Spatial variability in total and organic mercury levels in Antarctic krill *Euphausia superba* across the Scotia Sea

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**A B S T R A C T**

Total and organic mercury concentrations were determined for males, females and juveniles of *Euphausia superba* collected at three discrete locations in the Scotia Sea (South Orkney Islands, South Georgia and Antarctic Polar Front) to assess spatial mercury variability in Antarctic krill. There was clear geographic differentiation in mercury concentrations, with specimens from the South Orkney Islands having total mercury concentrations 5 to 7 times higher than Antarctic krill from South Georgia and the Antarctic Polar Front. Mercury did not appear to accumulate with life-stage since juveniles had higher concentrations of total mercury (0.071 μg g⁻¹ from South Orkney Islands; 0.014 μg g⁻¹ from South Georgia) than adults (0.054 μg g⁻¹ in females and 0.048 μg g⁻¹ in males from South Orkney Islands; 0.006 μg g⁻¹ in females and 0.007 μg g⁻¹ in males from South Georgia). Results suggest that females may use egg laying as a mechanism to excrete mercury, with eggs having higher concentrations than the corresponding somatic tissue. Organic mercury makes up a minor percentage of total mercury (15–37%) with the percentage being greater in adults than in juveniles. When compared to euphausiids from other parts of the world, the concentration of mercury in Antarctic krill is within the same range, or higher, highlighting the global distribution of this contaminant. Given the high potential for biomagnification of mercury through food webs, concentrations in Antarctic krill may have deleterious effects on long-lived Antarctic krill predators.

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**1. Introduction**

Mercury contamination in the environment has been acknowledged as a global problem, the production and use of this element is nowadays very strictly regulated and limited (Selin, 2009; UNEP, 2013). Pathways of dispersion through ecosystems, including in the Antarctic, of this long-range contaminant are complex (Streets et al., 2009). Interplay between the distinctive Antarctic atmosphere and the seasonal sea-ice cycle in the Southern Ocean generates a unique combination of environmental factors that can explain why the remote Southern Ocean has some of the highest reported concentrations of organic mercury (i.e. compounds containing covalent bonds between carbon and mercury) in open waters (Cossa et al., 2011). Due to its high affinity for proteins (Bustamante et al., 2006), organic mercury is the most toxic form of the element (Clarkson, 1992). It accumulates in aquatic organisms and biomagnifies within food webs, being toxic for top predators (Ackerman et al., 2014; Chouvelon et al., 2012; Coelho et al., 2010; Dehn et al., 2006) with consequencies at the population level (Goutte et al., 2014a, 2014b). Wandering albatrosses, *Diomedea exulans*, are an example of this biomagnification effect in
Antarctica, as it was found that they had some of the highest concentration of total mercury (from now on noted as mercury) in marine birds (up to 24.80 ± 8.61 μg g⁻¹ dry weight) (Cherel et al., 2018; Tavares et al., 2013).

In the Southern Ocean, Antarctic krill, *Euphausia superba*, is a key species in the marine food webs connecting primary producers and higher predators (Everson, 2000). It has an estimated biomass of around 379 million tonnes (Atkinson et al., 2009) and being the main food for many vertebrates (Murphy et al., 2007; Xavier and Peck, 2015). For example, minke whales, *Balaenoptera acutorostrata*, and Crab eater seals, *Lobodon carcinophaga*, feed almost exclusively (>95%) on Antarctic krill (Adam, 2005; Armstrong and Siegfried, 1991; Croll and Tershy, 1998; Perrin et al., 2008). Chinstrap penguins, *Pygoscelis antarctica*, Gentoo penguins, *Pygoscelis papua*, and other species of penguins, in the Southern Ocean, also feed mostly on Antarctic krill (Dimitrijevic et al., 2018; Xavier et al., 2018) with values around 1.2 kg d⁻¹ (Croll and Tershy, 1998). Finally, Antarctic krill is the most harvested species in the Southern Ocean, with >260 000 tonnes fished in 2016, regulated under the Convention for the Conservation of Antarctic Living Resources (Nicol et al., 2000; CCAMLR, 2017).

In the context of environmental change (Constable et al., 2014; Costas, 2013; Gutt et al., 2015), it is important to evaluate the impact accumulation dynamics, in an effort towards the preservation of presumably less impacted environments such as Antarctica with the presence of contaminants like mercury, particularly in a remote and pristine Southern Ocean with warmer waters (Dong et al., 2006). Under this context, understanding the pathway of this contaminant through Southern Ocean such an important prey as Antarctic krill is crucial to better understand the ecosystem. Assessing the levels of organic mercury in Antarctic krill. Content in Antarctic krill. Assessing the levels of organic mercury in Antarctic krill.

