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Lead, cadmium and mercury in the blood of the blue-footed booby (*Sula nebouxii*) from the coast of Sinaloa, Gulf of California, Mexico

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ABSTRACT

We used blood samples of the Blue-footed Booby, considering sex (female and male) and age-class (adult and chick) of individuals at different breeding stages during two breeding seasons (2010–2011 and 2011–2012) in Isla El Rancho, Sinaloa, to determine lead, cadmium, and mercury concentrations. Lead and cadmium concentrations were below our detection limit (0.05 and 0.36 ppm, respectively). A higher concentration of mercury was found in early stages of breeding, likely related to changes in mercury environmental availability. Mercury concentrations in adults did not relate with their breeding output. Males and adults had higher mercury concentration than females and chicks. We provide information of temporal, sex and age-related variations in the concentrations of mercury in blood of the Blue-footed Booby.

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1. Introduction

Heavy metal concentrations are increasing in the marine environment as a result of their anthropogenic use in agriculture and industry worldwide (Nriagu and Pacyna, 1988). In the Gulf of California, agriculture, aquaculture, mining, and harbor activities result in the transport of materials from the watershed to coastal areas (Frías-Espericueta et al., 2014; Montaña-Ley and Páez-Osuna, 2012; Páez-Osuna and Osuna-Martínez, 2015; Ruiz-Fernández et al., 2009). In Sinaloa, the occurrence of lead (Pb), cadmium (Cd) and mercury (Hg) has been reported in coastal organisms (Frías-Espericueta et al., 2014; Ruelas-Inzunza and Páez-Osuna, 2007; Ruelas-Inzunza et al., 2011). In particular, concentrations of Pb, Cd and Hg in some fish are above the limits considered as safe for human consumption (Ruelas-Inzunza and Páez-Osuna, 2007; Ruelas-Inzunza et al., 2011). Moreover, seasonal variations of Pb, Cd and Hg concentrations were reported in mangrove oysters (*Crassostrea corteziensis* and *Crassostrea palmula*) as a consequence of the effect of upwelling (Páez-Osuna and Osuna-Martínez, 2015). Though strong seasonal variations of oceanographic conditions

occur in Gulf of California, to our knowledge, seasonality of heavy metal concentrations in fish-eating organisms has not been studied. Seabirds are good indicators of contamination in the marine environment as they accumulate contaminants thru their food web (Burger and Gochfeld, 2004). Identifying seasonal variability of heavy metals in piscivorous organisms may improve our understanding of elemental fluctuations in the environment, and eventually help to prevent health risks associated to fish consumption.

Seabirds can experience sublethal effects by heavy metal contamination. Effects of Cd, Pb and Hg in birds range from anatomic deformities in their offspring to damage in reproductive organs of adults (Eisler, 2010). Individuals with large concentrations of Hg in blood have been found to skip reproduction (Tartu et al., 2013), and experience a reduced breeding success (Evers et al., 2008; Goutte et al., 2014). These sublethal effects can provide early alerts to environmental degradation derived of heavy metals. Moreover, individuals within a population can vary considerably in their heavy metals concentration, thus it is necessary to consider sex and age-class to fully understand the dynamics of contaminants (Robinson et al., 2012), and to identify groups of concern from a demographic perspective (Goutte et al., 2014). Sex-related differences in heavy metals concentration can be attributed to sex-specific diet or metabolism (Robinson et al., 2012). In herring gulls (*Larus argentatus*), excretion of metals through eggs results in lower Hg concentration in females (Lewis et al., 1993). However, in penguins, eggs were not considered an important elimination route of heavy metals (Honda et al., 1986), and in skuas, females and males had similar

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Hg concentrations in blood (Goutte et al., 2014). In giant petrels (*Macronectes halli* and *Mastigoproctus giganteus*) Cd concentrations were significantly higher in females (more pelagic foraging habits) than in males (more scavenging habits) (González-Solís et al., 2002). Then, intersexual differences in heavy metals have been documented in several species, but without a homogeneous pattern, and possibly with several mechanisms involved. In chicks, heavy metals can experience a dilution in blood as a consequence of gaining weight (Ackerman et al., 2011; Wayland et al., 2002), eliminating metals through feathers (Condon and Cristol, 2009), or being fed on smaller prey than adults that contain lower heavy metal concentrations (Evers et al., 2005).

