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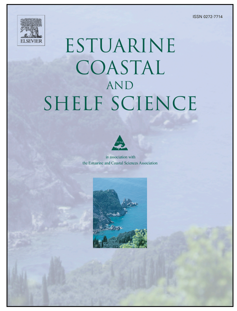

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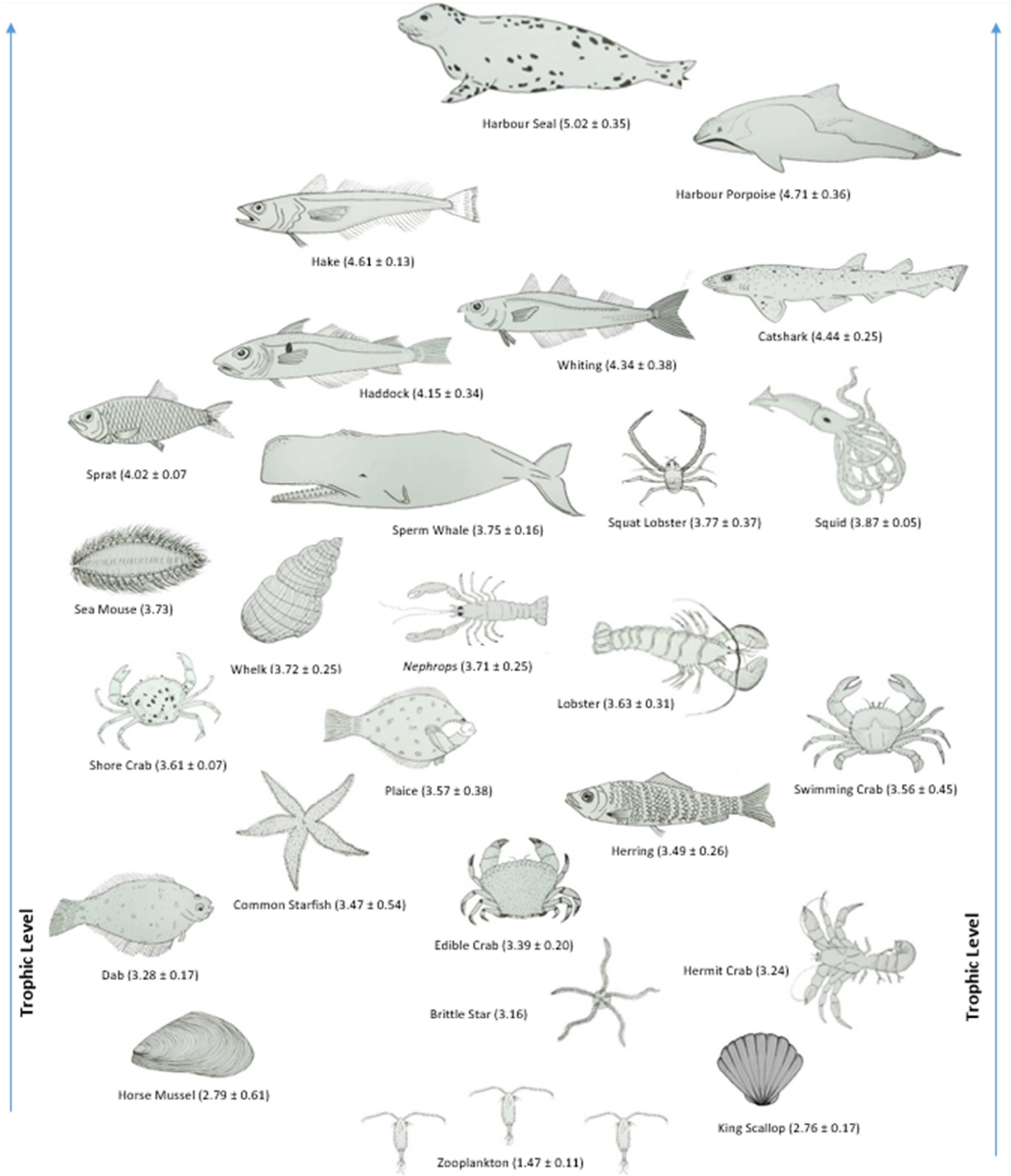
Harbour Seal (5.02 ± 0.35)
Harbour Porpoise (4.71 ± 0.36)
Hake (4.61 ± 0.13)
Catshark (4.44 ± 0.25)
Whiting (4.34 ± 0.38)
Haddock (4.15 ± 0.34)
Sprat (4.02 ± 0.07
Sperm Whale (3.75 ± 0.16)
Squat Lobster (3.77 ± 0.37)
Squid (3.87 ± 0.05)
Sea Mouse (3.73)
Whelk (3.72 ± 0.25)
Nephrops (3.71 ± 0.25)
Lobster (3.63 ± 0.31)
Shore Crab (3.61 ± 0.07)
Plaice (3.57 ± 0.38)
Swimming Crab (3.56 ± 0.45)
Herring (3.49 ± 0.26)
Common Starfish (3.47 ± 0.54)
Edible Crab (3.39 ± 0.20)
Dab (3.28 ± 0.17)
Hermit Crab (3.24)
Brittle Star (3.16)
Horse Mussel (2.79 ± 0.61)
King Scallop (2.76 ± 0.17)
Zooplankton (1.47 ± 0.11)
Trophic Level
Trophic Level

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.

1. Introduction

Habitats and species are exposed to a range of pressures, one of which is organic and inorganic contaminants. 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 and Environmental Assessment Criteria (Robinson et al., 2017). The species which meet the sampling criteria presented in the OSPAR Coordinated Environmental Monitoring Programme (CEMP) Guidelines for Monitoring Contaminants in Biota (OSPAR, 2018) include specific shellfish, flatfish and round fish, 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. Estimating the contaminant concentration using Trophic Magnification Factors (TMFs) permits an assessment of a wider range of species. However, establishing impact on the wider marine food web requires an understanding of trophic level structure, feeding patterns and nutritional relationships (Burkhard, 2003; MIME, 2016). There are limited amounts of high-quality trophic level data available covering the diverse marine species inhabiting Scottish waters for which detail inorganic and organic contaminant concentrations is also available.

Food webs support groups of short and/or complex food chains composed of organisms at a variety of trophic levels (Briand and Cohen, 1987). A food chain is a biotic interaction describing 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, and on up to larger predators such as fish and marine mammals (Jacob et al., 2011; Ashok, 2016). The trophic level describes the position that an organism occupies in a food chain (Thompson et al., 2007). There will be natural within-species variation in the trophic level as individuals may feed at more than one level and some species occupy different trophic levels through progressive life stages (Giraldo et al., 2016; Davis et al., 2012).

Previous studies on food web dynamics in the North Sea have incorporated limited diversity within each trophic level. For example, a study by Frederiksen et al (2006) looked at the trophic interactions present in a food chain (phytoplankton, zooplankton, sand eel larvae and seabirds) in the North Sea.

Individual consumer dynamics (type and length of food chains) contribute to variability in environmental impact assessments of environmental contaminants. For example, individuals of one species may not be at a constant trophic level due to variation in age, sex, location and habitat, seasonal and dietary differences (Kousteni et al., 2017). Contaminants such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and trace metals such as mercury enter the marine environment primarily from anthropogenic sources (Del Vento and Dachs, 2007). Some are resistant to metabolic biotransformation and can biomagnify up the food web (Copat et al., 2013; Lavandier et al., 2019). Therefore, contaminant concentrations detected in marine organisms can be strongly influenced by their trophic level. Theoretical or assigned trophic levels for species are used to model and estimate biomagnification of persistent contaminants within food webs and therefore should be both accurate and capture the diversity known to exist within species (Cardoso et al., 2013; Reum, Williams and Harvey, 2017). Studies on food web characteristics can be used to improve the understanding and modelling of contaminant transfer and to establish accurate assessments of the impact of such contaminants on organisms at all trophic levels on a large scale (Kim et al., 2016).

