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Halogenated persistent organic pollutants in relation to trophic level in deep sea fish

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ABSTRACT

The bioaccumulation of persistent organic pollutants (POPs) in deep sea fish from the Rockall fishing area was investigated. Predator and prey species were analysed for stable isotopes, fatty acids, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs). $\delta^{15}N$ indicated that black scabbard was at the highest trophic level and the prey the lowest. The fatty acid signatures indicated that black scabbard and black dogfish fed at a higher trophic level compared to the roundnose grenadier. PCBs and PBDEs were detected in the liver of all three predator species. PCB concentrations were significantly higher in the roundnose grenadier, possibly due to their longer life span. PCB concentrations were compared to OSPAR assessment criteria, concentrations were above background but below Environmental Assessment Criteria for all but one congener. PCB concentrations were below food safety levels in the flesh, but exceeded the limit for liver in the roundnose grenadier and black dogfish.

1. Introduction

To the north-west of Scotland lies a deep water ecosystem that supports a diverse and abundant deep sea fish population. A deep water fish is defined as one that lives most of its life cycle at depths of more than 400 m (Gordon et al., 1995). Scottish deep water fisheries are located along the Rockall Trough and Faroe-Shetland Channel at depths of between 500 and 1500 m. Commercial interest in deep water fish started in the 1970s, but since the turn of the century has decreased due to declining landings and regulation (Neat and Burns, 2010). More than 130 deep water fish species are found in waters to the west of the UK and approximately 12 of these are fished commercially including roundnose grenadier (*Coryphaenoides rupestris*), black scabbard (*Aphanopus carbo*) and black dogfish (*Centroscyllium fabricii*). Roundnose grenadier and black scabbard are targeted for the consumer market, whilst black dogfish are mainly obtained through by-catch but are used in the production of fishmeal.

There is concern that deep waters may act as a sink for persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs). Fish will primarily accumulate POPs through their diet. Fish can also uptake POPs present in the water column via their gills. This will not, however, be a significant route as the concentration of POPs in water will be low as they are hydrophobic in nature. Many deep water fish species are long lived (e.g. >10 years) and, therefore, have the potential to accumulate significant concentrations of contaminants.

PCBs and PBDEs are persistent, bioaccumulative and toxic (PBT) chemicals and for these reasons pose serious concerns to human health which has resulted in their inclusion on the OSPAR List of Chemicals for Priority Action (OSPAR, 2001a,b). Both contaminant groups were used in high volumes. It was estimated that across the globe 1.3 million tonnes of PCB compounds have been produced since they were first introduced in the 1930s (Breivik et al., 2007). PBDEs were first used in the 1970s and between 1970 and 2000 it has been estimated that approximately 12,000–15,000 tonnes of penta-BDEs were produced in Europe (Prevedouros et al., 2004). Historically, the main sources of PCBs to the marine environment include energy production, combustion industries, production processes and waste (landfill, incineration, waste treatment and disposal). Although the production and use of PCBs have been banned in Western Europe since the 1970s, they still enter the marine environment following the destruction and disposal of industrial plants and equipment, or from emissions from old electrical equipment (for example present in landfill sites). Since the ban on the production and use of PBDE formulations in Europe in 2004, the main sources of PBDEs will be from the disposal of products containing these chemicals.

Due to the concerns about the accumulation of contaminants in deep water fish, a number of studies have investigated the presence of POPs in these species. Compared to shallow water species, deep water species were found to have an increased burden of the more highly chlorinated PCBs, indicating the accumulation of anthropogenic pollutants may be a concern for the deep water ecosystem (Mormede and Davies, 2002). More recently (2006–2008 inclusive) roundnose grenadier, black scabbard and black dogfish, collected from the continental shelf slope to the west of Scotland from depths ranging between 600 and 1700 m were analysed for PCBs and PBDEs (Webster et al., 2009, 2011a). Halogenated POPs were detected in all three species, confirming that these contaminants are transported to the Scottish deep water environment, probably as a result of atmospheric deposition.

Trophic position can influence POP concentrations. Fatty acid and stable isotope analyses can be used to investigate feeding patterns of fish, birds and marine mammals and are valuable tools in examining trophic relationships (Dalsgaard et al., 2003). These techniques have the advantage over stomach content analysis as they can be applied to any fish and provide information on an average diet integrated over a period of time, rather than a snapshot of their most recent meal. Stable isotope ratios are also influenced by the diet. An enrichment of the nitrogen isotope ratios occurs with increasing trophic level and, therefore, allows the position of a species in the food web to be assessed (DeNiro and Epstein, 1981). Nitrogen isotope ratios ($^{15}N/^{14}N$ expressed as $\delta^{15}N$, see Experimental Section) show a stepwise enrichment between trophic levels of about 3.8‰ and therefore $\delta^{15}N$ can be used as an indicator of trophic level (Hobson et al., 2002). Similarly, the carbon isotope ratio ($^{13}C/^{12}C$ expressed as $\delta^{13}C$, see Experimental Section) increases the higher the animal is in the food chain, however compared to the increase in $\delta^{15}N$ this change is small with an increase of 0–1‰ between prey and predator (during trophic transfers). However, $\delta^{13}C$ can be used to identify sources of carbon at the base of the food web.

The fatty acid composition of both storage and structural lipids is indicative of the diet. As such, fatty acid profiles can be used to provide information on diet. The fatty acid profiles of primary producers will be passed up the food chain. Although the profile will be modified at each step of the food chain (by metabolism or biosynthesis of fatty acids), some features will be retained and can be used as fatty acid trophic markers (FATM) (Dalsgaard et al., 2003). Some fatty acids and fatty acid ratios can be used as biomarkers for species at different trophic levels. In recent years the complementary methods of isotope ratio determination and fatty acid signature analysis have gained increased application in marine foraging ecology.

The three species (black scabbard, roundnose grenadier and black dogfish) previously analysed for POPs (Webster et al., 2009, 2011a) were the focus of this study. In addition, potential prey species were collected and all samples analysed for POPs, fatty acids and stable isotopes. Black scabbard is a relatively fast-growing species achieving a maximum length of around 150 cm at an age of 8–10 years (Morales-Nin and Sena-Carvalho, 1996). Most black scabbard found at Scottish latitudes are immature and spawning is thought to take place around the island of Madeira off the west coast of north Africa. Observation of stomach contents suggests that their diet consists of crustaceans, fish and, particularly, squid (Gordon and Mauchline, 1990). The roundnose grenadier is thought to live for more than 50 years, maturing around 10 years old (Bergstad, 1990; Allain and Lorance, 2000). The spawning period for roundnose grenadier occurs between May and November and spawning adults are found at Scottish latitudes. Individuals grow to over 100 cm in length. Roundnose grenadiers are generalists, feeding on copepods, amphipods and fish (Mauchline and Gordon, 1986). Stomach content analysis of roundnose grenadier from the Skagerrak identified 96% of the identifiable stomach remains as being crustacean (copepods to brachyurans) (Bergstad et al., 2003). The diet of roundnose grenadier collected from the Mid-Atlantic ridge was found to consist of cephalopods, pelagic shrimps, fish and copepods (Bergstad et al., 2010). Copepods were also found to have a significant contribution to the diet of roundnose grenadier from the Rockall Trough (Mauchline and Gordon, 1986 and Gordon and Mauchline, 1990). Little is known about the life-history of the black dogfish. However, observations of stomach contents suggest they feed on crustaceans, cephalopods and small fish (Froese and Pauly, 2007). Similar to other deep water sharks, black dogfish have a large liver with a high squalene content, this being required to maintain buoyancy (Deprez et al., 1990).

The three fish species so far described are reported to occupy slightly different trophic levels. Black scabbard have a long maximum lifespan (>30 years) and are reported to occupy a high trophic level[1] (4.5). Roundnose grenadier occupy a relatively low trophic level (3.5). They have a maximum life-span of more than 50 years. The black dog fish occupies a trophic level of 3.9 (Froese and Pauly, 2007).

Assessing the diet and trophic position of Scottish deep water fish is important in developing our understanding of the ecology of deep water food webs and may contribute to explaining some of the variability in contaminant concentrations observed in deep water fish. This paper investigates the occurrence of persistent organic pollutants (PCBs and PBDEs) in a range of deep water fish species and examines the concentrations in the context of trophic position as inferred from fatty acid signature analysis and $^{15}N/^{14}N$ and $^{13}C/^{12}C$ isotope ratios.

2. Materials and methods

2.1. Sample collection

Building on surveys conducted during the period 2006–2008 (Webster et al., 2009, 2011a), three species of deep water fish were collected by the research vessel MRV *Scotia* from the Rockall fishing area, to the west of Scotland (Fig. 1), in September 2009 and again in August and September 2011 and 2012. Black scabbard ($n = 33$ individual fish) were caught at six different locations (Fig. 1 – the specific black scabbard locations are not specified on the map) at depths of 725 or 1260 m. The full length of the black scabbard ranged from 73 to 106 cm, similar to sizes reported from the previous work (Webster et al., 2009, 2011a). There was considerable overlap in the depth zones the three species were collected from. However, the black scabbard tended to be found in the shallower waters compared to the roundnose grenadier and black dogfish. The roundnose grenadier ($n = 43$ individual fish), were collected at six different locations, at depths of between 600 m and 1800 m. The length (nose to anal fin) of the roundnose grenadier ranged from 11 to 24 cm (equivalent to 48 to 104 cm full length). This is similar to those collected in 2006–2008 (Webster et al., 2009, 2011a). Black dogfish ($n = 20$) were collected at depths of between 725 m and 1500 m. Individual fish lengths (30–47 cm, mean = 41 cm) were similar to fish collected in 2008 (33–49 cm, mean 42 cm). However, the fish collected in 2006 (length range, 32–46 cm, mean = 37 cm) were significantly ($p < 0.001$) smaller compared to those caught in the more recent years. For the roundnose grenadier and black scabbard, both the muscle and liver

[1] The trophic level is the position of the organism in the food chain and can range from 1 (Plants and algae make their own food and are called primary producers) to 5 (Apex predators which have no predators and are at the top of the food chain), primary producers will have a trophic level of 1 and marine mammals and humans a trophic level of 5.

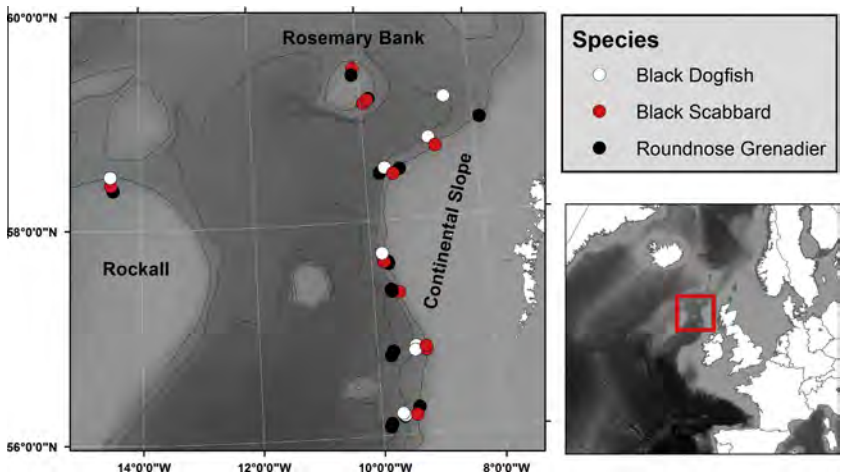

Fig. 1. Location of deep water fish sites on the west of Scotland sampled in September 2009 and August and September 2011 and 2012 from the MRV *Scotia*.

tissue were analysed for PCBs, PBDEs, fatty acid composition and stable isotopes. Sufficient muscle tissue could not be obtained from the black dogfish due to the limited amount of muscle tissue and the small size of the fish; this made these fish impracticable to fillet. The lipid rich liver tissue of these fish accumulates POPs, making this matrix the preferred choice for analysis of lipophilic contaminants. Furthermore, the liver constitutes the metabolically active storage reservoir and will therefore be most influenced by changes in the diet of the fish and thus intake of fatty acids. Hence, this is the recommended tissue when investigating prey-predator relationships in these species. Additional information can, however, be provided on the longer-term variations in diet by the analysis of the muscle.

In 2011 and 2012, ten possible prey species were collected; blue whiting (*Micromesistius poutassou*), greater argentine (*Argentina silus*), Goiter blacksmelt (*Bathylagus euryops*), lanternfish (*Lampanyctus macdonaldi*), Bean's bigscale (*Scopelogadus beanie*), horse mackerel (*Trachurus trachurus*), poor cod (*Trisopterus minutus*), silvery pout (*Gadiculus argenteus thori*), shrimp (infraorder: *Caridea*) and squid (order: *Teuthida*). These species were collected at depths ranging from 500 m to 2000 m. As marine predators generally consume their prey whole, the fatty acid composition was determined for the whole prey species except for the squid where the soft body was used.

At time of capture, all biota samples were wrapped separately in aluminium foil and stored at −20 ± 5 °C prior to analysis.

2.2. Fatty acid and stable isotope analysis

2.2.1. Lipid extraction

Lipid was extracted from the liver and flesh (where available) of the three species of deep water fish using the method of Bligh and Dyer (1959) as modified by Hanson and Olley (1963) and used extensively in the authors' laboratory (Stowasser et al., 2006, 2009). Butyl hydroxyl toluene (BHT) was added to the tissue samples prior to homogenising with a mixture of methanol, chloroform and water (2:2:1.8 v/v). The organic and aqueous layers were separated by centrifugation. The organic layer was recovered and the extract evaporated to dryness by rotary evaporation. The lipid was re-suspended in *iso*-hexane (2 ml) and stored at −20 °C, prior to trans-esterification (see below).

The protein residue left after lipid extraction was air dried on a filter paper, freeze dried, and then ground to a fine powder using a mortar and pestle in preparation for stable isotope analysis.

2.2.2. Trans-esterification of lipids extracted from fish tissue

The method outlined here describes the trans-esterification of the lipid extracted from deep water fish tissue. An appropriate volume of lipid extract (equivalent to a maximum of 50 mg lipid) was transferred into clean, solvent rinsed test tubes. The *iso*-hexane was removed by evaporating under charcoal scrubbed nitrogen. Distilled toluene (1 ml) and sulphuric acid in methanol (1%, 2 ml) were added to each vial. The mixture was shaken vigorously and heated, using a heating block, at 50 °C overnight. The mixture was allowed to cool before HPLC grade water (5 ml) containing sodium chloride (5% w/v) was added. The resulting fatty acid methyl esters were extracted using *iso*-hexane (2 × 5 ml). The combined organic layers were washed with 2% potassium bicarbonate (5 ml) before being dried over anhydrous sodium sulphate (5 g). Prior to analysis by gas chromatography-flame ionisation detection (GC-FID) the extracts were diluted to give an approximate lipid concentration of 1 mg ml^{-1}.

2.2.3. Determination of fatty acid methyl esters by gas chromatography-flame ionisation detection (GC-FID)

Trans-esterified lipids (fatty acid methyl esters (FAMEs)) were analysed on an Agilent 6890 (HP 6890) gas chromatograph (Agilent Technologies, Berkshire, UK) with flame ionisation detection (GC-FID) and equipped with a cool, on-column injector. An Agilent DB-23 fused silica column (30 m × 0.2 mm id) coated with a 0.25 μm film of 50% cyanopropyl (Crawford Scientific, Strathaven, UK) was used for the separation of the FAMEs. Injections (1 μl) were made at 60 °C and the temperature ramped at 25 °C min^{-1} up to 150 °C and then at 1 °C min^{-1} to 200 °C. The temperature was held constant for 10 min before a final temperature elevation at 5 °C min^{-1} to 230 °C where it was held for 5 min. The detector was set at 300 °C and nitrogen was used as the carrier gas (1 ml min^{-1}). Thirty FAMEs were investigated: 14:0, 14:1(n−5), 15:0, 16:0, 16:1(n−7), 16:2, 16:3, 16:4, 17:0, 18:0, 18:1(n−9), 18:1(n−7), 18:2(n−6), 18:3(n−3), 18:3(n−6), 18:4(n−3), 20:0, 20:1(n−9), 20:1(n−11), 20:2(n−6), 20:3(n−3), 20:4(n−6), 20:4(n−3), 20:5(n−3), 21:5(n−3), 22:0, 22:1(n−9,11), 22:5(n−3), 22:6(n−3) and 24:1(n−9). The data was collected via a PE Nelson 610 link box and processed using Perkin–Elmer Turbochrom Navigator version 6.1.0.2:GO7 software (Perkin Elmer, Beaconsfield, UK). The normalised area percentages were calculated for each of the 30 individual FAMEs as a percentage of the total area for the 30 FAMEs. A Restek Marine Oil FAME standard was analysed with each batch of samples to confirm the GC retention times. EO23 fish oil, a long-standing reference material which has been used to

confirm FAME retention times for many years (McGill and Moffat, 1992), was analysed for FAMEs along with each batch and was used as a check on the retention times. A cod liver oil laboratory reference material (LRM) was esterified and analysed with each batch of samples as a check on the esterification procedure. The data obtained from the LRMs were transferred onto NWA Quality Analyst, and control charts were produced with warning and action limits.

2.2.4. Stable isotope analysis

Approximately 0.75 ± 0.15 mg of ground fish flesh or fish liver tissue (de-lipified and freeze dried, as detailed above) was loaded into a 6 × 4 mm tin capsule and combusted in an Integra CN Isotope Ratio Mass Spectrometer (Sercon Ltd, Crewe, UK). Samples, together with a laboratory reference material (de-lipified fish flesh), were analysed in an automated sequential procedure. A certified reference material, USGS 40 (L-glutamic acid; US Geological Survey), was used as an internal standard for the stable isotope measurements.

Results are expressed in the standard δ unit notation as parts per thousand (‰) difference from a standard reference material, calculated as follows:

$\delta X = ((R_{sample}/R_{reference}) - 1) \times 10^3$, where X is ^{13}C or ^{15}N and $R = {}^{13}C/{}^{12}C$ for carbon and $^{15}N/^{14}N$ for nitrogen and are reported relative to international standards, VPDB (Vienna Pee Dee Belemnite) for carbon and atmospheric nitrogen for nitrogen.

Analytical precision of the instrument throughout the different batch analyses was <0.4‰ for ^{15}N and <0.7‰ for ^{13}C.

2.3. Lipid determination

The total lipid content was determined according to the method of Smedes (1999). The biota sample (liver, 0.2–0.5 g; muscle/whole fish, 2–5 g) was weighed into a centrifuge tube and *iso*-propanol (18 ml) and cyclohexane (20 ml) added. The sample was homogenised then de-ionised water (~13–22 ml, depending on the moisture content of the sample) was added and the mixture homogenised. Centrifugation was used to separate the organic extract from the particulate material. A second extraction was carried out with 13% (v/v) *iso*-propanol in cyclohexane. The two extracts were combined and the solvent removed by rotary evaporation before drying in an oven at 80 °C (±5 °C) for one hour. The weight of residue was determined and the lipid content calculated as % wet weight.

2.4. Determination of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs)

2.4.1. Pressurised liquid extraction (PLE)

PCBs and PBDEs were extracted as described by Webster et al. (2009, 2011a). Briefly, samples were mixed with sodium sulphate and spiked with appropriate internal standards (PCBs: ^{13}C-CB28, ^{13}C-CB52, ^{13}C-CB101, ^{13}C-CB153, ^{13}C-CB138, ^{13}C-CB156, ^{13}C-CB180, ^{13}C-CB189, ^{13}C-CB194 and ^{13}C-CB209; PBDEs: FBDE160[2]) prior to pressurised liquid extraction (PLE). Solvent washed PLE cells (100 ml) were packed as follows: solvent washed filter paper, pre-washed sodium sulphate (10 g), 5% deactivated alumina (30 g), solvent washed filter paper and the biota/sodium sulphate mixture prepared as above. Samples were extracted by PLE using an ASE 300 (Dionex Ltd., Camberley, Surrey, UK) at a temperature of 100 °C and a pressure of 1500 psi. The extraction solvent was *iso*-hexane.

[2] A fluorinated PBDE where one of the bromines is replaced with a fluorine.

Special precautions were required when analysing PBDEs due to their sensitivity to UV light. Specifically, incoming light was minimised in the laboratory by placing UV filters over the windows.

2.5. Extract clean-up for polybrominated diphenyl ether (PBDE) and polychlorinated biphenyl (PCB) analysis

Following PLE, the extract was split in two, one half for PBDE analysis and the other for PCB analysis. The extract for PCB analysis was concentrated by Syncore (fitted with flushback module) to ~0.5 ml and passed through silica columns. The concentrated extracts were analysed for PCBs by gas chromatography (GC)-electron impact mass spectrometry (EIMS).

The remaining extract was also passed through silica columns and transferred to Syncore tubes and the volume reduced to ~0.5 ml at 30 °C (Syncore, fitted with flushback module), before transferring, with washings, to pre-weighed crimp top, amber glass GC vials. The extracts were concentrated further under a stream of nitrogen to approximately 0.5 ml before analysis of PBDEs by gas chromatography–electron capture negative ionisation mass spectrometry (GC–ECNIMS).

2.6. Additional clean-up for samples with a high squalene content

An additional clean-up step was required for the extracts from black dogfish liver to remove the squalene. Gel permeation chromatography (GPC) was used to separate the PBDEs from the squalene. Two GPC columns (Phenogel 10 μm, 50 A, 300 × 21.20 mm, Phenomenex, UK) were connected in series and eluted with 1:1 (v/v) dichloromethane (DCM)/*iso*-hexane. Squalene eluted in the 130–160 ml fraction and the PBDEs in the 160–230 ml fraction. The PBDE fraction was collected, concentrated by Syncore and solvent exchanged to *iso*-hexane before analysis by GC-ECNIMS.

2.7. Determination of polychlorinated biphenyls (PCBs) by gas chromatography–electron impact mass spectrometry (GC–EIMS)

The concentration and composition of 28 ortho CB congeners (CB31, 28, 52, 44, 49, 70, 74, 110, 101, 99, 97, 149, 118, 132, 153, 105, 157, 137, 138, 158, 183, 128, 156, 180, 187, 189, 170, 194) were determined by GC–MS in electron impact mode using an HP6890 Series gas chromatograph interfaced with an HP5975 MSD, fitted with a cool, on-column injector and a 50 m × 0.22 mm × 25 μm SGE HT-8 column (SGE, Milton Keynes, UK). Temperature programmes have previously been described (Webster et al., 2009, 2011a).

2.8. Analysis of PBDEs by gas chromatography–electron capture negative ionisation mass spectrometry (GC–ECNIMS)

The concentration and composition of the PBDEs, specifically BDE28, 47, 66, 85, 99, 100, 153, 154 and 183, were determined by GC-ECNIMS using an HP6890 Series gas chromatograph interfaced with an HP5973N MSD, fitted with a cool on-column injector. A Thames Restek STX-500 column (STX-500, 30 m × 0.25 mm i.d., 0.15 μm film thickness, Thames Restek, Buckinghamshire, UK) was utilised, fitted with a Thames Restek Siltek (0.53 mm i.d.) 5 m guard column. Oven and injector temperature programmes have previously been described (Webster et al., 2009, 2011a). The carrier gas was helium and methane was used as the reagent gas. The MS was set for selective ion monitoring (SIM), ions monitored were *m/z* 78.9 and 80.9 (ions equating to bromine) for all PBDEs.

2.9. Quality control

The PCB and PBDE methods are accredited by the United Kingdom Accreditation Service (UKAS) to ISO 17025. All methods were validated by the replicate analysis of standards and samples, and through spiking experiments or analysis of certified reference materials (CRMs). Limits of detection (LoDs) were determined through the repeat analysis of a low spiked sample and the LoD calculated from 4.65 × standard deviation (SD) of the mean concentration. LoDs were dependent on the sample size. The replicate analysis of standards on separate days gave coefficient of variation (CV%) of ~3% for PCBs analysed by GC–MS. Recoveries of greater than 75% were achieved for PCB spiked biota and CRMs. LoDs were around 0.5 μg kg^{-1} wet weight for the fish liver samples (~0.2–0.5 g) and, around 0.05 μg kg^{-1} wet weight for fish muscle samples (2–5 g). LoDs for the tri- to hepta-BDEs in biota were between 0.05 and 0.07 μg kg^{-1} wet weight (10 g samples) and recoveries were >75%.

Internal quality control procedures incorporated the use of a LRM for all determinants, and also a certified reference material (CRM) for PCBs, in each batch of samples. Procedural blanks were performed with each batch of samples, and the final concentration adjusted accordingly. The data obtained from the LRM and CRM were transferred onto NWA Quality Analyst and Shewhart charts were produced with warning and action limits being drawn at ±2× and ±3× the standard deviation of the mean, respectively. CRM data was accepted if recoveries were between 80 and 120% of the certified concentration. Quality assurance was further demonstrated through successful participation in the QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) Laboratory Performance Studies.

2.10. Statistical analysis

Data were analysed for normality (Ryan-Joiner test) and non-normal data was transformed (Johnson transformation), using Minitab 15. Analysis of variance (ANOVA) at the 95% confidence level, with Tukey's pair-wise comparisons, on the normalised data was used to assess significant differences between the different fish species, and compare concentrations between years and depths. Principal component analysis (PCA) was used to look at the variations in the fatty acid profiles and identify the fatty acids responsible for any detected differences.

3. Results

3.1. Stable isotopes

Isotope ratios (δ^{15}N and δ^{13}C) were determined on the delipified and dried tissue of the fish collected in 2008 (stored samples which were previously analysed for contaminants, Webster et al., 2009, 2011a), 2009, 2011 and 2012 (Table 1). Lipid correction, either by extraction of the lipids or mathematical corrections is recommended when determining stable isotopes, particularly for δ^{13}C, as it removes the variability associated with varying lipid content (Logan et al., 2009). δ^{13}C is more depleted in lipids relative to proteins and carbohydrates, and therefore tissues with a high lipid content, such as deep sea fish liver, will have lower δ^{13}C compared to tissue with a lower lipid content.

Of all the deep sea species, δ^{15}N was significantly ($p < 0.05$) more enriched in the black scabbard liver and muscle in all four years (2008, 2009, 2011, 2012) with means ranging from 13.4‰ (muscle, 2012) to 14.9‰ (liver, 2008) (Fig. 2, Table 1). The δ^{15}N of roundnose grenadier liver and flesh ranged from 11.1‰ (liver, 2009) to 12.6‰ (flesh, 2009) and for black dogfish liver from 9.7‰ (2011) to 12.5‰ (2009). There was no significant difference in δ^{15}N between roundnose grenadier and black dogfish liver. In 2011 δ^{15}N was significantly lower in the black dogfish liver compared to all other years, almost 3‰ lower ($p < 0.05$, ANOVA, Tukey). δ^{15}N was significantly more depleted ($p < 0.05$, ANOVA, Tukey) in the prey species (2011) compared to predator (roundnose grenadier, black dogfish and black scabbard) liver and flesh with a mean value of 9.72‰ (SD = 1.6, $n = 28$) in 2011 and 9.54‰ (SD = 1.84, $n = 31$) in 2012 (Fig. 2), there was no significant difference between the prey fish, shrimp and squid.

Black scabbard liver gave mean δ^{13}C values of −19.7‰ (2008, 2011 and 2012) and −18.1‰ (2009) (Table 1). Roundnose grenadier liver gave mean values of between −23.8‰ (2008) and −20.1‰ (2012) whilst black dogfish liver gave mean values of between −23.2‰ (2011) and −18.7‰ (2009). δ^{13}C was significantly higher (less depleted) ($p < 0.05$, ANOVA, Tukey) in black scabbard liver compared to the liver of roundnose grenadier and black dogfish. There was no significant difference in δ^{13}C between the black dogfish and roundnose grenadier liver ($p < 0.05$, ANOVA, Tukey). For the flesh tissue, δ^{13}C values of between −19.0‰ (2011) and −18.3‰ (2009) were obtained for black scabbard while for the roundnose grenadier, δ^{13}C values ranged between −19.5‰ (2011) and −18.7‰ (2009). Prey species collected in 2011 and 2012 gave a mean δ^{13}C of −20.0‰ (SD = 0.95, $n = 28$) and −18.9‰ (SD = 0.58, $n = 32$), respectively (Fig. 2b).

Roundnose grenadier flesh was more enriched in δ^{13}C and δ^{15}N compared to the liver and in black scabbard δ^{13}C only was more enriched in the flesh ($p < 0.05$, ANOVA, Tukey), for black dogfish only the liver was collected (Table 1).

3.2. Total lipid content

The total lipid content of the flesh and liver of the deep water fish collected in 2009, 2011 and 2012 is presented in Table 2, along with previous years data (2006–2008, inclusive, Webster et al., 2009, 2011a) for comparison. The lipid content was significantly higher in the black dogfish and roundnose grenadier liver compared to the black scabbard liver. The average annual lipid content for the black scabbard ranged from 8.6% to 11.9% whilst for the roundnose grenadier and black dogfish the average lipid was between 51.8% and 74.7% (excluding the black dogfish data from 2006). There was no significant difference in the liver lipid content between the three most recent years (2009, 2011 and 2012) and for roundnose grenadier and black scabbard the lipid content was similar to previous years (2006–2008, Webster et al., 2009, 2011a). For black dogfish, the lipid content was consistent with that observed in 2008 but higher than in 2006. The 2006 liver samples were pooled due to the small liver size (0.4 g to 2.2 g). However, in 2008, 2009, 2011 and 2012 the liver was sufficiently large (30.4–89.65 g) for individuals to be analysed. Similar to the 2008 samples, an additional clean-up step had to be introduced for the PBDE analysis of the black dogfish collected in 2009, 2011 and 2012 due to their high squalene content (see experimental and PBDE section), this was not required in the 2006 samples due to the low lipid content and correspondingly lower squalene content (Webster et al., 2009, 2011a).

The lipid content of both black scabbard flesh and roundnose grenadier flesh was low, with one exception (2007 black scabbard, Table 2) the mean was less than 2%. In contrast to the liver, the flesh lipid content was significantly lower ($p < 0.05$, ANOVA) in roundnose grenadier compared to the black scabbard (Table 2), across all 6 years. The mean lipid content for roundnose grenadier was 0.45% (SD = 0.22%, $n = 22$) in 2009, 0.46% (SD = 0.23, $n = 7$) in 2011 and 0.34% in 2012 (SD = 0.13, $n = 12$), whilst the black scabbard flesh gave means of 1.43% (SD = 1.1%, $n = 19$), 0.91%

Table 1
Mean ± SD for the stable isotope ratios ($^{15}N/^{14}N$ and $^{13}C/^{12}C$ expressed as $\delta^{15}N$ and $\delta^{13}C$) of liver and flesh from three deep sea fish collected in 2008, 2009, 2011 and 2012. The number of samples (n) was the same for $\delta^{13}C$ as for $\delta^{15}N$.

Species	Year	$\delta^{15}N$ (‰)		$\delta^{13}C$(‰)	
		Liver	Flesh	Liver	Flesh
Black dogfish	2008	12.2 ± 0.98, n = 15		−21.9 ± 0.69	
	2009	12.5 ± 0.55, n = 5		−18.7 ± 0.91	
	2011	9.7 ± 1.5, n = 7		−23.2 ± 1.9	
	2012	11.0 ± 1.0, n = 8		−20.5 ± 1.8	
Roundnose grenadier	2008	11.5 ± 0.81, n = 10	12.5 ± 0.44, n = 7	−23.8 ± 0.70	−18.9 ± 0.68
	2009	11.1 ± 1.5, n = 24	12.6 ± 0.87, n = 27	−20.8 ± 0.80	−18.7 ± 0.47
	2011	11.5 ± 0. 78, n = 7	11.9 ± 1.0, n = 7	−22.9 ±.2.1	−19.5 ± 0.97
	2012	11.4 ± 1.1, n = 12	11.3 ± 0. 48 (n = 12)	−20.1 ± 0. 98	−18.1 ± 0.95
Black scabbard	2008	14.9 ± 0.37, n = 10	14.5 ± 0.23, n = 4	−19.7 ± 0.71	−18.8 ± 0.70
	2009	14.7 ± 0.54, n = 19	14.5 ± 0.70, n = 21	−18.1 ± 0.52	−18.3 ± 0.32
	2011	13.9 ± 0.56, n = 6	13.6 ± 0.71	−19.7 ± 1.1	−19.0 ± 0.36
	2012	13.0 ± 1.6, n = 8	13.4 ± 0. 71, n = 8	−19.7 ± 1.6	−17.3 ± 0.48

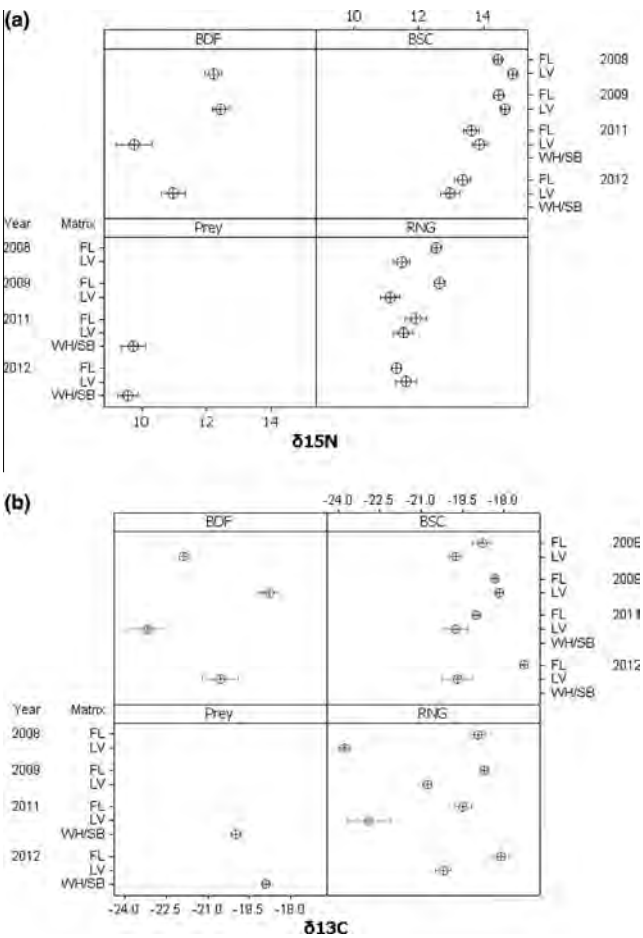

Fig. 2. Stable isotope ratios, (a) $\delta^{15}N$ (‰) and (b) $\delta^{13}C$ (‰) in the liver (LV) and flesh (FL) of black scabbard (BSC), black dogfish (BDF) and roundnose grenadier (RNG) collected in 2008, 2009, 2011 and 2012 from the Rockall fishing area to the west of Scotland. Possible prey species were collected in 2011 and 2012. Fish and shrimp samples were analysed whole (WH) whilst for squid the soft body (SB) was analysed. The mean (circle) and standard error are shown.

(SD = 0.72, n = 6) and 1.37% (SD = 1.32, n = 9) in 2009, 2011 and 2012, respectively.

3.3. Fatty acids

The flesh and liver tissue of roundnose grenadier, black scabbard, black dogfish (liver only) collected in 2008 (stored samples, samples previously analysed for contaminants and data published, Webster et al., 2009, 2011a), 2009 2011 and 2012 and prey species (whole or soft body) collected in 2011 and 2012 were analysed for fatty acids. The major fatty acids in the liver were 16:0 (>10%), 18:1(n−9) (>15%), 20:1(n−9) (>8%) and 22:1 (>5%). In the flesh 16:0 (>15%), 18:1(n−9) (>9%), 20:5 (>5%) and 22:6(n−3) (>25%) were the dominant fatty acids whilst in the prey (whole and soft body) 16:0(n−9) (>12%), 18:1(n−9) (>5%), 20:5 (>5%) and 22:6(n−3) (>25%), were the main fatty acids. These six fatty acids accounted for between 64% and 85% of the total fatty acids in all samples (Fig. 3). Mono-unsaturated fatty acids (MUFA) constituted more than half of the total fatty acids in the liver, accounting for 55%, 69% and 61% in the black scabbard, black dogfish and roundnose grenadier, respectively. The proportion of MUFAs has been reported to increase with increasing size and lipid content of fish (Stowasser et al., 2009), the black dogfish and roundnose grenadier also had a higher lipid content than black scabbard. The flesh of both predator species (black scabbard and roundnose grenadier) contained a higher proportion of polyunsaturated fatty acids (PUFAs) than the liver, accounting for an average of 50% in the roundnose grenadier flesh, 41% in the black scabbard flesh. The high proportion of PUFAs in fish flesh tissue of fish with a low flesh lipid content is not unusual as structural lipids in fish tend to have a greater proportion of PUFAs. The prey species (whole and soft body) contained a high proportion (mean of 42% for whole and soft body prey) of PUFAs. Docosahexaenoic acid (DHA, 22:6(n−3)) was the dominant PUFA, with a normalised area% of more than 20% in most predator flesh (Fig. 3). The high proportion of PUFAs in fish flesh is not unexpected as these are the main component of phospholipids, which is the predominant lipid class in flesh tissue. For the prey species EPA and DHA were the dominant PUFAs in all species except the lanternfish where 20:3 also had a significant contribution. Phytoplankton are the dominant producers of PUFA, therefore, lower trophic level organisms will have a higher proportion of PUFAs.

The predominant fatty acid in the black scabbard and black dogfish liver was 18:1(n−9), accounting for 32.0% and 28.3% of the normalised total fatty acid content, respectively (Fig. 3). A high proportion of 18:1(n−9) and a low 18:1(n−7)/18:1(n−9) ratio (<0.6) is often used as an indicator of a more carnivorous diet (Falk-Petersen et al., 2000; Dalsgaard et al., 2003 and Petursdottir et al., 2008). The 18:1(n−7)/18:1(n−9) ratio was <0.6 in all three species and was significantly lower in the black scabbard (mean = 0.15, SD = 0.06) and black dogfish (mean = 0.14, SD = 0.03) compared to the roundnose grenadier (mean = 0.34, SD = 0.09) (p < 0.05, ANOVA, Tukey). 18:1(n−9) was also one of the main fatty acids in roundnose grenadier liver, although lower than for black scabbard and black dogfish, accounting for an average of 17.1% of the fatty acid content.

Table 2
Lipid content (%) and concentrations for ΣICES7 PCBs (μg kg^{-1} lipid weight) in liver and muscle from three deep water fish species collected from Rockall during September 2009, 2011 and 2012. Previously published data for samples collected between 2006 and 2008 is also shown for comparison (Webster et al., 2011a). SD, standard deviation.

