Trophic ecology drives trace element concentrations in the Antarctic octopod community

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HIGHLIGHTS

- Stable isotope values vary among species with *Adelieledone polymorpha*, having highest values.
- Variations in trace element concentrations suggest different feeding ecology.
- Antarctic incirrate octopods are likely vectors of As, Cd and Hg to their predators.
- Sex influences Cd, Co, Cu, Fe, Pb and Zn concentrations.

GRAPHICAL ABSTRACT
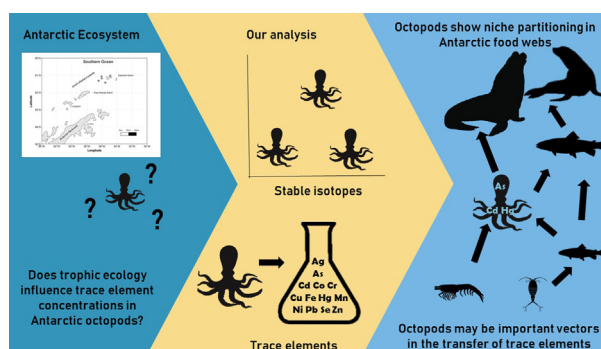
ABSTRACT

Despite the Antarctic Ocean being considered a pristine environment, elevated trace element concentrations have been reported in many marine organisms. The Antarctic Ocean is particularly vulnerable to climate change, which can also affect the bioaccumulation of trace element concentrations in biota. While Antarctic octopods are key components of the regional food webs as prey for a variety of predators (e.g., seals, fish, and seabirds), their contamination state by trace elements remains largely unknown. This study investigated the trace element concentrations in relation to the trophic ecology in Antarctic octopods. Stable isotope values (δ^{13}C and δ^{15}N) and trace element concentrations (Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V, and Zn) were measured in eight different species (*Adelieledone polymorpha*, *Pareledone aequipapillae*, *P. albimaculata*, *P. aurata*, *P. charcoti*, *P. cornuta*, *P. felix*, and *P. turqueti*) sampled near Elephant Island, close to the Antarctic Peninsula. Stable isotopes of δ^{15}N varied among species, with significant differences between *A. polymorpha* and *P. aurata* suggesting potential niche segregation. Trace element concentrations also differed among species and with sampling depth, which likely reflects their trophic ecology. The data presented in this study provides the first insight into the trace element concentrations for these endemic octopods in this vulnerable habitat and their stable isotope values.

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1. Introduction

Antarctica is recognized for its stable environment, sheltering numerous endemic species, and unique geography, which makes it especially susceptible to climate change as highlighted by increasing temperatures (Gille, 2002) and CO_2 saturation. It is the southernmost

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continent and contains 90% of Earth's ice masses, which make up about 70% of Earth's freshwater reservoir (Bentley and Giovinetto, 1992). The surrounding Antarctic Ocean plays a crucial role in Earth's oceanic circulation by connecting three main oceans— the Atlantic, Indian, and Pacific Oceans—and contains up to 6% of the world's oceanic volume (Eakins and Sharman, 2010). In addition, the high levels of atmospheric transport and the connectivity of ocean currents make the Antarctic Ocean susceptible to inorganic pollutants (Potapowicz et al., 2019). Elevated concentrations of trace elements (in particular Cd and Hg) have been reported in Antarctic crustaceans (Keil et al., 2008; Petri and Zauke, 1993), fish (Bargagli et al., 2000; Bustamante et al., 2003; Goutte et al., 2015), seabirds (Blévin et al., 2013; Carravieri et al., 2013, Carravieri et al., 2014), and marine mammals (Andrade et al., 2007; Honda et al., 1987). For Subantarctic and Antarctic cephalopods, some studies demonstrated moderate Hg levels and very high Cd concentrations (Bustamante et al., 1998b; Matias et al., 2019; Trevizani et al., 2018; Seco et al., 2020). Global cephalopod studies have demonstrated their remarkable bioaccumulation potential for trace elements, such as Ag, Cd, and Hg (Bustamante et al., 2006; Martin and Flegal, 1975; Miramand and Bentley, 1992). Although the trace element concentrations in benthic octopods from temperate waters have been previously studied (e.g., Chouvelon et al., 2011; Raimundo et al., 2005; Rjeibi et al., 2014), comparative data from the Southern Ocean is lacking for most benthic octopods.

Cephalopods are key components of the Antarctic ecosystem (Collins and Rodhouse, 2006; Xavier et al., 2018). Benthic octopods have a role as both predator and prey, and are crucial in the transfer of energy and trace elements in the Antarctic food web, linking low trophic level consumers to high-level predators (Allcock, 1997; Allcock, 2005; Allcock et al., 2001; Daly, 1996; Piatkowski et al., 1998, 2003; Strugnell et al., 2017). A wide variety of predators feed on benthic octopods, for example: elephant seals (*Mirounga leonine*, Burdman et al., 2015; Daneri et al., 2000; Rodhouse et al., 1992), Weddell seals (*Leptonychotes weddellii*, Acevedo et al., 2015; Casaux et al., 1997; Lipinski and Woyciechowski, 1981; Negri et al., 2016), Patagonian toothfish (*Dissostichus eleginoides*, Xavier et al., 2002), and the black-browed albatross (*Thalassarche melanophris*, Xavier and Croxall, 2007). In turn, benthic octopods prey on crustaceans, polychaetes, bivalves, gastropods (Daly, 1996), and amphipods (Daneri et al., 2000; Piatkowski et al., 2003). Clear differences in the feeding ecology among octopod species were proposed based on the interspecific variation observed in beak morphology (Matias et al., 2019; Schwarz et al., 2019) and stomach content analyses (Büring, 2019; Daly, 1996; Piatkowski et al., 2003). These differences suggest that Antarctic benthic octopods occupy different trophic niches, despite their geographic co-occurrence.

The purpose of the present study is to investigate the role that the eight benthic octopod species, collected from near Elephant Island, might play in the transfer of trace elements in this pristine Antarctic ecosystem. We tested two hypotheses: 1) we expect to see differences between individuals of different sizes, due to ontogenetic shifts in feeding, as well as between the mantle and digestive gland tissues, the two main storage organs for trace elements in cephalopods. Furthermore, the relative trophic position of each octopod species (assessed through the δ^{15}N in mantle as a proxy) and their feeding habitat (δ^{13}C in mantle) should differ at the intraspecific and interspecific levels; and 2) we expect to see niche partitioning reflected by interspecific differences in the stable isotope values. The trophic ecology is supposed to mainly drive the trace element contamination differences recorded in individuals' tissues.

