# Habitat use and nursery evaluation for the longnose stingray *Hypanus guttatus* (Bloch & Schneider, 1801) using vertebral microchemistry

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**Abstract :**

Here, we use vertebral microchemistry to investigate the habitat use patterns of the longnose stingray Hypanus guttatus in the northern and northeastern regions of Brazil, and to evaluate the existence of potential nurseries of the species. Samples were collected in Maranhão, Rio Grande do Norte, and Pernambuco states between 2008 and 2019. Trace element concentrations of Ba:Ca, Mg:Ca, Mn:Ca, and Sr.:Ca in vertebrae were determined through laser ablation inductively coupled plasma mass spectrometry. We used one-step permutational ANOVAs to evaluate multi and single-element differences in element:Ca ratios among life stages and sexes across and within sites. Element signatures differed among all sites for both single and the multi-element settings. However, there were few differences among life stages within sites, except for Mn:Ca between life stages for Maranhão states, and between sexes for Sr.:Ca for both Pernambuco and Rio Grande do Norte, and Ba:Ca for Rio Grande do Norte. Furthermore, all multi and single-element analyses across locations were significant and highlight the strong differences by sites. We also performed a non-metric multidimensional scaling analysis, which demonstrated the strong differences for samples from Rio Grande do Norte and the other two sites. By analyzing the lifetime transects of each sample, we observed that there was little variation in all element:Ca ratios within sites. Therefore, we argue that the longnose stingray likely does not display extensive habitat use shifts and fulfills much of its life cycle within each area. Finally, we obtained no evidence of nurseries for any site and suggest this is likely a pattern across the species distribution. Future studies investigating habitat use with samples from larger specimens (disc width > 90 cm, > 15 years) are needed to better understand the species habitat use patterns across all size ranges.

1 INTRODUCTION

The longnose stingray *Hypanus guttatus* (Dasyatidae) is a tropical batoid belonging to the order Myliobatiformes, which accounts for 43% of all batoids and for more than 22% of all known chondrichthyan species (Aschliman et al., 2012; Weigmann, 2016). The species is a demersal stingray that can reach up to 200 cm disc width and occurs from the Western Central to the Southwest Atlantic Oceans, as well as in the Caribbean Islands to 70 m depth (Carlson et al., 2020). In fisheries on the northern and northeastern coasts of Brazil, which lay apart about 2000 km, *H. guttatus* is the most frequently caught batoid species (Gianeti et al., 2019a; Menni and Lessa, 1998; Yokota and Lessa, 2006) and supplies a substantial portion of the growing elasmobranch meat trade in the region (Gemaque et al., 2017). Due to an exploitation shift, this species, which used to be discarded, has been increasingly exploited in recent years.

Overall, published information on *H. guttatus* is scant, but most data available come from the northern and northeastern coasts in Brazil. Females of *Hypanus guttatus* reach maturity at older ages than males – 7 and 5 years, respectively (mean DW = 51.3 cm for females and 43.5 cm for males) (Gianeti et al., 2019a). Mean size at birth is 16 cm DW for both sexes and the species displays relatively fast growth but males grow faster (k = 0.219) than females (k = 0.112) (Gianeti et al., 2019a). Furthermore, the gestation through matrotrophic histotrophy lasts around five months (Yokota and Lessa, 2006), and fecundity is low (1-5 embryos per litter) (Gianeti et al., 2019a; Melo, 2016). The species can tolerate a wide range of salinities (Grant et al., 2019; Thorson, 1983) and is exploited across large areas and diverse fisheries.

Indeed, the longnose stingray is targeted by artisanal and semi-industrial fisheries in northern and northeastern Brazil, comprising a substantial proportion of the bycatch

for the pink shrimp (*Farfantepenaeus subtilis*) (11%) and the Laulao catfish (*Brachyplatystoma vaillantii*) (36%) fisheries, respectively (Oliveira et al., 2004; Asano-Filho et al., 2004). Although fishing pressure is strong and has increased in the last decades (SEAP/MMA, 2010), the species' conservation status, which is accounted for when building national public policies, is of Least Concern (LC) in Brazil (ICMBio/MMA, 2016). In contrast, its global status by the International Union for the Conservation of Nature (IUCN) is Near Threatened (NT), but almost qualifying for Vulnerable in South America (Carlson et al., 2020). These factors highlight the need for information on its habitat use before declines are detected.

Vertebral microchemistry has been increasingly applied to study elasmobranch habitat use with promising results for management (Feitosa et al., 2020; Lewis et al., 2016; Mohan et al., 2018; Tillett et al., 2011). A useful application of this technique uses element ratios relative to Calcium (Ca) as proxies for the habitats that individuals use along their life span (McMillan et al., 2017; Pontual and Geffen, 2002). For example, higher concentrations of Barium (Ba) in vertebrae may denote the use of freshwater habitats, while higher Strontium (Sr) may indicate presence in saltwater (McMillan et al., 2017; Smith et al., 2013, 2016). In addition, Magnesium (Mg) and Manganese (Mn) have been inversely correlated with low temperatures and dissolved oxygen in the water, respectively (Limburg et al., 2015; Limburg and Casini, 2018; Smith et al., 2013). However, there are conflicting results for different elasmobranch species (Pistevos et al., 2019; Smith et al., 2013).

Nevertheless, there are still many caveats for this technique, including the lack of geographical precision on habitat use (Walther, 2019), physiological interference on element concentrations (Elsdon and Gillanders, 2006), and environmental noise, especially for estuarine species (Feitosa et al., 2020). However, it has become a highly

useful tool for investigating existence of nurseries in teleost and elasmobranch fishes (Santana et al., 2018; Tillett et al., 2011).

Therefore, we analyzed trace elements from vertebrae of *H. guttatus* with two goals: (1) to explore its habitat use patterns between life stages and sexes, and (2) to infer if each of our three studied areas along the northern and northeastern coasts represent nurseries according to the specific nursery criteria developed for batoids (Martins et al., 2018). We further compare trace element concentrations between sampling sites to check for specific signatures of estuarine and strictly marine habitats. This is the first study in the world to employ vertebral microchemistry to investigate habitat use patterns and the existence of nurseries in batoids.

## 2. MATERIALS & METHODS

### 2.1 Sampling area

Samples were collected in an extensive and heterogeneous area comprising Brazil's northern coast at Maranhão state (Brazilian Amazon Coast), and two locations in the northeastern coast (Rio Grande do Norte and Pernambuco states) (Figure 1). All areas are extremely different from each other, as the northern coast has a highly indented coastline influenced by the rivers from the Amazon biome (Lessa, 1997). In contrast, Pernambuco's coast has limited river runoff and a narrow continental platform (~30 nm), while oceanic water predominates on the Rio Grande do Norte state's coast due to the scarcity of estuaries. Nevertheless, all sampled areas share an equatorial climate with little temperature variation throughout the year (average sea surface temperature 28 ºC), and seasonal variations restricted to rainfall (Souza-Filho, 2005).

