

Response to Anonymous Reviewers

Reviewer#1

COMMENT: The manuscript “Insights into deep-sea food webs and global environmental gradients revealed by stable isotopes ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$) and fatty acids trophic biomarkers” is the first attempt to summarize data on the use of stable isotopes and fatty acids as trophic markers for deep-sea ecosystems. The authors thoroughly analyze the practical aspects of application of these methods and suggest using the standardized methods in order to generate more reliable global predictions. Almost all currently available information was presented in this analysis. In general, the authors have shown convincingly the variations in fatty acid composition and isotope ratio of marine animals along the latitudinal and bathymetric gradients. The main drawbacks of this study are not directly related to the authors’ efforts and are associated with the scarcity of studies in the polar and, especially, tropical regions. Therefore, data on the tropical region can be considered as preliminary. I think that this manuscript is appropriate for publication by the “Biogeosciences” and should be of high interest to ecologists.

REPLY: Many thanks to the anonymous referee #1 for the positive feedback suggesting the publication of this study. Specifically, my co-authors and I are glad the referee #1 was able to recognize the value of our study, while understanding its caveats derived from the limited number of investigations in certain areas of the globe.

Reviewer#2

COMMENT 1: The manuscript “Reviews and syntheses: Insights into deep-sea food webs and global environmental gradients revealed by stable isotopes (15N, 13C) and fatty acids trophic biomarkers” is an important contribution to the field of trophic ecology of deep-sea ecosystems. I like table 1, where the authors compared in detail the advantages and disadvantages of gut content, SI and FA analyses, and I highly appreciate the attempt to assess potential latitudinal and bathymetric trends in SI and FA.

REPLY: My coauthors and I would like to thank you for the insightful feedback and suggestions.

COMMENT 2: I know that the dataset is very sparse, but apparently the keywords, the authors used, did not identify all literature published about this topic. I therefore listed several papers that should be included into the data analysis to increase its explanatory power. Gontikaki et al. 2011 (Deep-Sea Research I), Jeffreys et al. 2013 (Plos One) (mainly a tracer study, but it also includes natural abundance SI data), Jeffreys et al. 2015 (Biogeosciences), Kiyashko et al. 2014 (MEPS), Levin et al. 1999 (MEPS) (tracer study, but also natural abundance SI data), Lin et al. 2014 (MEPS), Mincks et al. 2008 (Deep-Sea Research II), Moens et al. 2007 (Polar Biology), Quiroga et al. 2014 (MEPS), Sweetman & Witte 2008 (Deep-Sea Research I) (tracer study, but also natural abundance SI data), Veit-Köhler et al. 2013 (Progress in Oceanography). There are likely more papers published, but these were the ones that came to my mind. Since the selection process for these papers does not follow the procedure described in the manuscript, the authors could include them under the term ‘additional sources’.

REPLY: Thank you for providing new references that our previous search did not find. All the suggestions provided were carefully examined. While we used data from a number of them, some studies were not considered relevant to our analysis as they were either experimental (e.g. Levin et al. 1999, Gontikaki et al. 2011) or dealt with meiofauna (e.g. Veit-Köhler et al 2013)/foraminifera (Jeffreys et al. 2015). In addition, a few new studies were found after conducting a final search; therefore, 6 more studies were added to our data set for analysis. Results from updated statistical analyses are presented,

along with updated version of Fig. 1, 2, and 3; nonetheless, conclusions have remained unchanged.

COMMENT 3: I also miss information about the geological feature, i.e., whether samples were taken in canyons, at open slopes, in plains, etc. I assume that especially in canyons the SI composition of detritus that reaches the seafloor will be very different from plains at similar depth due to the faster transport of detritus down the canyon. This factor should also be investigated in the statistical analysis.

REPLY: While this comment was pertinent and valuable, we eventually decided not to run new statistical analyses considering varying geological features because our primary goal was to assess variations at a global spatial scale, as it has been stated a few times throughout the text (e.g. see lines 48, 334, 367 of edited manuscript). Including 'Geographical feature' as a variable would have narrowed down our focus. Indeed, for the same aforementioned reason, we had originally excluded other variables, whether environmental (e.g. season) or biological (e.g. size, trophic group), from our assessment. Furthermore, by including the 'Geological feature' parameter to our analysis, all the studies conducted in pelagic habitats would have been automatically excluded, thus reducing sample size. In addition, the records would have been biased towards areas more commonly represented (e.g. slope) than others (e.g. ridges, trenches). Nonetheless, we added this additional source of variation (Environmental) in Table 2.

COMMENT 4: The authors could mention earlier in the study that they explicitly excluded chemosynthetic studies. I know that it is mentioned in the MM section, but when I started reading the manuscript, I quickly went to the supplement to see which studies were included and I missed the chemosynthetic studies. Of course, this is absolutely related to the way I read the paper, but I could imagine that I am not the only one and that other readers would also like to know already in the abstract (or at the end of the introduction), that chemosynthetic studies were not included.

REPLY: Agreed. This information has been added in the Abstract (line 7 of edited manuscript), Introduction (line 47), as well as the Materials and Methods (line 339) and Conclusions (lines 514-515).

COMMENT 5: Table 1: The authors stated that the interpretation of gut content analysis is relatively easily, and that prey items cannot be taxonomically misidentified. I disagree here, as I think that it depends on the grade of digestion: Strongly digested prey items might not be identifiable.

REPLY: We completely agree with this comment. Depending on the digestion level, it is more or less possible to identify a prey item. The sentence in the Table has been adjusted accordingly.

COMMENT 6: Minor technical things in table 1: There is a 'may' missing in line 3 of gut content analysis. It should be 'Small sample sizes may lower representativity of diet'.

REPLY: Added.

COMMENT 7: Please spell out tech and med. Do you mean technology or technique, methods, or something else?

REPLY: Both terms have been spelled out accordingly in Table 1.

COMMENT 8: Fig. 2 and 3: The author reported the sample size as $n = 33-1470$ and $n = 7-212$, respectively. I suggest reporting the sample size per latitude instead of a range. This helps the reader to interpret the results and see where the data are specifically sparse.

REPLY: Samples sizes in Fig. 2 and 3 have now been reported by latitude, as suggested.

