Mercury levels in Southern Ocean Squid: variability over the last decade

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Abstract

The concentrations of total and proportions of organic mercury were measured in tissues of 355 individuals of 8 species of Southern Ocean squid (*Alluroteuthis antarcticus*, *Bathyteuthis abyssicola*, *Filippovia knipovitchi, Galiteuthis glacialis*, *Gonatus antarcticus, Kondakovia longimana, Psychroteuthis glacialis* and *Slosarczykovia circumantarctica)*. Squid were caught around South Georgia (Scotia Sea) during 5 cruises, between the austral summers of 2006/07 to 2016/17 to evaluate temporal changes in bioaccumulation and tissue partitioning. Total mercury concentrations varied between 4 ng g^{-1} and 804 ng g^{-1} among all tissues. Net accumulation of mercury in muscle with size was observed in *A. antarcticus, B. abyssicola* and *P. glacialis,* but no relationship was found for *S. circumantarctica* and lower concentrations were observed in larger individuals of *G. glacialis.* Muscle tissues had the highest mercury concentrations in the majority of species, except for *F. knipovitchi* for which the digestive gland contained highest concentrations. In terms of the percentage of organic mercury relative to total mercury in tissues, muscle always contained the highest values (67% to 97%), followed by the digestive gland (22% to 38%). Lowest organic mercury percentages were found consistently in the gills (9% to 19%), suggesting only low levels of incorporation through the dissolved pathway and/or a limited redistribution of dietary organic mercury towards this tissue. Overall, results are indicative of a decreasing trend of mercury concentrations in the majority of analysed species over the last decade. As cephalopods are an important Southern Ocean trophic link between primary consumers and top predators, these changes suggest decreasing mercury levels in lower trophic levels (i.e. squid prey) and an alleviation of the mercury burden on higher predators that consume squid.

Key-words: Organic mercury; Muscle; Gills; Digestive gland; Tissue allocation; Temporal trends.

Introduction

Evidence suggest that coleoid cephalopods, such as squid and octopods, are one of the groups that are benefitting from environmental change (Halpern et al., 2008), with some cephalopod populations being on the rise at a variety of locations worldwide (Arkhipkin et al., 2015; Doubleday et al., 2016). Within the Southern Ocean, cephalopods are important links between primary consumers and top predators (Clarke, 1996; Collins and Rodhouse, 2006; Seco et al., 2015). They prey mainly on crustaceans (Kear, 1992; Xavier et al., 2018) and are eaten by a wide range of predators including fish, penguins, albatrosses, seals and whales (Mikhalev et al., 1981; Split, 1995; Xavier and Cherel, 2009). In the Southern Ocean, the waters around South Georgia host a variety of squid species. *Galiteuthis glacialis, Gonatus antarcticus, Filippovia knipovitchi, Kondakovia longimana* and *Psychroteuthis glacialis* are oceanic species and some of the most important prey for several predators (such as seabirds, seals and whales (Xavier and Cherel, 2009)), both by number and by mass (Xavier et al., 2018). *Alluroteuthis antarcticus* and *Slosarczykovia circumantarctica* are taken by a wide range of predators although not in high numbers (Collins and Rodhouse, 2006; Xavier and Cherel, 2009). *Bathyteuthis abyssicola* is a deep-sea squid which is rarely found in the diet of predators. Despite their important role in the Antarctic ecosystem, there is still a lack of knowledge about their ecology (Clarke, 1983; Collins and Rodhouse, 2006; Xavier et al., 2018). Moreover, very few studies have focused on the ecotoxicological aspects of Southern Ocean cephalopods

(Anderson et al., 2009; Bustamante et al., 1998; Cipro et al., 2018). Their focal position within Southern Ocean food webs means that cephalopods are likely to be vectors of contaminants and could be valuable bioindicators of ecosystem contamination.

Mercury is one of the contaminants that has been acknowledged as a global toxicity problem (Selin, 2009; UNEP, 2013). Due to its high affinity to proteins (Bloom, 1992), mercury is highly bioaccumulative, becoming toxic for marine organisms higher up the food chain (Ackerman et al., 2014; Coelho et al., 2010; Dehn et al., 2006). Furthermore, it biomagnifies along food webs, putting long-lived top predators particularly at risk (e.g. (Goutte et al., 2014; Tartu et al., 2014; Tavares et al., 2013). Indeed, there is already evidence that major cephalopod predators have high levels of mercury in their tissues (e.g. Bustamante et al., 2003; Fontaine et al., 2014; Tavares et al., 2013). In terms of bioavailability within foodwebs, organic mercury (due to its high affinity to Sulphur-based protein groups) is a particularly toxic form of this element which demands further attention.

In a warming world, in which Antarctica is one of the most rapidly changing and vulnerable areas (IPCC, 2013; Rintoul et al., 2018; Turner et al., 2014), mercury is becoming more bioavailable due to the combined influence of increased temperature and depletion of oxygen, which favour methylation of the element by microorganisms (Cossa, 2013). It is therefore important to evaluate the impact that these changes have on the bioavailability of mercury in the Southern Ocean, along with any potential temporal trends in bioaccumulation. Fast growing, short-lived (i.e. generally 1-2 year life cycles) organisms such as squid (Arkhipkin, 2004; Boyle and Rodhouse, 2005) are likely to be responsive bioindicators of contaminant variability over time and space.

Cephalopods bioaccumulate mercury from two main sources: seawater and food. Some mercury uptake can occur through the gills during respiration, from seawater, but mercury in prey is considered to be the main intake pathway (Lacoue-Labarthe et al., 2009). To assess the relative importance of the two pathways in the bioaccumulation of mercury, we analysed mercury in three different tissues: 1) muscle, which represents most of the body weight of the animal and is expected to accumulate high levels of organic mercury (Bustamante et al., 2006); 2) gills, responsible for respiration and subjected to a constant water flow, so likely to be a pathway of incorporation of dissolved Hg from seawater; and 3) digestive gland, which is most affected by the dietary pathway (Bustamante et al., 2006; Penicaud et al., 2017; Pierce et al., 2008) and plays a major role in both the metabolism and detoxification of contaminants such as mercury (Bustamante et al., 2006).

