# Mercury contamination in the tropical seabird community from Clipperton Island, eastern Pacific Ocean

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### Abstract :

Mercury (Hg) pollution is a global problem affecting remote areas of the open ocean, but the bioaccumulation of this neurotoxic pollutant in tropical top predators remains poorly documented. The objective of this study was to determine Hg contamination of the seabird community nesting on Clipperton Island using blood and feathers to investigate short and longer-term contamination, respectively. We examined the significance of various factors (species, sex, feeding habitat [ $\delta$ 13C] and trophic position [ $\delta$ 15N]) on Hg concentrations in six seabird species. Among species, Great Frigatebirds had the highest Hg concentrations in blood and feathers, boobies had intermediate values, and Brown Noddies and Sooty Terns the lowest. At the interspecific level, although  $\delta 13C$  values segregated boobies from frigatebirds and noddies/terns, Hg concentrations were explained by neither  $\delta$ 13C nor  $\delta$ 15N values. At the intraspecific level, both Hg concentrations in blood and feathers show relatively small variations (16-32 and 26-74%, respectively), suggesting that feeding ecology had low seasonal variation among individuals. Despite most species being sexually dimorphic, differences in Hg contamination according to sex was detected only in Brown Boobies during the breeding period. Indeed, female Brown Boobies feed at a higher trophic level and in a different area than males during this period, resulting in higher blood Hg concentrations. The present study also shows that most of the seabirds sampled at Clipperton Island had little or no exposure to Hg toxicity, with 30% in the no risk category and 70% in the low risk category.

Keywords : Metal, Bioaccumulation, Biomonitoring, Stable isotopes, Trophic ecology, Brown Boobies

### 1. Introduction

Due to its atmospheric residence time of up to a year (Schroeder and Munthe 1998, Sprovieri et al. 2010), mercury (Hg) is dispersed by atmospheric currents at the global scale and deposited in all ecosystems. As a consequence, extremely remote areas, including the open ocean, are contaminated by this ubiquitous pollutant (Fitzgerald et al. 1998), and compared to pre-industrial times, Hg concentrations in marine surface waters have tripled (Lamborg et al. 2014). Once deposited in the sea, Hg is subject to methylation by microorganisms (e.g., Benoit et al. 2002, Gilmour et al. 2013, Yu et al. 2013, Hsu-Kim et al. 2013), resulting in the production of methylmercury (MeHg). Due to its high assimilation efficiency and high affinity for proteins, MeHg bioaccumulates in marine organisms (concentrations increase over time in their tissues) and biomagnifies in the food chain (concentrations increase at each trophic level) up to apex predators, resulting in elevated concentrations in top predators, such as fish, seabirds, and marine mammals (Eagles-Smith et al. 2018). Thus, long-lived marine top predators exhibit highly elevated concentrations of Hg in their tissues (e.g., Muirhead and Furness 1988, Stewart al. 1999, Renedo et al. 2021a).

The strong toxicity of MeHg poses a problem for all living organisms, with neurological, physiological, immunological, and behavioural impacts reported in vertebrates (Tan et al. 2009, Wolfe et al. 1998, Evers 2018). In seabirds, Hg may affect fundamental fitness traits, such as reproduction and lifespan (e.g., Whitney and Cristol 2017, Tartu et al. 2015), with negative consequences at the population level (Evers et al. 2008, Goutte et al. 2014a,b). Due to their elevated position in marine food webs, seabirds are prone to accumulate high levels of Hg, which can have harmful effects. Seabirds are also considered as relevant bioindicators of Hg contamination of marine ecosystems (e.g., Furness and Camphuysen 1997, Monteiro and Furness 1995). They are usefull organisms to investigate Hg availability in the open ocean

marine food webs (e.g., Albert et al. 2019, Carravieri et al. 2017). Therefore, they are particularly relevant to monitor remote areas where Hg is only documented during oceanographic cruises, along shipping lanes, or at research stations –leaving many areas of the ocean without data (Cossa et al. 2011, Hammerschmidt and Bowman, 2012).

By combining the analysis of Hg in different tissues of seabirds, like blood and feathers which allow non-lethal sampling, local and large-scale contamination by Hg can be documented (e.g., Cherel et al. 2018, Pollet et al. 2022). The half-life of Hg in blood is in the range of a few weeks, therefore, it provides information on short-term Hg exposure (weeks to months), allowing the assessment of local contamination around the colonies (Monteiro and Furness 2001). Conversely, Hg concentrations in feathers of adult individuals are commonly considered to indicate integrated accumulation between two successive moults, i.e., long-term exposure encompassing the year-long foraging range of birds (Braune and Gaskin 1987, Burger 1993).

Food is the main route for Hg exposure in seabirds (Burger and Gochfeld 2004, Atwell et al. 1998), and trophic ecology (i.e., feeding habitat and diet) represents a major driver of intraand inter-specific variations of Hg concentrations (Bearhop et al. 2000, Anderson et al. 2009, Carravieri et al. 2014a). Hence, the use of seabirds to investigate the contamination of water masses by Hg requires knowledge of their trophic ecology, whether by direct methods, such as biologging that allows determining precisely their feeding areas, or by indirect methods, such as the use of trophic tracers (stable isotopes of carbon and nitrogen) that provide information on their foraging habitat and trophic position, respectively (Hobson et al. 1994, Fort et al. 2014).

