

High cadmium and mercury concentrations in the tissues of the orange-back flying squid, *Sthenoteuthis pteropus*, from the tropical Eastern Atlantic

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ABSTRACT

The orange-back flying squid, *Sthenoteuthis pteropus*, plays an important role in the eastern tropical Atlantic Ocean (ETA) pelagic food web, as both predator and prey. Specimens of *S. pteropus* were caught off the Cape Verde Islands and concentrations of Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V, and Zn were measured in the digestive gland. Among the analysed elements, Cd showed the highest average concentration with values among the highest ever recorded in cephalopods. In addition to the digestive gland, Hg concentrations were also analysed in the buccal mass and mantle tissue. Among the three tissues, buccal mass showed the highest Hg concentrations. In females, Hg concentrations in the buccal mass were positively correlated with stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and mantle length, showing both bioaccumulation with age and bioamplification along the trophic levels. High Cd and Hg concentrations in the digestive gland and muscle respectively would lead to elevated exposure of squid-eating top predators such as yellowfin tuna, swordfish or dolphinfish, which are commercially harvested for human consumption. This study provides a deeper understanding of the trace element contamination in an abundant and ecologically important, but poorly studied pelagic squid in the ETA.

1. Introduction

Non-essential elements, such as silver (Ag), cadmium (Cd), mercury (Hg) and lead (Pb), are usually present in the marine environment at trace concentrations (Moore and Ramamoorthy, 2012) and have toxic effects at high concentrations (Järup and Åkesson, 2009). Anthropogenic activities like agriculture, industry, and mining can elevate concentrations of these elements to levels that can affect the health of the marine community (Ansari et al., 2004; Amiard et al., 2006). In contrast to organic pollutants, trace elements remain in the ecosystem permanently and are not degraded or eliminated (Rainbow, 1995). Once introduced into ecosystems, they bioaccumulate in marine organisms and are transferred along food webs, some of them reaching high concentrations at higher trophic levels (Fisher and Reinfelder, 1995). Habitat, diet, life cycle and ontogenic stages are among the main drivers of the contamination of an organism (Förstner and Wittmann, 2012).

Cephalopods play a pivotal role in marine food webs (Piatkowski et al., 2001), either as prey for marine mammals (Clarke, 1996; Klages, 1996), seabirds (Croxall and Prince, 1996) and predatory fishes (Smale,

1996), or as predators of crustaceans, small fishes and other cephalopods (Rodhouse and Nigmatullin, 1996). They have become an indicator species for studying marine ecosystems as over the last few decades, global cephalopod populations and abundances are increasing. This proliferation is likely a result of global climate change, overfishing of apex predators, or a combination of both (Doubleday et al., 2016). They are also known to rapidly accumulate both organic and inorganic contaminants in their tissues (Penicaud et al., 2017; Rodrigo and Costa, 2017). Cephalopods are therefore considered biological vectors of contaminants in marine food webs (Bustamante et al., 1998). Although it is known that trace element concentrations of cephalopods are influenced by life cycle, diet and habitat (Bustamante et al., 2006; Choy et al., 2009; Chauvelon et al., 2011), the level of contamination of cephalopod species in many oceanic regions remains widely unknown as most studies concentrate on commercial coastal species. It is therefore particularly relevant to determine the levels of contaminants and to understand the explaining drivers in abundant oceanic cephalopod species, as they are important components in the food chain.

The “orange-back squid” (*Sthenoteuthis pteropus*, Steenstrup 1855, Ommastrephidae) is highly abundant within the epi- and mesopelagic

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zones of the eastern tropical Atlantic Ocean (ETA) (Arkhipkin and Mikheev, 1992; Zuev and Nikolsky, 1993). *Sthenoteuthis pteropus* shows strong sexual size dimorphism, high fecundity, long intermittent spawning, active feeding, and exhibits somatic growth during spawning (Arkhipkin and Mikheev, 1992; Zuev and Nikolsky, 1993; Laptikhovskiy and Nigmatullin, 2005). *Sthenoteuthis pteropus* is a good model squid as it shares these traits with many other ommastrephids (Zuev and Nikolsky, 1993), the family of oceanic squids that is intensively fished all over the world. *Sthenoteuthis pteropus* occurs within the Tropical Atlantic Ocean, with the eastern Atlantic representing one of the main large-scale productive zones where abundances can exceed 250 kg/km², roughly 750 individuals/km² (Zuyev et al., 2002; Arkhipkin and Mikheev, 1992). They constitute an important prey for swordfish (*Xiphias gladius*), dolphinfish (*Coryphaena hippurus*), yellowfin tuna (*Thunnus albacares*) and oceanic whitetip shark (*Carcharhinus longimanus*) (Hernández-García, 1995; Zuyev et al., 2002). As active predator, *S. pteropus* feeds intensely on myctophid fishes, crustaceans (such as copepods and euphausiids), and other cephalopods (Zuyev et al., 2002; Merten et al., 2017). Late juveniles and adult individuals of the genus *Sthenoteuthis* undergo daily migration to the epipelagic layer at night for feeding and descend to 800–1200 m in the morning (Zuyev et al., 2002). *Sthenoteuthis pteropus* has been recorded from oxygen minimum zones, and hypoxia has been proposed to be one of the most important factors in speciation of species of this genus (Moiseev, 1991; Shulman et al., 2002; Zuyev et al., 2002). Another species of the same genus, *S. oualaniensis*, has been recorded feeding in areas of the Arabian Sea with strong hypoxia (Snyder, 1998; Zuyev et al., 2002). Deep oxygen-depleted parts of the ocean are known to exhibit higher organic Hg concentrations (Cossa et al., 1997; Choy et al., 2009), therefore diel vertical migration by *S. pteropus* might increase their exposure to trace elements as they venture into the deep sea.

