esta população relativamente pequena apresentou alta taxa de sobrevivência, baixa probabilidade de emigração temporária e probabilidade de captura e abundância variáveis. A partir dos nossos modelos que consideraram critérios biológicos relevantes, fornecemos estimativas de parâmetros chave que auxiliarão a compreensão da ecologia e da história de vida de cavalos-marinhos. Nossa expectativa é que este método passe a ser amplamente utilizado em outras populações, em um esforço coletivo para avaliar o estado de conservação desta espécie.

**Palavras-chave:** Desenho Robusto, Ecologia de Populações, Probabilidade de Captura, Probabilidade de Emigração, Taxa de Sobrevivência.

## Introduction

Comprehending how populations interact with ecological and evolutionary factors is a primary motivation in population ecology. An essential step towards this goal is the quantification of parameters of wild populations, which also provides the baseline information to assess the conservation status of

a species (Lebreton *et al.*, 1992; Dulvy *et al.*, 2004). Mark-recapture techniques provide outstanding tools for estimating these parameters across taxa (*e.g.* Bjørndal *et al.*, 2003; Hammond, Anthony, 2006; Rotheray *et al.*, 2014). By tracking the capture history of marked individuals in a local population, one can accurately estimate abundance, survival, movements — to name but a few parameters (*e.g.* White, Burnham, 1999).

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The number of individuals in a given population (*i.e.*, abundance) essentially reflects the balance between addition through birth and immigration, and losses through deaths and emigration (Williams *et al.*, 2002). Traditionally, estimates of abundance were based on mark-recapture models of closed populations, which assume negligible addition, loss, and movement of individuals between subpopulations. However, this assumption does not hold true in many cases, especially in studies spanning over large spatiotemporal scales (Kendall *et al.*, 1997). Too often, individuals are part of meta-populations in which emigration and immigration exert a strong influence in the balance of individuals over time (Hanski, 1998). To account for this influence, models of closed and open populations were combined (Pollock, 1982; Kendall *et al.*, 1997), allowing precise estimates of abundance, temporary emigration, and survival probabilities in mark-recapture studies. This inclusive model, known as Pollock's Robust Design (Kendall *et al.*, 1997), has been successfully applied to a wide range of species in different habitats (*e.g.* Frick *et al.*, 2010; Cantor *et al.*, 2012; Wayne *et al.*, 2016), providing essential information about population ecology. Besides being important for understanding basic life history parameters, this framework has been proven to be valuable for assessing population trends of threatened species, required to guide conservation initiatives.

Pipefishes, pipehorses, seahorses and seadragons belong to the Syngnathidae family, which comprises around 300 species of characteristic long-snouted fish with fused jaws and male brooding, inhabiting temperate and tropical coastal waters (Hamilton *et al.*, 2017). The mark-recapture approach has been successfully employed to study life history traits, behavior and movement patterns of some of these species (*e.g.* Connolly *et al.*, 2002; Bell *et al.*, 2003; Silveira, 2005; Curtis, Vincent, 2006; Caldwell *et al.*, 2011). Despite such effort, key population parameters remain poorly known for most Syngnathids, hindering a broader understanding of how dynamic their natural populations can be over time. To date, few studies have quantified the parameters that allow evaluation of population trends, such as abundance, survival, immigration and emigration (but see Sanchez-Camara *et al.*, 2006). This gap is critical given that natural populations of some of these species seem to be declining (Martin-Smith, Vincent, 2005).

The major causes of decline in populations of Syngnathidae species are the intensive exploitation for traditional medicine and the aquarium industry, and the disturbance of natural habitats by a myriad of human coastal activities (Lourie *et al.*, 2004). Amongst the species that are particularly vulnerable to anthropogenic threats are the seahorses of the genus *Hippocampus* Rafinesque, 1810, because they are coastal, have small home ranges and tend to be highly associated with the substrate (Foster, Vincent, 2004). This alarming situation led to the inclusion of 38 *Hippocampus* species in IUCN's Red List in 1996 (IUCN, 2016), as well as the addition of the genus in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora in 2004 (CITES, 2016).

