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► **To cite this version:**

Jessica Kojadinovic, Christine H. Jackson, Yves Cherel, George D. Jackson, Paco Bustamante. Multi-elemental concentrations in the tissues of the oceanic squid *Todarodes filippovae* from Tasmania and the southern Indian Ocean. *Ecotoxicology and Environmental Safety*, 2011, 74 (5), pp.1238-1249. 10.1016/j.ecoenv.2011.03.015 . hal-00599931

**HAL Id: hal-00599931**

**<https://hal.science/hal-00599931>**

Submitted on 11 Jun 2011

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Multi-elemental concentrations in the tissues of the oceanic squid *Todarodes filippovae* from Tasmania and the southern Indian Ocean

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**Abstract :** This study investigates 14 elements (Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V and Zn) in the tissues of the oceanic ommastrephid squid *Todarodes filippovae* from waters surrounding Ile Amsterdam (southern Indian Ocean) and Tasmania (Australia). As for other cephalopod species, the digestive gland and branchial hearts showed the highest concentrations of many elements (Ag, Cd, Se, V and Zn, and Cr and Ni respectively) highlighting their role in bioaccumulation and detoxification processes. With the exception of As and Hg, the muscles showed relatively low trace element concentrations. Squid size was positively correlated to Ag, As, Cd, Hg and Zn concentrations in Tasmanian squid and negatively correlated to all but Hg and Zn concentrations in Ile Amsterdam squid. Furthermore, no differences in elemental concentrations were noted between sexes. There were, however, some differences between mated and non-mated females from Tasmania. Comparing elemental concentrations in squid from both islands, higher concentrations of Cd, Co, Cr, Ni, Pb and V in squid sampled in Ile Amsterdam reflect different exposure conditions. When considering *T. filippovae* as a dietary resource for humans it should be noted that, given their Hg content, squids from Ile Amsterdam are not recommended for consumption on a regular basis. Moreover, regardless of the squid's origin, digestive glands should be avoided as Cd and Hg concentrations were above the European Union authorized limits in these organs.

**Key words:** Cephalopod; Southern Indian Ocean; Tasmania; Metals; Bioaccumulation; Consumption guidelines.

## **1 Introduction**

Trace elements in cephalopods have received increasing attention in recent decades, particularly in Europe and in Japan, as these molluscs play a major role both as predator and prey in marine ecosystems (Boyle and Rodhouse, 2005), and are known to accumulate large quantities of Cd that can be transferred to higher trophic levels (e.g. Bustamante et al., 1998a; Dorneles et al., 2007). Cephalopods also represent an increasing resource for humans (Piatkowski et al., 2001). Historically, the consumption of cephalopod products has been highest in countries of south-east Asia (e.g. Japan) and these countries still predominate in fisheries for cephalopods as well as in developing new technologies for catching them. In European waters, cephalopods have traditionally been a minor catch, although locally important in some southern European countries such as Portugal, Italy, Spain and Greece where cephalopods are high in local diets (Payne et al., 2006). However their importance to European fisheries is increasing with the decline in traditional finfish

stocks across the globe. For instance in the English Channel between 1983 and 2003, total catches of cephalopods increased by almost 300%, from 8000 to 23000 tonnes (Payne et al., 2006). Cephalopod fisheries are also becoming more important in other parts of the world, with the example of total cephalopod catches in Chile increasing by more than 5000% from 69 tonnes in 1978 to 3503 tonnes in 1996 (Rocha and Vega, 2003). This global fishery effort is likely to further increase in the next decade and species from remote areas could represent new fishery targets.

Besides their important ecological role, cephalopods display relatively short life spans, typically from one to two years in most of the targeted species. For this reason, they are of major interest for monitoring variations of pollutant concentrations in the environment as the accumulated concentrations in the cephalopod tissues reflect the bioavailability and metal variations in their immediate environment over a relatively short time scale (Rossi et al., 1993; Miramand et al., 2006). Indeed trace element analyses in marine organisms are commonly used to reflect the ambient concentrations, providing an integrated information over time according to the biological half life of elements in animals' tissues (e.g. Bustamante et al., 2008; Hédouin et al., 2009). This approach is particularly useful when trace elements are present at very low concentrations in the environment as it occurs in the Austral Ocean (Honda et al., 1987; Fitzwater et al., 2000; Sañudo-Wilhelmy et al., 2002) and to compare different locations (Rainbow, 1995; Warnau et al., 1998).

Most of the studies concerning metal bioaccumulation have focused on species from the northern hemisphere, while very little data has been reported for cephalopods in the southern hemisphere. To the best of our knowledge, these studies are limited to selected metals such as Cd, Cu and Zn (e.g. Bustamante et al., 1998b; Dorneles et al., 2007). In the Southern Ocean the importance of cephalopods in trophic webs is exacerbated as squid can occupy the ecological niche of epipelagic fish (Rodhouse and White, 1995).

Although species of the genus *Todarodes* are particularly common in the northern part of this ocean, there is a paucity of biological information for the genus, particularly in relation to metal concentrations. Similarly there are surprisingly few data on metals for the well studied Japanese squid, *Todarodes pacificus* (Tanaka et al., 1983; Ichihashi et al., 2001b; Oikawa et al., 2003; Kim et al., 2008). Records of metal concentrations in *T. filippovae*, which has a vast but remote distribution zone, are to our knowledge nonexistent.

This paper describes the bioaccumulation and tissue distribution of 14 trace elements (Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V and Zn) in *T. filippovae* from waters neighboring the Subtropical Front<sup>1</sup> in the northern part of this species' distribution zone. These elements were selected as they represent, on one hand common essential elements accumulated by marine organisms, on the other hand non-essential elements the most likely to be hazardous to the squid's health and to that of its predators, including humans. The uniformity of elemental concentrations within this area is tested by comparing the results from two sampling zones located in the southern Indian Ocean and the southern Pacific Ocean (waters surrounding Ile Amsterdam and Tasmania, respectively) where *T. filippovae* appears to be the most abundant ommastrephid species (Dunning, 1993). Furthermore, because *T. filippovae* is believed to have some fishery potential, the elemental concentrations obtained in this study are analyzed within the framework of metal concentrations associated with health risks to humans.

## 2 Materials and methods

### 2.1 *Todarodes filippovae*

*Todarodes filippovae* (Adam 1975) is a large epipelagic ommastrephid squid that can reach 50 cm (mantle length), although it commonly measures between 20 and 40 cm (Rodhouse, 1998). It is characterized by a circum-polar distribution in the Southern Ocean from sub-Antarctic waters to slightly north of the Subtropical Front (south of 35°73 S), in association with high velocity currents (Dunning and Wormuth, 1998; Jackson et al., 2007). This oceanic species occurs mostly between the surface and 200 m in depth, in the open ocean up to the continental slope, and appears not to extend into shelf waters (Rodhouse, 1998). Its life cycle is thought to be about a year. Spawning occurs in the Tasman Sea and off South Africa and probably takes place in the austral autumn and winter (Rodhouse, 1998; Jackson et al., 2007). *Todarodes* species are opportunistic and will feed on fish as well as squid, or even have cannibalistic tendencies (Boyle and Rodhouse, 2005). Indeed, *T.*

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<sup>1</sup> Subtropical Front: A zone of enhanced meridional gradients of sea surface temperature and salinity in the poleward part of the subtropical convergence. A commonly used criterion that is found at the latitude at which the salinity, at a depth of 100 m, drops below 34.9 practical salinity units. In the Southern Hemisphere, the subtropical front can be traced from 40° S at the east coast of South America across the Atlantic into the Indian Ocean and across the Great Australian Bight, where it shifts to 45° S to pass south of Tasmania and reach the southern tip of New Zealand's South Island. It continues in the Pacific Ocean from the Chatham Rise east of New Zealand near

*filippovae* feeds on myctophids and squid in Tasmanian waters (Jackson et al., 2007). *Todarodes* species are likely to be an important part of the ecosystem in the Southern Ocean as a number of vertebrates prey on them including marine mammals (such as the sperm whale *Physeter macrocephalus* and the southern elephant seal *Mirounga leonina*), fish (e.g. the haddock *Merluccius australis*) and seabirds (e.g. albatrosses *Diomedea melanophrys*) (Rodhouse, 1998; Cherel et al., 2002; Jackson et al., 2007). Although research on species identification, stock structure, and most aspects of its biology is still needed, *T. filippovae* is believed to have some fishery potential, with commercially viable catch rates having been reported in the Tasman Sea (Rodhouse, 1998). First taken as bycatch to the Japanese jig fishery for *Nototodarus sloani* off New Zealand and southern Australia, it started being caught in commercial quantities off northeast Tasmania in 1978. In this region, it is the most abundant ommastrephid in the Subtropical Front and in slope waters off southeastern Australia (Dunning, 1993).