In this context, the aim of this study was to investigate the concentrations of selected trace elements (i.e., Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V, and Zn) in the most abundant ommastrephid squid species in the ETA, *S. pteropus*. The digestive gland was selected to investigate the levels of these elements as it represents the storage organ of many contaminants, including trace elements (Penicaud et al., 2017; Rodrigo and Costa, 2017). We also considered Hg in muscular tissues, i.e. the mantle muscle and the buccal mass. Indeed, the mantle muscle represents the main reservoir of Hg in cephalopods (Bustamante et al., 2006). Moreover, establishing the Hg concentrations relationship between both tissues would allow using the buccal mass, one of the last remaining tissue in the stomach contents of predators, to infer Hg concentrations in the squids. In order to determine the influence of intrinsic and extrinsic factors of trace element concentrations in *S. pteropus*, trace elements have been analysed considering the effects of body size (reflecting age) and the stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) used as biochemical tracers of the cephalopod habitat and trophic level, respectively.

2. Material and methods

2.1. Study area and sampling

Specimens were collected during a research cruise on the German RV *Maria S. Merian* (MSM49). In total, 61 specimens of *S. pteropus* (Cephalopoda: Ommastrephidae) were collected. The sampling area was between 12–18°N and 20–24°W around the Republic of Cape Verde in the eastern tropical Atlantic (ETA) and included seven different sampling stations (Christiansen et al., 2016, Fig. 1.). Specimens were collected by fishing rods using squid jigs. Specimens were killed immediately by decapitation. Data were collected on board for sex, maturity stage (following Arkhipkin, 1992), size and weight. Specimens were dissected on board and tissue samples (mantle, buccal mass, and digestive gland) were collected. Specimens comprised 12 males (dorsal mantle length, DML = 184–210 cm; Body weight BW = 133–400 g)

and 49 females (DML: 188–475 cm; BM: 120–4400 g). Tissue samples of buccal mass and digestive gland were not available for all specimens as these were later additions to the sampling protocol. Aliquots of tissues were stored at – 80 °C in individual plastic vials for later analysis.

2.1.1. Analytical procedure

Concentrations of Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V, and Zn were determined for digestive gland samples (n = 26) as this organ is known to be the main trace element storage organ in cephalopods (see the review of Penicaud et al., 2017). Additionally, because approx. 80% of the total Hg accumulated is associated with muscle tissue in cephalopods (Bustamante et al., 2006), Hg concentrations were also measured in samples of mantle muscle (n = 61) and buccal mass (n = 26), a muscle tissue often found in stomach content of predators. Tissue samples were freeze-dried for 48 h, ground into powder, and homogenized prior to trace element analyses.

Sample aliquots (ranging from 10 to 300 mg dry weight) were digested in a 3:1 mixture of 65% HNO₃ (Merck, suprapur quality) and 37% HCl (Merck, suprapur quality). After overnight acidic digestion, mineralization of samples was completed by heating for 30 min in a microwave with a maximum temperature of 105 °C. The trace element concentrations were then 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) and lobster hepatopancreas (TORT-3, National Research Council, Canada) were treated and analysed in the same way as the other samples. Recoveries of the elements ranged from 83% to 122% (n = 9). The detection limits for Ag, Co, Cd, Pb were 0.025 µg g⁻¹, Fe was 5 µg g⁻¹, Cu and Se were 0.125 µg g⁻¹, Ni was 0.05 µg g⁻¹, and V was 0.5 µg g⁻¹, based on 200 mg of sample material diluted in a volume of 50 ml. Results for the trace element concentrations are expressed in µg g⁻¹ dry weight (dw).

Total Hg concentrations (hereafter Hg concentrations) were measured using an Advanced Mercury Analyser (ALTEC AMA 254 with a detection limit of 0.05 ng) on homogenized dry tissue (1–2 mg dw) as described in Bustamante et al. (2006). For every 10 samples, one CRM TORT-2 (lobster hepatopancreas; National Research Council, Canada) was analysed. Recovery was 98% and results for Hg were also expressed in µg g⁻¹ relatively to the dw.

2.2. Isotopic analysis

2.2.1. Stable isotope analysis

Stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) were measured in mantle muscle tissue, as it is a common reference tissue in food web studies that use these biochemical tracers (Hobson and Welch, 1992; Pinnegar and Polunin, 1999). Lipids were extracted using chloroform. Isotopic value of ratios of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were measured using an elemental analyser system (NA 1110, Thermo, Milan, Italy) connected to a temperature-controlled gas chromatography oven (SRI 9300, SRI Instruments, Torrance, CA, USA), which contained a column for permanent gases. Separated sample gases and the reference gases N₂ and CO₂ were transferred via a ConFloIII™ interface (Thermo Fisher Scientific, Bremen, Germany) to the isotope ratio mass spectrometer (Delta^{plus} Advantage, Thermo Fisher Scientific). The measured isotopic values are given as δ values in per mil deviation (‰) from the standard reference material Vienna PeeDee Belemnite (VPDB) and atmospheric nitrogen following Eq. (1), where δX refers to ¹³C or ¹⁵N and R represents the ratio of the heavy isotope to the light isotope (¹³C/¹²C or ¹⁵N/¹⁴N).

