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**Influence of sexual dimorphism on stable isotopes and trace element concentrations in the greater hooked squid *Moroteuthopsis ingens* from New Zealand waters**

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## **Abstract**

The Chatham Rise, one of the highest offshore-primary production regions in New Zealand waters, hosts a great abundance and diversity of deep-sea cephalopods including the greater hooked squid, *Moroteuthopsis ingens*. Stable isotope ratios of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) and trace element concentrations (Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Pb, Se, V, and Zn) were assessed in female and male specimens of different size classes (89–563 mm mantle length). Values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were overall higher in females and  $\delta^{13}\text{C}$  was further influenced by size and sex. Both muscular mantle (the largest fraction of the total body mass) and digestive gland (the known main storage organ for Ag, Cd, Cu and Zn in many cephalopods) tissues were analysed. Higher levels of Cd were observed in males than in females. A positive effect was found between size and Hg concentrations, which could be related to the ontogenetic descent of larger specimens into deeper waters, where they are exposed to higher Hg concentrations, and/or dietary shifts toward Hg-enriched prey with increasing size. This study provides trace element data for this abundant and ecologically important species, and further reveals higher trace element concentrations (especially Hg) in *M. ingens* from the Chatham Rise, compared to specimens from the sub-Antarctic zone.

**Keywords:** Cephalopods; biomonitoring; bioaccumulation; metals; Onychoteuthidae

## **Introduction**

Trace elements occur naturally at varying concentrations in the marine environment. They are persistent and do not degrade but are taken up by marine organisms, and their biogeochemical cycling can be strongly influenced by their transfer through food webs (Wang and Fisher, 1998). Concentrations of trace elements recorded in biota are influenced by both natural (e.g., geothermal, volcanic activity or upwelling events; Langston, 2017) and anthropogenic sources (e.g. agriculture, industrial production or mining; Tchounwou et al., 2012). High concentrations of Cd and Hg have been previously measured in marine organisms (Bryan, 1984; Eisler, 2009), even from remote or pristine environments (Bustamante et al. 2003, Cipro et al. 2018). For example, anomalously high Cd concentrations were reported from polar oceans (Frew, 1995; Gault-Ringold et al., 2012), and also found in high-latitude biota (e.g., Jöst and Zauke, 2008; Keil et al., 2008; Petri and Zauke, 1993; Zauke et al., 1999). In the case of Hg, which mainly occurs in marine organisms as methyl-Hg (its organic form), biomagnification has been reported along food webs, reaching highest concentrations in apex predators (Cherel et al., 2018; Eagles-Smith et al. 2018; Fisher and Reinfelder, 1995; Rainbow, 1995).

Cephalopods are crucial members of the pelagic food webs, as they are consumed by a variety of apex predators (Boyle and Rodhouse, 2005; de la Chesnais et al., 2019), and are also the most frequently observed predators in bathypelagic and mesopelagic food webs themselves (Choy et al., 2017). Cephalopods have the ability to accumulate high concentrations of trace elements, particularly Ag, Cd, Cu, and Zn, in their digestive glands (e.g, Martin and Flegal, 1975; Miramand et al., 2006; Miramand and Bentley, 1992), and have been suggested as a major vector for Cd to their predators (Bustamante et al., 1998a).

The warty or greater hooked squid, *Moroteuthopsis ingens* (Smith, 1881), formerly known as *Moroteuthis* or *Onykia ingens* (the genus placement recently changed; Bolstad et al., 2018), is a deep-sea sub-Antarctic species that shows a strong sexual dimorphism, with females reaching about five times the weight of males (Bolstad, 2006; Jackson, 1995). Its high abundance and its central position in marine food webs highlight its ecological importance in sub-Antarctic waters. Indeed, *M. ingens* is a mid-trophic species, and its diet shifts with ontogeny (Arkhipkin and Laptikhovsky, 2010). In early life stages it consumes planktonic crustaceans and small *Doryteuthis gahi* (formerly *Loligo gahi*); later it feeds primarily on squid (*D. gahi* and *Histioteuthis atlantica*), followed by myctophid fish (*Gymnoscopelus* sp., *Salilota australis*), crustaceans (Munidae, Euphausiacea), and chaetognaths (Cherel and Duhamel, 2003; McArthur et al., 2003; Rosas-Luis et al., 2014). In addition, cannibalism has been reported in this species (Ibáñez and Keyl, 2010). Beside its role as a predator, a variety of marine animals feed on *M. ingens*, such as the wandering albatross, *Diomedea exulans* (Cherel et al., 2017), Patagonian toothfish, *Dissostichus eleginoides* (Xavier et al., 2002), southern elephant seal, *Mirounga leonina* (Green and Burton, 1993), and sperm whale, *Physeter macrocephalus* (Clarke and Roper, 1998). Finally, *Moroteuthopsis ingens* is a frequent bycatch species in commercial trawling (Cherel and Duhamel, 2003; Jackson, 1995), but without commercial interest due to the stored ammonium in its tissues used for buoyancy, making it unpalatable to humans (Jackson et al., 2000; Lu and Williams, 1994).

Within New Zealand waters, *M. ingens* has been reported between the subtropical and Antarctic polar fronts, and in high abundances on the Chatham Rise (Jackson, 1995, Jackson et al., 1998; Fig 1.) This rise is a submarine plateau extending about 800 km eastward from New Zealand's South Island (Carter, 1980), and is overlain by the southern subtropical front, where warmer northern waters mix with cooler southern waters. This area has New Zealand's highest offshore primary production and biomass (Murphy et al., 2001; Probert and McKnight, 1993). A diverse assemblage of cephalopods

occurs on the Chatham Rise, some in considerable abundance, including the southern arrow squid, *Nototodarus sloanii*, the red squid, *Ommastrephes bartramii*, the giant squid, *Architeuthis dux*, the rugose hooked squid, *Onykia robsoni*, as well as *M. ingens* (Pinkerton, 2011).

To date, studies on trace element concentrations from New Zealand waters have primarily focussed on either commercially important species, such as arrow squids, *Nototodarus* spp. (Lischka et al., 2019b, 2020), greenshell mussels, *Perna canaliculus* (Whyte et al., 2009), finfish species such as snapper, *Chrysophrys auratus* (Brooks and Rumsey, 1974), and seabirds such as grey-faced petrels, *Pterodroma gouldi* (Lyver et al., 2017). Trace element concentrations in cephalopods, including *M. ingens*, remain understudied despite their ecological importance as both predator and prey. Currently, only a single study has been undertaken on trace elements in this species from New Zealand waters using beaks as environmental tracers (Northern et al., 2019), but no studies have examined contaminant concentrations in the soft parts (including the digestive gland and the mantle tissue) which represent most of the squid's biomass. The digestive gland is the main storage organ for a variety of trace elements, containing up to 98% of the whole body burden of Cd in squids (Bustamante et al. 2002a). The muscular mantle tissue represents approximately half of the total weight of the squid and has been reported to contain up to 95% of the whole Hg body burden in cephalopods (Bustamante et al. 2006). Although the digestive gland and mantle tissue have been analysed for trace elements in *M. ingens* from other parts of the Southern Ocean (south of the Subtropical Front; McArthur et al., 2003, Cipro et al. 2018), comparative data from New Zealand are needed in order to understand the cycling of certain elements within different food webs and the potential role of this squid as a vector of trace elements.

