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### ► To cite this version:

Ricardo Matias, Hugo Guímaro, Paco Bustamante, José Seco, Nesho Chipev, et al.. Mercury biomagnification in an Antarctic food web of Antarctic Peninsula. Environmental Pollution, 2022, 304, pp.119199. 10.1016/j.envpol.2022.119199. hal-03768790

## HAL Id: hal-03768790 https://hal.science/hal-03768790v1

Submitted on 4 Sep 2022

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### Mercury biomagnification in an Antarctic food web of Antarctic Peninsula

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Abstract: Under the climate change context, warming Southern Ocean waters may allow mercury (Hg) to become more bioavailable to the Antarctic marine food web (i.e., ice-stored Hg release and higher methylation rates by microorganisms), whose biomagnification processes are poorly documented. Biomagnification of Hg in the food web of the Antarctic Peninsula, one of world's fastest warming regions, was examined using carbon ( $\delta^{13}$ C) and nitrogen ( $\delta^{15}$ N) stable isotope ratios for estimating feeding habitat and trophic levels, respectively. The stable isotope signatures and total Hg (T-Hg) concentrations were measured in Antarctic krill Euphausia superba and several Antarctic predator species, including seabirds (gentoo penguins Pygoscelis papua, chinstrap penguins Pygoscelis antarcticus, brown skuas Stercorarius antarcticus, kelp gulls Larus dominicanus, southern giant petrels Macronectes giganteus) and marine mammals (southern elephant seals *Mirounga leonina*). Significant differences in  $\delta^{13}$ C values among species were noted with a great overlap between seabird species and M. leonina. As expected, significant differences in  $\delta^{15}$ N values among species were found due to interspecific variations in diet related to their trophic position within the marine food web. The lowest Hg concentrations were registered in *E. superba*  $(0.007 \pm 0.008 \ \mu g \cdot g^{-1})$  and the highest values in *M. giganteus* (12.090 \pm 14.177 \ \mu g \cdot g^{-1}). Additionally, a significant positive relationship was found between Hg concentrations and trophic levels (reflected by logarithm-transformed T-Hg values), biomagnifying nearly 2 times its concentrations each level. Our results support that trophic interactions are the major pathways for Hg biomagnification in Southern Ocean ecosystems and warn about an increase in the effects of Hg on long-lived (and high trophic level) Antarctic predators under climate change in the future.

**Keywords:** Trophodynamics; Trace metals; Stable Isotopes; Livingston Island; Trophic Magnification Factors; Southern Ocean.

#### Introduction

Marine pollution is a growing environmental problem in today's world (Häder et al., 2020). In Antarctica and the Southern Ocean, the effects of anthropogenic activities are visible and threatening the sustainability of this region's delicate ecosystems (Lohmann et al., 2006; Gutt et al., 2020). Mercury (Hg) is a non-essential metal known for its toxicity even at low concentrations, particularly in its methylated form (MeHg), and it has been acknowledged as a global toxicity problem in aquatic ecosystems (Jackson, 1998). Once thought to be pristine, the Antarctic region constitutes a sink for various pollutants such as Hg which condensate in colder regions after evaporating and undergo long-range transportation from lower latitudes (Dommergue et al., 2010; Angot et al., 2016). Consequently, the Southern Ocean present some of the highest MeHg concentrations ever recorded in open waters (Cossa et al., 2011). Even though anthropogenic emissions are negligible (Aronson et al., 2011), the volcanic activity in Antarctic Peninsula significantly contributes for a higher Hg bioavailability in this region (de Ferro et al., 2014). Besides the above-mentioned pathways, the release of Hg stored in sea ice also contributes to its availability for microbial methylation (Cossa, 2013; Gionfriddo et al., 2016). When in marine environment, Hg can be methylated by microorganisms and then bioconcentrated by phytoplankton and bioaccumulated by organisms along the food chain (Bargagli et al., 1998). MeHg in prey is bioavailable for predators and strongly retained in marine organisms once assimilated, it leads to the stepwise increase of Hg concentrations along the food chain (so called biomagnification) and to its bioaccumulation with age in organisms (Wiener et al., 2007; Tavares et al., 2013). Previous studies have demonstrated a trend for increasing Hg concentrations from producers < primary consumers < secondary consumers (Dehn et al., 2006; Eagles-Smith et al., 2018; Rodrigues et al., 2019). In the Southern Ocean, previous studies have focused on describing Hg concentrations in specific taxonomic groups (Szefer et al., 1993; Seco et al., 2020), even finding higher Hg concentrations than expected relative to other organisms from more polluted regions, particularly in long-lived predators (Tavares et al., 2013; Goutte et al., 2014; Cherel et al., 2018).