Lipids, including fatty acids (FAs), are an important source of energy in marine ecosystems and are involved in several biochemical pathways (Ibarguren, et al., 2014). FA profiles in storage and structural lipids are indicative of an organisms' likely prey (Galloway et al., 2013). FA profiles of primary producers pass up the food chain and are modified at each trophic level through metabolism and biosynthesis, however specific FAs are conserved (Sikorski, 1990). FA signatures known as "fatty acid trophic markers" (FATMs) can therefore be used to provide information about the trophic level and diet of an organism (Dalsgaard et al., 2003; Parrish et al., 2000). Connelly et al (2014) found FATMs to be a powerful tool, predicting marine taxa with 99% accuracy.

Previous studies have used FAs as biomarkers for trophic level indication in marine mammals (Guerrero et al., 2016; Budge et al., 2008), shark (Pethybridge, Daley and Nichols, 2011), fish (Würzberg et al., 2011; Olsen et al., 2015), invertebrates (Allan et al., 2010; Rabei et al., 2018; Soler-Membrives, Rossi and Munilla, 2011) and zooplankton (Deschutter et al., 2019; Gonçalves et al., 2012). 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).

The SI ratios $\delta^{15}N$ and $\delta^{13}C$ are influenced by diet and are useful for identifying broad sources of primary production and differentiating benthic and pelagic trophic pathways (Park et al., 2018). When using SI ratios to analyse diet composition, there is typically a slight enrichment in the heavier isotope between producer/prey and consumer due to preferential metabolism of the lighter isotopic forms of carbon and nitrogen (Post, 2002; McCutchan et al., 2003; DeNiro and Epstein, 1981). The $^{13}C/^{12}C$ ($\delta^{13}C$) ratio enrichment between each trophic level (0–1‰) 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 (France., 1995). The ratio of $^{15}N/^{14}N$ ($\delta^{15}N$) enriches by 3.4 - 3.8‰ (Fry and Sherr, 1984; Hobson and Welch, 1992) 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 concentration values (European Commission, 2014). Current studies on contaminant transfer continue to use 3.4 ‰ as a fixed value (Annette et al., 2018).

Although SI analysis of $\delta^{15}N$ and $\delta^{13}C$ is highly effective at trophic level determination, it can fail to discriminate between isotopically similar sources and only provides two-dimensional discrimination (Farias, 2014). To better understand the trophic ecology of marine biota, coupling both FA and SI analysis will likely be more effective and provide more nuanced information (Couturier, 2013). A study by Young et al (2018) found that the analysis of $\delta^{15}N$ and $\delta^{13}C$ were limited in distinguishing among a diverse group of prey species, as most of the prey had similar $\delta^{15}N$ ranges. FA profiles were able to resolve four separate prey groups with clarity, providing a temporal contrast to the stomach content "snapshot".

In this study, we use a combination of FA signatures and SI ratios to identify the trophic level, feeding patterns and nutritional relationships between a variety of species and classes within the Scottish marine food web. Future work will present the inorganic and organic contaminant data and the calculated TMFs for the species detailed in this paper. Comparisons of measured concentrations will be made against recalculated assessment criteria.

2. Experimental Procedure and Data Analysis

2.1. Sample Collection and Preparation

Seven fish species, one shark species and fourteen invertebrate species were collected from nine locations around Scotland between 2015 and 2017, using the MRV *Scotia* and MRV *Alba na Mara* (Figure 1), during December-February of each sampling year. Sampling was opportunistic during an environmental assessment cruise. Areas 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). Fish, shark and invertebrates were used for FA and SI analysis. King scallops were collected from different locations around Scotland in 2018. They provided the baseline data for the SI calculation (Equation 1; section 2.5.2).

Bottom trawling was conducted using a BT 137 GOV 50 mm mesh 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 tissue for analysis (depending on species, tissue type, size and sampling location), packaged and stored at - 20 °C.

Preparation resulted in five tissue types (whole, muscle, liver, soft body, brown meat). 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 with lengths of 4–6 cm. (Table 1).

Marine mammal blubber samples were collected by the Scottish Marine Animal Strandings Scheme (SMASS; Scotland's Rural College, Inverness, Scotland) from eight locations (green circles, Figure 1) between 2012 and 2016. Sperm whale, harbour seal and harbour porpoise were selected due to their differing diets and metabolic capabilities (Boon et al., 1997). A cross sectional strip of blubber was removed from the cranial insertion of the dorsal fin to the ventral midline following internationally standardised protocols (Kuiken and Garcia-Hartmann., 1991). Blubber and skin were separated, and then blubber stored at -20°C prior to FA and SI analysis. Individuals were obtained from different regions and varied in age and decomposition state (Table S2).

Calanus finmarchicus/helgolandicus and *Pseudocalanus minutus-elongatus* (zooplankton) were collected from Stonehaven (Figure 1) in 2018 using 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 prior to FA and SI analysis.

2.2. Lipid Extraction, Trans-esterification and Instrumental Analysis

Lipid extraction and trans-esterification was carried out as reported in Webster et al (2014). Further analytical details are provided in the supplementary information.

FAME extracts were diluted and vialled prior to analysis by gas chromatography-flame ionisation detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS) to give an approximate FAME concentration of 1 mg/mL. Further analytical details are provided in the Supplementary Information.

GC-FID analysis was carried out as reported in Stowasser et al (2009). Further details are provided in the Supplementary Information.

GC-MS was used to analyse five fatty alcohol/fatty acid (FAl/FA) co-eluting peaks: FAl14:0/FA15:0, FAl16:0/FA17:0, FAl18:0/FA18:3(n-3), FAl20:0/FA20:4(n-6), and FAl20:1(n-9)/FA20:3(n-3) to establish whether the FAl or FA was present/dominating the peak observed in the FID chromatogram. Samples with a significantly higher coeluting FA normalised area % for the above peaks were identified and analysed using GC-MS. If the peak was identified as FAl, the normalised area % was eliminated from the GC-FID profile. 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.

Laboratory reference materials (LRMs) and procedural blanks were esterified and analysed with each batch of samples as part of the internal quality control process for all determinants. Full details of quality control procedures are provided in the Supplementary Information.

2.3. Stable Isotope Analysis

Analysis of the SI ratios $\delta^{15}N$ and $\delta^{13}C$ was carried out using the method described in Mayor et al (2013) utilising an Integra CN Isotope Ratio Mass Spectrometer (Sercon Ltd, Crewe, UK). Full analytical details are provided in the Supplementary Information.

2.4. Trophic Level Determination

2.4.1. Fatty Acid Trophic Markers (FATMs)

The trophic marker ratio 20:5(n-3)/22:6(n-3) can be used as an indication of the degree of carnivory (Dalsgaard et al., 2003; El-Sabaawi and Dower, 2009). The lower the 20:5(n-3)/22:6(n-3) ratio, the higher the indicative trophic level. The ratios of 18:1(n−7)/18:1(n−9) and 16:1(n-7)/16:0 can also be used as indicators of a more carnivorous diet. The lower the 18:1(n−7)/18:1(n−9) (<0.6) and 16:1(n-7)/16:0 ratios, the higher the trophic level (Stübing and Hagen, 2003).

2.4.2. Stable Isotope Ratios

Isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) were determined for the dried and de-lipified tissue of the various samples (Table 1 and Table 3). $\delta^{13}C$ is significantly more depleted in lipid relative to carbohydrates and proteins (Logan and Miller, 2009). Therefore, tissue with a higher lipid content such as liver, brown meat and blubber will not have SI ratios truly representative of diet and feeding patterns. De-lipified tissue or mathematical corrections are therefore used for SI analysis as it reduces the variation associated with lipid content (Clark, Horstmann and Misarti, 2019).

The $\delta^{15}N$ from the baseline species (in this study, King Scallop) was used with the value for the test organism to give the trophic level (Equation 1; MIME, 2016). This method is currently recommended by OSPAR for the trophic adjustment of contaminant monitoring data (OSPAR Commission, 2016).

$$\text{Trophic Level} = (\delta^{15}N(\text{species}) - \delta^{15}N(\text{baseline})) / 3.4 + TL_{baseline} \qquad \textbf{(Equation 1)}$$

$\delta^{15}N$(species) is the measured nitrogen isotope ratio of the sample species; $\delta^{15}N$(baseline) is the measured nitrogen isotope ratio of the baseline species. The mean enrichment per trophic level of $\delta^{15}N$ is 3.4‰ and $TL_{baseline}$ is the trophic level of the baseline species. King scallop (*Pecten maximus*) was used as the baseline species as they are likely to be part of the same food web as the other samples (Figure 1). King scallops are assumed to be herbivorous/detritivorous and consequently feeding at trophic level 2 which is assigned as the baseline value (Pinnegar et al., 2002).

