Reviews and syntheses: Insights into deep-sea food webs and global environmental gradients revealed by stable isotope ($\delta^{15}N$, $\delta^{13}C$) and fatty acid trophic biomarkers

Camilla Parzanini[1], Christopher C. Parrish[1], Jean-François Hamel[2], Annie Mercier[1]

[1]Department of Ocean Sciences, Memorial University, St. John's, NL, Canada

[2]Society for Exploration and Valuing of the Environment (SEVE), St. Philips, NL, Canada

Correspondence to: Camilla Parzanini (cparzanini@ryerson.ca). Current address: Department of Chemistry and Biology, Ryerson University, Toronto, ON, Canada

Abstract. Biochemical markers developed initially for food-web studies of terrestrial and shallow-water environments have only recently been applied to deep-sea ecosystems (i.e. in the early 2000s). For the first time since their implementation, this review took a close look at the existing literature in the field of deep-sea trophic ecology to synthesize current knowledge. Furthermore, it provided an opportunity for a preliminary analysis of global geographic (i.e. latitudinal, along a depth gradient) trends in the isotopic ($\delta^{15}N$, $\delta^{13}C$) and fatty acid composition of deep-sea macro- and megafauna from heterotrophic systems. Results revealed significant relationships along the latitudinal and bathymetric gradients. Deep-sea animals sampled at temperate and polar latitudes displayed lower isotopic ratios and greater proportions of essential ω3 long-chain polyunsaturated fatty acids (LC-PUFA) than did tropical counterparts. Furthermore, $\delta^{15}N$ and $\delta^{13}C$ ratios as well as proportions of arachidonic acid increased with increasing depth. Since similar latitudinal trends in the isotopic and fatty acid composition were found in surface water phytoplankton and particulate organic matter, these results highlight the link across latitudes between surface primary production and deep-water communities. Because global climate change may affect quantity and quality (e.g. levels of essential ω3 PUFA) of surface primary productivity, and by extension those of its downward flux, the dietary intake of deep-sea organisms may likely be altered. In addition, because essential ω3 PUFA play a major role in the response to temperature variations, climate change may interfere with the ability of deep-sea species to cope with potential temperature shifts. Importantly, methodological disparities were highlighted that prevented in-depth analyses, indicating that further studies should be conducted using standardized methods in order to generate more reliable global predictions.

1 Introduction

1.1 Historical background of biochemical biomarkers in deep-sea food-web studies

While the use of biochemical biomarkers in marine food-web studies has a long and successful tradition in shallow-water ecosystems, starting from the 1970s with the use of stable isotopes (McConnaughey and McRoy, 1979) and lipids (Lee et al., 1971), their application in deep-water environments is relatively new (e.g. Iken et al., 2001; Polunin et al., 2001; Howell et al., 2003). Undoubtedly, technological advances made over the past few decades have allowed the exploration of ever deeper ecosystems with more refined techniques. Iken et al. (2001) were among the first to provide a comprehensive analysis of a deep-sea food web, which was sampled at a depth of ~4840 m at the Porcupine Abyssal Plain (PAP, Northeast Atlantic), by using bulk stable N and C isotope ratios ($\delta^{15}N$ and $\delta^{13}C$ respectively) as trophic markers. In the same year, Polunin et al. (2001) used the same approach to study the trophic relationships of a slope megafaunal assemblage collected off the Balearic Islands (western Mediterranean). Since these first two investigations, several others have been carried out across different oceanic regions and climes, such as the Canadian Arctic (Iken et al., 2005), the Arabian Sea (Jeffreys et al., 2009), and the Sea of Japan (Kharlamenko et al., 2013). Furthermore, over the past decade, it has become evident that the simultaneous use of different trophic markers (e.g. $\delta^{15}N$, $\delta^{13}C$, and fatty acids, FA) and techniques (e.g. bulk or compound specific isotope analysis, as well as FA, gut content and morphometric analyses) provides a more complete picture of trophic structure and dynamics. Indeed, while the first investigations relied on a single method (Iken et al., 2001; Polunin et al., 2001; Howell et al., 2003), the latest trend in deep-sea food-web studies favours an integrative approach, which maximizes the efficiency of each technique, while increasing the resolution of the investigation (e.g. Stowasser et al., 2009; Parzanini et al., 2017).

For the first time since the implementation of trophic markers in studies of deep-sea food webs, this review synthesizes current knowledge in this growing field of research, mainly focusing

on heterotrophic ecosystems (i.e. relying on photosynthetic primary production). In addition, it provides a preliminary overview of large-scale geographic trends from the analysis of isotopic and FA data for macro- and megafauna, along with guidance for future investigations. In particular, the present contribution i) briefly defines various trophic biomarkers and their respective advantages; ii) describes deep-sea food webs, based on examples from the literature; iii) lists the sources of variation among the different studies to highlight pitfalls and gaps; and iv) provides a preliminary quantitative analysis across studies by using relevant datasets.

1.2 Comparison of major trophic markers

The analysis of gut contents was among the first techniques (together with *in situ* observation of feeding behaviors) applied in trophic ecology and food-web studies in aquatic systems (Gartner et al., 1997; Michener and Kaufman, 2007). Subsequently, other methods were developed as alternative or supplementary means of studying diet and feeding behaviors within the same ecosystems. Among them, the use of biochemical markers as trophic tracers rapidly grew in popularity in food-web ecology, since it is relatively simple and should overcome many of the issues ascribed to gut content analysis (Michener and Kaufman, 2007). In this regard, Table 1 lists strengths and drawbacks of gut content analysis and of the two most popular biochemical techniques, i.e. bulk stable isotope and FA analyses. For instance, bulk stable isotope and FA analyses may, theoretically, be performed on any species, regardless of feeding mode and food sources, whereas gut content analysis can only be applied to those organisms characterized by a sufficiently large and full stomach. Except in cases where individuals are too small and have to be analyzed whole, biochemical analyses are typically conducted on target tissues (e.g. muscle) that provide long-term dietary data and reduce intra-individual variability (Table 1). In addition, the use of biochemical tracers requires shorter processing times than gut content analysis. Thanks to this integrative approach and faster output, the application of food-web tracers has been particularly helpful in deep-sea studies, which are often plagued by financial and logistical constraints. Furthermore, due its relative ease of use, it has favoured the analysis of wider sets of taxa/feeding

guilds, primary producers included, rather than focusing on one or a few focal groups. However, the interpretation of isotopic and FA data is complex, and both techniques require dedicated and sophisticated instrumentation (e.g. gas chromatograph, mass spectrometer) and knowledge of intrinsic sources of variations (see Sect. 1.4). Although each method needs a sufficient sample size, only gut content analysis may provide direct and clear taxonomic evidence of the diet (Table 1). Therefore, as stated above, the latest trend in trophic ecology advocates a multifaceted approach, on the understanding that each technique may offer unique and valuable data.

The principle behind the use of food-web tracers is that the biochemical signature of consumers reflects that of their diet. Among them, $\delta^{15}N$ and $\delta^{13}C$ are the most popular. While the former is used to study trophic positions and dietary sources, with an enrichment factor of 2-4‰ between a consumer and its food (Minagawa and Wada, 1984); the latter undergoes little fractionation (<1‰) and, therefore, is used to distinguish primary food sources (McConnaughey and McRoy, 1979). For further details, refer to Sulzman (2007) and Michener and Kaufman (2007) who have provided extensive reviews on the chemistry behind stable isotopes and their use as food-web tracers, respectively. In addition, sterols, FA and amino acids, which are important constituents of lipids (for the former two) and proteins (for the latter), have successfully been used to study trophic relationships and dietary sources in deep-water systems (Howell et al., 2003; Drazen et al. 2008a, 2008b). Their use is based on the principle that certain FA and amino acids are considered essential for animals, being required for optimal fitness. However, most species cannot synthesize these essential compounds *de novo* and, therefore, they must gain them through their diet. Indeed, only primary producers and a few consumers possess the enzymatic apparatus to synthesize essential FA and amino acids *de novo*. Conversely, a few taxa are unable to synthesize sterols *de novo*, which are critical for them; therefore, they have to acquire these essential sterols through diet (Martin-Creuzburg and Von Elert, 2009). Because sterols, FA, and amino acids undergo little or no alteration when consumed, it is possible to detect dietary sources within the consumers' tissues (Parrish et al., 2000). The isotopic signature of amino acids can also be used to study trophic position through compound specific analysis ($\delta^{15}N$), as some of these

100 acids show trophic enrichment (Bradley et al., 2015). Detailed information about FA analysis was
101 outside the scope of this study, and is provided by Parrish (2009) and Iverson (2009); whereas the
102 use of sterols as food-web tracers was outlined in Martin-Creuzburg and Von Elert (2009) and
103 Parrish et al. (2000). McClelland and Montoya (2002) and Larsen et al. (2009), conversely, discuss
104 the use of amino acids as trophic biomarkers.

105 **1.3 Understanding deep-sea food webs through biochemical markers**

106 As there is no photosynthetically-derived primary production in the deep sea, deep-water
107 ecosystems are mostly heterotrophic (Gage, 2003), and may hence largely rely on particulate
108 organic matter (POM) that passively sinks from the surface waters as a primary source of nutrients
109 (Hudson et al., 2004). Nonetheless, food can also be actively transported down by those animals
110 that carry out vertical diel migrations through the water column (Trueman et al., 2014); it can also
111 be provided by the occasional fall of large animal carcasses (Smith and Baco, 2003); and/or by
112 lateral inputs, from inland and shelf areas towards abyssal offshore regions (Pfannkuche, 2005).
113 Although most of the deep-water ecosystems are heterotrophic, a few, such as hydrothermal vents
114 and cold seeps, are fuelled by chemical energy (e.g. methane, hydrogen sulfide) and rely on
115 chemosynthetic microorganisms for the production of organic matter. Each of these primary food
116 sources has a specific isotopic composition and biochemical signature, resulting from a
117 combination of chemical and physical processes reflective of its origin. By knowing the composition
118 of the food source(s) that fuel(s) a given food web, it is possible to re-construct its trophic structure
119 and dynamics. Conversely, by measuring the signatures of the food-web components, it is possible
120 to assess food sources on which they rely. For instance, Iken et al. (2001) showed that
121 phytodetritus was the primary energy input of the deep-sea benthic community at PAP, and also
122 defined two different trophic pathways: a pelagic and isotopically lighter one in which sinking POM
123 and small pelagic prey constituted the main food sources; and a benthic and more isotopically
124 enriched trophic pathway, fuelled by degraded sedimented POM. In fact, once POM settles on the
125 seafloor, it undergoes continuous degradation by microbes and is reworked through bioturbation

126 and feeding activities, thus leading to a more isotopically enriched material relative to the sinking
127 one (Iken et al., 2001). Depending on the primary food source they relied on, benthic organisms at
128 PAP were thus characterized by either lower or higher values of $\delta^{15}N$. Similar scenarios of dual
129 trophic pathways characterizing benthic systems were also found by Iken et al. (2005) in the
130 Canadian Arctic; Drazen et al. (2008b) in the North Pacific; Reid et al. (2012) within the benthic
131 community sampled on the mid-Atlantic Ridge; Valls et al. (2014) in the western Mediterranean;
132 and Parzanini et al. (2017) in the Northwest Atlantic. Moreover, Kharlamenko et al. (2013) used
133 both stable isotopes and FA to study the dietary sources of benthic invertebrates collected along
134 the continental slope (500-1600 m depth) in the Sea of Japan. The authors recognized different
135 trophic pathways (i.e. planktonic, benthic, microbial) and dietary sources by using biochemical
136 tracers; and they proposed a strong link with the primary production of the surface waters, as the
137 FA composition of the deep-sea echinoderms and mollusks was similar to that of the shallow-water
138 counterparts.

139 As POM sinks through the water column, its $\delta^{15}N$ increases, reflecting the preferential
140 assimilation of the lighter isotope, ^{14}N by microbes; in particular, a gradient in POM $\delta^{15}N$ has been
141 detected with depth, where POM at greater depths is more enriched (Altabet et al., 1999). For this
142 reason, Mintenbeck et al. (2007) carried out a study in the high-Antarctic Weddell Sea to assess
143 whether this gradient was reflected in the isotopic signature of POM consumers sampled at 50-
144 1600 m. In this regard, only those organisms feeding directly on sinking POM (e.g. suspension
145 feeders) showed increasing values of $\delta^{15}N$ with depth, whereas the increase was less evident for
146 the deposit feeders (Mintenbeck et al., 2007). Similar results for suspension feeders were obtained
147 by Bergmann et al. (2009) who analyzed a benthic food web sampled at the deep-water
148 observatory HAUSGARTEN, west of Svalbard (Arctic), between 1300 and 5600 m depth.
149 Conversely, deposit feeders exhibited a negative trend along the bathymetric gradient in terms of
150 $\delta^{15}N$, and predator/scavengers were not affected. In another study, Sherwood et al. (2008) did not
151 detect any relationships with depth in the $\delta^{15}N$ values measured from cold-water corals collected on
152 a slope environment in the Northwest Atlantic. Among the explanations suggested for these

153 inconsistencies and differences among feeding groups, Mintenbeck et al. (2007) and Sherwood et
154 al. (2008) included feeding preferences with respect to the size and sinking velocity of POM.
155 According to these authors, only those organisms feeding on small particles of sinking POM should
156 reflect a bathymetric gradient in $\delta^{15}N$. In fact, small-sized particles sink at a lower velocity and,
157 therefore, experience high rates of degradation, with more evident changes in $\delta^{15}N$ (Mintenbeck et
158 al., 2007). Based on these findings, depth-stratified sampling should ideally be conducted when
159 studying a system characterized by a bathymetric gradient, as it would prevent biases in the
160 interpretation of the isotopic data.

