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# **Trace elements in invertebrates and fish from Kerguelen waters, southern Indian Ocean**

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# **ABSTRACT:**

Given the lack of background data on essential and non-essential trace elements in invertebrates and fish known to be the predominant prey of marine mammals and seabirds breeding at the Kerguelen Islands, this study intends to provide these results of great influence for predators in higher trophic levels. To this end, plankton organisms (9 species/4 phyla), mollusks (2 bivalves and 2 squid species) and fishes (8 benthic and 10 pelagic species) from Kerguelen waters were analysed for cadmium (Cd), copper (Cu), mercury (Hg), and zinc (Zn). Individual concentrations of non-essential elements (particularly Cd) showed larger variation in comparison with essential ones due to homeostasis. Cd ranged over 4 orders of magnitude; however, Hg ranged only 1, without significant correlation to trophic level. Instead, ecological parameters (benthic/mesopelagic habitat and feeding ecology) showed a more important influence on the results. Concerning seashore organisms, bivalves collected inside the Gulf of Morbihan had higher Cd concentrations compared to those from the Kerguelen shelf, suggesting a local source of Cd, such as runoff water from bird colonies. Comparison with literature showed metal concentrations in invertebrates and fishes from Kerguelen Islands somewhat lower than in the Antarctic area, with Hg prevailing in benthic species and Cd in pelagic ones. In contrast to Hg, Cd values of squids, jellyfish and the amphipod *Themisto gaudichaudii* were significantly higher than all other species. Finally, top predators foraging in this area that can be subject to potentially high Hg and Cd exposure through their diet at Kerguelen are reviewed.

**Keywords:** plankton; myctophid; food web; metals; trace elements; Kerguelen

## **INTRODUCTION**

The Southern Ocean constitutes a particular environment for marine biota where human inputs of metals are supposed to be very low. Several essential elements such as iron (Fe) or copper (Cu) are poorly concentrated and/or bioavailable in these waters and could therefore act as limiting factors for phytoplankton (e.g. Coale, 1991). In higher trophic level organisms, low Cu and zinc (Zn) concentrations in regard to organisms from temperate regions have been found in crustaceans and mollusks (e.g. Bustamante et al. 1998a, 2003; Petri and Zauke 1993; Rainbow 1989), raising the question of how can these organisms cope with essential element supposed deficiency. Several hypotheses have been suggested to answer this question such as a relatively higher efficiency of mechanisms of element uptake compared to similar organisms from nondeficient areas and/or the replacement of essential elements by non-essential ones in biochemical reactions or in enzymes. To date, such replacement has only been evidenced for Zn substitution by Cd in carbonic anhydrase from marine diatoms (Lane and Morel 2000; Lane et al. 2005; Xu et al. 2008). In this context, the interactions between essential and non-essential elements in invertebrates and fish from the Southern Ocean appear poorly documented and, moreover, baseline values necessary for the study of such interactions are even scarcer, if not inexistent in the literature.

Despite the comparatively low inputs of non-essential elements, very high concentrations of Cd and Hg have been reported in several organisms from distinct trophic levels in Antarctic and subantarctic environments (Sanchez-Hernandez 2000; Bustamante et al. 2003; Dos Santos et al. 2006; Bargagli 2008). Population growth and industrial development in several countries of the Southern Hemisphere are changing the global pattern of persistent anthropogenic contaminants and new classes of chemicals have already been detected in the Southern Ocean (e.g. Bargagli 2008; Von Waldow 2010; Carravieri et al. 2014a). Very high concentrations of both Cd and Hg were also found in the tissues of top predators such as seabirds and marine mammals from the Southern Ocean (see the review of Sanchez-Hernandez, 2000). These high trophic level vertebrates are mainly exposed to trace elements through their food (Aguilar et al. 1999; Muirhead and Furness 1988) and some specific prey highly contribute to the exposure to a given element. For example, cephalopod consumption is well-known to provide elevated concentrations of Cd under a bioavailable form (Bustamante et al. 1998b, 2002) and mesopelagic fish contain high amounts of methyl-Hg (e.g. Chouvelon et al. 2012; Monteiro et al. 1996). However, there is only a few data on invertebrates and fish to provide background for explaining the high Cd and Hg concentrations in the top predators from the Southern Ocean. Therefore, it is of major concern to provide data about lower trophic level organisms they feed on to give a more comprehensive and evidence supported basis to their contamination pattern. In addition to that, age, trophic position, sex, size among other ecological parameters likely play a role in trace elements concentration in these prey organisms (e.g. Dehn et al 2006; Locarnini and Presley 1995; McIntyre et al. 2007).

Situated near the Polar Front, the Kerguelen Islands are a particularly important area for breeding seabirds and for mammals (see Guinet et al. 1996). Specifically, this archipelago hosts a large and highly diverse avian assemblage (35 different breeding species according to Weimerskirch et al. 1989) and 13 species of marine mammals (3 mysticetes, 7 odontocetes and 3 pinnipeds; Borsa 1997). According to their reproduction strategies, many seabird species catch their prey in the highly productive waters around the Archipelago and feed on a few key species of marine organisms, including some crustaceans (e.g. euphausiids, hyperiids, copepods), fish (e.g. myctophids, notothenioids) and cephalopods (e.g. oceanic squids) (Bocher et al. 2001; Cherel and Hobson 2005; Cherel et al. 2010; Guinet et al. 1996). Their exposure to contaminants and that of their offspring is therefore determined by the concentrations in these

lower trophic level organisms consumed specifically in this area, at least during the breeding period.

