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Total and organic Hg concentrations in cephalopods from the North Eastern Atlantic waters: Influence of geographical origin and feeding ecology

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Abstract: Total (T–Hg) and organic (O–Hg) mercury concentrations and tissue distribution were examined in 20 species of cephalopods (n = 278) from the north eastern Atlantic waters, i.e. from the Bay of Biscay to the Faroe Islands. Concentrations of T–Hg in whole cephalopods showed elevated variations among species, i.e. from 40 to 3560 ng g^{-1} dwt, but a low variability within each species (mean CV% = 39%). With the exception of oceanic squids, the digestive gland globally displayed higher T–Hg concentrations than the remaining tissues. In contrast, O–Hg concentrations determined in selected species were generally higher in the remaining tissues. Despite higher T–Hg concentrations, the digestive gland weakly contributed to the total body burden of both T–Hg and O–Hg (< 25% and < 15%, respectively). In fact, from 75% to 95% of the T–Hg and O–Hg were contained in the muscular remaining tissues. Therefore, O–Hg may have a strong affinity to proteins in cephalopods. Sex and size only significantly influenced the bioaccumulation of Hg for the Loliginidae family. T–Hg and O–Hg concentrations were also influenced by geographical origin: Celtic Sea > Bay of Biscay > Faroe Islands, corresponding to the seawater Hg concentrations in these areas. In the Faroe Islands and the Celtic Sea, benthic cephalopods contained significant higher Hg concentrations compared to pelagic ones. This suggests that diet is not the main pathway of Hg uptake in cephalopods as pelagic species were expected to be more exposed to O–Hg through fish consumption than benthic ones.

Keywords: Bioaccumulation; Speciation; Body distribution; Squid; Octopus; Cuttlefish

INTRODUCTION

Transfer of metals in marine food webs appears to be mainly directed by their physico-chemical forms, which therefore defines their bioavailability to the upper level (Wallace & Lopez 1997). Among metals of environmental concern, mercury (Hg) is readily methylated by micro-organisms, bioaccumulates in marine biota and consistently biomagnifies through the food chain, predators showing higher tissue concentrations than in their prey.

Among organic Hg compounds, methyl Hg is the most stable form in the marine environment but also the most toxic one to organisms (Cossa et al. 1990). Hg methylation generally increases its toxicity as a result of its enhanced penetration through lipid membranes (Boudou et al. 1983). In top predators such as Humans, the nervous system is the critical organ for chronic Hg exposure and methyl Hg can react directly with important receptors (WHO, 1990). Overall, Hg is considered as the most toxic metal since the accident of the Minnamata Bay in Japan and seafood consumption constitutes the main ingestion source of Hg in Man (GESAMP, 1986).

A wealth of scientific information on Hg in fish is available due tot the fact that (1) they are important components of marine, estuarine and freshwater ecosystems and (2) they constitute a major food source for Man. Even if Hg concentrations in fish may vary according to their trophic level and age (Honda et al. 1983, Clarkson et al. 1988, Riisgård & Hansens 1990), virtually all the Hg (i.e. > 95%) in fish is methylated (Bloom 1992) and is therefore bioavailable for upper trophic levels. Hence, fish consumption is an important source of dietary Hg (Svensson et al. 1992) and is of special health concern (Clarkson 1990).

In contrast to fish, information on Hg in cephalopod tissues is scarce. Most of the studies about total Hg concentrations has mainly addressed on edible tissues (e.g. Nagakura et al. 1974, Stoepler et al. 1979, Cappon & Smith 1982, Buzina et al. 1989, Cappon 1990, Sapunar

et al. 1989, Plessi et al. 2001) and very few studies have discussed the ratio between organic and inorganic Hg in cephalopods (i.e. Buzina et al. 1989, Cappon & Smith 1982). Contradictory results have been published in the current literature, as for instance Nagakura et al (1974) showed a higher percentage of O-Hg in cephalopod tissues whereas Cappon (1990) found higher inorganic Hg in octopus and squid flesh. Therefore, the assessment of the chemical state of Hg is of ground importance since this strongly determines the bioavailability of this metal to the upper trophic level (Cossa et al. 1990).

Several studies concerning other heavy metals in cephalopods tissues have demonstrated the major role of the digestive gland in metal metabolism, as this organ is deeply involved in assimilation processes, detoxification and storage of both essential and non-essential metals (e.g. Martin & Flegal 1975, Smith et al. 1984, Miramand & Bentley 1992, Bustamante et al. 2000, 2002ab, 2004). Moreover, cephalopods also accumulate organic contaminants such as organotin, organochlorine pesticide or PCBs in the digestive gland (Tanabe et al. 1984, Kawano et al. 1986, Yamada et al. 1997, Weisbrod et al. 2000, 2001, Ueno et al. 2003, Danis et al. 2005). Conversely, very little information is available concerning Hg in the digestive gland of cephalopods and mainly focuses on common octopus (e.g. Renzoni et al. 1973, Raimundo et al. 2003, Seixas et al. 2005).

Cephalopods are widespread in a great variety of marine habitats from coastal waters to very deep ocean environments. They are benthic (e.g. octopus), nectobenthic (cuttlefish, bottlesquid), neritic and oceanic (mainly squids), and constitute a primary food source for many marine predators such as fish, marine mammals or seabirds (Clarke 1996, Croxall & Prince 1996, Klages 1996, Smale 1996). It follows that they could represent an important vector of contaminant to the consumers as shown for Cd (Bustamante et al. 1998). However, estimations of top predator exposures to Hg are limited because of a poor knowledge about

Hg levels and bioavailability in this prey. Cephalopods are also extensively fished and consumed by humans (Amaratunga 1983) and thus, are also of direct concern to public health. In this framework, the present study aims at providing baseline data on Hg concentrations in cephalopods for a large range of species from the North-eastern Atlantic waters and at investigating their variations according to biological and geographical factors. To this end, total and organic Hg concentrations have been determined in the digestive gland and remaining muscular tissues of several squid, cuttlefish, and octopus species caught at various latitudes, i.e. from the Bay of Biscay to the Faroe Islands. We then discuss the observed variations of the Hg concentration and its body distribution and finally show the correlation between organic and inorganic Hg.