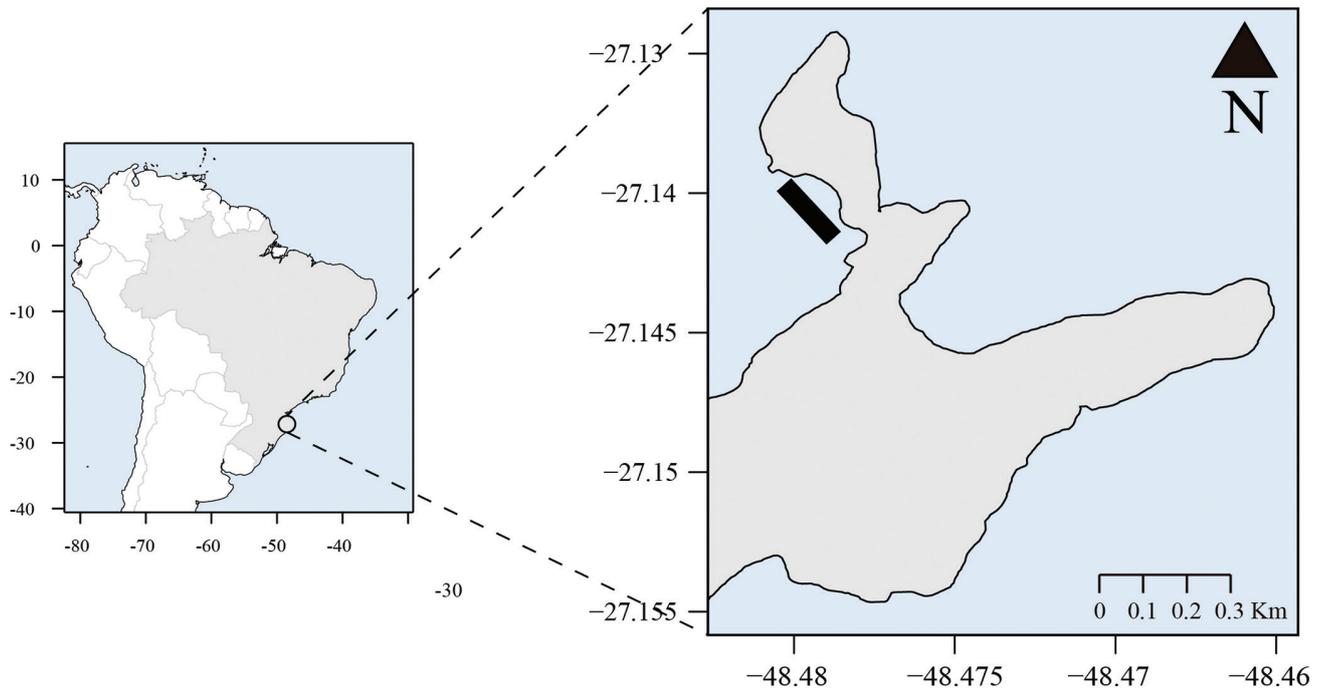
Eight species of this genus occur in the Atlantic Ocean. One of the most widespread is the longsnout seahorse *Hippocampus reidi* Ginsburg, 1933, which ranges from the Caribbean to the southern coast of Brazil (Teske *et al.*, 2007). This species is abundant in reef and estuarine environments along the coast (Silveira, 2011), but their population dynamics remain mostly unknown (but see Silveira, 2005; Mai, Velasco, 2011; Silveira *et al.*, 2016). The study of natural populations is limited to the tropical northeastern Brazilian coast (Silveira, 2005; Mai, Rosa, 2009; Mai, Velasco, 2011), while in the south subtropical coast, studies are much rarer and restricted to artificial environments (*e.g.* Freitas, Velastin, 2010). There has been a single effort to characterize natural populations of *H. reidi* along the Brazilian coast (Rosa *et al.*, 2007). This lack of basic population parameters estimation is the primary factor preventing a proper assessment of the conservation status of the species, which still figures as Data Deficient (DD) status on the IUCN's Red List.

