Universidade de Aveiro Departamento de Biologia
2020

José Sérgio Maurício Seco

Acumulação de mercúrio na cadeia alimentar do mar de Scotia, Oceano Antártico

Mercury accumulation in the food web of the Scotia Sea, Southern Ocean

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em (Ciência, Tecnologia e Gestão do Mar), realizada sob a orientação científica da Professora Doutora Maria Eduarda Pereira, Professora associada do Departamento de Química da Universidade de Aveiro e do Professor Doutor Andrew Stuart Brierley, Professor da *School of Biology* da Universidade de St. Andrews.

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palavras-chave Elementos traço, Contaminação, Poluentes, Tendências temporais, Antártica

resumo

O objetivo desta tese foi compreender os processos de bioacumulação e biomagnificação do mercúrio (Hg) na cadeia alimentar do Oceano Antártico (OA) e avaliar o efeito do tamanho e habitat do organismo na acumulação de Hg, durante a última década. Tecidos de várias espécies de diferentes níveis tróficos foram analisados em amostras recolhidas de vários anos de amostragem (2006/07 - 2016/17) de áreas com características distintas. Diferentes padrões de acumulação foram encontrados: os juvenis do krill antártico apresentaram maiores concentrações de Hg que os adultos; nas lulas, a concentração de Hg aumentou com o tamanho individual em algumas espécies (*Alluroteuthis antarcticus*), diminuiu em outras (*Galiteuthis glacialis*) e em outras não houve relação óbvia (*Bathyteuthis abyssicola*). Nos mictofídeos, houve um aumento da concentração de Hg com o tamanho dos peixes, com exceção das fêmeas de *Electrona antarctica*.

As proporções de Hg orgânico variaram entre os grupos, de relativamente baixas (15-37%) em krill a virtual 100% em todos os tecidos de mictofídeos. Quanto ao Hg em tecidos, o músculo das lulas foi o tecido que apresentou maior Hg, seguido pela glândula digestiva e brânquias. Hg nos mictofídeos foi mais elevado no fígado e no coração do que nos músculos ou brânquias.

Há diferenças geográficas na concentração de Hg no krill, indivíduos da Órcades do Sul têm níveis de Hg 5 a 7 vezes superiores aos da Geórgia do Sul. Esta variação geográfica não foi encontrada nos mictofídeos.

Como esperado, ao avaliar o Hg ao longo da cadeia alimentar, *POM* apresentou os níveis mais baixos de Hg, seguidos de uma concentração crescente no zooplâncton, lulas, mictofídeos, peixes nototenídeos e aves marinhas. Os predadores exibiram variabilidade nos níveis de Hg que correspondiam ao nível trófico das suas dietas, com níveis mais baixos de $\delta_{15}N$ correspondendo a concentrações mais baixas de Hg. Os grupos do meio da cadeia alimentar (lulas e mictofídeos) mostraram uma tendência decrescente no nível de Hg na última década, mas esse decréscimo não se refletiu nos principais predadores, para os quais os níveis de Hg foram mais altos em 2016/17 do que em 2007/08. Essa diferença entre os anos pode ser devida a uma diminuição na abundância de krill naquele ano, o que exigiria uma mudança na dieta dos predadores de krill para mictofídeos, que têm níveis de Hg maiores. Esta tese revelou detalhes da contaminação por Hg na biota do OA enfatizando o papel do transporte atmosférico na contaminação global de mercúrio. O aquecimento regional atual pode levar ao aumento da disponibilidade de Hg no AO. Os glaciares a derreter, libertam contaminantes que foram retidos após a precipitação atmosférica. As mudanças climáticas, a poluição e a crescente pressão da pesca estão a exercer uma pressão crescente sobre os ecossistemas marinhos de OA e os recursos vivos que eles contêm.

keywords Trace elements, Contamination, Pollutants, Temporal trends, Antarctica

abstract

The aim of the work presented in this thesis was to understand processes of bioaccumulation and biomagnification of mercury (Hg) in a Southern Ocean (SO) food web, and to evaluate the effect of organism size and habitat in Hg accumulation during the last decade. To do this, tissues of various species occupying different trophic levels were analysed in samples collected over various sampling years (2006/07 and 2016/17) from areas with distinctive environmental characteristics.

Different accumulation patterns were found: Antarctic krill juveniles had higher Hg concentrations than adults; in squid, Hg concentration increased with individual size in one species (*Alluroteuthis antarcticus*), decreased in another (*Galiteuthis glacialis*), and in another still, there was no obvious relationship (*Bathyteuthis abyssicola*); for myctophid fish there was a consistent increase of Hg concentration with fish size, with the exception of *Electrona antarctica* females.

Proportions of organic Hg also varied between trophic groups, from relatively low (15-37%) in krill to virtual 100% in all myctophid tissues.

Regarding Hg tissue allocation, squid muscle was the tissue that had highest Hg, followed by digestive gland and gills. Myctophids' Hg concentrations were higher in the liver and heart than in muscle or gills.

Geographic differences in Hg concentration in krill were found, with individuals from the South Orkney having Hg levels 5 to 7 times higher than South Georgia: this geographic variation was not found in myctophids.

As expected, when evaluating Hg along the food web, POM spell out had the lowest Hg levels, followed in increasing concentration by zooplankton, squid, myctophid, notothenid fish and seabirds. Predators exhibited variability in Hg levels which corresponding to the trophic level of their diets, with lower δ_{15}N levels corresponding to lower Hg concentrations. The mid food web groups (squid and myctophids) showed a decreasing trend in Hg level over the last decade, but that difference was not reflected in top predators, for which Hg levels were higher in 2016/17 than in 2007/08. This difference between years may be due to a decrease in the abundance of krill that year, which would have necessitated a change by krill predators to myctophids, a higher Hg body burden prey. This thesis revealed details of Hg contamination in SO biota, emphasizing the role of atmospheric transportation in global mercury contamination. Present-day regional warming may lead to increasing Hg availability in the SO as glacial melt is releasing contaminants previously trapped following atmospheric precipitation. Climate change, pollution and growing fishing pressure are together placing increased pressure on SO marine ecosystems and the living resources they contain.

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1. – General Introduction

World mercury problem

Mercury is a naturally occurring trace metal but is one of the most known hazardous elements (ATSDR 2019). Can be found as elemental mercury and as inorganic and organic compounds, the latter of which is the most toxic form life. Due to its affinity to thiol groups of proteins, methylmercury (an organic form of mercury) is hard for organisms to excrete and highly bioaccumulative (Bustamante et al., 2006; J. Zhang et al., 2004). There is no mechanism to degrade mercury into harmless products and it is therefore a persistent pollutant.

Natural sources of mercury are mainly rock erosion, geothermic and volcanic activity, but these make a relatively small contribution to the total global release of mercury to the atmosphere. Natural sources release between 76 to 300 Mg/year, while anthrophonic activities release about 2000 Mg/year (Streets et al., 2017). Gold mining, coal-fired power plants, production of cement, chemical and pharmaceutical industry and mercury electrodes used in the chlor-alkali industry, are the main activities that contribute to global mercury emission (Streets et al., 2017).

It was only recently, around 1950, after the Minamata major disaster, the high toxicity of this element was acknowledged, being the toxicological effects of mercury thereafter known as the Minamata disease. Acute cases of mercury poisoning in humans affected vision, hearing, sense of smell and taste, ataxic gait, dysarthria, loss of movement coordination and psychiatric disorders. Children born to mother exposed to methylmercury, showed substantial spongiosis of the cerebral cortex, a feature characteristic of the effects of Minamata disease in foetus. Chronic exposure to mercury is associated to cardiovascular diseases, anaemia, developmental abnormalities, neurobehavioral disorders, kidney and liver damage, and in some cases, cancer (Zahir et al., 2005). Mercury poising can eventually be fatal, in Minamata 1,784 humans dead directly related to mercury contamination. The poisoning of Minamata habitant was caused by the biomagnification of mercury along the food webs, mercury was intake by humans through consumption of fish with very high mercury concentrations.

Thereafter, a plan to increase the regulation for anthropogenic emission of mercury was put in place. In 2013 the Minamata Convention on mercury was created, with the goal of protecting human health and the environment from anthropogenic mercury emissions (Evers et al., 2016). Yet the levels of mercury released to the

atmosphere are still very concerning, because atmospheric mercury has a lifetime of about 1 year, making it easily transport in a global scale through atmospheric currents. Plus, deposited mercury can still cycle through soils, snow, rivers, lakes and oceans until it is absorbed by biota or deposited in benthic sediments, although this mechanism can take from decades to millennia (Streets et al., 2019).

Mercury biogeochemical cycle

The biogeochemical mercury cycle is very complex due to its multiple inter-shift phases, atmospheric, terrestrial, aquatic and biotic. This element is particularly reactive when moving in the environment. Figure 1.1 as a simplify illustration of the mercury biogeochemical cycle with special focus in the transportation to the Southern Ocean marine ecosystem.

Mercury in the atmosphere

The mercury found in the atmosphere is in the vapor phase, and it is mainly in the form of elemental gaseous mercury (Hg^0), with sometimes a small percentage of momomethylmercury (MMHg). Atmospheric mercury is relatively inert, which in turn allows this Hg^0 to have lifetime of about 1 year, making it easily transport in a global scale through atmospheric currents, including the distance Southern Ocean ecosystem. Mercury precipitates down to the surface of the planet by two mechanisms. The dry deposition associated with atmospheric particle, through the conversion from the gas phase to the particle. But the wet route is responsible for the majority of mercury deposition, due to process of mercury oxidation, with Hg^{2+}, becoming more soluble and precipitating associated with raindrops or snowflakes.

Mercury in the water column

Wet and dry depositions from the atmosphere are the main source of mercury into the surfaces waters of remote locations, like the Southern Ocean (Cossa et al., 2011). In areas closers to anthropogenic sources the aquatic system can be contaminated directedly by runoff of for instance gold mines that use mercury to extract gold from the ore.

In the aquatic environment, mercury undertakes several forms, transforming into each other depending on environmental variables [e.g., redox potential, salinity, pH, dissolved oxygen and particulate organic matter (POM); (Pereira, 1997)]. The forms $HgCl_4^{2-}$ and $HgOH^-$ dominate in the oxidation conditions, whereas sulfur-related forms (HgS_2^- and CH_3HgS^-) prevail in the reduction conditions. In the intermediate conditions, the alkyl forms of mercury, MeHgCl and EtHgCl, can most often be found (Kabata-Pendias, 2007). Higher concentrations of Cl^- ions, which form stable complexes with mercury, such as $HgCl_3^-$, $HgCl_2^-$, $HgCl_4^{2-}$, or $HgBrCl^-$, lead to increased dissolution of solid phases of mercury (Grassi and Netti, 2000). Depending on their stability, different mercury forms in the dissolved fraction can be classified as reactive or non-reactive. Reactive dissolve mercury forms include all the inorganic mercury, organic forms and dissolved elemental mercury (Hg^0), all this forms are bioavailable to be incorporated by biota. Non-reactive forms on the other hand comprises the mercury complexes that do not bond with organic matter, although it can also be availed to biota, the processes is slower and less efficient than when compared with reactive forms (Firzgerald et al., 1997).

Methylation is one of the most biologically relevant processes in the mercury biogeochemical cycle, since methylated mercury is the most toxic form of mercury and is highly bioaccumulative along the marine food webs. Methylation can, chemically, result from abiotic and biotic processes, although in environment methylation is only possible through biotic transformation. Sulfate-reducing bacteria are the most likely facilitators of the mercury methylation process. This bacteria transform the Hg^{2+} into the highly reactive dimethylmercury (DMHg), that in it turn will be decomposed into monomethylmercury (MMHg). The methylation processes can be influenced by environmental factors such as pH, temperature, salinity, redox potential, the presence of sulphate or sulphide, availability of organic carbon (Beckvar et al., 1996). In environments with relative low satiny (fresh water systems) the predominant form of methylmercury would be a hydroxide CH_3HgOH, on the other hand, in marine ecosystems (high salinity) mercury forms complex with chloride forms will dominate ($HgCl_3^-$ and $HgCl_4^{2-}$).

The reduction of Hg^{2+} back to elemental gaseous mercury and the degradation of organic mercury compounds are natural mechanisms of mercury detoxification. Specific bacteria and able to grow and develop in the presence of mercury. This strain of bacteria are resilient to the harmful toxicity of Hg^{2+} due to the presence of

organomercurial lyase show also show resistance to organic forms of mercury (Walsh et al., 1988). Lyase and mercury reductase are the enzymes responsible for the resistance to organic forms. Hg^{2+} reduction can also take place without the presence of microorganism, however this degradation of the organic forms by an abiotic process is extremely slow and can be neglected in the overall mercury cycle in the ecosystem (Baltisberger et al., 1979). So after being transform it back to Hg^0, either by a biotic of abiotic process, due to its low water solubility and high vapor pressure, Hg^0 can be released back to the atmosphere. The exchange between the atmospheric and the aquatic compartment is an active and dynamic cycle. Hg^{2+} is deposit (dry or wet) in the water column from the atmospheric transported Hg^0, while aquatic Hg^{2+} undertakes biotic or abiotic reduction back to Hg^0 and resealed to the atmosphere.

Summarizing the mercury cycle in the water column, starts with the oxidation and deposition of atmospheric mercury into the sea surface. There mercury is either reduced and re-emitted again to the atmosphere or bound to mineral or organic compounds, or adsorbed/absorbed by phytoplankton in the photic zone. Sinking inorganic mercury can be biomethylated in the water column. Contrariwise demethylation process also occur, so methylmercury concentrations reflect the net effect of methylation – demethylation. Deposition into sediments constitutes the final stage of mercury sinking in the open ocean environment.

Mercury in benthic sediments

Mercury in sediments derives essentially from the deposition of particulate organic matter. The fixation of mercury in sediments depends on the presence of iron and manganese oxides and oxyhydroxides, sulfides and organic matter (Canário et al., 2003). Also the form in whish mercury is available depends on pH, ionic strength and redox-potential that will control the mercury absorption, adsorption and retention (Ramalhosa, 2002).

In the anoxic conditions, the main forms of mercury are in HgS, HgS_2H_2 and CH_3HgS. The deposition rates of this forms of mercury is correlated with the content of organic matter and iron oxide ions, as its solubility increases with the content of sulfite ions (Boszke et al., 2002). Regarding organic mercury forms, abiotic methylation can be promoted by the organic matter found in the anoxic layers of sediments.

The redeposition of mercury from benthic sediments to the water column can occurs as result of diffusion, but the main factor is the activity of benthonic biota. As the oxic conditions in the surface layer of sediments limits the upward mercury efflux from the anoxic layers. Considerable amount of methylmercury can exit in the pore waters of anoxic sediments layers, but the superficial oxic layer works like a geochemical barrier, promoting bacterial or catalytical demethylations, preventing the diffusion of methylmercury to the water column (Gagnon et al., 1996). This oxic layer is crucial to reduce the amount of methylmercury available to be incorporated by the benthonic fauna. Although mercury trapped in the sediments can be remobilized to the water column, through desorption processes (Cardoso et al., 2008), due to the effect of benthonic biota and primarily due to event of high turbidity (like strong upwelling events (Marshall and Speer, 2012)).

Mercury in aquatic biota

Mercury can be incorporated by biota by two main pathways, the dissolved fraction through direct adsorption to body surfaces (e.g., fish and squid gills), and particulate intake (Wang and Fisher, 1999). The proportion from each route will vary among species and with the bioavailability of mercury in water column and diet. Nondietary pathways of mercury accumulation refer to the increment of mercury in the dissolved state, by biota from the water column. This accumulation processes occurs mainly through passive diffusion at the surface of biological membranes [gills and skin; (Monteiro et al., 1996)]. The adsorptive properties of mercury also depend on the water chemistry (pH, salinity) that also influence mercury transformation processes (Laporte et al., 1997).

Mercury is a non-essential element as it does not have a role in any metabolic activity. Being, however, very toxic to organisms, even at low concentrations (Zahir et al., 2005), in some cases can have harmful effect to public health, as in the previous example of the Minamata disaster. The risk is particularly high for populations where fish plays and important role of their alimentation. The mercury limits for fish to be sold for human consumption were set at 0.5 mg kg^{-1} wet weight by several organizations (World health organization; European Food Safety Authority; United States Food and Drug Administration). However, for large predatory fish like, sharks, swordfish, tunas, and for the specific case of the Southern Ocean, toothfish, the limit was set at 1 mg kg-

$^{-1}$ ww. The allowance for larger fish to be sold with higher mercury levels, was only settled to not affect the fishery industry, as this fish would typically have concentrations higher than 0.5 mg kg^{-1} ww, due to the phenomenon of bioaccumulation and biomagnification. This legislation does not take in account the ecotoxicological risks associated with the ingestion of higher quantities of mercury.

Bioaccumulation is the process by which a substance concentration increases in an organism (Lacoue-Labarthe et al., 2009). Bioaccumulation occurs when the intake of mercury is higher that the excretion rates and detoxification processes. It starts with mercury intake, either by direct contact, by respiration (through the gills) or by assimilation (ingestion) and subsequence accumulation in tissues or organs. Benthonic species are special vulnerable to mercury accumulation due to their feeding ecology and living grounds and so they are more exposed to this contaminant. Specially species that take shelter in the benthic sediments as they will be in direct contact and ingesting mercury deposited and bioavailable in the anoxic zone of the sediments. Benthonic detritivores organisms will have a major intake of mercury through the dietary pathway as they feed in the sediment organic matter (Zhong and Wang, 2006).

Methylmercury, the major organic form of mercury found in biota, as a high affinity to proteins (Bustamante et al., 2006), being therefore easily accumulated in biota and biomagnified along food webs (Chouvelon et al., 2012; Coelho et al., 2010). Consequently levels of mercury and proportion of organic mercury tend to increase with trophic level and individual longevity. In higher trophic levels, some species can present proportion of almost 100% organic mercury (Bloom, 1992). The increment of the organic fraction is due to the fact that inorganic mercury is excreted with more ease and also the organic form is the one that is mostly transported in trophic transference.

When the bioaccumulation process is mainly due to intake of contaminant from the environment, the process can be called bioconcentration (Morel et al., 1998), on the other hand if the accumulated substance intake is mainly through the dietary path it is called biomagnification (Bloom, 1992). Biomagnification, is the increasing concentration levels of a determined substance, like mercury, in organisms at along the trophic steps. The persistence nature of mercury, which as low degradation and excretion rates, promotes the progressively greater concentration of this contaminant along food web (Bargagli et al., 1998; Gray, 2002). So due to the biomagnification

process, higher mercury levels in an organism will exceed the concentration of its of its prey (Bloom, 1992).

There are several substances that bioaccumulate but only a few have biomagnification capabilities. The insecticide DDT was the first substance to be describe as capable of bioaccumulate through food webs (M. S. Evans et al., 1991). The bioaccumlative substance are divided in two main groups: 1) persistent organic pollutants (POPs), this novel organic substances are not easily degraded or excreted, because organism lack previous exposure and have thus not developed specific detoxification and excretion processes for this contaminants; 2) trace metals namely mercury, arsenic, cadmium, copper, lead and others can also bioaccumulate. Some organisms will have excretion mechanisms for these elements, although when the

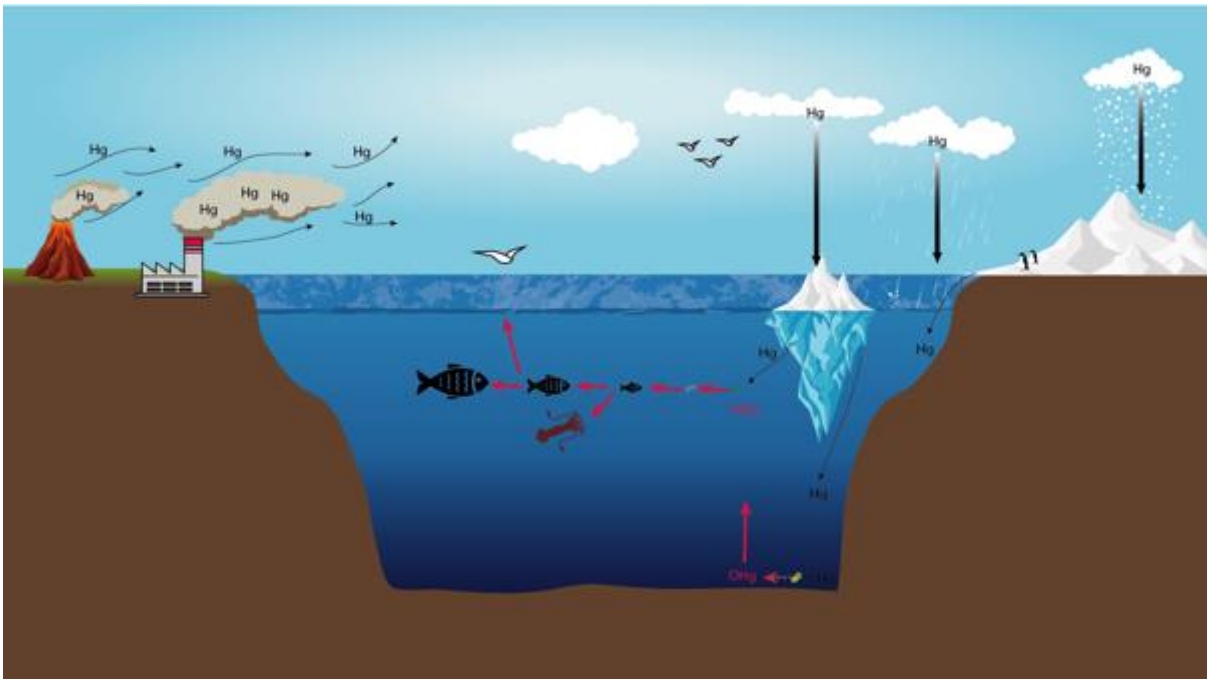

Figure 1.1 - Schematic representation of mercury biochemical cycle.

intake of the contaminate is higher than the detoxification rate, it will be available to magnify to the next trophic level.

Methylmercury will play a fundamental role in the phenomenon of bioaccumulation and biomagnification in organisms. As mercury increases at a define rate, depending on the ecosystem and species characteristics (like growth rates and number of trophic links), with every trophic step (Lavoie et al., 2013) – biomagnification. Plus the well know relation between mercury and size or age, where concentrations increase with body mass and older individuals normally have higher concentrations as they accumulate mercury along their life – bioaccumulation. There are some

exceptions, as some species found mechanism to reduce their levels of contamination. This detoxification strategies can be the synthesis of metal-binding proteins that reduce the toxicity of mercury (Amiard et al., 2006) or by the excretion of mercury with feathers or with the production of eggs (Pedro et al., 2015).

Mercury in Antarctica and the Southern Ocean

Even though the Antarctic continent became geographically isolated about 30 Ma ago by the formation of the Drake Passage, which separates the Antarctic Peninsula from South America [Figure 1.2 (Convey et al., 2009)], contaminants like Hg still reach this remote ecosystem.

Global industrialization and particularly the rapid development of Southern hemisphere countries in the last decades had a huge impact in the Antarctic environment (Bargagli, 2008). Contaminants are transported from its source to the "pristine" Antarctica by oceanic but mainly by atmospheric currents (Beyer and Matthies, 2001).

Total mercury concentrations in the surfaces waters increased towards the south pole reaching a maximum of 44.4 pmol L^{-1} below the sea ice (Cossa, 2013). Proportion of methylmercury in relation to total mercury are higher in the Southern Ocean, up to 78% (Cossa, 2013) than in other oceans, up to 10% in the Atlantic (Mason et al., 1998; Mason and Sullivan, 1999) and about 15% in the Pacific Ocean (Gill and Fitzgerald, 1988; Laurier et al., 2004). The distinctive atmosphere with the single Southern Ocean plus the sea ice, creates a particular combination that can explain why does the remote Antarctic Ocean have some of the highest concentrations of methylmercury and organic form of mercury in open waters (Cossa et al., 2011; Mason et al., 2012). The presence of halogens in the atmosphere promotes oxidation and deposition of atmospheric mercury to the Southern Ocean water or to be incorporated in sea ice or glaciers. This inorganic mercury sinks to the hypoxic zone, richer in organic matter, supply methylation bacteria that transform mercury into methylmercury. In the Southern Ocean, as expected, total mercury concentration are higher at the deeper water than at the surface or intermediate waters (Cossa et al., 2011). Methylmercury concentrations are low in the surface mixed layer and increased with depth to an intermediate maximum. As reported for other oceanic water columns (Heimbürger et al., 2010; Mason and Fitzgerald, 1990), the highest methylmercury

levels are measured in the oxygen minimum zone (Cossa et al., 2011). Upwelling of deep waters will transport some of the methylmercury to the pelagic zone to be bioavailable to be incorporated in the food web (Cossa, 2013).

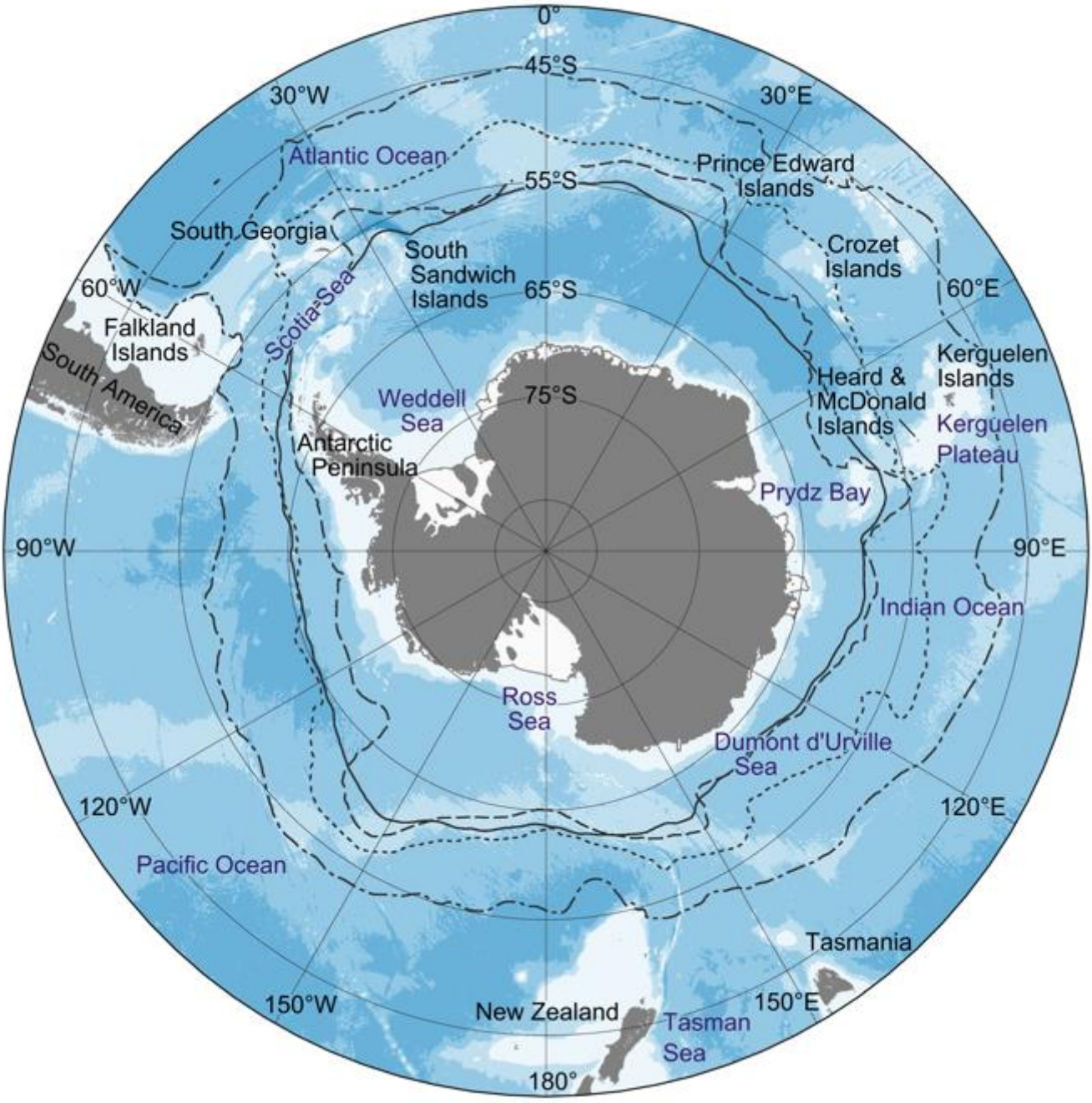

Figure 1.2 - Map of Antarctica and the Southern Ocean [Xavier et al. (2016)].

Southern Ocean food web

The Southern Ocean is one of the most productive areas of the planet, producing energy that will be distributed across all the ocean through the thermohaline circulation. The cold water can dissolve more oxygen (up to 1.8 times more than tropical waters), the upwelling currents bring up nutrients to the microalgae and the long periods of light (during the austral summer) provide the favourable conditions for a very high primary production (A. Clarke et al., 2008; Xavier and Peck, 2015). The major limiting factors for the microalgae growth in the Southern Ocean is the micronutrient iron (Kaiser and Attrill, 2011). Due this limitation, micro algae blooms tend to occur next to iron sources, like melting glaciers or areas with strong upwelling currents.