The northeastern coast has mangrove areas composing roughly 2% of the total mangrove cover in Brazil, while the northern coast comprises 77% (Diniz et al., 2019). These characteristics make the comparison between these areas with trace elements more

compelling, since concentrations are likely to differ based on abiotic characteristics, thus reducing the inherent environmental noise.

2.2 Sampling design

Vertebrae of *Hypanus guttatus* were opportunistically sampled from landings in three sites: Raposa (Maranhão state; n = 13; DW ranged from 38.4 to 76 cm) on the northern coast; Caiçara do Norte (Rio Grande do Norte state; n = 7; DW ranged from 52.4 to 80 cm), and Itamaracá (Pernambuco state; n = 14; DW ranged from 32.6 to 86.2 cm) on the northeastern coast to a total of 34 samples (Supplementary information 1). Blocks of vertebrae behind the posterior synarcual plate were removed, and either stored frozen (n = 7) or embedded in formaldehyde 4% solution for 24 hours (n = 27). Then, samples subjected to formaldehyde were kept in 70% ethanol for another 24 hours and air dried for long term storage. Although most samples were subjected to this chemical treatment, the only trace element known to be affected by formaldehyde is Mg, which is predicted to decrease slightly (Mohan et al. 2017). However, this likely had little to no effect on our results, since samples subjected to this treatment came from all areas and the Mg:Ca ratios obtained are much more distinct than they would due to the chemical process alone.

2.3 Ethics statement

All samples were collected from specimens landed and traded at local markets, and thus no animal was killed by any of the authors nor specifically for this study. Therefore, no ethics committee approval was necessary. Furthermore, sampling abided to the Brazilian environmental laws under the license 49663-1 from SISBIO/IBAMA.

2.4 Vertebrae processing

For the vertebral microchemistry analysis, we removed the connecting tissue of two vertebrae per specimen, which were air dried for 48 hours at room temperature. For processing, dried vertebrae were embedded in polyester resin and air-dried for 48 hours

at room temperature. Subsequently, vertebrae were transversely sectioned with a low speed diamond Isomet™ (Buehler) saw. Translucent and opaque ring pairs were considered to be formed annually (Gianeti et al., 2019a). The distance from each growth band to the focus was measured to obtain the exact positioning of element concentrations determined by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). This enabled us to establish the profile for each element analyzed per year of life.

Since specimens had different ages and the information on habitat use per life stage is much more meaningful than on an annual basis, we analyze the data by life stages, following the age and growth information for both sexes (Gianeti et al., 2019a). The neonate phase was considered to range from the birthmark to the end of the first year of life, followed by the juvenile phase between the start of the second year of life to the fourth ring pair in males and to the sixth in females. Adult reads were considered to begin on the fifth year in males and from the seventh year in females.

Prior to analysis by the LA-ICP-MS, vertebrae sections were polished with silicon carbide paper (no 8000), washed ultrasonically with hydrogen peroxide at 30% for 10 min, rinsed and cleaned ultrasonically again with ultrapure water for 40 min (Milli-Q, Millipore). Cleaned samples were air-dried for 48 hours and stored individually in hermetic plastic bags until laser ablation. Vertebrae were submitted to an initial ablation run, and after to the laser ablation run itself. Both runs were carried out linearly from the focus to vertebral edge along the *corpus calcareum* as shown in Figure 2.

A frequency quadrupled Q-switched pulse 213 nm Nd:YAG laser ablation system (LSX-266 Teledyne CETAC Technologies, USA) coupled to an ELAN DRC II (PerkinElmer Sciex, USA) inductively coupled plasma mass spectrometer (LA-ICP-MS) was used. The laser was operated following the same settings used by Feitosa et al. (2020) with a pulse frequency of 20 Hz, a scan speed of 30 µm s$^{-1}$, 50 µm spot diameter, and an

energy output of 0.2 to 0.3 mJ per pulse. The ablated material was conducted through a Teflon-coated tube into the ICP-MS using Argon as a carrier gas (1.20 L min$^{-1}$). The plasma was operated at 1,300 W nominal powers with outer and intermediate gas glows of 15.0 and 1.1 L min$^{-1}$, respectively. Calcium was used as an internal standard to compensate possible variations due to differences in quantity of material ablated, thus quantification of each element was performed as element:Ca ratios.

The NIST 612 (National Institute of Standards and Technology) certified reference material was employed as the external standard to obtain signal intensity (counts per second – CPS) values for each element. Limit of detection (LOD) values were calculated following Longerich et al. (1996). The external standard NIST 612 was analyzed once for each two vertebrae ablated to correct for instrument drift. Measurements were taken at the *Laboratório de Espectrometria Atômica* (Universidade Federal de Santa Maria – UFSM, Brazil) and at the Marine Biodiversity, Exploitation and Conservation Research Institute for Development (MARBEC/IRD – *Université de Montpellier*, France).

2.5 Data analyses

Elements assayed included $^{24}$Mg, $^{43}$Ca, $^{55}$Mn, $^{86}$Sr, and $^{138}$Ba. The raw data in CPS were first cleaned of outliers, defined as any value greater than three times the interquartile distance, during both the blank and the ablation windows. Outliers were checked manually and removed through interpolation with the neighboring data points. Element:Ca ratios were calculated for each element with raw CPS and transformed into concentrations in ppm following Longerich et al. (1996). We used a calcium concentration of 35% to convert element reads from CPS to ppm, which were then scaled with square root transformation to enable statistical comparisons in the same scale, since raw concentrations differed in several orders of magnitude. The average relative standard

deviation (%RSD) for the NIST 612 glass plates during data collection were as follows: $^{24}Mg = 5.63\%$; $^{55}Mn = 2.85\%$; $^{86}Sr = 5.41\%$; $^{138}Ba = 4.46\%$.

The data analyses followed Zuur et al. (2010). First, initial data exploration and check for normality distributions. Since no element had normal distributions, we used medians per life stage for each sample in the statistical tests. Second, the largest female specimen from each site was chosen as a representative to follow element:Ca ratios across lifetime. We chose this visualization because the evaluation of movements from mature females provides a specific understanding of habitat use for the most important individuals for population maintenance (Hixon et al., 2014).