Aiming to better understand the basic ecology and life history of this poorly known species, we assessed key population parameters of longsnout seahorses *in situ*, in a natural subtropical environment. By combining mark-recapture methods for open and closed populations, we estimate survival probabilities, abundance, movements, sex ratio and size of individuals. Our study provides important empirical ecological information that could be used as a baseline for larger-scale population assessments aiming to clarify the definition of the conservation status of this species.

## Material and Methods

**Study site.** We carried out a mark-recapture experiment in the rocky shores of Sepultura beach (27°08'S 48°28'W), southern Brazil (Fig. 1). The beach is relatively protected from waves, providing conditions for shallow water organisms including many fish and invertebrate species. The local benthic community is predominantly composed of the sessile *Sargassum* algae, which seahorses are commonly associated with (Coston-Clements *et al.*, 1991).

**Data sampling.** For a full year (April 2011 to April 2012), we searched for seahorses during free diving (snorkeling) surveys. *Sargassum* banks occurred at the maximum depth of three meters, making SCUBA diving unnecessary. Two researchers actively searching for seahorses during 1 hour (totaling 2 hours per occasion) throughout the entire study area (1,800 m<sup>2</sup>) defined a capture occasion. Each animal we located was placed into a plastic pot with water and substrate to minimize stress during measurements. On the beach, the following individual biological data were recorded: sex, color, and length (linear measurement from the top of the head to the tail, Lourie *et al.*, 2004). The presence (male) or absence of the pouch (female) in specimens longer than 10.1 cm determined sex. Individuals smaller than 10.0 cm were considered juveniles (Silveira, 2005).



**Fig. 1.** Sampling area location (black stripe) at Sepultura beach on the southern coast of Brazil.

We marked all individuals in the first rings of the trunk with numbered collars. We placed the collars so as not to harm the animal, keeping a clearance to avoid injury. This method is commonly used in mark-recapture studies with Syngnathidae, with little influence on its natural behavior (Caldwell *et al.*, 2011). After marking, individuals were released in the same place of capture. We assessed the efficiency of our sampling effort with a discovery curve built with sample-based rarefaction methods (Gotelli, Colwell, 2001) in R environment (R Development Core Team 2016). An asymptotic discovery curve was diagnostic of sufficient sampling effort.

**Mark-recapture modelling procedures.** We organized the mark-recapture data over a six-month period between November 2011 and April 2012, totaling 24 sampling days. The previous six months (April–November 2011) consisted of a pilot sampling effort, with only one capture occasion per month. We analyzed the binary matrix of capture history of all marked individual in the MARK program (version number 8.1; White, Burnham, 1999).

Our sampling effort was designed to model population parameters using the Pollock's Robust Design (Pollock, 1982; Kendall *et al.*, 1997). This framework considers primary and secondary sampling occasions to combine open and closed population models. We then divided our sampling occasions among the six months and 24 days as follows: each month was considered a primary period in which we had four days of sampling, regarded as the secondary periods. We concentrated these four sampling days in the first 15 days of the month and spaced them by three days. This approach allowed us to consider the population within

secondary occasions as closed, that is, the three-day intervals between these sampling occasions were short enough so we could assume no changes in the population, allowing accurate abundance estimates ( $N$ ).

Between the primary occasions, we considered the population as open, assuming that individuals could enter or leave the population between months. This approach enabled the estimation of apparent survival probabilities ( $\Phi$ ) and capture and recapture probabilities ( $p$  and  $c$ ). The temporary emigration estimates ( $\gamma'$  and  $\gamma''$ ) were made possible by analyzing the set of primary and secondary occasions following Kendall, Nichols (1995). We used a conditional parameterization of the Robust-Design models, also known as "Huggins closed model" (Huggins, 1989). The Huggins robust-design closed model compute abundance as a derived parameter. In other words, the statistical model does not contain abundances as parameters. Huggins parameterization allows including individual covariates and is more stable in recognizing parameters.

