



Mercury and stable isotopes portray colony-specific foraging grounds in southern rockhopper penguins over the Patagonian Shelf

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ABSTRACT

Mercury pollution is a serious global environmental issue and the characterization of its distribution and its driving forces should be urgently included in research agendas. We report unusually high mercury (Hg) concentrations (>5 µg/g) along with stable isotopes values in feathers of southern rockhopper penguins (*Eudyptes chrysocome*) from colonies in the Southwest Atlantic Ocean. We found a highly heterogeneous prevalence of Hg throughout the study area and over a three-fold higher mean Hg concentration in southernmost colonies. Variation in Hg concentrations among colonies is primarily explained by site, rather than by trophic position. We provide further support to the existence of a Hg hotspot in the food web of the Patagonian Shelf and spatially restrict it to the southern tip of South America. Our findings highlight the need for regional and colony-based seabird conservation management when high local variability and plasticity in foraging habits is evident.

1. Introduction

Mercury (Hg) is a globally pervasive pollutant with no biological function that continues to increase in the surface of the global ocean due to anthropogenic perturbations (Lamborg et al., 2014). Once dissolved in the ocean, inorganic Hg is transformed into the organic bioavailable and most toxic form of methylmercury, which is mainly deposited in sediments and coastal environments (Helmrich et al., 2021; Chen et al., 2008) and is the main bioaccumulated and biomagnified compound in higher trophic levels (Renedo et al., 2020; Evers et al., 2005). Seabirds forage at high trophic position (TP) and tend to accumulate higher concentrations of Hg (Albert et al., 2019). In general, impacts of Hg on

reproduction and physiological functions have been described for North American birds at blood concentrations of approximately 1 µg/g, while substantial impairments arise at approximately 2 µg/g (Ackerman et al., 2016). While TP can explain and predict the risk of exposure to harmful concentrations of Hg (e.g. Gatt et al., 2020), foraging habitat also drives bioaccumulation as Hg bioavailability can significantly vary with local environmental conditions (e.g. Brasso and Polito, 2013). Thus, the risk of exposure to Hg in many predators is driven by a combination of factors including local environmental conditions in their preferred foraging habitat, TP, and prey preferences (Peterson et al., 2015).

Over the past decades, stable isotopic composition of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) in seabird tissues have been widely used to outline

Abbreviations: Hg, Mercury; TP, Trophic position.

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trophic relationships between species and populations and to infer foraging areas (Inger and Bearhop, 2008). In particular, the concurrent use of stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and Hg analyses has been widely used to determine how foraging ecology may influence Hg exposure (Thompson et al., 1998). In species with broad but scattered distributions and in which sampling proves challenging, such as seabirds, the understanding of such ecological tracers and their correlation provide a unique opportunity to get insights into these complex ecological interactions (e.g. Pinzone et al., 2019).

Low Hg concentrations, below adverse effect thresholds, have been recorded in most penguin species throughout their distribution in the southern hemisphere (Brasso et al., 2015; Carravieri et al., 2016; Espejo et al., 2017). However, some populations have been identified to have elevated concentrations approaching levels associated with adverse effects (Brasso et al., 2015; Dodino et al., 2022). It is of particular interest when elevated Hg concentrations in a population appear unrelated to diet or TP, suggesting a localized increase in the bioavailability of Hg in the area in which the individuals forage.

Despite a relatively low TP compared to other penguin species, elevated Hg concentrations have been reported in feathers from southern rockhopper penguin (*Eudyptes chrysocome*) populations in the Southwestern Atlantic Ocean suggesting the existence of a potential environmental Hg hotspot (Brasso et al., 2015). This region, the Patagonian Shelf Large Marine Ecosystem (hereafter Patagonian Shelf, Heileman, 2008), is characterized by a set of diverse physical and environmental conditions: a large latitudinal shelf break, complex coastal areas, the Malvinas Basin, and the mixing of nutrient-rich polar water masses advected by the Malvinas Current (Guihou et al., 2020). The environmental complexity of this region and its extensive frontal

systems drives high productivity, which in turn attracts several seabird species to feed and breed (Acha et al., 2004). For these reasons, it is important to further investigate spatial patterns regarding the extent of this hotspot, the potential source(s) of Hg in this location, and the implications for its affected food web. This is particularly relevant for endangered top predators, such as the southern rockhopper penguin, currently assessed as Vulnerable by the IUCN (BirdLife International, 2022).

