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Differences in mercury (THg) levels in Brown booby (*Sula leucogaster*) feathers from two environmentally distinct Brazilian archipelagos

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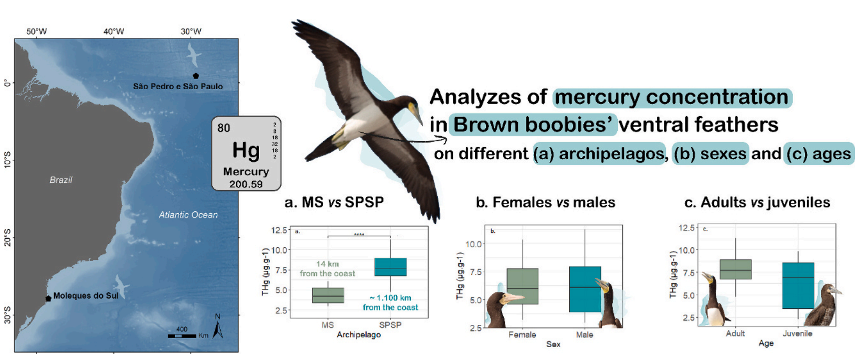
HIGHLIGHTS

- We investigated mercury levels in Brown booby feathers from two archipelagos.
- We also tested for intersexual and age-related differences in mercury levels.
- Higher mercury concentrations were found in the tropical/oceanic archipelago.
- No significant differences were found concerning sex or age.
- Differences were attributed to environmental features and local diet.

GRAPHICAL ABSTRACT



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ABSTRACT

Mercury pollution is a matter of global concern due to its detrimental effects on ecosystems and human well-being. Seabirds generally occupy high levels within trophic chains and are often used as valuable indicators of marine pollution, including mercury contamination. We examined the concentrations of total mercury (THg) in the ventral feathers of Brown boobies (*Sula leucogaster*) from two distinct Brazilian archipelagos: one coastal and subtropical (Moleques do Sul) and one tropical and oceanic (São Pedro and São Paulo). We hypothesized that there would be differentiation in mercury levels between these archipelagos due to differences in geographical location and environmental conditions, where higher Hg levels would be found in Brown boobies from the coastal one since they are more exposed to anthropogenic sources from the continent. Additionally, we also investigated whether there were any differences in mercury levels based on sex and age. We found significantly higher THg levels in Brown boobies from São Pedro and São Paulo compared to those from Moleques do Sul, indicating differential levels of exposure to mercury sources. No significant differences between sexes or age classes, although juvenile individuals showed the lowest values. We suggest that the THg higher levels found in individuals from São Pedro e São Paulo can be due to the geological peculiarities of this archipelago, formed by mantle peridotites considered mercury hotspots. Our findings emphasize the importance of considering spatial and environmental factors in mercury biomonitoring and highlight the potential use of seabird feathers as a proxy for mercury contamination in marine environments.

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1. Introduction

Mercury (Hg) is a non-essential metal naturally distributed worldwide, originating from both natural (e.g. volcanoes, geothermal) and anthropogenic (e.g. fossil fuel-fired power plants, mining) sources (Pirrone et al., 2010; Travnikov et al., 2017; Dastoor et al., 2022). Despite human activities having accelerated the redistribution of metals in the environment, especially since the Industrial Revolution, the main determinants of metal distribution over the Earth's surface are still natural geochemistry processes of the underlying and local rocks (Garrett, 2000). Globally, mercury accumulation is concentrated in areas with specific geological and tectonic features (Gustin et al., 2000). These include plate tectonic boundaries (Yin et al., 2024), recent volcanic activity (Geyman et al., 2023), precious and base metal mineralization and areas of increased heat flow in the Earth's crust (Gustin et al., 2000). Areas of arc crustal rocks and mantle peridotites are considered mercury hotspots (Canil et al., 2015; Yin et al., 2024).

Furthermore, anthropogenic sources also play an important role in the geographical distribution of mercury emissions. In the year 2000, for instance, total mercury emissions from all anthropogenic sources worldwide were approximately 2190 tons, two-thirds of which came from the burning of fossil fuels (Pacyna et al., 2006). Africa and Asia together produce more than half of global mercury emissions from anthropogenic sources (Pirrone et al., 2010). In South Africa there is intense activity of gold mining and coal combustion in power plants (Dabrowski et al., 2008; Leaner et al., 2009), while in Asian countries (e.g. China, India), other activities such as non-ferrous production, stationary combustion, electrical and electronic manufacturing industry are also expressive sources of mercury emission into the atmosphere (Streets et al., 2005; Mukherjee et al., 2009; Pirrone et al., 2010).

Due to its volatility, atmospheric circulation is the main pathway for Hg transportation, although it can also be dispersed through water, such as ocean currents and river runoffs (Travnikov et al., 2017; Dastoor et al., 2022). It is considered one of the most toxic elements to living organisms, especially when it is subjected to the methylation process by microorganisms' action and assumes its organic form methylHg (MeHg) (Clarkson, 1997; Fitzgerald et al., 2007). In food webs, Hg biomagnifies from low to high trophic levels, occurring at higher levels in predator species such as seabirds. It incorporates into food webs through particulate organic matter (POM). It accumulates in organic tissues due to its high affinity for protein, being retained in organisms' bodies rather than excreted, thus accumulating throughout the following levels of the trophic web (Bloom, 1992; Kidd et al., 2012; Calle et al., 2015; Seco et al., 2021; Matias et al., 2022). For instance, several studies have reported a positive relationship between nitrogen stable isotopes ratios – a common proxy for trophic level and trophic width niche used in ecology – and Hg concentration, indicating that species occupying higher levels in the trophic chain are likely to exhibit higher mercury levels in their tissues (Bisi et al., 2012; Di Beneditto et al., 2012; Coelho et al., 2013; Hilgendag et al., 2022), as well as a larger and more variable Hg concentrations in generalist species (wider trophic niche) than in specialist ones (narrower trophic niche) (Pinzone et al., 2019).