2. Material and methods

2.1. Sample collection

Specimens were collected during a research cruise of the German research vessel 'Polarstern' (ANT-XXVIII/4) in March/April 2012 near Elephant Island, South Shetland Islands (Lucassen, 2012). The sampling area ranged from 61°04′ to 61°37′ S and 55°01 to 56°17′ W. Samples were caught in depths of 109–323 m by bottom otter trawling (OTB, Fig. 1; Table 1). A total of 60 individuals from eight different species (Table 1) were subsampled from approximately 800 collected specimens in total (Lucassen, 2012). For each specimen, the sampling depth (m), the mantle and total length (mm), and the sex (male, female, or sex unknown [indet./juvenile]) was noted. Specimens were stored at −40 °C until they were analyzed for trace elements, stable isotopes, and genetics.

2.2. Species identification

Specimens were initially morphologically identified to species level on board of the research vessel, and identifications were verified genetically for specimens that could not be confidently identified to species. When an identification was uncertain, a tissue snip was taken from the arm and stored frozen for later DNA analysis. DNA was extracted using EconoSpin columns (Epoch Life Science) with QIAGEN reagents, following protocols for the DNeasy Blood & 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 PCR (Macrogen, Korea). Sequences were edited in CodonCode Aligner (CodonCode Corp., Dedham, MA, USA) and uploaded to the Barcode of Life Data System (Ratnasingham and Hebert, 2007) 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.

2.3. Stable isotope analysis

Carbon and nitrogen stable isotopes were measured from freeze-dried tissue samples (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) following Lischka et al. (2018). Stable isotope values were calculated using the following formula: δ^{13}C or δ^{15}N = [(R$_{sample}$/R$_{standard}$) − 1] × 10^3, where R is ^{13}C/^{12}C or ^{15}N/^{14}N; all results are expressed in the ‰ unit notation as a deviation of the standard (Vienna Pee Dee Belemnite for δ^{13}C and N$_2$ in air for δ^{15}N). Internal laboratory standards (acetanilide and peptone) were used to assess the analytical precision, which was <0.10‰ for δ^{13}C and <0.15‰ for δ^{15}N.

2.4. Trace element analysis

Digestive gland and mantle tissue samples were freeze-dried for 48–72 h and ground into a homogenous powder. The water content in the octopod species (measured in *P. charcoti*, as an example) was 68± 8% in the digestive gland, and 79±3% in the mantle tissue. Sample aliquots were prepared with ~200 mg dry weight (dw) of the tissue samples and digested overnight in a 3:1 mixture of 65% HNO$_3$ (Merck, suprapur quality) and 37% HCl (Merck, suprapur quality). The acidic digestion was followed by a mineralization, where samples were heated for 30 min in a Milestone microwave (maximum temperature of 105 °C). Trace element concentrations (Ag, As, Cd, Co, Cr, Cu, Fe, Mn, 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 by optical emission spectroscopy (ICP-OES)—with a Varian Vista-Pro ICP—following Lucia et al. (2016). Dogfish liver (DOLT-4, National Research Council, Canada), lobster hepatopancreas (TORT-3, NRCC), and clam muscle tissue (IAEA-461, International Atomic Energy Agency, Austria) were used as certified reference materials (CRMs). CRMs, together with procedural blanks, were treated and analyzed in the same way as the samples. Recoveries of the elements ranged from
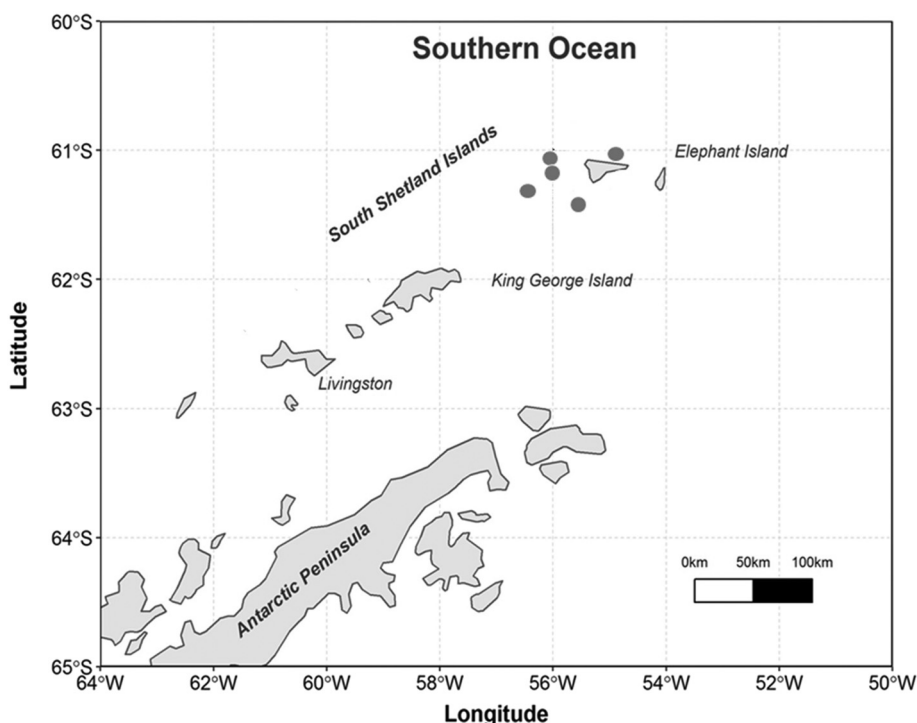
Fig. 1. Sampling locality of octopod specimens used in this study (grey circles), adapted from Schwarz et al., 2019.

85 to 105% (n=9). The detection limits for Ag, Cd, Co, Cr, and Pb were 0.025 µg g^{-1}, Ni was 0.05 µg g^{-1}, Cu, Mn, and Se were 0.125 µg g^{-1}, As was 0.25 µg g^{-1}, V was 0.5 µg g^{-1}, and Fe and Zn were 5 µg g^{-1}, and based on 200 mg of sample material diluted in a volume of 50 ml. The Cd concentrations was only measured in the octopod digestive gland tissue and not in the mantle tissue due to a potential diffusion effect (Francesconi et al., 1993; Lischka et al., 2020a).