Species	Year	Range	Mean	Median	SD	Range	Mean	Median	SD
Liver lipid content (%)						*Flesh lipid content (%)*			
Black dogfish	2006	3.9–7.6 (n = 4 pools)	5.7	5.8	1.3				
	2008	55.2–88.7 (n = 15)	74.2	75.6	9.3				
	2009	59.8–74.5 (n = 5)	66.6	66.8	5.5				
	2011	69.0–80.8 (n = 7)	74.7	72.7	4.7				
	2012	69.1–77.9 (n = 8)	73.5	73.8	3.1				
Black scabbard	2006	9.9–26.5 (n = 9)	13.7	11.5	5.2	0.44–2.12 (n = 5)	1.07	0.80	0.74
	2007	5.9–23.3 (n = 10)	15.7	16.1	5.8	0.59–6.40 (n = 10)	2.26	1.35	1.96
	2008	5.3–26.1 (n = 13)	11.4	9.9	5.8	0.42–5.00 (n = 5)	1.61	1.01	1.90
	2009	4.1–16.9 (n = 19)	8.6	8.2	3.3	0.20–5.30 (n = 19)	1.43	1.20	1.10
	2011	6.5–18.1 (n = 6)	11.9	11.8	3.9	0.22–2.25 (n = 6)	0.91	0.86	0.72
	2012	5.2–18.7 (n = 8)	11.0	10.5	4.9	0.46–4.20 (n = 8)	1.37	0.66	1.32
Roundnose grenadier	2006	28.0–83.0 (n = 18)	57.8	58.3	16.5	0.39–2.70 (n = 10)	0.92	0.73	0.66
	2007	48.1–86.3 (n = 20)	73.0	72.1	8.9	0.52–4.20 (n = 20)	1.20	0.85	0.98
	2008	38.5–81.3 (n = 15)	62.9	61.9	11.8	0.34–1.51 (n = 12)	0.55	0.42	0.34
	2009	44.6–83.1 (n = 24)	61.8	61.8	9.9	0.20–1.30 (n = 24)	0.45	0.40	0.22
	2011	46.9–75.9 (n = 7)	60.3	58.7	11.3	0.26–0.90 (n = 7)	0.46	0.45	0.23
	2012	22.3–80.1 (n = 12)	51.8	55.6	16.5	0.21–0.67 (n = 12)	0.34	0.34	0.13
Liver ΣICES7 PCB concentration (μg kg^{-1} lipid weight)						*Flesh ΣICES7 PCB concentration (μg kg^{-1} lipid weight)*			
Black dogfish	2006	111–260 (n = 4) pools)	188	191	79.2				
	2008	131–935 (n = 15)	280	235	255				
	2009	284–518 (n = 5)	426	452	92.6				
	2011	227–343 (n = 7)	300	304	39.3				
	2012	227–421 (n = 8)	338	336	72.7				
Black scabbard	2006	130–760 (n = 9))	463	470	193	251–1,285 (n = 5)	583	497	405
	2007	155–606 (n = 10)	380	345	156	185–1,131 (n = 10)	599	581	298
	2008	59.3–703 (n = 13)	308	294	214	164–714 (n = 5)	447	389	213
	2009	58.7–621 (n = 19)	267	293	150	41.7–670 (n = 19)	283	281	175
	2011	148–961 (n = 6)	521	426	301	<LoD – 1966 (n = 6)	613	331	739
	2012	158–765 (n = 8)	291	200	216	<LoD – 273 (n = 8)	91.6	<LoD	116
Roundnose grenadier	2006	177–3475 (n = 18)	792	472	893	52.0–1671 (n = 10)	610	325	607
	2007	44.4–1,106 (n = 20)	284	235	235	65.4–846 (n = 20)	331	322	293
	2008	175–1,767 (n = 15)	601	534	429	<LoD – 658 (n = 12)	138	7.1	221
	2009	90.3–1,263 (n = 24)	471	314	334	<LoD – 207 (n = 24)	36.0	<LoD	67.3
	2011	77–1,621 (n = 7)	690	467	609	<LoD – 2155 (n = 7)	696	469	782
	2012	110–3587 (n = 12)	946	507	1075	<LoD – 605 (n = 12)	110	<LoD	194

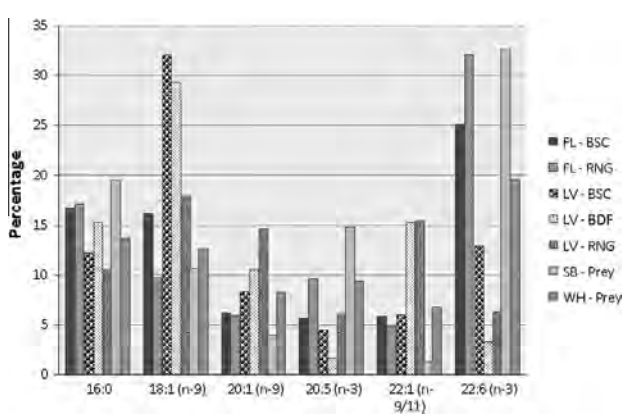

Fig. 3. Fatty acid signature for major fatty acids in predator flesh (FL) and liver (LV) of black scabbard (BSC), black dogfish (BDF) and roundnose grenadier (RNG) and of the prey species (whole, WH; SB, soft body).

The copepod markers, 20:1(n−9) and 22:1(n−9, 11)[3], gave a similar normalised area percentage in the roundnose grenadier liver, accounting for 14.2% and 15.2% of the fatty acid content. The 18:1(n−7)/18:1(n−9) ratio for the flesh of the roundnose grenadier and the black scabbard were similar to the liver, with the roundnose grenadier ratio being significantly higher (p < 0.05, ANOVA, Tukey). For the prey species (fish, shrimp and squid) 18:1(n−7)/18:1(n−9) was also significantly higher compared to the black scabbard and black dogfish with a mean of 0.24 (SD = 0.04).

The ratio of eicosapentaenoic acid (EPA, 20:5(n−3)) to docosahexaenoic acid (DHA, 22:6(n−3)) can potentially be used as an index of carnivory (Dalsgaard et al., 2003 and El-Sabaawi and Dower, 2009). DHA is conserved through the food web, therefore the ratio is lower at higher trophic levels, (Dalsgaard et al., 2003 and Petursdottir et al., 2008). EPA/DHA ratios were significantly lower (p < 0.05, ANOVA, Tukey) in the black scabbard and black dogfish liver compared to the roundnose grenadier liver with means of 0.36 (SD = 0.10, n = 42), 0.56 (SD = 0.30, n = 36) and 0.93 (SD = 0.40, n = 52), respectively. In the flesh tissue this ratio was lower than the liver due to the high proportion of 22:6(n−3) found in flesh tissue, with a mean of 0.31 (SD = 0.09) for roundnose grenadier and 0.23 (SD = 0.05) for black scabbard. EPA/DHA ratios in the prey species ranged from 0.34 to 1.34 and were highest in the squid. Squid has previously been reported to have a high% of 22:6(n−3) (Drazen et al., 2009 and Stowasser et al., 2006).

PCA showed separation in the fatty acids signatures of the liver of all three predator species, and from the prey (whole and soft body) (Fig. 4a). The prey species were more dispersed, however this included ten different species. Both the black scabbard and black dogfish had a positive factor two indicating higher proportions of MUFAs (18:1(n−9) and 24:1), 16:0 and 18:0, compared to the roundnose grenadier. The positive factor 1 of the roundnose grenadier showed that this species had higher percentages of 16:1, PUFAs (18:3, 20:3(n−3), 20:4(n−3)) and the copepod markers, 20:1 and 22:1. The majority of prey species had a negative factor 2 indicating a higher proportion of 14:0 and PUFAs (18:4, 20:5,

[3] 22:1(n−11) is the copepod marker. However, isomers were not separated during GC-FID analysis.

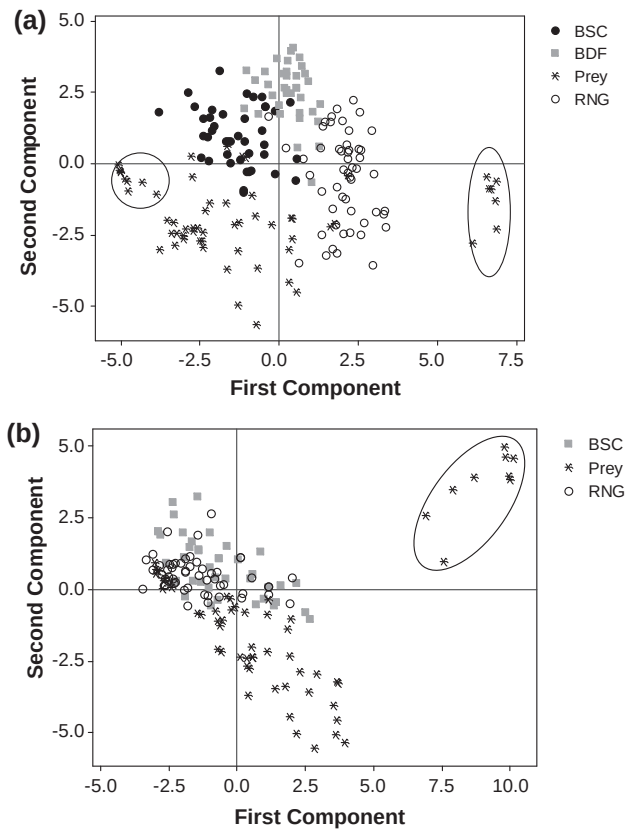

Fig. 4. PCA of fatty acids (normalised area %) in (a) liver and (b) flesh of black scabbard (BSC), black dogfish (BDF, liver only) and roundnose grenadier (RNG). Both figures include the prey species (whole tissue). Lanternfish are circled on the right of the plot and squid on the left.

22:5, and 22:6). The lanternfish were separated from the prey and predator species (but closest to the roundnose grenadier), having a highly positive factor 1, indicating a higher proportion of 16:1, PUFAs (16:3, 18:3, 18:4, 20:3($n-3$), 20:4($n-3$), 22:6) and the copepod markers, 20:1 and 22:1. Squid were also grouped together due to their negative factors 1 and 2, indicating a high proportion of PUFAs (especially 22:6), 18:0 and 16:0. PCA of the fatty acid profile of each predator species individually showed no separation either by sex or location.

PCA saw a separation between the fatty acid profiles of the flesh of the predator and prey species (whole and soft body) (Fig. 4b). The prey species had a higher proportion of PUFAs compared to the flesh of the predator species. The lanternfish were again grouped separately from the other prey species and predator flesh, due to their very high proportion of MUFAs. However, the roundnose grenadier and black scabbard flesh were not clearly separated.

3.4. Polychlorinated biphenyls (PCBs)

PCB concentrations were normalised to the lipid content (%) to take into account the different lipid content of the liver of the different species. Twenty-eight PCBs were measured including the ICES (International Council for the Exploration of the Seas) 7 PCBs. The ICES7 PCBs were recommended by the European Community Bureau of Reference; these PCBs were selected as indicators due to their relatively high concentrations in technical mixtures and their wide chlorination range. The ICES7 PCBs are the most frequently measured PCB grouping and are often used as indicators in environmental monitoring. Concentrations for the sum of the ICES7 PCBs ($\sum$ICES7 PCB) are summarised in Table 2 for the liver and flesh of each of the three species. Additional PCBs were also measured in liver and flesh due to their toxicity and occurrence in the marine environment. Concentrations for $\sum PCB_{28}$ follow the same pattern as concentrations for ΣICES7 PCBs and were approximately 2.5 times the ΣICES7 PCB concentration. PCB data for deep sea fish collected between 2006 and 2008 has previously been reported (Webster et al., 2009, 2011a). PCBs were detected in the liver of all three species collected in 2009, 2011 and 2012, and ranged from 58.7 μg kg^{-1} lipid weight for the sum of the ICES7 PCBs in black scabbard to 3587 μg kg^{-1} lipid weight in roundnose grenadier. Concentrations were mainly <500 μg kg^{-1} lipid weight (or <1250 μg kg^{-1} lipid weight for $\sum PCB_{28}$). Twenty-three of the ninety-five fish liver collected in between 2009 and 2012 inclusive gave $\sum$ICES7 PCBs concentrations >500 μg kg^{-1} lipid weight (eighteen roundnose grenadier, four black scabbard and one black dogfish). Concentrations were significantly higher in the roundnose grenadier liver ($p < 0.05$, ANOVA, Tukey). No temporal trends were detected in the PCB concentrations in the liver of the three species across all years (including previously reported data, Webster et al., 2011a) ($p > 0.05$, ANOVA) (Fig. 5). PCBs were also detected in roundnose grenadier and black scabbard flesh (Table 2). Concentrations were below the limit of detection (LoD) for a number of congeners, and for twenty out of the forty-three roundnose grenadier and three black scabbard concentrations were below the LoD for all congeners. Concentrations were not significantly different in the flesh of the two species, or from previous years ($p < 0.05$, ANOVA, Tukey). This is typical of fish species which tend to have lower lipid content in the flesh tissue. Some of the samples varied from <LoD to greater than 1000 μg kg^{-1} lipid weight with a mean >500 μg kg^{-1} lipid weight.

No increase in PCB concentration with depth was observed in the three predator species collected between 2006 and 2012. Black scabbard were collected at depths ranging between 725 m or 1260 m, roundnose grenadiers at depths between 600 m and 1800 m and black dogfish were caught at depths of 725 m or 1500 m. However, no correlation with the PCB concentration and depth in either liver or flesh was observed and there was no obvious spatial pattern in the concentrations observed across the Rockall fishing area.

A number (ten species, sixty samples) of possible prey species were collected and analysed whole (except for squid where the soft body was analysed) for PCBs. PCBs concentrations were low and were below the detection limit for all congeners in twenty of the samples. Highest concentrations were found in the lanternfish and the Bean's Bigscale, which gave mean concentrations for the ICES7 PCBs of 137 μg kg^{-1} lipid weight (SD = 62.0 μg kg^{-1} lipid weight, $n = 9$) and 106 μg kg^{-1} lipid weight (SD = 26.3 μg kg^{-1} lipid weight, $n = 3$), respectively. However, concentrations were significantly lower in all the prey species compared to the predator species ($p < 0.05$, ANOVA, Tukey).

3.5. Comparison with assessment criteria

Standards have been set by the European Union for dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs, this includes limits for fish flesh and liver (EC 1259/2011). Maximum limits for the sum of the ICES6 PCBs (CB28, 52, 101, 138, 153 and 180) are 75 μg kg^{-1} wet weight for fish flesh and 200 μg kg^{-1} wet weight for fish liver. Concentrations above this limit would render the product unsuitable for human consumption. None of the flesh samples gave concentrations greater than 75 μg kg^{-1} wet weight for the sum of the ICES6 PCBs. Therefore, there is no risk to human health from consumption of these deep water fish. However, almost half of the 2009, 2011 and 2012 roundnose grenadier (21 out of 43) and black dogfish liver samples (10 out of 20)

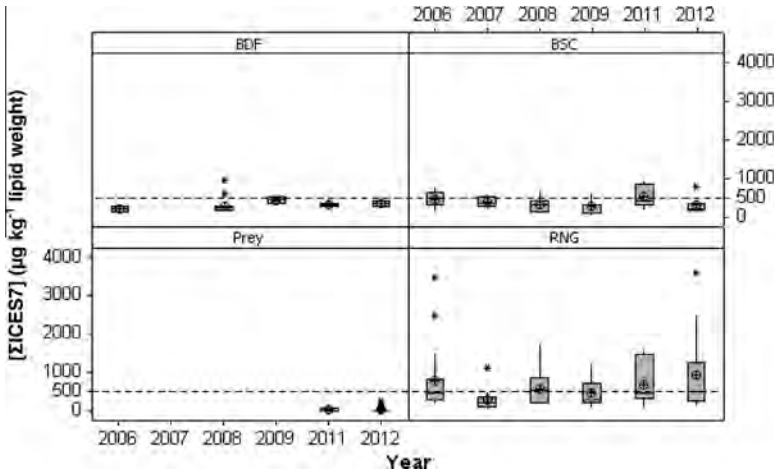

Fig. 5. Concentrations (µg kg^{-1} lipid weight) for ΣICES7 PCBs in the liver of black scabbard (BSC), black dogfish (BDF) and roundnose grenadier (RNG) collected in 2009, 2011 and 2012 from the Rockall fishing area to the west of Scotland. Previously published data from 2006 to 2008 is also shown for comparison (Webster et al., 2009 and Webster et al., 2011a). PCBs were also measured in a range of prey species (whole) collected in 2011 and 2012. Concentrations of <500 µg kg^{-1} lipid weight for the ΣICES7 PCBs have previously been found in plaice liver collected at remote, reference sites. The box represents the interquartile range, the circle is the mean concentration and asterisks are outliers.

gave concentrations for the sum of the ICES6 PCBs above 200 µg kg^{-1} wet weight. Although food safety levels were exceeded it is unlikely that the liver of these species will be for the consumer market.

The data obtained were also compared to the assessment criteria adopted by OSPAR for use in the Quality Status Report 2010 (OSPAR, 2010; Table 3). Background assessment concentrations (BACs) and Environmental Assessment Criteria (EACs) were primarily developed for the assessment of contaminant concentrations in shallow water fish such as plaice. The mean PCB concentrations in the deep water fish and approximate upper 95% confidence limit on the mean (mean + ($t_{df,0.95}$ × SE[4])) were calculated to enable comparisons to BACs (µg kg^{-1} wet weight) and the EAC (µg kg^{-1} lipid weight) to be made (Fig. 6a and b). Where PCB concentrations were below the LoD, half the LoD value was used in the calculation. Mean concentrations in all three species were above BACs for all ICES7 PCBs, with upper confidence bound concentrations in the roundnose grenadier for the more chlorinated PCBs being more than one hundred times the BAC (Fig. 6a). PCB concentrations in shallow water fish liver, even from offshore sites away from point sources, are rarely below BACs (Webster et al., 2005, 2011b).

Time averaged PCB concentrations in all three species only exceeded the EAC^{passive} for CB118 (Fig. 6b). Shallow water fish from Scottish waters were also found to exceed the EAC^{passive} for CB118, even in areas remote from industrial and urban activity and with ΣICES7 PCB concentrations <500 µg kg^{-1} lipid weight (Webster et al., 2005, 2011b). To aggregate results for PCB congeners within a traffic light assessment system, the rule that was adopted was that if 2 congeners were classed as 'red' then the overall PCB classification would be 'red' (Webster et al., 2011b). This approach, used by OSPAR in its assessment of hazardous substances in the marine environment, minimises the potential of unusual or outlying data from misleadingly influencing assessments. If the same rule was applied to deep water species, PCB concentrations would be considered to be acceptable (green) as only concentrations for CB118 above the EAC^{passive}.

3.6. PCB profiles

By examining the distribution of PCBs it can be possible to distinguish PCB sources. Uptake of PCBs *via* food is likely to result in similar absorption for the various congeners present in the food; hence the pattern will be determined by the diet (Hoekstra et al., 2003; Pastor et al., 1996; Hope et al., 1997). PCBs can be metabolised by the cytochrome P450 group of enzymes. The age, sex and species of fish can affect the extent to which PCBs are metabolised. Most of the less chlorinated PCB congeners (tri- and tetrachloro) and some of the more highly chlorinated PCBs can be metabolised by fish. Therefore, the more chlorinated PCBs (⩾6 chlorines) tend to be dominant in fish as they are less volatile, more lipophilic and more resistant to metabolic and microbial degradation. Furthermore, a number of studies of deep water fish have shown a higher proportion of the more highly chlorinated PCBs compared to shallow water species (Mormede and Davies, 2002; Storelli et al., 2004, 2007; Storelli et al., 2009).

A high proportion of the higher chlorinated PCBs was observed in the liver of all 3 species collected in 2009, 2011 and 2012 which is similar to the 2006–2008 data reported previously (Webster et al., 2009, 2011a). Based on the 28 PCBs measured, the hexa-PCBs

Table 3
Summary of OSPAR assessment criteria for PCBs in fish liver. The BAC is the first transition point (blue/green transition) while the second transition point (green/red transition) is defined by the EAC. BC, background concentration; LC, low concentration; BAC, background assessment concentration, EAC, Environmental Assessment Criteria derived from the EAC for sediment using data from passive sampling. It should be noted that the units for the BAC and EAC are different with BAC being reported on the basis of (µg kg^{-1} wet weight) and EAC on the basis of (µg kg^{-1} lipid weight).

Congener	BC/LC	Assessment criteria	
		BAC (µg kg^{-1} wet weight)	EAC (µg kg^{-1} lipid weight)
CB28	0.0/0.05	0.10	64
CB52	0.0/0.05	0.08	108
CB101	0.0/0.05	0.08	120
CB118	0.0/0.05	0.10	24
CB138	0.0/0.05	0.09	316
CB153	0.0/0.05	0.10	1600
CB180	0.0/0.05	0.11	480

[4] Standard error of the mean.