The use of quantitative Hg analysis coupled with other techniques, such as stable isotope analysis (SIA), can provide a multi–dimensional view of Hg links with ecological traits within marine ecosystems (Lavoie *et al.*, 2013). In order to assess Hg concentrations within a food web, it is essential to assess the trophic levels of their various organisms. Indeed, to our knowledge few studies are available on Hg biomagnification within Antarctic Peninsula food webs (Cipro *et al.*, 2017), a region that is affected by several threats such as anthropogenic pollution (Tin *et al.*, 2009; Aronson *et al.*, 2011) and climate change impacts (Constable *et al.*, 2014; Gutt *et al.*, 2020; Pertierra *et al.*, 2020). Stable isotope ratios of carbon ( $^{13}C/^{12}C$ ,  $\delta^{13}C$ ) and nitrogen ( $^{15}N/^{14}N$ ,  $\delta^{15}N$ ) are a very useful method to determine feeding habitat and trophic level of organisms, respectively (Cherel & Hobson, 2005; Zimmer *et al.*, 2007). In a marine context, the  $\delta^{13}C$  signatures enable the characterization of the organisms' preferential habitat as it varies according to latitude, depth and coast proximity (Cherel & Hobson, 2007; Jaeger *et al.*, 2010).  $\delta^{15}N$  signatures can be used as an indicator of consumers' trophic position as  $^{15}N$  values consistently increase from lower to higher trophic levels within a range of 2.5–3.4 ‰ per trophic level (Minagawa & Wada, 1984). According

to the considered tissues and their respective stable isotope turn-overs, stable isotopes can provide short– and long–term environmental signatures of the individual organisms (Ceia *et al.*, 2012) and become a useful tool for reconstructing trophic interactions, elucidating patterns of resource allocation and constructing food webs (Hobson & Welch, 1992; Cherel & Hobson, 2005). Even though several studies have addressed the biomagnification of Hg within food webs resorting to  $\delta^{15}$ N values as a proxy of trophic levels, only recently these have been used to determine Hg biomagnification along trophic levels and pathways in Southern Ocean food webs (Anderson *et al.*, 2009; Cipro *et al.*, 2017; Seco *et al.*, 2021).

In this study, we analysed total Hg concentrations,  $\delta^{13}$ C and  $\delta^{15}$ N values in the tissues of key functional organisms from Livingston Island, South Shetlands Archipelago (Figure 1), from zooplankton to top predators foraging in marine waters around the west Antarctic Peninsula (Muelbert *et al.*, 2013; Herman *et al.*, 2017). The main objectives of this study were to: 1) characterise the Hg concentrations in the considered organisms and their trophic levels; 2) evaluate the Hg biomagnification levels in the marine food web; and 3) discuss Hg pathways taking place in ecosystems around west Antarctic Peninsula.

#### Materials & Methods

#### Study area and species

The study area was Livingston Island (62°36′S, 60°30′W), which is the second largest island in the South Shetland Archipelago, in maritime Antarctica (Fig. 1). More specifically, samples were collected at Hanna Point, Miers Bluff Point and near shore close to the Bulgarian Antarctic base St. Kliment Ohridski research station, during January 2011 (austral summertime). Regarding climate, average air temperatures are slightly above 0 °C during summer months, dropping below –25 °C during winter. For that reason, ice-free areas in the island host colonies of various seabird and seal species.

