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Short Communication

Where are the juveniles of the gray sea cucumber *Holothuria* (*Halodeima*) *grisea*?

Rosana Aquino-Souza[1] & **José Gerardo Ferreira Gomes-Filho**[1]
[1]Universidade Federal do Delta do Parnaíba, Parnaíba, Piauí, Brazil
Corresponding author: José Gerardo Ferreira Gomes-Filho (gerardogomes@ufpi.edu.br)

ABSTRACT. The sea cucumber *Holothuria* (*Halodeima*) *grisea* Selenka, 1867 is distributed from Florida (USA) to southern Brazil. Juveniles of this species are very hardly found in the wild. *H.* (*H.*) *grisea* is very common on sandstone reefs in some localities from the northern coast of Brazil. Because seagrass beds are a conspicuous feature in some of these localities, the authors have speculated that juveniles live in these habitats. Following this species' reproduction period, seagrass beds adjacent to sandstone reefs were inspected in search of juveniles from December to May. Sea cucumbers were found in one of them. Individuals were collected from two areas within the seagrass bed and two areas within neighboring rocky shores, and their contracted body lengths were measured. Mean sizes were significantly smaller in the seagrass areas than in reef areas (*post-hoc* Tuckey test, $P < 0.05$). Within the seagrass bed, more than 70% of individuals were smaller than 7.5 cm, whereas 84.9 and 93.3% in the two reef areas were larger than 7.5 cm -the first report of an *H.* (*H.*) *grisea* population dominated by juveniles. Therefore, seagrass meadows should be looked at as possible sources of juveniles for adult populations of *H.* (*H.*) *grisea* in the tropical western Atlantic. Also, given the rarity of juvenile sightings for this species anywhere within its distribution range, the results are very meaningful for future research on its ecology and local and regional fishery management.

Keywords: *Holothuria* (*Halodeima*) *grisea*; Aspidochirotida; reproduction period; size distribution; size segregation; habitat preference

Sea cucumbers (Echinodermata: Holothuroidea) are important food sources in the Asian market. Many populations worldwide have been depleted because of over-exploitation to supply these markets (Anderson et al. 2011, Purcell et al. 2013). *Holothuria* (*Halodeima*) *grisea* Selenka, 1867 is distributed from Florida (USA) to southern Brazil and belongs to the family Holothuriidae (Tommasi 1969, Hendler et al. 1995, Mendes et al. 2006, Gondim et al. 2013). Many commercially exploited holothurian species belong to this family (Purcell et al. 2012). Therefore, *H.* (*H.*) *grisea* is a good substitute for over-exploited sea cucumber species in the global market. Its exploitation in some localities on the northern coast of Brazil has been documented (Souza-Júnior et al. 2017, Ponte & Feitosa 2019), and the population of at least one fishing site has been depleted (Ponte & Feitosa 2019). Its exploitation in Colombia is also mentioned by Pérez et al. (2018).

Despite its economic importance and conspicuous presence in benthic communities, many aspects of sea cucumber biology and ecology have yet to be well studied because their settlement and nursery sites are largely unknown to biologists. Juveniles of most sea cucumber species and populations have never been observed in great numbers in the wild, as highlighted by Shiell (2004). From a worldwide compilation of anecdotal observations of juvenile sea cucumbers, Shiell (2004) drew attention to the fact that most juvenile observations were made in the same habitat as the adults. The author stressed that one of the reasons for the rarity of juvenile observations in the wild could

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be their cryptic behavior living alongside adults. Cryptic behavior of juveniles found naturally in the adult site has been demonstrated for at least one species, *Parastichopus californicus* (Cameron & Fankboner 1989). Additionally, there is direct evidence of natural larval settlement amongst adults in *P. californicus* (Cameron & Fankboner 1989), *Psolus fabricii* (Jennings & Hunt 2010), and on top of adults in *Holothuria floridana* (Rogers et al. 2021). Shiell (2004, 2005) mentions a conflict between his compiled data (showing the presence of adults during juvenile sightings) and previous evidence of size-related distribution for some species.

