

# Squid as a vector for trace element bioaccumulation in New Zealand's food webs

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### **Attestation of Authorship**

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgments), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.



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## **List of acronyms**

BMDL– benchmark dose level

dw– dry weight

EEZ– Exclusive Economic Zone

ENSO– El Niño Southern Oscillation

ML– Mantle length

NZ– New Zealand

STFZ– subtropical front zone

TACC– Total Allowable Commercial Catch

PMTI– provisional tolerable monthly intake

PWTI– provisional tolerable weekly intake

ww– wet weight

## **Co-authored work**

This thesis has been realised with the help of several colleagues. Individual contributions to the chapters are listed for each chapter.

### **Chapter 2**

This chapter was co-authored with Chris Pook, Kathrin Bolstad, Jennifer Pannell and Heather Braid.

The author of this thesis contributed 80% to this manuscript (design of the study, dissection of the samples, conduction of the lab work, writing of the manuscript). Chris Pook (5%) and Kathrin Bolstad (5%) contributed to the manuscript by helping with the study design and proof-reading, Jennifer Pannell helped with creating the study map and the statistical analysis (5%) and Heather Braid contributed with specimen collection and proof-reading (5%).

### **Chapter 3**

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The author of this thesis contributed 80% to this manuscript (design of the study, dissection of the samples, conduction of the lab work, writing of the manuscript). Chris Pook (4%) contributed to the manuscript by helping with the study design and proof-reading, Jennifer Pannell helped with creating the study map and the statistical analysis (4%) and Heather Braid contributed with specimen collection (4%), Sally Gaw (4%) and Kathrin Bolstad (4%) helped with proof-reading and critical input.

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The author of this thesis contributed 80% to this manuscript (design of the study, dissection of the samples, conduction of the lab work, writing of the manuscript). Heather Braid contributed with sample

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## Chapter 6

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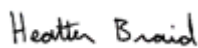
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*‘Dans les champs de l’observation le hasard ne favorise que les esprits préparés.’ [Louis Pasteur]*

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## Abstract

Cephalopods are known for their high bioaccumulation capacity of trace elements, particularly for Cd and Hg. With growing interests for alternative marine protein sources, due to overfishing and climate change, the importance of cephalopods for human consumption is increasing, which in turn creates additional pressures on already stressed trophic systems. Arrow squid (*Nototodarus gouldi* and *N. sloanii*) play a pivotal role in New Zealand's food web: both as prey for a plethora of marine mammals, seabirds and fish species and as one of New Zealand's main fishery targets for human consumption. The greater-hooked squid (*Moroteuthopsis ingens*) are also abundant and ecologically important, but have been less well studied as they have highly ammoniacal tissues and are not commercially fished. Due to their commercial and/or ecological importance, and the opportunity to compare squids of muscular body composition with those that are highly ammoniacal, these three species were selected as focus organisms.

New Zealand waters are active regions, shaped by geothermal and volcanic activities, which are natural sources for trace elements. Concentration data for trace elements in Southern Hemisphere cephalopods are currently sparse, and this study aims to fill the existing knowledge gaps. In order to investigate the role of squids in the transfer of trace elements within New Zealand's waters, a series of four complementary studies were undertaken. First, trace element concentrations (As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Mn, Pb, U, V, and Zn) were assessed in different tissue types of *Nototodarus sloanii* from the Chatham Rise to analyse the bioaccumulation in edible muscular tissues and other organs. Arrow squid are an important vector of particularly Cd to top predators which was revealed by high concentrations in the digestive gland tissue. Next, the total body burden of the squids was calculated and toxicological and dietary consequences for predators (including humans) were assessed. Mean observed concentrations in the muscular tissues, which are consumed by humans, and simulated *in-vitro* digestion analysis, showed that concentrations were overall below the maximum permissible levels for the toxic elements As, Cd, Hg and Pb. However, a contamination of edible mantle tissue with digestive gland fluids was observed in market arrow squid, which might pose a risk for human consumers.

Third, regional patterns of trace element concentrations were assessed in arrow squid samples (both *Nototodarus* species) from multiple sampling locations within New Zealand's Exclusive Economic Zone. Regional differences were particularly significant for As and Hg, with the West Coast exhibiting the highest overall concentrations (max. mantle concentrations, As= 59.58  $\mu\text{g g}^{-1}$  dw; Hg= 2.13  $\mu\text{g g}^{-1}$  dw), and for Cd with the highest measured concentrations in the Taranaki region (digestive gland Cd= 788.46  $\mu\text{g g}^{-1}$  dw).

Fourth, the muscular, non-ammoniacal arrow squid trace element concentrations were compared with an ammoniacal, non-commercially fished species, *Moroteuthopsis ingens*, from the Chatham Rise. Concentrations of Hg were higher in female *M. ingens*, likely due to their deeper habitat and thus

a higher Hg exposure when compared with arrow squid. Otherwise, no major differences in trace element whole-body burden or storage within the mantle and digestive gland were observed, suggesting that the ammoniacal nature of the tissues does not affect trace element bioaccumulation.

Finally, trace element concentrations were measured in one of the arrow squid's main predators, the long-finned pilot whale, *Globicephala melas edwardii*, representing one of the first trace element studies on cetaceans in New Zealand waters and the first globally for this subspecies. Analysis of blubber, kidney, liver, muscle revealed extremely high kidney Cd and liver Hg concentrations. Based on trace element analysis of arrow squid from the cetaceans' stomachs and estimated intake calculations, arrow squid seem to be a vector of trace elements, in particular Cd, to this apex predator. This thesis establishes a baseline for arrow squid, *Moroteuthopsis ingens* and *Globicephala melas edwardii* trace element concentrations for future measurements of toxins within New Zealand's waters. It further investigates potential impacts of arrow squid consumption on humans and strongly suggests that they can be considered important vectors in terms of trace element transfer to top predators. The data included in this thesis have implications for New Zealand's food security, commercial squid stock management, and conservation of charismatic megafauna and the broader marine community.

## **Chapter 1: General Introduction**

### **Trace elements in the marine environment**

There is a growing need to study marine trace element concentrations since they are involved in marine ecosystem dynamics and carbon cycling (SCOR, 2007). Non-essential elements, such as silver (Ag), cadmium (Cd), mercury (Hg) and lead (Pb) have toxic effects on humans and/or other organisms at high concentrations but are usually present in the marine environment at trace concentrations (Järup & Åkesson, 2009; Moore & Ramamoorthy, 2012). Trace element concentrations can be influenced by a range of anthropogenic activities as well as natural geological processes (Tchounwou et al., 2012). Anthropogenic activities such as mining (Brewer et al., 2012), hydrocarbon extraction (Killops et al., 1994) and pollutants, e.g. antifoulants (Morrisey et al., 2000), can elevate concentrations of these elements to levels that can affect the health of marine communities (Amiard, et al., 2006; Ansari et al., 2004). Pelagic trace elemental profiles are also influenced by natural sources such as volcanism, hydrothermal vents, climate (temperature, precipitation), physical mixing processes or upwelling events (Mart et al., 1982; Reimann & de Caritat, 2005).

Changing oceanic conditions, influenced by climate change, can affect elemental cycles in the global oceans (Henderson et al., 2018). Ocean acidification, the decrease in pH associated with increased carbon dioxide, has been associated with a greater bioavailability of a potentially toxic form of Cu (2+) (Avendaño et al. 2016). Changes in oceanic pH have also been correlated with the bioavailability of Fe in the global oceans (Shi et al., 2010). Although a decrease in pH causes an increase in the bioavailable forms of Fe, it is also associated with the binding to ligands, which can actually lead to a net decrease in the Fe availability to phytoplankton (Avendaño et al. 2016; Liu & Millero, 2002). Iron is a limiting factor for phytoplankton growth, the driver of primary productivity in the global oceans. A decrease in phytoplankton growth can ultimately affect food web dynamics at multiple levels (e.g., seafood stocks, Friedland et al., 2012; Runge, 1988). However, the effects of ocean acidification on phytoplankton growth are currently unclear (Shi et al., 2010).

Increasing sea surface temperatures can affect stress tolerance in marine biota (Rathore & Khangarot, 2002; Yang & Chen, 1996). A study by Sokolova & Lannig (2008) described the effects of changes in sea surface temperature on trace element handling in marine ectotherms (e.g. fish and molluscs). Their findings indicated that increased temperature interfered with aerobic metabolism (i.e., mitochondrial functioning). Due to the correlation between increased physiological stress and higher temperatures, organisms have a reduced capacity to handle high toxic trace element concentrations. Oceanic warming has been linked to the depletion of dissolved oxygen in the oceans (Stramma et al., 2008), expanding 'oxygen minimum zones' in various oceanic regions (e.g., Helm et al., 2011; Stramma et al., 2012). Oxygen minimum zones might function as a sink for Cd and lead to Cd anomalies in the bathypelagic

(Janssen et al., 2014). Research outcomes that link trace element concentrations to scenarios of global change all rely on baseline data. The analysis of trace element concentrations in biota is crucial for understanding and monitoring changes in our global oceans, but baseline data have only recently begun to be collected for many marine organisms, and the majority remain unknown.

### **Trace elements in New Zealand's oceans**

New Zealand waters are considered relatively pristine, in comparison to other oceanographic regions like the English Channel, which has a long history of industrial pollution (Bryan & Langston, 1992; Nriagu & Pacyna, 1988). However, there is a paucity of quantitative data on this subject. Studies on trace elements in New Zealand's marine environment to date have mainly focussed on coastal sediments (Abraham & Parker, 2002), harbour regions (Glasby et al., 1988; Stoffers et al., 1986) and freshwater inputs (Hickey & Clements, 1998). Anthropogenic pollution of marine environments is commonly attributed to mining activities (Ellis et al., 2017; Pozebon et al., 2005; Sadiq et al., 2003), long-distance transport of smoke, dust, and particulates from biomass burning or fossil-fuel power stations (Ranville et al., 2010; Zhang et al., 1992).

Apart from anthropogenic influences, geological activity is a significant natural source of trace elements in the marine environment (Giggenbach & Glasby, 1977). The New Zealand seafloor topography is highly complex, with ridges, trenches and hydrothermal vents (de Ronde et al., 2003; Heath, 1985), which can influence the concentration of trace elements in the immediate vicinity. For example, deep ocean floor sediments around the Lowe Howe Rise contained elevated As levels (mean concentration  $\sim 6.5 \mu\text{g g}^{-1}$ ; Radke et al., 2011). The effect of volcanism on trace elements was shown by a study along the Bay of Plenty, where an enrichment in Ag, As, Au, Hg, Pb, and Sb was identified (Stoffers et al., 1999). The elevated concentrations levels were related to the 'offshore extension of the Taupo volcanic zone' in that area, influencing the marine concentrations (Stoffers et al., 1999).

Other contributors for trace elements are physical oceanographic processes. Within New Zealand waters, the Chatham Rise is an extension of the continental shelf east of the South Island. In this area, subtropical water encounters sub-Antarctic water, which results in a subtropical front zone (STFZ) situated over the Chatham Rise (Sutton, 2001). The cooler, nutrient-rich sub-Antarctic surface water mixes with the warmer, less dense, subtropical water (Ellwood & Maher, 2002). This creates a region with high primary productivity, which is the major source of New Zealand's fisheries (Clark & O'Driscoll, 2003). It has been shown that Fe concentrations differ along the STF, with the highest values measured north of the mixing zone (Tian et al., 2006). Measurements from the STF close to the Otago continental shelf showed the highest Fe concentrations over the central shelf, with a decrease across the mixing zone of the STF. Variation in As was also measured in that area, with higher concentrations of

dissolved As measured along the Chatham Rise compared to waters north and south of the rise (Ellwood & Maher, 2002). Apart from the local variations within New Zealand's Exclusive Economic Zone (EEZ), the marine trace element concentrations are also influenced by the proximity to the Southern Ocean. The STF located along the Chatham Rise is described as the northern boundary of the Southern Ocean (Pakhomov & McQuaid, 1996). As a result, the biogeochemistry of New Zealand's waters is a varying mixture of the Southern and Pacific Oceans and the Tasman sea. It is important to understand potential fluxes between both ocean bodies and their associated fauna because the Southern Ocean plays a major part in the storage of heat and CO<sub>2</sub> fixation (Sabine et al., 2004).

### **Trace elements in New Zealand's marine taxa**

Within marine organisms, some trace elements, e.g., As, Cd, Pb, and U, can bioaccumulate over time (Bhupander & Mukherjee, 2011; Goyer, 1989; Pavlakis et al., 1996). Other trace elements, such as an organic form of Hg, methyl-Hg, are known for their biomagnification capacities in marine apex predators, and have been reported at concentrations that could be hazardous to human consumers (Fisher & Reinfelder, 1995).

To date, studies on trace element concentrations in New Zealand's marine taxa have focused primarily on vertebrates and commercially important invertebrates. Examples include commercial fish species (Brooks & Rumsey, 1974; Fenaughty et al., 1988; Kalish et al., 1996; Vlieg et al., 1991), seabirds (Bond & Lavers, 2011; Lock et al., 1992; Lyver et al., 2017; Stewart et al., 1999) and mussels (Chandurvelan et al., 2015; Kennedy, 1986; Marsden et al., 2014; Peake et al., 2010; Pridmore et al., 1990; Vlieg et al., 1991; Whyte et al., 2009). A summary of some trace element concentrations across marine taxa within New Zealand waters is presented in Table 1. A discussion of some notable findings, and their implications for human consumption (where relevant), follows.

Cadmium is a carcinogen and is known to cause kidney and liver damage in terrestrial mammals (including humans) when ingested in high concentrations (Järup et al., 1998). Greenshell mussels (*Perna canaliculus*) from sites in the Bay of Islands were analysed for Cd concentrations (Whyte et al., 2009). The concentrations were compared to the provisional tolerable weekly intake (PTWI), which is an estimate of the maximum amount of a substance that a person can be exposed to per week without encountering adverse health effects (Joint FAO/WHO Expert Committee on Food Additives [JEFCA], 2013). It was concluded that, for a 'typical diet' (70 kg adult consuming <0.65 kg mussel flesh a week), the amount of Cd ingested would not exceed the PTWI of 7 µg kg body weight<sup>-1</sup> week<sup>-1</sup> (Whyte et al., 2009). However, the consumption of mussels collected from one sampling site, Urapukapuka Island, (0.75 µg Cd g<sup>-1</sup> dry weight [dw]), would pose a risk to the average adult (Whyte et al., 2009). Elevated Cd concentrations in edible molluscs have also been reported in other regions. For example, dredge

scallops (*Pecten novaezelandiae*) sampled from the Chatham Islands contained high whole-body Cd concentrations ( $65 \mu\text{g g}^{-1} \text{ dw}$ ) and particularly high digestive gland concentrations ( $3,985 \pm 1,108 \mu\text{g g}^{-1} \text{ dw}$ ; Peake et al., 2010). Those concentrations were correlated with water currents of the STF and their associated primary productivity, which is linked to enriched Cd concentrations (Frew & Hunter, 1995). Cadmium is transported with the ocean currents, leading to higher concentrations in areas affected by these currents, e.g. Chatham Islands (Peake et al., 2010).

To date, there have been few reports of trace element concentrations in mesozooplankton, such as copepods. Copepods are an important component of marine food webs since they are prey for many taxa, including the early life stages of cephalopods (Passarella & Hopkins, 1991). They are a significant vector for trace element transfer (Reinfelder et al., 1993). Anomalously high Cd concentrations have been described in marine invertebrates from the Southern Ocean and were referred to as the ‘polar cadmium anomaly’ (Bargagli et al., 1996; Kahle & Zauke, 2002; Petri & Zauke, 1993). Marine upwelling events, such as the upwelling of deeper Antarctic waters, can increase Cd concentrations in the surface waters which results in an increased uptake of Cd by phytoplankton and zooplankton (Honda et al., 1987; Kahle & Zauke, 2002). For example, ratios of Cd:P and Zn:P in copepods from sub-Antarctic waters east of New Zealand were almost twice as high as those measured in subtropical samples (Mackie & Hunter, 2005).

The most abundant organic form of Hg, methylmercury (MeHg), is the main bioavailable species of Hg in fish and cephalopods (Bloom, 1992; Bustamante et al., 2006). Methylmercury is known for its toxicity in mammals (including humans), where it can lead to neurological disorders and affect the kidneys, liver, and immune system (Booth & Zeller, 2005). Most Hg in cephalopod muscle tissue is stored in its methylated form (Bustamante et al., 2006; Table 3). Very high Hg concentrations were measured in the muscle tissue of black cardinalfish (*Epigonus telescopus*) from the Challenger Plateau, West coast of the North Island (Table 1; Tracey, 1993). These concentrations would just exceed the PTWI set by the World Health Organization (WHO) of  $1 \mu\text{g kg}^{-1} \text{ bodyweight}^{-1} \text{ week}^{-1}$  (70 kg adult consuming  $<0.51 \text{ kg}$  muscle tissue a week; WHO, 2010b). Mercury concentrations in the muscle tissue of orange roughy (*Hoplostethus atlanticus*) caught along the Chatham Rise showed mean concentrations of  $0.49 \mu\text{g g}^{-1} \text{ ww}$ . The latter concentration exceeds the PTWI for a 70 kg adult consuming  $>0.15 \text{ kg}$  muscle tissue a week (van den Broek & Tracey, 1981).

Seabirds seem to show a dose-response relationship towards Hg, meaning that increased concentrations in their prey lead to a corresponding increased concentration in the birds (Monteiro & Furness, 1995). The diet of grey-faced petrels seems to consist largely of cephalopods: 58% Cranchiidae and Histioteuthidae by weight (Imber, 1973), which suggests that they play a role in the birds’ Hg bioaccumulation. Feathers of petrels and shearwaters collected North of the North Island had high Hg

concentrations ( $36.48 \mu\text{g g}^{-1} \text{ dw}$ , Lyver et al., 2017; Table 1). For grey-faced petrels (*Pterodroma gouldi*), the mean concentration was  $36.48 \pm 9.59 \mu\text{g g}^{-1} \text{ dw}$  which represents one of the highest Hg concentrations measured in seabirds within the last 25 years.

### Trace element concentrations in cephalopods

Within the pelagic food web, most squids are generalist predators as well as a major prey item for fish, seabirds and marine mammals (Boyle & Rodhouse, 2005; Clarke, 1996a,b). This position makes squids an excellent study organism in terms of accumulation of trace elements through the food web. Furthermore, as many squid species, particularly of the family Ommastrephidae, are consumed by humans, studying their trace element bioaccumulation is important in terms of food security (see Page 14). With the increasing human reliance on oceanic protein, cephalopods are of growing interest for fisheries (Caddy & Rodhouse, 1998). Their abundance, alongside the decline of smaller fish species' stocks, is increasing (Doubleday et al., 2016; Jereb et al., 2015). For example, in parts of the Antarctic Ocean, cephalopods have been reported to increasingly fill the ecological role of declining stocks of mesopelagic fish (Rodhouse & White, 1995). Growing consumption of cephalopod species has led to a broader investigation of trace elements in the tissues of commercially targeted species (e.g., Rjeibi et al., 2015, 2014; Storelli et al., 2005; Storelli et al., 2010; Tables 2, 3), and a recent review on trace element concentrations in cephalopods highlighted their high trace element bioaccumulation capacities, especially for squids of the family Ommastrephidae (Penicaud et al., 2017). Species of this family have been suggested as a vector for Cd in particular to apex predators (Bustamante et al., 1998a). Squid tend to accumulate trace elements and other toxins in the digestive gland (Bustamante et al., 2002a; Koyama et al., 2000; Rodrigo & Costa, 2017), a lipid-rich, liver-like organ that is typically not consumed by humans (although it is consumed by other predators). For example, Cd concentrations of  $\sim 3,711 \pm 2,094 \mu\text{g g}^{-1} \text{ dw}$  have been measured in the digestive gland of the shortfin squid *Illex argentinus* from the South-west Atlantic Ocean off Argentina (Dorneles et al., 2007) and from the northern Argentinian coast ( $1,213 \pm 295 \mu\text{g g}^{-1} \text{ dw}$ , Gerpe et al., 2000). Most recently, the pelagic ommastrephid *Sthenotheuthis pteropus* from the Eastern Tropical Atlantic showed both elevated Cd and Hg concentrations (Lischka et al., 2018; Table 2).

In the Southern Hemisphere, where fewer commercial fisheries operate overall, few trace element biomonitoring studies have been conducted on cephalopods (see Table 2). Those that have been conducted have focussed on commercial species, despite the known abundance and ecological importance of other families such as the Onychoteuthidae (including *M. ingens*). To date trace element concentrations, including Cd, were reported in the digestive gland of *Nototodarus gouldi* from Port Philip Bay in Australia (Finger & Smith, 1987; Table 2) and from the muscle tissue of *Nototodarus* sp. caught within New Zealand's EEZ (Vlieg et al., 1991; Table 2).

Table 1. Concentrations of elements of most health concern (As, Cd, Hg, Pb) in selected taxa from New Zealand's EEZ. All concentrations are in  $\mu\text{g g}^{-1}$  dw.

Species	Tissue	As	Cd	Hg	Pb	Location	Study
Mollusca (Bivalvia)							
<i>Austrovenus stutchburyi</i> (cockle)	whole body	14.8–48.4	0.18–0.41		0.31–2.75	Christchurch	Marsden et al., 2014
<i>Chlamys delicatula</i> (queen scallop)	whole body		65			Otago Coastal Shelf	Peake et al., 2010
<i>Haliotis iris</i> (Paua)	muscle	10.9*	1.35*			Hawke's Bay	Vlieg et al., 1991
<i>Pecten novaezelandiae</i> (dredge scallop)			332			Chatham Islands	Peake et al., 2010
<i>Perna canaliculus</i> (Greenshell mussel)	muscle	14.85*	3.75*			Bay of Islands Urapukapuka	Whyte et al., 2009
<i>Perna canaliculus</i> (Greenshell mussel)	muscle	11.25*	0.55*			Bay of Islands Opuā Marina	Whyte et al., 2009
<i>Perna canaliculus</i> (Greenshell mussel)	muscle	8.55*	0.35*			Bay of Islands Waitangi Bridge	Whyte et al., 2009
<i>Perna canaliculus</i> (Greenshell mussel)	muscle	7.8*	0.45*			Bay of Islands Opuā Wharf	Whyte et al., 2009
Mollusca (Cephalopoda)							
<i>Nototodarus</i> sp. (arrow squid)	muscle	21*	1.8*			Golden Bay	Vlieg et al., 1991
<i>Nototodarus sloanii</i> (Southern arrow squid)	muscle			0.35*		Auckland Islands	van den Broek & Tracey, 1981
<i>Nototodarus sloanii</i> (Southern arrow squid)	muscle			0.2*		Southland	van den Broek & Tracey, 1981
<i>Nototodarus sloanii</i> (Southern arrow squid)	muscle			0.25*		East Coast South Island	van den Broek & Tracey, 1981
<i>Sepioteuthis bilineata</i> (broad squid)	muscle	19.5*	NA			Golden Bay	Vlieg et al., 1991
Cartilaginous fish (Chondrichthyes)							
<i>Isurus oxyrinchus</i> (Mako shark)	muscle	22.15*	NA	7.9*		Louisville Seamount Chain	Vlieg et al., 1993
<i>Lamna nasus</i> (Porbeagle shark)	muscle	11.5*	NA	3.4*		Louisville Seamount Chain	Vlieg et al., 1993
Bony fish (Osteichthyes)							
<i>Epigonus telescopus</i> (Black cardinalfish)	muscle			4.90*		Challenger Plateau	Tracey, 1993
<i>Gasterochisma melampus</i> (Butterfly tuna)	muscle			1.65*		Kermadecs area	Vlieg et al., 1993
<i>Hoplostethus atlanticus</i> (Orange roughy)	muscle			1.75*		Chatham Islands	van den Broek & Tracey, 1981
<i>Lamoris guttatus</i> (Moonfish)	muscle			2.15*		Kermadecs area	Vlieg et al., 1993
<i>Macruronus novaezelandiae</i> (NZ hoki)	muscle			0.35*		Auckland Islands	van den Broek & Tracey, 1981
<i>Pseudocyttus maculatus</i> (Smooth oreo)	muscle		0.45*		s	Chatham Islands	van den Broek & Tracey, 1981
<i>Thunnus alalunga</i> (Albacore tuna)	muscle	12.7*	NA	2.45*		Kermadecs area	Vlieg et al., 1993
<i>Xiphias gladius</i> (Swordfish)	muscle			4.4*		Kermadecs area	Vlieg et al., 1993
Birds (Aves)							
<i>Pelecanoides urinatrix</i> (common diving petrel)	feather	0.08	0.02	3.36	0.18	North Island	Lyver et al., 2017
<i>Pterodroma gouldi</i> (grey-faced petrel)	feather	0.04	0.02	36.48	0.05	North Island	Lyver et al., 2017
<i>Puffinus assimilis</i> (little shearwater)	feather	0.11	0.08	4.26	0.04	North Island	Lyver et al., 2017
<i>Puffinus carneipes</i> (Flesh-footed shearwater)	feather	1.229	0.071	7.466	0.347	Kauwahaia island	Bond and Lavers, 2011
<i>Puffinus carneipes</i> (Flesh-footed shearwater)	feather	3.629	0.059	8.007	0.492	Lady Alice island	Lyver et al., 2017
<i>Puffinus gavia</i> (fluttering shearwater)	feather	0.19	0.02	2.6	0.06	North Island	Lyver et al., 2017
Mammalia							
<i>Delphinus</i> sp. (common dolphin)	kidney		56.67			Hauraki Gulf	Stockin et al., 2007
	liver			176.67		Hauraki Gulf	Stockin et al., 2007
<i>Arctocephalus forsteri</i> (NZ fur seal)	faeces	5.29	94.51			Snares Islands	Wing et al., 2017
<i>Phocarcus hookeri</i> (Hooker's sea lion)	faeces	0.91	2.85			Auckland Islands	Wing et al., 2017
		1.76	13.68			Snares Islands	Wing et al., 2017

\* wet weight converted to dry weights, assuming a water content of 80% for molluscs, fish and mammals



Table 2. Concentrations of Cd in digestive glands of ommastrephid squids (mean±standard deviation, sd, in  $\mu\text{g g}^{-1}$  dw).

Species	mean ± sd	Region	Reference
<i>Illex argentinus</i>	5014.50±2830.00*	Central South Brazil Bight	Dorneles et al., 2007
<i>Illex argentinus</i>	92.50±NA	Argentina	Falandysz, 1988
<i>Illex argentinus</i>	5.14±1.54	Patagonia	Gerpe et al., 2000
<i>Illex argentinus</i>	0.60±NA*	Argentina	Storelli et al., 2012
<i>Illex argentinus</i>	144.80±65.00	Argentina	Kurihara et al., 1993
<i>Illex coindetii</i>	14.40±7.55*	Adriatic Sea	Storelli & Marcotrigiano, 1999
<i>Nototodarus gouldi</i>	50.00±25.00	Bass Strait, Australia	Smith et al., 1984
<i>Nototodarus gouldi</i>	33.00±30.00	Port Phillip Bay, Australia	Finger & Smith, 1987
<i>Ommastrephes bartramii</i>	826.50±369.10	Japanese Waters	Kurihara, et al., 1993
<i>Ommastrephes bartramii</i>	286.79±194.39	Southern California	Martin & Flegal, 1975
<i>Sthenoteuthis oualaniensis</i>	0.13±NA	Sea of Japan	Ichihashi et al., 2001
<i>Sthenoteuthis pteropus</i>	747.80±279.36	Eastern Tropical Atlantic	Lischka et al., 2018
<i>Todarodes filippovae</i>	246.00±187.00	Indian Ocean	Kojadinovic et al., 2011
<i>Todarodes filippovae</i>	98.50±67.20	Tasmania	Kojadinovic, et al., 2011
<i>Todarodes pacificus</i>	16.68±NA	Sea of Japan	Ishizaki et al., 1970
<i>Todarodes sagittatus</i>	13.84±5.96*	Bay of Biscay	Bustamante et al., 1998a
<i>Todarodes sagittatus</i>	18.00±12.00	Bay of Biscay	Chouvelon et al., 2011

\* wet weight converted to dry weights, assuming a water content of 80% for molluscs

Table 3. Concentration of total Hg (THg) and organic, methyl-Hg (MeHg) in tissues (Digestive gland, DG; muscle, M) of selected non-ammoniacal and ammoniacal squid species (mean ± standard deviation [sd] in  $\mu\text{g g}^{-1}$  dw).

Species	Tissue	THg±sd	MeHg± sd	%MeHg	Ammonia	Region	Study
Oegopsida							
<i>Alluroteuthis antarcticus</i>	DG	0.04±0.002	0.002± 0.016	38%	Yes	South Georgia, Antarctica	Seco et al., 2020
	M	0.07±0.004	0.004± 0.052	80%			
<i>Architeuthis dux</i>	DG	0.47±0.13			Yes	Bay of Biscay	Bustamante et al., 2008
	M	2.07±0.19					
<i>Filippovia knipovitchi</i>	DG	0.64±0.01	0.098± 0.144	22%	Yes	South Georgia, Antarctica	Seco et al., 2020
	M	0.08±0.03	0.028± 0.053	67%			
<i>Gonatus antarcticus</i>	M	0.16±0.06	0.058± 0.158	97%	No	South Georgia, Antarctica	Seco et al., 2020
<i>Gonatus fabricii</i>	DG	0.05±0.02			No	Greenland	Lischka et al., 2019a
	M	0.11±0.07					
<i>Moroteuthopsis longimana</i>	DG	0.05±0.02	0.021± 0.011	25%	Yes	South Georgia, Antarctica	Seco et al., 2020
	M	0.08±0.02	0.023± 0.078	95%			
<i>Psychroteuthis glacialis</i>	DG	0.03±0.006	0.006± 0.006	24%	No	South Georgia, Antarctica	Seco et al., 2020
	M	0.08±0.02	0.018± 0.062	74%			
<i>Sthenoteuthis pteropus</i>	DG	0.43±0.18			No	Eastern Tropical Atlantic	Lischka et al., 2018
	M	0.33±0.14					
<i>Todaropsis eblanae</i>	DG	0.13±0.10			No	British waters	Pierce et al., 2008
	M	0.21±0.20					
<i>Todarodes sagittatus</i>	DG	0.28±0.11			No	British waters	Pierce et al., 2008
	M	0.43±0.20					
Myopsida							
<i>Alloteuthis subulata</i>	DG	0.07±0.01			No	British waters	Pierce et al., 2008
	M	0.10±0.01					

## Introduction to study species

This thesis analysed trace element concentrations in four key species of New Zealand's pelagic food web: three of the most abundant and ecologically important squid species, and one of their cetacean predators. The main focus was on arrow squids (*Nototodarus gouldi* and *N. sloanii*), belonging to the family Ommastrephidae. Arrow squids are one of New Zealand's main commercial fishery targets and play a pivotal role in the diets of many apex predators. Their tissue concentrations were assessed and considered in the context of human consumption. Next, arrow squid trace element concentrations were compared to concentrations of the ammoniacal (non-commercial) deep-sea hooked squid *Moroteuthopsis ingens*, which is one of the other most abundant squid species within New Zealand's EEZ and the Southern Ocean (Jackson et al., 2000), and inhabits deeper waters than arrow squids. Finally, trace element concentrations in one of the region's main arrow squid predators, the long-finned pilot whale (*Globicephala melas edwardii*), were assessed.

### Arrow squids

Arrow squids are fished throughout the EEZ using fishing lines that are lowered from a fishing vessel, a method called 'jigging' (MPI, 2019). Squid are usually caught between January and May at night time, attracted by fishing vessels' powerful lights. In some regions, trawling is also used. Apart from the Auckland Islands fishery, both arrow squid species are managed as one species (despite *N. sloanii* being endemic) with a single aggregate Total Allowable Commercial Catch (TACC). The arrow squid fishery is one of the largest commercial fisheries in New Zealand with a TACC of 82,120 tons for the year 2018 throughout New Zealand's EEZ (MPI, 2019), and a value of \$95 million when last posted more than 10 years ago (2008; MPI, 2020). Apart from their role in the diet of New Zealanders, arrow squids are important in the pelagic food web as predator and prey (Pethybridge et al., 2012). In New Zealand waters, they are known to represent a main prey item for long-finned pilot whales, *Globicephala melas* (Beatson et al., 2007ab), Buller's mollymawk, *Diomedea bulleri*, (West & Imber, 1986), the brown fur seal, *Arctocephalus pusillus doriferus* (Emami-Khoyi et al., 2016), the rare and endemic New Zealand sea lion, *Phocarctos hookeri* (Childerhouse et al., 2001), Buller's albatross, *Diomedea bulleri bulleri* (James & Stahl, 2000) and orange roughy, *Hoplostethus atlanticus* (Jones, 2007).

Arrow squids are generalist predators that feed on a wide range of prey, which consists mainly of fish, cephalopods and crustaceans (Braley et al., 2010; Dunn, 2009). Pearlsides (*Maurolicus* sp.) as well as lantern fish (*Lampanyctodes hectoris*) are frequently reported prey items from their stomach contents (Dunn, 2009; Pethybridge et al., 2012). Crustaceans (Isopoda, Amphipoda) tend to represent a frequent but less important food component in the diet of arrow squid (Pethybridge et al., 2012). The food intake

of female and male *N. gouldi* specimens seem to be largely comparable (O’Sullivan & Cullen, 1983) although females appear to feed more on cephalopods and crustaceans than males do (Dunn, 2009). Both *Nototodarus gouldi* and *N. sloanii* are neritic– oceanic species that are most abundant in the continental shelves and only infrequently occur offshore (Uozumi, 1998; Uozumi & Förch, 1995). Whereas *N. sloanii* is found in waters shallower than 600m, with greatest abundance in water shallower than 300m (Chen, 1998; Jackson et al., 2000), *N. gouldi* shows greatest abundance in shelf waters of 50 – 200 m (Uozumi, 1998). Adult *N. sloanii* are usually found within and south of the STF whereas adult *N. gouldi* are commonly found north of the STF (Smith et al., 1981). Both species co-occur along the Golden Bay and the North-West of the South Island (Fig. 1), although hybridisation has not been reported. Unlike other ommastrephid squids (Anderson & Rodhouse, 2001), arrow squid tend to be less migratory and do not seem to follow clear migration patterns, according to tagging studies (Dunning & Förch, 1998; Stark et al., 2005).

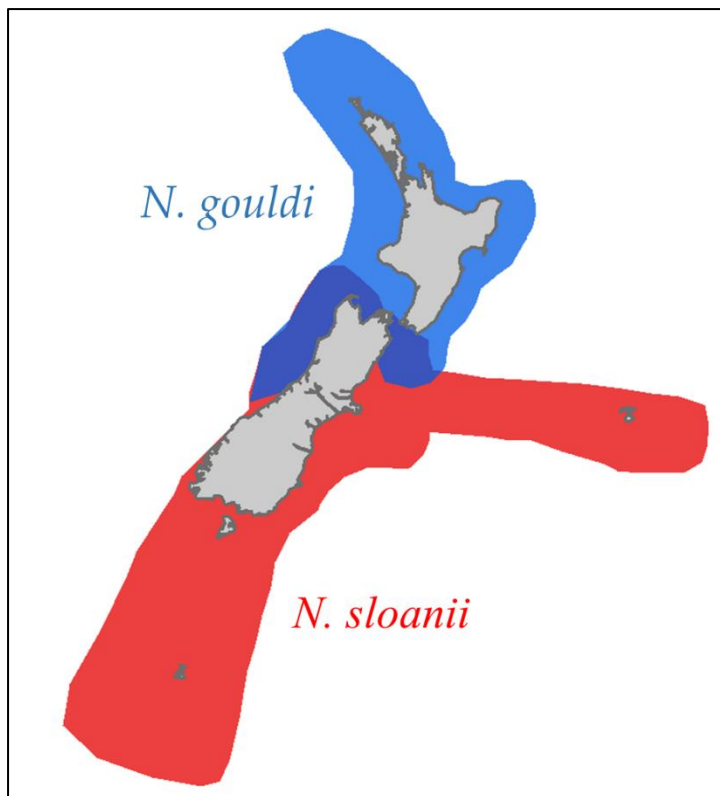


Fig. 1. Distribution map of New Zealand’s arrow squid species (*Nototodarus gouldi* in blue and *N. sloanii* in red). The dark blue colour represents their overlapping distribution range.

#### *Moroteuthopsis ingens*

After the endemic *N. sloanii*, the deep-sea greater hooked squid *Moroteuthopsis ingens*, belonging to the family Onychoteuthidae, is the most abundant squid species in Southern New Zealand waters (Jackson et al., 2000). This species is an important prey item in the diets of cetaceans such as sperm whales (Clarke, 1980), long-finned pilot whales (Clarke, 1996a; Mansilla et al., 2012) and bottlenose

whales (Sekiguchi et al., 1992). It has also been reported from stomach contents of New Zealand hoki (*Macruronus novaezelandiae*; Connell et al., 2010). However, in comparison with arrow squids, much less is known about *M. ingens*' biology (Arkhipkin & Laptikhovsky, 2010). This is because they are not of commercial interest due to their unpalatable ammonium content. The effects, if any, of this difference in body chemistry (compared with the highly muscular *Nototodarus* species) on their trace element accumulation remain unknown. Around New Zealand waters *M. ingens* has been reported to feed on teleost fishes such as myctophids (lanternfish) and stromiids (dragonfish) as well as other squids (Jackson et al., 1998). A shift in diet with growth has been described from specimens caught around the Patagonian Shelf, where large individuals feed mostly on other squid and smaller individuals tend to prey on crustaceans (Rosas-Luis et al., 2014). An ontogenetic change in diet may also have implications for trace element accumulation, so comparative data from New Zealand may provide insight into this aspect of their biology. A sexual dimorphism in size, which affects its habitat choice, has been described in this species, which might influence trace element bioaccumulation patterns, but lay outside the scope of previous studies (McArthur et al., 2003, Cipro et al. 2018). Trace elements in *M. ingens* from the Southern Ocean have been analysed in digestive gland and mantle tissue (McArthur et al., 2003, Cipro et al. 2018), while data from New Zealand waters are currently lacking. Acquiring trace element bioaccumulation data for this species from New Zealand waters will allow for a wider comparison between regions.

#### *Globicephala melas edwardii*

The long-finned pilot whale, *Globicephala melas* (family Delphinidae), is found worldwide but comprises two geographically distinct subspecies. In the northern hemisphere, *G. melas melas* is known to prefer deep, open ocean waters, particularly over continental shelves or continental slopes (Bloch et al., 2003; Minton et al., 2018), and follows its prey along continental shelf waters during the summer and autumn months (Reeves, 2003). In the Southern Hemisphere *G. melas edwardii* is widely distributed (Oremus et al., 2009) but its abundance, population structures and distribution patterns remain more poorly known, especially in New Zealand waters (Betty, 2019; Betty et al., 2020). Mass strandings are relatively frequent and have been well documented around New Zealand and Australian coastlines (Betty, 2019). This species is of cultural importance to *tangata whenua* (New Zealand's indigenous people).

Long-finned pilot whales are apex predators, with regional dietary differences between the two subspecies (Beatson 2007ab; Gannon et al., 1997). These differences have implications for their potential vectors for trace elements. While the diet of *G. m. edwardii* is composed of a high proportion of arrow squid (97.9% Beatson et al., 2007a), the Northern Hemisphere subspecies *G. m. melas* feeds on a variety of species (e.g., Atlantic mackerel *Scomber scombrus*, Atlantic herring *Clupea harengus*, and long-finned squid *Loligo pealei*). Both subspecies are globally known for mass stranding events,

the causes of which are not well understood (Geraci & Lounsbury, 2005). Their health can be impacted by toxic trace elements, which have their main entry pathways through diet (Gajdosechova et al., 2016). Because the diet of *G. m. edwardii* is nearly exclusively composed of arrow squid, this predator–prey combination represents an ideal model for trophic transfer.

### **Impact on humans**

Trace elements can have toxic effects on humans in high concentrations; in particular As, Cd, Hg and Pb were defined by the World Health Organization (WHO) as the four trace elements of most concern for human health (WHO, 2010a). This makes them—together with air pollution, asbestos, benzene, dioxin, excess fluoride and hazardous pesticides—the WHO’s top ten priority in terms of globally hazardous substances.

Arsenic is defined as a metalloid as it possesses both characteristics of a metal as well as a non-metal (Bentley & Chasteen, 2002). When As is combined with other elements such as O or S, it is present as inorganic As (iAs), which shows the most toxic effect on humans (Hughes, 2002; Jomova et al., 2011), including carcinogenic and genotoxic effects (Hall, 2002). Most commonly, humans are exposed to As through industrial exposure (Wang et al., 2007) or diet (Jomova et al., 2011). The main reported sources of dietary As intake for humans are mushrooms, rice, poultry and seafood (Jomova et al., 2011; EFSA, 2009). However, most As in seafood, including cephalopods (Devesa et al., 2001; Muñoz et al., 2000), is present as arsenobetaine which is considered less toxic to humans (Adams et al., 1994; Borak & Hosgood, 2007; Edmonds et al., 1977).

High Cd exposure can lead to bone, kidney and lung damage (Bernard & Lauwerys, 1986; Nicholson et al., 1983; Nicholson & Osborn, 1983). The most severe form of chronic Cd poisoning in humans has been manifested in the ‘itai–itai’ disease, which can lead to kidney failure and painful bone softening (Inaba et al., 2005). Apart from exposure through occupational hazard, e.g., in mining or smelting, diet and smoking are the main exposure pathways for Cd in humans (Pan et al., 2010). For example, soils can be enriched in Cd after the treatment with phosphorous fertilisers (Williams & David, 1973). Edible plants can selectively take up Cd from the soils (Welch & Norvell, 1999), and represent a main contributor to Cd intake through diet. Bioaccumulation of Cd is also reported in seafood, with particularly high concentrations found in molluscs, including cephalopods (Bustamante et al., 1998a,b; Wright & Welbourn, 1994), and crustaceans (Noël et al., 2011).

In particular, methylmercury (MeHg) is toxic and can lead to impaired neurological development (Booth & Zeller, 2005; Mergler et al., 2007). ‘Minamata’ disease is the best-known example of severe Hg poisoning, which affected a population in Japan after consumption of shellfish and fish from MeHg contaminated waters (Eto, 2000). In mammals, including humans, the excretion of MeHg mainly takes

place through bile and faeces (Hong et al., 2012). However, breast milk is also a significant pathway for MeHg excretion and thereby a significant source for newborns (Bose-O'Reilly et al., 2008). For example, a study in New Zealand highlighted the effects of prenatal Hg transfer from a mother with high hair Hg concentrations ( $86 \mu\text{g g}^{-1} \text{dw}$ ) to her child, including disorders in intelligence quotient and motor skills (Crump et al., 1998). Methylmercury biomagnifies through the marine foodweb, reaching highest concentrations in predatory fishes and cetaceans (e.g., Bosch et al., 2016; Caurant et al., 1996; de Pinho et al., 2002). As a result, certain countries have guidelines for fish consumption; i.e., Food Standards Australia New Zealand (FSANZ) advised restricted consumption of billfish and shark to one serving per week because of potentially high concentrations of MeHg (FSANZ, 2016). This toxic trace element presents a uniquely potent hazard and emphasis lies on the exposure of vulnerable demographics, pregnant women and children (FSANZ, 2011, 2016).

Lead is another element of human health concern; prolonged exposure can have effects on the central nervous system, the kidneys and the gastrointestinal tract (Altmann et al., 1993; Bernard & Becker, 1988). The principal exposure pathways of Pb to humans are air and diet (Hammond, 1977). Additives of Pb in fuel have been characterised as one of the main sources for this metal to urban environments, and are banned in most western countries (Mielke et al., 2011; Tong et al., 2000). In comparison to internationally reported levels, the dietary exposure of adults to Pb in New Zealand is low (MPI, 2018).

With the increasing global consumption of cephalopods (Hunsicker et al., 2010; Mouritsen & Styrbæk, 2018), and their known bioaccumulation patterns for Cd and Hg (Bustamante et al., 1998a, Penicaud et al., 2017), identification and awareness of their trace elemental burdens are important. Arrow squid fisheries in New Zealand are increasing (MPI, 2019), but they are not currently addressed in the New Zealand Total Diet Study (NZTDS; MPI, 2018). Analysing their trace element concentrations is thus a timely and critical step enabling their safety as a food item to be assessed. Such a risk assessment involves the comparison of measured concentrations in squid mantle tissue to the currently defined PWTi to identify potential risk factors associated with the consumption of arrow squid.

## Thesis objectives and significance

This thesis aims to gain insight into dynamics of trace element accumulation within New Zealand's pelagic food web. The trace elements analysed in this thesis were chosen to obtain a baseline reading since these have been commonly analysed in cephalopod (e.g., Bustamante et al., 2000,2008; Ichihashi et al., 2001; Kojadinovic et al., 2011) and cetacean (e.g., Bustamante et al., 2003; Law et al., 2001) biomonitoring studies. Specifically, the non-essential elements (As, Cd, Hg, Sb, U, and Pb) were analysed because of their known toxicity to marine biota (Rainbow, 1995, 2007) and their effects on humans (WHO, 2010b). The role of squids as a potential vector for trace elements was analysed, with the goal to provide baseline information concerning health and safety in terms of arrow squid consumption for human consumers, and of assessing bioaccumulation in non-human organisms within pelagic food webs, including the squids themselves (three species) and a cetacean predator. This aim was accomplished through the following objectives and means, each comprising one study and chapter (Fig. 2):

- Chapter 2: Which trace elements are present, in which tissues (and quantities), in arrow squids from New Zealand waters? How can we robustly estimate whole-body burden of these trace elements?  
*Method summary: (1) Quantification of trace elements (Cd, Co, Cr, Cu, Fe, Ni, U, Zn) in 180 specimens and nine tissue types of N. sloanii from the Chatham Rise.*
- Chapter 3: Based on the results of Chapter 2, do As, Cd, Hg and Pb concentrations in arrow squid from the Chatham Rise pose a risk to human consumers?  
*Method summary: (1) Quantification of As, Cd, Hg and Pb, the elements of most concern, in 30 specimens and nine tissue types of N. sloanii from the Chatham Rise. (2) Simulating human digestion of squid specimens in-vitro to estimate bioavailability of squid trace elements following human consumption.*
- Chapter 4: Do trace element concentrations in arrow squid vary regionally and are they influenced by size or sex?  
*Method summary: (1) Analysing data for patterns in trace element distribution, controlling for biotic and abiotic factors across seven sampling locations within New Zealand's EEZ.*
- Chapter 5: How do trace element concentrations in arrow squids compare with those in an ammoniacal species (*Moroteuthopsis ingens*) found in similar regions?  
*Method summary: (1) Assessment of trace element concentration and trophic position of M. ingens from the Chatham Rise through stable isotope analysis.*

- Chapter 6: What trace element concentrations can be observed in different tissues of *Globicephala m. edwardii*, a main predator of arrow squid? Can arrow squid be considered as their vector for trace elements?

*Method summary: (1) Assessment of trace element concentrations in G. m. edwardii tissues. (2) Analysing arrow squid tissue from G. m. edwardii stomach contents to estimate trophic transfer of trace elements.*

Little is currently known about concentrations or bioaccumulation of trace elements in New Zealand cephalopods and the food webs to which they contribute. Therefore, this project creates a knowledge base to develop further studies and research questions. In particular, global change issues such as climate change (ocean acidification) or pollution (e.g. through seabed mining) could be addressed, including the measured concentrations as baseline data.

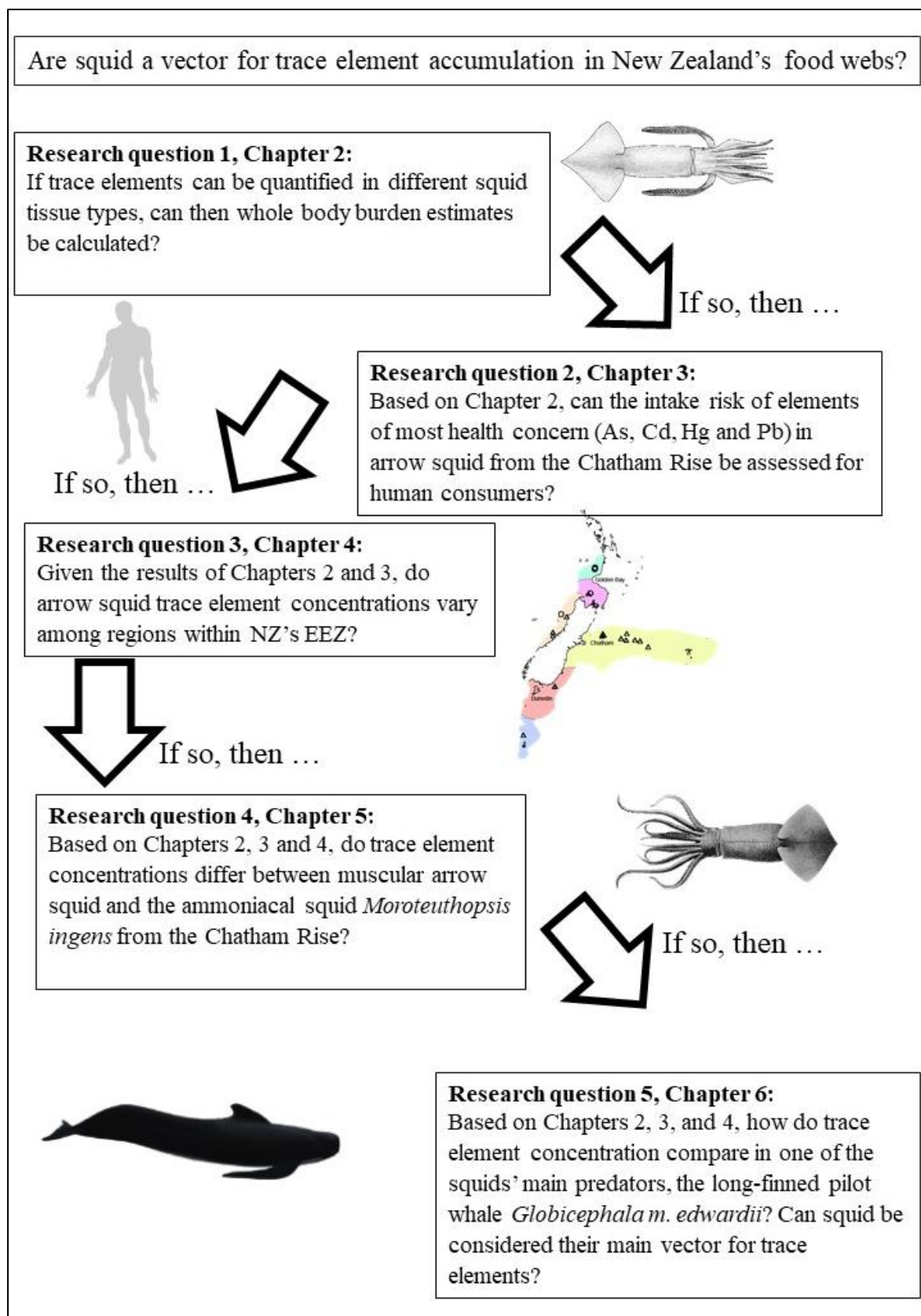


Fig. 2. Conceptual diagram of the main research question addressed in each chapter and the relationship (flow) among the chapters.

## **Chapter 2: Tissue distribution of trace elements in arrow squid (*Nototodarus sloanii* [Gray, 1849]) from the Chatham Rise, New Zealand**

*This chapter has been published as part of:*

Lischka, A., Pook, C. J., Bolstad, K. S. R, Pannell, J L. & Braid, H.E (2019). Metal composition of arrow squid (*Nototodarus sloanii* [Gray 1849]) from the Chatham Rise, New Zealand: implications for human consumption. *Environmental Science and Pollution Research* 26 (12): 11975-11987. <https://doi-org.ezproxy.aut.ac.nz/10.1007/s11356-019-04510-w>  
*The human risk assessment has been integrated into Chapter 3.*

### **Abstract**

Cephalopods are important in the diets of humans and many other apex predators, and can play a central role in the bioaccumulation of trace elements. In this study, trace element concentrations were analysed in the commercially and ecologically important southern arrow squid, *Nototodarus sloanii* (Gray, 1849), from a heavily targeted fisheries area on New Zealand's Chatham Rise. A variety of tissue types were compared to assess the bioaccumulation in edible tissues (mantle and arms) and other organs (digestive gland, kidney, and hearts). Although trace element concentrations varied among tissue types, the highest concentrations were found in the digestive gland (for Cd, Cu, Fe, Ni, Zn) and branchial hearts (for Cr, U). This chapter reports the first Cd concentrations for *N. sloanii* from the Chatham Rise, with mean values of 3.11  $\mu\text{g g}^{-1}$  in the mantle and 102.53  $\mu\text{g g}^{-1}$  in the digestive gland. These data suggest that mantle tissue, as the largest part of the squid, and digestive gland, as the primary organ for trace element accumulation, can be used to estimate the animal's total body burden for all trace elements analysed. The toxicological and dietary consequences for predators (including humans) feeding on arrow squid can be inferred from measurements of mass and trace element concentration in these tissues. These are the first recorded baseline data for trace element concentrations for any squid in this oceanic region.

### **Introduction**

Both essential and non-essential trace elements have been shown to accumulate through marine food webs (Rainbow, 2007), with Cd and Hg reaching high concentrations in high-level predators (Storelli, 2008). Several studies have shown that cephalopods can also accumulate considerable concentrations of both organic and inorganic contaminants in their tissues (e.g. Costa & Rodrigo, 2017, Gerpe et al., 2000). A recent review of trace element concentrations in cephalopods highlighted their high trace element bioaccumulation capacities, which was especially clear in the family Ommastrephidae Steenstrup, 1857 (Penicaud et al. 2017). Many ommastrephids are commercially fished, with total catch rates increasing worldwide (e.g., Arkhipkin et al., 2015). In accordance, there is a growing interest in analysing trace element concentrations in commercially targeted squid species (e.g. Rjeibi et al., 2014,

2015; Storelli et al., 2005, 2010). However, for some oceanic areas, information regarding trace element concentrations in cephalopods remains scarce.

In New Zealand (NZ) waters, arrow squids (*Nototodarus gouldi* [McCoy, 1888] and *N. sloanii* [Gray, 1849]) form the largest commercial cephalopod fishery (McGregor & Large, 2015). Trace element accumulation in these species is important because of their abundance and pivotal role in the marine food web both as predator and prey (Pethybridge et al., 2012). *Nototodarus gouldi* is commonly found north of the STF with greatest abundance in shelf waters at 50–200 m, and is not usually found on the Chatham Rise (Smith et al., 1981; Uozumi, 1998). In comparison, *N. sloanii* is distributed throughout the Chatham Rise and extends south of the STF into sub-Antarctic waters, and west of the South Island of New Zealand as well as to the east and west of the South Island of New Zealand (Jackson et al., 2000). This species occurs in waters shallower than 600 m, with the greatest abundance in water shallower than 300 m (Chen, 1998; Jackson et al., 2000).

Arrow squids are important in the diets of many apex predators, representing the main prey item for long-finned pilot whales, *Globicephala melas* (Beatson & O'Shea, 2009), Buller's mollymawk, *Diomedea bulleri* (James & Stahl, 2000; West & Imber, 1986), the Australian fur seal, *Arctocephalus pusillus doriferus* (Emami-Khoyi et al., 2016) and the endangered and endemic NZ sea lion, *Phocarcos hookeri* (Childerhouse et al., 2001). Arrow squids have also been recorded from the stomach contents of orange roughy, *Hoplostethus atlanticus* (Jones, 2007). Arrow squids are generalist predators, mainly feeding on cephalopods, crustaceans, and fish, with pearlsheds (*Maurolicus* sp.) and lantern fish (*Lampanyctodes hectoris*) frequently reported from their stomach contents (Dunn, 2009; Pethybridge et al., 2012). Crustaceans (Isopoda, Amphipoda) tend to represent a frequent, but less important, food component in the diet of mature arrow squids (Pethybridge et al., 2012). Due to their ubiquitous position in pelagic food webs, they could represent a key biological vector in the transfer of toxins through marine food webs, which has been suggested for other oceanic squid species (Bustamante et al., 1998a).

