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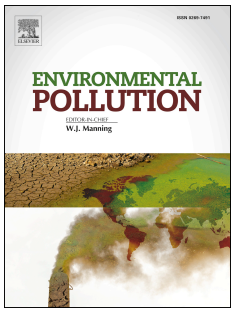


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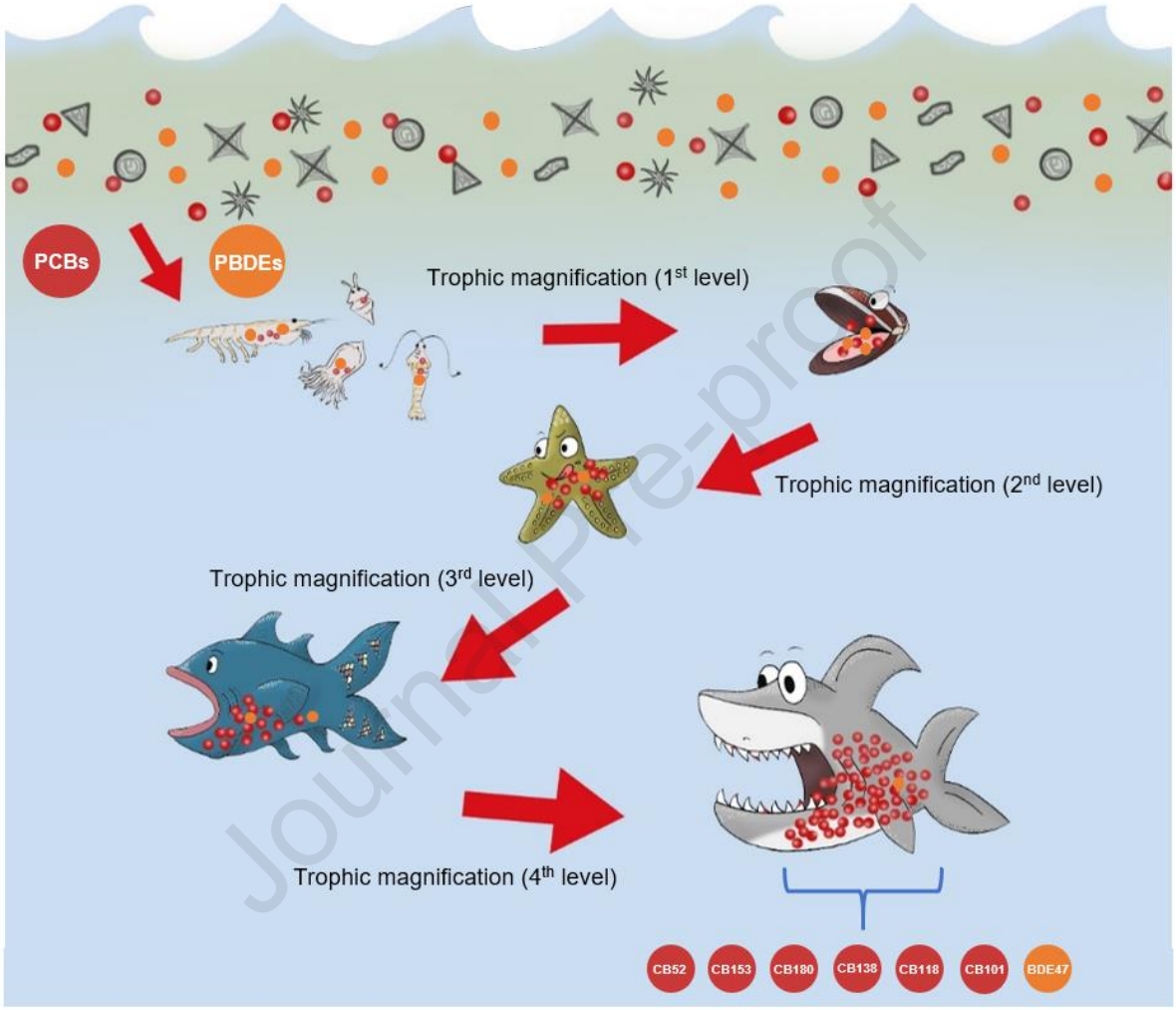
# Graphical abstract



# The concentration and biomagnification of PCBs and PBDEs across four trophic levels in a marine food web

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## Abstract

Contracting Parties to the OSPAR Convention for the Protection of the Maine Environment of the North-East Atlantic are required to undertake monitoring and assessment of both inorganic and organic contaminants. There is a requirement to assess contaminants across different trophic levels on an ecosystem-specific basis. However, this is currently constrained by the availability of relevant samples to cover the full range of trophic levels. This study investigates the variability (inter- and intra- species variation) of the concentrations and distributions of thirty-two polychlorinated biphenyl (PCB) congeners and nine polybrominated diphenyl ether (PBDE) congeners in twenty-six species covering four trophic levels from different geographic locations around Scotland. Trophic magnification factors (TMFs) were calculated using a traditional method and a balanced method for both the ICES-7 PCBs and BDE47, to refine and improve the application of TMFs to assess and predict biomagnification risk to biota in the marine environment. There were clear differences in congener percentage distribution between sample categories and species, with differences influenced by physiological processes and eco-biological parameters. Trophic magnification was found to occur for the ICES-7 PCBs and BDE47 using the traditional method, with the highest degree of trophic magnification reported for CB52. An unbalanced dataset was found to influence the calculated TMF and in some cases, the overall conclusion of the trophic transfer of PCB and PBDE congeners. The balanced method is highly recommended for calculating TMFs to ensure that the TMF is a true indication of the biomagnification potential, particularly when conducting regional comparisons for which sampling requirements are difficult to achieve.

## Key words

Biomagnification, Assessment, Scotland, Contaminants, Persistent organic pollutants, Trophic magnification factor

# Introduction

Persistent organic pollutants (POPs) represent a large category of heterogeneous organic compounds including polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs). PCBs and PBDEs are ubiquitous environmental contaminants and are classified as POPs by the Stockholm Convention due to their persistence, bioaccumulation in the environment and toxicity to humans and wildlife (Kaw and Kannan, 2017; Stockholm Convention, 2019). Both PCBs and PBDEs are included on the OSPAR list of chemicals for priority action (OSPAR Commission, 2019).

PCBs were extensively used as heat exchange fluids in a wide range of electric and electronic devices including transformers and capacitors (Lavandier *et al*., 2019). PBDEs were commercially available in three technical mixtures; penta-, octa- and deca- BDEs and were widely used in numerous polymer-based commercial and household products such as textiles, furniture and electronics as fire retardants (Shaw and Kannan, 2009; Chang *et al*., 2020).

Production of PCBs, which began in 1928 (OSPAR Commission, 2019), was banned in United States in 1979, in the United Kingdom in 1981, and in the rest of the European Union in 1987 (NOAA, 2021). PBDEs were first produced commercially in the 1970s (CDC, 2017). Octa- and penta-PBDE mixtures were banned in 2004 whilst deca-BDE was phased out of production by 2013. Although production and use of PCBs and PBDEs are now banned, they continue to enter the marine environment by leaching from landfill sites (electrical waste and furniture), industrial wastewaters and as waste incineration by-products through mechanisms such as direct spillage or discharge, atmospheric transport (wet and dry deposition), re-suspension of sediments during storms and diffusive air–water exchange (Del Vento and Dachs, 2007; Ma *et al*, 2018; Chakraborty *et al*, 2022; Luarte *et al*, 2022).

The bioaccumulative nature of many organic contaminants and their transfer to high trophic level organisms has received substantial attention (Cresson *et al*, 2016; Corsolini and Sarà, 2017; An *et al*, 2020; Yu *et al*, 2020; Won *et al*., 2020; Xie *et al*, 2020; Guo *et al*, 2021). PCBs and PBDEs reach their highest concentrations in marine mammals, which in many cases, have a lower capacity to metabolise organohalogen compounds compared to terrestrial mammals, although this is species dependent (Krahn *et al*., 2009; Jepson *et al*., 2016). Toxic effects of organohalogen compounds are also known to occur in lower trophic level organisms. For example, a study by Feng *et al*., (2019) found that Chinese mitten crabs fed a PCB supplemented diet had significantly lower weight gain than those fed a control diet (without PCB supplementation).

To achieve the United Kingdom Marine Strategy vision of “good environmental status”, with clean, healthy, safe, productive and biologically diverse oceans and seas, the sources and pathways of contaminants to the ocean, their concentrations and biological effects in the marine environment must be monitored and assessed (UKMMAS, 2022). The Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) uses two assessment criteria, based on the concentrations of seven PCB congeners (the ICES 7 PCBs (ICES, 2013)), to assess the consequences of the varying concentration of PCBs in biota: Background Assessment Concentration (BAC) and Environmental Assessment Criteria (EAC) (OSPAR, 2014). Concentrations below BACs represent measured concentrations that are near background levels for naturally occurring substances and close to zero for synthetic substances such as PCBs and PBDEs (Moffat *et al*., 2020). EACs represent the contaminant concentration in the environment below which no chronic effects are expected to occur in marine species (OSPAR, 2009). Currently, there are no EACs available for the assessment of PBDEs in sediment or biota (OSPAR, 2020a). Alternative assessment criteria that could be used for PBDE status assessments are the Canadian Federal Environmental Quality Guidelines (FEQGs) for sediment and biota (OSPAR, 2020b).

In more recent years, there has been an increasing interest in ‘ecosystem-based assessments’ (Marine Scotland, 2020; Moffat *et al*, 2020 and ICES, 2022). This requires determination of concentration of contaminants at different trophic levels. However, obtaining relevant samples for analyses is a challenge in marine systems. Trophic magnification factors (TMFs) are useful in characterising the bioaccumulation potential of a chemical and are increasingly used to quantify biomagnification and represent the average diet-to-consumer transfer of a chemical through food webs (Borgå *et al*., 2012; An *et al*, 2020; Wang *et al*, 2021). However, the selection of a TMF for a given substance is critical, due to the variability existing within ecosystems (factors relating to geographic region, physiology and metabolism, etc). In order to apply TMFs and investigate whether the main driver of bioaccumulation is trophic level or not, the cause of variability within sample categories (inter- and intra- species variation) on an ecosystem-basis must be established to determine the reliability of the calculated TMF (Mcleod *et al*, 2014; Madgett *et al*, 2019). Unlike essential metals and metalloids, there are no bodily requirements for organic contaminants and a large proportion of body burden will more likely be a direct result of trophic transfer rather than exposure (Gupta *et al*, 2018).

It is well established that factors such as sex, tissue type, reproductive status, metabolism, geographic location and feeding ecology influence the PCB (Filmann *et al*., 2007; Jepson *et al*., 2016; Williams *et al*., 2020) and PBDE (Weijs *et al*., 2008; Rotander *et al*., 2012) profiles of marine species. For example, different marine mammal species and even different

cetaceans are able to more readily detoxify certain PCB congeners. Those that are less able to are more vulnerable to accumulation, particularly to the dioxin-type PCB congeners including CBs 77, 81, 126 and 169 (Boon, 1992; Boon, 1997; Evans, 2011; Mendez-Fernandez *et al*., 2017). This variability in metabolic capacity associated with different marine mammal species will influence body burden levels and the concentrations of individual contaminants, with some being metabolizable and others being metabolically stable (Boon *et al*, 1997; Williams *et al*, 2020). This will in turn influence the calculated TMF of the associated congener. Fish and invertebrates, covering a range of trophic positions, have also been found to accumulate PCBs and PBDEs, where body burden can be driven by dietary absorption, tissue type, geographic location, metabolic capacity and maturation state across different species (Buckman *et al*., 2006; Johnson *et al*., 2007; Szlinder-Richert, 2009; Tian, Zhu and Liu, 2010; Zhang *et al*., 2016).

In this study, we examine the variability of concentrations (inter- and intra- species variation) of thirty-two PCB congeners and nine PBDE congeners. Biomagnification or otherwise of these chemicals was then investigated in the specific food web being studied. This was followed by consideration of whether or not the application of TMFs to describe biomagnification is appropriate for a consistent, trophic specific biota assessment.

To investigate this, samples of marine biota were divided into nineteen sample categories (refer to Madgett *et al.*, (2019) for the categorisation of twenty-six species using fatty acid (FA) and stable isotope ratio analysis (SI)). The samples were collected from four biogeographic regions around Scotland, United Kingdom and were used to investigate the relationship between PCB and PBDE concentrations and key influencing factors on accumulation (trophic level, region, sample categorisation and physiological features). TMFs were calculated using both traditional and balanced methods, as described in Borgå *et al*., (2012); Brisebois, (2013); and Madgett *et al* (2021).

# Experimental Procedure and Data Analysis

## Sample Collection and Preparation

Sample collection and preparation is discussed in detail in Madgett *et al*., (2019, 2021). In summary, 211 samples, covering seven fish species (haddock, whiting, hake, plaice, dab, herring and sprat), one shark species (small-spotted catshark) and thirteen invertebrate species (horse mussel, brittle star, hermit crab, edible crab, common starfish, swimming crab, shore crab, European lobster, *Nephrops*, whelk, sea mouse, squat lobster and veined squid) were collected from nine locations, covering four biogeographic regions around Scotland, United Kingdom between 2015 and 2017 during December and February (Figure 1).

Sample preparation resulted in five tissue types (whole animal, muscle, liver, soft body and brown meat). Further sampling information describing the sampling locations, species collected, number of individuals collected per species, number of individuals per pool and sample matrices is presented in Table 1. Further information on the treatment of specific species can be found in Madgett *et al*. (2019).

*Calanus* spp. and *Pseudocalanus* spp. were collected from a site 3 nautical miles east of Stonehaven on the east coast of Scotland (Figure 1) in 2018. A 1 m ring net, with a 350 µm mesh and a non-filtering cod end was used to minimise damage to the animals which were stored on the deck in 15 L plastic buckets, out of the wind and sunlight, until arrival at the Marine Laboratory. The target herbivorous species were isolated using a Zeiss Stemi-11 stereomicroscope and stored at -20˚C (Madgett *et al*., 2019).