Third, multi and single-element one-step PERMANOVAs were performed from a resemblance matrix calculated with Euclidean distances and 1,000 permutations to assess differences between life stages and sexes (fixed factors) for each site. Furthermore, a multi-element pairwise PERMANOVA was carried out to evaluate the degree of significant differences between study sites. This analysis is one of the most robust non-parametric statistical tests and can deal with unbalanced designs with few concerns (Anderson, 2017; Anderson & Walsh, 2013). Due to the significant results obtained between sites, we also did a pairwise PERMANOVA to evaluate differences between each pair of sites. Fourth, a non-metric dimensional scaling (NMDS) analysis was performed with Euclidian distances to visualize potential groupings according to site study. All PERMANOVAs were performed in the PRIMER6 software with an $\alpha = 0.05$, while exploratory analyses, plots, and the NMDS were made in the R software version 4.0.3 (R Core Team, 2020) with the tidyverse (Wickham et al., 2019), vegan (Oksanen et al., 2019), patchwork (Pedersen, 2020), zoo (Zeileis & Grothendieck, 2005), and magick packages (Ooms, 2021).

## 3. RESULTS

All elements remained above the LOD in all 34 samples. After calculating the element:Ca ratios, Mg:Ca was the most abundant ratio followed by Sr:Ca, Ba:Ca, and Mn:Ca, respectively (Figure 3). Element:Ca ratios were the lowest and least variable in samples from Rio Grande do Norte and the highest and more variable in Pernambuco, except for Mn:Ca, which had its greatest range in samples from Maranhão (Table 1). Among the element:Ca ratios calculated, Mg:Ca had the largest overall range, followed by Sr:Ca, Mn:Ca and Ba:Ca (Figure 3). Regarding life stages, Ba:Ca, Mg:Ca, and Sr:Ca had the greatest variations in the neonate phase for Pernambuco, while Mn:Ca varied the most for neonates from Maranhão (Table 1). This pattern remained for the juvenile and adult life stages, with samples from Pernambuco having the largest ranges for all element:Ca ratios. The only exception was Ba:Ca for adults from Maranhão, which yielded the greatest variation between locations (Figure 3).

Furthermore, our data demonstrated that the largest females from each site had contrasting habitat use patterns. Generally, the representative female from Rio Grande do Norte showed little to no variation for all element:Ca ratios during its life (Figure 4a-d). The only exception was for Ba:Ca, which showed strong fluctuations during the first years of life and a decrease at the end of the juvenile life stage, followed by stability at low concentrations (Figure 4a). Representative samples from the other sites showed contrasting patterns, with the Ba:Ca concentrations from Maranhão decreasing slowly in a consistent pattern, while the sample from Pernambuco showed an increasing trend throughout life (Figure 4a).

The sample from Pernambuco also showed a similar pattern for Sr:Ca but concentrations started to increase at the end of the juvenile phase and remained higher than before until the end of life (Figure 4b). In contrast, the sample from Maranhão showed a decreasing trend in Sr:Ca concentrations in the interphase between the juvenile

and adult stages. Regarding the Mg:Ca ratios, samples from each site had drastic differences in concentration scales, with Pernambuco having more than four times Mg than Rio Grande do Norte (Figure 4c). Nevertheless, trends were similar throughout life stages between Maranhão and Rio Grande do Norte, while the sample from Pernambuco showed an increasing trend starting from the end of the juvenile life stage and continuing until capture (Figure 4c).

Finally, Mn:Ca ratios were considerably distinct for each study site (Figure 4d). For example, the representative sample from Pernambuco showed strong fluctuations during the early juvenile phase and culminating in a steep increase at the end of the juvenile phase followed by another steep decline in early adulthood. Both samples from Maranhão and Rio Grande do Norte remained stable at low concentrations during the juvenile phase, but started to increase at the end of the juvenile phase, reaching five times more Mn:Ca when compared to the first point before the trend began. In contrast, the sample from Rio Grande do Norte showed a slight increase at the beginning of the adult phase followed by a subsequent decline to levels similar of the juvenile phase.

Likewise, most of the multi and single-element PERMANOVAs demonstrate the lack of overall differences between the biological variables studied. Significant values for the PERMANOVA between sites were obtained for multi and all single-element tests with location as a fixed factor, as well as between life stages for Ba:Ca ratios (Table 2). When PERMANOVAs were performed for within-site interactions, statistically significant differences were obtained only between life stages for Mn:Ca in Maranhão, and by sexes for Sr:Ca and Ba:Ca in Rio Grande do Norte (Table 3). In Pernambuco, a significant difference was found only by sexes for Sr:Ca. Even though there seems to be a strong difference between certain samples from Pernambuco (Supporting information 2), these might not have been significant because of the small sample size. Nevertheless,

the plots on the Supporting information 2 show an overall lack of variation between samples for all elements in each site.

Since we obtained significant values for all PERMANOVAs with sampling site as a fixed factor, we performed a pairwise PERMANOVA to assess which locations differed between one another and in what degree. All locations yielded significant results, with samples from Rio Grande do Norte being significantly different from all other places (PE x RN: t = 13.2, $p$ = 0.001; MA x RN: t = 10.82, $p$ = 0.001), and Maranhão and Pernambuco with a slightly smaller but still significant difference between them (MA x PE: t = 3.38, $p$ = 0.002). Finally, average distances between and within sites in the pairwise PERMANOVA were higher between Rio Grande do Norte and the other two sites (Distances: PE x RN = 23.8; MA x RN = 17.95; MA x PE = 9.86), and lower within Rio Grande do Norte (1.75), followed by Maranhão (7.66), and Pernambuco (9.24).

Indeed, the NMDS analysis of the multi-element signatures reinforce the small within-group differences for animals from Rio Grande do Norte when compared to those from Maranhão and Pernambuco (Figure 5). If we consider the NMDS, the PERMANOVAs, and the ranges provided in Table 1, the differences between areas are striking. Even though Maranhão and Pernambuco showed statistically significant values between each other in the pairwise PERMANOVA, most of their points were grouped together in the NMDS, irrespective of sex and life stage. Furthermore, the NMDS results reinforce the within group differences for each site obtained with the pairwise PERMANOVA, since most values from Maranhão and Pernambuco are mixed and those from Rio Grande do Norte are not. Despite the small sample size, the low stress value supports the reliability of this ordination (Clarke, 1993). Nevertheless, the lack of samples from larger specimens (DW > 90 cm) hampers conclusions about the types of habitat where those individuals are found.

## 4. DISCUSSION

Here we provide a general understanding of *Hypanus guttatus* habitat use characteristics across a major portion of its geographic distribution range in Brazil. We obtained overwhelming evidence for the uniqueness of the trace-element patterns of Rio Grande do Norte state's waters, as well as the high variation in trace-element signature profiles for Pernambuco and Maranhão states. We consider that, although these two locations are over 2,000 km apart, these strong variations are likely related to the type of habitat where specimens reside, since samples from Rio Grande do Norte came from areas predominantly influenced by saltwater and those from Maranhão and Pernambuco are from large estuaries. Furthermore, the species showed similar patterns within each area, with little variation in element:Ca ratios throughout life, which likely reflects high site fidelity and that most of the life cycle is fulfilled in the same area.