Within the NZ Exclusive Economic Zone (EEZ), arrow squids are abundant on the Chatham Rise (Uozumi, 1998). The Chatham Rise is an elongated submarine platform that extends ~800 km from the central east coast of NZ's South Island to the Chatham Islands, with water depths of up to 2000 m (Carter, 1980). In this area, subtropical water encounters sub-Antarctic water, resulting in a subtropical front zone situated over the Chatham Rise (Sutton, 2001). In this zone, the cooler, nutrient-rich sub-Antarctic surface water mixes with the warmer, less dense, subtropical water (Chiswell et al., 2015), forming a highly productive area (Bradford-Grieve, 1983). The region has supported several major commercial fisheries since 1978 (Clark & O'Driscoll, 2003; Probert et al., 1997), with a range of target species including hoki (*Macruronus novaezelandiae*), orange roughy (*Hoplostethus atlanticus*), ling (*Genypterus blacodes*), and the southern arrow squid, *N. sloanii* (Murphy et al., 2001). The arrow squid

fishery is one of the largest commercial fisheries in NZ, with a Total Allowable Commercial Catch of 82,120 tonnes for the year 2018 (MPI, 2019).

Due to the important ecological and economic role of *N. sloanii* within the NZ EEZ, the aim of the present study was to analyse trace element concentrations and tissue distribution in this species. This study provides a first insight into trace element concentrations in *N. sloanii*, an important component of the Chatham Rise ecosystem.

## Material and Methods

### *Study area and sampling*

Specimens of *N. sloanii* were collected in January 2012, 2014, and 2016 during trawl surveys on the Research Vessel *Tangaroa*, with depths ranging from 200–800 m. Arrow squid were collected between  $-43^{\circ} 03'$  to  $-44^{\circ} 34'$  S, and  $-174^{\circ} 77'$  to  $-179^{\circ} 42'$  E, from 23 stations (Fig. 3). Specimens were caught by bottom trawling. Upon capture, sex, size (dorsal mantle length), and weight were measured on board and specimens were frozen at  $-20^{\circ}\text{C}$ . In total, 180 specimens were included in the trace element analysis. The dorsal mantle length ranged from 130 to 373 mm. For all 180 specimens, mantle tissue was analysed because it represents the most commonly consumed part of the squid, which can be used to infer health implications for humans. In order to analyse the distribution of the trace elements among tissues, samples were taken from the digestive gland ( $n=77$ ), first arm pair ( $n=51$ ), gonads (including ovaries [ $n_{\text{♀}} = 16$ ] and testes [ $n_{\text{♂}}=19$ ]), left tentacle ( $n=52$ ), kidney ( $n=33$ ), branchial ( $n=44$ ) and systemic hearts ( $n=47$ ), and fins ( $n=37$ ). In total, 257 tissue samples from female specimens and 299 tissue samples from male specimens were analysed, for a total of 556 observations.

### *Trace element analysis and procedures*

Essential trace elements—Copper (Cu), Chromium (Cr), Iron (Fe), Nickel (Ni), and Zinc (Zn)—and non-essential trace elements—Cadmium (Cd) and Uranium (U)—were analysed in all tissues with Microwave Plasma-Atomic Emission Spectrometry (MP-AES 4200 Agilent Technologies, USA). Prior to analysis, each sample was freeze-dried for 48 hours (Alpha 2-4 LDplus, Martin Christ Germany), ground into powder, and homogenised.

Samples (~100–300 mg dry weight [dw]) were digested in a 3:1 mixture of 70%  $\text{HNO}_3$  (Merck, suprapur quality) and 37%  $\text{HCl}$  (Merck, suprapur quality). Acidic digestion took place in a microwave digestion system (Multiwave GO, Anton Paar GmbH, Austria) at  $105^{\circ}\text{C}$  for 50 minutes. Samples were diluted to a volume of 30 or 50 ml, depending on the sample weight, with Milli-Q water.

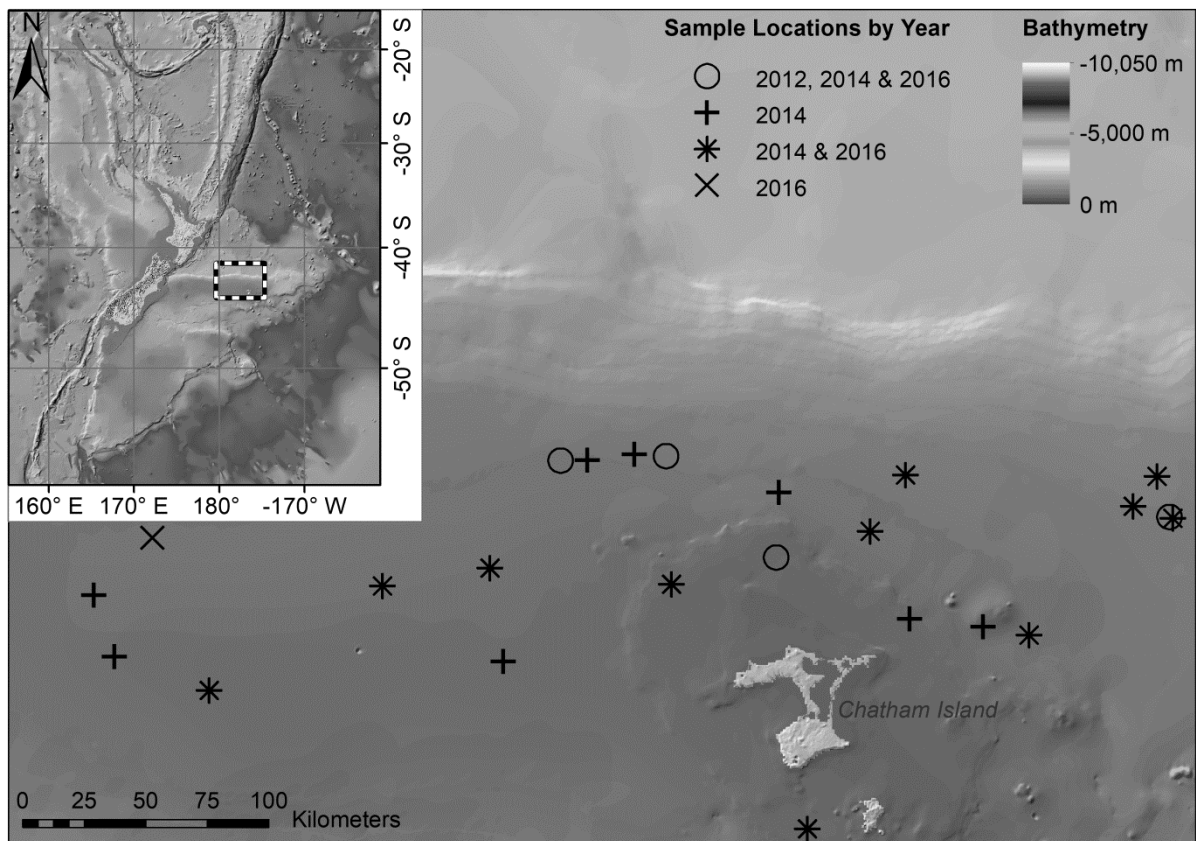


Fig. 3. Sampling stations of *N. sloanii* on the Chatham Rise, NZ. Symbols indicate sampling stations and correspond to sampling year. Basemap: 'New Zealand Region Bathymetry', 1:4,000,000 scale at 46°S on Mercator projection, sourced from NIWA (National Institute of Water and Atmospheric Research, Wellington, New Zealand).

Lobster hepatopancreas certified reference material (CRM; TORT-3, National Research Council, Canada), together with the procedural blanks, were treated and analysed in the same way as the other samples during each digestion and analysis. Recoveries of the elements ranged from 71 to 118% ( $n=7$ , based on 300 mg CRM, Table 4). Results for the trace element concentrations are expressed in  $\mu\text{g g}^{-1}$  dw. The Limit of Detection of the MP-AES was calculated as  $3 \times$  the standard deviation of the reagent blanks. The detection limits were calculated for Cd ( $0.10 \mu\text{g g}^{-1}$ ), Cr ( $0.08 \mu\text{g g}^{-1}$ ), Cu ( $0.06 \mu\text{g g}^{-1}$ ), Fe ( $0.16 \mu\text{g g}^{-1}$ ), Ni ( $0.04 \mu\text{g g}^{-1}$ ), U ( $0.0009 \mu\text{g g}^{-1}$ ), and Zn ( $0.09 \mu\text{g g}^{-1}$ ). A subset of the samples also analysed by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700, x-lens model) for method validation, and no significant difference between that and the MP-AES data was found (paired t-test:  $df=34$ ,  $p=0.5563$ ,  $t=-0.57916$ , data not shown).

#### Statistical analysis

A principal component analysis (PCA) was used to examine differences in overall trace element concentrations between tissues. The dataset consisted of all seven trace element concentrations from 257 tissue samples (mantle and digestive gland). Ordination biplots were produced, showing the differences in trace element concentrations between digestive gland and mantle tissues (R version 3.1.0,

R Core Team 2017, R package ‘ggbiplot’, Vu, 2011). These two tissues were chosen because the digestive gland is the main storage organ for many trace elements and the mantle is important for human consumption. Correlations were checked among the different trace element concentrations and the size of the specimens. Correlations were visualised in a correlogram using the R package ‘corrgram’ (R version 3.1.0, R Core Team, 2017; R package ‘corrgram’, Wright, 2012).

Table 4. Element concentrations ( $\mu\text{g g}^{-1}$ ) and recovery (%) of the certified reference material (TORT-3) analysed in this chapter, based on 300 mg ( $n=14$ ).

Element	Certified value (TORT-3) $\mu\text{g g}^{-1}$	Measured value $\mu\text{g g}^{-1}$	Recovery (%)
Cd	42.3 $\pm$ 1.8	39.5 $\pm$ 2.8	93
Cr	1.95 $\pm$ 0.24	1.8 $\pm$ 0.3	94
Cu	497.0 $\pm$ 22.0	451.7 $\pm$ 26.4	91
Fe	179.0 $\pm$ 8.0	167.9 $\pm$ 7.3	94
Ni	5.3 $\pm$ 0.24	5.6 $\pm$ 0.3	106
Zn	136.0 $\pm$ 6.0	132.1 $\pm$ 8.3	97

Generalised linear mixed-effect models in R were used to determine whether the concentrations of each of the seven elements were predicted by tissue type, dorsal mantle length, sex, or catch year. Prior to analysis, missing concentration data (assumed to be below the detection limit of the MP-AES) was set to the lowest measured value of the corresponding element multiplied by 0.5 and trace element concentrations were z-transformed (Graf, 2004). Variance homogeneity and normality of model errors were checked by diagnostic plots (residuals vs. fitted values, quantile-quantile plots). Using the package lme4 (Bates et al., 2015), one model per element was fitted, with each predictor variable as a fixed effect, and a nested random term of individual squid within sampling station to account for pseudo-replication. Since females were generally larger than males, a size and sex interaction term was also included in the models (sex $\times$ size).

A hypothesis-testing approach was taken in model fitting in order to determine whether size, sex, or year explained the variance in trace element concentrations once tissue type was accounted for, and to determine the relative contributions of each variable. For each element, a tissue-only model and a full model containing all variables were compared to an intercept-only model. Akaike’s information criteria (AIC) were used for model evaluation and comparison, using analysis of covariance (ANCOVA) to calculate significant reduction in AIC between models (Burnham & Anderson, 2004; Zuur et al., 2009) and conditional and marginal pseudo  $r^2$  to infer the explanatory power of the models (Nakagawa & Schielzeth, 2013).

## Results

### *Distribution of trace elements across tissues*

Trace element concentrations ranged from 0.001 to 4843.17  $\mu\text{g g}^{-1}$  dw across all nine tissue types (digestive gland, arms, gonads, tentacles, kidneys, and hearts, Table 5). Trace element concentrations were highly variable and showed clear differences among tissues (Fig. 4). Among the tissues analysed, the highest trace element concentrations were measured in the digestive gland, the kidneys, and the hearts. The digestive gland contained the highest observed concentrations of Cd, Cu, and Ni (including the maximum value observed for any trace element in the present study: Cu 4843.17  $\mu\text{g g}^{-1}$ ), while the lowest concentrations of the same trace elements (and of uranium) were measured in the arms and gonads. The branchial hearts showed the highest Cr and U concentrations amongst all tissues, followed by the kidney and the systemic heart (Fig. 4, Table 5). Overall, Cu was the most abundant trace element measured. The concentration of Zn was mostly consistent across tissue types, with the highest levels found in the digestive gland. In most of the tissues analysed, relative average concentrations were: Cu>Fe>Zn>Cd>Ni>Cr>U (Table 5).

### *Exploratory analysis*

Exploratory analyses showed that Cd was positively correlated with Cu ( $r=0.7$ ,  $p<0.001$ ), Fe ( $r=0.51$ ,  $p<0.001$ ) and Zn ( $r=0.49$ ,  $p<0.001$ ), while U concentrations showed a positive correlation with Cr ( $r=0.5$ ,  $p<0.001$ , Fig. 5). Ordination results showed clear differences in overall trace element concentrations between the mantle and digestive gland tissue (Fig. 6). Principle Component (PC) 1 was largely driven by the concentrations of Cd, Cu, Fe, U and Zn, whereas PC2 was driven mostly by Cr and Ni. Nickel data showed greater variation in the PCA than the other elements. Differences between the two tissues were mainly influenced by the elements Cd, Cu, Fe, U and Zn, for which concentrations were orders of magnitudes higher in the digestive gland compared to the mantle tissue. The first axis of the PCA explained 45.8% of the variance, the second axis 17.8 % (Fig. 6).

### *Generalised Linear Mixed-Effects Models*

Tissue was a highly significant predictor of element concentration in all models (Table 6), with the highest concentrations usually found in the digestive gland. Sex was a significant factor for Cr and Cu, with males exhibiting slightly higher Cr concentrations than females and higher Cu concentrations measured in females. Size was only a significant factor in the Cd model, where smaller individuals showed higher Cd concentrations (Table 6). However, no significant interaction effects between sex and size were observed for any element. For all trace elements except U and Zn, the sampling year was highly significant (Table 6).

The highest mean mantle concentrations varied by year, with Cd exhibiting highest mean concentrations in 2016, while the mean concentrations for Fe and Ni were highest in 2014 (Appendix Fig. 1). Especially for Cd, mean concentration in the mantle tissue almost doubled from 2012 (1.66  $\mu\text{g g}^{-1}$  dw) and 2014 (2.93  $\mu\text{g g}^{-1}$  dw) to 2016 (4.72  $\mu\text{g g}^{-1}$  dw).

For all models, the full model explained equal or more variance than the tissue-only or intercept-only models according to marginal and conditional  $r^2$  (Table 7). The Cd, Cu and Fe models had relatively high marginal  $r^2$  ( $> 0.5$ ) and exhibited little difference between marginal and conditional  $r^2$ . This indicates that the predictor variables explain the majority of the variation, with little variation associated with the random effects (individual squids or sampling stations). The other models (Cr, Ni, U) showed much lower marginal  $r^2$  indicating that the fixed effects explain much less of the variation. However, for these models, the conditional  $r^2$  is also quite low ( $<0.5$ ), indicating that this was not attributable to the random effects either.

Table 5. Summary of the seven trace element concentrations (minimum, mean, and maximum) for the nine different tissue types analysed in *N. sloanii* from the Chatham Rise. Tissue abbreviations are as follows: arm (A), branchial heart (BH), digestive gland (DG), fin (F), gonad (G, including ovaries and testes), kidney (K), mantle (M), systemic heart (SH), tentacle (T).

Element	A		BH		DG		F		G		K		M		SH		T	
	mean± sd	min- max	mean± sd	min- max	mean± sd	min- max	mean± sd	min- max	mean± sd	min- max	mean± sd	min- max	mean± sd	min- max	mean± sd	min- max	mean± sd	min- max
Cd	1.11± 0.89	0.24- 3.64	10.7± 11.09	0.36- 65.8	103± 86	8.48- 444	1.09± 0.85	0.22- 3.78	2.08± 2.4	0.36- 10.62	46.66± 41.35	7.51- 197	3.11± 3.53	0.14- 18.32	17.25± 19.89	0.64- 105	1.3± 1.25	0.23- 5.99
Cr	0.38± 0.41	0.08- 1.97	1.45± 0.9	0.35- 3.36	0.32± 0.29	0.08- 1.21	0.39± 0.23	0.09- 0.84	0.34± 0.22	0.13- 0.74	1.04± 1.33	0.09- 3.98	0.47± 0.4	0.09- 1.64	1.25± 1.31	0.12- 4.61	0.35± 0.19	0.08- 0.85
Cu	15.8± 7.85	2.59- 32.5	245± 176	42.44- 1225	592± 764	9.97- 4843	21.58± 8.02	7.59- 53.42	27.61± 20.02	7.06- 88.13	351± 386	41.53- 1673	29.17± 22.92	5.89- 176	206± 105	36.35- 470	21.13± 9.57	6.36- 53.77
Fe	25.53± 25.51	0.67- 129	169± 140	17.67- 844	328± 138	106.8- 707	14.98± 9.9	3.41- 39.94	20.95± 10.38	6.12- 48.21	74.11± 45.13	7.1- 215	20.89± 25.83	1.27- 231	64.42± 55.5	4.98- 375	42.54± 49.22	0.29- 229
Ni	0.48± 0.3	0.10- 1.27	1.25± 1.21	0.04- 4.69	1.46± 1.16	0.1- 5.27	0.88± 0.76	0.08- 2.06	0.95± 0.89	0.04- 2.41	0.62± 0.57	0.04- 1.85	1.005± 0.69	0.04- 3.77	0.79± 0.6	0.05- 1.95	0.78± 0.69	0.05- 2.78
U	0.02± 0.01	0.01- 0.04	0.05± 0.05	0.001- 0.21	0.03± 0.02	0.001- 0.08	0.02± 0.01	0.001- 0.06	0.02± 0.01	0.001- 0.06	0.02± 0.02	0.01- 0.1	0.02± 0.02	0.001- 0.13	0.03± 0.03	0.001- 0.15	0.02± 0.01	0.001- 0.06
Zn	72.24± 13.45	34.9- 123	95± 93	5.22- 563	111± 80	20.17- 489	55.28± 10.54	33.12- 75.73	79.69± 37.08	21.02- 155	90.16± 47.48	43.93- 254	61.09± 12.46	29.72- 110	90.67± 27.84	11.74- 189	65.9± 15.83	31.08- 134

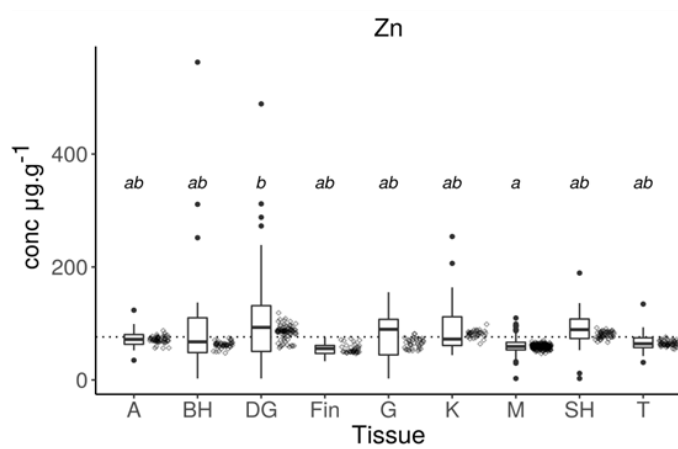
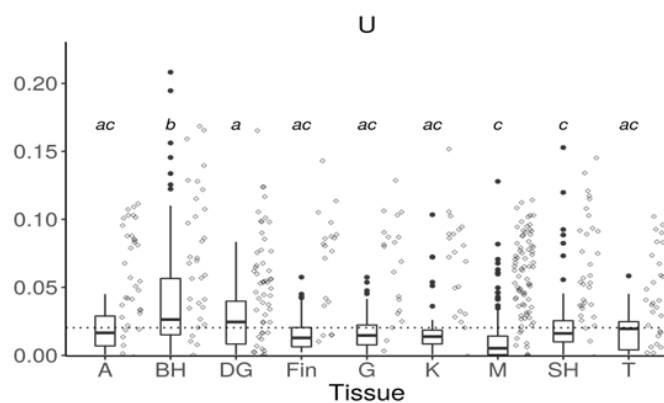
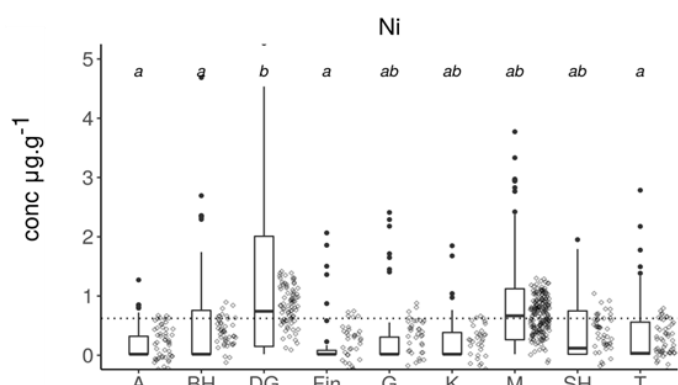
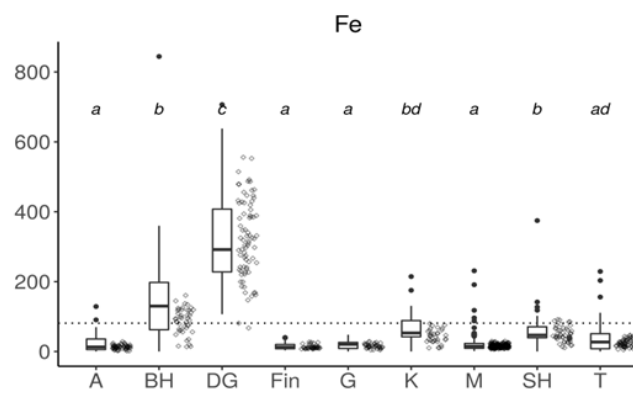
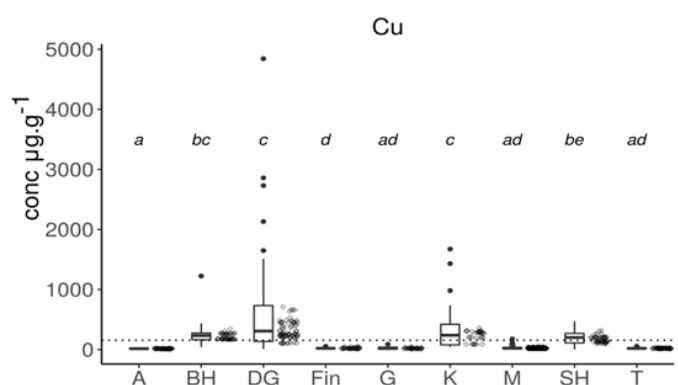
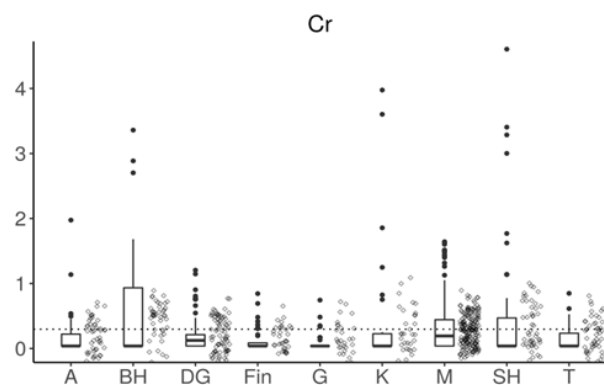
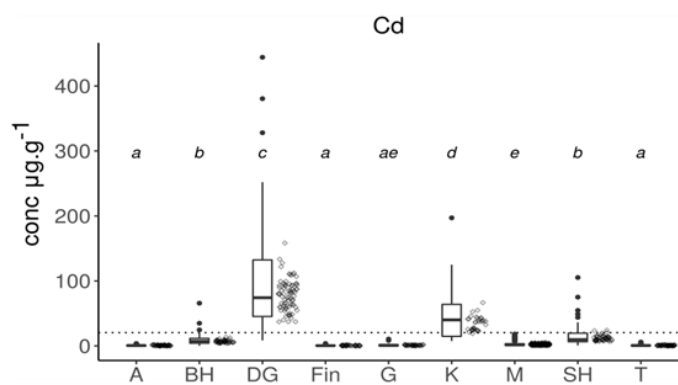


Fig. 4. Boxplots of element concentrations of *N. sloanii* from the Chatham Rise in the different tissues. Dark dots indicate outliers of the raw data and hollow dots the predicted values from the models. Mean element concentrations are represented by dashed lines. Significant differences between tissues (according to pairwise comparison) are indicated with letters in italics, tissues that are not significantly different are grouped together, with the same letter, different letters equal  $p < 0.001$  \*\*\*. Tissue abbreviations as following: arm (A), branchial heart (BH), digestive gland (DG), fin (F), gonad (G, ovaries and testes), kidney (K), mantle (M), systemic heart (SH), and tentacle (T). Outliers ( $> 2$  sd from the mean) are displayed as dots. Element abbreviation as following: Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Nickel (Ni), Uranium (U) and Zinc (Zn).

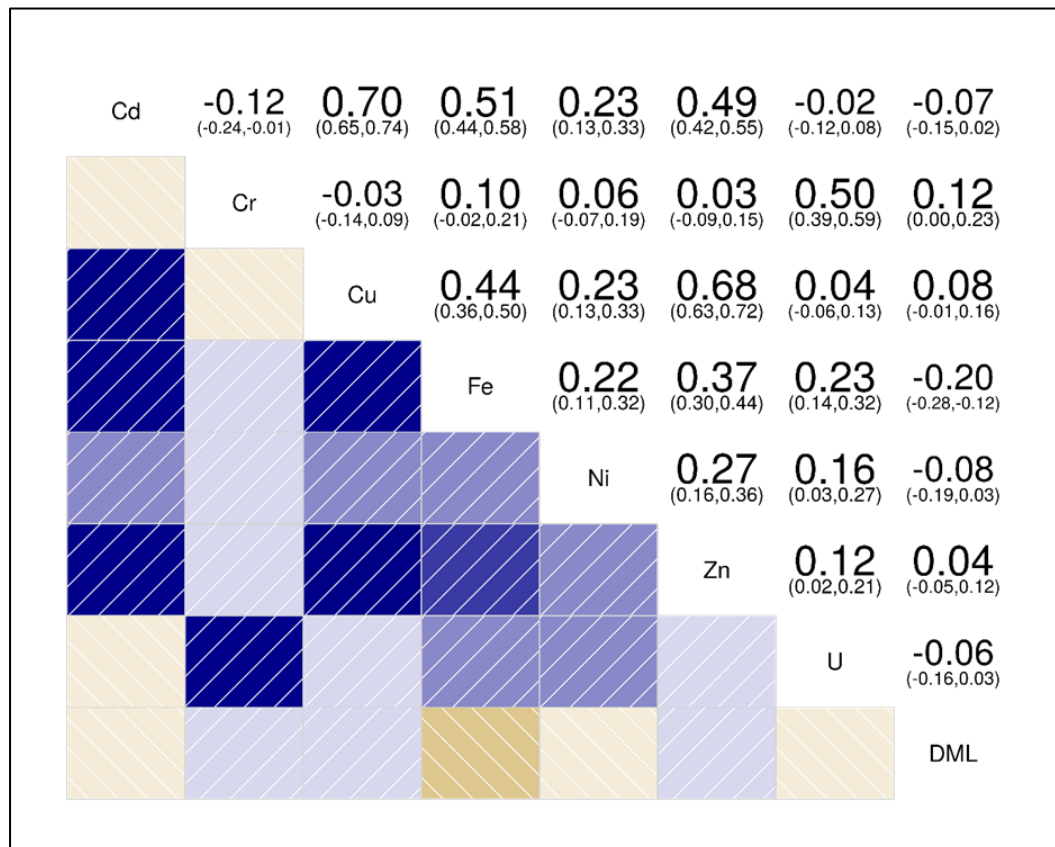


Fig. 5. Correlation matrix of the analysed trace elements in *N. sloanii*, Chatham Rise, including dorsal mantle length (DML in mm). Trace element concentrations are in  $\mu\text{g g}^{-1}$  dw. Confidence intervals (95%) are indicated in brackets. Positive correlations are indicated in blue, negative correlations in red colour. Darker colours symbolise a stronger correlation.

Table 6. Variables included in the full generalised linear mixed-effect models for *N. sloanii* on New Zealand's Chatham Rise. Models are fitted for each element, and their associated F-values according to analysis of covariance performed on the model's significance ( $p < 0.001$ ) is indicated with asterisks \*\*\*.

Factor	Cd	Cr	Cu	Fe	Ni	U	Zn
Tissue	226.16 ***	4.62 ***	102.37 ***	71.10 ***	9.24 ***	10.37 ***	3.83 ***
Sex	0.04	18.46 ***	2.42 ***	0.42	2.43	2.91	1.49
Size (DML)	28.18 ***	0.29	0.83	6.58	6.23	3.24	0.91
Year	29.03 ***	23.15 ***	12.60 ***	11.96 ***	12.24 ***	6.76	0.06
Sex:Size	0.24	0.00003	0.21	0.06	1.26	0.27	2.68

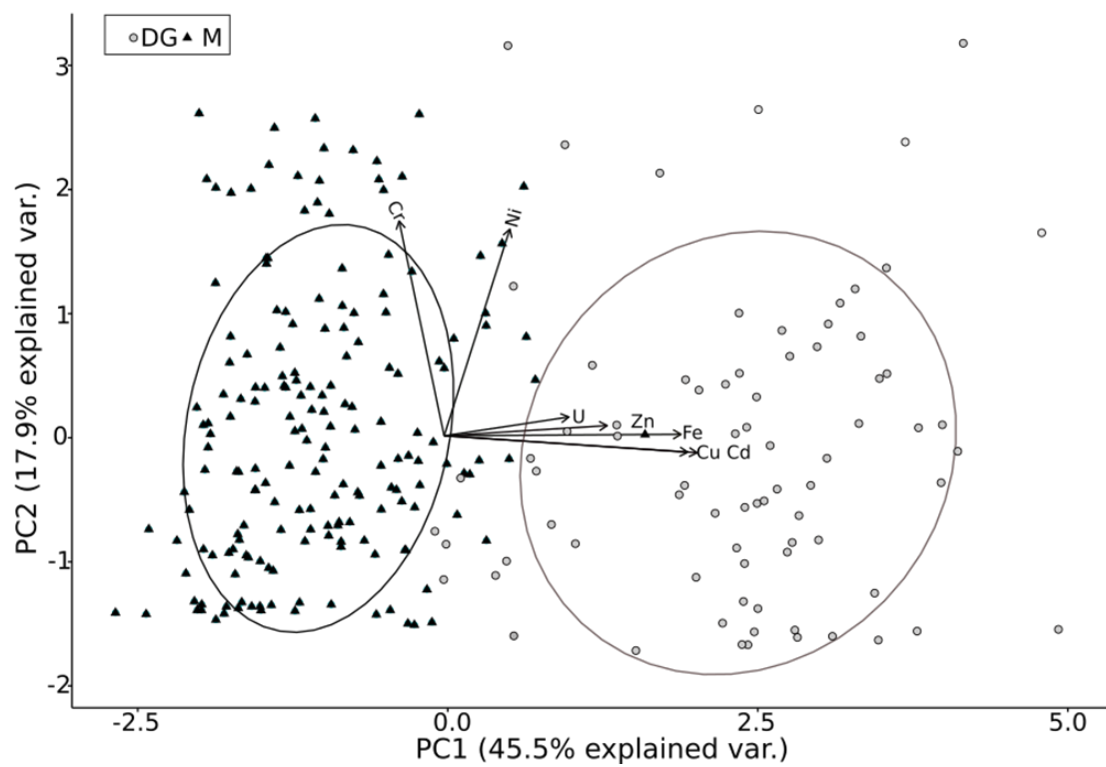


Fig. 6. Principal component analysis (PCA) biplot (R package 'ggbiplot', Vu, 2011) showing element concentrations in mantle (M) and digestive gland (DG) tissues for *N. sloanii* on New Zealand's Chatham Rise. Arrows indicate element loadings along PC1 and PC2 axes. Ellipses indicate 95% confidence interval.

Table 7. Comparison of generalised linear mixed-effect models of trace element concentrations for *N. sloanii* from New Zealand's Chatham Rise. Models include intercept-only, tissue-only and full models. Degrees of freedom (df), Akaike's information criteria (AIC), conditional and marginal pseudo  $r^2$  ( $Pr^2$ ) given. Significance of model improvement (based on AIC) relative to intercept-only model, according analysis of variance (ANOVA), indicated by asterisk (\*), where \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .

Element	Model	df	AIC	$Pr^2$ marginal	$Pr^2$ conditional
Cd	Intercept only	4	672.99	0.00	0.33
Cd	Tissue	12	-46.22 ***	0.76	0.83
Cd	Full	17	-115.62 ***	0.80	0.82
Cr	Intercept only	4	764.36	0.00	0.16
Cr	Tissue	12	747.99 ***	0.08	0.23
Cr	Full	17	698.02 ***	0.17	0.27
Cu	Intercept only	4	653.06	0.00	0.40
Cu	Tissue	12	185.04 ***	0.63	0.71
Cu	Full	17	166.40 ***	0.65	0.72
Fe	Intercept only	4	697.7	0.00	0.18
Fe	Tissue	12	379.91 ***	0.51	0.68
Fe	Full	17	359.52 ***	0.54	0.67
Ni	Intercept only	4	779.37	0.00	0.14
Ni	Tissue	12	730.76 ***	0.13	0.20
Ni	Full	17	706.04 ***	0.18	0.24
U	Intercept only	4	657.28	0.00	0.23
U	Tissue	12	602.1 ***	0.17	0.41
U	Full	17	594.04 **	0.19	0.41
Zn	Intercept only	4	801.57	0.00	0.10
Zn	Tissue	12	787.21 ***	0.07	0.18
Zn	Full	17	791.56	0.08	0.19

## Discussion

A great variation in the trace element concentrations of *N. sloanii* from the Chatham Rise was measured. Depending on the trace element, some of that variation can be explained by sex, size, and sampling year. Trace element concentrations varied substantially among tissue types, with the highest concentrations in the digestive gland and the hearts. Lowest concentrations were measured in the gonads, arms, and tentacle tissue. Trace elements such as Cd and Cu were concentrated in the digestive gland, whereas Cr and U were mainly accumulated in the branchial hearts.

### *Trace element concentrations across tissues*

Trace element concentrations in different tissues varied greatly in arrow squid, which is consistent with previous studies on pelagic squids (Gerpe et al., 2000; Rjeibi et al., 2015). After the digestive gland, trace element concentrations were highest in the kidneys, followed by the branchial hearts (especially for U), and systemic hearts. The branchial hearts have been suggested to be involved in trace element excretion and storage processes (Bustamante et al., 2002a), which could explain the relatively high trace element concentrations. The branchial heart tissue contains rhogocytes, which are cells that participate in haemolymph detoxification as they detoxify and eliminate substances such as damaged haemocyanin (Beuerlein et al., 2002). Rhogocytes contain an abundance of a pigment called adenochrome, which has a notable affinity for heavy elements, such as U and Pb, causing them to accumulate excessively in the branchial heart tissue (Miramand & Guary, 1981). Rhogocytes are also believed to participate in haemocyanin metabolism and could be the reason for the elevated Cu concentrations, but processes are not yet fully understood (Penicaud et al., 2017).

Relatively low trace element concentrations were found in the arms, fin, gonad, and tentacle tissue, which are likely due to a detoxification process that led to a sequestration of trace elements in the digestive gland and other organs involved in these processes, such as the branchial hearts or kidneys (Penicaud et al., 2017). Trace element concentrations are generally higher in the digestive gland compared to mantle tissue (Penicaud et al., 2017), and the digestive gland in the samples from the present chapter contained the highest mean concentrations for most of the analysed trace elements (Cd, Cu, Fe, Ni, and Zn; Fig. 4; Table 5). Finger & Smith (1987) reported mean Cd concentrations in the digestive gland of *N. gouldi* from Port Philip Bay in Australia, of  $33 \mu\text{g g}^{-1} \text{ dw}$  (converted from ww), which is one third of the concentrations found in the present study. The digestive gland only contributes ~7% to the body weight of an arrow squid, but stores ~90% of the whole Cd content (Appendix Table 1). This finding is consistent with other studies that report that Cd is mainly sequestered in the digestive gland of other ommastrephid species (Bustamante et al., 1998a, 2002a). The Cd concentrations of the other tissues are negligible due to their small weights and contributions to the total Cd body burden. Therefore, I suggest that digestive gland tissue (as the organ of main storage) and mantle tissue (as it is the tissue that is potentially consumed by humans) could be analysed to infer whole body burden and trophic transfer. Furthermore, arrow squid around NZ's EEZ should be analysed in order to make assumptions about trace elemental fluxes and the possible impact on predators and human consumers.

### *Correlations*

Strong correlations were found in the present study between Cd and the essential elements Cu and Zn. Similar correlations have also been described in *N. gouldi* from waters off the Bass Strait, Australia by Smith et al. (1984), who described that because these three elements are involved in biochemical processes essential in haemocyanin synthesis, their presence is correlated. Their co-occurrence might

be due to detoxification processes for immobilisation of excess metal ions (Smith et al., 1984). In addition, Cu and Zn are cofactors in digestive gland enzyme systems and therefore co-occur (Bustamante et al., 2002a). In the environment, Cu, Cd, and Zn usually occur as divalent cations (2+) and show high affinity for sulfhydryl groups of enzymes and metalloproteins (Viarengo & Nott, 1993). Through binding to metalloproteins, excessive metals that have penetrated a cell can be bound so detoxification processes can take place (Jebali et al., 2008).

#### *Size effect and sex*

The negative correlation observed between specimen size and Cd concentrations in the mantle tissue could have been caused by multiple factors. An ontogenetic diet shift, as observed by Dunn et al. (2009), could lead to an increased uptake of prey with lower Cd concentrations in larger specimens. Furthermore, the presence of detoxification mechanisms, supposedly activated by a threshold concentration, can lead to decreased concentrations in mature individuals (Bustamante et al., 2002b; Gerpe et al., 2000; Miramand et al., 2006). Additionally, the allometric changes (increase in overall size), could explain the negative correlation of Cd concentrations with size, which means that metal intake is lower in respect to growth rate (Lischka et al., 2018; Uozumi & Ohara, 1993; Fig. 5).

Concentrations of Cr and Cu varied by sex, a trend that was particularly apparent in Cu in the gonads (Table 6, Fig. 7). Sex differences have been previously reported for Cu in cephalopods (Gerpe et al., 2000, Miramand & Bentley, 1992), and could be because female reproductive organs go through a prolonged modification phase and have a higher mass (Gerpe et al., 2000). Furthermore, Cu might be required for egg production, as reported for *Loligo forbesi* (Craig, 2001).

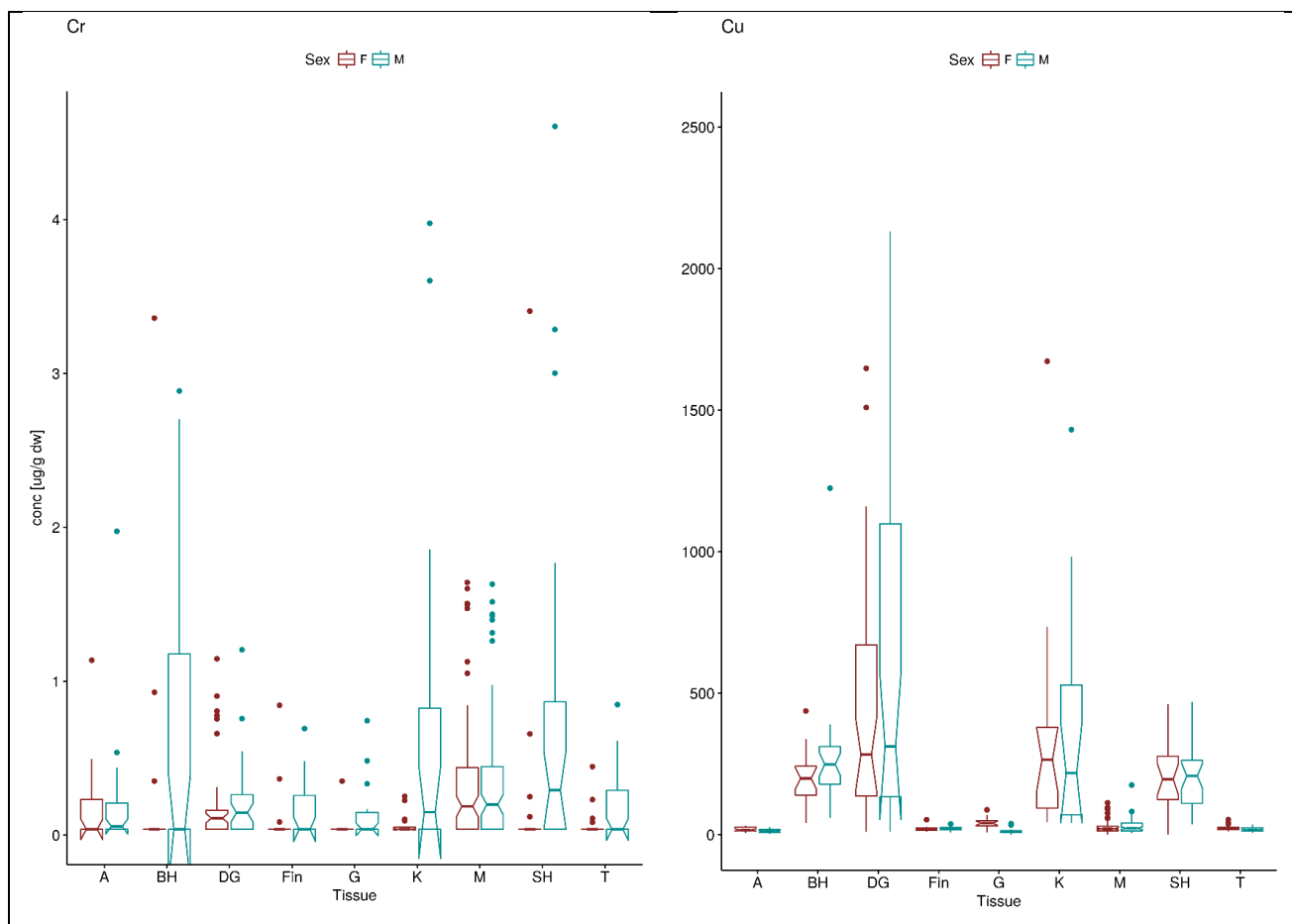


Fig. 7. Boxplots of Cr and Cu concentrations for each tissue in both sexes (females=red, males=blue) of *N. sloanii* from the Chatham Rise, NZ. Tissue abbreviations as following: arm (A), branchial heart (BH), digestive gland (DG), fin (F), gonad (G, ovaries and testes), kidney (K), mantle (M), systemic heart (SH), and tentacle (T). Outliers (>2sd from the mean) are displayed as dots.

#### *Annual differences in trace element concentrations*

Sampling year had a significant effect on almost all element concentrations, with the exception of U and Zn (Table 6; Appendix Fig. 1). This increase in concentration could be linked to the El Niño Southern Oscillation event that took place in 2014–2016 (Tenzer & Wiart, 2018). The uptake and retention of trace elements by marine organisms is highly dynamic and there are many reasons why they might vary from year to year (International Atomic Energy Agency [IAEA], 2004); nonetheless, the El Niño event is likely to be a major factor (Tenzer & Wiart, 2018). The productivity of the Chatham Rise is driven by cold, nutrient- and trace-element-rich water flowing up from the Antarctic Circumpolar Current and by inputs of terrigenous dust transported by wind from NZ's South Island and from Australia (Jaeschke et al., 2017; Ramadyan, 2017). During El Niño events, both of these mechanisms intensify, and primary productivity is likely to have increased over the course of the sampling period (Marx et al., 2009). The annual variation in the Cd concentrations can be explained by the fact that this element is reported to bioaccumulate up food chains from phytoplankton to

cephalopods (IAEA, 2004). Bioaccumulation trends for other trace elements are less clearly understood and may show different annual trends in the food web (IAEA, 2004).

It is possible that some other source of trace elements has driven the annual changes we have observed. For example, Cd of igneous origin may occur naturally and in temporally varying concentrations (Davey et al., 1998). The abundance of commercial shipping in the region, mostly fishing vessels, may contribute to dissolved Cu or Fe concentrations, but it is unlikely that annual fluctuations in activity are of a magnitude sufficient to drive the changes observed. Within NZ's EEZ, Cd concentrations of  $0.9 \mu\text{g g}^{-1}$  dw (converted from ww) were previously measured in the muscular tissue of arrow squid (*Nototodarus* sp., Vlieg et al., 1991, Fig. 8). Mean Cd concentrations measured in similar tissues in this chapter (arms, tentacles, and mantle) were 346% higher, although the results for 2012 were similar to the levels in Vlieg et al. (1991). Locality differences may explain the variation measured between the two studies. This observed difference could be important concerning human consumption because specimens from different sampling locations might exhibit lower Cd concentrations than others. Increasing sea water temperatures could influence pelagic Cd concentrations, by affecting metal uptake in invertebrates (Sokolova & Lannig, 2008). Furthermore, open water Oxygen Minimum Zones have been identified as a sink for Cd concentrations in particular (Janssen et al., 2014). Certain squid species, such as *Dosidicus gigas*, migrate through—and spend certain amounts of time in—those oxygen depleted areas, which might affect their exposure and uptake of Cd concentrations (Rosa & Seibel, 2010). Oxygen Minimum Zones have been previously reported from the Chatham Rise (Cobianchi et al., 2015). Studying the migration patterns of *N. sloanii* in relation to oxygen depleted areas would be helpful in further exploring their Cd metabolism.

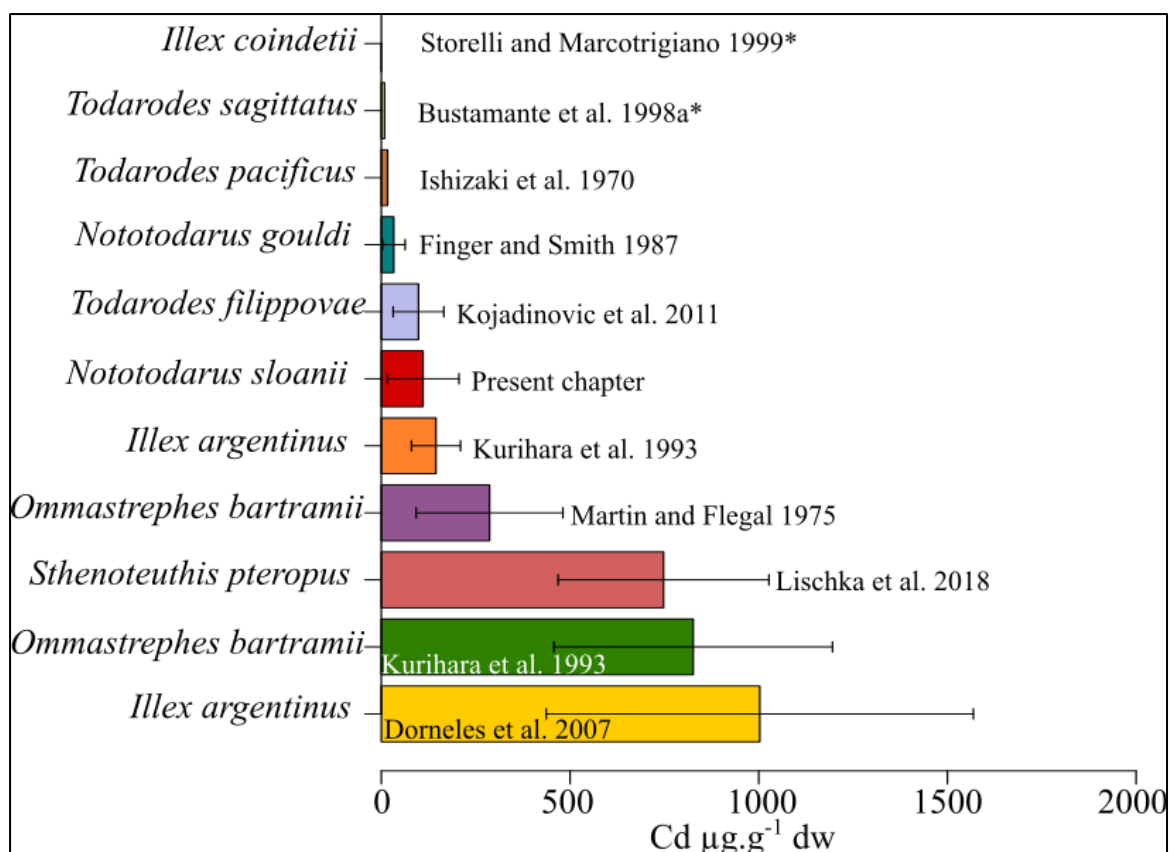


Fig. 8. Bar plot comparing reported Cd concentrations (conc) in different ommastrephid species globally. The references for the data are presented on the plots. Asterisks indicate that concentration has been converted from wet weight (ww) to dry weight (dw).

## Conclusion

This study is the first report of trace element concentrations across a wide range of tissues for the endemic NZ species *N. sloanii*. Its position in the food web of the Chatham Rise makes it an important vector for trophic transfer of trace elements to predators such as seabirds, pinnipeds, cetaceans, and humans. This study indicates that trace element concentrations are generally highest in the digestive gland and that, while the muscular mantle makes up the greatest proportion of the body's tissue, it contains relatively low concentrations of trace elements. Additional studies analysing both tissues in order to assess possible impacts for marine predators as well as human consumers are recommended. A human risk assessment will be addressed in the following Chapters 3 and 4.

### **Chapter 3: Assessment of trace elements in the tissues of arrow squid (*Nototodarus sloanii*) from the Chatham Rise: bioaccessibility and human health implications**

*This chapter is based on the following published article:*

Lischka, A., Pook, C. J., Pannell, J. L., Braid, H. E., Gaw, S., & Bolstad, K. S. R. (2020). Distribution of trace elements in the tissues of arrow squid (*Nototodarus sloanii*) from the Chatham Rise, New Zealand: Human health implications. *Fisheries Research*, 221, 105383.

<https://doi.org/10.1016/j.fishres.2019.105383>

*The in-vitro digestion study, integrated with Chapter 4, was published in Environmental Pollution.*

#### **Abstract**

The southern arrow squid, *Nototodarus sloanii* (Ommastrephidae), is a key species in New Zealand's pelagic ecosystem and is commercially fished. Specimens in this study were caught along the Chatham Rise, one of New Zealand's most heavily targeted fisheries areas. Nine different tissue types were analysed from 15 female and 15 male specimens for 13 trace elements (As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Mn, Pb, Sb, V, and Zn). Trace element concentrations varied among tissue types with the highest concentrations observed in the digestive gland, the branchial hearts, and the kidney. The mean observed concentrations of As, Cd, Hg, and Pb in the muscular tissues were less than their respective maximum permissible levels for seafood consumption for adults. However, the analysis of market arrow squid showed that Cd contamination from the digestive gland to the mantle tissue occurs and can potentially pose a threat to human consumers. To assess the bioaccessibility of these trace elements to humans, an *in-vitro* digestion simulation experiment was conducted, revealing bioaccessible concentrations overall below the maximum levels presently considered tolerable for human consumption. The *in-vitro* digestion had no observable effect upon Cd concentrations in arrow squid mantle tissue (implying that the measured concentrations are bioaccessible to humans), and Cd has the highest potential impact on marine food webs and for human health. Regular testing of the potential exposure of Cd in human diets is recommended.

## Introduction

Trace elements are a natural component in the marine environment but can occur at elevated levels due to local geochemistry or anthropogenic inputs (Langston, 2017; Tchounwou et al., 2012). Trace elements, such as arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb), can accumulate through marine food webs, posing a risk to human consumers and other apex predators (Kim et al., 2016; Wu et al., 2016). While Food Standards Australia New Zealand (FSANZ, 2016) has established maximum tolerable concentrations for As, Cd, Hg and Pb in selected seafood (mussels and fish) and the New Zealand Total Diet study (NZTDS) measured these four elements in seafood, maximum tolerable concentrations for commercially fished cephalopods in this region have not been assessed. Cephalopods are known to accumulate trace elements in their tissues, in particular Cd and Hg, and have been suggested as a potential vector for the transfer of trace elements to apex predators, including humans (Bustamante et al., 1998a; Pastorelli et al., 2012; Penicaud et al. 2017; Rjeibi et al., 2015; Rodrigo & Costa, 2017; Storelli, 2008).

In the South Pacific, arrow squids (*Nototodarus gouldi* [McCoy, 1888] and *N. sloanii* [Gray, 1849]) are important components of marine ecosystems due to their abundance and role as predators and prey. Arrow squids prey on fish (e.g., pearlsides and lantern fish), other cephalopods, and crustaceans (Dunn, 2009; Pethybridge et al., 2012) and are also a major food source for long-finned pilot whales (*G. m. edwardii*, Beatson & O'Shea, 2009), buller's mollymawk (*Diomedea bulleri*, James & Stahl, 2000), the Australian fur seal (*Arctocephalus pusillus doriferus*, Emami-Khoyi et al., 2016), and the New Zealand sea lion (*Phocarctos hookeri*, Childerhouse et al., 2001). Of the cephalopod fisheries in New Zealand, the arrow squid fishery is the largest, with a Total Allowable Commercial Catch of 82,120 tonnes for 2018 (Ministry for Primary Industries, MPI, 2019). Much of this catch is taken on the Chatham Rise, an oceanic plateau that extends about 800 km east of the South Island (Carter, 1980); *N. sloanii* is the only arrow squid species in this area (Uozumi, 1998). The Chatham Rise has New Zealand's highest level of off-shore primary production, making it one of New Zealand's most productive fishing grounds (Murphy et al., 2001).

This chapter reports baseline trace element concentrations in *N. sloanii*, including As, Cd, Hg, and Pb (the trace elements identified by the World Health Organization's as four of the ten major pollutants; WHO, 2010b). Trace element concentration in terms of benchmark doses used by the New Zealand Total Diet Study (MPI, 2018) are also evaluated. Monitoring of these trace elements is important, as arrow squid are one of New Zealand's most commercially exploited species (MPI, 2019). However, the concentrations of trace elements in food items alone do not necessarily reflect their bioavailability to humans when consumed (Laparra et al., 2003; Zia et al., 2011). This chapter also analyses the bioaccessibility of trace elements through *in-vitro* simulated digestion (Versantvoort et al., 2005), in

the interest of informing human consumers about potential toxicological hazards associated with their food intake (e.g., Metian et al., 2009; Waisberg et al., 2004).

## Material and Methods

Specimens of *N. sloanii* were caught by bottom trawling in January 2014 and 2016 during fishery surveys on the Research Vessel *Tangaroa* (National Institute for Water and Atmospheric Research, Ltd. [NIWA]), at depths of 200–800 m. Arrow squid were collected between 43° 03' and 43° 85' S, and 174° 77' and 179° 42' E, from 11 stations (Fig. 9). In total, 30 specimens (15 females and 15 males) were included in the trace element analysis. For the distribution analysis of the trace elements among different tissues, samples were taken from the first arm pair, digestive gland, fins, gonads (including ovaries and testes), kidney, mantle, left tentacle, and the branchial hearts including appendages and systemic hearts. In total, 280 tissue samples were analysed.

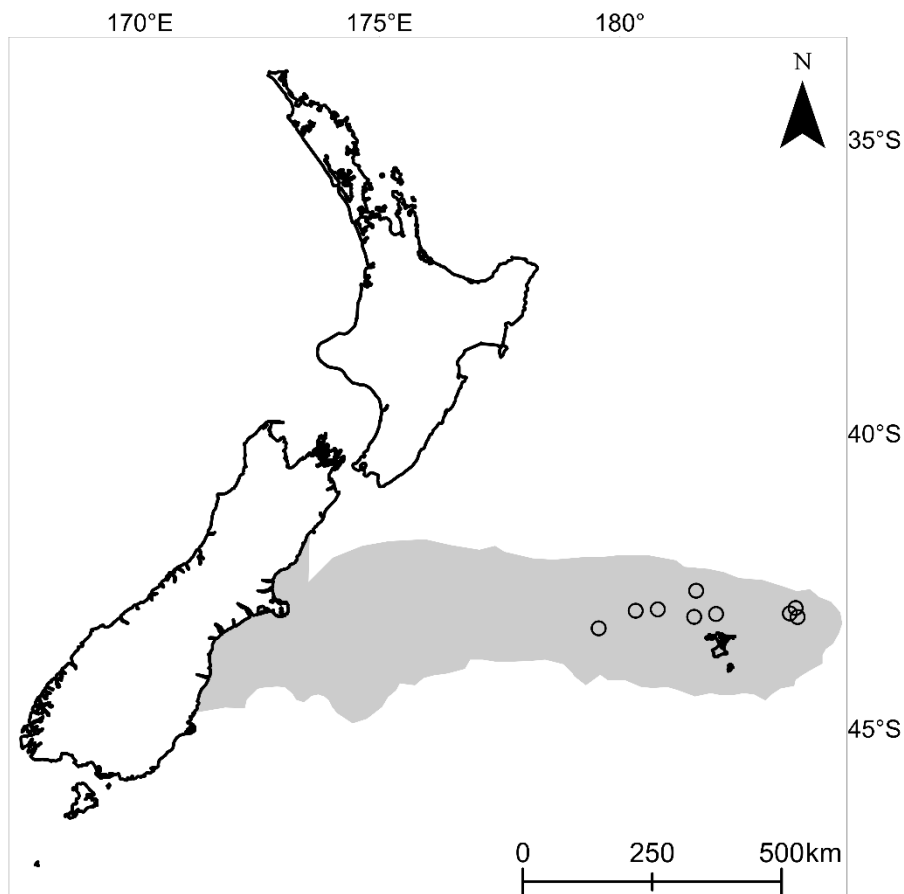


Fig. 9. Map of the Chatham Rise showing sampling stations (hollow circles) for the arrow squid (*Nototodarus sloanii*) specimens analysed in the present chapter. The grey shaded area represents the approximate extent of the Rise.