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

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1 **Abstract.** Biochemical markers developed initially for food-web studies of terrestrial and shallow-
2 water environments have only recently been applied to deep-sea ecosystems (i.e. in the early
3 2000s). For the first time since their implementation, this review took a close look at the existing
4 literature in the field of deep-sea trophic ecology to synthesize current knowledge. Furthermore, it
5 provided an opportunity for a preliminary analysis of global geographic (i.e. latitudinal, along a
6 depth gradient) trends in the isotopic ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$) and fatty acid composition of deep-sea
7 taxa-macro- and megafauna from heterotrophic systems. Results revealed significant relationships
8 along the latitudinal and bathymetric gradients. Deep-sea animals sampled at temperate and polar
9 latitudes displayed lower isotopic ratios and greater proportions of essential ω 3 long-chain
10 polyunsaturated fatty acids (LC-PUFA) than did tropical counterparts. Furthermore, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$
11 ratios as well as proportions of arachidonic acid increased with increasing depth. Since similar
12 latitudinal trends in the isotopic and fatty acid composition were found in surface water
13 phytoplankton and particulate organic matter, these results highlight the link across latitudes
14 between surface primary production and deep-water communities. Because global climate change
15 may affect quantity and quality (e.g. levels of essential ω 3 PUFA) of surface primary productivity,
16 and by extension those of its downward flux, the dietary intake of deep-sea organisms may likely be
17 altered. In addition, because essential ω 3 PUFA play a major role in the response to temperature
18 variations, climate change may interfere with the ability of deep-sea species to cope with potential
19 temperature shifts. Importantly, methodological disparities were highlighted that prevented in-depth
20 analyses, indicating that further studies should be conducted using standardized methods in order
21 to generate more reliable global predictions.

22 **1 Introduction**

23 **1.1 Historical background of biochemical biomarkers in deep-sea food-web studies**

24 While the use of biochemical biomarkers in marine food-web studies has a long and successful
25 tradition in shallow-water ecosystems, starting from the 1970s with the use of stable isotopes
26 (McConnaughey and McRoy, 1979) and lipids (Lee et al., 1971), their application in deep-water
27 environments is relatively new (e.g. Iken et al., 2001; Polunin et al., 2001; Howell et al., 2003).
28 Undoubtedly, technological advances made over the past few decades have allowed the
29 exploration of ever deeper ecosystems with more refined techniques. Iken et al. (2001) were
30 among the first to provide thea comprehensive analysis of a deep-sea food web, which was
31 sampled at a depth of ~4840 m at the Porcupine Abyssal Plain (PAP, Northeast Atlantic), by using
32 bulk stable N and C isotope ratios ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ respectively) as trophic markers. In the same
33 year, Polunin et al. (2001) used the same approach to study the trophic relationships of a slope
34 megafaunal assemblage collected off the Balearic Islands (western Mediterranean). Since these
35 first two investigations, several others have been carried out across different oceanic regions and
36 climes, such as the Canadian Arctic (Iken et al., 2005), the Arabian Sea (Jeffreys et al., 2009), and
37 the Sea of Japan (Kharlamenko et al., 2013). Furthermore, over the past decade, it has become
38 evident that the simultaneous use of different trophic markers (e.g. $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and fatty acids, FA)
39 and techniques (e.g. bulk or compound specific isotope analysis, as well as FA, gut content and
40 morphometric analyses) provides a more complete picture of trophic structure and dynamics.
41 Indeed, while the first investigations relied on a single method (Iken et al., 2001; Polunin et al.,
42 2001; Howell et al., 2003), the latest trend in deep-sea food-web studies favours an integrative
43 approach, which maximizes the efficiency of each technique, while increasing the resolution of the
44 investigation (e.g. Stowasser et al., 2009; Parzanini et al., 2017).

45 For the first time since the implementation of trophic markers in studies of deep-sea food
46 webs two decades ago, this review synthesizes current knowledge in this growing field of research.

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

54 **1.2 Comparison of major trophic markers**

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

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

80 The principle behind the use of food-web tracers is that the biochemical signature of
81 consumers reflects that of their diet. Among them, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ are the most popular. While the
82 former is used to study trophic positions and dietary sources, with an enrichment factor of 2-4‰
83 between a consumer and its food (Minagawa and Wada, 1984); the latter undergoes little
84 fractionation (<1‰) and, therefore, is used to distinguish primary food sources (McConnaughey
85 and McRoy, 1979). For further details, refer to Sulzman (2007) and Michener and Kaufman (2007)
86 who have provided extensive reviews on the chemistry behind stable isotopes and their use as
87 food-web tracers, respectively. In addition, sterols, FA and amino acids, which are important
88 constituents of lipids (for the former two) and proteins (for the latter), have successfully been used
89 to study trophic relationships and dietary sources in deep-water systems (Howell et al., 2003;
90 Drazen et al. 2008a, 2008b). Their use is based on the principle that certain FA and amino acids
91 are considered essential for animals, being required for optimal fitness. However, most species
92 cannot synthesize these essential compounds *de novo* and, therefore, they must gain them through
93 their diet. Indeed, only primary producers and a few consumers possess the enzymatic apparatus
94 to synthesize essential FA and amino acids *de novo*. Conversely, a few taxa are unable to
95 synthesize sterols *de novo*, which are critical for them; therefore, they have to acquire these
96 essential sterols through diet (Martin-Creuzburg and Von Elert, 2009). Because sterols, FA, and
97 amino acids undergo little or no alteration when consumed, it is possible to detect dietary sources
98 within the consumers' tissues (Parrish et al., 2000). The isotopic signature of amino acids can also
99 be used to study trophic position through compound specific analysis ($\delta^{15}\text{N}$), 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 $\delta^{15}\text{N}$. 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 $\delta^{15}\text{N}$ increases, reflecting the preferential
140 assimilation of the lighter isotope, ^{14}N by microbes; in particular, a gradient in POM $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$, and predator/scavengers were not affected. In another study, Sherwood et al. (2008) did not
151 detect any relationships with depth in the $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$. In fact, small-sized particles sink at a lower velocity and,
157 therefore, experience high rates of degradation, with more evident changes in $\delta^{15}\text{N}$ (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 $\delta^{13}\text{C}$ values), but they were located at different trophic levels (i.e. exhibiting a wide range of $\delta^{15}\text{N}$).
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

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

192 **1.4 Sources of variation across studies**

193 When comparing studies relying on biochemical analysis, there are numerous sources of variation,
194 which may influence results and findings, and also prevent the detection of similarities and general
195 trends. However, their importance may depend on the scale of the investigation (i.e. local, regional,
196 or global). In this section, the main sources of variation are illustrated and explained by type (Table
197 2).

