Contaminants in deep water fish

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Summary

Metals, PCBs and pesticides were measured in various organs of 13 deep water fish species caught between 80 and 4000m depth in 5 locations in the eastern North Atlantic and one in the eastern South Atlantic. The results were investigated in relation to biological and environmental factors.

The levels of metals and organic contaminants in muscle tissue were generally below the EU regulatory limits for food, although up to 10% of individuals were above. Livers were not suitable for human consumption and, with the forthcoming EU regulations on PCBs and dioxins, will not be suitable as fish oil or fish meal either.

Metal levels in deep water fish were similar to those in shallow water fish, apart from Cu, Cd and Hg, which were higher. Levels were similar in both hemispheres, but differences between areas were found. Top predators, benthic feeders and species below 2000m depth were more contaminated. The preferred organ of accumulation of each metal as well as correlations with depth were species dependent. Hg and Cd were positively correlated with length, showing bioaccumulation, whilst As was negatively correlated with length as it is adsorbed from the gills rather than food.

Organic contaminants were evenly distributed throughout the body's lipids, by equilibrium with the concentrations in the surrounding water. Inter-species differences were attributed to differences in phospholipid compositions. Levels of organic contaminants were substantially lower in the southern hemisphere. Compared to shallow water fish, deep water species presented an increased burden of p,p'-DDT, due to their lesser ability to metabolise this compound; and of higher chlorinated biphenyls, due to selective transport to the deep sea. Differences between water masses were visible from the HCH isomer composition. PCB concentrations presented a seesaw pattern with the log of the octanol-water coefficient rather than a linear relationship, which was attributed to the specific steric effect of each congener.

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Declaration

I declare that the following thesis is a record of the work carried out by myself in the Marine Laboratory, Aberdeen, in conjunction with the University of Glasgow. It has not been accepted in any previous application for a higher degree. All sources of information have been acknowledged by the appropriate references.

Sophie Mormede

Preface

This volume presents the work carried out by myself during my PhD research at the Marine Laboratory in Aberdeen, entitled "Contaminants in deep water fish".

In the first chapter I have given a brief introduction on the contaminants I have quantified in the study and on the deep sea environment in general, and set the aims of the study. The second chapter summarises available information on the toxicity of the chemicals I was interested in, both to animals and humans, as well as known toxicity mechanisms and transfer through the marine food chain. Chapter 3 gives more information on the species studied and the analytical methods used throughout this thesis. Chapter 4 summarises the results obtained over the three years.

Chapter 5 addresses the health issues raised by the presence of these contaminants in human food. I detail present European regulations that apply to these contaminants in food and foodstuffs, and their implications for deep water fish consumption. Chapters 6 and 7 are publications I have submitted during the course of my thesis: Chapter 6 is an in-depth analysis of the data obtained for monkfish and black scabbardfish in particular, with comparisons to literature and biological meanings where found. Chapter 7 concentrates on the changes in contaminant levels in deep water fish with depth and location.

Finally, Chapter 8 and 9 contains in depth discussion on the results of the whole thesis. Chapter 8 focuses on metals and Chapter 9 on organic contaminants. Chapter 10 pulls together the most important findings of the thesis.

A summary, table of contents, list of figures, tables and references are also provided. The raw concentrations measured in each individual fish are included in a CD attached to the inside back cover, metals expressed in mg/kg wet weight and organic contaminants in μg/kg lipid weight. A paper copy is not provided of this appendix because of the size of the data set. A list of fish species and their abbreviations is included in appendix 2.

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Chapter 1
Introduction

With the development and acceleration of industrialisation, man has been using and producing an increasing variety and quantity of anthropogenic elements and compounds such as metals and organic contaminants. Some of these are highly stable and/or not easily degraded, as is the case for polychlorinated biphenyls (PCBs), pesticides and metals, which will be the main focus of the present study. They are called conservative pollutants because they are not subject to bacterial attack, or if they are, degradation is so long that they are considered as persistent additions to the environment.

The European Union defines aquatic pollution as "the discharge by man, directly or indirectly, of substances or energy into the aquatic environment, the results of which are such as to cause hazards to human health, harm to living resources and to aquatic ecosystems, damage to amenities or interference with other legitimate uses of water" (Crompton, 1997). The presence of contaminants in fish is of concern because they may affect the health and reproduction of fish and may accumulate in humans when consumed and, in turn, affect human health. Some toxicological effects on fish of a selection of contaminants are summarised in table 1.1. The toxicity of contaminants to humans forms the grounds of EU and international regulations on acceptable levels of contaminants in food and foodstuffs, and will be examined in chapter 5.

The transport of metals and organic contaminants to the marine environment, as well as their toxicity to organisms and humans, will be addressed in chapter 2. General physical and biological characteristics of the deep sea environment as well as levels of its contamination by metals and organic compounds will be addressed in the following sections.

Table 1.1: Some toxicological effects of selected contaminants.

mercury	Nerve toxin. Particularly damaging to developing young. Reduces plant growth. In fish, damages gills, disrupts gut absorption and chemoreception. Strongly bioaccumulative.
cadmium	Affects growth and larval development. Upsets ionic control and calcium metabolism in fish. Accumulates in kidney and liver of higher animals and disrupts calcium and vitamin D metabolism.
lead	Damages central nervous system, particularly when the brain is growing. Accumulates in gills, liver and kidney of fish and reduces larval survival.
PCBs	Highly accumulative. Effects depend on the chemical structure, coplanar forms are most toxic. Generally immunosuppressive, some are carcinogenic and/or hormone disruptors.
dioxins and furans	Carcinogenic, immunosuppressive, disrupt reproduction. Generally more toxic than coplanar PCBs
HCBs	Inhibit haem synthesis and cause porphyria
DDT	Hormone disruptor, affects liver enzymes, causes eggshell thinning in birds.
toxaphene	Affects nervous system, particularly in fish which become hyperactive and lose balance control.
chlordane	Neurotoxin, carcinogenic, disrupts immune and reproductive system.
dieldrin	Carcinogenic.
PAHs	Wide range of substances, some are carcinogenic and/or mutagenic.

1.1. The deep sea environment

The seabed covers two-thirds of the surface of the Earth, of which 99% lies beyond the shallow margins of the continents, and most lies under more than 2km of water. Therefore, the deep sea constitutes the most typical environment of the earth. However, because of its remoteness, which induces difficulties in observing and sampling deep sea organisms, it has been little studied until recently.

1.1.1. Characteristics of deep sea fauna

The interest in deep water fisheries has increased markedly in the last decade, particularly in the North Atlantic. Work on the biology and ecology of these species is leading to a better understanding of stock size, production and behaviour (eg Gordon, 1985; Gordon and Duncan, 1987; Savvatimsky, 1993).

The fauna of the deep sea has been studied in detail (eg Gordon and Mauchline, 1990). There is a steady increase in the numerical abundance of fauna with increasing depth to a peak at 1250m depth, a large part of which is attributable to the deep sea eel *Synaphobranchus kaupi*. Below 1250m, numerical abundance declines with depth. The changes in biomass follow a similar pattern with depth, but the biomass attributable to *S. kaupi* is small and alepocephalids, especially *Alepocephalus bairdii*, become an important component of the biomass. A few species dominate the biomass in each bathymetric zone in the Rockall Trough and have been tabulated by Gordon and Mauchline (1990) (table 1.2).

About half, by numbers, of all the fish feed on benthopelagic prey, with benthic feeders accounting for about 30% of fish. Only small numbers feed on pelagic organisms. The diet of the deep sea megafauna depends on the depth at which it lives. Fish are dominant in the diet of the biomass at 500 to 1000m depth, which corresponds to the highest biomass of piscivorous predators such as the squalid sharks, the black scabbardfish (*Aphanopus carbo*) and the larger gadid fishes such as the blue ling (*Molva dypterygia*). Below 1000m depth, fish are no longer the dominant component of the biomass diet, but remain important, especially when, in terms of energy, a single fish can be several orders of magnitude greater than, for example, a copepod. Moreover, the presence of species such as blue whiting (*Micromesistius poutassou*) in the diet of some of the deeper species suggests that these species may be scavenging food-falls from the upper pelagic zone (Gordon and Mauchline, 1990).

The main concerns over chemical contamination in deep water fish arise because many of the deep water species are long lived and tend to feed at higher trophic levels than their shallow water counterparts (Gordon *et al.*, 1995). Hence they

present a higher potential for accumulation of contaminants and in particular conservative contaminants.

Table 1.2: Species which make up at least 90% of the total biomass in each bathymetric zone in the Rockall Trough. Species in bold are studied in the present work.

zone (m)	Species	%		%
500	***Aphanopus carbo***	**24.5**	*Lepidion eques*	
	Chimaera monstrosa	24.2	***Merluccius merluccius***	**3.2**
	Coryphaenoides rupestris	**11.1**	*Centrophorus squamosus*	2.8
	Deania calceus	8.2	***Lophius piscatorius***	**2.4**
	Phycis blennoides	5.6	*Molva molva*	2.4
	Argentina silus	4.4		2.1
750	***Coryphaenoides rupestris***	**30.4**	*Lepidion eques*	2.7
	Deania calceus	24.1	*Brosme brosme*	2.1
	Chimaera monstrosa	6.9	*Centroscymnus coelolepis*	2.0
	Centroscymnus crepidater	5.8	*Nezumia aequalis*	1.8
	Aphanopus carbo	**4.6**	*Mora moro*	1.7
	Molva dypterygia dypterygia	**3.2**	*Raja nidarosiensis*	1.7
	Centrophorus squamosus	2.8	*Epigonus telescopus*	1.6
1000	*Alepocephalus bairdii*	41.2	*Chimaera monstrosa*	
	Coryphaenoides rupestris	**19.6**	***Molva dypterygia dypterygia***	**4.5**
	Centroscymnus coelolepis	12.7	*Deania calceus*	3.8
	Aphanopus carbo	**8.1**		2.3
1250	*Alepocephalus bairdii*	53.3	*Centroscymnus coelolepis*	
	Coryphaenoides rupestris	**28.8**	*Trachyrhynchus murrayi*	2.8
	Aphanopus carbo	**2.9**		2.1
1500	***Coryphaenoides rupestris***	**41.6**	*Trachyrhyncus murrayi*	3.5
	Alepocephalus bairdii	39.4	*Synaphobrancus kaupi*	3.1
	Centroscymnus coelolepis	3.9		
1750	***Coryphaenoides rupestris***	**35.2**	*Etmopterus princeps*	6.4
	Synaphobranchus kaupi	11.7	*Cataetyx laticeps*	4.3
	Coryphaenoides guentheri	9.3	*Hydrolagus affinis*	3.2
	Alepocephalus bairdii	8.6	*Coryphaenoides mediterranea*	2.8
	Hydrolagus sp.	8.1	*Coelorhynchus labiatus*	2.8
2000	*Antimora rostrata*	60.9	*Alepocephalus agassizii*	8.6
	Coryphaenoides guentheri	15.8	*Synaphobranchus kaupi*	5.9
2250	*Coryphaenoides guentheri*	36.1	*Narcetes stomias*	5.5
	Antimora rostrata	19.5	*Halosauropsis macrochir*	3.6
	Spectrunculus grandis	15.0	*Coryphaenoides mediterranea*	3.0
	Alepocephalus agassizii	9.0		
2500	*Antimora rostrata*	41.6	***Histiobranchus bathybius***	**7.9**
	Coryphaenoides armatus	23.7	*Spectrunculus grandis*	3.2
	Coryphaenoides guenther	12.5	*Alepocephalus australis*	3.1
2900	***Coryphaenoides armatus***	**81.2**	***Histiobranchus bathybius***	**11.1**

1.1.2. Hydrography of the Rockall Trough area

The deep sea is composed of layers of water masses of different temperature and salinity characteristics originating from the poles (Ballschmiter, 1992; Cromwell, 2000). The deep sea water masses are made of the arctic and antarctic convergences, which carry high latitude surface water to the depth of the oceans. Therefore, any pollutant input by deposition in these areas, as well as those contained in the surface water masses at the poles, will be carried into the deep sea.

The hydrography of the Rockall Trough has been studied in detail by Ellett *et al.* (1986). Two main water masses occupy the Rockall Trough. The upper water mass, which extends from the surface to about 1500m depth, derives from the North Atlantic Central Water. Beneath these depths, the lower water mostly originates from the Labrador Sea. However, influences from other water masses are apparent. The water masses are composed of the Eastern North Atlantic Water between the surface and 200m depth, the Subarctic Intermediate Water between 200 and 400m depth, the North Atlantic Central Water between 400 and 900m depth, the Gulf of Gibraltar Water between 900 and 1400m depth, and the Labrador Sea Water below that depth. Although boundaries are discrete and present both water temperature and salinity differences, the depth of these boundaries vary according to environmental factors such as the season or the weather. A map of the areas sampled and their bathymetries is reproduced in fig 3.1.

1.2 Metals

1.2.1. Background

Metals are considered to be the oldest toxins, known to humans since the Stone Age. Lead was mined in ancient Egypt before 2000BC, as a by-product of smelting silver. Arsenic, used for the decoration of tombs in Egypt, was recovered while melting copper; and tin was mentioned by Theophratus of Erebus (370-287BC). Antimony was used medicinally as far back as 4000BC. Hippocrates was the first

philosopher/physician to describe the symptoms of abdominal colic in a man who extracted metals from soil (Clark, 1997).

The advent of industrialisation has associated many occupational diseases with a variety of toxic metals, in addition to their role as environmental pollutants. However, many metals have therapeutic applications and some of them are essential nutrients (see chapter 2 for details).

Although 80 of the 105 elements in the periodic table are metals, fewer than 30 have been reported as toxic to humans, and even less are detectable in fish. The present study will focus on Mn, Cu, Zn, As, Se, Cd and Hg. All of these were found in detectable amounts in deep sea fish and are toxic in various concentrations. All but Cd and Hg are essential metals, necessary for life in small amounts (see chapter 2 for details).

1.2.2. Metals in deep water fish

Metals in the environment are of both anthropogenic and natural origins, and it is often difficult to distinguish between these two inputs. Moreover, in comparison with shelf species, relatively little is known about the "natural" levels of trace metals in deep water species. Deep water fisheries are growing in importance, and there is increasing interest in quantifying the level of contamination of the deep sea environment.

Few data are available on trace metal contamination in deep-sea fish (Cross *et al.*, 1973; Leatherland *et al.*, 1973; Morris, 1975; Windom *et al.*, 1987; Bonwick *et al.*, 1990; Vas *et al.*, 1993; Forlin *et al.*, 1996; Takahashi *et al.*, 1997; Cronin *et al.*, 1998; Mormede and Davies, 1998 and 2001a). Most of these studies concentrate on levels of metals in muscle tissue only, without any broader picture of the accumulation processes and distribution of the metals in the other tissues of individual fish.

Among the six most recent papers related to metals in deep-sea fish, three of them did not study the same metals as in the present study. Bonwick *et al.* (1990)

qualitatively studied the methallothionein in liver from deep-sea sharks, which is suggested to play a role in the detoxification of non-essential metals and in the regulation of essential metals. Forlin *et al.* (1996) studied the effects of contaminants in roundnose grenadiers (*Coryphaenoides rupestris*) and measured metals in mussels (*Mytilus edulis*). Takahashi *et al.* (1997) studied butyltin residues in deep-sea organisms collected from Suruga By, Japan, finding up to 980 $\mu g\ kg^{-1}$ wet weight in deep-sea fish, which is comparable to reported values from other developed countries, and close to estimated effective chronic toxicity end-points. The TBT/3BT ratio was higher in deep-sea fish than in the shallow water related species, suggesting an enhanced risk to deep-sea organisms because of the higher toxicity of TBT than other butyltins.

Table 1.3 summarises the available data on metals in muscle tissue of deep water fish species. Windom *et al.* (1987) studied Al, Cd, Co, Cu, Fe, Pb, Mn and Ni in muscle in *Coryphaenoides armatus* from North Atlantic and North Pacific. Vas *et al.* (1993) measured Cu, Mn and Ni in liver, gills and muscle in deep-sea fish from the Rockall Trough, North Atlantic. Cronin *et al.* (1998) studied Hg, Zn, Cu, Cd and Pb in muscle of deep-sea fish from the Rockall trough as well. Mormede and Davies (1998 and 2001a) measured Cd, Cu, Pb, Hg and Zn in muscle tissue, liver and gills of three deep-sea fishes (*Nezumia aequalis, Lepidion eques* and *Raja fyllae*) as well as Cr and Ni, which were not detected. Windom *et al.* (1993) reported their data in $mg\ kg^{-1}$ dry weight whereas the others reported it on a wet weight basis. The wet to dry weight coefficient for the muscle tissue of the species studied is not exactly known, but assumed to be around 6 for grenadiers (Hoc *et al.*, unpublished data).

Table 1.3: Median and range of metal concentrations in deep water fish muscle tissue, expressed in mg kg^{-1} wet weight apart from results from Windom *et al.* (1987), which are expressed in mg kg^{-1} dry weight (ND: not detected).

Study	species	cadmium	copper	lead	mercury	zinc
Windom *et al.* 1987	*C. armatus* Atlantic Pacific	 0.027±0.025 0.025±0.012	 0.74±0.42 1.09±0.45	 0.012±0.011 0.016±0.007		
Vas *et al.* 1993	*C. rupestris* *C. guentheri* *A. rostrata* *S. bathybius* *C. armatus*		0.53 0.12 0.26 <0.02 <0.02			
Cronin *et al.* 1998	*C. rupestris*	ND–0.01 0.002	0.03-0.54 0.08	ND-0.06 0.004	0.02-0.28 0.07	1.7-2.9 2.2
	M. berglax	ND-0.21 0.010	ND-0.24 0.01	0.003-0.04 0.010	0.15-0.88 0.34	2.8-3.9 3.2
	H. atlanticus	ND-0.01 0.010	0.04-0.19 0.09	ND-0.66 0.010	0.11-0.86 0.42	2.0-3.4 2.7
	C. mediter-ranea	ND-0.07 0.020	ND-0.89 0.48	0.07-2.40 0.72	0.02-0.34 0.07	2.6-8.5 5.0
	C. labiatus	0.01-0.41 0.020	0.33-0.70 0.40	0.31-0.97 0.57	0.12-0.50 0.17	5.0-10.6 6.7
	N. armatus	0.01-0.13 0.010	0.20-0.60 0.31	0.07-0.44 0.17	0.19-0.65 0.38	4.2-4.9 4.4
Brown & Balls 1997 shallow water	cod	<0.001 <0.001	<0.02-0.36 0.10	<0.02 < 0.02	0.02-0.42 0.10	2.93-9.19 3.65
	haddock	<0.001-0.005 <0.001	<0.02-0.42 0.11	<0.01-0.03 <0.01	0.01-0.16 0.12	0.01-7.36 3.30
	skate	<0.001-0.012 0.001	0.02-0.24 0.10	<0.01-0.02 <0.02	0.01-0.22 0.06	2.29-4.82 3.58
Mormede *et al.* 2001a	*N. aequalis*	0.002-0.010 0.004	0.17-0.37 0.21	ND-0.024 0.005	0.035-0.532 0.150	3.06-5.79 3.91
	L. eques	0.003-0.013 0.005	0.13-0.24 0.17	ND-0.011 0.002	0.038-0.398 0.077	2.16-3.56 2.62
	R. fyllae	0.008-0.027 0.012	0.22-0.83 0.33	ND-0.044 0.027	0.044-0.410 0.129	4.57-6.15 5.53

1.3. Organic contaminants

1.3.1. Background

Plants, the world's main source of food, are affected by different pests and by competition from weeds. From the 800,000 known species of insects, about 10,000 can cause extensive economic losses, and approximately 30% of the 3000 known nematodes commonly attack crop plants. Almost 1,800 of the 30,000 weeds around the world pose serious concerns in crop production. Between 10 and 30% of the

world's food crops is destroyed by pests and plant diseases, with much higher losses in emerging countries (Moffat and Whittle, 1999).

As a result of scientific advance, pesticides have been developed over the last five decades as extremely important aids to world agricultural production. Pesticides are chemical agents, and are classified according to the target pest, eg insecticides, fungicides, molluscicides, bactericides or herbicides.

The idea of combating pests by the use of chemicals is not new. For example, the fumigant value of burning sulphur was widely appreciated in ancient Greece and Rome, as was the use of soda and olive oil for the treatment of legume seeds and the application of arsenic against field pests. The first systematic studies into the use of chemicals for crop protection were initiated in the middle of the nineteenth century. But the 1930s can be considered as the real beginning of the modern era of synthetic organic pesticides. In 1939, the powerful insecticidal properties of DDT were discovered and this compound became the most widely used single insecticide in the world (Moffat and Whittle).

1.3.2. Structure of some PCBs and pesticides

Like metals, chlorinated hydrocarbons are conservative pollutants, hence persistent additions to the marine environment. However, unlike metals, most of these compounds are man-made and do not occur naturally.

The higher molecular weight chlorinated hydrocarbons have been a matter of particular concern because, unlike the low molecular weight compounds, they enter marine ecosystems and accumulate in animal tissues, particularly fatty tissues. These chlorinated hydrocarbons include several classes of pesticides and the polychlorinated biphenyls.

An international treaty to control persistent organic pollutants (POPs) was signed in May 2001 at a UN convention in Stockholm. Known as the "dirty dozen", POPs comprise 12 toxic pesticides and industrial chemicals, including DDT and dioxins. (New Scientist, 28th April 2001 no 2288). A new meeting has now been planned for

17-22 June 2002 untitled "Sixth session of the Intergovernmental Negotiating Committee for an International Legal Binding Instrument for Implementing International Action on Certain Persistent Organic Pollutants" and will be held in Geneva, Switzerland (www.un.org).

Descriptions of PCBs and pesticides have been extensively detailed in the literature (eg Crompton, 1997; Clark, 1997; Moffat and Whittle, 1999). The chemical structures of PCBs and selected pesticides are shown in fig 1.1.

1.3.2.1. Pesticides

- DDT: although DDT (dichlorodiphenyltrichloroethane) was known in the last century, it was not introduced as an insecticide until 1939. In many ways it is an ideal pesticide: it is extremely toxic to insects, but very much less toxic to other animals and is very persistent, with a half-life in the soil of about 10 years. It continues to exert its insecticidal properties for a very long time, and is relatively cheap.

- DDD (dichlorodiphenyldichloroethane) is a derivative of DDT resulting from the loss of a chlorine atom from the $-CCl_3$ group in the DDT molecule. It has some toxicity to insects and is less toxic than DDT to fish. For this reason it is occasionally used as an insecticide in situations where its low toxicity to fish is important. It can be excreted by many organisms and rarely accumulates in them.

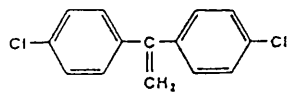
- DDE (dichlorodiphenylethane) is another derivative of DDT, resulting from the loss all the chlorine atoms from the $-CCl_3$ group in the DDT molecule. It has low toxicity to insects and is not used as a pesticide. Most of the chlorinated hydrocarbons in the sea, and 80% of those in marine organisms, is in the form of DDE and presumably nearly all of it has been derived from the breakdown of DDT.

- Commercial DDT is a mixture of DDT, DDE and DDD, with DDT predominating. DDT has now been almost completely superseded by less persistent insecticides in the developed world, but it continues to be extensively

used in developing countries because it is effective, safe to handle and relatively cheap.

- "Drins": this group of interrelated insecticides includes aldrin, dieldrin, endrin, heptachlor and so on. They are all extremely persistent and their degradation products are also toxic. For example, heptachlor degrades to heptachlor epoxide, which is even more toxic than the parent compound, and aldrin degrades to dieldrin. These pesticides were used as seed dressings and in situations where their high toxicity and persistence were required. Their chief disadvantage is that they are toxic to mammals as well as being persistent and subject to accumulation. They were largely withdrawn during the 1970s, although they continued to be used to mothproof textiles, carpets and fleeces, and for some minor agricultural purposes, until the late 1980s. Although now used only in very small quantities because of their persistence, they are widespread in the environment and continue to leach out of agricultural land into water courses and the sea.

- Hexachlorobenzene (HCB): this product was formerly widely used as a soil fumigant and as seed dressing and in wood preservatives. It has been largely replaced as a soil fumigant, but continues to be used in agriculture and for other purposes. For example, it is used as a fluxing agent in aluminium smelting and also occurs as a by-product in the manufacture of carbon tetrachloride, pentachlorophenol and vinyl chloride. There are, therefore, numerous routes by which it may reach the sea. It is relatively insoluble in water and most of the HCB in the sea is attached to sediment particles. It is highly persistent.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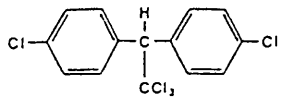
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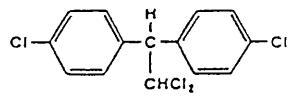
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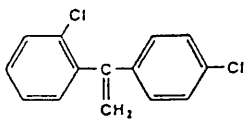
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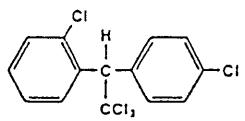
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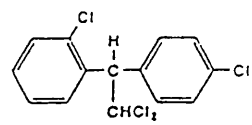
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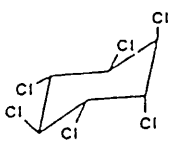
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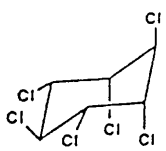
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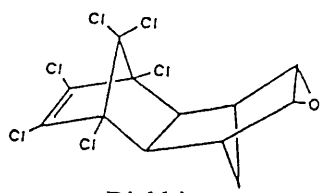
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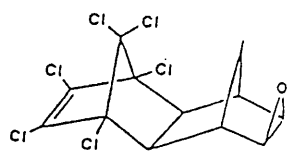
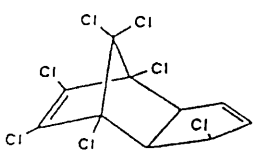
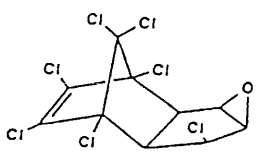
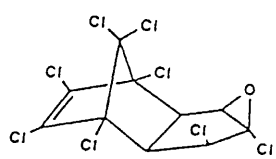
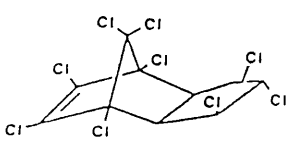
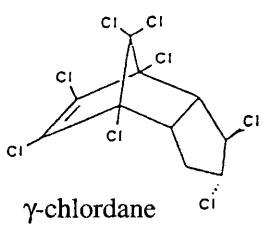

Endrin

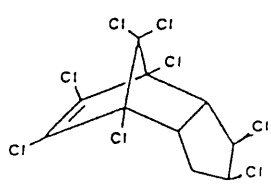

Heptachlor

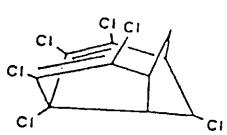

Heptachlor Epoxide

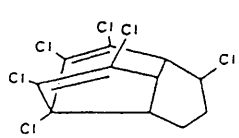

Oxychlordane


Trans-Nonachlor

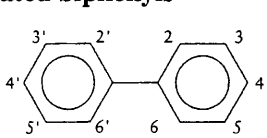

γ-chlordane


α-chlordane


γ-chlordene


α-chlordene

Polychlorinated biphenyls



Biphenyl substitution nomenclature

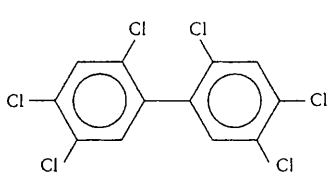

PCB 153
(2,4,5,2',4',5'-Hexachlorobiphenyl)

Fig 1.1: Structures of PCBs and pesticides measured in the present study

1.3.2.2. Polychlorobiphenyls

Polychlorobiphenyls (PCBs) have been in use since the early 1930s. They are not pesticides but have been used in electrical equipment, in the manufacture of paints, plastics, adhesives, coating compounds and pressure-sensitive copying paper. They are chemically very stable, they resist chemical attack and act as flame retardants. They have been used in fluid drive systems and as dielectrics in transformers and large capacitors. Commercial PCBs are produced to physical specifications and contain a mixture of congeners. Following concern about the environmental impact of chlorinated hydrocarbon pesticides, a number of steps were also taken to reduce the use of PCBs as well. All PCBs should have been collected and safely destroyed by 1999. However, up to the time when the use of PCBs was restricted in the mid 1980s, total world production had amounted to more than a million tons, most of which has been dispersed in the environment where it continues to cause concern.

Polychlorinated biphenyls are a related group of 209 congeners with the empirical formula $C_{12}H_{10-n}Cl_n$, where n takes a value between 1 and 10. Congeners have been numbered by Ballschmiter and Zell (1980) according to their chemical structure (see fig 1.1 and table 1.4).

Table 1.4: Substitution patterns of the selected PCB congeners studied

congener number	substitution pattern	congener number	substitution pattern
trichlorobiphenyls		**hexachlorobiphenyls**	
28	2,4,4'	128	2,2',3,3',4,4'
31	2,4',5	138	2,2',3,4,4',5'
tetrachlorobiphenyls		149	2,2',3,4',5',6
44	2,2'3,3'	153	2,2',4,4',5,5'
49	2,2'4,5'	156	2,3,3',4,4',5
52	2,2',5,5'	157	2,3,3',4,4',5'
70	2,3',4',5	158	2,3,3',4,4',6
74	2,4,4',5	**heptachlorobiphenyls**	
pentachlorobiphenyls		170	2,2',3,3',4,4',5
101	2,2',4,5,5'	180	2,2',3,4,4',5,5'
105	2,3,3',4,4'	187	2,2',3,4',5,5',6
110	2,3,3',4',6	189	2,3,3',4,4',5,5'
114	2,3,4,4',5	**octachlorobiphenyls**	
118	2,3',4,4',5	194	2,2',3,3',4,4',5,5'

1.3.3. PCBs and pesticides in deep water fish

Organic contaminants such as polychlorobiphenyls and pesticides are persistent and semivolatile, and hence have been detected in a wide range of environmental media, including biota (eg Risebrough *et al.*, 1968; Fowler, 1990; Tanabe *et al.*, 1994). They can now be found in remote parts of the globe, such as the Arctic (Hargrave *et al.*, 1992).

Until recently, very few studies have reported concentrations of organochlorine compounds in deep sea organisms (Barber and Warlen, 1979; Kramer *et al.*, 1984; Fischer *et al.*, 1991), probably as result of the combination of the low commercial interest of the species at the time, and the need for specialised fishing gear. Increasing concern that the deep sea might act as an ultimate sink for such contaminants and the greater importance of deep sea commercial fisheries around the word, have resulted in more studies being conducted (eg Lee *et al.*, 1997; Berg *et al.*, 1997 and 1998; Takahashi *et al.*, 1998; Froescheis *et al.*, 2000; Looser *et al.*, 2000; Sole *et al.*, 2001; Mormede and Davies, 2001c).

Berg *et al.* (1998) concluded that deep fjords might act as a trap for organochlorine contaminants. Froescheis *et al.* (2000) and Looser *et al.* (2000) showed that the concentrations of these pollutants in bottom dweller fish at depths greater than 800m was between 10 and 17 times more than in related surface species. This result highlighted transport processes of persistent organochlorinated compounds to the deep sea biophase, both vertically and from the poles. Table 1.5 summarises some of the data available in the literature on organic contaminants in deep water fish.

Table 1.5: Organochlorine concentrations in muscle (FL), liver (LV), kidney (KD), swimbladder (SB), mesenteric adipose tissue (MA), intestine content (IC) and stomach content (SC) in various deep water fish species.

reference	units	Species	organ	ΣCB	ΣDDT	p,p'-DDE	p,p'-DDD	p,p'-DDT
Meith-Avcin 1973	mg kg^{-1} wet weight	blue hake	FL		5400			
Barber 1979	1972 1973 1974 mg kg^{-1} wet weight	*Antimora rostrata*	LV		5.43 3.81 11.93	1.45 1.66 5.19	0.62 0.40 1.16	3.15 1.62 5.21
Kramer 1984	µg kg^{-1} lipid weight	black scabbardfish	LV	5800	9190	3030	1100	5060
Stegeman 1986	mg kg^{-1} wet weight	*C. armatus*	FL	2700 360				
Hale 1991	µg kg^{-1} wet weight	*Latimeria chalumnae*	LV KD SB MA FL	89 77 330 280 38	210 77 840 750 9.4	120 46 520 490 6.0	12 2.3 42 23 0.27	79 29 280 240 3.1
Hargrave 1992	mg kg^{-1} lipid weight	*L. Frigidus*		2300	1500			
Lee 1997	µg kg^{-1} lipid weight	dogfish cusk eel scorpion fish dories green eyes argentines	LV IC SC FL FL FL FL FL	1000 690 560 1900 1200 780 720 450	910 350 160 51 770 340 130 130			
Berg 1997	µg kg^{-1} lipid weight	halibut rabbit fish redfish wolf-fish grenadier dogfish blue hake tusk	LV LV LV LV LV LV LV LV	559 306 947 110 1058 545 1156 939	473 426 652 70 390 955 1446 992	302 249 282 39 19 232 680 421	27 49 75 4.5 99 111 182 140	102 93 243 19 246 566 475 324
Looser 2000	µg kg^{-1} lipid weight	grenadier halibut	FL FL		1090 220	640 95	170 52	242 42
Froescheis 2000	µg kg^{-1} lipid weight	grenadier halibut	FL FL	850 195				

1.4. Aims and objectives

The aims of the present study were to determine the influence of biological and environmental factors on the concentration and distribution of inorganic and organic contaminants in deep water fish species. The objectives were as follows:

- to generate a large data set on the concentrations of metals and organic contaminants in various organs of commercial and non-commercial deep water fish species, covering various locations and depths of the eastern North Atlantic
- to check the concentrations found against those available in the literature
- to investigate potential health issues arising from the consumption of deep water fish species, by relating levels of contaminants found in fish to the EU guidelines on contaminant levels in food and foodstuff
- to investigate the distribution of contaminants in various organs of individuals in each species, and link the findings with specific species biology (such as habitat or feeding) and metabolism (such as accumulation or detoxification)
- to investigate differences in concentrations and distribution of contaminants between species, location and depth of capture
- to investigate the use of fish as biomarkers of the water mass in which they live by relating levels and distribution of contaminants in fish from different water masses

Chapter 2
Toxicity and fate of metals and organic contaminants

2.1. Metal pollution in the marine ecosystem

Pollution of the ecosystem has always occurred to some extent or other. For example, over the whole of prehistory and still to some extent today, the eruption of volcanoes has led to the large-scale contamination of the ecosystem. In more recent times, the lead-smelting activities of the Romans caused pollution, as is indicated by the presence of lead-rich layers of sediments in Yorkshire, and this is perhaps the earliest example of man-made chemical pollution (Clark, 1997).

Metal pollution of the environment and its effects on human health have been a concern for decades, starting with the Minamata disaster in the 1950s (Moffat and Whittle, 1999). It still presents major environmental and health issues, as evidenced by the vast amount of publications and textbooks still published on that subject. The following discussion has been based mainly on the following references: Tessier and Turner (1995), Clark (1997), Crompton (1997), and Moffat and Whittle (1999) and will address the basic mechanisms of metal intake by organisms and their toxicity to cells, fish and humans. Factors controlling metal concentrations in fish will also be investigated.

2.1.1. Mechanisms of metal intake and toxicity

The mechanisms of intake of trace metals by marine organisms vary between metals, and attempts have been made to group metals that show similar properties. Metals can be classified into essential and non-essential metals or, alternatively, in class A,

B and borderline metals. Once these groups have been defined, some specific uptake routes and the case of organomercury will be detailed.

2.1.1.1. Essential and non-essential trace metals

The essential nature of a metal or metalloid is established by two criteria. (1) Does the absence or deficiency of the element result in the impairment of life processes? And (2), the impairment can be corrected only by the maintenance or addition of physiological levels of the metal and not by other metals? There are approximately 17 essential metals: Na, K, Ca and Mg which are required in relatively large amounts; As, Cr, Co, Cu, Fe, Mn, Mo, Ni, Se, Si, Sn, V and Zn which are required in trace (0.001 to 1mmol kg^{-1} wet weight) or ultra-trace levels (<1μmol kg^{-1} wet weight). Both essential and non-essential metals can exhibit toxic effects.

2.1.1.2. Class A, B and borderline metals

Class A metals are ionised in solution, forming cations with a closed shell configuration and an inert gas electron configuration. Such ions include K^+, Li^+, Ba^{2+}, Ca^{2+}, Mg^{2+} and Al^{3+}. They are non-polarisable, highly stable and form weak complexes in solution via electrostatic bonding.

Borderline metals present ligand-binding characteristics intermediate between the two classes. Such elements are Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+} and Cr^{2+} for example. Many of the borderline metals are required in trace quantities. Although deemed essential, they can also show toxic effects when in excess. The biological roles of these metals can be divided in three categories: steric (such as Zn in DNA binding proteins), catalytic (eg Zn, but such roles are unclear), or oxydative/reductive (eg Fe, Mn, Cu and Mo).

Class B metals are highly polarisable, hence form covalent bonds. This is the case of Ag^+, Au^+, Tl^+, Hg^{2+}, Bi^{3+}, Cd^{2+} and Cu^+. With the exception of copper, they are all non-essential and generally toxic, even at low doses. Class B metals are non-essential and toxic for two reasons. First, the class B metals are relatively rare in terms of their natural geological abundance and their required use in biological systems would

cause problems in terms of acquisition and storage. Secondly, these metals show high reactivity and lack of binding specificity to organic ligands.

2.1.1.3. Specific uptake routes

Some specific uptake routes in fish are detailed below:

- Mn^{2+} is taken up through Ca^{2+} channels. Co^{2+} slightly inhibits the uptake, but Ca^{2+}, Mg^{2+} and Fe^{2+} do not compete.

- Copper is taken up through the stomach and the small intestine. Uptake is passive and transport is determined by the chemical gradient, possibly via a common site with zinc. The free metal ion dictates the bioavailability and toxicity of the metal. The process is a simple or facilitated diffusion as saturation is not observed at the concentrations of copper available in the active forms.

- Zinc uptake takes place through the small intestine via passive diffusion. The rapid saturable process at physiological concentrations of zinc suggests a finite set of binding sites with specific recognition of zinc. Cd^{2+} and Mn^{2+} inhibit Zn^{2+} intake, whereas Co^{2+}, Cu^{2+} and Ni^{2+} do not.

- For cadmium, direct proportionality between external and internal Cd^{2+} concentration was demonstrated in the gills of *Mytilus edulis* (Carpene and George, 1981). In this case, the process is a facilitated diffusion without competition from Ca^{2+} or Mn^{2+}. In mammals, cadmium does not readily cross the intestinal barrier and at most, 1 to 5% is absorbed from food. Ca^{2+}, Zn^{2+} and La^{2+} depress Cd^{2+} absorption from food. Most of the cadmium absorbed is lost following desquamation of the epithelial cells. However inefficient its absorption, cadmium is very toxic because of its long biological half-life, which is approximately 20 years (Tessier and Turner, 1995).

- Lipid soluble metals are ions such as Cu^{2+}, Ag^{2+}, Cr^{2+}, Zn^{2+} and Cd^{2+}, which are likely to form covalent complexes and have enhanced membrane permeability. Metal speciation can also modify apparent permeability, for example $HgCl_2$ passes across membranes over a million times faster than ions such as Na^{+}.

- Anionic metals are metals occurring in an anionic form such as chromate (CrO_4^{2-}) or arsenic, and go through anion channels. Cr(VI) as dichromate or chromate ions is more toxic and more readily absorbed than Cr(III). Uptake happens through the gills.

- Organometals constitute a separate category, which is detailed in the following section.

2.1.1.4. Organomercury

The most important source of organometals in the aquatic environment is their direct input from synthesised products used in pest control and from industries using organometals in their processes. Fortunately, most organometals prepared as catalysts, especially those with cobalt, nickel and platinium, are "air-sensitive" and readily oxidised or hydrolysed in an aqueous medium. Thus, numerous organometals induce limited adverse effects in marine organisms due to their short half-life in the environment. However, some of them such as organotin and organolead compounds persist in the environment and are now widespread in living organisms.

The second major source of organometals to the environment is the action of living organisms themselves on some metallic ions dissolved in water or adsorbed onto mineral substrates, such as the methylation of Hg(II). The production of methylmercury in the environment is considered to be a biological process occurring in water, soil and sediments via the bacterial methylation of inorganic mercury. High mercury levels in fish and shellfish is still a major environmental and health problem. The major threat comes from the neurotoxicity of methylmercury, which is quickly taken up but slowly excreted by organisms. For example, over 90% of mercury in fish is present as methylmercury. Fish do not methylate or demethylate mercury, but bacteria strains isolated from fish intestines do have methylation and demethylation capacities (Rudd *et al.*, 1980).

In freshwater lakes, mercury methylation can be affected by acid rain. In environments not affected by acid rain such as estuarine and coastal sediments, mercury methylation seems to be controlled more by the sulphide content than by mercury levels and organic contents in the sediments. As sulphur concentrations are

high in marine sediments and sulphate-reducing bacteria play an efficient role in methylation of mercury, relatively high concentrations of methylmercury may be expected in coastal and estuarine sediments.

In addition to bacterial demethylation, the removal of monomethylmercury from contaminated anoxic sediment can occur via an abiotic sulphide-mediated route:

$$2\ CH_3Hg^+ + S^{2-} \rightarrow (CH_3Hg)_2S \rightarrow (CH_3)_2Hg\ (\text{volatile}) + HgS$$

Concentrations of monomethylmercury are very low in surface waters, from below 0.02 to 0.10ng l^{-1} with values up to 0.50ng l^{-1} in polluted areas. Precipitation is an important direct source of methylmercury in freshwater ecosystems but not in the open ocean waters, where atmospheric deposition is a source of inorganic mercury only (Tessier and Turner, 1995). In ocean waters, the maximum concentrations of mono and dimethylmercury are below the thermocline at depths between 300 and 500m; no detectable dimethylmercury and very low concentrations of monomethylmercury are found in the surface mixed layer of the ocean. Hence there is production of both mono and dimethylmercury in subthermocline waters, and their removal or chemical transformation in surface waters. Biological methylation is particularly efficient in low-oxygen environments, and therefore important in subthermocline waters where oxygen concentrations are below 100μM. In a similar way, demethylation accounts for the loss of dimethylmercury in more oxygenated waters (Mason and Fitzgerald, 1990).

Virtually all mercury in fish and seafood is in the monomethylmercury form. A relationship between mercury concentration and size has been observed in some cases, being stronger in slow-growing species (mainly predators) than fast-growing species (prey) (Driscoll *et al.*, 1994). In a marine food chain experiment, increasing mercury concentration with both age and trophic levels was observed (Riisgard and Hansen, 1990).

2.1.2. Trophic transfer of metals in the marine environment

Phytoplankton can concentrate some metals from seawater and are the principal food source for most marine herbivores. Hence they play a crucial role in introducing metals into marine food webs. Similarly, metal concentrations in tissue of diverse invertebrate species often correlate with metal contamination in sediments (Tessier and Turner, 1995).

Marine organisms can accumulate trace metals from the dissolved phase and from ingested food. Elements accumulated by marine organisms from ingested food are initially taken up by cells in the gut and then distributed to other tissues. A trace metal accumulated mainly from ingested food can be expected to become concentrated in an animal's internal soft tissues rather than exoskeletons or exposed surface tissues such as gills. Therefore, if present at toxic levels, trace metals accumulated from food will affect internal tissues first. As internal tissues are recycled upon death or transferred to the next level of the food web through carnivory, so are the associated trace metals. On the other hand, trace metals that are absorbed from the dissolved phase are often associated with refractory components of marine organisms such as phytoplankton cells or zooplankton exoskeletons. These refractory components can act as vectors for elements to the deep sea by themselves or, as they are largely not assimilated in the marine planktivores, as part of the sinking faecal pellets.

Any trace metal concentration in an animal's body is proportional to the concentration of metal binding ligands specific to this particular metal present in the animal's body. Ligands can be present either at the surface of certain organs such as the gills or throughout an organ. If ligands are present at the surface of certain organs, the concentration of these ligands, although increasing in numbers in bigger fish, will be inversely proportional to the length cubed or to the weight of the fish. On the other hand, ligands present throughout an organ will be proportional to the length/weight of the fish. Hence trace metals whose concentration is inversely related to animal mass are mainly accumulated via adsorption of dissolved ions, with uptake proportional to the number of surface ligands. Trace element concentrations remaining constant or increasing as an animal grows are proportional to the amount

of metal binding ligands in an animal's entire body, access to which is enhanced by digestive processes.

Because herbivorous zooplankton tend to package many of the non-essential particle-reactive metals into waste products (eg faecal matter, cast houses, crustacean exoskeletons released during molting), these herbivorous animals may serve as a break in the trophic transfer of metals from lower food-chain organisms to carnivores. Because unassimilated metals are released by these herbivores in the form of fast-sinking debris, the carnivores that ingest herbivores do not ingest appreciable amounts of the unassimilated metals, and their principal source of these metals must be from the dissolved phase. Thus, the trophic transfer of metals that are not assimilated by herbivores probably decreases greatly after the phytoplankton – herbivore link. This is clearly reflected in the apparent lack of food-chain magnification of metals (apart from mercury) although it does not necessarily mean lack of trophic transfer of metals. Concentration factors of diverse metals are typically highest in phytoplankton and lowest in fish (Bernhard and Andreae, 1984).

Generally, metals that are assimilated by animals from their food (particularly biologically essential metals) are biologically recycled in surface waters and tend to follow organic carbon in decomposing debris. Therefore, these metals, which include many of the transition metals, should have longer retention times in surface waters than non-biologically essential metals. In contrast, metals that are not assimilated by animals are packaged in faecal pellets and other waste products, which can sink rapidly in the deep sea, at sinking rates of between 1 and 1000m/day depending on the nature of the waste, and should have short residence times in surface waters.

2.1.3. Biological factors influencing metal concentrations in aquatic organisms

2.1.3.1. Biological mechanisms

Biological mechanisms influencing metal concentrations in aquatic organisms can be separated in various categories: uptake, accumulation and elimination, and regulation mechanisms.

- **Uptake.** Aquatic plants derive metals almost exclusively from the aqueous phase. For the majority of animal species, metal uptake occurs from a combination of water and food (including sediment) in varying degrees. For many dissolved class B and transition metals, passive entry into organisms takes place along a diffusion gradient which is maintained by sequestering influxing metals with intracellular ligands, some of which may be inducible (such as metallothioneins). Uptake is proportional to the free ion concentration or activity. Uptake takes place across the whole body surface in soft-bodied organisms, and across the digestive epithelium and respiratory structures such as gills in all organisms.

- **Accumulation and elimination**. Having entered the organism, further transfer of metals to sites of higher binding strength may occur, resulting in accumulation. Subsequent storage of metals takes place in the tissues which are particularly rich in, or capable of synthesising relatively large quantities of, metal binding ligands such as granules or metallothioneins. Elimination is often passive, by processes such as desorption of adsorbed metals from external surfaces, desquamation of epithelial cells or through permeable membranes, especially the gills. The kidneys of some bivalves also hold significant concentrations of metal-rich granules, which are probably passed into the urine for elimination.

- **Regulation**. There are two basic strategies by which organisms may attempt to limit problems caused by an excess influx of metal: by elimination (or exclusion) or by storage of surplus metal in an inert form. The latter option is often reversible in the case of essential metals, mobilisation occurring in response to metabolic requirements. There is evidence that marine fish are able to regulate

copper, zinc and possibly iron and manganese concentrations in muscle tissue. Thus, even though copper is assimilated in the viscera in a dose- and time-dependant manner, copper concentrations in the muscle of plaice (*Pleuronectes platessa*) remain unchanged (Saward *et al.*, 1975). It would appear that metal levels in fish muscle and perhaps some other tissues are, with the exception of mercury, relatively unresponsive to contamination when compared to many invertebrates.

In general, metals having an essential biochemical role, such as copper, zinc and iron are regulated, while for non-essential metals such as mercury, cadmium and silver there is little control of accumulation. Mercury is virtually the only metal biomagnified along food chains. This is attributed to the exceptionally slow elimination rate of methylmercury in most organisms and its subsequent transfer and retention at higher trophic levels.

2.1.3.2. Factors influencing uptake

Uptake of metals in each individual marine species along the food chain depends on a variety of factors:

- Quality of food: the importance of food quality in determining metal accumulation is demonstrated in carnivores such as plaice, which retains 80 to 93% of methylmercury from a diet of *Nereis*, but only 4 to 42% from *Mytilus* (Pentreath, 1976).

- Habitat/behaviour: food is an important source of metals in fish. A detailed investigation using plaice highlights the importance of the diet as the primary source of heavy metal uptake. Input from water represents less than 1% of the zinc, manganese, iron and cobalt contribution from food (Pentreath, 1973). The route of uptake can influence the final tissue distribution of metals. In salmonids, for example, dietary cadmium is retained principally in the gut, kidney and liver, while exposure to dissolved cadmium results in accumulation mainly in the gills and kidney (Segner, 1987). Although diet is usually the primary source of metals in many fish, concentrations of metals in certain tissues tend to increase in

proportion to increasing exposure concentrations in water, such as lead and cadmium.

- Selective feeding: small organisms, because of morphological constraints, ingest smaller particles which are often metal enriched due to their higher surface to volume ratio, while large individuals ingest a wide size range of items, including less enriched larger particles. Therefore, differences in metal burdens may be exacerbated by a size-mediated shift in diet.

2.1.3.3. Relationships between metal concentrations and biological factors

The effects of growth are highly species, metal and sometimes site specific. It has been long established for a broad range of organisms including bivalves, crustaceans and fish that accumulation of non-essential metals such as mercury and cadmium during short-term exposures is most rapid in smaller individuals.

Metal-size relationships in fish, for elements other than mercury are less consistent. In a study of copper, zinc, lead, cadmium, mercury and arsenic in 8 tropical fish species, only 15 correlations of 108 were significant in muscle tissue, 6 positive and 9 negative, and mercury was the only element for which all correlations were positive. For other tissues, correlations were significant in 28 of 196 cases. More than half of these involved copper, zinc and cadmium in liver and kidney, indicating the tendency for such tissues to accumulate and store these metals (Powell *et al.*, 1981). Similar results were found in other studies, including deep sea fish studies (eg Cronin *et al.*, 1998; Mormede and Davies, 1998 and 2001a).

A number of studies also reveal differences in trace metal behaviour and concentrations between male and female fish. Loss of copper and cadmium from female marine teleost (*Blennius pholis*) occurs during spawning. Female gonad tissue of many fish species contains more zinc and in some cases copper than males, which is then shed during spawning (Shackley *et al.*, 1981). Mercury content is also highly dependent on sex, appearing to accumulate faster in males than females.

2.1.4. Metal toxicity in marine organisms

It is generally assumed that metals exert their toxicological activity through their inappropriate non-specific binding on physiologically important molecules. No target is entirely metal-specific and no metal appears to be target specific. The binding process elicits a different toxicological response symptomatic of the function of the target molecule and the biochemical pathway it controls or influences. At the physiological and behavioural levels, these cellular changes are manifested in many ways. The response depends on the target molecule, dosage and duration of exposure, but also on the life-stage of the animal at the time of exposure, for example, juveniles are often more susceptible.

Metal detoxification represents a cellular or physiological process that ameliorates, reduces or eliminates metal-induced toxic effects. Metal toxicity can be defined as the impairment of biological function caused by the uptake of a particular metal.

2.1.4.1. Effects of toxicity on marine populations

Trace metal contamination of the aquatic environment occurs as a result of many natural and anthropogenic activities. Adverse effects of the metal occur when a compensatory mechanism is overwhelmed or when compensation/detoxification imposes secondary costs (table 2.1).

Table 2.1: Cascade of effects from the biochemical level of organisation to the population level. This continuum of effects defines the toxicity process in nature (from Tessier and Turner, 1995).

organisational level	secondary effect	primary effect
molecular/biochemical	detoxification: lysosomes, metallothioneins ↓ detoxification overwhelmed	← bioaccumulation ↘ change or disruption of biochemical processes
physiological	detoxification: acclimate, adapt reproduction cycle ↓ compensation overwhelmed	↙ ↘ physiological stress: weak individuals, inhibit reproduction, vulnerability to stress
whole organism	detoxification: adult survival ↓ compensation overwhelmed	↙ ↘ individuals cannot survive or reproduce
population	detoxification: tolerance, immigration, age structure ↓ compensation overwhelmed	↙ ↘ species absent where it could be present

2.1.4.2. Toxic effects of metals in fish

The toxicity of a wide variety of metals in marine fish is well documented. Most toxicity evaluations have been conducted in tank experiments by the measurement of the median lethal concentration (LC_{50}), which is the concentration calculated to cause mortality of 50% of the population. Metals have mainly been tested separately (but not all metals have been tested in fish for LC_{50}). The following statements are drawn from Tessier and Turner (1995).

- Ni is relatively non-toxic to fish, less toxic in saline water (LC_{50}=35,000μg l^{-1}) than in non-saline water (4-day LC_{50}=10,000μg l^{-1}). Toxicity is greater at higher water temperatures.

- Se is particularly toxic to invertebrates (4-day LC_{50}=2,900 to >10,000μg l^{-1}).

- V: LC_{50}>10,000μg l^{-1} both in fish and invertebrates.

- Cr in fish, LC_{50}=12,400 to 190,000μg l^{-1}.

- As: different arsenic species have different levels of toxicity and bioavailability. The Arsenic in Food Regulations (1959) states that foodstuffs must not contain more than 1mg kg^{-1} wet weight of total arsenic with the exception of fish. It is accepted that arsenic in fish and shellfish is mainly organically bound, hence less toxic than the inorganic arsenic. Fish are less susceptible (4-day LC_{50}=15,000 to 28,000μg l^{-1}) than invertebrates (4-day LC_{50}=400μg l^{-1}).

- Zn: marine life is relatively resistant to zinc at all life stages (4-day LC_{50}=66,420 to 76,950μg l^{-1} in crab for example).

- Pb: mollusc larvae are particularly sensitive to lead, abnormal development occurring upon 2 days' exposure to 400μg l^{-1} lead. In fish, lead damages the central nervous system, particularly when the brain is growing, accumulates in the gill, liver and kidneys and reduces larval survival.

- Cu: complexion agents are assumed to reduce the biological availability of copper and minimise its toxic effect. Juvenile fish are more sensitive to copper than adults. For example, in mollusc, 4-day LC_{50}=300 and 30,000μg l^{-1} in young and adult mollusc respectively.

- Cd: fish are relatively resistant to cadmium with a 4-day LC_{50} of 6,400 to 16,400μg l^{-1}. However, cadmium intoxication in fish has been shown to affect growth and larval development, upset ionic control and calcium metabolism, accumulate in kidneys and liver of higher animals and disrupt calcium and vitamin D metabolism.

- Hg: fish embryos undergo damage when exposed to 32μg l^{-1} mercury for 32 days. Organomercury compounds are more toxic than metallic mercury. Methylmercury has been stated to be neurotoxic, particularly damaging to developing young. Some of the toxic effects on fish include liver abnormalities, carcenogenesis and kidney damage.

However, fish mortality can occur at much lower levels than the median lethal concentrations, which will affect populations. On 4 to 14 days exposure, some metal concentrations above which fish mortality can occur are the following: 10 to 100μg l^{-1} for mercury and copper, 100 to 1,000 μg l^{-1} for cadmium and nickel, 1 to 10mg l^{-1} for zinc, lead and arsenic, and 10 to 100mg l^{-1} for chromium.

2.1.5. Toxicity of metals to humans

Humans can be subject to non-negligible amounts of metal contamination, mainly from their food. Metal intoxication in humans is well documented, both at chronic or acute exposure, and forms the basis of the justification for international food regulations. The following description of toxic effects of some metals on humans is based on Clark (1997) and Moffat and Whittle (1999).

- Inorganic mercury: the toxicity of mercury to humans has been known for centuries. Mad hatters in Victorian England got their name from the convulsions and loss of neuromuscular co-ordination symptomatic of chronic poisoning from mercury used in the treatment of felt in hat manufacture. Effects of inorganic mercury at low exposure levels are primarily neurological and renal. However, elemental mercury can be readily excreted, and although it is dangerous to those exposed to it occupationally, it is not a risk for the general public.

- Organic mercury exposure in humans occurs mainly through the consumption of fish and shellfish. This was brought to light by an outbreak of methylmercury poisoning in the small Japanese coastal town of Minamata after an accidental discharge of large quantities of mercury catalyst. Organic mercury intoxication leads to cellular and neuronal damage, and is generally called the Minamata

disease. Clinical manifestations of its neurotoxic effects are parathaesia, neurathenia, vision and hearing loss, and finally coma and death.

- Cadmium achieved notoriety in the aftermath of the Minamata disaster with a major human poisoning incident when released by the activities of the "Kamioka Mine" through waste water used for irrigation of rice fields in the Jinzu River Valley in Japan. The osseous illness is known as itai-itai (ouch-ouch) disease because afflicted patients continually moan owing to severe internal pains. As cadmium accumulates mainly in bones, chronic exposure induces ostheoporosis, but also liver and kidney damage. Acute toxicity results in nausea, vomiting and abdominal pains, but recovery is rapid. Cadmium is considered as a Category 1 (human) carcinogen primarily on the basis of its induction of pulmonary tumours from cadmium inhalation, but this does not appear to be a hazard arising from marine pollution.

- Copper: humans are not at risk of copper poisoning from fish and seafood because although the lethal dose is about 100mg, the human taste threshold for copper is low (5 to 7.6ppm) and the taste is repulsive.

- Lead: the main effects of lead on humans include damage to the nervous system in children and peripheral neuropathy and hypertension in adults. However, lead contamination of the sea and marine products are of little general concern due to very low levels.

- Arsenic: the toxicity of inorganic arsenic in humans is well-known, with a lethal dose of 50 to 300mg depending on the individual. Inhalation of inorganic arsenic by workers in smelters has been associated with respiratory cancers. Chronic ingestion, as from drinking waters, has been associated with bladder, liver and kidney cancers, and possibly with skin cancer. Arsenic exists as the toxic trivalent form (arsenic trioxide, As_2O_3, sodium arsenite, etc.), as the less toxic pentavalent form (arsenic pentoxide, arsenic acid, lead arsenate, calcium arsenate, etc.), and as numerous organic forms (arsenilic acid, bimethyl arsenate, etc.). Potency depends on the chemical form of the element, with As(III) being the most potent, followed by As(V), then monomethylarsenate and finally dimethylarsenate. The predominant form of arsenic that exists in the edible parts

of animals is the organic form, either as arsenobetaine or arsenochlorine, which have shown to have no toxic effects in animal or humans.

- Selenium at low concentrations is an essential element, but at high doses causes toxicity to humans and animals, including embryotoxicity, teratogenicity and mutagenicity. Data from China indicate that daily intake below 20μg selenium leads to Keshan disease, an endemic cardiomyopathy, first reported in Keshan County, China in 1935, due to consumption of maize and rice grown in selenium-deficient soils. The role of selenium deficiency as a cancer risk factor is still debated because the evidence is inconsistent and insufficient.

The toxicity of most of these metals to humans is widely acknowledged, as is the importance of minimising intake of metals by humans, particularly via food. Therefore, levels of these metals in human food are subject to numerous national and international regulations throughout the world. Details of these regulations and of the performance of fish against these regulations will be detailed in chapter 5.

2.2. PCB pollution in the marine environment

The first recording of the effects of polyhalogenated hydrocarbons (PHHs), which include polychlorobiphenyls (PCBs), was at the end of the nineteenth century. During the production of caustic potash by the electrolysis of potassium chloride, workers suffered from dermatitis, which was identified as the contamination from PHHs formed by the reaction of the chlorine and the pitch tar lining of the electrolytic cell. The widespread manufacture and use of PHHs in the 1960s and 1970s meant that, in addition to occupational exposure, there was the risk of severe environmental contamination close to the sites of manufacture and usage. The main uses of PCBs were as dielectric fluids in capacitors and transformers, hydraulic fluids, heat transfer fluids, additives in paint and carbonless copy paper.

The early benefits of the PHHs in both agriculture and industry were quickly tempered by acute and chronic effects of these materials in the environment and in humans. During the late 1960s, there was a growing awareness that the persistence, toxicity and rapid redistribution of these chlorinated compounds to sites remote from any production or use meant that low-level long-term contamination of all compartments of the environment and the food chain was inevitable. The apolarity and lipophilicity of PCBs also meant that these compounds could be bio-accumulated in fatty tissue through the food web. It took over a decade to reduce output and place restrictions on the use and disposal of these PHHs (Tanabe, 1988).

Numerous incidents have occurred, such as in 1968, when rice oil contaminated with PCBs caused an outbreak of "yusho" disease in Japan. Conditions similar to "yusho" have been observed in the Yucheng in Taiwan in 1979 to 1981. Incidents have also happened in the Hudson River, when General Electric discharged an effluent contaminated with PCBs. PCBs are no longer used in open systems in most developed countries, eg as additives in paints. Manufacture and new applications have now been banned or severely curtailed.

The following discussion has been based on the following references: Waid (1986), Clark (1997) and Moffat & Whittle (1999). Although this section concentrates on

PCBs, DDTs will also be included in the discussion as, in many cases, the two groups behave in a similar way.

2.2.1. Mechanisms of toxicity

One of the biological responses most frequently studied following exposure to PCBs is the induction of the hepatic microsomal mixed-function oxidase system, cytochrome P450, which is essential for the oxidative detoxification of a broad variety of xenobiotic chemicals. Some of the enzymes of the P450 system can be induced by xenobiotics, which is regarded as a strategy of the organism to cope with toxic chemicals (Moffat and Whittle, 1999).

Although individual PCBs are structurally similar, they bind to different cytosolic receptors, the binding site depending on the molecular shape, which results from the different chlorine substitution pattern. The complex formed interacts with DNA to alter a specific gene expression of the P450, which depends on the CB structure. Subsequently, the different groups present different toxic responses. These are sometimes referred to as Specific Qualitative Structure-Activity Relationships (QSAR).

The group presenting the highest toxicity comprises PCBs with a planar configuration. They are also called dioxin-like PCBs because they present a configuration similar to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 3-methylcholanthrene (3-MC) and therefore present similar toxicity. These PCBs are CB 77, 126 and 169 and are known as 3-MC type inducers. CB 101, 128, 138, 149, 153, 170, 180 and 194 present a similar spatial configuration to phenobarbital (PB) and are called PB type inducers. CB 105, 118 and 156 present structural characteristics intermediate between the two previous groups. Finally, CB 29, 48 and 52 present a lower lipophilicity, do not induce P450 enzymes and exert neurotoxicity via an unknown mechanism (McKenzie, 1999).

2.2.2. Inputs of PCBs in the marine environment

A variety of related compounds are usually produced in the manufacture of chlorinated hydrocarbons, only one of which is the desired product. These unwanted hydrocarbons are extracted and were formerly dumped or incinerated at sea in considerable quantities.

In his review of PCBs in aquatic environments, Tanabe (1988) estimated that 31% of the total PCBs produced globally had reached terrestrial or aquatic environments by 1988. However, only 4% of the total amount produced had been destroyed through high-temperature incineration, and the remaining 65% was still in use, mostly in ageing electrical equipment, or had been land-filled, mostly with inadequate containment.

The commercial production of PCBs has now ceased in most developed countries. However, as a rough estimate, about 800,000 tons of PCBs are being used on land. This amount corresponds to more than three times the current total PCB load in the open ocean environment. Therefore the large amount of land-stocked PCBs holds a crucial key for the future trend of PCB contamination in the open ocean environment. As PCBs are not produced in large quantities any longer, their main source comes from leaching of landfills and incinerators. These will subsequently be transported to the sea by river. For example, over 3 tons/year of PCBs enter the North Sea from river inputs, mainly from the Rhine. Sewage sludge may also contain appreciable quantities of chlorinated hydrocarbons and, if dumped at sea, represents an additional source of contamination.

2.2.3. Fate of PCBs in the marine environment

PCBs are extremely insoluble in water, with a saturation concentration of no more than 1μg l^{-1}, but they are soluble in fats and adsorb strongly onto particles. Chlorinated hydrocarbons adsorbed onto inorganic particles may ultimately be carried to the seabed, which then acts as a sink. However, suspended or resuspended particles, if they are of a suitable size or density, can be ingested by filter-feeding

animals, and PCBs may enter food chains by this route. Halogenated hydrocarbons, most particularly PCBs, DDT and its derivatives, now occur in all organisms in all environments, and a considerable proportion of these compounds in the sea is in the bodies of marine organisms and will continue to circulate within food webs.

Concentrations of PCBs in open ocean water are generally in the few ng l^{-1} range or less. Atlantic waters revealed relatively higher concentrations than the other oceans (mean concentration of up to 1ng l^{-1} in the North and South Atlantic against 0.5 in the North Pacific and 0.12ng l^{-1} in the South Pacific for example, from Waid, 1986). This seems to be due to the extensive use of PCBs in the US and European countries. Much lower concentrations of PCBs were found in southern oceans. PCB levels in surface waters were generally lower in the southern hemisphere than the northern hemisphere and much higher concentrations were observed in the mid-latitudes of the northern hemisphere (Waid, 1986).

The transport of PCBs and DDTs in open ocean waters has also been demonstrated by their detection in deep sea fish, sediments and subsurface waters. The descending surface waters at both poles form the oceanic deep currents, which extend well beyond the equator. In the southern hemisphere, these ocean bed currents, which are fed from the west wind drift circulating around the South Pole, flow towards the north, whereas water from the North Polar Sea flows southwards (Ballschmiter, 1992). However, the transport of xenobiotics in the depths of the oceans takes place not only through the downwards flows of water that occur mainly in the polar regions, but also by the fall-out of organic matter in the oceans.

The vertical transport of these chemicals is strongly associated with sinking particles in the water column. Less water soluble organochlorines such as PCBs and DDTs are rapidly transported into deeper layers of water columns by sinking particles, whereas relatively soluble organochlorines such as HCHs are more slowly scavenged from surface to deeper layers. As transport of PCBs via the poles is less congener specific, bottom living fish should show a relatively greater proportion of higher chlorinated biphenyls than surface living species (Waid, 1986).

2.2.4. Biological mechanisms of PCBs in animals

PCBs are known for their persistency as their half-lives are of the order of several years. Moreover, bioaccumulation factors range between 100 and 10000 in fish and crustaceans. Hence high concentrations are likely to be found in animals (Clark, 1997).

2.2.4.1. Storage and accumulation

PCBs are excreted slowly and tend to accumulate in the body. Because they are lipid soluble, they occur in much higher concentrations in fatty tissues than in other tissues. This introduces two risks. First, in times of poor feeding such as at the end of the winter, animals mobilise and use their fat reserves, which can increase the concentrations of chlorinated hydrocarbons circulating in the body, possibly to a dangerous level. And as with other bioaccumulating contaminants, there is a possibility of transmission through food-webs and biomagnification, as has been established in a number of animals (Goerke *et al.*, 1979).

Bioaccumulation factors for organochlorines in fish vary widely between species and compounds, between 100 and 10000, with each species presenting its own profile of organochlorine accumulation. The distribution of organochlorines within an animal's tissue is not uniform either: the brain and the liver both acquire the highest concentrations and eliminate them most slowly. The brain does accumulate at a different rate from most other organs because it is composed of different phospholipids from those found in other organs (Andersson *et al.*, 1998). Since organochlorines are eliminated slowly from the body, they may be expected to show biomagnification in food chains (Clark, 1997).

2.2.4.2. Biological effects in animals

Because organochlorines are stored in the fatty tissue of the body, they become biologically available and exert their influence when the fat tissues are metabolised. Animals may therefore acquire a considerable body burden of halogenated

hydrocarbons, but show no ill effects except in conditions of starvation such as in late winter, when fat reserves are mobilised.

Exposure of different test animals to varying doses to PCDDs, PCDFs and PCBs have induced neurobehavioural changes, reproductive and immunotoxicological abnormalities, reduced hepatic vitamin A storage, vitamin K deficiencies and dermal effects (Liem and Theelen, 1997).

Some PCBs, and a variety of non-halogenated organic compounds are now known to show oestrogenic activity in a range of vertebrates. There is also a possibility that falling fertility of humans in developed countries and decreasing sperm counts may be the result of dietary exposure to oestrogenic substances, such as PCBs and pesticides in food. The total intake of PCBs in μg/person/day has been estimated at 14 in Finland, 6.9 in Australia, 6.8 in Sweden, 1.6 in the USA, 0.86 in India, 0.53 in the UK and 0.09 in Canada (Moffat and Whittle, 1999).

2.2.4.3. Biological effects in fish

Uptake of PCBs by aquatic organisms could occur by three mechanisms: absorption of PCBs from water through the gills, similar uptake through the epidermis, or consumption of contaminated food. There is no significant information indicating that uptake through the epidermis is of general importance. On the other hand, there is sound evidence that all aquatic organisms studied in aquaria can absorb PCBs directly from water. Bioconcentration of PCBs by fish from water in natural systems has also been shown to occur. The main route of uptake by aquatic organisms has been shown to be absorption through the gills since the gills represent the active membrane surface for water exchange. Once absorbed by the gills, the PCBs are then partitioned into the blood and from blood to tissues (eg Mayer et al., 1977; Waid, 1986; Phillips, 1995).

A study of PCB levels in various organisms in Puget Sound by Clayton *et al.* (1977) demonstrated that all biota in that ecosystem had similar PCB levels, when normalised to lipid content. This indicates that PCB in their food was not the controlling factor. It has been suggested that the accumulation of PCBs by aquatic organisms is a passive physicochemical process and that uptake is predominantly

governed by equilibrium partitioning of the PCBs between the organism and ambient water. Nevertheless, the assimilation of PCBs from ingested food occurs by partition across the lipoprotein membrane lining the gut into the bloodstream, but is usually of much less significance.

The significance of biomagnification of persistent residues as compared to direct uptake from the water has been the subject of a considerable debate. Several authors have concluded that, for pelagic biota at the lower trophic levels, food chain biomagnification is not a controlling factor in attaining observed PCB levels. Bioaccumulation is predominantly controlled by equilibrium partitioning of the chemical between the internal lipids of the biota and ambient water.

Marine fish also appear to be very sensitive to a number of organochlorines. The 96h LC_{50} for DDT can be as low as 0.4μg l^{-1} and for dieldrin ranges between 0.9 and 34μg l^{-1} for a variety of teleosts (Clark, 1997).

2.2.4.4. Environmental impacts

During the 1960s, there were increasing signs that the widespread and intensive use of pesticides, most obviously DDT, was having unforeseen and unwelcome consequences for the environment. For example, in 1964, fish were found to be dying in an area around the marine outfall of a Danish factory manufacturing the pesticide parathion. The Laguna Madre, which was a productive lagoon system on the coast of Texas, became heavily contaminated with pesticides from neighbouring agricultural land and the fish catch fell abruptly after 1964 (Clark, 1997).

Good evidence of the damaging effect of organochlorine on wildlife was demonstrated in 1967 by Dr D.A. Ratcliffe with the sharp decline of the population of the peregrine falcon *Falco peregrinus*. This was due to reproductive failure as birds were laying eggs with abnormally thin shells, a large proportion of which were broken during incubation.

Chlorinated hydrocarbons, and more particularly PCBs, have been held responsible for the decline of some seal and sea lion populations, and may also have affected

otters in the Baltic. High levels of PCBs in the animals have been responsible for a failure of reproduction.

Many xenobiotic compounds are reported to have the potential to disrupt endocrine development in wildlife and laboratory animals, and a number of these compounds are PCBs. Exposure to endocrine-disrupting chemicals is associated with abnormal thyroid function, decreased fertility and defeminisation and demasculinisation in fish and birds. Furthermore, breast cancer in women may be related to human exposure to endocrine disruptors (Moffat and Whittle, 1999).

2.2.5. Toxicity of PCBs to humans

In 1968, rice oil contaminated with PCBs caused an outbreak of "yusho" disease in Japan. In all, 1200 people were affected and suffered darkening of the skin, enlargement of the hair follicles and eruptions of the skin resembling acne. A majority developed respiratory difficulties, which persisted for several years. However, there has been no confirmed case of illness resulting from ingesting PCB residues through consumption of marine organisms.

In western industrialised countries, 90% of the human intake of PCBs is via food consumption, and particularly through consumption of fish, meat and milk products. Fish consumption, although relatively small in volume compared to that of meat and milk products, can contribute up to 50% of the intake of PCBs. Some social groups may be exposed to higher PCB intakes as a result of their particular diet.

Moreover, Dulfer (1996) demonstrated that PCBs in fatty food are very well absorbed in an *in vitro* system that simulates human intestines. There is also near complete gastro-intestinal absorption of PCBs by breast-fed babies.

Until recently, the toxicity of PCBs to humans did not give rise to concerns, as levels in food were believed to be low. However, with rising concentrations of PCBs in food and feedstuff, EU regulations on the concentrations of dioxins and dioxin-like PCBs in food are being reviewed. These are discussed in more detail in chapter 5.

2.3. Pesticide pollution in the marine environment

Studies of pesticide residues in the human diet began in the 1960s. Following alarming findings on environmental hazards resulting from their use, such as their accumulation in fatty tissues throughout the food chain and subsequent endocrine disruption, DDT and some other persistent organochlorine insecticides (eg aldrin and dieldrin), were banned from use all over the world at the beginning of the 1970s. The discovery of some subtle long-term effects of some other pesticides in the environment on non-target organisms, such as humans, resulted in cancellation in many countries of the registration of several other pesticides such as toxaphene and dinoseb.

Pesticides can be classified according to their target pest, amongst which are insecticides, fungicides, herbicides, dessicants, defoliants for example. Organochlorines such as aldrin, dieldrin, DDT, chlordane, endrin, HCH and heptachlor are used as insecticides.

2.3.1. Physicochemical properties of pesticides

Pesticides possess a wide range of physicochemical properties due to their diverse chemical structures. Relationships between some characteristics of pesticides and their behaviour both in environmental compartments and during food processing are summarised below.

- Water solubility provides insight in the fate of pesticides in the environment as well as into losses during processing of residue-containing crops. Highly soluble pesticides are likely to be easily removed by water from the surface of the plant and, in the soil they do not tend to partition. Such polar compounds are likely to biodegrade, their rate of hydrolysis and oxidation being typically higher than less soluble pesticides. On the other hand, substances possessing good solubility are less likely to volatilise from water, inducing insignificant losses from aqueous solutions.

- Vapour pressure also influences the volatilisation from water of the pesticide. Substances with vapour pressures less than ca 1.10^{-7}mPa will be associated mostly with particulate matter. In food processing, substances with high vapour pressures will be lost to some extent due to volatilisation.

- Octanol-water partition coefficient (K_{ow}) has been shown to correlate well with adsorption of pesticides to soil and sediments. Unless other factors such as rapid biodegradation operate, compounds with high K_{ow} values, such as DDT, tend to bio-accumulate in the fat portion of organisms. A compound is designated as fat-soluble when K_{ow} exceeds 4 (with several exceptions) and is not so designated when K_{ow} is less than 3.

- Soil sorption coefficient (K_{oc}) is reported to describe soil sorption, which is the main process affecting pesticide pollution potential in terrestrial ecosystems. It is calculated by measuring the ratio K_d of the sorbed to soluble concentrations of the pesticide after equilibration of the pesticide in a water-soil slurry and then dividing by the weight fraction of organic carbon in soil, $K_{oc}=K_d/F_{oc}$. Pesticides that are strongly sorbed by soil particles are likely to be more persistent because they are protected from degradation and volatilisation by the binding. Sorbed pesticides will not easily leach to ground water; they will wash off the field only under erosive conditions, ie attached to moving soil particles.

- Bioconcentration factor (BCF) is reported for hydrophobic chemicalssuch as organochlorines, which have a tendancy to partition from the water column and bioconcentrate in aquatic organisms. BCF is calculated as the concentration of the chemical in the organism at equilibrium divided by the concentration of the chemical in water.

Table 2.2: Physiochemical properties of persistent organochlorine hydrocarbons (NA not available)

pesticide	water solubility (mg l^{-1} at 20°C)	vapour pressure (mPa at 20°C)	Kow	Koc	half-life in soil (days)	half-life in air (days)	log BCF (fish)
pp-DDT	$1.7*10^{-3}$	$1.5*10^{-4}$	$1.0*10^{6}$	$2.4*10^{5}$	700-5000	3	4.468
pp-DDE	$1.3*10^{-3}$	$1.8*10^{-5}$	$5.8*10^{5}$	$1.0*10^{6}$	NA	NA	NA
HCB	$5.0*10^{-3}$	$2.5*10^{-3}$	$2.0*10^{5}$	$1.4*10^{4}$	1300	627	4.342
aldrin	$2.7*10^{-2}$	$1.0*10^{-5}$	$3.2*10^{6}$	$6.7*10^{3}$	53	0.16	4.029
dieldrin	$1.9*10^{-1}$	$4.0*10^{-4}$	$2.1*10^{4}$	NA	2550	2.2	4.100

2.3.2. Biological fate of pesticides

2.3.2.1. Fate in the environment

On average, 35 to 50% of pesticides is deposited on soil immediately after spraying, and between 10 and 20% is subject to short-term or long-range drift (Moffat and Whittle, 1999). Long-range transport of some pesticides occurs via the atmosphere in the form of vapour and/or sorbed on solid particles: eg persistent chlorinated pesticides and related compounds such as HCB, DDT, dieldrin, aldrin, and toxaphene. Thus contamination of food chains at remote places may occur due to use and/or emissions elsewhere.

In general, the factors responsible for pesticide degradation can be classified as chemical, physical or biological. Light and heat are the physical agents of primary importance. Chemical reactions can be caused by various agents, mainly by water as the breakdown of most pesticides occurs in solution. Microorganisms such as bacteria and fungi represent the most important groups of pesticide degraders in soil and water. Plants, invertebrates and vertebrates are further degradation agents. The last group possesses the most sophisticated enzymatic system capable of biodegrading xenobiotics. These systems are most effective in birds and mammals where the spectrum of transformation reactions is very broad and the rates of detoxification and elimination are typically high.

2.3.2.2. Inputs to the marine environment

The principal source of widespread pesticide contamination is from the agricultural use of these compounds. Aerial transport is the main route by which they reach the sea (over 80%) (Clark, 1997). All organochlorine pesticides volatilise and, particularly in the tropics where they are still used in large quantities, climatic conditions favour their release to the atmosphere. Aerial transport has resulted in the world-wide distribution of organochlorine pesticides. DDT is an entirely man-made substance, and does not occur naturally; it has been in use only since 1940. Yet within 20 years, DDT and its residues (particularly DDE and DDD) have pervaded the entire biosphere. Even king penguins *Aptenodytes patagonicus* in Antarctica, several thousand kilometres from any place where DDT has been used, contain detectable traces (Clark, 1997).

Although the total burden of pesticides carried to the sea by rivers is small compared with aerial inputs, it may be locally damaging. The use of DDT and "drins" (aldrin, dieldrin, etc) was phased out in western European states in the early 1970s, but elevated levels of these chlorinated hydrocarbons are still recorded near the mouths of the major rivers (Sole *et al.*, 2001).

2.3.2.3. Fate in organisms

All living organisms possess defence mechanisms protecting them from small quantities of diverse foreign compounds including pesticides, such as excretion and/or accumulation in less-toxic form. In higher animals, pesticide metabolites are transported in the bloodstream, which leads to their eventual excretion. In general, pesticides are transformed in a detoxification process resulting in less toxic substances. Nevertheless, some products may also be toxic to the target pest, such as DDT and its product dicofol. Several pesticide degradation products are relatively persistent and may be of concern as contaminants of environmental resources. For example, major degradation products of DDT such as DDE and DDD are typical of contaminants that may accumulate in the human food chain.

2.3.3. Toxicity of pesticides to humans

Although designed to control pests, pesticides can also be toxic to non-target organisms, including humans, since many species from insect to man share the same basic enzymes, hormones and other biochemical systems.

As described for PCBs, fat-soluble substances (eg organochlorines such as DDT, HCH and other persistent substances) are accumulated in the body and, when stored in fatty tissues, they are not readily metabolised. However, in times of poor nutrition or relative starvation, the deposits of these compounds are mobilised and they are released into the bloodstream, with the possibility of toxic effects if concentrations reach a high enough level. In general, within the body, pesticides may be metabolised, stored in fat, or excreted unchanged. Metabolism will normally make the pesticide more soluble and thus more easily excretable (eg fat-soluble pyrethroid insecticides are hydrolysed to water-soluble compounds).

Dieldrin, DDT and γ-HCH, many of which are manufactured in the developed countries, continue to be used on a large scale in the developing world, particularly in China and India. As a result, γ-HCH residues in human milk are higher in India than anywhere else in the world. Whether or not this is harming the population has not been determined (Moffat and Whittle, 1999).

One of the recent concerns about the risk posed by pesticides to humans is their ability to alter or disrupt the body's hormone or endocrine system. Exposure to some notoriously persistent, bioaccumulative, organochlorine pesticides (such as DDT and metabolites, toxaphene, mirex, dieldrin and heptachlor), implicated in disruption of hormones, occurs primarily through foods such as meat, dairy products and fish. Therefore, concentrations of pesticides in fish harvested for human consumption are of particular concern.

Chapter 3
Materials and methods

3.1. Materials

A wide variety of deep sea fish species were sampled between September 1998 and September 2000, in numerous locations. Within species and location, individuals were sampled in as wide a size-range as available.

3.1.1. Sampling sites

Samples were collected by various means: either by sampling at sea during research cruises, recovering samples collected by other people during research cruises, sampling on fish markets, or by recovering samples collected by other people during market samplings. The sampling locations are pinpointed on fig 3.1 and the total number of fish per species per location is summarised in fig 3.2. The details of individual samplings are as follows:

- 22 to 30 September 1998 on the Fisheries Research Vessel Scotia (FRS-MLA), 38 monkfish (*Lophius piscatorius*) and 54 black scabbardfish (*Aphanopus carbo*) were sampled in the Rockall Trough

- 20 October to 6 November 1998 on the Fisheries Research Vessel Scotia, 44 black scabbardfish, 15 blue ling (*Molva dyperygia*), 9 blue whiting (*Micromesistius poutassou*), 11 hake (*Merluccius merluccius*) and 10 roundnose grenadier (*Coryphaenoides rupestris*) were sampled in the Rockall Trough

- 14 April to 3 May 1999 on the Navire Oceanographique Thalassa (IFREMER), 66 black scabbardfish, 29 orange roughy (*Hoplostethus atlanticus*), 33 roundnose grenadier, 16 *Bathysaurus ferox* and 15 blue ling were sampled in the Rockall Trough and Meriadzec

- 22 October 1999 in a local harbour of Madeira (Portugal), 25 black scabbardfish were collected

- 13 November 1999 in the harbour of Sesimbra (Portugal), 32 black scabbardfish were collected

- 20 January to 12 February 2000 on a South African research cruise, 8 kingklip (*Genypterus capensis*), 10 *Lophius vomerinus*, 13 *Merluccius capensis* and 11 *Merluccius paradoxus* were sampled at about 31°S and 17°W, off South Africa

- 3 to 16 September 2000 on the Fisheries Research Vessel Scotia, 34 blue whiting, 54 black scabbardfish, 66 roundnose grenadier and 38 orange roughy were collected from the Rockall Trough

- 15 September to 10 October 2000 on the Royal Research Ship Discovery, 24 *Coryphaenoides armatus* and 12 *Histobranchus bathybius* were sampled from the Porcupine Bank

The list of all species studied, along with their latin names and abbreviation codes is included in appendix 2.

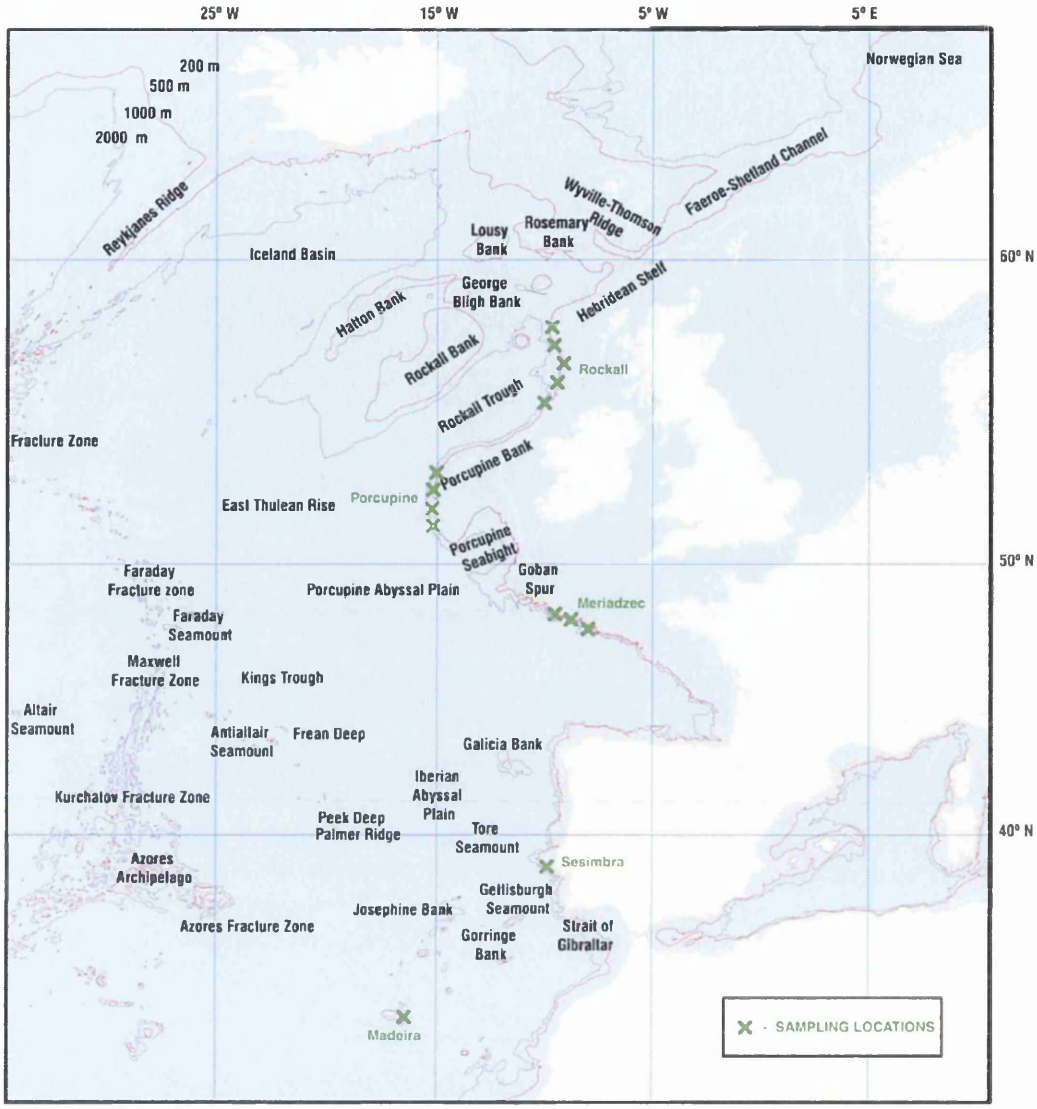


Fig 3.1: Bathymetry and sampling locations in the eastern North Atlantic (modified from www.ospar.org).