Prior to trace element analysis, freeze-dried, homogenised samples (~100–300 mg dry weight [dw]) were digested in a 3:1 mixture of 70% HNO<sub>3</sub> (Merck, suprapur quality) and 37% HCl (Merck, suprapur quality). Acidic digestion took place in a microwave digestion system (Multiwave GO, Anton Paar

GmbH, Austria) at 105°C for 50 minutes. Samples were diluted to a volume of 30 or 50 ml (depending on sample weight), with Milli-Q water. An additional threefold dilution with 2% HNO<sub>3</sub> was performed for the inductively coupled plasma mass spectrometry (ICP-MS) analysis.

Trace element concentrations (As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Mn, Pb, Sb, V, and Zn) were analysed by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS, Agilent Technologies 7500 series, CA, USA) at the University of Canterbury. Detection limits ( $\mu\text{g g}^{-1}$  dw) were calculated as 3 $\times$  standard deviation of the blank: As (0.007), Cd (0.02), Co (0.005), Cr (0.076), Cu (0.24), Fe (1.39), Hg (0.0001), Mn (0.12), Ni (0.13), Pb (0.05), Sb (0.01), V (0.04), and Zn (1.81). The quality of the analysis was assured by measuring five blanks, duplicate samples, and lobster hepatopancreas certified reference material (CRM; TORT-3, National Research Council, Canada). Mean recoveries ranged between 81 and 118 % (for As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Mn, Sb, V, and Zn) and 130% (for Pb).

In order to test for potential Cd contamination of the muscular tissue from the digestive gland during storage processes, market arrow squid tissues were analysed ( $n=3$ ). To test for such contamination effects, Cd concentrations of visually stained mantle tissue were compared to non-stained muscular tissue (Fig. 10).

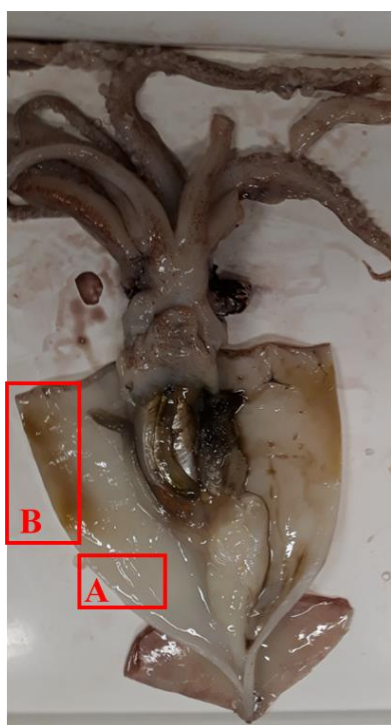


Fig. 10. Market arrow squid (*Nototodarus gouldi*) analysed in this chapter. A) Tissue sample of unstained mantle tissue; B) Tissue sample of stained mantle tissue.

The mean and maximum concentrations of As, Cd, Hg, and Pb in arrow squid mantle tissue were compared to the maximum allowable concentrations in food defined by the FSANZ (2016) and to the benchmark dose level (BMDL). The BMDL is used by the WHO, the European Food Safety Authority

(EFSA), and the New Zealand Total Diet Study (MPI, 2018) for food safety assessments. A benchmark dose (BMD) is the concentration at which a change in an adverse response can be measured (Filipsson et al., 2003), and the BMDL is the lower 95% confidence limit of that concentration. Inorganic arsenic concentrations [iAs] were calculated from total arsenic concentrations [tAs], measured by ICP-MS, with conversion factors (0.376% present as iAs in tAs measured) from the existing literature (Devesa et al., 2001; Muñoz et al., 2000).

The *in-vitro* digestions were performed following Minekus et al. (2014). Freeze-dried, homogenised mantle tissue ( $n=7$ ) underwent a simulated human digestion cycle with three phases (mimicking the digestion processes in the mouth, stomach, and intestine) at 37°C. All enzymes were purchased from Sigma Aldrich, USA. The simulated digestion fluids were prepared with electrolyte stock solutions, enzymes, and ultrapure water following Minekus et al. (2014). The simulated digestion cycle was as follows: 1) simulated salivary fluid and  $\alpha$ -amylase (EC 3.2.1.1) were mixed with 5 g of the sample at pH=7 and incubated for 5 minutes; 2) simulated gastric fluid and pepsin (EC 3.4.23.1) were added to the sample and left in a shaking water bath for two hours at pH=3; and 3) simulated intestinal fluid, the digestive enzymes pancreatin (EC 232.468.9), lipase (EC 3.1.1.3), and bile (EC 232.369.0) were added at pH=3 and left in the water bath for three hours. After the digestion cycle, 5 ml of supernatant was removed, adjusted to pH=4.5, centrifuged, and stored at -80°C until further trace element analysis. The supernatant contains the bioaccessible fraction of the trace elements, which becomes available for absorption in humans (Versantvoort et al., 2005). Prior to ICP-MS analysis, *in-vitro* digested samples were thawed, and diluted 21-fold with 1% nitric acid. CRMs and blanks were processed following the *in-vitro* digestion protocols outlined above. A paired *t*-test was conducted to test for differences between *in-vitro* digested and undigested samples.

Heatmaps were generated using Euclidean distance and Ward hierarchical clustering using the software MetaboAnalyst v2.0 (Chong & Xia, 2018). Missing values were estimated by replacing them with half of the minimum concentration value for the respective element. The samples were normalised and transformed using auto-scaling, mean centred, and divided by the standard deviation. A principal component analysis (PCA) was used to examine differences in overall trace element concentrations among tissues based on the auto-scaled sample set. Ordination biplots were produced that showed differences between muscular tissue (arms, fins, mantle, and tentacles) and the organs (digestive gland, gonads, branchial and systemic hearts, and kidney). The PCA was performed in R version 3.1.0 (R Core Team, 2017) using the package 'ggbiplot' (Vu, 2011).

## Results and Discussion

This is the first baseline tissue trace element concentration data reported for arrow squid (*N. sloanii*) that includes all four trace elements included in the World Health Organization's *Ten chemicals of major public health concern* (WHO, 2010a). Overall, observed concentrations of trace elements analysed followed the order: Cu>Zn>Cd>Fe>As>V>Mn>Cr>Pb>Co>Ni>Hg (Table 8). The heatmap showed a clustering of trace element concentrations within tissue groups with a clear distinction on the first order of clustering (Fig. 11). Two distinct tissue groups were identified: the “muscular tissue group”, which contained muscular tissue and the gonads; and the “organ group”, consisting of the remaining organs (Fig. 11). Two groups of trace elements were identified: one contained Cd, Cu, Co, Fe and V; and the other consisted of As, Cr, Hg, Mn, Pb, Sb and Zn (Fig. 11). Both trace element groups had lower concentrations in the muscular tissue group than in the organ group. Within the organs, the concentrations of trace element group one were highest in the digestive gland, and trace element group two showed highest concentrations in the branchial hearts.

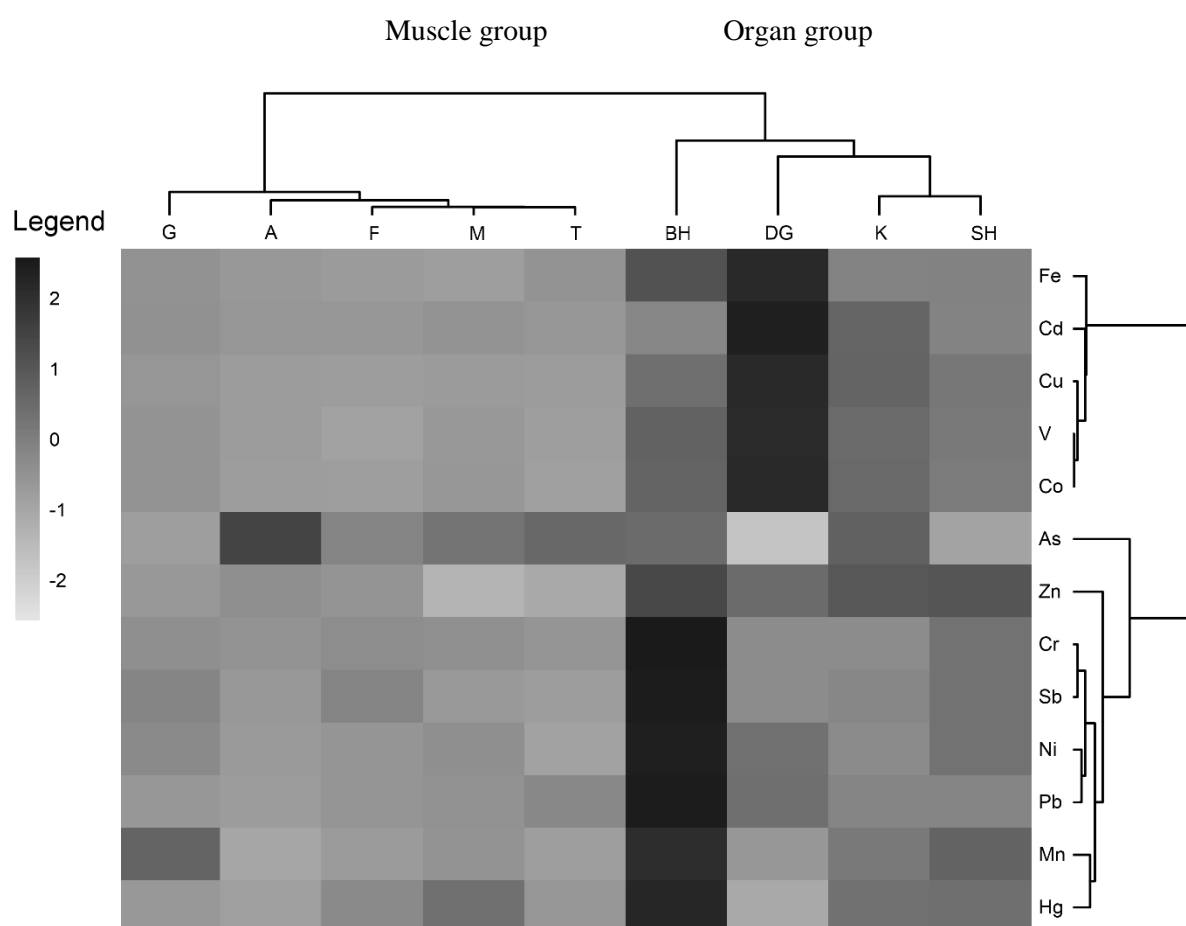


Fig. 11. Heatmap showing the auto-scaled trace element concentrations in the different tissues of *N. sloanii* from the Chatham Rise. Darker shades represent higher concentrations; pale shades, lower concentrations. Hierarchical clustering is indicated by brackets. The organs are abbreviated as follows: arm (A), branchial heart (BH), digestive gland (DG), fin (F), gonads (G), kidney (K), mantle (M), systemic heart (SH), and tentacles (T).

Table 8. Trace element concentrations (mean±standard deviation [sd], minimum, and maximum) for the nine different tissue types analysed ( $n=30$ ) of *N. sloanii* from the Chatham Rise, NZ. Concentrations are in  $\mu\text{g g}^{-1}$  dw. Tissue abbreviations are as follows: arm (A), branchial heart (BH), digestive gland (DG), fin (F), gonad (G, including ovaries and testes), kidney (K), mantle (M), systemic heart (SH), and tentacle (T).

	A		BH		DG		F		G		K		M		SH		T	
	<i>mean±sd</i>	<i>min–max</i>	<i>mean±sd</i>	<i>min–max</i>	<i>mean±sd</i>	<i>min–max</i>	<i>mean±sd</i>	<i>min–max</i>	<i>mean±sd</i>	<i>min–max</i>	<i>mean±sd</i>	<i>min–max</i>	<i>mean±sd</i>	<i>min–max</i>	<i>mean±sd</i>	<i>min–max</i>	<i>mean±sd</i>	<i>min–max</i>
As	17.26± 9.81	5.58– 54.26	16.32±7.8 6	2.27– 41.09	14.15±5.2 6	5.68– 29.31	15.71±6.2 7	6.93– 30.70	15.06±8.9 8	4.37– 38.93	16.57±5.7 9	7.36– 29.43	16.10±6.7 1	5.97– 32.50	14.46±6.9 7	0.29– 29.02	16.42±6.6 3	9.65– 37.08
Cd	0.43±0.42	0.04–1.60	13.18± 14.35	0.79– 65.78	89.04±11 3.50	11.35– 476.51	0.73±0.41 1.61	0.16–	5.82±5.85	0.30– 22.66	37.88±48. 02	0.13– 206.10	0.65±0.47	0.09–1.49	15.49±12. 92	0.99– 51.46	0.51±0.42	0.08–1.47
Cr	0.28±0.13	0.11–0.54	1.86±2.11	0.41– 10.00	0.31±0.26	0.02–1.30	0.35±0.49	0.05–2.57	0.28±0.27	0.01–1.12	0.31±0.33	0.01–1.30	0.27±0.33	0.02–1.82	0.63±0.60	0.11–2.22	0.20±0.15	0.07–0.70
Co	0.06±0.13	0.01–0.75	0.62±0.40	0.05–1.88	1.19±1.16	0.26–4.62	0.06±0.03	0.02–0.16	0.16±0.11	0.03–0.40	0.57±0.56	0.02–1.98	0.12±0.14	0.02–0.59	0.38±0.25	0.02–1.37	0.04±0.02	0.02–0.10
Cu	21.72± 28.79	7.03– 163.76	225.62± 111.50	43.74– 461.80	545.67± 867.70	7.73– 3981.00	17.56±7.8 2	6.39– 31.39	51.54±43. 79	6.78– 249.61	278.03± 354.20	12.86– 1597.60	24.05±23. 73	6.91– 100.00	185.5± 122.31	0.37– 550.68	20.55±7.8 3	12.41– 56.05
Fe	26.75± 29.59	2.04– 147.34	149.73± 76.07	16.83– 348.08	222.85±8 3.32	81.50– 461.03	20.33±11. 70	8.48– 69.51	37.36±24. 13	5.46– 90.18	63.16± 60.77	6.02– 247.16	16.35±11. 71	4.53– 58.20	63.19±47. 94	3.84– 280.87	34.24±36. 80	4.93– 186.16
Hg	0.26±0.16	0.02–0.65	0.70±0.46	0.08– 1.96	0.21±0.15	0.04–0.58	0.34±0.24	0.05–0.77	0.28±0.19	0.01–0.71	0.38±0.21	0.09–0.99	0.40±0.29	0.01–0.89	0.44±0.27	0.08–0.99	0.26± 0.19	0.02–0.74
Ni	0.36±0.35	0.13–1.57	2.56±2.95	0.34– 14.50	0.99±0.72	0.18–3.87	0.38±0.28	0.03–1.15	0.55±0.50	0.04–2.42	0.56±0.37	0.07–1.90	0.45±0.49	0.01–2.23	0.94±0.65	0.19–2.44	0.14± 0.13	0.13–0.58
Mn	1.06±1.04	0.48–6.41	5.71±10.9 7	0.74– 10.10	1.64±0.76	0.76–4.17	1.47±1.13	0.65–6.67	3.62±2.67	1.15– 10.97	2.80±1.85	0.68–9.82	1.77±0.60	0.85–3.00	3.54±1.54	0.05–9.07	1.36±0.71	0.54–3.31
Pb	0.14±0.07	0.05–0.30	0.99±1.07	0.12– 4.14	0.41±1.04	0.03–5.85	0.15±0.13	0.02–0.61	0.14±0.06	0.03–0.33	0.26±0.22	0.04–1.02	0.18±0.18	0.03–0.83	0.25±0.17	0.03–0.71	0.24±0.25	0.02–1.08
Sb	0.07±0.07	0.01–0.28	0.37±0.22	0.01–0.94	0.09±0.11	0.02–0.53	0.13±0.22	0.02–0.97	0.13±0.13	0.01–0.46	0.12±0.12	0.01–0.38	0.07±0.05	0.01–0.18	0.16±0.13	0.01–0.51	0.05±0.03	0.02–0.13
V	0.20±0.40	0.05–2.15	1.13±1.24	0.12– 6.19	2.03±4.58	0.17– 24.10	0.11±0.10	0.00–0.44	0.34±0.62	0.01–3.36	1.00±2.63	0.02– 13.39	0.30±0.63	0.07–2.50	0.73±1.41	0.03–7.66	0.15±0.14	0.05–0.62
Zn	74.81± 32.70	50.06– 239.47	108.55± 50.91	12.60– 289.54	91.59± 103.52	19.38– 480.91	72.16±23. 02	42.54– 120.72	70.17±43. 31	28.62– 169.89	101.88±5 2.63	37.83– 270.08	56.54±7.7 7	41.35– 70.91	99.18±44. 04	4.14– 264.72	61.99±8.6 1	47.51– 79.91

The first two axes of the PCA explained 41.9% of the variation in trace element concentrations (Fig. 12). Variation in Cd, Co, Cu, Fe, and Mn were the strongest drivers of PC1 whereas PC2 was primarily driven by differences in As, Hg, and Zn (Fig. 12). Muscular tissue was largely clustered in the mid-range of trace element concentrations, while organs varied greatly within and among tissues, and exhibited trace element concentrations toward the lower and upper extremes of both PCA axes (Fig. 12). On average, trace element concentrations were higher in organs compared to muscular tissue (Table 8, Fig. 12).

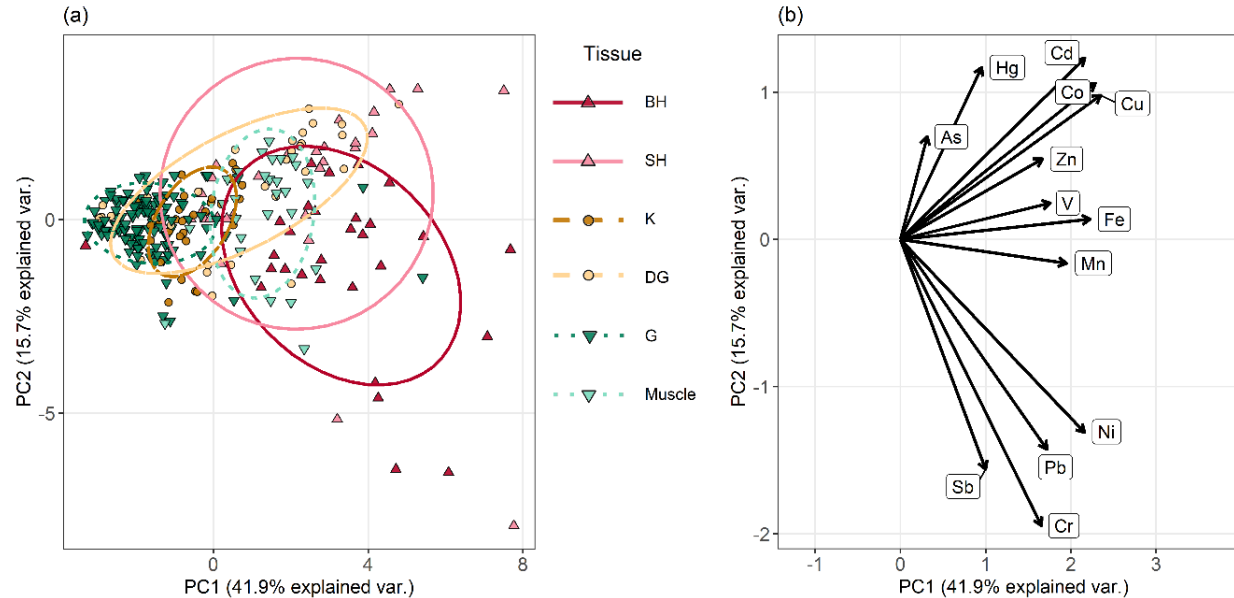


Fig. 12. Principal component analysis (PCA) presenting the elemental concentrations in *N. sloanii* from NZ's Chatham Rise. (a) Muscle tissue and organs. (b) Element loadings along PC1 and PC2, shown by arrows. The organs are abbreviated as follows: branchial heart (BH), systemic heart (SH), kidney (K), digestive gland (DG), and gonads (G). Ellipses indicate the 95% confidence interval around tissue groupings.

The findings for the elements of most health concern (As, Cd, Hg, and Pb) are discussed in terms of their benchmark dose level and maximum permissible levels, as well as the estimated intakes for adults, children and infants in New Zealand (Table 9).

Total As concentrations (mean) ranged from 14.15 to 17.26  $\mu\text{g g}^{-1}$  dw in the analysed tissues (Table 8). Concentrations were marginally higher in the arm tissue, although overall As concentrations did not vary greatly among the different tissues (Table 8). Similar As tissue distribution was reported in another ommastrephid squid, *Todarodes pacificus* (Ninh et al., 2007). In seafood, most As is present as arsenobetaine (Borak & Hosgood, 2007), which is considered harmless to humans (Adams et al., 1994; Edmonds et al., 1977). Inorganic Arsenic (iAs) is much more toxic to humans (Hughes, 2002) and chronic As poisoning can lead to a variety of cancers and peripheral neuropathy (Hall, 2002). Herein, concentrations of iAs are estimated to be very low (mean of 0.03–0.09  $\mu\text{g g}^{-1}$  wet weight [ww]) in the mantle tissue, well below the maximum allowable level in shellfish of 1  $\mu\text{g g}^{-1}$  ww (Table 9).

Table 9. *Nototodarus sloanii* mantle tissue concentrations (mean and maximum) for total As (tAs), Cd, Hg and Pb ( $\mu\text{g g}^{-1}$  ww) compared to the maximum allowable levels in shellfish as defined by Food Standards Australia and New Zealand (FSANZ, 2008); the benchmark dose level (BMDL) and tolerable weekly intake (TWI)<sup>3</sup> for NZ children (8kg) and adults (70kg). The mean and maximum measured concentrations are compared to the estimated weekly exposure of elements in New Zealand adults and infants<sup>2</sup>. Portion size refers to a meal of 120g squid mantle tissue.

	tAs	Cd	Hg	Pb
<b>Guidelines</b>				
Maximum allowable levels in shellfish ( $\mu\text{g g}^{-1}$ )		2.00	0.50	2.00
Maximum allowable levels in shellfish ( $\mu\text{g g}^{-1}$ ) per portion week <sup>-1</sup>		240.00	60.00	240.00
TWI ( $\mu\text{g kg}^{-1} \text{bw}^{-1} \text{week}^{-1}$ )		2.50	4.00 <sup>4</sup>	
TWI for 70kg adult. week <sup>-1</sup>		175.00	280.00	
TWI for 8kg child. week <sup>-1</sup>		20.00	32.00	
BMDL ( $\mu\text{g kg}^{-1} \text{bw}^{-1} \text{week}^{-1}$ ) <sup>1</sup>	N.A.	15.68	N.A.	4.20
BMDL for 70kg adult week <sup>-1</sup>		1098.00		294.00
BMDL for 8kg child week <sup>-1</sup>		125.44		33.60
<b>Empirical data</b>				
Estimated weekly exposure adult (>25 years in $\mu\text{g kg}^{-1} \text{bw}^{-1} \text{week}^{-1}$ ) <sup>2</sup>	12.40	12.20	0.30	0.55
Estimated weekly exposure 70 kg adult week <sup>-1</sup>	868.00	854.00	21.00	38.50
Estimated weekly exposure infants (6-12 months $\mu\text{g kg}^{-1} \text{bw}^{-1} \text{week}^{-1}$ ) <sup>2</sup>	12.00	12.00	2.30	2.90
Estimated weekly exposure 8 kg child week <sup>-1</sup>	96.00	96.00	18.46	23.20
<b>This study</b>				
Mean measured mantle concentration ( $\mu\text{g g}^{-1}$ wet weight)	3.22	0.13	0.08	0.04

Maximum measured mantle concentration ( $\mu\text{g g}^{-1}$ wet weight)	6.50	0.30	0.18	0.17
Estimated intake by portion with mean concentration (120g mantle tissue; $\mu\text{g g}^{-1}$ ww)		15.60	9.60	4.32
Estimated intake by portion with maximum concentration (120g mantle tissue; $\mu\text{g g}^{-1}$ ww)		35.76	21.36	19.92
% of estimated weekly exposure adult (one portion week <sup>-1</sup> ) <sup>2</sup>		1.83	45.71	11.22
% of estimated weekly exposure infant (one portion week <sup>-1</sup> ) <sup>2</sup>		37.25	115.73	85.86

<sup>1</sup> The BMDL are calculated from  $\mu\text{g kg}^{-1}$  bw.day<sup>-1</sup> to  $\mu\text{g kg}^{-1}$  bw week<sup>-1</sup>.

<sup>2</sup> Concentrations extracted from the New Zealand Total Diet Study (MPI, 2018)

<sup>3</sup> European Food Safety Authority (EFSA, 2012a,b)

<sup>4</sup> For inorganic Hg

The highest concentrations of Cd (11.35–476.51  $\mu\text{g g}^{-1}$  dw) were observed in the digestive gland tissue (Table 8, Fig. 11). This observation is consistent with previous studies comparing trace element concentrations in different tissues of other ommastrephids, e.g., *Illex argentinus* (Dorneles et al., 2007), *Todarodes filippovae* (Kojadinovic et al., 2011), and *Sthenoteuthis pteropus* (Lischka et al., 2018). The higher Cd concentration measured in the digestive gland could affect squid predators because they consume the whole animal. However, human consumers in New Zealand generally only consume the muscular tissue of squids, where lower concentrations were found than in the organs (Table 8). This chapter suggests that, based on the mean mantle concentration, consuming a single portion of arrow squid mantle tissue will not exceed the maximum allowable level for shellfish as defined by the FSANZ (2016, Table 9). Cadmium is carcinogenic and can cause human kidney and liver damage when ingested in high concentrations (Järup et al., 1998). The maximum concentration for Cd measured in the mantle tissue was 1.49  $\mu\text{g g}^{-1}$  dw (equals 0.30  $\mu\text{g g}^{-1}$  ww), which remains below the maximum permissible concentration for this element (Table 9). Comparably low Cd concentrations have been reported in arrow squid (identified as '*Nototodarus* sp.') muscle tissue from the Cook Strait (west of the present sampling area, (Vlieg et al., 1991) and in *N. sloanii* from the Chatham Rise (Chapter 2). However, the analysis of fresh purchased market arrow squid (*N. gouldi*) observed that Cd diffusion from the digestive gland contaminated the adjacent mantle tissue (21.88  $\mu\text{g g}^{-1}$  dw, approx. 4.38  $\mu\text{g g}^{-1}$  ww), which has the potential to pose a risk to human consumers (Fig. 10, Table 10).

Table 10. Comparison of Cd ( $\mu\text{g g}^{-1}$  dw) concentrations measured in *N. gouldi* from unstained [M(A)] and stained mantle muscle [M(B)] and the other tissues: arm (A), branchial heart (BH), digestive gland (DG), fin (F), gonad (G= testes), kidney (K), mantle (M), systemic heart (SH), and tentacle (T)) of fresh male arrow squid (*Nototodarus gouldi*).

Sample ID	A	BH	DG	F	G	K	M(A)	M (B)	SH	T
Market Squid 1	1.872	18.89	133.64	1.69	0.97	40.03	1.39	21.31	9.51	5.33
Market Squid 2	1.48	9.50	176.75	0.44	0.54	105.39	0.58	11.61	17.89	0.33
Market Squid 3	0.73	17.66	168.59	5.57	1.18	179.36	0.72	21.88	29.68	0.50

Additionally, in some cultures small squids are eaten whole, and in other cultures the digestive gland (kimo, shiokara) is considered a delicacy (Kojadinovic et al., 2011; Kurihara 1993). The Cd concentrations found in the digestive gland of NZ arrow squids makes them unsafe for human consumption in this way, because the average measured concentrations would exceed the maximum allowable levels of Cd in shellfish by 1233% (FSANZ, 2016). However, this study focussed on the bioaccessibility of Cd, which does not consider the full absorption and thus bioavailability through the intestinal barrier. Further studies that focus on the bioavailability of Cd through squid consumption are necessary before further conclusions can be drawn on the risk to humans.

The simulated *in-vitro* digestion resulted in bioaccessible fractions of Cu, Mn and Zn that ranged between 46 and 89% of the total concentration (Table 11). The bioaccessible Hg concentration ranged between 0.58 and 1.92% which could mean that this element was not extracted. For Cd, Co and Fe the bioaccessible concentration was equivalent to the total concentration (Table 11). Those results (Table 11; Appendix Fig. 2) indicated that Co, Cd and Fe in the mantle tissue are not significantly affected during the digestion process (Table 11). This means that the total observed amount of those trace element concentrations is bioaccessible through ingestion (but the bioavailability remains unknown). The reduced concentrations of Cu, Hg, Mn and Zn after the digestion process could be explained by a higher absorption of the trace elements to the squid tissue at higher pH (representative of the intestinal phase; Waisberg et al., 2004). Although the concentrations of Cd were below the tolerable limits, the analysis of the fresh market squid reported Cd contamination of mantle tissue by digestive gland diffusion in freshly caught arrow squid, which led to a significant increase of the mantle Cd concentrations (Table 10). An *in-vitro* digestion analysis of contaminated mantle tissue could reveal potential risks for human consumption and could be interesting for future studies. Overall, the concentrations of trace elements measured in this study represent a simulation and only reveal the bioaccessible fraction of the elements, which does not consider the full absorption

and thus bioavailability through the intestinal barrier (Dean, 2007; Peijnenburg & Jager, 2003). Further studies that focus on the bioavailability of Cd through squid consumption are necessary before further conclusions can be drawn on the risk to humans.

Table 11. Trace element concentrations before and after the completed *in-vitro* simulated digestion of *N. sloanii* mantle tissue ( $\mu\text{g g}^{-1}$  dw), used to assess the bioaccessibility of trace elements during human digestion A significant increase ( $\uparrow$ ) or decrease ( $\downarrow$ ) in concentration after the paired *t*-test is indicated by arrows ( $p<0.001$   $\uparrow\uparrow\uparrow$ ,  $p<0.01$   $\uparrow\uparrow$ ,  $p<0.05$   $\uparrow$ ).

	Before digestion		After digestion		
	mean $\pm$ sd	min–max	mean $\pm$ sd	min–max	
Cd	0.67 $\pm$ 0.48	0.15– 1.35	0.38 $\pm$ 0.30	0.04– 0.89	
Co	0.06 $\pm$ 0.04	0.02– 0.13	0.05 $\pm$ 0.05	0.01– 0.14	
Cu	17.5 $\pm$ 12.9	8.42– 46.23	15.5 $\pm$ 13.7	7.41– 46.1	$\downarrow\downarrow$
Fe	10.9 $\pm$ 4.22	5.31– 16.0	9.56 $\pm$ 3.57	6.16– 14.6	
Hg	0.52 $\pm$ 0.19	0.18– 0.75	0.01 $\pm$ 0.01	0.003– 0.01	$\downarrow\downarrow\downarrow$
Mn	1.78 $\pm$ 0.76	1.01– 3.00	1.25 $\pm$ 0.75	0.53– 2.61	$\downarrow\downarrow$
Zn	61.8 $\pm$ 4.86	55.5– 70.2	28.2 $\pm$ 6.54	21.4– 38.3	$\downarrow\downarrow\downarrow$

Mercury concentrations ranged from 0.01 to 1.96  $\mu\text{g g}^{-1}$  dw and were similar to those reported in other squid species, e.g., *Architeuthis dux* (Bustamante et al., 2008), *Sthenoteuthis pteropus* (Lischka et al., 2018) and *Todarodes sagittatus* (Pierce et al., 2008). The highest concentrations of Hg were found in the branchial hearts, followed by the muscular tissues. The affinity of Hg to the sulfhydryl groups of proteins has been previously reported (e.g., Bloom, 1992; Bustamante et al., 2006) and could be the reason for its elevated concentration in the muscular tissue. The branchial hearts and their appendages are reported to have circulatory and excretory functions, which might contribute to elevated Hg concentrations in those tissues (Miramand & Bentley, 1992). Mercury is especially toxic in its organic form (methylmercury [MeHg])—which is the main bioavailable form of Hg in fish and cephalopods (Bloom, 1992; Bustamante et al., 2006)—and can lead to neurological disorders and affect the human kidneys, liver, and immune system (Booth & Zeller, 2005). The FSANZ advises a restricted consumption of billfish and shark to one serving per week because certain fish species, especially predatory fishes such as swordfish (*Xiphias gladius*), can

contain high concentrations of Hg (e.g., Bosch et al., 2016; de Pinho et al., 2002). Overall, the Hg concentrations reported in this study for arrow squid were well below the maximum tolerable levels defined by FSANZ.

The maximum Pb concentration measured in arrow squid was  $5.85 \mu\text{g g}^{-1} \text{ dw}$  in the digestive gland (Table 8). Lead concentrations varied among tissues with highest concentrations measured in the branchial hearts and the digestive gland and lowest concentrations in the muscular tissue (Table 8). Similarly, Pb concentrations were reported to be lowest in the muscular tissue of *Todarodes filippovae* (Kojadinovic et al., 2011), *Todarodes pacificus* (Ichihashi et al., 2001) or *Sthenoteuthis pteropus* (Lischka et al., 2018). The Pb mantle concentrations measured in this chapter were well below the maximum tolerable concentrations and did not exceed the BMDL of  $0.6 \mu\text{g kg}^{-1} \text{ bw}^{-1} \cdot \text{day}^{-1}$ . Although low in acute toxicity, Pb can damage the kidneys, the gastrointestinal tract, and the central nervous system over longer exposure (Jangid et al., 2012). In general, the dietary exposure of adults to Pb in New Zealand is low, compared with internationally reported levels (MPI, 2018).

## Conclusion

This study has provided baseline data on the tissue distribution and concentrations of the most physiologically and toxicologically important trace elements in arrow squid from the Chatham Rise, New Zealand, where high volumes of this species are commercially exploited. In addition, these baseline data describing concentrations in multiple tissues are of particular relevance to marine predators of arrow squid, as they consume the entire squid. This chapter focused on the concentrations of As, Cd, Hg, and Pb in order to determine potential implications for human health. Although ubiquitous in the marine environment, the concentrations of these trace elements in the arrow squid sampled lie well below the maximum allowable levels. However, members of vulnerable demographics, such as infants, young children, pregnant women, and people with an enriched Cd intake (e.g., smokers) might need to monitor their arrow squid consumption (Järup & Åkesson, 2009). *In-vitro* digestion of the mantle tissue had no observable effect upon bioaccessible Cd concentrations, indicating that the Cd mantle tissue concentrations are representative of the amount available for human absorption, when humans consume arrow squid. Because of the diffusion of Cd from the digestive gland to the edible mantle tissue in *N. Gouldii*, it is possible that Cd levels in market squid exceed maximum permissible limits. Future studies should examine fresh market squid samples of other species to see whether this phenomenon is ubiquitous across all commercially-fished squids. Due to the importance of seafood in the diet of New Zealanders a broader monitoring of Cd in seafood, particularly of arrow squid, is proposed. This chapter also

highlighted that the contamination of muscular tissue by the digestive gland might be non-specific to the species and other commercially-fished squid species should be investigated further.

## Chapter 4: Regional assessment of trace element concentrations in arrow squids (*Nototodarus gouldi*, *N. sloanii*) from New Zealand waters: impact on food web and human consumers

*This chapter is based on the following published article:*

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### Abstract

The New Zealand arrow squids, *Nototodarus gouldi* and *N. sloanii*, play an important role in the marine food web, and are both economically important fishery species. This study compares trace element concentrations (As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Mn, Pb, U, V, and Zn) in these animals from different fishing locations within New Zealand's Exclusive Economic Zone (Auckland Islands, Chatham Rise, Dunedin, Golden Bay, Taranaki, and West Coast of the South Island). Muscular mantle (the tissue usually consumed by humans) and digestive gland tissue (the primary organ for trace element accumulation) concentrations were compared among regions, revealing size, species and sampling location effects. Overall, *N. gouldi* had higher concentrations of As and Zn. The Taranaki region had the highest concentrations for Cd, Co, Cu and Zn in both tissues analysed. A human risk assessment revealed that mean mantle concentrations of As, Cd, Hg and Pb in this study lie overall below the maximum levels presently considered tolerable for human consumption. Digestive gland concentrations in particular indicate that arrow squids can be considered important vectors for trace element transfer to top predators (mean concentration for Cd:  $123 \pm 148 \mu\text{g g}^{-1} \text{ dw}$ ), with region-specific differences.

### Introduction

Cephalopods play a key role within marine ecosystems as both prey and active predators (Clarke, 1996; Santos et al., 2001). They have a high ecological importance for the energy transfer within marine food webs (Coll et al., 2013) and as vectors of trace elements (Bustamante et al., 1998). Human overharvesting of finfish, combined with changing oceanic temperatures, has led to increased abundance of, and fishing effort for, certain cephalopod species (Doubleday et

al., 2016; Rodhouse and White, 1995). Cephalopods accumulate high concentrations of some trace elements and have been suggested as an important vector in the transport and bioaccumulation of toxins within food webs (Bustamante et al., 1998). With the increasing popularity of cephalopods as a food source for humans, there is a growing need to study trace element concentrations in commercially fished species (Galitsopoulou et al., 2009; Rjeibi et al., 2014; Storelli, 2008; Storelli & Marcotrigiano, 1999). Notably, some recent studies have reported especially high concentrations of Cd and Hg in oceanic squids (e.g., Dorneles et al., 2007; Lischka et al., 2018). Nonetheless, data are lacking for certain commercially fished squid species, particularly deep-sea and oceanic species. Trace elements are ubiquitous in marine environments and concentrations can be influenced by anthropogenic sources (e.g., agriculture, burning fossil fuels, industrial discharges, mining, shipping, smelters, road run-off or waste deposition; Langston, 2017; Tchounwou et al., 2012) or natural sources (e.g., geothermal activity or dissolution, erosion and mobilisation of terrigenous dust, mineral deposits, riverine discharge or volcanism; Jaeschke et al., 2017; Rainbow, 2007; 1995, Ramadyan, 2017).

In New Zealand waters, “arrow” squids are one of the most heavily fished groups (Hurst et al., 2012), with an annual total allowable commercial catch of 82,120 t for 2018 (MPI 2019). New Zealand has two arrow squid species: *Nototodarus gouldi* (McCoy, 1888), and the endemic *N. sloanii* (Gray, 1849; Jackson et al., 2000). Both are neritic-oceanic species and occur from 50 to 600m (Chen, 1998). While *N. gouldi* is typically distributed around the east and west coasts of the North Island and the northeast coast of the South Island (Uozumi, 1998; Uozumi & Förch, 1995), *N. sloanii*, the Southern arrow squid, is most abundant south of the subtropical front in the sub-Antarctic waters east of the South Island, and westward to the Chatham Rise (Chen, 1998). Compared with other ommastrephid squids, arrow squids tend to be less migratory and do not follow clear migration patterns in tagging studies (Dunning & Förch, 1998; Stark et al., 2005). Arrow squids are consumed by a variety of predators, including long-finned pilot whales, *Globicephala melas* (Beatson & O’Shea 2009), the endemic New Zealand sea lion, *Phocarctos hookeri* (Childerhouse et al. 2001), Buller’s albatross, *Diomedea bulleri bulleri* (James & Stahl, 2000), orange roughy, *Hoplostethus atlanticus* (Jones, 2007), and might represent a significant vector for trace element transfer.

Beyond their central position in pelagic food webs, arrow squids play an important role in human diets (MPI, 2019), making them excellent study organisms for trace element accumulation. Most studies to date have focussed on trace element concentrations in *N. sloanii* from one of New Zealand’s main fishing areas, the Chatham Rise (Chapters 2, 3) or Golden Bay (e.g., van den Broek & Tracey, 1981; Vlieg et al. 1991). Expanding on previous studies focussing on arrow squids from single regions within New Zealand’s Exclusive Economic Zone (EEZ), the present chapter aims to compare trace element concentrations in both

New Zealand arrow squid species across six different fishing areas. Previously, the reported trace element concentrations in the edible muscular tissues for the elements of greatest human concern (As, Cd, Hg, Pb; WHO, 2010a) have fallen below the maximum tolerable levels for humans (Chapters 2, 3). In this chapter, regions/fisheries with trace element concentrations potentially influenced by a range of anthropogenic and natural sources (Golden Bay, Taranaki, West Coast) are compared to rather pristine areas (Auckland Islands, Chatham Rise, Dunedin). For example, the Taranaki region (Fig. 13f) is known for intense agriculture (Abraham, 2018), a dormant volcano (Hoernle et al., 2006), and oil extraction (Pitt, 2016), whereas the West Coast has a long history of mining for coal and gold (Davies et al., 2011). The analysis of arrow squids from different regions might reveal local differences in trace element concentrations, with implications for fisheries management and public health choices.

## Material and Methods

### Sampling

Specimens of *N. gouldi* and *N. sloanii* were collected opportunistically by bottom trawl in the austral summer months (January and February) from 2013 to 2016, during fishery surveys on the Research Vessels *Tangaroa* and *Kaharoa* (National Institute for Water and Atmospheric Research, Ltd [NIWA]). Trawls were conducted throughout New Zealand's Exclusive Economic Zone (Fig. 13f) at depths of 200–800 m. In total, 118 specimens were analysed from six different locations (Table 12). Tissue samples were taken from each specimen for trace element analysis from the digestive gland (the main storage organ for certain trace elements) and the mantle tissue (relevance for human consumption) from 59 females and 59 males (119–349 mm dorsal mantle length [DML]; Table 12). For the specimens collected from the Auckland Islands, only digestive gland tissue was available.

Table 12. Sample details for arrow squids used in this study (*Nototodarus gouldi* and *N. sloanii*) from the six different sampling locations in the New Zealand exclusive economic zone (EEZ). Sample size (*n*) for males and females, dorsal mantle length (DML) range, and numbers of specimens of each species are included.

Location	Coordinates	Specimen ( <i>n</i> )		DML [mm]	<i>N. gouldi</i> ( <i>n</i> )	<i>N. sloanii</i> ( <i>n</i> )
		♀	♂			
Auckland Islands	50°0' S, 166°1' E	10	10	168–244		20
Chatham Rise	43°0' –43°9' S, 174°77' – 179°42' E	15	15	194–336		30
Dunedin	46°8' S, 169°9' E	10	10	178–276		20
Golden Bay	40°3' S 173°6' E	8	8	228–314	9	7

Taranaki	38°5' S –38°6', 174°0' E	8	8	175–349	16	
West Coast	41°7'– 43°2' S, 169°9'– 171°3' E	8	8	119–280	11	5

### *Trace element analysis*

Tissue samples were freeze-dried, homogenised (~300 mg dry weight [dw]), and digested in a 3:1 mixture of 70% HNO<sub>3</sub> (Merck, suprapur quality) and 37% HCl (Merck, suprapur quality). Acidic digestion took place in a microwave digestion system (Multiwave GO, Anton Paar GmbH, Austria) at 105°C for 50 minutes. Samples were diluted with Milli-Q water to a volume of 50 ml. For the inductively coupled plasma mass spectrometry (ICP-MS) analysis, an additional dilution with 2% HNO<sub>3</sub>, 0.1% L-Cysteine and 0.5% HCl was performed (Aldridge et al., 2017). Concentrations of As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Mn, Pb, and Zn were analysed by ICP-MS (Agilent Technologies 7500 series, CA, USA). Detection limits (µg g<sup>-1</sup> dw) were calculated as 3x standard deviation of the blank: As (0.007), Cd (0.02), Co (0.005), Cr (0.076), Cu (0.24), Fe (1.39), Hg (0.001), Mn (0.12), Ni (0.13), Pb (0.05), and Zn (1.81). The quality of the analysis was assured by measuring five blanks, duplicate samples, and lobster hepatopancreas certified reference material (CRM; TORT-3, National Research Council, Canada). Mean recoveries ranged from 89 to 115%, except for Cr (142%) and Pb (156%, Appendix Table 2). The concentrations for U were measured by microwave plasma atomic emission spectroscopy (MP-AES 4200 Agilent Technologies, Australia) with a detection limit of 0.001 µg g<sup>-1</sup> dw and a recovery rate for TORT-3 of 105%.

### *Statistical analysis*

For exploratory analysis, a principal component analysis (PCA) was applied to examine differences in overall trace element concentrations among regions for digestive gland and mantle tissue (R version 3.1.0, Ihaka & Gentleman, 1996; R Core Team, 2017; package 'ggbiplot', Vu, 2011). Data for the PCA were normalised and z-transformed (mean centred and divided by the standard deviation). Missing concentration data, assumed below the detection limit of the ICP-MS, was set to the lowest measured value of the corresponding element multiplied by 0.5 (Scholz et al., 2005).

Generalised linear models (GLM) were applied in R (package 'MASS', Ripley et al., 2013) to determine whether sex, size, species, and sampling region influenced trace element concentrations. One model per trace element was fitted against raw concentration data for both the digestive gland and mantle tissue. The GLMs were fitted with a negative binomial distribution and the logit link function; then, the variables size, sex, region × species were added sequentially. Species and region are confounded because neither species occurs at all sampling locations (Table 12), and thus was added as an interaction term. Data

exploration, model fitting and evaluation were conducted following Zuur et al. (2009). Model fit was estimated using residual deviance compared with an intercept-only model; decreasing deviance was interpreted as a goodness of fit (Guisan & Zimmermann, 2000). In order to check for differences in trace element concentrations among regions, Tukey's post-hoc tests were applied ('glht' function, R package 'multcomp', Hothorn et al., 2016) and the *p*-values were adjusted to account for multiple testing following Benjamini & Hochberg (1995).

## Results

Overall, mean trace element concentrations in the digestive gland decreased in the following order: Cu>Fe>Zn>Cd>As>Mn>Co>Ni>V>Cr>Pb>Hg>U. Mean trace element concentrations in the mantle tissue followed the order: Zn>Cu>As>Fe>Mn>Cd>Ni>Co>Hg>Cr>V>Pb>U (Table 14). With the exception of As and Hg, trace element concentrations were higher in the digestive gland than in the mantle tissue.

In general, sex had no significant effect on the trace element concentrations according to the GLMs, with the exception of Ni concentrations in the digestive gland (*p*=0.02). Female digestive gland samples had higher Ni concentrations (mean=1.38±1.48 µg g<sup>-1</sup> dw) than male samples (mean=0.91±0.77 µg g<sup>-1</sup> dw).

Size had significant effects on the trace element concentrations in the GLMs of As, Cd, Co, Cu, Fe, and Zn concentrations (

,  $p < 0.05$ ). Digestive gland and mantle Fe concentrations decreased with increasing size (coefficient from GLM  $\sim -0.0003$ ). Arsenic concentrations decreased with size in the digestive gland (coefficient from GLM  $= -0.0004$ ) and increased in the mantle tissue (coefficient from GLM  $= 0.001$ ,

). In the mantle tissue samples, Cd and Cu concentrations decreased with size, whereas Cd concentrations increased with size in the digestive gland tissue (

). No significant relationships were observed between Hg concentration and size.

#### *Regional and species differences*

The highest concentrations of As, both in digestive gland and mantle tissue, were measured in the West Coast samples ( $41.3 \pm 11.9 \mu\text{g g}^{-1} \text{ dw}$ ; Appendix Table 3, Fig. 13a), and Hg was highest in both West Coast and Taranaki samples (Appendix Table 3). Taranaki samples contained the highest concentrations of Cd, Co, Cu, Fe, Mn, Pb, and Zn (Appendix Table 3). The greatest mantle and digestive gland concentrations of Cr and Ni were measured in the Dunedin samples, while the highest U concentrations were measured in the Chatham Rise samples (Appendix Table 3).

Table 13. GLM output for the digestive gland and the mantle models of trace element concentrations of arrow squids (*Nototodarus gouldi* and *N. sloanii*) from NZ waters. 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 dorsal mantle length (DML) are indicated with arrows ↑↑↑ 0.001, ↑↑ 0.01, ↑ 0.05).

<b>Digestive gland</b>												
	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	U	Zn
Size	↓↓↓	↑↑↑	↑↑↑		↓	↓↓↓						↑↑
Sex									*			
Region	***	***	***		***	***	*	**	***			***
Species	***											***
Region × Species	***	*										
<b>Mantle</b>												
	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	U	Zn
Size	↓↓↓	↓			↓↓↓	↓↓↓						
Sex												
Region	***		**		***	***	*					***
Species	***		*									
Region × Species	***		*			***						***

Table 14. Overall concentrations of trace elements (mean  $\pm$  standard deviation, minimum, and maximum) observed in the mantle ( $n=118$ ) and digestive gland tissue ( $n=118$ ) of *Nototodarus gouldi* and *N. sloanii* from New Zealand waters. Concentrations are in  $\mu\text{g g}^{-1}$  dw.

<i>N. gouldi</i>					<i>N. sloanii</i>			
Digestive gland			Mantle		Digestive gland		Mantle	
	mean $\pm$ sd	min–max	mean $\pm$ sd	min–max	mean $\pm$ sd	min–max	mean $\pm$ sd	min–max
As	29.4 $\pm$ 14.3	9.09–54.9	29.9 $\pm$ 17.7	7.48–59.6	11.3 $\pm$ 5.08	5.68–29.3	11.9 $\pm$ 9.54	3.03–40.7
Cd	194 $\pm$ 214	8.79–788	0.81 $\pm$ 0.51	0.17–1.82	89.3 $\pm$ 86.3	11.4–476	0.65 $\pm$ 0.46	0.09–1.58
Co	1.99 $\pm$ 1.31	0.36–4.47	0.50 $\pm$ 0.65	0.05–2.34	0.89 $\pm$ 0.87	0.20–4.62	0.36 $\pm$ 0.56	0.05–1.83
Cr	0.35 $\pm$ 0.18	0.10–0.87	0.26 $\pm$ 0.13	0.08–0.66	0.35 $\pm$ 0.26	0.09–1.33	0.32 $\pm$ 0.36	0.08–2.79
Cu	1185 $\pm$ 1008	172–4456	25.4 $\pm$ 18.3	7.50–106	352 $\pm$ 581	9.48–3981	23.3 $\pm$ 18	6.43–100
Fe	346 $\pm$ 231	75.7–1444	14.9 $\pm$ 11.6	4.11–57.0	186 $\pm$ 94.9	58.8–461	15.6 $\pm$ 9.28	4.53–58.2
Hg	0.44 $\pm$ 0.26	0.05–0.99	0.52 $\pm$ 0.41	0.09–2.13	0.14 $\pm$ 0.12	0.01–0.58	0.28 $\pm$ 0.22	0.01–0.89
Mn	2.61 $\pm$ 1.35	0.98–7.52	1.54 $\pm$ 0.84	0.64–4.67	1.54 $\pm$ 0.74	0.53–4.79	1.59 $\pm$ 0.82	0.82–6.67
Ni	1.05 $\pm$ 0.65	0.25–2.85	0.37 $\pm$ 0.20	0.14–1.13	1.17 $\pm$ 1.37	0.13–7.73	0.64 $\pm$ 0.67	0.14–3.87
Pb	0.56 $\pm$ 0.29	0.22–1.32	0.21 $\pm$ 0.32	0.06–2.03	0.26 $\pm$ 0.29	0.05–2.19	0.27 $\pm$ 0.50	0.05–4.16
U	0.03 $\pm$ 0.01	0.02–0.05	0.02 $\pm$ 0.008	0.02–0.03	0.03 $\pm$ 0.01	0.01–0.07	0.02 $\pm$ 0.02	0.008–0.13
Zn	351 $\pm$ 307	64.9–1575	64.2 $\pm$ 8.89	49.6–87.3	77.7 $\pm$ 68.6	13.3–480	55.4 $\pm$ 11.8	41.4–137

Trace element concentrations measured in digestive gland tissue showed clear regional differences when PCA was applied (Fig. 14a). The first and second PCA axes explained 36.9 % and 15.7% of the variance, respectively. The first principal component (PC) 1 was mainly driven by decreasing Cd, Co, Cu, Mn, Pb, and Zn, while PC 2 was mainly driven by increasing As and Hg concentrations. Taranaki and West Coast samples both formed distinct, separate groups, with the Taranaki samples being characterised by higher Cd, Co, Cu, Fe, Mn, Pb and Zn concentrations and the West Coast group being driven by high As and Hg concentrations; samples from all other regions were clustered together (Fig. 14a). For the mantle tissue PCA, the first and second PCA axes explained 22.8 % and 15.8% of the variance, respectively (Fig. 14b). The higher As and Hg concentrations in the West Coast samples showed a clear grouping (Fig. 14b). The Taranaki mantle samples also showed a clear grouping, which was driven by increased Cd concentrations (Fig. 14b). Increasing Fe, Cu, Mn and V were mainly driving PC 1, whereas PC 2 was mainly driven by increasing As, Cd and Hg concentrations.

In the GLMs, region had a highly significant effect on both digestive gland and mantle tissue concentrations of As, Cd, Co, Cu, Fe, Mn, Ni, and Zn (

), with particularly high concentrations of Cd in the Taranaki region and high As concentrations for the West Coast (Fig. 13a,b). No significant differences in Hg concentrations were observed among regions (

). Species, and the interaction term of region and species, had a significant effect on As, Cd, Fe and Zn concentrations, with *N. gouldi* generally showing higher concentrations (

).

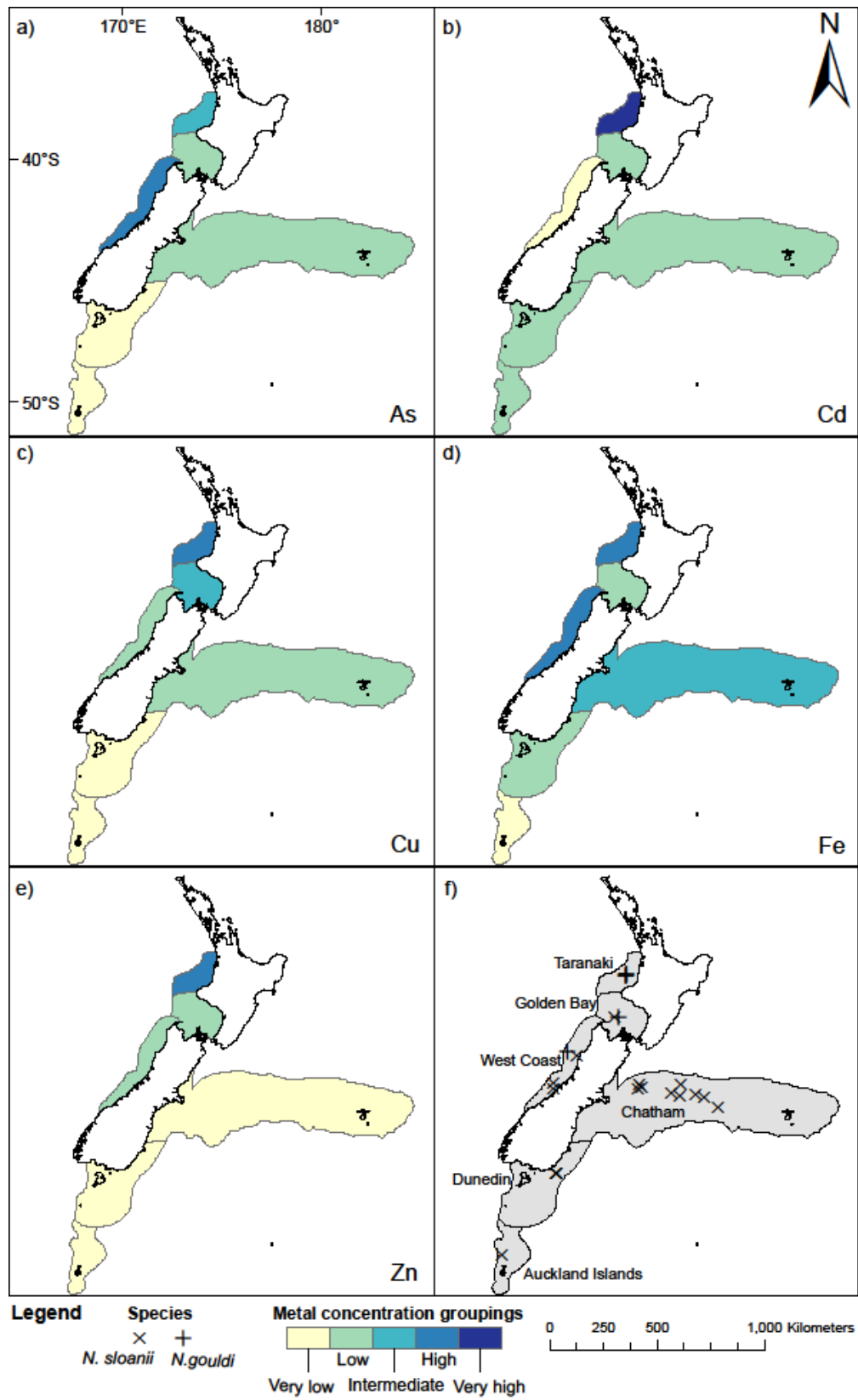


Fig. 13 a-e. Map of sampling area for arrow squid specimens (*Nototodarus gouldi* and *N. sloanii*.) with contour plots of the elements where region had a significant effect in the GLMs. Areas with similar metal concentration

(very low to very high) share the same colours (see legend). f: represented are the sampling locations for the six different sampling areas.