In this context, this study aims to provide information on trace element levels and distribution in *M. ingens* tissues and to analyse the possible influence of the sexual dimorphism on the observed

concentration patterns. Our specific objectives were to 1) assess the trophic ecology, trophic magnification and feeding habitat of *M. ingens* through stable isotope analysis of nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ), respectively; 2) analyse trace element concentrations in the digestive gland and the muscular mantle tissue; 3) contrast the trace element concentrations of *M. ingens* from New Zealand waters with specimens from other areas in the Southern Ocean; and 4) compare the trace element concentrations of *M. ingens*, as an ammoniacal species, with those observed in *N. sloanii*, a co-occurring non-ammoniacal species, thereby examining potential differences in the physiological strategies for trace element storage.

## Material and Methods

### *Sample collection*

All specimens of *M. ingens* were opportunistically collected during a trawl survey (using a hoki bottom trawl) in January and February 2016 by the Research Vessel *Tangaroa* (National Institute for Water and Atmospheric Research, Ltd. [NIWA]). Trawling depths ranged from 800 to 1300 m on the Chatham Rise in New Zealand ( $42^{\circ} 55'$  to  $45^{\circ} 07'$  S, and  $174^{\circ} 15'$  to  $179^{\circ} 58'$  E, Fig. 1). In total, 84 specimens, including 25 males (89–470 mm in mantle length [ML]) and 59 females (98–563 mm ML, with  $n=15 > 400\text{mm ML}$ ; Table S1) were collected from 26 stations and frozen at  $-20^{\circ}\text{C}$  until examination. Upon dissection, specimens were sexed, measured and weighed.

### *Stable isotope analysis*

Stable isotope values of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) were analysed in freeze-dried mantle tissue (0.2–0.4 mg) with a continuous flow mass spectrometer (Delta V Plus with a ConFlo IV interface, Thermo Scientific, Bremen, Germany) coupled to an elemental analyser (Flash 2000, Thermo Scientific, Milan, Italy). Results are expressed in the ‰ unit notation as deviations from standards

(Vienna Pee Dee Belemnite for  $\delta^{13}\text{C}$  and  $\text{N}_2$  in air for  $\delta^{15}\text{N}$ ) following the formula:  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N} = [(\text{R}_{\text{sample}}/\text{R}_{\text{standard}}) - 1] \times 10^3$ , where R is  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ , respectively. The analytical precision, based on internal laboratory standards (acetanilide and peptone), was  $<0.10\text{‰}$  for  $\delta^{13}\text{C}$  and  $<0.15\text{‰}$  for  $\delta^{15}\text{N}$ .

### *Trace element analysis*

Freeze-dried tissue samples of digestive gland and mantle tissue were homogenised. Tissue samples (~200 mg dry weight [dw]) were digested overnight in a 3:1 mixture of 65%  $\text{HNO}_3$  (Merck, suprapur quality) and 37%  $\text{HCl}$  (Merck, suprapur quality). Mineralisation followed by heating the samples for 30 min in a Milestone microwave (maximum temperature of  $105^\circ\text{C}$ ). Concentrations of 12 trace elements (Ag, As, Cd, Co, Cr, Cu, Fe, Ni, Pb, Se, V, and Zn) were measured by inductively coupled plasma mass spectroscopy (ICP-MS) with a Thermo Fisher Scientific X Series 2 and optical emission spectroscopy (OES) with a Varian Vista-Pro following Lucia et al (2016). Procedural blanks and certified reference materials were treated and analysed in the same way as the other samples. Element recoveries ranged from 91 to 118% ( $n=9$ ) for lobster hepatopancreas (TORT-3, National Research Council, Canada NRCC) and from 78 to 107% for dogfish liver (DOLT-5, NRCC). The detection limits for Ag, Cd, Co, Mn and Pb were  $0.01\ \mu\text{g g}^{-1}$ ; As was  $10.22\ \mu\text{g g}^{-1}$ ; Cr was  $0.1\ \mu\text{g g}^{-1}$ ; Fe and Zn were  $4.09\ \mu\text{g g}^{-1}$ ; Cu was  $1.02\ \mu\text{g g}^{-1}$ ; Ni was  $0.04\ \mu\text{g g}^{-1}$ ; Se was  $20.43\ \mu\text{g g}^{-1}$  and V was  $2.04\ \mu\text{g g}^{-1}$  (based on 200 mg of sample material diluted in a volume of 50 ml). Results for trace element concentrations are further expressed in  $\mu\text{g g}^{-1}$  dw.

Mercury concentrations were measured by an Advanced Mercury Analyser (ALTEC AMA 254) for dried, homogenised digestive gland and mantle tissue (1–2 mg dw) as described in Bustamante et al. (2006). All analyses were repeated two or three times until a relative standard deviation  $<10\%$  was obtained. The accuracy was controlled by the analysis of certified reference material (CRM) of



DOLT-5, with recoveries of 98% ( $n=18$ ). The detection limit of the AMA was 0.05 ng.

### *Interaction between Hg and Se*

Selenium has been suggested to have a protective role against Hg toxicity (Cuvin-Aralar and Furness, 1991) and an excess of Hg in relation to Se in the tissue is indicated by a Hg:Se molar ratio  $>1$  (Ralston et al., 2008). Molar ratios were assessed for both tissue types by converting measured concentrations to  $\text{nmol g}^{-1}$  using the molecular weight of 200.59 for Hg and 78.96 for Se (Lischka et al., 2019a).

### *Statistical analysis*

All statistical analyses were conducted with the software R (version 3.1.0, R Core Team, 2018). To assess whether size and sex influenced stable isotope values in the mantle tissue, analysis of covariance (ANCOVA) was performed on log-standardised  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values to check for interaction between size (ML), sex and  $\delta^{15}\text{N}/\delta^{13}\text{C}$  respectively. To test whether tissue type, size,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and/or sex influenced the trace element concentrations, generalised linear models (GLMs) with a negative binomial distribution and logit link function were applied (GLM, package 'MASS', Ripley et al., 2013). One model per trace element was fitted against non-transformed concentration and the variables were added sequentially; sex and size were added as an interaction term since females grow larger than males, confounding size as an independent variable. The variable sex was divided in three categories: females, large females ( $>400$  mm) and males. Relationships between Hg and Se were assessed using pairwise nonparametric Spearman correlations ('corr.test' function of the 'corrgram' package, Wright, 2012).

## Results

The  $\delta^{13}\text{C}$  values were higher (sex;  $p < 0.001$ ) in females ( $-19.6 \pm 1.1$  ‰), followed by males ( $-20.5 \pm 1.1$  ‰), with size ( $p < 0.001$ ) and  $\delta^{15}\text{N}$  ( $p < 0.001$ ) showing significant effects (Fig. 2, Table S2). The  $\delta^{15}\text{N}$  values were overall higher (sex;  $p < 0.01$ ) in females ( $+11.5 \pm 1.3$  ‰), followed by males ( $+10.6 \pm 1.3$  ‰), with  $\delta^{13}\text{C}$  ( $p < 0.01$ ) showing a significant effect (Fig. 2, Table S2).