## 2.2 Sampling and preparation

*T. filippovae* were sampled from two locations, approximately 5 000 km apart, both situated slightly north of the Subtropical Front, in the northern part of the species distributional zone. The first location, in the Indian Ocean, lies roughly centered between the southern tip of Africa and Australia. Twenty four individuals were collected from the research vessel *La Curieuse* during a cruise undertaken in April 2000. Squids were taken from 22 pelagic trawls (13 - 380 m) during five consecutive nights in oceanic waters between 44°S, 76°E, and Saint Paul and Amsterdam islands (38°S, 78°105 E). Secondly, in the south Pacific Ocean, samples were obtained from waters around Tasmania (42°00'S, 147°107 00'E) which is situated off the south-east corner of the Australian mainland. Thirty two specimens were trawled in waters south-east of Tasmania in February/March

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40° S and reaches the west coast of South America near 30° S (American Meteorology Society, 2010; Wikimedia, 2010).

and July/August 2005. Samples were frozen in plastic bags, at  $-20^{\circ}\text{C}$ , on board the sampling vessels.

Individual squid were defrosted and dorsal mantle length (ML) was measured to the nearest millimeter (mm). The total weight of each squid as well as the weight of the ovary or testis, digestive gland, gills and branchial hearts were weighed to the nearest gram (g). Significantly larger cephalopods were collected from Tasmanian waters (Table 1). Individuals were also sexed and females were examined as to whether they had recently mated. Subsamples of mantle muscle and digestive gland as well as the gills and branchial hearts were removed from all squid then freeze-dried, blended and ground to a fine powder to prepare for elemental determination. A subsample of the gonad was also removed from the Tasmanian squid and prepared in the same way.

### 2.3 Metal analysis

The metal analyses in samples from both study sites were realized simultaneously in the same batches of analyses. The total Hg concentrations in the powder obtained from the tissues were determined by analyzing Hg directly with an Advanced Mercury Analyzer (ALTEC AMA 254) on aliquots ranging from 5 to 50 mg of dry sample weighed to the nearest 0.01 mg (Bustamante et al., 2006b). The analysis of Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, V and Zn required an extra step in the preparation protocol. From 20 to 300 mg of each sample were microwave digested in a mixture of 3 ml of suprapure nitric acid (VWR/Merck) and 1 ml of suprapure chloridric acid (VWR/Merck), and then diluted to 25 ml with deionized water. These 13 elements were then analyzed by Inductively Coupled Plasma Atomic Emission Spectrometry (Varian Vista-Pro ICP-AES) and Mass Spectrometry (ICP-MS II Series Thermo Fisher Scientific) in all tissues but the gonads. To avoid metal contamination, all glass and plastic utensils used were washed with detergent, soaked in a bath of mixed nitric ( $35 \text{ mL.l}^{-1}$ ) and chlorhydric ( $50 \text{ mL.l}^{-1}$ ) acids for a

minimum of 24 h, rinsed 3 times in deionized (Milli-Q quality) water and dried in an oven at 50°C before use.

Accuracy and reproducibility of the preparation were tested by preparing analytical blanks and replicates of lobster hepatopancreas (TORT-2) and dog-fish liver (DOLT-3) reference standards (National Research Council, Canada) along with each set of samples. Recovery rates were respectively equal to  $91 \pm 9\%$  for Ag,  $95 \pm 23\%$  for As,  $92 \pm 7\%$  for Cd,  $83 \pm 8\%$  for Co,  $105 \pm 40\%$  for Cr,  $94 \pm 8\%$  for Cu,  $87 \pm 8\%$  for Fe,  $100 \pm 3\%$  for Hg,  $96 \pm 14\%$  for Mn,  $95 \pm 15\%$  for Ni,  $109 \pm 23\%$  for Pb,  $154 \pm 23\%$  for Se,  $96 \pm 7\%$  for V, and  $101 \pm 12\%$  for Zn. Element concentrations are expressed in  $\mu\text{g}\cdot\text{g}^{-1}$  of dry weight (dw).

## 2.4 Data analysis

Besides classical numerical summaries and plots, the 149 following statistical analyses were performed at the 95% confidence level while carefully observing the p-value in the analysis. First, t-tests were used to assess whether the squid sampled in Tasmania were significantly different from those sampled in Amsterdam in terms of the biological measurements (Table 1). Second, one-way ANOVAs for paired samples were used to assess whether there were significant differences between concentrations of the same metal in up to five tissues. In addition, in order to test the significance of differences in As and Cu concentrations between gills and the other tissues of Tasmanian squid, we performed paired t-tests and adjusted their p-values using Bonferroni's correction (which is known to be a conservative method). Third, ANCOVAs, using the log-transformed mantle length as covariate, were used to test the effect of sex (in Amsterdam squid), of the mated status (in Tasmania squid) and of the geographic site on the log-transformed elemental concentrations.

## 3 Results



This section documents the concentrations of trace elements in the tissues of *T. filippovae* caught in Ile Amsterdam and Tasmanian waters (see Tables 2 and 3, and Figures 1, 2 and 3), as well as describes factors that were shown to influence trace element concentrations in this species. However, for some elements, concentrations were below the detection limits. In such cases (“< DL” symbol in Tables 2 and 3) the results were not included in any further analyzes.

### 3.1 Organotropism

The digestive gland contained the highest concentrations of Ag, Cd and Zn of all the tissues analyzed, irrespective of geographic location. Similarly, the highest concentrations of Co and Cu as well as Fe were found in the digestive gland of squid from Ile Amsterdam and Tasmania respectively (Figures 1, 2 and 3). The branchial hearts had the highest concentrations of Cr and Ni in both locations along with the highest concentrations of Hg and Fe in Ile Amsterdam squid and Co in Tasmanian squid. However, the gills were the major accumulation tissues for As, Cu and Mn in *T. filippovae* caught around Tasmania (for As  $p < 0.01$  between gills and digestive gland and  $p < 0.001$  between gills and muscle; for Cu  $p < 0.001$  between gills each other tissues: branchial hearts, digestive gland and muscle; for Mn  $p < 0.01$  between gills and muscle). On the other hand, the highest concentrations of Hg in Tasmanian squid were found in the muscle. For the three tissues in which there were detectible concentrations of As, the highest amounts were found in the gills of the Tasmanian squid and in the muscle of the Ile Amsterdam squid. Furthermore, detectible concentrations of Pb were highest in the gills of Tasmanian squid but for Ile Amsterdam squid, the digestive gland had the highest concentration of Pb. Levels of Se and V were highest in the digestive glands from both of the study areas. Overall, the digestive gland stands out as the tissue of greatest importance in terms of elemental accumulation followed by the branchial hearts, the gills and the muscle.

### 3.2 Squid biological variables

Among the measurements taken on each individual (viz. mantle length, total weight, ovary weight, digestive gland weight, gills weight and branchial heart weight), mantle length was found to be the most correlated to the concentrations of trace elements (Table 4). Moreover mantle length was very well correlated to the weight measurements (data not shown) and was thus chosen as a covariate when testing (by means of ANCOVAs) the effect of sex, mated status and geographic site on elemental concentrations.

The influence of gender was tested only in Ile Amsterdam as there were no males among the squid collected in Tasmania. Jackson et al. (2007) have noted that males seemed better at avoiding the trawl nets than females. No significant difference in elemental concentrations was detected between male and female squid of equal mantle length (Table 5).

Mated females were found only from Tasmanian waters. The comparison of elemental concentrations between mated and non-mated females of similar sizes showed no significant differences in the branchial hearts, gills or gonads. However, some weak evidence was found for As and Cu in the muscle, and for Cr and Mn in the digestive glands (Table 5). There were lower concentrations of Cu and higher concentrations of As, Cr and Mn in mated females (data not shown).