More recent sightings of sea cucumber juveniles in the wild have followed the same pattern observed by Shiel (2004, 2005), with observations restricted to one or very few individuals (Bourjon & Morcel 2016, Bourjon & Desvignes 2018, Desbiens & Wolfe 2020). Exceptions to this are very rare, as the discovery of higher numbers of juveniles of *Holothuria leucospilota* (Setyastuti et al. 2018) and *Stichopus* cf. *vastus* (Kinch 2012, Tanita et al. 2021). Available data is increasing, which will help detect general patterns about stage-specific habitat use in sea cucumbers. There is growing evidence suggesting that settlement outside adult sites or habitats is a very common feature of many sea cucumber's life cycles (Bulteell et al. 1992, Medeiros-Bergen & Miles 1997, Reichenbach 1999, Mercier et al. 2000a,b, Eriksson et al. 2012, Gadhavi et al. 2014, Palazzo et al. 2016, Palomar-Abesamis et al. 2017, Wolfe & Byrne 2017, Rogers et al. 2018, Setyastuti et al. 2018, Tanita et al. 2021). Most juvenile observations compiled by Shiell (2004) likely occurred in the adult habitat because this is where researchers normally carry on their work. This possibility points out the importance of recording observations of juvenile sea cucumbers outside known adult areas and taking advantage of such events to test size-related distribution hypotheses using natural experiments.

To our knowledge, no published data have reported a settlement site or a juvenile habitat of *H.* (*H.*) *grisea* in the wild anywhere within its geographic range, despite its large geographic distribution. Reichenbach (1999) found seagrass beds as a nursery habitat for the congeneric species *H. fuscogilva*. Mercier et al. (2000a, b) found a strong preference for *Holothuria scabra* to settle on seagrass leaves in Solomon Islands. These findings led us to search for earlier stages of *H.* (*H.*) *grisea* within *Halodule wrightii* Ascherson, 1868 beds, in an environmentally protected area in northern Brazil. This seagrass forms extensive meadows in this region. These meadows are located adjacent to sandstone reefs. The aims of this paper are: a) to report the finding of a population of *H.* (*H.*) *grisea* in a seagrass bed in northern Brazil, b) to analyze the mean size and size frequency distribution of sea cucumbers collected from the seagrass bed and adjacent reefs, and c) to discuss whether this seagrass bed is a juvenile site for this species.

The study area is in a tropical region near the Equator parallel, on the northern coast of Brazil, with no major variations in air temperature throughout the year (22 to 33ºC). The weather in the region is semi-arid, with a marked rainy season from January to June. The mean annual seawater surface temperature is 27.9ºC. Seagrass meadows explored in this study were situated between two major rivers, the Parnaíba River, which forms a Delta (02º52’12”S, 41º39’15”W Igaraçu River mouth), and the Ubatuba River (02º45’06”S, 41º15’03”W Ubatuba River mouth) (Fig. 1).

The extent between these two rivers corresponds to the coastline of the State of Piauí, situated within a protected area; the Área de Proteção Ambiental Delta do Parnaíba (APA Delta do Parnaíba). This coastline stretch includes a variety of natural marine and estuarine habitats, including mangrove forests, sandy beaches, and reefs. Reefs are formed by rocky outcrops mainly composed of sandstone; seagrasses are common around these sandstone reefs. Sea cucumbers analyzed in our study were found in Barra Grande Beach (Fig. 1), where sandy bottoms surround reefs of varying sizes with or without seagrass cover. Barra Grande Beach is located near the mouth of the Cumurupim River (Fig. 1) and is a tourist site.

H. (*H.*) *grisea* is a free-spawning, gonochoric species that shows a discrete reproduction period along the Brazilian coastline (Bueno et al. 2015, Leite-Castro et al. 2016). Following the known reproduction period of *H.* (*H.*) *grisea* in the region, which is from December to May (Leite-Castro et al. 2016), we searched for sea cucumbers in seagrass beds in shallow areas of the sublittoral fringe. These searches were performed around four localities (Fig. 1), where sandstone intertidal reefs are abundant, and adult sea cucumbers were easily encountered under its boulders. Visual searches of the seafloor surface under the seagrass were performed by sweeping the full extension of beds without disturbing or digging the substratum. Searches were performed by leaning over the seagrass bed and looking for sea cucumbers under the leaves in shallow areas where seagrass beds have partially emerged and by snorkeling in deeper areas (up to 1.5 m) where seagrass beds were completely submerged. Sea cucumbers were only found in one of the four seagrass