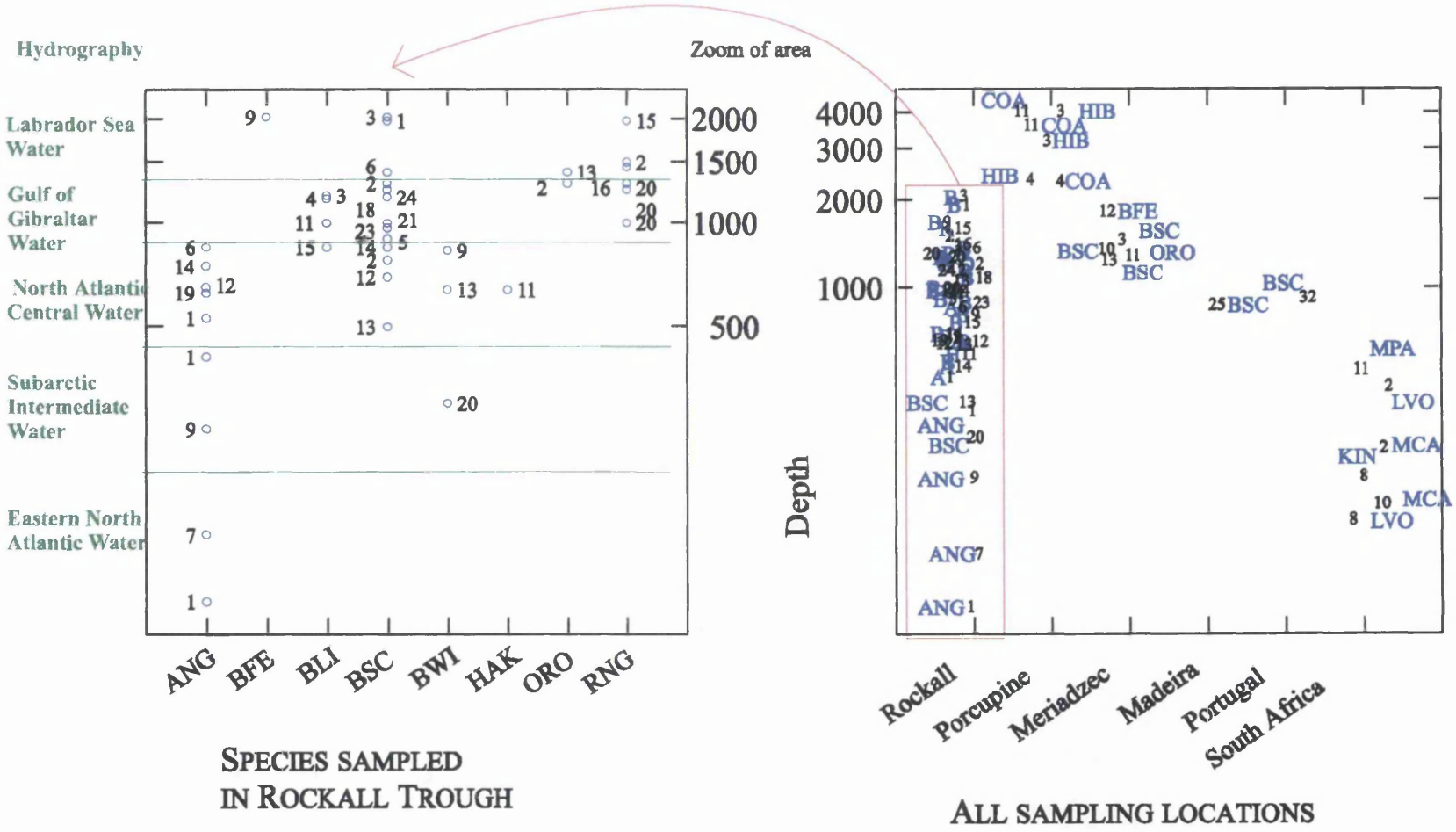


Fig 3.2: Number of samples collected by location, species and depth: monkfish (ANG), B. ferox (BFE), blue ling (BLI), black scabbardfish (BSC), blue whiting (BWI), C. armatus (COA), hake (HAK), H. bathybius (HIB), kingklip (KIN), L. vomerinus (LVO), M. capensis (MCA), M. paradoxus (MPA), orane roughy (ORO) and roundnose grenadier (RNG).

3.1.2. Description of fish species

- ***Aphanopus carbo* – black scabbardfish**

Fig 3.3: Black scabbardfish (photo www.sams.ac.uk)

Adult black scabbardfish are benthopelagic, living on the continental slope or underwater rises at about 200 to 1600m depth, whilst juveniles are mesopelagic. They migrate to midwater at night. They are distributed in the eastern Atlantic, from the Denmark Strait to Madeira and western North Africa. They, or closely related species, are also known from many localities in the Atlantic, Indian and Pacific oceans. They are caught with special deepwater lines off Madeira and, to a lesser extent, off Portugal; and by deep water trawls west of Scotland. Black scabbardfish is a relatively fast growing species, living up to 8 years. They become mature at 80 to 85cm standard length. They are carnivorous, feeding on fish, squid and crustaceans (www.ipimar.pt/basblack).

- ***Bathysaurus ferox***

Fig 3.4: *B. ferox* (photo courtesy of A. Carpentier, IFREMER)

Fishes of the lizard fish genus *Bathysaurus* are amongst the most characteristic of the ocean floor at depths below 1000m. *B. ferox* are circumglobal except beneath polar waters, at depths of 1000 to 2500m and temperatures between 3 and 4°C. They grow to about 40 to 60cm in length, with a maximum recorded at 83.5cm and 7kg.

Their trophic role as top predators in an energy-poor ecosystem dictates low population densities with wide spacing of individuals. *B. ferox* have a

predominantly piscivorous diet of demersal and bathypelagic fishes, supplemented by occasional large benthic crustacea. Individuals rest motionless on the substrate and capture prey by lunging forward in a sudden rapid burst (Sulak *et al.*, 1985).

- ***Coryphaenoides armatus***

This species is caught in demersal trawls over a depth range of 2000 to 3000m, but it has a wide depth range extending to 4000m depth. Small individuals feed on copepods and amphipods supplemented by mysids, while larger individuals feed on an increased proportion of smaller fish. *C. armatus* appears to feed in the water column immediately above the sea bed rather than on organisms on the surface of the sediment (Mauchline and Gordon, 1984b).

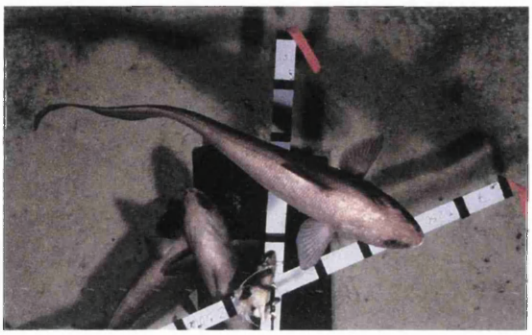


Fig 3.5: *C. armatus* (photo courtesy of C. Henriques, Aberdeen University, Oceanlab)

- **Coryphaenoides rupestris – roundnose grenadier**

Macrouridae are the most widespread family of fish occurring on the continental slope of the North Atlantic, and the roundnose grenadier is the most heavily exploited.

Roundnose grenadier reaches lengths in excess of 100cm, although up to 50% of it consists of its whip-like tail. It is benthopelagic, feeding on copepods and amphipods, and becomes more piscivorous as it grows. It occurs at 180 to 2200m depth, but off north west of Ireland it is normally present at 600 to 1800m depth. It occurs along the continental slope from North Africa, Europe, Iceland, Greenland, Canada to the south east of the United States, generally between 37 and 66°N, and occasionally as far

Fig 3.6: Roundnose grenadier (photo www.sams.ac.uk)

south as 20°N (Kelly *et al.*, 1997).

Gordon (1979) suggested that juveniles are demersal at about 1000m depth on the slope. As they grow, they move down the slope to about 1800m depth as they grow. Nearing maturity, they migrate back up so that mature females are at about 1000 to 1200m depth and males are shallower.

- ***Genypterus capensis* – kingklip**

Kingklip are deep water benthic fish, endemic to southern Africa. Their distribution extends from north of Walvis Bay on the west coast of southern Africa to the east of Port Elizabeth on the South African south coast. The genus *Genypterus* is found only in the southern hemisphere temperate waters, and is widely known as ling. Kingklip are long-lived, slow-growing fish and have been aged to 24 years (Punt and Japp, 1994).

Fig 3.7: Kingklip (illustrations by Chris Van Dusen; courtesy of Seafood Business Magazine www.newmex.com)

- ***Hoplostethus atlanticus* – orange roughy**

In the Atlantic, orange roughy is widely distributed from Europe to South Africa in the east, but is apparently restricted to the Gulf of Maine in the west of the Atlantic. There is little genetic differentiation between the populations from the eastern North Atlantic, the south west Pacific and the Tasman Sea.

On the slopes to the west of the British Isles, orange roughy is most common between depths of 1000 and 1200m. There are no differences in the length/frequency of orange roughy between the 1000 and 1250m bathymetric zones in the Rockall Trough. The principal components of its diet are decapods and mysids, with fish, cephalopods and amphipods of lesser importance (Gordon and Duncan, 1987).

Fig 3.8: Orange roughy (photo courtesy of A. Carpentier, IFREMER)

- ***Histiobranchus bathybius***

H. bathybius occurs benthopelagically over the continental rise and abyss of the world ocean, primarily beneath temperate and subpolar surface waters. Smaller fish occur in shallower regions. They are caught from 1800m depth, but are more abundant below 2400m depth, with a size range between 7 and 137cm total length (Karmovskaya and Merrett, 1998). *H. bathybius* feeds benthopelagically rather than benthically, with possible scavenging. Blue whiting occurred in the stomachs of *H. bathybius* at depths of 2200 to 2900m in April, which may have been scavenged (Mauchline and Gordon, 1991).

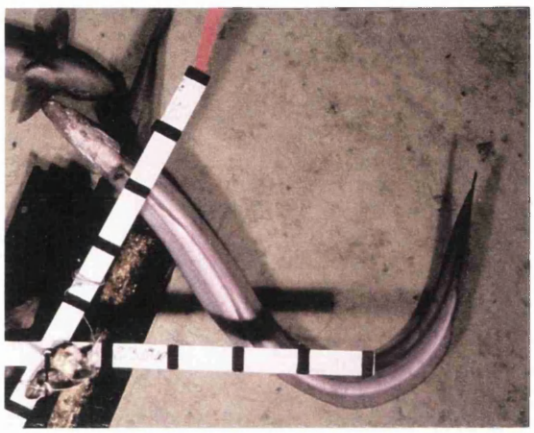


Fig 3.9: *H. bathybius* (photo courtesy of C. Henriques, Aberdeen University, Oceanlab)

- ***Lophius piscatorius* – monkfish, anglerfish**

Monkfish is a benthic species, inhabiting relatively shallow inshore waters to 500m depth. It is distributed in the Mediterranean, Black Sea, eastern North Atlantic from the Strait of Gibraltar to the south western Barents Sea (Whitehead *et al.*, 1984).

Fig 3.10: Monkfish (photo www.sams.ac.uk)

Gadoids, in particular whiting and Norway pout, and also *Nephrops* are the main components of monkfish diet. Size-related differences in diet are observed, with *Nephrops*, cod and whiting being favoured by adult monkfish (Crozier, 1985).

- ***Lophius vomerinus***

L. vomerinus is distributed in the eastern South Atlantic and western India, as well as off South Africa. It is a bathydemersal species, feeding on fish such as hake, with a depth range of 150 to 350m (Froese and Pauly, 1998).

- ***Merluccius capensis* and *M. paradoxus*, Cape hake**

Cape hake is made of two species: the shallow water species *M. capensis* and the deep water species *M. paradoxus*. *M. capensis* is usually caught on the south coast and the west coast of South Africa in waters shallower than 300m depth. *M. paradoxus* is caught on the west coast at depths of 200 to 600m, but there is an overlap between the two species at depths between 150 and 450m. Both species feed on benthic crustaceans and finfish (Osborne *et al.*, 1999).

- ***Merluccius merluccius* – hake**

Fig 3.11: Hake (illustrations by Chris Van Dusen; courtesy of Seafood Business Magazine www.newmex.com)

Hake is a gadiform fish widely distributed from the Norwegian coasts and Iceland to Mauritanian waters, as well as in the Mediterranean and Black Seas. It is usually found between 70 and 370m depth, but may also occur within a wider depth range, to 1000m depth. Its diet in the North East Atlantic is almost entirely composed of blue whiting, whiting, Norway pout and herring (Lucio *et al.*, 1997).

- ***Micromesistius poutassou* - blue whiting**

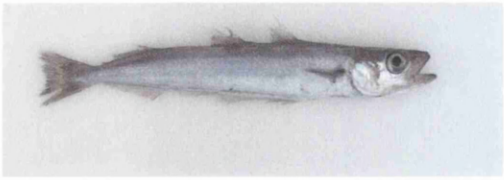


Fig 3.12: Blue whiting (photo courtesy of A. Carpentier, IFREMER)

Blue whiting occurs in the Rockall Trough primarily as a migrant passing through the region. It is found between 450 and 720m depth, and occasionally as deep as 1000m. Its diet is pelagic, mainly composed of euphausiids, with other pelagic crustaceans and cephalopods (Mauchline and Gordon, 1984).

- ***Molva dypterygia* – blue ling**

Fig 3.13: Blue ling (photo courtesy of K. Peach, FRS MLA)

Blue ling is distributed in the eastern North Atlantic from Iceland down to Morrocco, as well as in the Mediterranean. It is usually caught at depths below 300m, down to 800m and feeds on crustaceans and fish, with predominance of silver pout and blue whiting (Whitehead *et al.*, 1984; Thomas, 1997).

3.2. Methods

The fish were usually dissected onboard soon after being caught, although in some cases whole frozen fish were taken back to shore, thawed and then dissected. Muscle (without skin), liver, and in some cases gonad and/or gill tissue were sampled, individually wrapped in aluminium foil and frozen at -20°C. Each fish was given a single identification number. Date, location and depth of capture were recorded, together with species, length, weight, sex and, when possible, maturity stage of the fish.

Once ashore, individual samples were transferred to -20°C freezers, then thoroughly defrosted and homogenised just before the analysis. Each sample was analysed individually for metals. In the case of organic contaminants, after an exploratory study on all organs, mainly livers were analysed. Moreover, in order to decrease the within-site variance and analysis time, samples were pooled by species, date, location/depth of capture, length and sex by groups of no more than five individuals.

3.2.1. Metal analysis

The metal analysis was conducted using two different techniques. Analysis before the year 2000 was carried out on flame and graphite furnace Atomic Absorption Spectroscopy (AAS), analysing the samples for Cu, Cr, Zn, As, Cd, Hg, and Pb. From 2000 onwards, work was carried out on a newly acquired Inductively Coupled Plasma – Mass Spectrometer (ICP-MS), analysing the samples for (up to) Li, Be, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Cd, Hg, Pb, Bi, U.

3.2.1.1. Digestion methods

The digestion of samples was historically carried out in open flasks. However, this digestion proved insufficient for ICP-MS as it lead to high matrix interferences on some elements such as Co, Cr, Mn and Ni that could not be easily corrected (see fig 3.14 and 3.15). Hence a microwave digestion method was subsequently developed, reducing those interferences to acceptable levels.

The open flask digestion method consists of overnight digestion of sample in nitric acid followed by evaporation to 5ml and further dilution. Each digestion is conducted with ca 0.5g of homogenised liver tissue or ca 5g of homogenised muscle, gonad or gill tissue. The sample is weighed exactly in a silica open flask, to which 25ml of Analar concentrated nitric acid is added. Up to 42 samples can be digested in a single batch, alongside a blank and ca 0.5g of Certified Reference Material (CRM) Dorm2 (dogfish muscle, National Research Council, Canada). The silica flasks are then heated overnight (or for at least 8 hours) on a hotplate at ca 60EC. The flasks are further heated to ca 200°C and samples evaporated to ca 5ml. The resulting digests are quantitatively transferred in 25ml plastic vials, made up to 25ml with ultra-pure distilled water (12.8μΩ) and labelled accordingly. The empty silica flasks are rinsed with concentrated nitric acid then ultra-pure distilled water before being used for the next batch.

The microwave digestion method consists of a 35 minutes digestion under high temperature and pressure (maxima of 300°C and 75bar). Each digestion is conducted on the Perkin Elmer Anton Paar Multiwave with ca 0.5g of homogenised sample in all cases. Only 6 samples can be digested at one time; a blank and ca 0.5g of the Dorm2 CRM are analysed on every day of digestion and at least every 40 samples. Each sample is weighed exactly in the teflon microwave high pressure flask, to which 5ml of Aristar concentrated nitric acid is added. The flasks are then sealed and introduced in the Anton Paar microwave. The samples are digested under the following program:

Table 3.1: Microwave digestion program

start power (W)	**time (min)**	**end power (W)**	**fan (0 to 3)**
100	5	600	1
600	5	600	1
1000	10	1000	1
0	15	0	3

Once the program is finished, the vials are opened following the manufacturer's procedure. The digests are then quantitatively transferred in 25ml plastic vials, made up to 25ml with ultra-pure distilled water (12.8μΩ) and labelled accordingly. The empty high-pressure flasks are rinsed with ultra-pure distilled water before the next

run. At the end of each day of use, the flasks are cleaned under pressure, by digesting 5ml of Aristar nitric acid in each flask as follows:

Table 3.2: Microwave vials cleaning program

start power (W)	time (min)	end power (W)	fan (0 to 3)
1000	20	1000	1
0	10	0	3

Once the program is finished, the vials are opened following the manufacturer's procedure. The digests are discarded and the pressure flasks rinsed with ultra-pure distilled water before being stored.

3.2.1.2. Flame Atomic Absorption Spectroscopy

Before the ICP-MS was purchased by the laboratory, copper, chromium, nickel and zinc were analysed by flame AAS on the Perkin Elmer AA5000 under conditions optimised during my MSc project (Mormede, 1998). All samples showed levels of chromium and nickel under the limits of detection.

In flame atomic absorption spectrometry, the sample, either as a liquid or in solution, is sprayed by a pneumatic nebuliser into a flame. In the flame, the solution droplets are first dried, then thermally decomposed and finally dissociated into free atoms that absorb light at their characteristic wavelengths. These processes are dependent upon the temperature and the chemistry of the flame and must occur within milliseconds as the sample passes through the flame. During that time, combustion products of the flame and decomposition products are present in large quantities, leading to well-known interferences: transport, chemical, ionisation, matrix, spectral and background interferences (Harris, 1995).

This instrument possesses a background correction by deuterium lamp. The background corrector was used for all elements analysed except for copper. The flame used was air / acetylene, optimised for each element, with a 6cm Perkin-Elmer flat burner head. The slit width used was 0.7nm for copper, chromium and zinc, reduced to 0.2nm for nickel. None of the elements analysed is considered as easily ionisable so it was not necessary to add any modifier to avoid ionisation interferences. A spoiler

rather than an impact bead was used in the nebuliser chamber to reduce memory effects.

Every day, the performance of the instrument was checked for the element to be analysed with a standard against tabulated data (see table 3.3) and optimised in terms of burner head, lamp and nebuliser positions if necessary. At the start of every analysis, a calibration curve of at least six different concentrations was constructed. A middle concentration was subsequently checked every six samples, and both a high and low concentrations every 12 samples to check for possible drift. The data obtained from these repeated analyses of standards has been used to determine the limit of detection of the analyses (see paragraph 3.2.1.5.).

Table 3.3: Conditions of use of the flame atomic absorption spectrometer

	current (mA)	wave-length (nm)	air flow (l min^{-1})	acetylene flow (l min^{-1})	slit width (nm)	inte-gration time (s)	Expan-sion	linear range (up to)	typical response (absorbance units)
Zn	12	213.9	25	15	0.7	1	0	3 ppm	0.5 ppm = 0.120
Cr	13	357.9	20	23	0.2	2	5	5 ppm	2.0 ppm = 0.100
Cu	8	324.7	25	15	0.7	2	10	5 ppm	5.0 ppm = 0.250
Ni	15	232.3	25	15	0.2	1	0	2 ppm	2.0 ppm = 0.050

3.2.1.3. Graphite Furnace Atomic Absorption Spectrometry

Before the ICP-MS was purchased by the laboratory, arsenic, cadmium and lead were analysed by graphite furnace AAS on the Perkin Elmer Zeeman 3030 under conditions optimised during my MSc project (Mormede, 1998).

The graphite furnace AAS is based on the same physical mechanism as the flame AAS (see paragraph 3.2.1.2.). However, in the furnace, the sample is dispensed into a small graphite tube, which is heated electrically. The temperature is increased stepwise to separate the processes of drying, ashing and atomising. During the two first steps, an inert gas stream removes solvent and matrix vapours. During the atomisation, the gas stream is shut off so that the free atoms remain in the light beam for several seconds. This analysis time is far longer than in the flame thus a much

higher concentration of atoms are stimulated to absorption, leading to increased sensitivity, and lower limits of detection with very small sample volumes.

A modifier is always used with traditional graphite furnace AAS. It is a solution, usually characteristic of the analyte, which combines with the analyte so as to allow a higher ashing temperature without losing the element of interest.

The instrument used is equipped with a pyrocoated graphite tube, a L'Vov platform and a Zeeman background corrector. Each condition of use has been optimised for each element of interest: the wavelength, the volumes of sample and matrix modifier to introduce, which modifier to use and the temperature programming have all been optimised using the Dorm2 certified reference materials (see table 3.4).

Every morning, the instrument's response to predetermined standards was checked against quoted values and the instrument optimised if necessary. At the start of every analysis, a calibration curve of at least six different concentrations was constructed. Subsequently, a middle concentration was analysed every ten samples, and a blank analysed every batch of 40 analysis to check for possible drift. The multiple analysis of the standards has also been used to calculate the limit of detection for each element (see paragraph 3.2.1.5.).

Table 3.4: Conditions of use of the graphite furnace AAS

	current (mA)	**wavelength (nm)**	**delay time (s)**	**reading time (s)**	**amount sample (µl)**	**amount modifier (µl)**	**dilution**	**modifier**	**slit width (nm)**
As	460	193.7	1.1	3	5	20	50	Pd #	0.7
Cd	8	228.8	0	3	15	15		*	0.7
Pb	460	283.3	0	3.5	10	10		Pd #	0.7

*: $Mg(NO_3)_2$-$(NH_4)_2HPO_4$ modifier (Weltz and Schlemmer, 1986)

Pd 5000 ppm

The temperature programs were optimised for each element during my MSc project (Mormede, 1998). They were subsequently used without change for this work (tables 3.5 to 3.7).

Table 3.5: Temperature program for arsenic

Step	temperature (°C)	ramp (s)	hold (s)	gas flow (ml min^{-1})
Drying	80	3	15	300
	130	15	15	300
Ashing	1300	10	30	300
atomising	2300	0	5	0
cleaning	2650	1	1	300

Table 3.6: Temperature program for cadmium

step	temperature (°C)	ramp (s)	hold (s)	gas flow (ml min^{-1})
drying	80	5	15	300
	130	10	25	300
ashing	900	10	20	300
atomising	1500	0	3	0
cleaning	2650	1	5	300

Table 3.7: Temperature program for lead

step	temperature (°C)	ramp (s)	hold (s)	gas flow (ml min^{-1})
drying	80	5	15	300
	130	15	35	300
ashing	1000	5	10	300
atomising	2000	0	5	0
cleaning	2650	1	7	300

3.2.1.4. Inductively Coupled Plasma – Mass Spectrometer

The Perkin Elmer Elan 6100 DRC (dynamic reaction cell) ICP-MS, purchased in June 1999, replaced both flame and graphite furnace AAS. Elements analysed were (up to) Li, Be, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Cd, Hg, Pb, Bi, U. The method development on ICP-MS was carried out by Jerome Barraque during his MSc project under my supervision (Barraque, 2000).

In the ICP-MS, the solution is sprayed by a nebuliser into a high temperature radio frequency excited argon plasma (6000EC). The excited atoms are then directed through a sampler cone and a skimmer cone to a mass spectrometer. The main advantage of ICP-MS over AAS methods is that a single scan can quantify all elements of interest. The limit of detection of the ICP-MS normally lies between those of graphite furnace AAS and flame AAS (see paragraph 3.2.1.5. for details).

The dynamic reaction cell was not used for the samples of interest because it did not provide better results than the normal mode and slowed the analyses down. The ICP-MS was optimised every day with a 10µg l^{-1} multi-element solution to the following standard: Mg counts s^{-1} > 40,000; Rh counts s^{-1} > 200,000; In counts s^{-1} > 300,000; Pb counts s^{-1} > 200,000; precision better than 1%, CeO/Ce < 0.03, background level count s^{-1} (mass 220) < 30.

The optimised method presents a pump flow of 20rpm with 40s flush time, 30s read delay and 30s wash delay. The dual detector is used, as is the autolens. Correction equations are used to take into account any spectral interferences (in most cases) plus, in the case of Cr^{52} and Zn^{64}, the correction factors to matrix interference of C^{12} and S^{34} respectively (which have been determined by Barraque, 2000). The reading parameters are as follows: peak hopping, 60 sweeps per reading, 1 reading per replicate, 3 replicates per analysis. The dwell time per atomic mass unit (AMU) and correction equations were optimised per element (table 3.8).

Table 3.8. ICP-MS method

element	internal standard	calibration range (ppb)	dwell time (ms)	integration time (ms)	equation
Li^{7}	Sc	0-100	25	1500	
Be^{9}	Sc	0-100	25	1500	
V^{51}	Sc	0-100	25	1500	
Cr^{52}	Sc	0-100	25	1500	$-0.00324*C^{12}$
Mn^{55}	Sc	0-100	25	1500	
Co^{59}	Ge	0-100	25	1500	
Ni^{58}	Ge	0-100	25	1500	$-0.003053*Fe^{56}$
Cu^{63}	Ge	0-100	25	1500	
Zn^{64}	Ge	0-100	25	1500	$-0.035313*Ni^{60}$ $-0.0087*S^{34}$
As^{75}	Rh	0-100	50	1500	
Se^{80}	Rh	0-100	50	1500	$-1.00896*Kr^{83}$
Cd^{114}	Rh	0-100	50	1500	$-0.040033*Sn^{118}$
Hg^{202}	Rh	0-10	25	1500	
Pb^{208}	Rh	0-100	50	1500	
Bi^{209}	Rh	0-100	25	1500	
U^{238}	Rh	0-100	25	1500	

Samples are diluted 10 times, and the internal standards added, respectively 100ppb Rh, 200ppb Ge, 100ppb Sc, 5ppm Au (to stabilise the mercury).

3.2.1.5. Limits of detection and ranges

The limit of detection was defined as 4.6 times the standard deviation of seven replicates of the standard representing 10% of the calibration curve range.

In the case of ICP-MS, all limits of detection for one element in various organs were the same as ca 0.5g of sample was used at all times. However, in the case of AAS studies, sample weight varied, ca 0.5g for liver tissue (which was more reactive) and ca 5g for other tissues. Different limits of detection therefore applied, depending on the sample type.

Table 3.9: Limits of detection for the metal analysis

Method	**AAS**		**ICP-MS**	
Element	In solution (ppb)	In the sample (mg kg^{-1} wet weight) Liver – other tissues	In solution (ppb)	In the sample (mg kg^{-1} wet weight)
Li			2.36	0.047
Be			1.88	0.038
V			1.70	0.034
Cr	60	3 – 0.3	1.86	0.037
Mn			1.77	0.035
Co			1.61	0.032
Ni	40	2 – 0.2	2.12	0.042
Cu	20	1 –0.1	1.80	0.036
Zn	20	1 –0.1	1.47	0.029
As	5	0.25 – 0.025	1.67	0.033
Se			1.91	0.038
Cd	5	0.25 – 0.025	1.56	0.031
Hg	5	0.25- 0.025	0.29	0.006
Pb	5	0.25 – 0.025	1.64	0.033
Bi			1.59	0.032
U			1.60	0.032

Table 3.10: Ranges of concentrations of metals in all samples (mg kg^{-1} wet weight)

Element	**Mean**	**Minimum**	**Maximum**
Li	0.04	uld	1.08
Be	0.003	uld	0.22
V	0.19	uld	3.97
Cr	0.48	uld	27.56
Mn	0.86	uld	6.06
Co	0.02	uld	0.56
Ni	0.84	uld	33.01
Cu	4.64	uld	80.86
Zn	25.83	uld	166.37
As	5.94	uld	177.43
Se	1.43	uld	30.56
Cd	2.17	uld	65.59
Hg	0.56	uld	44.90
Pb	0.03	uld	0.81
Bi	0.007	uld	0.29
U	0.006	uld	0.10

uld: under limit of detection

2.2.1.6. Quality control

These methods are accredited by the United Kingdom Accreditation Service (UKAS) and are regularly validated through successful participation in national and international intercalibration exercises (Pedersen *et al.*, 1997 and www.quasimeme.marlab.ac.uk).

The accuracy of the data was monitored by the use of the Certified Reference Material Dorm 2 (dogfish muscle, National Research Council, Canada) in every batch of analysis. The concentrations obtained for the CRM were monitored on shewart charts and checked against the certified values (see fig 3.14 and 3.15). If the results were unsatisfactory for a specific element and batch, the results for this element on this batch are discarded and that batch was either re-analysed or abandoned for that specific element.

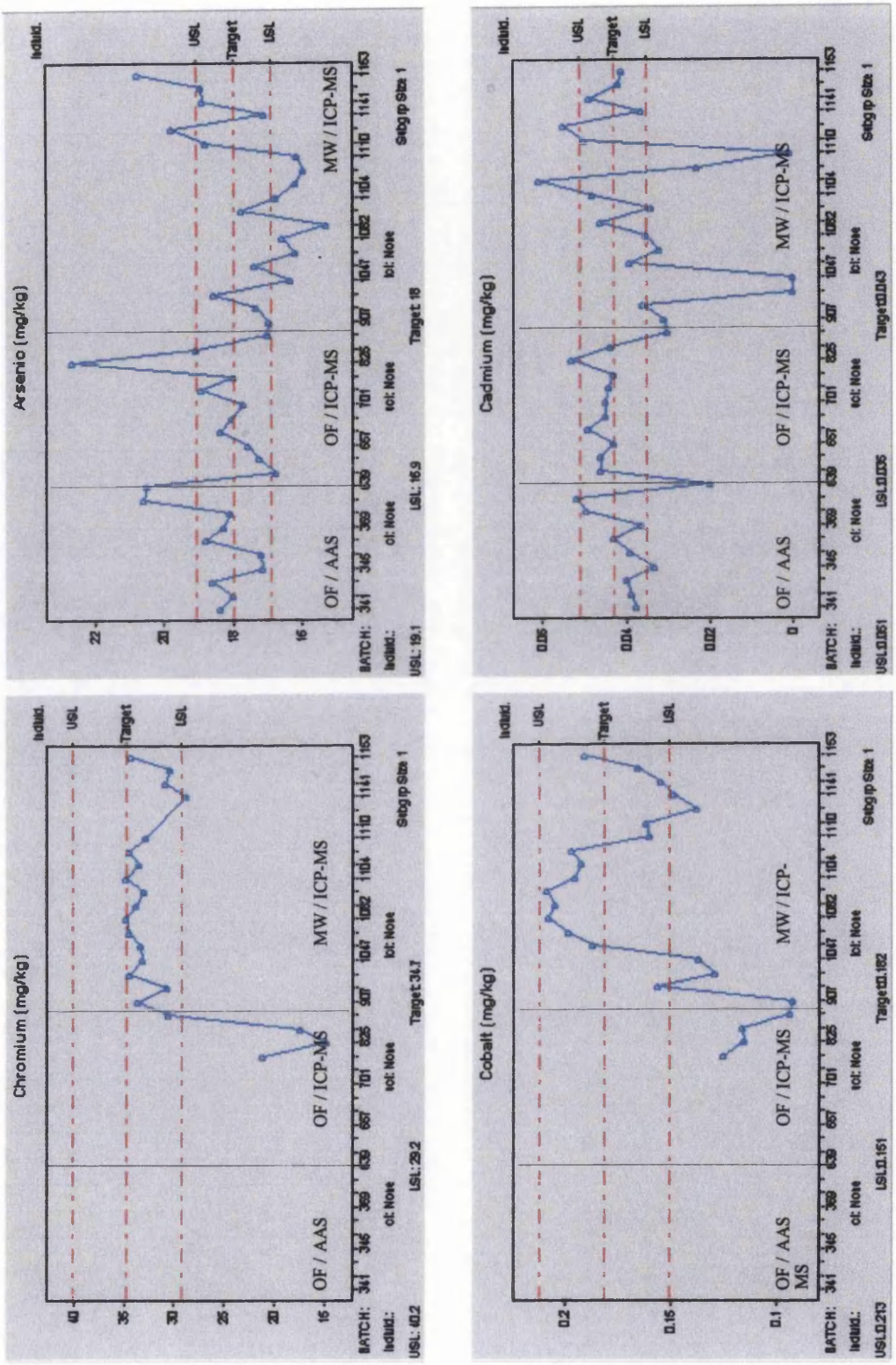


Fig 3.14: Shewart charts for As, Cd, Co, Cr levels (mg kg^{-1}) in DORM2, including certified value and 95% tolerance limits (digestion method: OF: open flask, MW: microwave; analysis by AAS or ICP-MS).

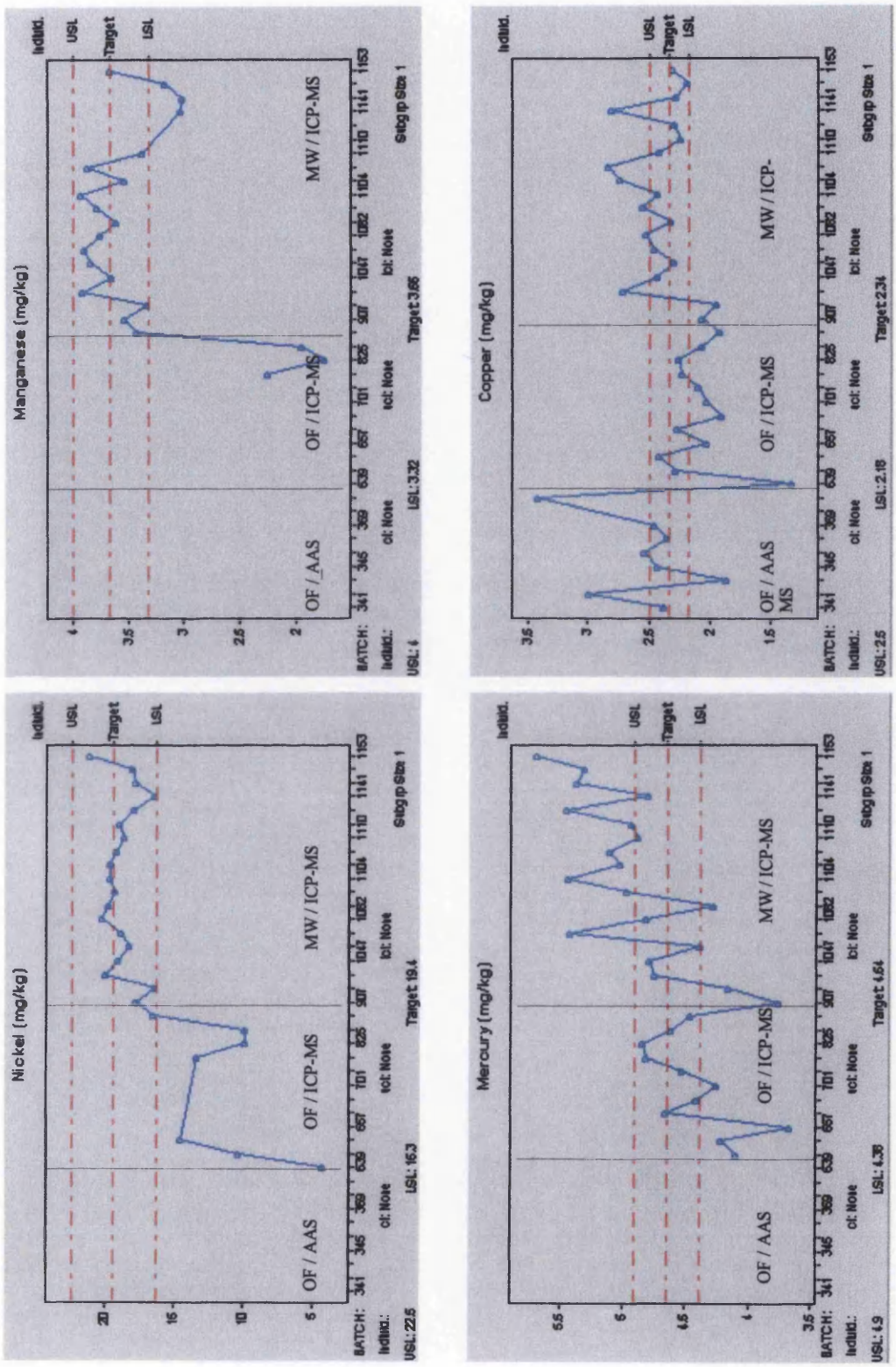


Fig 3.14: Shewart charts for Cu, Hg, Mn, Ni levels (mg kg^{-1}) in DORM2, including certified value and 95% tolerance limits (digestion method: OF: open flask, MW: microwave; analysis by AAS or ICP-MS).

3.2.2. PCB and pesticide analysis

Concentrations of HCB, CBs 28, 31, 44, 49, 52, 70, 74, 101, 105, 110, 114, 118, 128, 138, 149, 153, 156, 157, 158, 170, 180, 187, 189, 194 were measured, and the sum of all these CBs was calculated as ΣCB. The following pesticides were also determined: α-HCH, γ-HCH, α- chlordene, γ-chlordene, aldrin, heptachlor-epoxide, α-chlordane, γ-chlordane, oxychlordane, transnonachlor, heptachlor, endrin, dieldrin, o,p'-DDD, p,p'-DDD, o,p'-DDT, p,p'-DDT, o,p'-DDE, p,p'-DDE. Σchlordane was calculated as the sum of α- chlordene, γ-chlordene, heptachlor-epoxide, α-chlordane, γ-chlordane, oxychlordane, transnonachlor and heptachlor. ΣDDT was calculated as the sum of o,p'-DDD, p,p'-DDD, o,p'-DDT, p,p'-DDT, o,p'-DDE and p,p'-DDE. Limits of detection were calculated separately for each sample, about 1.5pg kg^{-1} wet weight, ie 0.03μg kg^{-1} lipid weight for all tissues and all determinands.

3.2.2.1. Extraction

10 samples can be analysed in any single batch, each made of up to 5g of liver or 10g of muscle, gonad or gill tissue of the previously homogenised pools. The exact weight of each sample is recorded. Each sample is subsequently dried by grinding with anhydrous sodium sulphate, using 10 times more sodium sulphate than sample, and allowed to dry for a minimum of two hours. Anhydrous sodium sulphate is prepared beforehand by heating sodium sulphate at 400°C for at least four hours and no more than six hours, cooled down in a dessicator then stored in a dry cabinet.

The samples are quantitatively transferred to extraction thimbles, and ca 0.5ml of CB209 (weighed exactly) is added to monitor recovery. New thimbles are extracted with methyl tert-butyl ether (MTBE) by soxhlet extraction over heated mantles for at least four hours. Previously used thimbles are re-used without prior cleaning procedure until the control blank increases or a new set of samples is started, in which cases new thimbles are then used. In each batch, a control blank made of anhydrous sodium sulphate, and a laboratory reference material (LRM110) added to anhydrous sodium sulphate, are extracted alongside the samples, with ca 0.5g of CB209 solution as well. The thimbles and their contents are extracted by soxhlet extraction over heated mantles with 300ml MTBE for at least 8 hours, and usually overnight.

At the end of the extraction, the samples are allowed to cool down. The volume of solvent recovered from the extraction is then quantified using a measuring cylinder. The thimbles are allowed to dry before being emptied of their content for re-use in the next batch. 20ml of each solvent sample is quantitatively transferred in pre-dried and pre-weighed evaporating dish, and allowed to evaporate to dryness on a hot plate set at 45EC. The evaporating dishes are allowed to cool in a dessicator, then re-weighed. The residue weighed is the amount of lipid in the 20ml aliquot evaporated, from which the lipid content (in %) of the sample can be derived by the following formula:

lipid content =(weight of lipid*volume of extract) / (initial sample mass*20) * 100

3.2.2.2. Clean-up and separation

Clean-up and separation require deactivated 5% alumina and 3% silica. Detailed preparation procedures can be found in paragraph 3.2.2.3.

The following step is a clean-up procedure through a 6g alumina column in order to get rid of the lipid content of the samples. Such a column can absorb up to 0.2g of lipid, hence only a fraction of the extract can be used. The amount to be used is determined by the following formula:

volume of extract = 4 / weight of lipid in the 20ml sub-sample

If that volume is greater than the total volume of the extract, then the whole of the remaining extract is used. The fraction of extract determined is noted then evaporated by turbo-vap evaporation to 0.5ml. A quantitative transfer into hexane is achieved by adding 10ml of hexane to the 0.5ml extract then evaporating it to 0.5ml.

The 0.5ml hexane fraction recovered is then added to the top of a 6g alumina column. Once it has absorbed onto the column, the turbo-vap tube that has contained the fraction is rinsed with 1ml of hexane, which is then added to the column. The tube is rinsed a second time in the same way. The appropriate amount of hexane is subsequently added to the column (between 100 and 150ml, see paragraph 3.2.2.3. for determination). The entire fraction is recovered.

Once all the solvent has gone through the column, the fraction recovered is evaporated to 0.5ml using the turbo-vap evaporator. That fraction is then added to a 3g alumina column and the tube rinsed as previously. A further 100ml of hexane is added to the column. Two fractions are recovered, the first up to ca 5.5ml (see paragraph 3.2.2.3. for determination) containing the PCBs and some pesticides, and the second one containing the remaining of the pesticides. The second fraction is kept as such.

The first fraction is evaporated to 1ml using a pressured air blow-down system then added to a 3g silica column. The tube is rinsed twice with hexane as previously, then 25ml of hexane is added to the column. Two fractions are recovered again, the first one up to ca 7ml (see paragraph 3.2.2.3.) containing all PCBs, some p,p'-DDE and some p,p'-DDT; and the second one containing the remaining pesticides. The second fractions from both the alumina and the silica columns are added together to form the pesticides fraction.

Both fractions are then evaporated to ca 0.5ml using either the blow down apparatus or the turbo-vap evaporator. 0.5ml of a D6/D16 mixture is added to all samples and weighed exactly. This constitutes the internal standard for the GC. 7ml of iso-octane is also added to all fractions, which are then evaporated to 0.4±0.1ml. These final fractions are vialled in GC vials at as near as 0.347g as possible (which represents exactly 0.5ml iso-octane) by adding iso-octane if necessary, recording the exact weight and labelling accordingly.

3.2.2.3. Preparation of alumina and silica gels

5% alumina and 3% silica gels are needed for the clean-up and separation procedures. They need to be prepared and tested before use and are only usable within one month of the preparation date.

About 400g of aluminium oxide is heated to 400°C for at least four hours and no more than six hours. It is then allowed to cool down in a dessicator until cool enough to handle. Alumina is separated in three equal fractions in round flasks, to which 5% by weight of ultra-pure distilled water is added. The flasks are subsequently shaken

for at least one hour. Eventually all fractions are re-united and the whole alumina is shaken for a further five minutes.

A similar procedure is used for silica gel, with the differences that silica is heated to 600°C, and 3% by weight of ultra-pure distilled water is added. Both deactivated alumina and silica are then tested and calibrated for the separation of PCBs and pesticides into two different fractions.

The 6g alumina column is tested for recovery of dieldrin, which needs to be over 95%. For that purpose, 0.5ml (weighed exactly) of a specific test mixture for alumina containing dieldrin, p,p'-DDT and a-HCH is added to a 6g alumina column. Once this mixture is absorbed onto the column, between 100 to 150ml of hexane is added to the column, being the required volume in the previous batch. The whole of the solvent is then recovered as a single fraction, transferred to iso-octane, vialled and analysed following the same procedure as for the samples. 0.5ml of the test mixture is directly added to 0.5ml iso-octane and vialled to provide a check standard. The dieldrin recovery is calculated from the comparison of the concentrations in the two samples. If it is below 95%, the same procedure is repeated with a higher hexane volume until dieldrin recovery reaches 95%.

The 3g alumina column is tested for separation between p,p'-DDT and α-HCH. All p,p'-DDT needs to be in the first fraction and all α-HCH in the second. For that purpose, 0.5ml (weighed exactly) of the same test mixture is added on a 3g alumina column. Once this mixture is absorbed onto the column, 100ml of hexane is added to the column. The following fractions are recovered: 0-4ml, 4-4.5ml, 4.5-5ml, 5-5.5ml, 5.5-6ml, 6–6.5ml, 6.5–7ml, 7-7.5ml, 7.5-8ml, and the remaining fraction. Each fraction is treated as a single sample, transferred to iso-octane, vialled and analysed following the same procedure as for the samples. The alumina fraction cut is then determined from the GC results, usually at about 5.5ml.

The 3g silica column is tested for separation of p,p'-DDE and p,p'-DDT, most of the p,p'-DDE needing to be in the first fraction and all p,p'-DDT in the second. For that purpose, 0.5ml (weighed exactly) of a test mixture for silica containing p,p'-DDE, o,p'-DDT and p,p'-DDT is added on a 3g silica column. Once this test mixture is absorbed onto the column, 25ml of hexane is added to the column. The following

fractions are recovered: 0-5ml, 5-6ml, 6–6.5ml, 6.5–7ml, 7-7.5ml, 7.5-8ml, 8-8.5ml, 8.5-9ml, and the remaining fraction. Each fraction is treated as a single sample, transferred to iso-octane, vialled and analysed following the same procedure as for samples. The silica fraction cut is then determined from the GC results, usually at about 7ml.

Both deactivated alumina and silica are then stored in brown glass jars and labelled with the batch number, preparation and expiry dates, volume of solvent needed and fraction volumes.

3.2.2.4. Gas chromatography analysis

Instrumental analysis is performed by capillary column gas chromatography with an electron capture detector. PCBs are analysed on Perkin Elmer ECD gas chromatography fitted with 50m*0.25mm I.D. CP-Sil 8 column and 2.5m*0.53mm I.D. uncoated deactivated precolumn, programmable on-column (POC) injector with hourglass inset and autosampler. The oven temperature program is 80°C for 1min, then 3°C min^{-1} to 270°C, then held for 12min. Injector temperature starts at 120°C for 0.2min, then 200°C min^{-1} to 270°C then held for 70min. The detector is held at 270°C.

Pesticides are analysed on VARIAN 3500 ECD gas chromatography fitted with 50m*0.25mm I.D. CP-Sil 8 column and 2.5m*0.32mm I.D. uncoated deactivated precolumn, septum programmable injector with glass insert and 8200 CX autosampler. The oven temperature program is 80°C to 180°C at 15°C min^{-1}, then held for 6min, then to 290°C at 3°C min^{-1} where it is held for 20min. Injector and detector temperatures are the same as above.

See fig 3.16 and 3.17 respectively for typical PCB and pesticide chromatograms of standards, and fig 3.18 to 3.21 for PCB and pesticide chromatograms of the liver of a 108cm black scabbardfish from Rockall Trough and Madeira.

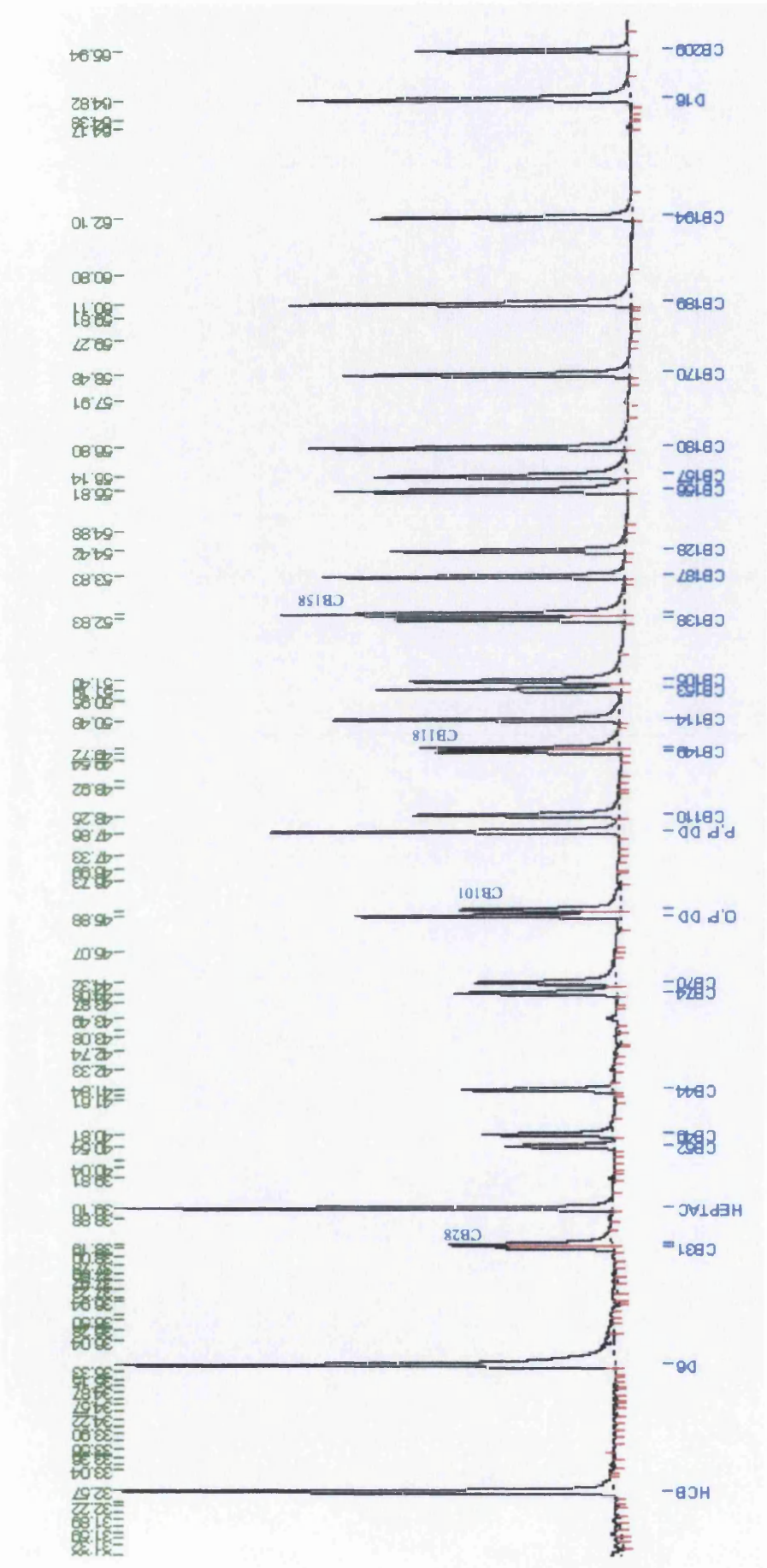


Fig 3.16: Chromatogram of the 0.1ppm PCB standard

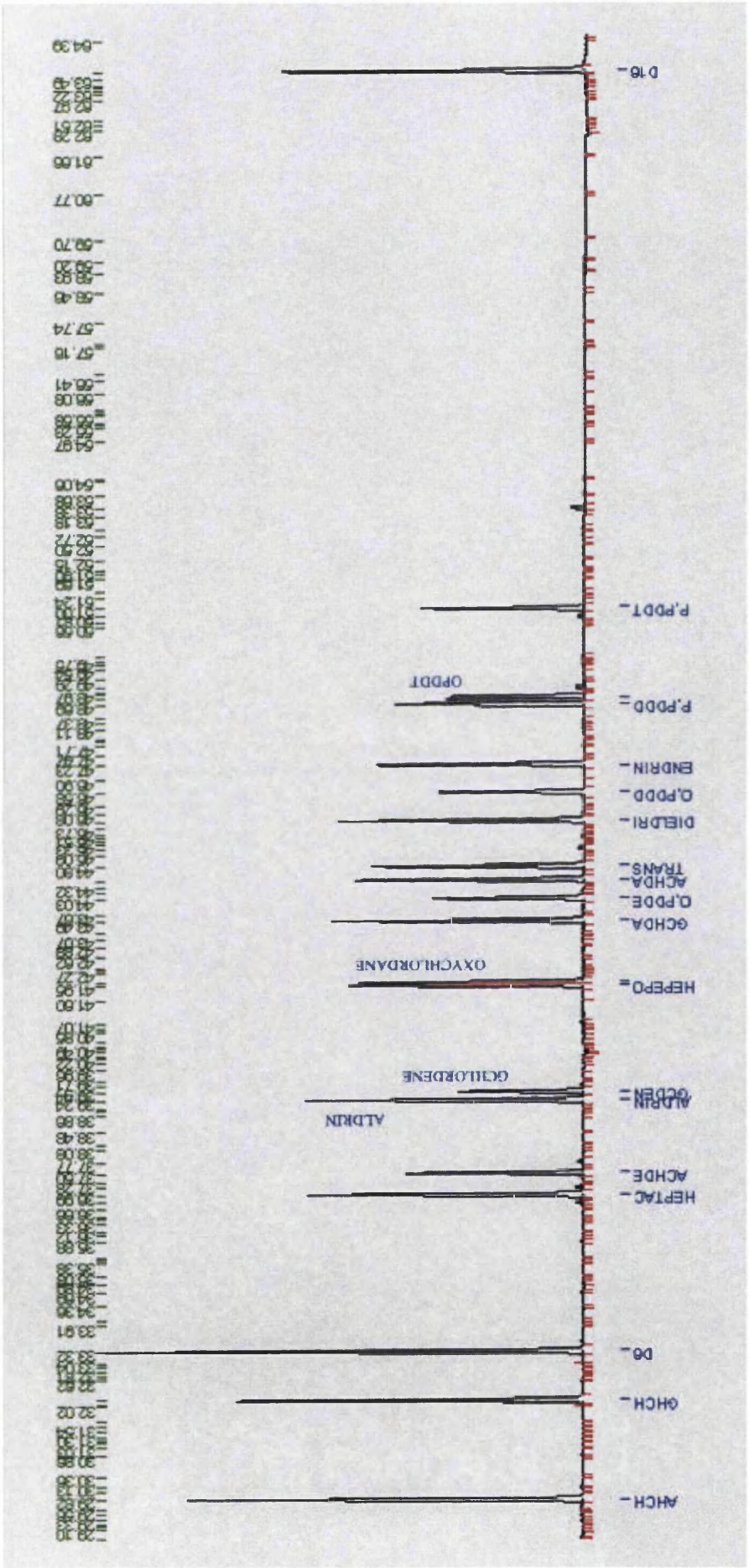


Fig 3.17: Chromatogram of the 0.1ppm pesticide standard

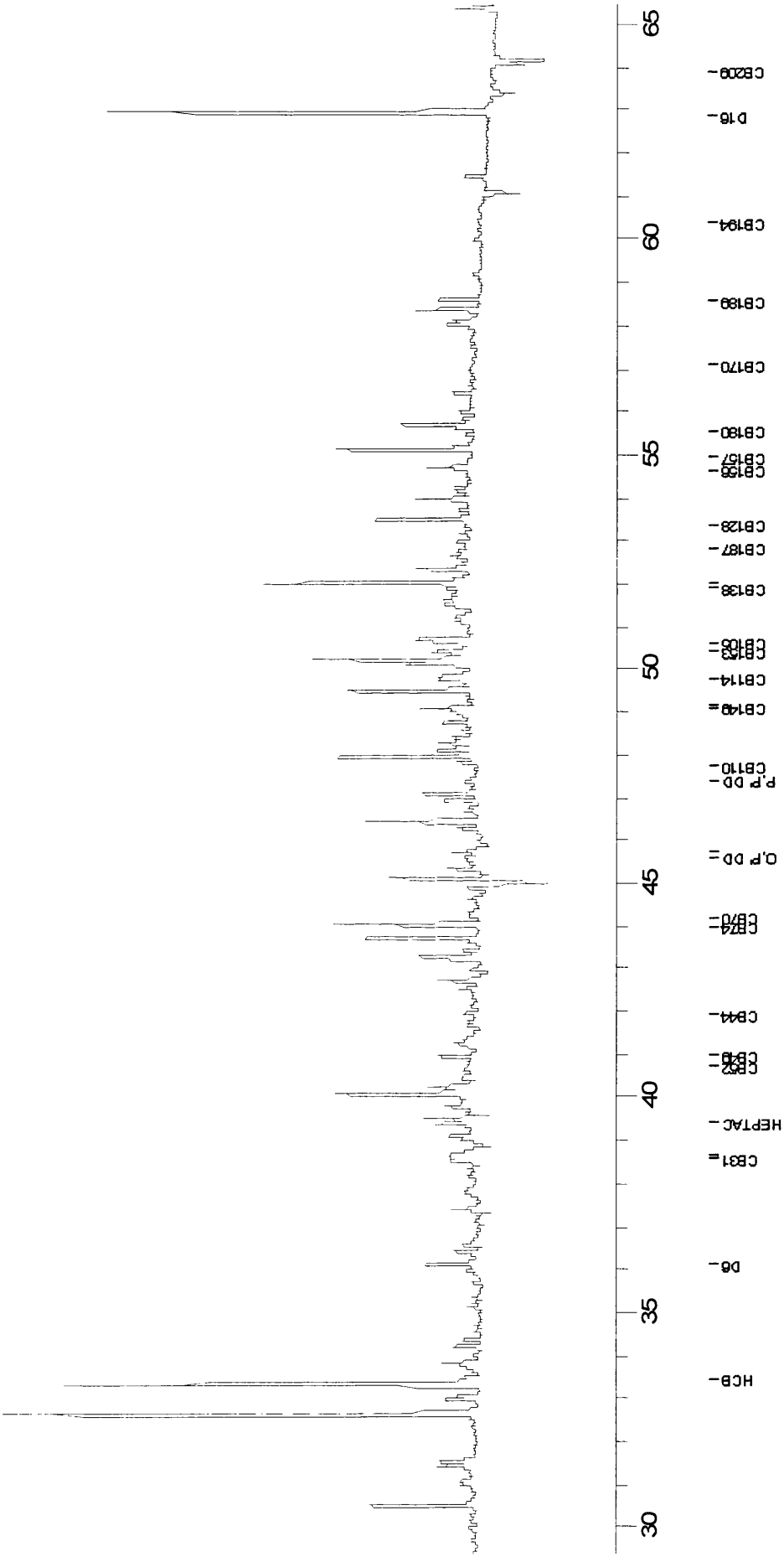


Fig 3.18: PCB chromatogram of a 108.8cm black scabbardfish from the Rockall Trough

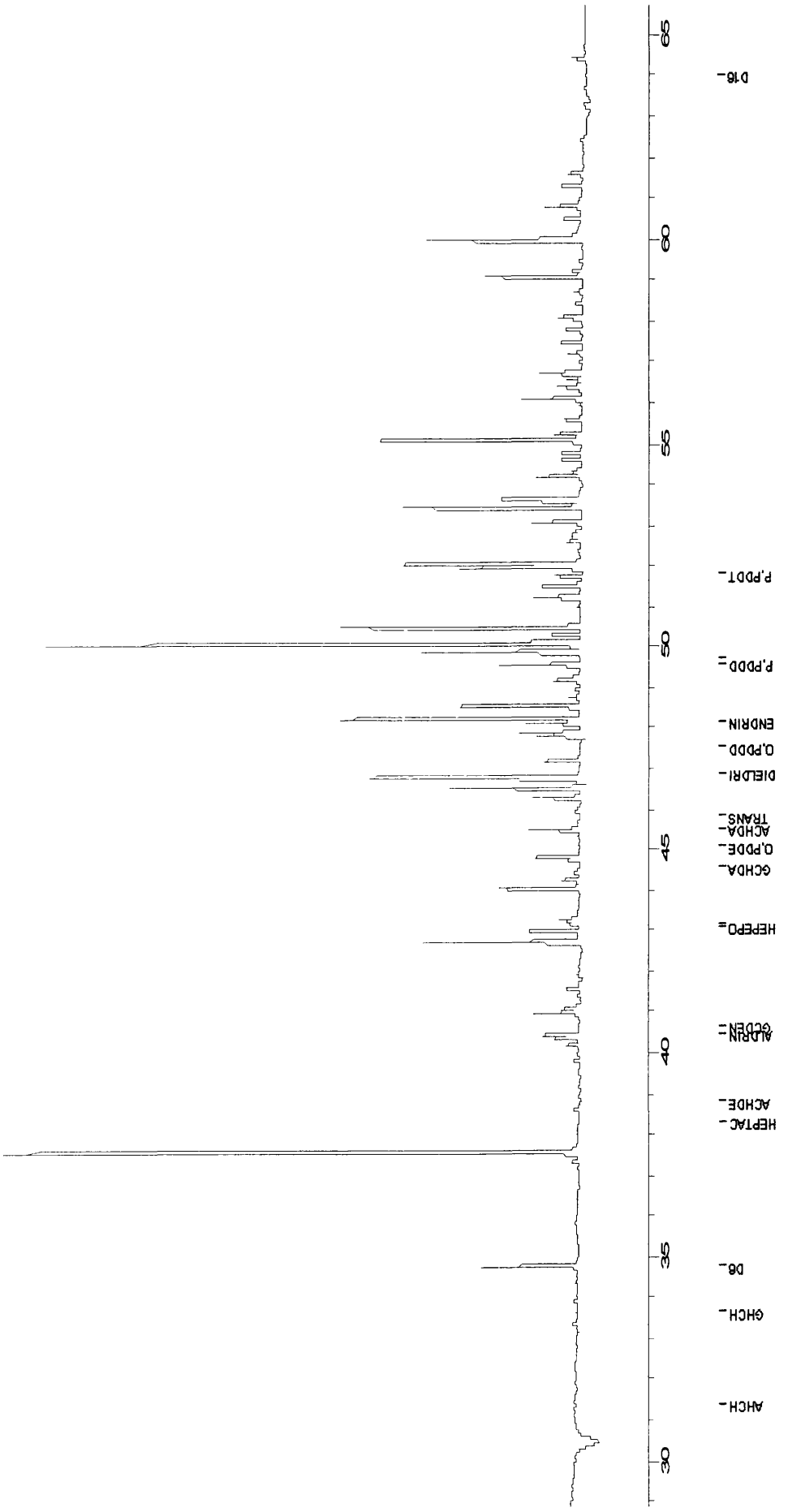


Fig 3.19: Pesticide chromatogram of a 108.8cm black scabbardfish from the Rockall Trough

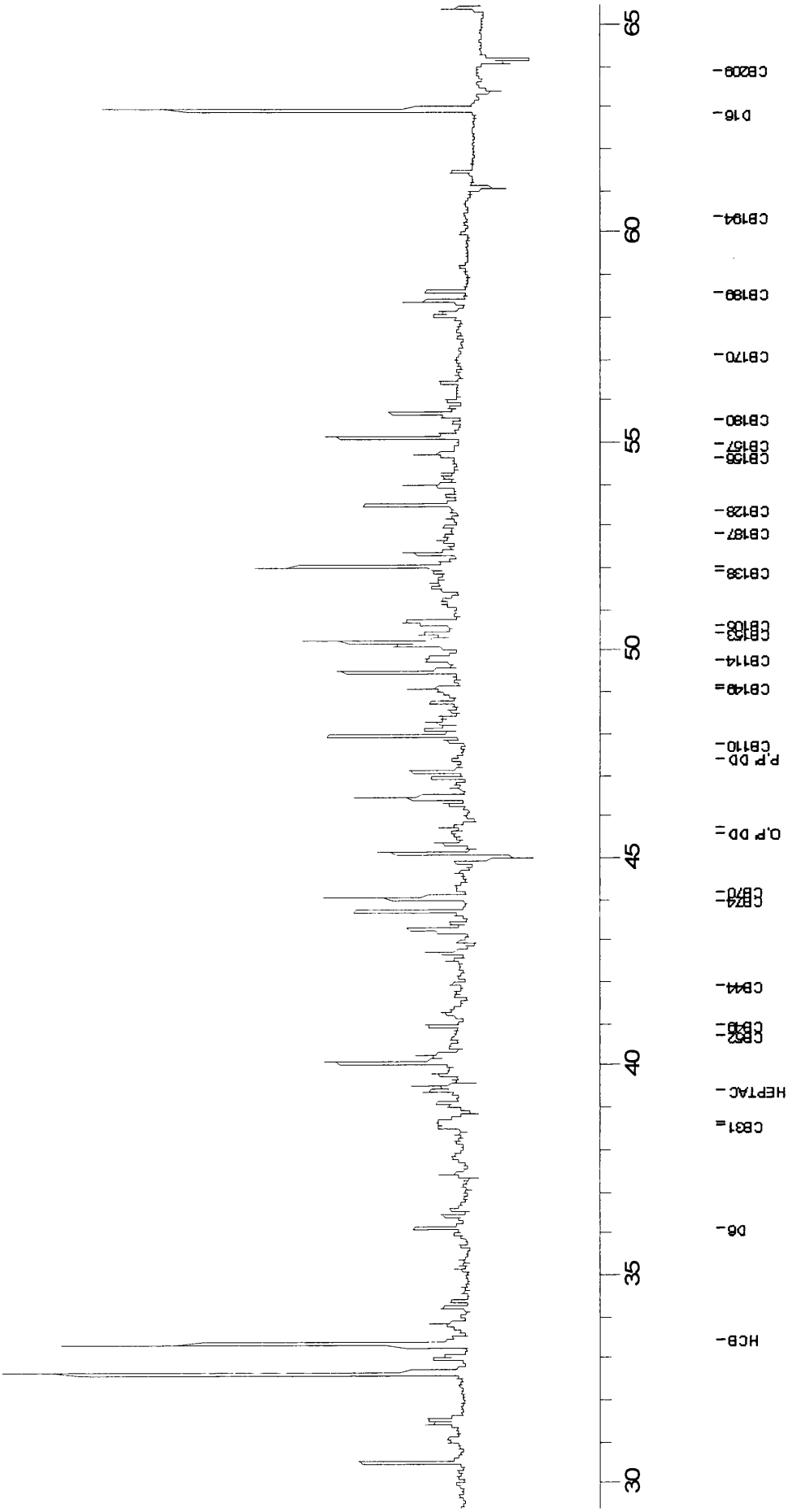


Fig 3.20: PCB chromatogram of a 108.8cm black scabbardfish from Madeira

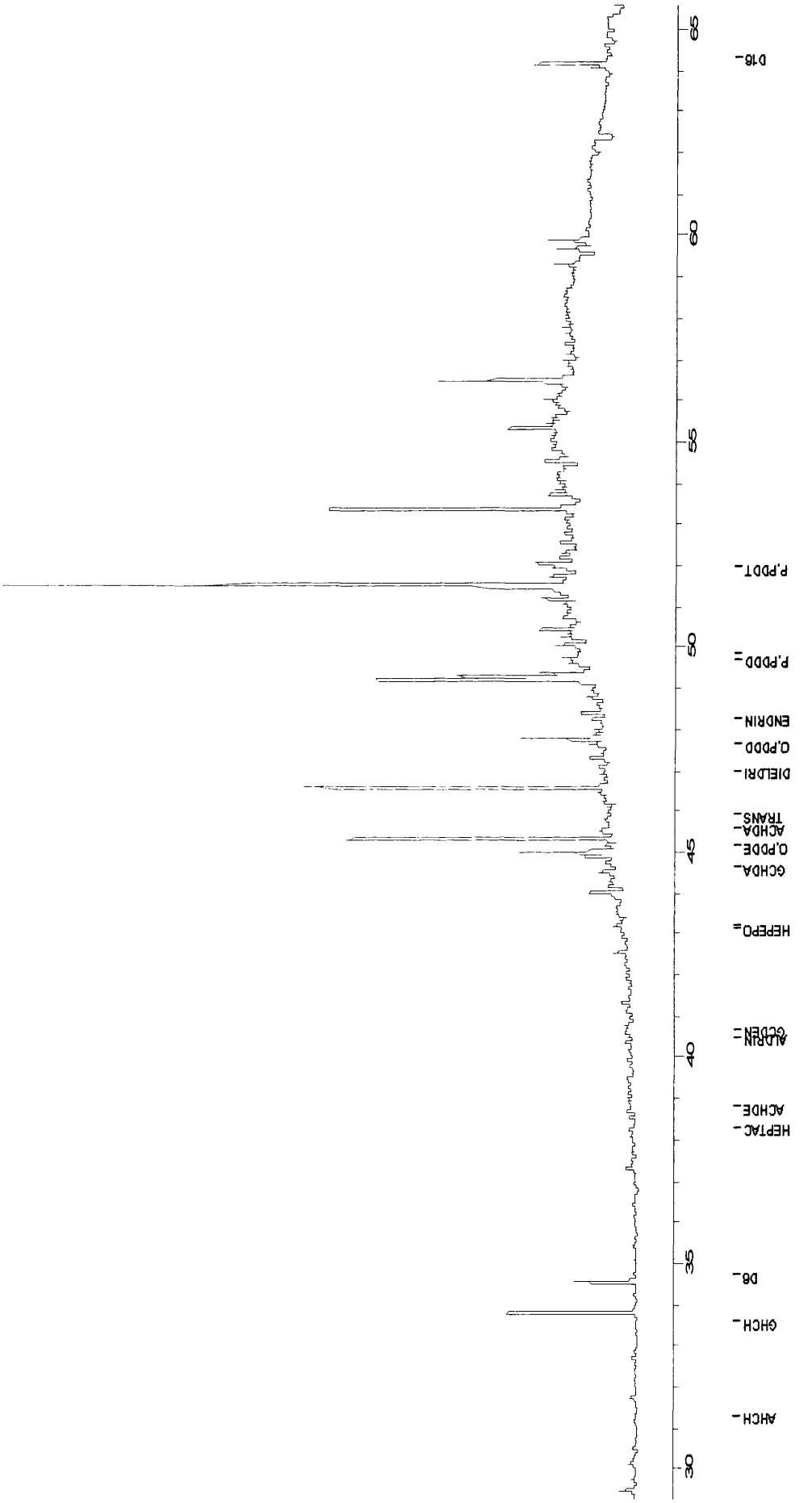


Fig 3.21: Pesticide chromatogram of a 108.8cm black scabbardish from Madeira

3.2.2.5. Limits of detection and quality control

Limits of detection were calculated separately for each sample, about 1.5μg kg^{-1} wet weight, ie 0.03μg kg^{-1} lipid weight for all tissues for all determinands. Recoveries of CB209 were typically between 85 and 115% for blank, LRM110 and most samples. However, in some cases, recoveries in samples were much higher, up to 400%, which is probably due to the presence of CB209 in the tissue analysed, hence those values were ignored as long as the recoveries for the blank and LRM samples in the same batch were within 85-115% recovery.

These methods are accredited by the United Kingdom Accreditation Service (UKAS) and are regularly validated through successful participation in national and international intercalibration exercises (Boer and Wells, 1997 and www.quasimeme.marlab.ac.uk).

The accuracy of the data was monitored by the use of the Laboratory Reference Materials (LRM110) in every batch of analysis, and the recovery was monitored in each sample by the addition of an internal standard (CB209) prior to extraction. The PCB and pesticide concentrations obtained for the LRM110 were monitored on shewart charts and batches re-analysed if the results were unsatisfactory (see fig 3.18 and 3.19).

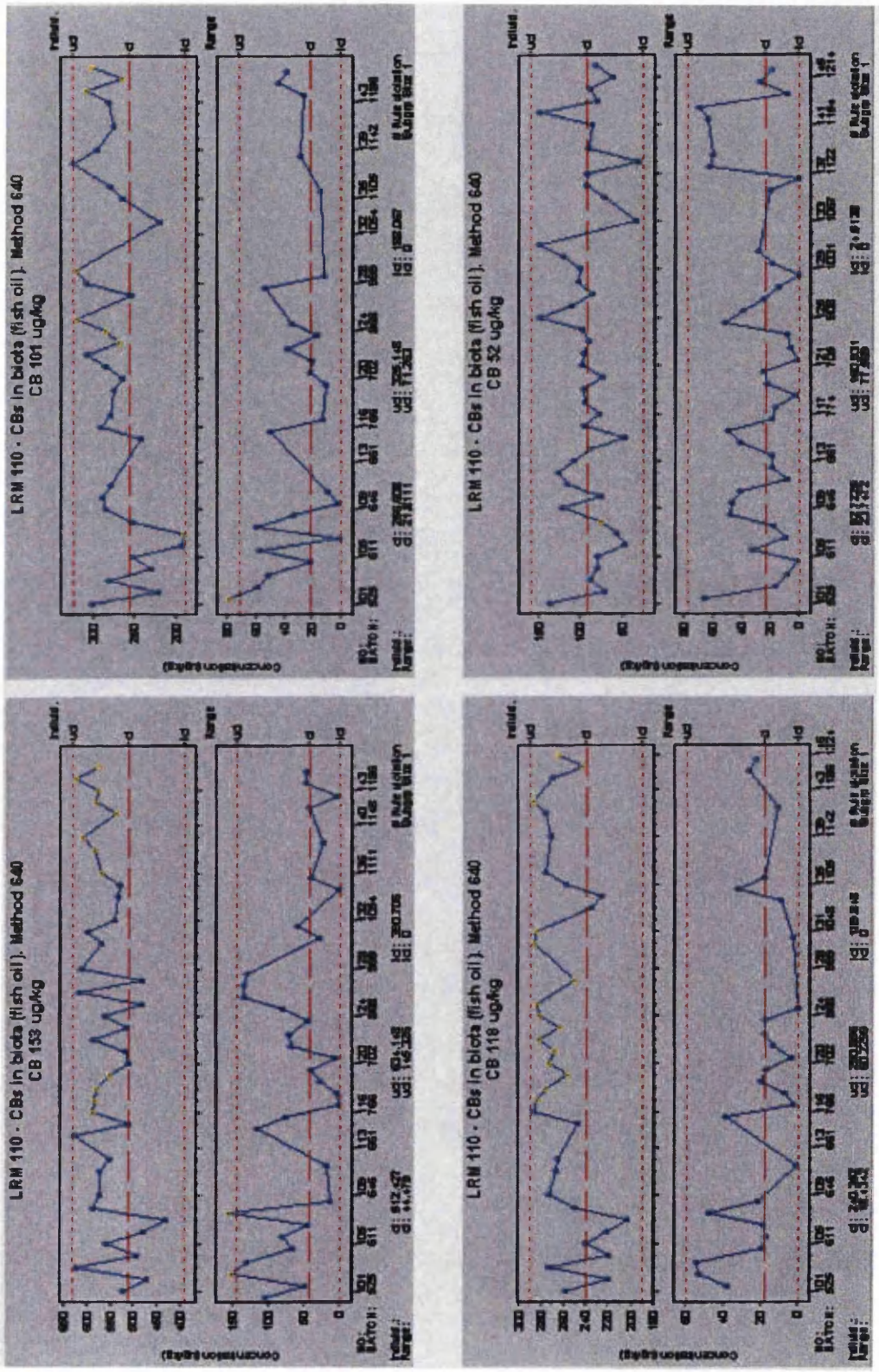


Fig 3.22: Shewart charts for CB52, 101, 118 and 153 concentrations ($\mu g\ kg^{-1}$ wet weight) in LRM110, including certified value and 95% tolerance limits.

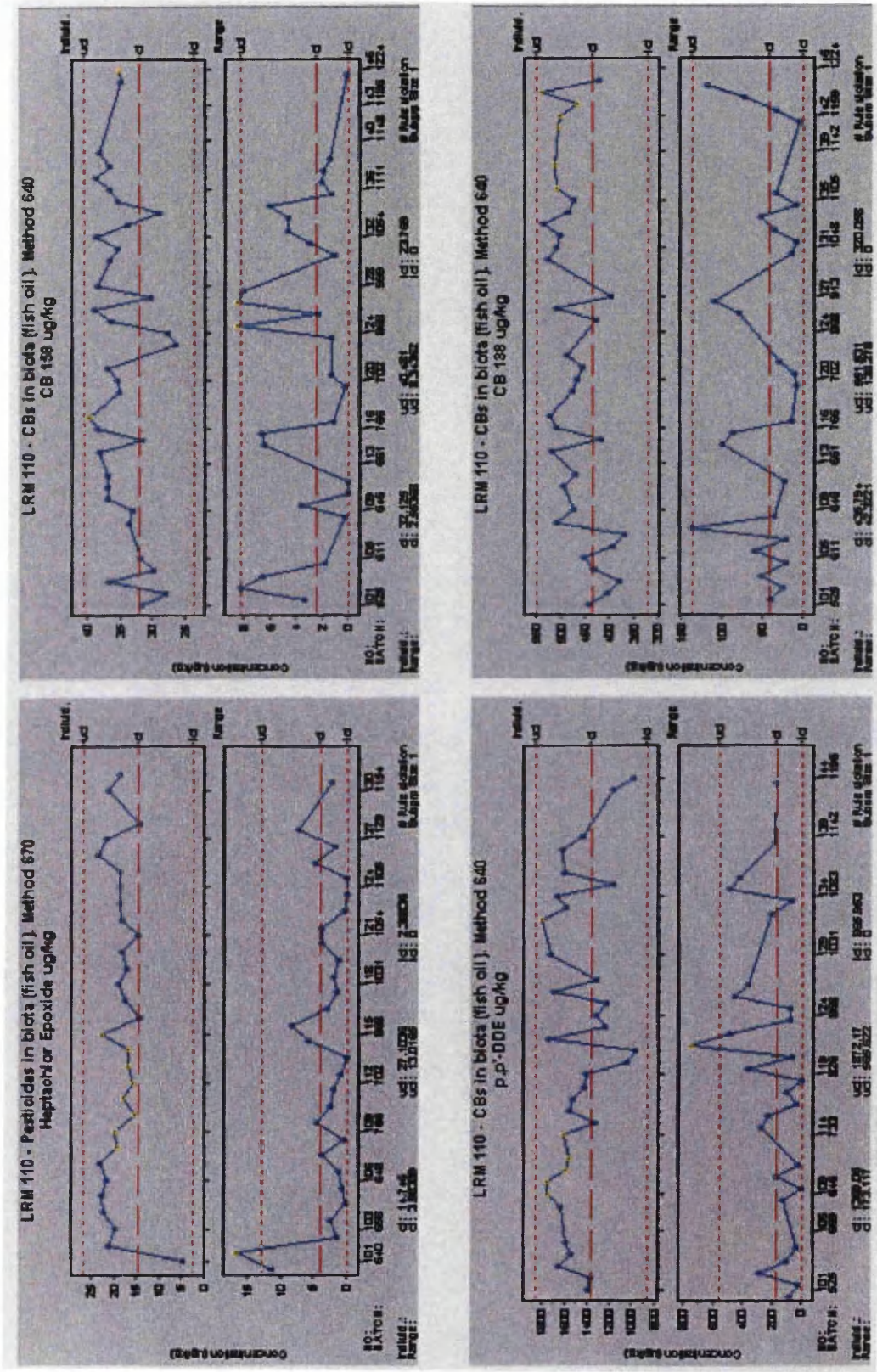


Fig 3.23: Shewart charts for CB138, 158, p,p'-DDE and heptachlor epoxide concentrations ($\mu g\ kg^{-1}$ wet weight) in LRM110, including certified value and 95% tolerance limits.

Chapter 4
Results

In the present chapter, results of the measurements of concentrations of contaminants in deep water fish are presented. All statements are supported by statistical analysis of the data appropriate for their non-normal distribution, ie Mann-Whitney and Spearman's tests. A few samples presented very high concentrations of one or a few elements compared to the rest of the samples. These outliers were not plotted on the graphs for ease of reading. However they were retained in the calculations of the means, and hence there are occasionally apparent differences between the tables and figures.

The analytical results have been separated into groups according to the species analysed (13 different species analysed), the location of sampling (Rockall Trough, Porcupine Bank, Meriadzec, Madeira, Sesimbra or South Africa), the discrete depth range from which the samples were obtained and, finally, the organ sampled (muscle, liver, gonad and/or gill tissue). In total, 39 distinct groups were formed, each presenting levels of contaminants in one or more organs. Sex differentiation was not made at this point.

The species analysed were: monkfish (*Lophius piscatorius*), *Bathysaurus ferox*, blue ling (*Molva dypterygia*), black scabbardfish (*Aphanopus carbo*), blue whiting (*Micromesistius poutassou*), *Coryphaenoides armatus*, hake (*Merluccius merluccius*), *Histiobranchus bathybius*, kingklip (*Genypterus capensis*), *Lophius vomerinus*, *Merluccius capensis*, *Merluccius paradoxus,* orange roughy (*Hoplostethus atlanticus*) and roundnose grenadier (*Coryphaenoides rupestris*).

The results are summarised in tables, as mean and standard deviation of the concentration of the element of interest, and in figures as box-and-whisker plots. The central horizontal line of the box-and-whisker plot marks the median value. The edges of the box, called hinges, mark the first and third quartiles. The median splits the ordered batch of numbers in half, and the hinges split the remaining halves in half again – that is, the central 50% of the values fall within the range of the box. Hspread

is the absolute value of the difference between the values of the outer hinges. The whiskers show the range of values that fall within 1.5Hspread of the hinges. Inner and outer fences are defined as follows:

lower inner fence=lower hinge-(1.5*(median-lower hinge))
upper inner fence=upper hinge+(1.5*(upper hinge-median))
lower outer fence=lower hinge-(3*(median-lower hinge))
upper outer fence=upper hinge+(3*(upper hinge-median))

Values outside the inner fences are plotted as asterisks. Values outside the outer fences, called far outside values, are plotted as empty circles (Systat, 1996).

4.1. Metals concentrations

Each individual sample was analysed for a range of metals, comprising some or all of the following: lithium, beryllium, vanadium, chromium, manganese, cobalt, nickel, copper, zinc, arsenic, selenium, cadmium, mercury, lead, bismuth and uranium, by methods described in chapter 3 above.

The results for metals are summarised by group for all elements in tables 4.1 to 4.5 and by element and group in figs 4.1 to 4.16. In total, 1407 samples were analysed for the range of metals.

