AN INTEGRATED APPROACH TO STUDYING THE TROPHIC ECOLOGY OF A DEEP-SEA FAUNAL ASSEMBLAGE FROM THE NORTHWEST ATLANTIC

by

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Abstract

Despite being the largest ecosystem on Earth, the deep sea is still poorly known. Since the study of food webs allows a better understanding of ecosystems, the current research aimed to provide new insights into trophic relationships and element cycling within a deep-water faunal assemblage sampled in deep-sea areas of eastern Canada (Northwest Atlantic). The faunal assemblage consisted of a broad array of deep-sea taxa (143 species representing 8 phyla) collected within a tight window in space and time (100 km radius, 7 days), but across a large depth range (~1000 m) off insular Newfoundland. Functional diversity was studied along the bathymetric gradient. The integrated use of stable isotope, lipid, elemental, morphometric, and gut content analyses was crucial in obtaining an overall picture of the food web analyzed. Specifically, two major trophic pathways were recognized within the faunal assemblage: a pelagic pathway, relying on sinking organic matter (OM) as the primary food source; and a benthic pathway, in which settled OM constituted the base. A key role in energy and nutrient cycling was highlighted for pelagic vertical migrators and deep-water benthic communities. Vertical migrators actively provide inputs of food to benthic communities; benthic communities bioaccumulate certain energetic and nutritive compounds, and transfer them along the food web. Moreover, type and amount of lipids reflected not only dietary sources, but also environmental conditions typical of the deep sea. Large proportions of wax esters detected in certain species likely provide them with long-term energy reserves in a food-depleted environment. In addition, while the unsaturation level of phospholipid fatty acids increased, sterols diminished along the bathymetric gradient. This finding was interpreted to reflect adaptations of deep-water organisms to cope with increasing pressure and decreasing temperature with depth. Lastly, a preliminary

analysis of the literature showed the existence of latitudinal trends in the isotopic and fatty acid composition of deep-sea benthic organisms, which exhibited lower C isotope ratios and higher proportions of ω3 fatty acids at temperate and polar latitudes than at tropical ones. This investigation raises concerns about potential effects of global climate change on deep-water communities, and about standardizing analytical methods to enable comparisons.

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“As for me, I am tormented with an everlasting itch for things remote. I love to sail forbidden seas, and land on barbarous coasts”

Herman Melville

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List of Abbreviations and Symbols

%C	Proportion of elemental carbon
%N	Proportion of elemental nitrogen
ARA	Arachidonic acid
C:N	Elemental carbon to nitrogen ratio by mass
$C:N_{mol}$	Molar elemental carbon to nitrogen ratio
CV	Coefficient of variation
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FA	Fatty acid(s)
FFA	Free fatty acid(s)
MUFA	Monounsaturated fatty acid(s)
PL	Phospholipid(s)
PL:ST	Phospholipid to sterol ratio
PUFA	Polyunsaturated fatty acid(s)
SAT	Saturated fatty acid(s)
ST	Sterol(s)
TAG	Triacylglycerol(s)
TAG:ST	Triacylglycerol to sterol ratio
TMF	Trophic multiplication factor
TP	Trophic position
VLCFA	Very long chain fatty acid(s)
WE/SE	Wax ester(s) and steryl ester(s)
$\delta^{13}C$	Stable carbon isotope ratio
$\delta^{13}C_n$	Lipid-normalized stable carbon isotope ratio
$\delta^{15}N$	Stable nitrogen isotope ratio
ω3-FA	ω3 fatty acid(s)
ω6-FA	ω6 fatty acid(s)

Fatty acid nomenclature: omega (ω) reference system

Following the ω reference system, the number of carbons, number of double bonds, and position of the double bond closest to the omega carbon/methyl group identify each fatty acid. For example, molecules of eicosapentaenoic acid, reported as 20:5ω3 according to this system, are made up of 20 atoms of carbon, and have 5 double bonds in position 3 relative to the methyl group.

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Chapter 1 : **Introduction and Overview**

The deep sea: main features

The deep sea is commonly defined as the portion of ocean that resides below the shelf break, which generally occurs around 200 m, and where a transition from shallow- to deep-water fauna has been observed (Thistle 2003; Ramirez-llodra et al. 2011). By covering more than 65% of the Earth's surface (Herring 2001; Thistle 2003) and occupying a volume of 1368 x 10^6 km^3 (Ramirez-llodra et al. 2011), the deep ocean is considered the largest ecosystem on the planet (Gage and Tyler 1991; Herring 2001; Pfannkuche 2005; Table 1-1); however, it is still poorly explored and understood. In fact, despite considerable advances in technology made over the last few decades, less than 5% of the deep ocean bottom has so far been mapped and studied (Ramirez-Llodra et al. 2010; Table 1-1).

Overall, the deep sea is characterized by unique environmental conditions, which sustain distinctive and sometimes peculiar lifeforms and habitats (Herring 2001; Ramirez-Llodra et al. 2010). For one, the deep-sea environment covers a wide depth range (0.2-11 km; Herring 2001), and many other abiotic factors and processes vary along with it. In this regard, pressure increases by 1 atm every 10 m and, therefore, varies markedly along the broad depth gradient, i.e. from 20 atm at the shelf break to >1000 atm at trench depths (Thistle 2003). Conversely, temperature generally decreases with increasing depth, and it usually stabilizes at 1-2°C below ~800 m (Thistle 2003). Likewise, light intensity diminishes with depth, and solar wavelengths typically do not penetrate below ~1000 m in clear waters, which marks the limit of the twilight or disphotic zone; below this limit, the deep sea is completely dark (Warrant and Locket 2004).