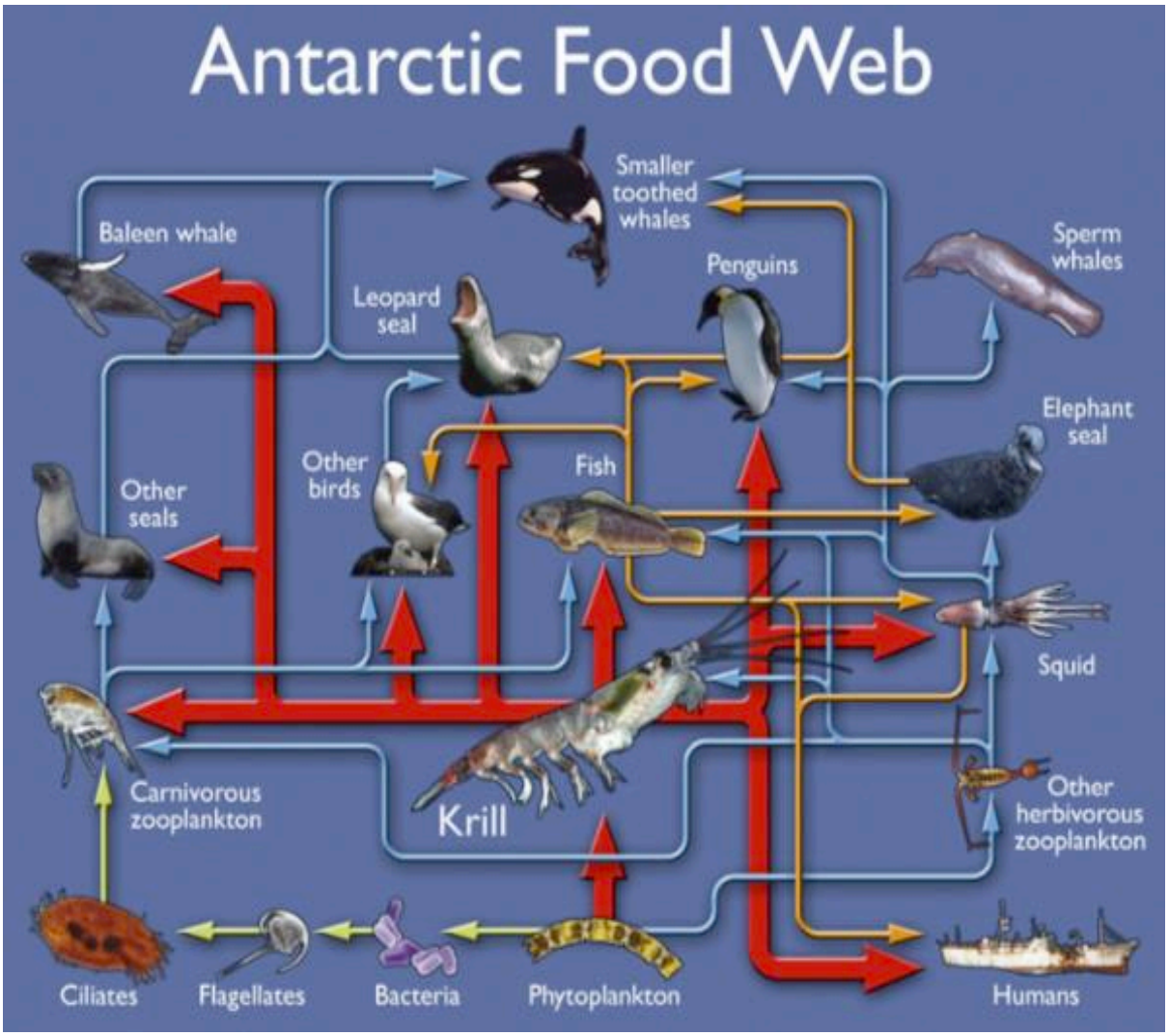

Figure 1.3 - Schematic representation of the Antarctic marine food web (Xavier and Peck 2015).

Figure 1.4 - Antarctic krill, *Euphausia superba.*

Polar food webs are normally less complex than lower latitudes ones (Lavoie et al., 2013). In the Southern Ocean (Figure 1.3), one species is mostly responsible for the trophic step between primary producers and top predators, Antarctic krill *Euphausia superba* (Murphy et al., 2007). Antarctic krill (Figure 1.4) is a key species in this ecosystem, it is the most abundant species, stocks are estimated around 379 million tonnes (Atkinson et al., 2009). It is eaten by a wide range of predators being the main prey for many vertebrates [e.g.: Crabeater seals, *Lobodon carcinophaga* > 95% by mass (Adam, 2005); minke whales, *Balaenoptera acutorostrata* > 95% (Armstrong and Siegfried, 1991); Macaroni penguins, *Eudyptes chrysolophus* > 89.8 (Croxall et al., 1999); black-browed albatrosses *Thalassarche chrysostoma* > 60% (Xavier et al., 2003c); Antarctic fur seals *Arctocephalus gazella* > 50%; (Xavier and Peck, 2015)]. But this dependence in one species means that the Southern Ocean food web is fragile, as any changes in Antarctic krill abundance or distribution can affect all the trophic levels (Murphy et al., 2007). Indeed there are already evidences that in “bad” Antarctic krill years, low abundance, the reproductive successes of predators is highly affected (Fielding et al., 2012; Xavier et al., 2003c; 2003a). There are, of course, other zooplankton species in the Southern Ocean that provide energy to higher trophic levels in such “bad” Antarctic krill years (Murphy et al., 2007), such as the hyperiid amphipod

Themisto gaudichaudii but apparently not in higher abundance to sustain predators populations (Xavier et al., 2017).

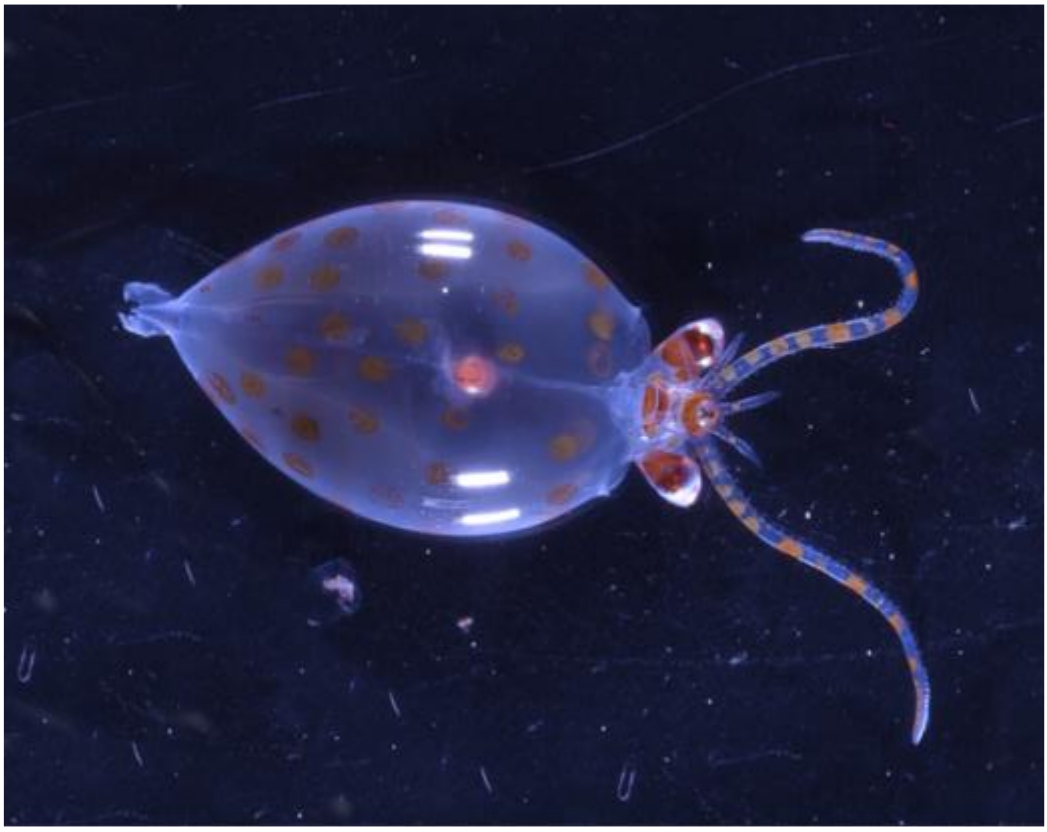

Figure 1.5 - Southern Ocean squid, *Galiteuthis glacialis.*

Mid trophic levels in the Southern Ocean food web also have an alternative energy path to predators, in which second consumers is mainly constituted by cephalopods (Figure 1.5) and mesopelagic fish [e.g. myctophids (Murphy et al., 2007)]. Antarctic squid feed mainly on zooplankton (Antarctic krill or other crustaceans), small mesopelagic fish or even other smaller squid. While squid are typically pelagic, octopods occupy the benthic ecosystem, feeding mostly on benthic crustaceans, some fish and even other cephalopods (Matias et al., 2019). These organisms are predated by several species including fish, penguins, albatrosses, seals and whales (Mikhalev et al., 1981; Split, 1995; Xavier and Cherel, 2009).

Myctophidae or lanternfish (Figure 1.6) are the largest and most important species of mesopelagic fish of the ocean, either by total mass [estimated mass of 70-200 Mt (Catul et al., 2010)] or species diversity. Myctophids have the second most important role in the energy transfer across the food web, after Antarctic krill. The different species prey on wide range of crustaceans (Antarctic krill and others) amphipods, copepods and other zooplankton (Lourenço et al., 2017; Pakhomov et al., 1996; Saunders et al., 2018). Myctophids are prey of large predatory fish (Fenaughty

Figure 1.6 - Southern Ocean myctophid, *Electrona antarctica.*

et al., 2003; Stevens et al., 2012), cephalopods (Cherel and Duhamel, 2003; Olson and J. W. Young, 2006; Rodhouse et al., 1992a), marine mammals (Newland et al., 2011) and seabirds (Xavier et al., 2003c).

Penguins, seals, whales and albatrosses (Figure 1.7) are the top predators of the Southern Ocean, as some fish species (e.g.: Antarctic toothfish, *D. mawsoni*) and squid (e.g.: Colossal squid, *Mesonychoteuthis hamiltoni*) species. These predators feed on a wide range of prey, from Antarctic krill (main source of energy), fish, cephalopods, to even other predators. Killer whales, *Orcinus orca* feed on fish, squids, penguins and seals, some bird species like the Brown skua, *Stercorarius antarcticus* and Northern, *Macronectes halli*, or Southern Giant petrel, *Macronectes giganteus* prey other birds chicks, seal cubs and carrion (Reinhardt et al., 1997; Xavier and Peck, 2015).

Most predator species have a circumpolar distribution, but they have different habitat preferences. Sea ice plays also an important role in the distribution of predators. Emperor penguins, *Aptenodytes forsteri*, Antarctic petrels, *Thalassoica antarctica*; Weddell seals, *Leptonychotes weddellii* are some of the species that live near the Antarctic continent, so on the sea ice/glaciers regions. Others species like the Wandering albatrosses, *Diomedea exulans*; Gentoo penguin, *Pygoscelis papua* live in

the open oceanic areas to north closer to the Antarctic Polar front (Xavier and Peck, 2015).

Figure 1.7 - Black browed albatross, *Thalassarche melanophris.*

Mercury in the Southern Ocean food web

Within the Southern Ocean food web, marine organisms, whether algae, invertebrates or vertebrates, can accumulate mercury in their tissues and its concentrations were found to be much higher than environmental levels, principally in top predators (deMoreno et al., 1997; Tavares et al., 2013). Mercury concentrations increase along successive trophic levels, from primary producers to top predators, indicating that mercury is highly bioaccumulative and it easily biomagnifies along food webs (Figure 1.3). It is absorbed by micro algae, that then are eaten in high quantities by crustaceans, squid or fish, that in its turn are eaten and transfers their mercury burden to bigger fish, seals, birds, or other top predators, a clear relationship between mercury levels and trophic position (Lavoie et al., 2013). This phenomenon of biomagnification of mercury had been consistently observed in marine food webs (Lavoie et al., 2013), although this process had not been studied for the Southern Ocean food web, on a multi trophic level perspective.

Primary producers incorporated mercury mainly through absorption, this proportion decreases along the trophic levels, with more mercury intake from the diets. Mercury incorporation also varies between tissues, with some organs having higher concentrations than others. One of the organism mechanisms to avoid high mercury levels in vital organs is to allocate this element in tissues that are excreted, like hair, feathers and eggs, or to distribute mercury to less fragile tissues, like muscle.

Southern Ocean seabirds have some of the highest mercury levels ever registered in biota, with feather of Wandering albatross Diomedea exulans getting as high as 95 $\mu g\ g^{-1}$ (Bustamante et al., 2016; Cherel et al., 2018; Tavares et al., 2013), this species is a top predators and has high longevity, putting it at risk of high mercury contamination levels (Goutte et al., 2014a). Other seabirds species have also relative high levels (Cherel et al., 2018), varying according to species-specific diets and trophic levels. This very high mercury levels in seabirds are affecting populations, in some cases the concentrations are so high that they stop reproducing (Goutte et al., 2014b). Southern Ocean organisms have very slow growth rates, mainly due to the effect of cold waters, slow metabolic activity will therefore influence the rates of mercury excretion, meaning that generally polar food web would have higher mercury bioaccumulation factors than food webs from lower latitudes (Lavoie et al., 2013).

Thesis Outlines and Objectives

This thesis is structured in four science chapters, each one composed by one manuscript submitted, in preparation, under review or already published on major scientific journals and a general discussion. Each chapter aims to answer group specific questions regarding mercury distribution in Southern Ocean biota and chapter 6 does an overall view of mercury accumulation in all the groups interlinking it with stable isotopes analysis to define the pathways of this contaminate across the food web.

The main general objective of my thesis was to first describe the mercury levels in several Southern Ocean species, across different taxonomic groups. To after be able to identify mercury accumulation patterns, either related with biological factors, like maturity, sex or size, or with abiotic factors like habitat and sampling year. With the final goal of evaluate the pathway of mercury accumulation in a Southern Ocean ecosystem.

Chapter 3. Spatial variability in total and organic mercury levels in Antarctic krill Euphausia superba across the Scotia Sea

In this chapter total and organic mercury were analysed and compared in Antarctic krill from three different locations: the South Orkney Islands, an Antarctic island group which experiences winter sea ice, South Georgia, a sub-Antarctic island free of sea ice, and the Antarctic Polar Front, a transition area from the Southern Ocean to the Atlantic Ocean with warmer waters. And to asses if mercury bioaccumulated differently among life stages (eggs, juveniles, adults) and sexes (males and females).

The hypothesis tested in this chapter were: Antarctic krill has the same mercury concentration across different habitats; Antarctic krill accumulates mercury equally across life stages and sex.

In this chapter, that produced the following scientific article, I did all the sampling collection (except for the females form JR177 (in 2007/08) that were collected by G. Tarling and G. Stowasser. All the laboratorial analysis were performed by me. The writing and preparation of the manuscript was made by me, with comments and suggestion from all the co-authors.

Seco J, Xavier JC, Coelho JP, Pereira B, Tarling G, Pardal MA, Bustamante P, Stowasser G, Brierley AS, Pereira ME (2019) Spatial variability in total and organic mercury levels in Antarctic krill *Euphausia superba* across the Scotia Sea. Environ Pollut 247:332–339. doi: 10.1016/j.envpol.2019.01.031

Chapter 4. Mercury levels in Southern Ocean Squid: variability over the last decade

In the second chapter total and organic mercury of different tissues (muscle, gills and digestive gland) of 8 Southern Ocean squid from different sampling years (from 2006/07 to 2016/17) were analysed. The main goals of this chapter were: 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).

The hypothesis tested in this chapter were: Different species of Antarctic squid have similar levels of mercury; Mercury in Antarctic squid increases with size; Mercury in Antarctic squid is allocated equally across tissues(muscle, digestive gland and gills); Mercury in Antarctic squid increased over the last decade.

In this chapter, that produced the following scientific article, I collected the samples on JR16003, samples from JR161, JR177, JR200 and SG13 were previously collected by collaborators. All the laboratorial analysis were performed by me. The writing and preparation of the manuscript was made by me, with comments and suggestion from all the co-authors.

Seco J, Xavier JC, Brierley AS, Bustamante P, Coelho JP, Gregory S, Fielding S, Pardal MA, Pereira B, Stowasser G, Tarling GA, Pereira ME (2020) Mercury levels in Southern Ocean squid: Variability over the last decade. Chemosphere 239:124785. doi: 10.1016/j.chemosphere.2019.124785

Chapter 5. Main drivers of mercury levels in Southern Ocean Lantern fish Myctophidae

In the third chapter, total and organic mercury concentrations were measured in different tissues of several myctophids species in 10 years apart sampling years. With the main goal to evaluate spatial, temporal, inter-specific and ontogenetic patterns in this group.

The hypothesis tested in this chapter were: Different species of Antarctic myctophids have similar levels of mercury; Mercury in Antarctic myctophids increases with size; Mercury in myctophids squid is allocated equally across tissues (muscle, liver, heart and gills); Mercury in Antarctic myctophids increased over the last decade.

In this chapter, that produced the following scientific article, I collected the samples on JR15004 and JR16003, samples from JR177 were previously collected by collaborators. All the laboratorial analysis were performed by me. The writing and preparation of the manuscript was made by me, with comments and suggestion from all the co-authors.

Seco J, Xavier JC, Brierley AS, Bustamante P, Coelho JP, Saunders RA, Ferreira N, Gregory S, Fielding S, Pardal MA, Pereira B, Stowasser G, Viana T, Tarling GA, Pereira ME (2020) Main drivers of mercury levels in Southern Ocean Lantern fish Myctophidae. Environmental pollution. doi: 10.1016/j.envpol.2020.114711

Chapter 6. Temporal variability of mercury biomagnification in the Southern Ocean food web

In the final and fourth chapter, the present chapter investigated the relationships between mercury concentrations and $\delta_{13}C$ and $\delta_{15}N$ of 12 species of zooplankton, 10 species of pelagic fish (myctophids) and squid, 6 species of benthopelagic fish and 8 species of seabirds. The majority of the species were captured in two non-consecutive, 10 years apart, sampling years. The goals are, 1) to describe the mercury distribution along the Southern Ocean food web and 2) to evaluate if there are any variations on the mercury pathway to top predators over the last decade.

The hypothesis tested in this chapter were: Mercury levels are higher in species with higher $\delta_{15}N$; Mercury levels on the Southern ocean food web increased over the last decade.

In this chapter, that produced the following scientific article, I collected the samples on JR16003, samples from JR177 and seabirds samples were previously collected by collaborators. All the laboratorial analysis were performed by me. The writing and preparation of the manuscript was made by me, with comments and suggestion from all the co-authors.

Seco J, Xavier JC, Bustamante P, Coelho JP, Saunders RA, Ferreira N, Gregory S, Fielding S, Pardal MA, Pereira B, Stowasser G, Viana T, Tarling GA, Pereira ME, Brierley AS (in preparation) Temporal variability of mercury biomagnification in the Southern Ocean food web

2. – Study area and Mercury determination

Study area – Scotia sea

Within the Southern Ocean, the Scotia Sea is located east of the Antarctic peninsula, circumscribed by South Georgia, South Sandwich Islands and the South Orkneys, also known as the Scotia arc (Figure 1.2). This sea is a transitional water mass, between the South Atlantic Ocean to the Scotia arc, having therefore a latitudinal gradient of water temperature. The Scotia sea is an heterogenic ecosystem, as it is crossed by the Antarctic circumpolar current, to the south of the front, waters are colder and have the characteristic winter sea ice. To the north waters get warmer to lower latitudes, getting to the transition to sub-Antarctic waters in the Polar front.

It is a small portion of the Southern Ocean, comprising only 0.4% (900 000 km²) of this vast ocean. Although, it is one of the most productive areas of the Southern Ocean (Holm-Hansen et al., 2004). As mentioned above, the lack of iron, an essential micronutrient for photosynthesis, is a grow limitation all across the Southern Ocean (Martin and Fitzwater, 1988), however when this element is available productivity arises. This is the case of the Scotia Sea with extensive microalgae bloom supported until late summer by macronutrients and iron resupplied from the shelf edge, shelf–sediment interactions and vertical mixing of deep waters (Korb et al., 2008). Being this region the largest contributor for the carbon dioxide in the Southern Ocean (Schlitzer, 2002). Due to the high productivity this region of the Southern Ocean houses big populations of several Antarctic top predators, like whales, seals, penguins, flying birds and predatory fish, looking for a forage hotspot. As reported before, fisherman follow predators for fishing hotspots (Bertrand et al., 2007), this is also the case in the Scotia Sea (Murphy et al., 2007). It is one of the main fishing areas for all the exploited species from the Southern Ocean, Antarctic krill, *Euphausia superba*, Antarctic toothfish *Dissostichus mawsoni*, Patagonian toothfish *Dissostichus eleginoides*, and Mackerel icefish *Champsocephalus gunnari* (Agnew, 2004).

Mercury determination

There are several processes and equipment that are able to quantify mercury [e.g., (Bendl et al., 2006; García et al., 1994; Rahman et al., 2000; Uthe et al., 1970)]. In the present thesis, total mercury will be quantified through atomic absorption spectroscopy after thermal decomposition of samples, using a LECO AMA-254

(Advanced Mercury Analyser). This efficient mercury specific methodology, requires minimum sample handling to run the analysis, as non-prior chemical digestion or sample pre-treatment is needed. Samples just need to be frizzed dried (to remove the water) and homogenised to powder. Tissue sample are weight with on a precision scale, placed in a pre-cleaned nickel combustion boat and inserted in a quartz combustion catalytic tube (cobalt oxalate and a mixture of manganese oxide, cobalt and calcium acetate), where the sample is dried at 120 ºC during 10 seconds. After the short desideration processes, samples are combusted at 680-700 ºC during 150 seconds with a constant flux of pure oxygen (200mL min^{-1}). The combustion gases are removed by a Mn_3O_4 and CaO catallactic column. The mercury vapor (Hg_0) is then collected by a gold amalgator and after a delay time of 45 seconds the amalgator is heated to 900ºC to remove the mercury that is transported to a cuvette at 120 ºC prior to the analysis by atomic absorption spectroscopy using a silicon diode detector at 253.6 nm [Figure 2.1; (Costley et al., 2000)]. The two LECO AMA-254 at the laboratory have different internal calibration curves. One equipment has a calibration range from 0.1 to 30 and 100 – 500ng of mercury. The second is equipped with a more sensitive optic cell, with a calibration range from 0.1 to 8 and 10 -200ng of mercury.

Quantification of organic mercury in biological samples is preformed via an acid

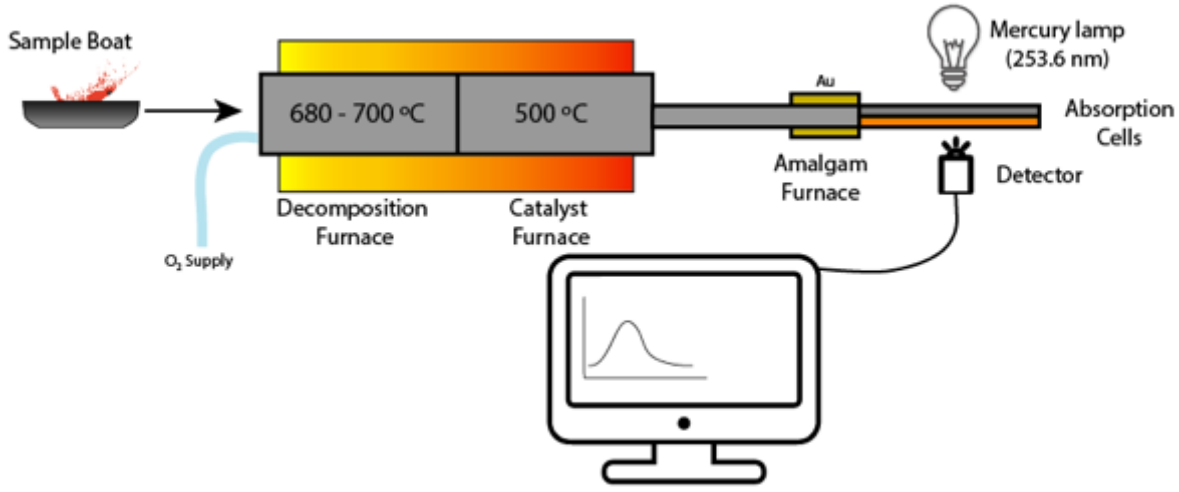

Figure 2.1 - Schematic representation of the AMA-254 atomic absorption spectrometer.

leaching with a mixture of KBr, H_2SO_4 and $CuSO_4$ prior to an extraction with toluene (Válega et al., 2006). At room temperature, about 200mg of sample are weight into 50

mL polypropylene centrifuge tubes, where after 5 mL of KBr (18%) in H_2SO_4 and 1 mL of $CuSO_4$ (1 mol L^{-1}). After a leaching period of 15 minutes, 5 mL of toluene are added, followed by 15 minutes of vigorous agitation, to extract the organic mercury fraction. Samples are centrifugated during 15 minutes at 4000 rpm, to separate the organic phase, from where 3 mL are pipetted and stored in a glass flask. The extraction process is repeated once more, but in the second run, 5 mL of the organic phase are collected, at the end a total of 8 mL of the organic extract are store in the flask. The organic mercury compound retained in the toluene are back-extracted into 5 mL of an aqueous sodium thiosulphate solution 0.002 mol L^{-1} ($Na_2S_2O_3$). Quantification of liquid aliquots is carried out in the aqueous sodium thiosulphate fraction, using the LECO AMA-254. The methodology in the LECO AMA-254 will be similar to the total mercury analysis, with the only difference being on the drying time, that for liquid samples will be given in seconds by the volume (μL) to be analysed times 0.7 (e.g.: a volume of 0.5μL will need a drying time of 350 seconds). This methodology was described my Válega et al. (2006). Although this method does not specify different forms of organic mercury, dimethylmercury or ethylmercury have been detected rarely in sediments and biota, since they are readily lost from the aquatic systems and are not considered highly bioavailable for uptake by aquatic organisms, so this method will mainly quantify methylmercury. Even tough that virtually all the mercury quantified with this technic is 100% methylmercury, the analytical method employed is not specific to methylmercury and therefore we cannot use another term than organic mercury.

Quality assurance

To avoid possible errors that could have been induced during the collection of samples, preservation, handling and chemical procedures (in the case of organic analysis) and in during the mercury analysis and to ensure that the presented mercury concentration reflected the its true value 3 quality assurance producers always have to be take in account: precision, accuracy and limits of detection

Precision is assessed through the repeatability of replicate analysis. Acceptance criteria is established by a coefficient of variance inferior to 10%. The coefficient of variance is given by the equation [(standard deviation/ mean) x 100]. All the samples will be analysed in duplicate, if the coefficient of variance is higher than 10%, samples will be re-analysed until the acceptance level is reached.

To determine the accuracy, certified reference materials (CRM) as similar as possible to the type of matrix to be analysed. Certified reference materials are described in table 2.1. The internal calibration of the equipment (LECO AMA-254) is checked on a daily basis using the certified reference materials. The mercury recovery is given by the equation [(Obtained Hg/ Certified Hg) x 100]. Final results will be corrected according to the daily recoveries obtain from CRM, this procedures avoids the daily variation on the equipment. This normalization of the mercury concentrations allows the comparison between samples analysed in different days (different recuperation percentages). Catalytic tube will need to be replaced when the CRM determined values are no longer within the certified confidence interval. As the internal calibration curves of the AMA-254 provide different recovery efficiency, CRM mercury levels need to be within the same range as the samples that are going to be analysed. Plus blank runs were performed between each sample or CRM analysis, being repeated if in some cases there was a memory reflection of the previous analysis.

Analytical limit of detection is considered the lowest amount of a substance or element that can be statistically distinguished from the absence of that substance or element (a blank value) (MacDougall and Crummett, 1980). Limits of detection are determined differently depending on the analytical method to be used. Detection limits are obtained through the average of blank measurements times 3 standard deviations.

Table 2.1 - Total and Methylmercury in $\mu g\ g^{-1}$, respective recuperation percentages source of certified reference materials (CRM) available with similar biological matrix. na - non applicable.