161 Deep-water systems are generally characterized by a limited food supply, as the quantity of
162 food being transferred from the surface to the bottom diminishes with increasing depth (Gage,
163 2003). In addition, in temperate areas, food arrives as intermittent pulses, following the spring and
164 late summer blooms of primary (and secondary) productivity. For this reason, deep-water benthic
165 communities can only rely on fresh, high-quality phytodetritus within short temporal windows
166 following algal blooms; whereas reworked and resuspended POM fuels these communities for the
167 rest of the year (Lampitt, 1985). Deep-sea benthic organisms have hence developed adaptations
168 and strategies to increase their feeding success and minimize competition for food, including
169 trophic niche expansion and specialization. In this regard, certain benthic taxa (e.g. pennatulacean
170 corals, hexactinellid sponges) and/or feeding groups (e.g. suspension and deposit feeders) at PAP
171 showed vertical extension of their trophic niches (i.e. omnivory) which, according to Iken et al.
172 (2001), was most likely driven by a strong competition for food. In other words, some species
173 belonging to the same taxon or feeding guild shared similar food sources (i.e. exhibiting similar
174 $\delta^{13}C$ values), but they were located at different trophic levels (i.e. exhibiting a wide range of $\delta^{15}N$).
175 Similarly, Jeffreys et al. (2009) reported trophic niche expansion among and within feeding guilds
176 sampled between 140 and 1400 m depth, at the Pakistan margin (Arabian Sea). Pennatulacean
177 corals and other sestonivorous cnidarians, for example, displayed the greatest niche expansion;
178 they fed not only on POM, but also on small invertebrates (e.g. zooplankton). Moreover, ophiuroids,
179 which are typically selective deposit feeders, switched to an omnivorous diet under food-limited

conditions (Jeffreys et al., 2009). Apart from trophic niche expansion, Iken et al. (2001) proposed that specialization on certain food items represented another adaptation developed by benthic organisms at PAP to mitigate competition for food. Holothuroid echinoderms, for instance, were thought to accomplish food specialization through a combination of different factors involving changes in morphology, mobility, and digestive abilities (Iken et al., 2001). Further examples of trophic niche segregation and food partitioning, as strategies to minimize competition, were also reported for deep-sea demersal fishes in the Northwest Mediterranean Sea (Papiol et al., 2013) and for asteroid echinoderms in the Northwest Atlantic (Gale et al., 2013). Howell et al. (2003) detected trophic niche expansion across different species of deep-sea asteroids (1053-4840 m) by analyzing their FA composition. In particular, multivariate analysis of FA proportions discriminated three different feeding guilds among the asteroids analysed, including mud ingesters, predators/scavengers, and suspension feeders.

1.4 Sources of variation across studies

When comparing studies relying on biochemical analysis, there are numerous sources of variation, which may influence results and findings, and also prevent the detection of similarities and general trends. However, their importance may depend on the scale of the investigation (i.e. local, regional, or global). In this section, the main sources of variation are illustrated and explained by type (Table 2).

1.4.1 Biological sources

Age, size, and sex, whether related to diet, determine natural intraspecific variability in the isotopic and FA compositions of organisms, which may affect data interpretation of small spatial scale investigations. At a basic level, sessile and sedentary taxa typically experience a transition from a pelagic to a benthic lifestyle between the larval and the juvenile stage (Rieger, 1994). Research has also shown that certain deep-sea fish experience changes in diet with age, typically with younger individuals preying upon benthic organisms and adults feeding on prey that are larger and of

benthopelagic origin (Mauchline and Gordon, 1984; Eliassen and Jobling, 1985). Stowasser et al. (2009) combined stable isotope analysis (SIA) and FA analysis to detect ontogenetic shifts in the diet of the fish *Coryphaenoides armatus* and *Antimora rostrata*, collected at depths between 785 and 4814 m at PAP (Northeast Atlantic). By looking at their biochemical composition, the two species switched from active predation to scavenging with increasing size. Similar results are reported in Drazen et al. (2008c) for macrourid fish species from the eastern North Pacific. Conversely, although Reid et al. (2013) detected size-related trends in the $\delta^{13}C$ of deep-water fish collected from the Mid-Atlantic Ridge at 2400-2750 m depth, the authors were not able to distinguish whether these results were due to ontogenetic changes in diet or merely to an effect of increasing size, within the size-range sampled. Moreover, $\delta^{15}N$ and trophic position may increase with body size in adult shallow-water fish, as larger predatory fish ingest larger, more isotopically enriched prey (Badalamenti et al., 2002; Galván et al., 2010).

The potential influence of sex as a source of variation in biomarker studies has not received as much attention and remains ambiguous. Nonetheless, Boyle et al. (2012) studied whether diet and trophic position varied between sexes in deep-sea fish species collected at 55 -1280 m depth in the eastern North Pacific using gut content and stable isotope analysis of muscle tissue. The authors did not detect any difference between sexes, but variations in trophic position were encountered when analyzing fish of different sizes (Boyle et al., 2012). An investigation of the oceanic squid *Todarodes filippovae* sampled within a depth range of 13-380 m in the southwestern Indian Ocean by Cherel et al. (2009), revealed that females had higher values of $\delta^{15}N$, and thus occupied a higher trophic position. However, because *T. filippovae* exhibits sexual dimorphism in body size, this difference was ultimately shown to be driven by size, i.e. no $\delta^{15}N$-variations were detected when females and males of similar sizes were compared (Cherel et al., 2009). Sex may constitute a source of variation in relation to diet in those species that exhibit extreme cases of sexual dimorphism, as in deep-sea anglerfish (Shine, 1989). However, investigation of the role of sex on intraspecific variability will need to be carried out across a broader taxonomic scope before drawing generalizations.

1.4.2 Environmental sources

Larger-scale (e.g. regional, global) comparative studies among deep-sea habitats are complicated by the wide bathymetric ranges they may occupy, anywhere between 200 and ~11 000 m depth. Depth may constitute a major driver of variation of $\delta^{15}N$ and $\delta^{13}C$ in deep-sea organisms for two main reasons. First, as mentioned earlier, biodegradation processes occurring within the water column may favour the enrichment of POM as it sinks, thus influencing the stable isotope composition of those organisms that directly feed on it (Mintenbeck et al., 2007; Bergmann et al., 2009). Second, size-based trends and shifts in diet, hence in the isotopic composition, with depth have been reported for deep-sea demersal fish (Collins et al., 2005; Mindel et al., 2016a, 2016b). Likewise, deep-sea species may exhibit different lipid and FA compositions along a bathymetric gradient, reflecting physiological adaptations to changing temperature and pressure with depth (Parzanini et al., 2018b).

Geographic location (e.g. latitude) and season, linked to level/type of surface primary production, nitrogen supply dynamics, as well as temperature, are also important factors to consider when comparing studies, as large-scale temporal and spatial differences may be detected in the organisms' isotopic composition. Stowasser et al. (2009), for instance, combined stable isotope and FA acid analyses to study seasonal variations in the diet of 5 species of demersal fish collected between 785 and 4814 m in the Northeast Atlantic. The authors found overall that stable isotope and FA composition of fish varied temporally, and that these differences most likely reflected timing and strength of food inputs sinking from surface waters. However, not all the species (e.g. *Coryphaenoides armatus*) exhibited a strong seasonality in their biochemical composition, probably due to the high trophic position of the species and the length of the food web analyzed obscuring the effects of the seasonal POM inputs (Stowasser et al., 2009). Colombo et al. (2016) detected a latitudinal gradient in the FA composition of marine species, with higher levels of ω3-polyunsaturated fatty acids in organisms collected at polar and temperate regions in comparison to tropical ones. Large-scale geographic effects will be further explored below, in the

exploratory analytical section; however, Fig. 1 shows where food-web studies accomplished via biochemical tracers have been carried out in heterotrophic ecosystems, highlighting important geographic heterogeneity, especially the limited number of investigations in the southern hemisphere.

1.4.3 Analytical sources

Several aspects of the SIA methodology can generate variability among studies, including type(s) of tissue chosen for analysis, as well as sample treatment and storage, thus influencing interpretation of small-scale investigations. For instance, lipids have lower ^{13}C in comparison to proteins and carbohydrates (DeNiro and Epstein, 1977), lipid-rich tissues hence display lower $\delta^{13}C$ values. In addition, there are tissues, such as liver in fish and gonads in other taxa, which are characterized by higher turnover rates of lipids than others (e.g. white muscle), and hence incorporate information only on the recent diet. To avoid biases caused by the presence of lipids in tissues, several approaches may be used. Stowasser et al. (2009) and Boyle et al. (2012), for example, opted to extract lipid from the tissues prior to analysis, whereas Sherwood et al. (2008), Fanelli et al. (2011a, 2011b) and Papiol et al. (2013) applied a mathematical correction to their $\delta^{13}C$ data, based on the elemental C to N ratio (C:N) characterizing the samples. Other authors, such as Polunin et al. (2001) and Carlier et al. (2009), did not apply any treatment. In the case of mathematical corrections, two equations are currently used for deep-sea organisms, those proposed by Post et al. (2007) and Hoffman and Sutton (2010). Since lipid extraction increases values of $\delta^{15}N$ in deep-sea fish muscle tissue (Hoffman and Sutton, 2010), this practice is not recommended. Conversely, mathematical corrections seem to be preferable when dealing with lipids, and they have already been applied in several studies, including those mentioned above.

Some marine organisms, such as corals and echinoderms, contain carbonate skeletal elements. Since inorganic carbonate has higher $\delta^{13}C$ values than other fractions (Pinnegar and Polunin, 1999), it is a widespread practice to acidify these types of samples. Variations occur when acidification is executed on samples that are simultaneously run for $\delta^{15}N$ and $\delta^{13}C$, as the treatment

may affect $\delta^{15}N$ data (Bunn et al., 1995). Whenever feasible, depending on both financial constraints and the sizes of the organisms, processing samples separately for each isotope would therefore be advisable, as in Carlier et al. (2009), Sherwood et al. (2008), and Papiol et al. (2013).

The tissues of elasmobranchs (e.g. sharks, rays) contain urea and trimethylamine oxide, which are both ^{15}N-depleted; therefore, their presence may affect stable isotope data (Hussey et al., 2012; Kim and Koch, 2012; Churchill et al., 2015). As for the inorganic carbonate issue, there is no agreement among studies. Nonetheless, the removal of urea prior to analysis or the use of arithmetic corrections are among the most common solutions applied to deal with the presence of these compounds. In addition, the former seems to be the more commonly recommended and performed, as the application of mathematical corrections requires the calculation of species-specific discrimination factors, which is not always feasible (Hussey et al., 2012).

Sample storage is also crucial to obtain reliable data, since non-optimal preservation methods may compromise the outcome of the investigation. Regarding the storage temperature, while biological samples for gut content and stable isotope analysis are commonly frozen at -20°C, if not processed soon after their collection; those for lipid analysis are either stored at -80°C (recommended) or at -20°C prior to further processing in the lab. Since storage at -20°C might not completely prevent lipid degradation, especially if samples are analyzed after several years, rapid initial processing of samples and vacuum packing may reduce potential issues when freezing at -80°C is not logistically feasible. In addition, freezing is highly recommended over chemical storage for stable isotope analysis, as there is evidence that formalin/ethanol considerably alters the isotopic ratios in biological tissues (Arrington and Winemiller, 2002; Syväranta et al., 2011; Xu et al., 2011).

2 Preliminary comparative analysis

The study of large-scale trends in biological variables (e.g. distribution, biochemical composition, biodiversity) may not only help understand general functioning and structure of ecosystems, but it may also allow us to make predictions and support conservation initiatives. While several studies

310 already exist on large-scale distribution and biodiversity patterns of deep-sea species (Rex et al.,
311 1993; Stuart et al., 2003; Ramirez-Llodra et al., 2010), a similar approach has yet to be applied to
312 trophodynamics. This preliminary analysis detected global spatial trends (i.e. along latitudinal and
313 depth gradients) in the isotopic and FA composition of deep-water animals for the first time since
314 the application of biochemical tracers to the study of trophic ecology in the deep sea.

