INTER- AND INTRA-SPECIFIC VARIATION OF MERCURY LEVELS IN MUSCLE OF CEPHALOPODS FROM THE AZORES

LUIS R. MONTEIRO, FILIPE M. PORTEIRO & JOÃO M. GONÇALVES

ARQUIPÉLAGO

MONTEIRO, LUIS R., FILIPE M. PORTEIRO & JOÃO M. GONÇALVES 1992. Inter- and intra-specific variation of mercury levels in muscle of cephalopods from the Azores. - *Arquipélago*. Life and Earth Sciences 10: 13-22. Angra do Heroísmo. ISSN 0870-6581.

Levels of total mercury were determined in the mantle muscle tissue of 184 cephalopods belonging to four species. Mean levels were significantly higher in *Loligo forbesi* (108 ng.g^{-1} $\pm$ 7.7 *S.E.*) than in *Octopus vulgaris* (64 ng.g^{-1} $\pm$ 6.0 *S.E.*), *Ommastrephes bartrami* (47 ng.g^{-1} $\pm$ 8.3 *S.E.*) and *Todarodes sagittatus* (50 ng.g^{-1} $\pm$ 8.5 *S.E.*). Intra-specific variability in mercury levels was investigated in relation to size, sex, locality and season of capture. The mercury concentrations increased exponentially with cephalopod size. There was a significant difference in the mercury-length and mercury-weight relationships between the sexes in *L. forbesi*, the slopes being higher in females than in males. Mercury levels in *O. vulgaris* were related to locality, being significantly higher at coastal stations under urban influence than in remote coastal stations. Significant seasonal variations in mercury levels were detected in small to medium sized *L. forbesi*.

MONTEIRO, LUIS R., FILIPE M. PORTEIRO & JOÃO M. GONÇALVES 1992. Variação inter- e intra-específica dos níveis de mercúrio no músculo de cefalópodes dos Açores. - *Arquipélago*. Ciências da Natureza 10: 13-22. Angra do Heroísmo. ISSN 0870-6581.

Os níveis de mercúrio total em tecido muscular do manto foram determinados em 184 cefalópodes pertencentes a quatro espécies. Os níveis médios foram significativamente mais elevados em *Loligo forbesi* (108 ng.g^{-1} $\pm$ 7.7 *S.E.*) do que em *Octopus vulgaris* (64 ng.g^{-1} $\pm$ 6.0 *S.E.*), *Ommastrephes bartrami* (47 ng.g^{-1} $\pm$ 8.3 *S.E.*) e *Todarodes sagittatus* (50 ng.g^{-1} $\pm$ 8.5 *S.E.*). A variabilidade intra-específica dos níveis de mercúrio foi estudada em relação com o tamanho e sexo dos indivíduos, a localidade e data de captura. As concentrações de mercúrio aumentam exponencialmente com o tamanho dos cefalópodes. Os declives das relações mercúrio-comprimento e mercúrio-peso diferem significativamente entre sexos de *L. forbesi*, sendo mais elevados nas fêmeas do que nos machos. Os níveis de mercúrio em *O. vulgaris* diferem significativamente entre localidades, sendo mais elevados em estações sob influência de aglomerados urbanos do que em estações de regiões remotas. Os níveis de mercúrio variam sazonalmente de forma significativa em especimens de tamanho pequeno a médio de *L. forbesi*.

Luis R. Monteiro, Filipe M. Porteiro, João M. Gonçalves. Departamento de Oceanografia e Pescas, Universidade dos Açores, P - 9900 Horta, Portugal.

INTRODUCTION

Molluscs are well known for their ability to concentrate trace elements (PHILLIPS 1980). However, most research has been limited to bivalves and gastropods with comparatively little on members of the class Cephalopoda (squids, octopuses and sepias). Cephalopods are active carnivores exhibiting rapid growth and short life spans (see BOYLE 1983, 1987). They are unique among multicellular organisms in their ability to sustain true exponential growth for a significant portion of their life cycle (FORSYTHE & VAN HEUKELEM 1987; MANGOLD 1989). Cephalopods play an important role in oceanic and coastal food webs (AMARATUNGA 1983) and in some parts of the world are important as food resources (see RATHJEN & VOSS 1987).