The aim of this study is to evaluate colony-specific foraging ecology (diet and foraging habitat use) in southern rockhopper penguins in order to understand the risk of exposure to Hg throughout the Patagonian Shelf. We analyzed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values together with Hg concentrations in feathers from adult rockhoppers collected in six colonies. This species has been recently proposed as an independent sister species of the eastern rockhopper penguin (*Eudyptes filholi*) (Frugone et al., 2021; Frugone et al., 2018), which compromises even more its conservation status. Moreover, two intraspecific genetic clusters have been identified for southern rockhoppers, which also differ in their foraging behavior (Lois et al., 2020). This scenario allows us to test whether these ecological tracers ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) co-vary among colonies and genetic clusters, which could provide explanatory power for documented Hg concentrations. While we expect Hg concentration and TP to be correlated because of biomagnification, spatial differences in baseline Hg could potentially override this correlation. All in all, we look for our results to provide support for the use of colony-specific information to inform vulnerability reports and the creation of conservation priorities for this species.

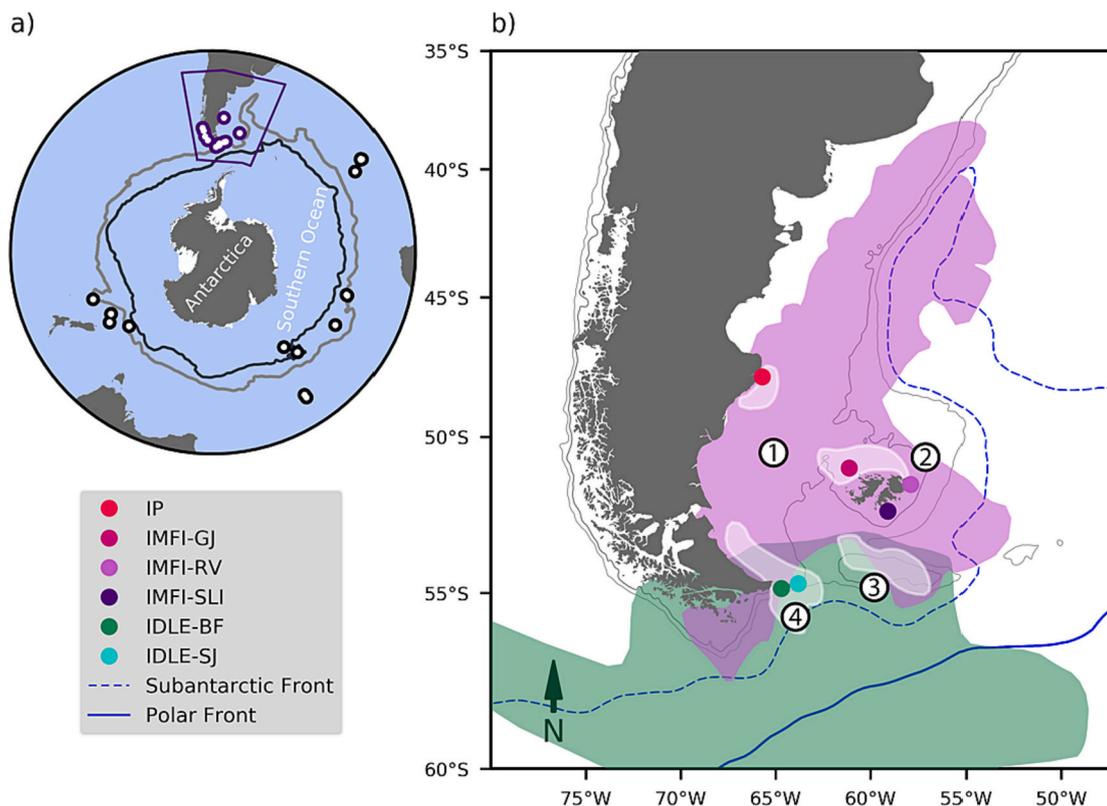


Fig. 1. a) Study area location in the context of global rockhopper penguin colonies. Sampled colonies (b) color-coded following genetic clusters described by Lois et al. (2020). Northern cluster: close to continental shore - Isla Pingüino (IP), within Islas Malvinas/Falkland Islands archipelago - Grand Jason (IM/FI-GJ), Rookery Valley (IM/FI-RV), Sea Lion Island (IM/FI-SLI). Southern cluster: within Isla de los Estados archipelago - Bahía Franklin (IDLE-BF), Cabo San Juan (IDLE-SJ). Winter foraging core areas for the species highlighted in white, IDLE individuals wintering range in light green, and IM/FI's range in light purple (Pütz et al., 2006). Numbers represent the areas with bibliographical compilation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for baseline estimation: Mid-Shelf (1), IM/FI (2), Burdwood Bank (3) and IDLE (4) (see Methods). 200 and 1000 m isobaths plotted in light gray (NOAA National Geophysical Data Center, 2009). Southern Ocean fronts plotted in blue (Kim and Orsi, 2014). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Methods

