



Trace element analysis reveals bioaccumulation in the squid *Gonatus fabricii* from polar regions of the Atlantic Ocean[☆]

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ABSTRACT

The boreoatlantic gonate squid (*Gonatus fabricii*) represents important prey for top predators—such as marine mammals, seabirds and fish—and is also an efficient predator of crustaceans and fish. *Gonatus fabricii* is the most abundant cephalopod in the northern Atlantic and Arctic Ocean but the trace element accumulation of this ecologically important species is unknown. In this study, trace element concentrations (Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn) were analysed from the mantle muscle and the digestive gland tissue of juveniles, adult females, and adult males that were captured south of Disko Island off West-Greenland. To assess the feeding habitat and trophic position of this species, stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) were measured in their muscle tissue. Mercury concentrations were positively correlated with size (mantle length) and trophic position. The Hg/Se ratio was assessed because Se has been suggested to play a protective role against Hg toxicity and showed a molar surplus of Se relative to Hg. Cadmium concentrations in the digestive gland were negatively correlated with size and trophic position ($\delta^{15}\text{N}$), which suggested a dietary shift from Cd-rich crustaceans towards Cd-poor fish during ontogeny. This study provides trace element concentration data for *G. fabricii* from Greenlandic waters, which represents baseline data for a northern cephalopod species. Within West-Greenland waters, *G. fabricii* appears to be an important vector for the transfer of Cd in the Arctic pelagic food web.

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1. Introduction

Trace elements—such as arsenic, cadmium, lead or mercury—have important implications for human health and are indicators for environmental pollution (Tchounwou et al., 2012). In the marine environment, most metals and metalloids occur naturally at low concentrations (Langston, 1990). However, their levels can be increased by natural phenomena (such as atmospheric deposition, soil erosion or volcanic activity (Boutron et al., 1994) and by anthropogenic activities (such as mining, river discharges or industrial wastes (e.g., Gao and Chen, 2012; García et al., 2008).

The Arctic Ocean has been suggested to be particularly vulnerable to trace element contamination because it acts as a sink for various contaminants that are transported north through the

atmosphere, rivers and oceanic currents (Barrie et al., 1992; McConnell and Edwards, 2008; Muir et al., 1992). Arctic marine mammals, fish and birds have been the focus of several trace element concentration studies and long-term biomonitoring programs (e.g., AMAP, 2018; Becker, 2000; Campbell et al., 2005; Dehn et al., 2006; Dietz et al., 1996; Macdonald and Sprague, 1988; Zauke et al., 1999). However, to the best of our knowledge, cephalopods have not been included in these previous studies, although they have been proposed as vectors of contaminants, particularly Cd and Hg, to marine top predators (Bustamante et al., 1998, 2006). Indeed, cephalopods play a pivotal role in the Arctic marine ecosystem as both predators and prey (Nesis, 1965, 2001; Gardiner and Dick, 2010). Their distribution has been correlated to the occurrence of predators, such as toothed whales (Bjørke, 2001), the northern fulmar, *Fulmarus glacialis* (Savinov et al., 2003), and the Greenland halibut, *Reinhardtius hippoglossoides* (Orr and Bowering, 1997). The northern distribution ranges of some cephalopod species appear to have been expanded by warming Arctic waters (Gardiner and Dick, 2010; Gilly, 2005; Golikov et al., 2013). In addition, increased

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abundance can result in a shift in the predator's diet from fish to squid, changing the contaminant exposure accordingly (Dehn et al., 2006). Therefore, it is fundamentally important to consider cephalopods as a major vector in the trace element transfer along trophic food webs linking their trace element concentrations to their ecological role.

The boreoatlantic gonate squid *Gonatus fabricii* (Lichtenstein, 1818) is the most abundant squid in the northern Atlantic and Arctic Oceans (Kristensen, 1983; Nesis, 2001; Zumholz and Frandsen, 2006; Gardiner and Dick, 2010; Golikov et al., 2018) and represents the only squid species that spends its entire life cycle of around two years, in the Arctic Ocean (Golikov et al., 2018; Kristensen, 1984; Nesis, 1971). It shows a vertical distribution that covers a broad depth range, with early life stages occurring from the surface to about 1000 m depth, and more mature stages down to 3000 m (Kristensen, 1983, 1984; Nesis, 1965; Piatkowski and Wieland, 1993; Wiborg et al., 1982). This ontogenetic descent has also been inferred from variations in Sr/Ca ratios in the statoliths of *G. fabricii* captured off West Greenland, which suggests a migration of adult squids into deeper and colder waters (Zumholz et al., 2007). The species is believed to spawn near the bottom of the continental slopes off West Greenland and northern Norway (Arkhipkin and Bjørke, 1999; Kristensen, 1984) and spawned eggs are likely carried ('brooded') by the female in the water column as observed for the sister species *G. onyx* in the Pacific (Bjørke et al., 1997; Seibel et al., 2005).

Gonatus fabricii plays an important role in the energy transfer from epipelagic to meso- and bathypelagic layers through its vertical migration (Gardiner and Dick, 2010; Kristensen, 1984). Although both juvenile and adult *G. fabricii* prey on macroplanktonic crustaceans (Kristensen, 1984; Nesis, 1965; Sennikov et al., 1989), their diet shifts during maturation from invertebrates (i.e., amphipods, copepods, euphausiids, pteropods, and chaetognaths) to fish (e.g., capelin, Arctic cod, redfish, and lanternfish) and other cephalopods (Sennikov et al., 1989; Wiborg et al., 1984). In the Arctic marine food web, *G. fabricii* is a major prey item for seals, various cetaceans, seabirds, and deep-sea fishes (Gardiner and Dick, 2010). Sperm whales alone are estimated to consume 1.5 million tonnes of *G. fabricii* annually in the northern Atlantic (Bjørke, 2001). Squid prey that was estimated from cephalopod beaks in stomach contents of sperm whales that stranded along the coasts of the North Atlantic consisted of up to 95% of *G. fabricii* (Bjørke and Gjørseter, 2004; Ijsseldijk et al., 2018; Martin and Clarke, 1986; Santos et al., 1999). Narwhales, *Monodon monoceros*, observed in West Greenland waters during autumn almost exclusively fed on *G. fabricii* (Laidre and Heide-Jørgensen, 2005). Furthermore, *G. fabricii* plays an economic role because it is used as bait in Greenland's long-line and trap fisheries (Frandsen and Wieland, 2004), which make up around 85% of Greenland's economic exports (Lund, 2018).

