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# Trace elements in Antarctic fish species and the influence of foraging habitats and dietary habits on mercury levels



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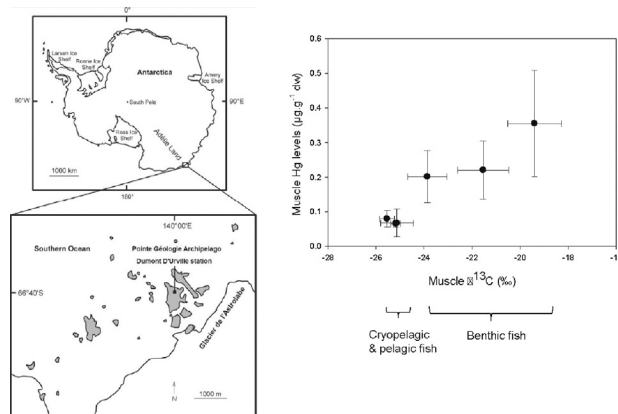
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## HIGHLIGHTS

- Trace elements and stable isotopes were analyzed in seven Antarctic fish species.
- Levels of trace elements in liver and in muscle differed among species.
- Hg load was higher in benthic fish than in cryopelagic and pelagic fish.
- These findings could be due to the high methylation rate of Hg in the sediment.

## GRAPHICAL ABSTRACT



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## ABSTRACT

This study aims at describing and interpreting concentration profiles of trace elements in seven Antarctic fish species (N = 132 specimens) off Adélie Land. Ichthyofauna plays a key role in the Antarctic ecosystem, as they occupy various ecological niches, including cryopelagic (ice-associated), pelagic, and benthic habitats. Firstly, trace element levels in the studied specimens were similar to those previously observed in fish from the Southern Ocean. Apart from manganese and zinc, concentrations of arsenic, cadmium, copper, iron, mercury (Hg), nickel, selenium and silver differed among fish species. Muscle δ<sup>13</sup>C and δ<sup>15</sup>N values were determined to investigate whether the fish foraging habitats and dietary habits could explain Hg levels. Species and foraging habitat (δ<sup>13</sup>C) were strong predictors for variations of Hg concentrations in muscle tissues. The highest Hg contamination was found in shallow benthic fish compared to cryopelagic and pelagic fish. This pattern was likely due to the methylation of Hg in the coastal sediment and the photodemethylation by ultraviolet radiation in surface waters.

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## 1. Introduction

Trace elements, from natural and anthropogenic sources are increasingly released in the environment (Sen and Peucker-Ehrenbrink, 2012) and are ultimately deposited in the ocean. Long-range transport to the open ocean happens through oceanic currents and atmospheric circulation, followed by wet and dry deposition processes (SCOR, 2007). Moreover the ocean plays a critical role in the biogeochemical cycle of trace metals, through chemical and biological reactions in the water column (Morel and Price, 2003; SCOR, 2007). For instance, mercury (Hg) is deposited through the atmosphere in its inorganic form (HgII) and is methylated (Me–Hg) in the ocean, thereby being more bioavailable for marine biota. In that respect, marine ecosystems could be particularly exposed to toxic metals, even in remote and isolated areas (Fitzgerald et al., 1998; Ebinghaus et al., 2002).

Although the contamination pattern of Arctic marine biota by trace elements has been well described (Atwell et al., 1998; AMAP, 1998, 2011), such investigations have been less abundant in the Southern Ocean (de Moreno et al., 1997; Sanchez-Hernandez, 2000; Bargagli, 2008). Moderate to high levels of trace metals have been reported in Antarctic and subantarctic zooplankton (Rainbow, 1989; Petri and Zauke, 1993), benthic octopuses (Bustamante et al., 1998), fish (Honda et al., 1983; Bargagli et al., 1998a,b; Marquez et al., 1998; Bustamante et al., 2003), seabirds (Blévin et al., 2013; Carravieri et al., 2014a) and marine mammals (Szefer et al., 1993). This could raise matters of environmental concerns, since some metals, such as Hg, cadmium (Cd) and lead (Pb), cause health problems in vertebrates, including endocrine disruption, DNA damage, immunotoxicity and reprotoxicity (UNEP, 2010a,b; Wolfe et al., 1998). On the other hand, some elements (e.g. copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), zinc (Zn)) play essential biological roles, often as cofactors or part of cofactors in enzymes and structural elements of proteins.

