# The significance of cephalopod beaks in marine ecology studies: Can we use beaks for DNA analyses and mercury contamination assessment?

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ABSTRACT

Cephalopod beaks found in the diet of predators have been a major source of scientific information. In this study, we evaluated the usefulness of DNA and contaminants analysis (total mercury- T-Hg) in cephalopod beaks in order to assess their applicability as tools in marine ecology studies. We concluded that, when applying DNA techniques to cephalopod beaks from Antarctic squid species, when using flesh attached to those beaks, it was possible to obtain DNA and to successfully identify cephalopod species; DNA was not found on the beaks themselves. This study also showed that it is possible to obtain information on T-Hg concentrations in beaks: the T-Hg concentrations found in the beaks were 6 to 46 times lower than in the flesh of the same cephalopod species. More research on the relationships of mercury concentrations in cephalopod beaks (and other tissues), intra- and inter- specifically, are needed in the future.

CAPSULE ABSTRACT: DNA and contaminants analyses for the first time in cephalopods beaks showed that flesh attached to beaks allows DNA species ID and beaks had 6-46 times less total mercury than flesh.

## 1. Introduction

Cephalopods (Mollusca: Cephalopoda) are widely recognized as playing a pivotal role in many marine ecosystems, being consumed by a wide range of predators (Boyle and Rodhouse, 2005; Clarke, 1996b; Hoving et al., 2014; Xavier et al., 2015; Xavier and Cherel, 2009). Their beaks are well known to resist digestion and can stay in predator stomachs for days, weeks or even months (Ashmole and Ashmole, 1967; Duffy and Jackson, 1986; Furness et al., 1984; Gales and Cheal, 1992; Jackson and Ryan, 1986;

47 Votier et al., 2003; Xavier et al., 2005). More than 28 000 beaks have been found in the
48 stomach of a single sperm whale (Akimushkin, 1955; Clarke, 1977).
49 In 1962, Malcolm Clarke showed the importance of cephalopod beaks for marine
50 ecology (Clarke, 1962), as cephalopod soft bodies are rarely found in the stomach of their
51 predators (Clarke, 1977; Clarke, 1980b). Back then, little was known about interactions of
52 cephalopods with top predators, in particular the relevance of each cephalopod species in
53 the diet of top predators. Consequently, the construction of reliable food webs including
54 cephalopods then was difficult if not impossible. The efforts of Malcolm Clarke and
55 colleagues catapulted our ability to understand diet composition of predators that feed on
56 cephalopods by using their beaks (Cherel and Klages, 1998; Clarke, 1986, 1996a, b;
57 Croxall and Prince, 1996; Klages, 1996; Smale, 1996).
58 Cephalopod beaks in the diet of top predators have been acknowledged as good
59 tools for a variety of studies on marine ecology. They can provide information on size,
60 frequency of occurrence and mass of cephalopods that are part of a top predator's diet
61 (Clarke, 1980b; Xavier et al., 2005). Beak data analyses have been used to monitor
62 seasonal and annual changes in availability (Xavier et al., 2013; Xavier et al., 2003;
63 Xavier et al., 2007b), to aid fisheries assessment and management (Xavier et al., 2007b),
64 to assess potential competition between predators (Xavier and Croxall, 2007) and to
65 evaluate the amount of potential scavenging both by a predator (Croxall and Prince, 1994),
66 or to recognize a new species in a given area (Clarke et al., 2002). Information regarding
67 age (Clarke, 1965; Perales-Raya et al., 2014; Perales-Raya et al., 2010), growth,
68 reproduction (Clarke, 1980b, 1993; Hernández-Garcia et al., 1998; Jarre et al., 1991),
69 distribution (Clarke, 1980b; Clarke et al., 2002; Liu et al., 2015; Xavier et al., 2002a;
70 Xavier et al., 2006; Xavier et al., 2002b; Xavier et al., 2014), paleontology (Clarke and
71 Maddock, 1988), feeding ecology, behavior (Castro and Hernández-Garcia, 1995; Franco-