In addition to the samples described above, blubber from three marine mammal species was collected by the Scottish Marine Animal Strandings Scheme (SMASS; Institute of Biodiversity Animal Health & Comparative Medicine, University of Glasgow) from eight locations (green circles, Figure 1) between 2012 and 2016. Sperm whale (number of individuals = 5), harbour seal (number of individuals = 10) and harbour porpoise (number of individuals = 18) were selected due to their differing diets and metabolic capabilities. Blubber and skin samples taken just cranial to the dorsal fin were separated, wrapped in food-grade aluminium foil and stored at -20°C. Individuals were obtained from different regions and varied in age and decomposition state (Madgett *et al*., 2019).

## Lipid determination

The total lipid content of samples was determined according to the method of Smedes (1999) as described in Webster *et al*., (2011a &b). Briefly, the biota sample 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) added and the mixture homogenised again. The solvent layer was collected and a second extraction of the aqueous layer was carried out with 13% (v/v) *iso*-propanol in cyclohexane. The two (organic) extracts were combined, and the solvent removed by rotary evaporation before the residue was dried in an oven at 80˚C (± 5 ˚C) for one hour. The weight of residue was determined, and the lipid content calculated.

## Determination of PCB and PBDE concentrations in marine biota

The determination of PCBs and PBDEs, summarised below, was carried out as reported in Méndez-*Fernandez et al*., (2017) and Webster *et al*., (2011a and b).

*Pressurised liquid extraction (PLE) of the samples*

Tissue samples, whole body samples or blubber were extracted by PLE in an Accelerated Solvent Extraction (ASE) 300 system, (Dionex Ltd., Camberley, Surrey, UK) using compressed nitrogen. The $^{13}$C labelled PCB internal standard mix) was added to all samples (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) together with the PBDE internal standard (fluoro-BDE160) prior to PLE. The extraction solvent was *iso*-hexane.

*Clean-up of the extract*

Once the samples had been prepared and extracted by PLE, the extract was split in two, one half for PCB analysis and the other for PBDE analysis. A silica column clean-up (3 g silica and a 60-micron mesh size) was performed to separate the PCBs from any organochlorine pesticides (OCPs) that might have been present. The first eluted fraction (volume determined previously by a split test) was collected for analysis. The remaining fraction that might have contain OCPs was discarded. For PBDEs, the entire eluant from the silica column was collected for analysis.

The extracts were reduced to 0.5 ± 0.2 mL using a Syncore and transferred, with washings, to a GC amber glass vial with insert.

*Quantification of PCBs by gas chromatography–electron impact mass spectrometry (GC–EIMS)*

The concentration and composition of thirty-two PCB congeners: CB28, CB31, CB52, CB49, CB44, CB74, CB70, CB101, CB99, CB97, CB110, CB123, CB118, CB105, CB114, CB149, CB153, CB132, CB137, CB138, CB158, CB128, CB156, CB167, CB157, CB187, CB183, CB180, CB170, CB189, CB194, CB209 were determined using a Hewlett Packard 5975B GC-MS in electron impact (EI) mode, fitted with a 50 m x 0.22 mm HT-8 column and on-column injector (SGE, Milton Keynes, UK) as detailed in Méndez-Fernandez *et al*., (2017) and Webster *et al*., (2011a and b).

The MS was set for selective ion monitoring (SIM) with a dwell time of 50 ms. Calibration standards containing all thirty-two PCB congeners were analysed relative to $^{13}C$ labelled PCB internal standards, covering the concentration range of 0.6–500 ng/mL. Correlation coefficients of at least 0.99 were achieved for all PCBs.

*Quantification of PBDEs by gas chromatography–electron capture negative ionisation mass spectrometry (GC–ECNIMS)*

The concentration and composition of nine PBDE congeners: BDE28, BDE47, BDE66, BDE100, BDE99, BDE85, BDE154, BDE153 and BDE183 were analysed using a fluorinated PBDE internal standard on an HP6890 Series GC interfaced with a 5973 MSD in chemical ionisation mode. The GC was fitted with a Restek RTX1614 column (15 m x 0.25 mm i.d., 0.10 µm film thickness: Thames Restek, Buckinghamshire) with an automated cool on-column injector (HP7673 auto injector). Seven calibration standards, with nominal concentrations ranging from 0.2 to 500 ng/mL were run with each batch of samples and a new calibration curve constructed for each batch. Correlation coefficients of at least 0.99 were achieved.

## Trophic magnification factor calculation

Trophic magnification factors were calculated as outlined in Madgett *et al.,* (2021), based on linear regressions of log-transformed concentrations versus trophic level, which were previously determined from $\delta^{15}N$ for the species under analysis as detailed in Madgett *et al.,* (2019).

## Quality Control

Analyses at Marine Scotland Science were conducted within a laboratory accredited to ISO-17025 by the UK Accreditation Service (UKAS). All analytical batches included the analysis of blanks and a laboratory reference material (LRM; cod liver oil), with the results recorded on Shewhart control charts. Warning and control limits were set at two- and three-times standard deviation respectively. Limits of detection (LoDs) and Limits of Quantification (LoQ) were determined through the repeat analysis of a low spiked sample and the LoD calculated as 4.65 x standard deviation (SD) of the mean concentration and the LoQ 10 x standard deviation (SD) of the mean concentration. LoDs and LoQs were dependent on the sample size used in the extraction and therefore were higher for liver and blubber samples, where a smaller sample size was extracted. The LoDs for PCBs ranged from 0.05 µg/kg to 1.34 µg/kg in fish liver, 0.03 µg/kg to 0.33 µg/kg in fish muscle and 0.043 µg/kg to 0.28 µg/kg in shellfish (covering the blubber and zooplankton). The LoQs for PCBs ranged from 0.12 µg/kg to 2.33 µg/kg in fish liver, 0.07 µg/kg to 0.50 µg/kg in fish muscle and 0.03 µg/kg to 0.61 µg/kg in shellfish. The LoDs for PBDEs ranged from 0.12 µg/kg to 0.34 µg/kg in fish liver, 0.01 µg/kg to 0.06 µg/kg in fish muscle and 0.01 µg/kg – 0.16 µg/kg in shellfish. The LoQs for PBDEs ranged from 0.25 µg/kg to 0.73 µg/kg in fish liver, 0.01 µg/kg to 0.13 µg/kg in fish muscle and 0.02 µg/kg to 0.13 µg/kg in shellfish. The replicate analysis of standards on separate days gave coefficient of variation (CV%) of ~3% for PCBs and PBDEs analysed by GC–MS. Recoveries of greater than 75% were achieved for PCB and PBDE spiked biota and CRMs. External quality assurance was confirmed through successful participation in the Quality Assurance of Information on Marine Environmental Monitoring in Europe (QUASIMEME) proficiency testing scheme

## Data analysis

Statistical analysis was undertaken on Minitab 17®. The normality of the data distribution for PCB and PBDE concentrations were examined using the Ryan-Joiner test and data logarithmically transformed where appropriate. Analysis of Variance (ANOVA) at the 95% confidence level, with Tukey's pair-wise comparisons was carried out to establish significant differences in logarithmically transformed PCB and PBDE concentrations (µg/kg lipid weight (lw)) between species, categories and regions. Principal component analysis (PCA) was applied to PCB concentrations normalised to the concentration of CB153 to remove the variance associated with differences in absolute values of concentration between samples and produce relative contaminant patterns (Méndez-Fernandez *et al.*, 2017). CB153 was selected due to its resistance to biotransformation and dominance in aquatic PCB profiles

(Bodin *et al*, 2008; Batang *et al*, 2016; Weijs *et al*, 2020a; Romanić *et al*, 2021). PCA was used in R Studio (version 3.6.2) to investigate variations in PCB patterns. Pearson's correlation was used to measure the linear correlation between PCB and PBDE concentrations with potential influencing variables such as age, length and weight. Microsoft Office Excel was used to create bar charts for PCB and PBDE congener proportions and concentrations and regional comparisons and plotting the $Log_{10}$ [PCB/PBDE concentration] against trophic level (traditional and balanced methods). Values <LoD were not included in the analysis.

# Results and Discussion

## Some general considerations about the individual congener concentrations and of specific congener grouping concentrations for both PCBs and PBDEs

The lipid content (%) and concentration range (µg/kg lipid weight) of the ΣICES-7 PCBs, $\Sigma PCB_{32}$ and $\Sigma PBDE_{9}$ in the muscle, liver, homogenised whole, brown meat, soft body and blubber samples from eighteen of the nineteen sample categories (PCB and PBDE concentrations in the zooplankton were below the LoD) are presented in Table 2. Individual congener concentrations are shown in Tables S.1 (PCBs) and S.2 (PBDEs). PCB and PBDE concentrations were normalised to the lipid content (%) to account for the different lipid content of the various tissues studied and are therefore presented on a lipid weight (lw) basis. Tissue-specific differences in PCB concentrations have been observed due to the lipophilic nature of POPs, where the higher the lipid content the higher the absolute organic pollutant concentration (Lema *et al*., 2007; Lavandier *et al*., 2013; Brázová, Hanzelová and Šalamún, 2015).

The concentration of $\Sigma PCB_{32}$ across the reported sample categories ranged from <LoD (when all congeners were less than the congener specific LoD) in demersal invertebrates to 139,800 µg/kg lw in harbour seal blubber (Table 2). The recalcitrant, metabolically stable CB153 was the most abundant congener in the sample categories, with only demersal invertebrates muscle samples and flatfish muscle samples showing mean concentrations <LoD for all samples (which for CB153 was 0.22 µg/kg lw for fish muscle and 0.07 µg/kg lw for shellfish, Table S.1). CB153 is a well-studied congener, generally exhibiting the highest concentration in marine biota (Pérez-Fernández, Viñas and Besada, 2019).

The maximum concentration of $\Sigma PBDE_9$ across eighteen of the nineteen sample categories (concentrations were <LoD for all nine congeners for zooplankton) was 1,888 µg/kg lw detected in sperm whale blubber (Table 2). BDE47 was the most abundant congener in the sample categories (Table S.2), and is the most studied congener, reported to accumulate in crustaceans, fish and marine mammals (Hale et al., 2003; Gaion *et al*., 2021). A study by Pérez-Fuentetaja *et al*., (2015) found that out of ten PBDE congeners, BDE47 had the highest concentration and TMF in a food web composed of multiple invertebrates and fish species.

Seven ICES PCBs (ICES-7) were recommended for monitoring by the European Community Bureau of Reference and selected as indicators of wider PCB contamination (ICES, 2013). The ICES-7 PCBs have a wide chlorination range and represent ~20% by weight of the PCBs present in commercial mixtures (Kennedy, 2017). $\Sigma PCB_{32}$ for the samples were approximately twice the ICES-7 PCB concentration, except in the case where concentrations were low (for example flatfish muscle with a maximum ICES-7 concentration of <LoD and maximum $\Sigma PCB_{32}$ of 40.91 µg/kg lw), and the ICES-7 were the only PCBs detected (Table 2).

Due to the large number of PCB congeners, PCA was used to study the inter- and intra-variability of PCBs associated with sample category, species, region and physiological parameters. PCA was not conducted for PBDEs as BDE47 dominated the majority of the profiles while concentration of other congeners was low with many having a concentration <LoD (Table 2).

## Marine Mammals

### *$\Sigma PCB_{32}$ and $\Sigma PBDE_9$ concentrations*

The three marine mammal species covered in this section (harbour seal, harbour porpoise and sperm whale) represent a trophic level range of 3.75 – 5.02 (Madgett *et al*., 2019). Marine mammals had significantly higher concentrations of $\Sigma PCB_{32}$ and $\Sigma PBDE_9$ in their blubber than the other sample categories (Table 2) ($p < 0.05$, ANOVA, Tukey). It is well established that there is a positive correlation between trophic level, calculated from $\delta^{15}N$, and PCB concentrations in marine food webs (Kobayashi *et al*., 2015; Verheart *et al*., 2017; Masset *et al*., 2019). In this study, harbour seal and harbour porpoise had higher mean trophic levels (Madgett *et al*., 2019) and significantly higher detected $\Sigma PCB_{32}$ concentrations in their blubber than sperm whale ($p < 0.05$, ANOVA, Tukey; Table 2).

CB153 had the highest congener concentration in the marine mammal categories (Table S.1). CB153 is a hexa-chlorinated congener and is one of the most persistent PCB congeners in marine mammals (Williams *et al*., 2020). It is metabolically stable and less likely to be

transferred from females to offspring via reproductive processes (gestation and lactation) compared to the higher chlorinated congeners that have similar metabolic stability (Weijs *et al*., 2009). The number of congeners and degree of chlorination of the dominant seven congeners differed in the blubber of each of the marine mammal species studied. CB153 and CB138 were consistently the highest and second highest concentration respectively in all three species (Figure 2). However, there was quite a lot of variation in the subsequent five congeners with decreasing concentration. CB118, a dioxin-like CB, was only in the top seven, in terms of relative concentration, for sperm whales (Figure 2). The ICES-7 congeners only contributed three of the dominant congeners in harbour seal and harbour porpoise and five of the dominant congeners in sperm whale. This observation brings into question the reliability of using only the ICES-7 PCBs as indicators of wider PCB contamination in marine mammals.

There was less of a trophic level relationship within the marine mammal category for $\Sigma PBDE_9$; sperm whale had a significantly higher $\Sigma PBDE_9$ in their blubber than harbour seal and harbour porpoise. The concentration range for sperm whale was 139.4 – 1,888 µg/kg lw ($p < 0.05$, ANOVA, Tukey) (Table 2) while that for harbour seal and harbour porpoise was 21.75 – 638.2 µg/kg lw and 38.76 – 778.8 µg/kg lw respectively.

There was a wide concentration range of $\Sigma PCB_{32}$ and $\Sigma PBDE_9$ in all three marine mammal species (Table 2). To reduce the variability associated with sex, age and reproductive status, the blubber of only male marine mammals was analysed in this study and available regional and physiological information fully investigated to determine whether they contributed to the within-species concentration and congener proportion variation.