Sample aliquots (1–2 mg) of the dried homogenized digestive gland and mantle tissue were used to analyse Hg using an Advanced Mercury Analyser (ALTEC AMA 254, with a detection limit > 0.05 ng) as described in Bustamante et al. (2006). For every 10 samples, one standard sample of certified reference material DOLT 5 (Dogfish liver; NRCC) was analyzed with a recovery of 109 ± 2%. Results for all trace element concentrations are expressed in µg g^{-1} dw.

2.5. Statistical analyses

All statistical analyses were conducted with the statistical software R (R Core Team, 2017; Ihaka and Gentleman, 1996). The species *Pareledone felix* (n=2) and *P. turqueti* (n=1) were excluded from all statistical analysis and the interspecific comparisons due to their small sample sizes.

Analysis of covariance (ANCOVA) was performed on log-standardised δ^{15}N and δ^{13}C values to check if these were influenced by size (total length), sampling depth, sex (female, sex unknown [indet./ juvenile], male), and species.

Interspecific differences in stable isotope values were assessed with Tukey's post-hoc tests ('glht' function, R package 'multcomp', Hothorn et al., 2016). Adjustment of the p-values was conducted following Benjamini and Hochberg (1995).

A principal component analysis (PCA) was applied as an exploratory tool to examine differences in overall trace element concentrations among species for the digestive gland tissue. Prior to the PCA, data was normalised and z-transformed (auto-scaled, mean centred, and divided by the standard deviation).

To test whether tissue type, sampling depth, species, size (as total length), "sex", δ^{15}N and the residuals of δ^{13}C had an influence on trace element concentrations, generalised linear models (GLMs) with a negative binomial distribution and logit link function were applied (GLM, package 'MASS', Ripley, 2011). The fit of the models was confirmed by analysing the residuals. One model per trace element was fitted against non-transformed concentrations and the variables were added sequentially. Since the variables δ^{13}C and δ^{15}N were correlated (Pearson's r = 0.71), only the residuals of the correlation between δ^{13}C and δ^{15}N

Table 1

Specimen data for the eight octopod species included in this study. Acronyms are defined as follows: sample size (n), mean ± standard deviation (SD), mantle length (ML; mm), total length (TL; mm), and number of sex indetermined (Juvenile, J), female (♀) and male (♂) specimen.

| Species	Sampling Depth (m)	n	ML Mean ± SD	ML min-max	TL Mean ± SD	TL min-max	J	♀	♂
Adelieledone polymorpha	293–323	15	75.1 16.9	50–109	175.1 43.9	110–265		7	8
Pareledone aequipapillae	295–320	4	38.5 ± 4.3	33–44	86.8 19.1	67–110	2	2	
Pareledone albimaculata	295–320	5	45.4 21.1	24–82	101.6 21.7	70–128		4	1
Pareledone aurata	150–178	7	58.4 26.2	28–100	142.4 ± 43.4	84–213		4	3
Pareledone charcoti	109–118	22	49.3 7.7	35–63	112.7 13.1	89–145		11	11
Pareledone cornuta	288–307	4	46.2 6.3	38–54	104.0 18.1	80–127	1		3
Pareledone felix	288–307	2	49.5 17.9	34–65	125.0 63.5	70–180	1	1	
Pareledone turqueti	288–307	1	46		112				1
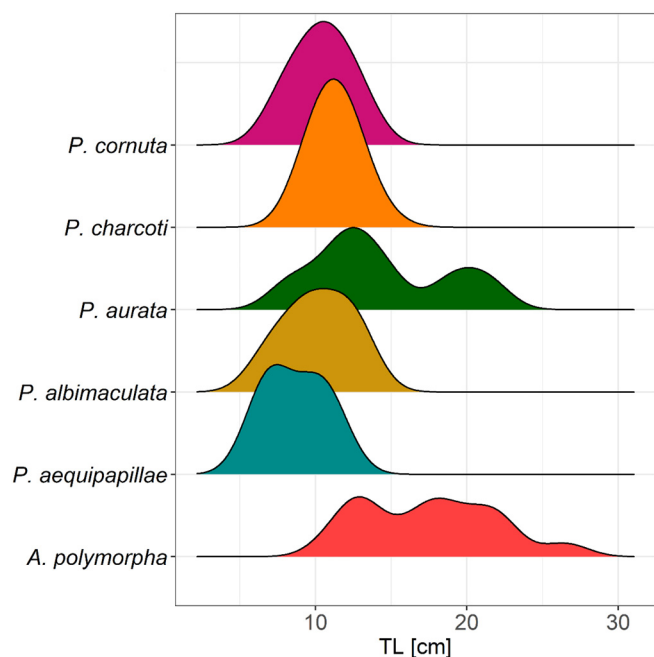
Fig. 2. Frequencies of sizes for the analyzed octopod specimens analyzed in our study (only including species with $n > 3$) from near Elephant Island, Antarctic Ocean.

were included in the models. Because size is affected by species, these variables were included as an interaction term in the models.

ANCOVAs were performed to see whether "sex", size, and/or stable isotopes had an influence on trace element concentrations for *P. charcoti*, because this species had a balanced sample size (11 females, 11 males). This model was chosen because the residuals of the *P. charcoti* dataset were normally distributed.

3. Results

3.1. Species identifications

Sixty specimens were morphologically identified to the species level (*Adelieledone polymorpha, Pareledone aequipapillae, P. albimaculata, P. aurata, P. charcoti, P. cornuta, Pareledone felix,* and *P. turqueti*; Table 1).

Table 2
Analysis of covariance (ANCOVA) for the linear models fitted to the δ^{13}C and δ^{15}N values of the mantle tissue from the different species used in this study. Abbreviations are defined as follows: TL = total length, Df = degrees of freedom, and 'depth' the sampling depth. Df – degrees of freedom.