Ichthyofauna plays a key role in the Antarctic ecosystems, as they constitute a link between lower (copepods, euphausiids) and higher (seabirds, seals) levels of the trophic web (La Mesa et al., 2004). Moreover Antarctic fish occupy most of the available ecological niches, including cryopelagic (ice-associated), pelagic, benthic and epibenthic habitats (La Mesa et al., 2004; Cherel et al., 2011). Antarctic fish species are thus appropriate to assess the respective contribution of foraging habitat and diet in both the inter- and intra-specific variations of trace element concentrations. As a result of bioaccumulation and bioamplification processes within trophic webs (Atwell et al., 1998; Morel et al., 1998; Bargagli et al., 1998a), the concentrations of biomagnifiable trace element are supposed to increase with higher trophic level organisms. In addition, foraging habitat may shape the levels of exposure to trace metals. Due to the high Hg bioavailability in coastal bottom water around the Antarctic shelf (Fitzgerald et al., 2007), benthic fish species are expected to have higher Hg levels than species foraging in the water column and underneath the sea ice.

The present study was carried out in the Pointe Géologie archipelago, Adélie Land, Antarctica, which contains a rich marine ecosystem with a high density of epibenthic organisms, pelagic fish, seabirds and marine mammals (Micol and Jouventin, 2001; Gutt et al., 2007). The first goal of this study was to describe the concentrations of 13 trace elements in the liver (silver (Ag), arsenic (As), Cd, cobalt (Co), chromium (Cr), Cu, Fe, Mn, Ni, selenium (Se), vanadium (V), and Zn) and in the muscle (Hg) of seven Antarctic fish species (N = 132 specimens) off Adélie Land. The second objective aims at exploring the influence of feeding strategies in driving Hg variations in Antarctic fish, by interpreting the ratios of stable isotopes of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) in fish muscle. In the marine environment, the  $\delta^{13}\text{C}$  values are mainly used to indicate inshore versus offshore, or pelagic versus benthic contribution to food intake. Moreover, consumers are typically enriched in  $^{15}\text{N}$  relative to their food and consequently the  $\delta^{15}\text{N}$  values serve as indicators of a dietary habits and trophic position (Vanderklift and Ponsard, 2003).

## 2. Materials and methods

### 2.1. Study area, species and sample collection

This study was carried out from the 24th of April 2010 to 17th of January 2012 in the Pointe Géologie Archipelago (Adélie Land, Antarctica, 66°40' S, 140°01' E). Table 1 provides general information about the samples analyzed in this study. *Pleuragramma antarcticum* is the dominant pelagic fish in high-Antarctic waters (La Mesa et al., 2004; La Mesa and Eastman, 2012) and was collected over the continental slope (66°18'S and 141°56'E) from the Research Icebreaker Astro-labe using an Isaacs-Kidd Midwater Trawl (IKMT). The six other species were caught near-shore underneath the sea ice and up to 87-meter-deep in the vicinity of the station. The cryopelagic (Hoshiai et al., 1989) fish *Pagothenia borchgrevinki* were caught in cracks in the sea ice, using fishing rods at a depth of less than one meter. Most of the benthic species (*Notothenia coriiceps*, *Trematomus bernacchii*, *Trematomus hansonii*, *Trematomus pennellii*, Gon and Heemstra, 1990) were caught by using fish traps on the sea bottom, or by paying out line until we felt contact with the bottom and then reeled in just enough to keep our lure from hanging bottom. The fish *Trematomus newnesii*, known to feed in the water column or at the undersurface of ice in near shore shallow areas (Gon and Heemstra, 1990), was collected in the water column using a fishing rod. Each fish was identified to the species level based on morphological criteria. Individuals were measured (total length; nearest mm) and weighed (nearest g). These morphometric measures were not taken for the 9 *P. antarcticum*, because they had head or caudal fin removed while fishing with the IKMT net. Samples of white muscle (N = 129) and liver (N = 75) were excised and immediately frozen at  $-80\text{ }^{\circ}\text{C}$  until analysis in the laboratory. Since amounts of liver samples were limited, it was not possible to analyze both Hg and the 12 other elements in liver samples. Hence, Hg concentrations were determined in muscle samples and the 12 other elements in liver samples.