In this study, we evaluate the concentrations of total and organic mercury in 8 different squid species (*A. antarcticus*, *B. abyssicola*, *F. knipovitchi, G. glacialis*, *G. antarcticus, K. longimana, P. glacialis*, *S. circumantarctica)* from South Georgia between the austral summers of 2006/07 and 2016/17. These species were selected due to their different ecological roles in the Southern Ocean ecosystem (Xavier et al., 2018). The specific objectives of this study are: 1) to evaluate the accumulation pattern along with size (a proxy of age) of Antarctic squid, 2) to understand the partitioning of total and organic mercury in different tissues (muscles, gills and digestive gland) and 3) to assess variability and trends in total and organic mercury concentrations of these species over a 10 year period (2006/07 to 2016/17).

Material and methods

115 *Sampling*

116 South Georgia is a sub-Antarctic island located in the southwest Atlantic (Figure
117 1). Water temperatures around this area vary from -0.95 ºC in winter to 1.75 ºC in
118 summer. South Georgia is a highly productive area of the Southern Ocean, therefore
119 it holds large populations of seabirds, marine mammals and it is one of the most
120 important Southern Ocean fishing areas (Collins et al., 2004; Murphy et al., 2007).

121 The samples were collected from the Scotia Sea, around South Georgia (Figure
122 1), during scientific cruises during the austral summer of 2006/07 (on board of the
123 Royal Research Ship (RRS) *James Clark Ross* (JCR): cruise JR161 [October –
124 December 2006], 2007/08 - JR177 [December 2007 – February 2008], 2008/09 -
125 JR200 [March – April 2009], Fishing Vessel (FV) *Sil* research survey SG13 [13
126 January 2013]) and RRS JCR cruise JR16003 (December 2016 – January 2017),
127 2016/17.

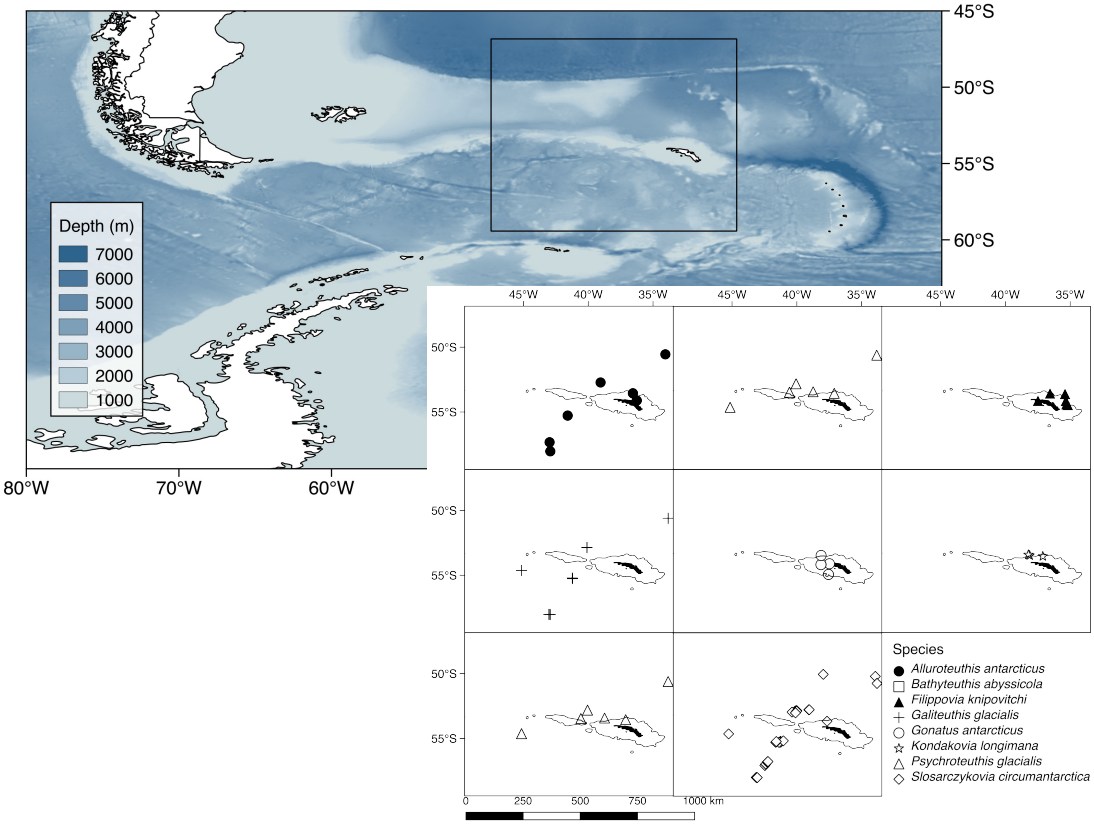

Figure 1. Sampling sites and distribution of species captured around South Georgia across all sampling years. Lines represent 1000m isobath.

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129 On board the RRS *JCR,* samples were collected using an 8 or 25 m² mouth-
130 opening Rectangular Midwater Trawl (RMT8 - mesh size reducing from 4.5 mm to 2.5
131 mm in the cod end; RMT25 - mesh size reducing from 8 mm to 4.5 mm in the cod end
132 (Roe and Shale, 1979)). The nets were rigged with two opening/closing nets that could
133 be remotely opened and closed at different depths. Samples were collected from
134 1000m deep to surface. Cephalopods were identified (Nesis, 1987; Xavier and Cherel,
135 2009), measured and weighed on board. Samples were preserved individually in
136 separate ziplock bags at -20 ºC for later laboratory analyses.