2.5. Data Analysis

FAs profiles within class categories (Table 2) were investigated with principal component analysis (PCA) in the R statistical environment (R version 3.1.2) and Analysis of Variance (ANOVA) at the 95% confidence level, with Tukey's pair-wise comparisons. Once factors influencing the FA profile were identified, sub-categories were made within each class for analysis to minimise within-group variation. ANOVA at the 95% confidence level, with Tukey's pair-wise comparisons was used to establish significant differences in enrichment of $\delta^{15}N$ and $\delta^{13}C$ between species and categories and Pearson's correlation was used to measure the linear correlation between $\delta^{15}N$ and $\delta^{13}C$ with potential influencing variables such as age, length and weight.

3. Results and Discussion

3.1. Fatty Acid Profiles

Principal component analysis (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 present in the study, the taxonomic rank of class was initially selected for grouping species to allow easier visualisation (Table 2). A clear dispersion of the samples was achieved based on their taxonomic class (Figure 2b). There were 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 to reduce the FA variation.

The analysis of each class revealed that the FA profile was found to vary with tissue type and water column feeding zone (benthic/demersal/pelagic feeding). Previous studies have found lipid class and FA profiles to be tissue-specific due to the underlying physiological differences between tissue types (Meyer et al., 2017; Aras et al., 2003).

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). The finalised categories and category mean normalised area % of each of the 31 FAMEs, accounting for tissue type and water column zone, are shown in Table S2. Classification was adapted to incorporate these influencing factors.

3.1.1. Marine Mammals (mammalia)

Mammalia were more negatively correlated to the first principal component when samples were grouped on the basis of class alone (Figure 2b) 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). PCA was applied to the marine mammal samples on a species basis to study the differences between the FA profiles of the three species (Figure 3a and b). Although sample numbers are smaller in comparison to harbour porpoise and harbour seal, sperm whale possess the least variable FA profile in this dataset (Figure 3b) and were separated from the other marine mammals. Separation is due to the significantly higher proportion ($p<0.001$ ANOVA, Tukey) of 18:1(n-9) and lower proportion of 22:6(n-3) in comparison to harbour seal and harbour porpoise blubber. 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, 2004). 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 correlates with the data from this study; these three FAs account for over 60% of the FAs present.

The three marine mammal species contained a significantly higher proportion of the FA marker 18:1(n-9) compared to other organisms ($p<0.001$ ANOVA, Tukey). The peak assigned as 18:1(n-9) might include a small amount of 18:1(n-11), as these two isomers could not be separated. 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 3b) but are generally separated by species across PC1 and PC2 (Figure 3b). The degree of variation of 18:1(n-9), 16:0 and 24:1(n-9) was largest in harbour seals, each possessing a standard deviation (SD) of >5, suggesting that harbour seal diet is highly variable, although sampling location did not influence FA profiles. Harbour porpoise are more negatively correlated to PC2 (Figure 3b) than the other mammalia species. This is due to the higher proportion of MUFAs 16:1(n-7) and 14:1(n-5) and the dienoic acid 18:2(n-6), ($p<0.001$ ANOVA, Tukey), in their blubber, supporting findings from other studies on harbour porpoise around Scotland where 16:1(n-7) and 18:1(n-9) were the most predominant FAs (Learmonth., 2003). 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, year and age (all listed on Table S2) were investigated but were not found to influence the data ($p>0.05$).

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. PCA (Figure 4a 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 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) ($p<0.001$ 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 4b) due to a lower proportion of 22:6(n-3), which again is consistent with their physiology (Njinkouéa et al., 2008).

Flatfish liver contained 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 S2). 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) ($p<0.001$ ANOVA, Tukey). 22:6(n-3) was significantly higher in plaice liver than dab liver ($p<0.001$ ANOVA, Tukey) as observed in the PCA score plot (Figure 4b). Sampling location (Table 1), average length (ranging from 198-350 mm), average weight (ranging from 82.60-508.0 g) and average age (ranging from 3.4–10.0 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, the FA profiles in this study suggest there can be sufficient differences in their diets leading to a clear distinction in their tissue FA profiles (Gibson et al., 2015).

The FAs 22:1(n-11) and 22:6(n-3) within demersal roundfish liver showed the largest variation 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 ($p<0.001$ ANOVA, Tukey), suggesting dietary differences between the species. This is consistent with the pattern variation observed using PCA (Figure 4b, PC1 = -5 to +5).

Pelagic roundfish muscle and liver (herring) is negatively correlated with PC1 (Figure 4b) 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 (Keinänen et al., 2017).

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 5a and b) showing considerable variation for the benthic invertebrates whole, muscle and soft body FA profiles (Figure 5b). 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 (Russell and Nichols, 1999; Meijer et al., 1984). 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 %). Sargent et al (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) than the other contributing species of whole benthic invertebrates ($p<0.001$ ANOVA, Tukey). Previous studies have found 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).

A single sea mouse sample is separated from the others in the category and is grouped with the benthic invertebrates muscle category. 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 3 sites in the Clyde (Hunterston, Pladda and Holy Loch; Table 1). The two sample pools more negatively correlated to PC1 (Figure 5b) 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 suggests that starfish in Pladda were consuming 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. Further influences such as average pool length (ranging from 161.7 – 396.0 mm) and average pool weight (ranging from 35.0 – 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 5b) due to the higher proportion of 22:6(n-3) and 16:0. 22:6(n-3) and 16:0 are the most characteristic FAs for squid (Phillips, Nichols and Jackson, 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 was found to have a significantly higher mean normalised area % (38.28 ± 0.16 %) of 22:6(n-3), than in the other invertebrate categories ($p<0.005$ ANOVA, Tukey).

Benthic invertebrates soft body sample pools gave rise to the most dispersed category (Figure 5b) 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 second group, composed of horse mussel (n=2), swimming crabs (n=6) and shore crabs (n=2) is more negatively correlated to PC2 and is widely dispersed, suggesting a range of feeding patterns.

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). 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 2b), 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 S2). *Pseudocalanus minutus* and *Calanus finmarchicus* 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.

3.2 Fatty Acid Trophic Markers (FATMs)

FATM analysis is based on the observation that the FA profiles of primary producers can be passed up the food chain and 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) ($p<0.001$ ANOVA, Tukey). This does not agree with other studies as zooplankton is a primary consumer 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 ($p<0.001$ 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.

3.3. Stable Isotopes Ratios

Sample pools (fish, shark, invertebrates and zooplankton) and individuals (marine mammals) were segregated on the basis of their SI enrichment ($p<0.001$ ANOVA, Tukey). Isotopic enrichment 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 (with 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 FA analysis, significant differences in $\delta^{15}N$ and $\delta^{13}C$ between groups of sample pools (fish, shark, invertebrates and zooplankton) and individuals (marine mammals) were observed (Table 3 and Figure 6). At a species level, the $\delta^{15}N$ 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 blubber. Mean $\delta^{13}C$ values across the 19 designated categories ranged from -19.37 ± 0.02 ‰ in demersal invertebrates muscle pools to -14.48 ± 2.99 26 ‰ in benthic invertebrates whole pools (Table 3).

3.3.1. Marine Mammals

The mean and range of $\delta^{13}C$ in harbour seal (-16.36 ± 2.02 ‰) and harbour porpoise (-16.48 ± 1.05 ‰) compared to sperm whale (-14.60 ± 0.46 ‰) (Table 3) 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 on Figure 3b due to significant variation of FAs such as 18:1(n-9), 16:1(n-7) and 22:6(n-3). Although harbour seal sample numbers are low, variables such as geographic location of stranding, year, age, length and girth (Table S1) had no significant influence on the $\delta^{13}C$ ($p<0.001$ 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 harbour seal scat, Wilson and Hammond (2016) found that sand eel was an important component in their diet in Shetland, Orkney, Moray Firth and South East Scotland. Although sand eel populations were facing a rapid decline, they made up to 70% of the diet across all seasons. Sand eel is a planktivorous primary consumer with a low enrichment of $\delta^{13}C$ (Sarà et al., 2010). The within species variation of harbour seal $\delta^{13}C$ in this study (-16.36 ± 2.02 ‰) suggests sand eel was not making up a majority of their diet. Seals enriched in $\delta^{13}C$ could potentially be feeding directly on $\delta^{13}C$ rich organisms 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, Table 3). Harbour seals have been reported to consume a mixture of benthic invertebrates (Perrin et al., 2009).

