Variation of 210-polonium in the cephalopod community from the Bay of Biscay, North-East Atlantic

Paco Bustamante a,*, Aniel Guillen-Arruebarruena b, Thomas Lacoue-Labarthe b, Tiphaine Chouvelon c, d, Jéréme Spitz e, f, Michel Warnau b, Carlos M. Alonso Hernandez b, f

a Littoral Environnement et Sociétés (LIENSs), UMR 7266 CNRS-La Rochelle Université, 2 rue Olympe de Gouges, 17000, La Rochelle, France
b Centro de Estudios Ambientales de Cienfuegos, AP5, Ciudad Nuclear, Cienfuegos, Cuba
c Observatoire Pelagis, UAR 3462 CNRS-La Rochelle Université, 5 allées de l’Océan, 17000, La Rochelle, France
d Ifremer, Unité Contamination Chimique des Écosystèmes Marins (CCEM), Centre Atlantique, Rue de l’île d’Yeu, BP 21105, 44311, Nantes, France
e Centre d’Etudes Biologiques de Chizé (CEBC), UMR 7372 CNRS-La Rochelle Université, 405 Route de Prise la Charrière, 79360, Villiers-en-Bois, France
f International Atomic Energy Agency – Environment Laboratories (IAEA-EL), 4 Quai Antoine 1er, MC-98000, Monaco

ARTICLE INFO

Keywords:
Bioaccumulation
Polonium
Squid
Octopus
Cuttlefish
Trophic transfer

ABSTRACT

Among natural radionuclides, 210Po is the major contributor to the radiation dose received by marine organisms. In cephalopods, 210Po is concentrated in the digestive gland, which contains over 90% of the whole-body burden of the nuclide. Although previous studies showed that 210Po was taken up independently of 210Pb, its parent nuclide, very little is known about the factors influencing its levels in cephalopods. To the best of our knowledge, no studies investigated 210Po levels in different species at the same time. In the present study, 210Po was analysed in the digestive gland of 62 individuals from 11 species representing a large range of feeding ecologies and habitats, including squids, cuttlefish and octopus species from coastal to deep-oceanic habitats. Among species, the highest activity was measured in Loligo vulgaris (5720 ± 3606 Bq/kg) and the lowest in T. megalops (188 Bq/kg). However, considering the habitats (benthic vs pelagic and neritic vs oceanic), no significant differences appeared. At the species level, no differences between sexes were found so both sexes were plotted together to test the size effect for species with at least 8 individuals (i.e., Eledone cirrhosa, L. vulgaris, L. forbesi and Sepia officinalis). In the first three species, 210Po levels decreased significantly with increasing size or weight but not in S. officinalis. In squid, this could be related to ontogenetic changes in diet from a high proportion of crustaceans (high Po content) in small individuals to fish (low Po content) in larger individuals, while the high dietary plasticity of S. officinalis at all stages of its life cycle could explain the lack of decrease in 210Po with size. In comparison to the few data from the literature, the levels of 210Po concentrations in the cephalopod community of the Bay of Biscay were overall in the same range than those reported in other cephalopods, varying across 4 orders of magnitude. Further studies are needed to understand the mechanism of retention in the cephalopod digestive gland.

1. Introduction

Cephalopods have the ability to concentrate contaminants in their tissues to very high concentrations (e.g., Penicaud et al., 2017; Rodrigo and Costa 2017). In this respect, the digestive gland appears to be a key organ in the metabolism of organic, metallic and radioactive contaminants (e.g., Martin and Hegal, 1975; Ueda et al., 1979; Guary and Fowler 1982; Bustamante et al., 2002a; Ueno et al., 2003). Thus, this organ is deeply involved in the processes of assimilation, detoxification and retention of contaminants, being the storage tissue for organochlorinated compounds and trace elements (e.g., Tanabe et al., 1984; Miramand and Bentley, 1992; Bustamante et al., 2002b; Danis et al., 2005). The digestive gland of cephalopods also concentrates natural radionuclides such as polonium 210 (210Po) to very high levels compared to the other elements (Heyraud and Cherry, 1979; Smith et al., 1984; Waska et al., 2008). This organ contains over 90% of the whole-body burden of the nuclide in cephalopods, which is the most dominant radionuclide within the marine tropic pathway that results in 210Po