MATERIAL & METHODS

Sampling and sample preparation

The sampling of cephalopods includes 278 individuals belonging to 20 species (see Table 1), which were caught by trawl between 1996 and 2003 in the waters from the Bay of Biscay, the Celtic Sea, the Scottish waters and the Faroe Islands waters.

Cephalopods were fished either by scientific or commercial vessels. Collected specimens were immediately frozen on board in individual plastic bags. In the laboratory, each individual was weighed and measured (mantle length, total length), and the sex determined. The origin, number of individuals and body weight of each species of cephalopods are given in Table 1. In most of the individuals, the digestive gland was dissected to be treated separately. Therefore, Hg concentrations in the whole animals were calculated according to the concentrations in the digestive gland and in the remaining tissues. The latter are in fact

mainly composed by muscle tissues (arms, fins and mantle accounting for 70 to 80% of the body weight).

Analytical procedure

Separated tissue samples were either dried to a constant weight for several days at 60°C or freeze-dried and then homogenised in a motar and porcelain pestle. For T-Hg, two aliquots ranging from 10 to 50 mg of dried material were directly analysed in an Advanced Mercury Analyser spectrophotometer (Altec AMA 254). Hg determination involved evaporation of the metal by progressive heating until 800°C was reached and then held under oxygen atmosphere for 3 min, and subsequent amalgamation on a gold-net. Afterwards, the net was heated to liberate the collected Hg, which was then measured by atomic absorption spectrophotometry. The same procedure was performed for the analysis of O-Hg after extraction adapted from Uthe et al. (1972). O-Hg was extracted from 2 aliquots of approx. 500 mg of each homogenised dry sample using 2 ml of acidic sodium bromide (30% NaBr in 4N H_2SO_4), 4 ml of cupric sulfate (2.5% $CuSO_4$ in milli-Q quality water) and 10 ml of toluene under agitation for 10 min in glass flasks. The organic phase was then separated and centrifuged 10 min at 1500 G to eliminate residues of the tissue material. The supernatants were used for the analysis in the AMA.

Quality control

Mercury analyses were ran with respect to a thorough quality control program including analyses of reference materials dogfish liver DOLT-2 and lobster hepatopancreas TORT-2 purchased from the National Research Council, Canada. These standards were treated and analysed under the same conditions as the samples. The results were in good agreement with the certified values (Table 2). Detection limits ($ng.g^{-1}$ dry wt) were 5 for total Hg and 20 for organic Hg. Metal concentrations in tissues are also reported in $ng.g^{-1}$ dry wt.

Data analyses

Prior to statistical analyses, Hg concentrations and mantle length were tested for normality and equality of variances. Comparisons of Hg concentrations between tissues within each species were tested by t-test. Gender variations of Hg concentrations with size were tested by ANCOVA. Comparisons of Hg concentrations among several species were performed using one-way ANOVA followed by Tukey multiple comparison test. Statistical analyses were performed using XLStat 7.0. The significance for statistical analyses was always set at $\alpha = 0.05$.

RESULTS

Levels of concentrations and tissue distribution

T-Hg has been determined in both the digestive glands and the remaining tissues of 278 cephalopods sampled in the north-eastern Atlantic waters and its concentrations in the whole individuals have been calculated accordingly (Table 3). T-Hg in the whole cephalopods varies greatly among species, from 40 to 3560 $ng.g^{-1}$ dwt but generally displays a low variability in each species. Indeed, the coefficients of variation (CV) of T-Hg concentrations in the whole individuals, calculated as the standard deviation divided by the mean multiplied by 100, vary from 14 to 98% (mean CV% = 39%).

With a few exceptions concerning oceanic squids (Cranchidae, Histioteuthidae and Ommastrephidae), T-Hg concentrations are higher in the digestive gland of cephalopods than in the remaining tissues (Table 3). This difference is significant ($P < 0.05$) for various species from the Bay of Biscay (the squid *Loligo vulgaris*, the bobtail squid *Rossia Macrosoma*, the cuttlefishes *Sepia elegans*, *S. officinalis* and *S. orbignyana*, and the octopuses *Bathypolypus*

baierii, B. sponsalis, *Eledone cirrhosa*, *Octopus vulgaris* and *O. salutii*), for *Bathypolypus baierii* from West Scotland, and for *Loligo forbesi* from the Faroe Islands. Between the two compartments analysed, the remaining tissues contain most of the T-Hg burdens whichever the species, varying from 60 to 95% of the total body burden (Fig. 1).

Organic Hg (O-Hg) has been determined in the digestive gland and in the remaining tissues of selected species (Table 4). As *L. forbesi, Todarodes sagittatus*, and *E. cirrhosa* were found in three sampling areas (i.e. Bay of Biscay, Celtic Sea and Faroe Islands), these species have been retained to carry out further geographical comparisons. In addition, O-Hg has been determined in the remaining tissues of the other cephalopod species. Regardless of the origin and independently of the levels of the concentrations, O-Hg is strongly correlated with T-Hg in the remaining tissues of the species analysed (Fig. 2). The remaining tissues contain a high percentage of O-Hg representing 84 to 98% of the total O-Hg in the organism. Therefore, as muscular remaining tissues constitute the main proportion of cephalopod body mass (i.e. around 90%), they contain the greatest fraction of the whole body burden, of which 67 to 83% of the T-Hg is under organic form in this compartment (Table 4).

In the digestive gland, the percentage of O-Hg given relatively to T-Hg is lower and varies widely among species, i.e. from 22 to 65 %. However, when considering the total body burden of O-Hg, the digestive gland always contains less than 16% of O-Hg for all the considered species (Table 4).