315 Latitudinal gradients have been detected in $\delta^{13}C$ of plankton and POM collected from
316 surface waters in both the southern and northern hemispheres, with decreasing values towards the
317 polar regions (Sackett et al., 1965; Rau et al. 1982; Francois et al., 1993). Both environmental (e.g.
318 temperature, nutrient supply) and biological (e.g. plankton metabolism) factors have been proposed
319 to explain such trends (Rau et al., 1982; Francois et al., 1993). The stable N isotope signature of
320 surface primary production may also vary regionally, depending on the nutrient (mainly N) supply to
321 the phytoplankton, as well as its community structure and cell size (Choy et al., 2015; Hetherington
322 et al., 2017). Oligotrophic areas, characterized by marked oxygen minimum zones and by high
323 denitrification rates, such as the eastern tropical Pacific Ocean, typically have higher $\delta^{15}N$ values
324 (Hetherington et al., 2017). In addition, latitudinal trends have been detected in the FA composition
325 of marine organisms, which tend to have higher levels of essential ω3 long-chain polyunsaturated
326 fatty acids (LC-PUFA) in the polar and temperate regions in comparison to the tropical ones
327 (Colombo et al., 2016). As POM is the main food source of most deep-sea food webs (Gage, 2003;
328 Hudson et al., 2004), we hypothesized that a) similar latitudinal gradients exist in the isotopic and
329 essential PUFA composition of deep-water organisms; and that b) the strength of these trends
330 varied among organisms from different habitats, i.e. pelagic, demersal, and benthic, as diversely
331 dependant on POM. Furthermore, as both isotopic and lipid composition of POM and as deep-sea
332 taxa varied along a depth gradient in the deep North Pacific (Lewis, 1967; Altabet et al., 1999),
333 North Atlantic (Polunin et al., 2001; Parzanini et al., 2018a, 2018b, 2017) and Arctic Ocean
334 (Bergmann et al., 2008), we hypothesized that similar trends could be extended to the global scale.

2.1 Materials and methods

2.1.1 Data set

This analysis focused on studies that used either bulk stable isotope or FA analysis, or a combination of them, to infer trophic relationships of deep-water macro- and megafauna, as well as to study deep-sea food webs, from heterotrophic ecosystems. Experimental studies, as well as investigations on chemosynthetic habitats (e.g. hydrothermal vents) were excluded *a priori* to avoid possible biases. In fact, these habitats are fuelled by primary dietary sources, e.g. methane, whose isotopic and FA composition is substantially different than that of POM (Rau and Hedges, 1979; Saito and Osako, 2007). Table 3 outlines the full data set collated for the present analysis, which includes 52 different studies. The literature search was carried out through Scopus and Google Scholar portals using the following key words: stable isotopes, fatty acids, food webs, deep sea, trophic ecology, and trophic relationships. Additional sources provided by an anonymous referee were also included. These studies were used to analyze global trends in $\delta^{15}N$, $\delta^{13}C$, and the essential arachidonic (ARA, 20:4ω6), eicosapentaenoic (EPA; 20:5ω3) and docosahexaenoic (DHA, 22:6ω3) acids across deep-water communities. ARA, EPA, and DHA are the most important nutrients in aquatic ecosystems, required by organisms for optimal health (Parrish 2009), as well as excellent trophic biomarkers. In fact, whereas EPA and DHA are typically used as biomarkers in diatoms and dinoflagellates respectively (Parrish, 2013), in the deep sea, ARA is associated with microorganisms from the sediment (Howell et al. 2003). Our study focused on these three FA since they are present in all the organisms under analysis.

2.1.2 Variables considered

Each species from each investigation was sorted by latitude (i.e. tropical, 0 - 30°; temperate, 30 - 60°; and polar, 60 - 90°), habitat (i.e. pelagic, demersal, and benthic) depth at collection (i.e. mesopelagic, 200 – 1000 m; bathypelagic, 1000 – 4000 m; and abyssopelagic, >4000 m, for pelagic species; bathyal 200 - 4000 m; abyssal, 4000 - 6000 m; and hadal, > 6000 m, for benthic

species), and phylum (i.e. Annelida, Arthropoda, Brachiopoda, Bryozoa, Chaetognatha, Chordata, Cnidaria, Hemichordata, Echinodermata, Mollusca, Nematoda, Nemertea, Porifera, and Sipuncula). Information about species habitat was either obtained through WoRMS and FishBase online databases or was already included in the source paper. In addition, species were labelled as "meso-bathypelagic" and "bathyal-abyssal", if the depth at collection was not specified further, but the whole set of samples for a study was collected within those zones. In the current analysis, tissue type, acidification treatment, sampling season, sex, and age were not considered as variables, because i) they were assumed to not play a major role in global-scale investigations and/or ii) this information was not always provided. In addition, tests were performed on lipid-corrected and uncorrected $\delta^{13}C$ data pooled together. For analyses regarding stable isotope composition ($\delta^{15}N$, $\delta^{13}C$), data were obtained from Iken et al. (2005), Mincks et al. (2008), Bergmann et al. (2009), Quiroga et al. (2014), and van Øevelen et al. (2018), for polar regions; Iken et al. (2001), Madurell et al. (2008), Sherwood et al. (2008), Carlier et al. (2009), Fanelli et al. (2009), Stowasser et al. (2009), Fanelli et al. (2011a, 2011b), Boyle et al. (2012), Reid et al. (2012), Fanelli et al. (2013), Gale et al. (2013), Kharlamenko et al. (2013), Papiol et al. (2013), Reid et al. (2013), Tecchio et al. (2013), Kiyashko et al. (2014), Trueman et al. (2014), Valls et al. (2014a, 2014b), Kopp et al. (2018), Parzanini et al. (2017), Preciado et al. (2017) and Parzanini et al. (2018a), for temperate latitudes; and Jeffreys et al. (2009), Churchill et al. (2015), Shipley et al. (2017), and Richards et al. (2019), for tropical regions (Table S1). FA composition (ARA, EPA, and DHA) data were collected from Pétursdóttir et al. (2008a, 2008b), and Würzberg et al. (2011a, 2011b, 2011c), for polar areas; Lewis (1967), Howell et al. (2003), Hudson et al. (2004), Økland et al. (2005), Drazen et al. (2008a, 2008b), Stowasser et al. (2009), Murdukhovich et al. (2018), Parzanini et al. (2018a), Salvo et al. (2018), van Øevelen et al. (2018), and Kharlamenko et al. (2018), for temperate regions; and Jeffreys et al. (2009) and Shi et al. (2018), for tropical regions (Table S2).

2.2 Statistical analysis

Comparisons among multiple groups of deep-sea organisms were run through t-tests and oneway analysis of variance (ANOVA). In particular, isotopic (i.e. $\delta^{15}N$, $\delta^{13}C$) and FA (i.e. ARA, EPA and DHA) data were compared across organisms from different latitudes (i.e. tropical, temperate and polar), habitats (i.e. pelagic, demersal, benthic), and collection depths (i.e. mesopelagic, bathypelagic, meso-bathypelagic, abyssopelagic, bathyal, bathyal-abyssal, abyssal, and hadal) to detect any significant differences. When the normality assumption was violated, Mann-Whitney rank sum test, Kruskal-Wallis oneway ANOVA on ranks, and Dunn's method pairwise comparisons were performed instead. In addition, multivariate statistics, i.e. principal coordinate analysis (PCO) and permutational MANOVA (PERMANOVA) were used to study the variability in the isotopic and FA composition of deep-water organisms across different latitudes, habitats, collection depths, and phyla. In addition, a distance based linear model (DistLM) was run to assess which of these four factors contributed the most to such a variability. PCO, PERMANOVA, and DistLM were run on resemblance matrices, based on Euclidean distance for the isotopic data, and Bray-Curtis for the FA data. Data were not normalized or transformed prior to analysis. Univariate statistics was conducted using Sigmaplot 12.5, while PCO, PERMANOVA and DistLM were run through Primer 7.0 with the add-on package PERMANOVA+ (Clarke and Gorley, 2006).

2.3 Results

Analyses revealed both latitudinal and depth-related trends for isotopic and essential FA composition. In particular, mean values (±SD) of $\delta^{15}N$ and $\delta^{13}C$ were significantly lower in deep-sea fauna sampled at high latitudes than in that collected at low latitudes ($\delta^{15}N$, ANOVA on Ranks, H = 35.6, $p \leq 0.001$; $\delta^{13}C$, ANOVA on Ranks, H = 277.9, $p \leq 0.001$; Fig. 2). Conversely, no difference was detected across latitudes in terms of ARA, but mean proportions (±SD) of EPA and DHA were significantly greater at polar latitudes than at temperate and tropical areas (EPA, ANOVA on Ranks, H = 11.4, p = 0.003; DHA, ANOVA on Ranks, H = 63.6, $p \leq 0.001$; Fig. 3). Similarly, PERMANOVA

410 detected significant differences across latitudes in terms of both stable isotopes [Pseudo-F = 81.4,
411 $p(perm) = 0.0001$] and essential FA [Pseudo-F =11.0, $p(perm) = 0.0001$].

412 When deep-water species were analyzed separately according to their habitat, the same
413 latitudinal trend in the isotopic composition were shown for deep-water benthic species ($\delta^{15}N$,
414 ANOVA on Ranks, $H = 40.5$, $p \leq 0.001$; $\delta^{13}C$, ANOVA on Ranks, $H = 171.2$, $p \leq 0.001$); whereas,
415 for demersal and pelagic species, only the $\delta^{13}C$ ratios were significantly lower at higher latitudes
416 (ANOVA on Ranks, $H = 105.7$, $p \leq 0.001$, for demersal species; ANOVA on Ranks, $H = 11.5$, $p =$
417 0.003, for pelagic species). PERMANOVA showed that the isotopic composition of deep-sea
418 animals was indeed statistically different across the three habitats [Pseudo-F = 112.6, $p(perm) =$
419 0.0001], and benthic and demersal species had higher stable N and C isotope ratios than the
420 pelagic counterparts ($p < 0.05$). Conversely, only benthic and pelagic species revealed a latitudinal
421 gradient in their essential FA composition (EPA, ANOVA on Ranks, $H = 12.1$, $p = 0.002$; DHA,
422 ANOVA on Ranks, $H = 43.6$, $p \leq 0.001$, for benthic species; EPA, ANOVA, $H = 6.4$, $p = 0.011$, for
423 pelagic taxa). In this regard, pelagic, demersal, and benthic taxa had a different essential FA
424 composition (ARA, ANOVA on Ranks, $H = 39.7$, $p \leq 0.001$; EPA, ANOVA on Ranks, $H = 12.5$, $p =$
425 0.002; DHA, ANOVA on Ranks, $H = 76.9$, $p \leq 0.001$; Pseudo-F =19.7, $p(perm) = 0.0001$). Benthic
426 species had the highest proportions of ARA and EPA ($p < 0.05$); while demersal species had the
427 highest levels of DHA, although similar to those of pelagic species.

428 While mean values of both stable N and C isotope ratios significantly increased with depth
429 for benthic and demersal species ($\delta^{15}N$, ANOVA on Ranks, $H = 63.9$, $p \leq 0.001$; $\delta^{13}C$, ANOVA on
430 Ranks, $H = 126.2$, $p \leq 0.001$), only $\delta^{13}C$ ratios showed the same trend in pelagic taxa (ANOVA on
431 Ranks, $H = 125.5$, $p \leq 0.001$). Proportions of EPA significantly decreased along the bathymetric
432 gradient for pelagic taxa (ANOVA on Ranks, $H = 12.3$, $p = 0.002$), and levels of ARA were
433 significantly higher at abyssal depths for benthic and demersal species (ANOVA on Ranks, $H =$
434 39.7, $p \leq 0.001$). In addition, for benthic and demersal fauna, levels of $\delta^{15}N$, $\delta^{13}C$, and ARA
435 increased for benthic and demersal organisms with increasing depth ($\delta^{15}N$, ANOVA on Ranks, $H =$
436 84.7, $p \leq 0.001$; $\delta^{13}C$, ANOVA on Ranks, $H = 105.0$, $p \leq 0.001$; ARA, ANOVA on Ranks, $H = 22.8$,

437 $p \leq 0.001$). PERMANOVA revealed significant differences in the isotopic [Pseudo-F = 74.6, *p*(*perm*)
438 = 0.0001] and essential FA composition [Pseudo-F = 8.6, *p*(*perm*) = 0.0001] across collection
439 depths.

440 Among the four variables considered (i.e. latitude, habitat, collection depth, and phylum),
441 analyses revealed that 'habitat' and 'phylum' were the most important factors influencing the
442 variability of the stable isotope (respectively 12 and 9%; DistLM, *adjusted* R^2 = 0.4) and FA
443 (respectively 8 and 11%; DistLM, *adjusted* R^2 = 0.3) composition of deep-water organisms (Fig. 4).

444 ## 2.4 Discussion

445 The present analysis shows for the first time, the existence of a) latitudinal trends in both stable
446 isotope and essential FA composition of deep-sea organisms, with decreasing $\delta^{13}C$ ratios and
447 increasing ω3 LC-PUFA towards the poles; b) global bathymetric trends in the isotopic composition
448 of deep-water fauna for which mean levels of $\delta^{15}N$, $\delta^{13}C$, and ARA increased with increasing depth.
449 In addition, it provides further evidence of the link, across latitudes and depth, between surface
450 primary production of the surface waters and the deep-water consumers. The present findings
451 generally align with reports of decreasing values of $\delta^{13}C$ in surface-waters plankton and POM
452 towards the polar regions, in both the southern and northern hemisphere (Sackett et al., 1965; Rau
453 et al., 1982; Francois et al., 1993), as well as of increasing POM isotopic ratios along a bathymetric
454 gradient (Altabet et al., 1999). They also agree with Colombo et al. (2016) who noticed that
455 proportions of ω3 LC-PUFA were higher in marine organisms from polar and temperate regions in
456 comparison to tropical regions, and with Parzanini et al. (2018a) who detected increasing
457 proportions of ARA along a slope area in the deep Northwest Atlantic.