The trace element concentrations decreased in the following order: 1) in the digestive gland as  $\text{Fe} > \text{Cd} > \text{Zn} > \text{Cu} > \text{Se} > \text{As} > \text{V} > \text{Ag} > \text{Pb} > \text{Ni} > \text{Hg} > \text{Co} > \text{Cr}$ ; and 2) in the mantle as  $\text{Zn} > \text{Se} > \text{Fe} > \text{As} > \text{Cu} > \text{V} > \text{Ni} > \text{Cd} > \text{Hg} > \text{Cr} > \text{Ag} > \text{Co} > \text{Pb}$  (Table 1). The overall highest concentration for Cd was observed in the digestive gland of a male specimen (377 mm ML) with  $1030 \mu\text{g g}^{-1}$  dw (Table 1).

The Hg:Se molar ratio varied from 0.001 to 0.028 for both muscle and digestive gland. A significant positive relationship was detected between Hg and Se in the digestive gland ( $R = 0.80$ ,  $p = 0.001$ ), but not in the mantle ( $R = 0.35$ ,  $p = 0.24$ ).

Tissue type had a significant effect on Ag, Cd, Cu, Fe, Hg, Pb, and Zn concentrations according to the GLMs (Table 2). In both female and male specimens, concentrations of Hg and Zn were significantly higher in the mantle tissue, while Ag, Cd, Cu, Fe, and Pb concentrations were highest in the digestive gland (Table 1). In both tissues, size had a positive effect on Ag ( $p < 0.001$ ), Hg ( $p < 0.001$ , Fig. 3), Se ( $p < 0.01$ ) and Zn ( $p < 0.001$ ) concentrations, whereas a negative effect was observed for Cd ( $p < 0.05$ ) and Pb concentrations ( $p < 0.001$ , Table 2). The digestive gland concentrations of Pb in smaller individuals of both sexes ( $< 200$  mm) were on average 3.1 times higher than those observed in larger individuals (Tables 1 and 2). The  $\delta^{13}\text{C}$  values had significant effects on Ag, Cd (Fig. 4), Cu, Pb and Zn concentrations while  $\delta^{15}\text{N}$  showed significant effects on Cu, Fe, Pb and Zn concentrations in both tissues (Table 2). Sex effects were observed for Cd, Cu, Fe ( $p < 0.001$ ) and Zn ( $p < 0.05$ ). The

interaction term 'Sex×Size' showed a significant effect in Cu and Zn ( $p < 0.001$ ) concentrations (Table 2).

## Discussion

The present study measured stable isotope values and trace element concentrations in one of the most abundant oegopsid squids in sub-Antarctic waters, the onychoteuthid *M. ingens*. Stable isotope values showed a large overlap, but also some differences, between sexes (Fig. 2), with large females having both higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (Fig 2). On average, the values were similar to mantle tissue values of specimens sampled from the Kerguelen Islands (average  $\delta^{13}\text{C}$ :  $-20.1 \pm 0.4$  ‰;  $\delta^{15}\text{N}$ :  $+10.0 \pm 0.4$  ‰; Cherel et al., 2008). Specimens sampled from shallower depths (168–285m) showed slightly higher  $\delta^{13}\text{C}$  ( $-18.59 \pm 0.76$  ‰), but similar  $\delta^{15}\text{N}$  values ( $+11.48 \pm 1.19$  ‰; Rosas-Luis et al., 2016). The increased  $\delta^{13}\text{C}$  values could correspond to increased primary production in the shallower water layers (Faul et al., 2000), as the carbon signature is representative of the habitat of an individual (Chouvelon et al., 2011). The present results indicate that males and small-to-mid-sized females (ML 59–400 mm) primarily forage in similar habitats, while larger females (ML >400 mm) occupy a different, deeper habitat, indicated by their positive  $\delta^{15}\text{N}$  values. This result suggests that females move towards a deeper habitat with growth (Fig. 2). This ontogenetic migration in females, resulting in a change in diet, has been previously reported in this species (Jackson and Mladenov, 1994).

Similar to other oegopsids (e.g., Bustamante et al. 2008, Kojadinovic et al., 2011), most trace element concentrations were higher in the digestive gland than in the mantle tissue. The digestive gland can detoxify and store particular elements, such as Ag, Cd, Cu, and Fe (Bustamante et al., 2002b, 2004; Finger and Smith, 1987; Penicaud et al., 2017; Rodrigo and Costa, 2017). This has been previously described in both non-ammoniacal squids *Sthenoteuthis oualaniensis* (Wu et al., 2017), *S. pteropus* (Lischka et al., 2018), *Todarodes filippovae* (Kojadinovic et al., 2011), and *T. pacificus* (Ichihashi et

al., 2001), and ammoniacal squids such as *A. dux* (Bustamante et al., 2008) or ammonium-containing squids, e.g., *Cranchia scabra*, *Galiteuthis armata*, *Helicocranchia pfefferi* and *Leachia atlantica*, (Rafael, 2017). This suggests that ammonium metabolism may not have a major influence on global metal detoxification and storage strategies in squids. The digestive gland functioning is likely not affected by the buoyant ammonia stored in the muscular tissues (Voight et al., 1995). Despite a limited body of studies published on other squid families, our results suggest that bioaccumulation processes should be consistent across squid families.

### *Cadmium*

The highest Cd concentrations were measured in the digestive gland of *M. ingens* (Table 2). Size had a negative effect on Cd concentrations and sex showed a significant effect, with the highest concentrations found in the digestive gland of males (Tables 1 and 2). A similar decrease in Cd concentrations with size has been observed in several other squid species including *L. forbesi*, *L. vulgaris*, *T. sagittatus*, and *S. pteropus* (e.g. Miramand et al., 2006, Dorneles et al., 2007, Chouvelon et al., 2011; Lischka et al., 2018), which could be explained by an ontogenetic diet shift toward prey with lower Cd concentrations (Lischka et al., 2018). Female *M. ingens* are believed to have a growth rate twice as high as males, and to migrate into deeper waters with maturity, possibly related to egg deposition (Jackson, 1997; Jackson et al., 2000), which could also contribute to the lowering of Cd (a dilution effect with size; Lischka et al., 2018). In contrast, this type of ontogenetic descent has not been observed for males so far. The difference in the Cd levels between the sexes could be related to a shift in habitat and correlated dietary shift (Arkhipkin and Laptikhovsky, 2010; Jackson et al., 1997), highlighted by the significantly different  $\delta^{13}\text{C}$  values (Fig. 4). Oceanic Cd concentrations vary across the Chatham Rise and the upwelling of low Cd sub-Antarctic waters to intermediate depths (200 – 400 m) has been previously suggested (Frew, 1995; Frew and Hunter, 1995). As sex-specific habitats

are suggested by the  $\delta^{13}\text{C}$  values (Table 1), the bathypelagic feeding behaviour of female *M. ingens* could explain the lower Cd concentrations measured in specimens of this sex at a given size. Furthermore, in mature and spent (post-spawning) females, the mantle tissue degrades, resulting in a thin-walled mantle (Jackson and Mladenov, 1994). Spent females appear to cease feeding (Arkhipkin and Laptikhovsky, 2010), which could result in a reduced uptake of Cd through diet when compared to males.