In this context, the present study was conducted to document selected trace element concentrations in zooplankton, mollusks and fishes around the Kerguelen Islands to provide understanding on the degree of metal contamination in low trophic level organisms being the prey of seabirds and marine mammals. To this end, Cd, Cu, Hg and Zn have been analysed in organisms belonging to different phyla of pelagic invertebrates and in 17 coastal and oceanic fish species to cover the main categories of prey species of the Kerguelen seabird community and of the marine mammals foraging in these waters. The non-essential elements Cd and Hg constitute the main metals of concern for wild vertebrates because of their known toxicity (Scheuhammer 1987; Tan et al. 2009; Tartu et al. 2013). In turn, Cu and Zn can be disturbed by the interaction of Cd and Hg on their regulation proteins such as the metallothioneins (e.g. Øverjordet et al. 2015). The levels of these trace elements were compared within the benthic and pelagic food webs and were globally compared with similar organisms from other marine ecosystems, when available. Finally, the significance of key species in contaminants transfer towards top predators was examined.

#### **MATERIALS AND METHODS**

# *Sampling of organisms*

Pelagic and benthic organisms were successively collected in the waters surrounding the Kerguelen Island Archipelago (Figure 1) during the austral summers from 1997 to 1999. Information on these organisms are summarised in Table 1. In coastal waters, pelagic zooplankton mainly constituted by hyperiid amphipods (*Themisto gaudichaudii*) and copepods (*Paraeuchaeta antarctica*), was sampled inside the Morbihan Gulf in March 1997 with an ORInet (2 m², 1 mm mesh aperture). In this area, several benthic fish species (mostly Notothenidae) were also collected by net fishing overnight (Table 1). Outside the gulf, hyperiid amphipods (*T. gaudichaudii*), euphausiids (*Euphausia vallentini*, *E. frigida* and *E. triacantha*) and six myctophid species were collected in the eastern part of the peri-insular shelf in February 1998, using a IYGPT trawl (International Young Gadoid Pelagic Trawl; opening: 12 x 7 m) with 10 mm mesh size in the cod-end. Other pelagic material (gelatinous plankton, planktonic crustaceans, squid and fish) were sampled during February 1999 on cruises of the RV "La Curieuse". Benthic fish were collected by bottom trawl used for commercial fishery in the Southern Kerguelen shelf. The samples of the copepod *Thysanoessa* sp. were obtained from stomach contents of the South Georgian diving petrel collected during investigations on the diet of this species (Bocher et al. 2003) and potential effects of partial digestion must be taken into account.Mussels were collected by hand on the shore during low tides in January 1999. Immediately after collection, the organisms were separated by species, then sex and size or age classes whenever possible (*Euphausia vallentini* above and below 25mm, and *Champsocephalus gunnari* adults and juveniles respectively), and frozen in plastic bags or vials. Then, samples were stored at –20°C until analysis. All organisms were analysed whole, except the mussels, which had their shells removed.

## *Sample preparation and analysis*

Length (mm) and mass (g) of fish and squids were thoroughly determined, as well as the sex, whenever possible (i.e., when size and maturity allowed so), and their gut content was removed. In the case of myctophids, otoliths were taken out to ensure identification of the species. Fish and squids were systematically treated individually, except *Harpagifer* sp. (12 individuals resulted in 4 samples). In contrast, all other invertebrates were pooled, except the jellyfish, which were treated individually. Pooling was primarily made because of analytical reasons, in order to gather enough mass to attend protocol specifications. Sample characteristics, i.e. family, species, length, weight and sex (for squid, fish and some crustacean species), are given in Table 1. Trophic level is assessed based on the personal data of the co-authors, mostly based on stomach contents. Six of the seventeen fish species had also their trophic levels determined by stable isotope ratio of nitrogen published in other studies (Cherel et al. 2010). Since the difference between these two methods was lower than one trophic level, only the former will be further considered during data interpretation.

Samples were dried for two to three days at 50°C to a constant weight and then homogenised. Next, 2 aliquots of approx. 100 to 300 mg each (according to availability) of homogenised dry sample were digested with 5 ml of 65% HNO<sub>3</sub> and 0.3 ml of 70% HClO<sub>4</sub> during 72 h at 80°C. When the solution was clear, acids were evaporated and the obtained residues were dissolved in 10 ml 0.3 N nitric acid. Cd, Cu and Zn were analysed using a flame and graphite furnace atomic absorption spectrophotometer Varian 250 Plus with deuterium background correction. For Hg, aliquots ranging from 5 to 20 mg of dried material were analysed directly in an Advanced Mercury Analyser spectrophotometer (Altec AMA 254). Hg determination involved evaporation of Hg by progressive heating until 700°C were reached and then held under oxygen atmosphere for 3 min, followed by an amalgamation on a gold-net. Afterwards, the net was heated to liberate the collected mercury, subsequently measured by UV atomic absorption spectrophotometry.

Quality assurance was assessed using dogfish liver DOLT-2 (NRCC) and dogfish muscle DORM-2 (NRCC) as reference materials. Such standards were analysed and treated under the same conditions as the samples, with errors in regard to the certified values remaining below 5% in both cases. Detection limits were 0.004 for Cd, 0.5 for Cu, 0.005 for Hg, and 3 µg

 $g^{-1}$  dry weight (dw) for Zn. All trace element concentrations in Kerguelen Islands marine organisms are reported in  $\mu gg^{-1}dw$  unless stated otherwise. Water contents allowing recalculations of the metal concentrations from dw to wet weight (ww) are given in Table 1.

# *Statistical analyses*

Statistical analyses were performed in Microsoft Excel 2007 and Statsoft Statistica 11 and 12. Before analyses, data were checked for normality of distribution and homogeneity of variances using Shapiro-Wilk and Brown-Forsythe tests, respectively, followed by ANOVA and Posthoc Tukey HSD. Spearman's or Pearson's correlations are used to assess the degree of monotonic or linear dependence, respectively, between two variables. At-test was used to assess whether female and male datasets differed when available. All statistically significant results were set at  $\alpha$ =0.05 and all presented correlations should be assumed significant unless stated otherwise.