458 Water temperature, in combination with other abiotic (e.g. oceanographic and
459 biogeochemical processes, nutrient supply) and biological factors (e.g. species metabolism,
460 taxonomic composition of deep-water communities, microbial remineralization processes) seems to
461 play a role in these trends (Rau et al., 1982; Francois et al., 1993; Altabet et al., 1999; Colombo et
462 al., 2016). In particular, water temperature influences isotopic fractionation processes and, typically,

463 higher fractionation is associated with lower temperatures (Sackett et al., 1965). High fractionation
464 rates are also linked to the pronounced denitrification activities characterizing oligotrophic areas
465 such as observed in some areas of the tropics (Hetherington et al., 2017). This may explain the
466 higher $\delta^{15}N$ ratios of the deep-sea organisms from the tropical latitudes analyzed in this study.
467 Furthermore, water temperature affects membrane fluidity, and lower temperatures decrease the
468 fluidity of cell membrane (Parrish, 2013; Colombo et al., 2016). Thus, in order to maintain normal
469 membrane function and condition, i.e. health, ectotherms may counteract variations in water
470 temperature by readjusting their FA composition (Cossins and Lee, 1985; Parrish, 2013). For
471 example, larger proportions of long chain unsaturated FA (e.g. ARA, EPA) within the lipid bilayer
472 help increase membrane fluidity (Parrish 2013), as these molecules are characterized by a higher
473 flexibility (DeLong and Yayanos, 1985; Colombo et al., 2016).

474 Trends in the isotopic and FA composition of deep-sea organisms were also seen along a
475 depth gradient. As a proxy for water temperature as well as nutrient supply, depth may influence
476 biochemical composition of marine consumers (Parzanini et al., 2018a, 2018b). POM becomes
477 more isotopically enriched while sinking to deeper depth due to microbial degradation (Altabet et
478 al., 1999). Thus, the isotopic composition of deep-water organisms which feed on POM may vary
479 accordingly (Mintenbeck et al., 2007). In the present analysis, levels of ARA were globally higher at
480 deeper depths, similar to the study by Parzanini et al. (2018a), which may be due to i) a higher
481 reliance of deeper-dwelling organisms on the benthic-detrital trophic pathway; and/or ii) the need to
482 maintain membrane fluidity at low temperatures via increasing the unsaturation levels of membrane
483 phospholipids.

484 Finding latitudinal trends in the biochemical composition of deep-water organisms that
485 mirror results from shallow depths provides further evidence of the link between the two systems, in
486 that deep-sea benthic communities rely on POM sinking from the surface water as a primary food
487 source (Gage, 2003; Hudson et al., 2004). Close dependence of deep-sea food webs on near-
488 surface processes raises important concerns. According to the latest climate estimates, both air
489 and water temperatures have been rising, and continue to increase; and seawater pH has already

dropped by 0.1 units due to large CO_2 emissions, and is expected to decrease further (IPCC, 2017). Furthermore, models predict that increasing surface water temperature will favor stratification, while reducing vertical mixing as well as enhancing variability in the transport of primary production and energy (i.e. carbon) transport to the deep sea (Smith et al., 2009; Jones et al., 2014; Sweetman et al., 2017). At the same time, deep-water benthic biomass is expected to decrease due to the increasing variability in the food supply, which may in turn affect health and functioning of benthic ecosystems, as well as global biogeochemical cycles (Jones et al., 2014). Hixson and Arts (2016) showed that the FA composition of the six most common fresh- and salt-water phytoplankton species responded to temperature and, specifically, that their ω3 PUFA levels decreased with increasing temperature. Not only do ω3 PUFA, such as EPA and DHA, play an important role in the response to temperature variations in aquatic systems, but they are also essential nutrients and are highly required by aquatic organisms for optimal growth and health (Parrish, 2009). A case in point, Rossoll et al. (2012) showed experimentally that growth and reproduction of the copepod *Acartia tonsa* were severely compromised by the alteration of FA content and composition of its primary food source, the diatom *Thalassiosira pseudonana*, exposed to high CO_2 levels. The present investigation, therefore, suggests that changes in amounts and composition of surface production could also result in changes in essential nutrients and biomarkers in deep-sea benthic organisms that feed on it, with possible cascading effects throughout deep-water food webs. Such variations may alter nutrient intake of deep-sea benthic organisms, as well as trophodynamics; and they may also influence species' abilities to cope with deep cold waters.

3 Conclusions

This investigation provides a first summary of the information available on deep-sea food webs inferred by bulk stable isotope and FA analyses, providing guidance for future studies and a glimpse at global-scale patterns in the biochemical composition of deep-water organisms from heterotrophic ecosystems. Food-web tracers represent a powerful tool that can help elucidate the

structure and dynamics of food webs from shallow to deeper waters, and support management initiatives. However, this tool is even more effective when combined with other techniques (e.g. gut content analysis), as each method provides uniquely valuable data. When comparing studies, it emerges that there are multiple sources of variations, whether biological, environmental, and/or analytical. Depending on the scale of the investigation, these differences are more or less susceptible to biases, suggesting that they have to be considered and acknowledged when attempting cross-comparisons even though they may be contextually acceptable. The preliminary analysis conducted here detected latitudinal and bathymetric trends in the isotopic and FA composition of deep-sea species. In light of global climate change and the link between surface production and deep-sea communities, changes in amounts and composition of surface production may influence the essential nutrient intake (e.g. ω3 PUFA) of deep-water organisms. Because ω3 PUFA are involved in the response to temperature variations in ectotherms, climate change may also affect the ability of these species to cope with potential temperature shifts. However, more studies are required to help detect global trends, especially in those areas that are still poorly understood (most deep-sea areas) or not yet investigated (e.g. in the southern hemisphere). In addition, it is necessary to standardize analytical methods to limit their influence and help compensate for natural variability.

Data availability

All data used for analysis can be found as supplementary material, in Table S1 and S2.

Table S1. Dataset applied to analyze trends in the isotopic composition of deep-sea animals.

Table S2. Dataset applied to analyze trends in the essential FA composition of deep-sea animals.

Author contribution

All the authors contributed to the manuscript conceptualization and methodology. CP was responsible of data curation, formal analysis, investigation, and in writing the original draft of the manuscript. CCP, JH, and AM reviewed and edited the draft. Lastly, CCP and AM provided supervision, as well as funds to this project.

Competing interests

The authors declare that they have no conflict of interest.

Acknowledgements

The authors acknowledge the Natural Science and Engineering Research Council of Canada (NSERC) Discovery Grant (Grant no. 311406 to A. Mercier and 105379 to C.C. Parrish) and Canada Foundation for Innovation (CFI) Leaders Opportunity Fund (Grant no. 11231 to A. Mercier) for funding. The authors also want to thank Jeff Drazen, Paul Snelgrove, Patrick Gagnon, E. Montgomery and K. Bøe for providing ideas in the development and improvement of this manuscript, as well as two anonymous reviewers for insightful comments.

References

Altabet, M. A., Pilskaln, C., Thunell, R., Pride, C., Sigman, D., Chavez, F., and Francois, R.: The nitrogen isotope biogeochemistry of sinking particles from the margin of the Eastern North Pacific, Deep-Sea Res. Pt. I, 46, 655-679, https://doi.org/10.1016/S0967-0637(98)00084-3, 1999.

Arrington, D. A., and Winemiller, K. O.: Preservation effects on stable isotope analysis of fish muscle, Trans. Am. Fish Soc., 131, 337-342, https://doi.org/10.1577/1548-8659(2002)131<0337:PEOSIA>2.0.CO;2, 2002.

Badalamenti, F., D'Anna, G., Pinnegar, J., and Polunin, N.: Size-related trophodynamic changes in three target fish species recovering from intensive trawling, Mar. Biol., 141, 561-570, https://doi.org/10.1007/s00227-002-0844-3, 2002.

Bergmann, M., Dannheim, J., Bauerfeind, E., and Klages, M.: Trophic relationships along a bathymetric gradient at the deep-sea observatory HAUSGARTEN, Deep-Sea Res. Pt. I., 56, 408-424, https://doi.org/10.1016/j.dsr.2008.10.004, 2009.

Boyle, M., Ebert, D., and Cailliet, G.: Stable-isotope analysis of a deep-sea benthic-fish assemblage: evidence of an enriched benthic food web, J. Fish Biol., 80, 1485-1507, https://doi.org/10.1111/j.1095-8649.2012.03243.x, 2012.

Bradley, C. J., Wallsgrove, N. J., Choy, C. A., Drazen, J. C., Hetherington, E. D., Hoen, D. K., and Popp, B. N.: Trophic position estimates of marine teleosts using amino acid compound specific isotopic analysis, Limnol. Oceanogr-Meth., 13, 476-493, https://doi.org/10.1002/lom3.10041, 2015.

Bunn, S., Loneragan, N., and Kempster, M.: Effects of acid washing on stable isotope ratios of C and N in penaeid shrimp and seagrass: implications for food-web studies using multiple stable isotopes, Limnol. Oceanogr., 40, 622-625, https://doi.org/10.4319/lo.1995.40.3.0622, 1995.

Carlier, A., Le Guilloux, E., Olu, K., Sarrazin, J., Mastrototaro, F., Taviani, M. and Clavier, J.: Trophic relationships in a deep Mediterranean cold-water coral bank (Santa Maria di Leuca, Ionian Sea), Mar. Ecol. Prog. Ser., 397, 125-137, https://doi.org/10.3354/meps08361, 2009.

Cherel, Y., Fontaine, C., Jackson, G. D., Jackson, C. H., and Richard, P.: Tissue, ontogenic and sex-related differences in $\delta^{13}C$ and $\delta^{15}N$ values of the oceanic squid *Todarodes filippovae* (Cephalopoda: Ommastrephidae), Mar. Biol., 156, 699-708, https://doi.org/10.1007/s00227-008-1121-x, 2009.

Choy, C. A., Popp, B. N., Hannides, C. C., and Drazen, J. C.: Trophic structure and food resources of epipelagic and mesopelagic fishes in the North Pacific Subtropical Gyre ecosystem inferred from nitrogen isotopic compositions, Limnol. Oceanogr., 60(4), 1156-1171, https://doi.org/10.1002/lno.10085, 2015.

Churchill, D. A., Heithaus, M. R., Vaudo, J. J., Grubbs, R. D., Gastrich, K., and Castro, J. I.: Trophic interactions of common elasmobranchs in deep-sea communities of the Gulf of Mexico revealed through stable isotope and stomach content analysis, Deep-Sea Res. Pt. II., 115, 92-102, https://doi.org/10.1016/j.dsr2.2014.10.011,2015.

Clarke, K., and Gorley, R. PRIMER Plymouth, UK: PRIMERE Ltd, 2006.

Collins, M., Bailey, D., Ruxton, G., and Priede, I.: Trends in body size across an environmental gradient: a differential response in scavenging and non-scavenging demersal deep-sea fish, P. Roy. Soc. B-Biol. Sci., 272, 2051-2057, https://doi.org/10.1098/rspb.2005.3189, 2005.

Colombo, S. M., Wacker, A., Parrish, C. C., Kainz, M. J., and Arts, M. T.: A fundamental dichotomy in long-chain polyunsaturated fatty acid abundance between and within marine and terrestrial ecosystems, Environm. Rev., 25, 163-174, https://doi.org/10.1139/er-2016-0062, 2016.

Cossins, A., and Lee, J.: The adaptation of membrane structure and lipid composition to cold, in: Circulation, respiration, and metabolism, edited by Gylles, R., Springer, Berlin, Heidelberg, Germany, 543-552, 1985.

DeLong, E. G., and Yayanos, A. A.: Adaptation of the membrane lipids of a deep-sea bacterium to changes in hydrostatic pressure, Science, 228, 1101-1104, 1985.

DeNiro, M. J., and Epstein, S.: Mechanism of carbon isotope fractionation associated with lipid synthesis, Science, 197, 261-263, 1977.

Drazen, J. C., Phleger, C. F., Guest, M. A., and Nichols, P. D.: Lipid, sterols and fatty acid composition of abyssal holoturians and ophiuroids from the North-East Pacific Ocean: food web implications, Comp. Biochem. Phys. B., 151(1), 79-87, https://doi.org/10.1016/j.cbpb.2008.05.013, 2008a.

Drazen, J. C., Phleger, C. F., Guest, M. A., and Nichols, P. D.: Lipid, sterols and fatty acids of abyssal polychaetes, crustaceans, and a cnidarian from the northeast Pacific Ocean: food web implications, Mar. Ecol. Prog. Ser., 372, 157-167, https://doi.org/10.3354/meps07707, 2008b.

Drazen, J. C., Popp, B. N., Choy, C. A., Clemente, T., Forest, L. D., and Smith, K. L.: Bypassing the abyssal benthic food web: Macrourid diet in the eastern North Pacific inferred from stomach content and stable isotopes analyses, Limnol. Oceanogr., 53, 2644-2654, https://doi.org/10.4319/lo.2008.53.6.2644, 2008c.

Drazen, J. C., Phleger, C. F., Guest, M. A., and Nichols, P. D.: Lipid composition and diet inferences in abyssal macrourids of the eastern North Pacific, Mar. Ecol. Prog. Ser., 387, 1-14. https://doi.org/10.3354/meps08106, 2009.

Eliassen, J. E., and Jobling, M. Food of the roughhead grenadier, *Macrourus berglax*, Lacepede in North Norwegian waters, J. Fish Biol., 26, 367-376, https://doi.org/10.1111/j.1095-8649.1985.tb04276.x, 1985.