We built 10 candidate models to estimate the following parameters: capture probability within ( $p'$ ) and between ( $p$ ) secondary periods, survival probabilities ( $\Phi$ ), temporary emigration probabilities ( $\gamma''$ ) between primary periods (*i.e.*, the probability that an individual emigrant remained outside the study area ( $\gamma'$ ) during subsequent primary periods; Kendall, Nichols, 1995; Kendall *et al.*, 1997) and abundance ( $N$ ) of marked individuals within each pair of secondary periods. We also tested different movement patterns, considering completely random ( $\gamma'' = \gamma'$ ) or Markovian emigration models ( $\gamma'' \neq \gamma'$ ), or no emigration models ( $\gamma'' = \gamma' = 0$ ) (see Kendall *et al.*, 1997). Candidate models were built assuming, or not, the time-dependence

(t) or group (g) (sex – female, male or juveniles) effects in the parameters of interest ( $p'$ ,  $p$ ,  $\phi$ ,  $\gamma$ ). Since our method for capturing and marking individuals could be considered as invasive (Correia *et al.*, 2014), we also tried the model with behavioral responses ( $p \neq c$ ).

We used the quasi-Akaike's Information Criterion adjusted for small sample size and extra binomial variation (QAICc; Burnham, Anderson *et al.*, 1992) to select the most parsimonious model (*i.e.*, the model that best explains the variation of data with the smaller number of parameters). To incorporate the uncertainty of the model selection process into our parameter estimates, we used the model averaging method. Differences in AICc values were used to compute the model weights, which were then used to determine the weighted average of the parameters based on the relative importance of the models using MARK.

**Model assumptions.** Multiple samples mark-recapture estimates of population parameters depend on the following assumptions (Otis *et al.*, 1978; Seber, 1982): (i) animals do not lose their marks during the experiment; (ii) all marks are correctly recorded in each recapture event; (iii) each animal has an equal and constant capture probability; (iv) each animal has an equal and constant survival probability. For closed models, we also assume (v) the population does not change in composition during the sampling periods. Violation of these assumptions causes sampling heterogeneity, resulting in extra-binomial variation, or overdispersion (Williams *et al.*, 2002). We tried to reduce variations by discarding the data on poorly marked (*i.e.*, tags with unidentifiable scraped numbers) seahorses from our sampling subset, only including the identifiable collars in our capture-recapture dataset (limiting violations of assumptions *i* and *ii*). Since there are no appropriate methods for testing the goodness-of-fit (GOF) in RF models (White, Burnham, 1999), we measured overdispersion of the data using a general CJS model, based on primary periods collapsed from the Robust Design matrix, estimating the variance inflation factor ( $\hat{c}$ ) by *Bootstrapping* with 1000 iterations (White, Burnham, 1999). Where  $\hat{c}$  was  $> 1$ , the estimated value was used to adjust the models.

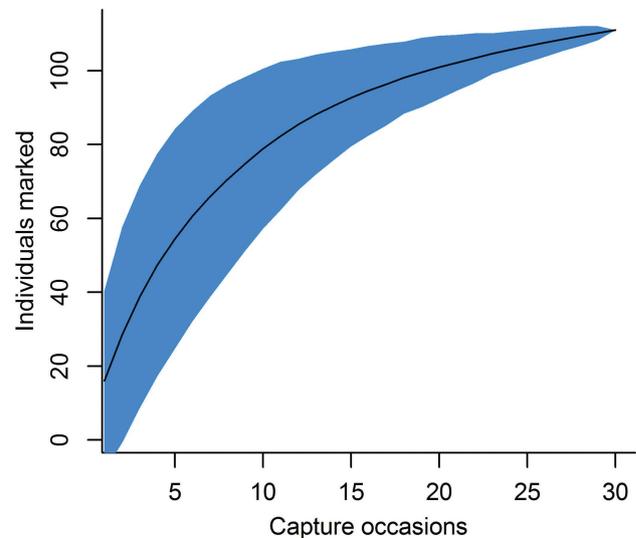
**Analysis of other population aspects.** To estimate sex ratio, we have only used the data on sexually mature individuals. This is because juveniles were rarely sighted due to their small sizes and had no secondary sexual characters that were visually identifiable. We used a chi-square test to verify if there were differences between the number of males and females.