$$\delta X = \left[\frac{R_{\text{Sample}}}{R_{\text{Standard}}} - 1 \right] \times 1000 \quad (1)$$

Laboratory gas cylinders of CO₂ and N₂ were used as working

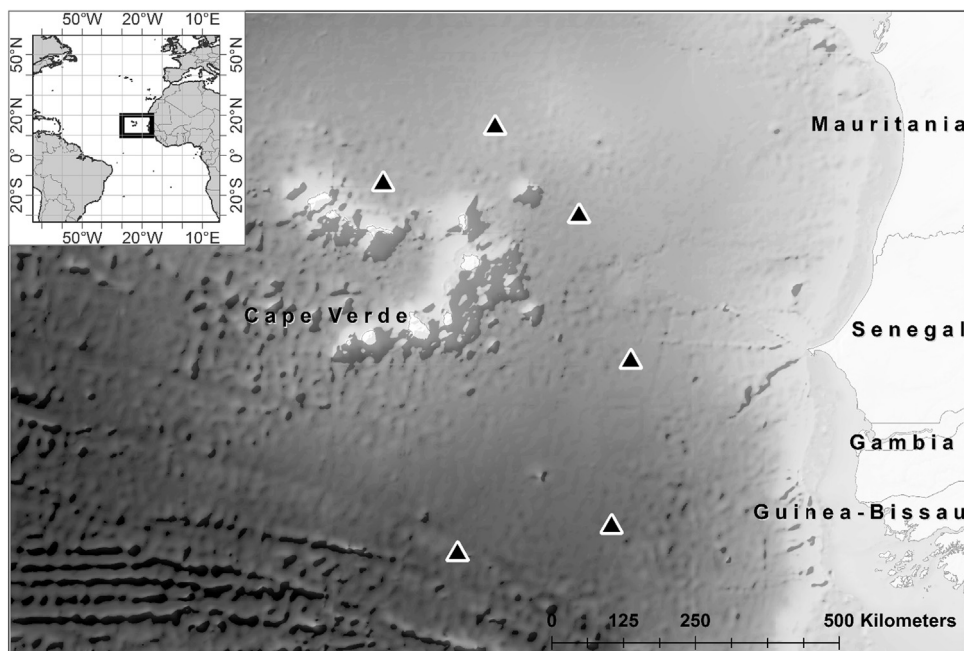


Fig. 1. Map of sampling area including the stations where specimens were jigged by hand.

standards and calibrated against primary solid standards (IAEA-N1, -N2, -N3, USGS24, NBS22). The lab standard acetanilide used to estimate C and N content for each sample series was measured every seventh sample and had a standard deviation of $\pm 0.16\%$ for $\delta^{15}\text{N}$ and $\pm 0.39\%$ for $\delta^{13}\text{C}$.

2.2.2. Data analyses

Data exploration and analyses were conducted in base R version 3.3.3 (R Core Team, 2017). Prior to analysis, data exploration was conducted following Zuur et al. (2010) to test for assumptions and for collinearity of explanatory variables. Correlations between variables were visualized in a correlogram with the R package ‘*corrgram*’ (Wright, 2012; Fig. 1. Suppl. material).

Informed by the results of the data exploration, Cd concentrations in the digestive gland and Hg concentrations in the mantle, digestive gland and buccal mass were selected for analysis. Log-standardised mean Cd and Hg concentrations were chosen as response variables, and body size (DML) and stable isotope ratios were chosen as explanatory variables. Due to the small sample size of digestive glands collected from male specimens ($n = 3$), male data were excluded from most analyses. Only for Hg in the mantle tissue was male data included in the analysis ($n = 12$) and sex included as an explanatory variable. Analyses of covariance (ANCOVA) were conducted to test for effects of body size and stable isotope ratios on Cd and Hg concentrations, as well as to check for interactions between explanatory variables. Variables were added to the ANCOVA in the following order: $\delta^{15}\text{N}$, sex, DML, to test whether there was a sex difference once the stable isotope values were accounted for as well as a size difference once sex was accounted for.

Next, generalised linear models were applied to the Hg and Cd data, using the same set of response and explanatory variables. Candidate models were compared using model AIC (Akaike Information Criterion) and the best models selected. Residual plots were checked for homogeneity, normality and independence of residuals. Because body size and stable isotope values are confounded, only the explanatory variable with the higher explanatory power was included in the final linear model.

3. Results

3.1. Trace element concentrations in the digestive gland

Among the 14 trace elements analysed in the digestive gland, the highest concentrations were found for Cd (217–1411 $\mu\text{g g}^{-1}$ dw), Cu (9–828 $\mu\text{g g}^{-1}$ dw), Fe (127–798 $\mu\text{g g}^{-1}$ dw), Zn (72–552 $\mu\text{g g}^{-1}$ dw), and Se (22–86 $\mu\text{g g}^{-1}$ dw) (Table 1). Trace elements ranged between 0.09 and 1411 $\mu\text{g g}^{-1}$ dw in the digestive gland and mantle of both sexes, with the following average concentrations in descending order: Cd > Fe > Zn > Cu > Se > Co > As > Ag \approx Ni > Mn > V > Cr > Hg \approx Pb. Negative correlations with size (DML) were found for Cd, Co, Cu, and Fe concentrations within the correlation graph (Fig. S1, Suppl. material).