Seabirds, as wide-ranging predator species, are often used as monitors of the marine environment since they reflect the conditions of the previous trophic levels within the food chain (Furness and Camphuysen, 1997; Rajpar et al., 2018). They play a crucial role in regulating food webs and can also provide information on the ecosystem's quality, such as pollution levels in ecosystems (Burger and Gochfeld, 2000; Camphuysen, 2006; Carravieri et al., 2013; Cusset et al., 2023). Feathers are commonly used to sample Hg levels because they are non-invasive and useful biotracers of Hg exposure since they are one of the main excretory pathways of this non-essential element in a bird's body (Burger, 1994; Bottini et al., 2021; Bighetti et al., 2022). As they are inert tissue, they represent contamination at the time they were synthesized by the bird, during the short period of feather growth when it is connected to the bloodstream (Burger and Gochfeld, 2000). Feathers are likely to show significantly higher Hg concentrations than blood, with a narrower range of values at the interspecific scale and a higher variation at the specific scale (Bustamante et al., 2023). Molting in most seabirds occurs annually and alternates between parts of the body, so evaluating different types of feathers (i.e. ventral, dorsal, wing) provides information on the contamination levels at different periods of the bird's annual cycle (Carravieri et al., 2013; Bighetti et al., 2022).

The contamination of seabirds by Hg occurs through the ingestion of contaminated prey. It may cause damage to animal health, as it can cause neurological and endocrine disruptions, as well as negatively affect the immune system (Tartu et al., 2013; Mallory and Braune, 2018; Chastel et al., 2022; Ibañez et al., 2024). Chastel et al. (2022) proposed toxicity benchmarks for seabird feathers by converting previously established parameters for blood (Ackerman et al., 2016) to other tissues, and categorized health risk values into the following categories: no risk (<1.62 μg g^{-1}), low risk (1.62–4.53 μg g^{-1}), moderate risk (4.53–9.14 μg g^{-1}), high risk (9.14–10.99 μg g^{-1}), and severe risk (>10.9 μg g^{-1}). For comparison, the threshold exposure recommended by the World Health Organization (2007, 2008) for scalp human hair (pregnant woman) is <1 μg g^{-1}.

It has been demonstrated that Hg contamination could lead to a decline in gonadotropin-releasing hormone (GnRH) release, which is involved in gonadal maturation, sex steroid secretion, and the onset of reproduction, thus impairing seabird breeding behavior (Tartu et al., 2013; Provencher et al., 2016). Moreover, in some Antarctic seabirds, it was observed that high Hg concentrations influence parental behavior, leading to a lower commitment to incubate eggs (Tartu et al., 2015). Neurotoxic effects of Hg on seabird brain regions responsible for geomagnetic information and biomechanics control can also affect navigation, flight, and ultimately migration performance (Seewagen, 2020). Hg contamination can also cause negative effects on body condition in some bird species, such as fat stores and body mass relative to individual's size (Moura et al., 2018; Ackerman et al., 2019), which are known to be determinant biological traits influencing migration performance and breeding success for seabirds (Chastel et al., 1995; Duijns et al., 2017).

The Brown booby *Sula leucogaster* (Boddaert, 1783) is a widely distributed pantropical seabird that breeds on islands between 27°S and 25°N (Birdlife International, 2024). The species exhibit a high degree of trophic plasticity according to local prey availability (Jacoby et al., 2023), and a high degree of sexual dimorphism in body size, where females can be up to 38 % heavier than males (Nelson, 1978; Lewis et al., 2005; Nunes et al., 2017) and perform longer foraging trips (Miller et al., 2018; Correia et al., 2021). Foraging behavior consists mainly in plunge-dives, generally up to 2 m depth, feeding mostly on bony fish, and to a lesser extent on squids and crustaceans (Del Hoyo et al., 1996; Branco et al., 2005; Correia et al., 2021). In this study, we aimed to determine and compare total mercury (THg) concentration in feathers of Brown boobies from two environmentally distinct Brazilian archipelagos, as well as to test for intersexual and age-related differences. We hypothesize that (1) Brown boobies from the coastal archipelago would show higher Hg levels since they are more exposed to anthropogenic sources from the continent, (2) females would show lower Hg levels since they excrete part of the mercury burden through egg production and laying and (3) adults would show higher levels than juveniles since they accumulate Hg in their tissues for a longer time due to the bioaccumulation process.