### 3.3 Geography

The size-normalized comparison of the squid sampled in Ile Amsterdam and in Tasmania indicated significant differences in concentrations of all elements but Mn and Se, although not for every

element/tissue combination (Table 5). Levels were higher in Ile Amsterdam than in Tasmania for Cd, Cr, Ni and Pb in all tissues, and equal or higher for Co and V according to the tissue (Tables 2 and 3 and Figures 1, 2 and 3). On the contrary, concentrations were higher in Tasmania for As in all tissues, and equal or higher for Cu, Hg and Zn according to the tissue. The results for Ag and Fe were opposite in different tissues. For Ag, concentrations in the digestive gland were highest in squid from Ile Amsterdam whereas, in the gills they were highest in squid from Tasmania. For Fe, concentrations in the branchial hearts were highest in squid from Ile Amsterdam whereas, in the digestive gland, they were highest in squid from Tasmania. Overall, particularly large differences in concentrations between both sites were observed for Cd, Co, Cr, Cu in the gills, Fe in the branchial hearts and the digestive gland, for Hg in the muscle tissue and for Ni in the branchial hearts (Figures 1, 2 and 3).

#### 4 Discussion

Trace element concentrations in cephalopods have received increasing attention over the last decades as these molluscs play a major role both as predators and prey in marine ecosystems, and are widely consumed by man (e.g. Boyle and Rodhouse, 2005; Pierce et al., 2008). Previous studies have demonstrated their ability to rapidly accumulate high concentrations of trace elements in their tissues making them good bioindicators of environmental levels but also a potential source of human intoxication (e.g. Storelli et al., 2006, 2010; Pernice et al., 2009). As in most marine animals, concentrations may vary with biological factors specific to an individual, a population or a species, or with environmental factors (Pierce et al., 2008). The impact of such factors on the elemental concentrations found in *Todarodes filippovae* are discussed hereafter in the aim to facilitate the future use of this species as a bioindicator of environmental element levels. Finally, the results are regarded from a human health perspective.

#### 4.1 Metal distribution in the tissues

Many studies highlight that in cephalopods, the digestive gland has a central role in the bioaccumulation of trace elements, particularly for toxic metals such as Ag and Cd (see e.g. Martin and Flegal, 1975; Ichihashi et al., 2001a; Bustamante et al., 2002b, 2004a; Miramand et al., 2006). The branchial hearts are considered as excretion tissues, allowing the depuration and/or the storage of various elements (e.g. Nardi and Steinberg, 1974; Guary et al., 1981; Bustamante et al., 2002b). Consequently, in most cephalopod species, these two tissues contain the highest concentrations of many trace elements. This was also the case in this study of *T. filippovae*, irrespective of location, particularly in relation to Ag, Cd, Se, V and Zn, and Cr and Ni that were concentrated in the digestive gland and the branchial hearts respectively. It is interesting to note that, in many marine animals, Ag, Cd, Cu, Hg and Zn bind to metallothioneins as detoxification and homeostasis processes (Anan et al., 2002; Bustamante et al., 2004a). Although these phenomena are not well documented in cephalopods (Tanaka et al., 1983; Craig and Overnell, 2003; Bustamante et al., 2006a), the joint concentration of Ag, Cd, Cu and Zn in the digestive gland of *T. filippovae* may well be an indication that detoxification mechanisms exist to hinder the toxic action of both essential and non-essential metals in this species. The strongest metal concentration correlations were observed in the digestive gland between Ag, Cd, Cu and Zn in squid from Amsterdam (correlation coefficients ranging from 0.69 to 0.89, with 266 p-values <0.001), and between Ag and Cu in squid from Tasmania ( $r=0.79$ ,  $p<0.001$ ). The correlations cited above may be the sign of the joint binding of these metals to metallothioneins.

Another alternative route for metal elimination in female squid is the transfer of metals to their eggs, as this has been observed in experimental conditions for Ag, Se and Zn (Lacoue-Labarthe et al., 2008). No significant differences in Cd and Hg concentrations between mated and non-mated females were observed in this study suggesting that these two elements are not likely to be excreted through the eggs in *T. filippovae*. The transfer to the eggs might however be specific to certain

elements such as essential ones (Lacoue-Labarthe et al., 2008). Levels of Cu in muscle were found to be significantly lower in mated individuals than in non-mated ones suggesting a transfer of Cu from the muscle to the gonads in the mated females as this element is essential for the development of the hatchlings (Villanueva and Bustamante, 2006).

In individuals originating from Tasmania, the gills concentrated most of the As, Cu and Mn. The majority of the elements present in cephalopods are assumed to be incorporated by the diet as these carnivorous animals have a high feeding rate (Bustamante et al., 2002b; Villanueva and Bustamante, 2006; Bustamante et al., 2006c). However direct uptake of elements from seawater through the gills is also a known phenomenon in cephalopods (Bustamante et al., 2004b, 2006c). It has been verified in different species for elements such as Ag, Cd and Zn (Ueda et al., 1985; Koyama et al., 2000; Bustamante et al., 2002a, 2004b) and suggested for elements such as Cr, Fe and Ni (Miramand et al., 2006; Bustamante et al., 2008). It appears from the present results that seawater may also be a direct source of As and Mn in some cephalopods under certain conditions (in Ile Amsterdam the gills were not the target tissue for As). It would be interesting to further pursue this hypothesis as, to the best of our knowledge, there exist no experimental studies on the absorption of these elements via the gills. The results for Cu were quite striking, with concentrations, two, three and 58 fold higher in the gills than in the branchial hearts, the digestive glands and the muscles, respectively. Copper is however usually present in low concentrations in seawater (Watanabe et al., 1997). The most straightforward explanation for such a gill-oriented accumulation of this essential element is its association with haemocyanin. Indeed, Cu works as a respiratory pigment in haemocyanin, which is the typical dioxygen carrier for molluscs and crustaceans that represents 98% of blood protein in cephalopods (Ghiretti, 1966; D'Aniello et al., 1986). Due to the respiratory function of the gills, it would appear highly likely to observe a greater presence of haemocyanin and hence Cu in the gills.

The muscle tissue of *T. filippovae* was characterized by the lowest concentrations of most trace elements. This result is consistent with data reported for other cephalopod species (Miramand and Bentley, 1992; Storelli and Marcotrigiano, 1999; Ichihashi et al., 2001b; Bustamante et al., 2008). There were two exceptions, for As and Hg, which were accumulated mostly in the muscle of cephalopods from Ile Amsterdam and Tasmania, respectively. Arsenic has been shown to have a tropism towards muscular tissues in cephalopods (Pernice et al., 2009), but few data exist on its speciation. The As accumulated in the muscle tissue of *T. filippovae* could be mostly present in its organic form, hence non toxic, but further research would need to be conducted to corroborate this possibility. A Hg organotropism towards muscle has also previously been described by various authors including (Bustamante et al., 2006b), who related it to the existence of a Hg excretion function of the digestive gland and/or a preferential redistribution of Hg (mostly in the organic form) to muscular tissues where it binds to sulphhydryl groups of proteins. Such chemical transformations are also well described in fish in which virtually 100% of the Hg is in the methylated form (Bloom, 1992). Since food appears as the main pathway of exposure for Hg (Lacoue-Labarthe et al., 2009b) and *T. filippovae* mainly feeds on mesopelagic fish in both areas (Jackson et al., 2007, Cherel unpublished data), the reasons for such a difference in the accumulation processes between *T. filippovae* from Tasmania (Hg mostly accumulated in the muscle) and those from Ile Amsterdam (Hg mostly accumulated in the branchial hearts) appear unclear.

## 4.2 Influence of biological variables

The potential impact of certain biological factors are considered here, such as body and organ measurements, sex and whether or not the individual had mated that year.