The Blue-footed Booby (*Sula nebouxi*) inhabits the Gulf of California, and is associated with arid islands near upwelling waters (Nelson, 1978). Blue-footed Booby is a piscivorous seabird that, by plunge diving, prey mainly on sardines and anchovies in the first 3–4 m of water column (Zavalaga et al., 2007; Castillo-Guerrero and Mellink, 2011; Ancona et al., 2012). At the study site, with a multi-source Bayesian mixing model, the diet composition of adult birds varied throughout the breeding season, but the primary prey were Pacific anchovy (*Cetengraulis mysticetus*) and common halfbeak (*Hyporhamphus unifasciatus*), with a combined contribution of 50–60% (González-Medina, 2016). During foraging trips distance traveled is regularly <30 km (Weimerskirch et al., 2009). Breeding season and colony sites of this seabird is predictable due to its high philopatry (Kim et al., 2007). The Blue-footed Booby is a sexually dimorphic seabird, with females heavier (30–32%) and larger (5–10%) than males (Torres and Drummond, 1999). Both sexes forage in the same areas (Zavalaga et al., 2007) and have similar diet composition (Ancona et al., 2012; Castillo-Guerrero and Mellink, 2011), but females can consume larger fishes than males (Castillo-Guerrero and Mellink, 2011; Zavalaga et al., 2007). Larger fish often have higher heavy metal concentration than smaller fish (Eisler, 1987). Blue-footed Booby chicks grow from 50 g at hatchlings to 1500–2000 g at 65 days old; after 80 days they have juvenile plumage (Nelson, 2005). Characteristics of the Blue-footed Booby make this species a good candidate to assess heavy metal concentrations in the environment and to the best of our knowledge, there are no studies available on heavy metals concentrations in seabirds like the Blue-footed Booby in the region. The objectives of the study were: 1) to assess variations of heavy metal concentrations over time (within and among seasons) in blood of breeding adults of the Blue-footed Booby, 2) to assess heavy metal concentrations in relation with their breeding participation (laying or not laying eggs), and numbers of eggs and chicks, and 3) to evaluate differences of heavy metal concentration in blood according to sex and age.

2. Methods

Bahía Santa María (24°50'–25°10'N and 107°55'–108°20'W) is a coastal lagoon system in Sinaloa, northwestern Mexico, surrounded by agriculture and aquaculture areas (Montaño-Ley and Paéz-Osuna, 2012), and receives wastewater from its drainage basin and Culiacan City (Páez-Osuna et al., 2007). Isla El Rancho, located in the north part of Bahía Santa María, harbors a colony of approximately 3000 pairs of the Blue-footed Booby. We visited the colony during the breeding season (November to May) in 2010–2011 and 2011–2012 to collect blood samples of breeding adults and chicks. In 2010–2011, 119 blood samples of adults were obtained from 63 nests: 60 samples from parents during incubation, 34 from parents during early rearing (chicks of 4–5 weeks old), and 25 from parents during late rearing (chicks of 10–12 weeks old). In 2011–2012, 124 blood samples of adults from 27 nests were collected (repeated measures): 48 from parents during courtship, 27 from parents during incubation, 25 from parents from early rearing (chicks of 4–5 weeks old), and 24 from parents during late rearing (chicks of 10–12 weeks old). We also collected blood samples of chicks, 32 individuals in 2010–2011 and 15 individuals in

2011–2012. Chicks were 85.7 ± 7.4 days old and had reached their asymptotic growth, thus they were in the pre-fledging stage. Chicks were classified as females or males using the length of their ulna at a mean age of 80 days, when it reached an asymptotic, bimodal distribution that did not overlap between the sexes (Drummond et al., 1991; Torres and Drummond, 1997). In both breeding seasons, we assigned a number to each nests and check it each visit to register the laying date, the number of eggs laid, and chicks raised. Blood samples were extracted from the brachial vein with a 25-gauge syringe, collected in vacutainer tubes, and then frozen.