2. Material and methods

2.1. Study area

Moleques do Sul (MS) (27°51′S, 48°25′W) is a coastal archipelago located 14 km from the South American continent. Maior Island, where samples were collected, has an area of 9.86 ha and is formed by granite rocks, overall alkaline granitoids of post-tectonic nature, and has its surface predominantly covered by bush and herbaceous vegetation (Bege and Pauli, 1989; Salvador and Fernandez, 2008; Tomazzoli and Pellerin, 2015; Corrêa, 2016). Granite is an intrusive igneous rock, formed primarily by the slow solidification of magma beneath the Earth's surface (Schmidt, 2023). Although granite itself is not a significant source of mercury, some of the minerals that may be present in it, such as quartz and certain feldspars, may contain traces of mercury, and weathering of exposed granites can release mercury contained in tiny amounts in the minerals (Gerlach, 1989; Stein et al., 1996; Zhu et al., 2001).

São Pedro e São Paulo (SPSP) (00°55′N, 29°20′W) is a pelagic archipelago located 1100 km from South America and 1824 km from Africa, has a total area of 1.3 ha and its entire surface is covered by plutonic rocks. It is the only archipelago in the Atlantic Ocean that is not formed by volcanic rocks, but by rocks originating from the Earth's mantle and the only one in the world that maintains a direct link with the Earth's mantle. Bonatti (1990) describes SPSP archipelago not as a sample of the sub-oceanic mantle but as a genuine remnant of the subcontinental mantle. This remnant was left behind and subsequently tectonically uplifted during the opening of the Equatorial Atlantic Ocean. Belmont Island, where this study was conducted, comprises Peridotite mylonites and Serpentized Peridotite mylonites (Campos et al., 2022).

Whereas MS is under the continuous influence of the Brazilian Current, seasonally affected by the upwelling of cold waters from the Malvinas Current, SPSP is inserted in the Equatorial System of Marine Currents, under the direct influence of the South-Equatorial Current, which flows superficially in the EW direction, and the submerged Equatorial Current, which flows in the opposite direction (WE) at a depth between 60 and 100 m (Castro and Miranda, 1998; Costa Campos et al., 2005; Knoppers et al., 2009).

2.2. Sampling

We sampled ventral feathers from a total of 58 Brown boobies: 21 in MS, in September 2022, and 37 in SPSP, in June 2022. From MS, all sampled individuals were breeding adults during chick rearing (11 females and 10 males). From SPSP, 21 individuals (10 females, 11 males) were breeding adults during chick rearing, and 16 were juveniles. Birds were captured directly from the nest using a hand net and 8 ventral feathers were sampled manually from each individual and then stored in plastic bags until chemical analysis. We chose to sample ventral feathers following Bighetti et al. (2022), which demonstrated that ventral feathers are the most appropriate for this analysis because they show the highest Hg concentration and do not impair the seabird's flight. Bird sampling and manipulation were approved by a federal license SISBIO 73286–4, state license IMA 31562–2022 and by the Ethics Committee on Animal Use CEUA 14.2019.

2.3. Sample preparation

Feathers were initially subjected to a washing process being rinsed with abundant distilled water inside a glass Petri dish to remove any remaining external residue from the sampling site. After washing, feathers were dried in a laboratory oven at 25 °C overnight and then stored in ziploc plastic bags at room temperature, protected from light until analyses for mercury determination.

2.4. Analytical techniques for quantifying trace elements

Analyses for mercury determination were performed in the Research Reactor Center (CERPq) at IPEN/CNEN (Instituto de Pesquisas Energéticas e Nucleares). Due to their small size, feathers were not cut before analyses. We weighed approximately 0.05 g aliquots of ventral feathers and 0.10 g aliquots of the certified reference materials IAEA 085 (Human Hair), IPEN TM-1 (Mussel tissue), and IPEN CO-1 (Fish tissue) in perfluoroalkoxy (PFA) digestion vessels, using an analytical balance with 0.0001-g resolution. Then, we added 10 mL of concentrated P. A. nitric acid (HNO_3) and kept it at rest for approximately 7 h at room temperature in a laboratory hood. We also prepared a regent blank, consisting of 10 mL HNO_3, for every digestion batch. The samples were taken to the microwave oven (MARS-6/CEM) and digested using the equipment pre-established Animal Tissue method. The method uses a technology to control temperature and pressure inside the digestion vessels, adjusting the power output from 900 to 1050 W in order to keep samples at 200 °C for 15 min. After complete digestion and cooling of the digestion vessels at room temperature, the samples were increased to 25 mL with Milli-Q water, in order to have a less concentrated acid solution in a known volume. To quantify Hg in each sample, we used a 2-mL aliquot of the digested solution diluted to 10 mL with Milli-Q water. This procedure is necessary to favor the redox reaction that occurs in the spectrometer. Then we quantified THg by Cold Vapor Generation Atomic Absorption Spectrometry (CV AAS) in the Perkin-Elmer FIMS spectrometer (*Flow Injection Hg System*) using a 3 % (m/v) $SnCl_2$ solution as the Hg^{2+} reducing agent. We used a calibration curve prepared using five Hg solutions (Perkin Elmer) from 0.5 to 5.0 ng mL^{-1} to calculate sample concentrations. Accuracy was checked by assessing the following certified reference materials (CRM): IAEA 085 (Human Hair), (23.6 ± 3.5 μg g^{-1}); IPEN CO-1 (Fish tissue), (1.02 ± 0.06 μg g^{-1}); and IPEN TM-1 (Mussel tissue), (0.14 ± 0.03 μg g^{-1}). These results are mean values and standard deviations of four determinations. Recovery values were 115 %, 85 % and 116 %, respectively.