$\Sigma PCB_{32}$ and $\Sigma PBDE_9$ concentrations for the three species the Irish Sea (Clyde and Solway) biogeographic region were higher than from the other three biogeographic regions from which samples were obtained. However, on a species basis, there was no statistically significant difference in $\Sigma PCB_{32}$ concentration between the regions for harbour seal (Irish Sea (Clyde and Solway) n=2; Minches and Western Scotland n=2; Northern North Sea n=6) and harbour porpoise (Irish Sea (Clyde and Solway) n=5, Minches and Western Scotland n=5, Northern North Sea n=6 and Scottish Continental Shelf n=2) ($p > 0.05$ ANOVA, Tukey). This may in part be due to a lack of statistical power resulting from the low sample sizes for each category when examined on a regional basis. Only harbour porpoise from the Minches and Western Scotland (Region 3, Figure 3) had a significantly lower $\Sigma PBDE_9$ concentration in their blubber (84.25 ± 37.94 µg/kg lw; n=5) ($p < 0.05$, ANOVA, Tukey) than those from the Scottish Continental Shelf (193.0 ± 58.22 µg/kg lw; n=2), Northern North Sea (300.6 ± 151.5 µg/kg lw; n=6) and Irish Sea (1,129 ± 1,443 µg/kg lw; n=5) (Figure 3). A regional assessment was not conducted on sperm whale as they are a highly migratory species.

Pearson's correlation analysis revealed that there was no significant relationship between animal length and weight with $\Sigma PCB_{32}$ concentration ($p>0.05$) and no significant difference between stranding year and reproductive status for all three mammal species ($p > 0.05$) (physiological information available in Madgett *et al*., (2019)).

*PCB congener proportion*

Harbour porpoise, harbour seal and sperm whale are clearly separated when PCA was conducted on marine mammal PCB concentrations normalised to CB153 (Figure 4a). The first two principal components (PCs) of the PCA accounted for 72% of the PCB ratio variability. To determine whether species and/or biogeographic region are contributing to the variance associated with these species, PCA was also conducted to investigate biogeographic regional differences (Figure 4b). Figure 4b shows that there is a high degree of dispersion across both components, suggesting that there is no biogeographic regional influence on the PCB congener profile across the three categories. Sperm whales were stranded within two biogeographic regions – Minches and Western Scotland (blue dots Figure 4b) and the Northern North Sea (green dots on Figure 4b), identified as the five tightly clustered points at +10 on PC1 (Figure 4a). Male sperm whales are migratory and have one of the widest global distributions of any marine mammal species. The PCB congener profile in sperm whale is therefore not a true reflection of a specific region, but a general average of PCB composition across the migratory route, adjusted for age.

Sperm whale was more positively correlated to the first component due to the higher proportion of the lower chlorinated PCBs, CB49, 44, 74, 101, 118 (Figure 4a) suggesting a lower metabolic capacity to biotransform these compounds compared to other marine mammal species, or lower concentration-dependant induction of metabolising enzymes. Sperm whale is the largest of the species studied with the slowest and least developed metabolism (Nomiyama *et al*., 2016) and will therefore have a less 'metabolically weathered' profile, where the relative abundance of degraded forms of pollutants increases with age in males (not females due to reproductive transfer, mainly through lactation). Cephalopod feeders and oceanic species such as sperm whale have previously been found to have a higher proportion of less chlorinated congeners (i.e., tri, tetra- and penta-CBs) in their blubber due to their lower biotransformation capacity (Méndez-Fernandez *et al*., 2014).

Madgett *et al.* (2019) used a combination of fatty acid (FA) signatures and stable isotope (SI) ratios to identify the trophic level, feeding patterns and nutritional relationships between the species described in this study. Sperm whales were found to possess the least variable FA profile in the dataset and were separated from the other marine mammals due to having a

significantly different feeding pattern, which corresponds to their different PCB profiles compared to harbour seals and harbour porpoise (Figure 4a).

Harbour seal data was more positively correlated to the second component than harbour porpoise due to the higher proportion of metabolically stable hepta- (CB180) and octa- (CB194) chlorinated congeners and lower proportions of CB52 and 101 (Figure 4a), whilst harbour porpoise contain a larger proportion of the hexa-chlorinated congeners CB149, 138 and 153 (Figure 4a). This confirms previous reports (Boon *et al*., (1997), Hobbs *et al*., (2002), Weijs *et al*., (2009) and Méndez-Fernandez *et al*., (2017)) that harbour seals have an enhanced ability to metabolise lower chlorinated PCB congeners (e.g., CB52 and CB101), and CB149, compared to harbour porpoise. Other than CB153, CB138 and CB149 have previously been reported in the UK as the most prevalent congeners in harbour porpoise blubber (Weijs *et al*., 2008; Williams *et al*., 2020), which is similar to the data reported in this study (Table S.1).

Harbour seal and harbour porpoise are more dispersed across the second component in the score plot than sperm whale (Figure 4a), suggesting within-species ecological and biological parameters as potential explanatory variables. The analysis of $\delta^{13}C$ and FA profiles in Madgett *et al*. (2019) revealed that harbour seal and harbour porpoise have a more variable dietary pattern and/or feeding location than sperm whale. The mean trophic level calculated for sperm whale was 3.75 ± 0.16. This was significantly lower than the mean trophic level calculated for harbour seal (5.02 ± 0.35) and harbour porpoise (4.71 ± 0.36) (Madgett *et al*., 2019). The difference in trophic level is likely a factor contributing to the significant difference in $\Sigma PCB_{32}$ concentration (Table 2) and congener proportion in sperm whale (Figure 4a).

As well as metabolism and feeding ecology, location could be a contributing factor to congener proportion. The concentration of PCBs may differ according to the distance from the source (Fontaine *et al*., 2007), with highly halogenated congener concentrations decreasing with distance from the source as the lighter congeners are more volatile and capable of being transported over a longer distance (Stemmler and Lammel, 2012; Das *et al*., 2017). Sperm whales have one of the widest distributions of all marine mammals and can be found worldwide, inhabiting and foraging in deep offshore areas (Johnson, 2013). Sperm whales would therefore be further from primary contaminant sources than harbour seal and harbour porpoise (which inhabit coastal waters) and the higher proportion of lower chlorinated congeners in sperm whale is likely due to the more efficient long-range transport of lower chlorinated PCBs through both atmosphere and water (Beyer *et al*., 2000) combined with a lower metabolic capacity to biotransform particular congeners e.g., CB52 and CB101, compared to other marine mammal species.

Cross referencing the contaminant concentrations to FA and stable isotope (SI) data for all three marine mammal species (Madgett *et al*., 2019) it can be inferred that as well as metabolic capacity, diet is a contributor to PCB concentration and congener proportion in marine mammals. This association between PCB pattern and feeding ecology agrees with the findings by Mendez-Fernandez *et al*., (2017), where PCB patterns were identified as tracers for studying the feeding ecology, sources of contamination and population structure in odontocetes (toothed whales) from the Northwest Iberian Peninsula.

**Shark and fish**

*$\Sigma PCB_{32}$ and $\Sigma PBDE_9$ concentrations*

The ten categories covered in this section (demersal shark liver, demersal shark muscle, demersal roundfish whole, demersal roundfish liver, demersal roundfish muscle, pelagic roundfish whole, pelagic roundfish liver, pelagic roundfish muscle, flatfish muscle, flatfish liver) represent a trophic level range of 3.28 – 4.61 (Madgett *et al*., 2019). Flatfish liver had significantly lower concentrations of $\Sigma PCB_{32}$ and $\Sigma PBDE_9$ than demersal shark liver, three demersal roundfish categories and the three pelagic roundfish categories (Table 2; $p < 0.05$, ANOVA, Tukey). This was anticipated, as all flatfish sample pools in this study were collected from less industrialised areas such as Burra Haaf (n=7), Moray Firth (n=3) and the Solway Firth (n=2) (Figure S.1). Pelagic roundfish liver pools had a significantly higher $\Sigma PBDE_9$ (8.759 – 106.7 lw) than the other shark and fish categories ($p < 0.05$, ANOVA, Tukey) although the highest concentration was determined for one of the pools of flatfish liver (131.8 µg/kg lw).

As well as a category influence, there was also a regional influence on all the fish species and catshark liver categories, where sample pools collected from the Irish Sea (Clyde and Solway) biogeographic region (particularly the Clyde) had a significantly higher mean concentration of $\Sigma PCB_{32}$ and $\Sigma PBDE_9$ than those from the Northern North Sea and Scottish Continental Shelf ($p < 0.05$, ANOVA, Tukey). This agrees with the previous findings of Webster *et al*., (2007) and Scotland's Marine Assessment 2020 (Moffat *et al.,* 2020). In both cases, the conclusion was that around Scotland, the highest concentrations of PCBs and PBDEs occur in the Irish Sea (Clyde and Solway) biogeographic region (due to most sites being in the Firth of Clyde, an industrial area).

The Clyde has received significant direct inputs from both dumping and industrial effluents (pollution sources) in part because of its significant enclosed, coastal location which is quite distinct to those from further offshore such as the Scottish Continental Shelf. Between 1961

474 and 1992, Holy Loch, a site within the Clyde, was used to refit US nuclear-powered
475 submarines (Edwards, 1997) and was home to up to ten submarines, a floating dry dock and
476 a depot ship. Before clean-up, a quarter of the surface area of the floor of the loch was covered
477 in waste, resulting in 130,000 cubic metres of dangerous debris. The Ministry of Defence
478 (MoD) employed Environmental Resources Management (ERM) to carry out an environmental
479 survey of the Holy Loch sediments which found elevated PCB concentrations (15 congeners)
480 of up to 864 µg/kg dw (ERM, 1997). Another study by Miller, Pirie and Redshaw, (2000) found
481 the ∑ICES-7 concentration (µg/kg dw) in mussels collected before and after the initial phase
482 of the debris removal operation showed little change with concentrations in the region of 4.8–
483 19.4 µg/kg dry weight.

484 Whiting was the only species where physiology was found to influence $\Sigma PCB_{32}$ concentrations.
485 The length of the fish ranged from 162.0 – 356.0 mm, weight from 56.60 – 556.3 g, age from
486 1.4 – 6.6 years and trophic level from 3.65 – 4.65 (Madgett *et al*., 2019). Pearson's correlation
487 analysis revealed a significant relationship between $\Sigma PCB_{32}$ concentration and length, age,
488 weight and trophic level ($p<0.05$); the larger, older and heavier the fish, the higher the $\Sigma PCB_{32}$
489 concentration. This was anticipated, as size is equivalent to age and thus length of exposure.
490 It has been shown that factors other than the trophic position can play a role in the
491 biomagnification of PCBs in fish. A study by Burreau *et al*., (2006) found that biomagnification
492 in fish can also be dependent on the body size (weight), probably due to the slower clearance
493 rate of PCBs in larger individuals.

494 $\Sigma PBDE_9$ concentrations in demersal roundfish liver were highly variable (although much lower
495 than $\Sigma PCB_{32}$ concentrations), ranging from 2.14 – 47.54 µg/kg lw. The physiological variables
496 of trophic level, age, weight and length were not found to significantly influence $\Sigma PBDE_9$
497 concentration ($p>0.05$) in fish and catshark species.
498

499 *PCB congener proportion*

500 PCA was conducted on demersal shark liver and fish (pelagic, demersal and flatfish) liver PCB
501 concentrations normalised to CB153. Pooled flatfish liver (light green), pooled demersal shark
502 liver (blue), pooled demersal roundfish liver (red) and pooled pelagic roundfish liver (dark
503 green) showed a degree of separation on the score plot (Figure 5). The first two principal
504 components of the PCA accounted for 56% of the PCB ratio variability.

505 Demersal shark liver possesses the least variable PCB profile and form a tight cluster on the
506 PCA score plot (Figure 5). This has a corollary with the FA distribution of demersal shark
507 where there was little variation in feeding pattern identified within the species (Madgett *et al*,

2019) suggesting that, like marine mammals, PCB patterns could potentially be used as tracers for studying feeding ecology.

All flatfish liver sample pools were negatively correlated to the first component with fewer PCB congeners detected than in the other fish and shark categories (Table S.1). Flatfish are bottom-feeding fish, living in close contact with sediments where and are known to accumulate a variety of contaminants (Amiard-Triquet, Amiard and Rainbow, 2016). Higher chlorinated congeners are known to adsorb to sediments, which act as a sink for numerous organic compounds and free particles (Van der Oost, Beyer and Vermuelen, 2003). Benthic feeders such as flatfish are therefore widely used in offshore marine monitoring programmes due to their close association with sediment bound contaminants and less pronounced migration, thus being more likely to represent the area in which they are caught. All flatfish sample pools in this project were collected from less industrialised, offshore sites such as Burra Haaf (n=7), Moray Firth (n=3) and the Solway Firth (n=2).

There are two pelagic roundfish liver sample pools not clustered together on Figure 5, having different PCB profiles. The two points are the individuals comprising the two herring sample pools that were collected from Holy Loch which is in the Irish Sea (Clyde and Solway) biogeographic region. They had similar average pool trophic levels (3.49 ± 0.26) and feeding pattern, as inferred from their FA profiles (Madgett *et al*., 2019), similar average pool length (231 mm and 264 mm) and similar average pool weight (96.4 g and 98.8 g).

There were two demersal roundfish liver samples more positively correlated to the first component with a higher proportion of CB44, 52, 74, 99 138 and 158 (circled in red on Figure 5). Hake are at a higher trophic level (4.20 ± 0.13) than whiting and haddock (3.91 ± 0.39 and 3.73 ± 0.35) respectively (Madgett *et al*., 2019). The difference in congener proportion is likely due to the different species-specific metabolic capacities existing within the demersal roundfish category.

There was considerable variation in PC1 scores for the demersal roundfish liver sample category (Figure 5). To determine whether species and/or biogeographic region is contributing to the variance associated with this category, PCA was conducted on these variables (Figure S.2a and b). The fish species selected for this study are not highly migratory.

Although grouping samples on a species level separated hake from whiting and haddock (Figure S.2a), there is still a considerable spread across the score plot for haddock and whiting, suggesting a regional influence on congener proportion. The PCB profiles across the demersal roundfish liver biogeographic sampling locations were analysed (Figure S.2b). Fish collected from the Scottish Continental Shelf (5 whiting pools and 1 haddock pool) had the least variable PCB profiles and form a tight cluster on the PCA score plot due to having a

higher proportion of CB138 and 118 in their liver compared to those collected from the Irish Sea (Clyde and Solway) and Northern North Sea (Figure S.2b). Samples collected from the Irish Sea and Northern North Sea are spread across both components, but when biogeographic region was investigated, there appears to be a localised influence on species from the Holy Loch, composed of hake (n=2), whiting (n=1) and haddock (n=1) (Figure circled in red on S.2b). The proportion of higher chlorinated PCBs from Holy Loch is unsurprising as sampling locations at this site are closer to a highly contaminated, more industrialised area than the Pladda and the Solway Firth sites, whereas samples collected at other sites will be closer to a 'background' profile.