2.1. Study area

During the breeding season 2016–2017 (November–January), we visited six colonies in the Patagonian Shelf (Fig. 1, Supplementary Table 1), encompassing two previously reported genetic clusters for the species (Lois et al., 2020). We will follow the southern and northern groups proposed therein. Within the northern group we sampled Rookery Valley (RV), Grand Jason (GJ) and Sea Lion Island (SLI), all three in Islas Malvinas/Falkland Islands (IM/FI); and a small, recently founded (ca. 1980) colony in Isla Pingüino (IP), Santa Cruz, Argentina (Gandini et al., 2017). The southern group is represented by two colonies facing opposing coasts on Isla de los Estados (IDLE), Argentina: San Juan de Salvamento (SJ) and Bahía Franklin (Fr).

2.2. Feather sampling

We collected back feathers from 72 adult southern rockhopper penguins. We focused on feather sampling because it is not invasive and an effective tissue for monitoring Hg (Carravieri et al., 2013). Penguins undergo a catastrophic feather molt which occurs during late summer in which all body feathers are lost and replaced within a short period of time. This allows the employment of any body feather to estimate Hg and stable isotopes ratio (Brasso et al., 2013). During the approximately three-week molting period penguins stay on land and fast, and thus the newly grown feathers mainly integrate Hg uptake from recent ingestion, but also Hg accumulated since the previous molt in endogenous tissues mobilized due to molting combined with nutritive stress (Furness et al., 1986; Renedo et al., 2018; Squadrone et al., 2019; Quillfeldt et al., 2022). Carbon and nitrogen stable isotopes compositions integrate the foraging trip that penguins undertake before the molt, a difference in integration time which has raised concern due to its lack of consideration in several bird studies (Bond, 2010). The sampled feathers used in this study correspond to Hg accumulation since the previous molting period (i.e. February 2015–February 2016), with a higher prevalence of the pre-molt foraging trip exposure (Late January–early February 2016). We restrict our inferences by acknowledging that Hg integrates a longer time than stable isotopes, but we also rest assured that the catastrophic feather molt in this species ascertains the origin and time of production of each measured feather.