2.5. Statistical analyses

We tested mean values of THg concentrations for significant differences according to archipelago, sex, and age. To assess differences between archipelagos, we used only adult individuals to avoid age bias. For evaluating intersexual differences, we used all adult individuals from both archipelagos who were visually sexed during sampling. We tested for intersexual differences separately for each archipelago, as well as for all individuals from both archipelagos together. To assess differences between adults and juveniles, we only used individuals from SPSP to avoid biased results from asymmetric age ratios. Prior to analysis, we tested data for normality and homogeneity of variances using Shapiro-Wilk and F tests, respectively. Then, we ran Student's *t*-test and Welch's t-test to evaluate significant differences between groups. All statistical procedures were performed in R 4.3.0 using the packages "dplyr" and "rstatix", and graphics were produced through the packages "ggpubr" and "ggplot2" (R Core Team, 2021).

3. Results

Mercury (THg) concentration in Brown booby feathers ranged between 2.23 and 11.28 μg g^{-1}, with a mean of 6.29 ± 2.31 μg g^{-1}. The THg concentration means observed for each archipelago, sex, and age can be found in Table 1. We found significant differences between adult individuals from the two archipelagos, where Brown boobies from SPSP showed higher THg levels ($t = -8.06$, df = 42, $p < 0.05$). Brown boobies from SPSP showed a mean of 7.18 ± 2.18 μg g^{-1}, while those from MS showed a mean of 4.33 ± 1.07 μg g^{-1} (Fig. 1). However, we found no significant differences between males and females nor for individuals from both archipelagos together ($t = 0.40$, df = 42, $p > 0.05$), nor separately for individuals from SPSP ($t = 0.53$, df = 21, $p > 0.05$) and from MS ($t = 1.96$, df = 17, $p > 0.05$). We also found no significant differences between adults and juveniles from SPSP ($t = 1.97$, df = 22, $p > 0.05$).

4. Discussion

The Hg levels in Brown booby feathers found in this study are the highest concentrations among studies carried out on Brazilian islands to

Table 1
Total mercury (THg) concentration in ventral feathers of Brown boobies (*Sula leucogaster*) from São Pedro e São Paulo ($n = 37$) and Moleques do Sul ($n = 21$) archipelagos in Brazil, sampled in June and September 2022, respectively. Values are expressed as mean ± standard deviation (μg g^{-1}).

	Adults ($n = 42$)			Juveniles ($n = 16$)
	Males	Females	Both sexes	
Moleques do Sul	3.86 ± 0.96	4.72 ± 1.00	4.33 ± 1.05	–
São Pedro e São Paulo	7.60 ± 1.64	7.97 ± 1.71	7.76 ± 1.64	7.11 ± 2.39
Both archipelagos	6.05 ± 2.20	6.20 ± 2.22	6.20 ± 2.22	–

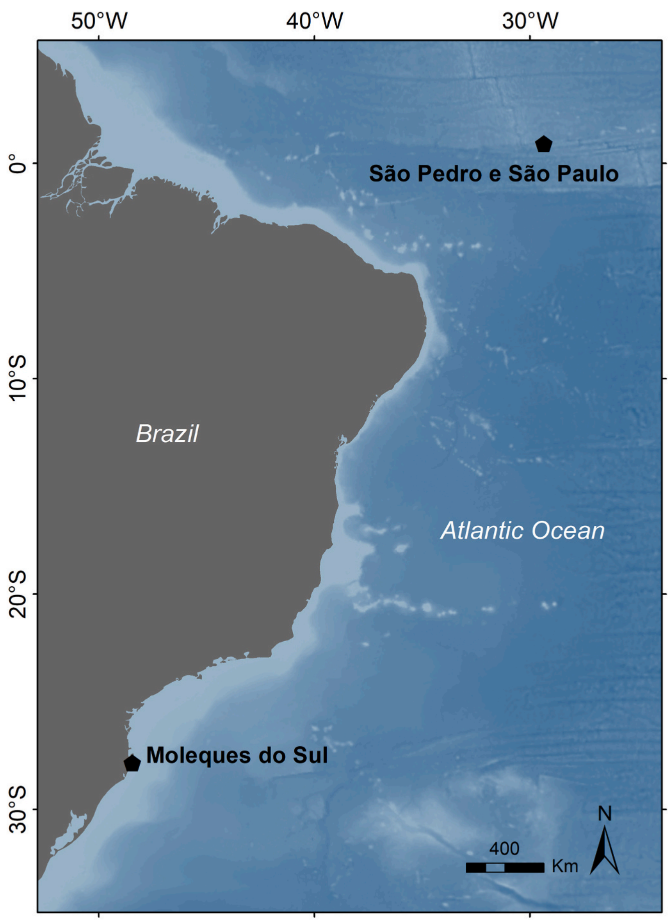

Fig. 1. Location of Moleques do Sul/BR and São Pedro e São Paulo/BR archipelagos, where Brown booby (*Sula leucogaster*) feathers were sampled in September 2022 and June 2022, respectively. The blue shade represents bathymetry (lighter = shallower, darker = deeper).

date (Table 2). We attribute, in general, such high levels to (1) the type of feather analyzed and (2) the geographic location of SPSP compared to other Brazilian islands, and (3) the geological formation of the archipelagos.