#### **RESULTS**

Metal concentrations in invertebrates and fishes from the Kerguelen Island waters are presented in Table 2. Cu, Hg, and Zn concentrations showed, in a general way, a lesser degree of variability when compared to Cd. Indeed, Cu, Hg and Zn average concentrations vary one order of magnitude each whereas Cd ranged over three orders of magnitude.

Regarding the influence of biological factors in the results, the only crustacean species for which gender comparison was possible was *Paraeuchaeta antarctica*. All four trace elements presented significant differences between males and females, these later showing significantly higher Cd ( $p<0.001$ ), Cu ( $p=0.004$ ) and Zn ( $p=0.015$ ) levels than males. This difference can be attributed to their considerably higher length and weight (Table 1). Concerning Hg, significantly higher (*p*=0.037) concentrations were found in males than in females.

In cephalopods, there were no differences on metal concentrations between males and females (for *Todarodes angolensis* Cd, *pt-test* = 0.149 and Hg, *pt-test* = 0.867; for *Moroteuthis ingens* Cd, *pt-test* = 0.206 and Hg, *pt-test* = 0.923); moreover, no correlation (neither Pearson's nor Spearman's) between biometrics and metal concentrations were found. In fish, metal concentrations showed no significant difference between the sexes nor correlation with the biometric parameters (total length and total weight) with a few exceptions concerning Cd and Hg. Cd correlated significantly with total length in *L. squamifrons* (*r*=0.723, *p*=0.018) and with both length and weight in *G. fraseri* (*r*=0.704, *p*=0.005 and 0.862, *p*<0.001, respectively). Interestingly, a significant negative correlation of Cd with both biometric parameters was found for juvenile *C. gunnari* (*r*=-0.702, *p*=0.023 and -0.636, *p*=0.048) and *Protomyctophum tenisoni*  $(r=0.549, p=0.034$  and  $-0.627, p=0.012$ ). In turn, Hg correlated (Spearman's rank) significantly with total length in *Gymnoscopelus piabilis* (*r*=0.591, *p*=0.033) and *Lepidonotothen squamifrons* (*r*=0.670, *p*=0.034); with weight in *Protomyctophum bolini* (*r*=0.697, *p*=0.004)

and with both length and weight (shown respectively) in *Channichthys rhinoceratus* (*r*=0.843, *p*=0.02 and *r*=0.802, *p*=0.07), *G. fraseri* (*r*=0.771, *p*=0.001 for both), *Notothenia rossii* (*r*=0.554, *p*=0.049 and *r*=0.577, *p*=0.038) and *Zanclorhynchus spinifer* (*r*=0.959, *p*=0.002 and  $r=0.965$ ,  $p=0.002$ ). Finally, in regard to between-elements relationships, Cd and Hg levels correlated with one another significantly in *Champsocephalus gunnari* (total, *r*=0.947, *p*<0.001) and *G. fraseri* (*r*=0.843, *p*<0.001).

Concerning Bivalves, two mussel species (the blue mussel, *Mytilus edulis desolationis* and the Magellan mussel, *Aulacomya atra*) sampled in four different locations: (Foch, Cap Noir, Port-aux-Français and Mayes Island) were analysed in the present study. A significant negative correlation (Spearman's rank) between Cd and Cu (*r*=-0.725, *p*=0.039) was found in the blue mussel, and also in the Magellan mussel ( $r=0.798$ ,  $p=0.004$ ). This latter species presented yet significant correlation between Cu and total weight (*r*=-0.682, *p*=0.042), Cd and total weight (*r*=0.673, *p*=0.048) and Cd and Hg (*r*=-0.702, *p*=0.034). When all samples (both species) are considered together, there is a significant correlation between Cd and total weight (*r*=0.547,  $p=0.007$ ) and also a significant negative correlation between Cd and Cu ( $r=0.537$ ,  $p=0.009$ ).

#### **DISCUSSION**

The main objective of this study was to provide baseline levels in a wide range of phyla which constitute the prey for high trophic level organisms such as large fish, seabirds, and marine mammals. The species collected in the present study represent a wide range of ecological groups from the pelagic/benthic and neritic/oceanic communities from the Kerguelen Island waters. The size of the collected organisms falls within the range of the size preyed by top predators from this area and more specifically, from the large seabird community that includes 35 species (Weimerskirch et al. 1989).

# *Influence of biological factors on metal concentrations*

Among factors influencing metal concentrations, sex differences were found for the crustacean *Paraeuchaeta antarctica*. Higher concentrations of Cd, Cu and Zn in female *P. antarctica* might be due to the fact that metals are highly retained by copepods and Cd, Cu and Zn bioaccumulation with the size is likely to have played a major role in this difference, not necessarily *via* diet, but also possibly via the dissolved phase, in a passive way (Wang et al. 1996; Wang and Fisher 1998) or else *via* a possible remobilisation due to sexual maturation, as further discussed. In contrast to Cd, Cu and Zn, higher Hg concentrations in males is likely due to the fact that the sampled females could have already reached sexual maturity size and have excreted Hg in the laid eggs. Indeed, maternal transfer may act as a major pathway for Hg(II) and MeHg elimination in crustaceans (Tsui and Wang 2004). The size of the specimens analysed here ranged from 5 to 10 mm, whereas this species presents CV (last copepodite stage) at 6.5±0.3 mm and CVI (adults) at 8.7±0.4 mm (Bocher et al. 2002), therefore the sampleset likely contained sexually mature individuals.