2.3. Mercury and stable isotopes analyses

We analyzed both Hg concentration and carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope values of the feathers. In short, 4–6 feathers from each individual were cleaned using a 2:1 chloroform-methanol solution to remove any exogenously deposited oils or contaminants, air-dried for 24 h, and then cut into small fragments. We subsampled 10 mg of these fragments to measure total Hg using a Nippon MA-3000 Direct Mercury Analyzer at Weber State University (Ogden, UT, USA). In order to measure accuracy, each set of 20 samples was preceded and followed by two method blanks (i.e. an empty sample boat measure), two sample blanks (i.e. distilled water), and two samples of standard reference material (TORT-3 lobster hepatopancreas, $0.292 \pm 0.022 \mu\text{g/g}$ Hg, National Research Council Canada). Mean percent recovery for the standard reference material was $102.8 \pm 7.6 \%$ ($n = 16$). The relative significant difference among standard reference material samples was 7.4% ($n = 16$) and the detection limit of the assay was 0.0015 ng Hg , with no samples falling below this limit. A second 0.6 mg subsample of feather was weighed into a tin capsule and analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values using a Costech ECS4010 elemental analyzer coupled to a Thermo-Fisher Delta Plus XP or Delta V Advantage continuous-flow stable isotope ratio mass spectrometer (CFIRMS) at Louisiana State University (Baton Rouge, LA, USA). Stable isotope values were normalized on a two-point scale using glutamic acid reference materials

(i.e. USGS-40: $\delta^{13}\text{C} = -26.4 \text{ ‰}$, $\delta^{15}\text{N} = -4.5 \text{ ‰}$; USGS-41: $\delta^{13}\text{C} = 37.6 \text{ ‰}$, $\delta^{15}\text{N} = 47.6 \text{ ‰}$). Sample precision based on repeated reference materials were 0.1 ‰ both for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Stable isotope values were calculated with the following Eq. (1) and are expressed in standard delta (δ) notation in per mil units (‰):

$$\delta X = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000 \quad (1)$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. The R_{standard} values were based on Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}\text{C}$ and atmospheric N_2 (AIR) for $\delta^{15}\text{N}$ values.

2.4. Data analysis

In order to assess differences between colonies we first examined the distribution of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ values and Hg concentration in feather samples. We thus decided to implement separate generalized least square models (GLS) in R environment (R Core Team, 2020, version 4.1) with *varIdent* variance function (*nlme* package; Pinheiro et al., 2022), because of the unbalanced sample sizes and the lack of homoscedasticity. We assigned each tracer as a response variable for each model, and the colony as an explanatory variable. We then conducted a *posteriori* Games-Howell test for multiple comparisons between colonies.

We gathered $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ published baseline values to refer each sample to the closest available baseline references (Supplementary Table 2, Riccialdelli et al. in prep, Quillfeldt et al., 2010, Ciancio et al., 2008). Southern rockhopper penguins mainly prey on offshore pelagic prey, especially during pre-molt and non-breeding season (Pütz et al., 2013). We thus compiled offshore pelagic herbivores represented in rockhopper penguin's diet (Raya Rey and Schiavini, 2005; Raya Rey et al., 2007; Clausen and Pütz, 2002; Pütz et al., 2001). We geographically clustered these baselines values within sampling regions (see Fig. 1). Additionally, wintering grounds were characterized following previous telemetry studies on the species (Pütz et al., 2006; Ratcliffe et al., 2014).

To estimate TP, we used *trophicPosition* package (version 0.7.7, Quezada-Romegialli et al., 2018) in R software (R Core Team, 2020, version 4.1). This package estimates TP through a Bayesian approach, avoiding limitations of other methods by including variability in baseline input and propagating sampling error in the estimation of the model parameters. When two baselines are provided, this package also produces an estimation of the proportion of each baseline used for the estimation of TP (i.e. alpha). We separated the data in three distinct geographical groups (IM/FI, IP and IDLE). For each one, we used both a local and a distant or a relatively offshore baseline to account for possible effects of regional variability and the mobility of the rockhopper penguin. We used the baseline compilation previously mentioned, assuming a trophic level two (i.e., primary consumers) to build the baselines. We conservatively set up the default trophic discrimination factor (Post, 2002). To estimate alpha and TP, we used the *multiSpeciesTP* function with the *twoBaselinesFull* model, since two clusters (IM/FI and IDLE) included more than one sampling site. We use 50,000 iterations as adaptive sampling; 50,000 iterations were discarded as burn-in and we ran 50,000 iterations as posterior sampling. We evaluated differences in TP between colonies using the *credibilityIntervals* function (Quezada-Romegialli et al., 2018).