* Corresponding author. Littoral Environnement et Sociétés (LIENSs), UMR 7266 CNRS-La Rochelle Université, 2 rue Olympe de Gouges, 17000, La Rochelle, France.
E-mail address: pbustama@univ-lr.fr (P. Bustamante).

https://doi.org/10.1016/j.jenvrad.2023.107265
Received 12 April 2023; Received in revised form 19 July 2023; Accepted 3 August 2023
0265-931X/© 2023 Elsevier Ltd. All rights reserved.
accumulation in marine organisms (Carvalho and Fowler, 1993). Previous studies showed that $^{210}\text{Po}$ was taken up independently of $^{210}\text{Pb}$, its parent nuclide (Smith et al., 1984; Fowler 2011), but very little is known about the factors influencing $^{210}\text{Po}$ levels in cephalopods. In planktonic crustaceans as well as in fish, size appears a key factor determining $^{210}\text{Po}$ levels in the organisms (Cherry and Heyraud 1988, 1991; Strady et al., 2015). In cephalopods, the relationships with size do not appear so clear, with negative and positive relationships considering muscle or whole organisms, respectively (Heyraud et al., 1994). More broadly, little information is available concerning $^{210}\text{Po}$ in cephalopods as investigations to date mainly focused on a limited number of commercial species such as the common cuttlefish Sepia officinalis and the European squid Loligo vulgaris (e.g., Heyraud and Cherry 1979; Smith et al., 1984; Waska et al., 2008).

Cephalopods are a class of marine molluscs that are widespread in diverse marine habitats from polar to tropical environments and from coastal waters to the deep-sea (Boyle and Rodhouse, 2005). Thus, they occupy benthic (e.g. octopus), nektobenthic (cuttlefish, bottlesquid), neritic and oceanic (mainly squids) habitats from the surface to deep waters and have a large variety of trophic niches. Importantly, they constitute a primary food source for many marine predators such as fish, marine mammals or seabirds, for instance the swordfish Xiphias gladius, the long-finned pilot whale Globicephala melas or the wandering albatross Diomedea exulans (Clarke 1996; Croxall and Prince 1996; Klaages 1996). Due to their key position in marine food webs, they represent a major vector of contaminants to their predators, as previously shown for cadmium (Bustamante et al., 1998). However, estimation of marine predator exposure to $^{210}\text{Po}$ following predation on cephalopods is limited due to paucity of knowledge regarding their tissue-burden levels and bioavailability as prey for higher trophic organisms. In humans, exposure to $^{210}\text{Po}$ through the consumption of cephalopods is generally limited, given that polonium concentrations are relatively low in the edible parts (i.e. muscles) compared with other edible seafood (e.g. oysters, mussels, scallops, all of whose soft tissues are consumed; Belivermis & et al., 2019; Bustamante et al., 2002c). Similarly, only the flesh of fish is consumed contributing to a low polonium intake for human consumers (Kilic et al., 2018). However, unlike fish gut, the digestive gland of cephalopods is consumed in some countries such as in Japan where it is used for making a widely consumed dish named shiokara or consumed directly from very fresh squids. Thus, the consumption of cephalopod digestive gland may also be of direct concern to public health and more knowledge and research into the contents and potential risks to human consumers from consuming $^{210}\text{Po}$ from cephalopods is required.