198 **1.4.1 Biological sources**

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

205 benthopelagic origin (Mauchline and Gordon, 1984; Eliassen and Jobling, 1985). Stowasser et al.
206 (2009) combined stable isotope analysis (SIA) and FA analysis to detect ontogenetic shifts in the
207 diet of the fish *Coryphaenoides armatus* and *Antimora rostrata*, collected at depths between 785
208 and 4814 m at PAP (Northeast Atlantic). By looking at their biochemical composition, the two
209 species switched from active predation to scavenging with increasing size. Similar results are
210 reported in Drazen et al. (2008c) for macrourid fish species from the eastern North Pacific.
211 Conversely, although Reid et al. (2013) detected size-related trends in the $\delta^{13}\text{C}$ of deep-water fish
212 collected from the Mid-Atlantic Ridge at 2400-2750 m depth, the authors were not able to
213 distinguish whether these results were due to ontogenetic changes in diet or merely to an effect of
214 increasing size, within the size-range sampled. Moreover, $\delta^{15}\text{N}$ and trophic position may increase
215 with body size in adult shallow-water fish, as larger predatory fish ingest larger, more isotopically
216 enriched prey (Badalamenti et al., 2002; Galván et al., 2010).

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

232 1.4.2 Environmental sources

233 Larger-scale (e.g. regional, global) comparative studies among deep-sea habitats are complicated
234 by the wide bathymetric ranges they may occupy, anywhere between 200 and ~11 000 m depth.
235 Depth may constitute a major driver of variation of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in deep-sea organisms for two
236 main reasons. First, as mentioned earlier, biodegradation processes occurring within the water
237 column may favour the enrichment of POM as it sinks, thus influencing the stable isotope
238 composition of those organisms that directly feed on it (Mintenbeck et al., 2007; Bergmann et al.,
239 2009). Second, size-based trends and shifts in diet, hence in the isotopic composition, with depth
240 have been reported for deep-sea demersal fish (Collins et al., 2005; Mindel et al., 2016a, 2016b).
241 Likewise, deep-sea species may exhibit different lipid and FA compositions along a bathymetric
242 gradient, reflecting physiological adaptations to changing temperature and pressure with depth
243 (Parzanini et al., 2018b).

244 Geographic location (e.g. latitude) and season, linked to level/type of surface primary
245 production, nitrogen supply dynamics, as well as temperature, are also important factors to
246 consider when comparing studies, as large-scale temporal and spatial differences may be detected
247 in the organisms' isotopic composition. Stowasser et al. (2009), for instance, combined stable
248 isotope and FA acid analyses to study seasonal variations in the diet of 5 species of demersal fish
249 collected between 785 and 4814 m in the Northeast Atlantic. The authors found overall that stable
250 isotope and FA composition of fish varied temporally, and that these differences most likely
251 reflected timing and strength of food inputs sinking from surface waters. However, not all the
252 species (e.g. *Coryphaenoides armatus*) exhibited a strong seasonality in their biochemical
253 composition, probably due to the high trophic position of the species and the length of the food web
254 analyzed obscuring the effects of the seasonal POM inputs (Stowasser et al., 2009). Colombo et al.
255 (2016) detected a latitudinal gradient in the FA composition of marine species, with higher levels of
256 ω 3-polyunsaturated fatty acids in organisms collected at polar and temperate regions in
257 comparison to tropical ones. Large-scale geographic effects will be further explored below, in the

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

262 **1.4.3 Analytical sources**

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

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

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

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

295 Sample storage is also crucial to obtain reliable data, since non-optimal preservation
296 methods may compromise the outcome of the investigation. Regarding the storage temperature,
297 while biological samples for gut content and stable isotope analysis are commonly frozen at -20°C ,
298 if not processed soon after their collection; those for lipid analysis are either stored at -80°C
299 (recommended) or at -20°C prior to further processing in the lab. Since storage at -20°C might not
300 completely prevent lipid degradation, especially if samples are analyzed after several years, rapid
301 initial processing of samples and vacuum packing may reduce potential issues when freezing at -
302 80°C is not logistically feasible. In addition, freezing is highly recommended over chemical storage
303 for stable isotope analysis, as there is evidence that formalin/ethanol considerably alters the
304 isotopic ratios in biological tissues (Arrington and Winemiller, 2002; Syväranta et al., 2011; Xu et
305 al., 2011).

306 **2 Preliminary comparative analysis**

307 The study of large-scale trends in biological variables (e.g. distribution, biochemical composition,
308 biodiversity) may not only help understand general functioning and structure of ecosystems, but it
309 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 $\delta^{13}\text{C}$ 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 $\delta^{15}\text{N}$ 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 ω_3 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.

335 2.1 Materials and methods

336 2.1.1 Data set

337 This analysis focused on studies that used either bulk stable isotope or FA analysis, or a
338 combination of them, to infer trophic relationships of deep-water ~~organisms~~macro- and megafauna,
339 as well as to study deep-sea food webs. ~~Studies, from heterotrophic ecosystems. Experimental~~
340 studies, as well as investigations on chemosynthetic habitats (e.g. hydrothermal vents) were
341 excluded *a priori* to avoid possible biases. In fact, these habitats are fuelled by primary dietary
342 sources, e.g. methane, whose isotopic and FA composition is substantially different than that of
343 POM (Rau and Hedges, 1979; Saito and Osako, 2007). Table 3 outlines the full data set collated
344 for the present analysis, which includes 4552 different studies. The literature search was carried out
345 through Scopus and Google Scholar portals using the following key words: stable isotopes, fatty
346 acids, food webs, deep sea, trophic ecology, and trophic relationships. Additional sources provided
347 by an anonymous referee were also included. These studies were used to analyze global trends in
348 $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and the essential arachidonic (ARA, 20:4 ω 6), eicosapentaenoic (EPA; 20:5 ω 3) and
349 docosahexaenoic (DHA, 22:6 ω 3) acids across deep-water communities. ARA, EPA, and DHA are
350 the most important nutrients in aquatic ecosystems, required by organisms for optimal health
351 (Parrish 2009), as well as excellent trophic biomarkers. In fact, whereas EPA and DHA are typically
352 used as biomarkers in diatoms and dinoflagellates respectively (Parrish, 2013), in the deep sea,
353 ARA is associated with microorganisms from the sediment (Howell et al. 2003). Our study focused
354 on these three FA since they are present in all the organisms under analysis.