Elevated Cd levels observed in Southern Ocean cephalopods have been linked to the Cd anomaly in subpolar systems (Bustamante et al., 1998b; Cipro et al., 2018). A study from the Kerguelen Islands (Cipro et al., 2018) reported similar Cd concentrations in whole *M. ingens* compared to the mean digestive gland concentrations reported in this study (with the exception of the high Cd concentrations measured in males, Table 1). Compared to other squids, the mean digestive gland Cd concentrations in the female specimens ( $52.9 \pm 102.8 \mu\text{g g}^{-1} \text{ dw}$ ) were comparable to concentrations measured in *T. filippovae* from Tasmania ( $98.5 \pm 67.2 \mu\text{g g}^{-1} \text{ dw}$ , Kojadinovic et al., 2011) and *N. sloanii* from the Chatham Rise ( $89.0 \pm 113.5 \mu\text{g g}^{-1} \text{ dw}$ , Lischka et al., 2020). In particular, male *M. ingens* exhibited similar Cd concentrations ( $165.9 \pm 266.8 \mu\text{g g}^{-1} \text{ dw}$ ) to those reported for other oceanic squids, which might support the different exposure level between males and females, probably linked to a sex-specific difference in habitat, as suggested by the differences in  $\delta^{13}\text{C}$  values. However, the  $\delta^{15}\text{N}$  values do not support the effect of trophic magnification and further studies are needed to confirm this hypothesis.

Although the health effects of high Cd exposure for squids are not yet fully understood, Cd exposure has been linked to kidney and liver damage in humans and mammals (Gallien et al. 2001; Nicholson et al., 1983; Nicholson and Osborn, 1983). Some of the observed deleterious effects on cephalopods to date include altered digestive processes and/or increased oxidative stress on the embryonal development, as observed in the common cuttlefish *Sepia officinalis* or in the common octopus

*Octopus vulgaris* (Lacoue-Labarthe et al., 2010a; Nicosia et al., 2015). Further research on the effects of Cd toxicity in cephalopods is needed to discuss our results in that respect.

### *Mercury and selenium*

The mantle tissue of *M. ingens* had higher concentrations of Hg compared to the digestive gland (Tables 1 and 2). Observed Hg concentrations are consistent with previous studies on different squid species from the following families: Ommastrephidae, Onychoteuthidae, Gonatidae, Architeuthidae, and the myopsid family Loliginidae (Bustamante et al., 2000, 2008; Pierce et al., 2008). In cephalopods, Hg is mainly incorporated through the diet as methyl-Hg and might be partially demethylated in the digestive gland and excreted rapidly under its inorganic form (Lacoue-Labarthe et al., 2009). However, most of the Hg is redistributed to the muscular tissues, where it is stored as a result of the strong affinity of methyl-Hg for sulfhydryl protein groups (Bloom, 1992; Bustamante et al., 2006; Chauvelon et al., 2011). Therefore, Hg bioaccumulates with age in squid muscle tissue, which is reflected in the observed positive effect of size on Hg concentrations (Table 2, Fig. 2). In several cephalopod species, the bioaccumulation of Hg is also size-related, showing an increase with size (Bustamante et al., 2006). This has also been previously analysed in the curled octopus *Eledone cirrhosa* (Rossi et al., 1993) and the European flying squid *T. sagittatus* (Chauvelon et al., 2011). Other studies showed opposite trends, for example decreasing Hg concentrations with size were observed in *L. vulgaris* (Rjeibi et al., 2014) and *Galiteuthis glacialis* (Seco et al., 2020), whereas no effect of size on Hg concentrations was observed in *Psychroteuthis glacialis* (Seco et al., 2020). The Hg concentrations can be also enhanced by a shift in diet that occurs with growth (e.g., Barghigiani et al., 2000; Blum, 2011; Chauvelon et al., 2011); however, no shift in  $\delta^{15}\text{N}$  (which would suggest a change in trophic position) was observed for *M. ingens* in the present study. Furthermore, deep-sea organisms have been reported to contain higher Hg concentrations relative to epipelagic animals

(Chouvelon et al., 2012; Choy et al., 2017; Monteiro et al., 1996), which also contributes to an increased exposure of Hg for squids migrating towards deeper waters. Further investigations are needed to validate this hypothesis, including the use of stable isotopes of Hg (e.g., Blum et al., 2013; Renedo et al., 2018).

The positive correlation of Hg and Se concentrations in the digestive gland of *M. ingens* could indicate the involvement of Se in Hg detoxification processes, which has been previously proposed for fish (Yang et al., 2010), seabirds (Carravieri et al., 2017), and marine mammals (Cuvin-Aralar and Furness, 1991; Dietz et al., 2000). Although no mechanism of Hg and Se coprecipitation has yet been demonstrated in cephalopods, it has been proposed in *O. vulgaris* (Raimundo et al., 2010b). Selenium concentrations measured in the present study were overall comparable to those reported for *A. dux* (Bustamante et al., 2008) and *T. filippovae* (Kojadinovic et al., 2011). The positive size effect on Se concentrations (Table 2) strengthens the hypothesis of the involvement of this element in Hg detoxification, as the storage of both Hg and Se is dependent on growth and might highlight their similar bioaccumulation patterns.

Specimens of *M. ingens* from higher latitudes—e.g., from Macquarie Island (54°S)—had slightly lower mean Hg concentrations (0.06–0.13  $\mu\text{g g}^{-1}$  dw; McArthur et al., 2003), compared to the specimens analysed in this study. Similarly, mantle concentrations of Hg in *M. ingens* from the Kerguelen Islands (49°S) ( $0.11 \pm 0.08 \mu\text{g g}^{-1}$  dw, Cipro et al., 2018) were slightly lower than those observed from the Chatham Rise (40–43°S). Concentration differences could be linked to geochemical variation, since concentrations appear to vary with latitude in *M. ingens*. A latitudinal gradient in Hg concentrations has also been observed in seabirds (e.g., *Pelecanoides urinatrix*, *D. exulans*), with specimens from lower latitudes exhibiting lower concentrations when compared to higher-latitude specimens (Blévin et al., 2013; Carravieri et al., 2014, 2017, 2020). This effect could

be explained by the increasing complexity in food webs with decreasing latitude (Carravieri et al., 2014, 2017). Thereby, the higher Hg concentrations in *M. ingens* from the Chatham Rise could be due to an increased complexity of the Chatham Rise food web when compared to the higher latitude food webs (e.g., Macquarie and Kerguelen Islands), or due to other factors, such as differences in primary productivity, which influence Hg concentrations in prey organisms, temperature and solar radiation (Carravieri, 2014; Fitzgerald et al., 2007). The subtropical front crosses the Chatham Rise and is known for its high primary productivity (Frew and Hunter, 1995), which could be reflected in the specimens' trace elemental concentrations. In the Chatham Rise individuals, the highest Hg concentrations were measured in female *M. ingens* ( $2.42 \mu\text{g g}^{-1} \text{ dw}$ ), and were higher than those reported in *N. sloanii* from the same area ( $0.01\text{--}0.89 \mu\text{g g}^{-1} \text{ dw}$ , Lischka et al., 2020). This interspecies difference could be partly explained by different habitats, diets, sizes and/or physiology —*N. sloanii* is reported to generally occupy shallower waters (around 300m) than female *M. ingens*, which are found in deeper and colder waters ( $>800\text{m}$ , Arkhipkin and Laptikhovskiy, 2010), a segregation corroborated by the the  $\delta^{13}\text{C}$  values observed in this study.