CRM	Matrix	Total mercury	Recuperation (%)	Methylmercury	Recuperation (%)	Source
TORT-2	Lobster hematopancreas	0.27	84 – 90	0.152	78 – 82	National Research Council of Canada
TORT-3	Lobster hematopancreas	0.292	82 – 102	0.137	85 – 105	National Research Council of Canada
NIST 2976	Mussel tissue	0.061	78 – 92	0.028	na	National Institute of Standards and Technology
ERM-CE278K	Mussel tissue	0.071	87 – 97	na	na	Joint Research Centre
DORM-4	Fish protein	0.412	83 – 104	0.355	91 – 107	National Research Council of Canada
ERM-BB422	Fish protein	0.601	91 – 105	na	na	Joint Research Centre

3. – Spatial variability of mercury levels in Antarctic krill *Euphausia superba* across the Scotia Sea

Abstract

Total and organic mercury concentrations were determined for males, females and juveniles of Euphausia superba collected at three discrete locations in the Scotia Sea (the South Orkney Islands, South Georgia and the Antarctic Polar Front) to assess spatial mercury variability in Antarctic krill. There was clear geographic differentiation in mercury concentrations, with specimens from the South Orkneys 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; 0.015 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 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 to 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.

Key words: Food-web; Eggs; Organic Mercury; Southern Ocean, Antarctica

Introduction

Mercury contamination in the environment has been acknowledged as a global problem, and 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 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 consequences at the population level (Goutte et al., 2014b; 2014a). Wandering albatrosses 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 379million 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 Crabeater seals, *Lobodon carcinophaga,* feed almost exclusively (>95 %) on Antarctic krill (Adam, 2005; Armstrong and Siegfried, 1991; Croll and Tershy, 1998; Dimitrijević et al., 2018; 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 (Dimitrijević et al., 2018; Xavier et al., 2018b) 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; Tou et al. 2007; CCAMLR 2017).

In the context of environmental change (Constable et al., 2014; Cossa, 2013; Gutt et al., 2015), it is important to evaluate the impact of contaminants like mercury, particularly in a remote and presumably less impacted environments such as Antarctica with the associated risk to Southern Ocean top predators. This approach will contribute to a more in-depth knowledge of mercury bioaccumulation dynamics, in an effort towards the preservation of Antarctica ecosystems into the future (Rintoul et al., 2018; Seewagen, 2010). Despite the major role of Antarctic krill in the Southern Ocean, there are only a few studies reporting mercury concentrations in this region (Bargagli et al., 1998; Brasso et al., 2012b; Locarnini and Presley, 1995; Moren et al., 2006). Indeed, to my knowledge, no studies have ever analysed organic mercury content in Antarctic krill. Assessing the levels of organic mercury in such an important prey as Antarctic krill is crucial to better understand the pathway of this contaminant through Southern Ocean food webs. In this context, this study compares the total and organic mercury of Antarctic krill from three different locations: the South Orkney Islands, an Antarctic island group which experiences winter sea ice (Murphy et al., 1995); South Georgia, a sub-Antarctic island free of sea ice (A. D. Rogers et al., 2015); and the Antarctic Polar Front, a transition area from the Southern Ocean to the Atlantic Ocean with warmer waters (Dong et al., 2006). Under this context, differences among life stages (eggs, juveniles, adults) and sexes (males and females), were assessed and interpreted in the scope of a possible biomagnification of mercury in the Antarctic trophic web.

Material and methods

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 (Figure 3.1) 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 m^2 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 -20ºC (JR15004 and JR16003) or on vials in ethanol (for JR177) (Fort et al., 2016).

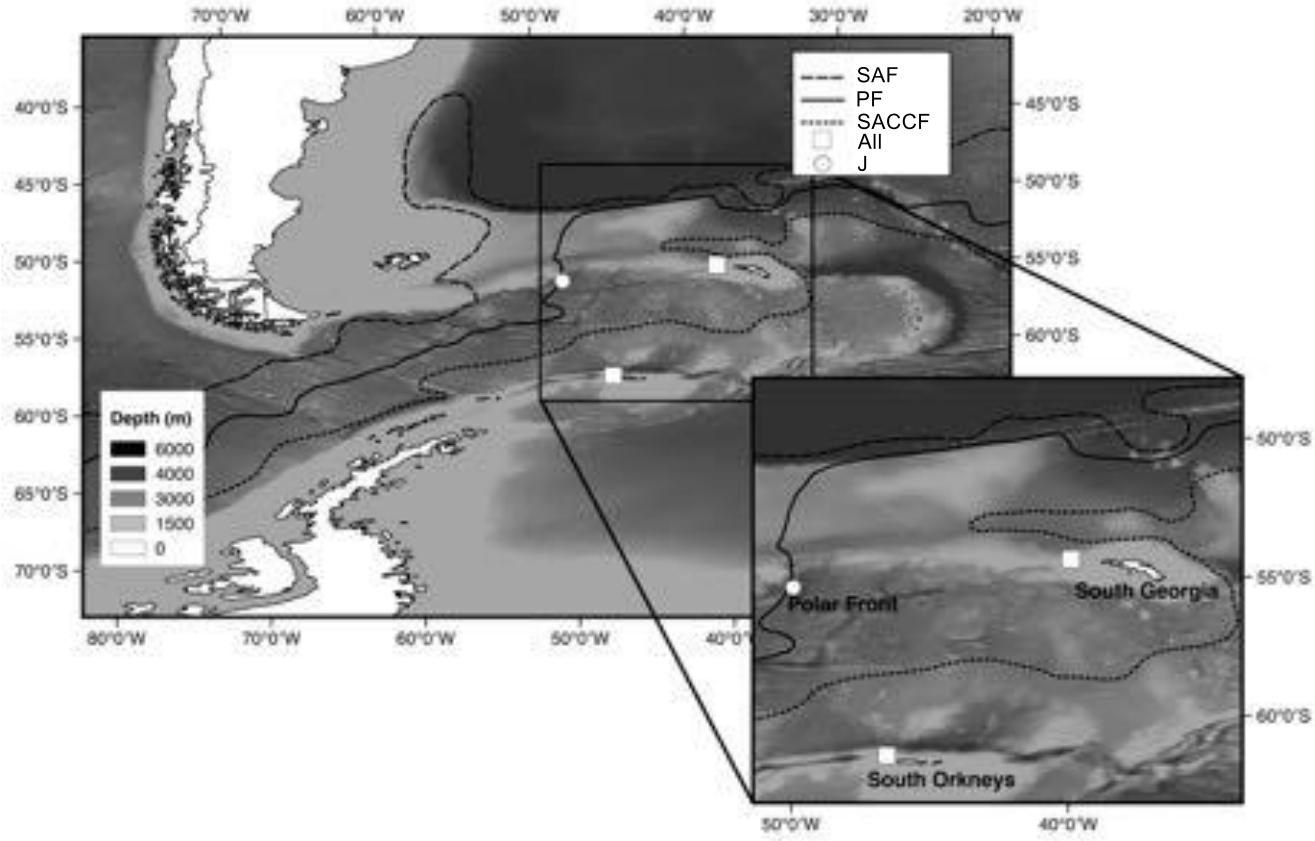

Figure 3.1 - Sampling sites of Antarctic krill (white square – samples of juveniles, females and males; white dot – samples of juveniles) and general positions of the Subantarctic Front (SAF), Polar Front (PF) and the Southern boundary of the Antarctic Circumpolar Current Front (SACCF) (Sallé et al., 2008).

Laboratory procedures

Prior to the mercury analysis, all samples were freeze-dried for at least 24 hours. 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., 2008). Organic mercury was determined through digestion with a mixture of 18 % potassium bromide (KBr) in 5 % sulfuric acid (H_2SO_4), 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 102 % (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 be 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 0.00001 $\mu g\ g^{-1}$ of absolute mercury and 0.004 $\mu g\ g^{-1}$ for organic mercury. All concentration data are expressed subsequently in $\mu g\ g^{-1}$ dry weight.

Statistical analysis

Wilcoxon test 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 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.

Results

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 (Figure 3.2): 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, $H_3 = 41.03$, p< 0.001) than those collected at South Georgia and 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).

Total mercury concentrations in Antarctic krill according to life stage

There were significant differences (Wilcoxon signed rank test, Z= -3.351p = 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 (Figure 3.2). 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) from the same region. 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 a (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 ($Y = -0.0124*X – 1.525$, $R_2 = 0.46$, $F_{1, 43} = 36.41$, $p < 0.001$ from South Georgia; $Y = -0.01072*X - 0.8675$, $R_2 = 0.2746$, $F_{1, 52} = 19.69$, $p < 0.001$ from South Orkney Islands) meaning that bigger individuals had lower mercury concentrations (Figure 3.1). 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.

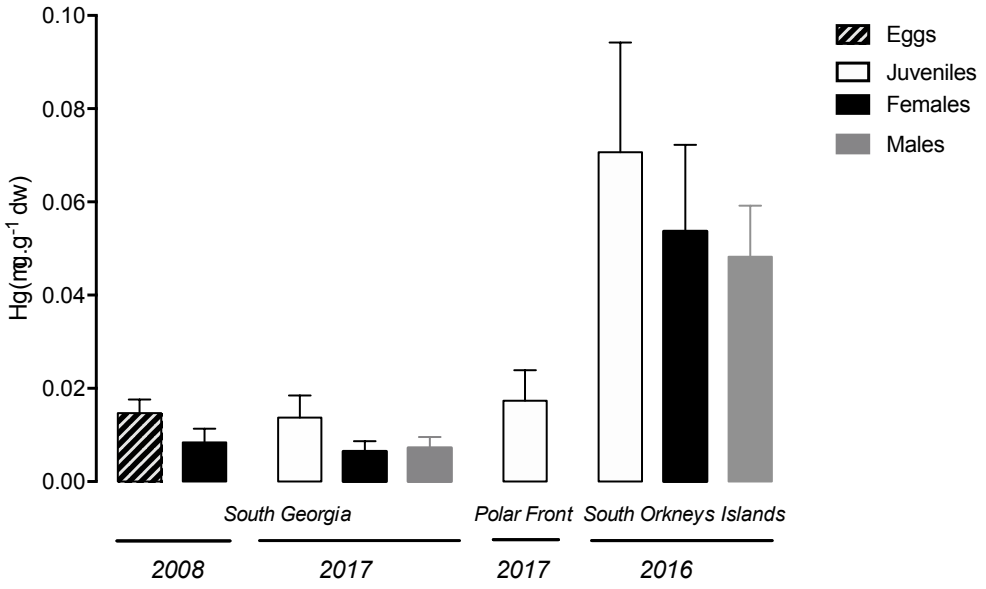

Figure 3.1 – Total mercury concentrations (μg g^{-1} dw) in Antarctic Krill (*Euphausia superba*) collected around South Georgia and at the Polar Front in the austral summer of 2017, and around the South Orkneys during the summer of 2016. Bars show the mean. Error bar is 1 standard deviation.

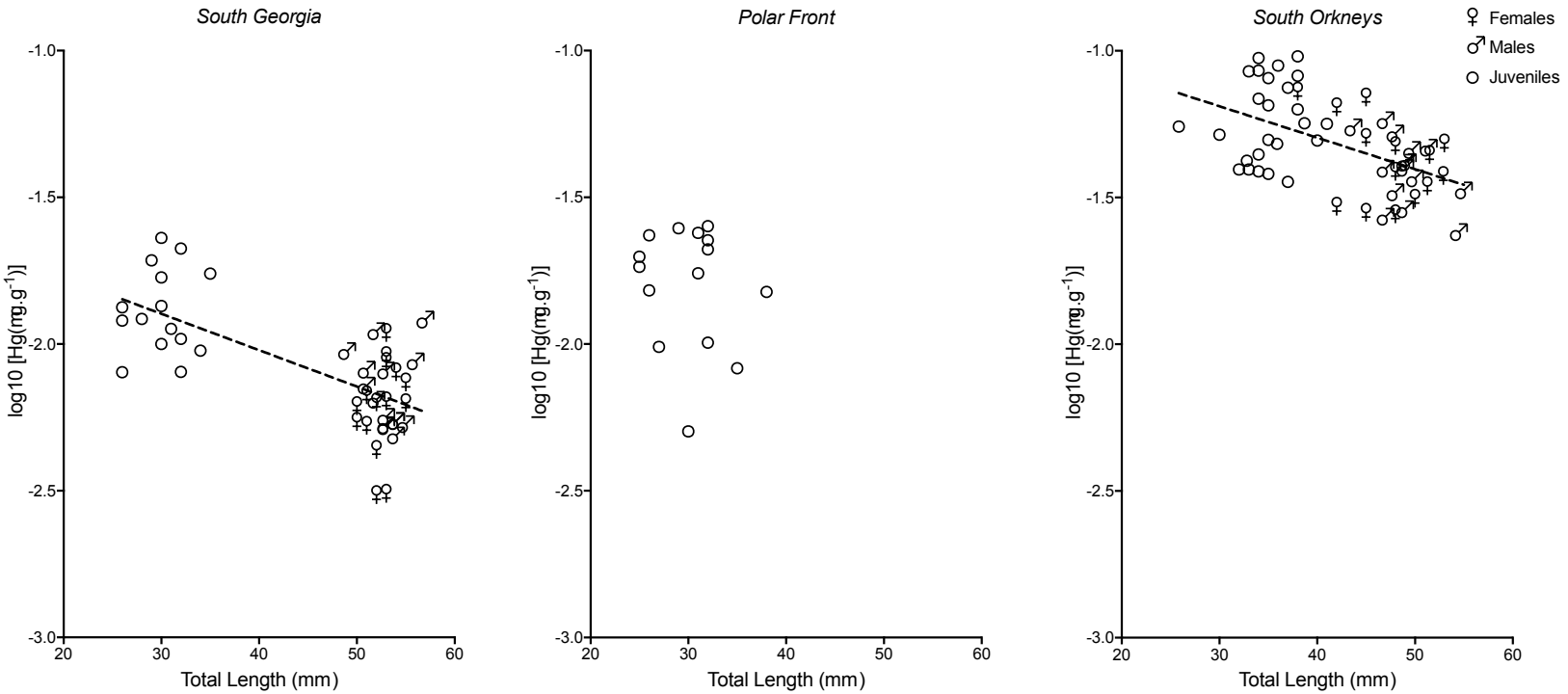

Figure 3.2 – Total mercury concentration (μg g_{-1} dw) on a log10 scale versus total length (mm) for individual Antarctic krill (*Euphausia superba*) by maturity stage and sex respectively. Data are shown separately for krill collected around South Georgia ($Y = -0.0124*X - 1.525$, $R_2 = 0.46$, $F_{1, 43} = 36.41$, $p < 0.001$), the Antarctic Polar Front (both in the austral summer of 2016/17) and the South Orkney Islands ($Y = -0.01072*X - 0.8675$, $R_2 = 0.2746$, $F_{1, 52} = 19.69$, $p < 0.001$; summer of 2015/16).

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 (t_{28}= 0.9323, p= 0.4; Figure 3.2). There were also no differences in mercury concentration between sexes in the samples collected from the South Orkney Islands (t_{27} = 0.917, p= 0.4; Figure 3.2).

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 3.1) (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 3.1). Adults had slightly higher organic mercury percentages than juveniles (Table 3.1).

Table 3.1 – Organic mercury (OHg) and total mercury (THg) concentrations in samples of Antarctic krill (*Euphausia superba*) collected from different locations in the Scotia Sea during the austral summers of 2015/16 and 2016/17. Average ± Standard deviation

Location	Year	Sex / Maturity	Number	OHg (ug g^{-1} dw)	THg (ug g^{-1} dw)	%OrgHg
South Orkney Islands	2016	Juvenile	20	0.008 ± 0.003	0.051 ± 0.016	15%
South Orkney Islands	2016	Female	20	0.008 ± 0.002	0.052 ± 0.022	16%
South Orkney Islands	2016	Male	20	0.008 ± 0.003	0.040 ± 0.014	21%
South Georgia	2017	Juvenile	20	0.008 ± 0.002	0.024 ± 0.006	29%
South Georgia	2017	Female	20	0.002 ± 0.0002	0.006 ± 0.0003	37%
South Georgia	2017	Male	20	0.003 ± 0.0001	0.007 ± 0.0004	36%
Antarctic Polar Front	2017	Juvenile	20	0.005 ± 0.001	0.014 ± 0.005	35%

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 my knowledge this is the first study to determine organic mercury concentrations in Antarctic krill.

Total mercury concentrations according to geographic areas

Antarctic krill from South Orkney Islands 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 differences 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; A. 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 (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 D. N. 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 my 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 Georgia and the Antarctic Peninsula (Brasso et al., 2012a; Cipro et al., 2016; Locarnini and Presley, 1995), but similar to those at the South Orkney Islands (Table 3.2).

Other possible explanations for the higher mercury contamination in Antarctic krill from the South Orkney Islands could be the proximity to active volcanoes, which are well-known sources of mercury (Varekamp and Buseck, 1981; Zambardi et al., 2009). Several volcanoes have recently been reported in the Antarctic Peninsula (van Wyk de Vries et al., 2018), which is closer to the South Orkney Islands than to the other two sampling sites in the present study. Nevertheless, the uptake of mercury from such sources is likely to be variable given that previous studies measuring mercury concentrations in Antarctic krill from the Antarctic Peninsula measured levels that were lower than those specifically in the South Orkney Islands Antarctic krill population reported here (Brasso et al., 2012a; Locarnini and Presley, 1995; Moren et al., 2006) (Table 3.2). Mercury body burdens in Antarctic krill may also be related to food availability (Chen and Folt, 2005). Phytoplankton blooms, which are a main source of mercury to krill, are spatially and temporally variable in the Southern Ocean and have a large influence on Antarctic krill growth (Atkinson et al., 2006; Cuzin-Roudy, 2000). Accordingly, the dynamics and availability of food between locations will probably have a significant effect on the mercury bioavailability, intake and bioaccumulation in Antarctic krill.

In comparison with other krill species around the world (Table 3.2), there are examples where the concentration of mercury is lower, for instance, species from the Order Euphausiacea in the Hudson bay (Canada) (Foster et al., 2012) and *Euphausia pacifica* in the Californian Current (Sydeman and Jarman, 1998) than in some of my samples. Mercury concentrations in euphausiids from more industrialized European regions (Chouvelon et al., 2012; Leatherland et al., 1973; Minganti et al., 1996) and the Arctic (Ritterhoff and Zauke, 1997) are nevertheless considerably higher than in Antarctic krill (Table 3.2). Higher concentrations are also evident in euphausiid populations in the sub-Antarctic Kerguelen Islands (Cipro et al., 2018b) which, like the Southern Ocean, is likely to result from remote atmospheric sources (Cossa et al., 2011).

Total mercury concentration according to life stage and sex

Mercury concentration in Antarctic krill unexpectedly 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 pre-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 bio-magnification 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 the subantarctic krill *Euphausia vallentini* (Cipro et al., 2018b). 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 (T. A. Johnston et al., 2001; Schofield et al., 1994),

Table 3.2 – Total mercury concentrations (μg g^{-1} dw) in different species of Antarctic krill around the world from published data and this study (mean ± standard deviation).

Species	Hg (μg g^{-1})	Location	Reference
Euphausia frigida	0.023 ± 0.002	Kerguelen Islands	Cipro et al. (2017)
Euphausia pacifica, Thysanoessa spinifera	0.030	Californian Current	Sydeman et al (1998)
Euphausia superba	0.008 ± 0.002	Antarctic Peninsula	Brasso 2012
Euphausia superba	0.008	Krill food	Moren 2006
Euphausia superba	0.018 ± 0.005	King George Island	Cipro et al. (2016)
Euphausia superba	0.013 to 0.049	Antarctic Peninsula	Locarnini (1995)
Euphausia superba	0.077 ± 0.026	Ross Sea	Bargali 1998
Euphausia superba (Adult)	0.007 ±0.002	South Georgia	This study
Euphausia superba (Adult)	0.051 ± 0.015	South Orkneys	This study
Euphausia superba (Female)	0.008 ± 0.003	South Georgia	This study
Euphausia superba (Juvenile)	0.014 ± 0.004	South Georgia	This study
Euphausia superba (Juvenile)	0.017 ± 0.006	Polar Front	This study
Euphausia superba (Juvenile)	0.071 ± 0.023	South Orkneys	This study
Euphausia triacantha	0.036 ± 0.006	Kerguelen Islands	Cipro et al. (2017)
Euphausia vallentini (Large 25-30mm)	0.017 ± 0.001	Kerguelen Islands	Cipro et al. (2017)
Euphausia vallentini (Small 16-24mm)	0.042 ± 0.003	Kerguelen Islands	Cipro et al. (2017)
Euphausiaceae	0.023 ± 0.004	Hudson Bay (Canada)	Foster et al. (2012)
Meganyctiphanes norvegica	0.130 ± 0.004	Arctic	Ritterhoff et al. (1997)
Meganyctiphanes norvegica	0.172 ± 0.014	Bay of Biscay	Chouvelon et al (2012)
Meganyctiphanes norvegica	0.250	South of Portugal	Leatherland et al. (1973)
Meganyctiphanes norvegica	0.490	Mediterranean	Minganti et al (1996)
Thysanoessa inermis	0.120 ± 0.004	Arctic	Ritterhoff et al. (1997)
Thysanoessa sp.	0.067 ± 0.031	Kerguelen Islands	Cipro et al. (2017)

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.

Organic mercury

Concentrations of the highly toxic, organic form of mercury were between 0.002 and 0.008 ug g^{-1} dw, with the higher concentrations being found in both the South Orkneys 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, 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 consumption can explain how some Antarctic seabirds have particularly high concentrations of mercury (Tavares et al., 2013).

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 a growth dilution effect and also elimination through gonadic tissue (eggs and spermatophores).

The observed spatial differences suggest that Antarctic krill reflects differential 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, this results stress the need to put into action pollutant monitoring programs to evaluate the sources, pathways and effects of contaminants in remote ecosystems.

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

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 (M. R. Clarke, 1996; Collins and Rodhouse, 2006; Seco et al., 2015). They prey mainly on crustaceans (Kear, 1992; Xavier et al., 2018a) 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., 2018a). *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 (M. Clarke, 1983; Collins and Rodhouse, 2006; Xavier et al., 2018a). 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., 2018b). 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., 2014b; 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 9 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., 2018a). 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

Sampling

South Georgia is a sub-Antarctic island located in the southwest Atlantic (Figure 4.1). Water temperatures around this area vary from -0.95 ºC in winter to 1.75 ºC in summer. South Georgia is a highly productive area of the Southern Ocean, therefore it holds large populations of seabirds, marine mammals and it is one of the most important Southern Ocean fishing areas (Collins et al., 2004; Murphy et al., 2007).

The samples were collected from the Scotia Sea, around South Georgia (Figure 4.1), during scientific cruises during the austral summer of 2006/07 (on board of the Royal Research Ship (RRS) *James Clark Ross* (JCR): cruise JR161 [October – December 2006], 2007/08 - JR177 [December 2007 – February 2008], 2008/09 - JR200 [March – April 2009], Fishing Vessel (FV) *Sil* research survey SG13 [13

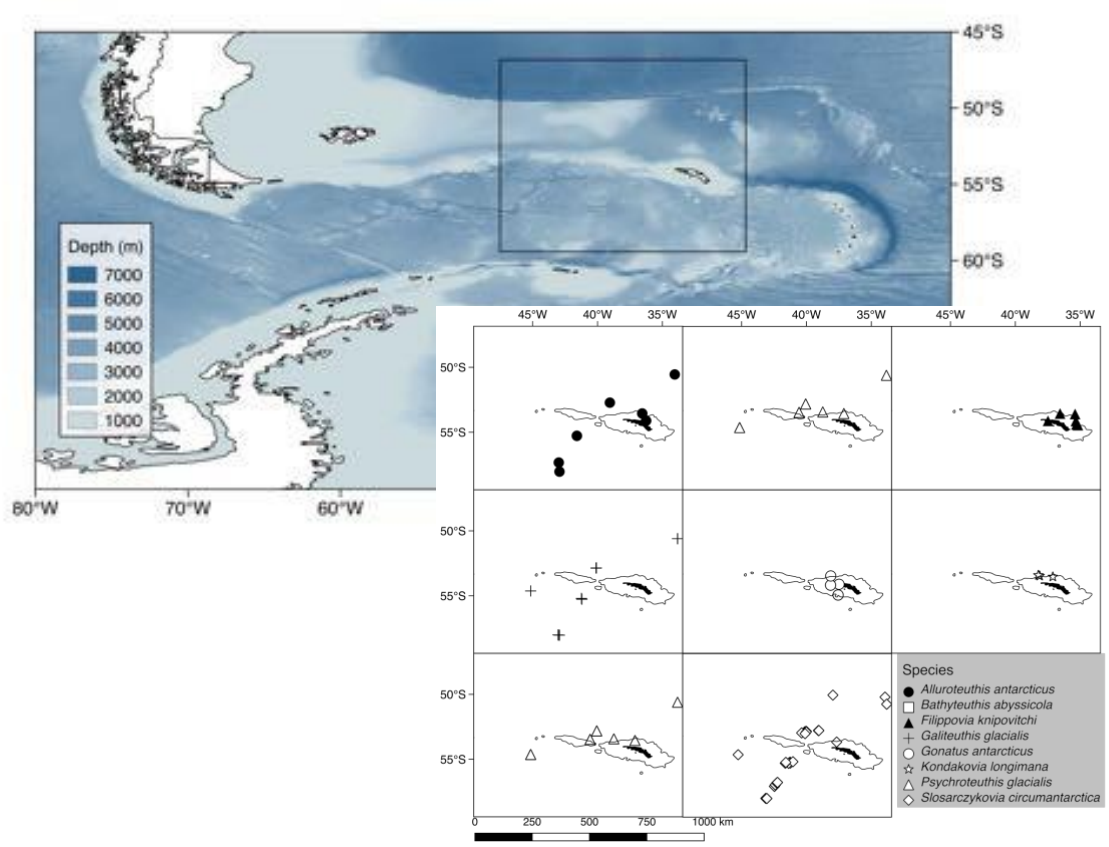

Figure 4.1. Sampling sites and distribution of species captured around South Georgia across all sampling years. Lines represent 1000m isobath.

January 2013]) and RRS JCR cruise JR16003 (December 2016 – January 2017), 2016/17.

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

The samples collected by FV *Sil* were obtained from bottom trawls using an FP120 trawl net with a standard steel bobbin rig, conducted at tow speeds between 3.1 and 4.1 knots over a distance of between 1.25 and 2.1 nautical miles, dependent on the prevailing sea conditions and bottom topography. Whenever possible, samples were identified on board but, in some cases, identification was not possible. In each case, individuals were frozen at -20 ºC for later laboratory processing.

Laboratory procedures

All samples were checked for identification (using cephalopod beaks to confirm identification where there was any doubt (Xavier and Cherel, 2009)), measured and weighed again. When the measurement of the mantle length (ML) of the individual was not possible, allometric equations were used, based on beak size (Xavier and Cherel, 2009). An effort was made to collect samples of muscle, gills and digestive gland in all individuals, although the digestive gland was destroyed in some specimens.