355 2.1.2 Variables considered

356 Each species from each investigation was sorted by latitude (i.e. tropical, 0 - 30°; temperate, 30 -
357 60°; and polar, 60 - 90°), habitat (i.e. pelagic, demersal, and benthic) depth at collection (i.e.
358 mesopelagic, 200 – 1000 m; bathypelagic, 1000 – 4000 m; and abyssopelagic, >4000 m,
359 ~~whether for~~ pelagic species; bathyal 200 - 4000 m; ~~and~~ abyssal, >4000 m, ~~whether-~~ 6000 m; and

360 [hadal, > 6000 m, for](#) benthic species), and phylum (i.e. Annelida, Arthropoda, Brachiopoda,
361 [Bryozoa, Chaetognatha,](#) Chordata, Cnidaria, [Hemichordata,](#) Echinodermata, Mollusca, Nematoda,
362 [Nemertea,](#) Porifera, and Sipuncula). Information about species habitat was either obtained through
363 WoRMS and FishBase online databases or was already included in the source paper. In addition,
364 species were labelled as “meso-bathypelagic” and “bathyal-abyssal”, if the depth at collection was
365 not specified further, but the whole set of samples for a study was collected within those zones. In
366 the current analysis, tissue type, acidification treatment, sampling season, sex, and age were not
367 considered as variables, because i) they were assumed to not play a major role in global-scale
368 investigations and/or ii) this information was not always provided. In addition, tests were performed
369 on lipid-corrected and uncorrected $\delta^{13}\text{C}$ data pooled together. For analyses regarding stable
370 isotope composition ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$), ~~from polar to tropical regions,~~ data were obtained from Iken et al.
371 (2005), [Mincks et al. \(2008\),](#) Bergmann et al. (2009), [Quiroga et al. \(2014\), and](#) van [Oevelen et al.](#)
372 ~~(2018);~~, [for polar regions;](#) Iken et al. (2001), Madurell et al. (2008), Sherwood et al. (2008), Carlier
373 et al. (2009), Fanelli et al. (2009), Stowasser et al. (2009), Fanelli et al. (2011a, 2011b), Boyle et al.
374 (2012), Reid et al. (2012), Fanelli et al. (2013), Gale et al. (2013), Kharlamenko et al. (2013), Papiol
375 et al. (2013), Reid et al. (2013), Tecchio et al. (2013), [Kiyashko et al. \(2014\),](#) Trueman et al. (2014),
376 Valls et al. (2014a, 2014b), Kopp et al. (2018), Parzanini et al. (2017), Preciado et al. (2017);) [and](#)
377 [Parzanini et al. \(2018a\);](#), [for temperate latitudes; and](#) Jeffreys et al. (2009), Churchill et al. (2015),
378 ~~and~~ Shipley et al. (2017);), [and Richards et al. \(2019\), for tropical regions](#) (Table S1). FA
379 composition (ARA, EPA, and DHA) data were collected from Pétursdóttir et al. (2008a, 2008b), [and](#)
380 Würzberg et al. (2011a, 2011b, 2011c);), [for polar areas;](#) Lewis (1967), Howell et al. (2003),
381 Hudson et al. (2004), Økland et al. (2005), Drazen et al. (2008a, 2008b), Stowasser et al. (2009),
382 Murdukhovich et al. (2018), Parzanini et al. (2018a), Salvo et al. (2018), van [Oevelen et al.](#)
383 ~~(2018);~~, [and Kharlamenko et al. \(2018\), for temperate regions;](#) and Jeffreys et al. (2009) [and Shi et](#)
384 [al. \(2018\), for tropical regions](#) (Table S2).

385 2.2 Statistical analysis

386 Comparisons among multiple groups of deep-sea organisms were run through t-tests and oneway
387 analysis of variance (ANOVA). In particular, isotopic (i.e. $\delta^{15}\text{N}$, $\delta^{13}\text{C}$) and FA (i.e. ARA, EPA and
388 DHA) data were compared across organisms from different latitudes (i.e. tropical, temperate and
389 polar), habitats (i.e. pelagic, demersal, benthic), and collection depths (i.e. mesopelagic,
390 bathypelagic, meso-bathypelagic, abyssopelagic, bathyal, bathyal-abyssal, ~~and~~-abyssal, and hadal)
391 to detect any significant differences. When the normality assumption was violated, Mann-Whitney
392 rank sum test, Kruskal-Wallis oneway ANOVA on ranks, and Dunn's method pairwise comparisons
393 were performed instead. In addition, multivariate statistics, i.e. principal coordinate analysis (PCO)
394 and permutational MANOVA (PERMANOVA) were used to study the variability in the isotopic and
395 FA composition of deep-water organisms across different latitudes, habitats, collection depths, and
396 phyla. In addition, a distance based linear model (DistLM) was run to assess which of these four
397 factors contributed the most to such a variability. PCO, PERMANOVA, and DistLM were run on
398 resemblance matrices, based on Euclidean distance for the isotopic data, and Bray-Curtis for the
399 FA data. Data were not normalized or transformed prior to analysis. Univariate statistics was
400 conducted using Sigmaplot 12.5, while PCO, PERMANOVA and DistLM were run through Primer
401 7.0 with the add-on package PERMANOVA+ (Clarke and Gorley, 2006).

402 2.3 Results

403 Analyses revealed both latitudinal and depth-related trends for isotopic and essential FA
404 composition. In particular, mean values ($\pm\text{SD}$) of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were significantly lower in deep-sea
405 fauna sampled at high latitudes than in that collected at low latitudes ($\delta^{15}\text{N}$, ANOVA on Ranks, $H =$
406 69.135.6, $p \leq 0.001$; $\delta^{13}\text{C}$, ANOVA on Ranks, $H =$ 196.6277.9, $p \leq 0.001$; Fig. 2). Conversely, no
407 difference was detected across latitudes in terms of ARA, but mean proportions ($\pm\text{SD}$) of EPA and
408 DHA were significantly greater at polar latitudes than at temperate and tropical areas (EPA,
409 ANOVA on Ranks, $H =$ 10.511.4, $p = 0.005003$; DHA, ANOVA on Ranks, $H =$ 52.063.6, $p \leq 0.001$;

410 Fig. 3). Similarly, PERMANOVA detected significant differences across latitudes in terms of both
411 stable isotopes [Pseudo-F = ~~67.081.4~~, $p(\text{perm}) = 0.0001$] and essential FA [Pseudo-F = ~~9.411.0~~,
412 $p(\text{perm}) = 0.0001$].

413 When deep-water species were analyzed separately, according to their habitat, the same
414 latitudinal trend in the isotopic composition were shown for deep-water benthic species ($\delta^{15}\text{N}$,
415 ANOVA on Ranks, $H = \text{6440.5}$, $p \leq 0.001$; $\delta^{13}\text{C}$, ANOVA on Ranks, $H = \text{443171.2}$, $p \leq 0.001$);
416 whereas, for demersal and pelagic species, only the $\delta^{13}\text{C}$ ratios were significantly lower at higher
417 latitudes (ANOVA on Ranks, $H = \text{97.9105.7}$, $p \leq 0.001$; ~~$t_{434} = -4.0$, for demersal species; ANOVA on~~
418 ~~Ranks, $H = 11.5$, $p \leq 0.001 = 0.003$, for pelagic species). PERMANOVA showed that the isotopic~~
419 composition of deep-sea animals was indeed statistically different across the three habitats
420 [Pseudo-F = ~~125.7112.6~~, $p(\text{perm}) = 0.0001$], and benthic and demersal species had higher stable N
421 and C isotope ratios than the pelagic counterparts ($p < 0.05$). Conversely, only benthic and pelagic
422 species revealed a latitudinal gradient in their essential FA composition (EPA, ANOVA on Ranks, H
423 = ~~40.212.1~~, $p = 0.006002$; DHA, ANOVA on Ranks, $H = \text{35.543.6}$, $p \leq 0.001$, for benthic species;
424 EPA, ANOVA, $H = 6.4$, $p = 0.011$, for pelagic taxa). In this regard, pelagic, demersal, and benthic
425 taxa had a different essential FA composition (ARA, ANOVA on Ranks, $H = \text{35.039.7}$, $p \leq 0.001$;
426 EPA, ANOVA on Ranks, $H = 12.5$, $p = 0.002$; DHA, ANOVA on Ranks, $H = \text{70.876.9}$, $p \leq 0.001$);
427 ~~Pseudo-F = 19.7, $p(\text{perm}) = 0.0001$). Benthic species had the highest proportions of ARA and EPA~~
428 ~~($p < 0.05$);~~ while demersal species had the highest levels of DHA, although similar to those of
429 pelagic species.