### 2.2. Trace element analyses

Freeze-dried samples were powdered and homogenized. The total Hg concentrations in the muscle were determined by analyzing Hg directly with an Advanced Mercury Analyzer (ALTEC AMA 254) on aliquots ranging from 5 to 50 mg of freeze-dried sample weighed to the nearest 0.01 mg (Bustamante et al., 2006). All analyses were repeated 2–3 times for each sample until having a relative standard deviation <10%. The analysis of Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Se, V and Zn were determined in liver samples according to Kojadinovic et al. (2011). Briefly, 60 to 300 mg samples were microwave digested in a mixture of 6 mL of 65% HNO<sub>3</sub> (VWR Quality SUPRAPUR) and 2 mL of 30% HCl (VWR Quality SUPRAPUR), except for the samples with a weight below 100 mg: 3 mL HNO<sub>3</sub> and 1 mL HCl. Then the samples were diluted to 50 mL (25 mL for the samples with a weight below 100 mg) with ultrapure water. The 12 elements were then analyzed by Inductively Coupled Plasma Atomic Emission Spectrometry (Varian Vista-Pro ICP-OES) and Mass Spectrometry (ICP-MS II Series Thermo Fisher Scientific). To avoid trace element contamination, all glass and plastic utensils used were soaked in a bath of nitric acid (50 mL in 2 L) for a minimum of 48 h, rinsed in ultrapure water and dried under a laminar hood before use. Accuracy and reproducibility of the preparation were tested by preparing analytical blanks and replicates of lobster hepatopancreas (TORT-2) and dog-fish liver (DOLT-3) reference standards (National Research Council, Canada) along with each set of samples. Results for the certified reference materials were in good agreement with the certified values and recovery rates varied from 83% to 109%. The detection limits ( $\mu\text{g} \cdot \text{g}^{-1}$  dry wt) were 0.005 (Hg), 0.02 (Ag, Cd, Co, Cr), 0.1 (Cu, Mn, Se), 0.2 (As), 0.3 (Ni), 0.33 (V), and 3.3 (Fe, Zn). Trace element concentrations are expressed in  $\mu\text{g} \cdot \text{g}^{-1}$  dry weight (dw).

**Table 1**

Sample data, including scientific names of species, sample size (N), sampling period (start and end, dd-mm-yy), habitat, total water depth (in m), sample collection depth, fishing gear, total length (TL, mean  $\pm$  SD, in mm) and mass (mean  $\pm$  SD, in g).

Species	N	Sampling period	Habitat	Water depth (m)	Fishing gear	TL (mm)	Mass (g)
<i>Pagothenia borchgrevinki</i>	28	21-09-10 22-12-11	Cryopelagic	1	Fishing rod	131 $\pm$ 24	26.8 $\pm$ 26.1
<i>Pleuragramma antarcticum</i>	9	18-01-11	Pelagic	200–250	IKMT net	NA	NA
<i>Notothenia coriiceps</i>	45	21-02-11 17-01-12	Benthic	15–87	Fish trap, fishing rod	242 $\pm$ 39	201 $\pm$ 105
<i>Trematomus bernacchii</i>	20	24-03-11 02-12-11	Benthic	5–38	Fish trap, fishing rod	192 $\pm$ 41	104 $\pm$ 65
<i>Trematomus hansonii</i>	9	21-02-11 22-11-11	Benthic	38–87	Fish trap	231 $\pm$ 67	226 $\pm$ 120
<i>Trematomus newnesi</i>	20	24-04-10 03-10-11	Semipelagic	5–15	Fishing rod	167 $\pm$ 25	50.8 $\pm$ 22
<i>Trematomus pennellii</i>	1	21-02-11	Benthic	87	Fish trap	228	170

### 2.3. Stable isotope analyses

Stable isotopes were determined in muscle samples. After being freeze-dried, samples were ground to a fine powder, lipids were extracted using cyclohexane and carbonates were removed using 1 N HCl. The low C:N mass ratio of the delipidated samples (<4.0; Post et al., 2007) allowed comparison of the carbon isotopic signature without any deleterious effect due to different lipid contents among individuals and species (Table 1). Relative abundance of  $^{13}\text{C}$  and  $^{15}\text{N}$  were determined with a continuous flow mass spectrometer (Thermo Scientific Delta V Advantage) coupled to an elemental analyzer (Thermo Scientific Flash EA 1112) as detailed in Chouvelon et al. (2012). Results are presented in the  $\delta$  notation relative to Vienna PeeDee Belemnite and atmospheric  $\text{N}_2$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively. Based on replicate measurements of internal laboratory standards (acetanilide), the experimental precision is  $\pm 0.15$  and  $\pm 0.20\%$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively.

### 2.4. Statistical analyses

All statistical analyses were performed using R software version 3.2.0 (R Development Core Team, 2014). Only trace element concentrations that were above the limit of quantification (LoQ) in at least 70% of individuals were included in statistical analyses. Hence Co, Cr and V were not further considered. For the other trace elements, concentrations below the LoQ were substituted using  $0.5 \cdot \text{LoQ}$  to avoid missing values distorting the statistical outcomes. *T. pennellii* was not included in the statistical analyses because only one specimen was collected. Data were first checked for normality and homogeneity of variances and were log-transformed if necessary. Generalized linear models (GLM) with a normal distribution and an identity-link function were used to test the effects of species and length on log-transformed trace element concentrations. Terms were added sequentially. If necessary, post-hoc tests were conducted using Tukey multiple comparisons of means or pairwise comparisons using Wilcoxon rank sum test and Bonferroni correction.