Sperm whale blubber had a significantly less enriched $\delta^{15}N$ (13.36 ± 0.53 ‰) and significantly more enriched $\delta^{13}C$ (-14.60 ± 0.46 ‰) compared to harbour seal and harbour porpoise ($p<0.001$ ANOVA, Tukey). Sperm whale blubber shows the least variation in SI ratios (SD <1 of the mammal species studied, suggesting little variation in the species feeding pattern, which is in agreement with the sperm whale FA data. The $\delta^{15}N$ enrichment observed for cephalopods (13.75 ± 0.18 ‰) in this study (demersal invertebrates muscle) was not significantly different when compared with the sperm whale, but 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). The sperm whale samples in this study were all male and SI ratio data from other studies in the Pacific based on stomach content analysis found that adult males fed more frequently on fish and dogfish where adult females fed on giant squid (Flinn et al., 2002). The significantly higher enrichment of $\delta^{13}C$ in relation to the other marine mammals and other species has 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). Other studies in the Pacific have found that sperm whales (male and female) have a higher fish intake than squid in waters of high latitudes than those of low latitudes (Rice, 1989) which would increase the $\delta^{15}N$ and $\delta^{13}C$ ratios.

3.3.2 Fish and Catshark

The pelagic fish in this study included sprat (n=3) and herring (n=2) 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 et al., 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 be more enriched in $\delta^{15}N$ than pelagic roundfish (herring liver and muscle) and flatfish (dab liver and muscle and plaice liver and muscle), suggesting a species/tissue influence on SI ratios.

The $\delta^{13}C$ was significantly lower in flatfish liver (-19.01 ± 0.78 ‰; n=12) than pelagic roundfish whole (-18.45 ± 0.38 ‰; n=3), pelagic roundfish muscle (-18.03 ± 0.17 ‰; n=2), flatfish muscle (-18.03 ± 0.40 %; n=12) and pelagic roundfish liver (-17.65 ± 0.28 %; n=2) (p<0.001 ANOVA, Tukey), 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 SI ratios in muscle provides a long-term dietary indicator compared to liver (Hesslein et al., 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. Average pool age (ranging from 3.4-10.0 years), length (198.0-350.0 mm) and weight (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) were significantly less enriched in $\delta^{15}N$ (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)). This suggests plaice habituating in Burra Haaf have a less carnivorous diet than those from the Moray Firth and Solway. 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) ($p<0.001$ 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) ($p<0.001$ 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 $\delta^{15}N$ 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 be significantly more enriched in $\delta^{15}N$ and $\delta^{13}C$ ($p<0.001$ ANOVA, Tukey) than flatfish and pelagic roundfish (combined overall matrices demonstrated in Figure 6). 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). Forty-four catsharks (resulting in 12 sample pools; Table 1) were collected from four locations from west Scotland: Solway and the Clyde (Pladda, Hunterston and Holy Loch; Figure 1). Sampling location was not found to influence the SI ratios. Average weight was found to significantly influence the $\delta^{15}N$ in catshark muscle, ($p<0.05$) where the heavier the catshark pool, the more enriched the $\delta^{15}N$, indicating that larger catshark are feeding higher up the food chain than smaller catshark. Average pool length, another indicator of age, was found to significantly influence the $\delta^{13}C$ in catshark liver ($p<0.05$): the smaller the catshark, the less enriched the $\delta^{13}C$; 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 ($p<0.005$), supporting the $\delta^{15}N$ and $\delta^{13}C$ data showing larger catshark larger are more carnivorous. Catshark liver sample pools taken in 2016 from Solway and Pladda were significantly less enriched in $\delta^{13}C$ (-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) ($p<0.001$ ANOVA, Tukey). Collection year also influenced the $\delta^{15}N$ in muscle tissue where catshark muscle sample pools collected in 2016 were significantly less enriched in $\delta^{15}N$ (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) ($p<0.001$ 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. None of the FATMs supported this data, with no significant differences found in catfish liver between the three years ($p>0.05$ ANOVA, Tukey).

The $\delta^{15}N$ enrichment of demersal roundfish muscle (15.42 ± 1.13 ‰) and liver (14.51 ± 1.15 ‰) in this study was not significantly higher than the demersal shark isotope ratios which suggest 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 selachiformes (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 shark muscle and liver.

For whiting 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 more enriched the $\delta^{15}N$, indicating bigger, 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 less enriched ($p<0.001$ 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) were significantly less enriched in $\delta^{15}N$ than sample pools collected from the other sampling points ($p<0.001$ ANOVA, Tukey) suggesting a spatial influence on diet.

3.3.3. Benthic and Demersal Invertebrates

Benthic and demersal invertebrates (muscle, whole and brown meat from crustaceans) gave a range of $\delta^{15}N$ and $\delta^{13}C$ values (Figure 6). 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 et al., 1991). Along with horse mussel, king scallops were found to be significantly less enriched in $\delta^{15}N$ than the other benthic invertebrate species ($p<0.005$ 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 SI ratio results from this study position king scallop as the lowest trophic level benthic invertebrate in the Scottish marine food web. This species was therefore used as the baseline for trophic level calculations.

Brittle star was significantly more enriched in $\delta^{13}C$ than the other categories ($p<0.001$ ANOVA, Tukey), with a value of -6.26 ‰, however there was only one pool of brittle star. This is higher than previously reported $\delta^{13}C$ values in brittle star from around Britain (Scotland and the English Channel) (McKenzie et al., 2000; Leroux et al., 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 be significantly more enriched with $\delta^{13}C$ than the other categories ($p<0.001$ ANOVA, Tukey). Benthic microalgae and kelp have a higher carbon isotopic ratio 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 an enrichment of $\delta^{13}C$ 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 study, common starfish had a significantly higher $\delta^{13}C$ than other benthic species collected from the offshore Moray Firth, suggesting this species feeds on organisms with a different primary carbon source.

When the $\delta^{15}N$ was investigated within the starfish species, sample pools from Pladda (Clyde) had a significantly lower average isotope ratio (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). 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.

3.3.4. Zooplankton

Zooplankton possessed a significantly lower $\delta^{15}N$ (5.62 ± 0.38 ‰) enrichment in comparison to the other sample categories, positioning *Pseudocalanus minutus* and *Calanus finmarchicus* at the bottom of the food web investigated (Figure 6; note: no phytoplankton were examined in this study). 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 et al., 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 study have plankton as their primary carbon source at the base of the food web.

3.4. Trophic Level

Trophic level was calculated using Equation 1 described in section 2.5. Based on the trophic level data obtained for each species 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 1. Trophic level ranges from 1.12 ± 0.11 in zooplankton to 4.66 ± 0.34 in harbour seal (Figure 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 lower 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) in agreement with the trophic level obtained using $\delta^{15}N$. The positioning of higher trophic level organisms by FATM however were incorrect (on the basis of SI data), with demersal shark 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. Marine mammals have a lower proportion of 22:6(n-3) due to MUFAs dominating the FA profile (Table S2). The tissue-specific nature of FA profiles has been found to influence trophic level indication. 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). This emphasises that care that must be taken when interpreting the FA data.

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 study and SI ratio analysis was able to ascribe the trophic levels of twenty-six species collected between 2012-2018 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.

211 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 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 while and 18:1(n−7)/18:1(n−9) gave 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 (Fishbase), as that will increase the uncertainty of the assessment.

In 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 of 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 study will permit 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.