## Results

**Population characterization.** We captured and marked 111 individuals of *H. reidi*, out of which 49 were males, 51 females, and 11 juveniles. The population showed sex ratio of 1:1, with no significant difference between sexes ( $X^2=0.02$ ,  $df=1$ ,  $p=0.88$ ). Body size ranged from 6.0 to 15.5

cm (mean  $\pm$  SD; males= $10.30 \pm 1.85$  cm; females= $10.70 \pm 1.74$  cm). The smallest male with forming pouch was 6.6 cm length. Juveniles and reproductively active animals with pregnant pouch were found throughout the entire duration of the studied year, and two couples were spotted mating during our mark-recapture sampling occasions. All animals were found associated with *Sargassum* macroalgae.

**Robust Design results.** There were 336 recapture events throughout the mark-recapture sampling occasions. The discovery curve (Fig. 2) tended to be asymptotic, with narrower 95% confidence intervals towards the end of the study, indicating that sampling effort was sufficient to mark most of the individuals in the population.

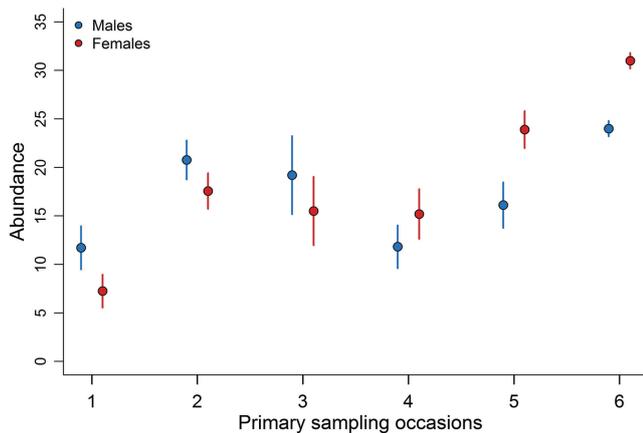


**Fig. 2.** Sample-based discovery curve of marked *Hippocampus reidi* at Sepultura beach. The black curve represents the rarefaction estimate and blue area the 95% confidence intervals.

Tab. 1. provides all candidate models ranked (adjusted by a  $\hat{c}$  of 2.41) according to their lowest quasi-Akaike Information Criterion (QAICc). The most parsimonious model considered random temporary emigration with no time-dependence ( $\gamma'(\cdot)=\gamma''(\cdot)$ ); constant apparent survival probabilities with no time-dependence and group effect ( $\phi(\cdot)$ ); and capture probability equal to recapture probability and time-dependent over primary and secondary periods ( $p(ts)=c(ts)$ ). With this model, the survival rate was estimated to be relatively high ( $\phi=0.83 \pm 0.05$  SE - 95% CI=0.71 – 0.91) and emigration probability to be low throughout the study ( $\gamma''=0.19 \pm 0.08$  SE - 95% CI=0.07 – 0.42). Capture and recapture probabilities were variable, and ranged between  $0.08 \pm 0.07$  SE and  $0.76 \pm 0.09$  SE. Likewise, abundance (N) varied throughout the study and ranged from  $12 \pm 2.23$  SE to  $24 \pm 0.8$  SE males and  $7 \pm 1.7$  SE to  $31 \pm 0.8$  SE females in primary capture occasions (Fig. 3). Based on these abundance estimates, we estimated densities ranging from 0.3 to 1.7 individuals/100m<sup>2</sup> at Sepultura beach.