### 4.2.1 Size-related elemental concentrations

The most obvious result was the correlation between the size (viz. mantle length) of *T. filippovae* and the trace element concentrations found in its tissues (Table 4). Such an observation is common across a large spectrum of marine species. Most often, given the bioaccumulating properties of trace elements, their concentrations tend to increase with the size (and presumably age) of the individual. This was the case for Ag, As, Cd, Hg and Zn in Tasmanian squid and for Hg and Zn in Ile Amsterdam squid. On the contrary, for all the other elements tested in squid from Ile Amsterdam, concentrations decreased with squid size. This denotes a greater tendency of these elements to accumulate in small sized animals rather than in larger ones. A plausible explanation for the negative correlations between these metals and squid size could be attributed to the marked modification in *T. filippovae's* diet during its life cycle. It is common for squid to experience a drastic change in diet during ontogeny, young squid consuming mainly crustaceans and older squid living primarily on fish (Pierce et al., 2008). This hypothesis concurs with recent findings stemming from isotopic studies on the very same animals used in this study, indicating the existence of dietary shifts from lower to higher trophic levels during growth (Cherel et al., 2009). Another explanation could be the existence of a “dilution” effect linked to the rapid increase of tissue mass due to growth (that is very rapid in cephalopods) with regards to proportionally lower intake of trace elements (Pierce et al., 2008).

#### 4.2.2 Sex and mated status

Contrary to expectations, no differences in metal concentrations were noted between sexes in spite of differential growth rates enabling females to reach greater mantle lengths and weights than males (Jackson et al., 2007; Cherel et al., 2009). This suggests, however, that both sexes share the same physiology regarding uptake and depuration of trace elements, as previously suggested during exposure to radioisotopes of a range of metals in *Sepia officinalis* (Bustamante et al., 2002b, 2004b,

2006c; Lacoue-Labarthe et al., 2009a). Additionally, this also suggests that males and females of similar sizes have a similar diet as the trophic pathway is the most important one for the majority of trace elements (Bustamante et al., 2002a; Lacoue-Labarthe et al., 2009a). Among females however, differences in muscular Cu concentrations and concentrations of Cr and Mn in the digestive gland, seem linked to the reproductive state (Table 5). Some of these differences could be due to a redistribution of these essential elements within the organism of the mated individuals.

#### 4.3 Influence of geography

Among environmental factors, the geographical origin often has a considerable impact on trace element concentrations in cephalopods. The environment dictates the quantity of trace elements available, either directly by water absorption or indirectly through the ingestion of impregnated prey items.

For a recent review of metal concentrations in different cephalopod species from various origins refer to Bustamante et al. (2008), Table 3. Overall, the metal concentrations measured in the digestive gland of *T. filippovae* during this study are within the spectrum of values found in the digestive gland of cephalopods worldwide, often towards the lower end, specially for Pb and V. With the exception of Cd, the concentrations of non-essential elements resemble particularly those measured in the closest related species, *T. pacificus* and *Stenoteuthis oualaniensis*, sampled off Japan (western Pacific Ocean). Cadmium concentrations in Ile Amsterdam were higher than in most other cephalopods and similar to another ommastrephid species, *Ommastrephes bartrami*, sampled off the California coast (eastern Pacific Ocean). It is noteworthy that the concentrations of Cr and Ni reported here are among a very small body of information on these metals in cephalopods. In comparison, Ni and Cr concentrations in the digestive gland of *T. filippovae* were 3 to 30, respectively 5 to 31, times lower than concentrations measured in nautilus species from New



Caledonia and the Vanuatu archipelago (Bustamante et al., 2000; Pernice et al., 2009). However, the concentrations of essential elements seem to be more variable among species in relation to the specific physiological needs of each animal rather than purely dependent on the environment. For instance, Pernice et al. (2009) showed that nautilus living in an environment enriched in Ni (from Ni mining in New Caledonia) did not have higher Ni concentrations than a closely related nautilus species living in an environment not Ni-enriched (Vanuatu archipelago).

In this study, *T. filippovae* showed significantly different elemental concentrations according to their location. Higher concentrations of Cd, Cr, Ni and Pb (and to a lesser extent Co and V) in squid sampled in Ile Amsterdam compared to those caught in Tasmanian waters could be an indication that these elements are present in higher concentrations and/or more available in the Ile Amsterdam environment. On the contrary, concentrations of As (and to a lesser extent Cu, Hg and Zn) were highest in squid from Tasmania suggesting higher bioavailability of As (and potentially Cu, Hg and Zn) in the Tasman sea. Differences in trophic behaviors and diets of *T. filippovae* between locations, if they exist, could also explain some of the site-related differences in metal concentrations.

#### 4.4 *T. filippovae* as a resource for man, a health viewpoint

As mentioned previously *T. filippovae* is likely to be an important part of the ecosystem as it is preyed upon by a number of vertebrates, including man. Moreover, stocks of more broadly targeted squid species undergo great fluctuations in abundance from year to year and will potentially be declining under an increasing pressure as global fisheries look to new populations to exploit (Boyle and Rodhouse, 2005). From this standpoint, *T. filippovae* could soon become a new targeted resource and the object of much larger fisheries than it is today. Consequently, it is of interest to relate the metal concentrations given here to concentrations associated to health risks in humans. Although they lack uniformity, guideline values are available for cephalopod consumers. The

European Union has set regulations for Cd, Hg and Pb (European Commission, legislations 2001/22/CE) and the United Nations has set regulations for As in addition to the elements mentioned above. Silver, Cr and V are very seldom reported in mollusc studies and have not been appointed international legal concentration limits. International legal thresholds are also nonexistent for essential elements even though, in excess, these elements can be toxic.

Mercury is of particular concern for human health. High exposures to Hg, dependent on its form, can cause disruption of the nervous system, damage to brain functions, gastrointestinal tract and kidneys, DNA and chromosomal damage, allergic reactions and negative reproductive effects (such as sperm damage, birth defects and miscarriages) (Clarkson et al., 2003). Inorganic mercury occurring naturally, or from pollution, is converted to methyl-mercury (MeHg) by microorganisms and is biomagnified up the food chain. Consequently, a large percentage of Hg is present as toxic MeHg in the edible portions of squids consumed by man. The most widely established guideline value for Hg concentrations in cephalopods is  $0.5\mu\text{g}\cdot\text{g}^{-1}$  ww. This value, as those that will be given hereafter for Cd, Pb and As, corresponds to the guideline value for whole gutted animals which, for squid, corresponds mostly to muscle tissue. We have thus compared only the muscular Hg concentrations to this recommended limit and found that they exceeded  $0.5\mu\text{g}\cdot\text{g}^{-1}$  ww in 52% of cases in Ile Amsterdam and in 9% of cases in Tasmania. The theories according to which “exposure to MeHg exceeding guideline levels leads to adverse effects” or “maternal MeHg exposures are directly associated with adverse child development” should be considered with a critical eye as they are somewhat incomplete. First, as for any metal, unless the dose absorbed is extremely high, the exposure must be prolonged for adverse effects to appear. Second, the toxicity of a metal may be decreased by its interaction with other elements, such as Se in the case of Hg. Se is a nutritionally essential element that is present in all foods, but is particularly abundant in ocean fish and squid. A sufficient intake of Se offers a protective effect against Hg by their mutual irreversible binding (Parizek and Ostadalova, 1971; Iwata et al., 1973; Ohi et al., 1976; Whanger, 1992). For a detailed

explanation of these biochemical processes refer to Ralston (2008). In order to better assess the true risk linked to Hg exposure, Kaneko and Ralston (2007) have proposed a new measure of seafood safety that takes the protective role of Se into account. It is called the Selenium Health Benefit Value (Se-HBV) and is based on the relative quantity of Hg versus Se. Using this index, the authors conclude that most varieties of ocean fish (Hg:Se ratio around 1:5) are safe to eat. Squids also tend to be rich in Se. In this study, the large differences in the levels of the detection limits for Hg and Se prevent Hg:Se ratio comparisons in the muscle tissue. However, relatively large amounts of Se were measured in other organs: Hg:Se ratios in the digestive gland were 1:27 and 1:41, in Tasmanian and Amsterdam squid respectively, and around 1:3 in gills in both locations. The toxicity of Hg in the studied squid might thus be lesser than what appears from a simple comparison to consumption guidelines. The use of Se-HBV should be considered in further studies dealing with Hg toxicity in relation to squid consumption. It is however important not to forget that, although Se offers protection from the toxic effects of Hg, overexposure to Se can also cause damaging effects including serious neurologic, endocrine, and dermatologic effects (Agency for Toxic Substances and Disease Registry, 2003).