Fanelli, E., Cartes, J. E., Rumolo, P., and Sprovieri, M.: Food-web structure and trophodynamics of mesopelagic–suprabenthic bathyal macrofauna of the Algerian Basin based on stable isotopes of carbon and nitrogen, Deep-Sea Res. Pt. I., 56(9), 1504-1520, https://doi.org/10.1016/j.dsr.2009.04.004, 2009.

Fanelli, E., Cartes, J. E., and Papiol, V.: Food web structure of deep-sea macrozooplankton and micronekton off the Catalan slope: insight from stable isotopes, J. Marine Syst., 87, 79-89, https://doi.org/10.1016/j.jmarsys.2011.03.003, 2011a.

Fanelli, E., Papiol, V., Cartes, J. E., Rumolo, P., Brunet, C., and Sprovieri, M.: Food web structure of the epibenthic and infaunal invertebrates on the Catalan slope (NW Mediterranean): evidence from $\delta^{13}C$ and $\delta^{15}N$ analysis, Deep-Sea Res. Pt. I, 58, 98-109, https://doi.org/10.1016/j.dsr.2010.12.005, 2011b.

Fanelli, E., Papiol, V., Cartes, J. E., Rumolo, P., and López-Pérez, C.: Trophic webs of deep-sea megafauna on mainland and insular slopes of the NW Mediterranean: a comparison by stable isotope analysis, Mar. Ecol. Prog. Ser., 490, 199-221, https://doi.org/10.3354/meps10430, 2013.

Francois, R., Altabet, M. A., Goericke, R., McCorkle, D. C., Brunet, C., and Poisson, A.: Changes in the $\delta^{13}C$ of surface water particulate organic matter across the subtropical convergence in

643 the SW Indian Ocean, Global Biogeochem. Cy., 7, 627-644.
644 https://doi.org/10.1029/93GB01277, 1993.

645 FishBase: http://www.fishbase.org, last access: February 2019.

646 Gage, J. D.: Food inputs, utilization, carbon flow and energetics, in: Ecosystems of the deep
647 oceans, edited by Tyler, P. A., Elsevier Science B.V., Amsterdam, The Netherlands, 313-
648 382, 2003.

649 Gale, K. S., Hamel, J-F., and Mercier, A.: Trophic ecology of deep-sea Asteroidea (Echinodermata)
650 from eastern Canada, Deep-Sea Res. Pt. I, 80, 25-36,
651 https://doi.org/10.1016/j.dsr.2013.05.016, 2013.

652 Galván, D., Sweeting, C., and Reid, W.: Power of stable isotope techniques to detect size-based
653 feeding in marine fishes, Mar. Ecol. Prog. Ser., 407, 271-278,
654 https://doi.org/10.3354/meps08528, 2010.

655 Gartner, J. V., Crabtree, R. E., and Sulak, K. J.: Feeding at depth, Fish physiology, 16, 115-193,
656 https://doi.org/10.1016/S1546-5098(08)60229-0, 1997.

657 Hamoutene, D., Puestow, T., Miller-Banoub, J., and Wareham, V.: Main lipid classes in some
658 species of deep-sea corals in the Newfoundland and Labrador region (Northwest Atlantic
659 Ocean), Coral Reefs, 27(1), 237-246, https://doi.org/10.1007/s00338-007-0318-7, 2008.

660 Hetherington, E. D., Olson, R. J., Drazen, J. C., Lennert-Cody, C. E., Balance, L. T., Kaufmann, R.
661 S., and Popp, B. N.: Spatial food-web structure in the eastern tropical Pacific Ocean based
662 on compound-specific nitrogen isotope analysis of amino acids, Limnol. Oceanogr., 62(2),
663 541-560, https://doi.org/10.1002/lno.10443, 2017.

664 Hixson, S. M., and Arts, M. T.: Climate warming is predicted to reduce omega-3, long-chain,
665 polyunsaturated fatty acid production in phytoplankton, Global Change Biol., 22, 2744-2755,
666 https://doi.org/10.1111/gcb.13295, 2016.

667 Hoffman, J. C., and Sutton, T. T.: Lipid correction for carbon stable isotope analysis of deep-sea
668 fishes, Deep-Sea Res. Pt. I, 57, 956-964, https://doi.org/10.1016/j.dsr.2010.05.003, 2010.

669 Howell, K. L., Pond, D. W., Billett, D. S., and Tyler, P. A.: Feeding ecology of deep-sea seastars
670 (Echinodermata: Asteroidea): a fatty-acid biomarker approach, Mar. Ecol. Prog. Ser., 255,
671 193-206, 2003.

672 Hudson, I. R., Pond, D. W., Billett, D. S., Tyler, P. A., Lampitt, R. S., and Wolff, G. A.: Temporal
673 variations in fatty acid composition of deep-sea holothurians: evidence of bentho-pelagic
674 coupling, Mar. Ecol. Prog. Ser., 281, 109-120, https://doi.org/10.3354/meps281109, 2004.

675 Hussey, N., MacNeil, M., Olin, J., McMeans, B., Kinney, M., Chapman, D., and Fisk, A.: Stable
676 isotopes and elasmobranchs: tissue types, methods, applications and assumptions, J. Fish
677 Biol., 80, 1449-1484, https://doi.org/10.1111/j.1095-8649.2012.03251.x, 2012.

678 Iken, K., Brey, T., Wand, U., Voigt, J., and Junghans, P.: Food web structure of the benthic
679 community at the Porcupine Abyssal Plain (NE Atlantic): a stable isotope analysis, Prog.
680 Oceanogr., 50, 383-405, https://doi.org/10.1016/S0079-6611(01)00062-3, 2001.

681 Iken, K., Bluhm, B., and Gradinger, R.: Food web structure in the high Arctic Canada Basin:
682 evidence from $\delta^{13}C$ and $\delta^{15}N$ analysis. Polar Biol.. 28, 238-249,
683 https://doi.org/10.1007/s00300-004-0669-2, 2005.

684 IPCC, sixth assessment Rep., http://www.ipcc.ch/, last access: February 2019, 2017.

685 Iverson, S. J.: Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative
686 determination, in: Lipids in aquatic ecosystems, edited by Arts, M. T., Brett, M. T., and

687 Kainz, M. J., Springer, New York, NY, 281-308, https://doi.org/10.1007/978-0-387-89366-
688 2_12, 2009.

689 Jeffreys, R. M., Wolff, G. A., and Murty, S. J.: The trophic ecology of key megafaunal species at the
690 Pakistan Margin: evidence from stable isotopes and lipid biomarkers, Deep-Sea Res. Pt. I,
691 56, 1816-1833, https://doi.org/10.1016/j.dsr.2009.05.001, 2009.

692 Jones, D. O., Yool, A., Wei, C. L., Henson, S. A., Ruhl, H. A., Watson, R. A., and Gehlen, M.:
693 Global reductions in seafloor biomass in response to climate change, Global Change Biol.,
694 20(6), 1861-1872, https://doi.org/10.1111/gcb.12480, 2014.

695 Kharlamenko, V. I., Brandt, A., Kiyashko, S. I., and Würzberg, L.: Trophic relationship of benthic
696 invertebrate fauna from the continental slope of the Sea of Japan, Deep-Sea Res. Pt. II, 86,
697 34-42, https://doi.org/10.1016/j.dsr2.2012.08.007, 2013.

698 Kharlamenko, V.I., Maiorova, A.S., and Ermolenko, E.V.: Fatty acid composition as an indicator of
699 the trophic position of abyssal megabenthic deposit feeders in the Kuril Basin of the Sea of
700 Okhotsk. Deep-Sea Res. Pt. II, 154, 374-382, https://doi.org/10.1016/j.dsr2.2018.03.005,
701 2018.

702 Kim, S. L., and Koch, P. L.: Methods to collect, preserve, and prepare elasmobranch tissues for
703 stable isotope analysis, Environ. Biol. Fish, 95, 53-63, https://doi.org/10.1007/s10641-011-
704 9860-9, 2012.

705 Kiyashko, S.I., Kharlamenko, V.I., Sanamyan, K., Alalykina, I.L., and Würzberg, L.: Trophic
706 structure of the abyssal benthic community in the Sea of Japan inferred from stable isotope
707 and fatty acid analyses, Mar. Ecol. Prog. Ser., 500, 121-137,
708 https://doi.org/10.3354/meps10663, 2014.

709 Kopp, D., Robert, M., and Pawlowski, L.: Characterization of food web structure of the upper
710 continental slope of the Celtic Sea highlighting the trophic ecology of five deep-sea fishes, J.
711 Appl. Ichthyol., 34(1), 73-80, https://doi.org/10.1111/jai.13544, 2018.

712 Lampitt, R.: Evidence for the seasonal deposition of detritus to the deep-sea floor and its
713 subsequent resuspension. Deep-Sea Res., 32, 885-897, https://doi.org/10.1016/0198-
714 0149(85)90034-2, 1985.

715 Larsen, T., Taylor, D. L., Leigh, M. B., and O'Brien, D. M.: Stable isotope fingerprinting: a novel
716 method for identifying plant, fungal, or bacterial origins of amino acids, Ecology, 90, 3526-
717 3535, https://doi.org/10.1890/08-1695.1, 2009.

718 Lee, R., Nevenzel, J., and Paffenhöfer, G-A. Importance of wax esters and other lipids in the
719 marine food chain: phytoplankton and copepods, Mar. Biol., 9, 99-108,
720 https://doi.org/10.1007/BF00348249, 1971.

721 Lewis, R. W. Fatty acid composition of some marine animals from various depths, J. Fish Res.
722 Board. Can., 24(5), 1101-1115, 1967.

723 Madurell, T., Fanelli, E., and Cartes, J. E.: Isotopic composition of carbon and nitrogen of
724 suprabenthic fauna in the NW Balearic Islands (western Mediterranean), J. Marine
725 Syst., 71(3-4), 336-345, https://doi.org/10.1016/j.jmarsys.2007.03.006, 2008.

726 Martin-Creuzburg, D., and Von Elert, E.: Ecological significance of sterols in aquatic food webs, in:
727 Lipids in aquatic ecosystems, edited by Kainz, M., Brett, M. T., and Arts, M. T., Springer,
728 New York, NY, 43-64. https://doi.org/10.1007/978-0-387-89366-2_3, 2009.

729 Mauchline, J., and Gordon, J.: Diets and bathymetric distributions of the macrourid fish of the
730 Rockall Trough, northeastern Atlantic Ocean, Mar. Biol., 81, 107-121,
731 https://doi.org/10.1007/BF00393109, 1984.

McClelland, J. W., and Montoya, J. P.: Trophic relationships and the nitrogen isotopic composition of amino acids in plankton, Ecology, 83, 2173-2180, 2002.

McConnaughey, T., and McRoy, C.: Food-web structure and the fractionation of carbon isotopes in the Bering Sea, Mar. Biol., 53, 257-262, https://doi.org/10.1007/BF00952434, 1979.

Michener, R. H., and Kaufman, L.: Stable isotope ratios as tracers in marine food webs: an update, in: Stable isotopes in ecology and environmental science, edited by Michener, R., and Lajtha, K., Blackwell Publishing Ltd, Oxford, UK, 238-282, 2007.

Minagawa, M., and Wada, E. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age, Geochim. Cosmochim. Acta, 48, 1135-1140, https://doi.org/10.1016/0016-7037(84)90204-7, 1984.

Mincks, S.L., Smith, C.R., Jeffreys, R.M., and Sumida, P.Y.: Trophic structure on the West Antarctic Peninsula shelf: detritivory and benthic inertia revealed by $\delta^{13}C$ and $\delta^{15}N$ analysis. Deep-Sea Res. Pt. II, 55(22-23), 2502-2514, https://doi.org/10.1016/j.dsr2.2008.06.009, 2008.

Mindel, B. L., Neat, F. C., Trueman, C. N., Webb, T. J., and Blanchard, J. L.: Functional, size and taxonomic diversity of fish along a depth gradient in the deep sea, PeerJ, 4, e2387, https://doi.org/10.7717/peerj.2387, 2016a.

Mindel, B. L., Webb, T. J., Neat, F. C., and Blanchard, J. L.: A trait-based metric sheds new light on the nature of the body size–depth relationship in the deep sea, J. Anim. Ecol., 85(2), 427-436, https://doi.org/10.1111/1365-2656.12471, 2016b.

Mintenbeck, K., Jacob, U., Knust, R., Arntz, W., and Brey, T.: Depth-dependence in stable isotope ratio $\delta^{15}N$ of benthic POM consumers: the role of particle dynamics and organism trophic guild, Deep-Sea Res. Pt. I, 54, 1015-1023, https://doi.org/10.1016/j.dsr.2007.03.005, 2007.

Mordukhovich, V. V., Kiyashko, S. I., Kharlamenko, V. I., and Fadeeva, N. P.: Determination of food sources for nematodes in the Kuril Basin and eastern slope of the Kuril Islands by stable isotope and fatty acid analyses, Deep-Sea Res. Pt. II, https://doi.org/10.1016/j.dsr2.2018.01.003, 2018.

Økland, H. M., Stoknes, I.S., Remme, J. F., Kjerstad, M., and Synnes, M.: Proximate composition, fatty acid and lipid class composition of the muscle from deep-sea teleosts and elasmobranchs, Comp. Biochem. Phys. B, 140(3), 437-43, https://doi.org/10.1016/j.cbpc.2004.11.008, 2005.