After being dissected, the collected tissues were frozen in sterilised decontaminated plastic vials and freeze-dried for at least 48 hours. Dried 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., 2008). Organic mercury was determined through digestion with a mixture of 18 % potassium bromide (KBr) in 5 % sulphuric acid (H_2SO_4), followed by extraction of organic mercury into toluene (C_7H_8) and back-extraction with an aqueous solution of thiosulphate ($Na_2S_2O_3$), as described in (Válega

et al., 2006). Where there was low individual mass (less than 200 mg), samples were aggregated in groups of the same species, with similar sizes and collected from the same location. Analytical quality control was performed using three certified reference 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

Total mercury concentrations in Antarctic squid muscle

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

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 4.2). 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* ($Y = 2.432*X + 47.83$,

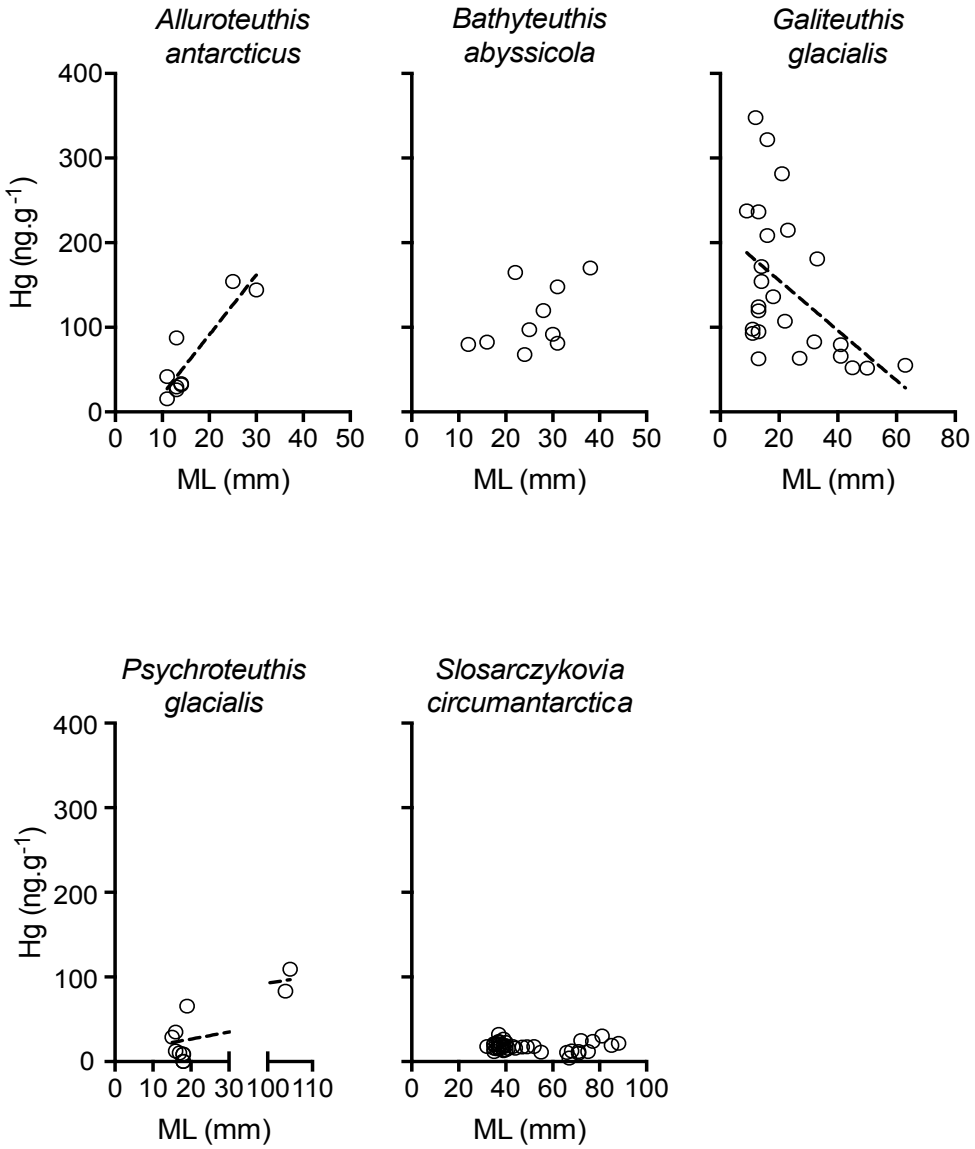

Figure 4.2 – Total mercury concentration (ng g^{-1} dw) versus mantle length (ML; mm) in individual Antarctic squid collected around South Georgia in the austral summer of 2007/08 (except *Psychroteuthis glacialis* 2016/17). Regression equations are given in the text.

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)).

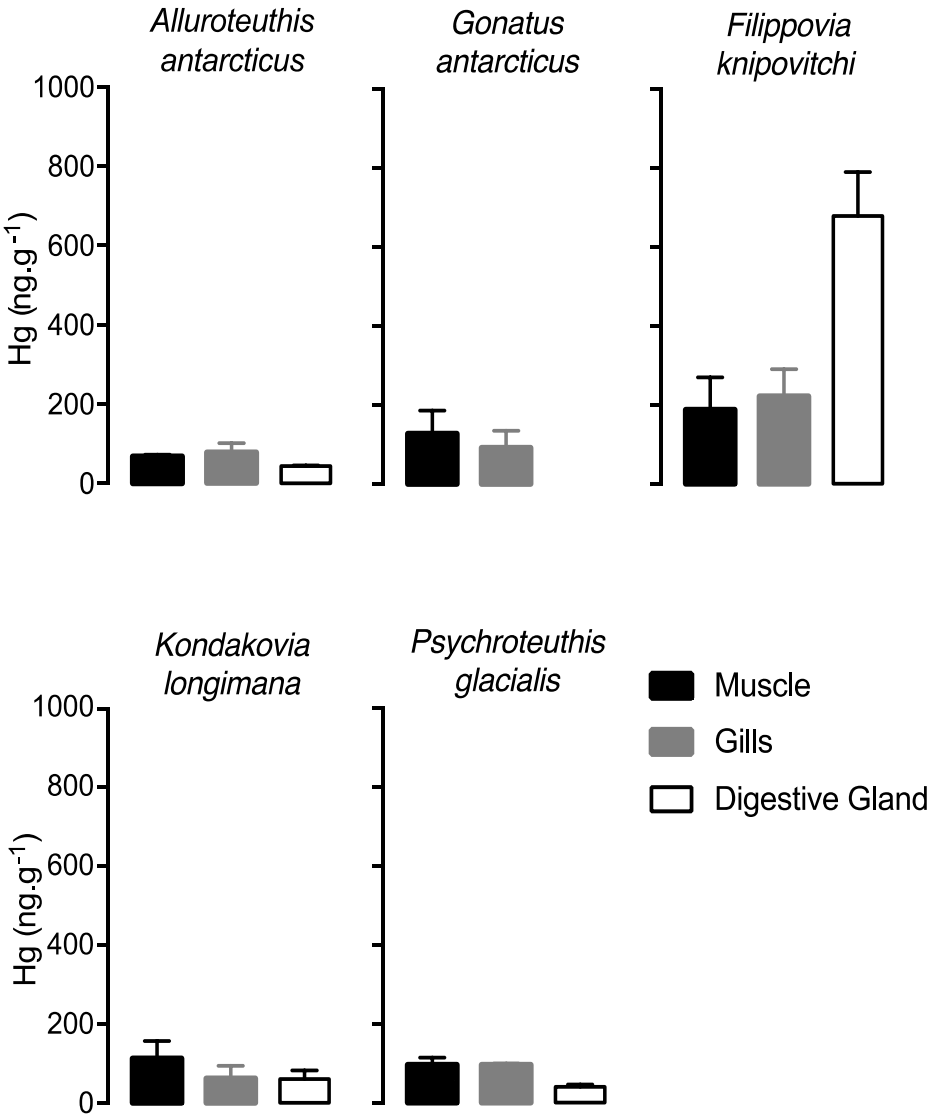

Figure 4.3. Total mercury concentrations (Mean ± SD, ng g_{-1} dw) in different tissues (muscle, gills and digestive gland) of Antarctic squid collected around South Georgia in the austral summer of 2012/2013. No digestive gland tissue was available for G. *antarcticus*.

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: χ^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: χ^2 (2) = 3, p= 0.5; χ^2 (2) = 3, p= 0.5; χ^2 (2) = 3, p= 0.5; *A. antarcticus*, *K. longimana* and *P. glacialis,* respectively; Figure 4.3). 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).

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 4.4).

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 4.4). 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).

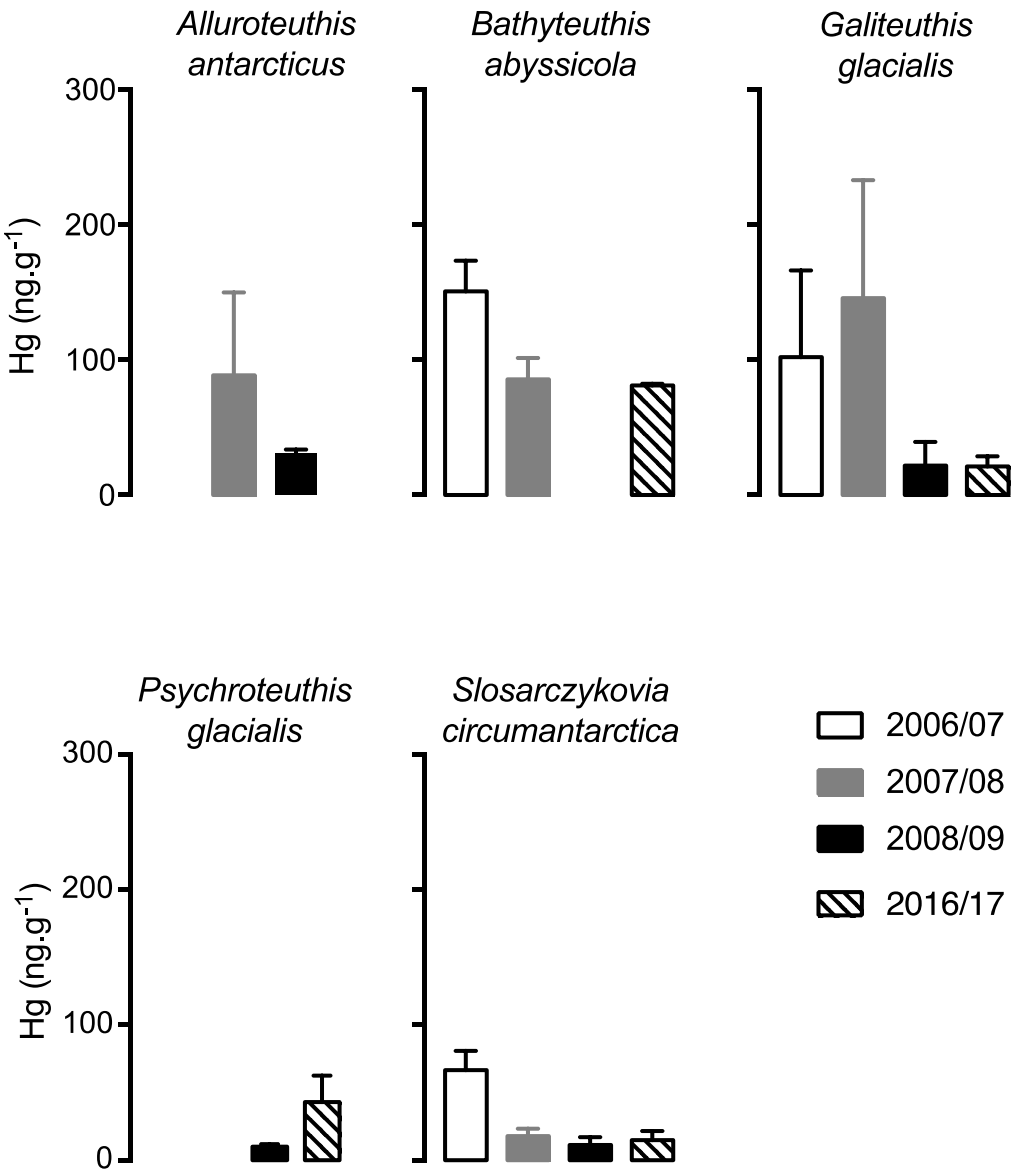

Figure 4.4- Total mercury concentrations (Mean ± SD, ng g_{-1} dw) in the muscles of Antarctic squid from the Southern Ocean collected around South Georgia in the austral summers of 2006/07, 2007/08, 2008/09 and 2016/17.

Organic mercury concentrations and proportions in Antarctic squid

Regarding the percentage of organic mercury relative to total mercury in the different tissues of the five squid species sampled in 2013 (SG13 samples; Table 4.1),

muscle had the highest values (67% to 97%) in all the analysed species, followed by the digestive gland (22% to 38%) and the gills (9% to 19%).

Organic mercury concentrations were also analysed in the muscle of three species (*B. abyssicola, G. glacialis* and *S. circumantarctica,* Table 4.2) across sampling years. Concentrations varied from 2 ng g^{-1} to 84 ng g^{-1}, constituting between 25% to 77% of total mercury. *S. circumantarctica* was the only species where organic mercury 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 4.1 – 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%

Table 4.2 – 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., 2018b; McArthur et al., 2003), to the best of my 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 4.3), 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 *G. glacialis* was negative, 3) Mercury concentration did not appear to be related to size in *B. abyssicola* or *S. circumantarctica*.

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

Tissue allocation of mercury in Antarctic squid

For four species (*A. antarcticus, G. antarcticus K. longimana* and *P. glacialis*), there were no differences in total mercury concentrations between tissues. However, the fraction of organic mercury was always higher in muscle (from 67% to 97%) than in the digestive gland (from 22% to 38%) and gills (9% to 19%). While presently the reasons for the mercury distribution patterns are still a matter of debate, the partitioning of total mercury among tissues is consistent with previous studies on oceanic squid (Cranchidae, Histioteuthidae and Ommastrephidae (Bustamante et al., 2006)), suggesting some degree of equilibrium between contaminant accumulation and excretion rates, as well as efficient redistribution of total mercury between tissues. In most studies of cephalopod species however, the digestive gland had higher total mercury concentrations than other tissues (Bustamante et al., 2006; Pierce et al., 2008; Raimundo et al., 2010b), as we observed in *F. knipovitchi* (Figure 4.4). The high rates of absorption and assimilation of trace metals in the digestive gland (Bustamante et al., 2002; Miramand and Bentley, 1992; Raimundo et al., 2004) make this organ a major pathway of incorporation for contaminants into cephalopods (Bustamante et al., 2002), and reflects the predominant role of the dietary pathway for mercury bioaccumulation. The lower concentrations of organic mercury in the digestive gland is in accordance with the detoxification of organic mercury by demethylation that is suspected to occur in this organ (Bustamante et al., 2006; Penicaud et al., 2017). As this form of mercury is incorporated from the diet, a fraction is likely to be demethylated in the digestive gland, to allow its excretion (Lacoue-Labarthe et al., 2009), and a portion will be circulated, trapped and stored in muscle tissues. Because organic mercury has a strong affinity for the sulphydryl groups of proteins, its accumulation in muscle tissues is favoured (Bloom, 1992; Leaner and Mason, 2004). In fish muscles, for instance, it may be tightly bound by carbon-mercury and sulphydryl linkages (Ruelas-Inzunza et al., 2003). As muscles comprise the highest mass fraction of cephalopods, and given the known affinity of organic mercury to muscular proteins (Bloom, 1992), high concentrations and proportions of organic mercury are to be expected in this tissue. The present results also highlight the high bioavailability of mercury within the Southern Ocean food web (Chouvelon et al., 2012; Clarkson, 1992; Dehn et al., 2006).

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

Temporal trends of mercury concentrations in Antarctic squid

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

Squid are considered an r-selected species (Pianka, 1970): they have a fast growth rate, are semelparous (reproduce only once) and are short lived ($\sim<$ 1–2 years) (Arkhipkin, 2004; Boyle and Rodhouse, 2005; Xavier et al., 2018a), although, some large squid species, like *K. longimana* may live longer (Jarre et al., 1991; Lynnes and Rodhouse, 2002). These characteristics make them responsive bioindicators with which would be possible to monitor trends in mercury concentrations over time, as they will reflect rapidly any changes in the bioavailability of this contaminant.

Data from the squid species *A. antarcticus*, *B abyssicola, G. glacialis* and *S. circumantarctica* appears to indicate a decreasing trend of mercury concentration along time, although further monitoring is required to confirm this pattern. This suggestive pattern of declining concentrations of mercury in the majority of species is consistent with the decreasing trend of atmospheric mercury over the last decade (Soerensen et al., 2012) as a consequence of the reduction of worldwide anthropogenic emissions of mercury (Streets et al., 2017; Y. Zhang et al., 2016). The decrease of mercury in the global atmosphere could also mean a reduction in mercury

deposition levels in the study area. Comparing this results with a previous study in the same region (Anderson et al., 2009) who sampled in 2001/02 and analysed specimens with atomic fluorescence spectrophotometry, a general reduction in the concentration of mercury can also be observed: *G. glacialis* had a mercury concentration in 2001/02 (230 ± 70 ng g_{-1}) (Anderson et al., 2009) that was more than twice as high as present results from 2006/07 (100 ± 60 ng g_{-1}), 1.5 times when compared with 2007/08 (150 ± 90 ng g_{-1}) and more than ten times when compared with 2008/09 or 2016/17 (20 ± 10 ng g_{-1}; 20 ± 7 ng g_{-1}); in *G. antarcticus* (600 ± 2 ng g_{-1}) the difference was bigger, with mercury concentrations being 5 times higher in 2001/02 than in present results for 2013. *P. glacialis* had concentration 4 times higher (180 ± 110 ng g_{-1}) in 2001/02 than my observations in 2008/09. In the family Onychoteuthidae, the concentrations of mercury in 2001/02 (100 ± 20 ng g_{-1} in *F. knipovitchi;* 160 ± 90 ng g_{-1} in *K. longimana*) were similar to my results for 2013.

The pattern of mercury bioaccumulation in species is influenced by specific traits such as dietary preference, ingestion, excretion, and growth rate. Prevailing environmental conditions may also enhance or reduce contaminant bioavailability to cephalopods. Habitat use has a major effect on mercury accumulation in organisms, as sites contaminated by mercury will likely have higher bioavailability of this toxic element. All samples were collected in the Scotia Sea, around South Georgia (Figure 4.1), which is known to be a fairly stable ecosystem, which should mean lower mercury variation between sampling sites.

The majority of the study species have a wide range of vertical distributions: the depth of occurrence of *A. antarcticus* is normally at 800-900 m (Rodhouse, 1988), *G. glacialis*, at 600-1000 m (Roper and R. E. Young, 1975), *G. antarcticus,* at 250-928 m (Collins et al., 2004; Roper et al., 1984), *F. knipovitchi,* at 480-760 m (Collins et al., 2004), *K. longimana,* at 300-900 m (Collins et al., 2004; Xavier et al., 2002), *P. glacialis,* at 275-928 m (Collins et al., 2004; Xavier et al., 2002) and *S. circumantarctica,* at 487-928 m (Collins et al., 2004). An outlier amongst these is *B. abyssicola*, which is a deep-sea squid and occurs at a depth of 1000-1500 m (Roper and R. E. Young, 1975). Higher mercury levels were expected in species from deep habitats as increasing depth raises mercury concentrations in fish (Choy et al., 2009; Le Bourg et al., 2019; Monteiro et al., 1996). However, that was not the case of the present study, as mercury concentrations in the tissues of the deep-sea squid *B.*

abyssicola were within the same range as the majority of other analysed species (see Figure 4.4). The lack of difference between species may be explained by the frequent diel vertical migration associated with cephalopods (Norman et al., 2014) in order to feed and to avoid 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., 2018a; Xavier and Cherel, 2009) and it is presently not possible to evaluate the effect of diet on mercury bioaccumulation in the studied species.

This 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.

5. – Main drivers of mercury levels in Southern Ocean Lantern fish Myctophidae

Abstract

Myctophids are the most abundant fish group in the Southern Ocean pelagic ecosystem and are an important link in the Antarctic marine food web. Due to this major ecological role, evaluating the level of mercury (Hg) contamination in myctophids is important for understanding the trophic pathway of this harmful contaminant. The concentrations and proportions of both total and organic mercury were determined in muscle, gill, heart and liver tissue of 9 myctophid species to quantify tissue partitioning variability between species. Hg concentrations were higher in the liver and heart than in muscle and gills, but the proportion of organic Hg was almost 100% in all tissues, indicating that the main uptake route for Hg is through the diet. Most of the species analysed have similar vertical and horizontal distributions, and similar feeding modes/prey. Geographical and temporal variability of Hg concentrations was examined using samples from 3 different years (2007/08, 2015/16 and 2016/17) and 2 locations (South Georgia and South Orkneys Islands). Results suggest a decreasing trend in Hg contamination over the last decade, which is in agreement with a previous study on squid from the same regions. There was no significant variability in mercury concentration between the different sampling locations. Hg levels were also consistent with values reported in the literature for myctophids around the world, showing low geographic variability. A positive relationship between fish size and Hg concentration was found for most species, with the exception of Electrona antarctica females: egg laying may be an elimination route for Hg. Myctophids collectively may comprise a Southern Ocean mercury ‘reserve’ of ≈ 1.82 metric tonnes.

Keywords: Trace element; metal; bioaccumulation; mesopelagic fish; Antarctic

Introduction

Knowledge of our oceans and their organisms is fragmentary, particularly for deep-sea habitats (Cvitanovic et al., 2015). One group of organisms that are particularly understudied is mesopelagic fish (Catul et al., 2010). These are one of the most diverse marine vertebrates, with around 900 species, whose biomass is likely very high, although uncertain: estimates of biomass range between 1.8 and 16 gigatonnes (Proud et al., 2018). As a consequence of their high biomass, mesopelagic fish are important components of oceanic ecosystems and global biogeochemical cycles (Irigoien et al., 2014). They are crucial in the transfer of energy through oceanic food webs, linking primary consumers and macro-zooplankton to higher predators (Saunders et al., 2019), and contribute as well to the export of carbon from the sea surface to mesopelagic deep-sea depths (the so-called biological carbon pump) through their extensive vertical migrations (Irigoien et al., 2014; Smith et al., 2011). However, major uncertainties still remain regarding the ecology of these fishes (St John et al., 2016). Within the mesopelagic fishes, lanternfish (Family Myctophidae; hereafter myctophids), are considered the most abundant and diverse family, with ~250 species globally and a conservatively estimated biomass of 550-660 million tonnes (Bone et al., 1995; Gjøsaeter and Kawaguchi, 1980).

In the Southern Ocean, myctophids are an important part of the oceanic food web (Murphy et al., 2007), as they consume mainly planktonic crustaceans (Lourenço et al., 2017; Pakhomov et al., 1996; Saunders et al., 2018) and are a major food source for a range of marine predators, including large predatory fish (Fenaughty et al., 2003; Stevens et al., 2012), cephalopods (Cherel and Duhamel, 2003; Olson and J. W. Young, 2006; K. L. Phillips et al., 2001; Rodhouse et al., 1992b), marine mammals (Newland et al., 2011) and seabirds (Xavier et al., 2003b). In this region, many myctophid species have broadly overlapping horizontal and vertical distribution patterns [between 0 – 1000m depth (Saunders et al., 2018)], and typically migrate from depths below ~400 m to feed on zooplankton in the near-surface (top 200 m) regions of the water column (Pakhomov et al., 1996). Trophodynamic studies in the Southern Ocean have shown that the diet of the biomass-dominant species vary spatially, seasonally, inter-specifically and ontogenetically, suggesting that most myctophids are opportunistic feeders with a broad dietary range, preying on the most available food resources at their disposal (Cherel et al., 2010; Pakhomov et al., 1996;

Saunders et al., 2018; Stowasser et al., 2012). This, in turn, potentially makes them good bioindicators of contamination in the local marine environment.

Mercury (Hg) is one of the most know hazard elements and it has a global dispersion (Selin, 2009; UNEP, 2013). Hg has a long-range dispersion capacity (Streets et al., 2019) so despite the great distances between Antarctica and the anthropogenic Hg sources, relatively high Hg concentrations can reach the Antarctic ecosystem (Cossa et al., 2011). For example, strong upwelling currents in the Southern Ocean, and seasonal sea ice cover at high latitudes, create conditions that favour Hg methylation leading to increased organic Hg (O-Hg) concentrations (Cossa et al., 2011). Such a methylation enhanced Hg bioavailability to marine organisms as O-Hg is bioaccumulative, due to the strong bond with proteins (Bloom, 1992). It is, therefore, a very toxic form to biota. Its detrimental effects include changing biochemical processes, damaging cells and tissues, and reducing reproductive success in fish (Sandheinrich and Wiener, 2011; Scheuhammer et al., 2015). Furthermore, O-Hg biomagnifies along the food web, putting long-lived apex predatory species at high toxic risk and populations to depletion from intensive, or prolonged exposure to this contaminant. For example, studies of predatory birds (e.g: *Gavia immer, Haliaeetus leucocephalus*) found that exposure to Hg altered neurotransmitter concentrations (Scheuhammer et al., 2015; 2007), increased oxidative stress (Hoffman et al., 2011) and decreased reproductive success (Eagles-Smith et al., 2018; Goutte et al., 2014b).

In mid-trophic level fish such as myctophids, Hg can be accumulated biologically via two main pathways: absorption from the environment through the surfaces involved in respiration (e.g. gills and skin) and absorption through ingestion of contaminated prey. Whilst physical absorption can be an important pathway, particularly under high environmental exposure levels, the majority of Hg is absorbed through food intake (G. R. Phillips and Buhler, 1978). Although many studies have examined the general diets and trophodynamics of myctophids across the globe (Hudson et al., 2014; Olivar et al., 2018; Van Noord et al., 2016), only a few studies have investigated Hg bioaccumulation in these fish at a global scale (Blum et al., 2013; Chouvelon et al., 2012; Gibbs et al., 1974; Lahaye et al., 2006; Martins et al., 2006; Monteiro et al., 1996; Windom et al., 1973), with only two studies focusing on the Southern Ocean (Bustamante et al., 2003; Cipro et al., 2018b). Due to their crucial role in the Southern Ocean pelagic food web, evaluating the level of Hg contamination

in this group is important to understand the trophic pathway of this contaminant. Such studies are also essential for establishing robust baselines for future environmental monitoring that will inform potential ecosystem management strategies in the context of the Minamata convention objectives (Gustin et al. 2016).

Hg concentrations were measured in myctophids collected across the Scotia Sea, one of the most productive regions of the Southern Ocean (Holm-Hansen et al., 2004), and looked for spatial, temporal, inter-specific and ontogenetic patterns. The Scotia Sea houses globally important populations of higher predator species such as penguins, flying birds, seals and whales (Murphy et al., 2007) and important commercial fisheries (Constable et al., 2000) so understanding Hg pathways is important. Specifically, total mercury (T-Hg) concentrations was assessed in four tissues (muscle, gills, heart and liver) and organic Hg (O-Hg) in the muscle of the nine biomass-dominant myctophids (*Electrona antarctica, Electrona carlsbergi, Gymnoscopelus braueri, Gymnoscopelus nicholsi, Gymnoscopelus opisthopterus, Gymnoscopelus fraseri*, *Protomyctophum bolini*, *Krefftichthys anderssoni* and *Nannobrachium achirus*) from two regionally distinct food webs in the Scotia Sea (South Georgia and South Orkneys Islands) in different years (2007/08, 2015/16 and 2016/17). These regions are characterised by different environmental conditions, zooplankton population dynamics and myctophid community composition/structure, as well as different higher predator-prey dynamics, facilitating an interesting examination into how Hg may affect different components of the overall Southern Ocean ecosystem. This study therefore provides important insights on T-Hg and O-Hg accumulation in Southern Ocean myctophids that are essential for monitoring the health of the Southern Ocean food web in a resource management context.

Material and methods

Sampling

Myctophids were caught on three multidisciplinary research cruises at the *RRS James Clark Ross* around South Georgia (north of the SACC) and the South Orkney Islands (south of the SACC) during austral summer between 2007/08 and 2016/17 (Figure 5.1). The surveys around South Georgia were undertaken between December 2007 and February 2008 and December 2016 and January 2017, whilst the survey around the South Orkneys islands was undertaken between December 2015 and January 2016.All samples were caught during the night period and at a depth between 0 and 1000 m, using two Rectangular Midwater Trawl net, a RMT8 and RMT25 (Piatkowski et al., 1994; Roe and Shale, 1979)]. Myctophids were identified onboard to species level, measured to the nearest mm using standard length (SL). Individuals were then frozen at -20ºC for subsequent laboratory analyses, with each fish species preserved separately in different plastic bags.

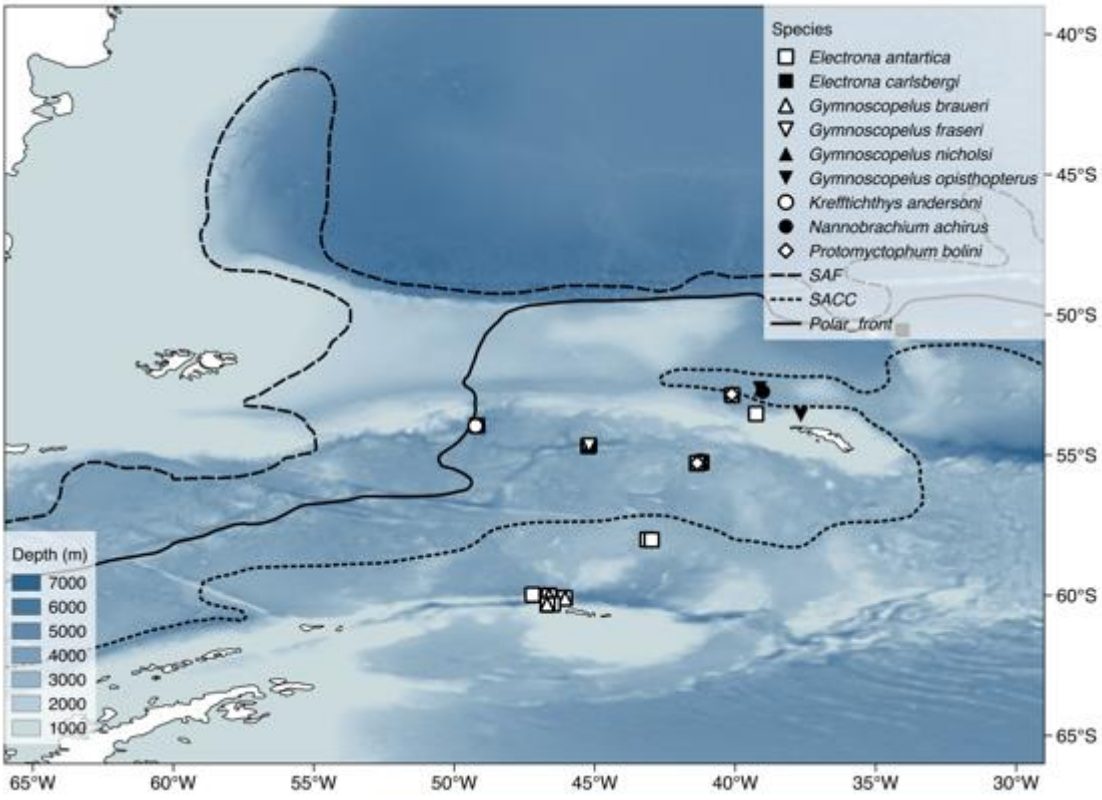

Figure 5.1. Sampling sites and distributions of species captured around the Scotia Sea across all sampling years. SAF – Sub Antarctic Front; SACC - Southern boundary of the Antarctic Circumpolar Current Front.