**Tab. 1.** Robust Design candidate models to estimate survival probability ( $\phi$ ), capture probability ( $p$ ) and temporary emigration probability ( $\gamma$ ) for *Hippocampus reidi*. Models are ranked by the lowest quasi-Akaike's Information Criterion with a 2<sup>nd</sup>-order correction for small sample sizes (QAICc values). Notation: (.) constant; (t) time-dependence; (g) group effect; ( $p=c$ ) no recapture effect; ( $\gamma''=\gamma'=0$ ) no emigration models; ( $\gamma''=\gamma'$ ) random emigration models; ( $\gamma''\gamma'$ ) Markovian emigration models.

Model	QAICc	Q $\Delta$ AICc	AICc Weights	Likelihood	Parameters	QDeviance
1) $\phi(.) \gamma'(.)=\gamma''(.) p(ts)=c(ts)$	271.67	0.00	0.882	1.000	36	268.35
2) $\phi(g) \gamma'(.)=\gamma''(.) p(ts)=c(ts)$	277.33	5.65	0.052	0.059	39	266.75
3) $\phi(.) \gamma'(.)\gamma''(.) p(ts)=c(ts)$	278.05	6.37	0.036	0.041	39	267.47
4) $\phi(.) \gamma'(.)=\gamma''(.)=0 p(ts)=c(ts)$	279.00	7.32	0.022	0.025	37	273.27
5) $\phi(.) \gamma'(g) \gamma''(g) p(ts)=c(ts)$	281.84	10.16	0.005	0.006	41	266.36
6) $\phi(t) \gamma'(.)=\gamma''(.) p(ts)=c(ts)$	285.15	13.47	0.001	0.001	42	267.19
7) $\phi(g*t) \gamma'(.)=\gamma''(.) p(ts)=c(ts)$	294.27	22.60	0.000	0.000	47	263.76
8) $\phi(.) \gamma'(.)=\gamma''(.) p(ts) c(ts)$	304.42	32.75	0.000	0.000	56	250.44
9) $\phi(.) \gamma'(.)=\gamma''(.) p(g*ts)=c(g*ts)$	321.43	49.75	0.000	0.000	62	251.13
10) $\phi(.) \gamma'(.)=\gamma''(.) p(g*ts) c(g*ts)$	409.69	138.01	0.000	0.000	98	228.70



**Fig. 3.** Abundance estimates for males (Blue) and females (Red) of longsnout seahorses, *Hippocampus reidi*, for all primary sampling occasions. Whiskers represent Standard Errors.

## Discussion

Our results provide a snapshot of a longer-term local population dynamics of *H. reidi* and represent a valuable reference line to improve our understanding of the ecology of this seahorse species. Taken together, the high survival probability between secondary periods and the low emigration probabilities suggest site fidelity, highlighting the importance of the area for seahorses. By estimating parameters of a longsnout seahorse population for the first time, our study shows how mark-recapture experiments using Pollock's Robust Design can help to characterize local population dynamics of seahorses, which we hope will inspire similar studies with other populations throughout the species distribution.

Our studied population shows similar sex ratio, the proportion of juveniles, and body size when compared to other seahorse populations (Foster, Vincent, 2004). The 1:1 sex ratio suggests a monogamic mating system, as seen among most seahorse species (Foster, Vincent, 2004). Monogamy

might be feasible to increase reproductive success, given that seahorses usually live in low densities, have restricted mobility, and are cryptic to avoid predation (Vincent, Sadler, 1995; Foster, Vincent, 2004). The few populations with unequal sex ratio among seahorses (Bell *et al.*, 2003; Martin-Smith, Vincent, 2005) may reflect overexploitation of individuals for commercial purposes (Rosa *et al.*, 2007). The average body size of our studied individuals lay within the range recorded for other populations along the Brazilian coast (6.1 to 14.2 cm; Rosa *et al.*, 2007). The slightly larger body size of females—also known for other seahorse species (Foster, Vincent, 2004)—may be positively correlated with offspring production (Vincent, Giles, 2003).