In the laboratory, blood samples were lyophilized for 72 h (133×10^{-3} mBar and -49 °C). Samples (0.25 g) were predigested overnight with nitric acid (trace metal grade) using Savillex Teflon vessels. Digestion was made on a hot plate at 120 °C for 3 h. Analyses were carried out by atomic absorption spectrophotometry in a Varian SpectrAA220 equipment with a deuterium background correction. Graphite furnace atomic absorption spectrophotometry (GFAAS) was used for Pb and Cd measurements. For total Hg, analyses were carried out by reducing Hg compounds in solution samples using SnCl₂ (Loring and Rantala, 1995), measurements were made by cold vapor atomic absorption spectrophotometry (CVAAS). Detection limits were 0.05 ppm for Cd; 0.36 ppm for Pb, and 0.07 ppm for Hg. Heavy metal concentrations are expressed as ppm on a dry weight basis. To check for contamination, blanks were read every 15 samples. The quality of the analytical method was assessed by trace metal determination of certified material DOLT-4. Readings of Cd, Pb, and Hg of certified material were acceptable (recoveries: Hg 92%, Cd 98%, and Pb 92%).

Sea surface temperature (SST) was used as a proxy of upwelling, which can influence heavy metal availability in the environment (Páez-Osuna and Osuna-Martínez, 2015). SST grid data was obtained from satellite Aqua MODIS 0.0125 degrees (<http://coastwatch.pfeg.noaa.gov/>). QGIS 2.6.1 software was used to calculate the monthly SST average on a 30 km radius from the colony according to foraging area for this species (Weimerskirch et al., 2009).

To assess variations of heavy metal concentrations during the breeding season in blood of adults of the Blue-footed Booby, generalized linear models (GLM) were performed to test the effect of the breeding stages (courtship, incubation, early rearing and late rearing), the SST (sea surface temperature), and the date when the sample was collected on heavy metal concentrations of blood of adults. GLM analyses were performed separated by breeding season, because sampling methodology was different (In 2010–2011, each sample was obtained from different individual, whereas in 2011–2012, samples were obtained from the same individuals in each breeding stage (repeated measures)), therefore, in 2011–2012 analyses 'individual' was included as a random factor. Previous to the analyses, covariates were tested in multiple regressions. Larger individuals are expected to dilute heavy metal concentrations in their blood (Wayland et al., 2002), thus individuals body mass was considered a covariate. Laying date is related with experience and age of parents (Arnold et al., 2004), and this may influence Hg ingestion and accumulation (Evers et al., 2008), so laying date was used as a covariate. Only significant covariates were included in analyses.

To assess heavy metal concentrations in relation with breeding participation (laying or not laying an egg), and numbers of eggs and chicks, generalized linear models (GLM) were performed to test the effect of sex (females and males), the current breeding output (breeding participation, clutch size, or number of chicks), the interaction of sex and breeding output, and the breeding season (2010–2011 or 2011–2012). Analyses were performed by each breeding stage separately. To assess heavy metal concentration in blood according to age-class, chicks and adults samples from simultaneous periods were used. A two-way analysis of variance (ANOVA) was performed to test the effect of the sex (females and males), the age-class (chicks and adults), and the interaction sex and age class in Hg concentration in blood. For these analyses, no covariates were included, as they were already tested in

the general GLM. We report means (\pm standard error). Significant results were considered at $P < 0.05$. All statistical analyses were performed using Statistica Software (StatSoft, Inc.).

3. Results

Pb and Cd concentrations were under detection limits of 0.36 and 0.05, respectively. Hg was in 300 of 301 analyses (99.6%) over the detection limit of 0.07 ppm. Although data were analyzed by each breeding season with different model structure (2010–2011 included body mass as covariate $F_{1,113} = 9.58$, $P < 0.01$, $r^2 = 0.07$; 2011–2012 individual as random factor $F_{48,70} = 3.03$, $P < 0.01$ and not covariates), Hg blood concentrations were related to date in both breeding seasons. Higher Hg blood concentration occurs early in the breeding season and then decreases gradually toward the end of the breeding season (2010–2011: $F_{1,110} = 10.94$, $P < 0.01$; 2011–2012: $F_{1,70} = 5.34$, $P = 0.02$; Fig. 1). In both breeding seasons, the breeding stage (2010–2011: $F_{2,110} = 0.02$, $P = 0.97$; 2011–2012: $F_{3,70} = 0.72$, $P = 0.54$), and the SST (2010–2011: $F_{1,110} = 0.19$, $P = 0.65$; 2011–2012: $F_{1,70} = 2.42$, $P = 0.12$) were not significantly related with blood Hg concentrations.