*Environmental assessment*

Demersal fish and flatfish are often used in environmental monitoring programmes and the contaminants are measured in the liver (Webster *et al*., 2014b). Flatfish and mussels are classed as "indicator species" for monitoring uptake and accumulation of hydrophobic contaminants in the marine environment and are representative of the regional quality status due to their limited mobility and contact with sea floor sediments in comparison to other species (Webster *et al*., 2007).

The concentrations of seven PCB congeners (ICES-7) in all fish liver samples (Figure 4a) and fish liver samples originating from the Irish Sea (Clyde and Solway) biogeographic region (Figure 4b) were compared to OSPAR's EACs. Only the EAC of CB118 was exceeded by demersal roundfish (liver, muscle and whole) and pelagic roundfish (whole) (Figures 6a and b). CB118 is the most toxic congener of the ICES-7 PCBs, being mono-ortho chlorine substituted and able to obtain an approximately planar configuration and therefore capable of exhibiting dioxin-like toxicity which relies on such a planar molecular configuration (OSPAR, 2021). Lyons *et al*., (2017) previously found CB118 in dab livers to exceed the EAC at 10 sites in the Central North Sea and Moffat *et al*., (2020) found that CB118 gave a regional mean concentration above the EAC for sediment and biota in the Irish Sea (Clyde and Solway). Fish liver from the Irish Sea do, however, have higher concentrations of the heavier PCBs 138, 153 and 180 (Figure 6b) compared to fish liver from the Northern North Sea and Scottish Continental Shelf, but do not exceed the EACs.

FEQGs provide benchmarks for the quality of the environment and are available for the six individual PBDE congeners described above in water, sediment and biota. FEQGs assess whether concentrations are likely to cause harm to marine organisms via the water or sediment, or where chemicals may bioaccumulate, and are currently being trialled for the OSPAR MIME (the Working Group on Monitoring and on Trends and Effects of Substances

in the Marine Environment) status assessment of PBDEs in sediment and biota (OSPAR, 2020c). Biota FEQG is expressed on a %ww basis, which fails to account for potential differences in the uptake of PBDEs due to differences in the lipid content of different monitoring species and tissues. The FEQGs were adjusted by MIME to a %lw basis by assuming the whole fish used in the toxicity trials had a 5% lipid content and multiplying the FEQGs (on a ww basis) by 20. None of the PBDE concentrations in each of the species matrix combinations exceeded the FEQG on this basis (Table 3).

**Invertebrates**

*$\Sigma PCB_{32}$ and $\Sigma PBDE_9$ concentrations*

The five categories in this section (demersal invertebrates, benthic invertebrates whole, benthic invertebrates muscle, benthic invertebrates brown meat, benthic invertebrates soft body) represent a trophic level range of 3.24 - 3.87. PCBs were not detected in demersal invertebrates muscle (squid) (Table 2). Benthic invertebrates muscle had a significantly higher $\Sigma PCB_{32}$ than the other invertebrates categories, ranging from 26.83 – 797.8 µg/kg lw (n=13) (Table 2). There was however no significant difference of $\Sigma PCB_{32}$ concentration between the four species making up the benthic invertebrates muscle category (squat lobster, *Nephrops*, edible crab, European lobster, hermit crab) ($p < 0.05$, ANOVA, Tukey). $\Sigma PCB_{32}$ concentrations (µg/kg lw) detected in the majority of invertebrates collected from the Holy Loch were higher than those detected in samples from other regions, in agreement with previous findings (Webster *et al*., 2014a). This data provides an indication of species-specific and localised regional influence on $\Sigma PCB_{32}$ in invertebrate species, but a higher sample number would be required for a comprehensive analysis.

Common starfish (n=9 pools) had the largest degree of variation in their $\Sigma PCB_{32}$ concentration, ranging from <LoD in the Northern North Sea (Moray Firth) to 1,418 µg/kg lw in the Irish Sea (Solway Firth). Some echinoderm species, including common starfish, in direct contact with the sediment have been shown to be valuable indicators of contamination (Knickmeyer, Landgraff and Steinhart, 1992; Schweitzer, Bay and Suffet, 2000; Lin and Davis, 2018). Studies in the North Sea have found a strong relationship between the concentrations of PCBs from the sediments and those in starfish, suggesting a direct accumulation from the sediment (Coteur *et al*., 2003). Common starfish also vary significantly in their trophic level, ranging from 2.67 - 4.13. The two starfish sample pools with the highest concentration of $\Sigma PCB_{32}$ had the highest trophic level values (3.97 and 4.13), were collected from Holy Loch and were not

612 significantly larger in size or weight to the other sample pools ($p>0.05$) (Madgett *et al*., 2019).
613 This suggests both a trophic and localised regional influence on PCB concentrations in this
614 species.

615 $\Sigma PBDE_9$ was <LoD for the benthic invertebrates muscle category and was significantly higher
616 in benthic invertebrates whole pools (common starfish=9, sea mouse=1, brittle star=1) (<LoD
617 – 124.5 µg/kg lw). There was no regional influence on any of the benthic invertebrates
618 categories or species, likely due to the low number of individuals with detected concentrations.

619

620 *PCB congener proportion*

621 PCA carried out on PCB congener profiles for the benthic invertebrates categories showed
622 considerable spread across both principal components, with substantial within-group and
623 between-group variation (Figure 7a). All PCB congener concentrations in demersal
624 invertebrates (squid) were below the LoD and concentrations of CB114 and 189 were below
625 the LoD for all invertebrate samples. Demersal invertebrates and CB114 and CB189 were
626 therefore not included in the multivariate analysis.

627 The first two principal components of the PCA explained 50% of the variability present in the
628 dataset. All four categories are spread across the first component (Figure 7a). A similar
629 pattern was found in Madgett *et al*., (2019), where considerable variation for the benthic
630 invertebrates whole, muscle and soft body FA profiles suggested highly variable feeding
631 patterns. PCA was conducted on species (Figure 7b) and biogeographic region (Figure 7c)
632 to determine whether these factors contribute to the observed variation (Figure 5b).

633 The benthic invertebrates whole samples negatively correlated to the second component and
634 pooled common starfish (dark blue on Figure 7b) are spread across the first component (from
635 -2 to +3). Starfish are positively correlated to the first component, having higher proportions
636 of hexa-chlorinated congeners (CB138, CB149). Four out of the nine starfish sample pools
637 are separated on the first component. Figure 7c shows that these samples were collected
638 from the Irish Sea (Clyde and Solway) biogeographic region, consisting of the only two pools
639 collected from Holy Loch (furthest from the cluster), one from Hunterston and one from the
640 Solway Firth, and two sample pools collected from Pladda. The three sample pools in the
641 main cluster were collected from the Moray Firth in the Northern North Sea. This suggests
642 that as well as concentration, there is a localised regional influence on congener proportion in
643 common starfish which has the potential of influencing the calculated TMF on a regional basis.

644 Benthic invertebrates soft body PCB congener patterns are also highly variable (Figure 7a).
645 The two benthic invertebrates soft body sample pools which are more positively correlated to
646 the first and second components (circled in green, Figure 7a) were identified as shore crab
647 (Figure 7b), containing a higher proportion of hepta-chlorinated congeners (CB187, CB183)
648 than the other invertebrate species, possibly due to being collected from Tancred Bank in the
649 Northern North Sea close to a highly industrialised area. In Madgett *et al* (2019), all
650 contributing species to the benthic invertebrates soft body category could be separated due
651 to their differing FA profiles (Figure 7b). Samples collected from the Northern North Sea and
652 Irish Sea are highly dispersed across both components of the PCA score plot (Figure 7c),
653 suggesting more of a species influence on congener proportion in the benthic invertebrates
654 soft body category than geographical variation.

655 Two *Nephrops* sample pools (circled in dark blue on Figure 7a) were separated from the other
656 benthic invertebrate muscle sample pools (including the other four *Nephrops* sample pools).
657 These two sample pools contain a higher proportion of penta-chlorinated congeners (CB101,
658 99, 110, 118) and hexa-chlorinated congeners (CB149, 132), although six out of the seven
659 *Nephrops* sample pools were collected from the Irish Sea Biogeographic Region. As observed
660 in other species, the two separated pools were collected from Holy Loch, further suggesting a
661 localised regional influence on PCB congener proportion but not $\Sigma PCB_{32}$ concentration
662 ($p>0.05$).

663

664 **Trophic magnification**

665 Trophic magnification was investigated using the ICES-7 PCBs and BDE47 in marine mammal
666 blubber, shark and fish (demersal roundfish, pelagic roundfish and flatfish) liver and benthic
667 invertebrates (whole, muscle, soft body, brown meat). The ICES-7 PCBs have been selected
668 as being representative of the range of PCB congeners detected in environmental matrices
669 and represent substances with a range of physico-chemical properties, prevalence and
670 metabolic stability. They are included in most, if not all, PCB monitoring programmes.
671 However, for marine mammals it should be noted that the results from this study do cast some
672 doubt on the pertinence of using the ICES 7 PCBs for some species. BDE47 was the only
673 congener with detectable concentrations in more than ten benthic invertebrate sample pools,
674 ensuring the inclusion of several lower-trophic-level taxa (several different benthic invertebrate
675 families) and a reasonable balance with respect to sample numbers of lower- versus higher-
676 trophic-level organisms (as per the guidance by Kidd *et al*., 2018).

To determine whether biomagnification for these substances occurs in the studied food web and to establish whether the application of TMFs is appropriate in this context, TMFs for individual congeners were calculated using two methods described in Madgett *et al*., (2021): the "traditional method" using the slope of logarithmically transformed (to base 10) concentrations of POPs versus trophic levels of organisms in the food web (Borgå *et al*., 2012), and the "balanced method" which is used to overcome the issue of unbalanced sampling, using the slope of geometric mean concentrations and trophic levels rather than concentrations and trophic levels of each individual organism (Brisebois, 2013).

Sperm whales are highly migratory, undertaking large seasonal migrations for feeding (Arctic) and breeding (near the equator), often passing through waters to the north and west of Scotland in the process (Marine Scotland, 2016). This was evident from FA and SI analysis (Madgett *et al*., 2019), which showed that sperm whales had a different feeding location and/ or diet to harbour seal and harbour porpoise. Although sperm whales are part of the Scottish marine ecology, for the purpose of TMF calculations for regional based assessments they are not classed as a "fixed" species around Scottish waters and so have not been included in the calculation of TMFs in this study. Harbour seal and harbour porpoise have been identified as good indicators of coastal pollution as they generally remain in coastal waters and don't undergo large-scale migrations (Weijs *et al*., 2020b).

The plots used to determine TMFs for CB180 are included in the text (Figures 8-10), but those for CB153, 138, 118, 101, 52 and 28 and BDE47 are presented in the supplementary information (Figures S.3 to S.23). The regression summary for the determination of TMF using both the traditional method (Borgå *et al*., 2012; OSPAR, 2016) and balanced method (Brisebois, 2013) is shown in Table S.3 and calculated TMFs for the ICES-7 PCBs and BDE47 are shown in Tables 4 and 5.

TMFs using ecosystem specific data have not previously been reported in Scottish waters. CB52 had the highest TMF value which was 2.6 times greater than the TMF of CB180. This was unexpected, as CB180 is the highest chlorinated congener in this study. However, the TMF for CB180 in this study is higher than reported globally, where Rüdel *et al*., (2020) reported a range of 1.2 - 4.6 in pelagic and benthopelagic food webs in Italy, Norway, Finland and Canada; and An *et al*., (2020) reported a TMF of 1.06 from a food web composed of invertebrates and fish in Ulson Bay, Korea. The value for CB52 is also much higher than reported globally. Houde *et al*., (2008) reported TMFs ranging from 0.8 – 4.5 (n=20) in lake trout, forage fish, and invertebrates in Canada; Brisebois, (2013) reported TMFs of 1.06 (traditional method) and 1.38 (balanced method) in a food web composed of zooplankton, benthos and fish in the Netherlands; and Kobayashi *et al*., (2019) reported TMFs of 1.7 for a

benthic food web and 3.4 for a pelagic food web in Tokyo Bay. Variations in cytochrome P450 enzyme (CYPs) distribution and function between animal groups could result in differential metabolism of certain contaminants. Koenig, Fernández and Solé, (2012) have found that differential CYP patterns have contributed to differences in PCB accumulation profiles between species. Ortho-substituted PCBs (such as CB52) are preferentially metabolised by CYP2B isoenzymes. CB52 is more metabolically stable than the other congeners (Boon *et al*., 1992; Boon *et al*., 1997). Due to the differential expression of the CYP2B enzyme between species, harbour seal and harbour porpoise, which appear to express this enzyme to a greater extent than other marine mammal species, have an enhanced ability to metabolise CB52 (harbour seal are more genetically adapted for this than harbour porpoise). Fish, on the other hand, do not express this enzyme (James and Kleinow, 2014). This difference in metabolic capacity between harbour seal and harbour porpoise is apparent in Figures S.15 and b, where harbour porpoise has a noticeably higher concentration in relation to trophic level than harbour seal.

The TMF of CB52 was more than two times higher using the balanced method than the traditional method, showing that an unbalanced dataset (different number of samples at each trophic level/category) influences the calculated TMF for CB52 (Table 4). An unbalanced dataset was also found to influence the TMF of CB28, where biomagnification was found to occur using the traditional method but trophic dilution was identified using the balanced method (Table 4). CB28 is the lowest chlorinated PCB analysed in this study and is (relatively) more water soluble, volatile and more likely to biodegrade abiotically and biotically than the other PCBs studied (Beyer and Biziuk, 2009).