Table 4.1: Mean and standard deviation of trace metals concentrations (mg kg^{-1} wet weight) in muscle (FL), gonad (GD) and liver (LV) tissues of monkfish (ANG) and roundnose grenadier (RNG) from the Rockall Trough at various depths.

species	location	depth range (m)	organ	statistics	fish analysed	length (cm)	weight (g)	Li	Be	V	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Cd	Hg	Pb	Bi	U
ANG	Rockall	80-125	FL	Mean	9	52.9	2340.8	uld	uld	uld	0.24	0.07	uld	uld	0.05	3.55	12.01	0.44		0.104	uld	uld	uld
				std		10.5	1323.9	0.01	<0.01	<0.01	0.08	0.05	<0.01	0.02	0.04	0.84	3.42	0.12		0.060	0.01	<0.01	<0.01
			GD	Mean	9	52.9	2340.8	0.06	uld	uld	0.30	0.28	uld	uld	0.89	23.96	1.58	0.72	uld	uld	uld	uld	uld
				std		10.5	1323.9	0.02	<0.01	<0.01	0.17	0.07	<0.01	0.03	0.56	15.20	0.52	0.33	0.01	0.008	0.05	<0.01	<0.01
			LV	Mean	8	54.6	2519.6	uld	uld	uld	0.25	0.66	0.06	uld	13.76	22.33	3.06	2.03	0.38	0.185	uld	uld	uld
				std		9.8	1293.9	<0.01	<0.01	0.01	0.10	0.17	0.02	0.03	15.04	8.46	0.46	0.40	0.39	0.089	<0.01	0.01	<0.01
		250	FL	Mean	9	54.2	2502.0	uld	uld	uld	3.52	0.37	uld	2.23	0.56	13.95	7.28	0.81	uld	0.671	uld	uld	uld
				std		6.6	908.4	0.02	<0.01	0.04	9.04	0.83	0.06	6.65	0.98	15.67	5.64	0.47	0.01	1.739	0.03	<0.01	<0.01
			GD	Mean	9	54.2	2502.0	uld	uld	uld	0.73	0.26	uld	uld	1.42	23.75	2.58	1.04	0.04	0.068	uld	uld	uld
				std		6.6	908.4	0.02	<0.01	0.01	1.42	0.14	0.01	0.08	1.36	15.33	1.32	0.35	0.05	0.076	0.01	<0.01	<0.01
			LV	Mean	9	54.2	2502.0	uld	uld	uld	0.68	0.24	uld	uld	5.15	10.28	6.64	1.01	0.13	0.154	uld	uld	uld
				std		6.6	908.4	0.01	<0.01	0.02	1.20	0.21	0.03	0.09	8.18	6.68	3.19	0.47	0.14	0.069	0.03	0.02	<0.01
		400-700	FL	Mean	37	64.0	4605.5	uld	uld	uld	0.17	0.26	uld	uld	0.33	14.35	6.24	0.62	uld	0.033	uld	uld	uld
				std		0.1	7629.4	0.01	<0.01	<0.01	0.05	0.16	<0.01	<0.01	0.27	7.86	4.93	0.24	0.01	0.018	0.01	<0.01	<0.01
			GD	Mean	35	64.2	4680.5	uld	uld	uld	0.28	0.35	0.06	uld	5.91	17.37	3.44	1.20	0.15	0.225	uld	uld	uld
				std		0.1	7629.4	<0.01	<0.01	0.01	0.11	0.16	0.03	0.01	13.43	6.95	2.89	0.56	0.42	0.132	0.02	<0.01	<0.01
			GL	Mean	22	62.6	3481.8								0.56		1.46		uld		uld		
				std		0.1	7629.4								0.06		0.70		0.02		0.06		
			LV	Mean	35	64.3	4726.4	uld	uld	uld	0.23	0.07	uld	uld	7.02	4.14	9.26	0.37	0.13	0.238	uld	uld	uld
				std		14.7	7629.4	0.01	<0.01	<0.01	0.05	0.01	<0.01	0.01	9.98	0.81	7.60	0.22	0.24	0.125	0.02	0.01	<0.01
		700-800	FL	Mean	7	82.7	10872.6								0.15		10.64		uld		uld		
				std		29.1	11345.8								0.02		5.63		0.02		<0.01		
			GD	Mean	7	82.7	10872.6								1.48		1.76		uld		uld		
				std		29.1	11345.8								1.03		0.60		<0.01		0.01		
			GL	Mean	7	82.7	10872.6								0.53		1.69		uld		uld		
				std		29.1	11345.8								0.23		0.85		0.01		0.01		
			LV	Mean	7	82.7	10872.6								8.01		7.65		0.50		uld		
				std		29.1	11345.8								5.13		1.17		0.55		0.01		

Table 4.1 (continued): Mean and standard deviation of trace metals concentrations (mg kg^{-1} wet weight) in muscle (FL), gonad (GD) and liver (LV) tissues of monkfish (ANG) and roundnose grenadier (RNG) from the Rockall Trough at various depths.

species	location	depth range (m)	organ	statistics	fish analysed	length (cm)	weight (g)	Li	Be	V	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Cd	Hg	Pb	Bi	U
ANG	**Rockall**	**850**	**FL**	**Mean**	6	67.2	4476.2								0.14		9.73		uld		uld		
				std		15.6	2670.5								0.03		5.28		0.01		<0.01		
			GD	**Mean**	6	67.2	4476.2								0.70		30.67		uld		uld		
				std		15.6	2670.5								0.67		71.90		<0.01		<0.01		
			GL	**Mean**	6	67.2	4476.2								0.63		1.53		uld		uld		
				std		15.6	2670.5								0.14		1.38		0.02		0.01		
			LV	**Mean**	6	67.2	4476.2								9.72		6.19		0.43		uld		
				std		15.6	2670.5								7.39		4.34		0.33		0.01		
RNG	**Rockall**	**1000**	**FL**	**Mean**	19	18.4	1122.6	uld	uld	uld	0.63	0.08	uld	uld	0.09	1.52	8.54	0.30	uld	0.053	uld		uld
				std		2.7	403.9	0.02	<0.01	0.01	0.12	0.05	<0.01	0.02	0.03	0.32	3.80	0.07	0.01	0.057	0.02		<0.01
			LV	**Mean**	20	18.4	1112.5	0.05	uld	0.20	0.62	0.63	uld	0.10	1.96	27.27	15.36	0.82	2.31	0.030	uld	uld	uld
				std		2.7	395.8	0.03	0.01	0.24	0.27	0.33	0.01	0.08	1.11	10.75	10.76	0.43	2.12	0.055	0.01	0.02	0.01
		1250	**FL**	**Mean**	21	16.4	781.2	0.06	uld	uld	0.53	0.05	uld	uld	0.10	1.69	4.55	0.28	uld	0.037	uld	uld	uld
				std		2.3	330.8	0.03	<0.01	<0.01	0.13	0.01	<0.01	0.01	0.04	0.21	1.30	0.06	<0.01	0.042	<0.01	0.01	<0.01
			LV	**Mean**	20	16.5	790.0	0.14	uld	0.13	0.45	0.36	uld	0.12	4.38	27.91	11.86	0.91	2.59	0.032	uld	uld	uld
				std	0	2.4	336.8	0.09	<0.01	0.20	0.22	0.19	0.01	0.09	2.30	15.85	9.38	0.59	2.51	0.043	<0.01	0.01	<0.01
		1300	**LV**	**Mean**	16	15.9	726.9	uld	uld	0.13	uld	0.21	uld	uld	3.18	31.13	21.55	1.11	3.45	0.058	uld	uld	uld
				std		2.6	352.5	0.01	<0.01	0.16	0.01	0.13	0.01	0.01	1.92	9.15	15.68	0.50	7.13	0.089	0.01	0.01	<0.01
		1450-	**FL**	**Mean**	22	16.5	686.1	0.14	uld	uld	0.57	0.05	uld	uld	0.11	1.64	4.29	0.30	uld	0.007	uld	uld	uld
		1500		**std**		1.7	188.8	0.13	<0.01	<0.01	0.14	0.03	<0.01	0.02	0.06	0.40	1.39	0.06	0.02	0.015	0.01	0.01	<0.01
			LV	**Mean**	22	16.5	686.1	0.21	uld	0.22	0.46	0.43	uld	0.14	4.27	27.10	18.72	1.02	4.24	0.031	uld	uld	uld
				std		1.7	188.8	0.23	<0.01	0.32	0.17	0.24	0.02	0.17	2.63	12.52	17.44	0.77	5.99	0.079	0.01	0.01	0.01
		1974	**LV**	**Mean**	15	15.3	572.3	uld	uld	0.24	0.05	0.27	uld	uld	6.67	31.15	16.10	1.50	3.47	0.080	uld	uld	uld
				std		1.9	226.0	0.01	<0.01	0.17	0.04	0.16	0.02	0.01	5.07	13.13	9.94	1.04	2.29	0.053	0.01	0.01	<0.01

Table 4.2: Mean and standard deviation of trace metals concentrations (mg kg^{-1} wet weight) in muscle (FL), gill (GIL), gonad (GD) and liver (LV) tissues of black scabbardfish (BSC) at various locations and various depths in the North East Atlantic.

species	location	depth range	organ	statistics	fish analysed	length (cm)	weight (g)	Li	Be	V	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Cd	Hg	Pb	Bi	U
BSC	Rockall	500	FL	Mean	15	92.1	1057.0	uld	uld	uld	0.52	0.11	uld	uld	0.12	1.90	1.37	0.33	uld	0.279	uld	uld	uld
				Std		7.2	365.1	<0.01	<0.01	<0.01	0.12	0.02	<0.01	<0.01	0.05	0.19	0.51	0.05	0.03	0.145	0.01	<0.01	<0.01
			LV	Mean	15	92.1	1057.0	uld	uld	1.61	0.52	1.98	0.08	uld	9.37	44.20	8.78	2.13	5.51	0.576	uld	uld	uld
				Std		7.2	365.1	0.01	<0.01	0.74	0.15	0.58	0.03	0.05	5.63	15.59	3.60	0.53	2.89	0.397	0.02	0.01	<0.01
		690-790	FL	Mean	13	97.8	1517.4								0.11	2.75	1.16		uld		uld		
				Std		8.9	933.3								0.02	0.40	0.62		<0.01		<0.01		
			GL	Mean	13	97.8	1517.4								0.92	13.04	1.10		uld		uld		
				Std		8.9	933.3								0.13	1.29	0.70		0.01		<0.01		
			LV	Mean	14	97.7	1703.6								7.84	61.48	6.14		5.63		uld		
				Std		8.6	1135.5								2.21	13.29	4.72		2.68		0.01		
		850	FL	Mean	14	96.2	2539.9								0.14	2.85	2.84		uld		uld		
				Std		11.8	1693.9								0.04	0.38	6.82		<0.01		0.02		
			GL	Mean	14	96.2	2539.9								0.99	13.75	1.11		uld		0.11		
				Std		11.8	1693.9								0.17	2.66	0.74		0.02		0.11		
			LV	Mean	14	96.2	2539.9								14.85	68.25	5.87		9.73		0.04		
				Std		11.8	1693.9								10.19	17.74	5.76		4.31		0.03		
		890-966	FL	Mean	29	82.2	736.9							uld	0.13	2.92	1.61	0.44	uld	0.187	uld		
				Std		12.0	430.5							<0.01	0.06	0.36	1.01	0.01	<0.01	0.025	0.01		
			GL	Mean	26	82.3	744.4								0.79	12.48	1.15		0.05		uld		
				Std		12.6	451.4								0.18	1.48	1.22		0.03		0.01		
			LV	Mean	28	82.0	731.9							uld	16.94	63.76	10.06	2.53	8.14	0.198	0.07		
				Std		12.1	437.5							0.01	8.23	15.46	9.17	0.06	3.88	0.076	0.09		
		1000	FL	Mean	21	85.0	796.0	uld	uld	uld	0.43	0.11	uld	uld	0.10	2.00	1.60	0.35	uld	0.205	uld	uld	uld
				Std		14.3	267.6	<0.01	<0.01	<0.01	0.12	0.03	<0.01	<0.01	0.03	0.25	1.47	0.06	0.01	0.080	0.02	<0.01	<0.01
			LV	Mean	21	85.0	796.0	uld	uld	1.39	0.46	2.03	0.09	0.05	14.86	57.99	6.22	2.02	8.13	0.297	uld	uld	uld
				Std		14.3	267.6	0.01	<0.01	0.90	0.17	0.34	0.03	0.04	9.30	12.93	4.26	0.48	6.79	0.124	0.02	<0.01	<0.01

Table 4.2 (continued): Mean and standard deviation of trace metals concentrations (mg kg^{-1} wet weight) in muscle (FL), gill (GIL), gonad (GD) and liver (LV) tissues of black scabbardfish (BSC) at various locations and various depths in the North East Atlantic.

species	location	depth range	organ	statistics	fish analysed	length (cm)	weight (g)	Li	Be	V	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Cd	Hg	Pb	Bi	U
BSC	Rockall	1130-1210	FL	Mean	25	99.5	1545.2							uld	0.17	2.86	1.40	0.51	uld	0.357	0.07		
				Std		8.1	972.5							<0.01	0.11	1.03	0.74	0.15	0.01	0.140	0.14		
			LV	Mean	24	100.2	1584.2							0.28	23.47	67.17	9.45	3.18	12.98	0.564	uld		
				Std		7.6	973.2							0.05	14.90	18.79	7.51	0.52	9.14	0.241	0.01		
		1250-1300	FL	Mean	19	95.2	1146.1	uld	uld	uld	0.44	0.09	uld	2.67	0.18	3.47	1.67	0.43	uld	0.354	0.05	uld	uld
				Std		9.5	366.4	0.01	<0.01	<0.01	0.07	0.02	0.03	5.56	0.12	3.40	0.75	0.14	0.02	0.146	0.11	<0.01	<0.01
			LV	Mean	18	95.2	1150.6	uld	uld	1.27	0.34	1.45	0.09	3.09	24.42	70.07	9.73	2.84	11.05	0.494	0.07	uld	uld
				Std		9.8	376.4	0.02	<0.01	0.36	0.08	0.18	0.05	5.93	11.71	18.82	5.87	0.83	5.49	0.290	0.12	0.01	<0.01
		1400	FL	Mean	6	100.2	1520.0							uld	0.15	2.79	1.85	0.52	uld	0.413	uld		
				Std		13.4	654.6							<0.01	0.08	0.33	0.93	0.08	<0.01	0.147	0.02		
			GD	Mean	5	103.6	1681.0							uld	0.51	83.78	2.67	0.86	0.06	0.101	0.07		
				Std		11.7	584.2								0.10	20.89	1.65	0.18	0.03	0.063	0.15		
			LV	Mean	6	100.2	1520.0							0.09	14.80	69.00	11.83	2.81	9.21	0.579	0.10		
				Std		13.4	654.6							0.13	4.76	11.23	6.97	0.55	5.77	0.258	0.24		
		1970-2025	FL	Mean	4	91.5	1085.0							uld	0.19	3.42	1.83	0.43	uld	0.193	uld		
				Std		7.9	443.9							<0.01	0.09	0.62	0.39	0.19	<0.01	0.172	0.02		
			GD	Mean	2	96.5	1362.5							0.13	1.16	122.00	4.33	1.49	0.22	0.081	uld		
				Std		9.2	526.8							0.02	0.62	15.89	1.95	0.23	0.04	0.114	<0.01		
			LV	Mean	4	91.5	1085.0							0.27	16.93	71.32	9.68	3.17	10.87	0.387	uld		
				Std		7.9	443.9							0.15	7.29	11.09	5.60	0.64	6.78	0.292	0.01		
	Meriadzec	1140-1150	FL	Mean	14	104.5	1661.4							uld	0.05	2.57	1.78	0.52	uld	0.686	uld		
				Std		10.8	624.9							0.03	0.01	0.64	1.25	0.06	0.01	0.318	0.01		
			GD	Mean	9	106.7	1758.9							uld	0.94	103.93	2.40	1.16	0.10	0.153	uld		
				Std		10.7	676.1							0.01	0.31	17.95	1.76	0.31	0.05	0.111	0.01		
			LV	Mean	13	104.9	1687.7							0.40	17.60	83.58	7.90	3.88	11.53	1.692	0.11		
				Std		11.1	642.3							0.18	10.05	30.21	6.23	1.26	5.75	1.527	0.22		

Table 4.2 (continued): Mean and standard deviation of trace metals concentrations (mg kg^{-1} wet weight) in muscle (FL), gill (GIL), gonad (GD) and liver (LV) tissues of black scabbardfish (BSC) at various locations and various depths in the North East Atlantic.

species	location	depth range	organ	statistics	fish analysed	length (cm)	weight (g)	Li	Be	V	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Cd	Hg	Pb	Bi	U
BSC	Meriadzec	1190-1290	FL	Mean	15	108.4	1816.7							uld	0.05	2.26	2.49	0.49	uld	0.618	uld		
				Std		9.9	556.5							<0.01	0.02	0.23	1.58	0.10	<0.01	0.253	0.03		
			GD	Mean	13	108.6	1813.1							0.09	0.66	105.32	3.46	1.01	0.06	0.127	uld		
				Std		10.6	596.2							0.22	0.06	24.08	2.11	0.21	0.05	0.081	0.01		
			LV	Mean	13	108.2	1759.2							0.37	26.80	93.18	13.19	3.28	12.13	1.519	0.06		
				Std		10.4	574.4							0.24	16.02	37.83	9.45	0.81	11.20	0.867	0.08		
	Sesimbra	1000	FL	Mean	32	104.8	1483.4								0.13	4.12	1.58	0.41	uld	0.507	uld		
				Std		9.5	499.8								0.10	1.10	0.85	0.09	<0.01	0.175	0.01		
			LV	Mean	32	104.9	1482.5								10.70	65.92	3.79	2.49	8.91	1.259	uld		
				Std		9.4	500.4								8.78	26.87	2.25	0.81	5.57	0.877	0.03		
	Madeira	1000	FL	Mean	25	116.8	2207.4								uld	3.69	2.11	1.08	uld	0.814	uld		
				Std		5.4	405.7								0.09	1.29	1.14	0.10	<0.01	0.245	<0.01		
			LV	Mean	25	116.8	2207.4								1<0.01	63.89	5.12	3.88	13.56	4.040	0.05		
				Std		5.4	405.7								8.57	24.53	3.34	1.62	9.93	4.141	0.05		

Table 4.3: Mean and standard deviation of trace metals concentrations (mg kg^{-1} wet weight) in muscle (FL), gonad (GD) and liver (LV) tissues of *B. ferox* (BFE), blue ling (BLI), blue whiting (BWI), hake (HAK) and orange roughy (ORO) from the North East Atlantic at various depths.

species	location	depth range (m)	organ	statistics	fish analysed	length (cm)	weight (g)	Li	Be	V	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Cd	Hg	Pb	Bi	U
BFE	Meriadzec	1960-2025	FL	Mean	12	65.3	1222.9		uld		0.30	0.27	uld	0.05	0.06	2.75	7.10	0.58	uld	2.176	0.03		uld
				Std		3.6	180.4		0.01		0.30	0.34	<0.01	0.12	0.08	0.90	5.94	0.08	0.01	0.539	0.06		<0.01
			GD	Mean	10	66.1	1247.5		uld		0.22	0.75	uld	uld	0.58	34.25	5.92	1.39	0.66	0.934	0.12		uld
				Std		3.4	189.1		<0.01		0.04	1.38	<0.01	0.01	0.15	11.11	4.79	0.33	2.06	0.347	0.16		<0.01
			LV	Mean	12	65.3	1222.9		uld		0.65	0.13	uld	0.08	0.96	8.81	4.90	2.23	0.36	1.708	uld		uld
				Std		3.6	180.4		<0.01		0.81	0.07	<0.01	0.05	0.65	2.69	4.51	1.17	0.22	0.944	<0.01		<0.01
	Rockall	1960-2025	FL	Mean	9	63.9	1505.0		uld		0.03	0.14	uld	uld	0.07	2.26	6.18	1.68	uld	1.151	0.08		0.06
				Std		1.8	188.9		<0.01		0.03	0.08	<0.01	<0.01	0.03	0.22	1.40	0.46	<0.01	0.300	0.01		<0.01
			GD	Mean	9	63.9	1505.0		uld		uld	0.32	uld	uld	1.53	47.21	2.04	2.12	uld	0.395	0.08		0.06
				Std		1.8	188.9		<0.01		0.01	0.06	<0.01	0.03	2.77	3.37	0.59	0.13	<0.01	0.163	0.03		<0.01
			LV	Mean	9	63.9	1505.0		uld		0.60	0.08	uld	0.06	0.95	11.60	1.83	2.31	0.03	0.318	0.03		uld
				Std		1.8	188.9		<0.01		0.29	0.03	<0.01	0.04	0.23	2.80	0.38	0.54	0.02	0.081	0.02		<0.01
BLI	Rockall	800-900	FL	Mean	15	92.6	3851.8								0.18		7.53		uld		uld		
				Std		20.7	3259.6								0.09		3.21		<0.01		<0.01		
			LV	Mean	15	92.6	3851.8								3.81		5.12		0.33		0.04		
				Std		20.7	3259.6								1.80		7.88		0.47		0.08		
		1000	FL	Mean	11	97.8	3667.3		uld		0.05	0.09	uld	uld	0.29	4.41	4.66	0.29	uld	0.365	0.08		0.06
				Std		11.4	1318.4		<0.01		0.03	0.03	<0.01	<0.01	0.19	1.10	2.73	0.02	<0.01	0.142	0.02		<0.01
			GD	Mean	11	97.8	3667.3		uld		uld	0.14	uld	0.54	0.79	45.20	6.49	0.74	uld	0.116	0.08		0.06
				Std		11.4	1318.4		<0.01		0.04	0.05	<0.01	1.74	0.53	29.42	7.14	0.16	0.01	0.063	0.02		<0.01
			LV	Mean	11	97.8	3667.3		uld		0.26	0.69	uld	uld	6.54	20.54	6.52	1.92	1.07	0.183	0.05		0.03
				Std		11.4	1318.4		<0.01		0.12	0.30	<0.01	0.02	3.11	4.02	3.25	0.47	2.03	0.120	0.04		0.03
		1100-1200	FL	Mean	6	101.2	3750.8		uld		uld	0.09	uld	uld	0.22	4.47	2.91	0.31	uld	0.399	0.08		0.06
				Std		13.3	1799.7		<0.01		0.02	0.05	<0.01	0.01	0.05	0.67	1.91	0.03	<0.01	0.131	0.02		<0.01
			GD	Mean	6	101.2	3750.8		uld		uld	0.23	uld	uld	0.68	42.73	3.58	0.82	uld	0.150	0.08		0.06
				Std		13.3	1799.7		<0.01		0.02	0.12	<0.01	0.01	0.22	23.91	2.04	0.20	<0.01	0.067	0.03		<0.01
			LV	Mean	7	99.4	3542.1		uld		0.54	0.64	uld	0.03	5.53	18.05	5.59	1.78	0.53	0.165	0.03		uld
				Std		12.9	1733.2		<0.01		0.07	0.31	<0.01	0.02	2.18	4.60	2.15	0.49	0.51	0.130	0.02		<0.01

Table 4.3 (continued): Mean and standard deviation of trace metals concentrations (mg kg^{-1} wet weight) in muscle (FL), gonad (GD) and liver (LV) tissues of *B. ferox* (BFE), blue ling (BLI), blue whiting (BWI), hake (HAK) and orange roughy (ORO) from the North East Atlantic at various depths.

species	location	depth range	organ	Statistics	fish analysed	length (cm)	weight (g)	Li	Be	V	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Cd	Hg	Pb	Bi	U
BWH	Rockall	300	FL	Mean	20	26.8	117.5	uld	uld	uld			0.06	11.25	0.45	4.28	3.97	0.53	uld	0.054	0.21	uld	uld
				Std		1.4	23.9	0.01	<0.01	<0.01			0.02	4.13	0.19	0.64	1.56	0.17	0.01	0.030	0.07	<0.01	<0.01
			LV	Mean	20	26.8	117.5	uld	uld	0.08			0.21	15.40	5.42	26.16	8.60	1.90	0.59	uld	0.27	uld	uld
				Std		1.4	23.9	0.01	<0.01	0.04			0.10	6.38	2.87	9.72	3.27	0.79	0.31	0.008	0.09	<0.01	<0.01
		640	FL	Mean	13	32.5	213.5	uld	uld	uld			uld	0.73	0.20	3.53	5.29	0.54	uld	0.088	uld	uld	uld
				Std		2.2	50.9	0.01	<0.01	<0.01			<0.01	0.42	0.05	0.99	1.80	0.15	<0.01	0.044	0.02	0.01	<0.01
			LV	Mean	13	32.5	213.5	uld	uld	0.03			0.05	1.05	6.17	21.98	10.30	0.85	0.56	0.013	uld	uld	uld
				Std		2.2	50.9	0.01	<0.01	0.03			0.03	0.66	3.11	6.49	5.71	0.62	0.58	0.024	0.01	0.01	<0.01
		830	FL	Mean	9	29.2	172.1								0.31		2.47		4.70		uld		
				Std		4.3	78.5								0.10		1.93		13.63		0.01		
			LV	Mean	9	29.2	172.1								4.45		5.96		0.64		uld		
				Std		4.3	78.5								2.34		3.80		0.44		0.02		
HAK	Rockall	640	FL	Mean	11	65.3	2158.5								0.30		1.24		0.03		uld		
				Std		13.9	1631.9								0.11		0.96		0.03		0.02		
			LV	Mean	11	65.3	2158.5								7.61		3.20		0.54		0.04		
				Std		13.9	1631.9								5.13		2.28		0.50		0.04		
ORO	Meriad zec	1290-1300	FL	Mean	11	42.7	1462.3	uld	uld	uld	uld	uld	uld	uld	0.14	1.68	0.60	0.72	uld	0.517	uld	uld	uld
				Std		8.3	789.3	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	0.01	0.02	0.38	0.26	0.21	<0.01	0.369	<0.01	<0.01	<0.01
			GD	Mean	5	46.0	1811.0	uld	uld	0.05	uld	0.18	uld	uld	0.49	99.72	1.25	2.43	0.03	0.298	uld	uld	uld
				Std		7.8	884.6	<0.01	<0.01	0.09	<0.01	0.07	<0.01	<0.01	0.05	22.13	0.92	0.32	0.04	0.560	<0.01	<0.01	0.01
			LV	Mean	11	42.7	1462.3	0.04	uld	0.49	0.03	0.40	0.04	uld	8.32	34.55	3.39	6.50	14.12	5.143	0.05	uld	uld
				Std		8.3	789.3	0.01	<0.01	1.06	0.09	0.14	0.02	<0.01	13.90	7.59	1.05	8.03	17.43	13.192	0.03	<0.01	0.03
	Rockall	1400	FL	Mean	19	28.2	501.6	uld	uld	uld	0.15	0.03	uld	uld	0.09	1.67	0.42	0.49	uld	0.195	uld	uld	uld
				Std		8.4	396.0	0.02	0.01	<0.01	0.11	0.09	<0.01	0.01	0.05	0.36	0.12	0.10	<0.01	0.080	<0.01	<0.01	<0.01
			GD	Mean	8	34.9	806.3	0.04	uld	uld	0.17	0.24	uld	0.05	0.92	59.57	0.71	2.02	uld	0.016	0.05	uld	uld
				Std		4.5	332.7	0.01	<0.01	<0.01	0.14	0.07	0.01	0.10	0.89	22.46	0.14	0.72	0.02	0.026	0.04	<0.01	<0.01
			LV	Mean	17	29.2	545.0	0.05	0.04	0.07	0.49	0.50	uld	0.13	5.71	18.90	3.15	2.37	3.31	0.304	0.03	uld	uld
				Std		8.1	396.2	0.01	0.06	0.02	0.69	0.14	0.02	0.29	2.66	3.66	1.04	0.81	1.49	0.134	0.09	<0.01	<0.01

Table 4.4: Mean and standard deviation of trace metals concentrations (mg kg^{-1} wet weight) in muscle (FL) and liver (LV) tissues of *C. armatus* (COA) and *H. bathybius* (HIB) from the Porcupine Bank at various depths.

species	location	depth range (m)	organ	statistics	fish analysed	length (cm)	weight (g)	Li	Be	V	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Cd	Hg	Pb	Bi	U
COA	Porcupine	2450	FL	Mean	4	431.3	351.3	uld	uld	uld	0.11	0.31	uld	uld	0.35	3.21	16.59	0.73	uld	0.203	uld	uld	uld
				Std		54.1	140.9	0.01	<0.01	<0.01	0.02	0.24	<0.01	<0.01	0.12	0.32	4.90	0.27	0.003	0.071	<0.01	<0.01	<0.01
			LV	Mean	4	431.3	351.3	uld	uld	0.06	0.12	0.90	0.09	uld	7.01	33.66	14.67	3.77	1.882	0.161	0.12	uld	uld
				Std		54.1	140.9	<0.01		0.04	0.03	0.36	0.02	0.01	4.56	9.19	15.21	1.09	1.78	0.063	0.23		
		3100	FL	Mean	10	490.0	512.7	uld	uld	uld	0.13	0.16	uld	uld	0.13	3.38	24.80	0.78	uld	0.299	uld	uld	uld
				Std		69.8	248.1	<0.01	<0.01	<0.01	0.03	0.06	<0.01	0.01	0.04	0.53	7.45	0.30	0.001	0.090	0.01	<0.01	<0.01
			LV	Mean	10	490.0	512.7	0.03	uld	0.08	0.17	0.57	0.06	0.06	4.92	32.87	16.17	5.01	1.570	0.235	0.04	uld	uld
				Std		69.8	248.1	0.01	<0.01	0.03	0.09	0.25	0.02	0.10	2.95	10.33	10.03	3.59	0.909	0.096	0.02	0.01	<0.01
		4000	FL	Mean	10	614.0	1666.5	uld	uld	uld			uld	uld	0.22	6.64	12.70	1.05	0.006	0.814	uld	uld	uld
				Std		243.5	1186.2	0.01	<0.01	<0.01			<0.01	<0.01	0.15	6.04	6.67	0.31	0.013	0.504	<0.01	<0.01	<0.01
			LV	Mean	10	614.0	1666.5	0.03	uld	0.08			0.05	uld	5.29	51.46	20.12	9.80	3.014	1.034	0.04	uld	uld
				Std		243.5	1186.2	0.01	<0.01	0.04			0.04	<0.01	2.47	37.37	13.02	8.23	1.499	0.969	0.08	0.01	<0.01
HIB	Porcupine	2450	FL	Mean	6	689.2	662.5	0.03	uld	uld	0.12	0.18	uld	uld	0.18	2.85	15.79	0.59	0.020	1.240	uld	uld	uld
				Std		174.2	412.1	0.02	<0.01	<0.01	0.04	0.13	<0.01	<0.01	0.05	0.42	9.17	0.22	0.011	0.731	0.01	<0.01	<0.01
			LV	Mean	4	730.0	779.5	0.03	uld	0.06	0.10	0.69	0.03	uld	20.71	29.39	5.08	5.34	3.962	4.422	0.08	uld	uld
				Std		117.5	383.3	0.01	<0.01	0.06	0.07	0.19	0.01	<0.01	12.29	2.96	5.06	2.57	3.545	4.447	0.13	0.01	0.02
		3100	FL	Mean	3	746.7	726.7	uld	uld	uld	0.04	0.05	uld	uld	0.27	3.75	22.98	0.74	0.016	0.861	0.03	uld	uld
				Std		56.9	238.9	<0.01	<0.01	<0.01	<0.01	0.08	<0.01	<0.01	0.14	0.59	4.16	0.18	0.001	0.177	0.02	<0.01	<0.01
			LV	Mean	3	746.7	726.7	0.04	uld	uld	0.04	0.53	0.03	uld	65.69	43.19	4.81	7.05	7.093	1.815	0.04	uld	uld
				Std		56.9	238.9	0.02	<0.01	0.01	0.02	0.15	0.01	<0.01	13.95	1.45	1.58	2.51	4.224	0.451	0.02	<0.01	<0.01
		4000	FL	Mean	2	590.0	294.5	0.05	uld	0.03	0.18	0.36	uld	uld	0.78	3.04	21.26	0.87	0.032	1.654	0.40	uld	uld
				Std		28.3	72.8	0.03	<0.01	0.01	0.01	0.21	<0.01	<0.01	0.14	0.11	8.66	0.14	0.034	0.813	0.55	<0.01	<0.01
			LV	Mean	2	590.0	294.5	0.05	uld	0.05	0.12	0.80	0.04	0.86	33.27	57.67	6.10	7.01	8.628	6.379	0.03	uld	uld
				Std		28.3	72.8	0.04	<0.01	0.01	0.08	0.47	0.01	1.22	11.97	11.61	3.33	2.73	2.158	2.113	0.04	<0.01	0.01

Table 4.5: Mean and standard deviation of trace metals concentrations (mg kg^{-1} wet weight) in muscle (FL) and liver (LV) tissues of kingklip (KIN), *L. vomerinus* (LVO), *M. capensis* (MCA) and *M. paradoxus* (MPA) from South Africa at various depths.

Species	Location	Depth range (m)	organ	statistics	fish analysed	length (cm)	weight (g)	Li	Be	V	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Cd	Hg	Pb	Bi	U
KIN	**South**		**FL**	**Mean**	**8**	40.4	302.5				0.38	0.10	uld	uld	0.52	3.72	1.45	0.27	uld	0.042	uld	0.09	
	Africa			**Std**		5.1	125.3				0.09	0.05	<0.01	<0.01	0.39	0.40	0.27	0.03	0.02	0.025	<0.01	0.12	
			LV	**Mean**	**8**	40.4	302.5				0.30	1.44	uld	uld	14.79	16.01	1.02	0.75	0.72	0.019	uld	0.04	
				Std		5.1	125.3				0.10	0.33	<0.01	<0.01	17.19	4.81	0.12	0.21	0.96	0.013	<0.01	0.08	
LVO	**South**		**FL**	**Mean**	**10**	40.4	1135.8				0.53	0.07	uld	uld	0.43	3.23	2.85	0.39	0.03	0.085	uld	0.09	
	Africa			**Std**		7.8	622.0				0.17	0.04	<0.01	0.04	0.37	1.38	1.19	0.09	0.06	0.045	<0.01	0.10	
			LV	**Mean**	**10**	40.4	1135.8				0.57	0.51	uld	0.12	13.77	18.77	1.75	1.04	1.85	0.075	uld	0.09	
				Std		7.8	622.0				0.44	0.14	0.01	0.31	6.88	3.31	0.30	0.26	1.49	0.025	0.05	0.09	
MCA	**South**		**FL**	**Mean**	**13**	36.5	479.0	uld	uld	uld	0.80	0.13	uld	0.03	0.28	2.63	1.15	0.30	uld	0.036	uld	uld	uld
	Africa			**Std**		12.2	493.0	0.01	<0.01	<0.01	0.25	0.06	<0.01	0.09	0.08	0.52	0.36	0.06	0.03	0.053	0.06	0.02	<0.01
			LV	**Mean**	**13**	36.5	479.0	0.03	uld	0.04	0.68	3.42	uld	0.05	8.11	21.90	1.64	0.60	0.51	0.033	uld	uld	uld
				Std		12.2	493.0	0.02	<0.01	0.03	0.21	1.38	0.03	0.10	5.13	5.29	0.25	0.13	0.30	0.050	0.01	<0.01	<0.01
MPA	**South**		**FL**	**Mean**	**11**	42.5	732.3	uld	uld	uld	0.96	0.13	uld	uld	0.24	2.47	1.25	0.26	uld	0.127	uld	uld	uld
	Africa			**Std**		13.4	894.6	0.01	<0.01	0.01	0.13	0.06	<0.01	0.02	0.06	0.41	0.56	0.06	0.02	0.168	0.01	<0.01	<0.01
			LV	**Mean**	**11**	42.5	732.3	uld	uld	0.03	0.70	2.42	0.05	0.07	4.01	20.73	2.37	0.69	0.93	0.054	uld	uld	uld
				Std		13.4	894.6	0.01	<0.01	0.03	0.30	1.02	0.03	0.04	1.70	7.50	0.38	0.24	0.93	0.105	<0.01	<0.01	<0.01

4.1.1. Lithium (fig 4.1)

Most individual samples presented lithium concentrations below the limit of detection (0.047mg kg^{-1} wet weight), as were most mean lithium concentrations when grouped by species, location and depth of capture. All South African samples contained lithium concentrations below the limit of detection, as did all *B. ferox*, blue ling and hake samples, all but two blue whiting samples and all but three black scabbardfish samples. The only groups in which lithium concentrations were commonly above the limit of detection were monkfish in shallow waters (80 to 125m depth), orange roughy, *H. bathybius* and roundnose grenadier, all sampled at depths greater than 1200m.

All but two groups presented mean concentration under the limit of detection for muscle tissue, *H. bathybius* from 4000m in the Porcupine Bank (0.05±0.03mg kg^{-1}; as mean±standard deviation throughout the chapter) and roundnose grenadier at 1250 to 1450m depth in the Rockall Trough (respectively 0.06±0.03 and 0.14±0.13mg kg^{-1} wet weight). One group presented mean lithium concentrations in gonad above the limit of detection, namely monkfish at 80 to 125m (0.06± 0.02mg kg^{-1} wet weight). Two groups presented mean lithium concentrations in livers above the limit of detection, namely orange roughy caught at 1400m in the Rockall Trough (0.05±0.01mg kg^{-1}), and in *H. bathybius* caught at 4000m (0.05±0.04mg kg^{-1} wet weight) from the Porcupine Bank.

Orange roughy was the only species presenting significantly lower concentrations of lithium in muscle than liver tissue ($p=0.014$). Monkfish presented decreasing lithium concentration in gonad tissue with depth ($p=0.001$) but not in muscle and liver tissues ($p>0.05$).

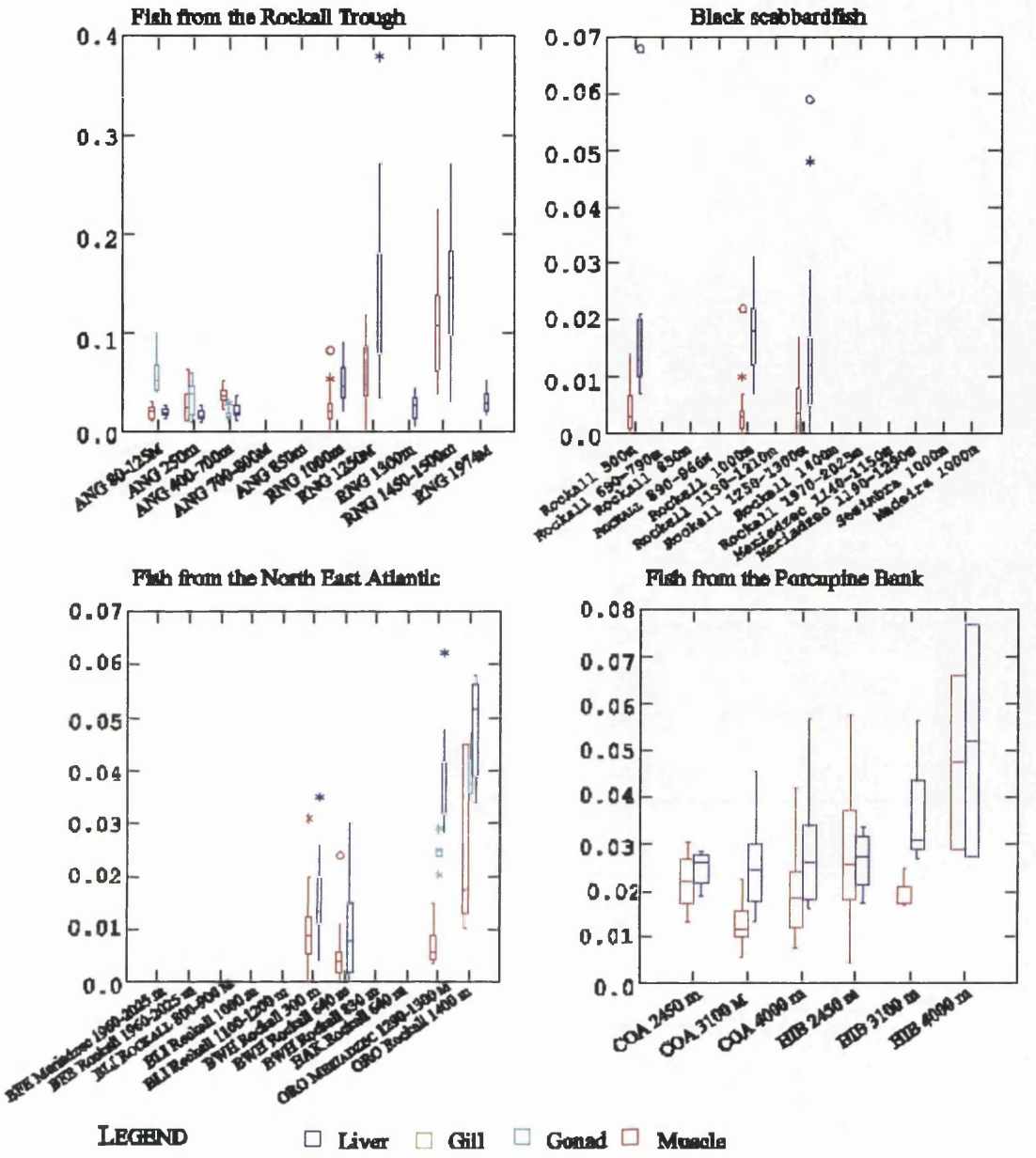


Fig 4.1: Li distribution in muscle, gill, gonad and liver tissues of monkfish (ANG), *B. ferox* (BFE), blue ling (BLI), blue whiting (BWI), *H. bathybius* (HIB), orange roughy (ORO) and roundnose grenadier (RNG) at various locations and depths, expressed in $mg.kg^{-1}$ wet weight (limit of detection $0.047mg.kg^{-1}$ wet weight).

4.1.2. Beryllium (fig 4.2)

All mean beryllium concentrations were below the limit of detection (0.038mg kg^{-1} wet weight), and only a handful of samples presented concentrations higher than the limit of detection, three monkfish liver tissue and one muscle tissue, a few orange roughy livers and one liver of a *C. armatus*. These data will not be considered further.

4.1.3. Vanadium (fig 4.3)

Mean vanadium concentrations were above the limit of detection in livers of black scabbardfish, blue whiting, orange roughy, *C. armatus, H. bathybius, M. capensis, M. paradoxus* and roundnose grenadier. Apart from these species, only monkfish presented some livers with vanadium concentrations above limit of detection, and was the only species presenting any gonad tissue with vanadium concentrations above the limit of detection. All muscle and most gonad tissue analysed for all species presented low vanadium levels, well below the limit of detection.

Blue whiting, orange roughy, *C. armatus*, *H. bathybius, M. capensis* and *M. paradoxus* presented very low levels of vanadium in liver, with mean concentrations ranging from 0.03 to 0.08mg kg^{-1} wet weight. Roundnose grenadier presented intermediate vanadium concentrations, typically 0.1-0.2mg kg^{-1} wet weight throughout the depth range (1000-2000m). Black scabbardfish presented much higher concentrations, with mean values in liver tissues between 1.27 and 1.61mg kg^{-1} wet weight. Black scabbardfish presented similar vanadium concentrations and ranges throughout the sampling depth ranges, i.e. from 500 to 1300m depth.

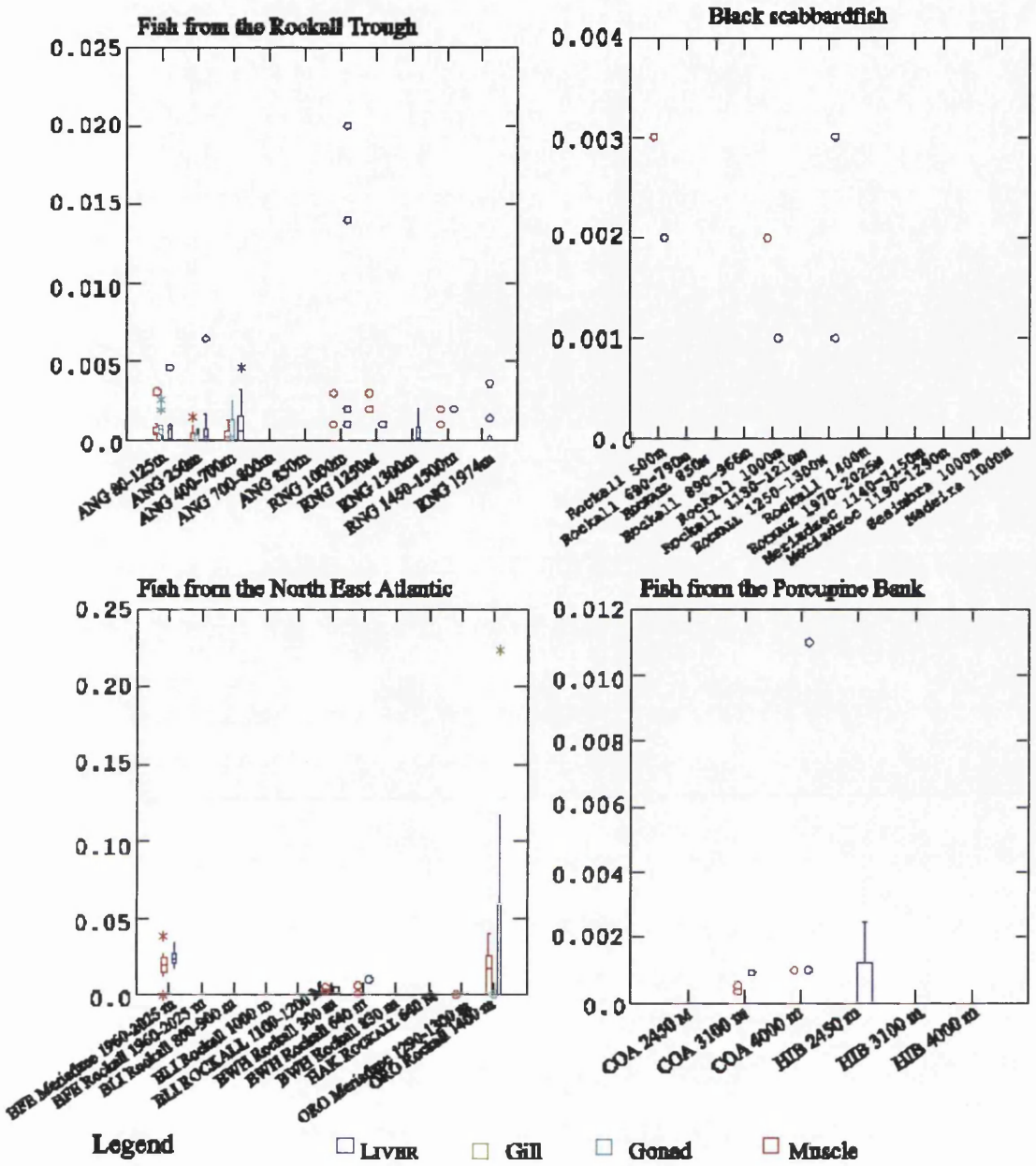


Fig 4.2: Be distribution in muscle, gill, gonad and liver tissues of monkfish (ANG), *B. ferox* (BFE), blue ling (BLI), blue whiting (BWI), *H. bathybius* (HIB), orange roughy (ORO) and roundnose grenadier (RNG) at various locations and depths, expressed in $mg.kg^{-1}$ wet weight (limit of detection $0.047mg.kg^{-1}$ wet weight).

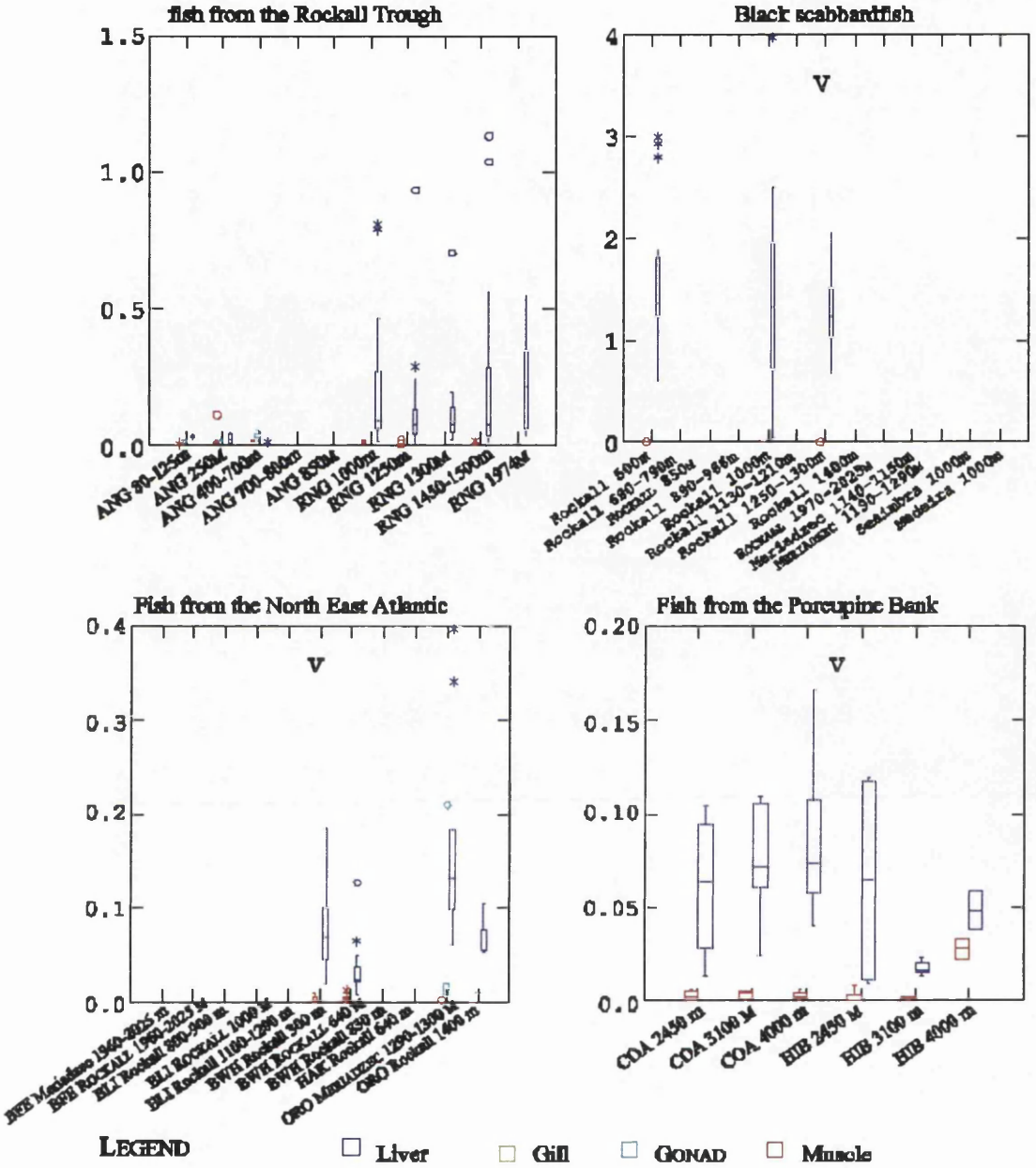


Fig 4.3: V distribution in muscle, gill, gonad and liver tissues of monkfish (ANG), *B. ferox* (BFE), blue ling (BLI), blue whiting (BWI), *H. bathybius* (HIB), orange roughy (ORO) and roundnose grenadier (RNG) at various locations and depths, expressed in $mg.kg^{-1}$ wet weight (limit of detection $0.047mg.kg^{-1}$ wet weight).

4.1.4. Chromium (fig 4.4)

All species presented low chromium levels, with most samples containing <1mg kg^{-1} wet weight, and only a few outliers above 1mg kg^{-1} wet weight. The highest single concentration was found in muscle tissue of a monkfish caught at 250m depth in the Rockall Trough, presenting a chromium concentration of ca 4.5mg kg^{-1} wet weight (which was not plotted on fig 4.4 to avoid distorting the scale of the figure).

Concentration ranges were similar in all organs in each individual group, showing no accumulation in a specific organ. In a similar way, no trend was significant with depth, as concentration ranges overlapped ($p>0.05$).

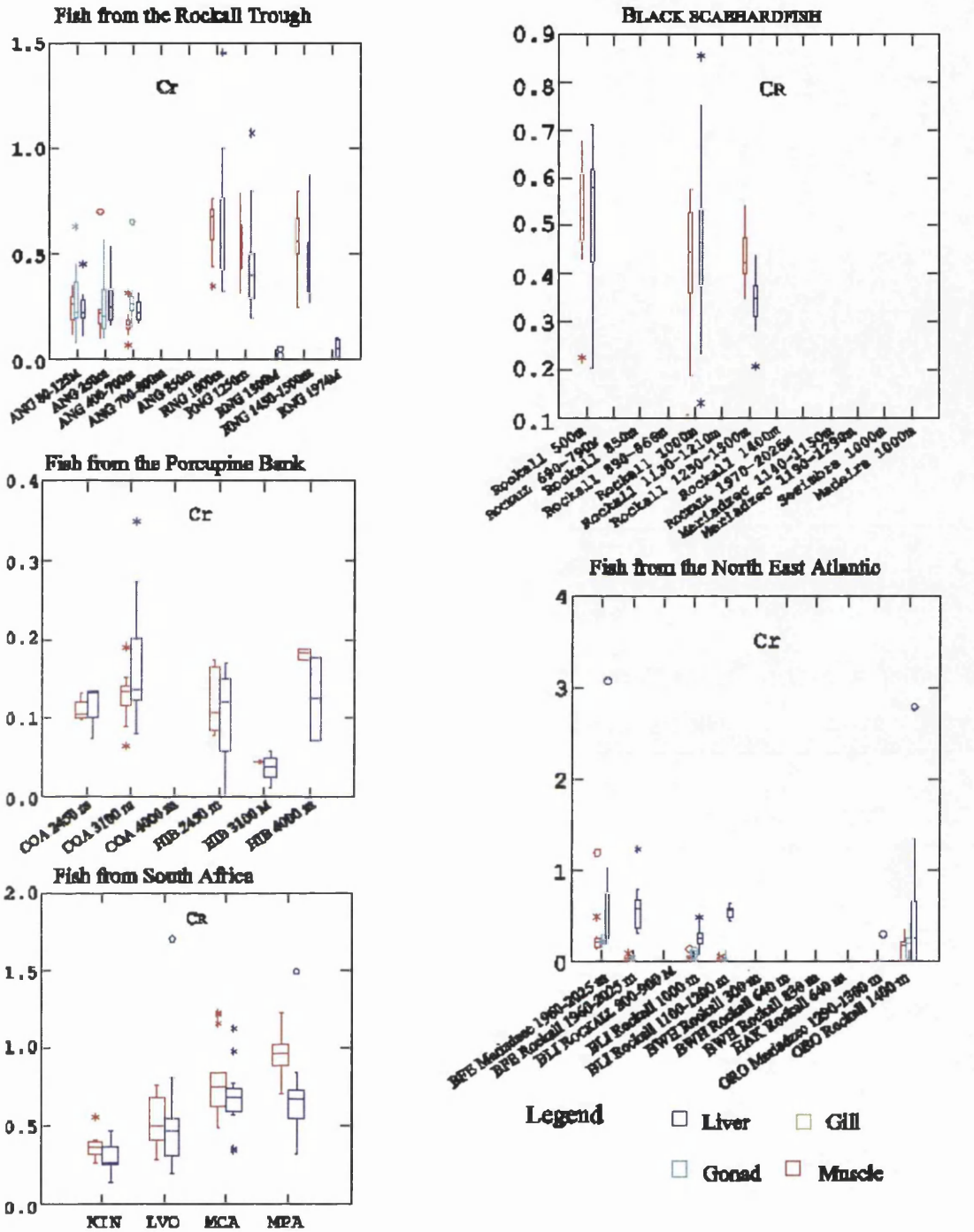


Fig 4.4: Cr distribution in muscle, gill, gonad and liver tissues of monkfish (ANG), *B. ferox* (BFE), blue ling (BLI), blue whiting (BWI), *H. bathybius* (HIB), kingklip (KIN), *L. vomerinus* (LVO), *M capensis* (MCA), *M. paradoxus* (MPA), orange roughy (ORO) and roundnose grenadier (RNG) at various locations and depths, expressed in $mg.kg^{-1}$ wet weight (limit of detection $0.047mg.kg^{-1}$ wet weight).

4.1.5. Manganese (fig 4.5)

Mean manganese concentrations in muscle tissue were typically within the 0.03-0.3mg kg^{-1} wet weight range. The highest manganese concentrations were generally found in liver tissues, with highest means in species sampled in South Africa (ca 3mg kg^{-1} wet weight) followed by black scabbardfish (ca 2mg kg^{-1} wet weight). All other species presented mean manganese concentrations in livers below 1mg kg^{-1} wet weight. In all fish species, apart from monkfish and *B. ferox*, manganese levels were much lower in muscle than in gonad, than in liver tissues, by up to a factor of ten ($p<0.005$ for all species).

In monkfish from 80 to 125m depth however, the mean and range of manganese concentrations were significantly higher ($p<0.005$) in liver (0.66±0.17mg kg^{-1} wet weight) than gonad than gill tissue (respectively 0.28±0.17 and 0.07±0.05mg kg^{-1} wet weight). At 250m depth, all three organs sampled presented a similar range of manganese concentrations (ca 0.25mg kg^{-1} wet weight), lower than at 80 to 125m depth. At the 400 to 700m depth range, the mean and range of manganese concentrations dropped in liver tissues to 0.07±0.01mg kg^{-1} wet weight, but did not change in muscle and gonad tissues (ca 0.26mg kg^{-1} wet weight).

In *B. ferox*, the mean manganese concentration was similar in gonad, muscle and liver ($p>0.05$). Similar patterns and ranges were found from both Rockall Trough and Meriadzec sampling sites (both at ca 2000m depth) and differences between the two sampling sites were not significant ($p>0.05$).

Apart from monkfish and roundnose grenadier, no significant trend was found with sampling depths or location within species, as manganese concentrations covered similar ranges in single species for various locations and depths ($p>0.05$ in all cases). Monkfish presented decreasing manganese concentrations in liver with depth but increasing in muscle tissue ($p<0.001$ in both cases). Roundnose grenadier presented decreasing concentrations in both muscle and liver tissue with depth ($p=0.001$).

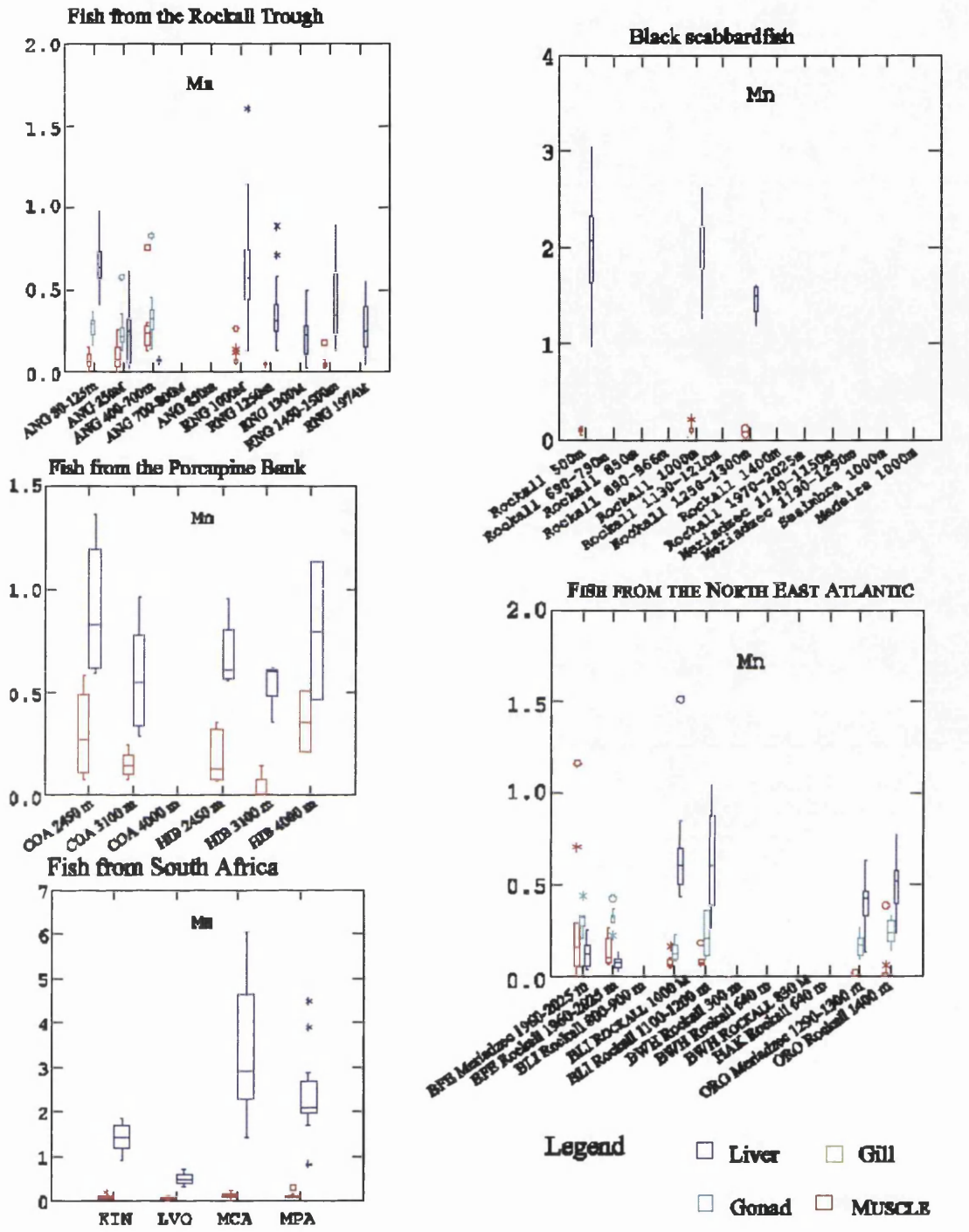


Fig 4.5: Mn distribution in muscle, gill, gonad and liver tissues of monkfish (ANG), *B. ferox* (BFE), blue ling (BLI), blue whiting (BWI), *H. bathybius* (HIB), kingklip (KIN), *L. vomerinus* (LVO), *M capensis* (MCA), *M. paradoxus* (MPA), orange roughy (ORO) and roundnose grenadier (RNG) at various locations and depths, expressed in $mg.kg^{-1}$ wet weight (limit of detection $0.047mg.kg^{-1}$ wet weight).

4.1.6. Cobalt (fig 4.6)

Most mean cobalt concentrations were below the limit of detection (0.032mg kg^{-1} wet weight). All samples of gonad tissue analysed presented concentrations below the limit of detection, as did most muscle tissue samples. Only monkfish caught in the Rockall Trough at 250m depth presented a mean concentration of cobalt in muscle tissue above the limit of detection (2.23±6.65mg kg^{-1} wet weight). Concentrations were higher in liver than muscle tissue in all species ($p<0.05$ in all cases).

All concentrations in *B ferox*, blue ling, hake, kingklip, *L. vomerinus* and roundnose grenadier were below limits of detection for all organs. Maximum mean cobalt concentrations in livers were found in blue whiting at 300m depth (0.21±0.1mg kg^{-1} wet weight), then black scabbardfish and *C. armatus* at ca 0.09mg kg^{-1} wet weight, then *H. bathybius* and *M. paradoxus* at ca 0.05mg kg^{-1} wet weight.

The concentration of cobalt in monkfish gonad tissue increased with depth, but decreased in liver ($p<0.001$ in both cases). *C. armatus* also showed decreasing mean cobalt concentration in liver with depth ($p=0.018$), from 0.09 to 0.05mg kg^{-1} wet weight between 250 and 4000m depth; as did blue whiting ($p<0.001$), with mean cobalt concentrations of 0.21±0.1 and 0.05±0.03mg kg^{-1} wet weight at 300 and 600m depth respectively. However, black scabbardfish, roundnose grenadier and *H. bathybius* did not show the same pattern, with relatively constant concentrations and ranges in liver tissue throughout the sampling depths ($p>0.05$).

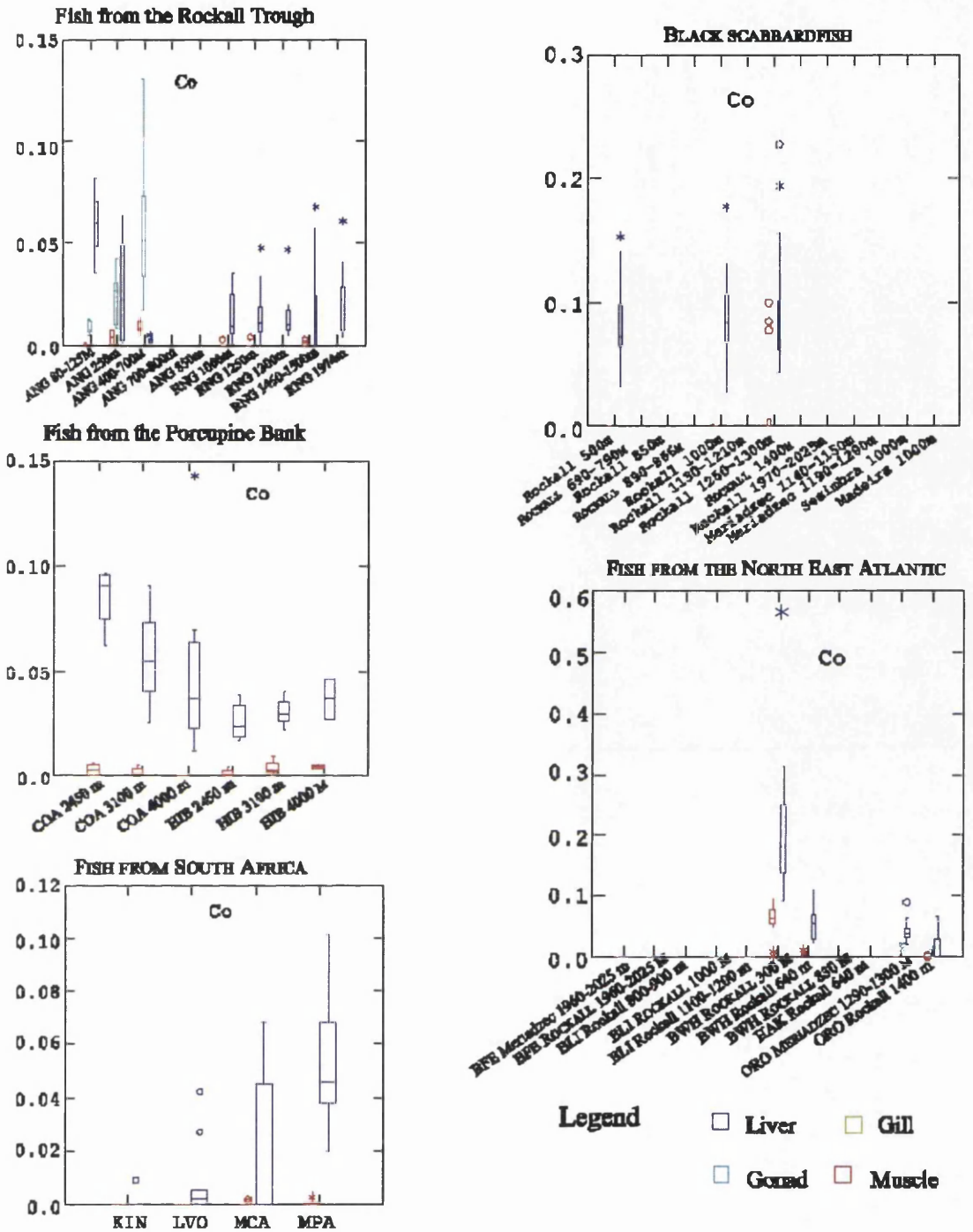


Fig 4.6: Co distribution in muscle, gill, gonad and liver tissues of monkfish (ANG), *B. ferox* (BFE), blue ling (BLI), blue whiting (BWI), *H. bathybius* (HIB), kingklip (KIN), *L. vomerinus* (LVO), *M capensis* (MCA), *M. paradoxus* (MPA), orange roughy (ORO) and roundnose grenadier (RNG) at various locations and depths, expressed in $mg.kg^{-1}$ wet weight (limit of detection 0.047$mg.kg^{-1}$ wet weight).

4.1.7. Nickel (fig 4.7)

Most samples contained very low levels of nickel, with a few higher mean values due to high concentration outliers, such as in the case of black scabbardfish (with a highest value of ca 18mg kg^{-1} wet weight), monkfish and *H. bathybius*. Apart from these cases, all fish species presented mean nickel concentration under the limit of detection for muscle and gonad tissue, and in most species for liver tissues as well.

L. vomerinus, *M. capensis*, *M. paradoxus*, *C. armatus*, *B. ferox*, blue ling and orange roughy all presented mean nickel concentrations in liver tissues below 0.1mg kg^{-1}. Roundnose grenadier presented mean nickel concentrations in liver tissues of ca 0.1mg kg^{-1} wet weight. Black scabbardfish presented mean nickel concentrations in liver tissues between ca 0.1 and 0.4mg kg^{-1} wet weight. In all species, liver presented higher concentration than muscle tissue ($p<0.05$ in all cases).

Blue whiting and roundnose grenadier presented nickel levels in muscle and liver tissues increasing with depth ($p<0.001$). For example, the mean nickel concentration in blue whiting at 300m depth was respectively 11.25±4.13 and 15.40±6.30mg kg^{-1} wet weight in muscle and liver tissues, and was respectively 0.73±0.42 and 1.05±0.66mg kg^{-1} wet weight in muscle and liver tissues at 640m depth. Black scabbardfish presented increasing levels of nickel with depth ($p<0.001$), and also higher concentrations in Meriadzec than the Rockall Trough ($p<0.001$). On the other hand, orange roughy presented the opposite trend, with higher nickel concentrations in the Rockall Trough than in Meriadzec ($p=0.005$).

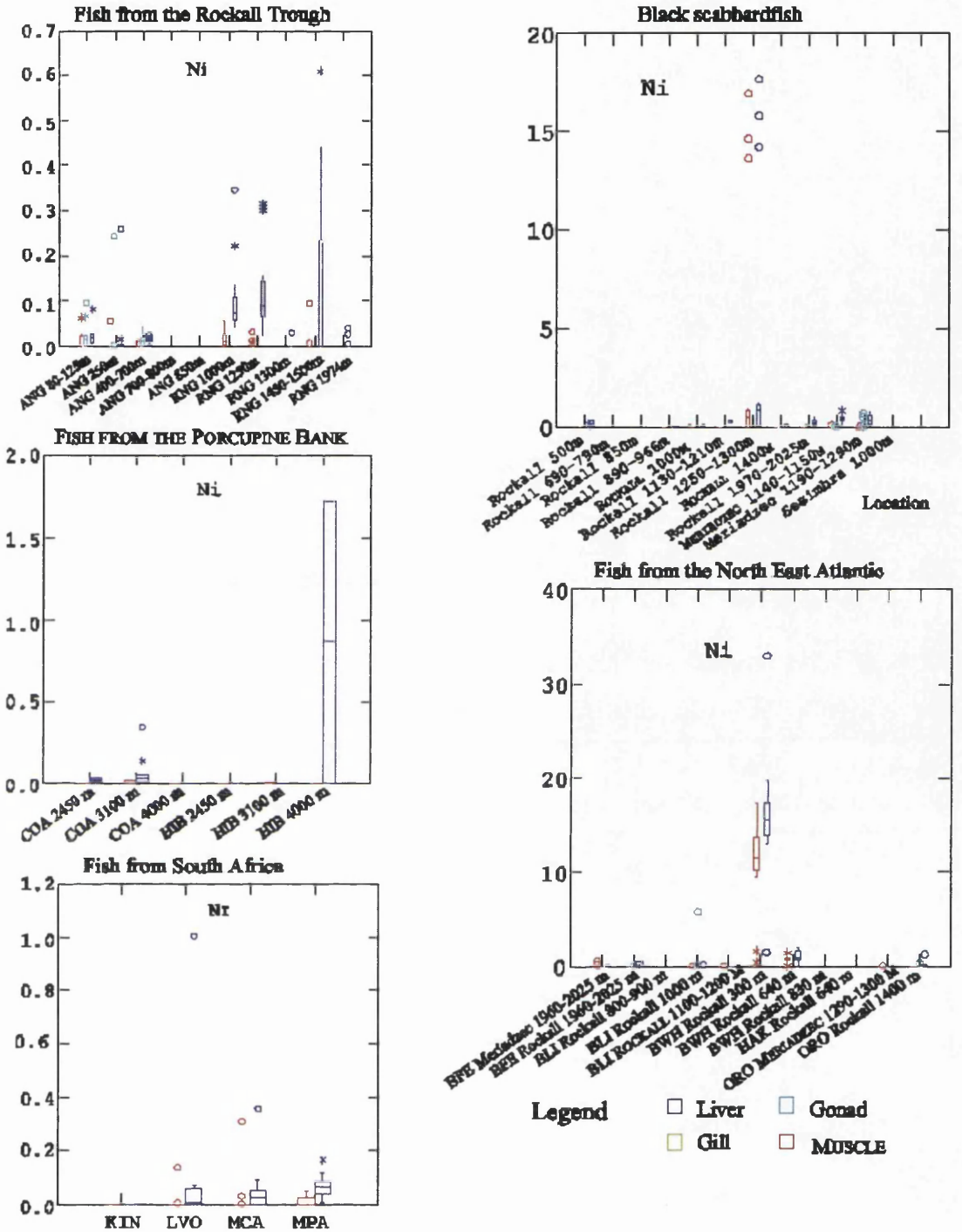


Fig 4.7: Ni distribution in muscle, gill, gonad and liver tissues of monkfish (ANG), *B. ferox* (BFE), blue ling (BLI), blue whiting (BWI), *H. bathybius* (HIB), kingklip (KIN), *L. vomerinus* (LVO), *M capensis* (MCA), *M. paradoxus* (MPA), orange roughy (ORO) and roundnose grenadier (RNG) at various locations and depths, expressed in mg.kg^{-1} wet weight (limit of detection 0.047mg.kg^{-1} wet weight).

4.1.8. Copper (fig 4.8)

All fish species at all sampling sites and depths presented mean copper concentrations in muscle tissue below 0.5mg kg^{-1} wet weight, up to 1.5mg kg^{-1} wet weight in gonad tissue, and substantially higher concentrations in liver tissues (as high as 65mg kg^{-1} wet weight in the case of *H. bathybius*). Copper ranges in liver tissues were large in most species.

B. ferox presented the lowest copper mean concentration in livers, with ca 1.5mg kg^{-1} wet weight at both locations and about 2000m depth. Roundnose grenadier presented mean copper concentrations in liver between 1.5 and 6mg kg^{-1} wet weight. Monkfish, blue ling, blue whiting, hake, orange roughy, *C. armatus*, *H. bathybius*, *M. capensis* and *M. paradoxus* all presented mean copper concentrations in liver between 5 and 8mg kg^{-1}, with overlapping ranges. Kingklip and *L. vomerinus* showed higher mean copper concentrations in liver tissues of about 14mg kg^{-1} wet weight. Black scabbardfish presented mean copper concentrations in livers ranging between 10 and 25mg kg^{-1} wet weight with large overlapping concentration ranges and maximum values up to 60mg kg^{-1} wet weight.

H. bathybius, black scabbardfish, monkfish and roundnose grenadier presented increasing concentrations of copper with depth ($p<0.001$ in all cases).

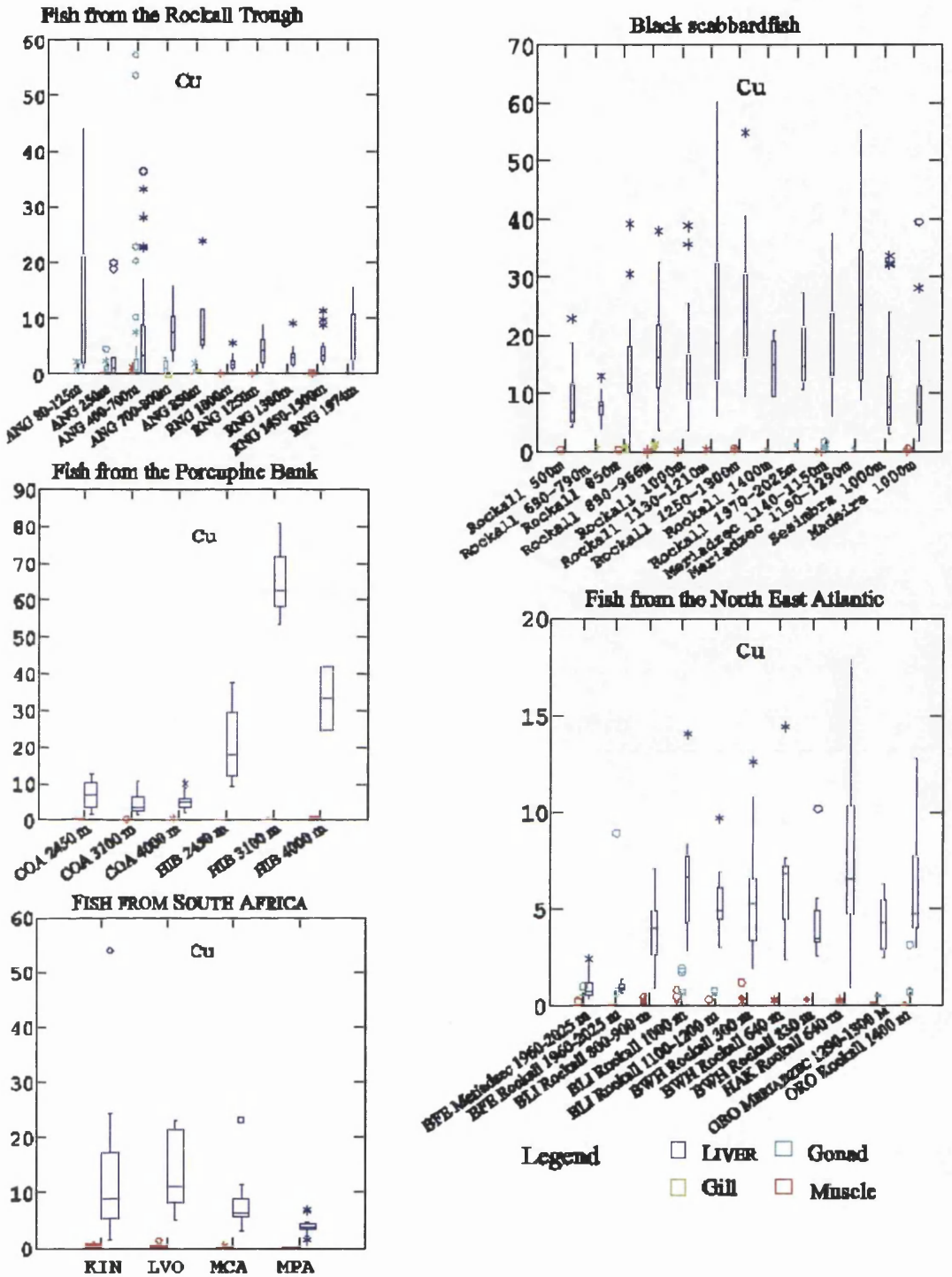


Fig 4.8: Cu distribution in muscle, gill, gonad and liver tissues of monkfish (ANG), *B. ferox* (BFE), blue ling (BLI), blue whiting (BWI), *H. bathybius* (HIB), kingklip (KIN), *L. vomerinus* (LVO), *M capensis* (MCA), *M. paradoxus* (MPA), orange roughy (ORO) and roundnose grenadier (RNG) at various locations and depths, expressed in $mg.kg^{-1}$ wet weight (limit of detection $0.047mg.kg^{-1}$ wet weight).

4.1.9. Zinc (fig 4.9)

The lowest levels of zinc were found in muscle tissue, with mean concentrations never exceeding 5mg kg^{-1} wet weight in any fish species and significantly lower than found in liver ($p<0.05$ in all cases). Gill tissue also presented lower zinc concentrations than livers in black scabbardfish ($p<0.001$), at ca 13mg kg^{-1} wet weight. All species presented higher zinc concentrations in gonad than liver tissues ($p<0.05$), with the most striking example in the case of *B. ferox*, which presented mean zinc concentrations of ca 40mg kg^{-1} wet weight in gonad and 9mg kg^{-1} wet weight in liver tissue ($p<0.001$). Lowest mean zinc concentrations in liver (ca 9mg kg^{-1} wet weight) were found in *B. ferox*, then in fish sampled from South Africa (between 16 and 20mg kg^{-1} wet weight). Mean zinc concentrations in *H. bathybius*, *C. armatus*, orange roughy, blue whiting and roundnose grenadier ranged between 25 and 50mg kg^{-1} wet weight, with a large spread of values.

Black scabbardfish presented the highest mean zinc concentrations in livers and gonads, ranging respectively from ca 60 to 90 and from ca 85 to 120mg kg^{-1} wet weight, and increasing with depth ($p=0.002$). *H. bathybius* also presented zinc levels in livers increasing with depth ($p<0.001$), with mean concentrations at ca 30, 43 and 58mg kg^{-1} wet weight at respectively 2500, 3100 and 4000m depth. However, *C. armatus*, which was sampled at the same time and depths as *H. bathybius*, did not show such a trend, but presented similar mean concentrations and large scatter of zinc concentrations at the various sampling depths ($p>0.05$). Monkfish also presented increasing zinc levels in muscle tissue with depth ($p<0.001$), the opposite trend with depth in liver ($p<0.001$) and no trend in gonad tissue ($p>0.05$).

Orange roughy presented significantly higher levels of zinc in livers and gonads in individuals sampled in Meriadzec than in fish obtained from the Rockall Trough ($p<0.001$), with mean concentrations in gonad of ca 100 and 60mg kg^{-1} wet weight respectively; and, in livers, ca 35 and 20mg kg^{-1} wet weight respectively. Black scabbardfish also presented a similar trend, with higher zinc concentrations in Meriadzec than at Rockall ($p=0.001$) but similar concentrations at Rockall, Madeira and Sesimbra ($p>0.05$).

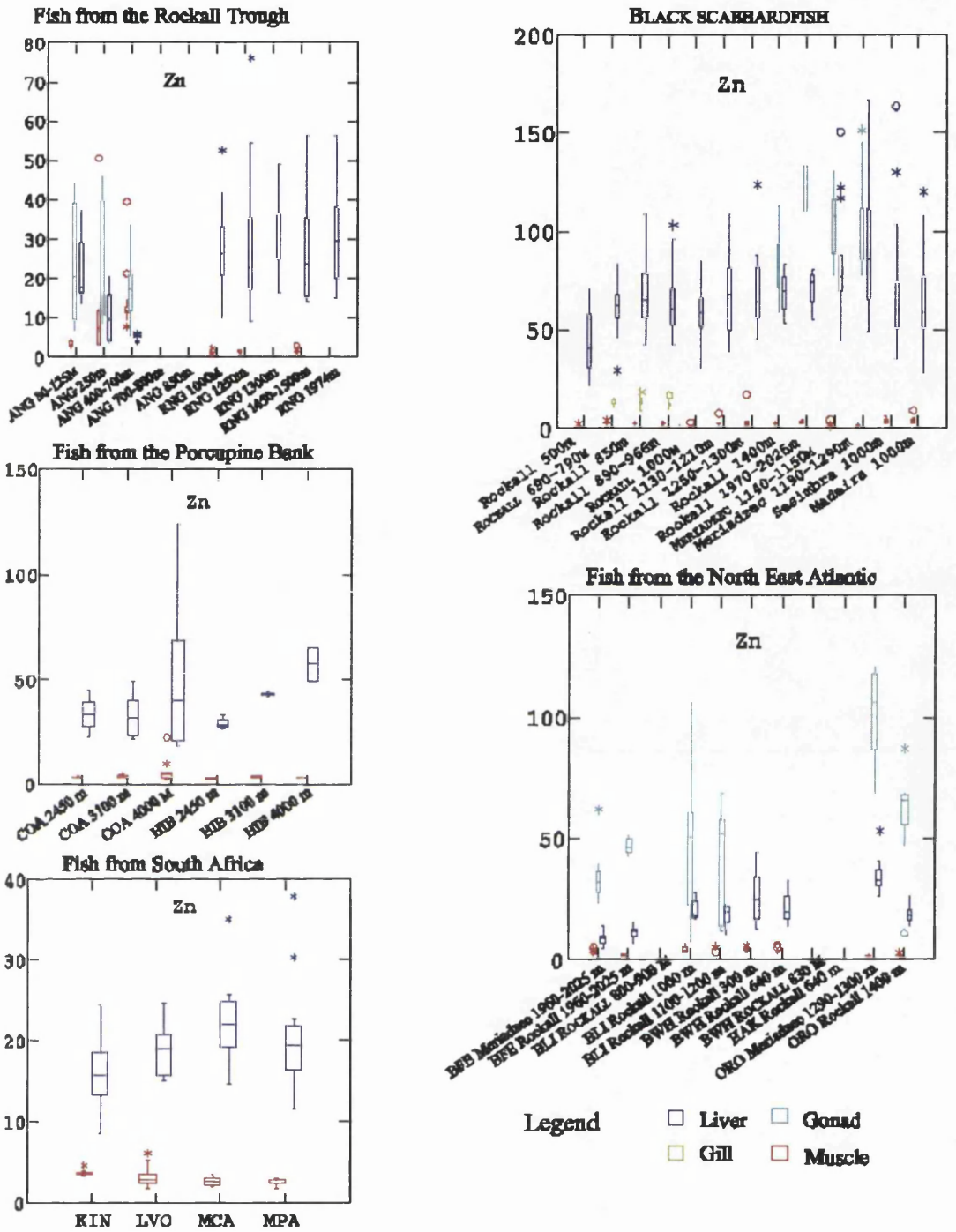


Fig 4.9: Zn distribution in muscle, gill, gonad and liver tissues of monkfish (ANG), *B. ferox* (BFE), blue ling (BLI), blue whiting (BWI), *H. bathybius* (HIB), kingklip (KIN), *L. vomerinus* (LVO), *M capensis* (MCA), *M. paradoxus* (MPA), orange roughy (ORO) and roundnose grenadier (RNG) at various locations and depths, expressed in $mg.kg^{-1}$ wet weight (limit of detection $0.047 mg.kg^{-1}$ wet weight).

4.1.10. Arsenic (fig 4.10)

Highest arsenic levels were found in muscle tissue of *H. bathybius* and muscle and liver tissue of *C. armatus*, which presented mean arsenic concentrations between 15 and 25mg kg^{-1} wet weight. Liver tissue of *H. bathybius* presented much lower arsenic concentrations, typically between 5 and 6mg kg^{-1} wet weight. Other species showed mean arsenic concentrations in muscle and liver tissue between those two ranges. Gonad and gill tissues of all species showed mean arsenic concentrations between 1 and 2mg kg^{-1} wet weight.

Patterns between organs differed from species to species. For example, kingklip and *L. vomerinus* showed higher arsenic levels in muscle than liver tissue, whilst *M. capensis* and *M. paradoxus* showed the opposite trend ($p<0.001$ in all cases), all fish having been sampled in South Africa at similar depths, and all presenting arsenic mean concentrations between 1 and 2mg kg^{-1} wet weight. In the North East Atlantic, orange roughy showed higher arsenic levels in livers than gonad than muscle tissue, as did black scabbardfish, blue whiting and roundnose grenadier ($p<0.001$ in all cases). However, monkfish, *H. bathybius* and *B. ferox* showed higher arsenic levels in muscle than liver tissue ($p<0.005$ in all cases).

Arsenic concentrations in gonad tissue in monkfish showed no trend with depth ($p>0.05$), with mean arsenic concentrations ranging from 7 to 10 and 1.5 to 3.5mg kg^{-1} wet weight respectively, with large variances. However, arsenic concentrations in liver tissue decreased with depth, and increased in muscle tissue with depth ($p<0.001$ in both cases). Blue whiting presented a similar pattern in arsenic concentrations in both muscle and liver tissues, increasing between 300 and 640m depth, then decreasing at 830m depth.

Black scabbardfish and roundnose grenadier did not present such a pattern with depth ($p>0.05$). Arsenic concentrations in black scabbardfish livers were higher in the Rockall Trough and Meriadzec than in Madeira and Sesimbra ($p<0.001$), with no significant differences between Rockall and Meriadzec or Madeira and Sesimbra ($p>0.05$). *B. ferox* presented higher concentrations of arsenic in Meriadzec than the Rockall Trough ($p=0.007$).

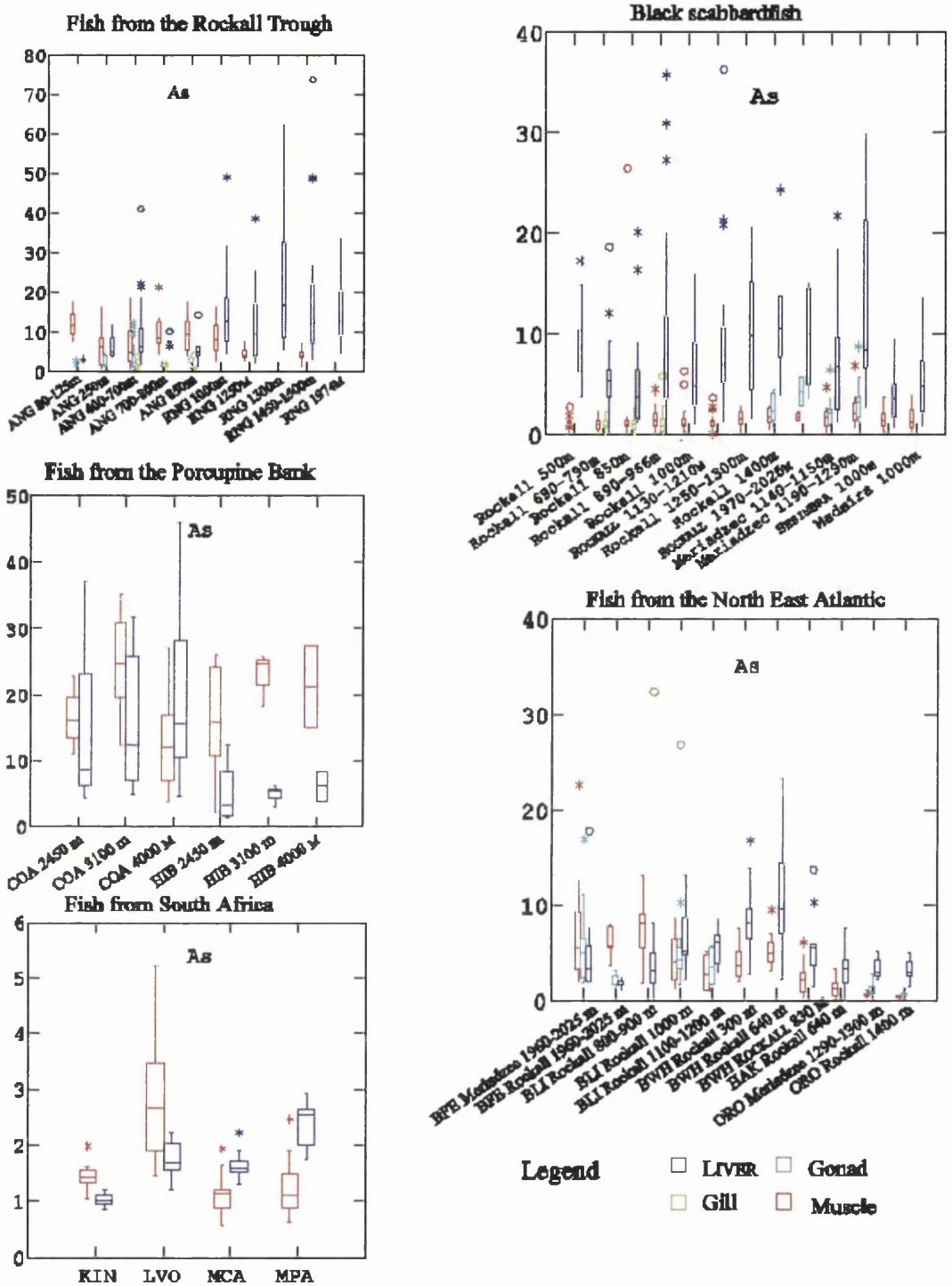


Fig 4.10: As distribution in muscle, gill, gonad and liver tissues of monkfish (ANG), *B. ferox* (BFE), blue ling (BLI), blue whiting (BWI), *H. bathybius* (HIB), kingklip (KIN), *L. vomerinus* (LVO), *M capensis* (MCA), *M. paradoxus* (MPA), orange roughy (ORO) and roundnose grenadier (RNG) at various locations and depths, expressed in $mg.kg^{-1}$ wet weight (limit of detection $0.047mg.kg^{-1}$ wet weight).

4.1.11. Selenium (fig 4.11)

Mean selenium concentrations ranged between ca 0.3 and 0.7mg kg^{-1} wet weight in muscle tissue, between ca 0.7 and 2mg kg^{-1} wet weight in gonad tissue, and between ca 0.4 and 9mg kg^{-1} wet weight in liver tissue, with the highest levels in *C. armatus* and *H. bathybius*. Apart from monkfish, all species showed lower selenium levels in muscle than gonad, than liver tissue ($p<0.001$ in all cases).

In monkfish gonad, selenium levels increased with depth ($p=0.001$), from ca 0.7 to 1.2mg kg^{-1} wet weight between 80 and 700m depth; and decreased in liver with depth ($p<0.001$), from ca 2 to 0.4mg kg^{-1} wet weight over the same depth range. Selenium levels in blue whiting liver tissue decreased with depth ($p=0.015$), from ca 2 to 0.8mg kg^{-1} wet weight between 300 and 640m depth, whilst remaining constant in muscle tissue at ca 0.5mg kg^{-1} wet weight ($p>0.05$). Selenium levels in livers of black scabbardfish, *C. armatus* and *H. bathybius* also increased with depth ($p<0.001$, 0.019 and 0.044 respectively).

Black scabbardfish sampled in the Rockall Trough presented lower selenium concentrations than the same species sampled at Meriadzec ($p<0.001$), respectively between ca 2.5 and 3mg kg^{-1} wet weight, and between ca 3.3 and 3.9mg kg^{-1} wet weight mean selenium concentrations. Fish sampled in Sesimbra presented lower levels than in Madeira ($p<0.001$), which presented levels similar to those at Meriadzec ($p>0.05$). Orange roughy also presented higher selenium concentrations in Meriadzec than in the Rockall Trough ($p<0.001$). However, *B. ferox* did not show a similar pattern ($p>0.05$).