Hg concentrations in blood were not related to breeding output at any stage. Hg in blood was not related with laying or not laying an egg ($F_{1,44} = 1.16$, $P = 0.28$), neither with the clutch size ($F_{2,63} = 1.15$, $P = 0.22$), nor with the number of chicks hatched or raised to fledgling ($F_{1,49} = 2.67$, $P = 0.10$, and $F_{1,42} = 1.49$, $P = 0.22$, respectively), and the

interaction terms were not significant ($P > 0.10$). Hg concentration was higher in 2010–2011 than in 2011–2012 in incubation ($F_{1,63} = 23.48$, $P < 0.01$), early rearing ($F_{1,49} = 29.37$, $P < 0.01$), and late rearing ($F_{1,42} = 11.83$, $P < 0.01$, Fig. 2). Males had larger Hg concentration than females during courtship ($F_{1,44} = 6.99$, $P = 0.01$), incubation ($F_{1,63} = 4.42$, $P = 0.04$) and late rearing ($F_{1,42} = 9.55$, $P < 0.01$, Fig. 2). No intersexual differences were detected during early rearing ($F_{1,49} = 2.59$, $P = 0.11$).

The interaction sex-age class was significant ($F_{1,86} = 13.68$, $P < 0.01$), Hg concentrations in blood of adults were higher than in chicks, adult males had significantly higher Hg concentrations than adult females, without sex-related differences in chicks (Fig. 3).

4. Discussion

In the Blue-footed Booby Cd and Pb were below the limit of detection whereas Hg levels in blood showed variations according to breeding season, date, sex, and age-class. In adults, Hg levels had no relation with reproductive parameters.

In other studies in the area, Cd and Pb have been measured in fish (*Mugil cephalus*, *Diapterus* spp. and *Lutjanus* spp.; Frías-Espéricueta et al., 2014), sharks (*Sphyrna lewini* and *Carcharhinus leucas*, Ruelas-Inzunza and Páez-Osuna, 2007), and oysters (*C. corteziensis* and *C. palmula*, Páez-Osuna and Osuna-Martínez, 2015) reaching levels greater than the maximum permissible limits in some species (Frías-Espéricueta et al., 2014; Ruelas-Inzunza and Páez-Osuna, 2007). It is possible that these elements are absent or occurring at low concentrations in the prey that the Blue-footed Booby consumes (mainly Pacific anchovy and common halfbeak), or are efficiently transported toward target tissues where they are stored and are not free to circulate in blood (Becker et al., 2003; Carravieri et al., 2014). It has been reported that some pelagic fishes have low Cd concentrations (Thompson, 1990). Similarly, Pb is accumulated more in sediments than in biota, thus it is less likely to be biomagnified (Ruelas-Inzunza and Páez-Osuna, 2008). Adverse effects had been detected even at levels of 0.01–0.09 ppm of Pb in blood of birds (Eisler, 2010), and Cd in blood occur in birds at levels as low as 1 ppb (García-Fernández et al., 1996). These levels are below our detection, thus it is needed a different analytical technique to evaluate Pb and Cd exposure in our area.