3. Results

We report a more than three-fold higher mean total Hg concentration in colonies in the southern group than the northern and also found significant differences between both groups in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Fig. 2, Supplementary Table 1). When evaluated between colonies, differences are also significant in $\delta^{13}\text{C}$ ($F_{5,66} = 50.4$, $p < 0.001$), $\delta^{15}\text{N}$ values ($F_{1,66} = 143.5$, $p < 0.001$) and Hg concentration ($F_{5,66} = 101.3$, $p < 0.001$). A decreasing North to South trend is evident for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

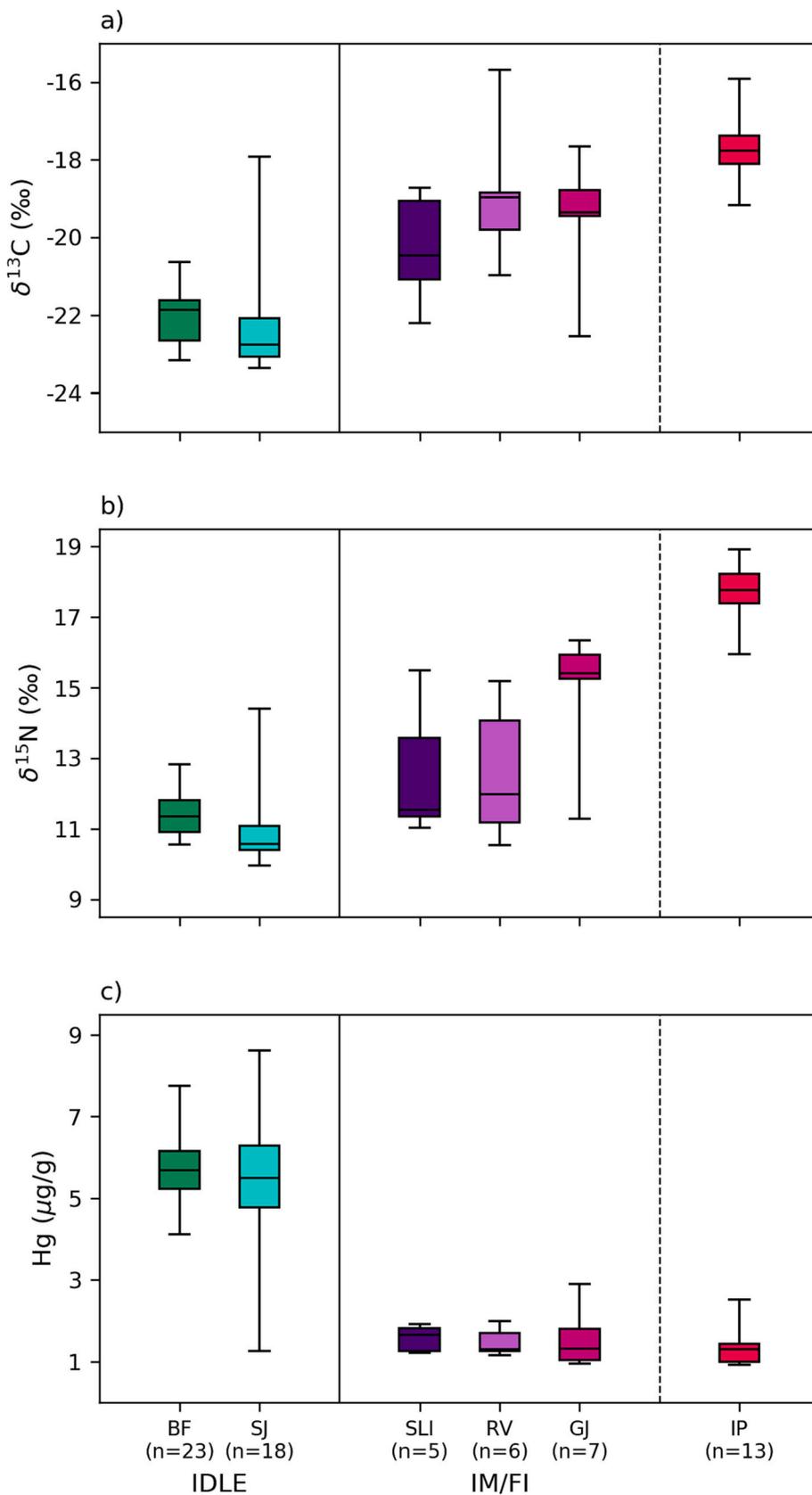


Fig. 2. $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and Hg concentration for adult rockhopper penguin feathers by colony. The solid vertical line separates north and south genetic clusters and the dashed vertical line separates the recently founded IP colony within the north cluster. Each box extends from the lower to upper quartile values with a line at the median and the whiskers represent the whole range.

Southernmost colonies, IDLE-BF and IDLE-SJ had the lowest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and, at the same time, the highest Hg concentrations (Fig. 2).

TP estimates were calculated by offering two baselines for all rockhopper penguin colonies. Confidence intervals highly overlap between colonies and medians range from approximately 3 to 4.5. IM/FI-GJ shows the lowest TP, while IP, the northernmost and recently founded colony within the same genetic cluster, shows the highest TP. Overall, TP does not respond to genetic cluster nor correlates with Hg concentrations for rockhopper penguins over the Patagonian Shelf (Fig. 3).

The methodological approach followed in this work allows the estimation of the proportion of baseline used for the Bayesian TP estimation, which in our case were set to a local source and a more remote/offshore source. Colony-specific confidence intervals for this proportion (alpha) are higher than 0.5 (Fig. 4), which indicate a higher prevalence of local food sources. IP presents a lower alpha value, but it also presents the largest confidence interval, which ranges from 0.03 to 0.95.