Among natural radionuclides, $^{210}\text{Po}$ is the major contributor to the radiation dose received by marine organisms. In addition to its high specific activity and assimilation efficiency (Fowler 2011; Belivermis et al., 2019), $^{210}\text{Po}$ is a high energy alpha particle emitter (5.3 MeV; Cherry and Shannon, 1974) that can cause intense ionization of tissues. It can thus account for until 80% of the committed efficient dose due to $^{210}\text{Po}$ and the tracer $^{210}\text{Po}$ yield tracer (ca. 50 mBq; $T_{1/2} = 102$ years) to permanently, and polonium was allowed to deposit spontaneously onto the acidification of tissues, which is considered to be the major source of toxic effects. The so-called $^{210}\text{Po}$ solution was evaporated near to dryness to remove the acids, a step which is considered to be the major source of toxic effects. The so-called $^{210}\text{Po}$ solution was evaporated near to dryness to remove the acids, a step which is considered to be the major source of toxic effects.

### 2. Materials and methods

#### 2.1. Study area

Fieldwork was carried out in the Bay of Biscay (from 1 to 10° W and from 43 to 48° N) opening onto the North-East Atlantic Ocean between France and Spain (Fig. 1). The Bay of Biscay is a very large bay of more than 220 000 km² with a continental shelf extending more than 200 km offshore in the north of the Bay but only 10 km in the south. Two main river plumes, i.e. the Loire and the Gironde, influence its hydrological structure (Planque et al., 2004; Puillat et al., 2004). The Bay of Biscay is home to a rich fauna including many protected species, such as sharks, rays, seabirds and marine mammals (Lassalle et al., 2014). It is subject to numerous anthropogenic activities such as extensive fishery activity (Lorance et al., 2009; OSPAR, 2010).

#### 2.2. Sampling and sample preparation

Sampling was carried out in October–November 2011 during the EVOHÉ (“Evolution Halieutique de l’Ouest de l’Europe”; https://doi.org/10.17660/1104066) groundfish survey conducted by Ifremer (“Institut Français de Recherche pour l’Exploitation de la Mer”). During this survey, bottom and pelagic trawls were performed on the continental shelf, on the slope and in the canyons indenting the continental slope to specifically collect neritic, oceanic and deep-sea organisms.

For the present study, selected cephalopods included 62 individuals belonging to 11 species (Table 1). Cephalopod species were caught in various habitats (inshore vs. offshore waters, pelagic vs. benthic or demersal domains). They belong to six squid species: the veined squid Loligo forbesi, the European squid Loligo vulgaris, the flying squid Todarodes sagittatus, the lesser flying squid Todaropsis eblanae, the Atlantic cranch squid Teuthowenia megalops, the reverse jewel squid Histiotethis reversa; one bobtail species: the stout bobtail Rossia macrosona; one cuttlefish species: the common cuttlefish Sepia officinalis; and three octopus species: the common octopus Octopus vulgaris, the spider octopus O. salutii, the horned octopus Eledone cirrhosa.

Each individual was frozen at −20 °C on board and brought back to LIENs laboratory. They were then thawed and dissected to remove the digestive gland. Wet weight (g), dorsal mantle length (mm) for all specimens and total length (for octopuses, in mm) were determined as well as the sex by direct observation of the gonads.

Following dissection, digestive gland samples were frozen at −20 °C in individual plastic bags and freeze-dried for 48h. Freeze-dried samples were ground into a fine powder with porcelain mortar and pestle. The homogenised samples were then stored individually in plastic vials.