137 The samples collected by FV *Sil* were obtained from bottom trawls using an
138 FP120 trawl net with a standard steel bobbin rig, conducted at tow speeds between
139 3.1 and 4.1 knots over a distance of between 1.25 and 2.1 nautical miles, dependent

140 on the prevailing sea conditions and bottom topography. Whenever possible, samples
141 were identified on board but, in some cases, identification was not possible. In each
142 case, individuals were frozen at -20 ºC for later laboratory processing.
143
144 *Laboratory procedures*
145 All samples were checked for identification (using cephalopod beaks to confirm
146 identification where there was any doubt (Xavier and Cherel, 2009)), measured and
147 weighed again. When the measurement of the mantle length (ML) of the individual
148 was not possible, allometric equations were used, based on beak size (Xavier and
149 Cherel, 2009). An effort was made to collect samples of muscle, gills and digestive
150 gland in all individuals, although the digestive gland was destroyed in some
151 specimens.
152 After being dissected, the collected tissues were frozen in sterilised
153 decontaminated plastic vials and freeze-dried for at least 48 hours. Dried tissues were
154 homogenized and analysed for total mercury by thermal decomposition atomic
155 absorption spectrometry with gold amalgamation, using a LECO AMA-254 (Advanced
156 mercury analyser) following (Coelho et al., 2008). Organic mercury was determined
157 through digestion with a mixture of 18 % potassium bromide (KBr) in 5 % sulphuric
158 acid (H_2SO_4), followed by extraction of organic mercury into toluene (C_7H_8) and back-
159 extraction with an aqueous solution of thiosulphate ($Na_2S_2O_3$), as described in (Válega
160 et al., 2006). Where there was low individual mass (less than 200 mg), samples were
161 aggregated in groups of the same species, with similar sizes and collected from the
162 same location. Analytical quality control was performed using three certified reference
163 materials (CRM): NIST 2976 mussel tissue, ERM-CE278K mussel tissues and TORT-

3 lobster hepatopancreas. The obtained values (mean ± SD) for the whole of the CRM analyses ranged from 82 to 105 % (NIST 2976: 85 ± 7%; ERM-CE278K: 92 ± 5%; TORT-3: 93 ± 8 %). Analyses were performed in duplicate, and the coefficient of variation between replicates never exceeded 10%. TORT-3 was also used to validate organic mercury analyses, with an extraction efficiency of 95 ± 10 %. The limit of detection for this analytical method was 0.01 ng of absolute mercury. All concentration data are expressed as a function of dry weight (dw).

Statistical analysis

All analyses were performed using the R software version 3.4.2 (R Core Team, 2013). Correlations were determined between the mercury concentration and the mantle length. Hg levels were tested for normality using Shappiro-Wilk normality test and homogeneity using a Bartlett's test. Friedman tests were used to compare Hg values between the different tissues (muscle, gills, digestive gland) and a Wilcoxon Signed Ranks Test for the tissues of *G. antarcticus* (muscle, gills). Wilcoxon rank test and Kruskal–Wallis test were used to compare Hg in muscle tissue between different years, followed by Dunn's test multiple comparison test.

All values are presented as mean ± SD. The significance level for statistical analyses was always set at $\alpha = 5\%$.