2. Material and methods

2.1. Sampling

Antarctic krill *Euphausia superba* were collected from the British research vessel RRS *James Clark Ross* during the austral summers of 2007/08, 2015/16 and 2016/17 (cruises JR177, JR15004 and JR16003 respectively). The three cruises sampled three areas of the Scotia Sea (Fig. 1) with different oceanic characteristics. JR16003 had one sampling point at the Antarctic Polar Front. Both JR16003 and JR177 sampled predominantly around South Georgia, and JR15004 sampled around the South Orkney Islands.

Samples were collected from the water column using an 8 m² mouth-opening Rectangular Midwater Trawl (RMT8; mesh size reducing from 4.5 mm to 2.5 mm in the cod end) (Roe and Shale, 1979). The net was rigged with two nets that could be remotely opened and closed at different depths. The RMT8 was used to target particularly Antarctic krill swarms and other layers of interest (e.g. fish layers) identified by the vessel scientific echosounder system (i.e. Simrad EK60/EK80 operating between 38 and 200 kHz).

Antarctic krill in the catches were identified and total length (TL) of each individual was measured, from the anterior edge of the eye to the tip of the telson and rounded down (Morris et al., 1992). Sex and maturity stage were determined with reference to the presence of a petasma (males), thelycum (females) or absent (juveniles; individuals without visible external sexual characteristics) (Ross and Quetin, 2000). Samples were either preserved in sample bags at −20 °C (JR15004 and JR16003) or on vials in ethanol (for JR177) (Fort et al., 2016).

2.2. Laboratory procedures

Prior to the mercury analysis, all samples were freeze-dried for at least 24 h. The eggs of females (Maturity stage III) (Ross and Quetin, 2000) from JR177 (South Georgia) were removed under the microscope before freeze-drying.

Dried individuals and tissues were homogenized and analysed for total mercury by thermal decomposition atomic absorption spectrometry with gold amalgamation, using a LECO AMA-254 (Advanced mercury analyser) following Coelho et al. (2018). Organic mercury was determined through digestion with a mixture of 18% potassium bromide (KBr) in 5% sulfuric acid (H₂SO₄), followed by extraction of organic mercury into toluene as described in Válega et al. (2006). Analytical quality control was performed using certified reference material (CRM; in this case TORT-2 and TORT-3 [Lobster hepatopancreas, National Research Council, Canada]). The obtained values (mean ± SD) for the whole of the CRM analyses ranged from 81 to 100% (TORT-2: 87 ± 3%, n = 41; TORT-3: 90 ± 8%, n = 27), results were corrected using the daily recovery efficiency of CRMs. The mass of CRM used for quality control analyses was adjusted to within the range of total mercury (in ng) present in the samples. Analyses were performed in duplicate, blanks were analysed at the beginning of each set of samples and the coefficient of variation between replicates never exceeded 10%. CRMs were also used to validate organic mercury analyses, with an extraction efficiency of 80 ± 2% and 98 ± 5%, respectively. The limit of detection for this analytical method is 0.01 ng g⁻¹ of total mercury and 4 ng g⁻¹ for organic mercury. All concentration data are expressed subsequently in μg g⁻¹ dry weight.

2.3. Statistical analysis

Wilcoxon tests were used to investigate whether there were any differences in mercury concentrations between females and males, between eggs and females, or between sampling sites. Kruskall-Wallis tests were performed to examine if there were statistical differences between sex/maturity and location. Linear regressions were calculated to examine possible relationships between Antarctic krill length and individual mercury concentration. All analyses were performed using the R software version 3.4.2 (R Core Team, 2013). All values are presented as mean ± SD.

3. Results

3.1. Total mercury concentrations in Antarctic krill according to geographic areas

Total mercury concentrations varied between 0.054 ± 0.018 μg g⁻¹ in females, 0.048 ± 0.011 μg g⁻¹ in males and 0.071 ± 0.023 μg g⁻¹ in juveniles from the South Orkney Islands to 0.006 ± 0.002 μg g⁻¹ in females, 0.007 ± 0.002 μg g⁻¹ in males and
0.014 ± 0.005 μg g⁻¹ in juveniles from the South Georgia and 0.017 ± 0.006 μg g⁻¹ in juveniles from the Antarctic Polar Front.