Cadmium is a stable, ubiquitous toxic metal, not abundant in its pure state in the environment. It mainly enters the humans organism via diet and inhalation (The Environmental Bureau of Investigation, 2009). Although the European Commission threshold concentration set for this metal in cephalopods is double that of Hg ( $1 \mu\text{g}\cdot\text{g}^{-1}$  ww), Cd is also highly toxic (e.g. Jarup, 2003). It mainly causes kidney diseases but can also affect the liver, lungs, bone, immune system, blood and nervous system following chronic inhalation or oral exposure, and is possibly carcinogenic (United States Environmental Protection Agency, 2007b). In the muscle of *T. filippovae* all concentrations were under this guideline value. The  $1 \mu\text{g}\cdot\text{g}^{-1}$  ww: guideline value for Cd refers to gutted squid, hence without their digestive glands. However, cephalopods tend to accumulate Cd mainly in their digestive gland, and in greater concentrations than most other marine zoological groups (Cognetti,

1992). Moreover, in Japan the digestive gland, called kimo, is essential for making a widely consumed dish named shiokara. It is thus noteworthy that in the present study 100% of digestive glands from both study sites showed higher concentrations than the authorized  $1 \mu\text{g}\cdot\text{g}^{-1}$  ww.

Lead can accumulate in bone and affects practically all systems within the body (e.g. central nervous system, kidneys and blood cells) (United States Environmental Protection Agency, 2007a). The guideline value adopted by the European Commission for Pb in cephalopods is  $1 \mu\text{g}\cdot\text{g}^{-1}$  ww. None of the individuals sampled had concentrations reaching this value. The Pb permissible value given above is more than 10 times higher than the maximum Pb concentration in muscle recorded during this study.

Arsenic is usually less of an issue in marine organisms than the metals previously discussed as, contrary to Hg, organic forms of As are less toxic than its inorganic forms. Marine organisms are able to convert inorganic As into organic As compounds (Lunde, 1977). In fact, numerous studies on animals have indicated that As is an essential nutrient although its physiological role is open to conjecture (Uthus, 1992). Symptoms of acute intoxication in humans by inorganic As include severe gastrointestinal disorders, hepatic and renal failure, and cardiovascular disturbances, whereas chronic exposure causes skin pigmentation, hyperkeratosis, and cancers in the lung, bladder, liver, and kidney as well as skin (Gorby, 1994). The guideline value adopted by the World Health Organization for As in cephalopods is  $2 \mu\text{g}\cdot\text{g}^{-1}$  ww.. This value was not exceeded in the muscle of *T. filippovae* from this study. Moreover, the figures from this study refer to measures of total As, part of which is likely to be present in a non-toxic form. In a recent study on market fishes and squids, Lin et al. (2008) found that 87% of As in muscle was arsenobetaine, a form of As which is non-toxic and non-carcinogenic to humans, and is rapidly excreted through urine after ingestion.

The dietary implications that stem from this study in terms of human consumption are the following. On one hand, As and Pb concentrations in *T. filippovae* caught around Ile Amsterdam or Tasmania are of no concern to human health. On the other hand, given the substantially high percentage (52%) of individuals from Ile Amsterdam accumulating muscular Hg past the health guideline concentration, a frequent and regular consumption of *T. filippovae* originating from this area is not recommended. Moreover, regardless of the squid's origin, digestive glands should be avoided as Cd and Hg concentrations were past the authorized limits in all, or nearly all, the digestive glands analyzed. It should also be mentioned that fetuses, infants, and children are more vulnerable to Hg, Cd, Pb and As exposure than adults since these metals are more easily absorbed into growing bodies, they pass biological barriers (e.g. into the brain) more easily and children's brains and nervous systems are more sensitive to their damaging effects. Parents should thus be particularly aware of the quantity of seafood products, including *T. filippovae*, eaten by their children, and pregnant women should of course closely monitor their diet.

## 5. Conclusion

This study of the bioaccumulation and tissue distribution of 14 trace elements in *T. filippovae* from the southern Indian and Pacific oceans has not only confirmed a number of trace element behaviors observed in other cephalopod species from distinct locations, but has also highlighted patterns specific to this species in these areas. The digestive gland and branchial hearts of *T. filippovae* contained the highest concentrations of most trace elements whereas the muscle tissue was characterized by the lowest concentrations, irrespective of where the squid were caught. This familiar pattern in trace elemental studies was somewhat contradicted here by the exceptions of two elements, As and Hg, that showed clear organotropisms towards muscle. Furthermore, the size of the squid was the most influential biological factor, followed by its geographic origin. Higher concentrations of Cd, Cr, Ni and Pb were noted in squid sampled in Ile Amsterdam compared to

those caught in Tasmanian waters. Contrarily, concentrations of As were highest in squid from Tasmania. With respect to other cephalopod studies, metal concentrations of *T. filippovae* were overall within the spectrum of values found in cephalopods worldwide, often towards the lower end. However in the comparison of metal concentrations to human health consumption guidelines the major conclusion is that *T. filippovae* squid from both, Tasmania and Ile Amsterdam, contain levels of Hg and Cd often exceeding the recommended guidelines, especially in their digestive glands. Nevertheless, the non-negligible concentrations of Se also detected in these animals are to be kept in mind when considering Hg results because of the antagonistic effect of Se on Hg toxicity (Parizek and Ostadalova, 1971; Iwata et al., 1973; Ohi et al., 1976; Whanger, 1992; Ralston, 2008). The toxicity of other non-essential elements such as Ag and Cd is also probably diminished by their binding to metallothioneins.

## 6. Acknowledgments

We are grateful to G. Duhamel, P. Pruvost, the crew of the research vessel La Curieuse and the crew of the Adriatic Pearl from Tasmania who participated in the sampling effort. The field work at Amsterdam was supported financially and logistically by the Institut Polaire Français Paul Emile Victor (IPEV, Programme N°558 109, H. Weimerskirch). The analytical investigations were supported financially by the LIENSs (University of La Rochelle) and by a Hermon Slade grant awarded to G.D. Jackson. We also thank the referees for their helpful comments on the manuscript.

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## Caption to figures

Fig. 1. Levels of trace elements ( $\mu\text{g.g}^{-1}$  dw) in *Todarodes filippovae* tissues from Tasmania and Amsterdam (DG: Digestive Gland; BrH: Branchial Hearts). To assess whether the differences in concentrations were significant, one-way ANOVAs for paired samples were performed. The corresponding p-values are given above the boxplots.

Fig. 2. Continued from Figure 2. Levels of trace elements ( $\mu\text{g.g}^{-1}$  dw) in *Todarodes filippovae* tissues from Tasmania and Amsterdam (DG: Digestive Gland; BrH: Branchial Hearts). To assess whether the differences in concentrations were significant, one-way ANOVAs for paired samples were performed. The corresponding p-values are given above the boxplots.

Fig. 3. Continued from Figure 3. Levels of trace elements ( $\mu\text{g.g}^{-1}$  dw) in *Todarodes filippovae* tissues from Tasmania and Amsterdam (DG: Digestive Gland; BrH: Branchial Hearts). To assess whether the differences in concentrations were significant, one-way ANOVAs for paired samples were performed. The corresponding p-values are given above the boxplots.