In this study, we investigated blood and feather Hg concentrations from six seabird species breeding on Clipperton Island, a remote atoll in the eastern tropical Pacific Ocean, being more than 1,000 km from Mexico and more than 5,000 km from Hawaiif. We used  $\delta^{13}$ C and  $\delta^{15}$ N values in both tissues as trophic tracers of the foraging habitat and trophic position of birds during breeding, and outside the breeding period, respectively (Cherel et al. 2008). Blood and feathers from adults were used to investigate intra- and inter-specific differences in Hg exposure, and to assess the contamination of the area at local (blood Hg) and regional (feather Hg) scales. As most seabird species considered in this study show sexual dimorphism with potential foraging segregation between males and females (e.g., Brown Booby; Mancini et al. 2023), sexes were considered separately in order to determine if the different foraging strategies between sexes is reflected in Hg concentrations in the short- (blood) and long-term (feathers). Specifically, we tested the following hypotheses: (i) stable isotope values of tropical seabirds will show significant variation as a result of variable primary production around the Clipperton Atoll (Willett et al. 2006) despite a relatively poor diversity of tropical seabird foraging methods and prey diversity; (ii) blood and feather Hg concentrations are expected to reflect ecological segregation between species with with those having the highest  $\delta^{15}$ N values exhibiting the highest Hg concentrations between males and females as a result of expected sexual different Hg concentrations between males and females as a result of expected sexual different Hg concentrations between males and females as a result of expected sexual different Hg concentrations between males and females as a result of expected sexual different Hg concentrations between males and females as a result of expected sexual differences in foraging strategies.

### 2. Materials and methods

# 2.1. Study sites and sampling procedure

Fieldwork was carried out at Clipperton Island (10.3° N, 109.2° W) in the central eastern Pacific (Fig. 1) in January–March, 2005. Clipperton Island is the only coral atoll in the eastern Pacific, with a circular ring-shaped aspect, roughly 3 km in diameter and 100–300 m wide. The island is uninhabited, and constitutes the nesting place for thousands of seabirds of 13 different species (Weimerskirch et al. 2009). Notably, it holds the largest Masked Booby *Sula dactylatra* colony in the world, with ~120 000 breeding individuals (Pitman et al. 2005).

Six species were sampled: the Masked Booby (n=24), Brown Booby (Sula leucogaster;n=20), Red-footed Booby (Sula sula; n=20), Great Frigatebird (Fregata minor; n

= 9), Sooty Tern (*Onychoprion fuscata*; n=10), and Brown Noddy (*Anous stolidus*; n=6). For each individual, a blood sample (~ 1 ml) was taken from the brachial vein with a heparinized syringe, and stored in a 1.5 mL Eppendorf tube with 70% ethanol. Sections of the secondary flight feathers N°1 to 3 (two to three per individual) were collected and stored in individual plastic bags for subsequent laboratory analysis.

#### 2.2. Molecular sexing

Molecular sexing was conducted at the Centre d'Etudes Biologiques de Chizé (CEBC), France. Birds were sexed from whole blood by polymerase chain reaction amplification of part of two highly conserved genes (CHD) present in sexual chromosomes, as described in Fridolfsson and Ellegren (1999).

# 2.3. Mercury and stable isotope analyses

Total Hg and isotopic analyses were carried out on whole blood and feathers at the Littoral, Environment and Societies (LIENSs) laboratory. After evaporating the ethanol at room temperature under a fume hood, blood was freeze-dried and homogenised prior to its analyses. Feathers were washed twice with a mixture of chloroform: methanol (2:1) to remove dirt and contaminants, rinsed in methanol and then dried at 45°C for 48 h. The dried feathers were then cut into very fine pieces with stainless steel scissors. For each individual, feathers were pooled and homogenised together in order to provide integrated Hg and isotopic values (Carravieri et al. 2014b).

Aliquots weighing between 1.4 and 4.8 mg for blood and 0.8 and 2.6 mg for feathers were analysed for Hg quantification with an Altec AMA 254 spectrophotometer, as described in Chouvelon et al. (2009). All analyses were carried out in duplicates or triplicates until having a relative standard deviation <10%. Blanks and Certified reference material (CRM) DOLT-5

(Dogfish liver, NRC, Canada; certified Hg concentration:  $0.44 \pm 0.18 \ \mu g \ g^{-1}$  dry weight) were measured all along the analytical sets (at the beginning and the end of each set, and every 10 samples). The CRM mass was adjusted to get similar amounts of Hg as in the samples. Our results for CRM were in good agreement with the certified values with recoveries of 98.3  $\pm$  0.7%. The limit of detection (LOD) of the AMA was 0.1 ng. Results for Hg are further presented in  $\mu g \ g^{-1}$  relatively to the dry weight (dw).

Blood isotopic values are representative of the diet during the 3–4 weeks preceding sampling (Bearhop et al., 2002), while those in feathers correspond to exposure during foraging at the time of their synthesis (Cherel et al. 2018). From 0.2–0.5 mg of blood and feather aliquots were packed in a tin cup for isotopic analyses, respectively. Carbon and nitrogen stable isotope values ( $\delta^{13}$ C and  $\delta^{15}$ N) were determined with a continuous flow mass spectrometer (Thermo Scientific Delta V Advantage) coupled to an elemental analyser (Thermo Scientific Flash EA 1112). Isotopic data were defined by following equation:

$$\delta^{15}$$
N or  $\delta^{13}$ C (‰) =((R<sub>sample</sub>/R<sub>standard</sub>)-1) x 1000

where R is <sup>15</sup>N/<sup>14</sup>N or <sup>13</sup>C/<sup>12</sup>C. Results are presented in  $\delta$  notation relative to Vienna PeeDee Belemnite and atmospheric N<sub>2</sub> (air) for carbon and nitrogen, respectively. Internal laboratory standards (USGS-61 and USGS-63) were used to check accuracy. Measurement errors were <0.15 ‰ for both  $\delta$ <sup>13</sup>C and  $\delta$ <sup>15</sup>N values.