72 Santos and Vidal, 2014), spawning areas (Cherel and Weimerskirch, 1999), post-
73 spawning mortality (Xavier and Croxall, 2007), sexual dimorphism (Bolstad, 2006;
74 Cherel et al., 2009a; Jackson, 1995), biomass estimations, cephalopod consumption
75 (Clarke, 1987; Clarke, 1983; Clarke et al., 2002; Santos et al., 2001; Xavier et al., 2007b)
76 and predator migrations (Clarke and Stevens, 1974) can also be provided by studying
77 cephalopod beaks. Recent stable isotope analyses of beaks enabled the determination of
78 habitat preferences and trophic levels for a wide range of cephalopods (Cherel et al.,
79 2011; Cherel and Hobson, 2005; Cherel et al., 2009b; Guerra et al., 2010). Also,
80 cephalopod beaks exhibit unique characteristics with mechanical properties that can be
81 applied to engineering and biomaterial research (Dilly and Nixon, 1976; Miserez et al.,
82 2007; Miserez et al., 2008; Uyeno and Kier, 2005).
83 Despite the countless applications of cephalopod beaks in marine ecology studies,
84 DNA-based identification and chemical contamination assessments have not yet been
85 evaluated. DNA has been used as an important tool to identify and discover new
86 cephalopod species as well as gain insights into their ecology and evolution (Allcock et al.,
87 2014; Strugnell et al., 2009; Strugnell and Lindgren, 2007; Xavier et al., 2015). Studies
88 using DNA for the identification of cephalopods in stomach contents have also been
89 conducted (Strugnell and Lindgren, 2007), relying on DNA extraction from tissues of
90 recently consumed cephalopods (Strugnell et al., 2005).
91 Another application not commonly applied to beaks is contaminants assessment.
92 Mercury is listed as one the most hazardous substances, with all chemical forms
93 (elemental, inorganic and organic) exhibiting toxicological characteristics, and thus
94 increasingly raising environmental concerns. Once mercury enters the marine ecosystems
95 it can be easily methylated by bacteria, which accelerates bioaccumulation and
96 biomagnification along food webs, ultimately concentrating in top predators (Wiener et al.,

2007). The methylation process increases toxicity with methylmercury being the most toxic form. Mercury uptake occurs mainly through diet (Mieiro et al., 2012) and it is accumulated in specific tissues (e.g. Muscle tissue stores most as methylmercury (Bustamante et al., 2006; Mieiro et al., 2011)). To our knowledge, no studies so far explored the possibility of using beaks to assess environmental and ecological relevant mercury concentrations.

Our study aims to use cephalopod beaks from squid that occur in the Southern Ocean (here defined as south of the subtropical front) in order to: (1) Apply DNA barcoding to both beaks and muscle tissue attached to the beaks to assess its feasibility for cephalopod identification; (2) Assess the utility of beaks to evaluate total mercury accumulation in cephalopods by comparing concentrations in beaks and muscle; (3) Discuss the future applicability of DNA barcoding and mercury analysis in ecological studies of cephalopods.

## 2. Material and methods

### *2.1 DNA analyses*

Cephalopod lower beaks of two of the most common species in top predators diets (i.e. *Kondakovia longimana* and *Moroteuthis knipovitchi*; see Xavier and Cherel 2009) were collected from stomach contents of grey headed *Thalassarche chrysostoma* and black-browed *T. melanophrys* albatrosses breeding at Bird Island, South Georgia, following Xavier et al. (2003), Guerreiro *et al*. (2015) and Alvito *et al*. (2015). Lower beaks samples from adult Southern Ocean squid were fixed in ethanol (70–90%) and stored at –20 °C until DNA extractions were carried out. At the laboratory, the beaks were then macerated and proteinase K (20 μg/mL) was added overnight. DNA extraction was

121 performed using the JETFLEX Genomic DNA Purification Kit (Genomed, Germany).
122 DNA yield was quantified using NanoDrop equipment (Thermo Scientific, USA).
123 For DNA analyses of tissue samples that were attached to cephalopod beaks (i.e.
124 from buccal mass), from more squid species common in the diet of top predators
125 (*Galiteuthis glacialis*, *Psychroteuthis glacialis, Gonatus antarcticus* and *Alluroteuthis*
126 *antarcticus*). DNA extraction was done by using a Glass Fiber Plate DNA Extraction
127 method (Ivanova et al., 2006).
128 The primer pair LCO1490_t1 and HCO2198_t1 was used to amplify a 658 bp
129 fragment of the COI gene. Samples which did not amplify successfully were re-run using
130 a combination of overlapping primer sets: C_LepFolF,MLepR2 and MLepF1,C_LepFolR.
131 The PCR thermal regime for all primer sets was: initial denaturing at 94 °C for 1 min; five
132 cycles at 94 °C for 1 min, 45 °C for 1.5 min and 72 °C for 1.5 min; 35 cycles of 94 °C for
133 1 min, 50 °C for 1.5 min and 72 °C for 1 min followed by a final cycle at 72 °C for 5 min.
134 Each PCR product was cleaned by Sephadex. Prior to sequencing, the clean PCR product
135 was diluted 1:10 with sterile water and 2-5 μL of it was sequenced in both directions
136 using ABI 3730xl automated DNA sequencers. All sequences and supporting information
137 have been deposited in the Barcode of Life Datasystems (BOLD) database (Ratnasingham
138 and Hebert, 2007) in the project DIETA, and were submitted to GenBank (Accession
139 numbers are given in Table 1).
140
141 *2.2 Mercury analyses*
142 Cephalopod lower beaks of some of the most important cephalopod species in top
143 predator diets (*Galiteuthis glacialis*, *Gonatus antarcticus*, *Kondakovia longimana*,
144 *Moroteuthis knipovitchi* and *Psychroteuthis glacialis;* see Xavier and Cherel 2009) were
145 collected from stomach contents of albatrosses breeding at Bird Island, South Georgia as

