Bioaccumulation of persistent organic pollutants and trace metals in Scottish marine food webs and their relationship with trophic level and fatty acid signatures.

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Bioaccumulation of Persistent Organic Pollutants and Trace Metals in Scottish Marine Food Webs and their Relationship with Trophic Level and Fatty Acid Signatures

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Declaration

I declare that the work presented in this thesis is my own, except where otherwise acknowledged, and has not been submitted in any form for another degree or qualification at any other academic institution.

Information derived from published or unpublished work of others has been acknowledged in the text and a list of references is given.

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List of abbreviations

ANOVA	Analysis of Variance
ASE	Accelerated Solvent Extraction
BAC	Background Assessment Concentration
BC	Background Concentration
BDE	Brominated Diphenyl Ether
CB	Chlorinated Biphenyl
CEMP	Coordinated Environmental Monitoring Programme
CRM	Certified Reference Material
CSEMP	Clean and Safe Seas Environmental Monitoring Programme
EAC	Environmental Assessment Criteria
EC	European Commission
EQS	Environmental Quality Standards
EI	Electron Impact
ERM	Environmental Resources Management
EU	European Union
EV	Electron Volt
FA	Fatty Acid
FAl	Fatty Alcohol
FAME	Fatty Acid Methyl Ester
FATM	Fatty Acid Trophic Marker
FBDE	Fluorinated Brominated Diphenyl Ether
FEQG	Federal Environmental Quality Guidelines
GC	Gas Chromatography
GC-ECNIMS	Gas Chromatography – Electron Capture Negative Ionisation Mass Spectrometry
GC-EIMS	Gas Chromatography – Electron Impact Mass Spectrometry
GC-FID	Gas Chromatography – Flame Ionisation Detection
GC-MS	Gas Chromatography – Mass Spectrometry
GES	Good Environmental Status
IAEA	International Atomic Energy Agency
ICES	International Council for the Exploration of the Sea
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
id	Internal Diameter

JAMP	Joint Assessment & Monitoring Programme
LoD	Limit of Detection
LRM	Laboratory Reference Material
lw	Lipid Weight
MERMAN	Marine Environment Monitoring and Assessment National (Database)
MIME	Working Group on the Monitoring and on Trends and Effects of Substances in the Marine Environment
MSS	Marine Scotland Science
MoD	Ministry of Defence
MRV	Marine Research Vessel
MS	Mass Spectrometry
MUFA	Monounsaturated Fatty Acid
m/z	Mass/Charge
OCP	Organochlorine Pesticides
PAH	Polycyclic Aromatic Hydrocarbon
PBDE	Polybrominated Diphenyl Ether
PCA	Principal Component Analysis
PCB	Polychlorinated Biphenyl
PLE	Pressurised Liquid Extraction
POP	Persistent Organic Pollutant
PSI	Pounds per Square Inch
PUFA	Polyunsaturated Fatty Acid
SCA	Stomach Content Analysis
SEPA	Scottish Environment Protection Agency
SD	Standard Deviation
SI	Stable Isotope
SIA	Stable Isotope Analysis
SIM	Selective Ion Monitoring
SFA	Saturated Fatty Acid
SMASS	Scottish Marine Animal Strandings Scheme
TBT	Tributyltin
THg	Total Mercury
TMF	Trophic Magnification Factor
UKAS	United Kingdom Accreditation Service
ww	Wet Weight

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Summary

There is a global programme of action in place for the protection of the marine environment to ensure our seas are clean and safe. One of the biggest threats to our oceans is man-made pollution and it is the responsibility of governments to conduct assessments to advise policy. Across the North-East Atlantic, Contracting Parties to the OSPAR Convention for the Protection of the Maine Environment of the North-East Atlantic, including the United Kingdom, are required to undertake monitoring and assessment of contaminants. The assessment utilises assessment criteria, including Background Assessment Concentrations (BAC) and Environmental Assessment Criteria (EAC). Guidelines for monitoring contaminants in biota include specific shellfish, flatfish and roundfish, as well as seabird eggs. Extending the assessment to other species has considerable merit, but such species may, for example, be more difficult to sample, with generic trophic level values obtained from literature and databases adding additional uncertainty to assessments. Currently, assessment criteria for organic and inorganic contaminants either do not account for secondary poisoning as a route of exposure, or a proxy is used due to the lack of ecotoxicological data available. Secondary poisoning is a result of biomagnification, which can be expressed as the trophic magnification factor (TMF; the average increase in concentration per trophic level).

Fatty acid (FA) signatures and stable isotope (SI) ratios were used to develop an understanding of Scottish marine food web ecology and reliably ascribe trophic levels to a wide range of species. Analysis was conducted on 215 samples from different locations around Scotland which comprised of seven fish species, one shark species, fourteen marine invertebrate species, three marine mammal species and two zooplankton species. The concentrations of three priority heavy metals and six additional trace metals and metalloids, thirty-two PCB congeners and nine PBDE congeners were determined to investigate the relationship between concentration and potential influencing factors (trophic level, region, sample categorisation and physiological features). TMFs were calculated using two methods on selected PCB and PBDE congeners and metals and metalloids possessing a significant trophic relationship. It was concluded that ecosystem specific TMFs can be used as a reliable tool, permitting the assessment of a wider range of species, but a reasonable balance with respect to sample numbers of lower- versus higher-trophic level organisms is highly recommended when calculating TMFs.

Key words: Trophic level, Fatty acids, Stable Isotopes, PCBs, PBDEs, Trophic magnification, Bioaccumulate, Contaminant, Assessment, Scotland

Chapter 1

General Introduction

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1.1 Introduction

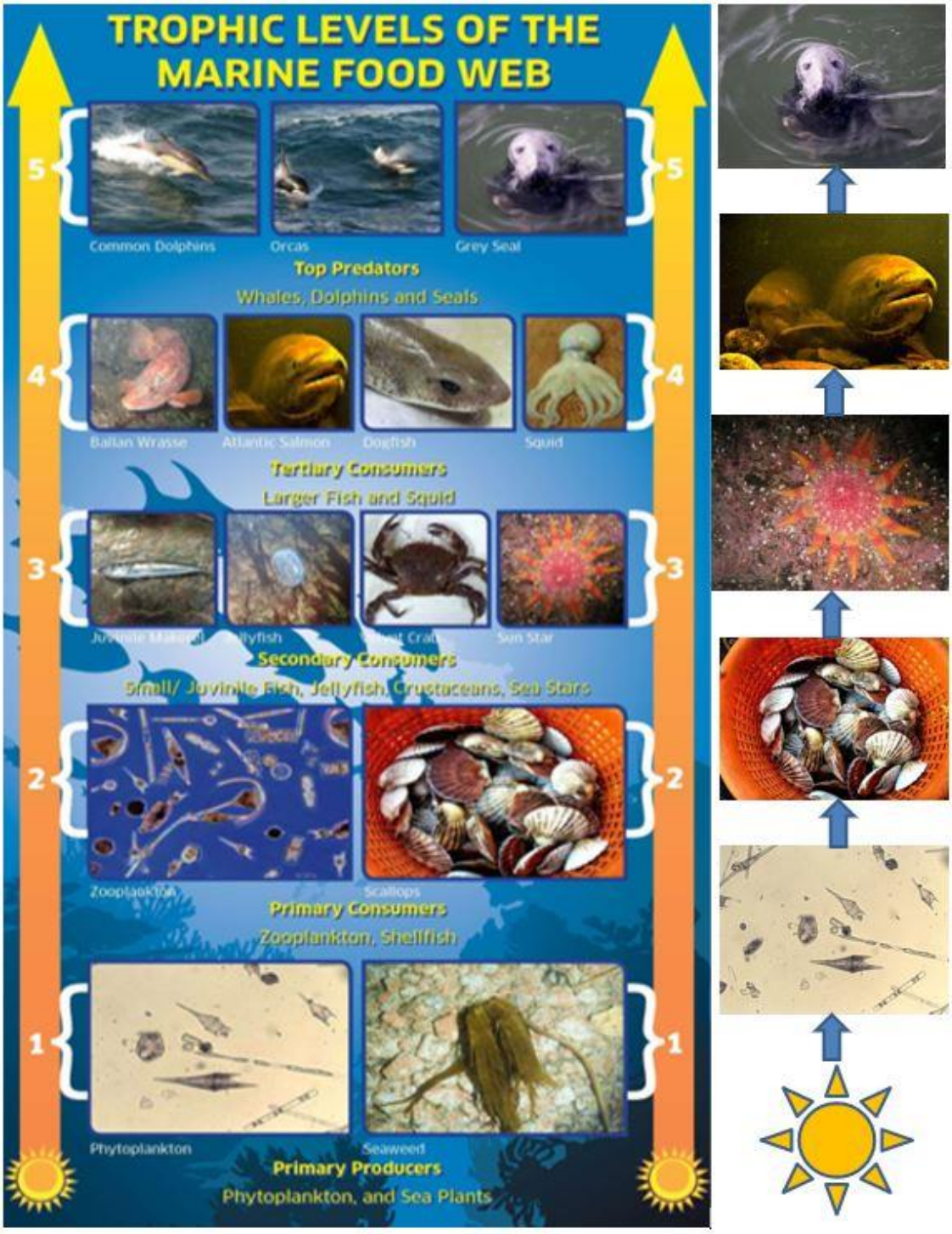
Scotland's seas cover an area six times that of the Scottish landmass and support a rich and abundant variety of marine life and habitats. This includes over 120 species of fish, both demersal and pelagic, rich cold-water coral communities, algal communities, aggregations of sponges as well as the varied marine mammals and seabirds, all of which contribute to maintaining the balance of the natural environment and rely on clean and healthy seas to flourish.

The most significant pressure influencing the marine environment is human activity (Halpern *et al*., 2019). There is proven evidence that ecosystem components have been, and continue to be, impacted through activities such as overfishing, aquaculture, land-based pollution and transport (Derraik, 2002). The seas' economic contribution to Scotland cannot, however, be overstated, netting billions of pounds from oil and gas, transport, fishing, and in more recent years tourism, including eco-tourism (The Scottish Government, 2019). Other activities that have impacted the marine environment include the disposal of treated industrial effluent and urban wastewater, leisure and recreational use and developments in the renewable energy sector (Dolman and Simmons, 2010). There are actions in place for EU marine waters to mitigate the effects of many pressures on the marine environment to achieve the aim of having "clean, healthy, safe, productive and biologically diverse marine and coastal environments, managed to meet the long-term needs of nature and people". These include the development and improvement of monitoring, management, scientific research and the raising of awareness through education.

1.2 Ecosystems and food webs

The term "ecosystem" was first used in print in 1935 by A.G. Tansley in his paper describing vegetational concepts and terms (Tansley, 1935). He expanded on the concept of a system or "biome" which describes the whole community of organisms inhabiting a particular region. Tansley stressed the importance of the interactions between biotic and abiotic elements forming one physical system. He concluded that "the ecosystem may be formally defined as the system composed of physical-chemical-biological processes active within a space-time unit of any magnitude" (Lindeman, 1942). Subsequently the term 'trophic level' was established, categorising organisms depending on their energy level and nutritional requirements.

Ecosystems are therefore defined by food webs which describe a network of energy flow composed of overlapping and interconnecting food chains. A food chain describes one possible path that energy and nutrients may take as they move from primary producers (autotrophs) who produce their own food and energy (photoautotrophs and chemoautotrophs) to consumers (heterotrophs) that feed upon them (Jacob *et al*., 2011; Ashok, 2016). Food webs support short and/or complex food chains (Figure 1.1) composed of a variety of trophic levels (Briand and Cohen, 1987). The position an organism occupies in a food chain is represented using a level followed by numerical value from 1, primary producers, to 5, apex predators (Pavluk and Vaate, 2008). For a single species there will be a natural variation in its trophic level as individuals or populations may feed at more than one level. In addition, the life stages of some species occupy different trophic levels (Davis *et al*., 2012: Giraldo *et al*., 2016).



Marine Scotland, 2015

Figure 1.1: The major components of an aquatic food web showing the network of feeding relationships (from primary producers to apex predators) existing among species in a marine community and their trophic level. A food chain represents the movement of energy through a group of biota. The numbers 1 – 5 represent the trophic levels.

1.2.1 Fatty acids

Establishing the impact of contaminant concentration on the wider marine food web requires an understanding of trophic level structure, feeding patterns and nutritional relationships (Burkhard, 2003; MIME, 2016). Lipids, including fatty acids (FAs) are an important source of energy in marine ecosystems and are involved in several biochemical pathways (Ibarguren, López and Escribá, 2014). When lipids, such as triacylglycerols, are digested in marine organisms, FAs are released and absorbed, but not degraded. The FAs of interest exist as long lipid-carboxylic acid chains and FA profiles in storage and structural lipids and are indicative of an organisms' average, long-term feeding pattern. The FA profiles of primary producers are passed up the food chain and modified at each trophic level through biological processes such as metabolism and biosynthesis, but there are recognised patterns due to the conservation of specific FAs (Sikorski, 2006).

To describe the structure of a FA molecule, the length of the carbon chain (number of carbons), the number and position of double bonds present and the position of the first double bond relative to the methyl terminal must be considered. An example of a polyunsaturated FA (PUFA) is 22:6(n-3) (docosahexaenoic acid; DHA) which is shown in Figure 1.2.

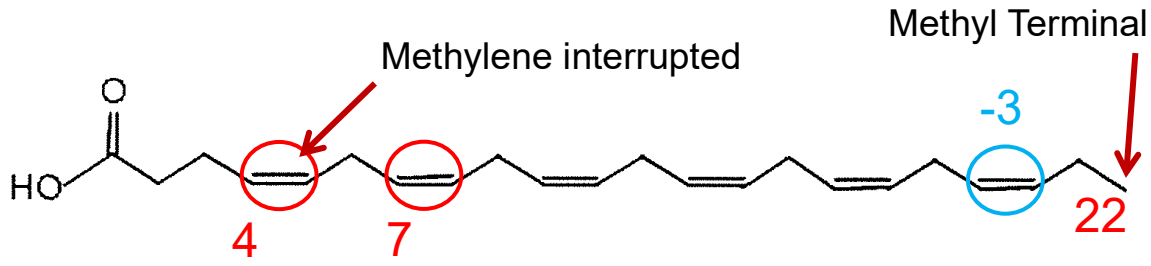


Figure 1.2: The polyunsaturated fatty acid (PUFA) *cis*-4,7,10,13,16,19-docosahexaenoic acid (22:6(n-3)) containing six double bonds in its hydrocarbon chain. The nomenclature numbers from the carboxylic acid while the (n-3) specifies the position of the first double bond relative to the methyl terminal.

The FA composition of marine lipids is more complex than those found in terrestrial plants and animals. The carbon chain is generally from 14 to 24 carbons and marine FAs are particularly high in unsaturated compounds. Most of the PUFAs of fish lipids occur as the n-3 type whereas the n-6 type only makes up a small percentage (Colombo *et al*., 2016). Fish lipids can also contain FAs with an odd number of carbons in the chain such as 15 and 17. Two important FAs in marine organisms are all-*cis*-5,8,11,14,17-eicosapentaenoic acid (20:5(n-3), EPA), which is synthesised by marine algae, and all-*cis*-4,7,10,13,16,19-docosahexaenoic acid (22:6(n-3), DHA) which is synthesised by zooplankton (Budge, Iverson and Koopman, 2006). The feeding habits of marine

organisms can be determined using the ratio of these FAs; organisms feeding on zooplankton will contain a higher proportion of 22:6(n-3) in their lipids than 20:5(n-3). Specific signatures like these are known as “fatty acid trophic markers” (FATM) which can be used to indicate the trophic level and diet of an organism (Dalsgaard *et al.*, 2003). Connelly, Deibel and Parrish (2014) found FATMs to be a powerful tool, predicting marine taxa with 99% accuracy. FA analysis has advantages over stomach content analysis (SCA) in that it provides information on time-averaged feeding patterns and can be applied to many different species. For example, a study by Varela *et al.*, (2019) found FA analysis to be more effective in segregating skipjack tuna geographical groups (three Spanish marine regions) than SCA, and a study by Pethybridge, Daley and Nichols (2011) found FA analysis of 16 co-occurring shark species and chimeras to detect relative diet variation, diet specialisation, niche partitioning within species and developmental shifts in diet which SCA could not detect. This study also found that FA analysis required less specimens and was less destructive than SCA techniques, was more cost effective and tangible for species that are of high conservation concern and are logistically difficult to obtain (especially deep-water species).

Previous studies have used FAs as biomarkers for trophic level indication in marine mammals (Budge *et al*., 2008; Guerrero *et al*., 2016), shark (Pethybridge, Daley and Nichols, 2011), fish (Würzberg *et al.*, 2011; Olsen *et al.,* 2015), invertebrates (Allan *et al.,* 2010; Soler-Membrives, Rossi and Munilla, 2011; Rabei *et al*., 2018) and zooplankton (Gonçalves *et al*., 2012; Deschutter *et al.,* 2019). However, these biomarkers can be affected by an organism's ability to metabolise and transform FAs which may vary within and between species at the same or similar trophic levels. They should therefore be used with caution or in conjunction with other quantitative techniques for identifying trophic level such as stable isotopes (SI) (Alfaro *et al.,* 2006).

1.2.2 Stable isotope ratios

Isotopes are atoms of the same element that have different numbers of neutrons but the same number of protons and electrons (Kendall and Doctor, 2003). Although the atomic number remains the same, the difference in the number of neutrons between the various isotopes of an element means that the various isotopes have different weights. The superscript number to the left of the element abbreviation indicates the number of protons plus neutrons in the isotope. Isotopes of the same element share the same chemical character, whilst isotopologues, containing at least one stable isotope, have different

physical properties (melting points, boiling points etc.). The nuclei of some isotopes are unstable and radioactive (Stewart, 2009).

The elements C, N, S, H, and O all have more than one isotope. Carbon, for example, has three naturally occurring isotopes: ^{12}C (carbon-12), ^{13}C (carbon-13) and ^{14}C (carbon-14), where ^{14}C is radioactive and gives out beta ray and ^{12}C and ^{13}C are classed as stable isotopes, with ^{12}C the more abundant of the two amounting to 98.93 % of carbon. Stable isotopes (SI) are therefore defined as non-radioactive forms of atoms (Waring and Running, 2007).

Stable isotope analysis (SIA) has emerged as a common tool in ecology and has proven especially useful in the study of animal diet, habitat use, movement, and physiology (Newsome, Clementz and Koch, 2010). The ratio of SI is expressed for those with relative abundances affected by isotope fractionation in nature. Stable isotopic abundances of ^{15}N and ^{13}C in biochemical compounds in animals, as expressed by $\delta^{15}N$ and $\delta^{13}C$ values, are influenced by diet. Analysis of these isotopic signatures can be indicative of trophic position. The positive shift of 0 to +1 ‰ in mixed tissue from one trophic level up to the next is too small for precise determination of trophic level (Hobson *et al.,* 2002) but can be used to establish diet and general feeding habits; for example, phytoplankton tends to be more depleted in ^{13}C than benthic primary producers such as eukaryotic algae and cyanobacteria possibly due to reduced water turbulence (France, 1995). There is however a large degree of intraspecific variation due to factors such as species, region and season. For example, a study by Gearing *et al.,* (1984) found the ^{13}C from phytoplankton varied with taxon and size, ranging from -20.3 ± 0.6 ‰ to -22.2 ± 0.6 ‰ and a study by Wada *et al*., (2012) reported the natural abundances of ^{15}N and ^{13}C for cyanobacteria which was characterised by extremely low $\delta^{15}N$ and widely ranging $\delta^{13}C$ (-25 to -3 ‰).

The $\delta^{15}N$ has a positive shift of 3.4 - 3.8 ‰ (DeNiro and Epstein, 1981; Fry and Sherr, 1984; Hobson and Welch, 1992; Post, 2002; McCutchan *et al*., 2003) with each increasing trophic level, allowing more accurate identification of trophic position. A fixed value of 3.4 ‰ is commonly used to estimate relative species trophic level and food web structure in additive food web structure models. A study by Hussey *et al*., (2014) suggests, however, that consumer discrimination is not constant between trophic levels but decreases (narrows) with increasing dietary $\delta^{15}N$. It is suggested that failure to take this into account using a 'scaled' model rather than an additive model results in the underestimation of the trophic level of top predators and leads to the compression of food web length contrary to field data. Despite this, the "narrowing effect" is not currently

considered in trophic level adjustments as more data is required to establish a procedure which has the potential to alter the recalculated assessment values of organic and inorganic contaminants in the marine environment (European Commission, 2014). Current studies on contaminant transfer continue to use 3.4 ‰ as a fixed value (Fliedner *et al.,* 2018).

SIA is widely used to estimate food web trophic levels and dietary composition. A study by Jansen *et al.,* (2012) on marine mammals, found that the analysis of $\delta^{13}C$ and $\delta^{15}N$ in muscle and bone samples collected from 157 stranded porpoises and 30 prey species along the Dutch coast revealed geographic differences in isotopic composition in prey and identified dietary patterns, foraging areas and seasonal groupings in harbour porpoise. In fish, a study by Croizier *et al*., (2019) caught around Africa, found that in the muscle of 132 fish from 23 different species, $\delta^{13}C$ differentiated between demersal/coastal species and reflected the use of estuarine habitat rather than offshore pelagic signatures. They found a positive correlation between mercury (Hg) concentration and $\delta^{13}C$, suggesting Hg exposure in individuals is a result of the involvement of the coastal estuarine environment and sediments. Within invertebrates, a study in India by Bouillon *et al.,* (2002) found a distinct spatial gradient in consumer $\delta^{13}C$ values in benthic invertebrate species between bay regions with varying distances from mangroves (22 sites). They found that mangrove-derived and other terrestrial carbon is not a significant food source for benthic invertebrate communities in this ecosystem during the pre-monsoon period, with a marked selectivity for pelagic and benthic microalgal food sources. The $\delta^{15}N$ was found to be a useful indicator of trophic level but overlap between $\delta^{15}N$ values of presumed low and higher trophic levels did occur due to differences in inorganic nitrogen sources and availability; Also, in zooplankton, a study by School *et al.,* (2018) used SIA and FA analysis to examine how the variability at the base of the food web affects trophic interactions between primary producers and copepod consumers. They found that relative contributions of autotrophic and heterotrophic fractions in the zooplankton determined the SI signal of the $\delta^{15}N$ in copepods, showing the complexity of trophic relations in planktonic food-webs.

The combination of FA analysis and SIA has proven to be a more powerful tool to determine trophic interactions in complex food webs (van der Bank *et al.,* 2011; Falk-Petersen and Gislason, 2012; Gaillard *et al*., 2017). The advantage of this combined approach is mainly attributed to the fact that FAs are more specific to dietary source than stable carbon isotopes, particularly when differences in the $\delta^{13}C$ of different carbon sources are small (El-Sabaawi *et al.,* 2009). For example, a study by McMeans, Arts

and Fisk, (2015) combined the techniques of SIA and FA trophic markers whilst analysing Hg contamination in Greenland Sharks. They found that the Log_{10}THg (total mercury) increased significantly with trophic position (calculated using δ^{15}N) and regressions between shark THg and individual FA proportions revealed that 18:1(n-9) explained a significant amount of the variability of Log_{10}THg in shark. It was concluded that Hg bioaccumulated in this species and concentrations were within the previously reported range for warm-blooded Arctic predators and a better understanding of feeding behaviour and food web characteristics was achieved using both techniques compared to each in isolation. Another study by Young *et al*., (2018) found that the analysis of δ^{15}N and δ^{13}C was limited in distinguishing a diverse group of prey species (including fish, cephalopods, and crustaceans), as most of the prey had similar δ^{15}N ranges. FA profiles were able to resolve four separate prey groups with clarity, providing a temporal contrast to the stomach content "snapshot".

1.3 Contaminants

Understanding the transport and fate of contaminants in marine ecosystems and their potential effects on aquatic organisms is critical for hazard assessment, especially in areas where agriculture or urbanisation are dominant. Hazardous materials are defined as 'accumulative substances' with the ability to inflict harmful effects on marine life and ultimately humanity (OSPAR, 2009a). Contaminants can often reach concentrations that threaten aquatic life due to their persistent and bioaccumulative properties, resulting in the disruption of biological processes, reproductive abnormalities, alterations in development and behaviour and mortality (Crump and Trudeau, 2009; Murphy *et al*., 2015). These contaminants can either be of an organic or inorganic nature.

1.3.1 Persistent organic pollutants

Persistent organic pollutants (POPs) represent a vast category of synthetic 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). The Stockholm Convention is a global environmental treaty, signed in 2001 and effective from May 2004, that aims to eliminate or restrict the production and use of persistent organic pollutants (United Nations, 2015). PCBs have a long half-life (2 months – 30 years) dependent upon their chemical structure

(Sinkkonen and Paasivirta, 2000) and poor solubility in water. Environmental fate and transport behaviour can be predicted from the octanol-water partitioning coefficient (K_{ow}), typically expressed in logarithmic form, which is a measure of how a chemical will partition in a solution of a polar and non-polar solvent (Cicilio, 2013). The Log K_{ow} value of PCBs has been reported to have a range of 4-9 in 209 congeners (Hawker and Connell, 1988) and PBDEs reported to have a range of 3-6 in 23 congeners (Bao, You and Zeng, 2011) indicating hydrophobic character. OSPAR define persistent pollutants as having a half-life of greater ≥50 days and hydrophobic as having a Log K_{ow} ≥4 (OSPAR, 2019). Due to their persistent and lipophilic properties, PCBs and PBDEs bioaccumulate in various lower trophic organisms such as plankton, moving up the food chain through bivalve molluscs, fish, reptiles, marine mammals, birds, and terrestrial mammals (Kodavanti, 2017).

PCBs make up a group of congeners composed of 209 individual chlorinated biphenyl rings based on the number and position of chlorine atoms attached to the ring structure (Figure 1.3). Each carbon is numbered, and the position of the chlorine atom(s) provides the basis for the systematic numbering system proposed by Ballschmitter and Zell (1980). This system is widely accepted and known as *Ballschmitter nomenclature*.

PCBs are categorised under two major groups based on their toxic potential: the dioxin-like PCBs, which share common toxicity mechanisms with dioxins (highly toxic range of compounds produced as a by-product in some manufacturing processes), and the non-dioxin-like PCBs (Renieri *et al*., 2019). Dioxin-like PCBs have no more than one chlorine atom at the ortho-position (polychlorinated non-ortho and mono-ortho biphenyls) and the molecules can rotate and adopt a coplanar structure, while non-dioxin PCBs have two or more of the ortho-positions in the biphenyl molecules occupied by chlorine molecule and the two phenyl rings are consequently not in the same plane (Ssebugere *et al.*, 2019). The planar conformation of the non- and mono-ortho PCBs (as with dioxins and other dioxin-like compounds) is a requirement for high affinity binding of such the compounds to the aryl hydrocarbon receptor (AhR) binding pocket (Zhang *et al.,* 2012). AhR is a widely expressed nuclear transcription factor found in the cytosol; and the binding of a suitable ligand to the receptor induces translocation of the ligand-receptor complex into the nucleus, where further downstream signalling occurs leading to a range of harmful biological effects which have been previously reviewed (Okey *et al*., 1994). Non-ortho PCBs are normally found at much lower concentrations when compared to the ortho-PCBs but are more biologically active. The rest of the PCBs, with non-planar

conformation due to chlorine substitution at ortho position, do not bind to the AhR and are referred to as non-dioxin-like PCBs (Viluksela *et al.*, 2012).

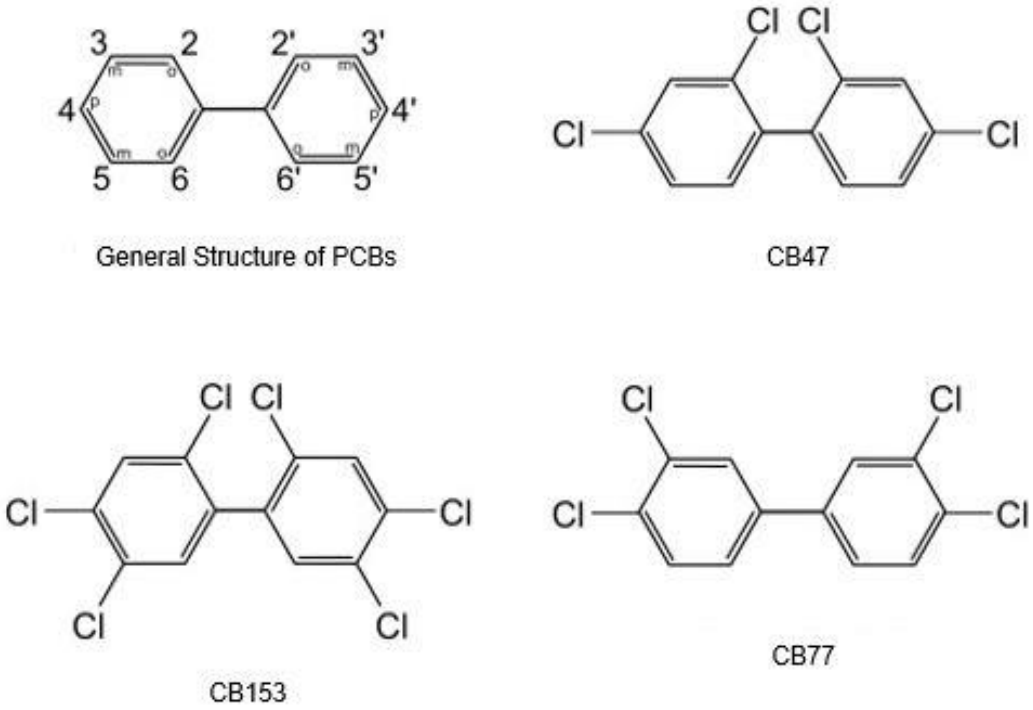


Figure 1.3: General structure of PCBs and structures of selected CB congeners. The numbers in the generalised structure indicate the position of the chlorine atoms. The letters (o), (m), and (p) indicate ortho, meta-, and para- substitutions for chlorine side groups. Non-dioxin like CB47 and CB153, as well as dioxin-like CB77 are shown here as examples (Kodavanti and Loganathan, 2014).

PBDEs are a group (209 congeners) of brominated flame retardants. PBDEs consist of a diphenyl ether, with 1–10 bromine atoms substituted on the rings and are classified according to the average number of bromine atoms in the molecule (Figure 1.4). The number and position of bromine atoms on the diphenyl ring structure determines the congener identification number. Highly brominated PBDEs (>5 bromine atoms) can undergo de-bromination in the environment to form lower brominated PBDEs. This can result in the production of congeners with increased toxicity (Crawford and Quinn, 2017). Although PBDE congeners belong to the same chemical class of compounds, their distinct structures display different chemical properties (Manchester-Neesvig, Volters and Sonzogni, 2001), giving rise to different toxic effects. Toxic effects include disrupting several endocrine mechanisms, which culminate in neurotoxicity, sexual dysfunction, hepatotoxicity and even liver cell death by apoptosis (Souza *et al*., 2016). For example, PBDEs are structurally similar to thyroid hormones and thereby have the ability to bind

to thyroid hormone receptors and thyroxin transporting molecules (transthyretin). The toxicity of metabolically transformed hydroxylated PBDEs (OH-PBDEs) exceeds that of PBDEs, showing a greater affinity for hormone receptors than PBDEs (Legradi *et al*., 2017).

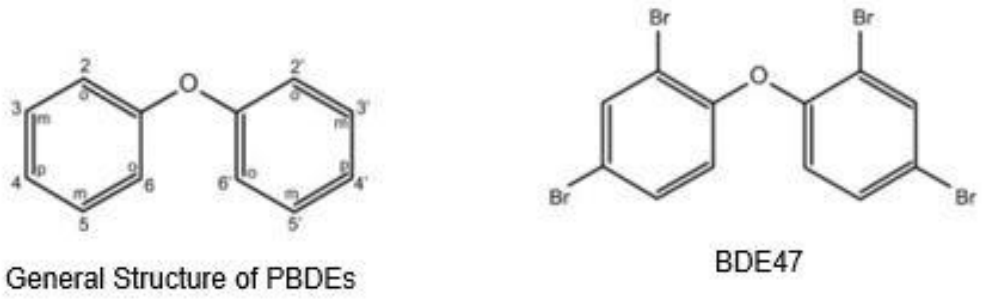


Figure 1.4: General structure of PBDEs and structures of selected BDE congener, BDE47, as an example. The numbers in the generalized structure of PBDE indicate the position of the bromine atoms. The letters (o), (m), and (p) indicate ortho-, meta-, and para- substitutions for bromine side groups (Kodavanti and Loganathan, 2014).

PBDEs are commercially available in three technical mixtures; penta-, octa- and deca-BDEs and are widely used in numerous polymer-based commercial and household products such as textiles, furniture and electronics. These compounds were used to meet increasingly strict fire safety standards by increasing flame ignition resistance (Shaw and Kannan, 2009; Chang *et al*., 2020). Annual global production of PBDEs is estimated to be around 67,125 metric tonnes (13% penta-, 5.7% octa- and 82% deca-BDEs) (Arias, 1992; BSEF, 2003). The European Commission (EC) brought a proposal to the European Union (EU) in 2001 that would ban the use of penta- and octa-brominated diphenyl ethers (BDE) fire retardants over the concern of human and environmental health. The EU voted to accept this proposal and banned the use of penta- and octa-BDEs by August 2004 and extended the ban to the use of deca-BDE by January 2006 (Siddiqi, Laessig and Reed, 2003).

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). Production of PCBs was however, banned in Western Europe and North America in the late 1970s (Henretig, 2009). Octa- and penta-PBDE mixtures were banned in 2004 and deca-BDE was later phased out of production by 2013 and is now prohibited.

Current sources of PCB and PBDE pollution include leaching from landfills (electrical waste and furniture) and incineration of waste producing dangerous by-products. These can enter the marine environment 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). Both PCBs and PBDEs have the ability to bioaccumulate in fatty tissues and the persistent nature of these compounds (chemically stable and resistant to abiotic and biotic degradation) can result in their bioaccumulation and biomagnification in marine food webs. PCBs and PBDEs reach their highest concentrations in marine mammals, which in many cases, have a lower capacity to metabolise and excrete organohalogen compounds as compared to terrestrial mammals, although this is species dependent (Krahn *et al*., 2009). Orcas (killer whales; *Orcinus orca*) are recognised as the most contaminated animals on this planet. An individual referred to as "Lulu" by researchers, stranded on the Isle of Tiree, on the west coast of Scotland in 2016 and is recognised as one of the most contaminated worldwide. Analysis conducted by CEFAS found 957 mg/kg lw (lipid weight) of the sum of 25 PCB congeners (SMASS, 2016; SRUC, 2017). This is 80 times higher than the accepted total PCB toxicity threshold for marine mammals (11 mg/kg lw), where a toxicity threshold of 17 mg/kg in the blubber of marine animals has been reported as leading to reproductive impacts (Kannan *et al.,* 2000). Toxic effects are also known to occur in lower trophic level organisms, for example, a study by Tomy *et al*., (2004) found that lake trout dosed with a mixture of 13 PBDE congeners had depressed levels of circulating free thyroxine and 3,5,3′-triiodothyronine in their plasma, which are responsible for regulating body temperature, metabolism, and heart rate.

1.3.2 Trace metals and metalloids

The chemical elements can be broadly divided into metals, metalloids and non-metals according to their shared physical and chemical properties. Metals are good conductors of heat and electricity, have low ionisation energies and electronegativities and form alloys with other metals. Metalloids occupy a position between metals and non-metals and are semi-conductive and allow a moderate transmission of heat, maintaining a solid but brittle state of matter (Anastas and Maertens, 2018). Elements classed as metalloids include arsenic (As) and selenium (Se) which are widely distributed in minerals, soil, water, atmosphere, and biological tissues (Reis and Duarte, 2019). Metals and metalloids are naturally present in the environment due to erosion (removal) of underlying rocks, volcanic emissions and weathering (breakdown) of rocks and soils, however their extraction, production, use and release anthropogenically can lead to the increase of their environmental levels to concentrations that may be toxic to biota (Richir and Gobert, 2016).

Metals and metalloids can be divided into two categories – essential and non-essential. Essential elements are required in organisms for normal bodily functions and are depleted by a variety of metabolic processes utilising energy. Examples include the cofactor zinc (Zn), which is used in over 100 enzyme reactions, chromium (Cr) which is involved in lipid metabolism and required for maintaining normal glucose metabolism; and cobalt (Co), which is a key component of vitamin B12, a coenzyme in a number of cellular processes including the oxidation of FAs and the synthesis of deoxyribonucleic acid (DNA) (Pouill *et al*., 2017). Essential metals and metalloids are however toxic at a threshold concentration, above which adverse biological effects begin to occur (Scheuhammer *et al*., 2015). Factors affecting toxicity include those affecting the organism's ability to handle and detoxify accumulated elements and those influencing metal uptake, which varies between and within species (Rainbow, Phillips and Depledge, 1990). For example, a study by Hédouin *et al.,* (2010) analysed Ni accumulation in clams and oysters. Although both bivalve species were shown to efficiently assimilate Ni ingested with their food (especially clams) and retain it very efficiently (especially oysters), they displayed different bioaccumulation behaviour for Ni suggesting different environmental interactions and/or physiological ability.

Heavy metals are defined as “naturally occurring metals having an atomic mass number above 20 and an elemental density greater than 5 g/cm^3 (Ali and Khan, 2018b). Non-essential metalloids and heavy metals persist in the marine environment for millions of years and move through various biogeochemical cycles and unlike organic chemicals, the majority of metals cannot be easily metabolised into less toxic compounds (Morel and Price, 2003).

The most common heavy metal and metalloid pollutants are Cr, nickel (Ni), copper (Cu), Zn, cadmium (Cd), lead (Pb), Hg and As, with the non-essential elements Hg, Pb, Cd and As of the greatest environmental concern. Non-essential elements produce toxic effects in plants and animals even at very low concentrations, particularly when ingested over time (Tchounwou *et al.,* 2014). These elements are biomagnified to higher trophic levels (marine top predators) including marine mammals and seabirds, with total Hg, in particular, reported to have an estimated biomagnification rate of 6.0 ± 3.7 times for each trophic level in polar marine food webs (Lavoie *et al.,* 2013; Ali and Khan, 2018a) and 5.4 times for each trophic level in tropical marine food webs (Kehrig *et al.,* 2013).

Hg, considered the most toxic heavy metal, exists in nature as an elemental or metallic form, in inorganic salts and as organomercurial compounds and has different bioavailabilities and toxicities associated with them. After its release into the

environment (soil and water), inorganic Hg is acted upon by bacteria, leading to its transformation to methylmercury (MeHg). Of the different chemical forms of Hg, MeHg is the most toxic and abundant in the marine food web (Maage *et al*., 2017). Because it is lipophilic in nature, MeHg can easily permeate across biomembranes such as the blood–brain barrier into the central nervous system (CNS) causing sensory and motor deficits and behavioural impairment, resulting in animals becoming anorexic and lethargic (Krishna *et al*., 2003). Inorganic Hg follows a non-uniform pattern of distribution, accumulating mainly in the kidneys; thereby, causing acute renal failure (Jan *et al*., 2015). Hg is also easily transferred across the placenta (Wagemann *et al*., 1988) and thus concentrates in the foetal brain, resulting in development alterations and often death (Wolfe, Schwarzbach and Sulaiman, 1998).

1.4 Contaminant monitoring

The monitoring of contaminants in the marine environment is vital to guide the conservation, protection and sustainable management of marine ecosystems. The oldest intergovernmental organisation associated with the marine environment is the International Council for the Exploration of the Sea (ICES) established in 1902. This organisation is concerned with marine and fisheries science and is still a leading scientific forum for the coordination of research by 20-member countries (ICES, 2020) including the United Kingdom (UK). ICES was formed to provide decision makers with the best available science to make informed choices on the sustainable use of the marine environment and ecosystems, particularly on marine policy and management issues.

The OSPAR Commission is the Regional Sea Convention for the North-East Atlantic. The Hazardous Substances and Eutrophication Strategies of the OSPAR Convention cover pollution sources and matters relating to the protection of the marine environment in respect of nutrients and hazardous substances. It was adopted in Paris, France in 1992 and replaced the Convention for the Prevention of Marine Pollution by Dumping from Ships (Oslo convention) adopted in 1972 and the Convention for the Prevention of Marine Pollution from Land-Based Sources (Paris convention) adopted in 1974 (JNCC, 2013). The convention was signed by 15 European countries with the aim of reducing, preventing, and if possible, eliminating pollution from anthropogenic sources (e.g. industrial discharges, offshore installations and from atmospheric deposition (OSPAR, 2000).

The European Union (EU) Water Framework Directive (WFD; 2000/60/EC) was adopted in 2000 to reduce (eliminate or achieve concentrations near background values for naturally occurring substances) the emissions of hazardous substances to water (Fliedner *et al*., 2016). It was concluded that the improvement of water quality in surface (including coastal) and ground water must be monitored by EU Member States and a standardised approach developed for the sampling, analysis and monitoring of water. Coastal waters and transitional waters (estuaries) are included and three types of monitoring were established: surveillance monitoring (river basins), operational monitoring (water bodies) and investigative monitoring (when thresholds are unknown, or objectives not met during surveillance monitoring) (WFD, 2008).

After extensive consultation, the EU Marine Strategy Framework Directive (MSFD; 2008/56/EC) was adopted in 2008 with the aim to have European marine waters achieving or progressing to 'Good Environmental Status' (GES) by 2020. The Directive establishes European marine regions and sub-regions on the basis of geographical and environmental criteria. The Directive lists four European marine regions – the Baltic Sea, the North-East Atlantic Ocean, the Mediterranean Sea and the Black Sea – located within the geographical boundaries of the existing Regional Sea Conventions. The four European Regional Sea Conventions are: The Convention for the Protection of the Marine Environment in the North-East Atlantic of 1992 – the OSPAR Convention (OSPAR), The Convention on the Protection of the Marine Environment in the Baltic Sea Area of 1992 - the Helsinki Convention (HELCOM), The Convention for the Protection of Marine Environment and the Coastal Region of the Mediterranean of 1995– the Barcelona Convention (UNEP-MAP) and The Convention for the Protection of the Black Sea of 1992 – the Bucharest Convention. Each EU member state is required to develop a continually updated marine strategy. The MSFD created a list of eleven (11) qualitative descriptors of environmental status for GES to be achieved by 2020. Descriptor 8 "Concentrations of contaminants are at levels not giving rise to pollution effects" is specifically relevant to this project and is assessed mostly using Background Assessment Concentrations (BACs) and Environmental Assessment Criteria (EACs) or proxys for these assessment criteria.

In May 2008, OSPAR Contracting Parties (CP) started preparations on a collective approach on the regional aspects of the implementation of the MSFD (Directive 2008/56/EC). The OSPAR maritime area encompasses the MSFD 'North-East Atlantic Ocean' Region, and in particular its sub-regions 'Greater North Sea', 'Celtic Seas', 'Bay of Biscay and 'Iberian Coast'. To address Descriptor 8 of the MSFD, OSPAR affirmed

its strategy on hazardous substances with the ultimate aim of achieving concentrations in the marine environment near background values for naturally occurring substances and close to zero for synthetic substances (OSPAR, 2012).

To achieve the OSPAR's vision of a clean, healthy and biologically diverse North-East Atlantic Ocean, used sustainably, monitoring of how the seas function is required to determine whether the Programme of Measures the OSPAR Contracting Parties agree to take are having the intended effect. The OSPAR common indicators assess the changes in populations of marine mammals, seabirds, fish, changes in the phyto- and zoo-plankton communities, benthic habitats and food webs (OSPAR, 2014a). Common indicators have been developed to provide regionally comparable assessment outputs across the OSPAR maritime area. Parameters can be assessed against a threshold value or assessment value to establish whether or not biological effects on marine biota are likely.

Developing a shared data and information system between the EU and the Regional Sea Conventions is vital for achieving an improved scientific understanding of the marine environment, to contribute to the periodic review of policy objectives and associated targets and indicators. Monitoring and assessment based on scientific knowledge of the seas is the basis for the management of human activities in our seas. OSPAR's Joint Assessment & Monitoring Programme (JAMP) describes the strategy, themes and products that OSPAR Contracting Parties are committed to deliver, through collaborative efforts in OSPAR (OSPAR, 2014b). A number of JAMP guidelines were implemented, including the adoption of the revised Coordinated Environmental Monitoring Programme (CEMP) in 2016, designed to deliver comparable data from across the OSPAR Maritime Area to support OSPAR assessments; indicating the extent of contamination of fish, shellfish and sediments with hazardous substances and the intensity of their biological effects. The data collected under the OSPAR CEMP for North-East Atlantic contaminants in biota, sediment and water are quality controlled and hosted at ICES.

The CEMP is divided into the following themes reflecting the different issues that OSPAR is addressing under its thematic objectives, as set out in the JAMP:

Theme A: Cross Cutting Components (ocean acidification),

Theme B: Biodiversity and Ecosystems,

Theme E: Eutrophication,

Theme H: Hazardous Substances,

Theme O: Offshore Oil and Gas Industry; and

Theme R: Radioactive Substances.

These data are assessed annually by the OSPAR Working Group on Monitoring and on Trends and Effects of Substances in the Marine Environment (MIME) to assess the effectiveness of measures to reduce releases of hazardous substances to the environment (OSPAR, 2013). CEMP monitoring is suitable to track contaminants which accumulate through the food chain in marine organisms, but which cannot easily be detected in seawater. Therefore, CEMP assessment results may lead to different conclusions about the chemical quality status than water-based monitoring under the WFD.

OSPAR's monitoring work on hazardous substances comprises of the monitoring and assessment of the sources and pathways of contaminants and their concentrations and effects in the marine environment. Data supporting the aims of Theme H of the CEMP are to be measured on a mandatory basis and include the following components: heavy metals Cd, Hg and Pb in biota and sediment, PCB congeners - CB28, CB52, CB101, CB118, CB138, CB153, and CB180 in biota and sediment, polycyclic aromatic hydrocarbons (PAHs) - anthracene, benz[*a*]anthracene, benzo[*ghi*]perylene, benzo[*a*]pyrene, chrysene, fluoranthene, ideno[1,2,3-*cd*]pyrene, pyrene and phenanthrene in biota and sediment; brominated flame retardants hexabromocyclododecane (HBCD) and PBDE congeners - BDE28, BDE47, BDE66, BDE85, BDE99, BDE100, BDE153, BDE154 and BDE183 in biota and sediment, and BDE 209 in sediment; and tributyl tin (TBT)-specific biological effects and TBT in sediment or biota. Monitoring of TBT concentrations in the marine environment in either sediments or biota carried out in parallel with monitoring of TBT-specific biological effects.

The Clean Seas Environmental Monitoring Programme (CSEMP) is the main UK monitoring programme for contaminants in sediment and biota and, in Scotland, is undertaken by Marine Scotland Science (MSS) and the Scottish Environment Protection Agency (SEPA). Since 1999, as part of CSEMP, sediment, water and fish samples have been collected for nutrients, contaminants and biological effects monitoring for coastal, estuarine and offshore areas. PAHs, PCBs, PBDEs and trace metals are measured in sediment and biota, and inorganic nutrients and salinity measured in water samples

(Webster *et al*., 2007). The programme provides quality assured data annually to the UK Marine Environment Monitoring and Assessment National (MERMAN) database and from there on to international databases maintained by ICES. This fulfils the UK's commitment to specific European Directives and its requirements under the OSPAR Hazardous Substances and Eutrophication Strategies.

1.5 Assessment criteria

In order to achieve the aim of GES adopted across European Member States, Descriptor 8 must be addressed (Law *et al*., 2010). Contaminant concentrations and their biological effects need to be assessed in environmental samples by comparison to assessment criteria (ICES, 2011). As described above, this descriptor is being addressed by monitoring programmes such as CSEMP (conducted by MSS and SEPA) and the WFD (conducted by SEPA).

OSPAR developed the indicators of BACs and EACs for specific contaminants in biota and sediment. Background Concentrations (BC) represent concentrations of hazardous substances found in remote sites of “pristine” condition with no anthropogenic (industrial developments) and oceanographic influences (OSPAR, 2010). Due to the movement of compounds by ocean currents and long-range atmospheric transport, areas representing a true background situation do not exist. BACs combine BCs with precautionary statistics during environmental assessments to account for natural and analytical variability (OSPAR, 2009b). The concentration of a contaminant is considered “near background” if the upper confidence limit of a given data set is significantly below the BAC. The EAC represent the contaminant concentration in sediment and biota below which no chronic effects are expected to occur. For threshold definitions and effect categories, ecotoxicological data is essential in the establishment of EACs.

Hydrophobic substances, such as PCBs and PBDEs, are hardly detectable in water even using the most advanced techniques, but accumulate in biota (Eljarrat and Barceló, 2018). For this reason, the Directive 2008/105/EU established Environmental Quality Standards (EQS) for biota for a number of chemical pollutants, below which no harmful effects are expected to occur in wildlife, or humans. These EQSs serve as a benchmark to decide whether specific measures are required.

There are several standards for the same chemical depending on the environmental matrix and overall goal. For fish, EQS are set at trophic level 4 as this value represents

a specific contaminant concentration at which birds and mammals are protected against the effects via secondary poisoning (European Commission, 2014). Contamination is usually evaluated by analysing muscle fillets relative to human health exposure or whole fish relative to wildlife exposure (European Commission, 2014; Amiard and Amiard-Triquet, 2015).

A number of studies have shown that diverse ecosystems are contaminated by pollutants (Voorspoels *et al.,* 2004; Hylland and Vethaak, 2012; Manju *et al*., 2020; Hermabessiere *et al*., 2020) with those animals in higher trophic levels (e.g. cetacean) in particular containing concentrations that are among the highest found in the oceans (Schlingermann *et al*., 2020). It is currently unknown whether species at all trophic levels are at risk from specific pollutant concentrations, so data must be normalised with the use of trophic magnification factors (TMFs). A TMF is a metric of contaminant biomagnification through the food web, indicating the average increase in concentration of chemicals per trophic level. A value >1 indicates biomagnification and a value <1 indicates trophic dilution (Hallanger *et al.,* 2010). For example, a study by Romero-Romero *et al.*, (2017) found that the TMF for the prevalent and recalcitrant PCB congener CB153 in the pelagic food web (spanning four trophic levels) was 6.2 or 2.2, depending on whether homeotherm top predators were included in the analysis, indicating trophic magnification of this congener in the food web. Another study by Kim *et al*., (2012) reported a TMF of 2.5 for the magnification of Hg using fish species, polychaetes, bivalves, crustaceans and cephalopods, indicating trophic magnification.

1.6 Aims and objectives

To achieve GES, monitoring changes in the dynamics of marine ecosystems is important when studying the effects of human activities on marine systems to improve scientific knowledge and determine the effectiveness of current monitoring practices (policy objectives and targets). For organic contaminants, assessment values applicable to OSPAR monitoring data for temporal trends and the status of PBDEs in biota need to be developed, and a strategy is needed to make data from different monitoring species comparable. Secondary poisoning was not considered in the development of the EAC for PCBs, and because high PCB concentrations have been identified in cetaceans, EACs need to be developed for the purpose of protection against secondary poisoning. For heavy metals, there is a lack of ecotoxicological data for developing new assessment criteria based on the European Union WFD or OSPAR EAC principles. Currently,

OSPAR assessment criteria for metals are BACs and the European Commission maximum levels in foodstuffs (proxy). The European Commission has derived an EQS for Hg in fish (20 µg/kg ww) which is lower than current OSPAR BAC for Hg in fish (35 µg/kg ww). There is a lack of trophic level data and ecosystem specific TMFs to address these knowledge gaps, with generic trophic level values obtained from literature and databases, adding additional uncertainty to assessments. Trophic level studies in Scottish waters have to date not yet covered the large species diversity or regions present in the marine food web, focussing more on the trophic interactions in individual food chains composed of a few species (Schoo *et al.,* 2018) or food webs with a maximum of three trophic levels (Jennings *et al.,* 2002; Morissette, Christensen and Pauly, 2012; Jayasinghe, Amarasinghe and Newton, 2017).

The biota monitoring community typically faces the questions of what species to choose, physiological features such as size, tissue type, whether to pool samples or analyse individuals, converting data from one matrix to another and how to assess compliance with target values. Monitoring data on biota contamination concentrations need to be adjusted to a standard trophic level for consistency and comparability across member states using the appropriate TMF. The selection of a TMF value for a given substance is a critical issue as trophic magnification can show considerable variation, not only relating to ecosystem characteristics and the biology and ecology of organisms, but also the experimental design and statistical methods used for TMF calculation, including region selection, trophic level range and how to treat unbalanced sampling, limit of detection (LoD), data normalisation (wet weight/lipid weight/dry weight), non-normal data distribution and outliers.

The objectives for this work are:

1. To contribute high-quality trophic level data covering the diverse marine species inhabiting Scottish waters;
2. To determine the concentration of contaminants (PCBs, PBDEs and trace metals and metalloids) in marine species covering a wide trophic level range within the Scottish marine food web; and
3. To contribute to the wider development of TMFs for organic and inorganic contaminants with a high level of confidence and worldwide applicability.

The findings of this project will contribute to the further development of assessment criteria representing organisms at all trophic levels in the marine food web, incorporating secondary poisoning, where necessary, as an accumulation route. Chapter 2 describes

the materials and methods used in this project, including sample collection and preparation and analysis. Chapter 3 describes the determination of feeding patterns and trophic levels existing in the Scottish marine food web, addressing the first objective. Chapters 4 (PCBs and PBDEs) and 5 (metals/metalloids) focus on the second and third objectives, where the concentration of thirty-two PCBs, nine PBDEs and nine metals/metalloids were determined, identifying the cause of variability within and between sample categories (inter- and intra- species variation) in relation to feeding patterns. TMFs were calculated on selected PCBs, PBDEs and metals/metalloids using two different methods. Chapter 6 describes the conclusions and future work, followed by the Appendix and published work.

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Chapter 2

Experimental procedure and data analysis

2.1 Materials

Reagents

All chemicals used were of analytical grade or better. *iso*-Hexane, ethyl acetate, dichloromethane, chloroform, *iso*-propanol, cyclohexane, toluene, de-ionised water and acetone were purchased from Rathburn Chemicals Ltd, Walkerburn, UK. Sulphuric acid, nitric acid, hydrochloric acid, butylated hydroxytoluene, sodium chloride, potassium hydrogen carbonate, sodium sulphate anhydrous granular, silica gel and alumina were obtained from VWR International Ltd, Lutterworth, UK.

Reference Materials

For FA and alcohol (FAl) analysis, Laboratory Reference Materials (LRM), LRM 173 (Cod liver oil) and LRM 145 (Orange Roughy oil) were prepared in-house. EO23 fish oil (prepared in-house), Marine Oil FAME Mix from Restek, USA and FAl standard from Nu-Chek Prep, Inc., USA were used for quality control checks and establishing retention times. A powdered de-lipified fish muscle LRM was prepared in-house and two Certified Reference Materials (CRMs), namely CRMs USGS 40 and USGS 41 (L-glutamic acid) were produced by the US Geological Survey and purchased from the International Atomic Energy Agency (IAEA), Vienna, Austria for use with the SIA. For the analysis of PCBs and PBDEs, individual labelled internal standard solutions (^{13}C ortho PCBs) and, custom mix standard solution (ortho CBs) for calibration and system suitability checks were purchased from Cambridge Isotope Laboratories and Ultra Scientific, both distributed by LGC Standards Ltd, Teddington, UK. PBDE individual standard solutions and fluorinated BDE160 (internal standard) were purchased from Greyhound Chromatography, Birkenhead, UK.

DORM-4 fish protein CRM, TORT-3 lobster hepatopancreas CRM, DOLT-5 dogfish Liver CRM for trace metals/metalloid analysis were purchased from the National Research Council of Canada, Halifax, Canada. Individual Ge, Sb, Au, Sc, Ir (each 1,000 ppm), Bi, Rh, and Hg (each 10 ppm) standard solutions and a multi-element standard solution (10 ppm) were obtained from Inorganic Ventures, USA.

2.2 Sample collection and preparation

2.1.1 Fish, catshark and marine invertebrates

Seven fish species, one shark species and fourteen invertebrate species were collected from nine locations around Scotland between 2015 and 2017. The MRV *Scotia* and MRV *Alba na Mara* were used to collect these biota (Figure 2.1) during December-February. Sampling was opportunistic on-board a marine environmental assessment cruise. The areas explored were a mixture of urbanised and industrialised estuarine locations (Clyde: Holy Loch, Pladda, Hunterston; Forth: Tancred Bank) and more offshore locations (Moray Firth, Burra Haaf, Montrose Bank, Solway Firth, NE Dunbar) (Figure 2.1). King scallops were originally collected as reference samples for other studies and were analysed for SI ratios only. Samples were selected based on being able to access a reasonable quantity, location and historical contaminant data, covering three assessment regions (Figure 2.2). The assessment regions around Scotland used in this project are designated based on physical and biological features (UKMMAS, 2010). These include the regions: Irish Sea, Minches and Western Scotland, Northern North Sea and Scottish Continental Shelf (Figure 2.2). They have been used in a variety of marine assessments (e.g. Charting Progress 2 (a comprehensive report on the state of the UK seas) and for Marine Strategy Framework Directive reporting purposes) to improve our understanding of the environment, managing and collecting data within a structured and co-ordinated approach. The Regions have been revised in line with Scotland's National Marine Plan which was published in 2015 and due to be refreshed by March 2021.

Bottom trawling was conducted using a BT 137 GOV 50 mm mesh size net (wingspread: 20 m, headline height: 5 m, length: 71 m) with attached blinder. Samples were collected in 40-135 m depth of water. All individual fish, shark and invertebrates were dissected, pooled to ensure sufficient sample quantity for analysis (depending on species, tissue type, size and sampling location), appropriately packaged and stored at -20 °C prior to analysis.

Figure 2.1: Sample 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, Montrose Bank, Moray Firth, Burra Haaf, Holy Loch, Hunterston, Pladda, Outer Firth of Forth (North East (NE) Dunbar) and Solway Firth (black circles). Marine mammal samples were collected from strandings between 2012-2016 and the individual stranded animals (small green circles) were collected from eight regions around Scotland (green text): Fife, Lothian, Tayside, Grampian, Highland, Orkney, Western Isles, and Strathclyde. King scallops were collected from ten offshore sites around Scotland (purple circles). Zooplankton were collected from the Scottish Observatory site off Stonehaven from the RV *Temora* in 2017 (red circle).

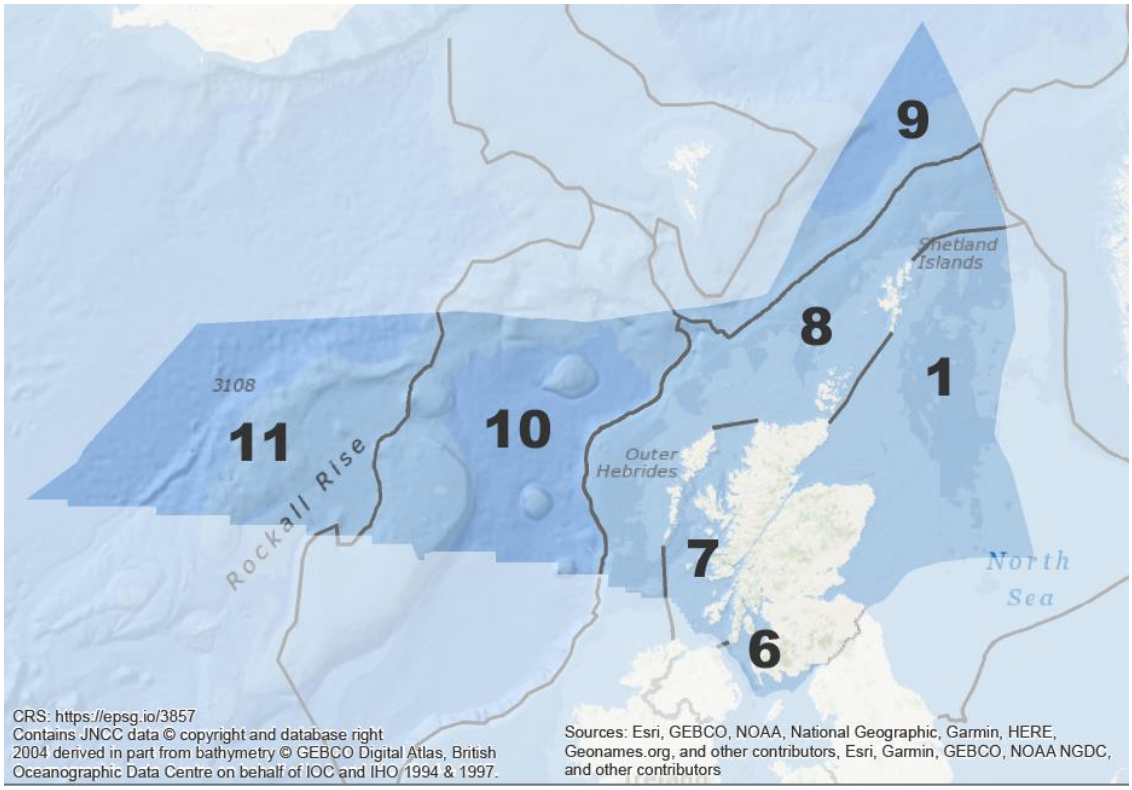


Figure 2.2: Map of the biogeographic regions around Scotland defined by the review of marine nature conservation (JNCC, 2004) and used for assessment purposes in this project. 1: Northern North Sea, 6: Irish Sea, 7: Minches and West Scotland, 8: Scottish Continental Shelf, 9: Faroe-Shetland Channel. 10: Rockall Trough and Bank, 11: Atlantic North-West Approaches (biogeographic regions are currently in review).

Sample preparation resulted in five tissue types (whole animal, muscle, liver, soft body, brown meat) and sample pools composed of three to six individuals for fish, catshark, common starfish, king scallop and squid. The remaining invertebrates ranged from twenty to one hundred individuals per pool (Table 2.1). The length was recorded for all fish and catshark and whole animal weight was recorded for individuals except squat lobsters, swimming crabs, hermit crabs, shore crabs, *Nephrops* and brittle star where the overall pool was weighed (due to the small size and large quantity of individuals).

Samples were homogenised using a blade homogeniser prior to freezing. Liver and muscle were dissected from the fish and catshark and homogenised. Brown meat and muscle were dissected from edible crab and lobster and separately homogenised. Otoliths were extracted from fish collected from one cruise in 2016 (36 individuals including haddock, whiting, plaice and dab) and stored in sealed plastic vials. Otoliths were sent to a specialist analyst for microstructure examination for age determination. Small fish (<120 mm) were homogenised whole. Common starfish and brittle star were homogenised whole (including their exoskeleton). Sea mouse was homogenised whole.

King scallop, horse mussel, whelk, swimming crab and shore crab had their exoskeletons discarded and the soft body was homogenised. Squat lobster, *Nephrops* and hermit crab had their muscular tails isolated and then homogenised. Squid mantle, which is composed of a muscular framework of connective tissue fibres, was dissected, homogenised and classified as muscle tissue.

Table 2.1: Sample pools collected from each of the five environmental monitoring survey cruises from nine areas (covering three biogeographic regions) around Scotland. n = number of matrix specific sample pools associated to that particular species and sampling point (total n=167). The specific locations are identified in Figure 2.1. King scallops were originally collected as reference samples for other studies and are not included in this table (n=10). There are two sample pools per fish and shark as both muscle and liver were dissected. Smaller fish were homogenised whole.

Biogeographic Region	Sampling Location	Species Collected	Number of Individuals Collected	Number of Sample Pools	Matrix
Northern North Sea	Tancred Bank	Shore Crab (*Carcinus maenas*)	27	2	Soft Body (n=2)
	North East Dunbar	Haddock (*Melanogrammus aeglefinus*)	36	6	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	2	Muscle (n=1), Liver (n=1)
		Whiting (*Merlangius merlangus*)	10	4	Muscle (n=2), Liver (n=2)
		Edible Crab (*Cancer pagurus*)	14	2	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	8	Muscle (n=4), Liver (n=4)
		Plaice (*Pleuronectes platessa*)	15	6	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)
Scottish Continental Shelf	Burra Haaf	Haddock (*Melanogrammus aeglefinus*)	5	2	Muscle (n=1), Liver (n=1)
		Whiting (*Merlangius merlangus*)	20	10	Muscle (n=5), Liver (n=5)
		Plaice (*Pleuronectes platessa*)	17	8	Muscle (n=4), Liver (n=4)
		Dab (*Limanda limanda*)	15	6	Muscle (n=3), Liver (n=3)
		Squid (*Loligo forbesii*)	5	1	Muscle (n=1)
		Hermit Crab *(Pagurus bernhardus)*	10	1	Muscle (n=1)
		Nephrops (*Nephrops norvegicus*)	53	1	Muscle (n=1)
Irish Sea	Holy Loch	Catshark *(Scyliorhinus canicula)*	15	8	Muscle (n=4), Liver (n=4)
		Haddock (*Melanogrammus aeglefinus*)	10	4	Muscle (n=2), Liver (n=2)
		Hake (*Merluccius merluccius*)	7	4	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	4	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	6	Muscle (n=3), Liver (n=3)
		Haddock (*Melanogrammus aeglefinus*)	21	5	Muscle (n=1), Liver (n=1), Whole (n=3)
		Whiting (*Merlangius merlangus*)	25	12	Muscle (n=6), Liver (n=6)
		Herring (*Clupea harengus*)	10	4	Muscle (n=2), Liver (n=2)
		Common Starfish (*Asterias rubens*)	10	2	Whole (n=2)
		Horse Mussel (*Modiolus modiolus)*	6	1	Soft Body (n=1)
		Whelk (*Buccinum undatum)*	4	1	Soft Body (n=1)
		European Lobster (*Homarus gammarus*)	9	2	Muscle (n=1), Brown Meat (n=1)
	Solway Firth	Catshark *(Scyliorhinus canicula)*	13	6	Muscle (n=3), Liver (n=3)
		Haddock (*Melanogrammus aeglefinus*)	8	4	Muscle (n=2), Liver (n=2)
		Whiting (*Merlangius merlangus*)	15	5	Muscle (n=2), Liver (n=2), Whole (n=1)
		Plaice (*Pleuronectes platessa*)	8	4	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	2	Muscle (n=1), Brown Meat (n=1)
		Sea Mouse (*Aphrodita aculeata*)	33	1	Whole (n=1)

2.1.2 Marine mammals

Blubber samples from three marine mammal species were collected by the Scottish Marine Animal Strandings Scheme (SMASS; Scotland's Rural College, Inverness, Scotland) from eight locations (green circles, Figure 2.1) between 2012 and 2016. Sperm whale, harbour seal and harbour porpoise were selected due to their differing diets and metabolic capabilities. A cross sectional strip of blubber was removed following internationally standardised protocols (Kuiken and Garcia-Hartmann, 1991). Blubber and skin were separated, and samples stored at -20°C. Individuals were obtained from different regions and varied in age and decomposition state (Table 2.2).

Table 2.2: Marine mammal samples from Scottish waters. N/A, not available. The location of individual animal strandings are presented on Figure 2.1 (small green circles). n= number of individuals.

Harbour Porpoise (*Phocoena phocoena*; n=18)	
Length (cm)	133-163 (n=18)
Weight (Kg)	33.0-59.5 (n=10), N/A (n=8)
Girth (cm)	71-111 (n=16), N/A (n=2)
Dorsal Blubber Thickness (mm)	10-39 (n=16), N/A (n=2)
Age	Adult (n=15), Juvenile (n=2), N/A (n=1)
Sex	Male (n=18)
Decomposition State	Freshly Dead (n=5), Slight Decomposition (n=5), Moderate Decomposition (n=7), Moderate-Advanced Decomposition (n=1)
Year Stranded	2014 (n=9), 2015 (n=4), 2016 (n=5)
Area Stranded	Strathclyde (n=5), Highland (n=4), Grampian (n=3), Tayside (n=1), Lothian (n=1), Western Isles (n=1), Fife (n=1), Orkney (n=2)
Cause of Death	Physical Trauma (n=5), Live Stranding (n=3), Encephalitis (n=1), Neoplasia (n=1), Bacterial Infection/Septicaemia (n=1), N/A (n=7)
Harbour Seal (*Phoca vitulina*; n=10)	
Length (cm)	153-189 (n=10)
Weight (Kg)	55.5-92.5 (n=7), N/A (n=3)
Girth (cm)	39-151 (n=10)
Dorsal Blubber Thickness (mm)	13 (n=1), N/A (n=9)
Age	Adult (n=9), Juvenile (n=1)
Sex	Male (n=10)
Decomposition State	Freshly Dead (n=3), Slight Decomposition (n=2), Moderate Decomposition (n=3), Advanced Decomposition (n=1), N/A (n=1)

Year Stranded	2012 (n=4), 2014 (n=3), 2015 (n=2), 2016 (n=1)
Area Stranded	Highland (n=2), Fife (n=3), Grampian (n=1), Lothian (n=1), Tayside (n=1), Strathclyde (n=2)
Cause of Death	Physical Trauma (n=7), Chronic Peritonitis (n=1), Pneumonia (n=1), N/A (n=1)
Sperm Whale (*Physeter macrocephalus*; n=5)	
Length (cm)	1183-1524 (n=5)
Weight (Kg)	26,060 (n=1), N/A (n=4)
Girth (cm)	610-722 (n=2), N/A (n=3)
Dorsal Blubber Thickness (mm)	98-110 (n=2), N/A (n=3)
Age	Adult (n=1), Subadult (n=3), N/A (n=1)
Sex	Male (n=5)
Decomposition State	Freshly Dead (n=1), Slight Decomposition (n=1), Moderate Decomposition (n=1), Moderate-Advanced Decomposition (n=2)
Year Stranded	2012 (n=1), 2013 (n=1), 2014 (n=2), 2015 (n=1)
Area Stranded	Highland (n=1), Western Isles (n=3), Lothian (n=1)
Cause of Death	Live Stranding (n=2), Physical Trauma (n=1), N/A (n=2)

2.1.3 Zooplankton

Calanus spp. (Figure 2.3) and *Pseudocalanus spp.* were collected from Stonehaven (Figure 2.1) in 2018 from the MRV *Temora*. 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 in 15 L, plastic buckets out of wind and sunlight until arrival at the laboratory. The target herbivorous species were isolated using a Zeiss Stemi-11 stereomicroscope and stored at -20˚C.

Figure 2.3: *Calanus* copepods at 20 x magnification in sea water under a Zeiss Stemi-11 stereomicroscope. Photograph taken during the copepod selection process (species confirmed by a zooplankton ecologist at the Marine Laboratory, Aberdeen). *Calanus spp.* is typically 2–4 millimetres long and *Pseudocalanus spp. is* typically 0.8 to 1.5 millimetres long. The white oval outline highlights a single *Calanus* copepod.

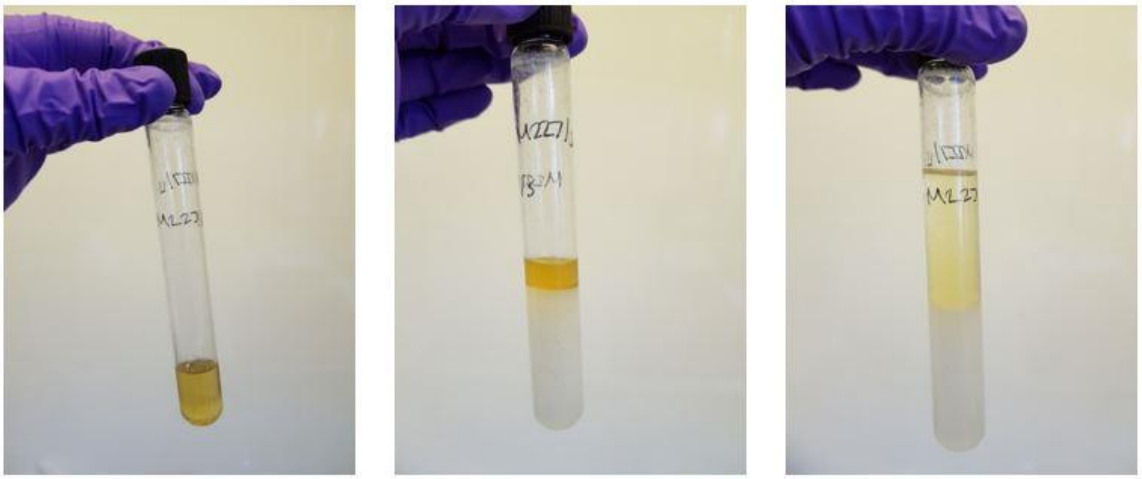
2.3 Lipid extraction and trans-esterification

Lipid extraction and trans-esterification were carried out as reported in Webster *et al*. (2014). Blood vessels were not removed prior to lipid extraction due to the minor contribution blood vessels would make to the results. Blubber for example is approximately 50:50 by weight collagen: fat than blood vessels. Collagen protein would therefore dominate $\delta^{15}N$ and $\delta^{13}C$. However, it is acknowledged that the comparison of blubber $\delta^{15}N$ and $\delta^{13}C$ to those of muscle and liver should be done with caution.

Lipid was extracted from sample pools, blubber and copepods into a chloroform-methanol-water mixture (2:2:1.8 v/v/v) based on the method of Bligh and Dyer (1959) modified by Hanson and Olley (1963). Methanol, chloroform and deionised water were added to the sample pools which were homogenised on ice using an ultra-turrax blender. Centrifugation using a SL-40R, Thermo Scientific, USA centrifuge at 1,800 rpm at 0 ˚C for 20 minutes separated the organic and aqueous layers. The organic layer was recovered and evaporated to dryness. The lipid was re-suspended in *iso*-Hexane and stored at -20 ˚C until required for analysis. The protein residue that formed between the organic and aqueous layers comprised of a mixture of proteins, including cellular protein, collagen and smooth muscle protein from blood vessels. The mixed protein pellet was air dried overnight on a filter paper, freeze dried, and ground to a fine powder using a mortar and pestle in preparation for SI analysis.

Iso-Hexane was removed from a calculated volume of the extracted lipid by evaporating under charcoal scrubbed nitrogen. The lipid extract was dissolved in a test tube with toluene (1 mL for fish tissue, invertebrates, blubber and associated LRMs and 0.1 mL for zooplankton) mixed with 1% v/v sulphuric acid in methanol (2 mL for fish tissue, invertebrates, blubber and associated LRMs and 0.2 mL for zooplankton) and two BHT crystals. The test tubes were placed in a heat block (copepod extracts were placed on top of the heat block) set at 50 ˚C for a minimum of twelve hours (maximum of eighteen hours). The samples (Figure 2.4a) were allowed to cool prior to the addition of sodium chloride (5% w/v) in HPLC grade water (5 mL for fish tissue, invertebrates, blubber and associated LRMs and 0.5 mL for the zooplankton) (Figure 2.4b), followed by *iso*-Hexane, to each sample (5 mL for fish tissue, invertebrates, blubber and associated LRMs and 0.5 mL for the zooplankton (Figure 2.4c)). The upper, solvent layer containing the fatty acid methyl esters (FAMEs) was extracted twice with *iso*-hexane and the combined organic layers were washed with 2% w/v potassium bicarbonate in HPLC grade water

(4 mL for fish tissue, invertebrates, blubber and associated LRMs and 0.4 mL for the zooplankton). The washed organic extract was dried over anhydrous sodium sulphate.



(a) (b) (c)

Figure 2.4: (a) The trans-esterified lipid extract. (b) The trans-esterified lipid extract (top layer) after the addition of 5% w/v sodium chloride in water (bottom layer). (c): The trans-esterified lipid extract (top layer) after the addition of 5% w/v sodium chloride in water (bottom layer) and *iso*-Hexane (top layer combined with the trans-esterified lipid extract).

2.4 Lipid determination

The total lipid content was determined according to the method of Smedes (1999). The biota sample was weighed into a 250 mL centrifuge tube and *iso*-propanol (18 mL) and cyclohexane (20 mL) added. The sample was homogenised over ice (nominal speed setting 13,500 rpm) for two minutes using a calibrated timer. The appropriate volume of de-ionised water was added (~13–22 mL, depending on the moisture content of the sample) to the mixture and homogenised for a further minute using a calibrated timer. Moisture content was provided as a table of approximate values for different tissue types. Samples were subsequently centrifuged at 0 ºC and a speed of 1,800 rpm, for ten minutes, where the organic extract was separated from the particulate material. A portion (10 mL) of the organic phase was transferred to a 50 mL round bottom flask.

A second extraction on the same particulate matter was carried out using 13% (v/v) *iso*-propanol in cyclohexane, homogenisation and centrifugation as described above. A portion (10 mL) of the organic phase was combined with the previous extract and the solvent subsequently removed by rotary evaporation at 75 ºC 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.

2.5 Stable isotope analysis

Measurement of $\delta^{15}N$ and $\delta^{13}C$ values was carried out using the method described in Mayor *et al*., (2013).

Approximately 0.60 ± 0.10 mg of ground, de-lipified mixed protein material, (see Section 2.3), was loaded into a 6 × 4 mm tin capsule and compressed using tweezers. The compressed capsules were then loaded into the autosampler of an Integra CN Isotope Ratio Mass Spectrometer (IRMS) (Sercon Ltd, Crewe, UK), combusted and analysed in an automated sequential procedure. The internationally accepted CRMs USGS 40 and USGS 41 (L-glutamic acid; US Geological Survey) were used as internal analytical standards at the beginning, middle, and end of the analysis sequence to enable satisfactory scale correction and correction of drift with time. The best method to obtain both repeatable and reproducible results is scale normalisation based on multi-point isotopic calibration, whose isotopic composition are significantly different to envelope or bracket the anticipated SI composition of the samples (Meier-Augenstein, 2018; Meier-Augenstein and Schimmelmann, 2019). The true sample value was calculated using Equation 2.1:

$$\delta_{true} = m * \delta_{measured} + b \qquad \textbf{Equation 2.1}$$

The slope of the regression line (m) is referred to as the “stretch factor” and the intercept (b) as the “shift”.

$$\delta E = (R_{sample} - R_{standard})/R_{standard} = (R_{sample}/R_{standard}) - 1 \qquad \textbf{Equation 2.2}$$

R_{sample} is the measured isotope ratio of the heavier isotope of a given chemical element E in a sample over the lighter isotope of the same element. $R_{standard}$ is the contemporaneously measured isotope ratio for the chosen standard.

While isotope ratios are measured by IRMS instruments, for light elements like carbon and nitrogen, the results of these measurements are reported as stable isotopic abundance values using the delta notation. A delta value (δ) is an isotopic abundance value relative to an international scale reference point and is derived using Equation 2.2. Results were reported in this project using the standard δ unit notation as parts per thousand (‰) difference from a standard reference material. These ratios are reported relative to international standards: atmospheric nitrogen for nitrogen and Vienna Pee Dee Belemnite (VPDB) for carbon.

2.6 Trophic level determination

The $\delta^{15}N$ from the baseline species (King Scallop (*Pecten maximus*))) was used with the $\delta^{15}N$ value for the test organism to give the trophic level (Equation (2.3); MIME, 2016). This method is currently recommended by OSPAR for the trophic adjustment of contaminant monitoring data (OSPAR Commission, 2016).

$$\text{Trophic Level} = (\delta^{15}N(\text{species}) - \delta^{15}N(\text{baseline})) / 3.4 + TL_{baseline} \quad \textbf{Equation 2.3}$$

$\delta^{15}N$(species) is the nitrogen isotopic abundance value of the sample species; $\delta^{15}N$(baseline) is the nitrogen isotopic abundance value of the baseline species (in this case King Scallop). The mean shift per trophic level of $\delta^{15}N$ is 3.4 ‰ and $TL_{baseline}$ is the trophic level of the baseline species. King scallop was used as the baseline species as they are as they are an integral component of Scottish marine food webs (Figure 1.1). King scallops are herbivorous/detritivores and consequently feeding at trophic level 2 which was assigned as the baseline value ($TL_{baseline}$ in equation 2.3; Pinnegar *et al.,* 2002).

2.7 Gas chromatography with flame ionisation detection (GC-FID) and gas chromatography with mass spectrometry (GC-MS)

The FAME extracts were diluted with *iso*-Hexane and vialled prior to analysis by GC-FID and GC-MS to give an approximate total FAME concentration of 1 mg/mL. Thirty-two FAMEs (Table 2.3) were determined using an HP Agilent 6890 GC-FID.

GC-FID analysis was carried out as reported in Stowasser *et al*., (2009). 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. Cool on-column injection using a Hewlett Packard 7673 automatic injector of 1.0 µL of sample was carried onto and through the GC column by nitrogen at a flow rate of 1.0 mL/min using a specific oven temperature profile: the GC oven temperature was ramped from 60 °C at 25 °C min^{-1}, to 150 °C, followed by 1 °C min^{-1} up to 200 °C and held for 10 minutes, then ramped at 10 °C min^{-1} to 220 °C and finally held at 220 °C for 5 minutes.

The area responses (µV.s) from the FID was summed for the individual compounds listed in Table 2.3. The normalised area percentages were calculated for each of the FAMEs as a percentage of the combined area for the compounds/groups of compounds.

Baseline separation could not be achieved for the positional isomers of eicosenoic acid (20:1), and so the normalised area percentages were calculated based on the summed area for thirty-one individual FAs and the combined peak area for the positional isomers for 20:1.

A small number of samples with a significantly higher coeluting FA normalised area % were identified and analysed using GC-MS. These samples were also known to contain both traiacylglycerols and wax esters. On preparing the FAMEs, the wax esters will hyrolyse to give a FAs and a FAls. The FAls will co-extract with the FAs and will also pass through the methylation process. Samples were analysed using the same column, injection method and oven profile as for the GC-FID. The five FAl/ FA coeluting peaks: FAl14:0/FA15:0, FAl16:0/FA17:0, FAl18:0/18:3(n-3), FAl20:0/FA20:4 (n-6), and FAl20:1(n-9)/FA20:3(n-3) were analysed to establish whether the FAl or FA was present/dominating the peak observed in the FID chromatogram. If the peak was identified as FAl, the area % was removed from the normalised area percent calculation for the FAs. If the FAl and FA were both present, the ratio of the peak area was determined and applied to the corresponding peak area from GC-FID and data re-normalised.

Table 2.3: A list of the 32 FAs investigated in this study. These include saturated, monounsaturated and polyunsaturated FAs. The peak areas (and thus normalised area percentage) of the positional isomers 20:1(n-9) and 20:1(n-11) are reported as a single value.

Systematic name	Common Name	Abbreviation
Saturated		
tetradecanoic acid	myristic acid	14:0
pentadecanoic acid	-	15:0
hexadecanoic acid	palmitic acid	16:0
heptadecanoic acid	margaric acid	17:0
octadecanoic acid	stearic acid	18:0
eicosanoic acid	arachidic acid	20:0
docosanoic acid	behenic acid	22:0
tetracosanoic acid	lignoceric acid	24:0
Monounsaturated		
cis-9-tetradecenoic acid	myristoleic acid	14:1(n-5)
cis-9-hexadecenoic acid	palmitoleic acid	16:1(n-7)
cis-9-octadecenoic acid	oleic acid	18:1(n-9)
cis-11-octadecenoic acid	*cis*-vaccenic acid	18-1(n-7)
cis-9-eicosenoic acid	gadoleic acid	20:1(n-11)
cis-11-eicosenoic acid	gondoic acid	20:1(n-9)
cis- 11-docosenoic acid	cetoleic acid	22:1(n-11)
cis-13-docosenoic acid	erucic acid	22:1(n-9)
cis-15-tetracosenoic acid	nervonic acid	24:1(n-9)
Polyunsaturated		
cis-7,10-hexadecadienoic acid	-	16:2(n-6)
cis-7,10,13-hexadecatrienoic acid	-	16:3(n-3)
cis-4,7,10,13-hexadecatetraenoic acid	-	16:4(n-3)
cis-9,12-octadecadienoic acid	linoleic acid	18:2(n-6)
cis-6,9,12-octadecatrienoic acid	γ -linolenic acid	18:3(n-6)
cis-9,12,15-octadecatrienoic acid	α -linolenic acid	18:3(n-3)
cis-6,9,12,15-octadecatetraenoic acid	stearidonic acid	18:4(n-3)
cis-11,14-eicosadienoic acid	-	20:2(n-6)
cis-11,14,17-eicosatrienoic acid	-	20:3(n-3)
cis-5,8,11,14-eicosatetraenoic acid	arachidonic acid	20:4(n-6)
cis-8,11,14,17-eicosatetraenoic acid	-	20:4(n-3)
cis-5,8,11,14,17-eicosapentaenoic acid	timnodonic acid	20:5(n-3)
cis-6,9,12,15,18-heneicosapentaenoic acid	-	21:5(n-3)
cis-7,10,13,16,19-docosapentaenoic acid	clupanodonic acid	22:5(n-3)
cis-4,7,10,13,16,19-docosahexaenoic acid	cervonic acid	22:6(n-3)

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

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

2.8.1 Pressurised liquid extraction (PLE) by Accelerated Solvent Extraction (ASE)

Solvent washed (*iso*-Hexane and dichloromethane) sodium sulphate (40 g) was added to the sample (muscle, soft body, whole = 5 g, blubber, brown meat, liver and zooplankton = 0.25 g) to aid drying. Subsequently, 250 µL of the PCB internal standard 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; and 100 µL of the PBDE internal standard: fluoro-BDE160 prior to PLE. Extraction cells (100 mL) were solvent washed and packed with: 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) using compressed nitrogen.

The ASE was initially rinsed with acetone and *iso*-Hexane (3 times) and rinsed with *iso*-Hexane between samples. The settings selected for the extraction PCBs and PBDEs is described in Table 2.4:

Table 2.4: ASE 300 settings for the extraction of PCBs and PBDEs

Pressure	1,500 psi
Temperature	100 °C
Heat	5 min
Static time	5 min
Flush	50 sec
Purge	120 sec
Number of cycles	2
Extracting solvent	*iso*-Hexane

2.8.2 Extract clean-up for PCB and PBDE analysis

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 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 containing OCPs was discarded. For PBDEs, the entire eluant was collected for analysis.

The PCB extract was evaporated to 0.5 ± 0.2 mL at 30 °C, using a Syncore system (fitted with a flushback module). Solvent washed glass columns were filled with 3.0 ± 0.2 g silica, topped up with 1 to 2 cm sodium sulphate. The reduced *iso*-Hexane extract was transferred to the top of the column, followed by 20 mL *iso*-Hexane and allowed to adsorb. Once the 20 mL *iso*-Hexane passed through the column, the extract was reduced to 0.5 ± 0.2 mL using a Syncore and transferred, with washings, to a GC amber glass vial with insert. The extract was concentrated to 50 ± 5 µL under a stream of charcoal scrubbed nitrogen and analysed for PCBs by GC-electron impact mass spectrometry (EIMS).

The PBDE extract was passed through silica columns (prepared as described above) followed by 30 mL *iso*-Hexane and transferred to Syncore tubes and the 30 mL volume reduced to 0.5 ± 0.2 mL at 30 °C. The extract was transferred, with washings, to a pre-weighed crimp top, amber glass GC vial and concentrated further under a stream of nitrogen to approximately 50 ± 5 µL before analysis of PBDEs by gas chromatography–electron capture negative ionisation mass spectrometry (GC-ECNIMS).

2.8.3 Determination 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-MSD 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). 1.0 µL of sample was carried onto and through the GC column by helium at a flow rate of 0.5 mL/min and pressure of 15 psi using a specific oven temperature profile: the GC oven temperature was held at 80 °C for 1 minute, followed by 20 °C min^{-1} up to 170 °C and held for 7.5 minutes, then ramped at 3 °C min^{-1} to 290 °C and held for 10 minutes, and finally ramped at 50 °C min^{-1} to 320 °C and held for 10 minutes. The sample sequence consisted of samples, reference material, procedural blank, two calibration solutions to check for calibration drift and an *iso*-Hexane blank at the end of the sequence to remove/check for any residual contamination.