In cephalopods, the lack of difference in Cd and Hg concentrations between males and females is surprising considering the sexual dimorphism in both species, females reaching larger sizes than males. Indeed, sexual dimorphism and ontogenic effects can influence in metal concentrations in cephalopods (e.g. Pierce et al., 2008; Chouvelon et al., 2011). The absence of variation of metal concentrations in both cephalopod species is likely due to the limited number of specimens of each sex and limited size range for both *T. angolensis* and *M. ingens*. Nevertheless, metal concentrations found in both squid species were among the highest for all the species from the present study, especially for Cd. Cephalopods are considered as superbioaccumulators of many trace elements and their capacity to bioaccumulate remarkable Cd levels was already shown in several environments (Dorneles et al. 2007; Martin and Flegal 1975; Miramand and Bentley 1992; Miramand and Guary 1980), including Kerguelen waters

(Bustamante et al. 1998b). Even though the specific proteins involved in Cd bioaccumulation are not fully known, its significant correlation (Spearman's rank) with Zn (r=0.902) in *T. angolensis* (the highest Cd level) suggests the involvement of MTLP in Cd detoxification. For Hg as well, cephalopods were among the most contaminated species. Within this group, Hg is mainly under the highly bioavailable organic form (Bustamante et al. 2006) and therefore they represent a significant source of this element for their predators.

In fish, the significant negative correlation of Cd with both biometric parameters (size and weight) for *C. gunnari* and *P. tenisoni* could be due to a higher Cd exposure in earlier life stages (due to diet, habitat or maternal transfer), with a growth dilution effect taking place and concentrations decreasing thereafter. Since benthic organisms present, in a general way, overall lower Cd values when compared to pelagic ones (Fig. 2), this is in accordance with previous studies describing *C. gunnari* as bentho-pelagic, but with a shift from the pelagic to the benthic environment as the fish grow (Kock 2005a, b). Concerning Hg, the correlations found between this element and biometrics are likely related to the bioaccumulation of Hg as the fish grow. Moreover, benthic species presented mostly higher Hg levels than pelagic ones (Fig. 2). In sediments, organic carbon and microbial activity play an important role on Hg bioavailability as microorganisms highly contributes to the methylation of inorganic Hg (Andersson et al. 1990), enhancing the exposure of benthic species to Hg. In regard to Cd and Hg positive significant correlations with size and weight in *C. gunnari* and *G. fraseri*, it would mean that these species are simultaneously exposed to sources of both contaminants. This could be due to the consumption 1) of one specific prey that presents high concentrations of Cd and Hg or 2) of several prey that present conversely high concentrations of Cd or Hg. The second hypothesis seem more likely in regard to *C. gunnari*, since its diet in a subantarctic environment (South Georgia Archipelago) comprised a large proportion of Antarctic krill *Euphausia superba*, knowing that Euphausids had comparatively high Hg levels (See Table 2) and, more

importantly, the hyperiid amphipod *Themisto gaudichaudii* (with very high Cd levels) as the most frequent prey (Kock et al., 1994). In regard to *G. fraseri*, it feeds mainly on copepods and to a lesser extent, on the Euphausid *Thysanoessa* spp. (Saunders et al. 2015), both presenting high Cd and Hg concentrations (Table 2). Therefore it seems reasonable to state that the first one of the previous hypotheses would be more likely in this case.

The influence of the trophic position on metal concentrations is examined in Figure 2, which presents the concentrations of Cd and Hg stratified by trophic level. In this regard, stable isotopes ratios of nitrogen bring slightly superior data for trophic levels (TLs) of six fish species included in the present study (Cherel et al. 2010), all of them inferior to one trophic level: 0.9 TL for *Protomyctophum bolini*, 0.7 TL for *Gymnoscopelus nicholsi*, 0.5 TL for *P. tenisoni*, 0.3 TL for *Electrona antarctica* and *G. fraseri* and finally 0.2 TL for *G. piabilis*. The difference between these TLs obtained by different methods was negligible and did not change the stratification of concentrations along the TLs.

Cd concentrations ranged over 4 orders of magnitude, i.e. from 0.063  $\mu$ g g<sup>-1</sup> in *Gobionotothen acuta* to 79.4  $\mu$ g g<sup>-1</sup> in *T. angolensis* across the different trophic levels, interestingly, a relatively similar pattern to the one reported for an Arctic marine food web (Macdonald and Sprague 1988). Moreover, a large intraspecific variation is shown by different taxa as well, such as in salps and specially crustaceans (Table 2). However, no significant correlation between Cd concentrations and the trophic level of the species was found in our sampling, suggesting that Cd is not biomagnified within our sampling set. However, it is important to remark that the increase in trophic level within our sample set does not always infer a direct food-consumer link between the sampled organisms, so biomagnification *strictu sensu* must be regarded with caution. Moreover, samples were taken in different years and interannual and local differences are possible as well. Also, the transfer of Cd appears to be more related to the species rather than to the trophic level itself, as previously shown in other environments (Miramand et al. 1999; Pigeot et al. 2006). This specificity is likely related to Cd bioaccumulation capacities which are particularly elevated for some taxa such as the hyperiid amphipods *Themisto* sp. (e.g., Ritterhoff and Zauke 1997) and cephalopods (e.g., Bustamante et al.1998a, 2002; Dorneles et al. 2007, Penicaud et al. 2017), or particularly weak as in most marine fish species (Kojadinovic et al. 2007; Wang 2002).