### *Lead*

Overall, the highest Pb concentrations were measured in the digestive gland of male specimens (Tables 1, 2). Size had a significant negative effect on Pb concentrations, with smaller individuals exhibiting the highest Pb concentrations (Table 2). Both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values also had significant effects on the Pb concentrations, suggesting that the Pb differences could be habitat related, linked to the ontogenetic descent observed in *M. ingens* (Arkhipkin and Laptikhovskiy, 2010). Concentrations of Pb in the open ocean are predicted to reach their maximum concentrations in near-surface layers (Henderson and Maier-Reimer, 2002), where smaller individuals, including small males, feed (Arkhipkin and Laptikhovskiy, 2010). In contrast, large female *M. ingens* tend to migrate to, and then reside in, deeper waters (Arkhipkin and Laptikhovskiy, 2010; Jackson, 1997), where they might be



exposed to lower Pb concentrations. In addition, Pb has been reported to bioaccumulate along the food web (Wang, 2002), with higher concentrations observed in individuals feeding at lower trophic levels, which aligns with the lower  $\delta^{15}\text{N}$  values observed in males in this study (Tables 1, 2). The cycling of Pb in New Zealand offshore waters remains understudied and future research on Pb concentrations found in different water layers and their associated fauna are necessary for understanding the bioaccumulation and distribution of this metal.

### *Silver*

The concentrations of Ag were overall highest in the digestive gland (Tables 1, 2), which suggests a high storage potential of this organ for Ag. Although few reports on Ag storage in cephalopods have been published, its duration appears to vary widely among groups, with a relatively short half-life of Ag [7 days] observed in the common cuttlefish *Sepia officinalis* (Bustamante et al., 2004). Concentrations of Ag in squid can reach high concentrations, which seems to indicate a stronger retention of this metal in their digestive gland when compared to the mantle tissue (e.g., *A. dux*, Bustamante et al., 2008; *T. filippovae*, Kojadinovic et al., 2011). In this study, both size and  $\delta^{13}\text{C}$  had a significant positive effect on Ag concentrations (Table 2); this could indicate that specimens are exposed to higher Ag concentrations as they migrate to greater depths, which are enriched in Ag (Zhang et al., 2004). Further investigations into the uptake, detoxification, and retention mechanisms of Ag, as well as its distribution in pelagic waters, are needed to clarify these processes in squids.

### *Zinc*

The tissue distribution of Zn found herein was comparable to that observed for the boreoatlantic armhook squid *Gonatus fabricii*, where Zn concentrations were higher in the mantle compared to the

digestive gland tissue (Lischka et al., 2019a). Unbound Zn in the digestive gland cells is believed to induce metallothionein production, which plays an important part in the detoxification process of Cd in cephalopods (Bustamante et al., 2002b; Miramand and Bentley, 1992). Metallothionein has however been rarely quantified in cephalopods (see Bustamante et al., 2002a; Craig and Overnell, 2003; Raimundo, 2010a). The observed distribution pattern for Zn, with higher concentrations in the mantle than digestive gland, could be explained as: 1) Cd and Zn may compete for binding sites in the digestive gland, and a stronger affinity of Cd to the binding sites could lead to a coaccumulation of Zn; or 2) differences due to ammoniacal metabolism, which is supported by similar distribution pattern in the ammonium-containing *C. scabra* or *L. atlantica* (Rafael, 2017).

Larger female *M. ingens* individuals had higher Zn concentrations (Table 2), which could indicate a bioaccumulation pattern for this element. Similar relationships have been described in *A. dux* (Bustamante et al., 2008, ammoniacal) and *G. fabricii* (Lischka et al., 2019a, non-ammoniacal). In addition, Zn concentrations were affected by the interaction between sex and size, with larger females exhibiting higher Zn concentrations in the digestive gland relative to smaller males (Tables 1 and 2). This is consistent with Zn concentrations reported for mature Argentine shortfin squids (*Illex argentinus*, non-ammoniacal), where higher levels were observed in mature females than in mature males (Gerpe et al., 2000). The sex difference could be related to Zn cycling in the global oceans, where surface waters (and their associated fauna) exhibit lower concentrations compared to deeper waters (Conway and John, 2014; Lohan et al., 2002; Zhao et al., 2014). Because mature females of *M. ingens* are expected to feed in deeper waters (Jackson, 1997), they are more likely to be exposed to higher Zn concentrations from their diet. The significant effect of stable isotope values observed on Zn concentrations support this hypothesis. Another explanation could be that the maturation of the female gonads requires higher amounts of Zn for the synthesis of the vitellus, which results in a higher bioaccumulation of Zn (Lacoue-Labarthe et al., 2010b, 2016). Some essential elements (such as Cu and Zn) are reportedly deficient in the Southern Ocean (Petri and Zauke, 1993, Bustamante et al.,

1998b), which could result in a super accumulation of Cd at low Cu and Zn levels.

## Conclusion

This study reports sex-specific differences in trace element concentrations in *M. ingens* from the Chatham Rise, New Zealand, with males exhibiting significantly higher Cd concentrations than females. Significant observed differences in  $\delta^{13}\text{C}$  values suggest that the Cd bioaccumulation in *M. ingens* is likely influenced by diet, with notable differences observed across different habitats. Large female *M. ingens* are known to migrate into deeper waters, where they likely consume less Cd-rich prey (i.e., fish) than males, which remain in shallower waters and feed on crustaceans known to be rich in Cd (Arkhipkin and Laptikhovsky, 2010). Overall, Cd concentrations in male *M. ingens* were comparable to those observed in both sexes of *N. sloanii*, from the same locality (Lischka et al., 2019b). Size showed significant effects on Ag, Hg, Se, and Zn concentrations, which suggests that these elements bioaccumulate with growth. The high Hg concentrations measured in large females might indicate that their diet includes Hg-enriched prey. Furthermore, the Hg concentrations measured in our female specimens from the Chatham Rise were higher than those observed in *N. sloanii* from the same location and in *M. ingens* from lower latitudes, which indicates a difference in habitat and diet. As has been reported in non-ammoniacal squids, trace element concentrations between the mantle and digestive gland of *M. ingens* differed. Overall, no clear trace element concentration differences between the ammoniacal *M. ingens* and non-ammoniacal squid species were observed, suggesting that the stored ammonium does not impact trace element bioaccumulation patterns. However, there is currently a paucity of trace element data for ammoniacal squids and further comparisons between the trace element-handling of ammoniacal and non-ammoniacal squid species is needed to confirm this hypothesis. Based on the measured Cd and Hg concentrations, *M. ingens* might represent a significant vector of these elements towards New Zealand's pelagic predators, since this species constitutes an important prey item.