430 While mean values of both stable N and C isotope ratios significantly increased with depth
431 ~~(for benthic and demersal species~~ ($\delta^{15}\text{N}$, ANOVA on Ranks, $H = \text{446.463.9}$, $p \leq 0.001$; $\delta^{13}\text{C}$,
432 ANOVA on Ranks, $H = \text{422.7126.2}$, $p \leq 0.001$), ~~proportions only~~ $\delta^{13}\text{C}$ ratios showed the same trend
433 in pelagic taxa (ANOVA on Ranks, $H = 125.5$, $p \leq 0.001$). Proportions of EPA significantly
434 decreased along the bathymetric gradient for pelagic species taxa (ANOVA on Ranks, $H = 12.3$, $p =$
435 0.002), and levels of ARA were significantly higher at abyssal depths for benthic and demersal
436 species (ANOVA on Ranks, $H = 39.7$, $p \leq 0.001$). In addition, for benthic and demersal fauna,

437 levels of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and ARA increased for benthic and demersal organisms with increasing depth
438 ($\delta^{15}\text{N}$, ANOVA on Ranks, $H = 84.7$, $p \leq 0.001$; $\delta^{13}\text{C}$, ANOVA on Ranks, $H = 105.0$, $p \leq 0.001$; ARA,
439 ANOVA on Ranks, $H = 22.8$, $p \leq 0.001$). PERMANOVA revealed significant differences in the
440 isotopic [Pseudo-F = 89.574.6, $p(\text{perm}) = 0.0001$] and essential FA composition [Pseudo-F =
441 7.38.6, $p(\text{perm}) = 0.0001$] across collection depths.

442 Among the four variables considered (i.e. latitude, habitat, collection depth, and phylum),
443 analyses revealed that 'habitat' and 'phylum' were the most important factors influencing the
444 variability of the stable isotope (respectively 13.12 and 9%; DistLM, *adjusted R*² = 0.4) and FA
445 (respectively 8 and 12.11%; DistLM, *adjusted R*² = 0.3) composition of deep-water organisms (Fig.
446 4).

447 **2.4 Discussion**

448 The present analysis shows for the first time, the existence of a) latitudinal trends in both stable
449 isotope and essential FA composition of deep-sea organisms, with decreasing $\delta^{13}\text{C}$ ratios and
450 increasing $\omega 3$ LC-PUFA towards the poles; b) global bathymetric trends in the isotopic composition
451 of deep-water fauna for which mean levels of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and ARA increased with increasing depth.
452 In addition, it provides further evidence of the link, across latitudes and depth, between surface
453 primary production of the surface waters and the deep-water consumers. The present findings
454 generally align with reports of decreasing values of $\delta^{13}\text{C}$ in surface-waters plankton and POM
455 towards the polar regions, in both the southern and northern hemisphere (Sackett et al., 1965; Rau
456 et al., 1982; Francois et al., 1993), as well as of increasing POM isotopic ratios along a bathymetric
457 gradient (Altabet et al., 1999). They also agree with Colombo et al. (2016) who noticed that
458 proportions of $\omega 3$ LC-PUFA were higher in marine organisms from polar and temperate regions in
459 comparison to tropical regions, and with Parzanini et al. (2018a) who detected increasing
460 proportions of ARA along a slope area in the deep Northwest Atlantic.

461 Water temperature, in combination with other abiotic (e.g. oceanographic and
462 biogeochemical processes, nutrient supply) and biological factors (e.g. species metabolism,

463 taxonomic composition of deep-water communities, microbial remineralization processes) seems to
464 play a role in these trends (Rau et al., 1982; Francois et al., 1993; Altabet et al., 1999; Colombo et
465 al., 2016). In particular, water temperature influences isotopic fractionation processes and, typically,
466 higher fractionation is associated with lower temperatures (Sackett et al., 1965). High fractionation
467 rates are also linked to the pronounced denitrification activities characterizing oligotrophic areas
468 such as observed in some areas of the tropics (Hetherington et al., 2017). This may explain the
469 higher $\delta^{15}\text{N}$ ratios of the deep-sea organisms from the tropical latitudes analyzed in this study.
470 Furthermore, water temperature affects membrane fluidity, and lower temperatures decrease the
471 fluidity of cell membrane (Parrish, 2013; Colombo et al., 2016). Thus, in order to maintain normal
472 membrane function and condition, i.e. health, ectotherms may counteract variations in water
473 temperature by readjusting their FA composition (Cossins and Lee, 1985; Parrish, 2013). For
474 example, larger proportions of long chain unsaturated FA (e.g. ARA, EPA) within the lipid bilayer
475 help increase membrane fluidity (Parrish 2013), as these molecules are characterized by a higher
476 flexibility (DeLong and Yayanos, 1985; Colombo et al., 2016).