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6. Declaration of Interest

Declaration of Interest: none

7. References

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Tables

Sampling Location	Species Collected	Number of Individuals Collected	Number of Sample Pools	Tissue Type
Tancred Bank	Shore Crab (*Carcinus maenas*)	27	2	Soft Body (n=2)
North East Dunbar	Haddock (*Melanogrammus aeglefinus*)	36	4	Muscle (n=2), Liver (n=2), Whole (n=2)
	Swimming Crab (*Liocarcinus depurator*)	68	2	Soft Body (n=2)
Montrose Bank	Haddock (*Melanogrammus aeglefinus*)	5	1	Muscle (n=1), Liver (n=1)
	Whiting (*Merlangius merlangus*)	10	2	Muscle (n=2), Liver (n=2)
	Edible Crab (*Cancer pagurus*)	14	1	Muscle (n=1), Brown Meat (n=1)
	Squat Lobster (*Munida rugosa*)	8	1	Muscle (n=1)
	Swimming Crab (*Liocarcinus depurator*)	31	1	Soft Body (n=1)
Moray Firth	Haddock (*Melanogrammus aeglefinus*)	20	4	Muscle (n=4), Liver (n=4)
	Plaice (*Pleuronectes platessa*)	15	3	Muscle (n=3), Liver (n=3)
	Squid (*Loligo forbesii*)	5	1	Muscle (n=1)
	Common Starfish (*Asterias rubens*)	16	3	Whole (n=3)
	Nephrops (*Nephrops norvegicus*)	28	1	Muscle (n=1)
	Brittle Star (*Ophiura ophiura*)	96	1	Whole (n=1)
Burra Haaf	Haddock (*Melanogrammus aeglefinus*)	5	1	Muscle (n=1), Liver (n=1)
	Whiting (*Merlangius merlangus*)	20	5	Muscle (n=5), Liver (n=5)
	Plaice (*Pleuronectes platessa*)	17	4	Muscle (n=4), Liver (n=4)
	Dab (*Limanda limanda*)	15	3	Muscle (n=3), Liver (n=3)
	Squid (*Loligo forbesii*)	5	1	Muscle (n=1)
	Hermit Crab *(Pagurus bernhardus)*	10	1	Muscle (n=1)
	Nephrops (*Nephrops norvegicus*)	53	1	Muscle (n=1)
Holy Loch	Catshark *(Scyliorhinus canicula)*	8	4	Muscle (n=4), Liver (n=4)

Sampling Location	Species Collected	Number of Individuals Collected	Number of Sample Pools	Tissue Type
	Haddock (*Melanogrammus aeglefinus*)	10	2	Muscle (n=2), Liver (n=2)
	Hake (*Merluccius merluccius*)	7	2	Muscle (n=2), Liver (n=2)
	Common Starfish (*Asterias rubens*)	10	2	Whole (n=2)
	Squat Lobster *(Munida rugosa)*	44	1	Muscle (n=1)
	Nephrops (*Nephrops norvegicus*)	73	2	Muscle (n=2)
	Whelk (*Buccinum undatum)*	12	4	Soft Body (n=4)
	Swimming Crab (*Liocarcinus depurator*)	64	2	Soft Body (n=2)
	Horse Mussel (*Modiolus modiolus)*	8	1	Soft Body (n=1)
Hunterston	Catshark *(Scyliorhinus canicula)*	10	2	Muscle (n=2), Liver (n=2)
	Common Starfish (*Asterias rubens*)	10	1	Whole (n=1)
	Nephrops (*Nephrops norvegicus*)	71	2	Muscle (n=2)
	Squat Lobster *(Munida rugosa)*	31	1	Muscle (n=1)
	Swimming Crab (*Liocarcinus depurator*)	34	1	Soft Body (n=1)
Pladda	Catshark *(Scyliorhinus canicula)*	13	3	Muscle (n=3), Liver (n=3)
	Haddock (*Melanogrammus aeglefinus*)	21	4	Muscle (n=1), Liver (n=1), Whole (n=3)
	Whiting (*Merlangius merlangus*)	25	6	Muscle (n=6), Liver (n=6)
	Herring (*Clupea harengus*)	10	2	Muscle (n=2), Liver (n=2)
	Common Starfish (*Asterias rubens*)	10	2	Whole (n=2)
	Lobster (*Homarus gammarus*)	4	1	Muscle (n=1), Brown Meat (n=1)
	Horse Mussel (*Modiolus modiolus)*	6	1	Soft Body (n=1)
	Whelk (*Buccinum undatum)*	4	1	Soft Body (n=1)
Solway Firth	Catshark *(Scyliorhinus canicula)*	13	3	Muscle (n=3), Liver (n=3)
	Haddock (*Melanogrammus aeglefinus*)	8	3	Muscle (n=3), Liver (n=3)
	Whiting (*Merlangius merlangus*)	15	2	Muscle (n=1), Liver (n=1), Whole (n=1)
	Plaice (*Pleuronectes platessa*)	8	2	Muscle (n=2), Liver (n=2)

Sampling Location	Species Collected	Number of Individuals Collected	Number of Sample Pools	Tissue Type
	Sprat (*Sprattus sprattus*)	149	3	Whole (n=3)
	Common Starfish (*Asterias rubens*)	3	1	Whole (n=1)
	Whelk (*Buccinum undatum)*	20	2	Soft Body (n=2)
	Edible Crab (*Cancer pagurus*)	14	1	Muscle (n=1), Brown Meat (n=1)
	Sea Mouse (*Aphrodita aculeata*)	33	1	Whole (n=1)

Table 1: Sample pools collected from each of the five environmental monitoring survey cruises from nine areas around Scotland. n = number of tissue specific sample pools associated to that particular species and sampling point. The specific locations are identified in Figure 1.

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*	*Pseudocalanus*					

Table 2: The eleven sample classes their associated species.

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)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Harbour Seal Blubber	Harbour seal (n=10)	10	1.46 ± 0.67	0.23 ± 0.05	0.43 ± 0.12	17.69 ± 1.19	-16.36 ± 2.02
Harbour Porpoise Blubber	Harbour porpoise (n=18)	18	2.41 ± 0.78	0.13 ± 0.10	0.43 ± 0.15	16.62 ± 1.22	-16.48 ± 1.05
Sperm Whale Blubber	Sperm whale (n=5)	5	2.36 ± 0.69	0.10 ± 0.02	0.56 ± 0.30	13.36 ± 0.53	-14.60 ± 0.46
Pelagic Roundfish Whole	Sprat (n=3)	3	0.38 ± 0.02	0.44 ± 0.04	0.58 ± 0.09	14.26 ± 0.23	-18.45 ± 0.38
Pelagic Roundfish Muscle	Herring (n=2)	2	0.23 ± 0.04	0.19 ± 0.02	0.50 ± 0.08	13.37 ± 0.01	-18.03 ± 0.19
Pelagic Roundfish Liver	Herring (n=2)	2	0.10 ± 0.01	0.55 ± 0.06	0.38 ± 0.01	11.60 ± 0.00	-17.65 ± 0.28
Demersal Shark Muscle	Catshark (n=12)	12	0.23 ± 0.05	0.53 ± 0.11	0.32 ± 0.09	16.12 ± 0.86	-17.13 ± 0.57
Demersal Shark Liver	Catshark (n=12)	12	0.45 ± 0.08	0.55 ± 0.11	0.43 ± 0.07	15.26 ± 0.58	-17.53 ± 0.55
Demersal Roundfish Whole	Whiting (n=1), Haddock (n=5)	6	0.24 ± 0.07	0.53 ± 0.13	0.66 ± 0.23	15.65 ± 0.37	-17.58 ± 0.31
Demersal Roundfish Muscle	Whiting (n=14), Hake (n=2), Haddock (n=14)	30	0.23 ± 0.08	0.46 ± 0.17	0.55 ± 0.24	15.42 ± 1.13	-17.75 ± 0.57
Demersal Roundfish Liver	Whiting (n=14), Hake (n=2), Haddock (n=14)	30	0.40 ± 0.13	0.42 ± 0.15	0.80 ± 0.31	14.51 ± 1.15	-18.45 ± 0.65
Flatfish Muscle	Plaice (n=9), Dab (n=3)	12	0.30 ± 0.07	0.54 ± 0.13	0.90 ± 0.29	12.98 ± 1.21	-18.03 ± 0.40
Flatfish Liver	Plaice (n=9), Dab (n=3)	12	0.55 ± 0.18	0.52 ± 0.22	0.63 ± 0.18	12.03 ± 1.06	-19.01 ± 0.78
Demersal Invertebrates Muscle	Squid (n=2)	2	0.13 ± 0.01	0.54 ± 0.02	0.34 ± 0.01	13.75 ± 0.18	-19.37 ± 0.02
Benthic Invertebrates Whole	Common starfish (n=9), Brittle star (n=1), Sea mouse (n=1)	11	0.30 ± 0.13	4.48 ± 2.71	2.79 ± 1.93	12.22 ± 1.71	-14.48 ± 2.99
Benthic Invertebrates Muscle	Edible crab (n=2), Lobster (n=1), Squat lobster (n=3), Hermit crab (n=1),	13	0.44 ± 0.15	0.66 ± 0.59	1.34 ± 0.38	13.13 ± 1.01	-17.48 ± 0.49

