



Squid beaks as a proxy for mercury concentrations in muscle of the giant warty squid *Moroteuthopsis longimana*

Sara Lopes-Santos^a, José C. Xavier^{a,b}, José Seco^a, João P. Coelho^c, Philip R. Hollyman^{b,d}, Eduarda Pereira^e, Richard A. Phillips^b, José P. Queirós^{a,b,*}

^a University of Coimbra, Marine and Environmental Sciences Centre (MARE)/ Aquatic Research Network (ARNET), Department of Life Sciences, 3000-456, Coimbra, Portugal

^b British Antarctic Survey (BAS), Natural Environment Research Council (NERC), High Cross, Madingley Road, CB3 0ET, Cambridge, United Kingdom

^c Laboratory for Innovation and Sustainability of Marine Biological Resources (ECOMARE), Centre for Environmental and Marine Studies (CESAM), Department of Biology, University of Aveiro, Estrada do Porto de Pesca Costeira, 3830-565 Gafanha da Nazaré, Portugal

^d School of Ocean Sciences, Bangor University, Askew Street, Menai Bridge, LL59 5AB, United Kingdom

^e Departamento de Química & Laboratório Central de Análises, Laboratório Associado para a Química Verde (LAQV – REQUIMTE), Universidade de Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal

ARTICLE INFO

Keywords:

Antarctica
Cephalopods
Hg
Onychoteuthidae
Scotia Sea
Southern Ocean.

ABSTRACT

Cephalopods play a major role in marine food webs as both predators and prey. Although most of the Hg in cephalopods is present in the muscle, most studies on its accumulation by predators are based on concentrations in beaks. Here, using upper and lower beaks and buccal masses of *Moroteuthopsis longimana*, we evaluated the relationship between Hg concentrations in different cephalopod tissues. Hg concentrations in muscle tissue ($329.9 \pm 166.4 \text{ ng.g}^{-1} \text{ dw}$) were ≈ 100 -fold higher than in different sections of the upper ($3.5 \pm 1.4 \text{ ng.g}^{-1} \text{ dw}$) and lower ($3.5 \pm 1.0 \text{ ng.g}^{-1} \text{ dw}$) beaks. A positive linear relationship was found between the Hg in the beak wing and in the muscle. Hg concentrations in the wing are therefore a useful proxy for the total Hg body burden, and their analysis provides a means of assessing the levels, transport and fate of Hg in marine ecosystems.

1. Introduction

Mercury (Hg) is a naturally occurring metal but whose concentrations have increased in the environment due to anthropogenic activities such as mining and the burning of fossil fuels (Streets et al., 2017). Hg is a toxic element, especially in its organic form, causing adverse reproductive, neurological, immunological, physiological or behavioural effects in wildlife and humans (Bustamante et al., 2006; Goutte et al., 2014; Kershaw and Hall, 2019; Sunderland, 2007). Due to its high affinity to proteins and high bioavailability, organisms accumulate Hg throughout their life (Bustamante et al., 2006; Seco et al., 2020). Marine organisms intake most of their Hg from their prey, and to a lesser extent from surrounding seawater (Seco et al., 2021a). Hg bioaccumulates throughout the life of organisms (Bustamante et al., 2016; Cai et al., 2007), and biomagnifies in food webs to reach high concentrations in some top predators (Matias et al., 2022; Mills et al., 2020; Seco et al., 2021a). To understand the threat of Hg for higher predators, it is

important to monitor and evaluate concentrations in organisms at mid trophic levels, as they are major links in the Hg trophic transference.

The polar regions are a sink for Hg (Angot et al., 2016). Although Antarctica is largely free of human activities that release Hg, it is transported from lower latitudes into the region by atmospheric currents where it then condenses and precipitates (Lean et al., 2005). Indeed, higher concentrations of Hg have been measured in the Southern Ocean than in open water closer to anthropogenic sources (Cossa et al., 2011). Hg concentrations in some Southern Ocean organisms are also higher than in other oceans (Cherel et al., 2018; Lischka et al., 2021; Seco et al., 2021b), with previous studies showing an increase in the recent decades (Carravieri et al., 2016; Mills et al., 2020; Queirós et al., 2020b). Many studies have measured Hg concentrations in marine predators in the Southern Ocean, including seabirds (e.g. Bustamante et al., 2016; Carravieri et al., 2017; Cherel et al., 2018; Cusset et al., 2023; Mills et al., 2020), marine mammals (e.g. Barragán-Barrera et al., 2024; Celis et al., 2022) and fishes (e.g. Cipro et al., 2018; Goutte et al., 2015; Queirós

* Corresponding author. University of Coimbra, Marine and Environmental Sciences Centre (MARE)/ Aquatic Research Network (ARNET), Department of Life Sciences, 3000-456, Coimbra, Portugal.

E-mail address: jqueiros@student.uc.pt (J.P. Queirós).

<https://doi.org/10.1016/j.marenvres.2024.106841>

Received 7 October 2024; Received in revised form 27 October 2024; Accepted 8 November 2024

Available online 16 November 2024

0141-1136/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

et al., 2020b; Seco et al., 2020). However, few have measured Hg in cephalopods, despite their importance as both predators and prey (e.g. Lischka et al., 2023; Queirós et al., 2020a; Seco et al., 2021a, 2020).

Southern Ocean squid are difficult to catch using scientific nets, especially larger specimens, due to their fast movements and avoidance behaviours (Rodhouse, 2013). As consequence, most studies focusing on Southern Ocean cephalopods used beaks obtained from diet samples of their predators (Xavier et al., 2022). Cephalopod beaks are chitinous structures that resist to digestion; as such, they accumulate in the stomachs of predators and can be sampled when regurgitated in a bolus (pellet) or when the animal dies (Xavier et al., 2022). As beak shape differs between species, and the beak grows throughout the life of the animal, it is possible to identify species from beak morphology and estimate the size of the individual (Clarke, 1986; Xavier et al., 2022; Xavier and Cherel, 2021). Chemical analyses can also be applied to beaks, including stable isotope analyses that provide proxies for habitat and trophic level, and analyses of trace elements, including Hg (Matias et al., 2019; Northern et al., 2019; Queirós et al., 2020a; Xavier et al., 2016). Previous studies show that Hg concentrations vary between different tissues (Xavier et al., 2016), with higher concentrations (≈ 10 -fold) in muscle than beaks (Bustamante et al., 2006; Matias et al., 2019; Xavier et al., 2016). As the mantle forms the bulk of the cephalopod body, muscle is the most relevant tissue for uptake of Hg to predators (Ahmad et al., 2015). Although several studies have measured Hg concentrations in muscle and beaks, they did not determine if concentrations in both tissues were correlated (Northern et al., 2019; Queirós et al., 2020a; Seco et al., 2020; Xavier et al., 2016).