#### 2.3. Radionuclide determination

Subsamples of ~1 g dry weight (dw) of cephalopod digestive gland were spiked with $^{209}\text{Po}$ yield tracer (ca. 50 mBq; $T_{1/2} = 102$ years) to assess the chemical yield and calculate the activity concentration of $^{209}\text{Po}$. The internal standard was prepared with a certified standard solution of $^{209}\text{Po}$ (Eckert & Ziegler® Isotope Products). They were then digested adding 2 mL of 65% HNO$_3$ and 2 mL of 30% H$_2$O$_2$ in round bottom flasks in a hot plate with temperature control (80 °C). The solution was evaporated near to dryness to remove the acids, a step which was repeated 3 more times. The residues were then dissolved in 5 mL 0.5M HCl. Ascorbic acid was added until the yellow colour faded permanently, and polonium was allowed to deposit spontaneously onto silver discs for 8 h (Flynn, 1968). Measurements were performed with a high-resolution alpha spectrometer (CANBERRA) with passivated implanted planar silicon (PIPS) detectors with an active area of 450 mm$^2$ and 17.6 keV nominal resolution. Samples were measured until at least 400 counts were observed for individual $^{210}\text{Po}$ peak. The characteristic peak areas of the alpha particles from $^{210}\text{Po}$ and the tracer $^{209}\text{Po}$ activity at the energies of 5.30 MeV and 4.88 MeV were used in the analysis of the sample spectra. The Certified Reference Material (CRM) IAEA-437 (Mussel from Mediterranean Sea) was used for accuracy control. CRM samples were processed following the same way as the cephalopod samples. The recovery obtained for $^{210}\text{Po}$ in the CRM was 90 ± 3%.
2.4. Statistical analysis

Descriptive statistics were applied using Minitab 13.1. Differences between species, habitats, and between males and females were determined using Mann-Whitney U test. Size (mantle length), weight (total body mass) and sex were considered as factors of variation in the species with more than 8 individuals (Loligo vulgaris, L. forbesi, Eledone cirrhosa, and Sepia officinalis) and their effects were checked using ANCOVA on log transformed values. The significance for statistical analyses was always set at $\alpha = 0.05$.

3. Results

3.1. Levels of $^{210}$Po in cephalopod digestive gland

The concentrations of $^{210}$Po in the digestive gland of 11 species of cephalopods from the Bay of Biscay are given in Table 1. Concentrations of $^{210}$Po varied widely across the dataset, i.e. from 188 Bq kg$^{-1}$ dw in the mesopelagic squid Teuthowenia megalops to 11853 Bq kg$^{-1}$ dw in the neritic squid Loligo vulgaris (Table 1). On average, L. vulgaris contained the highest concentrations of $^{210}$Po with $5720 \pm 3606$ Bq kg$^{-1}$ dw on average. More generally, the two species of loliginids (i.e. L. forbesi and L. vulgaris) showed the highest concentrations and the mesopelagic squids the lowest, whereas benthic and necto-benthic species had intermediate levels (Fig. 2). Considering the habitats (benthic vs pelagic and neritic vs oceanic), there was no significant difference ($p > 0.05$).

3.2. Influence of size and sex on $^{210}$Po concentrations

The variations of $^{210}$Po concentrations in the digestive gland of cephalopods with size (mantle length) according to the sex were tested for the species with more than 8 individuals, i.e. Loligo forbesi, L. vulgaris, L. vulgaris, L. forbesi, Eledone cirrhosa, Sepia officinalis, and Sepiidae.
Sepia officinalis and Eledone cirrhosa. There was no difference due to sex in the four species. In these species, males and females when considered to assess size effect.

$^{210}$Po concentrations significantly varied with size in only three species over four, i.e. $L.$ forbesi ($n = 10$), $L.$ vulgaris ($n = 10$) and $Eledone$ cirrhosa ($n = 17$), but not in $Sepia$ officinalis ($n = 8$; Fig. 3).