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

487 Finding latitudinal trends in the biochemical composition of deep-water organisms that
488 mirror results from shallow depths provides further evidence of the link between the two systems, in
489 that deep-sea benthic communities rely on POM sinking from the surface water as a primary food

490 source (Gage, 2003; Hudson et al., 2004). Close dependence of deep-sea food webs on near-
491 surface processes raises important concerns. According to the latest climate estimates, both air
492 and water temperatures have been rising, and continue to increase; and seawater pH has already
493 dropped by 0.1 units due to large CO₂ emissions, and is expected to decrease further (IPCC,
494 2017). Furthermore, models predict that increasing surface water temperature will favor
495 stratification, while reducing vertical mixing as well as enhancing variability in the transport of
496 primary production and energy (i.e. carbon) transport to the deep sea (Smith et al., 2009; Jones et
497 al., 2014; Sweetman et al., 2017). At the same time, deep-water benthic biomass is expected to
498 decrease due to the increasing variability in the food supply, which may in turn affect health and
499 functioning of benthic ecosystems, as well as global biogeochemical cycles (Jones et al., 2014).
500 Hixson and Arts (2016) showed that the FA composition of the six most common fresh- and salt-
501 water phytoplankton species responded to temperature and, specifically, that their ω₃ PUFA levels
502 decreased with increasing temperature. Not only do ω₃ PUFA, such as EPA and DHA, play an
503 important role in the response to temperature variations in aquatic systems, but they are also
504 essential nutrients and are highly required by aquatic organisms for optimal growth and health
505 (Parrish, 2009). A case in point, Rossoll et al. (2012) showed experimentally that growth and
506 reproduction of the copepod *Acartia tonsa* were severely compromised by the alteration of FA
507 content and composition of its primary food source, the diatom *Thalassiosira pseudonana*, exposed
508 to high CO₂ levels. The present investigation, therefore, suggests that changes in amounts and
509 composition of surface production could also result in changes in essential nutrients and
510 biomarkers in deep-sea benthic organisms that feed on it, with possible cascading effects
511 throughout deep-water food webs. Such variations may alter nutrient intake of deep-sea benthic
512 organisms, as well as trophodynamics; and they may also influence species' abilities to cope with
513 deep cold waters.

514 **3 Conclusions**

515 This investigation provides a first summary of the information available on deep-sea food webs
516 inferred by bulk stable isotope and FA analyses, providing guidance for future studies and a
517 glimpse at global-scale patterns in the biochemical composition of deep-water organisms-from
518 heterotrophic ecosystems. Food-web tracers represent a powerful tool that can help elucidate the
519 structure and dynamics of food webs from shallow to deeper waters, and support management
520 initiatives. However, this tool is even more effective when combined with other techniques (e.g. gut
521 content analysis), as each method provides uniquely valuable data. When comparing studies, it
522 emerges that there are multiple sources of variations, whether biological, environmental, and/or
523 analytical. Depending on the scale of the investigation, these differences are more or less
524 susceptible to biases, suggesting that they have to be considered and acknowledged when
525 attempting cross-comparisons even though they may be contextually acceptable. The preliminary
526 analysis conducted here detected latitudinal and bathymetric trends in the isotopic and FA
527 composition of deep-sea species. In light of global climate change and the link between surface
528 production and deep-sea communities, changes in amounts and composition of surface production
529 may influence the essential nutrient intake (e.g. ω 3 PUFA) of deep-water organisms. Because ω 3
530 PUFA are involved in the response to temperature variations in ectotherms, climate change may
531 also affect the ability of these species to cope with potential temperature shifts. However, more
532 studies are required to help detect global trends, especially in those areas that are still poorly
533 understood (most deep-sea areas) or not yet investigated (e.g. in the southern hemisphere). In
534 addition, it is necessary to standardize analytical methods to limit their influence and help
535 compensate for natural variability.

536 Data availability

537 [All data used for analysis can be found as supplementary material, in Table S1 and S2.](#)

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

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

542 **Author contribution**

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

547 **Competing interests**

548 The authors declare that they have no conflict of interest.

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924 **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 <u>may</u> 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. ontogenetic 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, <u>unless food is highly digested</u> , 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 tech, med technology, medium/high cost	Medium tech, med technology, medium/high cost

925 **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
-	-	<u>Geological feature (e.g. shelf, slope, canyon, plain, trench)</u>

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928 **Table 3** List of trophic ecology studies in deep-sea heterotrophic systems, ~~that have been~~ carried
 929 out using stable isotopes (bulk) and lipids (including FA) as food-web tracers. Experimental studies
 930 were excluded a priori. Reference, method(s) applied, latitude, sampling depth, ocean region, and
 931 taxa analyzed are reported for each study. Polar latitudes include investigations between 60 - 90°
 932 N/S, whereas temperate and tropical latitudes represent studies carried out within 0 - 30° N and 30
 933 - 60° N, 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, sipunculans, and tunicates
<u>Quiroga et al. 2014</u>	<u>Stable isotopes</u>	<u>Polar</u>	<u>250-322</u>	<u>Weddell Sea</u>	<u>Benthic annelids, crustaceans, bryozoans, tunicates, cnidarians, echinoderms, molluscs, nemertea worms, sponges and sipunculans.</u>
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
<u>Mincks et al. 2008</u>	<u>Stable isotopes</u>	<u>Polar</u>	<u>550-650</u>	<u>Bellinghausen Sea</u>	<u>Benthic annelids, cnidarians, echinoderms, molluscs, sponges, and demersal fish</u>
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 Benthic cnidarians, crustaceans, echinoderms, echiurans, mollusks, and polychaetes; pelagic crustaceans
Iken et al. 2005	Stable isotopes	Polar	800-2082	High Arctic Canadian Basin	Mesopelagic crustaceans and fish
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, echinoderms, echinoderms, fish, mollusks, nematodes, polychaetes, 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
Shiple 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.