The Antarctic krill (*Euphausia superba* Dana, 1850) was selected as it is keystone species in the Southern Ocean food web, linking between primary producers and predators. In addition, the

Samples were collected in the South Bay of Livingston Island (62°36'S, 60°30'W), part of the South Shetland Archipelago (Figure 1), specifically at Hanna Point, Miers Bluff Point and near shore close to the Bulgarian Antarctic base St. Kliment Ohridski research station, during the austral summer (January) of 2011. A wide taxonomic range of species were collected from Antarctic krill *Euphausia superba* (n = 62, pooled samples with specimens collected; from the stomachs of dissected chinstrap penguin *Pygoscelis antarcticus*, black rockcod *Notothenia coriiceps* and marbled rockcod *N. rossii*) to several predator species – gentoo penguin *Pygoscelis papua* (n = 30; feathers), *P. antarcticus* (n = 29; feathers), brown skua *Stercorarius antarcticus* (n = 5; feathers), kelp gull *Larus dominicanus* (n = 13; feathers), southern giant petrel *Macronectes giganteus* (n = 15; feathers) and southern elephant seals *Mirounga leonina* (n = 15; hair). All samples were preserved in individual bags at room temperature, with the exception of *E. superba, which* was kept at –20°C. Despite being metabolic inert, feathers are the main excretion route for Hg bioaccumulated in internal tissues until the moult. Their Hg concentrations,  $\delta^{13}$ C and  $\delta^{15}$ N values do not vary much over time and are generally in line with the diet and foraging habitat of seabirds, providing a perspective on the species Hg annual exposure (Carravieri *et al.*, 2014, Albert et al. 2019). For example, even though *S. antarcticus* is a migratory species (Kopp *et al.*, 2011; Krietsch *et al.*, 2017), their feathers should provide in a great extent information on the environment where they breed. However, we acknowledge that some exposure to Hg might be from outside areas.

The SIA protocol was performed in the MAREFOZ laboratory in Figueira da Foz, Portugal. Samples of *E. superba* were ground into fine powder (after removal of exoskeletons) and its lipidic content extracted using cyclohexane, due to its depletion in <sup>13</sup>C relative to other tissue components. Feather and hair samples were cleaned of surface contaminants using 2:1 solution of chloroform–methanol and air dried in an oven at 60°C for 48h. Thereafter, samples were cut with a stainless–steel scissor into small fragments and discarding the calamus in feathers. After homogenising all samples, approximately 0.35 mg of each sample was analysed for  $\delta^{13}$ C and  $\delta^{15}$ N values using a Continuous Flow Isotope Mass Spectrometer (CF–IRMS) coupled with an elemental analyser. Ratios were calculated using the formula,  $\delta X = [(R_{sample}/R_{Standard}) - 1] \times 1000$ , considering the standardised values of Vienna–PeeDee Belemnite (V–PDB) and atmospheric N<sub>2</sub> for  $\delta^{13}$ C and  $\delta^{15}$ N values, respectively (Hobson & Welch, 1992).

The Hg analysis was performed in the CESAM laboratory in University of Aveiro, Portugal. For the Hg analysis were used a smaller number of specimens of *E. superba*, *P. papua* and *P. antarcticus* than for SIA (see *n* size in Table 2). The total Hg concentrations were determined to the nearest 0.01 ng by thermal decomposition atomic absorption spectrometry with gold amalgamation, using an Advanced Mercury Analyser AMA LECO 254, following Coelho *et al.* (2010). Analyses were done several times until the coefficient of variation was lower than 10%. Accuracy was checked using certified reference materials (CRM) at the beginning of each set of samples. The CRM used and respective recoveries were dogfish muscle DORM–2 (98.1 ± 1.2%), mussel tissue NIST2976 (100.1 ± 12.6%) and lobster hepatopancreas TORT–2 (100.3 ± 9.8%). All Hg data is expressed as a function of dry weight ( $\mu g \cdot g^{-1} dw$ ).