The lack of biomagnification is apparently repeated for Hg in our set of samples, contrary to what is previously reported for local avifauna (Blévin et al. 2013; Carravieri et al. 2014b). It is important to remark that Hg biomagnification concerns its main organic form, i.e. methyl-Hg, due to its high bioavailability (e.g. Kannan et al. 1998) whereas our analyses were made only for total Hg. A much higher assimilation efficiency is displayed for methyl-Hg than for inorganic Hg and a slight variation in this property may determine whether or not some of the element is biomagnified (Reinfelder et al. 1998). Yet, the proportion of methyl-Hg is poorly documented for low trophic level prey in the Southern Ocean. This issue clearly deserves further research in this ecologically important and representative oceanographic area.

# *Sessile organisms as indicators of possible secondary metal sources*

In mussels, the positive correlation between Cd and weight and the negative correlation between Cd and Cu suggest that as they grow, the homeostatically controlled Cu is proportionally surpassed by the bioaccumulative Cd in their organisms, likely bound to metallothioneins (Klaassen et al. 1999). Indeed, mussels produce Cd-induced metallothioneins (Mackay et al. 1993) and Cd can displace essential metals such as Cu and Zn normally associated to these proteins (Amiard et al. 2006). Tukey post-hoc tests resulted in separated groups for Cd (raw data for average lot length and mass and TE concentrations are available as Online Resource 1). One group containing samples from Foch, Cap Noir and Port-aux-Français;

and the second one, from Port-aux-Français and Mayes (always in crescent order). This result suggests some local Cd source enrichment inside the Gulf of Morbihan, where Port-aux-Français, the largest human settlement in the area (between 45 in austral winter and 120 people in austral summer) and Mayes are both located. Conversely, both other locations (Foch and Cap Noir) are exposed to the open ocean, in the northern shore. This is apparently contrary to the *T. gaudichaudii* data (Table 2), which presented levels for continental shelf samples three times higher than coastal ones. However, the average individual weight from the continental shelf samples was around three times the one from coastal samples, therefore there is likely an influence from age and, thus, from bioaccumulation. A strong effect of age is confirmed by a previous study on the closely related species *T. libellula* from the Greenland Sea (Ritterhoff and Zauke 1997). Nevertheless, a growth dilution effect should be expected for the continental shelf samples, which leaves two hypotheses: whether exposure is increased for larger individuals, or the influence of local Cd sources within the Gulf of Morbihan is not as important to *T. gaudichaudii* as it is for mussels. Since literature presents related species (*T. japonica* and probably *T. gaudichaudii* as well) switching from herbivory to carnivory during their life cycles (Pakhomov and Perissinotto 1996), the first hypothesis remains more likely, corroborating the existence of a local Cd secondary source.

Using fish for comparison did not prove to be helpful, since the only two species collected inside the Gulf were benthic (*N. rossii* and *P. magellanica*) and there was an evident bias in Cd levels towards pelagic species, as stated above, possibly because of Cd enriched upwelled waters around the archipelago(Bustamante et al. 1998b, 2003). For coastal sites inside the Morbihan Bay, another source can be suspected. Indeed, Mayes provides diverse and highly suitable breeding sites for large colonies of burrowing petrels, which breed there in very high densities (Weimerskirch et al. 1989), with up to 6 burrows per square metre (Mougeot et al. 1998). Seabird faeces can alter the trace metal composition in soils (Headley 1996) and therefore, colonies might work as secondary trace metal sources (see Choy et al. 2010; Espejo et al. 2014). Water percolating bird colonies has already been shown to have an important role in the exposure of terrestrial flora to contaminants in Antarctic lands (Cipro et al. 2011). It is therefore plausible to assume an analogous effect for the mussels when this water reaches the sea. Nevertheless, this subject deserves further investigation.

#### *Comparison with similar ecosystems in other regions*

Table 3 presents a comparison of the obtained results with those from the literature despite the scarcity of data for equivalent organisms in similar environments, which reinforces the need for studies such as this one. Therefore taxonomic, geographic and ecological differences might present some bias that has to be taken into account when interpreting the data.

Having said that, the jellyfish in our work showed Cd values at least one order of magnitude higher than its counterparts from North-eastern Atlantic waters (Caurant et al. 1999). Its Zn concentrations were around three times higher as well, not characterising the coaccumulation of Cd and Zn seen in other cases. To the best of our knowledge, the presence of metallothioneins in jellyfish has not been reported yet.

For Ctenophora, our results are somewhat closer to the ones for the North Atlantic Ocean than the ones for the Mediterranean, suggesting an influence from local conditions. Tunicates presented results for Cd and Cu in a reasonable agreement with the ones from the literature.

Crustaceans, in turn, showed some differences: in a general way, *Euphausia* spp. values of Cd, Cu and Zn are lower than in organisms from Antarctica and *Thysanoessa* spp. values for Cd were higher than in organisms from the Bering Sea, closer to pollution sources than Kerguelen Islands (Zauke et al. 2003). So, the difference might be due to a species-specific reason or in a lesser extent, to the influence of water masses dynamics

As for mollusks, bivalves presented lower values when compared to Antarctic filter feeders, even if the previously discussed influence of a local source is considered (Ahn et al. 1996; Bargagli 2001). Cephalopods, on the other hand, presented similar Hg levels when compared to another subantarctic environment, Macquarie Island (McArthur et al. 2003) or slightly superior when compared to branchial hearts concentrations from samples collected off Amsterdam Islands (Kojadinovic et al. 2011), which is located north of the Polar Front. Care must be taken when comparing digestive gland data (Kojadinovic et al. 2011) to muscle or even whole organism data, since this organ might present higher concentrations, even if it does not greatly contribute to the total Hg burden in some oceanic squids (Bustamante et al. 2006).