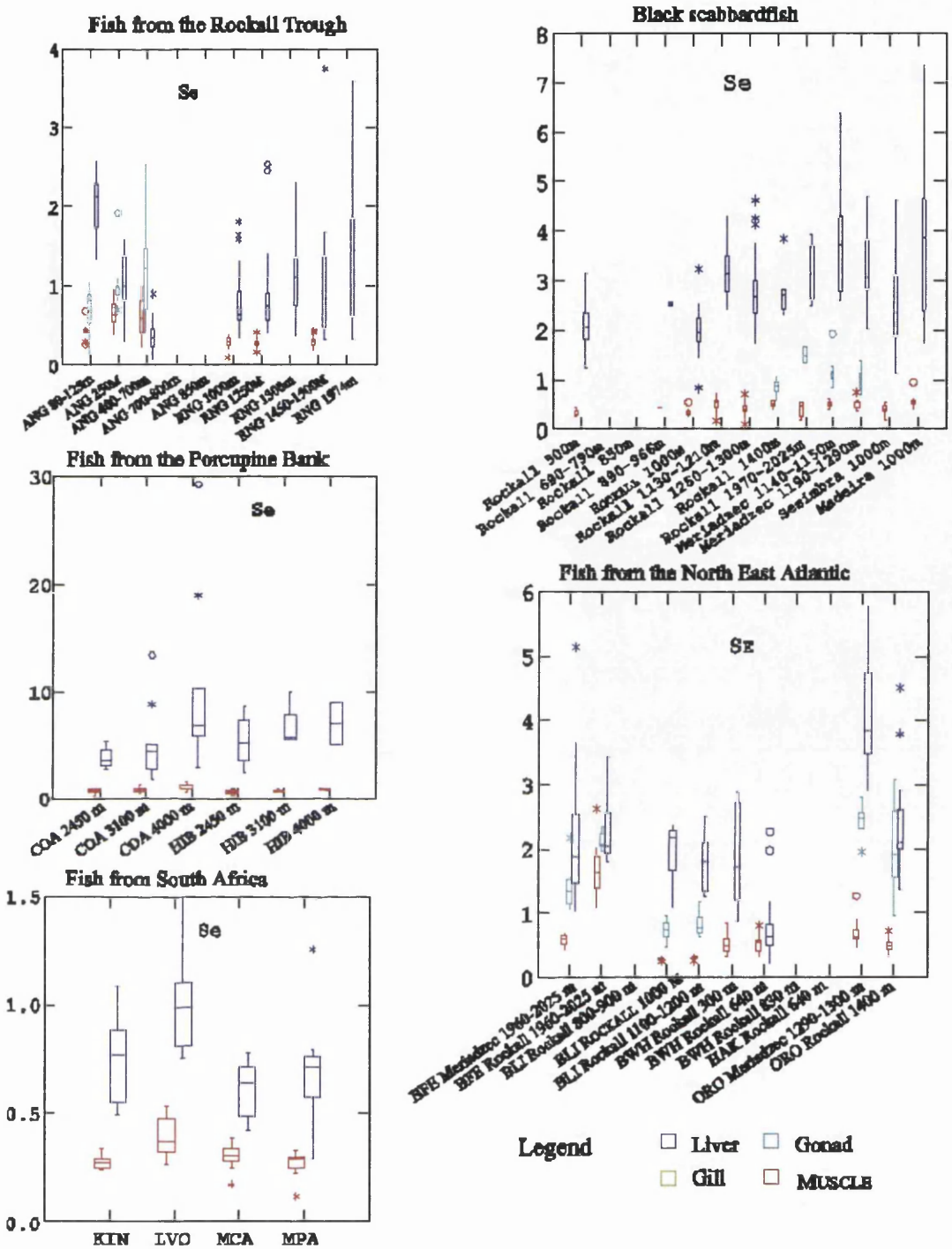


Fig 4.11: Se distribution in muscle, gill, gonad and liver tissues of monkfish (ANG), *B. ferox* (BFE), blue ling (BLI), blue whiting (BWI), *H. bathybius* (HIB), kingklip (KIN), *L. vomerinus* (LVO), *M capensis* (MCA), *M. paradoxus* (MPA), orange roughy (ORO) and roundnose grenadier (RNG) at various locations and depths, expressed in $mg.kg^{-1}$ wet weight (limit of detection $0.047mg.kg^{-1}$ wet weight).

4.1.12. Cadmium (fig 4.12)

All muscle and gill tissue presented very low levels of cadmium, with mean concentrations below the limit of detection (0.031mg kg^{-1} wet weight). Mean concentrations in liver tissue ranged from between 0.1 and 0.5mg kg^{-1} wet weight in monkfish tissue to between 12 and 15mg kg^{-1} wet weight in black scabbardfish and orange roughy. Monkfish, *B. ferox*, blue ling, blue whiting and hake presented very low levels in liver tissue. Species originating from South Africa presented mean cadmium concentrations in liver between 0.5 and 1.7mg kg^{-1} wet weight, with overlapping ranges. Roundnose grenadier presented mean cadmium concentrations in liver between 2.3 and 4.3mg kg^{-1} wet weight with a wide range. Liver presented higher cadmium levels than muscle tissue for all species ($p<0.001$ for all species), and for black scabbardfish, liver tissue presented higher cadmium levels than gonad than gill than muscle tissue ($p<0.001$ in all cases).

H. bathybius showed increasing cadmium concentrations with depth ($p=0.016$), ranging from 4 to 8.8mg kg^{-1} wet weight between 2500 and 4000m depth. *C. armatus*, sampled at similar depths did not show a similar pattern ($p>0.05$). Black scabbardfish also presented such a pattern ($p<0.001$).

Concentrations in livers of orange roughy differed with sampling site ($p<0.001$), being higher in fish sampled in Meriadzec (14±17mg kg^{-1} wet weight) than in the Rockall Trough (3.3±1.5mg kg^{-1} wet weight). A similar pattern was present in *B. ferox* sampled from the two locations ($p<0.001$) but not in black scabbardfish ($p>0.05$).

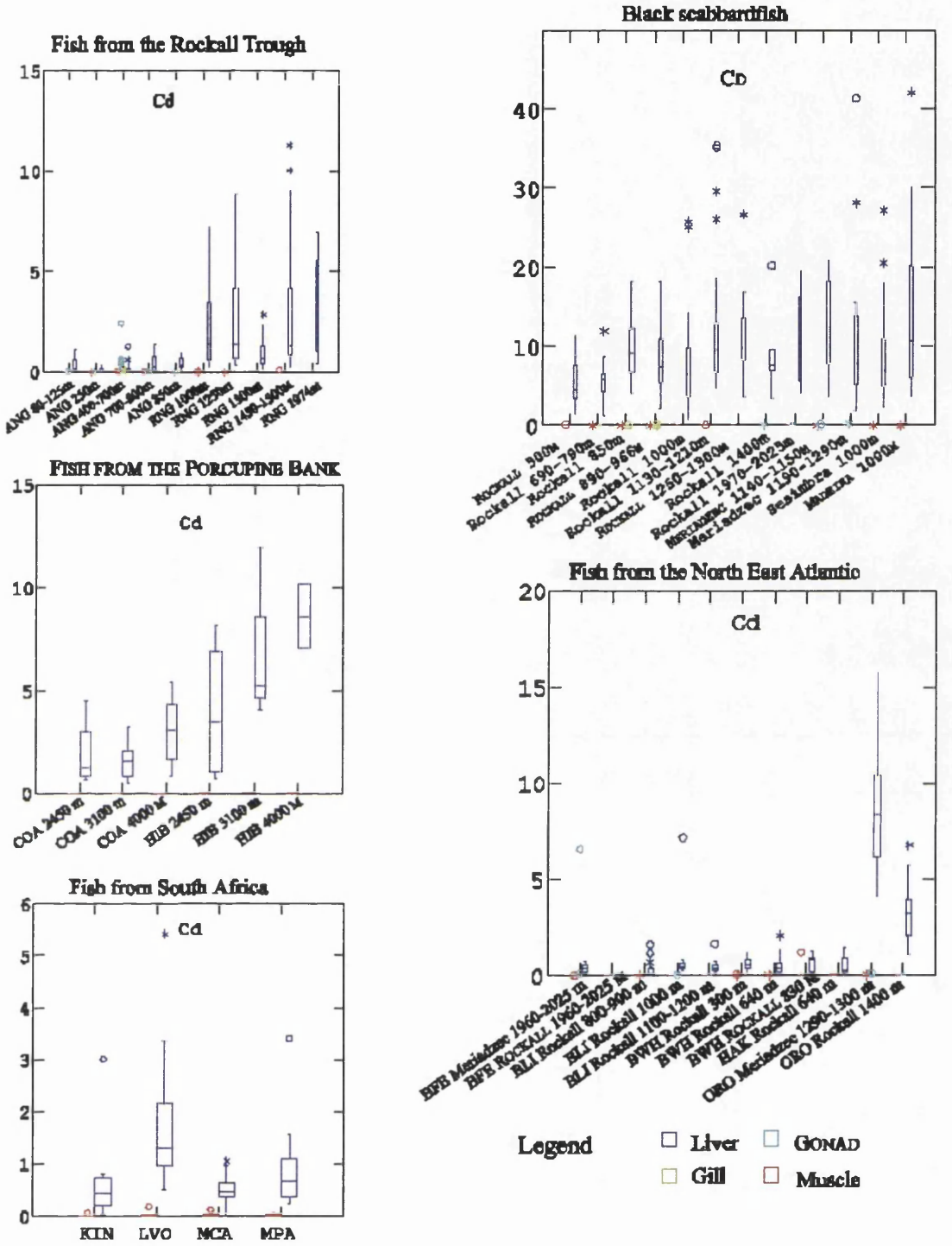


Fig 4.12: Cd distribution in muscle, gill, gonad and liver tissues of monkfish (ANG), *B. ferox* (BFE), blue ling (BLI), blue whiting (BWI), *H. bathybius* (HIB), kingklip (KIN), *L. vomerinus* (LVO), *M capensis* (MCA), *M. paradoxus* (MPA), orange roughy (ORO) and roundnose grenadier (RNG) at various locations and depths, expressed in $mg.kg^{-1}$ wet weight (limit of detection $0.047mg.kg^{-1}$ wet weight).

4.1.13. Total mercury (fig 4.13)

The highest mean mercury concentrations were found in *H. bathybius* livers, at ca 6.3mg kg^{-1} wet weight. Monkfish, blue ling, blue whiting, kingklip, *L. vomerinus*, *M. capensis, M. paradoxus* and roundnose grenadier exhibited mean concentrations below 0.2mg kg^{-1} wet weight in all organs.

The distribution of mercury between organs differed between species, with higher levels in muscle than liver tissue in the case of *B. ferox* ($p=0.008$), blue ling ($p<0.001$), blue whiting ($p<0.001$) and species from South Africa ($p<0.001$); similar levels in *C. armatus* and roundnose grenadier ($p>0.05$), and higher levels in livers for the other species ($p<0.05$).

C. armatus showed an increase in mercury concentrations between 2500 and 4000m depth ($p<0.001$), increasing from 0.2 to 0.8mg kg^{-1} wet weight in muscle tissue and from 0.16 to 1.03mg kg^{-1} wet weight in livers. Roundnose grenadier showed a similar trend although not as strong ($p=0.043$), no other species presented increase of mercury with depth ($p>0.05$).

All species sampled both in Rockall Trough and Meriadzec presented higher levels of mercury in all organs at the latter location ($p<0.001$ for all species). For example, mean mercury concentrations in *B. ferox* livers increased from 0.3 to 1.1mg kg^{-1} wet weight, in orange roughy from 0.3 to 5mg kg^{-1} wet weight and in black scabbardfish from 0.5 to 1.5mg kg^{-1} wet weight. Black scabbardfish livers presented higher concentrations in Madeira than Sesimbra ($p<0.001$), which in turn presented similar levels to Meriadzec ($p>0.05$).

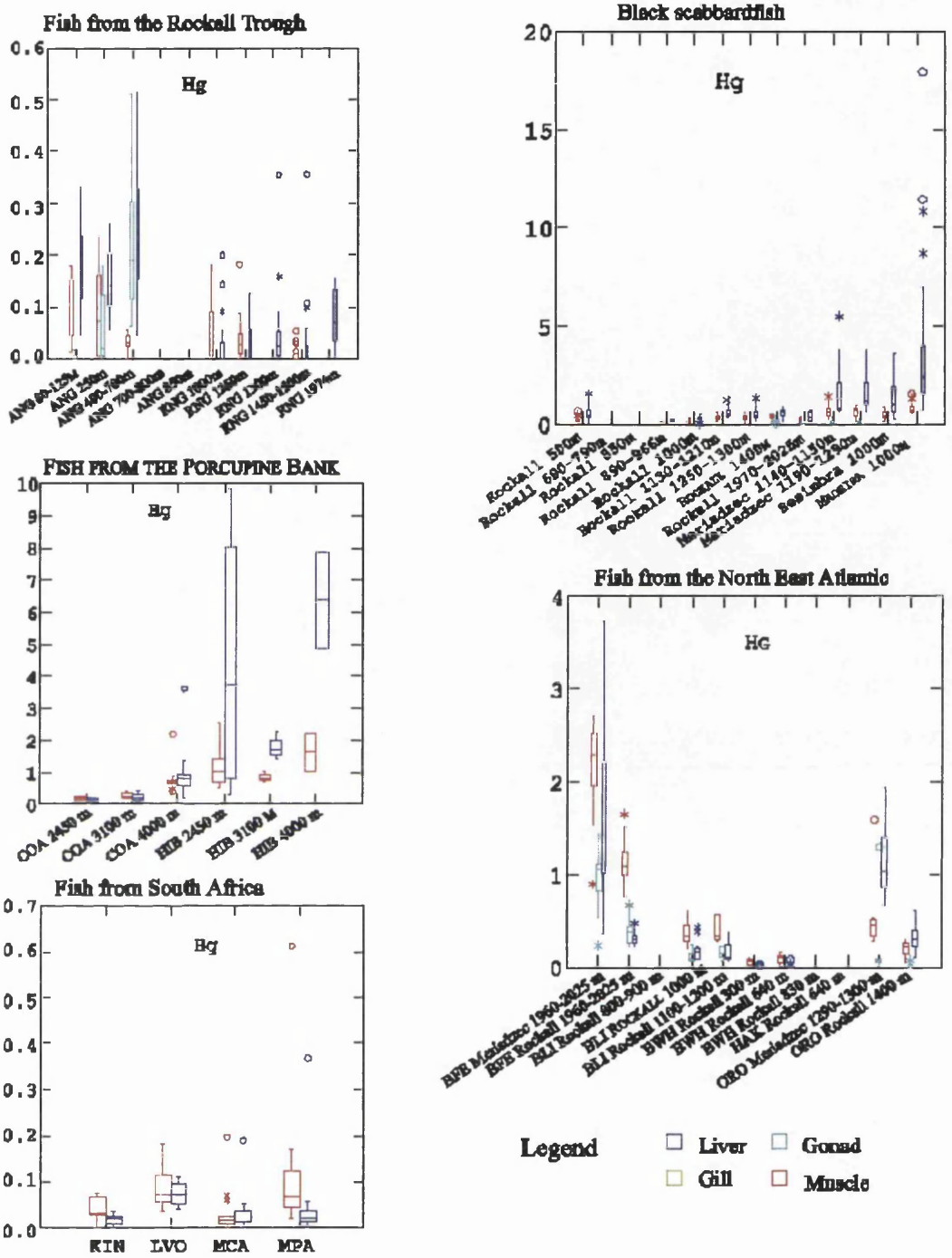


Fig 4.13: Hg distribution in muscle, gill, gonad and liver tissues of monkfish (ANG), *B. ferox* (BFE), blue ling (BLI), blue whiting (BWI), *H. bathybius* (HIB), kingklip (KIN), *L. vomerinus* (LVO), *M capensis* (MCA), *M. paradoxus* (MPA), orange roughy (ORO) and roundnose grenadier (RNG) at various locations and depths, expressed in $mg.kg^{-1}$ wet weight (limit of detection $0.047mg.kg^{-1}$ wet weight).

4.1.14. Lead (fig 4.14)

Lead levels were very low, with mean concentrations under the limit of detection (0.033mg kg^{-1} wet weight) in most cases. Black scabbardfish, orange roughy, *C. armatus* and *H. bathybius* showed mean concentrations in livers reaching 0.05mg kg^{-1}, but these were due to a small number of high values.

Lead levels in *B. ferox* and blue ling were above the limit of detection, with mean concentrations of about 0.08 and 0.03mg kg^{-1} wet weight in liver and muscle tissue respectively. The highest concentrations were reached in muscle tissue of blue whiting, with a mean lead concentration of ca 0.2mg kg^{-1} wet weight in individuals sampled at 300m depth. However, the lead levels were below the limit of detection in muscle tissue of blue whiting sampled below 300m depth and in liver tissues from all depths.

4.1.15. Bismuth (fig 4.15)

In all fish sampled in the North East Atlantic, bismuth levels were below the limit of detection (0.032mg kg^{-1} wet weight), apart from 7 isolated cases, where it reached values of up to 0.065 mg kg^{-1} wet weight in the liver of a roundnose grenadier individual.

Amongst the species sampled in South Africa, *M. capensis* and *M. paradoxus* also presented levels and mean concentrations of bismuth below the limit of detection. Kingklip and *L. vomerinus* showed mean bismuth concentrations of ca 0.09mg kg^{-1} wet weight.

4.1.16. Uranium (fig 4.16)

Amongst all species sampled, only *B. ferox* and blue ling sampled in the Rockall Trough presented mean uranium concentrations above the limit of detection (0.032mg kg^{-1} wet weight): at 0.06mg kg^{-1} in both muscle and gonad tissue. All other species sampled showed uranium concentrations below the limit of detection.

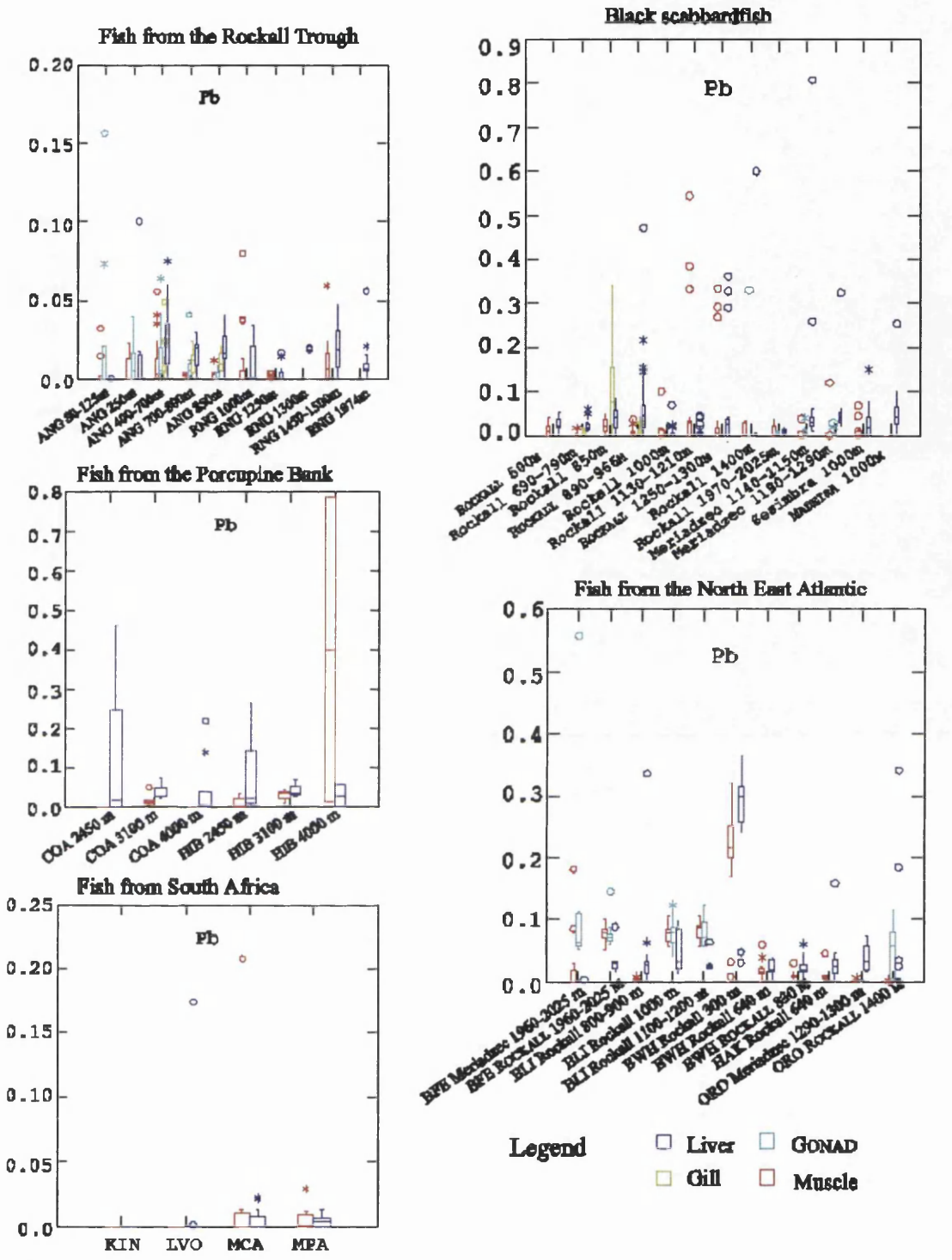


Fig 4.14: Pb distribution in muscle, gill, gonad and liver tissues of monkfish (ANG), *B. ferox* (BFE), blue ling (BLI), blue whiting (BWI), *H. bathybius* (HIB), kingklip (KIN), *L. vomerinus* (LVO), *M capensis* (MCA), *M. paradoxus* (MPA), orange roughy (ORO) and roundnose grenadier (RNG) at various locations and depths, expressed in $mg.kg^{-1}$ wet weight (limit of detection $0.047mg.kg^{-1}$ wet weight).

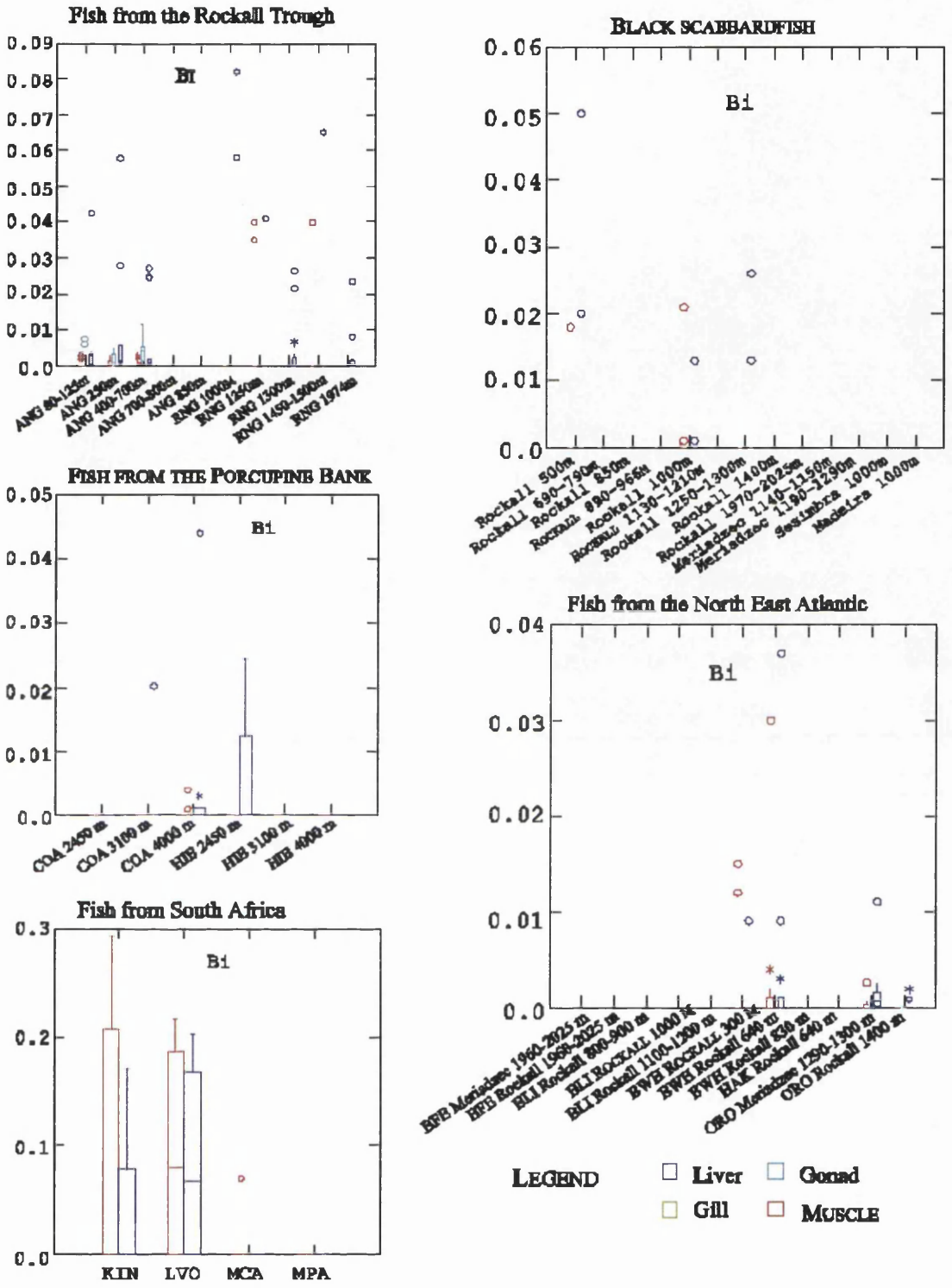


Fig 4.15: Bi distribution in muscle, gill, gonad and liver tissues of monkfish (ANG), *B. ferox* (BFE), blue ling (BLI), blue whiting (BWI), *H. bathybius* (HIB), kingklip (KIN), *L. vomerinus* (LVO), *M capensis* (MCA), *M. paradoxus* (MPA), orange roughy (ORO) and roundnose grenadier (RNG) at various locations and depths, expressed in $mg.kg^{-1}$ wet weight (limit of detection $0.047mg.kg^{-1}$ wet weight).

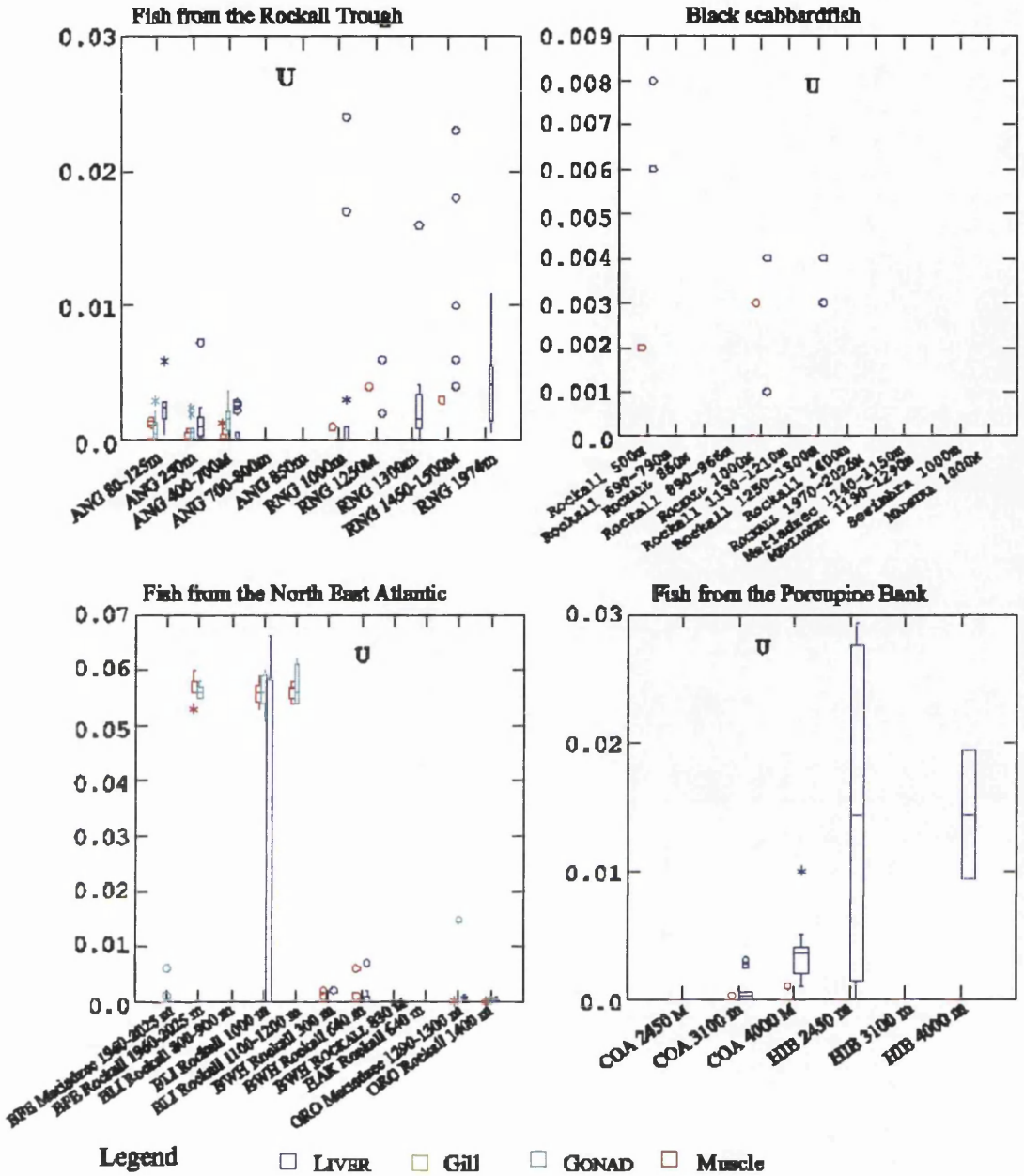


Fig 4.16: U distribution in muscle, gill, gonad and liver tissues of monkfish (ANG), *B. ferox* (BFE), blue ling (BLI), blue whiting (BWI), *H. bathybius* (HIB), orange roughy (ORO) and roundnose grenadier (RNG) at various locations and depths, expressed in $mg.kg^{-1}$ wet weight (limit of detection $0.047mg.kg^{-1}$ wet weight).

4.1.17. Conclusions

Most deep sea fish species studied presented very low levels of Li, Be, V, Cr, Co, Ni, Pb, Bi and U. However, black scabbardfish presented strikingly higher concentrations of vanadium and blue whiting higher levels of cobalt and nickel. These elements will not be studied during further statistical analysis.

In most species, manganese, copper and selenium accumulated mainly in liver tissue whilst zinc accumulated mostly in gonad tissue. The preferred organs for accumulation of arsenic and cadmium are species-dependant.

Different metals in different species showed different trends with depth. For example, copper, zinc, selenium and cadmium increased with depth in black scabbardfish and *H. bathybius*. *C. armatus*, sampled at similar locations and depths. *H. bathybius* presented increasing mercury and selenium with depth, and decreasing cobalt. Black scabbardfish also presented increasing nickel concentrations with depth, whilst the reverse was found in roundnose grenadier, alongside manganese and nickel. Roundnose grenadier also presented increasing copper and mercury with depth.

Monkfish differed from other species in that the metals patterns with depth were organ dependent. For example, manganese and zinc decreased with depth in liver tissue but increased in muscle tissue. Cobalt and selenium decreased in liver tissue but increased in gonad tissue. Mercury increased in gonad with depth whilst decreasing in muscle tissue. Copper increased with depth only in muscle tissue, as did arsenic in liver tissue, and chromium decreased with depth only in muscle tissue.

In a similar fashion, the location of capture has an important influence on metal concentrations. For example, fish species sampled in Meriadzec showed higher levels of most metals than the same species sampled in Rockall Trough. Black scabbardfish presented higher levels of copper and arsenic in Meriadzec and the Rockall Trough than in Sesimbra and Madeira, whereas selenium and mercury showed the opposite trend.

4.2. Organic contaminants

Individual fish were pooled by groups of no more than 5 fish in order to reduce overall variance of the data. After a preliminary study of all organs, only livers were analysed for 24 PCBs and 20 pesticides.

The results are summarised per group for all organic contaminants in table 4.6. The distribution of ICES7CB, ΣCB, ΣDDT and Σchlordane are expressed in figs 4.17 to 4.20 respectively. The ΣCB/ΣDDT and p,p'-DDE/ΣDDT ratios are showed in fig 4.21. The PCB distribution is investigated in fig 4.22. In total, 309 samples were analysed for PCBs and pesticides.

Apparent discrepancies between tables and figures originate from the fact that tables quote mean concentrations whereas figures quote median, and that, in some cases, some outliers have not been plotted to avoid distorting the scale.

Table 4.6: Mean CB and pesticide concentrations in the species sampled (μg kg^{-1} lipid weight; uld: under limit of detection) – see appendix 2 for codes.

species	location	organ	depth range (m)	no cases	length (cm)	weight (g)	% lipid	CB31	CB28	CB52	CB49	CB44	CB74	CB70	CB101	CB110	CB149	CB118	CB114	CB153	CB105
ANG	Rockall	FL	530-626	7	67.1	4258.4	0.5	3.7	0.8	33.0	7.8	11.1	5.1	12.2	22.6	15.6	9.6	11.0	145	30.8	77.8
			850	5	75.9	8814.6	0.4	4.9	6.2	94.4	10.3	40.6	6.4	50.3	93.7	67.0	59.6	59.4	uld	210	28.1
		LV	80-250	12	53.6	2445.6	33.8	0.3	2.9	0.7	1.0	uld	4.9	uld	3.5	uld	10.7	20.9	2.1	125	7.8
			590-650	15	70.3	6956.1	48.9	1.6	0.2	0.7	0.5	uld	3.6	0.9	1.9	0.5	14.4	17.5	2.6	167	8.3
			770-850	7	77.8	10389.8	37.8	1.2	2.3	2.1	0.9	uld	9.9	uld	27.4	3.7	74.7	61.3	7.4	414	32.5
BFE	Rockall	LV	2025	3	64.9	1537.7	66.8	7.6	2.7	uld	uld	uld	uld	uld	uld	uld	0.3	31.5	5.0	239	14.1
	Meriadzec	LV	1960	4	66.9	1291.5	56.9	7.5	7.6	16.4	uld	uld	54.4	uld	158	11.6	120	367	30.2	2090	142
BLI	Rockall	LV	1000-1200	7	98.8	3682.6	59.8	7.4	9.6	24.4	9.6	3.8	15.7	2.9	45.9	0.9	54.6	111	12.8	273	35.8
BSC	Rockall	FL	850-900	12	88.8	1304.1	2.6	14.1	15.4	258	20.5	35.5	13.7	64.7	102	79.0	58.5	86.3	0.5	139	14.8
			1189	7	100.7	1747.6	4.3	3.5	4.8	19.9	4.7	5.4	4.9	8.1	29.8	8.4	36.4	40.4	8.2	127	13.7
			2025	2	91.5	1085.0	3.7	10.3	4.1	uld	uld	uld	uld	uld	uld	uld	15.4	15.3	3.3	92.5	6.6
		GIL	850-1130	14	88.8	1299.8	4.7	6.3	5.9	62.1	9.3	14.9	7.2	28.8	37.3	25.4	113	29.1	2.8	75.7	6.6
		LV	500	5	96.3	625.1	12.3	4.2	5.3	20.9	7.2	3.1	9.4	6.0	48.0	6.4	41.9	51.9	3.8	137	18.6
			850-900	11	87.6	1136.2	10.9	0.5	0.6	uld	0.6	uld	0.6	1.5	1.7	2.3	1.9	4.1	13.2	64.1	4.0
			1000	5	88.1	84.3	9.7	5.0	6.9	14.8	4.6	1.7	8.4	5.4	36.2	6.1	31.4	35.0	2.1	89.3	10.9
			1189	7	100.7	1747.6	13.7	0.8	uld	uld	uld	uld	0.4	uld	uld	uld	27.5	30.8	11.4	130	11.7
			1250-1400	8	90.6	225.3	12.1	6.2	5.2	7.8	3.7	2.2	18.0	4.4	43.5	2.4	34.5	48.6	4.8	175	14.7
			2025	2	91.5	1085.0	16.1	2.7	uld	uld	uld	uld	uld	uld	uld	uld	1.1	uld	2.4	76.3	uld
	Meriadzec	FL	1195	7	107.2	1764.8	1.0	14.7	21.4	78.4	19.0	32.8	40.5	30.8	85.9	28.8	74.1	83.7	8.7	258	31.1
		GD	1195	7	107.2	1764.8	2.6	10.6	12.8	54.9	13.8	16.1	15.4	24.4	63.1	17.1	53.9	56.4	4.6	216	17.2
		LV	1140-1195	7	107.2	1764.8	12.9	3.8	2.0	8.5	3.3	1.8	4.9	2.8	22.2	1.2	65.4	68.8	6.1	295	25.5
	Sesimbra	FL	1000	12	105.4	1476.7	3.9	1.1	8.1	16.4	5.3	0.9	20.6	2.4	91.5	4.8	197	181	13.6	726	66.3
		LV	1000	12	105.4	1476.7	13.5	1.6	7.3	21.8	6.7	1.5	16.3	2.2	94.7	3.4	138	131	11.3	473	43.7
	Madeira	FL	1000	6	119.6	2403.6	0.5	62.9	15.4	30.4	7.7	15.8	28.5	11.4	182	10.1	288	291	18.0	1060	56.2
		LV	1000	5	120.2	2446.0	8.2	3.8	4.4	15.8	5.2	2.0	23.3	2.4	149	uld	183	201	15.4	1140	56.3
BWI	Rockall	LV	300	7	27.0	30.5	58.5	0.5	0.3	1.5	0.5	0.9	0.7	0.4	10.4	3.9	12.2	12.8	1.2	74.6	2.8
			640	5	32.7	21.9	62.9	3.2	0.3	uld	1.2	uld	2.7	0.5	16.2	6.2	22.3	22.4	11.8	82.6	10.0
COA	Porcupine	LV	2450	8	25.8	405.7	31.1	11.2	14.8	169	39.9	47.3	55.8	112	18.1	198	90.5	128	22.8	399	56.7
			3100	7	17.3	512.5	43.7	0.5	0.5	9.9	1.9	4.4	9.7	2.7	uld	2.1	2.9	85.6	65.1	912	44.6
			4000	5	70.2	314.0	50.6	4.2	uld	3.8	uld	uld	18.7	uld	2.1	uld	31.9	201	30.2	1600	81.9
HIB	Porcupine	LV	3100	3	35.0	726.7	19.5	1.0	2.4	31.8	6.6	14.0	12.5	7.7	uld	5.8	42.2	73.4	35.3	236	31.8
			4000	1	27.7	294.5	22.3	19.5	26.3	249	47.5	80.2	49.1	97.0	241	223	102	168	5.9	313	62.0
KIN	South Africa	LV	273	6	40.7	312.6	18.4	3.8	0.1	uld	0.6	uld	uld	uld	uld	uld	uld	1.2	0.2	15.5	uld
LVO	South Africa	LV	180-430	6	40.3	1172.6	23.6	uld	uld	uld	uld	uld	uld	uld	uld	uld	0.5	10.0	0.4	19.9	0.1
MCA	South Africa	LV	180-270	10	37.9	545.9	24.7	2.0	1.5	14.8	2.7	5.8	1.5	2.5	7.1	2.1	3.2	25.3	1.6	37.0	3.8
MPA	South Africa	LV	430	9	43.6	802.3	32.9	0.2	0.1	7.4	2.4	1.6	2.1	4.1	8.4	6.5	3.3	10.4	1.4	19.8	0.9
ORO	Rockall	LV	1300-1400	7	31.5	642.3	27.5	8.9	12.1	35.4	6.2	6.2	16.7	10.3	60.1	19.9	55.3	74.2	7.9	225	25.1
	Meriadzec	LV	1290	6	43.4	1501.3	21.7	5.8	8.6	18.6	5.6	1.5	11.8	4.3	42.0	4.3	48.5	60.3	7.2	208	23.9
RNG	Rockall	LV	1000	7	18.0	1060.4	51.4	uld	uld	uld	uld	uld	6.7	uld	uld	uld	8.1	41.2	3.9	249	16.9
			1250	8	16.5	850.1	51.8	uld	uld	1.1	0.4	uld	14.6	uld	10.4	1.0	14.5	100	4.8	384	40.6
			1300	11	15.9	717.0	55.4	0.6	0.3	19.0	1.0	0.7	14.9	0.2	20.9	3.0	32.2	78.6	3.4	197	26.0
			1450-1500	9	16.6	708.3	48.6	2.3	2.1	23.1	3.8	1.0	16.6	1.6	25.7	4.9	24.3	101	5.1	395	43.9
			1974	9	14.9	527.2	48.9	0.4	0.6	23.8	1.3	1.4	19.4	3.3	29.3	6.8	42.9	128	6.6	760	54.1

Table 4.6 (cont.): Mean CB and pesticide concentrations in the species sampled (μg kg^{-1} lipid weight; uld: under limit of detection) – see appendix 2 for codes.

species	location	organ	depth range (m)	no cases	CB138	CB158	CB187	CB128	CB156	CB157	CB180	CB170	CB189	CB194	7CB	ΣCB	α-HCH	γ-HCH	HCB	α-cdane	γ-cdane
ANG	Rockall	FL	530-626	7	14.1	1.8	37.7	4.2	1.5	13.8	15.6	2.6	0.1	0.9	134	485	uld	73.5	2.3	2.7	0.1
			850	5	122	uld	75.6	29.1	5.9	uld	76.6	19.2	uld	uld	663	1060	uld	28.3	3.7	24.5	uld
		LV	80-250	12	62.2	2.4	25.1	9.3	6.7	2.6	42.1	14.7	0.9	6.0	258	352	4.8	2.3	26.5	28.6	5.5
			590-650	15	83.3	5.5	39.9	14.3	9.0	3.0	59.7	21.2	0.8	8.0	330	464	2.5	0.4	19.4	44.8	7.9
			770-850	7	237	13.1	116	43.3	24.2	7.6	155	55.6	1.8	23.1	900	1320	0.8	uld	23.0	35.8	4.7
BFE	Rockall	LV	2025	3	164	8.6	74.5	15.6	14.2	7.7	146	44.6	1.2	22.4	583	800	3.4	1.9	35.8	11.3	2.8
	Meriadzec	LV	1960	4	1240	47.7	509	96.7	92.9	35.0	798	299	10.9	160	4680	6300	3.7	1.5	39.8	27.3	16.7
BLI	Rockall	LV	1000-1200	7	210	13.0	90.5	33.4	16.7	7.2	118	39.1	1.2	15.7	792	1150	4.4	2.1	54.7	93.6	20.5
BSC	Rockall	FL	850-900	12	110	3.7	41.8	13.8	7.4	0.9	52.5	18.1	uld	4.7	740	1150	1.5	5.1	26.9	44.4	10.8
			1189	7	95.1	6.5	52.1	15.5	6.9	3.1	64.2	20.7	uld	7.4	423	586	3.6	1.7	29.0	57.8	14.1
			2025	2	72.8	4.8	40.7	12.4	5.8	2.8	48.5	9.6	5.6	5.3	241	356	4.1	uld	31.0	56.0	14.3
		GIL	850-1130	14	45.4	1.3	32.3	6.2	2.1	0.5	40.5	14.8	uld	4.9	299	572	1.5	4.0	22.8	39.2	13.8
		LV	500	5	107	5.3	50.9	13.2	6.3	3.4	63.3	22.6	0.4	7.8	437	644	2.8	2.7	28.2	47.1	15.3
			850-900	11	27.3	1.9	35.5	3.5	1.3	5.6	39.6	15.1	uld	5.8	139	231	uld	uld	22.7	39.7	10.6
			1000	5	70.4	3.9	32.3	8.6	3.7	2.2	36.9	11.3	uld	5.7	288	433	5.9	5.5	33.1	54.0	17.4
			1189	7	90.2	5.3	47.8	15.6	5.7	3.3	70.4	22.8	0.4	10.2	322	484	3.0	uld	34.5	63.0	13.7
			1250-1400	8	114	4.0	53.9	11.6	6.3	3.0	55.9	18.0	0.9	6.2	450	645	3.0	9.4	25.3	43.5	19.7
			2025	2	48.8	3.6	37.0	7.0	4.7	2.6	46.5	14.7	uld	6.3	171	254	3.2	uld	25.1	42.5	9.7
	Meriadzec	FL	1195	7	208	6.7	105	29.3	13.4	5.5	130	49.5	0.7	17.7	873	1370	0.9	5.9	24.0	48.1	13.4
		GD	1195	7	165	3.4	89.7	21.5	11.8	4.2	117	39.7	0.7	14.7	683	1040	0.5	3.4	15.4	39.4	9.6
		LV	1144-1195	7	191	18.9	122	26.0	16.1	5.6	168	57.0	1.2	21.7	775	1140	0.6	1.6	23.5	56.8	14.1
	Sesimbra	FL	1000	12	536	19.7	318	71.2	39.5	14.6	388	143	2.5	42.1	1960	2910	uld	0.6	36.4	128	38.0
		LV	1000	12	343	14.0	217	42.9	25.8	10.1	279	96.3	2.0	35.2	1360	2020	0.6	0.6	22.0	70.3	24.4
	Madeira	FL	1000	6	866	47.6	484	46.7	64.5	31.0	611	225	5.1	81.4	3030	4540	uld	uld	40.3	163	42.4
		LV	1000	5	729	26.4	227	43.9	43.0	21.4	431	138	3.9	60.5	2660	3530	2.2	uld	19.2	112	37.3
BWI	Rockall	LV	300	7	36.2	1.6	13.1	3.3	2.2	0.9	17.2	5.4	uld	1.7	152	204	3.1	1.5	8.3	14.3	2.5
			640	5	67.1	4.1	28.0	11.5	4.5	1.8	28.9	10.6	0.2	3.7	217	340	3.4	3.8	20.2	62.2	13.0
COA	Porcupine	LV	2450	8	332	66.9	160	26.1	24.4	21.5	129	76.3	1.5	44.0	1190	2250	3.4	6.8	33.9	39.1	17.7
			3100	7	68.2	5.3	202	3.0	29.3	18.6	348	103	3.1	46.9	1420	1970	0.7	0.6	19.5	16.4	1.9
			4000	5	1220	27.8	958	15.0	65.7	42.1	841	285	8.0	130	3870	5570	0.0	4.3	25.2	39.0	5.2
HIB	Porcupine	LV	3100	3	68.8	44.7	80.0	19.3	15.1	10.0	75.8	26.4	2.2	20.2	488	863	1.1	2.5	21.2	17.8	11.9
			4000	1	296	18.9	138	37.9	24.0	15.2	178	62.5	uld	31.4	1470	2490	0.6	5.6	30.8	55.3	81.5
KIN	South Africa	LV	273	6	9.2	2.4	6.9	2.9	1.7	1.5	6.1	2.5	0.0	0.6	32.2	55.4	0.4	1.9	4.0	2.5	6.2
LVO	South Africa	LV	180-430	6	8.6	1.7	7.9	3.8	2.0	1.3	11.6	3.9	0.1	1.6	50.1	73.6	1.1	3.0	9.1	4.1	5.6
MCA	South Africa	LV	180-270	10	22.3	4.5	13.1	6.7	4.0	1.8	17.2	5.5	0.1	2.1	125	188	0.5	4.1	6.0	6.0	3.4
MPA	South Africa	LV	430	9	11.4	2.8	5.0	0.9	1.9	0.8	8.7	5.3	0.1	1.8	66.3	107	3.3	4.8	15.3	8.2	8.7
ORO	Rockall	LV	1300-1400	7	167	8.5	56.5	6.2	10.7	4.6	83.2	24.0	1.6	9.8	657	935	11.3	5.0	35.3	34.9	6.2
	Meriadzec	LV	1290	6	146	6.7	75.2	6.4	10.9	4.8	103	36.4	1.4	11.8	586	853	7.3	12.3	23.4	23.9	6.9
RNG	Rockall	LV	1000	7	179	10.2	53.9	21.3	13.0	5.9	110	38.6	0.5	14.3	579	772	4.9	5.9	31.2	9.0	13.9
			1250	8	292	15.2	82.1	39.1	19.1	7.7	148	55.4	0.7	19.8	936	1250	5.4	2.6	39.4	12.7	8.2
			1300	11	162	8.6	47.5	24.2	11.2	4.6	83.5	29.2	0.7	8.5	562	778	5.2	1.2	27.1	10.6	1.7
			1450-1500	9	285	13.8	70.7	38.0	18.9	7.6	154	57.5	0.8	21.5	986	1320	5.6	2.9	36.9	10.7	5.4
			1974	9	467	21.7	75.0	68.2	31.4	11.3	261	85.1	2.3	32.7	1670	2130	6.7	2.3	30.0	9.1	1.4

Table 4.6 (cont.): Mean CB and pesticide concentrations in the species sampled (μg kg^{-1} lipid weight; uld: under limit of detection) – see appendix 2 for codes.

species	location	organ	depth range (m)	no cases	α-cdene	γ-cdene	oxy cdane	hepta chlor	hepoxy cdane	t-non achlor	dieldrin	aldrin	endrin	opDDD	ppDDD	opDDT	ppDDT	opDDE	ppDDE	ΣDDT	Σcdane	ΣCB/ ΣDDT	,p'DDE /ΣDDT
ANG	Rockall	FL	530-626	7	uld	3.1	1.5	uld	0.2	5.1	8.0	uld	2.9	0.1	10.6	0.2	2.5	11.3	44.1	68.7	12.6	7.07	0.64
			850	5	uld	uld	uld	uld	uld	42.1	34.7	uld	21.9	uld	67.4	uld	34.7	51.0	311	464	66.6	2.28	0.67
		LV	80-250	12	0.4	0.3	8.9	3.5	5.8	48.1	39.7	0.1	35.3	1.8	34.3	4.2	20.5	11.7	115	187	101	1.88	0.61
			590-650	15	0.0	0.3	12.5	1.7	5.5	84.5	42.4	0.0	49.1	4.5	82.5	5.0	51.6	26.1	229	397	157	1.17	0.57
			770-850	7	uld	uld	10.7	uld	4.6	75.5	40.2	uld	32.5	uld	105	6.1	45.4	36.9	443	636	131	2.07	0.70
BFE	Rockall	LV	2025	3	0.2	uld	6.1	11.0	0.9	62.6	12.1	0.5	30.5	1.4	73.8	44.8	140	uld	412	663	94.9	1.19	0.61
	Meriadzec	LV	1960	4	3.2	uld	52.4	6.2	uld	402	46.2	3.2	230	2.2	1050	244	1220	43.2	1230	3790	508	1.66	0.32
BLI	Rockall	LV	1000-1200	7	2.0	6.0	11.6	1.8	2.0	169	41.7	2.9	101	4.7	141	42.2	221	24.5	579	1010	306	1.14	0.57
BSC	Rockall	FL	850-900	12	uld	uld	2.8	2.1	0.3	67.0	22.6	uld	36.3	uld	79.0	16.8	89.1	32.8	330	547	127	2.11	0.60
			1189	7	uld	0.7	8.4	1.2	2.8	94.5	33.1	1.1	59.6	2.6	105	13.0	163	13.0	417	714	179	0.82	0.58
			2025	2	uld	uld	7.9	uld	3.7	86.7	33.6	1.7	58.5	uld	98.3	24.1	144	uld	327	594	169	0.60	0.55
		GIL	850-1130	14	uld	0.9	5.1	2.0	1.5	61.4	20.9	uld	38.6	uld	71.0	11.7	83.5	18.5	235	420	124	1.36	0.56
		LV	500	5	uld	10.4	7.5	0.8	1.1	118	20.1	1.9	52.2	uld	59.7	39.3	160	14.0	344	617	200	1.04	0.56
			850-900	11	0.5	2.7	3.9	uld	1.2	56.7	36.7	0.5	39.2	1.9	84.3	9.7	86.7	16.8	234	433	115	0.53	0.54
			1000	5	2.1	4.7	8.4	3.6	3.2	74.6	29.5	1.7	59.2	uld	55.3	54.0	119	18.3	231	478	168	0.91	0.48
			1189	7	1.3	14.0	7.5	1.1	2.6	103	42.0	2.8	63.0	3.1	116	11.6	162	3.6	460	756	206	0.64	0.61
			1250-1400	8	5.5	21.6	6.0	2.8	1.3	79.9	21.7	2.4	49.1	0.4	57.1	23.0	133	11.5	412	636	180	1.01	0.65
			2025	2	2.8	19.8	5.8	uld	2.8	68.0	32.9	1.6	42.4	0.6	69.4	18.1	106	uld	277	471	151	0.54	0.59
	Meriadzec	FL	1195	7	uld	1.1	8.0	16.5	1.6	98.4	17.2	1.9	64.3	4.2	97.9	24.5	282	14.7	507	931	187	1.48	0.54
		GD	1195	7	uld	0.8	6.6	8.8	1.2	92.2	14.9	2.4	60.5	3.5	79.4	17.1	243	16.1	383	742	159	1.41	0.52
		LV	1144-1195	7	1.0	7.9	9.7	6.1	1.5	118	32.4	1.0	77.6	1.4	123	22.4	286	7.4	608	1050	215	1.09	0.58
	Sesimbra	FL	1000	12	uld	uld	16.5	8.2	3.6	226	32.9	1.6	143	uld	282	56.9	594	13.1	1490	2430	420	1.20	0.61
		LV	1000	12	0.9	4.2	11.3	5.6	3.3	119	26.7	1.1	69.4	uld	175	18.6	264	18.8	1030	1500	239	1.34	0.68
	Madeira	FL	1000	6	uld	uld	17.9	18.7	1.1	373	60.6	0.9	392	2.2	306	299	1890	30.3	1740	4270	617	1.06	0.41
		LV	1000	5	2.7	4.1	16.1	2.0	uld	211	23.2	1.9	266	8.7	261	147	952	23.8	752	2150	385	1.64	0.35
BWI	Rockall	LV	300	7	uld	0.1	1.1	0.4	0.8	34.8	12.4	uld	15.0	0.1	20.3	5.9	36.0	4.1	158	220	54.0	0.91	0.70
			640	5	0.6	3.2	10.1	0.1	4.2	101	33.4	0.4	60.9	6.2	68.6	16.7	102	28.5	288	510	195	0.67	0.56
COA	Porcupine	LV	2450	8	1.7	14.5	7.5	0.8	1.0	96.4	15.0	1.7	61.7	0.3	108	53.2	368	138	1310	1970	166	1.14	0.66
			3100	7	0.4	3.1	13.1	2.2	0.8	110	16.3	0.8	69.1	4.0	150	45.3	345	14.2	1430	1990	148	0.99	0.72
			4000	5	0.5	5.0	45.0	0.2	1.4	324	30.5	1.0	138	4.1	541	212	645	14.4	1500	2920	420	1.91	0.51
HIB	Porcupine	LV	3100	3	0.7	9.5	5.9	5.2	0.6	95.3	9.7	2.3	47.5	4.9	99.2	39.7	410	87.8	890	1530	147	0.56	0.58
			4000	1	10.3	79.8	5.0	uld	uld	155	11.9	7.9	61.6	uld	117	94.8	622	45.0	1720	2590	376	0.96	0.66
KIN	South	LV	273	6	1.5	3.3	0.5	2.0	0.0	23.2	4.5	0.7	4.1	0.1	12.7	9.5	6.0	uld	87.3	108	39.4	0.48	0.76
LVO	South	LV	180-430	6	0.8	2.4	0.0	1.9	uld	15.7	4.4	0.4	7.6	0.2	13.6	8.4	10.8	uld	94.5	120	30.5	0.58	0.74
MCA	South	LV	180-270	10	0.4	0.7	0.1	1.7	uld	9.5	5.0	1.9	5.3	0.2	25.2	17.3	11.0	28.5	133	211	21.9	0.87	0.62
MPA	South	LV	430	9	1.7	1.1	1.0	1.3	0.4	27.9	7.7	1.6	11.2	0.5	24.9	11.9	32.1	2.7	108	170	49.5	0.60	0.60
ORO	Rockall	LV	1300-1400	7	9.1	3.0	28.4	4.1	0.7	48.2	51.6	4.2	78.7	2.6	147	103	151	15.9	551	971	135	0.96	0.57
	Meriadzec	LV	1290	6	25.5	6.6	11.0	4.7	0.1	35.5	31.3	4.5	46.2	2.6	138	49.0	125	12.2	361	687	114	1.24	0.52
RNG	Rockall	LV	1000	7	3.2	11.5	35.0	2.0	1.9	65.1	29.3	3.6	91.8	0.0	144	15.3	213	uld	770	1120	142	0.68	0.67
			1250	8	0.0	3.5	23.6	1.3	3.0	21.2	29.3	0.8	105	uld	204	102	342	uld	1420	2050	73.5	0.60	0.69
			1300	11	1.8	1.0	33.7	1.6	2.2	47.5	33.1	1.8	182	0.6	185	149	325	4.9	645	1310	100	0.59	0.49
			1450-1500	9	0.2	7.1	45.3	0.9	1.1	57.5	34.7	2.2	109	uld	233	74.1	421	5.8	1040	1770	128	0.74	0.59
			1974	9	1.3	1.2	41.4	1.0	2.3	38.0	30.6	1.2	185.6	4.8	275.5	192.5	629.1	4.7	1084.9	2191.5	95.7	0.97	0.50

4.2.1. ICES7CB (fig 4.17)

ICES7CB is the sum of CB28, 52, 101, 118, 153, 138 and 180. These particular CBs have been chosen by the International Council for the Exploration of the Sea (ICES) because they are all persistent and present different degrees of chlorination. Therefore the concentration of these 7 CBs in any organism will be representative of its contamination in the 209 CBs.

The lowest levels of ICES7CB were found in fish sampled in South Africa, with mean concentrations in liver tissue ranging from ca 30 to 125μg kg^{-1} lipid weight. Highest mean concentration was found in *B. ferox* livers from Meriadzec at ca 4680 μg kg^{-1} lipid weight.

B. ferox presented higher levels in Meriadzec than in the Rockall Trough ($p=0.037$), up to 10 times higher. Black scabbardfish also presented higher ICES7CB in Meriadzec than the Rockall Trough ($p=0.001$). However, orange roughy did not show such a trend, with similar ICES7CB concentrations in Meriadzec and Rockall samples ($p>0.05$).

Monkfish presented higher levels in liver than muscle tissue ($p=0.030$). However, in black scabbardfish, muscle presented higher concentration than liver ($p=0.001$). In monkfish, concentrations in muscle tissue increased with depth ($p=0.027$) but not in liver tissue ($p>0.05$). Roundnose grenadier also presented increasing mean concentrations with depth, but in two discrete groups. Concentrations increased between 1000 and 1250m depth, then levels at 1300m were similar to the levels found at 1000m depth, and increased again from 1300 to 2000m depth. No other species presented significant increase or decrease in ICES7CB concentration with depth.

ICES7CB concentrations in black scabbardfish were significantly lower at Rockall Trough than at Meriadzec ($p<0.001$), concentrations were similar at Meriadzec, Sesimbra and Madeira ($p>0.05$ in each case).

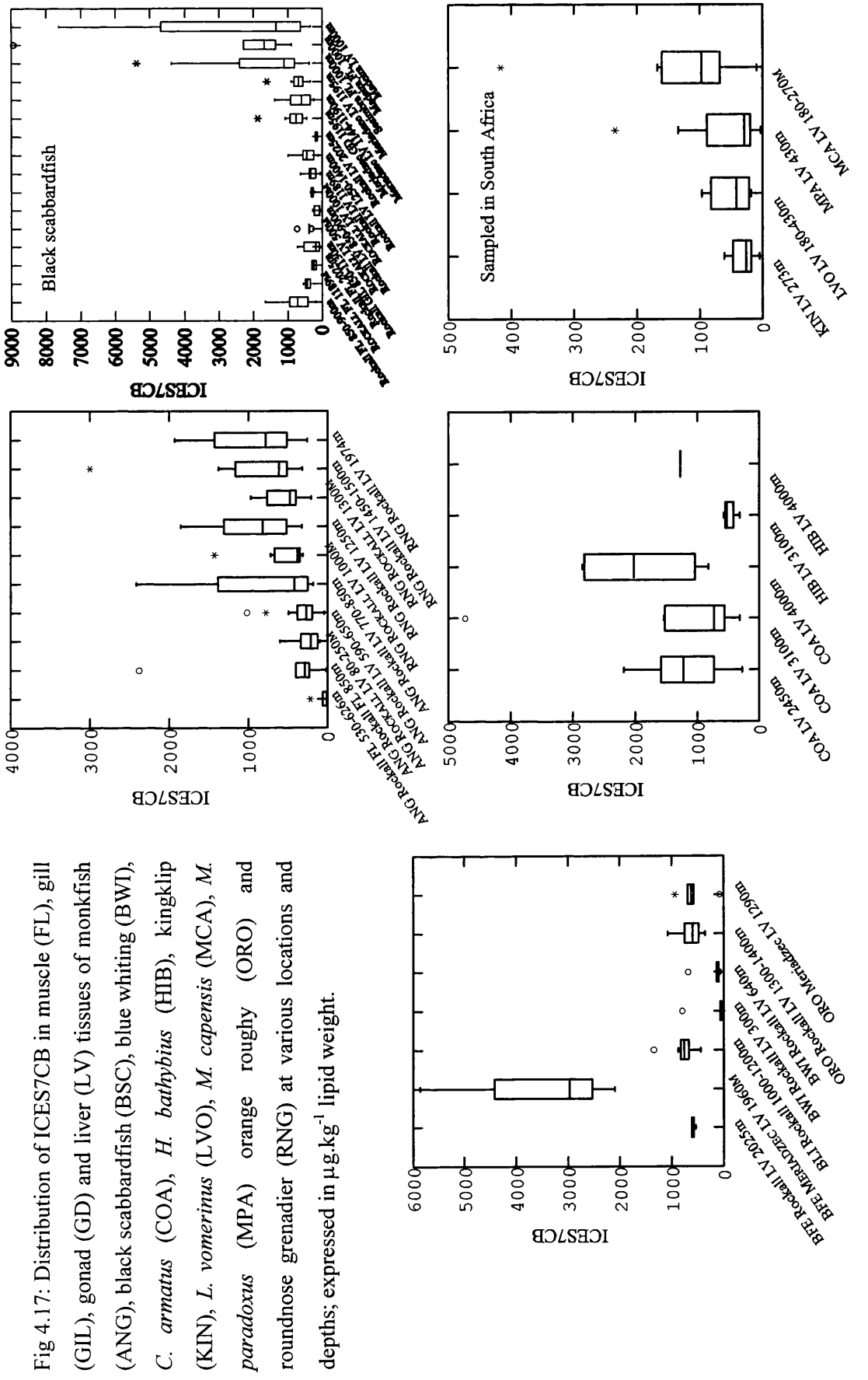


Fig 4.17: Distribution of ICES7CB in muscle (FL), gill (GIL), gonad (GD) and liver (LV) tissues of monkfish (ANG), black scabbardfish (BSC), blue whiting (BWI), *C. armatus* (COA), *H. bathybius* (HIB), kingklip (KIN), *L. vomerimus* (LVO), *M. capensis* (MCA), *M. paradoxus* (MPA) orange roughy (ORO) and roundnose grenadier (RNG) at various locations and depths; expressed in $\mu g.kg^{-1}$ lipid weight.

4.2.2. ΣCB (fig 4.18)

ΣCB is the sum of all CBs determined (CBs28, 31, 44, 49, 52, 70, 74, 101, 105, 110, 114, 118, 128, 138, 149, 153, 156, 157, 158, 170, 180, 187, 189 and 194).

The lowest levels of ΣCB were found in fish sampled in South Africa, with mean concentrations in liver tissue ranging from ca 55 to 188μg kg^{-1} lipid weight. Highest mean concentration was found in *B. ferox* livers from Meriadzec at ca 6300 μg kg^{-1} lipid weight.

B. ferox presented higher levels in Meriadzec than the Rockall Trough ($p=0.037$), up to 10 times higher. Black scabbardfish also presented higher ΣCB concentrations in Meriadzec than the Rockall Trough ($p<0.001$). However, orange roughy did not show such a trend, with similar ΣCB concentrations in Meriadzec and Rockall samples ($p>0.05$).

Monkfish presented higher levels in livers than muscle tissue ($p=0.020$). However, in black scabbardfish, muscle tissue presented higher ΣCB concentration than liver tissue at all depths and locations ($p=0.001$).

Roundnose grenadier presented increasing mean concentrations with depth, but in two discrete groups. Concentrations increased between 1000 and 1250m depth. Levels at 1300m were similar to 1000m depth, and increased again to 2000m depth. No other species presented significant increase or decrease in ΣCB concentration with depth ($p>0.05$ in all cases).

ΣCB concentrations in black scabbardfish were significantly lower at Rockall Trough than at Meriadzec ($p<0.001$), concentrations were similar at Meriadzec, Sesimbra and Madeira ($p>0.05$ in each case).

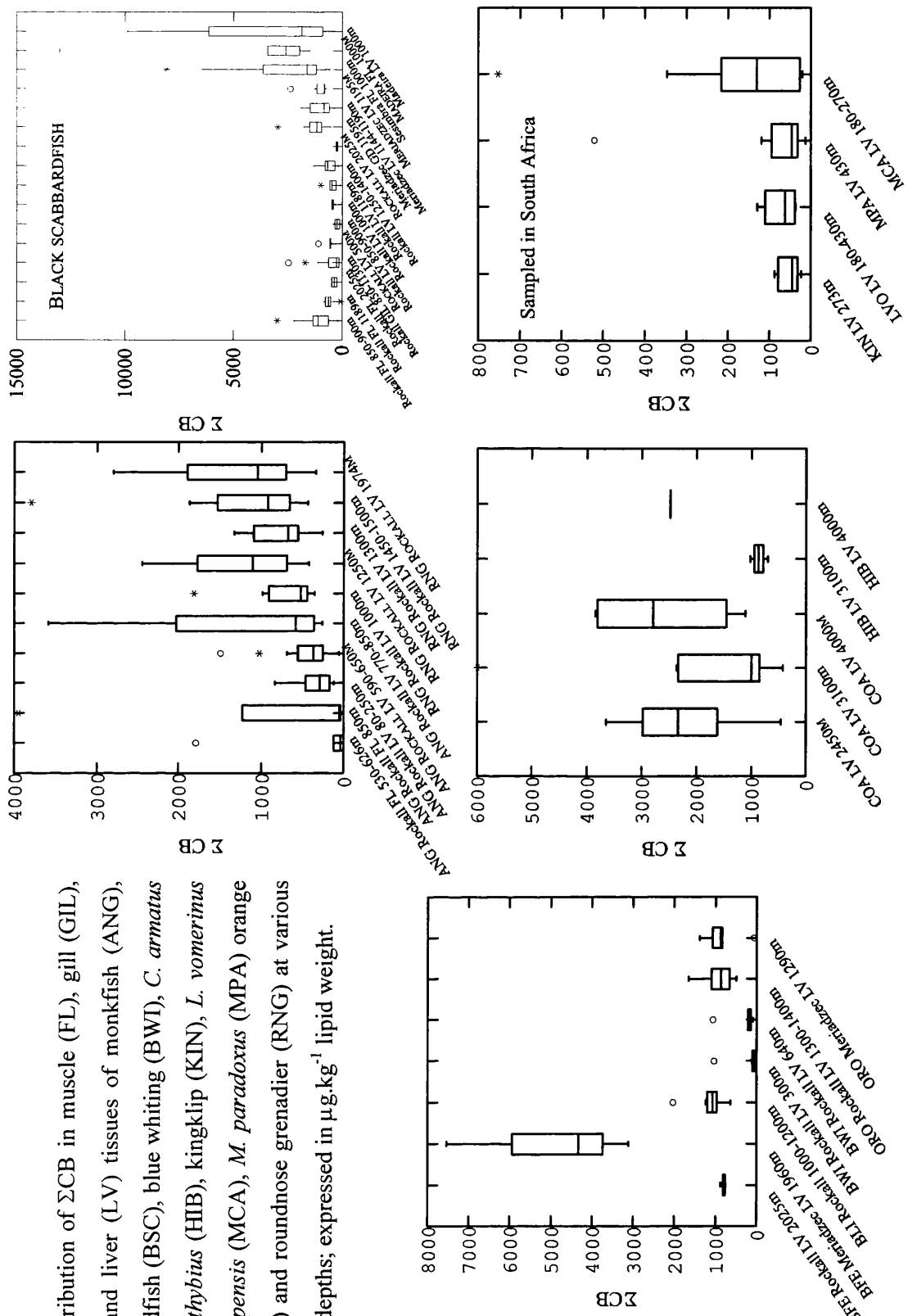


Fig 4.18: Distribution of ΣCB in muscle (FL), gill (GIL), gonad (GD) and liver (LV) tissues of monkfish (ANG), black scabbardfish (BSC), blue whiting (BWI), *C. armatus* (COA), *H. bathybius* (HIB), kingklip (KIN), *L. vomerimus* (LVO), *M. capensis* (MCA), *M. paradoxus* (MPA) orange roughy (ORO) and roundnose grenadier (RNG) at various locations and depths; expressed in $\mu g.kg^{-1}$ lipid weight.

4.2.3. ΣDDT (fig 4.19)

ΣDDT is the sum of o,p'-DDD, p,p'-DDD, o,p'-DDT, p,p'-DDT, o,p'-DDE and p,p'-DDE.

The lowest levels of ΣDDT were found in fish sampled in South Africa, with mean concentrations in liver tissue ranging from ca 110 to 210μg kg^{-1} lipid weight. Highest mean concentrations were found in muscle tissue of black scabbardfish sampled in Madeira, at ca 4270μg kg^{-1} lipid weight, then in *B. ferox* livers from Meriadzec at ca 3800μg kg^{-1} lipid weight.

B. ferox presented higher levels in Meriadzec than the Rockall Trough ($p=0.034$), up to 5 times higher. Black scabbardfish presented a similar trend ($p<0.001$). However, orange roughy did not present such a trend ($p>0.05$).

Monkfish showed higher levels in livers than muscle tissue ($p=0.002$), but black scabbardfish did not ($p>0.05$). In monkfish, concentrations increased with depth, in both muscle ($p=0.028$) and liver tissue ($p=0.015$), with for example ca 180 and 635 μg kg^{-1} lipid weight in livers between ca 80-250 and 770-850m depth respectively. Blue whiting presented a similar trend ($p=0.019$), with higher ΣDDT mean concentration at 640 than 300m depth (ca 500 and 220 μg kg^{-1} lipid weight respectively). Black scabbardfish also presented increasing concentrations with depth ($p=0.016$). Roundnose grenadier also presented increasing concentrations with depth, but in two discrete groups. Concentratins increased between 1000 and 1250m depth. Levels at 1300m were similar to 1000m depth, and increased again to 2000m depth. No other species presented significant pattern with depth.

ΣDDT concentrations in black scabbardfish were significantly lower at Rockall Trough than at Meriadzec ($p<0.001$), concentrations were similar at Meriadzec, Sesimbra and Madeira ($p>0.05$ in each case).

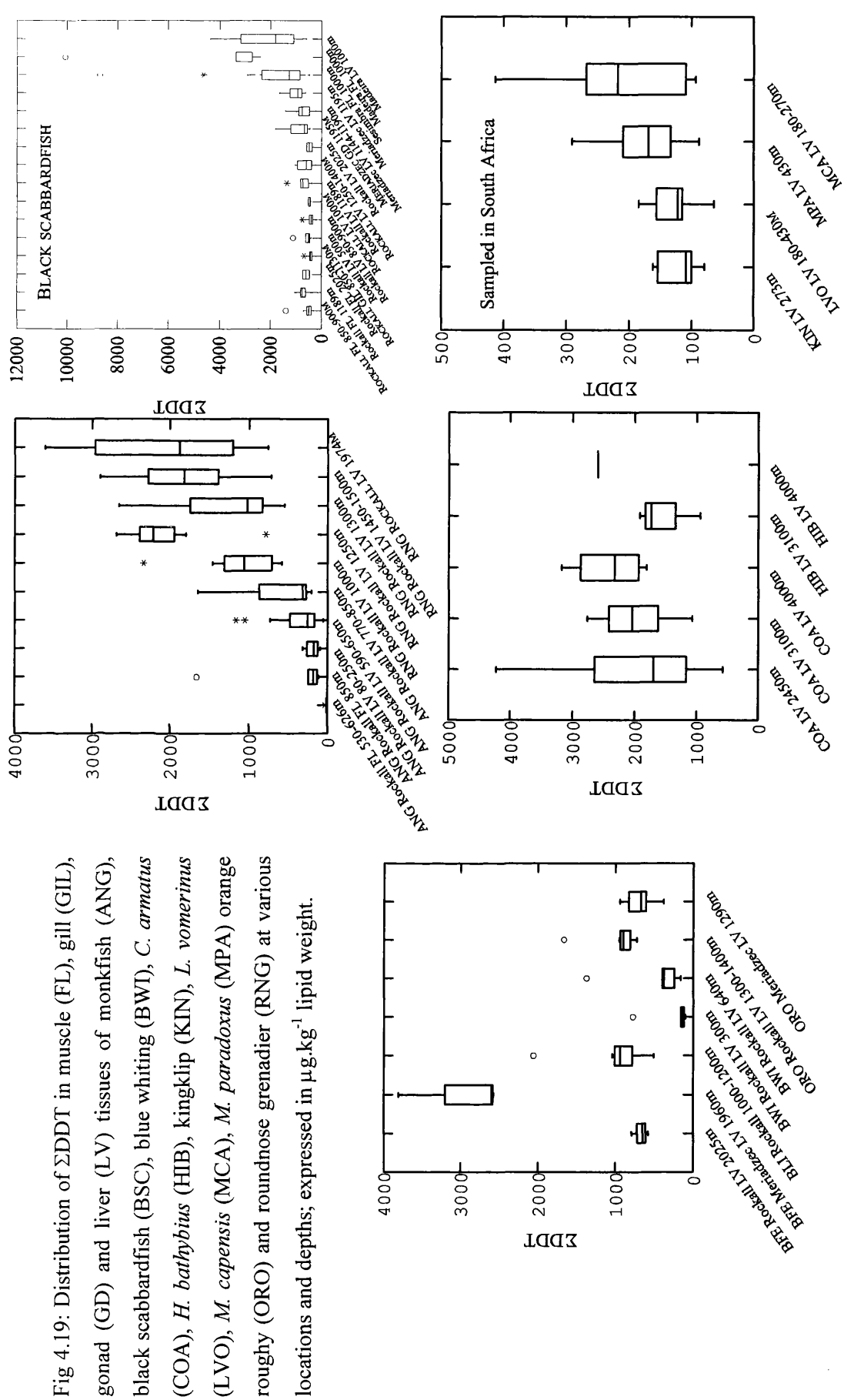


Fig 4.19: Distribution of ΣDDT in muscle (FL), gill (GIL), gonad (GD) and liver (LV) tissues of monkfish (ANG), black scabbardfish (BSC), blue whiting (BWI), *C. armatus* (COA), *H. bathybius* (HIB), kingklip (KIN), *L. vomerinus* (LVO), *M. capensis* (MCA), *M. paradoxus* (MPA) orange roughy (ORO) and roundnose grenadier (RNG) at various locations and depths; expressed in $\mu g.kg^{-1}$ lipid weight.

4.2.4. ΣChlordane (fig 4.20)

Σchlordane is the sum of α- chlordene, γ-chlordene, heptachlor-epoxide, α-chlordane, γ-chlordane, oxychlordane, transnonachlor and heptachlor.

The lowest levels of Σchlordane were found in fish sampled in South Africa, with mean concentrations in liver tissue ranging from ca 20 to 50μg kg^{-1} lipid weight. Highest mean concentration was found in muscle tissue of black scabbardfish sampled in Madeira, at ca 620μg kg^{-1} lipid weight.

B. ferox presented higher levels in Meriadzec than Rockall ($p=0.037$), up to 5 times higher. However, neither black scabbardfish nor orange roughy present such a pattern, with similar Σchlordane concentrations in Rockall and Meriadzec ($p>0.05$ in both cases).

Monkfish presented higher levels in livers than muscle tissue ($p<0.001$), whilst they were similar in muscle and livers of black scabbardfish ($p>0.05$). In blue whiting, concentrations increased with depth in liver tissues ($p=0.004$), with higher mean Σchlordane concentrations at 640 than 300m depth (ca 134 and 114 μg kg^{-1} lipid weight respectively). However, roundnose grenadier presented decreasing mean concentrations with depth, but in two discrete groups. Concentrations decreased between 1000 and 1250m depth. Levels at 1300m were similar to 1000m depth, and decreased again to 2000m depth, which is the reversed pattern to that found for ICES7CB, ΣCB and ΣDDT. No other species presented significant pattern with depth ($p>0.05$ in all cases).

In the case of black scabbardfish, mean Σchlordane concentrations did not show any specific trend with depth in liver or muscle tissues ($p>0.05$). Mean Σchlordane concentration in black scabbardfish did not increase significantly southwards ($p>0.05$ in all cases).

In general, ΣChlordane presented similar trends to ICES7CB, ΣCB and ΣDDT.

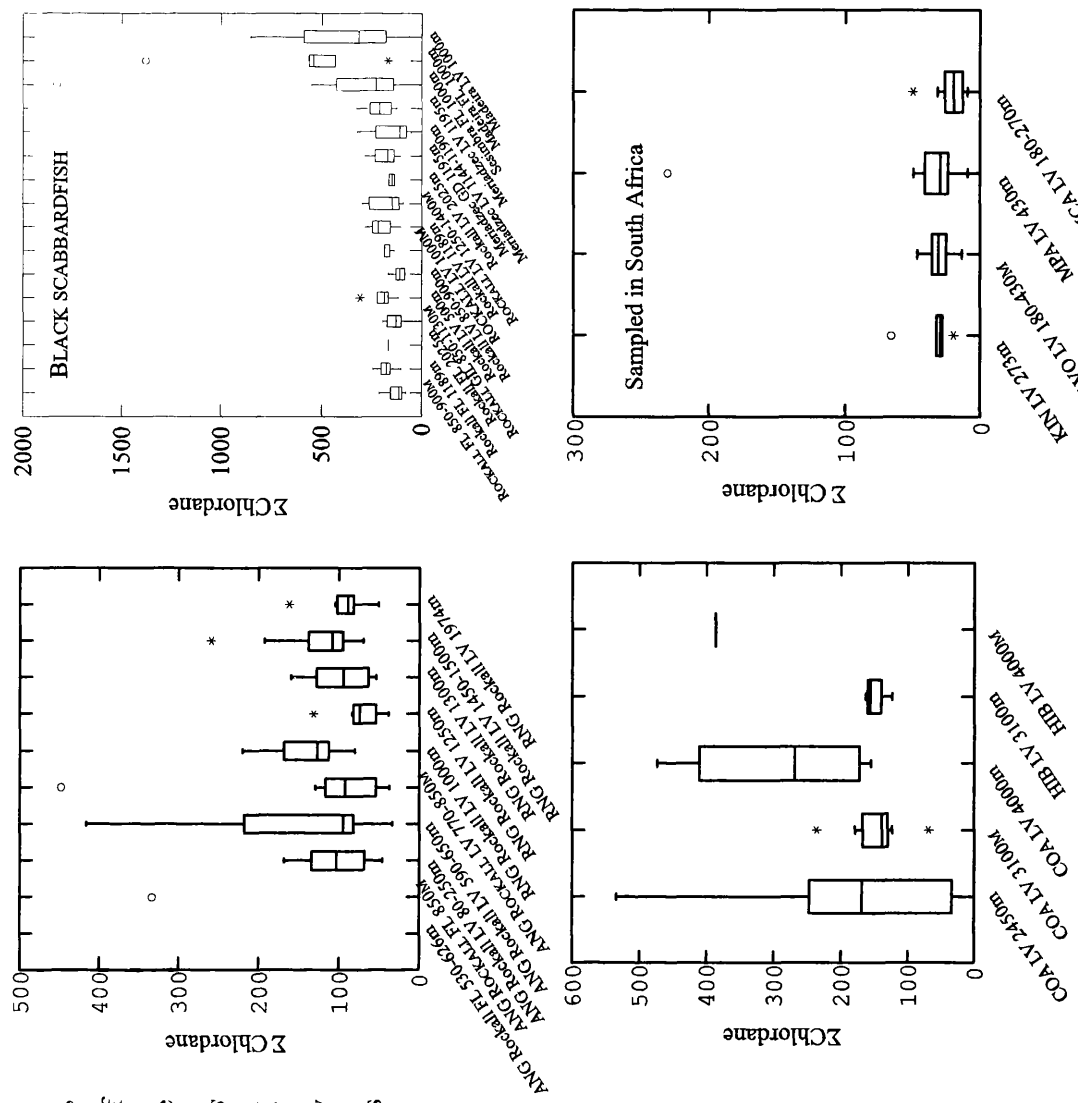


Fig 4.20: Distribution of ΣChlordane in muscle (FL), gill (GIL), gonad (GD) and liver (LV) tissues of monkfish (ANG), black scabbardfish (BSC), blue whiting (BWI), *C. armatus* (COA), *H. bathybius* (HIB), kingklip (KIN), *L. vomerinus* (LVO), *M. capensis* (MCA), *M. paradoxus* (MPA) orange roughy (ORO) and roundnose grenadier (RNG) at various locations and depths; expressed in $\mu g.kg^{-1}$ lipid weight.

4.2.5. ΣCB/ΣDDT and p,p'-DDE/ΣDDT ratios (fig 4.21)

The highest ΣCB/ΣDDT ratio was found in monkfish muscle from 530m depth, at ca 7. The lowest values were found in South African samples, with a ratio ranging between 0.48 and 0.86. The p,p'-DDE/ΣDDT ratio presented a typical value of ca 0.6, with higher values in fish sampled in South Africa (between 0.6 and 0.76). *B. ferox* sampled in Meriadzec and black scabbardfish sampled in Madeira at 2000m depth presented striking low values at ca 0.3.

Both ratios were similar in muscle and liver tissue of monkfish ($p>0.05$). In black scabbardfish, the ΣCB/ΣDDT ratio was higher in liver than muscle ($p=0.022$).

The samples presenting a ΣCB/ΣDDT ratio below 1 were the following: black scabbardfish muscle and liver tissues from the Rockall Trough and muscle tissue from Madeira; blue whiting, *H. bathybius*, all species caught in South Africa and all roundnose grenadier tissues. All other samples presented ratios above 1, ie higher concentration of ΣCB than ΣDDT. The ΣCB/ΣDDT ratio is an indicator of the provenance of the chemicals (Tanabe *et al.*, 1997). A ratio <1 represents a landmass with less industrialisation where DDT is still used for agricultural purposes, and a ratio >1 represents industrialised pollution where DDT has been banned in agricultural applications but where PCBs remain in use in the industrial sector.

Black scabbardfish did not present an increase in either ratio from Rockall to Meriadzec, Sesimbra and Madeira ($p>0.05$ in all cases). However, *B. ferox* did present an increase in the p,p'-DDE/ΣDDT ratio from Madeira to the Rockall Trough, which is the reverse from the ΣDDT pattern.

Monkfish presented an increase in the p,p'-DDE/ΣDDT ratio with depth ($p=0.001$), whilst blue whiting and roundnose grenadier presented a decrease of the p,p'-DDE/ΣDDT ratio with depth ($p=0.019$ and 0.001 respectively). Other species did not present any significant trend.

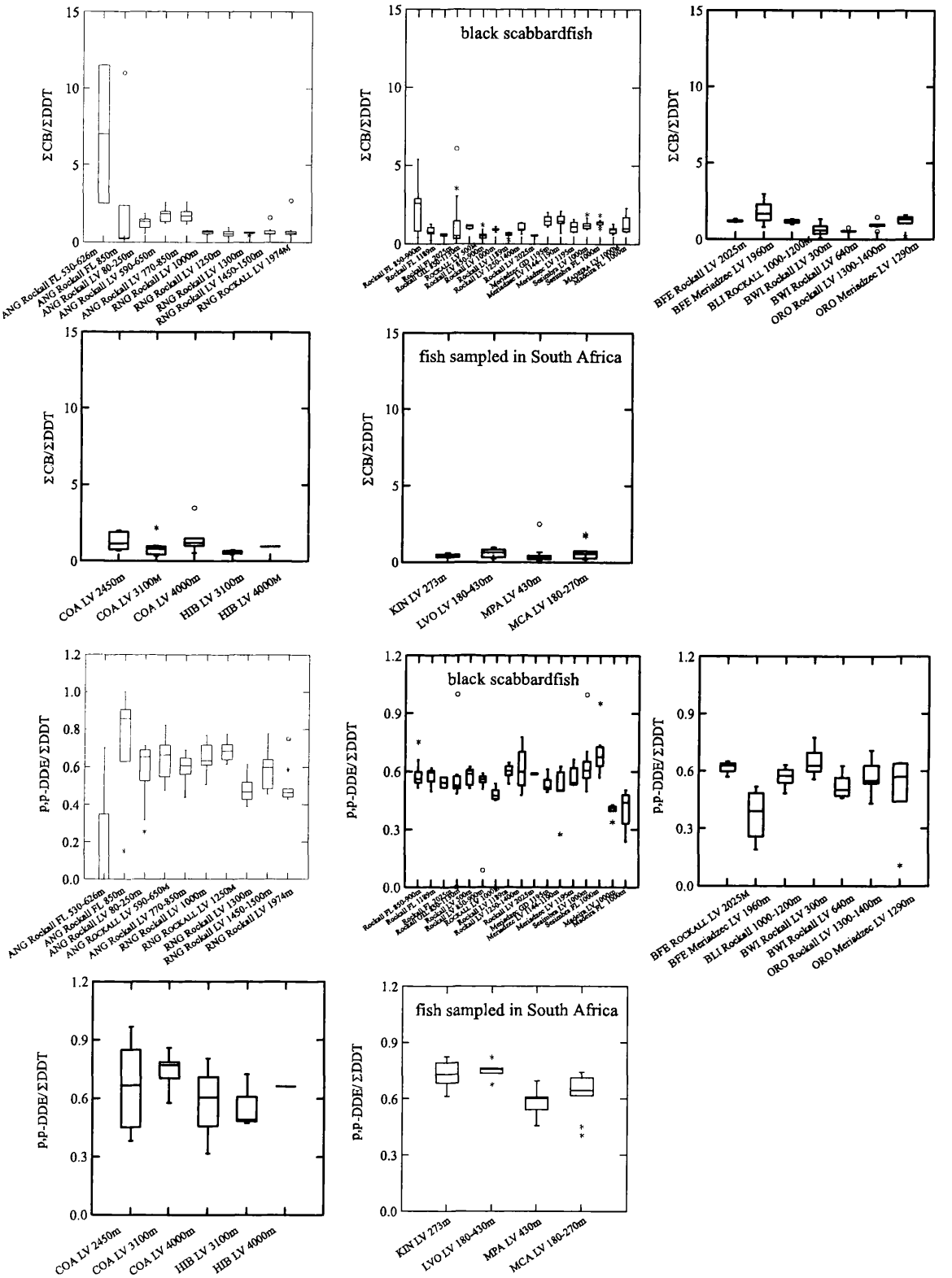


Fig 4.21: Distribution of the ΣCB/ΣDDT and p,p'-DDE/ΣDDT factors in muscle (FL), gill (GIL), gonad (GD) and liver (LV) tissues of monkfish (ANG), *B. ferox* (BFE), blue ling (BLI), black scabbardfish (BSC), blue whiting (BWI), *C. armatus* (COA), *H. bathybius* (HIB), kingklip (KIN), *L. vomerinus* (LVO), *M. capensis* (MCA), *M. paradoxus* (MPA), orange roughy (ORO) and roundnose grenadier (RNG) at various locations and depths.

4.2.6. CB distribution (fig 4.22)

PCBs were separated in discrete groups by degree of chlorination: trichlorinated (CB28 and 31), tetrachlorinated (CB44, 49, 52, 70 and 74), pentachlorinated (CB101, 105, 110, 114 and 118), hexachlorinated (CB128, 138, 149, 153, 156, 157 and 158), heptachlorinated (CB170, 180, 187 and 189) and octachlorinated (CB194) biphenyls.