Linear relationship between logarithm–transformed Hg concentrations (log<sub>10</sub>[Hg]) and <sup>15</sup>N values was used to evaluate Hg biomagnification along trophic levels according to Nfon *et al.* (2009). The trophic magnification factor (TMF) was calculated using the formula:

#### $TMF = 10^{b}$

where *b* is the slope of the linear relationship between the  $\log_{10}[Hg]$  and  $\delta^{15}N$  values, also known as trophic magnification slope (TMS). TMF represents the increase of Hg concentrations per trophic level (TL). The TL of a consumer was estimated by:

#### TL<sub>Consumer</sub>= $(\delta^{15}N_{Consumer} - \delta^{15}N_{Baseline})/\Delta^{15}N + \lambda$

where TL<sub>consumer</sub> is the trophic level of a given consumer and  $\delta^{15}N_{consumer}$  its  $\delta^{15}N$  values (Lavoie *et al.*, 2013). The  $\delta^{15}N_{Baseline}$  is the  $\delta^{15}N$  values for the baseline organism – in this study, *E. superba* – and its trophic level ( $\lambda$ , TL = 2 for primary consumers).  $\Delta^{15}N$  is the trophic discrimination factor used for <sup>15</sup>N equal to 3.4 ‰, which is the most frequently used for marine studies (Borgå *et al.*, 2012).

The data normality was assessed using Shapiro–Wilks's test, and statistical differences among species' Hg concentrations,  $\delta^{13}$ C and  $\delta^{15}$ N values were assessed by Kruskal–Wallis test and Dunn's post hoc test for multiple species' comparison. *P*–values were considered statistically significant when *p* < 0.05.

All the calculations were performed using GraphPad (La Jolla, California, USA) and R statistical software (R Core Team, 2020).

#### Results

#### Carbon and nitrogen stable isotopes across the marine food web of Antarctic Peninsula

Species differed significantly (Kruskal–Wallis test; H = 129.4, p < 0.001) regarding  $\delta^{13}$ C values, with *E. superba* presenting the lowest mean values (-25.30 ± 1.29 ‰, n = 62) and significantly differing from the rest of the species. *P. papua* values of  $\delta^{13}$ C (-22.78 ± 0.77 ‰, n = 30) only significantly differed from *E. superba* values (p < 0.001). Also,  $\delta^{13}$ C values of *P. antarcticus* (-23.36 ± 0.67 ‰, n = 29) were significantly lower than the values registered in *S. antarcticus* (-17.89 ± 1.77 ‰, n = 5; p = 0.011), *L. dominicanus* (-21.68 ± 1.09 ‰, n = 13; p = 0.050), *M. giganteus* (-20.30 ± 2.23 ‰, n = 15; p = 0.001) and *M. leonina* (-21.81 ± 0.35 ‰, n = 15; p = 0.006), which not differed between them. Only the  $\delta^{13}$ C values of *E. superba* (p < 0.001) and *P. antarcticus* were significantly lower from *S. antarcticus*.

Significant differences were also found among species in  $\delta^{15}$ N values (Kruskal–Wallis test; H = 149.7, p < 0.001). As expected, the lowest  $\delta^{15}$ N values found in *E. superba* (5.20 ± 0.72‰, n = 62) differed significantly from the rest of the species. An intermediate level of  $\delta^{15}$ N values was occupied by *P. papua* (8.69 ± 0.82 ‰, n = 30) and *P. antarcticus* (8.66 ± 0.88 ‰, n = 29), both differing from the higher values registered in *L. dominicanus* (p = 0.013 and p = 0.009, respectively; 13.49 ± 1.28‰) and *M. giganteus* (p = 0.002 and p = 0.001, respectively; 14.29 ± 1.68 ‰). Both penguin species'  $\delta^{15}$ N values did not differed from *M. leonina*. Only, *E. superba*  $\delta^{15}$ N values differed from *S. antarcticus* (p < 0.001).