Pearson or Spearman correlation rank tests were used to test relationships between muscle Hg concentrations and  $\delta^{13}\text{C}$  (or  $\delta^{15}\text{N}$ ) values, or between trace element concentrations. Since stable isotopes were measured in muscle samples, we did not perform a correlation test between the 12 trace elements measured in liver samples and stable isotopes. Since  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were significantly correlated (Spearman's rank correlation,  $\rho = 0.877$ ,  $p < 0.001$ ), these explanatory variables were not included in the same models. GLM with a normal distribution and an identity-link function were used to test the effects of species, foraging habitat or dietary habits (inferred from muscle  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, respectively) and the interaction between species and foraging habitat (or dietary habits) on log-transformed Hg concentrations in

muscle. Model selection was based on Akaike's Information Criteria adjusted for small sample sizes (AICc). The model with the lowest AICc value was considered to be the most accurate. Models with AICc values differing by less than 2 have a similar level of support in the data, and the model including the least number of parameters was regarded as the most accurate, according to the principle of parsimony (Burnham and Anderson, 2002).

### 3. Results

Trace element concentrations for each fish species were detailed in Tables 2 and 3. Concentrations of trace elements in liver, except for Mn and As, were strongly and positively correlated (Table 4). By contrast, concentrations of Hg in muscle were not correlated to other trace elements, except a strong correlation between Hg in muscle and Cd in liver (Table 4).

Ag, As, Cd, Cu, Fe, Hg, Ni and Se, but not Mn and Zn, significantly differed among Antarctic fish species (Table 5). Significantly higher Hg levels ( $p < 0.05$  for all post-hoc tests) were found in *T. bernacchii* compared to *P. antarcticum*, *P. borchgrevinki*, *T. newnesi*, and *N. coriiceps*; and in *N. coriiceps* and *T. hansonii* compared to *P. antarcticum*, *P. borchgrevinki*, and *T. newnesi*.

After taking into account species-differences, the majority of trace element concentrations were not related to fish length. Concentrations of As, Cd, Hg and Se significantly increased with fish length (Table 5). It should however be noticed that slopes' estimates ranged between 0.002 and 0.008 (Table 5) and were thus closed to 0. Since standard errors were even smaller (0.001 for all tests), we concluded that concentrations of As, Cd, Se in liver and of Hg in muscle slightly increased with length in Antarctic fish.

Hg concentrations in muscle were significantly and positively correlated to both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in muscles (Spearman correlation,  $\rho = 0.719$  and  $0.714$ , respectively,  $p < 0.001$  for both tests). Models were ranked to disentangle the influence of species, foraging habitat ( $\delta^{13}\text{C}$ ), dietary habits ( $\delta^{15}\text{N}$ ) and interactions on Hg concentrations in fish muscles (Table 6). Models including species as covariate (models 1–5, Table 6) had the highest AICc, corroborating the strong species-differences in Hg concentrations. In addition, models including  $\delta^{13}\text{C}$  values as covariate (models 1 and 2, Table 6, Fig. 1) had the highest AICc weight (91%) and thus presented the best fit to the data set. Models including species and  $\delta^{15}\text{N}$  values as covariates (models 4 and 5, Table 6, Fig. 2) had a lower AICc than the model including only species and did not explain well the variation in Hg levels (AICc weight: 2%). Model 1 (Table 6) described Hg concentrations as a function of species,  $\delta^{13}\text{C}$ , and the interaction between species and  $\delta^{13}\text{C}$ , had the highest level of support in the data, but its AICc value differed by less than 2 with model 2 (no interaction effect). According to the principle of parsimony, the most accurate model is model 2: Hg concentrations in muscles were

**Table 2**  
Mean  $\pm$  standard deviation of stable isotopes ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) in muscle and mean  $\pm$  standard deviation and range Hg in muscle ( $\mu\text{g} \cdot \text{g}^{-1}$  dw) of 129 specimens representing seven different species.

Species	N	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Hg ( $\mu\text{g} \cdot \text{g}^{-1}$ dw)
<i>Pagothenia borchgrevinki</i>	28	$-25.1 \pm 0.7$	$10.0 \pm 0.4$	$0.067 \pm 0.040$ [0.021; 0.239]
<i>Pleuragramma antarcticum</i>	9	$-25.2 \pm 0.2$	$10.8 \pm 0.2$	$0.065 \pm 0.009$ [0.047; 0.080]
<i>Notothenia coriiceps</i>	45	$-21.5 \pm 1.1$	$11.2 \pm 0.4$	$0.221 \pm 0.085$ [0.061; 0.428]
<i>Trematomus bernacchii</i>	20	$-19.4 \pm 1.1$	$12.10 \pm 0.6$	$0.355 \pm 0.153$ [0.151; 0.796]
<i>Trematomus hansonii</i>	6	$-23.9 \pm 0.8$	$11.1 \pm 0.6$	$0.202 \pm 0.076$ [0.153; 0.350]
<i>Trematomus newnesi</i>	20	$-25.5 \pm 0.3$	$9.9 \pm 0.3$	$0.079 \pm 0.024$ [0.055; 0.141]
<i>Trematomus pennellii</i>	1	$-22.0$	11.1	0.279

**Table 3**  
Mean  $\pm$  standard deviation and range of trace elements in liver ( $\mu\text{g} \cdot \text{g}^{-1}$  dw) in 75 specimens representing six different species.

