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Original Article

Mercury in Brown Booby (*Sula leucogaster*) Feathers from the São Pedro and São Paulo Archipelago as a Tool for Environmental Biomonitoring

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Abstract

Seabirds are important bioindicators of environmental quality as their feathers contain records of metal concentration. Among the metals, mercury (Hg) is toxic, causing damage to the organism even in small concentrations. The objective of this study is to determine and evaluate the total Hg levels in brown booby feathers in the São Pedro and São Paulo archipelago (SPSPA) (0°55'02''N; 29°20'42''W), comparing to literature levels of metal in feathers of different Sula species in several locations from Brazil and elsewhere in the world. To determine Hg levels, the study uses the methodology of partial digestion of collected feathers, followed by the analysis by inductively coupled plasma optical emission spectrometry (ICP-OES). The average value of the Hg concentration was 2.6 ± 3.5 mg kg⁻¹¹, which is below the threshold for the occurrence of adverse effects due to mercury toxicity in seabirds. For the statistical analysis of comparison between sexes of the SPSPA population, and for comparison between species of the genus Sula in different places around the world, this study uses Analysis of Variance (ANOVA), followed by the Tukey test. There was no significant difference between sexes in Hg concentration. However, there was a significant difference between the compared sites, demonstrating that the SPSPA has a low Hg concentration, differing only from Daphne Island, Galapagos a region with high Hg atmospheric deposition due to its climatic conditions. Between the species of the genus Sula, the study detected a significant difference in the accumulation of Hg. The species S. leucogaster differed from the species S. granti, which is also associated with Galapagos. The other species displayed accumulation of Hg resembling that of S. leucogaster, confirming a similarity between their nourishment habits and their trophic niches. The results support future Hg monitoring programs in this important Marine Conservation Unit and Environmental Protection Area.

Keywords: Seabirds, ICP-OES, Oceanic Island, Sula leucogaster

INTRODUCTION

Seabirds are considered important indicators of environmental quality. They are top animals in the food chain and travel great distances to obtain food; because of that, their diet integrates a large part of the food web over a wide area (Camphuysen, 1997). When they feed, the metals accumulated in the tissues of the prey are absorbed by the birds, being distributed within the organism according to their affinity with the tissues (Peterson et al., 2019). The feathers are enriched in mercury (Hg): the sulfhydryl, an active compound in their tissue, binds to the metals present in the bloodstream and, after the feather's growth ceases, the blood passage is interrupted and the feather records the concentration of the metals in the organism during this period (Burger and Gochfeld, 1992). A study by Thompson and Furness (1989) demonstrates that more than 90% of the mercury found in the feathers of several seabirds is in the form of methylmercury. Methylmercury, even at low concentrations, has a high level of toxicity, being able to block, displace or disfigure essential functional groups in biomolecules and enzymes (Toma, 2015).

The brown booby (*Sula leucogaster*) is a strictly marine bird of the Sulidae family, widely distributed in all ocean basins in tropical and subtropical regions (Nelson, 2005). It usually uses islands along the continental shelf for feeding and reproduction, being classified as a species of Least Concern in the International Union for Conservation of Nature's (IUCN) Preservation Status (IUCN, 2020). The population of brown boobies in the São Pedro and São Paulo Archipelago (SPSPA) has increased over the last decade, having evolved from 377 individuals in 2001, to 588 individuals in 2015 (Nunes *et al.*, 2018). Barbosa Filho and Vooren (2009) characterize the species as not very migratory in the region since the population has low annual variance; however, the daily variation of the brown boobies in the SPSPA is significant, seeing as they spend the night in the archipelago and go out to sea to feed during the day. Nunes et al. (2018) estimated that feeding trips last an average of 57 minutes, with an average distance of 7.1 km from the colony. Adult brown boobies cover an average total distance of 27 km per day, alternating male and female in the care of the nest. Their diet is mainly composed of teleost (99.1%), in particular, the flying fishes (90.6%) (Both and Freitas, 2001). In general, species of the genus Sula feed on flying fish and squid, which makes it possible to compare mercury concentrations between different species (Schreiber and Hensley, 1976; García and López-Victoria, 2008). Despite being an oligotrophic region, the main limitation of the species is the quality of the nesting area, with higher mortality during the egg-laying period (Barbosa Filho and Vooren, 2001). This characteristic can generate a hierarchical organization in the choice of place for nesting based on the individual's body size, which explains why the resident population of the SPSPA has larger body size compared to populations from other locations in the world (Nunes et al., 2018).