4. Discussion

In this study we show that regardless of their TP, southern rockhopper penguins' exposure to Hg is largely driven by site, which can be explained by differences in Hg baseline in the specific foraging areas used by each colony. The relatively high variation in stable isotope values between IM/FI and IP colonies contrast with their negligible variation in Hg. This further suggests that Hg exposure is relatively independent of TP, but related to baseline concentrations at foraging grounds. Although no correlation between Hg prevalence and TP was found, bioaccumulation is still evident in this system through the higher Hg burden of the main terrestrial predator of southern rockhopper penguins, the striated caracara (*Phalacrocorax australis*) (Balza et al., 2021). Considering the high correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in this study (see Fig. 2 and Supplementary Fig. 1), Hg turns into an informative and relatively independent ecological tracer, which may offer further information on the foraging ecology of this species. In fact, two genetic clusters reported for rockhopper penguins in the Patagonian Shelf are consistent with the differences found in Hg prevalence, which reinforces the foraging segregation of these clusters (Lois et al., 2020). The high regional and local variability in foraging habits results in sharp intra-specific genetic structure with potential differences in the exposure to heavy metals, which should be considered in future vulnerability assessments of rockhopper penguin species. In particular, Hg mean

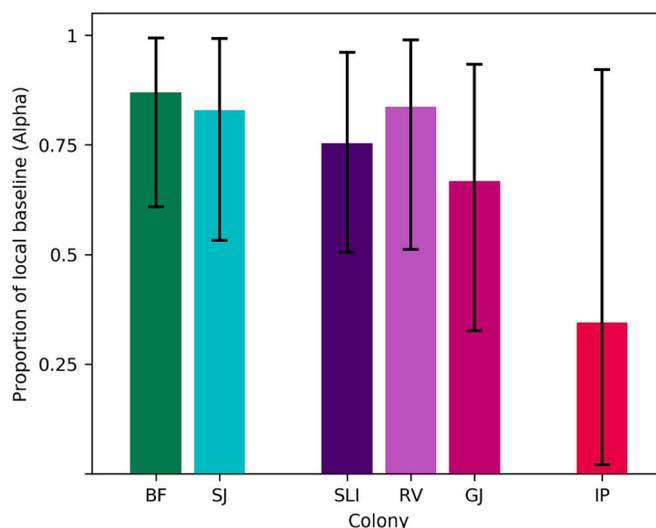


Fig. 4. Proportion of baseline (alpha) used in TP analysis expressed as local over distant-offshore krill baseline.