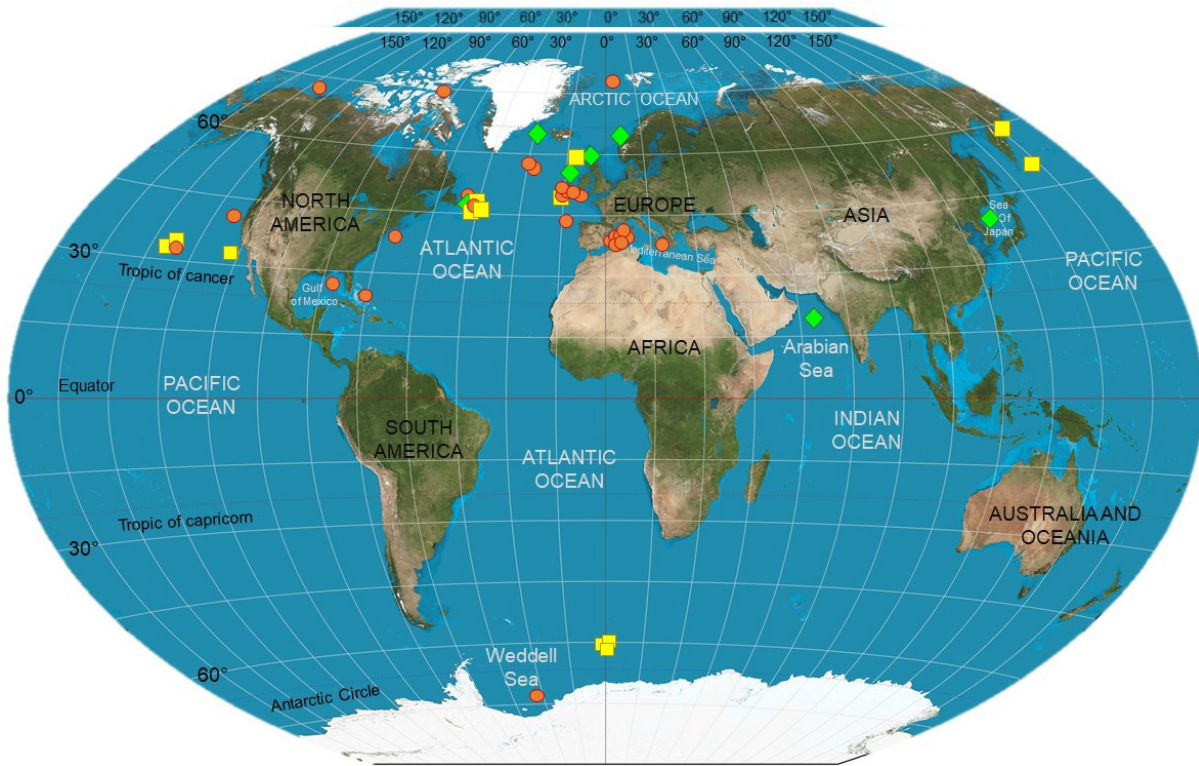
935 **Figures caption**

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

940 **Fig. 2.** Stable N and C isotopic composition of deep-sea animals across latitudes. Mean values of
941 $\delta^{15}\text{N}$ (blue circles above) and $\delta^{13}\text{C}$ (orange circles below) (‰) measured in deep-sea organisms
942 across polar, temperate, and tropical latitudes. Bars represent standard deviation (~~polar, n = 33–~~
943 ~~1479), and a letter code indicates significant differences ($p < 0.05$) across latitudes.~~235; temperate,
944 n = 1468; tropical, n = 41).

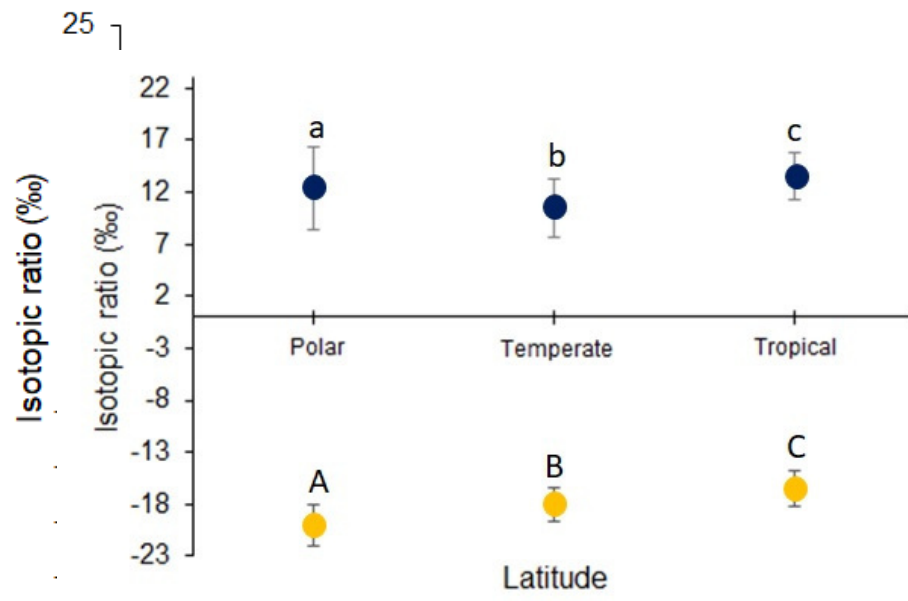
945 **Fig. 3.** Essential FA composition of deep-sea animals across latitudes. Mean proportions of
946 essential FA measured in the tissues of deep-sea animals from polar (blue bars), temperate
947 (orange diagonal striped bars), and tropical (green vertical striped bars) latitudes. Bars represent
948 standard deviation (~~n = 7–212), and a letter code indicates significant differences ($p < 0.05$)~~
949 ~~across latitudes.~~polar, n = 176; temperate, n = 227; tropical, n = 11).

950 **Fig. 4.** Differences in terms of biochemical compositions among deep-sea animals from various
951 habitats. Principal coordinate analysis plots representing differences in terms of isotopic (above)
952 and essential FA composition (below) of deep-water species. In both cases, the variable 'habitat'
953 resulted one of the most important factors, contributing ~~43~~12 and 8% respectively to the variability
954 in the biochemical composition of the deep-sea species.
955