146 well as Patagonian toothfish *Dissostichus eleginoides* from the South Sandwich Islands,
147 following Xavier *et al*. (2002b), Xavier et al. (2003) and Seco *et al*. (2015). At the
148 laboratory, all beaks were ground to a fine powder using liquid nitrogen for further
149 analyses of mercury concentrations. Total mercury (T-Hg) was determined by atomic
150 absorption spectrometry (AAS) with thermal decomposition and gold amalgamation,
151 using an Advanced Mercury Analyser (AMA) LECO 254 (Costley et al., 2000). This
152 method does not require previous sample treatment, and also allows for a small sample
153 mass to be used. In this case, an average of 36mg per beak replicate was used for Hg
154 determinations. The limit of detection of the AMA – LECO 254 analyzer is 0.01 ng of
155 mercury. Accuracy and precision of the analytical methodology for T-Hg determinations
156 were assessed by daily replicate analysis of certified reference materials (CRM), namely
157 Tort-2 (lobster hepatopancreas). Precision of the method was always better than 9% (n=
158 9), with a recovery efficiency of 105 ± 7% (n= 27).
159
160 *2.3 Statistical analyses*
161 For cephalopod beaks that could be identified to species level we used allometric
162 equations to convert lower beak size to mantle length (ML) and body mass (g), in Xavier
163 and Cherel (2009). After assessing the normality of the data, non-parametric tests were
164 used to assess relationships between T-Hg and ML/body mass. Values on statistics are
165 given as means ± standard deviation unless if stated.
166
167 **3. Results**
168 *3.1 DNA extraction and sequencing analysis*
169 A total of 20 clean cephalopod lower beaks, with no visible tissue, were used for
170 DNA extraction, with 10 beaks belonging to *Kondakovia longimana* (10.9 ± 0.9 mm

171 Lower Rostral Length (LRL); range: 8.9 – 12.0 mm LRL) and 10 beaks belonging to
172 *Moroteuthis knipovitchi* (4.6 ± 0.5 mm LRL; range: 3.9 – 5.4 mm LRL). With the
173 methods applied, it was not possible to retrieve any DNA. Another set of cephalopod
174 beaks with visible flesh attached (i.e. buccal mass), were used to retrieve DNA for COI
175 gene amplification. The buccal mass flesh used was identified as *K. longimana* (n=10),
176 *Galiteuthis glacialis* (n=1), *M. knipovitchi* (n=6), *Psychroteuthis glacialis* (n=1), *Gonatus*
177 *antarcticus* (n=1) and *Alluroteuthis antarcticus* (n=2). This DNA barcoding confirmed the
178 identification of all species by beak morphology (Xavier and Cherel, 2009).
179
180 *3.2 Mercury concentrations*
181 The total mercury (T-Hg) levels of lower beaks from five squid species of the
182 Southern Ocean were obtained (Table 2, Figure 1). Concentrations ranged from 0.004 (*K.*
183 *longimana* and *G. glacialis*) to 0.047 mg $kg^{-1}$ dry weight (*M. knipovitchi*), indicating low
184 mercury concentrations in beaks. There were significant interspecific differences in T-Hg
185 concentrations (Kruskall-Wallis $H=14.56$, $p<0.01$) between *P. glacialis* and *K. longimana*
186 (Dunn's test $Q=3.11$ $p<0.05$). The average T-Hg concentration found in species with
187 larger beaks (*K. longimana*) was similar to species with smaller beaks (*G. glacialis*; Table
188 2, Figure 1) but with the highest estimated ML (Table 3). *M. knipovitchi, P. glacialis* and
189 *G. glacialis* showed a higher intra-species variability while T-Hg levels in beaks of
190 individuals of *G. antarcticus* were more consistent (Table 2, Figure 1). No correlation was
191 found between T-Hg concentration and the lower rostral length (Spearman correlation
192 $\rho=0.06$ $p=0.77$) or with the body mass (Spearman correlation $\rho= 0.009$ $p=0.96$) of the
193 studied species. However, there was a negative correlation between T-Hg concentration
194 and the mantle length (Spearman correlation $\rho=-0.487$ $p=0.02$). When comparing the T-
195 Hg concentration of lower beaks (present data) with those in flesh/muscle (Anderson et

196 al., 2009) for the same cephalopod species from the same region of the Southern Ocean
197 (Atlantic sector, around South Georgia), the levels found in the beaks were significantly
198 lower than those found in flesh/muscle (Mann-Whitney U=0.00; $p<0.01$). The species
199 showing least variability in T-Hg concentration in both studies were *G. antarcticus* and *K.*
200 *longimana*.
201
202 **4. Discussion**
203 Given the difficulty to capture cephalopods, the use of recovered beaks from
204 stomach contents from cephalopod predators has been widely used in ecological studies,
205 particularly for the purpose of species identification (Clarke, 1980a; Clarke, 1986; Xavier
206 and Cherel, 2009). However, there are only a few experts in the world trained to do this
207 kind of identification (Clarke, 1986; Xavier et al., 2007a). In this study, we assessed the
208 utility of a molecular approach, using DNA recovered from tissues attached to the beaks.
209 We also assessed the utility of beaks to obtain information on mercury concentration in
210 cephalopods.
211
212 *4.1 DNA extraction and sequencing analyses*
213 This study showed that it was possible to extract DNA directly from flesh attached
214 to the beaks (i.e. from buccal mass), but not from the beaks themselves. The reason for
215 the latter is likely caused by the beak's composition. They do not contain living cells
216 (Miserez et al., 2010), and any residue tissue on their surface will be digested after a
217 longer time in a predator's stomach. Larger buccal mass tissue bits attached to the beak
218 contain enough DNA for further analysis and may allow using DNA barcoding to
219 determine the species. We chose only species whose beaks could also be identified using
220 beak morphology (Xavier and Cherel, 2009) in order to test if there is correspondence