### 2.4. Statistical analyses

Statistical analyses were performed with *R* (R Studio, Inc; version 1.0.153). First, the normality of the data was examined via a Shapiro-Wilk test. Measurements of Hg as a response variable did not show a normal distribution, thus, nonparametric Kruskal-Wallis tests were performed. For all tests, the significance level was set at the threshold of  $\alpha$ =0.05. Values are presented as means ± standard deviation (SD).

# 3. Results

3.1. Inter- and intra-specific variations of Hg and stable isotopes

Mercury and stable isotopes have been measured in 84 blood and 89 feather samples from seabirds breeding on Clipperton Island. In blood, Hg concentrations varied significantly among species, ranging from an average concentration of  $0.36 \pm 0.11 \,\mu g \, g^{-1}$  dw in the Brown Noddy to  $2.42 \pm 0.49 \,\mu g \, g^{-1}$  dw in the Great Frigatebird (Table 1, Fig. 2). Across species, blood Hg concentrations showed relatively low variations at the species level, with coefficient of variations of 16% in the Masked Booby to 32% in the Brown Noddy. In feathers, Hg concentrations were significantly higher than in blood, but with a narrower range of values at the interspecific scale and a higher variation at the specific scale (Fig. 2). Thus, the Sooty Tern had the lowest average feather Hg concentrations with  $1.50 \pm 1.10 \,\mu g \, g^{-1}$  dw, and the Great Frigatebird had the highest with  $4.50 \pm 2.50 \,\mu g \, g^{-1}$  dw (Table S1). Coefficients of variation regarding feather Hg concentrations varied from 26% in the Masked Booby to 74% in the Sooty Tern. Despite this higher variation than in blood, there was a significant correlation between Hg concentrations in blood and in feathers (Pearson, r=0.46; df=80; p<0.001).

Blood and feather  $\delta^{13}$ C and  $\delta^{15}$ N values revealed that the six sympatric seabird species from Clipperton Island were segregated by their overall isotopic values (Fig. 3). Thus,  $\delta^{13}$ C values in the blood and feathers separated the three species of boobies from the other species, with boobies having average  $\delta^{13}$ C values  $\geq$ -17.0 and -15.8 ‰ in blood and feathers, respectively. The species were not segregated by  $\delta^{15}$ N values in blood and feathers (Fig. 3), and boobies showed small variation in  $\delta^{15}$ N values (coefficients of variation <2%) compared to the other species (coefficients of variation variyng from 7% in the Great Frigatebird to 16% in the Sooty Tern). 4.2. Sex-related variations of Hg and stable isotopes

There was no significant sex-related difference in blood and feather Hg concentrations in seabirds from Clipperton Island, with the exception of the Brown Booby for blood (Fig. S1). In this species, females had higher blood Hg concentrations  $(1.25 \pm 0.20 \ \mu g \ g^{-1} \ dw)$  than males  $(0.81 \pm 0.08 \ \mu g \ g^{-1} \ dw; Table 1, Fig. 4)$ . Females also had significant higher  $\delta^{13}C$  and  $\delta^{15}N$  values than males in blood, but no significant difference was found in feathers (Fig. 5).

### 4. Discussion

This study investigates for the first time concentrations of Hg in a suite of seabirds breeding on Clipperton Island. The measurement of Hg in blood and feathers in this seabird community provides a valuable opportunity to study the Hg availability in oceanic waters exploited by species with different trophic ecologies. This appears particularly relevant in the context of the evaluation of the effectiveness of the Minamata Convention, which aims to document Hg contamination at large spatio-temporal scale (Evers et al. 2016, Gustin et al. 2016). The use of bioindicators, such as seabirds, to assess and monitor Hg concentrations in the environment is particularly suitable for large-scale spatial and long-term monitoring (Furness and Camphuysen 1997).

Tissue Hg concentrations in Clipperton Island seabirds were similar to those of other tropical birds, but lower than those of subantarctic birds, such as skuas, albatrosses, and petrels (e.g., Becker et al. 2002, Anderson et al. 2009, Carravieri et al. 2014a). More specifically, on Clipperton Island, we found a relatively small intraspecific variation in blood and feather Hg concentrations depending on different explanatory factors (species, trophic ecology, sex). No differences in blood and feather Hg concentrations was found between males and females, with

the exception of blood in the Brown Booby, reflecting sexual segregation in trophic ecology in this species over the short-term. Concerning the risks associated with Hg contamination, most species were at no or low risk of Hg toxicity (Ackerman et al. 2016, Chastel et al. 2022).