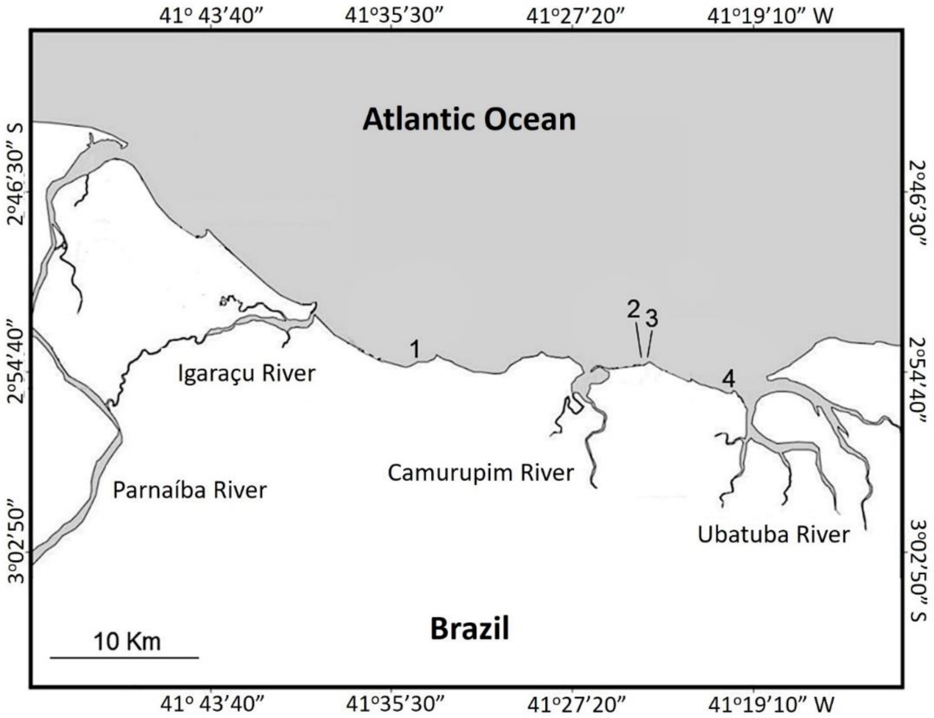

Figure 1. Map of the studied region. Numbers indicate the sites of the inspected seagrass beds. 1) Praia do Coqueiro (02º53'54"S, 41º34'17"W), 2) Barra Grande (02º53'52"S, 41º24'28"W), 3) Cemitério (02º54'12"S, 41º24'05"W), 4) Morro Branco (02º55'12"S, 41º20'30"W). Juveniles of sea cucumbers *Holothuria* (*Halodeima*) *grisea* were found only at site 2.

beds examined at Barra Grande Beach (site 2; Fig. 1). These were found on May 25, 2017, during flood tide, which precluded further sampling on that date.

A standardized sampling of the seafloor surface on seagrass and adjacent sandstone reefs was performed during the next suitable daytime spring tide, in June 2017, on the site where sea cucumbers had been found inhabiting seagrass (site 2). Three researchers recorded all the sea cucumbers within four meters wide areas parallel to the shoreline. Researchers positioned themselves parallel to each other while moving forward for 30 min, ensuring the whole seafloor surface of the sampled area was carefully inspected. The searches were time-limited to avoid the flooding tide. Two areas were sampled within the seagrass bed (named seagrass 1 and 2), and two areas were sampled in adjacent sandstone reefs (named reefs 1 and 2), as shown in Figure 2. At the end of each search, the final length of the sampled area was measured. Because the tide was very low when the search was performed, seagrass leaves were laid on the shore and had to be uplifted to check the sediment surface below. In the reef areas, sea cucumbers live under the boulders. Hence all boulders within the inspected area were turned over to search for sea cucumbers under them. Debris was removed to permit a better view, and only the superficial layer of the substrate was searched. All sea cucumbers within the sampling areas were counted in both habitats, and their contracted lengths were measured to the nearest mm. The density of individuals was estimated considering the total size of each sampled area. Data collected were used for descriptive statistics. An ANOVA ($\alpha = 0.05$) was performed to test the null hypothesis of no mean size (length) difference between individuals collected from different areas. Length data were transformed to obtain homogenous variance prior to ANOVA (Levene $P = 0.461$). A *post-hoc* Tuckey test ($\alpha = 0.05$) was performed to test pairwise differences.