4.1. Differences between archipelagos

We found a significant difference in THg concentration between adult Brown boobies from MS and SPSP, where birds from the latter showed the highest values. Such results may be explained by two main factors: (1) distinct environmental features (e.g. oceanographic, atmospheric) from these two locations and (2) differences in Brown booby diet between these two archipelagos.

Table 2
THg mercury concentrations found in Brown booby feathers from Brazilian islands. Values are expressed as mean ± standard deviation (when available) and ordered by latitude (from South to North).

Author (year)	Archipelago	Coordinates	Sampling year	Feather	Hg (μg. g^{-1})
This study	Moleques do Sul	27°51′S, 48°25′W	2022	Ventral	4.33 ± 1.07
Bighetti et al., 2022	Santana	22°24′S, 41°42′W	2019	Ventral	6.46 ± 1.19
Bighetti et al., 2022	Santana	22°24′S, 41°42′W	2019	Wing S1	5.85 ± 1.10
Bighetti et al., 2022	Santana	22°24′S, 41°42′W	2019	Tail R6	2.94 ± 0.45
Bighetti et al., 2022	Santana	22°24′S, 41°42′W	2019	Dorsal	4.52 ± 1.33
Bighetti et al., 2021	Santana	22°24′S, 41°42′W	2018	S1, contour	2.68 ± 0.78
Nunes et al., 2022	Abrolhos	17°59′S, 38°42′W	2011	Contour	2.34 ±?
Nunes et al., 2022	Abrolhos	17°59′S, 38°42′W	2019	Contour	0.03 ±?
Bauer et al., 2024	Abrolhos	17°59′S, 38°42′W	2019–2022	Contour	0.94 ± 1.31
Benedetti et al., 2022	São Pedro e São Paulo	00°55′N, 29°20′W	2017	P1, S8, breast	2.60 ± 3.50
This study	São Pedro e São Paulo	00°55′N, 29°20′W	2022	Ventral	7.77 ± 2.18

4.1.1. Environmental features

São Pedro and São Paulo Archipelago and the Moleques do Sul Archipelago are located in different hemispheres (Northern and Southern, respectively) and are consequently inserted in different oceanographic/atmospheric systems that can reflect on the transport and availability of mercury. SPSP is a tropical pelagic archipelago located near the equator in the North Atlantic Ocean (00°55′N, 29°20′W), which is under the direct influence of the South-Equatorial Current in its surface waters and the Equatorial Current at depths between 60 and 100 m (Castro and Miranda, 1998; Costa Campos et al., 2005; Knoppers et al., 2009). Lamborg et al. (2014) show evidence that North Atlantic waters are more enriched in Hg than South Atlantic waters at the thermocline zone (100–1000 m depth), and anomalously enriched relative to deep waters of the South Atlantic, Southern, and Pacific oceans, probably due to anthropogenic mercury inputs. Furthermore, some studies suggest that oceanic regions can show higher levels of Hg when compared to coastal regions due to atmospheric deposition and oceanic circulation, and although the mercury cycle is similar in coastal and oceanic environments, there are more chemical species of mercury dissolved in oceanic waters (Fitzgerald et al., 2007; USEPA 2020).

Atmospheric deposition is the main pathway of mercury into the ocean, so knowledge of atmospheric circulation patterns is crucial to better understanding mercury dispersion across oceanic regions (Dastoor and Larocque, 2004; Lamborg et al., 2014; Soerensen et al., 2014). Soerensen et al. (2014) found higher mean Hg^0 (an elemental form of mercury) concentration in the northern hemisphere in both atmospheric and oceanic environments, with a pronounced peak at lower latitudes (near the Equator), a pattern consistent with other studies reporting enrichment of atmospheric mercury in the northern hemisphere deriving from anthropogenic sources (Fitzgerald, 1986; Slemr, 1996; Kuss et al., 2011). The SPSP is under the influence of the Intertropical Convergence Zone (ITCZ), where northeast and southeast trade winds converge (Soares et al., 2009). Both northeast and southeast trade winds come from zones of high anthropogenic mercury emission rates (e.g. South Africa, China, and India) deriving from activities such as coal and oil combustion, artisanal gold mining, non-ferrous metal production, and cement production (Streets et al., 2005; Dabrowski et al., 2008; Leaner et al., 2009; Mukherjee et al., 2009; Pirrone et al., 2010).

Another issue that must be considered to explain the high Hg concentration found in Brown booby feathers from SPSP is the geological composition of this archipelago, which may represent a natural source of mercury. The SPSP archipelago is formed by mantle peridotite rocks originating from the subcrustal oceanic mantle, where the oceanic mantle is exposed directly on the surface without any coverage by the oceanic crust of basaltic composition (Motoki et al., 2009). The mantle is considered the largest Hg reservoir on Earth, since it represents ~67 % of the planet's mass (Lodders and Fegley, 1998), therefore it could represent a significant source of mercury emissions in the SPSP archipelago. Heavy metals are considered geogenic contaminants, that is, harmful elements that occur naturally in geological materials and originate from natural processes such as weathering, erosion, and geologic formations (Gwenzi, 2021). Peridotite mylonites and Serpentized Peridotite mylonites, which are the mantle-originated rocks that compound the archipelago, are known as geogenic hotspots (Canil et al., 2015; Yin et al., 2024). Furthermore, natural environmental processes such as weathering (the process by which rocks and minerals break down or dissolve over time) and mercury biogeochemical cycling (the transferring mechanisms of mercury across the atmosphere, ecosystems, and the lithosphere) enhance Hg distribution and availability from these natural sources (Yin et al., 2024). However, knowledge about mercury and other heavy metals concentrations in the peridotite rocks of the SPSP archipelago is still unavailable.