The ASPSP is the smallest and one of most isolated group of Brazilian oceanic islands located 1010 km off the northeast coast (Vaske-Jr *et al.*, 2010). Composed of 15 rocky islets, its minerals are of plutonic origin, which shows that its formation was given from the elevation of the Meso-Atlantic mountain range, unlike the other oceanic islands that in general have volcanic origin, causing the ASPSP to have a unique geological formation in the world (Campos *et al.*, 2005; Vaske-Jr *et al.*, 2010). Figure 1 illustrates the study region, highlighting Belmonte Island, where the samples were collected, where 88% of the archipelago's boobies nest (Barbosa Filho and Vooren, 2001).

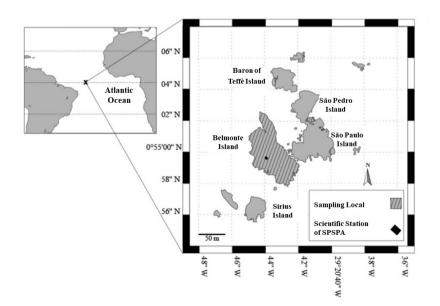


Figure 1. São Pedro São Paulo Archipelago with emphasis on the sample collection site

Globally, several studies explore the use of seabirds as indicators of metal contamination (Becker et al., 2016; Lyver et al., 2017; Tsipoura et al., 2017; Carravieri et al., 2014, 2018). In Brazil, studies on contaminants in seabirds are mostly carried out with birds accidentally caught in longline nets or found dead in the coastal region (Barbieri et al., 2007, 2010; Vega et al., 2010; Carvalho et al., 2013), being little performed in breeding colonies in archipelagos. Furthermore, studies carried out with the species S. leucogaster are scarce: Dolci et al. (2017) analyzed metals in feathers and eggshells of S. leucogaster in the National Marine Park of Currais Island (south of Brazil); Cunha et al. (2012) and Mello et al. (2012) characterized persistent organic pollutants in S. leucogaster individuals from SPSPA. Also in the SPSPA, Costa et al. (2009) and Rosa et al. (2018) analyzed mercury in muscle tissue and liver of Thunnus albacares and in feathers of S. *leucogaster*, respectively. The results of the studies converge: the first presents Hg concentrations below the limit stipulated by the National Health Surveillance Agency (ANVISA, 2013) for fish (0.5 mg kg⁻¹, in muscles). While the second presents an average concentration below the limit of occurrence of adverse effects by mercury toxicity in seabirds of 5 mg kg-11in feathers, defined by Schreiber and Burger (2002).

Thus, the present study aims to evaluate the levels of Hg in feathers of brown boobies from SPSPA, verifying whether the accumulation of Hg is influenced by the sex of the birds, in addition to performing a comparison with previous studies of *S. leucogaster* and of other species of the genus *Sula* in different locations around the world, highlighting the importance of monitoring a region that apparently does not have significant interference from anthropogenic action.

METHODS

Sampling was carried out at the SPSPA in January 2017. The individual capture of the brown booby was performed manually with the approach of a researcher from the area of reproduction of the species. All birds were handled in accordance with the World Health Organization regiment, identifying the sex and stage of maturity of the individuals. With the aid of scissors, the feathers (P1- 1st primary; S8 - 8th secondary and 4 breast feathers) of each individual were collected and then stored in plastic envelopes. All the material collected was frozen at -20°C for laboratory analysis, adding 26 samples of feathers from individuals of brown boobies, so that each one has about 4 whole feathers and 2 pieces of flight feathers. Despite the different types of feathers collected, in order to obtain a minimum mass for chemical analyses, all feathers were used without distinction for digestion.

To prepare the samples, the feathers were cleaned with 10% hydrogen peroxide (H_2O_2) and Milli-Q water, dried in an oven at 60 °C, triturated with the aid of tweezers and scissors. The samples were weighed, and transferred to teflon tubes for the mercury extraction process.