There was a clear differentiation in mercury concentrations between the three locations (Fig. 2): adult Antarctic krill from the South Orkney Islands had concentrations of mercury about 7 times higher in females (Wilcoxon rank sum test, W = 120, p < 0.001) and males (Wilcoxon rank sum test, W = 120, p < 0.001) than adult Antarctic krill from South Georgia, and juveniles showed concentrations around 5 times higher in the South Orkney Islands (Kruskall-Wallis, H₃ = 41.03, p < 0.001) than those collected at South Georgia and at the Antarctic Polar Front. Juveniles from the northern locations (South Georgia and Antarctic Polar Front) had similar mercury concentrations (Wilcoxon rank sum test, W = 192, p = 0.093).

3.2. Total mercury concentrations in Antarctic krill according to life stage

There were significant differences (Wilcoxon signed rank test, Z = -3.351, p = 0.001) between the mercury concentrations in the eggs (0.015 ± 0.002 μg g⁻¹) and the corresponding female somatic tissue (0.008 ± 0.003 μg g⁻¹) from South Georgia (Fig. 2). There were no significant differences (Wilcoxon rank sum test, W = 189, p = 0.071) between the females sampled in 2007/08 and 2016/17 at South Georgia (0.007 ± 0.002 μg g⁻¹). Juveniles caught around South Georgia (0.014 ± 0.005 μg g⁻¹) had significantly higher mean concentration of mercury than adults (0.007 ± 0.002 μg g⁻¹; Kruskall-Wallis H = 41.031, p < 0.01). Juveniles and eggs from South Georgia also had similar concentrations (Wilcoxon rank sum test, W = 205, p = 0.254). Like in juveniles from South Georgia, juveniles caught at the South Orkney Islands (0.071 ± 0.024 μg g⁻¹) also had significantly higher mercury concentrations than adults (0.051 ± 0.015 μg g⁻¹; Kruskall-Wallis H = 10.048, p = 0.07).

Significant negative correlations of mercury concentration with body size was common to both the South Orkney Islands and South Georgia (Y = -0.0124X - 1.525, R² = 0.46, F₁,₄₃ = 36.41, p < 0.001 from South Georgia; Y = -0.01072X - 0.8675, R² = 0.2746, F₁,₅₂ = 19.69, p < 0.001 from South Orkney Islands) meaning that bigger individuals had lower mercury concentrations (Fig. 3). It was not possible to discern if such a relationship also existed at the Antarctic Polar Front, since only juveniles were found at this location.

3.3. Total mercury concentrations in Antarctic krill according to sex

Concentrations of mercury in adult females (0.054 ± 0.018 μg g⁻¹) and males (0.048 ± 0.011 μg g⁻¹) from South Georgia were similar (Wilcoxon signed rank test, W = 216, p = 0.5; Fig. 2). There were also no differences in mercury concentration between...
sexes in the samples collected from the South Orkney Islands (Wilcoxon signed rank test, \( W = 199, p = 0.6 \); Fig. 2).

### 3.4. Organic mercury in Antarctic krill

Adult Antarctic krill from the South Orkney Islands had higher concentrations of organic mercury than adults from South Georgia (Table 1) (for both males and females), but concentrations in juveniles were similar between the two locations. While no significant differences between juveniles, males and females were observed in the South Orkney Islands, juveniles in South Georgia had higher organic mercury concentrations than adults.

Organic mercury percentages in Antarctic krill were lower in the South Orkney Islands (15% in juveniles, 16% in females and 21% in males) than at South Georgia (29% in juveniles, 37% in females and 36% in males) and the Antarctic Polar Front (35% in juveniles; Table 1).

### 4. Discussion

Despite some studies reporting mercury levels in Antarctic krill (Bargagli et al., 1998; Brasso et al., 2012b; Locarnini and Presley, 1995; Moren et al., 2006), there has remained a gap in knowledge regarding variability in mercury concentration by size, gender and location. Furthermore, to our knowledge this is the first study to determine organic mercury concentrations in Antarctic krill.