Influence of size and sex

The variations of T-Hg concentrations with size in whole-body cephalopods according to the sex have been tested by ANCOVA only for the largest samples (i.e. more than 10 individuals). Hg concentrations significantly vary with size only for a few species, all belonging to the Loliginidae family (Table 5). Results generally do not show any significant

differences due to sex but *Loligo forbesi* from the Faroe Islands and from the Celtic Sea exhibit a significant interaction between size and sex, meaning that the bioaccumulation rate of Hg is different between sexes, females showing the highest values.

Interspecific and geographical variations

Fig. 3 compares the T-Hg concentrations in the whole individuals of *E. cirrhosa*, *L. forbesi* and *T. sagittatus* between the Bay of Biscay, the Celtic Sea and the Faroe Islands. Because of their origin, T-Hg concentrations greatly vary within each species, i.e. from 80 to 743 $ng.g^{-1}$ dry wt for *E. cirrhosa*, from 100 to 458 $ng.g^{-1}$ dry wt for *L. forbesi* and from 40 to 879 $ng.g^{-1}$ dry wt for *T. sagittatus*. Significant differences ($P<0.05$) of T-Hg concentrations in whole individuals are hence found among such areas for each species. Indeed, the cephalopods from the Faroe Islands exhibit the lowest values whereas the highest are found in those from the Irish waters (Fig. 3). A similar pattern is observed for O-Hg in the remaining tissues because of its correlation with T-Hg (Fig. 2).

Figure 4 compares the T-Hg concentrations between benthic and pelagic cephalopods from the Bay of Biscay, the Celtic Sea and the Faroe Islands. Benthic cephalopods display higher T-Hg concentrations in the Faroe Islands and the Celtic Sea ($F=5.39$ $P=0.024$ and $F=23.39$ $P<0.001$, respectively) compared to pelagic ones (Fig. 4). However, such is not the case for cephalopods from the Bay of Biscay ($F=2.1$ $P=0.150$).

DISCUSSION

The distribution of Hg was considered between two separated body compartments, the digestive gland, and the remaining tissues of cephalopods (Figure 2). The digestive gland was first selected as this organ plays a major role in the energetic metabolism of cephalopods

(Boucaud-Camou & Boucher-Rodoni 1983) and is known to accumulate metal in large amounts (e.g. Martin & Flegal 1975, Finger & Smith 1987, Miramand & Bentley 1992, Bustamante et al. 2000). The second compartment included the rest of the animal (around 90% of the total body weight). This compartment is mainly constituted by muscles, which could also store Hg in significant amounts (e.g. Renzoni et al. 1973, Cappon & Smith 1982, Cappon 1990, Monteiro et al 1992, Seixas et al. 2005).

Although cephalopods have been demonstrated to have special capabilities to concentrate heavy metals at various levels in their tissues, dissimilar information on Hg concentrations in cephalopods and on their variations has been reported (see Table 6 for review). Overall, Hg concentrations have been mainly studied in a few cephalopod species for human health purposes but most of these works generally remained limited to edible tissues, i.e. mantle muscle, arms and fins. Very little data consider various species at the same time or over a large area (Stoepler et al. 1979, Monteiro et al. 1992). To the best of our knowledge, this study is the first one to investigate Hg concentrations and tissue distribution in a wide range of cephalopod species including benthic and pelagic species living in neritic and oceanic habitats over a large geographical scale.

T-Hg concentrations in cephalopods from the north-eastern Atlantic waters were globally within the same range of concentrations as other related species from the south West Atlantic and the Japanese waters (Table 6). In contrast, higher T-Hg concentrations have been reported for cephalopods from the Tyrrhenian and Adriatic Seas (e.g. Renzoni et al. 1973, Storelli & Marcotrigiano 1999). Higher Hg concentrations in Mediterranean organisms are typically explained by high temperature and absence of solar radiation in the deep environment that favors a high methylation rate. Moreover, natural sources of Hg in the Mediterranean Sea may contribute to Hg enrichment through the benthic food webs, as it constitutes the richest natural reserve of this element (Bacci 1989).

Regardless of the species and the origin, it is striking to note that the coefficients of variation of T-Hg concentrations in the whole individuals are relatively low for a toxic element (i.e. always < 98%). Similar coefficients of variation have been previously reported for essential Cu and Zn which might be under homeostatic control (Bustamante 1998), suggesting also a relative ability of cephalopods to regulate Hg. However, T- and O-Hg concentrations varied between the digestive gland and the remaining tissues, the former generally showing higher T-Hg but lower O-Hg concentrations (Tables 3 & 4). The digestive gland plays a major role in the digestive processes and also in the detoxification of xenobiotics in cephalopods: on the one hand, the digestive gland synthesizes the digestive enzymes and is a place of absorption and assimilation of nutrients (Boucaud-Camou & Boucher-Rodoni 1983, Boucher-Rodoni et al. 1987); on the other hand, this organ is involved in the storage and detoxification of several trace elements such as Ag, Cd, Cu, or Zn (e.g. Miramand & Guary, 1980, Finger & Smith, 1987, Miramand & Bentley, 1992, Bustamante et al. 2002ab, 2004) and organic compounds such as PCBs or TBT (Yamada et al. 1997, Ueno et al. 2003, Danis et al. 2005). Sources of metals to cephalopods are 1) seawater, as it passes through the skin and through the gills during respiration, and 2) food, which probably represents the main pathway for most of transition and heavy metals (Koyama et al. 2000, Bustamante et al. 2002a, 2004). However, it is difficult to ascertain the relative importance of each pathways of uptake for Hg, which can be influenced by the speciation of this metal in both seawater and prey. Moreover, to the best of our knowledge, neither inorganic nor organic Hg uptake has been studied in cephalopods and would be particularly interesting to be investigated in the future.