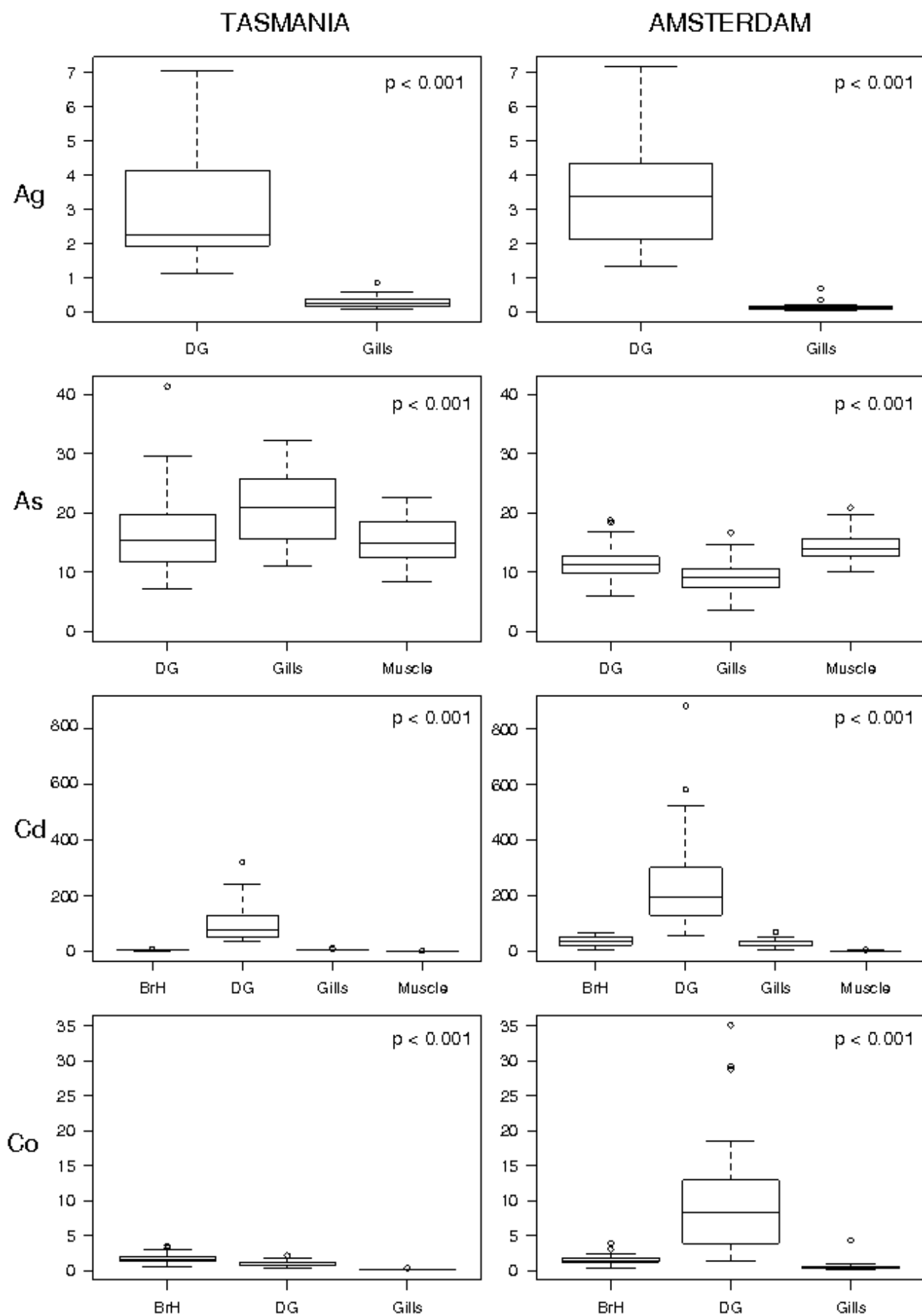


Fig. 1



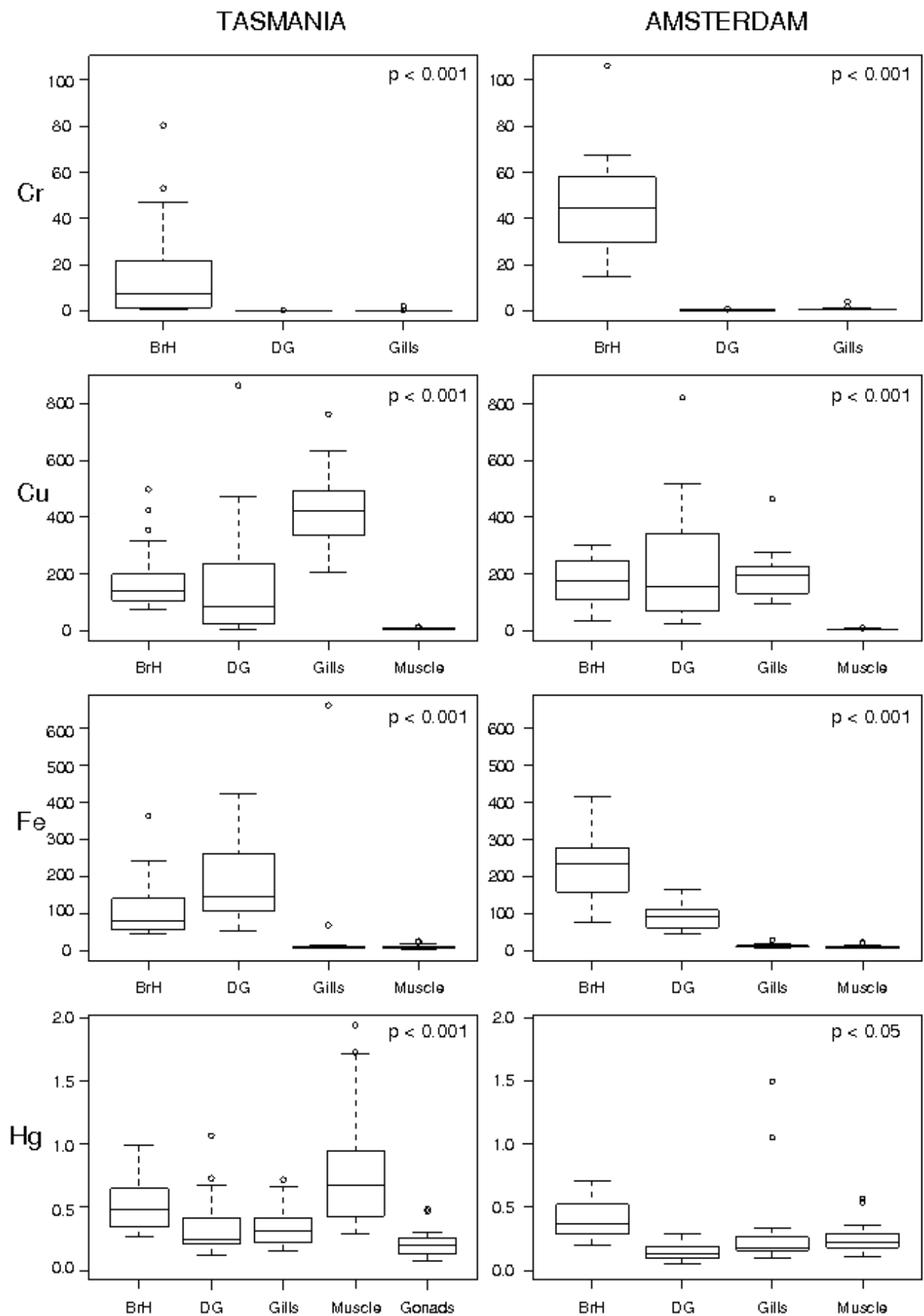


Fig. 2

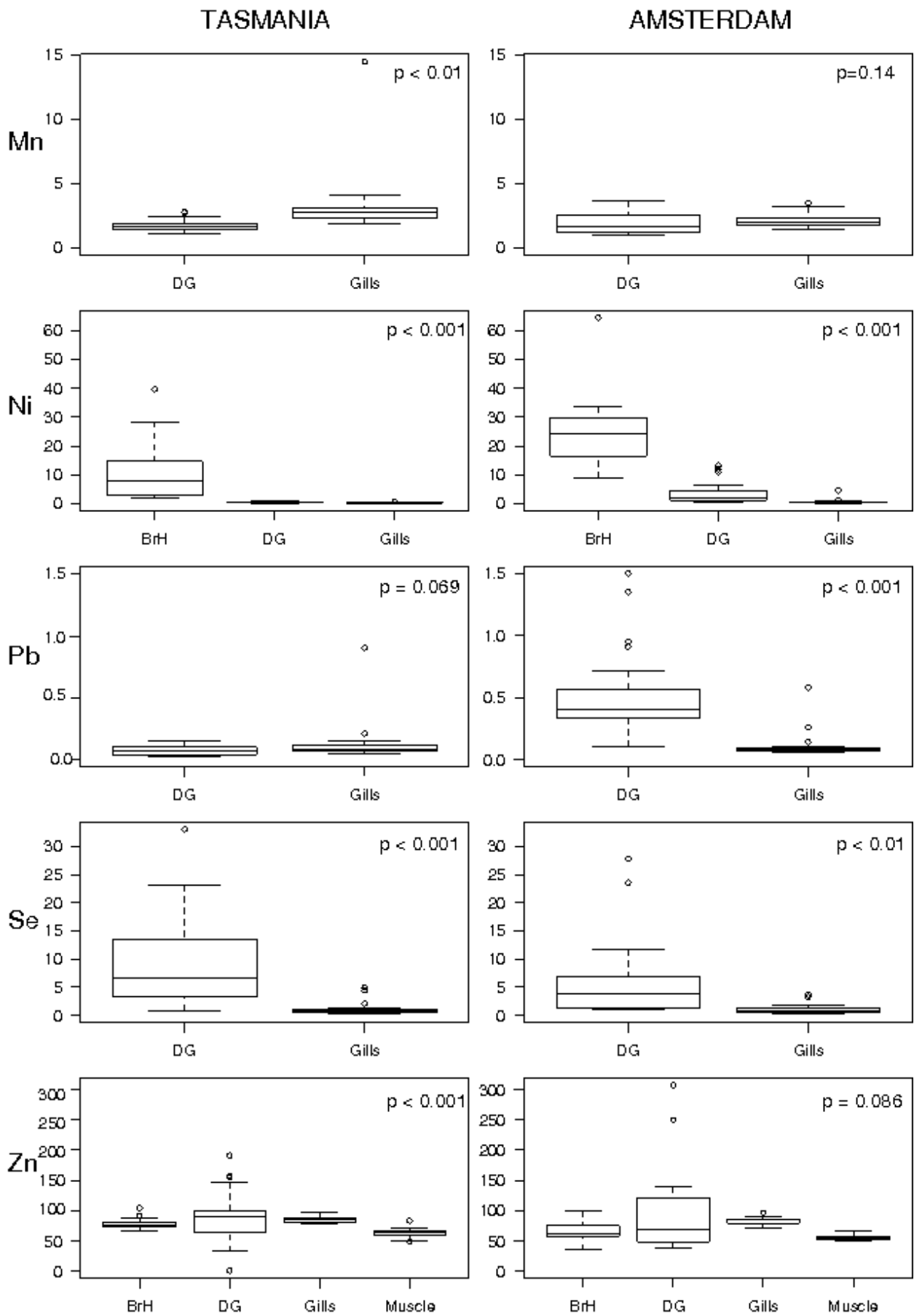


Fig. 3

Table 1. Biological measurements obtained from the *Todarodes filippovae* samples. The Tasmanian squid were comprised only of females, while in Amsterdam, out of 24, 17 were females and 7 were males. To assess whether there were significant differences in the measurements of squid from each location, 2-sample *t*-tests were performed. The resulting *p*-values are given in the last column.

	Tasmania			Amsterdam			<i>p</i> -value
	n	Mean $\pm$ SD	Range	n	Mean $\pm$ SD	Range	
Mantle length (mm)	32	447 $\pm$ 42.9	[375, 538]	24	341 $\pm$ 48.5	[251, 430]	< 0.001
Total weight (g)	32	2290 $\pm$ 881	[1040, 4470]	24	825 $\pm$ 394	[240, 1830]	< 0.001
Ovary weight (g)	32	109 $\pm$ 75.4	[8.54, 237]	17	4.97 $\pm$ 2.4	[1.08, 10.6]	< 0.001
Digestive gland weight (g)	32	188 $\pm$ 72.9	[47.5, 369]	23	80.3 $\pm$ 67	[13.2, 260]	< 0.001
Gill weight (g)	32	29.7 $\pm$ 10.4	[12.7, 56.5]	19	10.7 $\pm$ 2.25	[7.09, 16.1]	< 0.001
Branchial heart weight (g)	32	2.84 $\pm$ 0.69	[1.86, 4.47]	15	1.04 $\pm$ 0.26	[0.71, 1.5]	< 0.001

Table 2

Trace element concentrations ( $\mu\text{g}\cdot\text{g}^{-1}$  d.w.) for each site, given as means, standard deviations and ranges of the metal concentrations. The symbol < DL indicates concentrations below detection limits.

Organ	Metal	n	Ternmama		n	Amsterdam	
			Mean $\pm$ SD	Range		Mean $\pm$ SD	Range
Branchial hearts	Ag	32	< DL		15	< DL	
	As	32	< DL		15	< DL	
	Cd	32	2.64 $\pm$ 1.74	[0.95, 7.14]	15	34.3 $\pm$ 19.7	[1.57, 64.7]
	Co	32	1.70 $\pm$ 0.68	[0.51, 3.51]	15	1.61 $\pm$ 0.92	[0.30, 3.89]
