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SHORT NOTES

First reports of trace element bioaccumulation in the Antarctic deep-sea squid *Psychroteuthis glacialis*

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Abstract

Trophic interactions in the Antarctic Ocean are likely to be affected by changing environmental conditions. Some of these impacts can be observed, and predicted, by monitoring trace element concentrations in the tissues of animals at certain trophic levels. The 'glacial' squid (*Psychroteuthis glacialis*) is an ideal indicator species for measuring trace element bioaccumulation in the Ross Sea because it plays a central role in local marine food webs. Trace elements (Al, As, Cd, Co, Cu, Fe, Hg, Ni, Mn, Pb, U, V, and Zn) were measured in mantle and digestive gland tissues of 57 *P. glacialis* specimens, including juvenile and mature individuals. Significant differences in Al, As, Cd, Cu, Fe, Mn, V, and Zn concentrations were observed across life stages, with juveniles generally having the highest concentrations. As the bioaccumulation of most trace elements is influenced by diet, our results suggest different feeding patterns between juvenile and mature *P. glacialis*. In turn, it is likely that the life stage of *P. glacialis* individuals consumed by predators will determine trace element exposure higher up the trophic web. Overall, this Antarctic squid appears to be influenced by the trace element cycling in the Ross Sea and contains lower concentrations of trace elements than have been observed in squids in warmer waters.

Keywords Ross sea · Antarctic squid · Biomonitoring · Baseline studies · Bioindicators · Trace elements

Introduction

The Ross Sea is considered to be the most productive and biodiverse region of the Southern Ocean (Smith et al. 2012). Changing environmental conditions have impacted this pristine ecosystem over the past five decades through drastic sea-ice reduction and altered deep-sea circulation (Smith et al. 2012, 2014). Noticeable changes have been reported in the Ross Sea food web, including the foundational phytoplankton blooms (Orsi and Wiederwohl 2009), which are highly influenced by iron and the availability of other essential trace elements (Feng et al. 2010).

Trace elements are ubiquitous in the marine environment (Anderson 2020), although concentrations vary among oceanic regions, and some are influenced by depth (e.g., Hg (Choy et al. 2009) and Pb (Henderson and Maier-Reimer 2002). Sources of trace elements in the world's ocean can be either anthropogenic or naturally influenced (Tchounwou et al., 2012). The remote Southern Ocean is considered to be isolated from human inputs of trace elements. Several trace elements occur only at naturally low levels (e.g., Cu or Fe) within this region, and are a limiting factor for phytoplankton blooms (Grotti et al. 2008; Loscher et al. 1997; Martin et al. 1990), while others occur in very high concentrations (e.g., Hg, Cossa et al. 2011). These blooms, in turn, impact all trophic levels in the Ross Sea (Pinkerton and Bradford-Grieve 2014).

Some Southern Ocean marine invertebrates have been reported to have highly efficient trace element bioaccumulation rates (Cipro et al. 2018), resulting in anomalously high concentrations at surprisingly low trophic levels, as has been observed for Cd in crustaceans such as amphipods (e.g., Duquesne et al. 2000; Bargagli et al. 1996), and also cephalopods (e.g., Bustamante et al. 1998a), bivalves (Mauri et al. 1990) and fish (Bustamante et al. 2003). These high Cd concentrations are hypothesized to result from co-accumulation of the limiting essential elements such as Cu and

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Fe (Petri and Zauke 1993; Koyama et al. 2000; Bustamante et al. 2002). Cephalopods in particular have high bioaccumulation capacities. For example, Cd and Cu were reported in high concentrations in *Todarodes sagittatus* (Bustamante et al. 1998b), and these trace elements have recently been suggested as biomarkers of overall trace element availability in marine ecosystems (Seco et al. 2020). In particular, pelagic squids have been used as a proxy to assess Hg concentrations in different ecosystems including the Southern Ocean (Seco et al. 2020).