By analyzing each element:Ca ratios specifically, we obtained statistically significant results for Mg:Ca solely among locations (Table 2). When considering trends for all samples, Mg:Ca tends to remain at a somewhat constant trend throughout life irrespective of study site. Recent evidences highlight that Mg is poorly correlated with environmental concentrations and that it is significantly affected by dietary differences and growth processes in teleost fishes (Hüssy et al., 2020). Therefore, we argue that the strong discrepancies in Mg:Ca concentrations between areas might be better explained by differences in the prey types rather than habitat use, since diet compositions are distinct between Maranhão, Rio Grande do Norte, and Pernambuco (Carvalho-Neta & Almeida, 2001; Gianeti, Yokota, et al., 2019; Queiroz, 2017). Furthermore, we suggest that small increasing trends in Mg:Ca for the representative females are likely related to physiological processes involved in sexual maturity (Aoba et al., 1992; Okumura and Kitano, 1986).

In contrast, Mn is considered as a reliable element to reflect environmental concentrations, especially considering hypoxic zones and proximity to mangrove areas (Hüssy et al., 2020; Limburg et al., 2015; Limburg & Casini, 2018; Paillon et al., 2014). In our study, Mn:Ca ratios from Rio Grande do Norte were much lower than any other site, which is further supported by the species reliance on estuaries and coastal areas during its life cycle (Gianeti et al., 2019; Queiroz, 2017). Therefore, the smaller concentrations of Mn in samples from Rio Grande do Norte may be related to the lack of estuarine zones. Likewise, the larger values of Mn for Maranhão and Pernambuco states are probably related to the proximity of estuaries and mangrove forests.

On a different trend, Sr:Ca ratios were mostly constant between the different sites. However, our within-site PERMANOVAs yielded statistically significant values between sexes for both Pernambuco and Rio Grande do Norte states. Indeed, *H. guttatus* seems to display sex segregation in some areas, with sex ratios skewed towards males in Colombia (Cordovés et al., 2009), and females in northeastern Brazil and Venezuela (Silva et al., 2018; Tagliafico et al., 2013). In Maranhão state, the sex ratio is balanced (Nunes et al., 2005), which corroborates the lack of differences between sexes we found. Furthermore, despite the lack of statistical differences between life stages, fisheries catch data are consistent with ontogenetic habitat partitioning at some level, with larger individuals caught in deeper areas than neonates and juveniles (Yokota and Lessa, 2006). Therefore, we consider that habitat partitioning between life stages and sex segregation are behaviors dependent on the ecological features of each habitat, rather than an overall pattern for the species.

While Sr reflects presence of saltwater, high Ba:Ca ratios reflect proximity to freshwater runoff (McMillan et al., 2017). Indeed, our data demonstrate that Ba:Ca ratios were much higher for areas with freshwater influence, than for the samples from Rio

Grande do Norte. Nevertheless, Ba:Ca ratios did not yield significant values within sites except between sexes for Rio Grande do Norte. Indeed, females have a larger variance for Ba:Ca than males in Rio Grande do Norte for all life stages, which might either suggest stronger roaming behavior or be due to the small sample size obtained rather than actual sex-specific habitat uses. Our results also suggest that Ba:Ca was the most representative element:Ca ratio for the samples from Maranhão (Figure 5), which reinforce the use of habitats where freshwater influence is strong.

Indeed, *Hypanus guttatus* has a high capacity of sustaining salinity gradients throughout ontogenetic development (Thorson, 1983), which grants its euryhaline status (Grant et al., 2019). In fact, Thorson (1983) hypothesized that neonates and juveniles of *H. guttatus* have small tolerances to low salinities and that the capacity to become euryhaline developed throughout ontogeny. This hypothesis is based on elevated catches of females with near-term embryos in high salinity waters and the absence of neonates in low salinity environments in the Colombian and Venezuelan coasts. In Pernambuco, neonates, juveniles, and females with near-term embryos are most frequently caught in higher salinity areas, while young and adults are distributed in both habitats (Santos et al., 2016). Therefore, this catch pattern and the small ratios of Mn:Ca during the first year of life shown in Figure 4d, reinforce Thorson (1983)'s hypothesis.

Yet, individuals of *Hypanus guttatus* from Maranhão seem to follow a slightly different pattern, considering the lack of segregation between small and large specimens, and males and females (Carvalho-Neta and Almeida, 2001; Nunes et al., 2005). Typically, estuaries in Maranhão and the Brazilian Amazon coast are highly productive (Smith & Demaster, 1996), thus offering a substantial amount of prey (Carvalho-Neta & Almeida, 2001). This feature probably enables a larger carrying capacity to sustain both juvenile and adult phases in the same habitat throughout life. This hypothesis is supported by the

similarity in prey items between sexes and life stages in Maranhão (Carvalho-Neta and Almeida, 2001), which is the opposite of what Queiroz (2017) and Gianeti et al. (2019b) found for Pernambuco and Rio Grande do Norte states, respectively. Therefore, we suggest that this species likely adapts its habitat use to the specific features of each habitat along its geographical distribution.

Following Simpfendorfer & Milward (1993)'s criteria on elasmobranch nurseries, Yokota and Lessa (2006) categorized the coast of Rio Grande do Norte as a primary nursery for *H. guttatus*, where parturition occurs and the young also live. However, due to the updates on the elasmobranch nursery criteria developed by Heupel et al. (2007), there is no evidence of nurseries for *H. guttatus*. This is because the updated criteria include segregation between neonates, juveniles, and adults (Beck et al. 2001), philopatric behavior by pregnant females, higher abundances of neonates and juveniles than in other places, and that early life stages remain in a specific area for long periods (Heupel et al., 2007; Martins et al., 2018).

*Hypanus guttatus* does not seem to fit several of these criteria due to a potentially asynchronous reproductive cycle (Tagliafico et al., 2013), and the possible lack of extensive movements based on other *Hypanus* species (Schwanck et al., 2020; Tilley et al., 2013) and our habitat use data. Therefore, *H. guttatus* probably follows the habitat use patterns of coastal sharks described by Knip et al. (2010), with individuals fulfilling their whole life cycle in a relatively small area. Despite the evidence of ontogenetic habitat partitioning based on depth gradients in Caiçara do Norte (Yokota and Lessa 2006), corresponding movements were not evidenced in vertebrae profiles due to the homogeneous features of the water. These include overall high temperature (for the three sites), nearly constant salinity (Rio Grande do Norte), reduced freshwater runoff (in Pernambuco) (Ekau and Knoppers, 1999), and the low number of adults in samples

limited to shallower depths in the three sites. These limitations may have obscured trace elements profiles, as has also been the case for a small coastal shark in Maranhão state (Feitosa et al., 2020).