Most studies that have measured Hg concentrations in seabird feathers have focused on body feathers, which have the advantage that they are easy to collect and they can be pooled to obtain an average value with a reduced variability (Carravieri et al. 2014b, Peterson et al. 2019). Body feathers reflect long-term contamination to Hg, typically one year in most species (Braune and Gaskin 1987, Burger 1993). In studies on Hg in the feathers of tropical birds, body feathers have also been the main focus, although flight feathers have been considered and compared with body feathers in a few cases (e.g., Gochfeld 1980, Bighetti et al. 2021a). In seabirds overall, depending on the moult pattern, flight feathers may have lower (moulted after body feathers; Braune and Gaskin, 1987) or higher (moulted before body feathers; Furness et al. 1986) Hg concentrations. Due to the lack of information on the moulting patterns in most of the species from the Clipperton Island community, it is difficult to compare Hg concentrations in flight feathers obtained in this study with those from other sites involving other feather types (i.e., body feathers). Nevertheless, these concentrations appear higher than those reported in the tropical seabird communities from the western Indian Ocean, which showed lower feather Hg concentrations (from 0.05 to 1.5 µg g<sup>-1</sup> dw; Catry et al., 2008; Kojadinovic et al., 2007) than at Clipperton Island (from 1.50 to 4.60  $\mu$ g g<sup>-1</sup> dw; Table S1). In the nearby Galápagos Islands, several identical or closely related species, including two frigatebirds and three boobies, showed higher feather Hg concentrations than the Clipperton community (i.e., between 5.23 and 7.32 μg g<sup>-1</sup> dw for the Great Frigatebird, and between 6.25 and 9.80 μg g<sup>-1</sup> dw for the Red-footed Booby; Zarn et al. 2020). In addition, there was no difference in feather Hg concentrations between frigatebirds and boobies in the Galápagos Islands, unlike in our study. Interestingly,  $\delta^{13}$ C values between Great Frigatebirds and Red-footed boobies were in the same range in the

Galápagos, suggesting that there is no segregation in feeding habitats between these two species (see discussion below on this aspect). The timing of plumage moult therefore appears to be a key element to consider for comparing data between sites and between species (Albert et al. 2019). It would be useful to use chick feathers to monitor Hg in future investigations, as chick feathers reflect Hg contamination over a shorter period of time, i.e., the chick-rearing period (Blévin et al. 2013).

In contrast to feathers, information provided by blood allow a direct comparison of Hg levels in seabirds from the Clipperton Island community with those from other sites (Table 2). Blood Hg concentrations in the present study are in line with other works on the same or closely related species, with the Laridae having the lowest concentrations, Sulidae intermediate values, and Fregatidae the highest levels. This general trend is affected by local conditions and diet, however. For example, the Peruvian Booby breeding on Pescadores Island had low blood Hg concentrations as they primarily forage on anchovies (Barbraud et al. 2018), which display low Hg concentrations as a result of biodilution in the very high biomass in the Humboldt Current (Le Croizier et al. 2022). A review of literature reveals that very high concentrations of Hg have been reported in the blood of Sooty Terns from the Dry Tortugas in Florida. These values are unexpected and merit further investigation at the seabird community level to determine if the Laridae < Sulidae < Fregatidae pattern is verified, or if high blood Hg concentrations in the Sooty Tern is linked to its feeding ecology in the area.

The biomagnification process illustrates why diet is one of the main factors explaining Hg contamination in top consumers, including seabirds (Monteiro et al. 1998, Stewart et al. 1999, Carravieria et al. 2014a). Seabirds at Clipperton Island feed in flocks, depending on schools of dolphins and tuna to drive their prey to the surface waters (Au and Pitman 1986). In this tropical region, flocks of Sooty Tern forage in areas of low productivity, whereas flocks of boobies forage in areas of high productivity, which can be explained by differential energetic

costs of flight and competition (Ballance et al. 1997). These different productivity zones should have different isotopic values and also different Hg concentrations. A zone of high production occurred near Clipperton Island every year from January to March, coinciding with the reproductive peak of most seabird species (Willett et al. 2006, Weimerskirch et al. 2008). While most tropical seabirds reproduce all year long (Nelson 1978), a relative synchronicity is at work at Clipperton Island. This is particularly the case for Masked Boobies, which represent the most numerous seabird species on the atoll (Pitman et al. 2005). In this region of the Pacific Ocean, Hg concentrations in seabirds may vary because of the productivity which is influenced by climatic events such as El Niño/La Niña Southern Oscillations (ENSO). ENSO was shown to shift isotopic baselines in the Humboldt Current food web, but without any detected effect on Hg exposure of seabirds (Renedo et al. 2021b). Fishing pressure is another factor that can affect Hg concentrations in seabirds. Indeed, the catch of large predators, such as tunas, may reduce the accessibility of prey for some surface-feeding seabirds, such as the Sooty Tern. In the south tropical Atlantic Ocean, the population of Sooty Terns nesting on Ascension Island dramatically decreased between the 1940s and the 1970s (by 84%, from 3.32 million birds in 1958, to 350,000 individuals in 2013), and feather analysis of stable isotopes in museum and contemporary specimens over a period of 140 years showed a shift in Sooty Tern diet as a result of industrial tuna fisheries (Reynolds et al. 2019). Such a dietary change caused a 59% increase in Sooty Tern feather Hg concentrations, from 1.2  $\mu$ g g<sup>-1</sup> in 1920 to 2.0  $\mu$ g g<sup>-1</sup> in 2020 (Cusset et al. 2023).