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, covering the concentration range of 0.6–500 ng/mL. Example target and qualifier ions or selected PCB congeners are shown in table A.1. The average response was used to compute a calibration curve, which shows the relation between amount ratio and response ratio (C^{13} labelled PCB internal standards with responses and known concentrations), enabling the system to quantify unknown component amounts. Correlation coefficients of at least 0.99 were achieved for all PCBs.

2.8.4 Determination 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 an HP6890 Series GC interfaced with a 5973 MSD in chemical ionisation mode, 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) using a specific oven temperature programme: Injections (1 µL) were made at 120 °C and the oven temperature held constant for 2 minutes, followed by a ramp of 15 °C min^{-1} up to 205 °C. This was followed by a ramp at 6 °C min^{-1} to 330 °C and held for 4 minutes. Seven calibration standards, with nominal concentrations of 500, 300, 100, 50, 10, 2 and 0.2 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. The MS was set for SIM mode at 70 eV with a dwell time of 100 ms. Ions monitored were m/z 78.9 and 80.9 (ions for bromine) for all PBDEs. The average response was used to compute a calibration curve, which shows the relation between amount ratio and response ratio (using a fluorinated PBDE internal standard), enabling the system to quantify unknown component amounts.

2.9 Determination of trace metals and metalloids

2.9.1 Sample digestion

Samples were digested and analysed as reported in Robinson, *et al.* (2017). Briefly, homogenised biota (0.5 g for fish and shark muscle and whole and invertebrates soft body, whole and muscle and 0.3 g for fish and shark liver and invertebrates brown meat),

whole zooplankton copepods (0.3 g), blubber sections (0.3 g) and associated CRMs (0.2 g) were digested overnight using 2.5 mL of nitric acid (Aristar grade) and 3.5 mL hydrogen peroxide (VWR Merck, Lutterworth, UK; Suprapure grade), followed by a digestion programme on a Berghof Speedwave Xpert Microwave Digestion System which took the temperature to 70 ºC over 2 minutes, after which the temperature was kept constant for 5 minutes, prior to ramping to 210 ºC over 13 minutes. After 25 minutes at 210 ºC, the system was cooled to 50 ºC and maintained at that temperature for 20 minutes. Each digestion run included one procedural blank and one CRM (NRCC Canada). The CRM was selected based on the digested tissue type and included TORT-3 (shellfish hepatopancreas), DORM-4 (fish muscle) and DOLT-5 (fish liver). After digestion, the vessels were allowed to cool to room temperature before each sample was diluted to 25.0 mL with ultra-pure water using a volumetric flask.

2.9.2 Determination of trace metals and metalloids by inductively coupled plasma mass spectrometry (ICP-MS)

The digests were diluted a further 5-fold using a solution containing ultra-pure water, concentrated hydrochloric acid (cHCl) and gold (Au) to remove the memory effects in the determination of Hg (Robinson *et al*., 2017). Calibration standards were made containing concentrations of trace elements Hg, Fe, Zn, Cu, As, Pb, Cd, Se, and Au which were determined using an Agilent Technologies 7700x inductively coupled plasma mass spectrometer (ICP-MS) equipped with a peristaltic pump and AS-90/91 autosampler, Micromist nebuliser, Peltier-cooled quartz modified Scott spray chamber and quartz torch. Analytical standards and tuning solutions were obtained from Essex Laboratories (UK).

ICP-MS was operated in standard mode (axial mode). Germanium internal standard (*m/z* 72) was used to monitor and correct for any instrumental drift. Plasma correction was carried out whenever any part of the sample introduction system (e.g. spray-chamber, nebuliser, torch, cones, or lens assembly) had been changed/cleaned, and at least every six months. A tuning check was carried out before each batch using the criteria described in Table 2.5.

Table 2.5: Tuning criteria for the ICP-MS prior to analysis

	m/z	**Acceptance criteria**
Precision	7, 89, 205	<5% RSD
Sensitivity	7	>1000
	89	>3500
	205	>2000
Oxide ratio	156/140	<1.5
Double charged ratio	70/140	<5
	69/138	<3

2.10 Quality control

All glassware was solvent washed with acetone and *iso*-Hexane to avoid contamination. Due to the photo degradative nature of PBDEs when exposed to UV light, UV filters were placed over the laboratory windows to minimise incoming light. For internal quality control, an LRM (used in all methods), CRM (used for SI and trace metals/metalloid methods) and procedural blank was analysed in each batch of samples (maximum 12), with data adjusted accordingly. A vial of *iso*-Hexane was run on the GC-FID and GC-MS before each sample batch to check for the presence of any contamination on the systems; the batch was rejected and re-run if any peaks were detected. The data obtained from the LRM was transferred onto NWA Quality Analyst (version 5.2) and Shewhart charts were produced with warning and action limits being drawn at ± 2× and ± 3× the standard deviation of the mean, respectively. The PCB, PBDE and trace metals/metalloids methods were accredited by the United Kingdom Accreditation Service (UKAS) to ISO 17025. LoDs were determined for PCBs, PBDEs and metals and metalloids through the repeat analysis of a low spiked sample and the LoD calculated from 4.65 × standard deviation (SD) of the mean concentration. The LoDs were determined for FAs using 3:1 signal to noise ratio, in that the peak is three times greater than the background noise on the GC using a Restek Marine FAME standard. Quality assurance for contaminants analysis was further demonstrated through successful participation in the QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) Laboratory Performance Studies. It is procedure within a government laboratory for a data manager to review all batches to ensure all quality control and data records have been applied and are of an appropriate standard.

2.11 Data analysis

The normality of the data distribution for PCB, PBDE and trace metal/metalloid concentrations were tested using the Ryan-Joiner test on Minitab 17 and data logarithmically transformed. Statistical analysis was undertaken on Minitab 17 using Analysis of Variance (ANOVA) at the 95% confidence level, with Tukey's pair-wise comparisons to establish significant differences in FAs (normalised area %), $\delta^{15}N$ and $\delta^{13}C$ (‰), logarithmically transformed PCB and PBDE concentrations (µg/kg lipid weight) and logarithmically transformed trace metal and metalloid concentration (µg/kg wet weight) between species, categories and regions. Due to the substantial number of FAs and PCBs, principal component analysis (PCA) was used in R Studio (version 3.6.2) to investigate variations in FA and PCB patterns. Mixed effects model analysis on Minitab 17 was used to determine the interaction and significant relationships existing between the logarithmically transformed trace metals/metalloid concentration (µg/kg wet weight) and investigated variables (sample category, trophic level and region). Pearson's correlation on Minitab 17 was used to measure the linear correlation between $\delta^{15}N$ and $\delta^{13}C$, PCB and PBDE concentrations and trace metals/metalloid concentration with potential influencing variables such as age, length and weight. Box plots were developed on Minitab 17 to show the logarithmically transformed concentration differences of PCBs, PBDEs and trace metals/metalloid in categories, species, and regions in order to visualise concentration differences between categories and to show data outliers. Microsoft Office Excel was used to create charts and diagrams for each chapter (scatter plot for SI analysis, bar charts for PCB and PBDE congener proportions and concentration and regional comparisons, pie charts and Venn diagram for metals and metalloid analysis and plotting the Log_{10} [PCB, PBDE and trace metal/metalloid concentration] against trophic level (traditional and balanced methods).

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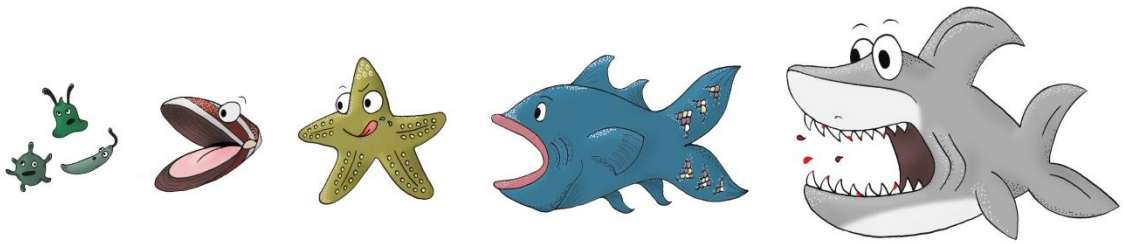
Chapter 3

Scottish Marine Food Web Dynamics: An Assessment of Four Trophic Levels using Fatty Acid Signatures and Stable Isotopic Composition



Anneka Madgett, 2018

3.1 Introduction

Complex characteristics present in marine food webs (type and length of food chains) contribute to the variability observed in environmental assessments of the impact of environmental contaminants on the marine environment. For example, individuals of one species may not be at a constant trophic level due to factors such as age, sex, location, season and diet (Kousteni, Karachieand and Megalofonou, 2017). Some contaminants, resistant to metabolic biotransformation, can biomagnify up the food web and are therefore influenced by trophic level. The characterisation of trophic markers, feeding patterns and predator prey relationships is therefore important in developing our understanding of marine food web ecology (Kelly and Scheibling, 2012). Monitoring changes in the dynamics of marine ecosystems is particularly important when studying the effects of human activities on marine systems. Trophic level studies in Scottish waters have to date not yet covered the large species diversity present in the marine food web.

The aim of this chapter is to contribute high-quality trophic level data covering the diverse marine species inhabiting Scottish waters. A combination of FA signatures and SI ratios will be used to identify the trophic level, feeding patterns and nutritional relationships between a variety of species and classes within the Scottish marine food web. Such data will ultimately permit the calculation of TMFs, which will be used to consider the harmful effects of contaminant bioaccumulation in the development of environmental impact assessment criteria.

3.2 Materials and methods

Detailed information on materials and methods utilised in this chapter are discussed in Chapter 2, Sections 2.3, 2.5, 2.6 and 2.7.

3.3 Results and discussion

3.3.1 Fatty acid profiles

PCA was used to study the inter- and intra-class variability of FA profiles and to identify the FAs responsible for any differentiation. PCA was applied to the pooled samples (fish, shark, invertebrates, zooplankton) and individuals (marine mammals). Due to the large

number of species included in the study, the taxonomic class was initially selected for grouping species to allow easier visualisation of the data (Table 3.1). The first two principal components accounted for 34% of the FA variability.

Table 3.1: The eleven taxonomic classes and their associated species included in this study.

Class	Contributing Species						
Mammalia	Harbour Porpoise	Sperm Whale	Harbour Seal				
Chondrichthyes	Catshark						
Actinopterygii	Whiting	Haddock	Hake	Plaice	Dab	Herring	Sprat
Cephalopoda	Squid						
Malacostraca	Edible Crab	Lobster	Squat Lobster	Swimming Crab	Shore Crab	Hermit Crab	*Nephrops*
Asteroidea	Common Starfish						
Gastropoda	Whelk						
Ophiuroidea	Brittle Star						
Bivalvia	Horse Mussel	King Scallop					
Polychaeta	Sea Mouse						
Hexanauplia	*Calanus* spp.	*Pseudocalanus* spp.					

A clear dispersion of the samples in the PCA biplots was achieved based on their taxonomic class (Figure 3.1b) demonstrating that there were measurable differences in FA profiles between classes and observable variation within classes. This dispersion suggested that a more specific classification system was required to account for factors other than class likely to be influencing the FA profile.

The FA profile was found to vary with tissue type (Figure 3.2) and water column feeding zone (benthic/demersal/pelagic feeding) (Figure 3.3). Marine mammals were not included in Figure 3.3 as they inhabit both pelagic and demersal habitats. This agrees with the findings from previous studies, where FA profiles are reported to be tissue-specific due to the underlying physiological differences between tissue types (Aras, Haluloulu, and Ayik., 2003; Meyer *et al*., 2017).

As well as tissue type, species within each class were influenced by the water column zone inhabited by organisms as feeding patterns vary between zones (benthic/demersal/pelagic) (Figure 3.3). The finalised nineteen categories and category mean normalised area % of the thirty-one FAMEs, accounting for tissue type and water column zone, are shown in Table 3.2. Classification was adapted to incorporate these influencing factors.

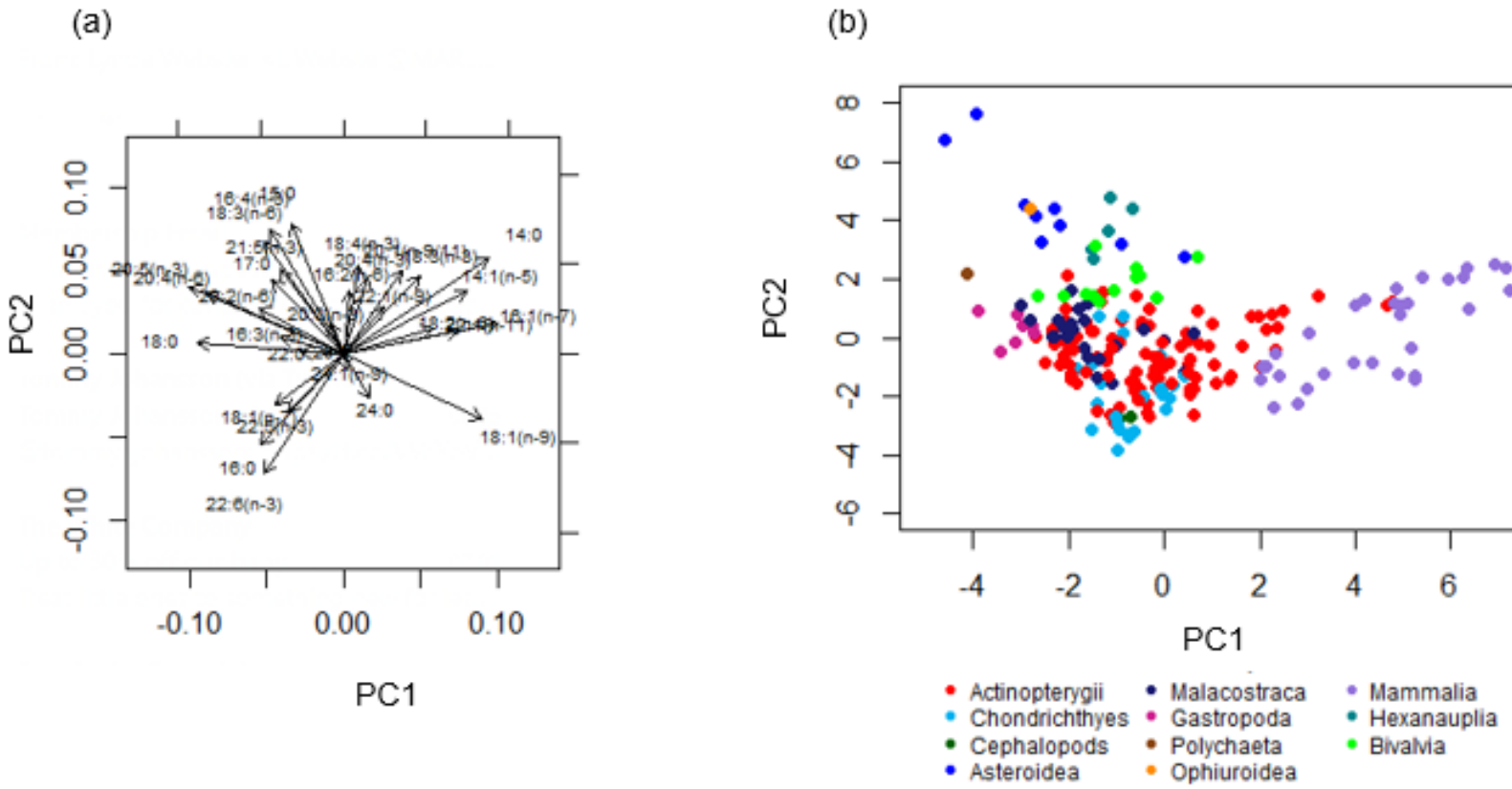


Figure 3.1: (a) PCA loading plot and (b) PCA score plot, both demonstrating variation in the FA profiles (normalised area percentages) for the muscle, liver, homogenised whole, brown meat, soft body and blubber pools across the eleven classes. The first two principal components accounted for 34% of the FA variability.

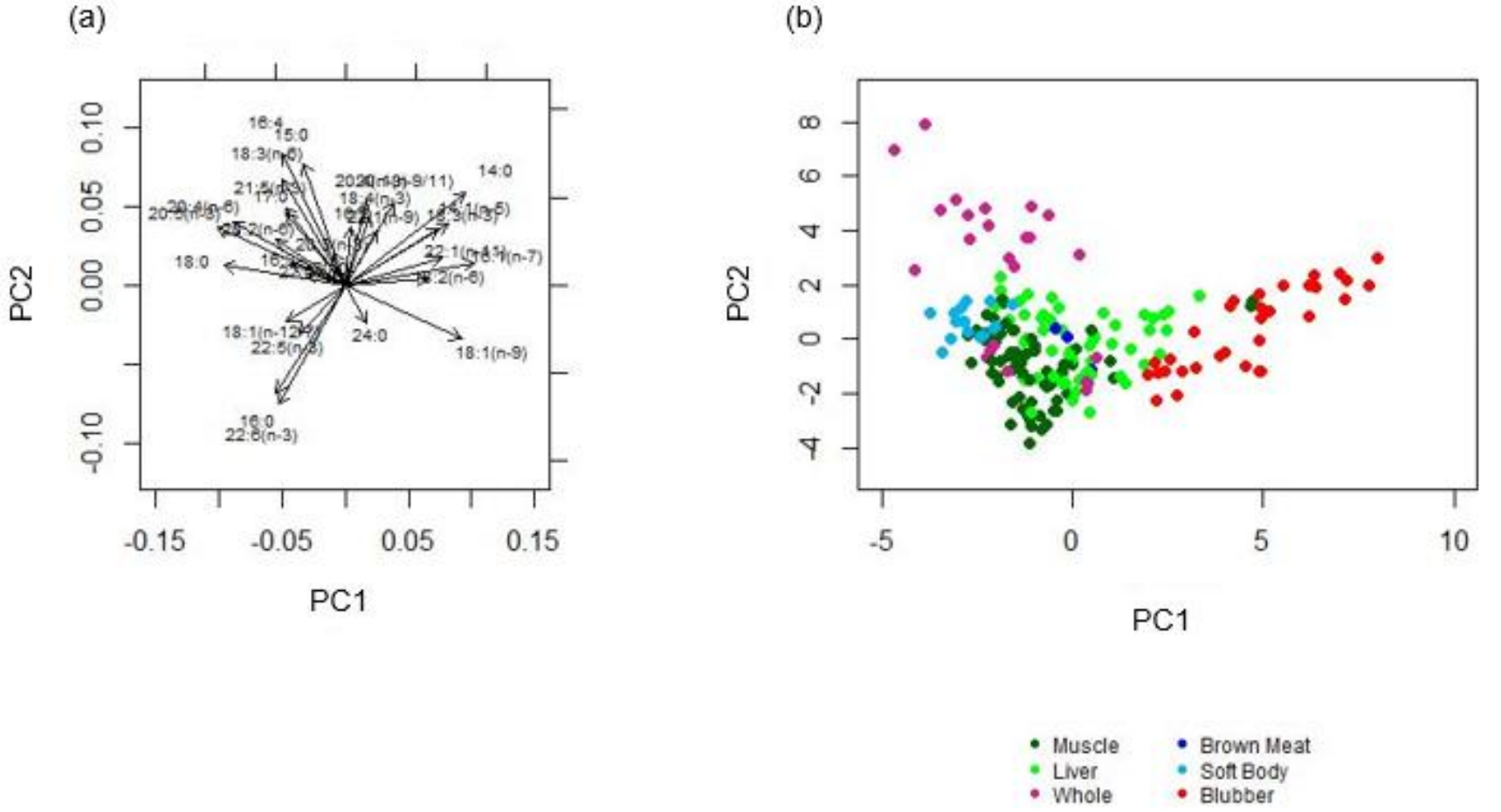


Figure 3.2: (a) PCA loading plot and (b) PCA score plot, both demonstrating variation in the FA profiles (normalised area percentages) across the six tissue types: muscle, liver, homogenised whole, brown meat, soft body and blubber pools.

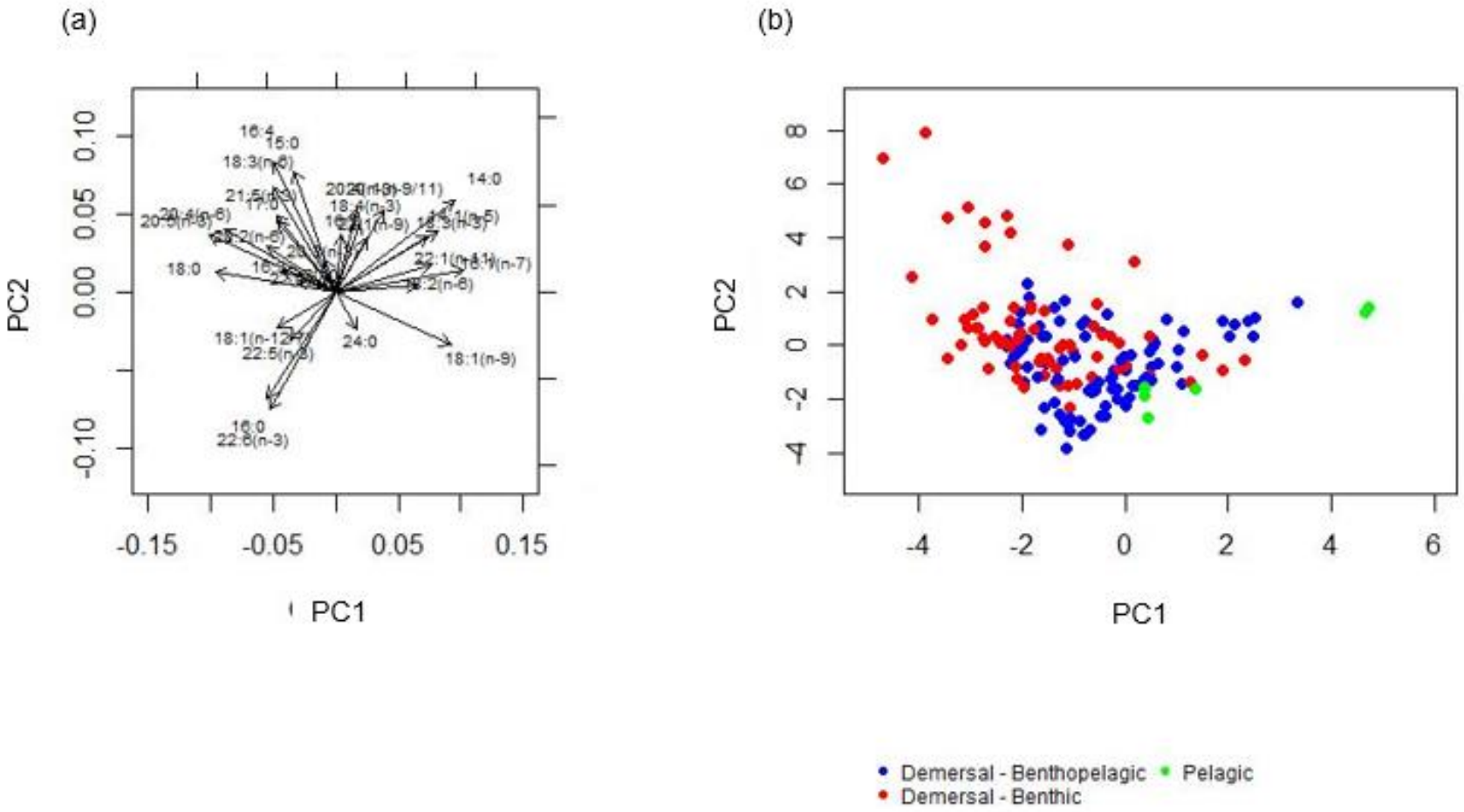


Figure 3.3: (a) PCA loading plot and (b) PCA score plot, both demonstrating variation in the FA profiles (normalised area percentages) across three water column depths: pelagic, benthopelagic and benthic.

Table 3.2: The mean ± 1 standard deviation of normalised area % of each FA and total SFA, MUFA and PUFA in the profile of the nineteen sample categories identified on the basis of PCA of the FA profiles (Figures 3.1b – 3.3b). LoD not shown as data is displayed as normalised area %.

Category	Sample Number	14:0	14:1(n-5)	15:0	16:0	16:1(n-7)	16:2(n-6)
Harbour Seal Blubber	10	3.96 ± 1.14	0.27 ± 0.20	0.40 ± 0.13	12.41 ± 5.17	15.22 ± 3.09	0.37 ± 0.14
Harbour Porpoise Blubber	18	12.22 ± 3.54	1.53 ± 0.53	0.79 ± 0.13	9.52 ± 0.89	22.48 ± 5.84	0.74 ± 0.15
Sperm Whale Blubber	5	5.98 ± 0.46	0.23 ± 0.09	0.97 ± 0.20	9.07 ± 1.53	20.94 ± 5.31	0.89 ± 0.18
Pelagic Roundfish Whole	3	5.21 ± 0.05	0.05 ± 0.01	0.70 ± 0.01	19.87 ± 0.27	7.55 ± 0.45	0.42 ± 0.01
Pelagic Roundfish Muscle	2	7.77 ± 0.02	0.25 ± 0.01	0.43 ± 0.01	14.21 ± 0.05	3.33 ± 0.53	0.35 ± 0.03
Pelagic Roundfish Liver	2	3.43 ± 0.14	0.43 ± 0.11	0.47 ± 0.01	20.39 ± 1.67	2.07 ± 0.03	0.17 ± 0.01
Demersal Shark Muscle	12	1.74 ± 0.49	0.23 ± 0.14	0.40 ± 0.09	20.82 ± 1.38	4.86 ± 1.10	0.23 ± 0.18
Demersal Shark Liver	12	2.37 ± 0.30	0.13 ± 0.09	0.58 ± 0.07	16.54 ± 1.70	7.44 ± 1.14	0.34 ± 0.11
Demersal Roundfish Whole	6	1.99 ± 0.99	0.12 ± 0.06	0.72 ± 0.06	15.25 ± 1.84	3.82 ± 1.42	0.77 ± 0.24
Demersal Roundfish Muscle	30	2.03 ± 0.56	0.13 ± 0.13	0.51 ± 0.18	16.12 ± 1.21	3.67 ± 1.31	0.75 ± 0.30
Demersal Roundfish Liver	30	3.79 ± 1.07	0.15 ± 0.10	0.63 ± 0.22	15.15 ± 1.61	5.99 ± 1.84	0.77 ± 0.26
Flatfish Muscle	12	2.52 ± 0.77	0.19 ± 0.09	0.66 ± 0.14	18.28 ± 1.13	5.47 ± 1.41	1.09 ± 0.16
Flatfish Liver	12	3.15 ± 0.87	0.53 ± 0.20	0.67 ± 0.20	18.68 ± 2.19	10.54 ± 4.15	1.05 ± 0.27
Demersal Invertebrates Muscle	2	3.65 ± 0.18	0.49 ± 0.10	0.48 ± 0.03	24.00 ± 0.21	3.17 ± 0.19	<LoD
Benthic Invertebrates Whole	11	6.19 ± 3.18	0.36 ± 0.14	1.02 ± 0.55	10.65 ± 3.65	3.07 ± 1.23	0.50 ± 0.59
Benthic Invertebrates Muscle	13	1.48 ± 0.76	0.23 ± 0.15	0.79 ± 0.21	14.81 ± 2.77	6.19 ± 1.09	0.39 ± 0.27
Benthic Invertebrates Brown Meat	3	2.23 ± 0.70	0.24 ± 0.04	0.77 ± 0.19	13.84 ± 0.53	12.17 ± 4.65	0.21 ± 0.29
Benthic Invertebrates Soft Body	17	2.15 ± 0.53	0.25 ± 0.13	0.80 ± 0.38	15.72 ± 2.28	5.10 ± 2.28	0.38 ± 0.42
Zooplankton Whole	5	5.86 ± 0.83	0.37 ± 0.30	1.10 ± 0.09	15.62 ± 1.08	6.94 ± 0.13	1.28 ± 0.04

Table 3.2 (continued): The mean ± 1 standard deviation of normalised area % of each FA and total SFA, MUFA and PUFA in the profile of the nineteen sample categories identified on the basis of PCA of the FA profiles (Figures 3.1b – 3.3b). LoD not shown as data is displayed as normalised area %.

Category	Sample Number	17:0	16:3(n-3)	16:4(n-3)	18:0	18:1(n-9)	18:1(n-7)
Harbour Seal Blubber	10	0.31 ± 0.08	0.13 ± 0.13	0.16 ± 0.13	1.61 ± 0.81	23.18 ± 5.06	5.34 ± 1.57
Harbour Porpoise Blubber	18	0.32 ± 0.10	0.15 ± 0.10	0.18 ± 0.10	1.26 ± 0.53	21.54 ± 2.62	2.88 ± 2.53
Sperm Whale Blubber	5	<LoD	0.53 ± 0.19	0.17 ± 0.09	1.40 ± 0.35	36.68 ± 1.99	3.56 ± 0.58
Pelagic Roundfish Whole	3	0.35 ± 0.05	0.37 ± 0.02	0.30 ± 0.05	3.51 ± 0.21	23.06 ± 1.30	10.12 ± 0.46
Pelagic Roundfish Muscle	2	0.23 ± 0.02	0.16 ± 0.07	0.22 ± 0.06	1.51 ± 0.06	8.83 ± 1.27	1.63 ± 0.04
Pelagic Roundfish Liver	2	0.37 ± 0.15	<LoD	0.10 ± 0.10	2.95 ± 0.24	8.41 ± 0.39	4.58 ± 0.32
Demersal Shark Muscle	12	0.72 ± 0.30	0.30 ± 0.30	0.46 ± 0.35	4.86 ± 0.85	12.08 ± 1.77	6.29 ± 1.01
Demersal Shark Liver	12	0.83 ± 0.25	0.32 ± 0.26	0.21 ± 0.11	3.74 ± 0.65	13.72 ± 1.78	7.50 ± 1.63
Demersal Roundfish Whole	6	1.00 ± 0.25	0.49 ± 0.16	0.65 ± 0.34	6.10 ± 0.98	11.90 ± 3.43	5.88 ± 0.65
Demersal Roundfish Muscle	30	0.63 ± 0.25	0.38 ± 0.21	0.19 ± 0.12	4.81 ± 0.84	11.19 ± 1.67	5.19 ± 1.98
Demersal Roundfish Liver	30	0.67 ± 0.37	0.55 ± 0.31	0.29 ± 0.13	4.36 ± 1.36	15.10 ± 2.39	6.34 ± 2.23
Flatfish Muscle	12	0.74 ± 0.22	0.58 ± 0.14	0.35 ± 0.21	4.44 ± 0.67	7.82 ± 1.92	4.04 ± 0.60
Flatfish Liver	12	0.78 ± 0.37	0.84 ± 0.34	0.05 ± 0.10	3.40 ± 1.25	15.39 ± 7.74	6.49 ± 1.36
Demersal Invertebrates Muscle	2	0.59 ± 0.06	0.11 ± 0.01	0.56 ± 0.01	3.48 ± 0.15	3.32 ± 0.23	1.78 ± 0.06
Benthic Invertebrates Whole	11	0.72 ± 0.29	0.17 ± 0.27	8.13 ± 2.25	7.58 ± 3.12	1.97 ± 1.44	5.71 ± 0.89
Benthic Invertebrates Muscle	13	0.95 ± 0.44	0.74 ± 0.18	1.18 ± 1.19	4.22 ± 1.67	12.46 ± 3.46	6.38 ± 1.27
Benthic Invertebrates Brown Meat	3	0.53 ± 0.12	0.76 ± 0.14	1.35 ± 0.97	3.80 ± 0.09	14.91 ± 2.35	9.23 ± 0.52
Benthic Invertebrates Soft Body	17	0.94 ± 0.46	0.58 ± 0.47	2.20 ± 1.29	6.53 ± 1.85	6.83 ± 2.91	6.03 ± 1.72
Zooplankton Whole	5	4.67 ± 0.71	0.52 ± 0.51	1.91 ± 0.36	1.75 ± 0.19	4.35 ± 0.71	1.90 ± 0.21

Table 3.2 (continued): The mean ± 1 standard deviation of normalised area % of each FA and total SFA, MUFA and PUFA in the profile of the nineteen sample categories identified on the basis of PCA of the FA profiles (Figures 3.1b – 3.3b). LoD not shown as data is displayed as normalised area %.

Category	Sample Number	18:2(n-6)	18:3(n-6)	18:3(n-3)	18:4(n-3)	20:0	20:1(n-9)
Harbour Seal Blubber	10	1.56 ± 0.37	0.08 ± 0.08	0.87 ± 0.27	1.11 ± 0.64	0.04 ± 0.03	3.59 ± 1.61
Harbour Porpoise Blubber	18	1.81 ± 0.37	0.03 ± 0.03	1.16 ± 0.27	0.95 ± 0.40	0.03 ± 0.03	4.11 ± 1.64
Sperm Whale Blubber	5	0.94 ± 0.20	<LoD	<LoD	0.50 ± 0.27	0.93 ± 0.47	9.16 ± 3.13
Pelagic Roundfish Whole	3	0.68 ± 0.06	0.05 ± 0.01	0.05 ± 0.03	1.16 ± 0.05	0.22 ± 0.11	1.04 ± 0.06
Pelagic Roundfish Muscle	2	1.39 ± 0.02	0.03 ± 0.01	0.92 ± 0.08	1.60 ± 0.03	0.12 ± 0.11	12.57 ± 0.14
Pelagic Roundfish Liver	2	1.43 ± 0.29	<LoD	0.33 ± 0.33	0.80 ± 0.03	<LoD	5.81 ± 0.91
Demersal Shark Muscle	12	0.94 ± 0.14	0.11 ± 0.12	0.32 ± 0.26	0.48 ± 0.20	0.10 ± 0.10	1.12 ± 0.37
Demersal Shark Liver	12	1.02 ± 0.20	0.23 ± 0.22	0.62 ± 0.26	1.03 ± 0.39	0.15 ± 0.13	1.94 ± 0.60
Demersal Roundfish Whole	6	0.94 ± 0.13	0.15 ± 0.11	0.45 ± 0.22	0.59 ± 0.42	0.15 ± 0.07	1.22 ± 0.40
Demersal Roundfish Muscle	30	0.93 ± 0.35	0.17 ± 0.15	0.42 ± 0.24	0.90 ± 0.32	0.11 ± 0.09	2.21 ± 1.60
Demersal Roundfish Liver	30	1.31 ± 0.28	0.28 ± 0.26	0.66 ± 0.38	1.53 ± 0.53	0.14 ± 0.06	4.42 ± 3.08
Flatfish Muscle	12	0.82 ± 0.48	0.13 ± 0.11	0.26 ± 0.19	0.56 ± 0.16	0.13 ± 0.07	1.25 ± 0.29
Flatfish Liver	12	1.24 ± 0.60	0.30 ± 0.24	0.33 ± 0.18	0.48 ± 0.12	0.12 ± 0.09	2.07 ± 0.51
Demersal Invertebrates Muscle	2	0.22 ± 0.00	<LoD	0.14 ± 0.00	0.17 ± 0.01	<LoD	3.22 ± 0.27
Benthic Invertebrates Whole	11	0.26 ± 0.39	0.51 ± 0.40	0.35 ± 0.25	0.70 ± 0.28	0.15 ± 0.15	10.82 ± 5.75
Benthic Invertebrates Muscle	13	1.17 ± 0.24	0.23 ± 0.16	0.38 ± 0.19	0.59 ± 0.80	0.51 ± 0.25	1.77 ± 1.61
Benthic Invertebrates Brown Meat	3	1.58 ± 0.58	0.06 ± 0.05	0.54 ± 0.20	0.48 ± 0.11	0.21 ± 0.03	2.61 ± 0.55
Benthic Invertebrates Soft Body	17	1.36 ± 0.43	0.20 ± 0.18	0.32 ± 0.21	0.69 ± 0.70	0.21 ± 0.18	2.45 ± 1.47
Zooplankton Whole	5	0.74 ± 0.09	0.32 ± 0.13	0.34 ± 0.03	2.47 ± 1.32	0.21 ± 0.09	0.69 ± 0.42

Table 3.2 (continued): The mean ± 1 standard deviation of normalised area % of each FA and total SFA, MUFA and PUFA in the profile of the nineteen sample categories identified on the basis of PCA of the FA profiles (Figures 3.1b – 3.3b). LoD not shown as data is displayed as normalised area %.

Category	Sample Number	20:2(n-6)	20:3(n-3)	20:4(n-6)	20:4(n-3)	20:5(n-3)	22:0
Harbour Seal Blubber	10	0.18 ± 0.12	0.07 ± 0.10	0.86 ± 0.38	0.49 ± 0.25	5.37 ± 1.55	0.01 ± 0.02
Harbour Porpoise Blubber	18	0.06 ± 0.06	0.04 ± 0.04	0.28 ± 0.17	0.65 ± 0.35	2.78 ± 1.63	0.02 ± 0.03
Sperm Whale Blubber	5	0.06 ± 0.12	<LoD	<LoD	0.08 ± 0.12	0.21 ± 0.28	0.04 ± 0.04
Pelagic Roundfish Whole	3	0.13 ± 0.05	0.01 ± 0.02	0.47 ± 0.06	0.34 ± 0.03	7.71 ± 1.21	0.04 ± 0.03
Pelagic Roundfish Muscle	2	0.05 ± 0.05	0.11 ± 0.00	0.44 ± 0.07	0.41 ± 0.01	4.83 ± 0.08	0.03 ± 0.01
Pelagic Roundfish Liver	2	0.05 ± 0.02	<LoD	0.79 ± 0.57	0.44 ± 0.21	10.39 ± 0.80	0.09 ± 0.09
Demersal Shark Muscle	12	0.21 ± 0.20	0.06 ± 0.06	3.69 ± 0.78	0.41 ± 0.17	7.94 ± 1.35	0.10 ± 0.09
Demersal Shark Liver	12	0.51 ± 0.14	0.11 ± 0.09	1.63 ± 0.49	0.67 ± 0.18	9.58 ± 1.38	0.33 ± 0.81
Demersal Roundfish Whole	6	0.51 ± 0.12	0.14 ± 0.09	4.20 ± 1.91	0.37 ± 0.12	14.48 ± 3.18	0.20 ± 0.15
Demersal Roundfish Muscle	30	0.39 ± 0.19	0.10 ± 0.08	2.56 ± 1.20	0.48 ± 0.13	13.54 ± 2.76	0.10 ± 0.14
Demersal Roundfish Liver	30	0.45 ± 0.10	0.15 ± 0.06	1.39 ± 0.97	0.66 ± 0.16	11.91 ± 2.65	0.06 ± 0.07
Flatfish Muscle	12	0.28 ± 0.14	0.08 ± 0.15	4.73 ± 1.64	0.43 ± 0.08	18.24 ± 3.08	0.11 ± 0.11
Flatfish Liver	12	0.35 ± 0.02	0.10 ± 0.07	2.37 ± 1.16	0.49 ± 0.14	9.34 ± 0.52	0.04 ± 0.07
Demersal Invertebrates Muscle	2	0.16 ± 0.54	0.18 ± 0.00	0.88 ± 0.07	0.15 ± 0.02	13.04 ± 4.66	<LoD
Benthic Invertebrates Whole	11	1.32 ± 0.55	0.07 ± 0.22	7.95 ± 3.79	0.67 ± 0.39	17.94 ± 4.66	0.21 ± 0.31
Benthic Invertebrates Muscle	13	0.78 ± 0.42	0.11 ± 0.12	4.09 ± 1.00	0.26 ± 0.18	20.78 ± 5.63	0.24 ± 0.24
Benthic Invertebrates Brown Meat	3	1.37 ± 0.53	0.38 ± 0.23	2.96 ± 0.47	0.33 ± 0.06	11.03 ± 2.13	0.07 ± 0.10
Benthic Invertebrates Soft Body	17	2.30 ± 1.92	0.12 ± 0.23	5.36 ± 1.80	0.32 ± 0.21	20.83 ± 2.01	0.11 ± 0.09
Zooplankton Whole	5	0.07 ± 0.02	2.28 ± 1.56	0.43 ± 0.14	0.91 ± 0.13	23.39 ± 1.34	0.04 ± 0.05

Table 3.2 (continued): The mean ± 1 standard deviation of normalised area % of each FA and total SFA, MUFA and PUFA in the profile of the nineteen sample categories identified on the basis of PCA of the FA profiles (Figures 3.1b – 3.3b). LoD not shown as data is displayed as normalised area %.

Category	Sample Number	22:1(n-11)	22:1(n-9)	21:5(n-3)	24:0	22:5(n-3)	22:6(n-3)
Harbour Seal Blubber	10	1.47 ± 1.32	0.12 ± 0.18	0.29 ± 0.15	0.18 ± 0.13	5.36 ± 1.34	12.46 ± 1.93
Harbour Porpoise Blubber	18	5.12 ± 2.81	0.20 ± 0.22	0.11 ± 0.11	0.08 ± 0.06	1.92 ± 1.15	6.85 ± 3.70
Sperm Whale Blubber	5	7.43 ± 1.52	0.41 ± 0.34	0.07 ± 0.13	0.03 ± 0.02	0.09 ± 0.12	0.32 ± 0.36
Pelagic Roundfish Whole	3	1.65 ± 0.44	0.26 ± 0.21	0.24 ± 0.16	<LoD	0.88 ± 0.02	13.20 ± 0.14
Pelagic Roundfish Muscle	2	25.38 ± 0.26	1.30 ± 0.01	0.14 ± 0.09	0.19 ± 0.03	0.68 ± 0.03	9.88 ± 1.73
Pelagic Roundfish Liver	2	6.72 ± 0.27	<LoD	0.02 ± 0.02	0.23 ± 0.23	1.15 ± 0.79	27.61 ± 1.51
Demersal Shark Muscle	12	0.50 ± 0.51	0.04 ± 0.11	0.21 ± 0.21	0.11 ± 0.15	4.87 ± 0.66	25.50 ± 3.99
Demersal Shark Liver	12	1.05 ± 0.76	0.10 ± 0.23	0.37 ± 0.23	0.22 ± 0.21	3.75 ± 1.00	22.21 ± 1.39
Demersal Roundfish Whole	6	0.65 ± 0.55	0.18 ± 0.13	0.30 ± 0.08	0.02 ± 0.03	2.62 ± 0.75	22.73 ± 3.62
Demersal Roundfish Muscle	30	1.61 ± 1.63	0.05 ± 0.12	0.28 ± 0.12	0.08 ± 0.29	2.05 ± 0.91	27.24 ± 6.73
Demersal Roundfish Liver	30	4.04 ± 3.47	0.11 ± 0.42	0.39 ± 0.11	0.05 ± 0.08	1.98 ± 0.69	16.09 ± 3.56
Flatfish Muscle	12	0.53 ± 0.45	0.01 ± 0.04	0.36 ± 0.10	<LoD	3.59 ± 0.70	21.52 ± 4.67
Flatfish Liver	12	0.43 ± 0.34	0.37 ± 0.29	0.36 ± 0.12	<LoD	2.11 ± 0.96	17.29 ± 9.62
Demersal Invertebrates Muscle	2	0.77 ± 0.41	<LoD	0.13 ± 0.00	<LoD	0.54 ± 0.04	38.28 ± 0.16
Benthic Invertebrates Whole	11	0.55 ± 0.45	0.57 ± 0.42	0.64 ± 0.78	<LoD	1.61 ± 0.94	8.50 ± 3.29
Benthic Invertebrates Muscle	13	1.24 ± 2.67	0.16 ± 0.25	0.30 ± 0.15	0.12 ± 0.38	1.60 ± 0.63	15.66 ± 1.98
Benthic Invertebrates Brown Meat	3	1.57 ± 0.92	0.33 ± 0.24	0.28 ± 0.08	<LoD	1.65 ± 0.06	13.99 ± 2.64
Benthic Invertebrates Soft Body	17	0.28 ± 0.24	0.04 ± 0.05	0.41 ± 0.20	0.04 ± 0.07	4.80 ± 3.02	12.57 ± 3.11
Zooplankton Whole	5	1.16 ± 0.84	<LoD	0.73 ± 0.07	0.01 ± 0.00	1.10 ± 0.13	17.37 ± 1.45

Table 3.2 (continued): The mean ± 1 standard deviation of normalised area % of each FA and total SFA, MUFA and PUFA in the profile of the nineteen sample categories identified on the basis of PCA of the FA profiles (Figures 3.1b – 3.3b). LoD not shown as data is displayed as normalised area %.

Category	Sample Number	24:1(n-9)	Total SFA	Total MUFA	Total PUFA
Harbour Seal Blubber	10	2.51 ± 5.15	18.85 ± 6.40	50.58 ± 8.10	30.19 ± 4.42
Harbour Porpoise Blubber	18	0.16 ± 0.09	24.36 ± 2.86	52.87 ± 6.60	22.01 ± 7.27
Sperm Whale Blubber	5	0.27 ± 0.29	17.45 ± 2.44	78.67 ± 2.54	3.87 ± 1.46
Pelagic Roundfish Whole	3	0.34 ± 0.18	30.13 ± 0.30	42.53 ± 0.86	26.88 ± 2.38
Pelagic Roundfish Muscle	2	1.01 ± 0.04	25.57 ± 0.13	27.80 ± 1.70	46.24 ± 1.17
Pelagic Roundfish Liver	2	0.77 ± 0.33	27.61 ± 2.21	22.14 ± 0.62	49.99 ± 1.59
Demersal Shark Muscle	12	0.29 ± 0.20	28.73 ± 1.50	25.15 ± 3.12	45.99 ± 2.75
Demersal Shark Liver	12	0.75 ± 1.57	24.38 ± 1.97	32.38 ± 3.89	42.80 ± 4.09
Demersal Roundfish Whole	6	1.42 ± 0.74	25.45 ± 1.71	25.31 ± 4.59	49.08 ± 3.16
Demersal Roundfish Muscle	30	1.18 ± 2.40	24.34 ± 1.27	25.30 ± 4.18	50.15 ± 6.11
Demersal Roundfish Liver	30	0.58 ± 0.30	24.91 ± 1.77	36.13 ± 3.86	38.82 ± 3.99
Flatfish Muscle	12	0.79 ± 0.14	26.87 ± 1.48	20.10 ± 3.82	53.03 ± 3.78
Flatfish Liver	12	0.65 ± 0.30	26.83 ± 1.79	36.47 ± 12.11	36.70 ± 4.49
Demersal Invertebrates Muscle	2	0.47 ± 0.05	32.21 ± 0.14	13.22 ± 0.49	54.56 ± 13.02
Benthic Invertebrates Whole	11	1.10 ± 0.44	26.53 ± 7.43	24.14 ± 5.29	49.33 ± 7.27
Benthic Invertebrates Muscle	13	0.18 ± 0.23	23.10 ± 3.27	28.97 ± 6.46	47.62 ± 5.84
Benthic Invertebrates Brown Meat	3	0.54 ± 0.28	21.45 ± 0.73	41.58 ± 4.10	36.97 ± 4.16
Benthic Invertebrates Soft Body	17	0.05 ± 0.08	26.56 ± 2.37	20.80 ± 4.95	52.64 ± 3.71
Zooplankton Whole	5	1.46 ± 0.19	33.10 ± 0.39	15.60 ± 0.58	50.05 ± 0.26

3.3.1.1 Marine mammals (mammalia)

Mammalia were more positively correlated to the first principal component when samples were grouped on the basis of taxonomic class alone (Figure 3.1b) due to a higher proportion of monounsaturated FAs (MUFAs) such as 16:1(n-7), 22:1(n-11), 18:1(n-9) and 14:1(n-5) and medium chain length PUFAs such as 18:2(n-6) in marine mammal blubber. PCA was therefore applied to the marine mammal samples on a species by species basis (Figure 3.4a and b). The first two principal components accounted for 52% of the FA variability.

Although sample numbers a of sperm whales is smaller than harbour porpoise and harbour seals, sperm whale possess the least variable FA profile in this dataset (Figure 3.4b). Sperm whale samples were separated from the other marine mammals in the PCA plots due to a significantly higher proportion of 18:1(n-9) and lower proportion of 22:6(n-3) ($p < 0.05$, ANOVA, Tukey). Sperm whales are long lived Odontoceti predators, inhabiting mesopelagic ecosystems and have a variable diet dependent on geographical region, sex and age (Best, 1999). In some oceanic areas, they feed primarily on bathypelagic and mesopelagic cephalopods (Ruiz-Cooley *et al*., 2004) whilst in others, such as Iceland, fish are the principal source of food (Roe, 1969). Previous studies on the lipid composition of sperm whales (male and female) collected from the Azores, found the main FA profile contributors in blubber to be 18:1(n-9), 16:1(n-7) and 16:0 (Walton *et al*., 2008), which agrees with the data from this study; these three FAs account for over 60% of the FAs present in blubber tested.

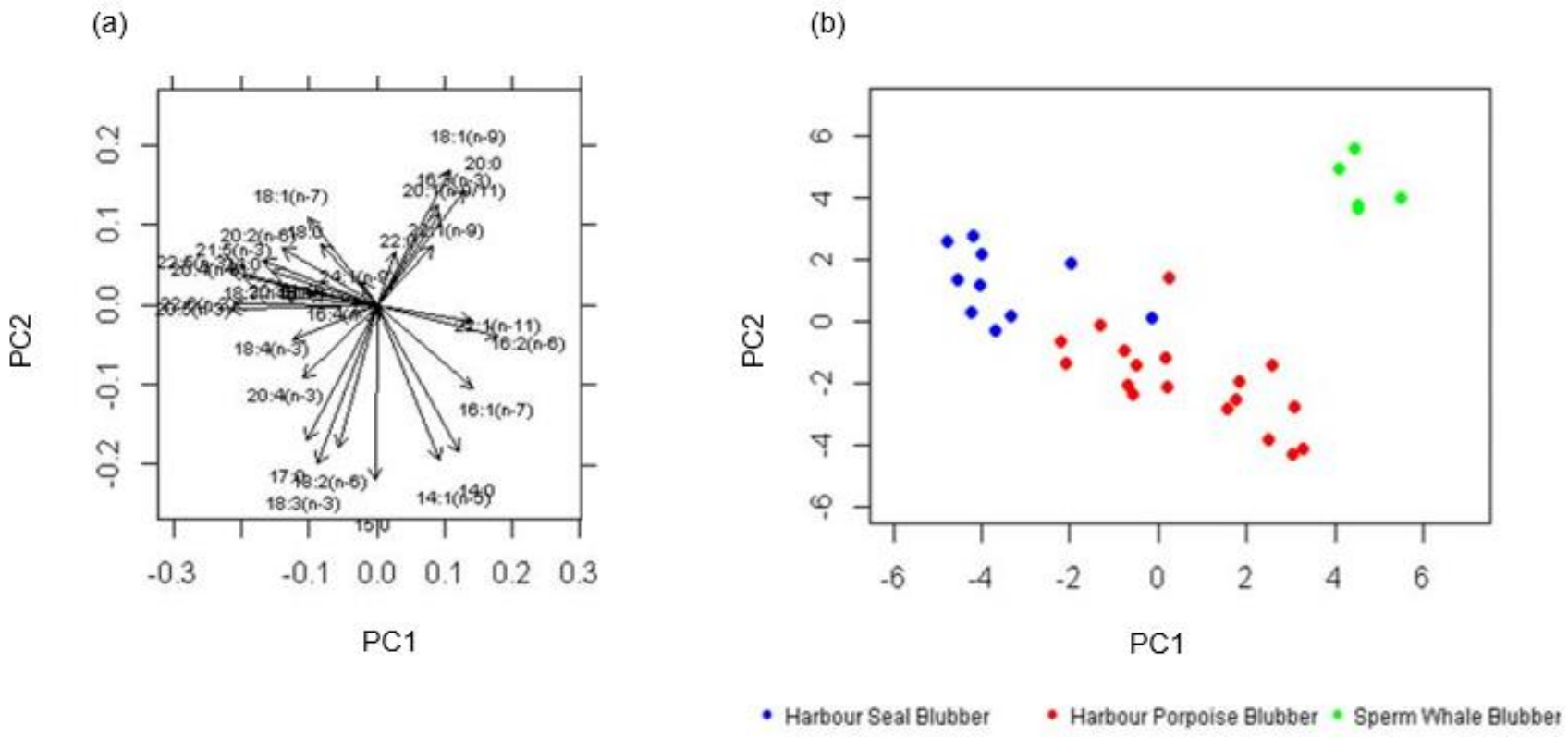


Figure 3.4: (a) PCA loading plot and (b): PCA score plot demonstrating variation in the FA profiles (normalised area percentages) across the three marine mammal species. Sperm whale blubber is well separated from the harbour porpoise and harbour seal blubber with the latter also showing a good degree of separation. As such it is appropriate to report on these as separate categories (see Table 3.2).

The three marine mammal species contained a significantly higher proportion of the FA marker 18:1(n-9) compared to other organisms in the study ($p < 0.05$, ANOVA, Tukey). This marker is reported to be an indicator of a carnivorous diet (Nelson *et al*., 2001) and the larger the accumulation, the more carnivorous the organism.

Harbour seal and harbour porpoise are widely dispersed on PC1 (Figure 3.4b) but are generally separated by species across PC1 and PC2 (Figure 3.4b). The degree of variation of 18:1(n-9), 16:0 and 24:1(n-9) was greatest in harbour seal, each possessing a standard deviation (SD) of >5, suggesting that harbour seal diet is highly variable. Harbour porpoise are more negatively correlated to PC2 (Figure 3.4b) than the other Mammalia species due to the higher proportion of MUFAs 16:1(n-7) and 14:1(n-5) and the dienoic acid 18:2(n-6), ($p < 0.05$, ANOVA, Tukey) in their blubber. This supports previous findings from harbour porpoise around Scotland where 16:1(n-7) and 18:1(n-9) were the most predominant FAs (Learmonth, 2003) detected. 16:1(n-7) is a diatom biomarker (Linder *et al.,* 2010) indicating harbour porpoise were likely feeding on pelagic fish or other planktonic feeding prey. There was significant variation ($SD > 3$) present for the FAs 14:0, 16:1(n-7) and 22:6(n-3). Potential influencing factors such as sampling location (biogeographic and localised), sampling year and age (all listed on Table 2.2) were investigated on harbour porpoise and harbour seal but were not found to significantly influence the data ($p > 0.05$). However, the one harbour seal sample at point 0,0 on Figure 3.4 is separate from the harbour seal cluster and when investigated further, was identified as the smallest and lightest individual (Table 2.2) with the lowest recorded ventral blubber thickness (11 mm in comparison to 15 – 105 mm in the other 9 samples). This was the only harbour seal individual to have a cause of death reported as “chronic peritonitis due to ingestion of foreign body (fishing gear)” and was found in an emaciated condition with poor blubber deposits. Seven individuals had a reported cause of death of “physical trauma”, one reported as “pneumonia” and one reported as “N/A”. There is also a harbour porpoise sample between point 0 and +2 on the second component which is separated from the main sample cluster. Unfortunately, other than sampling location and length, there was no additional information available on this specimen.

3.3.1.2 Fish (actinopterygii) and catshark (chondrichthyes)

The actinopterygii class was separated into eight sub-categories: demersal roundfish muscle, demersal roundfish liver, demersal roundfish whole (length < 120 mm), pelagic roundfish muscle, pelagic roundfish liver, pelagic roundfish whole, flatfish muscle and flatfish liver. The first two principal components accounted for 40% of the FA variability.

PCA (Figure 3.5a and b) showed that the demersal roundfish muscle, flatfish muscle, pelagic roundfish liver and demersal shark muscle were more negatively correlated to PC2 than other categories due to having a higher proportion of 22:6(n-3), 16:0 and 22:5(n-6). These categories possessed a significantly higher proportion of 22:6(n-3) ($p < 0.05$, ANOVA, Tukey) in comparison to the other categories. 22:6(n-3) is a common dominant FA in marine species required for growth and development, particularly to maintain the functional and structural integrity of cell membranes (Scott *et al.,* 2002). 22:6(n-3) is therefore higher in demersal fish muscle than liver due to the larger proportion of structural lipids. 22:6(n-3) is also characteristically higher in fish associated with the pelagic environment due to the predominant feeding on planktivorous prey (Cury *et al.*, 2000). Pelagic fish are likely to contain greater proportions of PUFAs associated to structural lipids, in their liver and MUFAs, associated to storage lipid, in their muscle tissue relative to the demersal species (Linder *et al.,* 2010). Demersal fish liver and pelagic muscle samples are positively correlated with PC2 (Figure 3.5b) due to a lower proportion of 22:6(n-3), which again is consistent with their physiology (Njinkoué *et al.,* 2002).

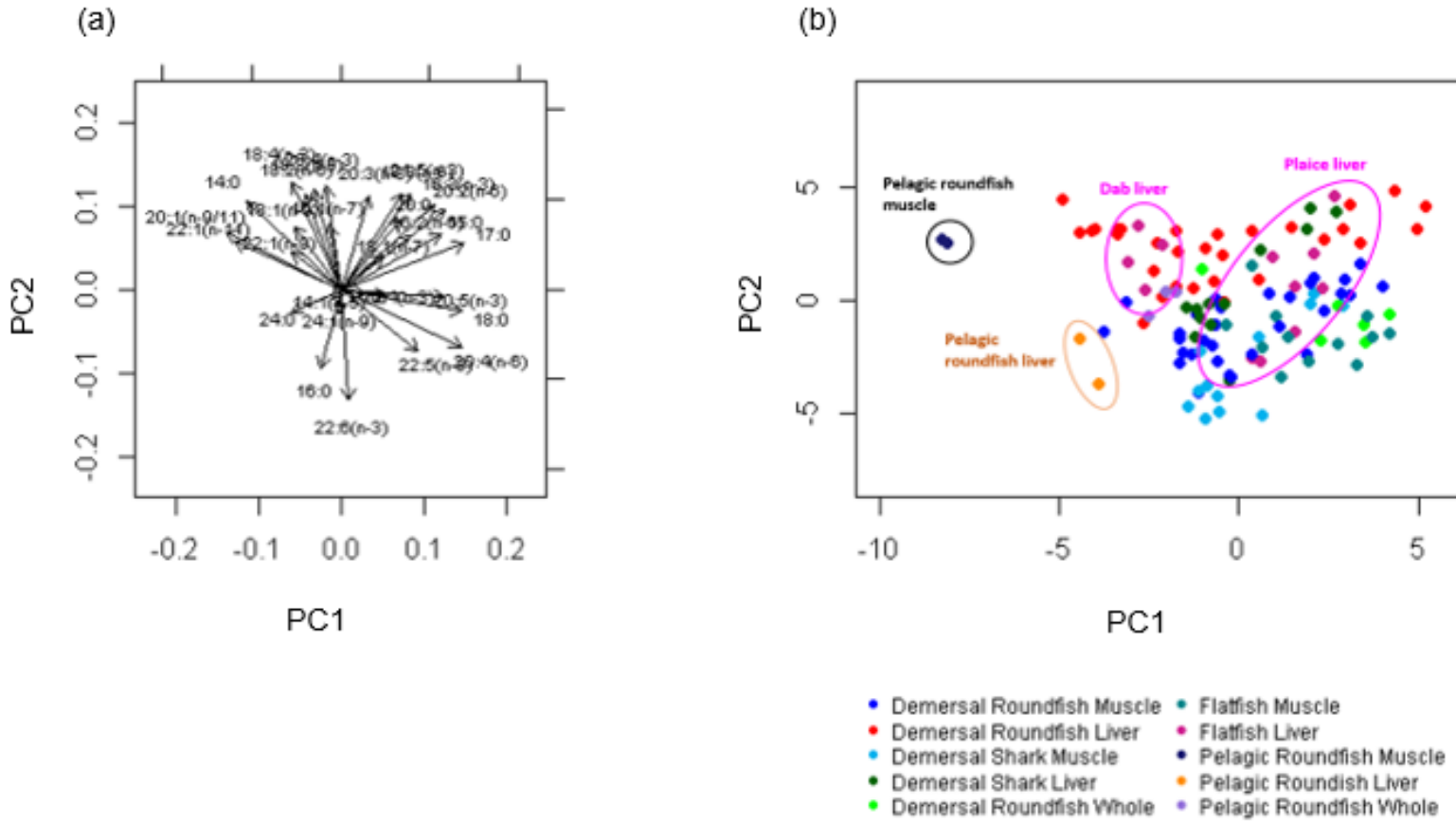


Figure 3.5: (a) PCA loading plot and (b) PCA score plot, demonstrating variation in the FA profiles (normalised area percentages) across the ten categories of fish and shark highlighting the group separation of pelagic fish muscle and liver due to differing proportions of MUFAs and plaice liver and muscle due to differing proportions of 18:1(n-9).

Flatfish liver showed the highest degree of variation of the MUFAs 16:1(n-7) and 18:1(n-9) (SD > 4) and PUFA 22:6(n-3) (SD > 9) in comparison to the other categories (Table 3.2). When flatfish liver was investigated, dab had significantly higher average proportions of 18:1(n-9) (26.39 ± 2.22%; n = 3) than plaice 18:1(n-9) (11.72 ± 5.30%; n = 9) ($p < 0.05$, ANOVA, Tukey) and 22:6(n-3) was significantly higher in plaice liver than dab liver ($p < 0.05$, ANOVA, Tukey), separating the species on the PCA score plot (Figure 3.5b). Sampling location (Table 2.1), average length (ranging from 198 to 350 mm), average weight (ranging from 82.60 to 508.0 g) and average age (ranging from 3.4 to 10.0 years) did not significantly influence the plaice FA data ($p > 0.05$), suggesting the within species variation for 22:6(n-3) is purely due to dietary differences. Flatfish are benthic organisms, feeding on a variety of zoobenthos including small crustaceans, bivalves, sand eels and polychaetes (Picton and Morrow, 2005). Although it has been reported that plaice and dab possess a similar diet of polychaetes and amphipods (Link *et al*., 2015), the FA profiles in this dataset suggest that differences in their diets are sufficient to result in a clear distinction in their tissue FA profiles.

The demersal roundfish liver category showed the largest degree of variation in the FA profile and are spread across PC1 from −5 to +5 (Figure 3.5). The FAs 22:1(n-11) and 22:6(n-3) within demersal roundfish liver showed the largest degree of variation (Table 3.2) and were influenced by the contributing species. Whiting liver has a significantly higher proportion of 22:1(n-11) and 22:6(n-3) compared to haddock liver and hake liver and hake a significantly higher proportion of 16:0 ($p < 0.05$, ANOVA, Tukey), suggesting dietary differences between the species. This is consistent with the pattern variation observed using PCA (Figure 3.3b, PC1 = −5 to +5). On a regional basis, haddock were the only demersal fish species collected from the Moray Firth (n=4) and hake from the Holy loch (n=2). Considering the species influence identified within the category, a larger sample number on a species basis across regions would be required for a comprehensive regional analysis.

Pelagic roundfish muscle and liver (herring) is negatively correlated with PC1 (Figure 3.5b) due to a higher proportion of MUFAs such as 20:1(n-9), 22:1(n-11) and 18:1(n-9). Monoenoic FAs are major characteristic components of pelagic fish tissue, whose lipids originate from their planktonic prey. 20:1(n-9), 22:1(n-11) and n-3 FAs are recognised copepod markers and higher proportions can be indicative of a copepod (zooplankton) enriched diet (Hiltunen, 2016). The dominant FA in pelagic roundfish whole (sprat) was 18:1(n-9), consistent with previous studies in the Baltic Sea and suggestive of a predominantly planktonic diet (Keinänen *et al*., 2017).

3.3.1.3 Benthic (malactostraca, bivalvia, asteroidea, ophiuroidea, polychaeta, gastropoda) and demersal (cephalopoda) invertebrates

PCA was applied to the benthic and demersal invertebrates FA data (Figure 3.6a and b) with considerable variation being observed (Figure 3.6b). The first two principal components accounted for 37% of the FA variability. The majority of benthic invertebrates whole (starfish and brittle star) are grouped together due to a higher proportion of saturated FAs (SFAs) including 14:0 and 18:0, MUFAs such as 20:1(n-9) and the PUFAs 20:4(n-6), 16:4(n-3) and 20:5(n-3) relative to demersal invertebrates. This corresponds with other studies where echinoderms contain a unique FA composition, characterized by proportionately higher 20:4(n-6) (Copeman and Parrish, 2003). 20:4(n-6) is indicative of benthic feeding and is a lipid required to induce maturation in starfish oocytes (Meijer, Guerrier and McClouf, 1984; Russell and Nichols, 1999). The variation in the proportion of 20:1(n-9) in the benthic invertebrates whole samples is due to the higher percentage in common starfish (*asteroidea*) (12.91 ± 3.99%; n = 9) compared to the other contributing species - brittle star (*ophiuroidea*) (2.67%) and sea mouse (*polychaeta*) (0.16%). A larger dataset is however required for a comprehensive analysis and comparison. Sargent, Falk-Petersen and Calder (1983) reported that common starfish can synthesise their own de novo 20:1 moieties (including 20:1(n-9)) which is required for bodily functions. Starfish and brittle star are more likely to feed upon molluscs and detritus than copepods. Brittle stars are significantly more enriched in 14:0 (12.86%; n = 1 pool; made up of 96 individuals) than the other contributing species of whole benthic invertebrates ($p < 0.05$, ANOVA, Tukey). Previous studies have found that saturated FAs such as 14:0 are ubiquitous among microalgae and are characteristic of calanoid species, suggesting brittle star are less carnivorous than the other benthic invertebrates in this study (Kopprio *et al.,* 2015). Although there is only one brittle star sample pool, this provides an indication of the feeding patterns of brittle star.

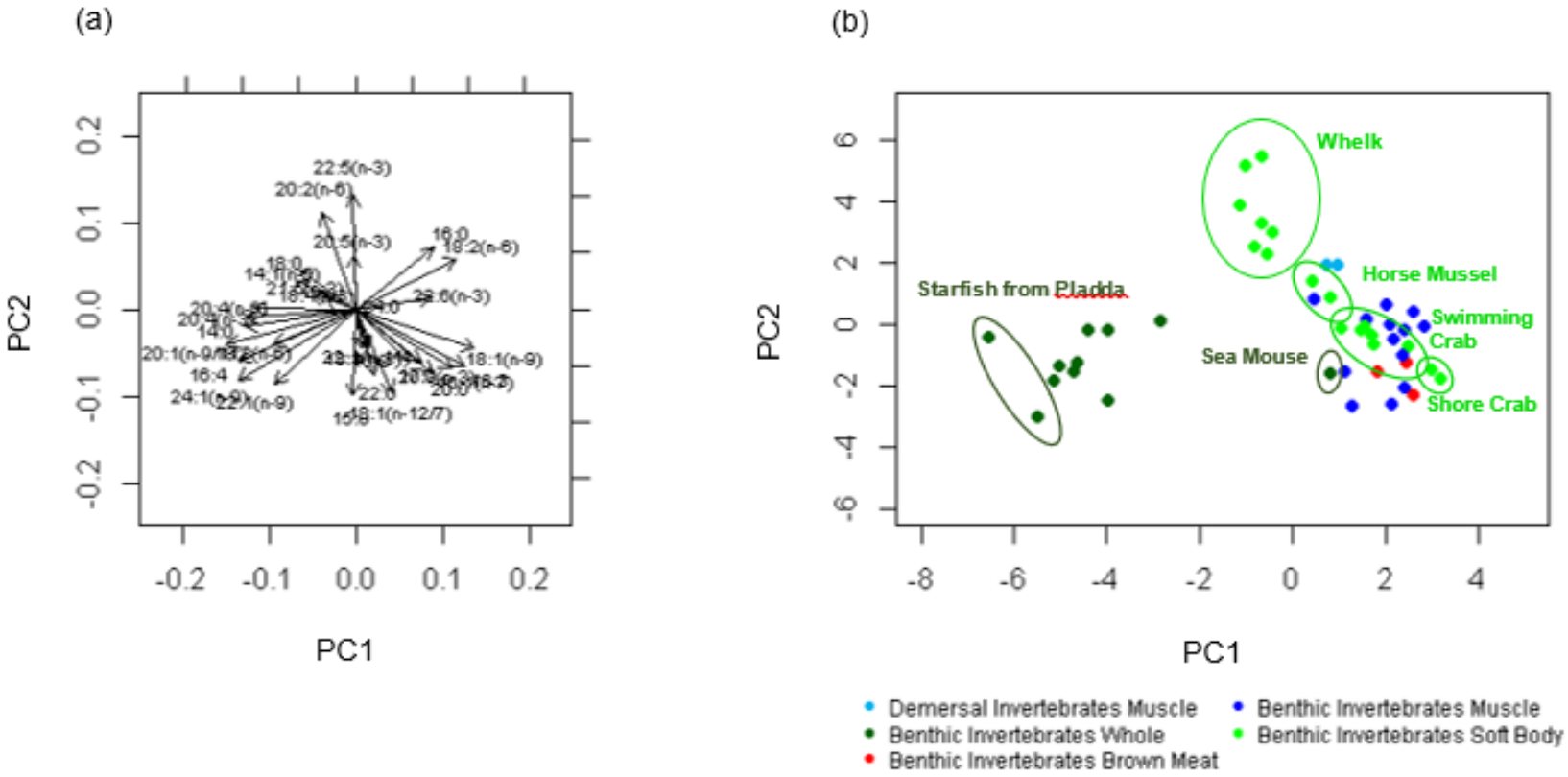


Figure 3.6: (a) PCA loading plot and (b) PCA score plot, demonstrating variation in the FA profiles (normalised area percentages) across the five categories of invertebrates highlighting the within-group separation of starfish collected from Pladda in comparison to the starfish group due to different proportions of 20:1(n-9), the separation of the four species in the invertebrates soft body category due to a contributing species FA profile influence and separation of one benthic invertebrates muscle sample pool , positioned with horse mussel.

A single sea mouse sample (made up of 33 individuals) is separated from the others in the category and is grouped with the benthic invertebrates muscle category (Figure 3.6). It is positively correlated to PC1 due to a lower proportion of the characteristic echinoderm markers of 20:1(n-9) and 20:4(n-6). Two common starfish sample pools are more negatively correlated to PC1 than the other common starfish pools. Starfish were collected from the Moray Firth, Solway and from three sites in the Clyde (Hunterston, Pladda and Holy Loch; Table 2.1). The two sample pools more negatively correlated to PC1 (Figure 3.6b) were collected from Pladda (lower Clyde) and had a higher normalised area % of the copepod marker 20:1(n-9) than the other starfish samples. This may suggest that starfish in Pladda have consumed a higher proportion of planktivorous feeding organisms compared to those in other sites, including those in the upper Clyde (Hunterston and Holy Loch) and the North East which possessed a different FA profile although this, of course, cannot be confirmed. Further influences such as average pool length (ranging from 161.7 to 396.0 mm) and average pool weight (ranging from 35.0 to 298.0 g) were investigated and were not found to influence the data ($p > 0.05$).

Demersal invertebrates (cephalopoda/squid; $n = 2$) are positively correlated to PC1 and negatively correlated to PC2 (Figure 3.6b) due to the higher proportion of 22:6(n-3) and 16:0. These FAs are the most characteristic FAs for squid (Phillips *et al.*, 2002) due to the much higher concentrations required for their rapid growth. For example, squid paralarvae require a high quantity of 22:6(n-3) during their rapid development (Navarro and Villanueva, 2000). Squid samples were found to have a significantly higher mean normalised area % ($38.28 \pm 0.16\%$) of 22:6(n-3), than other invertebrate categories ($p < 0.05$, ANOVA, Tukey).

Benthic invertebrates soft body sample pools gave rise to the most dispersed category (Figure 3.6b) and are spread across PC2 between −2 and +6. Whelk (*gastropoda*; n=7) contain very little variation in the species FA profile and are more positively correlated to PC2 than the other samples in the group. They have a higher proportion of the SFA 18:0 and PUFAs such as 20:2(n-6), 20:4(n-6) and 22:5(n-3). Gastropods (including whelk) are the most carnivorous in the category and are reported to feed on other benthic molluscs, worms and crustaceans (Chase, 2002). The other three species, composed of pools of horse mussel ($n = 2$), swimming crabs ($n = 6$) and shore crabs ($n = 2$), are more negatively correlated to PC2 and are separated on a species basis (Figure 3.6b), suggesting different feeding patterns between species but consistent feeding patterns within each species group. There is one benthic invertebrates muscle sample separated from the main category cluster and positioned with horse mussel (dark blue point within the green circle on Figure 3.6b). This is one squat lobster sample pool of three, and the

only pool collected from the Holy Loch (other two were collected from Hunterston and Pladda), suggesting different feeding patterns within the species group. A higher sample number is however required for a comprehensive analysis of the influence of sampling location on this species.

3.3.1.4 Zooplankton (hexanauplia)

Hexanauplia (zooplankton; n = 5) contain significant quantities of odd chain length SFAs such as 15:0 and 17:0 and the PUFAs 20:5(n-3) and 18:4(n-3). Both 20:5(n-3) and 18:4(n-3) are reported to be diatom and dinoflagellate phytoplankton markers, accumulating in the zooplankton primary consumer diet (Linder *et al*., 2010). Hexanauplia are positioned in-between the benthic invertebrates (*asteroidea* and *malacostraca*) and the more carnivorous Actinopterygii category (Figure 3.1b), suggesting they possess a similar feeding behaviour to these groups and have a more carnivorous feeding pattern due to higher proportions of 18:1(n-9) and 22:6(n-3) (Table 3.2). *Pseudocalanus Spp.* and *Calanus Spp.* are reported to perform diurnal vertical migrations, remaining in deeper water during the day and moving towards the surface at night to feed (Dale and Kaartvedt, 2000). There are variations of this behaviour at species, individual and population level. The water column depth and presence of predators might affect this behaviour and it has been found that predominantly herbivorous species are often detritovores (similar to the diet of echinoderms) when present in the benthopelagic environment (Mauchline *et al.,* 1998). They have been found to feed on a range of decomposing plants and animals which would classify the species as more carnivorous than a secondary consumer.

3.3.2 Fatty acid trophic markers (FATMs)

FATM analysis is based on the observation that the FA profiles of primary producers can be passed up food chains and can be retained at different trophic levels. Although modification of the profile occurs due to processes such as metabolism, certain FAs and FA ratios can be used as biomarkers for species with differing diets (Dalsgaard *et al*., 2003).

FATMs 20:5(n-3)/22:6(n-3) and 18:1(n−7)/18:1(n−9) were significantly higher in benthic invertebrate whole samples indicating organisms in this category are at a lower trophic level than the other categories (Table 3.3) ($p < 0.05$, ANOVA, Tukey). A larger sample is however required for a comprehensive analysis. These results do not agree with other

studies as zooplankton are primary consumers and therefore at a higher trophic level than invertebrates (Schulz and Yurista, 1999). The FATM 16:1(n-7)/16:0 was significantly higher in harbour porpoise blubber and sperm whale blubber ($p < 0.05$, ANOVA, Tukey) due to the characteristically higher proportion of diatom biomarker 16:1(n-7) in their profiles from their diet of pelagic fish or other planktonic prey. Although 16:1(n-7)/16:0 clearly indicates a diatom-based diet for this food chain, it is not appropriate as an indicator of trophic level due to the specific prey dietary characteristics.

Table 3.3: Mean (±standard deviation) FATM ratios 16:1(n-7)/16:0, 18:1(n−7)/18:1(n−9) and 20:5(n-3)/22:6(n-3) analysed in the nineteen chemotaxonomical sample categories. (n = the number of individuals for mammals and the number of pools for all other categories).

Category	Species	Number of Samples	16:1(n-7)/ 16:0	18:1(n−7)/ 18:1(n−9)	20:5(n-3)/ 22:6(n-3)
Harbour Seal Blubber	Harbour seal	10	1.46 ± 0.67	0.23 ± 0.05	0.43 ± 0.12
Harbour Porpoise Blubber	Harbour porpoise	18	2.41 ± 0.78	0.13 ± 0.10	0.43 ± 0.15
Sperm Whale Blubber	Sperm whale	5	2.36 ± 0.69	0.10 ± 0.02	0.56 ± 0.30
Pelagic Roundfish Whole	Sprat	3	0.38 ± 0.02	0.44 ± 0.04	0.58 ± 0.09
Pelagic Roundfish Muscle	Herring	2	0.23 ± 0.04	0.19 ± 0.02	0.50 ± 0.08
Pelagic Roundfish Liver	Herring	2	0.10 ± 0.01	0.55 ± 0.06	0.38 ± 0.01
Demersal Shark Muscle	Catshark	12	0.23 ± 0.05	0.53 ± 0.11	0.32 ± 0.09
Demersal Shark Liver	Catshark	12	0.45 ± 0.08	0.55 ± 0.11	0.43 ± 0.07
Demersal Roundfish Whole	Whiting, Haddock	6	0.24 ± 0.07	0.53 ± 0.13	0.66 ± 0.23
Demersal Roundfish Muscle	Whiting, Hake, Haddock	30	0.23 ± 0.08	0.46 ± 0.17	0.55 ± 0.24
Demersal Roundfish Liver	Whiting, Hake, Haddock	30	0.40 ± 0.13	0.42 ± 0.15	0.80 ± 0.31
Flatfish Muscle	Plaice, Dab	12	0.30 ± 0.07	0.54 ± 0.13	0.90 ± 0.29
Flatfish Liver	Plaice, Dab	12	0.55 ± 0.18	0.52 ± 0.22	0.63 ± 0.18
Demersal Invertebrates Muscle	Squid	2	0.13 ± 0.01	0.54 ± 0.02	0.34 ± 0.01
Benthic Invertebrates Whole	Common starfish, Brittle star, Sea mouse	11	0.30 ± 0.13	4.48 ± 2.71	2.79 ± 1.93
Benthic Invertebrates Muscle	Edible crab, European lobster, Squat lobster, Hermit crab, *Nephrops*	13	0.44 ± 0.15	0.66 ± 0.59	1.34 ± 0.38
Benthic Invertebrates Brown Meat	Edible crab, European lobster	3	0.87 ± 0.30	0.63 ± 0.10	0.79 ± 0.03
Benthic Invertebrates Soft Body	Swimming crab, Horse mussel, King scallops, Whelk, Shore crab	23	0.36 ± 0.16	1.15 ± 0.65	1.55 ± 0.67
Zooplankton Whole	*Calanus* and *Pseudocalanus*	5	0.46 ± 0.03	0.42 ± 0.09	1.36 ± 0.05

3.3.3 Stable isotopic composition

Sample pools (fish, shark, invertebrates and zooplankton) and individuals (marine mammals) were segregated on the basis of their isotopic signatures ($p < 0.05$, ANOVA, Tukey). Isotopic shift varies among tissue types (Lorrain *et al.,* 2002) with the liver providing information on short-term diet due to a faster metabolic turnover rate while muscle can provide information on the longer-term diet (Stowasser *et al.,* 2009). Contaminant accumulation differs between tissue types (due to differing lipid content) and the difference in dietary information can be used to study exposure (Webster *et al.,* 2014).

Using the sub-categories established by the earlier FA analysis, significant differences in $\delta^{15}N$ and $\delta^{13}C$ between groups of sample pools (fish muscle and liver, shark muscle and liver, invertebrates muscle, brown meat, soft body, whole) and zooplankton (whole)) and individuals (marine mammal blubber protein) were observed in this study (Table 3.4 and Figure 3.7). At a species level, $\delta^{15}N$ ranged from a mean of 5.62 ± 0.38 ‰ ($n = 5$ pools) in zooplankton to 17.69 ± 1.19 ‰ ($n = 10$ individuals) in harbour seal de-lipified blubber protein. Mean $\delta^{13}C$ values across the nineteen designated categories ranged from -19.37 ± 0.02 ‰ in demersal invertebrates muscle pools to -14.48 ± 2.99 ‰ in benthic invertebrates whole pools (Table 3.2). Comparing $\delta^{15}N$ and $\delta^{13}C$ on a like-for-like basis across tissue types must however be done with caution, as the tissue turnover rate for different protein pools vary. In the case of de-lipified protein blubber, up to 50 % of the blubber weight for weight is collagen (Lockyer, McConnell and Walters, 1984), which would therefore dominate the overall isotopic signature. Tables 3.5 and 3.6 show the mean (± standard deviation) $\delta^{15}N$ and $\delta^{13}C$, respectively, in each tissue type analysed across ten categories as a like-for-like comparison.

Table 3.4: Mean (±standard deviation) $\delta^{15}N$ and $\delta^{13}C$ analysed in the nineteen chemotaxonomical sample categories. $\delta^{15}N$ and $\delta^{13}C$ values are reported for de-lipified protein. (n = the number of individuals for mammals and the number of pools for all other categories).

Category	Species	Number of Samples	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Harbour Seal Blubber	Harbour seal	10	17.69 ± 1.19	-16.36 ± 2.02
Harbour Porpoise Blubber	Harbour porpoise	18	16.62 ± 1.22	-16.48 ± 1.05
Sperm Whale Blubber	Sperm whale	5	13.36 ± 0.53	-14.60 ± 0.46
Pelagic Roundfish Whole	Sprat	3	14.26 ± 0.23	-18.45 ± 0.38
Pelagic Roundfish Muscle	Herring	2	13.37 ± 0.01	-18.03 ± 0.19
Pelagic Roundfish Liver	Herring	2	11.60 ± 0.00	-17.65 ± 0.28
Demersal Shark Muscle	Catshark	12	16.12 ± 0.86	-17.13 ± 0.57
Demersal Shark Liver	Catshark	12	15.26 ± 0.58	-17.53 ± 0.55
Demersal Roundfish Whole	Whiting, Haddock	6	15.65 ± 0.37	-17.58 ± 0.31
Demersal Roundfish Muscle	Whiting, Hake, Haddock	30	15.42 ± 1.13	-17.75 ± 0.57
Demersal Roundfish Liver	Whiting, Hake, Haddock	30	14.51 ± 1.15	-18.45 ± 0.65
Flatfish Muscle	Plaice, Dab	12	12.98 ± 1.21	-18.03 ± 0.40
Flatfish Liver	Plaice, Dab	12	12.03 ± 1.06	-19.01 ± 0.78
Demersal Invertebrates Muscle	Squid	2	13.75 ± 0.18	-19.37 ± 0.02
Benthic Invertebrates Whole	Common starfish, Brittle star, Sea mouse	11	12.22 ± 1.71	-14.48 ± 2.99
Benthic Invertebrates Muscle	Edible crab, European lobster, Squat lobster, Hermit crab, *Nephrops*	13	13.13 ± 1.01	-17.48 ± 0.49
Benthic Invertebrates Brown Meat	Edible crab, European lobster	3	11.63 ± 0.19	-18.93 ± 0.75
Benthic Invertebrates Soft Body	Swimming crab, Horse mussel, King scallops, Whelk, Shore crab	23	11.75 ± 2.14	-18.08 ± 1.08
Zooplankton Whole	*Calanus* and *Pseudocalanus*	5	5.62 ± 0.38	-19.01 ± 0.41

Table 3.5: mean (±standard deviation) $\delta^{15}N$ in each de-lipified tissue type analysed across ten categories. (n = the number of individuals for mammals and the number of pools for all other categories).

Category	Number of Samples	Muscle	Liver	Blubber Protein	Whole	Soft Body	Brown Meat
		$\delta^{15}N$ (‰)	$\delta^{15}N$ (‰)	$\delta^{15}N$ (‰)	$\delta^{15}N$ (‰)	$\delta^{15}N$ (‰)	$\delta^{15}N$ (‰)
Harbour Seal	10			17.69 ± 1.19			
Harbour Porpoise	18			16.62 ± 1.22			
Sperm Whale	5			13.36 ± 0.53			
Pelagic Roundfish	7	13.37 ± 0.01	11.60 ± 0.00		14.26 ± 0.23		
Demersal Shark	24	16.12 ± 0.86	15.26 ± 0.58				
Demersal Roundfish	66	15.42 ± 1.13	14.51 ± 1.15		15.65 ± 0.37		
Flatfish	24	12.98 ± 1.21	12.03 ± 1.06				
Demersal Invertebrates	2	13.75 ± 0.18					
Benthic Invertebrates	50	13.13 ± 1.01			12.22 ± 1.71	11.75 ± 2.14	11.63 ± 0.19
Zooplankton	5				5.62 ± 0.38		

Table 3.6: Mean (±standard deviation) $\delta^{13}C$ in each de-lipified tissue type analysed across ten categories. (n = the number of individuals for mammals and the number of pools for all other categories).

Category	Number of Samples	Muscle	Liver	Blubber Protein	Whole	Soft Body	Brown Meat
		$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)
Harbour Seal	10			-16.36 ± 2.02			
Harbour Porpoise	18			-16.48 ± 1.05			
Sperm Whale	5			-14.60 ± 0.46			
Pelagic Roundfish	7	-18.03 ± 0.19	-17.65 ± 0.28		18.45 ± 0.38		
Demersal Shark	24	-17.13 ± 0.57	-17.53 ± 0.55				
Demersal Roundfish	66	-17.75 ± 0.57	-18.45 ± 0.65		-17.58 ± 0.31		
Flatfish	24	-18.03 ± 0.40	-19.01 ± 0.78				
Demersal Invertebrates	2	-19.37 ± 0.02					
Benthic Invertebrates	50	-17.48 ± 0.49			-14.48 ± 2.99	-18.08 ± 1.08	-18.93 ± 0.75
Zooplankton	5				-19.01 ± 0.41		

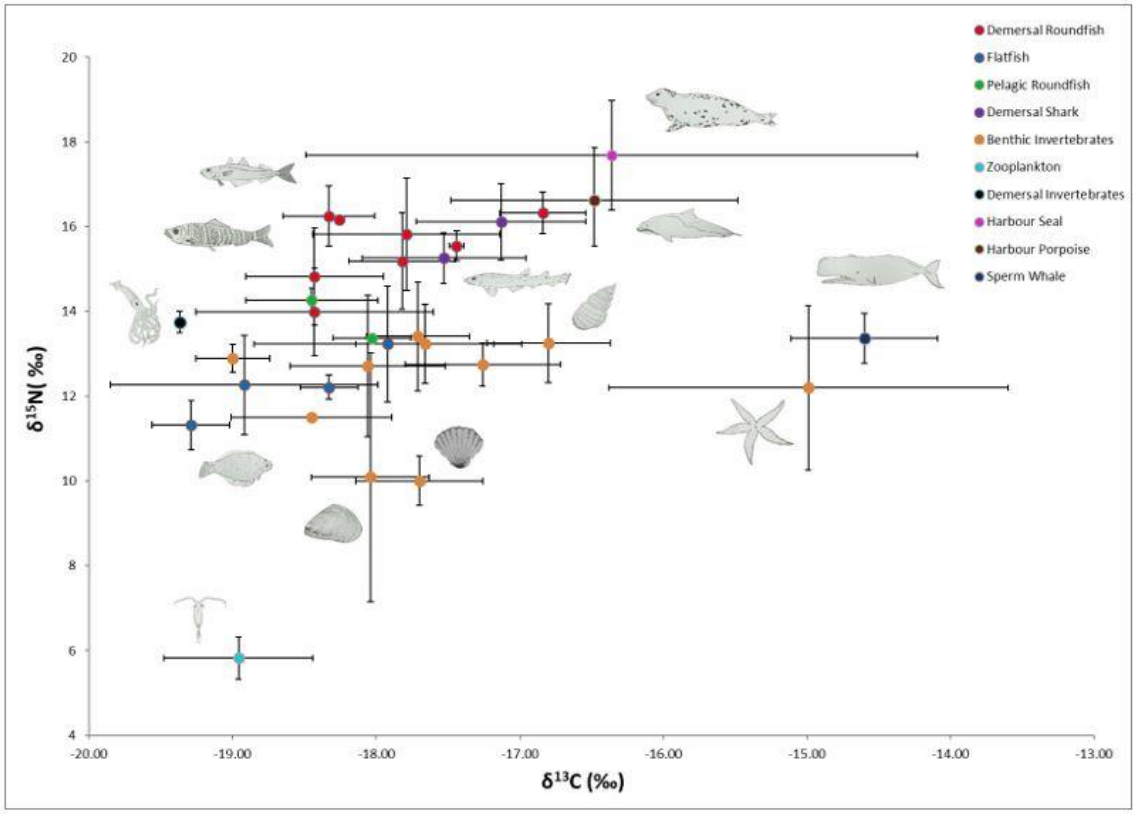


Figure 3.7: Scatter plot demonstrating the spread of $\delta^{15}N$ and $\delta^{13}C$ analysed in ten chemotaxonomical sample categories (combining tissue types for fish, shark and benthic invertebrates and excluding n = 1 samples). $\delta^{15}N$ and $\delta^{13}C$ values of different de-lipified tissues are being shown. The greater the $\delta^{15}N$ value the higher the trophic level. Differing $\delta^{13}C$ values, indicate different carbon sources at the base of the food web (benthic vs pelagic photosynthesis).