The mercury concentrations found in our study for SPSP Brown boobies (7.77 ± 2.18 μg.g^{-1}) were higher, but closer to those found in ventral feathers of Brown boobies in the Santana archipelago by Bighetti et al. (2022) (6.46 ± 1.19 μg.g^{-1}). Located in southeastern Brazil (closer to the mainland, <10 km from the coast), the Santana Archipelago is primarily composed of metamorphic rocks (gneiss and amphibolite), pegmatite dikes (plutonic rock) and diabase (subvolcanic rock) (Silva, 2007). A geological report published in 2010 by Geological Service of the State of Rio de Janeiro for the Santana Archipelago highlighted the presence of natural erosive processes on the metamorphic rock outcrops of Ilha do Francês. It is known that rock erosion is one of the natural sources of mercury into the environment, however, the rock formation and erosion does not appear to be the main source of mercury for the Santana Archipelago Brown boobies, because igneous rocks can contain low traces of mercury since their formation, usually ranging from 0.04 ppm to 0.5 ppm (Aston and Riley, 1972). In this case, perhaps the proximity to the continent and anthropogenic sources of mercury due to human activities may be exerting more influence on the amount found in the boobies.

The studies conducted in the Abrolhos Archipelago (Nunes et al., 2022, and Bauer et al., 2024), located in Northeast Brazil (70 km from the mainland) and characterized by volcanic rocks such as basalts and pyroclastic rocks, found much lower concentrations than those presented in this study, but this seems to be more related to the type of feather used (contour feathers), and therefore cannot be directly compared with the results found here from SPSP e MS (ventral feathers, known to be the type of feather with the highest concentrations of Hg in Brown boobies). Therefore, we highlight here that the results of mercury concentration in different populations of boobies must be analyzed using an integrated approach of (i) ecological factors (molting period, type of feather analyzed and movement of individuals in the population), (ii) geological (origin of the archipelago, rock composition) and (iii) geographic (location and influence of different ocean currents or anthropogenic pollution input).

Bighetti et al. (2022) tested differences in THg concentrations among ventral, dorsal, wing, and tail Brown booby feathers and found the highest concentrations in ventral ones, suggesting the use of these as suitable proxies of mercury concentration for the species, because despite showing expressive levels of the element, ventral feathers are easy to sample and do not impair flight performance. The molting strategy of Brown boobies' wing feathers is classified a as complex basic molt with a stepwise strategy (Howell, 2010). The molt of primaries and rectrices begins after chicks' hatch, but the molting schedule for body feathers including the ventral ones is not well-known (Howell, 2010; Schreiber and Norton, 2020). Due to the wide variation in THg concentration between feather types from the same individual, it is challenging to compare values among different locations or environments, impairing the comprehension of whether the source of variation is more related to local environmental factors or whether this variation is mostly derived from the type of feather evaluated in each study (Furness et al., 1986; Peterson et al., 2019; Low et al., 2020).

Brown booby at sea movements outside the breeding period are not known in most of their colonies. Kohno et al. (2019) tracked eight individuals with geolocators during the non-breeding period on Nakanokamishima island, Japan, and reported the shortest direct maximum distance from the colony varying from 574 to 4988 km, where 5 from the eight tracked individuals traveled <2000 km. For the Red-footed booby (*Sula sula*), a congener species, the mean maximum distance from colony reported in Raso Islet, Cabo Verde, during the non-breeding period was 55 km for males and 63 km for females (Almeida et al., 2021). In the same study, the mean maximum distance from the colony reported for Brown boobies was 42 km for males and 24 for females, but for this species, individuals were tracked during the breeding period, chick rearing specifically.

Considering that ventral feathers' molting period is not known (Howell, 2010; Schreiber and Norton, 2020), it is not possible to infer exactly when and where they were formed, and therefore the location at which these feathers reflect mercury exposure. However, since SPSP archipelago is a region with high prey availability for Brown boobies in the surroundings of the colony (Nunes and Bugoni, 2018), and since individuals do not travel long foraging trips during the breeding period when compared to other archipelagos. For instance, the mean maximum distance from colony reached by Brown boobies in SPSP was 7 km, while in different locations such as the Gulf of California and the in the Caribbean the mean maximum distances from colony reported were much higher: 28 km and 48 km, respectively (Weimerskirch et al., 2009; Soanes et al., 2015; Nunes and Bugoni, 2018). In the MS archipelago, the mean maximum distance from colony reported was 30 km (Benemann et al. Unpublished results). Thus, besides the absence of available information, we hypothesize that individuals from SPSP do not travel very long distances during the non-breeding period as well, but non-breeding movements of MS individuals completely unknown.