Laboratory procedures

In the laboratory, all specimens were re-measured and weighed to the nearest 0.01g before dissection. Sex and maturity of post-juveniles was determined (Hulley, 1990). For most specimens, the gills, heart and liver were extracted, as well as a sample of muscle (without the skin). For the 2 smallest species *Krefftichthys anderssoni* and *Protomyctophum bolini* organ dissection was not possible and only muscle was collected.

Samples were frozen in sterile containers and lyophilized during two days and ground to a fine powder for further analyses of Hg. T-Hg was determined by atomic absorption spectrometry (AAS) with thermal decomposition and gold amalgamation, using an Advanced Mercury Analyser LECO AMA-254. Quantification of O-Hg was performed through a chemical digestion described in (Válega et al., 2006). Where there was low individual mass (less than 200 mg), samples for O-Hg analyses were obtained by combining multiple individuals of the same species of similar sizes collected from the same location. Analytical precision and accuracy were determined by several daily analysis of following certified reference materials: DORM-4 (96 ± 13%) fish protein and ERM-BB422 (100 ± 4%). DORM-4 was used to certify O-Hg analyses, with an efficiency of 99 ± 8%. All analyses were repeated 2–3 times until having a relative standard deviation <10%. Detection limits for this technic is 0.01 ng of T-Hg and 0.004 μg g^{-1} for O-Hg. Hg concentrations are given as μg g^{-1} dw.

Statistical analysis

The relation between Hg concentration and myctophid standard length was evaluated by a correlation. Shappiro-Wilk and Bartlett's test were used to test the normality and homogeneity of the data, respectively. For the comparison of Hg in tissues (muscle, gills, heart and liver) Friedman tests were. To evaluate differences among years, locations and sexes Wilcoxon rank and Kruskal–Wallis tests were performed.

Results

Spatial and temporal variation in T-Hg concentrations in myctophids

Out of the 5 species collected across multiple years (Figure 5.2), statistically significant inter-annual differences in T-Hg concentration were found for *E. antarctica*. T-Hg concentrations in the muscle of the 8 analysed myctophid species sampled during 2007/08 varied between 0.026 μg g-1 (in *K. anderssoni*) and 0.418 μg g-1 (in *G. opisthopterus*; Figure 5.2; Table 5.1). In 2015/16, only 3 species were caught and T-Hg concentrations in the muscle ranged from 0.072 to 0.441 μg g-1, with the highest and lowest values observed in E. antarctica (Figure 5.2; Table 5.1). During 2016/17, T-Hg concentrations varied across the 6 species from 0.022 μg g-1 (*K. anderssoni*) to 0.424 μg g-1 (Gymnoscopelus fraseri; Figure 5.2; Table 5.1). Of the sampled myctophids, only 3 species (*E. antarctica*, *G. braueri* and *G. nicholsi*) were caught repeatedly each year. There were no significant differences in muscle T-Hg concentrations between years for *G. braueri* or *G. nicholsi* (Kruskal-Wallis test, H = 5.366, p = 0.068; Kruskal-Wallis test, H = 4.859, p = 0.0852, respectively), significantly lower T-Hg concentrations were for *E. antarctica* in 2016/17 than in 2007/08 and 2015/16 (Kruskal-Wallis test, H = 13.81, p = 0.001; Table 5.1). *Krefftichthys anderssoni* and *Protomyctophum bolini* were caught both in 2007/08 and 2016/17, with no significant differences in T-Hg muscle concentrations between years for either species (Mann-Whitney test, U = 43, p = 0.426; Mann-Whitney test, U = 66, p = 0.359).

To minimize the effect of temporal variation on the spatial analysis, comparisons were only performed within species between the 2 consecutive sampling years (2015/16 and 2016/17). During these surveys, only 3 species (*E. antarctica, G. braueri* and *G. nicholsi*) were caught consistently year on year at both South Georgia (North of the SACC) and the South Orkneys Islands (South of the SACC). There was no significant difference in T-Hg muscle concentrations in the two *Gymnoscopelus* species between the two locations (*G. braueri,* Kruskal-Wallis test, H = 5.366, p = 0.068; *G. nicholsi,* Kruskal-Wallis test, H = 4.859, p = 0.085). However, the concentrations of T-Hg in *E. antarctica* were significantly lower at the South Orkney Islands (0.126 ± 0.068 μg g^{-1}) than at South Georgia (0.216 ± 0.082 μg g^{-1}; Figure 5.3).

Table 5.1 – Number of analyzed individuals, standard length (mm), weight (g) and total mercury concentrations (Mean ± SD, min - max, μg g_{-1} dw) in different tissues (muscle, gills, heart and liver) of Southern Ocean myctophids collected in the Scotia sea in the austral summer of 2007/08, 2015/16 and 2016/17. n.a. not analysed.

Species			Tissues					Friedman test	
2007/08	n	Standard Length (mm)	Weight (g)	Muscle	Gills	Heart	Liver	X2	p
Electrona antarctica	16	72.6 ± 15.6 46 – 98	5.5 ± 3.4 1.1 - 12.7	0.21 ± 0.07 0.09 - 0.33	0.28 ± 0.05 0.19 - 0.37	0.59 ± 0.16 0.35 - 0.99	0.36 ± 0.16 0.18 - 0.80	30.5	<0.0001
Electrona carlsbergi	15	75.1 ± 4.0 67 – 81	6.0 ± 0.9 4.7 - 7.7	0.15 ± 0.03 0.11 - 0.18	0.31 ± 0.06 0.26 - 0.43	0.57 ± 0.12 0.42 - 0.80	0.29 ± 0.12 0.19 - 0.56	25.6	<0.0001
Gymnoscopelus braueri	5	102.2 ± 40.0 63 – 164	14 ± 16 2.0 - 38.8	0.16 ± 0.03 0.11 - 0.18	0.40 ± 0.12 0.24 - 0.56	0.77 ± 0.28 0.61 - 1.29	0.51 ± 0.12 0.37 - 0.62	14.0	<0.0001
Gymnoscopelus nicholsi	5	137.4 ± 11.3 124 - 153	28.3 ± 6.3 20.8 - 35.5	0.29 ± 0.12 0.18 - 0.47	0.25 ± 0.04 0.18 - 0.29	0.50 ± 0.08 0.42 - 0.62	0.59 ± 0.09 0.52 - 0.73	14.0	<0.0001
Gymnoscopelus opisthopterus	7	130.6 ± 19.8 109 - 164	24.0 ± 10.1 13.4 - 38.8	0.23 ± 0.12 0.11 - 0.34	0.14 ± 0.05 0.08 - 0.23	0.52 ± 0.08 0.41 - 0.62	0.42 ± 0.19 0.20 - 0.69	16.0	<0.0001
Nannobrachium achirus	6	129.2 ± 11.4 111 - 142	18.3 ± 5.4 10.8 - 24.7	0.11 ± 0.03 0.07 - 0.16	0.15 ± 0.041 0.10 - 0.20	0.24 ± 0.05 0.19 - 0.32	0.65 ± 0.23 0.34 - 0.94	17.0	<0.0001
Krefftichthys andersoni	10	37.9 ± 3.8 34 – 47	1.0 ± 1.8 0.3 – 6.0	0.04 ± 0.01 0.03 - 0.04	n.a.	n.a.	n.a.		
Protomyctophum bolini	10	42.3 ± 8.1 31 – 56	1.2 ± 0.7 0.5 - 2.6	0.09 ± 0.03 0.07 - 0.16	n.a.	n.a.	n.a.		
2015/16									
Electrona antarctica	36	80.2 ± 8.3 63 – 96	6.9 ± 2.6 3.1 - 14.2	0.22 ± 0.08 0.07 - 0.44	0.22 ± 0.06 0.11 - 0.37	0.49 ± 0.17 0.27 - 0.91	0.36 ± 0.14 0.09 - 0.79	79.6	<0.0001
Gymnoscopelus braueri	18	114.0 ± 9.1 94 – 127	11.9 ± 3.3 5.7 - 16.3	0.18 ± 0.03 0.13 - 0.22	0.17 ± 0.07 0.09 - 0.41	0.57 ± 0.11 0.43 - 0.82	0.58 ± 0.16 0.24 - 0.94	19.5	<0.0001
Gymnoscopelus nicholsi	9	142.6 ± 11.6 125 - 154	30.2 ± 5.8 20.0 – 38.7	0.17 ± 0.04 0.11 - 0.24	0.16 ± 0.02 0.14 - 0.19	0.31 ± 0.04 0.27 - 0.36	0.33 ± 0.05 0.26 - 0.38	41.7	<0.0001

2016/17									
Electrona antarctica	15	60.7 ± 16.4 39 - 83	3.3 ± 2.5 0.7 - 7.7	0.13 ± 0.07 0.06 - 0.30	0.07 ± 0.04 0.03 - 0.15	0.19 ± 0.13 0.07 - 0.48	0.15 ± 0.09 0.05 - 0.33	22.9	<0.0001
Gymnoscopelus braueri	7	92.0 ± 33.8 57 - 134	9.0 ± 9.2 1.1 - 24.7	0.12 ± 0.06 0.08 - 0.24	0.08 ± 0.03 0.05 - 0.14	0.41 ± 0.43 0.15 - 1.28	0.50 ± 0.30 0.20 - 0.99	17.0	<0.0001
Gymnoscopelus fraseri	8	74.9 ± 10.4 56 - 86	4.1 ± 1.7 1.5 - 6.5	0.22 ± 0.11 0.12 - 0.42	0.15 ± 0.06 0.10 - 0.26	0.39 ± 0.36 0.12 - 1.03	1.11 ± 0.64 0.25 - 2.11	22.9	<0.0001
Gymnoscopelus nicholsi	5	132.4 ± 3.2 129 - 137	21.1 ± 1.4 20 - 23.1	0.15 ± 0.02 0.12 - 0.18	0.10 ± 0.01 0.09 - 0.11	0.29 ± 0.07 0.20 - 0.38	0.39 ± 0.12 0.19 - 0.48	14.0	<0.0001
Krefftichthys andersoni	11	51.6 ± 12.4 40 - 70	1.87 ± 1.35 0.7 - 3.9	0.05 ± 0.04 0.02 - 0.14	n.a.	n.a.	n.a.		
Protomyctophum bolini	17	43.4 ± 10.2 28 - 57	1.24 ± 0.76 0.2 - 2.6	0.11 ± 0.03 0.07 - 0.20	n.a.	n.a.	n.a.		

Inter-specific variations in T-Hg concentrations in myctophids

Inter-specific variations in T-Hg concentrations in muscle were apparent for samples pooled by year and location. During 2008/09, the 3 species of Southern Ocean *Gymnoscopelus* (*G. braueri, G. nicholsi* and *G. opisthopterus*) and the two species of *Electrona* (*E. antarctica* and *E. carlsbergi*) had higher T-Hg concentrations than *K. anderssoni* and *P. bolini* (Kruskal-Wallis test, $H = 48.66$, $p < 0.0001$). However, the levels of T-Hg in *Nannobrachium achirus* were not significantly different from the other species caught during this time (Figure 5.2; Table 5.1).

No statistical differences were observed between T-Hg concentrations in any of the 3 fish species (*E. antarctica, G. braueri* and *G. nicholsi*) caught in 2015/16 (Kruskal-Wallis test, $H = 4.432$, $p = 0.109$). However, for fish caught in 2016/2017, *E. antarctica, G. fraseri, G. nicholsi* each had higher T-Hg concentrations than *K. anderssoni,* whilst no significant inter-specific differences were observed for either *G. braueri* and *P. bolini* (Kruskal-Wallis test, $H = 28.7$, $p < 0.0001$; Figure 5.2; Table 5.1).

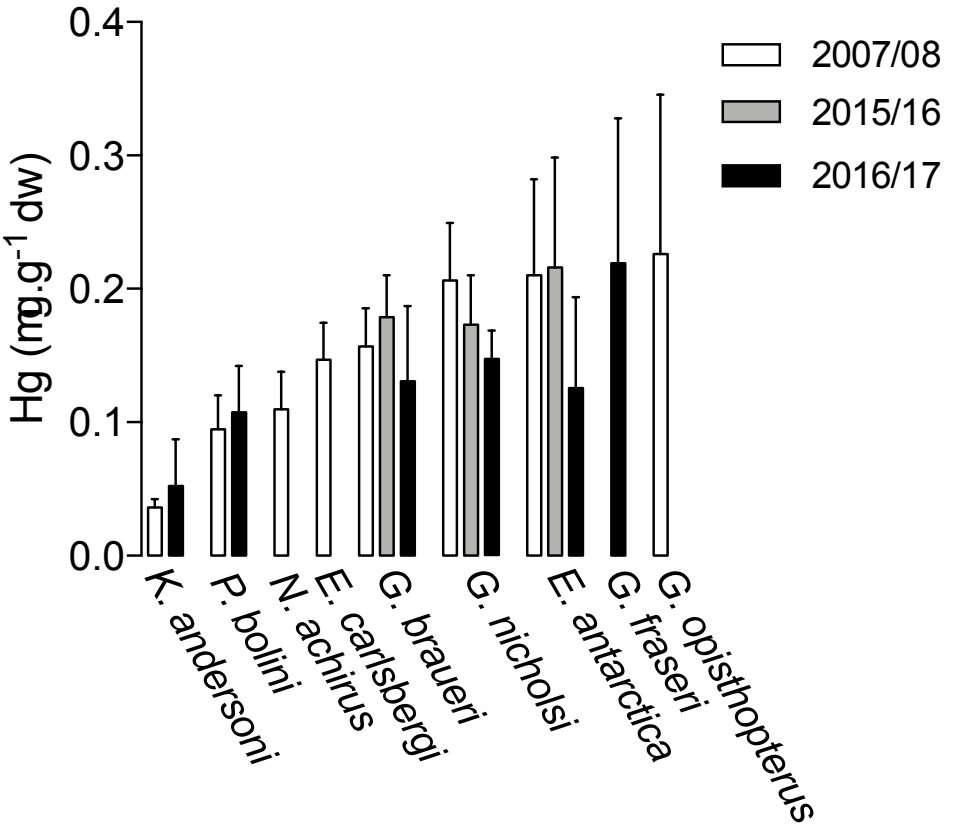

Figure 5.2 - Total mercury concentrations (Mean ± SD, μg g^{-1} dw) in the muscles of Southern Ocean myctophids, collected in the Scotia Sea in the austral summers of 2007/08, 2015/16 and 2016/17.

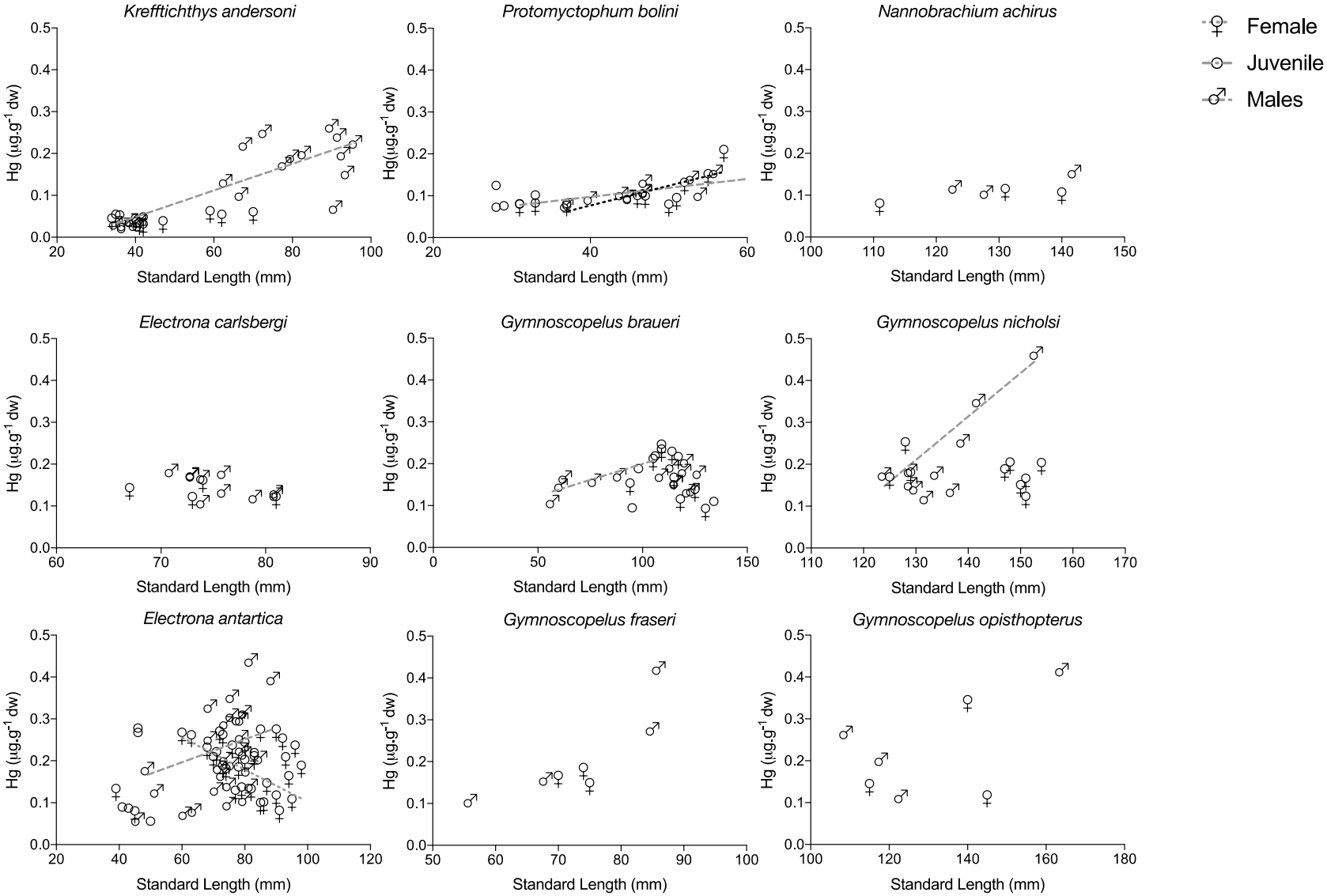

Figure 5.3 - Total mercury concentrations (Mean ± SD, μg g_{-1} dw) versus standard length (mm) in the muscles of Southern Ocean myctophids and significant regression lines, collected in the Scotia Sea in the austral summers of 2007/08, 2015/16 and 2016/17. Regression equation given in table 5.2.

Table 5.2 – Correlation equation, R_2, F, p value and class (A - increasing, B- not significant, C – decreasing) for the relation between T-Hg in muscle with standard length of Southern Ocean myctophids.

Species	Sex	Equation	R_2	F	p value	Class
Electrona antarctica	F	Y = -0.003829*X + 0.4854	0.41	10.5	0.005	C
Electrona antarctica	M	Y = 0.004988*X - 0.1489	0.26	9.7	0.004	A
Electrona antarctica	J	Y = 0.002929*X + 0.02343	0.01	0.02	0.882	B
Gymnoscopelus braueri	F	Y = -0.001069*X + 0.2878	0.17	4.1	0.056	B
Gymnoscopelus braueri	J	Y = 0.001529*X + 0.04665	0.77	16.9	0.009	A
Gymnoscopelus braueri	M	Y = -0.0006763*X + 0.2515	0.06	0.5	0.484	B
Gymnoscopelus fraseri	F	Y = 0.008816*X - 0.4084	0.82	8.9	0.096	B
Gymnoscopelus fraseri	M	Y = -0.001157*X + 0.2421	0.03	0.03	0.893	B
Gymnoscopelus nicholsi	M	Y = 0.001837*X + 0.04139	0.32	6.4	0.023	A
Gymnoscopelus nicholsi	F	Y = -0.0005184*X + 0.2394	0.03	0.3	0.578	B
Gymnoscopelus opisthopterus	F	Y = 0.001975*X - 0.06995	0.06	0.07	0.836	B
Gymnoscopelus opisthopterus	M	Y = 0.003837*X - 0.2421	0.54	2.3	0.268	B
Krefftichthys andersoni	M	Y = 0.003181*X - 0.07897	0.70	43.6	<0.001	A
Krefftichthys andersoni	F	Y = 0.0004552*X + 0.0175	0.29	3.7	0.086	B
Nannobrachium achirus	F	Y = 0.001056*X - 0.04281	0.74	2.7	0.344	B
Nannobrachium achirus	M	Y = 0.00228*X - 0.1709	0.77	3.3	0.320	B
Protomyctophum bolini	M	Y = 0.002139*X + 0.01184	0.54	23.7	<0.001	A
Protomyctophum bolini	J	Y = -0.001913*X + 0.1449	0.11	0.3	0.582	B
Protomyctophum bolini	F	Y = 0.004726*X - 0.1123	0.67	18.3	0.002	A

Gender-specific and ontogenetical variations in T-Hg concentrations

To increase the robustness of the analysis to gender- and size-related effects on T-Hg accumulation in myctophids, only samples with no significant differences across years were pooled for each species. Thus, samples were pooled across all years for every species, except *E. antarctica,* for which only samples from 2007/08 and 2015/16 were pooled.

No significant gender-related differences in T-Hg muscle concentrations were observed for any species other than *G. nicholsi* and *K. anderssoni,* in both of which males had higher T-Hg concentrations than females (Mann-Whitney test, $U = 41$, $p = 0.005$ and $U = 56$, $p = 0.017$, respectively),

Size- (and hence age-) related patterns in T-Hg concentration were evaluated for species where there was a sufficient sample size ($n > 5$) across their full expected size ranges (Figure 5.3; Table 5.2). Three patterns were noted between the different species/sexes: 1) No influence of size in T-Hg concentrations [in females of *G. braueri,* in males of *G. braueri, G. nicholsi, K. anderssoni* and juveniles of *P. bolini*]*;* 2) an increase of concentration of T-Hg with size [in males of *G. nicholsi, K anderssoni* and *P. bolini*, *E. antarctica* and *P. bolini* and in juveniles of *G. braueri*]; 3) and a decrease of T-Hg with size, was only found in females of *E. antarctica*.

Variations in T-Hg concentrations in the different tissues of Southern Ocean myctophids

Four different tissue types, muscle, gills, heart and liver, were analysed for T-Hg levels in each species, except K. anderssoni and P. bolini that were too small for us to achieve adequate extraction of these tissues (Table 5.1).

Overall, T-Hg concentrations varied between tissues, with different patterns of variation between species and years (Table 5.1). However, in the majority of the species analysed from 2007/08 and 2015/16 samples, the heart and liver had consistently higher T-Hg concentrations than the muscle and gills, which contained the lowest overall concentrations. In 2016/17 samples, gills had always lower concentration than muscle. Also, liver from G. fraseri was the only tissue to have an average T-Hg concentration greater than 1 μg g-1 (1.11 ± 0.644 μg g-1).

O-Hg concentrations in Southern Ocean myctophids

Concentrations of O-Hg in muscle of the analysed species ranged between 0.051 and 0.493 μg g-1. There were no significant differences in O-Hg concentrations between species (Kruskal-Wallis test, H = 6.428, p = 0.491; Table 5.3). The overall percentage of muscle O-Hg relative to muscle T-Hg was consistently greater than 75%, and close to 100% in all species (Table 5.2), indicating that the T-Hg found in myctophid muscle is predominantly in the organic form.

Table 5.3 – Total mercury (T-Hg) and organic mercury (O-Hg) concentrations (μg g^{-1} dw) and percentage of O-Hg in in the muscles of Southern Ocean myctophid, collected in the Scotia Sea in the austral summers of 2007/08, 2015/16 and 2016/17.

Species	T-Hg	O-Hg	%O-Hg
Electrona antartica	0.17 ± 0.07	0.141 ± 0.07	79 ± 9
Electrona carlsbergi	0.12 ± 0.01	0.107 ± 0.02	88 ± 8
Gymnoscopelus braueri	0.17 ± 0.06	0.114 ± 0.03	80 ± 11
Gymnoscopelus nicholsi	0.33 ± 0.14	0.331 ± 0.18	95 ± 17
Gymnoscopelus opisthopterus	0.12 ± 0.02	0.099 ± 0.02	79 ± 4
Gymnoscopelus fraseri	0.16 ± 0.004	0.157 ± 0.03	97 ± 18
Krefftichthys anderssoni	0.10 ± 0.04	0.096 ± 0.06	88 ± 13
Protomyctophum bolini	0.13 ± 0.02	0.123 ± 0.02	96 ± 2

Discussion

Despite their major role in the Southern Ocean food webs, Hg has been poorly investigated in Myctophids from the Southern Ocean. In this study, it was revealed that different intrinsic (e.g., sex, size) and extrinsic (e.g., year, sampling location) factors influence Hg levels in Myctophids. Furthermore, it was showed that they represent an important reservoir of bioavailable Hg across the Southern Ocean.

Spatial and temporal trends in muscle T-Hg concentration

In this study, it was examined for the first time short-term changes in T-Hg concentrations in the biomass-dominant myctophid community at South Georgia and the South Orkney Islands.

For most species, it were found no differences in T-Hg concentrations between sampling years, although the high standard deviation may mask the statistical difference of a possible decreasing trend shown in the species that were caught in all sampling years. *Electrona antarctica,* though, had statistically lower concentrations in 2016/17 than in the other years (see Figure 5.2). The lower T-Hg levels on the gills in 2016/17 than in 2007/08, may also suggest a lower bioavailability of Hg in the water. Such a decreasing pattern over time has been observed for squids from the same region at the same time scale ((Seco et al., 2020). Both declining pattern of T-Hg in squid and myctophid tissue concentrations along this 10-year period would suggest a global decrease in the bioavailability of Hg around South Georgia in the last decade. However, due to the comparatively shorter life spans of squid compared to myctophids (around 1 to 2 years in squid (Arkhipkin, 2004; Boyle and Rodhouse, 2005; Xavier et al., 2018a) verses ~ 4 to 6 years in myctophids (Gartner, 1991; Greely et al., 1999; Kawaguchi and Mauchline, 1982), acute changes in environmental pollutants are more likely to be reflected in these invertebrates due to a greater turnover in individuals within the population. In contrast, myctophids are likely to retain and integrate Hg contamination from the environment into their tissue over longer periods of exposure. Generally, longer-lived animals take longer to reflect any alteration in the habitat contaminants levels (Fränzle, 2006).

Habitat use has a major effect on Hg accumulation, since longer exposure in more contaminated areas will result in higher concentrations of this element in biota's

tissues (Desta et al., 2008; Le Bourg et al., 2019). In this study, little evidence was found of regional variation in T-Hg concentrations from the 3 species that were caught concurrently at South Georgia and the South Orkneys (*E. antarctica, G. braueri* and *G. nicholsi*), with regional differences only apparent for *E. antarctica. Electrona antarctica* specimens from South Georgia had lower concentrations of T-Hg than those from the South Orkneys, but samples from the South Orkneys Islands were, on average, 20 mm smaller, suggesting that the observed spatial pattern could reflect differences due to body size (i.e., bioaccumulation level) rather than differences in environmental factors. Indeed, other studies have shown that body size is typically positively correlated with T-Hg concentration in fish (Barghigiani et al., 2000; Bosch et al., 2016; Somers and Jackson, 1993); present study). However, *G. braueri* and *G. nicholsi* had similar T-Hg concentrations in both locations, regardless of body size, whilst an opposite trend was apparent for the pelagic euphausiid Antarctic krill (*Euphausia superba*), collected in the same surveys (Seco et al., 2019). In this last study, Antarctic krill collected around South Orkney Islands had higher T-Hg concentrations than those caught at South Georgia, a pattern attributed to the presence of sea ice around the South Orkney Islands. Both *G. braueri* and *G. nicholsi* feed on Antarctic krill at South Georgia and the South Orkneys [*G. braueri* 10% and *G. nicholsi* 25% of Index of relative importance (Saunders et al., 2018)], which suggests that the regional differences in Hg levels of this prey species should also be reflected in these myctophids if Hg accumulation by ingestion was the predominant pathway in the Scotia Sea [e.g. (Anderson et al., 2009; Paiva et al., 2008)]. However, the intake of T-Hg from other shorter-lived prey, such as copepods, small euphausiids and amphipods, and the long-term incorporation of Hg in the myctophid tissues might mask this krill-myctophid interaction on small spatial and temporal scales, such as those in the study of Seco et al. (2019). Myctophids migrate across the Scotia Sea (Saunders et al., 2018) and variability in time across this spatially extensive habitat may also mask any regional difference in Hg bioavailability. Such a question deserves further investigation to clarify the importance of spatial variation of Hg in myctophids and the role of their different prey in Hg bioaccumulation.