At the scale of a seabird community, stable isotopes represent a useful approach to investigate food partitioning between species (Hobson et al. 1994, Cherel and Carrouée 2022). Although isotopic differences were small among Clipperton Island seabirds (Fig. 2),  $\delta^{13}$ C values separated two groups – the Laridae and Fregatidae, and the Sulidae. This  $\delta^{13}$ C segregation in two different groups occurred both in the short term (i.e., during the breeding

period [blood values]) and in the long term (i.e., outside the period [feather values]). Such a segregation is not reflected in blood nor feather Hg concentrations, as the first group showed both the lowest (Sooty Tern and Brown Noddy) and highest Hg concentrations (Great Frigatebird; Fig. 1). Sebastiano et al. (2017) also reported that Magnificent Frigatebirds sampled during the breeding period had the highest blood Hg concentrations, and Sooty Terns the lowest, amongst six seabird species breeding in French Guiana. These authors showed that such elevated blood Hg concentrations were mainly explained by Hg biomagnification reflected by the highest  $\delta^{15}$ N values of frigatebirds. Such a difference in  $\delta^{15}$ N is not apparent in the Clipperton Island seabird community, suggesting that factors other than trophic position influence the contamination of seabirds in the short- (blood) and long-term (feathers). Further studies should consider Hg isotopic composition of both tissues to assess the dietary origin of MeHg contamination (Renedo et al. 2018, Le Croizier et al. 2021). For instance, blood  $\Delta^{199}$ Hg values were higher in feathers than in blood of Arctic seabirds, which reflects seasonal dietary changes and different integration times for MeHg exposure between tissues (Renedo et al. 2020).

Most seabird species from Clipperton Island are sexually dimorphic (Chardine and Morris 1989, Dearborn et al. 2001, Weimerskirch et al. 2006, 2009). This is interpreted as an adaptation to intraspecific competition, which expands the trophic niche of the species (Phillips et al. 2011, Gimenez et al. 2021, Mancini et al. 2023). Contrary to our predictions, isotopic values and Hg concentrations were not different in blood or feathers of males and females. The Brown Booby is nevertheless an exception since it showed that sexes have different blood stable isotope values and Hg concentrations. In this species, females are larger and heavier than males (Nelson 2005). At Clipperton Island, they showed trophic segregation, with females feeding on higher trophic level prey ( $\delta^{15}$ N) and in different areas ( $\delta^{13}$ C) than males, resulting in higher blood Hg concentrations in females. Such a sex-related trophic segregation did not seem to exist

outside of the breeding period, as the isotopic values did not differ between sexes in feathers, nor does Hg concentration (Table S1). For all the other species, the lack of significant differences between the stable isotopes of males and females in blood and feathers suggests that the feeding ecology remains very similar between the sexes regardless of season.

At high concentrations, Hg can pose a threat to the health of seabirds with effects on survival, reproduction, and demography (e.g., Chastel et al. 2022, Goutte et al. 2015, Tartu et al. 2013). For feathers, only some individuals (one Brown Booby, two Masked Boobies, and four Great Frigatebirds, i.e., less than 8% of the seabirds analysed) had concentrations above the toxicity levels of 5  $\mu$ g g<sup>-1</sup> dw (Eisler 1987). In blood, there were 20 individuals (25% of the seabirds analysed) below the lowest-observed effect levels of 1  $\mu$ g g<sup>-1</sup> dw, and all the remaining seabirds were in the low-risk range (i.e., blood Hg concentrations between 1 and 5  $\mu$ g g<sup>-1</sup> dw) according to Ackerman et al. (2016). Overall, these results indicate a low risk from Hg in both short- (blood) and long-term (feather). In a more precise assessment of the toxicological risk of Hg, selenium (Se) should nevertheless be considered as it has a protective effect against the toxicity of this metal (Cuvin-Aralar and Furness 1991, Ikemoto et al. 2004). In seabird populations with low concentrations of Hg and low concentrations of Se (e.g., skuas), a strong impact on reproduction has been demonstrated, whereas in populations with high concentrations of Hg and Se, Hg had only a very limited effect (Goutte et al. 2014b, Carravieri et al. 2020).

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	n	Blood Hg (µg g-1 dw)	Blood δ <sup>13</sup> C (‰)	Blood δ <sup>15</sup> N (‰)
Brown Booby (Sula leucogaster)	20	1.03 ± 0.27	-16.99 ± 0.17	$14.60 \pm 0.21$
Males	10	$0.81 \pm 0.08$	$-17.10 \pm 0.14$	$14.44 \pm 0.15$
Females	10	$1.25 \pm 0.20$	$-16.87 \pm 0.12$	$14.76 \pm 0.10$
Aasked Booby (Sula dactylatra)	20	1.67 ± 0.27	$-16.75 \pm 0.14$	$14.94 \pm 0.14$
Males	10	1.56 ± 0.23	$-16.81 \pm 0.14$	$14.84 \pm 0.11$
Females	10	1.78 ± 0.28	$-16.70 \pm 0.13$	$15.04 \pm 0.10$
Red-footed Booby (Sula sula)	20	$1.47 \pm 0.26$	$-17.02 \pm 0.10$	$14.78 \pm 0.10$
Males	11	$1.41 \pm 0.26$	$-17.02 \pm 0.10$	$14.82 \pm 0.09$
Females	9	1.55 ± 0.25	$-17.02 \pm 0.10$	$14.72 \pm 0.07$
Great Frigatebird ( <i>Fregata minor</i> )	9	2.42 ± 0.49	$-17.37 \pm 0.13$	$15.01 \pm 0.36$
Males	5	2.67 ± 0.48	-17.47 ± 0.06	14.85 ± 0.39
Females	4	$2.10 \pm 0.28$	$-17.24 \pm 0.08$	15.22±0.19
Brown Noddy ( <i>Anous stolidus</i> )	5	0.36 ± 0.11	$-17.50 \pm 0.18$	$14.50 \pm 0.16$
Males	2	$0.41 \pm 0.19$	-17.52 ± 0.36	$14.43 \pm 0.24$
Females	3	0.32 ± 0.06	$-17.49 \pm 0.06$	$14.55 \pm 0.11$
ooty Tern (Onychoprion fuscatus)	10	$0.62 \pm 0.14$	$-17.31 \pm 0.54$	$14.91 \pm 0.27$
Males	6	$0.66 \pm 0.14$	$-17.40 \pm 0.15$	$15.04 \pm 0.10$
Females	4	0.56 ± 0.14	-17.18 ± 0.89	14.71±0.34

Table 1. Blood Hg concentration and stable isotope values in males and females of six seabird species on the Clipperton Island. Values are means  $\pm$  SD.