Cephalopods have been suspected of concentrating mercury (e.g. STOEPPLER & al. 1979; GAJEWSKA & NABRZYSKI 1980; BARSKA & al. 1988; FALANDYSZ 1988, 1989) and other biologically non-essential and highly toxic heavy metals (PHILLIPS 1980). In general the knowledge of intrinsic

biotic (e.g. body size and sex) and extrinsic environmental (e.g. season and sampling locality) variables on the accumulation of mercury in cephalopods, remains inadequate.

The determination of mercury levels in muscle tissue of squid (*Loligo forbesi*, *Ommastrephes bartrami*, *Todarodes sagittatus*) and octopus (*Octopus vulgaris*) from Azorean waters were initiated as part of a study on the levels of metals in edible marine organisms. Levels of mercury were examined with respect to species, size, sex, locality and season. Elucidating these aspects is necessary to be able to interpret the results of baseline and/or trend-monitoring studies of pollutants in the marine environment.

MATERIAL AND METHODS

The cephalopods were caught in the waters of the Azores Archipelago, Central North Atlantic Ocean (Fig. 1). *Loligo forbesi* specimens were caught by jigging at depths between 215 to 270 m, from November 1990 to December 1991; *Octopus vulgaris* specimens were caught by hooking at depths between 0 to 10 m, from January to December 1990 (GONÇALVES 1991); additionally some specimens of the squid *Ommastrephes bartrami* and *Todarodes sagittatus* were caught by jigging machines at depths between 0 to 50 m, in June 1990. Sampling was specifically designed to ensure a complete range of sizes so that the mercury versus size relationships could be examined. Therefore a minimum of 4 specimens for each sex-species-length interval category (interval range: 5 cm for *L. forbesi*, 3 cm for *O. vulgaris*) were obtained. Additional samples containing the modal mantle length class of *O. vulgaris* (11-12 cm) were also obtained to study locality and seasonal effects on mercury levels in standard size specimens.

Freshly caught squid and octopus were frozen at -20°C. When required, specimens were de-

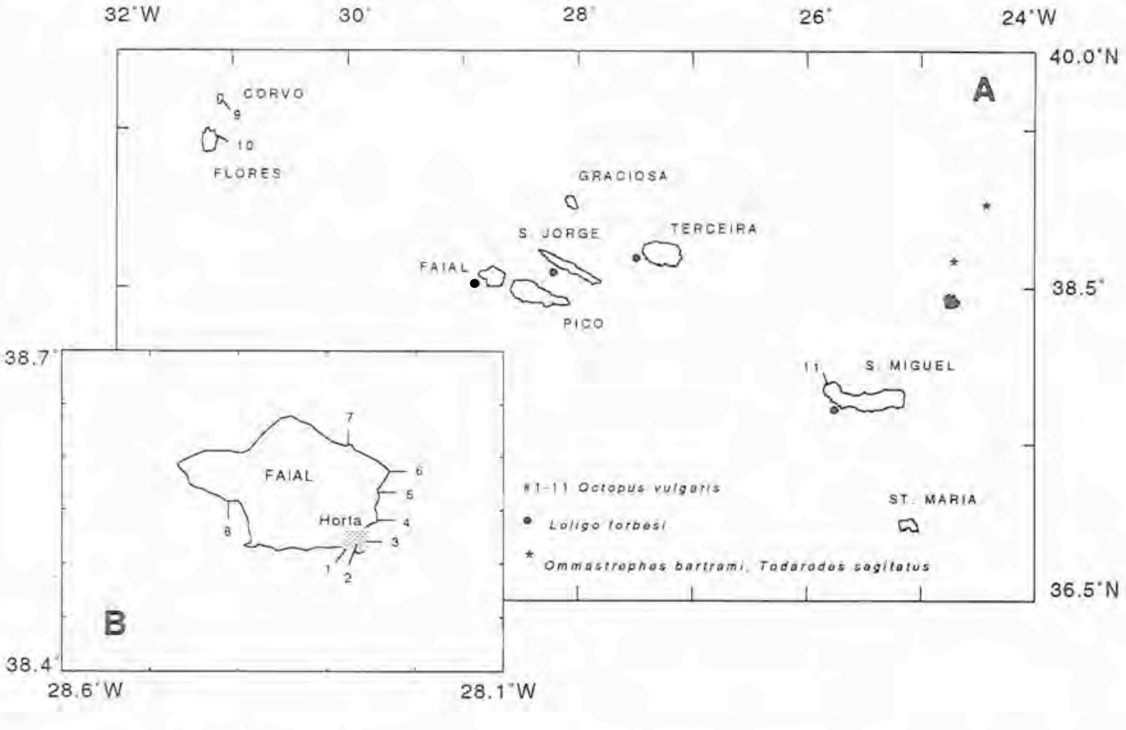

Fig. 1 - Geographical distribution of sampling localities within the (A) Azores Archipelago and in (B) Faial Island.