The overall aim of the present study was to assess the trace element accumulation and trophic position of *G. fabricii* within a polar region of the Atlantic Ocean. This was addressed through the following means:

- 1) the measurement of stable isotope values of carbon and nitrogen in muscle tissue to investigate shifts in the relative trophic position of *G. fabricii* during ontogeny;
- 2) the determination of trace element concentrations in the mantle muscle (>30% of the total mass of the squid) and the digestive gland (a key organ in the bioaccumulation and detoxification of contaminants; Penicaud et al., 2017) of juvenile and adult *G. fabricii* specimens;

- 3) the combination of trace element and stable isotope data to observe changes in trace element concentrations in conjunction with shifts in diet and feeding habitat.

2. Material and methods

2.1. Sample collection

Specimens of *Gonatus fabricii* were collected by the research vessel 'Paamiut' (Greenland Institute of Natural Resources) by midwater trawling in depths of 569–590 m, south of Disko Island off Western Greenland (69°23'N, 52°63'W) on 16 July 2005 (Fig. 1). A total of 45 specimens were stored at -40 °C, composed of 15 males (dorsal mantle length [DML]: 90–274 mm), 15 females (DML: 76–193 mm), and 15 juveniles (DML: 30–56 mm).

2.2. Stable isotope analysis

Carbon and nitrogen stable isotopes were analysed from subsamples (0.2–0.4 mg) of the freeze-dried mantle tissue 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 δ unit notation as deviations from standards (Vienna Pee Dee Belemnite for $\delta^{13}\text{C}$ and 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‰ for $\delta^{13}\text{C}$ and <0.15‰ for $\delta^{15}\text{N}$.

2.3. Trace element analysis

Prior to trace element analysis, tissue samples of digestive gland and mantle muscle were freeze-dried for 48 h and homogenised.

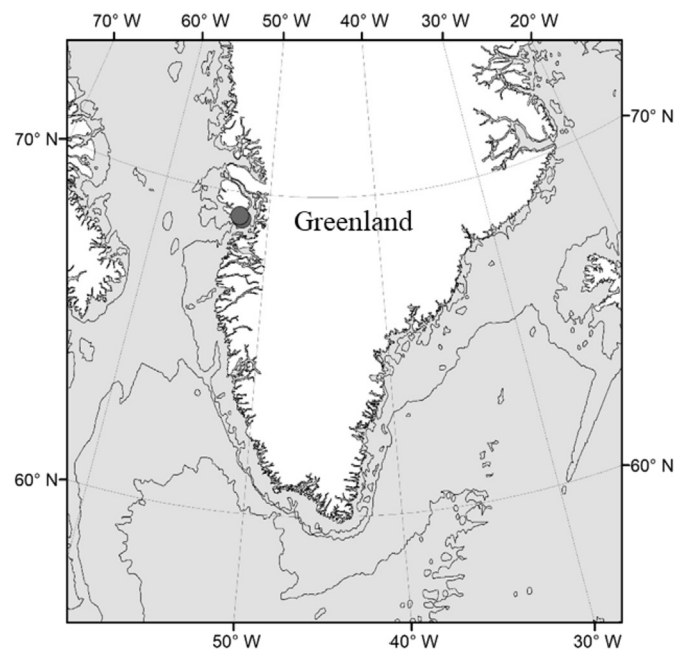


Fig. 1. Map of the sampling area during the cruise, the sampling station is indicated by a grey circle.

Water content ranged from 33.5 to 67.3% in the digestive gland and 72.9–89.4% in the mantle tissue. Sample aliquots (~200 mg dry weight [dw]) were digested overnight in a 3:1 mixture of 65% HNO₃ (Merck, suprapur quality) and 37% HCl (Merck, suprapur quality). This was followed by mineralization by heating the samples 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, and Zn) were measured by inductively coupled plasma mass spectroscopy (ICP-MS) (Thermo Fisher Scientific X Series 2) and optical emission spectroscopy (ICP-OES) (Varian Vista-Pro) following Lucia et al. (2016). Procedural blanks and certified reference materials (CRM)—dogfish liver (DOLT-4, National Research Council, Canada), lobster hepatopancreas (TORT-3, NRCC), and clam muscle tissue (IAEA461, International Atomic Energy Agency, Austria)—were treated and analysed in the same way as the samples. Recoveries of the elements ranged from 85 to 105% (n = 9). The detection limits for Ag, Cd, Co, Cr, and Pb were 0.025 µg.g⁻¹, As was 0.25 µg.g⁻¹, Fe and Zn were 5 µg.g⁻¹, Cu, Mn, and Se were 0.125 µg.g⁻¹ and Ni was 0.05 µg.g⁻¹, based on 200 mg. Cadmium concentrations were only measured in the digestive gland of specimens in order to prevent storage diffusion effects (Lischka et al., 2020; Bustamante et al., 2002; Francesconi et al., 1993).

Mercury concentrations were measured using an Advanced Mercury Analyser (ALTEC AMA 254, with a detection limit > 0.05 ng) on dried, homogenized digestive gland and mantle tissue (1–2 mg dw) as described in Bustamante et al. (2006). For every 10 samples, one standard sample of CRM DOLT 5 (Dogfish liver; NRCC) was analysed (recovery = 109%). The detection limit was 0.05 ng. Results for trace element concentrations are expressed in µg.g⁻¹ dw.

2.4. Mercury:selenium interaction

In order to assess Hg and Se ratios, measured concentrations were converted from µg.g⁻¹ dw into nmol.g⁻¹ using the molecular weight of 200.59 for Hg and 78.96 for Se. Ratios were assessed for both tissue types, as a Hg:Se ratio >1 indicates an excess of Hg in relation to Se in the tissue (Cuvin-Aralar and Furness, 1991; Ralston et al., 2008).

2.5. Statistical analysis

Most statistical analyses were conducted with R version 3.3.3 (Ihaka and Gentleman, 1996). Prior to data analysis, values below the detection limit were replaced by the lowest measured value of the corresponding element multiplied by 0.5. The samples were normalised and transformed using auto-scaling, mean centred, and divided by the standard deviation. Principal component analysis (PCA) plots were produced to examine differences in overall trace element concentrations among tissue types and maturation stage, using the package 'ggbiplot' (Vu, 2011). Correlations among trace element concentrations in the two tissues were assessed using the R package 'corrgram' (Wright, 2012). Significance of the variable collinearity was tested using pairwise nonparametric Spearman correlations ('corr.test' function of the 'corrgram' package, Wright, 2012). Using the software MetaboAnalyst v2.0 (<https://github.com/xia-lab/MetaboAnalystR>), heatmaps were generated using Euclidean distance and Ward hierarchical clustering.