The presence of pregnant individuals and couples in mating activities during our study agrees with suggestions that longsnout seahorses reproduce throughout the year (Rosa *et al.*, 2007; Mai, Velasco 2011; Silveira *et al.*, 2016). Moreover, reproductive activities suggest that the area provides conditions for the maintenance of the population. Despite being close to the subtropical limit of distribution of the species, this population does not seem to display seasonal reproductive activities. The low proportion of juveniles in this coastal population is somewhat expected (Moreau, Vincent, 2004; Martin-Smith, Vincent, 2005) since most of the juvenile phase of the species occurs in the pelagic environment (Curtis, Vincent, 2006).

By exhibiting a patchy distribution pattern, the *Hippocampus* genus tends to present low population densities (Lourie *et al.*, 2004; Foster, Vincent, 2004). In overfished locations, *H. reidi* present lower average densities, from as low as 0.18 individuals/100m<sup>2</sup> in the subtropical south to 6.6 individuals/100m<sup>2</sup> in the tropical northeast of the Brazilian coast (Rosa *et al.*, 2007). Our findings agree with such low density of subtropical populations near the southern limit of distribution of this species, although this is still a controversial ecological pattern given the lack of clearer empirical support (Sexton *et al.*, 2009).

Seahorses are mostly sedentary; they are incapable of active long-distance migration and show no large-scale

movements (Caldwell, Vincent, 2012; Boehm *et al.*, 2013). These features were evident in our models suggesting constant and random patterns of temporary emigration. Overall, our findings suggest that longsnout seahorses have strong site fidelity, with low emigration probability and high survival rates through time. The lifespan of this species in the wild was estimated to be around three years (Mai, Velasco, 2011); thus, our sampling effort was probably concentrated in a single cohort. The capture and recapture probabilities were variable both between and among primary occasions and might have been influenced by environmental conditions, such as water turbidity. As the water visibility was very variable in the sampling area, it may have affected capture probabilities through active visual searching. Nevertheless, abundance estimates were relatively constant after the first month of the mark-recapture experiment, which reinforces the robustness of our model estimates to parameter co-variation.

Although we focused on quantifying population parameters in a population, its local dynamics is coupled with a broader-scale dynamic, being influenced by other mechanisms such as dispersal, climate variability and biological interactions (Bjørnstad *et al.*, 1999). For instance, despite having small home ranges, being poor swimmers and being highly associated with the substrate, seahorses can perform long-distance dispersal by passively rafting in floating algal mats (Boehm *et al.*, 2013). This passive dispersal might exert a strong influence in the meta-population dynamics of seahorse species. Therefore, we suggest that future efforts for quantifying seahorse population trends should incorporate the possible influence of all these other variables to clarify their roles in the demographical dynamics.

Considering the lack of knowledge about seahorse populations in natural environments and the ongoing threats they are exposed to, our study provides baseline knowledge that aids the comprehension of the population ecology of longsnout seahorse *Hippocampus reidi*. Importantly, our study demonstrates how mark-recapture methods, such as Pollock's Robust Design, are suitable for studies with Syngnathids, and we recommend longer studies using this framework throughout the distribution of the species. Such future large-scale effort will improve our understanding of the ecology of natural populations of seahorses and, more specifically, provide the information that hitherto prevents the assessment of the conservation status of *H. reidi*.

### Acknowledgments

We thank all researchers who helped with field work, in particular Sergio R. Floeter for making his lab logistics available. We also thank the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) for licensing the study (authorization number 40311-2). This study was carried out in association with the Projeto Hippocampus, a non-for-profit organization supported by Petrobras Ambiental.

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Submitted 26 May, 2017

Accepted 26 November, 2017 by Franco Texeira de Mello