Cephalopods play an important role in the Ross Sea food web (Pinkerton et al. 2010). The deep-sea oegopsid 'glacial' squid *Psychroteuthis glacialis*, endemic to the waters south of the Antarctic polar front (Gröger et al. 2000), is an important prey item for a variety of predators such as seabirds—e.g., procellariiformes (Anderson et al. 2009), emperor penguins (*Aptenodytes forsteri*), and Adelie penguins (*Pygoscelis adeliae*, Offredo et al. 1985)—marine mammals—e.g., Weddell seals (*Leptonychotes weddellii*, Lake et al. 2003), elephant seals (*Mirounga leonina*, Daneri et al. 2000)—and Antarctic toothfish (*Dissostichus mawsoni*, Stevens et al. 2014). In light of its important trophic role, the present study is the first to investigate trace element concentrations in *P. glacialis*. Our specific aims were to: (1) analyze potential effects of sex (male, female) and maturity (juvenile, adult) on trace element concentrations; (2) assess trace element concentrations in both digestive gland and muscular mantle tissue; and (3) analyze whether squid size or sampling location may influence concentrations of trace elements.

Material and methods

Sample collection

Specimens of *Psychroteuthis glacialis* were collected by the Research Vessel *Tangaroa* (National Institute for Water and Atmospheric Research, Ltd [NIWA]) during one voyage (TAN1901) to the Ross Sea in January and February 2019. Samples were collected by bottom (demersal) trawls in depths of 600–1500 m and the sampling area ranged from 71°22′ to 76°02′ S and 169°13 to 177°14′ E (Fig. 1). In total, 57 individuals from 13 stations were analyzed for trace elements. The sample set consisted of 26 juveniles of undetermined sex (105–160 mm dorsal mantle length