Overall, although *H. guttatus* is currently not considered threatened, this likely lack of dispersion may create a problem for its long-term sustainability. The recent conservation status assessment by the IUCN considers this species to be Near Threatened globally and thought to be Vulnerable in tropical South America (Carlson et al., 2020). If our hypothesis of limited movements is confirmed in the future, each population can suffer substantial crashes due to fisheries pressure. Indeed, Rodrigues-Filho et al. (2020) found evidence of a considerable population structure for *H. guttatus* based on genetic data from the Brazilian Amazon coast. Since our data point towards a potential high degree of residency for all sites, this structuring could be a pattern for the species, which needs to be confirmed.

Despite the inherent caveats of trace-element analysis for understanding the habitat use of coastal species (Elsdon et al., 2008), the results from the multiple analyses employed here are complementary and make our conclusions more robust. However, due to the lack of samples from larger specimens (DW > 90 cm), which are mostly caught in trawl fisheries operating between 10 and 150 m depth at the Brazilian Amazon coast (Jablonski et al., 2006), we cannot draw conclusions regarding movements of older individuals. Nevertheless, we hypothesize that larger individuals are likely to remain in deeper areas of coastal platforms, such as the Amazon river estuary, for long periods. Such a hypothesis is beyond the scope of this study and is to be further tested using vertebral microchemistry and acoustic telemetry or capture-recapture studies with specimens captured at those depths. This is the first study employing vertebral microchemistry to analyze habitat use and the existence of nurseries for batoids in the

world, and we encourage its application for other tropical elasmobranchs, especially batoids.