At a global scale, the concentrations of T-Hg found in the literature for myctophids studied globally were consistent with those reported here (Table 5.4). The approximately uniform concentrations are unexpected given the large range of habitats and species sampled, each with different ecology, depth distribution, growth,

and diet. It seems that myctophids, in a global perspective, have similar T-Hg concentrations, despite their ostensible differences in physiology, biology and ecology. Comparisons among these studies have to be done with caution, however, as method differences such as sample preservation, analytical approach and number of samples may invalidate direct comparisons between studies.

Among the different studies on Hg in myctophids, to the best of my knowledge, there are only two other studies on Southern Ocean myctophids (Bustamante et al., 2003; Cipro et al., 2018b), both of which are on samples from the sub-Antarctic Kerguelen Islands (Indian Ocean sector). Thus, *E. antarctica* from the Kerguelen Islands had T-Hg concentrations between 2 and 4 times lower (0.066 ± 0.015 μg g^{-1}; (Cipro et al., 2018b) than in all sampled years/locations, despite the high degree of overlap in size ranges of the analysed specimens between studies. In contrast, the two co-occurring *Gymnoscopelus* species, G*. fraseri* and *G. nicholsi,* had similar values to those examined in these previous studies (Bustamante et al., 2003; Cipro et al., 2018a), for equivalent sized individuals. Although there are differences in diet and prey field between the two regions (South Georgia and Kerguelen), *E. antarctica* from Kerguelen Islands appear to feed more frequently on *Thysanoessa macrura* (Clarke et al., 2018), while individuals from South Georgia feed mainly on *E. superba* or *Themisto gaudichaudii* (Saunders et al., 2018), this difference in diet alone seems unlikely to explain the regional differences in T-Hg, as levels on *T. macrura* from Kerguelen Islands were similar to *E. superba* from the Scotia Sea (Cipro et al., 2018b; Seco et al., 2019), so similar concentrations would be expected. Furthermore, species-specific estimates of Hg levels across the whole prey field of myctophids from both Kerguelen Islands and the Scotia Sea are unknown, and the role of these taxa in the transfer of Hg to myctophids and upper trophic levels remains unclear. A possible lower fraction of O-Hg in the prey species from the Kerguelen Islands compared to their counterparts in the Scotia sea (Seco et al., 2019) could lead to a lower rate of Hg accumulation in myctophids in this region, given the shorter turnover of fish tissue, and the more limited trophic transfer potential of inorganic Hg compare to O-Hg. These results reinforce the importance of species-specific analyses of Hg levels, when trying to understand spatial patterns in myctophid Hg accumulation patterns through regional predator-prey interactions.

Gender-based and ontogenetical patterns in T-Hg concentration in Southern Ocean myctophids

Physiological and biological factors, such as sex and size are known to influence Hg concentration in fish (Bastos et al., 2016; Dang and Wang, 2012; Gewurtz et al., 2011; Le Bourg et al., 2019). When assessing the effect of body size in T-Hg concentration in the muscle of Southern Ocean myctophids, it was observed a general positive trend of increasing T-Hg with increasing size, except for E. antarctica females, for which this relationship was negative. In other species, such as G. braueri, G. braueri, G. nicholsi, K. anderssoni and P. bolini, the correlations were not significant (see Figure 5.3). The positive relationship between T-Hg and size, in fish, is well established (Dang and Wang, 2012; Gewurtz et al., 2011; Somers and Jackson, 1993), with a tendency for increased Hg bioaccumulation, mainly in muscle tissue, with age. Also, larger fish tend to feed on larger prey that usually bioaccumulate elevated Hg concentrations (Chouvelon et al., 2014), which coupled with the tendency for lower Hg excretion rates in larger fish, (Trudel and Rasmussen, 1997) results in higher concentrations in larger (older) individuals. This is also true for the present results, where T-Hg increases with size in males, however, females, generally show a trend for lower T-Hg accumulation rates with size than males. Thus, females in some myctophid species may have a Hg excretion system that does not occur in males. Egg laying is a well-known Hg elimination mechanism for most oviparous animals [e.g., arthropods (Bakker et al., 2017; Saxton et al., 2013), crustaceans (Seco et al., 2019), amphibians (Bergeron et al., 2010), fish (Khadra et al., 2019; Sackett et al., 2013) and in seabirds (Brasso et al., 2012b; Pedro et al., 2015)], and this mechanisms might explain the different bioaccumulation patterns between sexes.

Table 4 – Standard length (SL; average or range; in mm), total mercury (T-Hg) average and/or range concentrations (μg g^{-1} dw), sampling location and year and preservation method of global myctophids from published data.

Species	SL	T-Hg	Range	Location	Year	Preservation method	Reference
Benthosema glaciale	39 - 53	0.11	-	Gulf Stream	1993	Formaldehyde / Ethanol	Martins et al. 2006
Benthosema glaciale	39 - 53	0.14	-	Gulf Stream	1952	Formaldehyde / Ethanol	Martins et al. 2006
Benthosema glaciale	39 - 53	0.15	-	Gulf Stream	1971	Formaldehyde / Ethanol	Martins et al. 2006
Benthosema glaciale	39 - 53	0.17	-	Gulf Stream	1976	Formaldehyde / Ethanol	Martins et al. 2006
Benthosema glaciale	39 - 53	0.2	-	Gulf Stream	1936	Formaldehyde / Ethanol	Martins et al. 2006
Benthosema glaciale	39 - 53	0.22	-	Gulf Stream	1963	Formaldehyde / Ethanol	Martins et al. 2006
Benthosema glaciale	39 - 53	0.45	-	Gulf Stream	1942	Formaldehyde / Ethanol	Martins et al. 2006
Bolinichthys distofax	8.7 - 8.8		0.174 - 0.218	North Pacific Ocean	2007	Frozen	Blum et al. 2013
Bolinichthys distofax	4.9 - 7.8		0.037 - 0.040	North Pacific Ocean	2011	Frozen	Blum et al. 2013
Bolinichthys indicus	30 - 35	0.16	-	Bermuda	1974	Frozen	Gibbs et al. 1974
Bolinichthys indicus	-	0.2	-	Sargasso Sea	1973	Frozen	Windom et al. 1973
Bolinichthys longipes	3.9 - 4.2		0.015 - 0.042	North Pacific Ocean	2007	Frozen	Blum et al. 2013
Bolinichthys longipes	4.5		0.017	North Pacific Ocean	2011	Frozen	Blum et al. 2013
Ceratoscopelus rnaderensls	65 - 75	0.377 ± 0.009	0.318 - 0.423	Azores	1978	Ethanol	Monteiro et al. 1996
Ceratoscopelus warmingi	45 - 60	-	0.21 - 0.26	Bermuda	1974	Frozen	Gibbs et al. 1974

Ceratoscopelus warmingii	-	0.2	-	Sargasso Sea	1973	Frozen	Windom et al. 1973
Diaphus mollis	-	0.1	-	Sargasso Sea	1973	Frozen	Windom et al. 1973
Diaphus mollis	25 -30	0.11	-	Bermuda	1974	Frozen	Gibbs et al. 1974
Electrona antarctica	48 - 78	0.066 ± 0.015	0.046– 0.100	Kerguelen Island	1997-99	Frozen	Cipro et al. 2018
Electrona rissoni	68 - 90	0.323 ± 0.045	0.145- 0.533	Azores	1995	Ethanol	Monteiro et al. 1996
Gymnoscopelus fraseri	65 - 82	0.197 ± 0.101	0.094– 0.424	Kerguelen Island	1997-99	Frozen	Cipro et al. 2018
Gymnoscopelus nicholsi	129 - 164	0.137 ± 0.047	0.096– 0.200	Kerguelen Island	1997-99	Frozen	Cipro et al. 2018
Gymnoscopelus nicholsi	144 ± 15	0.205 ± 0.126	0.157- 0.297	Kerguelen Island	1998	Frozen	Bustamante et al. 2003
Gymnoscopelus piabilis	114 - 162	0.179 ± 0.078	0.067– 0.333	Kerguelen Island	1997-99	Frozen	Cipro et al. 2018
Gymnoscopelus piabilis	151 ± 11	0.310 ± 0.126	0.177- 0.475	Kerguelen Island	1998	Frozen	Bustamante et al. 2003
Hygophum hygomii	-	0.3	-	Sargasso Sea	1973	Frozen	Windom et al. 1973
Hygophum hygomii	45 - 55	-	0.18 - 0.31	Bermuda	1974	Frozen	Gibbs et al. 1974
Lampanyctus photonotus	45 - 60	-	0.16 - .21	Bermuda	1974	Frozen	Gibbs et al. 1974
Lampanyctus pusillus	-	0.3	-	Sargasso Sea	1973	Frozen	Windom et al. 1973
Lampanyctus pusillus	25 - 30	0.34	-	Bermuda	1974	Frozen	Gibbs et al. 1974
Lobianchia dofleini	-	0.2	-	Sargasso Sea	1973	Frozen	Windom et al. 1973
Lobianchia dofleini	20 - 25	-	0.2 - 0.27	Bermuda	1974	Frozen	Gibbs et al. 1974
Myctophum punctatum	70 - 83	0.320 ± 0.035	0.15 - 0.367	Azores	1994	Ethanol	Monteiro et al. 1996
Myctophum punctatum	71 ± 76	0.078 ± 0.024	0.063 – 0.121	Bay of Biscay	2001-10	Frozen	Chouvelon et al. 2012

Notoscopelus caudispinosus	60 - 75	0.24	-	Bermuda	1974	Frozen	Gibbs et al. 1974
Notoscopelus caudispinous	-	0.2	-	Sargasso Sea	1973	Frozen	Windom et al. 1973
Notoscopelus kroeyeri	93 ± 23	0.105 ± 0.080	0.029 – 0.210	Bay of Biscay	2001-03	Frozen	Lahaye et al. 2006
Protomyctophum bolini	49 - 58	0.086 ± 0.022	0.059– 0.135	Kerguelen Island	1997-99	Frozen	Cipro et al. 2018

Inter-specific variations in T-Hg concentrations in muscle of Southern Ocean myctophids

Species-specific traits like feeding ecology, vertical and horizontal distribution, metabolism or physiology play an important role in Hg accumulation. In the present study, however, size and sex seemed to more important drivers for Hg accumulation in myctophids, as the smallest species had the lower Hg concentrations in the muscle (*K. anderssoni* and *P. bolini*), whilst concentrations were broadly congruent within the larger species. The lack of an inter-specific signal may be due to overlap in the distribution and diet patterns in the studied community. Most of the analysed species feed upon the same zooplankton prey species, such as the copepods *Metridia* spp., *Rhincalanus gigas, Pleuromamma robusta* and *Calanoides acutus* and the euphausiid *Thysanoessa* spp. (Lourenço et al., 2017; Saunders et al., 2018), with the exception of *E. antarctica* that feeds mostly on *E. superba* and on the hyperiid amphipod *T. gaudichaudii* (Saunders et al., 2018). Most species were found across the Scotia Sea, with all species co-occurring in the northern Scotia Sea region (see Figure 5.1). The vertical distribution patterns were also broadly similar among most species with the greatest concentrations of fish occurring above 400 m, particular at night (Collins et al., 2012; Saunders et al., 2019; 2018). Specific details of metabolic and physiological characteristics of myctophid are still unknown but, due to the phylogenetic proximity of the analysed species, one would assume that there should not be significant differences on Hg accumulation or excretion mechanisms among these species.

Tissue allocation of Hg in the Southern Ocean myctophids

Significant differences in T-Hg levels were observed in muscle, heart, liver and gill tissues in all species examined. Heart and liver tissues consistently showed higher concentrations than muscle and gill tissues. Large variations in Hg concentrations in heart tissue was consistently observed between individuals, probably due to differences in blood volume inside of the heart chambers. The presence of blood fluid in the heart would decrease the overall Hg content in this organ, as Hg in fish blood was between 3 and 15 times lower than muscle in different fish species (Eilser, 2010; Hamada et al., 1977; Shultz and Crear, 1976).

The liver, as an organ responsible for detoxification and transformation of toxins (Maršálek et al., 2007; Yamashita et al., 2005), was expected to have high concentrations of T-Hg. Indeed, higher T-Hg levels occur in the liver of fish than in

muscle tissue (liver/muscle index), such that a high liver: muscle contamination ratio is regarded to be an appropriate bioindicator for highly contaminated habitats (D. W. Evans and Dodoo, 1993; Gonzalez et al., 2005; Havelková et al., 2008). Although the Southern Ocean is thought of as a fairly pristine environment, it is already known that due to the Hg atmospheric cycle and special conditions for Hg methylation processes, greater than expected O-Hg concentrations occur in the region (Cossa et al., 2011). It is likely that this bioavailability of O-Hg can be reflected on the high liver/muscle index. Present results are in agreement with a previous study in the Southern Ocean on the bald rockcod *Pagothenia borchgrevinki,* which also showed Hg concentrations in the liver to be twice that in muscle (Honda et al., 2014).

The main uptake route of T-Hg in fish is through the diet, with only low percentages (~10%) of the whole body burden T-Hg is waterborne Hg absorbed by the gills (G. R. Phillips and Buhler, 1978). Nevertheless, muscle comprises most of the fish weight and therefore is the tissue with the highest ecological relevance, as it will be the carrier for most of Hg to the next trophic level. Myctophids are an important prey to several Southern Ocean predators (Sabourenkov, 1991) and are also the most abundant mesopelagic fish family in the Southern Ocean (70-200 Mt) (Catul et al., 2010). Assuming the conservative lowest muscle concentration found in the present study, for all species, and the lowest biomass estimation, this would mean that ≈ 1.82t of Hg are potentially bioavailable to Southern Ocean predators, in this family of mesopelagic fish alone. It is therefore likely that myctophids may be viewed as “Hg light bulbs” in the Southern Ocean, given their significant role in Hg bioaccumulation and trophic transfer processes in this remote environment.

Conclusions

The present study shows that Hg levels in myctophids suggest a decreasing trend along the last decade, indication a possible lower bioavailability of this element. Little evidence of regional effect was shown, even when looking to a global scale, as myctophids species had the same Hg range, around the world. Also, the Hg concentration generally increased with body size, a proxy for age, with the exception of *E. antarctica* females, that had a decreasing pattern, suggesting that egg laying may be an elimination route for this contaminant. Regarding Hg tissue allocation, as expected, higher concentration were found in the liver and heart followed by muscle

and gills. Finally, results show that size, sex and time are the main drivers for Hg accumulation for Southern Ocean myctophids.

6. – Mercury biomagnification in a Southern Ocean food web

Abstract

Biomagnification of mercury (Hg) in the Scotia Sea food web of the Southern Ocean was examined using the stable isotope ratios of nitrogen (δ_{15}N) and carbon (δ_{13}C) as proxies for trophic level and habitat use respectively. Hg and stable isotopes were measured in particulate organic matter (POM), 12 species of zooplankton, 2 species of squid, 7 species of mesopelagic myctophid fish, 6 species of notothenioid fish and 8 species of seabirds, sampled in two years, nearly a decade apart (austral summers 2007/08 and 2016/17). Samples from different groups showed extensive overlap in δ_{13}C values, with the exception of the seabirds, as expected because feathers are more enriched in $_{13}$C than muscle or other organs. δ_{15}N showed an increasing trend across taxonomic groups in the order zooplankton < squid < myctophid fish < notothenioid fish < seabirds. There were also significant differences with taxonomic groups reflecting variations in diet. Hg increased with trophic level, with lowest values in POM (0.0005 ± 0.0002 μg g^{-1}) and highest values in seabird chicks (3.88 ± 2.41 μg g^{-1} in brown skuas *Stercorarius antarcticus*). Differences in Hg levels depended on diet; species with higher δ_{15}N also had higher Hg levels due to the ingestion of prey with more Hg. Hg concentrations tended to be lower in 2016/17 than in 2007/08 for the species at mid-trophic levels (squid and fish), which may be explained by a decline in anthropogenic emissions of Hg over the last decade. In contrast, the opposite trend was found in the top predators (i.e. seabirds), with higher levels in the 2016/2017 samples. This may be explained by a shift in the Scotia Sea marine food web, as reduced availability of a key prey, Antarctic krill *Euphausia superba*, may have forced seabirds to feed on alternative, higher trophic-level prey, such as myctophids, which have higher Hg burdens. These results reinforce the key role of Antarctic krill in this region of the Southern Ocean, and its importance for Hg uptake and its subsequent passage through the food web.

Keywords: Contaminants; Stable isotopes; Antarctic; Trophic web

Introduction

Antarctica and the Southern Ocean comprises an unique ecosystem, with very particular cold environmental characteristics. Due to the isolation and extreme weather conditions, much of this region has remained relatively untouched by humans, and it is often considered by the scientific community to be a natural laboratory (Walton, 2013). Given the lack of any local manufacturing industry, anthropogenic mercury (Hg) emissions from within Antarctica are negligible. Nevertheless, there is very long-range dispersal of Hg, and other pollutants, from outside the region, so high concentrations can be found in Southern Ocean waters (Cossa et al., 2011). The global distillation process leads to the atmospheric transportation of pollutants, in the form of vapour, to the cold regions of the planet (Arctic and Antarctica) where these condense and the contaminants precipitate in rain or snow (O'Driscoll et al., 2005). Indeed, Antarctica can be seen as a gigantic fridge where atmospherically transported pollutants are stored in the ice fields (Eisele et al., 2008), and becoming bioavailable when glaciers and icebergs melt (Mastromonaco et al., 2017).

Hg is one of most toxic elements known, and its organic form (methyl mercury, $[CH_3Hg]_+$), is particularly noxious (Clarkson, 1992). Due to its high affinity to proteins (Bloom, 1992), mercury is highly bioaccumulative and biomagnifies along food webs (Ackerman et al., 2014; Coelho et al., 2013; Dehn et al., 2006). Methyl mercury is assimilated more efficiently than inorganic mercury, and hence is accumulated rather than excreted; therefore, the concentrations in a consumer are greater than in its prey (Monteiro et al., 1996). Hg concentrations are higher in upper trophic levels of the food web, and may become toxic for long-lived top predators (Goutte et al., 2014; Tartu et al., 2014; Tavares et al., 2013). Some Southern Ocean predators have very high Hg concentrations; for instance, wandering albatrosses, *Diomedea exulans,* have amongst the highest reported feather Hg concentrations of any seabird (Anderson et al., 2009; Cherel et al., 2018; Thompson et al., 1993; Tavares et al., 2013)).

Analyses of stable isotopes of nitrogen ($\delta_{15}N$) and carbon ($\delta_{13}C$) provide proxies of trophic level and carbon source (habitat), respectively. $_{15}N$ become enriched in tissues in a consistent way, on average by +3.4‰ on each level in food chains (Minagawa and Wada, 1984). In contrast, because of its low enrichment (on average by 1‰ with each trophic level), $\delta_{13}C$ provides a useful tool to determine habitat, indicating the relative importance of feeding inshore *versus* offshore, in shallow *versus*

deep water, or in particular water masses given the broad latitudinal gradient in the Southern Ocean (Hobson et al., 1994). Although some studies have reported Hg concentrations in lower trophic-level organisms and top predators in the Southern Ocean (e.g. Anderson et al. 2009), none have evaluated Hg biomagnification across all levels in the food web from particulate organic matter (POM) upwards, and used δ_{15}N to determine trophic structure.

Antarctic krill (*Euphausia superba*) is a key component of the food web in the Southern Ocean (Murphy et al., 2007), and one of the main trophic links between primary producers and predators (Everson, 2000; Xavier and Peck, 2015; Xavier et al., 2018). However, recent studies showed that there are alternative energy pathways up the Southern Ocean food web in years of low Antarctic krill (e.g.: copepods – amphipods – myctophid fish – high predators ((Murphy et al., 2007; Saunders et al., 2019)). These alternative pathways are unlikely to support the same biomass of predators given the greater energy loss with more steps in the food chain (Barnes et al., 2010). Low Antarctic krill abundance in some years has decreased the reproductive performance of top predators (Xavier et al., 2003; 2017) suggesting that these alternative pathways cannot entirely replace those involving Antarctic krill.

In the present study, we measured Hg concentration, δ_{13}C and δ_{15}N in a suite of species to elucidate Hg dynamics within the food web in the Scotia Sea, one of the most productive regions of the Southern Ocean. POM and tissues from organisms from multiple trophic levels were analysed, including: zooplankton (12 species), squid (2 species), myctophid fish (7 species), notothenioid fish (6 species), and seabirds (8 species). The majority of the sampling was in two austral summers, 9 years apart (2007/08 and 2016/17). The main objectives of the current work were, 1) to describe Hg dynamics in the Scotia Sea food web, 2) evaluate the Hg biomagnification levels across the Scotia Sea food web and 3) to evaluate possible changes in the Hg pathway from POM to top predators over the last decade.

Materials and Methods

Sampling

Samples were collected during marine cruises around South Georgia by the research vessel RRS *James Clark Ross* during the austral summers of 2007/08 and 2016/17 (cruises JR177 and JR16003 respectively). Background concentrations of Hg

in POM was determined from water samples collected in Niskin bottles deployed on a CTD (conductivity, temperature, depth) rosette fired at the chlorophyll a maximum (30 to 76m) and at 500m. The depths were chosen in order to compare the highest values of POM with lower values below the euphotic zone. POM was obtained from 5L of water vacuum-filtered through glass fibre filters (GF/F Whatman, 47mm). Zooplankton, squid and myctophid fish species were collected using an 8 or 25 m² mouth-opening Rectangular Midwater Trawl [RMT8 - mesh size reducing from 4.5 mm to 2.5 mm in the cod end; RMT25 - mesh size reducing from 8 mm to 4.5 mm in the cod end (Roe and Shale, 1979)]. The nets were rigged with two opening/closing nets that could be opened and closed remotely at different depths. Cephalopods, myctophid fish and squid were identified using appropriate taxonomic keys (Gon and Heemstra, 1990; Hulley, 1981; Nesis, 1987; Xavier and Cherel, 2009), measured and weighed on board, and samples preserved individually in separate ziplock bags at -20ºC for later laboratory analyses. Zooplankton species were identified and total length (TL) of each individual measured (Morris et al., 1992). Samples were either preserved in sample bags at -20ºC (JR16003) or in vials of 70% ethanol (JR177) (Fort et al., 2016).

Notothenioid fish were also captured in the austral summer of 2016/17 by the Fishing Vessel (FV) *Sil* during research survey SG17. Samples were obtained from bottom trawls using an FP120 trawl net with a standard steel bobbin rig, towed at 3.1-4.1 knots over a distance of 1.25-2.1 nautical miles, dependent on the prevailing sea conditions and bottom topography. Whenever possible, samples were identified on board but, in some cases, identification was not possible and otherwise performed at the home laboratory. Individuals were frozen at -20ºC for later laboratory processing.

Feathers were collected from seabird chicks at Bird Island (54°00' S, 38°03' W), South Georgia in austral summers 2007/08 and 2016/17. Chicks were sampled rather than adults because Hg and stable isotope ratios in their tissues reflect those of prey consumed during the chick-rearing period (Moreno et al. 2016), and all feathers are grown over the same period, so Hg and stable isotope ratios can be compared directly.

Laboratory procedures

POM filters were digested with HNO_3 4 mol l^{-1} for determination of Hg concentration (for details, see Pato et al., 2010). Analyses were performed by cold-

vapour atomic fluorescence spectrometry (CV-AFS) using a PSA model Merlin 10.023 equipped with a PSA model 10.003 detector, with tin chloride as a reducing agent (2% in 10% HCl). Total dissolved mercury concentrations were measured after chemical decomposition of each sample with potassium persulfate and irradiation by a UV lamp. Following irradiation, the excess of oxidant was reduced with a hydroxylamine solution (Pato et al., 2010).

Zooplankton were analysed as whole individuals. Only muscle tissue (freeze-dried for > 24 h) of squid and fish were analysed, as total Hg in muscle is larger than in other tissues and hence the most important in terms of transfer to predators. Feathers were first cleaned to remove surface contaminants using a 2:1 chloroform:methanol solution followed by two methanol rinses, and then oven dried for 48 h at 50 ºC.

Dried individual zooplankton, muscle samples and feathers were homogenized and analysed for total Hg by thermal decomposition atomic absorption spectrometry with gold amalgamation, using a LECO AMA-254 (Advanced Mercury Analyser) at the University of Aveiro, following (Coelho et al., 2008). Analytical quality control was performed using certified reference materials (CRM): For zooplankton we used TORT-2: 87 ± 3 %; TORT-3: 90 ± 8 %; for squid, NIST 2976: 85 ± 7%; ERM-CE278K: 92 ± 5%; TORT-3: 93 ± 8 %; for myctophids, DORM-4: 96 ± 13%; ERM-BB422: 100 ± 4%; for notothenioid fish, ERM-BB422: 98 ± 7%; for seabirds, TORT-3: 99 ± 3%. The analyses were repeated in duplicate or triplicate until the relative standard deviation was <10% for multiple aliquots.

For isotopic analyses, ~0.4 mg subsample homogenates were weighed out in to tin cups. A continuous flow mass spectrometer (Thermo Scientific Delta V Advantage) was coupled to an elemental analyzer (Thermo Scientific Flash EA 1112), at either the LIENS or MAREFOZ laboratories, to measure δ_{13}C and δ_{15}N values, and feather carbon and nitrogen content, respectively. Stable isotope ratios are expressed using standard δ notation relative to carbonate Vienna PeeDee Belemnite and atmospheric nitrogen. The internal laboratory standards were acetanilide. Observed analytical errors were <0.10‰ for both δ_{13}C and δ_{15}N values.

Statistical analysis

All analyses were performed using the R software version 3.4.2 (R Core Team, 2013). Mercury levels, $\delta_{13}C$ and $\delta_{15}N$ were tested for normality using Shappiro-Wilk normality test, and homogeneity of variance was tested using Bartlett's test. Wilcoxon rank and Kruskal–Wallis tests were used to compare mercury, $\delta_{13}C$ and $\delta_{15}N$ between trophic group and species. Wilcoxon rank test were used to compare mercury, $\delta_{13}C$ and $\delta_{15}N$ between years. Correlations were examined between $Log_{10}Hg$ and $\delta_{15}N$.

All values are presented as mean ± SD. The significance level for statistical analyses was α = 5%.

Results

$\delta_{13}C$ as a proxy of habitat

$\delta_{13}C$ values for samples collected in 2007/08 and 2016/17 are given in Tables 1 and 2, respectively. Among whole zooplankton, $\delta_{13}C$ ranged from -25.64‰ in *Euphausia superba* to -20.48‰ in *Parandania boecki*. For secondary consumers (squid and myctophid fish), the range in muscle tissue was from -25.67‰ (*Electrona antarctica*) to -20.15‰ (*Slosarczykovia circumantarctica*), while for notothenioid fish, it was -23.8‰ in *Champsocephalus gunnari* and -20.6‰ in *Dissostichus eleginoides.* Values of $\delta_{13}C$ in seabird feathers tended to be higher, ranging from -22.82‰ in Antarctic prions (*Pachyptila desolata*) to -15.24‰ in brown skuas (*Stercorarius antarcticus*) (Table 1). There was no statistical difference in $\delta_{13}C$ values between trophic groups, with the exception of the seabirds (Kruskal-Wallis test, H = 50.23, p<0.0001, in 2007/08; H = 63.69, p < 0.0001 in 2016/17).

Within the seabird group, significant differences in $\delta_{13}C$ were found between brown skuas and northern giant petrels (*Macronectes halli*) in 2007/08 (Kruskal-Wallis test, H = 15.932, p = 0.007; Table 1). In 2016/17, significant differences were found brown skuas and Antarctic prions, and between brown skuas and blue petrels (*Halobaena caerulea*; Kruskal-Wallis test, H = 22.552, p = 0.00096; Table 2).

Within the notothenid fish, significant differences in $\delta_{13}C$ were found between *C. gunnari* and *Notothenia rossii* and between *C. gunnari* and *N. gibberifrons* (Kruskal-Wallis test, H = 24.794, p = 0.0002; Table 2). No significant differences in $\delta_{13}C$ were

observed between myctophid fish in 2007/08 (Kruskal-Wallis test, H = 16.141, p = 0.061; Table 1) whereas, in 2016/17, $\delta_{13}C$ values were significantly lower in *E. antarctica* than in *Gymnoscopelus nicholsi* (Kruskal-Wallis test, H = 16. 751, p = 0.0049, Table 2).

$\delta_{13}C$ values were no significantly different the both sampling years in both squid (Wilcoxon rank test, W=3, p = 0.25, in 2007/08; W=0, p = 0.2 in 2017) and zooplankton (Kruskal-Wallis test, H = 5.1538, p = 0.741, in 2007; H = 19.19, p = 0.1388 in 2016/17; Tables 1 & 2).

Table 6.1- Mercury concentration (Hg), $\delta_{13}C$ and $\delta_{15}N$ of different species caught in 2007/08 around South Georgia (Mean ± standard deviation).