Mercury extraction followed the methodology of Trevizani et al. (2016), adapted for the low masses available (on average 0.066 g), with 1.7 mL of concentrated nitric acid (HNO₃) being added, leaving to react for 8 hours, and 0.4 mL of hydrogen peroxide (H_2O_2) 30 %, allowing it to react for 15 hours. After this period, the samples were placed in the digester block, in a closed system, for 3 hours at 90°C. After this process, at room temperature, the samples were filtered and made up to 15 mL with Milli-Q water. The reference material Fish protein CRM certified for trace metals - National Research Council Canada (DORM-2) and a reagent blank were used for traceability and control of the method.

After Hg extraction, the samples were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES). The method recovery percentage, calculated from the DORM-2 analysis, was 80% (Table 1).

Table 1. Limit of detection of the method (LDM) for Hg, value certificate of Hg in DORM-2,

value of Hg obtained in the present study, and method recovery percentage.						
LDM (mg kg ⁻¹)	0.177					
DORM-2 (mg kg ⁻¹)	4.64 ± 0.26					
Present study (mg kg ⁻¹) (n=3)	3.73 ±0.59					
Recovery (%)	80.39					

The results were evaluated through statistical analysis using Microsoft Excel and Statistica software. Mean and standard deviation of Hg concentrations were calculated. The presence of outliers was verified using the Grubbs test. Normality was tested using the Shapiro-Wilk test, with a confidence interval of 95%. The p value rejected the hypothesis of normal distribution ($p = 4.4 \times 10^{-8}$); even after performing square and log transformations, the data did not show normality. Thus, the initial data were used to perform the analysis of variance (ANOVA), following the study by Underwood (1997): ANOVA is quite robust to non-normality, particularly in the case where experiments are large and/or the case where samples are balanced, ie, they are all of the same sample number, n, like in the present study.

To verify how the accumulation of Hg occurs in the species, t-test was performed between the concentration of Hg obtained in the feathers and the factor sex. To compare the Hg levels in feathers obtained in the present study with previous studies with *S. leucogaster* and other species in different places in Brazil and the world, ANOVA and Tukey's test were also performed. Different types of feathers store different amounts of mercury due to the difference in growth time between them; they also represent different periods of the animal's life, since breast feathers are changed every

few months, thus presenting lower average concentrations of mercury as well as less variation; while the others have an annual exchange, presenting higher levels of mercury and greater variation (Furness et al., 1986). With this in mind, a t-test was carried out to verify whether the type of feather used in this and others studies would interfere in the comparisons between locations and species. Such analyses make it possible to verify the influence of location, comparing isolated regions with urbanized/industrialized regions (Burger et al., 2009), also taking into account the species and possible influences of life habits in relation to the environment.

RESULTS

The sample set has the same number of females and males (n = 13) and most are adults, with the exception of 3 samples of sub-adult males. Mercury concentrations ranged between 0.6 and 16.6 mg kg⁻¹⁻¹, with an average of 2.6 ± 3.5 mg kg⁻¹⁻¹. The results showed two outliers with values of 16.6 and 10.7 mg kg⁻¹⁻¹ and an atypical value of 5.1 mg kg⁻¹⁻¹ (Figure 2). No significant difference was identified when comparing mercury concentrations between males and females using t-test (p = 0.32; Figure 2).

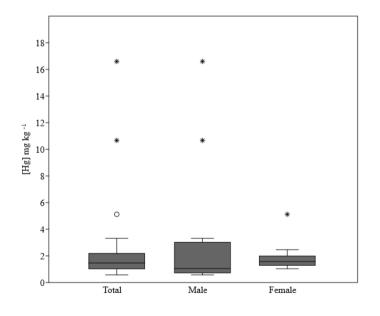


Figure 2. Box-plot of total mercury concentrations found in all individuals and male and female feathers of S. leucogaster from São Pedro São Paulo Archipelago. * Outliers ° Atypical value

In order to compare different locations to evaluate the concentration of mercury present in the brown booby population of the SPSPA, studies with mercury in feathers of *S. leucogaster* and other species of the genus *Sula* were searched in the literature (Table 2), as they have the same trophic niches and eating habits, such as obtaining food through diving and having fish and squid as their preferred prey (Schreiber and Burger, 2002; Table 2). Mercury levels in bird feathers differed significantly between different locations in Brazil and the world (p = 0.04) and the type of feathers does not significantly affect the comparison between studies (p = 0.53). It is noted that Daphne Island differs significantly from most Islands, except for the other islands of the Galapagos archipelago (North Seymour and Genovesa), the Ryukyu Islands, the Abrolhos archipelago and Midway Island, grouping the regions with the highest concentrations of

mercury (Table 3). The results were compared with studies of mercury in red-footed booby (*Sula sula*), peruvian booby (*Sula variegata*), Nazca booby (*Sula granti*), blue-footed booby (*Sula nebouxii*) and the masked booby (*Sula dactylatra*). The

Hg concentrations in the different species of the genus *Sula* also varied significantly (p = 0.008). The species *S. granti* was the only one to show a significant difference from the others, with the exception of *S. nebouxii* (Table 4).