between both methods. DNA barcoding confirmed the identification of species by beak morphology, which is a promising result as it provides researchers with two methods to choose from depending on the needs of their study. Surveys on the feeding ecology of cephalopod predators usually start with samples that contain clean beaks as well as beaks with flesh attached to them. A fair number of squid species found in the Southern Ocean (Rodhouse et al., 2014) have already been barcoded, and these sequences are publicly available through GenBank (Table 1) or BOLD. However, there are numerous species living in the Southern Ocean that still unknown to science, without a barcode sequence (Xavier et al., 2015; Xavier and Cherel, 2009; Xavier et al., 2014).

*4.2 Mercury concentrations*

Our study showed that it is possible to measure total mercury (T-Hg) concentration in cephalopod (lower) beaks, using a simple and easily accessible laboratory methodology. The total mercury concentrations found on the lower beaks of the studied cephalopod species were 6 to 46 times lower than those reported from muscle tissue of the same species in the Southern Ocean (see Table 2). Such results might be due to mercury organotropism (Bustamante et al., 2006; Jackson et al., 2007), since mercury accumulation is tissue-specific and muscle is known to harbour significant levels of mercury, mainly in organic form (Bustamante et al., 2006). Preferential accumulation of mercury in muscle tissue has also been reported for fish and is a protection mechanism that prevents mercury accumulation in other vital organs (e.g. brain) (Mieiro et al., 2011). Despite the proteinaceous nature of cephalopod beaks (beaks can have a protein content varying from 5% to 60% wet weight according to the pigmentation gradient (Miserez et al., 2008)), their slow growth rate (they are usually not replaced throughout a cephalopods relative short life), and mercury affinity for proteins, it seems that beaks are not a

structure with high accumulation potential (as the mercury values were very low; see results). In addition, the permanency of the beaks in the acidic contents of their predators' stomachs may induce the release of Hg due to the chelating action (i.e. chemical broke down activity) of acids, which may disrupt the Hg bonds to proteins (Hajeb and Jinap, 2009), and reduce Hg concentration in beaks.

Mercury concentrations in cephalopods depend on both biological and environmental factors such as size, lifestyle, food availability, growth rate and geographical origin (Bustamante et al., 2006; Pereira et al., 2009; Villanueva et al., 2002). With respect to size, this study did not show any relation between the T-Hg concentration in beaks and the lower rostral length (see results). In fact, larger beaks of *K. longimana*, showed similar T-Hg values when compared with species with smaller beaks, such as *G. glacialis.* This suggests that bioaccumulation of mercury in beaks does not seem to be dependent on body size of cephalopods, which is in agreement with previous studies on other tissues by Raimundo et al. (2009) who found comparable Hg concentrations (based on octopod digestible gland samples) among individuals of different age/size. The same result was obtained for the relationship between estimated body mass and T-Hg in squid beaks in our study.

In terms of assessing T-Hg and ML relationships, *K. longimana* can reach more than 1000 mm of mantle length (ML), whereas the other studied species generally have ML lower than 500 mm (Gröger et al., 2000; Lu and Williams, 1994; Lynnes and Rodhouse, 2002). For our study, the estimated ML of the specimens of *G. glacialis* and *K. longimana* were in the same range and had the highest ML registered, which may explain the similarity between the T-Hg concentrations found between these species. Both species showed the lower T-Hg burdens found in this study, possibly due to a somatic growth dilution of the metal, which can be corroborated by the negative correlation found

between ML and T-Hg; It has been shown that rapid growth can greatly reduce the mercury concentration in aquatic organisms by causing a greater than proportional gain in biomass relative to the metal concentration (Karimi et al., 2007).

Beaks from *M. knipovitchi* and *P. glacialis* showed T-Hg concentrations 3 times higher than *G. glacialis* and *K. longimana*, despite that their ML were lower, which is in line with the previous assumption that small species (with slower growth rate) may accumulate more mercury. *G. antarcticus* showed similar ML with *M. knipovitchi* and *P. glacialis*, but half of their T-Hg burden. This may be explained by the different feeding habits, different growth rates and distribution of these different species (Cherel et al., 2009a; Collins and Rodhouse, 2006 ; Pierce et al., 2008; Xavier et al., in press). In summary, there are no clear relationships between T-Hg with beak size and body mass, but there is a relationship between T-Hg and ML, emphasizing that this issue must be further investigated.