	Df	Sum of squares	Mean square	F value	Significance
δ^{13}C					
Depth	1	18.22	18.22	61.55	***
Species	8	42.52	5.31	20.72	***
Size (TL)	1	0.16	0.16	0.63	
Sex	2	1.89	0.94	3.68	*
δ^{15}N	1	2.39	2.38	9.30	**
Residuals	47	12.05	0.26		
δ^{15}N					
Depth	1	45.33	45.33	372	***
Species	8	52.02	6.50	53.72	***
Size (TL)	1	0.11	0.11	0.93	
Sex	2	0.02	0.01	0.09	
δ^{13}C	1	1.16	1.16	9.56	**
Residuals	47	5.69	0.12		

* $p < 0.05$ level of significance.
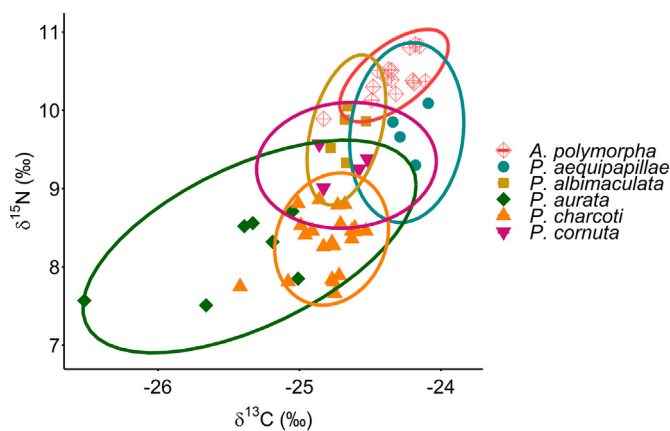** $p < 0.01$ level of significance.
*** $p < 0.001$ level of significance.

Fig. 3. Carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope values (‰) in eight octopod species from the genera *Adelieledone* and *Pareledone* collected near Elephant Island, Antarctic Ocean. Ellipses indicate the 95% confidence interval around the species groupings.

Among them, the identification of 14 specimens were confirmed with DNA barcoding, confirming a total of eight species (99.83–100% match in the BOLD database). The morphological species identification (size variations, Fig. 2) suggests that *P. aurata* and *P. albimaculata* might include more species than confirmed with DNA barcoding.

3.2. Stable isotopes

The δ^{13}C and δ^{15}N values varied significantly among the species (ANCOVA, $p < 0.001$; Table 2), indicating that the foraging habitats and diets differ among subantarctic octopods. On average, the highest δ^{13}C values were measured in *A. polymorpha*, *P. aequipapillae*, *P. felix* and *P. turqueti*, whereas *P. albimaculata, P. charcoti* and *P. cornuta* had similar (or overlapping) values, and *P. aurata* showed lowest δ^{13}C values (Fig. 3; Table S1). Statistically, only the δ^{13}C values of *A. polymorpha* and *P. aurata* differed significantly in the Tukey's post-hoc tests ($p < 0.001$). In addition, sex had a significant effect on δ^{13}C (Table 2), with males and juveniles exhibiting the highest values ($p < 0.05$, Table 2). Regarding the δ^{15}N values, *A. polymorpha* exhibited the highest average value (Table S1), followed by *P. aequipapillae*, *P. albimaculata*, *P. felix,* and *P. turqueti* (Fig. 3). Lower average δ^{15}N values were measured in *P. cornuta,* but the lowest δ^{15}N values were recorded in 29 individuals of *P. aurata* and *P. charcoti* (which differed significantly from all other species; Tukey's post hoc test, $p < 0.001$). Both δ^{13}C and δ^{15}N values are positively correlated (Pearson's r=0.71; $p < 0.05$) indicating that the highest δ^{13}C values were measured in specimens with the highest δ^{15}N values (Table 2). In addition, sampling depth had a significant effect on δ^{13}C and δ^{15}N values (Table 2), with the lowest δ^{13}C values and the highest δ^{15}N values measured in specimens sampled in deeper waters.

3.3. Trace elements

Average trace elements in the digestive gland were measured in decreasing order as follows: Fe> Cu> Zn> Cd> V> As> Ag> Se> Co> Mn> Ni> Pb> Cr> Hg (Table S1). In the mantle tissue, the average trace element concentrations decreased as follows: Zn> Cu> As> Fe> Se> V> Mn> Ni> Ag> Co> Hg> Cr> Pb (Table S1).

The PCA of the digestive gland tissue showed that Principal Component (PC) 1 explained 38.6% of the variance and was mainly driven by Ag, Cd, Co, Cu, Fe, Hg, and Pb, whereas PC2 explained 18.1% of the variance and was mainly driven by As, Cr, Mn, Ni, Se, and V (Fig. 4).

The specimens of *P. charcoti* formed a distinct cluster along the PC1 influenced by highest Ag, Cd, Co, Cu, Pb, and Zn concentrations recorded
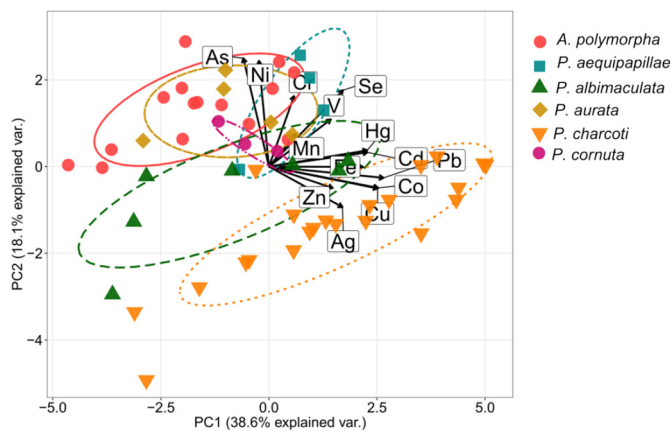
Fig. 4. Principal component analysis (PCA) of the digestive gland trace element concentrations of six octopod species of the genera *Adelieledone* and *Pareledone* collected near Elephant Island, Antarctic Ocean. Due to the low sampling number, *P. felix* and *P. turqueti* were excluded from the PCA.

in this species (see Table S1). The samples of *A. polymorpha*, *A. aequipapillae* and *A. albimaculata* formed a distinct group, characterised by higher As, Cr, and Ni concentrations. Overall, *P. albimaculata* displayed the highest concentrations of As and V (also supported by the GLMs, Table 3), *P. aequipapillae* the highest ones of Cr, Fe, Hg, and Mn (not confirmed by the GLMs), and *A. polymorpha* the highest Ni and Se concentrations (Table S1; supported by the GLMs, Table 3).