Trichlorinated biphenyls represented between 0 and 7% of the PCBs. Tetrachlorinated biphenyls represented between 0 and 20% of total PCBs with an outstanding value at 34% in muscle tissue of black scabbardfish from the Rockall Trough. Pentachlorinated biphenyls represented typically between 10 and 30% of the total PCBs, with low values in black scabbardfish muscle and liver tissues sampled at 2000m depth from the Rockall Trough. Hexachlorinated biphenyls represented between 30 and 60% of the total PCBs, heptachlorinated biphenyls between 20 and 30%, and octachlorinated biphenyls between 0 and 2%.

Patterns were very different in muscle and liver tissues of monkfish (all sampled between 25 and 800m depth) and in muscle, liver and gill of black scabbardfish sampled between 500 and 800m depth, with high percentage of hexachlorinated biphenyls in livers (typically 60%) but not in muscle or gill tissue (between 15 and 40%). However, with depth, this difference tended to disappear in black scabbardfish, with a rising percentage of hexachlorobiphenyl with depth in muscle tissue, to reach levels found in liver tissues.

The percentage of hexachlorinated biphenyls was lower in *H. bathybius* and *C. armatus* sampled in the Porcupine Bank at depths greater than 2000m than in the other species, which were sampled at depths smaller than 1500m.

C. armatus and blue whiting presented increasing percentage of heptachlorinated biphenyls with depth. Roundnose grenadier and *H. bathybius* presented the opposite trend, and monkfish and black scabbardfish no trend at all. These patterns will be discussed in details in chapter 9.

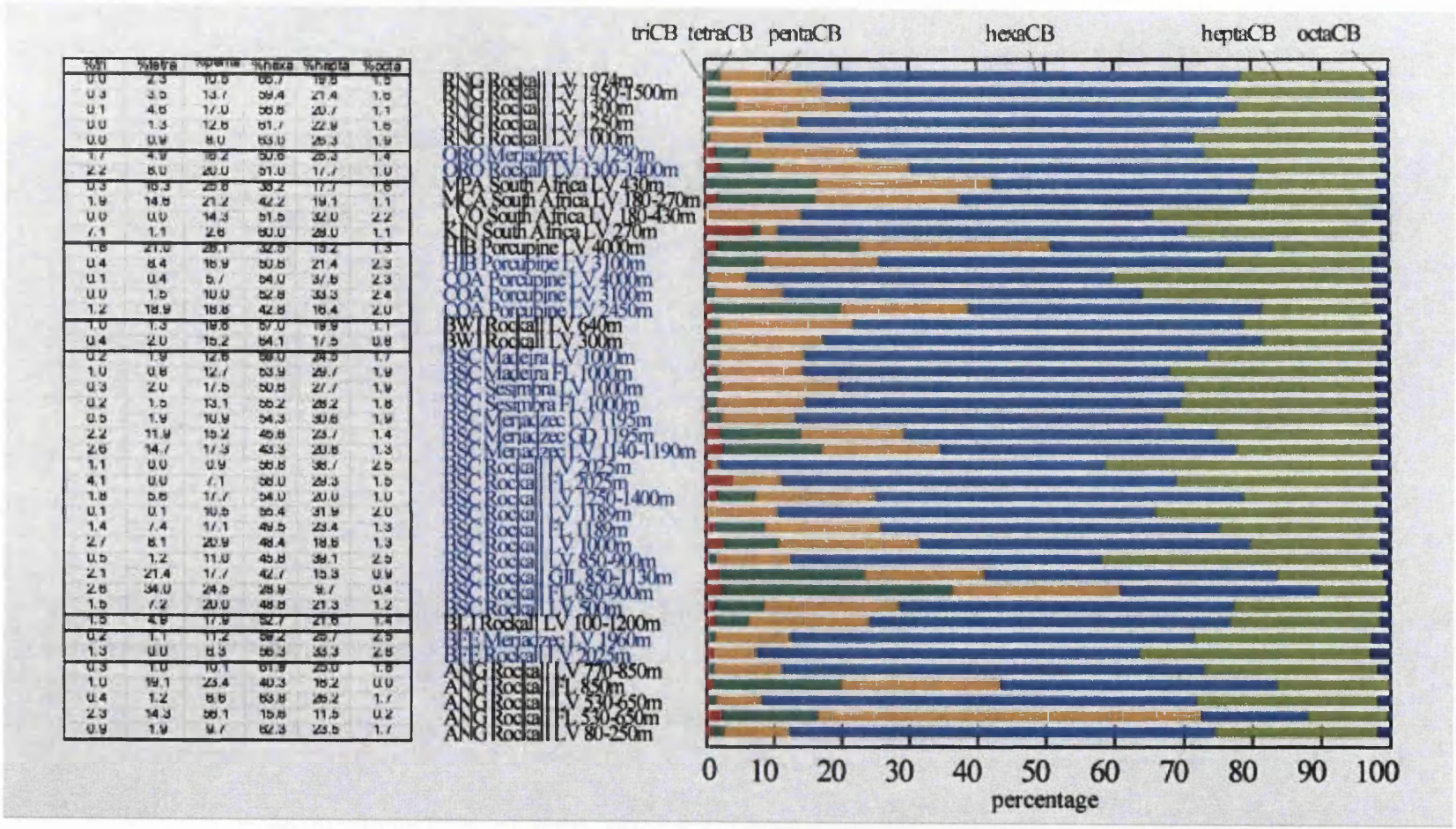


Fig 4.22: Contribution of the differently chlorinated CBs in muscle (FL), gill (GIL), gonad (GD) and liver (LV) tissues of monkfish (ANG), *B. ferox* (BFE), blue ling (BLI), black scabbardfish (BSC), blue whiting (BWI), *C. armatus* (COA), *H. bathybius* (HIB), kingklip (KIN), *L. vomerinus* (LVO), *M.. capensis* (MCA), *M. paradoxus* (MPA), orange roughy (ORO) and roundnose grenadier (RNG) at various locations and depths.

4.2.7. Conclusions

ICES7CB, ΣCB, ΣDDT and Σchlordane presented similar trends (sections 4.2.1. to 4.2.4.). Levels were higher in Meriadzec than in the Rockall Trough, but similar in Meraidzec, Madeira and Sesimbra; and higher in the North Atlantic than in the South Atlantic. Concentrations increased with depth in most cases.

ICES7CB and ΣCB presented very similar trends where ICES7CB was calculated as the sum of the 7 representative PCBs chosen by the ICES and ΣCB was calculated as the sum of the 25 PCBs which were available for determination in the Marine Laboratory. As they present similar trends, the ICES7CB is representative of the 25 PCBs measured in the present study and most probably of the 209 congeners as well.

PCB and pesticides concentrations and patterns with species and depth will be investigated in details in chapter 9.

Chapter 5
Health issues

In 1998, a study (Cronin *et al.*, 1998) of contaminants in deep sea fish showed some mercury concentrations failing limits imposed by some countries on food, and in general unusually high levels of contaminants. Two of the objectives of the present study were:

- to better define the risk of deep water fish failing to meet public health standards for heavy metals and organic contaminants
- to ensure the quality of deep water fish products in these respects

The previous chapter presented means and standard deviations of contaminant concentrations in the species studied. This chapter will emphasise those individual fish which present high levels of contaminants, their prevalence in a typical catch and, if and when possible, suggest ways of reducing the number of such individuals in commercial landings.

5.1. Metals

5.1.1. Guidelines on metal concentrations in food

Two publications set maximum levels of metal contamination in foodstuff and particularly in various fish tissue: the Ministry of Agriculture, Fisheries and Food (MAFF, 1995) and the European Commission (EC) Regulations (2001). The MAFF publication compiles food regulations from 1953 to 1993; the EC established new and stricter maximum levels for certain contaminants in foodstuff, which apply from 8 March 2001 (table 5.1).

Table 5.1: Maximum concentrations of metal contaminant concentrations in muscle and liver tissue of fish analysed during the present study compared with MAFF (1995) and EC (2001) regulatory limits, expressed in mg kg^{-1} wet weight.

element	maximum in muscle tissue	maximum in liver tissue	MAFF regulations	EC regulations
Cu	3.1	80.9	20	N/A
Zn	50.5	166.4	50	N/A
Cd	41.0	65.6	0.2	0.05
Hg	5.3	44.9	0.5-1	0.5-1
Pb	0.8	0.8	2	0.2

5.1.2. Discussion

The concentrations measured in fish during the present study were compared with the EC document where available, and otherwise with the MAFF guidelines (Table 5.2).

Table 5.2: Numbers and percentages of fish presenting metal concentrations above the strictest EC and MAFF regulations

	maximum (mg kg^{-1} wet weight)	number of samples above regulation	total samples analysed	% of samples above limits
in muscle				
Cu	3.1	0	577	0.0%
Zn	50.5	1	505	0.2%
Cd	41.0	13	571	2.3%
Hg	5.3	33	451	7.3%
Pb	0.8	23	577	4.0%
in liver				
Cu	80.9	83	597	14.0%
Zn	166.4	194	528	37.0%
Cd	65.6	561	597	94.0%
Hg	44.9	84	473	18.0%
Pb	0.8	33	597	5.0%

Out of the 597 liver samples analysed, only 32 did not exceed the EC (2001) regulations for one or more metals, which represents ca 5.4% of the number of fish analysed in this project. Livers are not commercially exploited in Scotland, but can be in other countries such as Portugal.

Up to 8% of fish muscle tissue presented levels of one or more metals above the regulations proposed by EC (2001): see table 5.3 for details.

Table 5.3: Percentage of fish presenting metal concentrations in muscle tissue higher than the MAFF (1995) and EC (2001) regulations.

Species	**location of capture**	**depth of capture (m)**	**% of samples above regulation**	**commercial interest?**
muscle tissue				
Monkfish	Rockall	80 to 850m	4.4% (3/68)	YES
B. ferox	Rockall	2000m	81.0% (17/21)	NO
blue ling	Rockall	1000 to 1200m	0.0% (0/32)	YES
black scabbardfish	Rockall	850 to 1140m	4.7% (7/146)	YES
	Meriadzec	1140m	10.3% (3/29)	YES (not by
	Madeira	1000m	12.0% (3/25)	Scottish fleet)
blue whiting	Rockall	300m	75.0% (15/20)	YES
		640m	0.0% (0/22)	YES
C. armatus	Porcupine	2500 to 4100m	4.2% (1/24)	NO
hake	Rockall	640m	18.1% (2/11)	YES
H. bathybius	Porcupine	2500 to 4100m	63.6% (7/11)	NO
kingklip *M. capensis* *M. paradoxus*	South Africa	170 to 450m	11.9% (5/42)	YES (not by Scottish fleet)
orange roughy	Rockall	1300 to 1400m	3.3% (1/30)	YES
roundnose grenadier	Rockall	1000 to 1500m	1.6% (1/61)	YES

Most species of commercial interest present very few individuals with metal concentrations above the regulations.

- Monkfish presented three individuals (4.4%) with metal concentration in muscle tissue above regulation, respectively mercury for a 56cm individual, zinc for a 41cm individual and cadmium for an 86cm individual. These three lengths are in the middle of the size range (41 to 132cm total length) and none of these metals increase in concentration with length. Hence no easy exclusion can be made to avoid these individuals who might present cadmium concentrations above regulation. However, the mean concentration in a catch of monkfish would be well within limits.

- Black scabbardfish presented one fish (0.7%) with high level of cadmium (85cm total length), and six (4.1%) with high levels of lead in muscle tissue

(respectively 93, 94 and 110cm total length). Levels of lead or cadmium do not increase with length or depth, but high levels are found in some specific individuals (fig 5.1). All were caught at depths greater than 1000m, although not all fish caught at depths greater than 1000m presented such high concentrations. The mean concentration of lead in black scabbardfish caught below 1000m was 0.07mg kg^{-1} wet weight, all fish were within the MAFF standard, and the mean concentration was well below the EC standard.

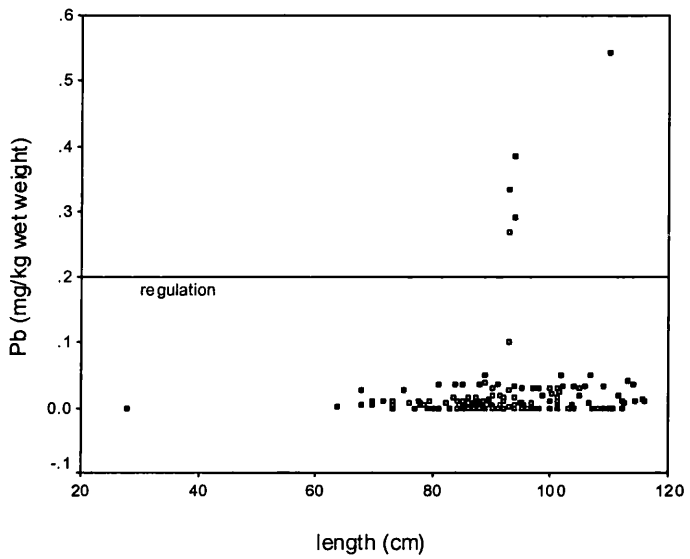


Fig 5.1: Lead concentrations in black scabbardfish from the Rockall Trough as a function of length

- Blue whiting presented fifteen individuals (75%) with lead concentrations in muscle tissue above the EC proposed regulation, all of which were caught at 300m depth. The waters at this depth are mostly composed of the Subarctic Intermediate Water. No fish exceeded the MAFF limit. All but two individuals caught at that depth presented high levels of lead although 25% were not above regulation limits. On the other hand, all individuals sampled at 640 or 830m depth presented very low levels of lead (fig 5.2). Moreover, blue whiting caught in the Rockall Trough are only migrating through the region. It is not clear why blue whiting show this pattern of lead contamination, or whether the pattern is persistent with location or time / season of capture as individuals have only been captured on two occasions for the present study: in October 1998 and September 2000. The current data suggests that not fishing blue whiting at 300m would significantly reduce the risk of fishing individuals with lead concentrations above

regulation. However, blue whiting probably move up and down the slope searching for food. Therefore, even though the present data is significant, not fishing shallower than 600m depth might not solve the problem.

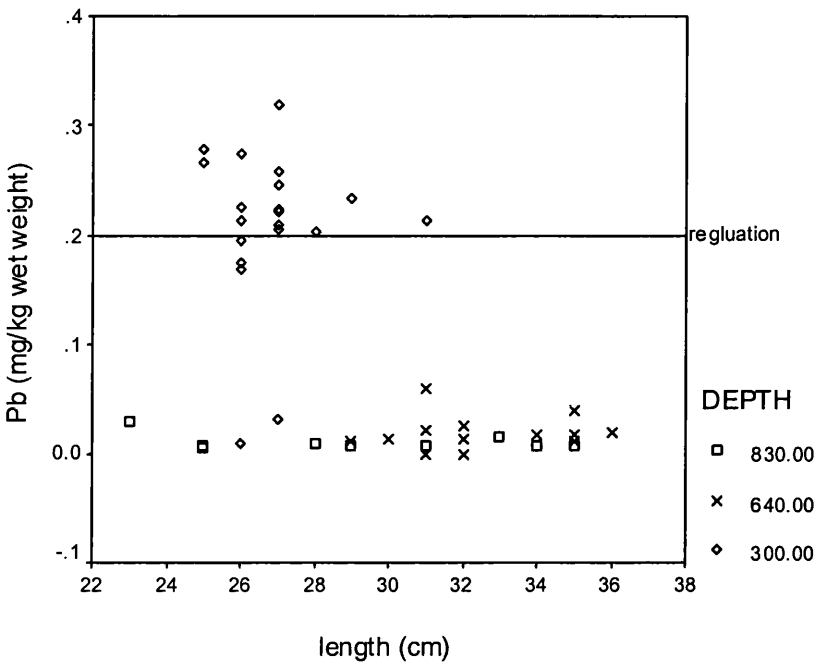


Fig 5.2: Lead concentrations in muscle tissue of blue whiting as a function of length (cm) and depth (m)

- Two out of 11 hake individuals (18%) presented concentrations of cadmium in muscle tissue higher than the regulation (fig 5.3). However, hake did not present increasing cadmium concentration with length and these two individuals were in the middle of the size range. No easy exclusion can be made to avoid these individuals who might present cadmium concentrations above regulation. No fish exceeded the MAFF limits.

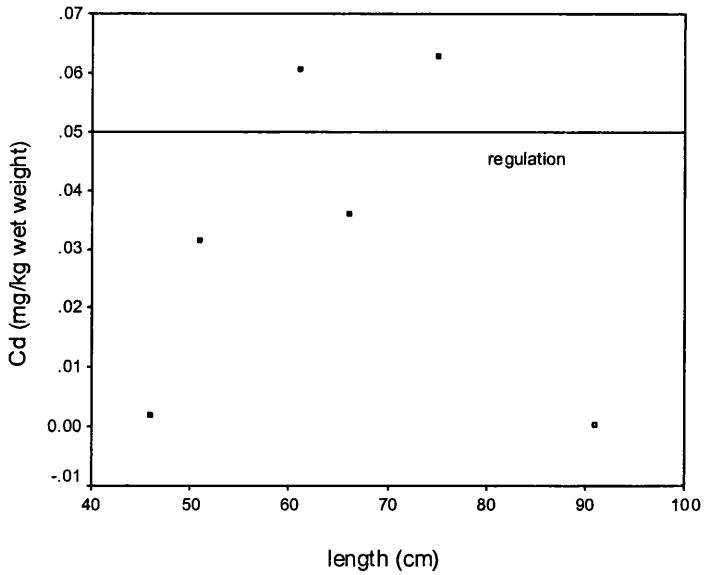


Fig 5.3: Cadmium concentrations in muscle tissue of hake as a function of length

- The only orange roughy specimen (3%) presenting metal concentrations in muscle tissue above regulation was 55.5cm total length, the biggest caught during the present study and contained high levels of mercury. Moreover, the concentration of mercury in orange roughy increased exponentially with length (fig 5.4). Considering the constant and coefficient of the exponential regression and their respective standard errors, only individuals bigger than 52.7cm total length might be expected to present mercury levels above regulations. Hence the risk of catching fish with levels of mercury above the regulation is not significant ($p<0.001$) in individuals smaller than 52.7cm total length. It is therefore advised to discard orange roughy individuals bigger than 52.7cm total length. However, the Scottish fleet does not fish deep enough to catch such large individuals, and the largest orange roughy landed in France measured 68cm (J.D.M. Gordon, pers.comm.). Therefore, discarding individuals over 52.7 cm in length would be of little financial repercussion, if any.

- A single individual roundnose grenadier (2%) presented cadmium concentration in muscle tissue above the regulation, in the middle of the length range (fig 5.5). Cadmium presented a very weak relationship with length and the individual with high cadmium concentration a strong outlier, which does not reflect the general level of cadmium contamination of this species.

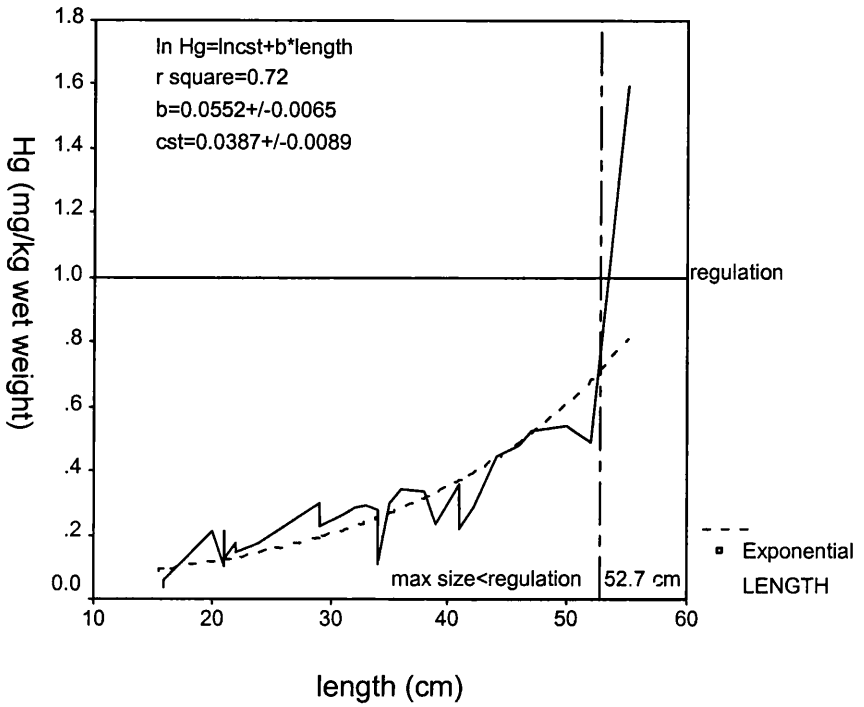


Fig 5.4: Mercury concentrations as a function of length in orange roughy

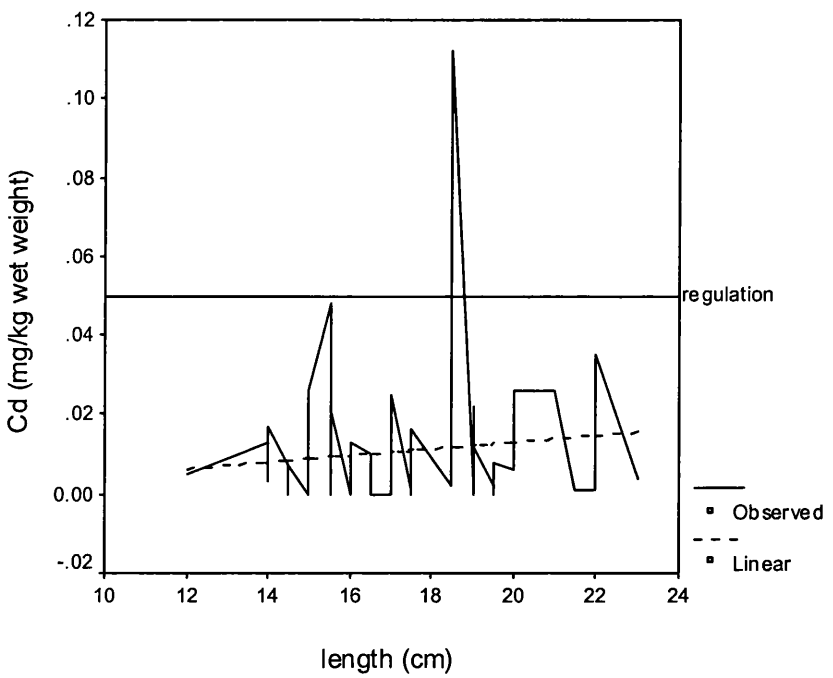


Fig 5.5: Cadmium concentrations as a function of length in muscle tissue of roundnose grenadier

- *B. ferox* and *H. bathybius*, both of no current commercial interest because both are found at depths greater than 2000m, presented a high proportion of fish with levels of metals higher than the limits. Hence, if deep sea fisheries are to move to

deeper grounds and new species in the future, heavy metal contamination might become a problem and would have to be thoroughly investigated.

5.1.3 Conclusions

The following recommendations are based on the previous discussion. Following these recommendations will not guarantee that 100% of fish caught will satisfy all the regulations concerning heavy metal concentrations but should significantly reduce the proportion of fish above EC regulations (2001), where it is feasible. Of all the fish sampled, only one individual monkfish presented metal concentrations above the MAFF regulatory limits, which was for zinc. Recommendations:

- black scabbardfish: if possible do not catch below 1000m depth to avoid breaching the EC regulations on cadmium and lead. Most black scabbardfish are caught shallower anyway.
- blue whiting: fish at 640m depth and deeper to avoid breaching the EC regulation on lead
- orange roughy: discard fish bigger than 52.7cm total length to avoid breaching the EC regulation on mercury
- livers: do not commercialise for human consumption as most of them present levels of a number of metals above EC and MAFF regulations.

No simple recommendations can be made to reduce the likelihood of individual monkfish, hake and roundnose grenadier exceeding the EC regulatory limits. The estimated risk of exceeding is 4, 18 and 2% respectively, although a relatively small number of hake were analysed.

All but one concentration were below the MAFF limits, and all the mean concentrations of metals in all these species were below the EC limits as well.

5.2. PCBs and pesticides

5.2.1. Guidelines on organic contaminants in food

The UK does not have maximum limits for PCBs and pesticides in foodstuffs. The main source of environmentally acceptable levels of PCBs and pesticides in fish comes from the OSPAR Commission (OSPAR, 1997). This organisation has set some Ecotoxicological Assessment Criteria (EAC) for a number of PCBs and pesticides in water, sediment, fish and mussel. The EAC for fish were set indicators for round and flat fish in European shelf waters. Some European and American regulations for levels in human food are also available in a JMG document (1992) and at www.epa.org respectively.

Table 5.4 summarises EAC and dietary regulations for PCBs and pesticides in round and flat fish such as plaice, cod, haddock and hake available through these documents, as well as the maximum concentrations of these contaminants obtained in the present study.

Table 5.4: Maximum concentrations of contaminants in muscle and liver tissue of fish analysed during the present study compared with Ecotoxicological Assessment Criteria and regulations, expressed in mg kg^{-1} wet weight

	maximum	**EAC**	**European dietary regulations (country)**	**US regulations (www.eap.com)**
in muscle				
Dieldrin	0.0047	0.005-0.05		0.3
γ-HCH	0.001	0.0005-0.005		
p,p'-DDE	0.212	0.005-0.05		5.0
ΣDDT	0.417		0.5 (Finland)	5.0
CB153	0.100	0.00025-0.0025	0.1 (Sweden)	
ICES7CB	1.849	0.001-0.01	1.0 (Norway)	
in liver				
Dieldrin	0.059	0.5		
γ-HCH	0.008	0.05		
p,p'-DDE	1.073	0.5		
ΣDDT	3.426		5 (Denmark, Germany)	
CB153	2.311	0.025		
ICES7CB	5.607	0.1	5 (Norway, Sweden)	

5.2.2. Discussion

5.2.2.1. Comparison with EAC

Table 5.5 summarises the number of fish analysed presenting concentrations higher than the EAC values.

Table 5.5: Number and percentage of fish above the Ecotoxicological Assessment Criteria

	maximum (mg kg^{-1} wet weight)	number of samples above EAC	total samples analysed	% of samples above regulation
in muscle				
Dieldrin	0.005	0	58	0.0%
γ-HCH	0.001	0	58	0.0%
p,p'-DDE	0.212	5	58	8.6%
ΣDDT	0.417	0	58	0.0%
CB153	0.100	30	58	51.7%
ICES7CB	1.849	28	58	48.3%
in liver				
Dieldrin	0.059	0	234	0.0%
γ-HCH	0.008	0	234	0.0%
p,p'-DDE	1.073	33	234	14.1%
ΣDDT	3.426	0	234	0.0%
CB153	2.311	151	234	64.5%
ICES7CB	5.607	126	234	53.8%

Although no fish showed values greater than the available food regulations, some contained concentrations greater than EAC. However, EAC "should be used to identify potential areas of concern and to indicate which substances could be considered as a priority, and should not be used as firm standards or as triggers for remedial action" (OSPAR, 1997).

The two contaminants of most concern (ie most frequently exceeding the EAC values) were CB153 and ICES7CB, with between 48 and 65% of samples above the EAC for both muscle and liver tissues. Table 5.6 indicates the percentage of fish above EAC per species and depth.

Table 5.6: Percentage of fish presenting levels of organic contaminants higher than the Ecotoxicological Assessment Criteria, per species and depth

species	Location	depth (m)	% of samples above EAC	commercial interest?
muscle tissue				
monkfish	Rockall	80 to 1000m	**8%**	YES
black scabbardfish	Rockall	850 to 1140m	**57%**	YES
	Meriadzec	1140m	**14%**	YES (but not
	Sesimbra	1000m	**92%**	by Scottish
	Madeira	1000m	**50%**	fleet)
liver tissue				
monkfish	Rockall	80 to 250m	**25%**	YES
		>500m	**71%**	YES
B. ferox	Rockall	2000m	**100%**	NO
blue ling	Rockall	1000 to 1200m	**100%**	YES
black scabbardfish	Rockall	850 to 1140m	**2%**	YES
	Meriadzec	1140m	**29%**	YES (but not
	Madeira	1000m	**38%**	by Scottish
	Sesimbra	1000m	**60%**	fleet)
blue whiting	Rockall	300 to 640m	**17%**	YES
C. armatus	Porcupine	2500 to 4100m	**100%**	NO
H. bathybius	Porcupine	2500 to 4100m	**75%**	NO
kingklip				YES (but not
M. capensis	South	170 to 450m	**0%**	by Scottish
M. paradoxus	Africa			fleet)
orange roughy	Rockall	1300 to 1400m	**84%**	YES
roundnose grenadier	Rockall	1000 to 2000m	**100%**	YES

Although Ecotoxicological Assessment Criteria (EAC) are only guideline concentrations and the UK does not currently enforce strict limits for these contaminants, most fish species presented a high proportion of individuals with contaminant concentrations in muscle tissue above the EAC.

B. ferox, *C. armatus* and *H. bathybius*, all collected deeper than 2000m, presented higher concentrations of contaminants than shallower species, with between 75 and 100% of individuals above EAC. Hence, species sampled below 2000m depth could generally present higher level of contaminants than species sampled shallower. In the case of fisheries moving to deeper waters and different species, background studies are advised to check the levels of contamination of potential new commercial species.

5.2.2.2. Comparison with tolerable weekly intake

The World Health Organisation has recently recommended a Tolerable Daily Intake (TDI) for PCBs of 1 to 4pg TEQ kg^{-1} body weight, where TEQ is the Toxic Equivalent (Department of Health, 1999). In November 2000, the European Scientific Committee on Food (SCF) fixed a temporary tolerable weekly intake (t-TWI) for dioxins and dioxin-like PCBs of 7pg TEQ kg^{-1} body weight (SANCO 1157/01). Moreover, the European Commission is currently drafting regulations on dioxin levels in all animal feed and human foodstuffs (SANCO/0384/01). Recommendations (SANCO/0385/01) include setting up similar regulations for PCB concentrations in all animal feed and human foodstuffs including fish oil, and gradually reducing the t-TWI. Stricter actions against fish meal and fish oil are recommended:

> "Emphasis should be put on reducing the impact of most contaminated feed materials eg fish meal and fish oil, on overall diet contamination. This could be achieved by substituting the most contaminated by lesser contaminated sources, by reducing their intrinsic contamination by using non (less) contaminated alternatives, continuing to meet the animal nutrient requirements" (SANCO/1157/01).

TEQ are calculated from the toxic equivalent factors (TEF), which were set up by the World Health Organisation (1997). Considering the temporary tolerable weekly intake of 7pgTEQ kg^{-1} body weight, an adult of 80kg would be able to consume up to 560pg TEQ weekly; and a 20kg child, 140pg TEQ weekly.

Total TEQ for PCBs could not be calculated precisely as important non-ortho PCBs (PCB 77, 81, 126 and 169) were not measured. Two different approaches have been used to overcome this problem. In the first instance, non-ortho CBs were considered negligible, and CB-TEQ approximated to the TEQ of mono-ortho CBs (fig 5.6 and 5.7). On the other hand, CB-TEQ was calculated from a conversion factor of CB153, assuming that TEQ CB = 0.526*CB153/100, factor established empirically from data on shelf-fish (G. Grewar, pers. comm.). However, this factor did not prove reliable in the case of the present study in deep sea fish as generated CB-TEQ concentrations 5 times lower than the ones calculated for muscle tissue, and 10 times higher for liver tissue, so will only provide outer limits.

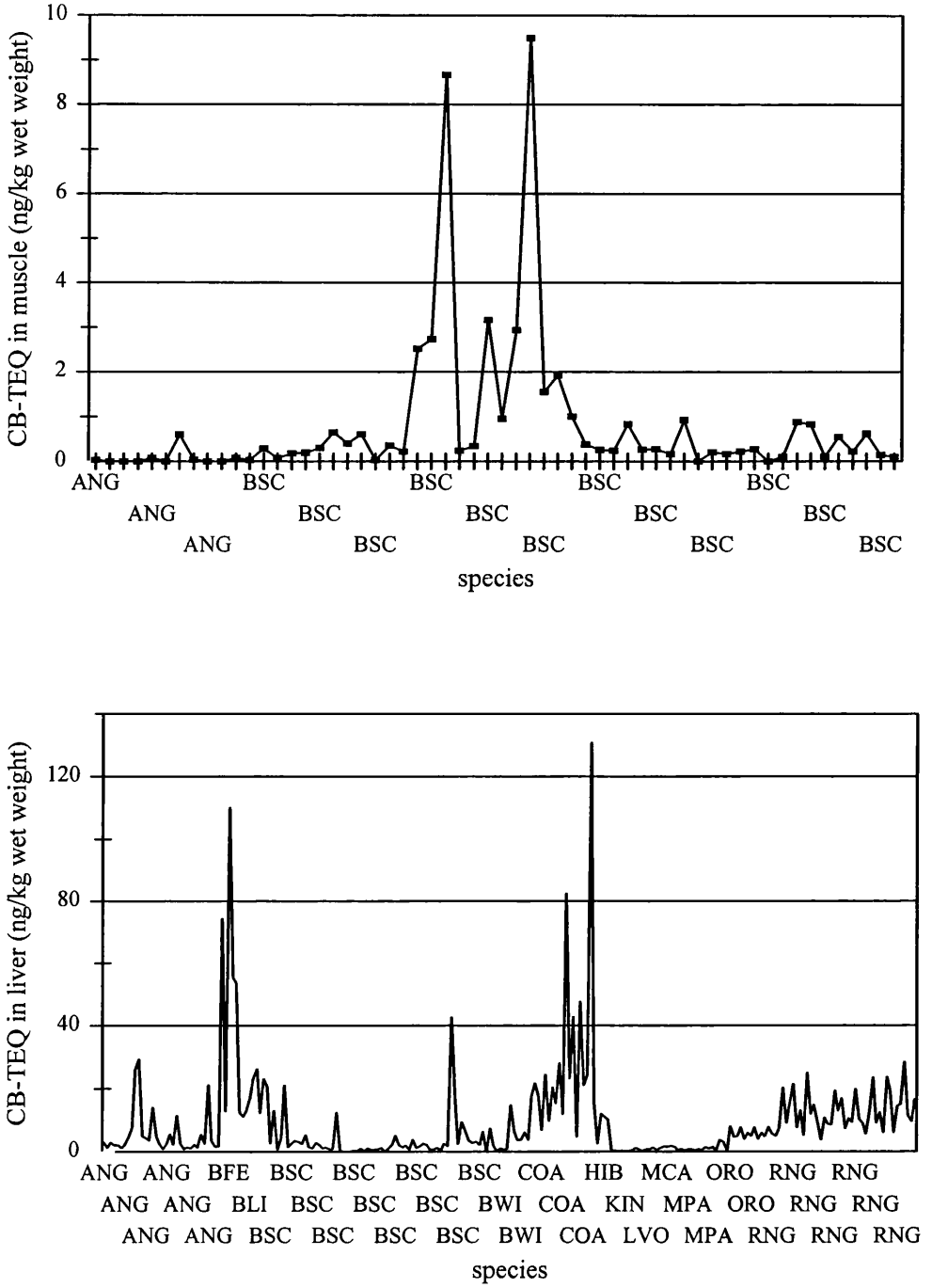


Fig 5.6: CB-TEQ (ng kg^{-1} wet weight of fish) based upon mono-ortho congeners in muscle and liver tissue of monkfish (ANG), *B. ferox* (BFE), blue ling (BLI), black scabbardfish (BSC), blue whiting (BWI), *C. armatus* (COA), *H. bathybius* (HIB), *L. vomerinus* (LVO), *M. capensis* (MCA), *M. paradoxus* (MPA), orange roughy (ORO) and roundnose grenadier (RNG).

In muscle tissue, the maximum TEQ is found in various black scabbardfish individuals, at about 10ng TEQ kg^{-1} wet weight of muscle. This means that an adult could consume only 56g of the most contaminated fish muscle weekly without exceeding the tolerable weekly intake; and a child, 14g. Supposing that a typical fish meal is constituted of 100g fresh weight of fish, these limitations represent a fish meal every other week for adults, and only one every 10 weeks for children. However, apart from these few black scabbardfish individuals, all of which were sampled in Sesimbra, the mean and maximum TEQ are below 1ng kg^{-1} wet weight. This level represents a weekly intake of at least 560g of fish for adults and of 140g for children, or 5 fish meals and one fish meal a week respectively.

In liver tissue, the maximum TEQ is found in *B. ferox*, *C. armatus* and *H. bathybius* at up to 130ng TEQ kg^{-1} wet weight of liver. However, none of these species are of current commercial interest at the present time as they live below 2000m depth. Apart from these species, the mean TEQ concentration is at ca 20ng TEQ kg^{-1} wet weight of liver. In these conditions, an adult consuming over 28g of liver weekly would exceed the tolerable weekly intake, and a child 7g. Moreover, if considering the TEQ calculated from CB153, this consumption would be reduced to 2.8 and 0.7g respectively. Hence, because of its PCB contamination, liver from these species is not suitable for human consumption.

Liver can also be commercialised as fish oil for animal feed. The PCB, and hence CB-TEQ, concentration in these products can be anticipated to be similar to these found in liver tissue when expressed on a lipid weight basis, as PCBs tend to accumulate in lipids. At a mean concentration of at least 20ng kg^{-1} wet weight CB-TEQ in liver tissue ie at least 50ng kg^{-1} lipid weight CB-TEQ, and potentially reaching concentrations ten times higher, fish oil from these deep sea fish species would most probably fail forthcoming contaminants controls even for use in animal feed.

5.2.3. Conclusions

From the present survey, apart from a few highly contaminated individuals, deep sea fish muscle tissue presents little risk to human consumption with regards to PCB and pesticide contamination and should not contravene existing UK and EU regulations or forthcoming EU regulations. However, if deep sea fisheries were to expand to the 2000m depth region and to new species, these should be checked for organic contaminants before commercialisation.

In the case of deep sea fish livers, the present study found most species unsuitable for human consumption, in the light of forthcoming EU regulations. Moreover, with the appearance of stricter EU regulations on fish meal and fish oil, deep sea fish livers are very likely to be unsuitable for such uses as well, although more research is probably needed to confirm this view.

A recent survey on organic contaminants in shallow water fish livers from Scottish waters (Webster *et al.*, 2000) has shown that shallow water fish species present similar or higher concentrations of PCBs in livers than deep water fish species, even in control sites. Hence the problem of the contamination of fish oil and fish meal is not only relevant to deep sea fish species, but also applies to shallow water species.

Further studies are advised, as well as a lookout for any future strict guidelines, if implemented.

Chapter 6
Pollutants in monkfish and black scabbardfish

Concentrations and patterns of metal and organic contaminants in monkfish and black scabbardfish, amongst other species, have been the subject of two publications and will be presented as such in the present chapter.

6.1. Heavy metal concentrations in commercial deep-sea fish from the Rockall Trough (*Continental Shelf Research*, 2001, 21: 899-916)

Levels and patterns of metals were published in the proceedings of the Atlantic Frontier Environmental Forum held in Aberdeen in August 1998, and reproduced in full in the present section. Earlier results were presented as a poster during that conference, but published elsewhere (Mormede and Davies, 1998, 2001a).

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Continental Shelf Research 21 (2001) 899–916

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Heavy metal concentrations in commercial deep-sea fish from the Rockall Trough

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Abstract

Samples of monkfish (*Lophius piscatorius*), black scabbard (*Aphanopus carbo*), blue ling (*Molva dypterygia*), blue whiting (*Micromesistius poutassou*) and hake (*Merluccius merluccius*) were obtained from 400 to 1150 m depth on the continental slope of Rockall Trough west of Scotland. Muscle, liver, gill and gonad tissue were analysed for arsenic, cadmium, copper, lead, mercury and zinc by various atomic absorption techniques. Median concentrations of arsenic in the muscle tissue ranged from 1.25 to 8.63 mg/kg wet weight; in liver tissue from 3.04 to 5.72 mg/kg wet weight; cadmium in muscle tissue from <0.002 to 0.034 mg/kg wet weight, in liver tissue from 0.11 to 6.98 mg/kg wet weight; copper in the muscle from 0.12 to 0.29 mg/kg wet weight, in the liver from 3.47 to 11.87 mg/kg wet weight; lead levels in muscle from <0.002 to 0.009 mg/kg wet weight, respectively, and in liver tissue <0.05 mg/kg wet weight for all species. In general, the concentrations are similar to those previously published on deep-sea fish, and higher or similar to those published for shallow water counterparts. All metal levels in black scabbard livers are much higher than in the other fish, and between 2 and 30 times higher than the limits of the European Dietary Standards and Guidelines. Differences in accumulation patterns between species and elements, as well as between organs are described using univariate and multivariate statistics (scatterplots, discriminant analysis, triangular plots).

Keywords: Accumulation; Contamination; Deep-sea fish; Discriminant analyis; Metals; Organ distribution

1. Introduction

Preceding studies (Mormede and Davies, 2001) have shown that heavy metals such as cadmium, mercury, lead, copper and zinc were found in relatively high concentrations in some deep-sea fish species (*Nezumia aequalis*, *Lepidion eques* and *Raja fyllae*). Some concern arose from

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that study, particularly in terms of safety for human consumption. The study showed interesting distribution and metabolisation patterns for the fish studied, and a need for more detailed work was indicated.

Deep-water fisheries are becoming more and more important, and there is a paucity of chemical monitoring of these recent fisheries and their products. The previous study was carried out on discarded fish of no commercial interest and small size. The current work has been carried out on commercial species.

This paper reports levels of arsenic, cadmium, copper, lead and zinc in various organs (muscle tissue, liver, gills, gonads) of the following commercial species: *Lophius piscatorius* (monkfish), *Aphanopus carbo* (black scabbard), *Molva dypterygia* (blue ling), *Micromesistius poutassou* (blue whiting) and *Merluccius merluccius* (hake).

2. Materials and methods

2.1. Species studied

All fish were collected in September and October 1998 in the Rockall Trough area (ICES statistical rectangles 41E0 to 46E1), during two deep-sea cruises of the research vessel FRV *Scotia*. The sampling took place between 56° and 59°N, at depths ranging from 400 to 1150 m. Thirty-eight monkfish were sampled in September, ranging from 43 to 122 cm total length, as well as 54 black scabbard ranging from 64 to 116 cm total length. Fifteen blue ling were sampled in October, ranging from 66 to 140 cm total length, along with nine blue whiting ranging from 23 to 35 cm total length, and nine hake ranging from 51 to 91 cm total length.

All fish are currently commercially exploited in the Rockall Trough area, by both French and Scottish fleets. The following species descriptions are based on Whitehead et al. (1984).

2.1.1. Lophius piscatorius (monkfish)

Habitat: benthic, from shallow, inshore waters to 500 m. *Diet*: mainly fish such as whiting and cod, *Nephrops* and occasionally sea birds. *Reproduction*: spawning from February to July. *Distribution*: Mediterranean, Black Sea, eastern North Atlantic from Straits of Gibraltar to south western Barents Sea.

2.1.2. Aphanopus carbo (black scabbard)

Habitat: oceanic benthopelagic on continental slope or underwater rises at about 200–1600 m, juveniles mesopelagic. Migrating to midwater at night. Caught commercially with special deep-water lines off Madeira and, to a lesser extent, Portugal. *Food*: cephalopods, fish, crustaceans. *Distribution*: eastern Atlantic, from Denmark Strait, Iceland and Norway to Madeira and western North Africa. Elsewhere, known from many localities in Atlantic, Indian and Pacific oceans.

2.1.3. Molva dypterygia (blue ling)

Habitat: deepwater, usually 350–500 m, rarely 200 and 1000 m. *Food*: crustaceans, fish. *Reproduction*: spring, usually from May to beginning of June at 500–600 to 1000 m. *Distribution*: north-eastern Atlantic from Iceland, western Barents Sea, Trondheim Fjord, Skagerrak, Kattegat

to Scotland, Ireland, Bay of Biscay and Morocco, also western Mediterranean. Elsewhere, western North Atlantic (rare).

2.1.4. Micromesistius poutassou (blue whiting)

Habitat: mesopelagic, over depths of 160–300 m in midwater, 30–400 m from the surface, also near the bottom at 180–400 m; immature live in shallow waters, occasionally inshore. *Food*: small crustaceans, amphipods, rarely fish. *Reproduction*: from February in the South to May in the North. *Distribution*: North Atlantic, from western Barents Sea, Spitzbergen, Iceland and Greenland, Skagerrak to Morocco, western Mediterranean, also western North Atlantic.

2.1.5. Merluccius merluccius (hake)

Habitat: midwater or at bottom, chiefly 100–300 m, at edge and slope of continental shelf; feeds mainly in midwater at night, returning to the bottom at daytime. *Food*: almost entirely fish, in deep-water blue whiting, in summer whiting. *Distribution*: north-eastern Atlantic, Mediterranean and Black Sea. Elsewhere, southwards recorded at Port-Etienne.

2.2. Methods

The fish were dissected onboard, on the day of capture. Muscle and liver were sampled from all fish; gills were also sampled from monkfish and black scabbards, and gonads from monkfish. The dissected organs were individually wrapped in aluminium foil and put in plastic bags to allow analysis for major trace metals and subsequently for organic contaminants. then frozen at −20°C onboard. Homogenised sub-samples of muscle tissue (ca. 10 g), liver (ca. 1 g), gills (ca. 5 g) and gonads (ca. 5 g) were digested in boiling nitric acid and made up to 25 ml with distilled water.

Copper and zinc were analysed by flame atomic absorption spectroscopy with deuterium background correction, the limits of detection were both of 0.02 ppm in solution; which represents a limit of detection of ca. 0.05, 1 and 0.5 mg/kg wet weight in muscle tissue, liver tissue and both gills and gonads tissue, respectively. Lead, cadmium and arsenic were analysed by graphite furnace atomic absorption spectroscopy with L'Vov platform, Zeeman background correction and palladium matrix modifier; the limits of detection were of 1 ppb for all three elements, which represents a limit of detection of ca. 0.002, 0.05 and 0.005 mg/kg wet weight in muscle tissue, liver tissue and both gills and gonads tissue, respectively.

The quality control of the data was provided throughout the analyses by the use of the certified reference material Dorm-2 (dogfish muscle, National Research Council, Canada) in every batch of 40 samples analysed (see Table 1).

3. Results

The median concentrations of trace metals for each species and each organ are summarised in Table 2 and Figs. 1–4. The quality control data are summarised in Table 1. All concentrations are calculated on a wet weight basis — but the control data which is in dry weight basis.

Table 1
Quality control data (concentrations expressed in mg/kg dry weight)

	As	Cd	Zn	Pb	Cu
	18.4	0.037	26.7	0.073	2.38
	18.0	0.039	27.6	0.061	2.43
	18.6	0.040	25.1	0.064	2.35
	17.1	0.039	26.4	0.063	2.46
	17.2	0.043	24.8		
	18.8	0.036	26.9		
	18.3	0.049			
	18.1				
Analysed	18.1 ± 0.6	0.040 ± 0.004	26.2 ± 1.0	0.065 ± 0.004	2.40 ± 0.04
Certified value	18.0 ± 1.1	0.043 ± 0.008	25.6 ± 2.3	0.065 ± 0.007	2.34 ± 0.16

Table 2
Concentration ranges and median of the five fish species (expressed in mg/kg wet weight)[a]

	Depth	Length	Weight	As	As	As	As	Cd	Cd	Cd	Cd
	(m)	(cm)	(g)	Muscle	Gonad	Gills	Liver	Muscle	Gonad	Gills	Liver
Monk ($n = 38$)											
Min	409.0	43.0	1278.0	2.70	0.38	0.16	1.44	<0.002	<0.005	<0.005	<0.05
Max	852.0	122.0	29000.0	21.47	3.65	4.19	14.33	0.041	0.034	0.064	1.34
Median	626.0	62.0	3030.0	8.63	1.63	1.29	5.72	<0.002	<0.005	0.021	0.19
SD	104.7	17.0	5736.7	4.77	0.70	0.84	2.43	0.008	0.009	0.014	0.36
Black scabbard ($n = 54$)											
Min	696.0	63.6	282.0	<0.02		<0.005	<0.05	<0.002		0.007	2.06
Max	1134.0	116.0	5928.0	26.49		5.87	35.79	0.017		0.120	18.24
Median	871.0	89.5	915.0	1.25		0.91	5.45	0.004		0.032	6.98
SD	90.8	13.6	1368.0	3.49		0.97	7.64	0.004		0.021	3.88
Blue ling ($n = 15$)											
Min	845.0	66.0	1000.0	1.84			<0.02	<0.002			<0.55
Max	866.0	140.0	13500.0	13.09			32.44	0.004			1.59
Median	866.0	88.0	2935.0	8.03			3.04	0.002			0.11
SD	9.3	20.0	3149.1	3.10			7.61	0.001			0.45
Blue whiting ($n = 9$)											
Min	830.0	23.0	87.0	0.37			1.52	0.001			0.06
Max	830.0	35.0	309.0	6.10			13.74	1.178			1.29
Median	830.0	29.0	166.0	2.24			5.49	0.022			0.85
SD	0.0	4.1	74.1	1.82			3.58	12.848			0.42
Hake ($n = 9$)											
Min	640.0	46.0	647.0	0.08			<0.05	<0.002			<0.05
Max	640.0	91.0	6102.0	3.30			7.59	0.063			1.43
Median	640.0	66.0	1659.0	1.37			3.29	0.034			0.22
SD	0.0	13.2	1556.0	0.92			2.17	0.025			0.47

[a]SD = standard deviation.

3.1. Median concentrations

Median arsenic concentrations varied from 1.25 to 8.63 mg/kg wet weight in the muscle, with the highest levels in monkfish and blue ling, from 0.91 to 1.29 mg/kg wet weight in the gills, and 3.29–5.72 mg/kg wet weight in the liver, with highest levels in monkfish, black scabbard and hake (see Table 2 and Fig. 2).

Median cadmium concentrations varied from 0.0007 to 0.034 mg/kg wet weight in the muscle, with the highest levels in blue whiting and blue ling, from 0.0215 to 0.0318 mg/kg wet weight in the gills, and 0.1065 to 6.9753 mg/kg wet weight in the liver, with highest levels in black scabbard, by a tenfold (see Table 2 and Fig. 3).

Median copper concentrations varied from 0.12 to 0.29 mg/kg wet weight in the muscle, with the highest levels in blue whiting and hake, 0.57 and 0.88 mg/kg wet weight in the gills of monks and black scabbard, respectively, and 3.47–11.87 mg/kg wet weight in the liver, with highest levels in black scabbard (see Table 2 and Fig. 4).

Cu	Cu	Cu	Cu	Pb	Pb	Pb	Pb	Zn	Zn	Zn
Muscle	Gonad	Gills	Liver	Muscle	Gonad	Gills	Liver	Muscle	Gills	Liver
0.06	<0.50	<0.50	1.45	<0.002	<0.005	<0.005	<0.05			
0.22	2.99	0.84	36.44	0.041	0.064	0.283	0.074			
0.15	0.56	0.57	6.53	<0.002	<0.005	0.006	<0.05			
0.03	0.64	0.12	9.00	0.007	0.012	0.046	0.018			
0.07		<0.50	<1.00	<0.002		<0.005	<0.05	2.12	9.06	29.42
0.27		1.35	39.05	0.051		0.342	0.471	3.90	18.85	108.70
0.12		0.88	11.87	0.009		0.013	<0.05	2.85	12.63	62.35
0.05		0.18	8.53	0.012		0.074	0.071	0.37	1.98	15.21
0.10			<1.00	<0.002			<0.05			
0.41			7.10	0.008			0.336			
0.15			3.95	0.003			<0.05			
0.08			1.73	0.002			0.080			
0.19			2.56	0.005			<0.05			
0.45			10.18	0.030			0.061			
0.29			3.47	0.008			<0.05			
0.09			2.21	0.007			0.018			
0.16			<1.00	<0.002			<0.05			
0.53			17.89	0.047			0.159			
0.27			6.50	0.008			<0.05			
0.10			4.89	0.016			0.042			

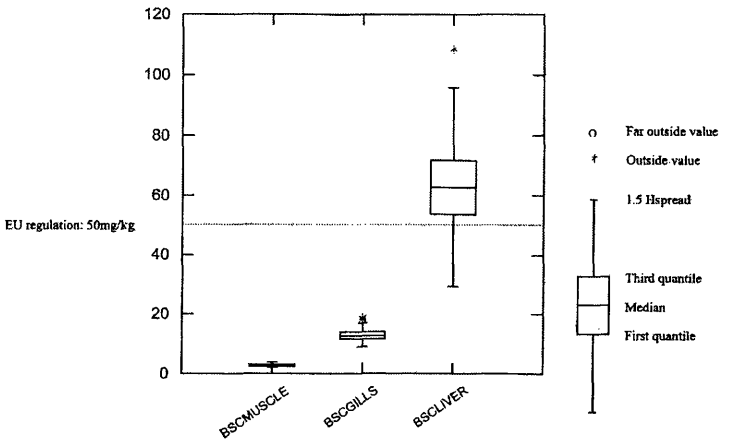

Fig. 1. Zn (mg/kg) distribution in black scabbard (BSC).

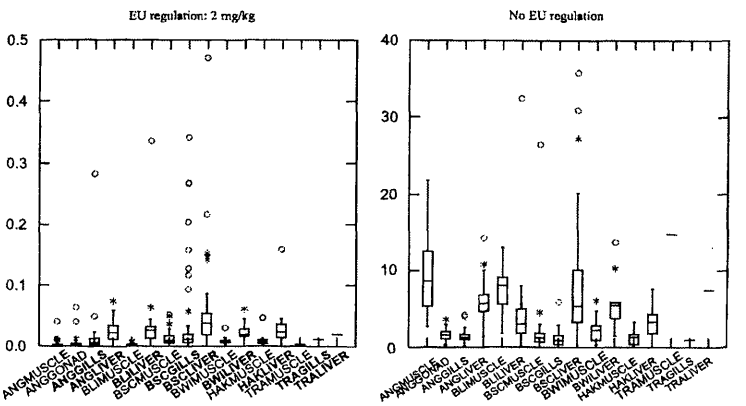

Fig. 2. Pb and As (mg/kg) distribution in various tissues of monkfish (ANG), blue ling (BLI), black scabbard (BSC), blue whiting (BWI), hake (HAK) and ray (TRA).

Median lead concentrations varied from 0.0016 to 0.0094 mg/kg wet weight in the muscle, with the highest levels in black scabbard, from 0.0064 to 0.0125 mg/kg wet weight in the gills, and 0.0196 to 0.0378 mg/kg wet weight in the liver, with highest levels in black scabbard (see Table 2 and Fig. 2).

Median levels of zinc in black scabbard were of 2.85, 12.63 and 62.35 mg/kg wet weight, respectively, in the muscle tissue, gills and liver (see Table 2 and Fig. 1).

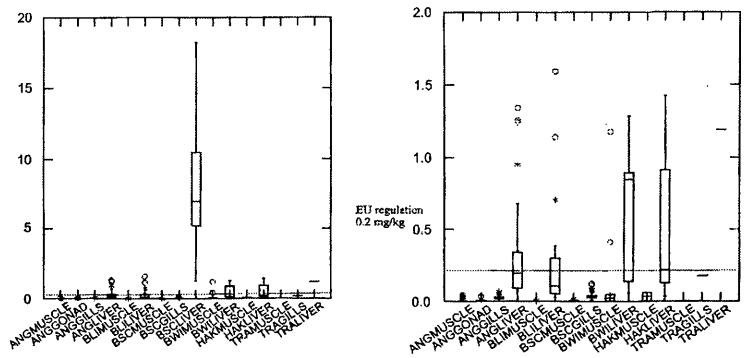

Fig. 3. Cd distributions (mg/kg) with and without the levels in the liver of black scabbard in various tissues of monkfish (ANG), blue ling (BLI), black scabbard (BSC), blue whiting (BWI), hake (HAK) and ray (TRA).

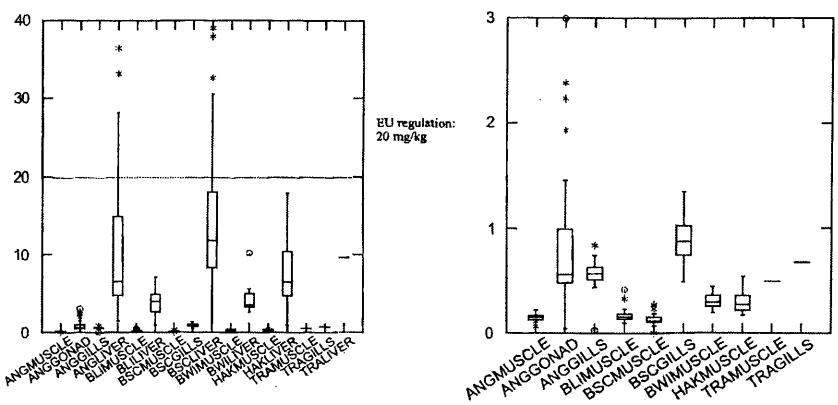

Fig. 4. Cu distributions (mg/kg) with and without levels in livers in various tissues of monkfish (ANG), blue ling (BLI), black scabbard (BSC), blue whiting (BWI), hake (HAK) and ray (TRA).

3.2. *Distribution between organs*

The highest levels of arsenic were found in muscle tissue for monkfish and blue ling, followed by livers then gonad and gills; and in liver, followed by muscle and gonads for the three other species. Levels of cadmium, copper and lead were highest in liver for all species, by a factor 10 at least compared to other organs, followed by gills, then gonads and eventually muscle.

3.3. Correlations

Matrices of Pearson's correlation coefficients for linear correlation between untransformed data on fish length and weight, and concentration of arsenic, cadmium, copper, lead and zinc were calculated for each species separately. The confidence levels of the correlations were calculated using Bonferroni's probabilities. The significant correlations are listed in Table 3.

In all five species, length and weight were very highly positively correlated. In monkfish, the level of copper in the gonads were very highly positively correlated with length and weight, and highly correlated with lead in gonads. The level of cadmium in gonads and in gills were positively correlated.

In black scabbard, cadmium in muscle and in gills were very highly negatively correlated with the length. All other correlations were positive. Cadmium in muscle was very highly correlated

Table 3
Summary of Pearson's correlation coefficients[a]

Species			Pearson's coefficient	Bonferroni's probability
Lophius piscatorius	Weight	Length	0.921	0.000
	Cu gonads	Length	0.711	0.000
		Weight	0.749	0.000
		Pb gonads	0.637	0.009
	Cd gonads	Cd gills	0.597	0.033
Alphanopus carbo	Weight	Length	0.631	0.000
	As gills	As liver	0.489	0.031
	Cd muscle	Length	−0.614	0.000
		As liver	0.523	0.009
		Cu muscle	0.634	0.000
		Pb liver	0.542	0.004
	Cd gills	Length	−0.570	0.001
		As liver	0.484	0.037
		Cd muscle	0.747	0.000
		Cu muscle	0.522	0.009
		Pb liver	0.650	0.000
	Cu gills	Weight	0.493	0.027
	Pb gills	Weight	0.726	0.000
		Pb muscle	0.551	0.003
		Zn gills	0.536	0.007
	Zn gills	Weight	0.568	0.001
	Zn muscle	Cu muscle	0.528	0.007
	Zn liver	Cd liver	0.479	0.044
Molva dypterygia	Length	Weight	0.953	0.000
Micromesistius poutassou	Length	Weight	0.969	0.001
Merluccius merluccius	Length	Weight	0.952	0.000

[a]The correlations confidences are based on Bonferroni's probabilities (SYSTAT, 1996): $p<0.001$, very highly correlated; $p<0.01$, highly correlated; $p<0.05$, correlated.

with copper in muscle, and highly correlated with both lead and arsenic in liver. Cadmium in gills was very highly correlated with cadmium in muscle and lead in liver, highly correlated with copper in muscle and correlated with arsenic in livers. Copper, lead and zinc in gills were all highly correlated to the weight. Lead in gills was also highly correlated with lead in muscle and zinc in gills. Zinc and copper in muscle were highly correlated, and zinc and cadmium in liver correlated.

In blue ling, blue whiting and hake, only length and weight were significantly correlated. No other correlations were evident probably due to the small number of fish sampled.

4. Discussion

4.1. Comparison with the European dietary standards and guidelines

Dietary standards and guidelines applicable in the UK for trace metals in fish have been summarised by MAFF (Ministry of Agriculture, Fisheries and Food, 1995) for cadmium, copper, lead, mercury and zinc. No guidelines exist for arsenic in fish.

Cadmium (Fig. 3): median levels of cadmium in livers of black scabbard were 30-fold higher than the guideline of 0.2 mg/kg wet weight, all individuals were above the limits and the most contaminated livers were about 100-fold above the limits. Medians of levels in monkfish, blue whiting and hake livers were also above the limits, as well as part of the levels in the livers of blue ling, but no value exceeded 2 mg/kg wet weight. Levels in the other organs, including flesh, were well under the European limits.

Copper (Fig. 4): only few specimens of monkfish and black scabbard presented livers with values above the European limit of 20 mg/kg wet weight. All other organs presented levels less than 2 mg/kg wet weight.

Lead (Fig. 2): all levels in all organs were lower than 0.5 mg/kg wet weight whereas the European guidelines quote 2 mg/kg wet weight as a safe limit.

Zinc (Fig. 1): almost all zinc levels in black scabbard liver were above the limit of 50 mg/kg wet weight, but muscle tissue and gills presented levels far under 20 mg/kg wet weight.

Overall, levels found in flesh were not of concern for human health, and most people do not consume livers. However black scabbard livers are considered as a delicacy in countries such as Portugal and Madeira, which arouses concern about the safety of the health of consumers.

4.2. Comparison with published data

Related publications on heavy metals in deep-sea fishes include Windom et al. (1987), Vas et al. (1993), Cronin et al. (1998) and Mormede and Davies (2001) (see Table 4).

The concentrations found in muscle tissue during this study are similar to those found by Windom et al.(1987) after applying a correction factor of six for dry-to-wet weight conversion. The results for muscle tissue from the other publications are summarised in Table 4. All concentrations are of the same order of magnitude, apart from cadmium sometimes present in very high concentrations in some deep-sea fishes.

Table 4
Comparison with previous data on metal concentration in fish muscle tissue (mg/kg wet weight)

Study	Species		As	Cd	Cu	Pb	Hg	Zn
Cronin et al. (1998)	*Coryphaenoides Rupestris*	Range		ND–0.01	0.03–0.54	ND–0.06	0.02–0.28	1.7–2.9
		Median		0.002	0.08	0.004	0.07	2.2
	Macrourus Berglax	Range		ND–0.21	ND–0.24	0.003–0.04	0.15–0.88	2.8–3.9
		Median		0.010	0.01	0.010	0.34	3.2
	Haplosethes Atlanticus	Range		ND–0.01	0.04–0.19	ND–0.66	0.11–0.86	2.0–3.4
		Median		0.010	0.09	0.010	0.42	2.7
	Coryphaenoides Mediterrana	Range		ND–0.07	ND–0.89	0.07–2.4	0.02–0.34	2.6–8.5
		Median		0.020	0.48	0.72	0.07	5.0
	Coelorhynus Labiatus	Range		0.01–0.41	0.33–0.70	0.31–0.97	0.12–0.50	5.0–10.6
		Median		0.020	0.40	0.57	0.17	6.7
	Nematonurus Armatus	Range		0.01–0.13	0.20–0.60	0.07–0.44	0.19–0.65	4.2–4.9
		Median		0.010	0.31	0.17	0.38	4.4
Brown and Balls (1997) shallow water study	Hake	Range	<0.03–2.39	<0.001–0.005	0.03–0.39	<0.01–0.07	0.01–0.28	1.61–5.90
		Median	1.18	<0.001	0.15	<0.01	0.03	3.58
	Ling	Range	1.30–11.6	<0.001–0.003	<0.02–0.27	<0.01–0.10	<0.03–0.15	<0.10–6.28
		Median	4.21	0.001	0.14	0.01	0.08	4.41
	Whiting	Range	0.50–22.2	<0.001–0.014	0.04–0.31	<0.01–0.11	0.02–0.37	2.18–5.16
		Median	4.27	0.001	0.14	<0.01	0.04	
	Cod	Range		<0.001–<0.001	<0.02–0.36	<0.02–<0.02	0.02–0.42	2.93–9.19
		Median		<0.001	0.110	<0.012	0.10	3.65
	Haddock	Range	0.04–28.1	<0.001–0.005	<0.02–0.42	<0.01–0.03	0.01–0.16	<0.01–7.36
		Median	8.71	<0.001	0.11	<0.01	0.12	3.30
	Skate	Range	11.2–20.3	<0.001–0.012	0.02–0.24	<0.01–0.02	0.01–0.22	2.29–4.82
		Median	14.6	0.001	0.10	<0.02	0.06	3.58
Mormede and Davies (in press)	*Nezumia Aequalis*	Range		0.002–0.010	0.17–0.37	ND–0.024	0.035–0.532	3.06–5.79
		Median		0.004	0.21	0.005	0.150	3.91
	Lepidion Eques	Range		0.003–0.013	0.13–0.24	ND–0.011	0.038–0.398	2.16–3.56
		Median		0.005	0.17	0.002	0.077	2.62
	Raja fyllae	Range		0.008–0.027	0.22–0.83	ND–0.044	0.044–0.410	4.57–6.15
		Median		0.012	0.33	0.027	0.129	5.53
Present study	Lophius Piscatorius	Range	2.70–21.47	<0.002–0.041	0.06–0.22	<0.002–0.041		
		Median	8.63	<0.002	0.15	<0.002		
	Aphanopus Carbo	Range	<0.002–26.49	<0.002–0.017	0.07–0.27	<0.002–0.052		2.12–3.90
		Median	1.25	0.004	0.12	0.009		2.85
	Blue ling	Range	1.84–13.09	<0.002–0.004	0.10–0.41	<0.002–0.008		
		Median	8.03	<0.002	0.15	0.003		
	Blue whiting	Range	0.37–6.10	<0.002–1.178	0.19–0.45	0.005–0.030		
		Median	2.24	0.022	0.29	0.008		
	Hake	Range	0.08–3.30	<0.002–0.062	0.16–0.54	<0.002–0.047		
		Median	1.37	0.034	0.27	0.008		

Compared to the results of Mormede and Davies (2001), some very high levels of cadmium are found in blue whiting and hake. This is also true when comparing with the data from Cronin et al. (1998) and a typical study of continental shelf species (Brown and Balls, 1997). Copper, zinc and lead levels are similar to those found in the previous study, although lead was found in relatively high concentrations in *Raja fyllae* (Mormede and Davies, 2001). Compared to the results found by Cronin et al. (1998), this study showed similar levels of copper and zinc although on the lower side and with a smaller range between species, while lead was much lower.

A comparison with the related shallow water species was conducted for hake, whiting and ling (Brown and Balls, 1997; see Table 4). In hake, the levels in the deep-sea samples was much higher than in its shallow water counterpart, where it was under the limit of detection. Copper was a little higher in deep-sea fishes as well, while arsenic and lead were quite similar. In ling, arsenic was higher in the deep-sea individuals by a factor of two, while cadmium, and lead were similar, and copper presented a similar median but a wider range in deep-sea fish. In whiting, the levels of arsenic in deep-sea samples was lower by a factor of two than in shallow water ones, thus much more cadmium and more copper and lead. The difference between arsenic in whiting and hake might be explained by the difference of habits between these two species. The tendency to find more cadmium in deep-water species was already highlighted, and is commonly explained by the fact that deep-water species live longer and feed at a higher trophic level than shallow-water counterparts (Gordon et al., 1995), thus are more likely to accumulate cadmium.

In this study in monkfish and blue ling, arsenic was mainly accumulated in muscle tissue, followed by liver, gonads and gills, while in the three other species the liver was first, followed by muscle tissue, gonad and gills. Its behaviour was different from the other elements but there are no published values for comparison. For all other elements and in all species, the highest concentrations were found in liver, higher by a factor 10 to those in gills, which were themselves higher than in gonads and in muscle tissue. This agrees with the results reported on copper by Vas et al. (1993) and by Mormede and Davies (2001) for copper and cadmium, but tends to differ from the results found by Mormede and Davies (2001) in zinc and lead. This may reflect inter-species differences.

All metals were present at statistically lower concentrations in gills in the present study than in the previous one (Mormede and Davies, 2001). Levels of cadmium in livers were also statistically lower for the monkfish, blue ling and hake, a little higher in the blue whiting but between 10 and 50 times higher in black scabbard. Copper was found on the higher side of the range for blue ling and blue whiting, 2–5 times higher in hake and monkfish and 10 times higher in black scabbard. Lead was very low in both studies and zinc was four times higher in black scabbard than in any other fish from the previous study. In general, the levels were about the same apart from the black scabbard which presented much higher levels of all metal concentrations in livers. This might be due to the trophic level of black scabbard as a top predator, being potentially exposed to higher levels of contaminations through its diet.

4.3. Accumulation processes

One anglerfish was dissected in more detail and the level of metals in the muscle tissue, liver, gonads, gills, spleen, kidneys, heart and gallbladder where determined (Table 5).

Table 5
Distribition of metal concentrations (mg/kg wet weight) in various organs of a female anglerfish

Type	As	Cd	Cu	Pb
Spleen	5.32	0.0046	0.79	0.0054
Liver	7.49	0.0856	7.39	0.0200
Kidney	3.91	0.0051	0.92	0.0077
Heart	16.25	0.0073	1.57	0.0013
Gills	2.26	0.0141	0.64	0.0124
Gonad	1.74	0.0016	2.99	0.0122
Gallbladder	4.65	0.0004	0.22	0.0048
Muscle	21.47	0.0417	0.12	0.0026

The main concentrations of arsenic were found in the muscle tissue and heart, then in liver, spleen, gallbladder and kidneys, with traces in the gonads and gills. Thus arsenic seems to be mainly accumulating in muscle organs such as the flesh and heart with some storing in other organs.

The highest concentrations of cadmium, copper and lead were found in liver, showing the detoxification and accumulation role of that organ. High copper and lead levels were then found in gonads which might excrete part of the toxic metals. Whilst there was very low levels of these metals in the muscle tissue, cadmium levels were the second highest after liver. Excess cadmium is probably stored in the muscle tissue, where it accumulates. Levels of cadmium in other organs were very low, apart from the gills which might be a route of uptake.

Copper levels in other organs were relatively low apart from a surprisingly high concentration in the heart whereas there was virtually none in the muscle tissue. Copper might be processed through blood and accumulate there.

Lead levels were more scattered in the various organs, by decreasing concentration orders: in the liver, gills, gonads, kidneys, spleen, gallbladder, muscle tissue and heart. There does not seem to be a particular accumulation process there.

4.4. Differentiation of the species by discriminant analysis

Discriminant analysis was used to try to separate the various species on the basis of their metal concentrations. A first separation between monkfish and black scabbard was attempted on the basis of levels of metals in muscle, liver and gills. Then all five species were separated with the levels of metals in muscle and liver organs only.

4.4.1. Separation of monkfish from black scabbard

By default analysis, i.e., using all the variates in the model, the separation of monkfish from black scabbard is already very good: the jackknifed classification provided 94% of monkfish and 98% of black scabbards properly classified. The principal component analysis showed a good separation as well (Fig. 5). Backwards stepwise modelling provided an increase of the monkfish properly classified to 97%, i.e., only one fish of each species was not properly classified. The classification functions after the modelling were as detailed in Table 6. As with all variables most of the fish were properly classified, all variables have some importance in differentiating the two

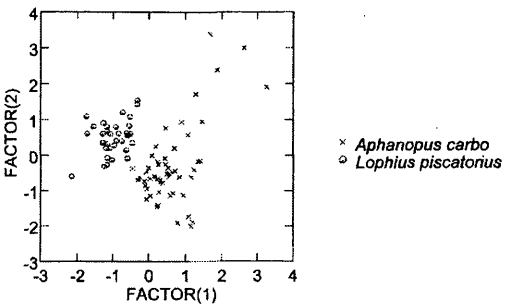

Fig. 5. Principal component analysis for the two species.

Table 6
Classification functions of monkfish and black scabbard

	Monkfish	Black scabbard
Constant	−53.226	−104.668
Depth	0.086	0.108
Length	0.763	1.259
Weight	−0.002	−0.004
As muscle	0.525	−0.090
Cd muscle	−83.320	259.649
Cd gills	102.531	198.134
Pb gills	−11.627	16.463

species. But the main ones are the physical variables (depth, length and weight) showing the huge difference in physiology and behaviour of the two species, but also levels of arsenic and cadmium in muscle and of cadmium and lead in gills. Apart from cadmium in gills, the other relevant variables have an opposite effect in the two species (Table 6).

4.4.2. Separation of all species

This attempted separation was made on the grounds of physical differences and on the levels of metals in muscle tissue and livers. The species were separated at 94% on the grounds of jackknifed classification (see Table 7 and Fig. 6). Backwards stepwise modelling did not provide any further separation.

The between groups F-matrix (Table 8) represents the distance between groups, computed from Mahalanobis D^2 statistics; the greater the number, the further apart the group means. Thus the species that is most differentiated from others is the black scabbard. This is probably due to the fact that it is a top predator whereas the others are of a lower trophic level. Blue whiting is also quite well separated from the other species, whilst the others are less clearly differentiated. This is confirmed by the classification where blue whiting and hake are properly classified whereas the others are less so.

Table 7
Jackknifed classification of the five species[a]

	ANG	BLI	BSC	BWI	HAK	% correct
ANG	31	1	1	0	2	89
BLI	0	13	1	0	0	93
BSC	0	2	52	0	0	96
BWI	0	0	0	9	0	100
HAK	0	0	0	0	6	100
Total	31	16	54	9	8	94

[a]ANG: anglerfish (monkfish); BLI: blue ling; BSC: black scabbard; BWI: blue whiting; HAK: hake.

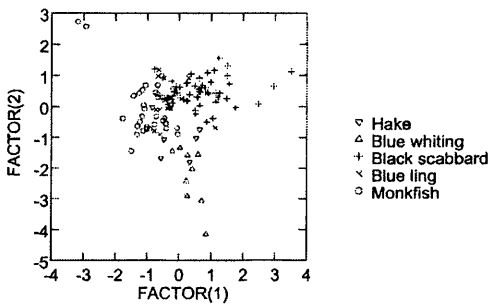

Fig. 6. Principal component analysis for the five species.

Table 8
Between groups F-statistics[a]

	ANG	BLI	BSC	BWI	HAK
ANG	0				
BLI	16.946	0			
BSC	50.959	8.992	0		
BWI	14.216	17.567	33.364	0	
HAK	4.860	8.095	10.025	5.987	0

[a]The classification functions are the following: ANG: anglerfish (monkfish); BLI: blue ling; BSC: black scabbard; BWI: blue whiting; HAK: hake.

Blue whiting presents an inverted response to cadmium and lead in liver compared with the other species, as well as hake and black scabbard to lead in muscle and hake to cadmium in liver. Weight and depth have a very small influence on the separation of the species.

4.5. Differences in organs' relative importance

4.5.1. Between monkfish and black scabbard

The relative importance of liver, gills and muscle tissue for the two species was plotted through triangular diagrams (Figs. 7 and 8). Each concentration is expressed as a percentage of the median concentration of the metal in the particular organ:

(concentration of metal M in muscle)/(median M concentration in muscle) = A, similarly for liver = B, and gills = C

These fractions are then summed within individuals and expressed as a percentage of this sum

$$A + B + C = D,$$

$$A_1 = (A/D)100, \quad B_1 = (B/D)100, \quad C_1 = (C/D)100.$$

The quantities plotted are A_1, B_1 and C_1, which are scaled from 0 to 100% on the sides of the triangular diagrams and read anticlockwise.

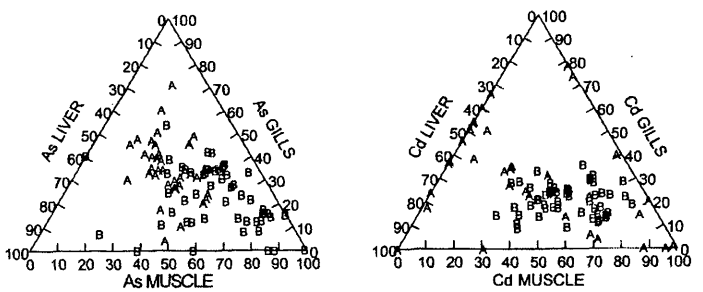

Fig. 7. As and Cd distribution between organs in monkfish (A) and black scabbard (B).

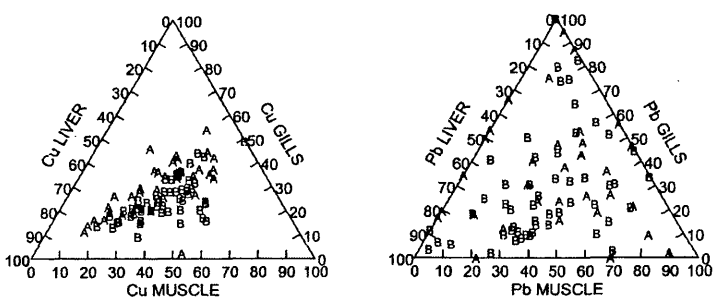

Fig. 8. Cu and Pb distribution between organs in monkfish (A) and black scabbard (B).

Copper and lead do not seem to present differences in the relative distribution within organs in the two species (see Fig. 8). Cadmium shows the black scabbard grouped towards the centre of the triangle, therefore distributed between organs, whereas monkfish are scattered on the sides, indicating that cadmium is held in one organ preferentially, with low levels either in the liver or the muscle tissue (see Fig. 7). Arsenic shows the best separation of the species, with black scabbards presenting lower levels in the gills compared to monkfish (see Fig. 7).

4.5.2. Between all five species

As only muscle and liver were analysed for all five species, the same process was applied to all data, but for a two dimension graph (Fig. 9). For each metal and species, the percentage of that metal in each of the two organs was plotted.

Copper is widely distributed for scabbard, monkfish and blue ling, with a tendency to have higher levels in the liver. A clear separation can be made between blue whiting with most copper in the muscle, and hake with most in the liver.

Lead is distributed throughout monkfish, ranging from 30 to 100% in the liver, whilst all other species presented the bulk of lead in the muscle. Arsenic covers most of the range for all species, not showing representative differences. Cadmium covers most of the range as well, but only for monkfish and black scabbard, the other species presenting the bulk of the cadmium in the muscle tissue (Table 9).

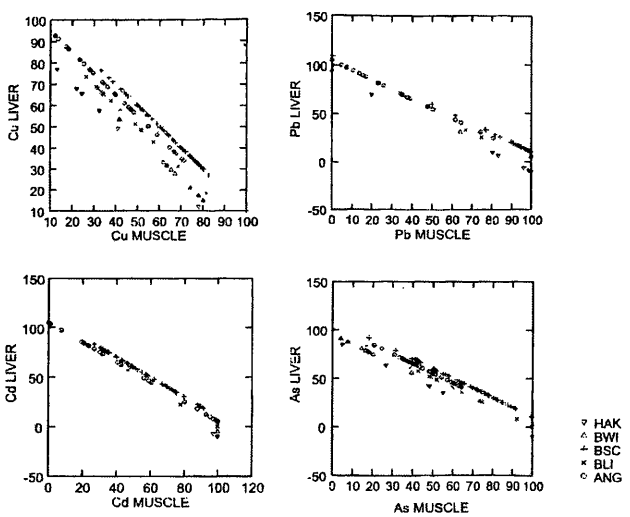

Fig. 9. Relative distribution of metals between organs.

Table 9
Classification functions for all five species[a]

	ANG	BLI	BSC	BWI	HAK
Constant	−68.539	−124.075	−123.706	−85.328	−75.100
Depth	0.112	0.150	0.144	0.135	0.107
Length	0.830	1.216	1.294	0.638	0.933
Weight	−0.003	−0.004	−0.005	−0.003	−0.003
As pmuscle	0.678	0.718	0.119	0.216	0.057
As liver	0.119	0.139	0.316	0.077	0.163
Cd muscle	−0.028	−0.050	−0.043	0.378	−0.009
Cd liver	0.014	0.009	0.002	0.002	−0.005
Cu muscle	66.390	87.671	66.098	117.413	97.452
Cu liver	−0.127	−0.314	−0.053	−0.261	−0.083
Pb muscle	−72.441	−84.775	56.524	−83.111	55.723
Pb liver	6.158	20.154	15.379	−10.256	10.328

[a]ANG: anglerfish (monkfish); BLI: blue ling: BSC: black scabbard; BWI: blue whiting; HAK: hake.

5. Summary

This paper presents new information on the concentrations of arsenic, cadmium, lead, copper and zinc in five commercial species of deep water fish — monkfish, black scabbard, blue ling, blue whiting and hake. The concentrations in muscle tissue are all well within EU limits for human consumption. The concentrations of cadmium, copper and zinc in fish liver are higher than in muscle tissue. In some cases, particularly cadmium and zinc in black scabbard livers, the concentrations exceed EU limits.

Discriminant analysis was used to separate the species on the basis of size of fish, the concentrations of metals and the depth of capture. Black scabbard was clearly separated from all other species by the procedure, possibly reflecting its status as a top predator. Hake, blue ling and monkfish were partially separated but some degree of overlap remained between these species.

Triangular diagrams were used to investigate patterns of accumulation of metals between organs in monkfish and black scabbard. Patterns were similar for copper and lead, but marked differences were found for cadmium and arsenic.

The data confirm the suitability of several species of deep-water fish as foodstuffs, However, the high concentrations of some metals in the liver, particularly of top predators such as black scabbard, indicate that accumulation to levels in excess of EU limits occurs. Assessments of dietary intake of trace metals in areas where fish livers form a significant component of the diet should take this into account in both typical diets and diets of critical groups.

This report has provided background information on the concentrations of six metals in deep-water fishes collected in the area of the Atlantic Frontier, where oil exploration is progressing and production can be anticipated to occur in the future. Experience in the North Sea and other shelf areas where oil is produced suggests that environmental contamination by metals arising from the oil exploitation is unlikely to be widespread. However, given the relatively poor understanding of

deep-water food webs and metabolic processes of deep-water fish, it will be advisable to maintain chemical monitoring of commercial fish species in the Frontier region.

Acknowledgements

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6.2. Polychlorobiphenyl and pesticide residues in monkfish *Lophius piscatorius* and black scabbard *Aphanopus carbo* from the Rockall Trough (*ICES Journal of Marine Science*, 2001, 58: 725-736)

Concentrations and patterns of organic contaminants were presented at the 1999 ICES Young Scientists' Conference in Gilleleje, Denmark, in November 1999. Subsequently, an extended abstract was published (Mormede and Davies, 2000). A paper is pulished (Mormede and Davies, 2001c), and is reproduced in full in the present section.

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Polychlorobiphenyl and pesticide residues in monkfish *Lophius piscatorius* and black scabbard *Aphanopus carbo* from the Rockall Trough

Sophie Mormede and Ian M. Davies

Mormede, S. and Davies, I. M. 2001. Polychlorobiphenyl and pesticide residues in monkfish *Lophius piscatorius* and black scabbard *Aphanopus carbo* from the Rockall Trough. – ICES Journal of Marine Science, 58: 725-736.

Chlorobiphenyl congeners (CBs) and pesticide residues were determined in various organs of monkfish (*Lophius piscatorius*) and black scabbard (*Aphanopus carbo*) caught 600–1150 m deep on the continental slope of the Rockall Trough, west of Scotland. Median concentrations of ΣCB (24 congeners), HCB, Σchlordane, ΣDDT and dieldrin ranged from 40–970, from 6–28, from 5–130, from 10–550 and from 5–36 μg kg lipid weight^{-1}, respectively, in the organs studied. There were no significant differences in the concentrations between organs (within species) on a lipid weight basis. Differences in the distribution of individual CB congeners between organs are described. The concentrations of all pesticides were linearly correlated in both species, as were some CBs in black scabbard. DDT and chlordane distribution patterns are broadly similar to those published elsewhere on deep-sea fish from the Northeastern Atlantic and off Japan. The different patterns of contaminant distributions between species permitted separation of the two species by discriminant analysis.

Key words: deep-sea fish, metabolism, PCB, pesticides, principal component analysis.

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Introduction

During the past few decades, contamination by anthropogenic organochlorine compounds has spread all over the globe and can now be detected in a wide range of environmental media, including fish (Tanabe *et al.*, 1994). Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), which are lipophilic and often resistant to biochemical degradation, have a tendency to accumulate in lipids in the marine biota.

The deep sea is no exception to this pattern, because it is generally considered as the ultimate sink for persistent organochlorines. Although the deep sea is distant from the primary sources of these compounds, most organochlorine compounds co-distil and are transported through the atmosphere to the poles. There they condense into the cold waters that subsequently provide the bulk of deep oceanic waters (Ballschmiter, 1992).

Few studies have reported concentrations of organochlorine compounds in deep-sea organisms (Barber and Warlen, 1979; Kramer *et al.*, 1984; Lee *et al.*, 1997; Berg *et al.*, 1997), probably because of the difficulty in obtaining samples and the relatively limited commercial interest (until relatively recently). This study was aimed at providing a consistent preliminary data set on levels of PCBs and OCPs in black scabbard (*Aphanopus carbo*) and monkfish (*Lophius piscatorius*) from the Rockall Trough, west of Scotland. Both species are commercially exploited in the area by both French and Scottish fishing fleets and are also of research interest. Monkfish are present in both shallow and deep water, permitting a comparison between levels of contamination without having to account for inter-species differences. Black scabbard are widely distributed in the North Atlantic, so permitting direct comparison between contaminants in fish caught in Scottish and, for instance, Portuguese waters.

Materials and methods

Samples of fish were collected in September and October 1998 in the area of the Rockall Trough, west of Scotland (ICES statistical rectangles 41E0–46E1), during two deep-sea cruises of the FRV "Scotia". Sampling took place between 56 and 59°N at depths ranging from 400

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to 1150 m. Totals of 38 monkfish, 43–122 cm total length, and 54 black scabbard, 64–116 cm total length, were sampled. The following species descriptions are based on those of Whitehead *et al.* (1984).

Lophius piscatorius (monkfish) – Habitat: benthic, from shallow, inshore waters to 500 m. Diet: mainly fish such as whiting and cod, *Nephrops* sp. and occasionally seabirds. Reproduction: spawn from February to July. Distribution: Mediterranean, Black Sea, eastern North Atlantic from Straits of Gibraltar to southwestern Barents Sea.

Aphanopus carbo (black scabbard) – Habitat: oceanic benthopelagic on continental slope or underwater rises at about 200–1600 m, juveniles mesopelagic. Migrate to midwater at night. Caught commercially with special deepwater lines off Madeira and, to a lesser extent, off Portugal; and by deep-water trawls west of Scotland. Diet: cephalopods, fish, crustaceans. Distribution: eastern Atlantic, from Denmark Strait, Iceland and Norway to Madeira and western North Africa. Known from many localities in Atlantic, Indian and Pacific oceans.

Fish were dissected onboard ship soon after being caught. Muscle tissue, livers and gills (the latter for black scabbard only) were sampled, wrapped in aluminium foil and frozen at −20°C. Once ashore, individual samples were thoroughly defrosted and homogenized in pools of no more than five fish, grouped by length. Pooled samples were dried by grinding with anhydrous sodium sulphate, followed by Soxhlet extraction with 300 ml of MTBE (methyl tert-butyl ether). After lipid determination, clean-up was achieved with one 6 g alumina column and keeping the whole fraction. Group separation was conducted with one 3 g alumina and one 3 g silica column; the fractions were at respectively approximately 5.5 and 7.5 ml (checked with every new absorbent batch). Instrumental analysis was performed by capillary column gas chromatography with an electron capture detector. PCBs were analysed on a Perkin Elmer ECD gas chromatograph fitted with a 50 m × 0.25 mm ID CP-Sil 8 column and a 2.5 m × 0.53 mm ID uncoated deactivated precolumn, programmable on-column (POC) injector with hourglass inset and autosampler. The oven temperature programme was 80°C for 1 min, then 3°C min^{-1} to 270°C, then held for 12 min. Injector temperature starts at 120°C for 0.2 min, 200°C min^{-1} to 270°C, then held for 70 min. The detector is held at 270°C.