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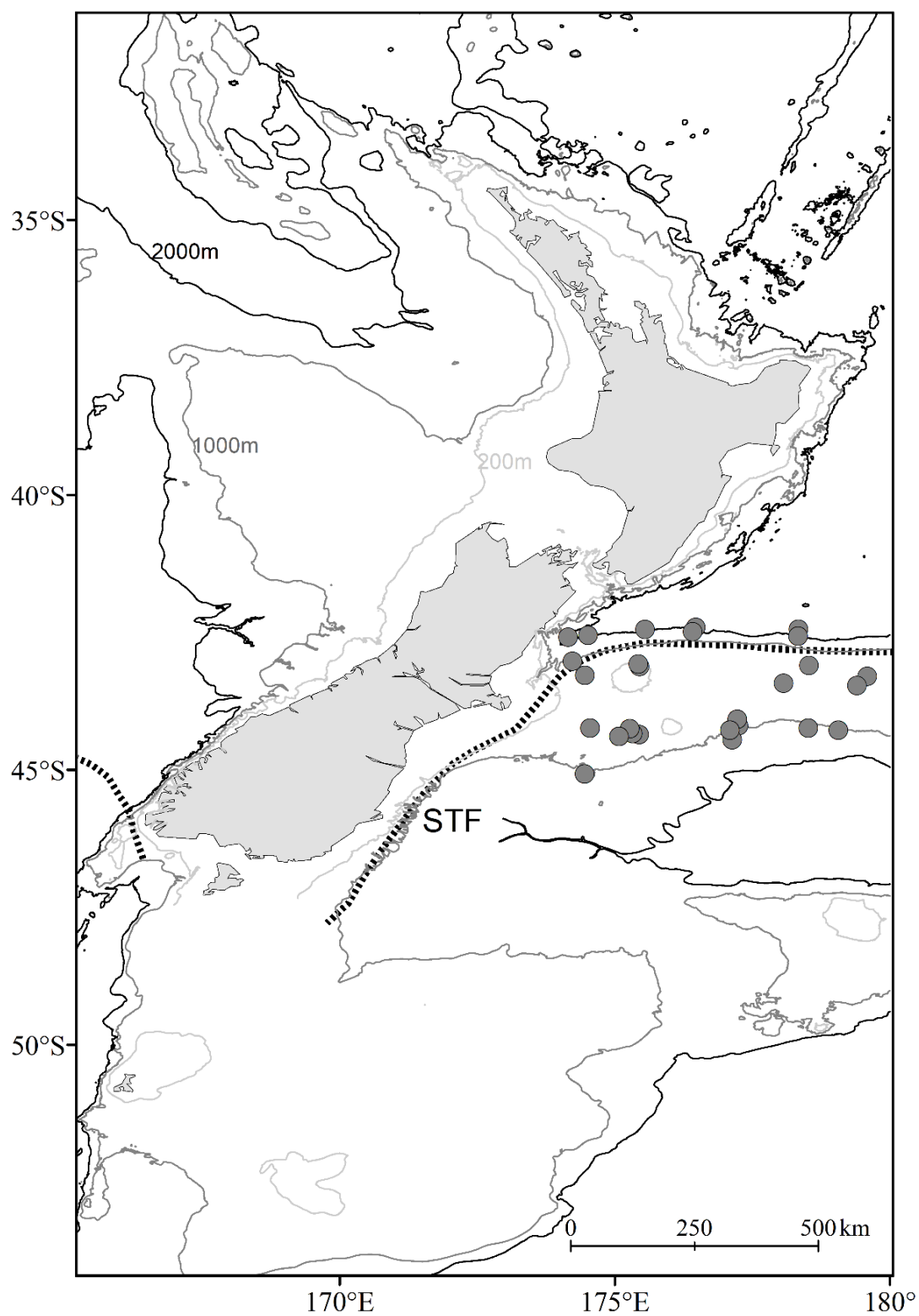
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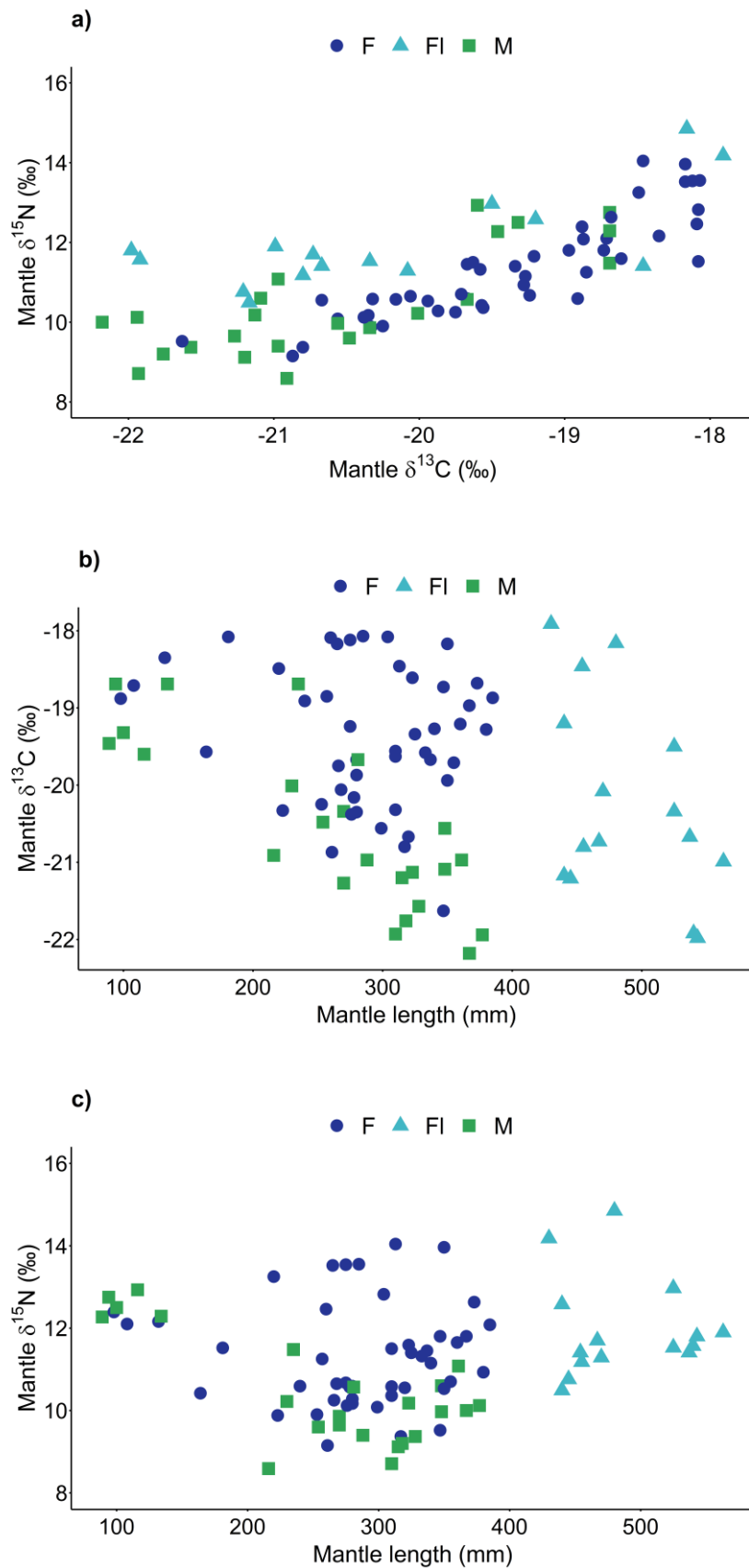
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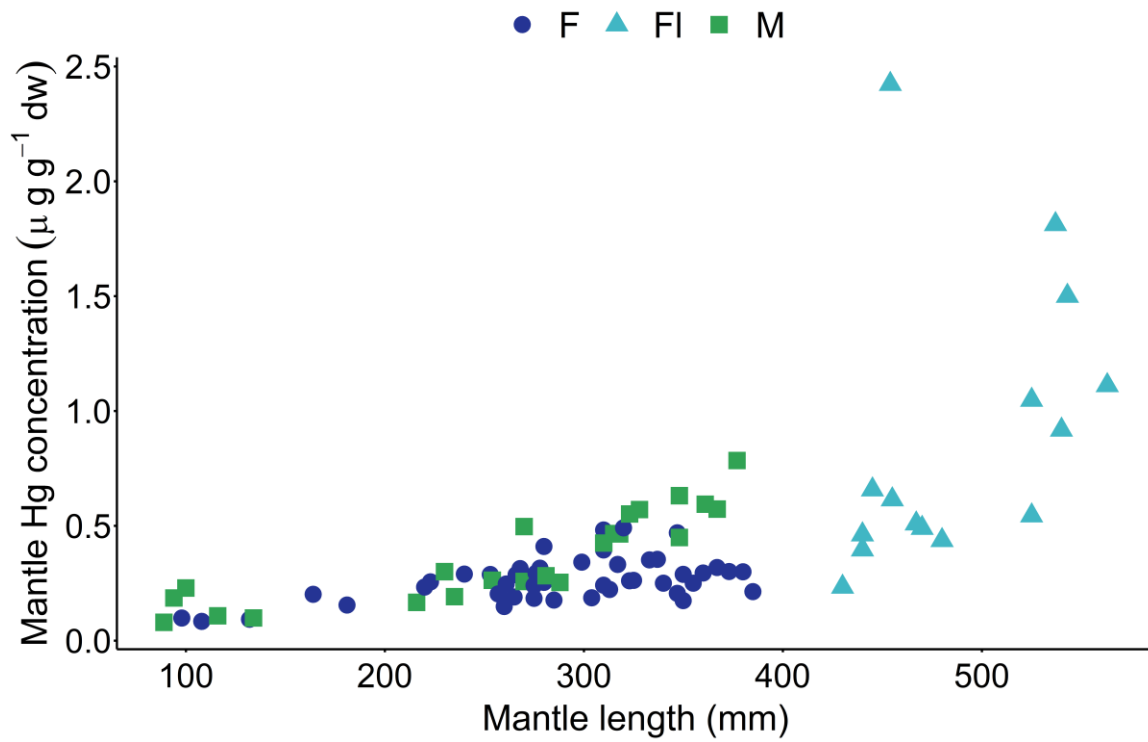