In the digestive gland, the fraction of O-Hg varied widely (i.e. from 20 to 60 %) but remained always lower to that determined in the remaining tissues (i.e. from 67 to 83%) (Table 4). Lower O-Hg contents in the digestive gland suggests that demethylation of O-Hg could occur in this organ like in fish liver (Lockhart et al. 1972, Boudou & Ribeyre 1983). However, it is

largely admitted that the biomagnification of Hg resembles that of hydrophobic organic pollutants rather than that of ionic metals, and it is generally thought to result from the lipid solubility of methyl Hg (Monteiro et al. 1996). High lipid contents in the digestive gland of cephalopods would therefore lead to high O-Hg concentrations. However, such prediction needs to consider Hg chemical properties. In fact, Hg has a low octanol-water distribution coefficient (K_{ow}) compared to other fat soluble compounds such as DDT or PCBs (Bienvenue et al. 1984, Veith et al. 1980). Unlike other hydrophobic compounds, O-Hg seems to have a strong affinity for the sulphydryl groups of certain proteins and resides in muscular proteins rather than in fat tissue in fish (Bloom 1992). Thus, a second reason for the low O-Hg contents in the digestive gland of cephalopods could be a preferential redistribution to muscular tissues. It is therefore not surprising that most of the Hg contained in the muscular remaining tissues was under organic form (Figure 2).

As for other metals, Hg concentrations in cephalopod may vary with biological and environmental factors such as age (size), sex, lifestyle and geographical origin (e.g. Monteiro et al. 1992, Bustamante et al. 1998, Raimundo et al. 2003, Seixas et al. 2005). It is striking that only the squids belonging to the Loliginidae family exhibited a significant increase of Hg concentrations with size and displayed different metal accumulation rates between sexes (Table 5). Despite lack of data on the influence of such intra-specific factors on Hg bioaccumulation, it is generally admitted that Hg concentrations in cephalopod tissues are positively correlated with size (Monteiro et al. 1992, Rossi et al. 1993, Storelli & Marcotrigiano 1999). The higher accumulation rate of Hg in *Loligo forbesi* females, probably due to the existence of physiological and/ or ecological inter-sex differences, has been previously cited by Monteiro et al. (1992). However, this paper does not focus on this issue and further research should be carried out on this aspect.

When considering the different species, Hg concentrations seem to be influenced by the lifestyle. Indeed, benthic cephalopods generally displayed significantly higher T-Hg and O-Hg concentrations than pelagic ones (Fig. 4). The only exception was the Bay of Biscay because of the different cuttlefish species showing lower Hg concentrations than octopus. The lower Hg concentrations in cuttlefish are likely due to the very low Hg concentrations found in the cuttlebone (Bustamante, unpublished data) leading to the dilution of the whole body Hg concentrations. Overall, benthic cephalopods exhibit more complex diets than pelagic ones and feed on polychets, crustaceans, molluscs, echinoderms and fish (Boyle 1990, McQuaid 1994). In contrast, squid mainly feed on fish and other cephalopod species (Nixon 1987, Rocha et al. 1993, Pierce et al. 1994). Such a pelagic diet would lead to higher O-Hg concentrations in pelagic cephalopods as their prey contain higher O-Hg concentrations than those of benthic ones (Cossa et al. 1990). Moreover, squids have higher feeding rates than octopus (Nixon 1987). Surprisingly, both oceanic and neritic squids displayed significant lower Hg concentrations compared to benthic octopus. It is therefore difficult to explain these results when only considering Hg exposure through the diet and it derives that trophic ecology is not the only factor controlling the intake of Hg in cephalopods. Because they live close to the sea floor, benthic cephalopods would be exposed to higher dissolved O-Hg concentrations resulting from the methylation by micro-organisms living in the sediment. Experiments to determine the affinity of dissolved O-Hg for cephalopods appear therefore necessary to assess this hypothesis.

The influence of the geographical area was considered using the three species of cephalopods (i.e. *E. cirrhosa*, *L. forbesi* and *T. sagittatus*) living between the Bay of Biscay (southern part) and the Faroe Islands (northern part) (Fig. 3). Our results indicated that geographical origin is an important factor contributing to both intra-specific and inter-specific variations of Hg concentrations. In fact, cephalopods from the Celtic Sea always displayed the highest T- and

O-Hg concentrations and those from the Faroe Islands the lowest (Fig. 3). Around Ireland, waters showed 10 to 100 times higher Hg concentrations compared to those from the Bay of Biscay (Garner 1978, Cossa & Noël 1987). Although Faroe Islands are poorly documented regarding Hg levels in seawater, it is likely that metal concentrations in this area would be very low according to the absence of heavy industry in this region. Assuming that cephalopods are short-lived species, they would be useful to provide information on the variation of Hg concentrations in seawater.

CONCLUSION

This study shows that Hg is mainly present under organic form in cephalopods, in which the metal is likely to be bound to proteins in muscles. Because O-Hg is highly bioavailable, cephalopods should therefore be considered as a significant source of Hg for consumers and predators. Among tissues, the digestive gland does not appear as a storage organ of Hg but may allow the partial demethylation of the organic form. In addition, our results suggest that the dissolved pathway could be an important route of uptake for Hg in cephalopods. On this basis, cephalopods would reflect the environmental concentrations of Hg. As they are short-lived and abundant, cephalopods would provide information on short-term variations of Hg concentrations in seawater. Therefore, cephalopods could be an interesting tool to monitor Hg contamination in the marine environment.

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Table 1. Characteristics of sampled cephalopods. ND: Not determined.

Family / Species	Sampling area	Sample Size	Mantle length (mm)	Fresh weight (g)	Sex	Water content (%) Digestive gland	Remaining tissues	Whole individual
Loliginidae								
Alloteuthis subulata	Bay of Biscay	15	152 ± 32	123 ± 57	7 ?, 8 ?	70 ± 3	78 ± 3	78 ± 2
Loligo forbesi	Faroe Islands	21	152 ± 36	115 ± 59	11 ?, 10 ?	73 ± 5	92 ± 2	78 ± 1
"	Celtic Sea	18	139 ± 26	88 ± 36	13 ?, 5 ?	69 ± 3	78 ± 1	78 ± 1
"	Bay of Biscay	12	119 ± 48	81 ± 81	8 ?, 4 ?	69 ± 5	78 ± 1	77 ± 1
L. vulgaris	Bay of Biscay	21	151 ± 47	117 ± 71	7 ?, 14 ?	71 ± 3	78 ± 3	78 ± 3
Ommastrephidae								
Illex coindetii	Bay of Biscay	22	130 ± 54	158 ± 191	11 ?, 11 ?	55 ± 7	80 ± 2	79 ± 2
Todarodes sagittatus	Faroe Islands	37	213 ± 21	203 ± 89	13 ?, 24 ?	46 ± 5	83 ± 4	75 ± 2
"	West Scotland	6	345 ± 50	1244 ± 906	2 ?, 4 ?	49 ± 6	78 ± 0	74 ± 2
"	Celtic Sea	5	449 ± 16	2682 ± 417	5 ?	32 ± 13	80 ± 2	76 ± 3
"	Bay of Biscay	5	98 ± 34	93 ± 112	4 ?, 1 ?	62 ± 12	84 ± 4	83 ± 4
Todaropsis eblanae	Bay of Biscay	9	101 ± 43	106 ± 112	5 ?, 4 ?	58 ± 7	80 ± 2	78 ± 3
Histioteuthidae								
Histioteuthis reversa	Bay of Biscay	6	38 ± 22	25 ± 47	2 ?, 4 ND	60 ± 8	87 ± 3	85 ± 3
Cranchidae								
Teuthowenia megalops	Bay of Biscay	1	180	40	ND	46	85	83
Sepiolidae								
Rossia macrosoma	Bay of Biscay	4	31 ± 4	16 ± 9	3 ?, 1 ?	71 ± 5	83 ± 1	81 ± 1
Sepiidae								
Sepia elegans	Bay of Biscay	14	60 ± 8	28 ± 11	4 ?, 10 ?	63 ± 6	76 ± 2	75 ± 2
S. officinalis	Celtic Sea	1	85	77	1 ?	70	78	78
"	Bay of Biscay	23	82 ± 26	96 ± 80	14 ?, 8 ?	64 ± 4	78 ± 1	78 ± 1
S. orbignyana	"	15	74 ± 10	57 ± 22	7 ?, 8 ?	60 ± 6	77 ± 2	76 ± 2
Octopodidae								
Bathypolypus baierii	West Scotland	2	44 ± 21	50 ± 51	1 ?, 1 ?	71 ± 2	85 ± 0	84 ± 0
"	Bay of Biscay	2	76 ± 0	203 ± 18	2 ?	73 ± 10	85 ± 0	85 ± 0
B. sponsalis	Bay of Biscay	5	41 ± 13	28 ± 15	1 ?, 4 ?	72 ± 5	85 ± 1	84 ± 1
Benthoctopus sp.	Celtic Sea	1	153	1500	1 ?	56	88	87
Eledone cirrhosa	Faroe Islands	4	100 ± 8	423 ± 105	4 ?	66 ± 6	93 ± 4	81 ± 1
"	Celtic Sea	2	51 ± 4	53 ± 23	1 ?, 1 ?	56 ± 14	82 ± 1	81 ± 1
"	Bay of Biscay	6	85 ± 16	201 ± 142	4 ?, 2 ?	53 ± 4	83 ± 1	81 ± 1
Octopus vulgaris	Bay of Biscay	13	115 ± 19	1388 ± 540	7 ?, 6 ?	58 ± 6	83 ± 1	82 ± 1
O. salutii	Bay of Biscay	6	58 ± 15	94 ± 75	3 ?, 3 ?	66 ± 8	82 ± 3	81 ± 4
Scaergus unicirrhus	Bay of Biscay	1	62	89	1 ?	60	80	79
Opisthoteuthidae								
Opisthoteuthis sp.	Celtic Sea	1	-	1111	1 ?	79	94	94

Table 2. Comparison of total and O-Hg concentrations (µg.g^{-1} dry wt) of certified standards from the NRCC determined in the present study with certified values.

Metal	DOLT-2 (n = 15)			TORT-2 (n = 15)		
	Certified values	Present study	Recovery	Certified values	Present study	Recovery
Total Hg	2.14 ± 0.28	2.13 ± 0.03	100%	0.270 ± 0.060	0.269 ± 0.006	100%
Organic Hg	0.693 ± 0.053	0.763 ± 0.021	109%	0.152 ± 0.060	0.156 ± 0.011	97%

Table 3. T-Hg concentrations (Mean ± SD; ng.g^{-1} dwt) in cephalopods from the North Eastern Atlantic. N: sample size

Family / Species	Sampling area	N	Digestive gland		Remaining tissues		Whole individual	
			Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Loliginidae								
Alloteuthis subulata	Bay of Biscay	15	ND	-	196 ± 40	121-262	ND	ND
Loligo forbesi	Faroe Islands	21	196 ± 40	121-262	136 ± 27	86-208	139 ± 27	87-211
"	Celtic Sea	18	382 ± 232	202-1030	260 ± 68	188-445	265 ± 74	188-458
"	Bay of Biscay	12	235 ± 104	165-512	179 ± 53	91-645	181 ± 151	95-640
L. vulgaris	Bay of Biscay	21	406 ± 171	113-681	264 ± 86	113-398	269 ± 88	115-404
Ommastrephidae								
Illex coindetii	Bay of Biscay	22	192 ± 76	81-357	193 ± 78	61-331	191 ± 75	63-333
Todarodes sagittatus	Faroe Islands	37	54 ± 16	25-91	71 ± 22	39-160	67 ± 17	35-116
"	West Scotland	6	318 ± 154	198-613	489 ± 245	251-945	440 ± 220	245-855
"	Celtic Sea	5	511 ± 552	186-1485	740 ± 208	424-991	660 ± 207	372-879
"	Bay of Biscay	5	168 ± 52	112-231	188 ± 89	73-289	178 ± 67	81-236
Todaropsis eblanae	Bay of Biscay	9	217 ± 108	120-463	281 ± 129	130-500	284 ± 120	140-497
Histioteuthidae								
Histioteuthis reversa	Bay of Biscay	6	88 ± 44	31-137	102 ± 31	65-147	99 ± 31	57-144
Cranchidae								
Teuthowenia megalops	Bay of Biscay	1	-	172	-	205	-	199
Sepiolidae								
Rossia macrosoma	Bay of Biscay	4	916 ± 83	310-2279	313 ± 190	52-743	394 ± 388	88-913
Sepiidae								
Sepia elegans	Bay of Biscay	14	614 ± 160	332-937	237 ± 92	90-406	265 ± 92	111-443
S. officinalis	Celtic Sea	1	-	650	-	980	-	960
"	Bay of Biscay	23	531 ± 274	196-1269	235 ± 105	98-596	262 ± 119	132-677
S. orbignyana	"	15	467 ± 161	241-757	118 ± 35	63-176	144 ± 34	90-195
Octopodidae								
Bathypolypus baierii	West Scotland	2	791± 239	622-961	415 ± 92	350-480	453 ± 104	379-526
"	Bay of Biscay	2	1650 ± 453	1330-1970	337 ± 71	287-387	424 ± 110	346-501
B. sponsalis	Bay of Biscay	5	1003 ± 233	796-1370	216 ± 32	185-269	304 ± 47	274-387
Benthoctopus sp.	Celtic Sea	1	-	1090	-	921	-	942
Eledone cirrhosa	Faroe Islands	4	184 ± 65	91-241	144 ± 71	79-234	146 ± 70	80-233
"	Celtic Sea	2	243 ± 168	124-362	504 ± 418	208-800	477 ± 390	201-753
"	Bay of Biscay	6	457 ± 36	422-524	298 ± 52	265-402	325 ± 47	295-419
Octopus vulgaris	Bay of Biscay	13	1141 ± 242	844-1690	381 ± 99	236-640	445 ± 92	297-662
O. salutii	Bay of Biscay	6	1950 ± 923	1001-3560	462 ± 161	302-767	617 ± 297	419-1207
Scaergus unicirrhus	Bay of Biscay	1	-	979	-	422	-	496
Opisthoteuthidae								
Opisthoteuthis sp.	Celtic Sea	1	-	610	-	993	-	971

ND: Not determined.

Table 4. O-Hg concentrations (Mean ± SD; $ng.g^{-1}$ dwt) and their relative contributions (% of the T-Hg) to tissue content and to whole body content in selected cephalopod species.

Species	Sampling area	N	O-Hg concentrations ($ng.g^{-1}$ dwt)			% of O-Hg / T-Hg concentration			% of whole body O-Hg	
			Digestive gland	Remaining tissues	Whole individual	Digestive gland	Remaining tissues	Whole individual	Digestive gland	Remaining tissue
Loligo forbesi	Faroe Islands	5	83 ± 17	110 ± 21	106 ± 19	40 ± 8	79 ± 8	72 ± 9	15 ± 1	85 ± 1
	Celtic Sea	5	181 ± 50	233 ± 69	272 ± 82	44 ± 18	86 ± 4	84 ± 4	3 ± 1	98 ± 1
	Bay of Biscay	5	153 ± 40	115 ± 38	136 ± 50	53 ± 2	77 ± 13	75 ± 12	5 ± 2	95 ± 2
Todarodes sagittatus	Faroe Islands	10	38 ± 11	82 ± 31	70 ± 18	60 ± 8	80 ± 12	74 ± 11	12 ± 4	88 ± 4
	Celtic Sea	5	171 ± 175	637 ± 234	531 ± 207	37 ± 12	84 ± 9	79 ± 7	6 ± 3	94 ± 3
	Bay of Biscay	3	93 ± 31	137 ± 78	126 ± 62	56 ± 8	68 ± 17	67 ± 15	16 ± 13	84 ± 13
Histioteuthis reversa	Bay of Biscay	1	63	143	119	46	98	83	7	93
Teuthowenia megalops	Bay of Biscay	1	63	192	166	37	94	84	5	95
Benthoctopus sp.	Celtic Sea	1	706	818	804	65	89	85	10	90
Eledone cirrhosa	Faroe Islands	4	102 ± 38	124 ± 55	123 ± 54	55 ± 7	88 ± 9	85 ± 8	14 ± 4	86 ± 4
	Celtic Sea	2	153 ± 138	469 ± 377	437 ± 349	57 ± 17	95 ± 3	93 ± 3	6 ± 1	94 ± 1
	Bay of Biscay	6	100 ± 38	267 ± 66	239 ± 39	22 ± 9	89 ± 7	73 ± 7	7 ± 3	93 ± 3
Scaergus unicirrhus	Bay of Biscay	1	352	413	405	36	98	82	16	84

Table 5. Sex and size (mantle length) variations of T-Hg concentrations in cephalopods from the North Eastern Atlantic waters (whole body): results of ANCOVA tests give the F-value and the associated probability (p) in brackets. Statistical significance is shown in bold face.

Location	Species	N	Size	Sex	Size * Sex
Faroe Islands					
	Loligo forbesi	21	**29.737 (<0.0001)**	4.255 (0.055)	**7.972 (0.012)**
	Todarodes sagittatus	37	2.461 (0.126)	0.547 (0.465)	0.828 (0.370)
Celtic Sea					
	Loligo forbesi	18	**20.811 (0.0004)**	0.010 (0.922)	**10.571 (0.006)**
Bay of Biscay					
	Loligo forbesi	12	2.114 (0.184)	0.001 (0.978)	0.037 (0.852)
	L. vulgaris	21	**13.048 (0.002)**	3.853 (0.066)	4.155 (0.057)
	Illex coindetii	22	3.025 (0.099)	0.275 (0.607)	0.949 (0.343)
	Sepia elegans	14	0.925 (0.359)	1.900 (0.198)	1.904 (0.198)
	S. officinalis	23	0.002 (0.964)	1.222 (0.283)	0.070 (0.794)
	S. orbignyana	15	2.530 (0.140)	0.038 (0.849)	0.001 (0.980)
	O. vulgaris	13	2.336 (0.161)	3.208 (0.107)	2.480 (0.150)

Table 6. Mean ± SD ($ng.g^{-1}$ dwt) of T-Hg and O-Hg concentrations and percentage of the O-Hg in the tissues of cephalopods from the literature.

Family Species	Sampling area	Tissue	N	T-Hg Mean ± SD	% O-Hg	Reference
Cephalopod	Adriatic Sea	Edible parts	37	528 ± 282*	-	Sapunar et al (1989)
Squid	Japan	Edible parts	6	120-1320*	39-64	Nagakura et al (1974)
Squid	United Sates	Edible parts	1	486*	35	Cappon & Smith (1982)
Loliginidae						
Loligo forbesi	Azores	Muscle	72	648 ± 396*	-	Monteiro et al. (1992)
L. patagonica	Argentina	Mantle	3	72*	-	Falandysz (1989)
Loligo vulgaris	Adriatic Sea	Edible parts	NA	1530*	63	Buzina et al. (1989)
Loligo vulgaris	North Morocco	Edible parts	10	1920*	-	Stoepler et al. (1979)
Loligo vulgaris	North Sea	Edible parts	10	600*	-	"
Loligo vulgaris	Adriatic Sea	Edible parts	NA	534 ± 42 *	-	Plessi et al. (2001)
Ommastrephidae						
Illex coindetii	Adriatic Sea	Muscle	15	17980 ± 906*	-	Storelli & Marcotrigiano (1999)
Illex coindetii	Adriatic Sea	Digestive gland	15	396 ± 165*	-	
Todarodes pacificus	Japan	Muscle	NA	510*[a]	-	Ichihaschi et al. (2001b)
Todarodes pacificus	Japan	Digestive gland	NA	149*[a]	-	"
Todarodes sagittatus	Azores	Muscle	2	300 ± 72*	-	Monteiro et al. (1992)
Ommastrephes bartrami	Azores	Muscle	14	282 ± 186*	-	"
Sthenoteuthis oualaniensis	Japan	Digestive gland	8	231*	-	Ichihashi et al. (2001a)
Sepiidae						
Sepia officinalis	Adriatic Sea	Edible parts	NA	1416*	83	Buzina et al. (1989)
Sepia officinalis	Sardinia	Edible parts	8	1200*	-	Stoepler et al. (1979)
Sepia officinalis	Adriatic Sea	Edible parts	34	930*	-	"
Sepia officinalis	North Morocco	Edible parts	15	750*	-	"
Sepia officinalis	North Sea	Edible parts	13	480*	-	"
Sepia officinalis	Adriatic Sea	Edible parts	NA	444 ± s *	-	Plessi et al. (2001)
Octopodidae						
Octopus	United States	Edible parts	1	1356	47	Cappon & Smith (1982)
Eledone moschata	Adriatic Sea	Edible parts	NA	138*	-	Plessi et al. (2001)
Eledone sp.	Mediterranean	Edible parts	3	2400*	-	Stoepler et al. (1979)
Octopus salutii	Adriatic Sea	Muscle	17	1620 ± 480*	-	Storelli & Marcotrigiano (1999)
Octopus salutii	Adriatic Sea	Digestive gland	17	2772 ± 1518*		
Octopus vulgaris	Tyrrhenian Sea	Muscle	5	9900 ± 3800*	-	Renzoni et al. (1973)
Octopus vulgaris	Tyrrhenian Sea	Digestive gland	5	367000 ± 274000*	-	"
Octopus vulgaris	Tyrrhenian Sea	Kidney	4	31100 ± 9400*	-	"
Octopus vulgaris	Tyrrhenian Sea	Gills	5	7000 ± 3300*	-	"
Octopus vulgaris	Tyrrhenian Sea	Gonad	5	5600 ± 1900*	-	"
Octopus vulgaris	Adriatic Sea	Edible parts	NA	3120	95	Buzina et al. (1989)
Octopus vulgaris	Azores	Muscle	96	384 ± 348*	-	Monteiro et al. (1992)
Octopus vulgaris	Sardinia	Edible parts	5	1728*	-	Stoepler et al. (1979)
Octopus vulgaris	North Morocco	Edible parts	6	690*	-	"
Octopus vulgaris	Adriatic Sea	Edible parts	4	690*	-	"

* calculated from fresh weight; NA: not available.

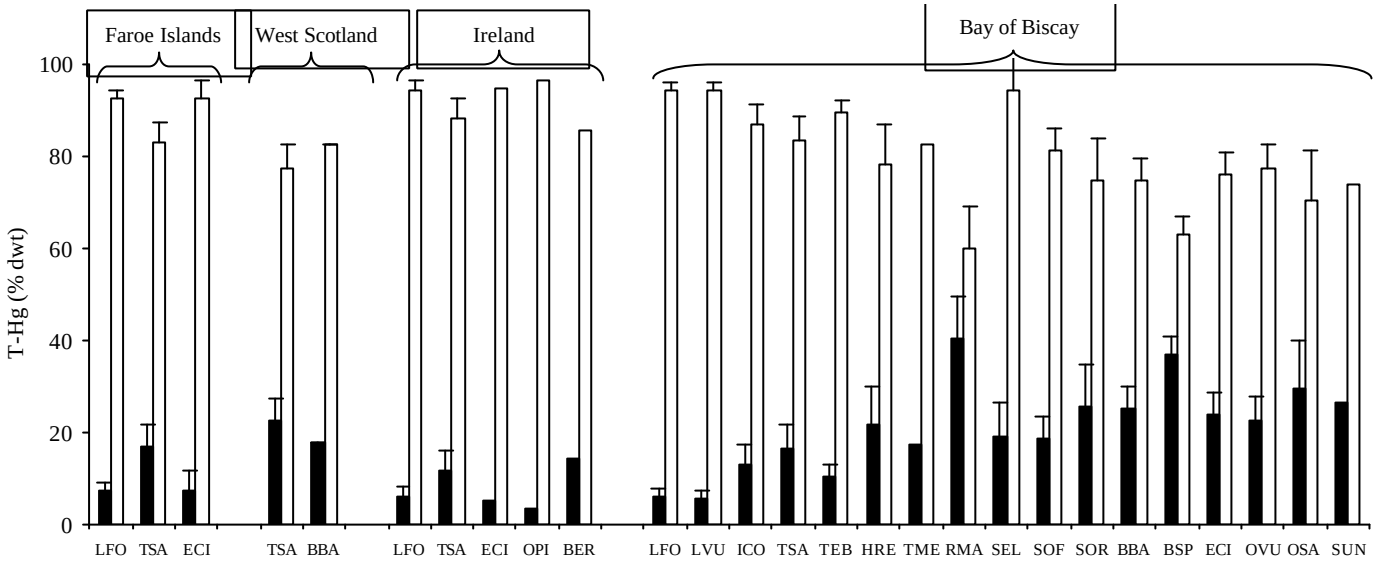

Figure 1. Percentage of the whole body burden of T-Hg (% dwt) contained in the digestive gland (black) and the remaining tissues (white) of cephalopods from the North Eastern Atlantic waters.

BBA : *Bathypolypus baierii* ; BER: *Benthoctopus sp.*; BSP: *Bathypolypus sponsalis* ; ECI: *Eledone cirrhosa*; HRE : *Histioteuthis reversa*; ICO: *Illex coindetii*; LFO : *Loligo forbesi*; LVU : *L. vulgaris*; OPI: *Opisthoteuthis* sp.; OSA : *Octopus salutii* ; OVU: *Octopus vulgaris*; RMA: *Rossia macrosoma* ; SEL: *Sepia elegans*; SOF: *S. officinalis*; SOR: *S. orbignyana*; SUN : *Scaergus unicirrhus*; TEB: *Todaropsis eblanae*; TME : *Teuthowenia megalops*; TSA: *Todarodes sagittatus.*

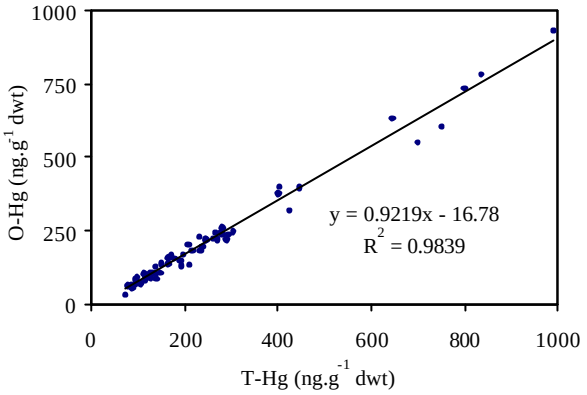

Fig. 2. Relationship between O-Hg and T-Hg (ng.g^{-1} dwt) in the remaining tissues of cephalopods from the North Eastern Atlantic waters.

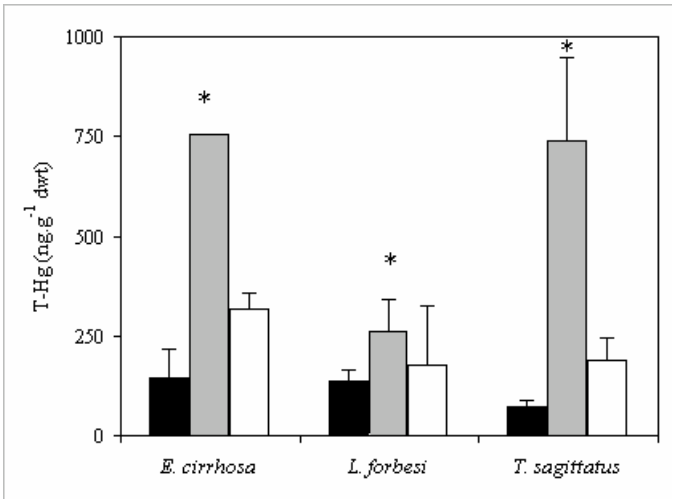

Fig. 3. Comparison of the T-Hg concentrations (Mean ± SD; $ng.g^{-1}$ dwt) in the whole body of *E. cirrhosa*, *L. forbesi* and *T. sagittatus* sampled in the Faroe Island (black), the Celtic Sea (grey) and the Bay of Biscay (white). * indicates a significant difference ($P_{ANOVA} < 0.05$) between the concentrations.

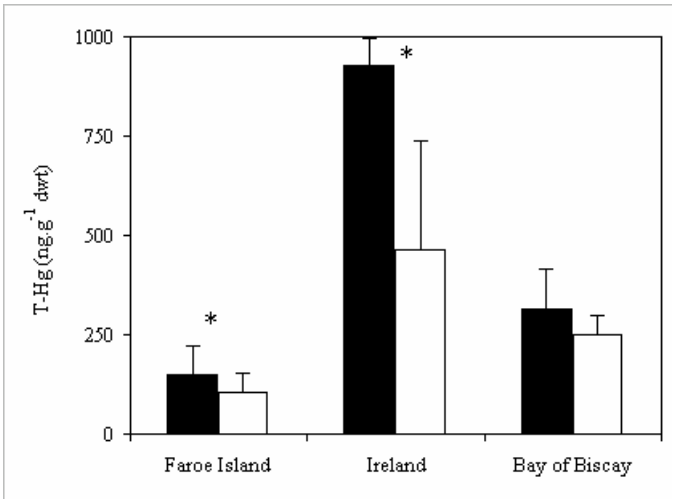

Fig. 4. Comparison of the T-Hg concentrations (Mean ± SD; ng.g^{-1} dwt) in benthic (black) and pelagic (white) cephalopods (whole body). * indicates a significant difference ($P_{ANOVA} < 0.05$) between the concentrations.