#### 4.1. Total mercury concentrations according to geographic areas

We found Antarctic krill from South Orkney Island had mercury body burdens 5 to 7 times higher than those from South Georgia and from the Antarctic Polar Front. Habitat differences may explain the variations in contamination levels between these three areas in the Southern Ocean. The average sea surface temperature around the South Orkney Islands is lower than in South Georgia (Barnes et al., 2005; Clarke and Leakey, 1996) and at the Antarctic Polar Front. This temperature gradient leads to an important ecosystem difference, promoting the presence of more winter ice in the South Orkney Islands than in South Georgia (Barnes et al., 2005; Clarke and Leakey, 1996) and at the Antarctic Polar Front. This temperature gradient leads to an important ecosystem difference, promoting the presence of more winter ice in the South Orkney Islands than in South Georgia (Atkinson et al., 2001). Ice formation can act as a buffer for mercury and other elements (Lindberg et al., 2002). Furthermore, the ice may act as a trap for contaminants precipitating from the atmosphere (Beyer and Matthies, 2001; Cossa et al., 2011), which are released into the water column upon ice melting (Brierley and Thomas, 2002; Geisz et al., 2008; Mastromonaco et al., 2017). In the Arctic, for instance, higher concentrations of mercury were measured in seawater under sea-ice, when compared with ice-free regions (Hintelmann et al., 2007) and higher concentrations of mercury were found under ice during spring (Mastromonaco et al., 2017). Additionally, depletion events promote higher precipitation rates of atmospheric mercury in colder areas, mainly during springtime, when halogen radicals oxidize the mercury (Ebinghaus et al., 2002; Lindberg et al., 2002). Indeed, these depletion events have been reported along and between regions of Antarctic sea-ice (Dommergue et al., 2010). Thus, higher depletion rates, sea ice formation and its melting may explain why there were more contaminants available to Antarctic krill around the South Orkney Islands than around South Georgia. Comparing our data with previous records of mercury in Antarctic krill, we see that samples from the Ross Sea, an area with winter sea ice (Bargagli et al., 1998), had higher concentrations than South

### Table 1

<table>
<thead>
<tr>
<th>Location</th>
<th>Year</th>
<th>Sex/Maturity</th>
<th>Number</th>
<th>( \text{OHg (mg g}^{-1} \text{dw)} )</th>
<th>( \text{THg (mg g}^{-1} \text{dw)} )</th>
<th>OHg (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>South Orkney Islands</td>
<td>2016</td>
<td>Juvenile</td>
<td>20</td>
<td>0.008 ± 0.003</td>
<td>0.051 ± 0.016</td>
<td>15%</td>
</tr>
<tr>
<td>South Orkney Islands</td>
<td>2016</td>
<td>Female</td>
<td>20</td>
<td>0.008 ± 0.002</td>
<td>0.052 ± 0.022</td>
<td>16%</td>
</tr>
<tr>
<td>South Orkney Islands</td>
<td>2016</td>
<td>Male</td>
<td>20</td>
<td>0.008 ± 0.003</td>
<td>0.040 ± 0.014</td>
<td>21%</td>
</tr>
<tr>
<td>South Georgia</td>
<td>2017</td>
<td>Juvenile</td>
<td>20</td>
<td>0.008 ± 0.002</td>
<td>0.024 ± 0.006</td>
<td>29%</td>
</tr>
<tr>
<td>South Georgia</td>
<td>2017</td>
<td>Female</td>
<td>20</td>
<td>0.002 ± 0.002</td>
<td>0.006 ± 0.003</td>
<td>37%</td>
</tr>
<tr>
<td>South Georgia</td>
<td>2017</td>
<td>Male</td>
<td>20</td>
<td>0.003 ± 0.001</td>
<td>0.007 ± 0.004</td>
<td>36%</td>
</tr>
<tr>
<td>Antarctic Polar Front</td>
<td>2017</td>
<td>Juvenile</td>
<td>20</td>
<td>0.005 ± 0.001</td>
<td>0.014 ± 0.005</td>
<td>35%</td>
</tr>
</tbody>
</table>
Decreased with age (see results). Since juveniles have a faster rate of growth compared to adults, one would otherwise expect burdens to be lower in juveniles through a growth dilution effect, as reported for Daphnia pulex (Karimi et al., 2007). Furthermore, juveniles have more frequent molting cycles compared to adults (Buchholz, 1991), and excretion ratios will probably be more efficient at these early stages. Somatic growth of Antarctic krill is programmed to slow once a certain age or maturity has been reached (Tarling et al., 2006), in order to divert considerable resources to reproductive tissue when reaching adulthood (Atkinson et al., 2006; Cuzin-Roudy, 2000). Adults also prey on higher trophic levels compared to juveniles (Atkinson et al., 2002) which should mean higher biomagnification potential, and therefore contrary to what was observed. The higher contaminant load of juveniles when compared with adults has, however, been reported in previous studies on Antarctic krill (Locarnini and Presley, 1995) as well as in the subantarctic krill Euphausia vallentini (Cipro et al., 2018). One mechanism that may explain this phenomenon is through egg laying, which has been reported as an important elimination route for mercury in several organisms such as birds (Brasso et al., 2012a; Pedro et al., 2015) and fish (Johnston et al., 2001; Schofield et al., 1994), and also previously hypothesized for crustaceans species (Coelho et al., 2008). In the present study, the higher mercury concentrations were found in Antarctic krill eggs when compared to corresponding somatic tissue, suggesting that egg laying maybe an elimination mechanism. However, males also have lower mercury burdens compared to juveniles which either rules out this hypothesis or indicates that males also eliminate mercury through their own gonadic tissue. Spermatophores are regularly produced and passed out of the body throughout the lifespan of males, although concentrations of mercury in these structures has yet to be measured.