	Nephrops (n=6)						
Benthic Invertebrates Brown Meat	Edible crab (n=2), Lobster (n=1)	3	0.87 ± 0.30	0.63 ± 0.10	0.79 ± 0.03	11.63 ± 0.19	-18.93 ± 0.75
Benthic Invertebrates Soft Body	Swimming crab (n=6), Horse mussel (n=2), King Scallop (n=10), Whelk (n=7), Shore crab (n=2)	23	0.33 ± 0.16	1.12 ± 0.75	1.80 ± 0.61	11.66 ± 1.84	-17.68 ± 0.74
Zooplankton Whole	*Calanus* and *Pseudocalanus*	5	0.46 ± 0.03	0.42 ± 0.09	1.36 ± 0.05	5.62 ± 0.38	-19.01 ± 0.41

Table 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) and mean (± standard deviation) stable isotope ratios $\delta^{15}N$ and $\delta^{13}C$ analysed in the nineteen chemotaxonomical sample categories. (n= the number of individuals for mammals and the number of pools for all other categories).

Figures

Figure 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, North East (NE) Dunbar and Solway Firth (yellow 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). Two zooplankton species were collected from the Scottish Observatory site off Stonehaven from the RV *Temora* in 2017 (red circle).

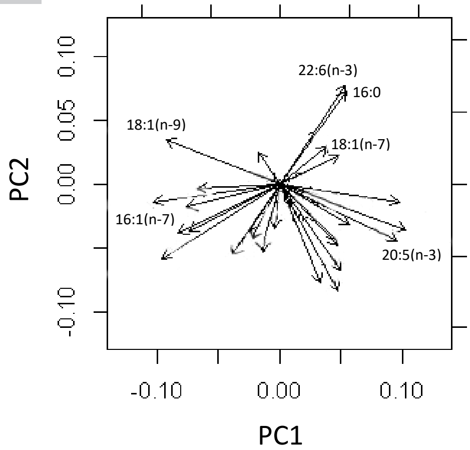

Figure 2a

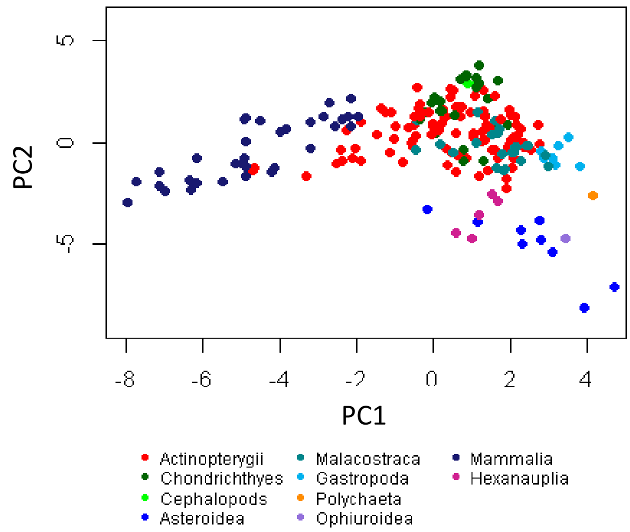

Figure 2b

Figure 2:(a) PCA loading plot 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 identified classes. The plot shows the 6 most abundant FAs accounting on average for >72 % of the profile. (b) PCA score plot 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.

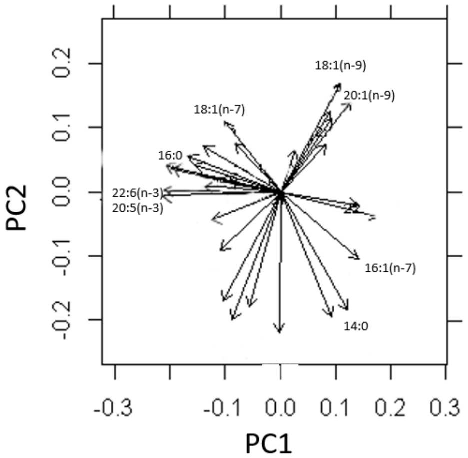

Figure 3a

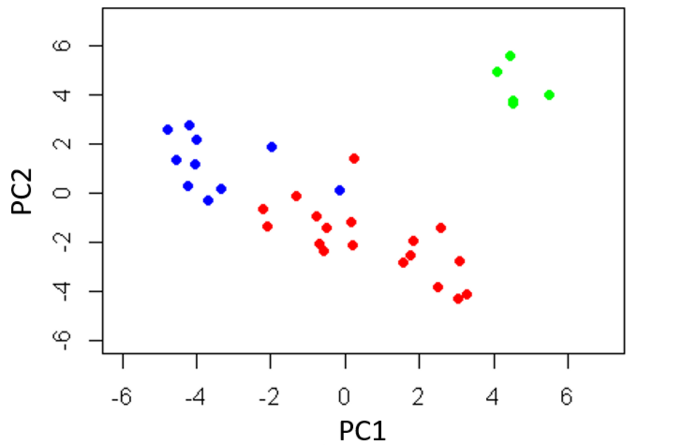

Figure 3b

Figure 3: (a) PCA loading plot demonstrating variation in the FA profiles (normalised area percentages) across the three marine mammal species. FAs labelled on the loading plot are those discussed in section 3.1.1. (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 S3).

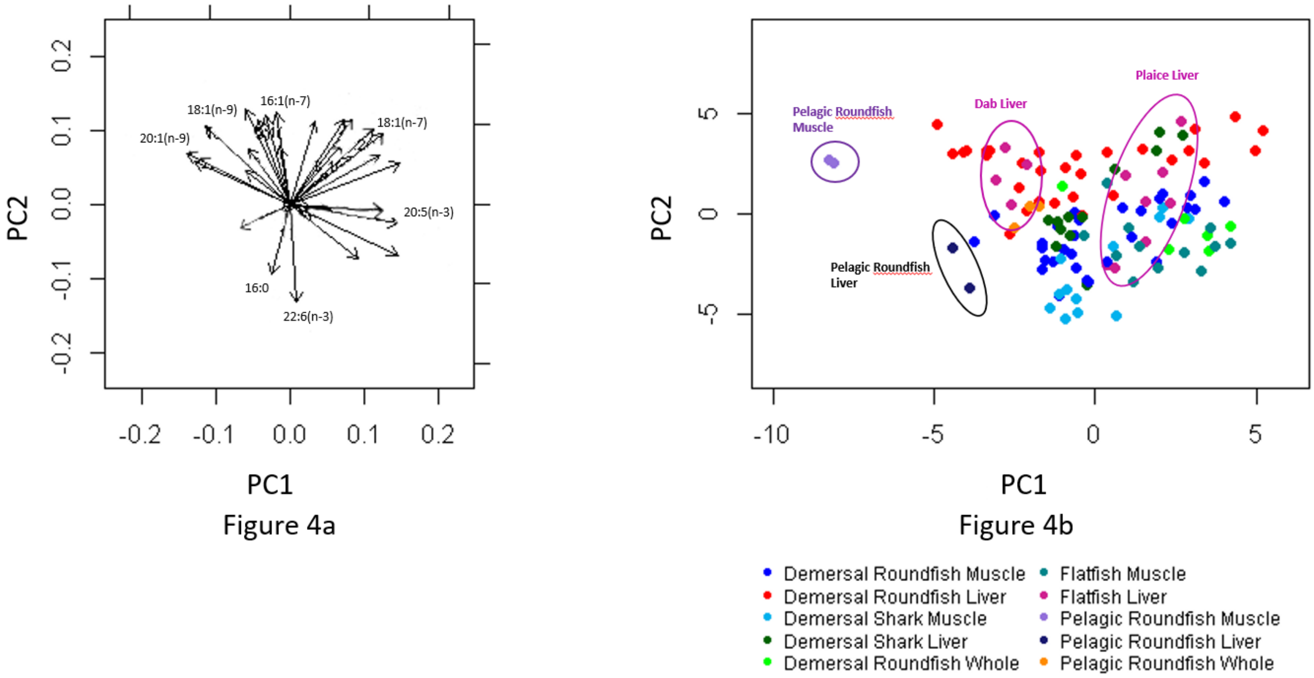

Figure 4: (a) PCA loading plot demonstrating variation in the FA profiles (normalised area percentages) across the ten categories of fish and shark highlighting the group separation of pelagic fish muscle and liver due to differing proportions of MUFAs and plaice liver and muscle due to differing proportions of 18:1(n-9). FAs labelled on the loading plot are those discussed in section 3.1.2. (b) PCA score plot demonstrating variation in the FA profiles (normalised area percentages) across the ten categories of fish and shark.