The relationship between trace element concentrations in the digestive gland and specimen size (using DML) evaluated through correlation are shown in Fig. S1, Suppl. material. With increasing specimen size, Cd ($r = -0.62$, $p < 0.01$) and Co ($r = -0.71$, $p < 0.001$, Fig. S1, Suppl. material) concentrations decreased. Similarly, concentrations of Fe ($r = -0.63$, $p < 0.05$) and Pb ($r = -0.55$, $p < 0.05$) significantly decreased with DML (Fig. S1, Suppl. material).

In the digestive gland, some trace elements were positively correlated to each other: Fe with Mn ($r = 0.92$, $p < 0.001$), Ni with Cr ($r = 0.71$, $p < 0.001$), Pb with Co ($r = 0.87$, $p < 0.001$), and Se with As ($r = 0.83$, $p < 0.001$) and Zn ($r = 0.59$, $p < 0.05$) (Fig. S1, Suppl. material).

ANCOVA results showed a strong positive relationship between $\delta^{15}\text{N}$ and body size (DML) on Cd concentrations in the digestive gland (Table 2). Body size was chosen as an explanatory variable in the linear model, with a small but highly significant negative effect explaining approximately 39% of the variation in Cd concentrations (Table 3).

3.2. Mercury distribution and concentrations

Among the different tissues analysed, Hg concentrations ranged from 0.14 to 1.18 $\mu\text{g g}^{-1}$ dw, with 0.14 to 0.86 $\mu\text{g g}^{-1}$ dw in the mantle, and 0.20 to 1.13 $\mu\text{g g}^{-1}$ in the digestive gland and 0.26 to 1.18 $\mu\text{g g}^{-1}$ dw in the buccal mass (Table 1). The buccal mass exhibited significantly higher ($F_{1,20} = 23.46$, $p < 0.001$) Hg concentrations compared to those in the other tissues, respectively. A correlation was detected

Table 1

Dorsal mantle length (DML), muscle stable isotope N and C values, muscle Hg ($\mu\text{g g}^{-1}$ dw), and trace elements concentrations ($\mu\text{g g}^{-1}$ dw) in the digestive gland of females, males and all individuals of the orange-back flying squid, *S. pteropus*. Values are presented as mean \pm standard deviation as well as the minimum and maximum concentrations.

Property	Females (n = 22)	Males (n = 3)	All (Total n = 26)
Digestive gland			
Ag $\mu\text{g g}^{-1}$ dw	9.58 \pm 3.52 3.02–15.87	11.96 \pm 3.19 8.47–14.73	9.86 \pm 3.44 3.02–15.87
As	19.6 \pm 12.3 7.8–65.0	10.1 \pm 1.6 9.0–11.8	18.3 \pm 11.7 7.8–65.0
Cd	724 \pm 295 217–1411	934 \pm 55 874–983	748 \pm 279 217–1411
Co	16.3 \pm 10.5 4.9–40.4	45.1 \pm 26.8 23.4–75.0	19.9 \pm 15.4 4.9–75.0
Cr	1.93 \pm 2.26 0.09–8.49	0.84 \pm 0.92 0.16–1.89	1.75 \pm 2.13 0.09–8.49
Cu	139 \pm 196 9.0–828	274 \pm 311 63–631	152 \pm 206 9.0–828
Fe	422 \pm 182 127–797	447 \pm 128 326–580	431 \pm 173 127–797
Mn	4.2 \pm 1.5 1.8–7.4	2.8 \pm 0.7 2.2–3.5	4.1 \pm 1.5 1.8–7.4
Ni	7.27 \pm 5.48 2.42–29.03	7.61 \pm 4.75 4.74–13.09	7.39 \pm 5.21 2.42–29.03
Pb	0.296 \pm 0.141 0.135–0.710	0.709 \pm 0.481 0.414–1.265	0.344 \pm 0.231 0.135–1.265
Se	45 \pm 17 22–86	53 \pm 9 47–63	46 \pm 16 22–86
V	2.49 \pm 1.39 0.41–5.41	1.56 \pm 0.61 1.06–2.24	2.72 \pm 2.11 0.41–5.41
Zn	201 \pm 114 87–552	88 \pm 20 72–111	187 \pm 111 72–552
Hg	0.45 \pm 0.19 0.20–1.13	0.32 \pm 0.11 0.23–0.44	0.43 \pm 0.18 0.20–1.13
DML mm	290 \pm 66 211–455	199 \pm 9 189–210	271 \pm 70 189–455
Buccal mass			
Hg $\mu\text{g g}^{-1}$ dw	0.51 \pm 0.20 0.26–1.18	0.34 \pm 0.04 0.31–0.38	0.48 \pm 0.19 0.26–1.18
Muscle			
$\delta^{15}\text{N}$ (%)	11.5 \pm 0.7 9.7–12.2	10.6 \pm 0.6 9.9–12.8	11.3 \pm 0.8 9.7–12.8
$\delta^{13}\text{C}$ (%)	–16.4 \pm 0.6 –17.324 to (–15.163)	–16.4 \pm 0.5 –16.874 to (–15.641)	–16.4 \pm 0.6 –17.324 to (–15.163)
Hg [†] $\mu\text{g g}^{-1}$ dw	0.33 \pm 0.13 0.15–0.86	0.21 \pm 0.05 0.14–0.34	0.33 \pm 0.14 0.14–0.86

Explanations: Values are expressed as mean \pm standard deviation and minimum range; DML – dorsal mantle length; $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ – muscle stable isotope values for nitrogen and carbon, respectively.