Hg concentrations in blood of Blue-footed Booby adults were among the levels known to cause adverse effects in some bird species (Eisler, 1987). Even so, we fail to detect a relation between Hg concentration and effects in their current breeding effort (laying or not laying an

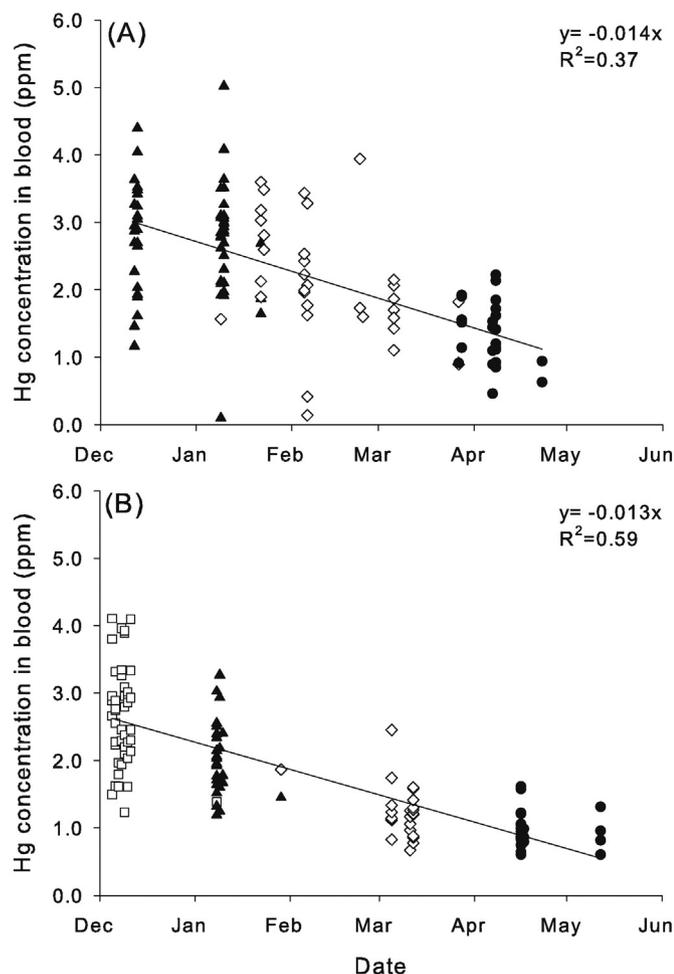


Fig. 1. Mercury (Hg) concentration in blood of adults of the Blue-footed Booby (*Sula nebouxi*) according to date in 2010–2011 (A) and 2011–2012 (B) breeding season in Isla El Rancho, Sinaloa, Mexico. Symbols indicate breeding stage: courtship (□), incubation (▲), early rearing (◇), and late rearing (●).

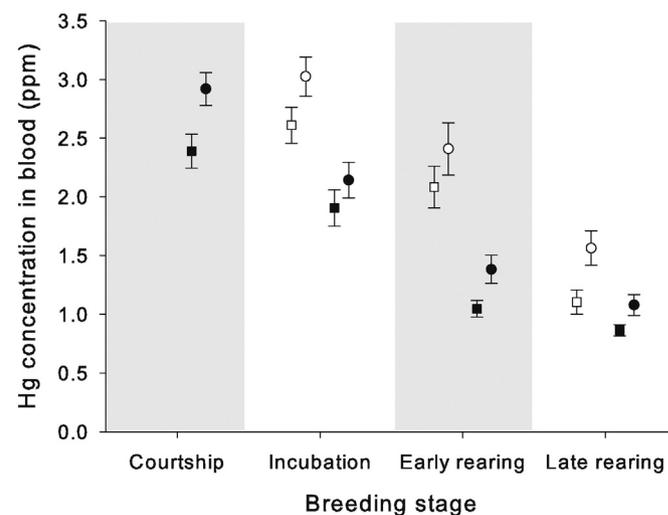


Fig. 2. Mean mercury (Hg) concentration (\pm standard error) in blood of adult male (○) and female (□) of the Blue-footed Booby (*Sula nebouxi*) during 2010–2011 (open symbols) and 2011–2012 (closed symbols), at Isla El Rancho, Sinaloa, Mexico.