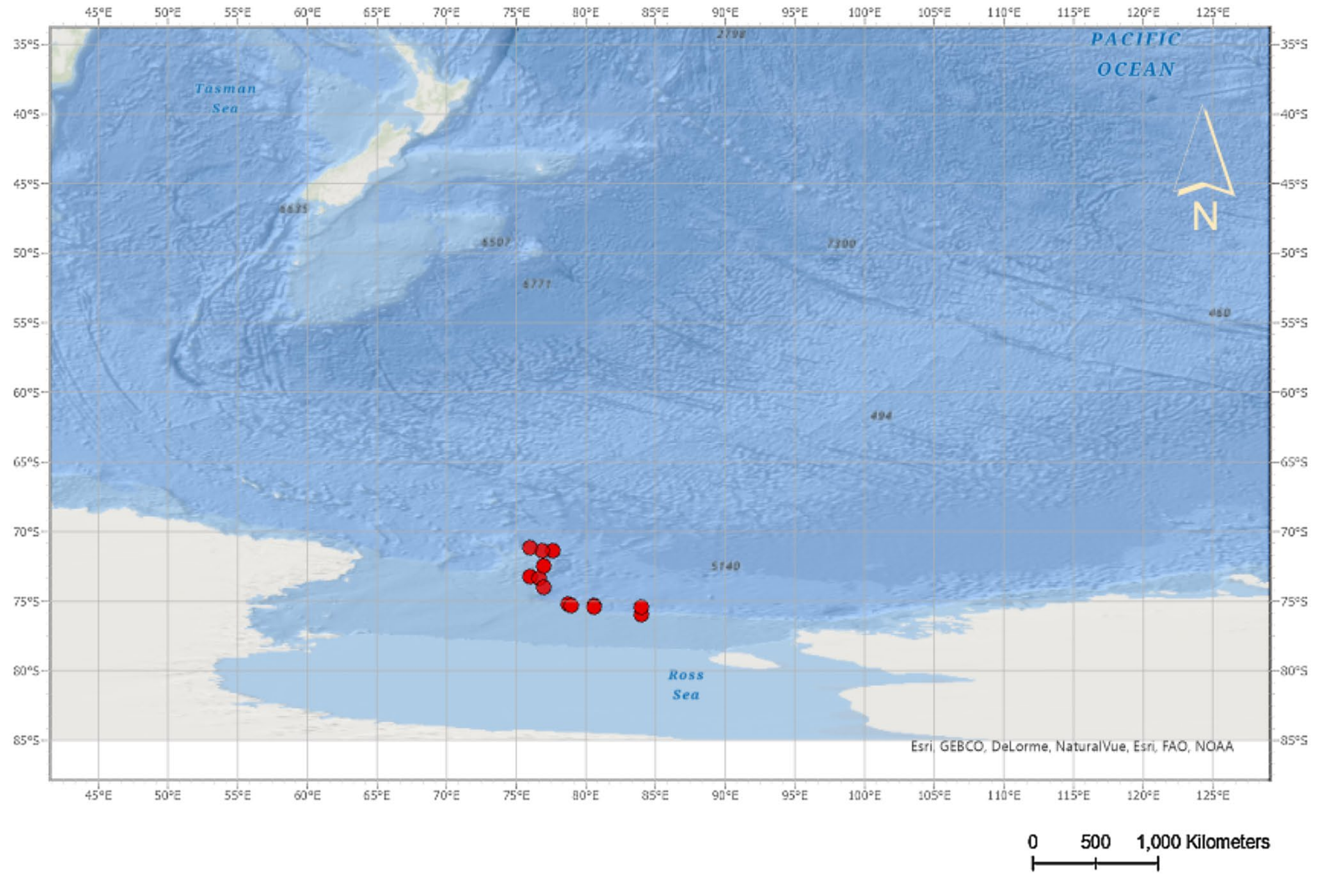


Fig. 1 Sampling locations of *Psychroteuthis glacialis* specimens collected in January 2019 (voyage TAN1901) in the Ross Sea, Antarctica

[DML]), 25 submature to mature females (143–405 mm DML), and 6 submature to mature males (144–335 mm DML). Specimens were stored frozen at –20° C until dissection and trace element analysis.

Trace element analysis

Digestive gland and mantle tissue samples were freeze-dried, homogenized with mortar and pestle, and ~ 100–300 mg dry weight (dw) of each sample was digested in a 3:1 mixture of 70% HNO_3 (Merck, suprapur quality) and 37% HCl (Merck, suprapur quality) in a microwave digestion system (Multiwave GO, Anton Paar GmbH, Austria) at 105 °C for 50 min. Following digestion, samples were diluted to a volume of 50 ml with Milli-Q water. Trace element analysis (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Mn, Pb, V, and Zn) was conducted by inductively coupled plasma mass spectroscopy (ICP-MS, Agilent Technologies 7500 series and Agilent 8900, CA, USA) at the University of Canterbury, Aotearoa New Zealand (Lischka et al. 2020a). The protocol for the Hg analysis followed Aldridge et al. (2017). 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). Uranium was analysed because of its potential relationship to sea-floor phosphorites (Baturin and Kochenov, 2001; Kolodny et al., 1970) and was measured by microwave plasma atomic emission spectroscopy (MP-AES 4200 Agilent Technologies, Australia) with a detection limit of 0.001 µg g^{-1} dw and a recovery rate for the certified reference material (QC1209; Sigma Aldrich) of 107%.

Detection limits (µg g^{-1} dw) were calculated as 3 × the mean standard deviation of the blanks and the solution concentration converted to a tissue concentration following Lischka et al. 2021a: Al (1.73), As (0.05), Cd (0.01), Co (0.01), Cr (0.77), Cu (0.52), Fe (1.70), Hg (0.020), Mn (0.10), Ni (0.10), Pb (0.04), Se (0.09), U (0.001), V (0.03), and Zn (1.27). With each ten samples, a blank and lobster hepatopancreas CRM (TORT-3, National Research Council, Canada, $n = 4$) was measured. Mean recoveries of trace elements ranged between 80 and 102%.

Chromium concentrations were removed from the analysis and manuscript as concentrations above 2 µg g^{-1} dw were frequently observed and are indicative of potential contamination of samples during sample storage or processing. We further removed Pb concentrations above 2 µg g^{-1} dw and Ni concentrations above 5 µg g^{-1} dw from the analysis since the observed values appear to be outliers based on a detailed comparison with other cephalopod trace element studies (e.g., Lischka et al. 2018; Lischka et al. 2021a, b).

Statistical analysis

Differences in trace element concentrations between the mantle and digestive gland tissue were visualized using principal component analysis (PCA) in R (Ihaka and Gentleman 1996; R Core Team 2017; package ‘ggbiplot’, Vu 2011). Prior to the PCA, concentration data were normalized and auto-scaled (mean centred and divided by the standard deviation). To test for relationships between trace elements, pairwise nonparametric Spearman correlations were applied (‘corr.test’ function of the ‘corrgram’ package, Wright 2012).