4.1.2. Diet related differences

Another factor that must be considered to evaluate the difference found in THg concentration between the two archipelagos is their diet. Brown booby diet varies widely between the two studied archipelagos: In MS, the species exhibits a high generalist foraging behavior, feeding on a wide variety of species from different taxa and inhabiting different strata of the water column (e.g. benthic, demersal, epipelagic), while in SPSP, Brown boobies are practically specialists on flying fish from the family Exocoetidae (Jacoby et al., 2023). In MS, which is a coastal archipelago, the diet of Brown boobies is predominantly based on fish from the Scianidae family, such as the Banded croaked (*Paralonchurus brasiliensis*), the Bigtooth corvina (*Isopisthus parvipinnis*) and the Stripped weakfish (*Cynoscion guatucupa*) (Branco et al., 2005, Benemann et al. Unpublished results). On the other hand, the main species that compound Brown boobies' diet in SPSP are the pelagic fish species Tropical two-wing flyingfish (*Exocoetus volitans*) and the Margined flyingfish (*Cheilopogon cyanopterus*) (Naves et al., 2002; Mancini and Bugoni, 2014; Benemann et al. Unpublished results).

When we look at the trophic level of the species mentioned above, we can observe that, in general, fish from the Scianidae family occupy a higher trophic level than flying fish. Using an isotopic approach in ecology, the trophic level of a species is often inferred through nitrogen ratios (δ^{15}N), where higher values indicate higher trophic positions (Post, 2002). In a study carried out in the Southwest Atlantic Ocean, it was reported δ^{15}N values from 16.1 ‰ to 18.5 ‰ (mean ± SD = 17.6 ± 0.5 ‰) for *C. guatucupa* (Viola et al., 2018). For *E. volitans* from the SPSP, Mancini and Bugoni (2014) reported δ^{15}N values varying from 9.7 ‰ to 10.4 ‰ (mean ± SD = 10.2 ± 0.5 ‰), suggesting that this flying fish species occupies a lower trophic level than *C. guatucupa*. Based on the Ecopath with Ecosim (EwE) model (Polovina, 1984), which is a tool for marine ecosystems modeling that is used, among other purposes, to estimate trophic levels, the trophic level of the Scianidae species *C. guatucupa*, *P. brasiliensis*, and *I. parvipinnis* were estimated at mean 3.7 ± 0.6, mean 3.4 ± 0.3 and mean 4.0 ± 0.4, respectively, while the Exocoetidae species *E. volitans* and *C. cyanopterus* were estimated at mean 3.0 ± 0.09 and mean 3.3 ± 0.38. respectively (FishBase, 2024). These estimates are based on Mathews (1993) where trophic level 1 is assigned to primary producers and detritus and represents further evidence that the fish consumed by Brown boobies from MS occupy higher trophic levels than those from SPSP. Controversially, such results are not in accordance with the premise that species occupying higher trophic levels in the trophic chain would exhibit higher Hg levels due to biomagnification (Kidd et al., 2012; Seco et al., 2021). Probably, local environmental factors exert a greater influence than prey trophic level; to test this hypothesis, an approach using stable isotopes would be interesting and could provide important results to relate mercury levels to prey trophic level and their relationship with the island's biotic and abiotic factors.

4.2. Sexual differences

Males and females had similar average concentrations of THg, which led us to reject the second research hypothesis. It was expected that female Brown boobies would show lower Hg concentrations since one of the main mercury excretion pathways is through egg production and laying (Robinson et al., 2012; Becker et al., 2002; Mills et al., 2022). Our results regarding the absence of sexual differences in Brown boobies' mercury levels are in accordance with Bighetti et al. (2021), which suggested that females' greater body mass (Lewis et al., 2005) and consumption of relatively larger prey than males (Bighetti et al., 2021) would compensate the amount of Hg excreted through eggs. Mancini et al., 2022, on the other hand, reported males foraging on prey of higher trophic positions than females in the Santana archipelago, which may indicate that males are consuming larger prey than females based on the assumption of a positive relationship between fish size and trophic position within the food chain (Romanuk et al., 2011). Benedetti et al. (2022) also found no differences in THg concentration between Brown booby sexes in the SPSP, attributing these results to the similarity in foraging behavior between males and females.

Despite the absence of significant differences between sexes, the difference in THg values between sexes was higher in the MS archipelago (Fig. 2, a and b). This result may be related to greater differentiation in diet between males and females in MS, since in this archipelago Brown boobies exhibit a much wider trophic niche than in SPSP, and although intersexual differences in diet were not tested yet in MS, females showed a wider trophic niche than males in the Santana archipelago, in which Brown boobies' diet is similar (Branco et al., 2005; Mancini et al., 2022). Bustamante et al. (2023) also reported significantly higher δ^{13}C and δ^{15}N values for females than males in blood Clipperton Island, Pacific Ocean, suggesting that females feed on higher trophic level prey and in different areas than males in that island.