Finally, our results show intra-species variations of T-Hg concentrations, being particularly higher in *M. knipovitchi* , *G. glacialis* and *P. glacialis* (see Results; Figure 1). Further studies will be needed to assess why such variations occur. They may be caused by various parameters related to the ecology of Southern Ocean cephalopods, such as biological (e.g. growth rate, size, sex, metabolic rate), ecological (e.g. feeding and habitat use) and environmental (mercury availability, primary productivity) factors (Chouvelon et al., 2012; Harmelin-Vivien et al., 2009).

The Antarctic seabed has been characterized as cold and thermally stable, without relevant changes in spatial or seasonal temperature (Xavier and Peck, 2015). As previously stated, mercury accumulation depends on a wide range of factors, namely abiotic factors, such as temperature, which not only affect the mercury cycle but also organism individual growth. Could mercury concentrations in Southern Ocean

296 cephalopods be different from elsewhere? Using T-Hg in muscle tissue as a measure,
297 there are no major differences between mercury concentrations in squid species from the
298 Southern Ocean (Anderson et al., 2009; McArthur et al., 2003) compared to
299 taxonomically close ones from the North Eastern Atlantic (Anderson et al., 2009;
300 Chouvelon et al., 2012), Adriatic and Mediterranean Sea (Perugini et al., 2009; Rjeibi et
301 al., 2015) and adjacent waters to Peninsular Malaysia (Ahmad et al., 2015) (Table 3),
302 which suggests comparable mercury levels in the aquatic environments of both areas. This
303 evidence reinforces mercury persistency and its global distribution.
304 In conclusion, when using DNA analyses, we can assess the identification of
305 cephalopods only when there is flesh attached to beaks, as it was not possible to obtain
306 DNA directly from the beaks using our methodology. However the success of DNA
307 barcoding in cases where tissue remnants were still attached to beaks provides researchers
308 with two tools that could be used in a complementary fashion to determine species
309 identities in the stomach content of cephalopod predators (i.e. in some studies you can
310 only be able to use DNA (only flesh available) while other studies only beaks are
311 available). It is possible to assess the mercury concentrations of cephalopod beaks and
312 despite the fact that T-Hg in beaks was lower than usually found in muscle tissue, beaks
313 could be a tool to assess marine contamination in a wide range of cephalopod species
314 (particularly oceanic squid species) that are more difficult to catch using traditional means
315 (nets) (Clarke, 1977; Xavier et al., 2015; Xavier et al., 2007a). Future studies in order to
316 suggest some relationship with cephalopod measurements (like the inverse relationship
317 with ML), studies should focus in testing the Hg concentrations with real measurements
318 obtained from different size/sex cephalopods (rather than estimations from allometric
319 equations).
320

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599 Table 1: Taxa of squid known to inhabit in Southern Ocean waters, following
600 Rodhouse *et al*. (2014; 19 species), that already have their respective COI Accession
601 number (* = species that were studied in this study).

| Species name | Accession number |
|---|---|
| *Alluroteuthis antarcticus** | AF131871 |
| *Bathyteuthis abyssicola* | AF000030 |
| *Batoteuthis skolops* | AY557527 |
| *Chiroteuthis veranyi* | AF000032 |
| *Galiteuthis* sp.* | KF309247 |
| *Gonatus antarcticus** | AY681064 |
| *Kondakovia* sp.* | EU735403 |
| *Martialia hyadesi* | AB270940 |
| *Mastigoteuthis psychrophila* | KC860979 |
| *Mesonychoteuthis hamiltoni* | EU735397 |
| *Moroteuthis ingens* | AB264119 |
| *Moroteuthis knipovitchi** | AF131875 |
| *Moroteuthis robsoni* | AB264117 |
| *Psychroteuthis glacialis** | AF131876 |
| *Todarodes filippovae* | AB270935 |

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614 Table 2: Total mercury concentration (mg $kg^{-1}$, dry weight; mean values, standard
615 deviation (SD), range and variation coefficient (%)) in cephalopod– beaks (present study)
616 and muscle (Anderson *et al*. 2009).
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| | **Beaks** | | | | | **Muscle** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | [Hg] | SD | Range | CV % | n | [Hg] | SD | Range | CV% |
| *Galiteuthis glacialis* | 4 | 0.008 | 0.004 | 0.004-0.011 | 45 | 3 | 0.23 | 0.07 | 0.18-0.31 | 30 |
| *Moroteuthis knipovitchi* | 5 | 0.025 | 0.015 | 0.009-0.047 | 59 | 4 | 0.16 | 0.09 | 0.07-0.29 | 58 |
| *Gonatus antarcticus* | 4 | 0.013 | 0.003 | 0.009-0.017 | 27 | 2 | 0.6 | 0.02 | 0.58-0.61 | 4 |
| *Psychroteuthis glacialis* | 5 | 0.029 | 0.011 | 0.018-0.042 | 37 | 2 | 0.18 | 0.11 | 0.10-0.25 | 61 |
| *Kondakovia longimana* | 6 | 0.008 | 0.003 | 0.004-0.013 | 34 | 2 | 0.1 | 0.02 | 0.08-0.11 | 22 |