Table 2. Mercury concentration (maximum – minimum; mean and standard deviation (SD) in mg kg⁻¹) in feathers of *S. leucogaster* and in feathers of different species of the genus *Sula* around the world, specifying the main type of feathers used in analyzes. n = sample number.

	Sampling			Majoritary Feather	Min- Max (mg	Mean (mg	SD	
Species	Year	Local	n	Туре	kg-1)	kg-1)	(mg kg ¹)	Reference
Sula leuco-								
gaster	2017	SPSPA	25	Breast	0.6 - 16.6	2.6	3.5	Current study
	2016	Ilha Bedout (Australia)	16	Breast	0.8 - 2.6	1.5	0.9	Lavers et. al (2020)
	2015	SPSPA	13	P1	-	4.2	1.4	Rosa et. al (2018)
	2015	Abrolhos (Brazil)	11	P1	-	6.4	2.9	Rosa et. al (2018)
	2015	Cagarras Islands (Brazil)	2	P1	-	1.9	0.7	Rosa et. al (2018)
	1996	Ryukyu Islands (Japan)	11	P1	0.7 - 4.3	2.9	1.0	Kim et. al (1996)
Sula dactyla-	1992	Johnston Atoll (Hawaii)	12	Breast	-	1.9	0.3	Burguer et. al (1992)
tra	2016	Bedout Island (Australia) Daphne (Galapagos Is-	20	Breast	1.6 - 2.8	3.2	0.6	Lavers et. al (2020)
Sula granti	2015	land) North Seymour (Galapa-	20	Breast	-	14.5	3.7	Zarn et. al. (2020)
Sula nebouxii	2015	gos Island) Genovesa (Galapagos	20	Breast	-	5.4	2.4	Zarn et. al. (2020)
Sula sula	2015	Island)	20	Breast	-	8.3	1.9	Zarn et. al. (2020)
	2003	Dongdao Island (China) Midway Island (North	2	Breast	0.8 - 1.2	1.6	0.6	Liu (2006) Burguer e Gochfeld
	1997	Pacific)	12	Breast	-	3.8	0.2	(2000)
	1992	Johnston Atoll (Hawaii)	12	Breast	-	3.5	0.3	Burguer et. al (1992) Ochoa-Acuna et. al
Sula variegata	1995	Pan de Azúcar (Chile)	1	Breast	-	2.5	-	(2002)
	1972	Villa El Salvador (Peru)	8	P1	-	0.8	0.5	Gochfeld (1980)

Table 3. Tukey test relating the place of collection of samples of studies taken from the bibliography and their respective average concentrations of mercury (JA = Johnston Atoll; RI = Ryukyu Island; BI = Bedout Island; SPSPA = São Pedro and São Paulo Archipelago; Ab = Abrolhos; CI = Cagarras Island; DaI = Daphne Island; NS = North Seymour; MI = Midway Island; DoI = Dongdao Island; GI = Genovesa Island; VeS = Villa El Salvador; PdA = Pan de Azúcar). Bold values = p < 0.05

	JA	RI	BI	SPSPA	Ab	СІ	DaI	NS	MI	DoI	GI	VeS	PdA
	0A	M	Ы	5151A			Dai	115		501		100	TuA
JA													
RI	1.0000												
BI	1.0000	1.0000											
SPS-	0.0000	1 0000	0.0055										
PA	0.9999	1.0000	0.9955										
Ab	0.5199	0.6788	0.4489	0.6740									
CI	0.9999	0.9997	1.0000	0.9859	0.4788								
DaI	0.0350	0.0545	0.0322	0.0409	0.1393	0.0435							
NS	0.7636	0.8893	0.6740	0.9118	0.9997	0.6788	0.1038						
MI	0.9980	0.9999	0.9859	1.0000	0.8710	0.9702	0.0678	0.9895					
DoI	0.9980	0.9976	0.9999	0.9584	0.4291	1.0000	0.0407	0.6140	0.9366				
GI	0.2365	0.3448	0.2063	0.3077	0.9702	0.2419	0.2593	0.8106	0.4788	0.2184			
VeS	0.9374	0.9494	0.9789	0.8012	0.3208	0.9994	0.0344	0.4617	0.7892	1.0000	0.1679		
PdA	1.0000	1.0000	1.0000	0.9998	0.5932	1.0000	0.0497	0.8106	0.9976	0.9999	0.2986	0.9845	