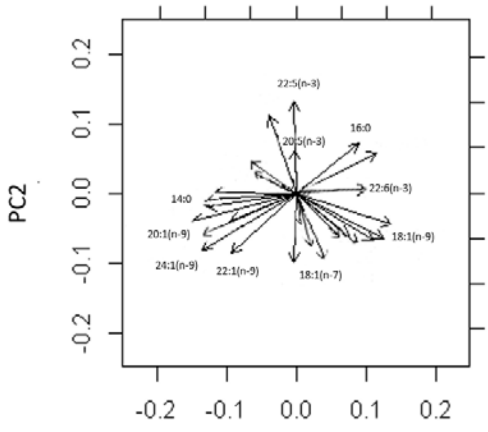

Figure 5a

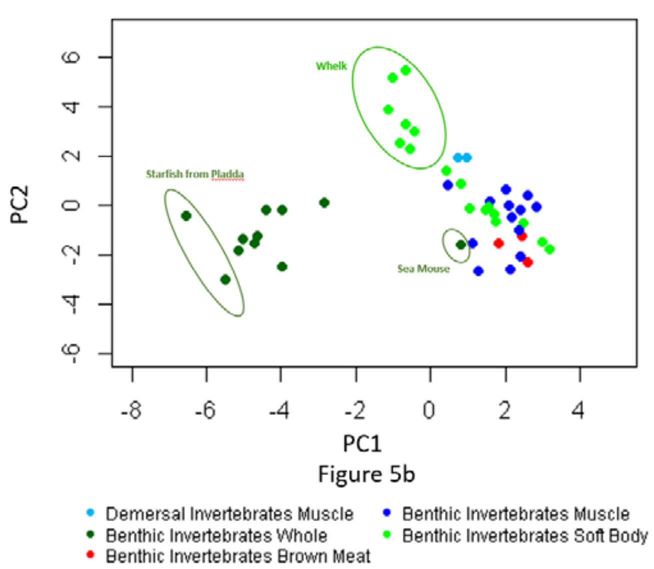

Figure 5b

Figure 5: (a) PCA loading 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) and the separation of the benthic invertebrates soft body category due to a contributing species FA profile influence. FAs labelled on the loading plot are those discussed in section 3.1.3. (b) PCA score plot demonstrating variation in the FA profiles (normalised area percentages) across the five categories of invertebrates.

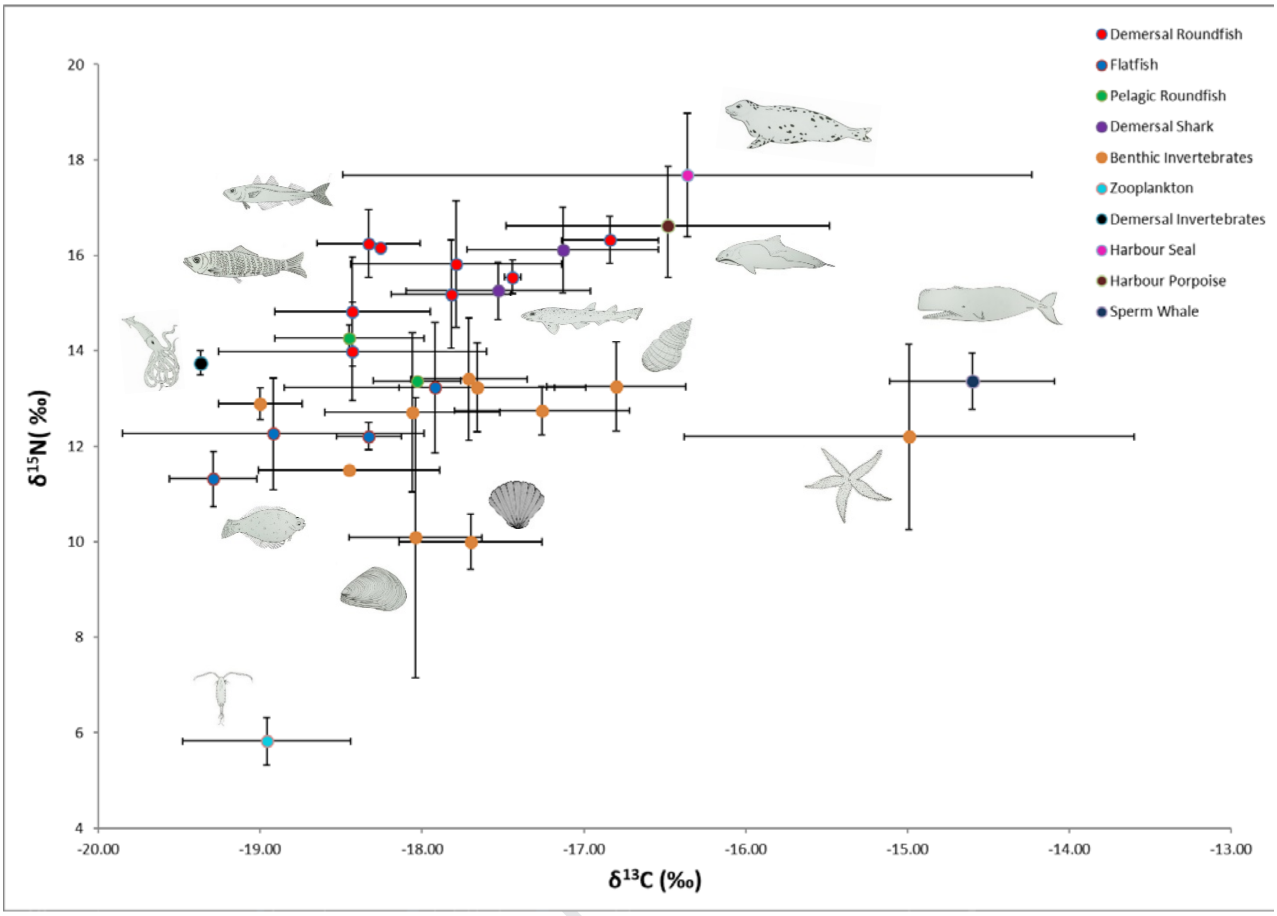

Figure 6: Scatter plot demonstrating the spread of mean stable isotope ratios $\delta^{15}N$ and $\delta^{13}C$ analysed in ten chemotaxonomical sample categories (not taking tissue type into account. Excluding n=1 samples). The greater the $\delta^{15}N$ value the higher the trophic level. Differing $\delta^{13}C$ values, indicate different carbon sources at the base of the food web (benthic vs pelagic photosynthesis).