frosted and dorsal mantle length (DML) was measured to the nearest 1 mm (0.5 cm for *O. vulgaris*) and total weight (TW) was measured to the nearest 0.1 g. The animals were dissected, sexed and a minimum of 10 g of mantle muscle tissue removed and sealed in a plastic bag and stored at -20°C.

For total mercury analysis the skinless muscle sample was macerated in a food cutter to produce a fine homogenous paste. An aliquot (1 to 2 g) of the homogenate was digested and mercury determined by cold vapor atomic spectrophotometry (ANON. 1975), using a Perkin-Elmer mercury analyzer system Coleman 50A. The detection limit of the method was 1 $ng.g^{-1}$. Precision and accuracy were lower than 10% (MONTEIRO & LOPES 1990) and were monitored through the study with replicate samples and standard reference materials of similar matrix (NBS, tuna muscle RM50; IAEA, fish muscle MA-A-2/TM). Samples weight loss, caused by dehydration in relation to the frost/defrost processes, was quantified: whole *O. vulgaris*, mean=2.1% ±0.01 *S.E.*, n=368; *O. vulgaris* samples, mean=8.2% +0.50 S.E., n=65; *L. forbesi* samples, mean=9.0% +0.45 S.E., n=61. However, concentration correction factors were not used, as is usual in these types of study. Mercury concentration values are in nanograms per gram on a wet weight basis ($ng.g^{-1}$ ww).

Prior to a full statistical analyses, mercury, length and weight data were tested for both normality (Kolmogorov-Smirnov one sample test) and homogeneity of variances (Bartlett box *F* test). Nonparametric single factor analyses of variance (Mann-Whitney and Kruskal Wallis tests) were used to investigate factor effects on mercury levels, length and weight. Total mercury concentrations were regressed against dorsal mantle length and total weight of cephalopods, using type I regression analysis fitted by the least squares method on logarithmically transformed data.

RESULTS

A summary of the statistics on mercury levels, length and weight for the four species of cephalopods studied is shown in Table 1. Highly significant inter-specific differences were apparent in mercury levels, length and weight (Kruskal Wallis tests, $P<0.001$), being higher in the specimens of *L. forbesi* than in the specimens of *Octopus vulgaris*, *Ommastrephes bartrami* and *Todarodes sagittatus*. However, the mercury levels observed in *O.vulgaris* showed the greatest intra-specific variability (see Table 1).

Mercury levels in *Loligo forbesi* were not significantly different between males and females (Mann-Whitney *U*-test, P=0.189), despite a highly significant difference in size between sexes (Mann-Whitney *U*-tests, $P<0.001$), with males being larger and heavier than females (Table 2). Also in *O. vulgaris* sexual differences in mercury levels were not detected (Mann-Whitney *U*-test, P=0.356).

Highly significant differences in mercury levels were detected in standard size *O. vulgaris* from different stations (Table 3; Kruskal Wallis test, $P<0.001$). Since, mercury levels did not vary significantly either among stations of urban origin

Table 1.
Mercury level (Hg), dorsal mantle length (DML) and total weight (TW) for *Loligo forbesi*, *Octopus vulgaris*, *Ommastrephes bartrami* and *Todarodes sagittatus* from the Azores.; X = mean; *S.E.* - standard error; Min.- minimum value; Max.- maximum value; CV - coeficient of variation.

species	n	Hg ($ng.g^{-1}$, ww)					DML (cm)				TW (g)			
		X	*S.E.*	Min.	Max.	CV (%)	X	*S.E.*	Min	Max	X	*S.E.*	Min	Max
Loligo forbesi	72	108	7.7	5	359	61	52	23.8	20	94	2438	202.6	261	8308
Octopus vulgaris	96	64	6.0	2	249	91	13	0.4	8	26	966	100.4	8	6110
Ommastrephes bartrami	14	47	8.3	19	122	66	17	2.0	10	33	223	91.5	21	954
Todarodes sagittatus	2	50	8.5	41	58	24	17	2.3	15	19	90	32.0	58	122

Table 2

Mercury level (Hg), dorsal mantle length (DML) and total weight (TW) for *Loligo forbesi, female* and male. X = mean ; *S.E.* - standard error; Min.- minimum value; Max.- maximum value.

sex	n	Hg ($ng.g^{-1}$, ww)				DML (cm)				TW (g)			
		X	*S.E.*	Min.	Max.	X	*S.E.*	Min.	Max.	X	*S.E.*	Min.	Max.
female	19	89	11.7	11	192	34	16.2	20	46	1013	103.4	261	1537
male	53	115	9.5	5	359	60	26.4	24	94	2942	237.2	371	8308

(Kruskal Wallis test, P=0.634) or among stations of remote origin (Kruskal Wallis test, P=0.396), data from each type of station were pooled (see Table 3). A 'locality' effect was apparent, with mercury levels 5 times higher occurring in stations under urban influence compared with stations from remote coastal regions (Mann-Whitney U-test, P<0.001). The variability of mercury levels is also higher in urban stations than in remote stations (see Table 3).

There were seasonal differences in mercury levels which were significant in immature *L. forbesi* (Mann-Whitney U-test test, P<0.03) and highly significant (Mann-Whitney U-test, P<0.001) in small to medium sized *L. forbesi* (DML<47 cm). The differences were caused by higher mercury levels in winter samples compared with autumn samples (Table 4). Mercury levels did not varied significantly (Kruskal Wallis test, P=0.489) during a one year cycle in standard sized *O. vulgaris* from urban stations (# 1, 2, 3; n=39).

The relationships between mercury level in muscle tissue (Hg) and dorsal mantle length (DML) or total weight (TW) conformed to a power function: Hg=a (DML or TW)^b, where a and b are constants. Parameters, correlation coefficients and significance of the ln(Hg) versus ln(DML or TW) simple regression relationships for *L. forbesi* female, *L. forbesi* male, *O. vulgaris* (specimens from urban stations) and *O. bartrami* are shown in Table 5. Plots of mercury level versus length relationships are given in Fig. 2. For *L.*

Table 3

Effect of locality on mercury levels ($ng.g^{-1}$ ww) in muscle tissue of standard size (DML = 11-12 cm) *Octopus vulgaris*. See Fig. 1 for location of stations. n= number of specimens analysed; X = mean; *S.E.* - standard error; Min.- minimum value; Max.- maximum value; CV - coeficient of variation.

station	type	location (island)	n	X	*S.E.*	Min	Max	CV(%)
1	urban	Pasteleiro (Faial)	34	89	10.8	11	237	
2	urban	Porto Pim (Faial)	2	43	12.5	30	55	
3	urban	Porto da Horta (Faial)	3	67	14.7	39	72	
4	remote	Espalamaca (Faial)	1	8				
5	remote	Pedro Miguel (Faial)	1	2				
6	remote	Ribeirinha (Faial)	3	19	3.0	13	22	
7	remote	Salão (Faial)	1	18				
8	remote	Varadouro (Faial)	6	16	3.9	5	29	
9	remote	Sta. Cruz (Flores)	3	17	3.8	13	25	
10	remote	Vila Nova (Corvo)	1	27				
11	remote	Mosteiros (S. Miguel)	2	24	1.5	22	25	
1-3	urban		39	85	9.7	11	237	60
4-11	remote		18	17	1.9	2	29	8

Table 4

Effect of season on mercury levels (ng.g-1 ww) in muscle tissue of small to medium sized *Loligo forbesi* (DML < 47 cm). X = mean; *S.E.* - standard error; Min.- minimum value; Max.- maximum value.

season	n	X	*S.E.*	Min.	Max.
Autumn	12	39	8.1	5	109
Winter	22	95	9.3	38	187

forbesi the slopes of the relationships differ significantly between sexes (lnHg vs. lnDML: *t*-test, $P<0.001$; lnHg vs. lnTW: *t*-test, $P<0.02$), being about twice as high in females as in males (Table 5, Fig. 2).

DISCUSSION

Inter-specific variation in mercury levels

The mean mercury levels in muscle tissue of the cephalopods studied varied between species: *O. vulgaris* < *O. bartrami* ≃ *T. sagittatus* < *L. forbesi*. For the purpose of this discussion the *O.vulgaris* samples of urban origin were excluded since they reflect local environmental contamination (see discussion below). The inter-specific variability may be due to differences in e.g. trophic level (YOUNG & al. 1980), growth (MONTEIRO & al. 1991) and extent of habitat contamination (COSSA & RONDEAU 1985), all of which are generally assumed to affect mercury levels in the tissues of marine organisms.

Both the octopus and squid studied appear to accumulate mercury levels of the same order of magnitude common to marine organisms from medium trophic levels (III to IV)(YOUNG & al. 1980). Cephalopods feed mainly on fish, crustacea and molluscs, but the relative importance of these groups varies widely between species (NIXON 1987). The existence of significant differences in mean mercury levels between squid and *O. vulgaris*, (which is almost a sixfold difference compared to *L. forbesi*), is probably due to a difference in diet. The rate of food ingestion differs widely between *Octopus* (16h) and *Loligo/Ommastrephes* (3-6h)(NIXON 1987). Squid are nektonic with a diet based primarily on fish

Table 5

Values for *a* and *b* for the regression equation Hg = *a* (DML or TW)^b for *Loligo forbesi* (female and male), *Octopus vulgaris* and *Ommastrephes bartrami.*

variable	species	n	*a*	*b*	*r*[1]	*P*
length (cm)	*L. forbesi* female	19	2.04×10^{-3}	2.980	0.927	< 0.0001
	L. forbesi male	53	2.78×10^{-1}	1.443	0.698	< 0.0001
	O. vulgaris	42	1.95	1.146	0.428	< 0.005
	O. bartrami	14	2.01	1.085	0.781	< 0.001
weight (g)	*L. forbesi* female	19	1.49×10^{-2}	1.245	0.928	< 0.0001
	L. forbesi male	53	4.03×10^{-1}	0.701	0.705	< 0.0001
	O. vulgaris	42	2.25	0.424	0.477	< 0.002
	O. bartrami	14	8.54	0.827	0.827	< 0.001

[1]Correlation coefficients between ln(mercury level) and ln(length or weight).

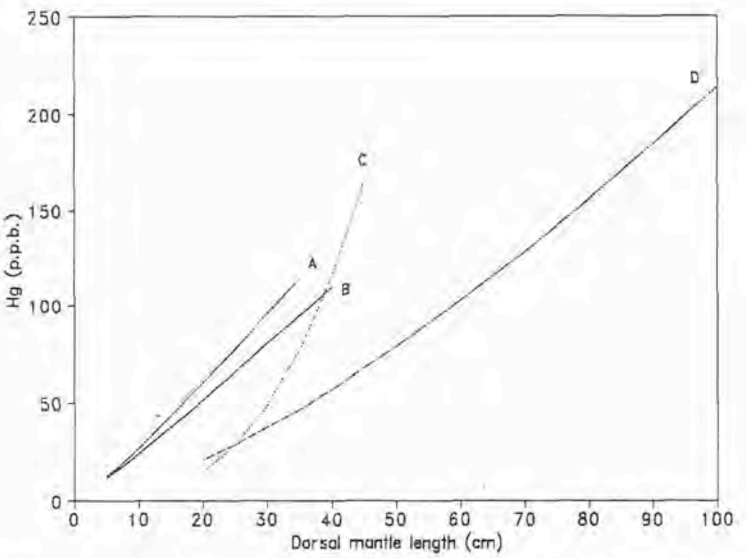

Fig. 2 - Relationships between mercury level in mantle muscle and dorsal mantle length for (A) *Octopus vulgaris*, (B) *Ommastrephes bartrami*, (C) *Loligo forbesi* female and (D) *L. forbesi* male.

(NIXON, 1987; relative frequency in *L. forbesi* =82%, MARTINS 1982) and could be assigned to the IV trophic level. *O. vulgaris* is benthic and preys primarily on crustaceans (relative frequency=80%, GUERRA 1978) and molluscs (GUERRA 1978) and could be assigned to the III-IV trophic level (MANGOLD 1983). Since mercury content is positively correlated with trophic level (YOUNG & al. 1980) the higher mercury levels in *L. forbesi* and the other squids can be interpreted as a consequence of biomagnification through the food web. The lower mercury levels observed in *O. bartrami* and *T. sagittatus* in relation to *L. forbesi* could not be attributed to inter-specific differences in feeding behaviour and related mercury intake. In fact, fish predominates in the diet of the three squid species and the rate of ingestion is similar in *Loligo* (4-6h at 18°C) and *Ommastrephes* (3-4h) (NIXON 1987).