From the four seagrass beds searched during our first efforts to locate individuals in vegetated midlittoral areas, *H.* (*H.*) *grisea* was found in only one of them (site 2) in Barra Grande, where further sampling was performed as described above. Between seagrass bed sampled areas, densities varied less than between sampled reef areas, and seagrass 1 had the highest density observed (3.295 ind m^{-2}). In comparison, the density of seagrass 2 was about half that value (1.604 ind m^{-2}). Reef 2 had the lowest density observed at this site, 0.147 ind m^{-2}. Reef 1, with a density of 1.919 ind m^{-2}, had a density more than 10x that of reef 2.

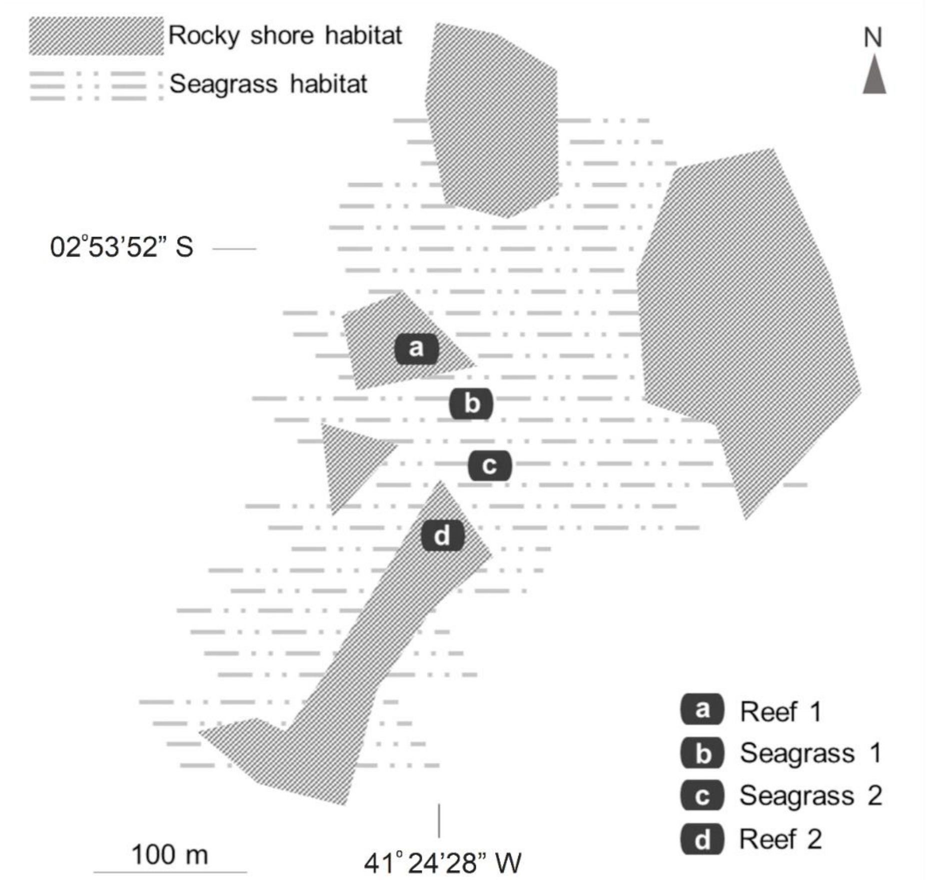

Figure 2. Schematic drawing of Barra Grande (site 2) reefs, seagrass bed, and sampled areas (reef 1 and 2, seagrass 1 and 2).