Species	N	Ag	As	Cd	Cu	Fe	Mn	Ni	Se	Zn
<i>Pagothenia borchgrevinki</i>	10	$0.206 \pm 0.123$ [0.066; 0.399]	$41.2 \pm 30.3$ [20.0; 124]	$2.413 \pm 1.054$ [0.957; 3.977]	$8.3 \pm 9.8$ [2.5; 35.3]	$86.8 \pm 68.7$ [45.2; 252]	$4.12 \pm 0.74$ [2.80; 5.50]	$2.88 \pm 5.58$ [0.12; 15.35]	$10.22 \pm 2.49$ [6.52; 14.84]	$132 \pm 80$ [69; 349]
<i>Notothenia coriiceps</i>	35	$1.168 \pm 0.917$ [0.249; 3.707]	$23.0 \pm 23.2$ [5.9; 144]	$9.032 \pm 3.215$ [4.552; 18.98]	$11.8 \pm 4.1$ [6.1; 21.3]	$508 \pm 186$ [186; 1073]	$3.46 \pm 0.89$ [2.14; 6.50]	$0.79 \pm 0.31$ [0.27; 1.66]	$11.13 \pm 2.39$ [6.23; 16.79]	$119 \pm 17$ [92; 175]
<i>Trematomus bernacchii</i>	16	$0.552 \pm 0.411$ [0.076; 1.514]	$26.7 \pm 12.9$ [10.6; 60.9]	$7.475 \pm 3.701$ [2.428; 14.50]	$11.7 \pm 6.3$ [4.4; 24.7]	$205 \pm 108$ [33.4; 446]	$3.56 \pm 0.94$ [2.35; 6.17]	$1.03 \pm 0.77$ [0.39; 3.00]	$9.48 \pm 1.81$ [5.74; 13.33]	$119 \pm 24$ [86; 189]
<i>Trematomus hansonii</i>	9	$0.264 \pm 0.427$ [0.025; 1.368]	$56.2 \pm 39.3$ [18.2; 153]	$4.445 \pm 2.699$ [0.963; 9.771]	$8.4 \pm 6.0$ [3.2; 18.8]	$142 \pm 82.4$ [37.1; 292]	$3.33 \pm 1.50$ [2.01; 6.79]	$0.23 \pm 0.29$ [0.06; 0.89]	$8.20 \pm 2.53$ [4.49; 13.38]	$102 \pm 27$ [71; 150]
<i>Trematomus newnesi</i>	4	$0.264 \pm 0.122$ [0.163; 0.399]	$17.9 \pm 4.6$ [11.9; 22.1]	$3.856 \pm 2.240$ [1.607; 6.958]	$10.6 \pm 5.1$ [6.8; 17.8]	$140 \pm 133$ [63.4; 294]	$3.12 \pm 0.48$ [2.49; 3.53]	$1.06 \pm 0.43$ [0.76; 1.68]	$9.94 \pm 1.32$ [8.75; 11.83]	$113 \pm 18$ [93; 129]
<i>Trematomus pennellii</i>	1	0.275	20.8	15.47	7.6	56.4	4.44	0.38	13.66	136

explained by species differences and increased with increasing  $\delta^{13}\text{C}$  values (Fig. 1).

## 4. Discussion

### 4.1. Comparison with other species and Antarctic areas

Our results fall within the range of trace metals levels for several fish species from the Antarctic Ocean (Sanchez-Hernandez, 2000). The present study could be further compared with a previous study that investigated the concentrations of trace elements in the liver, kidney and muscle of eight fish species from the subantarctic Kerguelen Islands (Bustamante et al., 2003). Antarctic fish (this study) had slightly lower Hg concentrations in muscle (ranges in  $\mu\text{g} \cdot \text{g}^{-1}$  dw; pelagic species: 0.02–0.24 vs 0.15–0.48; benthic species: 0.06–0.80 vs 0.03–1.51), similar Cd (0.95–19.0 vs 0.82–52.1), slightly higher Cu (2.45–35.3 vs 0.9–24.7), and higher Zn (68.6–349 vs 28.8–184) concentrations in liver than sub-Antarctic fish (Bustamante et al., 2003). The slight differences in trace element concentrations between fish species from Adélie Land and Kerguelen Island could be attributed to a geographical gradient in the oceanic distribution and bioavailability of trace elements (Cossa et al., 2011). Lower Hg levels in higher latitude organisms were

also recently observed in closely related seabird species (the South Polar Skua *Catharacta maccormicki* from Adélie Land vs the Brown Skua *Catharacta lonnbergi* from the subantarctic Kerguelen Archipelago, Goutte et al., 2014). Furthermore, wide-ranging wandering albatrosses showed a pronounced latitudinal effect, with birds feeding in northern subtropical waters having higher blood Hg concentrations than those feeding further south (Carravieri et al., 2014b).