Differences in mercury levels among the three species of squid studied could be attributed to the lack of large specimens of *O. bartrami* and *T. sagittatus* (DML up to 75 cm, ROPER & al. 1984) in our sampling, since the slopes of the mercury versus size relationships indicated similar rates of mercury accumulation in *L. forbesi* female and *O. bartrami*.

Habitat type had an important influence on the variability of mercury levels observed in *O. vulgaris*. Coastal benthic species are more likely to come into contact with mercury contamination from land-based sources thus producing a greater range in contamination levels. Mercury levels in neritic (*L. forbesi*) and oceanic (*O. bartrami* and *T. sagittatus*) species tend to reflect the evenly distributed background levels, thus giving a low variability.

Intra-specific variation in mercury levels

The results from this study show that size, sex, season and locality are variables which significantly determine the mercury levels in cephalopods.

Mercury levels in mantle muscle of *L. forbesi*, *O. vulgaris* and *O. bartrami* increase exponentially with size and consequently age. Studies concerning the influence of size on mercury levels in other molluscs are scarce (BOYDEN 1977) and several authors (cited in PHILLIPS 1980) refer to the lack of consistency in the relationships of mer-

cury versus size and age in bivalves under field conditions. Conversely, it is well known that mercury levels in fish muscle normally increase exponentially with fish size and age (e.g. WALKER 1977, MONTEIRO & LOPES 1990, MONTEIRO & al. 1991). Therefore, these results suggest that cephalopods exhibit pollutant kinetics much more similar to those of fish than those of other molluscs. This strongly supports the notion of biological similarities between cephalopods and fish (PACKARD 1972) rather than with other molluscs.

The higher rate of mercury accumulation in *L. forbesi* females may be due to the existence of some physiological and/or ecological inter-sex differences. Sexual dimorphism is a characteristic feature of this species (MARTINS 1982) but there is no evidence that males outlive females (FORSYTHE & VAN HEUKELEM 1987). FORSYTHE & HANLON (1989) suggested that immature females and males have the same size-at-age, but as the ovary of female starts to mature they gradually divert more energy from somatic to reproductive growth. Males, on the other hand, have a minor investment in reproductive tissue and continue to grow somatically. This leads to the hypothesis that the inter-sex differences in mercury accumulation were possibly due to metal concentration in mature females caused by growth levelling off at the onset of maturity, following the general pattern of metals being more highly concentrated in the tissues of the slower growing individuals (PHILLIPS 1980, MONTEIRO & al. 1991). Very little information concerning possible sex-based differences in mercury accumulation exists for molluscs. Similar sex-based differences in mercury versus size or age relationships have been observed in several fish species with differences in growth rates between sexes (MONTEIRO & LOPES 1990, MONTEIRO & al. 1991), however, this cannot be solely attributed to the higher growth rate of one sex with respect to the other. It must be emphasized that a more detailed knowledge of *L. forbesi* physiology and ecology is required before sexual difference in mercury accumulation kinetics may be fully interpreted.

Variability of mercury levels in standard size *O. vulgaris* is primarily due to locality. The most striking fact is the fivefold difference in mean mercury levels between areas under urban influence and remote coastal areas. This difference suggests that bioavailable mercury originated from land-based anthropogenic sources in the city of Horta reaches the species via food chain and/or coastal sediments. The variability of mercury levels in standard size *O. vulgaris* from areas under urban influence is also remarkably high when compared with the same variability in specimens from remote areas. This may be caused by individual variations in foraging behaviour (MATHER & O'DOR 1991), which possibly produces a wider range of exposure to mercury under urban influence compared to natural conditions.

Causes for seasonal variations in mercury levels of immature and small-medium sized *L. forbesi* specimens were not clear. The levels were lower in autumn than in winter, but the limited period of sampling (October-March) did not allow the detection of any pattern. The factors involved in seasonality of mercury and other pollutants in aquatic biota are complex and three major categories were identified (PHILLIPS 1980): *i*) pollutant delivery to the aquatic environment; *ii*) organism physiology, particularly the sexual cycle; *iii*) changes in the prevailing water quality parameters such as temperature and salinity. Nevertheless, this field remains open to further work. The results from much of the studies of mercury and other metals in molluscs are unanimous in concluding that seasonal variability in metal levels within sites is irregular and unpredictable. However, COSSA & RONDEAU (1985) detected a well defined pattern of seasonal variation in mercury content of the mussel *Mytilus edulis*, over two years, and argued that the discrepancies between the various studies probably result from poor sampling design (short periods and low sample sizes).