3.3.3.1 Marine mammals

The higher SD of $\delta^{13}C$ values in harbour seal blubber protein (−16.36 ± 2.02 ‰; n=10) and harbour porpoise blubber protein (−16.48 ± 1.05 ‰; n=18) compared to sperm whale blubber protein (−14.60 ± 0.46 ‰; n=5) (Tables 3.4 and 3.6) suggests a more variable dietary pattern and/or feeding location in the former two species than the latter. This agrees with the FA profile data where harbour seal and harbour porpoise were highly dispersed (Figure 3.4) due to significant variation of FAs such as 18:1(n-9), 16:1(n-7) and 22:6(n-3). Variables such as geographic location of stranding, year, age, length and girth (Table 2.2) had no significant influence on $\delta^{13}C$ ($p < 0.05$, ANOVA, Tukey) in harbour seal blubber protein. There were a range of decomposition states and causes of death (Table 2.2) but these were not found to significantly influence $\delta^{13}C$ and $\delta^{15}N$ ($p < 0.05$, ANOVA, Tukey). It can be concluded that the harbour seals in this study have a significantly variable $\delta^{13}C$ purely due to a diverse diet.

Through analysis of seal scat, Wilson and Hammond, (2016) identified that sand eel was an important component in the diet of harbour seals in Shetland, Orkney, Moray Firth and South East Scotland. Although sand eel populations were, at the time of sampling in that study, facing a rapid decline, they represented up to 70 % of the harbour seal diet across all seasons. Sand eel is a planktivorous consumer with a low $\delta^{13}C$ value. A study by Sarà, Pirro and Sprovieri, (2010) reported an average $\delta^{15}N$ of 9.3 ‰ and average $\delta^{13}C$ of -20.8 ‰ in seven individual sand eel samples collected from Faxaflói Bay, Iceland. The within species variation of harbour seal $\delta^{13}C$ in this study (−16.36 ± 2.02 ‰) suggests sand eel did not make up a majority of the diet of the seals sampled. Harbour seals have been reported to consume a mixture of benthic invertebrates (Perrin, Wursig and Thewissen, 2009). Individuals with a high $\delta^{13}C$ value could potentially be feeding directly on organisms with a high $\delta^{13}C$ value, such as echinoderms (common starfish and brittle star) which have been found to contain a significantly higher $\delta^{13}C$ than the other categories (benthic invertebrates whole, Tables 3.4 and 3.6).

Sperm whale blubber protein had a significantly lower mean $\delta^{15}N$ (13.36 ± 0.53 ‰) and significantly higher mean $\delta^{13}C$ (−14.60 ± 0.46 ‰) compared to harbour seal and harbour porpoise ($p < 0.05$, ANOVA, Tukey). Sperm whale blubber protein shows the least variation in SI ratios (SD < 1; Tables 3.4, 3.5 and 3.6) of the mammal species studied, suggesting little variation in the species feeding pattern, in agreement with FA data. The mean $\delta^{15}N$ value observed for cephalopods (13.75 ± 0.18 ‰) in this study (demersal invertebrates muscle) was not significantly different when compared with the sperm whale, but pooled squid sample numbers were too low to state a predator-prey relationship and perform a geographical comparison (Burra Haaf (Atlantic Ocean) n = 1, Moray Firth (North Sea) n = 1). All sperm whales sampled in this study were male and SI data from a long-term study conducted along the British Columbia Coast (Pacific) on 697 sperm whales, based on stomach content analysis, found that adult males fed more frequently on fish and catshark, whilst adult females fed on giant squid (Flinn *et al*., 2002). Sperm whales (male and female) sampled in waters of high latitudes of the Pacific were also found to have a higher intake of fish than squid than those inhabiting lower latitudes (Rice, 1989), a factor which would result in increased (more positive) $\delta^{15}N$ and $\delta^{13}C$ values. The significantly higher $\delta^{13}C$ value in sperm whales compared to other marine mammals and other marine species has also been reported in the North East Atlantic in other tissues such as teeth (Borrell *et al*., 2013) and skin (Ruiz-Cooley, Engelhaupt and Ortega-Ortiz, 2011).

3.3.3.2 Fish and catshark

The pelagic fish in this study included pools of sprat (n = 3) and herring (n = 2), both recognised as prey species for higher trophic level demersal fish such as cod (Köster *et al*., 2001). As strict consumers of plankton, sprat and herring compete for similar dietary resources (Casini, Cardinale and Arrheni, 2004). There is a difference in diet between young herring and adult fish, young fish feeding on phytoplankton and adults feeding primarily on holoplanktonic crustaceans (zooplankton). Pelagic roundfish whole (sprat) were found to have a higher mean $\delta^{15}N$ than pelagic roundfish (herring liver and muscle) and flatfish (dab liver and muscle and plaice liver and muscle), suggesting a species/tissue influence on relative abundance values.

Observed $\delta^{13}C$ values were significantly lower in flatfish liver protein (−19.01 ± 0.78 ‰; n = 12) than pelagic roundfish whole protein (−18.45 ± 0.38 ‰; n = 3), pelagic roundfish muscle protein (−18.03 ± 0.17 ‰; n = 2), flatfish muscle protein (−18.03 ± 0.40 %; n = 12) and pelagic roundfish liver protein (−17.65 ± 0.28 ‰; n = 2) ($p < 0.05$, ANOVA, Tukey; Table 3.4), suggesting both a tissue and dietary influence. Analysis of different tissues has the advantage of revealing the time scale of feeding patterns, where the slower turnover rate of stable isotopic composition in muscle provides a long-term dietary indicator compared to liver (Hesslein, Hallard and Ramlal, 1993). The difference between $\delta^{13}C$ in flatfish muscle and liver suggests a relatively recent change to the diet of the flatfish in this study. However, as previously stated, some of the observed differences in stable isotopic composition may reflect differences in tissue turn-over rates which would result in differences of apparent tropic level shifts. On a like-for-like basis, there was no significant difference between the shark and three fish categories (pelagic, demersal, flatfish) in their muscle protein $\delta^{15}N$ and $\delta^{13}C$ and liver protein $\delta^{13}C$. (Tables 3.4, 3.5 and 3.6). Pelagic roundfish liver protein however had a significantly lower $\delta^{15}N$ than the shark, demersal roundfish and flatfish liver protein (Table 3.5) ($p < 0.05$, ANOVA, Tukey).

Average pool (of individuals making up the pool) age (ranging from 3.4 to 10.0 years), length (ranging 198.0–350.0 mm) and weight (from 82.6–410.0 kg) were not significantly correlated ($p > 0.05$) with $\delta^{15}N$ or $\delta^{13}C$ in flatfish. Although sample size was limited from each location, when contributing species were analysed, plaice liver and muscle from Burra Haaf (n = 4) had a significantly lower mean $\delta^{15}N$ value (liver: 11.09 ± 0.39 ‰ (n = 4); muscle: 11.93 ± 0.53 ‰ (n = 4)) in comparison to those from the Moray Firth (liver: 13.13 ± 0.46 ‰ (n = 3), muscle: 13.80 ± 0.45 ‰ (n = 3)) and Solway (liver: 13.32 ± 0.36 ‰ (n = 2); muscle: 14.98 ± 0.22 ‰ (n = 2)) ($p < 0.05$, ANOVA, Tukey). When FATMs were

investigated at a species level only 20:5(n-3)/22:6(n-3) had a significant difference within plaice. Plaice muscle had a significantly lower ratio in Burra Haaf (0.79 ± 0.18; n = 4) and Moray Firth (0.91 ± 0.09; n = 3) in comparison to Solway (1.44 ± 0.25; n = 2) ($p < 0.05$, ANOVA, Tukey). Plaice liver had a significantly lower 20:5(n-3)/22:6(n-3) for plaice in Burra Haaf (0.42 ± 0.15; n = 4) than in Moray Firth (0.67 ± 0.03; n = 3) and Solway (0.80 ± 0.10; n = 2) ($p < 0.05$, ANOVA, Tukey). The FATM 20:5(n-3)/22:6(n-3) indicates that plaice had a more carnivorous diet in Burra Haaf, supporting the δ^{15}N data. There were insufficient sample numbers of dab to carry out a comprehensive regional analysis (n = 3 from the same location).

Demersal shark and demersal roundfish sample pools were found to have significantly higher mean δ^{15}N and δ^{13}C values ($p < 0.05$, ANOVA, Tukey) than flatfish and pelagic roundfish (combined overall matrices demonstrated in Figure 3.7). The small spotted catshark is reported as a mid-trophic level predator (Caut *et al.*, 2013) and is the most abundant shark species in the North Atlantic (Kousteni *et al*., 2014). In the Mediterranean and East Atlantic, catshark was found to feed on demersal fish and benthic crustaceans with diet appearing to vary spatially and ontogenetically (Barría, Navarro and Coll, 2017). In this study, fifty-one catsharks (resulting in twelve sample pools per tissue; Table 2.1) were collected from four locations from the Irish Sea: Solway and the Clyde (Pladda, Hunterston and Holy Loch; Figure 2.1). Sampling location was not found to influence the isotopic composition. Average weight was found to significantly influence δ^{15}N in catshark muscle, ($p < 0.05$) where the heavier the catshark pool, the higher than mean δ^{15}N value, indicating that larger catshark are feeding higher up the food chain than smaller catshark. Average pool length (average length of individuals making up the pool), another indicator of age, was found to significantly influence the δ^{13}C in catshark liver ($p < 0.05$): the smaller the catshark, the lower the δ^{13}C value; suggesting a different diet. When FATMs were investigated within the catshark species, only 20:5(n-3)/22:6(n-3) in catshark muscle was significantly influenced by length, where the larger the catshark the lower the ratio ($p < 0.05$), supporting the δ^{15}N and δ^{13}C data showing larger catshark larger are more carnivorous. Catshark liver sample pools taken in 2016 from Solway and Pladda had a significantly lower mean δ^{13}C (−18.16 ± 0.47 ‰; n = 4) than those collected in 2015 from Holy Loch, Solway and Hunterston (−17.35 ± 0.23 ‰; n = 6) and 2017 from Holy Loch and Pladda (−16.86 ± 0.04 ‰; n = 2) ($p < 0.05$, ANOVA, Tukey). Collection year also influenced the δ^{15}N in muscle tissue where catshark muscle sample pools collected in 2016 had a significantly lower δ^{15}N (15.05 ‰ ± 0.52 ‰; n = 4 pools) than those collected in 2015 (16.67 ‰ ± 0.43; n = 6 pools) and 2017 (16.64 ± 0.54 ‰; n = 2 pools) ($p < 0.05$, ANOVA,

Tukey). This suggests that the small spotted catshark collected in the 2016 sampling exercises were feeding more on lower trophic level benthic invertebrates with differing primary carbon sources in comparison to those collected during 2015 and 2017. There was no significant difference in sample lengths and weights between collection year ($p < 0.05$, ANOVA, Tukey). None of the FATMs supported this data, with no significant differences found in catfish liver between the three years ($p > 0.05$ ANOVA, Tukey).

$\delta^{15}N$ of demersal roundfish muscle (15.42 ± 1.13 ‰) and liver (14.51 ± 1.15 ‰) in this study was not significantly higher than the $\delta^{15}N$ of demersal catshark, suggesting that there is unlikely to be any significant predator-prey relationship ($p > 0.05$). This correlates with previous studies on the small spotted catshark where diet was closer to that of mid-level predator rajiformes (skates) than top predator selachimoformes (sharks) (Valls *et al*., 2011). This is supported by all three FATMs where no significant differences were present between demersal fish muscle and liver and demersal catshark muscle and liver.

Similar to the findings reported in Section 3.3.1.2 for FA analysis, the $\delta^{15}N$ was influenced by the contributing species in the demersal roundfish liver category, where Hake had a significantly higher mean $\delta^{15}N$ value (16.21 ± 0.62 ‰; n = 2), than whiting (14.82 ± 1.15 ‰; n = 14) and haddock (13.99 ± 0.10 ‰; n = 13) ($p < 0.05$, ANOVA, Tukey). For whiting only, there was a significant influence of age, length and weight on the $\delta^{15}N$ for all tissue types ($p < 0.05$). The higher the average pool age, length and weight of the sample pool, the higher the $\delta^{15}N$, indicating larger, older fish feed at a higher trophic level. Unlike the $\delta^{15}N$ values, there was no significant FATM variation present within the FA profile of demersal roundfish to indicate species dietary differences. When sampling location was investigated on the overall demersal roundfish category, it was found that species from the North East (Burra Haaf 14.68 ± 1.29 ‰; n = 6) and Moray Firth (14.22 ± 0.67 ‰; n = 4) were significantly lower ($p < 0.05$, ANOVA, Tukey) in $\delta^{15}N$ in their muscle tissue in comparison to those from the Clyde and West (Holy Loch (16.54 ± 0.50 ‰; n = 4), Pladda (16.47 ± 0.82 ‰; n = 7), Solway (16.32 ± 1.01 ‰; n = 4) and further South East (Outer Firth of Forth (15.08 ± 0.12 ‰; n = 2) and Montrose Bank (14.98 ± 0.70 ‰; n = 3). In demersal roundfish liver, sample pools collected from the Moray Firth (13.07 ± 0.39 ‰; n = 4) had a significantly lower mean $\delta^{15}N$ value than sample pools collected from the other sampling points ($p < 0.05$, ANOVA, Tukey) suggesting a spatial influence on diet. However, as described in 3.3.1.2, only haddock was collected from the Moray Firth and due to the species influence identified during FA analysis, a larger sample number across regions would be required for a comprehensive analysis.

3.3.3.3 Benthic and demersal invertebrates

Benthic and demersal invertebrates (muscle, whole and brown meat from crustaceans) presented with a range of $\delta^{15}N$ and $\delta^{13}C$ values (Table 3.4). The benthic invertebrate's data was the most variable for $\delta^{15}N$ (11.81 ± 1.90 ‰) due to the contributing bivalve species, king scallop (10.0 ± 0.58 ‰; n = 10) and horse mussel (10.09 ± 2.94 ‰; n = 2). King scallops are long-lived primary consumers situated at trophic level 2 and can grow to 150 mm or more (Ansell, Dao and Mason, 1991). Along with horse mussel, king scallops were found to have a significantly lower mean $\delta^{15}N$ value than the other benthic invertebrate species ($p < 0.05$, ANOVA, Tukey). They filter-feed on primary producers including bacteria, phytoplankton and meso-zooplankton and do not reflect short term fluctuations in the $\delta^{15}N$ due to their fast tissue turnover rate (Lehane and Davenport, 2002; Lorrain *et al*., 2002). The isotopic composition identified in this project position king scallop as the lowest trophic level benthic invertebrate in the Scottish marine food web as investigated. This species was therefore used as the baseline species for all trophic level calculations in this study.

Brittle star had a significantly higher mean $\delta^{13}C$ value than the other categories ($p < 0.05$, ANOVA, Tukey), with a value of −6.26 ‰, however there was only one pool of brittle star (containing 96 individuals). The $\delta^{13}C$ value observed is higher than previously reported in brittle star from around Britain (Scotland and the English Channel) (McKenzie *et al*., 2000; Leroux, Muths and Davoult, 2012) with values on average ranging from −17.00 to −20.00 ‰. When the species comprising only one pool were removed from the data set (brittle star, sea mouse, lobster (brown and white meat) and hermit crab), common starfish was found to have a significantly higher $\delta^{13}C$ value than the other categories ($p < 0.05$, ANOVA, Tukey). Benthic microalgae and kelp have a higher carbon isotopic value than phytoplankton which could be a possible carbon source at the base of the echinoderm food chain (France, 1995). Bioturbation of refractory organic matter (poorly biodegradable leftovers of organisms) in the sediment could also cause a higher $\delta^{13}C$ value if consumed by benthic primary consumers (Nadon and Himmelman, 2006; Kang *et al*., 2015). It can be concluded that the more complex and pelagic the food web, the more degraded material reaches the sea floor. In this project, common starfish had a significantly higher mean $\delta^{13}C$ value than other benthic species collected from the offshore Moray Firth, suggesting this species feeds on organisms with a different primary carbon source.

Influence on $\delta^{15}N$ values within the starfish species included localised region. Sample pools from Pladda (Clyde) had a significantly lower mean $\delta^{15}N$ (9.48 ± 0.23 ‰; n = 2)

than starfish from the other sites: Moray Firth (11.84 ± 0.84 ‰; n = 3), Hunterston (12.76 ‰ n = 1), Solway (13.91 ‰ n = 1) and Holy loch (14.35 ± 0.27 ‰ n = 2) ($p < 0.05$, ANOVA, Tukey). This is supported by the FA analysis where starfish from Pladda were found to have a different diet of planktonic feeding prey in comparison to the other starfish pools collected from other sites.

There was no significant species influence on the $\delta^{15}N$ of the benthic invertebrates soft body category ($p < 0.05$, ANOVA, Tukey) however, corresponding to the FA analysis, whelk had a significantly higher mean $\delta^{13}C$ (-16.90 ± 0.34 ‰; n=7) than horse mussel (- 18.18 ± 0.22 ‰; n=2), king scallop (-17.74 ± 0.44 ‰; n = 10), swimming crab (-18.30 ± 0.62 ‰; n=6) and shore crab (-19.09 ± 0.25 ‰; n=2). There was also a localised regional influence on this category where sample pools collected from the Holy Loch had a significantly higher mean $\delta^{15}N$ value (13.53 ± 0.38 ‰; n=7) than those collected from the Solway Firth (13.22 ± 0.31 ‰; n=2), Hunterston (13.20 ‰; n=1), Tancred Bank (12.82 ± 0.33 ‰; n=2), Outer Firth of Forth (12.79 ± 0.31 ‰; n=2), Pladda (9.75 ± 2.46 ‰; n=2) and Montrose Bank (9.53 ‰; n=1) ($p < 0.05$, ANOVA, Tukey). Although sample numbers are low, it does provide an indication of $\delta^{15}N$ between localised regions. There was no biogeographic or localised regional influence on $\delta^{13}C$. There was no within-category species or regional influence on the benthic invertebrates muscle category on both $\delta^{15}N$ and $\delta^{13}C$, which were also grouped together based on their FA profile (Figure 3.6), suggesting the contributing species (edible crab (n=2), European lobster (n=1), squat lobster (n=3), hermit crab (n=1), *Nephrops* (n=6) have a similar feeding pattern.

3.3.3.4 Zooplankton

Zooplankton possessed a significantly lower mean $\delta^{15}N$ (5.62 ± 0.38 ‰) value in comparison to the other sample categories, positioning *Pseudocalanus Spp*. and *Calanus* spp. at the bottom of the food web investigated (Figure 3.7; note: no phytoplankton were examined in this project). This does not correspond with the FATM data as 20:5(n-3)/22:6(n-3) and 18:1(n−7)/18:1(n−9) positioned benthic invertebrates whole as the lowest trophic level category. Many zooplankton are herbivorous and primarily feed on different forms of phytoplankton, including diatoms and dinoflagellates (Nejstgaard, Gismervik and Solberg, 1997). The $\delta^{13}C$ of zooplankton was not significantly different from a majority of the benthic invertebrate species, further suggesting that most of the benthic consumers in this project have plankton as their primary carbon source at the base of the food web.

3.3.4 Trophic level

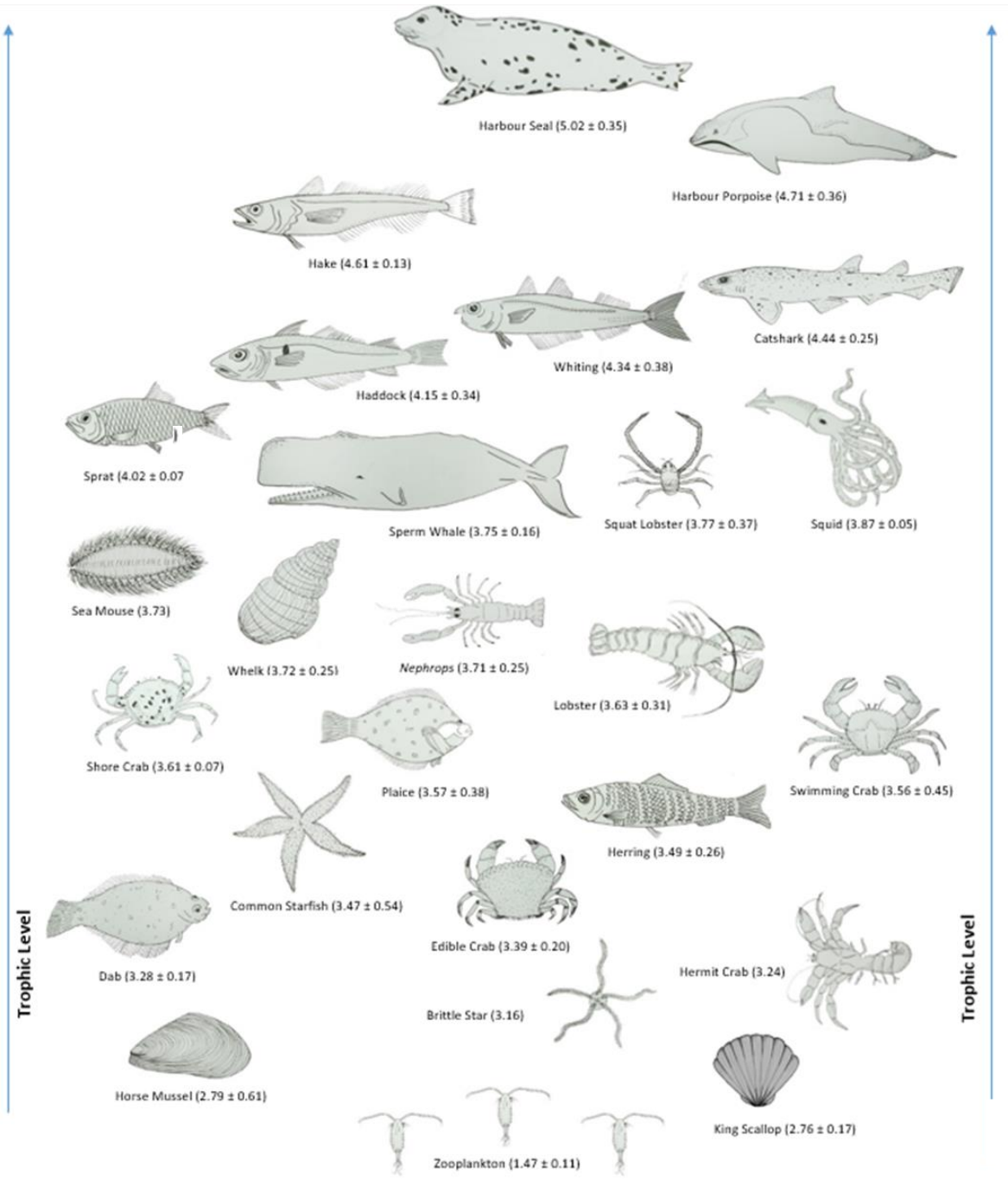
Trophic level was calculated using Equation 2.3 described in Section 2.6. Based on the trophic level data obtained for each species investigated using the $\delta^{15}N$ values, a Scottish marine food web diagram was developed. The mean trophic level for each species (combining tissue type for an overall value) was calculated using Equation 2.3. Trophic level ranged from 1.47 ± 0.11 in zooplankton to 5.02 ± 0.35 in harbour seal (Figure 3.8, Table 3.7). The majority of the species analysed sit between trophic level 3 and 4 with very few significant differences between the categories at these levels. If the "narrowing effect" mentioned in Hussey *et al.,* (2014) is incorporated in future trophic adjustment studies, the trophic level of predators would have a higher calculated value.

When compared to the trophic level indicated by the FATMs; 20:5(n-3)/22:6(n-3) was the most effective at predicting the trophic level of the lower trophic level organisms. Although not in the trophic level order obtained by SI analysis, benthic invertebrates whole, benthic invertebrates soft body, zooplankton whole and benthic invertebrates muscle were positioned at the bottom of the food web (ratio > 1; Table 3.7) in agreement with the trophic level obtained using $\delta^{15}N$. The positioning of higher trophic level organisms by FATM however were not equivalent to the SI data, with demersal catshark muscle positioned as the highest trophic level category due to a higher proportion of 22:6(n-3). A higher proportion of 22:6(n-3) is expected in muscle tissue due to the presence of structural lipid (Figure 3.2). Marine mammals have a lower proportion of 22:6(n-3) due to MUFAs dominating the FA profile (Table 3.2). The tissue-specific nature of FA profiles has been found to influence trophic level description. 18:1(n−7)/18:1(n−9) was more effective as an indicator of higher trophic level species, positioning the three marine mammal species and pelagic roundish muscle as the highest trophic level categories (ratio < 0.25; Table 3.7). This emphasises that care that must be taken when interpreting the FA data without accompanying SI data to corroborate the findings but shows that using both methods gives a clearer indication.

There is a considerable lack of trophic level data covering the diverse species range in Scottish waters, particularly for marine mammals and invertebrates. Trophic level studies have focused on commercial fish species, analysing the effect of fishing on marine ecosystems. For example, a study published by Jennings *et al.,* (2002) analysed the long-term trends in the trophic structure of the North Sea fish community. This study used Equation 2.3 to determine trophic level but used the value of 2.5, rather than 2.0, as a baseline trophic level. The trophic levels of the species included in Jennings *et al.*, (2002) were the lesser spotted catshark (4.3), whiting (5.3), haddock (4.7), herring (3.8),

plaice (4.5) and sprat (4.3). Whiting, haddock and plaice in the study were considerably higher for whiting, haddock and plaice than those calculated in this project (Figure 3.8). There are a variety of factors likely to be contributing to the differences in trophic level on a species basis. Firstly, the study by Jennings focused on fish muscle tissue from the North Sea, rather than a number of tissue types and sampling locations around Scotland. An example of this influence would be the sampling location of whiting in this project, where whiting muscle had a trophic level of 4.33 from the Northern North Sea and 4.74 from the Irish Sea; whereas whiting liver has a trophic level of 4.01 from the Northern North Sea and 4.38 from the Irish Sea. The study by Jennings *et al*., (2002) also used a different baseline value in their trophic level equation which would influence the trophic level result. In this project for example, the trophic level for whiting muscle would be 4.98 instead of 4.18 if a value of 2.5 was used in the equation. This demonstrates the current issues faced with calculating trophic level, where a consistent approach is required for a reliable comparison across studies and regions. As stated in Section 2.6, Equation 2.3 has been used in this project as it is the method currently recommended by OSPAR for the trophic adjustment of contaminant monitoring data.

The combined FA and SI analysis approach has been used in studies worldwide to assess feeding ecology. A study by Stowasser *et al*., (2009) investigated the trophic ecology of two of the dominant families of deep-sea fish. FA biomarkers reflected the seasonal influx from the photic zone though changes were species-specific and reflected the variation in prey availability and abundance. Biomarkers were found to successfully elucidate trophic specialisations in situations where conventional methods alone previously provided insufficient data. This combined approach has not been used to assess feeding patterns and trophic relationships for contaminant assessment purposes and has the potential to explain the variation currently encountered with trophic magnification factors.



Individual drawings by Anneka Madgett, 2018

Figure 3.8: Scottish marine food web diagram showing the mean trophic level (± SD) calculated from $\delta^{15}N$ for each species using Equation 2.3. Matrices within species have been combined to give an overall species trophic level. Primary producers (e.g. phytoplankton) are not included in this food web diagram as they were not investigated as part of this project.

Table 3.7: The mean trophic level (±standard deviation) calculated from $\delta^{15}N$ for each of the nineteen sample categories described in 3.3.1 using Equation 2.3.

Category	Number of Samples	Trophic Level
Harbour Seal Blubber	10	5.02 ± 0.35
Harbour Porpoise Blubber	18	4.71 ± 0.36
Sperm Whale Blubber	5	3.75 ± 0.16
Pelagic Roundfish Whole	3	4.02 ± 0.07
Pelagic Roundfish Muscle	2	3.75 ± 0.03
Pelagic Roundfish Liver	2	3.22 ± 0.01
Demersal Shark Muscle	12	4.55 ± 0.25
Demersal Shark Liver	12	4.30 ± 0.17
Demersal Roundfish Whole	6	4.43 ± 0.11
Demersal Roundfish Muscle	30	4.40 ± 0.36
Demersal Roundfish Liver	30	4.09 ± 0.35
Flatfish Muscle	12	3.64 ± 0.36
Flatfish Liver	12	3.36 ± 0.31
Demersal Invertebrates Muscle	2	3.87 ± 0.05
Benthic Invertebrates Whole	11	3.42 ± 0.50
Benthic Invertebrates Muscle	13	3.68 ± 0.30
Benthic Invertebrates Brown Meat	3	3.24 ± 0.06
Benthic Invertebrates Soft Body	17	3.53 ± 0.47
Zooplankton Whole	5	1.47 ± 0.11

3.4 Conclusions

A combined FA and SI analysis approach has further developed our understanding of trophic level ecology in the Scottish marine food web. FA analysis was able to provide an indication of the feeding patterns of many of the organisms sampled in this project and SI analysis was able to ascribe the trophic levels of twenty-six species collected between 2012 and 2018 from twenty-one sites around Scotland. These calculated trophic levels are required to calculate TMFs for a range of contaminants and perform a trophic level adjustment to normalise concentrations and allow the comparison of different species in different locations to international environmental impact assessment criteria.

215 samples were successfully categorised using FA chemotaxonomy into nineteen categories, accounting for the FA profile influences of tissue type and water column zone. Trophic level was calculated using the $\delta^{15}N$ and ranged from 1.47 ± 0.11 in zooplankton

to 5.02 ± 0.35 in harbour seal with samples from most species collected in this study positioned between trophic level 3 and 4. Interpretation of the FATMs, relative to the SI data, was complex with 20:5(n-3)/22:6(n-3) differentiating lower trophic level species and 18:1(n−7)/18:1(n−9) providing a better correlation with the SI data for higher trophic level species.

This study has demonstrated the complexity of marine systems where FA profiles and SI ratios of organisms at a single trophic level can have considerable variation due to factors such as species, tissue type, location, sampling year and physiological features such as size and age. It is therefore important not to use *generic* trophic levels and TMFs at the species level in trophic level adjustment of contaminant concentrations. Trophic levels need to be calculated for *each species* (in each location at an international scale) using SI analysis and not a theoretical or assigned trophic level value (e.g. Fishbase), as doing so will increase the uncertainty, and consequently the reliability, of the assessment.

In discussions of the wider marine food web, trophic level classifications and terminology such as “top predator” must be used with care. Furthermore, trophic level categorisation should use a multi-factorial approach (both FATM and SI) especially when investigating ecological dynamics. When conducting environmental assessments using TMFs, determinants such as species/class will not be consistent across all the categories due to regional and physiological influences. In order to conduct an effective marine contaminant environmental impact assessment, influencing factors need to be considered to fully understand the complex food chains existing within the marine food web. The trophic level data from this project will permit the calculation of geographically relevant TMFs for a range of contaminants which could be used in environmental status assessments and guide the management of human activities impacting on marine systems.

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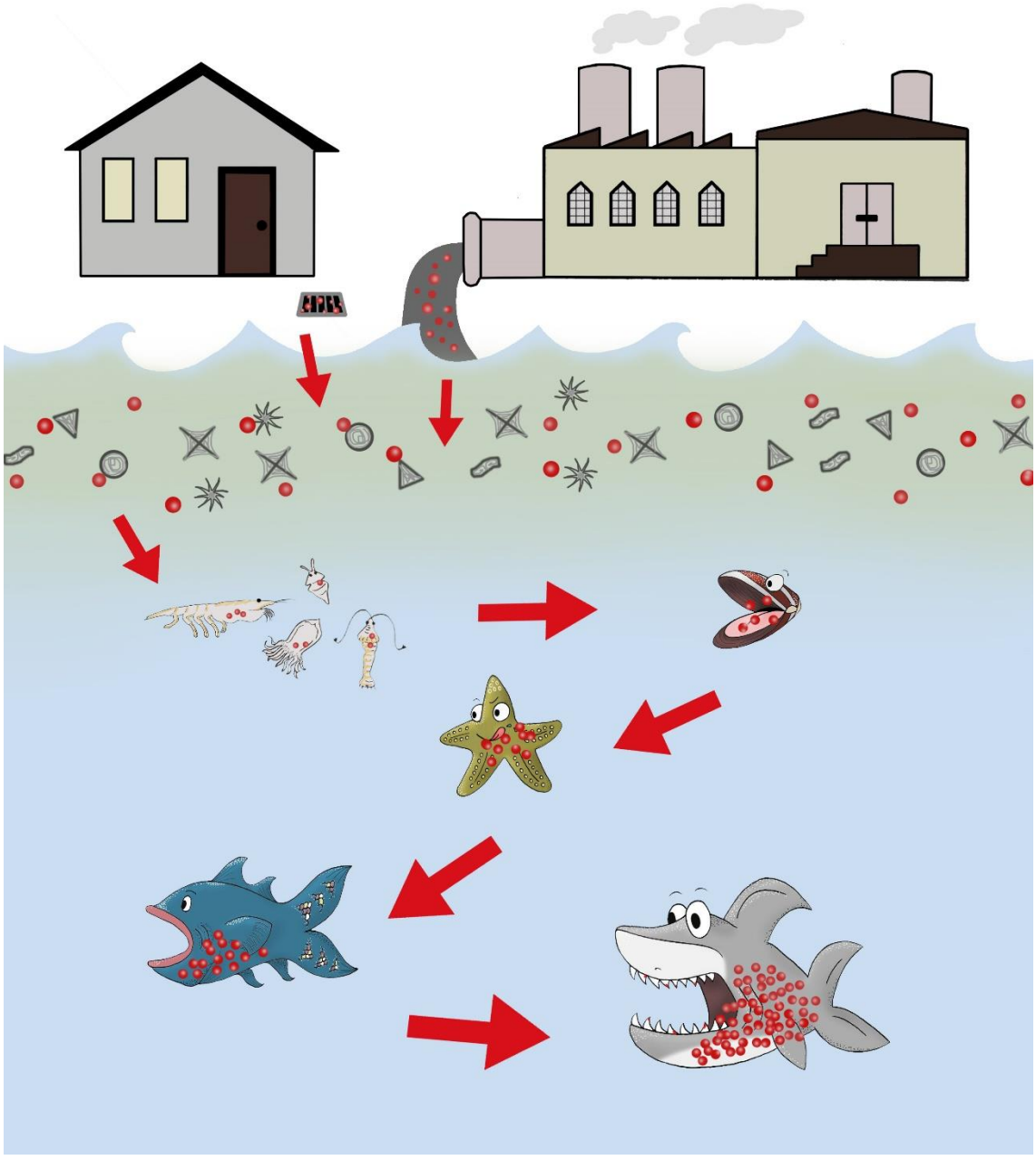
Chapter 4

The Concentration and Biomagnification of Polychlorinated Biphenyls (PCBs) and Polybrominated Diphenyl Ethers (PBDEs) Across Four Trophic Levels in the Marine Food Web



Anneka Madgett, 2020

4.1 Introduction

Although the ban and restrictions on the production and use of some POPs go back to the 1970s and 1980s, they continue to enter the environment through secondary sources such as leaching from waste disposal sites (European Commission, 2005). It is therefore of interest to analyse the temporal trends and current environmental concentrations of these POPs with respect to compliance.

Bioaccumulation of individual contaminants in marine species is dependent on factors such as diet, lipid content, location and region, trophic level and the species-dependant metabolic stability of those contaminants. PCBs and PBDEs are lipophilic and will accumulate to a greater extent in tissue with a higher lipid content such as liver, in the case of demersal fish. However, pelagic fish tend to store lipid just under the skin in the dark muscle, providing a more lipophilic environment. Demersal and flatfish are generally used in environmental monitoring programmes and the contaminants are therefore 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).

OSPAR uses two assessment criteria to assess PCB concentrations in biota: BACs and EACs. Concentrations below BACs represent measured concentrations that are near background levels for naturally occurring substances and close to zero for synthetic substances. 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 for the assessment of PBDEs in sediment or biota. Other assessment criteria available that could be used for PBDE status assessments include the WFD EQS for biota, and the Canadian Federal Environmental Quality Guidelines (FEQGs) for sediment and biota. PCB and PBDE concentrations are measured in biota (fish and mussels) annually (or every few years) from monitoring sites (OSPAR, 2009; OSPAR, 2016a).

The data collected under the CEMP for the North-East Atlantic for contaminants in biota, sediment and water are quality controlled and hosted by the ICES. These data are assessed annually by the OSPAR MIME. Seven ICES PCBs (Table 4.1) were recommended for monitoring by the European Union Community Bureau of Reference (Webster *et al*., 2014c). These congeners were selected as indicators of wider PCB

contamination and have been part of the CEMP since 1998. The ICES-7 PCBs have a wide chlorination range and represent ~20% by weight the PCBs present in commercial mixtures (Kennedy, 2017). Assessment criteria for PCBs apply to all fish and shellfish, although in practice shellfish are assessed on a wet weight basis to eliminate differences in wet weight which may be driven by other factors in invertebrates, and BACs and EACs for biota are converted to other bases (wet weight - ww, dry weight - dw or lipid weight - lw) using species-specific conversion factors.

Table 4.1: ICES-7 PCBs and associated BACs and EACs recommended for monitoring by the European Union Community Bureau of Reference (OSPAR, 2019). BACs are analysed in mussels and oysters on a dry weight (dw) basis and fish on a wet weight (ww) basis. EACs are analysed in fish and shellfish on a lipid weight (lw) basis.

	Background Assessment Concentration (BAC)		**Environmental Assessment Criteria (EAC)**
ICES-7 PCB	Mussels and Oysters (µg/kg dw)	Fish (µg/kg ww)	Fish and shellfish (µg/kg lw)
CB28	0.75	0.10	67
CB52	0.75	0.08	108
CB101	0.70	0.08	121
CB118	0.60	0.10	25
CB138	0.60	0.09	317
CB153	0.60	0.10	1,585
CB180	0.60	0.11	469

The European Commission Directive 2013/39/EU (European Commission, 2013) proposed biota EQS for PBDEs. Biota EQS for PBDEs was set at 0.0085 µg/kg ww and refers to the sum of the concentrations of six BDE congeners (BDE28, 47, 99, 100, 153 and 154) in fish, based on results from ecotoxicological studies on mice (Eljarrat and Barceló, 2018). There is currently no biota EQS for the ICES-7 PCBs.

The PBDE EQS is very low compared to typically reported environmental concentrations in biota (also below many monitoring laboratories detection limits) and most PBDE data for biota will exceed this concentration. The threshold is considered to be very precautionary and is currently in review by the EU Chemicals Working Group (OSPAR, 2020). Application of the EQS must also include a trophic level adjustment to trophic level 5 (for marine food webs); however, in practice this is currently not done. FEQGs

are available for individual PBDE congeners in water, sediment and biota and were derived from ecotoxicological testing. FEQGs assess whether concentrations are likely to cause harm to marine organisms via the water or sediment, or where chemicals may bioaccumulate. FEQGs are currently being trialled for the OSPAR MIME status assessment of PBDEs in sediment and biota (OSPAR, 2020).

The bioaccumulative nature of organic contaminants and their transfer to high trophic level organisms has received substantial attention, and pollutants can remain in an ecosystem despite a ban and/or reduction in use (Won *et al*., 2020). To achieve the aim of "good environmental status" with clean, healthy and productive seas, the sources and pathways of contaminants, their concentrations and effects in the marine environment must be monitored and assessed.

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). However, the selection of a TMF for a given substance is a critical issue, due to the variability existing within ecosystems (region, physiology, etc). In order to understand 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) must be established to determine the reliability of the calculated TMF.

It is well established that factors such as sex, tissue type, reproductive status, metabolism, location and feeding ecology influence the PCB (Helle *et al*., 1984; Filmann *et al*., 2007; Jepson *et al*., 2016; Williams *et al.,* 2020) and PBDE (Weijs *et al*., 2009; Rotander *et al*., 2012) profiles of marine mammals. For example, different marine mammals and even different cetaceans have been found to have differing capacities to metabolise PCB and PBDE congeners, with some species being more vulnerable than pinnipeds to certain pollutants, particularly the dioxin-type of PCB congeners (Boon, 1992; Boon, 1997; Evans, 2011). This variability in metabolic capacities associated with different marine mammal species will influence body burden levels and the relative concentrations of individual contaminants, and therefore the calculated TMF of the associated congener. As well as marine mammals, fish and invertebrates have been found to accumulate PCBs and PBDEs, where body burden can be driven by dietary absorption (feeding habits), tissue type, location, metabolic capacity and maturation state across different species (Buckman *et al.,* 2006; Johnson *et al*., 2006; Szlinder-Richert, 2009; Tian, Zhu and Liu, 2010; Zhang *et al*., 2016).

This chapter investigates the variability of the concentrations and distributions of thirty-two PCB congeners and nine PBDE congeners within and between nineteen sample categories from the Scottish marine food web (inter- and intra- species variation), with the justification for such categorisation having been previously discussed in Chapter 3. Based on this data, TMFs will be calculated for the ICES-7 PCBs and BDE47 (selection of congeners discussed in results and discussion) using both traditional and balanced methods (Borgå *et al*., 2012; Brisebois, 2013) to determine whether biomagnification occurs for specific contaminants in the food web and whether secondary poisoning via bioaccumulation should be considered when conducting environmental quality assessments.

4.2 Materials and methods

Detailed information on materials and methods utilised in this chapter are discussed in Chapter 2, Section 2.8.

4.3 Results and discussion

4.3.1 Lipid Content

PCB concentrations were normalised to the lipid content (%) to account for the different lipid content of the various tissues studied. 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 organic pollutant concentration (Lema *et al.*, 2007; Lavandier *et al*., 2013; Brázová, Hanzelová and Šalamún, 2015). Lipid normalisation minimises variability associated with changes in lipid content and facilitates comparisons across sampling species and locations. Generally, tissue contaminant burdens in two equally exposed organisms will vary proportionally to their lipid content (Hebert and Keenleyside, 1995; Heijden and Jonker, 2011).

The lipid content in the tissues of the eighteen of the nineteen sample categories are shown in Table 4.2. The lipid content was not determined in zooplankton as this is highly variable due to considerable seasonal, latitudinal, and taxonomic variance (Syväranta and Rautio, 2010) and PCB and PBDE concentrations in the zooplankton were below the LoD.

Table 4.2: Lipid content (%) in each of the eighteen of the nineteen sample categories (zooplankton are not included). Number of Samples = individuals for mammals and pools for all other categories. The shaded categories had a significantly higher lipid content than the unshaded categories ($p<0.05$).

Category	Sample Number	Lipid Content %
Harbour Seal blubber	10	61.90 – 95.58
Harbour Porpoise blubber	18	54.38 – 96.33
Sperm Whale blubber	5	26.18 – 63.19
Demersal Shark Muscle	12	0.36 – 1.99
Demersal Shark Liver	12	47.64 – 80.38
Pelagic Roundfish Muscle	2	2.65 – 5.85
Pelagic Roundfish Liver	2	0.45 – 1.37
Pelagic Roundfish Whole	3	6.17 – 7.15
Demersal Roundfish Muscle	30	0.61 – 1.96
Demersal Roundfish Liver	30	21.40 – 78.78
Demersal Roundfish Whole	6	0.87 – 3.01
Flatfish Muscle	12	0.35 – 0.92
Flatfish Liver	12	1.57 – 33.29
Demersal Invertebrates Muscle	2	2.10 – 2.66
Benthic Invertebrates Muscle	13	0.75 – 3.34
Benthic Invertebrates Soft Body	17	0.27 – 3.87
Benthic Invertebrates Whole	11	0.61 – 2.27
Benthic Invertebrates Brown Meat	3	8.57 – 26.43

Marine mammal blubber, demersal shark liver and demersal roundfish liver have a significantly higher lipid content than the other sample categories ($p < 0.05$, ANOVA, Tukey). This is expected, as blubber is composed of adipocytes and structural connective tissue fibres and serves the purpose of energy storage, insulation and buoyancy (Bagge *et al.*, 2012). Shark and fish (demersal and flatfish) liver were found to have a significantly higher lipid content than the muscle and whole tissue ($p < 0.05$, ANOVA, Tukey) and pelagic roundfish whole had a significantly higher lipid content than in the muscle and liver tissue ($p < 0.05$, ANOVA, Tukey). Pelagic roundfish in this study are, however, composed of two different species (herring for muscle and liver and sprat for whole) and the FA profile for pelagic roundfish whole is different to the FA profile of muscle and liver (Chapter 3: Figure 3.5), suggesting both a species and tissue influence on lipid content. The lipid content in fish muscle is highly variable, depending on species, age, diet, spawning season, and muscle type (Gehring, Davenport and Jaczynski, 2009). Bottom-dwelling ground fish such as haddock, hake, plaice and dab store lipids in their liver whereas pelagic fish such as herring, mackerel and sprat store lipids in their fat cells distributed in other body tissues (Huss, 1995). This corresponds with the FA analysis demonstrated in Figure 3.5 (Chapter 3) of this project, where pelagic roundfish contained

greater proportions of PUFAs associated to structural lipids, in their liver and MUFAs, associated to storage lipid, in their muscle tissue relative to the demersal species.

Benthic and demersal invertebrates muscle, whole and soft body samples had a lipid content ranging from 0.27 - 3.87% (Table 4.2). Benthic invertebrates brown meat had a significantly higher lipid content than the other invertebrates categories, ranging from 8.57 - 26.43% ($p < 0.05$, ANOVA, Tukey). Brown meat has a higher natural fat content than muscle tissue and the degree of variation is due to the within-category species differences of lipid content between edible crab (8.57 and 8.78%; n=2) and European lobster (26.43%; n=1) made up of 9 individuals).

4.3.2 Polychlorinated biphenyls (PCBs)

4.3.2.1 Concentrations and distributions

PCB concentrations were normalised to the lipid content (%) to account for the different tissues analysed. Table 4.3 shows the concentration (µg/kg lw) of each of the thirty-two PCB congeners tested, ΣICES-7 PCBs (CB28, 52, 101, 118, 138, 153, 180) and ΣPCB_{32} in eighteen of the nineteen sample categories (zooplankton were excluded as discussed previously).

Table 4.3: The concentration range (µg/kg lipid weight) of thirty-two CB congeners, ΣICES-7 PCBs and ΣPCB_{32} 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 2.1. Not all the LoD values are to four significant figures to account for precision. Values <LoD treated as zero when calculating the sum of CBs. ΣPCB_{32} and ΣICES-7 is expressed as the minimum sample concentration – maximum sample concentration within each category. The shaded columns indicate the ICES-7 PCB congeners.

Category	Sample Number	CB28	CB31	CB44	CB49	CB52	CB70
Harbour Seal	10	<0.11 - 18.62	<0.13 - 4.203	<0.19 - 5.381	0.820 - 101.9	4.816 - 632.6	0.952 - 11.45
Harbour Porpoise	18	<0.13 - 16.71	<0.11 - 2.843	1.093 - 15.94	3.013 - 104.6	18.31 - 2,450	<0.17 - 16.67
Sperm Whale	5	0.754 - 9.384	2.663 - 64.62	1.997 - 59.09	8.564 - 147.9	18.94 - 387.8	1.908 - 62.25
Demersal Shark Muscle	12	<0.03 - 13.79	<0.04	<0.23	<0.05	<0.09 - 0.198	<0.09 - 16.84
Demersal Shark Liver	12	4.082 - 36.15	<0.11 - 20.63	<0.19 - 17.47	<0.13 - 29.30	<0.24 - 40.97	<0.17 - 93.84
Pelagic Roundfish Muscle	2	<0.03 - 5.660	<0.04 - 4.151	<0.23	<0.05	0.331 - 0.353	4.615 - 10.19
Pelagic Roundfish Liver	2	<0.13	<0.11 - 22.63	<0.19	<0.13	<0.24 - 0.672	30.66 - 111.1
Pelagic Roundfish Whole	3	3.846 - 7.455	2.083 - 4.214	<0.23	3.846 - 7.618	0.617 - 1.080	7.371 - 12.642
Demersal Roundfish Muscle	30	<0.03 - 68.03	<0.04 - 52.46	<0.23 - 40.98	<0.05 - 66.39	<0.09 - 1.267	<0.09 - 74.59
Demersal Roundfish Liver	30	<0.13 - 72.50	<0.11 - 72.45	<0.19 - 63.96	<0.13 - 102.4	<0.24 - 106.3	0.729 - 99.46
Demersal Roundfish Whole	6	<0.03 - 15.95	<0.04	<0.23 - 18.61	<0.05 - 23.26	<0.09 - 1.256	<0.09 - 28.24
Flatfish Muscle	12	<0.03	<0.04	<0.23	<0.05	<0.09	<0.09
Flatfish Liver	12	<0.13 - 4.416	<0.11	<0.19	<0.13 - 10.85	<0.24	<0.17 - 4.935
Demersal Invertebrates Muscle	2	<0.03	<0.04	<0.23	<0.05	<0.09	<0.09
Benthic Invertebrates Muscle	13	<0.03 - 14.29	<0.04	<0.23	<0.05	<0.09	<0.09
Benthic Invertebrates Soft Body	17	<0.04 - 27.07	<0.03 - 7.520	<0.07	<0.03 - 24.81	<0.08 - 0.87	<0.06 - 25.56
Benthic Invertebrates Whole	11	<0.04 - 15.54	<0.03	<0.07 - 10.14	<0.03 - 30.05	<0.08 - 0.937	<0.06 - 37.31
Benthic Invertebrates Brown Meat	3	<0.04 - 2.733	<0.03 - 0.227	<0.07	<0.03	<0.08 - 0.253	<0.06 - 1.708

Table 4.3 (continued): The concentration range (µg/kg lipid weight) of thirty-two CB congeners, ΣICES-7 PCBs and ΣPCB_{32} 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 2.1. Not all the LoD values are to four significant figures to account for precision. Values <LoD treated as zero when calculating the sum of CBs. ΣPCB_{32} and ΣICES-7 is expressed as the minimum sample concentration – maximum sample concentration within each category. The shaded columns indicate the ICES-7 PCB congeners.

Category	Sample Number	CB74	CB97	CB99	CB101	CB105
Harbour Seal	10	4.772 - 223.7	0.820 - 14.89	72.98 - 6,509	16.28 - 794.0	4.168 - 104.0
Harbour Porpoise	18	3.664 - 83.67	1.628 - 12.59	27.85 - 5,418	21.16 - 539.1	8.295 - 181.2
Sperm Whale	5	11.38 - 221.8	14.13 - 240.6	40.14 - 669.3	49.19 - 885.2	14.47 - 281.7
Demersal Shark Muscle	12	<0.06 - 36.36	<0.08	<0.07 - 58.18	<0.11 - 37.93	<0.15 - 22.03
Demersal Shark Liver	12	8.139 - 158.3	<0.13 - 46.21	13.69 - 287.5	11.47 - 235.3	7.192 - 231.3
Pelagic Roundfish Muscle	2	3.761 - 5.660	<0.08	9.060 - 16.23	15.04 - 28.30	<0.15
Pelagic Roundfish Liver	2	<0.16 - 19.71	<0.13	<0.40 - 19.71	44.53 - 91.11	<0.20
Pelagic Roundfish Whole	3	4.968 - 8.266	7.371 - 11.67	13.14 - 21.88	23.24 - 38.25	6.891 - 10.53
Demersal Roundfish Muscle	30	<0.06 - 64.75	<0.08 - 25.41	<0.07 - 69.23	<0.11 - 123.5	<0.15 - 50.00
Demersal Roundfish Liver	30	<0.16 - 85.28	<0.13 - 42.17	2.696 - 131.3	2.628 - 217.6	1.899 - 69.56
Demersal Roundfish Whole	6	<0.06 - 21.59	<0.08 - 28.57	<0.07 - 53.16	<0.11 - 92.69	<0.15 - 28.24
Flatfish Muscle	12	<0.06	<0.08	<0.07	<0.11	<0.15
Flatfish Liver	12	<0.16 - 5.844	<0.13 - 16.67	<0.40 - 15.19	<0.45 - 19.09	<0.20 - 8.831
Demersal Invertebrates Muscle	2	<0.06	<0.08	<0.07	<0.11	<0.15
Benthic Invertebrates Muscle	12	<0.06 - 19.78	<0.08	<0.07 - 35.16	<0.11- 20.98	<0.15 – 23.17
Benthic Invertebrates Soft Body	19	<0.04 - 104.4	<0.07 - 38.35	<0.03 - 215.8	<0.05 - 100.6	<0.06 - 120.3
Benthic Invertebrates Whole	11	<0.04 - 79.27	<0.07 - 51.81	<0.03 - 93.78	<0.05 - 66.89	<0.06 - 45.95
Benthic Invertebrates Brown Meat	3	1.400 - 4.653	<0.07 - 0.757	6.651 - 14.00	0.795 - 3.417	5.018 - 11.39

Table 4.3 (continued): The concentration range (µg/kg lipid weight) of thirty-two CB congeners, ΣICES-7 PCBs and ΣPCB_{32} 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 2.1. Not all the LoD values are to four significant figures to account for precision. Values <LoD treated as zero when calculating the sum of CBs. ΣPCB_{32} and ΣICES-7 is expressed as the minimum sample concentration – maximum sample concentration within each category. The shaded columns indicate the ICES-7 PCB congeners.

Category	Sample Number	CB110	CB114	CB118	CB123	CB128
Harbour Seal	10	1.317 - 58.10	<0.11 - 169.3	11.78 - 242.1	<0.08	56.91 - 2,344
Harbour Porpoise	18	1.542 - 38.63	0.372 - 37.57	25.74 - 981.9	<0.08	18.68 - 1,527
Sperm Whale	5	25.09 - 489.7	1.331 - 20.51	60.66 - 1,105	0.910 - 17.19	14.62 - 211.3
Demersal Shark Muscle	12	<0.17 - 32.63	<0.05	<0.09 - 163.6	<0.06	<0.14 - 13.56
Demersal Shark Liver	12	9.389 - 246.9	<0.11 - 8.386	44.62 - 694.9	<0.08 - 12.10	17.59 - 203.9
Pelagic Roundfish Muscle	2	11.62 - 20.38	<0.05	12.99 - 24.15	<0.06	<0.14
Pelagic Roundfish Liver	2	<0.19 - 47.45	<0.11 - 10.95	<0.71 - 33.58	<0.08	<0.23
Pelagic Roundfish Whole	3	16.83 - 29.17	<0.05	21.15 - 32.58	2.885 - 4.862	8.974 - 13.13
Demersal Roundfish Muscle	30	<0.17 - 108.7	<0.05	<0.09 - 133.6	<0.06	<0.14 - 30.33
Demersal Roundfish Liver	30	<0.19 - 177.4	<0.11 - 3.041	6.00 - 197.8	<0.08 - 4.497	1.831 - 57.17
Demersal Roundfish Whole	6	<0.17 - 62.79	<0.05	12.00 - 85.56	<0.06	<0.14 - 36.67
Flatfish Muscle	12	<0.17	<0.05	<0.09	<0.06	<0.14
Flatfish Liver	12	<0.19 - 10.78	<0.11	<0.71 - 27.01	<0.08	<0.23 - 7.403
Demersal Invertebrates Muscle	2	<0.17	<0.05	<0.09	<0.06	<0.14
Benthic Invertebrates Muscle	12	<0.17 - 34.57	<0.05	<0.09 – 76.92	<0.06	<0.14 – 13.95
Benthic Invertebrates Soft Body	19	<0.11 - 73.41	<0.06	<0.07 - 338.6	<0.02 - 12.14	<0.04 - 89.47
Benthic Invertebrates Whole	11	<0.11 - 125.4	<0.06	<0.07 - 189.6	<0.02 - 41.45	<0.04 - 49.22
Benthic Invertebrates Brown Meat	3	<0.11 - 3.759	<0.06	14.24 - 35.11	<0.02 - 0.530	5.484 - 10.25

Table 4.3 (continued): The concentration range (µg/kg lipid weight) of thirty-two CB congeners, ΣICES-7 PCBs and ΣPCB_{32} 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 2.1. Not all the LoD values are to four significant figures to account for precision. Values <LoD treated as zero when calculating the sum of CBs. ΣPCB_{32} and ΣICES-7 is expressed as the minimum sample concentration – maximum sample concentration within each category. The shaded columns indicate the ICES-7 PCB congeners.

Category	Sample Number	CB132	CB137	CB138	CB149	CB153
Harbour Seal	10	<0.11 - 7,526	7.031 - 493.9	415.6 - 20,080	17.48 - 207.6	788.9 - 50,041
Harbour Porpoise	18	<0.11 - 1,725	2.687 - 447.2	93.39 - 20,670	48.79 - 8,183	183.2 - 35,440
Sperm Whale	5	31.57 - 481.9	5.902 - 96.34	83.18 - 1,417	56.60 - 991.3	160.9 - 2,636
Demersal Shark Muscle	12	<0.13 - 170.3	<0.02 - 10.91	<0.17 - 245.5	<0.07 - 74.55	<0.22 - 447.3
Demersal Shark Liver	12	<0.11 - 21.48	2.435 - 45.73	91.04 - 555.0	24.35 - 521.2	142.5 - 2,134
Pelagic Roundfish Muscle	2	<0.13	<0.02	21.71 - 40.75	24.96 - 46.42	36.58 - 68.68
Pelagic Roundfish Liver	2	<0.11	<0.09 - 5.839	94.89 - 166.7	36.50 - 186.7	81.02 - 235.6
Pelagic Roundfish Whole	3	5.128 - 14.42	1.282 - 1.538	38.78 - 59.97	32.37 - 51.38	55.93 - 86.71
Demersal Roundfish Muscle	30	<0.13 - 71.59	<0.02 - 8.197	<0.17 - 180.2	<0.07 - 182.1	<0.22 - 306.6
Demersal Roundfish Liver	30	<0.11 - 39.81	<0.09 - 11.19	11.21 - 323.4	<0.30 - 336.6	21.76 - 592.2
Demersal Roundfish Whole	6	<0.13 - 21.95	<0.02 - 5.556	28.50 - 200.0	<0.07 - 90.37	33.00 - 353.3
Flatfish Muscle	12	<0.13	<0.02	<0.17	<0.07	<0.22
Flatfish Liver	12	<0.11	<0.09 - 18.85	<0.85 - 197.5	<0.30 - 104.9	<1.34 - 303.3
Demersal Invertebrates Muscle	2	<0.13	<0.02	<0.17	<0.07	<0.22
Benthic Invertebrates Muscle	12	<0.13 – 236.3	<0.02	<0.17 – 68.02	<0.07 - 29.63	<0.22 – 178.0
Benthic Invertebrates Soft Body	19	<0.03 - 132.8	<0.03 - 18.35	<0.15 - 533.5	<0.05 - 272.2	<0.07 - 954.9
Benthic Invertebrates Whole	11	<0.03 - 22.80	<0.03 - 3.497	<0.15 - 126.8	<0.05 - 208.8	<0.07 - 223.6
Benthic Invertebrates Brown Meat	3	<0.03	<0.03 - 1.399	33.26 - 64.92	3.557 - 20.50	56.83 - 115.9

Table 4.3 (continued): The concentration range (µg/kg lipid weight) of thirty-two CB congeners, ΣICES-7 PCBs and ΣPCB_{32} 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 2.1. Not all the LoD values are to four significant figures to account for precision. Values <LoD treated as zero when calculating the sum of CBs. ΣPCB_{32} and ΣICES-7 is expressed as the minimum sample concentration – maximum sample concentration within each category. The shaded columns indicate the ICES-7 PCB congeners.

Category	Sample Number	CB156	CB157	CB158	CB167	CB170
Harbour Seal	10	9.510 - 355.10	2.323 - 52.52	7.345 - 377.3	<0.07 - 6.966	86.66 - 7,170
Harbour Porpoise	18	1.628 - 44.54	<0.05 - 7.705	3.827 - 677.6	1.797 - 47.52	33.61 - 4,668
Sperm Whale	5	9.141 - 139.8	1.487 - 26.81	6.39 - 100.9	4.238 - 79.06	28.33 - 405.6
Demersal Shark Muscle	12	<0.02 - 21.82	<0.04	<0.05 - 23.64	<0.04	<0.02 - 81.82
Demersal Shark Liver	12	8.131 - 99.56	1.808 - 20.63	6.023 - 90.48	2.179 - 54.05	19.40 - 375.9
Pelagic Roundfish Muscle	2	1.538 - 3.019	<0.04	<0.05	<0.04	6.325 - 10.94
Pelagic Roundfish Liver	2	<0.41 - 13.33	<0.05	<0.10	<0.07	39.41 - 111.1
Pelagic Roundfish Whole	3	3.357 - 5.515	<0.04	1.923 - 3.079	1.442 - 2.431	7.692 - 11.99
Demersal Roundfish Muscle	30	<0.02 - 22.13	<0.04	<0.05 - 19.67	<0.04 - 9.016	<0.02 - 63.11
Demersal Roundfish Liver	30	<0.10 - 31.80	<0.05 - 3.622	<0.10 - 31.91	0.483 - 12.38	<0.33 - 128.5
Demersal Roundfish Whole	6	<0.02 - 7.641	<0.04 - 4.319	<0.05 - 13.33	<0.04 - 11.11	<0.02 - 71.11
Flatfish Muscle	12	<0.02	<0.04	<0.05	<0.04	<0.02
Flatfish Liver	12	<0.41	<0.05	<0.10	<0.07 - 3.896	<0.33 - 15.19
Demersal Invertebrates Muscle	2	<0.02	<0.04	<0.05	<0.04	<0.02
Benthic Invertebrates Muscle	12	<0.02 – 9.890	<0.04	<0.05	<0.04	<0.02 – 29.63
Benthic Invertebrates Soft Body	19	<0.03 - 46.84	<0.04 - 7.595	<0.06 - 55.06	<0.06 - 24.06	<0.08 - 158.9
Benthic Invertebrates Whole	11	<0.03 - 11.19	<0.04	<0.06	<0.06	<0.08 - 29.55
Benthic Invertebrates Brown Meat	3	1.984 - 5.259	<0.04 - 1.892	<0.06 - 3.027	<0.06 - 4.692	8.751 - 12.49

Table 4.3 (continued): The concentration range (µg/kg lipid weight) of thirty-two CB congeners, ΣICES-7 PCBs and ΣPCB_{32} 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 2.1. Not all the LoD values are to four significant figures to account for precision. Values <LoD treated as zero when calculating the sum of CBs. ΣPCB_{32} and ΣICES-7 is expressed as the minimum sample concentration – maximum sample concentration within each category. The shaded columns indicate the ICES-7 PCB congeners.

Category	Sample Number	CB180	CB183	CB187	CB189	CB194
Harbour Seal	10	194.6 - 18,630	50.62 - 6,052	154.0 - 14,040	1.004 - 52.94	29.74 - 2.517
Harbour Porpoise	18	72.81 - 10,490	23.86 - 4,191	85.96 - 13,600	1.402 - 45.18	11.68 - 2,057
Sperm Whale	5	69.14 - 1,012	19.37 - 282.5	56.00 - 807.6	1.575 - 14.04	<0.34 - 26.81
Demersal Shark Muscle	12	<0.08 - 180.0	<0.03 - 65.45	<0.05 - 176.4	<0.05	<0.38
Demersal Shark Liver	12	50.05 - 955.1	12.41 - 333.7	35.18 - 910.2	<0.23 - 10.63	4.943 - 185.0
Pelagic Roundfish Muscle	2	15.21 - 26.42	5.641 - 10.19	22.22 - 41.51	<0.05	<0.38
Pelagic Roundfish Liver	2	39.42 - 111.1	<0.28 - 28.89	56.20 - 111.1	70.07 - 111.1	<0.34
Pelagic Roundfish Whole	3	<0.08 - 25.12	6.090 - 9.887	28.53 - 41.33	<0.05	<0.38
Demersal Roundfish Muscle	30	<0.08 - 169.2	<0.03 - 53.85	<0.05 - 119.4	<0.05	<0.38
Demersal Roundfish Liver	30	2.883 - 333.9	1.054 - 111.3	<0.56 - 236.8	<0.23 - 3.099	<0.34 - 75.98
Demersal Roundfish Whole	6	12.50 - 181.1	8.000 - 60.00	<0.05 - 79.73	<0.05	<0.38
Flatfish Muscle	12	<0.08	<0.03	<0.05 - 40.91	<0.05	<0.38
Flatfish Liver	12	<0.40 - 26.49	<0.28 - 10.78	<0.56 - 188.5	<0.23	<0.34
Demersal Invertebrates Muscle	2	<0.08	<0.03	<0.05	<0.05	<0.38
Benthic Invertebrates Muscle	12	<0.08 – 81.32	<0.03 – 18.68	<0.05 – 72.67	<0.05	<0.38
Benthic Invertebrates Soft Body	19	<0.06 - 524.1	<0.05 - 158.7	<0.05 - 462.0	<0.09	<0.28 - 79.11
Benthic Invertebrates Whole	11	<0.06 - 38.18	<0.05 - 10.91	<0.05 - 210.4	<0.09	<0.28
Benthic Invertebrates Brown Meat	3	17.27 - 31.06	6.378 - 7.567	29.02 - 41.12	<0.09	<0.28 - 4.465

Table 4.3 (continued): The concentration range (µg/kg lipid weight) of thirty-two CB congeners, ΣICES-7 PCBs and ΣPCB_{32} 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 2.1. Not all the LoD values are to four significant figures to account for precision. Values <LoD treated as zero when calculating the sum of CBs. ΣPCB_{32} and ΣICES-7 is expressed as the minimum sample concentration – maximum sample concentration within each category. The shaded columns indicate the ICES-7 PCB congeners.

Category	Sample Number	CB209	ICES-7	ΣPCB_{32}
Harbour Seal	10	4.297 - 70.99	1,439 - 90,640	1,965 - 139,800
Harbour Porpoise	18	1.091 - 180.0	417.9 - 71,200	754.3 - 114,500
Sperm Whale	5	1.243 - 7.057	462.0 - 7,630	821.1 - 13,520
Demersal Shark Muscle	12	<0.06	<0.03 - 1,036	<0.02 - 1,585
Demersal Shark Liver	12	<0.50 - 20.46	396.1 - 4,639	655.9 - 8,653
Pelagic Roundfish Muscle	2	<0.06	109.1 - 205.3	198.8 - 373.9
Pelagic Roundfish Liver	2	<0.50	337.9 - 604.4	668.6 - 1,202
Pelagic Roundfish Whole	3	<0.06 - 2.098	166.8 - 265.5	329.5 - 530.9
Demersal Roundfish Muscle	30	<0.06	<0.03 - 1,036	<0.02 - 1,858
Demersal Roundfish Liver	30	<0.50 - 6.601	40.90 - 1,684	57.91 - 3,065
Demersal Roundfish Whole	6	<0.06	141.5 - 820.0	160.5 - 1,164
Flatfish Muscle	12	<0.06	<0.03 - <0.22	<0.02 - 40.91
Flatfish Liver	12	<0.50	<0.11 - 586.9	<0.05 - 899.2
Demersal Invertebrates Muscle	2	<0.06	<0.03 - <0.22	<0.02
Benthic Invertebrates Muscle	12	<0.06	26.83 - 417.6	26.83 - 797.8
Benthic Invertebrates Soft Body	19	<0.08 - 19.08	<0.03 - 2,119	<0.03 - 3,888
Benthic Invertebrates Whole	11	<0.08 - 20.28	<0.03 - 555.9	<0.03 - 1,418
Benthic Invertebrates Brown Meat	3	<0.08	124.9 - 250.4	218.3 - 367.2

Thirty-two congeners have been analysed to provide as much data as possible for the assessment of a wide range of PCBs with varying lipophilicity and species-specific metabolic stability and for comparison to FA and SI data. The highest mean concentration of the ΣPCB_{32} was found in harbour seal blubber (Table 4.3). There was, however, a large degree of variation, with a range of 1,965 - 139,800 µg/kg lw, with the highest concentration being detected in an adult male sample. To reduce the variability associated with sex, age and reproductive status, the blubber of only male marine mammals in this study was analysed and available physiological information fully investigated to determine whether they contribute to the within-species concentration and congener proportion variation. The recalcitrant, metabolically stable PCB, CB153, was the most abundant congener in the sample categories, with only demersal invertebrates muscle and flatfish muscle showing mean concentrations <LoD.

Demersal invertebrates muscle was the only category to have ΣPCB_{32} <LoD. The highest total concentration for the sum of the ICES-7 PCBs was 90,640 µg/kg lw in harbour seal blubber. The range was 1,439 - 90,640 µg/kg lw (Table 4.3). Flatfish muscle and demersal invertebrates muscle had an ICES-7 PCB concentration <LoD and the lowest detected ICES-7 PCB concentration was reported in benthic invertebrates muscle, ranging from 26.83 - 417.6 µg/kg lw (Table 4.3). ΣPCB_{32} follow the same pattern as the concentrations for ICES-7 PCBs and were approximately 2 times the ICES-7 PCB concentration, except in the case where concentrations were low and the ICES-7 were only PCBs detected (Table 4.3).

Currently, concentrations of <500 µg/kg lw for the ICES-7 PCBs have been reported previously in plaice liver collected at remote, reference sites, and a concentration above this value is considered high (Webster *et al.,* 2011; Webster *et al.,* 2014a). As expected, the higher trophic level marine mammals have a higher ΣPCB_{32} and ICES-7 PCB concentration than the other sample categories. All three marine mammal species, demersal shark (muscle and liver), pelagic fish liver, demersal roundfish (muscle, liver and whole), flatfish liver and benthic invertebrates have maximum range concentrations >500 µg/kg lw and are therefore considered to have a high concentration of ICES-7 PCBs in their tissues (Table 4.3). Harbour seal blubber is the only category to have a concentration >500 µg/kg lw in all samples (10 individuals) (Table 4.3).

Figure 4.1 shows the sample categories with a ΣPCB_{32} concentration above 1,000 µg/kg lw. There is a sudden substantial increase in ΣPCB_{32} concentration (>25,000 µg/kg lw) in the top eight samples composed of harbour seal (n=2) and harbour porpoise (n=6). It is well established that there is a positive correlation between trophic

positions calculated from $\delta^{15}N$ and PCB concentrations in marine food webs (Kobayashi *et al.*, 2015; Verheart *et al*., 2017; Masset *et al*., 2019). This was confirmed using an ANOVA test, where harbour seal blubber and harbour porpoise blubber were found to have a significantly higher ΣPCB_{32} concentration (µg/kg lw) than the other sample categories ($p < 0.05$, ANOVA, Tukey). This corresponds with the findings in Chapter 3 (Figure 3.8; Table 3.1), where marine mammals, particularly harbour seals, had a significantly higher $\delta^{15}N$ and therefore the highest trophic level of all the species studied (5.02 ± 0.35). The number of harbour porpoise in the top eight samples is likely a result of their metabolic inability to biotransform some PCB congeners in comparison to harbour seal (Boon *et al.*, 1997; Hobbs *et al*., 2002; Weijs *et al*., 2009), which will likely influence the calculated TMF.

As well as inhabiting a lower trophic level, sperm whales will have a lower concentration of PCBs in their blubber due to their size. Sperm whales will carry larger masses of PCBs but due to their size, would have a lower ΣPCB_{32} concentration per unit mass of blubber. However, they also have a lower metabolic rate than seals and porpoises and appear to have a lower metabolic capacity to metabolise some PCBs compared to seals and porpoises (Wells, McKenzie and Ross, 1997).

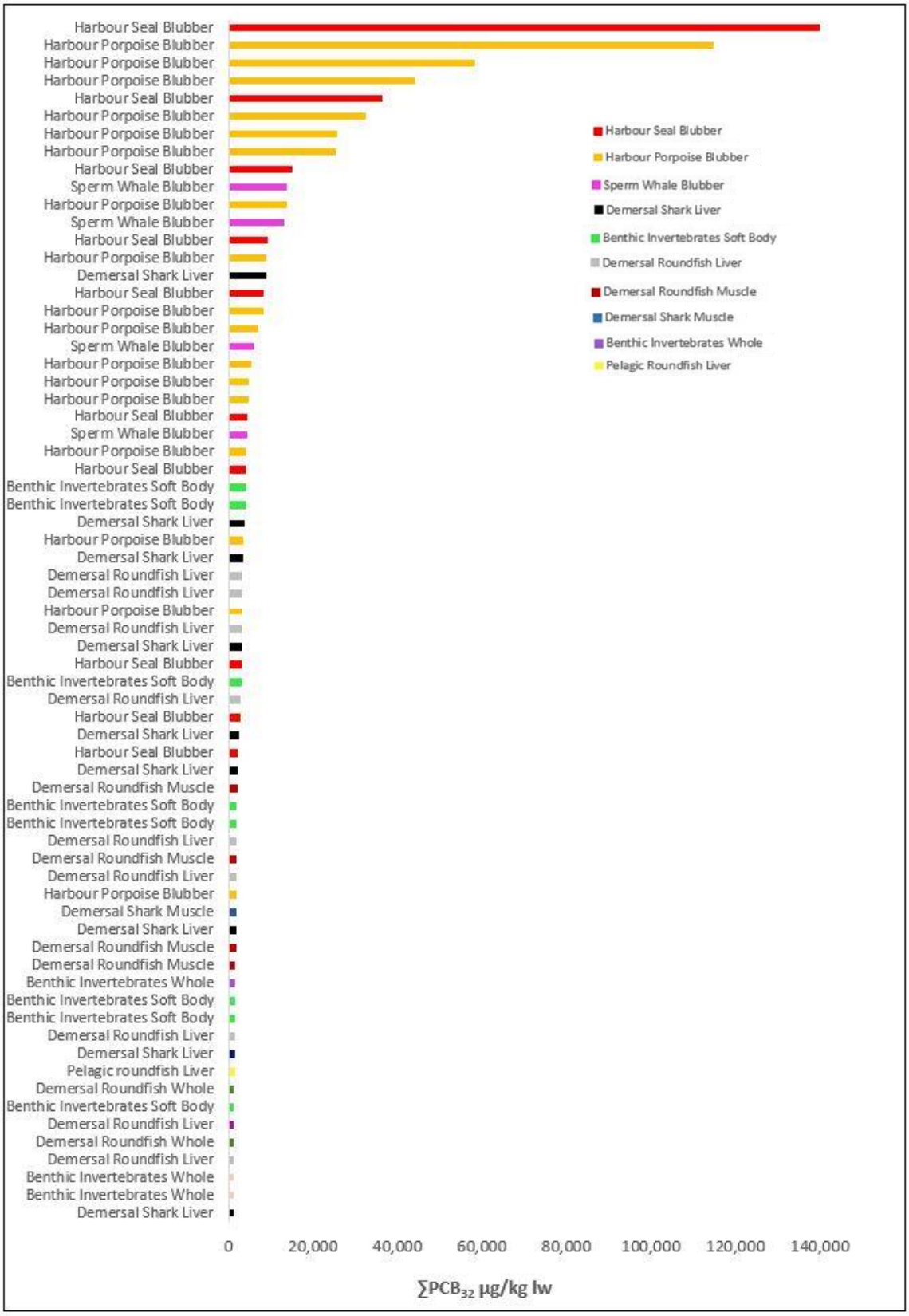


Figure 4.1: Sample categories (individuals for marine mammals and pools for all other categories) with a ΣPCB_{32} concentration above 1,000 µg/kg lw.

Lower and higher chlorinated congeners behave differently in the environment, with each congener exhibiting a different level of metabolic stability and toxicity dependent upon the species on which they are found (Burkhard, Armstrong and Andren, 1985; Ferreira, 2012). The most abundant PCB congener in marine mammals, shark, fish (pelagic and demersal) and benthic invertebrates was CB153, accounting for an average of between 36% of the ΣPCB_{32} profile in harbour seal blubber to 17% of the ΣPCB_{32} profile in pelagic roundfish liver. CB153 is a well-studied congener, generally exhibiting the highest concentration and correlates well with other analysed PCBs (Pérez-Fernández, Viñas and Besada, 2019). This is also the least toxic congener as shown by it having the highest EAC (Table 4.1). CB153, a hexa-chlorinated congener, is one of the most persistent PCB congeners in marine mammals. It is metabolically stable in all organisms and less likely to be transferred from females to offspring via reproductive processes (gestation and lactation) compared to higher chlorinated congeners which have similar metabolic stability (Weijs *et al.*, 2009) in agreement with the findings of this study (Table 4.3).

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 which was then discussed alongside concentration. This section is divided into three sub-sections: marine mammals, fish and shark and invertebrates to identify CB congeners responsible for any differentiation.

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 previously discussed resistance to biotransformation and dominance in aquatic profiles (Duinker *et al*., 1989; Boon *et al*., 1992; Wells and Echarri, 1992). The ratio was calculated as follows:

CB ratio = [CBx]/[CB153] **Equation 4.1**

Where [CBx] is the concentration of an individual CB congener x in the sample being analysed and
[CB153] is the concentration of CB153 in that sample.

Marine Mammals

Harbour porpoise (red), harbour seal (blue) and sperm whale (green) are clearly separated on Figure 4.2b. The first two principal components of the PCA accounted for 72% of the PCB ratio variability.

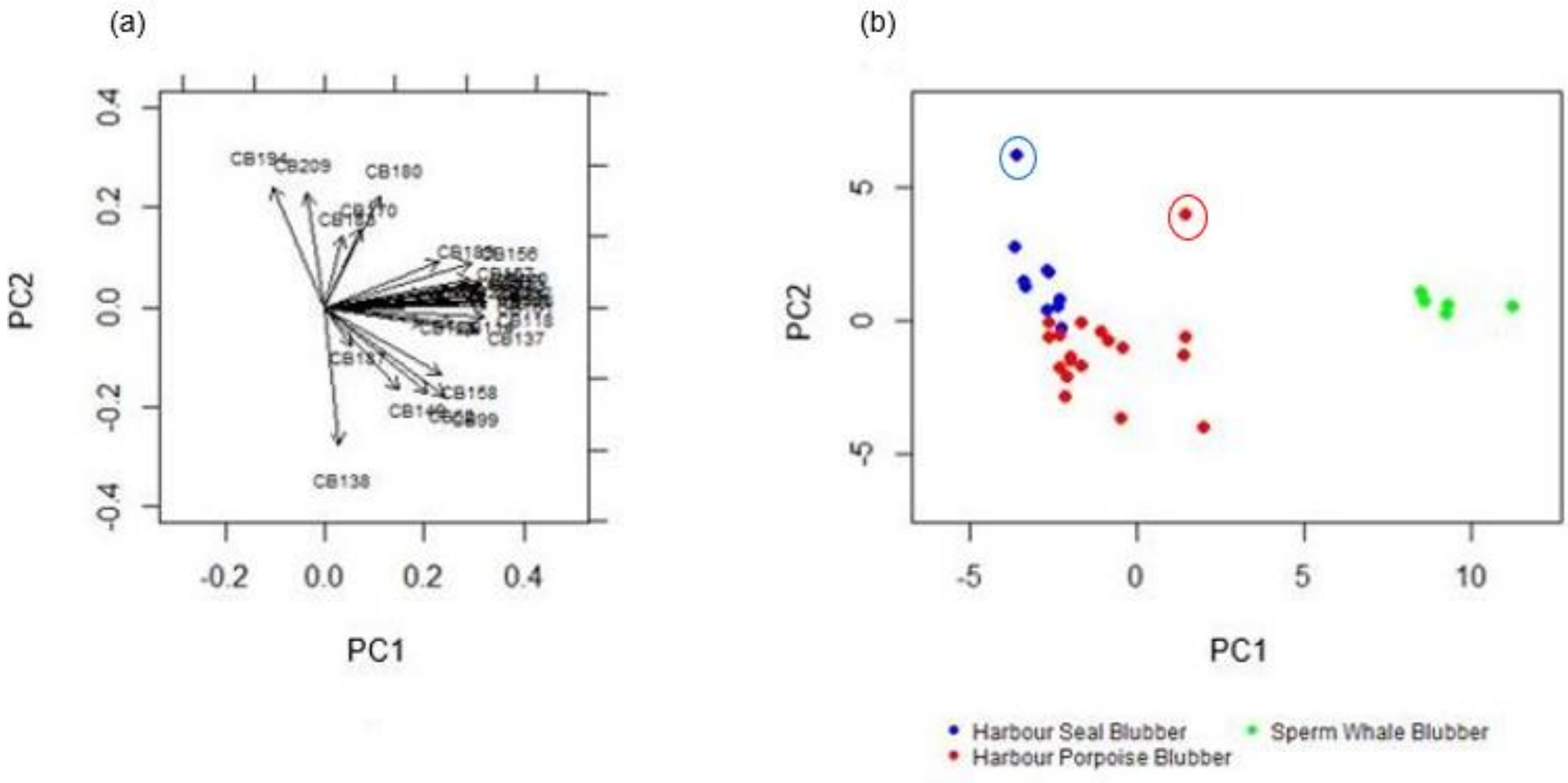


Figure 4.2: (a): PCA loading plot and (b): PCA score plot, both demonstrating variation in the PCB profiles (normalised to the concentration of CB153) across the three marine mammal species. The circled harbour seal and harbour porpoise samples highlight individuals with a different FA profile reported in Chapter 3 (Figure 3.4) and PCB profile in comparison to the other samples.

Although recalcitrant PCBs such as CB153 are the most abundant congeners in all three marine mammal species, sperm whale was more positively correlated to the first component due to the higher proportion of lower chlorinated PCBs (CB49, 44, 74, 101, 118) suggesting a lower metabolic capacity to biotransform these compounds or lower concentration-dependant induction of metabolising enzymes (Figure 4.2a and b). Sperm whale is the largest of the species studied with the slowest and least developed metabolism 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) (Aguilar and Borrell, 1988), which agrees with the findings of this study. Cephalopod feeders and oceanic species such as sperm whale, pilot whale and striped dolphin have previously been found to have a higher proportion of less chlorinated congeners (i.e. tri-, tetra- and penta-CBs) in their blubber (Méndez-Fernandez, 2014).

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, whilst harbour porpoise contain a larger proportion of hexa-chlorinated congeners (CB149, 138 and 153) (Figure 4.2a and b). This confirms previous data that harbour seals have an enhanced ability to metabolise lower chlorinated PCB congeners (e.g. CB52 and CB101), and CB149, compared to harbour porpoise, which agrees with the findings of Boon *et al.*, (1997), Hobbs *et al*., (2002) and Weijs *et al*., (2009). Other than CB153, CB138 and CB149 have been previously reported in the North Sea as the highest congener concentrations in harbour porpoise blubber (Weijs *et al.,* 2008), similar to the data reported in this study.

Sperm whale samples are tightly clustered on the PCA score plot (Figure 4.2b), showing very little within-group variation. Harbour seal and harbour porpoise are however more dispersed across the second component in the score plot, suggesting within-species ecological and biological parameters as potential explanatory variables. In Chapter 3 (Figure 3.4), sperm whale was 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. Harbour seal and harbour porpoise were widely dispersed on the first component of Figure 3.4 but were generally separated by species across both components, which corresponds with the PCB profiles shown in Figure 4.2b. The analysis of $\delta^{13}C$ in Chapter 3 also revealed that harbour seal and harbour porpoise have a more variable dietary pattern and/or feeding location than sperm whale, agreeing with

the FA analysis (Figure 3.7). The trophic level calculated for sperm whale was 3.75 ± 0.16, significantly lower than the trophic level calculated for harbour seal (5.02 ± 0.35) and harbour porpoise (4.71 ± 0.36); which would likely contribute to the significant difference in ΣPCB_{32} concentration (Table 4.3) and congener proportion in sperm whale (Figure 4.2a and b).

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 (Lailson *et al.*, 2010; 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 furthest from 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.

There is one harbour seal sample more positively correlated to the first component on Figure 4.2b than the other harbour seal samples (circled in blue). When this sample was investigated further, it was identified as the same individual discussed in Chapter 3 (Section 3.3.1.1; Figure 3.4) which had a different FA profile to the other harbour seal samples. This individual was the smallest and lightest individual with the lowest recorded ventral blubber thickness (suggesting malnourishment) and had a cause of death reported as “chronic peritonitis due to ingestion of foreign body (fishing gear) “. As well as a different congener proportion, this individual also had a significantly ($p < 0.05$) higher ΣPCB_{32} concentration of 139,800 µg/kg lw than the other nine samples (1,967 – 36,060 µg/kg lw). There is also one harbour porpoise sample more positively correlated to the second component on Figure 4.2b (circled in red) than the other harbour porpoise samples due to having a higher proportion of hepta - CBs such as CB180, 170 and 189 in its blubber. This is the same individual that was identified in Chapter 3 on Figure 3.4 with a different FA profile to the other harbour porpoise samples. This individual also had a significantly higher ΣPCB_{32} concentration of 114,500 µg/kg lw than the other nine samples with a range of 754.3 – 58,308 µg/kg lw ($p < 0.05$). Unfortunately, other than sampling location and length there was no additional information available, but this does

suggest a link between FA profile (feeding pattern) and PCB congener proportion as well as indicating enhanced metabolism of 'metabolisable' PCBs due to the concentration-dependent induction of the enzymes responsible for PCB metabolism (Boon *et al*., 1997).

It can therefore be concluded from cross referencing to FA and SI data for all three marine mammal species 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.

To determine whether species and/or biogeographic region is contributing to the variance associated with these species, PCA was conducted on biogeographic regional differences (Figure 4.3a and b).