	Cr	32	15.7 $\pm$ 19.9	[0.48, 80.4]	15	47.1 $\pm$ 29.7	[14.7, 106]
	Cu	32	179 $\pm$ 103	[75.1, 499]	15	179 $\pm$ 87.5	[35.9, 304]
	Fe	32	111 $\pm$ 73.4	[44.1, 364]	15	228 $\pm$ 86.6	[77, 415]
	Hg	16	0.51 $\pm$ 0.21	[0.26, 0.99]	15	0.42 $\pm$ 0.17	[0.20, 0.71]
	Mn	32	2.92 $\pm$ 1.15	[1.02, 6.80]	15	< DL	
	Ni	32	9.51 $\pm$ 9.86	[1.01, 39.6]	15	25.5 $\pm$ 13.4	[7.63, 64.4]
	Pb	32	< DL		15	< DL	
	Se	32	< DL		15	< DL	
	V	32	< DL		15	< DL	
Zn	32	77.8 $\pm$ 7.28	[65.9, 104]	15	65.6 $\pm$ 15.0	[35.0, 99.8]	
Digestive gland	Ag	30	3.04 $\pm$ 1.55	[1.11, 7.06]	24	3.40 $\pm$ 1.60	[1.33, 7.16]
	As	30	17.1 $\pm$ 7.16	[7.15, 41.3]	24	11.5 $\pm$ 3.07	[5.90, 18.7]
	Cd	30	98.5 $\pm$ 67.2	[33.7, 320]	24	246 $\pm$ 187	[53, 683]
	Co	30	0.92 $\pm$ 0.38	[0.31, 2.11]	24	10.5 $\pm$ 9.04	[1.30, 35]
	Cr	30	0.14 $\pm$ 0.05	[0.08, 0.28]	24	0.27 $\pm$ 0.16	[0.07, 0.87]
	Cu	30	137 $\pm$ 176	[4.95, 865]	24	218 $\pm$ 196	[24, 821]
	Fe	30	183 $\pm$ 105	[54.4, 425]	24	91.7 $\pm$ 32.1	[44.5, 166]
	Hg	30	0.33 $\pm$ 0.22	[0.12, 1.06]	24	0.14 $\pm$ 0.06	[0.06, 0.28]
	Mn	30	1.74 $\pm$ 0.45	[1.11, 2.81]	24	1.90 $\pm$ 0.78	[0.94, 3.67]
	Ni	30	0.55 $\pm$ 0.26	[0.2, 1.14]	24	3.54 $\pm$ 3.65	[0.41, 13.2]
	Pb	30	0.07 $\pm$ 0.04	[0.02, 0.15]	24	0.52 $\pm$ 0.34	[0.19, 1.50]
	Se	30	6.92 $\pm$ 7.85	[0.78, 33]	24	5.75 $\pm$ 6.83	[0.88, 27.8]
	V	30	0.59 $\pm$ 0.34	[0.21, 1.55]	24	0.91 $\pm$ 0.74	[0.22, 2.65]
Zn	30	88.5 $\pm$ 38.5	[1.2, 191]	24	94.3 $\pm$ 66.1	[37.8, 307]	

Table 3

Continued from Table 2. Trace element concentrations ( $\mu\text{g.g}^{-1}$  d.w.) for each site, given as means, standard deviations and ranges of the metal concentrations. The symbol < DL indicates concentrations below detection limits.