Pollution and monitoring

The mercury levels found in the cephalopods studied suggest that the level of contamination is low, both in the oceanic and in the coastal environments. All the mercury levels found were well below the guideline limit (1 $mg.kg^{-1}$ ww) for mercury in seafood fixed by the Portuguese Food Directorate. The metal levels found in the three squid species were similar to the levels reported for other oceanic and coastal squids from northeastern Atlantic (FALANDYSZ 1989) and

southwestern Atlantic (FALANDYSZ 1988, 1989), but were lower than the mercury levels reported in the smaller *Loligo vulgaris* (mean weight=97 grams) from the Mediterranean Sea by STOEPPLER & al. (1979)(mean=320 ng.g^{-1}, range=85-530, n=10). Only the mercury levels observed in *O. vulgaris* from urban areas indicated a moderate level of pollution in the coastal area around the city of Horta (Faial Island). However, these were considerably lower than the mercury levels in *O. vulgaris* from the Mediterranean (mean=288 ng.g^{-1}, range=80-710, n=5) (STOEPPLER & al. 1979).

Cephalopods have attracted least attention amongst molluscs as indicators of environmental contamination (PHILIPS 1980). However, they warrant further study as they can be easily sampled and are abundant. Cephalopods present bioconcentration factors of mercury at about 10^3 to 10^4 (cephalopod/water concentration), which suggests a limited ability to regulate such a pollutant. Due to rapid growth and a short-time span, regular sampling will provide information on short-term variations in mercury contamination. Other molluscs and fish used in these studies tend to be long-lived, therefore results of pollutant levels are averaged over longer time spans.

The worldwide distribution of *O. vulgaris* (WELLS 1978, NESIS 1987, MANGOLD 1983) makes this species suitable as the primary organism for the assessment and monitoring of mercury contamination in nearshore coastal environments. This is specially applicable in areas, like the Azores, where the widely used mussels (*Mytilus* spp.) are absent. This species fulfill the basic requirements of an indicator organism (PHILLIPS 1980; DEPLEDGE & RAINBOW 1990) and, as shown in this study, was sensitive enough to indicate small scale differences in mercury environmental contamination between remote and urban areas. Territoriality in this species (home ranges=100-200 m^2, up to six weeks; MATHER 1991), allows point sources of contamination to be elucidated and there are no problems with respect to sexual differences in mercury accumulation. We suggest the use of standard size specimens to increase the sensivity in biomonitor studies of mercury. Functional relationships to describe the mercury versus size dependence also provides an interesting possibility of comparison and analysis using the slopes, provided that adequate sample numbers and size ranges are used to develop satisfactory predictive models (BOYDEN 1977).

The oceanic squid species which are widely distributed in the open oceans, like *O. bartrami* and *T. sagittatus* (ROPER & al. 1984, NESIS 1987), were attractive for monitoring medium- to long-term temporal variations and large-scale geographical variations of mercury levels in the marine environment. They have attracted little attention as indicators of environmental contamination and deserve further study.

CONCLUSIONS

This study presents the first data on mercury levels and their natural variability in cephalopods from the Azores and Central North Atlantic. The importance of variables such as size, sex, locality and season on the accumulation of mercury by cephalopods is revealed. The concept that cephalopods exhibit pollutant kinetics similar to fish is suggested. Low to moderate mercury contamination was detected in the coastal environment close to a low level industrialized city of 7 thousand inhabitants. An argument for *Octopus vulgaris* as a bioindicator of mercury pollution in nearshore coastal environments is presented.

ACKNOWLEDGEMENTS

This research was financed by Regional Secretariat of Agriculture and Fisheries of the Azores. We thank Dr. Humberto Lopes, Luís Macedo and Leonilda Lopes for assistance in mercury determinations; Fátima Serpa, José Branco, Victor Rosa, and Fernando Goulart for assistance in cephalopod sampling; Dr. Helen Martins and Prof. Peter Boyle for permission to use biological material from the "Eurosquid" (EEC project, FAR M.A. 1.146); Prof. Michael Depledge, Dr. Helen Martins, Dr. Helder Silva and Dr. Richard Nash for comments and kind review of the manuscript.

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Accepted 30 June 1992