To detect whether trace element concentrations were influenced by tissue type, size, sex, or sampling location, generalised linear models (GLM) with a negative binomial distribution and the logit link function were applied in R (package ‘MASS’, Ripley et al. 2013). For each trace element, one model was fitted against non-transformed data. Variance homogeneity and distribution of the residuals was checked with diagnostic plots.

Results and discussion

Tissue distribution

Trace element concentrations of Co, Hg, and U did not vary significantly between mantle and digestive gland tissue (Tables 1, 2). While concentrations of Al, Fe, Mn, Ni, Pb, and Zn were significantly higher in the mantle tissue, As,

Table 1 Trace element concentrations (mean, standard deviation [sd], minimum, maximum) in digestive gland and mantle tissue of *Psychroteuthis glacialis* (µg g^{-1} dw)

	Digestive Gland		Mantle	
	mean ± sd	min– max	mean ± sd	min– max
Al	22.6 ± 26.4	4.25– 151	**31.6 ± 34.6**	11.3– 270
As	**7.83 ± 3.57**	1.57– 17.2	5.88 ± 2.68	2.31– 14.4
Cd	**19.0 ± 22.5**	2.83– 123	0.19 ± 0.32	0.01– 1.90
Co	0.23 ± 0.12	0.07– 0.55	0.12 ± 0.11	0.03– 0.54
Cu	**369 ± 220**	105– 1465	136 ± 243	12.0– 1245
Fe	45.4 ± 55.5	8.91– 374	**69.5 ± 59.8**	11.6– 309
Hg	0.17 ± 0.08	0.06– 0.47	0.13 ± 0.08	0.02– 0.49
Mn	3.29 ± 1.82	1.13– 11.4	**4.16 ± 2.08**	1.55– 9.97
Ni	**2.39 ± 1.32**	0.43– 4.78	2.1 ± 0.85	0.61– 4.03
Pb	0.12 ± 0.10	0.04– 0.53	**0.81 ± 0.51**	0.14– 1.95
U	0.03 ± 0.01	0.01– 0.08	0.02 ± 0.01	0.01– 0.06
V	**1.10 ± 0.76**	0.38– 3.92	0.23 ± 0.12	0.06– 0.84
Zn	56.0 ± 32.3	20.7– 256	**101 ± 62.3**	49.7– 370

Significantly higher concentrations (p-value ≤ 0.05) between the two tissues are highlighted in bold

Table 2 Generalised linear model (GLM) results for trace element models from tissues of *Psychroteuthis glacialis* specimens from the Ross Sea

	Al	As	Cd	Co	Cu	Fe	Hg	Mn	Ni	Pb	U	V	Zn
Tissue	***	***	***		***	***		*	***	***		***	***
Sex	**	**	***		***	***		***				*	***
DML						↓			*				
Location	***	***	***		*	***							

The *p*-values of the variables are shown according to likelihood ratio tests (*** 0.001, ** 0.01, * 0.05). Negative (↓) effects for the continuous variables dorsal mantle length (DML) are indicated by arrows

Cd, Cu, and V concentrations were significantly higher in the digestive gland tissue (Table 2; S.Fig. 1). This is comparable to other studies where higher concentrations of Cd and Cu were measured in the digestive gland (e.g., Bustamante et al. 2002; Lischka et al. 2018). The digestive gland is the known storage organ for Cd and Cu where detoxification processes take place (Penicaud et al. 2017). Therefore, higher concentrations can generally be expected than those observed in muscular mantle tissues.

Cadmium has been shown to bioaccumulate in cephalopods (Bustamante et al. 1998b); however, Cd concentrations have not been previously assessed in *P. glacialis*. In this study, mean Cd concentrations were 19 µg g^{-1} dw in the digestive gland and 0.19 µg g^{-1} dw in the mantle tissues (Table 1). These concentrations are lower overall than those reported in oegopsid species from other oceanic regions (e.g., Lischka et al. 2018; Table 3), such as *Sthenoteuthis pteropus* (Lischka et al. 2018) and *Todarodes sagittatus* (Bustamante et al. 2002). Cadmium concentrations fluctuate depending on the season in the Ross Sea (Corami et al. 2005), and Cd in surface waters is nearly depleted during the Antarctic summer months (Scarponi et al. 2000). It may be that Cd concentrations throughout the Ross Sea pelagic food web—especially during the austral summer—are accordingly lower, explaining the relatively low concentrations measured in *P. glacialis*. Comparative studies across