concentrations are over 5 $\mu\text{g/g}$ in IDLE colonies, three times higher than neighbour IM/FI archipelago, placing individuals at risk for adverse effects of Hg (Chastel et al., 2022; Evers et al., 2008).

Previous studies on seabirds highlight both foraging habitats and TP as relevant drivers of Hg concentrations at the regional scale between and even within species (Binkowski et al., 2021; Thébaud et al., 2021; Polito et al., 2016). In agreement with previous findings on the highly variable southern rockhopper penguins foraging strategies (Pütz et al., 2018; Pütz et al., 2013; Dehnhard et al., 2011), we found both high variability and a high degree of overlap in TP confidence intervals between colonies in the study area. Still, the underlying spatial negative North to South trend in modeled $\delta^{13}\text{C}$ values (Magozzi et al., 2017) and in *in-situ* observations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Lara et al., 2010) is evident though the stable isotopes ratio pattern found for rockhopper penguins in the present study (Fig. 2) and mirrored by other marine predators in the area (Forero et al., 2005; Dehnhard et al., 2011; Baylis et al., 2016; Ciancio et al., 2021; Drago et al., 2021). The high relative proportion of local baseline used for the TP modeling (Fig. 4) suggests that these birds mostly forage close to their nesting grounds during pre-molt, which aligns with foraging trips recorded through geolocation tags (Dodino et al. in prep), the relative contribution of offshore baseline in TP modeling is not negligible. For instance, the median relative contribution of the Burdwood Bank baseline is 16–35 % for IM/FI and 13–17 % for IDLE, supporting the idea of an area of foraging overlap among these two populations. Previous evidence of this overlap is provided by the tracks of tagged individuals in both archipelagos (Pütz et al., 2006, Green et al. in rev.).

As for the spatial scale of the outlying high Hg concentrations presented in this study, our findings support Brasso et al.'s (2015) proposal for a Hg exposure hotspot in the Patagonian Shelf. Globally, comparatively low Hg values have been reported for penguin species (Becker et al., 2016) with a latitudinal north to south negative trend (Carravieri et al., 2017; Mills et al., 2022). Off the coast of South America towards Antarctica and South Georgia, Hg concentration in seabirds decreases (Becker et al., 2016; Brasso et al., 2015; McKenzie et al., 2021). Recent studies in the Patagonian Shelf also reflect a contrasting North-South difference in Hg prevalence for Magellanic penguins (Quadri-Adrogué et al., 2022; Dodino et al., 2022). In agreement with previous results, which support an oceanic mesopelagic rather than a neritic epipelagic source for Hg in seabirds (Renado et al., 2020; Thébaud et al., 2021), Fioramonti et al. (2022) found a higher trophic transfer of Hg in oceanic waters of the Burdwood Bank than in coastal waters of Tierra del Fuego. Magellanic penguins, a species which forages closer to shore than

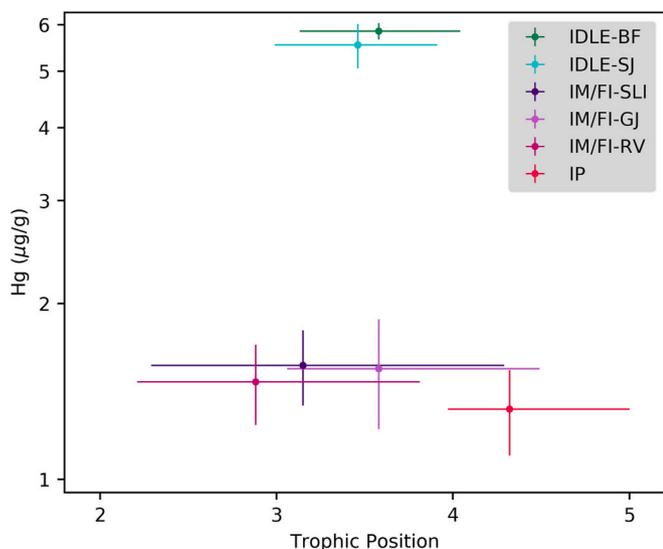


Fig. 3. Modeled TP and Hg concentration in southern rockhopper penguin feathers (y-axis has been log-scaled).