For benthic fish species, Antarctic organisms (Table 3) presented levels for both Cd and Hg one order of magnitude higher than in the present study. For pelagic ones, the only truly pelagic neritic fish present in all sizes throughout the water column is *Pleuragramma antarctica* (Wöhrmann et al. 1997). For this species, Brasso et al. (2014) present Hg data averaging 0.014  $\mu$ g g<sup>-1</sup> for juveniles and 0.021  $\mu$ g g<sup>-1</sup> for adults (for whole fish, after conversion to dry weight). This is an apparent contradiction to the trend previously discussed (Antarctic organisms with higher levels than subantarctic ones), however, this species feeds on a lower trophic level than the ones in Table 3 and also lives in shallower shelf waters (Giraldo et al. 2011; Pinkerton et al. 2013), which makes it less exposed to these contaminants. Nevertheless, Goutte et al. (2015) present an average of  $0.065 \mu g g^{-1}$  for *P. antarcticum* collected in Adelie Land, Antarctica, much similar to the concentration we found for *E. antarctica*.

Taking all the previous information into account, Kerguelen marine invertebrates and fish seemed to present lower metal concentrations when compared to other sub-Antarctic and especially to Antarctic environments, with the exception of some particular species, notably *Themisto gaudichaudii* (see also Beltcheva et al. 2011; Guynn and Peterson 2008; Hennig et al.

1985; Kahle and Zauke 2003; McArthur et al. 2003; Rainbow 1989; Stoeppler and Nürnberg 1979 and the comparison in Table 3).

# *Implications concerning the transfer to predators*

Because all of the species investigated here constitute to a wide extent, primary or secondary prey for seabirds breeding on the Kerguelen Islands, it is important to provide information on their exposure to trace elements. This is especially important for the non-essential Cd and Hg which can have toxic effects at different levels on wild vertebrates (e.g. Gallien et al. 2001; Tan et al. 2009; Goutte et al. 2014; Tartu et al. 2013). In addition to linking prey and predator, this section is intended to highlight the fact that some of the accumulation/magnification occur not only in high trophic levels.

In regard to Cd, the main homogenous group after Tukey HSD was composed by all species but *T. gaudichaudii*, jellyfish and both the cephalopods. *T. gaudichaudii* is an important part of local macrozooplankton and the main prey for local planktivorous seabirds (Bocher et al. 2001), therefore their main Cd source (Bocher et al. 2003). Jellyfish showed a Cd concentration one to two orders of magnitude higher than the fish, similar to the findings of Caurant et al.(1999). Jellyfish could therefore represent a vector for Cd transfer, since their energetic value is likely very low (Caurant et al. 1999) so their predators should ingest a large amount of them to satisfy their energetic needs. Cephalopods, in turn, may also function as vectors for Cd transfer to top predators (e.g., Bustamante et al. 1998a; Lahaye et al. 2005). This role is even more evident at higher latitudes as they showed somewhat higher Cd concentrations in Antarctic and subantarctic areas when compared to temperate and tropical waters (e.g. Dorneles et al. 2007; Kojadinovic et al. 2011; Pierce et al. 2008). Since cephalopods are present in the diet of several predators from Kerguelen Islands such as albatrosses or elephant seals (Cherel et al. 2000, 2004; Guinet et al. 1996; Lescroël et al. 2004), their role as Cd vectors is

evident. Reported concentrations in the kidney of seabirds from other subantarctic areas such as Gough Island in the South Atlantic Ocean clearly highlighted that seabirds feeding on squids displayed higher Cd concentrations that crustacean- and fish-feeder species (Muirhead and Furness 1988).

Hg, as previously presented, had a much less species-specific distribution, with less interspecific variation when compared to Cd, even though some stratification within the trophic levels could be detected. No statistical difference was found among all invertebrates taken together (Tukey HSD) and, concerning the fish, only *Notothenia rossii*, *Gymnoscopelus piabilis*, *G. fraseri* and *C. rhinoceratus* (in crescent Hg concentration) departed from a homogenous group that contained all the other species. The unicorn icefish *C. rhinoceratus* presented elevated Hg concentrations, which is consistent with its feeding ecology (Kock 2005a,b): indeed, the diet of juveniles is composed by crustaceans and then adults shift to forage on various notothenioids (mostly benthic) and mesopelagic fish in a lesser extent. In turn, mesopelagic fish, notably the myctophids, are among the most Hg contaminated. Therefore, predators relying on benthic or benthopelagic prey, as the gentoo penguin *Pygoscelis papua* (Lescroël and Bost 2005) or mesopelagic fish, such as elephant seals *Mirounga leonina* (Cherel et al. 2008) and the white-chinned petrel *Procellaria aequinoctialis* (Delord et al. 2010), would be highly exposed to Hg.

The present study highlights that not only top predators will be exposed to elevated amounts of Cd and Hg through their diet when consuming specific types of prey, but also lower trophic level organisms can be subject to the same effect. Zooplankton eating predators are exposed to Cd especially if they consume the amphipod *T. gaudichaudii* (i.e., *Halobaena caerulea*, *Pachyptila desolata*, *P. belcheri*, *Pelecanoides georgicus* and *P. urinatrix*, according to Bocher et al. 2003). Cephalopod eating species as the wandering albatross (*Diomedea exulans*) and the great-winged-petrel (*Pterodroma macroptera*) are also highly exposed to Cd but also to Hg which is consistent with the concentrations recorded in their internal tissues and their feathers (Anderson et al. 2009; Bustamante et al. 2016; Muirhead and Furness 1988; Stewart et al. 1999; Tavares et al. 2013) and also with blood (Anderson et al. 2010; Carravieri et al. 2014a). Finally, fish eating species, as the grey (*Procellaria cinerea*) or the white-chinnedpetrel (*Procellaria aequinoctialis*) are highly exposed (see Stewart et al. 1999; Anderson et al. 2009; Cipro et al. 2014) to Hg through the consumption of mesopelagic and benthic fish (Delord et al. 2010).