Results from one-way ANOVA test showed significant variations in mean length size between areas (Table 1). Among individuals collected from the seagrass bed areas, sizes ranged from 4.6 to 10 cm; among those collected from reefs, sizes varied from 5.0 to 14 cm in reef 1 and 6.5 to 16 cm in reef 2. The mean sizes (± standard deviation) of individuals collected from the seagrass bed areas (6.55 ± 1.12 and 6.77 ± 1.15 cm) were much lower than the mean size of individuals collected from reef areas (9.35 ± 2.05 and 12.37 ± 2.42 cm) (Fig. 3). These differences in size between seagrass areas and reef areas were statistically significant (Tuckey $P < 0.001$) (Fig. 3). Significant differences were also found between the two reef areas (Tuckey $P < 0.001$). In contrast, no significant difference in size was found between seagrass areas (Tuckey $P = 0.735$) (Fig. 3).

Length-frequency distribution was very similar among the seagrass bed areas (Fig. 4): around 60% of individuals ranged from 5.5 to 7.5 cm (62% for seagrass 1 and 60.5% for seagrass 2). Only around 25% were larger than 7.5 cm (25.9% for seagrass 1 and 27.9% for seagrass 2), and only one individual larger than 9.5 cm was found in each seagrass area. In contrast, 48.5% of individuals from reef area 1 and 93.3% of individuals from reef area 2 were larger than 9.5 cm (Fig. 4). Only one individual from the reefs was smaller than 5.5 cm. In contrast, around 12% of individuals from the seagrass (12.1% in seagrass 1 and 11.6% in seagrass 2) had body lengths within this interval (Fig. 4).

Table 1. Results of one-way ANOVA of length data (transformed to 1/x) of the sea cucumber *Holothuria* (*H.*) *grisea* on the northern Brazilian coast. SS: sum of squares, MS: mean square, DF: degrees of freedom.

Source	SS	MS	DF	F-value	*P*-value
Area	0.133	0.004435	3	67.22	< 0.001
Error	0.161	0.00066	244		
Total	0.294		247		

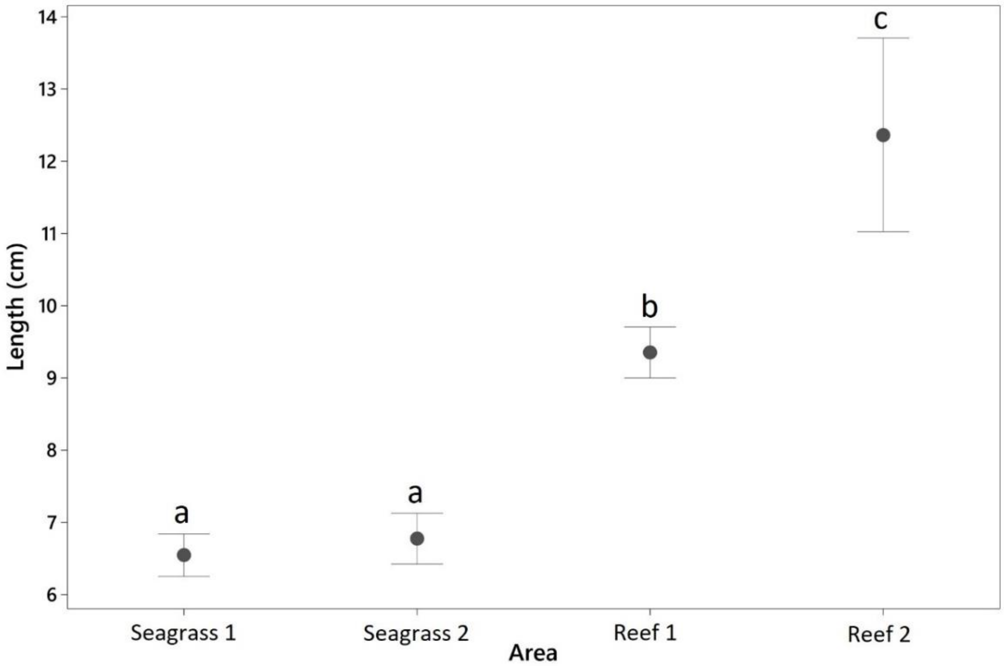

Figure 3. Mean length of *Holothuria* (*Halodeima*) *grisea* sea cucumbers at each sampled area in Barra Grande and results from *post-hoc* pairwise comparison. The same letters indicate no significant length difference (Tuckey, $P \geq 0.05$).