* Mantle tissue (n_♀ = 49, n_♂ = 12).

between Hg concentrations in both muscular tissues ($r = 0.906$). This linear relationship (Eq. (2), $p < 0.001$; $r^2 = 0.821$) could be used to predict mantle Hg concentrations in the instance of only buccal mass tissue being available, e.g., when beaks are sampled from predator stomachs.

Hg concentrations in the digestive gland were not related to body size, $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ (Table 3).

$$\text{Mantle (Hg)} = 0.7 \times \text{Buccal mass (Hg)} + 0.011 \quad (2)$$

The ANCOVA results showed a significant relationship between $\delta^{15}\text{N}$ values and Hg concentrations within the mantle tissue samples. After the stable isotope value as accounted for, sex showed a significant effect ($p < 0.05$) as well as DML ($p < 0.001$, Table 2). DML and $\delta^{15}\text{N}$ showed very large F-values: 63.088 and 46.321 respectively compared to sex (6.496; Table 2). DML was chosen as an explanatory variable in the linear model, with a highly significant negative effect explaining approximately 71% of variation in mantle Hg concentrations (Table 3).

Table 2

ANCOVA table for the linear models fitted to the Cd concentration in the digestive gland and Hg concentrations in the three different tissues.

	Df	Sum of squares	Mean square	F value	Significance
Cd Digestive gland					
$\delta^{15}\text{N}$	1	0.191	0.191	11.215	0.003**
DML	1	0.234	0.234	13.712	0.002**
Residuals	19	0.324	0.017		
Hg Mantle					
$\delta^{15}\text{N}$	1	0.028	0.028	63.088	< 0.001***
Sex	1	0.003	0.003	6.496	0.014*
DML	1	0.020	0.020	46.321	< 0.001***
Residuals	47	0.020	0.000		
Hg Buccal Mass					
$\delta^{15}\text{N}$	1	0.007	0.007	6.433	0.0213*
DML	1	4.4×10^{-5}	4.4×10^{-5}	0.041	0.842
Residuals	17	0.018	0.001		
$\delta^{15}\text{N}$	1	0.007	0.007	6.433	0.0213*

Explanations: Df – degrees of freedom. Asterisks show the level of significance.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

For the buccal mass tissue samples, ANCOVA results showed a slightly negative significant relation of $\delta^{15}\text{N}$ on Hg concentrations (Table 2). For the linear model, DML was chosen as the explanatory variable as it explained approximately 30.2% of the variation (Table 3). No significant effect of Hg concentration and the three explanatory variables was observed within the digestive gland tissue nor were any significant effects of $\delta^{13}\text{C}$ on Hg concentrations observed in the three tissue types.

4. Discussion

Although cephalopods are of growing interest to fisheries and their abundance in the oceans is increasing (Doubleday et al., 2016), knowledge of their role in bioaccumulation levels is still limited for most species, in particular for species inhabiting the open ocean and deep sea (see the review of Penicaud et al., 2017). This recent review on trace element concentrations in cephalopods highlighted their high Cd bioaccumulation capacity, especially for the ommastrephid squids, consistent with the finding from the present study. Nevertheless, most available data for this family has been recorded in specimens belonging to the genera *Todarodes* and *Illex* (e.g. Gerpe et al., 2000; Ichihashi et al., 2001a; Pierce et al., 2008; Kojadinovic et al., 2011). For the genus *Sthenoteuthis*, to the best of our knowledge, only two studies report on trace element concentrations, and they both deal with *S. oualaniensis* from the Pacific Ocean (Martin and Flegal, 1975; Ichihashi et al., 2001b). Thus, the present study reports the first data on trace elements in the orange-back flying squid *S. pteropus*, an oceanic squid from the tropical and subtropical Atlantic.

4.1. Cadmium concentrations in the digestive gland

Due to its key role in storage and detoxification of trace elements, the digestive gland of cephalopods generally presents very high concentrations of some of them (Penicaud et al., 2017). In *S. pteropus*, the digestive gland had Cd concentrations that are among the highest ever recorded for cephalopods. The only squid in which higher Cd values ($1003 \pm 566 \mu\text{g g}^{-1}$ ww/ c.a. $3711 \pm 2094 \mu\text{g g}^{-1}$ dw) were measured is *Illex argentinus* from the South-western Atlantic Ocean offshore Argentina (Dorneles et al., 2007). Thereby, the capacity of *S. pteropus* to bioaccumulate Cd at such elevated concentrations is noteworthy. Indeed, average Cd concentrations were higher than those of the essential elements Cu, Fe, and Zn (with values 5, 1.7, 4 times higher, respectively) which are usually present at elevated concentrations in cephalopods to cover their metabolic needs (e.g., Cu for the respiratory

Table 3

Coefficients, lower (2.5%) and upper (97.5%) confidence interval, p-value and coefficient of determination (R-square) for the linear models.