Due to the regional influence identified on $\Sigma PCB_{32}$ and $\Sigma PBDE_{9}$ concentration and congener proportions in marine mammals, fish and invertebrates collected from the Irish Sea (Clyde and Solway) biogeographic region, TMFs were investigated separately in this region. Regional variation on PCB and PBDE TMFs have been reported in other studies globally using only the traditional method (Bodin *et al*., 2008; Magalhães *et al*., 2017; Choo, Lee and Oh, 2019).

The TMF calculated from the Irish Sea Biogeographic Region food web was higher for CB180, 118, 52 and 28 than from the Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf using both methods, and higher for CB138, 153 and 101 using the balanced method only (Table 5). The regional influence on the calculated TMF was expected to be higher as some samples (fish and invertebrates species) collected from the Irish Sea Biogeographic Region were found to have a significantly higher concentration of $\Sigma PCB_{32}$ in their tissues than those from the other three regions. There is however a higher number of marine mammal samples and much fewer benthic invertebrate sample pools in the Northern

North Sea, Minches and Western Scotland and Scottish Continental Shelf (with concentrations above the LoD) compared to the Irish Sea Biogeographic Region, which has resulted in a steeper gradient and therefore higher calculated TMF. This emphasises the importance of a balanced dataset when calculating TMFs. The correlation was, however, not significant for CB28 in both regional comparisons ($p>0.05$) (Table S.3). This is likely due to the high concentration and ability of harbour seal to metabolise this congener. Metabolism has previously been shown to be concentration dependent, where the higher the concentration circulating in the plasma when fat is utilised, the more effectively the enzymes are induced resulting in greater metabolism (Weijs *et al.*, 2008).

The TMF of BDE47 calculated in this study is comparable to the TMFs reported in other studies using the traditional method. A study by Pérez-Fuentetaja *et al*., (2015) reported a TMF of 1.9 in a food web composed of multiple invertebrates and fish species, and a TMF of 4.2 when fish only were included using log transformed PBDE concentrations (lw) and $\delta^{15}N$ derived trophic level. A study by Shao *et al.,* (2016) reported a TMF of 3.3 for BDE47 in marine food webs from Bohai Bay, China composed of a variety of invertebrate and fish species spanning three trophic levels, using log transformed BDE concentrations (lw) and $\delta^{15}N$ derived trophic levels. Another study by Poma *et al*., (2014) based in Northern Italy reported a TMF of 1.8 for BDE47 in a food web composed of zooplankton and fish also using log transformed PBDE concentrations (lw) and $\delta^{15}N$ derived tropic level.

The TMF of BDE47 calculated in this study for the Irish Sea Biogeographic Region was higher using the balanced method than the traditional method, and vice versa for the Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf (Table 5). The calculated TMF was predicted to be higher in the Irish Sea Biogeographic Region as marine mammal and fish samples collected from that region had a higher concentration of $\Sigma PBDE_9$ in their tissues than those from the other three regions. A similar finding was reported for CBs 138, 153 and 101 due to sample imbalance. The geometric mean used for the balanced method TMF remedied this unbalanced proportion of trophic levels, providing a more representative TMF result of the studied regions. Our findings strongly support this approach when conducting a regional comparison.

# Conclusions

The aims of this study were to determine whether biomagnification of selected PCBs and PBDEs occurs in the specific food web being investigated and to establish whether the

application of TMFs to describe biomagnification is appropriate for a consistent, trophic specific biota assessment.

In order to calculate reliable TMFs representing the trophic transfer of PCB/PBDE congeners through the marine food web, sources of variability within sample categories (inter- and intra-species variation) must be identified and assessed. In this study, the concentrations and proportions of thirty-two PCBs and nine PBDEs in nineteen sample categories across four trophic levels were investigated in the Scottish marine food web.

There was a clear influence of trophic level and ecology on contaminant concentrations in the marine mammals categories, where the highest value of $\Sigma PCB_{32}$ reported in sperm whales was 1,888 µg/kg lw compared to 139,800 µg/kg lw $\Sigma PCB_{32}$ in harbour seals. Demersal invertebrates muscle had $\Sigma PCB_{32}$ concentration <LoD and benthic invertebrates muscle had $\Sigma PBDE_9$ concentration <LoD. PCB and PBDE concentrations in the zooplankton were < LoD. When the ICES-7 PCB concentrations in fish were compared to assessment criteria, only CB118 in all fish categories exceeded the relevant OSPAR EAC. Neither the PBDE congener concentrations exceeded the given FEQG values.

The variation of $\Sigma PCB_{32}$ and $\Sigma PBDE_9$ and congener proportion in shark and fish categories was due to their contributing species, feeding ecology, metabolic capacity, trophic level, and sampling location. Shark and fish had a higher proportion of lower chlorinated PCBs than marine mammals due to their lower metabolic capacity to biotransform these compounds. The metabolism of organic contaminants in fish is species-specific, and as such, this is a likely contributing factor to the variation observed in this study. Demersal shark also had the least variable PCB and PBDE profile, likely due to their consistent within-species feeding pattern identified in Madgett *et al*., (2019). Pelagic roundfish could be distinguished from shark and other fish categories, having a different PBDE profile, likely a result of their planktonic diet. Sampling location (biogeographic and localised) was found to influence the $\Sigma PCB_{32}$ in demersal species, where biogeographic region influenced the $\Sigma PBDE_9$ of the shark and fish categories. The benthic invertebrates categories had a similar level of variation in their PCB profile as their FA profiles, where considerable variation in the profiles suggest a highly variable feeding pattern between species. The concentration of $\Sigma PBDE_9$ in all invertebrate sample categories was low, with few congeners detected. Common starfish had a significantly higher concentration of $\Sigma PBDE_9$ indicating that the variation is species-specific within the benthic invertebrates categories for PBDEs, which corresponds with the FA, SI and PCB data. Selection of a broad range of species for inclusion in determining TMFs is therefore deemed to be important.

Trophic magnification was found to occur for the ICES-7 PCBs and BDE47 when using the traditional method, with the highest degree of trophic magnification reported for CB52.

An unbalanced dataset was found to influence the calculated TMF when conducting regional comparisons. CB153, 138, 101, 28 and BDE47 were found to have a higher TMF in the Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf using the traditional method in comparison to the Irish Sea, where the balanced method yielded a higher TMF in the Irish Sea than the other regions, which is more expected due to localised pollution inputs. This was due to the difference in sample numbers of invertebrates and marine mammals between the regions. CB28 gave the biggest TMF difference between the methods, where trophic magnification was reported in the Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf using the traditional method, and trophic dilution reported using the balanced method. For CB28 and BDE47, the correlation between geometric mean trophic level and geometric mean log concentration was not significant ($p>0.05$), suggesting that a larger dataset is required to examine the significance or otherwise of a relationship (these substances were not detected above the limit of detection in many of the samples analysed in this study).

Our findings show that feeding ecology does contribute to the variation identified in PCB and PBDE concentration and congener proportion across the sample categories and, along with other identified factors (sampling location, metabolic capacity etc.), can be used to identify the variation associated with calculated TMFs. An unbalanced dataset was found to influence the calculated TMF and in some cases, the overall conclusion of the trophic transfer of PCB and PBDE congeners. The balanced method is therefore highly recommended for calculating TMFs to ensure that the TMF is a true indication of the biomagnification potential, particularly when conducting regional comparisons for which sampling requirements are difficult to achieve.

## Credit author statement

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Tables

**Table 1:** Sample pools collected from each of the five environmental monitoring survey cruises from nine locations around Scotland, covering four biogeographic regions (Madgett *et al,* 2019). n = number of matrix specific sample pools associated to that particular species and sampling point. The sampling locations are demonstrated in Figure 1.

| Sampling Location | Species Collected | Number of Individuals Collected | Number of Sample Pools | Matrix |
|---|---|---|---|---|
| Tancred Bank | Shore Crab (*Carcinus maenas*) | 27 | 2 | Soft Body (n=2) |
| North East Dunbar | Haddock (*Melanogrammus aeglefinus*) | 36 | 4 | Muscle (n=2), Liver (n=2), Whole (n=2) |
| | Swimming Crab (*Liocarcinus depurator*) | 68 | 2 | Soft Body (n=2) |
| Montrose Bank | Haddock (*Melanogrammus aeglefinus*) | 5 | 1 | Muscle (n=1), Liver (n=1) |
| | Whiting (*Merlangius merlangus*) | 10 | 2 | Muscle (n=2), Liver (n=2) |
| | Edible Crab (*Cancer pagurus*) | 14 | 1 | Muscle (n=1), Brown Meat (n=1) |
| | Squat Lobster (*Munida rugosa*) | 8 | 1 | Muscle (n=1) |
| | Swimming Crab (*Liocarcinus depurator*) | 31 | 1 | Soft Body (n=1) |
| Moray Firth | Haddock (*Melanogrammus aeglefinus*) | 20 | 4 | Muscle (n=4), Liver (n=4) |
| | Plaice (*Pleuronectes platessa*) | 15 | 3 | Muscle (n=3), Liver (n=3) |
| | Squid (*Loligo forbesii*) | 5 | 1 | Muscle (n=1) |
| | Common Starfish (*Asterias rubens*) | 16 | 3 | Whole (n=3) |
| | *Nephrops* (*Nephrops norvegicus*) | 28 | 1 | Muscle (n=1) |
| | Brittle Star (*Ophiura ophiura*) | 96 | 1 | Whole (n=1) |
| Burra Haaf | Haddock (*Melanogrammus aeglefinus*) | 5 | 1 | Muscle (n=1), Liver (n=1) |
| | Whiting (*Merlangius merlangus*) | 20 | 5 | Muscle (n=5), Liver (n=5) |
| | Plaice (*Pleuronectes platessa*) | 17 | 4 | Muscle (n=4), Liver (n=4) |
| | Dab (*Limanda limanda*) | 15 | 3 | Muscle (n=3), Liver (n=3) |
| | Squid (*Loligo forbesii*) | 5 | 1 | Muscle (n=1) |

| Sampling Location | Species Collected | Number of Individuals Collected | Number of Sample Pools | Matrix |
|---|---|---|---|---|
| | Hermit Crab *(Pagurus bernhardus)* | 10 | 1 | Muscle (n=1) |
| | *Nephrops* (*Nephrops norvegicus*) | 53 | 1 | Muscle (n=1) |
| Holy Loch | Catshark *(Scyliorhinus canicula)* | 8 | 4 | Muscle (n=4), Liver (n=4) |
| | Haddock (*Melanogrammus aeglefinus*) | 10 | 2 | Muscle (n=2), Liver (n=2) |
| | Hake (*Merluccius merluccius*) | 7 | 2 | Muscle (n=2), Liver (n=2) |
| | Common Starfish (*Asterias rubens*) | 10 | 2 | Whole (n=2) |
| | Squat Lobster *(Munida rugosa)* | 44 | 1 | Muscle (n=1) |
| | *Nephrops* (*Nephrops norvegicus*) | 73 | 2 | Muscle (n=2) |
| | Whelk (*Buccinum undatum)* | 12 | 4 | Soft Body (n=4) |
| | Swimming Crab (*Liocarcinus depurator*) | 64 | 2 | Soft Body (n=2) |
| | Horse Mussel (*Modiolus modiolus)* | 8 | 1 | Soft Body (n=1) |
| Hunterston | Catshark *(Scyliorhinus canicula)* | 10 | 2 | Muscle (n=2), Liver (n=2) |
| | Common Starfish (*Asterias rubens*) | 10 | 1 | Whole (n=1) |
| | *Nephrops* (*Nephrops norvegicus*) | 71 | 2 | Muscle (n=2) |
| | Squat Lobster *(Munida rugosa)* | 31 | 1 | Muscle (n=1) |
| | Swimming Crab (*Liocarcinus depurator*) | 34 | 1 | Soft Body (n=1) |
| Pladda | Catshark *(Scyliorhinus canicula)* | 13 | 3 | Muscle (n=3), Liver (n=3) |
| | Haddock (*Melanogrammus aeglefinus*) | 21 | 4 | Muscle (n=1), Liver (n=1), Whole (n=3) |
| | Whiting (*Merlangius merlangus*) | 25 | 6 | Muscle (n=6), Liver (n=6) |
| | Herring (*Clupea harengus*) | 10 | 2 | Muscle (n=2), Liver (n=2) |
| | Common Starfish (*Asterias rubens*) | 10 | 2 | Whole (n=2) |
| | Lobster (*Homarus gammarus*) | 4 | 1 | Muscle (n=1), Brown Meat (n=1) |
| | Horse Mussel (*Modiolus modiolus)* | 6 | 1 | Soft Body (n=1) |
| | Whelk (*Buccinum undatum)* | 4 | 1 | Soft Body (n=1) |
| Solway Firth | Catshark *(Scyliorhinus canicula)* | 13 | 3 | Muscle (n=3), Liver (n=3) |

| Sampling Location | Species Collected | Number of Individuals Collected | Number of Sample Pools | Matrix |
|---|---|---|---|---|
| | Haddock (*Melanogrammus aeglefinus*) | 8 | 3 | Muscle (n=3), Liver (n=3) |
| | Whiting (*Merlangius merlangus*) | 15 | 2 | Muscle (n=1), Liver (n=1), Whole (n=1) |
| | Plaice (*Pleuronectes platessa*) | 8 | 2 | Muscle (n=2), Liver (n=2) |
| | Sprat (*Sprattus sprattus*) | 149 | 3 | Whole (n=3) |
| | Common Starfish (*Asterias rubens*) | 3 | 1 | Whole (n=1) |
| | Whelk (*Buccinum undatum)* | 20 | 2 | Soft Body (n=2) |
| | Edible Crab (*Cancer pagurus*) | 14 | 1 | Muscle (n=1), Brown Meat (n=1) |
| | Sea Mouse (*Aphrodita aculeata*) | 33 | 1 | Whole (n=1) |

**Table 2:** The lipid content (%) and concentration range (µg/kg lipid weight) for the ΣICES-7 PCBs, $\Sigma PCB_{32}$ and $\Sigma PBDE_9$ in the muscle, liver, homogenised whole, brown meat, soft body and blubber samples analysed across eighteen of the nineteen sample categories (not including zooplankton). Sample Number = individuals for mammals and pools for all other categories. Number of individuals per pool are referred to in Table 1. Not all the LoD values are to four significant figures to account for precision. Values <LoD were not included when calculating the sum of CBs. $\Sigma PCB_{32}$ and ΣICES-7 is expressed as the minimum sample concentration – maximum sample concentration within each category.