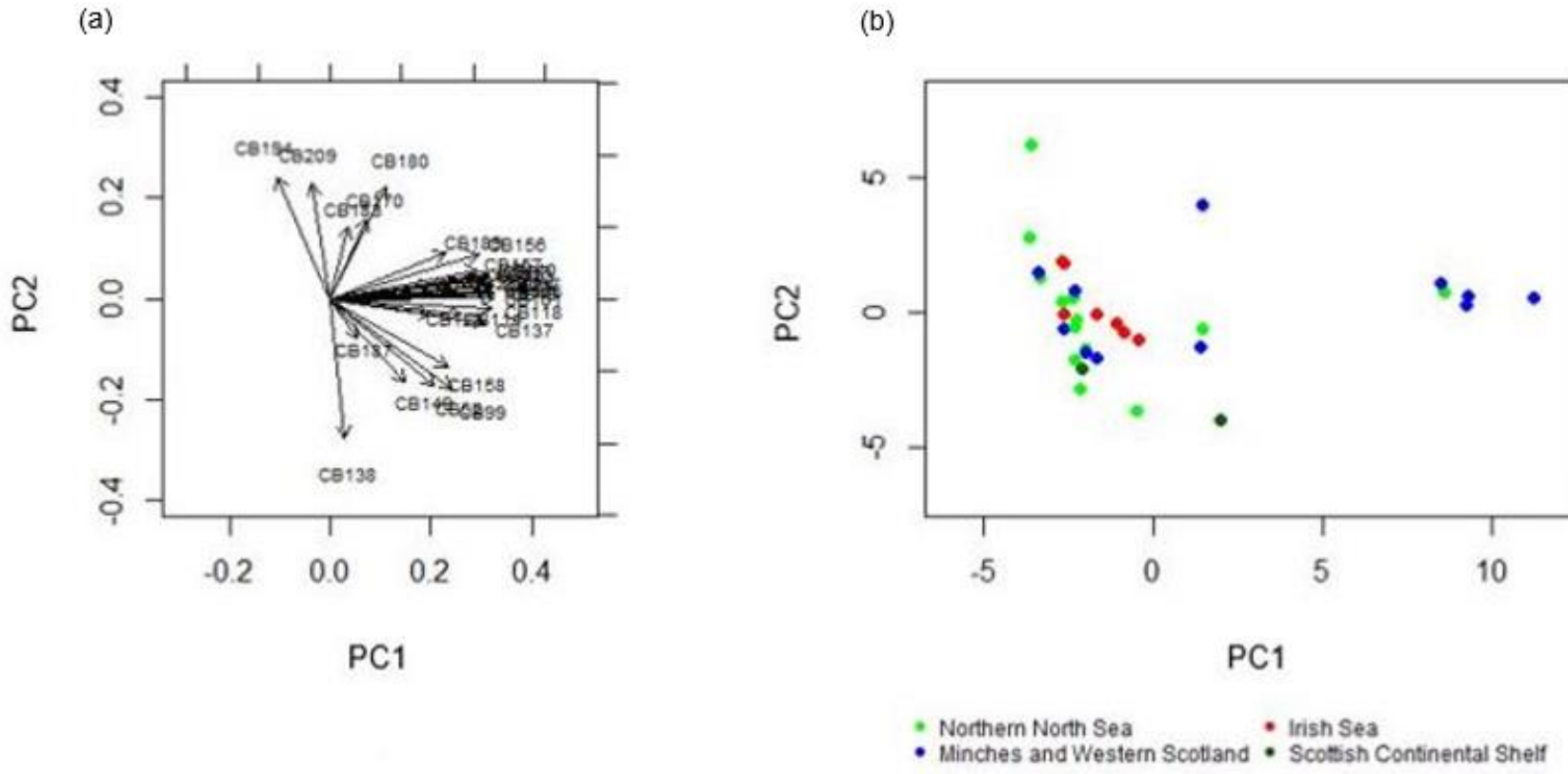


Figure 4.3: (a): PCA loading plot and (b): PCA score plot, both demonstrating variation in the PCB profiles (normalised to the concentration of CB153) across the four marine mammal biogeographic sampling locations. The five points more positively correlated to the first component at +10 are identified in Figure 4.2 as sperm whale, suggesting a species influence on the marine mammal categories rather than regional influence.

Figure 4.3a and 4.3b show 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 whale were stranded at two locations – Minches and Western Scotland (blue) and the Northern North Sea (green), identified as the five points on Figure 4.3b at +10 on PC1. 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 on a specific region, but an insight into the prey consumed during the migratory route and the contaminant loading of the prey.

ANOVA on ΣPCB_{32} in harbour seal and harbour porpoise (analysed separately) found that although concentrations in animals from the Irish Sea were higher, there was no statistically significant difference in concentration between the regions for harbour seal (Irish Sea n=2; Minches and Western Scotland n=2; Northern North Sea n=6) and harbour porpoise (Irish Sea n=5, Minches and Western Scotland n=5, Northern North Sea n=6 and Scottish Continental Shelf n=2) ($p > 0.05$ ANOVA, Tukey) and this may in part be due to a lack of statistical power due to the low sample sizes for each category. Factors potentially influencing the ΣPCB_{32} concentration in harbour seal and harbour porpoise include the stranding year, length, reproductive status and weight of the animal (Chapter 2; Table 2.2). Pearson's correlation analysis revealed that there was a significant relationship between harbour seal length and weight with ΣPCB_{32} concentration, where the smaller and lighter the animal, the higher the concentration ($p<0.05$). However, this was heavily influenced by the one malnourished seal specimen identified in Chapter 3 (Figure 3.4) and described above, having a significantly higher concentration of ΣPCB_{32} in its blubber than the other harbour seal samples. Once this sample was removed from the dataset, there was no significant relationship between harbour seal length and weight with ΣPCB_{32} concentration ($p > 0.05$) and no difference in the statistical analysis of ΣPCB_{32} between regions for the species. This demonstrates the importance of sample size for outlier identification and impact. There was also no correlation between length and weight on ΣPCB_{32} concentration for harbour porpoise and no significant difference between stranding year and reproductive status for all three mammal species ($p > 0.05$). This is an indication of both regional and physical factors on ΣPCB_{32} concentration in marine mammals. A larger sample size is however required for a more comprehensive analysis.

When focussing more specifically on the ICES-7 PCBs, which will be further assessed for trophic magnification, the differences in congener proportion are apparent between the marine mammals (Figure 4.4).

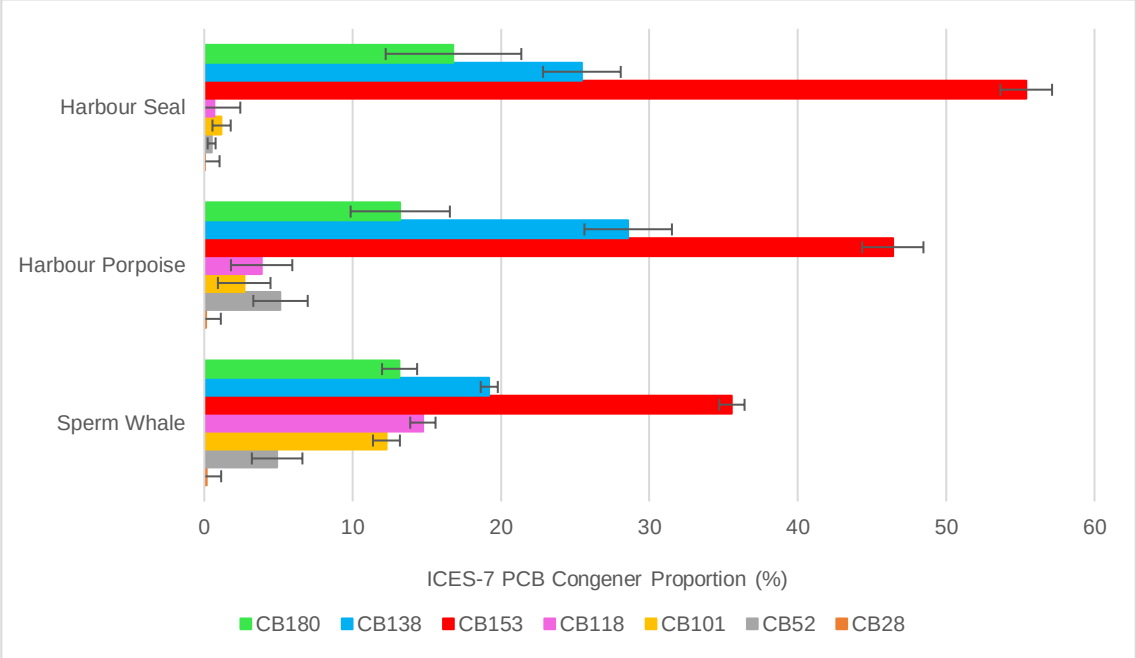


Figure 4.4: The mean proportion across the ICES-7 PCBs in individual harbour seal (n=10), harbour porpoise (n=18) and sperm whale (n=5) blubber samples, expressed as a percentage of the ∑ICES-7 concentration (µg/kg lw). Error bars are to one standard deviation. The number of chlorine atoms decrease from a maximum of 7 for CB180 to a minimum of 3 for CB28. The plot clearly illustrates the increased proportion of lower chlorinated congeners (CB118, CB101, CB52 and CB28) present in sperm whale.

Harbour seal was found to have consistent proportions of the ICES-7 PCBs in each sample with CB153 and 138 accounting for a range of 52 – 58% and 20 – 29% of the ICES-7 CB profile, respectively (Figure 4.4). Harbour porpoise and sperm whale had lower proportions of CB153 and CB138 and higher proportions of the lower chlorinated CB52, 101 and 118 than harbour seal, with the sum of these three congeners accounting for an average range of 6.0 - 24% in harbour porpoise, 31 – 35 % in sperm whale but only 1.2 – 3.8% in harbour seal (Figure 4.4). As previously described, this is due to harbour seals enhanced metabolism of lower chlorinated PCBs, particularly CB52 and 101, as well as CB149, relative to harbour porpoise and sperm whales. Sperm whale had a more distinctive profile, with the higher chlorinated CB153 only accounting for a range of 34 – 36% of the ICES-7 profile (Figure 4.4). This corresponds with the PCA on the thirty-two PCB congeners (Figure 4.2), where a higher proportion of lower chlorinated congeners is likely due to sperm whales lower metabolic efficiency, metabolic rate and

distance from contaminant sources (efficient long-range transport of lower chlorinated PCBs) than the other marine mammal species.

Shark and fish

PCA was conducted on demersal shark liver and fish liver PCB concentrations normalised to CB153. Pooled flatfish liver (light green), pooled demersal shark liver (blue), pooled demersal roundfish liver (red) and pooled pelagic roundfish liver (dark green) showed a degree of separation on the score plot (Figure 4.5b). The first two principal components of the PCA accounted for 56% of the PCB ratio variability.

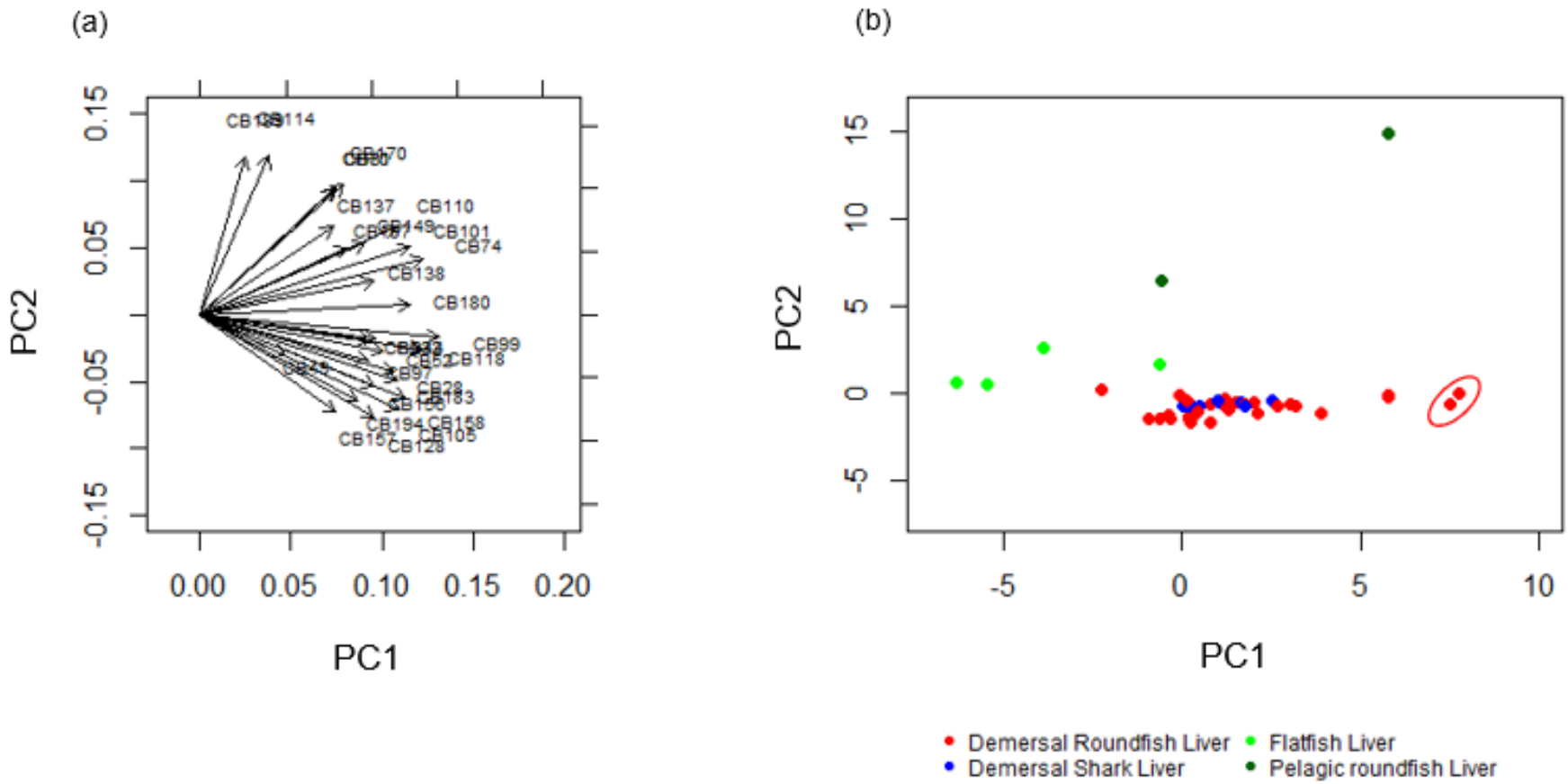


Figure 4.5: (a): PCA loading plot and (b): PCA score plot, both demonstrating variation in the PCB profiles (normalised to the concentration of CB153) across the shark and three fish liver sample categories. Hake sample pools (n=2) are separated from the demersal roundfish liver category and are circled in red.

Pelagic roundfish liver scored more positively on the second component due to having a higher proportion of CB189 than the other three categories (Figure 4.5a and b). A similar separation was also observed following the PCA analysis of FAs (Figure 3.5), where pelagic roundfish liver were separated from demersal roundfish liver, flatfish liver and demersal shark liver sample pools during the PCA analysis of FAs. This was due to the higher proportion of MUFAs in their profiles as a result of their planktonic diet. The two pelagic roundfish sample pools are however not clustered together when the variation in the PCB profiles across the four shark and fish liver sample categories were analysed, due to having different proportions of CB138 and 170 in their liver (Figure 4.5b). Both herring sample pools were collected from the same region (Holy Loch), had similar average pool trophic levels (3.49 ± 0.26) and feeding pattern (Chapter 3, Figure 3.5), similar average pool length (231 mm and 264 mm) and similar average pool weight (96.4 g and 98.8 g). Although this provides an insight to congener proportion in pelagic roundfish liver, a larger sample number is required for the analysis of ecological influences on the PCB profile.

There were two demersal roundfish samples more positively correlated to the second component with a higher proportion of lower chlorinated congeners in their liver, including CB44, 52, 74 and 99 (Figure 4.5). Hake are at a higher trophic level than haddock and whiting (Table 3.7) and this difference in congener proportion is likely due to the different species-specific metabolic capacities existing within the demersal roundfish category.

All flatfish liver sample pools were negatively correlated to the first component and have far fewer PCB congeners detected than the other fish and shark categories. Flatfish are bottom-feeding fish, living in close contact with sediments where they 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 it is caught. All flatfish sample pools in this project were collected from remote offshore sites such as Burra Haaf (n=7), Moray Firth (n=3) and the Solway Firth (n=2).

Demersal shark possesses the least variable PCB profile and form a tight cluster on the PCA score plot (Figure 4.5b). This is similar to the FA distribution of demersal shark where there was little variation in feeding pattern within the species (Figure 3.5b),

suggesting that like marine mammals, PCB patterns could potentially be used as tracers for studying the feeding ecology of sharks. The low degree of variation observed could also simply be due to the samples being collected from a single biogeographical region (Irish Sea).

There was a considerable variation in PC1 scores for the demersal roundfish sample category (Figure 4.5b). To determine whether species and/or biogeographic region is contributing to the variance associated with this category, PCA was conducted on these variables (Figures 4.6 and 4.7). Fish species selected for this study are not highly migratory (such as mackerel).

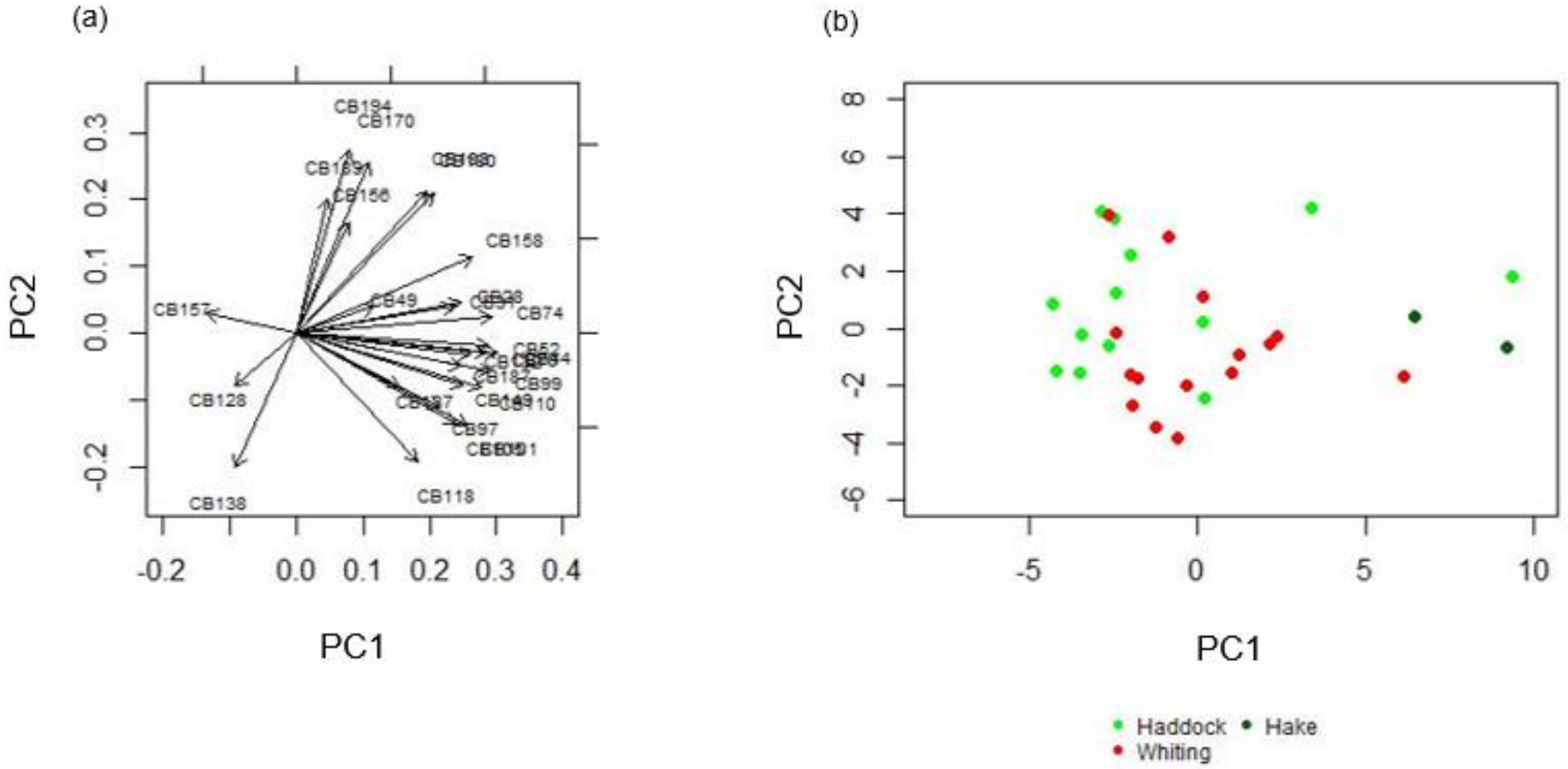


Figure 4.6: (a): PCA loading plot and (b): PCA score plot, both demonstrating variation in the PCB profiles (normalised to the concentration of CB153) across the three demersal roundfish liver species. Four sample pools (hake =2; haddock = 1, whiting = 1) are grouped together on the score plot (PC1 +5 - +10), suggesting that species is not the main influencing factor within the demersal roundfish category.

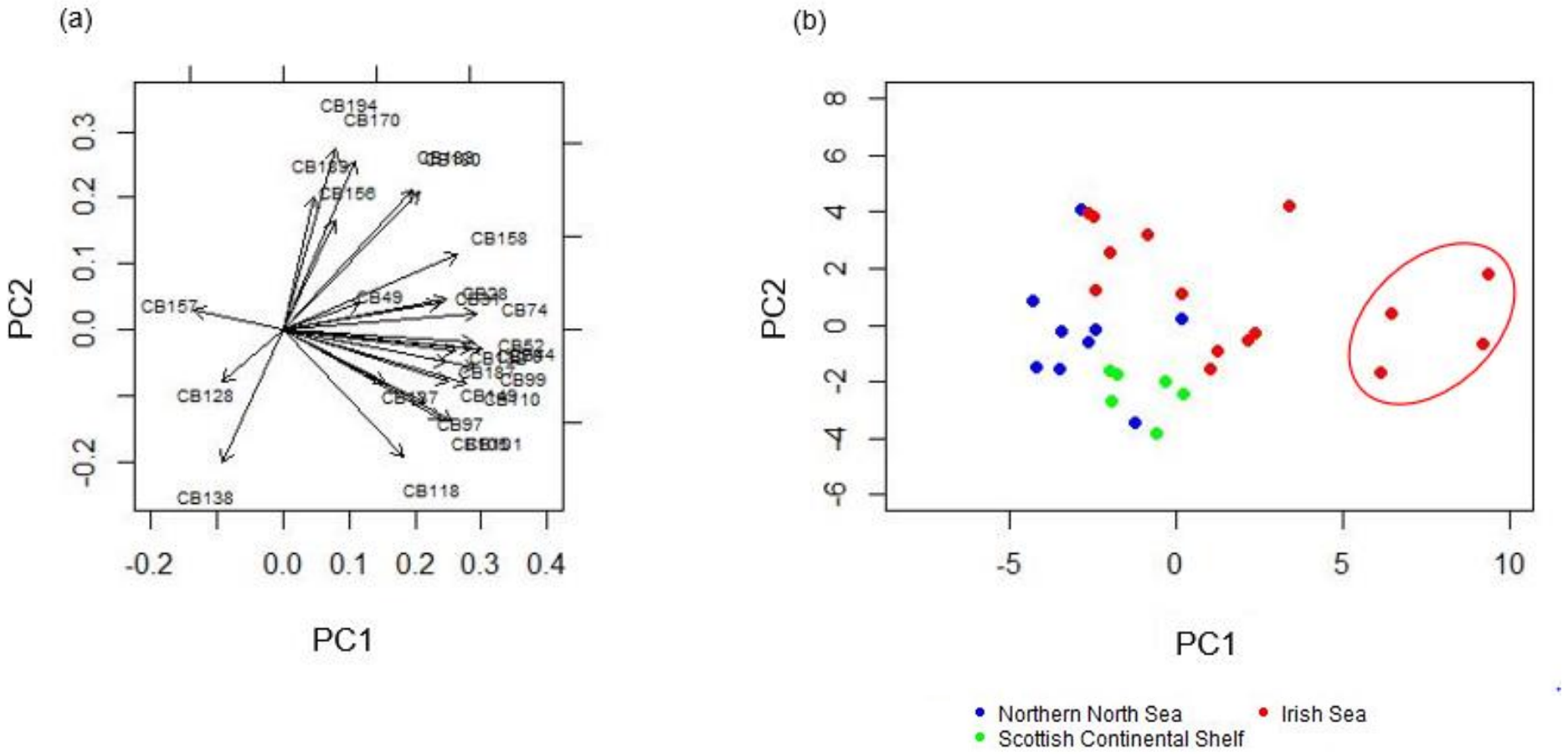


Figure 4.7: (a): PCA loading plot and (b): PCA score plot, both demonstrating variation in the PCB profiles (normalised to the concentration of CB153) across the demersal roundfish liver biogeographic sampling locations. The four sample pools identified in Figure 4.6b, circled in red, were collected from the Holy Loch, suggesting that localised sample collection area is a contributing factor to the variance associated within the demersal roundfish category.

Although grouping samples on a species level separated hake from whiting and haddock (Figure 4.6b), there is still a considerable spread across the score plot for haddock and whiting.

The PCB profiles across the demersal roundfish liver biogeographic sampling locations were analysed (Figure 4.7b). 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 and Northern North Sea (Figure 4.7a). 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 4.7b). This potential localised regional influence is investigated further below.

Demersal roundfish collected from Holy Loch (Irish Sea) were more positively correlated to the first component than those from the Solway Firth and Pladda, with a higher proportion of hepta-chlorinated congeners, including CB180, 183 and 170 (Figure 4.8a and b).

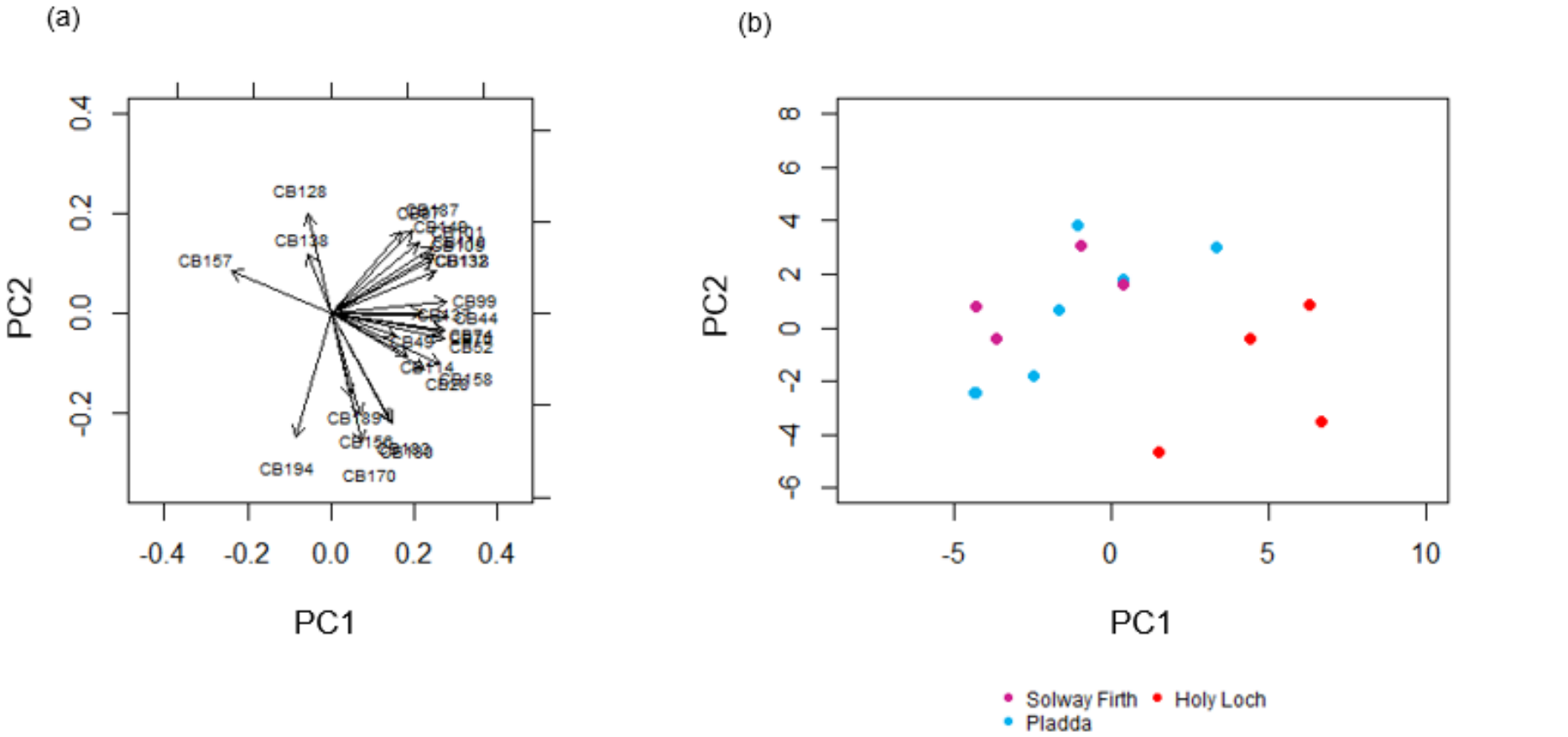


Figure 4.8: (a): PCA loading plot and (b): PCA score plot, both demonstrating variation in the PCB profiles (normalised to the concentration of CB153) across the demersal roundfish liver category localised regional sampling locations in the Irish Sea. Sample pools collected from the Holy Loch (n=4) are more positively correlated to the first component than sample pools collected from the other two regions

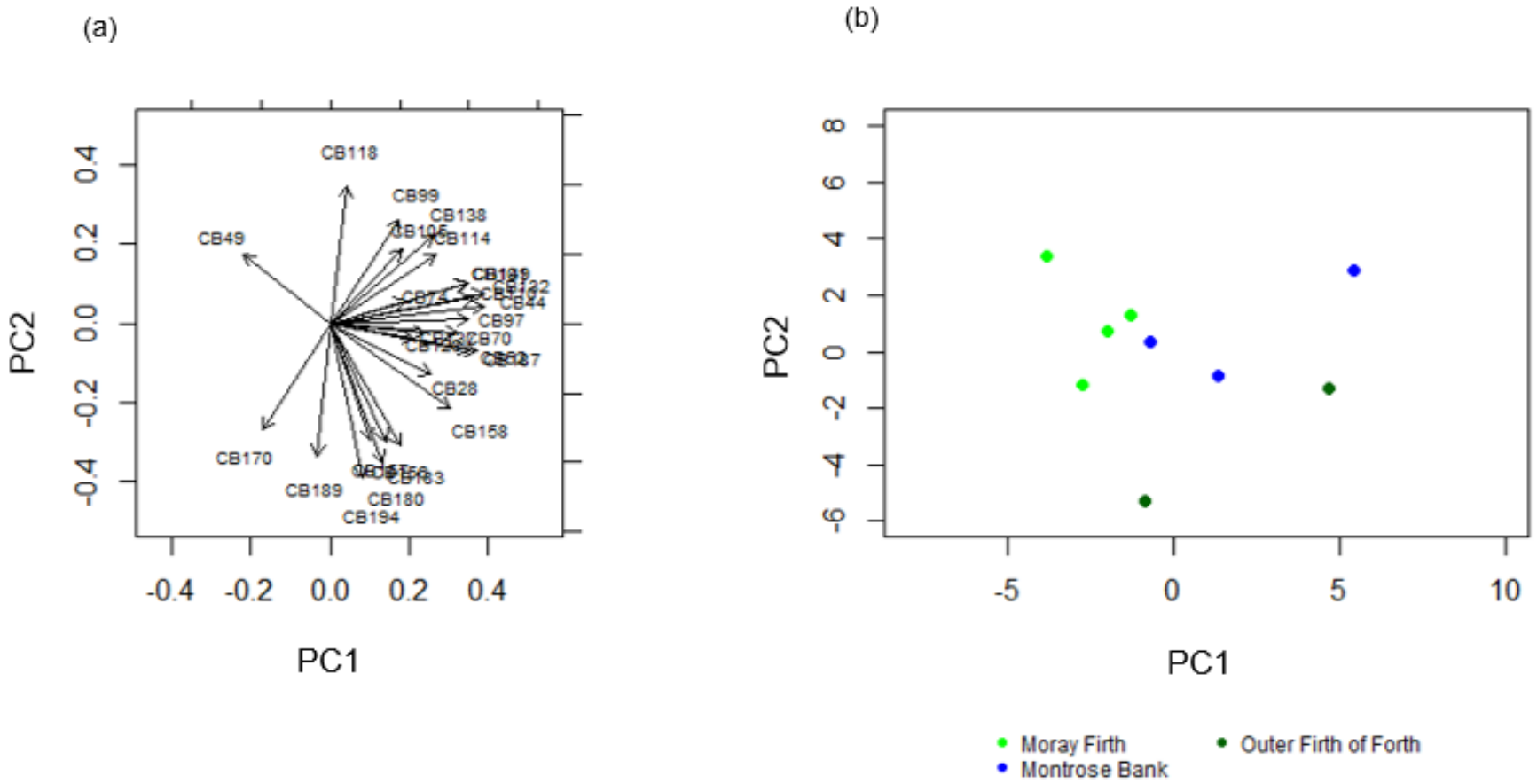


Figure 4.9: (a): PCA loading plot and (b): PCA score plot, both demonstrating variation in the PCB profiles (normalised to the concentration of CB153) across the demersal roundfish liver category localised regional sampling locations in the Northern North Sea. Sample pools collected from the Moray Firth (n=4), composed of haddock (n=4), are more negatively correlated to the first component than the other two regions.

The proportion of higher chlorinated PCBs from Holy Loch is unsurprising. Sampling locations in this region are closer to a highly contaminated, more industrialised area than the Pladda and the Solway Firth sites. In the Northern North Sea, fish collected from the Moray Firth (4 haddock pools) are clearly separated from the other two local regions and negatively correlated to the first component (Figure 4.9b).

It has been shown that factors other than the trophic position can also play a role in the biomagnification of PCBs in fish. A study by Burreau *et al.*, (2006) found that biomagnification in fish can also be dependent on the body size (weight), probably due to the slower clearance rate of PCBs in larger individuals. There was no biogeographic regional influence on the ΣPCB_{32} concentration in pooled haddock liver (Irish Sea: 342.7 - 3,065 µg/kg lw; n=5; Northern North Sea: 119.5 – 240.4 µg/kg lw; n=7; Scottish Continental Shelf: 211.5; µg/kg lw; n=1 composed of 5 individuals) ($p > 0.05$). ΣPCB_{32} concentration in sample pools collected from the Irish Sea Biogeographic Region did however have a large degree of variation and when regions were narrowed down further it was found that haddock from the Holy Loch had a higher concentration of ΣPCB_{32} (µg/kg lw) in their liver than those collected from the other locations within the Irish Sea Biogeographic Region (Pladda and Solway Firth), the Scottish Continental Shelf (Burra Haaf) and the Northern North Sea (Montrose Bank, Moray Firth and the Outer Firth of Forth) (Figure 4.10). This agrees with the findings by Webster *et al.,* (2005) where fish from the Holy Loch were significantly more contaminated than those from other sites in the Clyde (Skelmorlie, Hunterston and Irvine Bay). When the physiological variables of trophic level, age and size were considered, none of these variables were found to significantly influence ΣPCB_{32} concentration or congener proportion in haddock ($p>0.05$).

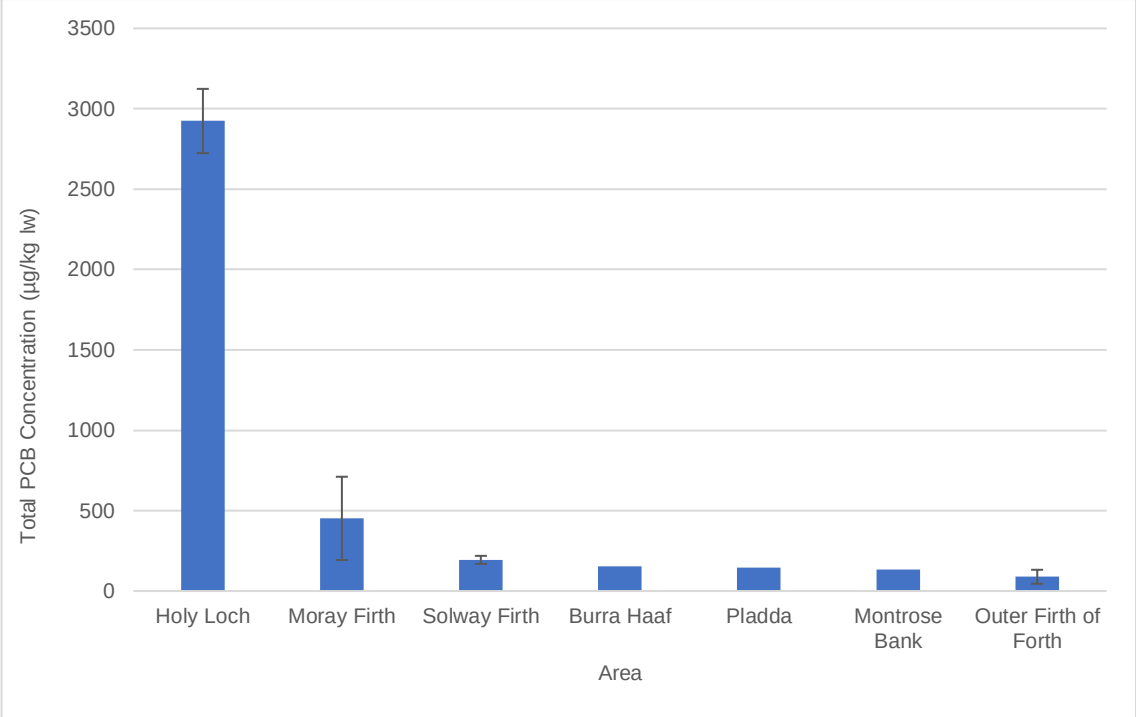


Figure 4.10: ΣPCB_{32} concentration in pooled haddock from the Holy Loch (n=2), Moray Firth (n=4), Solway Firth (n=2), Burra Haaf (n=1 composed of 5 individuals), Pladda (n=1 composed of 6 individuals), Montrose Bank (n=1 composed of 5 individuals) and the Outer Firth of Forth (n=2). Error bars are to one standard deviation. There was only one sample pool analysed from Burra Haaf, Pladda and Montrose Bank because of limited sample size.

Whiting sample pools collected from the Irish Sea had a significantly higher ΣPCB_{32} concentration in their liver (594.5 – 1,750 µg/kg lw; n=8) than those collected from the Northern North Sea (329.1 and 389.2 µg/kg lw; n=2) and Scottish Continental Shelf (82.4 – 399.6 µg/kg lw; n=5) ($p < 0.05$, ANOVA, Tukey). A larger sample number is however required for a comprehensive analysis.

Whiting length ranged from 162.0 – 356.0 mm, weight ranged from 56.60 – 556.3 g, age ranged from 1.4 – 6.6 years and trophic level ranged from 3.65 – 4.65. Pearson's correlation analysis revealed that there was a significant relationship between ΣPCB_{32} concentration and length, age and weight ($p<0.05$) where the larger, older and heavier the fish, the higher the concentration.

When focussing on the ICES-7 PCBs, which will be further assessed for trophic magnification, there are differences in congener proportion between the shark and fish categories (Figure 4.11). Demersal roundfish liver was found to have a regional influence (biogeographical) on congener proportion (Figures 4.8 and 4.9) which was further investigated (Figure 4.12).

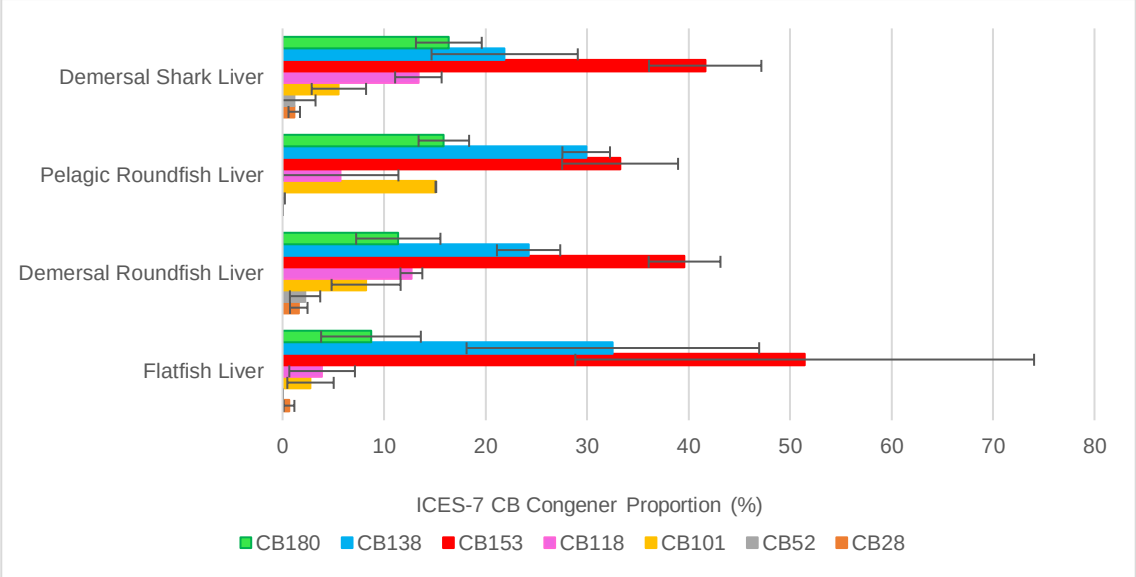


Figure 4.11: The mean contribution of PCBs across the ∑ICES-7 PCBs as measured in demersal shark liver, pelagic roundfish liver, demersal roundfish liver and flatfish liver sample pools, expressed as a percentage of the ΣICES-7 concentration (µg/kg lw). Error bars represent one standard deviation.

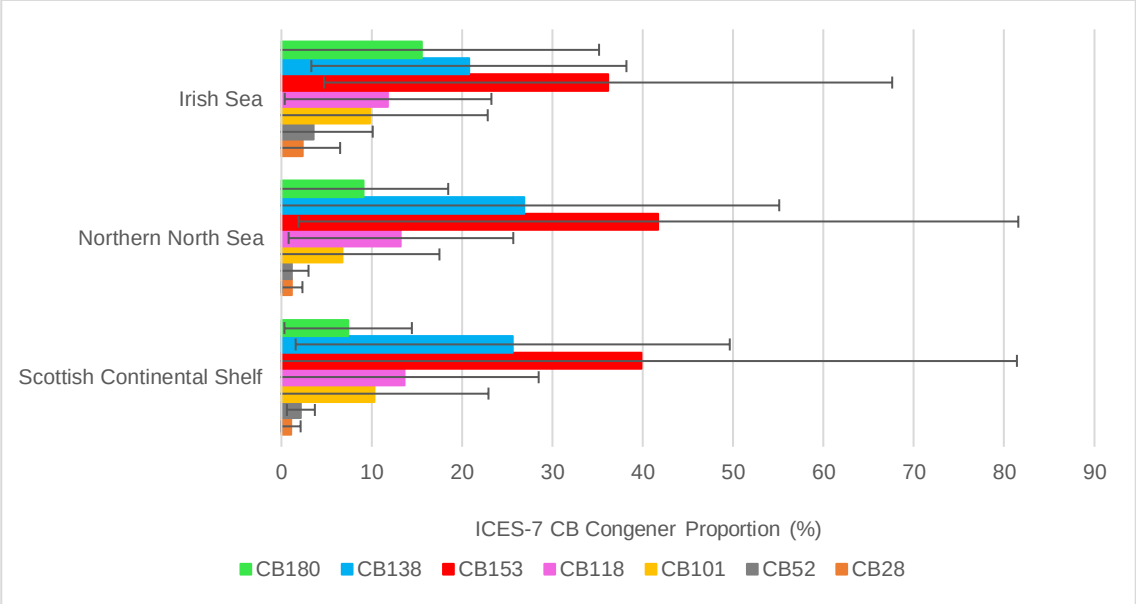


Figure 4.12: The mean contribution of PCBs across the ∑ICES-7 PCBs as measured in demersal shark liver collected from the Irish Sea (n=15), Northern North Sea (n=9) and Scottish Continental Shelf (n=6), expressed as a percentage of the ΣICES-7 concentration (µg/kg lw). Error bars are to one standard deviation.

CB153 and 138 dominate the profile in each of the four categories and shark and fish (demersal and pelagic) have a much higher proportion of the lower chlorinated PCBs in their ICES-7 profile (CB28, 52, 101, 118) than the higher trophic level harbour seal and

harbour porpoise (Figure 4.4). This was expected, as shark and fish are at a lower trophic level than harbour seal and harbour porpoise and have a lower metabolic capacity for metabolising lower chlorinated PCBs, especially CB52, CB101 and CB118 (Elskus *et al*., 1994).

Pelagic roundfish liver have a higher proportion of CB101 in their profile than the other three categories (Figure 4.11), ranging from 13 – 15%, in comparison to 3.1 – 15% in demersal roundfish, 2.8 – 13% in demersal shark and not detected – 8.3% in flatfish. There is a large degree of variation present within the flatfish category for CB153, with a range of not detected – 61%, as opposed to demersal shark (29 – 51%), demersal roundfish (34 – 42%) and pelagic roundfish (28 – 48%). This variation is likely due to the generally low flatfish sample concentrations. A greater proportion of higher chlorinated PCBs is expected in flatfish due to their constant association with sediment, where the higher chlorinated PCBs (with lower solubility) are known to adsorb to suspended particulate matter (Salem, Khaled and Nemr, 2013).

The main congener profile difference between the three sampled regions is that demersal roundfish from the Irish Sea have a marginally higher proportion of CB180 in their liver, ranging from 11 – 20% of the ICES-7 profile, in comparison to 6.8 – 15% in the Northern North Sea and 7.0 – 8.1% in the Scottish Continental Shelf (Figure 4.12). Sites in the Irish Sea (particularly those in the Clyde) are close to industrialised areas and biota inhabiting these sites were found to have the more highly chlorinated, hydrophobic PCBs in their tissues. These PCBs are known to be transported to a lesser extent through the water body and are more likely to accumulate in organic-rich sediments closer to the source.

ICES-7 PCB concentrations in all fish liver samples (Figure 4.13) and fish liver samples originating from the Irish Sea Biogeographic Region (Figure 4.14) were compared to OSPAR EACs. Only the EAC of CB118 is exceeded by demersal roundfish (liver, muscle and whole) and pelagic roundfish (whole) (Figures 4.13 and 4.14). Lyons *et al.,* (2017) previously found CB118 in dab livers to exceed the EAC at 10 sites in the Central North Sea and a study by Webster *et al.,* (2011) using samples collected from the Rockall Trough, to the west of Scotland, found that out of the ICES-7 PCBs, only CB118 in fish liver exceeded the EAC. Fish liver from the Irish Sea do, however, have higher concentrations of the heavier PCBs 138, 153 and 180 (Figure 4.14) compared to fish liver from the Northern North Sea and Scottish Continental Shelf. CB118 is the most toxic congener, capable of exhibiting dioxin-like toxicity.

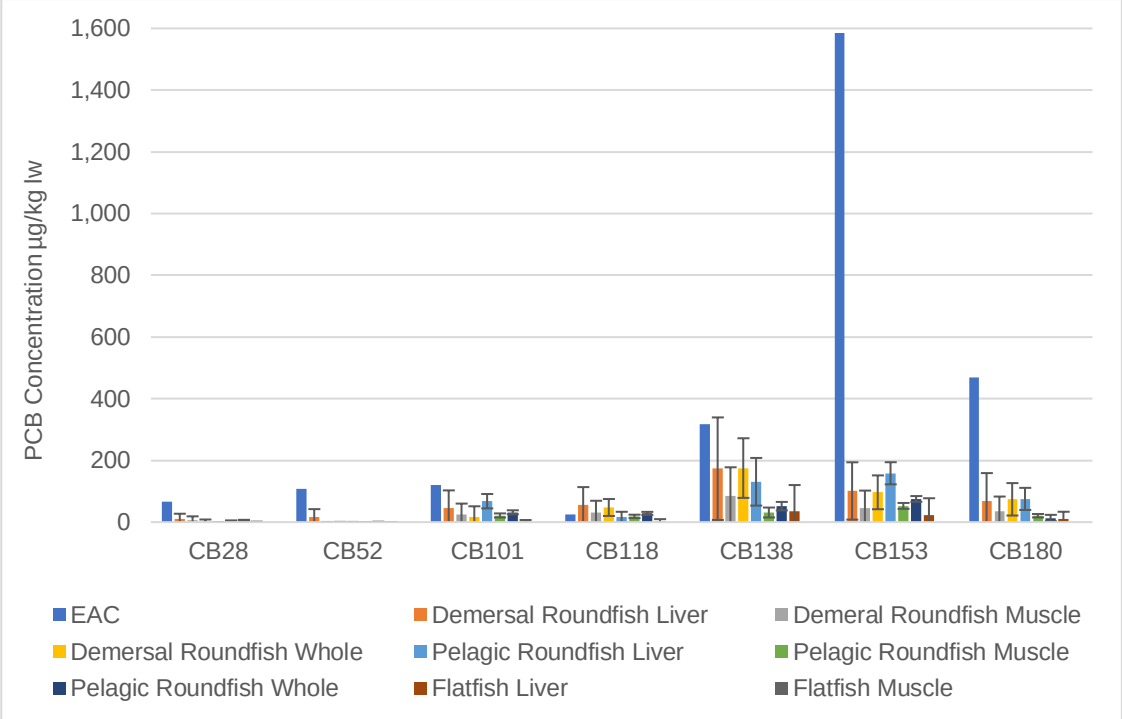


Figure 4.13: The concentrations of each ICES-7 PCB congener in pooled fish tissue (liver, muscle and whole) from all biogeographical regions (Irish Sea, Northern North Sea and Scottish Continental Shelf) in comparison to the Environmental Assessment Criteria (EAC) (µg/kg lw). Error bars represent one standard deviation.

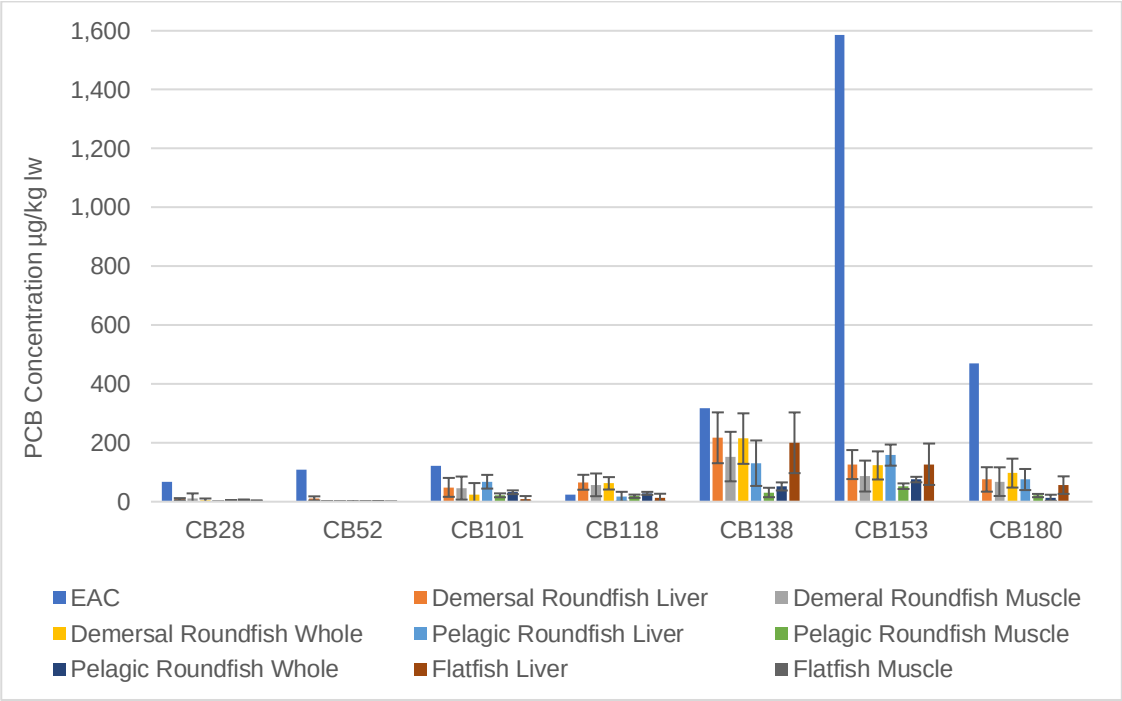


Figure 4.14: The concentrations of each ICES-7 PCB congener in pooled fish tissue (liver, muscle and whole) from the Irish Sea Biogeographic Region in comparison to the Environmental Assessment Criteria (EAC) concentration (µg/kg lw). Error bars represent one standard deviation.

Invertebrates

PCA carried out on PCB congener profiles for the benthic invertebrates categories showed considerable spread across both principal components, with substantial within-group and between-group variation (Figure 4.15b). All PCB congener concentrations in demersal invertebrates (squid) were below the LoD and concentrations of CB114 and 189 were below the LoD for all invertebrate samples and so congener profiles could not be calculated. Demersal invertebrates and CB114 and CB189 were therefore not included in the multivariate analysis.

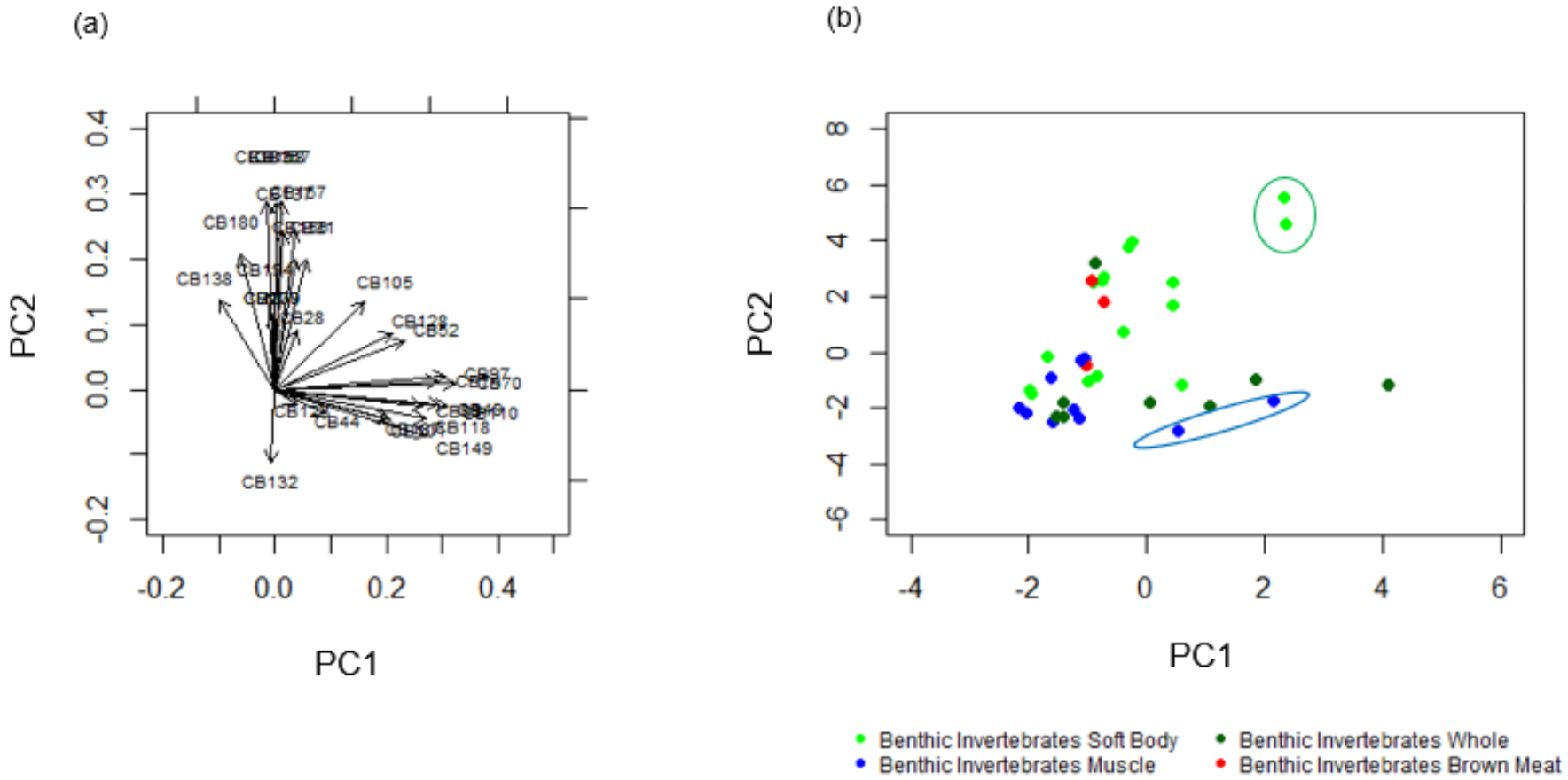


Figure 4.15: (a): PCA loading plot and (b): PCA score plot, both demonstrating variation in the PCB profiles (normalised to the concentration of CB153) across the four benthic invertebrates sample categories. Two shore crab sample pools are circled in green and two *Nephrops* sample pools are circled in dark blue. These are discussed below. CB114 and 189 were not included as they were < LoD in all samples. Similarly, squid are not included because the individual congeners were all < LoD.

The first two principal components of the PCA explained 50% of the variability present in the dataset. All four categories are spread across the first component ranging from -3 to +5 (Figure 4.15b). A similar pattern was found in Chapter 3 for FAs (Figure 3.6), where considerable variation for the benthic invertebrates whole, muscle and soft body FA profiles suggested highly variable feeding patterns.

PCA was conducted on species (Figure 4.16a and b) and biogeographic region (Figure 4.17a and b) to determine whether these factors contribute to the observed variation (Figure 4.14b).

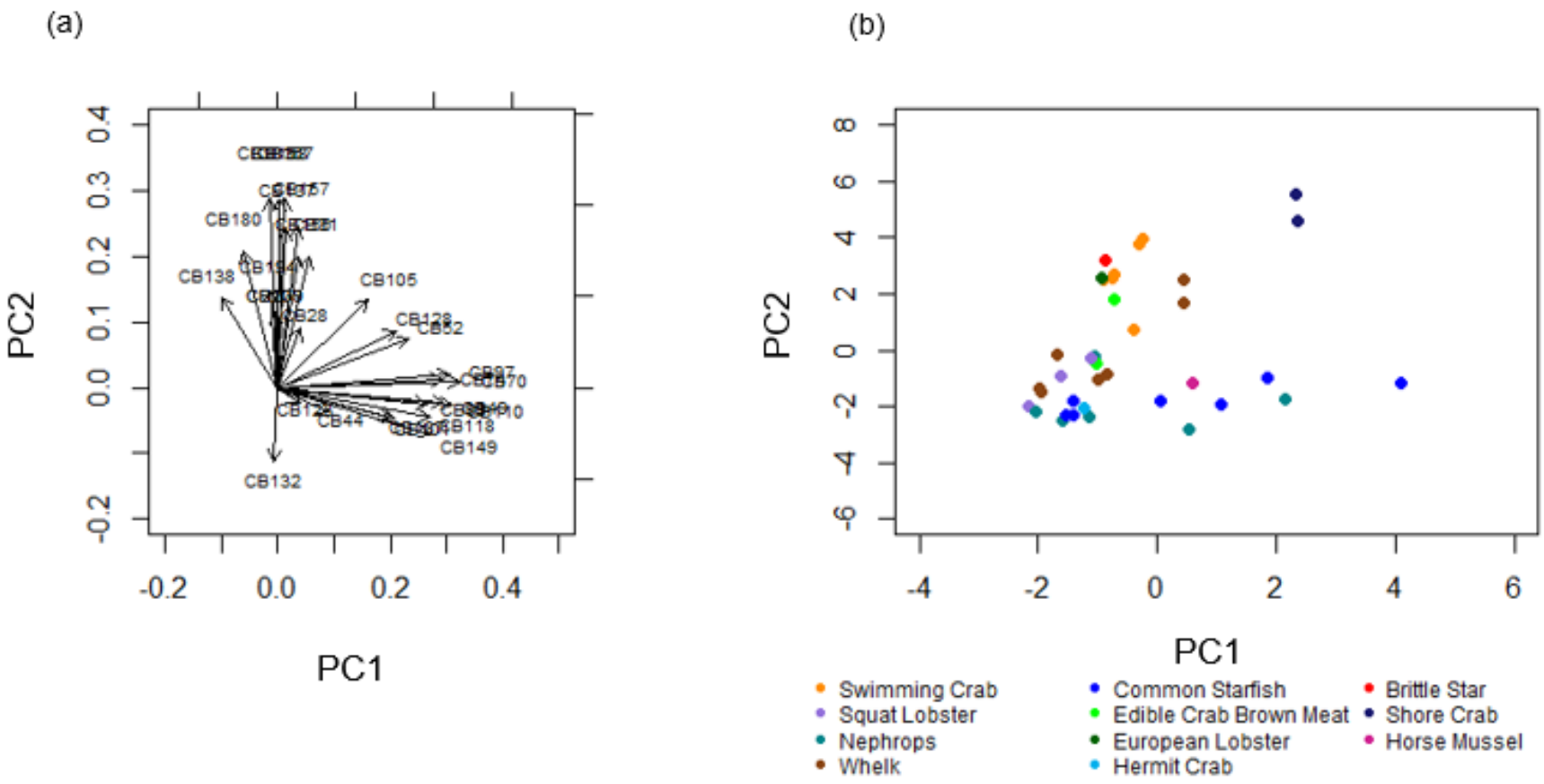


Figure 4.16: (a): PCA loading plot and (b): PCA score plot, both demonstrating variation in the PCB profiles (normalised to the concentration of CB153) across the eleven benthic invertebrates species.

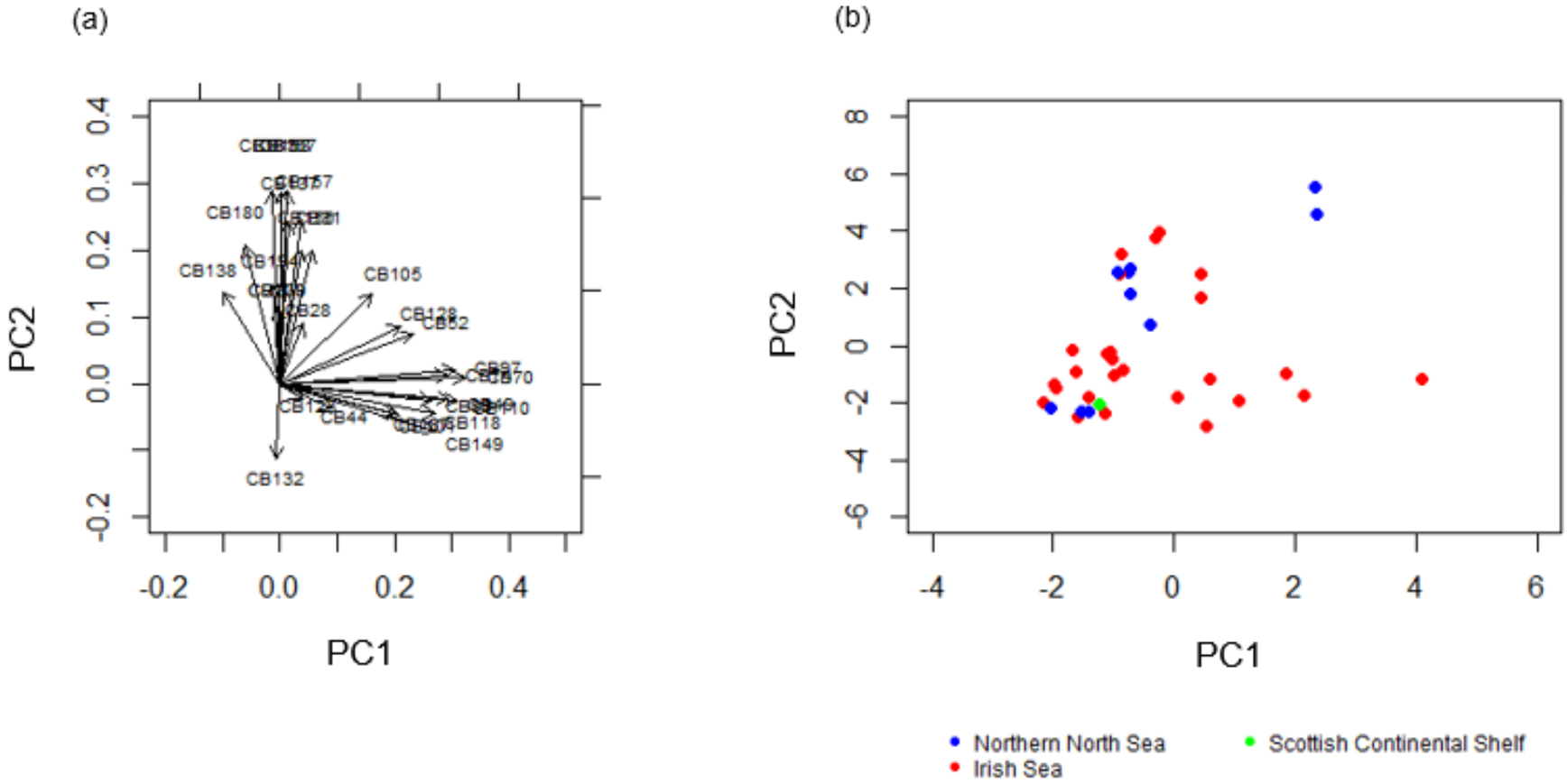


Figure 4.17: (a): PCA loading plot and (b): PCA score plot, both demonstrating variation in the PCB profiles (normalised to the concentration of CB153) across the three benthic invertebrates biogeographic sampling locations.

The benthic invertebrates whole samples negatively correlated to the second component and pooled common starfish (dark blue on Figure 4.16b) are spread across the first component (from -2 to +3). Starfish are positively correlated to the first component, having higher proportions of hexa-chlorinated congeners (CB138, CB149). Four out of the nine starfish sample pools are separated on the first component. Figure 4.17 shows that these samples were collected from the Irish Sea (Biogeographic Region), consisting of the only two pools collected from Holy Loch (furthest from the cluster), one from Hunterston and one from the Solway Firth, and two sample pools collected from Pladda. The three sample pools in the main cluster were collected from the Moray Firth in the Northern North Sea. This suggests that there is a localised regional influence on congener proportion in the benthic invertebrates whole category which has the potential of influencing the calculated TMF on a regional basis.

As well as the observed variability in PCB congener profiles, starfish have the largest degree of variation in their ΣPCB_{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 shown to be valuable indicators of contamination since they accumulate PCBs as a function of the contamination level of the environment (Knickmeyer, Landgraff and Steinhart, 1992; Schweitzer, Bay and Suffet, 2000). 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 trophic level values (3.97 and 4.13) were collected from Holy Loch and were not significantly larger in size or weight to the other sample pools ($p>0.05$).

Benthic invertebrates soft body PCB congener patterns are also highly variable (Figure 4.15). The two benthic invertebrates soft body sample pools which are more positively correlated to the first and second components (circled in green, Figure 4.15) were identified as shore crab, containing a higher proportion of hepta-chlorinated congeners (CB187, CB183) than the other invertebrate species, possibly due to being collected from Tancred Bank in the Northern North Sea close to a highly industrialised area (Chapter 2; Figure 2.1). In Chapter 3, all contributing species to the benthic invertebrates soft body category could be separated on Figure 3.6 due to their differing FA profiles. Samples collected from the Northern North Sea and Irish Sea are highly dispersed across both components of the PCA score plot (Figure 4.17), suggesting more of a

species influence on congener proportion in the benthic invertebrates soft body category than geographical variation.

Two *Nephrops* sample pools (circled in dark blue on Figure 4.15) were separated from the other benthic invertebrate muscle sample pools (including the other four *Nephrops* sample pools). These two sample pools contain a higher proportion of penta-chlorinated congeners (CB101, 99, 110, 118) and hexa-chlorinated congeners (CB149, 132). Although six out of the seven *Nephrops* sample pools were collected from the Irish Sea Biogeographic Region. As observed in other species, the two separated pools were collected from Holy Loch, further suggesting a localised regional influence on PCB congener proportion but not ΣPCB_{32} concentration ($p>0.05$).

In this study, both fish and invertebrates from Holy Loch contain significantly higher PCB concentrations and greater proportions of the higher chlorinated congeners. Between 1961 and 1992, Holy Loch was used to refit US nuclear-powered submarines (Edwards, 1997) and was home to up to ten submarines, a floating dry dock and a depot ship. Before clean-up, a quarter of the surface area of the floor of the loch was covered in waste, resulting in 130,000 cubic metres of dangerous debris. The Ministry of Defence (MoD) employed Environmental Resources Management (ERM) to carry out an environmental survey of the Holy Loch sediments which found elevated PCB concentrations (15 congeners) of up to 864 µg/kg dw (ERM, 1997). Another study by Miller, Pirie and Redshaw, (2000) found the ∑ICES-7 concentration (µg/kg dw) in mussels collected before and after the initial phase of the debris removal operation showed little change.

ΣPCB_{32} concentrations (µg/kg lw) detected in the majority of invertebrates collected from the Holy Loch are higher than those detected in samples from other regions (Figure 4.18) in agreement with previous findings (Webster *et al*., 2014a). Within the Firth of Clyde, PCB concentrations were significantly higher in the Holy Loch than in other sampling locations.

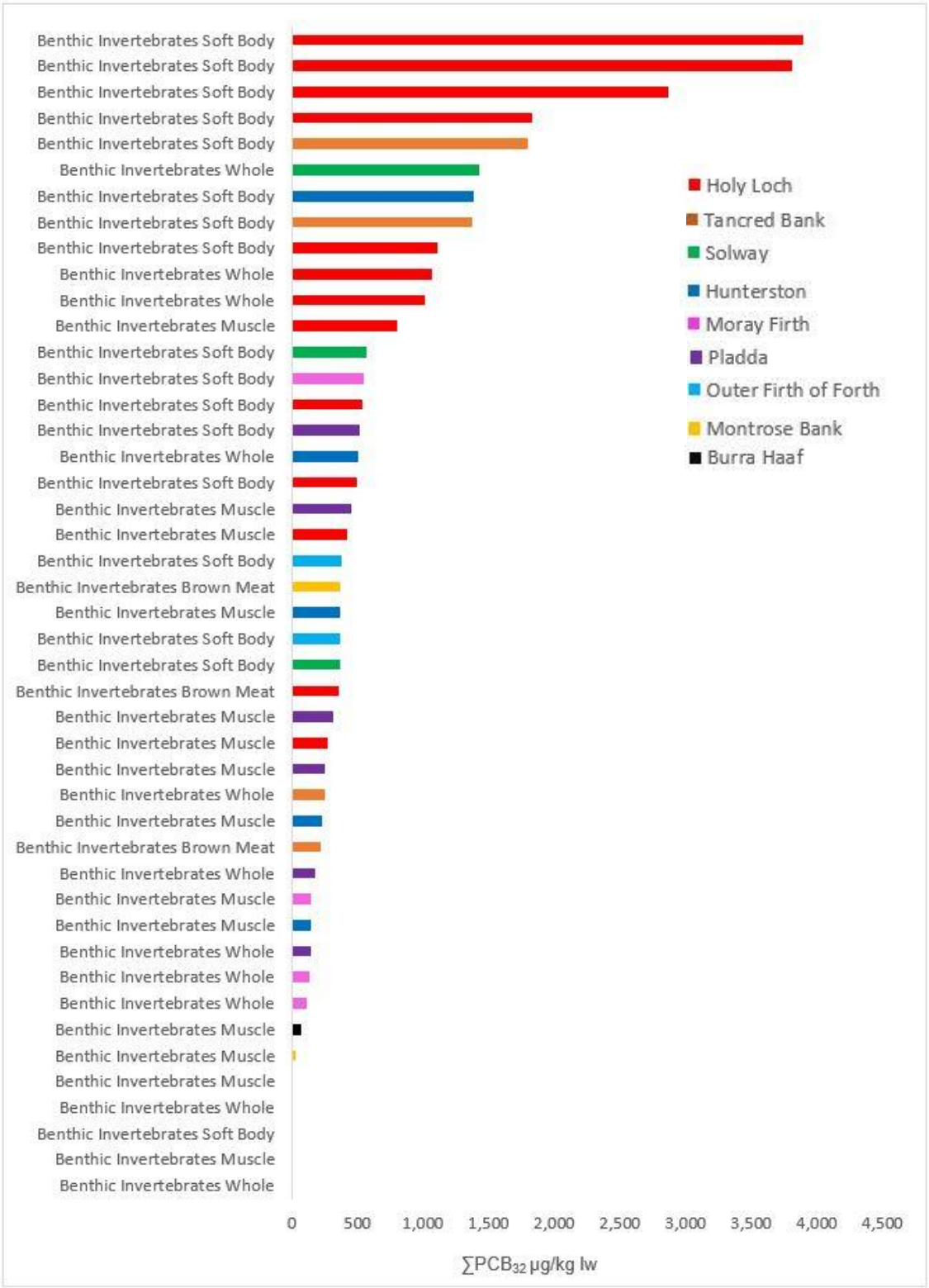


Figure 4.18: ΣPCB_{32} concentration (µg/kg lw) in each benthic invertebrate sample pool collected from nine locations around Scotland: Holy loch, Tancred Bank, Solway, Hunterston, Moray Firth, Pladda, Outer Firth of Forth, Montrose Bank and Burra Haaf (Figure 2.1). The last five samples on the Figure have a ΣPCB_{32} <LoD.

When focussing on the ICES-7 PCBs (Figure 4.19), there is considerable between- and within-category variation. The prevalent and recalcitrant CB153 and 138 dominate the profile of benthic invertebrates soft body, whole and brown meat.

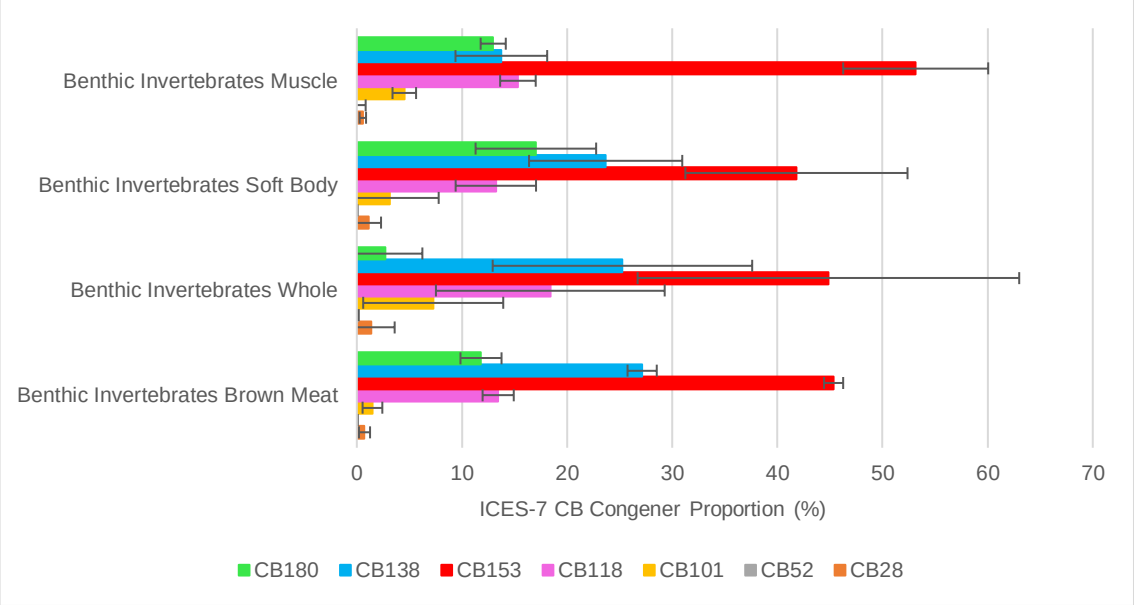


Figure 4.19: The mean proportion across the ICES-7 PCBs in benthic invertebrates muscle, benthic invertebrates whole, benthic invertebrates brown meat and benthic invertebrates soft body sample pools, expressed as a percentage of the ΣICES-7 concentration (µg/kg lw). Error bars are one standard deviation.

In order to understand TMFs and prove whether the main driver of bioaccumulation is trophic level, the cause of variability within sample categories must be established to determine the reliability of the calculated TMF. The findings from this section have identified inter- and intra- species variation in the marine mammals, shark and fish and invertebrates categories which will contribute to the variation in the calculated TMFs. ΣPCB_{32} concentration and congener proportion in marine mammals was influenced by trophic level, the metabolic capacity and feeding ecology of the species sampled, whereas in shark and fish, ΣPCB_{32} concentration and congener proportions were more influenced by species specific feeding ecology, sampling location, trophic level and physiological features such as length, age and weight in demersal species. ΣPCB_{32} concentration and congener proportion in benthic invertebrates was influenced by species-specific feeding ecology and sampling location. Such variables must be considered in the calculation of TMFs.

4.3.2.2 Trophic magnification of PCBs

Trophic magnification was investigated using the ICES-7 PCBs in marine mammal blubber, shark and fish (demersal roundfish, pelagic roundfish and flatfish) liver and benthic invertebrates (whole, muscle, soft body, brown meat). They have been selected as being representative of the range of PCB congeners detected in environmental matrices, and represent a range of physico-chemical properties, prevalence and metabolic stability. They are included in most, if not all, PCB monitoring programmes.

TMFs for individual congeners were calculated according to Borgå *et al.,* (2012), using the slope of logarithmically transformed (to base 10) concentrations of POPs versus trophic levels of organisms in the food web (Figures 4.20 – 4.23 and Figures A.1 – A.26). This is the traditional method of calculating the TMF of PCBs and PBDEs, used in studies worldwide (House *et al*., 2008; Walters *et al*., 2016; Romero-Romero *et al.,* 2017). Concentrations were normalised to the lipid content, which is widely recommended and practiced for assessing trophic magnification of PCBs (Borgå *et al.*, 2012; Burkhard *et al.*, 2013; OSPAR, 2016b). The Scottish marine food web diagram, that was developed from SI ratios (Chapter 3 of this thesis), provided the basis for the trophic level assessment which was then summarised by category (Table 3.7).

Considering the global diversity of habitats and species distribution, target species selection for the calculation of TMFs are not fixed. Lower trophic level organisms (zooplankton and benthic invertebrates) do, however, feed over a smaller area, and top predators can act as ecological integrators by consuming prey over relatively large geographical areas (McCann *et al.,* 2005). Migration and spatial heterogeneity in contaminant concentrations have been shown to be important factors influencing the magnitude and variation of TMFs (Kim *et al.,* 2016).

A recent paper by Kidd *et al.*, (2019) provided practical guidance for selecting or determining TMFs, stating that in order to have a higher level of confidence in the TMF values and their applicability, there must be:

- Inclusion of several lower-trophic-level taxa (several different benthic invertebrate families).
- Reasonable balance with respect to sample numbers of lower versus higher trophic level organisms.
- Measurements should be on organisms that are known to be linked by diet through the food web (FA and SI analysis).

- Lipid normalisation must be undertaken for organic contaminants to remove the effect of lipid content on PCB accumulation to allow the identification of trophic magnification itself.
- Data should only be analysed for TMFs on contaminant concentrations above the detection limit.
- Biota must be from the same food web and there must be a sufficient trophic level range.

In this project, FA analysis was used to determine the feeding patterns and SI analysis ($\delta^{15}N$) used to determine the trophic level. Thirteen lower trophic level invertebrate species were included and a sufficient trophic level range (four trophic levels) was utilised. All contaminant concentrations have been lipid normalised and samples with a concentration below the LoD have not been included in the calculation of TMFs. The only criteria difficult to achieve due to the opportunistic nature of sampling was the reasonable balance with respect to sample numbers of lower versus higher trophic level organisms. As a result, a similar number of samples for each trophic level (and all areas) could not be achieved.

An alternative method of TMF calculation known as the 'balanced method' is based on a regression of geometric mean concentrations and trophic levels, rather than concentrations and trophic levels of each individual organism. This methodology was explored and compared to the traditional method (Figures 4.20 – 4.23 and Figures A.1 – A.26) (Brisebois, 2013). Calculating the geometric mean reduces the influence of unbalanced sampling, i.e., a larger number of samples at certain trophic levels. To the best of this author's knowledge, there has only been one previous comparison between the two methods for PCB and PBDE TMF calculation (Brisebois, 2013).

As the true regional placement of marine mammals (samples were collected from strandings) is not known, TMFs were calculated using the traditional and balanced methods on the food webs that either included or excluded marine mammals. 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 during FA and SI analysis, where sperm whale had a significantly different FA profile (Figure 3.4), $\delta^{15}N$ and $\delta^{13}C$ (Figure 3.7) than harbour seal and harbour porpoise, supporting a different feeding location/diet. Although sperm whales are part of the Scottish marine food web, 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. Although the true regional placement of harbour seal and harbour porpoise samples obtained from strandings is not known, these species 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*., 2020).

Due to the regional influence identified on ΣPCB_{32} concentration and congener proportions in fish and invertebrates, sample pools collected from the Irish Sea (e.g. Figure 4.22) and sample pools collected from the Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf (e.g. Figure 4.23) were generated to investigate the effect on TMF in a localised, more contaminated region. Figures 4.20 – 4.23 show the plots for calculating the TMF of CB180 as an example. The plots for CB153, 138, 118, 101, 52 and 28 are provided in the Appendix (Figure A.1 to A.26). The regression summary for the determination of TMF using both the traditional method (Borgå *et al*., 2012; OSPAR, 2016b) and balanced method (Brisebois, 2013) is shown in Table 4.4 and calculated TMFs shown in Tables 4.5 and 4.6.

CB180

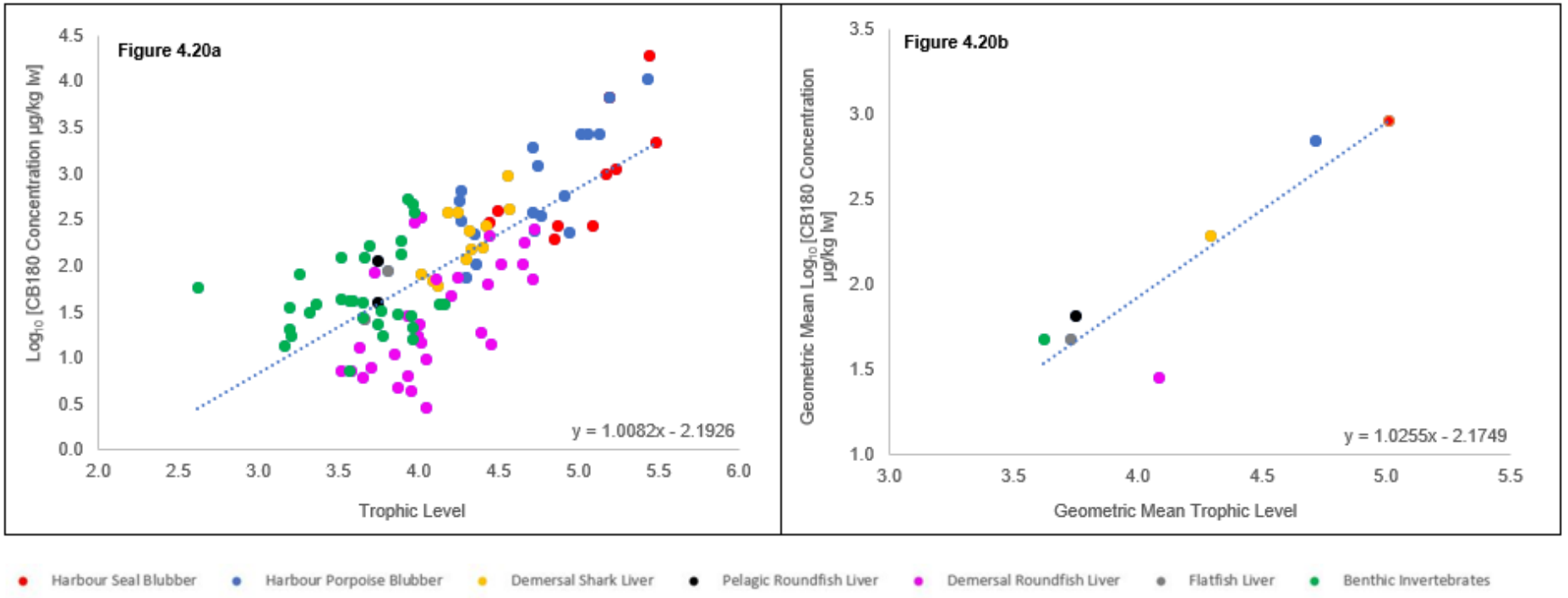


Figure 4.20: (a) Relationship between trophic level and logarithmically transformed CB180 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, 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

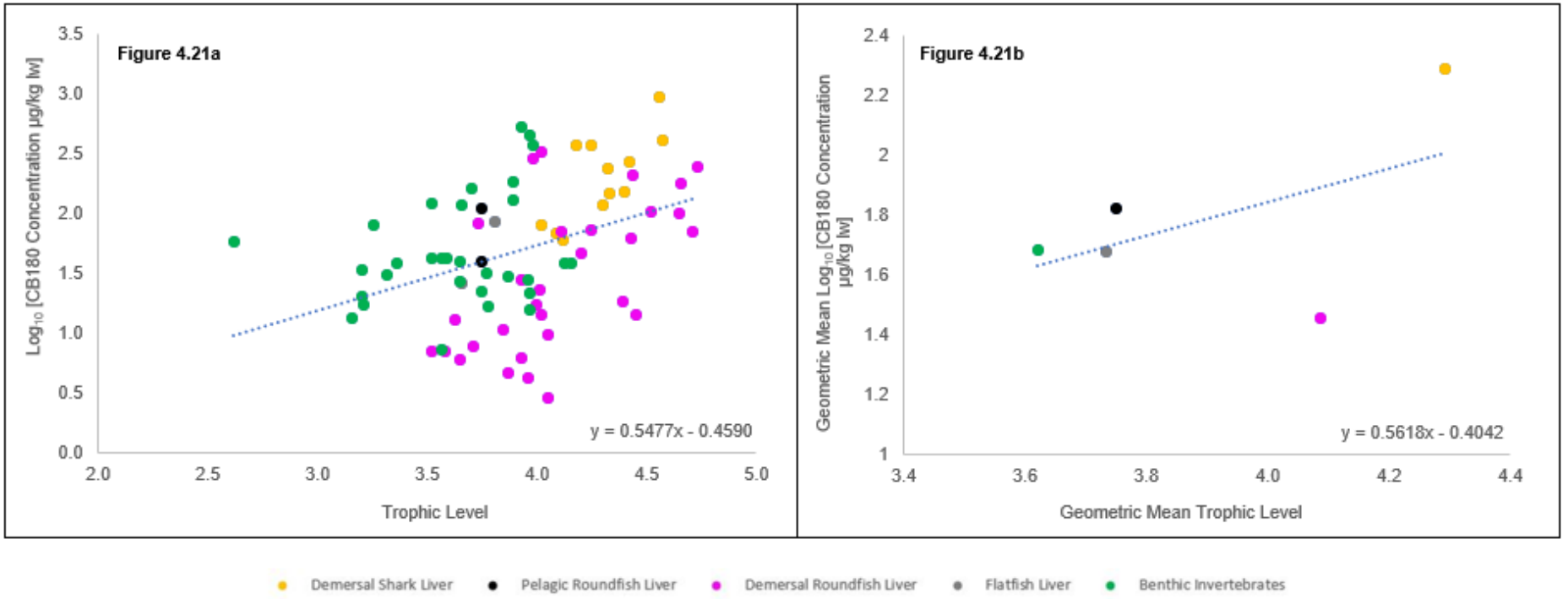


Figure 4.21: (a) Relationship between trophic level and logarithmically transformed CB180 concentration (µg/kg lw) in demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, muscle, brown meat, soft body (green) from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf. (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean CB180 concentration (µg/kg lw) in demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, muscle, brown meat, soft body) from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.

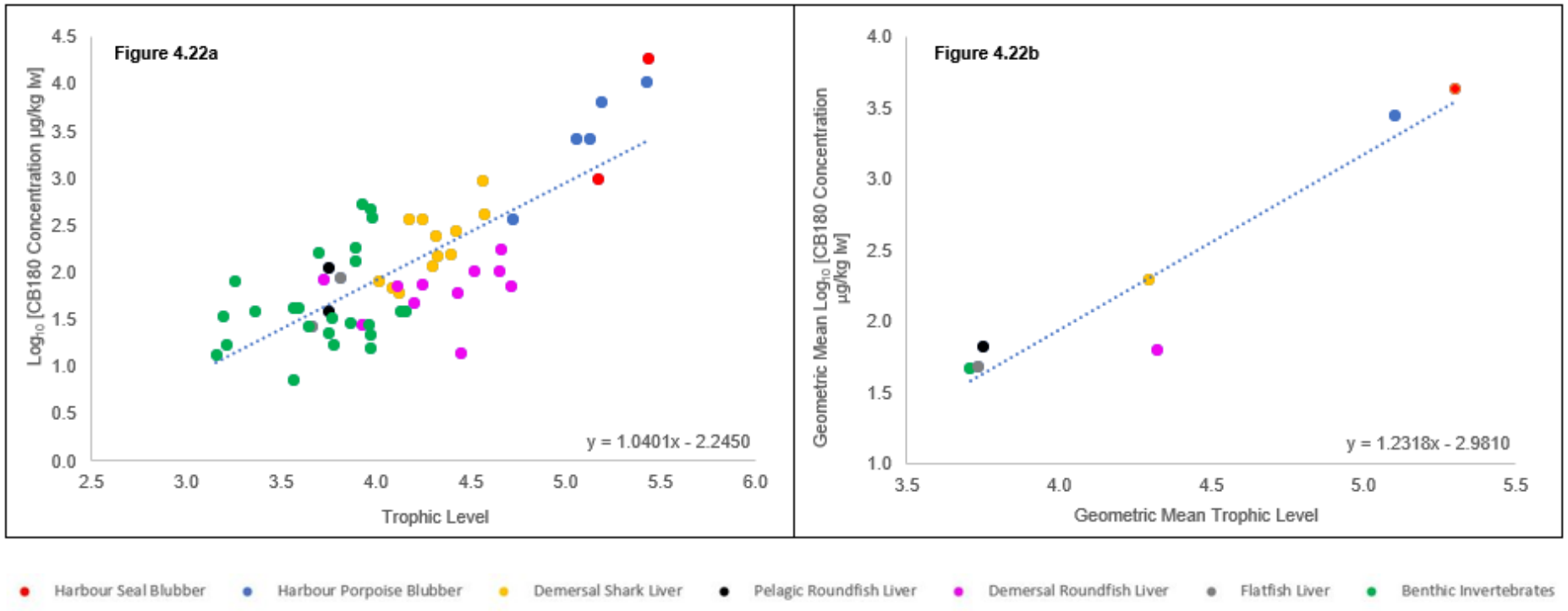


Figure 4.22: (a) Relationship between trophic level and logarithmically transformed CB180 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, 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.

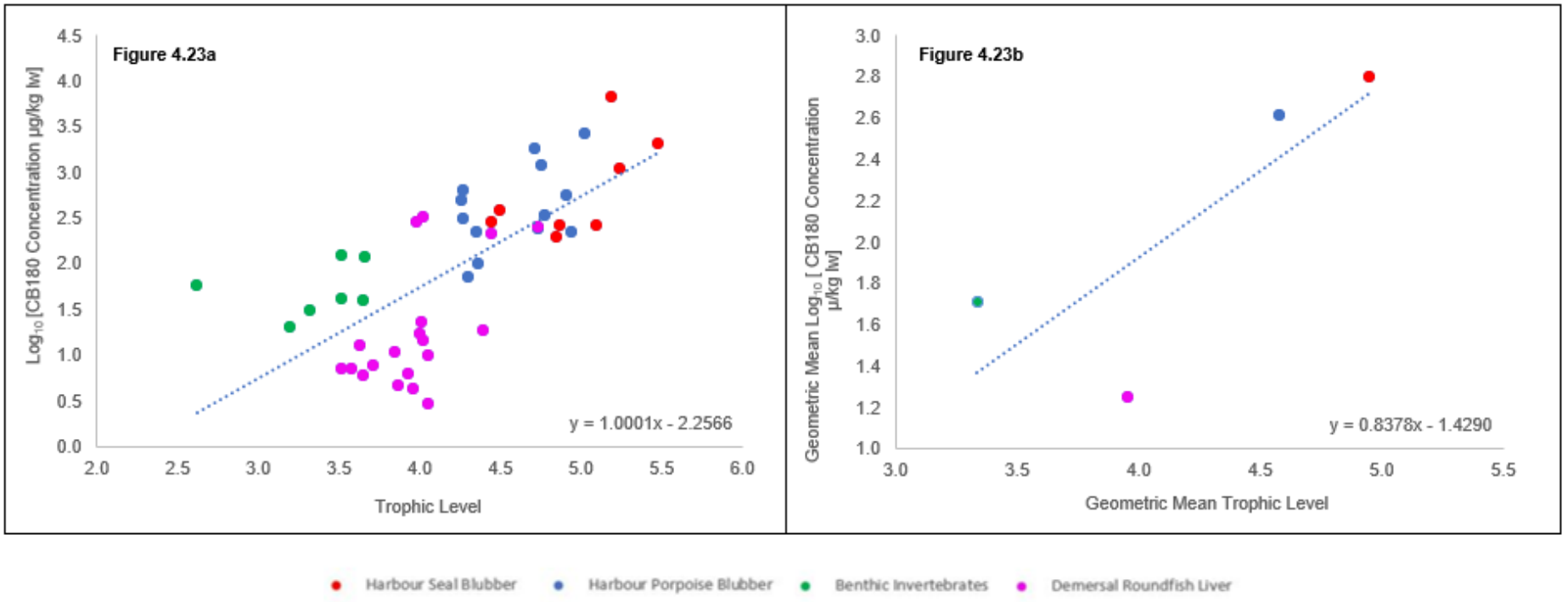


Figure 4.23: (a) Relationship between trophic level and logarithmically transformed CB180 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal roundfish liver and benthic invertebrate whole, muscle, brown meat, soft body (green) from the Northern North Sea, 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.

Table 4.4: Regression summary for the determination of TMF using both the traditional method (Borgå *et al.,* 2012; OSPAR, 2016b) and balanced method (Brisebois, 2013). For the traditional method, y = Log_{10} [CB Concentration µg/kg lw] and x = trophic level; whilst for the balanced method, y = Geometric mean Log_{10} [CB Concentration µg/kg lw] and x = Geometric mean trophic level.

PCB	Location	Regression Equation (p-value)		Sample categories
		Traditional Method	Balanced Method	
CB180	All trophic levels from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 1.0082x - 2.1926 (p<0.05)	y = 1.0255x - 2.1749 (p<0.05)	harbour seal blubber, harbour porpoise blubber, demersal shark liver, fish liver: demersal, flatfish, pelagic and benthic invertebrate whole, muscle, brown meat, soft body
	Shark, fish and invertebrates from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 0.5477x - 0.4590 (p<0.05)	y = 0.5618x - 0.4042 (p<0.05)	demersal shark liver, fish liver: demersal, flatfish, pelagic and benthic invertebrate whole, muscle, brown meat, soft body
	All trophic levels from the Irish Sea Biogeographic Region.	y = 1.0401x - 2.2450 (p<0.05)	y = 1.2318x - 2.9810 (p<0.05)	harbour seal blubber, harbour porpoise blubber, demersal shark liver, fish liver: demersal, flatfish, pelagic and benthic invertebrate whole, muscle, brown meat, soft body
	All trophic levels from the Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 1.0001x - 2.2566 (p<0.05)	y = 0.8378x - 1.4290 (p<0.05)	harbour seal blubber, harbour porpoise blubber, demersal roundfish liver and benthic invertebrate whole, muscle, brown meat, soft body
CB153	All trophic levels from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 0.9583x – 1.4724 (p<0.05)	y = 1.0744x – 1.8962 (p<0.05)	harbour seal blubber, harbour porpoise blubber, demersal shark liver, fish liver: demersal, flatfish, pelagic and benthic invertebrate whole, muscle, brown meat, soft body
	Shark, fish and invertebrates from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 0.5266x + 0.1036 (p<0.05)	y = 0.6526x – 0.3295 (p<0.05)	demersal shark liver, fish liver: demersal, flatfish, pelagic and benthic invertebrate whole, muscle, brown meat, soft body
	All trophic levels from the Irish Sea Biogeographic Region.	y = 0.9068x – 1.2074 (p<0.05)	y = 1.2618x – 2.7320 (p<0.05)	harbour seal blubber, harbour porpoise blubber, demersal shark liver, fish liver: demersal, flatfish, pelagic and benthic invertebrate whole, muscle, brown meat, soft body
	All trophic levels from the Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 1.0172 – 1.7783 (p<0.05)	y = 1.1627 – 2.2244 (p<0.05)	harbour seal blubber, harbour porpoise blubber, demersal roundfish liver and benthic invertebrate whole, muscle, brown meat, soft body

CB138	All trophic levels from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 0.9150x – 1.5211 (p<0.05)	y = 0.9624x – 1.6500 (p<0.05)	harbour seal blubber, harbour porpoise blubber, demersal shark liver, fish liver: demersal, flatfish, pelagic and benthic invertebrate whole, muscle, brown meat, soft body
	Shark, fish and invertebrates from the Irish Sea, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 0.4886x + 0.0278 (p<0.05)	y = 0.4943x + 0.0801 (p<0.05)	demersal shark liver, fish liver: demersal, flatfish, pelagic and benthic invertebrate whole, muscle, brown meat, soft body
	All trophic levels from the Irish Sea Biogeographic Region.	y = 0.8625x – 1.2635 (p<0.05)	y = 1.0936x – 2.1419 (p<0.05)	harbour seal blubber, harbour porpoise blubber, demersal shark liver, fish liver: demersal, flatfish, pelagic and benthic invertebrate whole, muscle, brown meat, soft body
	All trophic levels from the Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 9710x – 1.7995 (p<0.05)	y = 0.9940x – 1.8344 (p<0.05)	harbour seal blubber, harbour porpoise blubber, demersal roundfish liver and benthic invertebrate whole, muscle, brown meat, soft body
CB118	All trophic levels from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 0.4133x + 0.0545 (p<0.05)	y = 0.2717x + 0.6019 (p<0.05)	harbour seal blubber, harbour porpoise blubber, demersal shark liver, fish liver: demersal, flatfish, pelagic and benthic invertebrate whole, muscle, brown meat, soft body
	Shark, fish and invertebrates from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 0.5105x – 0.3063 (p<0.05)	y = 6674x – 0.9199 (p<0.05)	demersal shark liver, fish liver: demersal, flatfish, pelagic and benthic invertebrate whole, muscle, brown meat, soft body
	All trophic levels from the Irish Sea Biogeographic Region.	y = 0.5055x – 0.1705 (p<0.05)	y = 0.4400x – 0.0045 (p<0.05)	harbour seal blubber, harbour porpoise blubber, demersal shark liver, fish liver: demersal, flatfish, pelagic and benthic invertebrate whole, muscle, brown meat, soft body
	All trophic levels from the Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 0.3410x + 0.1819 (p<0.05)	y = 0.1821 + 0.8344 (p>0.05)	harbour seal blubber, harbour porpoise blubber, demersal roundfish liver and benthic invertebrate whole, muscle, brown meat, soft body
CB101	All trophic levels from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 0.6799x – 1.3205 (p<0.05)	y = 0.4018x – 0.0701 (p<0.05)	harbour seal blubber, harbour porpoise blubber, demersal shark liver, fish liver: demersal, flatfish, pelagic and benthic invertebrate whole, muscle, brown meat, soft body
	Shark, fish and invertebrates from the Irish Sea Biogeographic region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 0.7637x – 1.6527 (p<0.05)	y = 0.4028x – 0.0877 (p>0.05)	demersal shark liver, fish liver: demersal, flatfish, pelagic and benthic invertebrate whole, muscle, brown meat, soft body

	All trophic levels from the Irish Sea Biogeographic Region.	y = 0.6821x – 1.2050 (p<0.05)	y = 6389x – 0.9514 (p<0.05)	harbour seal blubber, harbour porpoise blubber, demersal shark liver, fish liver: demersal, flatfish, pelagic and benthic invertebrate whole, muscle, brown meat, soft body
	All trophic levels from the Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 0.7040x – 1.5590 (p<0.05)	0.5124x – 0.7445 (p<0.05)	harbour seal blubber, harbour porpoise blubber, demersal roundfish liver and benthic invertebrate whole, muscle, brown meat, soft body
CB52	All trophic levels from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 1.4219x – 5.0927 (p<0.05)	y = 1.6471x – 6.1983 (p<0.05)	harbour seal blubber, harbour porpoise blubber, demersal shark liver, fish liver: demersal, flatfish, pelagic and benthic invertebrate whole, muscle, brown meat, soft body
	Shark, fish and invertebrates from the Irish Sea Biogeographic region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 1.3010x – 4.7464 (p<0.05)	y = 1.9678x – 7.5062 (p<0.05)	demersal shark liver, fish liver: demersal, flatfish, pelagic and benthic invertebrate whole, muscle, brown meat, soft body
	All trophic levels from the Irish Sea Biogeographic Region.	y = 1.7901x – 6.8347 (p<0.05)	y = 1.8395x – 7.0780 (p<0.05)	harbour seal blubber, harbour porpoise blubber, demersal shark liver, fish liver: demersal, flatfish, pelagic and benthic invertebrate whole, muscle, brown meat, soft body
	All trophic levels from the Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 1.1016x – 3.5735 (p<0.05)	y = 1.2633x – 4.4003 (p<0.05)	harbour seal blubber, harbour porpoise blubber, demersal roundfish liver and benthic invertebrate whole, muscle, brown meat, soft body
CB28	All trophic levels from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 0.1115x + 0.3028 (p>0.05)	y = 0.1577x + 1.4251 (p>0.05)	harbour seal blubber, harbour porpoise blubber, demersal shark liver, fish liver: demersal, flatfish, pelagic and benthic invertebrate whole, muscle, brown meat, soft body
	Shark, fish and invertebrates from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 0.3400x – 0.5242 (p<0.05)	y = 0.0981x + 0.4585 (p>0.05)	demersal shark liver, fish liver: demersal, flatfish, pelagic and benthic invertebrate whole, muscle, brown meat, soft body
	All trophic levels from the Irish Sea Biogeographic Region.	y = 0.0609x + 0.7238 (p>0.05)	y = 0.0110x + 0.8776 (p>0.05)	harbour seal blubber, harbour porpoise blubber, demersal shark liver, fish liver: demersal, flatfish, pelagic and benthic invertebrate whole, muscle, brown meat, soft body
	All trophic levels from the Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	0.0266x + 0.4600 (p>0.05)	y = -0.2410x + 1.6260 (p>0.05)	harbour seal blubber, harbour porpoise blubber, demersal roundfish liver and benthic invertebrate whole, muscle, brown meat, soft body

Table 4.5: Calculated TMFs in a food web composed of marine mammals, shark, fish and invertebrates and a food web composed of shark, fish and invertebrates using the traditional and balanced methods. TMFs reported in Brisebois (2013) are also presented for a comparison.

	Traditional Method		Balanced Method		Brisebois, (2013)	
ICES-7 CBs	**Marine mammals, shark, fish, invertebrates**	**Shark, fish, invertebrates**	**Marine mammals, shark, fish, invertebrates**	**Shark, fish, invertebrates**	**Traditional Method**	**Balanced Method**
CB180	10	3.5	11	3.6	1.9	1.7
CB153	9.1	3.4	12	4.5	2.6	2.0
CB138	8.9	9.4	9.2	9.9	1.8	1.8
CB118	2.6	3.2	2.8	4.6	1.9	1.7
CB101	4.8	5.8	2.5	2.5	1.5	1.5
CB52	26	20	44	18	1.1	1.4
CB28	1.3	2.2	0.7	0.8	1.1	0.2

Table 4.6: 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	
ICES-7 CBs	**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

CB180, 153, 138, 118, 101 and 52 were found to biomagnify in all four scenarios using the traditional and balanced methods (Tables 4.5 and 4.6). The TMF including all trophic levels from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf was higher for CB180, 153 and 52 than when upper trophic level marine mammals are not included in the analysis using both methods (Table 4.5). Higher chlorinated PCBs are not metabolised by certain organisms and have longer half-lives, making them persistent to biodegradation (Wenaty *et al.,* 2019), resulting in a high degree of trophic magnification. For example, a study by Boon *et al*., (1997) reviewed the types of metabolic behaviour of several PCB congeners in five species of mammals and found that CB180 is highly resistant to biotransformation and consequently difficult to metabolise and Méndez-Fernandez *et al*., (2016) found a

positive relationship between $\delta^{15}N$ and concentration (µg/kg lw) for CB180 in harbour porpoises and bottlenose dolphins, suggesting the biomagnification of CB180 in the food web.

CB52 had the highest TMF value in all four scenarios using both methods (Tables 4.5 and 4.6). Variations in cytochrome P450 enzyme (CYPs) distribution and function between animal groups could result in differential metabolism for certain contaminants. Studies have found that differential CYP patterns have contributed to differences in PCB accumulation profiles between species (Koenig, Fernández and Solé, 2012). Ortho-substituted PCBs (such as CB52) are preferentially metabolised by CYP2B isoenzymes. CB52 is slightly harder to metabolise than the other congeners (Boon *et al*., 1992; Boon *et al*., 1997) and due to the differential expression of the CYP2B enzyme between species, harbour seal and harbour porpoise who 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). The difference in metabolic capacity between harbour seal and harbour porpoise is apparent in Figures A.19a and b, where harbour porpoise has a noticeably higher concentration in relation to trophic level than harbour seal. When marine mammals were removed from the analysis, the TMF decreased using the traditional method but increased using the balanced method.

The TMF of CB52 using all trophic levels was over two times higher using the balanced method than the traditional method, showing that an unbalanced dataset influences the calculated TMF for CB52 (Table 4.5). This however was not the case when shark, fish and invertebrates were analysed, confirming the imbalance lies with the marine mammal samples. An unbalanced dataset was also found to influence the TMF of CB28, where biomagnification was found to occur using the traditional method and trophic dilution using the balanced method when analysing all trophic levels and shark, fish, and invertebrates (Table 4.5). 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 (Beyer and Biziuk, 2009). The correlation was however not significant for CB28 when shark, fish and invertebrates were analysed using the traditional method and both of the CB28 TMFs calculated using the balanced method ($p>0.05$) (Table 4.4), suggesting that a larger dataset is required when calculating the TMF of lower chlorinated PCBs with less biomagnification ability, particularly when higher trophic level predators are not available and when the dataset is unbalanced. An unbalanced dataset

was also found to influence the assessment of CB101, where the correlation was not significant when shark, fish and invertebrates were analysed ($p>0.05$) (Table 4.4).

A study by House *et al*., (2008) calculated TMFs (traditional method) for CB180, 153, 138, 101 and 52 in a lake trout food web collected from seventeen lakes across Canada and in the north-eastern United States. The food web was composed of Lake trout and forage fish species, benthic invertebrates and zooplankton. Five PCB congeners were found to biomagnify in all seventeen locations. CB180 had the highest mean TMF, ranging from 1.6 – 8.0. CB52 had the lowest TMF, ranging from 1.0 – 2.7. This study reported a link between hydrophobicity and biomagnification, where higher TMFs were found for the most hydrophobic compounds (CB153, CB138, CB180), compared to those of intermediate and lower hydrophobicity. This agrees with the findings of this project for CB180, 153, 138, 118, 101 and 28, but not CB52.

A study by Brisebois, (2013) calculated the TMFs of the ICES-7 PCBs using the traditional method and balanced method in a food web composed of phytoplankton, zooplankton, benthic invertebrates and pelagic fish. Samples were collected from an estuary of the Scheldt River in southwestern Netherlands, an area known to be relatively polluted due to land based industrial activity on shore and in nearby Antwerp. The calculated TMFs from Brisebois, (2013) are shown in Table 4.5.

The TMFs calculated for substances in Brisebois, (2013) using the balanced method were consistent with TMFs calculated traditionally, with the exception of CB28, where the balanced method was described as “underestimating the substances biomagnifying ability”. It was concluded that there may not be a need to ensure a balance of trophic levels within the food web prior to determining the slope in the linear regression. This is in disagreement with the findings of this study. The TMFs calculated in this project are substantially higher compared to those calculated in Brisebois, (2013), with only CB28 having similar values to those in Table 4.4. The outcome of trophic magnification was the same, however with CB180, 153, 138, 118, 101 and 52 reported to biomagnify in the food web using both methods and CB28 found to biomagnify using the traditional method but trophic dilute (TMF <1) using the balanced method (Table 4.5).

It is apparent in Figures 4.20a (all calculated trophic levels using the traditional method) and 4.22a for CB180 (Irish Sea Biogeographic Region food web), that the single emaciated harbour seal sample identified as having a different FA profile (Figure 3.4), PCB congener profile (Figure 4.2) and a significantly higher $\sum PCB_{32}$ concentration in its blubber than the other harbour seal samples stands out and is potentially influencing the gradient. This was also identified in the equivalent plots for the other six PCB congeners

(A.1 – A.26). Another advantage to the balanced method of TMF determination is that the geometric mean is less sensitive to data outliers, which in often highly variable in environmental data and provides a more representative mean. This one harbour seal sample is a likely contributor to the higher traditionally calculated TMF in both scenarios. To investigate this further, CB153 was used as an example, where this point stands out on Figures A.1 (all trophic levels from all four locations) and A.3 (Irish Sea). The TMF including marine mammals, shark, fish, invertebrates from all four locations (Figure A.1) decreased from 9.1 to 8.4 when this sample was removed using the traditional method (Figure A.5a) but remains the same using the balanced method (12) (Figure A.5b), supporting that the balanced method is less sensitive to outliers. When marine mammals, shark, fish and invertebrates from the Irish Sea were analysed (Figure A.3), the TMF decreased from 8.1 to 6.9 using the traditional method (Figure A.6a) and decreased from 18 to 12 using the balanced method (Figure A.6b). There were fewer harbour seal samples collected from the Irish Sea (two) than the other three regions (eight). A larger sample number of harbour seal would be required to fully establish the impact of removing an outlier using both methods, as in this case there is only one other harbour seal sample available for analysis.

When conducting the regional comparison (Table 4.6), the TMF including all trophic levels from the Irish Sea Biogeographic Region was higher for CB180, 118, 52 and 28 than from the Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf using the both methods, and higher for CB138, 153 and 101 using the balanced method only. 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 ΣPCB_{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 shows the importance of a balanced dataset when calculating TMF. Our findings support this approach when conducting a regional comparison. The correlation was however not significant for CB28 in both regional comparisons and CB118 when all trophic levels were analysed from the Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf using both methods ($p>0.05$) (Table 4.6). This is likely due to the ability of harbour seal to metabolise this congener (Weijs *et al.*, 2007).

The trophic magnification of CB180 was reported by Bodin *et al*., (2008) in three regional invertebrate food webs in the Mediterranean Sea where the trophic levels included ranged from trophic level 2 (suspension feeders) to 3.9 (carnivore crustaceans). TMFs were 4.5, 16.8 and 4.4 for CB180 in each of the regions, using $\delta^{15}N$ derived trophic levels and logarithmically normalised (to lw) PCB congener concentrations.

Due to its high abundance in biota, CB153 is the most studied congener for bioaccumulation and biomagnification studies. A study by Romero-Romero *et al.,* (2017) determined the TMF for CB153 in the pelagic food web (spanning four trophic levels) was 6.2 or 2.2 with or without the inclusion of homeotherm top predators (cetaceans and seabirds) in the calculation. Using $\delta^{15}N$ derived trophic levels and logarithmically normalised (to lw) PCB congener concentrations, TMF values were highly influenced by the inclusion or exclusion of homeotherm top predators in the calculations (TMF = 6.2 with and TMF = 2.2 without). This finding is in agreement with the results of this study, where the inclusion of marine mammals influences the TMF of CB180, 153 and 52 calculated using the traditional and balanced methods due to the factors identified in Section 4.3.2.1 (inter- and intra- species metabolic capacity, feeding ecology and trophic level).

4.3.3 Polybrominated diphenyl ethers (PBDEs)

4.3.3.1 Concentrations and distributions

The concentration (µg/kg lw) of each PBDE congener and $\Sigma PBDE_9$ in eighteen of the nineteen sample categories (excluding zooplankton as discussed previously) is shown in Table 4.7 and Figure 4.24. Similar to PCBs, PBDE concentrations were normalised to the lipid content (%) to account for the different tissues and subsequently logarithmically transformed for ANOVA comparisons. The concentration of $\Sigma PBDE_9$ across eighteen of the nineteen sample categories was much lower than that of PCBs, with a concentration range of <LoD in benthic invertebrates muscle to 1,888 µg/kg lw in sperm whale blubber (Table 4.7). ANOVA carried out using the $\Sigma PBDE_9$ data revealed all marine mammals to have a significantly higher concentration in their blubber than the other sample categories (Figure 4.24) ($p < 0.05$, ANOVA, Tukey), with, as seen for PCBs, trophic level being a likely contributor. Due to BDE47 dominating a majority of the profiles and the low concentration of other congeners (many <LoD), PCA could not be conducted on sample categories.

The highest mean concentration of the $\Sigma PBDE_9$ was found in sperm whale blubber (Table 4.7). There was, however, a large degree of variation, with a range of 139.4 – 1,888 µg/kg lw, detected. As previously discussed, harbour porpoise and sperm whales accumulate a wider range of congeners compared to harbour seals due to their lower capacity for metabolising lower halogenated and less persistent PCB and PBDE congeners (Weijs *et al*., 2009). All marine mammals in this study were male and available physiological information fully investigated to determine whether they contribute to the within-species concentration and congener proportion variation.

Figure 4.25 shows the sample categories with a $\Sigma PBDE_9$ concentration above 30 µg/kg lw. Similar to Figure 4.1, there is a significant increase in $\Sigma PBDE_9$ concentration (>300.0 µg/kg lw) in the top eight samples composed of sperm whale (n=2), harbour seal (n=4) and harbour porpoise (n=3). The higher number of harbour porpoise in the top eight samples is likely a result of their metabolic inability to biotransform PBDEs in comparison to harbour seal (Boon *et al*., 1997; Hobbs *et al*., 2002; Weijs *et al*., 2009), which will likely influence the calculated TMF.

Table 4.7: The concentration range (µg/kg lipid weight) of nine BDE congeners and ΣPBDE$_9$ in the muscle, liver, homogenised whole, brown meat, soft body and blubber samples analysed across eighteen of the nineteen sample categories (not including zooplankton). Number of Samples = individuals for mammals and pools for all other categories. Number of individuals per pool are referred to in Table 2.1. Not all the LoD values are to four significant figures to account for precision. Values <LoD treated as zero when calculating the ΣPBDE$_9$. ΣPBDE$_9$ is expressed as the minimum sample concentration – maximum sample concentration within each category.

Category	Sample Number	BDE28	BDE47	BDE66	BDE100	BDE99
Harbour Seal	10	<0.18	14.54 - 302.7	<0.16	1.058 - 15.24	<0.12 - 109.3
Harbour Porpoise	18	<0.18 - 56.92	8.783 – 188.7	<0.16 - 12.32	<0.19 - 80.73	4.281 - 90.64
Sperm Whale	5	1.686 - 27.39	91.26 – 1,330	<0.16	6.488 - 75.19	18.01 - 274.8
Demersal Shark Muscle	12	<0.01	<0.06 - 32.63	<0.01	<0.04 - 5.263	<0.01
Demersal Shark Liver	12	<0.18 - 2.696	5.190 - 25.16	<0.16 - 0.144	<0.19 - 6.752	0.973 - 13.85
Pelagic Roundfish Muscle	2	<0.01	<0.06 - 2.906	<0.01	<0.04	<0.01
Pelagic Roundfish Liver	2	<0.18	<0.29 - 71.11	<0.16	8.759 - 22.22	<0.12
Pelagic Roundfish Whole	3	<0.01	3.846 - 7.293	<0.01	<0.04 - 1.399	<0.01 - 1.135
Demersal Roundfish Muscle	30	<0.01	<0.06 - 19.78	<0.01	<0.04	<0.01 - 5.495
Demersal Roundfish Liver	30	<0.18 - 0.724	1.255 - 30.75	<0.16 - 1.169	<0.19 - 6.796	<0.12 - 7.360
Demersal Roundfish Whole	6	<0.01 - 1.329	<0.06 - 22.59	<0.01 - 1.330	<0.04 - 5.980	<0.01 - 3.654
Flatfish Muscle	12	<0.01	<0.06 - 17.50	<0.01 - 3.261	<0.04 - 24.56	<0.01 - 9.783
Flatfish Liver	12	<0.18	<0.29 - 92.28	<0.16	<0.19 - 31.58	<0.12
Demersal Invertebrates Muscle	2	<0.01	<0.06 - 7.691	<0.01	<0.04	<0.01
Benthic Invertebrates Muscle	13	<0.01	<0.06	<0.01	<0.04	<0.01
Benthic Invertebrates Soft Body	17	<0.01	<0.06 - 24.62	<0.01	<0.04 - 6.329	<0.01 - 3.571
Benthic Invertebrates Whole	11	<0.01 - 10.88	<0.06 - 109.3	<0.01 - 2.073	<0.04 - 3.109	<0.01 - 2.899
Benthic Invertebrates Brown Meat	3	<0.01 - 0.303	<0.06 - 3.405	<0.01	<0.04 - 1.627	<0.01

Table 4.7 (continued): The concentration range (µg/kg lipid weight) of nine BDE congeners and ΣPBDE$_9$ in the muscle, liver, homogenised whole, brown meat, soft body and blubber samples analysed across eighteen of the nineteen sample categories (not including zooplankton). Number of Samples = individuals for mammals and pools for all other categories. Number of individuals per pool are referred to in Table 2.1. Not all the LoD values are to four significant figures to account for precision. Values <LoD treated as zero when calculating the ΣPBDE$_9$. ΣPBDE$_9$ is expressed as the minimum sample concentration – maximum sample concentration within each category.

Category	Sample Number	BDE85	BDE154	BDE153	BDE183	ΣPBDE$_9$
Harbour Seal	10	1.674 - 23.82	0.443 - 35.69	<0.15 - 149.7	<0.16 - 1.687	21.75 - 638.2
Harbour Porpoise	18	7.41 – 106.8	17.85 – 203.9	<0.15 – 117.3	<0.16 - 6.46	38.76 – 778.8
Sperm Whale	5	4.211 - 45.66	7.581 - 108.2	2.527 - 27.03	<0.16 - 0.111	139.4 - 1,888
Demersal Shark Muscle	12	<0.01 - 2.105	<0.02	<0.02	<0.01	<0.01 - 40.00
Demersal Shark Liver	12	<0.12 - 4.394	<0.34 - 4.572	0.603 - 10.63	<0.16 - 1.989	9.504 - 54.47
Pelagic Roundfish Muscle	2	0.342 - 1.132	<0.02	<0.02	<0.01	1.132 - 3.248
Pelagic Roundfish Liver	2	<0.12	<0.34 - 13.33	<0.15	<0.16	8.759 - 106.7
Pelagic Roundfish Whole	3	<0.01 - 5.315	<0.02 - 3.077	<0.02	<0.01	0.585 - 1.199
Demersal Roundfish Muscle	30	<0.01 - 5.195	<0.02 - 10.77	<0.02 - 6.593	<0.01	<0.01 - 35.165
Demersal Roundfish Liver	30	<0.12 - 3.768	<0.34 - 12.78	<0.15 - 1.221	<0.16 - 0.073	2.137 - 47.54
Demersal Roundfish Whole	6	<0.01 - 3.333	<0.02 - 2.326	<0.02	<0.01	<0.01 - 37.21
Flatfish Muscle	12	<0.01	<0.02 - 15.79	<0.02	<0.01	<0.01 - 28.26
Flatfish Liver	12	<0.12	<0.34 - 39.55	<0.15	<0.16	<0.01 - 131.8
Demersal Invertebrates Muscle	2	<0.01	<0.02 - 3.333	<0.02	<0.01	<0.01 - 10.95
Benthic Invertebrates Muscle	13	<0.01	<0.02	<0.02	<0.01	<0.01 - <0.06
Benthic Invertebrates Soft Body	17	<0.01	<0.02	<0.02	<0.01	<0.01 – 24.62
Benthic Invertebrates Whole	11	<0.01	<0.02 - 4.348	<0.02	<0.01 - 2.798	<0.01 – 124.5
Benthic Invertebrates Brown Meat	3	<0.01	<0.02	<0.02	<0.01	<0.01 - 5.335

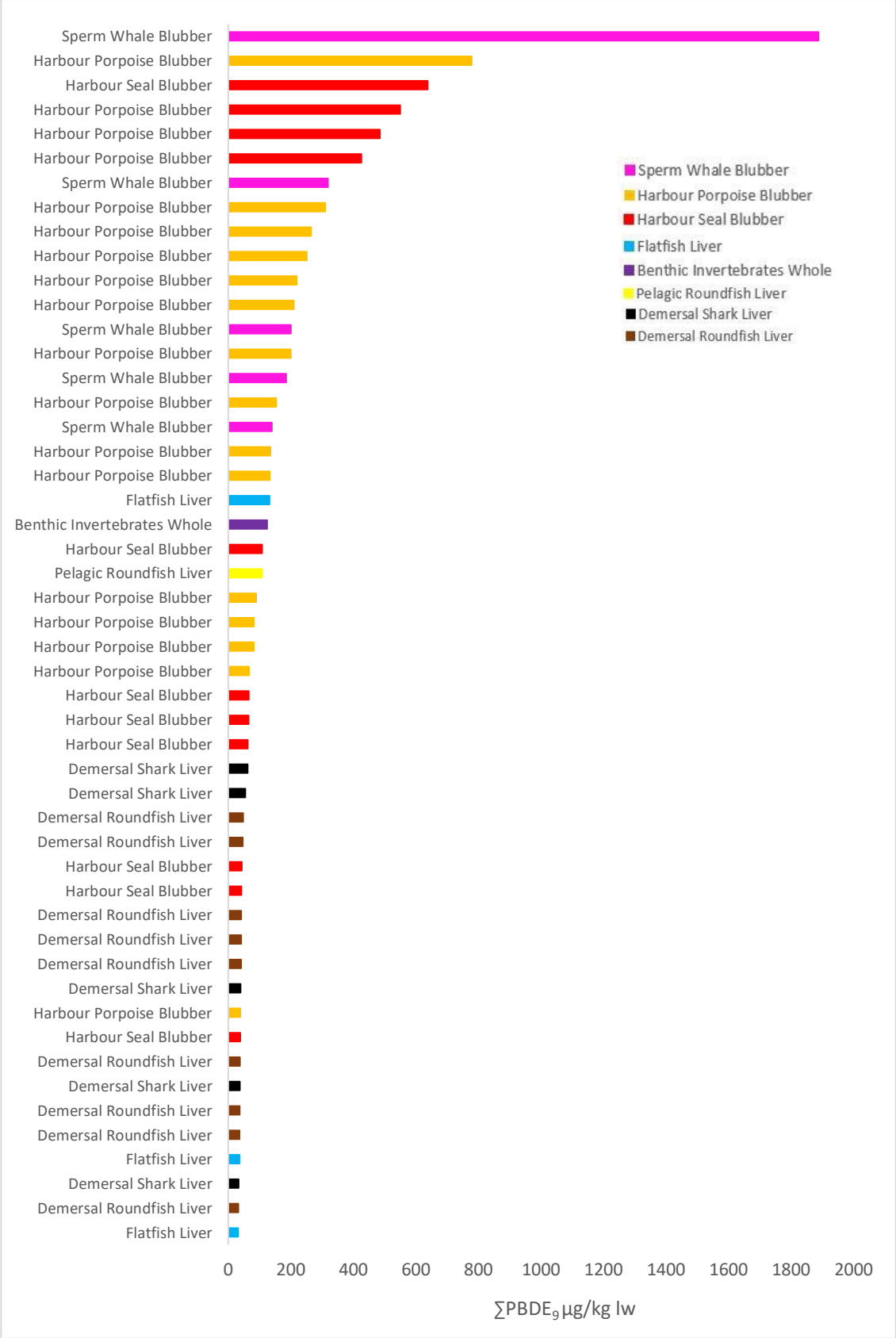


Figure 4.24. Sample categories (individuals for marine mammals and pools for all other categories) with a $\Sigma PBDE_9$ concentration above 30 µg/kg lw.

All three marine mammal categories have a large variation in their $\Sigma PBDE_9$ concentration (Table 4.7). Sperm whale samples have a concentration range of 139.4 - 1,888 µg/kg lw detected in their blubber (Table 4.7). Once the male subadult was removed from the comparison, the concentration ranged from 139.4 – 318.9 µg/kg lw (n=4). A regional assessment could not be conducted on sperm whale as they are highly migratory but when other potential factors were investigated further, this considerable degree of variation was not related to feeding patterns (Figure 3.4) or significantly influenced by length ($p>0.05$). Unfortunately, there was limited available information on body weight and age for a comparison, but a higher sample number would be required for a comprehensive analysis.

Harbour seal also has a considerable $\Sigma PBDE_9$ concentration range, with a concentration of 638.2 µg/kg lw in one individual in comparison to the range of 23.62 – 108.1 µg/kg lw identified in the other nine samples. This is the same emaciated individual discussed in Chapter 3 (Section 3.3.1.1; Figure 3.4) and earlier (Section 4.3.2.1; Figure 4.2) which had a different FA and PCB profile and significantly higher ΣPCB_{32} concentration than the other nine harbour seal samples, likely due to the smaller mass of blubber resulting in a higher concentration of $\Sigma PBDE_9$ per kg. The one harbour porpoise individual also identified as having a different FA and PCB profile and significantly higher ΣPCB_{32} concentration than the other seventeen samples was however not found to contribute to the variation identified in Table 4.7. Pearson's correlation showed that there was no significant influence on $\Sigma PBDE_9$ concentration from animal length and weight for harbour seal and harbour porpoise ($p>0.05$).

When region was investigated, harbour porpoise from the Minches and Western Scotland (n=5) had a significantly lower $\Sigma PBDE_9$ concentration in their blubber ($p < 0.05$, ANOVA, Tukey) than those from the Irish Sea (n=5), Northern North Sea (n=6) and Scottish Continental Shelf (n=2) (Figure 4.25). Although the $\Sigma PBDE_9$ concentration in harbour seal samples from the Irish Sea Biogeographic Region (both collected from Strathclyde) is higher (351.6 ± 286.6 µg/kg lw; n=2) than those collected from the Northern North Sea (54.10 ± 32.30 µg/kg lw; n=6) and Minches and Western Scotland (40.13 ± 2.380 µg/kg lw; n=2), the large concentration range in samples from the Irish Sea Biogeographic Region is due to the one individual identified with a different FA and PCB profile (Figure 3.4 and 4.2) and significantly higher ΣPCB_{32} and $\Sigma PBDE_9$ concentration in its blubber. Once this individual was removed from the regional comparison, similarly to PCBs, there was no significant difference of $\Sigma PBDE_9$ for harbour

seal and harbour porpoise (analysed separately) across their sampling regions ($p < 0.05$, ANOVA, Tukey).

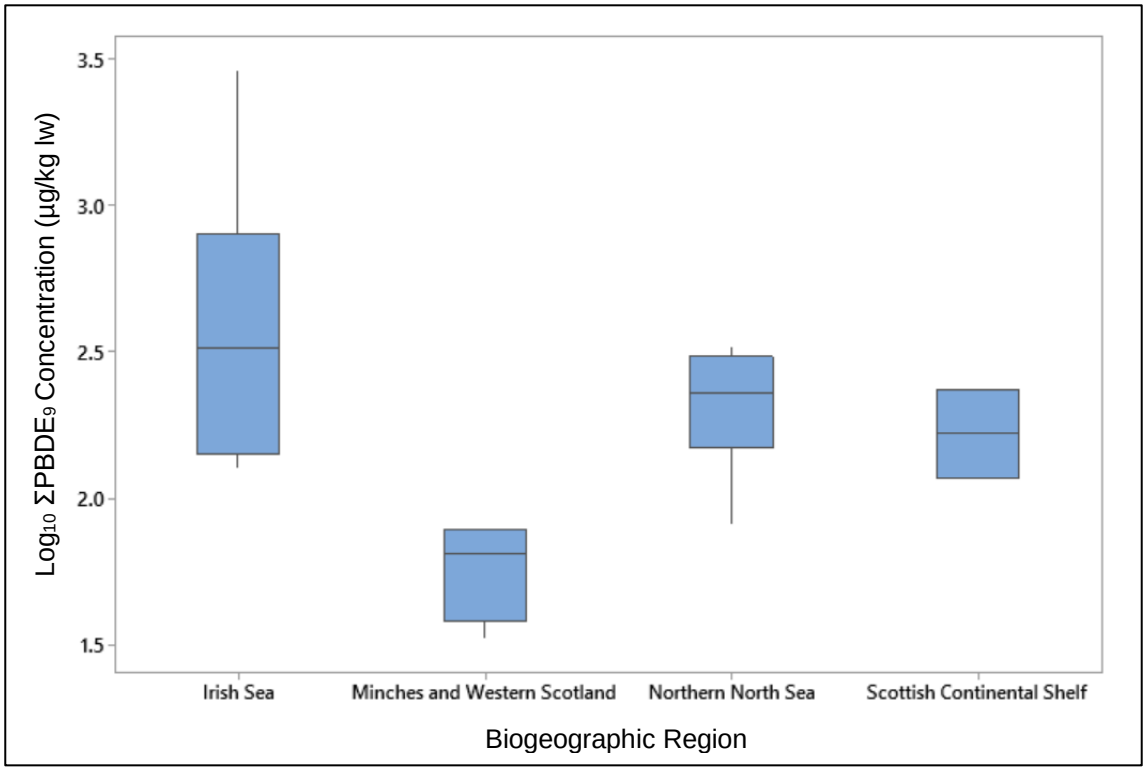


Figure 4.25: Box plot of Log_{10} $\Sigma PBDE_9$ concentration (µg/kg lw) in four Biogeographical Regions (Irish Sea n=5, Minches and Western Scotland n=5, Northern North Sea n=6, Scottish Continental Shelf n=2) where harbour porpoise samples were collected. Error bars are to one standard deviation.

According to published reports, PBDE levels in marine mammals may be generally one or more orders of magnitude higher than in invertebrates and fish collected from the corresponding sampling sites (Johnson-Restrepo *et al*., 2005). This agrees with the findings of this study (Table 4.7). Benthic invertebrates categories had very few congeners detected in comparison to fish and marine mammals, but common starfish had a significantly higher $\Sigma PBDE_9$ concentration of 124.5 µg/kg lw ($p < 0.05$, ANOVA, Tukey) compared to the other members of this category, suggesting a species-specific influence of $\Sigma PBDE_9$ accumulation. .

Comparing the fish and shark categories, flatfish liver had a significantly lower concentration of $\Sigma PBDE_9$ then demersal shark liver, demersal roundfish and pelagic roundfish (Table 4.7; $p < 0.05$, ANOVA, Tukey). As discussed previously, all flatfish sample pools in this project were collected from remote offshore sites such as Burra Haaf (n=7), Moray Firth (n=3) and the Solway Firth (n=2). As well as a category influence,

there was also a regional influence on the fish liver categories (Figure 4.26) were sample pools collected from the Irish Sea Biogeographic Region had a significantly higher mean concentration of $\Sigma PBDE_9$ in their tissues than those from the Northern North Sea and Scottish Continental Shelf ($p < 0.05$, ANOVA, Tukey). This was also found in Section 4.2.2 for PCBs, where shark and fish from the Irish Sea (particularly those in the Clyde) had a significantly higher ΣPCB_{32} concentration in their tissues due to being closer to an industrialised area (pollution sources) than those from further offshore sites such as the Scottish Continental Shelf. This agrees with the findings of Webster *et al*., (2007) and OSPAR's Intermediate Assessment 2017 (OSPAR, 2017) which found that around Scotland, the highest concentrations of PBDEs occur in the Irish Sea Biogeographic Region (due to most sites being in the Clyde, an industrial area). This was not the case for invertebrates, but a majority of sample pools had no detectable $\Sigma PBDE_9$ concentration.

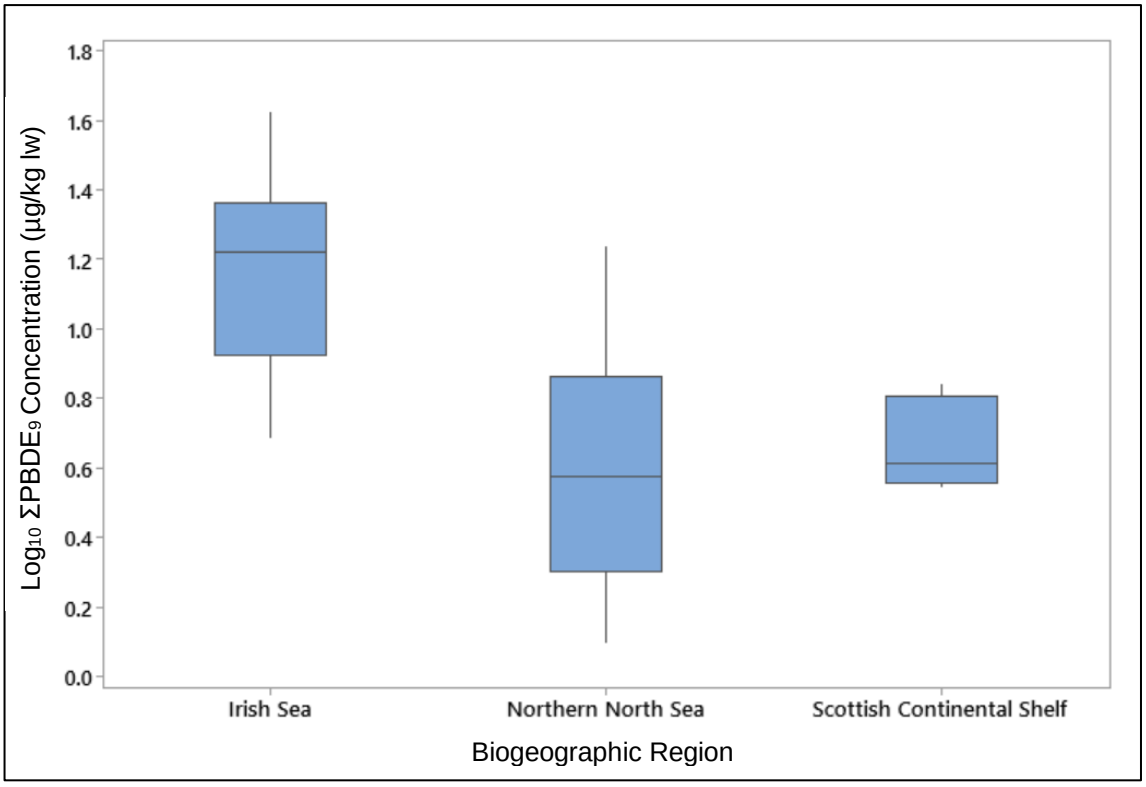


Figure 4.26: Box plot of Log_{10} $\Sigma PBDE_9$ concentration (µg/kg lw) in three Biogeographical Regions: (Irish Sea n=31; Northern North Sea n=12; Scottish Continental Shelf n=13) where shark and fish liver samples were collected. Error bars are to one standard deviation.

$\Sigma PBDE_9$ concentrations in demersal roundfish were highly variable, ranging from 2.14 – 47.54 µg/kg lw. Hake liver contained significantly higher $\Sigma PBDE_9$ concentrations (n=2) compared to whiting (n=15) and haddock (n=13) ($p < 0.05$, ANOVA, Tukey). Hake were

collected from the Irish Sea (Holy Loch), and as with the PCBs, the data suggests a localised regional influence on PBDE concentration. A larger sample number of single species across regions is required for a more comprehensive analysis. A study by Borga˚ *et al*., (2011) suggests that a minimum of 30 to 40 samples are likely needed to conduct a trophic magnification study, based on results from the power analysis following experimental designs similar to those in this project. When the physiological variables of trophic level, age and size were considered for haddock and whiting, none of these variables were found to significantly influence $\Sigma PBDE_9$ concentration ($p>0.05$).

When conducting an environmental assessment, WFD Quality Standards (QS) for human health and secondary poisoning were derived for the $\Sigma PBDE_6$ (BDE28, 47, 99, 100, 153, 154) in biota (fish), based on results from ecotoxicological studies on mice. The lower of these QS was assigned as the EQS, which for PBDEs was the human health QS based on fish muscle. The EQS (0.0085 µg kg^{-1} ww) is very low compared to typically reported environmental concentrations in biota, and most PBDE data for biota will exceed this concentration. In order to compare liver concentrations, an adjustment is required. Species-specific conversion factors (EQS is multiplied by the ratio of the lipid content in the liver to the typical lipid content in the muscle) enable a comparison of $\Sigma PBDE_6$ in liver tissue against the EQS. As predicted, the concentration of $\Sigma PBDE_6$ in all fish sample pools in this project exceeded the adjusted EQSs. Using a demersal roundfish sample pool as an example (haddock from the Outer Firth of Forth), the EQS would be multiplied by the ratio of the lipid content in the liver (68.13%) to the muscle (1.48%), becoming 0.0085 x 46.03 = 0.391 µg kg^{-1}. The $\Sigma PBDE_6$ in this sample pool was 9.908 µg kg^{-1} lw, exceeding the adjusted EQS.

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 status assessment of PBDEs in sediment and biota (OSPAR, 2020). 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. In contract to the EQS, none of the PBDE concentrations in each of the species matrix combinations exceeded the FEQG (Table 4.8).

Table 4.8: 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.

Category	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
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

Marine mammals

All nine PBDE congeners were detected in the blubber of the three marine mammal categories studied (Table 4.7). Differences in congener proportions (Figure 4.27) and concentration were observed between species.

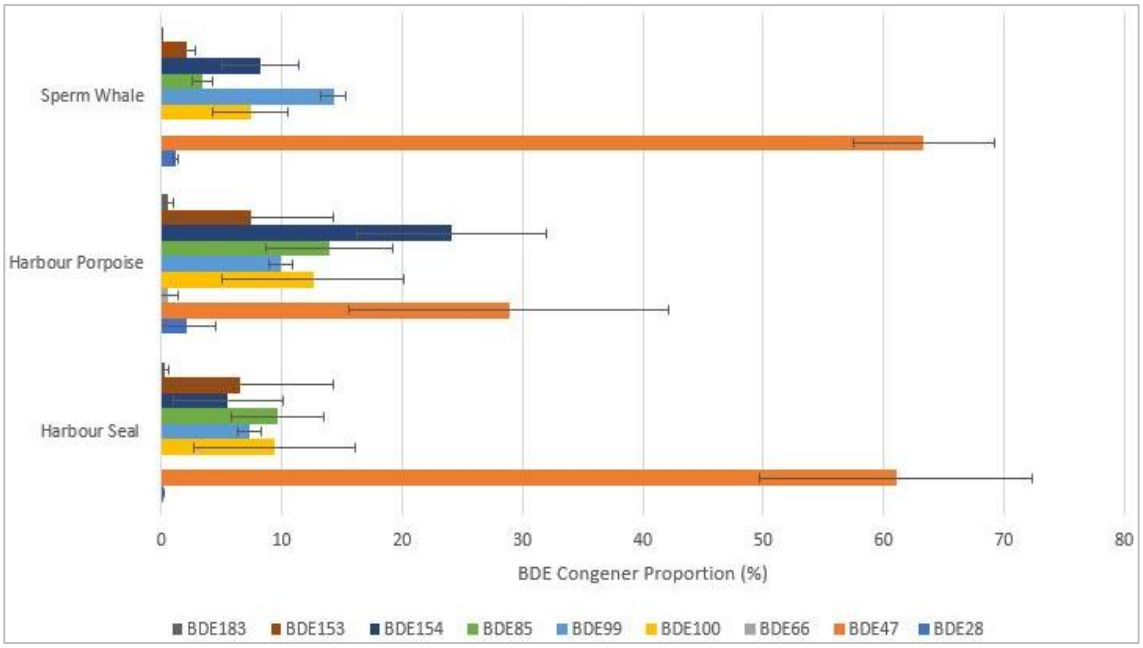


Figure 4.27: The mean proportion of each PBDE congener in individual harbour seal, harbour porpoise and sperm whale blubber samples, expressed as a percentage of the $\Sigma PBDE_9$ concentration. Error bars are to one standard deviation.

BDE47 is the most abundant congener in harbour seal and sperm whale, accounting for 46 – 83% and 56 – 70%, respectively. Harbour porpoise have higher proportions of the BDEs 85, 154 and 153 in comparison to harbour seal and sperm whale, accounting for 7.6 – 65% of the profile, as opposed to 2.1 – 29% in harbour seal and 7.2 – 15% in sperm whale and is the only marine mammal species with detectable concentrations of BDE66. The lower proportion of BDE47 in comparison to harbour seal has previously been reported in harbour porpoise from the Southern North Sea (Weijs *et al*., 2007) indicating that harbour porpoise are less genetically adapted than harbour seal to metabolise lower halogenated and less persistent PCB and PBDE congeners (Weijs *et al*., 2009).

Similar to the PCB profiles shown in Figures 4.2 and 4.4, sperm whales have a lower proportion of higher brominated congeners such as BDE153 and 154 (Figure 4.27) and less variation within congeners. This is likely due to sperm whales metabolic capacity, metabolic rate and distance from contaminant sources than the other marine mammal species. The PBDE profile described in this study corresponds to the PBDE profile reported in Bartelini *et al.,* (2018) in blubber collected from nine stranded sperm whales from the Adriatic Sea and Tuscany Coast (all male). BDE47 accounted for 65 – 70% of the profile, followed by BDE99, BDE100 and BDE154.

There is a higher degree of variation present for each PBDE congener in the harbour porpoise and harbour seal categories than sperm whale (Figure 4.27). Biogeographic Region was investigated as a potential contributing factor to the variation observed.

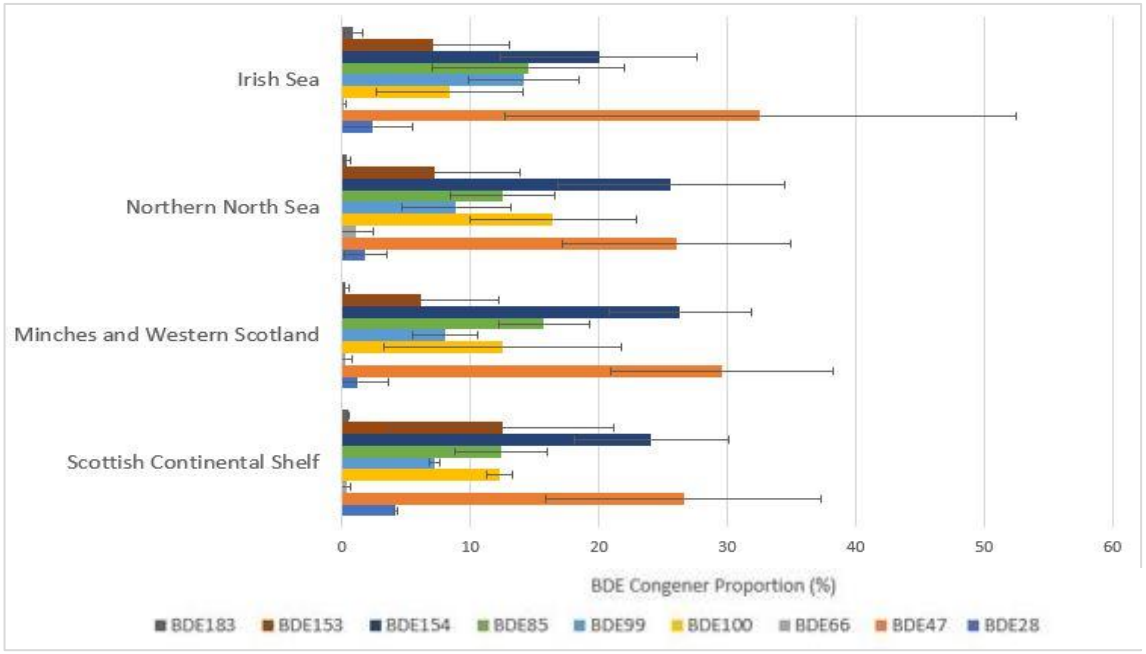


Figure 4.28: The mean proportion of each PBDE congener in the four Biogeographic Regions where individual harbour porpoise blubber samples were collected, expressed as a percentage of the $\Sigma PBDE_9$ concentration. Locations within the Irish Sea include Strathclyde (n=5). Locations within the Northern North Sea include Fife (n=1), Grampian (n=3), Lothian (n=1) and Tayside (n=1). Locations within the Minches and Western Scotland include the Highlands (n=4) and the Western Isles (n=1). Locations within the Scottish Continental Shelf include Orkney (n=2). Error bars are one standard deviation.

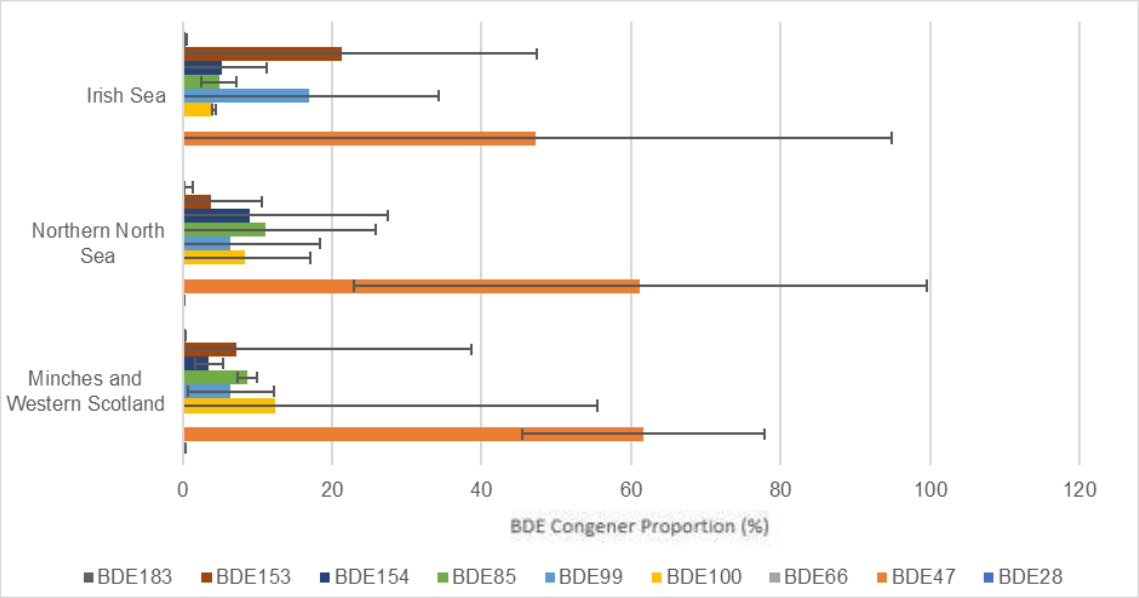


Figure 4.29: The mean proportion of each PBDE congener in the three Biogeographic Regions where individual harbour seal blubber samples were collected, expressed as a percentage of the $\Sigma PBDE_9$ concentration. Locations within the Irish Sea include Strathclyde (n=2). Locations within the Northern North Sea include Fife (n=3), Grampian (n=1), Lothian (n=1) and Tayside (n=1). Locations within the Minches and Western Scotland include the Highlands (n=2). Error bars are one standard deviation.

BDE47 and BDE154 are the most dominant congeners present in harbour porpoise collected from all four locations (Figure 4.28), accounting for 11 – 69% and 5.5 – 26% of the PBDE profile from animals stranded in the Irish Sea Biogeographic Region, 23 – 45% and 20 – 34% from those in the Minches and Western Scotland, 16 – 37% and 18 – 30% in those from the Scottish Continental Shelf and 13 – 38% and 12 – 37% in those collected from the Northern North Sea, respectively. Harbour porpoise from the Scottish Continental Shelf have a higher proportion of BDE153 in their blubber (Figure 4.28), ranging from 18 – 30% in comparison to those collected from the Minches and Western Scotland (not detected – 14%), Irish Sea Biogeographic Region (not detected – 15%) and Northern North Sea (not detected – 19%). This was unexpected as the Scottish Continental Shelf is away from an industrial area; higher brominated PBDE congeners adsorb to sediments (particles with high organic carbon contents) due to their hydrophobicity and are not transported long distances from pollution sources. This suggests that the greater accumulation of BDE153 in harbour porpoise (compared to harbour seal and sperm whale) is due to metabolic factors rather than geographic factors.

Harbour seals from the Irish Sea have a higher proportion of BDE99 and BDE153 in their blubber (Figure 4.29), ranging from 14 – 17% and not detected – 23%, than those from

the Northern North Sea (0.22 – 11.6% and not detected – 10.5%) and the Minches and Western Scotland (4.8 – 8.1% and not detected – 15%). Strathclyde is the only area within the Irish Sea Biogeographic Region where harbour seal and harbour porpoise samples were collected which is a highly contaminated site and close to an industrial area. Samples from the Minches and Western Scotland and Scottish Continental Shelf were expected to have a higher proportion of lower brominated congeners than the other two regions due to their higher volatility and ability for long-distance transport, suggesting that for harbour seal, there is more of a regional influence of PBDE congener proportion than in harbour porpoise.

The harbour seal individual identified with a different FA and PCB profile (Figure 3.4 and 4.2) and significantly higher ΣPCB_{32} and $\Sigma PBDE_{9}$ concentration is also likely contributing to the category congener proportion variation, and when investigated further (Figure 4.30), it was found that this individual (outlined in blue on Figure 4.30) had a much higher proportion of BDE153 and BDE99 than the other individuals, accounting for 23% and 17% respectively. The profile is also different from the other harbour seal sample collected from the same region (outlined in blue) where the individual has a much lower proportion of BDE85 (3.7%) and BDE100 (2.4%) than 16% and 27% respectively. It can also be identified from Figure 4.30 that the individual from Lothian (within the Northern North Sea Biogeographic Region) has a different profile from the other Northern North Sea localised regions (Grampian, Fife and Tayside), containing a higher proportion of BDE154.

There was a similar finding for the harbour porpoise individual (also from Strathclyde) identified with a different FA and PCB profile (Figure 3.4 and 4.2) and significantly higher ΣPCB_{32} concentration. This individual (outlined in blue on Figure 4.31) had a much higher proportion of BDE99 than the other individuals, accounting for 21% of the profile. Harbour porpoise from Strathclyde do however have a higher proportion of BDE99 (16 – 21%) in comparison to those from the other localised regions (4.0 – 13%). This individual also has no detected concentration of BDE100, which accounts for 4.0 – 28% of the profiles in other harbour porpoise (Figure 4.31). This within-category variation due to physiological condition and localised region would account for the variation established in the analysis of Biogeographic Regions.

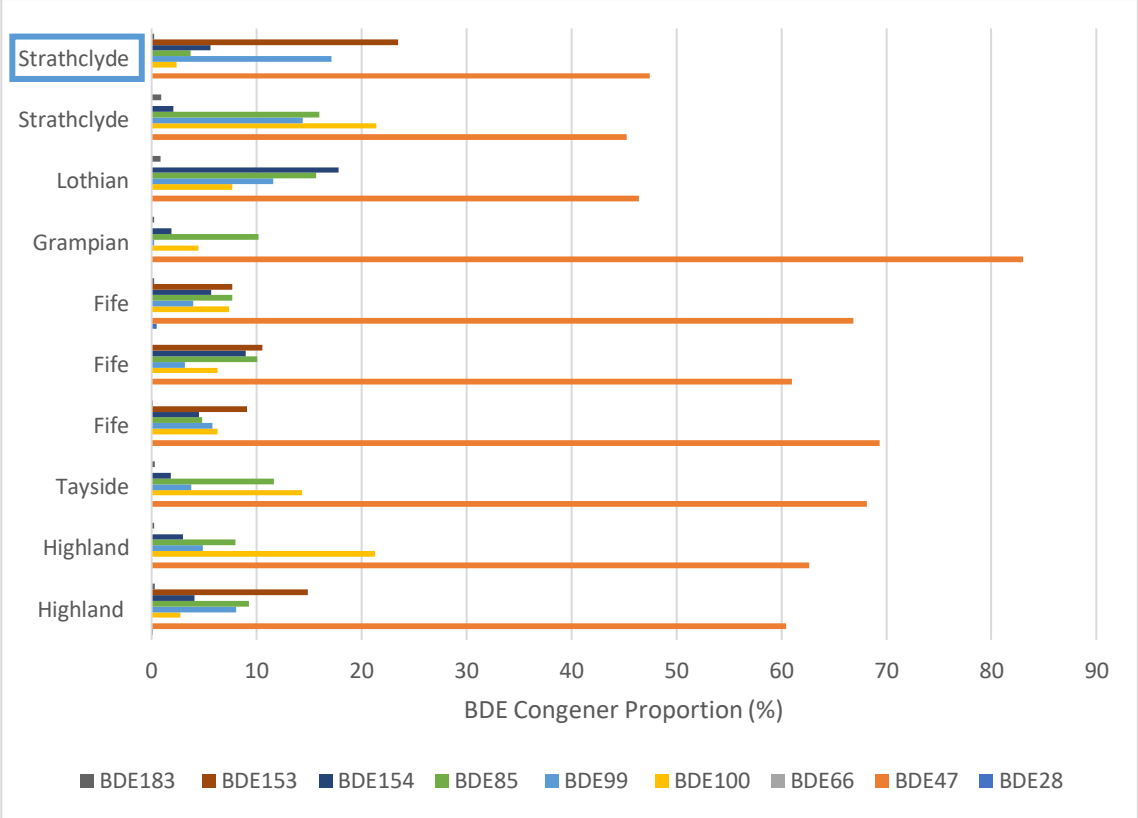


Figure 4.30: The mean proportion of each PBDE congener in each individual harbour seal sample from the three Biogeographic Regions (six localised regions) expressed as a percentage of the $\Sigma PBDE_9$ concentration. Locations within the Irish Sea Biogeographic region include Strathclyde (n=2). Locations within the Northern North Sea include Fife (n=3), Grampian (n=1), Lothian (n=1) and Tayside (n=1). Locations within the Minches and Western Scotland include the Highlands (n=2). Error bars are one standard deviation. The individual collected from Strathclyde with a different PBDE profile is outlined in blue.

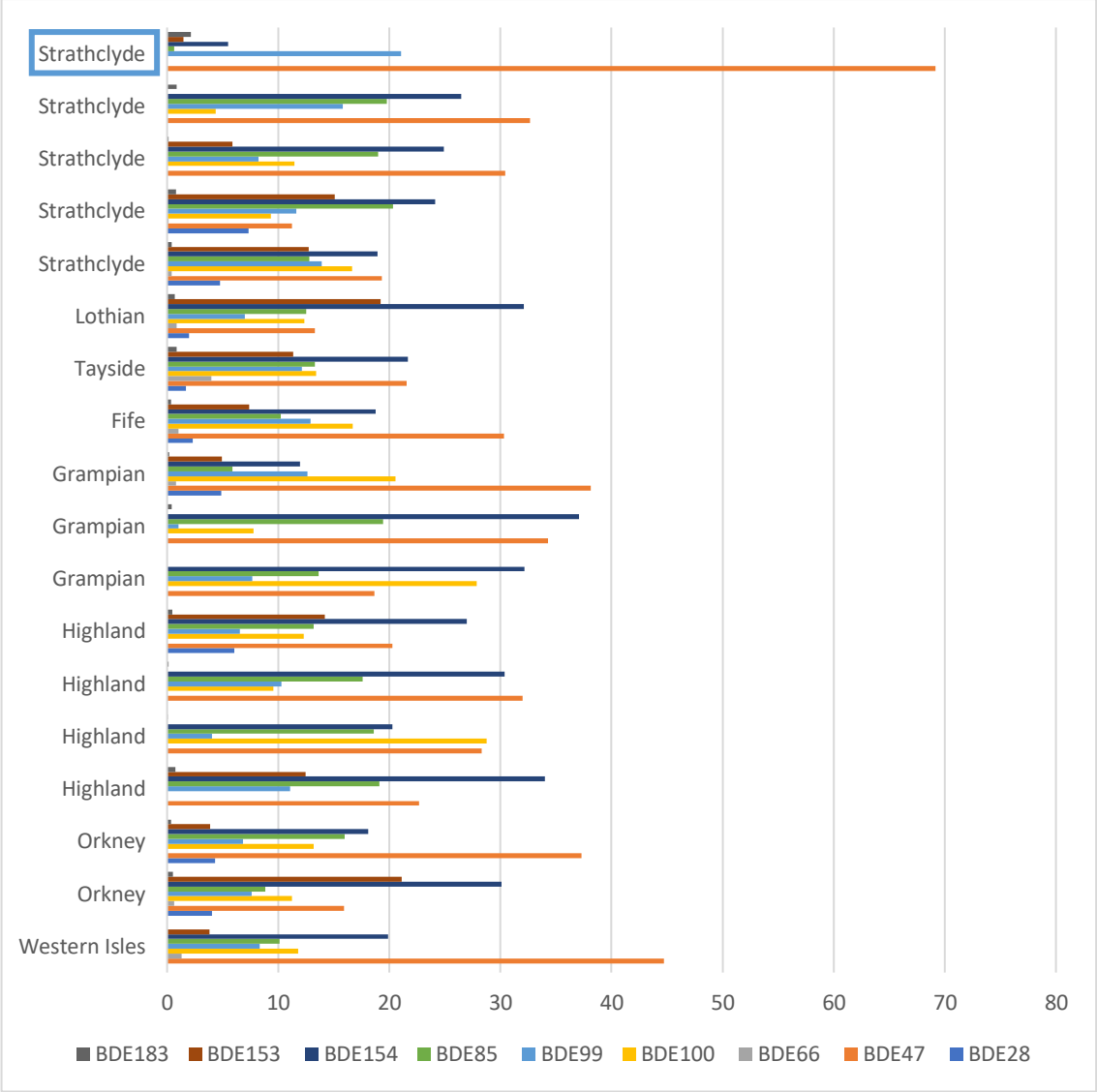


Figure 4.31: The mean proportion of each PBDE congener in each individual harbour porpoise sample from the four Biogeographic Regions (eight localised regions) expressed as a percentage of the $\Sigma PBDE_9$ concentration. Locations within the Irish Sea include Strathclyde (n=5). Locations within the Northern North Sea include Fife (n=1), Grampian (n=3), Lothian (n=1) and Tayside (n=1). Locations within the Minches and Western Scotland include the Highlands (n=4) and the Western Isles (n=1). Locations within the Scottish Continental Shelf include Orkney (n=2). Error bars are one standard deviation. The individual collected from Strathclyde with a different PBDE profile is outlined in blue.

Shark and fish

The four categories covered in this section represent a range of trophic levels which is reflected below, where PBDE congener proportion and concentration differ between and within the four categories.

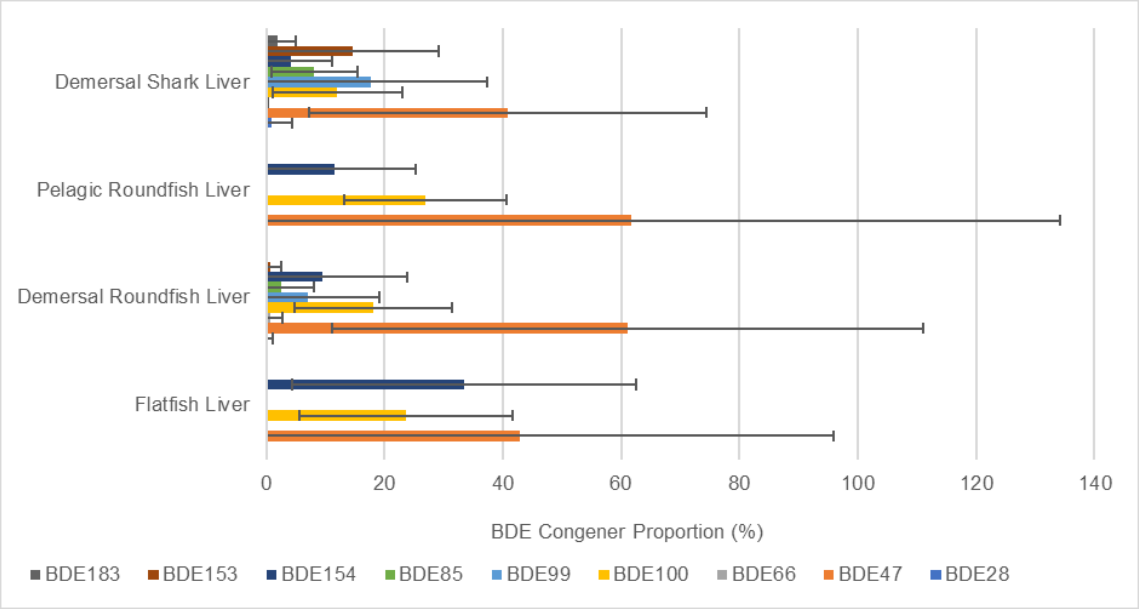


Figure 4.32: The mean proportion of each PBDE congener in pooled demersal shark liver, pelagic roundfish liver, demersal roundfish liver and flatfish liver samples, expressed as a percentage of the $\Sigma PBDE_9$ concentration. Error bars are one standard deviation.

BDE47 was the dominant congener in all four sample categories, accounting for a range of 29 – 55% of the congener profile in pooled demersal shark liver, not detected – 66% in pooled pelagic roundfish liver, 52 – 75% in pooled demersal roundfish liver and not detected – 70% in pooled flatfish liver. Pooled demersal shark liver has a higher proportion of BDE99 and 153 in in its congener profile than the other three categories (Figure 4.32), accounting for a range of 11 – 25% and 6.3 - 22%, respectively. This is expected, as demersal shark is at a higher trophic level than the other three categories, (Table 3.7) therefore accumulating higher brominated BDEs with a high bioaccumulation potential.

Metabolism likely plays an important role in influencing congener distributions of PBDEs in wild fish and previous studies have suggested that there may be species-specific differences in the metabolism of PBDEs among different fish (Browne *et al.,* 2009). The metabolism of PBDEs in mammals typically occurs via oxidative pathways, producing hydroxylated PBDEs and brominated phenols (Chen *et al.,* 2006; Stapleton *et al.,* 2009). Contrary to mammals, fish have not been shown to form oxidative metabolites of PBDEs but have instead demonstrated the ability to reductively de-brominate PBDEs (Stapleton *et al*., 2004; Stapleton, Letcher and Baker, 2004). Lower brominated congeners are often regarded as more toxic and have a higher biomagnification potential than higher brominated congeners. A study by Roberts *et al.,* (2011) investigated the metabolism of eleven individual PBDE congeners in three different fish species: rainbow trout, common

carp and Chinook salmon. It was found that metabolite formation rates were generally 10–100 times faster in carp than in trout and salmon and BDE47, 49, 101, 154, and 183 were the major metabolites observed in all three species, further suggesting a species-specific difference in the metabolism. Metabolism is likely to be a contributing factor to congener proportion in the fish categories in this study, particularly in the demersal roundfish category which is composed of three species - haddock, whiting and hake (Figure 4.32).

Demersal shark and pelagic roundfish were collected from the Irish Sea Biogeographic Region only and flatfish liver have very few samples with congeners detected across the three locations (e.g. only one sample from the Irish Sea had detectable PBDE congeners), so a regional analysis could not be conducted on these sample categories.

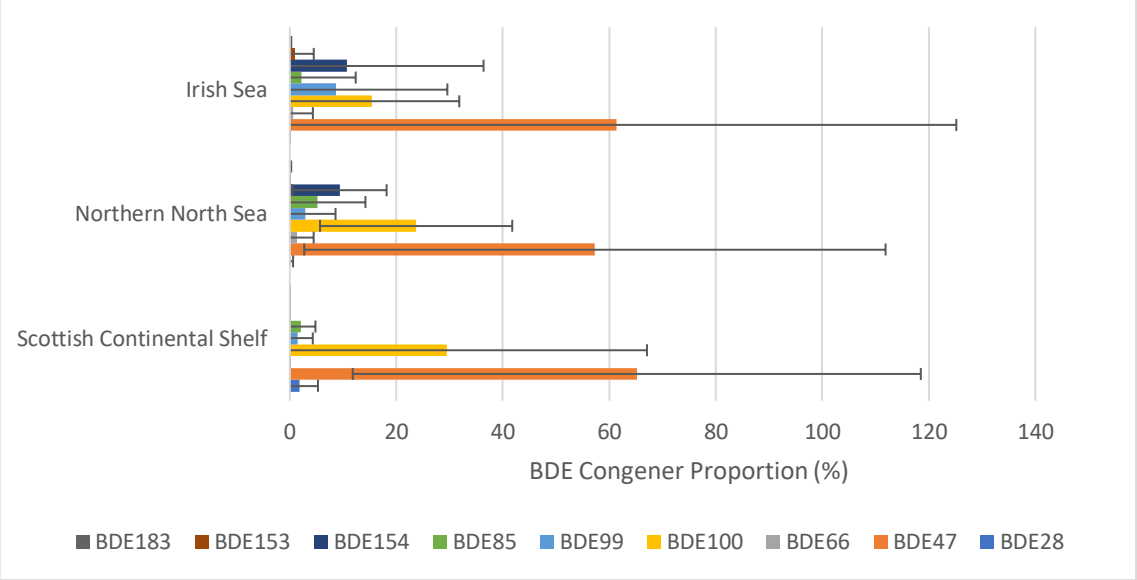


Figure 4.33: The mean proportion of each PBDE congener in the three Biogeographic Regions where pooled demersal roundfish liver samples were collected, expressed as a percentage of the $\Sigma PBDE_9$ concentration. Locations within the Irish Sea include the Holy Loch (n=4), Pladda (n=7) and the Solway Firth (n=4). Locations within the Northern North Sea include the Outer Firth of Forth (n=2), Montrose Bank (n=3) and the Moray Firth (n=4). Locations within the Scottish Continental Shelf include Burra Haaf (n=6). Error bars are to one standard deviation.

BDE47 was the dominant congener in the pooled demersal roundfish liver category across all three regions, accounting for a range of 45 – 73% of the profile from samples collected from the Irish Sea Biogeographic Region, 46 – 61% of the profile from samples collected from the Northern North Sea and 62 – 75% of the profile from samples collected from the Scottish Continental Shelf (Figure 4.33). Samples from the Irish Sea Biogeographic Region and Northern North Sea have a higher proportion of BDE99 in their profile, accounting for a range of not detected – 16%, and not detected – 15% in

compared to not detected – 5.1% in the Scottish Continental Shelf. BDE153 was only detected in sample pools collected from the Irish Sea Biogeographic Region and BDE28 was only detected in sample pools collected from the Scottish Continental Shelf. As previously discussed, samples from sites within the Irish Sea Biogeographic Region, closer to pollution sources in a highly industrial area, are more likely to have higher brominated congeners in their tissues due to the congeners inability for long-distance transport than further offshore sites such as the Scottish Continental Shelf, where more volatile lower brominated congeners are more prevalent.

Invertebrates

The four invertebrates categories contain a diverse range of species and tissue types. The concentration of $\Sigma PBDE_9$ in all invertebrate sample categories is low, with benthic invertebrates muscle having a concentration <LoD and detectable concentrations ranging from 1.351 – 122.3 µg/kg lw in five of the eleven benthic invertebrates whole samples (pooled common starfish). Most congeners have a concentration below or close to the LoD and it is therefore not appropriate to compare congener profiles. As reported in other studies (Bodin *et al*., 2007; Pizzini *et al*., 2015; Sutton *et al*., 2019), BDE47 has a concentration above the LoD more frequently than other congeners.

As expected, as observed for the PCBs, investigation of $\Sigma PBDE_9$ concentrations and profiles has identified inter- and intra- species variation in the marine mammals, shark and fish and invertebrates categories which will contribute to the reliability of calculated TMFs. $\Sigma PBDE_9$ concentration and congener proportion was influenced by trophic level, metabolic capacity and feeding ecology in marine mammals, whereas shark and fish had $\Sigma PBDE_9$ concentration and congener proportion influenced by species-specific feeding ecology, metabolism, sampling location and trophic level. $\Sigma PBDE_9$ concentration in the benthic invertebrates categories was low with few congeners detected, but a potential species-specific influence was identified with common starfish. These variables in each category/species must be considered in the calculation of TMFs.

4.3.4 Trophic magnification of PBDEs

Previous studies on the biomagnification of PBDEs through the food web have been contradictory. A study by Mizukawa *et al.,* (2009) found that PBDEs do not biomagnify as much as PCBs, with most congeners showing a negative or no correlation between concentration and trophic level, whilst the majority of studies have found TMFs of

particular PBDE congeners (BDE47, 66, 100, 99, 154, and 153) significantly greater than one (Yu *et al*., 2009; Choo, Lee and Oh, 2019).

Trophic magnification was investigated using the most abundant PBDE congener: BDE47 (Table 4.7) following the guidance recommended by Kidd *et al*., (2019). This was the only congener with detectable concentrations in more than ten benthic invertebrate sample pools, ensuring the inclusion of several lower-trophic-level taxa (several different benthic invertebrate families) and more of a reasonable balance with respect to sample numbers of lower- versus higher-trophic-level organisms (as per the guidance).

TMFs were calculated using the traditional and balanced methods on the food web both with and without marine mammals, the Irish Sea food Biogeographic Region web and the Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf food web. Figures 4.34 – 4.37 and A.27 – A.28 show the plots for calculating the TMF of BDE47. The regression summary for the determination of TMF using both the traditional method (Borgå *et al*., 2012; OSPAR, 2016b) and balanced method (Brisebois, 2013) is shown in Table 4.9 and calculated TMFs are shown in Tables 4.10 and 4.11.

BDE47

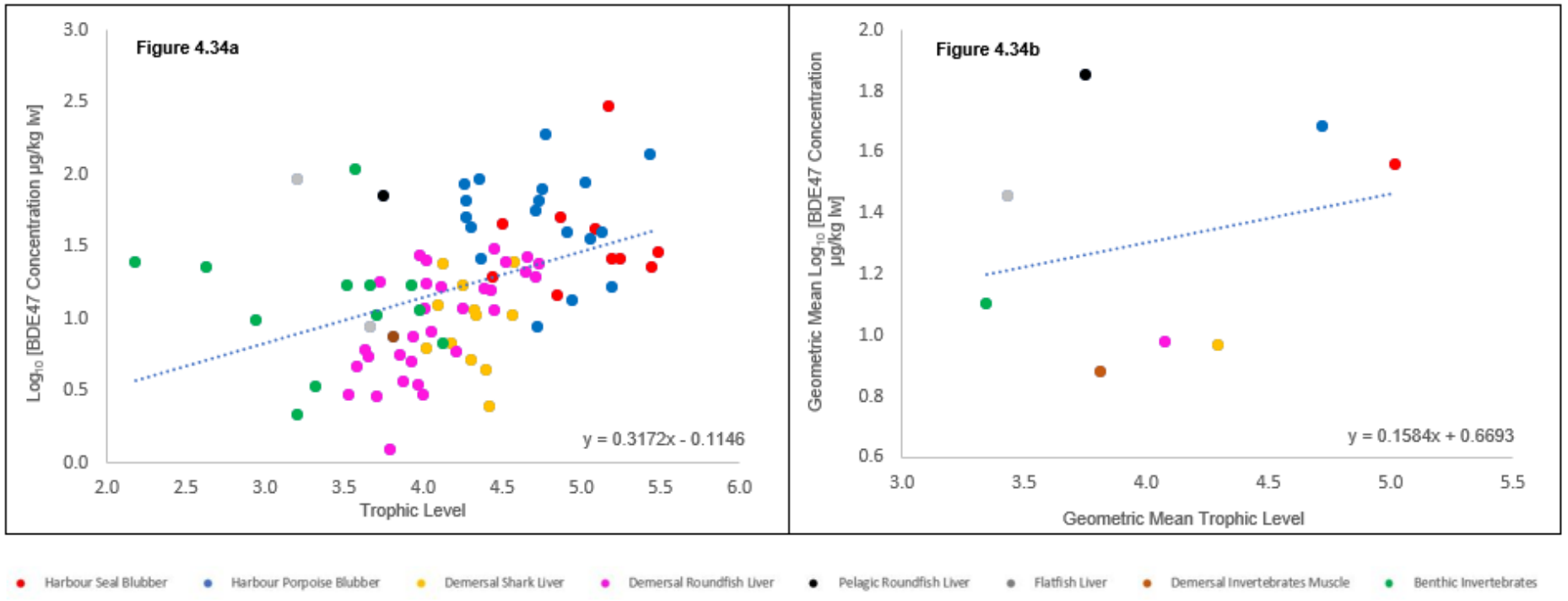


Figure 4.34: (a) Relationship between trophic level and logarithmically transformed BDE47 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black), demersal invertebrates (brown) and benthic invertebrate whole, 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 BDE47 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), demersal invertebrates (brown) and benthic invertebrate whole, brown meat, soft body (green) from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.

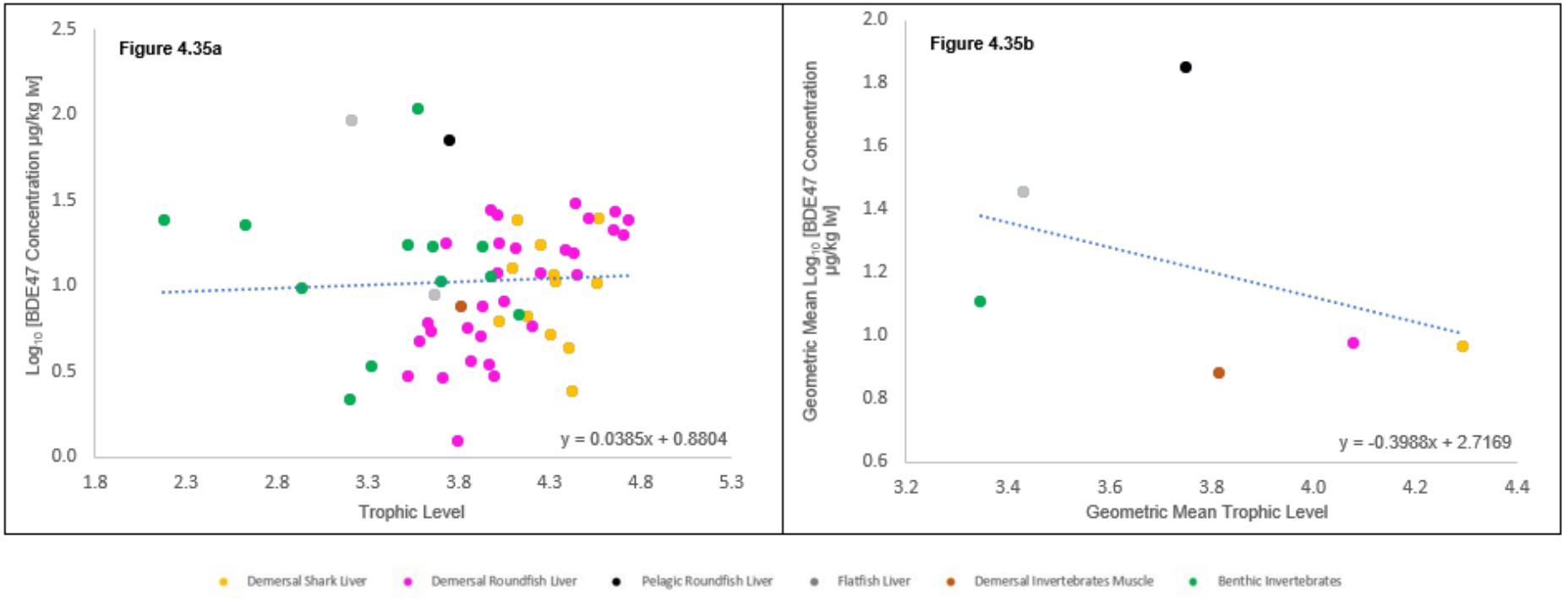


Figure 4.35: (a) Relationship between trophic level and logarithmically transformed BDE47 concentration (µg/kg lw) in demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black), demersal invertebrates (brown) and benthic invertebrate whole, brown meat, soft body (green) from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf. (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean BDE47 concentration (µg/kg lw) in demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black), demersal invertebrates (brown) and benthic invertebrate whole, brown meat, soft body (green) from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.