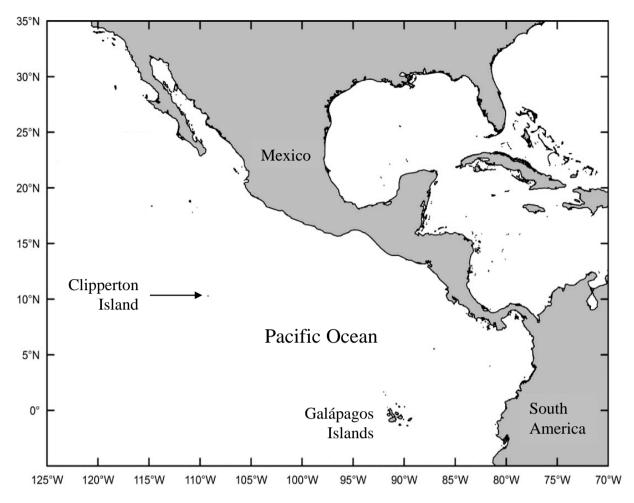


Fig. 1. Clipperton Island in the eastern tropical Pacific Ocean (adapted from Pitman et al. 2012)

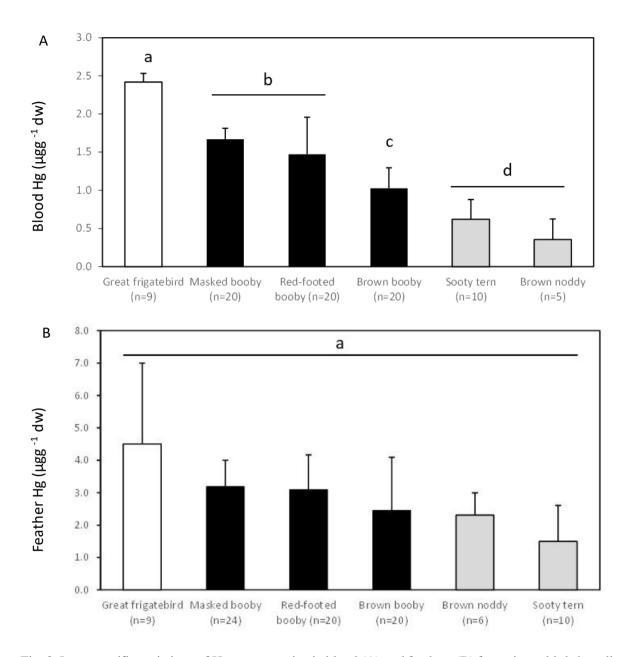


Fig. 2. Inter-specific variations of Hg concentration in blood (A) and feathers (B) from six seabirds breeding at the Clipperton Island. Species are presented according to taxonomic groups: Fregatidae (white), Sulidae (black) and Laridae (grey). Species sharing the same letter are not statistically different (Kruskal-Wallis, p < 0.05).

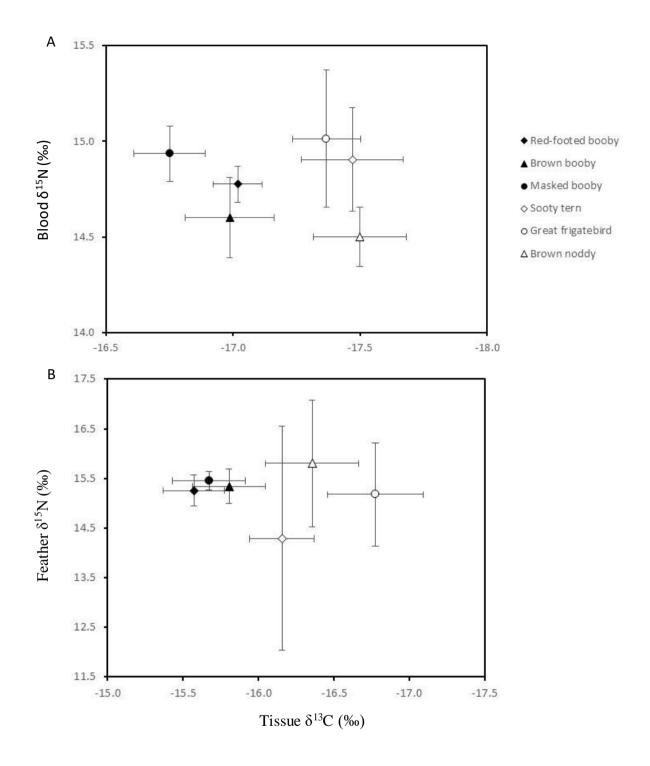


Fig. 3. Stable carbon and nitrogen isotope values of blood (upper panel) and feathers (lower panel) of adult seabirds on Clipperton Island. Values are means  $\pm$  SD. Isotopic scales are different in upper and lower panels.