Pesticides were analysed on a VARIAN 3500 ECD gas chromatograph fitted with a 50 m × 0.25 mm ID CP-Sil 8 column and a 2.5 m × 0.32 mm ID uncoated deactivated precolumn, septum programmable injector with glass insert and 8200 CX autosampler. The oven temperature programme was 80–180°C at 15°C min^{-1}, held for 6 min, then to 290°C at 3°C min^{-1}, where it was held for another 20 min. Injector and detector temperatures are the same as above.

This method has been validated through successful participation in national and international intercalibration exercises (QUASIMEME, De Boer and Wells, 1996). The accuracy of the data was monitored by the use of Laboratory Reference Materials (LRMs) in every batch analysed, and the recovery was monitored in each sample by the addition of an internal standard (CB209) prior to extraction.

Concentrations of HCB, CBs 28, 31, 44, 49, 52, 70, 74, 101, 105, 110, 114, 118, 128, 138, 149, 153, 156, 157, 158, 170, 180, 187, 189, and 194 were measured, and the sum of all these CBs was calculated as ΣCB. The following pesticides were also determined: α-HCH, γ-HCH, α-chlordene, γ-chlordene, aldrin, heptachlor-epoxide, α-chlordane, γ-chlordane, oxychlordane, transnonachlor, heptachlor, endrin, dieldrin, o,p'-DDD, p,p'-DDD, o,p'-DDT, p,p'-DDT, o,p'-DDE, p,p'-DDE. Σchlordane was calculated as the sum of α-chlordene, γ-chlordene, heptachlor-epoxide, α-chlordane, γ-chlordane, oxychlordane, transnonachlor and heptachlor. ΣDDT was calculated as the sum of o,p'-DDD, p,p'-DDD, o,p'-DDT, p,p'-DDT, o,p'-DDE and p,p'-DDE.

Limits of detection were calculated separately for each sample, being about 0.07, 0.3, and 1.5 μg kg^{-1} wet weight, respectively, for muscle tissue, gills, and liver, which represent 0.03 μg kg lipid $weight^{-1}$ for all tissues for all determinands.

Results

Median wet weight concentrations of ΣCB, HCB, Σchlordane, ΣDDT and dieldrin ranged from 0.27–174, from 0.037–8.37, from <0.005–39, from 0.15–145, and from 0.098–15.9 μg kg wet $weight^{-1}$, respectively, in the various organs of the two species studied. The compounds had statistically significantly greater wet concentrations ($p<0.05$) in livers than gills and muscle for both species. In contrast, the concentrations of contaminants expressed on a lipid weight basis (Figures 1 and 2, Table 1), where detected, were not statistically different ($p>0.05$ for all levels pairwise). In most cases, the ranges of concentrations are large: median concentrations of ΣCB, HCB, Σchlordane, ΣDDT and dieldrin ranged from 40–970, from 6–28, from 5–130, from 10–550 and from 5–36 μg kg lipid $weight^{-1}$, respectively, in the various organs of the two species studied.

One monkfish was more completely dissected and contaminant concentrations were determined in seven organs. Of the organs analysed (heart, gills, gonad, spleen, kidney, gall bladder, and muscle), the gonad contained the highest concentrations of ΣCB, HCB, Σchlordane, ΣDDT, endrin and dieldrin expressed on a wet weight basis, followed by the heart. However, expressed on a lipid weight basis, the levels tend to be

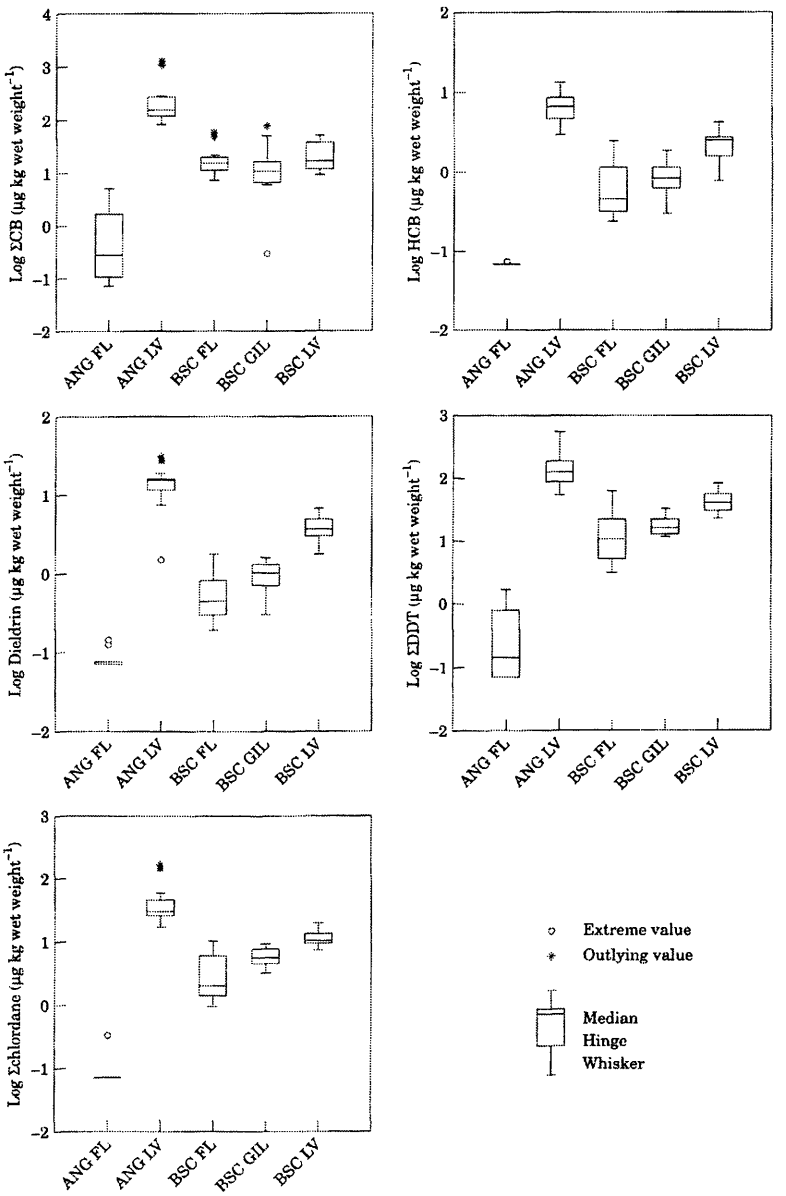

Figure 1. Concentrations by wet weight of organic contaminants in the muscle (FL), liver (LV), and gill (GIL) of monkfish (ANG) and black scabbard (BSC).

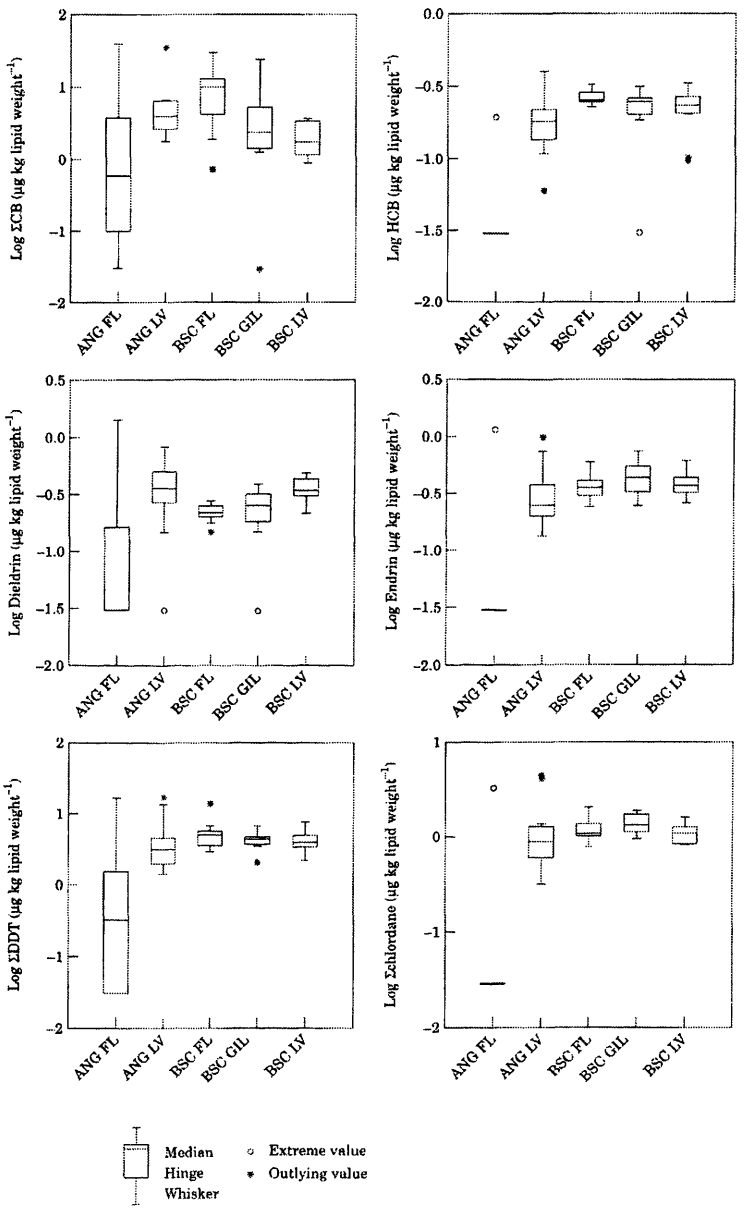

Figure 2. Concentrations by lipid weight of organic contaminants in the muscle (FL), liver (LV), and gill (GIL) of monkfish (ANG) and black scabbard (BSC).

Table 1. Median concentrations (and ranges) of selected organic contaminants in various organs of male and female monkfish and black scabbard (μg kg lipid weight^{-1}, except ΣTEQ-CB in pg kg lipid weight^{-1}).

Species/tissue	Sex	%Lipid	HCB	ΣCB	*a* HCH	γ-HCH	DIELDRIN	ENDRIN	ΣDDT	ΣChlordane	ΣTEQ-CB
Monkfish muscle	F	0.45	<6.1	650.6	<5.9	23.7	16.5	<5.9	70.6	<5.9	1.0
		(0.1–1.1)	(<6.1–<6.1)	(<5.9–3951.3)	(<5.9–<5.9)	(<15.9–153.7)	(<15.9–140.2)	(<5.9–109.3)	(<5.9–1661.4)	(<5.9–333.1)	(0.0–5.1)
		0.3	<8.0	39.0	<5.6	48.3	<5.6	<5.6	10.1	<5.6	0.0
	M	(0.1–1.2)	(<8.0–<8.0)	(<5.6–116.6)	(<5.6–<5.6)	(15.4–162.6)	(<5.6–<5.6)	(<5.6–<5.6)	(<5.6–238.3)	(<5.6–<5.6)	(0.0–0.4)
		35.1	17.4	491.9	<3.5	<3.5	41.2	27.0	375.8	99.3	1.0
Monkfish liver	F	(27.5–49.0)	(6.1–40.1)	(173.8–3589.9)	(<3.5–5.3)	(<3.5–<3.5)	(14.7–82.4)	(14.3–96.3)	(181.8–1652.7)	(37.0–448.3)	(0.3–8.0)
		43.0	18.2	275.4	<3.5	<3.5	26.4	22.0	205.5	62.2	0.6
	M	(37.6–57.3)	(10.9–23.7)	(248.4–651.6)	(<3.5–<3.5)	(<3.5–<3.5)	(<3.5–62.9)	(13.4–73.5)	(146.7–731.6)	(32.9–415.6)	(0.3–1.6)
		2.7	25.4	968.2	2.0	<3.5	22.0	34.5	473.9	114.1	1.3
Black scabbard muscle	F	(0.9–9.9)	(24.7–28.5)	(75.0–2200.1)	(<3.5–4.6)	(<3.5–20.8)	(17.6–27.6)	(25.6–59.8)	(325.6–1387.8)	(92.9–213.3)	(0.1–3.5)
		1.7	28.2	1313.6	<3.5	6.1	24.3	35.6	547.7	102.3	1.7
	M	(1.3–6.6)	(22.7–33.0)	(197.0–2986.4)	(<3.5–5.1)	(<3.5–15.2)	(14.8–26.7)	(23.7–47.2)	(297.2–684.1)	(80.4–170.2)	(0.2–3.9)
		3.3	25.2	358.0	<3.7	<3.7	21.3	33.4	391.3	131.8	0.5
Black scabbard gills	F	(2.5–9.1)	(<3.7–31.9)	(<3.7–1706.9)	(<3.7–6.5)	(<3.7–17.8)	(<3.7–39.2)	(<3.7–75.1)	(209.6–563.4)	(<3.7–196.6)	(0.0–2.0)
		3.7	24.0	163.1	<3.5	<3.5	27.3	43.4	456.6	129.5	0.2
	M	(2.6–9.5)	(18.5–27.0)	(128.4–2478.5)	(<3.5–<3.5)	(<3.5–9.3)	(16.6–35.1)	(23.4–60.2)	(358.8–687.7)	(103.6–193.2)	(0.1–1.9)
		11.4	23.0	139.8	<3.5	<3.5	32.4	37.8	384.5	111.3	0.2
Black scabbard liver	F	(6.9–14.2)	(9.7–31.0)	(99.2–315.8)	(<3.5–<3.5)	(<3.5–<3.5)	(21.9–43.9)	(28.5–43.9)	(219.7–630.9)	(84.5–150.8)	(0.1–0.6)
		10.8	24.2	364.3	<3.6	<3.6	36.4	36.5	409.6	110.5	0.2
	M	(5.8–13.9)	(20.5–33.3)	(90.8–384.9)	(<3.6–<3.6)	(<3.6–<3.6)	(32.4–49.3)	(26.3–62.0)	(355.8–755.1)	(84.0–169.6)	(0.1–0.8)

ΣCB=Σ(CB28, 31, 44, 49, 52, 70, 74, 101, 105, 110, 114, 118, 128, 149, 153, 156, 157, 158, 170, 180, 187, 189, 194, 209)
ΣTEQ-CB=TEF-CB105+TEF-CB118+TEF-CB156+TEF-CB170+TEF-CB180, using the TEFs calculated by Ahlborg *et al.* (1994) ΣDDT=p,p'-DDD+p,p'-DDE+p,p'-DDT+o,p-isomers
o,p-isomers=o,p'-DDD+o,p'-DDT+o,p'-DDE
Σchlordane=α-chlordane+γ-chlordane+trans-nonachlor+heptachlor+heptachlor epoxide.

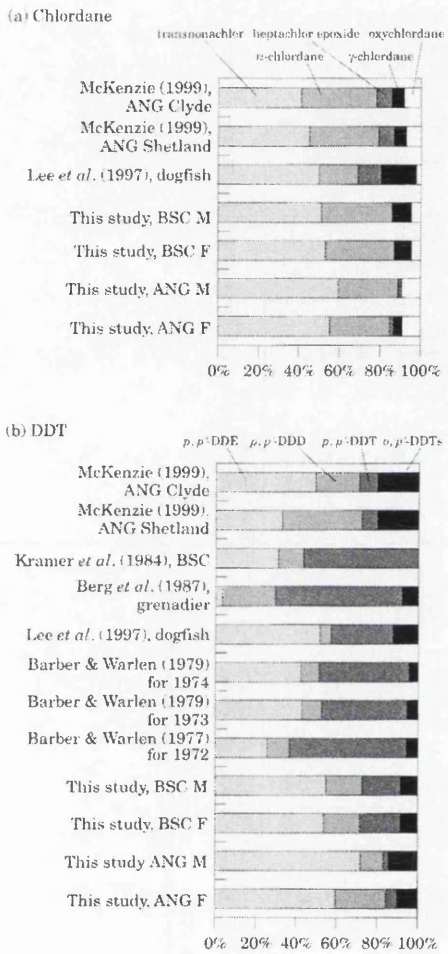

Figure 3. Composition of (a) chlordane and (b) DDT in male (M) and female (F) monkfish (ANG) and black scabbard (BSC) during this study and as recorded in the literature.

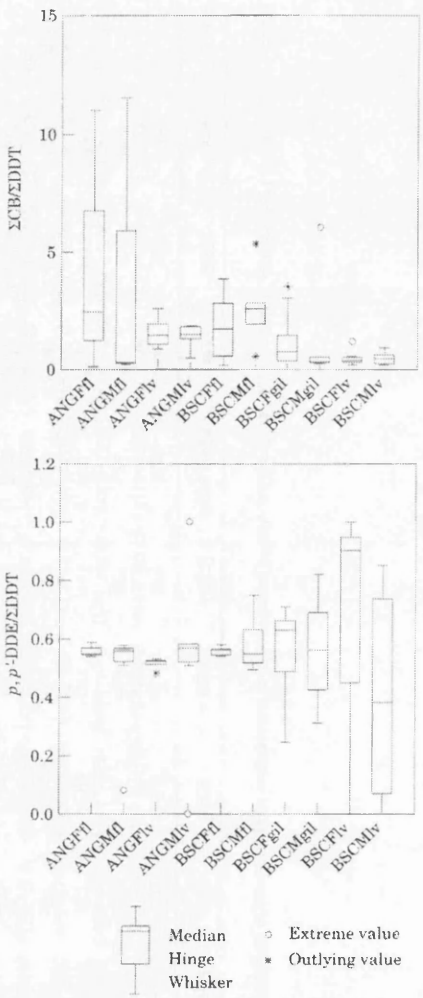

Figure 4. Dependence of ΣCB/ΣDDT and p,p'DDE/ΣDDT on the species, organ, and sex. ANG, monkfish; BSC, black scabbard; ml, muscle; lv, liver; gil, gill.

less different, with the exception of the gills and the gall bladder. Gills contained higher levels (lipid weight) of these contaminants than the other organs, which may suggest an important route of uptake of these contaminants by monkfish.

Σchlordane and ΣDDT compositions are detailed by species and organ in Figure 3, and ΣCB/ΣDDT and p,p'-DDE/ΣDDT ratios by species and organ in Figure 4.

The individual data (i.e. detailed CB and pesticide concentrations per individual fish) were examined for linear relationships between determinands using Pearson correlation coefficients. On a wet weight basis, few correlations were found, and those were mainly between length and weight in both species, between length and lipid in black scabbard, and between length/weight and

ΣCB/ΣDDT in monkfish. Although correlations between length/weight and contaminant concentration in monkfish suggest continuing accumulation with growth (age) of fish, a similar pattern was not observed in black scabbard. These correlations may reflect the migration of older monkfish to deeper water.

Toxic equivalents were calculated for the CBs (ΣTEQ-CB), using the toxic equivalent factors (TEFs) derived by Ahlborg *et al.* (1994) for the CBs measured. In both species, ΣTEQ-CB concentrations expressed in lipid weight were linearly related to ΣCB and CB153, expressed in lipid weight, although the slopes differed between species, being higher in monkfish than in black scabbard. ΣCB concentrations expressed in lipid weight were linearly related to the CB153 concentrations in both species. The gradients of the two correlations were different; higher for monkfish than for black scabbard, the latter showing surprisingly low CB153 concentrations. CB153 is very resistant to metabolism, so it can be used as a normalizing factor for CB levels in fish to reduce the variance attributable to naturally differing concentrations in different fish and to emphasize differences in CB accumulation patterns. The distribution of the RCBs (CB concentrations normalized to CB153) in the different organs indicated groups of compounds with different behaviours. For both species, RCBs 28, 31, 44, 49, 52, 70, 74, 101, and 110 are relatively concentrated in muscle tissue, when compared to the concentrations in the liver and the gill. SRCB and RCBs 118 and 149 show a similar pattern, but with slightly higher concentrations in the liver and the gill, although much lower than in muscle. RCBs 138, 180, and 187 are relatively concentrated in monkfish muscle tissue and in black scabbard liver and gill tissue. Finally, RHCB and RCBs 128, 156, 170, and 194 are present in relatively high concentration in black scabbard liver and gill tissue, though they are at very low concentrations in monkfish tissue (Figure 5). The low concentrations of contaminants in monkfish muscle reflect the very low lipid content and, in some cases, were close to the limit of detection of the system. However, this does not account for all differences between the CB patterns, with three main groups appearing: CBs accumulate mainly in the muscle of both species, in the liver of both species, or in muscle for one and in liver tissue for the other. The first two groups account for differences of accumulation between CBs, perhaps linked to their toxicity, whereas the third group shows a different accumulation/metabolization route between the species, though because the results have been normalized to CB153, they cannot be the result of different diets.

Normalized data were also examined for linear relationships. On a lipid weight basis, concentrations of most pesticides detected were positively correlated with each other in both monkfish and black scabbard. In black scabbard (but not in monkfish), a set of RCBs, namely RCBs 28, 31, 49, 70, 74, 101, 110, 118 and 149, were positively correlated with each other.

Discussion

All levels of contaminants in black scabbard and monkfish muscle and liver tissues were at least five times lower than the UK and strictest European dietary guidelines, so at present they do not present a health risk in the commercial use of these fish species (JMG, 1992).

McKenzie (1999) reported levels of CBs and OCPs in monkfish caught in shallow water around Scotland. Deep-water monkfish analysed during this study had levels of CBs and OCPs higher than those caught over shelf areas around Scotland, but lower than those from industrialized areas such as the Clyde Sea. HCB and ΣDDT were higher in deep-water fish than in shallow-water fish coming from both industrialized and offshore areas around Scotland (McKenzie, 1999).

The only authors reporting organic contaminant concentrations in deep-sea black scabbard are Kramer *et al.* (1984), who analysed one fish 106 cm long caught off Madeira at a depth of about 800 m. The levels they reported are 10–30 times higher than those measured during this study. Results documented from studies of other deep-water fish failed to cover the same species as the current study, so any comparisons would include inter-species differences, habitat and behaviour differences as well as location of capture, among other factors. Nevertheless, it is worth noting comparisons here. The levels found in the current study are similar to those published by Hale *et al.* (1991); Berg *et al.* (1997); Froescheis *et al.* (2000), and Looser *et al.* (2000). Further, they are similar to or lower than those published by Barber and Warlen (1979) for *Antimora rostrata* livers (after conversion of the present data to wet weight) and by Lee *et al.* (1997), and up to 100 times lower than those reported by Melzian *et al.* (1987) for deep-sea fish caught near former waste-disposal sites and a contaminated bay outflow in the eastern Pacific.

The chlordane composition of monkfish and black scabbard is similar to those found by Lee *et al.* (1997) in deep-sea organisms and by McKenzie (1999) in shallow-water monkfish (Figure 3), but with lower heptachlor epoxide. The DDT composition had much less p,p'-DDT than the other studies. The DDT composition reported by McKenzie (1999) for shallow-water monkfish was substantially different (Figure 3), suggesting that deep-sea fish may be exposed to a different pattern of contamination by DDT and its metabolites, and/or subject to different patterns of metabolism between fish living in shallow and deep water. The difference in patterns of DDT contamination may be due to shallow-water species tending to be contaminated by nearby coastal sources, whereas deep-sea contamination could

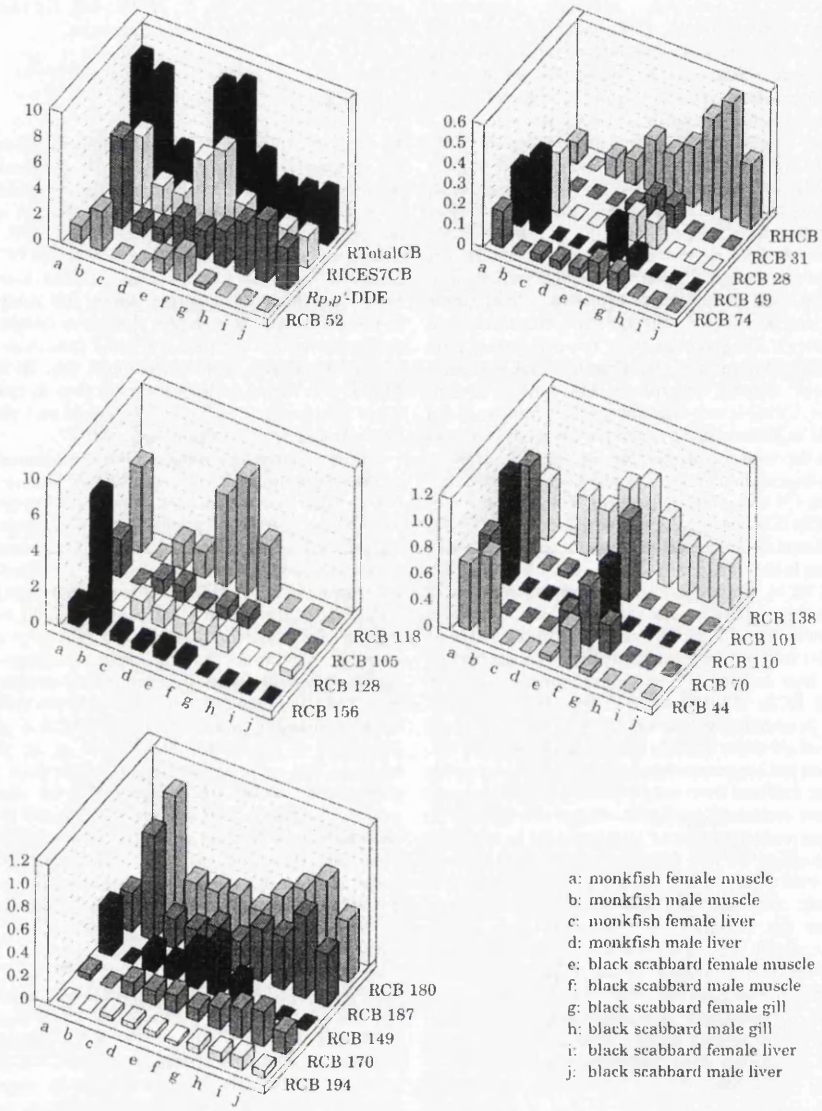

Figure 5. Distribution of different RCBs and p,p'-DDE in muscle liver and gill of monkfish and black scabbard (RCB expressed as CBx/CB153).

follow a more complex route (see the Introduction). On the other hand, some studies have shown that shallow-water fish can transform p,p'-DDT to p,p'-DDE (Uchida *et al.*, 1988). The close correlations between all DDT-related compounds in the present study suggest very little metabolism of these compounds in black scabbard and monkfish in deep water. If shallow-water fish also metabolize more effectively than deep-water fish, then differences in the DDT composition would be expected between the two groups.

DDT patterns have been more widely discussed in the literature as p,p'-DDE/ΣDDT ratios. The ratio in deep-sea fish seems to be generally between 0.6 and 0.7 in oceans surrounding industrialized countries in recent years (Hale *et al.*, 1991: Berg *et al.*, 1997; Lee *et al.*, 1997). Ratios were lower prior to 1979 (values of 0.3–0.4 were quoted by Barber and Warlen, 1979), probably reflecting subsequent prohibitions on the production of such chemicals in most industrialized countries. However, the processes moving volatile organic contaminants through the environment to the deep sea can be slow, and PCBs and pesticides persist in the environment long after bans. According to Ballschmiter (1992), it can take decades for organic contaminants to reach deep oceanic waters. He states that one mode of transportation is via the atmosphere to the poles, from where they precipitate into the cold waters: they ultimately dominate the deep sea. Other modes of transportation of these contaminants to the deep sea include the food chain or vertical transport (sinking of carcasses, the so-called deep-sea "snow"), but this mode is supposed to be of lesser importance than the poleward route (Ballschmiter, 1992). Tanabe (1988) estimated that only 31% of the PCBs produced had reached the environment, 4% had been destroyed through high-temperature incineration or by metabolic breakdown, and the remaining 65% were still in use (mostly in ageing electrical equipment) or had been landfilled, mostly inadequately. Considering the time lapse before these contaminants reach the deep sea, it is clear that the deep-sea environment is still highly vulnerable to fresh inputs of these pollutants, and continuing monitoring is required.

The ratio ΣCB/ΣDDT has been proposed as an indicator of the provenance of the chemicals (Tanabe *et al.*, 1997). A ratio <1 represents a landmass with less industrialization (mainly in the southern hemisphere) where DDT is still used for agricultural purposes, and a ratio >1 represents industrialized pollution where DDT has been banned in agricultural applications, but where CBs remain in use in the industrial sector. Monkfish have significantly higher ratios than black scabbard ($p<0.01$), and both species have higher ratios in muscle tissue than in liver and gill tissue (Table 2). These results are broadly consistent with the findings of Hale *et al.* (1991) and Lee *et al.* (1997). The difference between organs may be due to different patterns of metabolism in separate organs, meaning that compounds accumulate and equilibrate at different rates, or that in tissues with higher lipid content, there may be greater accumulation of ΣDDT relative to ΣCB. Pesticides in deep-sea fish may be metabolized very little, as indicated by the linear correlations between many pesticide concentrations. As liver tissue presents the lowest ΣCB/ΣDDT ratio of all organs studied, it may be metabolizing some CBs more than any other organ. Such differences between organs suggest that the use of this ratio as an indication of provenance may not be reliable in fish. It might be more reliable in environmental measurements where there is little potential for separation, fractionation, or degradation (such as in water, soils, etc.).

McKenzie (1999) reported a similar ΣCB/ΣDDT ratio for the shallow-water monkfish he studied, whether the animals came from either industrialized or offshore areas. Berg *et al.* (1997) reported various ratios for the eight deep-sea species he researched, ranging from 0.8 to 1.5, the differences perhaps being attributable to different behaviour of the various species. Melzian *et al.* (1987) showed that the same species caught in the same vicinity had a lower ratio at greater depth (1.0 at 500 m and 0.72 at 1000 m), perhaps reflecting different contaminant distributions between water masses. This may explain to some extent the differences between the results for monkfish and for black scabbard. Monkfish tend to live in shallow water when young and are caught deeper only when older, and 1000 m might be the extreme of its depth distribution. On the other hand, black scabbard are definitely deep-water species, and are seldom caught shallower than 500 m. The higher coefficient in monkfish than in black scabbard may therefore reflect the former's generally closer proximity to surface inputs closer to shore.

In monkfish, HCB, ΣCB and ΣDDT concentrations expressed in wet weight are positively correlated with fish length. This implies a gradual accumulation of these compounds in the body over time rather than a rapid equilibration to levels in the ambient environment. It may also reflect the changes of habitat of monkfish with age, living deeper when older (Whitehead *et al.*, 1984); concentrations of monkfish are higher in deep water. These trends are not shown in black scabbard, which do not have such a marked change in depth of habitat with age. Both species dispayed linear correlations between concentrations of most pesticides, suggesting little metabolic breakdown of these contaminants in deep-sea fish. In the case of black scabbard, a range of CB congeners, CB 28, 31, 49, 70, 74, 101, 110, 118, and 149, were also linearly correlated, supporting the same type of conclusion. These CBs were not correlated in the monkfish specimens, suggesting differing abilities to metabolize contaminants between species, even within the deep-sea environment.

Table 2. Summary of ΣCB/ΣDDT values in the literature for various organs in different fish species.

	Kramer *et al.* (1984)	Hale *et al.* (1991)	Berg *et al.* (1997)	Lee *et al.* (1997)	McKenzie (1999)	This study	
Capture location	Madeira	Madagascar	Greenland	Japan	Scotland	Scotland	
Species	*Aphanopus carbo*	*Lainneria chalumnae* (female–male)	Various (range)	*Deania calcea*	*Lophius piscatorius* (shallow water)	*Lophius piscatorius & Aphanopus carbo* (single *Lophius*)	
Muscle		4.04–0.82				6.5–2.2 (2.38)	
Liver	0.63	0.42	0.8–1.5	1.1	2.7	1.3–0.7 (2.06)	
Gonad						(2.91)	
Gills						–0.7 (2.59)	
Stomach				3.5			
Intestine				2.0			
Kidney		1.00					
Swimbladder		0.39–0.82				(2.26)	
Mesenteric adipose		0.37–0.63					
Gall bladder						(6.35)	
Spleen						(2.20)	

Differences between species

Principal component analysis (PCA) was used to attempt to distinguish between the species on the basis of their patterns of contaminants. Concentrations of lipid, CB153-normalized CBs and lipid-normalized pesticides in livers of both species were used. The PCA does not separate the species very well. Discriminant analysis was performed on the same data set to improve separation (Systat, 1996). Complete differential analysis gave a correct jack-knifed classification of 64% of monkfish and 50% of black scabbard. Backwards stepwise analysis gave a correct jack-knifed classification of 91% for monkfish and 100% for black scabbard, with a probability (p) of <0.001 of there being statistically different patterns in the two species. The variables used in the classification were, by order of importance, p,p'-DDE, CB44, CB194, CB28, CB70, CB74, and p,p'-DDT. Most of these CBs correspond to those displaying pairwise linear correlation in black scabbard but not in monkfish.

A differentiation by tissue was attempted within species. No statistically positive separation was achieved between the muscle and liver tissues in monkfish. In the case of black scabbard, a partial separation between organs was achieved (Figure 6). By discriminant analysis, respectively 91, 92, and 100% of muscle, gills, and liver tissues were properly classified. This shows differences in the accumulation/metabolization processes between the different organs in black scabbard.

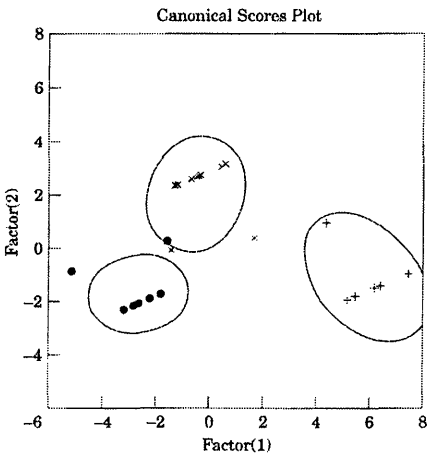

Figure 6. Discriminant score plot for muscle tissue (dots), liver (plus signs), and gill tissue (crosses) in black scabbard.

Conclusions

Median concentrations of ΣCB, HCB, Σchlordane, ΣDDT and dieldrin in black scabbard and monkfish ranged from 40–970, from 6–28, from 5–130, from 10–550 and from 5–36 μg kg lipid weight^{-1}, respectively.

Concentrations in various organs, expressed on a lipid basis, were not statistically different. The levels found were similar to those published elsewhere for deep-sea fish, but higher than those of shallow-water fish from Scottish coastal waters.

Individual CBs showed different distributions in the various organs. All pesticides were inter-correlated, as were some CBs in black scabbard. In both species ΣDDT was mainly composed of p,p'-DDE and chlordane of transnonachlor. The ratio of p,p'-DDE/ΣDDT approached 0.6 in both species and various organs. ΣCB/ΣDDT was significantly higher in monkfish than black scabbard, and higher in muscle than liver or gill, and ranged overall from 6.5 to 0.7. The species could be separated on the grounds of their levels of contaminants by differential analysis.

This study has pinpointed specific correlations between the contaminant concentrations, the species, depth of capture, and organs. More work clearly has to be done, including analysis of more species, a wider depth range, and different areas, to address questions relating to the source of the contaminants, their availability to deep-water fish, and possible metabolism of the contaminants by the fish.

Acknowledgements

This work was carried out as part of the PhD studies of S.M. through the University of Glasgow. The programme was jointly funded by the EU project Basblack (DG XIV 97-0084), Highlands and Islands Enterprise, Scottish Association for Marine Science, the University of Glasgow and Fisheries Research Services.

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Chapter 7
Horizontal and vertical distribution of contaminants

The effects of longitude and depth on the levels and patterns of contaminants have been the subject of two papers, prepared for external publication.

7.1. Mercury concentrations in black scabbardfish (*Aphanopus carbo*) commercially exploited in European waters (to be submitted to Environmental Science and Technology)

Levels of mercury in black scabbardfish have been studied in details during the EU Project BASBLACK (DGXIV-97/0084), in collaboration with various Scottish and Portuguese partners (see paper for details). Subsequently, the results have been collated and analysed collaboratively. This paper is in a final draft format, awaiting approval from all partners before being submitted to *Environmental Science and Technology* for publication.

The paper is reproduced in the present section.

Mercury concentrations in black scabbardfish (*Aphanopus carbo*) commercially exploited in European waters

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Abstract

Samples of black scabbardfish *(Aphanopus carbo)* have been collected from six locations in the North Eastern Atlantic; Rockall, Meriadzec, Sesimbra, Madeira and Azores. Individual fish have been measured, weighed, sexed and their maturity stage determined. Muscle, liver, gills and gonad tissues have been analysed for total mercury. Median concentrations in muscle were below 1 mg/kg, although some individuals amongst the higher maturity stages presented higher levels. Levels in gonads and gills were very low, with median total mercury concentrations below 0.33 and 0.19 mg/kg respectively. Levels in liver tissues were substantially higher. In all organs, females presented significantly higher concentrations than males, and concentrations increased with maturity stage.

Introduction

Aphanopus carbo (Lowe, 1839) is a benthopelagic trichiurid species inhabiting the continental shelf and slope between 180 and 1600m in depth (Parin, 1986). It is an elongated fish with a pointed head and long jaws bearing large fang-like teeth. A very long dorsal fin extends along most of the coppery black body. It can be distinguished from its co-genetic species by the number of dorsal fin spines and rays (Merrett and Haedrich, 1997). In English, it is known as black scabbardfish, in Portuguese as peixe-espada preto (mainland Portugal and Azores Islands) and as espada (Madeira Island), in Spanish as sable negro and in French as sabre noir.

The geographic distribution of black scabbardfish covers both the sides and underwater rises of the North Atlantic from Iceland and Norway, on both sides of the Mid-Atlantic Ridge, South to about 30EN (Canarias and Western North Africa) (Nakamura and Parin, 1993). Elsewhere, it is known from localities in the western Atlantic, Indian and Pacific Oceans (Parin, 1986). With the collapse and closure of some continental shelf fisheries, attention has turned to deep-water stocks on the continental slope. The wide distribution presents difficulties in regulating and assessing the ecological effects of commercial fisheries (Merrett and Haedrich, 1997).

Black scabbardfish matures at 80-85 cm (standard length) in Madeira (Merret and Haedrich, 1997). According to Nakamura and Parin (1993), it spawns to the west of the British Isles. Spawning and post spawning individuals have been found from November to April on Hatton Bank and in the vicinity of Porcupine Bank (Zilanov and Shepel, 1975) and two spawning individuals were found in the Rockall Trough during the period 1974 - 1980 (Ehrich, 1983). However, recent Spanish and Scottish investigations have failed to find any spawning fish to the west of the British Isles (A. Newton, pers. comm.). Spawners are mainly found in Madeira waters from October to December (Sena-Carvalho, 1988). A recent study suggests that black scabbardfish is a relatively rapidly growing species living up to eight years (Morales-Nin and Sena-Carvalho, 1996). Black scabbardfish grows to a maximum size of 145 cm standard length off Madeira.

The work reported here was carried out as part of the EU funded project BASBLACK: Environment and biology of deep-water species *Aphanopus carbo* in NE Atlantic: basis for its management (study project EC DG-XIV 97-0084). The institutes involved in this program were IPIMAR (Instituto de Investigação das Pescas e do Mar-The Portuguese Fisheries and Sea Research Institute), FRS ML (Fisheries Research Services Marine Laboratory, Aberdeen, UK), DOP (Departamento de Oceanografia e Pescas, University of Azores) and DSIP-DRP (Direcção de Serviços de Investigação das Pescas, the scientific branch of Madeira's Regional Directorate for Fisheries).

The main objective of BASBLACK was to provide the basis for the development and implementation of a program for the routine study and management of the black scabbardfish. Owing to the lack of biological and environmental information critical for the assessment and management of the species, special effort has been put into investigations of its biology, stock discrimination and habitat characterization (Gordo *et al.*, 2000). The project also included bioaccumulation studies, investigating the levels of heavy metals, pesticides (e.g. dieldrin, DDT and its derivatives), and polychlorobiphenyls. The present paper will discuss total mercury concentrations only. Other papers present the results of the investigations of other heavy metals, PCBs and pesticides (Mormede *et al.*, 2001b and 2001c).

The aim of this study was to establish mercury levels in several tissues of black scabbardfish in European waters and to compare these with current regulations and assessment criteria. The background concentration of mercury in fish muscle tissue is set between 0.01 and 0.07 mg/kg wet weight (OSPAR Commission 97/15/1). However, Gordon *et al.* (1995) suggested that, as a continental slope species, black scabbardfish tend to be top predators and to feed at higher trophic levels than their shelf counterparts. The potential for the accumulation of trace metals (particularly mercury) may consequently be greater in deepwater species. High concentrations of mercury in deep-sea predators were also found by Phillips (1980), Broek *et al.* (1981) and Cronin *et al.* (1998).

Materials and methods

Samples of muscle, liver, gonad and gill tissues from black scabbardfish were collected from different locations by the various participants. The number of samples analysed, as well as details of sampling sites and results of mercury analysis are summarised in table 1 and fig 1. All fish were assigned a maturity stage between 1 and 5 according to defined maturity scale (Gordo *et al.*, 2000). Only samples from Madeira and the Azores covered all maturity stages (1-5), whilst only maturity stages 1 and 2 were observed in fish off Scotland and mainland Portugal.

The analytical methods varied from laboratory to laboratory:

- IPIMAR: 325 black scabbardfish were sampled between July 1998 and April 2000, off Sesimbra, Portugal mainland. For each individual fish, total length, total weight, sex and maturation stage were recorded. Samples of muscle, liver, gonads and gills were removed, frozen to -30EC and then defrosted prior to analysis. Tissues were digested in boiling nitric acid and hydrogen peroxyde and made up to 50 ml with distilled water (Lawson and Kirkwood, 1982). Total mercury was determined by cold vapour atomic absorption spectrophotometry with a digital Backarach/Coleman Mercury Analyser System 50-D. Quality control of the data was provided through the use of certified reference materials (DORM-2, DOLT-2, dogfish muscle and liver, National Research Council of Canada, Ottawa) in every batch analysed.

- DSIP-DRP: Between October 1998 and January 2000, the DSIP-DGP had obtained 227 black scabbardfish. For each individual fish, total length, total weight, sex and maturation stage were recorded. Samples of muscle, liver, gonad and gills were frozen to -30EC and forwarded to IPIMAR for total mercury analysis.

- DOP: Levels of total mercury were determined in muscle, gonad, liver and gill of 96 specimens of *Aphanopus carbo* caught in the Azores in two different fishing periods, May/June 1998 and July/October 1999. The samples of various tissues were frozen at -30E and defrosted to 5EC for analysis. The samples were digested in sulphuric acid and potassium permanganate. This was followed by

total mercury determination by cold vapour atomic absorption spectrophotometry with a Perkin-Elmer Analyser System Coleman 50B. The limit of detection of the method, taken as twice the standard deviation of triplicate analyses of blanks, was 0.007μg. The mean blank (n=25) was 0.01 ± 0.004 μg. Biais of the method (expressed as relative error) was < 10%, and was monitored throughout the study with standards of inorganic mercury, and reference materials (dogfish muscle DORM1, National Research Council of Canada, Ottawa). Precision (reproducibility) of the method, expressed as the coefficient of variation of duplicates within and between batch, was within 10% for the total mercury determination in gonad, muscle and gill. In the case of the liver, the coefficient of variation (CV) of the total mercury concentration sometimes exceeded 10%. The analysis of these particular samples were repeated on different days.

- FRS ML: Black scabbardfish were sampled between September 1998 and October 1999 from the Rockall Trough, West of Ireland. Some samples were also obtained from West of France, Sesimbra and Madeira. For each individual fish, date, position and depth of capture as well as total length, total weight, maturity stage and sex were recorded. Muscle, liver and gonad tissues were sampled, individually wrapped in aluminium foil and frozen to -30EC. They were defrosted prior to analysis. Homogenised sub-samples of muscle tissue (ca 10g), liver (ca 1g) and gonads (ca 5g) were digested in boiling nitric acid and made up to 25 ml with distilled water. The samples were then diluted 20 times in 1% aristar nitric acid before being analysed by ICP-MS (Perkin-Elmer Elan 6100 DRC). Quality control of the data was provided through the use of the certified reference material DORM-2 (dogfish muscle, National Research Council, Canada) in every batch of samples analysed.

All laboratories participated in the international interlaboratory calibration exercises QUASIMEME (D. Boer and Wells, 1996)

Results

Results from all participants (mg/kg wet weight) are summarised in table 1 and fig 2 and 3. Mean mercury concentrations in muscle were all below 1 mg/kg apart from in some fish of high maturity stage. Mean concentrations in gonads did not exceed 0.33 mg/kg. Median concentrations in gills did not exceed 0.19 mg/kg. In livers, all mean concentrations were greater than 1 mg/kg apart from in samples collected west of Ireland where it was 0.55 mg/kg. These fish were small and in the first stages of maturation. The maximi values in livers were found in fish of highest maturity stage, with one individual presenting a total mercury concentration of 45.9 mg/kg. However, none of these individuals presenting high mercury values were classified as statistical outliers (see fig 2). In certain maturity stages, the mean and variances of the mercury concentrations are very high because of the presence of a small number of fish with particularly high concentrations of mercury.

Concentrations in the various organs were significantly different (standard t-test, $p=0.000$): levels in liver were higher than in muscle, gonad then gill. In the same way, the total mercury concentrations were significantly higher (standard t-test, $p=0.000$) in more advanced maturity stages (fig 2) in all three organs sampled. Within organs and considering all maturity stages together, the difference between sampling locations were significant ($p=0.000$), but was an artefact of sampling constraints, as only the Azores and Madeira presented fish with advanced maturity stages.

An analysis of variance was performed on all results to investigate the effects of maturity and sex on the concentration of mercury in each individual organ, using a two-way generalised linear model (fig 4). Anova testing was not used because of the unbalanced dataset. The effect of sex, maturity, and of the two combined, were statistically significant (see fig 4 for values) in all cases except for the combined factor for mercury in gills. The concentrations increased markedly with maturity stage. Higher concentrations were found in females than males. Concentrations in females were also more affected than concentrations in males by the maturity stage, ie there is a bigger difference between mercury concentrations in stages 1 and 5 in females than in males. The mercury concentrations increased linearly with the

maturity stage in all organs except in gonads, where it increased exponentially (fig 4).

Total length and total weight presented a strong exponential relationship, as is usually the case for fish. On the other hand, mercury concentrations in the various organs only showed weak relationships with length or weight, due to a high scatter of the values: exponential to length and linear to weight. Although the relationships were statistically significant (Bonferroni probability $p<0.05$), the variance was too large to draw any further conclusions.

Discussion

Within the European Union, the maximum recommended concentration of total mercury in edible tissues of fish is 0.5 mg/kg, with exception of some species, including black scabbardfish, for which it is 1 mg/kg (Commission Decision, 19 May 1993). For all maturity stages, the mean mercury concentrations in muscle tissue are under the 1 mg/kg limit. However, all maturity stages contained a proportion of fish with individually higher concentrations in muscle (fig 1).

Concentrations of mercury in gonad are of little practical toxicological concern as gonads are only occasionally consumed, even though occasional fish presented concentrations above the EU limits. The liver samples contained higher concentrations, with all but the lowest maturity stage presenting median concentrations above the EU limits. The livers of black scabbardfish have been regarded as a gastronomic delicacy in parts of Portugal where black scabbardfish constitutes an important component of the diet of some communities (Gaggi *et al.*, 1996; Renzoni *et al.*, 1998). However, as a consequence of Renzoni's work and other concerted actions by DSIP and IPIMAR, the Regional Government in Madeira has prohibited the consumption of black scabbardfish liver (Sena-Carvalho, pers. comm.). The results obtained during this study further confirm the basis for this action.

Costa and Monteiro (1997) showed that two study groups, one residing the Azores and the other in a coastal area of mainland Portugal, presented mean total mercury

concentrations of respectively 3.9 and 2.6 mg/kg fresh weight in hair. Analysis of human hair can be used to determine exposure to mercury because the mercury is deposited in the hair root and can remain in the hair for several years. The quantity of mercury deposited in the hair roots is linearly related to the concentration of mercury in the blood. Diet in the Azores comprises mainly deep-sea fish whilst diet on mainland Portugal consists mainly of epipelagic fish. Renzoni *et al.* (1998) reported similar results, showing that people in parts of Madeira exhibited enhanced mercury levels in hair and blood.

This report provides the most comprehensive evidence that black scabbardfish can contain mercury concentrations higher than the present EU regulatory limit, including in the edible muscle tissue. Effectively, the consumption of 200 g of black scabbardfish muscle tissue containing 1 mg/kg total mercury will result in the intake of 200 μg total mercury, which is the maximum total mercury recommended weekly intake (WHO, 1993). Hence, the risk of exceeding recommended mercury intake appears to be at its greatest in the communities where black scabbardfish occur regularly in the diet. This risk has been compounded in those areas where black scabbardfish livers have been traditionally consumed. The livers exhibit the highest levels of mercury (5 g of a fish liver presenting 40 mg/kg total mercury would meet the maximum recommended weekly intake). Concentrations are highest during the third quarter of the year when the species is spawning (ie maturity stages are high) The cycle of mercury in black scabbardfish is little understood, and details of the mechanisms linking maturation stage and mercury concentrations require further investigation.

The health risk of consuming deep-sea predators has also been highlighted by Monteiro et al. (1996). He found a four-fold increase in mercury accumulation between epipelagic and mesopelagic fish species, reporting total mercury concentration up to 0.377 mg/kg dry weight in deep-water fish species. Moreover, this contamination of the deep-sea fauna has also an influence on the rest of the ecosystem, as Monteiro et al. (1999) also found a similar four-fold increase in mercury accumulation in sea-bird feathers whether their diet is based on epipelagic of mesopelagic fish from the Portuguese Atlantic region, but without any significant

difference between the various locations studied (Azores, Madeira, Salvages and mainland Portugal).

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We would also like to thank Andrew Newton who organised the sampling in Scotland and reviewed the text.

Legends

Table 1: Summary of sampling information, including concentrations of total mercury in various organs of black scabbardfish expressed in mg/kg wet weight

Fig 1: Location of the sampling of black scabbardfish

Fig 2: Box plot of the concentrations of total mercury (mg/kg wet weight) in black scabbardfish per organ and maturity stage (within each organ, maturity stage from 1 to 5 increasing from left to right)

Fig 3: Box plot of the concentrations of total mercury (mg/kg wet weight) in black scabbardfish per organ and location (within each organ, respectively Azores (A), Ireland (I), Madeira, (M) Meriadzec (m) and Sesimbra (S) from left to right)

Fig 4: Analysis of variance: least square means in black scabbardfish livers as a function of sex and maturity

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Table 1

analysing Laboratory	sampling site	gear	no of fish analysed	maturity range	total length (cm) range	total weight (g) range	Hg liver mg/kg mean±std (range)	Hg muscle mg/kg mean±std (range)	Hg gonad mg/kg mean±std (range)	Hg gill mg/kg mean±std (range)
IPIMAR	Sesimbra	longline	325	1-2	73.5-136.5	685-3510	1.36±0.68 (0.20-4.95)	0.62±0.19 (0.20-1.58)	0.17±0.07 (0.04-0.55)	0.13±0.05 (0.03-0.39)
DSIP-DGP	Madeira	longline	227	1-5	56.4-137.5	178-3370	3.17±2.57 (0.08-42.59)	0.80±0.36 (0.11-6.29)	0.31±0.19 (0.01-2.09)	0.19±0.09 (0.01-0.89)
DOP	Azores	longline	96	1-5	62.5-137.0	273-3736	4.49±6.64 (0.43-45.90)	1.04±0.44 (0.27-2.19)	0.33±0.37 (0.04-2.74)	0.19±0.14 (0.04-0.77)
FRS ML	W France (Meriadzec)	trawl	26	1-2	87-124	850-2760	1.63±1.21 (0.63-5.54)	0.66±0.29 (0.15-1.42)	0.16±0.10 (0.05-0.40)	
FRS ML	W Ireland (Rockall)	trawl	38	1-2	74-114	440-2180	0.55±0.25 (0.16-1.24)	0.35±0.15 (0.11-0.60)	0.09±0.06 (0.03-0.22)	
FRS ML	Sesimbra	longline	30	1-2	88-123	740-2940	1.29±0.87 (0.22-3.61)	0.48±0.17 (0.10-0.85)		
FRS ML	Madeira	longline	25	1-5	108-131	1590-3461	4.03±4.06 (0.69-17.96)	0.80±0.24 (0.51-1.56)		

Fig 1

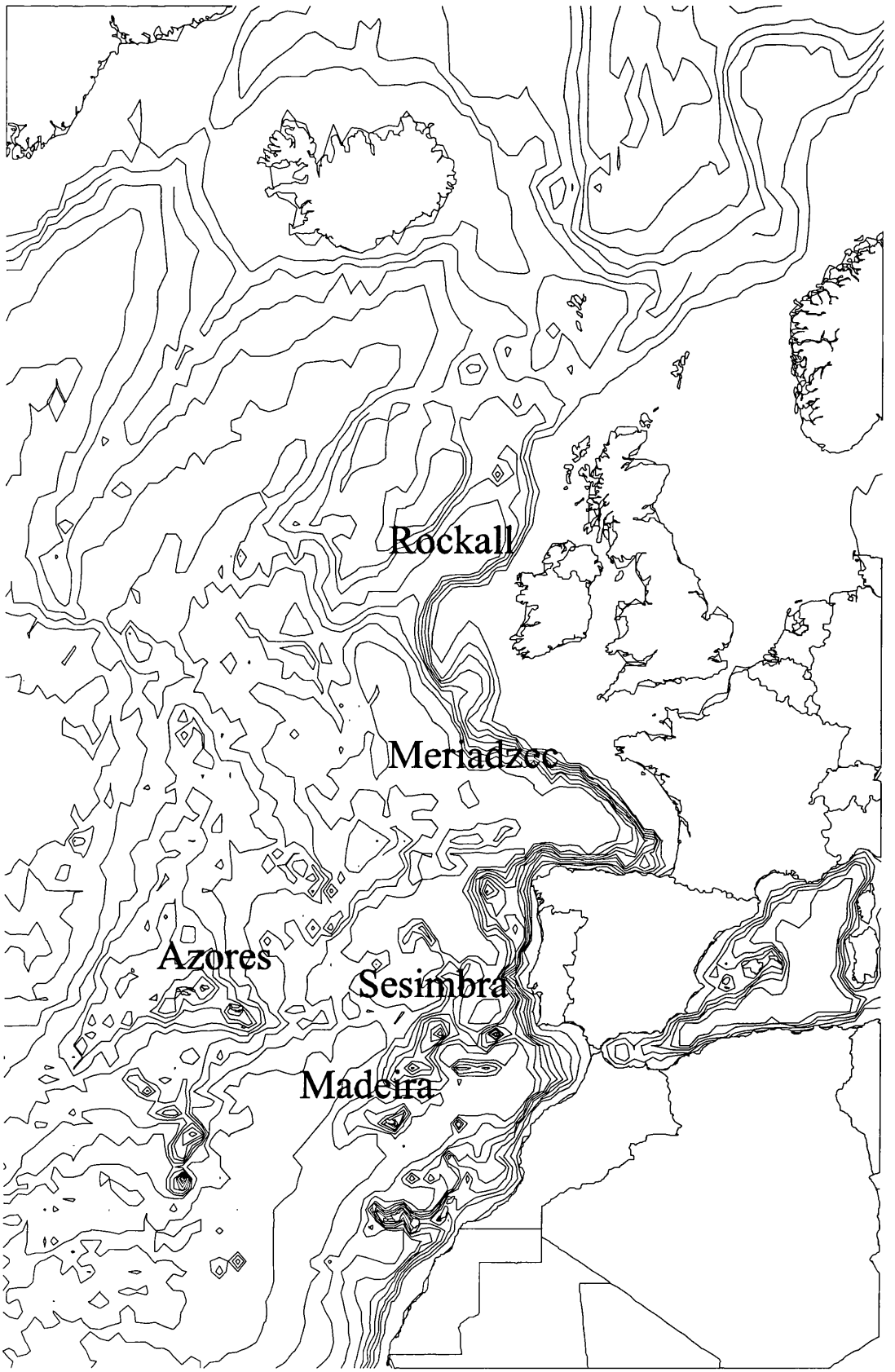


Fig 2

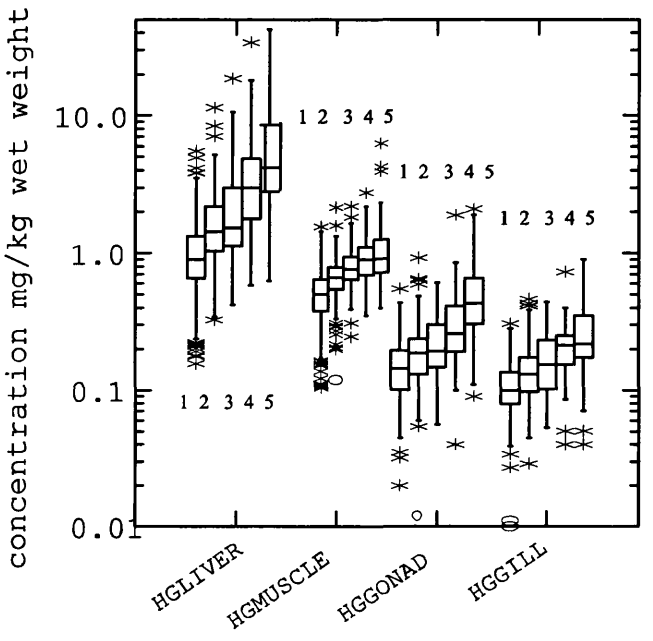
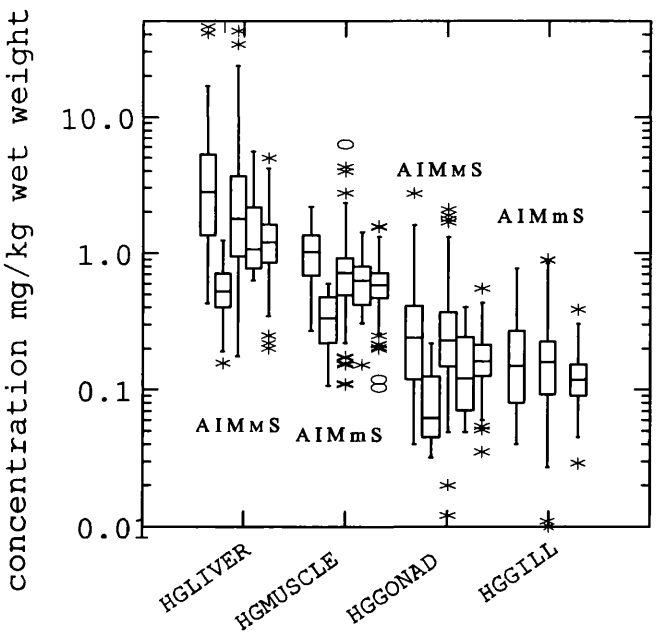


Fig 3



Legend

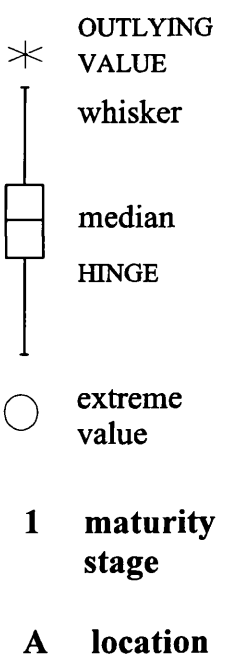
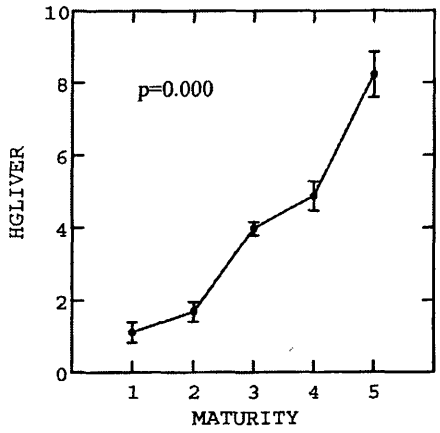
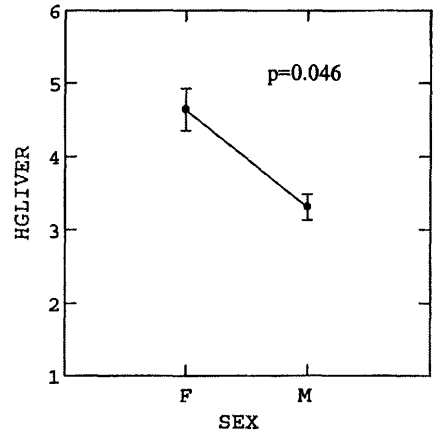


Fig 4





In other organs probabilities p:

	muscle	gonad	gill	liver
sex	0.000	0.015	0.004	0.046
maturity	0.000	0.000	0.000	0.000
sex*maturity	0.006	0.004	0.202	0.000

7.2. Horizontal and vertical distribution of organic contaminants in deep-sea fish species (submitted to *Chemosphere*)

The effects of longitude and depth on the concentrations and patterns of organic contaminants in deep sea fish have been the subject of a paper, which was submitted on 4th June 2001 to *Chemosphere* for publication and has been peer-reviewed.

The paper as submitted after peer review is reproduced in the present section.

Horizontal and vertical distribution of organic contaminants in deep-sea fish species

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Abstract

Polychlorobiphenyls (PCBs) and pesticides are persistent, semivolatile contaminants whose global circulation is now well established. One of their ultimate sink is the bio- and geo-phases in the deep-sea. We have investigated the distribution of selected PCBs and pesticides with depth and longitude in various deep-sea fish, namely black scabbardfish (*Aphanopus carbo*), orange roughy (*Hoplostethus atlanticus*), roundnose grenadier (*Coryphaenoides rupestris*) and *Bathysaurus ferox* from Madeira, Sesimbra, the Meriadzec Terrace and Rockall Trough in the North Eastern Atlantic at 1000 m depth, and at 2000 m depth where available.

In most species, males presented higher levels of contamination than females. This pattern was probably due to the females eliminating contaminants through egg production. Roundnose grenadier presented higher levels of contamination (mainly p,p'-DDE, CB 153, 138, 180, ΣCB(24) and p,p'-DDT) in fish caught at 2000 m than at 1000 m depth. Similarly, *Bathysaurus ferox*, which is found deeper than the other species studied, showed much higher levels of most CBs and pesticides (lipid normalised) than the other species. Concentrations were up to 10 times higher, and showed differences between water masses.

Keywords: PCB, pesticide, deep-sea, fish, distribution, water masses, Atlantic Ocean

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Introduction

Organic contaminants such as polychlorobiphenyls and pesticides are persistent and semivolatile, and hence have been detected in a wide range of environmental media, including biota (e.g. Risebrough et al., 1968; Fowler, 1990; Tanabe et al., 1994), and can now be found in remote parts of the globe, such as the Arctic (Hargrave et al., 1997).

Until recently, very few studies have reported concentrations of organochlorine compounds in deep-sea organisms (Barber and Warlen, 1979; Kramer et al., 1984), probably as a combination of the low commercial interest of the species at the time, and the need for specialised fishing gear. Increasing concern that the deep-sea might act as an ultimate sink for such contaminants and the greater importance of deep-sea commercial fisheries around the world, have resulted in more studies being conducted (Lee et al., 1997; Berg et al., 1997 and 1998; Takahashi et al., 1998; Froescheis et al., 2000; Looser et al., 2000; Mormede and Davies, 2001). Berg (1998) concluded that deep fjords might act as a trap for organochlorine contaminants. Froescheis et al. (2000) and Looser et al. (2000) showed that the concentrations of these pollutants in bottom dwellers at depths greater than 800 m was between 10 and 17 times more than in the related surface species, highlighting transport processes of persistent organochlorinated compounds to the deep-sea biophase, both vertical and from the poles.

Ballschmiter (1992) showed that PCB and pesticides contamination signatures in the Northern and Southern hemispheres were different, mainly due to differences in use of chemicals. For example, although DDT was banned in industrialised countries decades ago, it is still in use in developing countries. Similarly, α-HCH is used solely in the Northern hemisphere, whilst γ-HCH is much

preferred in the Southern hemisphere. Thus, the α-HCH/γ-HCH ratio can pinpoint the source of contamination. These differences are reflected in contaminant concentrations in the deep-sea water masses of the Atlantic, originating from the Arctic or Antarctic regions (Cromwell, 2000).

As the most rapid uptake of xenobiotic organic contaminants (up to 400-450 Dalton) by fish is via the gills rather than food (Randall et al., 1998), water mass characterisation is likely to be much more important than diet in explaining concentrations of contaminants such as PCBs and pesticides in fish. Hence fish could be used as biomarkers for water masses. In this study, we investigated the variations of contaminant concentration within the same deep-sea species from various locations of the North Eastern Atlantic, at two different depths. The hydrography of the Rockall Trough, one of the sampling locations, is well known, presenting two main water masses: the North Atlantic Central Water of the Western Atlantic between 0 and 1200 m depth, and the Labrador deep-sea water, below 1200 m depth (Ellett et al., 1986).

The aim of this study is to link hydrographic factors with contaminant concentrations in fish by differentiating patterns of contaminant distribution in fish from the same species (to avoid inter-species differences) caught at different depths.

Materials

Fish were collected during various deep-sea cruises using deep-sea trawls or long-lines (Sesimbra and Madeira only). The sampling locations were as follows: in the Rockall Trough at 1000m depth (EU statistic squares 43E0 to 47E4), west of

Ireland at 1000m depth (EU statistic squares 39D9 to 41E0), Meriadzec Terrace at 1000 and 2000m depth (EU statistic square 23E1), off Sesimbra harbour at 1000m depth (EU statistic square AM21) and off Madeira Island at 1000m depth (Fig 7). Twenty-nine orange roughy (*Hoplostethus atlanticus*) and 15 blue ling (*Molva dypterygia*) were collected in April 1999 from the Meriadzec Terrace (west of Brittany) and from west of Ireland at 1000 m depth. Thirty-three roundnose grenadier (*Coryphaenoides rupestris*) were collected from west of Ireland at 1000 m and 2000 m depths and 16 *Bathysaurus ferox* were taken from the two previous locations at 2000 m depth. Sixty-six black scabbardfish (*Aphanopus carbo*) were collected at 1000 m depth west of Ireland in April 1999 and at 1000m depth in the Rockall Trough, 25 were sampled 1000 m from Madeira and 32 from Sesimbra in October 1999. For split of samples in the various locations and depths, see Tables 1 to 3.

Description of species:

- Adult black scabbardfish are benthopelagic, living on the continental slope or underwater rises at about 200 to 1600m depth, whilst juveniles are mesopelagic. They migrate to midwater at night. They are distributed in the eastern Atlantic, from the Denmark Strait to Madeira and western North Africa. They, or closely related species, are also known from many localities in the Atlantic, Indian and Pacific oceans. They are caught with special deepwater lines off Madeira and, to a lesser extent, off Portugal; and by deep water trawls west of Scotland. Black scabbardfish is a relatively fast growing species, living up to 8 years. They become mature at 80 to 85cm standard length. They are carnivorous, feeding on fish, squid and crustaceans.
- Fishes of the lizard fish genus *Bathysaurus* are amongst the most characteristic of the ocean floor at depths below 1000m. *B. ferox* are

circumglobal except beneath polar waters, at depths of 1000 to 2500m and temperatures between 3 and 4°C. They grow to about 40 to 60cm in length, with a maximum recorded at 83.5cm and 7kg. Their trophic role as top predators in an energy-poor ecosystem dictates low population densities with wide spacing of individuals. *B. ferox* have a predominantly piscivorous diet of demersal and bathypelagic fishes, supplemented by occasional large benthic crustacea. Individuals rest motionless on the substrate and capture prey by lunging forward in a sudden rapid burst.

- Macrouridae are the most widespread family of fish occurring on the continental slope of the North Atlantic, and the roundnose grenadier is the most heavily exploited. Roundnose grenadier reaches lengths in excess of 100cm, although up to 50% of it consists of its whip-like tail. It is benthopelagic, feeding on copepods and amphipods, and becomes more piscivorous as it grows. It occurs at 180 to 2200m depth, but off north west of Ireland it is normally present at 600 to 1800m depth. It occurs along the continental slope from North Africa, Europe, Iceland, Greenland, Canada to the south east of the United States, generally between 37 and 66°N, and occasionally as far south as 20EN. Gordon (1979) suggested that juveniles are demersal at about 1000m depth on the slope. As they grow, they move down the slope to about 1800m depth as they grow. Nearing maturity, they migrate back up so that mature females are at about 1000 to 1200m depth and males are shallower.
- In the Atlantic, orange roughy is widely distributed from Europe to South Africa in the east, but is apparently restricted to the Gulf of Maine in the west of the Atlantic. There is little genetic differentiation between the populations from the eastern North Atlantic, the south west Pacific and the Tasman Sea. On

the slopes to the west of the British Isles, orange roughy is most common between depths of 1000 and 1200m. There are no differences in the length/frequency of orange roughy between the 1000 and 1250m bathymetric zones in the Rockall Trough. The principal components of its diet are decapods and mysids, with fish, cephalopods and amphipods of lesser importance.

- Blue ling is distributed in the eastern North Atlantic from Iceland down to Morrocco, as well as in the Mediterranean. It is usually caught at depths below 300m, down to 800m and feeds on crustaceans and fish, with predominance of silver pout and blue whiting (Whitehead *et al.*, 1984; Thomas, 1997).

Methods

The fish were dissected onboard soon after being caught. Livers were sampled, wrapped in aluminium foil and frozen at -20°C. Once ashore, individual samples were thoroughly defrosted and then homogenised in pools of no more than five fish, grouped by length. Pooled samples were dried by grinding with anhydrous sodium sulphate, followed by Soxhlet extraction with 300 ml MTBE (methyl tert-butyl ether). Lipid content was determined by weighing 20 ml of the extract in a dry evaporating dish, heating it to dryness at 45°C and weighing the lipid remains. After lipid determination, clean-up was achieved by using one 6 g alumina column and keeping the whole fraction. Group separation was conducted with one 3 g alumina and one 3 g silica column. The fractions kept were respectively 5.5 ml and 7.5 ml approximately (checked with every new absorbent batch).

Instrumental analysis was performed by capillary column gas chromatography with an electron capture detector. PCBs were analysed on Perkin Elmer ECD gas chromatograph fitted with 50 m*0.25 mm I.D. CP-Sil 8 column and

2.5 m*0.53 mm I.D. uncoated deactivated precolumn, programmable on-column (POC) injector with hourglass inset and autosampler. The oven temperature program was 80°C for 1 min, then 3°C/min to 270°C, then held for 12 min. Injector temperature started at 120°C for 0.2 min, then 200°C/min to 270°C then held for 70 min. The detector was held at 270°C.

Pesticides were analysed using a VARIAN 3500 ECD gas chromatograph fitted with 50 m*0.25 mm I.D. CP-Sil 8 column and 2.5 m*0.32 mm I.D. uncoated deactivated precolumn, septum programmable injector with glass insert and 8200 CX autosampler. The oven temperature program was 80°C to 180°C at 15°C/min, then held for 6 min, then to 290°C at 3°C/min where it was held for 20 min. Injector and detector temperatures were the same as above.

These methods are accredited by the United Kingdom Accreditation Service (UKAS) and are regularly validated through successful participation in national and international intercalibration exercises (QUASIMEME, Boer, D. and Wells, 1996). The accuracy of the data was monitored by the use of Laboratory Reference Materials (LRMs) in every batch of analysis, and the recovery was monitored in each sample by the addition of an internal standard (CB209) prior to extraction.

Concentrations of HCB, CBs 28, 31, 44, 49, 52, 70, 74, 101, 105, 110, 114, 118, 128, 138, 149, 153, 156, 157, 158, 170, 180, 187, 189 and 194 were measured, and the sum of all these CBs was calculated as ΣCB(24). The following pesticides were also determined: α-HCH, γ-HCH, α-chlordene, γ-chlordene, aldrin, heptachlor-epoxide, α-chlordane, γ-chlordane, oxychlordane, transnonachlor, heptachlor, endrin, dieldrin, o,p'-DDD, p,p'-DDD, o,p'-DDT, p,p'-DDT, o,p'-DDE and p,p'-DDE. Σchlordane was calculated as the sum of α-chlordene, γ-chlordene, heptachlor-epoxide, α-chlordane, γ-chlordane, oxychlordane, transnonachlor and heptachlor.

ΣDDT was calculated as the sum of o,p'-DDD, p,p'-DDD, o,p'-DDT, p,p'-DDT, o,p'-DDE and p,p'-DDE. Limits of detection were calculated separately for each sample, and were about 1.5 mg kg^{-1} wet weight, i.e. 0.03 mg kg^{-1} lipid weight, for all tissues for all determinands.

Results

Table 1, 2 and 3 present the mean concentrations and standard deviations of PCBs and pesticides in the different species from various locations; Fig. 1 and 2 present the distributions of levels of contaminants in all samples, separated by species. The various species were separated by differential analysis, as shown on Fig. 3. The chlordane and DDT compositions are described in Fig. 4, and the α-HCH/γ-HCH and ΣCB(24)/ΣDDT ratios expressed in Fig. 5.

Black scabbardfish presented higher levels of contamination in males than females from Madeira and Rockall Trough, but the reverse pattern in specimens from Sesimbra (Fig. 2). In general, levels of PCBs and pesticides were much higher in Madeira and Sesimbra samples than elsewhere, by up to a factor of 10. Male fish always showed higher concentrations of contaminants than female fish, apart from ΣCB(24) in orange roughy and Σchlordane in black scabbardfish. Roundnose grenadier presented higher levels of contamination, mainly p,p'-DDE, CB 153, CB 138, CB 180, ΣCB(24) and p,p'-DDT in fish caught at 2000 m than at 1000 m depth (Table 3). *Bathysaurus ferox*, which is found deeper than the other species studied, showed much higher levels of most CBs and pesticides (lipid normalised) than the other species; the concentrations were up to 10 times higher (Table 3).

The chlordane composition (Fig. 4) differed significantly between species only in the case of the *Bathysaurus ferox*, which presented a much higher proportion

of trans-nonachlor than the other species. The DDT composition showed a southerly decreasing percentage of p,p'-DDE, particularly in the case of black scabbardfish (Fig. 4). The HCH pattern appeared to show quite large variations, but these may be artefacts due to the very low concentrations of these contaminants. The ratio p,p'-DDE/ΣDDT was between 0.5 and 0.6 for most samples, but with significantly lower values for *Bathysaurus ferox*, at about 0.3 (see Fig. 5).

Discussion

i) Concentrations

The concentrations of PCBs and pesticides in various deep-sea fish species reported by Barber and Warlen (1979) and Melzian (1987 - off a waste disposal site) were much lower than those found in this study. Results reported by Berg et al. (1997, 1998), Hale et al. (1991), Lee et al. (1997) Takahashi et al. (1998), Froescheis et al. (2000), Looser et al. (2000) and Mormede et al. (in press) were all lower or similar to the concentrations in fish sampled at 1000 m during this study. Berg et al. (1998) reported higher concentrations of DDTs, which is probably due to the fact that the ban on DDTs did not occur in their study area before 1988. Looser et al. (2000) reported higher levels of most pesticides. Madeiran and Sesimbra samples, and all the orange roughy samples, showed much higher levels of CB and pesticides than reported in most of the previously cited studies.

Kramer et al. (1984) analysed one specimen of black scabbardfish, finding high levels of contaminants, hundreds of times higher than in shallow water fish from the same area. The black scabbardfish specimens sampled at the same location (Madeira) in the current study showed similar high concentrations. The fact that Madeiran samples showed such a high contamination level might be due to the

location, although it may well be due to the biology of the black scabbardfish: mature specimens were only collected at Maderia, and showed increasing concentration of total mercury with fish maturity (Mormede et al., in preparation).

Bathysaurus ferox showed much higher contamination levels, and also very differing chlordane composition. This might show the differences of contamination between the different water masses, as suggested by Ballschmiter (1992). Water masses at 1000 m and 2000 m are very likely to present different contaminant signatures, one originating from the North Atlantic Central Belt waters, and the other from the deep-water in the Labrador Sea (Ellett et al., 1986) This theory is being corroborated by the fact roundnose grenadier shows different contaminant levels at 1000 and at 2000 m.

The DDT and chlordane patterns were similar to those found in the other deep-sea studies, as well as the ΣCB(24)/ΣDDT ratio. The p,p'-DDE/ΣDDT ratio was similar to other published for most species, at between 0.5 and 0.6. However in the case of *Bathysaurus ferox*, it shows a low value of 0.3. Such low value has been reported by only Barber and Warlen (1979) for *Antimora rostrata* sampled East of the USA in 1972, and by Berg et al. (1997) for Portuguese dogfish sampled off Greenland. This shift in patterns from 1000 m depth strata in 1972 to Greenland in 1997 and now 2000 m depth might be representative of the water mass flows and show a good example of the delay between the actual pollution and arrival in the very deep-water masses.

In most cases, males presented higher contaminant burdens than females. This is probably because females can pass some of their contaminants burden to their offsprings via reproduction. Gonads, being very fatty tissues, present very high concentrations of PCBs and pesticides on a wet weight, which can then be lost

through the production of eggs. This mechanism has been shown previously in marine mammals (eg McKenzie, 1999) and fish (Phillips, 1995).

ii) Multivariate analysis

The species were separated by multivariate analysis (Fig. 3). All data (expressed on a lipid weight basis) were included in the analysis and separation was attempted by species only, without differentiating between sexes or locations of capture. The two first principal components explained 61% of the variance, and by backwards stepwise discriminant analysis (jackknife classification), 94% of the fish were properly classified in their corresponding species. The variables contributing to the differentiation of the species were (in decreasing order of importance) CB74, CB128, endrin, CB149, heptachlor, p,p'-DDD, CB28, CB138, CB153, then to a lesser extent γ-HCH, α-chlordane, α-chlordene, dieldrin, CB180, CB 31, CB 110, a-HCH. This separation reflects exposure to different patterns of contaminants, as well as the different ability of various species to metabolise individual contaminants. For example, mammals are known to metabolise PCBs to a higher extent than fish (eg: Risbrough et al., 1968).

iii) Depth variations

Variation of contaminant concentrations with depth was investigated by analysing the variance of results obtained for roundnose grenadier. This species was sampled in two different depths and for both sexes, so the sex and depth factors can be studied without introducing bias from differing species. ΣCB(24) showed significant sex and depth effects ($p=0.012$ and 0.003 respectively), showing a higher contamination in both males than females sampled at 2000 m than 1000 m. ΣDDT only showed a significant depth effect ($p=0.017$), with higher concentrations found in fish from 2000 m than 1000 m, but no significant differences between sexes. ΣChlordane

showed no significant differences. ΣCB(24)/ΣDDT showed sex, depth and sex*depth effects (p=0.007, 0.013 and 0.008 respectively), with higher ratios in males than females at 2000 m than at 1000 m, and bigger difference between males and females at 2000 m than 1000 m. The concentrations found for *Bathysaurus ferox* showed a similar trend, that is higher ΣCB(24), ΣDDT and ΣChlordane concentrations in males than females, but more marked for ΣCB(24) than the others.

In the Rockall Trough area, the deep-sea is made up by two main water masses. The water between the surface and *ca* 1500 m is mainly composed of North Atlantic Central Belt waters, whereas water below 1500 m is mainly derived from the deep-water in the Labrador Sea (Ellett et al., 1986). As PCBs and pesticides are mainly accumulated through the gills, the difference in contamination levels between the two roundnose grenadier samples probably reflects differences between these two water masses. As a reciprocate, as the two groups of grenadiers present different contaminants patterns and concentrations, this suggests that roundnose grenadier populations at 1000 m and 2000 m do not mix freely. Such behaviour patterns have not yet been demonstrated.

iv) Geographical variations

Black scabbardfish samples were obtained from five locations (Rockall, west of Ireland, Meriadzec, Sesimbra and Madeira), and therefore could be analysed for geographical variations. To study possible sex-linked variations, only fish from Rockall, Madeira and Sesimbra were included in the model (the other fish had not been sexed). ΣCB(24), ΣDDT and ΣChlordane showed a significant geographical variation (p=0.000, 0.000 and 0.010 respectively) but no significant sex variation. Sex effects were different in the various locations, with males showing higher

concentrations than females in Rockall and Madeira, but the other way around in Sesimbra.

Data from all sites for black scabbardfish were then analysed together, without differentiating between sexes. Once again, ΣCB(24), ΣDDT and ΣChlordane showed a significant geographical variation (p=0.001, 0.000 and 0.005 respectively). Concentrations increased significantly in a southerly direction (Fig. 6).

The ΣCB(24)/ΣDDT ratio in black scabbardfish from various locations increased in a southerly direction (Fig. 5), which shows that ΣCB(24) increased faster than ΣDDT in a southerly direction. (Fig. 5).

Conclusions

The concentrations of organic pollutants in deep-sea fish recorded in the present study are similar or higher than in other studies. In most species, females presented lower contaminant concentrations than males, probably due to the elimination process available to females, of losing contaminants through their eggs. Concentrations of most contaminants were higher in fish caught at 2000 m than at 1000 m in the Rockall Trough, probably expressing the differences in contamination of the water masses in which they live. In a similar way, concentrations of ΣCB(24), ΣDDT, Σchlordane and the ratio ΣCB(24)/ΣDDT in black scabbardfish increased in a southerly direction, from Rockall Trough to Portuguese locations.

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Tables and Figures Legends

Table 1: Concentrations of polychlorobiphenyls (PCBs) and pesticides in livers of black scabbardfish from various locations, expressed in μg kg^{-1} lipid weight, std = standard deviation; no sex is when sex could not be determined.

Table 2: Concentrations of polychlorobiphenyls (PCBs) and pesticides in livers of orange roughy and blue ling from various locations, expressed in μg kg^{-1} lipid weight, std = standard deviation.

Table 3: Concentrations of polychlorobiphenyls (PCBs) and pesticides in livers of roundnose grenadier and *Bathysaurus ferox* from various locations, expressed in μg kg^{-1} lipid weight, std = standard deviation.

Fig. 1: Distribution of ΣCB(24), ΣDDT and Σchlordane in livers of males (M) and females (F) *Bathysaurus ferox* (BFE), blue ling (BLI), black scabbardfish (BSC), orange roughy (ORO) and roundnose grenadier (RNG), expressed in μg kg^{-1} lipid weight.

Fig. 2: Distribution of ΣCB(24), ΣDDT and Σchlordane in livers of males (M) and females (F) black scabbardfish, expressed in μg kg^{-1} lipid weight, ? is undetermined sex.

Fig. 3: Discriminant score plot for five species; *Bathysaurus ferox* (BFE), blue ling (BLI), black scabbardfish (BSC), orange roughy (ORO) and roundnose grenadier (RNG).

Fig. 4: Chlordane and DDT composition in livers of *Bathysaurus ferox* (BFE), blue ling (BLI), black scabbardfish (BSC), orange roughy (ORO) and roundnose grenadier (RNG).