rockhoppers (Rosciano et al., 2016) also present an overall lower Hg burden following an inshore-offshore increase in Hg exposure (Dodino et al., 2022). When considered together, these findings spatially restrict the Hg hotspot in this region to an offshore area within Tierra del Fuego and the Burdwood Bank and not further south than the Polar Front.

Although so far there has been no biogeochemical proposal on the source of the uncharacteristically high Hg concentration around Tierra del Fuego archipelago, biological factors in Hg trophic transfer in the Southern Ocean water masses have been proposed as a relevant driver of this Hg hotspot (Fioramonti et al., 2022). As for a potential geological origin, Magellan-Austral is the youngest of the five productive oil basins in Argentina, where exploration and exploitation both inland and offshore have taken place since 1949 (Rosello et al., 2008). The Malvinas Basin hydrocarbon fields close to the Fuegian archipelago present long-term leakage history of both liquid and gaseous hydrocarbons (Baristead et al., 2012) with potential implications on Hg availability in its sediments. On the other hand, anthropogenic drivers are highly unlikely: industries, urbanization, an incipient historical artisanal gold exploitation and recent industrial mining, have little development and its spatial extent is highly local. All together, these potential sources present little support as drivers of the proposed regional hotspot, which calls for Hg prevalence to be further investigated in sediments, water column and island soil, as well as within the food web throughout the year (e.g. Quillfeldt et al., 2022). In the future, Hg stable isotopes composition (as in Renedo et al., 2020) could be used to unravel causes for these unusual Hg levels and tailor mitigation and/or adaptation policy in this region accordingly.

In general, seabird feather Hg concentrations have been reported to increase in the last decades in several regions of the Southern Ocean (Mills et al., 2020), which has been attributed to changes in global Hg anthropogenic emissions (Lamborg et al., 2014). However, contradictions have risen when lower Hg concentrations in modern samples relative to historic have been reported and explained by temporal changes in food web exposure, TP, or dietary composition changes (Gilmour et al., 2019). Further, differences in feather Hg concentrations in seabirds between age classes (Bighetti et al., 2021; Dodino et al., 2022) and sex (Mills et al., 2022) have been documented, highlighting the importance of exploring underlying mechanisms when examining spatio-temporal patterns of Hg prevalence. In this context, our findings raise awareness towards the southern tip of South America islands and their surrounding water masses and calls for regional long-term Hg monitoring and conservation planning in this area and for the species therein.

CRediT authorship contribution statement

Nicolás A. Lois: Conceptualization, Methodology, Investigation, Software, Data curation, Formal analysis, Validation, Visualization, Writing – original draft, Writing – review & editing. **Ulises Balza:** Conceptualization, Methodology, Investigation, Software, Data curation, Formal analysis, Writing – review & editing. **Rebecka Brasso:** Investigation, Writing – review & editing. **Samanta Dodino:** Software, Data curation, Formal analysis, Writing – review & editing. **Klemens Pütz:** Investigation, Funding acquisition, Writing – review & editing. **Michael J. Polito:** Investigation, Writing – review & editing. **Luciana Riccialdelli:** Resources, Writing – review & editing. **Javier Ciancio:** Resources, Writing – review & editing. **Petra Quillfeldt:** Resources, Writing – review & editing. **Bettina Mahler:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Andrea Raya Rey:** Conceptualization, Methodology, Investigation, Supervision, Funding acquisition, Writing – review & editing.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2022.114137>.

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