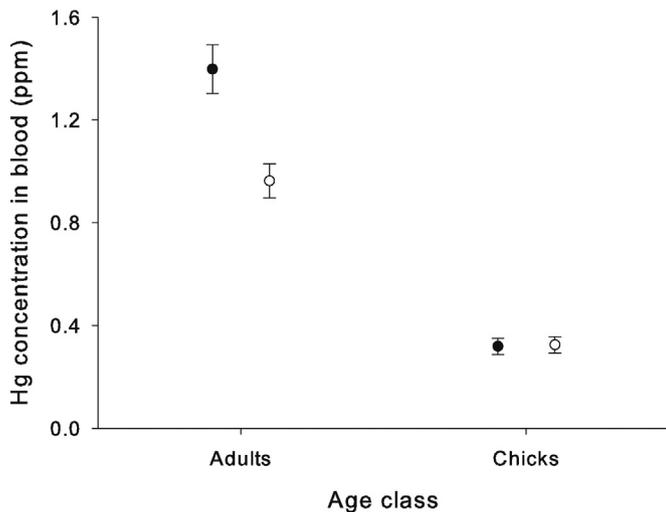


Fig. 3. Mean mercury (Hg) concentrations (\pm standard error) in blood of the Blue-footed Booby (*Sula nebouxi*) of male (●) and female (○) by each age-class (adults and chicks) at Isla El Rancho, Sinaloa, Mexico.

egg), or in the number of eggs and chicks raised to fledging. The levels of Hg in chicks and its consequences in their health were not evaluated. This does not imply that there are no adverse effects of Hg in the Blue-footed Booby. Adverse effects of heavy metals include an increase in the physiological stress in birds and/or increased susceptibility to diseases, which can lead to debilitation and death (Debacker et al., 1997). Piscivorous birds can cope with elevated Hg concentrations because they have mechanisms to deal with natural levels of Hg (e.g. demethylating mercury; Muirhead and Furness, 1988) that might be harmful to them; this issue is of concern in terms of toxicity, another protection process is provided by selenium that may be a binding site for demethylated Hg (Eagles-Smith et al., 2009; Thompson and Furness, 1989). Furthermore, the prey consumed by Blue-footed Booby are subject to commercial fisheries (Lluch-Belda et al., 2014), so high Hg concentration in this species is of concern from a commercial and ecological perspective.

The variations of Hg in blood of the Blue-footed Booby were not related with the breeding stage, and the reduction in blood concentration was similar in males and females before and after laying. Also the Blue-footed Booby do not molt wing feathers during the breeding season (Howell, 2010). This suggests that the gradual reduction in Hg concentrations in blood during the breeding season of the Blue-footed Booby is not related to intrinsic factors and is more likely related to its environmental availability. In marine environments, Hg has lower values at the sea surface and higher values at 100–500 m of depth (Strode et al., 2010), when upwelling occurs, cold waters enriched with Hg move up to the surface, where phytoplankton incorporate Hg into the food web (Bargagli et al., 1998). In the east margin of the Gulf of California, the upwelling occurs seasonally, and reaches its maximum intensity between December and January, when a persistent and effective pump of nutrient rich cold waters are driven to the surface (Lluch-Cota, 1999). Colder water temperatures in the Gulf of California are associated to strong upwelling (Lluch-Cota, 1999). Other marine species in the Gulf of California, such as the mussels (*Mytilus californianus*) also had their highest Hg concentrations in January (Lares et al., 2002). Moreover, the slightly colder 2010–2011 season (20.5 ± 3.2 °C) had higher concentrations of Hg than the slightly warmer 2011–2012 season (21.4 ± 3.7 °C). Afterwards, the lower Hg concentrations in blood of the Blue-footed Booby in subsequent months suggest a reduced availability of Hg plus its elimination in blood. Hg has a biological life of 2–3 months in blood of seabirds (Monteiro and Furness, 2001; Øverjordet et al., 2015). Nevertheless, there is the possibility that higher Hg concentrations in blood at the onset of breeding are associated to the

Blue-footed Booby feeding on areas with higher Hg availability previous the breeding season. In the Wandering albatross (*Diomedea exulans*) non-breeding foraging habitats caused Hg-biased concentration among individuals (Carravieri et al., 2014). Information about the non-breeding foraging areas of the Blue-footed Booby is lacking and merits further investigation.