Model	Coefficient name	Coefficient	2.5%	97.5%	p-value	R-square
Cd	Intercept	3.374	3.065	3.684		
Cd	DML	− 0.002	− 0.003	− 0.001	0.001**	0.390
HgM	Intercept	− 0.017	− 0.040	0.006		
HgM	DML	4.9×10^{-4}	4.1×10^{-4}	5.7×10^{-4}	< 0.001***	0.710
HgBM	Intercept	− 2.153	− 3.772	− 0.534		
HgBM	DML	0.009	0.0033	0.015	0.003**	0.302

Explanations: Asterisks show the level of significance: * p < 0.05.

** p < 0.01.

*** p < 0.001.

Table 4Cadmium concentrations ($\mu\text{g g}^{-1}$ dw) in muscle and digestive gland tissues from various squid species reported in previous studies. When available, the dorsal mantle length (DML) (mm) of the study animals is provided.

Taxa	Region	Mantle concentration	Digestive gland concentration	DML [mm]	Ref.
Myopsida					
<i>Alloteuthis subulata</i>	Bay of Biscay		$0.37 \pm 0.17^{\dagger}$		Bustamante et al. (1998)
<i>Loligo opalescens</i>	California		103.25 ± 25.81		Martin and Flegal (1975)
<i>Loligo forbesi</i>	English Channel		$0.46 \pm 0.30^{\dagger}$		Bustamante et al. (1998)
<i>Loligo vulgaris</i>	Bay of Biscay		0.03^{\dagger}		Bustamante et al. (1998)
Oegopsida					
<i>Illex argentinus</i>	Argentina	9.22 ± 4.15 to 21.8 ± 10.99*	3.26 ± 0.75 to 7.01 ± 2.33 [†]	183 ± 19 to 272 ± 34	Gerpe et al. (2000)
<i>Illex argentinus</i>	Argentina		0.6		Storelli et al. (2012)
<i>Illex argentinus</i>	Argentina		$1002.9 \pm 566^{\dagger}$		Dorneles et al. (2007)
<i>Illex argentinus</i>	Argentina		90–95		Falandysz (1988)
<i>Illex coindetti</i>	Adriatic Sea	0.07 ± 0.02	$0.12 \pm 0.05^{\dagger}$		Storelli and Marcotrigiano (1999)
<i>Illex coindetti</i>	Bay of Biscay	$0.220 \pm 0.026^{\dagger}$			Lahaye et al. (2005)
<i>Ommastrephes batramii</i>	Japanese Waters		826.5 ± 369.1		Kurihara et al. (1993)
<i>Ommastrephes batramii</i>	Pacific		286.79 ± 194.39		Martin and Flegal (1975)
<i>Nototodarus gouldi</i>	Australia		50 ± 25		Smith et al. (1984)
<i>Nototodarus gouldi</i>	Australia		33 ± 30		Finger and Smith (1987)
<i>Nototodarus sloanii</i>	New-Zealand		425		Takeuchi et al. (1979)
<i>Sthenoteuthis pteropus</i>	Eastern Tropical Atlantic		747.80 ± 279.36	255–455	This study
<i>Sthenoteuthis oualiensis</i>	Sea of Japan		0.125		Ichihashi et al. (2001a)
<i>Todarodes pacificus</i>	Sea of Japan	0.075–0.093 [†]	16.675	152–236	Ishizaki et al. (1970)
<i>Todarodes sagittatus</i>	Bay of Biscay		$8.41 \pm 5.99^{\dagger}$		Bustamante et al. (1998)
<i>Todarodes filipovae</i>	Indian Ocean	0.93 ± 0.79	246 ± 187	251–430	Kojadinovic et al. (2011)
<i>Todarodes filipovae</i>	Tasmania	0.20 ± 0.14	98.5 ± 67.2	375–538	Kojadinovic et al. (2011)
<i>Todaropsis eblanae</i>	Bay of Biscay	$0.216 \pm 0.147^{\dagger}$			Lahaye et al. (2005)
<i>Histioteuthis sp.</i>	Bay of Biscay		$0.50 \pm 0.146^{\dagger}$		Lahaye et al. (2005)
<i>Architeuthis dux</i>	Atlantic Spanish waters	1.86–3.32	0.32–1.561	600–1200	Bustamante et al. (2008)

* Values expressed in $\mu\text{g g}^{-1}$ wet weight.

pigment, haemocyanin; Penicaud et al., 2017). In other ommastrephid squid species, elevated Cd concentrations ($748 \pm 280 \mu\text{g g}^{-1}$ dw) have been documented as well (Table 4). High Cd concentrations have been reported for the neon flying squid *Ommastrephes batramii* (Kurihara et al., 1993), and the purpleback flying squid *S. oualiensis* from the Pacific Ocean, the Chinese Sea and the Japan Sea (Wu et al., 2017; Ichihashi et al., 2001b; Martin and Flegal, 1975). However, none of the aforementioned studies report concentrations as elevated as those found in our study.

Our results provide new insights into the remarkable capacity of these oceanic ommastrephids to bioaccumulate Cd. These exceptional concentrations recorded in *S. pteropus* raise therefore the question of the source of and the reasons explaining the Cd accumulated in the specimens.