Table 1

species	Black scabbardfish		Black scabbardfish				Black scabbardfish		Black scabbardfish				Black scabbardfish			
site	IRELAND		MADEIRA				MERIADZEC		ROCKALL				SESIMBRA			
sex			F		M				F		M		F		M	
no pools	10		2		3		7		7		5		7		5	
	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std
length (cm)	94.2	10.9	126	4.3	117.2	5.2	108	10.2	89.8	15.3	89.3	11.2	108	10.2	101	10.2
weight (g)	1093	496	3002	459	2052	311	1666	549	1387	1132	986	402	1713	560	1243	237
% lipid	13.2	2.8	8.2	0.6	7.6	0.7	11.1	3.1	11.4	2.5	10.8	2.7	13.9	2.9	10.8	5.2
HCB	29.5	12.8	5.3	5.3	30.3	3.9	19.2	8.3	23.0	7.1	24.2	4.9	23.1	10.7	16.2	5.5
CB 31	<0.03	6.5	<0.03	19.1	0.9	6.2	3.4	6.1	<0.03	<0.03	<0.03	2.1	10.3	4.5	2.9	11.0
CB 28	<0.03	4.1	2.7	7.9	5.1	7.1	0.1	3.5	<0.03	<0.03	<0.03	2.8	7.9	3.7	5.8	3.6
CB 52	<0.03	57.4	<0.03	36.0	28.8	76.6	<0.03	40.1	<0.03	<0.03	<0.03	<0.03	45.1	27.6	16.1	49.1
CB 49	<0.03	9.5	1.7	7.5	10.4	18.1	<0.03	8.4	<0.03	<0.03	<0.03	2.5	14.4	6.3	3.9	7.4
CB 44	<0.03	14.6	<0.03	8.2	<0.03	13.3	<0.03	11.0	<0.03	<0.03	<0.03	<0.03	5.7	8.8	<0.03	8.4
CB 74	<0.03	5.8	10.6	13.2	57.0	31.3	5.6	4.2	<0.03	<0.03	<0.03	2.8	18.6	13.0	2.9	57.0
CB 70	<0.03	20.3	<0.03	11.3	<0.03	22.7	<0.03	16.4	<0.03	<0.03	<0.03	6.6	15.0	18.8	<0.03	15.0
CB 101	<0.03	35.8	66.8	73.4	382	273.7	14.0	27.8	<0.03	<0.03	<0.03	7.7	123	102	4.9	415
CB 110	<0.03	23.2	<0.03	8.0	<0.03	25.1	<0.03	22.7	<0.03	<0.03	<0.03	10.1	16.9	34.8	<0.03	36.1
CB 149	17.1	19.1	104.3	76.3	430	296.9	56.4	24.6	<0.03	<0.03	<0.03	8.2	136	125	80.4	577
CB 118	14.8	24.3	98.2	75.6	497	301.0	54.6	36.0	<0.03	3.6	<0.03	13.9	124	114	51.3	984
CB 114	6.6	7.5	7.6	5.8	37.9	22.7	5.3	3.8	<0.03	41	5.2	5.3	11.8	7.3	10.7	44.0
CB 153	99	56	360	235	3515	1563	203	150	40	29	88	71	422	400	318	144
CB 105	7.3	10.9	29.3	25.0	155	93.2	19.4	14.7	<0.03	14.0	<0.03	1.6	38.9	41.7	17.8	178
CB 138	74	41.1	270	184	2115	1174	165	125	13.7	20.4	42.9	24.6	306	306	208	1203
CB 158	4.4	7.3	12.8	8.3	68.3	38.5	5.5	35.7	<0.03	7.3	<0.03	<0.03	12.8	14.0	6.0	60
CB 187	40	126	43.3	16.1	686	840	95.1	56.1	29.0	11.6	31.5	15.7	179	210	156	912
CB 128	11.0	7.8	15.9	24.5	124	82.6	23.6	17.9	<0.03	3.5	5.3	4.3	38.0	36.8	23.7	173
CB 156	5.8	2.1	20.1	14.7	113	69.9	10.2	11.4	<0.03	2.3	<0.03	1.7	20.5	26.5	13.8	124
CB 157	2.8	1.4	10.0	6.6	55.8	31.9	4.7	3.7	<0.03	16.0	<0.03	2.1	8.1	8.7	6.0	57.0

Table 1 (continued)

species	Black scabbardfish		Black scabbardfish				Black scabbardfish		Black scabbardfish				Black scabbardfish			
site	IRELAND		MADEIRA				MERIADZEC		ROCKALL				SESIMBRA			
sex			F		M				F		M		F		M	
no pools	10		2		3		7		7		5		7		5	
	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std
CB 180	56.6	27.6	218	140	1120	464	126	83.5	36.8	15.8	33.9	18.1	227	275	191	431
CB 170	17.1	10.0	79.3	54.3	337	192	42.1	31.8	13.6	4.2	12.5	5.7	76.5	106	63.5	534
CB 189	0.2	1.6	2.1	1.5	9.9	6.3	0.8	0.9	<0.03	<0.03	<0.03	<0.03	1.6	2.4	1.1	12.0
CB 194	6.7	5.1	30.6	20.5	157	97.2	16.1	11.8	4.9	2.0	5.3	1.8	26.5	36.3	21.2	154
ICES7CB	95.6	181	1010	753	7660	3780	563	362	91.1	63.7	239	99.8	1230	1200	769	4520
ΣCB	129	297	1370	1000	9880	5550	845	496	140	78.0	364	133	1850	1830	1090	7450
α-HCH	3.1	1.0	3.8	3.8	0.7	1.3	0.5	0.6	<0.03	<0.03	<0.03	<0.03	<0.03	0.4	0.3	2.2
γ-HCH	<0.03	2.8	<0.03	<0.03	<0.03	4.8	<0.03	4.9	<0.03	<0.03	<0.03	<0.03	0.9	1.8	<0.03	2.0
α-chlordene	0.7	1.8	3.5	3.4	1.0	1.1	0.1	2.0	<0.03	<0.03	<0.03	2.2	0.7	0.8	<0.03	2.5
Aldrin	1.1	2.6	2.1	2.0	2.9	2.4	<0.03	1.9	<0.03	2.1	<0.03	<0.03	1.5	1.1	<0.03	1.4
γ-chlordene	16.3	9.2	7.0	5.9	2.2	10.1	1.0	17.3	<0.03	4.4	<0.03	7.3	4.9	1.6	4.0	3.3
Hept.epoxide	3.0	1.3	0.3	0.3	<0.03	2.0	0.6	1.6	<0.03	2.1	<0.03	1.7	3.3	1.4	3.5	1.7
Oxychlordane	6.8	3.4	6.3	5.3	36.0	9.8	8.3	4.1	4.3	3.1	3.9	2.3	11.9	5.6	9.3	18.0
α-chlordane	13.4	3.4	24.2	14.4	68.0	33.6	14.8	5.2	9.0	4.2	11.5	3.5	21.8	17.3	14.8	45.7
γ-chlordane	51.3	18.4	61.0	44.4	215	81.3	56.4	19.0	38.0	6.4	40.7	11.3	73.9	45.1	39.3	158
T-nonachlor	82.7	29.8	89.0	57.0	530	305	118	41.0	59.3	14.0	54.4	20.7	112	69.1	62.0	422
Dieldrin	39.6	15.4	22.9	8.6	23.8	2.3	40.9	28.6	32.4	8.2	36.4	7.2	27.4	6.9	22.0	16.5
o,p-DDD	2.2	6.8	<0.03	3.4	18.7	12.1	<0.03	6.5	<0.03	1.7	<0.03	4.7	5.2	9.7	<0.03	38.3
Endrin	54.9	15.3	159	109	489	147	73.8	24.7	37.8	5.0	36.5	12.8	74.8	41.8	39.4	318
p,p'-DDD	91.5	41.9	144	98.6	577	250	123	50.7	74.8	9.7	88.9	29.3	226	131	98.1	466
o,p-DDT	12.5	5.9	75.2	54.8	317	147	22.1	9.8	9.1	3.9	11.3	3.9	13.7	15.4	10.1	594
p,p'-DDT	139	58.2	434	167	2350	1080	272	88.9	71.6	24.1	81.8	34.9	171	282	114	1630
o,p-DDE	<0.03	8.0	15.1	16.5	47.2	31.4	8.0	9.8	16.6	9.3	11.5	8.2	28.1	15.9	6.3	41.5
heptachlor	1.4	7.8	1.6	0.7	2.8	4.1	3.8	8.8	<0.03	<0.03	<0.03	<0.03	<0.03	5.6	8.8	5.4
ΣDDT	573	297	1310	713	4350	1672	943	358	384	115	410	145	1190	1320	839	2950
Σchlordane	173	58.1	193	131	855	442	213	66.4	111	21.7	110	32.0	221	134	143	645

Table 2

species	Orange roughy IRELAND				Orange roughy MERIADZEC				Blue ling IRELAND			
sex	F		M		F		M		F		M	
no pools	5		4		3		4		4		2	
	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std
length (cm)	32.0	6.2	27.9	9.2	43.5	7.2	39.5	7.6	104	11.1	87.1	3.6
weight (g)	59	367	384	368	1500	745	1130	561	4190	1480	2360	194
% lipid	27.8	6.3	14.6	15.7	22.7	3.5	19.7	9.9	59.3	6.2	66.2	0.4
HCB	35.8	4.8	29.8	7.2	24.2	3.9	29.8	17.8	52.6	19.1	45.2	2.0
CB 31	6.2	5.4	13.0	27.2	12.6	10.0	2.7	7.5	6.3	1.0	8.6	0.3
CB 28	14.6	7.8	8.3	15.8	5.8	3.5	13.6	10.7	10.2	1.4	9.5	1.7
CB 52	35.5	47.7	24.5	60.6	12.3	15.7	24.7	38.8	19.8	10.6	26.1	1.5
CB 49	7.0	10.4	<0.03	14.0	4.4	4.7	6.7	7.0	9.2	3.8	10.4	1.5
CB 44	4.3	11.4	<0.03	16.4	<0.03	2.9	1.8	9.0	3.6	8.2	-5.1	8.5
CB 74	13.0	6.2	17.5	75.8	13.1	1.1	14.3	14.1	13.6	8.6	15.7	0.2
CB 70	6.7	10.9	8.9	18.0	3.2	3.3	5.8	11.3	0.5	5.7	4.1	0.2
CB 101	59.9	27.3	52.9	113	39.2	6.9	61.2	73.1	26.4	20.1	73.7	8.7
CB 110	11.7	17.0	28.5	27.7	<0.03	5.7	7.8	17.2	0	8.1	0	1.1
CB 149	60.3	21.7	46.1	55.6	49.4	4.2	68.2	91.8	53.1	21.2	55.9	0.2
CB 118	74.6	37.4	58.9	242	64.9	5.6	80.5	81.6	100	52.9	116	6.0
CB 114	8.4	2.3	5.3	17.0	8.7	2.3	9.6	8.5	12.3	3.9	16.3	1.4
CB 153	224	86.5	194	756	213	22.3	277	287	254	95.0	284	9.3
CB 105	26.0	18.9	20.8	102	26.3	3.6	31.5	35.0	33.5	18.3	35.7	1.1
CB 138	166	59.4	147	573	155	10.7	197	20	188	84.4	216	8.7
CB 158	8.4	4.2	6.5	31.4	7.7	0.5	8.3	10.7	12.4	6.0	12.6	0.5
CB 187	66.5	18.8	61.8	347	75.2	8.5	99.9	132	82.0	36.4	87.5	1.0
CB 128	7.0	4.9	2.6	62.3	7.1	1.1	9.7	31.6	33.4	18.9	37.2	1.2
CB 156	10.9	2.2	11.0	55.1	11.5	1.0	14.1	13.4	16.1	7.6	16.4	0.8
CB 157	5.2	1.5	5.0	27.2	5.3	0.2	6.2	6.8	6.9	2.9	7.0	0.3
CB 180	85.7	26.0	88.8	488	117	8.2	130	140	119	61.4	111	6.7
CB 170	21.5	12.8	31.0	187	39.4	3.5	46.9	54.2	38.6	18.0	36.9	1.4

Table 2 (continued)

species	Orange roughy				Orange roughy				Blue ling			
site	IRELAND				MERIADZEC				IRELAND			
sex	F		M		F		M		F		M	
no pools	5		4		3		4		4		2	
	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std
CB 189	2.3	1.4	0.7	6.5	0.9	0.2	2.8	1.8	1.3	0.7	1.1	0.1
CB 194	9.9	3.1	11.6	92.8	13.2	1.0	14.8	16.0	15.7	9.2	12.7	0.4
ICES7CB	661	291	541	2240	607	71.0	783	828	703	317	836	42.5
ΣCB	938	419	758	3360	875	97.8	1130	1290	1030	479	1180	41.4
α-HCH	11.9	2.2	10.2	4.6	6.8	3.1	11.0	8.2	4.2	0.7	4.2	0.4
γ-HCH	5.0	3.6	11.8	7.4	4.6	13.1	10.4	6.4	2.0	1.5	2.0	0.7
α-chlordene	3.0	11.7	2.2	2.2	17.5	3.9	27.3	18.4	1.2	3.2	0.6	0.2
Aldrin	3.8	4.1	3.7	1.1	2.4	1.1	5.3	3.2	2.8	2.4	1.0	1.0
γ-chlordene	3.8	2.6	<0.03	0.5	0.3	3.2	6.4	6.4	0.5	11.9	1.6	1.6
Hept.epoxide	<0.03	2.0	<0.03	0.1	<0.03	0.3	<0.03	4.7	0.5	2.1	2.4	2.4
Oxychlordane	27.9	5.1	31.9	17.1	6.4	6.6	17.7	8.7	7.0	9.0	10.9	6.8
α-chlordane	2.9	2.8	14.2	7.5	5.8	9.1	3.8	1.3	20.9	7.4	22.1	4.7
γ-chlordane	35.6	19.4	43.9	21.1	1.4	5.7	34.8	20.1	85.1	26.5	116	12.2
T-nonachlor	50.2	18.7	74.7	224	20.6	8.4	61.4	29.0	130	51.2	200	2.4
Dieldrin	48.9	4.5	55.7	7.4	33.0	13.1	44.2	25.7	42.2	7.3	42.4	3.5
o,p-DDD	2.7	4.0	0.1	0.4	1.3	1.8	<0.03	5.1	0.8	11.0	0.6	0.5
Endrin	81.9	8.4	88.7	81.3	22.4	22.3	44.0	29.0	89.1	54.9	114	0.7
p,p'-DDD	125	15.6	187	616	139	33.4	148	39.5	125	79.3	129	0.7
o,p-DDT	100	22.0	96.7	48.4	55.8	24.7	79.5	51.1	9.6	42.4	75.1	68.6
p,p'-DDT	221	168	36.8	13370	19.6	99.5	115	115	201	118	234	8.3
o,p-DDE	15.5	5.8	21.5	24.1	11.9	6.7	17.9	7.4	24.3	7.4	26.2	2.5
heptachlor	4.6	1.5	6.2	2.1	4.6	1.7	4.1	1.2	2.3	0.8	1.5	0.6
ΣDDT	933	321	831	351	666	30.8	884	537	852	540	991	52.0
Σchlordane	139	30.0	164	256	82.7	18.8	165	57.4	270	69.8	356	26.6

Table 3

species	Roundnose grenadier								*Bathysaurus ferox*			
site	IRELAND 1000m				IRELAND 2000m				MERIADZEC		ROCKALL	
sex	F		M		F		M		F		F	
no pools	6		4		6		4		4		4	
	mean	std	mean	std	mean	std	mean	std	mean	std	Mean	std
length (cm)	15.8	2.1	15.8	3.5	15.3	1.4	14.8	2.7	67.4	3.5	64.0	2.3
weight (g)	690	203	652	481	545	195	412	236	1310	150	1550	146
% lipid	57.2	9.1	58.6	7.5	57.7	7.2	38.0	13.0	54.4	5.6	66.7	0.3
HCB	31.3	5.3	25.8	10.5	35.6	6.8	26.3	19.6	34.9	14.2	34.9	2.8
CB 31	<0.03	0.8	<0.03	3.0	<0.03	1.7	<0.03	1.7	4.8	10.1	3.2	6.4
CB 28	<0.03	1.5	<0.03	1.6	0.1	1.6	<0.03	1.5	<0.03	14.2	3.0	0.6
CB 52	4.3	17.3	25.6	19.1	5.2	7.6	56.1	1.5	1.8	28.3	<0.03	2.3
CB 49	<0.03	1.9	<0.03	2.4	<0.03	0.8	3.7	0.5	<0.03	1.7	<0.03	0.4
CB 44	<0.03	2.2	<0.03	3.5	<0.03	0.7	5.5	2.0	<0.03	1.6	<0.03	0.7
CB 74	11.9	3.6	16.2	6.5	13.5	3.0	28.3	5.8	23.0	62.4	<0.03	2.6
CB 70	<0.03	3.0	<0.03	2.9	<0.03	10.3	3.4	4.4	<0.03	4.3	<0.03	1.9
CB 101	3.6	13.5	20.2	20.8	12.0	14.4	51.8	11.4	149	32.6	<0.03	1.1
CB 110	<0.03	5.4	<0.03	7.8	<0.03	3.6	14.3	6.1	12.8	2.1	<0.03	2.6
CB 149	20.9	10.5	31.6	15.7	29.2	17.5	51.8	29.0	121	26.4	<0.03	1.9
CB 118	65.8	24.3	91.8	37.3	82.6	30.0	247	33.1	365	115	31.5	1.5
CB 114	0.8	1.9	4.3	2.6	5.1	3.1	11.4	5.9	29.0	9.3	5.1	0.3
CB 153	163	62.2	207	104	240	109	668	1620	2150	1120	249	17.7
CB 105	21.7	15.6	30.4	14.3	30.8	12.4	98.6	26.9	131	52.8	14.4	1.7
CB 138	138	50.8	174	80.3	196	91.9	587	674	926	795	167	9.1
CB 158	7.4	2.9	9.1	3.4	9.8	4.3	31.0	24.6	42.0	17.7	9.2	0.9
CB 187	45.9	15.5	55.9	21.9	68.4	26.3	103	52.7	484	126	94.7	41.8
CB 128	10.8	10.7	29.3	11.1	31.2	13.3	99.9	73.1	100	28.7	15.9	2.7
CB 156	10.9	4.0	12.2	5.4	13.7	6.9	46.2	33.7	91.0	27.9	14.6	1.0
CB 157	4.2	1.4	4.8	2.0	6.2	2.7	17.6	10.8	35.4	22.9	7.8	0.4
CB 180	85.7	26.1	82.5	37.2	110	58.3	358	336	794	333	145	4.7

Table 3 (continued)

species	Roundnose grenadier								*Bathysaurus ferox*			
site	IRELAND 1000m				IRELAND 2000m				MERIADZEC		ROCKALL	
sex	F		M		F		M		F		F	
no pools	6		4		6		4		4		4	
	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std
CB 170	27.5	9.6	28.8	14.1	37.8	20.1	118	105	316	85.2	44.8	1.9
CB 189	0.6	0.3	0.8	0.5	0.7	0.4	4.3	2.9	12.0	3.6	1.2	0.1
CB 194	9.6	5.7	9.1	4.7	7.6	7.1	53.0	48.8	164	49.9	23.0	1.6
Ices7CB	481	186	629	288	662	303	1980	2650	4380	2310	564	33.1
ΣCB	664	255	876	392	921	405	2790	2970	5900	2780	648	56.4
α-HCH	5.8	0.7	4.7	1.3	6.0	0.9	7.5	0.9	3.6	0.4	3.4	0.2
γ-HCH	1.3	0.3	1.0	0.4	2.0	0.9	2.5	0.7	1.4	0.2	1.8	0.1
α-chlordene	0.9	0.8	3.1	1.6	1.0	0.4	2.5	0.7	3.3	0.5	0.1	0.2
Aldrin	1.9	0.9	1.4	1.1	1.8	1.0	1.1	0.6	3.2	0.3	0.1	0.6
γ-chlordene	0.2	0.5	1.0	1.0	0.8	1.0	1.2	0.2	<0.03	<0.03	<0.03	<0.03
Hept.epoxide	2.8	1.5	1.4	1.8	2.4	0.8	1.8	1.5	<0.03	<0.03	1.0	0.3
Oxychlordane	27.0	14.9	39.9	16.7	31.2	9.5	49.2	18.9	55.8	10.3	8.4	3.4
α-chlordane	2.2	1.2	1.8	1.0	0.9	1.0	2.0	0.2	15.6	2.8	2.6	1.0
γ-chlordane	7.3	1.8	13.9	3.4	8.3	2.7	9.0	1.8	28.0	19.5	9.9	2.5
T-nonachlor	42.3	12.8	62.7	13.5	39.6	9.3	37.0	9.9	419	95.7	59.8	6.9
Dieldrin	30.7	6.2	32.8	10.4	30.1	3.1	28.2	4.5	46.2	1.6	12.4	1.3
o,p-DDD	<0.03	1.1	<0.03	3.1	3.3	1.5	7.7	1.4	<0.03	5.9	1.6	3.1
Endrin	153	51.0	209	65.1	156	37.4	204	69.6	231	40.1	28.1	5.6
p,p'-DDD	151	40.7	214	82.5	188	61.6	499	90.9	978	361	63.5	17.8
o,p-DDT	118	45.6	194	82.4	155	38.0	311	39.0	228	75.9	39.9	8.9
p,p'-DDT	291	58.5	323	209	342	302	1040	77.1	879	1290	125	28.6
o,p-DDE	1.0	2.9	8.3	3.6	3.1	1.3	8.1	2.3	44.1	15.2	<0.03	0.1
heptchlor	2.9	1.5	0.5	1.6	0.9	0.8	0.3	0.9	6.1	0.9	15.4	6.5
ΣDDT	1010	296	1500	779	1460	735	3260	254	3200	1470	638	88.2
Σchlordane	63.9	24.3	128	32.8	88.9	17.8	103	33.3	512	110	91.9	4.5

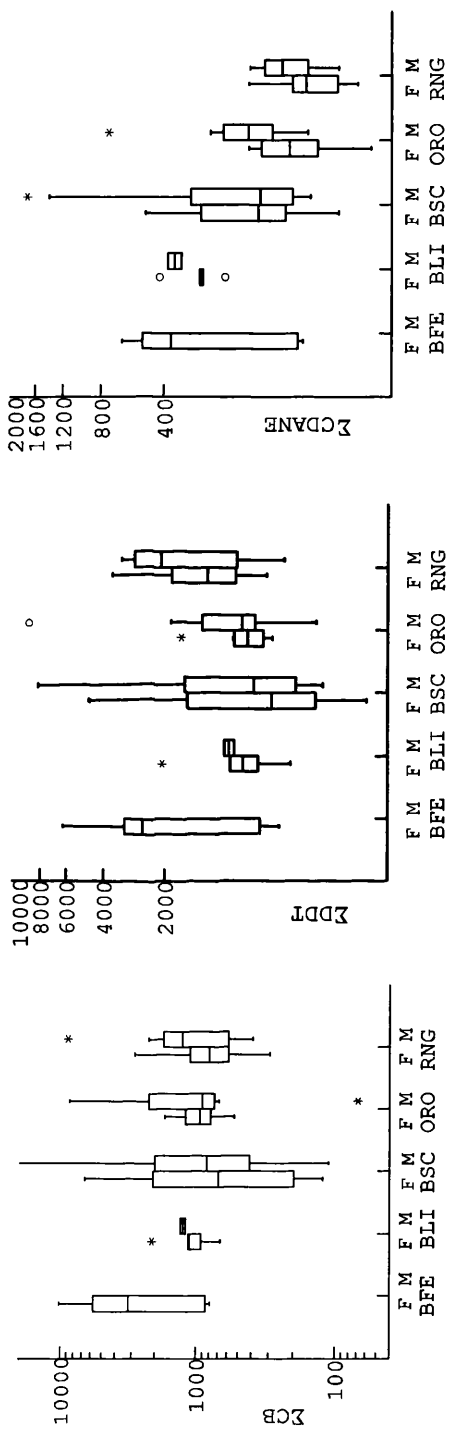

ΣCB
10000
1000
100
ΣDDT
10000
8000
6000
4000
2000
ΣCDANE
2000
1600
1200
800
400
F M F M F M F M F M
BFE BLI BSC ORO RNG

Fig 1

ΣCB
10000
1000
100
ΣDDT
5000
4000
3000
2000
1000
ΣCDANE
2000
1600
1200
800
400
IRELAND
MADEIRA
MERIADZEC
ROCKALL
SESIMBRA
? F M ? F M F M

Fig 2

Fig 3

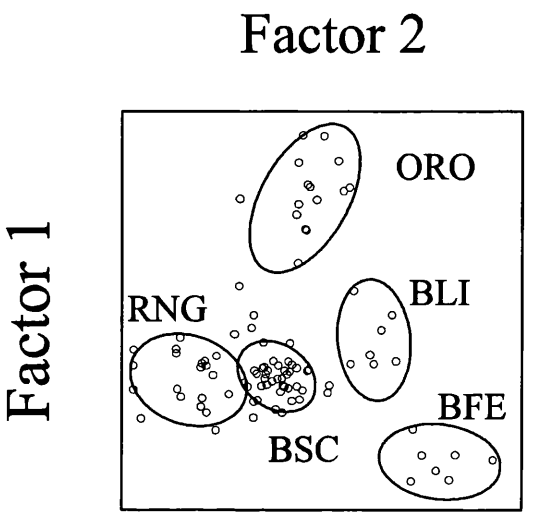
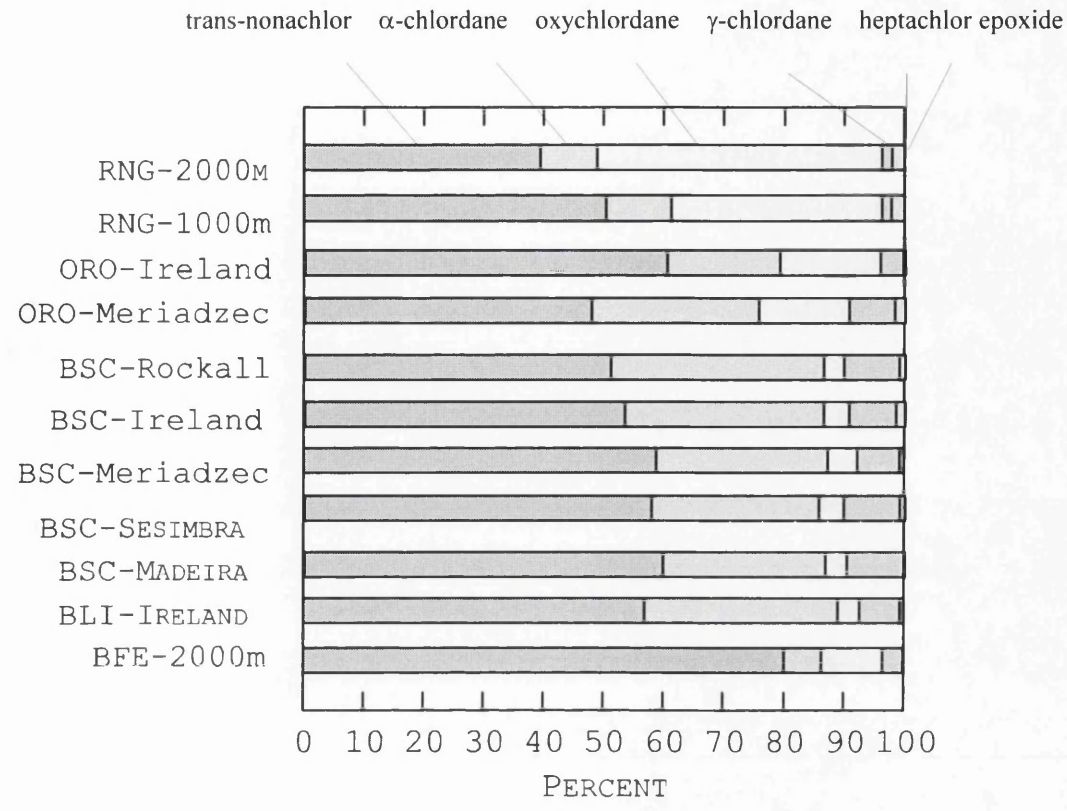


FIG 4


trans-nonachlor
α-chlordane
oxychlordane
γ-chlordane
heptachlor epoxide
RNG-2000M
RNG-1000m
ORO-Ireland
ORO-Meriadzec
BSC-Rockall
BSC-Ireland
BSC-Meriadzec
BSC-SESIMBRA
BSC-MADEIRA
BLI-IRELAND
BFE-2000m
0 10 20 30 40 50 60 70 80 90 100
PERCENT

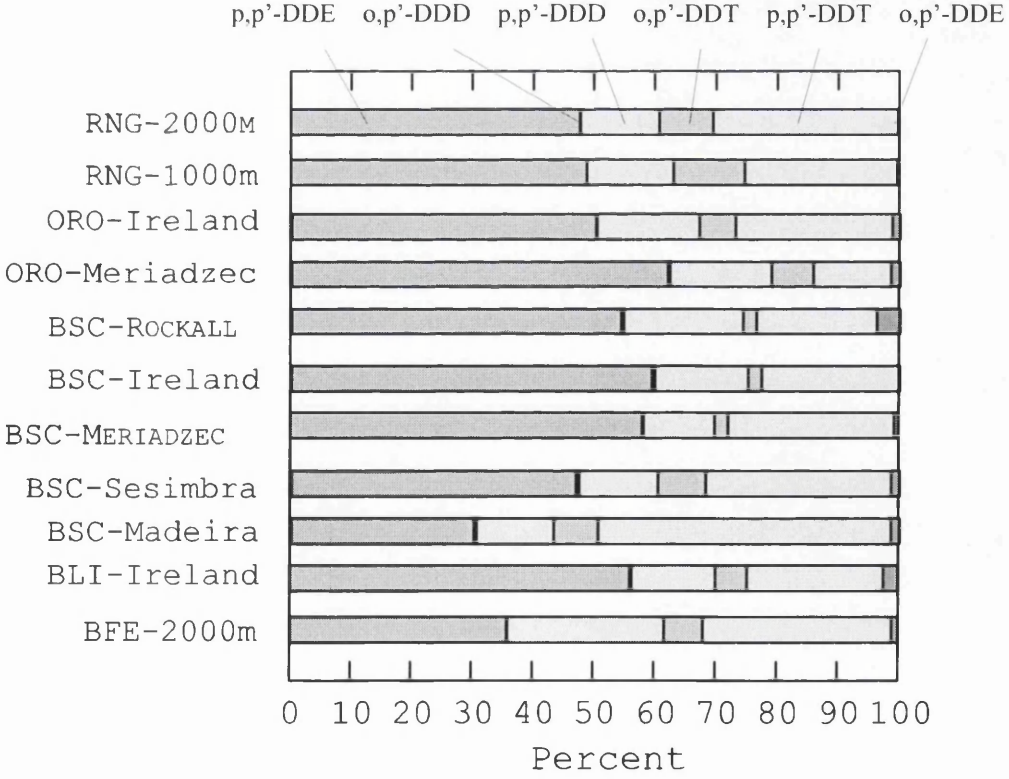

p,p'-DDE
o,p'-DDD
p,p'-DDD
o,p'-DDT
p,p'-DDT
o,p'-DDE
RNG-2000M
RNG-1000m
ORO-Ireland
ORO-Meriadzec
BSC-ROCKALL
BSC-Ireland
BSC-MERIADZEC
BSC-Sesimbra
BSC-Madeira
BLI-Ireland
BFE-2000m
0 10 20 30 40 50 60 70 80 90 100
Percent

Fig 5

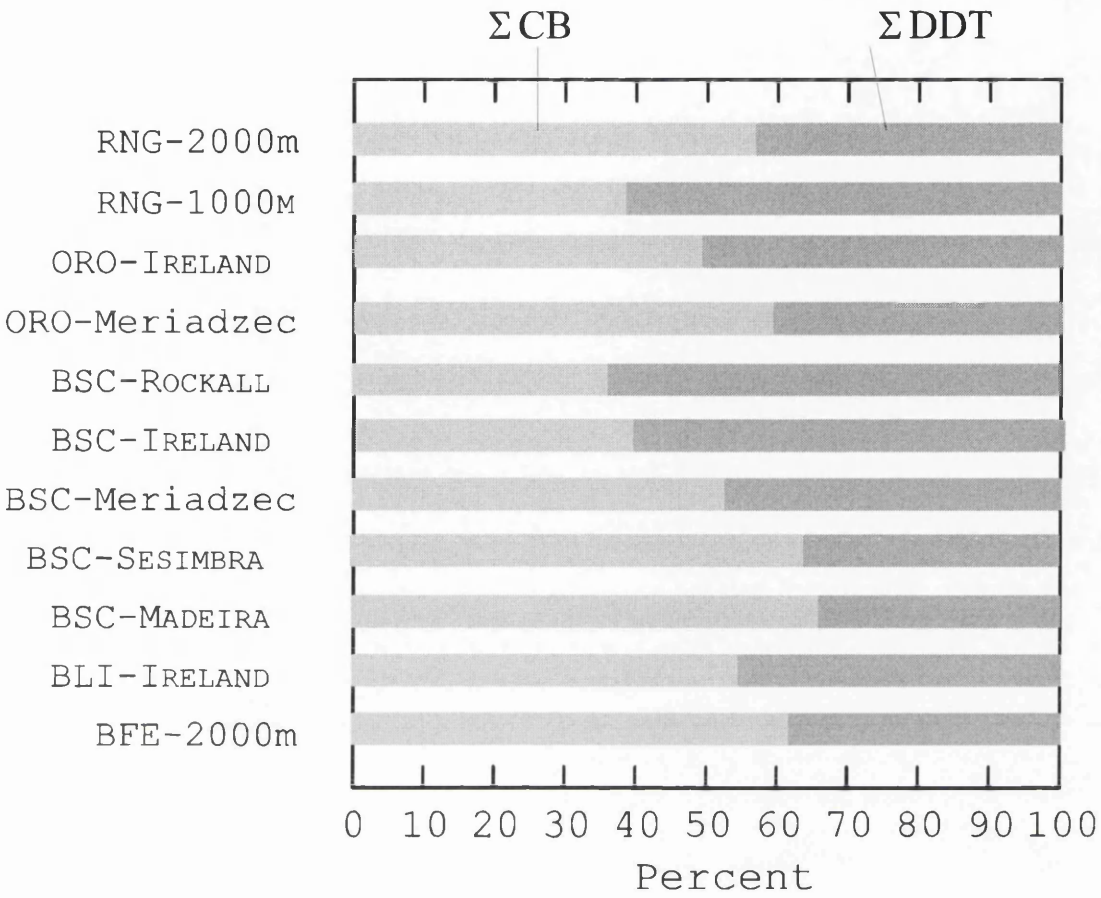

ΣCB
ΣDDT
RNG-2000m
RNG-1000M
ORO-IRELAND
ORO-Meriadzec
BSC-ROCKALL
BSC-IRELAND
BSC-Meriadzec
BSC-SESIMBRA
BSC-MADEIRA
BLI-IRELAND
BFE-2000m
0 10 20 30 40 50 60 70 80 90 100
Percent

FIG 6

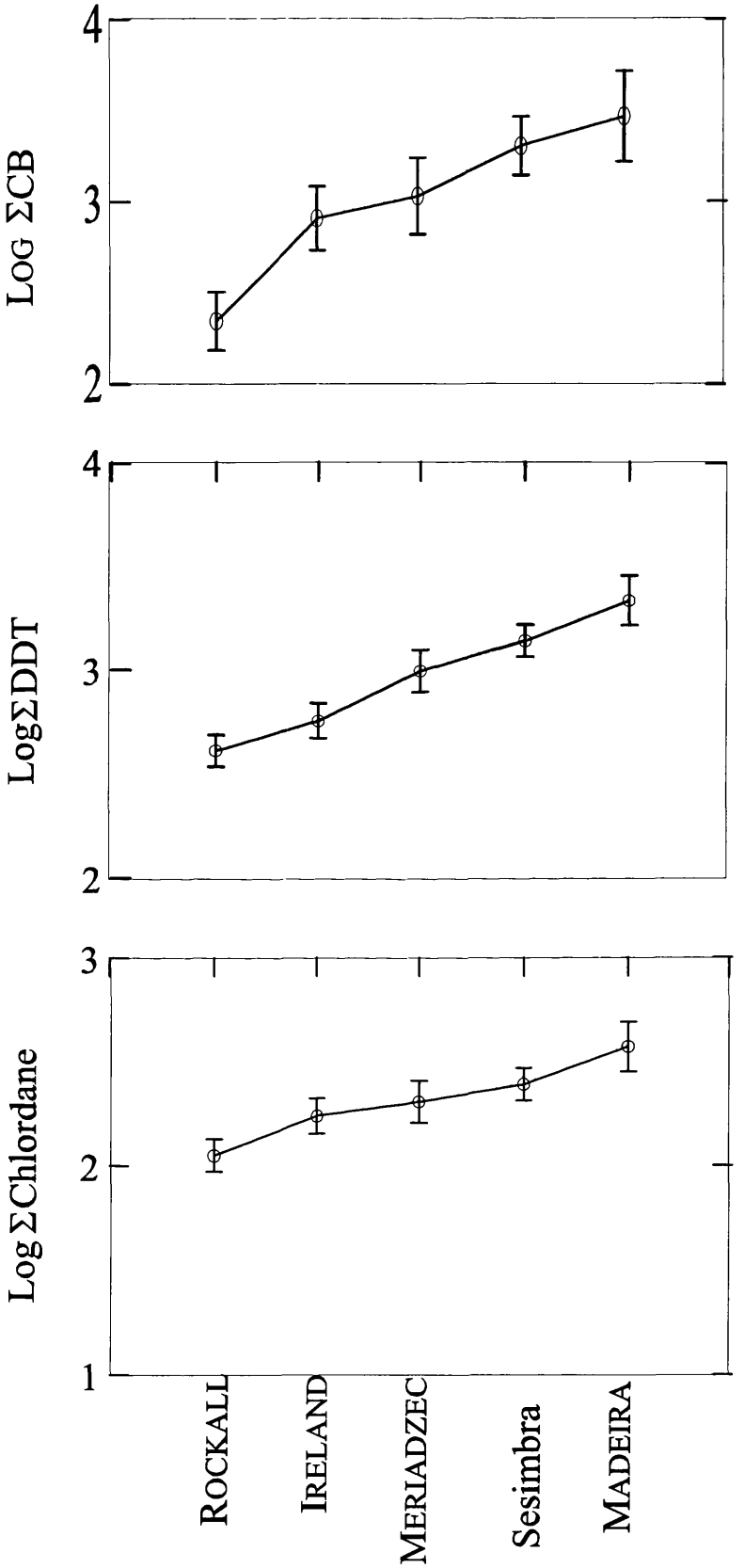
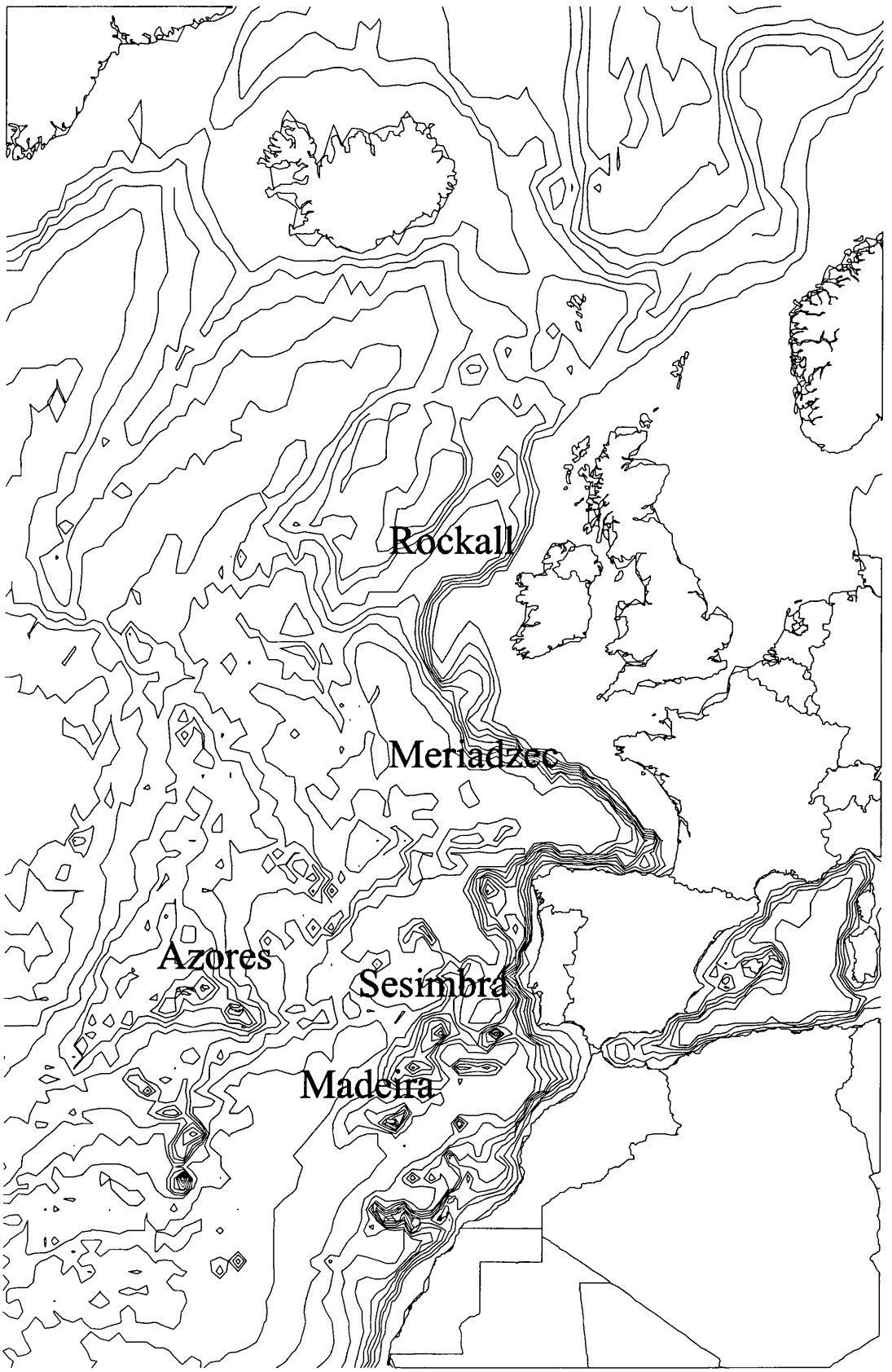

Log ΣCB
4
3
2
LogΣDDT
4
3
2
Log ΣChlordane
3
2
1
ROCKALL
IRELAND
MERIADZEC
Sesimbra
MADEIRA

Fig 7



Chapter 8
Effects of biological factors on metal concentrations

As has been shown in the sections 6.1 and 7.1 on monkfish and black scabbardfish, metal concentrations in fish are controlled by various biological and physiological factors, most of which are species dependant. Amongst those factors, the influence of the species, depth and other metal concentrations on the accumulation of metals will now be investigated in the whole range of the deep sea fish species studied.

All comparisons will be made on the grounds of statistical testing, most of which were conducted in chapter 4. Therefore the statistical significance of these comparisons will not be repeated in the present chapter. Mn, Cu, Zn, As, Se, Cd and Hg will be the only metals considered in the following discussion as the other metals presented very low concentrations in most of the deep sea fish species studied, often below the limits of detection.

8.1. Influence of the location of capture on metal concentrations

Three fish species have been sampled at various locations of the eastern North Atlantic, namely *B. ferox*, black scabbardfish and orange roughy in the Rockall Trough and Meriadzec areas. Moreover, black scabbardfish was also sampled in Madeira and Sesimbra.

Four species were also sampled in South Africa, off Cape Town, to investigate potential differences in fish contamination between the eastern part of North and South Atlantic.

8.1.1. Differences between the Rockall Trough and Meriadzec

Concentrations of mercury in all three species sampled in Meriadzec were higher than in the Rockall Trough for all three species, as was cadmium in *B. ferox* and orange roughy, selenium in black scabbardfish and orange roughy, and zinc in black scabbardfish and orange roughy. Arsenic also presented higher concentrations in *B. ferox* samples from Meriadzec than the Rockall Trough. Although the black scabbardfish and orange roughy individuals sampled in Meriadzec were significantly larger than those sampled in the Rockall Trough ($p<0.01$), this was not the case for the *B. ferox* individuals, hence length cannot account for all the differences between the two sampling positions.

Moreover, Cossa *et al.* (1991) studied mercury in fish species sampled in the Bay of Biscay, which is a shallow water site close to the Meriadzec deep water sampling site. Mercury concentrations reported in whiting and monkfish were higher than those measured in similar deep sea species in the Rockall Trough during the present study.

Therefore, the deep sea fauna in the Meriadzec area appears to be generally more contaminated in zinc, arsenic, selenium, cadmium and mercury than that in the Rockall Trough. This increase of metal burden in fish probably reflects the higher contamination of the Bay of Biscay than the Celtic Seas. Riverine and atmospheric inputs of various metals in the Bay of Biscay are documented as higher than those to the Celtic Seas (OSPAR Commission 2000, Regions IV and V), probably due to a higher industrialisation of the French West coast area compared to the Scottish West coast area. Moreover, the Meriadzec area presents a high density of litter in the sea and numerous unauthorised or accidental discharges of waste (OSPAR Commission 2000, Region IV).

8.1.2. Differences between the Rockall Trough, Meriadzec, Madeira and Sesimbra

Black scabbardfish individuals sampled in Madeira and Sesimbra presented similar levels of copper and arsenic, which in turn were lower than the levels found in individuals from the Rockall Trough and Meriadzec areas. On the other hand, selenium was present in higher concentrations in black scabbardfish caught in Sesimbra than Madeira than Meriadzec than the Rockall Trough. Mercury concentrations decreased from Madeira to Sesimbra, which were similar to Meriadzec, then decreased again to the Rockall Trough.

The increased mercury concentration in Madeira compared to the other locations has been attributed to the increased maturity stages of black scabbardfish in Madeira, which is the only place where mature black scabbardfish individuals were caught (see section 7.1).

Arsenic decreased with length in black scabbardfish and individuals were significantly larger in Madeira and Sesimbra than Meriadzec and the Rockall Trough. This can explain arsenic concentrations being lower in Madeira and Sesimbra than in Meriadzec and the Rockall Trough. Although copper was not statistically significantly correlated with length, lower concentrations tended to be found in larger individuals, which might explain, as for arsenic, decreased concentrations in Madeira and Sesimbra.

8.1.3. Difference between the northern and southern hemispheres

Four species were sampled in the deeper waters off the South African coast, namely kingklip, *L. vomerinus*, *M. capensis* and *M. paradoxus*. They were sampled at depths between 150 and 450m, which is significantly shallower than most of the sampling conducted in the eastern North Atlantic during the present study.

The contaminant concentrations, although varying greatly between species, were compared with those of North Atlantic species. In general in the present study, species originating from the south hemisphere presented similar metal concentrations

to the less contaminated deep sea species from the northern hemisphere, including copper, zinc, arsenic, selenium, cadmium and mercury. Manganese concentrations were similar to the most contaminated deep sea fish species of the northern hemisphere.

Metal levels were compared in hake from the Rockall Trough and *M. paradoxus* and *M. capensis* from South Africa in order to decrease inter-species variability, as they all belong to the *Merluccius* genus. Metal concentrations in these three species did not differ significantly in the northern and southern hemispheres. The present results suggest that metal contamination could be broadly similar in both eastern North and South Atlantic deep sea fish.

Mercury has also been studied in a variety of locations, including New Zealand (Broek *et al.*, 1981). In that study, hake presented similar to higher levels of mercury compared to the present study, and orange roughy exhibited similar levels in both studies. Windom *et al.* (1987) also compared *C. armatus* from the Atlantic and Pacific regions. Although levels were higher than these found during the present study, they presented a similar range between the Pacific and Atlantic areas, with significant differences only in cobalt, iron, manganese and nickel. These results tend to confirm the ones in the present study. Metal contamination of similar deep sea fish species seems to be of similar levels around the globe in non-contaminated areas.

8.2. Differences between species

As showed by the results obtained in chapter 4, even within a single location such as the Rockall Trough, concentrations of metals in the different organs of different fish species vary dramatically. Some of the factors accounting for these differences include the habitat, diet and specific metal accumulation processes of each species, as discussed in chapter 2.

Differences between species can be classified in two distinct categories. The first and most logical category consists of the differences in total concentrations of each metal in each organ of each species. It will be an expression of the levels of contaminants to which each species is exposed, either through food or directly available from

water, and of the accumulation and depuration processes available to the species. The second category will separate species on the grounds of their preferred organ of accumulation of each contaminant. This factor will reflect the differences in uptake and storage/depuration mechanisms between the various species. The two categories will be investigated separately.

8.2.1. Influence of the species on the levels of metals

Species coming from the same area and water mass, ie where they can be assumed to be subject to the same level of pollution from their environment, still present very different concentrations of metals. These concentrations should be higher in longer-lived species, in smaller fish species, at higher trophic levels (mainly for mercury) and in species living close to the sediments, amongst other factors (see chapter 2).

Black scabbardfish presented highest levels of Mn, Zn and Cd, and second highest of Cu and Se. In general all metal levels in black scabbardfish were amongst the highest of any deep sea species sampled in that area. Black scabbardfish is a top predator, and hence tend to accumulate metals over a longer period of time and from a diet enriched in metals and particularly mercury and cadmium through the food chain. Similar results have been shown in the Azorian ecosystem, with long-lived species and top predators presenting higher metal concentrations than species lower in the food chain, and in particular black scabbardfish (Monteiro *et al.*, 1996).

H. bathybius presented highest concentrations of copper, arsenic, selenium and mercury, and second highest of cadmium. This species lives significantly deeper than most other species studied and might be subject to a more contaminated environment. Because it lives deeper, it presents a slower metabolism and longer life span, which induces a higher scope for the accumulation of metals. However, this factor cannot account totally for the high concentration of copper as *C. armatus*, also sampled at such depths, presented high levels of selenium, but low levels of the other elements. Differences in diet might account for the differences in metal concentrations between these two species. *H. bathybius* is a benthic species, living on the sediment, feeding on benthic organisms and probably scavenging as well, whereas *C. armatus* lives and feed in the water column above the sediments.

Therefore, *H. bathybius* is subject to more contaminated food and environment. *B. ferox*, another benthic deep water species living below 2000m presented high concentrations of mercury only. Therefore, benthic species are generally more contaminated with metals than benthopelagic ones because they are subject to a diet of more contaminated prey.

Orange roughy also presented high levels of zinc, selenium and cadmium, but low in other elements such as arsenic. Orange roughy are long-lived fish, relatively small in size and carnivorous. Hence it could present increased factors of contamination, by longer accumulation time and by feeding on more contaminated prey.

All other species presented lower metal contamination.

8.2.2. Influence of the species on the distribution of metals in various organs

Monkfish individuals presented a pattern of metal concentrations in their organs dependent on the depth of capture. All other species presented higher concentrations of Mn, Cu, Zn, Se and Cd in liver than muscle tissue. The only species presenting higher arsenic concentrations in muscle than liver tissue were kingklip, *L. vomerinus*, monkfish, *H. bathybius* and *B. ferox*. In a similar way, fish sampled from South Africa, *B. ferox*, blue ling and blue whiting presented higher concentrations of mercury in muscle than in liver tissue.

As most elements behave in the same way, the influence of the species seems to be limited in the deep sea biota. This is probably due to the fact that the main availability of metals to the deep sea environment comes from food and not from water (see chapter 2). Physiological responses are also greatly limited in the deep sea environment due to physical and biological constraints (Merrett and Haedrich, 1997), and storage/elimination processes probably of less importance in deep sea fish species than in their shallow water counterparts. This theory is strengthened by the fact that most of the species with higher metal concentrations in muscle than liver tissue are shallower water species, such as the species from South Africa, monkfish, blue ling and blue whiting.

Gill tissue presented higher levels of cadmium and zinc than muscle tissue in black scabbardfish, but lower than liver tissue. Hence there might be a secondary source of cadmium and zinc from water and/or sediments by adsorption on the gills and absorption through the gills. Cadmium has been previously documented to adsorb on the gills (see chapter 2).

Gonad tissue presented concentrations of most metals intermediate between liver and muscle tissue concentrations apart from zinc, which presented highest concentrations in gonad tissue. Gonad can in some cases act as a metals regulator, with possible excretion with the loss of eggs or ovaries (see chapter 2).

Vas *et al.* (1993) showed different trends in similar fish species sampled in the Rockall Trough at similar depths, as in the present study. For example, roundnose grenadier presented highest concentration of manganese in gill and of copper in muscle tissue. However, concentrations in roundnose grenadier differed significantly between the two studies, the current one presenting copper levels in muscle 10 times higher than those reported by Vas *et al.* (1993), but five times lower in liver tissue. Manganese concentrations were also significantly higher in liver tissue in the present study. In Vas's study (1993), other species presented varying accumulation patterns with organs, with, for example, highest manganese concentrations in gill tissue of *Coryphaenoides guentheri* but in muscle tissue of *Antimora rostrata*.

8.3. Influence of depth on metal concentrations

8.3.1. Comparison with shallow water species

The concentrations of trace metals in the marine environment have been widely reported (eg Preston *et al.*, 1972; Clark, 1997; OSPAR Commission 2000). However, these studies have been largely confined to shallow water, with only a limited number of studies addressing the occurrence of metals in the deep sea until recently (Cross *et al.*, 1973; Leatherland *et al.*, 1973; Morris, 1975; Broek and Tracey, 1981). Until recently, the general belief was that the deep water regions were not exposed to

the same levels of pollution as coastal waters, nor the fish stocks from deep waters as exploited.

However, in recent years, studies of the contamination of the deep sea by metals have multiplied (Windom *et al.*, 1987; Bonwick *et al.*, 1990; Vas *et al.*, 1993; Forlin *et al.*, 1996; Takahashi *et al.*, 1997; Cronin *et al.*, 1998; Mormede and Davies, 1998 and 2001a). Two of the reasons are concern over levels of contaminants found in an environment supposed "pristine", and the development of deep water fisheries. In previous studies (Cronin *et al.*, 1998; Mormede and Davies, 1998), levels of contaminants in deep sea fish species from the Rockall Trough were found to be similar to those found in related shallow water species (eg Brown and Balls, 1997). Comparisons between the present study and the levels recorded by Brown and Balls (1997) shows a similar trend (table 8.1).

Table 8.1: Comparison between mean concentrations of contaminants in muscle tissue of shallow water and deep water species from the Rockall Trough area, expressed in mg kg^{-1} wet weight.

species	depth	Cu	Zn	As	Cd	Hg	Pb
species caught in shallow water, Brown and Balls (1997)							
monkfish	shallow	0.10	4.49		<0.001	0.06	<0.02
hake	shallow	0.15	3.58	1.18	<0.001	0.03	<0.01
ling	shallow	0.14	4.41	4.21	0.001	0.08	0.01
whiting	shallow	0.14	3.75	4.27	0.001	0.04	<0.01
species caught in deep water, present study							
monkfish	80m	0.05	3.55	12.01	<0.03	0.104	<0.03
	400m	0.33	14.35	6.24	<0.03	0.033	<0.03
hake	640m	0.30		1.24	0.03		<0.03
blue ling	1000m	0.29	4.41	4.66	<0.03	0.365	0.08
blue whiting	300m	0.45	4.28	3.97	<0.03	0.054	0.21
	640m	0.20	3.53	5.29	<0.03	0.088	<0.03
	830m	0.31		2.47			<0.03

In related species, copper was always present in higher concentrations in deeper water fish, as was mercury in two species and cadmium in hake. Zinc, arsenic and, in most cases, cadmium presented similar concentrations in shallow water and deep water individuals of related species.

Due to the specific accumulation of metals, biologically essential metals should have longer retention times in the surface water biota than non-biologically essential ones such as cadmium and mercury (see chapter 2). Hence the deep sea environment should present higher concentrations of non-essential metals than their shallow water counterparts, and the opposite trend for essential metals. In this study it is the case for mercury in some cases and cadmium in only one species. However, copper, an essential metal, is also present in higher concentrations in deep water species than their shallow water counterparts.

Dissolved metals show distinct profiles in the North Atlantic, with an increase with depth of cadmium, zinc, nickel and copper up to a plateau at about 2000m depth; and a decrease of manganese and lead with depth (Bruland and Franks, 1983). Copper is mainly taken up from food via the intestine in fish, hence deep water fish should not be directly affected by an increase of copper concentrations in sea water with depth. However, organisms lower in the trophic chain such as phytoplankton and zooplankton will be affected by this increase of copper in deeper water, with organisms living deeper being more contaminated in copper and other metals. Hence the whole food chain in deeper waters will be subject to higher levels of contamination of copper. Therefore fish sampled in deeper waters should present higher levels of copper than those caught in shallower waters. Within the present study and deep water species, the effects of depth on metal concentrations in each individual species will now be investigated.

8.3.2. Correlations between depth and metal concentrations

Correlations between depth and other factors were tested for 95% significance ($p<0.05$) using Spearman's statistics to account for the non-normal distribution of the data (table 8.2).

Table 8.2: Significant correlations between depth and other factors in muscle (FL), gill (GL), gonad (GD) and liver (LV) tissue of monkfish (ANG), blue ling (BLI), black scabbardfish (BSC), blue whiting (BWH), orange roughy (ORO) and roundnose grenadier (RNG), positive (+) or negative (-).

species	organ	length	weight	Mn	Cu	Zn	As	Se	Cd	Hg
ANG	FL	+	+	+	+	+				-
	GD	+	+					+		+
	GL									
	LV	+	+	-		-	+	-		
BLI	FL	+					-			
	GD									
	LV				+		+		+	
BSC	FL				+	+	+	+		+
	GL				-				+	
	LV				+	-		+	+	
BWH	FL	+	+		-	-				+
	LV	+	+					-		
ORO	FL	-	-	-		-				
	LV	-	-							
RNG	FL	-	-	-			-			-
	LV	-	-	-	+					+

Most deep sea fish species are believed to follow the older/deeper rule (Merret and Haedrich, 1997), ie younger individuals live in shallower waters, then migrate deeper as they grow older. This rule is followed by the data obtained during the present study for all species apart from orange roughy and roundnose grenadier. However, this apparent decrease in length when going deeper in these two species is probably an artefact of sampling conditions, as Gordon and Duncan (1987) did not find differences in the length-frequencies of orange roughy between the different bathymetric zones in the Rockall Trough.

As foreseen in chapter 2, the trends in metal concentrations with depth are highly dependent on the species and organ studied. A number of competing mechanisms are involved, amongst which:

- As most fish will be older in deeper waters, they will have had more time to accumulate metals, hence should present higher concentrations of bioaccumulating metals.

- But as fish will be older in deeper waters, they will be also larger. Hence they will ingest a wide size range of items rather than only smaller particles as smaller fish do. As smaller particles or organisms tend to be enriched in metals, older fish in deeper water should be subject to less contaminated food (see chapter 2). They will also feed on larger prey, which are higher in the food chain.

- As highlighted in the previous section, concentrations in cadmium, zinc, nickel and copper in water increase with depth to about 1500m for most of these metals. So fish in deeper waters might be subject to a more contaminated environment, even though direct uptake of metals from the water is supposed to be relatively unimportant in fish.

- The composition of the deep sea biota changes significantly between bathymetric zones (Gordon and Bergstad, 1992), hence a shift in diet with depth towards less fish and more other species such as copepods (Gordon and Mauchline, 1990), which are more contaminated in zinc (Vas *et al.*, 1993) and possibly other metals.

In each species, the importance of each of these factors will vary, which will explain the differences in correlations between species. For example, in muscle tissue of both monkfish and black scabbardfish species, depth presents positive correlations with numerous metals such as Mn, Cu, Zn, As, Se and Hg. This implies greater importance of the accumulation of metals with age rather than the shift in diet to less contaminated prey, which might be negligible. On the other hand, blue whiting, orange roughy and roundnose grenadier show a decrease in Cu, Zn, As or Hg with depth in muscle tissue. For these species, the main factor ruling metal accumulation is probably be the change in diet with length and depth towards bigger and less contaminated prey.

In numerous cases, accumulation is also opposite in different organs of a single species. For example, in monkfish, accumulation of Mn, Cu and Zn is positively correlated with depth in muscle tissue, but negatively in liver. Or in roundnose grenadier, mercury is negatively correlated with depth in muscle tissue and positively in liver. There might be a shift in accumulation/depuration processes with the change in diet and/or age.

However, apart from the change of diet with depth related to the availability of different prey species, the effects of depth can be difficult to separate from those of length as the two factors can be closely related in deep sea fish.

8.4. Influence of length on the levels of metals

Correlations between length and the metal concentrations were tested in the same way as previously, which is for 95% significance ($p<0.05$) using Spearman's statistics to account for the non-normal distribution of the data.

As explained in chapter 2, at constant trace metal uptake rate, trace metals whose concentrations is inversely related to animal mass are mainly accumulated via adsorption of dissolved ions, with uptake proportional to the number of surface ligands. Trace element concentrations remaining constant or increasing as an animal grows are proportional to the amount of metal binding ligands in an animal's entire body, and access is enhanced by digestive processes. In deep sea fish species, the factors detailed in the previous paragraph, such as a change in the size of the prey or in the quality of diet, will also influence the levels of metals to which these species are exposed. Any combination of these factors is possible in any single species and organ, hence the variability of responses in the various species (table 8.3).

Table 8.3: Significant correlations between length and metal concentrations in muscle (FL), gill (GL), gonad (GD) and liver (LV) tissue of monkfish (ANG), *B. ferox* (BFE), blue ling (BLI), black scabbardfish (BSC), blue whiting (BWH), orange roughy (ORO) and roundnose grenadier (RNG), positive (+) or negative (-).

species	organ	weight	Mn	Cu	Zn	As	Se	Cd	Hg
ANG	FL	+							
	GD	+							+
	GL	+							
	LV	+							+
BFE	FL	+				-		+	+
	GD								+
	LV	+					-		
BLI	FL	+	-			-			+
	GD	+				-			+
	LV	+						+	+
BSC	FL	+				-	+		+
	GL	+		+				-	
	LV	+			+		+	+	
BWH	FL	+		-	-				+
	LV	+			-		-		
ORO	FL	+			+	+			+
	LV	+						+	
RNG	FL	+					+		+
	LV	+							

The only metal positively correlated with length in all species in one or more organs was mercury, which confirms that it is the only metal bio-accumulating in the food chain and with age. This is due to the very high retention time of methylmercury in the body (see chapter 2).

Arsenic is the only metal consistently negatively correlated with length in several species (apart from orange roughy). Hence arsenic must be accumulated mainly via adsorption on the gills or the stomach and intestine rather than absorption from food.

Copper, zinc and selenium presented consistent correlations in any single species, but varying between species, showing the importance of the fish species in the accumulation and possible change of diet.

Cadmium presented positive correlation with length in several species, which is probably because cadmium is not an essential element, and probably bio-accumulated with time. However, it presented a negative correlation with length in gills of black scabbardfish. Cadmium might be partly adsorbed on the gills from water, as has been reported previously (see chapter 2).

8.5. Correlations between metals

In each fish species and organ, a large number of correlations were significant between the concentrations of various metals (table 8.4).

Table 8.4: Correlations between metal concentrations in muscle (fl), gill (gl), gonad (gd) and liver (lv) tissue of monkfish (ANG), *B. ferox* (BFE), blue ling (BLI), black scabbardfish (BSC), blue whiting (BWH), orange roughy (ORO) and roundnose grenadier (RNG), positive (+), negative (-) or not significant ( ).

	Mn	Cu	Zn	As	Se	Cd	Hg	species	organ
		+/ / /+	+/ / /+	-/ / /-	/ / /+	/ / /+	/ / /	ANG	fl/gl/gd/lv
		+/ /	+/-/	+/ /	+/ /	+/ /	-/ /+	BFE	fl/gd/lv
		/ /	/ /	+/ /	/ /	/ /	/ /	BLI	fl/gd/lv
Mn		+/ /	/ /	/ /	/ /	/ /	/ /	BSC	fl/gl/lv
		/	/	/	/	/	/	BWH	fl/lv
		/	/	/	/	/	/	ORO	fl/lv
		+/	/+	+/+	/+	/+	/+	RNG	fl/lv
			+/ / /+	-/+/+/-	+/ /+/+	/ /+/+	-/ /+/	ANG	fl/gl/gd/lv
			+/ /+	+/ /	+/ /+	+/ /	+/ /	BFE	fl/gd/lv
			/+/	/ /	/ /	/ /+	/ /+	BLI	fl/gd/lv
Cu			+/+/+	+/ /+	+/ /+	/ /+	/ /+	BSC	fl/gl/lv
			+/+	+/+	/+	/+	/	BWH	fl/lv
			/	/	+/	/	/	ORO	fl/lv
			+/+	/+	/+	/+	/+	RNG	fl/lv
				-/ / /-	+/ / /+	/ / /+	-/ / /	ANG	fl/gl/gd/lv
				+/ /	+/+/+	+/-/	+/-/	BFE	fl/gd/lv
				/ /+	/ /+	/ /	/ /	BLI	fl/gd/lv
Zn				+/ /+	+/ /+	/ /	/ /	BSC	fl/gl/lv
				/	/+	/+	/	BWH	fl/lv
				/+	/+	/+	/	ORO	fl/lv
				/+	/+	/+	/+	RNG	fl/lv
					/ /+/-	/ /+/-	+/ /+/+	ANG	fl/gl/gd/lv
					+/-/	+/ /+	+/ /+	BFE	fl/gd/lv
					/ /+	/ /+	/ /	BLI	fl/gd/lv
As					/ /+	/ /+	/ /	BSC	fl/gl/lv
					+/	/	+/+	BWH	fl/lv
					/	/	+/	ORO	fl/lv
					/+	/+	/+	RNG	fl/lv
						/ /+/+	/ /+/	ANG	fl/gl/gd/lv
						+/-/	+/-/	BFE	fl/gd/lv
						/ /	/ /	BLI	fl/gd/lv
Se						/ /+	+/ /	BSC	fl/gl/lv
						/+	+/	BWH	fl/lv
						/+	+/	ORO	fl/lv
						/+	/+	RNG	fl/lv
							/ /+/	ANG	fl/gl/gd/lv
							+/ /+	BFE	fl/gd/lv
							/ /	BLI	fl/gd/lv
Cd							/ /+	BSC	fl/gl/lv
							/	BWH	fl/lv
							/+	ORO	fl/lv
							/+	RNG	fl/lv

Blue ling, blue whiting, black scabbardfish and orange roughy presented the least correlations, hence accumulate metals to a lesser extent than other species. Therefore, these species have probably a better potential than others to depurate metals.

In most cases, copper was positively correlated with zinc, arsenic, selenium, cadmium and in some cases mercury. Supposing that fish follow a stable diet in terms of composition, they would be subject to a stable metal intake in relative proportions. On this basis, correlations of metal concentrations in fish means that depuration processes, if any, are not metal specific, otherwise metal levels would not be correlated. A few negative correlations were found in some organs, such as arsenic with manganese, copper and zinc in monkfish liver and muscle tissue, showing inhibition of one metal to the other for uptake. However, Cd and Mn have been reported to inhibit Zn uptake (see chapter 2), which is only found in *B. ferox* liver issue. None of the negative trends was reproducible in different species.

8.6. Conclusions

Metal concentrations in fish vary with species, location and depth of capture and length.

The Meriadzec area is more contaminated with heavy metals than the Rockall Trough area, as reflected by the concentrations of trace metals in respective fish populations (section 8.1.1.). However, Sesimbra and Madeira present higher contamination in mercury and cadmium only in black scabbardfish, which has been attributed to the higher maturity stages found in these locations (section 8.1.2.).

On the other hand, the eastern North and South Atlantic present very similar levels of contamination of deep water fish populations. Comparison with others studies in the Pacific Ocean also shows similar fish contamination in the deep sea (section 8.1.3.). This phenomenon might be explained by a regulation of concentrations in deep water species rather than similar levels of contamination throughout the globe.

Within the deep sea environment, top predators, benthic feeders and fish species living below 2000m depth are subject to more metal contamination (section 8.2.1.) because they are feeding on more contaminated prey higher in the food chain, living on the sediment surface or in a particularly contaminated biota respectively.

Fish sampled in deep waters present similar levels to shallow water counterparts, apart from copper, cadmium and mercury, which are present at higher concentrations in deep water fish species. Cadmium and mercury are non-essential metals, hence are excreted from shallow water organisms and are incorporated in marine snow and reach the deep sea faster than other elements. This explains the differences in concentrations between deep and shallow water fish. The copper concentration in the water column increases with depth hence is more biologically available to deep sea organisms, which explains its increased concentration in deep water fish (8.3.1.).

Within the deep sea, some correlations were made between depth and metal concentrations, positive or negative in similar numbers, showing varied responses depending on the fish species (section 8.3.2.).

Fewer correlations were made between length and metal concentrations, most of which were positive apart from arsenic. Mercury and, to some extent, cadmium were positively correlated with length in several species, showing the bio-accumulation in fish of these metals only. Arsenic was negatively correlated with length in several species, confirming that this metal is adsorbed onto the gills rather than absorbed from food and accumulated in fish (section 8.4.).

In most fish species apart from blue ling, blue whiting and orange roughy, most metals were correlated with one another. Therefore, depuration processes, are not metal specific in deep sea fish (section 8.5.).

Chapter 9
Effects of biological factors on organic contaminant concentrations

Organic contaminants such as PCBs and pesticides follow a different accumulation process from that of metals. As shown in the previous chapter, metal concentrations in fish are mainly accumulated from food, with increased copper and mercury burdens in deep sea fish compared to their shallow water counterparts. However, in the case of PCBs and pesticides, concentrations in fish are normally considered to be in equilibrium with those in the surrounding water, with exchange occurring across the gills and storage in lipid tissues throughout the body (Randall *et al.*, 1998). Hence concentrations and accumulation patterns should be different and reflect that uptake route.

The influence of species, depth, length and other factors on the concentrations of PCBs and pesticides in the fish species studied will now be investigated in a similar way as that for metals. All comparisons are made on a lipid weight basis apart from when stated, and on the grounds of statistical testing, most of which was conducted in chapter 4.

9.1. Influence of the location of capture

As the same fish were sampled for metal and organic analyses, the same species as for metals were obtained from the various locations. *B. ferox*, black scabbardfish and orange roughy were sampled in the Rockall Trough and Meriadzec areas. Black scabbardfish was also sampled in Madeira and Sesimbra. Four species were sampled in South Africa, off Cape Town, to investigate possible differences in fish contamination between the eastern North and South Atlantic.

9.1.1. Differences between the Rockall Trough and Meriadzec

B. ferox presented higher concentrations of 7CB, ΣCB, ΣDDT and ΣChlordane in Meriadzec than the Rockall Trough. On the other hand, the p,p'-DDE/ΣDDT ratio was significantly higher in the Rockall Trough than Madeira. Black scabbardfish also presented higher concentrations of 7CB, ΣCB and ΣDDT in Meriadzec than the Rockall Trough, but similar concentrations of ΣChlordane and ΣCB/ΣDDT and p,p'-DDE/ΣDDT ratios in both locations. Orange roughy did not present significant differences between the two locations in any of the concentrations or ratios.

B. ferox is a deeper-living species than orange roughy or black scabbardfish, and was sampled at 2000m depth against 1300m and 500 to 1300m depth respectively for the other two species. The deeper waters might be more contaminated in Meriadzec than the Rockall Trough, although the same pattern is not reproduced by the other two species. However, *B. ferox* is a bottom-living fish less subject to migration than the other two species and could reflect the higher deep water contamination in Meriadzec than the Rockall Trough. Black scabbardfish does not show a similar pattern. As this species is more likely to travel long distances within short periods of time, it therefore encounters different water masses and their respective contamination, hence the less distinct pattern. In the same way, orange roughy might also not reflect such differences in contamination, not because the waters it lives in are not different, but because the species itself moves between the two locations.

Hence the broad characteristics of the contamination pattern seems to be the same for PCBs and pesticides as for metals, with more contaminated deep water masses and biota in Meriadzec than the Rockall Trough. However, p,p'-DDE presented higher concentrations in *B. ferox* in the Rockall Trough than Meriadzec, as shown by the p,p'-DDE/ΣDDT ratio and the decrease of ΣDDT from Meriadzec to the Rockall Trough.

9.1.2. Differences between the Rockall Trough, Meriadzec, Madeira and Sesimbra

Black scabbardfish individuals did not present significantly different concentrations of organic contaminants in Meriadzec, Madeira and Sesimbra. Unlike for metals, the deep sea environment in Meriadzec, Madeira and Sesimbra seems to present similar contamination of PCBs and pesticides. This is not surprising as the water mass at ca 1000m depth is the same throughout that region, made up mainly with the Gulf of Gibraltar water (OSPAR Commission 2000, Region V) and organic contaminants in fish will reflect the levels of contaminants in the water rather than in the diet.

9.1.3. Differences between the northern and southern hemispheres

Four species were sampled in the deeper waters off the South African coast, namely kingklip, *L. vomerinus*, *M. capensis* and *M. paradoxus*. They were sampled at depths between 150 and 450m, which is significantly shallower than most of the samplings conducted in the eastern North Atlantic during the present study.

Concentrations of all PCBs and pesticides were significantly lower in individuals sampled in South Africa than in those sampled in the eastern North Atlantic, regardless of species, by a factor of up to 100. Moreover, Kelly & Campbell (1994) and Webster *et al.* (2000) reported PCB and pesticide concentrations in shallow water fish from the Rockall Trough area up to 100 times higher than those found in fish sampled in the eastern South Atlantic. Hence the differences in contamination between fish from the North and South Atlantic are not due to the depth of sampling but reflect a lesser level of pollution in the eastern South Atlantic compared to the eastern North Atlantic by organic compounds.

Froescheis *et al.* (2000) and Looser *et al.* (2000) studied shallow water and deep sea fish species from similar locations, both in the North and South Atlantic. Kingklip individuals originating from the eastern South Atlantic were analysed in both studies and presented higher concentrations of most organic contaminants than in the present study (apart from ΣChlordane which presented higher levels in the present study), by

a factor up to 10. However, hake species (*M. capensis* and *M. paradoxus*), also sampled in the eastern South Atlantic presented higher levels in the present study than in that of Froescheis *et al.* (2000) and Looser *et al.* (2000), although both were of the same order of magnitude. Grenadier individuals originating from the eastern North Atlantic were also analysed in both studies and presented similar levels.

Although from the present study we can conclude that the eastern North Atlantic is significantly more contaminated with PCBs and pesticides than the eastern South Atlantic, the previous authors concluded a similar contamination of both locations. However, deposition and flux of PCBs and pesticides have been reported significantly higher in the North Atlantic than the South Atlantic, apart from ΣDDT which were similar in both locations (OSPAR Commission 2000, Region V).

The transport of PCBs and pesticides in the environment is global, as they are volatilised from the site of production, use or discharge into the atmosphere. They are subsequently transported to the poles where they are precipitated on to the cold waters that will then form the deep water masses around the globe (Ballschmiter, 1992; Cromwell, 2000). However, the northern hemisphere remains the main user of PCBs and hence the main polluter, which would explain the large difference of contamination between the North and South Atlantic, up to a factor 100. On the other hand, the southern hemisphere remains the main user of pesticides, most of which have now been banned in industrialised countries, hence a smaller difference between the pesticide contamination levels of northern and southern hemisphere (see chapter 2).

9.2. Influence of the species

As already highlighted in the previous chapter with metal contamination, even within a single location such as the Rockall Trough, the contaminant levels in various fish individuals are highly dependant on the species. However, unlike metals, PCBs and pesticides do not accumulate in specific organs but evenly throughout the body lipids and as a consequence fatter organs present higher concentrations if expressed on a wet weight basis but not if expressed on a lipid weight basis. This was demonstrated

in chapter 6 and is generally quoted in the literature (eg Clayton *et al.*, 1977; Phillips, 1995). Therefore, this discussion will concentrate in the differences of organic contaminant concentrations between species from the Rockall Trough in livers only, expressed on a lipid weight basis.

The lowest ΣCB concentrations were found in blue whiting and monkfish caught in shallow water (between 60 and 650m depth). Black scabbardfish presented intermediate concentrations, as did *B. ferox*. Roundnose grenadier presented increasing concentrations with depth, up to the highest levels found in the present study, for *C. armatus* and *H. bathybius* (see chapter 4). In any single species, ΣCB concentrations increased with depth, which made comparisons difficult. However, even when using only individuals caught in the same depth range (eg 850 to 1000m depth), the differences in ΣCB concentrations between species were still statistically significant ($p<0.05$ in all cases).

9.2.1. Differences in PCB concentrations between species

In each discrete water mass, separation of the species on the basis of individual PCB concentrations was attempted using discriminant analysis.

9.2.1.1. Species differences within the North Atlantic Central Water

Discriminant analysis was used as the first two factors contained 75% of the information (fig 9.1). Backwards stepwise modelling highlighted the CBs whose concentration differed most between these species, which are CBs 49, 114, 156, 157 and 194 (table 9.1).

Differences between the species caught in that water mass were significant. Black scabbardfish presented opposite factors compared to blue whiting and monkfish, which in turns differed most on CBs 114 and 156. Monkfish presented higher concentrations of CB156 and 194 than black scabbardfish than blue whiting. On the other hand, black scabbardfish presented much higher concentrations of CB49 than the other two species.

Table 9.1: Backwards stepwise discriminant factors between species in the depth range 500 to 900m (North Atlantic Central Water) with PCB concentrations in livers expressed on a lipid weight basis

	F to remove	monkfish	black scabbardfish	blue whiting
(%) jackknifed classification		91	88	60
constant		-1.755	-6.125	-4.767
CB49	20.21	-0.178	1.379	-0.116
CB114	12.08	0.122	-0.161	0.701
CB156	44.69	0.267	-1.734	0.695
CB157	12.04	-0.314	0.513	-1.733
CB194	24.60	-0.106	1.618	-0.249

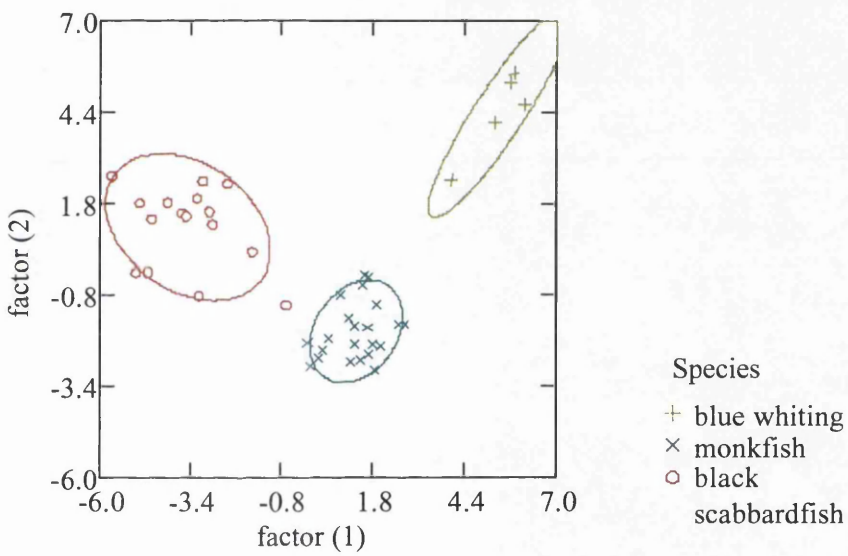


Fig 9.1: Discriminant analysis of species in the depth range 500 to 900m (North Atlantic Central Water) with PCB concentrations in livers expressed on a lipid weight basis.

9.2.1.2. Species differences within the Gulf of Gibraltar Water

Discriminant analysis was used, as the 2 first factors contained 70% of the information (fig 9.2). Backwards stepwise modelling highlighted the CBs whose concentration differed most between these species, which are CBs 28, 44, 70, 110, 114, 128, 138, 153, 156 and 157. CBs 114, 156 and 157 were present in separating

species in both depth ranges. However, in the Gulf o Gibraltar Water, CB28 and 114 were the most important factors, as expressed by the high F-to-remove value (table 9.2).

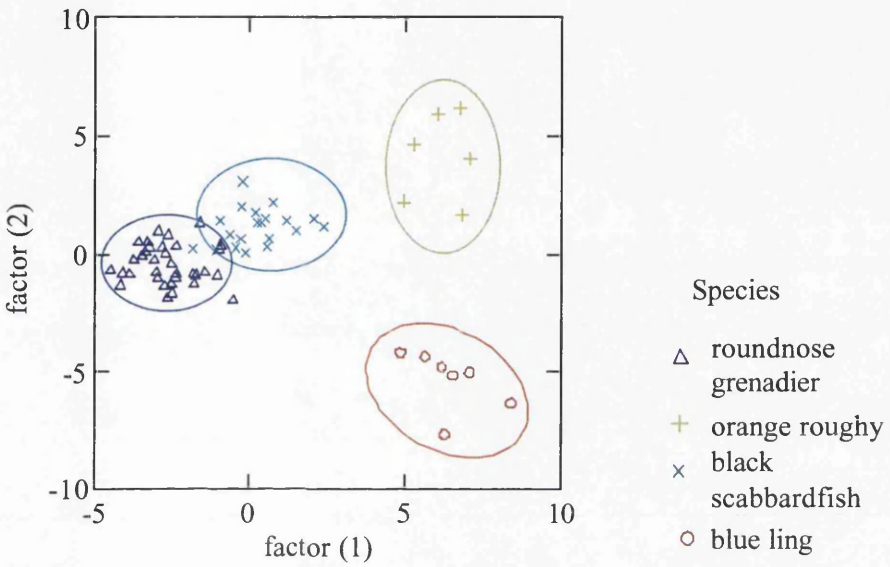


Fig 9.2: Discriminant analysis of species in the depth range 1000 to 1500m (Gulf of Gibraltar Water) with PCB concentrations in livers expressed on a lipid weight basis.

All four species were separated, although roundnose grenadier and black scabbardfish were to a lesser extent than blue ling and orange roughy. Orange roughy presented higher concentrations of CB28 than blue ling, than black scabbardfish than roundnose grenadier. Blue ling presented higher concentrations of CB114 than orange roughy than black scabbardfish than roundnose grenadier. And blue ling presented higher CB128 concentrations than roundnose grenadier than black scabbardfish than orange roughy.

Table 9.2: Backwards stepwise discriminant factors between species in the depth range 1000 to 1500m (Gulf of Gibraltar Water) with PCB concentrations in livers, expressed on a lipid weight basis

	F to remove	blue ling	black scabbardfish	orange roughy	roundnose grenadier
(%) jackknifed		100	90	86	94
constant		-32.177	-3.967	-31.905	-4.700
CB28	32.16	4.195	0.564	1.895	-0.062
CB44	10.27	1.746	-0.532	-1.660	-0.154
CB70	13.22	-2.704	0.262	0.517	0.050
CB110	6.21	-0.432	0.001	0.541	-0.059
CB114	24.57	1.354	0.518	2.049	-0.159
CB128	22.40	0.369	-0.176	-1.459	-0.046
CB138	10.54	-0.295	0.046	0.365	0.081
CB153	7.41	0.026	-0.21	-0.170	-0.044
CB156	18.94	5.657	-0.36	2.442	-0.314
CB157	8.39	-6.489	0.202	-5.141	1.347

9.2.1.3. Species differences within the Labrador Sea Water

Discriminant analysis was used as the 2 first factors contained 68% of the information (fig 9.3). Backwards stepwise modelling highlighted the CBs whose concentration differed most between these species, amongst which the most important were CBs 110, 170 and 138, as expressed by the high F-to-remove value (table 9.3). CBs 114, 156 and 157 were also present, as in the two previous water masses.

C. armatus presented highest concentrations of CB170, *H. bathybius* of CB31, and both species presented high concentrations of CB110. On the other hand, both *C. armatus* and roundnose grenadier presented higher concentrations of CB153 than *H. bathybius*.

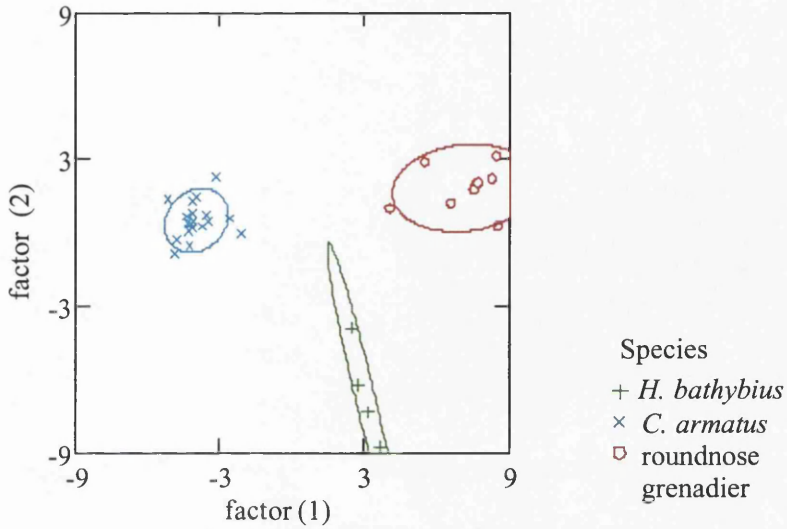


Fig 9.3: Discriminant analysis of species in the depth range 2000 to 4000m (Labrador Sea Water) with PCB concentrations in livers expressed on a lipid weight basis.

Table 9.3: Backwards stepwise discriminant factors between species in the depth range 2000 to 4000m (Labrador Sea Water) with PCB concentrations in livers, expressed on a lipid weight basis

	F to remove	*C. armatus*	*H. bathybius*	roundnose grenadier
(%) jackknifed classification		89	75	89
constant		-8.455	-31.372	-18.820
HCB	4.89	-0.026	-0.096	0.536
CB31	27.90	-1.812	4.048	3.912
CB74	16.39	-0.581	0.141	1.037
CB101	13.73	-0.007	0.267	-0.010
CB110	35.70	0.273	-0.580	-0.551
CB114	17.72	0.208	0.496	-0.378
CB128	14.18	-0.296	-0.816	0.385
CB138	18.57	0.042	0.079	-0.082
CB149	7.31	-0.078	0.426	0.145
CB153	16.18	-0.014	0.046	0.026
CB156	8.53	0.588	4.586	0.209
CB157	6.84	0.480	1.011	-1.492
CB170	32.12	-0.145	-1.590	0.330
CB180	6.72	0.019	-0.187	-0.107

9.2.1.4. Conclusions

Any PCB concentration in fish can be expressed as a function of its concentration in the food of the fish, of its concentration in the water in which the fish lives, and of the metabolism processes (eg degradation or excretion).

However, these contaminants are not supposed to accumulate in fish from their diet, but rather to equilibrate with the concentrations found in the water (Randall *et al.*, 1998), therefore we will consider the diet input negligible. The equilibrium between concentration in water and in the fish lipids is dictated by the octanol-water partition coefficient K_{ow} (eg Hawker and Connell, 1991; or see chapter 2 for details). We can then express a PCB concentration in fish as a function of its concentration in water, K_{ow}, and metabolism processes.

Farrington and Westall (1986) have shown that bioconcentration factors of PCB congeners in fish were poorly predicted by K_{ow}. The possible explanation they suggested was of a selective metabolism of different congeners, with different uptake and/or depuration rates due to specific chlorine configuration differences. Swackhamer and Hites (1988) have suggested that differences in uptake between species could be explained by lipid composition, ie the relative amounts of saturated and unsaturated fatty acids. The proportion of phospholipids in fish fat was also shown to affect the partitioning ability to PCBs in an experiment performed by Ewald and Larsson (1994). Therefore a PCB concentration in fish is a function of its concentration in the water, of the metabolism processes, and of the lipid-water partition coefficient, which we will note K_{lw} (equation 1). K_{lw} is itself a function of K_{ow} and of the lipid composition of the fish (equation 2).

$$[\text{CBx in fish}] = f([\text{CBx in water}], \text{metabolisation}, K_{lw}) \quad (1)$$

$$K_{lw} = f(K_{lw}, \text{lipid composition}) \quad (2)$$

In the present section, we have shown that within a single depth range, ie within a water mass of supposed uniform contamination, fish species differ in their levels of PCBs. In a previous study, benthic and pelagic fishes have been separated by their levels of contamination (McKenzie, 1999). Benthic fish live on the bottom of the

oceans, therefore are subject to higher contamination of PCBs in the water than those living in the water column, as they can be resuspended from the sediments. They exhibited higher PCB concentrations, with enhancement in higher chlorinated biphenyls (see previous sections). However, this approach cannot explain for all differences between species in the present study, as for example in the Labrador Sea Water (below 1500m depth), *C. armatus* and roundnose grenadier, both benthopelagic species and both of the *Coryphaenoides* genus, are well separated. However, their depth ranges might explain the differences, as roundnose grenadier occurs between 700 and 1800m depth and *C. armatus* below 2000m depth.

Therefore the differences between fish species must reflect not only a difference in habitat, but also a difference in the lipid composition of the various species, or a difference in the metabolism processes, or both.

9.2.2. Relative PCB concentrations

In order to evaluate metabolism differences between species, I have looked at their specific patterns of PCBs. The most common way to proceed is to study the relative PCB concentrations, obtained by dividing each PCB by a stable PCB congener. CB153 is usually used in fish studies, as it is very stable, easily detectable and very little metabolised by fish (McKenzie, 1999). In the present study, all but two individuals presented levels of CB153 well above the limits of detection. Therefore the relative concentration of PCBs were calculated, expressed as RCBx=CBx/CB153, eg RCB28=CB28/CB153.

However, as PCB concentrations differed between species within discrete water masses, so did their relative distributions. The principal component and discriminant analyses of CBs and RCBs in fish species within discrete water masses were very similar. They provided similar plots as in the previous section, and only slightly different CBs of importance for the stepwise modelling (table 9.4).

As CB and RCB composition do not differ significantly, all CBs must follow the same metabolisation and excretion processes as CB153. But this CB is very stable and not metabolised. Therefore, there can be very little metabolisation and excretion

of individual PCBs in deep water fish species. This might have been predicted as there is little metabolisation in shallow water species (eg Phillips, 1995), and deep water fish present a metabolism as much as 90% reduced compared to their shallow water counterparts (Gage and Tyler, 1991).

Table 9.4: In backwards stepwise discriminant analysis, F to remove factors between species in the various water masses with RCB concentrations in livers, values in red were of importance in the previous discriminant analysis.

water mass (depth range)	North Atlantic Central Water (400-900m)	Gulf of Gibraltar Water (1000-1500m)	Labrador Sea Water (2000-4000m)
species	monkfish, black scabbardfish, blue whiting	blue ling, black scabbardfish, orange roughy, roundnose grenadier	*C. armatus, H. bathybius*, roundnose grenadier
importance of first two factors (PCA)	59%	51%	57%
CB28		4.87	
CB49		5.95	
CB52	10.33		6.78
CB70	5.66	8.54	
CB101			39.82
CB105			13.76
CB110	7.14	4.19	
CB114	44.93	11.82	16.37
CB118		8.45	8.98
CB128		13.54	39.10
CB138		6.08	
CB149		12.77	
CB157	4.74		4.14
CB158	10.13		
CB170			18.19
CB187	15.68		
CB189		6.00	23.74

PCB concentration in fish is a function of its concentration in the water, of the metabolism processes, and of the lipid-water partition coefficient K_{lw} as previously established. It has now been shown that metabolism processes are negligible in deep water fish species, hence the PCB concentration is a function of its concentration in the water and of the lipid-water partition coefficient. Therefore, differences between species in a single water mass are probably partly due to differences in K_{lw} and hence in lipid composition.

9.2.3. Relationship between PCB concentration and K_{ow}

9.2.3.1. Pattern in fish in the present study

Hawker and Connell (1991) have shown that the log of the bioaccumulation factor K_B has a linear relationship to the log of K_{ow} for chlorinated aromatic hydrocarbons with log K_{ow} values between 2 and 6 (equation 3). With log K_{ow} between 6 and 9.5, they showed that the relationship became parabolic.

$$\log Kb = \log([\text{in organism}]/[\text{in water}]) = \log K_{ow} + \log(\text{lipid content}) \quad (3)$$

However, PCBs have log K_{ow} values between 5 and 8 (table 9.5, data from Hawker and Connell, 1986), which might explain why Farrington (1986) could not satisfactorily explain the bioaccumulation of PCBs from their log K_{ow} values.

Table 9.5: Log K_{ow} values and structures of the PCBs analysed in the present study.