Table 3: Total mercury concentration in cephalopod tissues from different sampling areas. N – sampling size; ML - mantle length (mean±SD or range (min-max)/ mm), Hg tissue T-Hg concentration (mean±SD (range)/mg $kg^{-1}$, dry weight). See exceptions (a-d) below.

| Species | Sampling area | N | ML | Hg beaks | | Hg flesh | | Hg digestive gland | | References |
|---|---|---|---|---|---|---|---|---|---|---|
| **Onychoteuthidae** | | | | | | | | | | |
| *Kondakovia longimana* | Southern Ocean | 6 | 554±37.7 | 0.008±0.003 | (0.007-0.013) | – | – | – | – | Present study |
| | Southern Ocean | 2 | – | – | – | 0.1±0.02 | (0.08-0.11) | – | – | Anderson et al. 2009 |
| *Moroteuthis knipovitchi* | Southern Ocean | 5 | 274±17.5 | 0.025±0.015 | (0.009-0.047) | – | – | – | – | Present study |
| | Southern Ocean | 4 | – | – | – | 0.16±0.09 | (0.07-0.29) | – | – | Anderson et al. 2009 |
| *Moroteuthis ingens* | Southern Ocean | 15 | 243-364 | – | – | 0.086±0.017 | (0.06-0.13) | – | – | McArthur et al. 2003 |
| **Gonatidae** | | | | | | | | | | |
| *Gonatus antarcticus* | Southern Ocean | 4 | 241±3.75 | 0.013±0.003 | (0.009-0.017) | – | – | – | – | Present study |
| | Southern Ocean | 2 | – | – | – | 0.6±0.02 | (0.58-0.61) | – | – | Anderson et al. 2009 |
| **Psychroteuthidae** | | | | | | | | | | |
| *Psychroteuthis glacialis* | Southern Ocean | 5 | 296±8.15 | 0.029±0.011 | (0.018-0.042) | – | – | – | – | Present study |
| | Southern Ocean | 2 | – | – | – | 0.18±0.11 | (0.10-0.25) | – | – | Anderson et al. 2009 |
| **Cranchiidae** | | | | | | | | | | |
| *Galiteuthis armata* | NE Atlantic | 3 | 252±91 | – | – | 0.252±0.041 | (0.206–0.284) | – | – | Chouvelon et al. 2012 |
| *Galiteuthis glacialis* | Southern Ocean | 4 | 425±21.5 | 0.008±0.004 | (0.04-0.11) | – | – | – | – | Present study |
| | Southern Ocean | 3 | – | – | – | 0.23±0.07 | (0.18-0.31) | – | – | Anderson et al. 2009 |
| *Teuthowenia megalops* | NE Atlantic | 4 | 134±12 | – | – | 0.150±0.033 | (0.111–0.192) | – | – | Chouvelon et al. 2012 |
| | NE Atlantic | 1 | 180 | – | – | – | 0.205 | – | 0.172 | Bustamante et al. 2006 |
| **Ommastrephidae** | | | | | | | | | | |
| *Illex coindetii* | NE Atlantic | 22 | 130±54 | – | – | 0.193±0.078 | (0.061–0.331) | 0.192±0.076 | (0.081–0.357) | Bustamante et al. 2006 |
| *Todaropsis eblanae* | NE Atlantic | 9 | 101±43 | – | – | 0.281±0.129 | (130–500) | 0.217±0.108 | (0.120–0.463) | Bustamante et al. 2006 |
| | NE Atlantic | 23 | 100±41 | – | – | 0.206±0.201 | – | 0.128±0.099 | – | Pierce et al. 2008 |
| *Todarodes sagittatus* | NE Atlantic | 22 | 260±42 | – | – | 0.324±0.380 | (0.139–1.998) | – | – | Chouvelon et al. 2012 |
| | NE Atlantic | 5 | 98±34 | – | – | 0.188±0.089 | (0.073–0.289) | 0.168±0.052 | (0.112–0.231) | Bustamante et al. 2006 |
| | NE Atlantic | 12 | 343±100 | – | – | 0.425±0.194 | – | 0.280±0.105 | – | Pierce et al. 2008 |