First, *S. pteropus* inhabits the Cape Verde Frontal Zone where the subtropical gyre and recirculation gyre converge, fostering a wind-driven coastal upwelling (Auger et al., 2015). These oceanographic conditions allow a cadmium and nutrient enrichment of water masses, especially in winter and spring (Horner et al., 2013). The high primary production that occurs in this zone leads to a high uptake and bioaccumulation of Cd from the upwelling source in biota (Auger et al., 2015). Finally, to a lesser extent, Saharan dust storms are known to

introduce nutrients and elements like Cd and Fe to the oligotrophic Atlantic Ocean. These storms foster the primary production and the potential integration of Cd in phytoplankton and subsequently into the food webs (Ridame et al., 2011; Kremling and Streu, 1993). This suggests that the high Cd concentrations observed in *S. pteropus* are derived from the naturally Cd-enriched environment around Cape Verde Islands.

Second, diet is considered as the main Cd bioaccumulation pathway for cephalopods (see the review of Penicaud et al., 2017 but also Bustamante et al., 2002a, Koyama et al., 2000), implying that the high concentrations observed may result from the consumption of peculiar prey enriched in Cd. *Sthenoteuthis pteropus* feeds on small squids (e.g. *Onychoteuthis banksii*, Euplotelidae) and myctophid fish in the upper epipelagic to mesopelagic layers (Zuev et al., 1985; Arkhipkin and Mikheev, 1992; Merten et al., 2017), which are known to have high Cd concentrations in their tissues compared to epipelagic prey (Lahaye et al., 2005; Bustamante et al., 2003; Cipro et al., 2018).

Third, the ommastrephids possess a particular physiological characteristic that affects their metabolism of trace elements resulting in high Cd concentrations. When comparing Cd concentrations reported in Ommastrephidae and Loliginidae (Table 4), the ommastrephid *Todarodes sagittatus* displayed higher Cd concentrations than the Loliginid

Loligo forbesi, which inhabits the same ecological niche in the Bay of Biscay (Chouvelon et al., 2012). The descriptions of the digestive gland cells of *T. sagittatus* showed, unlike those of *L. forbesi*, the typical ‘boule’ structure that is thought to contribute to the storage of large amounts of Cd (Boucaud-Camou and Yim, 1980; Boucher-Rodoni et al., 1987). *Sthenoteuthis pteropus* also possesses these structures, which could contribute to the high Cd accumulation in its digestive gland.

In addition, an association between concentrations of trace elements and size was observed for Cd (but also for Co, Cu, and Fe), where smaller male specimens exhibited the higher concentrations. Similar decreases in concentrations of some trace elements, including Cd, with individual size has been already observed for multiple cephalopod species e.g. *Loligo forbesi*, *L. vulgaris*, *Todarodes sagittatus* (e.g. Miramand et al., 2006; Dorneles et al., 2007; Chouvelon et al., 2011). This trend could result from three complementary explanations: 1) an ontogenic shift of the diet from Cd enriched prey to lesser contaminated ones. Specifically, the trophic regime of *S. pteropus* also changes with growth, switching from crustaceans to fish (Zuev and Nikolsky, 1993), the last generally showing lower Cd concentrations than crustaceans, including pelagic copepods (Chouvelon et al., 2011). This presumed diet change is also supported by the observed increase of $\delta^{15}\text{N}$ value with DML in the present study. 2) In addition, the decreasing Cd concentrations with DML could result from a significant growth dilution of the element within tissues considering the exceptional growth rate of this species, especially the females as they reach larger sizes than males (Takagi et al., 2002). 3) Finally, *S. pteropus* could exhibit detoxification processes that lead to decreased concentration levels with maturation (Bustamante et al., 2002b). However, Cd seem to be sequestered in the digestive gland (see the ‘boule’ structures mentioned above) for longer periods than other trace elements (Bustamante et al., 2002a). This could be one reason for the small negative effect of size observed on Cd concentrations.

Overall, the Cd bioaccumulation capacity in *S. pteropus* is likely to be mainly influenced by prey type (as shown by the $\delta^{15}\text{N}$ values from this study and Merten et al., 2017), which may be due to either the consumption of prey with high Cd concentrations, and/or the physiological processing capabilities of this species for Cd.

4.1.1. Mercury distribution in soft tissues

Mercury is an element of great concern because of its toxicity, especially under its methylated form. Indeed, Hg is methylated by marine microorganisms, bioaccumulates in marine taxa, and biomagnifies along the food chain reaching high concentrations in predators (Gray, 2002; Wang and Wong, 2003). Among marine organisms, most of the available information on Hg is related to top predators (mammals, birds and fish), mainly because they are relevant bioindicators of Hg contamination (e.g. Furness and Camphuysen, 1997) and more specifically for fish, because they constitute an important food source for humans (Booth and Zeller, 2005). Cephalopods have been suggested as the source of high Hg concentrations in fishes such as the wahoo *Acanthocybium solandri* and the swordfish *X. gladius* (e.g. Kojadinovic et al., 2006). However, information on Hg in cephalopod tissues is scarce, despite the fact that these molluscs occupy a pivotal position in food webs (Rodhouse et al., 2014).