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SUPPORTING INFORMATION

Supporting information 1: List of samples with biological information of each *Hypanus guttatus* specimen for per study site.

Supporting information 2: Lifetime line plots of single element:Ca ratios for all specimens from each site. Each line represents an individual. a – Ba:Ca, b = Mg:Ca, c = Mn:Ca, d = Sr:Ca.

AUTHOR CONTRIBUTIONS

RPL developed the study design, RPL, LMF and APNQ collected and processed samples in the laboratory, and analyzed the data, and wrote the manuscript. ML and VLD carried out the LA-ICP-MS analyses. All authors revised this manuscript equally.

SIGNIFICANCE STATEMENT

This is the first study investigating habitat use of a coastal stingray in Brazil, and the first to do so with vertebrae microchemistry in the world. Our study provides key information for the management of the most widely caught coastal batoid species in northern and northeastern Brazil. *Hypanus guttatus* may become an endangered species in the short term due to high fishing pressure and bycatch rates in both areas.

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SIGNIFICANCE STATEMENT

This is the first study investigating habitat use of a coastal stingray in Brazil, and the first to do so with vertebrae microchemistry in the world. Our study provides key information for the management of the most widely caught coastal batoid species in northern and northeastern Brazil. *Hypanus guttatus* may become an endangered species in the short term due to high fishing pressure and bycatch rates in both areas.
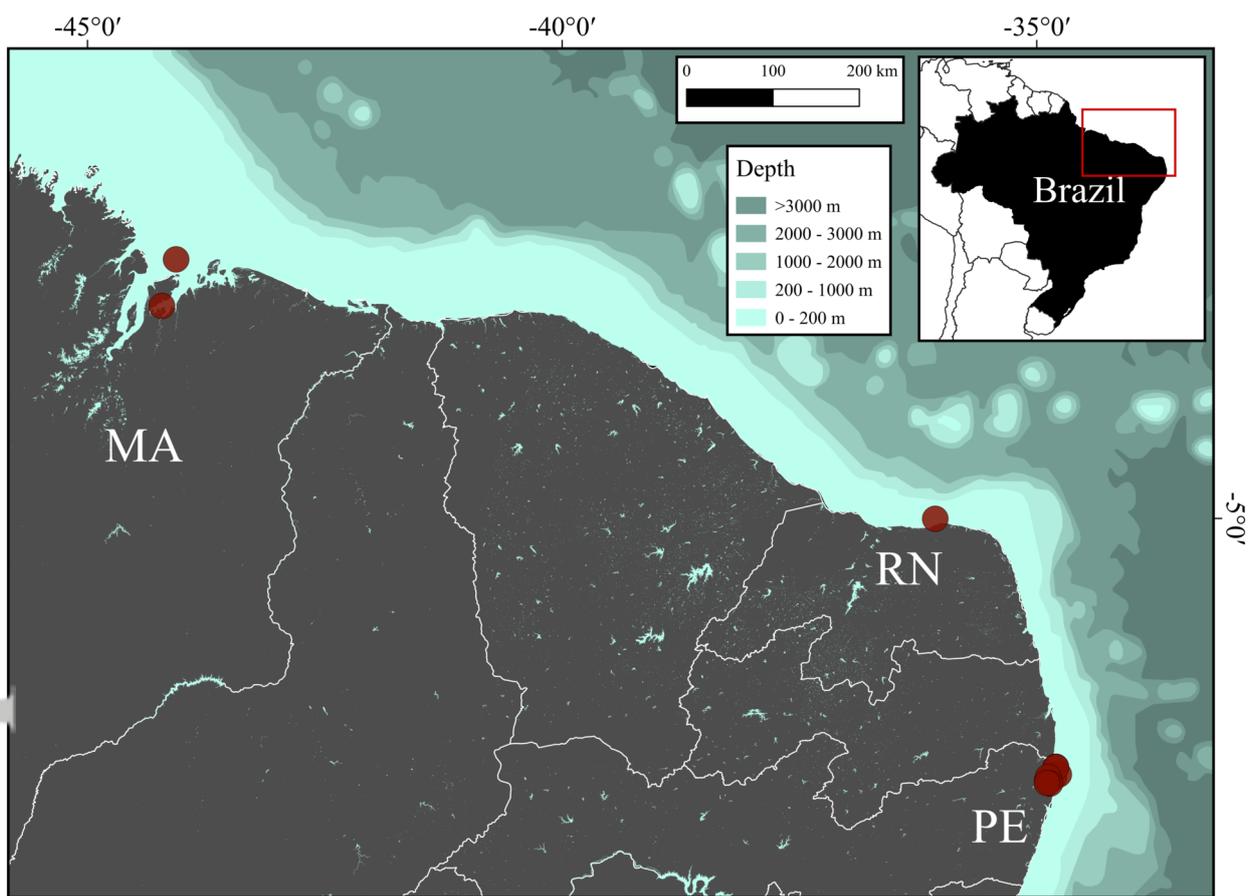
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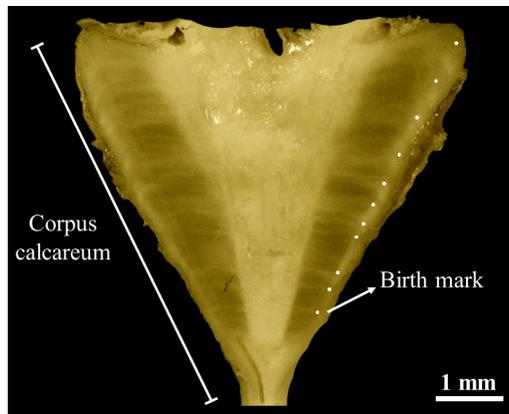
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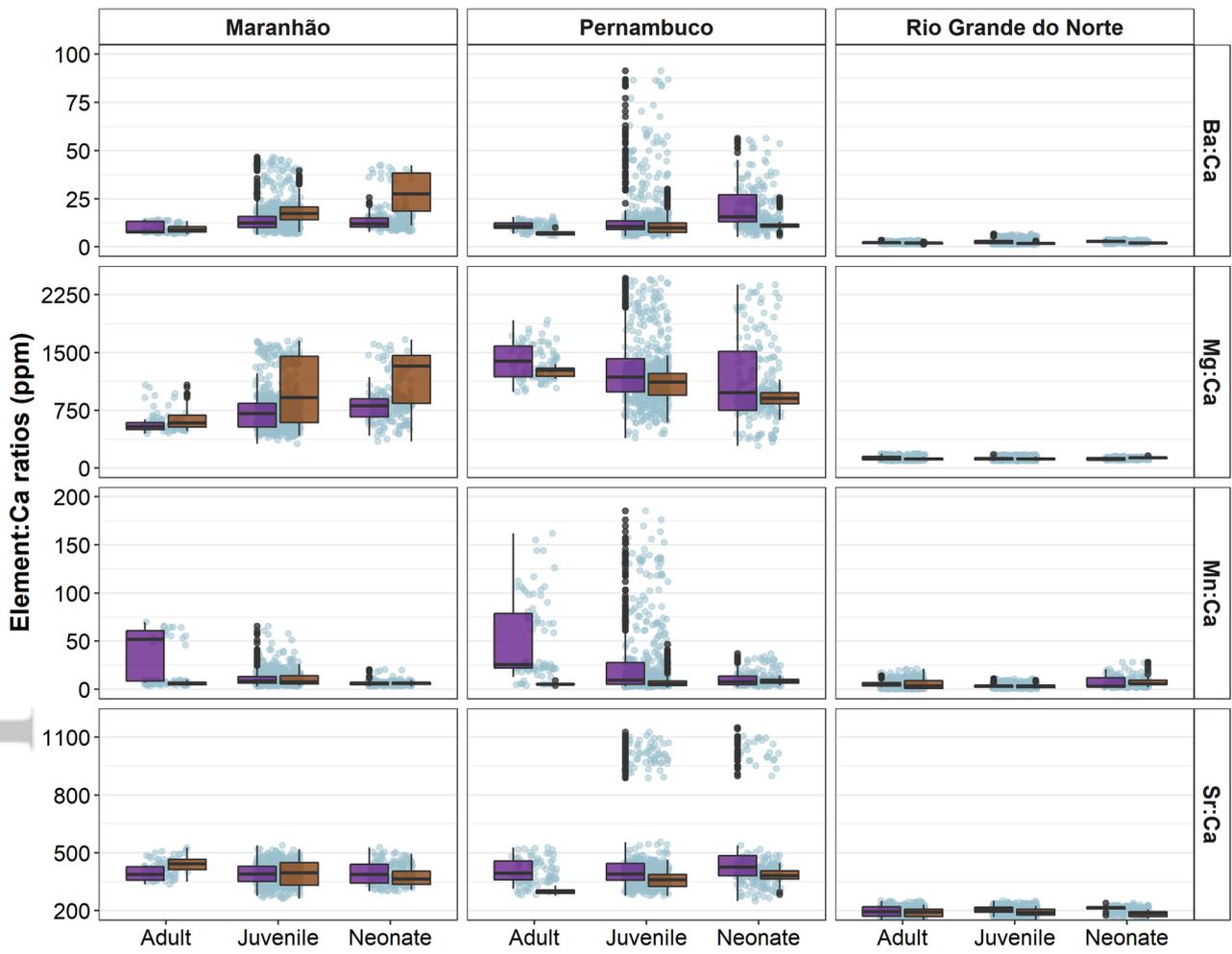
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Stress = 0.088
Non-metric fit = 0.992
Linear fit = 0.977