Table 3 Trace element concentrations for Cd (digestive gland [DG]) and Fe (mantle and digestive gland) reported to date in oegopsid squid species

Species	Cd DG	Fe Mantle	Fe DG	Sampling location	Study
Architeuthidae					
Architeuthis dux	65.8 ± 43.1			Bay of Biscay	Bustamante et al. 2008
Gonatidae					
Gonatus fabricii	35 ± 15	18.7	57.5	Western Greenland	Lischka et al. 2020a, b
Ommastrephidae					
Illex argentinus	1003 ± 566			Central South Brazil	Dorneles et al. 2007*
Illex coindetii	15 ± 5			Bay of Biscay	Bustamante et al. 2002
Nototodarus gouldi		15 ± 12	346 ± 231	New Zealand	Lischka et al. 2020a, b
Nototodarus gouldi	50 ± 25		745	South-East Australia	Smith et al., 1984
Nototodarus sloanii	111 ± 95	16 ± 9	186 ± 95	New Zealand	Lischka et al. 2020a, b
Ommastrephes bartrami	287 ± 202		399 ± 204	Southern California	Martin & Flegal 1975
Sthenoteuthis oualaniensis	782 ± 255		319 ± 67	Southern California	Martin & Flegal 1975
Sthenoteuthis oualaniensis	82		100	Ogasawara, Japan	Ichihashi et al. 2001
Sthenoteuthis pteropus	748 ± 279		431 ± 173	Eastern Tropical Atlantic	Lischka et al. 2018
Todarodes filippovae	246 ± 187	9.9 ± 4.8	92 ± 32	Indian Ocean	Kojadinovic et al. 2011
Todarodes filippovae	98.5 ± 67.2	9.7 ± 5.2	183 ± 105	Tasmania	Kojadinovic et al. 2011
Todarodes sagittatus	85 ± 37			Bay of Biscay	Bustamante et al. 2002
Todarodes sagittatus	18 ± 12			Bay of Biscay	Chouvelon et al. 2011
Onychoteuthidae					
Moroteuthopsis ingens (female)	53 ± 103	18 ± 27	233 ± 216	New Zealand	Lischka et al. 2020a, b
Psychroteuthidae					
Psychroteuthis glacialis	19 ± 23	**70 ± 60**	45 ± 56	Ross Sea	This Study

Concentrations are shown as the mean ± the standard deviation. All concentrations are in µg g^{-1} dry weight

*Converted from wet weight

different seasons would be helpful in understanding seasonal Cd fluctuations in marine organisms, but sampling of Antarctic cephalopods during the winter months remains challenging.

Iron, an essential element, is considered a limiting factor in the Southern Ocean (De Baar et al. 1995). In this study, Fe and Zn were measured at higher concentrations in the mantle compared to the digestive gland tissue (Tables 1, 3). The higher concentrations of Fe in the mantle compared to the digestive gland tissues contrast with findings for *Architeuthis dux* (Bustamante et al. 2008), *Moroteuthopsis ingens* (Lischka et al. 2020a), and *Todarodes filippovae* (Kojadinovic 2011). Iron has been reported to transfer from digestive gland tissues to mantle tissues in the squid *Doryteuthis patagonica* during freeze-thawing processes (Falandysz 1989). Since the squids analyzed in this study were freshly dissected, fluctuations between tissues are unlikely influenced by squid processing. It may be that the lower iron availability in the Southern Ocean, especially during the summer months, might influence tissue migrations in *P. glacialis* (Olson et al. 2000). However, the tissues analyzed in this study provide a single snapshot in time and further bi-annual monitoring is needed. Higher Zn concentrations in the mantle tissue compared to the digestive gland tissue were observed in other oceanic squid species, e.g., *M. ingens* (Lischka et al. 2020a) and *Gonatus fabricii* (Lischka et al. 2020b). This distribution pattern could be explained by a competition of Cd and Zn for binding sites in the digestive gland, leading to co-accumulation of Zn and a redistribution to muscular tissues (Lischka et al. 2020a).

Influence of sex and maturity on trace element concentrations

Concentrations of Al, As, Cd, Mn, V, and Zn were higher in juvenile than in mature male and female specimens (Fig. 2a–d, Table 2). Cadmium, Ni, Pb, and Zn have also been reported at higher concentrations in juvenile *Gonatus fabricii* than in mature specimens (Lischka et al. 2020b; Gerpe et al. 2000). It could be that dietary differences between juvenile and mature *P. glacialis* contribute to differences in trace element concentrations. Juvenile squids tend to have a more crustacean-rich diet compared to adult squids which mainly prey on fish (Bustamante et al. 1998b; Kear 1992). Since crustaceans are not known to regulate their intake of non-essential elements (unlike fishes), higher concentrations can be expected (Duquesne et al. 2000) and might contribute to the differences observed between juvenile and mature *P. glacialis*. Apart from dietary changes, changes in habitat might also influence those maturity related concentrations differences. Future studies focussing on the diet of *P. glacialis* in the Ross Sea might identify dietary preferences and feeding habits.

Mercury concentrations

The Hg concentrations measured in mantle (0.13 ± 0.08 μg g^{-1} dw) and digestive gland tissues (0.17 ± 0.08 μg g^{-1} dw; Table 1) were comparable to previously measured Hg concentrations in muscle tissues of this species (e.g., 0.18 ± 0.11 μg g^{-1} dw, Anderson et al. 2009). No relationship was observed between mantle length and Hg concentrations in this study.

While the present study focusses on trace elements in *P. glacialis* as a study organism, previous studies focussed on Hg in Antarctic seabird colonies and analysed *P. glacialis* Hg concentrations mainly in bird regurgitates (Anderson et al. 2009) or in comparison to other Antarctic cephalopods (Seco et al. 2020). In the latter study, as in our present findings, Hg concentrations did not differ between *P. glacialis* mantle and digestive gland tissues, but the overall concentrations of Hg were lower compared to this study (0.024 ± 0.021 μg g^{-1} dw; Seco et al. 2020). Differences between these two studies could be driven by sampling location or temporal fluctuations. Bioavailable mercury concentrations can vary among different Antarctic regions based on productivity, bathymetry, and temperature differences (Mason and Fitzgerald 1993; Cossa et al. 2011; Brasso and Polito 2013). For example, depending on the environmental factors, mercury “hot spots” have been previously reported from various regions of the Antarctic Ocean (Brasso and Polito 2013; Zheng et al. 2015). Future monitoring of Hg concentrations in Antarctic cephalopods may improve our understanding of mercury cycling within various regions of the Ross Sea.

Conclusion

This study provides the first comprehensive baseline data on trace element concentrations in an abundant mid-water cephalopod species of the Ross Sea. Juveniles generally had higher concentrations of trace elements than were observed in mature individuals. Overall, the trace element concentrations measured in this study were lower than have been reported in cephalopods from tropical and subtropical waters. In this study, Fe concentrations were lower than those reported in cephalopods from other oceanic regions, reflecting the generally low bioavailability of this element in the Ross Sea. Future studies should focus on Fe concentrations and compare the low Fe concentrations observed in this study to top predator concentrations, to improve our understanding of iron cycling in such naturally low parts of the ocean. If possible, samples should also be collected and analysed from other seasons, to investigate potential temporal variability in the trace element concentrations observed herein.

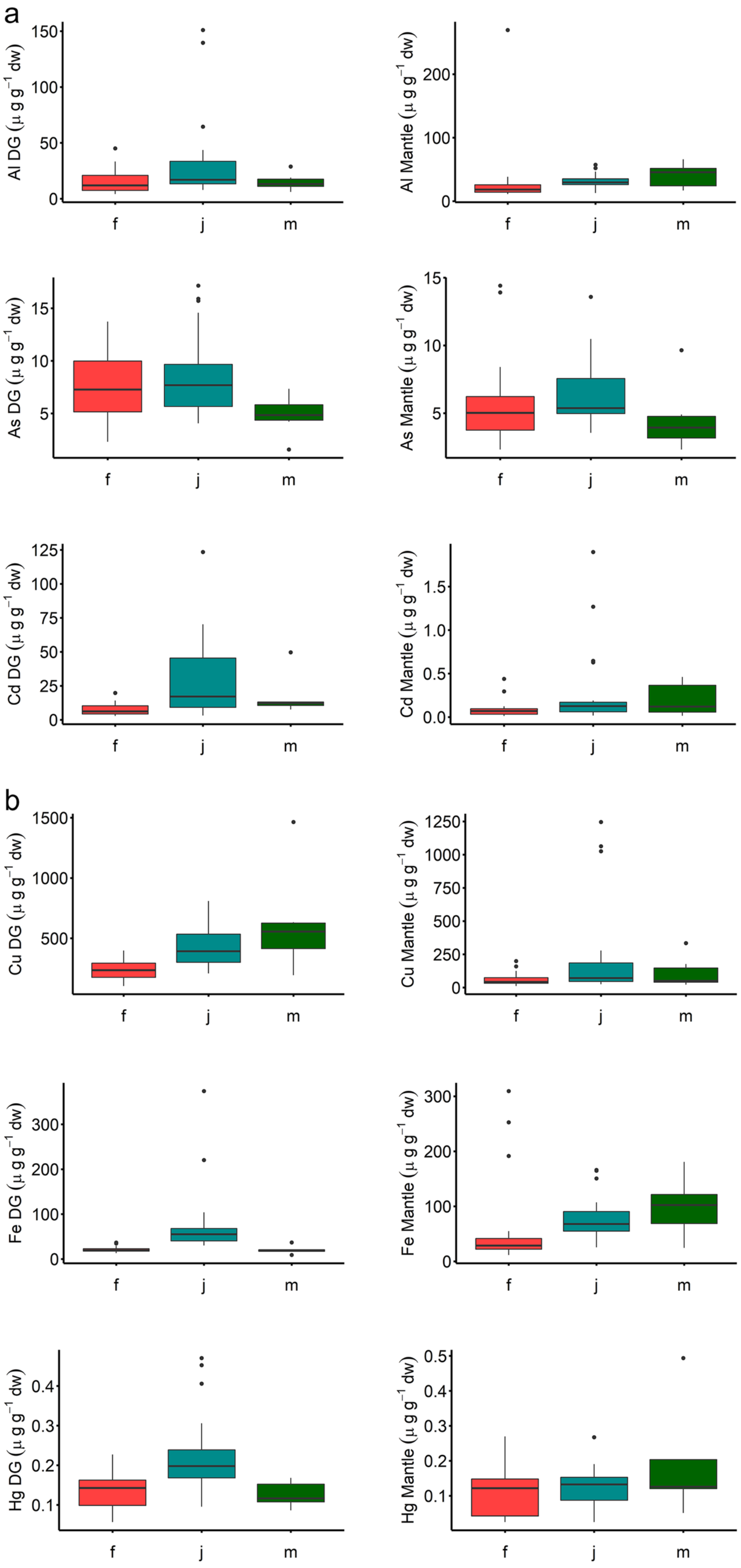
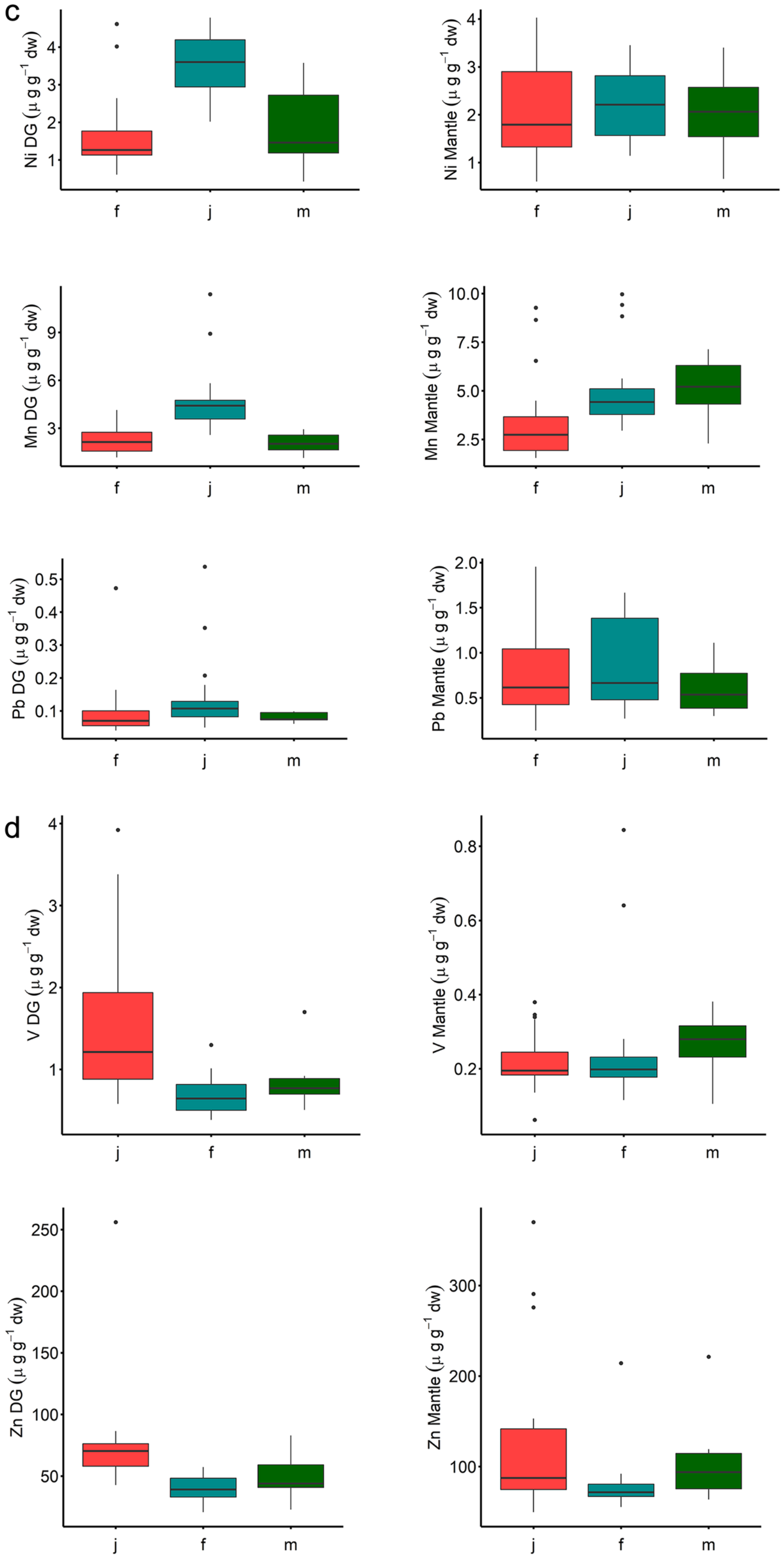


Fig. 2 Trace element concentrations in the digestive gland (DG) and mantle tissue of unsexed juvenile (J), and mature female (F) and male (M) *P. glacialis* specimens. The boxplots present the mean concentration as well as the 25th and the 75th percentile. 26 juveniles, 25 females and 6 males were included in this study

Fig. 2 (continued)



The data presented in this study will be important for future comparisons and for mapping trace element concentrations in Southern Ocean cephalopods, which are crucial members of Antarctic food webs. A comparison of different cephalopods from Antarctic waters would be useful in continuing to investigate trace element cycling in the Southern Ocean. This could help in identifying areas with high concentrations, enabling an assessment of potential contamination of different Antarctic habitats.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00227-023-04304-2.

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Authors' contribution statement All authors contributed to the study conception and design. Squid dissection, data collection and analysis were performed by Alexandra Lischka. All authors contributed to the drafting of the manuscript.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. All applicable international and national guidelines for sampling of cephalopods for the study have been followed and all necessary approvals have been obtained. The sampling conducted by the National Institute of Water and Atmospheric Research, Ltd (NIWA) was approved by the Ministry of Primary Industries (MPI) through an 'Antarctic Marine Living Resources' (AMLR) permit. An import permit as well as a Biosecurity clearance certificate (BACC number: B2019/52551) were also issued. The trace element study was funded by Auckland University of Technology (AUT) as well as by University of Canterbury.

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