Table 4. Tukey test relating the mean concentrations of mercury in feathers of species of the genus Sula. Bold values = p < 0.05

	Sula leucogaster	Sula dactylatra	Sula granti	Sula nebouxii	Sula sula	Sula variegata
Sula leucogaster						
Sula dactylatra	1.00					
Sula granti	0.00	0.03				
Sula nebouxii	0.89	0.97	0.09			
Sula sula	0.92	1.00	0.01	1.00		
Sula vanianata	0.95	0.99	0.01	0.69	0.69	
Sula variegata	0.95	0.99	0.01	0.69	0.69	

DISCUSSION

The Hg levels in feathers of S. leucogaster from the São Pedro São Paulo Archipelago mostly did not exceed the limit for adverse effects due to mercury toxicity in seabirds (5 mg kg⁻¹, in feathers) (Schreiber and Burger, 2002). Neves et al. (2015) described that the competition for space in the SPSPA can generate cases of cannibalism in the S. leucogaster population. That change of diet in some individuals can explain the high Hg values obtained in some individuals in the present study. Since seabirds have a higher trophic level when compared to fish and squid, which are the main prey of boobies. Comparing the present study with the previous study in the SPSPA (Rosa et al. 2018), it is seen that the previous study had higher mean mercury concentrations than the current one. The absence of a significant difference between Hg concentrations in the feathers of male and female brown boobies from SPSPA agrees with data from the previous study by Rosa et al. (2018). Robinson et al. (2012) report that the differentiation of mercury concentrations between sexes is mainly influenced by the type of prey, type of food and the region where food is obtained. The lack of differentiation between the sexes can be explained, therefore, by the equal length of the fishing journey between males and females in the S. leucogaster population of the SPSPA (Nunes et al., 2018). The equivalence of time to obtain food between the sexes can provide no food differentiation in the amount of fish caught or in the type of food, exposing both sexes to the same amount of Hg. However, this characteristic of equality between the sexes is not present in other populations of the genus Sula, such as the population of S. nebouxii in Mexico (Lerma et al., 2016).

Robinson *et al.* (2012) explain in depth the differentiation of mercury concentration by comparing blood levels between females and males, obtained in more than 50 studies with seabirds. They elucidate that many of the premises used to explain the concentration of Hg in the blood, such as the elimination of Hg through egg laying by the females, causing them to have lower concentrations of the element in the blood; or the differentiation of body size between the sexes, which relate greater weight to greater concentration; are not necessarily valid for all species of seabirds, like in the present study. The results of the present study were similar to those obtained by studies that qualified the levels of mercury in bird feathers as low, affirming the high environmental quality of the SPSPA. Among the sites of these studies are Bedout Island in Australia (Lavers *et al.*, 2020), Johnston Atoll in Hawaii (Burger *et al.*, 1992), Dongdao Island in China (Liu *et al.*, 2006) and National Pan de Azúcar of Chile (Ochoa-Acuna *et al.*, 2002), all considered natural reserves, with the exception of Johnston Atoll.

The comparison of Hg levels in feathers of birds of the genus *Sula* between different locations in Brazil and the world showed that there are differences between the locations presented in Table 1, with the SPSPA being significantly different from Daphne Island, Galapagos (Zarn *et al.*, 2020). The maximum concentrations of the element were found in the Galapagos Archipelago, which in 2015 this result is related to the effect of El Niño in the region, which favored greater deposition of atmospheric mercury in the ocean (Zarn *et al.*, 2020). Recently, studies have analyzed the influence of El Niño on the distribution and dispersion of atmospheric mercury (Gratz *et al.*, 2009; Brunke *et al.*, 2016; Slemr *et al.*, 2016), which can influence the concentration and disposition of the element in the ocean and, in turn, availability to marine biota.