Our data, obtained to compare the size structure of the Barra Grande population of *H.* (*H.*) *grisea* according to habitat, clearly demonstrate that the seagrass bed hosts a younger population compared to the population from surrounding reef bottoms. They are supported both by comparison of means and by size-frequency distributions. The absence or rarity of the smallest individuals in reefs, as they are abundant and predominant in an adjacent seagrass bed on the same sampling date, is evidence of an active mechanism of age-related habitat segregation. Based on these results, we propose that sea cucumbers in this locality prefer seagrass beds while young, where they can feed and grow more rapidly and with more protection from predators. While still growing, they initiate migration to sandstone reefs, where they reach their older stages. Our findings support that this seagrass bed is a juvenile site for *H.* (*H.*) *grisea* and, therefore, is important to the sustainability of local and regional populations. Ideally, these results should be replicated on other sites, but this is the only population of this species found on seagrass meadows. None of the other three seagrass beds searched had any sea cucumber population during the same period. However, other seagrass beds can also harbor young sea cucumbers.

Nonetheless, all four reefs searched were populated by *H.* (*H.*) *grisea*; this suggests a restricted distribution of juveniles instead of adult distribution. Slater et al. (2010) reported such localized distribution of juveniles for *Australostichopus mollis*. Rogers et al. (2018) also found the smallest juveniles of *Holothuria mexicana* to be restricted to only one site from the 14 sites sampled during their study. Juveniles of *Stichopus* cf. *vastus* were only sighted so far in specific sallow habitats (0.5 to 1.0 m) where adults were absent, either on seagrass associated with sand (Kinch 2012) or under corals and rubble (Tanita et al. 2021). Finally, Setyastuti et al. (2018) found juveniles restricted to shallow areas (up to 2 m) with seagrass and *Hypnea* sp., where no adults were found. A very restricted juvenile distribution may be the case for many other sea cucumber species, which would explain why young sea cucumbers are rarely sighted. Given the rarity of juvenile sightings for this species anywhere within its distribution range, results obtained during this study are very meaningful for future research on this species' ecology and its local and regional fishery management.

Our work adds to previous reports of preference for seagrass bottoms by sea cucumber juveniles, while other habitats, where older sea cucumbers are found, are available nearby (Reichenbach 1999, Eriksson et al. 2012, Palomar-Abesamis et al. 2017). Mercier et al. (2000a) found that newly-settled juveniles of *H. scabra* were found exclusively on seagrass leaves, where larvae settle (Mercier et al. 2000b), but older juveniles preferred other habitats. More data from a greater number

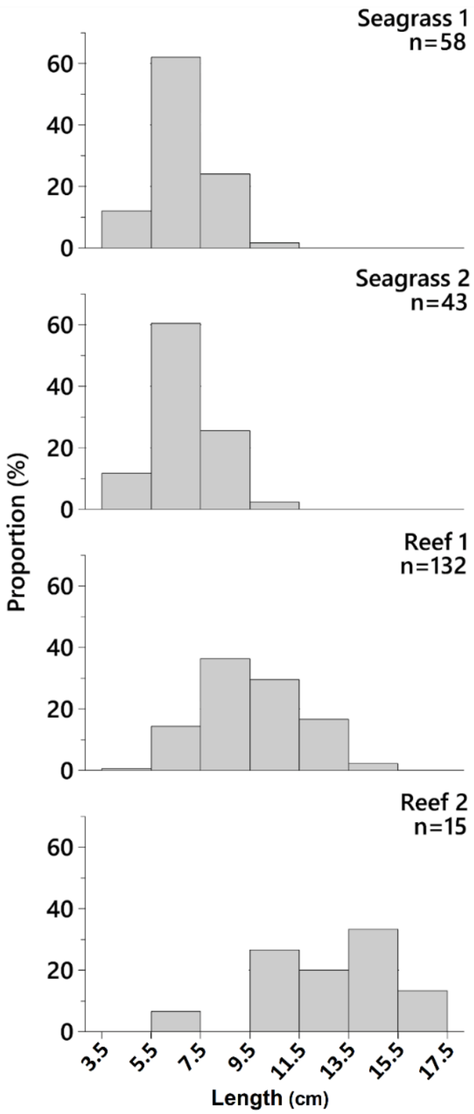

Figure 4. Length frequency distributions of *H. (H) grisea* at the sampled areas.