### 4.2. Species differences and relationships with length

Strong correlations between trace element concentrations in liver suggest that species had similar exposure and accumulation patterns for several trace elements. In contrast, poor correlations between Hg in muscle and other trace element in liver suggest differential Hg accumulation and/or distribution among tissues within organisms.

The concentrations of Hg in muscle, and of Ag, As, Cd, Cu, Fe, Ni, and Se in liver greatly differed among fish species collected off Adélie Land, Antarctica. Since all individuals were collected in the same area, the Pointe Géologie Archipelago and were not migratory species, regional discrepancies should be excluded. Other possible explanations include interspecific differences in i) exposure to trace elements, and especially in variation of these metals bioavailability across the water column (Cossa et al.,

**Table 4**  
Pearson or Spearman correlation matrix between trace element concentrations in Antarctic fish.

	Ag	As	Cd	Cu	Fe	Hg	Mn	Ni	Se
As	$-0.39^{***}$	–	–	–	–	–	–	–	–
Cd	$0.70^{***}$	$-0.19$	–	–	–	–	–	–	–
Cu	$0.70^{***}$	$-0.20$	$0.65^{***}$	–	–	–	–	–	–
Fe	$0.71^{***}$	$-0.32^{**}$	$0.69^{***}$	$0.57^{***}$	–	–	–	–	–
Hg	$0.25^*$	0.21	$0.47^{***}$	$0.25^*$	0.18	–	–	–	–
Mn	$-0.01$	0.10	0.02	0.14	$-0.07$	$-0.09$	–	–	–
Ni	$0.42^{***}$	$-0.19$	$0.28^*$	$0.65^{***}$	$0.43^{***}$	$-0.06$	0.10	–	–
Se	$0.51^{***}$	$0.24^*$	$0.46^{***}$	$0.49^{***}$	$0.46^{***}$	0.23	$0.34^{**}$	$0.50^{***}$	–
Zn	$0.48^{***}$	$-0.13$	$0.42^{***}$	$0.61^{***}$	$0.44^{***}$	0.03	$0.33^{**}$	$0.50^{***}$	$0.41^{***}$

\*  $p < 0.05$ .

\*\*  $p < 0.01$ .

\*\*\*  $p < 0.001$ .

**Table 5**  
Effects of species and length on log-transformed concentrations of trace elements in liver and muscle samples, using GLM with a normal distribution and an identity-link function. *P. antarcticum* and *T. pennellii* were excluded from these analyses due to the absence of morphometric data and low sample size respectively. When the correlation between a trace element concentration and length was significant, the slope's estimate and standard error (SE) was provided.

	Hg	Ag	As	Cd	Cu	Fe	Mn	Ni	Se	Zn
Species	$F_{4,113} = 98.78$ , $p < 0.005$	$F_{4,66} = 15.55$ , $p < 0.005$	$F_{4,69} = 10.58$ , $p < 0.005$	$F_{4,69} = 21.98$ , $p < 0.005$	$F_{4,69} = 4.298$ , $p = 0.004$	$F_{4,64} = 31.72$ , $p < 0.005$	$F_{4,69} = 1.759$ , $p = 0.147$	$F_{4,63} = 11.94$ , $p < 0.005$	$F_{4,69} = 4.768$ , $p = 0.002$	$F_{4,68} = 1.178$ , $p = 0.329$
Length	$F_{1,112} = 35.15$ , $p < 0.005$	$F_{1,65} = 1.268$ , $p = 0.264$	$F_{1,68} = 14.35$ , $p < 0.005$	$F_{1,68} = 3.92$ , $p = 0.052$	$F_{1,68} = 0.897$ , $p = 0.347$	$F_{1,63} = 0.169$ , $p = 0.683$	$F_{1,68} = 1.154$ , $p = 0.287$	$F_{1,62} = 1.659$ , $p = 0.203$	$F_{1,68} = 13.24$ , $p < 0.005$	$F_{1,67} = 0.131$ , $p = 0.719$
Slope ± SE	$0.005 \pm 0.001$		$0.008 \pm 0.001$	$0.002 \pm 0.001$					$0.002 \pm 0.001$	