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|---|---|---|---|---|---|---|---|---|---|---|
| | Adriatic Sea | 14 | – | – | – | 0.25±0.03[d] | (0.02-0.62) | – | – | Perugini et al. 2009 |
| **Histioteuthidae** | | | | | | | | | | |
| *Histioteuthis reversa* | NE Atlantic | 7 | 54±22 | – | – | 0.219±0.087 | (0.132–0.320) | – | – | Chouvelon et al. 2012 |
| | NE Atlantic | 6 | 38±22 | – | – | 0.102±0.031 | (0.065–0.147) | 0.088±0.044 | (0.031–0.137) | Bustamante et al. 2006 |
| **Loliginidae** | | | | | | | | | | |
| *Alloteuthis* sp. | NE Atlantic | 20 | 67±15 | – | – | 0.098±0.011 | – | 0.072±0.011 | – | Pierce et al. 2008 |
| *Alloteuthis subulata* | NE Atlantic | 15 | 152±32 | – | – | 0.196±0.040 | (0.121–0.262) | – | – | Bustamante et al. 2006 |
| *Loligo vulgaris* | NE Atlantic | 36 | 179±56 | – | – | 0.149±0.032 | (0.072–0.200) | – | – | Chouvelon et al. 2012 |
| | NE Atlantic | 21 | 151±47 | – | – | 0.264±0.086 | (0.113–0.398) | 0.406±0.171 | (0.113–0.681) | Bustamante et al. 2006 |
| | NE Atlantic | 10 | 130-420 | – | – | 0.05±0.02[d] | (0.02-0.08) | – | – | Lourenço et al. 2009 |
| | Mediterranean Sea | 95 | 120-256 | – | – | 0.072[c,d] | (0.030-0.95) | – | – | Rjeibi et al. 2015 |
| *Loligo forbesi* | NE Atlantic | 38 | 290±99 | – | – | 0.260±0.119 | (0.099–0.547) | – | – | Chouvelon et al. 2012 |
| | NE Atlantic | 12 | 119±48 | – | – | 0.179±0.053 | (0.091–0.645) | 0.235±0.104 | (0.165–0.512) | Bustamante et al. 2006 |
| | NE Atlantic | 101 | 129±78 | – | – | 0.153±0.081 | – | 0.216±0.176 | – | Pierce et al. 2008 |
| *Loligo duvaucelii* | Peninsular Malasya | 10 | 160-530[a] | – | – | 0.199±0.162[b] | (0.150-0.406) | – | – | Ahmad et al. 2015 |
| *Loligo uyii* | Peninsular Malasya | 4 | 240-384[a] | – | – | 0.249[b] | (0.099-0.324) | – | – | Ahmad et al. 2015 |
| *Loligo chinensis* | Peninsular Malasya | 7 | 306-600[a] | – | – | 0.275±0.122[b] | (0.158-0.309) | – | – | Ahmad et al. 2015 |
| *Loligo sibogae* | Peninsular Malasya | 6 | 217-612[a] | – | – | 0.364±0.507[b] | (0.194-1.506) | – | – | Ahmad et al. 2015 |
| *Loligo edulis* | Peninsular Malasya | 9 | 120-276[a] | – | – | 0.267±0.156[b] | (0.099-2.715) | – | – | Ahmad et al. 2015 |

[a] Possibly refers to the mantle length, but can also refers to total length (see Ahmad et al 2015)