**Figure 1.** Map of the Chatham Rise showing sampling stations of the *Moroteuthopsis ingens* specimens analysed in the present study. Depth bars include 200 m, 1000 m and 200 m. The subtropical front (STF) is indicated by a dashed line.

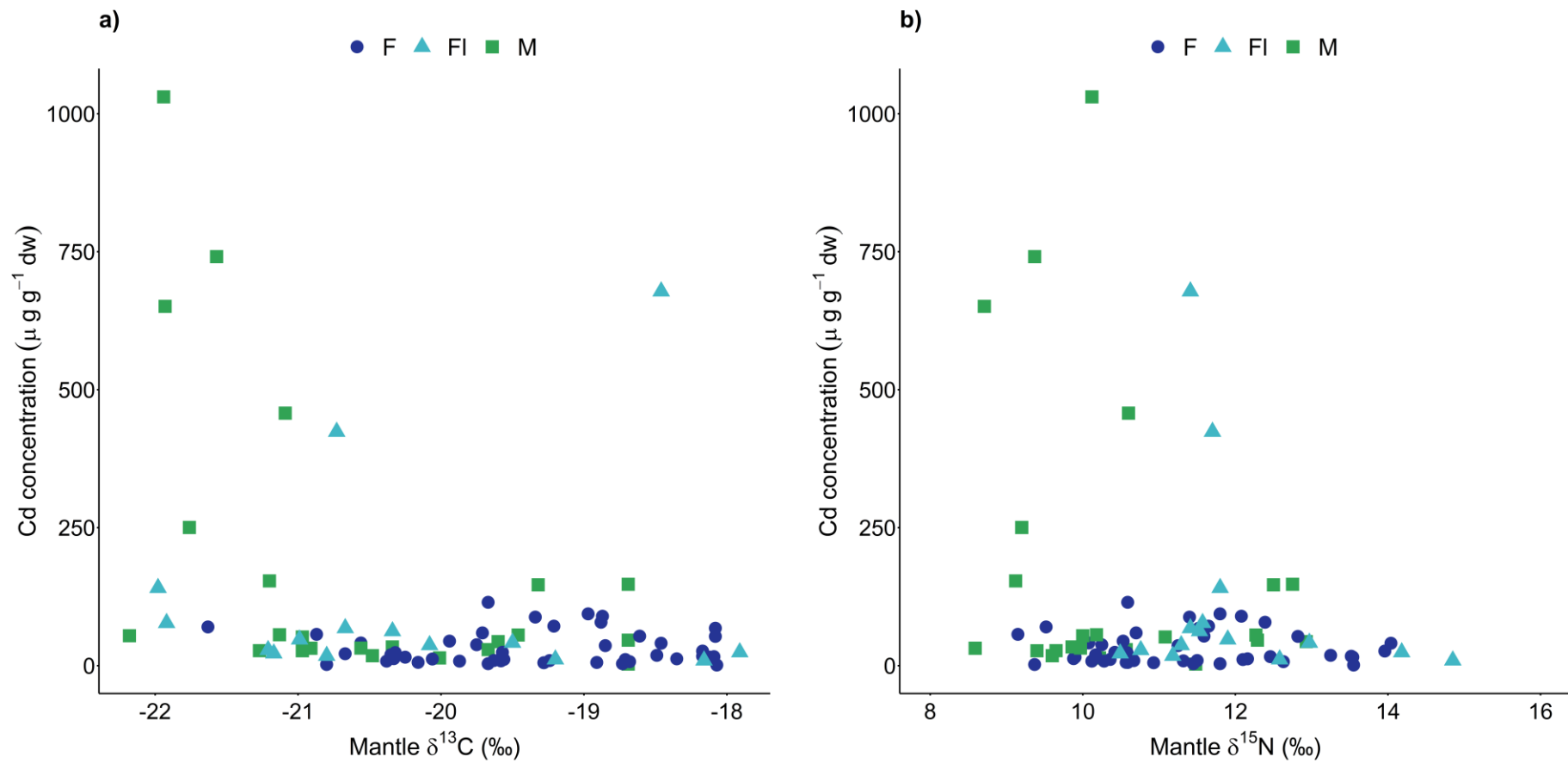


**Figure 2.** Relationship between a.)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , b.) mantle length (mm) and  $\delta^{13}\text{C}$ , and c.) Size and  $\delta^{15}\text{N}$  in the mantle tissue of female (F, n=44), large female (FI>400 mm, n=15) and male (M, n=25) *M. ingens* from the Chatham Rise, New Zealand.