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#### Caption to figures and table



**Figure 1** – Kerguelen location in the Southern Indian Ocean among the French Southern Lands (left) and detailed map (right).STZ, SAZ and AZ stand for, respectively, the subtropical, sub-Antarctic and Antarctic zones; whereas STF and PF stand for the subtropical and polar fronts. Taken from Cipro et al (2014).



Figure 2 – Hg (left, linear scale) and Cd (right, log scale) concentrations (µg g<sup>-1</sup> dw) stratified by trophic level. Benthic species in black, pelagic ones in white.



Table 1 - Sample characteristics together with water content in the whole organisms (for dry/wet wt metal concentrations conversion). P: individuals in pooled samples



**Fishes**

Group		${\bf N}$	Cd		Cu		Hg		Zn	
<b>Species</b>	Family		$Mean \pm SD$	Range	$Mean \pm SD$	Range	$Mean \pm SD$	Range	$Mean \pm SD$	Range
<b>Ctenophors</b>										
<i>Beroe</i> sp.	Beroidae	3 <sup>p</sup>	$0.077 \pm 0.009$	0.070-0.088	$6.7 \pm 0.2$	$6.4 - 6.9$	$0.054 \pm 0.010$	$0.045 - 0.065$	$642 \pm 58$	582-697
<b>Euphausids</b>										
Euphausia frigida	Euphausiidae	4P	$0.137 \pm 0.047$	0.109-0.207	$5.8 \pm 0.1$	$5.7 - 5.8$	$0.023 \pm 0.002$	$0.021 - 0.025$	$43 \pm 3$	$41 - 43$
E. triacantha		3 <sup>p</sup>	$0.289 \pm 0.025$	0.260-0.306	$14.9 \pm 6.7$	$4.3 - 19.8$	$0.036 \pm 0.006$	0.029-0.041	$55 \pm 2$	53-57
E. vallentini (small)		3 <sup>p</sup>	$0.500 \pm 0.217$	0.278-0.711	$17.8 \pm 2.1$	15.4-19.5	$0.042 \pm 0.003$	0.039-0.045	$58 \pm 9$	52-68
E. vallentini (large)		3 <sup>p</sup>	$0.659 \pm 0.093$	0.589-0.765	$30.0 \pm 1.0$	29.0-30.9	$0.017 \pm 0.001$	0.016-0.018	$39 \pm 3$	$37-43$
Thysanoessa sp.		8 <sup>p</sup>	$3.66 \pm 1.80$	1.38-6.26	$33.7 \pm 12.8$	12.8-45.4	$0.067 \pm 0.031$	$0.024 - 0.125$	$30 \pm 11$	$10 - 47$
Copepods										
Paraeuchaeta antarctica $(\mathcal{Q})$	Euchaetidae	3 <sup>p</sup>	$2.11\pm0.05$	2.06-2.16	$8.8 \pm 1.1$	7.7-9.8	$0.038 \pm 0.019$	0.023-0.060	$245 \pm 66$	197-321
P. antarctica $(\delta)$		3 <sup>p</sup>	$0.81 \pm 0.05$	0.78-0.87	$4.9 \pm 0.4$	$4.4 - 5.2$	$0.072 \pm 0.002$	0.071-0.074	$87 \pm 9$	77-92
<b>Amphipods</b>										
Themisto gaudichaudii(coast)	Hyperridae	4P	$28.5 \pm 7.4$	21.2-38.8	$12.5 \pm 0.7$	11.9-13.4	$0.026 \pm 0.005$	0.022-0.034	$52 \pm 3$	48-55
T. gaudichaudii (shelf)	Hyperridae	4P	$73.4 \pm 7.2$	70.4-81.7	$16.0 \pm 7.1$	11.8-24.2	$0.024\pm0.002$	0.023-0.027	$82 \pm 5$	80-88
<b>Tunicates</b>										
Salpa thompsoni	Salpidae	3 <sup>p</sup>	$0.624 \pm 0.066$	0.583-0.700	$19.9 \pm 5.5$	14.9-25.7	$0.033 \pm 0.003$	0.030-0.035	$969 \pm 142$	821-1104
$\epsilon\epsilon$		3 <sup>p</sup>	$2.154 \pm 0.135$	2.066-2.310	$7.7\pm1.8$	$5.6 - 9.0$	$0.015 \pm 0.001$	0.013-0.016	$367 \pm 82$	302-458
Cnidarians										
Jellyfish	Not determined	3	$29.0 \pm 30.8$	9.98-64.53	$7.4 \pm 0.9$	$6.8 - 9.4$	$0.081 \pm 0.015$	0.067-0.097	$353 \pm 305$	142-703
<b>Mollusks</b>										
Moroteuthis ingens	Onychoteuthidae	8	$29.6 \pm 20.0$	$6.0 - 60.0$	$39 \pm 14$	$23 - 61$	$0.099 \pm 0.063$	0.034-0.215	$66 \pm 20$	19-81
Todarodes angolensis	Ommastrephidae	11	$79.4 \pm 41.8$	17.5-172.3	$80 \pm 39$	50-194	$0.100 \pm 0.058$	0.057-0.270	$107 \pm 27$	59-158
Mytilus edulis desolationis	Mytilidae	12P	$6.50 \pm 5.83$	2.27-27.71	$6.23 \pm 1.95$	$3.57 - 9.08$	$0.273 \pm 0.187$	0.113-0.647	$83 \pm 25$	48.7-125
Aulacomya atra		<b>9P</b>	$9.11 \pm 4.79$	4.05-18.49	$8.49 \pm 2.08$	5.87-12.71	$0.208 \pm 0.116$	0.105-0.417	$135 \pm 39$	84.2-221
<b>Benthic fish</b>										
Channichthys rhinoceratus	Channichthyidae	13	$0.173 \pm 0.125$	0.022-0.484	$1.8 \pm 0.4$	$1.3 - 2.7$	$0.345 \pm 0.296$	0.058-0.870	$79 \pm 26$	50-128
Gobiotothen acuta	Notothenidae	1		0.063	$\overline{\phantom{a}}$	2.9		0.075	$\overline{\phantom{a}}$	88
Harpagifer sp.	Harpagiferidae	4 <sup>p</sup>	$0.404 \pm 0.091$	0.330-0.536	$3.5 \pm 0.45$	3.00-3.93	$0.230 \pm 0.084$	0.128-0.334	$107 \pm 19$	80-123
Lepidonotothen squamifrons	Notothenidae	10	$0.475 \pm 0.198$	0.232-0.808	$1.5 \pm 0.4$	$1.1 - 2.5$	$0.150 \pm 0.089$	0.063-0.271	$43 \pm 9$	$31 - 58$
Notothenia cyanobrancha				0.323	$\sim$	2.1		0.216		64