Mercury levels and its biomagnification levels in the food web of Antarctic Peninsula

Significant differences in Hg concentrations were found among species (Kruskal–Wallis test; H = 83.3, p < 0.001), with overall values ranging from 0.003  $\mu$ g·g<sup>-1</sup> dw in *E. superba* to 54.680  $\mu$ g·g<sup>-1</sup> dw in *M. giganteus* (Table 1). *E. superba* Hg concentrations were the lowest (0.007 ± 0.008  $\mu$ g·g<sup>-1</sup> dw), followed by *P. papua* (0.220 ± 0.092  $\mu$ g·g<sup>-1</sup> dw), *P. antarcticus* (0.665 ± 0.464  $\mu$ g·g<sup>-1</sup> dw), *S. antarcticus* (1.104 ± 0.785  $\mu$ g·g<sup>-1</sup> dw) and *M. leonina* (1.654 ± 0.734  $\mu$ g·g<sup>-1</sup> dw). The highest mean values were recorded in *L. dominicanus* (10.755 ± 6.750  $\mu$ g·g<sup>-1</sup> dw) and *M. giganteus* (12.090 ± 14.177  $\mu$ g·g<sup>-1</sup> dw; Table 1). The Hg concentrations in *E. superba* were significantly different from the ones found in *P. antarcticus* (p = 0.037), *L. dominicanus* (p < 0.001), *M. giganteus* (p < 0.001 and p = 0.002, respectively; Table 1).

A significant positive linear relationship ( $r^2 = 0.799$ , H = 362.1, p < 0.001) was found between the log<sub>10</sub>[Hg] and the  $\delta^{15}N$  values found in Antarctic species (Figure 2). The linear relationship can be translated by the equation log<sub>10</sub>[Hg] = 0.2902( $\delta^{15}N$ ) – 3.285, where 0.2902 represents the TMS value (Figure 2). Regarding TL and considering *E. superba* as our baseline value (TL = 2.00), TL values of secondary consumers were calculated *P. antarcticus* (3.06), *P. papua* (3.20), *M. leonina* (4.16), *S. antarcticus* (4.30), *L. dominicanus* (4.48) and *M. giganteus* (4.72). Between trophic levels, it was calculated a TMF equal to 1.95 ± 1.04 based on  $\delta^{15}N$  values for a finer analysis.

#### Discussion

#### Habitat evaluation

This research provided a snap-shot of habitat, trophic levels and Hg in an Antarctic Peninsula marine ecosystem by focusing on several species that co-habitat during the austral summer. As expected, the lowest  $\delta^{13}$ C values were found in *E. superba*, similar to those reported previously (Brault *et al.*, 2018) and were significantly lower than from top predators (seabirds and southern elephant seals). This may be attributed to not only due to the small enrichments occurring along the food web (on average +1‰ per trophic level; Post, 2002) but also due to different foraging ecology of these top predators. Moreover, stable isotope values can greatly vary among taxonomic groups and within organisms (Cherel *et al.*, 2014; Dimitrijević *et al.*, 2018), thus a careful interpretation of the results must be considered.

No significant differences in  $\delta^{13}$ C values were found among top predators, with the exception between *P. antarcticus* vs. *S. antarcticus*. Such specific differences might be explained not only by the different isotope turnover rates in tissues (i.e., rate at which tissues replace their isotopic signature by stable isotopes derived from diet; Hobson & Clark, 1992) but also by the very different species' trophic ecology: *P. antarcticus* feeds mostly on euphausiid, amphipod and myctophid fish species (Rombolá *et al.*, 2010) whereas *S. antarcticus* is highly opportunistic, feeding on carrion and even preying seabirds and pinnipeds (usually chicks, pups and sick/injured adults; (Hunter, 1983; Reinhardt *et al.*, 2000). Moreover, stable isotope signatures in feathers are in line with the ecology at their time of synthesis, so variations in  $\delta^{13}$ C can also be due to different predators' foraging areas as well as different annual migratory patterns to sub–Antarctic and subtropic regions, such as *S. antarcticus*. However, since our study focuses on understanding Hg dynamics along the food web,  $\delta^{13}$ C values were only considered as a general indication of species habitat ecology during sample collection.