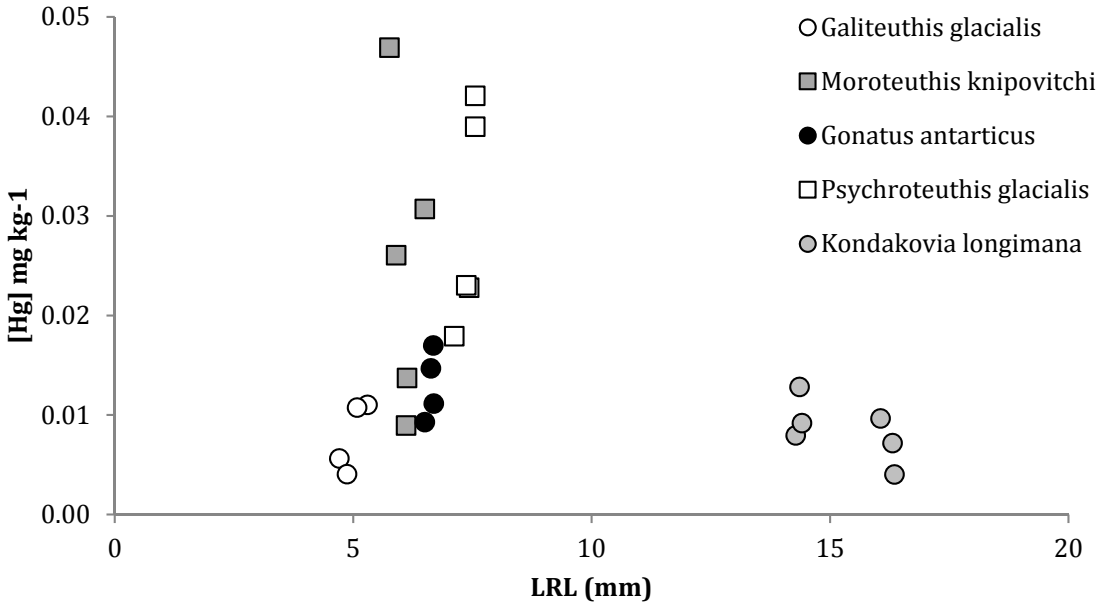
[b] Median±IQR

[c] Median

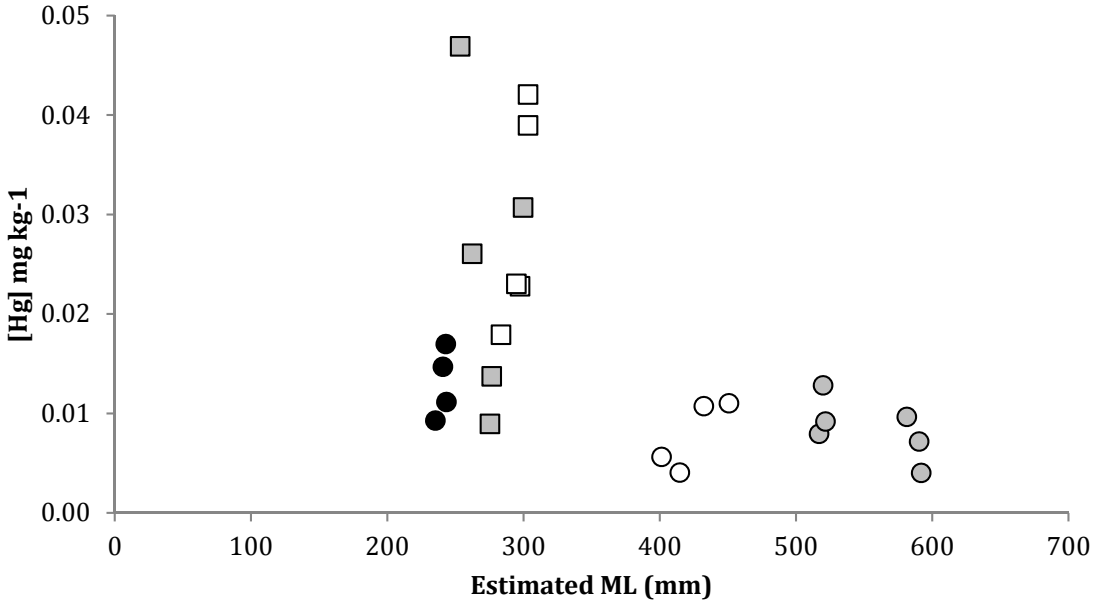
[d] T-Hg in mg $kg^{-1}$ wet weight; mean moisture content is indicated to be 78% in literature (Lourenço et al 2009; Rjeibi et al 2015)

Figure 1: Mercury concentration ([T-Hg]/ mg $kg^{-1}$, dry weight) obtained from beaks according to size dimensions: (a) lower rostral length (LRL/mm), (b) estimated mantle length (ML/mm) and (c) estimated body mass (M/g) of five southern ocean cephalopod species. This figure is for visual comparison rather than for determining trends as these different species have different morphologies, physiology, life histories and growth rates.

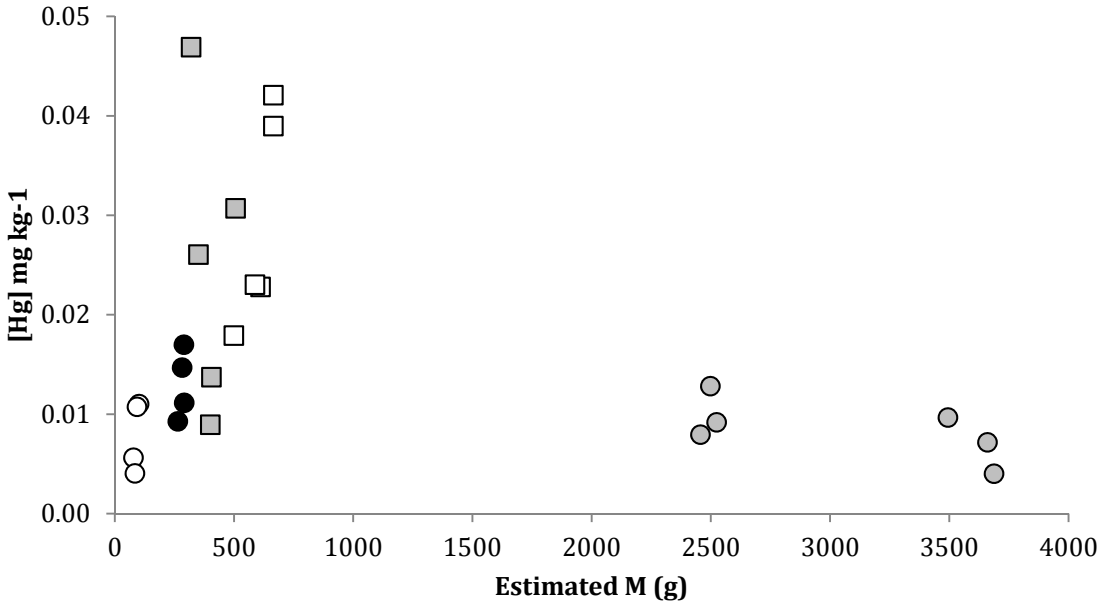
a)




b)



c)


0.05
0.04
0.03
0.02
0.01
0.00
[Hg] mg kg-1
0
500
1000
1500
2000
2500
3000
3500
4000
Estimated M (g)