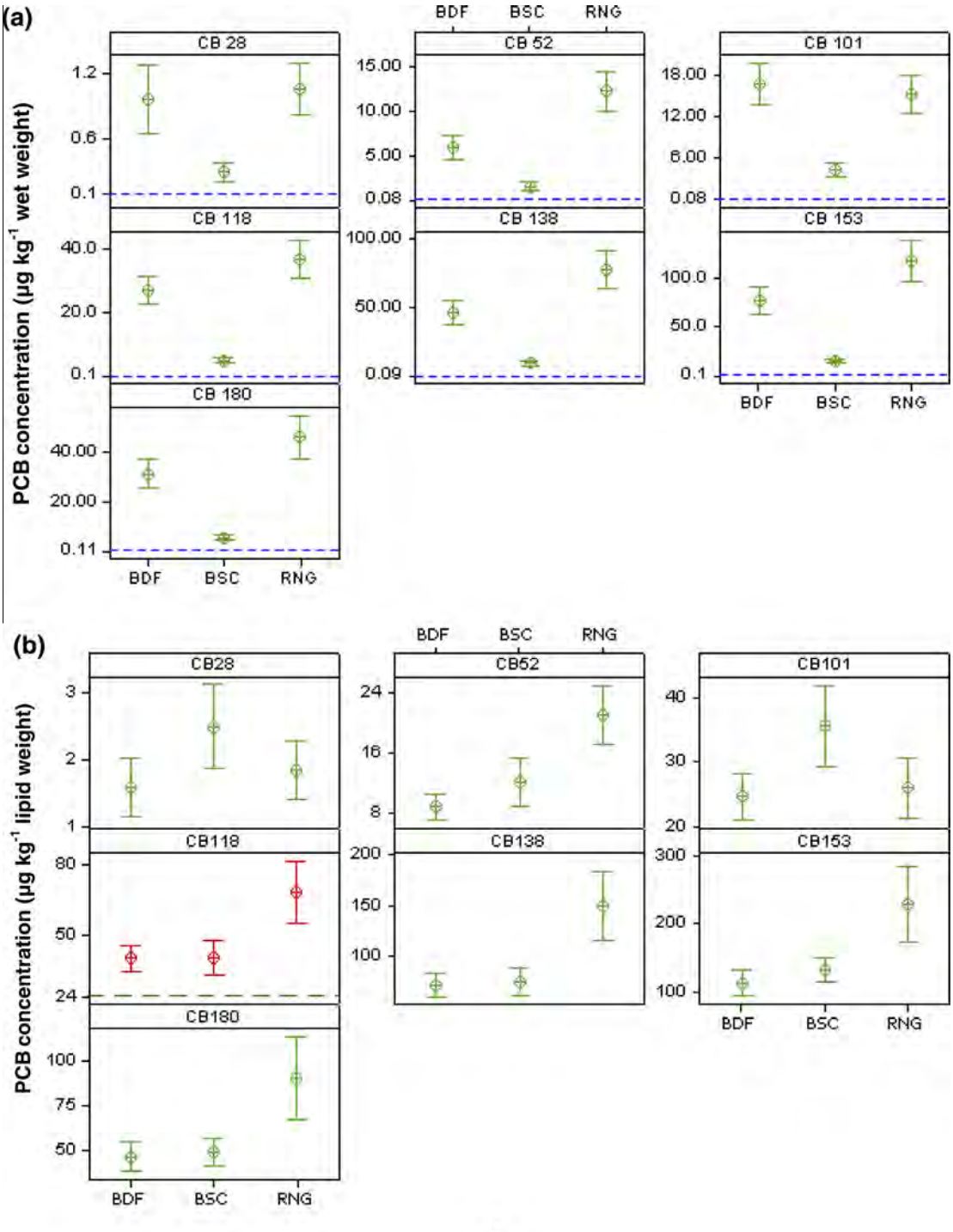

Fig. 6. Time-averaged interval plots of ICES7 PCBs in fish liver of black dogfish (BDF), black scabbard (BSC) and roundnose grenadier (RNG) collected between 2006 and 2012 (inclusive). (a) Individual congener concentrations reported as $\mu g\,kg^{-1}$ wet weight; the blue hashed line represents the OSPAR BAC and (b) individual congener concentrations reported as $\mu g\,kg^{-1}$ lipid weight; the green hashed line represents the $EAC^{passive}$ for CB118. (*Note:* the $EAC^{passive}$ was above the scale of the axis for all other congeners). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(CB132, CB137, CB149, CB138, CB153, CB128, CB156, CB157, CB158) accounted for 41–68% followed by the penta-PCBs (CB99, CB97, CB101, CB110, CB105, CB118) and hepta-PCBs (CB170, 180 and CB189) which accounted for 14–36% and 10–31%, respectively. The flesh also showed a higher proportion of the more chlorinated PCBs. However, the% composition was more variable. The greater proportion of more highly chlorinated PCBs is in agreement with other published studies on deep water fish. Mormede and Davies (2002) reported a high proportion of the hexa- and hepta-PCBs in black scabbard flesh and liver from Rockall, in this case accounting for 30–60% and 20–30%, respectively. A similarly higher proportion of more chlorinated PCBs was found in deep water fish, including black dogfish, collected from the west coast of Greenland (Berg et al., 1997). The black dogfish from this 2006 study contained 52.3% hexa-PCBs, 32.8% penta-PCBs and 9.1% hepta-PCBs (Berg et al., 1997). Storelli et al. also found the highly chlorinated PCBs

to be more abundant in deep sea fish from the Mediterranean (>50% hexa PCBs) (Storelli et al., 2004, 2007; Storelli et al., 2009).

Many of the PCBs were not detected in the prey species, but when they were present the profile was dominated by the tetra and penta-CBs. The hexa- and hepta-PCBs were detected less frequently than the lower chlorinated PCBs.

3.7. Polybrominated diphenyl ethers

Nine PBDE congeners (BDE28, BDE47, BDE66, BDE100, BDE99, BDE85, BDE154, BDE153 and BDE183) were measured in deep water fish as part of this study. PBDE concentrations (ΣPBDE9) were lower than the PCB concentrations in both liver and flesh (Table 4). Concentrations in all three predator species collected in 2009, 2011 and 2012 were low and were within the concentration range reported for the same species collected in 2006–2008 (Webster et al., 2009, 2011a), with annual means ranging from 3.6 $\mu g\ kg^{-1}$ lipid weight (black scabbard in 2009) to 38.3 $\mu g\ kg^{-1}$ lipid weight (black dogfish in 2009). Lowest concentrations were in the black scabbard, PBDEs were not detected in seventeen of the thirty-three black scabbard liver samples and concentrations were significantly lower compared to the roundnose grenadier and black dogfish ($p < 0.05$, ANOVA, Tukey). Similarly PBDE concentrations in the fish flesh were low with many congeners not being detected in both species. As seen in 2008 (Webster et al., 2011a), PBDEs were not detected in the flesh of roundnose grenadier in 2009 and 2012, PCB concentrations were also low with all congeners being below the LoD in 20 of the 36 2009 and 2012 flesh samples. There was no significant difference in the PBDE concentrations in flesh tissue between the two species.

PBDEs were significantly lower in the prey species, being below detection limits for all congeners in most samples (47 out of 60). As found for PCBs, PBDEs were detected most frequently in the lanternfish and the Bean's Bigscale, being detected in 11 out of the 13 samples; concentrations ranged from 2.2 $\mu g\ kg^{-1}$ lipid weight to 15.5 $\mu g\ kg^{-1}$ lipid weight.

Where PBDEs were detected, the PBDE profiles in both the flesh and liver were typical of other studies with BDE47, 99 and 100 dominating the profile (deWit et al., 2006; Allchin et al., 1999). BDE 183, often considered as a marker for the octa-mix PBDE formulation, was detected in only 1 (a black dogfish from 2009) of the 138 liver and flesh samples.

3.8. Discussion

$\delta^{15}N$ is more enriched at higher trophic levels with $\delta^{15}N$ reported to increase by approximately 3.8‰ with each trophic level (Hobson et al., 2002). The $\delta^{15}N$ values were highest in the black scabbard (liver and flesh), which is in agreement with their reported trophic levels (black scabbard - 4.5, black dogfish- 3.9 and roundnose grenadier- 3.5, (Froese and Pauly, 2007)). Within each species there was no significant change in $\delta^{15}N$ with fish length or water depth. However, there was a limited range of water depths and fish lengths for each species. In 2011, $\delta^{15}N$ (and also $\delta^{13}C$) was unusually low in the black dogfish, approximately 3‰ more depleted than seen in 2008 and 2009, corresponding to one trophic level. Such temporal differences can be attributed to the availability of food sources. However the fish size, lipid content and fatty acid profiles were similar to earlier years, no explanation could be found for this difference. As expected $\delta^{15}N$ was significantly lower in the prey species than the predators and more variable due to the range of species analysed.

$\delta^{13}C$ also increases up the food chain, although the differences are smaller (<1‰) and can be used to indicate if sources of primary production in the food chain are similar. The $\delta^{13}C$ of the non-lipid carbon sources (from delipified tissue) was significantly less depleted (difference of 2–3%) in black scabbard than black dogfish and roundnose grenadier liver. Although all three species occupy overlapping depth zones, the black scabbard tends to be found in shallower waters compared to the black dogfish and roundnose grenadier. Annual $\delta^{13}C$ means were highly variable in black dogfish liver and, as for $\delta^{15}N$, was unusually low in 2011. One possible explanation for this variability is that the dogfish diet varies more from year to year than the black scabbard and roundnose grenadier. The fatty acid profiles were not more variable for this species, however, the fatty acid profile is for the lipid fraction and $\delta^{13}C$ for the de-lipified tissue.

Analysis of liver provides information on short-term diet, and flesh on the long term diet. This is due to the differences in the turnover rates in the different tissues. Roundnose grenadier flesh was more enriched in $\delta^{13}C$ compared to the liver (Table 1). This perhaps indicates a difference in the short and long term diet. Stowasser et al. (2009) and Gaston and Suthers (2004) also reported higher $\delta^{13}C$ in the flesh of deep water fish compared to the liver. There was no clear explanation for this difference other than it might be a general feature of fish. However, there was no significant difference in $\delta^{13}C$ in the flesh and liver of the black scabbard, and for black dogfish only the liver was collected.

The fatty acid signatures of both prey and predator (flesh and liver) deep sea fish were typical of other fish species, containing a high proportion of 16:0, 18:1 and 22:6 (Dalsgaard et al., 2003 and Remme et al., 2006) (Fig. 3). The dominant fatty acid in both the black scabbard and black dogfish liver was 18:1(n−9). A high proportion of 18:1(n−9) has been reported as a characteristic of deep-sea organisms; 18:1(n−9) is the dominant fatty acids in carnivorous copepods (Remme et al., 2006). PCA showed there were differences in the fatty acid profiles of the liver of all three predator species. However, the fatty acid profiles and ratios of the black scabbard and black dogfish were most similar. The two ratios (18:1(n−7)/18:1(n−9) and EPA/DHA) assessed indicated that the black scabbard and black dogfish were at a higher trophic level and had a more piscivorous diet compared to the roundnose grenadier. Both ratios were significantly correlated with each other and negatively correlated with $\delta^{15}N$ ($p < 0.01$), indicating that the lower the ratio the higher the trophic position. El-Sabaawi and Dower also observed a significant correlation with fatty acid trophic markers (DHA/EPA and 18:1(n−9)/18:1(n−7)) and $\delta^{15}N$ in copepods. The roundnose grenadier liver also contained a high percentage (mean ~15%, Fig. 2) of MUFAs 20:1 and 22:1. These fatty acids reported to be biosynthesized by herbivorous, wax-ester storing/diapausing copepods and are therefore used as markers for *Calanus* copepods or fish that consume them (Dalsgaard et al., 2003 and Petursdottir et al., 2008). This is consistent with published studies on stomach content analysis which showed roundnose grenadier stomachs to contain copepods.