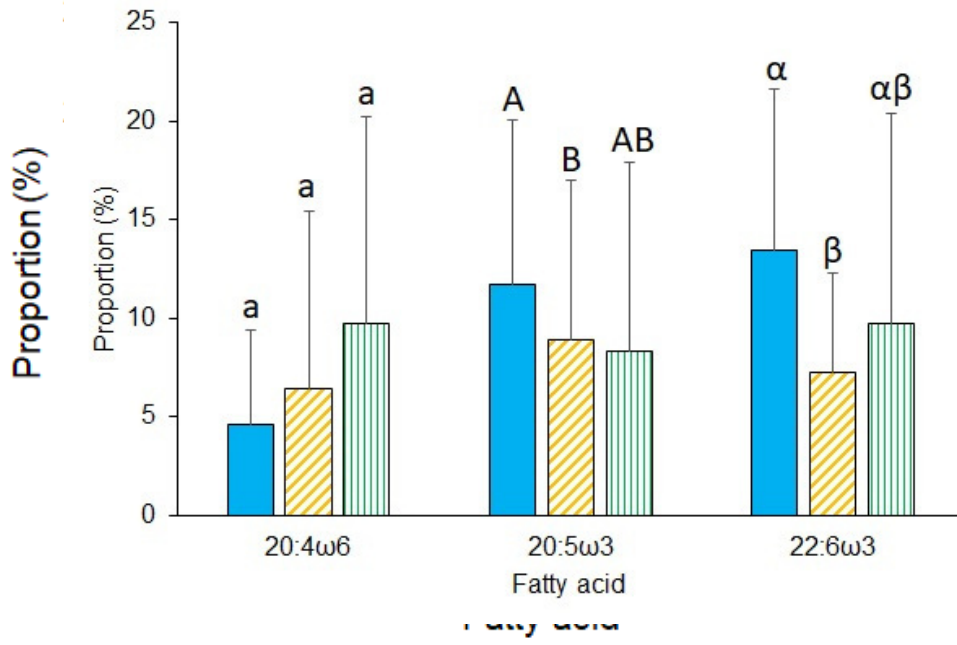


956 **Fig. 1.**

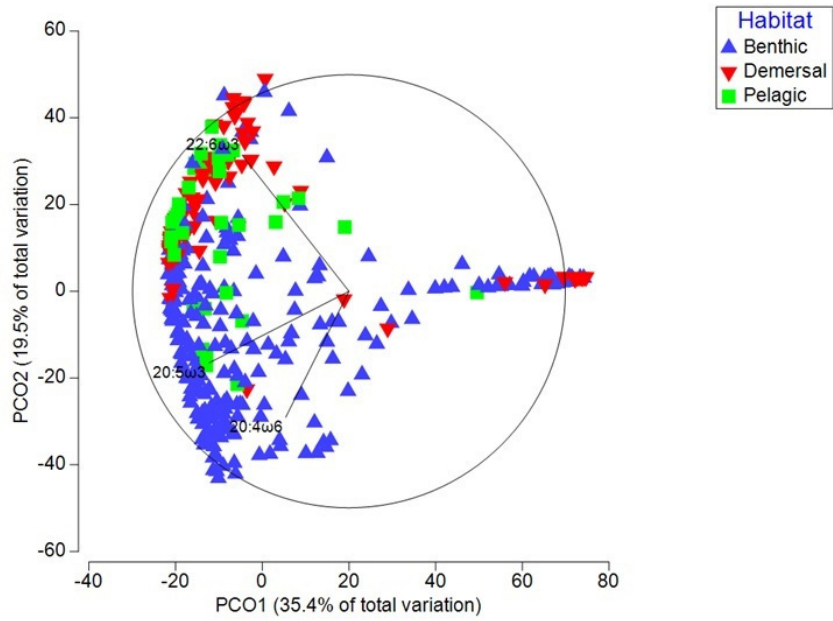
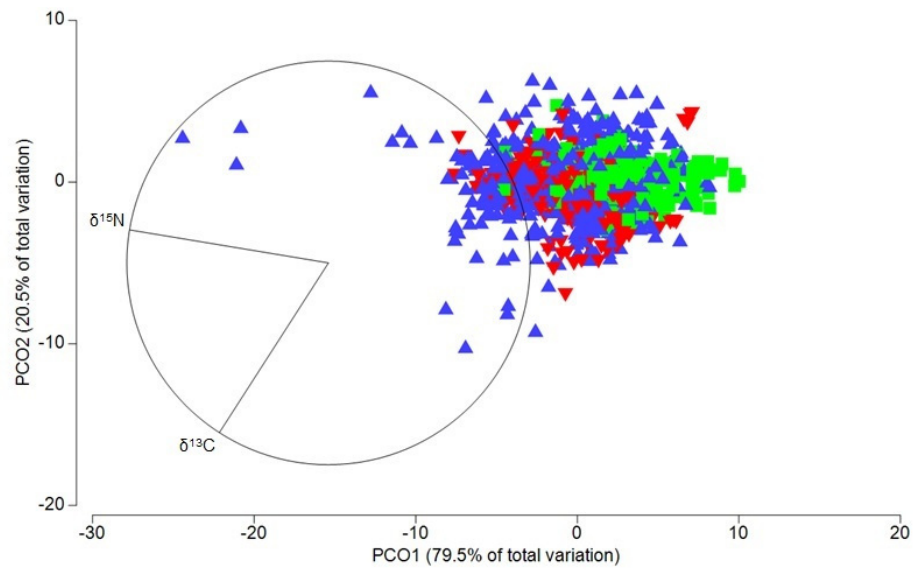
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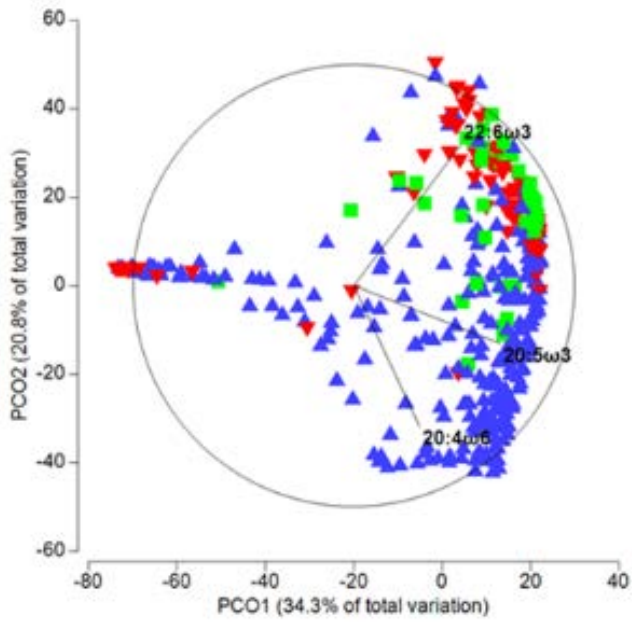
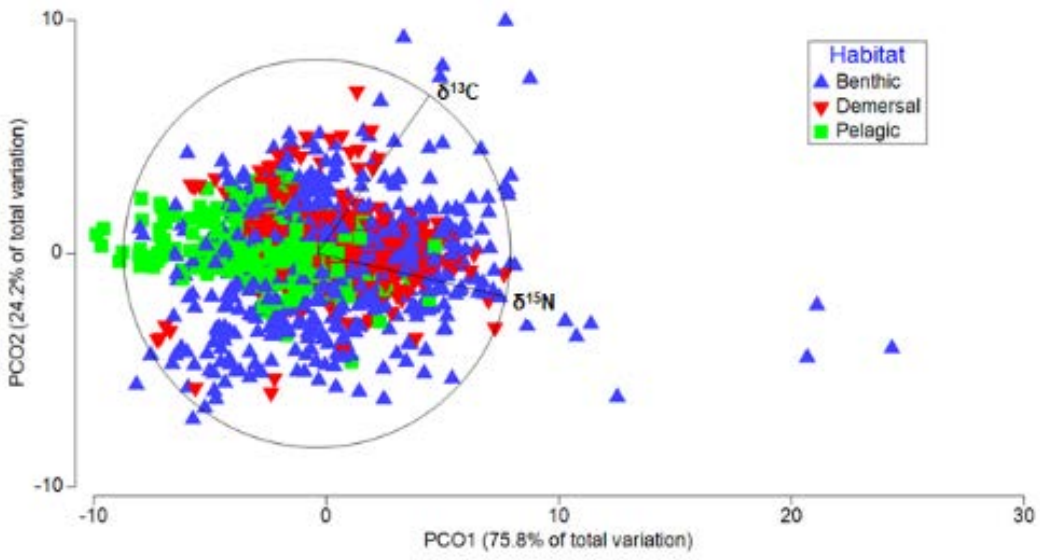


958 Fig. 2.



960 Fig. 3.





963 **Fig. 4.**

964

965