4.3. Organic mercury

We found concentrations of the highly toxic, organic forms of mercury of between 0.002 and 0.018 µg g⁻¹ dw, with the higher concentrations being found in both the South Orkney Islands and South Georgia, particularly in juveniles. Antarctic krill is the main prey for several Southern Ocean predators and it is estimated that more than half of its total biomass of 379 Mt is eaten by whales, decreased with age (see results). Since juveniles have a faster rate of growth compared to adults, one would otherwise expect burdens to be lower in juveniles through a growth dilution effect, as reported for Daphnia pulex (Karimi et al., 2007). Furthermore, juveniles have more frequent molting cycles compared to adults (Buchholz, 1991), and excretion ratios will probably be more efficient at these early stages. Somatic growth of Antarctic krill is programmed to slow once a certain age or maturity has been reached (Tarling et al., 2006), in order to divert considerable resources to reproductive tissue when reaching adulthood (Atkinson et al., 2006; Cuzin-Roudy, 2000). Adults also prey on higher trophic levels compared to juveniles (Atkinson et al., 2002) which should mean higher biomagnification potential, and therefore contrary to what was observed. The higher contaminant load of juveniles when compared with adults has, however, been reported in previous studies on Antarctic krill (Locarnini and Presley, 1995) as well as in the subantarctic krill Euphausia vallentini (Cipro et al., 2018). One mechanism that may explain this phenomenon is through egg laying, which has been reported as an important elimination route for mercury in several organisms such as birds (Brasso et al., 2012a; Pedro et al., 2015) and fish (Johnston et al., 2001; Schofield et al., 1994), and also previously hypothesized for crustaceans species (Coelho et al., 2008). In the present study, the higher mercury concentrations were found in Antarctic krill eggs when compared to corresponding somatic tissue, suggesting that egg laying maybe an elimination mechanism. However, males also have lower mercury burdens compared to juveniles which either rules out this hypothesis or indicates that males also eliminate mercury through their own gonadic tissue. Spermatophores are regularly produced and passed out of the body throughout the lifespan of males, although concentrations of mercury in these structures has yet to be measured.

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seals, seabirds, squid and fish (Atkinson et al., 2009). Assuming the lowest individual mercury concentrations measured by the present study, this would mean 1.33 t of mercury will be passed on from the consumption of Antarctic krill, of which 0.57 t will be in the organic form. However, the 1.33t of mercury potentially transferred in the trophic web is a conservative number, as it was calculated from the lowest concentration levels found in the present study, that is, at the same time the lowest concentration ever measured in the literature. So, it can be considered an underestimation. This organic mercury will be potentially bioaccumulated in the tissues of Antarctic krill predators and transferred towards upper food web predators leading to its biomagnification. Thus, it may reach concentrations that can affect the behaviour, reproductive success and even to reduce the survival of the top predators (Tan et al., 2009; Eagles-Smith et al., 2018). Such bioaccumulation of organic mercury from Antarctic krill accumulation can explain how some Antarctic seabirds have particularly high concentrations of mercury (Tavares et al., 2013).

5. Conclusions

The accumulation of mercury in Antarctic krill decreases with increasing body size and maturity. Juveniles have higher concentrations than adults which may be the result of mercury elimination through gonadic tissue (eggs and spermatophores). The observed spatial differences suggest that Antarctic krill reflects different contaminant bioavailability in the Southern Ocean, while further studies are needed to discern the most significant variables governing site-specific mercury bioaccumulation. The range of mercury concentrations reported in Antarctic krill are within the same range, or even higher, than other euphausiids from areas closer to the industrialized part of the world, highlighting mercury as a global pollutant.

Overall, our results stress the need to put into action pollutant monitoring programs to evaluate the sources, pathways and effects of contaminants in remote ecosystems.

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