The giant warty squid *Moroteuthopsis longimana* is an abundant Southern Ocean species with a circumpolar distribution in Antarctic and subantarctic waters (Cherel, 2020; Collins and Rodhouse, 2006; Xavier et al., 2018). It is a deep-sea species that can live at least 2 years and reach >2 m in total length (Lu and Williams, 1994; Lynnes and Rodhouse, 2002; Queirós et al., 2023). This species is one of the best studied squid species in the Southern Ocean, with previous studies analysing its diet, habitat, ecology, growth and reproduction (e.g. Abreu et al., 2020; Laptikhovsky and Xavier, 2017; Nemoto et al., 1988; Queirós et al., 2018). These studies showed that *M. longimana* presents an ontogenetic shift in diet and trophic position, changing from zooplankton to fish and squid as they grow, with the trophic position of adults remaining similar over the last 50 years (Abreu et al., 2020; Nemoto et al., 1988; Queirós et al., 2018); it presents a nearly constant growth throughout their life cycle, though with a period of faster growing in the Summer and/or Spring (Queirós et al., 2023); and it is an iteroparous species without a defined breeding season (Laptikhovsky et al., 2013; Queirós et al., 2023). Previous studies also analysed Hg concentrations in beaks, muscle and other tissues in this species testing, for example, how Hg concentrations vary between tissues and how it changes along the life of individuals (Anderson et al., 2009; Queirós et al., 2020a; Seco et al., 2020; Xavier et al., 2016). These studies found that Hg concentrations in the muscle (from 0.08 to 0.11 $\mu\text{g}\cdot\text{g}^{-1}$) can be ≈ 10 -fold higher than in entire beaks (from 0.004 to 0.013 $\mu\text{g}\cdot\text{g}^{-1}$), though these values were measured in different individuals from different studies (Anderson et al., 2009; Xavier et al., 2016). Furthermore, Seco et al. (2020) showed that Hg concentrations do not significantly vary between tissues, i.e. muscle, gills and digestive gland. Queirós et al. (2020a) sectioned upper beaks and showed that individuals of *M. longimana* bioaccumulate Hg throughout their life, with adults presenting ≈ 2 -fold higher concentrations than juveniles, and present a relationship with the trophic position. Due to the high knowledge about its life history and previous studies on Hg concentrations on beaks and muscle, *M. longimana* is a great model species to study Hg dynamics as we can relate any changes in Hg concentrations to major traits of its life cycle and previously knowledge on Hg concentrations across tissues. Furthermore, this species plays a key role in the Southern Ocean food-web as a major prey of top predators including seabirds, marine mammals and fish, highlighting the need to understand the role this species may have as a major Hg pathway to top

predators (Cherel and Duhamel, 2004; Clarke, 1980; Piatkowski and Pütz, 1994; Queirós et al., 2021, 2024; Xavier et al., 2003).

Building on the work of Xavier et al. (2016) and Queirós et al. (2020a), the main objective of our work was to establish the relationship between Hg concentrations in the beak and muscle of *M. longimana*. For that, we analysed muscle from buccal masses and both upper and lower beaks of *M. longimana* collected from the diet of Antarctic toothfish (*Dissostichus mawsoni*) to: (1) assess differences in Hg concentrations in different sections of upper and lower beaks, and muscle; (2) determine the relationship between Hg in beaks and muscle; and (3) evaluate bioaccumulation of Hg in *M. longimana*.

2. Materials and methods

2.1. Sample collection and preparation

Twenty-one buccal masses (with the respective upper and lower beaks) of *M. longimana* were sampled between 2019 and 2020 from the stomachs of Antarctic toothfish at the South Sandwich Islands (SSI). Buccal masses used in this study were chosen to include the largest range of beak sizes, i.e. we choose the largest and smallest beaks of the collection and we choose the maximum possible number of beaks available in this size range. Antarctic toothfish were captured onboard licensed fishing vessels operating during the toothfish fishing season at SSI and using an autoline-longline system baited with ommastrephidae squid (Fenaughty, 2008). Longlines were set between 950 and 2000 m deep. On board, stomachs were collected from the fish and frozen at -20 °C. In the laboratory, stomachs were defrosted and all prey items identified (see Queirós et al., 2024 for details in methodology). Beaks and buccal masses were identified using a cephalopod beak guide (Xavier and Cherel, 2021), and stored at 70% ethanol until further analysis. Both upper and lower beaks were extracted from the buccal mass and cleaned using 70% ethanol. The lower rostral length (LRL) was measured using a digital calliper (± 0.01 mm) (Fig. 1), and mantle length (ML, in mm) and mass (M, in g) estimated using published allometric equations ($\text{ML} = -22.348 + 37.318\text{LRL}$; $\text{M} = 0.713\text{LRL}^{3.152}$) (Xavier and Cherel, 2021).

The end of the hood (hereafter hood tip) from the upper beak, and the wing from the lower beak were separated from the remainder of the upper and lower beaks using a stainless steel scissors (Fig. 1). The wing and hood tip represent small portions of the beak that their removal has a negligible influence on Hg concentrations of the entire beak. A muscle sample (≈ 200 mg) of the buccal mass was also collected. Beak samples were dried in an oven at 60 °C for 24 h. Muscle samples were lyophilized at -86 °C for 36 h. After drying, all samples were ground into a fine powder using a mixer mill Retsch MM400 for 10 min with a 30 s^{-1} frequency.

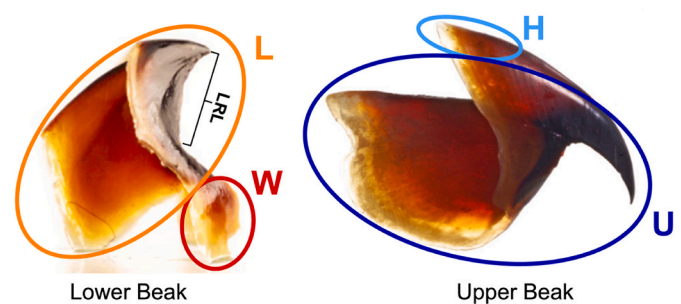


Fig. 1. *Moroteuthopsis longimana* beak sections analysed for Hg and lower rostral length (LRL). W: wing; H: hood tip; L: lower beak; U: upper beak. Beak photos from (Xavier and Cherel, 2021).

2.2. Mercury analysis

Hg concentrations were measured by thermal decomposition atomic absorption spectrometry with gold amalgamation using an Advanced Mercury Analyzer LECO AMA-245 (Detection limit of $0.00001 \mu\text{g}\cdot\text{g}^{-1}$, LECO Corporation, United States). This method does not require pre-treatment or digestion of the samples (Costley et al., 2000). The samples were weighed (wing: $\approx 12 \text{ mg}$; hood tip and muscle: $\approx 10 \text{ mg}$; upper and lower beak: $\approx 50 \text{ mg}$) and analysed in duplicate or triplicate, except for wing and hood tip which were only analysed once due to their low mass (Coefficient of Variation always $< 10\%$). Two certified reference materials (CRM) were used to evaluate the accuracy (mean \pm standard deviation) of the analytical method: ERM-CE278K mussel tissue ($n = 13$, $102 \pm 4\%$) and TORT-3 lobster hepatopancreas ($n = 33$, $94 \pm 3\%$). The CRM mass for quality control was adjusted to match total Hg concentrations in the samples. Blanks were analysed at the start of each sample set. All Hg concentrations are expressed in $\text{ng}\cdot\text{g}^{-1}$ dry weight (dw).

2.3. Statistical analysis

Statistical analyses were performed in GraphPad Prism v9.0.0 and considering a significance level of $\alpha = 5\%$. All analyses were preceded by tests for normality and homogeneity of variances. Differences in Hg concentrations between beak sections and muscle were tested using a Repeated Measures One-way ANOVA with a Greenhouse-Geisser correction, followed by a Tukey's multiple comparison test. Relationships of Hg concentrations in beak sections with muscle and of beak sections and muscle with lower rostral length were tested using linear regression. Two relationships were tested, one including all values and one excluding a outlier (extreme (high) Hg concentration in the wing that could not be confirmed as no further material was available (see above)). Aside from the high value measured in this wing, Hg concentrations measured in the hood tip, entire beaks (i.e. upper and lower) and in the buccal mass are within the range of values measured in the other studied individuals, supporting a potential analytical error in this sample (see raw data in Lopes-Santos et al., 2024).

3. Results

All beaks used in this study had fully dark wings, therefore belonging to adult individuals (Xavier and Chereil, 2021). The lower rostral length ranged between 13.0 and 21.2 mm, corresponding to mantle lengths of 461 to 767 mm, and masses of 2291 to 10743 g (Table 1). Overall, the highest Hg concentrations were measured in the muscle ($330 \pm 166 \text{ ng}\cdot\text{g}^{-1}$), being ≈ 100 -fold higher than in either upper or lower beaks ($3.5 \pm 1.4 \text{ ng}\cdot\text{g}^{-1}$ and $3.5 \pm 1.0 \text{ ng}\cdot\text{g}^{-1}$ respectively) (Table 1; Fig. 2). The highest Hg concentration was measured in the muscle of individual ML3 ($784 \text{ ng}\cdot\text{g}^{-1}$) and the lowest in the upper beak of individual ML7 ($1.4 \text{ ng}\cdot\text{g}^{-1}$) (Table 1). Mean Hg concentrations differed significantly between the beak sections and muscle ($F = 80.66$, $p < 0.0001$); all pairwise differences were significant except for wing vs hood tip, and upper beak vs lower beak (Fig. 2).

A significant positive relationship was found between Hg concentrations in the wing and muscle after the statistical outlier was removed ($\text{Hg}_{\text{muscle}} = 34.88 \text{ Hg}_{\text{wing}} + 0.12$; $n = 20$, $r = 0.551$, $p = 0.012$, $F = 7.845$) (Fig. 3). No other regressions between Hg in the various beak sections and muscle were significant (wing with outlier: $p = 0.189$, $F = 1.853$; hood tip: $p = 0.241$, $F = 1.462$; upper beak: $p = 0.236$, $F = 1.501$; lower beak: $p = 0.188$, $F = 1.187$). There were no significant linear relationships between lower rostral length and Hg concentrations in different beak sections or muscle (wing: $p = 0.060$; hood tip: $p = 0.978$; upper beak: $p = 0.977$; lower beak: $p = 0.519$; muscle: $p = 0.201$) (Fig. S1).

Table 1

Lower rostral length (LRL), estimated mantle length, estimated mass, and total mercury concentrations (Hg) of *Moroteuthopsis longimana* sampled between 2019 and 2020 from the stomachs of Antarctic toothfish at the South Sandwich Islands.

LRL (mm)	Mantle Length (mm)	Mass (g)	Hg ($\text{ng}\cdot\text{g}^{-1}$ dry weight)				Muscle
			Wing	Hood tip	Upper beak	Lower beak	
13.0	461	2291	11.3	4.2	3.1	3.3	748
13.6	484	2655	14.2	6.0	3.7	3.5	144
15.3	547	3834	8.8	7.7	4.9	4.2	390
15.4	551	3906	3.4	2.4	1.6	1.8	284
16.4	589	4784	4.4	9.4	3.1	2.3	267
16.6	598	5008	10.2	9.4	5.6	4.9	461
17.0	614	5429	3.3	2.6	1.7	2.5	372
17.1	615	5469	6.3	7.1	3.2	4.3	330
17.2	620	5611	5.4	4.4	3.0	2.9	267
17.3	624	5704	3.7	3.8	3.0	3.3	185
17.3	624	5715	8.6	7.1	5.7	4.6	568
17.3	624	5725	7.9	6.8	4.7	4.3	528
17.6	634	6000	3.5	5.8	2.0	1.9	416
17.7	637	6098	8.8	5.2	5.3	4.4	60
17.9	647	6385	3.1	1.6	1.4	2.0	206
18.0	648	6418	6.5	5.0	3.8	4.1	294
18.3	662	6833	7.6	7.6	4.6	3.8	312
18.8	681	7450	2.9	3.5	1.8	2.6	222
18.8	679	7400	4.9	4.0	3.2	2.6	86
19.0	687	7664	7.8	6.1	5.4	4.8	490
21.2	767	10743	8.4	7.0	3.1	4.4	298

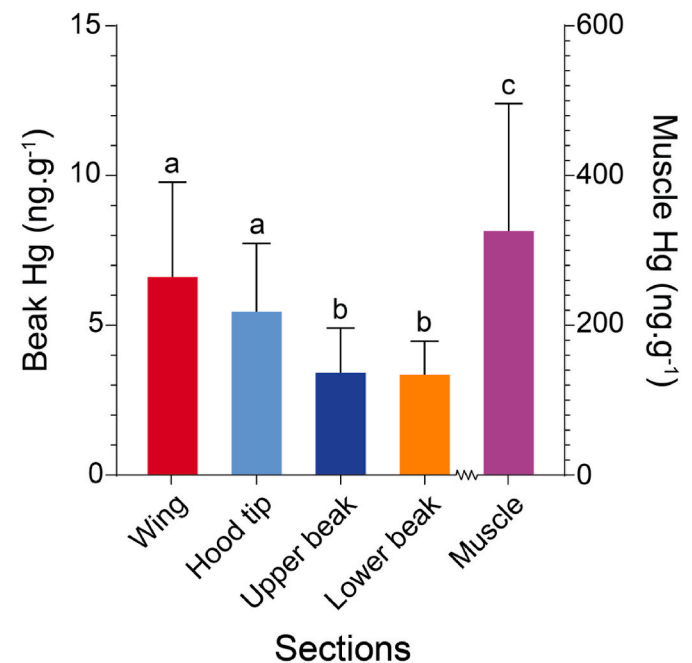


Fig. 2. Total mercury concentrations (Hg) in beak sections and muscle (buccal mass) of *Moroteuthopsis longimana* sampled between 2019 and 2020 from the stomachs of Antarctic toothfish at the South Sandwich Islands. Bars with different letters are statistically different. Left y-axis: wing, hood tip, upper beak and lower beak; Right y-axis: muscle. All concentrations are in dry weight. Values are mean \pm SD.

4. Discussion

4.1. Mercury levels in beaks and muscle of *Moroteuthopsis longimana*

We found significantly higher Hg concentrations in muscle tissue than in the beak of *Moroteuthopsis longimana*, which was expected as Hg has high affinity to proteins, and beaks have a lower protein content

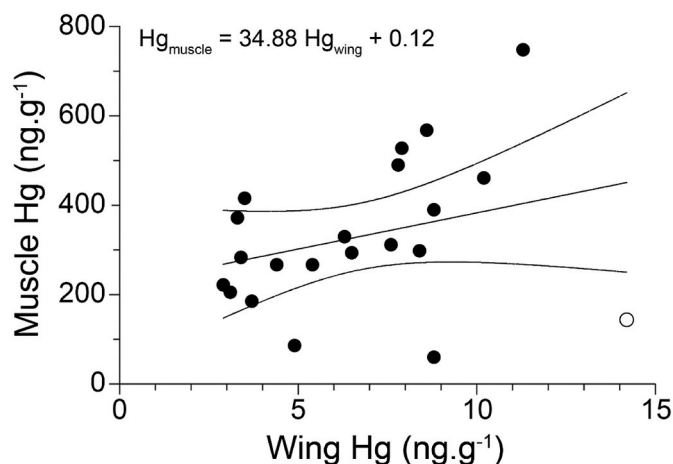


Fig. 3. Linear regression between mercury concentrations (Hg, dry weight) in the wing and muscle of *Moroteuthopsis longimana* sampled between 2019 and 2020 from the stomachs of Antarctic toothfish at the South Sandwich Islands. Dotted lines represent the 95% confidence interval. Linear regression: $n = 20$, $r = 0.551$, $p = 0.012$, $F = 7.845$. ○ is the outlier.

than muscle (Bustamante et al., 2006; Miserez et al., 2008). Our results showed that Hg concentrations in the muscle were ≈ 100 -fold higher than in the upper and lower beaks and ≈ 50 -fold higher than in the wing and hood tip. A previous study comparing beaks and muscle of *M. longimana* found this difference to be of just ≈ 10 -fold (Xavier et al., 2016). This is possibly because unlike previous studies that compared average Hg concentrations from different individuals (Xavier et al., 2016), we compared Hg in the beaks and muscle of the same specimens.

Hg concentrations found in the muscle in our study are amongst the highest ever measured in *M. longimana*, being ≈ 3 to 4-fold higher than in Anderson et al. (2009) and Seco et al. (2020) (Table 2). This could reflect the different sampling locations and possibly the different sizes of the studied individuals. Our samples were from the South Sandwich Islands, a volcanic archipelago within the winter sea-ice zone (Rogers et al., 2012; Thorpe and Murphy, 2022). Both volcanic activity and melting sea-ice are known to release mercury into the environment (Krabbenhoft and Sunderland, 2013; Mason et al., 2012). However, Hg concentrations were similar to those in the buccal mass of the benthic Antarctic octopod *Pareledone turqueti* (Matias et al., 2020). Hg levels in the Southern Ocean are known to increase with depth (Cossa et al., 2011). Furthermore, Hg concentrations are generally greater in benthic food-webs than in pelagic food-webs (Bustamante et al., 2006; Cipro et al., 2018). As such,

Table 2

Mercury (Hg) concentrations (mean \pm SD, dry weight) measured in *Moroteuthopsis longimana* in this and previous studies.

Tissue	n	Hg (ng.g ⁻¹)	References
Whole beak			
Lower	6	8.0 \pm 3.0	Xavier et al. (2016)
Lower	21	3.5 \pm 1.4	This study
Upper	21	3.5 \pm 1.4	This study
Beak sections			
Wing	21	6.7 \pm 3.1	This study
Hood tip	21	5.6 \pm 2.2	This study
Hood tip	4	10.1 \pm 2.4	Queirós et al. (2020a)
3 rd quarter of the hood	4	6.4 \pm 2.4	Queirós et al. (2020a)
2 nd quarter of the hood	4	6.0 \pm 2.0	Queirós et al. (2020a)
1 st quarter of the hood	4	5.2 \pm 2.8	Queirós et al. (2020a)
Tip of the rostrum	1	9.1	Queirós et al. (2020a)
Other tissues			
Muscle	2	100 \pm 20	Anderson et al. (2009)
Muscle	5	82 \pm 23	Seco et al. (2020)
Muscle	21	330 \pm 166	This study
Gills	5	75 \pm 32	Seco et al. (2020)
Digestive gland	5	45 \pm 21	Seco et al. (2020)

the high Hg levels in *M. longimana* may suggest that individuals studied here may have been feeding deeper in the water column and closer to the seafloor. Given its diet includes Antarctic krill (*Euphausia superba*), which is known to perform diel vertical migrations and sometimes occurs in deep waters near the sea bottom (Nemoto et al., 1985, 1988; Schmidt et al., 2011; Everson, 2000; Cuzin-Roudy et al., 2014), we may have sampled deep-water residents. This is likely as the buccal masses used in this study were collected from Antarctic toothfish which is a demersal species from the Southern Ocean deep-sea (Hanchet et al., 2015). There is also video footage of *M. longimana* feeding near the seafloor (unpublished data).

Although Hg concentrations in muscle were higher, those in the upper beak, lower beak, and hood tip in our study were lower than previously measured in *M. longimana* beaks from the diet of Patagonian (*D. eleginoides*) and Antarctic toothfish in the South Georgia and the South Sandwich Islands (Queirós et al., 2020a; Xavier et al., 2016) (Table 2). Such differences may arise from ontogenetic shifts in habitat of individuals, differences in feeding rates and/or differences in sampling years (Queirós et al., 2020a; Xavier et al., 2016).

Analysing different sections of the beaks provides information on different stages of the life cycle (Queirós et al., 2018, 2020a). Significant differences between all beak sections, except for wing vs the hood tip and upper beak vs lower beak, were expected since the wing and hood tip represent the same recent life period, and hence provide information on Hg incorporation in the last days before capture (Queirós et al., 2020a). The similarity in Hg concentrations in upper and lower beaks are expected as both beaks grow at the same rate throughout the individual's life, representing the long-term average for the individual (Perales-Raya et al., 2010; Queirós et al., 2018). Results from upper and lower beaks are therefore directly comparable in ecological studies as they provide similar information. Lower Hg concentrations in upper and lower beaks than the wing and hood tip were also expected. Much of the beak is synthesized during the juvenile life stage when Hg intake is lower, reducing the overall average (Queirós et al., 2020a). As such, we caution that Hg concentrations measured in whole beaks do not reflect exposure over the same timescale as tissues such as muscle, and Hg concentrations in the muscle, wings and hood tip better indicate recent Hg ingestion.

4.2. Relationship between Hg concentration in beaks and muscle

Most of the mercury that predators obtain from cephalopods is from the muscle (Ahmad et al., 2015), hence the advantage of establishing the relationship with Hg concentration in the beaks, which are easily accessed. We found a positive relationship between Hg concentrations in the wing – the part of the beak formed the most recently (Queirós et al., 2018) – and muscle, suggesting that this beak section can be used to estimate Hg concentrations in the muscle. Surprisingly, no linear relationship was found between Hg concentrations in the muscle and hood tip. The latter is the thinnest part of the beak, and so may be grown over an even shorter period of life than wing (Queirós et al., 2018), and presumably also the muscle. Another possible explanation is the inclusion of untanned portions of the beak, which have a different protein composition from the fully chitinised tanned beak (Miserez et al., 2008). More studies are needed to understand how the beak composition affect Hg concentrations.

Other studies can now use the equation provided here ($Hg_{muscle} = 34.88 Hg_{wing} + 0.12$), to estimate Hg concentrations in the muscle of *M. longimana* by analysing the beak wing. However, it is important to be cautious about potential differences in Hg concentrations between the muscle in the buccal mass and other muscular tissues, i.e. mantle and arms. A previous study analysing Hg concentrations in muscle from buccal masses and mantle of the orange-back flying squid (*Stenoteuthis pteropus*) in the tropical Eastern Atlantic Ocean found higher Hg concentrations in the buccal mass when compared to the mantle (Lischka

et al., 2018). Therefore, future studies should analyse the relationship between different muscular tissues in *M. longimana*. Furthermore, we analysed adults with fully-chitinised beaks, thus it should not be applied to beaks from juveniles or sub-adults with untanned sections that have a lower protein content (Miserez et al., 2008; Xavier and Cherel, 2021). Previous studies on two benthic octopods in the Southern Ocean, *P. turqueti* and *Adelieledone polymorpha* only found a relationship between Hg concentrations in the muscle and the beak for *A. polymorpha* (Matias et al., 2020). In addition, the equation differed from our study, indicating that such relationships may be species-specific. Previous studies analysing relationships in Hg concentrations between muscle and other tissues (e.g. digestive gland, optical lobes) also found different relations across species (e.g. Minet et al., 2021; Seco et al., 2020). Because Hg concentrations relationships between tissues seem to be species-specific, until further studies are made, the equation presented here should therefore only be used for adult *M. longimana*.

4.3. Relation between Hg concentration and squid size

Cephalopods are known to bioaccumulate trace metals, including Hg (Bustamante et al., 2008). We did not find a relationship between Hg concentrations in beak sections or muscle with the lower rostral length, which is a proxy for squid size and age, and nor did Xavier et al. (2016). In contrast, a previous study showed Hg concentrations in adult *M. longimana* being 2-fold higher than in juveniles, indicating bioaccumulation (Queirós et al., 2020a). Such difference between studies is probably related with the study of adult individuals in which the variation among individuals may be of much lower magnitude than the differences between adults and juveniles associated with the ontogenetic shift in diet in early life of this species (Queirós et al., 2018). This is another reason why caution should be exercised when interpreting Hg concentrations measured in whole beaks as they may not reflect exposure over the same timescale as muscle, and that levels in the wings or hood tip will provide a better indication of recent Hg ingestion.

In summary, this study provides an important tool to estimate Hg concentrations in *M. longimana* muscle by analysing the wings of lower beaks collected from the diet of predators. Our study also shows that the difference between beak Hg concentrations and the muscle can be of ≈ 100 -fold depending on the beak section that is analysed, i.e. the entire beak or just a section indicative of recent life. Our results also suggest that analysis of separate sections of the beak should provide better information to study Hg bioaccumulation in cephalopods than analysing entire beaks and test relationships with the lower rostral length (squid's size).

CRedit authorship contribution statement

Sara Lopes-Santos: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **José C. Xavier:** Writing – original draft, Supervision, Conceptualization. **José Seco:** Writing – original draft, Validation, Supervision, Conceptualization. **João P. Coelho:** Writing – original draft, Resources, Methodology. **Philip R. Hollyman:** Writing – original draft. **Eduarda Pereira:** Writing – original draft, Resources. **Richard A. Phillips:** Writing – original draft, Supervision. **José P. Queirós:** Writing – original draft, Supervision, Methodology, Conceptualization, Writing – review & editing.

Funding sources

JS salary was supported by FCT through an Individual Scientific Employment (2021/00624/CEECIND).

JPC salary was supported by FCT through an Individual Scientific Employment (2020/01778/CEECIND).

JPQ salary was supported by FCT through a PhD Scholarship co-financed by FSE (SFRH/BD/144320/2019).

The work was supported by FCT through national funds granted to

MARE (UIDB/04292/2020 and UIDP/04292/2020) and to Associate Laboratory ARNET (LA/P/0069/2020).

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.

Acknowledgments

Authors would like to thank observers on board Fishing Vessel San Aspiring and Fishing Vessel Nordic Prince, and the 2019 scientific team at King Edward Point Research Station for collecting the Antarctic toothfish stomachs and sorting the beaks used in this study. JPQ would like to thank Mark Belchier for the PhD supervision during the collection of samples. This study represents a contribution to the Ecosystems component of the Polar Science for Planet Earth Programme funded by the Natural Environment Research Council. JPQ was supported by FCT PhD Scholarship co-financed by FSE (SFRH/BD/144320/2019) and JS and JPC by an Individual Scientific Employment (2021/00624/CEECIND and 2020/01778/CEECIND). This work had the support of FCT through national funds granted to MARE (UIDB/04292/2020 and UIDP/04292/2020) and to Associate Laboratory ARNET (LA/P/0069/2020).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2024.106841>.

Data availability

Data used in this study is available at doi: [10.5281/zenodo.13119886](https://doi.org/10.5281/zenodo.13119886).

References

- Abreu, J., Phillips, R.A., Ceia, F.R., Ireland, L., Paiva, V.H., Xavier, J.C., 2020. Long-term changes in habitat and trophic level of Southern Ocean squid in relation to environmental conditions. *Sci. Rep.* 10, 15215. <https://doi.org/10.1038/s41598-020-72103-6>.
- Ahmad, N.I., Noh, M.F.M., Mahiyuddin, W.R.W., Jaafar, H., Ishak, I., Azmi, W.N.F.W., Veloo, Y., Mokhtar, F.A., 2015. The mercury levels in crustaceans and cephalopods from Peninsular Malaysia. *Environ. Sci. Pollut. Res.* 22, 12960–12974. <https://doi.org/10.1007/s11356-015-4415-9>.
- Anderson, O.R.J., Phillips, R.A., McDonald, R.A., Shore, R.F., McGill, R.A.R., Bearhop, S., 2009. Influence of trophic position and foraging range on mercury levels within a seabird community. *Mar. Ecol. Prog. Ser.* 375, 277–288. <https://doi.org/10.3354/meps07784>.
- Angot, H., Dastoor, A., De Simone, F., Gårdfeldt, K., Gencarelli, C.N., Hedgcock, I.M., Langer, S., Magand, O., Mastromonaco, M.N., Nordström, C., Pfaffhuber, K.A., Pirrone, N., Ryskov, A., Selin, N.E., Skov, H., Song, S., Sprovieri, F., Steffen, A., Toyota, K., Travnikov, O., Yang, X., Dommergue, A., 2016. Chemical cycling and deposition of atmospheric mercury in polar regions: review of recent measurements and comparison with models. *Atmos. Chem. Phys.* 16, 10735–10763. <https://doi.org/10.5194/acp-16-10735-2016>.
- Barragán-Barrera, D.C., Riet-Sapirza, F.G., Mojica-Moncada, D.F., Negrete, J., Curtosi, A., Bustamante, P., Caballero, S., Luna-Acosta, A., 2024. Sex-specific mercury levels in skin samples of southern elephant seals (*Mirounga leonina*) at Isla 25 de Mayo (King George Island), Antarctic Peninsula. *Mar. Mamm. Sci.* 40, 108–122. <https://doi.org/10.1111/mms.13058>.
- Bustamante, P., Carravieri, A., Goutte, A., Barbraud, C., Delord, K., Chastel, O., Weimerskirch, H., Cherel, Y., 2016. High feather mercury concentrations in the wandering albatross are related to sex, breeding status and trophic ecology with no demographic consequences. *Environ. Res.* 144, 1–10. <https://doi.org/10.1016/j.envres.2015.10.024>.
- Bustamante, P., Gonzalez, A.F., Rocha, F., Miramand, P., Guerra, A., 2008. Metal and metalloids concentrations in the giant squid *Architeuthis dux* from Iberian waters. *Mar. Environ. Res.* 66, 278–287. <https://doi.org/10.1016/j.marenvres.2008.04.003>.
- Bustamante, P., Lahaye, V., Durnez, C., Churlaud, C., Caurant, F., 2006. Total and organic Hg concentrations in cephalopods from the North Eastern Atlantic waters: influence of geographical origin and feeding ecology. *Sci. Total Environ.* 368, 585–596. <https://doi.org/10.1016/j.scitotenv.2006.01.038>.

- Cai, Y., Rooker, J.R., Gill, G.A., Turner, J.P., 2007. Bioaccumulation of mercury in pelagic fishes from the northern Gulf of Mexico. *Canadian J Fish Aquat Sci* 64, 458–469. <https://doi.org/10.1139/f07-017>.
- Carravieri, A., Cherel, Y., Brault-Favrou, M., Churlaud, C., Peluhet, L., Labadie, P., Budzinski, H., Chastel, O., Bustamante, P., 2017. From Antarctica to the subtropics: contrasted geographical concentrations of selenium, mercury, and persistent organic pollutants in skua chicks (*Catharacta* spp.). *Environ. Pollut.* 228, 464–473. <https://doi.org/10.1016/j.envpol.2017.05.053>.
- Carravieri, A., Cherel, Y., Jaeger, A., Churlaud, C., Bustamante, P., 2016. Penguins as bioindicators of mercury contamination in the southern Indian Ocean: geographical and temporal trends. *Environ. Pollut.* 213, 195–205. <https://doi.org/10.1016/j.envpol.2016.02.010>.
- Celis, J.E., Espejo, W., Chiang, G., Kitamura, D., Vergara, E., Kashiwada, S., O'Driscoll, N. J., 2022. Trace and rare earth elements in excreta of two species of marine mammals from South Shetland Islands, Antarctica. *Mar. Pollut. Bull.* 183, 114095. <https://doi.org/10.1016/j.marpolbul.2022.114095>.
- Cherel, Y., 2020. A review of Southern Ocean squids using nets and beaks. *Mar. Biodivers.* 50, 98. <https://doi.org/10.1007/s12526-020-01113-4>.
- Cherel, Y., Barbraud, C., Lahouinat, M., Jaeger, A., Jaquemet, S., Wanless, R.M., Phillips, R.A., Thompson, D.R., Bustamante, P., 2018. Accumulate or eliminate? Seasonal mercury dynamics in albatrosses, the most contaminated family of birds. *Environ. Pollut.* 241, 124–135. <https://doi.org/10.1016/j.envpol.2018.05.048>.
- Cherel, Y., Duhamel, G., 2004. Antarctic jaws: cephalopod prey of sharks in Kerguelen waters. *Deep-Sea Res. Part I Oceanogr. Res. Pap.* 51, 17–31. <https://doi.org/10.1016/j.dsr.2003.09.009>.
- Cipro, C.V.Z., Cherel, Y., Bocher, P., Caurant, F., Miramand, P., Bustamante, P., 2018. Trace elements in invertebrates and fish from Kerguelen waters, southern Indian Ocean. *Polar Biol.* 41, 175–191. <https://doi.org/10.1007/s00300-017-2180-6>.
- Clarke, M.R., 1986. *A handbook for the identification of cephalopod beaks*. Marine Bio. Clarendon Press, Oxford.
- Clarke, M.R., 1980. Cephalopoda in the diet of sperm whales of the Southern hemisphere and their bearing on sperm whale biology. In: *Discovery Reports*. Institute of Oceanographic Sciences, Plymouth, p. 324.
- Collins, M.A., Rodhouse, P.G.K., 2006. Southern Ocean cephalopods. *Adv. Mar. Biol.* 50, 191–265. [https://doi.org/10.1016/S0065-2881\(05\)50003-8](https://doi.org/10.1016/S0065-2881(05)50003-8).
- Cossa, D., Heimbürger, L.E., Lannuzel, D., Rintoul, S.R., Butler, E.C.V., Bowie, A.R., Averty, B., Watson, R.J., Remenyi, T., 2011. Mercury in the Southern Ocean. *Geochem. Cosmochim. Acta* 75, 4037–4052. <https://doi.org/10.1016/j.gca.2011.05.001>.
- Costley, C.T., Mossop, K.F., Dean, J.R., Garden, L.M., Marshall, J., Carroll, J., 2000. Determination of mercury in environmental and biological samples using pyrolysis atomic absorption spectrometry with gold amalgamation. *Anal. Chim. Acta* 405, 179–183. [https://doi.org/10.1016/S0003-2670\(99\)00742-4](https://doi.org/10.1016/S0003-2670(99)00742-4).
- Cusset, F., Bustamante, P., Carravieri, A., Bertin, C., Brasso, R., Corsi, I., Dunn, M., Emmerson, L., Guillou, G., Hart, T., Juárez, M., Kato, A., Machado-Gaye, A.L., Michelot, C., Olmastroni, S., Polito, M., Raclot, T., Santos, M., Schmidt, A., Southwell, C., Soutullo, A., Takahashi, A., Thiebaut, J.-B., Trathan, P., Vivion, P., Waluda, C., Fort, J., Cherel, Y., 2023. Circumpolar assessment of mercury contamination: the Adélie penguin as a bioindicator of Antarctic marine ecosystems. *Ecotoxicology* 32, 1024–1049. <https://doi.org/10.1007/s10646-023-02709-9>.
- Cuzin-Roudy, J., Irsson, J., Penot, F., Kawaguchi, S., Vallet, C., Southern Ocean Euphausiids, 2014. In: De Broyer, C., Koubbi, P., Griffiths, H., Raymond, B., D'Acoz, C., Van de Putte, A., Danis, B., David, B., Grant, S., Gutt, J., Held, C., Hossie, G., Huettmann, F., Post, A., Ropert-Coudert, Y. (Eds.), *Biogeographic atlas of the Southern Ocean*, The Scientific Committee on Antarctic Research. Cambridge, pp. 309–320.
- Everson, I., 2000. In: *Krill: Biology, Ecology and Fisheries*, first ed. Blackwell Science Ltd, United Kingdom.
- Fenaughty, J.M., 2008. The autoline system - an updated descriptive review of the method with recommendations to clarify CCAMLR conservation measures regulating longline fisheries within the convention area. Document WG-FSA-08/60. CCAMLR, Hobart, Australia 27.
- Goutte, A., Bustamante, P., Barbraud, C., Delord, K., Weimerskirch, H., Chastel, O., 2014. Demographic responses to mercury exposure in two closely related Antarctic top predators. *Ecology* 95, 1075–1086. <https://doi.org/10.1890/13-1229.1>.
- Goutte, A., Cherel, Y., Churlaud, C., Ponthus, J.P., Massé, G., Bustamante, P., 2015. Trace elements in Antarctic fish species and the influence of foraging habitats and dietary habits on mercury levels. *Sci. Total Environ.* 538, 743–749. <https://doi.org/10.1016/j.scitotenv.2015.08.103>.
- Hanchet, S., Dunn, A., Parker, S., Horn, P., Stevens, D., Mormede, S., 2015. The Antarctic toothfish (*Dissostichus mawsoni*): biology, ecology, and life history in the Ross Sea region. *Hydrobiologia* 761, 397–414. <https://doi.org/10.1007/s10750-015-2435-6>.
- Kershaw, J.L., Hall, A.J., 2019. Mercury in cetaceans: exposure, bioaccumulation and toxicity. *Sci. Total Environ.* 694, 133683. <https://doi.org/10.1016/j.scitotenv.2019.133683>.
- Krabbenhoft, D.P., Sunderland, E.M., 2013. Global change and mercury. *Science* 341, 1457–1458. <https://doi.org/10.1126/science.1242838>, 1979.
- Lapikhovskiy, V., Collins, M.A., Arkhipkin, A., 2013. First case of possible iteroparity among coleoid cephalopods: the giant warty squid *Kondakovia longimana*. *J. Molluscan Stud.* 79, 270–272. <https://doi.org/10.1093/mollus/eyt014>.
- Lapikhovskiy, V., Xavier, J.C., 2017. Dwarf males of giant warty squid *Kondakovia longimana* and a description of their spermatophores. *Polar Biol.* 2469–2474. <https://doi.org/10.1007/s00300-017-2158-4>.
- Lean, D., O'Driscoll, N., Rencz, A., 2005. The biogeochemistry and fate of mercury in the environment. In: Siegel, A., Siegel, H. (Eds.), *Metal Ions in Biological Systems*. Marcel Dekker, inc., New York, pp. 221–238. <https://doi.org/10.1201/9780824751999.ch9>.
- Lischka, A., Braid, H.E., Gaw, S., Bolstad, K.S.R., 2023. First reports of trace element bioaccumulation in the Antarctic deep-sea squid *Psychroteuthis glacialis*. *Mar. Biol.* 170, 163. <https://doi.org/10.1007/s00227-023-04304-2>.
- Lischka, A., Bustamante, P., Braid, H., Piatkowski, U., Lacoue-Labarthe, T., 2021. Trophic ecology drives trace element concentrations in the Antarctic octopod community. *Sci. Total Environ.* 768, 144373. <https://doi.org/10.1016/j.scitotenv.2020.144373>.
- Lischka, A., Lacoue-Labarthe, T., Hoving, H.J.T., JavidPour, J., Pannell, J.L., Merten, V., Churlaud, C., Bustamante, P., 2018. High cadmium and mercury concentrations in the tissues of the orange-back flying squid, *Sthenoteuthis pteropus*, from the tropical Eastern Atlantic. *Ecotoxicol. Environ. Saf.* 163, 323–330. <https://doi.org/10.1016/j.ecoenv.2018.07.087>.
- Lopes-Santos, S., Xavier, J.C., Seco, J., Coelho, J.P., Hollyman, P.R., Pereira, E., Phillips, R.A., Queirós, J.P., 2024. Total mercury concentrations in beaks and muscle of the giant warty squid *Moroteuthopsis longimana* (Southern Ocean). <https://doi.org/10.5281/zenodo.13119886> (Version 1, Data set) Zenodo.
- Lu, C.C., Williams, R., 1994. *Kondakovia longimana* filippova, 1972 (Cephalopoda: Onychoteuthidae) from the Indian Ocean sector of the Southern Ocean. *Antarct. Sci.* 6, 231–234. <https://doi.org/10.1017/S0954102094000350>.
- Lynnes, A.S., Rodhouse, P.G., 2002. A big mouthful for predators: the largest recorded specimen of *Kondakovia longimana* (Cephalopoda: Onychoteuthidae). *Bull. Mar. Sci.* 71, 1087–1090.
- Mason, R.P., Choi, A.L., Fitzgerald, W.F., Hammerschmidt, C.R., Lamborg, C.H., Soerensen, A.L., Sunderland, E.M., 2012. Mercury biogeochemical cycling in the ocean and policy implications. *Environ. Res.* 119, 101–117. <https://doi.org/10.1016/j.envres.2012.03.013>.
- Matias, R.S., Gregory, S., Ceia, F.R., Baeta, A., Seco, J., Rocha, M.S., Fernandes, E.M., Reis, R.L., Silva, T.H., Pereira, E., Piatkowski, U., Ramos, J.A., Xavier, J.C., 2019. Show your beaks and we tell you what you eat: different ecology in sympatric Antarctic benthic octopods under a climate change context. *Mar. Environ. Res.* 150, 104757. <https://doi.org/10.1016/j.marenvres.2019.104757>.
- Matias, R.S., Guimarães, H.R., Bustamante, P., Seco, J., Chipev, N., Fragão, J., Tavares, S., Ceia, F.R., Pereira, M.E., Barbosa, A., Xavier, J.C., 2022. Mercury biomagnification in an Antarctic food web of the Antarctic Peninsula. *Environ. Pollut.* 304, 119199. <https://doi.org/10.1016/j.envpol.2022.119199>.
- Matias, R.S., Seco, J., Gregory, S., Belchier, M., Pereira, M.E., Bustamante, P., Xavier, J.C., 2020. Antarctic octopod beaks as proxy for mercury concentrations in soft tissues. *Mar. Pollut. Bull.* 158, 111447. <https://doi.org/10.1016/j.marpolbul.2020.111447>.
- Mills, W.F., Bustamante, P., McGill, R.A.R., Anderson, O.R.J., Bearhop, S., Cherel, Y., Votier, S.C., Phillips, R.A., 2020. Mercury exposure in an endangered seabird: long-term changes and relationships with trophic ecology and breeding success. *Proc R Soc B: Biol. Sci.* 287, 20202683. <https://doi.org/10.1098/rspb.2020.2683>.
- Minet, A., Manceau, A., Valada-Mennuni, A., Brault-Favrou, M., Churlaud, C., Fort, J., Nguyen, T., Spitz, J., Bustamante, P., Lacoue-Labarthe, T., 2021. Mercury in the tissues of five cephalopods species: first data on the nervous system. *Sci. Total Environ.* 759, 143907. <https://doi.org/10.1016/j.scitotenv.2020.143907>.
- Miserez, A., Schneberk, T., Sun, C., Zok, F.W., Waite, J.H., 2008. The transition from stiff to compliant materials in squid beaks. *Science* 319, 1816–1819. <https://doi.org/10.1126/science.1154117>, 1979.
- Nemoto, T., Okiyama, M., Iwasaki, N., Kikuchi, T., 1988. Squid as predators on krill (*Euphausia superba*) and prey for sperm whales in the Southern Ocean. In: Sahrhage, D. (Ed.), *Antarctic Ocean and Resources Variability*. Springer-Verlag, Berlin Heidelberg, pp. 292–296. <https://doi.org/10.1007/978-3-642-73724-4>.
- Nemoto, T., Okiyama, M., Takahashi, M., 1985. Aspects of the roles of squid in food chains of marine Antarctic ecosystems. In: Siegfried, W., Condy, P., Laws, R. (Eds.), *Antarctic nutrient cycles and food webs*. Springer, Berlin Heidelberg, pp. 415–420. https://doi.org/10.1007/978-3-642-82275-9_58.
- Northern, T.J., Smith, A.M., McKinnon, J.F., Bolstad, K.S.R., 2019. Trace elements in beaks of greater hooked squid *Onkyia ingens*: opportunities for environmental tracing. *Molluscan Res.* 39, 29–34. <https://doi.org/10.1080/13235818.2018.1495604>.
- Perales-Raya, C., Bartolomé, A., García-Santamaría, M.T., Pascual-Alayón, P., Almansa, E., 2010. Age estimation obtained from analysis of octopus (*Octopus vulgaris* Cuvier, 1797) beaks: improvements and comparisons. *Fish. Res.* 106, 171–176. <https://doi.org/10.1016/j.fishres.2010.05.003>.
- Piatkowski, U., Pütz, K., 1994. Squid diet of emperor penguins (*Aptenodytes forsteri*) in the eastern Weddell Sea, Antarctica during late summer. *Antarct. Sci.* 6, 241–247. <https://doi.org/10.1017/S0954102094000374>.
- Queirós, J.P., Bartolomé, A., Piatkowski, U., Xavier, J.C., Perales-Raya, C., 2023. Age and growth estimation of Southern Ocean squid *Moroteuthopsis longimana*: can we use beaks collected from predators' stomachs? *Mar. Biol.* 170, 10. <https://doi.org/10.1007/s00227-022-04156-2>.
- Queirós, J.P., Bustamante, P., Cherel, Y., Coelho, J.P., Seco, J., Roberts, J., Pereira, E., Xavier, J.C., 2020a. Cephalopod beak sections used to trace mercury levels throughout the life of cephalopods: the giant warty squid *Moroteuthopsis longimana* as a case study. *Mar. Environ. Res.* 161, 105049. <https://doi.org/10.1016/j.marenvres.2020.105049>.
- Queirós, J.P., Cherel, Y., Ceia, F.R., Hilário, A., Roberts, J., Xavier, J.C., 2018. Ontogenic changes in habitat and trophic ecology in the Antarctic squid *Kondakovia longimana* derived from isotopic analysis on beaks. *Polar Biol.* 41, 2409–2421. <https://doi.org/10.1007/s00300-018-2376-4>.
- Queirós, J.P., Hill, S.L., Pinkerton, M., Vacchi, M., Coelho, J.P., Pereira, E., Ramos, J.A., Seco, J., Stevens, D.W., Xavier, J.C., 2020b. High mercury levels in antarctic toothfish *Dissostichus mawsoni* from the southwest pacific sector of the Southern Ocean. *Environ. Res.* 187, 109680. <https://doi.org/10.1016/j.envres.2020.109680>.

- Queirós, J.P., Ramos, J.A., Chereil, Y., Franzitta, M., Duarte, B., Rosa, R., Monteiro, F., Figueiredo, A., Strugnell, J.M., Fukuda, Y., Stevens, D.W., Xavier, J.C., 2021. Cephalopod fauna of the Pacific Southern Ocean using Antarctic toothfish (*Dissostichus mawsoni*) as biological samplers and fisheries bycatch specimens. *Deep-Sea Res. Part I Oceanogr. Res. Pap.* 174, 103571. <https://doi.org/10.1016/j.dsr.2021.103571>.
- Queirós, J.P., Xavier, J.C., Abreu, J., Collins, M.A., Belchier, M., Hollyman, P.R., 2024. What inhabits the South Sandwich Islands deep-sea? Biodiversity and biogeography of bathyal communities using predators as biological samplers. *Deep-Sea Res. Part I Oceanogr. Res. Pap.* 205, 104260. <https://doi.org/10.1016/j.dsr.2024.104260>.
- Rodhouse, P.G.K., 2013. Role of squid in the Southern Ocean pelagic ecosystem and the possible consequences of climate change. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 95, 129–138. <https://doi.org/10.1016/j.dsr2.2012.07.001>.
- Rogers, A.D., Tyler, P.A., Connelly, D.P., Copley, J.T., James, R., Larter, R.D., Linse, K., Mills, R.A., Garabato, A.N., Pancost, R.D., Pearce, D.A., Polunin, N.V., German, C.R., Shank, T., Boersch-Supan, P.H., Alker, B.J., Aquilina, A., Bennett, S.A., Clarke, A., Dinley, R.J., Graham, A.G., Green, D.R., Hawkes, J.A., Hepburn, L., Hilario, A., Huvenne, V.A., Marsh, L., Ramirez-Llodra, E., Reid, W.D., Roterman, C.N., Sweeting, C.J., Thatje, S., Zwirgmaier, K., 2012. The discovery of new deep-sea hydrothermal vent communities in the Southern Ocean and implications for biogeography. *PLoS Biol.* 10, e1001234. <https://doi.org/10.1371/journal.pbio.1001234>.
- Schmidt, K., Atkinson, A., Steigenberger, S., Fielding, S., Lindsay, M.C.M., Pond, D.W., Tarling, G.A., Klevjer, T.A., Allen, C.S., Nicol, S., Achterberg, E.P., 2011. Seabed foraging by Antarctic krill: Implications for stock assessment, benthic-pelagic coupling, and the vertical transfer of iron. *Limnol. Oceanogr.* 56, 1411–1428. <https://doi.org/10.4319/lo.2011.56.4.1411>.
- Seco, J., Aparício, S., Brierley, A.S., Bustamante, P., Ceia, F.R., Coelho, J.P., Phillips, R.A., Saunders, R.A., Fielding, S., Gregory, S., Matias, R., Pardal, M.A., Pereira, E., Stowasser, G., Tarling, G.A., Xavier, J.C., 2021a. Mercury biomagnification in a Southern Ocean food web. *Environ. Pollut.* 275, 116620. <https://doi.org/10.1016/j.envpol.2021.116620>.
- Seco, J., Freitas, R., Xavier, J.C., Bustamante, P., Coelho, J.P., Coppola, F., Saunders, R. A., Almeida, A., Fielding, S., Pardal, M.A., Stowasser, G., Pompeo, G., Tarling, G.A., Brierley, A.S., Pereira, E., 2021b. Oxidative stress, metabolic activity and mercury concentrations in Antarctic krill *Euphausia superba* and myctophid fish of the Southern Ocean. *Mar. Pollut. Bull.* 166, 112178. <https://doi.org/10.1016/j.marpolbul.2021.112178>.
- Seco, J., Xavier, J.C., Brierley, A.S., Bustamante, P., Coelho, J.P., Gregory, S., Fielding, S., Pardal, M.A., Pereira, B., Stowasser, G., Tarling, G.A., Pereira, E., 2020. Mercury levels in Southern Ocean squid: variability over the last decade. *Chemosphere* 239, 124785. <https://doi.org/10.1016/j.chemosphere.2019.124785>.
- Streets, D.G., Horowitz, H.M., Jacob, D.J., Lu, Z., Levin, L., ter Schure, A.F.H., Sunderland, E.M., 2017. Total mercury released to the environment by human activities. *Environ. Sci. Technol.* 51, 5969–5977. <https://doi.org/10.1021/acs.est.7b00451>.
- Sunderland, E.M., 2007. Mercury exposure from domestic and imported estuarine and marine fish in the U.S. Seafood market. *Environ. Health Perspect.* 115, 235–242. <https://doi.org/10.1289/ehp.9377>.
- Thorpe, S.E., Murphy, E.J., 2022. Spatial and temporal variability and connectivity of the marine environment of the South Sandwich Islands, Southern Ocean. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 198, 105057. <https://doi.org/10.1016/j.dsr2.2022.105057>.
- Xavier, J., Croxall, J., Trathan, P., Rodhouse, P., 2003. Inter-annual variation in the cephalopod component of the diet of the wandering albatross, *Diomedea exulans*, breeding at Bird Island, South Georgia. *Mar. Biol.* 142, 611–622. <https://doi.org/10.1007/s00227-002-0962-y>.
- Xavier, J.C., Chereil, Y., 2021. Cephalopod Beak Guide for the Southern Ocean: an Update on Taxonomy. *British Antarctic Survey, Cambridge*.
- Xavier, J.C., Chereil, Y., Allcock, L., Rosa, R., Sabirov, R.M., Blicher, M.E., Golikov, A.V., 2018. A review on the biodiversity, distribution and trophic role of cephalopods in the Arctic and Antarctic marine ecosystems under a changing ocean. *Mar. Biol.* 165, 93. <https://doi.org/10.1007/s00227-018-3352-9>.
- Xavier, J.C., Ferreira, S., Tavares, S., Santos, N., Míeiro, C.L., Trathan, P.N., Lourenço, S., Martinho, F., Steinke, D., Seco, J., Pereira, E., Pardal, M., Chereil, Y., 2016. The significance of cephalopod beaks in marine ecology studies: can we use beaks for DNA analyses and mercury contamination assessment? *Mar. Pollut. Bull.* 103, 220–226. <https://doi.org/10.1016/j.marpolbul.2015.12.016>.
- Xavier, J.C., Golikov, A.V., Queirós, J.P., Perales-Raya, C., Rosas-Luis, R., Abreu, J., Bello, G., Bustamante, P., Capaz, J.C., Dimkovikj, V.H., González, A.F., Guímaro, H., Guerra-Marrero, A., Gomes-Pereira, J.N., Hernández-Urcera, J., Kubodera, T., Laptikhovskiy, V., Lefkaditou, E., Lishchenko, F., Luna, A., Liu, B., Pierce, G.J., Pissarra, V., Reveillac, E., Romanov, E.V., Rosa, R., Roscian, M., Rose-Mann, L., Rouget, I., Sánchez, P., Sánchez-Márquez, A., Seixas, S., Souquet, L., Varela, J., Vidal, E.A.G., Chereil, Y., 2022. The significance of cephalopod beaks as a research tool: an update. *Front. Physiol.* 13, 1038064. <https://doi.org/10.3389/fphys.2022.1038064>.