Analyses of covariance (ANCOVA) were performed in R to check if concentrations of the trace elements (Ag, As, Cd, Hg, and Pb) in the two tissues were influenced by size, stable isotope values, or sex. Prior to the statistical tests, trace element concentrations were z-transformed (Graf, 2004) and diagnostic plots were used to check for variance homogeneity and normality of the residues. Explanatory variables were added (in the following order: DML, δ¹⁵N, sex,

δ¹³C) to see if the feeding habitat had an effect once size was accounted for. Analysis of variance (ANOVA) was conducted to test if there was a relationship between stable isotope values and sex/maturity stage (female, male, juvenile).

3. Results

3.1. Stable isotope values

The δ¹³C values were on average highest in mature males (−18.96‰), followed by females (−19.10‰), and juveniles (−20.60‰) (Table 1) and showed a distinct grouping with maturity stage (ANOVA, F-value = 339.66, p < 0.001); (Fig. 2). The δ¹⁵N values were significantly higher in females (12.75–15.15‰) and males (12.64–15.12‰), compared to juveniles (8.59–9.64‰) (ANOVA, F-value = 339.66, p < 0.001; Table 1, Fig. 3).

3.2. Trace element concentrations

Trace element concentrations in the digestive gland of mature *Gonatus fabricii* (females and males) followed the order Cu > Zn > Fe > Cd > As > Se > Ag > Ni > Mn > Co > Cr > Pb > Hg. Those measured in juveniles were found in the following order: Fe > Zn > Cd > Cu > As > Se > Ni > Mn > Cr > Co > Ag > Pb > Hg (Table 1). Between matures and juveniles, juveniles showed higher concentrations of Cd, Co, Cr, Fe, Mn, Ni, Pb, Se, and Zn in the digestive gland, while mature specimens had the highest concentrations of Ag and Cu (Table 1, Fig. S1).

Trace element concentrations in the mantle muscle of mature individuals followed the order Zn > Cu > As > Fe > Mn > Se > Ag > Mn > Ni > Cr > Hg > Ag > Co > Pb. Those found in juveniles ranged in the following order: Zn > Fe > Cu > As > Se > Mn > Ni > Cr > Co > Pb > Hg > Ag. The mature specimens exhibited the highest concentrations of As and Hg, with maximum concentrations measured in mantle tissue of males (Table 1, Fig. 2, Fig. S1). The PCA showed a distinction between tissue type and maturation stage (Fig. 2). The first axis of the PCA explained 45.6% of the variance, the second axis 27.6%. Principle component PC 1 was mainly driven by Cd, Co, Cr, Fe, Ni, Pb, Se, and Zn, while PC 2 was mainly driven by Ag, Cd, Co, and Cu.

3.3. Trace element correlations

The concentrations of Hg and Se in the mantle tissue showed a negative correlation ($r = -0.60, p < 0.001$). The molar ratio between Hg and Se in the digestive gland (mean = 0.007) and the mantle tissue (mean = 0.024) were well below 1 (Fig. 4a and b). A linear relationship between the Hg concentration and the molar ratio of Hg:Se was observed in the mantle tissue (Fig. 4b).

In the digestive gland tissue, Zn showed a positive correlation with Cd ($r = 0.37, p = 0.01$), while a negative relationship was found between Cu and Zn ($r = -0.65, p < 0.001$). Chromium and Ni were positively correlated both in the digestive gland ($r = 0.93, p < 0.001$) and mantle tissue ($r = 0.90, p < 0.001$).

3.4. Relationship between trace element concentrations and stable isotopes

ANCOVAs were performed to test which variables (δ¹⁵N, δ¹³C, size - or DML, and sex) influence the concentrations of the elements Ag, As, Cd, Cu, Hg, Pb, and Zn. Silver concentrations in both tissues were significantly influenced by δ¹⁵N, while the digestive gland was also affected by size (Table 2). Arsenic concentrations in both tissues showed a significant relationship with size and δ¹⁵N. Cadmium concentrations in the digestive gland showed a significant

Table 1
Summary of specimen data and trace elemental concentrations for *Gonatus fabricii* for females (n = 15), males (n = 15) and juveniles (n = 15). Presented are dorsal mantle length (DML in cm), muscle stable isotope $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (reported as ‰), and trace element concentrations (minimum, mean, and maximum) for digestive gland and mantle tissue (given in $\mu\text{g}\cdot\text{g}^{-1}$ dw).

	Female			Male			Juvenile		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
DML	12.80	7.60	19.30	17.14	9.00	27.40	4.06	3.00	5.60
$\delta^{13}\text{C}$	-19.10	-19.53	-18.77	-18.96	-19.35	-18.72	-20.60	-21.10	-19.98
$\delta^{15}\text{N}$	13.74	12.75	15.15	13.98	12.64	15.12	9.09	8.59	9.64
Digestive gland									
Ag	1.20	0.44	2.23	1.03	0.45	1.83	0.22	0.14	0.44
As	10.18	4.49	19.84	10.25	4.11	19.42	6.68	5.29	8.01
Cd	31.57	5.86	63.14	31.79	4.36	58.18	41.60	25.80	62.32
Co	0.17	0.08	0.29	0.12	0.05	0.18	0.29	0.13	0.63
Cr	0.10	0.09	0.10	0.12	0.09	0.41	1.68	0.10	11.56
Cu	124	72.7	192	138	67.6	223	14.1	7.66	50.0
Fe	57.5	22.4	136	42.8	20.1	92.4	207	99.5	320
Hg	0.05	0.01	0.09	0.06	0.01	0.11	0.04	0.02	0.06
Mn	1.01	0.82	1.29	0.85	0.51	1.21	2.45	1.18	5.45
Ni	1.16	0.48	2.06	0.86	0.26	1.78	4.49	1.24	19.21
Pb	0.08	0.03	0.15	0.05	0.03	0.12	0.22	0.09	0.37
Se	2.96	1.27	6.54	2.36	1.36	4.58	5.75	3.88	9.55
Zn	74.0	23.0	140	56.5	29.6	131	136	89.8	181
Mantle tissue									
Ag	0.09	0.01	0.19	0.08	0.02	0.26	0.03	0.01	0.05
As	22.31	11.65	51.07	26.30	11.30	35.39	6.06	5.57	6.81
Co	0.04	0.02	0.12	0.03	0.02	0.07	0.06	0.03	0.11
Cr	0.32	0.10	1.84	0.28	0.10	1.53	0.99	0.20	2.43
Cu	26.3	10.5	54.5	18.8	10.1	37.9	11.5	8.09	15.2
Fe	18.7	8.67	42.1	9.63	4.20	13.2	48.3	18.9	163
Hg	0.13	0.05	0.22	0.16	0.04	0.26	0.04	0.03	0.05
Mn	2.50	1.33	3.51	1.63	1.37	2.03	1.83	1.49	2.08
Ni	0.81	0.27	3.20	0.49	0.26	1.45	1.15	0.41	2.46
Pb	0.03	0.01	0.06	0.02	0.01	0.05	0.06	0.03	0.16
Se	1.80	1.34	2.09	1.78	1.43	2.35	2.23	1.87	2.92
Zn	90.6	41.6	131	54.0	44.4	65.3	91.7	80.1	107

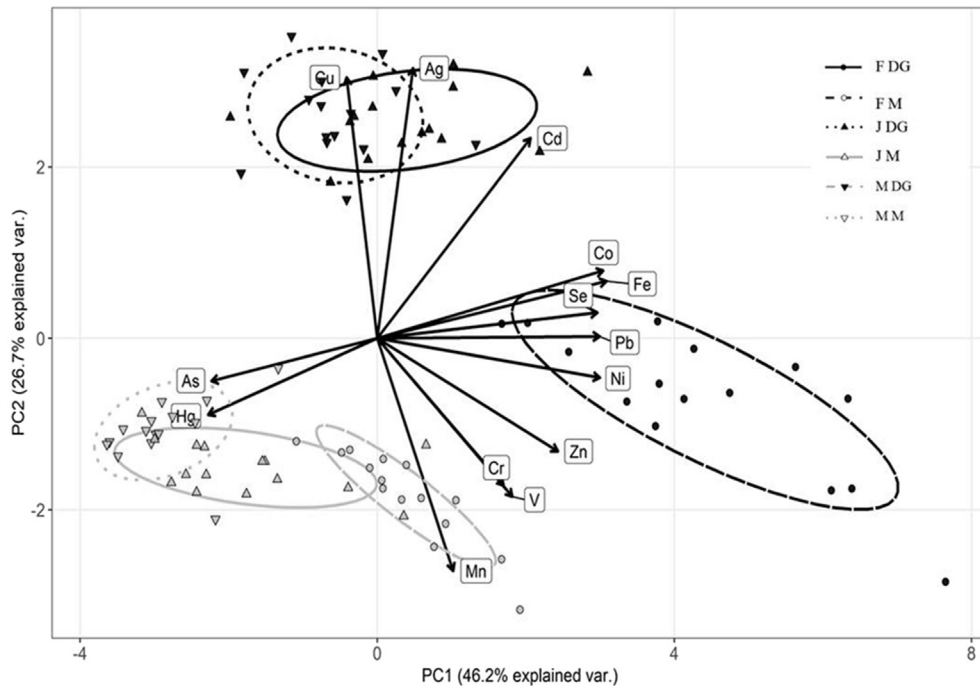


Fig. 2. Principal component analysis (PCA) presenting the trace element concentrations in digestive gland tissue. Element loadings along principle component PC1 and PC2 are represented by arrows. Abbreviations are the following: female digestive gland (F DG), female mantle (F M), juvenile digestive gland (J DG), juvenile mantle (J M), male digestive gland (M DG) and male mantle (M M). Ellipses indicate the 95% confidence interval around tissue/maturity stage groupings.

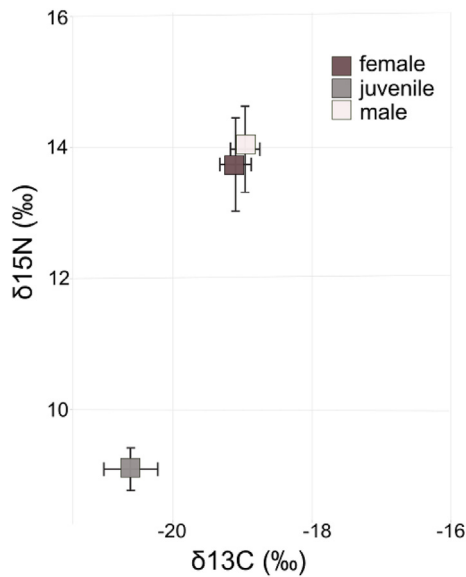


Fig. 3. Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope values (‰) in female, male and juvenile specimens.

relationship with stable isotope values and sex. Copper concentrations in the digestive gland were influenced by size, stable isotope values, and sex, while concentrations in the mantle tissue were only significantly influenced by $\delta^{15}\text{N}$. Mercury concentrations in both tissues showed a significant relationship with size and $\delta^{15}\text{N}$; however, Hg concentrations were higher in the mantle tissue and showed an additional relationship with sex. Both tissues showed significant relationships between Pb concentration with size and $\delta^{13}\text{C}$ (Table 2). Zinc concentrations were significantly correlated with size and sex in both tissues, while the digestive gland showed an additional relationship with $\delta^{15}\text{N}$.

4. Discussion

The trace element concentrations in deep-sea cephalopods, especially from high latitudes, are highly relevant in terms of bioaccumulation, due to the pivotal role that cephalopods play in marine ecosystems. The boreoatlantic gonate squid, *Gonatus fabricii*, is the most abundant cephalopod in the northern Atlantic Ocean and a key element in the oceanic food web and hence highly

relevant for the understanding of Arctic open-ocean ecology. We herein assess trace element concentrations in this cephalopod species from Greenland waters, which helps unravelling the bioaccumulation patterns within the sensitive Arctic ecosystem.

4.1. Stable isotopes

Stable isotopic signatures have been used for cephalopods to assess their trophic ecology (through $\delta^{15}\text{N}$) and the habitat use (through $\delta^{13}\text{C}$, Cherel and Hobson, 2005; Hobson, 1999). Nitrogen stable isotope values ($\delta^{15}\text{N}$) are considered to be directly related to diet and are therefore used as an indicator of trophic position (DeNiro and Epstein, 1978, 1981; Graham et al., 2010). Enriched $\delta^{15}\text{N}$ values indicate a higher trophic position of adult *G. fabricii* relative to juveniles (Fig. 3). This phenomenon has been well documented in fish (e.g., Chauvelon et al., 2014; Galván-Magaña et al., 2012), while fewer studies have focused on squid species (e.g., Chauvelon et al., 2011; Lischka et al., 2018; Merten et al., 2017). We found an increase in the $\delta^{15}\text{N}$ values associated with size, which was attributed to a shift in the trophic regime (i.e. predation of higher trophic levels; Kristensen, 1984), which is concomitant with a significant increase of the $\delta^{13}\text{C}$ values from immature to mature *G. fabricii* (Fig. 3).

Our distinct separation in the $\delta^{13}\text{C}$ values of mature individuals and juveniles indicates an ontogenetic habitat shift of *G. fabricii* (Fig. 3). The differences found between juvenile and mature *G. fabricii* for $\delta^{13}\text{C}$ are likely related to ontogenetic migration where older and larger specimens live deeper than juveniles (Kristensen, 1983; Nesis, 1965; Sennikov et al., 1989). Both these signatures are consistent with the known change in feeding habits as a result of ontogenetic migration with larger adults living in deeper waters (Nesis, 1965; Kristensen, 1983; Sennikov et al., 1989). Our results are consistent with the ontogenetic changes previously reported from stable isotope signatures in the beaks of *G. fabricii* (DML13–257 mm) captured off Greenland and in the Barents Sea (Golikov et al., 2018), and in elemental signatures in the statoliths (Zumholz et al., 2007).

4.2. Trace element concentrations

4.2.1. Cadmium

Compared to other taxa, cephalopods have a high capacity to accumulate Cd in elevated concentrations in the digestive gland (Penicaud et al., 2017). *Gonatus fabricii* shows intermediate Cd

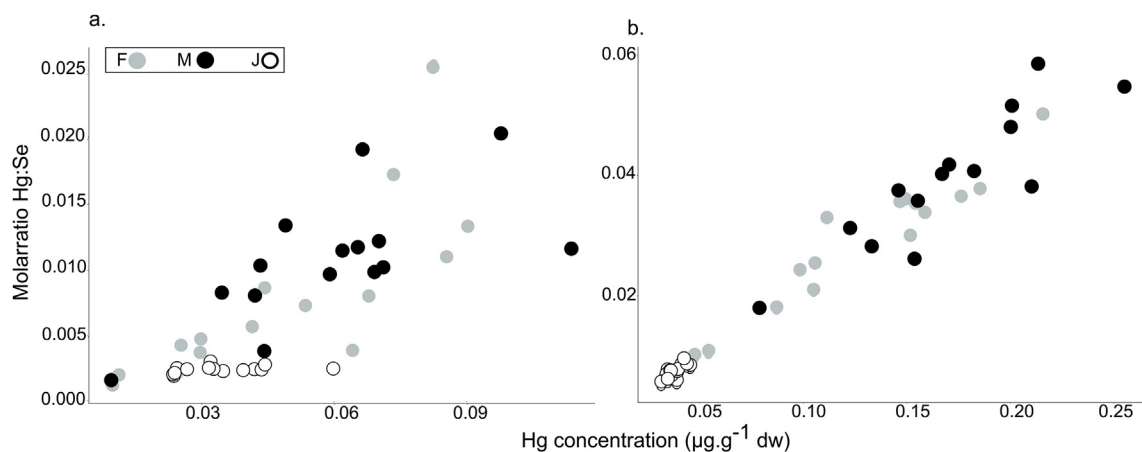


Fig. 4. Relationship between Hg concentrations ($\mu\text{g.g}^{-1}\text{ dw}$) and the molar ratio of Hg:Se in (a) digestive gland ($y = -0.002 + 0.19x$, $R^2 = 0.58$, $p < 0.001$) and (b) mantle tissue ($y = -0.002 + 0.24x$, $R^2 = 0.95$, $p < 0.001$) of female (F), male (M) and juvenile (J).¹

Table 2
Analysis of covariance (ANCOVA) for the linear models fitted to the trace element concentrations in the digestive gland and muscle tissues. Explanatory variables are as follows: dorsal mantle length (DML), $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (‰), and sex. Mantle tissue concentrations for Cd were excluded due to diffusion of the digestive gland concentrations to the mantle tissue during sample storage. Df represents the degrees of freedom. Asterisks show the level of significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	Df	Sum of squares	Mean square	F-value	Significance		Df	Sum of squares	Mean square	F-value	Significance	
Digestive gland						Mantle						
Ag												
DML	1	4.20	4.20	63.34	<0.001	***	1	0.40	0.40	2.28	0.14	
$\delta^{15}\text{N}$	1	3.89	3.89	58.62	<0.001	***	1	3.32	3.32	18.85	<0.001	***
Sex	2	0.14	0.07	1.06	0.35		2	0.19	0.10	0.55	0.58	
$\delta^{13}\text{C}$	1	0.18	0.18	2.77	0.10		1	0.23	0.23	1.29	0.26	
Residuals	39	2.59	0.07				39	6.86	0.18			
As												
DML	1	1.49	1.49	7.75	0.01	**	1	7.70	7.70	202.39	<0.001	***
$\delta^{15}\text{N}$	1	1.27	1.27	6.60	0.01	*	1	1.65	1.65	43.31	<0.001	***
Sex	2	0.59	0.29	1.53	0.23		2	0.15	0.07	1.92	0.16	
$\delta^{13}\text{C}$	1	0.17	0.17	0.87	0.36		1	0.02	0.02	0.40	0.53	
Residuals	39	7.49	0.19				39	1.48	0.04			
Cd												
DML	1	0.00	0.00	0.01	0.93							
$\delta^{15}\text{N}$	1	1.18	1.18	8.42	0.01	**						
Sex	2	3.65	1.83	13.03	<0.001	***						
$\delta^{13}\text{C}$	1	0.70	0.70	5.00	0.03	*						
Residuals	39	5.47	0.14									
Cu												
DML	1	6.42	6.42	270.98	<0.001	***	1	0.28	0.28	2.70	0.11	
$\delta^{15}\text{N}$	1	3.26	3.26	137.56	<0.001	***	1	6.02	6.02	57.46	0.00	***
Sex	2	0.35	0.18	7.44	0.002	**	2	0.56	0.28	2.67	0.08	
$\delta^{13}\text{C}$	1	0.05	0.05	2.01	0.16		1	0.06	0.06	0.56	0.46	
Residuals	39	0.92	0.02				39	4.08	0.10			
Hg												
DML	1	1.29	1.29	6.57	0.01	*	1	7.20	7.20	220.47	<0.001	***
$\delta^{15}\text{N}$	1	0.94	0.94	4.80	0.03	*	1	1.53	1.53	46.89	<0.001	***
Sex	2	0.84	0.42	2.14	0.13		2	0.89	0.45	13.65	<0.001	***
$\delta^{13}\text{C}$	1	0.29	0.29	1.47	0.23		1	0.10	0.10	3.01	0.09	
Residuals	39	7.65	0.20				39	1.27	0.03			
Pb												
DML	1	5.15	5.15	84.97	<0.001	***	1	4.78	4.78	41.24	<0.001	***
$\delta^{15}\text{N}$	1	1.57	1.57	25.83	<0.001	***	1	0.19	0.19	1.66	0.21	
Sex	2	0.20	0.10	1.65	0.21		2	0.14	0.07	0.61	0.55	
$\delta^{13}\text{C}$	1	1.71	1.71	28.27	0.00	***	1	1.37	1.37	11.83	0.001	**
Residuals	39	2.37	0.06				39	4.52	0.12			
Zn												
DML	1	4.18	4.18	42.66	<0.001	***	1	3.83	3.83	36.55	<0.001	***
$\delta^{15}\text{N}$	1	1.80	1.80	18.40	<0.001	***	1	0.01	0.01	0.09	0.77	
Sex	2	0.65	0.32	3.30	0.05	*	2	2.94	1.47	14.04	<0.001	***
$\delta^{13}\text{C}$	1	0.54	0.54	5.49	0.02	*	1	0.12	0.12	1.19	0.28	
Residuals	39	3.83	0.10				39	4.09	0.10			

concentrations, relative to the low levels found in the Loliginidae (Bustamante et al., 2002) and the high concentrations reported for oceanic Ommastrephidae (Gerpe et al., 2000; Lischka et al., 2018, 2019, Table 3). Although unusually high Cd concentrations have been previously reported in the subpolar waters of both hemispheres (Bustamante et al., 1998, Bustamante et al., 1998, 2003; Macdonald and Sprague, 1988; Petri and Zauke, 1993; Ritterhoff and Zauke, 1997), *G. fabricii* does not appear to bioaccumulate this trace element in those extreme concentrations. Physiological factors are likely responsible for this decreased Cd bioaccumulation relative to the Ommastrephidae. For example, the digestive gland of Ommastrephidae and Sepiidae, contrasting to Loliginidae, possesses a lysosomal system with specific cells ('boules' structures) that are thought to be involved in the storage of large amounts of Cd (Bustamante et al., 2002). The detoxification mechanisms in *G. fabricii* are not fully understood and future studies should focus on this aspect of their physiology.

The Cd concentrations found in *G. fabricii* individuals show a strong correlation with life stage (Tables 1 and 2; Fig. 2). Juveniles had higher Cd concentrations than adults, which could be linked to the ontogenetic change in diet that was revealed through the $\delta^{15}\text{N}$ values discussed above (Table 1). Indeed, diet has been suggested as

the main source for Cd accumulation in cephalopods (Penicaud et al., 2017). Juvenile *G. fabricii* have been reported to feed mainly on crustaceans in epipelagic waters, whereas adult specimens predominantly feed on deeper meso- and bathypelagic fishes (Bjørke and Gjosæter, 2004; Golikov et al., 2018; Nesis, 1965; Sennikov et al., 1989; Wiborg et al., 1984). Although Cd is strongly retained by cephalopods (Bustamante et al., 2002), there are two potential explanations for the Cd decrease with ontogeny. These include: 1) the diet of *G. fabricii* shifts from a Cd-rich diet (crustaceans) to a Cd-poor diet (fish); and/or 2) a fast growth rate in the juveniles results in a dilution of Cd in the growing tissues (Chouvelon et al., 2011). Predators that feed mainly on juvenile *G. fabricii* (e.g., Greenland halibut, *Reinhardtius hippoglossoides*, Dawe et al., 1998) could have an increased Cd intake relative to predators that preferentially feed on larger specimens (e.g., sperm whales *Physeter macrocephalus*, bottlenose whales *Hyperoodon ampullatus*, and narwhales *Monodon monoceros*, Bjørke, 2001; Laidre and Heide-Jørgensen, 2005).

Gonatus fabricii is an important prey item for top predators in the Arctic pelagic food web (Bjørke, 2001; Bluhm and Gradinger, 2008), which could make it an important vector for Cd. The diet of the harbour porpoise, *Phocoena phocoena*, off Greenland

Table 3
Comparison of digestive gland Cd concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ dw) reported for various squid species.

Species	Mean \pm SD	Sampling Location	Study
<i>Gonatus fabricii</i>	35 \pm 15	Disko Bay, Greenland	This study
<i>Architeuthis dux</i>	65.8 \pm 43.1	Bay of Biscay	Bustamante et al. (2008)
<i>Illex argentinus</i>	1003 \pm 566	Central South Brazil Bight	Dorneles et al., 2007 ^a
<i>Illex argentinus</i>	92.5	Argentina	Falandysz (1988)
<i>Illex argentinus</i>	5.1 \pm 1.5	Patagonia	Gerpe et al. (2000)
<i>Illex argentinus</i>	145 \pm 65	Argentina	Kurihara et al. (1993)
<i>Illex coindetii</i>	0.12 \pm 0.05	Adriatic Sea	Storelli and Marcotrigiano, 1999 ^a
<i>Illex coindetii</i>	15 \pm 5	Bay of Biscay	Bustamante et al. (2002)
<i>Nototodarus gouldi</i>	50 \pm 25	Bass Strait, Australia	Smith et al. (1984)
<i>Nototodarus gouldi</i>	33 \pm 30	Port Phillip Bay, Australia	Finger and Smith (1987)
<i>Nototodarus sloanii</i>	111 \pm 95	Chatham Rise, New Zealand	Lischka et al. (2019,2020)
<i>Ommastrephes bartramii</i>	827 \pm 369	Sea of Japan	Kurihara et al. (1993)
<i>Ommastrephes bartramii</i>	287 \pm 194	Southern California	Martin and Flegal (1975)
<i>Sthenoteuthis oualaniensis</i>	198	Japanese Sea	Ichihashi et al., 2001 ^a
<i>Sthenoteuthis pteropus</i>	748 \pm 279	Eastern Tropical Atlantic	Lischka et al. (2018)
<i>Todarodes filippovae</i>	246 \pm 187	Indian Ocean	Kojadinovic et al. (2011)
<i>Todarodes filippovae</i>	98.5 \pm 67.2	Tasmania	Kojadinovic et al. (2011)
<i>Todarodes pacificus</i>	16.7	Sea of Japan	Ishizaki et al. (1970)
<i>Todarodes sagittatus</i>	85 \pm 37	Bay of Biscay	Bustamante et al. (2002)
<i>Todarodes sagittatus</i>	18 \pm 12	Bay of Biscay	Chouvelon et al. (2011)

^a Concentrations have been converted from wet weight.

contained squid, which have been suggested as the main source of Cd (Szefer et al., 2002). However, the Cd concentrations in the digestive gland of *G. fabricii* reported herein (4.36–63.14 $\mu\text{g}\cdot\text{g}^{-1}$ dw; Table 1) are higher compared to those from the liver of *P. phocoena* from coastal Greenland (3.45 $\mu\text{g}\cdot\text{g}^{-1}$ dw; Szefer et al., 2002). Similarly, Cd concentrations in the Greenland shark, *Somniosus microcephalus* (which primarily feeds on mammals and fish), were lower on average (i.e., 10.7 \pm 4.87 $\mu\text{g}\cdot\text{g}^{-1}$; Corsolini et al., 2014) than those measured in *G. fabricii* (Table 1). The energetic requirements of ectothermic fish and endothermic mammals differ and could impact Cd exposure and bioaccumulation (Jeziarska and Witeska, 2006). In addition, the biological effects of cold Arctic waters can be significant and must be taken into account when comparing Cd accumulation in mammals and fishes (Sokolova and Lannig, 2008). There is a higher energetic cost associated with living in the Arctic compared to temperate oceans, and ingesting a large amount of squid could significantly contribute to the Cd exposure in predators (Bustamante et al., 1998a,b). Due to its importance in the diets of many predators (Bjørke, 2001) and high Cd concentrations (Table 1), *G. fabricii* from West-Greenland waters appear to be an important vector in the transfer of Cd in the Arctic pelagic food web.

4.2.2. Mercury

Mercury is a highly bioaccumulative metal and is known to cause neurological damage in various organisms including humans (Campbell et al., 2005). There is very little information on Hg concentrations in marine Arctic invertebrates (Fort et al., 2016) despite their key role in the Arctic food web. Our results for Hg concentrations in digestive gland (0.01–0.11 $\mu\text{g}\cdot\text{g}^{-1}$ dw) and mantle tissue (0.03–0.26 $\mu\text{g}\cdot\text{g}^{-1}$) were comparable to those reported for the magister armhooked squid *Beryteuthis magister*, a gonatid from the Bering Sea ($-0.21 \pm 0.11 \mu\text{g}\cdot\text{g}^{-1}$ dw, converted from ww) (Cyr et al., 2019). In both tissues, Hg concentrations showed a positive linear correlation with size and trophic level (Tables 1 and 2). A correlation between size/age and Hg concentration has been previously shown in fish (Monteiro et al., 1996; Scott, 1974) and several cephalopod species (Chouvelon et al., 2011; Lischka et al., 2018; Monteiro et al., 1992; Rossi et al., 1993; Storelli and Marcotrigiano, 1999). In addition, the link between trophic position and Hg concentrations in marine organisms is well documented (e.g., Phillips et al., 1980; Power et al., 2002), including cephalopods (Chouvelon et al., 2011). Furthermore, high concentrations

(-0.33 – $2.44 \mu\text{g}\cdot\text{g}^{-1}$ dw) of Hg in marine mammals (e.g., ringed seal *Phoca hispida*, harp seal *Phoca groenlandica*, harbour porpoise *Phocoena phocoena*, minke whale *Balaenoptera acutorostrata* or narwhale *Monodon monoceros*) were previously reported from the Arctic (Dietz et al., 2000).

Detoxification mechanisms for Hg that involve Se have been reported for marine animals (Chen et al., 2006; Huang et al., 1995; Ralston et al., 2008; Storelli and Marcotrigiano, 1999). With increasing Hg concentrations, and trophic level, Se concentrations decrease (Fig. 4), this has been also shown for flying fish (*Exocoetus volitans*) and mitre squid (*Uroteuthis chinensis*) (Wang et al., 2018). The mantle tissue of *G. fabricii* showed a negative correlation between molar Se and Hg (Fig. 4a and b). A bioreduction of Se concentrations with increasing trophic level has been well documented in the marine food web, but is still not fully understood (Stewart et al., 2010). The ontogenetic differences in Hg and Se concentrations observed herein could be explained by the dietary shift that occurs with maturation, with adult specimens feeding on Hg rich fishes and increasing their own Hg concentrations. It is assumed that a molar excess of Hg relative to Se indicates the storage of organic Hg in the tissues of marine taxa (Ralston et al., 2008). However, Se concentrations measured in our study for *G. fabricii* were several magnitudes higher than Hg concentrations, which is in concordance with data from other invertebrates from Greenland waters (Riget et al., 2007; Ritterhoff and Zauke, 1997). This could indicate an opposite trend where increased Hg concentrations lead to decreased Se concentrations, or that Hg is stored by binding to muscular proteins without Se being involved in its metabolism.

4.3. Lead

Lead concentrations of *G. fabricii* found in the present study were higher in the digestive gland (0.03–0.37 $\mu\text{g}\cdot\text{g}^{-1}$ dw) than in the muscular tissues (0.01–0.16 $\mu\text{g}\cdot\text{g}^{-1}$ dw), which showed a similar trend with concentrations previously reported for *Todarodes filippovae* (Kojadinovic et al., 2011). Lead appears to be mainly stored and detoxified in the digestive gland (Penicaud et al., 2017; Smith et al., 1984). Specimen size was significantly correlated with Pb concentrations in both digestive gland and muscle tissue, with higher concentrations found in juvenile specimens (Tables 1 and 2). This ontogenetic decrease in Pb concentrations can be explained by

the dietary shift that occurs with maturation and Pb dilution with growth, because the accumulation of Pb in cephalopods is associated with feeding habits (Villanueva and Bustamante, 2006) and the bioreduction of Pb within food webs (Wang, 2002).

4.4. Silver

Silver concentrations showed a positive linear relationship with size, maturation stage, and stable isotope values. These relationships suggest an accumulation of Ag with age and trophic position. High concentrations of Ag in cephalopods have been previously reported in e.g. *Ommastrephes bartrami* and *Sthenoteuthis oualaniensis* (Martin and Flegal, 1975) and are likely the result of the high bioaccumulation capacities of cephalopods for this metal (Bustamante et al., 2004). In concordance with the concentrations measured in our study, elevated Ag concentrations have often been observed together with high Cu concentrations, which are required for hemocyanin synthesis (Beuerlein et al., 2002; Martin and Flegal, 1975). The concentrations measured in this study are comparable to concentrations measured in the giant squid *Architeuthis dux* (Bustamante et al., 2008, Table 4). Silver naturally occurs in the Earth's crust and shows a high affinity to sulphur ligands in seawater (Bell and Kramer, 1999; Dehn et al., 2006). Cephalopods are known to take up Ag from seawater (Bustamante et al., 2004; Miramand et al., 2006). Because of the vertical migration, adult *G. fabricii* could be exposed to higher Ag concentrations in deeper waters (Boyé et al., 2012; Zhang et al., 2004), creating an indirect link between Ag concentrations and trophic level. Our results suggest that *G. fabricii* could be a vector for the bioaccumulation of Ag in the food web. However, more research is needed on the concentrations of Ag in other Arctic predators to better understand the bioaccumulation of this trace element in the pelagic food web.

4.5. Trace element correlations

The highest Cu concentrations were found in the digestive gland of *G. fabricii*, which is considered the main storage organ for this metal (Finger and Smith, 1987; Miramand and Bentley, 1992). We found a positive correlation between Cd, Cu and Zn in the digestive gland; however, the significance of this correlation varied between ontogenetic stages, with the highest correlation found between Cd and Zn in the digestive gland of juveniles. Copper and Zn are cofactors in digestive enzyme systems and are involved in hemocyanin synthesis (Bustamante et al., 2002; Smith et al., 1984). The role of Cu and Zn in the detoxification process of Cd in cephalopods has been previously discussed (e.g., Bustamante et al., 2002; Miramand and Bentley, 1992), and excessive metals in the digestive gland cells can be bound to metalloproteins (Jebali et al., 2008; Viarengo and Nott, 1993). In addition, the strong correlation found between Cd and Zn in juveniles likely indicates a stronger detoxification effect in the digestive gland, which is necessary due to the high Cd

concentrations in their diet, rather than an inefficient Cd detoxification method that improves with maturity.

A positive correlation was observed between trace element concentrations of Cr and Ni in the digestive gland. The association of these two trace elements could be linked with anthropogenic activities. Elevated Ni concentrations in marine biota have been linked with mining activities and natural erosion (Bustamante et al., 2000; Pernice et al., 2009). The correlation of these two trace elements could be associated with their main commercial use, because steel production uses Ni as an alloy with Cr and both metals are associated in ores (Cano et al., 2014; Sedriks, 1982). Therefore, an accumulation of Ni could likely result in a correlated accumulation of its associated metals (e.g., Co, Cr, Mn; Monniot et al., 1994). Both Ni and Cr can impact pelagic food webs through the bioconcentration in invertebrates and their predators (Campbell et al., 2005).

4.6. *Gonatus fabricii* as a vector of trace elements

Our results indicate that *Gonatus fabricii* is an important vector for the transfer of contaminants into the deep-sea pelagic food web due to its high abundance, its role as dominant prey and its ontogenetic migration into deeper waters. In addition, *G. fabricii* may also transfer contaminants to benthic and benthopelagic food webs via benthic-pelagic coupling. Gonatids undergo a single reproductive cycle (semelparous life strategy) (Boyle and Rodhouse, 2005; Laptikhovskiy et al., 2007). Females hold on to the eggs in the water column during an extensive brooding period (likely ~2 years) as has been observed for *G. onyx* in Monterey Canyon, off California at depths between 1,539 and 2,522 m (Seibel et al., 2005), which is followed by death. After death, Pacific gonatids sink to the seafloor where they represent an important food source for scavenging fauna (Hoving et al., 2017). Similarly, post-spawning carcasses of *G. fabricii* in the northern Atlantic are likely consumed by benthic scavenging fauna including fish (e.g., grenadiers, *Coryphaenoides* spp.; Martin and Christiansen, 1997) but *in situ* observations of gonatid carcasses remain undocumented from the Atlantic. Trace elements are accumulated with increasing trophic level in the deep pelagic ocean (Atwell et al., 1998; Campbell et al., 2005). The combination of ontogenetic migration, high abundance, terminal spawning, and the accumulation of trace elements along the pelagic food chain via consumption of meso- and bathypelagic fishes by *G. fabricii* suggests that significant amounts of Cd and Hg may be transported to the deep sea. Sinking carcasses of spent *G. fabricii* may then potentially introduce these contaminants to the benthic food web via benthic and benthopelagic scavengers. Future studies should focus on Cd and Hg concentrations in benthic scavengers of the northern seas to test this hypothesis.

Table 4
Comparison of digestive gland Ag concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ dw) reported for various squid species.

Species	Mean \pm SD	Sampling Location	Study
<i>Gonatus fabricii</i>	0.82 \pm 0.55	Disko Bay, Greenland	This study
<i>Architeuthis dux</i>	1.90 \pm 0.47	Bay of Biscay	Bustamante et al. (2008)
<i>Nototodarus gouldi</i>	3.3 \pm 1.4	Bass Strait, Australia	Smith et al. (1984)
<i>Ommastrephes bartrami</i>	12.1 \pm 8.6	Southern California	Martin and Flegal (1975)
<i>Sthenoteuthis oualaniensis</i>	24.1 \pm 10.9	Southern California	Martin and Flegal (1975)
<i>Sthenoteuthis pteropus</i>	9.86 \pm 3.44	Eastern Tropical Atlantic	Lischka et al. (2018)
<i>Todarodes filippovae</i>	3.04 \pm 1.55	Tasmania	Kojadinovic et al. (2011)
<i>Todarodes filippovae</i>	3.40 \pm 1.60	Indian Ocean	Kojadinovic et al. (2011)
<i>Todarodes pacificus</i>	1.86 ^a \pm NA	Sea of Japan	Ichihashi et al. (2001)

^a Concentrations have been converted from wet weight.

4.7. Conclusion

Overall, trace element concentrations measured in *G. fabricii*, collected off West Greenland, were on an intermediate level when compared to loliginid or ommastrephid squids. However, we found significant differences in trace element accumulation, in particular for Cd, with maturity stage and trophic position. Our findings suggest that concentrations of most trace elements vary with size in *G. fabricii* and support an ontogenetic change in diet. This implies that in the pelagic Arctic food web, the transfer of trace elements to predators depends on the size/age class of the squids. Predators that feed mainly on juvenile *G. fabricii* (e.g. Greenland halibut) might have an increased Cd intake compared to predators mainly feeding on adult specimens (e.g., sperm whales, bottlenose whales and narwhales). Conversely, Hg levels were higher in mature individuals and would have a stronger effect on predators that feed predominantly on mature *G. fabricii*. Future studies should be conducted on *G. fabricii* and other cephalopod species in Arctic waters in order to clarify our understanding of the element transfers in the marine Arctic food web, which will be crucial for tracing the bioaccumulation of contaminants in Arctic marine mammals, birds, and predatory fishes.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.envpol.2019.113389>.

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