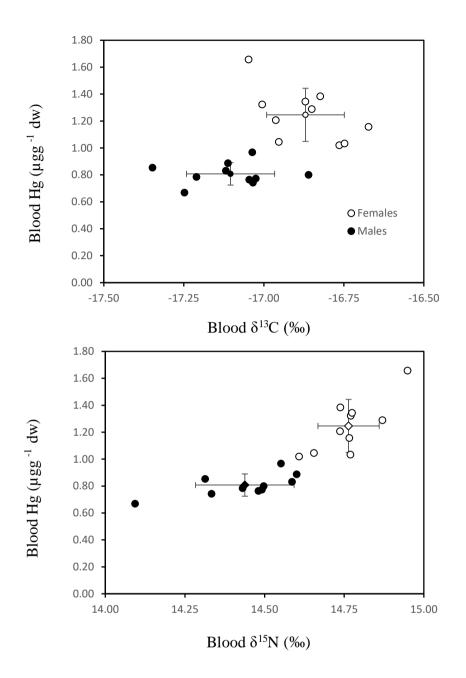


Fig 4. Blood Hg concentration as a function of blood  $\delta^{13}C$  (upper panel) and  $\delta^{15}N$  (lower panel) values in male and female Brown Boobies. Open and closed circles represent single individuals and diamonds represent mean values  $\pm$  SD.

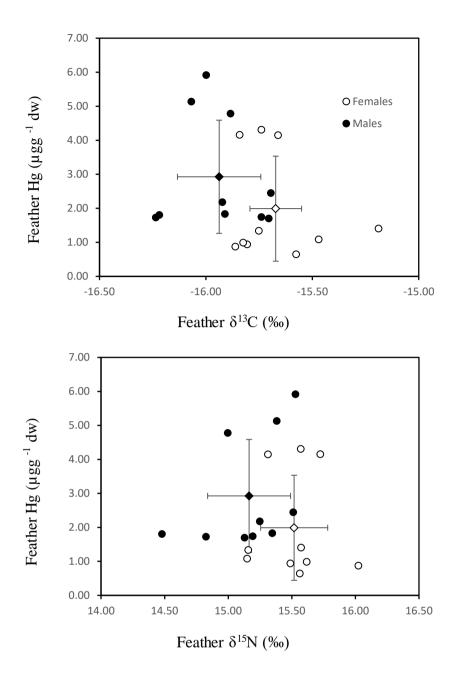


Fig. 5. Feather Hg concentration as a function of blood  $\delta^{13}C$  (upper panel) and  $\delta^{15}N$  (lower panel) values in male and female Brown Boobies. Open and closed circles represent single individuals. Diamonds represent mean values  $\pm$  SD.

Table 2. Review of blood Hg concentration of adult Sulidae, Fregatidae and Laridae from the intertropical area. Values are means  $\pm$  SD. Sex is indicated (M: males, F: females) when available.

Family and species	Site	Year	n	Blood Hg (µg g-1 dw)	Reference
Sulidae					
Brown Booby (Sula leucogaster)	Clipperton Atoll	2005	20	$1.03 \pm 0.27$	This study
	Santana Archipelago	2018	40	2.68 ± 0.78	Bighetti et al. 2021b
	Palmyra Atoll	2009-2014	10	2.49 ± 1.05	Gilmour et al. 2019
Masked Booby ( <i>Sula dactylatra</i> )	Clipperton Atoll	2005	20	1.67 ± 0.27	This study
	Layssan Island	2009-2014	3	$1.82 \pm 0.91$	Gilmour et al. 2019
	Tern Island	2009-2014	14	1.96 ± 1.25	Gilmour et al. 2019
Red-footed Booby (Sula sula)	Clipperton Atoll	2005	20	1.47 ± 0.26	This study
	New Caledonia	2015	7	0.92 ± 0.13	Le Croizier et al. 2022
	Brazil	2015	60	2.66 ± 1.61	Le Croizier et al. 2022
	Layssan Island	2009-2014	4	0.53 ± 0.01	Gilmour et al. 2019
	Tern Island	2009-2014	8	2.16 ± 0.43	Gilmour et al. 2019
Blue-footed Booby ( <i>Sula nebouxii</i> )	Isla El Rancho, Mexico	2010	16 (M)	2.41 ± 0.89	Lerma et al. 2016
	Isla El Rancho, Mexico	2010	15 (F)	2.08 ± 0.68	Lerma et al.2016
	Isla El Rancho	2011	13 (M)	$1.38 \pm 0.43$	Lerma et al. 2016
	Isla El Rancho, Mexico	2011	12 (F)	1.05 ± 0.24	Lerma et al. 2016
Peruvian Booby <i>(Sula variegata</i> ) #	Peru	2009	21	0.56 ± 0.09	0.56 ± 0.09 Le Croizier et al. 2022   0.66 ± 0.15 Le Croizier et al. 2022   0.32 ± 0.05 Le Croizier et al. 2022
	Peru	2010	16	0.66 ± 0.15	
	Peru	2011	7	0.32 ± 0.05	
	Peru	2012	29	0.62 ± 0.14	Le Croizier et al. 2022
	Peru	2013	13 17 0.67 ± 0.11 Le Croizier et	Le Croizier et al. 2022	
Fregatidae					
Great Frigatebird ( <i>Fregata minor</i> )	Clipperton Atoll	2005	9	2.42 ± 0.49	This study
	Layssan Island	2009-2014	5	1.68 ± 1.01*	Gilmour et al. 2019
	Palmyra Atoll	2009-2014	7	3.50 ± 1.34*	Gilmour et al. 2019
	Tern Island	2009-2014	7	4.46 ± 3.74*	Gilmour et al. 2019
Magnificent Frigatebird ( <i>Fregata magnificcens</i> )	French Guyana	2012	20	5.81 ± 1.27	Sebastiano et al. 2017
	Barbuda	2009-2014	15	4.41 ± 0.67*	Gilmour et al. 2019
Laridae					
Brown Noddy ( <i>Anous stolidus</i> )	Clipperton Atoll	2005	5	$0.36 \pm 0.11$	This study
	French Guyana	2012	20	$1.13 \pm 0.13$	Sebastiano et al. 2017
	<b>Cousin Island</b>	2005-2006	10	$0.54 \pm 0.06$	Catry et al. 2008
Lesser Noddy (Anous tenuirostris)	CousinIsland	2005-2006	10	$0.35 \pm 0.06$	Catry et al. 2008
Sooty Tern (Onychoprion fuscatus)	Clipperton Atoll	2005	10	10 0.62 ± 0.14 This study	
	Aride Island	2004	20	$0.59 \pm 0.40$	Author's unpublished data
	Dry Tortugas, Florida	1977 12 5.67 ± 2.51 Stoneburge	Stoneburger et al. 1980		
	French Guyana	2012	6	$0.85 \pm 0.18$	Sebastiano et al. 2017