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Figure captions

Figure 1: Sampling locations across northern and northeastern Brazil. MA – Maranhão state, RN – Rio Grande do Norte state, and PE – Pernambuco state.

Figure 2: Vertebrae section of *Hypanus guttatus* (Sample HGPE5 – DW= 86.2 cm) with the LA-ICP-MS transect along the *corpus calcareum*. White dots represent opaque age bands. The shaded line parallel to the *corpus calcareum* represents the path of the LA-ICP-MS.
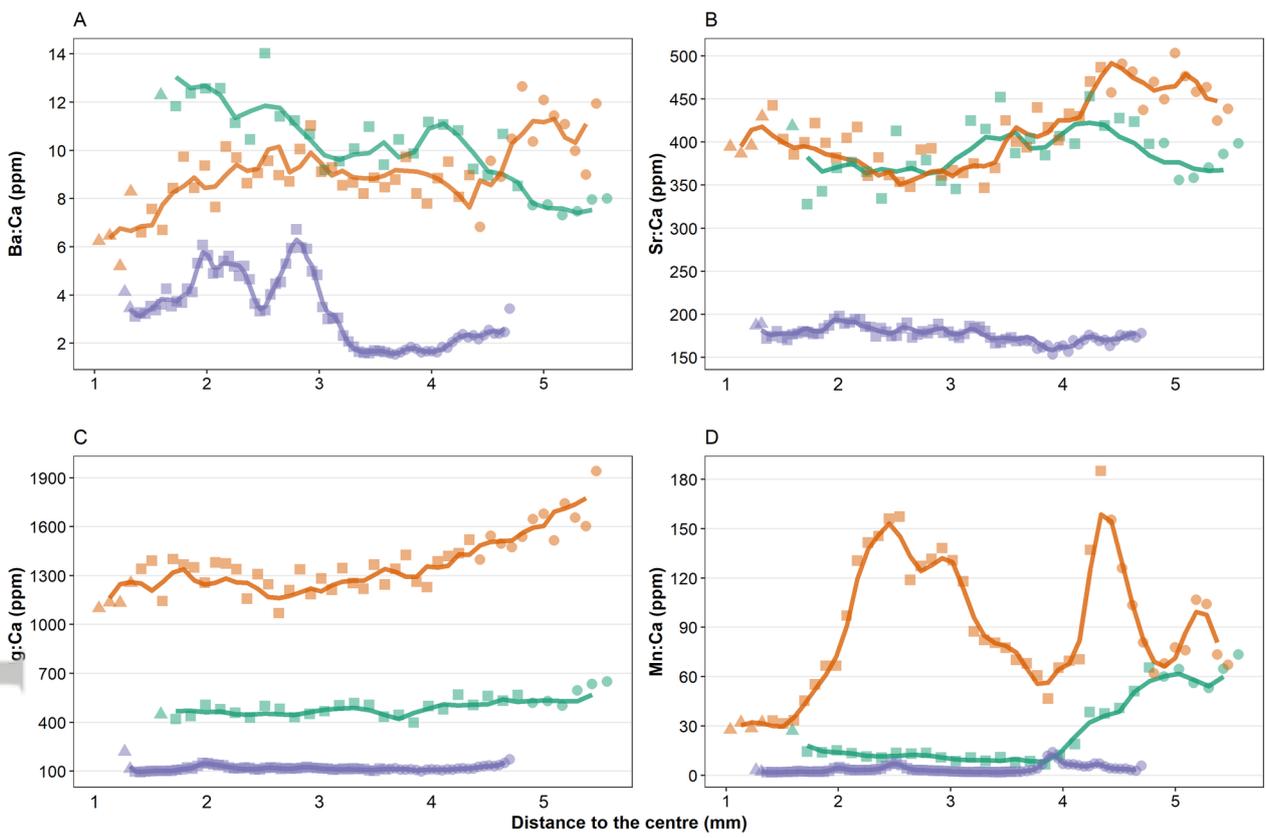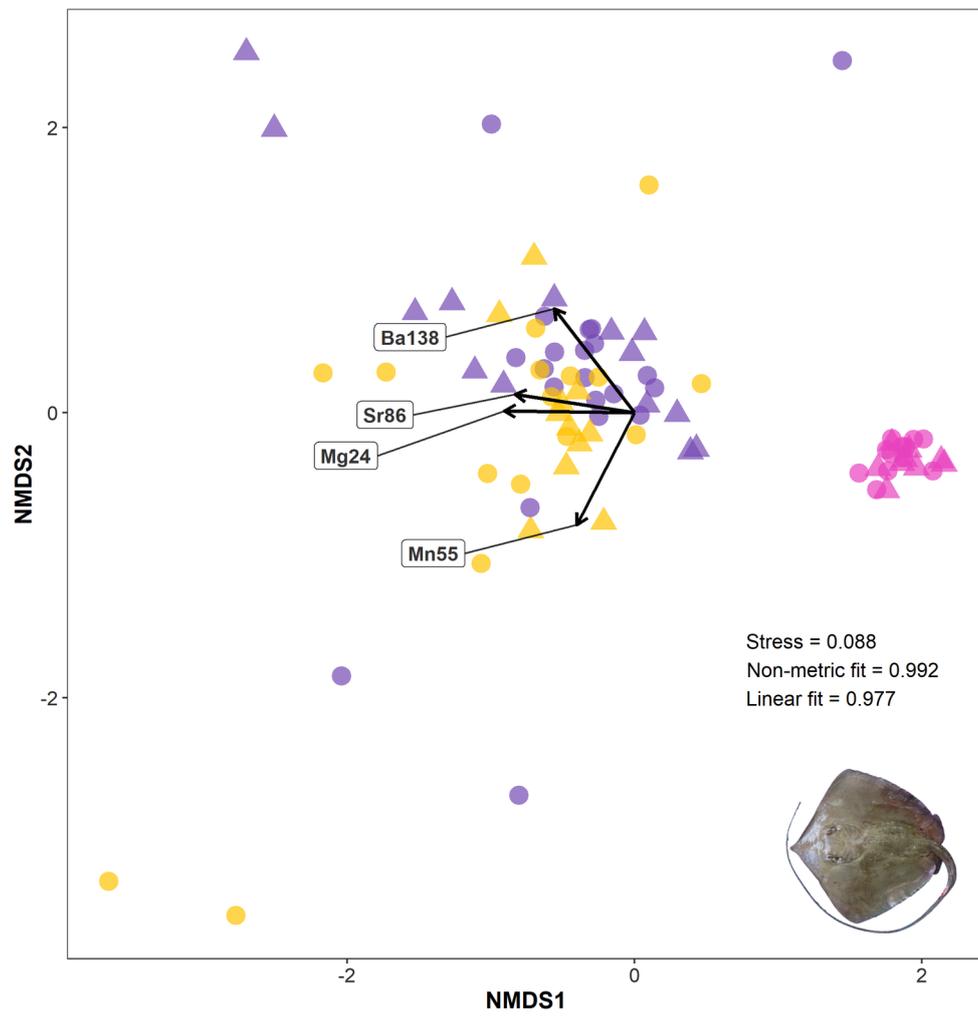
Figure 3: Exploratory analysis of element:Ca concentrations after removal of outliers for Ba, Mg, Mn, and Sr per left to right orientation of neonate, juvenile and adult life-stages and sampling site. Purple boxes represent data from females and brown boxes from males. Blue jitter are ratios from transect points for all samples.

Figure 4: Lifetime element:Ca concentrations (ppm) for the largest female from each site with seven-point rolling average (solid lines). A – Ba:Ca, B – Sr:Ca, C – Mg:Ca, D – Mn:Ca. Orange – HGPE08 (Pernambuco); Green – HGMA13 (Maranhão); Purple – HGRN233 (Rio Grande do Norte). Triangles represent element reads in the neonate portion of the vertebrae, while squares represent those at the juvenile portion, and circles are from the adult portion of the vertebrae.

Figure 5: Multi-element non-metric multidimensional scaling (NMDS) with location and sex as factors. Triangles represent males and circles represent females. Purple points depict samples from Maranhão, pink points are from Rio Grande do Norte, and yellow points are from Pernambuco. Arrows represent the participation of each element in the NMDS results.

Table 1: Ranges of element:Ca ratios (ppm) for each life stage and the three study sites.

| Life stage | Location | Ba:Ca | Mg:Ca | Mn:Ca | Sr:Ca |
|---|---|---|---|---|---|
| Neonate | Maranhão | 34.63 | 1666.13 | 37.51 | 351.21 |
| Juvenile | Maranhão | 40.84 | 1387.35 | 62.48 | 377.73 |
| Adult | Maranhão | 13.07 | 631.05 | 70.15 | 193.83 |
| Neonate | Pernambuco | 51.19 | 2094.22 | 34.82 | 899.65 |
| Juvenile | Pernambuco | 86.05 | 2145.98 | 183.07 | 865.4 |
| Adult | Pernambuco | 9.82 | 962.75 | 158.03 | 248.65 |
| Neonate | Rio Grande do Norte | 3.12 | 119.16 | 26.82 | 79.72 |
| Juvenile | Rio Grande do Norte | 5.54 | 85.31 | 10.72 | 98.66 |
| Adult | Rio Grande do Norte | 2.27 | 98.78 | 21.25 | 103.6 |

Table 2: Multi and single-element PERMANOVA with all sites gathered and sexes, life stages and locations as fixed factors. One or two asterisks (*) denote statistically significant values for *p*.