The GLMs analyses confirmed that the concentrations of As, Cd, Co, Fe, Ni, Pb, Se, V, and Zn significantly differed among species (Table 3) and that all concentrations, with the exception of As and Hg, are higher in the digestive gland compared to the mantle tissue. Arsenic tended to be more concentrated in the muscle than in the digestive gland in all species, whereas the Hg concentrations remained similar between the two tissues (Table S1). Sex had an impact on Cd, Co, Cu, Fe, and Pb concentrations in both tissues, with juveniles (sex indet.) and males generally exhibiting higher concentrations relative to females (Table 3, Fig. S1). Finally, the concentrations of Cu (Fig. S2) and Zn decreased significantly with increasing size, while Se significantly increased with size (Table 3).

The stable isotope values of $\delta^{15}N$ had a significant positive effect on Ag, As, and Se concentrations and a significant negative effect on Cd concentrations (Table 3). None of the analyzed variables had an effect on Hg concentrations. The residuals of $\delta^{13}C$ did not show a significant effect on any of the analyzed trace elements.

Table 3

Output of the generalised linear models (GLMs) for variables that significantly influence the trace element concentrations of the analyzed octopod species. Species included were: *A. polymorpha*, *P. aequipapillae*, *P. albimaculata*, *P. aurata*, *P. charcoti*, and *P. cornuta*. Negative ($\downarrow$) and positive ($\uparrow$) effects for the continuous variable size (total length [TL]) are indicated with arrows ($\uparrow\uparrow\uparrow = 0.001$, $\uparrow\uparrow = 0.01$, $\uparrow = 0.05$). For Cd, tissue type was not included in the GLM because it was not analyzed (NA). Models for each element included $\delta^{15}N$ and the residuals (Resid) of $\delta^{13}C$.

	Ag	As	Cd	Co	Cr	Cu	Fe	Hg	Ni	Pb	Se	V	Zn
Tissue type	***	***	NA	***	*	***	***		***	***	***	***	***
Species		***	***	**		*	***		***		**	***	***
Depth		***		***			***		***	**	***	*	
Size (TL)						$\downarrow\downarrow\downarrow$					$\uparrow$		$\downarrow$
Sex			***	***		**	*			*			
$\delta^{15}N$	$\uparrow$	$\uparrow\uparrow\uparrow$	$\downarrow$								$\uparrow$		
Resid ($\delta^{13}C$)													
Species × TL		**		*									

The p-values of the variables are shown according to likelihood ratio tests.
*** 0.001.
** 0.01.
* 0.05.

Sampling depth had a significant effect on Co, Fe, Ni, Pb, Se, and V concentrations (Table 3), which were highest in specimens sampled in shallower waters. In contrast, As concentrations reached the highest concentrations in individuals collected in deeper waters (Table 3).

3.4. Pareledone charcoti

Focusing on the sample set of *P. charcoti* ($n=11$ males and 11 females), the concentrations of Ag, Co, Cr, Cu, Fe, Ni, Pb, Se, V, and Zn differed significantly between digestive gland and mantle tissue, with highest concentrations measured in the digestive gland (Table S2). Significantly higher concentrations of Cd, Co, Cr, Cu, Fe, Ni, Pb, Se, and Zn were also measured in males (Table S2; Fig. S2) compared to females. The concentrations of Hg and Zn increased with increasing $\delta^{15}N$ values ($p < 0.05$; Table S2).

4. Discussion

Although cephalopods, particularly benthic octopods, are a key component of the Antarctic food web, little is known about their trophic ecology in the Southern Ocean (Seco et al., 2020). In this study, we report the stable isotope values and the concentrations of 14 trace elements in eight different octopod species sampled near Elephant Island. For the first time, stable isotopes and trace elements are linked together to describe niche segregation by feeding and habitat tracers, in these endemic octopod species. Our study demonstrated that shallower living octopods are distinguished from deeper living species by their habitat and diet, and that reflected on their isotopic signature and trace elemental burden. This clear segregation may have ecological repercussions for predators that feed on different species or that hunt at different depths.

4.1. Trophic ecology

The $\delta^{15}N$ signature is a reliable indicator of the trophic position in specific food webs (Cherel et al., 2009; Richert et al., 2015). A relative comparison of this proxy among the species the Antarctic and Subantarctic octopus assemblages investigated here reveals that *Paraeledone aurata* and *P. charcoti* are distinguished from the other species by their low $\delta^{15}N$ values (Fig. 3), suggesting a lower trophic position. These results are consistent with those from a recent study on the feeding ecology of Antarctic octopods from the same area, which reported higher $\delta^{15}N$ values in *A. polymorpha* and *P. aequipapillae* relative to *P. charcoti* (Büring, 2019). These differences were linked to their stomach content analysis, which revealed that octopods living at greater depths (e.g. *A. polymorpha*) consumed prey at higher trophic levels, such as fish. In contrast, species living in shallower waters, such as *P. charcoti*, base their diet on crustaceans and amphipods (Piatkowski et al., 2003). In line with this observation, our specimens of *P. aurata* and *P. charcoti* were collected at depths ranging from 109 to 178 m, whereas individuals from the other analyzed species were sampled below 288 m depth. The significant effect of sampling depth found in the ANCOVAs (Table 2) may indicate a spatial segregation of the octopod species with depth, probably driving a contrasting feeding ecology between *A. polymorpha* and *P. aurata*, reflected by the significant difference in $\delta^{15}N$ values (Table S1).

The carbon stable isotope signature can be used to assess habitat differences between species, since $\delta^{13}C$ values exhibit a latitudinal gradient, with highest values measured inshore relative to offshore habitats, and higher values in benthic species relative to pelagic species (Cherel et al., 2009; Chouvelon et al., 2011). Most of the species analyzed herein (i.e., *P. aequipapillae*, *P. albimaculata*, *P. charcoti*, *P. cornuta*, *P. felix* and *P. turqueti*) displayed a narrow range in $\delta^{13}C$ values (from −25.42 to −24.09), suggesting that they share an overlapping feeding habitat. No significant effect of $\delta^{13}C$ values independently of

the δ^{15}N values was measured in this study, likely due to the small δ^{13}C values variation, which never exceeded 2‰ (Table S1).

4.2. Arsenic

Most trace element concentrations in the digestive gland were generally higher than the mantle tissue, which is consistent with the detoxification and storage role of the digestive gland usually recognized in cephalopods, including octopods (e.g., Miramand and Bentley, 1992; Bustamante et al., 1998b; Seixas et al., 2005). However, As concentrations were higher in the mantle tissue than in the digestive gland (Table S1). This could reflect the affinity of arsenobetaine, the major As species in cephalopods (Taylor et al., 2017) to the proteins in the muscular tissue (Shen et al., 2013). This has been already described in other cephalopods, such as nautilus, *Nautilus macromphalus* (Bustamante et al., 2000), and arrow squid, *Nototodarus sloanii* (Lischka et al., 2020a).

Some trace elements are not well documented in cephalopods (e.g., Ag, As, and V), which makes the interpretation for those elements challenging. Nevertheless, As concentration data is available for certain cephalopod species (e.g., *Todarodes filippovae*, Kojadinovic et al., 2011). The As concentrations measured in all octopods in this study were higher (digestive gland As concentration average = 37.48 µg g^{-1} dw) relative to pelagic squid species from previous studies (e.g., *Gonatus fabricii*, 10.18 µg g^{-1} dw, Lischka et al., 2020b; *Sthenoteuthis pteropus*, 18.33 µg g^{-1} dw, Lischka et al., 2018), but comparable to octopod species from temperate environments (e.g., *Octopus vulgaris*; ~40 µg g^{-1} dw, Raimundo et al., 2010). This is congruent with the well-supported hypothesis that the benthic vs pelagic habitat is the main driver of As accumulation since As tends to be trapped in sediments (Sanders, 1980). In the present study, the deeper living *P. albimaculata* and *P. polymorpha* had the highest concentrations of As among the eight studied octopod species (Table S1; Fig. 4). Cephalopods mainly accumulate As through their diet (Kojadinovic et al., 2011), and since information about the feeding ecology of this species is currently not available, conclusions between diet and As exposure cannot be drawn. However, our results show that sampling depth and δ^{15}N values both positively influenced As concentrations (Table 3) likely reflecting that feeding at higher trophic levels results in higher As contamination. This may suggest a higher As bioavailability in octopod prey with depth and/or the biomagnification of the organic As compounds between prey and octopods (Kubota et al., 2001; Neff, 1997; Tu et al., 2011). Further dietary studies are needed to confirm this hypothesis.

4.3. Cadmium

The variations in Cd concentrations have been described for cephalopod species from different oceanic origins (Table 4). It is noteworthy that the Antarctic octopods in the present study display similar Cd concentrations with temperate species, such as *Octopus vulgaris* from the Mediterranean Sea (Miramand and Guary, 1980) and *Eledone cirrhosa* from the English Channel (Miramand and Bentley, 1992). However, the latter species displayed a higher variation in Cd concentrations with respect to its location when compared to *E. cirrhosa* from the Bay of Biscay (Chouvelon et al., 2011) and from the Faroe Islands (Bustamante et al., 1998a), which displayed at least 50% lower and 1800% higher Cd concentrations, respectively, compared to the *A. polymorpha* samples (Table 4). In contrast, it could be hypothesized that the reason that benthic octopods show lower concentrations of Cd within the pan-Antarctic region is due to the more homogenous and stable environment that characterizes this area.

The Cd concentrations measured in this study were relatively high and similar to concentrations from urbanized coasts (Table 4). This could be due to an enrichment of Cd in the surface layers because of upwelling events (Bustamante et al., 1998b). It could also be due to the limited bioavailability of essential elements such as Cu and Zn, leading

to an enhanced uptake of Cd (Gault-Ringold et al., 2012; Petri and Zauke, 1993). Therefore, organisms may have developed very efficient mechanisms of elemental uptake for Cu and Zn (Petri and Zauke, 1993). Because these mechanisms are probably not specific to Cu and Zn, Cd might be absorbed by the same physiological pathways (Penicaud et al., 2017). These high Cd concentrations might also be attributable to the 'Cd anomaly', which describes a latitudinal gradient of Cd concentration in marine invertebrates (including octopods), where higher Cd concentrations have been measured in marine organisms from subpolar areas. These descriptions include amphipods (Bargagli et al., 1996; Kahle and Zauke, 2002; Petri and Zauke, 1993), fish (Bustamante et al., 2003; Macdonald and Sprague, 1988; Zauke et al., 1999), and cephalopods (Bustamante et al., 1998a, Cipro et al., 2018).

There is a general paucity of data concerning trace elements in Antarctic octopods, but the high Cd concentrations in the digestive gland in *Graneledone gonzalezi* and *Benthoctopus thielei* from the subantarctic Kerguelen Islands (Bustamante et al., 1998b) are within the same range of the present values, suggesting that the Cd anomaly may occur in the South Shetland Islands area.

This study further reported significant differences in Cd concentration data among different species (GLMs; Table 3), with *P. charcoti* and *P. aurata* displaying the highest Cd concentrations (see also Fig. 4). These results suggest that Cd concentration differences, consistent with the δ^{15}N signature (Table 3), can be used to highlight the diet partitioning of octopod species. For example, *P. charcoti* has shown high Cd levels and their diet includes Cd richer prey (amphipods, crustaceans; Büring, 2019), while *A. polymorpha* showed lower Cd concentrations and is known to consume prey of higher trophic levels (e.g., fish; Büring, 2019) with lower Cd concentrations (Storelli and Marcotrigiano, 2004). Our results suggest that Cd concentration data might be used to discriminate and characterise the ecological niche of octopod species.

Apart from interspecific differences, significant differences in Cd concentrations were observed between the sexes in the GLMs, with the highest concentrations measured in male *P. charcoti* specimens relative to females of the same species (Table 3, Fig. S1). *Paraledone charcoti* was the only species in the present study that had a balanced sample set (11 females and 11 males), which was the reason other species could not be evaluated this way. Since cephalopods mainly take up Cd though their diet (Bustamante et al., 2002; Koyama et al., 2000), this difference could be due to diet composition, highlighting a niche partitioning between sexes (Table 3). Such a difference in diet between sexes was previously reported for *O. mimus* from Northern Chilean waters, with females having a higher food intake relative to males (Cortez et al., 1995). Therefore, the amount of ingested prey but also their type should influence Cd concentrations. In this respect, *P. charcoti* females might ingest more Cd-poor prey compared to males. To confirm sex differences in the diet of Antarctic benthic octopods, additional stomach content analysis, both morphologically and genetically, is necessary.

4.4. Mercury

The concentration of Hg measured in the mantle muscles of *P. turqueti* from the South Georgia coast was similar to the Hg concentrations measured in the present study (Matias et al., 2019, 2020). Worldwide, Hg concentrations fluctuated among octopod species and sampling locations, with the highest concentrations measured in *E. cirrhosa* from the Tyrrhenian Sea (Rossi et al., 1993; Barghigiani et al., 2000). The Hg concentrations in the present study were similar to those reported for *O. vulgaris* from the Portuguese Coast (Seixas et al., 2005), and *E. cirrhosa*, from the Bay of Biscay (Chouvelon et al., 2011; Table 4). Benthic species in the Octopodidae usually display higher Hg concentrations compared to other cephalopod families (Penicaud et al., 2017). The specimens analyzed in the present study showed lower Hg concentrations relative to pelagic Antarctic squids;

Table 4

Concentrations of Cd in the digestive gland and Hg (mean $\pm$ standard deviation [SD]) in muscular tissues of octopods from temperate and (sub-)Antarctic areas. All concentrations are presented in µg g^{-1} dw. Abbreviations as followed: ([A]) = arm muscles, ([M]) = mantle muscles. 'Year' indicates the tissue collection date.

Species	Cd in the digestive gland Mean $\pm$ SD	Hg in the muscle Mean $\pm$ SD	Location	Year	Reference
Antarctic species					
Adelieledone					
A. polymorpha	112 $\pm$ 44.4	0.56$\pm$ 0.13[M]	Antarctic Peninsula	2012	This study
A. polymorpha		0.322 $\pm$ 0.088[M]	South Georgia coast	2004	Matias et al., 2019
A. polymorpha		0.126 $\pm$ 0.032[M]	South Georgia region	2004	Matias et al., 2020
Benthoctopus					
B. thielei	215		Kerguelen Islands	1995	Bustamante et al., 1998b
Graneledone					
G. gonzalezi	369		Kerguelen Islands	1995	Bustamante et al., 1998b
Pareledone					
P. aequipapillae	80.92 $\pm$ 23.02	0.20$\pm$ 0.03[M]	Antarctic Peninsula	2012	This study
P. albimaculata	85.89 $\pm$ 43.27	0.15$\pm$ 0.02[M]	Antarctic Peninsula	2012	This study
P. annata	175$\pm$ 65.22	0.13$\pm$ 0.03[M]	Antarctic Peninsula	2012	This study
P. charcoti	152$\pm$ 87.33	0.41$\pm$ 0.22[M]	Antarctic Peninsula	2012	This study
P. cornuta	112 $\pm$ 11.98	0.12$\pm$ 0.01[M]	Antarctic Peninsula	2012	This study
P. felix	76.4 $\pm$ 1.76	0.39$\pm$ 0.03[M]	Antarctic Peninsula	2012	This study
P. subtilis	164 $\pm$ 140	0.19$\pm$ 0.04[M]	Antarctic Peninsula	2012	This study
P. turqueti	204	0.28[M]	Antarctic Peninsula	2012	This study
P. turqueti		0.434$\pm$ 0.128[M]	South Georgia Coast	2004	Matias et al., 2019
P. turqueti		0.196 $\pm$ 0.083 [M]	South Georgia region	2004	Matias et al., 2020
Temperate species					
Eledone					
E. cirrhosa	79.87 $\pm$ 4.38[*]		Cotentin Coast, English Channel	1987	Miramand and Bentley, 1992
E. cirrhosa	16.3 $\pm$ 9.6	0.34 $\pm$ 0.072[M]	Bay of Biscay	2005-2008	Chouvelon et al., 2011
E. cirrhosa		6.06 [M]	Tyrrhenian Sea	1999	Barghigiani et al., 2000
E. cirrhosa	2333 $\pm$ 1000[*]	0.144$\pm$ 0.071 [M]	Faroe Islands	1997	Bustamante et al., 1998a, 2006
Octopus					
O. vulgaris	50 $\pm$ 10		Monaco, Mediterranean Sea	1980	Miramand and Guary, 1980
O. vulgaris	94–185		Matosinhos, Portugal	2002	Raimundo et al., 2008
O. vulgaris	136–269		Northwest Coast, Portugal	2001	Raimundo et al., 2005
O. vulgaris	20–122		South Coast, Portugal	2001	Raimundo et al., 2005
O. vulgaris	65.8$\pm$ 24.2	0.13$\pm$ 0.02[M]	Sfax, Tunisia	2010	Rjeibi et al., 2014
	42.5$\pm$ 20.3	0.18$\pm$ 0.06 [M]	Bizerte, Tunisia		
	31.0$\pm$ 9.2	0.13$\pm$0.06 [M]	Monastir, Tunisia		
O. vulgaris		0.43 $\pm$ 0.13[A]	Cascais, Portugal	2002-2003	Seixas et al., 2005
O. vulgaris		0.213 $\pm$ 0.02[*M]	Azores	1990-1991	Monteiro et al., 1992

[*] Converted values from wet weight.

for example, Hg concentrations measured in the digestive gland of the squid *Kondakovia longimana* (0.045$\pm$ 0.021 µg g^{-1} dw; Seco et al., 2020) were ten-fold lower when compared to *A. polymorpha* (Table 4). This highlights the interspecific as well as inter-location variability of Hg concentrations. Surprisingly, none of the proxies of trophic and habitat ecology have an effect on Hg concentrations among species, whereas Hg levels usually increases with benthic habitat and trophic position (Chouvelon et al., 2012). This result is likely related to the close ecological niches shared by Antarctic octopods and indicate that Hg would be a pertinent proxy for the trophic position and the habitat (benthic/pelagic) at the cephalopod assemblage scale (i.e., including benthic octopods and pelagic squids) in future comparative ecological studies (e.g., Seco et al., 2020).

4.5. Lead

Lead is one of the major pollutants in oceanic systems (Boyle et al., 2014). In the present study, Pb concentrations in the digestive gland varied among species, with *P. charcoti* exhibiting significantly higher concentrations compared to the other species analyzed (Fig. S1). This finding is consistent with a previous study on Antarctic benthic invertebrate communities, which also showed significant variations of Pb concentrations among species (Majer et al., 2014). This variation was attributed to the anthropogenic pollution in the Admiralty Bay at King George Island. However, because Elephant Island is a near-pristine ecosystem, it is unlikely that Pb has reached high levels due to anthropogenic causes. Instead, the shallow-living *P. charcoti* might be exposed

to higher Pb concentrations due to diet, since it mainly feeds on invertebrates (Büring, 2019), which are known to bioaccumulate this element (Rainbow, 1997). Feeding at higher trophic levels should reduce the exposure to this element because it is bioreduced along the food webs (Michaels and Flegal, 1990). Furthermore, higher Pb concentrations are associated with near-surface water layers (Henderson and Maier-Reimer, 2002). The reasons for oceanic depth differences are not yet fully understood, and anthropogenic sources—e.g., aeolian dust from industrial applications—were described as a major contributor (Rosman et al., 1994; Sun and Xie, 2001). Although Pb concentrations in deeper waters of the Antarctic region of the Indian Ocean have increased over the last century, they are still lower when compared with oceanic regions that have higher regional anthropogenic emissions and slower vertical mixing rates (Echegoyen et al., 2014). Apart from the interspecific concentration differences found in the present study, Pb was generally higher in males and juveniles relative to females (Table 3, Fig. S1). A similar pattern has been observed in the squid *Gonatus fabricii* (from the Arctic ocean; Lischka et al., 2020b) and in *O. vulgaris* (from the Mediterranean Sea; Rjeibi et al., 2014), where Pb concentrations in the digestive gland were lowest in females and associated with feeding habits. An analysis of sex-specific dietary patterns is needed in order to make further conclusions about the effects of sex on Pb bioaccumulation.

4.6. Potential impacts on ecosystem

The shallower-living species *P. charcoti* had significantly higher concentrations of Ag, Cd, Co, Cu, Pb, and Zn, compared to all other species

analyzed in the present study (Fig. 4). These elevated concentrations could be related to the diet of *P. charcoti*, which is known to consist mainly of crustaceans (particularly amphipods; Piatkowski et al., 2003; Büring, 2019). In contrast, the larger *A. polymorpha* has a diet that contains fish, which generally exhibit lower trace element concentrations (particularly, the non-essential elements Ag, Cd, and Pb) than crustaceans (Chouvelon et al., 2011; Pierce et al., 2008). Since diet can be considered a major source for trace element exposure (Bustamante et al., 1998a), the measured differences in trace element concentrations among species in this study are likely due to differences in feeding habits and rates. One of the main findings of this study is the significant difference in $\delta^{15}N$ values found in *P. charcoti* and *P. aurata* (relative to all other analyzed species, which is supported by ecological tracers (trace elements, isotopic signature). In addition, based on the ranges of the $\delta^{15}N$ values and the significant effect of depth on $\delta^{15}N$ values (Table 2), the analyzed Antarctic octopod community seems to be a widely unique group with two segregated species, *P. charcoti* and *P. aurata*. This might likely reflect distinct ecological niches, driven by depth and leading to different diets. However, considering the narrow range of $\delta^{13}C$ variation among all species, the feeding patterns of the octopods investigated in our study do not seem differently enough to emphasize contrasted foraging habitats.

The species assessed in this study are consumed by a variety of Antarctic top predators (see Introduction; e.g., Casaux et al., 1997; Daneri et al., 2000; Lipinski and Woyciechowski, 1981; Rodhouse et al., 1992; Xavier et al., 2002), which are exposed to trace elements, sometimes in elevated concentrations, when they feed on the species analyzed in the present study. In particular, the high Cd concentrations measured in the digestive gland samples might represent a significant point source for predators (Bustamante et al., 2008). High Cd concentrations were measured in Antarctic seals and explained by dietary exposure, which includes benthic octopods, particularly the shallower *P. charcoti*, which exhibits the highest Cd concentrations found in the present study (Casaux et al., 1997; Malcolm et al., 1994; Szefer et al., 1994). Our study highlights that incirrate octopods represent a vector for trace elements, including Cd and Hg, to top predators foraging near Antarctic Elephant Island. This has been previously shown for other oceanic areas (Bustamante et al., 1998a; Penicaud et al., 2017). Pinnipeds such as the Antarctic fur seal *Arctocephalus gazella* and the Southern elephant seal *Mirounga leonina* might be exposed to elevated trace element concentrations due to their octopod-rich diet (Burdman et al., 2015; Casaux et al., 1998). Consistently, elevated Cd concentrations have been measured in liver and kidney tissues of *A. gazella*, which were associated with hepatotoxicity and nephrotoxicity (De Moreno et al., 1997; Malcolm et al., 1994). Overall, the extent of the toxic effects of trace elements on both predators and prey still remains understudied. In this context, it is necessary to provide information to allow risk assessment evaluations.

5. Conclusion

In this study, stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) and trace elements (Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V, and Zn) were assessed in an octopod assemblage collected near Elephant Island in the Antarctic Ocean. Significant differences were measured between sexes and species. For example, *P. aurata* and *P. charcoti* exhibited significantly lower $\delta^{15}N$ values compared to *A. polymorpha*, which indicates that they feed at a lower trophic level. This is consistent with the sampling depths of *P. aurata* and *P. charcoti*, which occurred in shallower waters than *A. polymorpha*. This suggests. a spatial segregation of the octopod species with depth, which could drive a contrasting feeding ecology. Furthermore, most trace element concentrations (with the exception of Hg) varied between species, suggesting different bioaccumulation patterns, which likely reflect trophic habitat discrimination. Our results are consistent with the few previous dietary studies on these species and highlight the influence of benthic Antarctic octopods in the transfer

of trace elements to their main predators, which is the first step in unravelling the complex interactions in this unique and irreplaceable environment.

CRediT authorship contribution statement

A. Lischka: Conceptualization, Visualization, Investigation, Methodology, Writing - original draft, Formal analysis. **P. Bustamante:** Conceptualization, Writing - review & editing. **H. Braid:** Data curation, Writing - review & editing. **U. Piatkowski:** Writing - review & editing. **T. Lacoue-Labarthe:** Conceptualization, Writing - review & editing.

Declaration of competing 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.144373.

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