CB	log Kow	Substitution pattern										number of Cl in position				no Cl
		2	2'	3	3'	4	4'	5	5'	6	6'	o (2+6)	m (3+5)	p (4)	m+p	
CB 28	5.670	x				x	x					1	0	2	2	3
CB 31	5.670	x					x	x				1	1	1	2	3
CB 44	5.750	x	x	x	x							2	2	0	2	4
CB 52	5.840	x	x					x	x			2	2	0	2	4
CB 49	5.850	x	x			x			x			2	1	1	2	4
CB 70	6.200	x			x		x	x				1	2	1	3	4
CB 74	6.200	x				x	x	x				1	1	2	3	4
CB 101	6.380	x	x			x		x	x			2	2	1	3	5
CB 110	6.480	x		x	x		x			x		2	2	1	3	5
CB 105	6.650	x		x	x	x	x					1	2	2	4	5
CB 114	6.650	x		x		x	x	x				1	2	2	4	5
CB 149	6.670	x	x	x			x		x	x		3	2	1	3	6
CB 118	6.740	x			x	x	x	x				1	2	2	4	5
CB 128	6.740	x	x	x	x	x	x					2	2	2	4	6
CB 138	6.830	x	x	x		x	x		x			2	2	2	4	6
CB 153	6.920	x	x			x	x	x	x			2	2	2	4	6
CB 158	7.020	x		x	x	x	x			x		2	2	2	4	6
CB 187	7.170	x	x	x			x	x	x	x		3	3	1	4	7
CB 156	7.180	x		x	x	x	x	x				1	3	2	5	6
CB 157	7.180	x		x	x	x	x		x			2	3	2	5	6
CB 170	7.270	x	x	x	x	x	x	x				2	3	2	5	7
CB 180	7.360	x	x	x		x	x	x	x			2	3	2	5	7
CB 189	7.710	x		x	x	x	x	x	x			1	4	2	6	7
CB 194	7.800	x	x	x	x	x	x	x	x			2	4	2	6	8

The bioaccumulation factor K_B represents the concentration in fish divided by the concentration in water (equation 3). Therefore, if log K_B is proportional to log K_{ow}, log of the CB concentration is too within any discrete water mass and species because the log of concentration in water will be constant (equation 4).

$$\log [\text{CBx in fish}] = \log K_{ow} + \log (\text{lipid content}) - \log [\text{CBx in water}] \quad (4)$$

Fig 9.4 represents the plots of the log of concentration of PCBs in each individual fish (plotted with the same symbol) as function of log K_{ow}. To exclude differences between species (shown in section 9.2.2.) and between water masses (shown in section 9.2.1.), individuals were plotted per species and water mass.

In fig 9.4, each individual is plotted with a different symbol. All species present similar patterns of log CB versus log K_{ow},, with smaller differences between individuals. Between individuals but within species, log CB is not linear to log K_{ow} throughout the K_{ow} range but presents a series of similar relationships within discrete log K_{ow} ranges of 0.5 increment: between 5.5 and 6, then between 6 and 6.5, and so on up to log K_{ow}=8. These discrete groupings of K_{ow} values cannot be explained by the chlorination degree of the PCBs as they are not correlated (see table 9.5), but only by the octanol-water partition coefficient. The section of log K_{ow} presenting the most data points is between 6.5 and 7, and is further studied (fig 9.5).

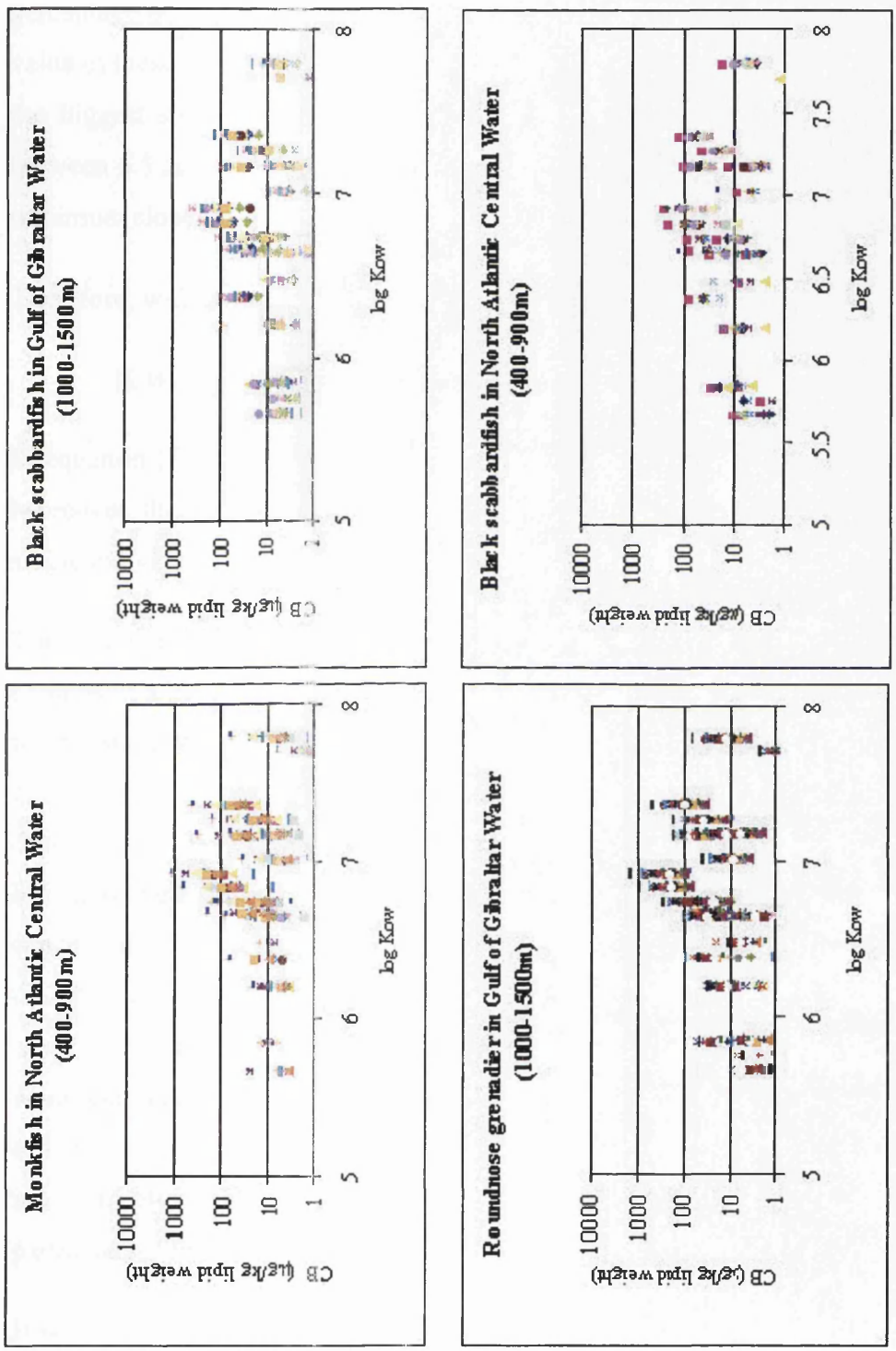


Fig 9.4: PCB concentrations in individual fish as a function of their octanol-water partition coefficient K_{ow}, grouped per fish species and discrete water masses. The PCBs are represented on the x-axis by their K_{ow} value and their concentration is quoted on the y-axis. Each differently coloured symbol represents the concentrations found in a single fish.

Within 0.5 increment of log K_{ow}, all individuals show a strong linear relationship between the PCB concentration and log K_{ow}. Each individual presents a different slope, although the value of the slope does not present a pattern with length of fish, percentage of lipid or sex. Moreover, between 0.5 log K_{ow} increments, although the value of these slopes change, the order of individuals does not, eg the individual with the biggest slope between 6 and 6.5 log K_{ow} will also present the biggest slope between 6.5 and 7. Between species, the slope values change, as does maximum and minimum slope values. This same pattern was found in muscle, gonad and gill tissue.

Therefore, within each 0.5 increment of log K_{ow} we have:

$$[CB] = a * \log K_{ow} + b \quad (5)$$

In equation (5), a and b are constants which depend on both species and individual. Moreover, these constants are different between 0.5 increments of log K_{ow}, even for a single individual.

These variations in slope values may be explained by the variations in lipid composition. A small variation between individual fish will result in a small variation in the slope whereas larger variations in lipid composition between species will induce larger variations of the slope. Therefore, factors a and b in equation (5) are dependant on the lipid composition, which accounts for differences between species, but also between individuals. Ewald and Larsson (1994) proved that PCB concentrations were higher in fish with lower percentage of phospholipids, therefore the change in slope is probably linked not only to the lipid composition, but also more specifically to the percentage of phospholipid. Similar results were found by Andersson *et al.* (1998). Phospholipids make up cell membranes and are a measure of the health of any organism. Therefore the phospholipid composition of lipids can have an effect on the lipid-water partition coefficient of xenobiotics and, in particular, of PCBs.

However, the breaks in linearity between 0.5 log K_{ow} increments are not explained by the differences between species or lipid composition.

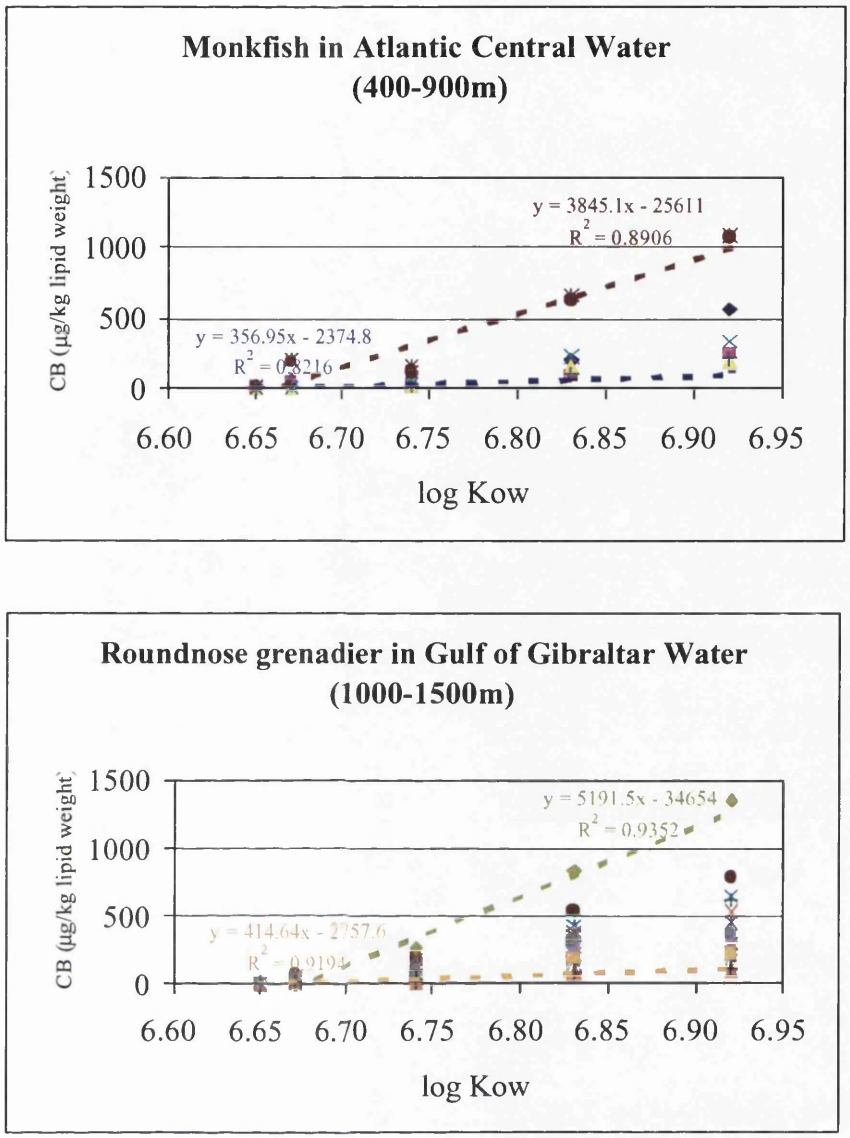


Fig 9.5: PCB concentrations in individual monkfish and roundnose grenadier as a function of the octanol-water partition coefficient K_{ow} within discrete water masses: zoom on log K_{ow} between 6.5 and 7.

9.2.3.2. Comparison with PCB mixture and water composition

The composition of commercial PCB mixtures was investigated (fig 9.6), with the percentage of PCB in each mixture taken from Erickson (1997). However, the individual composition of these commercial PCB mixtures cannot adequately explain the pattern of successive linear relationships found in fish even though this pattern can be found in part of these technical mixtures.

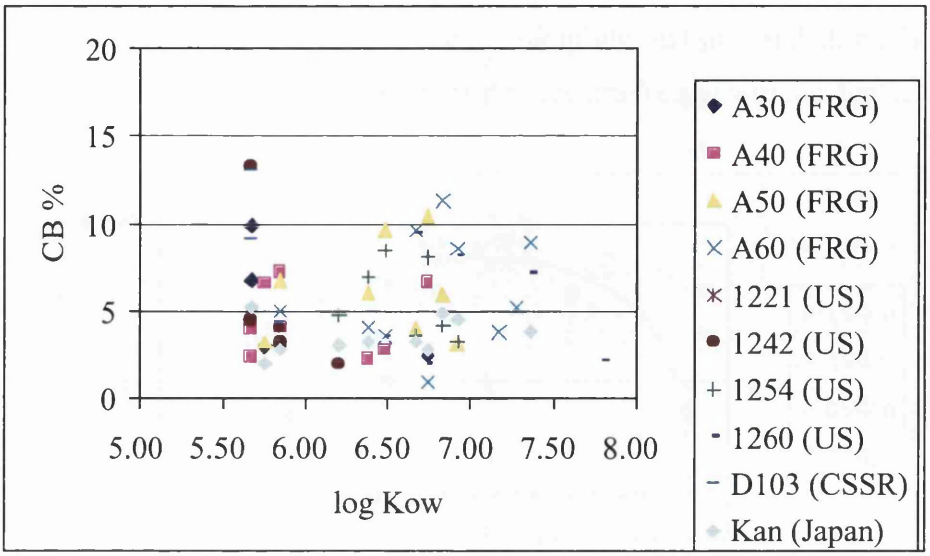


Fig 9.6: Composition of various commercial PCB mixtures as a function of log K_{ow}; FRG are compounds commercialised in France and Germany, and US in the United States.

If this pattern of PCBs in fish with K_{ow} is not linked to lipid composition or PCB mixtures, it might be linked to the pattern of contaminants in the water column. Kramer and Ballschmiter (1988) have reported PCB composition in deep waters of the western North Atlantic (33N65W). Fig 9.7 represents a plot of their concentrations at various depths.

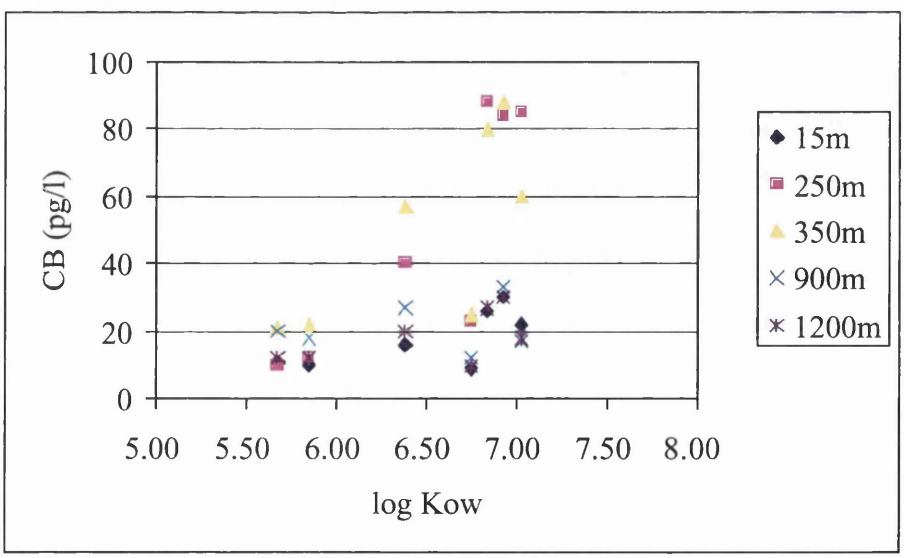


Fig 9.7: Concentration of individual PCBs in the water column as a function of log K_{ow}, data from Kramer and Ballschmiter (1988).

Schulz-Bull *et al.* (1998) have also reported PCB concentrations in deep waters in the northern North Atlantic, north of Scotland. They found that the PCB compositions were very similar at different sampling stations and throughout the depth range. Fig 9.8 represents a plot of their concentrations at various depths.

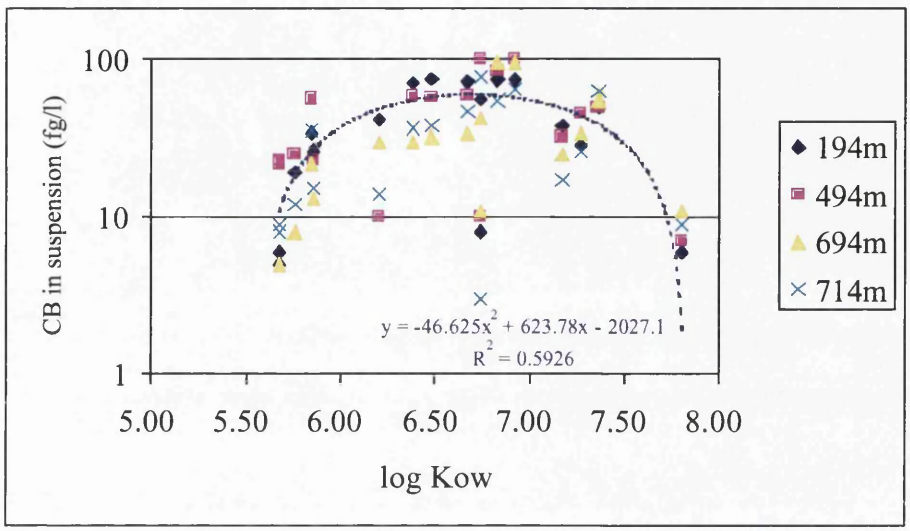


Fig 9.8: Concentration of individual PCBs in the water column as a function of log K_{ow}, data from Schulz-Bull *et al.* (1998).

None of these two data sets show a similar pattern to that found in deep water fish in the present study, but rather a parabolic relationship between PCB concentration and log K_{ow}, even though in each 0.5 log K_{ow} section there is some indication of an increase in PCB concentrations. This result is expected as PCBs with a low to intermediate log K_{ow} value are produced the most and PCBs with a high log K_{ow} value are larger and therefore have more difficulty going through the gills and therefore will be present at lower concentrations than predicted in the present model.

Therefore, the trend found in fish cannot be explained by a similar trend in the water, which would induce a linear relationship between the bioaccumulation factor and log K_{ow}.

9.2.3.3. Pattern in fish in other studies

Froescheis *et al.* (2000) measured individual PCBs in deep water fish from the North Atlantic. A plot of these concentrations against log K_{ow} showed a similar trend to the one obtained in the present study, although not as clear (fig 9.9). Only mean concentrations were available from the publication rather than PCB concentrations in individual fish, whose differences might explain the blurred picture.

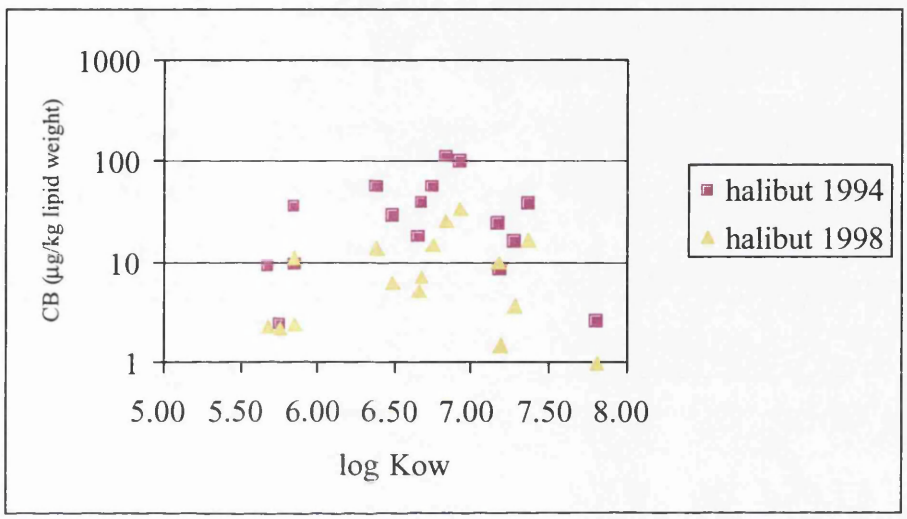


Fig 9.9: Concentration of individual PCBs in deep water fish as a function of log K_{ow}, data from Froescheis *et al.* (2000).

As has been shown before, linear relationships are found in each individual, with some scatter between individuals. Therefore, any relationship would be weaker if only mean concentrations were considered, as found in the previous example. In order to avoid such a problem, the same data analysis was conducted on shallow water plaice from Scottish waters (Webster *et al.*, 2000), on each individual (fig 9.10).

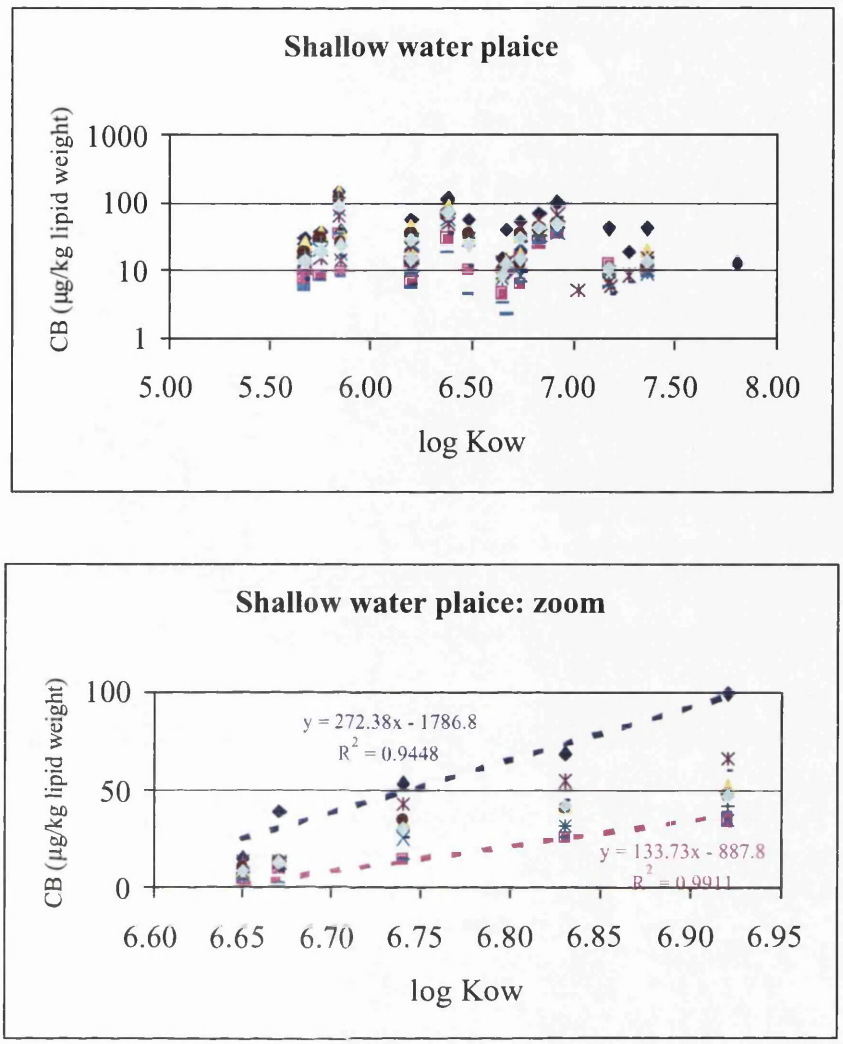


Fig 9.10: PCB concentrations in individual fish as a function of log K_{ow}, and zoom of the 6.5-7 region; data from Webster *et al.* (2000).

Not only did the whole log K_{ow} range in shallow water plaice present a strikingly similar pattern to that found in deep water fish the present study, but also similar linear relationships between PCB concentration and log K_{ow} in 0.5 log K_{ow} increments. The slopes calculated for shallow water plaice were much lower than those found in deep water fish, due to the higher level of contamination in deep water fish with PCBs (see section 9.3.1.1.).

Porte *et al.* (2000) studied deep water fish from the Mediterranean. They found an enrichment in CBs 153, 138, 187, 180 and 170, all of which have chlorine atoms in positions 2,4,5 in one or both rings, a substitution particularly recalcitrant to degradation. On the other hand, PCBs with H atoms in meta or para position (CBs

52, 101, 110, 151, 149 and 141) were depleted. They concluded that deep water fish are exposed to highly degraded PCB patterns. However, these trends were not found in the present data as, for example, CB114 and 153, both supposed to be recalcitrant, were present at very different concentrations. Therefore the ease of degradation of each individual PCB cannot explain the specific trend of PCB concentrations with the octanol-water partition coefficient. Moreover, the PCB patterns were the same in deep sea and shallow water fish, and therefore the PCBs are not more or less degraded between shallow water and deep water fish.

A similar data analysis was conducted on results from McKenzie (1999) on whitesided dolphin (fig 9.11). Although the general pattern presented an increase of PCB concentration within 0.5 log K_{ow} increments (see fig 9.11), these increases were not linear with the PCB concentration. Moreover, the general pattern resembled more that of PCB concentration in water (see fig 9.8), with a parabolic shape. Therefore, the PCB pattern found in dolphin blubber is more similar to that of surrounding waters.

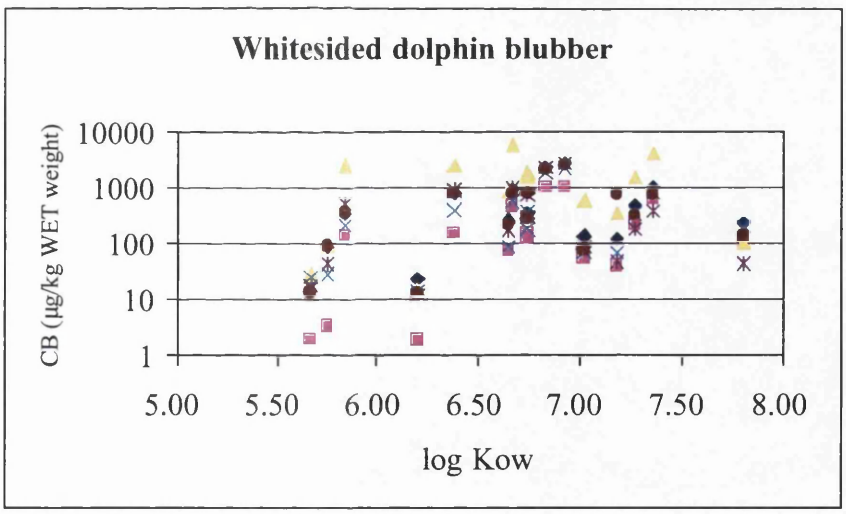


Fig 9.11: PCB concentrations in individual dolphin as a function of log K_{ow}, data from McKenzie (1999).

Therefore, as the pattern found in the present study is present both in shallow water and deep sea fish but not in marine mammals or in surrounding waters, differences might be due to the higher metabolising capacities of mammals compared with fish. A comparison between various data on fish from the literature and the water pattern obtained in fig 9.8 was made by plotting the relative PCB concentration as

CB/CB153*100 (fig 9.12). The patterns in fish are definitely different from those in water, and found both in different species and different studies, eliminating any possible artefact introduced during the sampling or analysis phase.

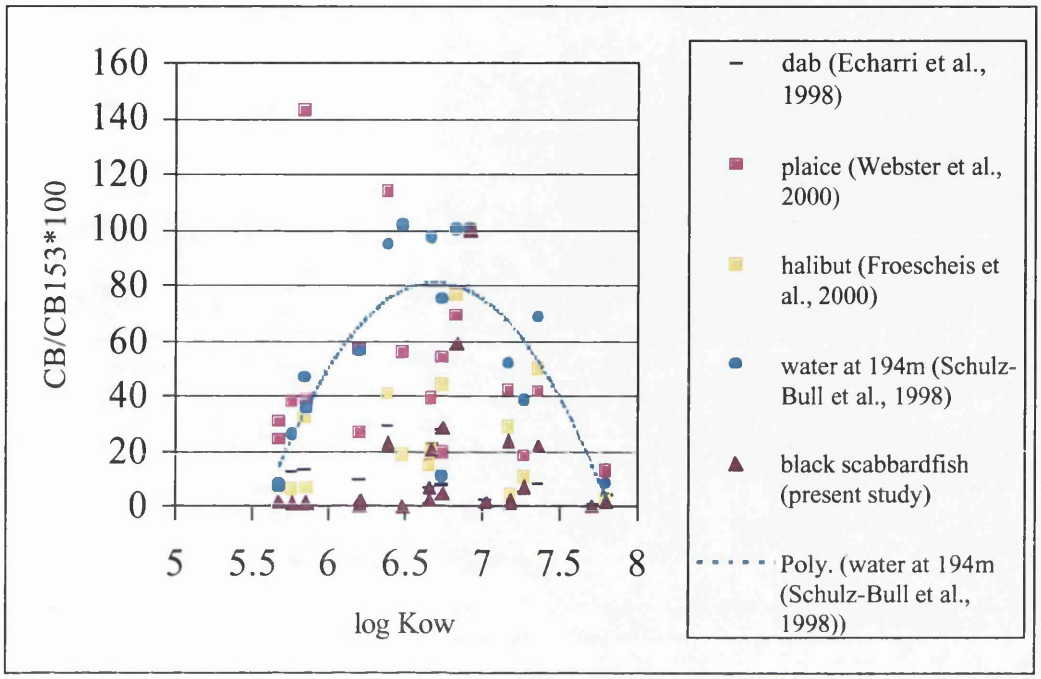


Fig 9.12: Comparison between PCB patterns in various fish species and studies, and with the pattern in water.

9.2.3.4. Structural effects of PCBs on concentrations

Some studies have concentrated on the effects of PCB structure on toxicity or bioaccumulation. Willman *et al.* (1997) found a good correlation between log K_{ow} and the bioconcentration factor in PCBs when log K_{ow} <6. Beyond that value, the steric effect became more significant in higher chlorinated congeners, which explained the loss of linearity, and the need for a more complex relationship depending on the ΣCB concentration.

In a similar way, Shain *et al.* (1991) studied the effects of PCB substitution patterns on their neurotoxicity. Congeners which resembled TCDD in structure were very poor neurotoxicants, highlighting different mechanisms from hepatotoxicity and immunotoxicity, as these were most sensitive to TCDD. Degree of chlorination did not correlate with toxicity, as in the present study it did not dictate the

concentrations. Congeners with ortho or para substitution were more potent, and that a chlorine in meta position decreased toxicity only if the congener was ortho and para substituted.

In the present study, a pattern emerges if congeners with a chlorine in position 6 (ie CB110, 149, 158 and 187) are disregarded. Chlorine substitution in ortho positions (ie 2,2',6 and 6') dominates the three dimensional structure of the congener, therefore will have a strong effect on the accumulation of the congener. This empirical description fits the theory that structure is the decisive factor to accumulation, as the four PCBs mentioned above do not fit the seesaw pattern detailed in above sections very well.

Out with these four congeners, within each linear pattern the number of chlorine in meta plus para positions stays constant while the number of chlorine in ortho position increases. In the following linear relationship the number of chlorine in meta plus para positions increases by one and the number of chlorine in ortho position goes back to 1 to increase again. PCB congeners with no chlorine in meta or para position were found to be more easily metabolised (eg Andersson et al., 1998). Although deep water fish are found to metabolise very little (see section 9.3), the number of chlorine in these position might dictate the half-life of the congeners in fish. As the ortho position has a strong impact on the shape of the molecule, it might rule the ease of absorption through the gills onto lipids. For example, Dunnivant and Elzerman (1988) found that the solubility and vapour pressure of PCB congeners increased with the degree of ortho-chlorination within homologous groups.

9.2.3.5. Quantitative structure-activity relationships of PCBs

Quantitative Structure-Activity Relationships (QSARs) and Quantitative Structure-Property Relationships (QSPRs) refer to mathematical models describing the relationships between the structure of chemicals and their activity, usually expressed as their toxicity to a specific function. In the case of PCBs, as well as polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), the function studied by Mekenyan *et al.* (1996) in QSARs was the aryl hydrocarbon receptor (AhR) as it has been correlated with many toxic responses such as thymic atrophy and

immunotoxicity. They found that the electron acceptor characteristics and steric descriptions were the most important factors in this QSAR.

Tupparainen and Ruuskanen (2000) also studied the QSARs of individual PCBs, PCDDs and PCDFs, by monitoring the AhR. They introduced the semi-empirical molecular orbital energies of the molecules, which are the eigenvalues of the electronic Schrödinger equation (EEVA). EEVA is a descriptor of molecular structure. It is suitable for "pure" electronic substituent effects ie for cases in which both hydrophobic and steric factors are of minor importance. The EEVA were very satisfactory for PCDDs and PCDFs, but poorer for PCBs, which is probably due to their non-planar structure, which partially hinders transmission of the electronic effects of the substituents.

Therefore, QSARs have only partially modelled the toxicity of PCBs, primarily because the steric effect was never fully considered in the models.

9.2.3.6. Steric effects in PCBs

Shaw and Connell (1984) found that the steric effect coefficient (SEC) multiplied by log K_{ow} correlated with bioaccumulation within groups of PCB isomers. This showed that the adsorption of PCBs is influenced by their stereochemistry, as foreseen in the previous section.

SEC of any individual congeners is calculated as the multiplication of the coefficients that apply to the molecule, or is 1 by default. The rules are as follows (from Shaw and Connell, 1984):

- three chlorines in the 2,2',6 positions — coefficient 0.80
- four chlorines in the 2,2',6,6' positions — coefficient 0.60
- two chlorines in the 2,6 or 2',6' positions — coefficient 0.85
- four chlorines adjacent — coefficient 0.80 (one ring)
  coefficient 0.60 (two rings)

• three chlorines adjacent	coefficient 0.95 (one ring) coefficient 0.90 (two rings)
• chlorine in 3 or 5 positions if adjacent to	coefficient 0.95 (1 in molecule)
chlorine in 2 or 6 positions	coefficient 0.90 (2 in molecule) coefficient 0.85 (3 in molecule) coefficient 0.80 (4 in molecule)
• chlorine in 3 or 5 positions not adjacent to chlorine	substract 0.02 from SEC for each chlorine in this position
• no chlorine in the 2,2',6,6' positions	SEC is 1 irrespective

These rules strengthen the previous theories concerning the effect of ortho-chlorination onto the structure of the PCB congeners. SECs were calculated for all PCBs studied in the present work and PCB concentrations plotted as a function of log Kow * SEC for a species of fish and the water concentrations (Schultz-Bull *et al.*, 1998) in fig 9.13.

Although Shaw and Connell (1984) predicted a linear relationship between the bioaccumulation factor and log K_{ow} * SEC, this pattern was not found in the present study. PCB concentrations were correlated with log K_{ow} * SEC for CB170, 187, 180, 138 and 153 only. These PCBs do not present the same chlorination degree or substitution pattern. Moreover, patterns in fish and water were completely different, showing that bioaccumulation would not be correlated either. Therefore SEC cannot explain the entirety of the pattern found in the present study or, more likely, is not detailed enough to consider such small differences in the steric effects, as the ortho effect is the main one considered there.

Shaw and Connell (1984) found that bioaccumulation of PCBs was correlated with their retention time, but Shain *et al.* (1991) found no correlation.

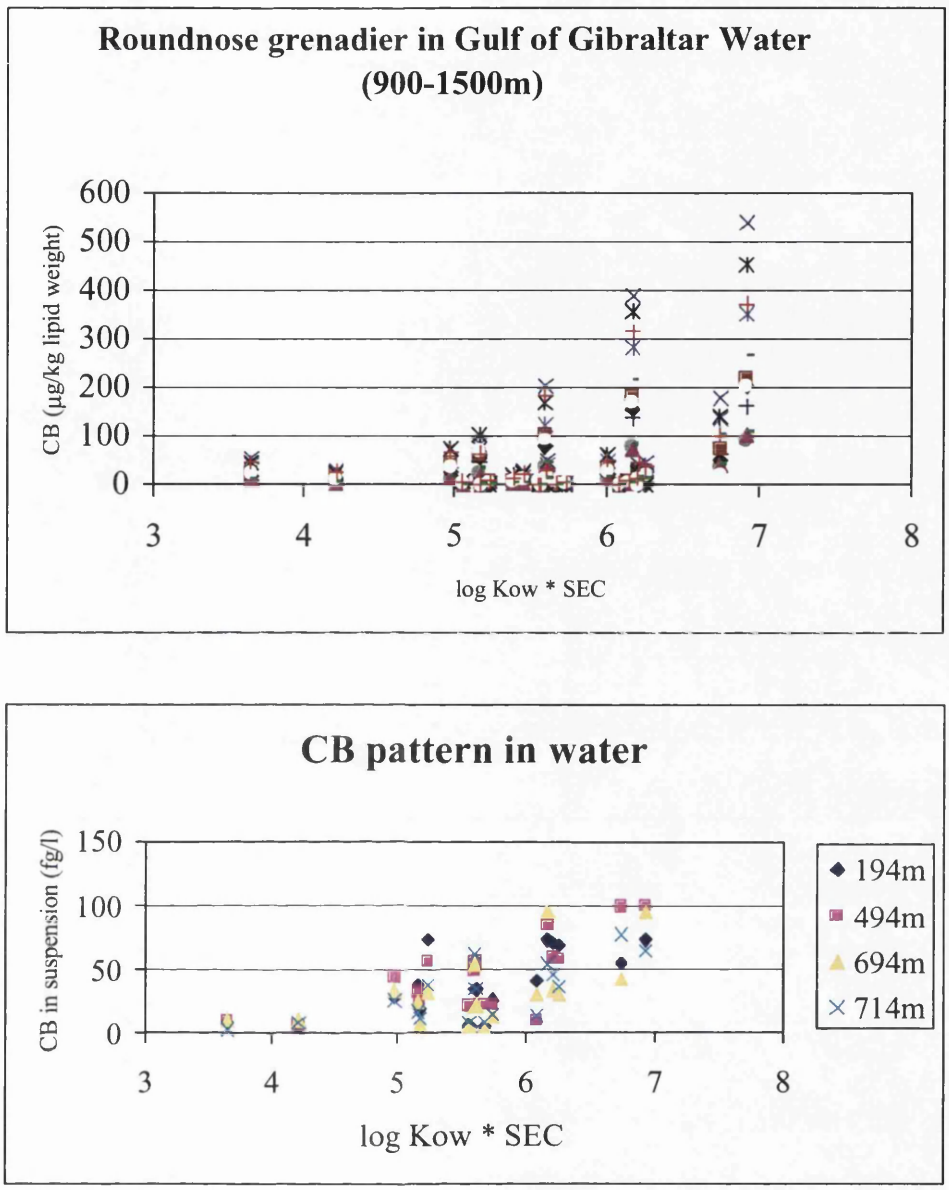


Fig 9.13: Patterns of PCBs in deep water fish (present study) and water (Schultz-Bull *et al.*, 1998) as a function of log K_{ow} * SEC.

Planar molecules are most efficiently adsorbed and adsorption decreases as the molecules become less planar. Moreover, ortho chlorine substitution in PCBs increases the angle of twist between rings thus reducing the planarity of the molecule. Therefore the increase in ortho-chlorine substitution within each linear group should decrease adsorption PCBs (Shaw and Connell, 1984). But in the present study, each seesaw group was made up with increasing ortho chlorination with increasing PCB concentrations. PCBs are available to fish from the water column, the bulk of which is adsorbed onto particulate matter in the North Atlantic

(Schulz-Bull *et al.*, 1998). The increase in ortho-chlorine substitution decreases the planarity and adsorption of these congeners onto particles in the water, hence probably induces a higher bioavailability to fish from the water.

9.2.3.7. Conclusions

The seesaw pattern of PCBs as a function of log K_{ow} found in the present study was also found in various other studies on fish, from both deep and shallow waters, but less so in mammals. The weaker pattern in mammals was attributed to their higher metabolism capacities for such compounds. This pattern was not linked to the pattern in commercial PCB mixtures, or to the PCB pattern in the surrounding waters. It was attributed to the specific steric characteristics of each individual PCB congener, and in particular their planarity. Each linear group was composed of PCBs of same meta- plus para-chlorination degree, thought to rule the half-life of PCBs in fish, and ortho-chlorination degree increasing with concentration, which rules the bioavailability of congeners in the water. However, few QSAR models specific to non-planar PCBs were found in the literature, particularly with special focus on the three dimensional structure, and future work needs done in that domain.

9.3. Influence of depth on PCB and pesticide concentrations and distributions

The influence of depth on PCB and pesticide concentrations and distributions in deep water fish can be demonstrated in several different ways. Amongst these are the differences between shallow water and deep sea fish contamination, the effect of depth on deep water fish contamination, and the effects of different water masses at different depths on fish contamination. These three approaches will be treated separately in the following sections.

9.3.1. Comparison with shallow water species

9.3.1.1. Comparison of concentrations

The PCB and pesticide concentrations and patterns in monkfish have been compared to shallow water individuals of similar species in chapter 6. To summarise, deep water monkfish analysed during the current study presented higher PCB and pesticide levels than those caught in shelf areas around Scotland, but lower than those from industrialised areas such as the Clyde. On the other hand, HCB and ΣDDT were higher in the deep water individuals than in any of the shallow water ones, coming from either industrialised or offshore areas.

Kelly and Campbell (1994) also studied persistent organochlorine contaminants in shallow water fish from Scottish waters, including whiting caught in the Minches, the eastern North Atlantic and in the Firth of Clyde. The first two sites represent relatively uncontaminated areas, whereas the Clyde is heavily industrialised and hence polluted. These results are compared with those obtained in the present study (table 9.6).

Table 9.6: Comparison between mean concentrations in livers of shallow water whiting (Kelly and Campbell, 1994) and deep water blue whiting (present study). The concentrations are expressed in μg kg^{-1} wet weight.

publication	present study		Kelly and Campbell (1994)		
location of capture	Rockall Trough (mean)		Minches (mean)	Atlantic (mean)	Clyde (range)
depth	300m	640m	shallow	shallow	shallow
ΣCB	119.3	213.9	124	181.0	1893-2601
HCB	4.8	12.7	6.8	6.7	5.7-10
γ-HCH	0.9	2.4	3.4	2.3	3.7-12
dieldrin	7.3	21.1	15.4	9.9	49-66
p,p'-DDE	92.7	181.2	20.0	22.4	93-233
p,p'-DDD	11.9	43.1	8.4	7.6	98-190
p,p'-DDT	21.1	64.2	4.1	5.4	28-43
ΣChlodane	31.6	122.5	12.3	15.7	31-58

HCB presented similar levels in individuals from all locations and depths. γ-HCH also presented similar to lower levels in deep water fish compared to the shallow water individuals. Deep water individuals presented similar levels of ΣCB and dieldrin in to those sampled in less contaminated shallow water areas, but lower than those found in individuals coming from the more contaminated Firth of Clyde. p,p'-DDE was present at lower levels in deep water individuals than in individuals from Minches and the Atlantic, and similar levels as in the Firth of Clyde. p,p'-DDD was present in deep water individuals at levels intermediate between those of shallow water individuals from less and more contaminated areas. Finally, p,p'-DDT and Σchlordane were present in highest concentrations in deep water fish.

Deeper water individuals generally presented higher concentrations of p,p'-DDT and lower concentrations of p,p'-DDD and p,p'-DDE. As DDT and DDE are very similar compounds, hence present similar chemical properties, transport of both chemicals in the marine environment and particularly to the deep sea should be similar as well. Therefore, we will assume for the following discussion that the DDT mixture in water is the same throughout the depth range. Some studies have shown that shallow water fish can transform p,p'-DDT to p,p'-DDD and p,p'-DDE (eg Uchida *et al.*, 1988). However, deep water fish present a slower metabolism than their shallow water counterparts, and can probably transform p,p'-DDT to p,p'-DDD and p,p'-DDE to a lesser extent, if at all. This could explain the increased proportions of p,p'-

DDT in deep water fish and lower proportions of p,p'-DDD and p,p'-DDE. Similar results were found for monkfish (see chapter 6).

9.3.1.2. Comparison of the distribution of PCBs

A comparison between the distribution of PCBs in shallow water whiting and deep sea blue whiting from Scottish waters was also conducted comparing data from the present study with the results quoted by Kelly and Campbell (1994). Each individual ICES7CB concentration was expressed as a percentage of the sum of all ICES7CB, which are CB28, 52, 101, 118, 153, 138 and 180 (table 9.7).

Table 9.7: Comparison between PCB distributions in livers of shallow water whiting (Kelly and Campbell, 1994) and deep water blue whiting (present study), expressed in % of the sum of the ICES7CB (CB28, 52, 101, 118, 153, 138 and 180).

location	% lipid	CB28	CB52	CB101	CB118	CB153	CB138	CB180
Minches	55.1	5.6	6.0	11.7	11.5	33.3	24.0	7.9
Atlantic	54.6	17.1	4.7	8.7	11.0	29.0	20.6	9.0
Clyde	52.6	1.0	3.4	10.9	11.1	34.2	25.7	13.7
300m	58.5	0.2	1.0	6.8	8.4	48.7	23.6	11.2
640m	62.9	0.1	0.0	7.4	10.3	38.0	30.8	13.3

PCBs reach the deep sea environment via two paths: precipitation at the poles and consequent transport to deeper waters, and marine snow, which is much quicker. During transport to deeper waters, very little selectivity of PCBs is achieved, as they will stay in the precipitated form they acquired at the poles, mainly adsorbed onto particles. However, in the case of sinking particles, less water soluble PCBs will be transported more rapidly to the deep water than more soluble PCBs, which will be more slowly scavenged to deeper waters. Schulz-Bull *et al.* (1998) have shown that PCBs with lower degree of chlorination were found preferentially in solution whereas those with a higher degree of chlorination were found preferentially in suspension, sinking faster to the deep waters. The higher the chlorination of PCBs, the less soluble they are, therefore they should be present in higher proportion in deep sea fish than shallow water ones (see chapter 2 for details). Kramer and Ballschmiter (1988) have also highlighted this pattern.

Individuals caught in deeper waters presented lower percentages of lower chlorinated biphenyls, up to CB118 (which is pentachlorinated), and higher percentages of higher chlorinated biphenyls. Fish sampled in the Clyde area presented an intermediate pattern, which is probably due to a different exposure to PCBs than that from less contaminated areas rather than a different process. Apart from the individuals sampled in the Clyde, these results are in agreement with the theoretical distribution pattern of PCBs, with a larger proportion of higher chlorinated biphenyls in deep sea fish compared to their shallow water counterparts.

Other studies of shallow water fish species from the same region presented a similar trend, with a lesser proportion of higher-chlorinated biphenyls (eg Webster *et al.*, 2000). We will now investigate the effects of depth on concentrations and distributions of PCBs and pesticides on each individual deep water species within the present study.

9.3.2. Correlations between depth and concentrations

Only muscle tissue of black scabbardfish was considered for statistical analysis, as no other species presented enough muscle tissue samples analysed for statistical significance.

In black scabbardfish muscle, depth was positively correlated with concentrations of HCB, α-chlordane, γ-chlordane, oxychlordane, aldrin, dieldrin, endrin, heptachlor-epoxide, trans-nonachlor, p,p'-DDT, o,p'-DDD, p,p'-DDD and Σchlordane; and negatively correlated with the concentration of o,p'-DDE. On the other hand, in black scabbardfish liver, depth was positively correlated with concentrations of α-HCH, α-chlordene, γ-chlordene, heptachlor and the percentage of hexachlorinated biphenyls; and negatively correlated with the concentration of o,p'-DDE.

In monkfish liver, depth was positively correlated with length, the percentage of lipids, the concentrations of o,p'-DDE and ΣDDT; and negatively correlated with the concentrations of heptachlor, o,p'-DDD and the percentage of tri and tetrachlorinated biphenyls.

In *C. armatus*, depth was positively correlated with the percentage of lipids and concentrations of oxychlordane, o,p'-DDD, p,p'-DDD and the percentage of hexa and heptachlorinated biphenyls; and negatively correlated with α-HCH, o,p'-DDE and the percentage of tri and pentachlorinated biphenyls.

Finally, in roundnose grenadier, depth was positively correlated with α-HCH, endrin, o,p'-DDT, p,p'-DDT, o,p'-DDD, p,p'-DDD, o,p'-DDE and the percentages of tri, tetr and pentachlorinated biphenyls; and negatively correlated with weight, the concentrations of γ-chlordane and the percentages of hexa, hepta and octachlorinated biphenyls.

In most species studied, depth was positively correlated with a number of pesticides and/or the percentages of higher chlorinated biphenyls (from pentachlorinated biphenyls), and negatively correlated with o,p'-DDE or o,p'-DDD and lower chlorinated biphenyls. In *C. armatus*, depth was also negatively correlated with α-HCH. These results show that what was true in the previous section for PCBs in shallow and deep water fish species is also true within deep water species at various depths. Therefore, within deep water fish, depth differences are significant enough to induce an increase in the proportion of higher chlorinated biphenyls. o,p'-DDE and o,p'-DDD seem to decrease in concentrations with depth.

However, in roundnose grenadier, the correlations were opposite from those found in other species. In that species, depth was also negatively correlated with weight, which is either an artefact of sampling or a strange behaviour specific to roundnose grenadier, as was suggested by Gordon (1979), and in the previous chapter on metals.

In black scabbardfish sampled between 500 and 1500m depth, numerous pesticides were positively correlated with depth. Water at 500m depth in the Rockall Trough is mainly composed of the North Atlantic Central Water, then at about 900m depth is composed of the Gulf of Gibraltar Water (Ellett *et al.*, 1986). This last water mass comes from a location where pesticides such as DDT are still in use, whereas they have already been banned in the northern hemisphere. Hence an increase in pesticides with depth at these latitudes is expected. The effects of water masses on organic contamination will be detailed further in the next section.

9.3.3. Effects of water masses on the concentrations and patterns of organic contaminants in fish

Fish do not accumulate organic contaminants through food but equilibrate with the concentrations in the surrounding waters. Therefore, the levels and patterns of organic contaminants in fish should reflect the contamination of the surrounding waters they live in, hence the water masses. However, this is tempered by numerous factors. Amongst others, fish move and migrate and might do so between different water masses; a fish sampled at a discrete depth does not necessarily live or spend most of its time there, and the depths of water masses can also vary slightly with season and climatic conditions. Unfortunately, the biology and behaviour of deep sea fish are difficult to assess. On the other hand, discrete water masses come with very distinct temperature profiles (Ellett *et al.*, 1986; OSPAR Commission 2000, Region V), which might control fish migration within that water mass.

It is assumed in the following discussion that fish sampled at a discrete depth actually live there most of their time and therefore their contaminant patterns represent the water mass found at that depth. In the Rockall Trough, water down to about 200m depth is mainly constituted of Eastern North Atlantic Water, then of Subarctic Intermediate Water down to about 400m depth, North Atlantic Central Water down to about 900m depth, Gulf of Gibraltar Water down to about 1400-1500m depth and of Labrador Sea Water below that (Ellett et al., 1986; see introduction for details). Various markers have been studied, such as the HCH or PCB compositions. These will be studied separately.

9.3.3.1. HCH composition as a marker of water masses

The composition of HCH, as reflected by the α-HCH/γ-HCH ratio has been suggested in numerous studies as a marker of different water masses (eg Tanabe and Tatsukawa, 1983; Fischer *et al.*, 1991; Ballschmiter, 1992). The hexachlorocyclohexane isomers fulfil most requirements for water mass markers: stability, sufficient high water solubility, an intermediate K_{ow} value resulting in a strongly reduced vertical particle-associated transport and the existence of two characteristically different technical products primarily used in different areas of the

world. α-HCH>> γ-HCH is characteristic of the northern hemisphere, and γ-HCH>> α-HCH for the southern hemisphere.

The use of fish as biomarkers has also been studied (Ballschmiter *et al.*, 1981; Fischer *et al.*, 1991), although the gradient in α-HCH/γ-HCH ratios was less pronounced than that found in water samples. In the present study, many samples presented levels of either or both α-HCH and γ-HCH lower than the detection limit. The following discussion will be based on fish livers only, and will not take into account individuals with levels under the limits of detection. Fig 9.14 summarises the results obtained.

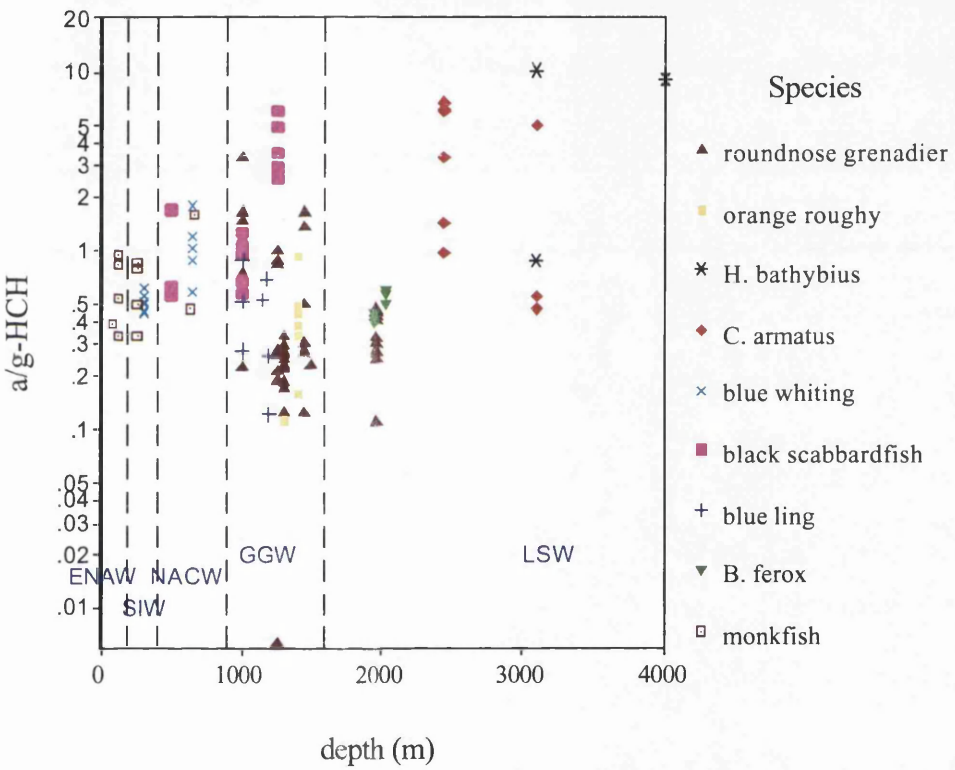


Fig 9.14: α-HCH/γ-HCH ratio in various fish species at different depths in the Rockall Trough, in relation to the water masses (ENAW: Eastern North Atlantic Water, SIW: Subarctic Intermediate Water, NACW: North Atlantic Central Water, GGW: Gulf of Gibraltar Water and LSW: Labrador Sea Water).

Monkfish was the only species present both in the Eastern North Atlantic Water and in the Subarctic Intermediate Water. Individuals from the two different water masses did not present differences in the α-HCH/γ-HCH ratio. These two water masses could be relatively similar in terms of HCH composition as they originate from a

similar area, which is distant from any important source of HCH. Moreover, any small differences might have been masked out by analytical uncertainties at the low levels of HCH in fish, and their lesser efficiency as biomarkers compared to water.

Blue whiting and, to a lesser extent, monkfish individuals presented increasing α-HCH/γ-HCH ratios between the Subarctic Intermediate Water and the North Atlantic Central Water. The North Atlantic Central Water mainly derives from the Western Atlantic and therefore should be enhanced in α-HCH rather than γ-HCH, as a result of the industrial pollution from North America. Hence the increase in α-HCH/γ-HCH ratio between these two water masses.

Black scabbardfish presented increasing α-HCH/γ-HCH ratios between the North Atlantic Central Water and the Gulf of Gibraltar Water. However, this result was not expected as the latter water mass should be enhanced in γ-HCH as a result of the use of lindane in the southern hemisphere. However, similar results were quoted by Fischer *et al.* (1991) for sea water, with an increase in the α-HCH/γ-HCH ratio between the North Atlantic Central Water and the North Atlantic Deep Water, supposing that the effects of the Gulf of Gibraltar Water were too weak to be perceived.

Roundnose grenadier, sampled at similar depths as black scabbardfish, presented an inverted trend, with an α-HCH/γ-HCH ratio higher in individuals caught at 1000m than below. However, this might be due to the biology of this species, where mature females are suspected to live below 1500m depth but migrating to about 1000m depth to spawn (Gordon, 1979). Therefore, large individuals caught at 1000m depth would actually live much deeper, hence their body burden of chemicals could represent the characteristics of a deeper water mass.

We can conclude that the various water masses in the Rockall Trough can be roughly separated from the α-HCH/γ-HCH ratio in fish. However, because of low levels of HCH in deep water fish and of the limited biology knowledge about these species, extreme care must be taken.

9.3.3.2. DDT composition as a marker of water masses

p,p'-DDE concentrations presented a general increase with depth, as described in section 9.3.2. But is that increase dependant on the water masses?

Kramer and Ballschmiter (1988) have reported varying p,p'-DDE and p,p'-DDT concentrations in the North Atlantic water column with depth and geographic location, but no trend or explanation was provided. Within a single location, the p,p'-DDE concentration increased from 250m to 900m depth, then decreased at 1200m depth; p,p'-DDT levels were below the limit of detection at all depths.

In the present study, p,p'-DDE, p,p'-DDD, o,p'-DDT and p,p'-DDT increased with depth (from 80 to 5000m depth) regardless of species, with $p<0.001$ in all cases for all the results without distinguishing species (eg fig 9.15). Moreover, the p,p'-DDE/ΣDDT ratio remained constant with depth. Therefore, the degree of accumulation of DDT and metabolites in fish is not dependent on the species.

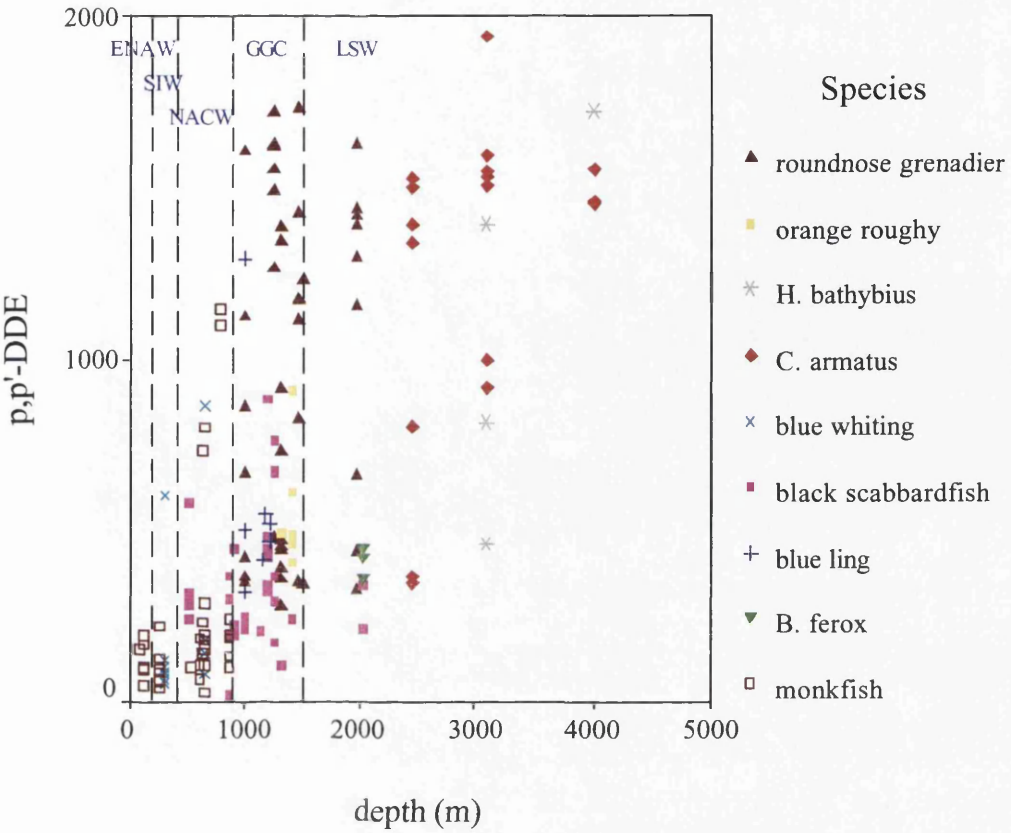


Fig 9.15: p,p'-DDE (µg kg^{-1} lipid weight) in various fish species at different depths in the Rockall Trough in relation to the water masses (ENAW: Eastern North Atlantic Water, SIW: Subarctic Intermediate Water, NACW: North Atlantic Central Water, GGW: Gulf of Gibraltar Water and LSW: Labrador Sea Water).

The increase of p,p'-DDE concentration with depth still follows to some extent the separation of water masses, with the biggest increase between the North Atlantic Central Water and the Gulf of Gibraltar Water. The Gulf of Gibraltar Water is expected to be enhanced in DDT and its metabolites, because countries on the south side of the Mediterranean basin still use DDT as pesticides. The Labrador Sea Water presents similar contamination as the Gulf of Gibraltar Water, as in the previous examples.

Interestingly, monkfish sampled between 1000 and 1500m depth present two very distinct groups, one with lower contamination and the other with an outstandingly high contamination level of p,p'-DDE, which distorts the linear relationship between p,p'-DDE concentration and depth. However, these highly contaminated individuals are probably the larger mature males and females from 1800m depth migrating to

shallower waters to breed, as supposed by Gordon (1979), and will be detailed in a subsequent section.

However, in the case of deep water fish species, both p,p'-DDE/ΣDDT and p,p'-DDE/p,p'-DDT ratios did not vary significantly with depth (eg fig 9.16). Therefore, within deep sea fish, the p,p'-DDE burden in fish increases as a consequence of its increase in the surrounding waters and not because of a lesser metabolisation of p,p'-DDT, as causes differences between shallow water and deep sea fish.

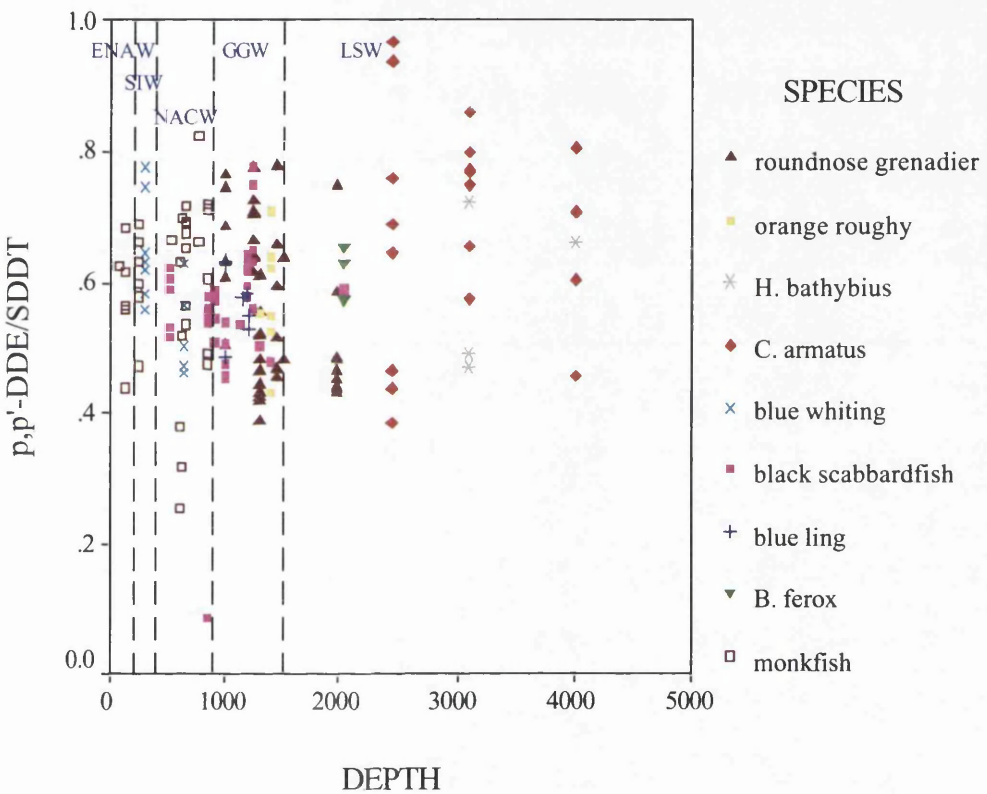


Fig 9.16: p,p'-DDE/ΣDDT ratio in various fish species at different depths in the Rockall Trough in relation to the water masses (ENAW: Eastern North Atlantic Water, SIW: Subarctic Intermediate Water, NACW: North Atlantic Central Water, GGW: Gulf of Gibraltar Water and LSW: Labrador Sea Water).

9.3.3.3. PCB composition as a marker of water masses

Previous studies have shown an enrichment of higher chlorinated biphenyls in deep-sea fish compared to their shallow water counterparts. This was explained by the particle-bound transport of more hydrophobic compounds from the surface of the

ocean to the deep-sea (Fischer and Ballschmiter, 1987; Kramer and Ballschmiter, 1988).

In the present study, as shown in section 9.3.2, there is also an increase of the proportion of higher chlorinated biphenyls and decrease of lower chlorinated biphenyls in some species. ΣCB show an increase with depth (from 80 to 5000m depth) regardless of species, with $p<0.001$ in all cases for all the results without distinguishing species (fig 9.17). Therefore, although the degree of accumulation of ΣCB in deep sea fish is dependent on the species, the trend is still to a general increase with depth and smaller differences between species.

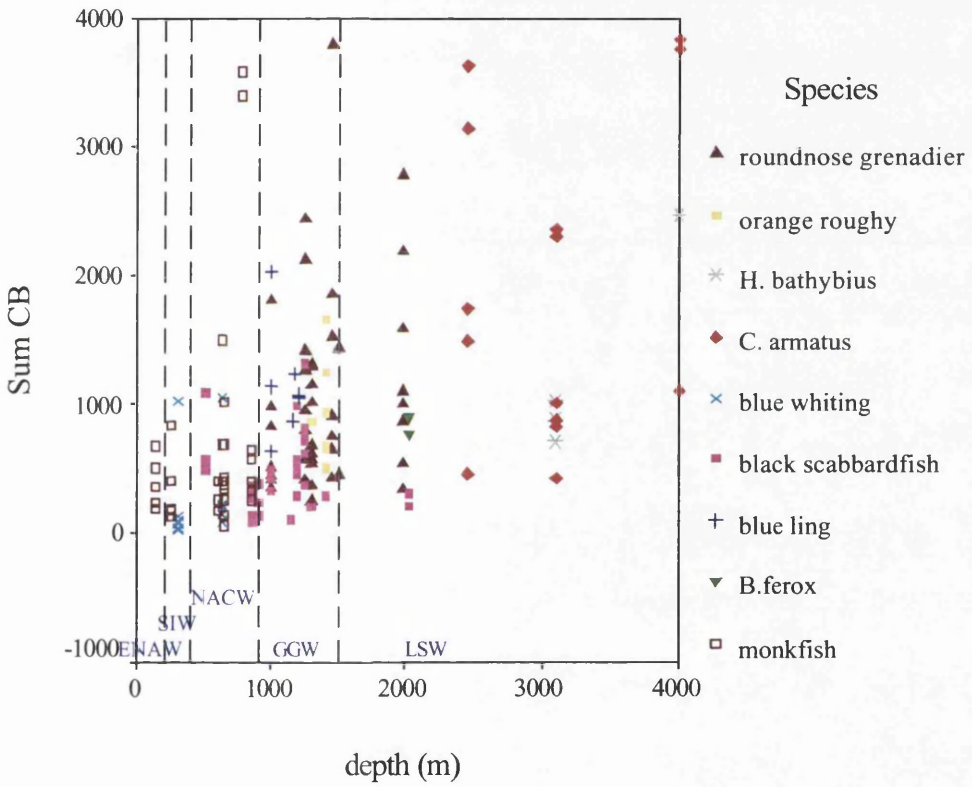


Fig 9.17: ΣCB (μg kg^{-1} lipid weight) in various fish species at different depths in the Rockall Trough in relation to the water masses (ENAW: Eastern North Atlantic Water, SIW: Subarctic Intermediate Water, NACW: North Atlantic Central Water, GGW: Gulf of Gibraltar Water and LSW: Labrador Sea Water).

In order to study the precise effect of water masses on the PCB distribution, discriminant analysis was used on single species overlapping different water masses covering black scabbardfish, monkfish and roundnose grenadier.

For black scabbardfish, discriminant analysis was used as the 2 first factors contained 65% of the information (fig 9.18). Backwards stepwise modelling highlighted the CBs whose concentration differed most between these locations, which are CB49, 149, 157, 158 and 170 (table 9.8).

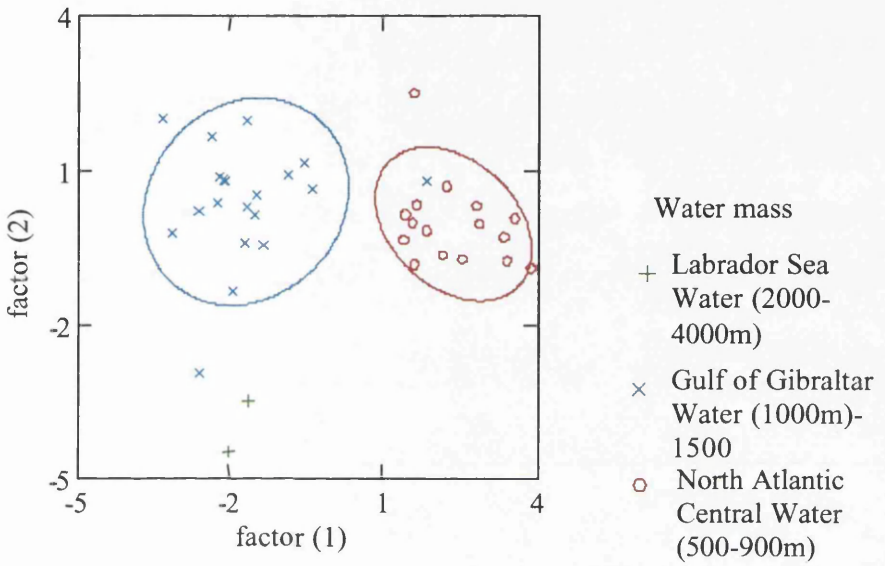


Fig 9.18: Discriminant analysis of depth ranges in black scabbardfish with PCB concentration in livers expressed on a lipid weight basis.

Table 9.8: Backwards stepwise discriminant factors between depth ranges in black scabbardfish with PCB concentrations in livers, expressed on a lipid weight basis

	F to remove	400-900m	1000-1500m	2000-4000m
(%) jackknifed		94	80	100
constant		-7.973	-4.236	-7.106
CB49	6.26	1.637	0.726	1.019
CB149	4.56	-0.259	-0.133	-0.353
CB157	7.47	0.462	-0.135	-0.619
CB158	7.16	-0.830	0.390	1.456
CB170	7.01	0.763	0.403	0.589

Monkfish also presented differences between samples from less than 400m depth and those from 400 to 900m depth. Backwards stepwise modelling highlighted the CBs

whose concentration differed most between these locations, which are all but CB189 and 194. Amongst the other PCBs, the most important were (in decreasing order according to F to remove values): CBs 31, 158, 70, 157 and 170. Individuals were properly (jackknife) classified at 67% for shallow water and 73% between 400 and 900m depth.

Roundnose grenadier also presented differences between individuals sampled in different water masses, namely Gulf of Gibraltar Water (900 to 1500m depth) and Labrador Sea Water (below 1500m depth). Backwards stepwise modelling highlighted the CBs whose concentration differed most between these locations, which are by decreasing order of importance according to F to remove values: CB 128, 138, 70, 49, 74 and 149.

Table 9.9 sumarises the influences of PCBs on separating the various water masses.

Table 9.9: PCBs of importance to differentiate water masses, and their relationship with depth (+: increase with depth, -: decrease with depth, =: equivalent, " " not of importance); ENAW: Eastern North Atlantic Water, SIW: Subarctic Intermediate Water, NACW: North Atlantic Central Water, GGW: Gulf of Gibraltar Water, LSW: Labrador Sea Water

water masses species	ENAW – SIW monkfish	SIW – NACW monkfish	NACW–GGW black scabbardfish	GGW – LSW roundnose grenadier
CB49	-	+	-	-
CB70	+	-		+
CB74	-	-		-
CB128	-	+		+
CB138	+	+		-
CB149	+	+	+	+
CB157	+	-	-	
CB158	-	+	+	
CB170	+	-	-	

CB74 showed a decrease with depth, and CB 149 an increase with depth, which corroborates the enhancement of higher chlorinated PCBs in the deeper environment. All other PCBs showed specific accumulation in the various water masses, although this was based on different species. However, CB49, 70 and 158 were very close to the limits of detection, if not under, therefore should not be considered.

9.4. Influence of length on the concentrations

In all species, length was positively correlated with the levels of most pesticides and the percentages of hexa and heptachlorinated biphenyls, and negatively correlated with o,p'-DDE and the percentages of tri and tetrachlorinated biphenyls. Moreover, most of these species present an older/deeper factor, ie live in deeper waters as they grow older. Therefore, as shown in previous sections, the depth at which a deep sea fish individual lives is the main factor determining its body burden in organic contaminants, rather than its species or length.

Interestingly, roundnose grenadier, which presented an inverted pattern with depth for both metals and organic contaminants, presents a similar pattern with length as the other species. This suggests that roundnose grenadier do live deeper when older, but follow specific migrations. These induced sampling larger individual at shallower waters and the subsequent apparent decrease of length when sampling deeper in the present data set. Gordon (1979) proposed that juveniles adopt the demersal mode of life at about 1000m depth on the slope. At this stage they might be one or two years old, having previously been pelagic. His hypothesis was that as they grow they move down the slope to about 1800m depth. Nearing maturity they migrate back up so that mature females are at about 1000 to 1200m depth and males are shallower. His theory would fit well with the trend found in this data set, as at ca 1000m depth the mature individuals normally living below 1500m depth and presenting the chemical characteristics of this water mass were probably sampled rather than the juveniles only, inducing the distortion in the results.

9.5 Correlations between the levels of organic contaminants

In all species, the concentrations of most organic contaminants (ΣCB, HCB, aldrin, endrin, dieldrin, ΣDDT and ΣChlordane) were positively correlated together, and none were negatively correlated. As these contaminants equilibrate between the water and the lipids within the body, their correlations only reflect the constant relative composition of organic contaminants of the water.

9.6 Conclusions

The Meriadzec area is more polluted with organic contaminants than the Rockall Trough area, as reflected by the contamination of respective fish populations (section 9.1.1.). However, Madeira and Sesimbra do not present increased contamination compared to the previous areas (section 9.1.2.). On the other hand, in the present study, the eastern South Atlantic presents a far lower organic contamination than the eastern North Atlantic (section 9.1.3.).

The levels of PCB and pesticides in fish are dictated by the depth at which they live rather than length of each individual, by equilibrium between the concentrations in the water and in the body's fatty tissues. However, differences between species were still detectable within individual PCB concentrations. Within individuals, organic contaminants are distributed throughout the body and depend on the lipid content of each organ rather than its metabolic function (section 9.2.).

PCB composition in both shallow water and deep sea fish presented a striking pattern, with PCB concentration proportional to the log of the octanol-water partition coefficient, but only within discrete log increments of 0.5. The seesaw pattern of PCBs as a function of log K_{ow} found in the present study was also found in various other studies on fish, from either deep or shallow waters, but less so in mammals. The weaker pattern in mammals was attributed to their higher metabolism capacities for such compounds. This pattern was not linked to the commercial PCB mixture pattern, or to the PCB pattern in the surrounding waters. It was attributed to the specific steric characteristics of each individual PCB congener, and in particular their

planarity. Each linear group was composed of PCBs of same meta- plus para-chlorination degree, thought to control the half-life of PCBs in fish, and ortho-chlorination degree increasing with concentration, which rules the bioavailability of congeners in the water. However, few QSAR models specific to non-planar PCBs were found in the literature, particularly with special focus on the three dimensional structure, and future work needs done in that domain.

Deep water fish species present an increased burden of p,p'-DDT and decreased burden of p,p'-DDD compared to their shallow water counterparts. Shallow water fish are able to metabolise p,p'-DDT to p,p'-DDD, but deep water fish have a much lower ability to do so because of their slower metabolism (section 9.3.1.1.).

Deep water fish also present an increased burden of higher chlorinated biphenyls such as hepta and hexchlorinated biphenyls, and a lower burden of lower chlorinated biphenyls such as tri and tetrachlorinated biphenyls compared to their shallow water counterparts. This is explained by the particle-bound transport of more hydrophobic compounds from the surface of the ocean to the deep-sea (section 9.3.1.2.).

Within deep water fish, DDT metabolites and ΣCB increase with depth (section 9.3.3.).

The α-HCH/γ-HCH ratio did to some extent differentiate the water masses present in the Rockall Trough. However, due to very low levels of HCH in these fish, the differences were not highly significant. Therefore, the use of HCH isomer ratios in fish as biomarkers of water masses is not recommended in the deep sea, unless the limits of detection of HCH isomers is very low. On the other hand, the DDT and PCB compositions did not reflect the differences in water masses but the increase in depth, as these are ruled by vertical transport rather than global transport from the hemispheres at least for PCBs (section 9.3.3.).

Finally, the peculiar migration pattern of roundnose grenadier was confirmed by the body burden and composition of organic contaminants in individual fish. Therefore, the levels and patterns of PCBs and pesticides can be used as a tool to help determining the behaviour of these deep water fish species, behaviour which is difficult to study by traditional means (section 9.4).

Chapter 10
Conclusions

The aims of the present study were to determine the influence of biological and environmental factors on the concentration and distribution of inorganic and organic contaminants in deep water fish species.

The conclusion can be separated into the following categories, which will then be presented separately:

- some potential human health issues arose from the levels of contaminants found in deep water fish, and some recommendations are made
- the effects of a wide variety of biological and environmental factors on metal concentrations in fish have been investigated
- the effects of a wide variety of biological and environmental factors on PCB and pesticide concentrations in fish have been investigated
- a specific pattern of PCB concentration in fish has been found and further investigated

10.1. Potential health issues

10.1.1. Metal concentrations

The metals of most concern for human health are copper, zinc, cadmium, mercury and lead, as reflected by the regulation of their concentrations in food around the world. Cadmium, mercury and lead presented most of the breach of regulations of the deep water fish analysed, with up to 7% of individuals above limits in muscle tissue and 90% above limits in liver tissue. However, mean concentrations of metals in muscle tissue of all species were below MAFF and EC limits.

Following a detailed investigation of the data, some recommendations were put forward in order to reduce the probability a percentage of the catch breaching EC regulations:

- do not catch black scabbardfish below 1000m depth
- catch blue whiting at 640m and deeper, but not shallower
- discard orange roughy individuals bigger than 52.7cm total length
- do not commercialise livers for human consumption

No simple exclusion rules were found to reduce the likelihood of individual monkfish, hake and roundnose grenadier exceeding regulatory limits. The estimated risk of exceeding is 4, 18 and 2% respectively, although a relatively small number of hake were analysed.

10.1.2. PCBs and pesticides

Following an increasing concern about the effects of PCBs on human health, the EU has recently issued new regulations on PCBs and dioxins in food and foodstuffs. However, apart from a few highly contaminated individuals, deep water fish muscle does not contravene existing or forthcoming regulations if weekly intake is kept to a maximum of 560g fresh weight of fish for adults and 140g for children. Nevertheless, if deep sea fisheries were to expand to the 2000m region and to new species, these should be checked before commercialisation as deeper species (not commercialised at the moment) presented high levels of contamination.

Livers of both shallow and deep water fish species are unsuitable for human consumption because their burdens of organic contaminants exceed the regulations. Moreover, forthcoming stricter EU regulations on fish meal and fish oil will make fish livers unsuitable for use in animal feed as well.

10.2. Effects of biological and environmental factors on metal concentrations

Metal concentrations in deep water fish were investigated in relation to biological and environmental factors such as the species, feeding and habitat, length, weight, location of capture and depth of capture. Levels in the various organs were also studied using a variety of statistical tools. The main conclusions reached follow:

- Most deep water fish species studied presented very low levels of Li, Be, V, Co, Ni, Pb, Bi and U, typically below 0.05 mg kg^{-1}.
- Black scabbardfish presented strikingly high concentrations of V (up to 1.6 mg kg^{-1} in liver tissue) and blue whiting of Ni (up to 15 mg kg^{-1} in liver tissue).
- Within the deep sea environment, top predators such as black scabbardfish, benthic feeders such as monkfish and species living below 2000m depth such as *H. bathybius* present higher levels of metals, hence are subject to higher level of contamination.
- The Meriadzec area is more contaminated by heavy metals than the Rockall Trough.
- Fish from Sesimbra and Madeira present lower levels of contamination, apart from mercury and cadmium in black scabbardfish, which is attributed to their maturity stages.
- The eastern North and South Atlantic present very similar levels of metal contamination of deep water fish species. Comparison with other studies in the Pacific Ocean shows a similar contamination of fish species by metals throughout the deep sea.
- Fish sampled in deep waters present similar levels of most metals to their shallow water counterparts, but higher copper, cadmium and mercury.
- Correlations between depth and metal concentrations are dependent on the species, which reflects their different intake and metabolism.

- The preferred organs of accumulation are both metal and species dependent, reflecting intake and metabolism specific to both metals and species.

- Mercury and cadmium are positively correlated with length in several species, due to the bio-accumulation of these metals in fish.

- Arsenic is negatively correlated with length in several species because it is adsorbed onto the gills rather than absorbed from food as most other metals.

- Metals are correlated with one another in most fish species (apart from blue ling, blue whiting and orange roughy). Therefore, depuration processes, if any, are not metal specific in deep water fish

10.3 Effects of biological and environmental factors on organic concentrations

Levels of PCBs and pesticides in deep water fish were investigated in relation to biological and environmental factors such as the species, feeding and habitat, length, weight, location of capture and depth of capture. The main conclusions reached follow:

- Most individual PCB congeners are found in quantifiable amounts in deep water fish species.

- Organic contaminants are evenly distributed throughout body and depend on the lipid content rather than the metabolic function of each organ.

- Levels of PCBs and pesticides in fish are dictated by the depth at which they live rather than the length or diet of each individual, through equilibrium between the concentrations in the water and in the body's fatty tissue.

- PCB concentrations in different species were significantly different. The concentration of PCBs is probably dependent on the percentage of phospholipids in the total lipid, which accounts for inter species and inter individual differences.

- The Meriadzec area is more polluted with organic contaminants than the Rockall Trough area.

- Fish from Sesimbra and Madeira present similar levels of pollution as Meriadzec.

- The eastern South Atlantic present far lower concentrations of PCBs in deep water fish because the southern hemisphere is less industrialised than the northern hemisphere. The differences in pesticide concentrations are smaller, as the southern hemisphere countries still use some of them for agricultural purposes.

- Deep water fish present higher proportion of p,p'-DDT and lower proportion of p,p'-DDD compared to their shallow water counterparts because of their lesser ability to metabolise p,p'-DDT into p,p'-DDD.

- Deep water fish present an increased burden of higher chlorinated biphenyls and decreased burden of lower chlorinated biphenyls compared to their shallow water counterparts because of the selective particle-bound transport of more hydrophobic compounds from the surface of the oceans to the deep sea.

- Concentrations of DDT metabolites and ΣCB increase with depth in deep water fish.

- The α-HCH/γ-HCH ratio does to some extent reflect the different water masses present in the Rockall Trough.

- Due to very low levels of HCH in these fish, these differences are not highly significant and therefore, the use of HCH ratios in fish as a biomarker of water masses is not recommended in the deep sea, unless the limit of detection of HCH isomers is very low.

- The DDT and PCB compositions do not reflect the differences in water masses but the increase with depth. This availability of groups is dominated by vertical transport rather than global transport from the hemispheres.

- The peculiar migration pattern of roundnose grenadier found by Gordon (1979) is confirmed by the body burden and composition of organic contaminants in its

individuals. Therefore, the levels and patterns of PCBs and pesticides can be used as indication of the behaviour of these deep water fish species, behaviour which is difficult to study by traditional means.

10.4. PCB pattern

The PCB composition of deep water fish presents a striking pattern, with PCB concentrations proportional to the log of the octanol-water partition coefficient, but only within discrete log increments of 0.5. This seesaw pattern of PCBs as a function of log K_{ow} was also present in data from various other studies on fish, from either deep or shallow waters, but less so in mammals. The weaker pattern in mammals was attributed to their higher metabolism capacities for such compounds.

This pattern is not closely linked to the composition of commercial PCB mixtures, or to the PCB pattern in the surrounding waters. It is attributed to the specific steric characteristics of each individual PCB congener, and in particular their planarity. Each linear group is composed of PCBs of same meta- plus para-chlorination degree, which is thought to determine the half-life of PCBs in fish; and the ortho-chlorination degree, which determines the bioavailability of congeners from the water.

10.5. Future work

The main aims of the present study were attained. However, future work needed in some of the areas investigated is obvious. Some of my suggestions follow.

- High burdens of metals and in particular mercury were found in black scabbardfish from Madeira. This was attributed to the presence of large, mature black scabbardfish from Madeira compared to other locations. Black scabbardfish have been found mature only in Madeiran waters. However, how mercury concentrations increase in livers with maturity has not been explained. I suggest a detailed modelling of the mercury (and other metals) cycle in all organs of black scabbardfish throughout the year, with the calculation of the distribution

of mercury in the various organs expressed in total quantity and percentage of body burden rather than in concentrations.

- The specific PCB pattern also needs further investigation. The present study lacks work on bioaccumulation factors as water samples were not collected. I suggest the in-depth investigation of PCB patterns including as many congeners as possible as most data available in the literature only present few congeners. One can either re-sample, or work on available data sets. Moreover, few QSARS models specific to non-planar PCBs were found in the literature, particularly with special focus on the three dimensional structure of individual congeners and future work needs done in that domain.

- PCB contamination of roundnose grenadier confirmed the biological suggestions made by Gordon (1979). However, a biased sampling towards a supposed deeper-older distribution blurs that picture. A sampling proportional to catches could confirm the findings. Moreover, chemical analysis is foreseen as a complementary tool to study the biology and behaviour of these deep water species and should be developed in that way.

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Appendix 1
Datasets

The dataset generated by the present study is large. In total, 1407 different samples have been analysed for up to 16 metals, and 309 different pooled samples have been analysed for 24 PCBs and 20 pesticides. Therefore, a printout of all data is not practicable. Instead is attached a CD with the complete datasets of all analyses of fish by individual organs for both metals and organic contaminants.

Results are expressed in mg kg^{-1} wet weight for metal (metalresults.pdf) and in mg kg^{-1} lipid weight for PCBs and pesticide (cbresults.pdf). Results under the limits of detection (as quoted in chapter 4) are noted uld and samples not analysed for a specific element are quoted NA. The results are sorted per species, location, depth and organ.

Each fish is numbered with a unique identification number, to allow for cross-referencing of concentrations of metals and organic contaminants in all the tissues analysed for each individual fish. Information on the location and depth of capture, length, weight, ... is also recorded. The species codes used are consistent with those used previously and referenced in Appendix 2.

Appendix 2

Abbreviations of Fish Species

- List of fish, with their abbreviation and Latin name.

Abbreviation	Common name	Latin name
ANG	monkfish	*Lophius piscatorius*
BFE		*Bathysaurus ferox*
BLI	blue ling	*Molva dypterygia*
BSC	black scabbardfish	*Aphanopus carbo*
BWI	blue whiting	*Micromesistius poutassou*
COA		*Coryphaenoides armatus*
HAK	hake	*Merluccius merluccius*
HIB		*Histiobranchus bathybius*
KIN	kingklip	*Genypterus capensis*
LVO		*Lophius vomerinus*
MCA	cape hake	*Merluccius capensis*
MPA	cape hake	*Merluccius paradoxus*
ORO	orange roughy	*Hoplostethus atlanticus*
RNG	roundnose grenadier	*Coryphaenoides rupestris*

- List of abbreviations of organs

FL	muscle
GD	gonad
GL	gill
LV	liver