Results

185 *Total mercury concentrations in Antarctic squid muscle*

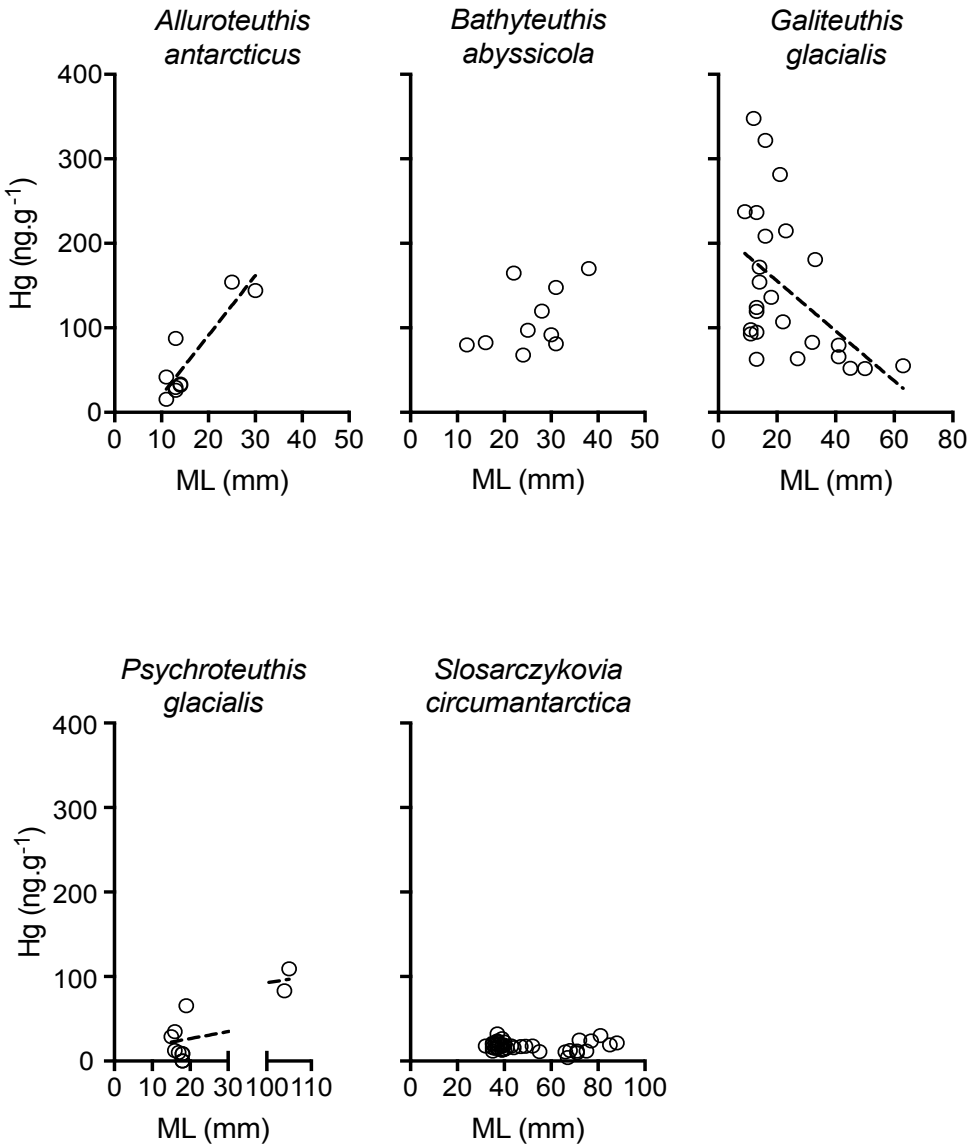

Figure 2 – Total mercury concentration (ng g^{-1} dw) versus mantle length (ML; mm) in individual Antarctic squid collected around South Georgia in the austral summer of 2007/08 (except *Psychroteuthis glacialis* 2016/17). Regression equations are given in the text.

186 Overall, total mercury values in muscle were: 63 ± 53 ng g^{-1} in *Alluroteuthis*
187 *antarcticus* (family Neoteuthidae), 110 ± 40 ng g^{-1} in *Bathyteuthis abyssicola* (family
188 Bathyteuthidae), 100 ± 80 ng g^{-1} in *Galiteuthis glacialis* (family Cranchiidae), 24 ± 21
189 ng g^{-1} in *Psychroteuthis glacialis* (family Psychroteuthidae) and 20 ± 20 ng g^{-1} in
190 *Slosarczykovia circumantarctica* (family Brachioteuthidae).

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Total mercury was analysed in the mantle muscle of squid species where there were sufficient individuals over a range of sizes to evaluate the pattern of bioaccumulation with size as a proxy for age (Figure 2). To avoid the effect of different years, samples were selected from the year with higher number of individual (2007/08 for *B. abyssicola, G. glacialis, A. antarcticus* and *S. circumantarctica*; 2016/17 for *P. glacialis*). Three patterns were noted: 1) an increasing concentration of total mercury with size [*A. antarcticus* ($Y = 7.074*X - 50.4$, $R^2 = 0.80$, $F_{1, 7} = 28.78$, $p = 0.001$) and *P. glacialis* ($Y = 0.8259*X + 10.1$, $R^2 = 0.74$, $F_{1, 7} = 19.68$, $p = 0.003$)]; 2) no change in total mercury concentration with size [*B. abyssicola* ($Y = 2.432*X + 47.83$, $R^2 = 0.24$, $F_{1, 8} = 2.56$, $p = 0.15$) and *S. circumantarctica* ($Y = -0.03111*X + 19.33$, $R^2 = 0.009$, $F_{1, 38} = 0.33$, $p = 0.56$)]; 3) a decrease in total mercury concentration with size (i.e. *G. glacialis* ($Y = -2.958*X + 214.8$, $R^2 = 0.2426$, $F_{1, 23} = 7.366$, $p = 0.012$)).

Differential tissue accumulation of total mercury in Antarctic squid

Analysis of mercury accumulation in different tissues was possible in four of the species (*A. antarcticus*, *F. knipovitchi, K. longimana* and *P. glacialis*). *F. knipovitchi* was the only species where there were significant differences between the tissues (Friedman test: $\chi^2 (2) = 7.6$, $p = 0.024$), with the digestive gland having a concentration 3 times higher than the muscle and gills. In the other three species, no statistical differences were observed between the mercury concentrations of the three tissues investigated (Friedman test: $\chi^2 (2) = 3$, $p = 0.5$; $\chi^2 (2) = 3$, $p = 0.5$; $\chi^2 (2) = 3$, $p = 0.5$; *A. antarcticus*, *K. longimana* and *P. glacialis,* respectively; Figure 3). For *G. antarcticus,* only the muscle and the gills were analysed, and no differences

between these two tissues were found (Wilcoxon Signed Ranks Test; Z= -1.826, p= 0.125).