Papiol, V., Cartes, J. E., Fanelli, E., and Rumolo, P.: Food web structure and seasonality of slope megafauna in the NW Mediterranean elucidated by stable isotopes: relationship with available food sources, J. Sea Res., 77, 53-69, https://doi.org/10.1016/j.seares.2012.10.002, 2013.

Parrish, C. C., Abrajano, T., Budge, S., Helleur, R., Hudson, E., Pulchan, K., and Ramos, C.: Lipid and phenolic biomarkers in marine ecosystems: analysis and applications, in: Marine chemistry , edited by Wangersky, P., Springer-Verlag, Berlin, Heidelberg, 193-223, 2000.

Parrish, C. C. Essential fatty acids in aquatic food webs, in: Lipids in aquatic ecosystems, edited by Arts, M. T., Brett, M. T., and Kainz, M. J., Springer New York, New York, NY, 309-326, 2009.

Parrish, C. C.: Lipids in marine ecosystems, ISRN Oceanography 2013, https://doi.org/ 10.5402/2013/604045, 2013.

Parzanini, C., Parrish, C. C., Hamel, J-F., and Mercier, A.: Trophic ecology of a deep-sea fish assemblage in the Northwest Atlantic, Mar. Biol., 164, 206, https://doi.org/10.1007/s00227-017-3236-4, 2017.

Parzanini, C., Parrish, C. C., Hamel, J-F., and Mercier, A.: Trophic relationships of deep-sea benthic invertebrates on a continental margin in the NW Atlantic inferred by stable isotope, elemental, and fatty acid composition, Prog. Oceanogr., 168, 279-295, https://doi.org/10.1016/j.pocean.2018.10.007, 2018a.

Parzanini, C., Parrish, C. C., Hamel, J-F., and Mercier, A.: Functional diversity and nutritional content in a deep-sea faunal assemblage through total lipid, lipid class, and fatty acid analyses, PLoS One, 13(11), e0207395, https://doi.org/10.1371/journal.pone.0207395, 2018b.

Pétursdóttir, H., Gislason, A., and Falk-Petersen, S.: Lipid classes and fatty acid compositions of muscle, liver and skull oil in deep-sea redfish *Sebastes mentella* over the Reykjanes Ridge, J. Fish Biol., 73(10), 2485-2496, https://doi.org/10.1111/j.1095-8649.2008.02100.x, 2008a.

Pétursdóttir, H., Gislason, A., Falk-Petersen, S., Hop, H., and Svavarsson, J.: Trophic interactions of the pelagic ecosystem over the Reykjanes Ridge as evaluated by fatty acid and stable isotope analyses, Deep-Sea Res. Pt. II, 55(1-2), 83-93, https://doi.org/10.1016/j.dsr2.2007.09.003, 2008b.

Pfannkuche, O.: Allochthonous deep-sea benthic communities: functioning and forcing, in: Interactions between macro-and microorganisms in marine sediments, edited by Kristensen, E., Ralf, R., and Kostka, E. J., American Geophysical Union, Washington DC, 251-266, 2005.

Pinnegar, J., and Polunin, N. Differential fractionation of $\delta^{13}C$ and $\delta^{15}N$ among fish tissues: implications for the study of trophic interactions, Funct. Ecol., 13, 225-231, https://doi.org/10.1046/j.1365-2435.1999.00301.x, 1999.

Polunin, N., Morales-Nin, B., Pawsey, W., Cartes, J., Pinnegar, J., and Moranta, J.: Feeding relationships in Mediterranean bathyal assemblages elucidated by stable nitrogen and carbon isotope data, Mar. Ecol. Prog. Ser., 220, 13-23, https://doi.org/10.3354/meps220013, 2001.

Post, D. M., Layman, C. A., Arrington, D.A., Takimoto, G., Quattrochi, J., and Montana, C. G.: Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses, Oecologia, 152, 179-189, https://doi.org/10.1007/s00442-006-0630-x, 2007.

Preciado, I., Cartes, J. E., Punzón, A., Frutos, I., López-López, L., and Serrano, A.: Food web functioning of the benthopelagic community in a deep-sea seamount based on diet and stable isotope analyses, Deep-Sea Res. Pt. II, 137, 56-68, https://doi.org/10.1016/j.dsr2.2016.07.013, 2017.

Quiroga, E., Gerdes, D., Montiel, A., Knust, R., and Jacob, U.: Normalized biomass size spectra in high Antarctic macrobenthic communities: linking trophic position and body size. Mar. Ecol. Prog. Ser., 506, 99-113, https://doi.org/10.3354/meps10807, 2014.

Ramirez-Llodra, E., Brandt, A., Danovaro, R., De Mol, B., Escobar, E., German, C., Levin, L., Arbizu, P., Menot, L., and Buhl-Mortensen, P.: Deep, diverse and definitely different: unique attributes of the world's largest ecosystem, Biogeosciences, 7, 2851-2899, https://doi.org/10.5194/bg-7-2851-2010, 2010.

Rau, G. H., and Hedges, J. I.: Carbon-13 depletion in a hydrothermal vent mussel: suggestion of a chemosynthetic food source, Science, 203, 648-649, https://doi.org/10.1126/science.203.4381.648, 1979.

Rau, G. H., Sweeney, R., and Kaplan, I.: Plankton ^{13}C:^{12}C ratio changes with latitude: differences between northern and southern oceans, Deep-Sea Res., 29, 1035-1039, https://doi.org/10.1016/0198-0149(82)90026-7, 1982.

824 Reid, W. D., Wigham, B. D., McGill, R. A., and Polunin, N. V.: Elucidating trophic pathways in
825 benthic deep-sea assemblages of the Mid-Atlantic Ridge north and south of the Charlie-
826 Gibbs Fracture Zone, Mar. Ecol. Prog. Ser., 463, 89-103,
827 https://doi.org/10.3354/meps09863, 2012.

828 Reid, W. D., Sweeting, C. J., Wigham, B. D., McGill, R. A., and Polunin, N. V.: High variability in
829 spatial and temporal size-based trophodynamics of deep-sea fishes from the Mid-Atlantic
830 Ridge elucidated by stable isotopes, Deep-Sea Res. Pt. II, 98, 412-420,
831 https://doi.org/10.1016/j.dsr2.2013.01.020, 2013.

832 Rex, M. A., Stuart, C. T., Hessler, R. R., Allen, J. A., Sanders, H. L., and Wilson, G. D.: Global-
833 scale latitudinal patterns of species diversity in the deep-sea benthos, Nature, 365, 636,
834 https://doi.org/10.1038/365636a0, 1993.

835 Richards, T.M., Gipson, E.E., Cook, A., Sutton, T.T., and Wells, R.D.: Trophic ecology of meso-and
836 bathypelagic predatory fishes in the Gulf of Mexico. ICES J. Mar. Sci, 76(3), 662-672,
837 https://doi.org/10.1093/icesjms/fsy074, 2019.

838 Rieger, R. M.: The biphasic life cycle—a central theme of metazoan evolution, Am. Zool., 34, 484-
839 491, https://doi.org/10.1093/icb/34.4.484, 1994.

840 Rossoll, D., Bermúdez, R., Hauss, H., Schulz, K. G., Riebesell, U., Sommer, U., and Winder, M.:
841 Ocean acidification-induced food quality deterioration constrains trophic transfer, PLoS One,
842 7, e34737, https://doi.org/10.1371/journal.pone.0034737, 2012.

843 Sackett, W. M., Eckelmann, W. R., Bender, M. L., and Bé, A. W.: Temperature dependence of
844 carbon isotope composition in marine plankton and sediments, Science, 148, 235-237,
845 https://doi.org/10.1126/science.148.3667.235, 1965.

846 Saito, H., and Osako, K.: Confirmation of a new food chain utilizing geothermal energy: unusual
847 fatty acids of a deep-sea bivalve, *Calyptogena phaseoliformis*, Limnol. Oceanogr., 52, 1910-
848 1918, https://doi.org/10.4319/lo.2007.52.5.1910, 2007.

849 Salvo, F., Hamoutene, D., Hayes, V. E. W., Edinger, E. N., and Parrish, C. C.: Investigation of
850 trophic ecology in Newfoundland cold-water deep-sea corals using lipid class and fatty acid
851 analyses, Coral Reefs, 37, 157-171, https://doi.org/10.1007/s00338-017-1644-z, 2018.

852 Sherwood, O. A., Jamieson, R. E., Edinger, E. N., and Wareham, V. E.: Stable C and N isotopic
853 composition of cold-water corals from the Newfoundland and Labrador continental slope:
854 examination of trophic, depth and spatial effects, Deep-Sea Res. Pt. I, 55, 1392-1402,
855 https://doi.org/10.1016/j.dsr.2008.05.013, 2008.

856 Shi, L., Xiao, W., Liu, Z., Pan, B., and Xu, Y.: Diet change of hadal amphipods revealed by fatty
857 acid profile: a close relationship with surface ocean. Mar. Environ. Res., 142, 250-256,
858 https://doi.org/10.1016/j.marenvres.2018.10.012, 2018.

859 Shine, R.: Ecological causes for the evolution of sexual dimorphism: a review of the evidence, Q.
860 Rev. Biol., 64, 419-461, 1989.

861 Shipley, O. N., Brooks, E. J., Madigan, D. J., Sweeting, C. J., and Grubbs, R. D.: Stable isotope
862 analysis in deep-sea chondrichthyans: recent challenges, ecological insights, and future
863 directions, Rev. Fish Biol. Fisher., 27(3), 481-497, https://doi.org/10.1007/s11160-017-9466-
864 1, 2017.

865 Smith, C. R., and Baco, A. R.: Ecology of whale falls at the deep-sea floor, Oceanogr. Mar. Biol.
866 Ann. Rev., 41, 311-354, 2003.

867 Smith, K. L., Ruhl, H. A., Bett, B. J., Billett, D. S., Lampitt, R. S., and Kaufmann, R. S.: Climate,
868 carbon cycling, and deep-ocean ecosystems, PNAS, 106(46), 19211-19218,
869 https://doi.org/10.1073/pnas.0908322106, 2009.

Stowasser, G., McAllen, R., Pierce, G., Collins, M., Moffat, C., Priede, I., and Pond, D. W.: Trophic position of deep-sea fish—assessment through fatty acid and stable isotope analyses, Deep-Sea Res. Pt. I, 56, 812-826, https://doi.org/10.1016/j.dsr.2008.12.016, 2009.

Stuart, C. T., Rex, M., and Etter, R. J.: Large-scale spatial and temporal patterns of deep-sea benthic species diversity, in: Ecosystems of the deep oceans, edited by Tyler, P. A., Elsevier, The Netherlands, 295-312, 2003.

Sulzman, E. W.: Stable isotope chemistry and measurement: a primer, in: Stable isotopes in ecology and environmental science, edited by Michener, R., and Lajtha, K., Blackwell Publishing Ltd, Oxford, UK, 1-21, 2007.

Sweetman, A. K., Thurber, A. R., Smith, C. R., Levin, L. A., Mora, C., Wei, C. L., Gooday, A. J., Jones, D. O., Rex, M., Yasuhara, M., and Ingels, J.: Major impacts of climate change on deep-sea benthic ecosystem, Elementa-Sci Anthrop., 5, 4, http://doi.org/10.1525/elementa.203, 2017.

Syväranta, J., Martino, A., Kopp, D., Céréghino, R., and Santoul, F.: Freezing and chemical preservatives alter the stable isotope values of carbon and nitrogen of the Asiatic clam (*Corbicula fluminea*), Hydrobiologia, 658, 383-388, https://doi.org/10.1007/s10750-010-0512-4, 2011.

Tecchio, S., van Oevelen, D., Soetaert, K., Navarro, J., and Ramírez-Llodra, E.: Trophic dynamics of deep-sea megabenthos are mediated by surface productivity, PloS One, 8(5), e63796, https://doi.org/10.1371/journal.pone.0063796, 2013.

Trueman, C., Johnston, G., O'Hea, B., and MacKenzie, K.: Trophic interactions of fish communities at midwater depths enhance long-term carbon storage and benthic production on continental slopes, Proc. R. Soc. B, 20140669, https://doi.org/10.1098/rspb.2014.0669, 2014.

Valls, M., Olivar, M. P., de Puelles, M. F., Molí, B., Bernal, A., and Sweeting, C. J.: Trophic structure of mesopelagic fishes in the western Mediterranean based on stable isotopes of carbon and nitrogen, J. Marine Syst., 138, 160-170, https://doi.org/10.1016/j.jmarsys.2014.04.007, 2014a.

Valls, M., Sweeting, C., Olivar, M., de Puelles, M. F., Pasqual, C., Polunin, N., and Quetglas, A.: Structure and dynamics of food webs in the water column on shelf and slope grounds of the western Mediterranean, J. Marine Syst., 138, 171-181, https://doi.org/10.1016/j.jmarsys.2014.04.002, 2014b.

van Oevelen, D., Duineveld, G. C., Lavaleye, M. S., Kutti, T., and Soetaert, K.: Trophic structure of cold-water coral communities revealed from the analysis of tissue isotopes and fatty acid composition, Mar. Biol. Res., 14(3), 287-306, https://doi.org/10.1080/17451000.2017.1398404, 2018.

WoRMS Editorial Board, World Register of Marine Species, available from http://www.marinespecies.org/, last access: February 2019.