Species	n	Hg (μg g^{-1})	$\delta_{15}N$ (‰)	$\delta_{13}C$ (‰)
Zooplankton				
Euphausia superba	20	0.04 ± 0.02	3.47 ± 2.45	-23.17 ± 2.86
Euphausia triacantha	20	0.03 ± 0.01	6.71 ± 0.55	-22.15 ± 1.47
Gigantocypris sp.	15	0.03 ± 0.01	8.81 ± 0.98	-22.27 ± 0.41
Parandania boecki	15	0.02 ± 0.01	8.34 ± 0.42	-21.90 ± 1.70
Sagitta sp.	3	0.06 ± 0.01	8.21 ± 0.84	-22.97 ± 1.03
Salpa thompsoni	10	0.03 ± 0.01	4.40 ± 0.22	-23.75 ± 1.32
Themisto gaudichaudii	20	0.04 ± 0.02	5.86 ± 0.56	-24.10 ± 0.45
Thysanoessa sp.	20	0.05 ± 0.01	6.94 ± 0.32	-23.99 ± 0.71
Tomopteris sp.	6	0.03 ± 0.01	7.25 ± 0.73	-22.64 ± 0.65
Squid				
Galiteuthis glacialis	5	0.09 ± 0.01	7.66 ± 1.49	-23.58 ± 1.05
Slosarczykovia circumantarctica	5	0.02 ± 0.01	6.72 ± 0.25	-21.64 ± 1.88
Myctophids				
Electrona antarctica	5	0.18 ± 0.09	8.64 ± 0.96	-24.91 ± 0.66
Electrona carlsbergi	5	0.14 ± 0.02	8.31 ± 1.12	-20.91 ± 0.83
Gymnoscopelus braueri	5	0.16 ± 0.03	9.77 ± 0.56	-23.63 ± 0.44
Gymnoscopelus nicholsi	5	0.29 ± 0.12	8.70 ± 0.94	-22.00 ± 1.14
Gymnoscopelus opisthopterus	5	0.16 ± 0.06	10.81 ± 0.15	-23.12 ± 0.96
Krefftichthys anderssoni	5	0.04 ± 0.01	8.21 ± 0.95	-20.96 ± 0.19
Protomyctophum bolini	5	0.09 ± 0.01	8.87 ± 0.88	-21.82 ± 2.13
Seabirds				
Diomedea exulans	5	3.28 ± 0.63	14.83 ± 0.61	-18.29 ± 0.42
Macronectes giganteus	5	0.85 ± 0.12	11.80 ± 0.33	-18.23 ± 0.66
Macronectes halli	5	0.47 ± 0.33	13.07 ± 0.56	-18.68 ± 0.87
Stercorarius antarcticus	5	2.44 ± 1.08	12.63 ± 0.54	-15.59 ± 0.32
Thalassarche chrysostoma	5	1.25 ± 0.45	12.06 ± 0.56	-18.22 ± 1.47
Thalassarche melanophris	5	1.18 ± 0.46	11.83 ± 0.19	-17.30 ± 0.93

Table 6.2- Mercury concentration (Hg), $\delta_{13}C$ and $\delta_{15}N$ of different species caught in 2016/17 around South Georgia (Mean ± standard deviation).

Species	n	Hg ($\mu g\ g^{-1}$)	$\delta_{15}N$ (‰)	$\delta_{13}C$ (‰)
POM	12	0.0005 ± 0.0002	n.a.	n.a.
Zooplankton				
Euphausia frigida	35	0.05 ± 0.01	5.94 ± 0.04	-21.16 ± 0.48
Euphausia spinifera	20	0.07 ± 0.02	5.05 ± 0.66	-22.79 ± 0.09
Euphausia superba	30	0.01 ± 0.03	3.38 ± 0.38	-24.98 ± 0.66
Euphausia triacantha	30	0.02 ± 0.03	7.08 ± 0.62	-22.72 ± 0.17
Euphausia vallentini	30	0.01 ± 0.03	3.00 ± 0.78	-21.6 ± 0.18
Gigantocypris sp.	20	0.07 ± 0.01	7.07 ± 1.09	-24.71 ± 0.35
Parandania boecki	30	0.12 ± 0.03	7.55 ± 0.28	-21.82 ± 1.90
Themisto gaudichaudii	40	0.06 ± 0.02	5.61 ± 0.79	-22.46 ± 0.52
Thysanoessa sp.	40	0.02 ± 0.01	5.62 ± 0.29	-23.99 ± 0.64
Squid				
Galiteuthis glacialis	5	0.02 ± 0.01	7.4 ± 0.09	-24.68 ± 0.49
Slosarczykovia circumantarctica	5	0.01 ± 0.01	6.23 ± 0.78	-23.13 ± 0.43
Myctophids				
Electrona antarctica	5	0.12 ± 0.07	7.41 ± 0.72	-24.54 ± 0.34
Gymnoscopelus braueri	5	0.12 ± 0.06	9.58 ± 0.97	-24.37 ± 0.61
Gymnoscopelus nicholsi	5	0.3 ± 0.17	9.73 ± 0.03	-20.74 ± 0.34
Krefftichthys anderssoni	5	0.05 ± 0.01	8.48 ± 0.5	-23.02 ± 0.1
Protomyctophum bolini	5	0.1 ± 0.03	7.98 ± 0.75	-23.43 ± 0.7
Benthopelagic fish				
Chaenocephalus aceratus	5	0.11 ± 0.02	10.79 ± 1.12	-21.66 ± 0.5
Champsocephalus gunnari	11	0.02 ± 0.01	9.18 ± 0.56	-22.95 ± 0.44
Dissostichus eleginoides	5	0.2 ± 0.06	11.86 ± 0.75	-21.76 ± 0.77
Notothenia gibberifrons	8	0.18 ± 0.07	11.3 ± 0.54	-19.93 ± 0.78
Notothenia rossii	8	0.18 ± 0.08	10.78 ± 1.67	-21.39 ± 0.58
Patagonotothen guntheri	5	0.1 ± 0.03	8.34 ± 0.56	-23.43 ± 0.32
Seabirds				
Halobaena caerulea	5	0.62 ± 0.23	9.25 ± 0.50	-21.14 ± 0.67
Macronectes giganteus	5	1.68 ± 0.27	11.52 ± 0.41	-20.46 ± 0.38
Macronectes halli	5	2.05 ± 0.8	11.58 ± 0.65	-19.69 ± 1.12
Pachyptila desolata	5	0.22 ± 0.14	8.59 ± 0.78	-21.58 ± 0.73
Stercorarius antarcticus	5	3.88 ± 2.41	11.88 ± 1.13	-18.79 ± 0.9
Thalassarche chrysostoma	5	1.43 ± 0.5	11.78 ± 0.67	-19.91 ± 0.4
Thalassarche melanophris	5	1.51 ± 0.46	11.52 ± 0.35	-20.32 ± 0.63

δ_{15}N as a proxy of trophic position

δ_{15}N values for each species in 2007/08 and 2016/17 are given in Tables 1 and 2, respectively. δ_{15}N was significantly higher in seabirds (7.69‰ in Antarctic prions to 15.5‰ in wandering albatrosses (*Diomedea exulans*) and notothenioid fish (only in 2016/17; 7.71‰ in *Patagonotothen guntheri* to 14.41‰ in *N. rossii*) than in the other taxonomic groups (Kruskal-Wallis test, $H = 59.184$, $p < 0.0001$, $H = 80.284$, $p < 0.0001$, respectively), where values decreased from myctophid fish (6.69‰ in *E. antarctica* to 10.91‰ in *G. opisthopterus*), to squid (5.68‰ in *S. circumantarctica* to 9.38‰ in *Galiteuthis glacialis*) and then zooplankton (1.73‰ in *E. superba* to 8.81‰ in *Gigantocypris* sp.).

In 2007/08, there were significant differences in δ_{15}N between seabird species, such as between wandering albatrosses and black-browed albatrosses (*Thalassarche melanophris*), between wandering albatrosses and grey-headed albatrosses (*Thalassarche melanophris*) and between wandering albatrosses and southern giant petrels (*M. giganteus*; Kruskal-Wallis test, $H = 20.979$, $p = 0.0008$, Table 1). In 2016/17, there were also differences among seabird species, between brown skuas and Antarctic prions and between Antarctic prions and grey-headed albatrosses (Kruskal-Wallis test, $H = 21.756$, $p = 0.00134$, Table 2).

δ_{15}N values also significantly differed between species of notothenioid fish (only sampled in 2016/17), namely between *C. gunnari* and *N. gibberifrons, C. gunnari* and *D. eleginoides, P. guntheri* and *N. gibberifrons* and between *P. guntheri* and *D. eleginoides* (Kruskal-Wallis test, $H = 25.847$, $p < 0.0001$, Table 2). No significant differences in δ_{15}N were observed between species of myctophid fish (Kruskal-Wallis test, $H = 7.9957$, $p = 0.2384$, in 2007/08; $H = 12.888$, $p = 0.2445$; Table 1 & 2) and squid (Wilcoxon rank test, $W=11$, $p = 0.3929$, in 2007/08; $W=6$, $p = 0.2$ in 2016/17; Tables 1 & 2) in either sampling years. Significant differences in δ_{15}N were only observed between two species of zooplankton; *E. superba* and *P. boecki* in 2007/08 (Kruskal-Wallis test, $H = 20.03$, $p = 0.01022$; Table 2).

Mercury concentrations

In both sampling years, seabirds had the highest Hg concentrations (0.12 μg g^{-1} in Antarctic prions to 7.17 μg g^{-1} in brown skuas), followed by myctophid fish (0.025

μg g^{-1} in *Krefftichthys anderssoni* to 0.352 μg g^{-1} in *G. nicholsi*) and notothenioid fish (0.007 μg g^{-1} in *C. gunnari* to 0.343 μg g^{-1} in *N rossii*), squid (0.012 μg g^{-1} in *S. circumantarctica* to 0.066 μg g^{-1} in *G. glacialis*) and zooplankton (0.006 μg g^{-1} in *E. superba* to 0.141 μg g^{-1} in *P. boecki*). POM had the lowest Hg concentrations (Kruskal-Wallis test, $H = 59.75$, $p < 0.0001$, in 2007/08; $H = 82.42$, $p < 0.0001$ in 2016/17).

Significant differences in Hg levels were observed among seabird species in 2007/08, between brown skuas - northern giant petrels and wandering albatrosses - northern giant petrels (Kruskal-Wallis test, $H = 22.621$, $p = 0.00039$; Table 1). In 2016/17, there were differences in Hg concentrations between brown skuas and Antarctic prions, northern giant petrels - Antarctic prions, southern giant petrels and Antarctic prions and brown skuas and blue petrel (Kruskal-Wallis test, $H = 24.175$, $p = 0.00048$; Table 2).

In notothenioid fish, there were also significant differences (Kruskal-Wallis test, $H = 27.795$, $p < 0.0001$) between the species *C. gunnari* and *N. gibberifrons, C. gunnari* and *N. rossii* and between *C. gunnari* and *D. eleginoides* (Table 2). Myctophids showed differences between the species *G. nicholsi* and *P. bolini* and *G. nicholsi* and *K. anderssoni* in both sampling years (Kruskal-Wallis test, $H = 15.317$, $p = 0.018$, in 2007; $H = 13.52$, $p = 0.019$ in 2016/17; Table 1 & 2). There were no significant differences in Hg levels between squid species in both years (Wilcoxon rank test, $W=15$, $p = 0.057$, in 2007; $W=4$, $p = 0.8$ in 2016/17) nor between zooplankton species in 2007/8 (Kruskal-Wallis test, $H = 7.4231$, $p = 0.492$). However, in 2016/17 there were significant differences between *E. superba* and *P. boecki* (Kruskal-Wallis test $H = 20.445$, $p = 0.0088$ in 2016/17).

Significant positive linear regressions were found between $\log_{10}$ (Hg) and δ_{15}N across all species in both years ($Y = 0.2668*X - 1.9276$, $r_s = 0.8756$, $p < 0.0001$ in 2007/8; $Y = 0.2001*X - 1.2826$, $r_s = 0.7326$, $p < 0.0001$ in 2016/17).

Comparison between years

Five of the eight seabird species were sampled in both years (grey-headed albatrosses, brown skuas, black-browed albatrosses, northern giant petrels and southern giant petrels). Significant differences between 2007/08 and 2016/17 in δ_{15}N were only found in northern giant petrels (Wilcoxon rank test, $W=25$, $p = 0.0079$), and

in δ_{13}C were apparent in grey-headed albatross (Wilcoxon rank test, W=25, p = 0.0079), brown skuas and southern giant petrels (Wilcoxon rank test, W=25, p = 0.0079). Hg concentrations were generally higher in 2016/17 than 2007/08 (Figure 2), but this was significant only for northern giant petrels (Wilcoxon rank test, W=0, p = 0.0079) and southern giant petrels (Wilcoxon rank test, W=0, p = 0.0079).

In myctophids, five of the seven species were caught in both sampling years (*E. antarctica, G. nicholsi, G. braueri, K. anderssoni* and *P. bolini*). δ_{15}N and δ_{13}C were similar in all species between the two sampling years. Unlike seabirds, Hg levels were lower in 2016/17 than 2007/08, being statistically lower in *E. antarctica (*Wilcoxon rank test, W=3, p = 0.002). Six species of zooplankton were caught in both sampling years (*E. triacantha, Parandania boecki, Gigantocypris sp., E. superba, Thysanoessa sp., Themisto gaudichaudii*). There were no differences between zooplankton species in δ_{15}N, δ_{13}C or Hg between years.

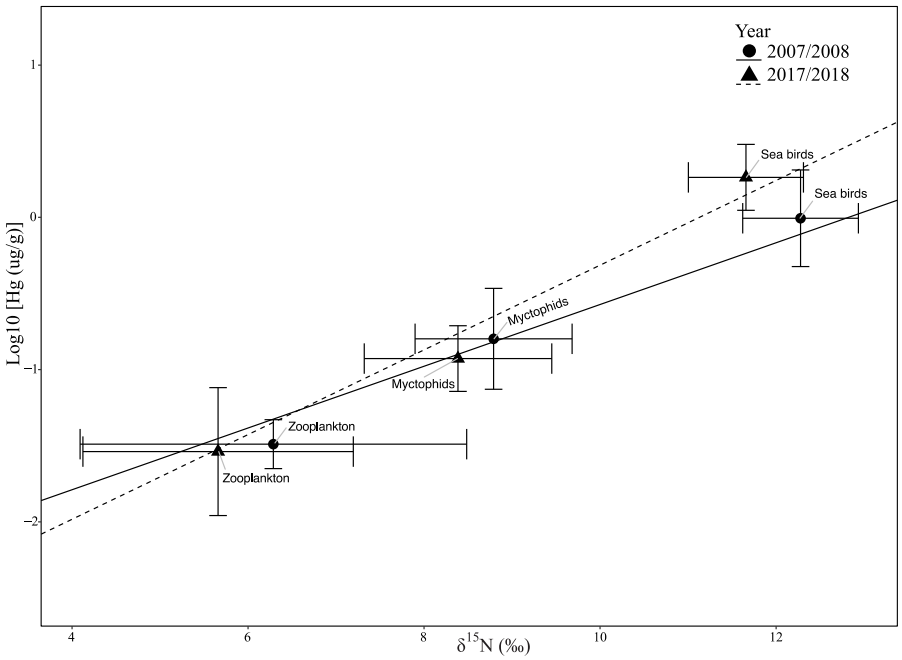

Figure 6.2 – Mercury concentration on a log 10 scale versus δ_{15}N for zooplankton, myctophids and seabirds that were caught in 2007/08 and in 2016/17.

There were significant positive linear regression between Log_{10} (Hg) and δ_{15}N in the species that were sampled in both years (Y = 0. 2028*X - 2.6008, r_s = 0. 8045477, p < 0.0001 in 2007/8; Y = 0.2782*X - 3.0960, r_s = 0.9081823, p < 0.0001 in 2016/17) (Fig. 2). The slope of the relationship was higher in 2016/17 (0.2782) than 2007/08 (0.2028).

Discussion

A number of studies have used stable nitrogen isotope ratios to understand the biomagnification of Hg within the food web in different ecosystems (Cabana and Rasmussen, 1994; Lavoie et al., 2013). To the best of our knowledge, this is the first study to evaluate the pathway of Hg in a Southern Ocean food web from POM to top predators such as seabirds.

Habitat evaluation

The first assumption when evaluating food web structure and links using stable isotope ratios is that all the analysed specimens share broadly the same habitat; this rules out the potential confounding effect of spatial variation in baseline $\delta^{15}N$. $\delta^{13}C$ was used here as a proxy for habitat (Kelly, 2000), and all taxonomic groups in our study had similar $\delta^{13}C$ values, with the exception of seabirds (Tables 1, 2). However, this difference was expected as feathers are more enriched in ^{13}C than blood, muscle and internal organs (Cherel et al., 2014; Kelly, 2000). In addition, based on tracking data during chick-rearing, all the sampled seabird species forage in the areas where mid-trophic-level species (myctophid, squid and zooplankton) are generally caught [wandering albatrosses (Jiménez et al., 2015); southern giant petrels and northern giant petrels (Granroth-Wilding and Phillips, 2018a); brown skuas (Carneiro et al., 2014); Antarctic prions and blue petrel (Navarro et al., 2013); black-browed albatrosses and grey-headed albatrosses (Phillips et al., 2004b)]. As expected, there were small differences in $\delta^{13}C$ among some species within taxonomic groups, indicating a limited degree of divergence in foraging habits, but this has no material effect on our conclusions about trophic level based on $\delta^{15}N$.

Trophic level in relation with mercury concentrations

The sampling provided two snapshots, 10 years apart, of Hg concentrations along the Scotia Sea food web, from POM to top predators. Values of Hg were the lowest in POM, at the base of the trophic web, followed by zooplankton, in which there was some variation as the species range from being predominantly herbivorous such as Antarctic krill (Quetin and Ross, 1991) to being predatory, such as *Themisto gaudichaudii* (Havermans et al., 2019). Squid and myctophid fish link zooplankton to

the top predators (seabirds) and, in terms of Hg dynamics, exhibit some degree of variation between individuals and species in Hg levels [for more details see (Seco et al., 2020) Seco et al. (unpublish data [myctophids])]. Generally, the highest Hg concentrations were found in the higher predators (notothenioid fish and seabirds). Our first clear inference is therefore that Hg levels increase along with δ_{15}N. Indeed, this relationship has been found in other studies in the region (Anderson et al., 2009). It partly reflects biomagnification through food chains, and partly bioaccumulation, as top predators usually live longer than lower trophic-level species and therefore accumulate more Hg. This does not pertain to seabird chicks, however, as they were only weeks to months old when sampled (Lavoie et al., 2013).

Trophic links to the two groups of top predators analysed here, seabirds and notothenioid fish (the latter taxa is sometimes considered to be mesopredators, as they can be predated by larger species), can be considered as two parallel paths in the Scotia Sea food web, because they occupy similar trophic levels. Feathers are the main pathway for Hg elimination in seabirds, and therefore levels of Hg are typically higher in this tissue than in muscle, at least for chicks (Ackerman et al., 2008; Bearhop et al., 2000; Thompson et al., 1990).

Based on our results, the seabirds can be differentiated into three different groups; *Wandering* albatrosses and brown skuas presented higher Hg concentrations than all the other species, followed by grey-headed albatrosses, black-browed albatrosses , southern giant petrels and northern giant petrels, with intermediate Hg levels, and Antarctic prions and blue petrel with the lowest levels. This can be explained largely by trophic level, given the positive correlation between Hg level and δ_{15}N. Wandering albatrosses feed on higher trophic level prey, mainly large fish and squid (Moreno et al., 2016), which had relatively high Hg levels in our study. *Brown skuas* have a diverse diet, feeding on Antarctic fur seals *Arctocephalus gazella* carrion (including placentae), seabirds and occasionally fish or squid which they obtain via kleptoparasitism (Phillips et al., 2004a). High diet diversity probably also explains the high variability in Hg and δ_{15}N in grey-headed albatrosses and black-browed albatrosses, which feed on fish, squid and crustaceans (Prince, 1980a). Northern giant petrels and southern giant petrels are more generalist, feeding both on carrion on land (from Antarctic fur seals and penguins) and Antarctic krill, squid and other seabirds at sea (Hunter, 1983). Their broad dietary niches, and sex differences in feeding habits

(males predominantly scavenge during the early-mid breeding season, whereas females mostly forage at sea; (Gonzáles-Solís et al., 2000; Granroth-Wilding and Phillips, 2018b) explains the large standard deviations of δ_{15}N and Hg in northern giant petrels from 2007/08. The smaller seabirds Antarctic prions and blue petrel feed mainly on zooplankton species, including *E. superba* (Prince, 1980b); the latter have lower Hg levels than fish and squid.

Differences were also found among the notothenioid fish species. Hg concentrations were lower in *C. gunnari* than all the other species. These differences, only in part reflect trophic level, as δ_{15}N was similar in *C. gunnari* and *P. guntheri*. Nor does the discrepancy appear to mirror what is known about diet, as both species are considered to feed mostly on euphausiids and amphipods (Collins et al., 2008; di Prisco et al., 1991). Furthermore, body size can also be discounted as *C. gunnari* were on average 67mm bigger than *P. guntheri*, and larger fish tend to have higher Hg concentrations in species with similar growth rates (Dang and Wang, 2012; Gewurtz et al., 2011). Instead, the low Hg concentrations seem more likely to relate to the highly specialized physiological characteristics of *C. gunnari*, including the absence of haemoglobin (Sidell and O'Brien, 2006), which is associated with lower metabolic rates (Johnston and Camm, 1987). However, this would need to be confirmed by further study.

As in seabirds, the notothenioid fish species at higher trophic levels had higher Hg concentrations. *D. eleginoides* feed mostly on fish, squid and crustaceans (Collins et al., 2010; 2007; Seco et al., 2015). In contrast, *N. gibberifrons* and *N. rossii* feed on diverse prey from algae to amphipods, euphausiids and other fish (Casaux et al., 1990), which would also explain the large variability in their δ_{15}N. The diet of *C. aceratus* is dominated by crustaceans and fish (Reid et al., 2007). Together, the results highlight the important role of diet in Hg bioaccumulation in these species.

The slope of the linear regression between log(Hg) concentration and δ_{15}N, also known as the trophic magnification slope (TSM), is an indicator of Hg biomagnification potential in a food web (Lavoie et al., 2013; Yoshinaga et al., 1992). Values of the TSM values for the Scotia Sea ecosystem were 0.267 for 2007/08 and 0.200 for 2016/17, which are both within the range of those previously reported for polar regions (0.21 ± 0.07; (Lavoie et al., 2013). Lavoie et al. (2013) suggested that the slower growth rates and slower Hg excretion rates, due to colder temperatures,

and the shorter food chains could lead to greater biomagnification of Hg in higher latitude compared to lower latitude regions.

Interannual variation of mercury concentration of the Southern Ocean food web

Comparing species sampled in both years, there was a decrease in Hg over time in the mid trophic-level groups, as reported in detail in (Seco et al., 2020) and Seco et al (unpublish data [myctophids]). The low ratio of organic Hg in zooplankton (Seco et al., 2019) may explain why the decrease in the mid trophic-level groups was not also apparent at the base of the food web. In general, Hg concentrations were higher in the seabirds sampled in 2016/17 than in 2007/08, which is the opposite trend to that for the mid trophic-levels (Seco et al., 2020). This was unexpected as Hg body burdens of predators should, in theory, reduce if there is less bioavailable Hg in their prey (Atwell et al., 1998).

An alternative hypothesis is that there was a change in the main trophic pathway (Ward et al., 2010). Differences in Hg levels in producers can directly influence Hg concentration in top predators, as well as changes in the productivity and food web structure (Ward et al., 2010). The Scotia Sea food web is centred on Antarctic krill (Murphy et al., 2007), the abundance of which can determine the reproductive success and survival of dependent predators (Grünbaum and Veit, 2003; Lynnes et al., 2004; Seyboth et al., 2016). In years with low Antarctic krill abundance, predators switch to alternative food sources such as salps, amphipods, myctophid fish or squid (Murphy et al., 2007). In our study, Antarctic krill had amongst the lowest Hg concentration, so if less available, predators likely switched to prey with higher Hg burdens. Abundance of Antarctic krill based on acoustic surveys to the northwest of South Georgia was much higher in 2007/08 than 2016/17 (Fielding et al., 2014), BAS unpublished data). Our results therefore illustrate the knock-on effect of variable Antarctic krill abundance on Hg dynamics, particularly the effect on higher predators throughout the food web. This is of particular concern as some studies suggest there has been a decadal decline in abundance of Antarctic krill in the Scotia Sea (Atkinson et al., 2004; (Hill et al., 2019)). In years of low Antarctic krill abundance, predators not only have to cope with the stress of reduced prey availability, but with a concomitant increase in Hg ingestion. Our results reinforce the crucial role of krill in the Southern

Ocean food web, not only as an energy source to predators but as a crucial link in trophic and contaminant pathways.

7. – General discussion

In this Chapter, an holistic overview of the previous chapters is given, as well as an integrative discussion of the major findings of all chapters.

Antarctica in a global context

Although remote, Antarctica and the Southern Ocean are key components of the planet system. The interlinks and dependencies between this region and the rest of the world go beyond the connecting oceanic and atmospheric currents, and include geopolitics and international agreements (Antarctic treaty), fisheries (CCAMLR), tourism (IATA) and international scientific collaboration (SCAR). To outline where the future will take the Earth, it is crucial to understand historical patterns, and Antarctic ice provides an exceptional window into planet Earth's past. Therefore, scientific research in Antarctica and the Southern Ocean are important for understanding the fate of our planet. In today's world, climate change, fishing and pollution are the main threats to the global oceans and to the fine balance of the Southern Ocean ecosystem. It is therefore important to evaluate how these pressures are presently affecting the Antarctic Environment, and how they may do so in the future.

Global temperatures are increasing, sea levels are rising, ocean circulation is altered, and these and other planetary physical changes are directly affecting marine ecosystems and organisms (Brierley and Kingsford, 2009). Global warming is one of the most recognized climate change impacts and its effects are being particularly evident in the Antarctic region (Mulvaney et al., 2012). Temperature in the Antarctic Peninsula has risen at a rate of + 1.09 ºC per decade from 1971 to 2000 (Turner et al., 2014). A projection looking 50 years into the future, even in a very optimistic scenario, predicts air temperature to rise + 0.9 ºC and Southern Ocean water + 0.7 ºC by 2070. If no changes are made to present climate politics, like the greenhouse gases emissions, temperature increase estimations go + 3 ºC in air temperature and + 1.9 ºC in water temperature by 2070 [Figure 7.1; (Rintoul et al., 2018)]. The raising temperatures have a considerable effect on the cryosphere, for instance in West Antarctica, where 24% of ice sheet by area is in a state of dynamic imbalance (Shepherd et al., 2019).

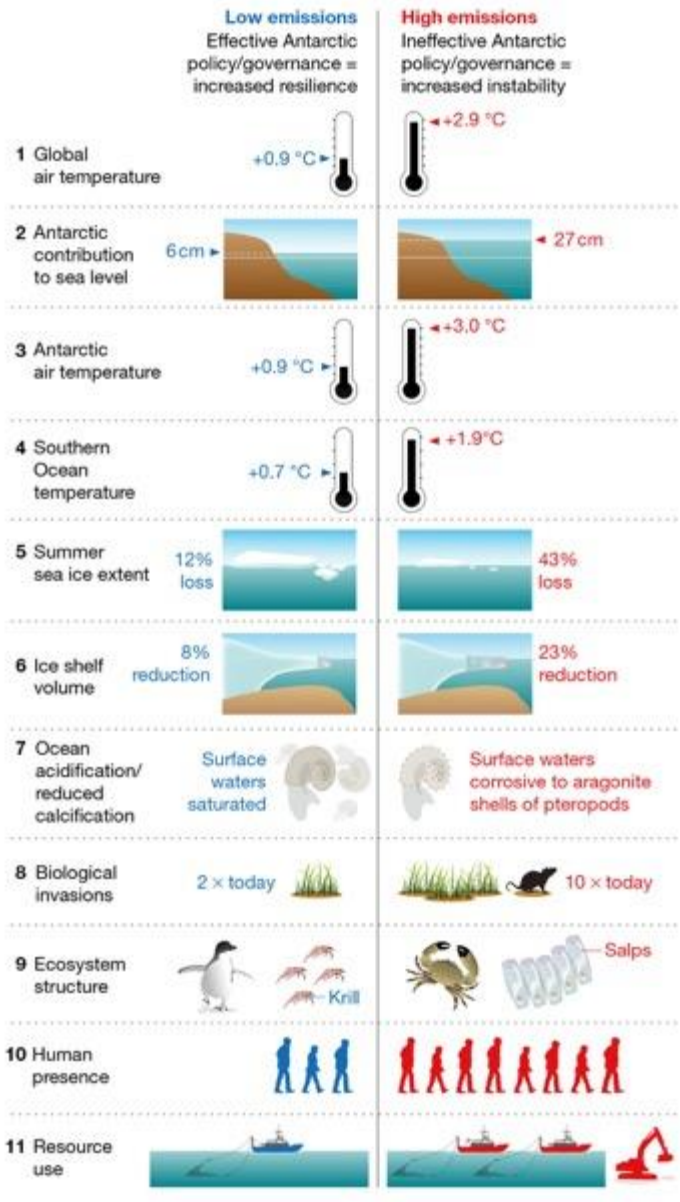

Figure 7.1. Illustration of Antarctica and the Southern Ocean in 2070, under two scenarios. Low greenhouse gas emission – blue; High emission – red. Illustration from Rintoul et al. (2018).