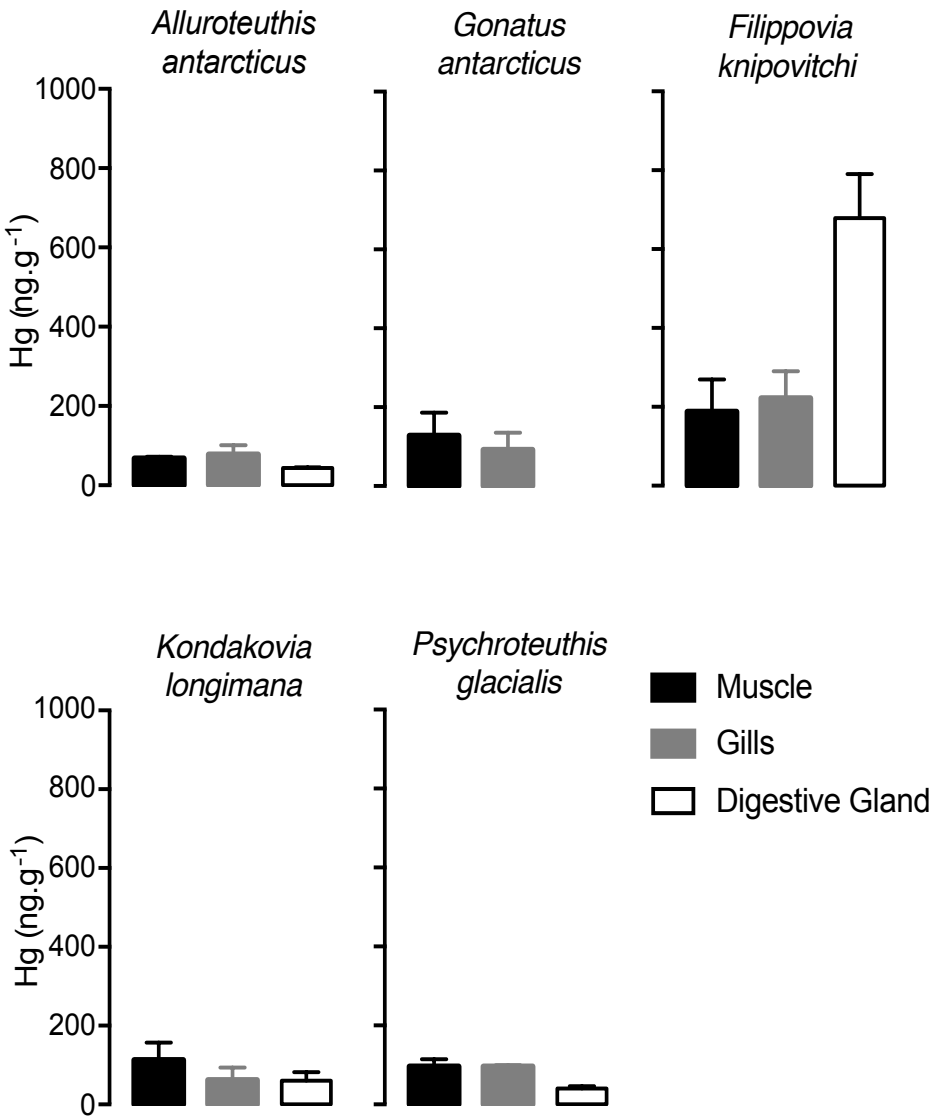

Figure 3. Total mercury concentrations (Mean ± SD, ng g^{-1} dw) in different tissues (muscle, gills and digestive gland) of Antarctic squid collected around South Georgia in the austral summer of 2012/2013. No digestive gland tissue was available for G*. antarcticus*.

Temporal trends of total mercury concentrations in muscle of Antarctic squid

Over the 10 year study period, there was a suggestive decreasing trend in mercury concentrations in the analysed species (Figure 4).

There were no differences in body size between the years for all the analysed species (*A. antarcticus,* Mann Whitney test, U = 9, p = 0.889; *B. abyssicola,* Kruskal–Wallis H= 1.754, p = 0.442; *G. glacialis,* Kruskal–Wallis H= 10.84, p = 0.442; *P. glacialis,* Mann Whitney test, U = 4.5, p = 0.8; *S. circumantarctica,* Kruskal–Wallis H= 65.46, p = 0.156). *A. antarcticus* and *P. glacialis* were each only caught in two of the austral seasons sampled. For *A. antarcticus*, total mercury was similar between the years 2007/08 (90 ± 60 ng g^{-1}) and 2008/09 (30 ± 3 ng g^{-1}; Wilcoxon rank test; W = 14, p = 0.19), as was *P. glacialis* between 2008/09 (10 ± 2 ng g^{-1}) and 2016/17 (40 ± 20 ng g^{-1}; Wilcoxon rank test; W = 10, p= 0.057). *B. abyssicola* were caught in three years, 2006/07 (150 ± 20 ng g^{-1}), 2007/08 (80 ± 10 ng g^{-1}) and 2016/17 (80 ± 1 ng g^{-1}), with individuals from 2006/07 having statistically higher concentrations of mercury than 2007/08 and 2016/17 (Kruskal–Wallis H= 6.709, p= 0.013; Figure 4). There were no differences between samples collected on the other two years. *G. glacialis* were caught on the four sampling cruises, 2006/07 (100 ± 60 ng g^{-1}), 20007/08 (150 ± 90 ng g^{-1}), 2008/09 (20 ± 10 ng g^{-1}) and 2016/17 (20 ± 7 ng g^{-1}). Mercury concentrations for this species were similar between the years 2006/07 and 2007/08 (Dunn's test Q = -8.77, p = 0.52) and also similar between the years 2008/09 and 2016/17 (Dunn's test Q = 0.057, p > 0.99), but were different between the two similarity groups (2006/07, 2007/08 and 2008/09, 2016/17 (Kruskal–Wallis H= 28.69, p< 0.001). *S. circumantarctica* was also caught in all the sampling years: 2006/07 (70 ± 10 ng g^{-1}), 2007/08 (20 ± 6 ng g^{-1}), 2008/09 (10 ± 6 ng g^{-1}) and 2016/17 (10 ± 7 ng g^{-1}); individuals

from 2006/07 had statistical higher concentration than the other 3 sampling years (Kruskal–Wallis H= 60.08, p< 0.001).