Würzberg, L., Peters, J., and Brandt, A.: Fatty acid patterns of Southern Ocean shelf and deep sea peracarid crustaceans and a possible food source, foraminiferans, Deep-Sea Res. Pt. II, 58, 2027-2035, https://doi.org/10.1016/j.dsr2.2011.05.013, 2011a.

Würzberg, L., Peters, J., Flores, H., and Brandt, A.: Demersal fishes from the Antarctic shelf and deep sea: A diet study based on fatty acid patterns and gut content analyses, Deep-Sea Res. Pt. II, 58(19), 2036-2042, https://doi.org/10.1016/j.dsr2.2011.05.012, 2011b.

Würzberg, L., Peters, J., Schüller, M., and Brandt, A.: Diet insights of deep-sea polychaetes derived from fatty acid analyses, Deep-Sea Res. Pt. II, 58, 153-162, https://doi.org/10.1016/j.dsr2.2010.10.014, 2011c.

Xu, J., Yang, Q., Zhang, M., Zhang, M., Xie, P., and Hansson, L-A.: Preservation effects on stable isotope ratios and consequences for the reconstruction of energetic pathways, Aquat. Ecol., 45, 483-492, https://doi.org/10.1007/s10452-011-9369-5, 2011.

Tables

Table 1 Comparison outlining the major strengths and drawbacks of gut content, stable isotope, and FA analysis.

Gut content analysis	Stable isotope analysis	FA analysis
Direct evidence of diet	Indirect evidence of diet (assumption validation required)	Indirect evidence of diet (assumption validation required)
Snap shot of the most recent meal	Integrative over time	Integrative over time
Small sample sizes may lower representativity of diet	Small sample sizes may lower representativity of diet	Small sample sizes may lower representativity of diet
Inter-individual variability can only be accounted for with appropriate sample size	Inter-individual variability minimized due to integrative nature	Inter-individual variability likely but minimized due to integrative nature
Temporal variability can only be accounted for with appropriate sample size	Temporal variability minimized due to integrative nature	Temporal variability minimized due to integrative nature
Partly dependent on sex in cases where there are dietary differences between sexes	Partly dependent on sex in cases where there are dietary differences between sexes	Partly dependent on sex in cases where there are dietary differences between sexes
May be sensitive to body size (e.g. onthogenetic dietary changes)	May be sensitive to body size, whether or not size influences diet	Dependent body size if size affects diet
Species with large stomachs and slow digestion rates are easier to study	Applies to all species, but requires enough material (see below)	Applies to all species, but requires enough material (see below)
The analysis cannot be carried out with empty stomachs	Independent of stomach fullness	Independent of stomach fullness
Digestion rates may bias contents recovered	Independent of digestion process	Independent of digestion process
Small specimens with small stomachs are more difficult to study	Small specimens may have to be pooled, guts included	Small specimens may have to be pooled, guts included
Only gut content is analyzed	Typically applied to target tissues	Typically applied to target tissues
Interpretation is relatively easy, unless food is highly digested, and the evidence obtained cannot be misinterpreted, taxonomically speaking	Data interpretation is complex (post-analysis mathematical corrections are often applied)	Data interpretation is complex (linked to FA biomarkers as food tracers)
Long processing time	Relatively short processing time	Relatively short processing time
Little instrumentation, low cost (unless high resolution scopes are used)	Medium technology, medium/high cost	Medium technology, medium/high cost

922 **Table 2** Sources of variations across studies, distinguished by type (i.e. biological, environmental, analytical).

Biological	Analytical	Environmental
Taxonomy	Sample gear	Depth
Sex	Sample storage	Season
Age	Sample treatment (e.g. Acidification of organisms containing carbonatic anatomical elements; Lipid removal; urea removal)	Primary productivity levels at surface
Size	Mathematical correction (i.e. whether applied and which one)	Latitude
Feeding habits	Tissue type	Temperature
General physiological condition		Ocean region
		Geological feature (e.g. shelf, slope, canyon, plain, trench)

923

924

925 **Table 3** List of trophic ecology studies in deep-sea heterotrophic systems, carried out using stable
926 isotopes (bulk) and lipids (including FA) as food-web tracers. Experimental studies were excluded *a*
927 *priori*. Reference, method(s) applied, latitude, sampling depth, ocean region, and taxa analyzed are
928 reported for each study. Polar latitudes include investigations between 60 - 90° N/S, whereas
929 temperate and tropical latitudes represent studies carried out within 0 - 30° N and 30 - 60° N,
930 respectively. References are ordered according to sampling depth(s).

References	Method(s)	Latitude	Depth (m)	Ocean region	Taxa analyzed
Mintenbeck et al. 2007	Stable isotopes	Polar	50-1600	Weddell Sea (Antarctic)	Benthic bryozoans, cnidarians, crustaceans, echinoderms, echiurans, mollusks, sponges, sipuncules, and tunicates
Quiroga et al. 2014	Stable isotopes	Polar	250-322	Weddell Sea	Benthic annelids, crustaceans, bryozoans, tunicates, cnidarians, echinoderms, molluscs, nemertea worms, sponges and sipunculans.
van Oevelen et al. 2018	Stable isotopes, Lipids	Polar/Temperate	270-850	Trænadjupet Trough (Norwegian continental shelf), Belgica Mounds (Porcupine Seabight)	Cold-water coral communities
Mincks et al. 2008	Stable isotopes	Polar	550-650	Bellinghausen Sea	Benthic annelids, cnidarians, echinoderms, molluscs, sponges, and demersal fish
Würzberg et al. 2011a	Lipids	Polar	600-5337	Weddell Sea (Antarctic)	Shelf and deep-sea peracarid crustaceans + foraminiferans
Würzberg et al. 2011b	Lipids, Gut contents	Polar	600-2150	Weddell Sea (Antarctic)	Demersal fish
Würzberg et al. 2011c	Lipids	Polar	600-5337	Weddell Sea (Antarctic)	Shelf and deep-sea polychaetes
Iken et al. 2005	Stable isotopes	Polar	800-2082	High Arctic Canadian Basin	Benthic cnidarians, crustaceans, echinoderms, echiurans, mollusks, and polychaetes; pelagic crustaceans
Pétursdóttir et al. 2008a	Stable isotopes, Lipids	Polar	1000-2000	Reykjanes Ridge (North Atlantic)	Mesopelagic crustaceans and fish
Pétursdóttir et al. 2008b	Stable isotopes, Lipids	Polar	1000-2001	Reykjanes Ridge (North Atlantic)	Mesopelagic crustaceans and fish
Bergmann et al. 2009	Stable isotopes	Polar	1300-5600	HAUSGARTEN observatory, west Svalbard (Arctic)	Benthic cnidarians, crustaceans, echiurans, echinoderms, mollusks, nemertean worms, polychaetes, priapulids, sponges, and tunicates; Demersal fish

Valls et al. 2014a	Stable isotopes	Temperate	40-400	Balearic Basin (western Mediterranean)	Mesopelagic fish and zooplankton
Sherwood et al. 2008	Stable isotopes	Temperate	47-1433	Northwest Atlantic	Cold-water corals
Hamoutene et al. 2008*	Lipids	Temperate	50-1500	Cape Chidley, and southern Grand Bank (Northwest Atlantic)	Cold-water corals
Boyle et al. 2012	Stable isotopes, Gut contents	Temperate	55-1280	eastern North Pacific	Benthic cnidarians, crustaceans, echinoderms, and mollusks; polychaetes; demersal fish
Polunin et al. 2001	Stable isotopes	Temperate	200-1800	Balearic Basin (western Mediterranean)	Demersal fish
Valls et al. 2014b	Stable isotopes	Temperate	250-850	Balearic Basin (western Mediterranean)	Hyperbenthic echinoderms and hyperbenthic/pelagic crustaceans, elasmobranchs and mollusks
Gale et al. 2013	Stable isotopes, Gut contents	Temperate	258-1418	Northwest Atlantic	Echinoderms
Carlier et al. 2009	Stable isotopes	Temperate	300-1100	Ionian Sea (central Mediterranean)	Cold-water coral community
Parzanini et al. 2018a	Stable isotopes, Lipids, Elemental	Temperate	310-1413	Northwest Atlantic	Slope cnidarians, crustaceans, echinoderms, fish, mollusks, sponges and tunicates
Parzanini et al. 2018b	Lipids	Temperate	310-1413	Northwest Atlantic	Slope cnidarians, crustaceans, echinoderms, fish, mollusks, sponges and tunicates
Parzanini et al. 2017	Stable isotopes, Gut contents, Morphometrics	Temperate	310-1413	Northwest Atlantic	Pelagic and demersal fish
Madurell et al. 2008	Stable isotopes	Temperate	350-780	Balearic Basin (western Mediterranean)	Suprabenthic crustaceans and fish
Kopp et al. 2018	Stable isotopes	Temperate	415-516	Celtic Sea (Northeast Atlantic)	Epifaunal crustaceans, mollusks, and fish
Papiol et al. 2013	Stable isotopes	Temperate	423-1175	Balearic Basin (western Mediterranean)	Benthopelagic crustaceans
Fanelli et al. 2013	Stable isotopes	Temperate	445-2198	Balearic Basin (western Mediterranean)	Slope crustaceans and mollusks
Økland et al. 2004	Lipids	Temperate	500-1600	Porcupine Bank and western continental slope (Northeast Atlantic)	Demersal fish
Trueman et al. 2014	Stable isotopes	Temperate	500-1500	Hatton Bank (Northeast Atlantic)	Demersal fish
Kharlamenko et al. 2013	Stable isotopes, Lipids	Temperate	500-1600	Sea of Japan	Echinoderms and mollusks
Preciado et al. 2017	Stable isotopes, Gut contents	Temperate	625-1800	Galicia Bank (Northeast Atlantic)	Demersal fish and pelagic/demersal crustaceans
Fanelli et al. 2009	Stable isotopes	Temperate	650-780	Algerian Basin (western Mediterranean)	Mesopelagic crustaceans and fish; benthic crustaceans
Fanelli et al. 2011a	Stable isotopes, Gut contents	Temperate	650-800	Balearic Basin (western Mediterranean)	Zooplankton and micronekton
Fanelli et al. 2011b	Stable isotopes	Temperate	650-1000	Balearic Basin (western Mediterranean)	Epibenthic/infaunal nemertin worms, polychaetes, sipuncules, mollusks, crustaceans, echinoderms

Salvo et al. 2017	Lipids	Temperate	770-1370	Northwest Atlantic	Cold water corals
Stowasser et al. 2009	Stable isotopes, Lipids, Gut contents	Temperate	785-4814	Porcupine Seabight and Abyssal Plain (Northeast Atlantic)	Moridae and Macrouridae fish
Hudson et al. 2004	Lipids	Temperate	800-4850	Porcupine Seabight and Abyssal Plain (Northeast Atlantic)	Holoturoids
Howell et al. 2003	Lipids	Temperate	1053-4840	Porcupine Abyssal Plain (Northeast Atlantic)	Asteroids
Tecchio et al. 2013	Stable isotopes	Temperate	1200-3000	Mediterranean Sea (western + central + eastern)	Zooplankton
Reid et al. 2012	Stable isotopes	Temperate	2400-2750	Mid-Atlantic Ridge (North Atlantic)	Benthic cnidarians, crustaceans, echinoderms,
Reid et al. 2013	Stable isotopes	Temperate	2404-2718	Mid-Atlantic Ridge (North Atlantic)	Deep-sea fish
Kiyashko et al. 2014	Stable isotopes	Temperate	2481-3666	Sea of Japan	Benthic annelids, crustaceans, ascidians, cnidarians, echinoderms, molluscs and sponges
Mordukhovich et al. 2018	Lipids	Temperate	3352-4722	Sea of Okhotsk and Pacific Ocean	Deep-sea macro-benthic nematodes
Kharlamenko et al. 2018	Lipids	temperate	>4000	Sea of Okhotsk	Benthic annelids, echinoderms, molluscs, and sipunculans
Drazen et al. 2008a	Lipids	Temperate	4100	eastern North Pacific	Ophiuroids and holoturoids
Drazen et al. 2008b	Lipids	Temperate	4100	eastern North Pacific	Cnidarians, polychaetes and crustaceans, demersal and pelagic crustaceans and fish
Drazen et al. 2008c*	Stable isotopes, Gut contents	Temperate	4100	eastern North Pacific	Macrourid fish
Drazen et al. 2009	Lipids	Temperate	4100	eastern North Pacific	Macrourid fish and cephalopods
Iken et al. 2001	Stable isotopes	Temperate	4840	Porcupine Abyssal Plain (Northeast Atlantic)	Demersal/Benthic cnidarians, crustaceans, echinoderms, echiurans, fish, mollusks, nematodes, polychaetas, sipuncules, and tunicates
Lewis, 1967	Lipids	Tropical	0-4000	Off San Diego and Baja California (eastern Pacific)	Demersal and pelagic crustaceans and fish
Jeffreys et al. 2009	Stable isotopes, Lipids	Tropical	140-1400	Arabian Sea	Crustaceans, cnidarians, and echinoderms
Churchill et al. 2015	Stable isotopes, Gut contents	Tropical	250-1200	south-central Gulf of Mexico, off Florida to Louisiana (western Atlantic)	Elasmobranchs
Shipley et al. 2017	Stable isotopes	Tropical/Polar	472-1024	Exuma Sound (The Bahamas), Lancaster Sound (Canadian Arctic)	Elasmobranchs
Richards et al. 2019	Stable isotopes	Tropical	1000-3000	Gulf of Mexico	Meso-bathypelagic fish
Shi et al. 2018	Lipids	Tropical	>6000 m	Pacific Ocean	Benthic amphipods

*The study was excluded from analyses because it did not meet the criteria outlined in Sect. 2.1.1 or did not include any data.