| Element | Factor | df | SS | MS | Pseudo-*F* | *p* | Var | SD |
|---|---|---|---|---|---|---|---|---|
| | Sex | 1 | 57.15 | 57.15 | 0.436 | 0.496 | -1.930 | -1.389 |
| Multi | Life stage | 2 | 401.58 | 200.79 | 1.563 | 0.196 | 2.759 | 1.661 |
| | Location | 2 | 7151.7 | 3575.9 | 81.137 | 0.001** | 129.54 | 11.381 |
| | Sex | 1 | 3.07 | 3.07 | 2.759 | 0.869 | -2.826 | -1.681 |
| Mg:Ca | Life stage | 2 | 337.99 | 169.00 | 1.557 | 0.219 | 2.305 | 1.518 |
| | Location | 2 | 6393.00 | 3196.5 | 97.22 | 0.001** | 116.03 | 10.772 |
| | Sex | 1 | 5.94 | 5.93 | 2.5094 | 0.134 | 9.321 | 0.305 |
| Mn:Ca | Life stage | 2 | 9.23 | 4.61 | 1.9602 | 0.148 | 8.622 | 0.293 |
| | Location | 2 | 27.30 | 13.65 | 6.4139 | 0.006* | 0.423 | 0.650 |
| | Sex | 1 | 46.06 | 46.06 | 2.9627 | 0.089 | 0.796 | 0.892 |
| Sr:Ca | Life stage | 2 | 41.41 | 20.71 | 1.3107 | 0.274 | 0.187 | 0.432 |
| | Location | 2 | 645.96 | 322.98 | 39.189 | 0.001** | 11.544 | 3.397 |
| Ba:Ca | Sex | 1 | 2.08 | 2.08 | 1.1312 | 0.279 | 6.314 | 7.946 |
| | Life stage | 2 | 12.94 | 6.47 | 3.7403 | 0.033* | 0.181 | 0.425 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Location | 2 | 85.49 | 42.75 | 51.901 | 0.001** | 1.537 | 1.240 | |

df, degrees of freedom; SS, sums of squares; MS, mean squares; Pseudo-*F*, test statistic; *p*, statistical significance of hypothesis tests; Var, estimated sizes of components of variation, which are analogous to ANOVA effect sizes; SD, square root of these values in Euclidian units.

Table 3: Multi and single-element PERMANOVAs per study site with sex and life stages as fixed factors. Asterisk (*) denotes statistically significant values for *p*.

| Element | Factor | df | SS | MS | Pseudo-*F* | *p* | Var | SD |
|---|---|---|---|---|---|---|---|---|
| | | | | Maranhão | | | | |
| Multi | Sex | 1 | 171.19 | 171.19 | 3.659 | 0.058 | 9.182 | 3.030 |
| | Life Stage | 2 | 62.81 | 31.41 | 0.600 | 0.594 | -2.169 | -1.473 |
| Mg:Ca | Sex | 1 | 166.44 | 166.44 | 4.030 | 0.059 | 9.237 | 3.039 |
| | Life stage | 2 | 38.03 | 19.01 | 0.401 | 0.696 | -2.938 | -1.714 |
| Mn:Ca | Sex | 1 | 1.07 | 1.07 | 0.499 | 0.525 | -7.924 | -0.281 |
| | Life stage | 2 | 17.01 | 8.50 | 5.146 | 0.010* | 0.710 | 0.843 |
| Sr:Ca | Sex | 1 | 1.81 | 1.81 | 0.671 | 0.453 | -6.529 | -0.255 |
| | Life stage | 2 | 6.25 | 3.13 | 1.189 | 0.334 | 5.167 | 0.227 |
| Ba:Ca | Sex | 1 | 1.87 | 1.87 | 2.867 | 0.091 | 9.012 | 0.301 |
| | Life stage | 2 | 1.52 | 0.76 | 1.103 | 0.358 | 7.384 | 8.593 |
| | | | | Pernambuco | | | | |
| Multi | Sex | 1 | 115.53 | 115.53 | 1.818 | 0.192 | 3.654 | 1.911 |
| | Life Stage | 2 | 76.59 | 38.29 | 0.569 | 0.630 | -3.214 | -1.793 |
| Mg:Ca | Sex | 1 | 39.22 | 39.22 | 0.938 | 0.334 | -0.181 | -0.426 |
| | Life stage | 2 | 50.35 | 25.17 | 0.587 | 0.564 | -1.961 | -1.401 |
| Mn:Ca | Sex | 1 | 5.06 | 5.06 | 1.584 | 0.229 | 0.131 | 0.368 |
| | Life stage | 2 | 12.46 | 6.23 | 2.045 | 0.142 | 0.352 | 0.594 |
| Sr:Ca | Sex | 1 | 67.18 | 67.18 | 3.885 | 0.047* | 3.515 | 1.875 |
| | Life stage | 2 | 12.04 | 6.02 | 0.303 | 0.819 | -1.534 | -1.238 |
| Ba:Ca | Sex | 1 | 4.07 | 4.07 | 2.943 | 0.093 | 0.189 | 0.435 |
| | Life stage | 2 | 1.74 | 0.87 | 0.575 | 0.583 | -7.121 | -0.267 |
| | | | | Rio Grande do Norte | | | | |
| Multi | Sex | 1 | 2.48 | 2.48 | 1.403 | 0.248 | 6.931 | 0.263 |
| | Life Stage | 2 | 1.81 | 0.902 | 0.474 | 0.827 | -0.143 | -0.378 |

| | | df | SS | MS | Pseudo-F | p | Var | SD |
|---|---|---|---|---|---|---|---|---|
| Mg:Ca | Sex | 1 | 0.107 | 0.11 | 0.137 | 0.711 | -6.574 | -0.256 |
| | Life stage | 2 | 0.56 | 0.28 | 0.348 | 0.725 | -7.474 | -0.273 |
| Mn:Ca | Sex | 1 | 3.41 | 3.41 | 7.009 | 0.784 | -4.394 | -0.209 |
| | Life stage | 2 | 1.04 | 0.51 | 1.132 | 0.347 | 8.661 | 9.306 |
| Sr:Ca | Sex | 1 | 2.06 | 2.06 | 4.446 | 0.037* | 0.155 | 0.394 |
| | Life stage | 2 | 9.63 | 4.81 | 8.053 | 0.920 | -7.856 | -0.280 |
| Ba:Ca | Sex | 1 | 0.28 | 0.28 | 8.223 | 0.009* | 2.377 | 0.154 |
| | Life stage | 2 | 0.12 | 5.75 | 1.283 | 0.319 | 1.809 | 4.254 |

df, degrees of freedom; SS, sums of squares; MS, mean squares; ; Pseudo-$F$, test statistic; $p$, statistical significance of hypothesis tests; Var, estimated sizes of components of variation, which are analogous to ANOVA effect sizes; SD, square root of these values in Euclidian units.