Organ	Metal	n	Tasmania		n	Amsterdam	
			Mean $\pm$ SD	Range		Mean $\pm$ SD	Range
Gills	Ag	32	0.3 $\pm$ 0.18	[0.09, 0.86]	19	0.16 $\pm$ 0.14	[0.04, 0.69]
	As	32	21.0 $\pm$ 5.75	[11.1, 32.3]	19	9.24 $\pm$ 3.13	[3.48, 16.7]
	Cd	32	4.37 $\pm$ 1.65	[2.29, 10.6]	19	27.5 $\pm$ 18.1	[5.23, 69.3]
	Co	32	0.13 $\pm$ 0.06	[0.06, 0.33]	19	0.63 $\pm$ 0.93	[0.16, 4.32]
	Cr	32	0.32 $\pm$ 0.32	[0.19, 2.02]	19	0.93 $\pm$ 0.82	[0.37, 4.02]
	Cu	32	416 $\pm$ 126	[205, 763]	19	199 $\pm$ 84	[96, 465]
	Fe	32	31.6 $\pm$ 116	[5.83, 663]	19	11.5 $\pm$ 5.03	[6.74, 28.8]
	Hg	32	0.34 $\pm$ 0.16	[0.16, 0.72]	19	0.31 $\pm$ 0.35	[0.10, 1.49]
	Mn	32	3.13 $\pm$ 2.13	[1.86, 14.4]	19	2.19 $\pm$ 0.59	[1.47, 3.48]
	Ni	32	0.30 $\pm$ 0.12	[0.15, 0.65]	19	0.75 $\pm$ 0.96	[0.16, 4.69]
	Pb	32	0.12 $\pm$ 0.15	[0.05, 0.90]	19	0.12 $\pm$ 0.12	[0.06, 0.58]
	Se	32	1.01 $\pm$ 1.03	[0.36, 4.53]	19	1.11 $\pm$ 0.56	[0.38, 3.69]
	V	32	< DL		19	0.08 $\pm$ 0.08	[0.04, 0.38]
	Zn	32	85.4 $\pm$ 5.1	[78.8, 96.8]	20	82.0 $\pm$ 6.2	[71.0, 96.9]
Muscle	Ag	32	< DL		24	< DL	
	As	30	15.3 $\pm$ 3.73	[8.4, 22.6]	23	14.4 $\pm$ 2.54	[10.2, 20.9]
	Cd	32	0.20 $\pm$ 0.14	[0.06, 0.70]	20	0.93 $\pm$ 0.79	[0.13, 3.66]
	Co	32	< DL		24	0.09 $\pm$ 0.08	[0.03, 0.36]
	Cr	32	< DL		24	< DL	
	Cu	30	7.30 $\pm$ 2.43	[2.90, 14.2]	23	6.36 $\pm$ 1.33	[4.60, 9.70]
	Fe	30	9.70 $\pm$ 5.21	[4.30, 25.5]	23	9.85 $\pm$ 4.76	[5.30, 22.1]
	Hg	32	0.76 $\pm$ 0.42	[0.28, 1.94]	24	0.26 $\pm$ 0.11	[0.11, 0.57]
	Mn	32	< DL		24	< DL	
	Ni	32	< DL		24	< DL	
	Pb	32	< DL		24	< DL	
	Se	32	< DL		24	< DL	
	V	32	< DL		24	< DL	
	Zn	30	63.1 $\pm$ 7.0	[49.3, 83.6]	23	55.6 $\pm$ 4.1	[50.4, 65.6]
Gonads	Hg	30	0.21 $\pm$ 0.10	[0.08, 0.48]			

Table 4

For each site, Pearson's linear correlation between the log-transformed elemental concentrations and the log-transformed mantle length.

Organ	Metal	Tasmania		Amsterdam	
		r	p-value	r	p-value
Branchial hearts	Cd	0.09	0.63	0.07	0.81
	Co	0.29	0.11	-0.04	0.9
	Cr	-0.22	0.23	-0.01	0.96
	Cu	-0.14	0.43	-0.42	0.12
	Fe	-0.21	0.26	-0.03	0.91
	Hg	0.44	0.09	0.42	0.12
	Mn	-0.28	0.12		
	Ni	-0.30	0.10	0.05	0.86
	Zn	0.36	0.04	-0.12	0.66
		Ag	0.28	0.13	-0.54
Digestive gland	As	0.42	0.02	-0.17	0.44
	Cd	-0.07	0.70	-0.46	0.02
	Co	-0.42	0.02	-0.59	< 0.01
	Cr	-0.07	0.72	-0.66	< 0.01
	Cu	0.04	0.86	-0.41	0.06
	Fe	-0.24	0.19	-0.66	< 0.01
	Hg	0.18	0.33	-0.36	0.09
	Mn	-0.23	0.22	-0.43	0.04
	Ni	-0.03	0.88	-0.64	< 0.01
	Pb	0.16	0.40	-0.62	< 0.01
	Se	0.22	0.26	-0.04	0.86
	V	0.06	0.81	-0.62	< 0.01
	Zn	-0.23	0.22	-0.34	0.10
Gills	Ag	0.70	< 0.01	-0.41	0.06
	As	0.19	0.30	0.12	0.62
	Cd	0.46	< 0.01	-0.47	0.04
	Co	-0.04	0.83	-0.47	0.04
	Cr	-0.14	0.46	-0.45	0.06
	Cu	-0.06	0.74	-0.19	0.43
	Fe	0.06	0.74	-0.51	0.03
	Hg	0.44	0.01	0.33	0.17
	Mn	0.03	0.87	0.04	0.88
	Ni	-0.23	0.21	-0.47	0.04
	Pb	0.28	0.12	0.13	0.61
	Se	-0.36	0.06	0.41	0.08
	V			-0.28	0.24
Zn	0.07	0.69	0.36	0.12	
Muscle	As	0.03	0.89	0.26	0.23
	Cd	0.23	0.20	-0.16	0.54
	Co			-0.33	0.11
	Cu	0.01	0.96	0.17	0.46
	Fe	0.23	0.21	-0.24	0.27
	Hg	0.65	< 0.01	0.55	< 0.01
	Zn	0.24	0.21	0.56	< 0.01
Gonads	Hg	0.40	0.16		

Table 5

Analysis of covariance results. The first (resp. second) column corresponds to the null hypothesis that there is no sex effect in Amsterdam (resp. no mated effect in Tasmania) on the log-transformed metal concentrations. The third column provides p-values corresponding to the null hypothesis that there is no site effect on the log-transformed metal concentrations. Mantle length (log-transformed) was used as a covariate in all three ANCOVAs.

Organ	Metal	Sex effect in Amsterdam	Mated effect in Tasmania	Site effect
Branchial hearts	Cd	0.74	0.23	< 0.01
	Co	0.37	0.93	0.65
	Cr	0.99	0.37	0.04
	Cu	0.77	0.36	0.29
	Fe	0.86	0.17	0.03
	Hg	0.22	0.10	0.76
	Mn		0.16	
	Ni	0.92	0.24	0.02
Digestive gland	Zn	0.40	0.94	0.02
	Ag	0.98	0.28	0.02
	As	0.26	0.12	< 0.01
	Cd	0.29	0.24	0.09
	Co	0.78	0.48	< 0.01
	Cr	0.63	0.03	0.04
	Cu	0.97	0.35	0.41
	Fe	0.65	0.83	< 0.01
	Hg	0.25	0.09	< 0.01
	Mn	0.87	0.01	0.17
	Ni	0.54	0.79	< 0.01
	Pb	0.70	0.63	< 0.01
Gills	Se	0.62	0.30	0.44
	V	0.54	0.54	0.02
	Zn	0.79	1.00	0.46
	Ag	0.60	0.92	< 0.01
	As	0.79	0.17	< 0.01
	Cd	0.88	0.94	< 0.01
	Co	0.84	0.10	< 0.01
	Cr	0.92	0.93	< 0.01
	Cu	0.76	0.21	< 0.01
	Fe	0.64	0.90	0.54
	Hg	0.96	0.21	0.83
	Mn	0.23	0.92	0.14
	Ni	0.90	0.38	0.18
Pb	0.94	0.48	0.37	
Se	0.94	0.65	0.04	
V	0.41			
Zn	0.40	0.53	0.64	
Muscle	As	0.32	0.06	0.92
	Cd	0.24	0.27	< 0.01
	Co	0.08		
	Cu	0.07	0.03	0.90
	Fe	0.13	0.99	0.26
	Hg	0.48	0.25	< 0.01
Gonads	Zn	0.04	0.07	0.46
	Hg		0.30	