Table 2 – Metal concentrations (µgg<sup>-1</sup> dry wt) of organisms from the Kerguelen Islands. Pooled samples are marked with a <sup>p</sup>



Taxa	Location	Cd	Cu	Hg	Zn	Reference	
Species							
<b>Cnidarians</b>							
Unidentified jellyfish	Southern Indian Ocean	$29.0 \pm 30.8$	$7.4 \pm 0.9$	$0.081 \pm 0.015$	$353 \pm 305$	Present study	
Pelagia noctiluca	Mediterranean Sea	0.4	2.0		46	Romeo et al. (1987)	
Velella velella	Mediterranean Sea	$1.2 \pm 0.5$	$5.7 \pm 0.7$		$118 \pm 34$		
$\alpha$	Mediterranean Sea	$2.7 \pm 0.9$	$9.3 \pm 1.9$		$100\pm18$		
Sagartia troglodytes	Seine estuary, France	0.06	5.8		238	Miramand et al. (2001)	
Ctenophora							
Beroe sp.	Southern Indian Ocean	$0.077 \pm 0.009$	$6.7 \pm 0.2$	$0.054 + 0.010$	$642 \pm 58$	Present study	
Beroe ovate	Mediterranean Sea	$1.4 \pm 0.4$	$2.8 \pm 0.8$		$29 \pm 9$	Romeo et al. (1992)	
$\alpha$	North Atlantic	0.16	2.0		52	Dubé (1982)	
<b>Tunicates</b>							
Salpa thompsoni	Southern Indian Ocean	$0.624 \pm 0.066$	$19.9 \pm 5.5$	$0.033 \pm 0.003$	$969 \pm 142$	Present study	
$\alpha$	Southern Indian Ocean	$2.154 \pm 0.135$	$7.7 \pm 1.8$	$0.015 \pm 0.001$	$367 \pm 82$	Present study	
S. maxima	Mediterranean Sea	$0.4 \pm 0.4$	$5.9 \pm 2.4$		$79 \pm 20$	Romeo et al. (1992)	
S. fusiformis	Mediterranean Sea	$1.1 \pm 1.0$	$6.6 \pm 4.9$		$64 \pm 28$	$\mathbf{u}$	
Euphausiacea							
Euphausia frigida	Southern Indian Ocean	0.109-0.207	$5.7 - 5.8$	$0.021 - 0.025$	$41 - 43$	Present study	
E. triacantha	Southern Indian Ocean	0.260-0.306	$4.3 - 19.8$	0.029-0.041	53-57	Present study	
$\alpha$	Southern Ocean	9.0	31	$\overline{a}$	402	Hennig et al., (1985)	
E. superba	Western Antarctic Peninsula	$0.13 - 0.75$	37.8-140	0.0131-0.0489	35.2-51.3	Locarnini and Presley (1995)	
E. vallentini	Southern Indian Ocean	0.278-0.765	15.4-30.9	0.016-0.045	$37 - 68$	Present study	
Thysanoessa longipes	Bering Sea	$0.36 - 1.14$			59-88	Hamanaka and Tsujita (1981)	
Thysanoessa sp.	Southern Indian Ocean	1.38-6.26	12.8-45.4	$0.024 - 0.125$	10-47	Present study	
Copepoda							
Paraeuchaeta antarctica	Southern Indian Ocean	$0.78 - 2.16$	$4.4 - 9.8$	0.023-0.074	77-321	Present study	
Calanus hyperboreus	Baffin Bay, Canadian Arctic	$1.62 \pm 0.79$	$1.55 \pm 0.57$	$0.025 \pm 0.017$	$17.73 \pm 0.65$	Campbell et al. (2005)	
Several species	Fram strait, Arctic	$0.32 - 0.75$	$4.0 - 7.5$	$0.31 - 0.68$	$79 - 351$	Ritterhoff and Zauke (1997)	
	Greenland Sea	$0.12 - 0.69$	$3.8 - 5.9$	$0.2 - 0.5$	$86 - 389$		
Pooled (Eurytemora affinis, Acartia	Seine estuary, high salinity	$0.9 \pm 0.4$	$15 \pm 6$		$260 \pm 68$	Miramand et al. (1998)	
clausi and Temora longicornis)	Seine estuary, low salinity	$5.5 \pm 2.1$	$47 + 27$	÷	$480 \pm 190$		
Several species	Weddel Sea, Antarctica	$2.3 - 14.4$				(Kahle and Zauke, 2003)	

**s** - Comparison of heavy metal concentrations in organisms from the Kerguelen Islands with those from the literature (range or mean  $\pm SD$ , in  $\mu$ g g<sup>-1</sup> dw).