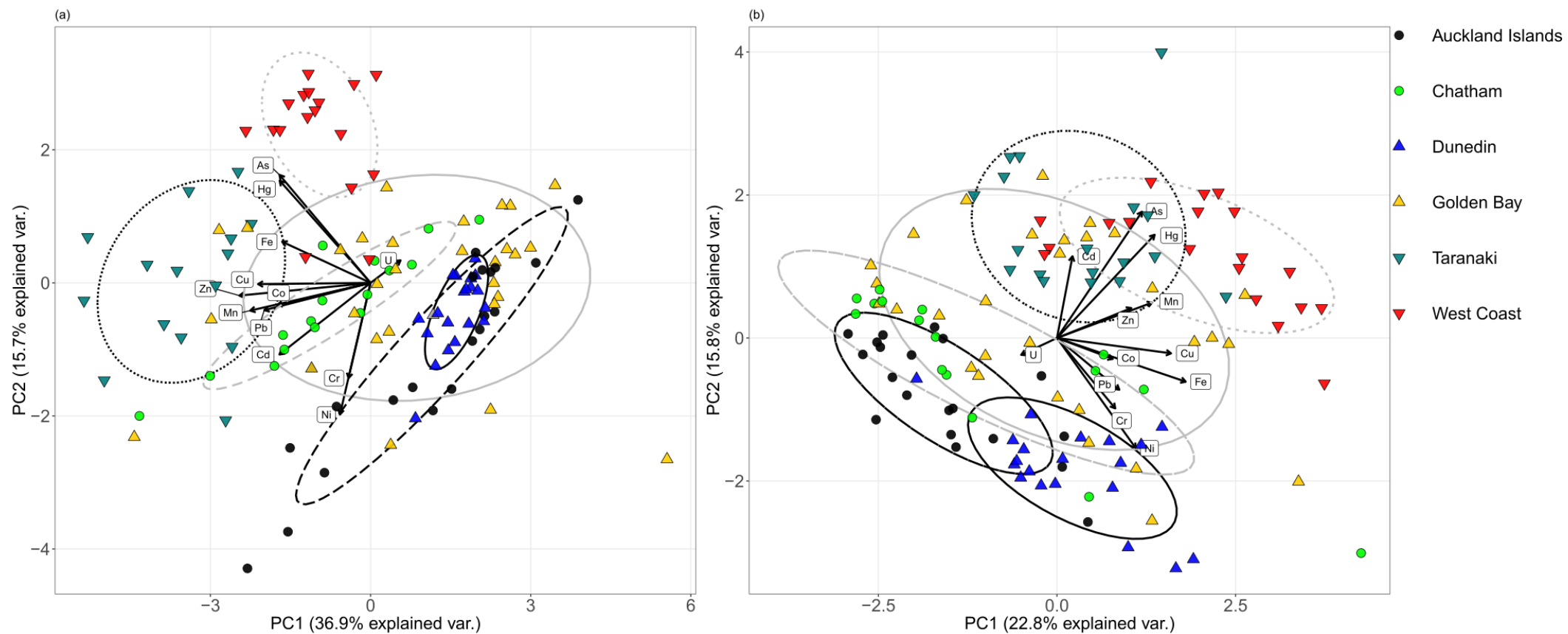


Fig. 14. Principal component analysis (PCA) of a.) digestive gland and b.) mantle tissue of *N. sloanii* from New Zealand's EEZ, according to sampling regions. Trace element loadings along PC1 and PC2 are presented by arrows.

## Discussion

Chapters 2 and 3 reported trace element concentrations in *Nototodarus sloanii* from the Chatham Rise. While those chapters focussed on a single locality and species, the present chapter analyses trace element concentrations in *N. gouldi* and *N. sloanii* from a variety of sampling locations throughout New Zealand's EEZ. This approach allows a comparison of areas where potential anthropogenic and natural sources might influence trace element concentrations, with implications for management decisions. At all sampling locations, the digestive gland had markedly higher trace element concentrations than the mantle tissue, as has been reported in the previous chapters (2 and 3) and by other authors (e.g., Ichihashi et al., 2001; Kojadinovic et al., 2011). In Southern arrow squid (*N. sloanii*), although the digestive gland makes up only ~8% of the total body mass, it contains ~90% of the total Cd (Chapter 3). This highlights the accumulation and detoxification abilities of this organ (e.g., Costa & Rodrigo, 2017; Penicaud et al., 2017). Our study focusses on mantle as well as digestive gland tissue because in New Zealand only squid muscular tissue is usually consumed by humans, whereas marine predators typically consume the entire animal and are therefore exposed to the total trace element body burden.

As previously reported (Chapters 2, 3), size had an observable effect on certain trace element concentrations (e.g., Cd, Cu, Fe) in the GLMs of the present arrow squid samples (

). A decrease of Cd with size was observed in the mantle tissue, while concentrations increased with size in the digestive gland (Table 14). The increase with size in the digestive gland possibly indicates the sequestration and accumulation of this element with size (Raimundo, 2005), while the negative size effect on Cd mantle concentrations could be explained by the observed dietary shift from Cd-rich crustaceans in juvenile squid to Cd-poorer fish in adults or dilution effects with growth (e.g., Chauvelon et al., 2011; Pierce et al., 2008). Apart from the biological parameters sex and size, the models used in this study analysed regional as well as species effects on trace element concentrations in arrow squids. For example, the concentrations of As, Fe and Zn were influenced by the interaction of region and species, with highest concentrations measured in *N. gouldi* from the West Coast (

, Appendix Table 3).

### *Regional and species differences*

*Nototodarus gouldi* and *N. sloanii* appear to occupy distinct, largely separate geographic ranges with a small overlapping zone (Uozumi & Förch, 1995). Thereby, most differences in trace element concentrations could be explained by a regional effect. Regional effects were observed for most trace elements, with particularly elevated concentrations of Cd, Co, Cu, Fe and Zn in the Taranaki region and high As, Fe and Hg concentrations in the West Coast. The potential sources for those concentration differences are discussed below, in sections for the West Coast and Taranaki. However, concentrations of As and Zn were also significantly influenced by the species with higher concentrations measured in *N. gouldi* (Table 14). This observation could be due to either physiological or dietary differences (Braley et al., 2010; Dunn, 2009), leading to species-specific bioaccumulation rates of those elements. The PCA (Fig. 14a,b) had a low rate of explanation (22.8 % of the variance explained by PC1), which highlights that region alone does not explain the high variability of trace element concentrations. The important drivers for As and Zn concentrations appear to be sex, size, and species. The physiological differences between the two species should therefore be further investigated.

### *West Coast*

The West Coast samples contained significantly higher levels of As and Fe (by orders of magnitude) than any of the other localities (Fig. 13; Table 14, Appendix Table 3). In marine sediments, As is strongly absorbed by Fe minerals, which means their concentrations are often linked (Edenborn et al., 1986; Neff, 1997). In the West Coast, As is primarily present in the form of arsenopyrite from gold-bearing ores (Haffert et al., 2010; Haffert & Craw, 2008; Hewlett et al., 2005; Pope et al., 2010) and Fe has been previously used for As neutralisation (Rait et al., 2010). However, the elevated levels of these elements could be linked with river discharges (Seixas et al., 2005) or acid mine drainage from mining of bituminous coals, which are primarily located in this region (Davies et al., 2011; Trumm et al., 2005). In addition, river run-offs from this region have been reported as particularly Fe-rich (Rait et al., 2010). As previously discussed, cephalopods likely take up trace elements through their diets (Bustamante et al., 2000, 2004; Koyama et al., 2000; Wang, 2002), and the As and Fe found in *N. spp.* specimens could therefore reflect the As- and Fe-enriched prey in this region. A regional biomonitoring study of another mollusc, the South Island green-lipped mussel, *Perna canaliculus*, reported elevated concentrations in specimens collected from West Coast beaches (e.g., Tauranga Bay, Chandurvelan et al., 2015).

### *Taranaki*

Specimens collected in Taranaki had significantly higher concentrations of Cd, Co, Cu, and Zn than those from other regions (Fig. 13, Appendix Table 3). The Cd concentrations observed in squid from this locality are also high relative to those reported for other pelagic cephalopods (e.g., Smith et al.,

1984; Bustamante et al., 2008), but comparable to concentrations described for *Ommastrephes bartramii* from Japanese waters (Kurihara et al., 1993, Chapter 1, Table 2). The Cd:Cu ratio (mean=0.04) was approximately twice as high as the other sample locations (mean~0.02). The co-occurrence between Cd and Cu has been reported in the digestive glands of other cephalopods (Bustamante et al., 2002a; Smith et al., 1984), and is due to the detoxification processes involved in the binding of excessive metals to metalloproteins (Jebali et al., 2008). The increased ratios might indicate higher detoxification activity for specimens exposed to higher Cd levels (e.g., Raimundo et al., 2005).

Elevated trace element concentrations could have many possible causes; for example, upwelling can lead to enhanced primary productivity (e.g., Auger et al., 2015; Pinedo-González et al., 2015). Upwelling has been linked to Cd-enriched waters in the Taranaki region (Bruland et al., 1978; Foster & Battaerd, 1985). Furthermore, volcanic emissions have been proposed as a source of trace elements in the atmosphere, soil, and marine environments (Buat-Ménard & Arnold, 1978; Hutton, 1983), and natural Cd can enter marine environments through the weathering of soils made of volcanic ash (Cronin et al., 1998; Deheyn et al., 2005; Hutton, 1983). Of the six sampling regions, Taranaki is the only dormant volcanic area (Hoernle et al., 2006); run-off from volcanic soils may be responsible for the increased trace element concentrations found in the specimens from this area. Algal blooms (Bargagli et al., 1996), and their link to the use of phosphorus-based fertilisers, can be a source of Cd (Francesconi et al., 1994; Glibert & Burkholder, 2006). For example, in the Taranaki region, along with the Waikato region, the highest mean soil Cd concentrations in agricultural land in New Zealand were measured (Abraham et al., 2018), which could influence coastal concentrations. In addition, the offshore waters of Taranaki may still experience anthropogenic pollution through mining activities (Vopel et al., 2017), such as hydraulic drilling (marine rigs) and offshore deep-water drilling, which have both been reported from the Taranaki region (Pitt, 2016). Drilling can disrupt sediments and expose marine organisms to higher Cd and other trace element concentrations (Ellis et al., 2017; Pozebon et al., 2005; Sadiq et al., 2003). This might explain why arrow squids from the Taranaki region accumulate higher Cd concentrations from their prey compared to arrow squids feeding in other study areas. However, with the current data, it is not possible to conclusively determine the source of the elevated trace elements found in this area.

#### *Impact on consumers and food web*

Differences were observed between species in the bioaccumulation patterns of As, Cu, Fe and Zn (Tables 13, 14). However, the consumption of either of the two species does not presently imply greater risks to human consumers, as concentrations lie below the established guidelines (Table 15).

In New Zealand, mantle tissue is the only part of arrow squids that is regularly consumed by humans. The World Health Organization has identified four elements of most concern for human health—As, Cd, Hg, and Pb (WHO, 2010a). Comparable to Chapter 3, in all mantle tissues samples analysed in this study, all four elements were below the maximum tolerable levels for these elements as defined by Food Standards Australia New Zealand (FSANZ, 2016) as well as below the tolerable weekly intake (TWI, EFSA, 2012ab) and the benchmark dose level (BMDL, Table 15). However, for children [5-6 years, ~20kg], the consumption of 120g arrow squid mantle tissue, with the maximum measured concentrations, would exceed the estimated weekly intake of Hg and Pb by orders of magnitude (Table 15). More studies are necessary to determine whether restricted consumption of arrow squid mantle tissue should be advised from the Taranaki region. In particular, guidelines for children and pregnant or breastfeeding women should be revised. Additionally, in some cultures small squids are eaten whole, and in other cultures the digestive gland (kimo, shiokara) is considered a delicacy (Kurihara, 1993; Kojadinovic et al., 2011). The Cd concentrations found in the digestive gland of NZ arrow squid makes them unsafe for human consumption in this way, as the average measured concentrations would exceed the maximum allowable levels of Cd in shellfish by 1233% (FSANZ, 2016).

Marine predators of arrow squid ingest the whole squid and are exposed to the high Cd concentrations in the digestive gland. Although the digestive gland constitutes only ~8% to the squid's body mass (Lischka et al., 2020), in the case of the high Taranaki samples, this equates to an estimated 407 µg Cd per digestive gland (dw converted to ww before calculation) for the average arrow squid (454g). Predatory fishes of arrow squids, such as the long-lived orange roughy (*Hoplostethus atlanticus*; Clark, 1999) and New Zealand hoki, *Macruronus novaezelandiae*, could therefore bioaccumulate Cd from squid. However, there is a general paucity of data regarding Cd concentrations in fish species caught in New Zealand; further analysis is necessary to fully understand the potential role of arrow squids as a vector for Cd. Such information is especially important in regions with enhanced primary productivity, which can be linked to high concentrations of trace elements, such as Cd (e.g., Auger et al., 2015; Pinedo-González et al., 2015).

Table 15. Arrow squids (*Nototodarus gouldi* and *N. sloanii*) mantle tissue concentrations (mean and maximum) for total As (tAs), Cd, Hg, and Pb (µg g<sup>-1</sup> ww) compared to the maximum allowable levels in shellfish as defined by Food Standards Australia and New Zealand (FSANZ, 2008). The benchmark dose level (BMDL) and tolerable weekly intake (TWI)<sup>3</sup> for children (8kg) and adults (70kg). The mean and maximum measured concentrations are compared to the estimated weekly exposure of elements in New Zealand adults and infants<sup>2</sup>. Portion size refers to a meal of 120g squid mantle tissue.

	tAs	Cd	Hg	Pb
<b>Guidelines</b>				
Maximum allowable levels in shellfish (µg g <sup>-1</sup> )		2.0	0.50	2.0

Maximum allowable levels in shellfish ( $\mu\text{g g}^{-1}$ ) per portion <sup>week</sup>		240	60.0	240
TWI ( $\mu\text{g kg}^{-1} \text{bw week}$ )		2.5	4.0 <sup>4</sup>	
TWI for 70kg adult <sup>week</sup>		175	280	
TWI for 8kg child <sup>week</sup>		20.0	32.0	
BMDL ( $\mu\text{g kg}^{-1} \text{bw week}$ ) <sup>1</sup>	N.A.	15.8 <sup>1</sup>	N.A.	4.20 <sup>1</sup>
BMDL for 70kg adult <sup>week</sup>		1098		294
BMDL for 8kg child <sup>week</sup>		125.44		33.6
<b>Empirical data</b>				
Estimated weekly exposure adult ( $>25$ years in $\mu\text{g kg}^{-1} \text{bw week}$ ) <sup>2</sup>	12.4	12.2	0.30	0.55
Estimated weekly exposure 70 kg adult <sup>week</sup>	868	854	21.0	38.5
Estimated weekly exposure children (5-6 years in $\mu\text{g kg}^{-1} \text{bw week}$ ) <sup>2</sup>	N.A.	3.05	0.75	1.13
Estimated weekly exposure 20kg child <sup>week</sup>	N.A.	61.0	15.0	22.6
<b>This study</b>				
Mean measured mantle concentration ( $\mu\text{g g}^{-1}$ wet weight)	3.52	0.14	0.07	0.05
Maximum measured mantle concentration ( $\mu\text{g g}^{-1}$ wet weight)	11.9	0.36	0.43	0.83
Estimated intake by portion with mean concentration (120g mantle tissue; $\mu\text{g}$ )		17.3	8.88	5.76
estimated intake by portion with maximum concentration (120g mantle tissue; $\mu\text{g}$ )		43.7	51.1	99.8
% of estimated weekly exposure adult (one portion <sup>week</sup> ) <sup>5</sup>		5.11	243	259
% of estimated weekly exposure children (one portion <sup>week</sup> ) <sup>5</sup>		71.6	341	442

<sup>1</sup> The BMDL are calculated from  $\mu\text{g kg}^{-1} \text{bw day}$  to  $\mu\text{g kg}^{-1} \text{bw week}$ .

<sup>2</sup> Concentrations extracted from the New Zealand Total Diet Study (MPI, 2018)

<sup>3</sup> European Food Safety Authority (EFSA, 2012ab)

<sup>4</sup> For inorganic Hg

<sup>5</sup> with max. measured concentration

Local marine mammals such as fur seals, *Arctocephalus forsteri* (Harcourt et al., 2002; Emami-Khoyi, 2016) also rely heavily on arrow squid in their diet (Childerhouse et al., 2001; Fraser & Lalas, 2004; Van Heezik, 1990). In one study, the stomach contents of nine stranded long-finned pilot whales (*Globicephala melas edwardii*) were almost exclusively composed of arrow squids (98%; Beatson & O'Shea, 2009). If these whales were feeding in the Taranaki region, an area they are known to frequent (Thompson & Abraham, 2009), they would be exposed to arrow squid highly rich in Cd (~121 mg Cd per day, Chapter 6.), which could bioaccumulate in their tissues. High Cd concentrations in stranded pilot whales have also been measured elsewhere in the world. For example, stranded pilot whales in Scotland (Gajdosechova et al., 2016) and the Faroe Islands (Caurant & Amiard-Triquet, 1995) had

kidney Cd concentrations exceeding the minimum adverse-effect levels established for humans (mean 74–78  $\mu\text{g g}^{-1}$  ww, Järup & Åkesson, 2009). These high concentrations have been well described for cetaceans and other marine mammals, but their effects are not fully understood (Das et al., 2003; Dietz et al., 2013). For example, a study on Arctic bowhead whales (*Balaena mysticetus*) showed a positive relationship between liver Cd concentrations and lung fibromuscular hyperplasia (Rosa et al., 2008). Similarly, a study of Atlantic long-finned pilot whales reported a significant correlation of high Cd concentrations with signs of cardio-vascular stress (Sonne et al., 2010). Within New Zealand's pelagic food web, arrow squids could be a main vector for the transfer of Cd. Hence, Cd concentration in one of the arrow squid's main predators, New Zealand's long-finned pilot whale, will be analysed in Chapter 6 to gain insight into the food web accumulation.

## Conclusion

Strong regional differences in the trace element concentrations of arrow squid (*N. gouldi* and *N. sloanii*) within New Zealand's Exclusive Economic Zone were observed. The findings indicate that the bioaccumulation of Cd and other trace elements can vary significantly among regions. Especially high Cd and Cu concentrations were measured in squids from the Taranaki region; anthropogenic and/or natural sources may drive these differences. Arrow squid caught from the West Coast of NZ's South Island contained elevated concentrations of As and Hg, which may be related to the acid mine drainage and gold mining activities. The measured concentrations of As, Cd, Hg and Pb were all below the maximum allowable concentrations as well as below the tolerable weekly intake and the benchmark dose level defined for those elements, in all mantle tissue samples analysed. Despite their relatively small contribution to the whole body burden, for marine predators, the high Cd levels measured in the digestive gland tissue in samples from Taranaki indicate that arrow squids may play an important role as vectors for Cd to apex predators, such as long-finned pilot whales. Analysis of toxic trace element accumulation in an important and abundant prey species in New Zealand's pelagic food web demonstrates their potential for bioaccumulation and raises questions about the consequences for their predators. However, these potential sources for the trace element concentrations remain speculative, and further investigation is needed.

## **Chapter 5: Trace element concentrations and stable isotopes in the greater-hooked squid *Moroteuthopsis ingens* from the Chatham Rise, New Zealand**

*This chapter is based on the following published article:*

Lischka, A., Braid, H. E., Bolstad, K. S. R., Lacoue-Labarthe, T., & Bustamante, P. (2020). Influence of sexual dimorphism on trace element concentrations and stable isotopes in the greater-hooked squid *Moroteuthopsis ingens* from New Zealand waters. *Marine Environmental Research* 179, 104976, <https://doi.org/10.1016/j.marenvres.2020.104976>:

### **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 measured 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 chapter 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.

### **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 & 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. 2003a, 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 & Zauke, 2008; Keil et al., 2008; Petri & 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 (Eagles-Smith et al. 2018; Fisher & Reinfelder, 1995; Rainbow, 1995).

Cephalopods are crucial members of the pelagic food webs, as they are consumed by a variety of apex predators (Boyle & 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 & Flegal, 1975; Miramand et al., 2006; Miramand & Bentley, 1992), and have been suggested as a major vector for Cd to their predators (Bustamante et al., 1998a).

The ‘greater hooked’ or ‘warty’ squid, *Moroteuthopsis ingens* (Smith, 1881), formerly known as *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, that migrates to deeper water with ontogeny (Fig 15), resulting in a shift in diet with ontogeny (Arkhipkin & 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 & Duhamel, 2003; McArthur et al., 2003; Rosas-Luis et al., 2014). In addition, cannibalism has been reported in this species (Ibáñez & Keyl, 2010). Besides 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 & Burton, 1993), and sperm whale, *Physeter macrocephalus* (Clarke & Roper, 1998; Mansilla et al., 2012). Finally, *Moroteuthopsis ingens* is a frequent bycatch species in commercial trawling (Cherel & 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 & Williams, 1994).

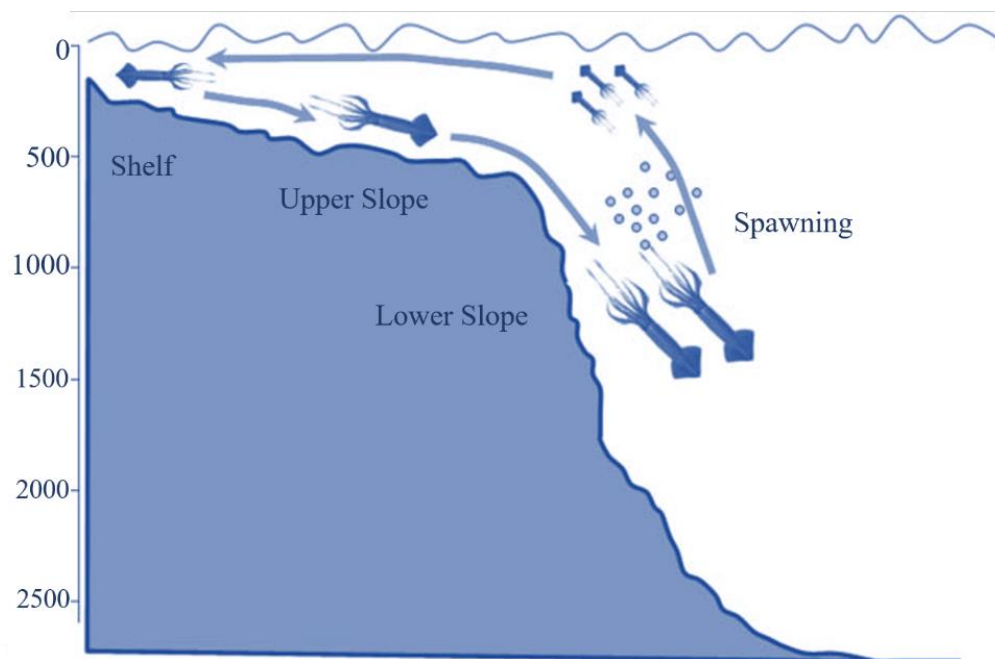


Fig. 15. Model of the life-cycle of *M. ingens* along the Patagonian shelf, adapted from Arkhipkin & Laptikhovsky, 2010).

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. 16). 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 & 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. (Chapters 2–4), greenshell mussels, *Perna canaliculus* (Whyte et al., 2009), finfish species such as snapper, *Chrysophrys auratus* (Brooks & 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 chapter aims to provide information on trace element levels and distribution in *M. ingens* tissues and to analyse the possible influence of sexual dimorphism on the observed concentration patterns. The 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°55' to 45°07' S, and 174°15' to 179°58' E, Fig. 16). In total, 84 specimens, including 25 males (89–470 mm in mantle length [ML]) and 59 females (98–563 mm ML; with  $n=15$  >400mm ML, Appendix Table 4) 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  $\delta^{15}\text{N} =$

$[(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$ , 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  $0.01\text{ }\mu\text{g g}^{-1}$ ; As was  $10.22\text{ }\mu\text{g g}^{-1}$ ; Cr was  $0.1\text{ }\mu\text{g g}^{-1}$ ; Fe and Zn were  $4.09\text{ }\mu\text{g g}^{-1}$ ; Cu was  $1.02\text{ }\mu\text{g g}^{-1}$ ; Ni was  $0.04\text{ }\mu\text{g g}^{-1}$ ; Se was  $20.43\text{ }\mu\text{g g}^{-1}$  and V was  $2.04\text{ }\mu\text{g g}^{-1}$  (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).

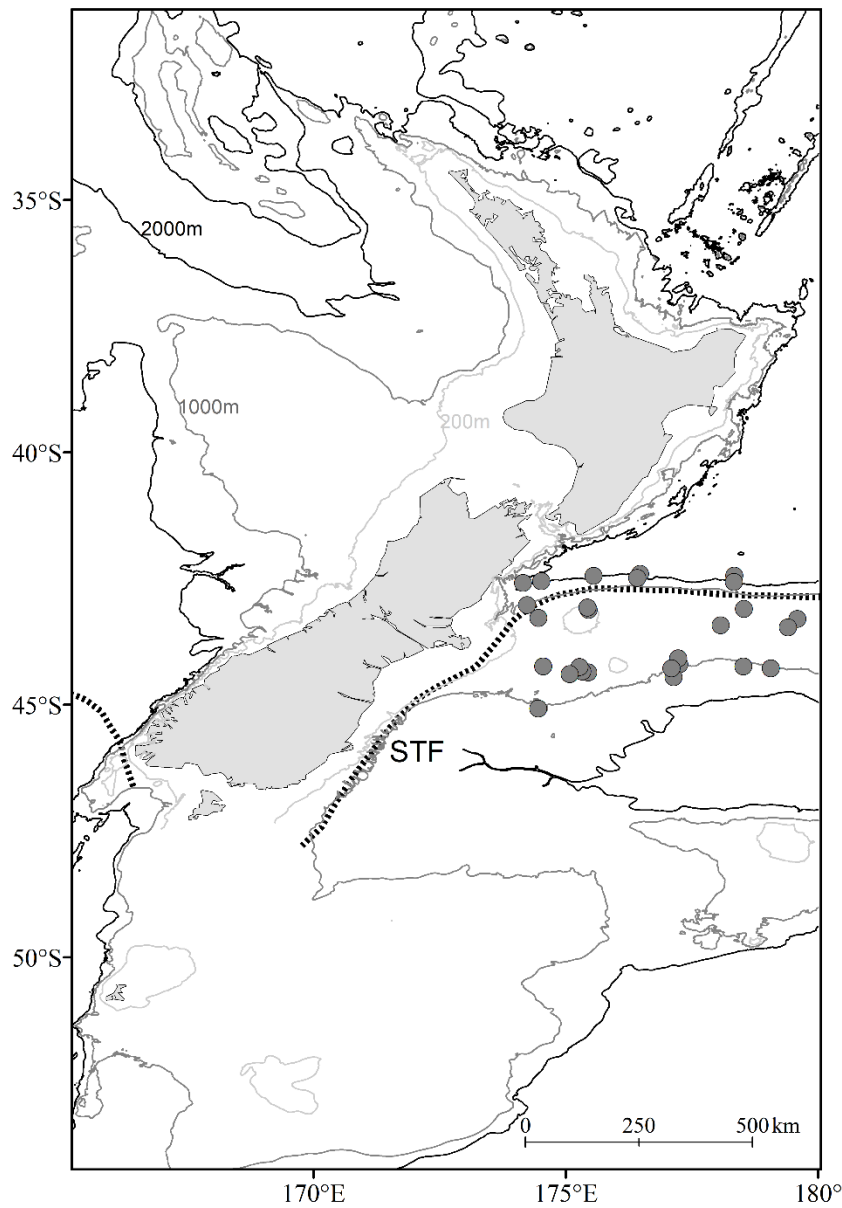


Fig. 16. Map of the Chatham Rise showing sampling stations of the *Moroteuthopsis ingens* specimens analysed in the present chapter. Depth bars include 200 m, 1000 m and 2000 m. The subtropical front (STF) is indicated by a dashed line.

### *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 ML) 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. 17; Appendix Table 5). 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. 17; Appendix Table 5).

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 16). The overall highest concentration for Cd was observed in the digestive gland of a male specimen (377 mm ML) with  $1030 \mu\text{g g}^{-1} \text{dw}$  (Table 16).

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 ( $R=0.35$ ,  $p=0.24$ ; Fig. 18).

Tissue type had a significant effect on Ag, Cd, Cu, Fe, Hg, Pb, and Zn concentrations according to the GLMs (Table 17). 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 16). In both tissues, size had a positive effect on Ag ( $p<0.001$ ), Hg ( $p<0.001$ , Fig. 19), 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 17). The digestive gland concentrations of Pb in smaller individuals of both sexes (< 200 mm ML) were on average 3.1 times higher than those observed in larger individuals (Tables 16, 17). The  $\delta^{13}\text{C}$  values had significant effects on Ag, Cd (Fig. 19), Cu, Pb and Zn concentrations, while  $\delta^{15}\text{N}$  showed significant effects on Cu, Fe, Pb and Zn concentrations in both tissues (Table 17). Sex effects were observed for Cd, Cu, Fe ( $p<0.001$ ) and Zn ( $p<0.05$ ). The interaction term ‘sex×size’ had a significant effect on Cu and Zn ( $p<0.001$ ) concentrations (Table 17).

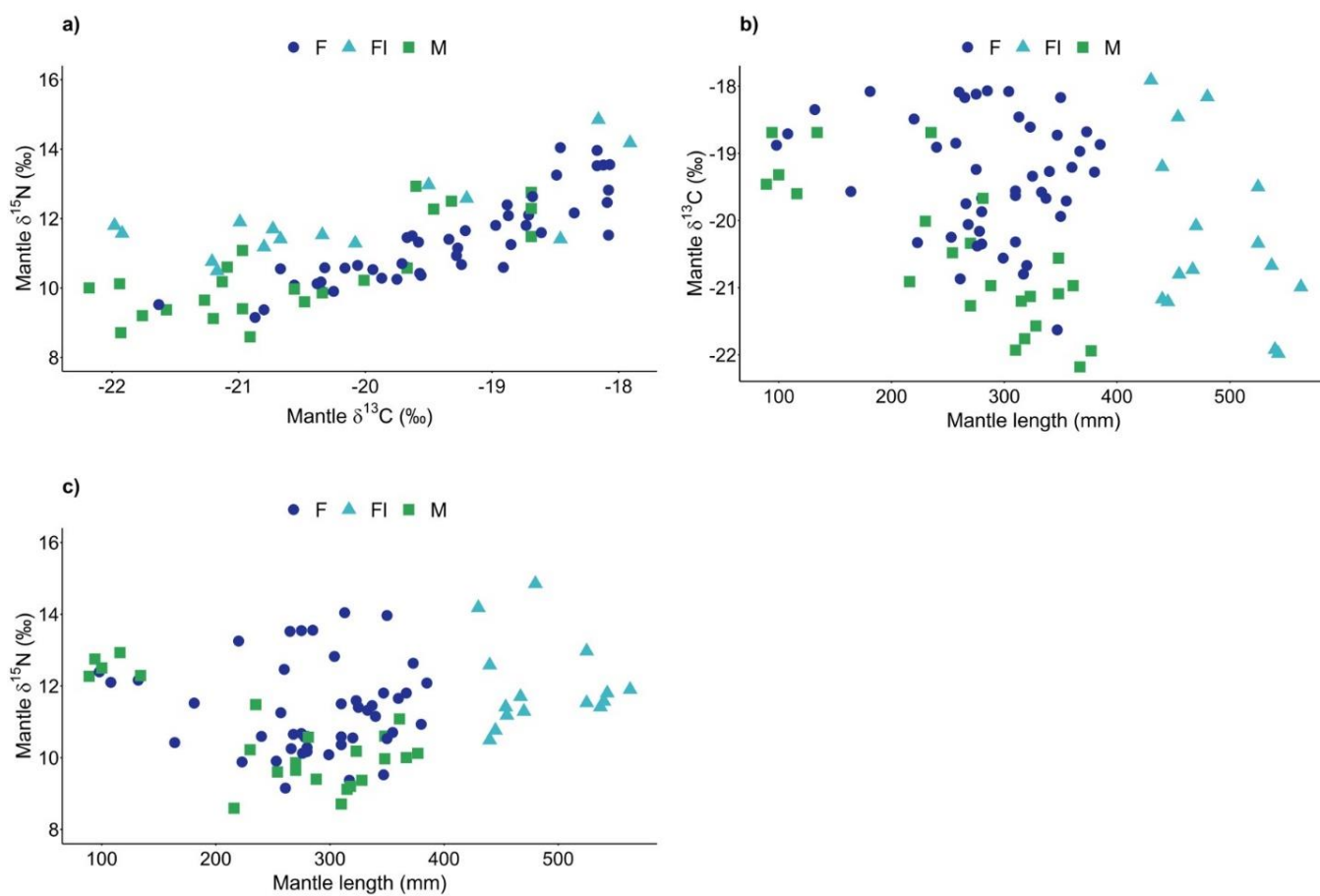


Fig. 17. Relationship between a.)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , b.) mantle length (mm) and  $\delta^{13}\text{C}$ , and c.) Size and  $\delta^{15}\text{N}$  in the mantle tissue of female (F,  $n=44$ ), large female (FI >400 mm ML,  $n=15$ ) and male (M,  $n=25$ ) *M. ingens* from the Chatham Rise, New Zealand.

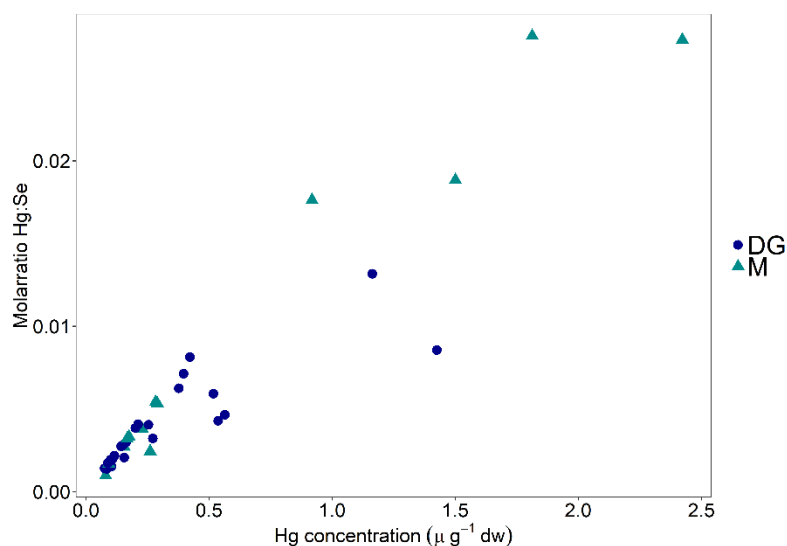


Fig. 18. Concentrations of Hg ( $\mu\text{g g}^{-1}\text{ dw}$ ) and the molar ratio of Hg:Se in the mantle tissue analysed of *M. ingens* from the Chatham Rise, New Zealand.

Table 16. 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 ‰. NA= Data not available.

	♀ Digestive gland (n=59)		♂ Digestive gland (n=25)		♀ Mantle (n=59)		♂ Mantle (n=25)	
Element	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	0.94-678.3	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	2.88-424.7	28.38 $\pm$ 34.92	4.86-176.4	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 $\pm$ 26.8	15.57-169	42.65 $\pm$ 12.25	26.34-76.19	66.88 $\pm$ 12.3	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	-22.1--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

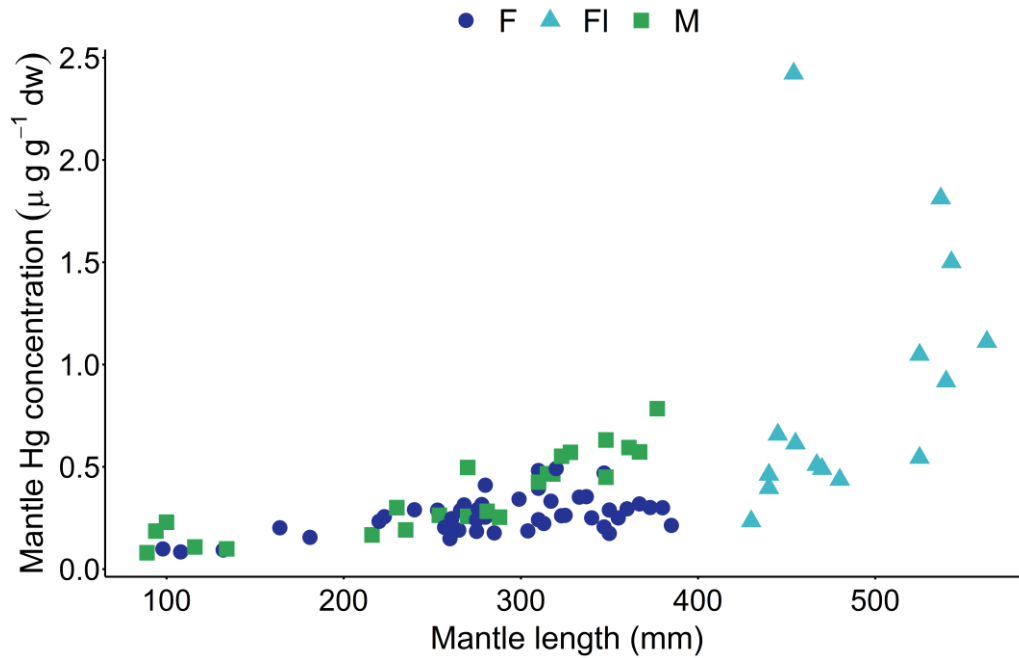


Fig. 19. Relationship between mantle length (mm) and Hg concentrations (in  $\mu\text{g g}^{-1}\text{ dw}$ ) in the mantle tissue of female (F,  $n=44$ ), large female (FI > 400 mm ML,  $n=15$ ) and male (M,  $n=25$ ) *M. ingens* from the Chatham Rise, New Zealand.

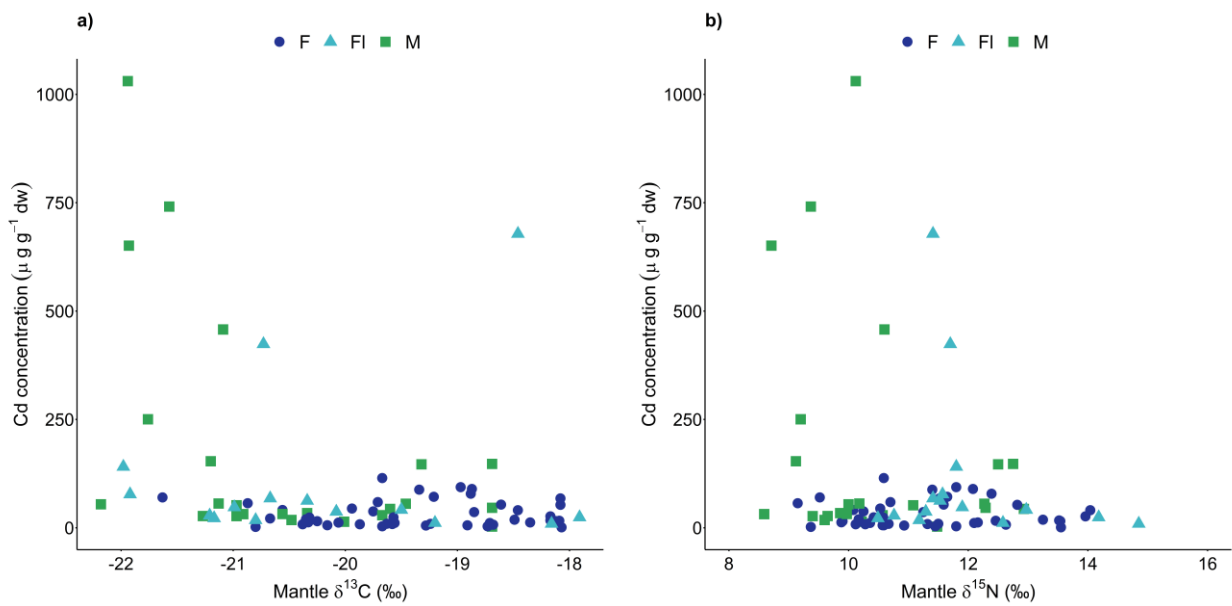


Fig. 20. Relationship between Cd concentration (in  $\mu\text{g g}^{-1}\text{ dw}$ ) in the digestive gland and a.)  $\delta^{13}\text{C}$  and b.)  $\delta^{15}\text{N}$  in the tissues of female (F,  $n=44$ ), large female (FI > 400 mm ML,  $n=15$ ) and male (M,  $n=25$ ) *M. ingens* from the Chatham Rise, New Zealand.

Table 17. 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						***							***

## Discussion

The present chapter 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, with large females having both higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (Fig 17). 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 (59–400 mm ML) primarily forage in similar habitats, while larger females (>400 mm ML) 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. 17). This ontogenetic migration in females, resulting in a change in diet, has been previously reported in this species (Jackson & 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, the 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 17). Size had a negative effect on Cd concentrations and sex showed a significant effect, with the highest concentrations observed in the digestive gland of males (Tables 16, 17). 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 & Laptikhovsky, 2010; Jackson et al., 1997), highlighted by the significantly different  $\delta^{13}\text{C}$  values (

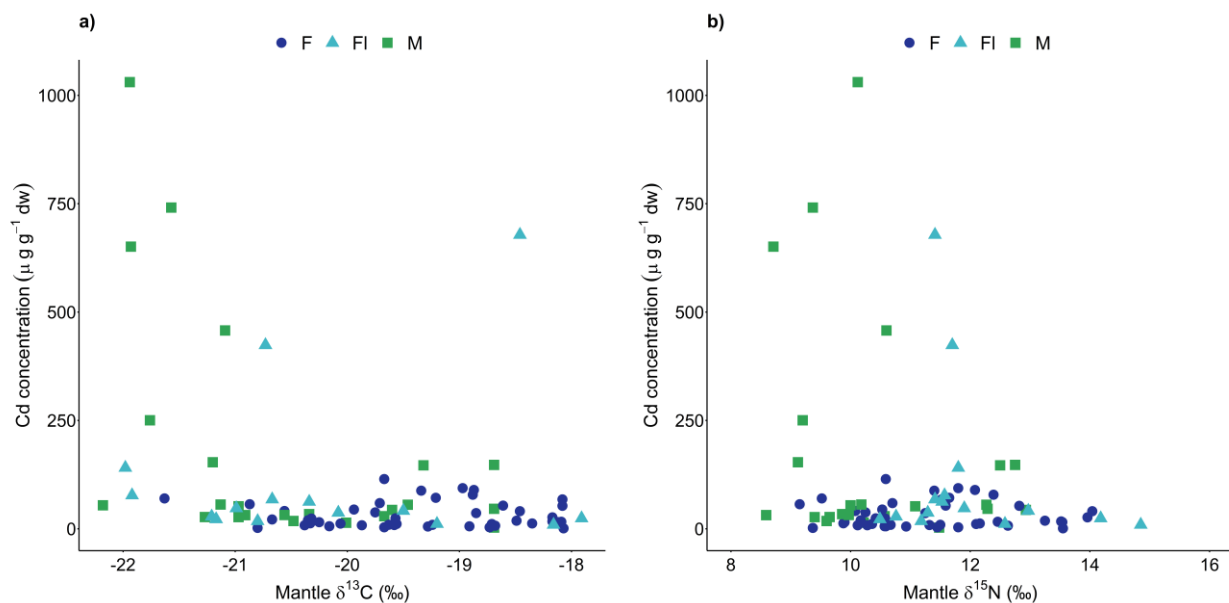


Fig. 20). Oceanic Cd concentrations vary across the Chatham Rise and the upwelling of low Cd sub-Antarctic waters to intermediate depths (200–400 m) has been previously suggested (Frew, 1995; Frew & Hunter, 1995). As sex-specific habitats are suggested by the  $\delta^{13}\text{C}$  values (Table 16), 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 & Mladenov, 1994). Spent females appear to cease feeding (Arkhipkin & 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 chapter (with the exception of the high Cd concentrations measured in males, Table 16). Compared to other squids, the mean digestive gland Cd concentrations in the female specimens ( $52.9 \pm 103 \mu\text{g g}^{-1} \text{ dw}$ ) 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 ( $89.0 \pm 114 \mu\text{g g}^{-1} \text{ dw}$ ; Chapter 3). In particular, male *M. ingens* exhibited similar Cd concentrations ( $166 \pm 267 \mu\text{g g}^{-1} \text{ dw}$ ) 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 the present results in that respect.

#### *Mercury and selenium*

The mantle tissue of *M. ingens* had higher concentrations of Hg compared to the digestive gland (Tables 16, 17). Observed Hg concentrations are consistent with previous studies on different squid species from the following families: Architeuthidae, Gonatidae, Ommastrephidae, Onychoteuthidae, 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; Chouvelon 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 17; Fig. 19). In several cephalopod species, the

bioaccumulation of Hg is also size related (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 *Todarodes sagittatus* (Chouvelon 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; Chouvelon 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 chapter. 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 (Blum et al., 2013; Renedo et al., 2018).

The positive correlation of Hg and Se concentrations in the digestive gland of *M. ingens* (Fig. 18) 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 (Civin-Aralar & 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 *Octopus 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 17) 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 ( $0.06\text{--}0.13\ \mu\text{g g}^{-1}\text{ dw}$ ; 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) ( $0.11 \pm 0.08\ \mu\text{g g}^{-1}\text{ dw}$ , 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  $\mu\text{g g}^{-1}$  dw), and were higher than those reported in *N. sloanii* from the same area (0.01–0.89  $\mu\text{g g}^{-1}$  dw, Chapters 3). 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 (>800m, Arkhipkin and Laptikhovsky, 2010), a segregation corroborated by the  $\delta^{13}\text{C}$  values observed in this chapter.

### Lead

Overall, the highest Pb concentrations were measured in the digestive gland of male specimens (Tables 16, 17). Size had a significant negative effect on Pb concentrations, with smaller individuals exhibiting the highest Pb concentrations (Table 17). Both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ratios 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 & Laptikhovsky, 2010). Concentrations of Pb in the open ocean are predicted to reach their maximum concentrations in near surface layers (Henderson & Maier-Reimer, 2002), where smaller individuals, including small males, feed (Arkhipkin & Laptikhovsky, 2010). In contrast, large female *M. ingens* tend to migrate to, and then reside in, deeper waters (Arkhipkin & Laptikhovsky, 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 chapter (Tables 16, 17). The cycling of Pb in New Zealand offshore waters remains understudied and future research on Pb concentrations measured 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 16, 17), 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 17), 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, 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 described 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 & Bentley, 1992). Metallothionein has, however, been rarely quantified in cephalopods (see Bustamante et al., 2002a; Craig & 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 individuals had higher Zn concentrations (Table 17), 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 than smaller males (Tables 16, 17). 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 & 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 ratios observed on Zn concentrations supports 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 & 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  $\delta^{13}\text{C}$  ratios 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. Overall, Cd concentrations in male *M. ingens* were comparable to those observed in both sexes of arrow squid, *N. sloanii*, from the same locality (Chapters 2, 3). 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 the female specimens from the Chatham Rise were higher than those observed in *N. sloanii* from the same location and *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.

## **Chapter 6: Arrow squids (*Nototodarus* spp.) as potential vectors for cadmium and mercury in long-finned pilot whales (*Globicephala melas edwardii*) from New Zealand waters**

*This chapter is based on the following article submitted to Marine Pollution Bulletin:*

*Lischka, A., Betty, E. L. Braid, H. E., Pook, C. J., Gaw, S., & Bolstad, K. S. R. Arrow squids (*Nototodarus* spp.) as potential vectors for cadmium and mercury in long-finned pilot whales (*Globicephala melas edwardii*) from New Zealand waters.*

### **Abstract**

The Southern Hemisphere long-finned pilot whale (*Globicephala melas edwardii*) is a top predator in the New Zealand pelagic food web, feeding predominantly on arrow squids (*Nototodarus* spp.). This study quantified trace element concentrations (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Mn, Pb, Se, V, and Zn) in four tissues of 21 *G. m. edwardii* individuals from two stranding sites in New Zealand. Maximum Cd and Hg concentrations were measured in liver and kidney, respectively. Sex, size, and age had significant effects on Cd and Hg. Selenium had a positive correlation with Cd and Hg in most tissues, suggesting a role of Se in Cd and Hg detoxification. Arrow squids from the whales' stomach contents were DNA barcoded and trace element concentrations were measured in the squid samples. Specimens were identified as *Nototodarus sloanii*; our results suggest their major role in Cd and Hg transfer to *G. m. edwardii*.

### **Introduction**

Marine biota can bioaccumulate certain trace elements, such as Cd or Hg, which are then transferred from prey to predators (Suedel et al., 1994). For example, the organic form of Hg, methyl-Hg, biomagnifies through the food web, reaching highest concentrations in apex predators, including the long-finned pilot whale (Gajdosechova et al., 2016; Gray, 2002). In marine ecosystems, environmental trace element concentrations vary based on regional differences and pollution levels (Tchounwou et al., 2012). Concentrations of trace elements are influenced both naturally, such as by geothermal activity, submarine volcanoes or sedimental disposition (Moore & Ramamoorthy, 2012), and anthropogenically, e.g. by mining, industrial waste disposal or agricultural run-off (Ansari et al., 2004).

Cetaceans are apex predators in marine ecosystems and have been the focus of some trace element studies (Das et al., 2003; de Carvalho et al., 2008; Kemper et al., 1994; Kershaw and Hall, 2019). For example, extremely high Cd and Hg concentrations in liver and kidney samples have been reported for North Atlantic long-finned pilot whales, *Globicephala melas melas*, Traill, 1809 (Caurant & Amiard-Triquet, 1995; Gajdosechova et al., 2016; Simmonds et al., 1994). Cetaceans appear to have a high tolerance for these elements, and, apparently, effective metal detoxification mechanisms, e.g., methyl-

Hg detoxification through the formation of Hg:Se complexes (Koeman et al., 1973). Such strategies seem to allow cetaceans to cope with these high concentrations, as measured in hunted and stranded individuals from the North Atlantic (Caurant et al., 1996). For humans, the Cd and Hg concentrations previously reported for *G. m. melas* would pose a serious health threat if consumed (Endo et al., 2005; Simmonds et al., 1994, 2002).

In the Southern Hemisphere, the subspecies *G. m. edwardii* (Smith, 1881) ranges throughout the southern South Pacific and Atlantic Oceans, with a southern range limit close to the Antarctic Polar Front (Olson, 2009). Southern Hemisphere long-finned pilot whales have a long history of mass stranding around the New Zealand coastline (Betty et al., 2020; Brabyn & McLean, 1992). In addition, this species is culturally significant in New Zealand because of its *taonga* (treasure) status to *tangata whenua* (New Zealand's indigenous people). However, despite frequent strandings of *G. m. edwardii* in the Southern Hemisphere, this subspecies has not been the subject of any trace element studies to date.

Diet is the main entry pathway for most trace elements in mammals, including cetaceans; further exposure can be through dermal absorption and inhalation (Nordberg et al., 2014). The known diet of *G. m. edwardii* in the Tasman Sea is largely composed of cephalopods (Beasley et al., 2019), similar to the reported diet of the North Atlantic subspecies (Gannon et al., 1997; Overholtz and Waring, 1991). For individuals stranded in New Zealand, arrow squids (*Nototodarus* spp.) comprise the bulk of reported prey (Beatson et al., 2007b; Beatson and O'Shea, 2009). Two species of arrow squids occur within New Zealand's Exclusive Economic Zone (EEZ): *N. gouldi* (McCoy, 1888), and *N. sloanii* (Gray, 1849; Uozumi, 1998). Gould's arrow squid, *N. gouldi*, is most abundant around the North Island's east and west coasts and the South Island's northwest coast (Uozumi and Förch, 1995), while the endemic southern arrow squid, *N. sloanii*, is found south of the subtropical front east of New Zealand's South Island (Chen, 1998). Previous studies on trace element concentrations in New Zealand arrow squids have demonstrated a high bioaccumulation capacity for Cd and other trace elements (Chapters 2–4). However, the bioaccumulation of trace elements in long-finned pilot whales from the Southern Hemisphere has not been previously studied, despite the importance of understanding the bioaccumulation pathways in marine food webs.

This chapter aimed to assess the burden of trace elements (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Mn, Pb, Se, V, and Zn) in *G. m. edwardii*. The objectives of the present study were to: 1) analyse the accumulation and distribution of trace elements within the blubber, kidney, liver, and muscle tissues of 21 stranded individuals and to test for the potential effects of age, sex, and total body length on these concentrations; 2) identify arrow squid species from the stomach contents of these cetaceans and test

the trace element burden in the squid specimens; and 3) combine predator and prey trace element concentration data to estimate the predator's total trace element burden.

## Material and Methods

### *Sample collection*

Samples and morphometric measurements were collected from *G. m. edwardii* individuals that had stranded and died in New Zealand at Farewell Spit (Golden Bay, January 2014) and West Ruggedy Beach (Stewart Island, February 2010; Fig. 21; Table 18). Following standard cetacean post-mortem examination procedures (Geraci & Lounsbury, 2005), samples of teeth, blubber, kidney, liver, and muscle were analysed from a total of 21 specimens (10 females and 11 males). Stomach contents were analysed from 10 individuals (5 females, 5 males) from the Stewart Island stranding. The decomposition status of all specimens analysed in this study was fresh to mild (classified after Geraci and Lounsbury, 2005), minimising any potentially confounding factors. All samples were stored at -20°C until further processing. From each of the ten *G. m. edwardii* specimens collected from Stewart Island, ten samples of squid mantle tissues were collected from the stomach (total  $n=100$ ) for trace element analysis and DNA barcoding.