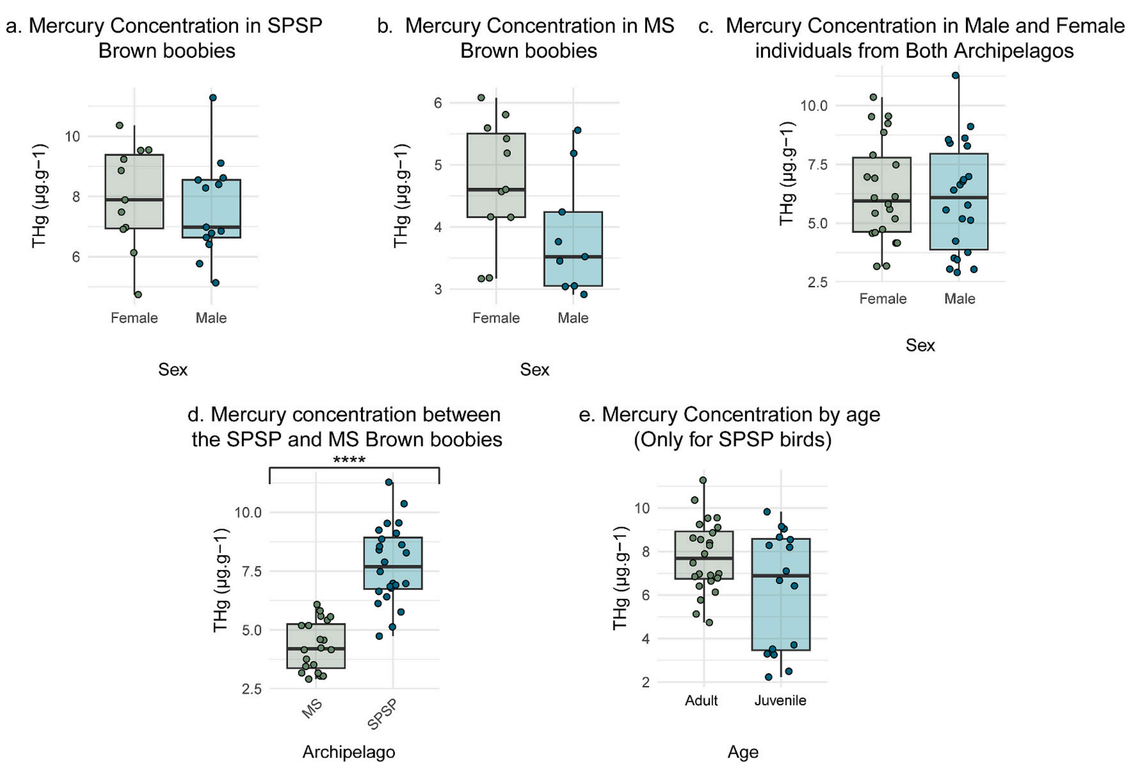

Fig. 2. Boxplots of total mercury (THg) concentration in ventral feathers of Brown boobies (*Sula leucogaster*) from the Moleques do Sul (n = 21) and São Pedro e São Paulo (n = 37) archipelagos in Brazil. a. Comparison between sexes from SPSP individuals; b. comparison between sexes from MS individuals; c. comparison between sexes of individuals from both archipelagos; d. comparison between adult individuals from both archipelagos and e. comparison between adults and juveniles from SPSP. The asterisks (****) represent a statistically significant difference ($p < 0.01$).

4.3. Age-related differences

In our study there were also no significant differences between adult and juvenile individuals, despite the assumption that adults would show higher concentrations due to bioaccumulation throughout the living years. These results contrast with those found by Bighetti et al. (2021) and Bighetti et al. (2022), who reported significantly higher THg and MeHg concentrations in adult Brown boobies. In a study conducted in Sinaloa, Mexico, Blue-footed booby (*Sula nebouxii*) nestlings showed higher Zinc (Zn) concentrations than adults, and the concentration decreased gradually with age (Lerma et al., 2020). The study by Padilha et al. (2018) also did not find significant differences in other bioaccumulative heavy metals (Cd and Cu) between adult and juvenile Brown boobies in the Cagarras archipelago. Despite our results indicating no significant differences in THg concentration between adult and juvenile Brown boobies, the lowest THg values were reported for juveniles, and we can observe a wider variance in THg concentrations in juvenile individuals than in adult ones (Fig. 2, Table 1). In this sense, the lack of significant difference in THg concentration between adults and juveniles in our study may be due to a small sample size ($n = 16$ juveniles).

5. Conclusion

We found no significant differences in THg concentration in Brown boobies' feathers between sexes and age classes, but rather between the two studied archipelagos. Higher values were found in feathers of Brown boobies from the SPSP, which is a tropical archipelago far from the coast off Brazil. While we present significant differences in mercury concentrations in Brown booby across different archipelagos and hemispheres, we understand that there are some limitations in the study that should be considered, such as the lack of knowledge about the molting period and movement during the non-breeding season of *Sula leucogaster*. That said, research is needed to investigate the molting and movement of Brown booby in the SPSP and MS Archipelagos so that we can better understand where these birds are becoming contaminated and whether this contamination reflects the surrounding areas of the archipelago or not. Our hypothesis is that the Hg concentrations found in the present study reflect exposure to Hg near the breeding colonies of the respective archipelagos.

Brazil is one of the signatory countries of the Minamata Convention, which is a global treaty aimed at protecting human health and the environment from the adverse effects of mercury. The conservation and sustainable use of the oceans and marine resources, for example, is one of the 17 Sustainable Development Goals of the United Nations (ODS 14) which foresees a series of actions including the reduction and prevention of marine ecosystems, to which Brazil is also committed. The high volatility and great potential for dispersion of mercury are intrinsic features that challenges its environmental monitoring once it can reach areas far from the emission sources. Seabirds show great potential as biomonitors of mercury in marine environments, and future studies should prioritize the standardization of methods to enable comparisons between different populations.

CRediT authorship contribution statement

Victória Renata Fontoura Benemann: Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Bianca Costa Ribeiro:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Edson Gonçalves Moreira:** Writing – review & editing, Supervision, Resources, Methodology, Formal analysis. **Maria Virginia Petry:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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