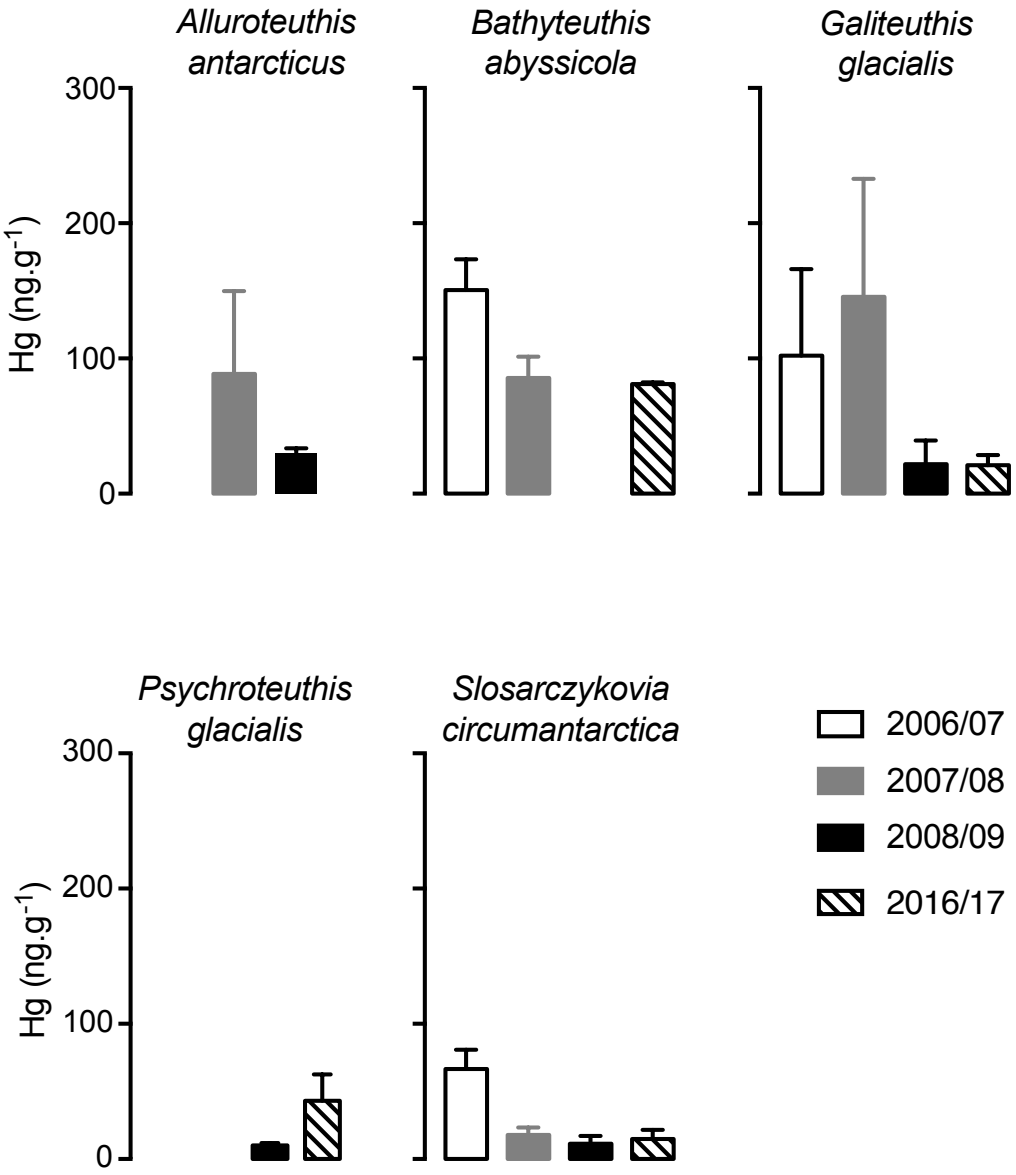

Figure 4- Total mercury concentrations (Mean ± SD, ng g^{-1} dw) in the muscles of Antarctic squid from the Southern Ocean collected around South Georgia in the austral summers of 2006/07, 2007/08, 2008/09 and 2016/17.

264 *Organic mercury concentrations and proportions in Antarctic squid*

265 Regarding the percentage of organic mercury relative to total mercury in the
266 different tissues of the five squid species sampled in 2013 (SG13 samples; Table 1),
267 muscle had the highest values (67% to 97%) in all the analysed species, followed by
268 the digestive gland (22% to 38%) and the gills (9% to 19%).

Table 1 – Total mercury (THg) and organic mercury (OHg) concentrations (ng g^{-1} dw) and percentage of OHg relative to THg in different tissues (digestive gland, gills and muscle) of Antarctic squid collected around South Georgia in the austral summer of 2013/14.

Species	*Tissue*	*THg*	*OHg*	*%OHg*
Alluroteuthis antarcticus	Digestive gland	42 ± 2	16 ± 3	38%
	Gills	60 ± 23	11 ± 2	18%
	Muscle	65 ± 4	52 ± 12	80%
Filippovia knipovitchi	Digestive gland	643 ± 98	144 ± 52	22%
	Gills	306 ± 68	57 ± 14	19%
	Muscle	79 ± 28	53 ± 9	67%
Gonatus antarcticus	Gills	89 ± 33	8 ± 2	10%
	Muscle	162 ± 58	158 ± 29	97%
Kondakovia longimana	Digestive gland	45 ± 21	11 ± 3	25%
	Gills	75 ± 32	9 ± 4	12%
	Muscle	82 ± 23	78 ± 14	95%
Psychroteuthis glacialis	Digestive gland	25 ± 6	6 ± 3	24%
	Gills	99 ± 3	9 ± 4	9%
	Muscle	83 ± 18	62 ± 22	74%

269

270 Organic mercury concentrations were also analysed in the muscle of three
271 species (*B. abyssicola, G. glacialis* and *S. circumantarctica,* Table 2) across sampling
272 years. Concentrations varied from 2 ng g^{-1} to 84 ng g^{-1}, constituting between 25% to
273 77% of total mercury. *S. circumantarctica* was the only species where organic mercury
274 was lower than 50% (40% in 2007/08; 47% in 2008/09; 25% in 2016/17). Although

organic mercury proportions differed significantly between species, there were no statistical differences between years.

Table 2 – Total mercury (THg) and organic mercury (OHg) concentrations (ng g^{-1} dw) and percentage of OHg relative to THg in the muscles of Antarctic squid collected around South Georgia in the austral summers of 2006/07, 2007/08, 2008/09 and 2015/16.