4. Discussion

The concentrations of $^{210}$Po were considered in the digestive gland of several species of cephalopods from the Bay of Biscay as this organ plays a major role in the metabolism of trace elements in these molluscs, and of this natural radionuclide in particular (Heyraud and Cherry 1979; Smith et al., 1984; Finger and Smith 1987). As for some trace elements such as Ag, Cd, Cu and Zn (Miramand and Bentley 1992; Kojadinovic et al., 2011), the digestive gland of cephalopods contains over 90% of the whole-body burden of the $^{210}$Po and the muscle tissues had only very low concentrations (Heyraud and Cherry 1979; Smith et al., 1984). But in contrast to Ag, Cd, Cu and Zn that have high affinity to thiols groups of the metallothioneins in cephalopods (Bustamante et al., 2002b), $^{210}$Po was rather associated with high molecular weight proteins (>70 kDa) in the digestive gland of the squid Nototodarus gouldi (Finger and Smith 1987). In this squid species, Smith et al. (1984) also pointed that there was no correlation between $^{210}$Po and these metals, further suggesting a minor role of metallothioneins in its bioaccumulation. In contrast, $^{210}$Po was shown correlate with Fe (Smith et al., 1984). Although there is only a limited number of studies that looked to molecular binding of $^{210}$Po in marine organisms, it appears that ferritin in fish (i.e. the Atlantic mackerel Scomber scombrus), bivalves (i.e. the Pacific oyster Crassostrea gigas) and crustaceans (i.e., the lobster Homarus gammarus) plays an important role in the accumulation of $^{210}$Po in the liver or digestive gland of these organisms (Durand et al., 1999, 2002), and the coaccumulation of both elements can support the binding of $^{210}$Po to ferritin in the digestive gland of cephalopods too. However, these authors also showed that metallothioneins in fish and hemocyanin in lobster bind $^{210}$Po to important extent. This highlights the need to further investigate specifically molecular binding of $^{210}$Po in more details, considering that the radionuclide subcellular distribution may vary among cephalopod taxa (see Penicaud et al., 2017).

The efficient retention of $^{210}$Po in this organ consistently lead to high concentrations in the different species investigated here and in those reported in the literature (Table 2). This review shows that all the species that have been analysed are species of commercial interest and consumed by humans, including octopus, cuttlefish and squids of the Ommastrephid and Loliginid families. To the best of our knowledge, our study is thus the first one to report values about the digestive gland $^{210}$Po concentrations in a wide range of cephalopod species, considering benthic and pelagic species living in neritic and oceanic habitats, including mesopelagic species. The effect of the habitat on trace element...
concentrations in cephalopods was significant for Cd and Hg in cephalopods from the same area, with benthic species having higher values compared to pelagic species showing at the same time the highest (Loliginids) and the lowest (Ommastrephids and mesopelagic squids) values (Fig. 2). The present review also shows that projections appeared to be higher in crustacean prey of squids than in fish (Heyraud and Cherry, 1979; Carvalho et al., 2011), likely due to the opportunistic feeding behaviour of common cuttlefish. This plasticity is supported by investigations of their diet trough stomach content analyses (Pinçon et al., 2000; Neves et al., 2009) as well as by the large variations in the ingestion rates between adult and juvenile stages (Ommastrephids and Loliginids (see Piccard et al., 1988)).

Among these biological factors, we have also considered the influence of sex and the size for the species with at least 8 individuals, i.e., the curled octopus E. cirrhosa, the common cuttlefish S. officinalis, the veined squid L. forbesi and the European squid L. vulgaris. We found no differences between 210Po concentrations of males and females. This result is consistent with previous studies on the Japanese flying squid Todarodes pacificus and on the arrow squid Nototodarus gouldii (Smith et al., 1984; Waska et al., 2008). This suggests that the physiological mechanisms of 210Po accumulation and elimination are not implied in the sexual maturation and gametogenesis.