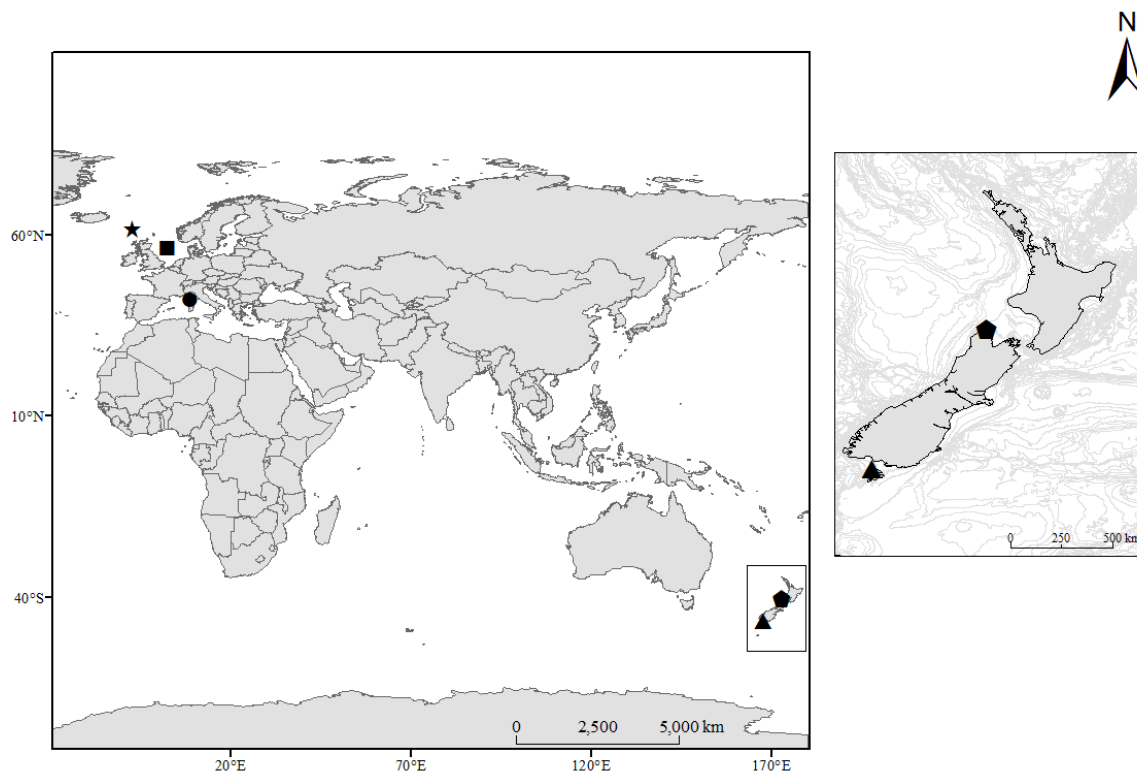


Fig. 21 Map of *Globicephala melas* specimens used for trace element analysis in previous and present studies. North Atlantic *G. m. melas* represented from the Faroe Islands (★, Caurant et al., 1996), Scotland (■, Gajdosechova et al., 2016), and Corsica (●, Frodello & Marchand, 2001). New Zealand specimens of *G. m. edwardii* used in this study were sampled during mass stranding events in Golden Bay (◆, 2014) and Stewart Island (▲, 2010).

Table 18. Details for *G. m. edwardii* specimens analysed during mass stranding events in New Zealand, including age and length (mean±standard deviation, minimum, and maximum).

Stranding Location	Sample size (n)	Size (m) mean±sd	min-max	Age (years) mean±sd	min-max	Stomachs analysed
<b>Farewell Spit, Golden Bay</b>	11					No
Female	5	3.95±0.40	3.26-4.36	14.6±9.9	3.5-28	
Male	6	4.45±1.09	2.76-5.56	10.7±6.1	2 - 18	
<b>West Ruggedy Beach, Stewart Island</b>	10					Yes
Female	5	4.23±0.35	3.64-4.60	20±7.8	13.5-32	
Male	5	4.17±0.47	3.54-4.90	9.1±4.2	4-14	

#### *Age estimation*

The ages of *G. m. edwardii* specimens were estimated by examining decalcified and stained tooth sections using a binocular microscope (10–40× magnification) and counting annual growth layer groups (GLGs) in the dentine, as defined by Perrin & Myrick (1980). Tooth preparation methods followed Betty et al. (2019). All age estimates were initially made ‘blind’ (with no biological information on the animal), with replicate counts made by at least two experienced readers.

#### *DNA barcoding*

DNA was extracted from the ingested squid mantle tissue using EconoSpin columns (Epoch Life Science) with QIAGEN reagents following protocols for the DNeasy Blood and Tissue Kit (QIAGEN). The mitochondrial gene cytochrome *c* oxidase subunit I (COI) was amplified using primers and protocols following Braid et al. (2014). The sequence reaction was performed using the primer HCO2198, which was the reverse primer used for the polymerase chain reaction (PCR, Macrogen, Korea). Sequences were edited in CodonCode Aligner (CodonCode Corp., Dedham, MA, USA). Sequences were uploaded to the Barcode of Life Data System (Ratnasingham & Hebert, 2007) public project titled ‘Stomach Contents from New Zealand Long-finned Pilot Whales’ (project code: GMSQX) and subsequently submitted to GenBank. All sequences were screened for potential contamination using GenBank’s Basic Local Alignment Search Tool (BLAST). Genetic identifications were made using the Full Database identification engine on BOLD.

#### *Trace element analysis*

Prior to trace element analysis, freeze dried tissue samples (~100–300 mg dry weight [dw]) were digested in a 3:1 mixture of 70% HNO<sub>3</sub> (Merck, suprapur quality) and 37% HCl (Merck, suprapur quality). Acidic digestion took place in a microwave digestion system (Multiwave GO, Anton Paar GmbH, Austria) at 105°C for 50 minutes and samples were diluted to a volume of 50 ml with Milli-Q water. Trace element concentrations (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Mn, Pb, Se, V, and Zn) were analysed by inductively coupled plasma mass spectroscopy (ICP-MS, Agilent Technologies 7500 series

and Agilent 8900, CA, USA) at the University of Canterbury, New Zealand. The Hg analysis was conducted after Aldrige et al. (2017). Detection limits ( $\mu\text{g g}^{-1}\text{ dw}$ ) were calculated as  $3\times$  standard deviation of the blank: Al (1.73), As (0.007), Cd (0.06), Co (0.01), Cr (0.09), Cu (0.36), Fe (1.39), Hg (0.045), Mn (0.024), Ni (0.09), Pb (0.04), Se (0.09), V (0.002), and Zn (0.3). The quality of the analysis was assured by measuring four blanks, duplicate samples, and lobster hepatopancreas certified reference material (CRM; TORT-3, National Research Council, Canada,  $n=4$ ). Mean recoveries ranged between 83–117%. The concentrations for U were measured by microwave plasma atomic emission spectroscopy (MP-AES 4200 Agilent Technologies, Australia) with a detection limit of  $0.001\text{ }\mu\text{g g}^{-1}\text{ dw}$  and a recovery rate for the CRM of 105%.

#### *Mercury:selenium interaction*

For the assessment of the Hg and Se ratio, measured concentrations were converted from  $\mu\text{g g}^{-1}\text{ dw}$  to  $\text{nmol.g}^{-1}$  using the molecular weight of 200.59 for Hg and 78.96 for Se. A Hg:Se ratio  $>1$  indicates an excess of Hg in relation to Se in the tissue (Cuvin-Aralar & Furness, 1991; Ralston et al., 2008).

#### *Body burden estimation*

The average long-finned pilot whale (ca. 4m total body length and 1000kg body mass) is estimated to require a minimum intake of 11 kg of food per day (Beatson et al., 2007b; Sergeant, 1962). The intake calculations were based on a diet almost exclusively comprised of arrow squid (97.9%; Beatson et al., 2007b), which equals 10.77 kg of arrow squid per day. Concentrations of As, Cd, Hg and Pb were extrapolated from total body burden results previously determined for New Zealand arrow squid samples (Chapter 3). The intake calculations were based on fresh *N. sloanii* trace element concentration data in order to avoid diffusion effects during digestion of the squid tissues. The mean trace element concentration for arrow squids was determined using the average concentration found in each tissue type (arm, digestive gland, fin, gonad, branchial and systemic hearts, kidney, mantle, and tentacle) as a relative proportion of the squid, for a squid with an average weight (453 g; Chapter 3). The reported ratio of total Hg to organic (methyl-) Hg in *G. m. melas* ranges from 2 to 5% in the liver and 83 to 85% in the muscle tissue (Caurant et al., 1996; Gajdosechova et al., 2016), which were used to extrapolate the Hg results of the present study.

#### *Statistical analysis*

Principal component analysis (PCA) was used to visualise trace element concentration among the different organs using the software R (Ihaka & Gentleman, 1996; R Core Team, 2017; package ‘ggbiplot’, Vu, 2011). Prior to analysis, values below the detection limit of the ICP-MS were estimated by replacing them with half of the minimum concentration value for the respective element. Concentration data were normalised and transformed using auto-scaling, mean centred, and then divided by the standard deviation.

Generalised linear models (GLM) with a negative binomial distribution and the logit link function were fitted in R (package ‘MASS’, Ripley et al., 2013). The models were applied to determine whether sex, size, age, and/or location of stranding (added sequentially) affected trace element concentrations. One model per trace element was fitted against non-transformed concentration data for the four organ types (blubber, kidney, liver, and muscle). Diagnostic plots were used to check for variance homogeneity and normality of the residues. Significant relationships ( $p < 0.001$ ) between trace element concentrations and effects in the models were visualised in plots. Pairwise nonparametric Spearman correlations were applied to test for relationships between trace element concentrations in the cetaceans and ingested squid specimens (‘corr.test’ function of the ‘corrgram’ package, Wright, 2012).

## Results

### *The samples*

The estimated age of the long-finned pilot whale specimens ranged from 2 to 32 years, based on growth layers in the teeth. Body length ranges showed overlap in sizes between females (3.26–4.6m) and males (2.76–5.56m).

### *Distribution across organs*

Trace element concentrations decreased in the following order in the four different organs analysed.

Blubber: Al>Fe>Zn>Cu>Mn>Hg>As>Cr>Se>Cd>Co>Ni>V>Pb>U;

kidney: Fe>Cd>Zn>Al>Se>Hg>Cu>Mn>As>Cr>Ni>Co>V>Pb>U;

liver: Fe>Hg>Zn>Se>Al>Cd>Cu>Mn>Cr>As>Ni>Pb>Co> V> U and

muscle: Fe>Zn>Al>Se>Hg>Cu>Cd> Mn>Cr>As>Ni>Co>V>Pb>U (Table 19).

The first principal component (PC) explained 42% of the variance (mainly driven by Cu, Cd, Hg, Mn, Se, and Zn), while the second PC explained 12.9% (driven by Al, As, Cr, U and V), and the third PC explained 8.8% (mainly driven by Fe, Ni and Pb, Fig. 22). Since concentrations of elements varied among organs (Fig. 22), the PCA alone did not explain the high variability, and GLMs were applied to identify factors influencing the concentrations (Table 20).

Trace element concentrations were higher in the kidney and liver compared to blubber and muscle (Fig. 22). The highest concentrations of Fe ( $1295 \mu\text{g g}^{-1} \text{ dw}$ ), Hg ( $705 \mu\text{g g}^{-1} \text{ dw}$ ) and Se ( $446.17 \mu\text{g g}^{-1} \text{ dw}$ ) were found in the liver, while the highest concentrations of Mn ( $5.54 \mu\text{g g}^{-1} \text{ dw}$ ) and Cd ( $614 \mu\text{g g}^{-1} \text{ dw}$ ) were found in the kidney (Fig. 22). The concentrations of Al, Cu and Zn were relatively high in both kidney and liver samples (Tables 19, 20). No significant differences were found among As, Co, Cr, Ni, Pb and U concentrations across the organ types (Table 20).

### Effects of sex, size age and sampling location

Females had higher concentrations of Cd, Hg and Se than males ( $p<0.01$ ). Concentrations of Al, Cd, Hg and Se increased with body length ( $p<0.01$ ,  $p<0.001$ ), while concentrations of Cu ( $p<0.05$ ), Hg ( $p<0.001$ ) and Se ( $p<0.01$ ) increased with age (Table 20, Fig. 24). Concentrations of Al and Fe were highest in the specimens sampled at Farewell Spit, Golden Bay (Table 20, Fig. 25).

### Stomach content results

DNA barcodes were successfully sequenced from 93 of the 100 squid mantles that were recovered of *G. m. edwardii* stomach contents from Stewart Island. The sequences ranged from 312 to 613bp and were all identified as *Nototodarus sloanii*. The remaining seven specimens failed to sequence.

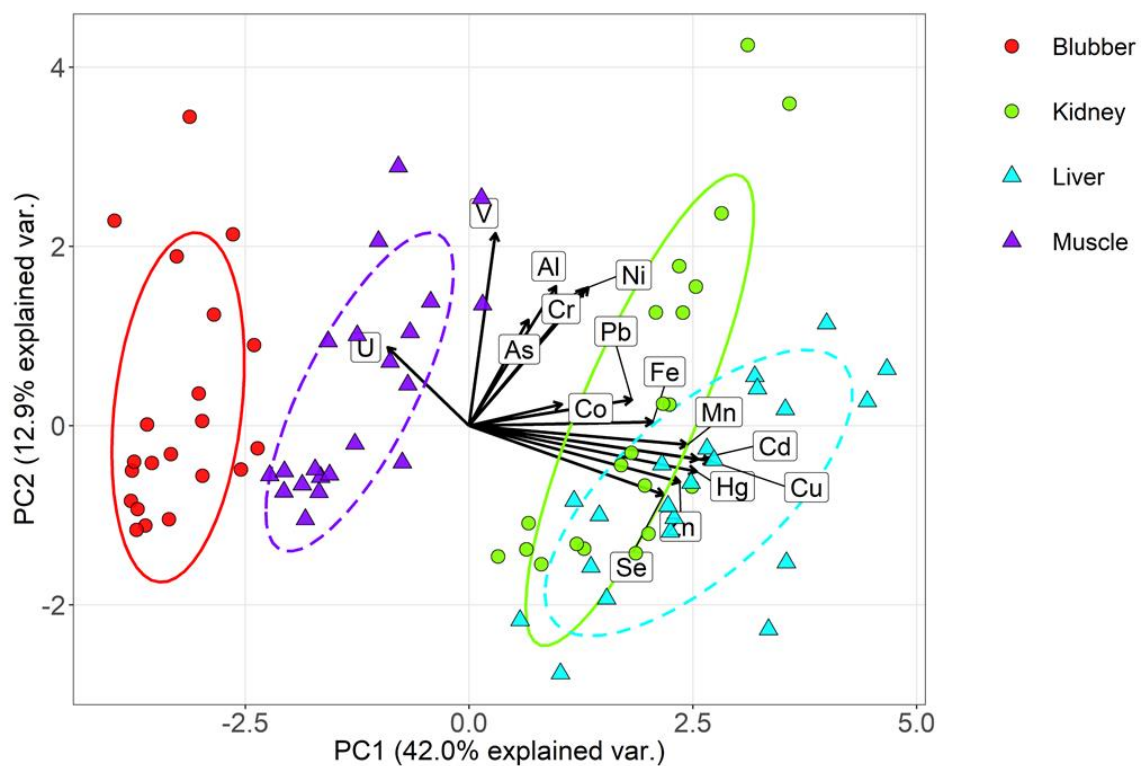


Fig. 22. Principal component analysis (PCA) presenting the trace element concentrations in blubber, kidney, liver, and muscle tissue of *G. melas edwardii* stranded in New Zealand waters. Element loadings along principle component (PC) 1 and PC 2 are represented by arrows. Ellipses indicate the 95% confidence interval around tissue/maturity stage groupings.

Trace element concentrations in the squid mantle samples decreased in the order  $Zn>Al>Fe>Cu>Cd>As>Mn>Cr>Ni>Hg>V>Pb>Co>U$  (Table 19). Concentrations of As, Cu, and Ni were higher in the squid mantle tissue than in the *G. m. edwardii* tissues, while Fe and Hg concentrations were lower in the squid samples (Table 19). Cadmium concentrations were higher in the squid mantle tissue than in the cetaceans' muscle tissue, but lower than the cetaceans' kidney concentrations. Cadmium concentrations in the cetaceans' kidney tissues were negatively correlated with the Cd concentrations

in the squid from the stomach contents (Pearson's  $r=-0.48$ ). In contrast, As concentrations in the squid samples were positively correlated with As concentrations in the cetaceans' muscle tissues (Pearson's  $r=0.31$ ).

#### *Estimated Body burden and trace element intake of G. m. edwardii*

Based on an estimated minimum diet of 10.7 kg arrow squid per day (equals ~24 individuals), *G. m. edwardii* have an estimated minimum average daily intake of 34.53 mg As, 14.72 mg Cd, 0.61 mg Hg, and 0.5 mg Pb from arrow squids alone, assuming a digestion cycle of less than 8 hours (Sergeant, 1962, 1969). However, the intake could reach concentrations as high as 66.01 mg As, 75.76 mg Cd, 1.24 mg Hg and 2.17 mg Pb, based on the maximum concentrations previously measured in arrow squids (Chapter 3). No significant difference for those elements was estimated for the consumption of *N. gouldi* or *N. sloanii* (Chapter 4).

Using the reported ratio of organic-Hg to total Hg (Caurant et al., 1996; Gajdosechova et al., 2016), the expected concentration of methyl-Hg could vary between 5.97 and 9.95  $\mu\text{g g}^{-1}$  dw in the liver and 0.96–0.99  $\mu\text{g g}^{-1}$  dw in the muscle tissue of *Globicephala* spp.

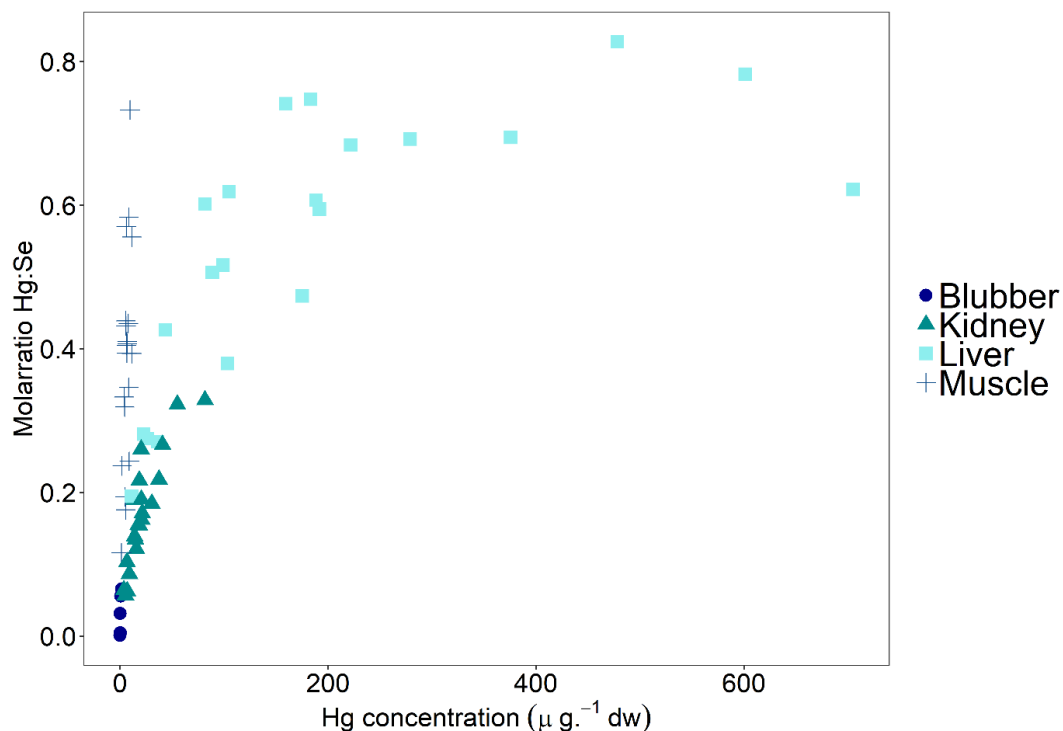


Fig. 23. Concentrations of Hg ( $\mu\text{g g}^{-1}$  dw) and the molar ratio of Hg:Se in the four tissues analysed from individuals of *G. melas edwardii* stranded in New Zealand waters.

Table 19. Trace element concentrations in  $\mu\text{g g}^{-1}$  dw (mean  $\pm$  standard deviation [sd], minimum, and maximum) for the four different organs of *G. m. edwardii*, stranded in New Zealand waters, and arrow squid (*Nototodarus sloanii*) retrieved from their stomach contents.

Element	Blubber		Kidney		Liver		Muscle
	mean $\pm$ sd	min–max	mean $\pm$ sd	min–max	mean $\pm$ sd	min–max	mean $\pm$ sd
Al	45.33 $\pm$ 41.48	4.73–139.96	85.85 $\pm$ 72.53	15.84–291.95	109.62 $\pm$ 152.62	1.99–654.01	45.54 $\pm$ 41.04
As	0.72 $\pm$ 0.21	0.39–1.28	1.04 $\pm$ 0.40	0.43–1.74	0.67 $\pm$ 0.37	0.23–1.79	0.71 $\pm$ 0.31
Cd	0.37 $\pm$ 0.34	0.07–1.55	280.45 $\pm$ 137.55	96.79–614.25	92.04 $\pm$ 46.07	19.03–187.91	1.16 $\pm$ 0.87
Co	0.24 $\pm$ 0.24	0.09–0.51	0.24 $\pm$ 0.19	0.08–0.69	0.13 $\pm$ 0.04	0.09–0.26	0.22 $\pm$ 0.11
Cr	0.42 $\pm$ 0.15	0.16–0.70	1.02 $\pm$ 0.75	0.12–2.99	0.82 $\pm$ 0.66	0.26–3.24	0.73 $\pm$ 0.55
Cu	1.08 $\pm$ 0.57	0.46–2.53	17.35 $\pm$ 4.24	12.21–27.96	15.81 $\pm$ 5.99	8.59–33.9	2.76 $\pm$ 0.56
Fe	43.48 $\pm$ 31.75	11.73–135.02	454.83 $\pm$ 143.31	247.13–792.55	674.36 $\pm$ 317.87	186.63–1294.51	507.83 $\pm$ 120.36
Hg	0.81 $\pm$ 0.96	0.08–2.82	22.57 $\pm$ 18.49	3.72–81.75	198.91 $\pm$ 191.94	11.24–704.88	6.93 $\pm$ 2.98
Mn	0.88 $\pm$ 1.43	0.15–6.84	4.22 $\pm$ 0.89	2.62–5.54	8.70 $\pm$ 2.99	5.45–19.59	0.98 $\pm$ 0.88
Ni	0.15 $\pm$ 0.04	0.09–0.21	0.40 $\pm$ 0.29	0.08–1.02	0.32 $\pm$ 0.26	0.08–1.04	0.36 $\pm$ 0.20
Pb	0.09 $\pm$ 0.05	0.05–0.19	0.10 $\pm$ 0.04	0.06–0.17	0.15 $\pm$ 0.07	0.07–0.28	0.07 $\pm$ 0.03
Se	17.05 $\pm$ 9.27	2.93–32.30	47.12 $\pm$ 17.34	22.83–97.89	124.22 $\pm$ 102.56	22.71–446.17	6.94 $\pm$ 2.96
U	0.01 $\pm$ 0.003	0.001–0.01	0.004 $\pm$ 0.002	0.001–0.01	0.004 $\pm$ 0.001	0.001–0.01	0.006 $\pm$ 0.005
V	0.10 $\pm$ 0.09	0.003–0.33	0.13 $\pm$ 0.11	0.03–0.36	0.04 $\pm$ 0.03	0.01–0.13	0.08 $\pm$ 0.04
Zn	20.68 $\pm$ 13.5	1.75–45.7	125.02 $\pm$ 23.02	93.08–181.86	127.96 $\pm$ 29	89.80–199.29	63.58 $\pm$ 33.98

Table 20. Generalised linear model (GLM) output for trace element models, with the  $p$ -values of the variables shown according to likelihood ratio tests (\*\*\* 0.001, \*\* 0.01, \* 0.05). Negative ( $\downarrow$ ) and positive ( $\uparrow$ ) effects for the continuous variables length and age are indicated by arrows.

	Al	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	S	U	V	Z
												e			n
Orga	***		***			***	***	***	***			*			**
n												*			*
												*			
Sex			**					**				*			
												*			
Lengt	$\uparrow\uparrow$		$\uparrow\uparrow\uparrow$					$\uparrow\uparrow\uparrow$				$\uparrow$			
h												$\uparrow$			
Age						$\uparrow$		$\uparrow\uparrow\uparrow$				$\uparrow$			
												$\uparrow$			
Locat	***						**								
ion															

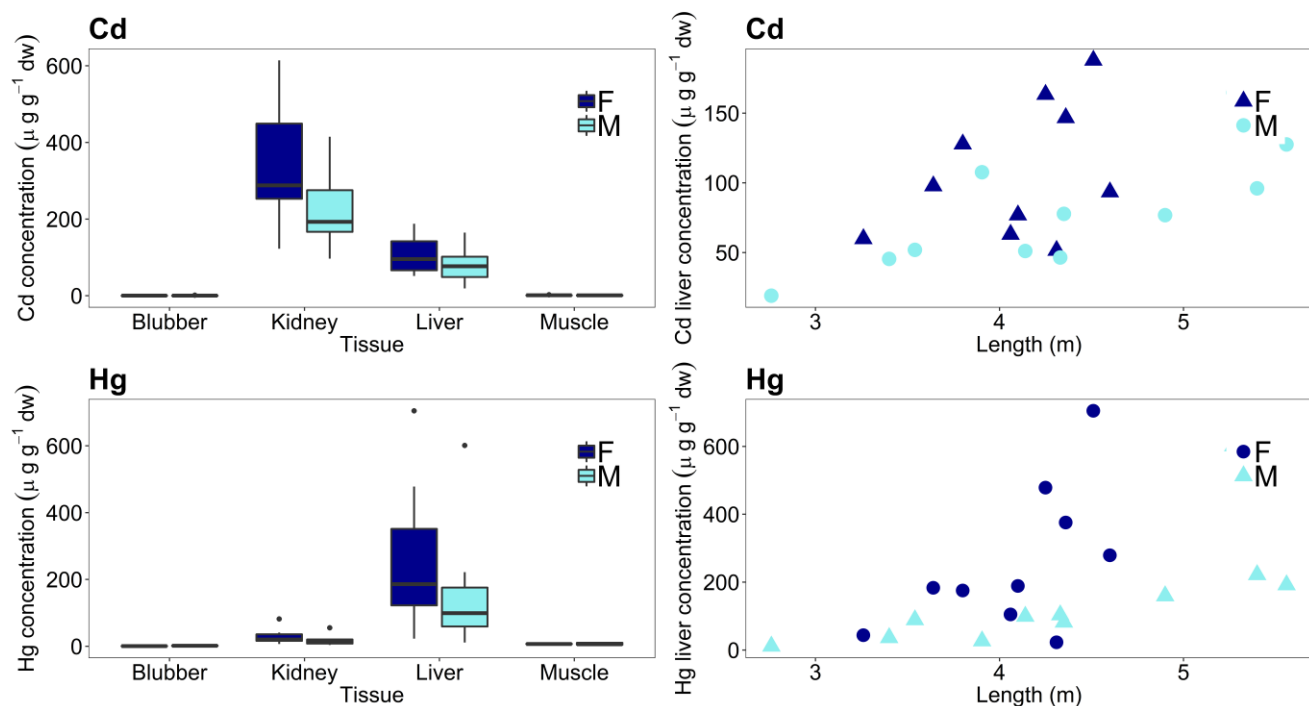


Fig. 24. Tissue distribution of Cd and Hg concentrations across organs of stranded *G. m. edwardii* stranded in New Zealand waters and relationships between total body length (m) and Cd and Hg concentrations.

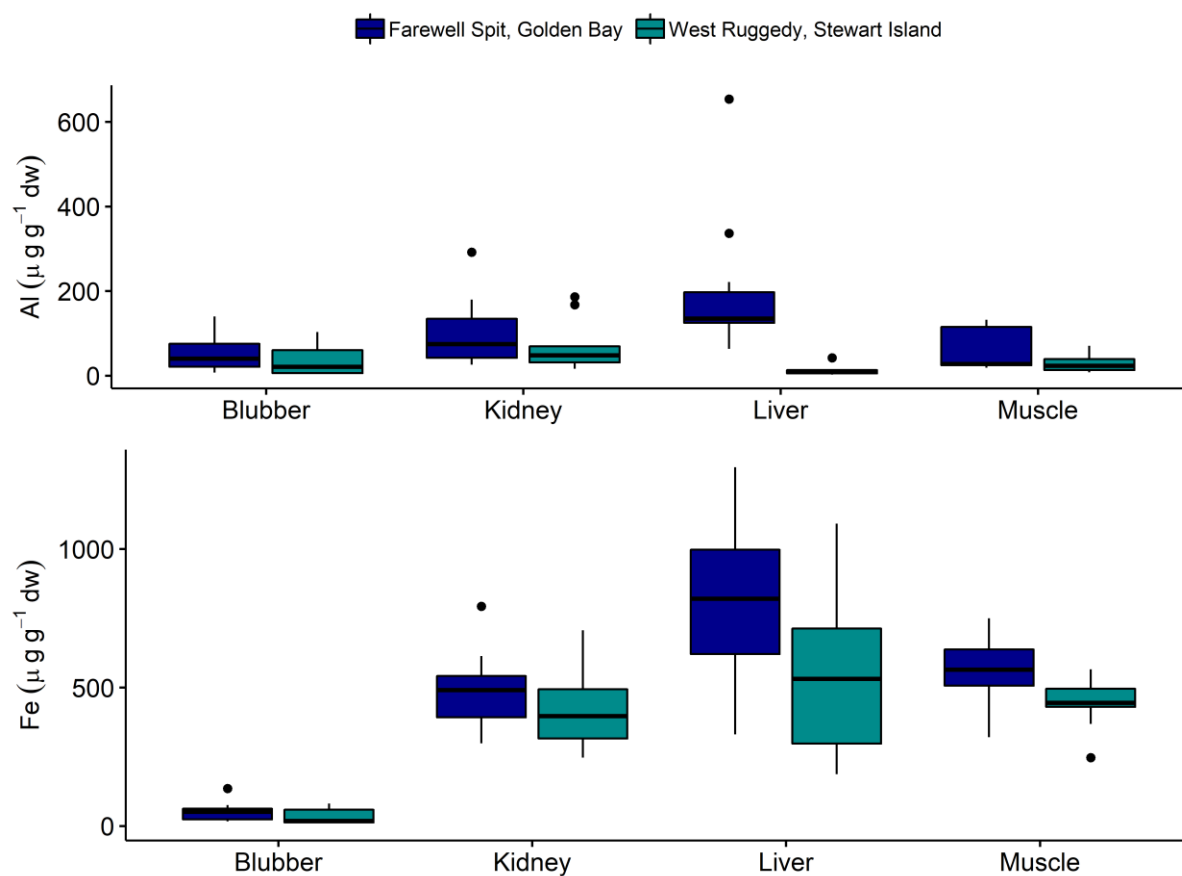


Fig. 25. Effect of sampling location on Al and Fe concentrations of *G. m. edwardii*, stranded in New Zealand waters.

## Discussion

Despite cetaceans' crucial role in New Zealand's pelagic food webs as apex predators, trace element concentrations of cetaceans in these waters remain largely unknown, with levels only previously reported for a single dolphin species (Stockin et al., 2007). This chapter reports trace element concentrations in *G. m. edwardii* from the Southern Hemisphere for the first time. The high kidney Cd and liver Hg concentrations measured in *G. m. edwardii* are the focus of this study because of their known high toxicity to mammals (Bernhoft et al., 2012; Nicholson et al., 1983). The analysis of prey items retrieved from *G. m. edwardii* stomach contents (*N. sloanii*) similarly identified elevated Cd concentrations. These high Cd concentrations, measured in both prey and predator, suggest that arrow squids, as a mayor prey source, are a significant vector for the bioaccumulation of these trace elements in *G. m. edwardii* within New Zealand's pelagic ecosystem.

### *Distribution of Cd, Hg and Se across organs*

Kidney samples contained the highest mean Cd concentrations ( $280.45 \pm 137.55 \mu\text{g g}^{-1} \text{ dw}$ ; Table 19), comparable to concentrations previously reported for *G. m. melas* ( $245.67\text{--}286.7 \mu\text{g g}^{-1} \text{ dw}$ ) from the North Atlantic (Caurant & Amiard-Triquet, 1995; Gajdosechova et al., 2016). The similarity in concentration magnitude suggests similar physiological strategies and exposure. The high concentrations of Cd in the kidney tissue could be due to a previously reported detoxification pathway involving metallothionein binding and mineral accretion with calcium and phosphorus (Gallien et al., 2001). For example, the kidney tissue of white-sided dolphins, *Lagenorhynchus acutus*, contains Cd-rich mineralised granules (Gallien et al., 2001). These are believed to be involved in the Cd detoxification process through immobilisation of excess Cd, similar to Hg- and Se-containing granules described from cetacean livers (Nigro & Leonzio, 1996). The high positive correlation between Cd and Se concentrations in the kidney, liver, and muscle tissues described in this chapter is similar to the findings of Lemos et al. (2013) on *Sotalia guianensis* (Guiana dolphin). Lemos et al. (2013) explained this finding by an increased demand for Se with high Cd concentrations. Furthermore, a protective effect of dietary Se against the Cd inhibition of glutathione reductase has been demonstrated in other mammals (rabbits; Uluşu et al., 2003; Valko et al., 2005).

The only other cetaceans evaluated for Cd concentrations in New Zealand waters is the common dolphin (*Delphinus* sp.; Stockin et al., 2007). Although the diet of this species also consists largely of arrow squid (Meynier et al., 2008), the kidney Cd levels in specimens sampled in the Hauraki Gulf, were considerably lower ( $56.7\text{--}173.4 \mu\text{g g}^{-1} \text{ dw}$ ) than *G. m. edwardii* evaluated in the present study (Stockin et al., 2007). This difference is unexpected because prey items have been suggested as the main exposure pathway for Cd in cetaceans (Bustamante et al., 1998a). However, the concentration differences between *G. m. edwardii* and the common dolphin in New Zealand waters could be explained

by: 1) the different habitat/region of the nearshore common dolphin and the more pelagic long-finned pilot whales, resulting in trace elemental concentration differences of their prey; and/or 2) and/or a difference in physiological properties as reported between *G. m. edwardii* and *Delphinus* sp. (as observed between the short-finned pilot whale *G. macrorhynchus* and the pygmy sperm whale *Kogia breviceps*; Bustamante et al., 2003), and/or 3.) consuming different prey species in addition to diets comprised largely of arrow squids.

The *G. m. edwardii* liver samples contained Hg and Se concentrations that were orders of magnitude higher than the other organs (Table 19). This is in agreement with previous studies, where the liver has been suggested as the main organ for demethylation of the toxic organic methyl-Hg to its non-toxic, inorganic mercuric selenide form (Caurant et al., 1996; Law et al., 2001; Martoja, 1980; Rawson et al., 1995). A Hg:Se ratio close to 1 has been suggested as an indicator for the presence of tiemannite granules, resulting from the demethylation of methyl-Hg in the liver with Se (Bustamante et al., 2003b; Koeman et al., 1973; Law et al., 2001). In this study, the measured Hg:Se ratio increased with increasing Hg concentrations, but slightly decreased in concentrations higher than 500  $\mu\text{g g}^{-1}$  dw (Fig. 23). An earlier study on striped dolphins, *Stenella coeruleoalba*, suggested that the demethylation process of methyl-Hg involving Se is efficient until a threshold of around 500  $\mu\text{g g}^{-1}$  dw is reached (Palmisano et al., 1995). The present measurements suggest a similar threshold (Fig. 23). However, because those high concentrations were only measured in livers of two specimens, an increased sample size is needed to further test this hypothesis. Overall, the Hg and Se liver concentrations observed in *G. m. edwardii* were comparable to previous reports for *G. m. melas* (from the Faroe Islands) and *Delphinus* sp. (from the Hauraki Gulf, New Zealand; Caurant et al., 1996; Stockin et al., 2007; Table 21). In contrast, the liver Hg concentrations in this study were orders of magnitude lower than *G. macrorhynchus* from New Caledonia (Bustamante et al., 2003b, Table 21). This observation could be due to differences in diet. *Globicephala macrorhynchus* mainly feeds on fish, which generally exhibit higher Hg concentrations (Bustamante et al., 2003b; Gannon et al., 1997), whereas *G. melas* spp. and *Delphinus* sp. are reported to mainly feed on cephalopods (Beatson et al., 2007a; Heide-Jørgensen et al., 2002).

#### *Effects of age, size and sex*

Mercury concentrations increased with age (Table 20), likely because this element is known to bioaccumulate in mammals over an individual's life span and is minimally excreted (e.g., through lactation; Atwell et al., 1998; Wolfe et al., 1998). Mercury concentrations also increased with size (i.e., total body length; Table 20, Fig. 24). The highest Hg concentrations were measured in larger individuals, which indicates that Hg bioaccumulates over the growth (and lifespan) of an individual and is therefore coupled with growth rate. An increase in Hg concentration with age has been previously reported in other cetacean species (Borrell et al., 1995; Endo et al., 2007; Lahaye et al., 2006; Siebert et al., 1999). Females had overall higher Hg concentrations than males (Table 20, Fig. 24). Similarly,

female *G. m. melas* (from the North Atlantic) and *G. macrorhynchus* (from New Caledonian waters) had trace element concentrations almost twice as high as males (Caurant et al., 1994; Table 21). The sex differences in trace element concentrations may reflect different feeding rates or metabolic capacities (Caurant et al., 1994; Endo et al., 2007). Similar effects were observed for the Se concentrations, which were generally higher in females and increased with age and size, which underlines the accumulation with age and the potential role of Se in Hg detoxification (Table 20). The growth rate in cetaceans, including long-finned pilot whales, is significantly higher in males than females (Betty, 2019; Bloch & Lockyer, 1989; Borrell et al., 1995; Endo et al., 2007; Zhou et al., 2001). Male long-finned pilot whales have a protracted growth period compared to females (Betty, 2019; Bloch et al., 1993), which could result in a dilution effect, meaning that both sexes are exposed to the same trace element concentrations, but the dilution rate is greater in males due to their larger body weight (André et al., 1990; Caurant et al., 1994). Dietary differences between the sexes could further affect their Hg and Se levels. Stomachs of lactating *G. m. melas* from the North Atlantic contained relatively more fish (Desportes & Mouritsen, 1993), which also had higher methyl-Hg concentrations, when compared to non-lactating females or males (Caurant et al., 1996). Fish consumption might cover the increased energetic needs of reproducing/lactating *G. m. melas* females and could contribute to the sex-specific differences in Hg concentrations (Caurant et al., 1996).

Consistent with Hg, the highest concentrations of Cd were measured in larger individuals (Table 20, Fig. 24). An ontogenetic shift in diet could result in different Cd bioaccumulation patterns over an individual's lifespan; as the results did not confirm an effect of age on Cd concentrations, this shift could rather be related to size or maturity stage (Table 20). Females generally had higher Cd concentrations than males (Tables 19, 20, Fig. 24). This effect could be explained with the protracted growth period of *G. m. edwardii* males (Betty et al., 2019; Caurant et al., 1994), leading to higher bioaccumulation rates in females. Sex differences in Cd bioaccumulation have also been reported for primates, including humans, with females generally exhibiting higher concentrations (Koizumi et al., 2008). The cause of this difference has been hypothesised as a slower Cd excretion rate (Koizumi et al., 2008) and the influence of iron deficiency on increased Cd absorption in females (Bartzokis et al., 2007; Bochud et al., 2018).

Table 21. Comparison of Cd and Hg in the kidneys, liver, and blubber tissues of small cetaceans from global sampling locations (mean concentrations in  $\mu\text{g g}^{-1}$  dw).

Species	Location	Year	Cd Kidney	Cd Liver	Hg Liver	Hg Blubber	Hg Muscle	Reference
<i>Globicephala</i>								
<i>G. melas edwardii</i>	New Zealand	2010–2014	280.50	92.00	198.9	0.81	1.16	Present study
<i>G. melas melas</i>	Faroe Islands	1986				0.77 <sup>a</sup>		Simmonds et al. (1994)
<i>G. melas melas</i>	Faroe Islands	1986	286.70 <sup>a</sup>	256.70 <sup>a</sup>				Caurant (1995)
<i>G. melas melas</i>	Faroe Islands	2007	120.00 <sup>a</sup>		460.00 <sup>a</sup>			Sonne et al. (2010)
<i>G. melas melas</i>	Scotland, UK	2012	245.67 <sup>ab</sup>	80.33	216.33		10.20	Gajdosechova et al. (2016)
<i>G. melas melas</i>	Mediterranean Sea	1993–1998	33.00	13.00			0.62	Frodello and Marchand (2001)
<i>G. melas melas</i>	North-West Iberia	2004–2013	122.00	24.40	127.00			Monteiro et al. (2017)
<i>G. melas melas</i>	North-West Iberia	2004–2008	100.00 <sup>a</sup>	27.67 <sup>a</sup>				Méndez-Fernandez et al. (2013)
<i>G. melas melas</i>	Massachusetts, USA	1990		26.27 <sup>a</sup>	134.33 <sup>a</sup>			Mackey et al. (1995)
<i>G. melas melas</i>	Newfoundland, Canada	1980	107.80	58.30	83.85		3.02	Muir et al. (1988)
<i>G. macrorhynchus</i> ♂	New Caledonia	1997		225.30	1411.00	11.00		Bustamante et al. (2003)
<i>G. macrorhynchus</i> ♀	New Caledonia	1997		464.40	1452.00	3.20		Bustamante et al. (2003)
<i>G. macrorhynchus</i>	West Indies, Atlantic	2016			2032.00		18.40	McCormack et al. (2019)
<i>Delphinus</i>								
<i>D. sp.</i>	Hauraki Gulf, New Zealand	2004	56.70 <sup>a</sup>		176.70 <sup>a</sup>			Stockin et al. (2007)
<i>Tursiops</i>								
<i>T. truncatus</i>	East Coast Australia	1995		6.30 <sup>a</sup>	54.50 <sup>a</sup>			Law et al. (2001)
<i>T. geophysus</i>	Argentina	1990		2.70 <sup>a</sup>	286.70 <sup>a</sup>			Marcovecchio et al. (1990)

<sup>a</sup>converted from wet weight to dry weight, based on 70% moisture content

<sup>b</sup>median concentration

One potentially important consideration is that the specimens analysed in this study died during mass stranding events, whereas the previously examined specimens of the North Atlantic subspecies (except in Gajdosechova et al., 2016), were based on drive-hunted specimens. Therefore, the stomach contents of the specimens used in this study might not be directly comparable to stomach contents reported from the North Atlantic subspecies. Although male *G. m. edwardii* have been found with fish remains in their stomach contents, this is rarely seen in females stranded on the New Zealand coast (EB, pers. obs.). Therefore, an in-depth analysis of the diet of *G. m. edwardii* is necessary and a better understanding of Cd and Hg levels in New Zealand's fish species is required to examine their role in the bioaccumulation pathways within this pelagic environment.

#### *Arrow squid as a vector for trace elements*

Trace element exposure for cetaceans is mainly through diet (Das et al., 2004), while dermal absorption (Wise et al., 2018) and inhalation (e.g., Savery et al., 2015; Wise et al., 2019) are also potential pathways (Nordberg et al., 2014). Dermal absorption is unlikely since the whales' skin represents a barrier (Bryan et al., 2010; Wise et al., 2018). In addition, the absorption of Cd from air pollution is highly unlikely in the New Zealand environment because this area is relatively isolated from any atmospheric Cd sources (McDowell et al., 2013). Therefore, we have focused on the potential for dietary exposure for Cd in *G. m. edwardii* in this study. DNA barcoding identified all arrow squids found in the *G. m. edwardii* stomachs in this study as *N. sloanii* (a species known to occur in the same areas as the stranding events; Chen, 1998; Uozumi and Förch, 1995). Due to the degraded nature of specimens recovered from stomach contents, morphological identifications are often not possible. In this case, the squid specimens were mantles only with no hard parts remaining. Furthermore, the two arrow squid species found in New Zealand waters cannot currently be differentiated by their beak shape (Liu et al., 2015), and morphological identifications rely on the structure of the male hectocotylus in males, and the sucker count (Smith et al., 1987). The DNA barcoding was highly successful and should be used in future studies as a method of identifying partially digested prey remains.

The Cd concentrations were highest in *G. m. edwardii* kidney tissue, followed by the stomach squid tissue (Table 19). The muscular tissue of *G. m. edwardii* contained the lowest Cd concentrations (Table 19). It should be noted that the trace element concentrations from the whales and their prey reflect different timescales. Prey remains provide a snapshot from the moment of digestion and might not be reflective of the average trace element concentrations in their diet. All trace elements analysed in the squid samples were exposed to a digestion process, which could have led to a diffusion of mobile elements, such as Cd, to the surrounding whale tissue (Francesconi et al., 1993; Chapter 3) or absorption during digestion could result in reduced concentrations of the squid samples. Therefore, the intake calculations of Cd for *G. m. edwardii* were based on fresh arrow squid data (Chapters 2, 3). The estimated daily intake of Cd for *G. m. edwardii* from arrow squid was between 14.72 and 75.76  $\mu\text{g}$

kg<sup>-1</sup>.day<sup>-1</sup>, which indicates that *N. sloanii* is the main vector for Cd as their concentrations correspond. Since cetaceans and other mammals, including humans, share a comparative biochemistry and toxicology, a comparison of intake rates of *G. m. edwardii* to those of New Zealanders was conducted. The average dietary Cd intake for a New Zealander varies from 0.16 to 0.23 µg kg<sup>-1</sup> day<sup>-1</sup> (MPI, 2018). However, if a human (scaled for a 70kg adult) were to take in Cd at the rate estimated herein from arrow squid in *G. m. edwardii* they would exceed the recommended daily maximum intake by at least 1470% and experience acute toxicity and likely suffer serious injury to their kidneys and bones (Bernard & Lauwerys, 1986; Roels et al., 1990). It should be noted, however, that the measured Cd concentrations do not reflect the bioavailable Cd fraction to the animal (Reeves and Chaney, 2008)—which is generally lower— but only the bioaccessible fraction. In general, an understanding of the bioavailability of Cd to mammals is needed and should be the focus of future studies (Reeves and Chaney, 2008).

Biomagnification through the food web could explain the high Hg concentrations found in *G. m. edwardii* in the present chapter. Relative to the squid samples recovered from their stomachs, *G. m. edwardii* specimens had higher Hg concentrations (Table 19). This was also true when compared with previously reported Hg concentrations in *Nototodarus* spp. (Chapters 2-4). The higher levels of Hg in *G. m. edwardii* likely reflects the biomagnification of this trace element in apex predators, who are exposed to higher Hg concentrations compared to organisms at lower trophic levels (Atwell et al., 1998; Lavoie et al., 2013). However, an assessment of the trophic position of both *G. m. edwardii* and *N. sloanii* with stable isotopes would increase the understanding of the biomagnification of Hg within New Zealand's food webs. Apart from biomagnification, whale falls are significant feeding grounds for scavenging fauna in the deep sea (e.g., Smith et al., 2003; Higgs et al., 2014) and might represent a Hg source for deep sea fauna.

The dietary source of Hg for *G. m. edwardii* could represent the specific exposure pathway for this element. Although Beatson et al. (2007a) previously suggested that arrow squids were the main food item for *G. m. edwardii*, a dietary analysis of non-stranded specimens may reveal different prey items. In the cetacean digestive tract, fish bones and otoliths are digested more quickly than squid beaks (Pierce and Boyle, 1991) and, therefore, it is possible that *G. m. edwardii* might feed largely on fish. A diet comprised mainly of fish could be the main source and explanation of the Hg concentrations measured because higher Hg concentrations have been reported in fish relative to cephalopods (Storelli and Marcotrigiano, 2004; Vlieg et al., 1991). However, this is not reflected in the recent literature on *G. m. edwardii* (Beatson et al 2007a; Beatson and O'Shea 2009). The diet of *G. m. edwardii* remains understudied, and studies rely on stranded individuals, which may not accurately reflect their typical diet. Future studies should focus on the use of molecular techniques, such as high-throughput sequencing, for a non-destructive dietary analysis of healthy individuals. Those studies would help

identifying potential sources of trace elements and might reveal whether or not arrow squid are their main prey.

The potential effects of the high Hg concentrations on marine mammals are not yet understood. It has been demonstrated that the oral bioavailability of methyl-Hg and inorganic Hg can have effects on mammals (e.g., renal toxicity in rats; Endo et al., 2003). The estimated daily intake of total Hg for *G. m. edwardii* through *N. sloanii* ranges from 0.61 to 1.24  $\mu\text{g}^{-1} \text{kg}^{-1} \text{day}^{-1}$  ( $\sim 0.51\text{--}1.03$  methyl-Hg  $\mu\text{g}^{-1} \text{kg}^{-1} \text{day}^{-1}$ ), while the average dietary intake of a New Zealander varies from 0.0009 to 0.001 methyl-Hg  $\mu\text{g}^{-1} \text{kg}^{-1} \text{day}^{-1}$  (MPI, 2018). The estimated methyl-Hg intake rates for *G. m. edwardii* would likely cause severe health effects in humans, such as neurotoxic damage and cardiovascular disturbances (Mergler et al., 2007).

## Conclusion

This chapter provides the first dataset on trace element concentrations in *G. m. edwardii* one of New Zealand's top predators and a taonga (cultural treasure) of the indigenous people of Aotearoa New Zealand—and its prey. Trace element concentrations measured in *G. m. edwardii* from two mass stranding events on the New Zealand coast were comparable to concentrations measured in *G. m. melas* harvested in North Atlantic waters. In *G. m. edwardii*, the liver appears to be the main storage organ for Hg, while the kidneys accumulated the highest Cd concentrations. Females had higher Cd and Hg concentrations than males, possibly linked to sex-specific energetic needs and dietary habits. While muscle tissue contained relatively low concentrations of most of the trace elements analysed in this study, observed muscular Hg concentrations could be hazardous for human consumption and might be a significant exposure for scavenging fauna. Furthermore, Hg concentrations were influenced by size and age, with larger and older individuals having higher concentrations. The concentrations of Cd and Hg were positively correlated with the Se concentrations, which suggested the potential role of Se in detoxification processes for this species. Arrow squid samples from the stomach contents of *G. m. edwardii* were identified as *N. sloanii* using DNA barcoding. Based on the known diet of *G. m. edwardii*, and Cd concentrations in both fresh arrow squid and specimens retrieved from their stomach contents, *N. sloanii* is the most likely vector for Cd bioaccumulation. Future studies on *G. m. edwardii* should focus on conducting pathological investigations in order to determine the role of trace elements in stranding events.

## Chapter 7: Overall discussion

### *Research summary*

The overall aim of this thesis was to gain insight into the dynamics of trace element accumulation within New Zealand's pelagic food webs and to test the hypothesis that squids are a vector for trace elements within these systems (Fig. 26). Four species were selected for analysis: the three squid species believed to be most locally abundant and ecologically important (including two species with high economic value), representing two disparate body chemistry categories (muscular and ammoniacal); and a cetacean species, with cultural importance to *tangata whenua*, reported to feed nearly exclusively on one of these species.

First, arrow squids (*Nototodarus gouldi* and *N. sloanii*) were investigated as vectors for trace element accumulation in pelagic food webs and in humans. An analysis of trace element distribution across a variety of tissues in *N. sloanii* from the Chatham Rise showed that the highest trace element concentrations (and highest absolute burden, e.g., 90% of Cd) occurred in the relatively small digestive gland (7% body weight). The mantle tissue makes up the greatest proportion of the animal's wet weight (30%) but carries a relatively small proportion of the trace element burden (e.g. 22% of total Cd, Chapter 2). In order to understand intake rates in humans during consumption, a simulated *in-vitro* human digestion experiment for *N. sloanii* was conducted (Chapter 3). These results indicated that, on average, New Zealanders are not exceeding the guideline threshold ingestion of As, Cd, Hg or Pb, through arrow squid consumption. However, the high Cd concentrations in some specimens from the Taranaki region (Chapter 4) and the contamination of edible mantle tissue with digestive gland fluids in market arrow squid (Chapter 3) highlight that consumers should be cautious with their squid intake. This includes vulnerable groups such as pregnant women, children, and any other people who consume above-average amounts of squid tissue. To identify differences in trace element bioaccumulation capacities between different body chemistry categories, the muscular *N. sloanii* from the Chatham Rise was compared with an ammoniacal squid species, *Moroteuthopsis ingens*, from the same region (Chapter 5). The results suggested that the stored ammonium had no observable impact upon trace element bioaccumulation patterns across species.

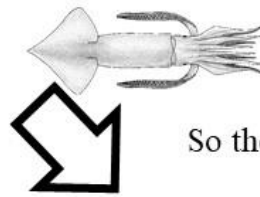
Finally, in order to identify trace element concentrations in an apex predator, long-finned pilot whale (*Globicephala melas edwardii*) tissues and *N. sloanii* from their stomach contents were analysed (Chapter 6). Particularly high Cd and Hg concentrations were measured in the kidneys and liver tissues—far above the levels considered toxic for humans. The high Cd concentrations observed in the long-finned pilot whales suggest arrow squid as their main vector (Chapter 6).

Are squid a vector for trace element accumulation in New Zealand's food webs?

**Research question 1, Chapter 2:**  
If trace elements can be quantified in different squid tissue types, can then whole body burden estimates be calculated?

Conclusions—Yes:

1. Trace element concentrations were robustly measured in New Zealand's arrow squids from nine different tissue types.
2. Whole body burden estimates can be calculated from the mantle tissue and the digestive gland concentrations.
3. Cd concentrations were particularly high in the digestive gland.



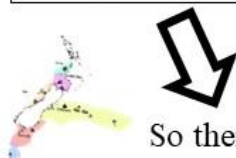
So then ...

**Research question 2, Chapter 3:**

Based on Chapter 2, can the intake risk of elements of most health concern (As, Cd, Hg and Pb) in arrow squid from the Chatham Rise be assessed for human consumers?

Conclusions — Yes:

1. The risk assessment concluded that all elements of concern were generally below the threshold intake levels for adults.
2. However, very high levels of Cd were identified in mantle tissue of fresh market squid contaminated by digestive fluids.
3. *In-vitro* simulated digestion of squid mantle tissue showed the complete bioaccessibility of consumed Cd for humans.



So then ...

**Research question 3, Chapter 4:**

Given the results of Chapters 2 and 3, do arrow squid trace element concentrations vary among regions within the NZ EEZ?

Conclusions — Yes:

1. As and Hg concentrations were highest in samples from the West Coast of South Island.
2. Cd concentrations were highest in Taranaki samples.
3. Overall mantle tissue did not exceed threshold levels for human consumption.



So then ...



So then ...



**Research question 5, Chapter 6:**

Based on Chapters 2, 3, and 4, how do trace element concentrations compare in a main arrow squid predator; the long-finned pilot whale *Globicephala m. edwardii*? Can squid be considered their main vector for trace elements?

Conclusions — Yes:

1. Hg and Cd concentrations were particularly high in pilot whale kidney and liver samples.
2. All squids from the whales' stomach contents were identified as *N. sloanii*.
3. Cd concentrations were likely influenced by consuming arrow squid, while Hg concentrations could be influenced by other prey (fish or other squid species such as *M. ingens*).



**Research question 4, Chapter 5:**

Based on Chapters 2, 3 and 4, do trace element concentrations differ between arrow squid and the ammoniacal squid *Moroteuthopsis ingens* from the Chatham Rise?

Conclusions — Yes:

1. Hg concentrations differ between the ammoniacal *M. ingens* and *Nototodarus* spp.
2. Marked differences in Cd and Hg concentrations were observed between sexes.
3. Male *M. ingens* and average *N. spp.* had similar Cd concentrations; female *M. ingens* were higher.

Fig. 26. A conceptual diagram of the main research conclusions of each chapter and their relationship.

## Bioaccumulation in specific taxa

Analysing trace element concentrations in marine biota not only provides important baseline data for a specific species, but also helps in understanding the transfer of trace elements within food webs. For example, measuring trace element concentrations in cephalopods can be used to assess the exposure that predators receive through their consumption. This exposure can vary significantly between humans (who usually selectively consume only the muscular tissues) and marine predators, which likely consume the whole squid, and are thus exposed to the full body burden. In all chapters of this thesis, Cd concentrations received considerable attention because of the remarkably high concentrations measured in the digestive gland of the squids (*N. gouldi*, *N. sloanii*, and *M. ingens*) and the kidneys of *G. m. edwardii*.

### *Arrow squids*

Studying the trace element tissue distribution in arrow squids was a powerful tool for understanding bioaccumulation patterns and for analysing the total body burden, which can then be used to assess the exposure of predators through arrow squid consumption. The results of the tissue distribution for arrow squids (Chapters 2, 3) suggested that the digestive gland and mantle concentrations can be used to extrapolate the risks for predators and humans respectively, and to estimate the whole-body burden without needing to measure concentrations in all tissue types in future studies. Based on these results, subsequent chapters only analysed trace element concentrations in the digestive gland and mantle tissue (Chapters 4 and 5). In Chapter 6, mantle tissue of *N. sloanii* was analysed from the stomach contents of *G. m. edwardii* because the digestive glands were partially dissolved. The identification of the trace element concentration in the different tissue types was the first of its kind to be performed in a southern Pacific squid species and provided valuable insights into their trace element bioaccumulation patterns, which were comparable to ommastrephid squids from the northern hemisphere (e.g., Gerpe et al., 2000; Rjeibi et al., 2015).