In the present study, Hg concentrations varied between 0.15 and 1.18 $\mu\text{g g}^{-1}$ dw among the three tissue types. The mean concentration of Hg in the mantle is consistent with previous Hg concentrations recorded in ommastrephids from different regions of the world (Penicaud et al., 2017). Buccal mass tissue showed the highest Hg concentrations in this study, followed by the less dense mantle muscle tissue and the digestive gland. The buccal mass is one of the most muscular parts in cephalopods because it connects the beaks, and controls the beak movement and shearing. Dense muscular body parts in cephalopods have been found to accumulate the highest concentrations of Hg (e.g. arm tissue in *Octopus vulgaris*; Seixas et al., 2005). In other ommastrephid species (*Illex coindetii*, *Todarodes sagittatus*, *Todaropsis eblanae*), the highest Hg

concentrations were also found in muscular tissues whereas the digestive gland displayed relatively low concentrations (Bustamante et al., 2006). A reason could be the transfer of Hg from the digestive gland to the more muscular body parts (Seixas et al., 2005; Bustamante et al., 2006). Organic Hg has a strong affinity for the sulfhydryl groups of proteins in muscular tissues (Cappon and Smith, 1981; Bloom, 1992). A second reason for lower Hg concentrations in the digestive gland could be related to the capacity of this organ to demethylate methylmercury into inorganic Hg that is then eliminated from the organisms relatively rapidly (Lacoue-Labarthe et al., 2009).

The strong relationship between mantle and buccal mass Hg concentrations implies that buccal tissues could be a useful proxy for inferring mantle concentrations in squids that are otherwise difficult to sample, e.g. by sampling undigested buccal tissues in the stomach contents of predatory fishes. However, the correlation detected in this study will not necessarily hold when extrapolated to other populations or species. Further studies should also consider the concentrations of Hg in the beaks, being the tissue the most resistant to digestive processes of predators (Xavier et al., 2016).

Interestingly, the Hg concentrations in the mantle tissue unlike in the digestive gland from this study are strongly associated with the body size even after accounting for variation in sex and/or trophic level, which is consistent with data reported in other pelagic cephalopods (Bustamante et al., 2006). This suggests that muscular body parts act as storage organs for Hg whereas the Hg in the digestive gland is regulated through detoxification/elimination processes. Other studies also show that, in cephalopods, Hg concentrations are positively correlated with age and size (Barghigiani et al., 2000; Pierce et al., 2008; Storelli and Marcotrigiano, 1999). In addition, females of *S. pteropus* displayed higher Hg concentrations than males. This difference appears to be highly influenced by prey (as the $\delta^{15}\text{N}$ values show) but also by sex, with a size effect nested within sex. At maturity females are generally larger than males in ommastrephids. Such female biased sexual size dimorphism effect on Hg concentrations is known for many other cephalopod species (e.g. Bustamante et al., 2006; Pierce et al., 2008). Larger (older) females have experienced longer exposure periods (Arkhipkin and Mikheev, 1992) or hunted for bigger prey, allowing Hg to bioaccumulate over time.

4.1.2. Mercury concentrations related to trophic tracers

Mercury is mainly accumulated through the trophic pathway (Bustamante et al., 2006; Lacoue-Labarthe et al., 2009). Nitrogen stable isotope ratios allow the characterization of the trophic level and the $\delta^{13}\text{C}$ signature can be used as an indicator of the feeding habitat (DeNiro and Epstein, 1978). Generally, carbon isotope ratios are used in marine ecology to differentiate between benthic/neritic and pelagic/oceanic (depleted in $\delta^{13}\text{C}$) species (France, 1995; Tamelander et al., 2006). The $\delta^{13}\text{C}$ values found in this study showed no trend with increasing DML, and therefore suggest that there is no segregation of the feeding habitat in this species regardless the size or the sex.

The tissues of predators are usually enriched in $\delta^{15}\text{N}$ in comparison to their prey (Ruiz-Cooley et al., 2013). Therefore, these values could be used as an indicator for the trophic position of an individual if stable isotope values of prey and primary producers are available (Merten et al., 2017). In this study, larger specimens showed the highest Hg concentrations and also higher $\delta^{15}\text{N}$ isotope ratios. As $\delta^{15}\text{N}$ values increase with size (approximately one trophic level, 3.4%, assuming a constant isotopic baseline; Minagawa and Wada, 1984), it is likely that the trophic position also increases with size and fosters the Hg uptake. Nevertheless, the results should be interpreted with caution since no values for the stable isotope values of the prey were available. The shift with size could be attributed, as described above, to a shift in diet in favour of prey of higher trophic level or larger prey sizes, which would lead to increased Hg exposure for squids (Chouvelon et al., 2011). In keeping with that, the higher Hg concentrations in large females might indicate different energy needs and thus the consumption of more prey

(Pierce et al., 2008; Chouvelon et al., 2011). Some small male specimens displayed relatively high $\delta^{15}\text{N}$ values but their Hg concentrations remained in the lower range of recorded Hg concentrations. This could be due to physiological differences between the two sexes as larger females have a higher consumption rate than males, which might lead to higher Hg exposure and therefore higher Hg concentrations in their tissues. Similar inter-sex differences have been observed in other cephalopods such as *Loligo forbesi* (Monteiro et al., 1992; Bustamante et al., 2006). The $\delta^{15}\text{N}$ content of the muscle tissue in this study reaches a plateau with DML > 40 cm, which suggests a stable trophic position (Merten et al., 2017).

5. Conclusion

Sthenoteuthis pteropus plays a crucial role in the ETA food web, and contains especially high concentrations of Cd and Hg, non-essential elements that can be transferred to top predators. The results from this study could have important implications for the economy and human health, because some species that prey on *S. pteropus*, such as yellowfin tuna, swordfish or dolphinfish, are commercially harvested for human consumption within the ETA. We encourage further studies focussing on trace element concentrations in prey and predators of *S. pteropus* in this region. This could give insight into assumptions about trophic transfer of trace elements within this oceanic region and its impact on human health.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2018.07.087.

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