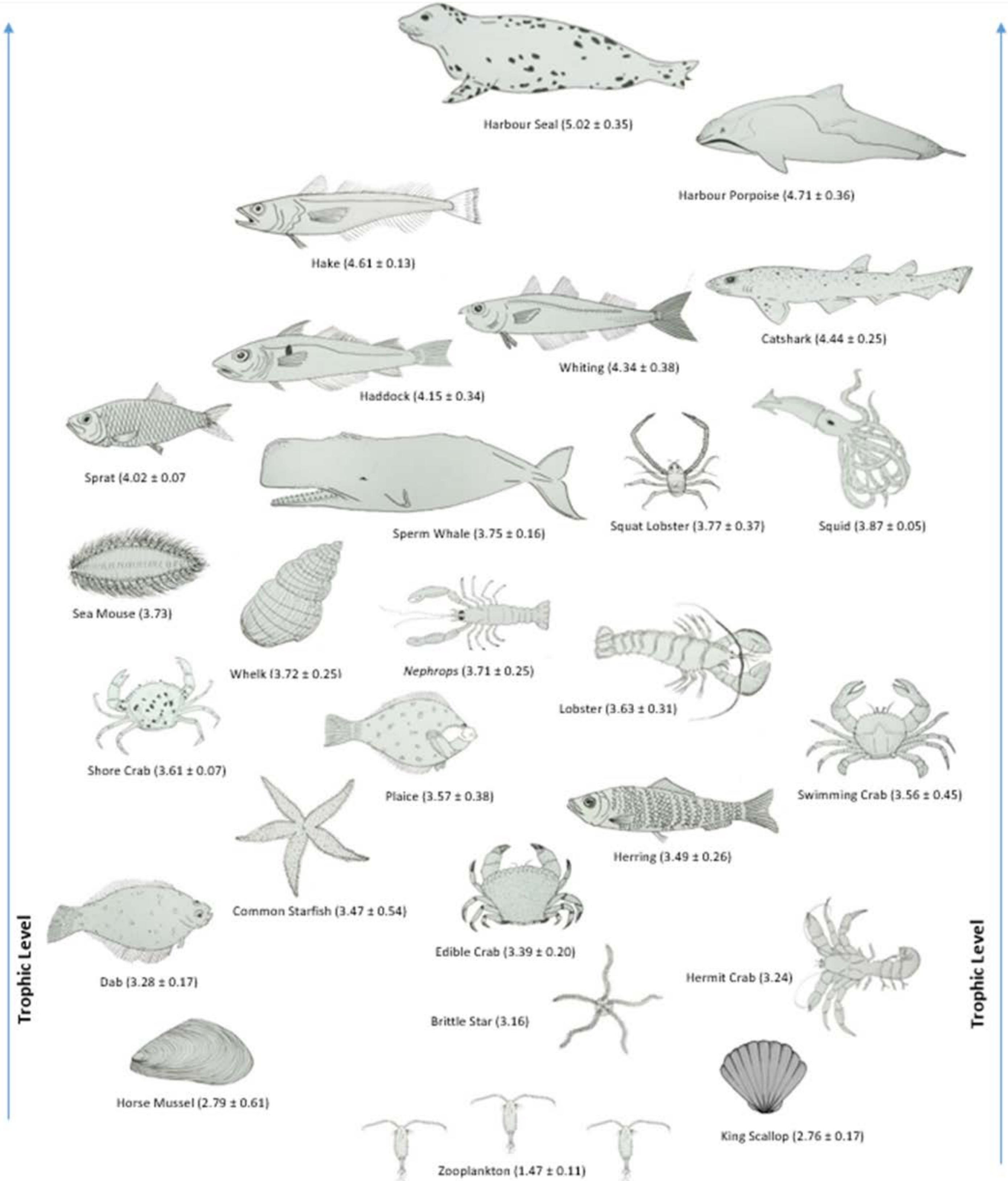

Figure 7: Scottish marine food web diagram showing the and mean trophic level (± standard deviation) calculated from $\delta^{15}N$ for each species using Equation 1. Matrices within species have been combined to give an overall species trophic level. Primary producers (e.g. phytoplankton) are not included in this food web diagram as they were not investigated as part of this study.

Highlights

- Trophic levels and feeding patterns within Scottish marine food webs were investigated
- The complexity in fatty acid profiles and stable isotope ratios is due to multiple influences
- Significant complexity can occur within a single trophic level
- Marine assessments must use a multi-factorial approach when investigating ecological dynamics
- Data will be used to determine contaminant trophic magnification factors

Supplementary Information

Understanding Marine Food Web Dynamics Using Fatty Acid Signatures and Stable Isotope Ratios: Improving Contaminant Impacts Assessments across Trophic Levels

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Sample Collection and Preparation: Pooling process and measurements taken of fish, shark and invertebrates (additional information).

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). Liver and muscle were dissected from the fish and catshark. Brown meat and muscle were dissected from edible crab and lobster. 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 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 homogenised. Squid mantle, which is composed of a muscular framework of connective tissue fibres, was classified as muscle tissue and homogenised. King scallops had their adductor muscle homogenised and used only for SI analysis.

Lipid Extraction, Trans-esterification and Instrumental Analysis

Lipid was extracted from fish, shark and invertebrate sample pools, individual marine mammal blubber and zooplankton pools 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). All solvents used were HPLC grade. The organic layer was recovered and evaporated to dryness and the lipid extract was trans-esterified to FAMEs in toluene mixed with 1% v/v sulphuric acid in methanol. The protein residue, formed between the organic and inorganic layers, was air-dried overnight on a filter paper, freeze dried, and ground to a fine powder for stable isotope (SI) analysis.

Thirty-two FAMEs were determined (although the two positional isomers for eicosenoic acid are reported together) using an HP Agilent 6890 GC-FID. Five FAMEs were further characterised using GC-MS (HP Agilent 6890 Series GC interfaced with an HP 5975 MSD) with automated on-column injection. Sample pools were chromatographed using a DB23 (polar) fused silica capillary column (30 m x 0.25 mm internal diameter; 0.15 µm film thickness).

In summary, 1.0 µL of sample was injected using cool on-column injection, nitrogen was used as the carrier gas at a flow rate of 1.0 mL/min using a specific oven temperature profile. The area responses (µV s) from the FID were summed for the compounds and normalised area percentages calculated for each of the FAMEs as a percentage of the combined area for the 31 compounds/groups of compounds. Data for the positional isomers of eicosenoic acid (20:1) were combined and so the normalised area percentages were calculated based on the summed area for 30 individual FAs (including the different positional isomers of 22:1) and the combined peak area for the positional isomers for 20:1.

Stable Isotope Analysis

Approximately 0.60 ± 0.10 mg of ground, de-lipified material was loaded into a 6 × 4 mm tin capsule and compressed using tweezers. The compressed capsules were loaded into the autosampler, combusted and analysed.

A LRM of de-lipified fish flesh and two certified reference materials were analysed with the samples. The certified reference materials 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. Analytical precision of the instrument throughout the different batch analyses was <0.3‰ for ^{15}N and <0.4 ‰ for ^{13}C.

Results are reported 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.

Quality Control

A vial of *iso*-hexane was run on the GC-FID or 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. To validate instrument performance, FA retention times were confirmed on the GC using a Restek Marine Oil FAME commercial standard and EO23 fish oil. EO23 is a reference material composed of a mixture of FAs, developed for calibration trials within the laboratory

(McGill and Moffat., 1992). The standards were run at the beginning and end of each batch, with EO23 run additionally every 8 samples, to continually check peak separation and retention times on the GC. Due to the high wax ester content of some samples, a commercial Nu-chek fatty alcohol (FAl) standard was analysed with each batch to establish whether co-elution of FAs and FAls was occurring.

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), Unknown (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
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=8), Pneumonia (n=1), N/A (n=1)
Sperm Whale (*Physeter macrocephalus*; n=5)	
Length (cm)	1183-1524
Weight (Kg)	26,060 (n=1), N/A (n=4)

Girth (cm)	610-722 (n=2), N/A (n=8)
Dorsal Blubber Thickness (mm)	98-110 (n=2), N/A (n=8)
Age	Adult (n=1), Subadult (n=3), Unknown (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)

Table S1: Marine mammal samples from Scottish waters used for fatty acid and stable isotope analysis. N/A, not available. The location of individual animal strandings are represented on Figure 1 by a small green circle.

Category	Sample Number	14:0	14:1(n-5)	15:0	16:0	16:1(n-7)	16:2
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	27	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 S2: 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 fatty acid profiles (Fig 2b – 5b).

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	27	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 S2 (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 fatty acid profiles (Fig 2b – 5b).

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	27	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 S2 (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 fatty acid profiles (Fig 2b – 5b).

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	27	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 S2 (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 fatty acid profiles (Fig 2b – 5b).

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	27	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 S2 (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 fatty acid profiles (Fig 2b – 5b).

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	27	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

Table S2 (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 fatty acid profiles (Fig 2b – 5b).