931

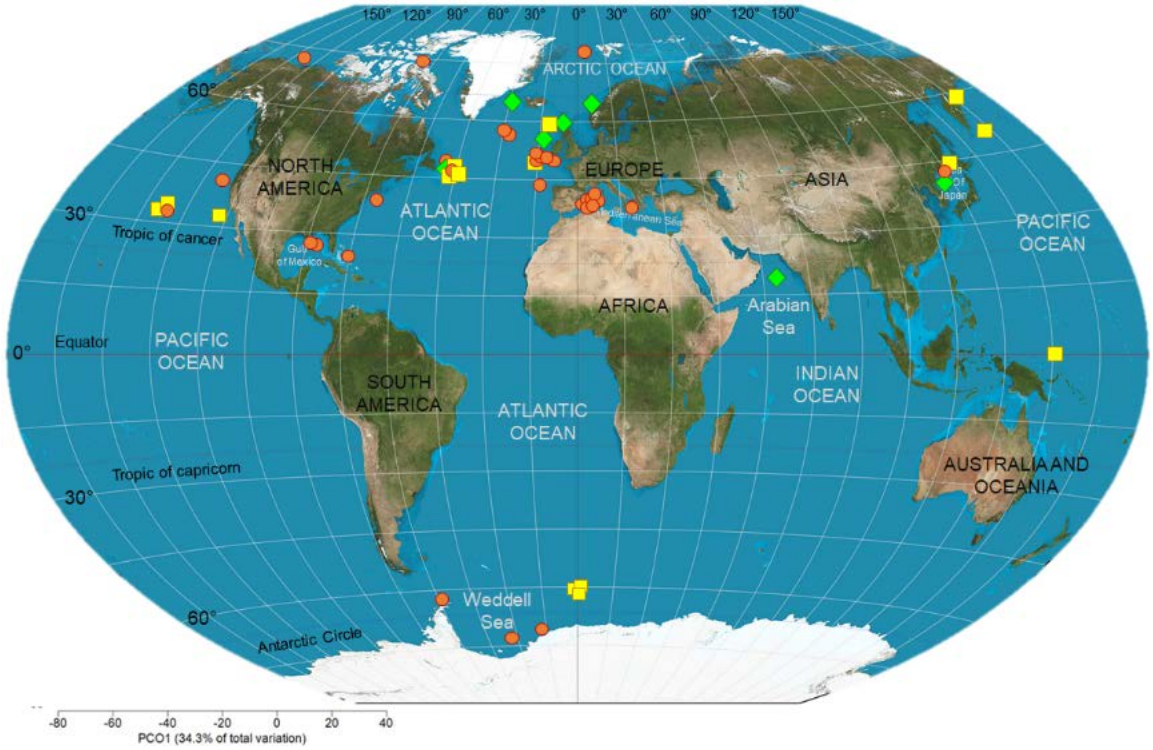
Figures caption

Fig. 1. Deep-sea biomarker studies in the world ocean. Symbols indicate where the studies listed in Table 2 have been carried out. In detail, red circles represent those investigations that have used stable isotopes as food web tracers; whereas yellow squares and green diamonds indicate those which used lipids and a combination of SIA and FA analysis, respectively.

Fig. 2. Stable N and C isotopic composition of deep-sea animals across latitudes. Mean values of $\delta^{15}N$ (blue circles above) and $\delta^{13}C$ (orange circles below) (‰) measured in deep-sea organisms across polar, temperate, and tropical latitudes. Bars represent standard deviation (polar, n = 235; temperate, n = 1469; tropical, n = 41).

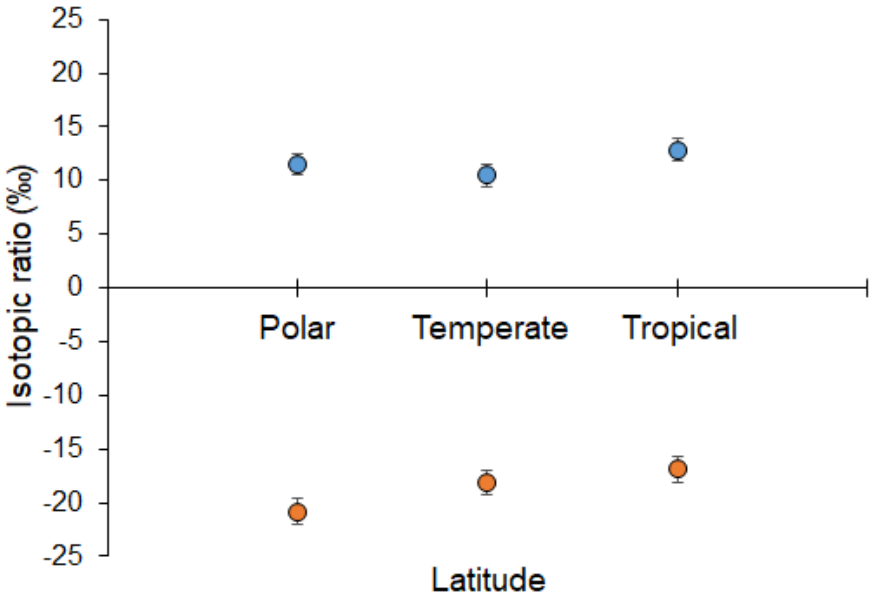
Fig. 3. Essential FA composition of deep-sea animals across latitudes. Mean proportions of essential FA measured in the tissues of deep-sea animals from polar (blue bars), temperate (orange diagonal striped bars), and tropical (green vertical striped bars) latitudes. Bars represent standard deviation (polar, n = 176; temperate, n = 227; tropical, n = 11).

Fig. 4. Differences in terms of biochemical compositions among deep-sea animals from various habitats. Principal coordinate analysis plots representing differences in terms of isotopic (above) and essential FA composition (below) of deep-water species. In both cases, the variable 'habitat' resulted one of the most important factors, contributing 12 and 8% respectively to the variability in the biochemical composition of the deep-sea species.

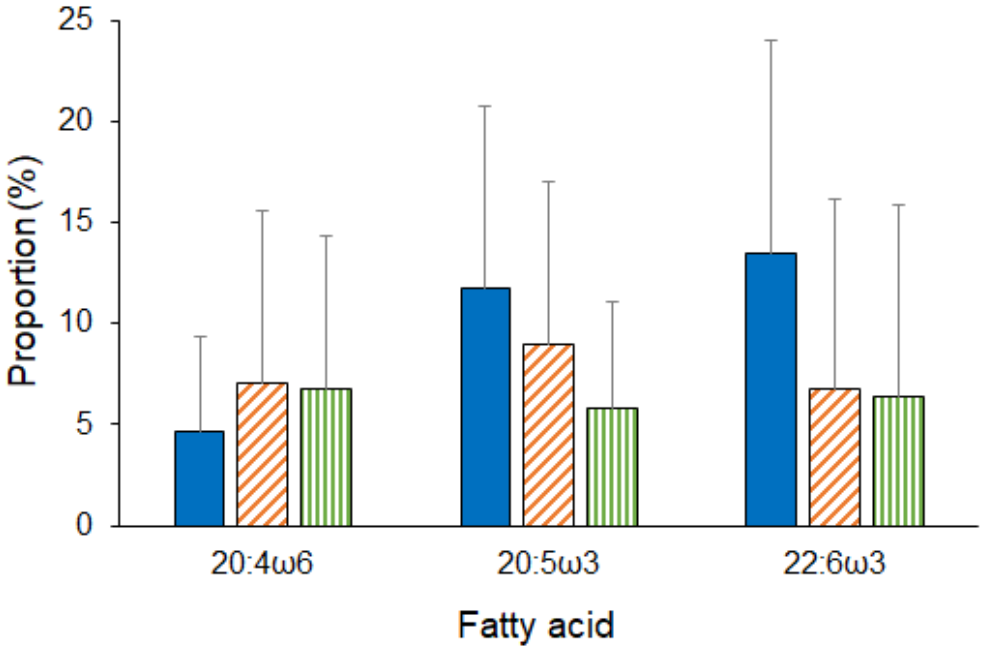

150° 120° 90° 60° 30° 0° 30° 60° 90° 120° 150°
ARCTIC OCEAN
60°
NORTH AMERICA
EUROPE
ASIA
ATLANTIC OCEAN
30°
Tropic of cancer
Gulf of Mexico
PACIFIC OCEAN
AFRICA
Arabian Sea
0°
Equator
PACIFIC OCEAN
SOUTH AMERICA
ATLANTIC OCEAN
INDIAN OCEAN
Tropic of capricorn
30°
AUSTRALIA AND OCEANIA
Weddell Sea
60°
Antarctic Circle
-80 -60 -40 -20 0 20 40
PCO1 (34.3% of total variation)

951 **Fig. 1.**

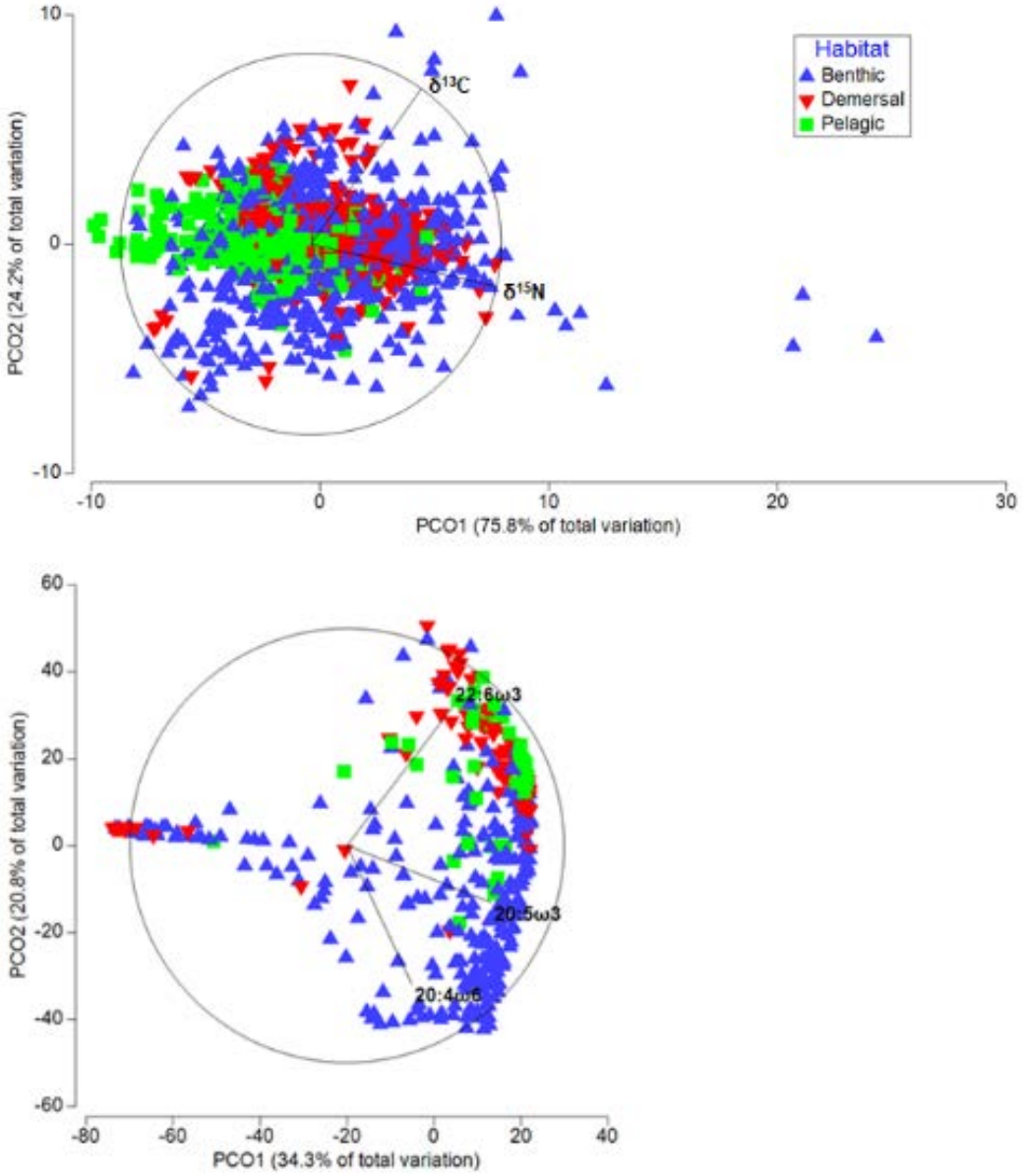
952


25
20
15
10
5
0
-5
-10
-15
-20
-25
Isotopic ratio (‰)
Polar
Temperate
Tropical
Latitude

953 **Fig. 2.**



954 **Fig. 3.**


Habitat
Benthic
Demersal
Pelagic
δ13C
δ15N
PCO2 (24.2% of total variation)
PCO1 (75.8% of total variation)
22:6ω3
20:5ω3
20:4ω6
PCO2 (20.8% of total variation)
PCO1 (34.3% of total variation)

955 **Fig. 4.**