Changing Southern Ocean ecosystems

Southern Ocean biota is showing the negative effects of global warming. Antarctic krill is one of the species that is reflecting this threatening alteration, with stocks either declining (Atkinson et al., 2004) or shifting southwards towards colder

water (Atkinson et al., 2019), and being replaced by other species (e.g. salps) in the food web of regions they have left.

The over exploration of marine resources by the fishing industry is an added pressure to biota that has already to cope with environmental stress caused by climate change. With fisheries around the world depleting (Pikitch, 2012), the fishing industry is looking into the potential of exploration of the Southern Ocean resources. Antarctic krill has always been a species of interest to fishing industry due to its high biomass, but a combination of improved fishing techniques and recently discovered uses for Antarctic krill oil (omega-3 nutritional supplements for humans) have increased the commercial interest in this species. This increased interest adds extra pressure in Antarctic krill populations at a time when populations appear already to be in a long-term decline (Atkinson et al., 2004). Antarctic krill fishing quota is set at 1% of total biomass [379 000 000 tonnes; (Atkinson et al., 2009)] but presently catch is about 0.3% of total biomass. Although the fishing catch is a relatively low percentage, with the present negative effects of climate change in the abundance of Antarctic krill, the sustainability of Antarctic krill is being revaluated. The Marine Stewardship Council (MSC), an international non-profit organisation that evaluates whether fisheries are 'sustainable', is presently re-assessing the Chilean Antarctic krill fishery (Nicol et al., 2012).

Mercury in the Southern Ocean fisheries

The majority of mercury taken in by humans comes from the consumption of seafood (Schartup et al., 2019). With more marine resources being exported from the Southern Ocean for direct or indirect human consumption, it is imperative to quantify the mercury concentrations in the exported species. Antarctic krill is harvested in the Southern Ocean to be consumed directly as krill meat or processed into omega-3 oil, and indirectly as krill meal to the aquaculture industry. The concentrations of total mercury and percentage of organic mercury were relatively low when compared with commercially fished species (e.g.: *Thunnus alalunga,* 1.37 μg g^{-1}, 100% (Storelli et al., 2002)**;** *Dicentrarchus labrax,* 1.14 μg g^{-1}, 74.5% (Coelho et al., 2013). However, the toxic effects of chronic exposure are felt even at low levels of mercury [e.g. neurological problems (Bakir et al., 1973); liver and kidney failure (Tan et al., 2009); cardiovascular disease (Virtanen et al., 2005)], and the high affinity of organic mercury

to proteins, making it very hard to excrete, lead to bioaccumulation in the tissues (Tan et al., 2009). It has to be noted that a long-term continuous consumption of Antarctic krill products as krill meat or krill omega-3 oil, may have a potential negative impact in human health.

On the other hand, toothfish species as predatory and long-lived species have relatively higher mercury concentrations [1.9 μg g^{-1} (Marko et al., 2014)]. Toothfish show different mercury concentration according to the fishing area (Marko et al., 2014), what may lead to market issues. With markets selling fish from different locations with wrong labelling, the consumer may eventually be misled to what species they are buying, from where and what are the expected mercury levels (Marko et al., 2014). Therefore, it is crucial in the first instance to stablish more restrictive rules to the correct identification of the selling product, and secondly to acknowledge and inform the consumers that mercury may be a problem.

This thesis in the context of the recent Antarctic Science agenda

In 2014, when this PhD project was starting to be shaped, a general plan was set out for required Antarctic science as part of the 'Antarctica and Southern Ocean science horizon scan' (Kennicutt et al., 2014). Several questions were raised in that document, including a couple regarding contaminants [Q52 & Q53 (Kennicutt et al., 2014)]. These scientific queries were drivers for this thesis, particularly the question "What is the exposure and response of Antarctic organisms and ecosystems to atmospheric contaminates (e.g. black carbon, mercury, sulphur, etc.), and are the distributions of these contaminants changing?" (Kennicutt et al., 2014).

5 years have now passed since these questions were raised by the scientific community, and this thesis brings new insights to better answer them. Our results show that all the analysed organisms have some degree of mercury contamination – contamination that even at low concentrations may cause significant effects (Lebel et al., 1998; Mahboob et al., 2001). Furthermore, in some cases Southern Ocean species appear to have higher mercury levels than similar species from areas closer to human populations (table 3.2, Chapter 3). This reveals that the Antarctic is not as "pristine" as perhaps thought (Szopińska et al., 2017). Organisms sampled closer to ice areas had significantly higher mercury concentrations, suggesting that sea ice plays an important role in the mercury cycle. Indeed, Antarctic sea ice and snowpack can work

as mercury sinks, mercury being trapped in it when it precipitates and released again, making it again bioavailable, on melting. A recent study estimated that up to 60 metric tonnes of mercury may be trapped annually on the Antarctic plateau (Spolaor et al., 2018). However, on a more optimistic note, in this thesis it was possible to establish a link between the decreasing trend in the global mercury atmospheric emission over the last decade (Y. Zhang et al., 2016) and decreasing concentrations in Southern Ocean organisms.

The declining levels of mercury in Antarctic marine biota reported in this thesis are a positive sign, meaning that this ecosystem is starting to be less impacted by atmospherically transported mercury contamination (Cossa et al., 2011), but this region of the planet may face a new source of mercury. As stated before, global warming is particularly affecting the Antarctic region (Mulvaney et al., 2012). Even on the most optimistic climate scenarios, air and water temperature will rise in the future (Rintoul et al., 2018), which will lead to higher rates of sea ice loss and glacial melt (Shepherd et al., 2019), and hence mercury release. This effect (release via ice melt) has previously reported for DDT (Dichlorodiphenyltrichloroethane), and Adélie penguins (*Pygoscelis adeliae*) at the Western Antarctic Peninsula, which have shown no decline in DDT levels since 1964 even though production was banned in 1972. Glacial meltwater is possibly the present source of DDT to Antarctic organisms (Geisz et al., 2008). Input of “glacier” mercury caused by global warming may supersede the decrease in atmospheric mercury emission, increasing the bioavailability of this contaminant to Antarctic organisms.

Some scientific advances allowed to answer the questions raised in 2014 by the scientific community regarding effects and distributions of mercury (Kennicutt et al., 2019). There is still, however, a knowledge gap regarding several contaminant-associated issues, such as the sources of contaminants deposited in the ice sheets and transported to the ocean, the base levels for other contaminants (e.g., trace elements like silver, lead and others), the interactions between contaminants (e.g., selenium reduces the toxicity effects of mercury), and how climate change will influence their distribution and availability. The present interest of the scientific community in the role of contaminants in Antarctica is highlighted by the recent creation of an action group in the Scientific Committee on Antarctic Research (SCAR), dedicated to the Input Pathways of Persistent organic pollutants to AntarCTica (ImPACT) (https://www.scar.org/science/impact/).

Discussion overview

This thesis provides total and organic mercury concentration baseline levels for several Southern Ocean species, some of which are first reports (see Chapters 2, 3 and 4). It also improves our understanding of the levels of mercury contamination in the Southern Ocean food web, at different temporal and spatial scales. The most relevant results encompass:

1) a decreased trend in mercury concentration in the mid trophic web (squid and myctophid fish) over the last decade (Chapters 4 and 5). The study of temporal trends of mercury accumulation is important to provide the accurate patterns of distribution of this contaminant. A decrease of mercury concentrations on the mid trophic web will mean lower bioavailability of this element to top predators;

2) the effect of low abundance of Antarctic krill (*Euphausia superba*) in increasing mercury accumulation in predators (Chapter 3). As Antarctic krill population declines, predators are forced to change their diet to feed more on myctophid fish, which results in a higher intake of mercury. Due to the effects of climate change, Southern Ocean predators will have the pressure to find enough prey and at the same time will be accumulating more mercury from the alternative prey;

3) description and quantification of the biomagnification pathways of mercury in a Southern Ocean food web.

Antarctic krill and zooplankton – primary consumers

In the marine food web, the first consumers group is constituted by zooplankton. Antarctic krill is a major component in the zooplankton group of the Southern Ocean, due to its large biomass, ecological importance and resource potential. However, there are other groups, like amphipods and copepods that also have an important role in the pelagic ecosystem.

Chapter 3 was focused exclusively on the evaluation of mercury concentration in different life stages of Antarctic krill across the Scotia Sea. The major findings of this chapter were: 1) Antarctic krill juveniles have higher mercury body burden than adults, which may be explained by females using egg laying to excrete mercury and to the higher food demand in early life stages; 2) Antarctic krill from oceanic regions

with sea ice have higher mercury concentrations. These two conclusions were later confirmed for another region of the Southern Ocean (Sontag et al., 2019); 3) despite the relative low mercury levels in Antarctic krill (0.006 to 0.071 μg g^{-1} dw), and due to its total mass (around 379 million tonnes) and importance for top predators, Antarctic krill has a major role in the bioavailability of mercury in the Southern Ocean, with an estimated mercury total mass of 1.33 tons.

In chapter 6, mercury was also analysed in other zooplankton species. Concentrations were relatively similar amongst zooplankton species (Tables 6.1 & 6.2, chapter 6), with the exception of *Parandania boecki* (in 2016/17) that had a mercury body burden 2 times higher than all the other zooplankton species. *P. boecki* is an omnivorous species, but specialized to prey Cnidaria (Moore and Rainbow, 1989), so dietary differences would not explain the difference in comparison with other zooplankton species. Also in 2007/08 the mercury concentration in *P. boecki* was similar to the other zooplankton species. The significant difference of mercury between years in *P. boecki* cannot be due to a trophic change, as this species had higher δ_{15}N, a proxy for trophic position, in the year that had lower mercury levels. The increase of more than 0.100 μg g^{-1} in the last 10 years on *P. boecki* is surprising, as there is no previous record of mercury levels in this amphipod to help to understand the specific bioaccumulation patterns, reiterating the importance of baseline studies.

Looking at the δ_{15}N signatures across zooplankton species (Tables 6.1 & 6.2, Chapter 6), it is possible to notice same degree of variation from 3.00 ‰ in *E. vallentini* to 8.81 ‰ in *Gigantocypris* sp., indicating that the feeding habits in this group vary across species, with some species being predominantly herbivorous while other may feed more on other small zooplankton and copepods (Atkinson et al., 2002; Quetin and Ross, 1991b). Normally, a variation in δ_{15}N, a proxy for trophic level, would also mean a difference of mercury levels among species as a result of the biomagnification process affecting Hg. Indeed, species feeding in higher trophic level (higher δ_{15}N) were expected to have higher mercury (Lavoie et al., 2013), although this was not verified in zooplankton, maybe because zooplankton incorporate a significant amount of mercury directly from seawater, neglecting the dietary contribution.

One of the main goals of this thesis was to evaluate habitat and temporal trends of mercury accumulation across different taxonomic groups of the Scotia sea. Habitat? is a main driver of mercury bioaccumulation, and a location with higher levels would,

in most cases, mean that the biota would have also higher levels than biota from a less contaminated area (Desta et al., 2008; Le Bourg et al., 2019).

Regional differences were found in Antarctic krill, with individuals from south of the southern boundary of the Antarctic Circumpolar Current Front (SACC), collected around South Orkney Islands, having mercury levels 5 to 7 times higher than Antarctic krill individuals collected north of the SACC, around South Georgia and at the Polar front (Chapter 2). Despite the one-year difference between the two sampling campaigns, this does not seem to explain such significant differences. The theory for the influence of sea ice presence/absence on mercury levels in Antarctic krill was later suggested in a study from the west side of the Antarctic peninsula (Sontag et al., 2019), supporting this inference across the Southern Ocean. The general conclusion for habitat differences is that colder areas of the Southern Ocean will have more mercury bioavailable. Antarctic krill was the only zooplankton species analysed between the two location, but it would be expected that this difference will be also noticed in the other zooplankton species.

Regarding time scales, Antarctic krill and other zooplankton species showed no differences along time, expect for *P. boecki,* as stated above. Zooplankton samples from 2007/08 had similar mercury body burden to individual from 2016/17. Life expectancy of zooplankton is about 5 to 10 years (Xavier and Peck, 2015), so a 10 year time scale would probably not reflect the effect of environmental difference in the mercury bioavailability as seen in squid (Chapter 3).

Squid and fish – the mid and meso trophic web

Squid and fish in the Southern Ocean are the secondary consumers/meso-predators of the Southern Ocean, depending on the size reach of the species or size of individuals. The small squid and myctophid fish feed mostly on zooplankton, having a dietary shift when they reach bigger sizes to predate other squid and fish besides the zooplankton (Bernal et al., 2012; Cherel and Hobson, 2007). Larger notothenioidei prey mainly on other fish or squid, with some species feeding also in zooplankton (Casaux et al., 1990; Collins et al., 2010b; Reid et al., 2007; Seco et al., 2015).

Results show that mercury concentrations in squid showed different patterns depending on the studied species; mercury increased with size in some squid species (*Alluroteuthis antarcticus* and *Psychroteuthis glacialis*) while in other squid species it

decreased (*Galiteuthis glacialis*). Furthermore, in some species, body size did not affect mercury accumulation (*Bathyteuthis abyssicola* and *Slosarczykovia circumantarctica*). These variable patterns across Southern Ocean squid species show that squid may have different mercury incorporation mechanisms. The metabolic activity and specific diets of the majority of this species are still poorly known (Collins and Rodhouse, 2006), which preclude any conclusions on what factors can be responsible for these inter-specific differences.

In myctophid fish, the size effect was more stable as the majority of the fish species studied had a mercury increasing pattern (e.g., *Gymnoscopelus braueri, Gymnoscopelus nicholsi, Krefftichthys anderson*i) with the exception of females of *Electrona antarctica*, but sexual differences will be discussed below. The role of size in fish mercury accumulation is a fairly well established process linked to bioaccumulation of Hg with age reflecting the high retention and low excretion of the metal (Dang and Wang, 2012; Gewurtz et al., 2011; Somers and Jackson, 1993).

Overall, results show that different taxonomic groups have a different mercury accumulation response to the effect of size. With Antarctic krill showing inverse patterns (Chapter 2), squid showing a strong species influence relation (Chapter 3), and the fish showing a more consistent bioaccumulation pattern along size (Chapter 4).

Sexual dimorphism may also influence growth rates, sex-related metabolic differences, behaviour, habitat use and feeding habits (McGee and Wainwright, 2013; Rennie et al., 2008; Saillant et al., 2003), which may result in differential mercury bioaccumulation. Egg laying plays also a role in the concentration of mercury across life stages, as females in some cases may use this mechanism as a mercury excretion route. This was previously reported for birds and fish (T. A. Johnston et al., 2001) and even possibly in crustaceans (Coelho et al., 2008).

Regarding the myctophid fish studied, females growth faster and larger than males in several species (e.g., *Electrona antarctica* (Greely et al., 1999)), so it would be expected that they accumulate greater mercury levels than males. Although the effect of sex in mercury accumulation in fish is known to be variable (Bastos et al., 2016), so different species may have distinct responses. In the present study, male *E. antarctica* had higher mercury levels than females (Chapter 4), as it was previously reported for other fish species [e.g., *Petromyzon marinus, Mullus barbatus, Sander vitreus*) (Madenjian et al., 2014; Polak-Juszczak, 2012; Stacy and Lepak, 2010)].

However, in other species (e.g., *Epinephelus diacanthus, Parastromateus niger, Alectis indicus*), no differences between sexes were observed (Abdolvand et al., 2014; Mela et al., 2013; Sonne et al., 2014) or even females had higher levels than males [i.e., *Zungaro zungaro, Sphyrna lewini, Lota lota* (Bastos et al., 2015; Bergés-Tiznado et al., 2015; Madenjian et al., 2015)]. Therefore, it seems that the effect of sex in myctophid fish from the Southern Ocean is also variable among species (Chapter 4). For *E. antarctica,* females and males were caught in the same hauls, suggesting that they inhabit the same areas and depths, and to the best of our knowledge, there are no studies reporting different feeding habits between sexes in any species of Southern Ocean myctophids. It therefore seems possible that the production of eggs (100 – 2000 eggs per spawn (Catul et al., 2010) may be the factor that better explains these differences found in *E. antarctica*.

Myctophid fish caught north and south of the SACC had similar mercury concentration levels (Chapter 4), contrasting with the results on Antarctic krill (Chapter 2). This lack of differences in mercury concentrations in myctophid fish may be due to the fact that myctophid populations migrate across the Scotia Sea (Saunders et al., 2014), probably masking the geographical differentiation found for Antarctic krill (Chapter 2). Although a puzzling conclusion was found when looking into myctophid fish from different locations elsewhere, mercury concentrations were similar among all the investigated species across different World Oceans (Table 4 chapter 4). It would have been expected to have some degree of variation, specially when looking into individuals that were collected from anthropogenic mercury sources (Lahaye et al., 2006) as was reported for krill species (Minganti et al., 1996) (Table 2 chapter 2). Overall, myctophid fish have a similar uptake of mercury across species and ecosystems around the World´s Oceans.

The evaluation of temporal scales of mercury concentrations in species in the mid trophic web of the Southern Ocean, analysed in squid (Chapter 3) and in myctophid fish (Chapter 4), showed a decreasing trend over the last decade. This tendency was most noticeable in squid, as these short-lived animals will reflect environmental change faster than animals with a longer live cycle (Fränzle, 2006). A temporal decrease on mercury levels in biota, suggests a decrease of mercury levels in the environment available to be incorporated (Y. Zhang et al., 2016) or a change in the oceanic methylation ratios. A decrease in atmospheric mercury has been observed (Soerensen et al., 2012), due the more restrict rules and regulations imposed to the

production and emission of mercury in the framework of the Minamata Convention, a decline in the anthropogenic emissions was registered (Streets et al., 2017; Y. Zhang et al., 2016). It therefore seems that the reduction in atmospheric mercury is driving a decline in mercury levels in Southern Ocean biota, as observed (see Chapters 3 and 4), although a monitoring plan should be put in place to confirm this trend.

Seabirds – top predators

In the present study, flying seabirds were the group of top predators investigated. This trophic group has very heterogenic feeding habits across species, from wandering albatrosses *Diomedea exulans* feeding in a range of squid and fish, brown skua *Stercorarius antarcticus* or giant petrels (*Macronectes* spp.) that scavenge/predate other birds and marine mammals, to Antarctic prion *Pachyptila desolata* or blue petrel *Halobaena caerulea* that feed mainly on zooplankton (Prince, 1980b). By analysing mercury concentrations in this group of diverse top predators, it was possible to assess the different pathways of this contaminant from different prey (Chapter 5). Seabirds chicks are particularly interesting as bioindicators because the mercury concentration in their feathers reflect primarily dietary sources from the local environment (Blévin et al. 2012). There are of course many other predators in the Southern Ocean, such as fish, whales, seals and other seabirds like penguins. Mercury concentrations were previously reported for fin whale *Balaenoptera physalus* (0.044 ± 0.019 *μ*g/g muscle) and Antarctic minke whale *Balaenoptera bonaerensis* (0.027 ± 0.021 *μ*g/g muscle) (Endo et al., 2012)], both mainly zooplankton feeders, which had lower mercury levels than the zooplankton feeding seabirds (i.e., *P. desolata* and *H. caerulea*). This difference may be due to the fact that in whales mercury concentrations were measured in the muscle tissue and was reported in wet weight, while in the seabirds was measured in feathers. In the fur of Antarctic seal such as the Crabeater seal *Lobodon carcinophaga* (0.26 ± 0.04 *μ*g/g), the Ross seal *Ommatophoca rossii* (0.48 *μ*g/g) and the Weddell seal *Leptonychotes weddellii* (2.09 ± 0.21 *μ*g/g) (Aubail et al., 2011), as in the case of the seabirds analysed in chapter 5, had different mercury concentrations across species. These differences will most certainly result from distinct dietary preferences between seal species: Crabeater seals feed almost exclusively on Antarctic krill (Adam, 2005) as *P. desolata* and *H. caerulea*, so had a lower mercury concentration than Ross seals, that eat mainly squid,

some fish and zooplankton (J. A. Thomas and T. Rogers, 2009) a diet similar to grey-headed albatrosses *Thalassarche chrysostoma* and black-browed albatrosses *T. melanophris* (Prince, 1980a). Higher levels were reported in Weddell seals, predominantly fish eaters (Burns et al., 1998), which have similar mercury concentrations to wandering albatrosses *D. exulans* (Chapter 5). In penguins, a similar relationship was found, with gentoo penguins *Pygoscelis papua* [0.97 ± 0.67 adults (Pedro et al., 2015)] in South Georgia prey in Antarctic krill and some fish (Xavier et al., 2018b), having a lower concentration than King penguin *Aptenodytes patagonicus* chicks (Carravieri et al., 2013) that feed mainly in myctophid fish (Olsson and North, 1997). These findings indicate, therefore, that the diet can be the main driver for mercury burden in marine mammals and seabirds in the Southern Ocean (Carravieri et al., 2014).

When looking into the mercury concentrations in feathers of seabird chicks around the world ocean (Table 1), the range of mercury concentrations are similar to the ones registered in species of the Southern Ocean. Even on species that habitat closer to more polluted areas, like in the Mediterranean (e.g., *Larus audouinii* from the Albrorán Island and *L. michahellis* from Tunisia), similar results were obtained (Table 1). It is also possible to notice the effect of diet in mercury accumulation, as species from the same areas had different mercury levels, depending on the diet (Monteiro et al., 1999; Stewart et al., 1997). Also, the effect of habitat was noted, as some seabird colonies from different locations had different mercury levels (Carravieri et al., 2016); (Abdennadher et al., 2010; Carravieri et al., 2017; Sanpera et al., 2007).

Another possible abiotic factor that may influence mercury levels is the time between sampling years, as the bioavailability of mercury in the habitat may change across temporal scales (Y. Zhang et al., 2016). As stated above, the tendency shown in squid and myctophid fish is that mercury is decreasing in the Southern Ocean. However, seabirds (Chapter 5) show an opposite pattern: chick feathers from 2016/17 had generally higher mercury concentrations than feathers from 2007/08. In this case, the differences are explained by a change in the food web in that 10-year time scale: with lower availability of Antarctic krill, seabirds had to feed more in higher trophic level prey, like myctophid fish (Murphy et al. 2007). Myctophid fish have higher mercury body burden than Antarctic krill, so predators will accumulate more mercury in years with low Antarctic krill abundance, such as 2016/17. This behaviour is evidence that

in seabirds, an enforced change in the diet had higher influence in mercury levels than the reduction of atmospheric emissions.

The decreasing trend of mercury levels in Southern Ocean mid trophic biota is an optimistic sign, as it would mean a reduction of bioavailable mercury to be incorporated by top predators. However, due to rising oceanic water temperatures, population of Antarctic krill are decreasing, putting Southern Ocean predators at risk (Klein et al. 2018). Not only predators will suffer for an extra pressure to forage, but will also intake more mercury.

Table 1. – Mercury concentration (μg g^{-1} dw) in chick feathers of different seabird species collected around the world (mean ± standard deviation).

Common name	Species	Local	Sampling year	Mercury	Reference
Cory's shearwater	*Calonectris borealis*	Azores	1993-1995	0.7 ± 0.2	Monteiro et al. (1999)
Great skua	*Catharacta skua*	Shetland	1994	4.15 ± 1.39	Stewart et al. (1997)
Black-headed gull	*Chroicocephalus ridibundus*	Baltic Coast	2009-2012	0.900 ± 0.263	Szumili-Pilarska et al. (2017)
Great black-backed gull	*Chroicocephalus ridibundus*	Baltic Coast	2009-2012	3.214 ± 1.468	Szumili-Pilarska et al. (2017)
Northern rockhopper penguin	*Eudyptes moseleyi*	Amsterdam Island	2007	0.34 ± 0.07	Carravieri et al (2016)
Herring gull	*Larus argentatus*	Baltic Coast	2009-2012	1.677 ± 1.911	Szumili-Pilarska et al. (2017)
Audouin's gull	*Larus audouinii*	Albrorán Island	2004	3.872	Sanpera et al. (2007)
Audouin's gull	*Larus audouinii*	Chafarinas Island	2004	3.165	Sanpera et al. (2007)
Audouin's gull	*Larus audouinii*	Ebro delta	2004	5.089	Sanpera et al. (2007)
Common gull	*Larus canus*	Baltic Coast	2009-2012	1.824 ± 1.066	Szumili-Pilarska et al. (2017)
Mediterranean gull	*Larus melanocephalus*	Axios Delta	2007	1.130 ± 0.201	Goutner et a. (2012)
Yellow-legged gull	*Larus michahellis*	Azores	1993-1995	2.3 ± 1	Monteiro et al. (1999)
Yellow-legged gull	*Larus michahellis*	Tunisia	2007	1.883 ± 0.452	Abdennadher et al. (2010)
European shag	*Phalacrocorax aristotelis*	Sagres (Portugal)	2004	1.071 ± 0.385	Moreno et al. (2011)
Arctic Skua	*Stercorarlus parasllic*	Shetland	1994	2.00 ± 0.91	Stewart et al. (1997)
Common tern	*Sterna hirundo*	Azores	1993-1995	1.5 ± 0.4	Monteiro et al. (1999)
Arctic tern	*Sterna paradjsaea*	Shetland	1994	0.69 ± 0.14	Stewart et al. (1997)
Guillemot	*Uria aalge*	Shetland	1994	1.24 ± 0.27	Stewart et al. (1997)

Future recommendations

As one hypothesis is tested, many other questions may arise, and that certainly happened during the course of this study. My work has raised numerous new questions. The first future study was identified while working in Chapter 2. As Antarctic krill females excrete mercury with egg laying, one would expect that females would have lower mercury levels than males. However, they had similar concentrations between sexes. The hypothesis raised is that Antarctic krill males excrete mercury during the production of their sexual tissue (spermatophores). The collection of mature Antarctic krill males could allow the evaluation of mercury concentration in the spermatophores and compare with values for somatic tissue. Complementary to this future study, it would be interesting to compare the high differences of mercury levels found between Antarctic krill from different habitats in the Scotia Sea, with other zooplankton species (e.g., copepods and amphipods).

A similar hypothesis was noted while working in Chapter 4, when female *E. antarctica* showed a decreasing pattern of mercury accumulation along size, suggesting that females may excrete mercury through egg laying. To test this hypothesis (i.e. “eggs of *E. antarctica* have higher mercury than their somatic tissues”), ovaries of *E. antarctica* would need to be collected from mature females.

Besides the specific questions raised along the chapters, the main future endeavour could focus in analysing other elements on the samples that were already collected and analysed during this study. Non-essential elements, such as silver (Ag), aluminium (Al), arsenic (As), cadmium (Cd), barium (Ba), beryllium (Be), mercury (Hg), lead (Pb), titanium (Ti) and uranium (U) are usually present in the marine environment at trace concentrations and have toxic effects at high concentrations. Other trace elements are essential, such as cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), manganese (Mn), nickel (Ni), selenium (Se), vanadium (V) and zinc (Zn), as they play a specific role in metabolism and are required by living organisms in small concentrations to have normal development (Bosch et al., 2015). However, essential trace elements may also have toxic effects at elevated exposure levels (Velusamy et al., 2014). To understand the level of trace elements contamination in the Southern Ocean food web, other elements besides mercury should be quantified. There are also interesting interactions between trace elements that may change their toxicity levels, that need to be evaluated for the Southern Ocean. For example, as the case of

selenium and mercury, where higher concentrations of the first mean lower toxicologic effects of mercury (Bjorklund et al., 2017).

Another type of analysis that has been lately used in mercury studies, is the measurements of mercury isotopes $\delta_{202}Hg$ (mass-dependent fractionation) and $\Delta_{199}Hg$ (mass-independent fractionation) as a tool to identify sources of mercury and biogeochemical processes within the different sections of the marine ecosystem. Mercury mass-dependent fractionation reflects different physical, chemical or biological processes (e.g., volatilization, reduction, methylation or demethylation, photochemical reactions, metabolic and trophic accumulation processes. As all of these process and combinations between processes will affect the ration of $\delta_{202}Hg$, it is possible to gather information regarding the processes and specific mercury reservoirs in the ecosystem. On the other hand, $\Delta_{199}Hg$ rations are set exclusively during the photochemical reactions. Mercury mass-independent fractionation is not affected by biological processes and its signatures is thus assumed to be preserved throughout the food web, meaning that this mercury stable isotope can be used as a conservative tracer of mercury from sources to predators.

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