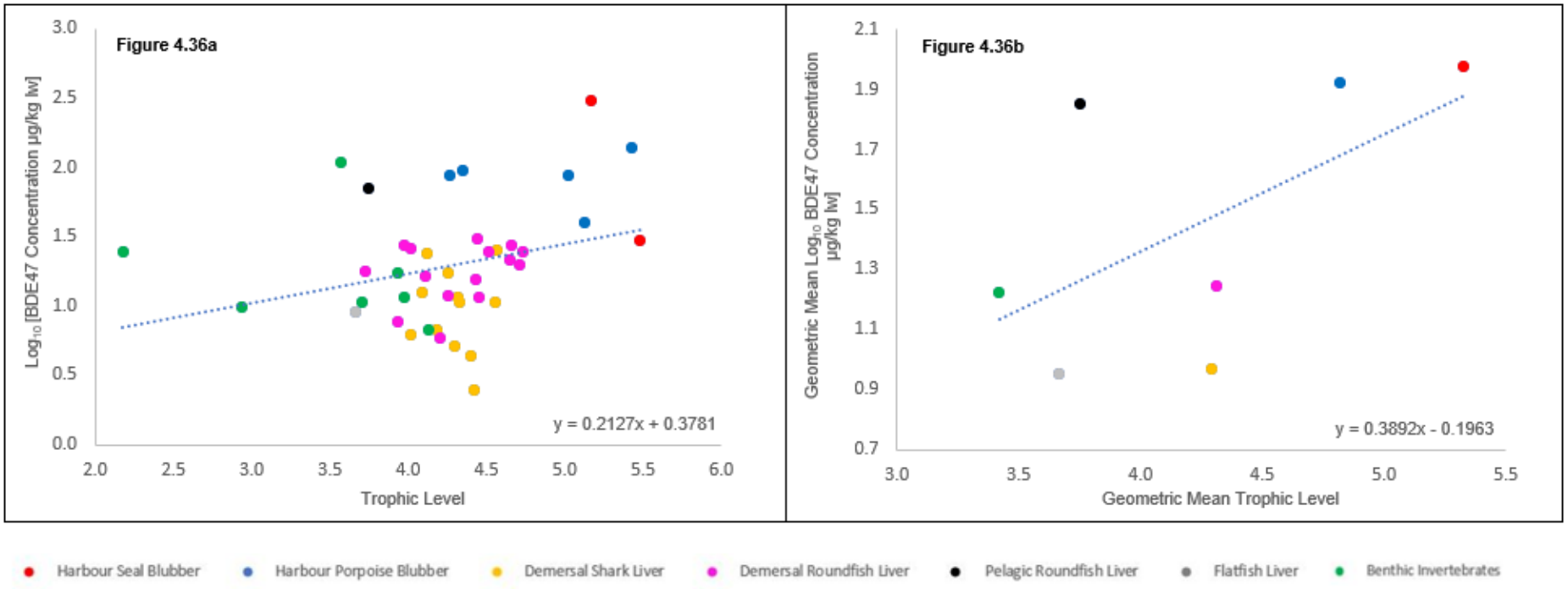


Figure 4.36: (a) Relationship between trophic level and logarithmically transformed BDE47 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, brown meat, soft body (green) from the Irish Sea Biogeographic Region (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean BDE47 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, brown meat, soft body (green) from the Irish Sea Biogeographic Region.

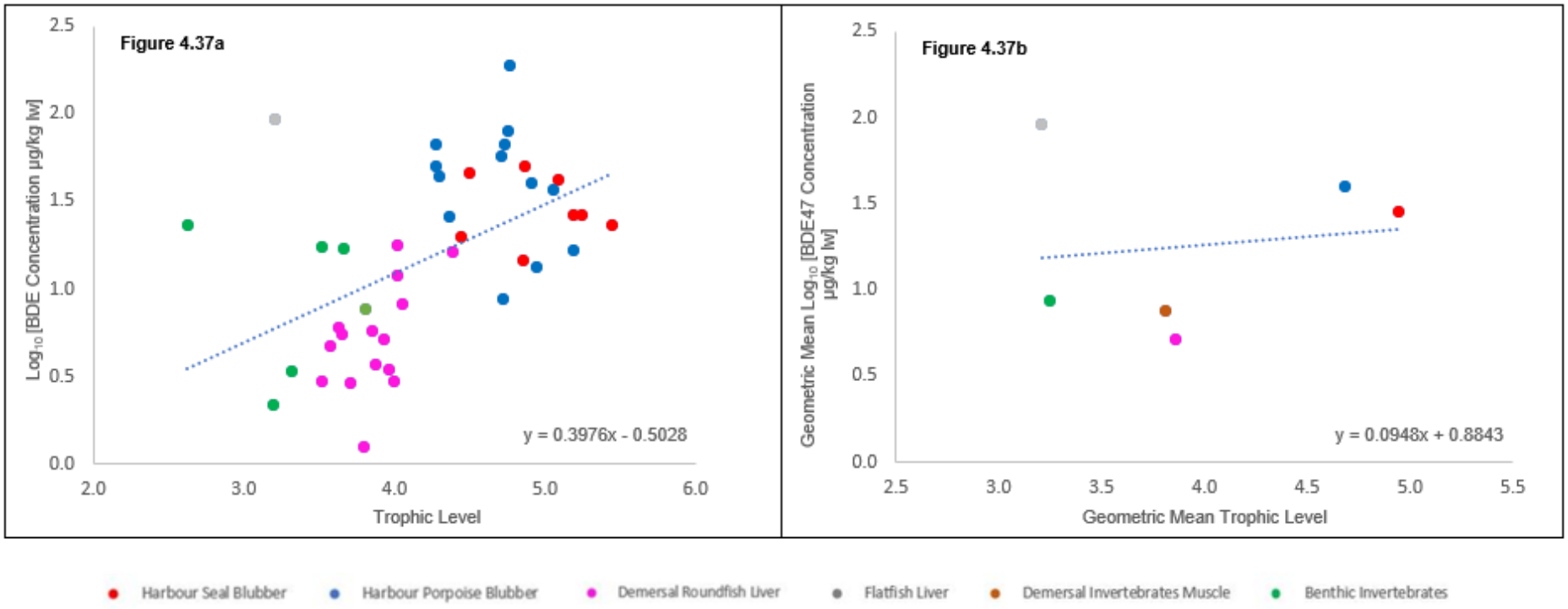


Figure 4.37: (a) Relationship between trophic level and logarithmically transformed BDE47 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), fish liver: demersal (pink), flatfish (grey), demersal imvertebrates and benthic invertebrate whole, brown meat, soft body (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 BDE47 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), fish liver: demersal (pink), flatfish (grey) and benthic invertebrate whole, brown meat, soft body (green) form the Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf.

Table 4.9: Regression summary for the determination of TMF using both the traditional method (Borgå *et al.,* 2012; OSPAR, 2016b) and balanced method (Brisebois, 2013). For the traditional method, y = Log_{10} [BDE Concentration µg/kg lw] and x = trophic level; whilst for the balanced method, y = Geometric mean Log_{10} [BDE47 Concentration µg/kg lw] and x = Geometric mean trophic level.

PBDE	Location	Regression Equation (p-value)		Sample categories
		Traditional Method	Balanced Method	
BDE47	All trophic levels from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 0.3172x – 0.1146 (p<0.05)	y = 0.1584x - 0.6693 (p>0.05)	harbour seal blubber, harbour porpoise blubber, demersal shark liver, fish liver: demersal, flatfish, pelagic, demersal invertebrates and benthic invertebrates whole, brown meat, soft body
	Shark, fish and invertebrates from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 0.0385x + 0.8804 (p<0.05)	y =-0.3988x + 2.7169 (p>0.05)	demersal shark liver, fish liver: demersal, flatfish, pelagic, demersal invertebrates and benthic invertebrates whole, brown meat, soft body
	All trophic levels from the Irish Sea Biogeographic Region.	y = 0.2127x + 0.3781 (p<0.05)	y = 0.3892x - 0.1963 (p>0.05)	harbour seal blubber, harbour porpoise blubber, demersal shark liver, fish liver: demersal, flatfish, pelagic and benthic invertebrates whole, brown meat, soft body, demersal invertebrates
	All trophic levels from the Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.	y = 0.3976x – 0.5028 (p<0.05)	y = 0.0948x + 0.8843 (p>0.05)	harbour seal blubber, harbour porpoise blubber, fish liver: demersal, flatfish, pelagic, demersal invertebrates and benthic invertebrates whole, brown meat, soft body

Table 4.10: Calculated TMFs in a food web composed of marine mammals, shark, fish and invertebrates and a food web composed of shark, fish and invertebrates using the traditional and balanced methods.

	Traditional Method		Balanced Method	
BDE	Marine mammals, shark, fish, invertebrates	Shark, fish, invertebrates	Marine mammals, shark, fish, invertebrates	Shark, fish, invertebrates
BDE47	2.1	1.1	1.4	0.4

Table 4.11: 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	
BDE	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
BDE47	1.6	2.5	2.5	1.2

Using the traditional method, BDE47 was found to biomagnify in a food web composed of all trophic levels (Table 4.10), a food web composed of shark, fish and invertebrates (Table 4.10) and the two regional comparisons (Table 4.11). The TMF was higher when marine mammals were included in the analysis (Table 4.10), suggesting that marine mammals accumulate a higher concentration of BDE47 than the lower trophic level organisms (shown in Figure 4.24). As previously discussed, a higher TMF when marine mammals are included is due to the increased proportion of higher brominated PBDE congeners in upper trophic level predators, which are highly persistent and lipophilic, consequently resulting in high concentrations in top predators (Shaw *et al*., 2009) as also observed with the higher chlorinated PCBs 180, 153 and 138. Although BDE47 was found to trophic dilute using the balanced method (Table 4.10), the correlation was not significant ($p>0.05$). This suggests that when a dataset is imbalanced, containing a higher number of lower trophic level organisms, the different degrees of trophic transfer within a sample category can affect the overall TMF result. The balanced method counteracts this difference in trophic level proportion, but a larger dataset is required to establish this with confidence.

The calculated TMF was lower in the Irish Sea Biogeographic Region food web for BDE47 using the traditional method (Table 4.11) than the Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf. There is however a higher sample number of marine mammals from the Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf than the Irish Sea Biogeographic Region. This

higher TMF value is therefore more likely due to the unbalanced dataset and the strong influence of marine mammal accumulation for this BDE congener.

The TMF of the Irish Sea Biogeographic Region food web was higher using the balanced method than the traditional, and vice versa for the Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf (Table 4.11). The calculated TMF was predicted to be higher in the Irish Sea Biogeographic Region as marine mammal (Figure 4.25) and fish (Figure 4.26) samples collected from the Irish Sea Biogeographic Region had a higher concentration of $\Sigma PBDE_9$ in their tissues than those from the other three regions. A similar finding was reported in Section 4.3.2.2 for CBs 138, 153 and 101, where it was concluded that a higher number of marine mammals 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, resulted in a steeper gradient using the traditional method and therefore higher calculated TMF. The geometric mean used for the balanced method TMF remediated this unbalanced proportion of trophic levels, providing a more representative TMF result of the studied regions.

As shown in Figure 4.34a (all calculated trophic levels using the traditional method) and 4.36a (Irish Sea Biogeographic Region food web), it is apparent that the single emaciated harbour seal sample identified as having a different FA profile to the other harbour seal samples and a significantly higher PCB and PBDE concentration in its blubber, has potentially influenced the gradient. Similar to CB153, when investigated further on BDE47, the TMF including marine mammals, shark, fish and invertebrates from all four locations (Figure 4.34) decreased from 2.1 to 1.9 when this sample was removed using the traditional method (Figure A.27a) but showed little change using the balanced method (decreased by 0.1) (Figure A.27b). When marine mammals, shark, fish and invertebrates from the Irish Sea were analysed (Figure 4.36), the TMF decreased from 1.6 to 1.4 using the traditional method (Figure A.28a) and decreased from 2.5 to 1.5 using the balanced method (Figure A.28b). As previously discussed, a larger sample number of harbour seal would be required to fully establish the impact of removing an outlier using both methods.

BDE47 is the most abundant PBDE in biota and is the most studied congener, reported to accumulate in crustaceans, fishes and marine mammals (Hale *et al*, 2003). A study by Pérez-Fuentetaja *et al*., (2015) found that out of ten PBDE congeners, BDE47 had the highest TMF in a food web composed of multiple invertebrates and fish species. The TMF was calculated to be 1.9 when all organisms were included and a TMF of 4.2 was

calculated 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 tropic level. 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 TMFs from these studies are all comparable to the TMFs calculated in this project using the traditional method only.

4.4 Conclusions

In order to calculate a 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.

The concentration of $\Sigma PBDE_9$ across the eighteen of the nineteen sample categories was much lower than that of PCBs with fewer congeners detected, with the highest value of 1,888 µg/kg lw quantified in sperm whale in comparison with 139,800 µg/kg lw ΣPCB_{32} detected in harbour seal. Demersal invertebrates muscle had ΣPCB_{32} concentration <LoD and benthic invertebrates muscle had $\Sigma PBDE_9$ concentration <LoD. When the ICES-7 PCB concentrations in fish were compared to assessment criteria, only CB118 in all fish categories exceeded OSPAR EACs. For the assessment of PBDEs, the $\Sigma PBDE_6$ concentration exceeded the adjusted EQS for each sample category. Neither of the congener concentrations exceed the given FEQG values.

The results presented in this chapter show clear differences in PCB and PBDE patterns between sample categories and species, with differences influenced by physiological processes (metabolism) and eco-biological parameters (region, length, weight, age, habitat and diet). ΣPCB_{32}, $\Sigma PBDE_9$ and congener proportions within the two pollutant classes in marine mammal categories were affected by species difference in metabolic capacity and feeding ecology. Feeding ecology also contributed to the highly variable PCB and PBDE profiles determined in harbour seals and harbour porpoise compared to sperm whales. Within-species variation could also be identified as a result of

physiological condition before death. It is concluded that such animals should be excluded from TMF calculations involving marine mammals.

The variation of ΣPCB_{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 PCB and brominated PBDE congeners than marine mammals due to their lower metabolic capacity to biotransform these compounds. The metabolism of organic contaminants in fish is species-specific, this is a likely contributing factor to variation in this study. The higher trophic level demersal shark had a significantly higher concentration of ΣPCB_{32} in their liver than fish and a higher proportion of higher brominated congeners with a high bioaccumulation potential. Demersal shark also had the least variable PCB and PBDE profile, likely due to their consistent within-species feeding pattern identified in Chapter 3. 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 ΣPCB_{32} in demersal species, where biogeographic region influenced the $\Sigma PBDE_9$ of the shark and fish categories. Benthic invertebrates categories had a similar PCB profile pattern 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 $\Sigma PBDE_9$ in its tissue 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 TMFs is therefore deemed to be important.

The TMF calculation methods were explored to overcome the issue of unbalanced sampling due to opportunistic environmental sampling on the calculation of TMFs: (i) the 'traditional method' of calculating TMFs using concentrations and trophic levels of each individual organism; and (ii) the 'balanced method', based on a regression of geometric mean concentrations and trophic levels. Trophic magnification was found to occur for the ICES-7 PCBs and BDE47 congener using the traditional method, with the highest degree of trophic magnification reported for CB52 (a result of the differential expression of the CYP2B enzyme between harbour seal and harbour porpoise and lack of expression of this enzyme in fish). CB180, 153, 52 and BDE47 were found to have a lower calculated TMF when marine mammals were removed from the dataset due to their identified resistance to biodegradation. The higher TMF of CB138, 118, 101 and

28 when marine mammals were not included in the analysis suggested that these congeners readily accumulate in lower trophic level organisms. An unbalanced dataset was found to influence the TMF of BDE47, where biomagnification occurred using the traditional method and trophic dilution occurred using the balanced method when shark, fish and invertebrates from the four regions were analysed. There was also a difference in the calculated TMFs between both methods for CB28, where trophic magnification was found to occur in all trophic levels from the four regions using the traditional method and trophic dilution using the balanced method and trophic magnification found to occur in the Irish Sea using the traditional method and trophic dilution using the balanced method.

An unbalanced dataset was also found to influence the 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. This was due to the difference in sample numbers of invertebrates and marine mammals between the regions. CB28 possessed 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 establish a significant relationship.

The findings from this study 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 TMF. 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 the TMF is a true indication of biomagnification potential, particularly when conducting regional comparisons when sampling requirements are difficult to achieve.

The data in this study contributes to the understanding of secondary poisoning of PCBs and PBDEs in the marine food web, providing evidence for the inclusion of this exposure mechanism in the development of assessment criteria. This data also contributes to the wider development of TMFs, where selected methods and identified factors influencing

the variation and magnitude of TMFs can be considered for the global diversity of habitats and species distribution.

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Chapter 5

The Concentration and Biomagnification of Trace Metals and Metalloids Across Four Trophic Levels in the Marine Food Web

Anneka Madgett, 2020

5.1 Introduction

The exposure of habitats and the organisms within them to inorganic contaminants, such as trace metals and metalloids, can lead to severe environmental damage and toxic effects (Raknuzzaman *et al.*, 2016; Gu *et al.,* 2017). Heavy metals such as Hg are known to bioaccumulate and biomagnify, leading to the elevated Hg concentrations often observed in high trophic level organisms, including marine predators and long-lived species (Endo *et al*., 2008). In addition to concerns related to environmental damage, such contamination poses a direct toxicological hazard for humans as consumers, for example, piscivorous fish (Damiano *et al*., 2011).

As for the previously discussed organic contaminants, there are a number of key environmental concentration thresholds for inorganic contaminants, created for the risk assessment of hazardous substances in aquatic biota. These include the OSPAR Commission's BACs and EACs and the EU derived EQS. BACs are reported as 35 µg/kg ww for Hg and 26 and 35 µg/kg ww for Cd and Pb. Currently there are no OSPAR EACs available for metals in fish or shellfish, therefore the EC food safety levels are used as EAC proxies for the metals Cd, Hg and Pb in biota (OSPAR, 2016a). The use of dietary standards is not ideal for assessing environmental risk and assessments using criteria should therefore be treated with caution when used to draw conclusions on the environmental impact of the presence of these contaminants in the marine food web.

The WFD was adopted by EU member states to achieve "good chemical status". Bioaccumulative chemicals such as Hg pose a threat to both aquatic wildlife and human health via the consumption of contaminated prey and are defined as "priority substances". Good chemical status will be achieved only when the concentrations of priority substances are maintained below their respective EQS (Tueros *et al*., 2009). EQSs for biota were set at the EU level, to protect against indirect effects and secondary poisoning. Secondary poisoning can arise from the uptake of contaminants from prey via the processes of bioaccumulation and biomagnification (Amiard and Amiard-Triquet, 2015). At present, an EU wide biota EQS value exists only for Hg (20 µg/kg ww) and is related specifically to trophic level four fish. The EQS is in place to protect freshwater and marine ecosystems from the adverse effects of chemicals and to protect human health as a result of dietary exposure to Hg. The EQS for Hg in the WFD is derived from available quality standards relating to the potential for secondary poisoning and is focussed on environmental quality within freshwater and estuarine environments.

An EQS value used in the context of the WFD is, however, not readily extendable to the marine environment and as such its use is acknowledged as an interim solution until a more appropriate approach can be defined and agreed. WFD biota guidance requires data to be adjusted to trophic level four to allow a comparison to the EQS($_{biota}$) (OSPAR, 2016b). However, this is not currently done due to a lack of trophic level information and ecosystem specific TMFs. Generic trophic levels and TMFs can be obtained from the literature and from databases such as Fishbase (Fishbase, 2019), however this approach inevitably adds additional uncertainty to assessments (OSPAR, 2016a). Ecosystem specific TMF and trophic level data are therefore required for the trophic level adjustment of Hg concentrations for comparison to assessment criteria (e.g. EQS).

The current TMF approach assumes that diet is the major route of contaminant exposure and trophic level is the main driver of their accumulation in the food web. As concluded in Chapter 3, in order to conduct effective environmental assessments using TMFs, factors influencing the accumulation within and between trophic levels must be considered (besides appropriate trophic level data). In doing so, our understanding of the complexity of marine systems and contaminant trophic transfer in increased, and the use of nominal TMFs or trophic levels derived from generic databases (e.g. Fishbase) can be reduced. For example, an understanding of the accumulation processes of multiple inorganic contaminants by a selected invertebrate is essential, as is knowledge of that organisms physiology. The metal could be utilised for essential metabolic purposes (Zn, Cu), excreted, stored in the body or could exert a toxic effect (Rainbow, 2002).

There are numerous ecological and physiological factors that might affect metal contamination within a particular organism or species: for example geographic location (Frodello and Marchand, 2001), feeding patterns (Azevedo *et al*., 2020), age and size (Mustafa and Guluzar, 2003; Farkas, Salánki and Specziár, 2011; Yi and Zhang, 2012), sex (Gewurtz, Bhavsar and Fletcher, 2011), the tissue type (Bilandžić *et al.,* 2012) and metabolic rates and capacity (Caurant, Navarro and Amiard, 1996). This chapter examines the variability of concentrations (inter- and intra- species variation) of three priority heavy metals (Hg, Cd and Pb) and six trace metals and metalloids (As, Ni, Se, Zn, Cu and Cr) in the nineteen biotic sample categories collected from different locations around Scotland. The aim of this chapter is to investigate the relationship between metal concentration within an organism or a sample category and key factors which influence those concentrations (trophic level, region, sample categorisation and physiological factors). TMFs were calculated on metals and metalloids identified as possessing a significant trophic relationship and TMFs using two methods as described in the previous

chapter (Borgå *et al*., 2012; Brisebois, 2013) to determine whether biomagnification occurs in the food web and to establish whether the application of TMFs is appropriate for the development of a consistent, trophic specific biota assessment criteria.

5.2 Materials and methods

Detailed information on materials and methods utilised in this chapter are discussed in Chapter 2, Section 2.9.

5.3 Results and discussion

The concentrations of three priority heavy metals (Hg, Cd and Pb) and additional trace metals and metalloids (metals; Cr, Cu, Ni and Zn, metalloids; As, Se) were measured in each of the nineteen sample categories (on a wet weight (ww) basis; Table 5.1) by ICP-MS following digestion as detailed in Chapter 2 (Section 2.9). Mixed effects model analysis, ANOVA Tukey and Pearson's correlation analysis were used to assess the significance of the influence of numerous physiological and ecological variables significantly influencing metal contamination (trophic level, region, sample category, within-category species, age, weight, collection year and length). This process established the analytes requiring further assessment of their potential for trophic dilution or biomagnification.

Table 5.1: Mean concentration range (µg/kg wet weight) of priority heavy metals Mercury (Hg), Lead (Pb) and Cadmium (Cd) and additional trace metals and metalloids Arsenic (As), Chromium (Cr), Copper (Cu), Nickel (Ni), Selenium (Se) and Zinc (Zn) in the muscle, liver, homogenised whole, brown meat, soft body and blubber samples analysed across the nineteen categories. Number of Samples = individuals for mammals and pools for all other categories. The number of individuals per pool are referred to in Table 2.1. Not all the LoD values are to four significant figures to account for instrumental precision.

Sample Category	Number of samples	Hg µg/kg	Pb µg/kg	Cd µg/kg	As µg/kg	Ni µg/kg
Harbour Seal Blubber	10	24.00 – 848.0	<13.6 – 230.0	<4.76 – 11.20	761.0 – 3,720	<8.93– 369.0
Harbour Porpoise Blubber	18	104.0 – 2,310	<13.6	<4.76	1,800 – 9,670	<8.93 – 425.0
Sperm Whale Blubber	5	153.0 - 580.0	18.0 – 127.0	50.90 – 238.0	221.0 – 1,080	16.50 – 85.30
Pelagic Roundfish Whole	3	37.20 – 42.10	<13.6 – 15.20	9.040 – 10.90	5,660 – 6,010	14.00 – 18.00
Pelagic Roundfish Muscle	2	43.30 – 50.60	<13.6	<4.76	4,330 – 6,040	<8.93
Pelagic Roundfish Liver	2	16.30 – 18.10	<13.6	32.80 – 39.20	3,260 – 4,930	16.20 – 21.60
Demersal Shark Muscle	12	262.0 – 990.0	<13.6 – 86.20	<4.76 – 16.10	15,200 – 25,500	<8.93 – 35.80
Demersal Shark Liver	12	24.70 – 201.0	<13.6 – 46.70	55.10 – 569.0	8,580 – 18,500	<8.93 – 20.70
Demersal Roundfish Whole	6	18.90 – 58.20	65.20 – 225.0	20.00 – 251.0	5,510 – 7,640	122.0 – 799.0
Demersal Roundfish Muscle	30	25.90 – 105.0	<13.6 – 50.70	<4.76 – 14.80	1,540 – 42,900	<8.93 – 86.10
Demersal Roundfish Liver	30	5.050 – 51.00	<13.6 – 329.0	4.830 – 179.0	2,410 – 21,000	9.99 – 458.0
Flatfish Muscle	12	20.10 – 341.0	<13.6 – 19.80	<4.76 – 9.460	5,380 – 23,200	<13.00 – 27.50
Flatfish Liver	12	26.20 – 278.0	15.40 – 627.0	63.00 – 645.0	2,940 – 29,300	19.20 – 101.0
Demersal Invertebrates Muscle	2	25.50 – 26.30	<13.6	17.00 ± 25.10	8,070 – 9,260	<8.93 – 20.70
Benthic Invertebrates Whole	11	22.20 – 127.0	194.0 – 8,870	100.0 – 486.0	2,280 – 17,000	131.0 – 1,760
Benthic Invertebrates Muscle	13	23.00 – 273.0	<13.6 – 183.0	24.80 – 300.0	4,590 – 26,200	<8.93 – 299.0
Benthic Invertebrates Brown Meat	3	62.00 – 80.40	<13.6 – 144.0	713.0 – 3,340	8,990 – 11,700	191.0 – 1,140
Benthic Invertebrates Soft Body	17	35.20 – 129.0	157.0 – 7,580	31.70 – 6,920	1,910 – 35,300	183.0 – 3,660
Zooplankton Whole	3	<3.16	63.31 – 112.4	99.76 – 248.7	530.7 – 630.3	107.7 – 148.8

Table 5.1 (continued): Mean concentration range (µg/kg wet weight) of priority heavy metals Mercury (Hg), Lead (Pb) and Cadmium (Cd) and additional trace metals and metalloids Arsenic (As), Chromium (Cr), Copper (Cu), Nickel (Ni), Selenium (Se) and Zinc (Zn) in the muscle, liver, homogenised whole, brown meat, soft body and blubber samples analysed across the nineteen categories. Number of Samples = individuals for mammals and pools for all other categories. The number of individuals per pool are referred to in Table 2.1. Not all the LoD values are to four significant figures to account for instrumental precision.

Sample Category	Number of samples	Cr µg/kg	Cu µg/kg	Se µg/kg	Zn µg/kg
Harbour Seal Blubber	10	<30.6 – 612.0	98.00 – 516.0	188.0 – 598.0	1,460 – 8,730
Harbour Porpoise Blubber	18	<5.20	<83.90 – 1,270	614.0 – 3,670	1,280 – 49,200
Sperm Whale Blubber	5	65.20 – 364.0	146.0 – 535.0	221.0 – 1,080	2,500 – 11,200
Pelagic Roundfish Whole	3	<30.6	478.0 – 625.0	379.0 – 388.0	15,100 – 17,600
Pelagic Roundfish Muscle	2	<30.6	416.0 – 500.0	336.0 – 348.0	2,070 - 2,110
Pelagic Roundfish Liver	2	<30.6	863.0 – 991.0	459.0 – 523.0	5,500 – 8,740
Demersal Shark Muscle	12	<30.6 – 109.0	213.0 – 469.0	315.0 – 747.0	6,160 – 13,100
Demersal Shark Liver	12	<30.6 – 306.0	805.0 – 6,660	352.0 – 903.0	3,550 – 11,100
Demersal Roundfish Whole	6	55.80 – 271.0	724.0 – 5,370	504.0 – 867.0	10,200 – 38,000
Demersal Roundfish Muscle	30	<30.6 – 115.0	147.0 – 501.0	331.0 – 2,780	2,940 – 14,100
Demersal Roundfish Liver	30	<30.6 – 462.0	864.0 – 4,790	246.0 – 1,770	10,100 – 36,500
Flatfish Muscle	12	<30.6 – 112.0	122.0 – 1,200	210.0 – 1,940	5,070 – 11,100
Flatfish Liver	12	<30.6 – 95.40	826.0 – 6,730	318.0 – 1,810	22,700 – 52,100
Demersal Invertebrates Muscle	2	<30.6	2,310 – 3,060	303.0 – 392.0	11,800 – 14,100
Benthic Invertebrates Whole	11	145.0 – 1,390	917.0 – 4,860	321.0 – 1,440	38,000 – 105,000
Benthic Invertebrates Muscle	13	<30.6 – 368.0	5,980 – 16,900	491.0 – 1,230	12,200 – 79,200
Benthic Invertebrates Brown Meat	3	<30.6 – 111.0	19,100 – 68,400	1,150 – 2,280	16,600 – 55,800
Benthic Invertebrates Soft Body	17	60.60 – 408.0	4,090 – 65,900	475.0 – 2,090	23,500 – 341,000
Zooplankton Whole	3	<30.6 – 42.53	106.2 – 858.84	240.7 – 357.2	4,429 – 11,946

Zn has the highest maximum concentration across the sample categories. The most abundant metals/metalloids are Zn, As and Cu, with concentrations above 40,000 µg/kg ww. The remaining have concentrations below 10,000 µg/kg ww (Table 5.1).

Figure 5.1 shows the proportion of each category making up the sample category profile for each metal/metalloid. Metal distribution (proportion) is not common across all sample categories, indicating category-specific accumulation. The highest sample category concentration of any metal/metalloid was 341,000 µg/kg ww for Zn found in benthic invertebrates soft body, although there was a wide inter-species variation in this category (Table 5.1). Whilst Cu, Se and Zn were found in all samples tested, Pb and Cr, were often not detectable (Table 5.1). Figure 5.1 shows that the benthic invertebrates soft body is the dominant category for Pb, Cd, Cu, Ni and Cu, with Cu being greater than 50%, suggesting that metal accumulation in these categories is not related to trophic level.

Of the priority heavy metals, preferential accumulation of Hg in harbour seal, harbour porpoise and demersal sharks can be seen on Figure 5.1. Cd tended to accumulate to a greater extent in the benthic invertebrates soft body category although there were large inter-species differences within the category, ranging from 31.70 µg/kg ww in swimming crab to 6,920 µg/kg ww in horse mussel (Table 5.1).

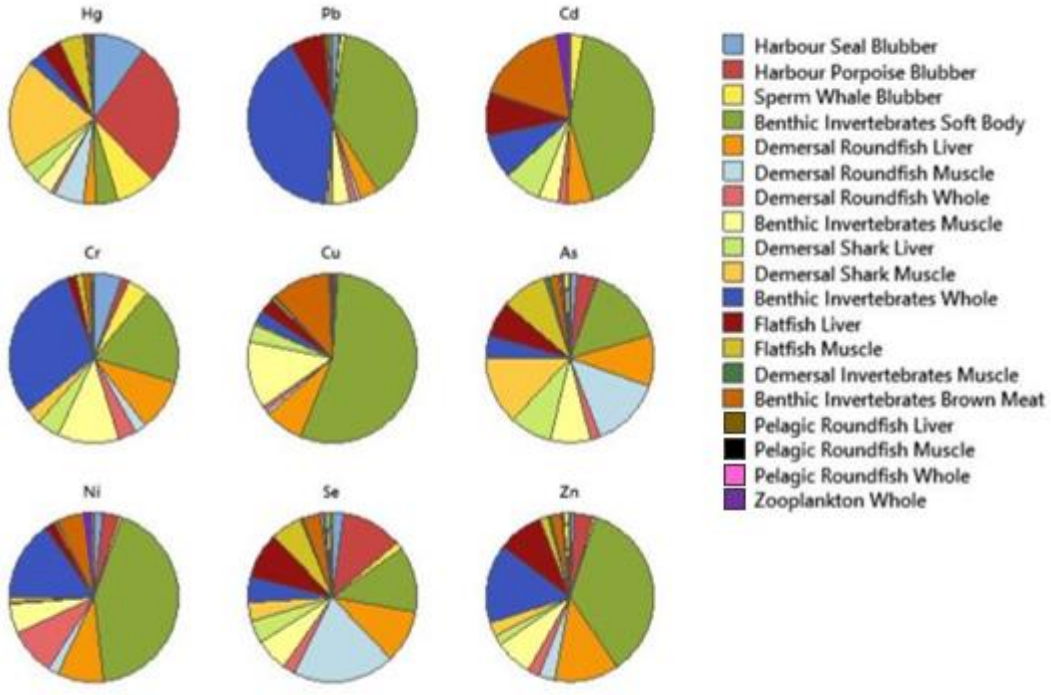

Figure 5.1: Pie charts showing the proportion of each category for making up the category profile for each metal/metalloid.

5.3.1 Tissue selection

Metal absorption by organisms and tissues dependant accumulation is influenced by a variety of factors, often directly relating to the physiology and metabolism of the animal concerned. Elemental concentrations between different tissues in a single organism or species can vary significantly due to tissue function and chemical affinity of contaminants to biomolecules (Aguilar, Borrell and Pastor, 1999). Therefore, before comparing sample categories and conducting mixed effects model analysis, it is necessary to select the appropriate tissue type for each shark and fish category.

5.3.1.1 Marine mammals

Previous studies on the distribution of heavy metals, trace metals and metalloids in marine mammals tissues have demonstrated that these substances preferentially accumulate in the liver (Hong *et al.,* 2012; Aubail *et al.,* 2013; Hansen *et al.,* 2015; Salgado, Más-Rosa and Azavedo, 2018). As a storage and detoxification organ, the liver is important in the binding of toxic, non-essential trace elements and the physiological regulation of essential trace elements (Hansen *et al*., 2015; Kershaw and Hall, 2019). However, for the marine mammals in this study, only blubber samples were available for testing.

A previous study on Hg tissue distribution in cetaceans (bottlenose dolphins, striped dolphins and Risso's dolphins) found concentrations in the liver to be 11- 34 times higher than in other tissues (brain, kidney, blubber, muscle, blood, skin, teeth and earplugs) (Bilandžić *et al.,* 2012). The same is true for the other trace metals and metalloids, which have also been found at higher concentrations in the liver of marine mammals than tissues such as kidney, heart, brain, lung and muscle (Baraj *et al.,* 2009; Martínez-López *et al*., 2019).

Blubber was, however, the only available tissue for marine mammals selected for inclusion in this project. As a result, marine mammal data for inorganic contaminants will not be included in the dataset when calculating TMFs. However, the data presented here contribute to developing a baseline for the recorded Hg concentrations present in blubber which can be used as an alternative when the liver is not available.

5.3.1.2 Shark and fish

Fish are exposed to inorganic Hg and MeHg in both water and food. Uptake of MeHg from diet accounts for approximately 80% to 90% of total uptake, with the remaining fraction coming from water (Hall *et al*., 1997; Hrenchuk *et al*., 2003). In fish muscle

tissue, >95% of Hg is present as MeHg (Bloom, 1992), most likely bound to cysteine residues in proteins (Harris, Pickering and George, 2011), making it a suitable tissue for the assessment of this Hg in fish. Cd and Pb is reported to accumulate in the liver (Agusa *et al.,* 2005; Bat *et al*., 2015). This is in agreement with this study, where concentrations of Hg in demersal shark muscle (262.0 – 990.0 µg/kg ww), pelagic roundfish muscle (43.30 – 50.60 µg/kg ww), demersal roundfish muscle (25.90 – 105.0 µg/kg ww) and flatfish muscle (20.10 – 341.0 µg/kg ww) were significantly higher than in the liver tissue (demersal shark liver 24.70 – 201.0 µg/kg ww; pelagic roundfish liver 16.30 – 18.10 µg/kg ww; demersal roundfish liver 5.050 – 51.00 µg/kg ww; flatfish liver 26.20 – 278.0 µg/kg ww; ($p<0.05$, ANOVA, Tukey); Table 5.1).

The distribution of As was similar to Hg, where demersal shark muscle (15,200 – 25,500 µg/kg ww), pelagic roundfish muscle (4,330 – 6,040 µg/kg ww), demersal roundfish muscle (1,540 – 42,900 µg/kg ww) and flatfish muscle (5,380 – 23,200 µg/kg ww) had a significantly higher concentration of As in their muscle tissue than in the liver (demersal shark liver 8,580 – 18,500 µg/kg ww; pelagic roundfish liver 3,260 – 4,930 µg/kg ww; demersal roundfish liver 2,410 – 21,000 µg/kg ww; flatfish liver 2,940 – 29,300 µg/kg ww; ($p<0.05$, ANOVA, Tukey); Table 5.1). Previous studies report mixed results on the tissue speciation of As. Gieter, *et al.,* (2002) found that As concentrations were higher in fish muscle and shellfish species from the North Sea, whilst studies by Suner, *et al*., (1999) and Gao, *et al.,* (2018) found that fish liver, where the biotransformation of inorganic As to organic As compounds takes place (e.g. methylarsenate), accumulated to a greater extent than in muscle tissue. In this study, mixed effects model analysis will use muscle concentrations of Hg and As of shark and fish (pelagic, demersal and flatfish) for assessment.

The concentration of Pb, Cd, Cr, Cu, Ni and Zn was significantly higher in demersal roundfish liver, flatfish liver and pelagic roundfish liver than the corresponding muscle tissue ($p<0.05$, ANOVA, Tukey); Table 5.1). Se concentrations were significantly higher in the muscle of demersal roundfish (331.0 – 2,780 µg/kg ww) compared to liver tissue (246.0 – 1,770 µg/kg ww). For pelagic roundfish, Se concentrations were significantly higher in liver tissue (459.0 – 523.0 µg/kg ww) than in muscle (336.0 – 348.0 µg/kg ww) ($p<0.05$, ANOVA, Tukey); Table 5.1). There was no significant difference in Se concentration between flatfish liver (318.0 – 1,810 µg/kg ww) and muscle (210.0 – 1,940 µg/kg ww) ($p<0.05$, ANOVA, Tukey); Table 5.1).

Demersal sharks had a different metal/metalloid tissue profile, where the concentration of Cd, Zn and Cu was significantly higher in the liver tissue than the corresponding

muscle tissue and there was no significant difference between the muscle and liver tissue on Pb, Cr, Ni and Se concentration ($p<0.05$, ANOVA, Tukey); Table 5.1).

Whole tissue was not available for demersal shark and flatfish for a comparison to other tissue types and categories. On this basis, mixed effects model analysis will be conducted in the liver tissue of shark and fish (pelagic, demersal and flatfish) for Pb, Cd, Se, Zn, Cu, Ni and Cr for consistency.

5.3.1.3 Invertebrates

Demersal invertebrates muscle, benthic invertebrates soft body and benthic invertebrates whole were analysed as the tissues are separated for sample quantity purposes and sample category includes a range of different species. Benthic invertebrates brown meat was extracted from European lobster (n=1; made up of 9 individuals) and edible crab (n=2). Brown meat is found in the shell cavity at the top of the crab and is composed of the hepatopancreas which has long been reported to contain relatively high metal concentrations (particularly Cd and Pb), generally well above levels measured in the muscle from legs and claws (Davies, 1981; Barrento *et al*., 2009a; Barrento *et al.,* 2009b; Noël *et al*., 2011; Bolam *et al.,* 2016). The presence of Cd in the crustacean brown meat is a public health concern in many countries worldwide. The assessment of the risks and benefits of human consumption remains challenging and controversial and, official legal exposure limits are still not in place (Ervik, Lierhagen, and Asimakopoulosb, 2020). Legal limits are, however, in place for white meat (muscle) (EU, 2004; EU, 2006; EU, 2011) and the EU maximum level for Cd in crustaceans, excluding the brown meat of crab and the head and thorax of lobster and similar large crustaceans, is 0.5 mg/kg ww. Both brown meat and muscle tissue were analysed due to the lack of tissue specific ecotoxicological data for assessment purposes.

5.2.2. Metal and metalloid concentrations and trophic magnification

TMFs were calculated using the traditional (Borgå *et al.,* 2012; OSPAR, 2016b) and balanced (Brisebois, 2013) methods descried in Chapter 4, Section 4.3.2.2, following the guidance by Kidd *et al*., (2019).

The pie chart showing the proportion of each category for making up the category profile of each metal/metalloid (Figure 5.1) indicated that of the three priority heavy metals, Hg distribution appeared to be more correlated with trophic level.

Mixed effects model analysis was used to determine the interaction between concentration and trophic level, sample category and region. As a result of this analysis, five metals were shown to have a significant relationship with trophic level (Zn, Hg, Cu, Ni and Cd) and will be considered for further analysis of trophic magnification/dilution. Metals whose accumulation is highly influenced by ecological and physiological factors (such as region and sample category) need to be assessed to reduce within category variation as much as possible when calculating TMFs.

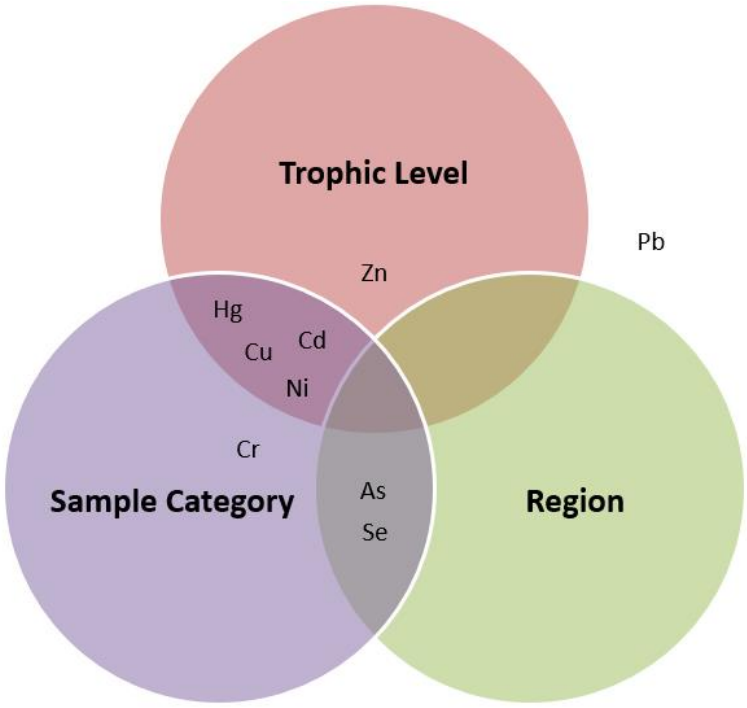

Figure 5.2: Venn diagram showing the output of mixed effects model analysis conducted on demersal shark, fish (demersal, pelagic, flatfish), invertebrates (benthic and demersal) and zooplankton, where the concentration of eight metals and metalloids (Zn, Hg, Cd, Cu, Ni, Cr, As and Se) (µg/kg ww) have a relationship with the three variables (trophic level, sample category and region). Pb sits outside all categories showing no relationship to the three variables. The greatest overlap was between Trophic Level and Sample Category.

Mixed effects model analysis revealed that Zn concentration had a statistically significant relationship with trophic level ($p<0.05$) while Hg, Cu, Ni and Cd concentrations had a statistically significant relationship with both sample category and trophic level ($p<0.05$). Cr had a statistically significant relationship with sample category while As and Se had a statistically significant relationship with sample category and region. There was no significant relationship between Pb concentration and sample category, trophic level or region ($p>0.05$).

Calculating the TMF of trace metals is more complex than that of PCBs and PBDEs, as metals such as Zn, Cu and Ni are essential elements and required for an organism's natural physiological processes and there will be interspecies differences (Rainbow, 2003). The sources of between and within category variance for metals shown to be associated with trophic level (Figure 5.2; Hg, Cd, Cu, Ni, and Zn) were identified. The muscle of shark and fish were used for comparisons for Hg and As and liver used for comparisons for Cd, Cu, Ni and Zn, based on the results from Section 5.2.1.

For metals with a trophic relationship (Figure 5.2), metal concentrations were plotted using both the traditional method (Log_{10} [metal concentration] against trophic level) and the balanced method (geometric mean Log_{10} [metal concentration] against geometric mean trophic level) to enable the determination of the TMF for that metal. To the author's knowledge, no previous studies have investigated the TMF of trace metals using the balanced method.

5.2.2.1 Mercury

The pie chart showing the proportion of each category for making up the category profile of each metal/metalloid (Figure 5.1) showed that of the priority heavy metals, Hg had a different distribution than the other eight metals and metalloids.

Figure 5.3 shows the logarithmically transformed Hg concentration in shark and fish muscle and demersal and benthic invertebrates. Demersal sharks, being a predatory species, have a significantly higher Hg concentration in their tissues than the other sample categories ($p<0.05$, ANOVA, Tukey) (Figure 5.3). Predatory species such as marine mammals and shark are good indicators for the monitoring of elemental contamination in the marine environment due to their high trophic level position and long lifespan (Bellante, *et al*., 2011). Most shark species accumulate high concentrations of Hg through their diet which then accumulates in their muscle tissue (Branco *et al*., 2007; Rumbold *et al.,* 2014). This data suggests the trophic relationship identified by the mixed model analysis is a biomagnification effect. Demersal sharks had little variation in trophic level (Figure 3.8; Table 3.7) and within-species dietary pattern (Chapter 3, Figures 3.5 and 3.7), which could explain the low degree of variation of Hg concentration in this category (Table 5.1).

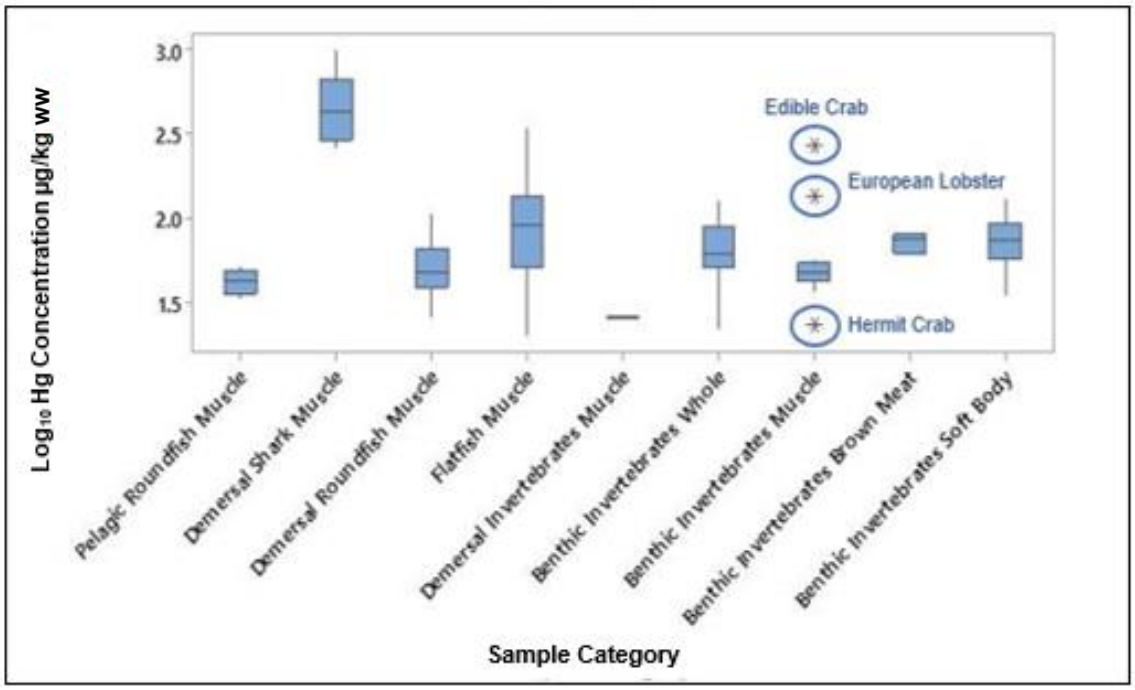

Figure 5.3: Box plot of Log_{10} Hg concentration (µg/kg ww) in nine sample categories (demersal shark and fish muscle, demersal invertebrates and benthic invertebrates). Marine mammals were not included in the analysis due to their selected tissue and zooplankton was not included in the analysis as the Hg concentration was below the LoD. One edible crab, one European lobster and one hermit crab sample pool were identified as data outliers ($p<0.05$). Error bars are to one standard deviation.

The flatfish muscle category has a significantly higher Hg concentration than the other fish and invertebrate categories ($p<0.05$, ANOVA, Tukey). This is to be expected as flatfish are directly exposed to near-shore seabed sediments which act as a sink for heavy metals from inputs such as river run-off and atmospheric depositions (Fergusson, 1990; Förstner and Müller, 1981; Glasby and Szefer, 1998; Szefer, 2002; França *et al.,* 2005).

Flatfish muscle had a larger Hg concentration range than the other fish muscle and invertebrates categories (Figure 5.3), ranging from 20.1 – 341.0 µg/kg ww; Table 5.1). On further investigation, it was found that flatfish from the Irish Sea Biogeographic Region (Solway Firth) had a significantly higher concentration of Hg in their muscle (158.0 – 341.0 µg/kg ww; n=2), than those from the Northern North Sea (Moray Firth) (89.80 – 131.0; n=3) and the Scottish Continental Shelf (Burra Haaf) (20.10 – 138.0 µg/kg ww; n=7) ($p<0.05$ ANOVA, Tukey; Figure 5.4). Although the sample size is small, this provides an indication of a localised regional influence on Hg concentration in flatfish. Physiological features such as average pool age, weight and length were not found to significantly influence the Hg concentration in the separate flatfish muscle and demersal roundfish muscle categories ($p>0.05$).

The Irish Sea Biogeographic Region (particularly the Clyde) is a contaminated region due to nearby industrial and wastewater discharges (UKMMAS, 2010). The OSPAR Intermediate Assessment 2017 (OSPAR, 2017) found that Hg concentrations in biota were at or above background concentrations (BAC: 35 µg/kg ww in fish) in all of the assessment areas (304 monitoring sites across 12 assessment areas), with the highest concentrations found in the Norwegian Trench, Northern North Sea, Southern North Sea and Irish Sea Biogeographic Region, at around twice the BAC. Eight out of the eleven flatfish sample pools in this project exceed the BAC; four sample pools from the Scottish Continental Shelf (50.90 – 87.80 µg/kg ww), two sample pools from the Irish Sea Biogeographic Region (173.0 – 278.0 µg/kg ww) and two sample pools from the Northern North Sea (67.70 – 77.70 µg/kg ww).

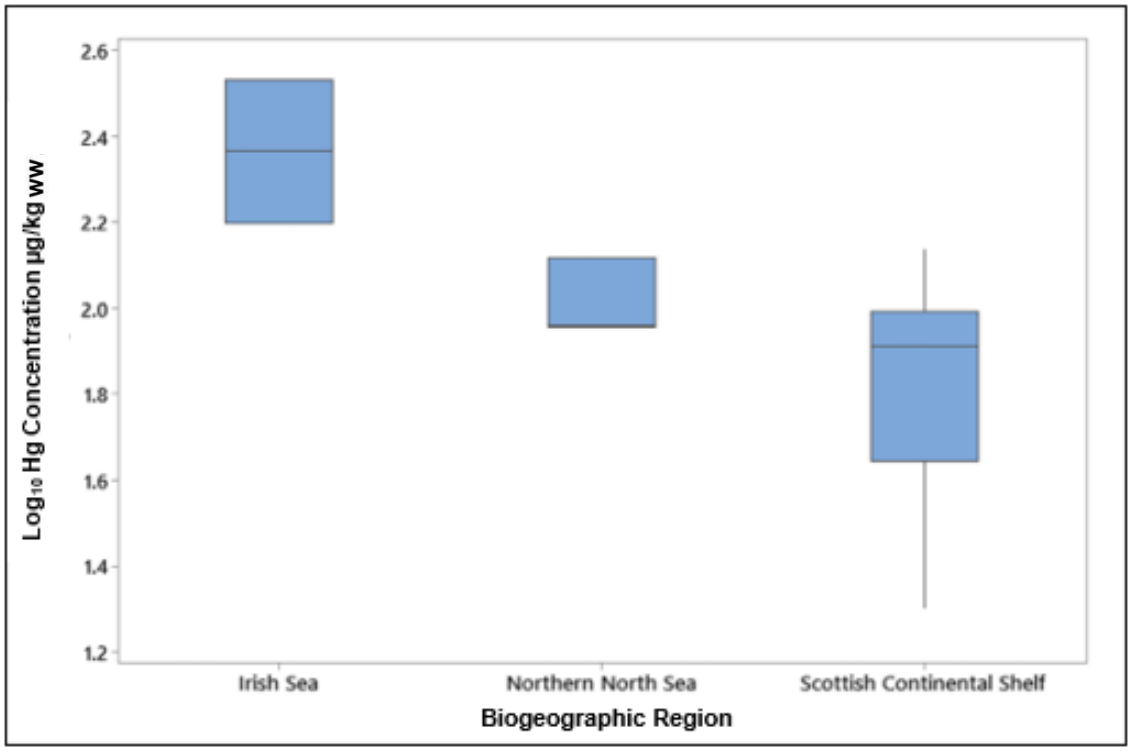

Figure 5.4: Box plot comparing the sample distributions of Log_{10} Hg Concentration µg/kg ww in each biogeographic region. Flatfish collected from the Irish Sea Biogeographic Region (n=2) have a significantly higher concentration of Hg in their muscle than those collected from the Northern North Sea (n=3) and Scottish Continental Shelf (n=7) ($p<0.05$). Error bars are to one standard deviation.

The EU wide biota EQS for Hg represents a concentration in fish at which birds and mammals are protected against effects of Hg via secondary poisoning i.e. the uptake of contaminants from prey. The maximum recommended concentration for Hg in biota of 20 µg/kg ww, expressed as total Hg, was set in Directive 2013/39/EU. The mean Hg concentration (ww) in the muscle tissue of demersal roundfish, pelagic roundfish and flatfish, the whole tissue of demersal roundfish and pelagic roundfish and liver tissue of

demersal roundfish and flatfish exceed the EQS concentration (Table 5.1 and Figure 5.5). It is recognised that the EQS for Hg refers only to whole fish and an accepted tissue-whole organism conversion factor is not currently available. Figure 5.5 shows that the only mean concentration found to be lower than the EQS was pelagic roundfish liver, with a mean concentration of 17.2 ± 0.9 µg/kg ww. The trophic adjusted Hg concentration for shark and fish muscle and invertebrates using calculated TMFs for comparison to EQS is discussed below (Table 5.3) in the trophic magnification Section.

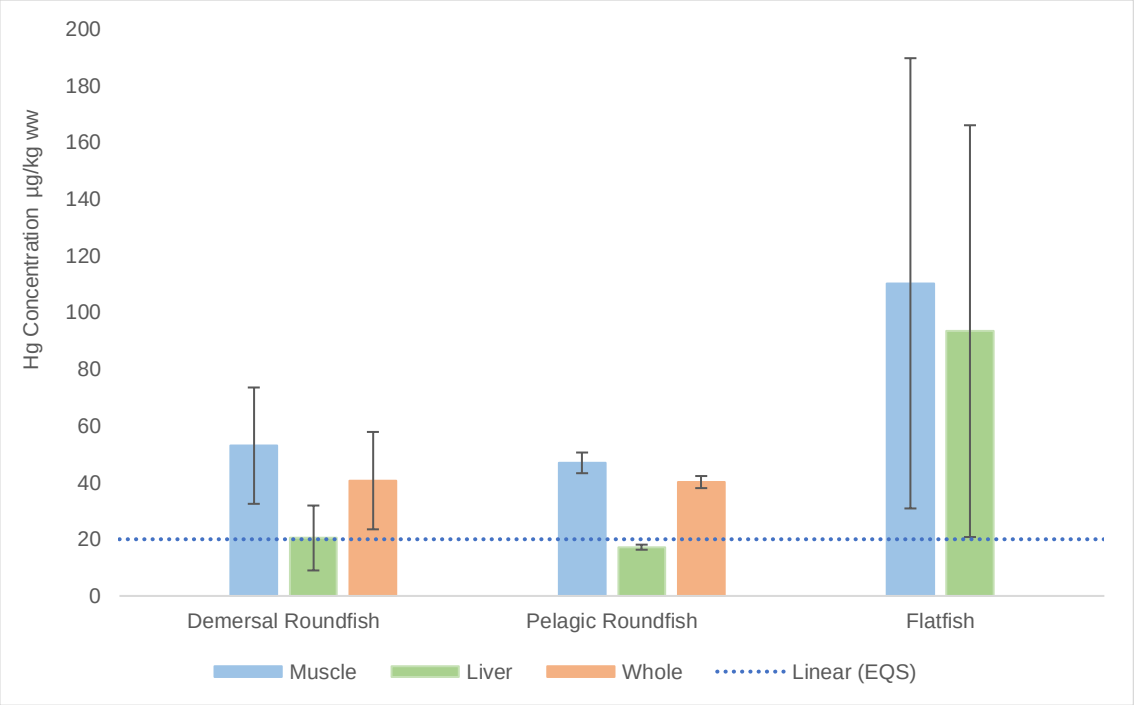

Figure 5.5: Bar graph showing the Hg concentration (µg/kg ww) in the tissue of each fish category (demersal roundfish, pelagic roundfish and flatfish) in comparison to the $EQS_{(biota)}$ (horizontal dashed trendline). Error bars are to one standard deviation. Flatfish were analysed using their muscle and liver tissues only as whole tissue was not available for this category.

When shark, fish and invertebrates were analysed (Figure 5.3), two outliers were identified within the benthic invertebrates muscle category ($p<0.05$). Pooled edible crab (n=1; made up of 14 individuals) and pooled European lobster (n=1; made up of 9 individuals) had a significantly higher concentration of Hg in their muscle than the other benthic invertebrate muscle category species ($p<0.05$, ANOVA, Tukey; Figure 5.6). Noël, *et al*., (2011) determined the Hg, Cd and Pb concentrations in white meat (muscle) and brown meat of crustaceans collected in France and found the concentration of Hg in three lobster brown meat and muscle samples to range from 43.00 – 65.00 µg/kg ww and 115.0 – 148.0 µg/kg ww, respectively. In this project, the concentration of Hg in the

one pooled lobster sample was comparable with brown meat, containing 62.00 µg/kg ww, and higher in white meat (muscle), containing 273.0 µg/kg ww.

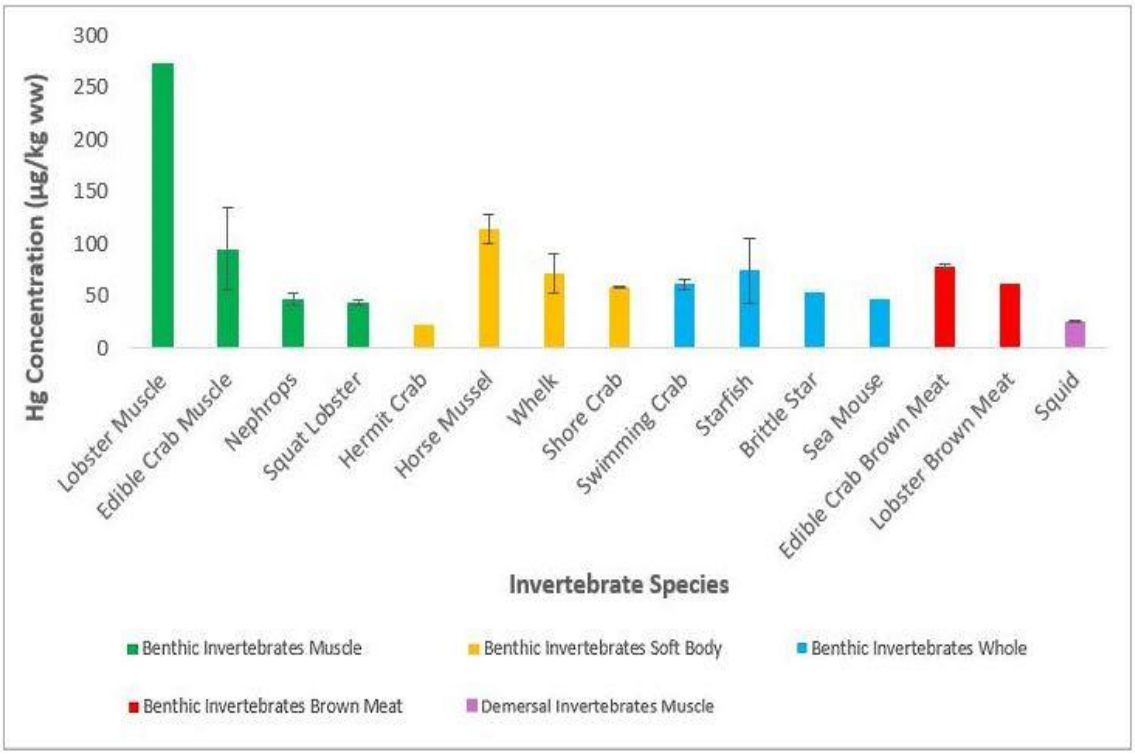

Figure 5.6: Bar graph showing the Hg concentration (µg/kg ww) in the tissue of each invertebrate species (benthic and demersal). Error bars are to one standard deviation. Lobster muscle, hermit crab, brittle star, sea mouse and lobster brown meat do not have error bars as there is only one sample pool available.

Mixed effects model analysis revealed a statistically significant relationship between Hg concentration and trophic level and sample category. Pooled demersal shark samples had a significantly higher liver concentration of Hg than the other fish and invetrebrate species (Figure 5.3) ($p<0.05$, ANOVA, Tukey). On this basis, the TMF of Hg will be calculated both including (Figures 5.7) and excluding (Figure 5.8) demersal shark muscle using both the traditional (a) and balanced (b) methods. This will determine whether Hg biomagnifies in the marine food web studied in this project and will identify categories that significantly influence the calculated TMF. The calculated TMF for Hg from Figures 5.7 – 5.8 are shown on Table 5.1.

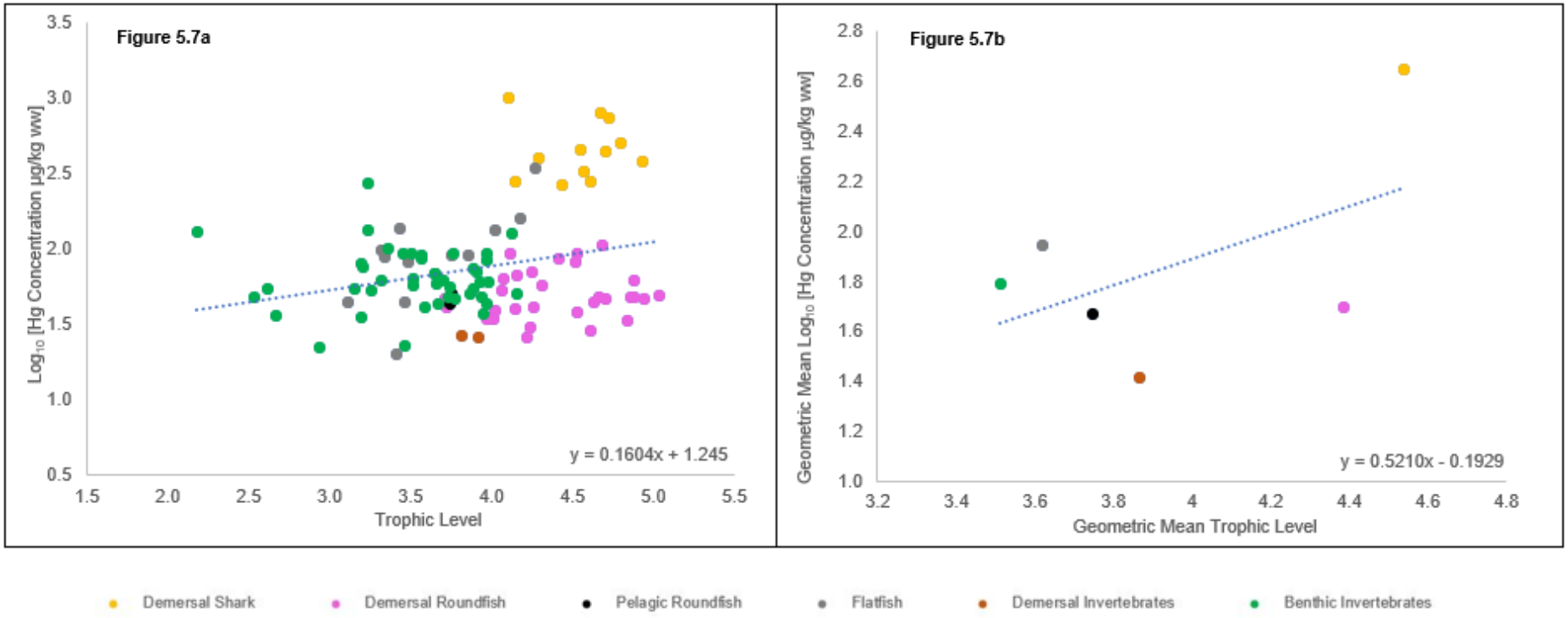

Figure 5.7: (a) Relationship between trophic level and logarithmically transformed Hg concentration (µg/kg ww) in demersal shark muscle (yellow), fish muscle: demersal (pink), flatfish (grey), pelagic (black) and Invertebrates: demersal invertebrate muscle (brown), and benthic invertebrate whole, muscle, brown meat, soft body (green). **(b)** Relationship between geometric mean trophic level and logarithmically transformed geometric mean Hg concentration (µg/kg ww) in demersal shark muscle (yellow), fish muscle: demersal (pink), flatfish (grey), pelagic (black) and Invertebrates: demersal invertebrate muscle (brown) and benthic invertebrate whole, muscle, brown meat, soft body (green).

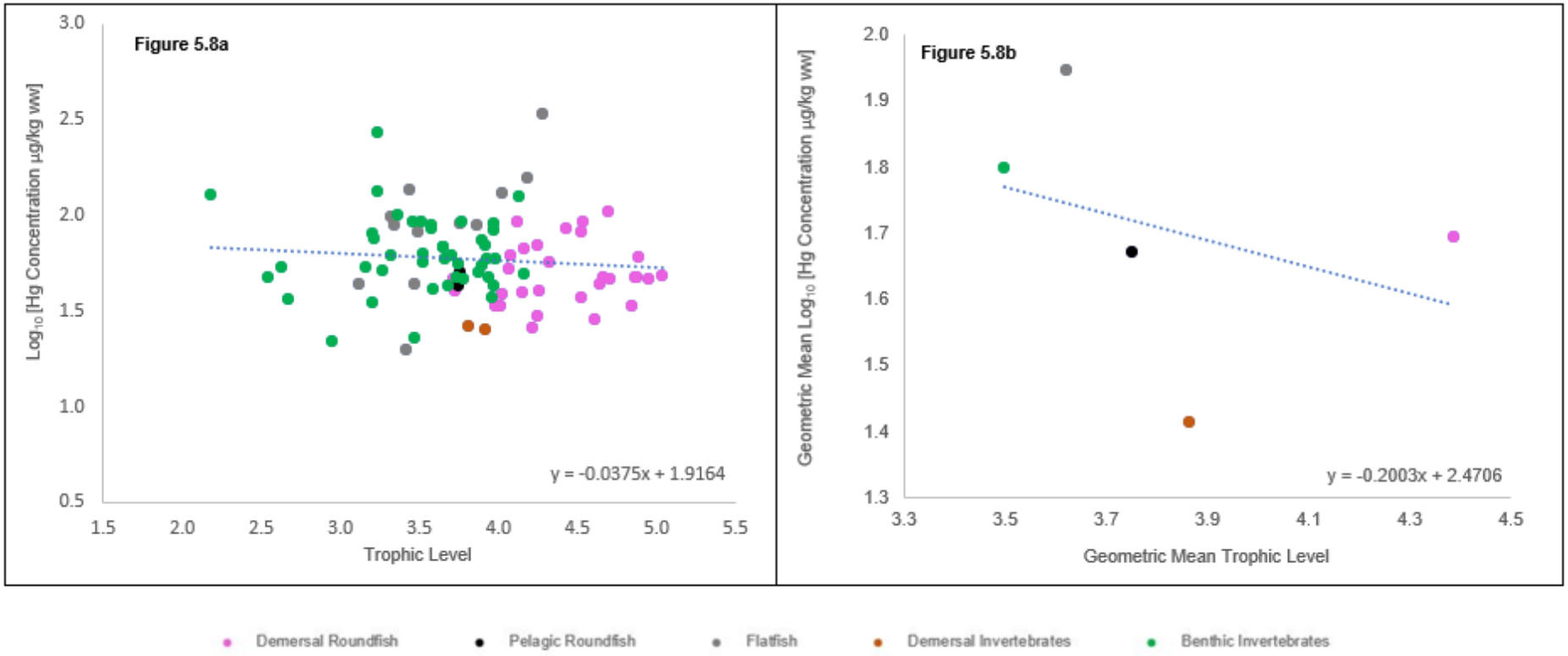

Figure 5.8: (a) Relationship between trophic level and logarithmically transformed Hg concentration (µg/kg ww) in fish muscle: demersal (pink), flatfish (grey), pelagic (black) and Invertebrates: demersal invertebrate muscle (brown) and benthic invertebrate whole, muscle, brown meat, soft body (green). **(b)** Relationship between geometric mean trophic level and logarithmically transformed geometric mean Hg concentration (µg/kg ww) in fish muscle: demersal (pink), flatfish (grey), pelagic (black) and Invertebrates: demersal invertebrate muscle (brown), and benthic invertebrate whole, muscle, brown meat, soft body (green).

Table 5.2: Calculated TMFs of Hg using the traditional and balanced methods in a food web composed of demersal shark, fish and invertebrates and a food web composed of fish and invertebrates.

Traditional method		Balanced method	
All trophic levels (p<0.05)	**Excluding demersal shark (p<0.05)**	**All trophic levels (p>0.05)**	**Excluding demersal shark (p>0.05)**
1.4	**0.9**	**3.3**	**0.6**

Hg was found to biomagnify when demersal shark, fish and invertebrates were analysed, using the traditional method and balanced method (Table 5.2). When upper trophic level demersal shark was not included in the analysis, the calculated TMF changed from trophic magnification to trophic dilution using both methods (Table 5.2). This shows the influence of the presence of higher trophic level predators when calculating the TMF of Hg and suggests that Hg does not biomagnify in a marine food web composed of lower trophic level organisms. Figure 5.8b shows that flatfish and benthic invertebrates have a much higher concentration of Hg in relation to trophic level in comparison to the other categories. A similar finding was reported by Kasper *et al*., (2009) where inorganic Hg concentrations decreased with the increase of trophic level (across four trophic levels), due to the feeding habits of detritivores being closely associated with the bottom sediment.

Although the overall biomagnification tendency is the same between both methods (Table 5.2), the balanced method TMFs were higher for Hg when shark, fish and invertebrates were analysed. There was also a larger difference between trophic magnification and dilution using the balanced method, showing the importance of a balanced dataset when calculating TMF, which from these findings is recommended when calculating the TMF of Hg in food webs composed of higher trophic level predators.

The correlation was however not significant (p>0.05) when using the balanced method for both calculated TMFs (Table 5.2), suggesting that a larger dataset is required to sufficiently test significance using a balanced dataset. A similar result was seen in Chapter 4 for CB101, 118 and BDE47, where the balanced method was found to influence the significance of the regression, and in some cases change the overall finding from biomagnification (using the traditional method) to trophic dilution (using the balanced method).

The TMFs reported in this Chapter using the traditional method are lower than what has been calculated in previous global studies, where an average TMF of 7.0 ± 4.9 was reported for a number of sites and ecosystems in Lavoie *et al*., (2013). A study in Mason

Bay, Korea (Kim *et al.,* 2012) reported a TMF of 2.5 for the magnification of Hg using fish species, polychaete, bivalves, crustacean and cephalopod and a TMF of 2.8 was reported in a study on the continental shelf where a food chain composed of zooplankton, crustaceans, all bony fish and squid groups (Pethybridge *et al*.,2012).

The latest EC guidance on the implementation of the biota EQS states that the EQS is set for animals of trophic level 4 and that concentration data should be corrected using TMFs before comparison (OSPAR, 2016b). The Hg EQS used presents difficulties as it is set at a level (20 µg/kg ww) which is close to or below the BAC of both the OSPAR region for mussels (18 µg/kg ww) and fish muscle (35 µg/kg ww) (Robinson *et al.,* 2017). The Hg concentration of shark and fish muscle and invertebrates was corrected (Equation 5.1; OSPAR, 2016b) using the TMF (all trophic levels to represent the food web) obtained by both methods for a comparison to the Hg EQS (Table 5.3).

$$Conc_{TL\text{-}adj} = Conc_{biota} * TMF^{(4-TL(x))} \qquad \textbf{Equation 5.1}$$

Table 5.3: The trophic adjusted (corrected) Hg concentrations using the TMFs obtained using the traditional method (1.4) and balanced method (3.3) analysing shark, fish and invertebrates (Table 5.2).

Category	Sample number	Mean trophic level (Table 3.7)	Mean Hg µg/kg ww	Trophic adjusted µg/kg ww TMF (1.4)	Trophic adjusted µg/kg ww TMF (3.3)
Demersal Shark Muscle	12	4.55	486.5	**403.8**	253.0
Pelagic Roundfish Muscle	2	3.75	46.95	51.18	63.38
Demersal Roundfish Muscle	30	4.40	53.02	46.13	32.87
Flatfish Muscle	12	3.64	110.3	124.6	169.9
Demersal Invertebrates Muscle	2	3.87	25.90	26.94	30.30
Benthic Invertebrates Whole	11	3.42	69.85	85.22	139.7
Benthic Invertebrates Muscle	13	3.68	69.08	76.68	101.5
Benthic Invertebrates Brown Meat	3	3.24	72.87	94.00	180.7
Benthic Invertebrates Soft Body	17	3.53	71.35	83.48	124.9

Table 5.3 shows that the original ww concentrations and the corrected concentrations using the TMFs obtained by both methods are all above the Hg biota EQS (20 µg/kg ww) (Table 5.3). The TMF calculated using the traditional method has only slightly adjusted the original ww concentrations but the balanced dataset has had more of an influence on the values, halving the original ww concentration of demersal shark and doubling the original ww concentration of benthic invertebrates brown meat. Although the current EQS for Hg is considered an interim solution to address the need for assessment criteria

until a more appropriate approach can be defined and agreed, this data has shown that trophic adjustment using a balanced dataset TMF has an influence on the adjusted concentration and is highly recommended for a true indication of secondary poisoning by biomagnification.

5.2.2.2 Cadmium

Figure 5.9 shows the logarithmically transformed Cd concentration in shark and fish liver, demersal and benthic invertebrates and zooplankton. Benthic invertebrates brown meat, soft body and zooplankton had a significantly higher Cd concentration in their tissues than the other sample categories ($p<0.05$, ANOVA, Tukey). This suggests a species-specific accumulation of Cd in the food web rather than concentrations as a result of biomagnification.

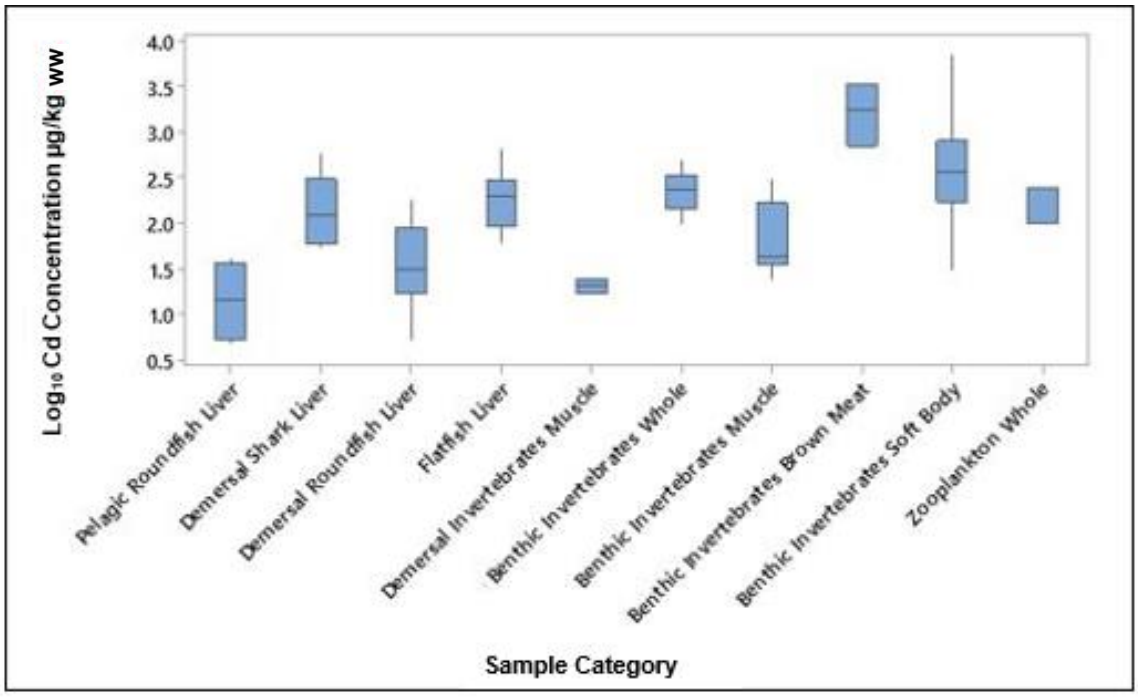

Figure 5.9: Box plot of Log_{10} Cd concentration (µg/kg ww) in ten sample categories (demersal shark, fish muscle, demersal invertebrates, benthic invertebrates and zooplankton (marine mammals were not included in the analysis due to their selected tissue). Error bars are to one standard deviation.

Figure 5.9 shows that benthic invertebrates soft body has a large degree of variation of Cd concentration in comparison to the other fish, invertebrates and zooplankton categories, ranging from 31.70 µg/kg in pooled swimming crab to 6,220 µg/kg in pooled horse mussel (Table 5.1).

A regional comparison on the benthic invertebrates soft category showed there was no regional influence within this category on both a biogeographic (Figure 5.10) or localised

level (Figure 5.11). A larger dataset is however required for a comprehensive analysis as there was only one benthic invertebrate soft body pool from Hunterston, Moray Firth and Pladda. There was a within category species influence on Cd concentration where horse mussel (n=2) had a significantly higher concentration of Cd in its tissues than whelk (n=7), swimming crab (n=6) and shore crab (n=2) ($p<0.05$, ANOVA, Tukey) (Figure 5.12).

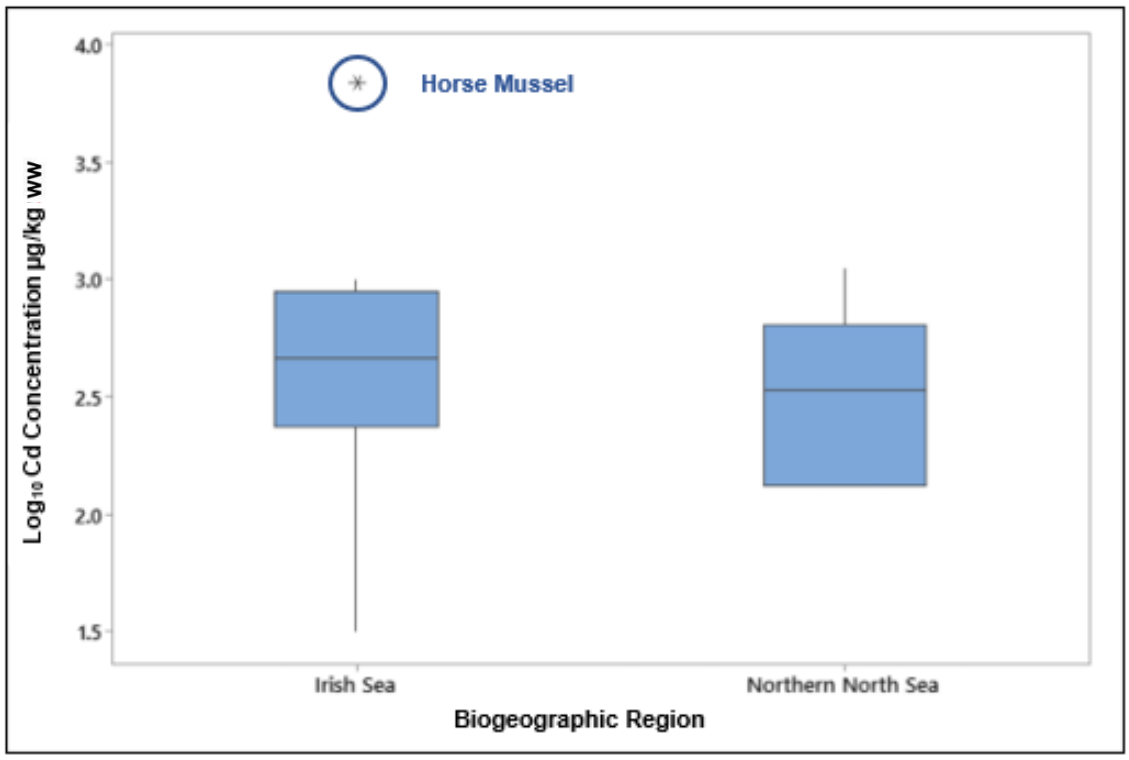

Figure 5.10: Box plot comparing the sample distributions of Log_{10} Cd concentration µg/kg ww in benthic invertebrates soft body across the two biogeographic regions. There was no significant difference of Log_{10} Cd concentration µg/kg ww in benthic invertebrates making up the soft body category collected from the Irish Sea Biogeographic Region (n=12) and the Northern North Sea (n=5) ($p>0.05$). Error bars are to one standard deviation.

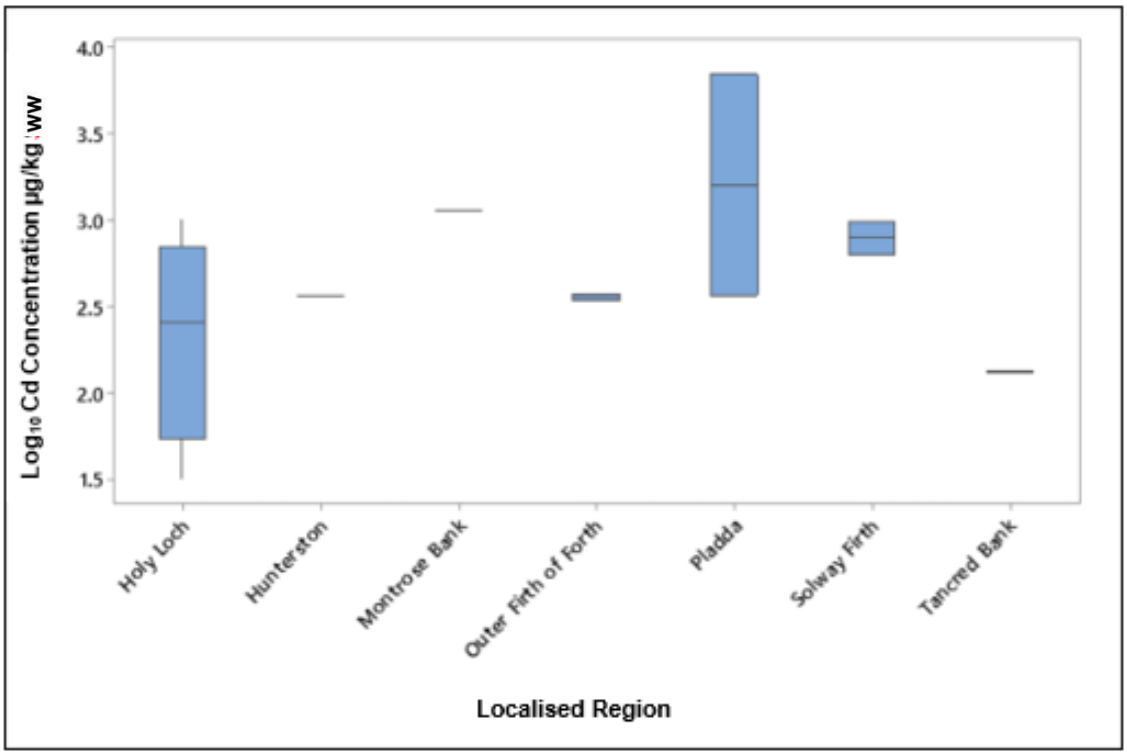

Figure 5.11: Box plot comparing the sample distributions of Log_{10} Cd concentration µg/kg ww in benthic invertebrates soft body across the seven localised regions. There was no significant difference of Log_{10} Cd concentration µg/kg ww in benthic invertebrates making up the soft body category collected from Holy Loch (n=7), Hunterston (n=1; made up of 34 individuals), Moray Firth (n=1; made up of 28 individuals), Outer Firth of Forth (n=2), Pladda (n=1; made up of 6 individuals), Solway Firth (n=3) and Tancred Bank (n=2) ($p>0.05$). Error bars are to one standard deviation.

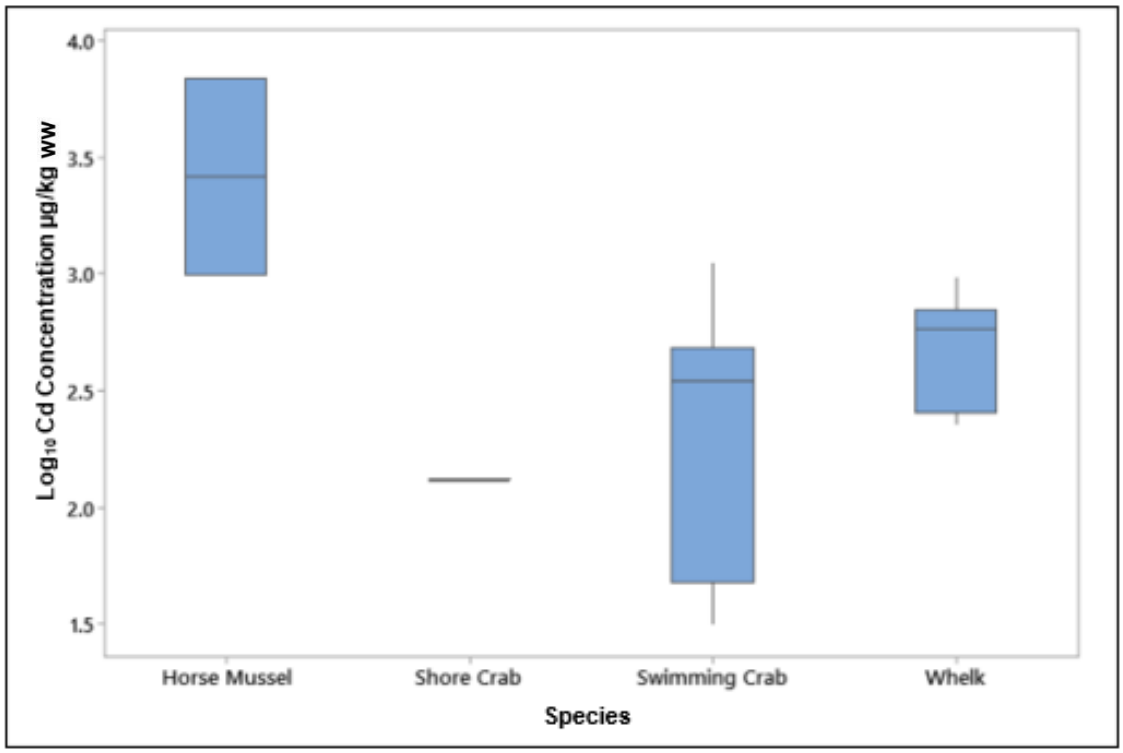

Figure 5.12: Box plot comparing the sample distributions of Log_{10} Cd concentration µg/kg ww across the four benthic invertebrates soft body species. Horse mussel (n=2) had a significantly higher concentration of Cd in its tissues than shore crab (2), swimming crab (6) and whelk (7) ($p<0.05$). Error bars are to one standard deviation.