The Hg levels verified in *S. leucogaster* from Abrolhos were higher than those found in the Cagarras Islands and in the SPSPA, this difference may be related to the fact that Abrolhos is closer to the mainland, compared to SPSPA (Rosa et al., 2020). On the other hand, in the Cagarras Islands, despite greater proximity to the coast, the large volume of suspended solids at the site reduces the availability of Hg to the biota, resulting in lower concentrations of Hg when compared to the other Brazilian islands in question (Rosa et al., 2020).

The higher average values of Hg in oceanic regions, when compared to regions located on the continental shelf, may be related to atmospheric deposition and oceanic circulation (USEPA, 2020). Fitzgerald *et al.* (2007) describe that the mercury cycle in coastal and oceanic environments are similar, however the first has more chemical species of the element dissolved in water. Chen *et al.*(2008) report that the main sources of mercury in the marine environment do not necessarily correspond to the collection sites of organisms with the highest concentrations of Hg, and the high spatial variation of Hg does not influence the distribution of bioaccumulation and biomagnification of the element in food webs from different places, concluding that physical and ecological processes are essential for the transport of mercury in the marine environment and for understanding biomagnification in oceanic food chains.

The mercury concentrations were different between species of the genus *Sula*, *S. granti* collection showed higher levels of Hg than the others, except for *S. nebouxii*, which can be explained by the fact that both collections took place in the Galapagos Islands. Zarn *et al.* (2020) justify that due to the effect of El Niño in the region, in addition to the greater deposition of atmospheric mercury in the ocean, changes in local primary productivity altered the strategy for obtaining food for the species *S. granti* from Daphne Island, making its diet more diverse, which explains its distinction of species. The similarity of results between *S. leucogaster*, *S. sula*, *S. variegata*, *S. nebouxii* and *S. dactylatra* demonstrates, as expected, the similarity of feeding behavior between them, since all of them prey on fish and squid through deep diving (Schreiber and Burger, 2002).

Studies on the seasonal variation of mercury concentration in seabird feathers are still poorly explored (Furness et al., 1986; Chen et al., 2008; Condon and Cristol, 2009) despite being fundamental for a better understanding of the process of bioaccumulation in the marine environment (Chen et al., 2008), also improving the prevention of fish contamination (Lerma et al., 2016). Research with stable isotopes of carbon and nitrogen is necessary to more accurately understand the process of biomagnification and bioaccumulation of mercury in the food web and marine biota, since these chemical species are excellent proxies in the investigation of seabird diets (Jiménez-Uzcátegui et al., 2019). Understanding the accumulation of Hg in different tissues and the relationship between them can elucidate the process of obtaining and eliminating the element in seabirds, facilitating, in the future, the comparison between studies with different methodologies. Therefore, future studies with Hg in seabirds from the SPSPA can be dedicated to knowing the temporal variation of Hg, taking into account seasonality and climatic events, such as El Niño, using different tissues, such as blood, feathers and eggs, which have non-invasive sampling, studying the stable isotopes of carbon and nitrogen in them.

CONCLUSION

The Hg concentrations obtained by the analysis of *S. leucogaster* feathers from the SPSPA demonstrate that the region has environmental quality and its population of brown boobies has concentrations below the limit for the occurrence of adverse effects due to mercury toxicity in seabirds. The sex of the individuals did not influence the concentration of Hg. The difference between the SPSPA brown booby and other species of the genus *Sula* in different regions was significant, with the population of *S. granti* from the Galapagos Islands

showing higher levels of Hg. The concentration of mercury in the marine environment suffers high dispersion by currents, making it a challenge to understand the patterns of bioaccumulation of the element in organisms. Monitoring the distribution and quantity of mercury in the SPSPA is extremely important to understand its role and influence on marine food webs, enabling the conservation and correct direction of management actions, prevention of mercury contamination of fish and, mainly, to ensure the environmental quality of this important Marine Conservation Unit and Environmental Protection Area, which enables the reproduction and feeding of the *S. leucogaster* species.

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AUTHOR CONTRIBUTION

B.B.: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Resources; Software; Validation; Visualization; Writing – original draft; Writing – review and editing.

T.H.T.: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Resources; Software; Supervision; Validation; Visualization; Writing – review and editing.

R.C.M.: Project Administration; Validation; Visualization.

M.V.P.: Methodology; Project administration; Validation; Visualization.

R.C.L.F.: Data curation; Investigation; Project administration; Supervision; Validation; Visualization; Writing – review and editing.

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