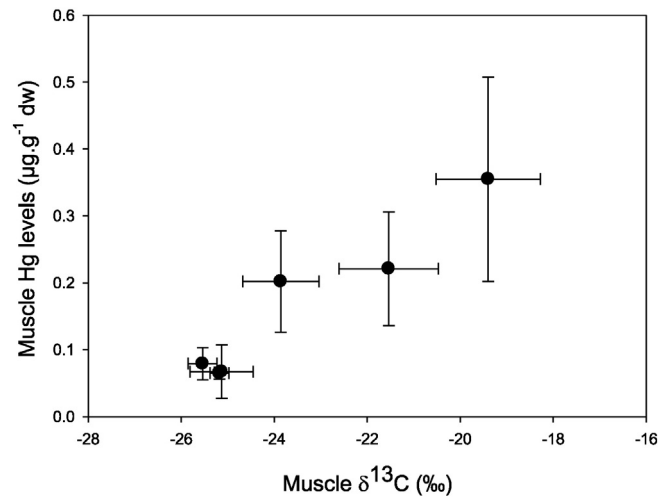
**Table 6**

AICc model ranking for Hg concentrations (log-transformed, N = 121) in muscle in relation to fish species (6 species, *T. pennellii* was not included because only one specimen was fished),  $\delta^{13}C$  (as a proxy of foraging habitat, inshore/benthic vs offshore/pelagic) and  $\delta^{15}N$  (as a proxy of dietary habits). Abbreviations: k: number of parameters; AICc: Akaike's Information Criteria adjusted for small sample-size values;  $\Delta AICc$ : difference between AICc and min(AICc); w(AICc): AICc weights, i.e. weight of evidence interpreted as a proportion.

Models	# models	K	AIC	AICc	$\Delta AICc$	w(AICc)
Species + $\delta^{13}C$ + species: $\delta^{13}C$	1	13	117.13	120.53	0	0.59
Species + $\delta^{13}C$	2	8	120.52	121.80	1.27	0.32
Species	3	7	123.90	124.89	4.36	0.07
Species + $\delta^{15}N$	4	8	125.75	127.04	6.51	0.02
Species + $\delta^{15}N$ + species: $\delta^{15}N$	5	13	132.51	135.91	15.38	0.00
$\delta^{13}C$	6	3	185.68	185.88	65.35	0.00
$\delta^{15}N$	7	3	192.25	192.46	71.93	0.00
Null	8	2	276.29	276.39	155.86	0.00

2011), ii) diet and trophic level according to biomagnification processes in Antarctic trophic web (Bargagli et al., 1998a), iii) mechanisms related to the kinetics of trace elements that are absorption, biotransformation, and excretion (e.g. Spry and Wood, 1989) and iv) individual parameters (sex, growth, body condition, life cycle, Al-Yousuf et al., 2000). The two last hypotheses were not tested due to the lack of appropriate individual information and the need for experimental monitoring. Because of food resource partitioning within Antarctic notothenioid fish assemblage (e.g. Barrera-Oro, 2003), low proportion of prey overlap among species may explain differences in trace element concentrations. Isotopic signatures were used to disentangle the respective contribution of foraging habitats and dietary habits in driving variations in muscle Hg concentrations.

Concerning an accumulation of trace elements during growth, this study highlighted a slight increase of As, Cd, Hg and Se concentrations with length and no significant relationship between Ag, Cu, Fe, Mn, Ni, Zn and length. In the open scientific literature, relationships between trace element levels and fish sizes are mostly non-significant or have negative slopes (e.g. Al-Yousuf et al., 2000; Canli and Atli, 2003; Merciai et al., 2014), except for Hg (e.g. Chauvelon et al., 2014). Since Me–Hg likely binds with sulfhydryl groups of muscular proteins, Hg shows slow elimination rates in muscles and accumulates with aging in fish (Chauvelon et al., 2014). Our data only reveal a slight increase of Hg with length, which could be due to a limited range of fish size and thus age.



**Fig. 1.** Positive correlation between muscle Hg concentrations (mean ± standard deviation,  $\mu g \cdot g^{-1} dw$ ) and  $\delta^{13}C$  (mean ± standard deviation, ‰) in fish species off Adélie Land, Antarctica.

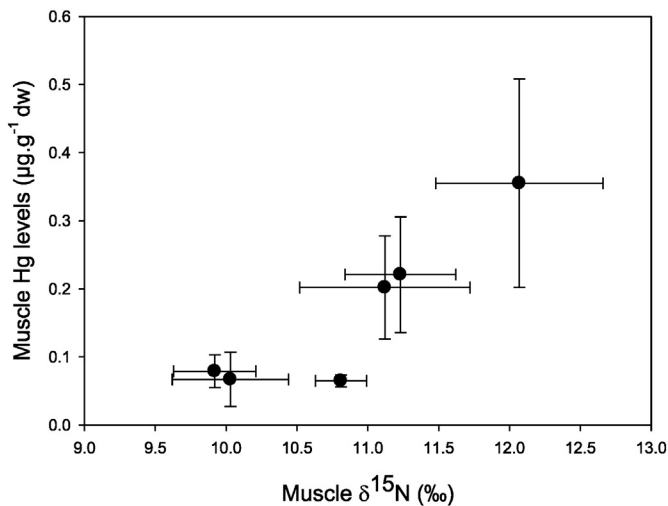


Fig. 2. Relationship between muscle Hg concentrations (mean  $\pm$  standard deviation,  $\mu\text{g}\cdot\text{g}^{-1}\text{ dw}$ ) and  $\delta^{15}\text{N}$  (mean  $\pm$  standard deviation, ‰) in fish species off Adélie Land, Antarctica.