The fatty acid profile of the black scabbard and roundnose grenadier flesh tissue showed a higher proportion of 22:6(n−3) and a lower proportion of 18:1(n−9) compared to the liver tissue. The high proportion of 22:6(n−3) is typical of fish flesh tissue where there is less storage lipid and a greater proportion of structural lipid. Although the ratios in the flesh of the black scabbard and roundnose grenadier were different from the liver, particularly for EPA/DHA, they showed a similar pattern and again supported the higher trophic level of the black scabbard compared to roundnose grenadier.

Due to the wider range of possible prey species analysed (ten different species including fish, shrimp and squid) the fatty acid signatures and ratios were more variable. The lanternfish had a distinctive fatty acid profile and were well separated from all species on the PCA plots. This was due to the high proportion of MUFAs (mean = 58%), including the copepod markers (20:1 and 22:1) compared to the other prey species. Squid gave a distinctive

Table 4
Concentration for the sum of 9 PBDE congeners (μg kg^{-1} lipid weight) in fish liver and flesh from three deep water fish species collected from Rockall during September 2009, 2011 and 2012. Previously published data for samples collected between 2006 and 2008 is also shown for comparison (Webster et al., 2011a). SD, standard deviation.

Species	Year	Range	Mean	Median	SD
Liver					
Black dogfish	2006	2.0–14.6 (n = 5)	8.8	11.4	5.5
	2008	10.3–110 (n = 15)	29.7	22.6	25.1
	2009	26.0–50.1 (n = 5)	38.3	39.3	8.6
	2011	16.6–38.4 (n = 7)	26.3	26.6	7.0
	2012	<LoD – 28.2 (n = 8)	13.8	11.9	10.0
Black scabbard	2006	3.7–51.4 (n = 10)	25.5	23.4	17.7
	2007	13.7–40.0 (n = 10)	25.8	26.6	8.2
	2008	<LoD – 21.5 (n = 13)	9.1	9.1	6.6
	2009	<LoD – 13.0 (n = 19)	3.6	<LoD	4.6
	2011	5.5–45.3 (n = 6)	17.0	9.5	15.3
	2012	<LoD – 10.7 (n = 8)	2.4	<LoD	3.9
Roundnose grenadier	2006	3.4–152 (n = 18)	46.0	39.4	36.0
	2007	3.8–59.8 (n = 20)	21.1	11.6	16.9
	2008	7.1–55.6 (n = 15)	21.0	14.9	13.2
	2009	6.5–58.6 (n = 24)	20.6	17.2	12.4
	2011	3.3–60.7 (n = 7)	26.8	19.6	20.2
	2012	6.5–76.9 (n = 12)	30.3	18.9	25.7
Flesh					
Black scabbard	2006	<LoD – 60.0 (n = 5)	30.5	29.3	28.4
	2007	15.0–99.0 (n = 10)	42.5	34.1	26.4
	2008	<LoD – 40.5 (n = 5)	10.7	<LoD	17.6
	2009	<LoD – 23.9 (n = 19)	5.7	1.9	7.6
	2011	<LoD – 46.0 (n = 6)	11.0	<LoD	18.9
	2012	<LoD – 8.5 (n = 8)	1.1	<LoD	3.0
Roundnose grenadier	2,006	<LoD – 217 (n = 9) 911)	33.6	39.4	36.0
	2007	<LoD – 26.7 (n = 12)	11.6	11.6	7.4
	2008	<LoD (n = 12)			
	2009	<LoD (n = 24)			
	2011	<LoD – 6.7 (n = 7)	1.0	<LoD	2.52
	2012	<LoD (n = 12)			

profile containing a high proportion (>30%) of 22:6 and therefore a low EPA/DHA ratio. Squid fed on fish have been reported to have high levels of 22:6 compared to those fed on crustaceans (Stowasser et al., 2006).

PCBs were detected in the liver and flesh of all three predator species collected in 2009, 2011 and 2012. Concentrations were below the detection limit more frequently in the flesh than in the liver. Compared to earlier years (2006–2008, Webster et al., 2011a) there has been little change in the PCB concentrations. The concentrations of PCBs were significantly higher in the liver of roundnose grenadier compared to the black scabbard and black dogfish. Previous work has shown that concentrations for ∑ICES7 PCBs in the liver of shallow water species from Scottish sites with limited industrial and urban activity are <500 μg kg^{-1} lipid weight. Concentrations in shallow water species from industrialised areas are normally >500 μg kg^{-1} lipid weight (Webster et al., 2005, 2011b), as such the value of 500 μg kg^{-1} lipid weight in liver has been taken as a transition point between typical background values and those greater than background. Concentration for ∑ICES7 PCBs were mostly <500 μg kg^{-1} lipid weight, with just twenty-three of the ninety-five fish liver (mainly roundnose grenadier) exceeding this concentration. It should be noted that in some instances, the concentration were into the thousands. There was, however, considerable variability and this may be due to the differences in age and diet. Clearly, there are some values which are above what might be regarded as typical background values. Other parameters have been used to assess PCB concentrations. For example, OSPAR has developed Background Assessment Concentrations and Environmental Assessment Criteria. Using these assessment tools it is apparent that PCB concentrations in all three species of deep water fish were typically above BACs, but as only CB118 exceeded the EAC concentrations would be classed as acceptable. Another tool is the EC maximum food levels for the ICES6 PCBs reported on a wet weight basis. These were exceeded in the liver of black dogfish and roundnose grenadier, but were below the EC maximum food level for flesh. The lipid content of the liver of both species is very high (means of 51.8% - 74.7%), therefore concentrations on a wet weight basis are higher than for other species with a lower lipid content. However, the liver tissue is not for the consumer market therefore there is no human health risk. Both the stable isotope ratios and FATM indicate that roundnose grenadier are at the lowest trophic level compared to black scabbard and black dogfish, therefore the higher concentrations in the liver are more likely due to the longer life span of this species. Furthermore the black scabbard collected from the deep waters to the west of Scotland are likely to be immature (<7 years). As such concentrations of POPs will be lower than in older fish.

PBDEs were detected in the roundnose grenadier, black scabbard and black dogfish liver but only occasionally in the flesh. Total PBDE concentrations were lower than for PCBs, many congeners were below the detection limits, but where they were detected, BDE47 was the major congener, which is typical of many other studies. The lower PBDE concentrations found reflects the fact that PBDEs were in use for a shorter time period, and were used in smaller volumes, compared to PCBs. In addition, PBDEs are less volatile than PCBs and will therefore have a lower potential to undergo long-range atmospheric transport. PBDE concentrations were significantly lower in the black scabbard liver compared to concentrations in the liver of the roundnose grenadier and black dogfish. Similar to the PCBs, PBDE concentrations were lower than found in shallow water species from more industrialised areas in Scotland such as the Clyde, where mean ΣPBDE9 concentrations in plaice liver ranged from 50.1 μg kg^{-1} lipid weight to 430 μg kg^{-1} lipid weight (Webster et al., 2011b). Currently there is only very limited published data on PBDEs in deep water fish. Ten different species of deep water fish (whole fish) from the Sulu Sea, western Pacific, collected at depths of up to 1,015 m were analysed for organohalogen contaminants, including PBDEs (Ramu et al.,

2006). Concentrations of all contaminants were very low with PBDE concentrations (sum of 14 congeners) ranging from 0.85 to 2.1 μg kg^{-1} lipid weight. In another study, PBDE concentrations reported in the liver of two deep sea fish species (hollowsnout grenadier and roughsnout grenadier) from the Mediterranean (Covaci et al., 2008) were similar to those found in the Scottish deep water fish from this study. Concentrations for the sum of 8 congeners (BDE 28, **49**, 47, 66, 100, 99, 154, 153 (bold number not determined in this study, but additionally this study analyses for BDE 85, 183) ranged from 11.8 to 27.3 μg kg^{-1} lipid weight ($n = 6$) in hollowsnout grenadier and from 3.2 to 7.0 μg kg^{-1} lipid weight ($n = 9$) in roughsnout grenadier. The lack of any temporal or spatial trends indicates that PCBs and, to a lesser extent, PBDEs, are widely distributed in deep waters to the west of Scotland. The most likely source of PCBs and PBDEs to the deep water trophic structure is from long range atmospheric transport.

PCB and PBDE concentrations were significantly lower in the prey species, often below detection limits, particularly for PBDEs. Of the ten prey species sampled in 2011 and 2012 highest PCB and PBDE concentrations were found in the lanternfish *(L. macdonaldi*) and the Bean's Bigscale (*S. beanie*), although still significantly lower than found in the predator species. The lanternfish and Bean's Bigscale are long lived compared to other prey species, with a life span of approximately 10 years. The proportion of the more highly chlorinated PCBs (hexa- and hepta-PCBs) was less than observed in the predator species. The more highly chlorinated PCBs are more resistant to metabolic and microbial degradation, therefore you might expect there to be a greater proportion in the longer-lived species.

4. Conclusions

Stable isotope and fatty acid analysis of prey and predator species was used to show differences in diet and trophic levels of both predator and possible prey species found in the deep waters to the west of Scotland. From the stable isotope analysis, the black scabbard were found to feed at the highest trophic level than the other two species investigated. Differences were observed in the fatty acid profiles of both prey and predator species, however the profiles were most similar for the black scabbard and black dogfish liver and indicated that these species were at a higher trophic level and had a more piscivorous diet compared to the roundnose grenadier and prey species. PCBs and, to a lesser extent, PBDEs were detected in the predator deep sea fish species. The most likely primary source of contaminants in this area is atmospheric deposition. The highest concentrations were found in the longest-lived species, roundnose grenadier. There was no correlation between the contaminant concentrations and the depths where the samples were collected. PCB and PBDE concentrations were significantly lower in the prey species, often below detection limits, but were detected most frequently in prey with the longest lifespan (lanternfish and Bean's Bigscale). Concentrations of the PCBs in all three species of deep water fish were typically above BACs, but only CB118 exceeded the EAC. PCB (sum of ICES6) concentrations in the deep sea fish flesh were below the EC maximum food level, therefore, consumption of the flesh from these deep water fish is unlikely to represent a risk to human health.

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