#### Relationships between mercury and trophic levels in the Antarctic Peninsula marine food web

The results show a positive relationship between Hg concentrations and  $\delta^{15}$ N values, as reported by previous studies in the Southern Ocean (Anderson *et al.*, 2009; Chiang *et al.*, 2021; Seco *et al.*, 2021). Based on our TL calculations, a total of three distinct trophic levels were defined throughout the species covered by this study. As noted in our results, *E. superba* showed the lowest  $\delta^{15}$ N values due to its primary consumer ecological role, mostly feeding on phytoplankton in open waters (Schmidt *et al.*, 2006). For that reason, *E. superba* occupies the 2<sup>nd</sup> trophic level among species studied. Both penguin species *P. papua* and *P. antarcticus* occupy the 3<sup>rd</sup> trophic level, presenting higher  $\delta^{15}$ N values and Hg concentrations than *E. superba*. These results were expected due to their known trophic ecology with a diet composed by zooplankton, particularly *E. superba*, fish and squid (Xavier *et al.*, 2018; Bertolin & Casaux, 2019).

Amongst top predators, *P. papua* and *P. antarcticus* showed the lowest  $\delta^{15}N$  values and Hg concentrations thus occupying a lower level relative to the species – *M. leonina*, *S. antarcticus*, *L. dominicanus* and *M. giganteus* –, which occupied the 4<sup>th</sup> and highest trophic level within our study (see TL values in results). Even though *M. leonina* and both penguin species present similar diets, the higher TL value reflected by higher  $\delta^{15}N$  values in *M. leonina* can be explained by a higher percentage of squid

prey in its diet (Bradshaw et al., 2003). Moreover, such results were expected as previous studies have reported some level of variation in Hg concentrations in seabird species influenced by diet and overall trophy ecology (Anderson *et al.*, 2009; Carravieri *et al.*, 2014). For instance, both penguin species *P. papua* and *P. antarcticus* stay all year round in Antarctic waters and are specialist species feeding mainly on krill whereas the other seabird species – *L. dominicanus*, *M. giganteus* and *S. antarcticus* – migrate and exhibit an opportunistic and scavenging foraging behaviour (Hunter, 1983; Reinhardt *et al.*, 2000; Silva *et al.*, 2001). *M. leonina* showed similar  $\delta^{15}$ N values and Hg concentrations relative to *L. dominicanus* and *M. giganteus*, despite the different nature of the tissues analysed and trophic ecology. In summary, the results of our study indicated a significant increase of Hg concentration of studied species according with their trophic position, thus indicating biomagnification in the pelagic Antarctic food web of Antarctic Peninsula.

#### Mercury pathways around Antarctic Peninsula and elsewhere

Some of the highest Hg concentrations worldwide have been recorded in marine Antarctic biota, particularly in seabirds from the Southern Ocean (Bargagli, 2008), such as the wandering albatross Diomedea exulans (from 3.8 up to 34.6  $\mu g \cdot g^{-1}$  in Cherel et al., 2018). In our study, the higher concentrations of Hg were reported in *M. giganteus* (from 1.9 up to 54.7  $\mu$ g·g<sup>-1</sup> in Table 1). The biomagnification of Hg has been addressed before by Bargagli and colleagues (1998), in the Ross Sea, reporting Hg to increase progressively and consistently in several species from different trophic levels. However, to our knowledge, few studies have investigated using stable isotopes to assess the influence of food web structure and diet on Hg concentrations in organisms of Antarctic Peninsula (Cipro et al., 2017). Our study reports a significantly positive linear relationship between  $\delta^{15}$ N values and Hg concentrations (TMS = 0.290, TMF = 1.950), similar to those registered by Seco and colleagues (2021) in Scotia Sea during the austral summers of 2007 (TMS = 0.203, TMF = 1.596) and 2016 (TMS = 0.278, TMF = 1.897). The differences in TMS values can be explained by different Hg bioavailability governed by the physiology of the organisms (Sontag et al., 2019), as well as site-specific variables, such as the proximity to the coast and ice shelves (Bargagli, 2016; Mastromonaco et al., 2017; Seco et al., 2019). Moreover, the northwest Antarctic Peninsula is a region with high volcanic activity (i.e., in Deception and Penguin islands; see Fig. 1), known natural emission sources of heavy metals (Dick, 1991). Our study supports that diet is a major pathway for Hg bioaccumulation in food webs but more studies are needed to fully understand how the taxonomic, geographic and ecological differences might influence processes driving Hg in the marine ecosystems of the Antarctic Peninsula.