| Category | Sample Number | Lipid Content % | ICES-7 | $\Sigma PCB_{32}$ | $\Sigma PBDE_9$ |
|---|---|---|---|---|---|
| Harbour Seal | 10 | 61.90 – 95.58 | 1,439 - 90,640 | 1,965 - 139,800 | 21.75 - 638.2 |
| Harbour Porpoise | 18 | 54.38 – 96.33 | 417.9 - 71,200 | 754.3 - 114,500 | 38.76 – 778.8 |
| Sperm Whale | 5 | 26.18 – 63.19 | 462.0 - 7,630 | 821.1 - 13,520 | 139.4 - 1,888 |
| Demersal Shark Muscle | 12 | 0.36 – 1.99 | <0.03 - 1,036 | <0.02 - 1,585 | <0.01 - 40.00 |
| Demersal Shark Liver | 12 | 47.64 – 80.38 | 396.1 - 4,639 | 655.9 - 8,653 | 9.504 - 54.47 |
| Pelagic Roundfish Muscle | 2 | 2.65 – 5.85 | 109.1 - 205.3 | 198.8 - 373.9 | 1.132 - 3.248 |
| Pelagic Roundfish Liver | 2 | 0.45 – 1.37 | 337.9 - 604.4 | 668.6 - 1,202 | 8.759 - 106.7 |
| Pelagic Roundfish Whole | 3 | 6.17 – 7.15 | 166.8 - 265.5 | 329.5 - 530.9 | 0.585 - 1.199 |
| Demersal Roundfish Muscle | 30 | 0.61 – 1.96 | <0.03 - 1,036 | <0.02 - 1,858 | <0.01 - 35.165 |
| Demersal Roundfish Liver | 30 | 21.40 – 78.78 | 40.90 - 1,684 | 57.91 - 3,065 | 2.137 - 47.54 |
| Demersal Roundfish Whole | 6 | 0.87 – 3.01 | 141.5 - 820.0 | 160.5 - 1,164 | <0.01 - 37.21 |
| Flatfish Muscle | 12 | 0.35 – 0.92 | <0.03 - <0.22 | <0.02 - 40.91 | <0.01 - 28.26 |
| Flatfish Liver | 12 | 1.57 – 33.29 | <0.11 - 586.9 | <0.05 - 899.2 | <0.01 - 131.8 |
| Demersal Invertebrates Muscle | 2 | 2.10 – 2.66 | <0.03 - <0.22 | <0.02 | <0.01 - 10.95 |
| Benthic Invertebrates Muscle | 13 | 0.75 – 3.34 | 26.83 - 417.6 | 26.83 - 797.8 | <0.01 - <0.06 |
| Benthic Invertebrates Soft Body | 17 | 0.27 – 3.87 | <0.03 - 2,119 | <0.03 - 3,888 | <0.01 – 24.62 |
| Benthic Invertebrates Whole | 11 | 0.61 – 2.27 | <0.03 - 555.9 | <0.03 - 1,418 | <0.01 – 124.5 |
| Benthic Invertebrates Brown Meat | 3 | 8.57 – 26.43 | 124.9 - 250.4 | 218.3 - 367.2 | <0.01 - 5.335 |

**Table 3:** The concentrations of BDE28, 47, 99, 100, 153 and 154 in pooled fish tissue (liver, muscle and whole) from all biogeographical regions (Irish Sea, Northern North Sea and Scottish Continental Shelf) in comparison to the Canadian Federal Environmental Quality Guidelines (FEQG) (µg/kg lw) for biota. The FEQG for fish has been normalised to 5% lipid (x 20, assuming a 5% lipid content). None of the PBDE concentrations in each of the species matrix combinations exceeded the FEQG. Demersal roundfish whole is not included as

| Category | Congener Concentrations and FEQG Values (µg/kg lw) | | | | | |
|---|---|---|---|---|---|---|
| | BDE28 | BDE47 | BDE99 | BDE100 | BDE153 | BDE154 |
| FEQG | 2400 | 880 | 20 | 20 | 80 | 80 |
| Pelagic Roundfish Muscle | <LoD | 1.453 ± 1.453 | <LoD | <LoD | <LoD | <LoD |
| Pelagic Roundfish Liver | <LoD | 35.56 ± 35.56 | 15.49 ± 6.732 | <LoD | 6.667 ± 6.667 | <LoD |
| Pelagic Roundfish Whole | <LoD | 5.485 ± 1.412 | 0.466 | 0.611 ± 0.467 | 1.620 ± 1.261 | <LoD |
| Demersal Roundfish Muscle | <LoD | 3.904 ± 6.683 | <LoD | 0.532 ± 1.405 | 1.964 ± 3.176 | 0.220 ± 1.184 |
| Demersal Roundfish Liver | 0.044 ± 0.152 | 12.91 ± 8.889 | 3.832 | 1.487 ± 2.131 | 1.991 ± 2.574 | 0.152 ± 0.326 |
| Demersal Roundfish Whole | <0.01 - 1.329 | <0.06 - 22.59 | <0.12 - 7.360 | <0.19 - 6.796 | <0.02 | <0.02 - 2.326 |
| Flatfish Muscle | <LoD | 2.726 ± 6.114 | 2.047 | 1.315 ± 3.041 | 2.703 ± 5.004 | <LoD |
| Flatfish Liver | <LoD | 8.437 ± 25.40 | 4.640 | <LoD | 6.570 ± 13.95 | <LoD |

**Table 4:** Calculated TMFs in a food web composed of marine mammals, shark, fish and invertebrates using the traditional and balanced methods.

| | Traditional Method | Balanced Method |
|---|---|---|
| **Congeners** | **Marine mammals, shark, fish, invertebrates** | **Marine mammals, shark, fish, invertebrates** |
| CB180 | 10 | 11 |
| CB153 | 9.1 | 12 |
| CB138 | 8.9 | 9.2 |
| CB118 | 2.6 | 2.8 |
| CB101 | 4.8 | 2.5 |
| CB52 | 26 | 44 |
| CB28 | 1.3 | 0.7 |
| BDE47 | 2.1 | 1.4 |

**Table 5:** Calculated TMFs in a food web in the Irish Sea Biogeographic Region and food web in the Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf using the traditional and balanced methods.

| | Traditional Method | | Balanced Method | |
|---|---|---|---|---|
| **Congeners** | **Irish Sea** | **Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf** | **Irish Sea** | **Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf** |
| CB180 | 11 | 10 | 17 | 6.9 |
| CB153 | 8.1 | 10 | 18 | 14 |
| CB138 | 7.3 | 9.4 | 12 | 9.9 |
| CB118 | 3.2 | 2.2 | 2.8 | 1.5 |
| CB101 | 4.8 | 5.1 | 4.4 | 3.3 |
| CB52 | 62 | 13 | 69 | 18 |
| CB28 | 1.2 | 1.1 | 1.0 | 0.6 |
| BDE47 | 1.6 | 2.5 | 2.5 | 1.2 |

# Figures


**Figure 1:** Sampling Sites: Fish, catshark and marine invertebrate samples were collected by the MRV *Scotia* and MRV *Alba na Mara* between 2015 and 2017 from Tancred Bank, Northeast (NE) Dunbar, Montrose Bank, Moray Firth, Burra Haaf, Holy Loch, Hunterston, Pladda and Solway Firth (black circles). Blue mussels were collected by hand from Loch Ewe, Loch Long and Lunderston Bay between 2013-2014 (purple circles). Marine mammal samples were collected from stranded animals between 2012-2016. The individual stranded animals (small green circles) were collected from eight regions around Scotland (green text): Lothian, Fife, Tayside, Grampian, Highland, Orkney, Western Isles, and Strathclyde. Two zooplankton species were collected from the Scottish Observatory site off Stonehaven from the RV *Temora* in 2017 (red circle).

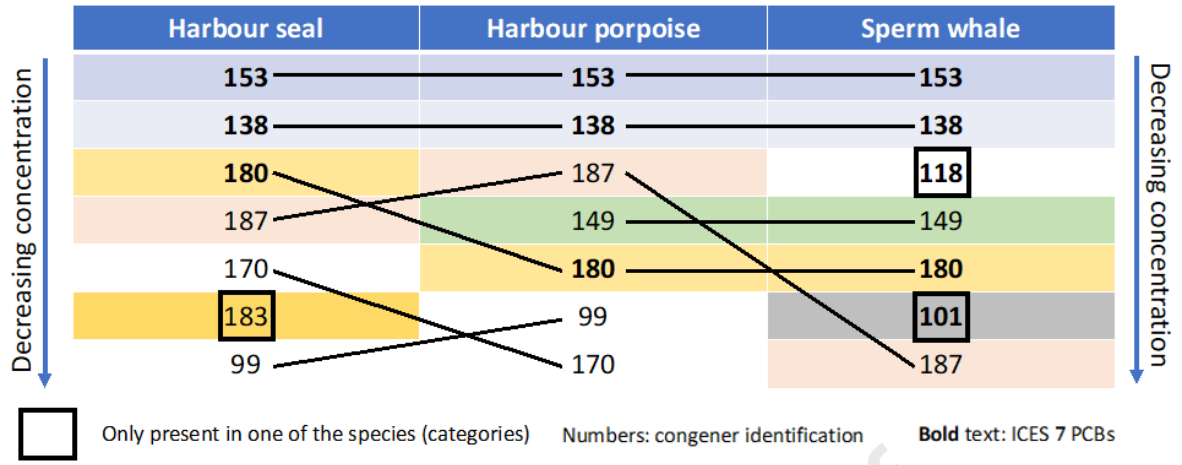



**Figure 2:** Top seven, in terms of relative average concentration, for CB congeners in harbour seal, harbour porpoise and sperm whale. CB183 was present in the top seven for harbour seal only. CB118 and CB101 were only in the top seven for sperm whale. The ICES-7 PCBs made up only three of the top seven for harbour seal and harbour porpoise, but five of the top seven for sperm whale.

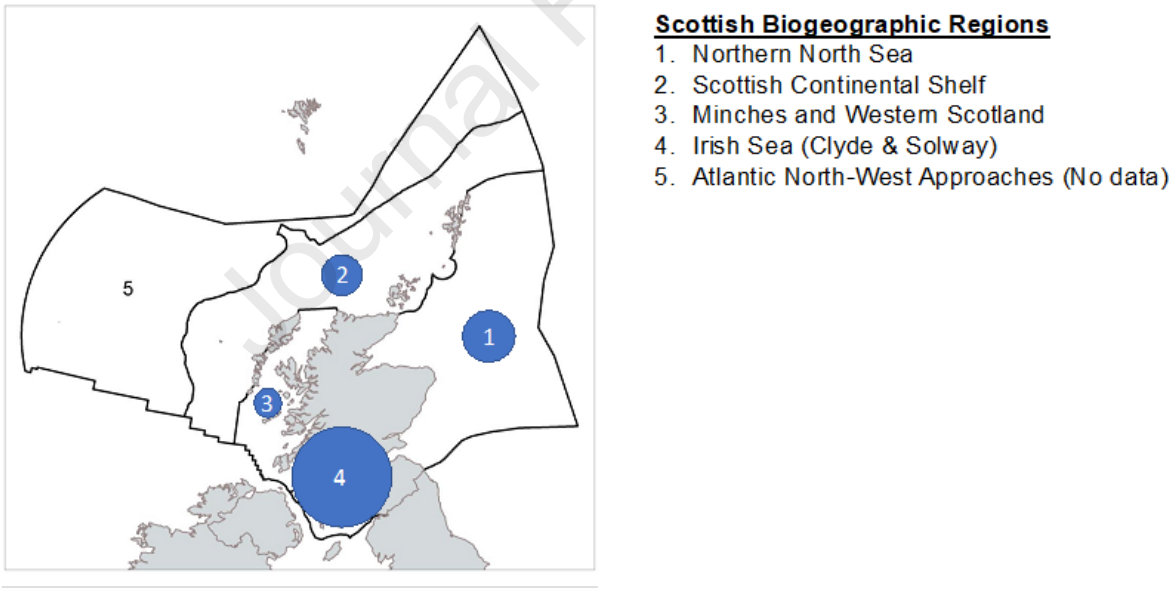
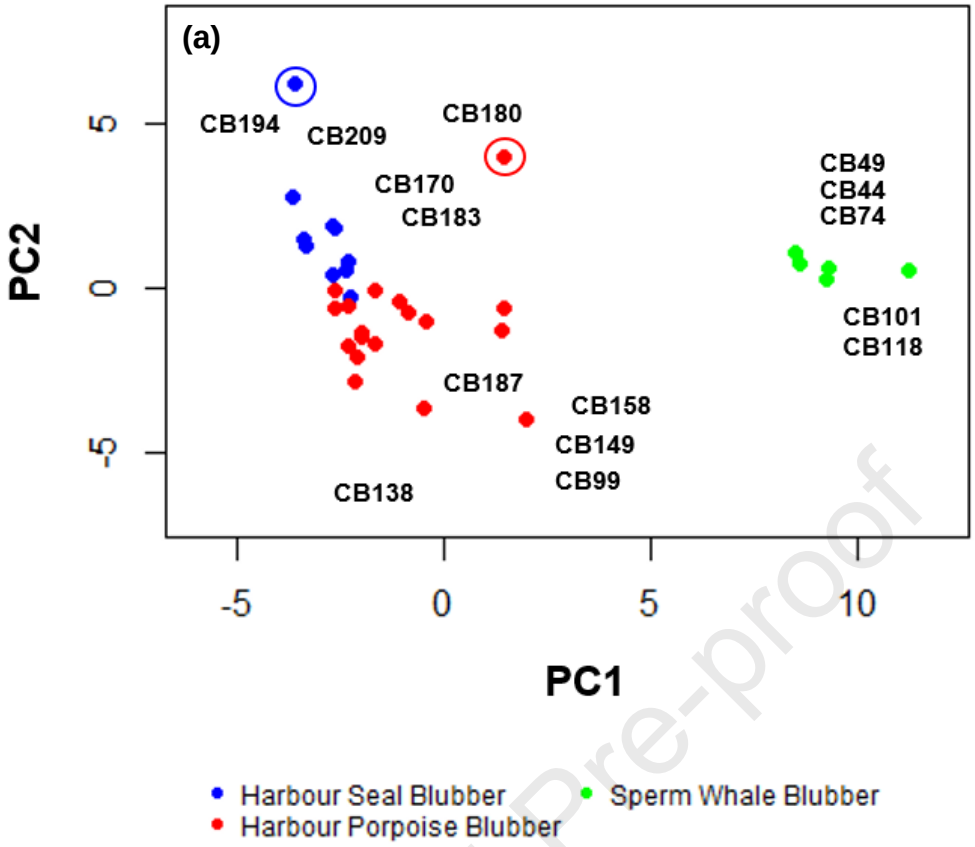



**Figure 3:** $\Sigma PBDE_9$ concentration for harbour porpoise presented on the basis of Scottish Biogeographic Region. Harbour porpoise from the Minches and Western Scotland (region 3 on the map) had a significantly lower $\Sigma PBDE_9$ concentration in their blubber (84.25 µg/kg lw ± 37.94 µg/kg lw; n=5) ($p < 0.05$, ANOVA, Tukey) than those from the Scottish Continental Shelf (193.0 ± 58.22 µg/kg lw; n=2; region 2 on the map), Northern North Sea (300.6 ± 151.5 µg/kg lw; n=6; region 1 on the map) and Irish Sea (1,129 ± 1,443 µg/kg lw; n=5; region 4 on the map). The circles represent the relative average concentration.