Horse mussels usually live part-buried in soft to coarse sediments and are larger in size than blue mussels, which are known to accumulate a high concentration of Cd and are considered a suitable indicator species of metal contamination (Andersen, Maage and Johannessen, 1996). Many horse mussels live for more than 25 years and some survive for 50 years, as opposed to blue mussels which have a lifespan of 2-3 years (SNH, 2019). Young horse mussels are predated on by crabs and starfish, but once they are over 6 cm long, they are relatively safe from these predators. This long-term exposure to sediment, significantly higher lifespan and more carnivorous behaviour will therefore result in a much higher accumulation of trace metals in comparison to blue mussel, which could explain the significantly higher concentration of Cd in this project. Due to predators feeding on smaller and younger animals with a lower concentration of trace metals, the trophic transfer of these metals would not be as high.

Studies from around the UK have found Cd concentrations in edible crab brown meat to vary. Overnell and Trewhella, (1979) reported Cd concentrations of up to 50,000 µg/kg in samples from Orkney and Shetland, whilst Falconer *et al.,* (1986) reported concentrations of up to 61,300 µg/kg in samples from sixteen areas around the Scottish coast. Similarly, Noël, *et al*., (2011) reported concentrations of up to 61,600 µg/kg with

a mean of 12,800 µg/kg in 40 edible crabs originating from France, the UK and Ireland. The concentrations of Cd in this study are much lower than these reported values, where pooled edible crab brown meat has a mean concentration of Cd of 2,565 µg/kg (n=2) and pooled lobster brown meat a concentration of 713 µg/kg (n=1; made up of 9 individuals).

Mixed effects model analysis revealed that there was a statistically significant relationship between Cd concentration and sample category and trophic level ($p<0.05$). Pooled benthic invertebrates brown meat, soft body and zooplankton had a significantly higher concentration of Cd in their tissues than the other categories (Figure 5.9) ($p<0.05$, ANOVA, Tukey). On this basis, the TMF of Cd will be calculated both including (Figures 5.13) and excluding (Figure 5.14) benthic invertebrates brown meat, soft body and zooplankton using both the traditional (a) and balanced (b) methods. This will determine whether Cd biomagnifies in the marine food web in this project and whether categories influence the calculated TMF. The calculated TMF for Cd from Figures 5.13 – 5.14 are shown on Table 5.4.

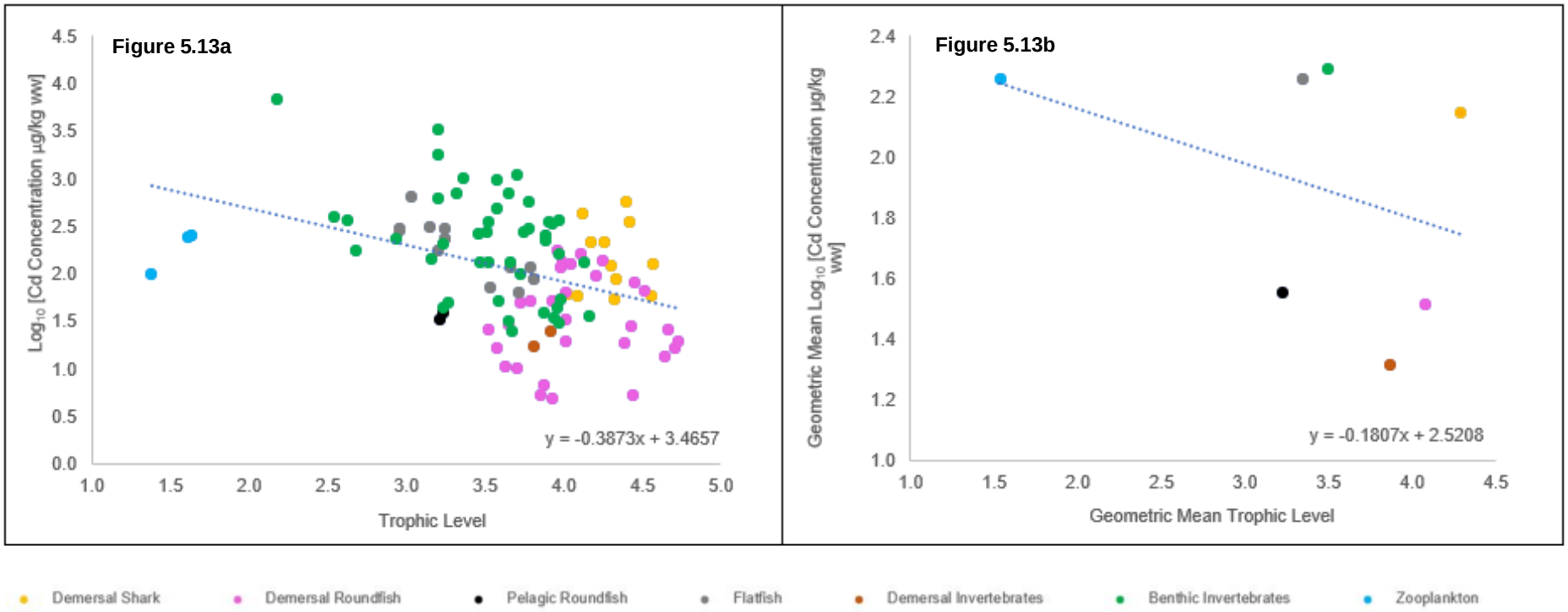

Figure 5.13: (a) Relationship between trophic level and logarithmically transformed Cd concentration (µg/kg ww) in demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black), Invertebrates: demersal invertebrate muscle (brown) and benthic invertebrate whole, muscle, brown meat, soft body (green) and zooplankton (blue). **(b)** Relationship between geometric mean trophic level and logarithmically transformed geometric mean Cd concentration (µg/kg ww) in demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black), Invertebrates: demersal invertebrate muscle (brown), and benthic invertebrate whole, muscle, brown meat, soft body (green) and zooplankton (blue).

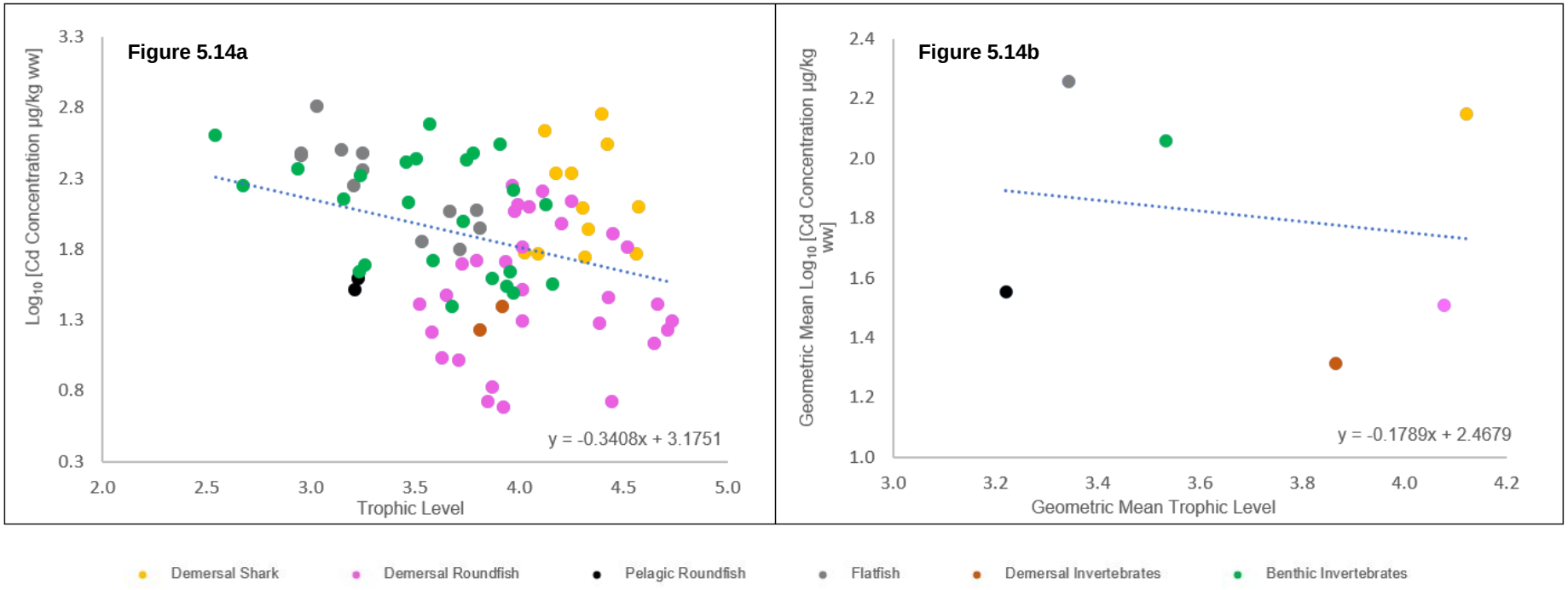

Figure 5.14: (a) Relationship between trophic level and logarithmically transformed Cd concentration (µg/kg ww) in demersal shark liver: fish: demersal (pink), flatfish (grey), pelagic (black), Invertebrates: demersal invertebrate muscle (brown), and benthic invertebrate whole, muscle (green). **(b)** Relationship between geometric mean trophic level and logarithmically transformed geometric mean Cd concentration (µg/kg ww) in demersal shark liver, fish liver: demersal (pink), flatfish (grey), pelagic (black), Invertebrates: demersal invertebrate muscle (brown), and benthic invertebrate whole, muscle (green).

Table 5.4: Calculated TMFs of Cd using the traditional and balanced methods in a food web composed of demersal shark, fish: pelagic, demersal and flatfish, invertebrates: demersal and benthic invertebrates whole, muscle, soft body and brown meat and zooplankton (all trophic levels) and a food web composed of demersal shark, fish: pelagic, demersal and flatfish and invertebrates: demersal, benthic invertebrates whole and muscle using the traditional method and balanced method.

Traditional method		Balanced method	
All trophic levels (p<0.05)	**Excluding benthic invertebrates brown meat, soft body and zooplankton (p<0.05)**	**All trophic levels (p>0.05)**	**Excluding benthic invertebrates brown meat, soft body and zooplankton (p>0.05)**
0.4	0.5	0.7	0.7

Cd was found to trophic dilute when all trophic levels (shark, fish, invertebrates and zooplankton) were analysed and when excluding benthic invertebrates brown meat, soft body and zooplankton using both the traditional and balanced methods (Table 5.4). The calculated TMFs using the balanced method were the same (0.7) when the categories containing species with a potential specific physiological basis for Cd were not included (Table 5.4). Although the trophic dilution tendency is slightly lower using the balanced method (closer to 1), overall trophic dilution is the same between both methods (traditional and balanced). This suggests that in the case of Cd, an unbalanced dataset does not influence the calculated TMF. Similar to Hg, the correlation was not significant (p>0.05) when using the balanced method for both calculated TMFs (Table 5.4).

Studies globally have found a trophic dilution effect of Cd through the food web. A study in the South China Sea on Cd and Pb in twelve marine organisms of differing trophic levels showed that these metals did not biomagnify (Gu, 2018) and a study in the Western Patagonia and Antarctic Pensinsula found that although Cd biomagnified in macroinvertebrates, significant trophic dilution occurred when higher trophic level organisms were assessed (Espejo *et al.,* 2018).

5.2.2.3 Copper

Figure 5.15 shows the logarithmically transformed Cu concentration in shark and fish liver, demersal and benthic invertebrates and zooplankton. Benthic invertebrates soft body and brown meat have a significantly higher concentration of Cu in their tissues than the other shark, fish and invertebrate categories (p<0.05, ANOVA, Tukey). Benthic invertebrates soft body has a concentration range of 4,090 µg/kg ww in horse mussel to 65,900 µg/kg ww in whelk, and benthic invertebrates brown meat has a concentration range of 19,100 - 68,400 µg/kg ww (Table 5.1). Similar to Cd, this suggests a species-

specific accumulation of Cu in the food web rather than concentrations as a result of biomagnification.

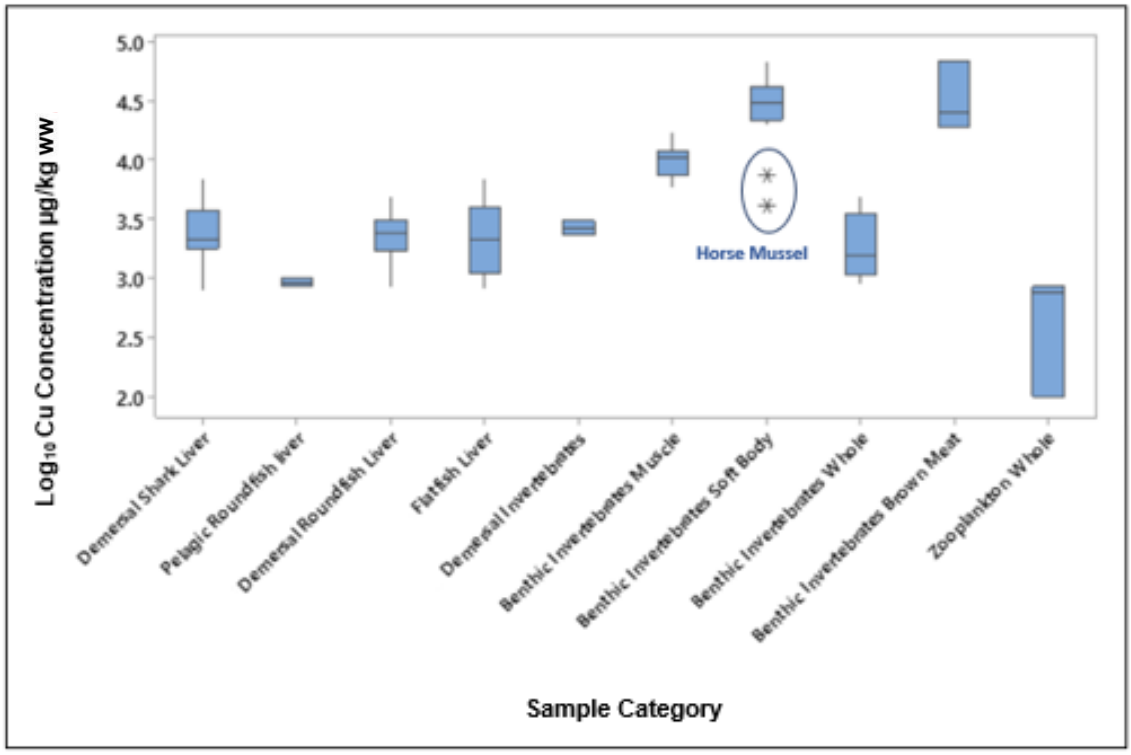

Figure 5.15: Box plot of Log_{10} Cu concentration (µg/kg ww) in ten sample categories (demersal shark, fish muscle, demersal invertebrates, benthic invertebrates and zooplankton (marine mammals were not included in the analysis due to their selected tissue. Two horse mussel sample pools were identified as data outliers ($p<0.05$). Error bars are to one standard deviation.

The hepatopancreas (making up the brown meat) has been found to show raised Cu concentrations, varying with moult cycle and associated bodily changes and blood concentration of oxygen binding haemolymph pigment haemocyanin (Rainbow, 2018), which could explain the significantly higher Cu concentration in brown meat in this project. Pooled whelk had the highest degree of variation of Cu, ranging from 21,000 - 65,900 µg/kg ww (n=7). It has been reported that molluscs can store Cu in granules, leading to elevated Cu concentrations (Marigómez, *et al*., 2002; Cheung and Wang, 2008). Higher trophic level organisms are however able to better regulate Cu to avoid lethal concentrations (Neff, 2002). In some molluscs (whelk in particular), Cu is especially important because it is responsible for the haemolymph pigment haemocyanin (Rainbow, 2018).

Two horse mussel sample pools (circled on Figure 5.15) have a significantly lower concentration of Cu in their tissue than the other benthic invertebrate soft body species ($p<0.05$, ANOVA, Tukey). Cu uptake is generally controlled by bivalves, but with different

efficiency from species to species (Pan and Wang, 2012). This could explain the low concentration of Cu in horse mussel in this project in comparison to the other benthic invertebrate soft body species (Figure 5.16), but a larger dataset will be required with a higher number of bivalve samples for a comprehensive analysis. Species contributing to benthic invertebrates soft body were separated based on their FA profiles in Figure 3.6. This suggests horse mussel having a significantly lower concentration of Cu could also be due to a similar feeding pattern in relation to Cu uptake.

There are no assessment criteria for detrimental effects available for fish or shellfish due to the fact that Cu is an essential element, and as such many organisms have some biological control over the uptake and release of Cu (OSPAR, 2016c).

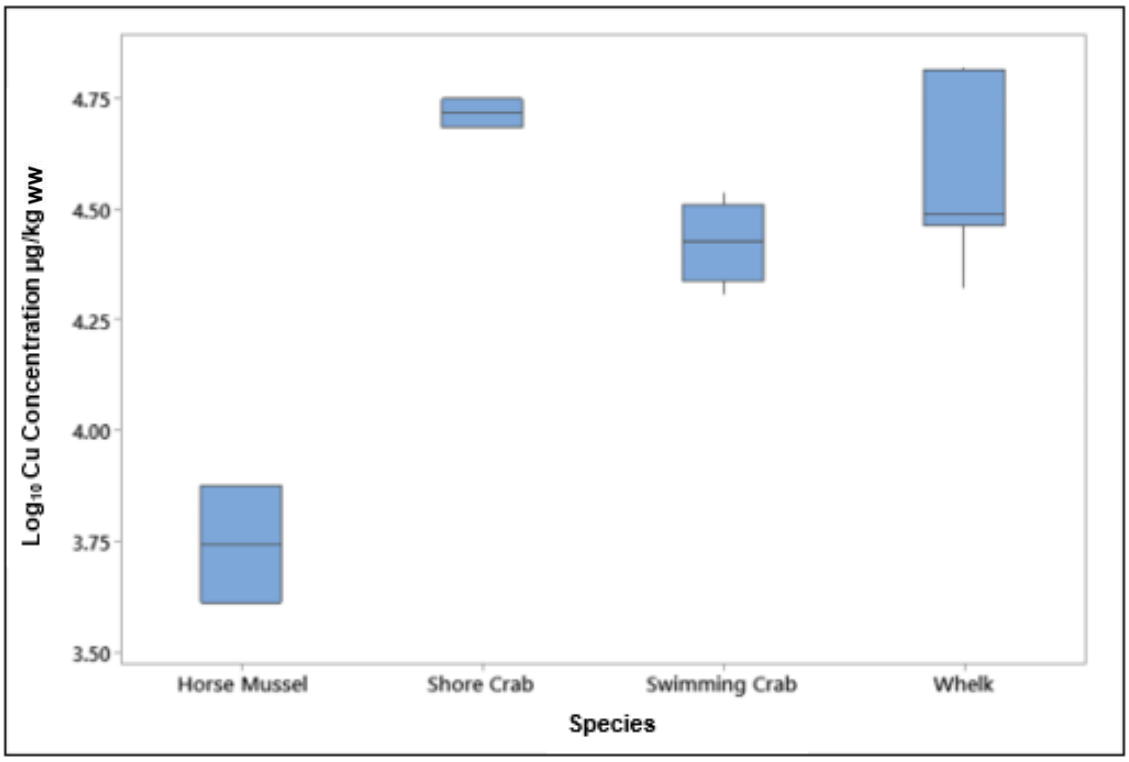

Figure 5.16: Box plot comparing the sample distributions of Log_{10} Cu concentration µg/kg ww across the four benthic invertebrates soft body species. Horse mussel (n=2) had a significantly lower concentration of Cu in its tissues than shore crab (2), swimming crab (6) and whelk (7) ($p<0.05$). Error bars are to one standard deviation.

Mixed effects model analysis revealed that there was a statistically significant relationship between Cu concentration and sample category and trophic level ($p<0.05$). Pooled benthic invertebrates soft body and brown meat had a significantly higher concentration of Cu in their tissues than the other categories (Figure 5.15) ($p<0.05$, ANOVA, Tukey). On this basis, the TMF of Cu will be calculated both including (Figures 5.17) and excluding (Figure 5.18) benthic invertebrates soft body and brown meat using

both the traditional (a) and balanced (b) methods. This will determine whether Cu biomagnifies in the marine food web in this project and if significant categories influence the calculated TMF. The calculated TMF for Cu from Figures 5.17 – 5.18 are shown on Table 5.5.

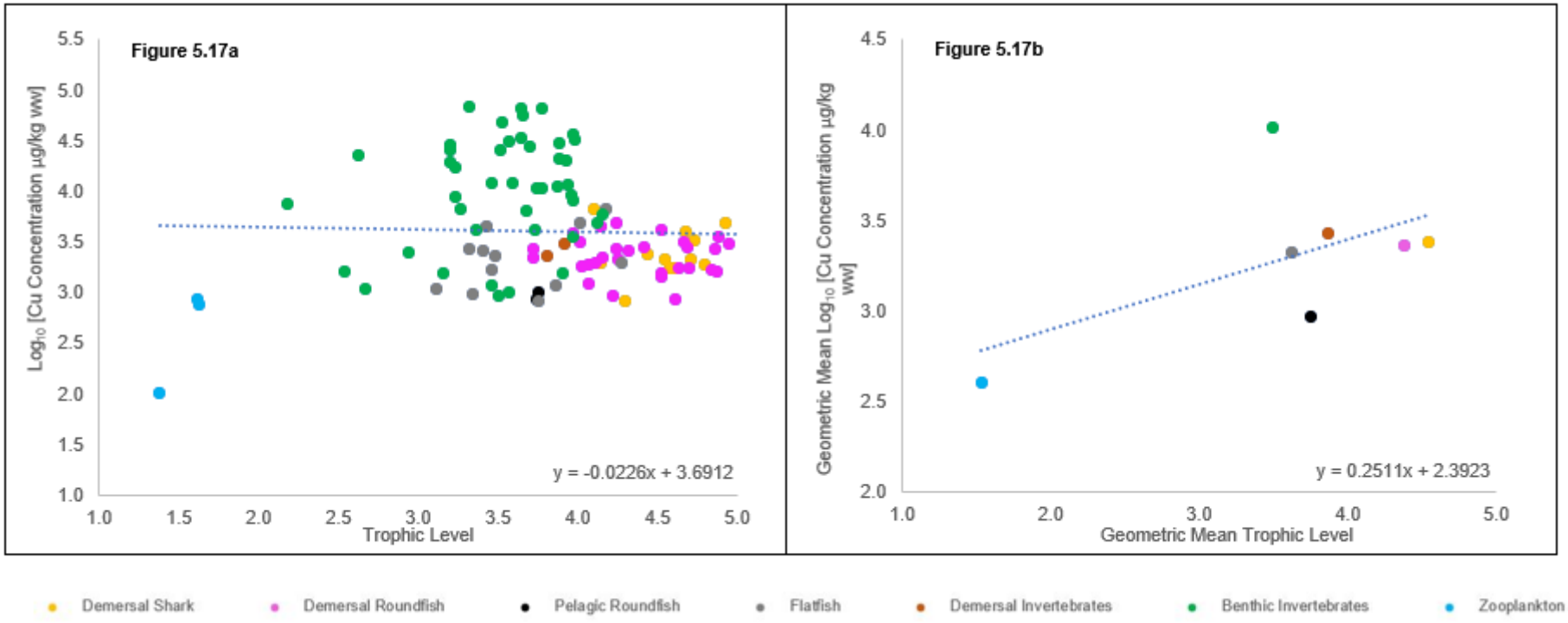

Figure 5.17: (a) Relationship between trophic level and logarithmically transformed Cu concentration (µg/kg ww) in shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black), Invertebrates: demersal invertebrate muscle (brown), and benthic invertebrate whole, muscle, brown meat, soft body (green) and zooplankton (blue). **(b)** Relationship between geometric mean trophic level and logarithmically transformed geometric mean Cu concentration (µg/kg ww) in shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black), Invertebrates: demersal invertebrate muscle (brown), and benthic invertebrate whole, muscle, brown meat, soft body (green) and zooplankton (blue).

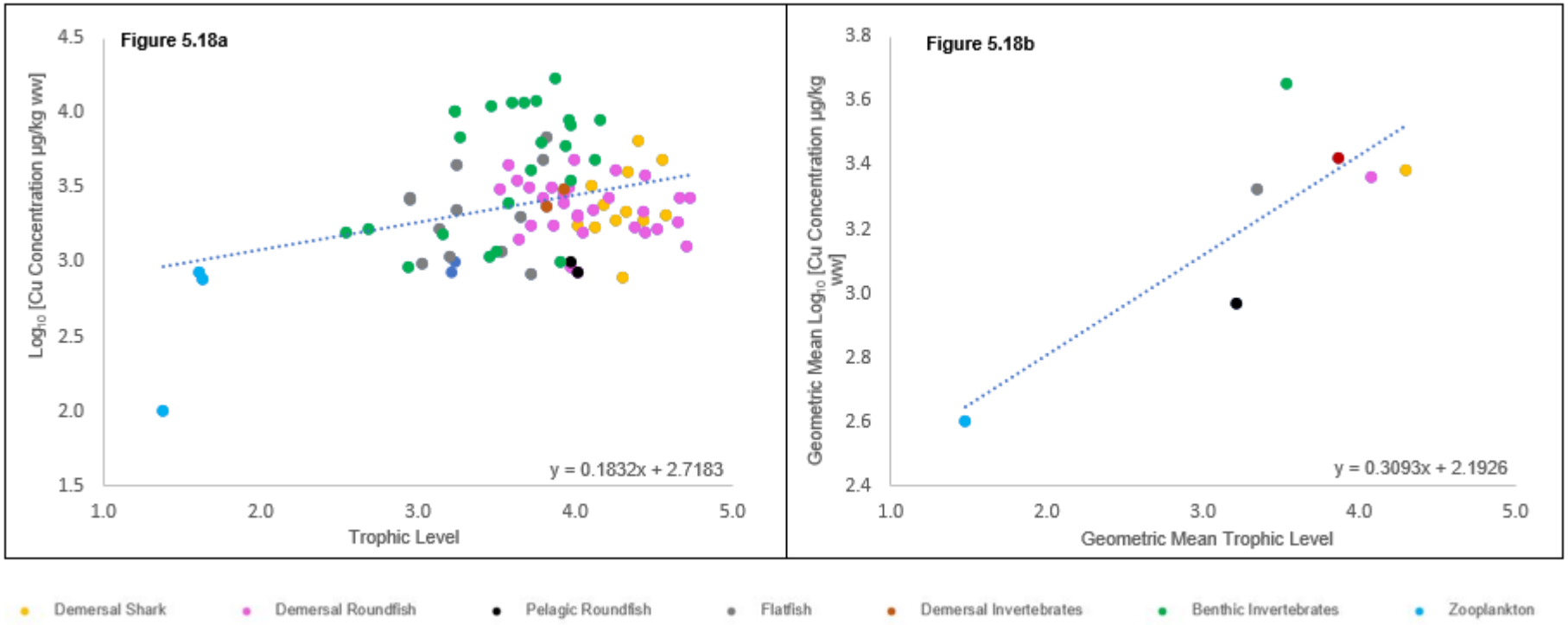

Figure 5.18: (a) Relationship between trophic level and logarithmically transformed Cu concentration (µg/kg ww) in demersal shark liver, fish liver: demersal (pink), flatfish (grey), pelagic (black) and Invertebrates: demersal invertebrate muscle (brown), and benthic invertebrate whole and muscle (green) and zooplankton (n=3). **(b)** Relationship between geometric mean trophic level and logarithmically transformed geometric mean Cu concentration (µg/kg ww) in fish muscle: demersal (pink), flatfish (grey), pelagic (black) and Invertebrates: demersal invertebrate muscle (brown), and benthic invertebrate whole and muscle (green) and zooplankton (n=3).

Table 5.5: Calculated TMFs of Cu using the traditional and balanced methods in a food web composed of demersal shark, fish: pelagic, demersal and flatfish, invertebrates: demersal and benthic invertebrates whole, muscle, soft body and brown meat and zooplankton (all trophic levels) and a food web composed of fish: pelagic, demersal and flatfish, invertebrates: demersal and benthic invertebrates whole and muscle and zooplankton.

Traditional method		Balanced method	
All trophic levels (p>0.05)	**Excluding benthic invertebrates soft body and brown meat (p<0.05)**	**All trophic levels (p>0.05)**	**Excluding benthic invertebrates soft body and brown meat (p<0.05)**
0.9	1.5	1.8	2.0

Cu was found to slightly trophic dilute in a food web composed of shark, fish, invertebrates and zooplankton when using the traditional method, but biomagnify using the balanced method (Table 5.5). Figure 5.17 shows that once the dataset was balanced, this difference in trophic transfer was due to zooplankton having a lower concentration of Cu in relation to its trophic level than the other sample categories. The calculated TMF was higher using both methods once benthic invertebrates soft body and brown meat were removed from the dataset (Table 5.5) which was expected, as the sample categories with a tendency to accumulate high concentrations of Cu have been removed from the analysis. The correlation was only significant ($p<0.05$) when using the balanced method and traditional method for the food web excluding benthic invertebrates soft body and brown meat from the analysis, suggesting that in this case, removing categories containing species with a specific physiological need for Cu has significantly reduced the variation associated with the calculated TMF. This highlights the importance of species selection and a balanced dataset when calculating the TMF of Cu, suggesting the different utilisation rates of Cu between trophic levels significantly influences the regression ($p<0.05$).

The trophic dilution of Cu has been established in other studies. A study by Schneider *et al.,* (2018) ranked mean Cu concentrations in the order: herbivores-suspension feeders > detritivores > autotrophs > carnivores, with calculated trophic dilution. Another study by Barwick and Maher (2003) found that Cu did not biomagnify in a temperate seagrass ecosystem, where lower trophic organisms such as molluscs and crustaceans accumulated high concentrations of Cu due to the essential nature of this trace metal.

5.2.2.4 Nickel

Figure 5.19 shows the logarithmically transformed Ni concentration in demersal shark and fish liver, demersal and benthic invertebrates and zooplankton. Benthic invertebrates soft body, brown meat, whole and zooplankton have a significantly higher concentration of Ni in their tissues than the other shark, fish and invertebrate categories ($p<0.05$, ANOVA, Tukey; Figure 5.19). Similar to Cd and Cu, this suggests a species-specific accumulation of Ni in the food web rather than concentrations as a result of biomagnification.

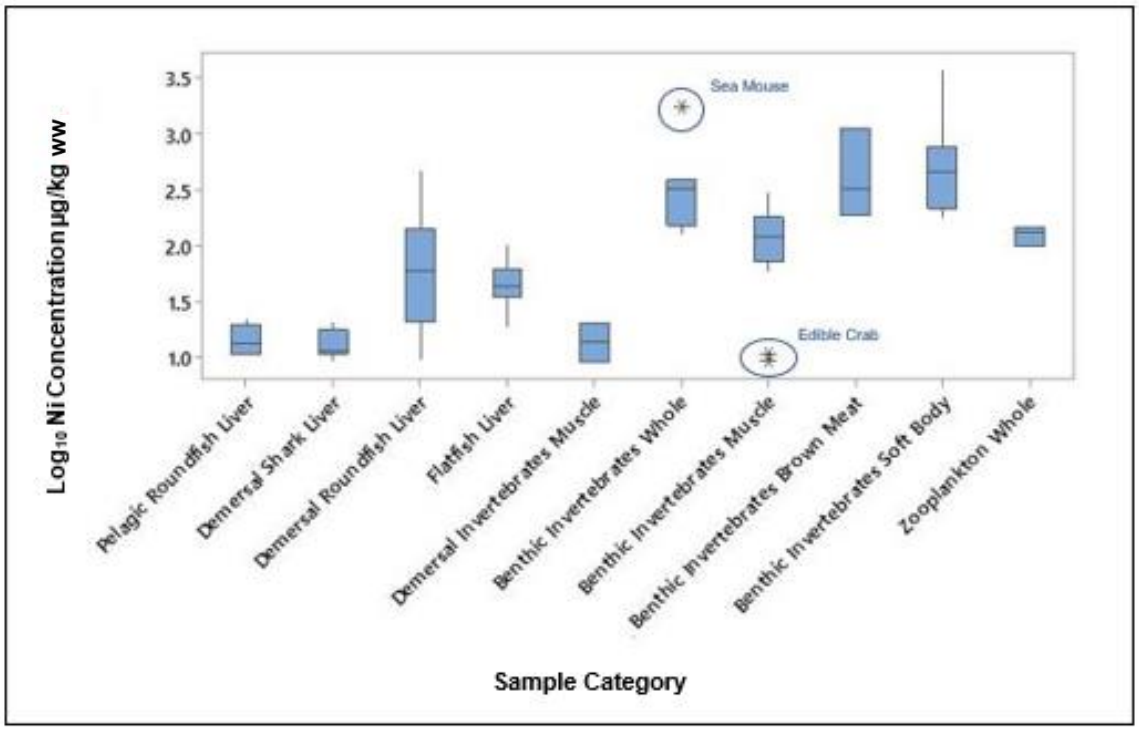

Figure 5.19: Box plot of Log_{10} Ni concentration (µg/kg ww) in ten sample categories (demersal shark, fish muscle, demersal invertebrates, benthic invertebrates and zooplankton (marine mammals were not included in the analysis due to their selected tissue. One sea mouse and two edible crab sample pools were identified as data outliers ($p<0.05$). Error bars are to one standard deviation.

Demersal roundfish liver has a larger degree of variation than the shark and other fish categories (Figure 5.19). In demersal roundfish liver, pooled whiting has the minimum category concentration of <13.00 µg/kg ww and the maximum category concentration of 458.0 µg/kg ww. When investigated further it was found that samples collected from the Irish Sea Biogeographic Region (n=8) had a higher concentration of Ni in their liver than those from the Northern North Sea (n=2) and Scottish Continental Shelf (n=5), but this was not significant ($p>0.05$) (Figure 5.20). Although the sample size is small, this provides an indication of a localised regional influence on Ni concentration in whiting. A larger dataset will however be required for a comprehensive analysis. Features such as

average pool age, weight, length and trophic level were not found to significantly influence the Ni concentration in whiting liver ($p>0.05$).

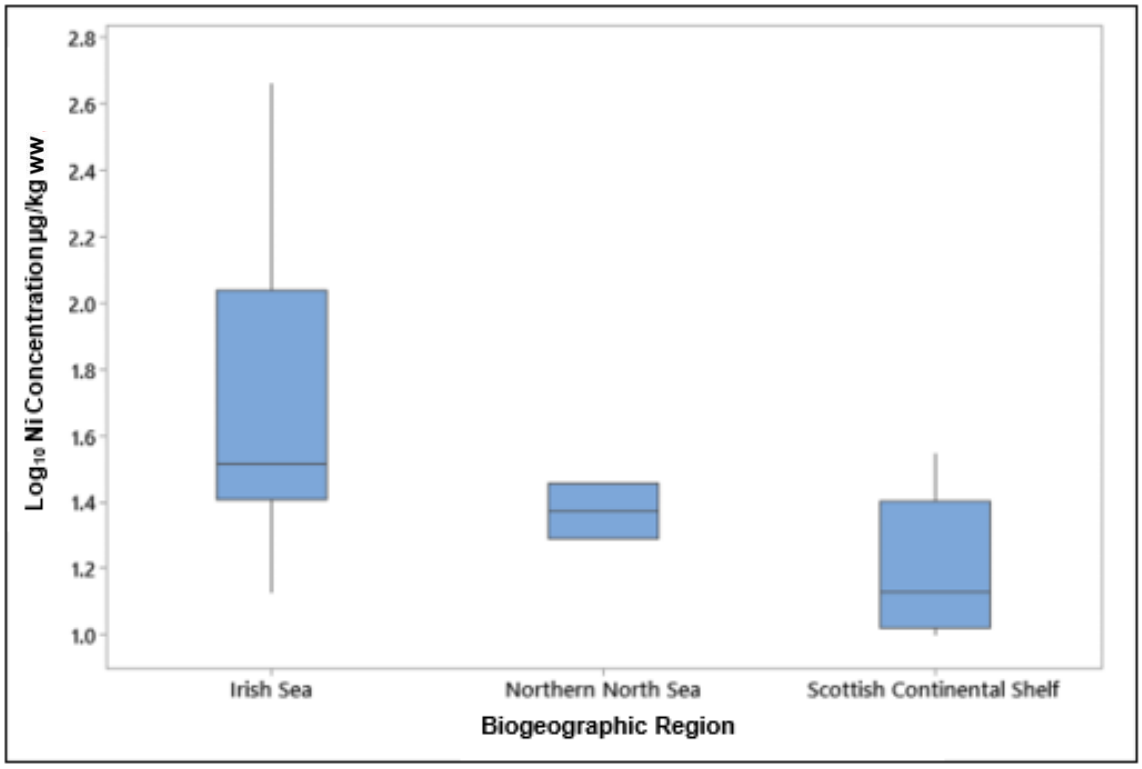

Figure 5.20: Box plot comparing the sample distributions of Log10 Ni concentration µg/kg ww in whiting liver across the three biogeographic regions. There was no significant difference of Log10 Ni concentration µg/kg ww in whiting liver making up the soft body category collected from the Irish Sea Biogeographic Region (n=8), Northern North Sea (n=2) and Scottish Continental Shelf (n=5) ($p>0.05$). Error bars are to one standard deviation.

There were three outliers identified on Figure 5.19 - one sea mouse sample pool and two edible crab muscle sample pools ($p<0.05$). To the authors knowledge there have been no previous studies investigating the accumulation of Ni in sea mouse. A study by Danje and Manoj., (2015) analysed the concentration of Cu, Ni, Zn and Cd in polychaete worms (*Nereididae*), mud skipper and mud crab from three sites in the Purna river estuary, India. It was reported that polychaete worms accumulated a higher concentration of Ni in their tissues than the Ni concentration in sediment, with samples collected from one site having five times the Ni concentration in their tissues than the sediment. Polychaete worms were found to have a a higher concentration of Ni in their tissues from all three sites than mud skipper and the chelipeds, walking legs and carapace of mud crab. There is only one sea mouse sample in this project (made up of 33 individuals), but this provides an indication of the accumulation ability of this species which requires further study with a larger dataset. It is also important to note that sea

mouse could be differentiated from the benthic invertebrates whole category during FA profile analysis, suggesting a different dietary pattern from starfish and brittle star (Figure 3.6).

The two edible crab muscle samples were collected from two different sites: the Irish Sea Biogeographic Region (Solway Firth) and Northern North Sea (Montrose bank), and the equivelant brown meat tissue from both sample pools had a higher concentration of Ni than the muscle tissue (322.0 – 1,140 µg/kg ww as opposed to 9.36 – 110.0 µg/kg ww). A higher concentration of metals is expected from the brown meat of crab, as it is taken from the soft body of the crab and is mainly composed of the gonads and hepatopancreas, known to contain high concentrations of trace metals due to the detoxifying nature of the hepatopancreas (Bolam and Bersuder, 2013a and b).

The concentration range present for both tissue types is however large, considering there are only two sample pools. This could be due to sample collection region, as the maximum Ni concentration in the range of both tissues was from the sample pool collected from the Irish Sea Biogeographic Region. When the benthic invertebrate muscle category is analysed as a whole, there was no significant regional influence ($p>0.05$), but this is likely due to the number of different species present in this category (5) each with differing accumulation rates depending on physiological requirements. Further study is required on a species level with a larger dataset to establish a regional influence on Ni concentration in invertebrates.

Benthic invertebrates soft body had a Ni concentration range of 183.0 µg/kg ww in pooled whelk to 3,660 µg/kg ww in pooled horse mussel. Pooled horse mussel was found to have the highest concentration of Ni in its tissues than the other invertberate species in ($p<0.05$, ANOVA, Tukey). As described in Section 5.2.2.2 for Cd, this accumulation is likely due to their constant exposure to sediment and long lifespan of 25-50 years.

The higher concentration and species-specific variation of Ni concentration in benthic invertebrates has been found in a number of studies. A study by Hédouin *et al.,* (2010) analysed Ni accumulation in clams and oysters. Although both bivalve species were shown to efficiently assimilate Ni ingested with their food (especially clams) and retain it very efficiently (especially oysters), they displayed different bioaccumulation behaviour for Ni suggesting different environmental interactions and/or physiological ability. The majority of studies have focussed on smaller, lower trophic level mussels (several species) as a target organism worldwide, concluding this species as an effective bio-indicator of Ni concentrations in sea water (Lu and Wang, 2018; Mejdoub *et al.,* 2018; Azizi *et al.,* 2018).

Mixed effects model analysis revealed that there was a statistically significant relationship between Ni concentration and sample category and trophic level ($p<0.05$). Pooled benthic invertebrates soft body, brown meat, whole and zooplankton had a significantly higher concentration of Ni in their tissues than the other categories (Figure 5.19) ($p<0.05$, ANOVA, Tukey). There are too few lower trophic level samples available to calculate TMF when pooled benthic invertebrates soft body, brown meat, whole and zooplankton are not included in the analysis (following the guidance by Kidd *et al.*, (2019). On this basis, the TMF of Ni was calculated for all trophic levels including these categories (Figure 5.21) using both the traditional (a) and balanced (b) methods. This will determine whether Ni biomagnifies in the marine food web in this project. The calculated TMF for Ni from Figure 5.21 are shown on Table 5.6.

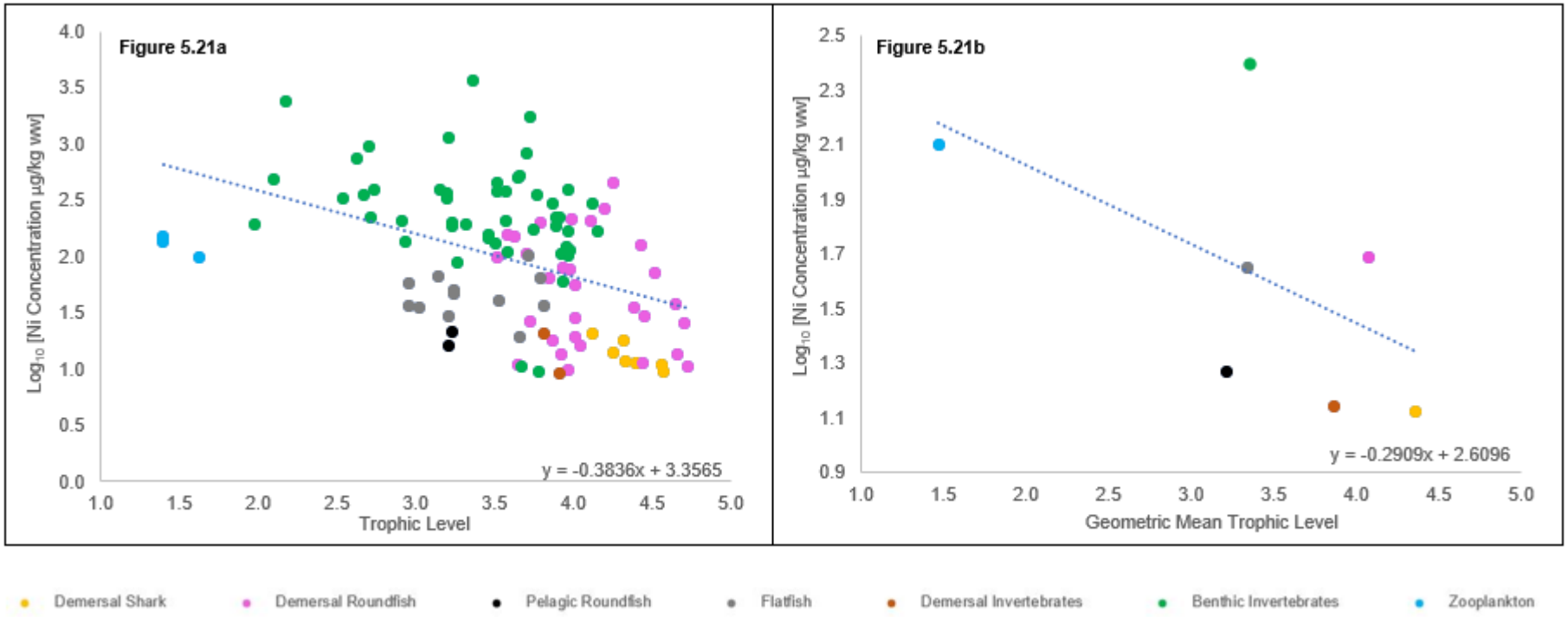

Figure 5.21: (a) Relationship between trophic level and logarithmically transformed Ni concentration (µg/kg ww) in demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black), Invertebrates: demersal invertebrate muscle (brown), and benthic invertebrate whole, muscle, brown meat, soft body (green) and zooplankton (blue). **(b)** Relationship between geometric mean trophic level and logarithmically transformed geometric mean Ni concentration (µg/kg ww) in demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black), Invertebrates: demersal invertebrate muscle (brown), and benthic invertebrate whole, muscle, brown meat, soft body (green) and zooplankton (blue).

Table 5.6: Calculated TMFs of Ni using the traditional and balanced methods in a food web composed of demersal shark, fish: pelagic, demersal and flatfish, invertebrates: demersal and benthic invertebrates whole, muscle, soft body and brown meat and zooplankton (all trophic levels).

Traditional Method	Balanced Method
All trophic levels (p>0.05)	**All trophic levels (p>0.05)**
0.4	0.5

Both of the calculated TMFs using the traditional method and balanced method found Ni to trophic dilute in the marine food web, suggesting a balanced data set does not influence the calculated TMF. The correlation was however not significant (p>0.05) when analysing all trophic levels using both methods, suggesting that a larger dataset is required to establish significance when analysing the trophic relationship of Ni.

There is relatively limited data available for the biomagnification/trophic dilution of Ni, but a study by Cardwell, *et al*., (2013) found that Ni generally does not biomagnify in food chains consisting of organisms occupying trophic level 3 and over. A study by Blewett and Leonard, (2017) found that organism physiology appears to be the main driver of the toxic impact of Ni rather than bioaccumulation but concluded that mechanisms of Ni toxicity in the marine environment are still not well understood.

5.2.2.5 Zinc

Figure 5.22 shows the logarithmically transformed Zn concentration in shark and fish liver, demersal and benthic invertebrates and zooplankton. Benthic invertebrates soft body, whole and brown meat have a significantly higher concentration of Zn in their tissues than the other sample categories ($p<0.05$, ANOVA, Tukey; Figure 5.22). Similar to Cd, Cu and Ni, this suggests a species-specific accumulation of Zn in the food web rather than a result of biomagnification.

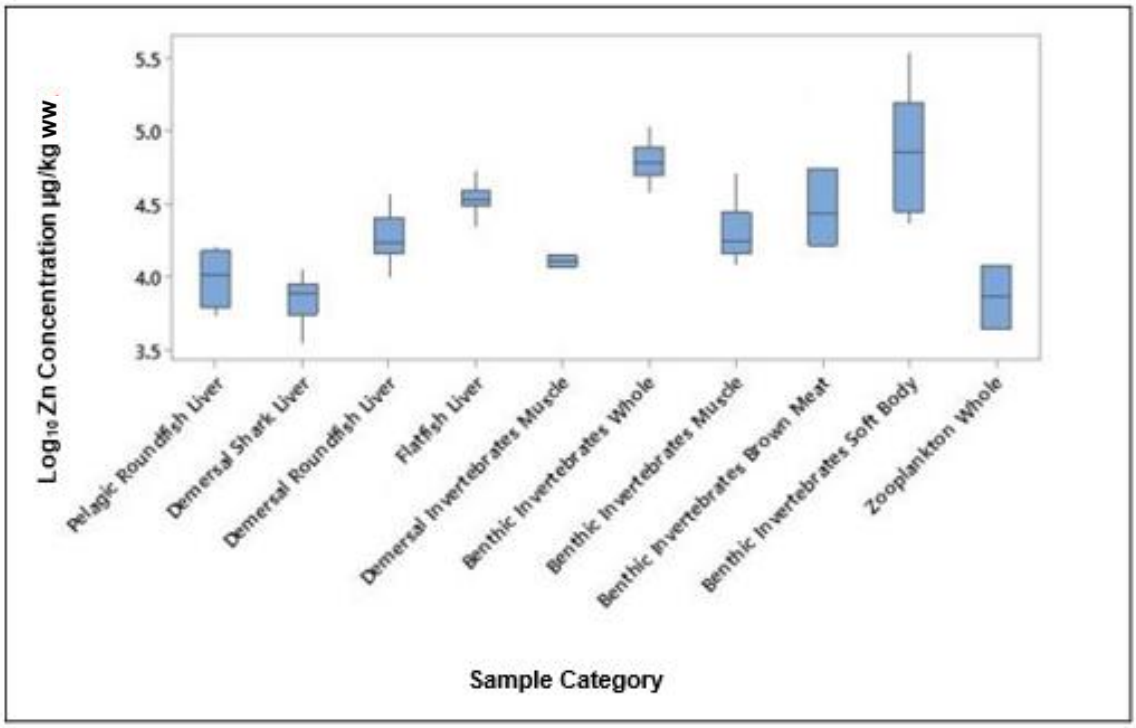

Figure 5.22: Box plot of Log_{10} Zn concentration (µg/kg ww) in ten sample categories (demersal shark and fish muscle, demersal invertebrates, benthic invertebrates and zooplankton (marine mammals were not included in the analysis due to their selected tissue). Error bars are to one standard deviation.

Figure 5.22 shows that benthic invertebrates soft body has a large degree of within category variation in comparison to the other demersal shark, fish and invertebrate sample categories, with a range of 23,500 µg/kg ww in swimming crab to 341,000 µg/kg ww in pooled horse mussel (Table 5.1). This supports a study by Chou *et al*., (2003) where the uptake of Zn by horse mussel was extremely high, suggesting this element may play a biological role in this species and correlates with the findings from Cd, Cu and Ni.

Edible crab (n=2) had a significantly higher concentration of Zn in their muscle tissue than the other benthic invertebrate muscle species, with concentrations of 61,500 and 79,200 µg/kg ww in comparison to 13,800 – 27,200 µg/kg ww, respectively. Unlike Ni,

the equivelant edible crab brown meat had a much lower concentration of Zn ranging from 27,500 – 55,800 µg/kg ww, suggesting a tissue specific accumulation. A study by Zhang *et al.,* (2019) investigated the concentration of Cu, Zn, Mn, Cd and Cr in three crab species from mangrove wetlands in China and compared their findings to those reported in eighteen species of crab from twelve studies worldwide. Signficant differences between tissue types were found in all three species, where concentrations of Cu and Cd were significantly higher in the hepatopancreas than in the muscle and carapace and Zn tended to be accumulated by muscle. When comparing metal data from twelve other studies, the metal concentrations in muscle tissues showed a trend of Zn > Mn ≥ Cu > Cd > Cr in all the species, while in the hepatopancreas, the trend varied with species. This agrees with the findings of Zhang *et al.,* (2019) and the findings of this project.

Mixed effects model analysis revealed that there was a statistically significant relationship between Zn concentration and trophic level ($p<0.05$). Pooled benthic invertebrates soft body, whole and brown meat have a significantly higher concentration of Zn in their tissues than the other sample categories (Figure 5.22) ($p<0.05$, ANOVA, Tukey). Similarly to Ni, there are too few lower trophic level samples available to calculate TMF when pooled benthic invertebrates soft body, brown meat and whole are not included in the analysis (following the guidance by Kidd *et al.,* (2019)). On this basis, the TMF of Zn was calculated for all trophic levels including these categories (Figure 5.23) using both the traditional (a) and balanced (b) methods. This will determine whether Zn biomagnifies in the marine food web in this project. The calculated TMF for Zn from Figures 5.23 are shown on Table 5.7.

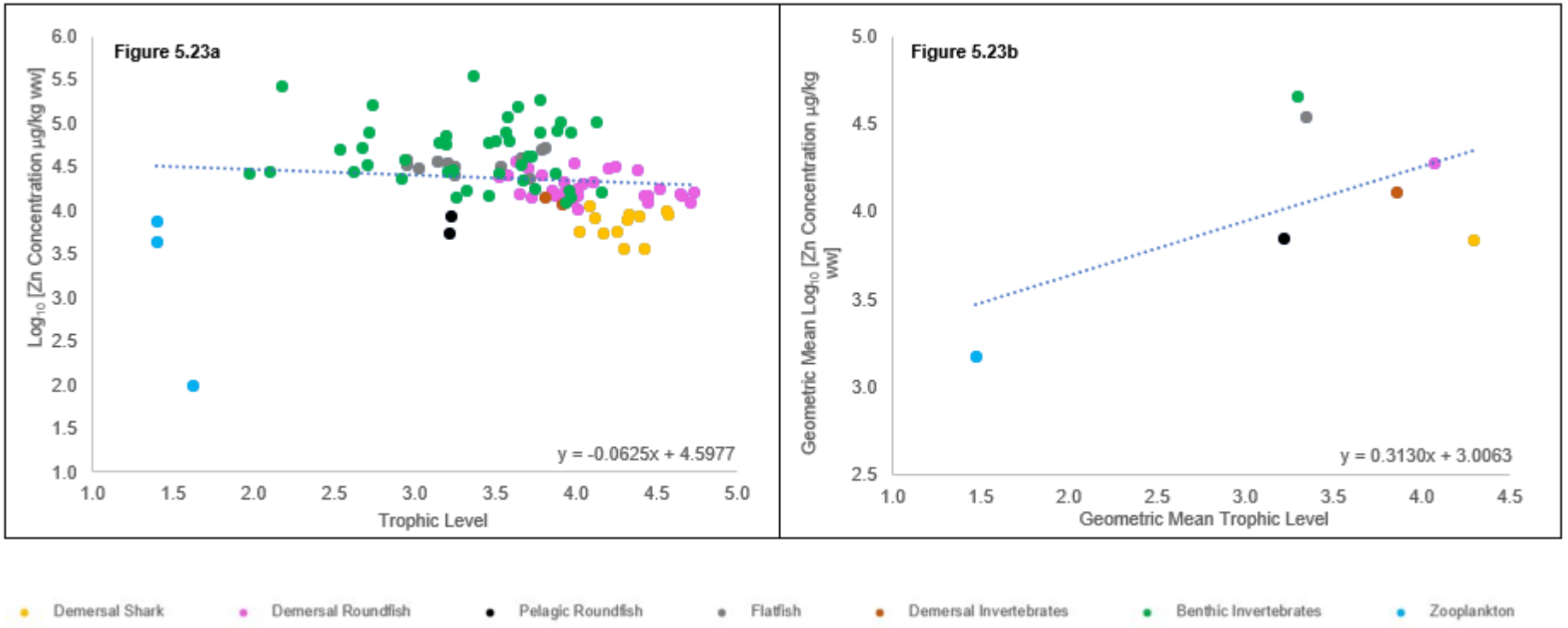

Figure 5.23: (a) Relationship between trophic level and logarithmically transformed Zn concentration (µg/kg ww) in demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black), Invertebrates: demersal invertebrate muscle (brown), and benthic invertebrate whole, muscle, brown meat, soft body (green) and zooplankton (blue). **(b)** Relationship between geometric mean trophic level and logarithmically transformed geometric mean Zn concentration (µg/kg ww) in demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black), Invertebrates: demersal invertebrate muscle (brown), and benthic invertebrate whole, muscle, brown meat, soft body (green) and zooplankton (blue).

Table 5.7: Calculated TMFs of Zn using the traditional and balanced methods in in a food web composed of demersal shark, fish: pelagic, demersal and flatfish, invertebrates: demersal and benthic invertebrates whole, muscle, soft body and brown meat and zooplankton (all trophic levels).

Traditional Method	Balanced Method
All trophic levels (p>0.05)	**All trophic levels (p>0.05)**
0.9	2.1

Zn was found to trophic dilute in the marine food web using the traditional method, but biomagnify when using the balanced method. Similar to Cu, this shows the importance of a balanced dataset when calculating the TMF of Zn, particularly when different utilisation rates of Zn exist between trophic levels (a higher utilisation rate when a dataset has more benthic invertebrates at a particular trophic level will influence the regression). Similar to Ni, the correlation was not significant (p>0.05) when analysing all trophic levels, suggesting that a larger dataset is required to establish significance when analysing the trophic relationship of Zn. A study by Cardwell *et al.,* (2013) found that Zn generally does not biomagnify through food chains consisting of primary producers, macroinvertebrate consumers, and fish occupying trophic level 3 and higher, which is similar to the food web in this study. The lack of trophic magnification has been the dominant finding for the trophic transfer of Zn, which is supported by a number of other studies worldwide (Barwick and Maher, 2003; Mathews and Fisher, 2008; Guo *et al*., 2016). Further studies are required to analyse the biomagnification of Zn using a balanced dataset.

5.4 Conclusion

This study examined the variability of concentrations (inter- and intra- species variation) of three priority heavy metals (Hg, Cd and Pb) and six additional trace metals and metalloids As, Ni, Se, Zn, Cu and Cr in the nineteen sample categories from different locations around Scotland. Mixed models analysis was used to determine the metals/metalloids with a trophic relationship and factors with the potential of influencing TMF variability identified.

None of the metals with a trophic relationship (Hg, Cd, Cu, Ni and Zn) had a significant relationship with biogeographic region. The findings from this project show that benthic invertebrates have a species-specific accumulation of Cd, Cu, Ni and Zn in the food web

rather than concentrations as a result of biomagnification, which can reduce the reliability of the calculated TMFs.

TMFs were calculated on those possessing a significant trophic relationship and TMFs calculated using two methods: traditional and balanced methods (Borgå *et al*., 2012; Brisebois, 2013) to determine whether biomagnification occurs in the food web and to establish whether the application of TMFs is appropriate for the development of a consistent, trophic specific biota assessment criteria.

When shark, fish, invertebrates and zooplankton were analysed, biomagnification was found to occur in the food web for Hg using both the traditional and balanced methods and Cu and Zn using the balanced method. This suggests that for Cu and Zn, the different utilisation rate of invertebrate species does influence the calculated TMF in an unbalanced dataset. When sample categories with a significantly different concentration of a metal were removed from the analysis, biomagnification was found to occur for Cu using the traditional method and balanced method. Removing these categories resulted in a change from biomagnification to trophic dilution for Hg, suggesting that biomagnification does not occur in a food web composed of lower trophic level organisms (invertebrates and fish). This also changed the result for Cu using the traditional method from trophic dilution to biomagnification, but as this is an unbalanced dataset, it could be a result of a lower number of invertebrates (benthic invertebrates soft body and brown meat were removed) accumulating a higher concentration of this metal in relation to their trophic level than the higher trophic level species. Due to three of the benthic invertebrate categories having a significantly higher concentration of Ni and Zn in their tissues than the other categories, there were too few lower trophic level samples available to calculate the TMF of a food web not including these categories. This does however show the higher accumulation of these metals by a majority of the invertebrates in this project in comparison to the other metals and sample categories. Cd and Ni were the only metals found to trophic dilute using both methods in all scenarios.

The concentration-trophic level correlation was significant ($p<0.05$) for Hg and Cd using the traditional method but not significant ($p>0.05$) when using the balanced method, suggesting that a larger dataset is required to sufficiently test significance using the balanced method. A similar result was seen in Chapter 4 for CB101, 118 and BDE47, where the balanced method was found to influence the significance of the regression, and in some cases change the overall finding from biomagnification (using the traditional method) to trophic dilution (using the balanced method). The correlation was significant ($p<0.05$) for Cu when categories with a significantly higher concentration in their tissues

(benthic invertebrates soft body and brown meat) were removed from the analysis using the traditional method and balanced method, suggesting that these categories were significantly influencing the trophic transfer of Cu in the marine food web. The correlation was not significant ($p>0.05$) for Ni and Zn in all the associated figures.

The data in this study contributes ecotoxicological data required to develop new assessment criteria based on the European Union WFD or OSPAR EAC principles and the wider development of TMFs. Although TMFs provide valuable information regarding bioaccumulation potential and should be incorporated into regulatory decision making, species selection and data treatment must be kept consistent in future studies. Due to the essential nature of trace metals, appropriate species selection is vital to ensure TMFs accurately represent the selected ecosystem. This study has shown that for Cu and Zn in particular, a balanced dataset has reduced the variation that species with a potential specific physiological basis have on the TMF and has changed the outcome of the trophic relationship from trophic dilution to biomagnification. The balanced method is therefore highly recommended to ensure that the TMF is a true indication of biomagnification potential.

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Chapter 6

Conclusions and future work

Alethea Madgett, 2014

Alethea Madgett, 2016

Monitoring and assessment efforts based on scientific knowledge are crucial for the management of human activities in our seas; to contribute to the periodic review of policy objectives and associated targets and indicators. To achieve the end goal of "good environmental status", it is important to understand the status and trends of human impact existing in our ever-changing oceans, coasts, and marine ecosystems.

With regards to the ecotoxicological assessment criteria for biota, secondary poisoning needs to be considered for contaminants that have biomagnification as a critical pathway. It has been established by researchers from academia, government, and industry that the actual bio-accumulative capacity of some chemical substances is not always identifiable using properties such as the K_{ow} (bioaccumulation factor, bioconcentration factor and biomagnification factor). TMFs have been suggested a reliable tool for the assessment of the bioaccumulation of substances that can be quantitatively measured in biota.

The aim of this project was to calculate TMFs for selected PCBs, PBDEs and trace metals, to allow the trophic level adjustment of existing assessment criteria and provide contaminant and trophic level data covering the diverse Scottish marine food web. An initial review of literature was undertaken (Chapter 1) whilst the methods applied in the thesis are described in Chapter 2. The thesis aim was achieved using three objectives: 1) To contribute high-quality trophic level data covering the diverse marine species inhabiting Scottish waters; 2) To determine the concentration of contaminants (PCBs, PBDEs and trace metals and metalloids) in marine species covering a wide trophic level range within the Scottish marine food web; and 3) To contribute to the wider development of TMFs for organic and inorganic contaminants with a high level of confidence and worldwide applicability.

First objective: To contribute high-quality trophic level data covering the diverse marine species inhabiting Scottish waters.

Chapter three focussed on the determination of feeding patterns and trophic levels existing in the Scottish marine food web, which was proven to be highly complex with organisms at a single trophic level having considerable variation. In assessing the contribution of contaminants to the overall pressure, measuring contaminants at a specific trophic level and then using TMFs to estimate concentrations at other trophic levels permits environmental assessments across the food web. Furthermore, it allows the adjustment of contaminant concentrations to a particular trophic level for subsequent comparison to assessment criteria. FA analysis was able to provide an indication of the

feeding patterns of many of the organisms sampled in this project and was used to categorise 215 samples into nineteen sample categories, accounting for the FA profile influences of tissue type and water column zone. SI analysis provided dietary information and ascribed the trophic levels of all twenty-six species. FATMs were used as trophic level indicators and with SI analysis, permitted identification of the mean trophic level of each species and determination of the feeding patterns and predator-prey relationships existing in the Scottish marine food web.

In the marine mammal category, the contributing three species could be differentiated by their FA profile. Harbour porpoise and harbour seal had a similar and highly variable diet and sperm whale had the least variable FA profile and were separated from other two species, suggesting a different feeding pattern. Individual marine mammal samples with different feeding patterns could be identified by analysis of their FA profiles. A harbour seal sample for example had a different FA profile to the other harbour seal samples and when further investigated, was found to be in a state of emaciation. This individual however could not be differentiated using SI analysis alone, showing the importance of having both techniques to fully understand the dynamics existing within a food web.

Corresponding to FA analysis, the three marine mammal species could also be differentiated based on their $\delta^{15}N$ and $\delta^{13}C$, where sperm whales had a lower $\delta^{15}N$ and higher $\delta^{13}C$ in their blubber protein compared to harbour seal and harbour porpoise. The range of $\delta^{15}N$ and $\delta^{13}C$ in harbour seal and harbour porpoise protein suggests a more variable dietary pattern and/or feeding location than sperm whale. Harbour seal were found to have the highest calculated trophic level in the food web of this project (5.02 ± 0.35), followed by harbour porpoise (4.71 ± 0.36). Sperm whale had a much lower trophic level of 3.75 ± 0.16, therefore classed as a mid-trophic level predator in this project.

In the shark and fish categories, FA and SI analysis was able to differentiate between tissue type, sample category, and species. There were no significant regional influences on the FA profiles for each sample category, but this is likely due to the number of species making up each category each with different feeding patterns. A larger dataset will be required for further research on a species level to investigate the influence of sampling location on the FA profile. Initially, FA analysis showed that muscle tissue of demersal shark, roundfish and flatfish and the liver tissue of pelagic roundfish had a higher proportion of PUFAs, which are components of structural lipids and characteristic to the physiology of these category tissue types. The demersal roundfish categories (muscle

and liver separately) were found to have a highly variable FA profile and $\delta^{15}N$ and $\delta^{13}C$. FA analysis showed that this variation was due to the dietary differences existing between the category species and SI analysis found that for whiting, there was a significant influence of age, length and weight on the $\delta^{15}N$ for all tissue types ($p < 0.05$). SI analysis also showed that there was a significant localised regional influence on the demersal roundfish liver category ($p<0.05$) and that there was unlikely to be a predator-prey relationship with demersal shark. Average weight was found to significantly influence the $\delta^{15}N$ in catshark muscle, ($p < 0.05$) indicating that larger catshark are feeding higher up the food chain than smaller catshark and average pool length was found to significantly influence the $\delta^{13}C$ in catshark liver ($p < 0.05$), suggesting a different diet. Sampling year was found to influence both the $\delta^{15}N$ and $\delta^{13}C$ of catshark, suggesting that the small spotted catshark collected in the 2016 sampling exercises were feeding more on lower trophic level benthic invertebrates with differing primary carbon sources in comparison to those collected during 2015 and 2017. Demersal shark had the highest calculated trophic level in its muscle tissue (4.55 ± 0.25) in comparison to the other shark and fish tissue categories, followed by demersal roundfish whole (4.43 ± 0.11), demersal roundfish muscle (4.40 ± 0.36), demersal shark liver (4.30 ± 0.17), demersal roundfish liver (4.09 ± 0.35), flatfish muscle (3.64 ± 0.36) and flatfish liver (3.36 ± 0.31).

There was considerable variation in the invertebrates FA profile and SI values. A majority of the benthic invertebrates whole category was separated and grouped together due to the more physically specific FA composition of echinoderms. All three species making up the category could however be differentiated due to the characteristic FAs required for bodily functions in common starfish (e.g. 20:4(n-6) required to induce maturation in starfish oocytes) and specific FAs associated with a less carnivorous diet of microalgae in brittle star. Sea mouse was separated from the echinoderm species and grouped with the benthic invertebrates muscle category, due to containing a higher proportion of FAs associated with a more carnivorous diet than common starfish and brittle star. Corresponding to FA analysis, brittle star and starfish had a higher $\delta^{13}C$ than the other categories, likely due to microalgae as possible carbon source at the base of the echinoderm food chain and/or bioturbation of refractory organic matter in the sediment. There was also a localised regional influence on common starfish, where those collected from the Moray Firth had a significantly higher $\delta^{13}C$ than other benthic species collected from the same location, suggesting this species feeds on organisms with a different primary carbon source. Also, two starfish samples were slightly separated from the others during PCA on the FA profiles, due to a localised regional

influence where those collected from Pladda had a different FA profile with a higher proportion of copepod biomarker than those collected from the in the upper Clyde (Hunterston and Holy Loch) and the North East, likely due to a higher degree of planktivorous feeding. A higher sample number is however required for brittle star, starfish and sea mouse for a more comprehensive analysis and further study required on the feeding patterns of these species. Benthic invertebrates soft body had the most variable FA profile out of the invertebrates categories due to the more carnivorous feeding pattern of whelk than horse mussel, swimming crab and shore crab, but all contributing species could be separated based on their FA profile. The SI data corresponded to this, where whelk had a higher $\delta^{13}C$ than the other contributing species, suggesting a different diet. There was a regional influence on the $\delta^{15}N$ within this category, where sample pools collected from Holy Loch had a significantly higher $\delta^{15}N$ than those collected from the Solway Firth, Hunterston, Tancred Bank, Outer Firth of Forth, Pladda and Montrose Bank. Demersal invertebrates muscle had the highest calculated trophic level (3.87 ± 0.05) in comparison to the other invertebrate species, followed by benthic invertebrates muscle (3.68 ± 0.30), benthic invertebrates soft body (3.53 ± 0.47), benthic invertebrates whole (3.42 ± 0.50) and benthic invertebrates brown meat (3.24 ± 0.06). Zooplankton had characteristic dinoflagellate phytoplankton markers, corresponding to a primary consumer diet and had the lowest calculated trophic level (1.47 ± 0.11) in the food web of this project.

To overcome the issue of uncertainty in environmental assessments using generic trophic levels and TMF data from literature and databases (e.g. Fishbase), Chapter 3 provided ecosystem-specific trophic level data covering the large species diversity or regions present in the Scottish marine food web. The trophic level data from this study permitted the calculation of TMFs for a range of contaminants which could be used in environmental status assessments and guide the management of human activities impacting on marine systems.

Second objective: To determine the concentration of contaminants (PCBs, PBDEs and trace metals and metalloids) in marine species covering a wide trophic level range within the Scottish marine food web.

The selection of a TMF for a given substance is a critical issue, due to the variability existing within ecosystems (region, physiology, etc). In order to understand TMFs and prove whether the main driver of bioaccumulation is trophic level, the cause of variability

within sample categories (inter- and intra- species variation) must be established to determine the reliability of the calculated TMF.

The variability of concentrations and distributions of thirty-two PCB congeners, nine PBDE congeners and nine metals and metalloids within and between nineteen sample categories (categorisation discussed in Chapter 3: first objective) from the Scottish marine food web was analysed. There were clear differences in PCB and PBDE patterns between different sample categories and species which were a consequence of the effect of a mixture of influencing factors such as physiological processes (metabolism) and eco-biological parameters (region, length, weight, age, habitat and diet).

There was much less of a link between the concentration of these metals and metalloids and the feeding pattern and trophic level relationships existing in the project marine food web than for PCBs and PBDEs. Metal distribution was not common across the sample categories, with Cu, Se and Zn detected in all the samples (individuals and pools). Five out of the nine metals and metalloids had a significant relationship with trophic level (Zn, Hg, Cu, Ni and Cd) ($p<0.05$).

There were differences in PCB congener proportion and concentration between the three marine mammal species. The highest $\sum PCB_{32}$ concentration compared to the other sample categories was found in harbour seal blubber and highest $\sum PBDE_{9}$ concentration was found in sperm whale. Sperm whale had very little within-group variation of PCB congener proportion in comparison to harbour seal and harbour porpoise and were separated from the other two species. This agrees with the findings of FA and SI analysis, where sperm whale was 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. As well as feeding ecology, the more distinct PCB and PBDE profiles of sperm whale are likely a result of the lower metabolic capacity to biotransform smaller congeners, size (lower concentration per unit mass) and distance from pollution sources (long-term transport of lower chlorinated and brominated congeners). Harbour seal and harbour porpoise had more variable PCB and PBDE profiles corresponding to their dietary pattern identified in Chapter 3 but could be differentiated from each other due to their differing metabolic capacity of lower chlorinated PCB congeners and CB149. Within-species variation could also be identified, where the individual emaciated harbour seal identified in Chapter 3 with a different FA profile was found to have a different PCB and PBDE profile compared to the other harbour seal samples and a significantly higher concentration of $\sum PCB_{32}$ and $\sum PBDE_{9}$ in its blubber. These findings indicate a strong

association between PCB pattern and feeding ecology and have the potential to influence TMF calculation.

The shark and fish categories were influenced by trophic level, species, diet, metabolism, sampling location and size. Shark and fish had a higher proportion of lower chlorinated and brominated congeners in their PCB and PBDE profiles than marine mammals due to their lower metabolic capacity for these lighter congeners. All four categories could be separated based on their PCB and PBDE congener profiles. Demersal shark liver had the highest $\sum PCB_{32}$ concentration and pelagic roundfish liver had the highest $\sum PBDE_9$ concentration out of the shark and fish categories. Demersal shark possessed the highest mean trophic level and least variable PCB and PBDE profile. This category also had the highest Hg concentration in its muscle in comparison to the other fish and invertebrate sample categories and a smaller Hg concentration range. This corresponds with the FA distribution and SI data, where there was little variation in feeding pattern within the species suggesting that diet is the major contributor to PCB, PBDE and Hg contamination in demersal shark.

Pelagic roundfish could be separated from the other shark and fish categories during PCA analysis of PCB profiles and had a different PBDE profile, likely due to their different diet of planktonic prey which was confirmed in Chapter 3 during FA profile analysis. Similar to the FA and SI results, there was a large degree of variation present in the PCB and PBDE profiles in the demersal roundfish category. On a species level, hake were found to have a different PCB profile in comparison to whiting and haddock, and all three species had PCB and PBDE profiles distinguishable based on their sampling location. This correlates to the SI result, where on a category and species level, there was a regional influence on the $\delta^{15}N$.

When PCB concentrations in the fish categories were compared to OSPAR EACs, only the EAC of CB118 was exceeded by demersal roundfish liver, muscle and whole and pelagic roundfish whole. For the assessment of PBDEs, the $\Sigma PBDE_6$ concentration exceeded the adjusted EQS for each sample category. Neither of the congener concentrations exceed the given FEQG values. The Hg concentration of shark and fish muscle and invertebrates was corrected using the TMF (both traditional and balanced method TMFs). It was found that trophic adjustment using a balanced dataset TMF had more of an influence on the adjusted concentration and is highly recommended for a true indication of secondary poisoning by biomagnification.

Flatfish had the highest concentration of Hg in their muscle and larger concentration range than the other fish categories due to a significant regional influence on Hg

concentration ($p<0.05$). This does not correlate with FA, SI and organic contaminant analysis where the demersal roundfish category had the largest degree of variation in feeding pattern and concentration range.

There was a large degree of variation present in the PCB profiles of the invertebrates categories which was found in FA analysis, where considerable variation for the benthic invertebrates whole, muscle and soft body FA profiles suggested highly variable feeding patterns. Common starfish had a large degree of variation in both their PCB congener proportion and concentration, where samples were separated based on their sampling location. FA analysis however found common starfish to group together and have very little variation in the FA profile suggesting a similar feeding pattern, however a $\delta^{13}C$ found a significant regional influence on the diet of this species ($p<0.05$). Benthic invertebrates soft body and muscle were also found to have highly variable PCB profiles which was also seen for their FA profiles. Within these categories there was both a significant species and regional influence on the PCB profiles ($p<0.05$). The concentration of $\sum PBDE_9$ in all invertebrate sample categories was low, with most congeners having a concentration below or close to the LoD.

Benthic invertebrates muscle had the largest degree of variation of Hg concentration than the other invertebrates categories due a significant within category species and regional influence. Cd, Cu, Ni and Zn were all significantly higher in benthic invertebrates categories which suggested that there was more of a species-specific accumulation of these metals in the food web rather than concentrations as a result of biomagnification. Benthic invertebrates brown meat had the highest concentration of Cd, Cu and Ni than the other sample categories due to the detoxifying nature of the hepatopancreas making up the brown meat. Benthic invertebrates soft body had a large concentration range for Cd, Cu, Ni and Zn in comparison to the other sample categories due to the specific physiological ability to accumulate Cd, Ni and Zn and control Cu uptake.

The data from this chapter provided ecosystem-specific information on the relationship between contaminant concentration within an organism or a sample category and key factors which influence those concentrations (trophic level, region, sample categorisation and physiological factors). The identified influencing factors were considered when TMFs were calculated on selected contaminants.

Third objective: To contribute to the wider development of TMFs for organic and inorganic contaminants with a high level of confidence and worldwide applicability.

Based on the data obtained from the first and second objectives, TMFs were calculated for the ICES-7 PCBs, BDE47 (selection of congeners discussed in results and discussion) and trace metals with a significant trophic level relationship (Hg, Cd, Cu, Ni and Zn) using two methods: traditional and balanced methods (Borgå *et al.*, 2012; Brisebois, 2013) to determine whether biomagnification occurs in the food web and whether secondary poisoning should be accounted for when conducting environmental quality assessments.

Biomagnification was found to occur for the ICES-7 PCBs and BDE47 congeners in a food web composed of marine mammals, demersal shark, fish and invertebrates using the traditional method, with the highest degree of trophic magnification reported for CB52. CB180, CB153, CB52 and BDE47 were found to have a lower calculated TMF when marine mammals were removed from the dataset due to their resistance to biodegradation. The higher TMF of CB138, CB118, CB101 and CB28 when marine mammals were not included in the analysis suggested that these congeners do not accumulate in upper trophic level marine mammals. Hg was found to biomagnify in a food web composed of demersal shark, fish and invertebrates using the traditional method, however when demersal shark was removed from the dataset, Hg was found to trophic dilute, suggesting that biomagnification does not occur in a food web composed of lower trophic level organisms (invertebrates and fish). Cu was found to biomagnify in the same food web as for Hg when sample categories with a specific physiological influence on concentration were removed (benthic invertebrates soft body and brown meat) using the traditional method.

When the 'balanced method' was used to calculate TMFs, an unbalanced dataset was found to influence some key findings. An unbalanced dataset was found to influence the TMF of BDE47, where biomagnification occurred using the traditional method and trophic dilution occurred using the balanced method when shark, fish and invertebrates from the four biogeographic regions were analysed. There was also a difference in the calculated TMFs between both methods for CB28, where trophic magnification was found to occur in all trophic levels from the four regions using the traditional method and trophic dilution using the balanced method. For trace metals, both Cu and Zn were found to biomagnify in the food web composed of demersal shark, fish, invertebrates and zooplankton using a balanced dataset but trophic dilute using the traditional method, suggesting that the

different utilisation rate of invertebrate species does influence the calculated TMF in an unbalanced dataset.

An unbalanced dataset was found to influence the TMF when conducting regional comparisons. CB153, CB138, CB101, CB28 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 Biogeographic Region, where the balanced method calculated a higher TMF in the Irish Sea Biogeographic Region than the other regions. This was due to the difference in sample numbers of invertebrates and marine mammals between the regions. CB28 possessed the largest 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.

The balanced method was found to influence the significance of the concentration-trophic level regression, where the correlation was significant ($p<0.05$) for CB28, BDE47, Hg and Cd using the traditional method but not significant ($p>0.05$) when using the balanced method. This suggests that a larger dataset is required to sufficiently test significance when calculating the TMF of lighter PCBs with less biomagnification ability, particularly when higher trophic level predators are not available and when the dataset is unbalanced. The balanced method also highlighted particular species-specific and regional-specific congener retention which was not clear using the traditional method, for example harbour porpoise from the Northern North Sea, Minches and western Scotland and the Scottish continental shelf food web had a much higher concentration of CB118 in their blubber than harbour seal due to their lower capacity to metabolise this congener, resulting in non-significant correlation ($p>0.05$) in comparison to the traditional method.

The findings from this study contribute to the wider development of TMFs for organic and inorganic contaminants with a high level of confidence and worldwide applicability. Feeding ecology contributed to the variation identified in PCB, PBDE and trace metal and metalloid concentration and PCB and PBDE congener proportion across the sample categories and, along with other identified factors, can be used to identify the variation associated with calculated TMF. An unbalanced dataset does influence the calculated TMF and in some cases, the overall conclusion of the trophic transfer of PCB and PBDE congeners and metals/metalloids. This method has proven particularly useful during regional comparisons when achieving a balanced dataset is difficult, there are data outliers and when concentrations are low (<LoD). Although TMFs provide valuable information regarding bioaccumulation potential and should be incorporated into

regulatory decision making, species selection and data treatment must be kept consistent in future studies. The balanced method is therefore highly recommended for calculating TMFs to ensure the TMF is a true indication of biomagnification potential.

Future work

The sampling in this project was opportunistic and although a diverse trophic level dataset was achieved representing the Scottish marine food web, to compare feeding patterns in species across different regions, a higher sample number is required for statistically robust results. The samples in this project were however pooled which does increase the confidence of the findings and provides a strong indication of the feeding patterns and trophic relationships existing in the project food web. Although grouping samples together based on their differences in FA profiles (separating classes, tissue types and water column depths) enabled a comparison of feeding patterns, there was still considerable variation in some of the categories (demersal roundfish and benthic invertebrates whole and soft body in particular), suggesting that future work should focus on species rather than groups. This also applies to contaminant analysis, where a large degree of variation was present within the same categories for PCBs and PBDEs, which correlated more to the feeding patterns and trophic relationships than trace metal concentrations, which were influenced more on a species level than regional. Due to the essential nature of trace metals, appropriate species and tissue selection is vital to ensure TMFs accurately represent the selected ecosystem.

It was recognised throughout the project that obtaining a larger sample number of individual species regions should also allow a regional comparison with a greater level of confidence. Borga˚ *et al.,* (2011) suggests that 30 to 40 samples are likely needed to conduct a trophic magnification study. The lack of sample numbers representing an assessment region has been encountered in Scotland's Marine Assessment 2020, where the Scottish Continental Shelf had insufficient number of time series or spread of stations for a regional status and trend assessment of PCB concentrations in sediment and biota; partly due to difficulties sampling in this area. A key finding from this project is that even with ecosystem specific trophic level and contaminant data, there was still a considerable level of variation present in the calculated TMF. If the recommended balanced method is used in the future for assessment purposes, it must be recognised that the method is time consuming, expensive, and careful balance must be struck between the analysis requirements and population numbers in a particular region.

Appendix

Table A.1: Example target and qualifier Ions, using SIM with a dwell time of 50 ms and molecular weight (MW) and chlorination level.

PCB	MW	Target Ion	Qualifier Ion	No Cl
^{13}C-CB28	**270**	268	270	3
CB31	**258**	258	256	3
CB28	**258**	256	258	3
^{13}C-CB52	**304**	304	302	4
CB52	**292**	292	290	4
CB49	**292**	292	290	4
CB44	**292**	292	290	4
CB74	**292**	292	290	4
CB70	**292**	292	290	4
^{13}C-CB101	**340**	338	340	5
CB101	**326**	326	328	5
CB99	**326**	326	328	5
CB97	**326**	326	328	5
CB110	**326**	326	328	5
CB123	**326**	326	328	5
CB118	**326**	326	328	5
CB105	**326**	326	328	5
CB114	**326**	326	328	5
^{13}C-CB153	**374**	372	374	6
CB149	**362**	360	362	6
CB153	**362**	360	362	6
CB132	**362**	360	362	6
CB137	**362**	360	362	6
^{13}C-CB138	**374**	372	374	6
CB138	**362**	360	362	6
CB158	**362**	360	362	6
CB128	**362**	360	362	6
^{13}C-CB156	**374**	372	374	6
CB156	**362**	362	360	6

CB167	**362**	360	362	6
CB157	**362**	360	362	6
^{13}C-CB180	**408**	406	408	7
CB187	**396**	394	396	7
CB183	**396**	394	396	7
CB180	**396**	394	396	7
CB170	**396**	394	396	7
13C-CB189	**406**	406	408	7
CB189	**396**	394	396	7
^{13}C - CB194	**442**	442	440	8
CB194	**430**	430	428	8
^{13}C-CB209	**510**	510	508	10
CB209	**498**	498	496	10

CB153

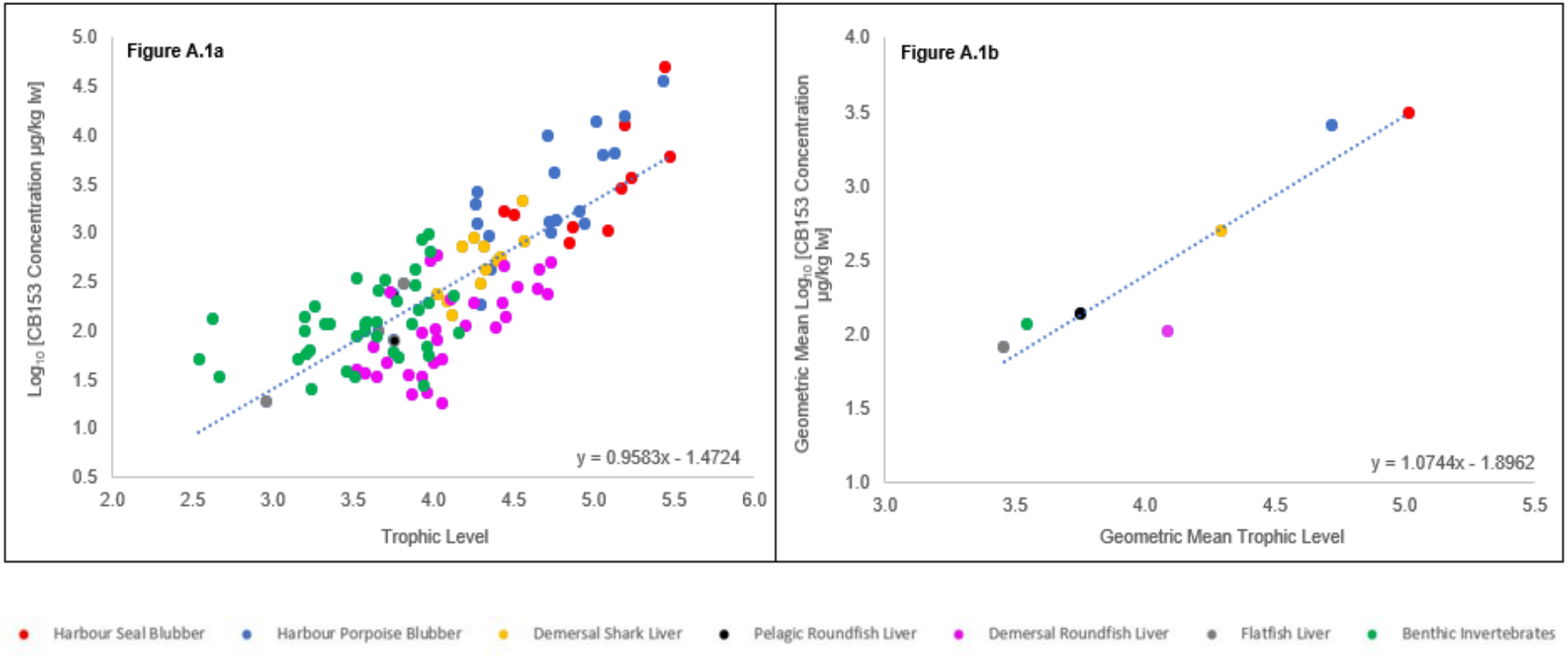

Figure A.1: (a) Relationship between trophic level and logarithmically transformed CB153 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, 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 CB153 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.