As the Antarctic Peninsula is one of the fastest warming regions in the Antarctic region (Amesbury *et al.*, 2017; Rintoul *et al.*, 2018), Hg concentrations in marine biota are expected to increase, and inflict higher levels of harm, in the next years under the current climate change context due to the enhanced Hg methylation and its bioavailability in low–oxygen subsurface waters, which are likely to increase as global sea temperatures rise (Cossa *et al.*, 2011; Blum *et al.*, 2013). For that reason, Hg impacts on the structure and functional dynamics of the Antarctic food webs are likely to increase, particularly in Antarctic top

predators already presenting high Hg concentrations relative to other regions (Gutt *et al.*, 2020; Mills *et al.*, 2020).

#### Conclusion

In sum, Hg biomagnification in the coastal environments of the South Shetland Islands, located in the northeast Antarctic Peninsula, has revealed high mercury burdens in top predators. Higher Hg levels in higher trophic level predators suggest biomagnification of Hg in Southern Ocean ecosystems. This suggests that, Hg concentrations increasing under a climate change context, may cause negative effects in Antarctic organisms, particularly long-lived predators, in the near future.

Acknowledgements: This study was supported by the Portuguese Foundation for Science and Technology (FCT) through a PhD grant to S. Tavares (SFRH/BD/48908/2008) and to José Seco (SRFH/PD/BD/113487), and to the transitory norm contract DL57/2016 to F. R. Ceia (DL57/2016/CP1370/CT90), co–founded by the European Social Fund and Portuguese Government National Funds. Moreover, this study benefited from the strategic program of Marine and Environmental Sciences Centre (MARE), financed by the FCT (UIDB/704292/2020). This paper represents a contribution to the Portuguese Polar Program PROPOLAR, ICED, SCAR EGBAMM and SCAR AnT–ERA, along with the Bulgarian and Spanish Antarctic Programmes. A special thank you to Dr. Dragomir Mateev and colleagues for the logistical support during fieldwork. We also thank the Institut Universitaire de France (IUF) for its support to P. Bustamante as a Senior Member.