\* wet weight converted to dry weight following a moisture content of 79.13% (Eagles-Smith et al., 2008)

# Data reported for blue-footed boobies correspond to the early chick-rearing period (Lerma et al. 2016)

	n	Feather Hg (µg g⁻¹ dw)	Feather $\delta^{13}$ C (‰)	Feather $\delta^{15}N$ (‰)
Brown Booby (Sula leucogaster)	20	2.46 ± 1.63	-15.80 ± 0.24	15.34±0.34
Males	10	2.93 ± 1.66	-15.94 ± 0.20	15.16±0.33
Females	10	1.99 ± 1.55	$-15.67 \pm 0.21$	15.52 ± 0.26
Masked Booby ( <i>Sula dactylatra</i> )	24	3.19 ± 0.82	-15.67 ± 0.24	15.45 ± 0.18
Males	11	2.62 ± 0.53	-15.67 ± 0.22	15.43 ± 0.25
Females	13	3.67 ± 0.71	$-15.68 \pm 0.27$	15.47 ± 0.10
Red-footed Booby (Sula sula)	20	$3.09 \pm 1.08$	-15.57 ± 0.20	15.26±0.31
Males	11	2.93 ± 0.99	-15.61 ± 0.25	15.16±0.33
Females	9	3.29 ± 1.20	$-15.53 \pm 0.13$	15.37±0.26
Great Frigatebird ( <i>Fregata minor</i> )	9	4.50 ± 2.50	-16.77 ± 0.32	15.17±1.03
Males	5	$4.41 \pm 2.40$	$-16.90 \pm 0.29$	14.81±0.87
Females	4	4.62 ± 2.99	$-16.62 \pm 0.31$	15.63 ± 1.16
Brown Noddy (Anous stolidus)	6	$2.18 \pm 0.69$	$-16.35 \pm 0.27$	15.64 ± 1.20
Males	3	2.34 ± 0.68	$-16.44 \pm 0.14$	14.81±0.88
Females	3	2.02 ± 0.80	$-16.26 \pm 0.38$	16.48±0.86
Gooty Tern (Onychoprion fuscatus)	10	$1.50 \pm 1.10$	$-16.16 \pm 0.21$	14.29 ± 2.25
Males	6	1.31 ± 0.65	-16.17 ± 0.26	13.72 ± 2.76
Females	4	1.78 ± 1.66	-16.13 ± 0.15	$15.15 \pm 0.96$

Table S1. Feather Hg concentration and stable isotope values in males and females of six seabird species breeding on Clipperton Island. Values are means  $\pm$  SD.

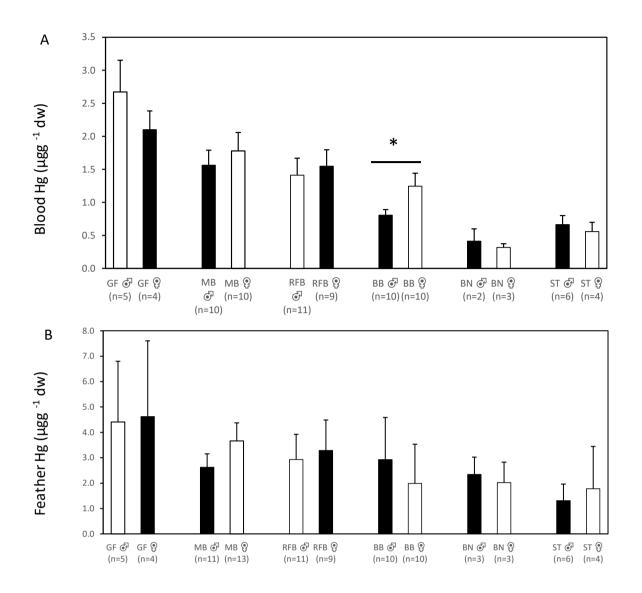


Fig S1. Male ( $\bigcirc$ ) and female ( $\bigcirc$ ) Hg concentrations (mean±SD, in µg.g<sup>-1</sup> dw) in blood (A) and feathers (B) from six seabird species breeding on Clipperton Island. Males are presented in white and females in black. The star indicates that males and females have statistically different concentrations (Kruskal-Wallis, p < 0.05).