**Figure 3.** Relationship between mantle length (mm) and Hg concentrations (in  $\mu\text{g.g}^{-1}\text{ dw}$ ) in the mantle tissue of female (F,  $n=44$ ), large female (FI  $>400\text{ mm}$ ,  $n=15$ ) and male (M,  $n=25$ ) *M. ingens* from the Chatham Rise, New Zealand.



**Figure 4.** Relationship between Cd concentration (in  $\mu\text{g}\cdot\text{g}^{-1}\text{ dw}$ ) in the digestive gland and a.)  $\delta^{13}\text{C}$  and b.)  $\delta^{15}\text{N}$  in the tissues of female (F,  $n=44$ ), large female (FI > 400 mm,  $n=15$ ) and male (M,  $n=25$ ) *M. ingens* from the Chatham Rise, New Zealand.

**Table 1.** Trace element concentrations (mean  $\pm$  standard deviation [SD], minimum, and maximum) in the digestive gland and mantle tissue, and stable isotope ratios ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) from the mantle tissue of *Moroteuthopsis ingens* from the Chatham Rise, New Zealand. Concentrations are given in  $\mu\text{g g}^{-1}$  dw and stable isotope values are expressed in ‰.

Element	♀ Digestive gland (n=59)		♂ Digestive gland (n=25)		♀ Mantle (n=59)		♂ Mantle (n=25)	
	mean $\pm$ SD	min-max	mean $\pm$ SD	min-max	mean $\pm$ SD	min-max	mean $\pm$ SD	min-max
Ag	1.63 $\pm$ 1.62	0.02-7.69	2.29 $\pm$ 2.06	0.07-10.72	0.04 $\pm$ 0.04	0.01-0.23	0.06 $\pm$ 0.04	0.01-0.31
As	11.10 $\pm$ 1.71	10.23-16.91	12.59 $\pm$ 2.03	10.29-14.9	13.66 $\pm$ 3.40	10.30-21.08	12.48 $\pm$ 2.58	10.25-16.75
Cd	52.90 $\pm$ 103	0.94-678	166 $\pm$ 267	2.70-1030	0.57 $\pm$ 0.51	0.11-2.06	0.99 $\pm$ 0.75	0.10-2.56
Co	0.15 $\pm$ 0.13	0.01-0.67	0.17 $\pm$ 0.11	0.06-0.54	0.06 $\pm$ 0.02	0.03-0.15	0.07 $\pm$ 0.04	0.04-0.16
Cr	0.11 $\pm$ 0.03	0.10-0.23	0.12 $\pm$ 0.03	0.10-0.20	0.15 $\pm$ 0.13	0.10-0.64	0.13 $\pm$ 0.06	0.10-0.31
Cu	29.84 $\pm$ 63.76	2.88-424	28.38 $\pm$ 34.92	4.86-176	8.37 $\pm$ 4.05	3.71-28.01	11.79 $\pm$ 7.46	4.27-32.99
Fe	233 $\pm$ 216	59.81-1514	264 $\pm$ 182	88.38-833	17.94 $\pm$ 26.98	4.65-173	24.15 $\pm$ 29.93	6.07-136
Hg	0.19 $\pm$ 0.23	0.06-1.43	0.17 $\pm$ 0.10	0.07-0.54	0.42 $\pm$ 0.41	0.08-2.42	0.37 $\pm$ 0.19	0.08-0.78
Ni	0.63 $\pm$ 0.66	0.04-3.54	0.62 $\pm$ 0.41	0.04-1.94	0.81 $\pm$ 0.24	0.49-1.64	0.93 $\pm$ 0.44	0.49-2.39
Pb	0.32 $\pm$ 0.62	0.01-3.07	0.82 $\pm$ 1.50	0.01-4.71	0.04 $\pm$ 0.04	0.01-0.27	0.03 $\pm$ 0.02	0.01-0.09
Se	26.78 $\pm$ 12.57	20.44-65.67	28.16 $\pm$ 8.97	18.15-49.43	26.75 $\pm$ 7.72	20.49-42.15	24.28 $\pm$ 5.54	20.50-32.24
V	2.19 $\pm$ 0.33	2.04-3.38	2.44 $\pm$ 0.40	2.06-2.98	2.56 $\pm$ 0.74	2.04-4.21	2.43 $\pm$ 0.55	2.05-3.22
Zn	44.55 $\pm$ 26.80	15.57-169	42.65 $\pm$ 12.25	26.34-76.19	66.88 $\pm$ 12.30	42.27-105	69.16 $\pm$ 15.05	55.03-106
$\delta^{13}\text{C}$	NA	NA	NA	NA	-19.57 $\pm$ 1.08	-21.98- -17.91	-20.47 $\pm$ 1.08	-22.10- -18.69
$\delta^{15}\text{N}$	NA	NA	NA	NA	+11.47 $\pm$ 1.28	+9.15- 14.85	+10.57 $\pm$ 1.34	+8.59- 12.93

**Table 2.** Output of the generalised linear models (GLMs), illustrating variables that significantly influence the trace element concentrations of *M. ingens* from the Chatham Rise, New Zealand. The *p*-values of the variables are shown according to likelihood ratio tests (\*\*\*) 0.001, \*\* 0.01, \* 0.05). Negative (↓) and positive (↑) effects for the continuous variable size are indicated with arrows (↑↑↑ 0.001, ↑↑ 0.01, ↑ 0.05).

	Ag	As	Cd	Co	Cr	Cu	Fe	Hg	Ni	Pb	Se	V	Zn
Tissue type	***		***			***	***	**		***			***
Size	↑↑↑		↓					↑↑↑		↓↓↓	↑↑		↑↑↑
$\delta^{13}\text{C}$	***		***			***				*			***
$\delta^{15}\text{N}$						***	***			***			**
Sex			***			***	***						*
Size×Sex						***							***

## Supplementary Materials

**Table S3.** Number of *M. ingens* specimens analysed from the Chatham Rise, New Zealand, according to their size classes (Mantle length, ML). Large females were classified as >400 mm.

Size class ML [mm]	Female ( <i>n</i> )	Male ( <i>n</i> )
<100	1	2
100-200	4	3
200-300	19	8
300-400	22	12
>400	13	

**Table S2.** Analysis of covariance (ANCOVA) table for the linear models fitted for the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the mantle tissue of *M. ingens* from the Chatham Rise, New Zealand. Df – degrees of freedom. Asterisks indicate the level of significance: \*  $p < 0.05$  \*\*  $p < 0.01$  \*\*\*  $p < 0.001$ .

	Df	Sum of squares	Mean square	F value	Significance
$\delta^{13}\text{C}$					
Size	1	2.11	2.11	34.37	***
Sex	2	5.38	2.69	43.72	***
$\delta^{15}\text{N}$	1	8.40	8.40	136.57	***
Residuals	79	4.86	0.06		
$\delta^{15}\text{N}$					
Size	1	0.07	0.07	0.96	
Sex	2	4.66	2.33	30.37	***
$\delta^{13}\text{C}$	1	9.95	9.95	129.59	***
Residuals	79	6.07	0.08		