In contrast to size, sex dramatically influenced 210Po concentrations in the digestive gland of cephalopods (Fig. 3). Such a decrease with size has often been reported for a variety of marine organisms including crustaceans, molluscs and fish (Fowler, 2011). As food is the dominant source of 210Po in marine organisms (Heyraud and Cherry, 1979; Cherry et al., 1983; Carvalho, 1990), this general decrease along size suggests a close relationship between 210Po concentration in these marine organisms and their food ingestion rates (Fowler, 2011). Also, it could result from a dilution of 210Po due to the fast growth of cephalopods and/or from a switch in the diet along ontogeny as shown for some metals such as Cd and Hg (Chouvelon et al., 2011). To the best of our knowledge, there was a single study that considered the influence of size on 210Po concentrations in cephalopods (Heyraud et al., 1994). These authors reported an increase of 210Po concentrations in whole squids from South Africa but they also considered unusual their own results as it was not following the decreasing paradigm. They suggested that a difference in diet between large and small squids could explain their unusual result. Variation of the diet with size in cephalopods is well documented (e.g., Nixon, 1987) and for instance, it results in higher Cd exposure of small sizes squids compared to adults, as the crustacean prey of the juveniles are Cd-enriched compared to Cd-poor fish (Penicaud et al., 2017). Thus, decreasing Cd concentrations with increasing body size was already reported for L. forbesi sampled in the Bay of Biscay and around the UK (Fowler et al., 2008; Chouvelon et al., 2011). In turn, 210Po concentrations appeared to be higher in crustacean prey of squids than in fish (Heyraud et al., 1994; Carvalho et al., 2011), which should lead to a decrease in 210Po values as observed in the present study. However, in our sampling, the common cuttlefish showed no significant variation of 210Po with size. Although this can be due to a limited number of individuals considered, a similar result has been reported for Cd in this species (Chouvelon et al., 2011), likely due to the opportunistic feeding behaviour of common cuttlefish. This plasticity is supported by investigations of their diet trough stomach content analyses (Pinçon et al., 2000; Neves et al., 2009) as well as by the large variations in the ingestion rates (a proxy of the trophic position) with size in this species, confirming that the common cuttlefish has a strong dietary plasticity at all stages of its life cycle (Chouvelon et al., 2011).

Cephalopods are preyed upon by many large predators such as tuna, swordfish, seabirds and marine mammals (Clarke, 1996, Croxall and Prince 1996; Snail 1996). The concentrations of Po in the muscle of the sperm whale (Balaenoptera borealis) from the Azores, which feeds exclusively on mesopelagic cephalopods, were 5.0 ± 0.2 Bq kg⁻¹ ww (Carvalho et al. 2011). This value is in line with the one reported in the muscles of bigeye tuna (3.0 ± 0.1 Bq kg⁻¹) from Madeira Islands (Carvalho et al., 2011), which feed on a variety of prey including both epipelagic and mesopelagic organisms and includes fish and squids in its diet (Duffy et al., 2017). However, epipelagic marine mammals such as common and white sided dolphins Delphinus delphis and Stenella coerulealba have higher concentrations of 210Po activity in their muscle tissue than sperm whales (Malta and Carvalho, 2011), likely because they include small pelagic fish and Loliginids in their diet. To the best of our knowledge, there is no data on large predators from the Bay of Biscay while there is a large community of fish, seabirds and marine mammals foraging in this highly productive area. Future studies should investigate Po in the tissues of top predators, to determine if those feeding mainly on cephalopods, and particularly on the neritic ones such as delphinids (Lahaye et al., 2005) are at risk due to high exposure to this radionuclide.
Declaration of competing interest

The authors declare they have no conflict of interest.

Data availability

Data will be made available on request.

Acknowledgments

Authors are very grateful to J.P. Léauté, M. Salaun and L. Pawlowski from Ifremer, as well as the crew of the R/V “Thalassa” for facilitating the sampling during the 2011 EVHOE survey (https://doi.org/10.17600/11040060). Thanks are due to F. Mercier his help in the sampling onboard. MW is an Honorary Senior Research Associate of the National Fund for Scientific Research (NFSR, Belgium). PB is an honorary senior member of the IUF (Institut Universitaire de France). This work benefitted from the French GDR “Aquatic Ecotoxicology” framework which aims at fostering stimulating scientific discussions and collaborations for more integrative approaches.

References

Rodrigo, A.P., Costa, P.M., 2017. The role of the cephalopod digestive gland in the storage and detoxification of marine pollutants. Front. Physiol. 8, 232
Strady, E., Hamelin-Vivien, M., Chiffoleau, J.-F., Veron, A., Troncyznki, J., Radakovitch, O., 2015. 210Po and 210Pb trophic transfer within the