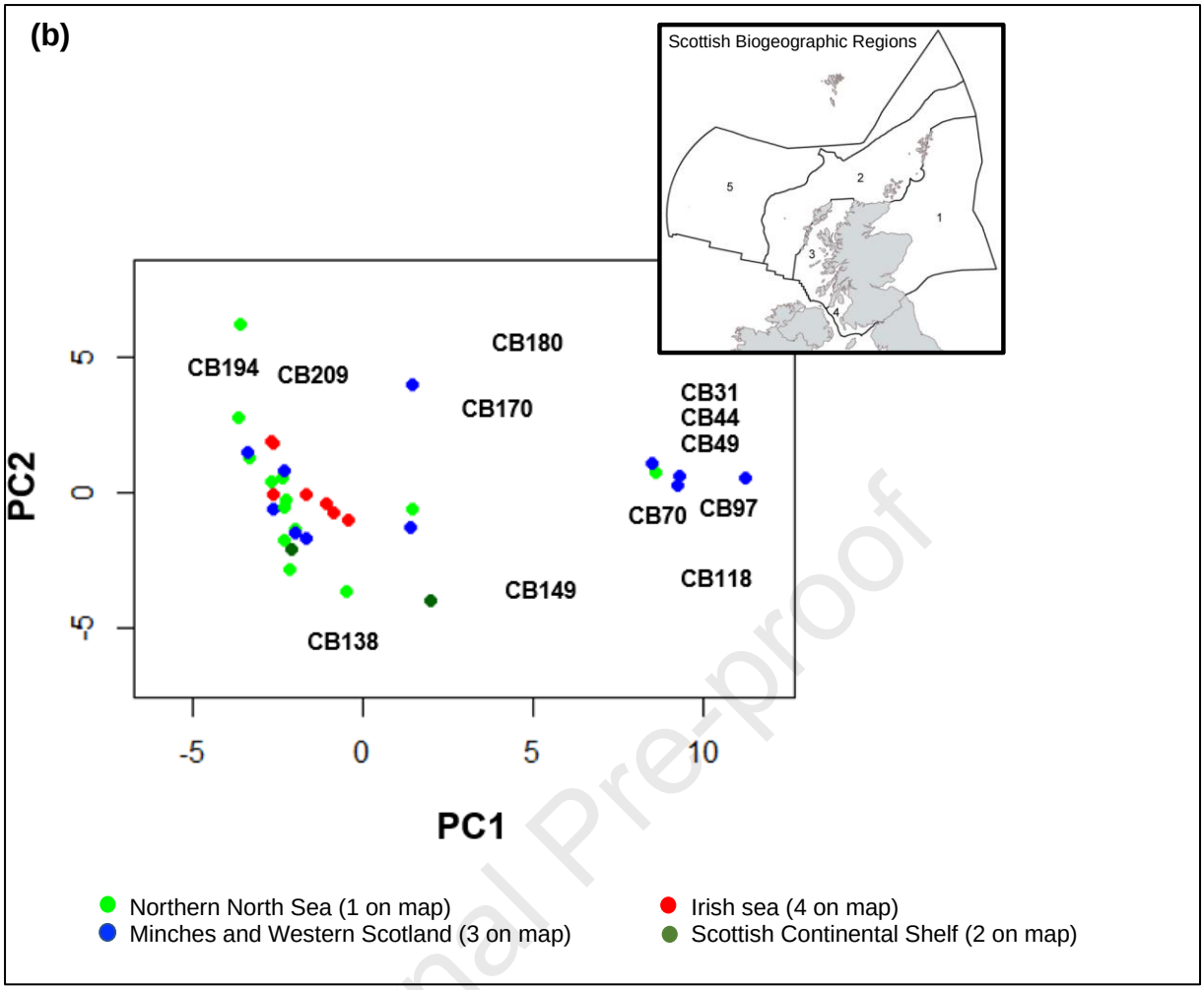



**Figure 4** PCA score plot (normalised to the concentration of CB153) demonstrating the **a)** variation in the PCB profiles across the three marine mammal species; **b)** variation in the PCB profiles across the four marine mammal biogeographic sampling locations which are shown in the map. The harbour seal and harbour porpoise samples that are circled on Figure 4a correspond to individuals with a different PCB profile and FA profile when compared to the other samples and as reported in Madgett *et al*., (2019). The sperm whale samples are well separated in the score plot from the harbour seal and harbour porpoise samples. Although there is some overlap between harbour seal and harbour porpoise it is limited to a few samples, with good separation for others due to the more negative positioning of the harbour porpoise samples with the second component. The five points more positively correlated to the first component at +10 on Figure 4b are identified in Figure 4a as sperm whale, suggesting a species influence on the marine mammal categories rather than regional influence.

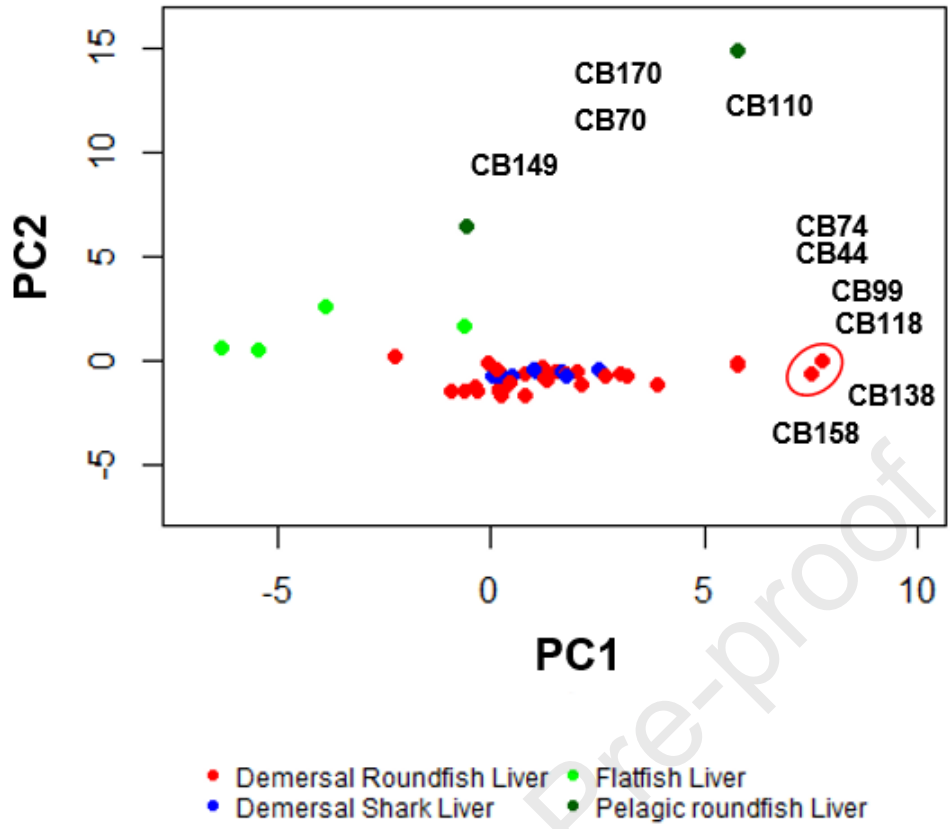



**Figure 5:** PCA score plot demonstrating the variation in the PCB profiles (normalised to the concentration of CB153) across the shark and three fish liver sample categories. Hake sample pools (n=2) are separated from the demersal roundfish liver category and are identified with a red ellipse. Ellipses drawn are illustrative only and have no statistical meaning.

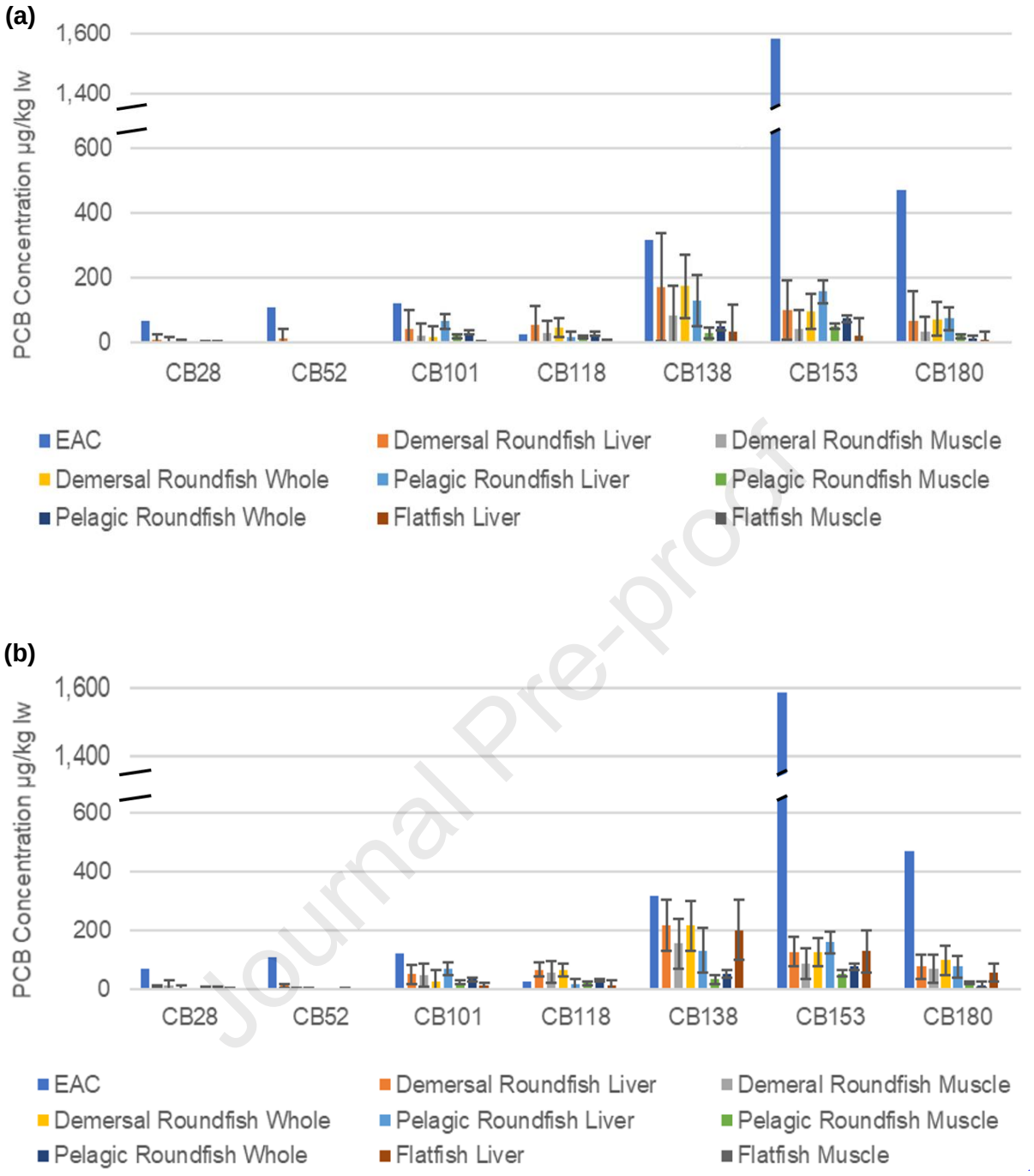
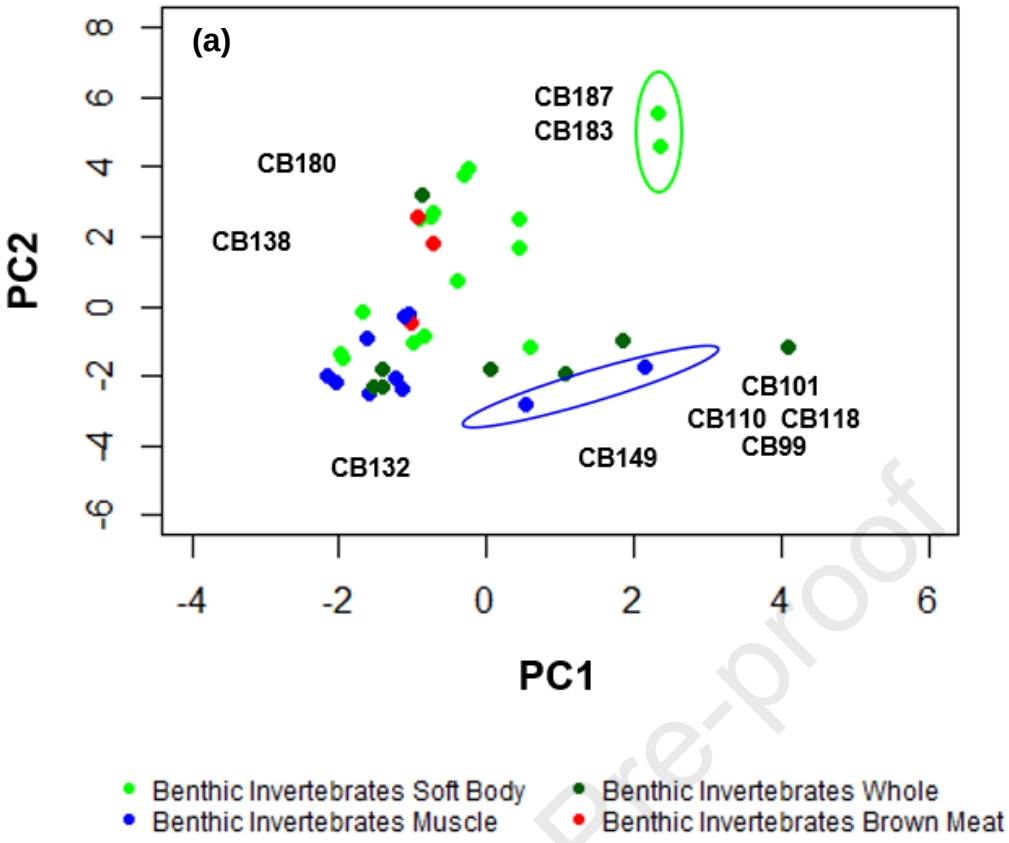
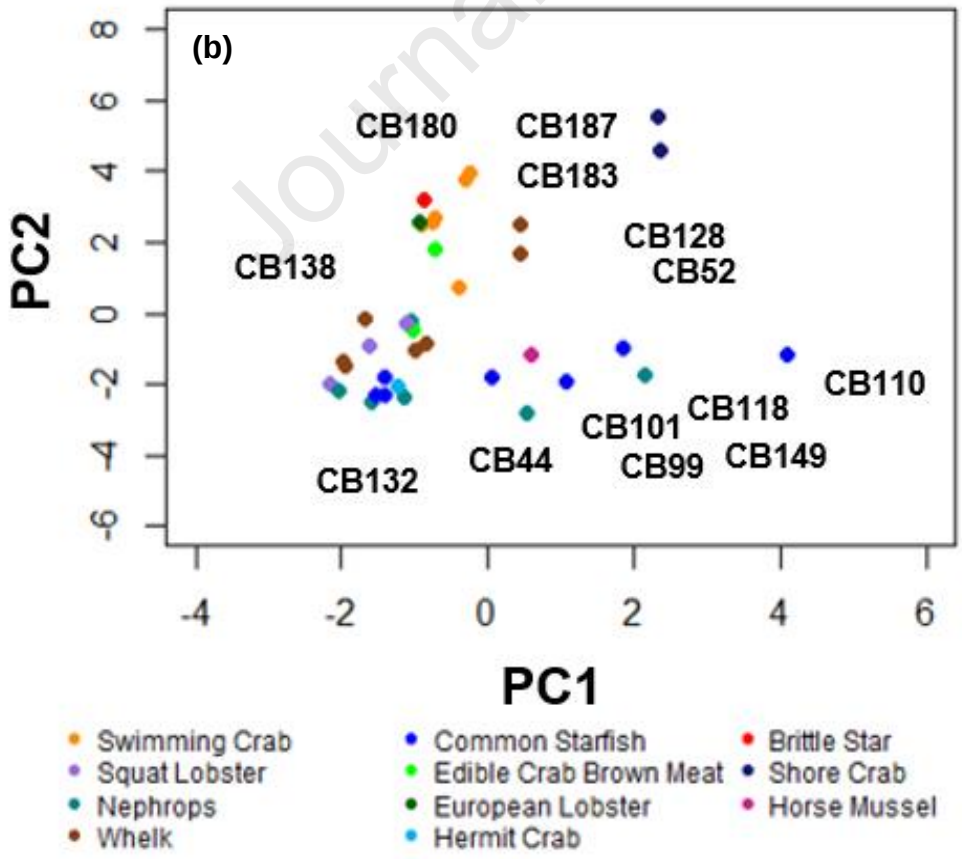