Species	*n*	*Year*	*THg*	*OHg*	*%OHg*
Bathyteuthis abyssicola	3	2006/07	103 ± 14	71 ± 12	69%
Galiteuthis glacialis	4	2006/07	40 ± 8	30 ± 5	75%
	4	2007/08	110 ± 26	60 ± 13	*55%*
	2	2008/09	26 ± 6	20 ± 5	77%
	2	2016/17	43 ± 2	24 ± 2	56%
Slosarczykovia circumantarctica	6	2007/08	20 ± 12	8 ± 5	40%
	4	2008/09	15 ± 5	7 + 3	47%
	2	2016/17	16 ± 7	4 ± 2	25%

Discussion

While some data exist on mercury concentrations in Antarctic cephalopods (Anderson et al., 2009; Cipro et al., 2018; McArthur et al., 2003), to the best of our knowledge, this is the first study to measure total and organic mercury concentrations, and to determine the percentage of organic mercury relative to total mercury in different tissues, across a range of Southern Ocean squid species.

Total mercury concentrations according to size

Biological and environmental factors such as size, sex, prey preferences and habitat are drivers for mercury concentrations in cephalopods (Bustamante et al., 2006; Monteiro et al., 1992; Storelli and Marcotrigiano, 1999). When looking into the relationship between size and contamination level in squid from South Georgia (Figure 3), it is possible to identify three different patterns: 1) *A. antarcticus* and *P. glacialis* had a positive correlation between contamination level and size, 2) The correlation for

292 *G. glacialis* was negative, 3) Mercury concentration did not appear to be related to size
293 in *B. abyssicola* or *S. circumantarctica*.

294 Significant variation and opposite patterns between size and mercury
295 concentrations in cephalopods have been reported previously. Mercury
296 concentrations increased with size in the veined squid *Loligo forbesi* (Chouvelon et
297 al., 2011; Monteiro et al., 1992; Pierce et al., 2008), the neon flying squid
298 *Ommastrephes bartramii* (Monteiro et al., 1992), the orange-back flying squid
299 *Sthenoteuthis pteropus* (Lischka et al., 2018), the common octopus *Octopus vulgaris*
300 (Rjeibi et al., 2014), the curled octopus *Eledone cirrhosa* (Rossi et al., 1993), the
301 common cuttlefish *Sepia officinalis* and *the European squid Loligo vulgaris* (Chouvelon
302 et al., 2011). However, in *O. vulgaris* (Raimundo et al., 2004; 2010a) and the greater
303 hooked *Onykia ingens* (McArthur et al., 2003), no relationships were found, while in
304 the spider octopus *Octopus salutii* (Storelli and Marcotrigiano, 1999), *O. vulgaris* and
305 *Loligo vulgaris* (Rjeibi et al., 2014) there were negative relationships. Hence, the effect
306 of size on mercury bioaccumulation in cephalopods is variable (Penicaud et al., 2017),
307 and is likely to be sensitive to fluctuating environmental conditions (see below).

308

309 *Tissue allocation of mercury in Antarctic squid*

310 For four species (*A. antarcticus, G. antarcticus K. longimana* and *P. glacialis*),
311 there were no differences in total mercury concentrations between tissues. However,
312 the fraction of organic mercury was always higher in muscle (from 67% to 97%) than
313 in the digestive gland (from 22% to 38%) and gills (9% to 19%). While presently the
314 reasons for the mercury distribution patterns are still a matter of debate, the
315 partitioning of total mercury among tissues is consistent with previous studies on

316 oceanic squid (Cranchidae, Histioteuthidae and Ommastrephidae (Bustamante et al.,
317 2006)), suggesting some degree of equilibrium between contaminant accumulation
318 and excretion rates, as well as efficient redistribution of total mercury between tissues.
319 In most studies of cephalopod species however, the digestive gland had higher total
320 mercury concentrations than other tissues (Bustamante et al., 2006; Pierce et al.,
321 2008; Raimundo et al., 2010b), as we observed in *F. knipovitchi* (Figure 4). The high
322 rates of absorption and assimilation of trace metals in the digestive gland (Bustamante
323 et al., 2002; Miramand and Bentley, 1992; Raimundo et al., 2004) make this organ a
324 major pathway of incorporation for contaminants into cephalopods (Bustamante et al.,
325 2002), and reflects the predominant role of the dietary pathway for mercury
326 bioaccumulation. The lower concentrations of organic mercury in the digestive gland
327 is in accordance with the detoxification of organic mercury by demethylation that is
328 suspected to occur in this organ (Bustamante et al., 2006; Penicaud et al., 2017). As
329 this form of mercury is incorporated from the diet, a fraction is likely to be demethylated
330 in the digestive gland, to allow its excretion (Lacoue-Labarthe et al., 2009), and a
331 portion will be circulated, trapped and stored in muscle tissues. Because organic
332 mercury has a strong affinity for the sulphydryl groups of proteins, its accumulation in
333 muscle tissues is favoured (Bloom, 1992; Leaner and Mason, 2004). In fish muscles,
334 for instance, it may be tightly bound by carbon-mercury and sulphydryl linkages
335 (Ruelas-Inzunza et al., 2003). As muscles comprise the highest mass fraction of
336 cephalopods, and given the known affinity of organic mercury to muscular proteins
337 (Bloom, 1992), high concentrations and proportions of organic mercury are to be
338 expected in this tissue. The present results also highlight the high bioavailability of

339 mercury within the Southern Ocean food web (Chouvelon et al., 2012; Clarkson, 1992;
340 Dehn et al., 2006).

341 Concentrations and proportions of organic mercury also show a considerable
342 variation between species. The high intra-species variation may be explained by the
343 dietary ontogenetic shift that occurs in cephalopods (Xavier et al., 2018) when they
344 change from feeding on small crustaceans (lower percentage of organic mercury
345 (Seco et al., 2019)) to prey at higher trophic levels (higher percentage of organic
346 mercury (Chouvelon et al., 2011)), however this can not be applied to *G. glacialis,* as
347 mercury in this species decreases in bigger individuals. Similarly, interspecific
348 differences are probably due to variations in diet (Boyle and Rodhouse, 2005),
349 although the trophic ecology of these cephalopods is still poorly known (Collins and
350 Rodhouse, 2006).