Trace element concentrations can show annual and regional variation (e.g., Pierce et al., 2008). Annual variation was reported in trace element concentrations in arrow squid (*N. sloanii*) from the Chatham Rise, where Cd concentrations in particular showed significant differences between years (Chapter 2). This sample set was ideal because it consisted of a single species, from a single locality, from multiple years and could therefore be used to assess the annual trace element concentration differences (Chapter 2). Unfortunately, annual variation could not be assessed in the interregional study because sampling year was confounded by sampling locations (Chapter 4). Examining the differences in trace element concentrations in arrow squids from different regions was necessary in order to better understand their role as vectors for trace elements in the New Zealand pelagic marine environment (Chapter 4). In Chapter 4, different sampling locations had widely disparate levels of trace elements, with especially

high Cd concentrations measured in arrow squids from the Taranaki area (Appendix Table 3); these were attributed to their prey items (Chapter 4), because diet is considered the main bioaccumulation pathway for Cd in cephalopods (e.g., through crustaceans that are enriched in Cd; Penicaud et al., 2017). The enriched concentrations in their prey are likely influenced by upwelling events, as frequently reported for this area, which can lead to enhanced primary productivity that is linked with increased Cd concentrations (Bruland et al., 1978; Foster & Battaerd, 1985). Other New Zealand monitoring studies, e.g. on dredge scallops (*Pecten novaezelandiae*, Peake et al., 2010), also measured Cd differences among sampling locations in New Zealand. Those differences were also mainly explained by upwelling events and their link to primary productivity (Peake et al., 2010). Because trace element concentrations vary among localities and over time, an annual monitoring programme that targets commercially fished stocks should be established. Such a programme should focus on New Zealand's most targeted arrow squid fisheries, such as the Chatham Rise, and regions with increased levels such as Taranaki. The biomonitoring of trace elements in fished arrow squid mantle tissue could help observe any potential fluxes in Cd concentrations and detect potential hazards to human consumers. In its absence, Cd levels may increase (annually or regionally) without human knowledge, and human consumers may be exposed to potentially high Cd concentrations under the erroneous assumption that they remain at the relatively safe levels reported here.

#### *Moroteuthopsis ingens*

Together with arrow squids, the ammoniacal *Moroteuthopsis ingens* is one of the most locally abundant species and is an important prey item in the diets of many predators within New Zealand's pelagic food webs (Jackson et al., 2000). The trace element analysis of this species has implications for its predators and suggested a high bioaccumulation capacity of Cd and Hg for this species, with the highest levels of Hg measured in large females (Chapter 5). In comparison, such a sexual disparity has not been reported from arrow squid (except for Cu and Cr, Chapter 2, Fig.7) and was not observed here in their trace element bioaccumulation patterns (Chapters 2, 3). The sexual difference in trace element levels in *M. ingens* appears linked to habitat, as indicated by the stable isotope ratios, which suggests that their main source of Hg was linked with habitat-related prey (Chapter 5). Large female *M. ingens* are known to migrate into deeper waters (Arkhipkin & Laptikhovsky, 2010), where they likely consume more Hg-enriched prey (e.g., fish) than males, which remain in shallower waters and feed on crustaceans (richer in Cd; Chapter 5). The effect of the sexual sex-specific diet and habitat shift on trace element concentrations in *M. ingens* is a novel finding and highlights the complexity of trace element bioaccumulation across different cephalopod species.

#### *Inter-species comparisons*

Although many previous biomonitoring studies of pelagic cephalopods have focussed on a single opportunistically caught species due to low sampling efforts (e.g., Bustamante et al., 2000, 2008;

Lischka et al., 2018, 2019a), the present study had the advantage of accessing and comparing two different cephalopod species from the same sampling region (*N. sloanii*, Chapters 2, 3 and *M. ingens*, Chapter 4). Interspecies differences in bioaccumulation of Cd have previously been observed in cephalopods (Rjeibi et al., 2014) as well as cnidarians (Karntanut & Pascoe, 2002) and was also confirmed between the present study species (Chapters 2–5). One of the main research questions in this thesis was the comparison of trace element bioaccumulation patterns between ammoniacal and non-ammoniacal squid species. Despite the observed regional variability, no clear trace element concentration differences between the ammoniacal *M. ingens* and non-ammoniacal squid species were observed (Table 22), suggesting that the stored ammonium does not impact trace element bioaccumulation patterns across species.

Trace element concentrations differed among the three cephalopod species analysed in this thesis and their sampling locations (Chapter 1, Table 1). In the Chatham Rise, the Cd concentrations in *N. sloanii* specimens (Chapters 2, 3) were similar to those reported for smaller *M. ingens* (Chapter 5), likely due to their similar feeding strategies and locality (Arkhipkin & Laptikhovsky, 2010; Uozumi, 1998). Locality effects, rather than species effects, were observed for Cd concentrations in *Nototodarus* spp. as different regions had different point sources for Cd (Chapter 4). For example, Cd concentrations in *N. gouldi* were almost twice as high in specimens sampled from along the Taranaki region and sampling locations of the West Coast of the South Island (Chapter 1, Table 1). Some of the sampling origins of *N. gouldi* and *N. sloanii* were confounded with their species occurrence; these species generally occur in separate locations (Chapter 1, Fig. 1). However, both species were sampled and analysed from the Golden Bay and the West Coast (Chapter 4, Table 12), where species differences were observed for As and Zn concentrations, with higher concentrations measured in *N. gouldi* (Chapter 4, Table 14). Fisheries New Zealand treats both species as a single species because they have similar physiology and diets (MPI, 2019; Uozumi, 1998). However, based on the species differences observed for As and Zn (Chapter 4), and the influence of sampling origin on Cd concentrations, it is recommended to treat *N. gouldi* and *N. sloanii* as separate species in fisheries management for their conservation and for human health implications.

The regionally measured trace element concentrations in the squids were assessed in a global context (Chapters 2, 4, 5). Among open-ocean squids, broad global differences in Cd concentrations appear to occur, with lower concentrations found in temperate-dwelling species relative to tropical taxa where extremely high concentrations have been measured (e.g., Dorneles et al., 2007; Lischka et al., 2018). On a global scale, Cd concentrations measured in the analysed species in this thesis were comparable to *Illex argentinus* from Argentinian waters (Kurihara et al., 1993) and *Todarodes filippovae* from Australian waters (Kojadinovic, et al., 2011; Table 22). The Hg concentrations measured in *M. ingens* in this thesis (Chapter 5) were comparable to *A. dux* from the Bay of Biscay and *S. pteropus* from the

Eastern Tropical Atlantic (Table 23), and higher than those reported in Antarctic species (Table 23). The lower concentrations measured in cephalopods from Antarctic waters could indicate lower Hg in seawater and the cephalopods' respective prey items (Seco et al., 2020). This thesis filled pre-existing knowledge gaps in terms of trace element concentrations for southern hemisphere squids (families Onychoteuthidae, Ommastrephidae). Because squids are known for their high bioaccumulation potential for trace elements (e.g., Bustamante et al., 1998a; Penicaud et al., 2017), the information in this thesis provides important context for the global assessment of trace elements in cephalopods, potentially using them as indicator species for increasing toxic trace elemental flux into food webs deriving from environmental changes.

Table 22. Concentration of Cd in the digestive glands of pelagic squids including the specimens analysed in this thesis (mean  $\pm$  standard deviation, sd, in  $\mu\text{g g}^{-1}$  dw).

Species	mean $\pm$ sd	Region	Reference
<b>Ommastrephidae</b>			
<i>Illex argentinus</i>	5014.50 $\pm$ 2830.00*	Central South Brazil Bight	Dorneles et al., 2007
<i>Illex argentinus</i>	92.50 $\pm$ NA	Argentina	Falandysz, 1988
<i>Illex argentinus</i>	5.14 $\pm$ 1.54	Patagonia	Gerpe et al., 2000
<i>Illex argentinus</i>	0.6 $\pm$ NA*	Argentina	Storelli et al., 2012
<i>Illex argentinus</i>	144.80 $\pm$ 65.00	Argentina	Kurihara et al., 1993
<i>Illex coindetii</i>	14.40 $\pm$ 7.55*	Adriatic Sea	Storelli & Marcotrigiano et al., 1999
<i>Nototodarus gouldi</i>	50.00 $\pm$ 25.00	Bass Strait, Australia	Smith et al., 1984
<i>Nototodarus gouldi</i>	33.00 $\pm$ 30.00	Port Phillip Bay, Australia	Finger & Smith, 1987
<i>Nototodarus gouldi</i>	194.05 $\pm$ 214.02	New Zealand	Chapter 4
<i>Nototodarus sloanii</i>	110.53 $\pm$ 95.15	Chatham Rise, New Zealand	Chapter 2
<i>Nototodarus sloanii</i>	89.04 $\pm$ 113.50	Chatham Rise, New Zealand	Chapter 3
<i>Nototodarus sloanii</i>	89.27 $\pm$ 86.34	New Zealand	Chapter 4
<i>Ommastrephes bartramii</i>	826.50 $\pm$ 369.10	Japanese Waters	Kurihara, et al., 1993
<i>Ommastrephes bartramii</i>	286.79 $\pm$ 194.39	Southern California	Martin & Flegal, 1975
<i>Sthenoteuthis oualaniensis</i>	0.13 $\pm$ NA	Sea of Japan	Ichihashi et al., 2001
<i>Sthenoteuthis pteropus</i>	747.80 $\pm$ 279.36	Eastern Tropical Atlantic	Lischka et al., 2018
<i>Todarodes filippovae</i>	246.00 $\pm$ 187.00	Indian Ocean	Kojadinovic et al., 2011
<i>Todarodes filippovae</i>	98.50 $\pm$ 67.20	Tasmania	Kojadinovic et al., 2011
<i>Todarodes pacificus</i>	16.68 $\pm$ NA	Sea of Japan	Ishizaki et al., 1970
<i>Todarodes sagittatus</i>	13.84 $\pm$ 5.96*	Bay of Biscay	Bustamante et al., 1998a
<i>Todarodes sagittatus</i>	18.00 $\pm$ 12.00	Bay of Biscay	Chouvelon et al., 2011
<b>Onychoteuthidae</b>			
<i>Moroteuthopsis ingens</i>	86.94 $\pm$ 175.80	Chatham Rise, NZ	Chapter 5
<i>Moroteuthopsis ingens</i>	29.60 $\pm$ 20.00	Kerguelen Islands	Cipro et al., 2017

\* wet weight converted to dry weights, assuming a water content of 80% for molluscs, fish and mammals

Table 23. Concentration of total Hg (THg) in tissues (digestive gland, DG; muscle (M) of selected non-ammoniacal and ammoniacal squid species (mean  $\pm$  standard deviation [sd] in  $\mu\text{g g}^{-1}$  dw) including the species analysed in this thesis.

Species	Tissue	THg± sd	Ammonia	Region	Study
Oegopsida					
<i>Alluroteuthis antarcticus</i>	DG	0.04± 0.002	Yes	South Georgia, Antarctica	Seco et al., 2020
	M	0.07± 0.004			
<i>Architeuthis dux</i>	DG	0.47 ± 0.13	Yes	Bay of Biscay	Bustamante et al., 2008
	M	2.07 ± 0.19			
<i>Filippovia knipovitchi</i>	DG	0.64± 0.10	Yes	South Georgia, Antarctica	Seco et al., 2020
	M	0.08± 0.03			
<i>Gonatus antarcticus</i>	M	0.16± 0.06	No	South Georgia, Antarctica	Seco et al., 2020
<i>Gonatus fabricii</i>	DG	0.05 ± 0.02	No	Greenland	Lischka et al., 2019a
	M	0.11 ± 0.07			
<i>Kondakovia longimana</i>	DG	0.05± 0.02	Yes	South Georgia, Antarctica	Seco et al., 2020
	M	0.08± 0.02			
<i>Moroteuthopsis ingens</i>	DG	0.18± 0.20	Yes	Chatham Rise, NZ	Chapter 5
	M	0.40± 0.36			
<i>Nototodarus gouldi</i>	DG	0.44± 0.26		NZ EEZ overall	Chapter 4
	M	0.52± 0.41			
<i>Nototodarus sloanii</i>	DG	0.14± 0.12		NZ EEZ overall	Chapter 4
	M	0.28± 0.22			
<i>Psychroteuthis glacialis</i>	DG	0.03± 0.01	No	South Georgia, Antarctica	Seco et al., 2020
	M	0.08± 0.02			
<i>Sthenoteuthis pteropus</i>	DG	0.43 ± 0.18	No	Eastern Tropical Atlantic	Lischka et al., 2018
	M	0.33 ± 0.14			
<i>Todaropsis eblanae</i>	DG	0.13 ± 0.10	No	British waters	Pierce et al., 2008
	M	0.21 ± 0.20			
<i>Todarodes sagittatus</i>	DG	0.28 ± 0.11	No	British waters	Pierce et al., 2008
	M	0.43 ± 0.20			
Myopsida					
<i>Alloteuthis subulata</i>	DG	0.07 ± 0.01	No	British waters	Pierce et al., 2008
	M	0.01 ± 0.01			

### *Globicephala melas edwardii*

Long-finned pilot whales (*G. m. edwardii*) in New Zealand waters are an important apex predator. Cadmium concentrations in *G. m. edwardii* were highest in the kidneys, and Hg concentrations were highest in the liver (Chapter 6); these results were comparable to those reported for *G. m. melas* specimens from the North Atlantic (Chapter 6, Table 21). Although lower than in the liver, the Hg concentrations observed in the muscular tissues of the specimens (Chapter 6, Table 19, Fig. 22) were still high enough to represent a potential human health hazard if consumed (Chapter 6). The concentrations of Cd and Hg increase with size and age with females generally having higher concentrations (Chapter 6, Table 20; Fig. 24). These observations suggest the bioaccumulation of Cd and Hg over the lifespan of the individuals, which corroborates previous studies (Caurant et al., 1994, 1996). No conclusion in terms of the stranded individuals' health could be made since necropsy data are not available. Necropsy data are recommended for future studies because it would help increase our

understanding of the potential health effects of trace element concentrations on *G. m. edwardii* and could help with identifying the causes of stranding events.

### **Impact on human consumers of arrow squid**

Global cephalopod fisheries are increasing (Doubleday et al., 2016) and, with the decline of global fish stocks (Rodhouse & White, 1995), human reliance on cephalopods for consumption will likely continue to increase, which has already been demonstrated by an increased fishing effort (FAO, 2020). Cephalopods appear to have a remarkable tolerance towards changing environmental conditions, such as increasing oxygen minimum zones (and their role as a sink for Cd in the world's oceans) (Janssen et al., 2014; Rosa & Seibel, 2010), and the increasing pressure on fish stocks (Rodhouse & White, 1995; Worm, 2016). However, the ecological performance of cephalopods may not be reliably extrapolated from reported population numbers, as these are usually extracted from fishing efforts (Doubleday et al., 2016). In the case of New Zealand's arrow squids, the reported catch overall increased from the year 2014 to 2018 (MPI, 2019).

Since catch rates for New Zealand's arrow squids are increasing, understanding the role of arrow squids as potential vectors for trace elements for New Zealand consumers is essential and formed the focus of this thesis (Chapters 3, 4). Identifying geographic areas where arrow squids with elevated levels of toxic elements have been fished may help inform decisions about concentrating future fisheries efforts on regions with stocks with lower trace element concentrations. In particular, at-risk groups (e.g., children, adolescents, and pregnant women) are often advised to act conservatively regarding potentially hazardous food items and should exercise caution in their exposure to arrow squids (Chapters 3, 4). However, the levels reported herein for trace elements of most health concern (As, Cd, Hg, and Pb) in mantle tissue of arrow squid evaluated in this thesis (Chapters 2–4) indicate that they do not appear to pose a risk to human consumers, if the tissue has not been contaminated by digestive gland fluids (see further discussion below).

In addition to the trace element levels found in food items, their bioaccessibility is another concern. In this thesis, concentrations of As, Cd, Hg, and Pb measured during the *in-vitro* digestion were all well below the maximum allowable levels in shellfish or the benchmark dose level (BMDL; Chapter 3, Table 9). While simulated *in-vitro* digestion is a useful method for determining the bioaccessibility of trace elements (Metian et al., 2009; Waisberg et al., 2004), this type of study has two inherent limitations. First, pH profiles are controlled throughout the simulated digestion phases (Minekus et al., 2014). However, this does not entirely reflect the conditions *in vivo*, which may fluctuate during each digestion step (Minekus et al., 2014). A link between pH and the bioaccessibility of Cd has been previously described in simulated digestion experiments, where the increased binding of Cd to plant material at

pH values above 3 has been observed (Waisberg et al., 2004). While *in-vitro* digestion studies usually focus on one food item (Minekus et al., 2014; Waisberg et al., 2004), real-life scenarios are usually much more complex, with meals consisting of multiple components (e.g., squid might be consumed in a salad). Specifically, the bioaccessibility and dissolution of a trace element can be influenced by other elements in the solution (Metian et al., 2009). For example, reductive processes have been described for Pb absorption in the intestine in the presence of other material (Laparra et al., 2003; Marschner et al., 2006), which might explain the unsuccessful detection of Pb in Chapter 3. Second, the simulated intestinal phase does not replicate the removal of water and certain digestive products, which can also alter trace element concentrations (Minekus et al., 2014). Therefore, trace element concentrations measured in the final step of the simulated *in-vitro* digestion (Chapter 3) are likely to differ from *in vivo* conditions. Although multiple limitations exist with the simulated *in-vitro* digestion, this method remains the most accurate available model of human digestion and provides valuable insights into the bioaccessibility of trace elements from diet (Minekus et al., 2014).

One novel observation was that cross-contamination from the digestive gland to the mantle tissue can significantly affect trace element concentrations. Although the trace element concentrations analysed in arrow squid mantle tissue (Chapters 2–4), including the *in-vitro* digestion (Chapter 3), were below the maximum levels presently considered tolerable for human consumption, a staining of the mantle tissue with digestive fluids from the digestive gland (Chapter 3, Fig. 10) was occasionally observed during the dissections of more than 320 squid specimens of various species (pers. obs.). This observation was also apparent in all squid ( $n=3$ ) purchased from the fish market for human consumption (Chapter 3, Table 10). Analyses of this contaminated tissue showed that trace element concentrations in the stained mantle parts were orders of magnitude higher than those measured in unstained tissue (Chapter 3, Table 10). New Zealand's Ministry for Primary Industries identified vegetables and shellfish as the main Cd contributors (MPI, 2018), but arrow squids were not included in the study. Based on the results of the stained mantle tissue, human consumers could potentially be at risk when exposed to these elevated mantle concentrations, since consuming a portion (120g) of the stained market squid tissue would exceed the maximum allowable level for Cd in shellfish by ~1233% ( $2.38 \mu\text{g g}^{-1}$  ww; Chapter 3, Table 8). Therefore, consumers need to pay careful attention to any staining on mantle tissue from the digestive gland and avoid those areas in order to not be exposed to potentially hazardous levels of Cd. It might be also advisable for squid harvesters and processors to review their handling procedures to minimise leakage of digestive gland fluids.

### **Impact on marine top predators**

Cephalopods have been suggested as vectors for trace elements to top predators (Bustamante et al., 1998a). Because they consume entire squids (rather than just the mantle), non-human predators are

exposed to the full body burden of trace element concentrations, including the high levels present in the digestive gland of squids (as measured in Chapters 2–5). The main known predators of the focus squid species (*N. gouldi*, *N. sloanii*, and *M. ingens*) in New Zealand waters are cetaceans (sperm whales [Clarke & Roper, 1998] and pilot whales [Beatson et al., 2007a,b]), seabirds (Cherel et al., 2017; James & Stahl, 2000), predatory fishes (Xavier et al., 2002; Emami-Khoyi et al., 2016), and seals (Green and Burton, 1993; Childerhouse et al., 2001). This section examines the potential role of squids as trace element vectors and has been divided into cetacean predators, and non-cetacean predators.

### *Cetaceans*

Cetaceans are apex predators in the New Zealand pelagic food web. This thesis suggests that, in at least one species (long-finned pilot whales), arrow squid are likely to be the primary vector of Cd, based on analysis of the cetaceans' tissues and their stomach contents (Chapter 6). Intake levels for *G. m. edwardii* were then estimated based on the average trace element concentrations measured in fresh arrow squid (based on Chapters 2–4), and arrow squid from their stomach contents (Chapter 6) and these data were combined with their estimated feeding rates (Beatson & O'Shea, 2009).

Analysing squid from the stomach contents of *G. m. edwardii* represented a unique opportunity to capture a snapshot of the cetaceans' prey contamination levels at the time of the stranding (Chapter 6). However, these concentrations alone cannot be used to assess the animals' uptake of trace elements through diet because not all tissues of the squid are digested at the same speed in the predator's stomach. For example, the viscera are digested more quickly than the muscle tissue, meaning that trace elements, such as Cd, from the digestive gland, might have already dissolved and were possibly already absorbed by the whale or contaminated the mantle tissue (Helm, 1984; Ugolev et al., 1983). Because the digestive gland of arrow squids contains higher levels of certain trace elements, it has the ability to contaminate mantle tissue (Chapter 3). Nevertheless, the analysis of trace elements in cephalopods from predator stomachs is particularly useful for cephalopod species that are rarely caught with nets, either because of net avoidance (Wormuth & Roper, 1983), or because they are of no commercial interest. Rare species are therefore recommended to be analysed as a whole organism (e.g., McArthur et al., 2003) because the contamination of mantle tissue with digestive fluids cannot be excluded. This method then addresses the squid's whole trace element body burden, rather than that of a localised tissue. In addition, for species where the tissue distribution of trace elements is known (e.g., *N. sloanii*, Chapters 2, 3), these data can be combined with the stomach sample data and allow the estimation of the trace element concentrations of the individual tissues. Hereby, the impact on predators that only consume certain tissues (e.g., humans), can be evaluated.

The estimation of trace element exposure in *G. m. edwardii* through arrow squid consumption might be influenced by interregional and annual variability. In particular, the high levels of Cd in the digestive

gland of arrow squid samples from the Taranaki region were remarkable (Chapter 4). In this region, Cd concentrations reached  $788.5 \mu\text{g g dw}^{-1}$  in the digestive gland, which can be extrapolated to a mean concentration of approximately 4.93mg Cd per whole squid (Chapter 4). Those concentrations would result in a daily intake for an average *G. m. edwardii* (based on a minimum diet of 24 arrow squid a day) of 118.32 mg Cd. The extrapolated intake with those maximum concentrations could reach concentrations of 1.29kg Cd over their lifespan (approx. 30 years). These estimated rates suggest that arrow squids are the most significant known source of Cd for long-finned pilot whales (Chapter 6). Since arrow squid can be considered the main pathway of Cd uptake in *G. m. edwardii*, any global change process that might increase Cd in their tissues might also have effects upon its predators.

*Moroteuthopsis ingens* has also been reported as a major prey item for *G. m. edwardii* in South Chilean waters (Mansilla et al., 2012). Although not yet reported as such, it might also be a prey item for *G. m. edwardii* in New Zealand waters, where it is known to form a large component in the diets of other locally foraging cetaceans such as sperm whales, *Physeter macrocephalus* (Clarke & Roper, 1998). Sperm whales that feed on *M. ingens* in New Zealand waters might be exposed to the high Hg concentrations measured in large *M. ingens* females (Chapter 5). By identifying trace element levels across a variety of *M. ingens* specimen, this thesis has laid the groundwork for future sperm whale studies that consider *M. ingens* as a vector for trace elements.

#### *Non-cetaceans*

Arrow squid (Chapters 2, 3) and *M. ingens* (Chapter 5) are also locally important in the diets of many non-cetaceans, including seabirds (Cherel et al., 2017; James & Stahl, 2000), fishes (Xavier et al., 2002; Emami-Khoyi et al., 2016) and seals (Green and Burton, 1993; Childerhouse et al., 2001). The trace element concentrations observed for *M. ingens* and *N. spp.* suggest that these squid species could also well be an important vector for trace elements in their predators.

In assessing the impact of squid consumption on apex predators, the regional variability in trace element concentrations should be considered as it may have different implications for their consumers. For example, less migratory predators with a very localised distribution, e.g. the New Zealand sea lion, *Phocartos hookeri*, from the Auckland Islands (Childerhouse & Gales, 1998), might primarily be exposed to the relatively low Cd levels measured in squid from this area, whereas seals from the Taranaki area could be chronically exposed to the higher Cd levels measured in arrow squid from this region. However, more migratory predators, such cetaceans (including *G. m. edwardii*) might experience a fluctuation in exposure to the localised high or low concentrations (Chapters 3, 4). This thesis aimed to provide novel baseline data (and has succeeded) that can now be used for comparison with future assessments of trace element levels in squid predators and improving understanding of trace element dynamics within New Zealand food webs. Such an assessment could help predict and monitor

climate-change-induced fluctuations in trace element concentrations for species that are considered vulnerable such as the New Zealand sea lion, *Phocarctos hookeri* (Chilvers, 2018), or the southern royal albatross, *Diomedea epomophora* (Waugh et al., 2005).

## Recommendations

1. Vulnerable consumers should limit arrow squid intake
2. Fisheries processors should assess handling procedures
3. Monitoring studies for trace elements by region and year

## Future research

In light of the increased fisheries effort for arrow squid and cephalopods in general, it is crucial to fully understand their role as vectors in the bioaccumulation pathways in New Zealand's marine environment. The detoxification mechanisms in cephalopods are still not fully understood but their high accumulation capacities for elements such as Ag and Cd are widely reported (Costa & Rodrigo, 2017; Penicaud et al., 2017). Future studies should assess the ecotoxicology of trace elements and other toxicants in cephalopods and their significance in terms of the squids' reproductive performance.

Arrow squids are important prey items for many top predators in New Zealand's pelagic food web. This thesis investigated and strongly suggests arrow squids as vectors for trace elements in long-finned pilot whales (*G. m. edwardii*). Future studies should analyse trace elements in other predators within New Zealand waters, such as the brown fur seal (*Arctocephalus forsteri*), hoki (*Macruronus novaezelandiae*), and orange roughy (*Hoplostethus atlanticus*), which have not been recently evaluated for trace element concentrations (van den Broek & Tracey, 1981). An analysis of these predators would provide further insight into trace element accumulation in New Zealand's pelagic food webs. The analysis of arrow squid from the Chatham Rise also showed annual variability in trace element concentrations (Chapter 2). I suggest a timeseries of trace element analyses in arrow squid and other marine organisms from the Chatham Rise in order to assess the annual variability of Cd concentrations, especially under the influence of changing oceanic conditions.

The *in-vitro* simulated digestion suggested that Cd concentrations are not significantly affected by the human digestive process, i.e., that all Cd contained within consumed muscle tissue is bioaccessible to human consumers (Chapter 3, Table 11). This was one of the main results of this study and is important when considering *N. sloanii* as a vector of Cd for humans. The exact processes that can explain the loss in trace element concentrations are not fully understood (e.g., Laparra et al., 2003) and further comparative analysis is necessary in order to obtain reliable results for the *in-vitro* digestion of As, Hg

and Pb. Future studies should conduct simulated digestion experiments with a broader range of As, Hg, Cd and Pb levels and also analyse muscular tissue contaminated with digestive fluids (as seen in Chapter 3, Fig. 10). The outcomes might have implications for food safety in terms of arrow squid consumption. Furthermore, the potential contamination of otherwise edible muscular mantle tissue by digestive fluids in arrow squid needs to be urgently investigated.

Several interesting trends in mercury concentrations were observed, with arrow squids lowest among the analysed species, and pilot whales highest. In fact, the high Hg concentrations observed in long-finned pilot whales were remarkable (Chapter 6) and raised the question of whether the low mercury concentrations in arrow squid could indeed accumulate to produce the levels measured in the whales, or whether additional prey species might be consumed—and thus contribute Hg—in quantities not presently reported (for example, higher Hg levels are usually observed in fish than in cephalopods; e.g., Storelli & Marcotrigiano, 2004). Female *M. ingens* also had higher Hg concentrations than *Nototodarus* spp., (Chapters 3,4), and have been reported as major prey items for *G. m. edwardii* in other regions such as Southern Chile (Mansilla et al., 2012), although this dynamic has not been reported in New Zealand. It could be that the importance of fish and other prey items (including other cephalopod species) has been previously underestimated in the diet of *G. m. edwardii* (Betty, pers. observation). I suggest future research that identifies prey from stranded *G. m. edwardii* stomach contents in New Zealand waters and also assesses Hg concentrations in their prey items to identify potential Hg sources. In addition, *M. ingens* is an important component in the diets of other locally foraging cetaceans such as sperm whales, *Physeter macrocephalus* (Clarke & Roper, 1998). While a global comparison measured second highest Hg concentrations in sperm whales from Australian waters (Savery et al., 2013), concentrations in this species from New Zealand waters have not been assessed. Identifying the levels of trace elements in *P. macrocephalus* from New Zealand waters would provide important context in assessing the role of *M. ingens* as a vector for trace elements.

Overall, our current understanding of trace element circulation in global oceanic environments remains relatively sparse. The analysis of stratified water samples worldwide could help in modelling those circulations and potentially identify reasons for the so called ‘Cd anomaly’ in the polar oceans (Bargagli et al., 1996; Chapter 1), the higher Hg concentrations in deeper waters (Choy et al., 2009) and the cycling of Ag in the global oceans (Gallon & Flegal, 2015). Furthermore, climate change and ocean acidification are affecting and will likely continue to affect all oceans, including New Zealand waters (Law et al., 2018a; Law, et al., 2018b). Ocean acidification can lead to altered trace element physiologies and toxicities in invertebrates, especially for pH-sensitive elements such as Cu or Fe (Avendaño et al., 2016; Lewis et al., 2016). Therefore, both baseline data and ongoing monitoring are crucial for understanding and predicting the potential influences of those changes on pelagic invertebrates, including cephalopods. This thesis has provided novel and important baseline data for

three cephalopod and one cetacean species (Chapters 2–6), which can be used to monitor potential changes over time. While these results provide important insights into several aspects of trace element bioaccumulation (especially Cd) within local pelagic food webs, plenty of work remains. Logical next steps include comparative analysis of other prey and predator species, and future studies reassessing these organisms (particularly those consumed by humans) to build a more complete picture of these processes through time.

## Conclusion

This work has supported the hypothesis that squids are important vectors for trace elements within New Zealand's food webs. This thesis significantly contributes to the current data available on trace elements in New Zealand pelagic food webs because it comprises the most comprehensive dataset to date on trace element concentrations and their tissue distribution in New Zealand arrow squids (*Nototodarus gouldi* and *N. sloanii*), greater-hooked squid (*Moroteuthopsis ingens*), and long-finned pilot whales (*Globicephala melas edwardii*). This is also the first study to conduct a regional assessment of trace element concentrations in arrow squids among multiple fishing locations of New Zealand's Exclusive Economic Zone (EEZ). The individual studies have confirmed that arrow squid are an important vector of trace elements within New Zealand foodwebs, and likely explain the high Cd concentrations measured in New Zealand's long-finned pilot whales. *Nototodarus sloanii* specimens extracted from the stomachs of stranded *G. m. edwardii* contained high trace element concentrations which make them a likely vector for trophic transfer, especially for Cd. The impact of arrow squid trace elements on human consumers was approached with the application of *in-vitro* digestion simulation, which is a novel approach for commercially fished ommastrephid cephalopods. Together with the estimations of intake rates, the concentrations of As, Cd, Hg, and Pb in the edible muscular parts of the arrow squids remain on average below the maximum permissible levels. However, the diffusion effect of digestive fluids on surrounding mantle tissue, as observed for purchased market squid, highlighted the possibility of exceeding those thresholds for Cd. Similar to other ommastrephid squids, Cd seemed to be the element of most concern in terms of arrow squids as they seem to accumulate this metal in high concentrations. This study provides baseline data on trace elements in the New Zealand pelagic food web, and will hopefully form the basis of future studies in this area.

## References

- Abraham, E. (2018). Cadmium in New Zealand agricultural soils. *New Zealand Journal of Agricultural Research*, 1–18. <https://doi.org/10.1080/00288233.2018.1547320>
- Abraham, G., & Parker, R. (2002). Heavy-metal contaminants in Tamaki Estuary: Impact of city development and growth, Auckland, New Zealand. *Environmental Geology*, 42(8), 883–890.
- Adams, M. A., Bolger, P. M., & Gunderson, E. L. (1994). Dietary intake and hazards of arsenic. *Arsenic Exposure and Health. Special Issue of Environmental Geochemistry and Health. Science and Technology Letters* 16, 41–50.
- Aldridge, S. P., Gormley, A. M., Gaw, S., Webb, S., Buxton, R. T., & Jones, C. J. (2017). Elevated mercury concentrations in the feathers of grey-faced petrels (*Pterodroma gouldi*) in New Zealand. *Marine Pollution Bulletin* 119(1), 195–203.
- Altmann, L., Weinsberg, F., Sveinsson, K., Lilienthal, H., Wiegand, H., & Winneke, G. (1993). Impairment of long-term potentiation and learning following chronic lead exposure. *Toxicology Letters*, 66(1), 105–112.
- Amiard, J. C., Amiard-Triquet, C., Barka, S., Pellerin, J., & Rainbow, P. S. (2006). Metallothioneins in aquatic invertebrates: Their role in metal detoxification and their use as biomarkers. *Aquatic Toxicology* 76, 160–202.
- Anderson, C. I., & Rodhouse, P. G. (2001). Life cycles, oceanography and variability: Ommastrephid squid in variable oceanographic environments. *Fisheries Research* 54(1), 133–143.
- André, J. M., Ribeyre, F., & Boudou, A. (1990). Mercury contamination levels and distribution in tissues and organs of delphinids (*Stenella attenuata*) from the eastern tropical Pacific, in relation to biological and ecological factors. *Marine Environmental Research* 30, 43–72.
- Ansari, T. M., Marr, I. L., & Tariq, N. (2004). Heavy metals in marine pollution perspective-a mini review. *Journal of Applied Sciences* 4, 1–20.
- Arkhipkin, A. I., & Laptikhovsky, V. V. (2010). Observation of penis elongation in *Onykia ingens*: Implications for spermatophore transfer in deep-water squid. *Journal of Molluscan Studies* 76(3), 299–300.
- Arkhipkin A. I., Rodhouse, P. G., Pierce, G. J., Sauer, W., Sakai, M., Allcock, L., Arguelles, ... & Chen, C. S. (2015). World squid fisheries. *Reviews in Fisheries Science & Aquaculture* 23(2), 92–252.
- Atwell, L., Hobson, K. A., & Welch, H. E. (1998). Biomagnification and bioaccumulation of mercury in an arctic marine food web: insights from stable nitrogen isotope analysis. *Canadian Journal of Fisheries and Aquatic Sciences* 55, 1114–1121.
- Auger, P. A., Machu, E., Gorgues, T., Grima, N., & Waeles, M. (2015). Comparative study of potential transfer of natural and anthropogenic cadmium to plankton communities in the North-West African upwelling. *Science of the Total Environment* 505, 870–888.
- Avendaño, L., Gledhill, M., Achterberg, E. P., Rérolle, V., & Schlosser, C. (2016). Influence of ocean acidification on the organic complexation of iron and copper in Northwest European shelf seas; a combined observational and model study. *Frontiers in Marine Science* 3, 58.
- Bargagli, R. (2000). Trace metals in Antarctica related to climate change and increasing human impact. *Reviews of Environmental Contamination and Toxicology* 166, 129–174.
- Bargagli, R., Nelli, L., Ancora, S., & Focardi, S. (1996). Elevated cadmium accumulation in marine organisms from Terra Nova Bay (Antarctica). *Polar Biology* 16(7), 513–520.
- Bargagli, R., Sanchez-Hernandez, J. C., Monaci, F., & Focardi, S. (2000). Environmental factors promoting bioaccumulation of Hg and Cd in Antarctic marine and terrestrial organisms. *Antarctic Ecosystems: Models for Wider Ecological Understanding* (Ed. W. Davison, C. Howard-Williams & P. Broady), 308–314.
- Barghigiani, C., Ristori, T., Biagi, F., & De Ranieri, S. (2000). Size related mercury accumulations in edible marine species from an area of the Northern Tyrrhenian Sea. *Water, Air, & Soil Pollution* 124, 169–176.
- Bartzokis, G., Tishler, T. A., Lu, P. H., Villablanca, P., Altshuler, L. L., Carter, M., Huang, D., Edwards, N., & Mintz, J. (2007). Brain ferritin iron may influence age-and gender-related risks of neurodegeneration. *Neurobiology of Aging* 28(3), 414–423.
- Bates, D. M., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using

- lme4. Journal of Statistical Software 67(1), 1–48.
- Baturin, G. N., & Kochenov, A. V. (2001). Uranium in phosphorites. Lithology and Mineral Resources 36(4), 303–321.
- Beasley, I., Cherel, Y., Robinson, S., Betty, E., Hagihara, R., & Gales, R. (2019). Stomach contents of long-finned pilot whales, *Globicephala melas* mass-stranded in Tasmania. PloS One 14, e0206747.
- Beatson, E., O'Shea, S., & Ogle, M. (2007a). First report on the stomach contents of long-finned pilot whales, *Globicephala melas*, stranded in New Zealand. Retrieved from <http://www.tandfonline.com/doi/abs/10.1080/03014220709510063>.
- Beatson, E., O'Shea, S., Stone, C., & Shortland, T. (2007b). Notes on New Zealand mammals 6. Second report on the stomach contents of long-finned pilot whales, *Globicephala melas*. New Zealand Journal of Zoology 34 (4), 359–362. <https://doi.org/10.1080/03014220709510063>.
- Beatson E., & O'Shea S. (2009). Stomach contents of long-finned pilot whales, *Globicephala melas*, mass-stranded on Farewell Spit, Golden Bay in 2005 and 2008. New Zealand Journal of Zoology 36, 47–58.
- Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B 57, 289–300.
- Bentley, R., & Chasteen, T. G. (2002). Microbial methylation of metalloids: Arsenic, antimony, and bismuth. Microbiology and Molecular Biology Reviews 66(2), 250–271.
- Bernard, A., & Lauwerys, R. (1986). Effects of cadmium exposure in humans. In Cadmium (pp. 135–177). Springer. Springer, Berlin, Heidelberg.
- Bernard, B. P., & Becker, C. E. (1988). Environmental lead exposure and the kidney. Journal of Toxicology: Clinical Toxicology 26(1–2), 1–34.
- Bernhoft, R. A. (2012). Mercury toxicity and treatment: a review of the literature. Journal of Environmental and Public Health, 460508, <http://dx.doi.org/10.1155/2012/460508>.
- Betty, E. L. (2019). Life History of the Long-finned Pilot Whale (*Globicephala melas edwardii*); Insights from strandings on the New Zealand Coast (PhD Thesis). Auckland University of Technology.
- Betty E. L., Bollard B., Murphy S., Ogle M., Hendriks H., Orams M.B., Stockin K.A. (2020). Using stranding records to inform conservation management of a data-poor cetacean species. Biodiversity and Conservation, 29(2) 643–665.
- Beuerlein, K., Ruth, P., Westermann, B., Löhr, S., & Schipp, R. (2002). Hemocyanin and the branchial heart complex of *Sepia officinalis*: are the hemocytes involved in hemocyanin metabolism of coleoid cephalopods? Cell and Tissue Research 310, 373–381.
- Bhupander, K., & Mukherjee, D. P. (2011). Assessment of human health risk for arsenic, copper, nickel, mercury and zinc in fish collected from tropical wetlands in India. Advances in Life Science and Technology, 2(1). Retrieved from [http://www.academia.edu/download/13095790/11.Assessment\\_of\\_Human\\_Health\\_Risk\\_for\\_Arsenic\\_\\_Copper\\_\\_Nickel\\_\\_Mercury\\_and\\_Zinc\\_in\\_Fish\\_Collected\\_from\\_Tropical\\_Wetlands\\_in\\_India.pdf](http://www.academia.edu/download/13095790/11.Assessment_of_Human_Health_Risk_for_Arsenic__Copper__Nickel__Mercury_and_Zinc_in_Fish_Collected_from_Tropical_Wetlands_in_India.pdf).
- Blévin, P., Carravieri, A., Jaeger, A., Chastel, O., Bustamante, P., & Cherel, Y. (2013). Wide range of mercury contamination in chicks of Southern Ocean seabirds. PLoS One 8(1), e54508.
- Bloch, D., & Heide-Jørgensen, M. P., Stefansson, E., Mikkelsen, B., Ofstad, L. H., Dietz, R., & Andersen, L. W. (2003). Short-term movements of long-finned pilot whales *Globicephala melas* around the Faroe Islands. Wildlife Biology 9(4), 47–59.
- Bloch, D., & Lockyer, C. (1989). Age-related parameters of the pilot whales off the Faroe Islands. Paper SC/41/SM15, Reports of the International Whaling Commission.
- Bloom, N. S. (1992). On the chemical form of mercury in edible fish and marine invertebrate tissue. Canadian Journal of Fisheries and Aquatic Sciences 49(5), 1010–1017.
- Blum, J. D. (2011). Marine chemistry: Marine mercury breakdown. Nature Geoscience 4, 139.
- Blum, J. D., Popp, B. N., Drzen, J. C., Choy, C. A., & Johnson, M. W. (2013). Methylmercury production below the mixed layer in the North Pacific Ocean. Nature Geoscience, 6(10), 879.
- Bochud, M., Jenny-Burri, J., Pruijm, M., Ponte, B., Guessous, I., Ehret, G., Petrovic, D., Dudler, V., Haldimann, M., Escher, G., & Dick, B. (2018). Urinary cadmium excretion is associated with

- increased synthesis of cortico-and sex steroids in a population study. *The Journal of Clinical Endocrinology & Metabolism* 103(2), 748–758.
- Bolstad, K. S. (2006). Sexual dimorphism in the beaks of *Moroteuthis ingens* Smith, 1881 (Cephalopoda: Oegopsida: Onychoteuthidae). *New Zealand Journal of Zoology* 33, 317–327.
- Bolstad, K. S., Braid, H. E., Strugnell, J. M., Lindgren, A. R., Lischka, A., Kubodera, T., Laptikhovsky, V. L., & Labiaga, A. R. (2018). A mitochondrial phylogeny of the family Onychoteuthidae (Cephalopoda: Oegopsida). *Molecular Phylogenetics and Evolution* 128, 88–97.
- Bond, A. L., & Lavers, J. L. (2011). Trace Element Concentrations in Feathers of Flesh-footed Shearwaters (*Puffinus carneipes*) from Across Their Breeding Range. *Archives of Environmental Contamination and Toxicology* 61(2), 318–326. <https://doi.org/10.1007/s00244-010-9605-3>.
- Booth, S., & Zeller, D. (2005). Mercury, Food Webs, and Marine Mammals: Implications of Diet and Climate Change for Human Health. *Environmental Health Perspectives* 113(5), 521–526. <https://doi.org/10.1289/ehp.7603>.
- Borak, J., & Hosgood, H. D. (2007). Seafood arsenic: implications for human risk assessment. *Regulatory Toxicology and Pharmacology* 47(2), 204–212.
- Borrell, A., Bloch, D., & Desportes, G. (1995). Age trends and reproductive transfer of organochlorine compounds in long-finned pilot whales from the Faroe Islands. *Environmental Pollution* 88, 283–292.
- Bosch, A. C., O'Neill, B., Sigge, G. O., Kerwath, S. E., & Hoffman, L. C. (2016). Mercury accumulation in Yellowfin tuna (*Thunnus albacares*) with regards to muscle type, muscle position and fish size. *Food Chemistry* 190, 351–356.
- Bose-O'Reilly, S., Lettmeier, B., Roider, G., Siebert, U., & Drasch, G. (2008). Mercury in breast milk—A health hazard for infants in gold mining areas? *International Journal of Hygiene and Environmental Health* 211(5–6), 615–623.
- Boyle, P., & Rodhouse, P. (2005). Cephalopods as prey. *Cephalopods—Ecology and Fisheries*. Blackwell Publishing, Oxford, 234–258.
- Brabyn, M. W., & McLean, I. G. (1992). Oceanography and coastal topography of herd-stranding sites for whales in New Zealand. *Journal of Mammalogy* 73(3), 469–476.
- Bradford-Grieve, J. M. (1983) Plankton and primary productivity in the vicinity of the Chatham Rise. *NZOI summary* 21, 1–14.
- Braid, H. E., McBride, P. D., & Bolstad, K. S. R. (2014). Molecular phylogenetic analysis of the squid family Mastigoteuthidae (Mollusca, Cephalopoda) based on three mitochondrial genes. *Hydrobiologia* 725, 145–164.
- Braley, M., Goldsworthy, S. D., Page, B., Steer, M., & Austin, J. J. (2010). Assessing morphological and DNA-based diet analysis techniques in a generalist predator, the arrow squid *Nototodarus gouldi*. *Molecular Ecology Resources* 10(3), 466–474.
- Brewer, D. T., Morello, E. B., Griffiths, S., Fry, G., Heales, D., Apte, S. C., Venables, ... & Pendrey, R. (2012). Impacts of gold mine waste disposal on a tropical pelagic ecosystem. *Marine Pollution Bulletin* 64(12), 2790–2806.
- Brooks, R. R., & Rumsey, D. (1974). Heavy metals in some New Zealand commercial sea fishes. *New Zealand Journal of Marine and Freshwater Research* 8(1), 155–166. <https://doi.org/10.1080/00288330.1974.9515495>.
- Brown, R. M., Newton, D., Pickford, C. J., & Sherlock, J. C. (1990). Human metabolism of arsenobetaine ingested with fish. *Human & Experimental Toxicology* 9, 41–46.
- Bruland, K. W., Knauer, G. A., & Martin, J. H. (1978). Cadmium in northeast Pacific waters 1. *Limnology and Oceanography* 23(4), 618–625.
- Bryan, G. W. (1984). Pollution due to heavy metals and their compounds. *Marine Ecology* 5, 1289–1431.
- Bryan, G. W., & Langston, W. J. (1992). Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries. *Environmental Pollution* 76(2), 89–131.
- Bryan, C. E., Christopher, S. J., McLellan, W. A., Schwacke, L. H., Wells, R. S. (2010). Application of ICP-MS to examining the utility of skin as a monitoring tissue for trace elements in bottlenose dolphin, *Tursiops truncatus*. *The Open Chemical and Biomedical Methods Journal* 3(1), 169–

- Buat-Menard, P., & Arnold, M. (1978). The heavy metal chemistry of atmospheric particulate matter emitted by Mount Etna Volcano. *Geophysical Research Letters* 5(4), 245–248.
- Burnham, K. P., & Anderson, D. R. (2004). Multimodel inference: understanding AIC and BIC in model selection. *Sociological Methods & Research* 33, 261–304.
- Bustamante, P., Bocher, P., Cherel, Y., Miramand, P., & Caurant, F. (2003a). Distribution of trace elements in the tissues of benthic and pelagic fish from the Kerguelen Islands. *Science of the Total Environment* 313(1–3), 25–39.
- Bustamante, P., Caurant, F., Fowler, S. W., & Miramand, P. (1998a). Cephalopods as a vector for the transfer of cadmium to top marine predators in the north-east Atlantic Ocean. *Science of the Total Environment* 220(1), 71–80.
- Bustamante, P., Cherel, Y., Caurant, F., & Miramand, P. (1998b). Cadmium, copper and zinc in octopuses from Kerguelen Islands, Southern Indian Ocean. *Polar Biology* 19, 264–271.
- Bustamante, P., Cosson, R. P., Gallien, I., Caurant, F., & Miramand, P. (2002a). Cadmium detoxification processes in the digestive gland of cephalopods in relation to accumulated cadmium concentrations. *Marine Environmental Research* 53, 227–241.
- Bustamante, P., Garrigue, C., Breau, L., Caurant, F., Dabin, W., Greaves, J., & Dodemont, R. (2003b). Trace elements in two odontocete species (*Kogia breviceps* and *Globicephala macrorhynchus*) stranded in New Caledonia (South Pacific). *Environmental Pollution* 124, 263–271.
- Bustamante, P., González, A. F., Rocha, F., Miramand, P., & Guerra, A. (2008). Metal and metalloid concentrations in the giant squid *Architeuthis dux* from Iberian waters. *Marine Environmental Research* 66, 278–287.
- Bustamante, P., Grigiono, S., Boucher-Rodoni, R., Caurant, F., & Miramand, P. (2000). Bioaccumulation of 12 trace elements in the tissues of the nautilus *Nautilus macromphalus* from New Caledonia. *Marine Pollution Bulletin* 8, 688–696.
- Bustamante, P., Lahaye, V., Durnez, C., Churlaud, C., & Caurant, F. (2006). Total and organic Hg concentrations in cephalopods from the North Eastern Atlantic waters: Influence of geographical origin and feeding ecology. *Science of the Total Environment* 368, 585–596.
- Bustamante, P., Teyssié, J.-L., Danis, B., Fowler, S., Miramand, P., Cotret, O., & Warnau, M. (2004). Uptake, transfer and distribution of silver and cobalt in tissues of the common cuttlefish *Sepia officinalis* at different stages of its life cycle. *Marine Ecology Progress Series* 269, 185–195.
- Bustamante, P., Teyssié, J.-L., Fowler, S.W., Cotret, O., Danis, B., Miramand, P., & Warnau, M. (2002b). Biokinetics of zinc and cadmium accumulation and depuration at different stages in the life cycle of the cuttlefish *Sepia officinalis*. *Marine Ecology Progress Series* 231, 167–177.
- Caddy, J. F., & Rodhouse, P. G. (1998). Cephalopod and groundfish landings: Evidence for ecological change in global fisheries? *Reviews in Fish Biology and Fisheries* 8, 431–444.
- Carravieri, A., Bustamante, P., Tartu, S., Meillère, A., Labadie, P., Budzinski, H., Peluhet, L., ... & Cherel, Y. (2014). Wandering albatrosses document latitudinal variations in the transfer of persistent organic pollutants and mercury to Southern Ocean predators. *Environmental Science & Technology* 48(24), 14746–14755.
- Carravieri, A., Cherel, Y., Brault-Favrou, M., Churlaud, C., Peluhet, L., Labadie, P., Budzinski, H., [...], & Bustamante, P. (2017). From Antarctica to the subtropics: Contrasted geographical concentrations of selenium, mercury, and persistent organic pollutants in skua chicks (*Catharacta* spp.). *Environmental Pollution* 228 464–473.
- Carravieri, A., Bustamante, P., Labadie, P., Budzinski, H., Chastel, O., & Cherel, Y. (2020). Trace elements and persistent organic pollutants in chicks of 13 seabird species from Antarctica to the subtropics. *Environment International* 134, 105225.
- Carter, L. (1980) New Zealand region bathymetry 1:6000000 (2nd ed.). NZ Oceanographic Institute Chart. Misc. Ser. 15. Dept. Sci. Ind. Res., Wellington, NZ.
- Caurant, F. (2013). Trace element trends & effects for small cetaceans in European waters. *Chemical Pollution and Marine Mammals* 59–93.
- Caurant, F., & Amiard-Triquet, C. (1995). Cadmium contamination in pilot whales *Globicephala melas*: source and potential hazard to the species. *Marine Pollution Bulletin* 30, 207–210.
- Caurant, F., Amiard, J.-C., Amiard-Triquet, C., & Sauriau, P.-G. (1994). Ecological and biological factors controlling the concentrations of trace elements (As, Cd, Cu, Hg, Se, Zn) in delphinids

- Globicephala melas* from the North Atlantic Ocean. Marine Ecology Progress Series 103(3), 207–219.
- Caurant, F., Navarro, M., & Amiard, J.-C. (1996). Mercury in pilot whales: possible limits to the detoxification process. Science of the Total Environment 186, 95–104.
- Chandurvelan, R., Marsden, I. D., Glover, C. N., & Gaw, S. (2015). Assessment of a mussel as a metal bioindicator of coastal contamination: Relationships between metal bioaccumulation and multiple biomarker responses. Science of the Total Environment 511, 663–675.
- Chen, X. (1998). The fishing ground of *Nototodarus sloanii* and its catch distribution around New Zealand waters. Journal of Shanghai Fisheries University 7, 93–101.
- Cherel, Y., Ducatez, S., Fontaine, C., Richard, P., & Guinet, C. (2008). Stable isotopes reveal the trophic position and mesopelagic fish diet of female southern elephant seals breeding on the Kerguelen Islands. Marine Ecology Progress Series 370, 239–247.
- Cherel, Y., & Duhamel, G. (2003). Diet of the squid *Moroteuthis ingens* (Teuthoidea: Onychoteuthidae) in the upper slope waters of the Kerguelen Islands. Marine Ecology Progress Series 250, 197–203.
- Cherel, Y., Xavier, J.C., de Grissac, S., Trouvé, C., & Weimerskirch, H. (2017). Feeding ecology, isotopic niche, and ingestion of fishery-related items of the wandering albatross *Diomedea exulans* at Kerguelen and Crozet Islands. Marine Ecology Progress Series 565, 197–215.
- Childerhouse, S., Dix, B., & Gales, N. (2001). Diet of New Zealand sea lions (*Phocarctos hookeri*) at the Auckland Islands. Wildlife Research 28(3), 291–298.
- Childerhouse, S., & Gales, N. (1998). Historical and modern distribution and abundance of the New Zealand sea lion *Phocarctos hookeri*. New Zealand Journal of Zoology 25(1), 1–16.
- Chilvers, B. L. (2018). Preliminary assessment of the foraging behaviour and population dynamics of a cryptic population of the endangered New Zealand sea lion. New Zealand Journal of Ecology 42(1), 48–57.
- Chiswell, S. M., Bostock, H. C., Sutton, P. J., & Williams, M. J. (2015). Physical oceanography of the deep seas around New Zealand: a review. New Zealand Journal of Marine and Freshwater Research 49(2), 286–317.
- Chong, J., Xia, J. (2018). MetaboAnalystR: an R package for flexible and reproducible analysis of metabolomics data. Bioinformatics 27, 4313–4314.
- Chouvelon, T., Spitz, J., Cherel, Y., Caurant, F., Sirmel, R., Mèndez-Fernandez, P., & Bustamante, P. (2011). Inter-specific and ontogenetic differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and Hg and Cd concentrations in cephalopods. Marine Ecology Progress Series 433, 107–120. <https://doi.org/10.3354/meps09159>.
- Chouvelon, T., Spitz, J., Caurant, F., Mèndez-Fernandez, P., Autier, J., Lassus-Débat, A., Chappuis, A., & Bustamante, P. (2012). Enhanced bioaccumulation of mercury in deep-sea fauna from the Bay of Biscay (north-east Atlantic) in relation to trophic positions identified by analysis of carbon and nitrogen stable isotopes. Deep-Sea Research 65, 113–124.
- Choy, C. A., Haddock, S. H., & Robison, B. H. (2017). Deep pelagic food web structure as revealed by in situ feeding observations. Proceedings of the Royal Society B: Biological Sciences 284, 20172116.
- Choy, C. A., Popp, B. N., Kaneko, J. J., & Drazen, J. C. (2009). The influence of depth on mercury levels in pelagic fishes and their prey. Proceedings of the National Academy of Sciences 106(33), 13865–13869.
- Cipro, C. V., Cherel, Y., Bocher, P., Caurant, F., Miramand, P., & Bustamante, P. (2018). Trace elements in invertebrates and fish from Kerguelen waters, southern Indian Ocean. Polar Biology 41, 175–191.
- Clark, M. (1999). Fisheries for orange roughy (*Hoplostethus atlanticus*) on seamounts in New Zealand. Oceanologica Acta 22(6), 593–602.
- Clark, M., & O'Driscoll, R. (2003). Deepwater fisheries and aspects of their impact on seamount habitat in New Zealand. Journal of Northwest Atlantic Fishery Science 31, 441–458.
- Clarke, M. R. (1980). Cephalopoda in the diet of sperm whales of the southern hemisphere and their bearing on sperm whale biology. Discovery Reports 37, 1–324.
- Clarke, M. R. (1996a). Cephalopods as prey. III. Cetaceans. Philosophical Transactions of the Royal Society of London B Biological Sciences 351, 1053–1065.