**Figure 6:** The concentrations of each ICES-7 PCB congener in pooled fish tissue (liver, muscle and whole) from **a)** all biogeographical regions (Irish Sea, Northern North Sea and Scottish Continental Shelf) and **b)** the Irish Sea (Clyde and Solway) biogeographic region in comparison to the Environmental Assessment Criteria (EAC) (µg/kg lw). Error bars represent one standard deviation.



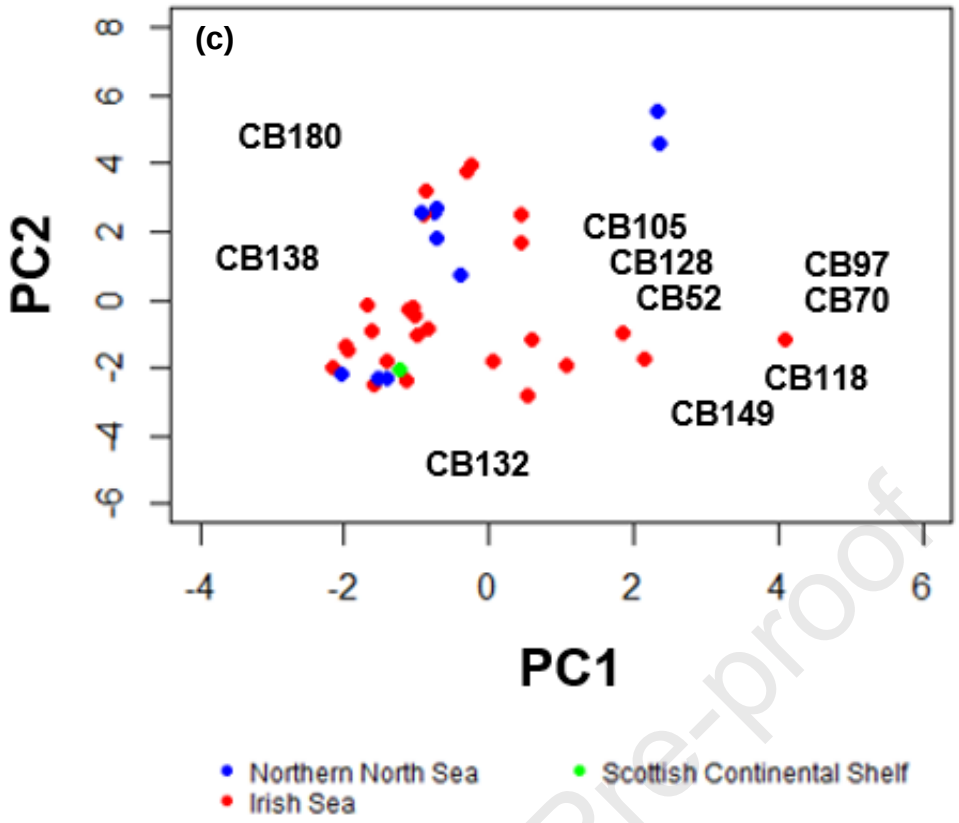



**Figure 7:** PCA score plot demonstrating the variation in the PCB profiles (normalised to the concentration of CB153) across the **a)** four benthic invertebrates sample categories; **b)** eleven benthic invertebrates species; **c)** three Biogeographic regions. Two shore crab sample pools are identified on Figure 7a with a green ellipse and two *Nephrops* sample pools are identified using a dark blue ellipse (discussed in main text). CB114 and 189 were not included as they were < LoD in all samples. Similarly, demersal invertebrates (squid) are not included because the individual congeners were all < LoD. Ellipses drawn are illustrative only and have no statistical meaning.

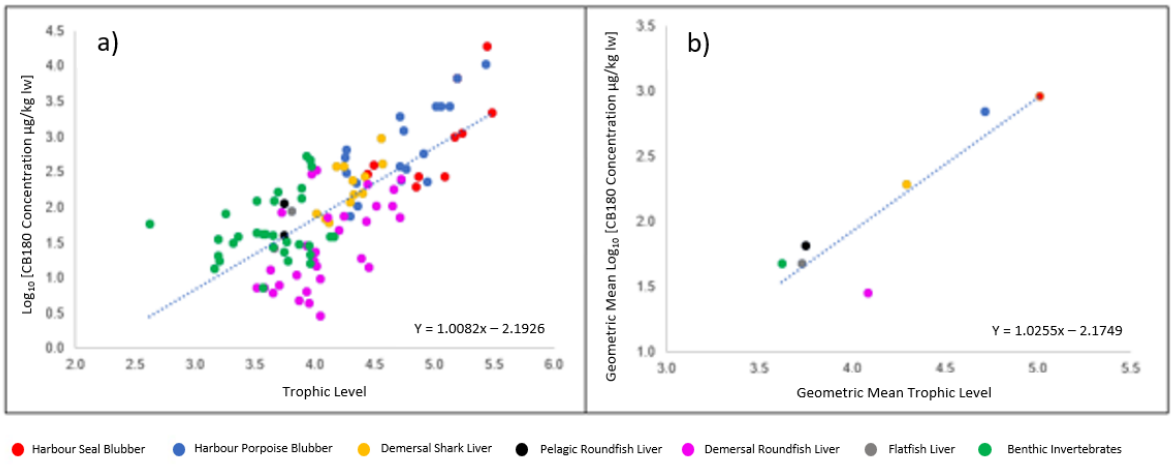



**Figure 8: (a)** Relationship between trophic level and logarithmically transformed CB180 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, muscle, brown meat, soft body (green) from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf. **(b)** Relationship between geometric mean trophic level and logarithmically transformed geometric mean CB180 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, muscle, brown meat, soft body (green) from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.

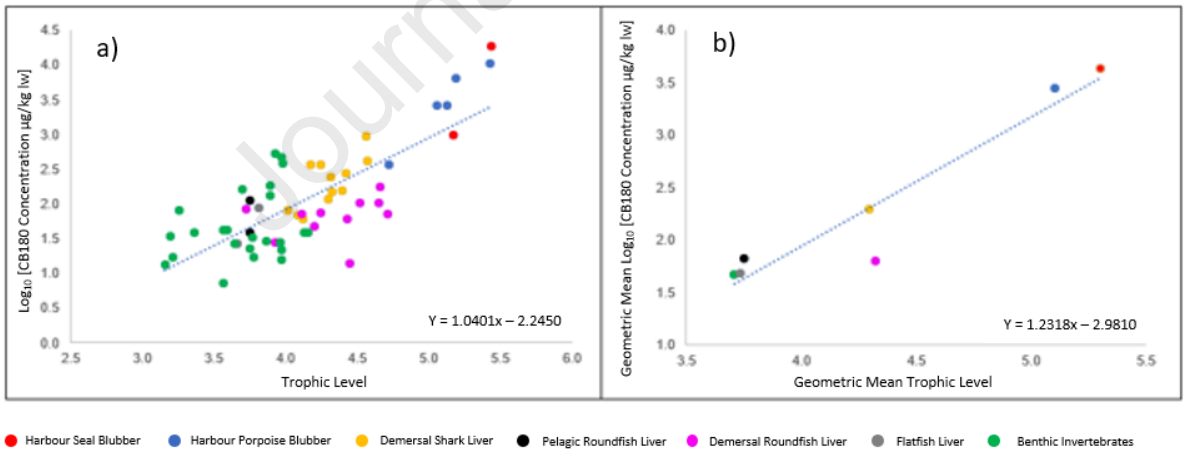



**Figure 9**: (a) Relationship between trophic level and logarithmically transformed CB180 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, muscle, brown meat, soft body (green) from the Irish Sea Biogeographic Region (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean CB180 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, muscle, brown meat, soft body (green) from the Irish Sea Biogeographic Region.

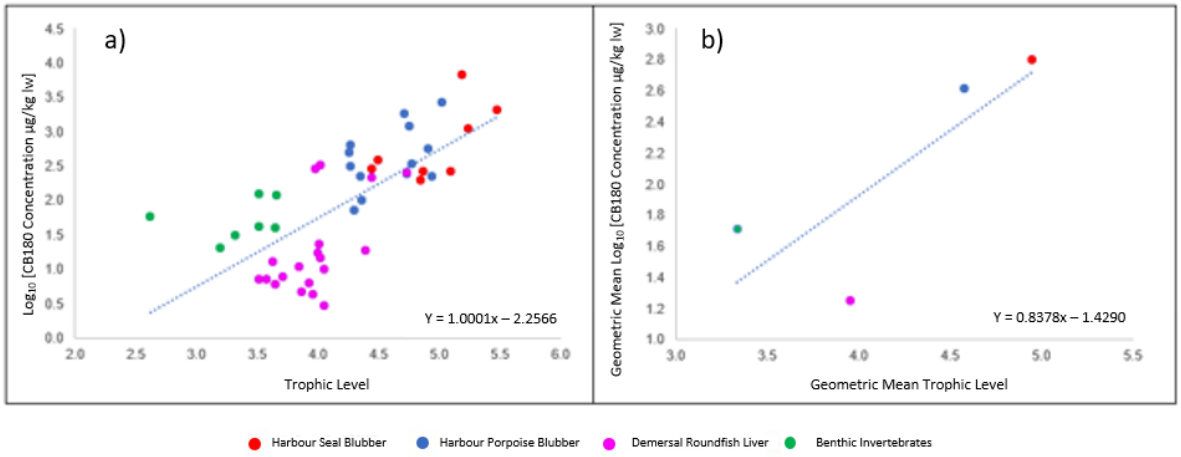



**Figure 10: (a)** Relationship between trophic level and logarithmically transformed CB180 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal roundfish liver and benthic invertebrate whole, muscle, brown meat, soft body (green) from the Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf. (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean CB180 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), demersal roundfish liver (pink) and benthic invertebrate whole, muscle, brown meat, soft body (green) form the Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf.

## Highlights

- All marine mammals, demersal and pelagic fish had detectable PCBs in their tissues.
- Diet contributed to PCB and PBDE concentration and congener variability.
- Trophic magnification was found to occur for the ICES-7 PCBs and BDE47.
- An unbalanced dataset was found to influence the calculated TMF.
- The TMF of CB52 calculated in this study was higher than reported globally.

**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: