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# Antarctic octopod beaks as proxy for mercury concentrations in soft tissues

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## Abstract:

As the role of mercury is poorly known in Southern Ocean biota, the total mercury (T-Hg) concentrations were evaluated in upper/lower beaks, digestive gland, gills and mantle muscle of *Adelieledone polymorpha* and *Pareledone turqueti*, two of the most abundant octopod species around South Georgia. Beaks had the lowest T-Hg concentrations (*A. polymorpha*: [T-Hg]<sub>Upper</sub> = 27.2±12.9 ng·g<sup>-1</sup> and [T-Hg]<sub>Lower</sub> = 27.5±20.0 ng·g<sup>-1</sup>; *P. turqueti*: [T-Hg]<sub>Upper</sub> = 34.6±13.9 ng·g<sup>-1</sup> and [T-Hg]<sub>Lower</sub> = 56.8±42.0 ng·g<sup>-1</sup>), followed by gills and muscle. The highest values were recorded in the digestive glands (*A. polymorpha*: 251.6±69.7 ng·g<sup>-1</sup>; *P. turqueti*: 347.0±177.0 ng·g<sup>-1</sup>). Significant relationships were found between the concentrations of T-Hg in the beaks and muscle of *A. polymorpha* (T-Hg in muscle is 10 times higher than in beaks). This study shows that beaks can be used as proxy for T-Hg in muscle for some octopod species, and a helpful tool for estimating total Hg body burden from beaks.

## Introduction

Mercury (Hg) is acknowledged as a global toxicity problem (Selin, 2009). Even the Southern Ocean has shown some of the highest Hg concentrations in open waters ever recorded (Cossa *et al.*, 2011; Hogg *et al.*, 2011; Shaw *et al.*, 2014). These Hg levels seem to result from a unique combination of high geological activity and physiochemical processes associated with the atmosphere, ocean and sea ice (Cossa *et al.*, 2011). Due to its high affinity to proteins (Bloom, 1992), Hg under its organometallic derivatives can irreversibly affect the well-functioning of entire food webs, bioaccumulating in organisms and biomagnifying along trophic levels with demonstrated adverse effects on top predators (Bargagli *et al.*, 1998; Goutte *et al.*, 2014; Tavares *et al.*, 2013).

Within the Southern Ocean food webs, cephalopods are known to play important roles as both mid-trophic predator and prey for numerous top predators, including seabirds (Alvito *et al.*, 2014; Casaux and Ramón, 2002; Cherel and Klages, 1998), marine mammals (Clarke, 1980; Negri *et al.*, 2016; Rodhouse *et al.*, 1992) and fish (Seco *et al.*, 2016; Smales, 1996; Xavier *et al.*, 2002). Since cephalopods are very elusive animals, researchers have still not fully addressed the existing gaps of knowledge regarding their ecology, including levels of contaminants such as Hg (Xavier *et al.*, 2016). To overcome the hard challenge of collecting cephalopods using traditional sampling methods (e.g. scientific nets), researchers have been collecting cephalopod beaks, sclerotized mouthparts, from the diet of top predators to study their ecology (Xavier and Cherel, 2009).

Only recently, researchers have been exploring the usage of beaks as a tool to evaluate Hg concentrations on cephalopods (Matias *et al.*, 2019; Nguyen *et al.*, 2018; Xavier *et al.*, 2016). The reason for that is beaks are capable of absorbing and creating strong links with Hg due to its high specificity for amino groups present in proteins (Barriada *et al.*, 2008), with Hg varying greatly as differences in beak pigmentation reflect different protein content (Miserez *et al.*, 2008). Similarly to nitrogen stable isotope signatures accompany the gradual formation of the beaks (Cherel & Hobson, 2005; Chouvelon *et al.*, 2011), it was also found recently that Hg increases from the rostrum towards the crest which is the most recent and metabolically active region of the beak (Queirós *et al.*, 2020).

Within Antarctic cephalopods, the octopod species *Adelieledone polymorpha* (Robson, 1930) and *Pareledone turqueti* (Joubin, 1905) are good bioindicators of the health of South Georgia's benthic ecosystems, as both species are short-lived and the most abundant cephalopod benthic species in the region (Barratt *et al.*, 2008; Collins *et al.*, 2004; Matias *et al.*, 2019). These two species occupy the same habitat throughout their lifecycle (i.e. sympatric) while exploiting different trophic niches in function of the adaptations in their digestive apparatus, such as their beak morphology (Allcock *et al.* 2003; Daly & Rodhouse, 1994; Matias *et al.*, 2019; Piatkowski *et al.*, 2003). Both species are also the main prey for some top predators (e.g. Blue-eyed shag *Phalacrocorax atriceps*, Southern elephant seal *Mirounga leonina*, Patagonian toothfish *Dissostichus eleginoides*, among others) breeding in the South Georgia region (Casaux and Ramón, 2002; Rodhouse *et al.*, 1992; Xavier *et al.*, 2002) thus understanding how Hg accumulates in the different tissues of *A. polymorpha* and *P. turqueti* can provide crucial information regarding Hg pathways in that region's ecosystems. As Hg has been found to accumulate differently in Southern Ocean squid tissues, with some species presenting higher Hg levels in the digestive gland and others in the muscles, highlighting existent knowledge gaps regarding Hg pathways in cephalopods (Pierce *et al.*, 2008; Seco *et al.*, 2020). The main aim of this study is 1) to quantify the total Hg (T-Hg) concentrations in upper/lower beaks, digestive gland, gills and mantle muscle of the octopods *A. polymorpha* and *P. turqueti*, 2) to compare T-Hg concentrations of tissues between both species, and 3) to discuss patterns and differences in T-Hg concentration in Antarctic octopods, in comparison to other octopod species elsewhere.

## Materials & Methods

Specimens from the Antarctic octopods *A. polymorpha* (n = 25) and *P. turqueti* (n = 20) were collected on board of the *Dorada* and *SIL* vessels during bottom trawling surveys in the South Georgia region, between January and February of 2004 (*A. polymorpha*: n = 9; *P. turqueti*: n = 10; adopted from Matias *et al.*, 2019), of 2013 (*A. polymorpha*: n = 6; *P. turqueti*: n = 5) and 2017 (*A. polymorpha*: n = 10; *P. turqueti*: n = 5). All the samples were preserved at -20°C throughout the study. At the laboratory, several tissues were sampled – digestive gland, gills and mantle muscle. However, in several specimens the digestive gland burst during the sampling process thus not being possible to collect. The weight (M, in g) and dorsal mantle length (DML, in mm) were measured for both *A. polymorpha* (M = 66.1 ± 32.7g, DML = 58.8 ± 13.1mm) and *P. turqueti* (M = 71.4 ± 29.2g, DML = 61.4 ± 11.4mm). All specimens were sub-adult. The fieldwork was carried out by a team of the British Antarctic Survey (BAS) on behalf of the Government of South Georgia and the South Sandwich Islands. The animal procedures used in this study were reviewed and approved by the joint BAS Cambridge University Animal Welfare and Ethical Committee.

At the laboratory, all samples were cleaned and both upper and lower beaks measured to the nearest of 0.01mm using a digital calliper for the upper beak's hood (UHL) and crest lengths (UCL), and lower beak's hood length (LHL). All tissue samples were lyophilized for 24h and then ground into fine powder for being analysed in an Advanced Mercury Analyzer LECO 254 (Xavier *et al.*, 2016). The recovery efficiency was calculated using mussel tissue (ERM – CE278K; 97 ± 16%) as certified reference material (CRM). All concentration data are expressed as a function of dry weight (ng·g<sup>-1</sup> dwt) with a detection limit of 0.01 ng.

The normality of data was assessed using Shapiro-Wilks tests and statistical analyses were performed accordingly. Comparing all individuals, differences between tissues were assessed through a Kruskal Wallis test and post-hoc Dunn's test, using 2013 and 2017 data. Regarding beaks versus muscles, the significance of the differences between T-Hg concentrations in the muscles and beaks were calculated with a one-way ANOVA and assessed using post-hoc Tukey test. After checking that data met all assumptions, a Pearson correlation and linear regression were used to study the relationship between T-Hg concentrations between muscles and beaks, and with its known beak measures. P values were considered statistically significant when p-values were lower than 0.05. All the calculations were performed using the GraphPad statistical software.

## Results

### *Total Hg in tissues from Adelleledone polymorpha*

The upper (27.21 ± 12.88 ng·g<sup>-1</sup>) and lower beaks (27.50 ± 20.00 ng·g<sup>-1</sup>) of *A. polymorpha* presented the lowest concentrations while the digestive gland (251.6 ± 69.71 ng·g<sup>-1</sup>) presented the highest T-Hg concentrations for this species (Table 1; Fig. 1). There were found significant differences of T-Hg concentrations between all tissues (H = 50.35, p < 0.001), with the exception between upper and lower beaks and, between T-Hg concentrations found in gills (117.1 ± 23.14 ng·g<sup>-1</sup>) and muscle (126.30 ± 31.97 ng·g<sup>-1</sup>).

Comparing the T-Hg between muscle and upper/lower beaks from *A. polymorpha*, T-Hg concentrations in muscle (231.90 ± 116.80 ng·g<sup>-1</sup>) were approximately 5 times higher than in the upper (42.53 ± 20.19 ng·g<sup>-1</sup>) and lower beaks (41.98 ± 19.84 ng·g<sup>-1</sup>; F<sub>2,45</sub> = 20.36, p < 0.001). The T-Hg concentrations in the upper and lower beaks increased proportionally to T-Hg in the muscle (Linear Regression: upper beaks to muscle - r<sup>2</sup> = 0.554, F<sub>1,14</sub> = 17.40, p < 0.001; lower beaks to muscle - r<sup>2</sup> = 0.436, F<sub>1,14</sub> = 10.80, p < 0.005). The relationships found are translated by the following equations, respectively (Fig. 3):

$$[T-Hg]_{Muscle} = \frac{-0.01270 + [T-Hg]_{Upper}}{0.1287}$$

$$[T-Hg]_{Muscle} = \frac{-0.01598 + [T-Hg]_{Lower}}{0.1121}$$

The relationships between upper/lower beaks with muscle were similar, as there was no significant difference between these two equations ( $F_{1,29} = 0.012$ ,  $p = 0.914$ ). Also, T-Hg concentrations in beaks is significantly related with UCL (Linear regression:  $r^2 = 0.236$ ,  $F_{1,14} = 4.327$ ,  $p = 0.024$ ). A linear regression was calculated and significant relationships were found between T-Hg concentrations in muscle and beak measurements – UHL ( $r^2 = 0.284$ ,  $F_{1,12} = 4.753$ ,  $p = 0.049$ ), UCL ( $r^2 = 0.573$ ,  $F_{1,12} = 16.10$ ,  $p = 0.002$ ) and LHL ( $r^2 = 0.608$ ,  $F_{1,12} = 18.61$ ,  $p = 0.001$ ). The relationships found are translated by the following equations, respectively (Fig. 4):

$$[T-Hg]_{Muscle} = \frac{-2.552 + UHL}{2.233}$$

$$[T-Hg]_{Muscle} = \frac{-6.321 + UCL}{8.978}$$

$$[T-Hg]_{Muscle} = \frac{-2.184 + LHL}{2.885}$$

#### *Total Hg in tissues from Pareledone turqueti*

Similarly, to *A. polymorpha*, the lowest T-Hg concentrations in *P. turqueti* were registered in the upper ( $34.62 \pm 13.92 \text{ ng}\cdot\text{g}^{-1}$ ) and lower beaks ( $56.78 \pm 42.03 \text{ ng}\cdot\text{g}^{-1}$ ) whereas the digestive gland ( $347.00 \pm 177.02 \text{ ng}\cdot\text{g}^{-1}$ ) presented the highest T-Hg concentrations. These results, were significantly different in relation to the other tissues analysed ( $H = 24.58$ ,  $p < 0.001$ ; Table 1; Fig. 1). No differences were found in between upper and lower beaks, and between T-Hg concentrations in gills ( $164.9 \pm 77.00 \text{ ng}\cdot\text{g}^{-1}$ ) and muscle ( $196.40 \pm 83.23 \text{ ng}\cdot\text{g}^{-1}$ ) were noted. Moreover, no significant effects on T-Hg related to size/mass were found.

The T-Hg concentrations in muscle ( $364.80 \pm 148.30 \text{ ng}\cdot\text{g}^{-1}$ ) were 10 times higher ( $H = 29.37$ ,  $p < 0.001$ ,  $n = 15$ ) than the T-Hg concentrations in upper ( $38.43 \pm 10.00 \text{ ng}\cdot\text{g}^{-1}$ ) and lower beaks ( $44.43 \pm 25.33 \text{ ng}\cdot\text{g}^{-1}$ ). No significant relationships were observed between the T-Hg concentrations in muscle and upper/lower beaks (see Table 2) or between T-Hg concentrations in muscle and upper/lower beak measurements (see Table 2).

#### *Comparison of total Hg in soft and hard tissues of Adelleledone polymorpha and Pareledone turqueti*

When comparing T-Hg between both species, *P. turqueti* presented higher concentrations in gills ( $t = 2.275$ ,  $df = 23$ ,  $p = 0.033$ ), muscle ( $t = 2.887$ ,  $df = 22$ ,  $p = 0.009$ ) and lower beak ( $U = 6.00$ ,  $p = 0.045$ ), than in *A. polymorpha*. No differences were found regarding T-Hg concentrations in the digestive gland and upper beaks between species. Even though both species beaks presented similar values, the T-Hg concentrations in *P. turqueti* digestive gland ( $CV = 48.39\%$ ), gills ( $CV = 44.28\%$ ) and muscle ( $CV = 50.09\%$ ) presented a higher variability, contrasting with the much narrower T-Hg concentrations registered in *A. polymorpha* ( $CV_{\text{Dig.Gland}} = 26.77\%$ ;  $CV_{\text{Gills}} = 19.08\%$  and  $CV_{\text{Muscle}} = 24.39\%$ ). Significant differences were also found between species' muscle T-Hg concentrations ( $t = 2.782$ ,  $df = 29$ ,  $p = 0.009$ ).

## Discussion

To the best of our knowledge, this is the first time that T-Hg specificity to tissue allocation was studied in the Antarctic octopods as well as establishing relationships between Hg levels in upper/lower beaks, digestive gland, gills and mantle muscle. Due to their beaks' frequent appearance in the diet of numerous predators of the South Georgia region, the Antarctic octopods *A. polymorpha* and *P. turqueti* accumulated T-Hg in their tissues will ultimately enable to roughly estimate both species contribution for T-Hg in Antarctic top predator exposures. Nevertheless, mercury bioaccumulation is a complex process still poorly understood and any interpretation of the results must take into account the different ecological and physiological processes taking place between species.

Data regarding Hg concentrations in Antarctic octopods is still scarce (Matias *et al.*, 2019) as previous studies mainly focused on squid species and analysed few tissues (Anderson *et al.*, 2009; Cipro *et al.*, 2018; Seco *et al.*, 2020; Xavier *et al.*, 2016). However, both *A. polymorpha* and *P. turqueti* had higher T-Hg concentrations relative to the data on T-Hg levels in Antarctic squids from previous studies (Table 3). These results are consistent with what is known for cephalopod ecology and Hg biochemistry, with benthic cephalopods presenting higher Hg concentrations due to a higher exposition to dissolved organic Hg concentrations resulting from the methylation by seafloor microorganisms and/or benthic prey enriched in organic Hg (Bargagli *et al.*, 1998; Bustamante *et al.*, 2006). Moreover, higher T-Hg concentrations have been documented in South Georgia top predators, such as seabirds which are known to heavily rely on cephalopods as food source (Becker *et al.*, 2002; Cherel *et al.*, 2018), highlighting the importance of addressing Hg bioaccumulation in cephalopods.

The digestive gland in *A. polymorpha* and *P. turqueti* was the tissue with higher T-Hg, as it has been previously reported in many cephalopods around the world (Bustamante *et al.*, 2006; Seixas *et al.*, 2005). This organ is known for being able to store high levels of various trace elements and organic for pollutants, playing a major role in the detoxification and elimination of such potentially harmful elements and compounds (Penicaud *et al.*, 2017; Seixas *et al.*, 2005). Dietary organic Hg is primarily incorporated in the digestive gland where it can be demethylated and/or translocated to the muscles, since Hg has a higher affinity for the sulfhydryl groups present in myocytes (Penicaud *et al.*, 2017; Raimundo *et al.*, 2010). For that reason, even though T-Hg concentrations were higher in the digestive gland, the muscles encompass more than 70% of a specimen's total body weight and it represents the biggest proportion of total T-Hg body burden (Bustamante *et al.*, 2006). The contrasting T-Hg levels between gills and digestive gland indicate that Hg intake might occur mainly via the digestive pathways and/or that the Hg incorporated through the gills is rapidly transferred towards other tissues (Lacoue-Labarthe *et al.*, 2009), as verified in Antarctic squid (Seco *et al.*, 2020). As expected, beaks presented the lowest concentrations relative to the other tissues (Xavier *et al.*, 2016), since Hg bioaccumulation in sclerotized tissues might be slower relative to soft tissues, due to their lower protein content (Barriada *et al.*, 2008). Even though cephalopod beak's T-Hg accumulation is still poorly understood (Matias *et al.*, 2019; Xavier *et al.*, 2016), the T-Hg concentrations in the muscles in *A. polymorpha* and *P. turqueti* were in the same range to the ones registered in other northern hemisphere octopod species, such as *Octopus vulgaris* (Bustamante *et al.*, 2006; Raimundo *et al.*, 2010; Seixas *et al.*, 2005).

One of the hypotheses raised that may explain the differences found in the T-Hg concentrations between the Antarctic octopods *A. polymorpha* and *P. turqueti* lies on the different trophic ecology features between these species (Piatkowski *et al.*, 2003). Feeding habits are known to influence T-Hg concentrations, as more contaminated prey would mean higher T-Hg levels in their predators due to Hg bioaccumulation (Anderson *et al.*, 2009; Paiva *et al.*, 2008). The wider range of T-Hg concentrations in *P. turqueti* (Fig. 1 and 2) can be due to its generalist feeding behaviour, as this species prey on a broad range of prey with different trophic positions, which will reflect in higher variability of T-Hg levels. On the other hand, the lower variability of T-Hg concentrations in *A. polymorpha* may be due to the specificity of its

trophic niche (Allcock et al., 2003; Daly and Rodhouse, 1994; Matias et al., 2019). The diet of both species seems to be mainly composed by amphipods and polychaetes even though the remains of fish and hard-shelled organisms were only identified on the diet of *P. turqueti*. (Daly, 1996; Piatkowski et al., 2001). Moreover, it is hypothesised that lower T-Hg concentrations in the gills of *A. polymorpha* can be an indication of an off-bottom lifestyle (Daly & Rodhouse, 1994; Yau et al., 2002), contrasting with *P. turqueti* and other benthic octopods which present higher Hg levels relative to more pelagic cephalopod species, possibly due to the higher exposition to Hg deposits and methylation (see above) in the seabed (Bustamante et al., 2006; Storelli et al., 2006; Wiener et al., 2007). Nonetheless, as cephalopod gills are still poorly documented and possibly present higher transferring rates to other tissues such as the digestive gland, because of its high vascularisation.

Beaks presented lower T-Hg concentrations when comparing with muscle or other tissues. A trend was observed in *A. polymorpha*, with bigger individuals (larger beaks) presenting higher concentrations of T-Hg in the muscle. This relationship may be explained by Hg absorption along individual's life and ontogenetic dietary shifts, with adults (bigger beak) preying on larger prey containing high Hg body burden (Chouvelon et al., 2011). In contrast, no strong relationships between beak size and T-Hg concentrations in the beak itself were found in this study, as only one significant relationship was found between the UCL and T-Hg concentrations in the upper beak of *A. polymorpha*. In addition, a positive relationship was found between the T-Hg concentrations in the beaks and muscle of *A. polymorpha*. Significant correlations between T-Hg concentrations in muscle and beaks have been reported in previous studies, especially for octopods (Nguyen et al., 2018). This main finding will help future studies to better understand Hg transfer along trophic levels and to evaluate the Hg contribution of this octopod species to Hg intake along the Southern Ocean food web. Since the only tissue found in the predators' diet are the beaks, and with the equations (see results) it will be possible to roughly estimate the amount of T-Hg that the predator ingested when eating the octopod.

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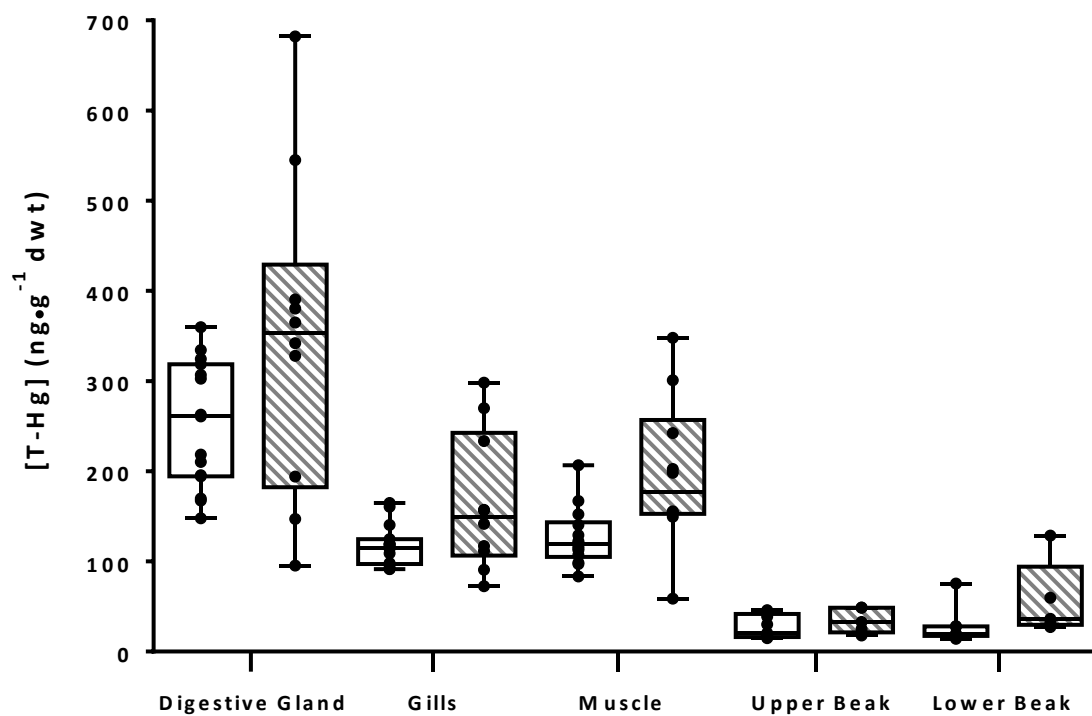


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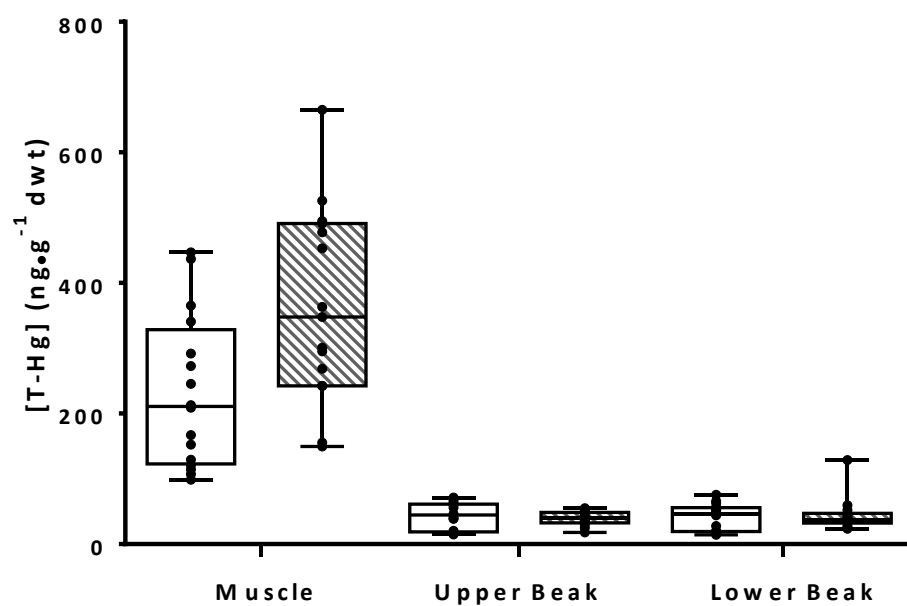
**Table 1.** Total mercury (T-Hg) concentrations in the upper/lower beaks, digestive gland, gills and muscle of *Adelieledone polymorpha* and *Pareledone turqueti*. Mean and standard deviation (SD) are given. Data from 2013 and 2017.

Species/ Tissues	[T-Hg] (in ng·g <sup>-1</sup> )		
	<i>n</i>	Mean ± SD	min – max
<b><i>Adelieledone polymorpha</i></b>			
Digestive gland	15	251.6 ± 69.7	147.7 – 359.6
Gill	15	117.1 ± 126.3	91.4 – 164.8
Muscle	14	126.3 ± 31.9	83.3 – 206.6
Upper beak	9*	27.2 ± 12.9	14.9 – 46.0
Lower beak	8*	27.5 ± 20.0	14.2 – 75.5
<b><i>Pareledone turqueti</i></b>			
Digestive gland	10	347.0 ± 177.0	95.4 – 682.2
Gill	10	164.9 ± 77.0	72.5 – 298.3
Muscle	10	196.4 ± 83.2	58.6 – 347.8
Upper beak	5*	34.6 ± 13.9	17.8 – 48.8
Lower beak	5*	56.7 ± 42.0	27.1 – 128.6

\* tissues only collected during 2017 survey



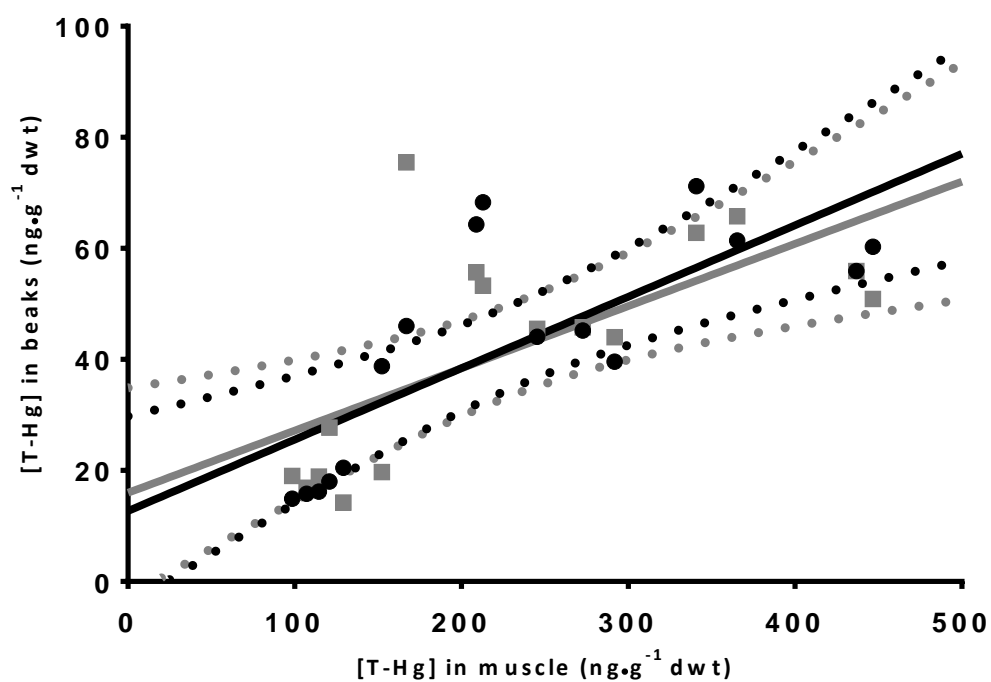
**Figure 1.** Boxplot of total mercury (T-Hg) concentrations found in the digestive gland, gill, muscle, upper and lower beak of *Adelieledone polymorpha* (blank-filled) and *Pareledone turqueti* (stripe-filled). Boxplot shows the median (line), 1<sup>st</sup>/3<sup>rd</sup> quartile (box), minimum/maximum (whiskers) and data values (black dots).



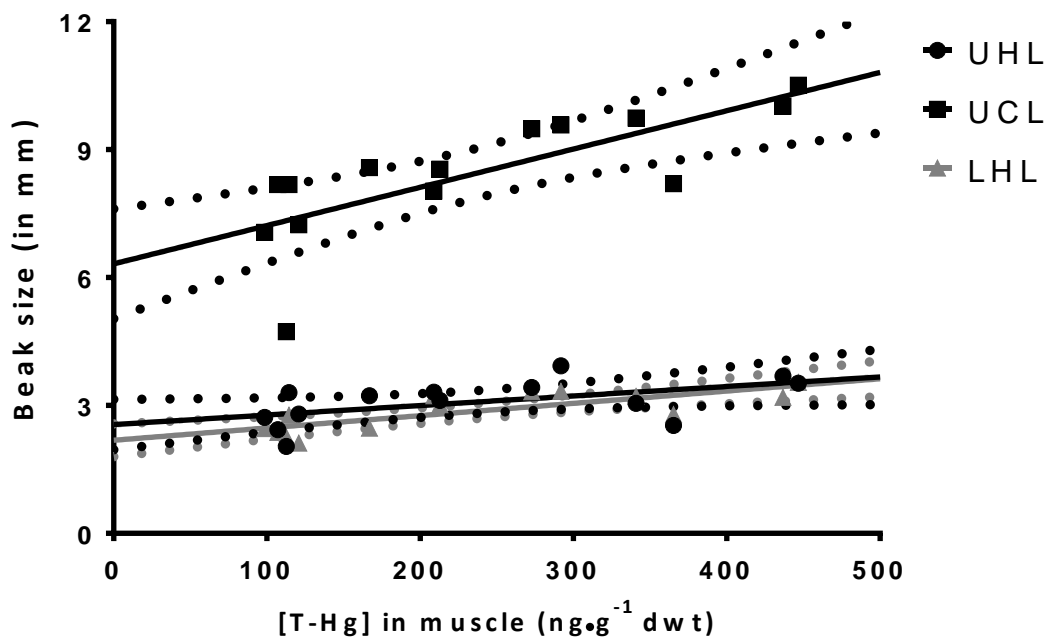
**Figure 2.** Total mercury (T-Hg) concentrations found in the muscle and beaks of *Adelieledone polymorpha* (dark grey) and *Pareledone turqueti* (light grey). Boxplot shows the median (line), 1<sup>st</sup>/3<sup>rd</sup> quartile (box), minimum/maximum (whiskers) and data values (black dots).

**Table 2.** Total mercury (T-Hg) concentrations in the muscle and in the upper and lower beaks of *Adelieledone polymorpha* and *Pareledone turqueti*. Mean and standard deviation (SD) are given. Legend: UHL – Upper beak’s hood length, UCL – Upper beak’s crest length and LHL – Lower beak’s hood length. Data from 2004 and 2017.

	<i>n</i>	[T-Hg] (ng·g <sup>-1</sup> )		Beak Size (mm)	
		Mean ± SD	min – max	Mean ± SD	min – max
<b><i>A. polymorpha</i></b>	16				
Muscle		231.9 ± 116.8	98.5 – 446.7		
Upper Beak		42.5 ± 20.2	14.9 – 71.2	<b>UHL:</b> 3.13 ± 0.42 <b>UCL:</b> 8.31 ± 1.77	2.43 – 3.93 3.01 – 10.51
Lower Beak		41.9 ± 19.8	14.2 – 75.5	<b>LHL:</b> 2.87 ± 0.43	2.12 – 3.54
<b><i>P. turqueti</i></b>	15				
Muscle		364.8 ± 148.3	149.6 – 665.0		
Upper Beak		38.4 ± 10.0	17.8 – 54.9	<b>UHL:</b> 5.43 ± 0.66 <b>UCL:</b> 12.72 ± 1.32	4.16 – 6.63 10.56 – 15.11
Lower Beak		44.4 ± 25.3	23.5 – 128.6	<b>LHL:</b> 4.11 ± 0.65	2.92 – 5.19



**Figure 3.** Linear regressions between the total mercury (T-Hg) concentrations in muscle and the T-Hg concentrations in the upper (black) and lower (grey) beaks of *Adelieledone polymorpha*. The dotted error bands represent the 95% confidence interval.



**Figure 4.** Relationship between T-Hg concentration in the muscle and known measures of the beak. From the beak, the upper hood (UHL) and upper crest lengths (UCL) data points are represented by the circle and square, respectively. The lower beak's hood length (LHL) data points are represented by the grey triangles. The lines represent the relationship between variables and the small dotted curves delineate the 95% confidence interval.

**Table 3.** Concentrations of total mercury (T-Hg) in several species of Antarctic cephalopods registered. Mean and standard deviation (SD) are given. The abbreviations of the regions are: SG – South Georgia Island, KE – Kerguelen Island and MC – Macquarie Island. Concentrations of T-Hg are expressed in  $\mu\text{g}\cdot\text{g}^{-1}$  for better comparison.

Species	n	Digestive Gland	n	Gills	n	Muscle	n	Upper Beak	n	Lower Beak	Region	Study
<b>Octopus</b>												
Megaleledonidae												
<i>Adelieledone polymorpha</i>	15	0.252 ± 0.070	15	0.117 ± 0.023	14	0.126 ± 0.032	9	0.027 ± 0.013	8	0.028 ± 0.020	SG	Current study
<i>Pareledone turqueti</i>	10	0.347 ± 0.177	10	0.165 ± 0.077	10	0.196 ± 0.083	5	0.035 ± 0.014	5	0.057 ± 0.042	SG	Current study
<b>Squids</b>												
Cranchiidae												
<i>Galiteuthis glacialis</i>	-	-	-	-	3	0.23 ± 0.08	-	-	-	-	SG	Anderson <i>et al.</i> (2009)
	-	-	-	-	-	-	-	-	4	0.008 ± 0.004	SG	Xavier <i>et al.</i> (2016)
Gonatidae												
	-	-	-	-	2	0.60 ± 0.02	-	-	-	-	SG	Anderson <i>et al.</i> (2009)
<i>Gonatus antarcticus</i>	-	-	-	-	-	-	-	-	4	0.013 ± 0.003	SG	Xavier <i>et al.</i> (2016)
	-	-	4	0.089 ± 0.033	4	0.162 ± 0.058	-	-	-	-	SG	Seco <i>et al.</i> , (2020)
Ommastrephidae												
<i>Martialia hyadesi</i>	-	-	-	-	2	0.05 ± 0.01	-	-	-	-	SG	Anderson <i>et al.</i> (2009)
<i>Todarodes angolensis</i>	-	-	-	-	11	0.100 ± 0.058	-	-	-	-	KE	Cipro <i>et al.</i> (2018)
Onychoteuthidae												
<i>Filippovia knipovitchi</i>	-	-	-	-	4	0.16 ± 0.09	-	-	-	-	SG	Anderson <i>et al.</i> (2009)
	-	-	-	-	-	-	-	-	5	0.025 ± 0.003	SG	Xavier <i>et al.</i> (2016)
	5	0.634 ± 0.098	5	0.306 ± 0.068	5	0.079 ± 0.028			-	-	SG	Seco <i>et al.</i> (2020)
<i>Moroteuthopsis ingens</i> *	-	-	-	-	8	0.099 ± 0.063	-	-	-	-	KE	Cipro <i>et al.</i> (2018)
	-	-	-	-	15	0.33 ± 0.12	-	-	-	-	MC	Mc Arthur <i>et al.</i> (2003)
<i>Moroteuthopsis longimana</i> *	-	-	-	-	2	0.10 ± 0.02	-	-	-	-	SG	Anderson <i>et al.</i> (2009)
	-	-	-	-	-	-	-	-	6	0.008 ± 0.003	SG	Xavier <i>et al.</i> (2016)
	5	0.045 ± 0.021	5	0.075 ± 0.032	5	0.082 ± 0.023	-	-	-	-	SG	Seco <i>et al.</i> (2020)
Neoteuthidae												
<i>Alluroteuthis antarcticus</i>	4	0.042 ± 0.002	4	0.060 ± 0.023	4	0.065 ± 0.004	-	-	-	-	SG	Seco <i>et al.</i> (2020)
Psychroteuthidae												
<i>Psychroteuthis glacialis</i>	3	0.025 ± 0.006	3	0.099 ± 0.003	3	0.083 ± 0.018	-	-	-	-	SG	Seco <i>et al.</i> (2020)

\*The species names are according to Bolstad *et al.* (2018).