- Clarke, M. R. (1996b). The role of cephalopods in the world's oceans: An introduction. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 351(1343), 979–983.
- Clarke, M. R., & Roper, C. F. E. (1998). Cephalopods represented by beaks in the stomach of a sperm whale stranded at Paekakariki, North Island, New Zealand. *African Journal of Marine Science* 20, 129–133.
- Cobianchi, M., Mancin, N., Lupi, C., Bordiga, M., & Bostock, H. C. (2015). Effects of oceanic circulation and volcanic ash-fall on calcite dissolution in bathyal sediments from the SW Pacific Ocean over the last 550 ka. *Palaeogeography, Palaeoclimatology, Palaeoecology* 429, 72–82.
- Coll, M., Navarro, J., Olson, R. J., & Christensen, V. (2013). Assessing the trophic position and ecological role of squids in marine ecosystems by means of food-web models. *Deep Sea Research Part II: Topical Studies in Oceanography* 95, 21–36.
- Connell, A. M., Dunn, M. R., & Forman, J. (2010). Diet and dietary variation of New Zealand hoki *Macruronus novaezelandiae*. *New Zealand Journal of Marine and Freshwater Research* 44(4), 289–308.
- Conway, T. M., & John, S. G. (2014). The biogeochemical cycling of zinc and zinc isotopes in the North Atlantic Ocean. *Global Biogeochemical Cycles* 28, 1111–1128.
- Costa, P. M., & Rodrigo, A.P. (2017). The Role of the Cephalopod Digestive Gland in the Storage and Detoxification of Marine Pollutants. *Frontiers in Physiology* 8, 232.
- Craig, S. (2001). Environmental conditions and yolk biochemistry: factors influencing embryonic development in the squid *Loligo forbesi* (Cephalopoda: Loliginidae) Steenstrup 1856. Dissertation, University of Aberdeen.
- Craig, S., & Overnell, J. (2003). Metals in squid, *Loligo forbesi*, adults, eggs and hatchlings. No evidence for a role for Cu-or Zn-metallothionein. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 134(3), 311–317.
- Cronin, S. J., Hedley, M. J., Neall, V. E., & Smith, R. G. (1998). Agronomic impact of tephra fallout from the 1995 and 1996 Ruapehu Volcano eruptions, New Zealand. *Environmental Geology* 34(1), 21–30.
- Crump, K. S., Kjellström, T., Shipp, A. M., Silvers, A., & Stewart, A. (1998). Influence of prenatal mercury exposure upon scholastic and psychological test performance: Benchmark analysis of a New Zealand cohort. *Risk Analysis* 18(6), 701–713.
- Cuvin-Aralar, M. L. A., & Furness, R. W. (1991). Mercury and selenium interaction: a review. *Ecotoxicology and Environmental Safety* 21, 348–364.
- Das, K., Debacker, V., Pillet, S., & Bouqueneau, J.-M. (2003). Heavy metals in marine mammals. *Toxicology of Marine Mammals* 3, 135–167.
- Das, K., Holsbeek, L., Browning, J., Siebert, U., Birkun Jr, A., & Bouqueneau, J. M. (2004). Trace metal and stable isotope measurements ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) in the harbour porpoise *Phocoena phocoena relicta* from the Black Sea. *Environmental Pollution* 131(2), 197–204.
- Davey, F. J., Henyey, T., Holbrook, W. S., Okaya, D., Stern, T. A., Melhuish, A., Henrys, S., ... & Uhrhammer, R. (1998). Preliminary results from a geophysical study across a modern, continent-continent collisional plate boundary—The Southern Alps, New Zealand. *Tectonophysics* 288(1–4), 221–235.
- Davies, H., Weber, P., Lindsay, P., Craw, D., & Pope, J. (2011). Characterisation of acid mine drainage in a high rainfall mountain environment, New Zealand. *Science of the Total Environment* 409(15), 2971–2980.
- Dean, J. R. (2007). Bioavailability, bioaccessibility and mobility of environmental contaminants (Vol. 32). John Wiley & Sons.
- de Carvalho, C. E. V., Di Benedetto, A. P. M., Souza, C. M. M., Ramos, R. M., & Rezende, C. E. (2008). Heavy metal distribution in two cetacean species from Rio de Janeiro State, south-eastern Brazil. *Journal of the Marine Biological Association of the United Kingdom* 88, 1117–1120.
- de la Chesnais, T., Fulton, E. A., Tracey, S. R., & Pecl, G. T. (2019). The ecological role of cephalopods and their representation in ecosystem models. *Reviews in Fish Biology and Fisheries* 29, 313–334.

- de Pinho, A. P., Guimarães, J. R. D., Martins, A. S., Costa, P. A. S., Olavo, G., & Valentin, J. (2002). Total mercury in muscle tissue of five shark species from Brazilian offshore waters: effects of feeding habit, sex, and length. *Environmental Research* 89(3), 250–258.
- de Ronde, C. E., Faure, K., Bray, C. J., Chappell, D. A., & Wright, I. C. (2003). Hydrothermal fluids associated with seafloor mineralization at two southern Kermadec arc volcanoes, offshore New Zealand. *Mineralium Deposita* 38(2), 217–233.
- Deheyn, D. D., Gendreau, P., Baldwin, R. J., & Latz, M. I. (2005). Evidence for enhanced bioavailability of trace elements in the marine ecosystem of Deception Island, a volcano in Antarctica. *Marine Environmental Research* 60(1), 1–33.
- Desportes, G., & Mouritsen, R. (1993). Preliminary results on the diet of long-finned pilot whales off the Faroe Islands. *Reports of the International Whaling Commission. Special Issue* 305–324.
- Devesa, V., Macho, M. L., Jalón, M., Urieta, I., Muñoz, O., Súaier, M. A., López, F., [...], & Montoro, R. (2001). Arsenic in cooked seafood products: Study on the effect of cooking on total and inorganic arsenic contents. *Journal of Agricultural and Food Chemistry* 49(8), 4132–4140.
- Dietz, R., Riget, F., & Born, E. W. (2000). An assessment of selenium to mercury in Greenland marine animals. *Science of the Total Environment* 245, 15–24.
- Dietz, R., Riget, F., & Johansen, P. (1996). Lead, cadmium, mercury and selenium in Greenland marine animals. *Science of the Total Environment* 186, 67–93.
- Dietz, R., Sonne, C., Basu, N., Braune, B., O'Hara, T., Letcher, R. J., Scheuhammer, T., Andersen, M., Andreasen, C., & Andriashek, D. (2013). What are the toxicological effects of mercury in Arctic biota? *Science of the Total Environment* 443, 775–790.
- Dorneles, P. R., Lailson-Brito, J., dos Santos, R. A., Silva da Costa, P. A., Malm, O., Azevedo, A. F., & Machado Torres, J. P. (2007). Cephalopods and cetaceans as indicators of offshore bioavailability of cadmium off Central South Brazil Bight. *Environmental Pollution* 148(1), 352–359. <https://doi.org/10.1016/j.envpol.2006.09.022>.
- Doubleday, Z. A., Prowse, T. A., Arkhipkin, A., Pierce, G. J., Semmens, J., Steer, M., Leporati, S. C., ... & Gillanders, B. M. (2016). Global proliferation of cephalopods. *Current Biology* 26(10), R406–R407.
- Dunn, M. R. (2009). Feeding habits of the ommastrephid squid *Nototodarus sloanii* on the Chatham Rise, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 43(5), 1103–1113.
- Dunning, M. C., & Förch, E. C. (1998). A review of the systematics, distribution, and biology of arrow squids of the genus *Nototodarus* Pfeffer, 1912 (Cephalopoda: Ommastrephidae). *Smithsonian Contributions to Zoology* 586, 393–404.
- Eagles-Smith, C. A., Silbergeld, E. K., Basu, N., Bustamante, P., Diaz-Barriga, F., Hopkins, W. A., Kidd, K. A., & Nyland, J. F. (2018). Modulators of mercury risk to wildlife and humans in the context of rapid global change. *Ambio* 47(2), 170–197.
- Edenborn, H. M., Belzile, N., Mucci, A., Lebel, J., & Silverberg, N. (1986). Observations on the diagenetic behavior of arsenic in a deep coastal sediment. *Biogeochemistry*, 2(4), 359–376.
- Edmonds, J. S., & Francesconi, K. A. (1977). Methylated arsenic from marine fauna. *Nature* 265(5593), 436.
- Edmonds, J. S., Francesconi, K. A., Cannon, J. R., Raston, C. L., Skelton, B. W., & White, A. H. (1997). Isolation, crystal structure and synthesis of arsenobetaine, the arsenical constituent of the western rock lobster *Panulirus longipes cygnus* George. *Tetrahedron Letters* 18, 1543–1546.
- EFSA, European Food Safety Authority (2009). Panel on Contaminants in the Food Chain (CONTAM). Scientific Opinion on arsenic in food. *EFSA Journal* 7(10), 1351.
- EFSA, European Food Safety Authority (2012a). Cadmium dietary exposure in the European population. *EFSA Journal* 10(1), 2551.
- EFSA, European Food Safety Authority (2012b). Panel on Contaminants in the Food Chain (CONTAM). Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. *EFSA Journal* 10(12), 2985.
- Eisler, R. (2009). *Compendium of Trace Metals and Marine Biota: Volume 1: Plants and Invertebrates*. Elsevier.
- Ellis, J. I., Clark, M. R., Rouse, H. L., & Lamarche, G. (2017). Environmental management frameworks for offshore mining: The New Zealand approach. *Marine Policy* 84, 178–192.

- Ellwood, M. J., & Maher, W. A. (2002). Arsenic and antimony species in surface transects and depth profiles across a frontal zone: The Chatham Rise, New Zealand. *Deep Sea Research Part I: Oceanographic Research Papers* 49(11), 1971–1981.
- Emami-Khoyi, A., Hartley, D. A., Paterson, A. M., Boren, L. J., Cruickshank, R. H., Ross, J. G., Murphy, E. C., & Else, T.-A. (2016). Identifying prey items from New Zealand fur seal (*Arctocephalus forsteri*) faeces using massive parallel sequencing. *Conservation Genetics Resources* 8(3), 343–352.
- Endo, T., Haraguchi, K., & Sakata, M. (2003). Renal toxicity in rats after oral administration of mercury-contaminated boiled whale livers marketed for human consumption. *Archives of Environmental Contamination and Toxicology* 44(3), 0412–0416.
- Endo, T., Hotta, Y., Haraguchi, K., & Sakata, M. (2005). Distribution and toxicity of mercury in rats after oral administration of mercury-contaminated whale red meat marketed for human consumption. *Chemosphere* 61, 1069–1073.
- Endo, T., Kimura, O., Hisamichi, Y., Minoshima, Y., & Haraguchi, K. (2007). Age-dependent accumulation of heavy metals in a pod of killer whales (*Orcinus orca*) stranded in the northern area of Japan. *Chemosphere* 67, 51–59.
- Eto, K. (2000). Minamata disease. *Neuropathology*, 20, 14–19.
- Falandysz, J. (1988). Trace metals in squid *Illex argentinus*. *Zeitschrift Für Lebensmittel-Untersuchung und Forschung*, 187(4), 359–361.
- FAO (2020, January 6<sup>th</sup>). Food and Agriculture Organization of the United Nations. *FAO Yearbook 2017: Fishery and Aquaculture Statistics*. Retrieved from: <http://www.fao.org/fishery/statistics/yearbook/en>.
- Faul, K. L., Ravelo, A. C., & Delaney, M. L. (2000). Reconstructions of upwelling, productivity, and photic zone depth in the eastern equatorial Pacific Ocean using planktonic foraminiferal stable isotopes and abundances. *Journal of Foraminiferal Research* 30, 110–125.
- Fenaughty, C. M., Tracey, D. M., & Lock, J. W. (1988). Heavy metal and organochlorine concentrations in New Zealand aquatic fish, crustaceans, and molluscs. *New Zealand Fisheries Data Report*. 1988.
- Filipsson, A. F., Sand, S., Nilsson, J., & Victorin, K. (2003). The benchmark dose method—Review of available models, and recommendations for application in health risk assessment. *Critical Reviews in Toxicology* 33(5), 505–542.
- Finger, J. M., & Smith, J. D. (1987). Molecular association of Cu, Zn, Cd and 210Po in the digestive gland of the squid *Nototodarus gouldi*. *Marine Biology* 95(1), 87–91.
- Fisher, N. S., & Reinfelder, J. R. (1995). The trophic transfer of metals in marine systems. *Metal speciation and bioavailability in aquatic systems* 3, 407–411.
- Fitzgerald, W.F., Lamborg, C.H., & Hammerschmidt, C.R. (2007). Marine biogeochemical cycling of mercury. *Chemical Reviews* 107(2), 641–662.
- Foster, B. A., & Battaerd, W. R. (1985). Distribution of zooplankton in a coastal upwelling in New Zealand. *New Zealand Journal of Marine and Freshwater Research*, 19(2), 213–226.
- Francesconi, K. A. (2010). Arsenic species in seafood: origin and human health implications. *Pure and Applied Chemistry* 82, 373–381.
- Francesconi, K. A., Moore, E. J., & Edmonds, J. S. (1994). Cadmium uptake from seawater and food by the western rock lobster *Panulirus cygnus*. *Bulletin of Environmental Contamination and Toxicology* 53(2), 219–223.
- Francesconi, K. A., Moore, E. J., & Joll, L. M. (1993). Cadmium in the saucer scallop, *Amusium balloti*, from western Australian waters: concentrations in adductor muscle and redistribution following frozen storage. *Marine and Freshwater Research* 44, 787–797.
- Fraser, M. M., & Lalas, C. (2004). Seasonal variation in the diet of blue penguins (*Eudyptula minor*) at Oamaru, New Zealand. *Notornis* 51, 7–15.
- Frew, R. D. (1995). Antarctic bottom water formation and the global cadmium to phosphorus relationship. *Geophysical Research Letters* 22, 2349–2352.
- Frew, R. D., & Hunter, K. A. (1995). Cadmium-phosphorus cycling at the subtropical convergence south of New Zealand. *Marine Chemistry* 51, 223–237.
- Friedland, K. D., Stock, C., Drinkwater, K. F., Link, J. S., Leaf, R. T., Shank, B. V., Rose, J. M., ... & Fogarty, M. J. (2012). Pathways between primary production and fisheries yields of large

- marine ecosystems. PLoS One 7(1), e28945.
- Frodello, J. P., & Marchand, B. (2001). Cadmium, copper, lead, and zinc in five toothed whale species of the Mediterranean Sea. *International Journal of Toxicology* 20, 339–343.
- FSANZ, Food Standards Australia New Zealand (2011, Sept. 11). Mercury in Fish. Food Standards Australia and New Zealand. Retrieved November 21, 2019, from <http://www.foodstandards.gov.au/consumer/chemicals/mercury>.
- FSANZ, Food Standards Australia New Zealand (2016, March 1<sup>st</sup>). Food Standards Australia New Zealand Food Standard Code-Schedule 19-Maximum Levels of Contaminants and Natural Toxicants. Retrieved from <http://www.foodstandards.govt.nz/code/Documents/Sched%2019%20Contaminant%20MLs%20v157.pdf>.
- Gajdosechova, Z., Brownlow, A., Cottin, N. T., Fernandes, M., Read, F. L., Urgast, D. S., Raab, A., [...], & Krupp, E. M. (2016). Possible link between Hg and Cd accumulation in the brain of long-finned pilot whales (*Globicephala melas*). *Science of the Total Environment* 545, 407–413.
- Galitsopoulou, A., Georgantelis, D., & Kontominas, M. G. (2009). Cadmium content in fresh and canned squid (*Loligo opalescens*) from the Pacific coastal waters of California (USA). *Food Additives and Contaminants: Part B* 2, 38–43. <https://doi.org/10.1080/02652030902824956>.
- Gallien I., Caurant F., Bordes M., Bustamante P., Miramand P., Fernandez B., Quillard N., & Babin P. (2001). Cadmium-containing granules in kidney tissue of the Atlantic white-sided dolphin (*Lagenorhynchus acutus*) off the Faroe Islands. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology* 130, 389–395.
- Gallon, C., & Flegal, A. R. (2015). Sources, fluxes, and biogeochemical cycling of silver in the oceans. In *Reviews of Environmental Contamination and Toxicology Volume 235* (pp. 27–48). Springer, Cham.
- Gannon, D. P., Read, A. J., Craddock, J. E., Frstrup, K. M., & Nicolas, J. R. (1997). Feeding ecology of long-finned pilot whales *Globicephala melas* in the western North Atlantic. *Marine Ecology Progress Series* 148, 1–10.
- Gault-Ringold, M., Adu, T., Stirling, C. H., Frew, R. D., & Hunter, K. A. (2012). Anomalous biogeochemical behavior of cadmium in subantarctic surface waters: mechanistic constraints from cadmium isotopes. *Earth and Planetary Science Letters* 341, 94–103.
- Geraci, J. R., & Lounsbury, V. J. (2005). *Marine mammals ashore: a field guide for strandings*. Second ed. National Aquarium, Baltimore.
- Gerpe, M. S., de Moreno, J. E. A., Moreno, V. J., & Patat, M. L. (2000). Cadmium, zinc and copper accumulation in the squid *Illex argentinus* from the Southwest Atlantic Ocean. *Marine Biology* 136, 1039–1044.
- Giggenbach, W. F., & Glasby, G. P. (1977). The influence of thermal activity on the trace metal distribution in marine sediments around White Island, New Zealand. *Bulletin of the New Zealand Department of Industrial Research* 218, 121–126.
- Glasby, G. P., Stoppers, P., Walter, P., Davis, K. R., & Renner, R. M. (1988). Heavy-metal pollution in Manukau and Waitemata Harbours, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 22(4), 595–611.
- Glibert, P. M., & Burkholder, J. M., (2006). The complex relationships between increases in fertilization of the earth, coastal eutrophication and proliferation of harmful algal blooms. In *Ecology of harmful algae* (pp. 341-354). Springer, Berlin, Heidelberg.
- Goessler, W., Rudorfer, A., Mackey, E. A., Becker, P. R., & Irgolic, K. J. (1998). Determination of arsenic compounds in marine mammals with high-performance liquid chromatography and an inductively coupled plasma mass spectrometer as element-specific detector. *Applied Organometallic Chemistry* 12, 491–501.
- Goyer, R. A. (1989). Mechanisms of lead and cadmium nephrotoxicity. *Toxicology Letters* 46(1), 153–162.
- Graf, U. (2004). z-Transformation. In: *Applied Laplace Transforms and z-Transforms for Scientists and Engineers*. Springer, 77–113
- Gray, J. E. (1849). *Catalogue of the Mollusca in the Collection of the British Museum*. Pt. 1. Cephalopoda Antepedia. British Museum, London.

- Gray, J. S. (2002). Biomagnification in marine systems: the perspective of an ecologist. *Marine Pollution Bulletin* 45, 46–52.
- Green, K., & Burton, H. R. (1993). Comparison of the stomach contents of southern elephant seals, *Mirounga leonina*, at Macquarie and Heard Islands. *Marine Mammal Science* 9, 10–22.
- Guisan, A., & Zimmermann, N. E. (2000). Predictive habitat distribution models in ecology. *Ecological Modelling* 135, 147–186.
- Haffert, L., & Craw, D. (2008). Processes of attenuation of dissolved arsenic downstream from historic gold mine sites, New Zealand. *Science of the Total Environment* 405, 286–300.
- Haffert, L., Craw, D., & Pope, J. (2010). Climatic and compositional controls on secondary arsenic mineral formation in high-arsenic mine wastes, South Island, New Zealand. *New Zealand Journal of Geology and Geophysics* 53, 91–101.
- Hall, A. H. (2002). Chronic arsenic poisoning. *Toxicology Letters*, 128(1–3), 69–72. [https://doi.org/10.1016/S0378-4274\(01\)00534-3](https://doi.org/10.1016/S0378-4274(01)00534-3).
- Hammond, P. B. (1977). Exposure of humans to lead. *Annual Review of Pharmacology and Toxicology* 17(1), 197–214.
- Heath, R. A. (1985). A review of the physical oceanography of the seas around New Zealand—1982. *New Zealand Journal of Marine and Freshwater Research* 19(1), 79–124.
- Heide-Jørgensen, M. P., Bloch, D., Stefansson, E., Mikkelsen, B., Ofstad, L. H., & Dietz, R. (2002). Diving behaviour of long-finned pilot whales *Globicephala melas* around the Faroe Islands. *Wildlife Biology* 8, 307–314.
- Helm, R. C. (1984). Rate of digestion in three species of pinnipeds. *Canadian Journal of Zoology* 62(9), 1751–1756.
- Helm, K. P., Bindoff, N. L., & Church, J. A. (2011). Observed decreases in oxygen content of the global ocean. *Geophysical Research Letters* 38(23), 23602.
- Henderson, G. M., Achterberg, E. P., & Bopp, L. (2018). Changing trace element cycles in the 21st century ocean. *Elements: An International Magazine of Mineralogy, Geochemistry, and Petrology* 14(6), 409–413.
- Henderson, G. M., & Maier-Reimer, E. (2002). Advection and removal of <sup>210</sup>Pb and stable Pb isotopes in the oceans: a general circulation model study. *Geochimica et Cosmochimica Acta* 66, 257–272.
- Hewlett, L., Craw, D., & Black, A. (2005). Comparison of arsenic and trace metal contents of discharges from adjacent coal and gold mines, Reefton, New Zealand. *Marine and Freshwater Research* 56, 983–995.
- Hickey, C. W., & Clements, W. H. (1998). Effects of heavy metals on benthic macroinvertebrate communities in New Zealand streams. *Environmental Toxicology and Chemistry* 17(11), 2338–2346.
- Higgs, N. D., Gates, A. R., & Jones, D. O. (2014). Fish food in the deep sea: revisiting the role of large food-falls. *PLoS One* 9(5), e96016.
- Hoernle, K., White, J. vd, van den Bogaard, P., Hauff, F., Coombs, D. S., Werner, R., Timm, C., ... & Cooper, A. F. (2006). Cenozoic intraplate volcanism on New Zealand: Upwelling induced by lithospheric removal. *Earth and Planetary Science Letters* 248(1–2), 350–367.
- Honda, K., Yamamoto, Y., & Tatsukawa, R. (1987). Distribution of heavy metals in Antarctic marine ecosystem. *Ninth Symposium on Polar Biology* 1, 184–197.
- Hong, Y.-S., Kim, Y.-M., & Lee, K.-E. (2012). Methylmercury exposure and health effects. *Journal of Preventive Medicine and Public Health* 45(6), 353.
- Hothorn, T., Bretz, F., Westfall, P., Heiberger, R. M., Schuetzenmeister, A., Scheibe, S., & Hothorn, M. T. (2016). Package ‘multcomp.’ Simultaneous inference in general parametric models. Project for Statistical Computing, Vienna, Austria.
- Hughes, M. F. (2002). Arsenic toxicity and potential mechanisms of action. *Toxicology Letters* 133(1), 1–16.
- Hunsicker, M. E., Essington, T. E., Watson, R., & Sumaila, U. R. (2010). The contribution of cephalopods to global marine fisheries: Can we have our squid and eat them too?: Contribution of cephalopods to fisheries. *Fish and Fisheries* 11(4), 421–438. <https://doi.org/10.1111/j.1467-2979.2010.00369.x>.

- Hurst R. J., Ballara S. L., MacGibbon D., & Triantafillos L. (2012). Fishery characterisation and standardised CPUE analyses for arrow squid (*Nototodarus gouldi* and *N. sloanii*), 1989-90 to 2007-08, and potential management approaches for southern fisheries. New Zealand Fisheries Assessment Report 47, 303.
- Hutton, M. (1983). Sources of cadmium in the environment. *Ecotoxicology and Environmental Safety* 7(1), 9–24.
- IAEA, International Atomic Energy Agency (2004). Sediment distribution coefficients and concentration factors for biota in the marine environment. Technical Reports Series No. 422, IAEA, Vienna, Austria.
- Ibáñez, C. M., & Keyl, F. (2010). Cannibalism in cephalopods. *Reviews in Fish Biology and Fisheries* 20, 123–136.
- Ichihashi, H., Kohno, H., Kannan, K., Tsumura, A., & Yamasaki, S. (2001). Multielemental Analysis of Purpleback Flying Squid Using High Resolution Inductively Coupled Plasma-Mass Spectrometry (HR ICP-MS). *Environmental Science & Technology* 35(15), 3103–3108. <https://doi.org/10.1021/es010653v>.
- Ihaka, R., & Gentleman, R., (1996). R: A language for data analysis and graphics. *Journal of Computational and Graphical Statistics* 5, 299–314.
- Ikemoto, T., Kunito, T., Tanaka, H., Baba, N., Miyazaki, N., & Tanabe, S. (2004). Detoxification Mechanism of Heavy Metals in Marine Mammals and Seabirds: Interaction of Selenium with Mercury, Silver, Copper, Zinc, and Cadmium in Liver. *Archives of Environmental Contamination and Toxicology* 47, 402–413. <https://doi.org/10.1007/s00244-004-3188-9>
- Imber, M. J. (1973). The food of grey-faced petrels (*Pterodroma macroptera gouldi* (Hutton)), with special reference to diurnal vertical migration of their prey. *The Journal of Animal Ecology* 42, 645–662.
- Inaba, T., Kobayashi, E., Suwazono, Y., Uetani, M., Oishi, M., Nakagawa, H., & Nogawa, K. (2005). Estimation of cumulative cadmium intake causing Itai–itai disease. *Toxicology Letters* 159(2), 192–201.
- Ishizaki, A., Fukushima, M., & Sakamoto, M. (1970). Distribution of Cd in Biological Materials Part 2. *Nippon Eiseigaku Zasshi (Japanese Journal of Hygiene)* 25(2), 207–222.
- Jackson, G. D. (1995). The use of beaks as tools for biomass estimation in the deepwater squid *Moroteuthis ingens* (Cephalopoda: Onychoteuthidae) in New Zealand waters. *Polar Biology* 15, 9–14.
- Jackson, G. D. (1997). Age, growth and maturation of the deepwater squid *Moroteuthis ingens* (Cephalopoda: Onychoteuthidae) in New Zealand waters. *Polar Biology* 17(3), 268–274.
- Jackson, G. D., McKinnon, J. F., Lalas, C., Ardern, R., & Buxton, N. G. (1998). Food spectrum of the deepwater squid *Moroteuthis ingens* (Cephalopoda: Onychoteuthidae) in New Zealand waters. *Polar Biology* 20(1), 56–65.
- Jackson, G. D., & Mladenov, P. V. (1994). Terminal spawning in the deepwater squid *Moroteuthis ingens* (Cephalopoda: Onychoteuthidae). *Journal of Zoology* 234(2), 189–201.
- Jackson, G. D., Shaw, A. G. P., & Lalas, C. (2000). Distribution and biomass of two squid species off southern New Zealand: *Nototodarus sloanii* and *Moroteuthis ingens*. *Polar Biology* 23(10), 699–705.
- Jaeschke, A., Wengler, M., Hefter, J., Ronge, T. A., Geibert, W., Mollenhauer, G., Gersonde, R., & Lamy, F. (2017). A biomarker perspective on dust, productivity, and sea surface temperature in the Pacific sector of the Southern Ocean. *Geochimica et Cosmochimica Acta* 204, 120–39.
- James, G. D., & Stahl, J.-C. (2000). Diet of southern Buller's albatross (*Diomedea bulleri bulleri*) and the importance of fishery discards during chick rearing. *New Zealand Journal of Marine and Freshwater Research* 34(3), 435–454.
- Jangid, A. P., John, P. J., Yadav, D., Mishra, S., & Sharma, P. (2012). Impact of chronic lead exposure on selected biological markers. *Indian Journal of Clinical Biochemistry* 27(1), 83–89.
- Janssen, D. J., Conway, T. M., John, S. G., Christian, J. R., Kramer, D. I., Pedersen, T. F., & Cullen, J. T. (2014). Undocumented water column sink for cadmium in open ocean oxygen-deficient zones. *Proceedings of the National Academy of Sciences* 111(19), 6888–6893.
- Järup, L., & Åkesson, A. (2009). Current status of cadmium as an environmental health problem. *Toxicology and Applied Pharmacology* 238(3), 201–208.

- Järup, L., Berglund, M., Elinder, C. G., Nordberg, G., & Vanter, M. (1998). Health effects of cadmium exposure—a review of the literature and a risk estimate. *Scandinavian Journal of Work, Environment & Health* 24, 1–51.
- Jebali, J., Banni, M., Gerbej H., Boussetta, H., López-Barea, J., & Alhama, J. (2008). Metallothionein induction by Cu, Cd and Hg in *Dicentrarchus labrax* liver: assessment by RP-HPLC with fluorescence detection and spectrophotometry. *Marine Environmental Research* 65, 358–363.
- Jereb, P., Allcock, A. L., Lefkaditou, E., Piatkowski, U., Hastie, L. C., & Pierce, G. J. (2015). Cephalopod biology and fisheries in Europe: II. Species Accounts. Retrieved from <http://oceanrep.geomar.de/29001/1/CRR%20325-1.pdf>.
- JEFCA, Joint FAO/WHO Expert Committee on Food Additives (2013). Expert Committee on Food Additives. Meeting, & World Health Organization. Evaluation of certain food additives and contaminants: seventy-seventh report of the Joint FAO/WHO Expert Committee on Food Additives No. 983; WHO Press: Geneva, Switzerland.
- Jomova, K., Jenisova, Z., Feszterova, M., Baros, S., Liska, J., Hudecova, D., Rhodes, C.J., & Valko, M., (2011). Arsenic: Toxicity, oxidative stress and human disease. *Journal of Applied Toxicology* 31(2), 95–107.
- Jones, M. R. L. (2007). Historic trawl data and recent information infers temporal change in the occurrence of squid in the diet of orange roughy (*Hoplostethus atlanticus* Collett) in New Zealand. *Reviews in Fish Biology and Fisheries* 17(2–3), 493–499.
- Jöst, C., & Zauke, G.-P. (2008). Trace metal concentrations in Antarctic sea spiders (Pycnogonida, Pantopoda). *Marine Pollution Bulletin* 56, 1396–1399.
- Kahle, J., & Zauke, G.-P. (2002). Bioaccumulation of trace metals in the copepod *Calanoides acutus* from the Weddell Sea (Antarctica): Comparison of two-compartment and hyperbolic toxicokinetic models. *Aquatic Toxicology* 59, 115–135.
- Kalish, J. M., Livingston, M. E., & Schofield, K. A. (1996). Trace elements in the otoliths of New Zealand blue grenadier (*Macruronus novaezelandiae*) as an aid to stock discrimination. *Marine and Freshwater Research* 47(3), 537–542.
- Karntanut, W., & Pascoe, D. (2002). The toxicity of copper, cadmium and zinc to four different Hydra (Cnidaria: Hydrozoa). *Chemosphere* 47(10), 1059–1064.
- Keil, S., De Broyer, C., & Zauke, G.-P. (2008). Significance and interspecific variability of accumulated trace metal concentrations in Antarctic benthic crustaceans. *International Review of Hydrobiology* 93, 106–126.
- Kemper, C., Gibbs, P., Obendorf, D., Marvanek, S., & Lenghaus, C. (1994). A review of heavy metal and organochlorine levels in marine mammals in Australia. *Science of the Total Environment* 154, 129–139.
- Kennedy, P. C. (1986). The use of molluscs for monitoring trace elements in the marine environment in New Zealand 1. The contribution of ingested sediment to the trace element concentrations in New Zealand molluscs. *New Zealand Journal of Marine and Freshwater Research* 20(4), 627–640.
- Kershaw, J. L., & Hall, A. J. (2019). Mercury in cetaceans: Exposure, bioaccumulation and toxicity. *Science of The Total Environment* 694, 1336. <https://doi.org/10.1016/j.scitotenv.2019.133683>.
- Killops, S. D., Woolhouse, A. D., Weston, R. J., & Cook, R. A. (1994). A geochemical appraisal of oil generation in the Taranaki Basin, New Zealand. *The American Association of Petroleum Geologists Bulletin* 78(10), 1560–1585.
- Kim, K.-H., Kabir, E., & Jahan, S. A. (2016). A review on the distribution of Hg in the environment and its human health impacts. *Journal of Hazardous Materials* 306, 376–385.
- Koeman, J. H., Peeters, W. H. M., Koudstaal-Hol, C. H. M., Tjioe, P. S., & De Goeij, J. J. M. (1973). Mercury-selenium correlations in marine mammals. *Nature* 245, 385.
- Koizumi, N., Murata, K., Hayashi, C., Nishio, H., & Goji, J. (2008). High cadmium accumulation among humans and primates: comparison across various mammalian species—a study from Japan. *Biological Trace Element Research* 121(3), 205–214.
- Kojadinovic, J., Jackson, C. H., Cherel, Y., Jackson, G. D., & Bustamante, P. (2011). Multi-elemental concentrations in the tissues of the oceanic squid *Todarodes filippovae* from Tasmania and the

- southern Indian Ocean. *Ecotoxicology and Environmental Safety* 74(5), 1238–1249. <https://doi.org/10.1016/j.ecoenv.2011.03.015>.
- Kolodny, Y., & Kaplan, I. R. (1970). Uranium isotopes in sea-floor phosphorites. *Geochimica et Cosmochimica Acta* 34(1), 3–24.
- Koyama, J., Nanamori, N., & Segawa, S. (2000). Bioaccumulation of waterborne and dietary cadmium by oval squid, *Sepioteuthis lessoniana*, and its distribution among organs. *Marine Pollution Bulletin* 40(11), 961–967.
- Kubota, R., Kunito, T., & Tanabe, S. (2001). Arsenic accumulation in the liver tissue of marine mammals. *Environmental Pollution* 115, 303–312.
- Kunito, T., Kubota, R., Fujihara, J., Agusa, T., & Tanabe, S. (2008). Arsenic in marine mammals, seabirds, and sea turtles, in: *Reviews of Environmental Contamination and Toxicology*. Springer, 31–69.
- Kurihara, H., Togawa, H., & Hatano, M. (1993). Concentration of cadmium in livers of several kinds of squids and an approach to its elimination. *Bulletin of the Faculty of Fisheries, Hokkaido University* 44(1), 32–38.
- Lacoue-Labarthe, T., Le Bihan, E., Borg, D., Koueta, N., & Bustamante, P. (2010a). Acid phosphatase and cathepsin activity in cuttlefish (*Sepia officinalis*) eggs: the effects of Ag, Cd, and Cu exposure. *ICES Journal of Marine Science*, 67(7), 1517–1523.
- Lacoue-Labarthe, T., Warnau, M., Oberhänsli, F., Teyssié, J. L., & Bustamante, P. (2010b). Contrasting accumulation biokinetics and distribution of <sup>241</sup>Am, Co, Cs, Mn and Zn during the whole development time of the eggs of the common cuttlefish, *Sepia officinalis*. *Journal of Experimental Marine Biology and Ecology*, 382(2), 131–138.
- Lacoue-Labarthe, T., Metian, M., Warnau, M., Oberhänsli, F., Rouleau, C., & Bustamante, P. (2009). Biokinetics of Hg and Pb accumulation in the encapsulated egg of the common cuttlefish *Sepia officinalis*: radiotracer experiments. *Science of the Total Environment* 407, 6188–6195.
- Lacoue-Labarthe, T., Le Pabic, C., & Bustamante, P. (2016). Ecotoxicology of early-life stages in the common cuttlefish *Sepia officinalis*: review and perspectives. *Vie Milieu* 66, 65–79.
- Lahaye, V., Bustamante, P., Dabin, W., Van Canneyt, O., Dhermain, F., Cesarini, C., Pierce, G. J., & Caurant, F. (2006). New insights from age determination on toxic element accumulation in striped and bottlenose dolphins from Atlantic and Mediterranean waters. *Marine Pollution Bulletin* 52, 1219–1230.
- Langston, W. J. (2017). Toxic effects of metals and the incidence of metal pollution in marine ecosystems. In *Heavy metals in the marine environment* (pp. 101–120). CRC Press.
- Laparra, J. M., Velez, D., Montoro, R., Barbera, R., & Farré, R. (2003). Estimation of arsenic bioaccessibility in edible seaweed by an in vitro digestion method. *Journal of Agricultural and Food Chemistry* 51(20), 6080–6085.
- Lavoie, R. A., Jardine, T. D., Chumchal, M. M., Kidd, K. A., & Campbell, L. M. (2013). Biomagnification of mercury in aquatic food webs: a worldwide metal-analysis. *Environmental Science & Technology* 47, 13385–13394.
- Law, R. J., Bennett, M. E., Blake, S. J., Allchin, C. R., Jones, B. R., & Spurrier, C. J. (2001). Metals and organochlorines in pelagic cetaceans stranded on the coasts of England and Wales. *Marine Pollution Bulletin* 42(6), 522–526.
- Law, C. S., Bell, J. J., Bostock, H. C., Cornwall, C. E., Cummings, V. J., Currie, K., Davy, S. K., ... & Lamare, M. (2018a). Ocean acidification in New Zealand waters: Trends and impacts. *New Zealand Journal of Marine and Freshwater Research* 52(2), 155–195.
- Law, C. S., Rickard, G. J., Mikaloff-Fletcher, S. E., Pinkerton, M. H., Behrens, E., Chiswell, S. M., & Currie, K. (2018b). Climate change projections for the surface ocean around New Zealand. *New Zealand Journal of Marine and Freshwater Research* 52(3), 309–335.
- Lemos, L. S., de Moura, J. F., Hauser-Davis, R. A., de Campos, R. C., & Siciliano, S. (2013). Small cetaceans found stranded or accidentally captured in southeastern Brazil: Bioindicators of essential and non-essential trace elements in the environment. *Ecotoxicology and Environmental Safety* 97, 166–175.
- Lewis, C., Ellis, R. P., Vernon, E., Elliot, K., Newbatt, S., & Wilson, R. W. (2016). Ocean acidification increases copper toxicity differentially in two key marine invertebrates with distinct acid-base responses. *Scientific Reports* 6, 21554.

- Lischka, A., Lacoue-Labarthe, T., Hoving, H. J. T., JavidPour, J., Pannell, J. L., Merten, V., Churlaud, C., & Bustamante, P. (2018). High cadmium and mercury concentrations in the tissues of the orange-back flying squid, *Sthenoteuthis pteropus*, from the tropical Eastern Atlantic. *Ecotoxicology and Environmental Safety* 163, 323–330. <https://doi.org/10.1016/j.ecoenv.2018.07.087>.
- Lischka, A., Lacoue-Labarthe, T., Bustamante, P., Piatkowski, U., & Hoving, H. J. T. (2019a). Trace element analysis reveals bioaccumulation in the squid *Gonatus fabricii* from polar regions of the Atlantic Ocean. *Environmental Pollution* 256, 113389. <https://doi.org/10.1016/j.envpol.2019.113389>.
- Lischka, A., Pook, C. J., Bolstad, K. S. R., Pannell, J. L., & Braid, H. E. (2019b). Metal composition of arrow squid (*Nototodarus sloanii* [Gray 1849]) from the Chatham Rise, New Zealand: Implications for human consumption. *Environmental Science and Pollution Research* 26(12), 11975–11987. <https://doi.org/10.1007/s11356-019-04510-w>.
- Lischka, A., Pook, C. J., Pannell, J. L., Braid, H. E., Gaw, S., & Bolstad, K. S. R. (2020). Distribution of trace elements in the tissues of arrow squid (*Nototodarus sloanii*) from the Chatham Rise, New Zealand: Human health implications. *Fisheries Research* 221, 105383. <https://doi.org/10.1016/j.fishres.2019.105383>.
- Liu, J.-Y., Chou, L.-S., & Chen, M.-H. (2015). Investigation of trophic level and niche partitioning of 7 cetacean species by stable isotopes, and cadmium and arsenic tissue concentrations in the western Pacific Ocean. *Marine Pollution Bulletin* 93, 270–277.
- Liu, X., & Millero, F. J. (2002). The solubility of iron in seawater. *Marine Chemistry*, 77(1), 43–54.
- Lock, J. W., Thompson, D. R., Furness, R. W., & Bartle, J. A. (1992). Metal concentrations in seabirds of the New Zealand region. *Environmental Pollution* 75(3), 289–300.
- Lohan, M. C., Statham, P. J., & Crawford, D. W. (2002). Total dissolved zinc in the upper water column of the subarctic North East Pacific. *Deep Sea Research Part II: Topical Studies in Oceanography* 49, 5793–5808.
- Lu, C. C., & Williams, R. (1994). *Kondakovia longimana* Filippova, 1972 (Cephalopoda: Onychoteuthidae) from the Indian Ocean sector of the Southern Ocean. *Antarctic Science* 6(2), 231–234.
- Lucia, M., Strøm, H., Bustamante, P., & Gabrielsen, G. W. (2016). Trace element concentrations in relation to the trophic behaviour of endangered Ivory Gulls (*Pagophila eburnea*) during their stay at a breeding site in Svalbard. *Archives of Environmental Contamination and Toxicology* 71, 518–529.
- Lyver, P. O. B., Aldridge, S. P., Gormley, A. M., Gaw, S., Webb, S., Buxton, R. T., & Jones, C. J. (2017). Elevated mercury concentrations in the feathers of grey-faced petrels (*Pterodroma gouldi*) in New Zealand. *Marine Pollution Bulletin* 119(1), 195–203.
- Mackey, E. A., Demiralp, R., Becker, P. R., Greenberg, R. R., Koster, B. J., & Wise, S. A. (1995). Trace element concentrations in cetacean liver tissues archived in the National Marine Mammal Tissue Bank. *Science of the Total Environment* 175(1), 25–41.
- Mackie, D. S., & Hunter, K. A. (2005). The partitioning of zinc, manganese, cadmium and selenium within copepods in oceanic waters east of New Zealand. *Marine and Freshwater Research* 56(1), 95–100.
- Mansilla, L., Olavarria, C., & Vega, M. A. (2012). Stomach contents of long-finned pilot whales (*Globicephala melas*) from southern Chile. *Polar Biology* 35(12), 1929–1933.
- Marcovecchio, J. E., Moreno, V. J., Bastida, R. O., Gerpe, M. S., & Rodríguez, D. H. (1990). Tissue distribution of heavy metals in small cetaceans from the Southwestern Atlantic Ocean. *Marine Pollution Bulletin* 21, 299–304.
- Marsden, I. D., Smith, B. D., & Rainbow, P. S. (2014). Effects of environmental and physiological variables on the accumulated concentrations of trace metals in the New Zealand cockle *Austrovenus stutchburyi*. *Science of the Total Environment* 470, 324–339.
- Marschner, B., Welge, P., Hack, A., Wittsiepe, J., & Wilhelm, M. (2006). Comparison of soil Pb in vitro bioaccessibility and in vivo bioavailability with Pb pools from a sequential soil extraction. *Environmental Science & Technology* 40(8), 2812–2818.

- Mart, L., Rützel, H., Klahre, P., Sipos, L., Platzek, U., Valenta, P., & Nürnberg, H. W. (1982). Comparative studies on the distribution of heavy metals in the oceans and coastal waters. *Science of the Total Environment* 26(1), 1–17.
- Martin, J. H., & Flegal, A. R. (1975). High copper concentrations in squid livers in association with elevated levels of silver, cadmium, and zinc. *Marine Biology* 30(1), 51–55.
- Martoja, R. (1980). Identification of tiemannite as a probable product of demethylation of mercury by selenium in cetaceans: a complement to the scheme of the biological cycle of mercury. *Vie et Milieu* 30, 7–10.
- Marx, S. K., McGowan, H. A., & Kamber, B.S. (2009). Long-range dust transport from eastern Australia: a proxy for Holocene aridity and ENSO-type climate variability. *Earth and Planetary Science Letters*, 282(1–4), 167–77.
- McArthur, T., Butler, E. C., & Jackson, G. D. (2003). Mercury in the marine food chain in the Southern Ocean at Macquarie Island: an analysis of a top predator, Patagonian toothfish (*Dissostichus eleginoides*) and a mid-trophic species, the warty squid (*Moroteuthis ingens*). *Polar Biology* 27, 1–5.
- McCormack, M. A., Fielding, R., Kiszka, J. J., Paz, V., Jackson, B. P., Bergfelt, D. R., & Dutton, J. (2019). Mercury and selenium concentrations, and selenium: Mercury molar ratios in small cetaceans taken off St. Vincent, West Indies. *Environmental Research* 181, 108908.
- McCoy F. (1888). *Natural History of Victoria. Prodrum of the Zoology of Victoria, figures and descriptions of the living species of all classes of the Victorian indigenous animals.* Melbourne and London 2(17), 255–257.
- McDowell, R. W., Taylor, M. D., & Stevenson, B. A. (2013). Natural background and anthropogenic contributions of cadmium to New Zealand soils. *Agriculture, Ecosystems & Environment* 165, 80–87.
- McGregor, V., & Large, K. (2015). Progress with in-season modelling of squid within New Zealand's EEZ and Management strategy evolution (MSE). Documents of South Pacific Regional Fisheries Management Organization 3rd meeting (SC-03–26).
- Meador, J. P., Varanasi, U., Robisch, P. A., & Chan, S. L. (1993). Toxic Metals in Pilot Whales (*Globicephala melaena*) from Standings in 1986 and 1990 on Cape Cod, Massachusetts. *Canadian Journal of Fisheries and Aquatic Sciences* 50, 2698–2706.
- Méndez-Fernandez, P., Pierce, G. J., Bustamante, P., Chouvelon, T., Ferreira, M., González, A. F., López, A., Read, F. L., Santos, M. B., Spitz, J., & Vingada, J. V. (2013). Ecological niche segregation among five toothed whale species off the NW Iberian Peninsula using ecological tracers as multi-approach. *Marine Biology* 160 (11), 2825–2840.
- Mergler, D., Anderson, H. A., Chan, L. H. M., Mahaffey, K. R., Murray, M., Sakamoto, M., & Stern, A. H. (2007). Methylmercury exposure and health effects in humans: A worldwide concern. *AMBIO: A Journal of the Human Environment* 36(1), 3–12.
- Metian, M., Charbonnier, L., Oberhänsli, F., Bustamante, P., Jeffree, R., Amiard, J. C., & Warnau, M. (2009). Assessment of metal, metalloid, and radionuclide bioaccessibility from mussels to human consumers, using centrifugation and simulated digestion methods coupled with radiotracer techniques. *Ecotoxicology and Environmental Safety* 72(5), 1499–1502.
- Meynier, L., Stockin, K. A., Bando, M. K. H., & Duignan, P. J. (2008). Stomach contents of common dolphin (*Delphinus* sp.) from New Zealand waters. *New Zealand Journal of Marine and Freshwater Research* 42, 257–268.
- Mielke, H. W., Laidlaw, M. A., & Gonzales, C. R. (2011). Estimation of leaded (Pb) gasoline's continuing material and health impacts on 90 US urbanized areas. *Environment International* 37(1), 248–257.
- Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., ... & Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food – an international consensus. *Food & Function* 5, 1113–1124.
- Minton, G., Reeves, R., & Braulik, G. (2018). *Globicephala melas*. The IUCN Red List of Threatened Species 2018: e.T9250A50356171. <http://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T9250A50356171.en>.
- Miramand, P., & Bentley, D. (1992). Concentration and distribution of heavy metals in tissues of two cephalopods, *Eledone cirrhosa* and *Sepia officinalis* from the French coast of the English

- Channel. *Marine Biology* 114:407–414.
- Miramand, P., Bustamante, P., Bentley, D., & Kouéta, N. (2006). Variation of heavy metal concentrations (Ag, Cd, Co, Cu, Fe, Pb, V, and Zn) during the life cycle of the common cuttlefish *Sepia officinalis*. *Science of the Total Environment* 361, 132–143.
- Miramand, P., & Guary, J. C. (1981). Association of americium-241 with adenochromes in the branchial hearts of the cephalopod *Octopus vulgaris*. *Mar Ecol Prog Ser* 4:127–129.
- Monteiro, L. R., & Furness, R. W. (1995). Seabirds as monitors of mercury in the marine environment. *Water, Air, & Soil Pollution* 80(1), 851–870.
- Monteiro, L. R., & Furness, R. W. (1995). Seabirds as monitors of mercury in the marine environment. In *Mercury as a Global Pollutant* (pp. 851–870). Springer, Dordrecht.
- Monteiro, L. R., Costa, V., Furness, R. W., & Santos, R. S. (1996). Mercury concentrations in prey fish indicate enhanced bioaccumulation in mesopelagic environments. *Marine Ecology Progress Series* 141, 21–25.
- Monteiro, S. S., Caurant, F., López, A., Cedeira, J., Ferreira, M., Vingada, J. V., Eira, C., & Méndez-Fernandez, P. (2017). Sympatric *Globicephala* species: feeding ecology and contamination status based on stable isotopes and trace elements. *Marine Ecology Progress Series* 563, 233–247.
- Moore, J. W., & Ramamoorthy, S. (2012). Heavy metals in natural waters: applied monitoring and impact assessment. Springer Science & Business Media.
- Morrisey, D. J., Gibbs, M. M., Pickmere, S. E., & Cole, R. G. (2000). Predicting impacts and recovery of marine-farm sites in Stewart Island, New Zealand, from the Findlay–Watling model. *Aquaculture* 185(3–4), 257–271.
- Mouritsen, O. G., & Styrbæk, K. (2018). Cephalopod gastronomy—A promise for the future. *Frontiers in Communication* 3, 38.
- MPI. (2018, May 24). New Zealand Total Diet Study. Retrieved from <https://www.mpi.govt.nz/dmsdocument/28976-2016-nz-total-diet-study-with-appendices-report>.
- MPI. (2019, Nov 11). Fisheries New Zealand. Retrieved from <https://fs.fish.govt.nz/Page.aspx?pk=5&tk=96&fpid=48>
- MPI. (2020, January 8<sup>th</sup>). Fisheries infosite. Retrieved from <https://fs.fish.govt.nz/Page.aspx?pk=5&fpid=48>.
- Muir, D. C. G., Wagemann, R., Grift, N. P., Norstrom, R. J., Simon, M., & Lien, J. (1988). Organochlorine chemical and heavy metal contaminants in white-beaked dolphins (*Lagenorhynchus albirostris*) and pilot whales (*Globicephala melaena*) from the coast of Newfoundland, Canada. *Archives of Environmental Contamination and Toxicology* 17, 613–629.
- Muñoz, O., Devesa, V., Suñer, M. A., Vélez, D., Montoro, R., Urieta, I., Macho, M. L., & Jalón, M. (2000). Total and inorganic arsenic in fresh and processed fish products. *Journal of Agricultural and Food Chemistry* 48(9), 4369–4376.
- Murphy, R. J., Pinkerton, M. H., Richardson, K. M., Bradford-Grieve, J. M., & Boyd, P. W. (2001). Phytoplankton distributions around New Zealand derived from SeaWiFS remotely-sensed ocean colour data. *New Zealand Journal Marine and Freshwater Research*, 35, 343–362.
- Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining  $R^2$  from generalized linear mixed-effects models. *Methods Ecology Evolution* 4, 133–142.
- Neff, J. M. (1997). Ecotoxicology of arsenic in the marine environment. *Environmental Toxicology and Chemistry: An International Journal* 16, 917–927.
- Nicholson, J. K., Kendall, M. D., & Osborn, D. (1983). Cadmium and mercury nephrotoxicity. *Nature* 304, 633.
- Nicholson, J. K., & Osborn, D. (1983). Kidney lesions in pelagic seabirds with high tissue levels of cadmium and mercury. *Journal of Zoology* 200, 99–118.
- Nicosia, A., Salamone, M., Mazzola, S., & Cuttitta, A. (2015). Transcriptional and biochemical effects of cadmium and manganese on the defense system of *Octopus vulgaris* paralarvae. *BioMed Research International*, 2015, Article ID 437328, 11 pages.
- Nigro, M., & Leonzio, C. (1996). Intracellular storage of mercury and selenium in different marine vertebrates. *Marine Ecology Progress Series* 135, 137–143.

- Ninh, T. D., Nagashima, Y., & Shiomi, K. (2007). Water-soluble and lipid-soluble arsenic compounds in Japanese flying squid *Todarodes pacificus*. *Journal of Agricultural and Food Chemistry* 55(8), 3196–3202.
- Noël, L., Chafey, C., Testu, C., Pinte, J., Velge, P., & Guérin, T. (2011). Contamination levels of lead, cadmium and mercury in imported and domestic lobsters and large crab species consumed in France: Differences between white and brown meat. *Journal of Food Composition and Analysis* 24(3), 368–375.
- Nordberg, G. F., Fowler, B. A., Nordberg, M. (2014). *Handbook on the Toxicology of Metals*. Academic Press. <https://doi.org/10.1016/B978-0-12-369413-3.X5052-6>.
- Northern, T. J., Smith, A. M., McKinnon, J. F., & Bolstad, K. S. R. (2019). Trace elements in beaks of greater hooked squid *Onykia ingens*: opportunities for environmental tracing. *Molluscan Research* 39, 29–34.
- Nriagu, J. O., & Pacyna, J. M. (1988). Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *Nature* 333(6169), 134–139.
- Olson, P. A. (2009). Pilot whales: *Globicephala melas* and *G. macrorhynchus*, in: *Encyclopedia of Marine Mammals*. Elsevier, 847–852.
- Oremus, M., Gales, R., Dalebout, M. L., Funahashi, N., Endo, T., Kage, T., Steel, D., & Baker, S. C. (2009). Worldwide mitochondrial DNA diversity and phylogeography of pilot whales (*Globicephala* spp.). *Biological Journal of the Linnean Society* 98(4), 729–744.
- O’Sullivan, D., & Cullen, J. M. (1983). Food of the squid *Nototodarus gouldi* in Bass Strait. *Marine and Freshwater Research* 34(2), 261–285.
- Overholtz, W. J., & Waring, G. T. (1991). Diet composition of pilot whales *Globicephala* sp. and common dolphins *Delphinus delphis* in the Mid-Atlantic Bight during spring 1989. *Fishery Bulletin* 89, 723–728.
- Pakhomov, E. A., & McQuaid, C. D. (1996). Distribution of surface zooplankton and seabirds across the Southern Ocean. *Polar Biology* 16(4), 271–286.
- Palmisano, F., Cardellicchio, N., & Zambonin, P. G. (1995). Speciation of mercury in dolphin liver: a two-stage mechanism for the demethylation accumulation process and role of selenium. *Marine Environmental Research* 40(2), 09–121.
- Pan, J., Plant, J. A., Voulvoulis, N., Oates, C. J., & Ihlenfeld, C. (2010). Cadmium levels in Europe: Implications for human health. *Environmental Geochemistry and Health* 32(1), 1–12.
- Park, J.-D., & Zheng, W. (2012). Human exposure and health effects of inorganic and elemental mercury. *Journal of Preventive Medicine and Public Health* 45, 344.
- Passarella, K. C., & Hopkins, T. L. (1991). Species composition and food habits of the micronektonic cephalopod assemblage in the eastern Gulf of Mexico. *Bulletin of Marine Science* 49(1–2), 638–659.
- Pastorelli, A. A., Baldini, M., Stacchini, P., Baldini, G., Morelli, S., Sagratella, E., Zaza, S., & Ciardullo, S. (2012). Human exposure to lead, cadmium and mercury through fish and seafood product consumption in Italy: A pilot evaluation. *Food Additives & Contaminants: Part A* 29(12), 1913–1921.
- Pavlakakis, N., Pollock, C. A., McLean, G., & Bartrop, R. (1996). Deliberate overdose of uranium: Toxicity and treatment. *Nephron* 72(2), 313–317.
- Peake, B. M., Marsden, I. D., Ashoka, S., & Bremner, G. (2010). Interspecific and geographical variation in trace metal concentrations of New Zealand scallops. *Journal of Shellfish Research* 29(2), 387–395.
- Peijnenburg, W., & Jager, T. (2003). Monitoring approaches to assess bioaccessibility and bioavailability of metals: Matrix issues. *Ecotoxicology and Environmental Safety* 56(1), 63–77.
- Penicaud, V., Lacoue-Labarthe, T., & Bustamante, P. (2017). Metal bioaccumulation and detoxification processes in cephalopods: A review. *Environmental Research* 155, 123–133. <https://doi.org/10.1016/j.envres.2017.02.003>.
- Perrin, W. F., & Myrick, A. C. eds. (1980). *Growth of Odontocetes and Sirenians: Problems in Age Determination: Proceedings of the International Conference on Determining Age of Odontocete Cetaceans [and Sirenians]*, La Jolla, California, September 5-19, 1978 (Vol. 3). International Whaling Commission.