of species and geographic regions are necessary to elucidate patterns of age-related habitat distribution in sea cucumbers. Questions remain as to what may explain the preference of older sea cucumbers for reefs. At the same time, seagrass beds are preferred by younger individuals when both habitats are available in the same site, in *H.* (*H.*) *grisea* and a few other species of sea cucumbers. However, our study needs to clarify whether larvae of *H.* (*H.*) *grisea* settle in the seagrass bed. In the laboratory, *H.* (*H.*) *grisea* larvae grew from metamorphosed juveniles to 5 cm juveniles in around five months (Leite-Castro 2016). If the same growth rate is found in the wild, the virtual absence of juveniles smaller than 5 cm would be explained by the lack of larval recruitment for at least five months prior to sampling (June). However, studies on the gametogenic cycle of this species in northern Brazil do not support this. According to Leite-Castro et al. (2016), gamete release happens until May. Therefore, if settlement occurs on this seagrass bed, much smaller individuals should have been found.

On the other hand, this hypothesis must be considered since many other factors besides larval availability in the plankton influence recruitment success. Besides, earlier stages may have a more cryptic behavior and were not detected by our search methodology, which did not include looking for individuals within the sediment. Also, larval settlement can occur in a different habitat type or site that has yet to be reached by our study and then migrate to this seagrass bed at a size of around 5 cm or less.

Natural populations of *H.* (*H.*) *grisea* with relevant numbers of juveniles <5 cm (contracted body) are still to be found, not only in this region but anywhere within its distribution range. Only one individual below this size was found in the present research. Pérez et al. (2018) found one individual that was 2.5 cm large. However, this was the only individual smaller than 5 cm found during their year-round study, from a total of 2766. Leite-Castro et al. (2016), working with a sandstone reef population of *H.* (*H.*) *grisea* during a year of monthly samplings, do not provide length data. However, they noted the absence of small sea cucumbers in their samples and the presence of mature gametes across all sizes found. Leite-Castro et al. (2016) remarks agree with Dias (2012) study, which performed four samplings at the same site and did not find any sea cucumber <6 cm. Other studies on *H.* (*H.*) *grisea* population biology (Mendes et al. 2006, Bueno et al. 2015) either have yet to measure size or have not reported the range of lengths found. In the later study (Bueno et al. 2015), similarly to Leite-Castro et al. (2016), all individuals found had mature gametes.

Our data reveal a seagrass bed that harbors the youngest population of *H.* (*H.*) *grisea* ever reported and younger than the rocky shores that surround it, having more than 70% of the population <7.5 cm. In all populations described earlier from other geographic locations (Dias 2012, Leite-Castro et al. 2016, Pérez et al. 2018), individuals <7.5 cm represented a very small proportion. Furthermore, in at least two studies, microscopic analysis of the gonads assures that all individuals could be regarded as adults and subadults (Bueno et al. 2015, Leite-Castro et al. 2016), being the first evidence of seagrass as a habitat for this species of juveniles, as has been shown for some other sea cucumber species. *H.* (*H.*) *grisea* occurs from Florida to southern Brazil. Despite this wide distribution, a juvenile site of this species has never been reported. Further work should focus on long-term and short-term processes and surveying other seagrass beds on the northern coast of Brazil to establish whether settlement happens within seagrass beds or in a habitat distinct from the adult and the older juvenile habitats. This work and that of other authors, and the rarity of small juvenile sightings, suggest that settlement sites and nursery sites of sea cucumber species are frequently very restricted and should be identified and protected. This work stresses the importance of a particular seagrass bed as a source of juvenile for adult populations.

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