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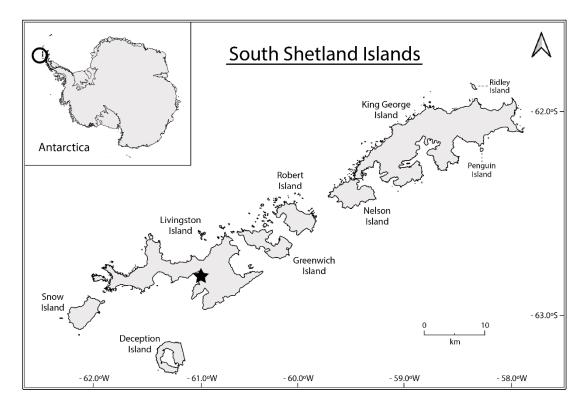
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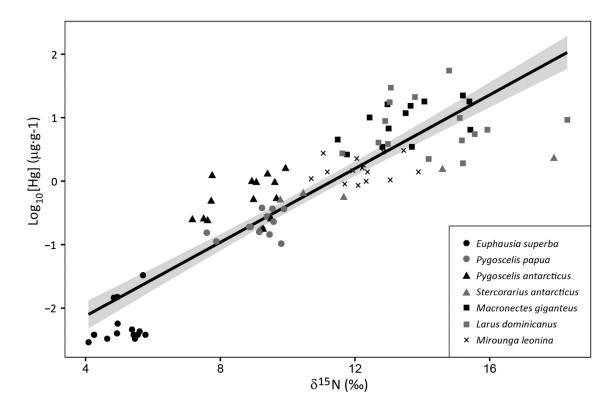
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Species	Tissues	n	[Hg] (μg·g <sup>-1</sup> dw) mean ± SD (min, max)	δ <sup>15</sup> N (‰) mean ± SD (min, max)	δ <sup>13</sup> C (‰) mean ± SD (min, max)	C/N mean ± SD (min, max)
Euphausia superba	whole	15	0.007 ± 0.008 (0.003, 0.015)	5.05 ± 0.56 (4.09, 5.78)	-24.80 ± 0.89 (-26.55, -23.51)	3.22 ± 0.07 (3.04, 3.31)
Pygoscelis papua	feathers	15	0.220 ± 0.092 (0.112, 0.380)	9.15 ± 0.64 (7.60, 9.89)	-22.97 ± 0.74 (-24.22, -21.54)	3.21 ± 0.20 (3.09, 3.93)
Pygoscelis antarcticus	feathers	15	0.665 ± 0.464 (0.239, 1.574)	8.66 ± 0.96 (7.23, 9.93)	-23.15 ± 0.62 (-24.19, -22.03)	3.18 ± 0.04 (3.10, 3.25)
Stercorarius antarcticus	feathers	5	1.104 ± 0.785 (0.516, 2.288)	12.88 ± 3.36 (9.78, 17.91)	-17.89 ± 1.77 (-19.93, -15.73)	3.14 ± 0.04 (3.10, 3.23)
Mirounga leonina	hair	15	1.654 ± 0.734 (0.867, 2.998)	12.20 ± 0.86 (10.72, 13.86)	-21.81 ± 0.35 (-22.61, -21.31)	3.18 ± 0.04 (3.13, 3.26)
Larus dominicanus	feathers	13	10.755 ± 6.750 (2.646, 22.486)	13.49 ± 1.28 (11.50, 15.42)	-21.68 ± 1.09 (-22.88, -19.34)	3.18 ± 0.04 (3.12, 3.23)
Macronectes giganteus	feathers	15	12.090 ± 14.177 (1.912, 54.680)	14.29 ± 1.69 (11.60, 18.30)	-20.30 ± 2.23 (-22.64, -16.37)	3.18 ± 0.04 (3.13, 3.26)

**Table 1.** Total mercury (Hg) concentrations, nitrogen ( $\delta^{15}N$ ) and carbon ( $\delta^{13}C$ ) stable isotope signatures, and C/N ratios registered of Antarctic species collected in the Livingston Island, South Shetland Archipelago in the west Antarctic Peninsula, during the austral summer of 2011.



**Figure 1.** Location of the Livingston Island (South Shetlands Archipelago) and the Bulgarian Antarctic base St. Kliment Ohridski research station (represented by a star), where the sampling for this study took place.



**Figure 2.** Linear relationship between logarithm-transformed total mercury (Hg) concentrations (in  $\mu g \cdot g^{-1}$  dw) and nitrogen stable isotope ratios ( $\delta^{15}N$ , in ‰) recorded in the studied Antarctic species: *Euphausia superba* (n = 15), *Pygoscelis papua* (n = 15), *P. antarcticus* (n = 15), *Stercorarius antarcticus* (n = 5), *Larus dominicanus* (n = 13), *Macronectes giganteus* (n = 15) and *Mirounga leonina* (n = 15). The relationship is given by the solid line (y = 0.2902x – 3.285) and the grey area represents the 95% confidence intervals.