- Pethybridge, H., Virtue, P., Casper, R., Yoshida, T., Green, C. P., Jackson, G., & Nichols, P. D. (2012). Seasonal variations in diet of arrow squid (*Nototodarus gouldi*): Stomach content and signature fatty acid analysis. *Journal of the Marine Biological Association of the United Kingdom* 92(1), 187–196.
- Petri, G., & Zauke, G.-P. (1993). Trace metals in crustaceans in the Antarctic Ocean. *Ambio-Journal of Human Environment Research and Management* 22(8), 529–536.
- Pierce, G. J., & Boyle, P. R. (1991). A review of methods for diet analysis in piscivorous marine mammals. *Oceanography and Marine Biology: An Annual Review* 29, 409–486.
- Pierce, G. J., Stowasser, G., Hastie, L. C., & Bustamante, P. (2008). Geographic, seasonal and ontogenetic variation in cadmium and mercury concentrations in squid (Cephalopoda: Teuthoidea) from UK waters. *Ecotoxicology and Environmental Safety* 70(3), 422–432.
- Pilsner, J. R., Lazarus, A. L., Nam, D.-H., Letcher, R. J., Sonne, C., Dietz, R., & Basu, N. (2010). Mercury-associated DNA hypomethylation in polar bear brains via the LUMInometric Methylation Assay: a sensitive method to study epigenetics in wildlife. *Molecular Ecology* 19, 307–314.
- Pinedo-González, P., West, A.J., Tovar-Sánchez, A., Duarte, C.M., Marañón, E., Cermeño, P., González, N., ... & López-Sandoval, D.C. (2015). Surface distribution of dissolved trace metals in the oligotrophic ocean and their influence on phytoplankton biomass and productivity. *Global Biogeochemical Cycles* 29 (10), 1763–1781.
- Pinkerton, M. H. (2011). A balanced trophic model of the Chatham Rise, New Zealand. Unpublished NIWA report. Retrieved November 6, 2019, from [https://niwa.co.nz/sites/niwa.co.nz/files/chatham-model\\_32.pdf](https://niwa.co.nz/sites/niwa.co.nz/files/chatham-model_32.pdf).
- Pitt, L. (2016). What's happening in Taranaki? Social workers and the environment. *Aotearoa New Zealand Social Work* 25(4), 52–61.
- Pope, J., Newman, N., Craw, D., Trumm, D., & Rait, R. (2010). Factors that influence coal mine drainage chemistry West Coast, South Island, New Zealand. *New Zealand Journal of Geology and Geophysics* 53, 115–128.
- Pozebon, D., Lima, E. C., Maia, S. M., & Fachel, J. M. (2005). Heavy metals contribution of non-aqueous fluids used in offshore oil drilling. *Fuel* 84, 53–61.
- Pridmore, R. D., Roper, D. S., & Hewitt, J. E. (1990). Variation in composition and condition of the Pacific oyster, *Crassostrea gigas*, along a pollution gradient in Manukau Harbour, New Zealand. *Marine Environmental Research* 30(3), 163–177.
- Probert, P. K., McKnight, D. G., & Grove, S. L. (1997). Benthic invertebrate bycatch from a deep-water trawl fishery, Chatham Rise, New Zealand. *Aquatic Conservation: Marine and Freshwater Ecosystems* 7, 27–40.
- Radke, L. C., Heap, A. D., Douglas, G., Nichol, S., Trafford, J., Li, J., & Przeslawski, R. (2011). A geochemical characterisation of deep-sea floor sediments of the northern Lord Howe Rise. *Deep Sea Research Part II: Topical Studies in Oceanography* 58(7), 909–921.
- Rafael, A. P. M.-H. (2017). Contaminants in deep-sea glass squids (Cranchiidae) from the eastern tropical Atlantic oxygen minimum zone (Master Thesis).
- Raimundo, J., Costa, P. M., Vale, C., Costa, M. H., & Moura, I. (2010a). Metallothioneins and trace elements in digestive gland, gills, kidney and gonads of *Octopus vulgaris*. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 152(2), 139–146.
- Raimundo, J., Pereira, P., Vale, C., & Caetano, M. (2005). Fe, Zn, Cu and Cd concentrations in the digestive gland and muscle tissues of *Octopus vulgaris* and *Sepia officinalis* from two coastal areas in Portugal. *Ciencias Marinas* 31, 243–251.
- Raimundo, J., Vale, C., Canário, J., Branco, V., & Moura, I. (2010b). Relations between mercury, methyl-mercury and selenium in tissues of *Octopus vulgaris* from the Portuguese Coast. *Environmental Pollution* 158, 2094–2100. <https://doi.org/10.1016/j.envpol.2010.03.005>.
- Raimundo, J., Vale, C., & Rosa, R. (2014). Trace element concentrations in the top predator jumbo squid (*Dosidicus gigas*) from the Gulf of California. *Ecotoxicology and Environmental Safety* 102, 179–186.
- Rainbow, P. S. (1995). Biomonitoring of heavy metal availability in the marine environment. *Marine Pollution Bulletin* 31, 183–192.

- Rainbow, P. S. (2007). Trace metal bioaccumulation: Models, metabolic availability and toxicity. *Environment International* 33, 576–582.
- Rait, R., Trumm, D., Pope, J., Craw, D., Newman, N., & MacKenzie, H. (2010). Adsorption of arsenic by iron rich precipitates from two coal mine drainage sites on the West Coast of New Zealand. *New Zealand Journal of Geology and Geophysics* 53, 177–193.
- Ralston, N. V., Ralston, C. R., Blackwell III, J. L., & Raymond, L. J. (2008). Dietary and tissue selenium in relation to methylmercury toxicity. *Neurotoxicology* 29, 802–811.
- Ramadyan, F. (2017). Spatial and Temporal Variability of Phytoplankton Productivity in the Subtropical Front around New Zealand. Dissertation, University of Otago.
- Ranville, M. A., Cutter, G. A., Buck, C. S., Landing, W. M., Cutter, L. S., Resing, J. A., & Flegal, A. R. (2010). Aeolian contamination of Se and Ag in the North Pacific from Asian fossil fuel combustion. *Environmental Science & Technology* 44(5), 1587–1593.
- Rathore R. S. & Khangarot, B. S. (2002). Effects of temperature on the sensitivity of sludge worm *Tubifex tubifex* Müller to selected heavy metals. *Ecotoxicology and Environmental Safety* 53, 27–36.
- Ratnasingham, S., & Hebert, P. D. (2007). BOLD: The Barcode of Life Data System (<http://www.barcodinglife.org>). *Molecular Ecology Notes* 7, 355–364.
- Rawson, A. J., Bradley, J. P., Teetsov, A., Rice, S. B., Haller, E. M., & Patton, G. W. (1995). A role for airborne particulates in high mercury levels of some cetaceans. *Ecotoxicology and Environmental Safety* 30, 309–314.
- Rawson, A. J., Patton, G. W., Hofmann, S., Pietra, G. G., & Johns, L. (1993). Liver abnormalities associated with chronic mercury accumulation in stranded atlantic bottlenose dolphins. *Ecotoxicology and Environmental Safety* 25, 41–47.
- R Core Team (2017). R: A Language and Environment for Statistical Computing. R Found. Stat. Comput., Vienna, Austria. Retrieved from <http://www.R-project.org>.
- Reeves, R. R. (2003). Dolphins, whales and porpoises: 2002-2010 conservation action plan for the world's cetaceans (Vol. 58). IUCN. <https://doi.org/10.2305/IUCN.CH.2003.SSC-AP.2.en>
- Reeves, P. G., Chaney, R. L. (2008). Bioavailability as an issue in risk assessment and management of food cadmium: A review. *Science of the Total Environment* 398(1–3), 13–19.
- Reimann, C., & de Caritat, P. (2005). Distinguishing between natural and anthropogenic sources for elements in the environment: Regional geochemical surveys versus enrichment factors. *Science of the Total Environment* 337(1–3), 91–107.
- Reinfelder, J. R., Fisher, N. S., Fowler, S. W., & Teyssié, J. L. (1993). Release rates of trace elements and protein from decomposing planktonic debris. 2. Copepod carcasses and sediment trap particulate matter. *Journal of Marine Research* 51(2), 423–442.
- Renedo, M., Amouroux, D., Pedrero, Z., Bustamante, P., & Cherel, Y. (2018). Identification of sources and bioaccumulation pathways of MeHg in subantarctic penguins: a stable isotopic investigation. *Scientific Reports* 8(1), 8865.
- Ripley, B., Venables, B., Bates, D. M., Hornik, K., Gebhardt, A., Firth, D., & Ripley, M. B. (2013). Package ‘mass.’ Cran R.
- Rjeibi, M., Metian, M., Hajji, T., Guyot, T., Chaouacha-Chékir, R. B., & Bustamante, P. (2014). Interspecific and geographical variations of trace metal concentrations in cephalopods from Tunisian waters. *Environmental Monitoring and Assessment* 186, 3767–3783.
- Rjeibi, M., Metian, M., Hajji, T., Guyot, T., Chaouacha-Chékir, R. B., & Bustamante, P. (2015). Seasonal Survey of Contaminants (Cd and Hg) and Micronutrients (Cu and Zn) in Edible Tissues of Cephalopods from Tunisia: Assessment of Risk and Nutritional Benefits: Cephalopods from Tunisia for human consumption. *Journal of Food Science*, 80(1), 199–206. <https://doi.org/10.1111/1750-3841.12711>
- Rodhouse, P. G., & White, M. G. (1995). Cephalopods occupy the ecological niche of epipelagic fish in the Antarctic Polar Frontal Zone. *The Biological Bulletin* 189(2), 77–80.
- Rodrigo, A. P., & Costa, P. M. (2017). The Role of the Cephalopod Digestive Gland in the Storage and Detoxification of Marine Pollutants. *Frontiers in Physiology* 8, 232.
- Roels, H. A., Lauwerys, R. R., Buchet, J.-P., Bernard, A. M., Lijnen, P., & Van Houte, G. (1990). Urinary kallikrein activity in workers exposed to cadmium, lead, or mercury vapour. *Occupational and Environmental Medicine* 47, 331–337.

- Rosa, C., Blake, J. E., Bratton, G. R., Dehn, L.-A., Gray, M. J., & O'Hara, T. M. (2008). Heavy metal and mineral concentrations and their relationship to histopathological findings in the bowhead whale (*Balaena mysticetus*). *Science of the Total Environment* 399, 165–178.
- Rosa, R., & Seibel, B. A. (2010). Metabolic physiology of the Humboldt squid, *Dosidicus gigas*: implications for vertical migration in a pronounced oxygen minimum zone. *Progress in Oceanography* 86, 72–80.
- Rosas-Luis, R., Navarro, J., Sánchez, P., & Del Río, J. L. (2016). Assessing the trophic ecology of three sympatric squid in the marine ecosystem off the Patagonian Shelf by combining stomach content and stable isotopic analyses. *Marine Biology Research* 12, 402–411.
- Rosas-Luis, R., Sánchez, P., Portela, J. M., & del Río, J. L. (2014). Feeding habits and trophic interactions of *Doryteuthis gahi*, *Illex argentinus* and *Onykia ingens* in the marine ecosystem off the Patagonian Shelf. *Fisheries Research* 152, 37–44.
- Rossi, A., Pellegrini, D., Belcari, P., & Barghigiani, C. (1993). Mercury in *Eledone cirrhosa* from the northern Tyrrhenian sea: Contents and relations with life cycle. *Marine Pollution Bulletin* 26, 683–686.
- Runge, J. A. (1988). Should we expect a relationship between primary production and fisheries? The role of copepod dynamics as a filter of trophic variability. In *Biology of Copepods* (pp. 61–71). Springer, Dordrecht.
- Sabine, C. L., Feely, R. A., Gruber, N., Key, R. M., Lee, K., Bullister, J. L., Wanninkhof, R., ... & Rios, A. F. (2004). The oceanic sink for anthropogenic CO<sub>2</sub>. *Science* 305(5682), 367–371.
- Sadiq, R., Husain, T., Bose, N., & Veitch, B. (2003). Distribution of heavy metals in sediment pore water due to offshore discharges: an ecological risk assessment. *Environmental Modelling & Software* 18, 451–461.
- Santos, M. B., Clarke, M. R., & Pierce, G. J. (2001). Assessing the importance of cephalopods in the diets of marine mammals and other top predators: problems and solutions. *Fisheries Research* 52, 121–139.
- Savery, L. C., Chen, T. L., Wise, J. T., Wise, S. S., Gianios Jr, C., Buonagurio, J., Perkins, C., ... & Wise, Sr J. P. (2015). Global assessment of cadmium concentrations in the skin of free-ranging sperm whales (*Physeter macrocephalus*). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 178, 136–144.
- Savery, L. C., Evers, D. C., Wise, S. S., Falank, C., Wise, J., Gianios Jr, C., Kerr, I., ... & Zheng, T. (2013). Global mercury and selenium concentrations in skin from free-ranging sperm whales (*Physeter macrocephalus*). *Science of the Total Environment* 450, 59–71.
- Scheuhammer, A. M., Basu, N., Burgess, N. M., Elliott, J. E., Campbell, G. D., Wayland, M., Champoux, L., & Rodrigue, J. (2008). Relationships among mercury, selenium, and neurochemical parameters in common loons (*Gavia immer*) and bald eagles (*Haliaeetus leucocephalus*). *Ecotoxicology* 17, 93–101.
- Scholz, M., Kaplan, F., Guy, C.L., Kopka, J., & Selbig, J. (2005). Non-linear PCA: a missing data approach. *Bioinformatics* 21(20), 3887–3895.
- SCOR Working Group (2007). GEOTRACES—An international study of the global marine biogeochemical cycles of trace elements and their isotopes. *Chemie der Erde-Geochemistry* 67(2), 85–131.
- Seco, J., Xavier, J. C., Brierley, A. S., Bustamante, P., Coelho, J. P., Gregory, S., Fielding, S., ... & Tarling, G. A. (2020). Mercury levels in Southern Ocean squid: Variability over the last decade. *Chemosphere* 239, 124785.
- Seixas, S., Bustamante, P., & Pierce, G. J. (2005). Interannual patterns of variation in concentrations of trace elements in arms of *Octopus vulgaris*. *Chemosphere* 59, 1113–1124.
- Sekiguchi, K., Klages, N. T. W., & Best, P. B. (1992). Comparative analysis of the diets of smaller odontocete cetaceans along the coast of southern Africa. *South African Journal of Marine Science* 12(1), 843–861.
- Sergeant, D. E. (1962). The biology of the pilot or pothead whale *Globicephala melaena* (Traill) in Newfoundland waters. *Journal of the Fisheries Research Board of Canada* 132, 1–84.
- Serrano, I. N., Ballesteros, M. T. L., Pacheco, S. S. F., Álvarez, S. I., & Colón, J. L. L. (2016). Total and speciated urinary arsenic levels in the Spanish population. *Science of The Total Environment* 571, 164–171.

- Shi, D., Xu, Y., Hopkinson, B. M., & Morel, F. M. (2010). Effect of ocean acidification on iron availability to marine phytoplankton. *Science* 327(5966), 676–679.
- Shiomi, K., Sugiyama, Y., Shimakura, K., & Nagashima, Y. (1996). Retention and biotransformation of arsenic compounds administered intraperitoneally to carp. *Fisheries Science* 62, 261–266.
- Siebert, U., Joiris, C., Holsbeek, L., Benke, H., Failing, K., Frese, K., & Petzinger, E. (1999). Potential Relation Between Mercury Concentrations and Necropsy Findings in Cetaceans from German Waters of the North and Baltic Seas. *Marine Pollution Bulletin* 38, 285–295.
- Simmonds, M. P., Haraguchi, K., Endo, T., Cipriano, F., Palumbi, S. R., & Troisi, G. M. (2002). Human health significance of organochlorine and mercury contaminants in Japanese whale meat. *Journal of Toxicology and Environmental Health, Part A* 65, 1211–1235.
- Simmonds, M. P., Johnston, P. A., French, M. C., Reeve, R., & Hutchinson, J. D. (1994). Organochlorines and mercury in pilot whale blubber consumed by Faroe islanders. *Science of the Total Environment* 149, 97–111.
- Smith, E. (1881). Account of the Zoological Collections made during the Survey of HMS ‘Alert’ in the Straits of Magellan and on the Coast of Patagonia. *Proceedings of the Zoological Society of London*. Wiley Online Library, 2–141.
- Smith, C. R., & Baco, A. R., (2003). Ecology of whale falls at the deep-sea floor. *Oceanography and Marine Biology* 41, 311–354.
- Smith, J. D., Plues, L., Heyraud, M., & Cherry, R. D. (1984). Concentrations of the elements Ag, Al, Ca, Cd, Cu, Fe, Mg, Mn, Pb and Zn, and the radionuclides <sup>210</sup>Pb and <sup>210</sup>Po in the digestive gland of the squid *Nototodarus gouldi*. *Marine Environmental Research* 13(1), 55–68.
- Smith, P. J., Mattlin, R. H., Roeleveld, M. A., & Okutanp, T. (1987). Arrow squids of the genus *Nototodarus* in New Zealand waters: systematics, biology, and fisheries. *New Zealand Journal of Marine and Freshwater Research* 21, 315–326.
- Smith, P. J., Roberts, P. E., & Hurst, R. J. (1981). Evidence for two species of arrow squid in the New Zealand fishery. *New Zealand Journal of Marine and Freshwater Research* 15(3), 247–253.
- Sokolova, I. M., & Lannig, G. (2008). Interactive effects of metal pollution and temperature on metabolism in aquatic ectotherms: Implications of global climate change. *Climate Research* 37, 181–201.
- Sonne, C., Dam, M., Leifsson, P. S., & Dietz, R. (2010). Liver and renal histopathology of North Atlantic long-finned pilot whales (*Globicephala melas*) contaminated with heavy metals and organochlorine compounds. *Toxicological & Environmental Chemistry* 92, 969–985.
- Stark, K. E., Jackson, G. D., & Lyle, J. M. (2005). Tracking arrow squid movements with an automated acoustic telemetry system. *Marine Ecology Progress Series* 299, 167–177.
- Steenstrup, J. (1857). Prof. Steenstrup foreviste og bestrev nogle nye blaeksprutter *Dosidicus Eschrichtii* Stp. Og *Onychoteuthis* (?). *Vid Medd Dansk Naturhist. Voren Kjöbenhavn* 1856–1857, 120–121.
- Stewart, C., Johnston, D. M., Leonard, G. S., Horwell, C. J., Thordarson, T., & Cronin, S. J. (2006). Contamination of water supplies by volcanic ashfall: a literature review and simple impact modelling. *Journal of Volcanology and Geothermal Research* 158, 296–306.
- Stewart, F. M., Phillips, R. A., Bartle, J. A., Craig, J., & Shooter, D. (1999). Influence of phylogeny, diet, moult schedule and sex on heavy metal concentrations in New Zealand Procellariiformes. *Marine Ecology Progress Series* 178, 295–305.
- Stockin, K. A., Law, R. J., Duignan, P. J., Jones, G. W., Porter, L., Mirimin, L., Meynier, L., & Orams, M. B. (2007). Trace elements, PCBs and organochlorine pesticides in New Zealand common dolphins (*Delphinus* sp.). *Science of the Total Environment* 387(1–3), 333–345.
- Stoffers, P., Glasby, G. P., Wilson, C. J., Davis, K. R., & Walter, P. (1986). Heavy metal pollution in Wellington Harbour. *New Zealand Journal of Marine and Freshwater Research* 20(3), 495–512. <https://doi.org/10.1080/00288330.1986.9516169>
- Stoffers, P., Hannington, M., Wright, I., Herzig, P., De Ronde, C., & Party, S. S. (1999). Elemental mercury at submarine hydrothermal vents in the Bay of Plenty, Taupo volcanic zone, New Zealand. *Geology* 27(10), 931–934.
- Stoneburner, D. L. (1978). Heavy metals in tissues of stranded short-finned pilot whales. *Science of the Total Environment* 9, 293–297.

- Storelli, M. M. (2008). Potential human health risks from metals (Hg, Cd, and Pb) and polychlorinated biphenyls (PCBs) via seafood consumption: Estimation of target hazard quotients (THQs) and toxic equivalents (TEQs). *Food and Chemical Toxicology* 46, 2782–2788.
- Storelli, M. M., Barone, G., & Marcotrigiano, G. O. (2005). Cadmium in cephalopod molluscs: Implications for public health. *Journal of Food Protection* 68(3), 577–580.
- Storelli, M. M., Garofalo, R., Giungato, D., & Giacomini-Stuffler, R. (2010). Intake of essential and non-essential elements from consumption of octopus, cuttlefish and squid. *Food Additives and Contaminants: Part B Surveillance* 3(1), 14–18.
- Storelli, M. M., & Marcotrigiano, G. O. (1999). Cadmium and total mercury in some cephalopods from the South Adriatic Sea (Italy). *Food Additives and Contaminants* 16(6), 261–265.
- Storelli, M. M., & Marcotrigiano, G. O. (2004). Content of mercury and cadmium in fish (*Thunnus alalunga*) and cephalopods (*Eledone moschata*) from the south-eastern Mediterranean Sea. *Food Additives and Contaminants* 21, 1051–1056. <https://doi.org/10.1080/02652030400023127>.
- Storelli, M. M., Normanno, G., Barone, G., Dambrosio, A., Errico, L., Garofalo, R., & Giacomini-Stuffler, R. (2012). Toxic metals (Hg, Cd, and Pb) in fishery products imported into Italy: Suitability for human consumption. *Journal of Food Protection* 75(1), 189–194.
- Stramma, L., Johnson, G. C., Sprintall, J., & Mohrholz, V. (2008). Expanding oxygen-minimum zones in the tropical oceans. *Science* 320(5876), 655–658.
- Stramma, L., Prince, E. D., Schmidtke, S., Luo, J., Hoolihan, J. P., Visbeck, M., Wallace, D. W., ... & Körtzinger, A. (2012). Expansion of oxygen minimum zones may reduce available habitat for tropical pelagic fishes. *Nature Climate Change* 2(1), 33.
- Suedel, B. C., Boraczek, J. A., Peddicord, R. K., Clifford, P. A., & Dillon, T. M. (1994). Trophic transfer and biomagnification potential of contaminants in aquatic ecosystems. In *Reviews of Environmental Contamination and Toxicology* (pp. 21–89). Springer, New York, NY.
- Sutton, P. (2001). Detailed structure of the subtropical front over Chatham Rise, east of New Zealand. *Journal of Geophysical Research: Oceans* 106(C12), 31045–31056.
- Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. In *Molecular, Clinical and Environmental Toxicology* (pp. 133–164). Springer.
- Tenzer, R., & Wiert, A. (2018). Evidence of the El Niño/La Niña Climatic Events in New Zealand Over the Last Century. *GSTF Journal of Geological Sciences (JGS)* 1(2), 28–38.
- Thompson, F. N., & Abraham, E. R. (2009). Dolphin Bycatch in New Zealand Trawl Fisheries, 1995–96 to 2006–07. Ministry of Fisheries, New Zealand.
- Tian, F., Frew, R. D., Sander, S., Hunter, K. A., & Ellwood, M. J. (2006). Organic iron (III) speciation in surface transects across a frontal zone: The Chatham Rise, New Zealand. *Marine and Freshwater Research* 57(5), 533–544.
- Tohyama, C., Shaikh, Z. A., Ellis, K. J., & Cohn, S. H. (1981). Metallothionein excretion in urine upon cadmium exposure: Its relationship with liver and kidney cadmium. *Toxicology* 22(3), 181–191.
- Tong, S., Schirnding, Y. E. von, & Prapamontol, T. (2000). Environmental lead exposure: A public health problem of global dimensions. *Bulletin of the World Health Organization* 78, 1068–1077.
- Tracey, D. M. (1993). Mercury levels in black cardinalfish (*Epigonus telescopus*). *New Zealand Journal of Marine and Freshwater Research* 27(2), 177–181. <https://doi.org/10.1080/00288330.1993.9516555>.
- Traill, T. S. (1809). Description of a new species of whale, *Delphinus melas*. *Journal of Natural Philosophy, Chemistry and the Arts* 22, 81–85.
- Trumm, D., Black, A., Gordon, K., Cavanagh, J., O'Halloran, K., & de Joux, A. (2005). Acid mine drainage assessment and remediation at an abandoned West Coast coal mine. In 'Metal contaminants in New Zealand, sources, treatments, and effects on ecology and human health', 317–342. New Zealand: Resolutionz Press.
- Ugolev, A. M., Egorova, V. V., Kuz'mina, V. V., & Grusdkov, A. A. (1983). Comparative-molecular characterization of membrane digestion in fish and mammals. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry* 76(3), 627–635.

- Ulusu, N. N., Acan, N. L., Turan, B., & Tezcan, E. F. (2003). Inhibition of glutathione reductase by cadmium ion in some rabbit tissues and the protective role of dietary selenium. *Biological Trace Element Research* 91(2), 151–156.
- Uozumi, Y. (1998). Fishery biology of arrow squids, *Nototodarus gouldi* and *N. sloanii* in New Zealand waters. *Bulletin of the National Research Institute of Far Seas Fisheries* 35(1), 111.
- Uozumi, Y., & Förch, E. (1995). Distribution of juvenile arrow squids *Nototodarus gouldi* and *N. sloanii* (Cephalopoda: Oegopsida) in New Zealand waters. *Fisheries Science* 61(4), 566–573.
- Uozumi, Y., & Ohara, H. (1993). Age and Growth of *Nototodarus sloanii* (Cephalopoda: Oegopsida) Based on Daily Increment Counts in Statoliths. *Nippon Suisan Gakkaishi* 59(9), 1469–1477.
- Valdes, J., Roman, D., Davila, P., Ortlieb, L., & Guinez, M. (2006). Seasonal variability of cadmium in a coastal upwelling system off northern Chile (Mejillones bay, 23° S). *Revista Chilena de Historia Natural* 79(4), 517–535.
- Valko, M. M. H. C. M., Morris, H., & Cronin, M. T. D. (2005). Metals, toxicity and oxidative stress. *Current Medicinal Chemistry* 12 (10), 1161–1208.
- van den Broek, W. L. F., & Tracey, D. M. (1981). Concentration and distribution of mercury in flesh of orange roughy (*Hoplostethus atlanticus*). *New Zealand Journal of Marine and Freshwater Research*, 15(3), 255–260. <https://doi.org/10.1080/00288330.1981.9515918>.
- Van Heezik, Y. (1990). Diets of yellow-eyed, Fiordland crested, and little blue penguins breeding sympatrically on Codfish Island, New Zealand. *New Zealand Journal of Zoology* 17, 543–548.
- Versantvoort, C. H. M., Oomen, A. G., Van de Kamp, E., Rompelberg, C. J. M., & Sips, A. J. A. M. (2005). Applicability of an in vitro digestion model in assessing the bioaccessibility of mycotoxins from food. *Food and Chemical Toxicology* 43, 31–40.
- Viarengo, A., & Nott, J. A. (1993). Mechanisms of heavy metal cation homeostasis in marine invertebrates. *Comparative Biochemistry and Physiology* 104C(3), 355–372.
- Villanueva, R., & Bustamante, P. (2006). Composition in essential and non-essential elements of early stages of cephalopods and dietary effects on the elemental profiles of *Octopus vulgaris* paralarvae. *Aquaculture* 261(1), 225–240.
- Vlieg, P., Lee, J., & Grace, N. D. (1991). Elemental concentration of marine and freshwater finfish, and shellfish from New Zealand waters. *Journal of Food Composition and Analysis* 4(2), 136–147.
- Vlieg, P., Murray, T., & Body, D. R. (1993). Nutritional data on six oceanic pelagic fish species from New Zealand waters. *Journal of Food Composition and Analysis* 6(1), 45–54.
- Voight, J. R., Pörtner, H. O., & O'Dor, R. K. (1995). A review of ammonia-mediated buoyancy in squids (cephalopoda: Teuthoidea). *Marine and Freshwater Behaviour and Physiology* 25(1–3), 193–203.
- Vopel, K., Pook, C., Wilson, P., & Robertson, J. (2017). Offshore iron sand extraction in New Zealand: Potential trace metal exposure of benthic and pelagic biota. *Marine Pollution Bulletin*.
- Vu, V. Q. (2011). ggbiplot: A ggplot2 based biplot. R Package, version 0.55.
- Waisberg, M., Black, W. D., Waisberg, C. M., & Hale, B. (2004). The effect of pH, time and dietary source of cadmium on the bioaccessibility and adsorption of cadmium to/from lettuce (*Lactuca sativa* L. cv. *Ostinata*). *Food and Chemical Toxicology* 42, 835–842.
- Wang, B., Luo, X., Yang, Y. M., Sun, W., Cane, M. A., Cai, W., Yeh, S. W., & Liu, J. (2019). Historical change of El Niño properties sheds light on future changes of extreme El Niño. *Proceedings of the National Academy of Sciences* 116(45), 22512–22517.
- Wang, W.-X. (2002). Cd and Se aqueous uptake and exposure of green mussels *Perna viridis*: influence of seston quantity. *Marine Ecology Progress Series* 226, 211–221.
- Wang, W.-X., & Fisher, N. S. (1998). Accumulation of trace elements in a marine copepod. *Limnology and Oceanography* 43, 273–283.
- Wang, S.-L., Chang, F.-H., Liou, S.-H., Wang, H.-J., Li, W.-F., & Hsieh, D. P. (2007). Inorganic arsenic exposure and its relation to metabolic syndrome in an industrial area of Taiwan. *Environment International* 33(6), 805–811.
- Waugh, S., Filippi, D., Fukuda, A., Suzuki, M., Higuchi, H., Setiawan, A., & Davis, L. (2005). Foraging of royal albatrosses, *Diomedea epomophora*, from the Otago Peninsula and its relationships to fisheries. *Canadian Journal of Fisheries and Aquatic Sciences* 62(6), 1410–1421.
- Welch, R. M., & Norvell, W. A. (1999). Mechanisms of cadmium uptake, translocation and deposition in plants. In *Cadmium in soils and plants* (pp. 125–150). Springer.

- West, J. A., & Imber, M. J. (1986). Some foods of Buller's mollymawk *Diomedea bulleri*. New Zealand Journal of Zoology 13(2), 169–174.
- WHO (2010a). Ten chemicals of major public health concern. Retrieved from [https://www.who.int/ipcs/assessment/public\\_health/chemicals\\_phc/en/](https://www.who.int/ipcs/assessment/public_health/chemicals_phc/en/).
- WHO (2010b). WHO human health risk assessment toolkit: chemical hazards (IPCS harmonization project document; no. 8.) International Programme on Chemical Safety. Geneva. Retrieved from [https://www.who.int/ipcs/methods/harmonization/areas/ra\\_toolkit/en/](https://www.who.int/ipcs/methods/harmonization/areas/ra_toolkit/en/).
- Wise Jr, J. P., Wise, J. T., Wise, C. F., Wise, S. S., Gianios Jr, C., Xie, H., Walter, R., Boswell, M., Zhu, C., Zheng, T., & Perkins, C. (2018). A three year study of metal levels in skin biopsies of whales in the Gulf of Mexico after the Deepwater Horizon oil crisis. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 205, 15–25.
- Wise Jr, J. P., Wise, J. T., Wise, C. F., Wise, S. S., Zhu, C., Browning, C. L., Zheng, T., Perkins, C., Gianios Jr, C., Xie, H., Wise Sr, J. P. (2019). Metal levels in whales from the Gulf of Maine: a one environmental health approach. Chemosphere 216, 653–660.
- Whyte, A. L., Hook, G. R., Greening, G. E., Gibbs-Smith, E., & Gardner, J. P. (2009). Human dietary exposure to heavy metals via the consumption of greenshell mussels (*Perna canaliculus* Gmelin 1791) from the Bay of Islands, northern New Zealand. Science of the Total Environment 407(14), 4348–4355.
- Williams, C. H., & David, D. J. (1973). The effect of superphosphate on the cadmium content of soils and plants. Soil Research 11(1), 43–56.
- Wing, S.R., Wing, L.C., Shatova, O.A. and Van Hale, R., 2017. Marine micronutrient vectors: seabirds, marine mammals and fishes ingest high concentrations of bioactive metals in the subantarctic island ecosystem. Marine Ecology Progress Series, 563, 13–23.
- Wolfe, M. F., Schwarzbach, S., & Sulaiman, R. A. (1998). Effects of mercury on wildlife: a comprehensive review. Environmental Toxicology and Chemistry 17, 146–160.
- Worm, B. (2016). Averting a global fisheries disaster. Proceedings of the National Academy of Sciences 113(18), 4895–4897.
- Wormuth, J. H., & Roper, C. F. (1983). Quantitative sampling of oceanic cephalopods by nets: problems and recommendations. Biological Oceanography 2(2-4), 357–377.
- Wright, D. A., & Welbourn, P. M. (1994). Cadmium in the aquatic environment: A review of ecological, physiological, and toxicological effects on biota. Environmental Reviews 2(2), 187–214.
- Wright, K. (2012). Corrgram: Plot a correlogram. R Package Version 1.
- Wu, X., Cobbina, S. J., Mao, G., Xu, H., Zhang, Z., & Yang, L. (2016). A review of toxicity and mechanisms of individual and mixtures of heavy metals in the environment. Environmental Science and Pollution Research 23(9), 8244–8259.
- Xavier, J., Rodhouse, P., Purves, M., Daw, T., Arata, J., & Pilling, G. (2002). Distribution of cephalopods recorded in the diet of the Patagonian toothfish (*Dissostichus eleginoides*) around South Georgia. Polar Biology 25, 323–330.
- Xavier, J. C., Rodhouse, P. G., Trathan, P. N., & Wood, A. G. (1999). A Geographical Information System (GIS) atlas of cephalopod distribution in the Southern Ocean. Antarctic Science 11(1), 61–62.
- Yang, D.-Y., Xu, Y., Chen, Y.-W., & Belzile, N. (2010). Inverse relationships between selenium and mercury in tissues of young walleye (*Stizosedion vitreum*) from Canadian boreal lakes. Science of the Total Environment 408, 1676–1683.
- Yang, H. N. & Chen, H. C. (1996). Uptake and elimination of cadmium by Japanese eel, *Anguilla japonica*, at various temperatures. Bulletin of Environmental Contamination and Toxicology 56, 670–676.
- Zauke, G.-P., Savinov, V. M., Ritterhoff, J., & Savinova, T. (1999). Heavy metals in fish from the Barents Sea (summer 1994). Science of the Total Environment 227, 161–173.
- Zhang, J., Huang, W. W., Liu, S. M., Liu, M. G., Yu, Q., & Wang, J. H. (1992). Transport of particulate heavy metals towards the China Sea: a preliminary study and comparison. Marine Chemistry 40(3-4), 161–178.
- Zhang, Y., Obata, H., & Nozaki, Y. (2004). Silver in the Pacific Ocean and the Bering Sea. Geochemical Journal, 38(6), 623–633.

- Zhao, Y., Vance, D., Abouchami, W., & De Baar, H. J. (2014). Biogeochemical cycling of zinc and its isotopes in the Southern Ocean. *Geochimica et Cosmochimica Acta* 125, 653–672.
- Zhou, J. L., Salvador, S. M., Liu, Y. P., & Sequeira, M. (2001). Heavy metals in the tissues of common dolphins (*Delphinus delphis*) stranded on the Portuguese coast. *Science of the Total Environment* 273, 61–76.
- Zia, M. H., Codling, E. E., Scheckel, K. G., & Chaney, R. L. (2011). *In vitro* and *in vivo* approaches for the measurement of oral bioavailability of lead (Pb) in contaminated soils: a review. *Environmental Pollution* 159(10), 2320–2327.
- Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). GLM and GAM for count data. In *Mixed effects models and extensions in ecology with R*. Springer Science, New York, NY, 209–243.

## Appendices

### Appendix A: Supplementary Tables

Appendix Table 1. Body burden calculations for the trace elements analysed in Chapter 2. Body burden calculation based on a subset of males (n=15) and females (n=15) of *N. sloanii* from the Chatham Rise, NZ. Body burden includes the mean weight (g) of each tissue fraction, the trace element concentrations\* in  $\mu\text{g g}^{-1}$  ww, the total trace element concentration in  $\mu\text{g}$  per tissue and percentual (%), and the contribution of each tissue to the total body weight (%). All element concentrations presented in  $\mu\text{g g}^{-1}$  ww (\*). Tissue abbreviations as follows: arm (A), branchial heart (BH), digestive gland (DG), F (fin), G (gonad, including ovaries and testes), kidney (K), mantle (M), systemic heart (SH), and tentacle (T).

Tissue	g wet weight	Tissue contribution bodyweight (%)*	As*	total As (ug)	% of total	Cd*	total Cd (ug)	% of total	Co*	total Co (ug)	% of total	Cr*	total Cr (ug)	% of total	Cu*	total Cu (ug)	% of total	Fe*	total Fe (ug)	% of total	Hg*	total Hg (ug)	% of total
Whole squid	453.65			906			765			15.22			17.28			5663			2783			20.41	
A	0.29	18.8	4.32	1.24	0.1	0.11	0.03	0.004	0.02	0.004	0.03	0.07	0.02	0.1	5.43	1.56	0.03	6.69	1.92	0.07	0.07	0.02	0.1
BH	1.41	0.04	4.08	5.75	0.6	3.30	4.64	0.6	0.16	0.22	1.43	0.47	0.65	3.8	56.41	79.43	1.4	37.43	52.71	1.89	0.18	0.25	1.2
DG	30.76	6.8	3.54	109	12.0	22.26	685	89.5	0.30	9.15	60.15	0.08	2.38	13.8	136	4197	74.1	55.71	1714	61.60	0.05	1.62	7.9
F	32.35	7.1	3.93	127.07	14.0	0.18	5.90	0.8	0.02	0.49	3.19	0.09	2.83	16.4	4.39	142.03	2.5	5.08	164	5.91	0.09	2.75	13.5
G	29.94	6.6	3.77	112.73	12.4	1.46	43.56	5.7	0.04	1.20	7.87	0.07	2.10	12.1	12.89	385.79	6.8	9.34	280	10.05	0.07	2.10	10.3
K	0.13	0.2	4.14	0.54	0.1	9.47	1.23	0.2	0.14	0.02	0.12	0.08	0.01	0.1	69.51	9.05	0.2	15.79	2.06	0.07	0.10	0.01	0.1
M	135.82	29.9	4.03	547	60.3	0.16	22.07	2.9	0.03	4.07	26.78	0.07	9.17	53.1	6.01	817	14.4	4.09	555	19.95	0.10	13.58	66.5
SH	0.66	0.1	3.62	2.40	0.3	3.87	2.57	0.3	0.10	0.06	0.41	0.16	0.10	0.6	46.38	30.80	0.5	15.80	10.49	0.38	0.11	0.07	0.4
T	0.25	5.7	4.11	1.02	0.1	0.13	0.03	0.004	0.01	0.002	0.02	0.05	0.01	0.1	5.14	1.28	0.02	8.56	2.13	0.08	0.07	0.02	0.1

Appendix Table 1 continued

	g wet weight	Tissue contribution bodyweight (%)*	Mn*	total Mn (ug)	%age of total	Ni*	total Ni (ug)	% of total	Pb*	total Pb (ug)	% of total	Sb*	total Sb (ug)	% of total	V*	total V (ug)	% of total	U*	total U (ug)	% of total	Zn*	total Zn (ug)	% of total
Whole squid	453.65			114.55			31.19			11.95			4.77			29.81			1.16			3800	
A	0.29	18.8	0.27	0.08	0.1	0.09	0.03	0.1	0.04	0.01	0.1	0.02	0.01	0.1	0.05	0.01	0.05	0.01	0.002	0.2	18.70	5.36	0.1
BH	1.41	0.04	1.43	2.01	1.8	0.64	0.90	2.9	0.25	0.35	2.9	0.09	0.13	2.7	0.28	0.40	1.3	0.01	0.01	0.6	27.14	38.21	1.0
DG	30.76	6.8	0.41	12.61	11.0	0.25	7.61	24.4	0.10	3.15	26.4	0.02	0.62	12.9	0.51	15.61	52.4	0.01	0.18	15.3	22.90	704	18.5
F	32.35	7.1	0.37	11.89	10.4	0.10	3.07	9.9	0.04	1.21	10.2	0.03	1.05	22.1	0.03	0.89	3.0	0.005	0.15	12.7	18.04	584	15.4
G	29.94	6.6	0.91	27.10	23.7	0.14	4.12	13.2	0.04	1.05	8.8	0.03	0.90	18.8	0.09	2.55	8.5	0.004	0.13	11.6	17.54	525	13.8
K	0.13	0.2	0.70	0.09	0.1	0.14	0.02	0.1	0.07	0.01	0.1	0.03	0.004	0.1	0.25	0.03	0.1	0.003	0.0004	0.0	25.47	3.32	0.1
M	135.82	29.9	0.44	60.10	52.5	0.11	15.28	49.0	0.05	6.11	51.1	0.02	2.04	42.7	0.08	10.19	34.2	0.01	0.69	59.2	14.14	1920	50.5
SH	0.66	0.1	0.89	0.59	0.5	0.24	0.16	0.5	0.06	0.04	0.3	0.04	0.02	0.5	0.18	0.12	0.4	0.005	0.00	0.3	24.80	16.47	0.4
T	0.25	5.7	0.08	0.1	0.04	0.01	0.03	0.06	0.01	0.1	0.01	0.003	0.1	0.04	0.01	0.03	0.01	0.001	0.1	15.50	3.85	0.1	
*Rest of organ contribution to total body weight: 25.8%																							

Appendix Table 2. Recovery concentrations ( $n=5$ ) for the analysis of the certified reference material (CRM, TORT 3) from Chapter 4 (mean  $\pm$  standard deviation).

Trace element	Measured concentration $\mu\text{g g}^{-1}\text{ dw}$	CRM value $\mu\text{g g}^{-1}\text{ dw}$	Recovery (%)
As	$64.95 \pm 1.92$	$59.5 \pm 3.8$	109
Cd	$37.54 \pm 1.28$	$42.3 \pm 1.8$	89
Co	$1.17 \pm 0.11$	1.06	111
Cr	$2.77 \pm 0.82$	$1.95 \pm 0.24$	142
Cu	$451.96 \pm 14.24$	$497 \pm 22$	91
Fe	$205.85 \pm 15.04$	$179 \pm 8$	115
Hg	$0.44 \pm 0.09$	$0.429 \pm 0.034$	102
Mn	$15.01 \pm 0.41$	$15.6 \pm 1.0$	96
Ni	$6.07 \pm 1.6$	$5.30 \pm 0.24$	115
Pb	$0.35 \pm 0.15$	$0.225 \pm 0.018$	156
V	$8.54 \pm 0.21$	$9.1 \pm 0.4$	94
Zn	$141.82 \pm 12.96$	$136 \pm 6$	104

Appendix Table 3. Concentrations of the trace elements (mean±standard deviation, minimum, and maximum) observed in the digestive gland ( $n=118$ ) and mantle tissue ( $n=118$ ) of arrow squids (*Nototodarus* spp.) sampled from six different regions within the New Zealand EEZ (Chapter 4). Concentrations are in  $\mu\text{g g}^{-1}$  dw.

Digestive gland												
Element	Auckland Islands		Chatham		Dunedin		Golden Bay		Taranaki		West Coast	
	mean±sd	min-max	mean±sd	min-max	mean±sd	min-max	mean±sd	min-max	mean±sd	min-max	mean±sd	min-max
As	7.65±0.76	5.75-8.96	14.16±5.26	5.68-29.31	8.00±1.06	6.26-10.08	13.59±2.66	9.09-19.3	25.27±7.03	16.13-39.61	41.27±11.87	14.7-54.88
Cd	101.04±44.68	25.25-186.07	191.64±114.6	11.35-476.51	72.51±82.77	11.95-334.55	125.73±73.32	38.11-264.9	371.14±216	105.42-788.46	21.75±14.77	8.79-58.33
Co	0.35±0.1	0.2-0.57	1.19±1.16	0.23-4.62	0.76±0.44	0.21-2.02	2.1±0.91	0.69-4.4	3.05±1.19	0.64-4.34	0.76±0.24	0.36-1.28
Cr	0.22±0.12	0.1-0.55	0.38±0.31	0.09-1.33	0.42±0.26	0.13-1.16	0.33±0.13	0.13-0.6	0.41±0.24	0.1-0.87	0.28±0.13	0.16-0.66
Cu	182.94±48.07	114.81-275.75	1546.46±867.19	79.48-3981.02	169.45±173.6	13.15-656.37	879.47±517.47	72.64-2103.5	1900.74±1176.89	172.2-4456.79	376.3±155.21	170.75-732.7
Fe	96.5±24.49	58.81-142.18	224.21±84.46	81.5-461.03	205.85±101.26	77.05-460.98	195.88±82.69	107.25-407.6	368.37±318.17	75.73-1444.06	394.17±115.54	190.05-621.62
Hg	0.09±0.04	0.02-0.17	0.21±0.15	0.04-0.58	0.1±0.08	0.01-0.24	0.12±0.07	0.02-0.2	0.46±0.26	0.07-0.99	0.56±0.19	0.22-0.77
Mn	1.31±0.21	0.99-1.65	1.65±0.77	0.76-4.17	1.5±1.08	0.53-4.79	2.03±0.8	1.1-4.1	3.31±1.56	1.28-7.52	1.96±0.84	0.98-4.46
Ni	0.54±0.2	0.24-1.00	1.24±1.42	0.18-7.73	2.08±1.75	0.19-6.07	1.02±0.67	0.13-2.01	1.25±0.69	0.41-2.85	0.51±0.15	0.25-0.75
Pb	0.22±0.12	0.08-0.55	0.21±0.19	0.03-0.74	0.37±0.49	0.07-2.19	0.37±0.25	0.13-1	0.74±0.31	0.25-1.32	0.35±0.12	0.14-0.57
U	0.02±0.01	0.02-0.03	0.04±0.02	0.01-0.07	0.02±0.003	0.02-0.03	0.02±0.005	0.02-0.03	0.03±0.004	0.02-0.03	0.03±0.01	0.02-0.05
V	1.05±0.61	0.29-2.57	0.92±0.89	0.17-4.11	0.56±0.36	0.18-1.55	0.35±0.23	0.16-0.9	0.45±0.16	0.2-0.77	0.29±0.05	0.22-0.39
Zn	68.81±14.26	45.48-15.12	90.99±103.94	13.28-480.91	67.2±46.29	28.19-181.93	187.48±135.6	43.84-498.2	574.26±354.39	124.03-1575.41	120.39±38.38	51.15-183.28
Mantle												
Element	Auckland Islands		Chatham		Dunedin		Golden Bay		Taranaki		West Coast	
	mean±sd	min-max	mean±sd	min-max	mean±sd	min-max	mean±sd	min-max	mean±sd	min-max	mean±sd	min-max
As	4.14±0.59	3.03-5.25	16.1±6.71	5.97-32.49	5.46±0.61	4.07-6.55	10.62±3.21	7.48-20.02	24.02±6.35	12.93-39.16	45.49±11.03	17.27-59.58
Cd	NA	NA	0.65±0.47	0.09-1.49	0.61±0.42	0.14-1.47	0.38±0.24	0.09-0.93	1.00 ± 0.46	0.38-1.82	1.02±0.49	0.18-1.77
Co	0.1±0.04	0.06-0.2	0.18±0.16	0.05-0.59	0.28±0.13	0.09-0.39	0.96±0.85	0.05-1.83	0.85 ± 0.94	0.05-2.34	0.29±0.25	0.09-0.94
Cr	0.3±0.15	0.14-0.81	0.29±0.33	0.08-1.82	0.42±0.57	0.08-2.79	0.26±0.16	0.08-0.66	0.23 ± 0.09	0.10-0.45	0.30±0.15	0.08-0.66
Cu	34.45±14.88	14.55-69.89	24.05±23.73	6.91-100.33	13.11±5.3	6.43-25.57	12.2±6.35	7.39-33.62	24.89 ± 22.47	9.69-106.65	32.71±9.82	18.86-55.2
Fe	18.26±9.18	9.66-46.11	16.35±11.71	4.53-58.18	11.36±4.38	5.49-22.1	8.7±4.77	4.11-17.58	10.38 ± 4.13	6.07-22.58	24.52±11.25	10.29-56.97
Hg	0.14±0.04	0.07-0.23	0.42±0.28	0.01-0.89	0.13±0.08	0.02-0.25	0.21±0.12	0.09-0.44	0.67 ± 0.53	0.21-2.13	0.53±0.13	0.31-0.84
Mn	1.41±0.24	1.09-2.02	1.77±0.60	0.85-3.00	1.08±0.16	0.82-1.33	1.31±0.75	0.72-3.61	1.79 ± 1.15	0.64-4.67	1.98±1.25	0.98-6.67
Ni	0.65±0.46	0.18-2.03	0.56±0.49	0.14-2.23	0.61±0.81	0.15-3.25	0.87±1.16	0.14-3.87	0.36 ± 0.10	0.20-0.51	0.37±0.22	0.19-1.13

Pb	0.23±0.23	0.06-1.02	0.18±0.18	0.03-0.83	0.2±0.25	0.05-0.89	0.52±0.99	0.08-4.16	0.19 ± 0.13	0.07-0.44	0.21±0.44	0.04-2.03
U	0.02±0.004	0.01-0.03	0.03±0.02	0.001-0.08	0.03±0.03	0.01-0.13	0.02±0.002	0.02-0.03	0.02 ± 0.002	0.02-0.02	0.02±0.01	0.01-0.03
V	0.26±0.08	0.16-0.44	0.24±0.09	0.17-0.43	NA	NA	NA	NA	NA	NA	0.29±0.09	0.22-0.53
Zn	57.75±19.85	44.57-137.39	56.54±7.77	41.35-70.91	50.72±6.62	41.86-64.38	57.59±6.19	49.59-69.73	65.36 ± 8.05	53.58-87.27	63.11±10.94	43.18-83.91

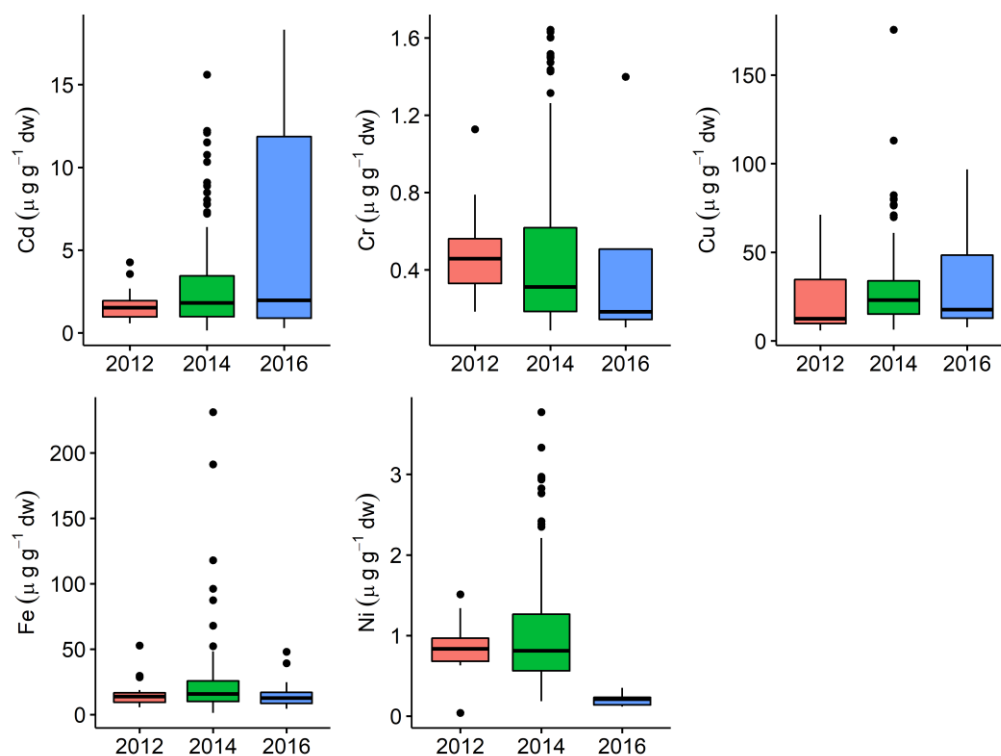
Appendix Table 4. Number of *M. ingens* specimens analysed from the Chatham Rise, New Zealand, according to their size classes (Mantle length, ML). Large females were classified as >400 mm.

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

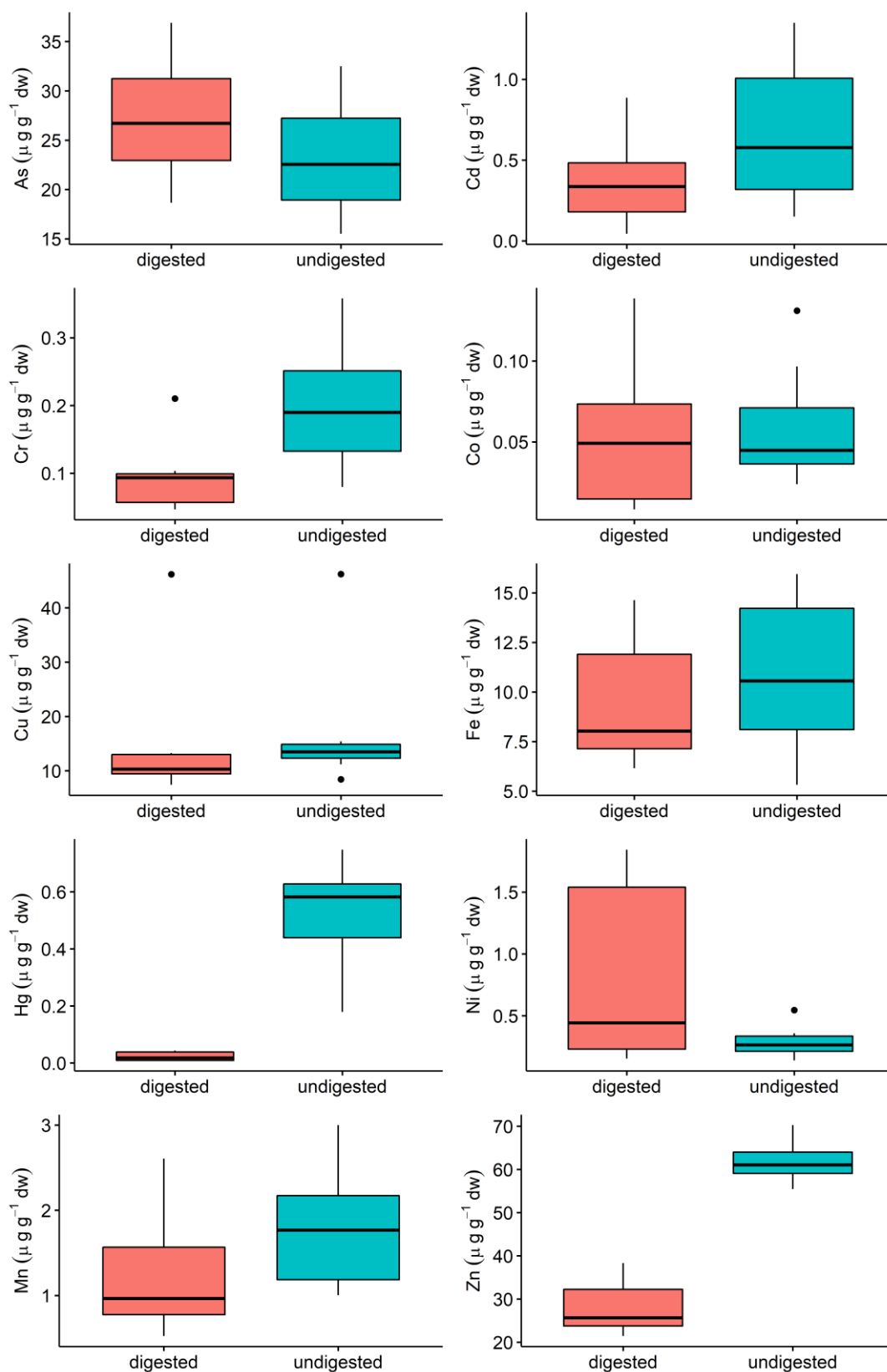
Appendix Table 5. Analysis of covariance (ANCOVA) table for the linear models fitted for the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the mantle tissue of *M. ingens* from the Chatham Rise, New Zealand. Df – degrees of freedom. Asterisks indicate the level of significance: \*  $p < 0.05$  \*\*  $p < 0.01$  \*\*\*  $p < 0.001$ .

	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		

## Appendix B: Supplementary Figures



Appendix Fig. 1. Interannual variation measured in chapter 2 between trace elements (Cd, Cr, Cu, Fe, Ni) mantle concentrations of *N. sloanii* from the Chatham Rise. Presented are the median and the 25% and 75% quantile



Appendix Fig. 2. Comparison of trace element concentrations in undigested (pink colour) and *in-vitro* digested samples (blue) of *N. sloanii*, Chatham Rise, NZ, from Chapter 3.