#### 4.3. Foraging habitats

We postulated that foraging habitat would shape Hg contamination in Antarctic ichthyofauna. In agreement with the hypothesis, the most contaminated fish were the shallow benthic species (*T. bernacchii*, *T. pennellii*, *N. coriiceps*, *T. hansonii*, Gon and Heemstra, 1990) and the less contaminated fish were the cryopelagic (*P. borchgrevinkii*, Hoshiai et al., 1989), pelagic (*P. antarcticum*, La Mesa et al., 2004; La Mesa and Eastman, 2012) and semipelagic (*T. newnesii*, Gon and Heemstra, 1990) species. Furthermore, muscle  $\delta^{13}\text{C}$  values were used to confirm the influence of foraging habitat on individual Hg concentrations. Except for *P. antarcticum*, all specimens were collected near-shore, so the  $\delta^{13}\text{C}$  values mainly reflect inshore/benthic versus offshore/pelagic contribution to food intake. Our results on  $\delta^{13}\text{C}$  values were consistent with previous investigations of the isotopic niches of fish species in Adélie Land (Cherel et al., 2011). Our model selection highlighted that Hg concentrations in muscles differed among species and increased with  $\delta^{13}\text{C}$  values. Three fish species shared low and highly similar profile of Hg and  $\delta^{13}\text{C}$  values: *P. antarcticum*, *P. borchgrevinkii* and *T. newnesii*. Although they differ in their ecological niche (pelagic, cryopelagic and semipelagic, respectively), they are all pelagic feeders, thus exhibiting low  $\delta^{13}\text{C}$  values and appeared to be poorly Hg-contaminated. *N. coriiceps* and *T. hansonii* exhibited non-significantly different Hg levels, although *N. coriiceps* were more benthic (higher  $\delta^{13}\text{C}$  values) than *T. hansonii*. At last, *T. bernacchii* had the highest Hg levels and the more benthic life style according to its  $\delta^{13}\text{C}$  values. This result strongly corroborates the hypothesis stating that Hg is more bioavailable for coastal benthic organisms, because of the production of Me-Hg in sediments (Fitzgerald et al., 2007). Moreover, photodemethylation by ultraviolet radiation provides a further mechanism for Me-Hg removal in surface waters (Lehnherr et al., 2011), and a lower Hg bioavailability for surface feeders, such as *P. antarcticum*, *P. borchgrevinkii* and *T. newnesii*. Hence foraging habitat strongly shapes Hg load in Antarctic ichthyofauna.

#### 4.4. Dietary habits

Since Hg is efficiently biomagnified along food webs (Atwell et al., 1998; Morel et al., 1998; Bargagli et al., 1998a), higher trophic level species were assumed to exhibit higher Hg levels than lower trophic level species. This pattern was clearly established for subantarctic seabird communities (Carravieri et al., 2014a). In the present study, fish Hg

concentrations increased with muscle  $\delta^{15}\text{N}$  values, but the strength of the correlation was not as pronounced as the link between Hg levels and  $\delta^{13}\text{C}$  values. This could be due to many non-mutually exclusive explanations. Firstly, the  $\delta^{15}\text{N}$  values in Antarctic fish species reflect not only trophic position, but also foraging habitats. Coastal/inshore benthic fish had higher  $^{15}\text{N}$  values than cryopelagic and pelagic consumers, mainly because baseline  $\delta^{15}\text{N}$  values are higher in inshore/benthic than offshore/pelagic ecosystems (e.g. Chauvelon et al., 2012). Secondly, as expected (Cherel et al., 2011),  $\delta^{15}\text{N}$  vary little among the studied Antarctic fish species [9.2–13.3‰]. This poor variability did not allow a clear relationship between Hg concentrations and trophic levels to be established. Thirdly, benthic fishes are considered as trophic generalists and opportunistic feeders (Gon and Heemstra, 1990; La Mesa et al., 2004), which results in poor inter-specific  $\delta^{15}\text{N}$  variations and thus precludes describing strong positive relationship between trophic levels and Hg levels.

To conclude, foraging habitat (benthic vs pelagic) and to a lesser extent, dietary habits and age, were strong predictors of variations in muscle Hg levels.

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