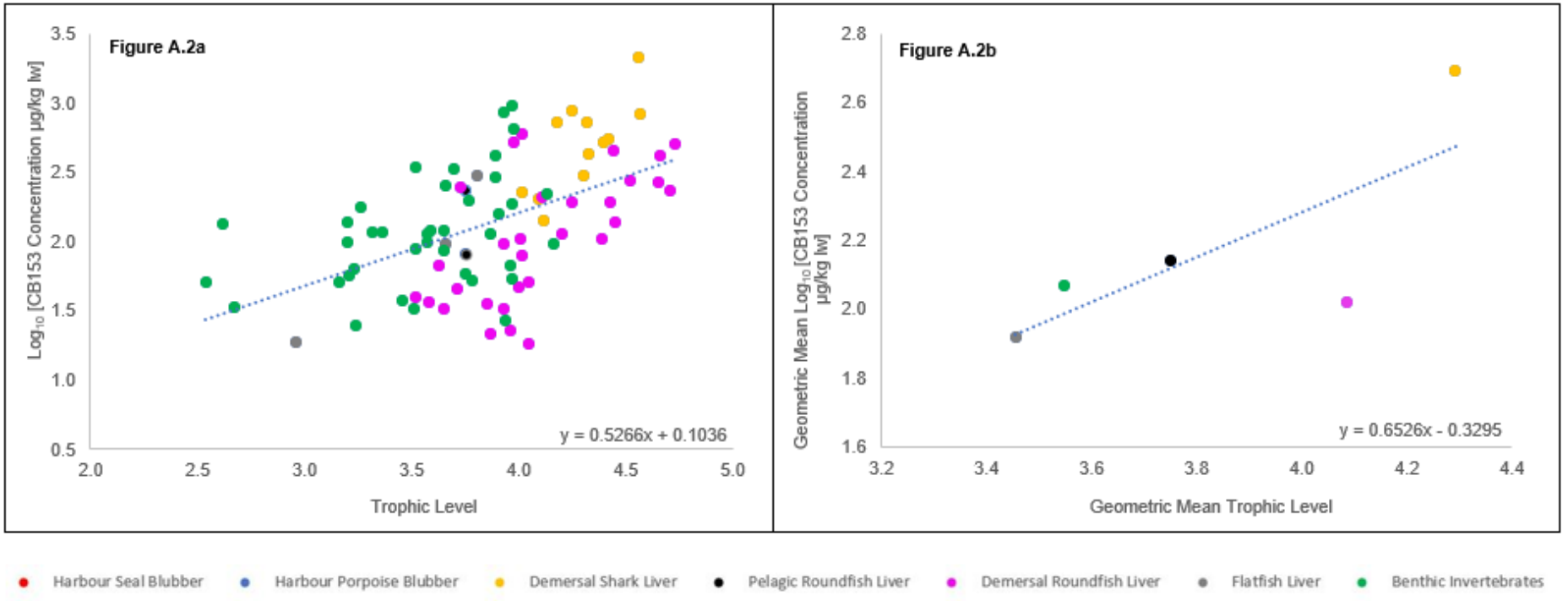

Figure A.2: (a) Relationship between trophic level and logarithmically transformed CB153 concentration (µg/kg lw) in demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, muscle, brown meat, soft body (green) from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf. (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean CB153 concentration (µg/kg lw) in demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, muscle, brown meat, soft bodyreen) from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.

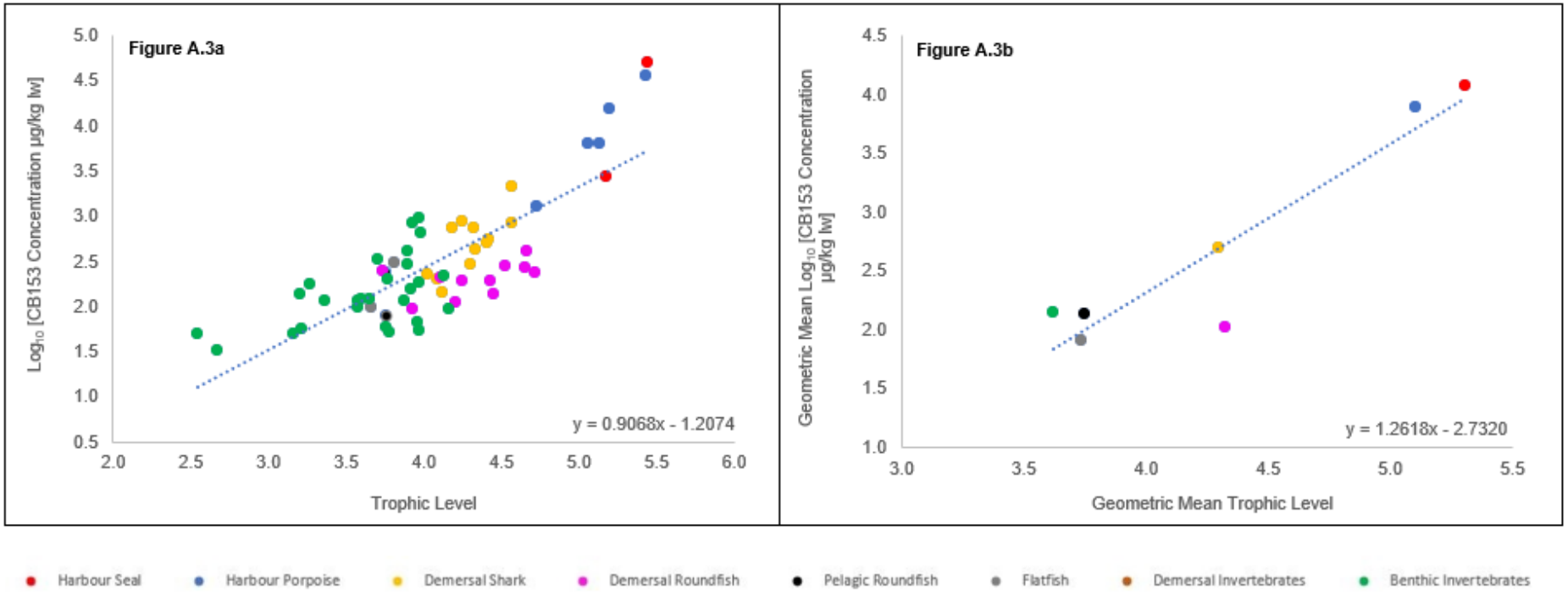

Figure A.3: (a) Relationship between trophic level and logarithmically transformed CB153 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, muscle, brown meat, soft body (green) from the Irish Sea Biogeographic Region (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean CB153 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.

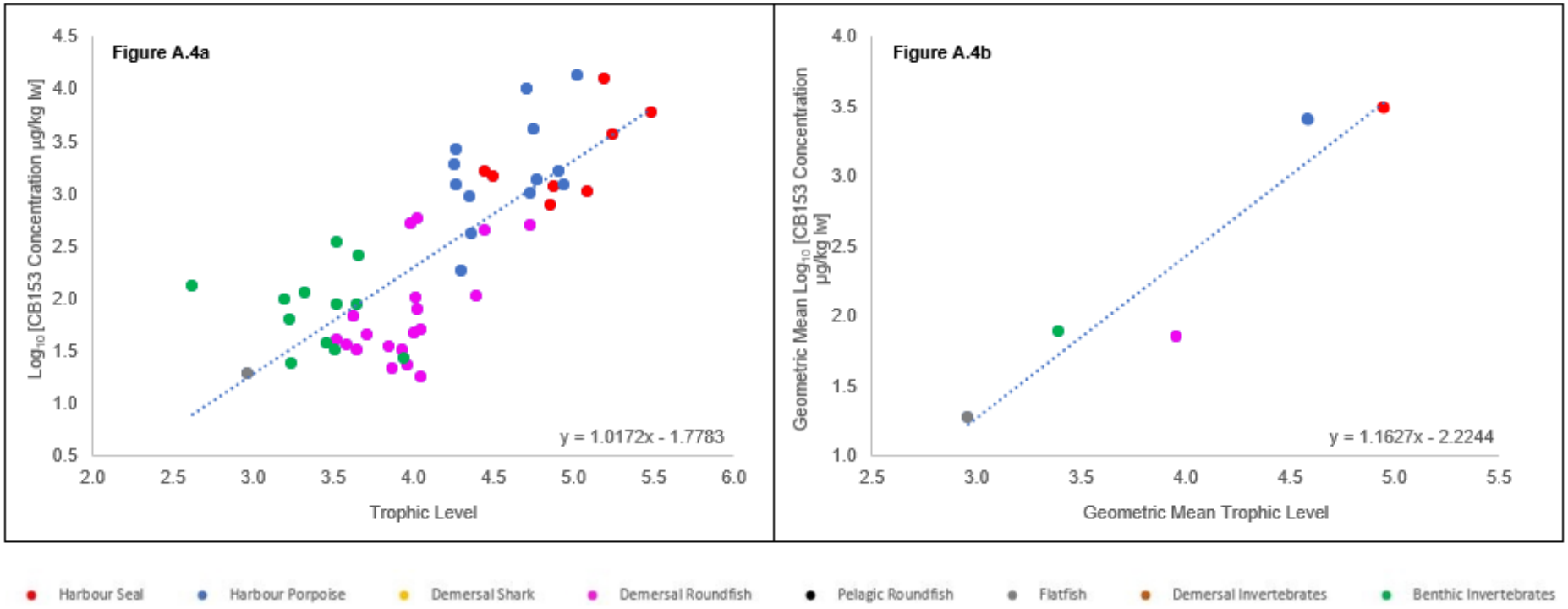

Figure A.4: (a) Relationship between trophic level and logarithmically transformed CB153 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal roundfish liver (pink), flatfish liver (grey) and benthic invertebrate whole, muscle, brown meat, soft body (green) 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 CB153 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), demersal roundfish liver (pink), flatfish liver (grey) and benthic invertebrate whole, muscle, brown meat, soft body (green) form the Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf.

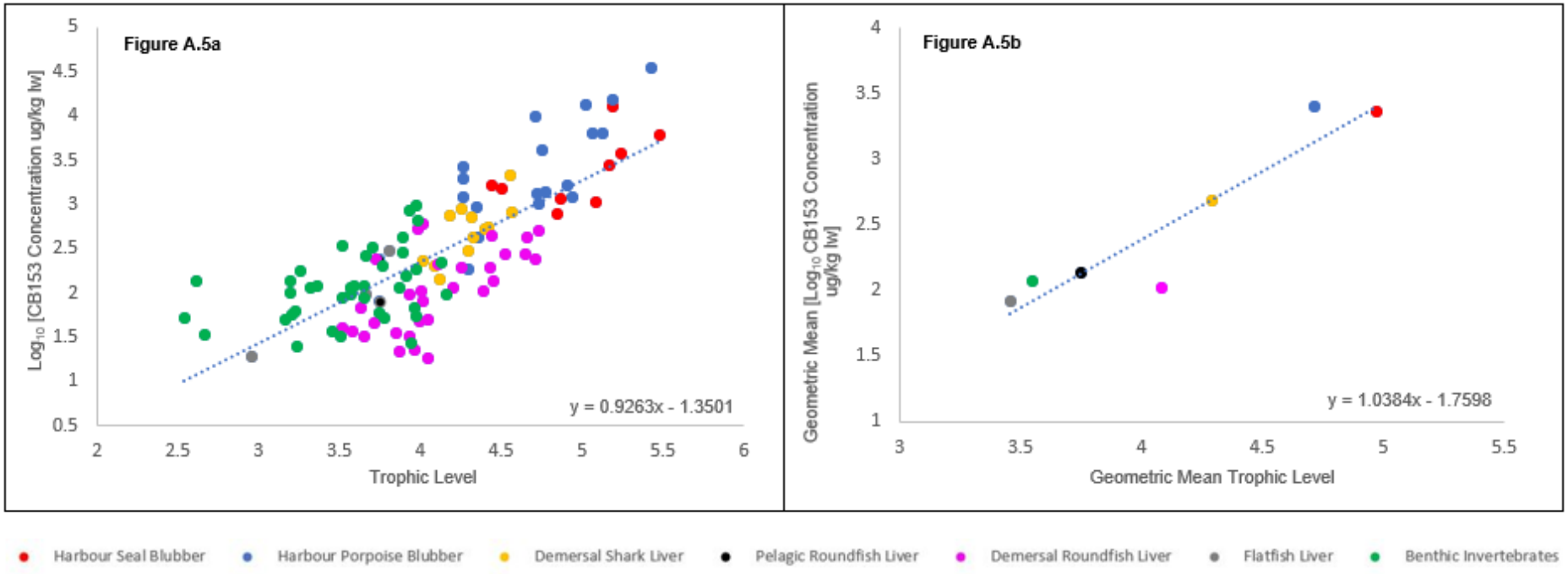

Figure A.5: (a) Relationship between trophic level and logarithmically transformed CB153 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, muscle, brown meat, soft body (green) from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf, excluding the emaciated harbour seal sample outlier. (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean CB153 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, excluding the emaciated harbour seal sample outlier.

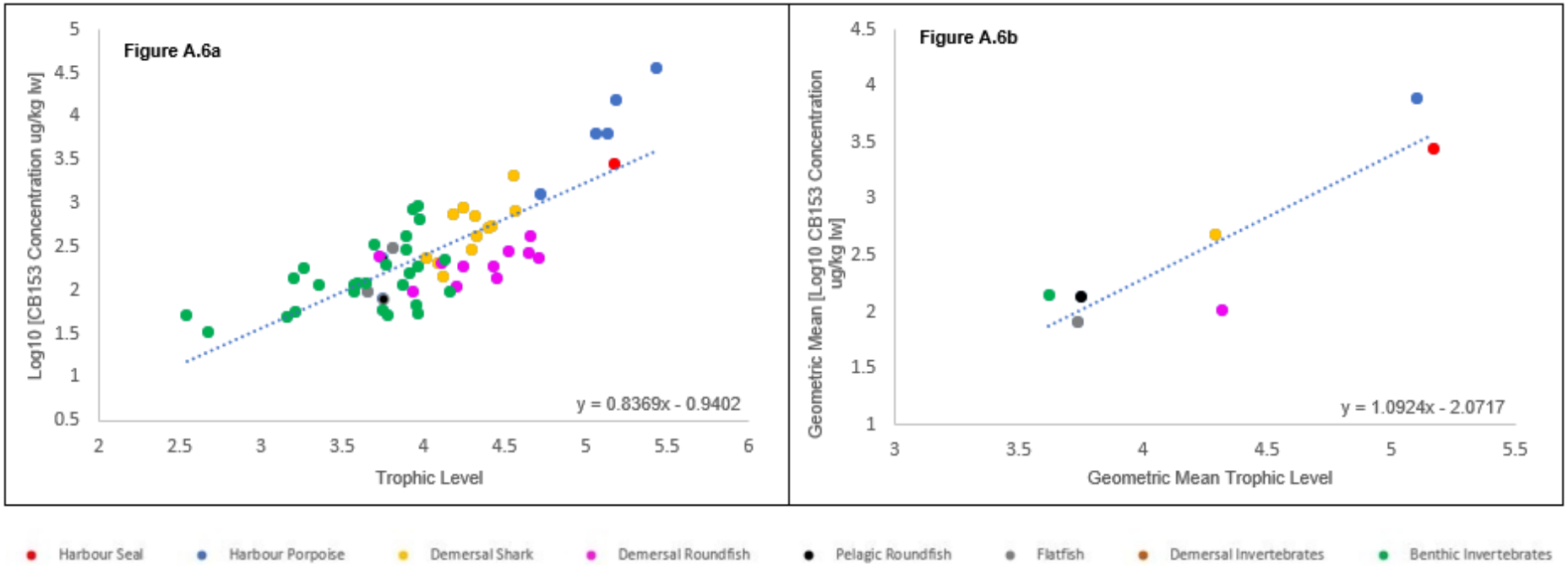

Figure A.6: (a) Relationship between trophic level and logarithmically transformed CB153 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, muscle, brown meat, soft body (green) from the Irish Sea Biogeographic Region, excluding the emaciated harbour seal sample outlier (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean CB153 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, excluding the emaciated harbour seal sample outlier.

CB138

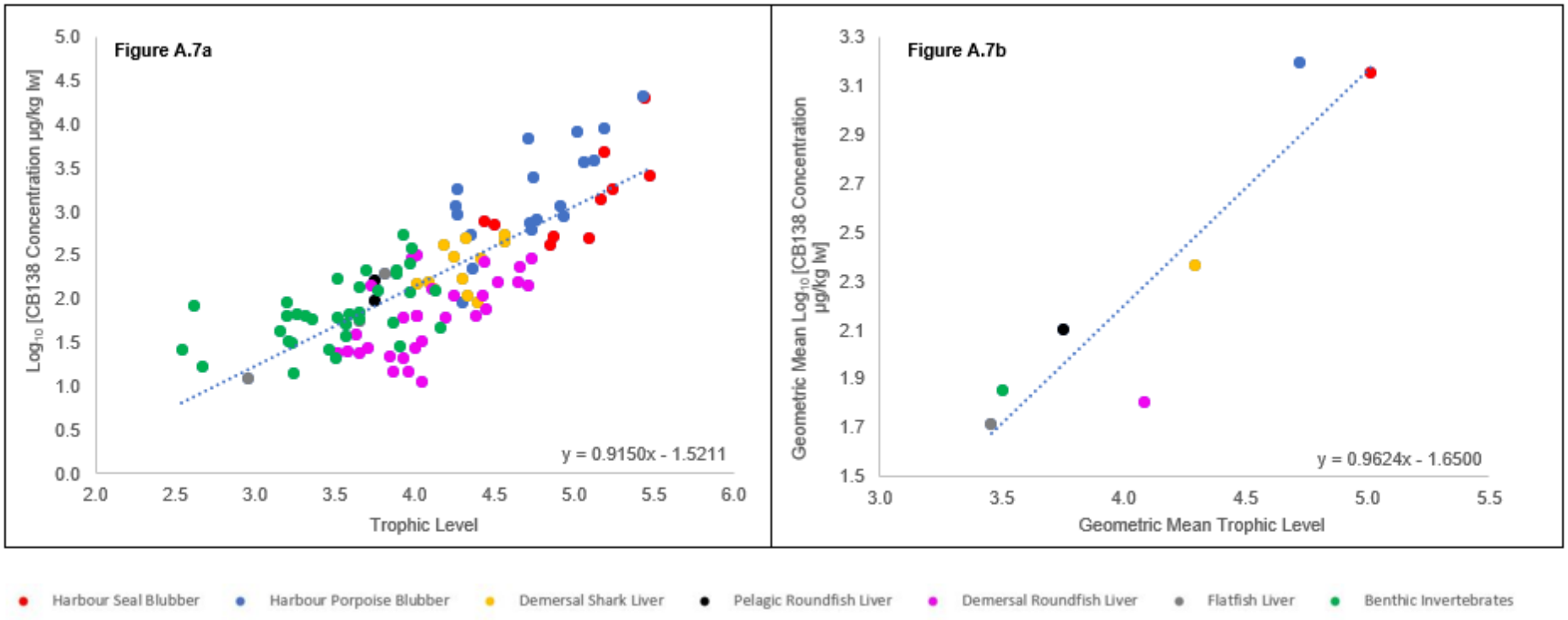

Figure A.7: (a) Relationship between trophic level and logarithmically transformed CB138 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, 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 CB138 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.

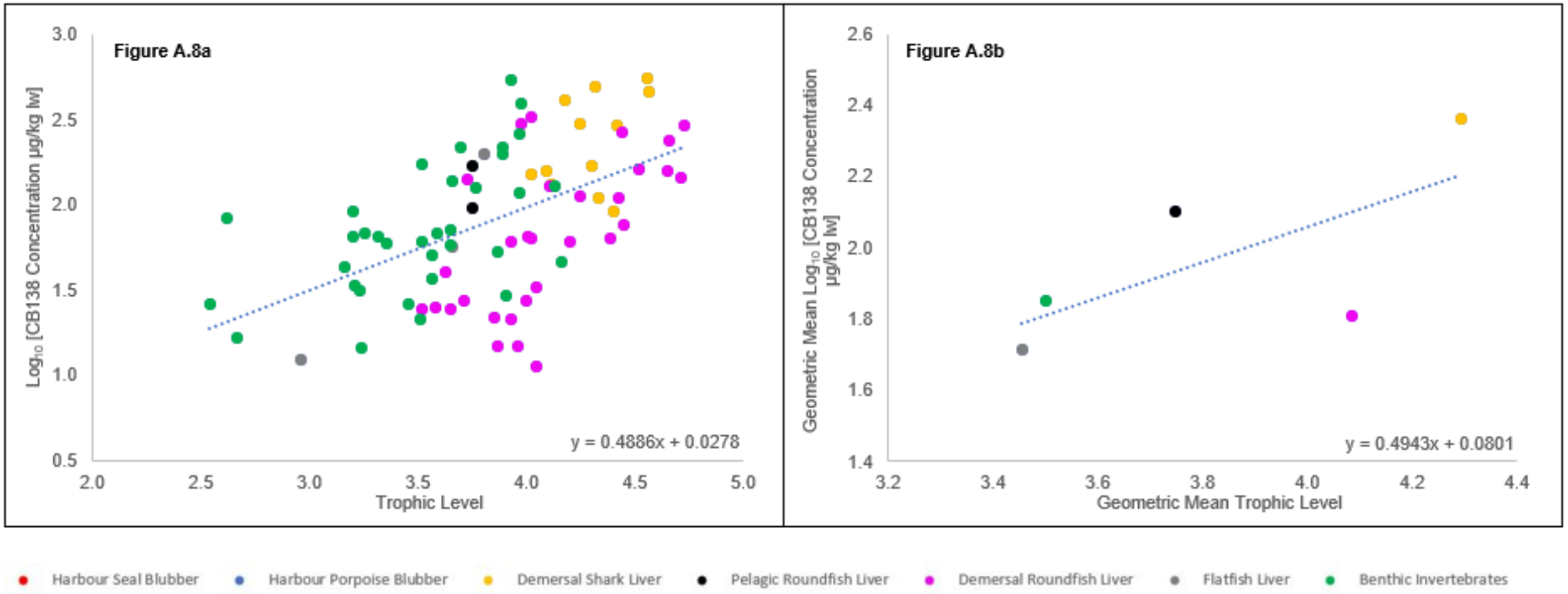

Figure A.8: (a) Relationship between trophic level and logarithmically transformed CB138 concentration (µg/kg lw) in demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, muscle, brown meat, soft body (green) from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf. (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean CB138 concentration (µg/kg lw) in 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.

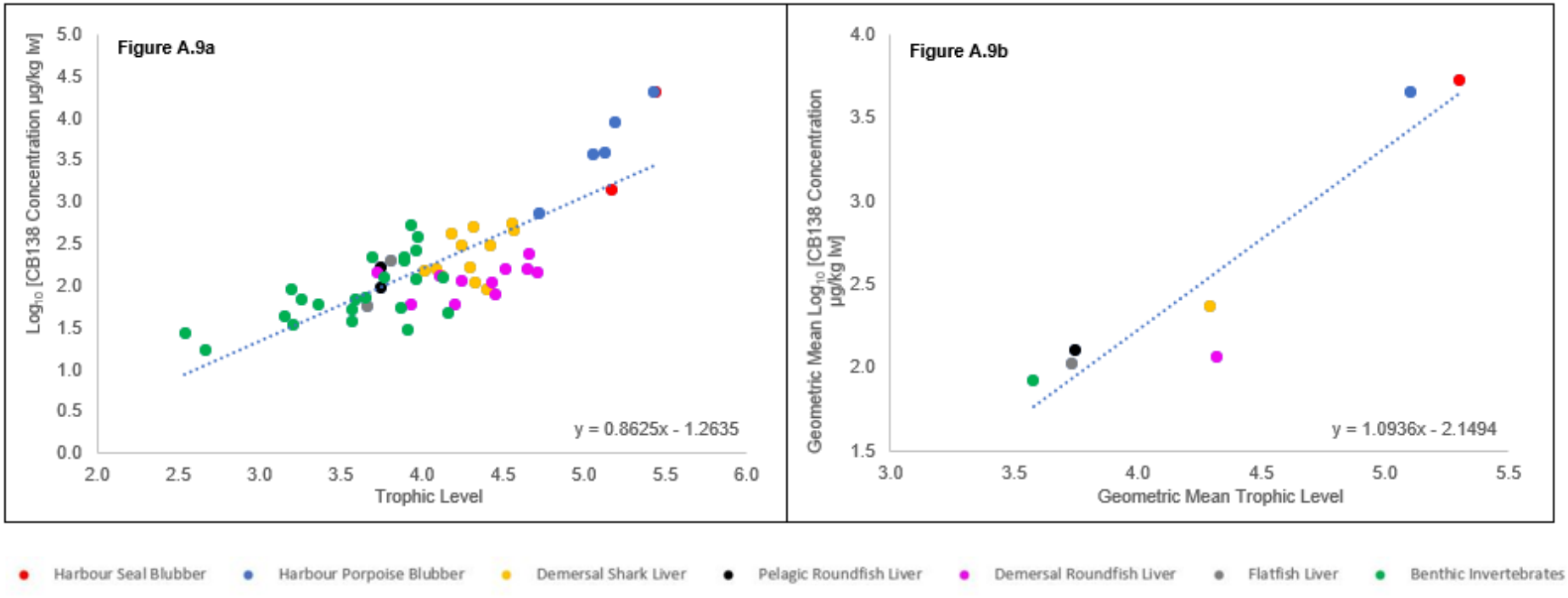

Figure A.9: (a) Relationship between trophic level and logarithmically transformed CB138 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, muscle, brown meat, soft body (green) from the Irish Sea Biogeographic Region (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean CB138 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.

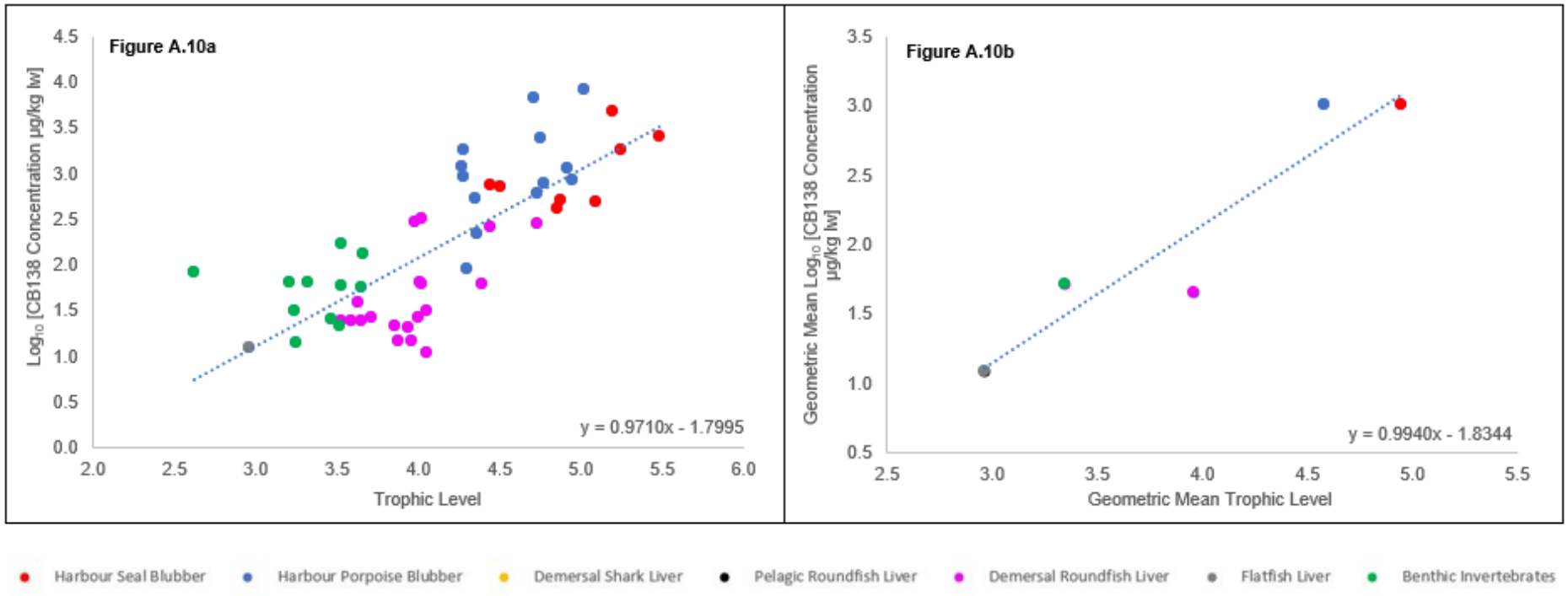

Figure A.10: (a) Relationship between trophic level and logarithmically transformed CB138 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal roundfish liver (pink), flatfish liver (grey) and benthic invertebrate whole, muscle, brown meat, soft body (green) 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 CB138 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), demersal roundfish liver (pink), flatfish liver (grey) and benthic invertebrate whole, muscle, brown meat, soft body (green) form the Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf.

CB118

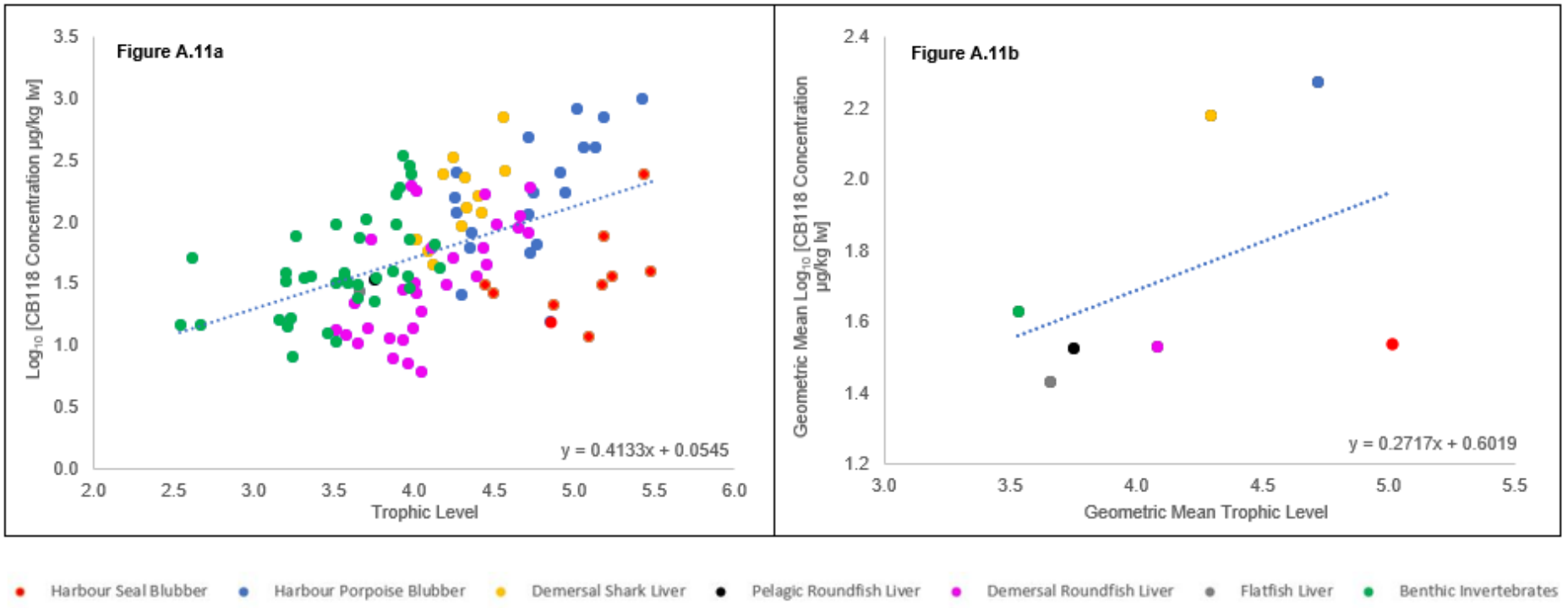

Figure A.11: (a) Relationship between trophic level and logarithmically transformed CB118 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, 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 CB118 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.

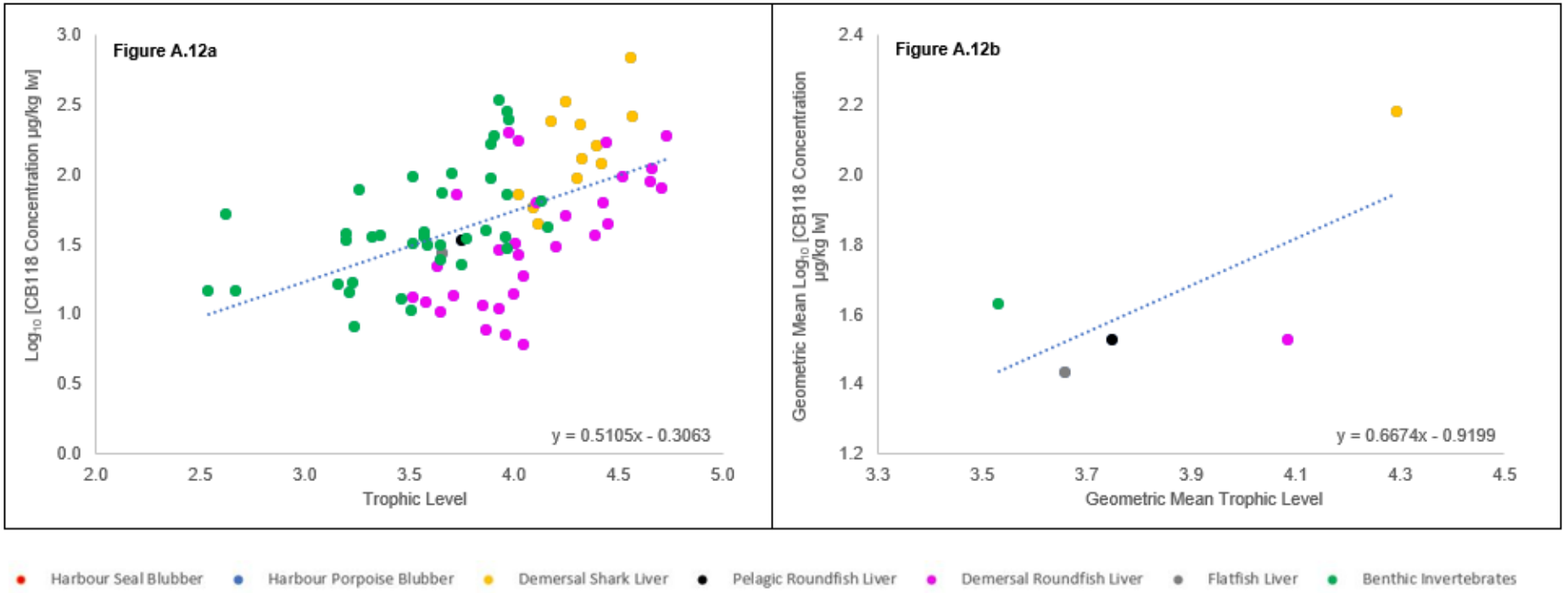

Figure A.12: (a) Relationship between trophic level and logarithmically transformed CB118 concentration (µg/kg lw) in demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, muscle, brown meat, soft body (green) from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf. (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean CB118 concentration (µg/kg lw) in 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.

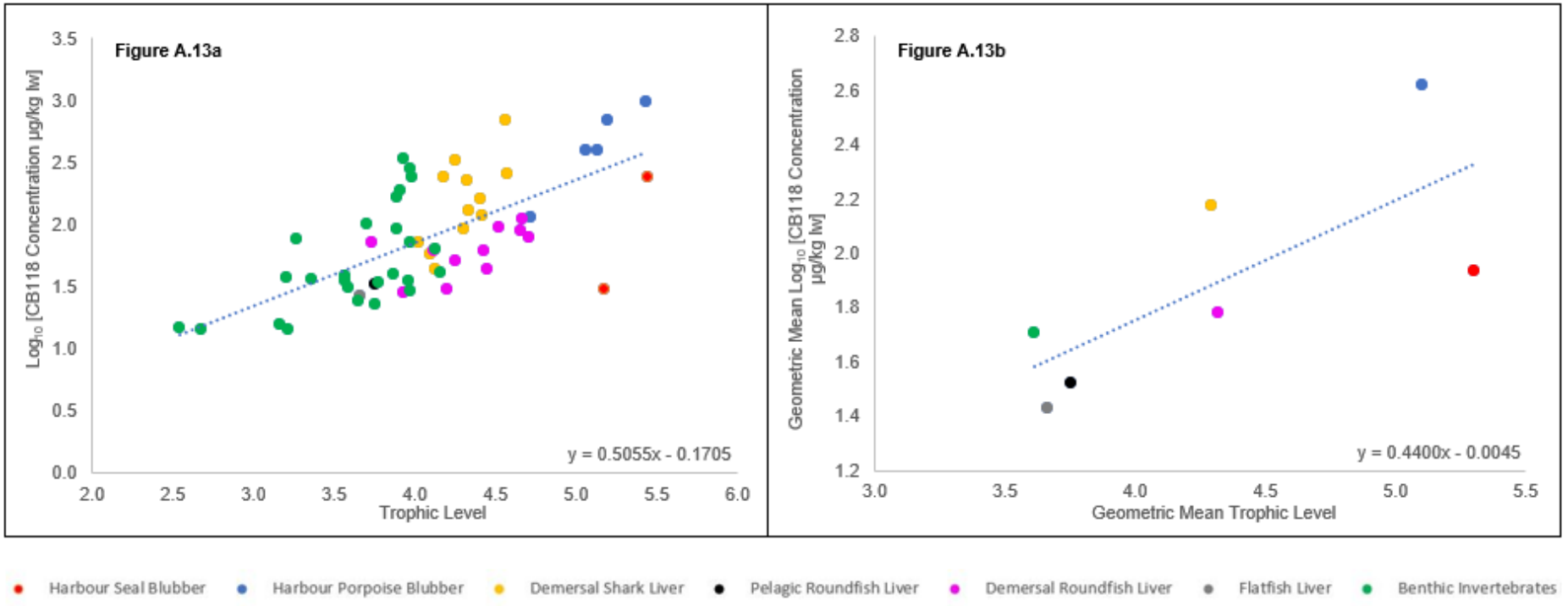

Figure A.13: (a) Relationship between trophic level and logarithmically transformed CB118 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, muscle, brown meat, soft body (green) from the Irish Sea Biogeographic Region (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean CB118 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.

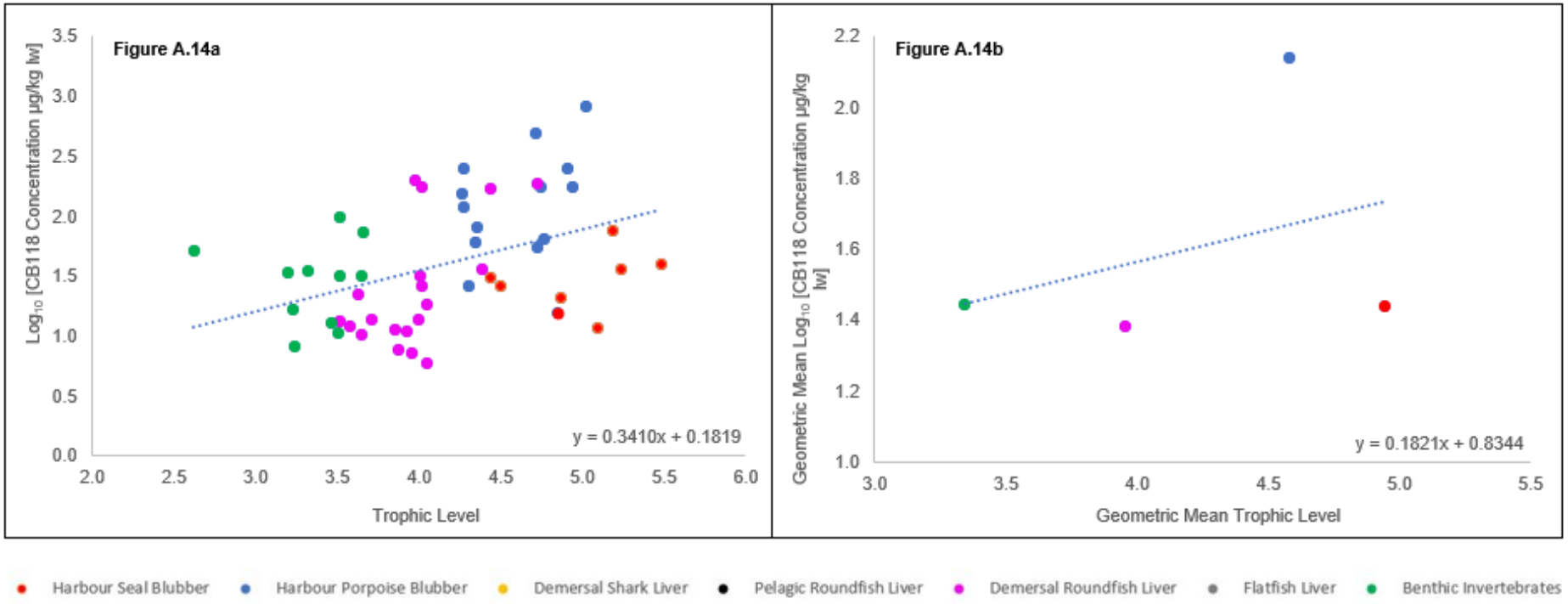

Figure A.14: (a) Relationship between trophic level and logarithmically transformed CB118 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal roundfish liver (pink) and benthic invertebrate whole, muscle, brown meat, soft body (green) from the Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf. (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean CB118 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.

CB101

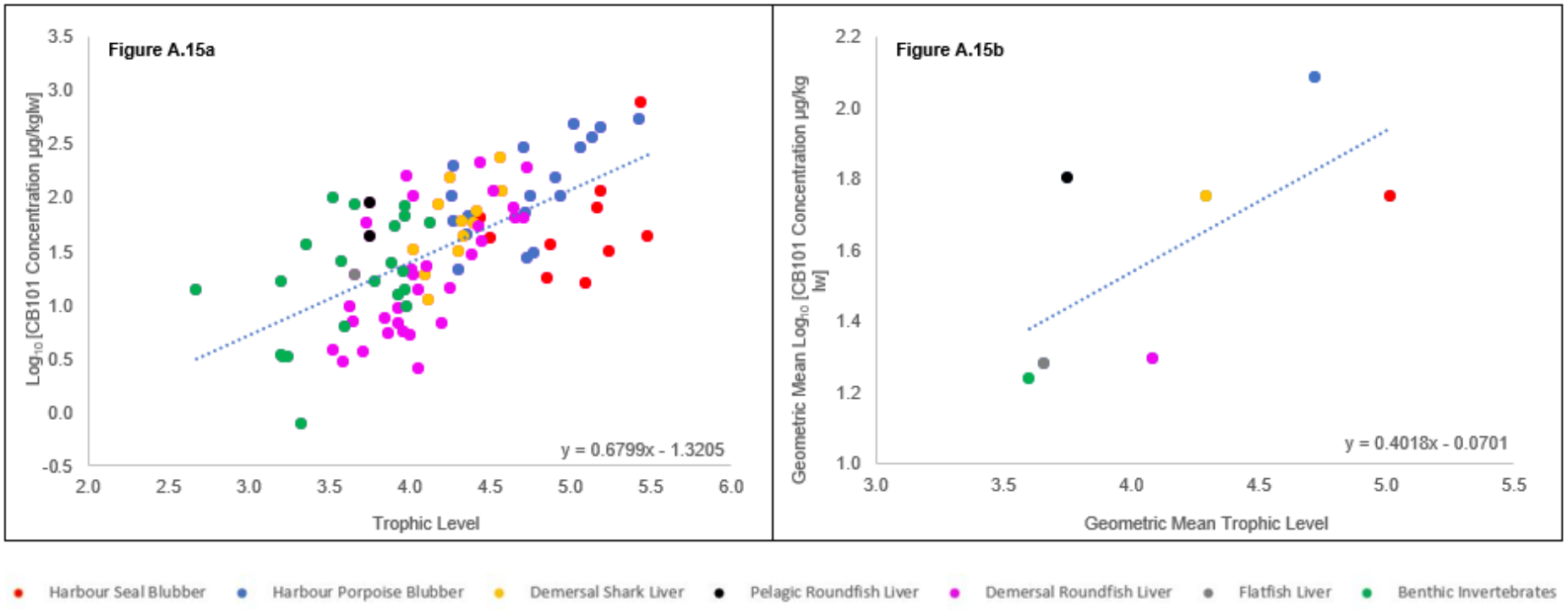

Figure A.15: (a) Relationship between trophic level and logarithmically transformed CB101 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, 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 CB101 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.

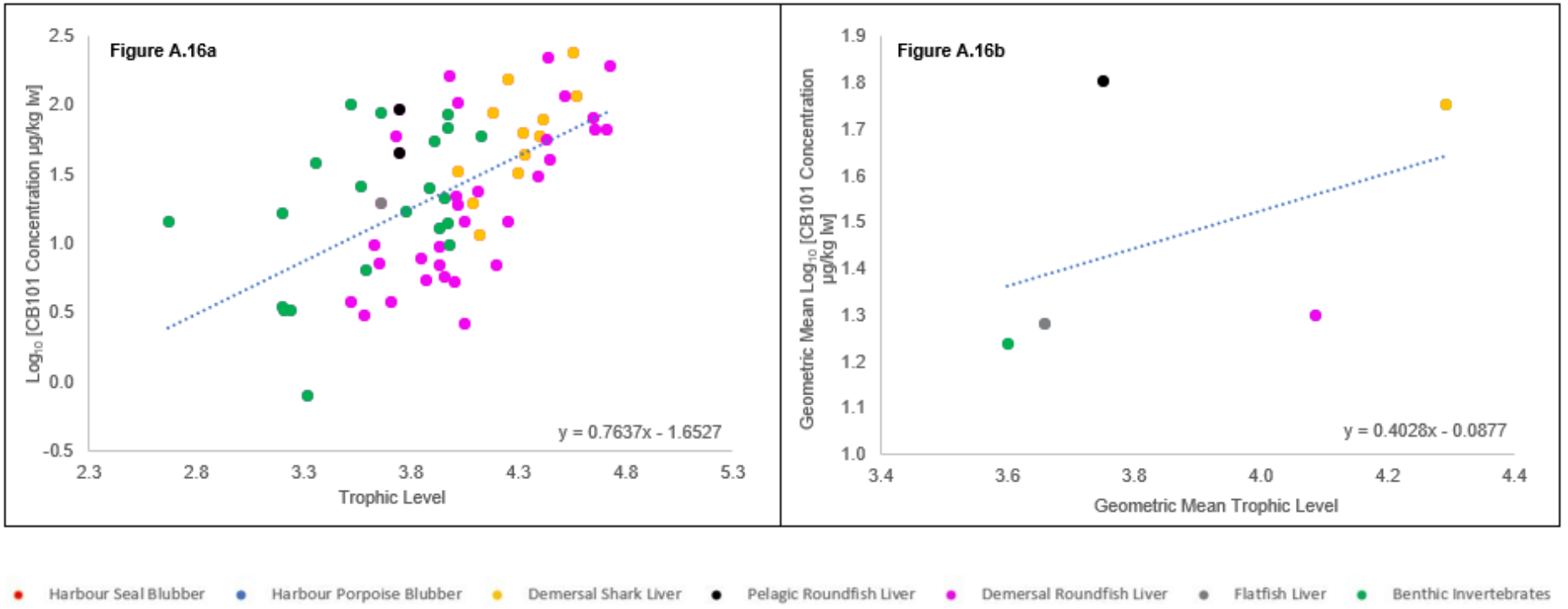

Figure A.16: (a) Relationship between trophic level and logarithmically transformed CB101 concentration (µg/kg lw) in demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, muscle, brown meat, soft body (green) from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf. (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean CB101 concentration (µg/kg lw) in 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.

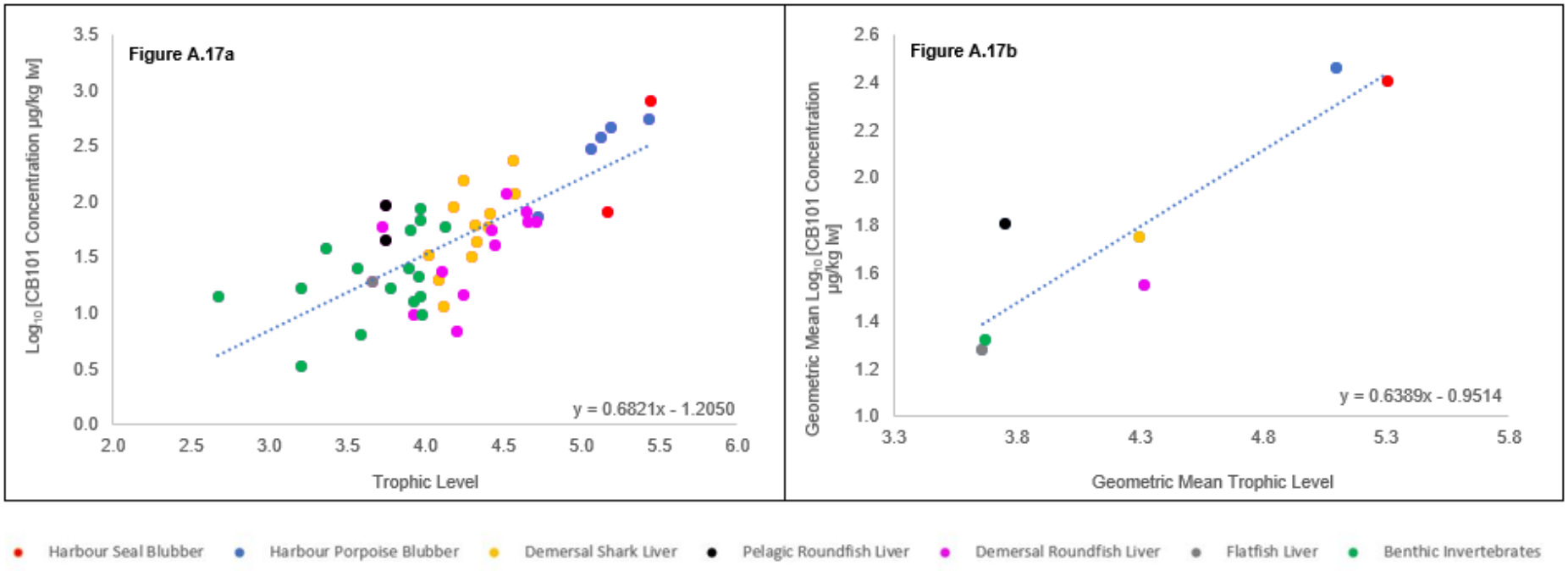

Figure A.17: (a) Relationship between trophic level and logarithmically transformed CB101 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, muscle, brown meat, soft body (green) from the Irish Sea Biogeographic Region (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean CB101 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.

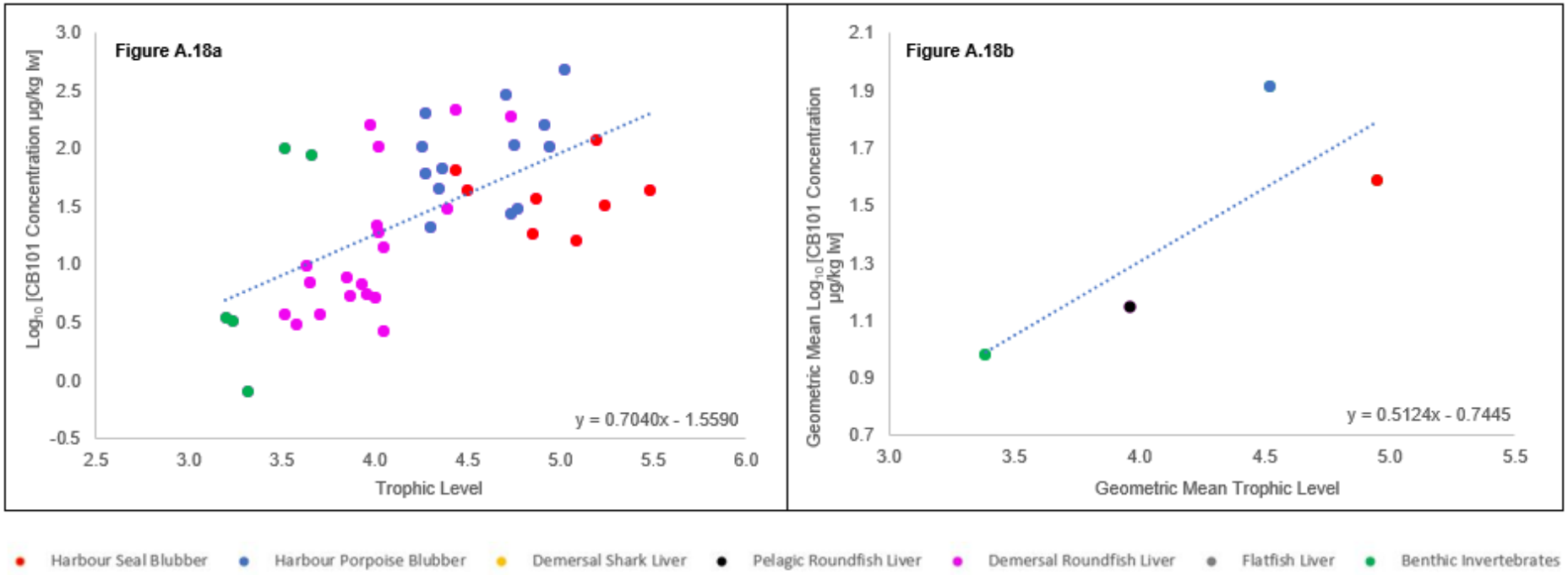

Figure A.18: (a) Relationship between trophic level and logarithmically transformed CB101 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal roundfish liver (pink), pelagic roundfish (black) and benthic invertebrate whole, muscle, brown meat, soft body (green) 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 CB101 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), demersal roundfish liver (pink), pelagic roundfish (black) and benthic invertebrate whole, muscle, brown meat, soft body (green) form the Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf.

CB52

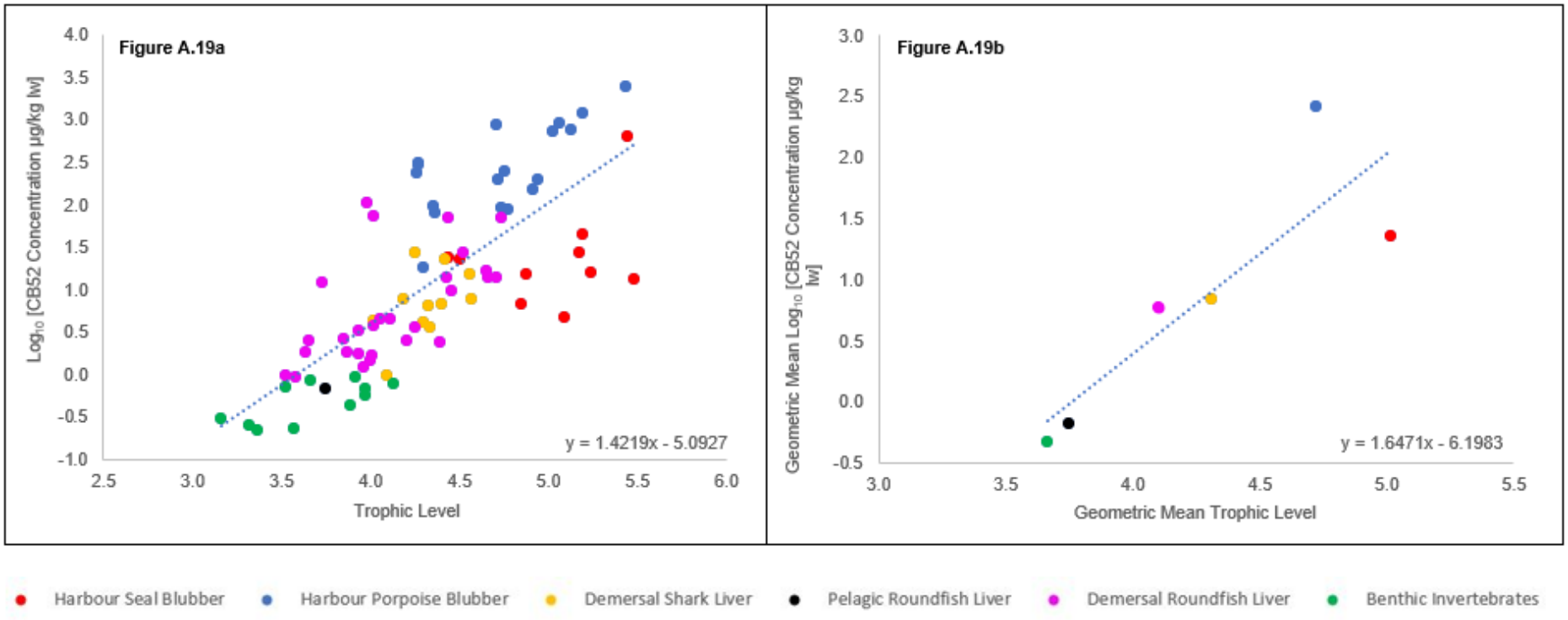

Figure A.19: (a) Relationship between trophic level and logarithmically transformed CB52 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, 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 CB52 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, brown meat, soft body (green) from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.

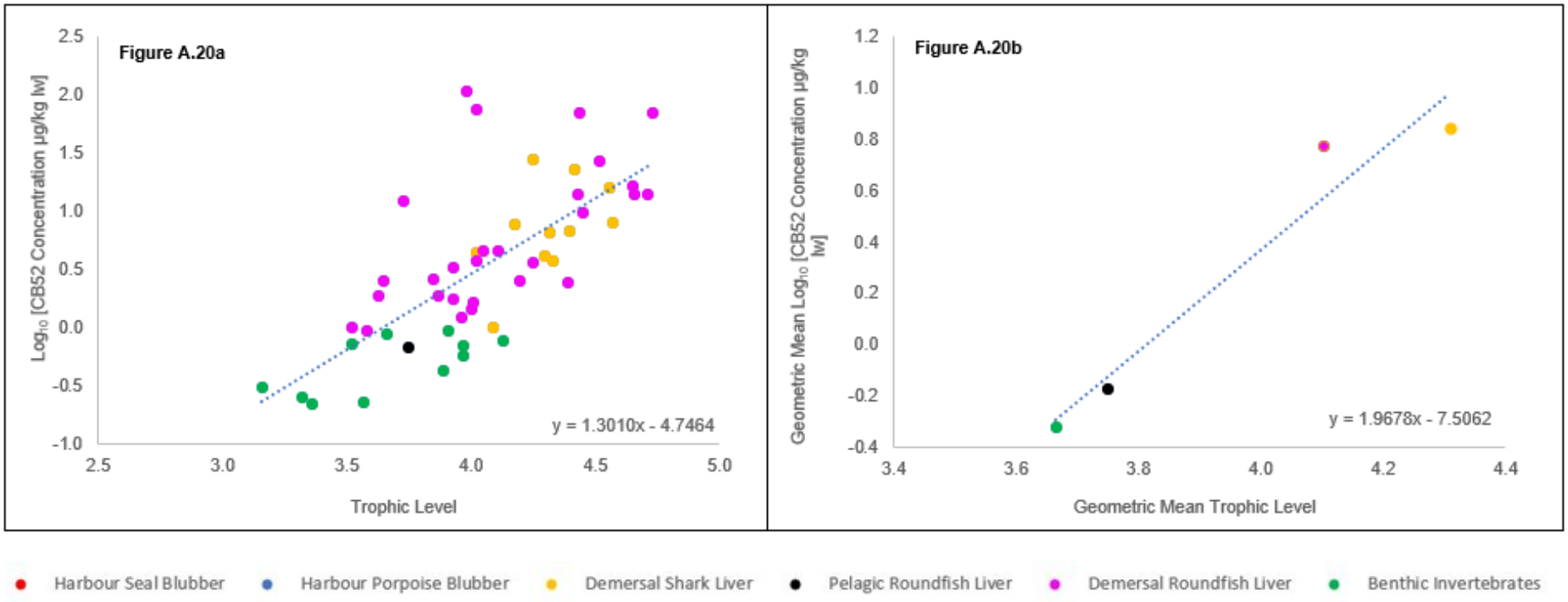

Figure A.20: (a) Relationship between trophic level and logarithmically transformed CB52 concentration (µg/kg lw) in demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, brown meat, soft body (green) from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf. (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean CB52 concentration (µg/kg lw) in demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, brown meat, soft body (green) from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.

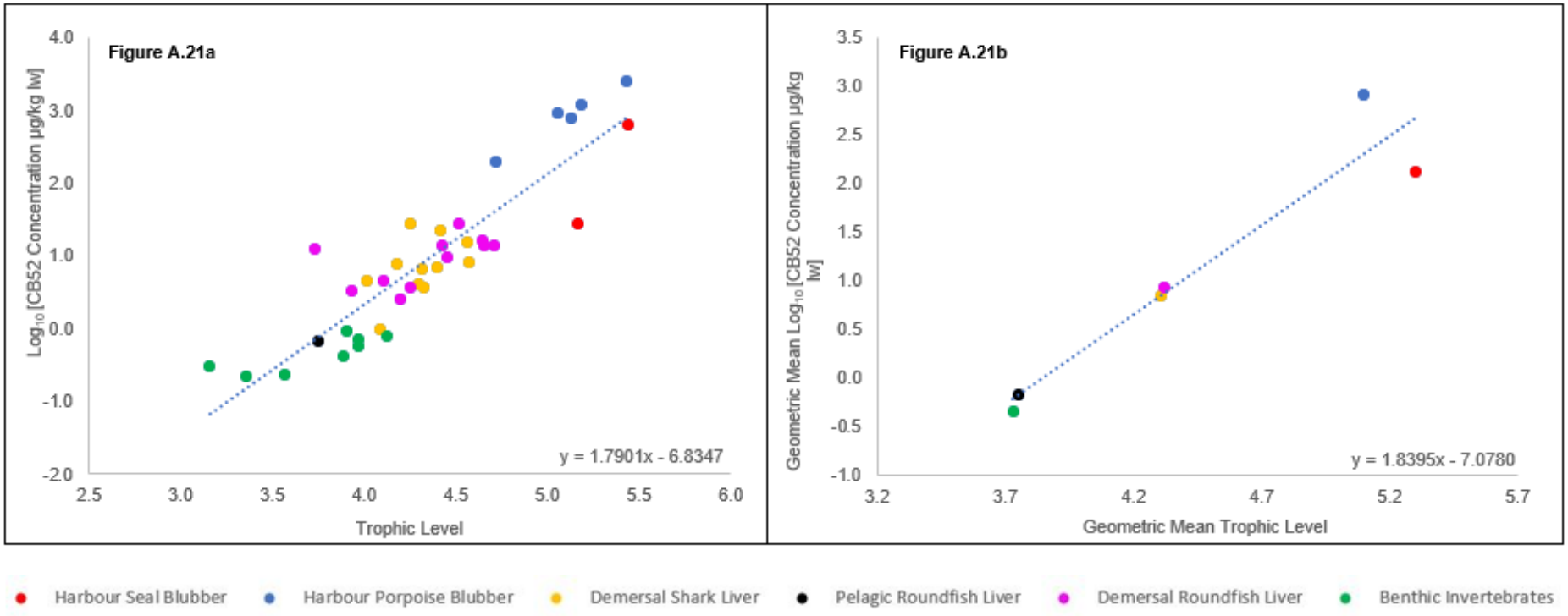

Figure A.21: (a) Relationship between trophic level and logarithmically transformed CB52 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, brown meat, soft body (green) from the Irish Sea Biogeographic Region (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean CB52 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, brown meat, soft body (green) from the Irish Sea Biogeographic Region.

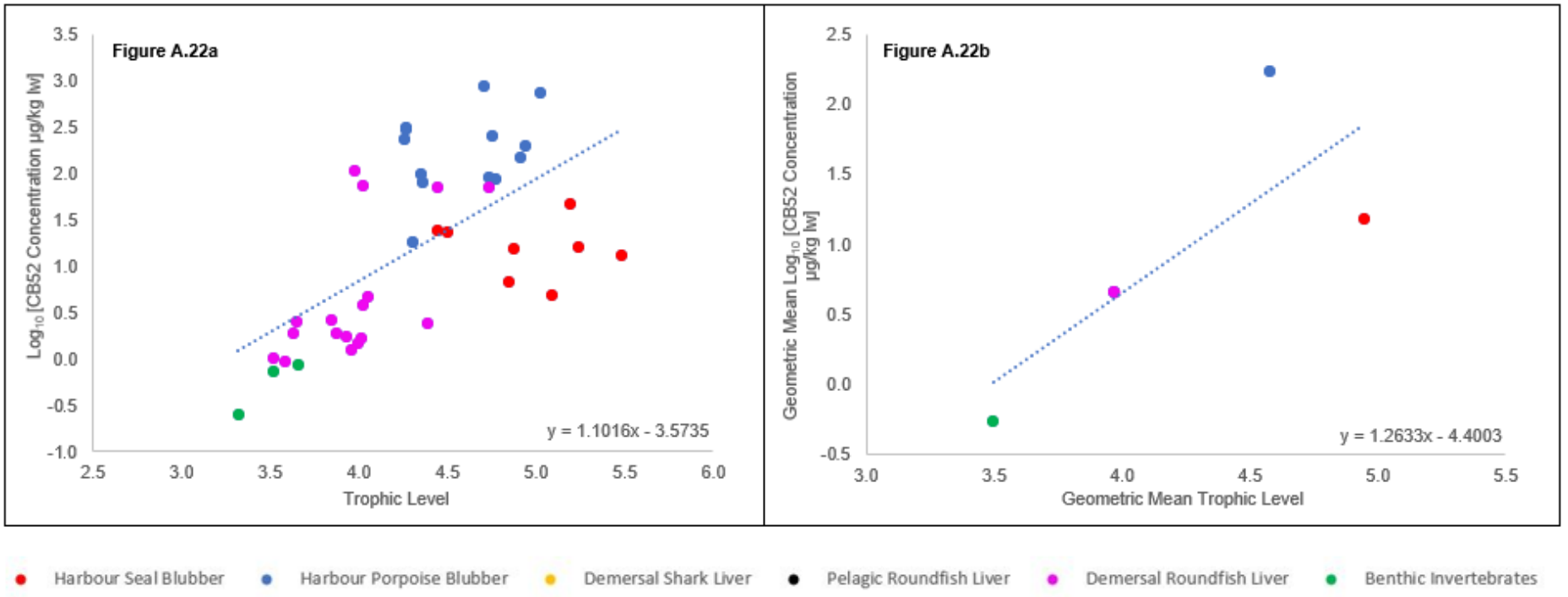

Figure A.22: (a) Relationship between trophic level and logarithmically transformed CB52 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal roundfish liver (pink), and benthic invertebrate whole, brown meat, soft body (green) from the Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf. (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean CB52 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal roundfish liver (pink) and benthic invertebrate whole, brown meat, soft body (green) form the Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf.

CB28

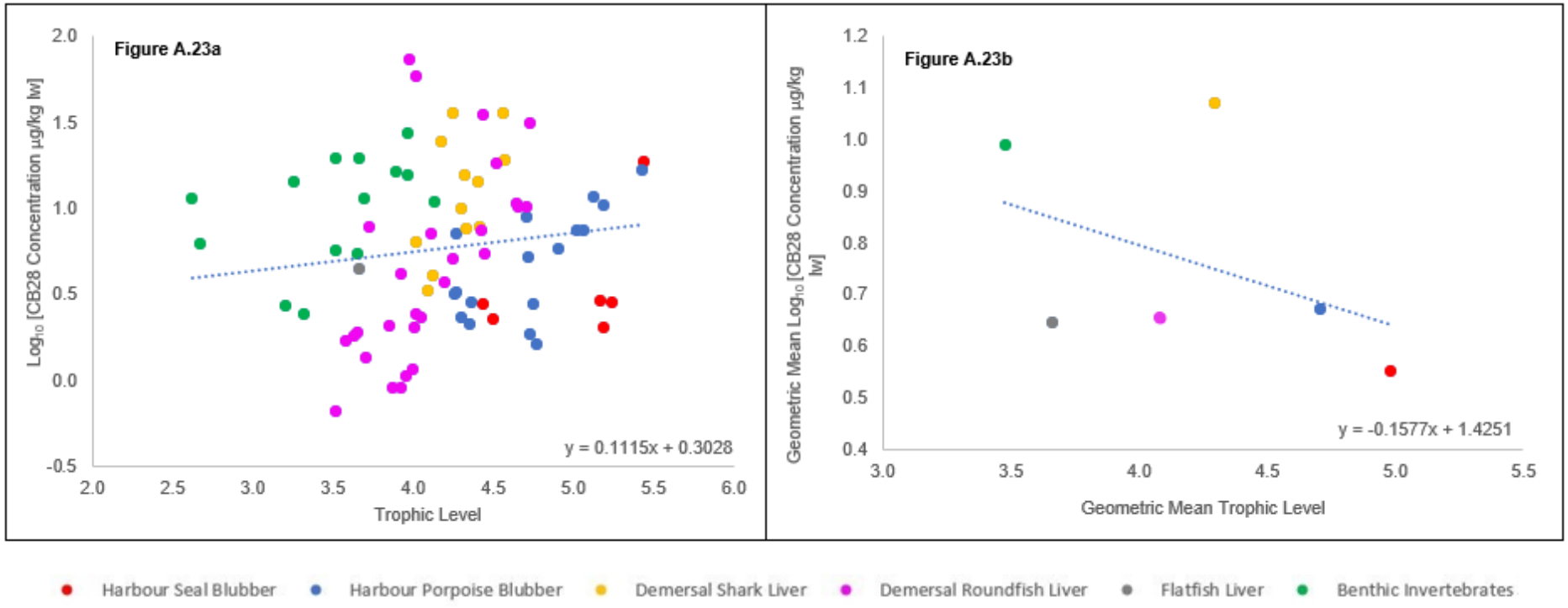

Figure A.23: (a) Relationship between trophic level and logarithmically transformed CB28 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey) and benthic invertebrate whole, brown meat, soft 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 CB28 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey) and benthic invertebrate whole, brown meat, soft body (green) from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.

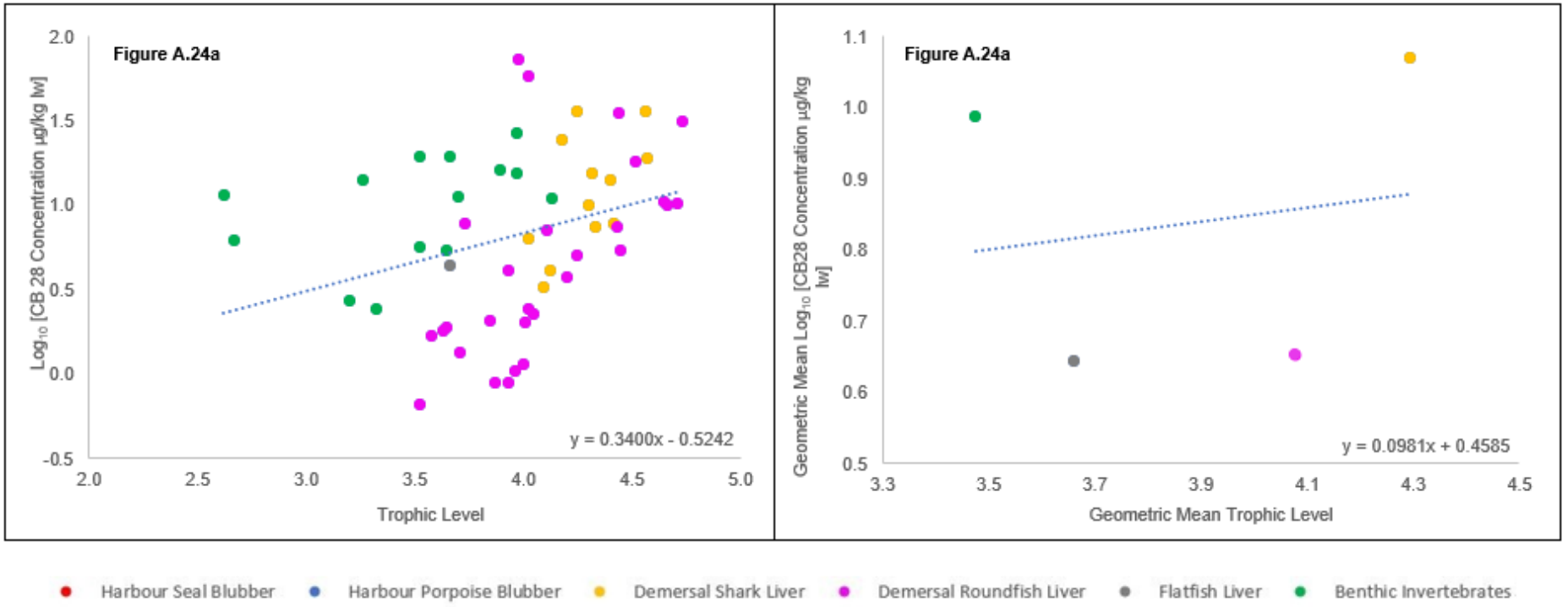

Figure A.24: (a) Relationship between trophic level and logarithmically transformed CB28 concentration (µg/kg lw) in demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey) and benthic invertebrate whole, brown meat, soft body (green) from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf. (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean CB28 concentration (µg/kg lw) in demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey) and benthic invertebrate whole, brown meat, soft body (green) from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf.

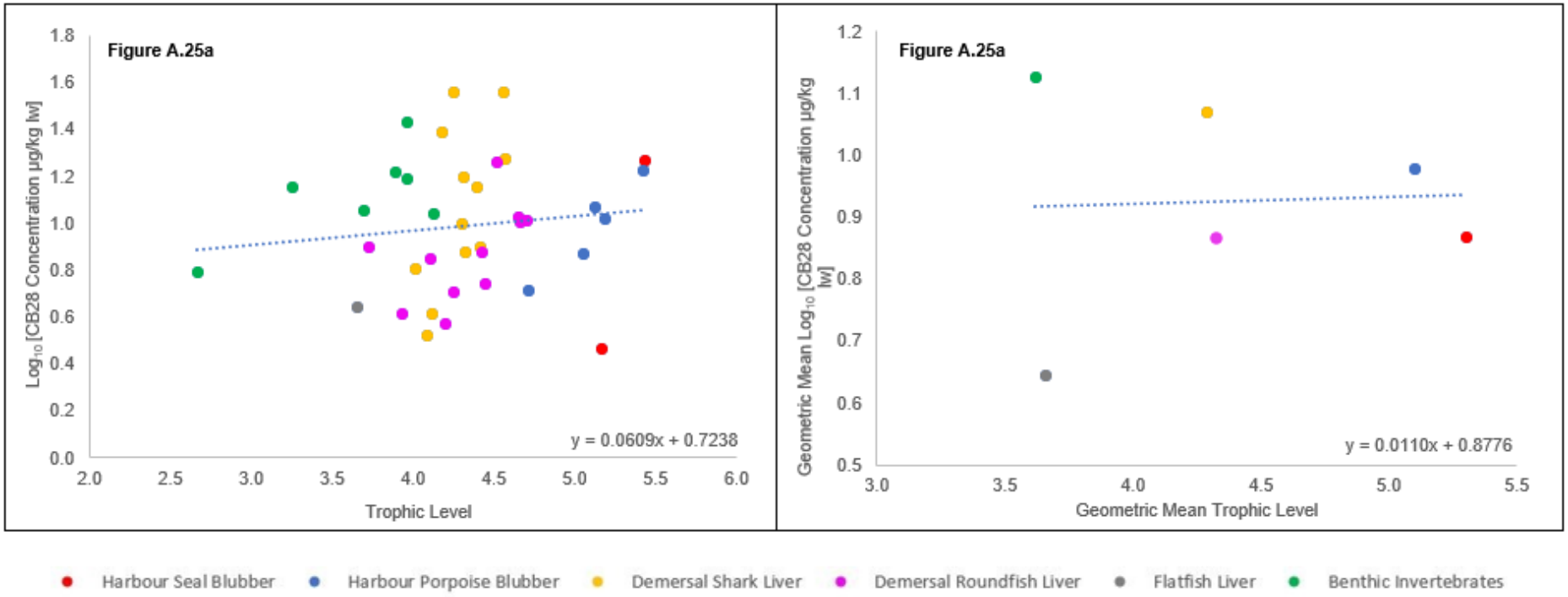

Figure A.25: (a) Relationship between trophic level and logarithmically transformed CB28 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey) and benthic invertebrate whole, brown meat, soft body (green) from the Irish Sea Biogeographic Region (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean CB28 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey) and benthic invertebrate whole, brown meat, soft body (green) from the Irish Sea Biogeographic Region.

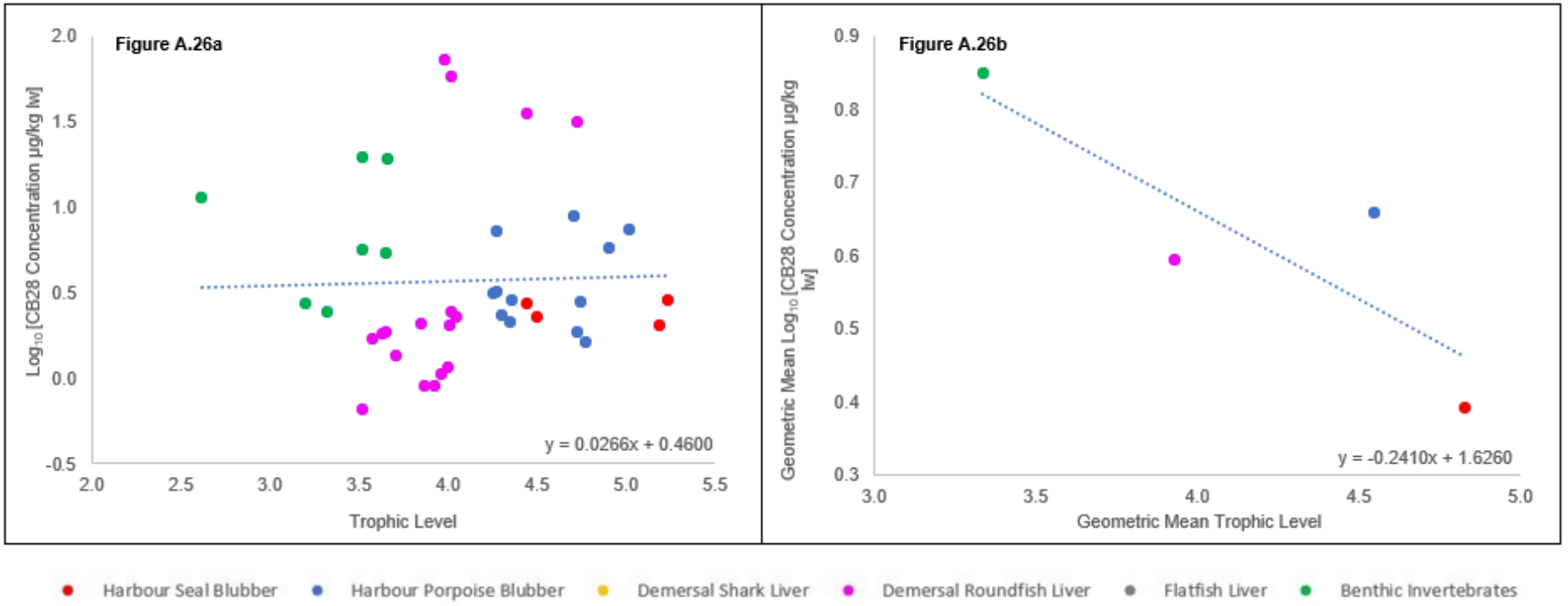

Figure A.26: (a) Relationship between trophic level and logarithmically transformed CB28 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal roundfish liver (pink), and benthic invertebrate whole, brown meat, soft body (green) from the Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf. (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean CB28 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal roundfish liver (pink) and benthic invertebrate whole, brown meat, soft body (green) form the Northern North Sea, Minches and Western Scotland and Scottish Continental Shelf.

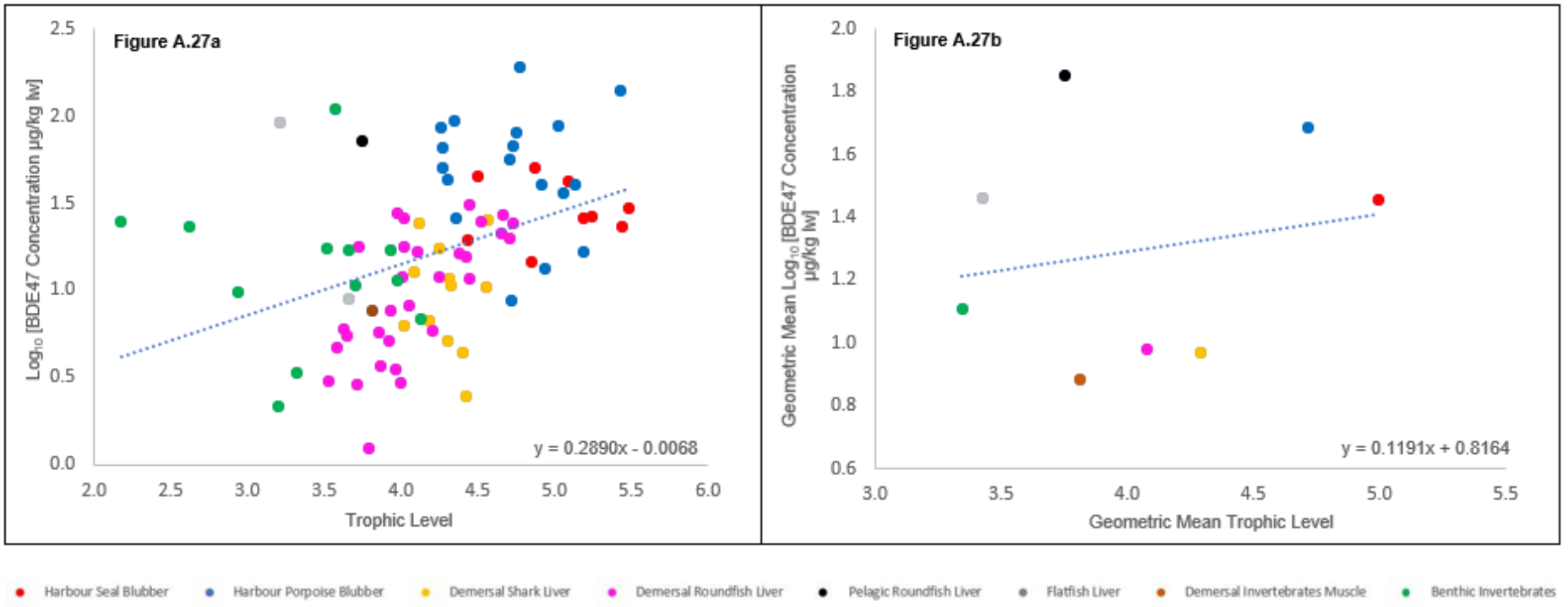

Figure A.27: (a) Relationship between trophic level and logarithmically transformed BDE47 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black), demersal invertebrates (brown) and benthic invertebrate whole, brown meat, soft body (green) from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf, excluding the emaciated harbour seal sample outlier. (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean BDE47 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), demersal invertebrates (brown) and benthic invertebrate whole, brown meat, soft body (green) from the Irish Sea Biogeographic Region, Northern North Sea, Minches and Western Scotland and the Scottish Continental Shelf, excluding the emaciated harbour seal outlier.

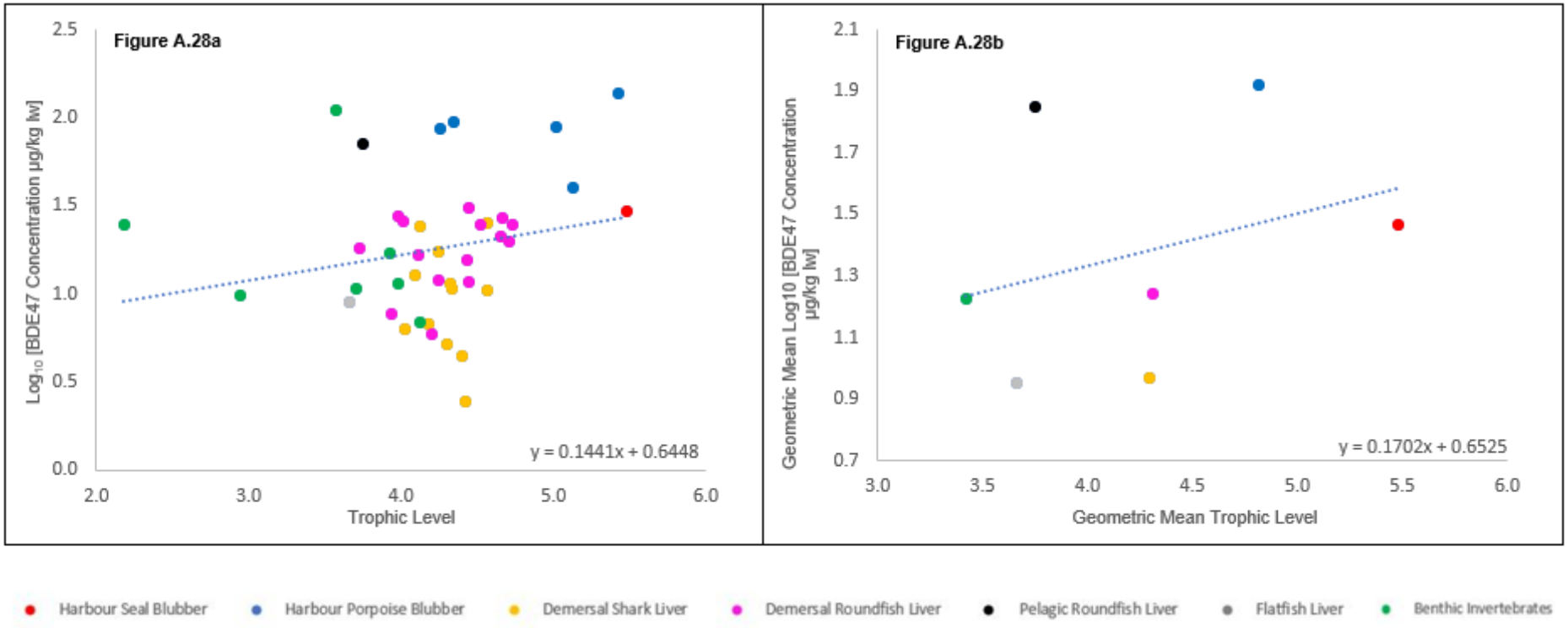

Figure A.28: (a) Relationship between trophic level and logarithmically transformed BDE47 concentration (µg/kg lw) in harbour seal blubber (red), harbour porpoise blubber (blue), demersal shark liver (yellow), fish liver: demersal (pink), flatfish (grey), pelagic (black) and benthic invertebrate whole, brown meat, soft body (green) from the Irish Sea Biogeographic Region, excluding the emaciated harbour seal sample outlier. (b) Relationship between geometric mean trophic level and logarithmically transformed geometric mean BDE47 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, brown meat, soft body (green) from the Irish Sea Biogeographic Region, excluding the emaciated harbour seal sample outlier.

Publication

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Understanding marine food web dynamics using fatty acid signatures and stable isotope ratios: Improving contaminant impacts assessments across trophic levels

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ABSTRACT

Scotland's marine food webs support a diversity of species and habitats. They contribute to maintaining the balance of the natural environment. Previous studies show that these ecosystems are contaminated by persistent organic pollutants and trace metals; with animals in higher trophic levels (e.g. cetaceans and pinnipeds) containing concentrations that are among the highest found in the ocean. Contaminants represent one of many pressures to which species and habitats are exposed. In assessing the contribution of contaminants to the overall pressure, measuring contaminants at a specific trophic level and then using trophic magnification factors (TMFs) to estimate concentrations at other trophic levels permits assessments across the food web, as well as allowing the adjustment of contaminant concentrations to a particular trophic level for comparison to assessment criteria. Fatty acid (FA) signatures and stable isotope (SI) ratios were used to develop a picture of Scottish marine food web ecology and reliably ascribe trophic levels to a wide range of species. Fatty acid trophic markers (FATMs) were used as trophic level indicators and with SI analysis, permitted identification of the mean trophic level of each species and determination of the feeding patterns and predator-prey relationships existing in the Scottish marine food web. Two hundred and eleven (211) samples comprising of seven fish species, one shark species, fourteen marine invertebrate species, three marine mammal species and two zooplankton species from different locations around Scotland were found to have mean trophic levels ranging from 1.47 ± 0.11 in zooplankton to 5.02 ± 0.35 in harbour seal. Fatty acid profile showed specific dietary information which differed between the eleven taxonomic classes and twenty-seven species. The organic and inorganic contaminant concentrations of the species for which trophic level has been determined, together with TMFs, will be reported in future papers.