351

352 *Temporal trends of mercury concentrations in Antarctic squid*

353 Our study analysed samples obtained over a 10 year period (between 2006/07
354 and 2016/17), although only two species were captured in all sampling seasons (*G.*
355 *glacialis, S. circumantarctica*) and the number of individuals captured for some species
356 was relatively low despite the sustained sampling effort. Squid have a highly
357 developed sensory system and can swim fast, while sampling nets are relatively small
358 and slow, so most adult squid can avoid capture (Clarke, 1977; Xavier et al., 2002).

359 Squid are considered an r-selected species (Pianka, 1970): they have a fast
360 growth rate, are semelparous (reproduce only once) and are short lived (~< 1–2 years)
361 (Arkhipkin, 2004; Boyle and Rodhouse, 2005; Xavier et al., 2018), although, some
362 large squid species, like *K. longimana* may live longer (Jarre et al., 1991; Lynnes and

363 Rodhouse, 2002). These characteristics make them responsive bioindicators with
364 which would be possible to monitor trends in mercury concentrations over time, as
365 they will reflect rapidly any changes in the bioavailability of this contaminant.

366 Data from the squid species *A. antarcticus*, *B abyssicola, G. glacialis* and *S.*
367 *circumantarctica* appears to indicate a decreasing trend of mercury concentration
368 along time, although further monitoring is required to confirm this pattern. This
369 suggestive pattern of declining concentrations of mercury in the majority of species is
370 consistent with the decreasing trend of atmospheric mercury over the last decade
371 (Soerensen et al., 2012) as a consequence of the reduction of worldwide
372 anthropogenic emissions of mercury (Streets et al., 2017; Zhang et al., 2016). The
373 decrease of mercury in the global atmosphere could also mean a reduction in mercury
374 deposition levels in our study area. Comparing our results with a previous study in the
375 same region (Anderson et al., 2009) who sampled in 2001/02 and analysed specimens
376 with atomic fluorescence spectrophotometry, a general reduction in the concentration
377 of mercury can also be observed: *G. glacialis* had a mercury concentration in 2001/02
378 (230 $\pm$ 70 ng g^{-1}) (Anderson et al., 2009) that was more than twice as high as our
379 results from 2006/07 (100 $\pm$ 60 ng g^{-1}), 1.5 times when compared with 2007/08 (150 $\pm$
380 90 ng g^{-1}) and more than ten times when compared with 2008/09 or 2016/17 (20 $\pm$ 10
381 ng g^{-1}; 20 $\pm$ 7 ng g^{-1}); in *G. antarcticus* (600 $\pm$ 2 ng g^{-1}) the difference was bigger, with
382 mercury concentrations being 5 times higher in 2001/02 than our results for 2013. *P.*
383 *glacialis* had concentration 4 times higher (180 $\pm$ 110 ng g^{-1}) in 2001/02 than our
384 observations in 2008/09. In the family Onychoteuthidae, the concentrations of mercury
385 in 2001/02 (100 $\pm$ 20 ng g^{-1} in *F. knipovitchi;* 160 $\pm$ 90 ng g^{-1} in *K. longimana*) were
386 similar to our results for 2013.

387 The pattern of mercury bioaccumulation in species is influenced by specific
388 traits such as dietary preference, ingestion, excretion, and growth rate. Prevailing
389 environmental conditions may also enhance or reduce contaminant bioavailability to
390 cephalopods. Habitat use has a major effect on mercury accumulation in organisms,
391 as sites contaminated by mercury will likely have higher bioavailability of this toxic
392 element. All of our samples were collected in the Scotia Sea, around South Georgia
393 (Figure 1), which is known to be a fairly stable ecosystem, which should mean lower
394 mercury variation between sampling sites.
395 The majority of our study species have a wide range of vertical distributions:
396 the depth of occurrence of *A. antarcticus* is normally at 800-900 m (Rodhouse, 1988),
397 *G. glacialis*, at 600-1000 m (Roper and Young, 1975), *G. antarcticus,* at 250-928 m
398 (Collins et al., 2004; Roper et al., 1984), *F. knipovitchi,* at 480-760 m (Collins et al.,
399 2004), *K. longimana,* at 300-900 m (Collins et al., 2004; Xavier et al., 2002), *P.*
400 *glacialis,* at 275-928 m (Collins et al., 2004; Xavier et al., 2002) and *S.*
401 *circumantarctica,* at 487-928 m (Collins et al., 2004). An outlier amongst these is *B.*
402 *abyssicola*, which is a deep-sea squid and occurs at a depth of 1000-1500 m (Roper
403 and Young, 1975). Higher mercury levels were expected in species from deep habitats
404 as increasing depth raises mercury concentrations in fish (Choy et al., 2009; Le Bourg
405 et al., 2019; Monteiro et al., 1996). However, that was not the case in our study, as
406 mercury concentrations in the tissues of the deep-sea squid *B. abyssicola* were within
407 the same range as the majority of other analysed species (see Figure 4). The lack of
408 difference between species may be explained by the frequent diel vertical migration
409 associated with cephalopods (Norman et al., 2014) in order to feed and to avoid
410 predation. The differences in mercury concentrations between years may be partially

explained by shifts in the abundance and contaminant loads of prey items (Chouvelon et al., 2011; Paiva et al., 2008). However, there is still a lack of data on the diets of the studied species (Collins and Rodhouse, 2006; Xavier et al., 2018; Xavier and Cherel, 2009) and it is presently not possible to evaluate the effect of diet on mercury bioaccumulation in our study species.

Our results suggest a decreasing trend of total mercury concentration in most of the South Georgia squid species analysed over the last decade, with a stable proportion of organic mercury. Considering that cephalopods are a major link between primary consumer and top predators, these changes possibly reflect a drop in mercury bioavailability in lower trophic levels and suggests that mercury intake by squid predators may have decreased.

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