We found a consistent sex-related difference in Hg concentration, with males having higher Hg concentrations than females during the breeding season. In males and females, the Hg concentration decreased in a similar magnitude, without differences before and after egg laying. The possible Hg excretion through eggs seems not enough to explain the sex-related difference in Hg blood concentrations. Inter-sex diet differences have been reported for Blue-footed Booby, females can consume larger fishes than males (Castillo-Guerrero and Mellink, 2011; Zavalaga et al., 2007) which was expected to derive in larger Hg concentrations, the opposite to results. At the same study site, with the use of stable isotopes, the $\delta^{15}\text{N}$ values (indicator of trophic position of consumers, Bearhop et al., 2004) showed a similar temporal pattern between sexes: nitrogen isotope values of adults became more enriched in ^{15}N after the offspring hatched, with males more enriched than females during some periods (González-Medina, 2016). Despite differences in $\delta^{15}\text{N}$, both sexes show a decrease in the consumption of Pacific anchovy and increase in that of the common halfbeak between courtship and chick rearing (Gonzalez-Medina 2016). Then, diet differences seem unlikely to explain the consistent pattern of intersex difference in Hg. We lack of information about the physiological mechanisms than can promote sex-related differences in Hg concentrations (Robinson et al., 2012), and as mentioned before, about the non-breeding distribution of the Blue-footed Booby of the Gulf of California, making it difficult to draw conclusions about factors driving the sex-related differences in Hg concentrations. Nevertheless, in the Blue-footed Booby, males arrive earlier than females to their breeding colonies to prospect, select and defend a territory (Nelson, 1978). There were temporal variations in the Hg availability, being at higher concentrations at early stages of breeding. Thus, males could be exposed to higher Hg concentrations than females because they arrive earlier to the colony, when Hg is more available, and this mismatch between sexes be maintained during the breeding season.

The low Hg concentration in chicks suggests that the growing and molting process can dilute and eliminate Hg burdens in chicks (Ackerman et al., 2011; Condon and Cristol, 2009; Wayland et al., 2002). Blue-footed Booby chicks went from 50 g in recently hatched chicks to as far as 2100 g when they were sampled, and they had already completed the juvenile plumage molt, this implies the growth of down and replacement by juvenile plumage. On the other hand, Blue-footed Booby adults have an irregular and long wing molting process (Nelson, 1978; Howell, 2010). Therefore, adults lack of the acute Hg elimination mechanism that chicks have. Female chicks were heavier than male chicks and it was expected a higher Hg dilution in female chicks, but this did not occur. Instead, females and male chicks had similar Hg concentrations. This result may account that feather molting is a stronger force eliminating Hg in blood of this seabird than weight gaining alone. In shearwater chicks (*Calonectris diomedea*), the excretion percentages into the final plumage varied between 42 and 60% of intake (Monteiro and Furness, 2001).

5. Conclusion

These results should be considered as a baseline for Hg concentrations in the blood of the Blue-footed Booby because is the first study, and one of the few reporting concentrations in a tropical seabird species. For Pb and Cd, more sensitive analytical technique is needed. There were not a relation between Hg concentrations and breeding effort. We reported seasonal and annual variations in Hg blood concentrations of Blue-footed Booby. Males had higher Hg concentration than females, but sex-related differences did not occur in chicks. This suggests that

sexes consume different proportions of prey items and/or some physiological difference occur after maturation in the dynamic compartmental model. Chicks had lower concentration of Hg than adults, which can be related with Hg dilution while weight gaining and molting. We highlight the lack of information to explain with certitude the mechanisms that cause these temporal and intersex differences in Hg concentrations. This study shows the importance of factors as temporality, seasonality, sex and age of the individuals affecting Hg concentrations. Moreover, this information can be useful for different sectors involved in environmental issues; e.g., the Minamata Convention has established that the signatory countries must develop monitoring programs of Hg in diverse vertebrates, including birds, and the Blue-footed Booby may be an excellent sentinel of metal fluctuations in coastal ecosystems. From the view of global change, this type of seabirds may give valuable information on the changing patterns of metal accumulation in marine biota resulting from changes associated to water temperature variations and associated changes of productivity and ecosystem alterations.

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