The absence of light precludes photosynthetic primary production; hence, deep-water ecosystems are mainly allochthonous and heterotrophic (Gage 2003; Pfannkuche

2005). However, a few exceptions exist. A well-known example is represented by seep and vent communities, which are fueled by chemical compounds, such as methane (CH_4) and hydrogen sulfide (H_2S), released through the oceanic crust. In addition, a species of deep-sea green sulfur bacteria (Chlorobiaceae), recently isolated from a vent site in the Pacific, has shown the potential for photosynthesis using faint light of geothermal origin (Beatty et al. 2005). In general, deep bottom-dwelling communities rely on exogenous inputs of food (Fig. 1-1) from vertical, i.e. downwards from surface waters, and/or lateral, i.e. from the margins, transport through physical processes (Pfannkuche 2005). Specifically, phytodetritus in the form of marine snow (i.e. flocculent aggregates of diverse origin, such as dead animals or parts thereof, exudates and fecal pellets, as well as phytoplankton cells and associated bacteria) represents the major food source to deep benthic communities (Gage 2003). In addition, organic material is actively transported down by organisms undergoing diel vertical migration (DVM). The DVM community is composed of zooplanktonic and nektonic organisms that swim up to the surface and feed upon phytoplankton at night, and swim back to deeper depths (~1000 m) during the day (Trueman et al. 2014). Larger food falls of whale or shark carcasses also make an important local/transient contribution to the downward flux of organic carbon (Smith 1985). However, since less than 2% of the net primary production reaches the seafloor below 2000 m (Buesseler et al. 2007) because organic matter undergoes degradation during its downward transportation, the deep sea is considered an oligotrophic environment (Ramirez-Llodra et al. 2010).

Food webs and nutrient cycling

Food webs describe the networks of feeding/trophic interactions within an ecosystem, in which consumers rely on a diverse set of prey or food sources (Govenar 2012). Both

energy (i.e. carbon) and material (i.e. biomass) are transferred along food webs. In an attempt to better represent a complex system, the definition of food web has been periodically infused with new concepts and theories, and revised several times over the last century, as summarized below.

For the first time in 1927, the ecologist and zoologist Charles S. Elton introduced the notion of “food chain”, which was defined as a simple, binary (i.e. presence or absence) depiction of the trophic interactions inside a system (Thompson et al. 2012), where consumers were feeding on only one type of food source or prey (Paine 1980). In 1942, Raymond L. Lindeman proposed a more dynamic perspective of the food chain-model proposed by Elton (Paine 1980). Not only did Lindeman recognize the existence of discrete groups of organisms exerting a similar functional role, or “trophic levels”; he also suggested that each level was dependent on the previous as a food-source, and that solar energy was transferred from the lowest (i.e. producers) to the highest level (i.e. final consumers) of a given food chain (Lindeman 1942). However, both these early theories did not take into account omnivory, which represents the possibility of consumers feeding upon different sources and trophic levels (Paine 1988). In this regard, Vander Zanden and Rasmussen (1996) suggested “trophic position” as a better descriptor of the functional role of taxa within the complex network of feeding interactions of an ecosystem, or food web. The authors introduced “trophic position” as quantitative means to measure the hierarchical role of species within food webs (Hussey et al. 2014), and species with similar trophic positions belong to the same “trophic guilds” (Vander Zanden and Rasmussen 1996).

While energy and organic matter move throughout food webs, several processes, such as respiration, excretion, and death determine the release of both organic and inorganic compounds into the surrounding environment (Pomeroy et al. 2007).

Microorganisms such as bacteria, viruses, and protists, which together constitute the so-called “microbial loop”, play a major role in marine food-web dynamics and functioning (Azam et al. 1983), and hence constitute a fundamental part of food-web theory. Not only do such microorganisms recycle essential compounds (Pomeroy et al. 2007), they also modulate the amount of energy that flows throughout food webs (Pomeroy et al. 2007; Govenar 2012), and are vital to those ecosystems where local environmental conditions prevent classic photosynthetic processes from occurring. In reduced environments, for example, chemoautotrophic bacteria are responsible for converting inorganic energy sources (e.g. H_2S) into products that can be utilized by the rest of the community (Govenar 2012).

While they remain simplified models, food webs provide valuable depictions of the complex network of species interactions, and thereby represent a key tool in our bid to understand communities and ecosystems (Paine 1988; Thompson et al. 2012).

Analysis of food webs and trophic interactions

Over the last few decades, there has been growing attention devoted to trophic interactions and food webs in environmental conservation management (Sala and Sugihara 2005; Rombouts et al. 2013). In fact, food webs provide information that has greatly improved our knowledge of communities and ecosystems (Thompson et al. 2012). Specifically, analyses focus on the understanding of two main features: the structure of food webs, for example through the number of trophic links and/or levels; and their dynamics, which deals with the quantification of biomass and energy flows. While these features are often interconnected (Rombouts et al. 2013), they both have been shown to affect ecosystem function and stability (MacArthur 1955; Strogatz 2001;

Thompson et al. 2012), and hence are of major importance in food-web theory. Table 1-2 lists further examples of descriptors of food web structure.

Several techniques have been developed to study trophic structure and dynamics at different biological scales (i.e. from species to ecosystem level). Since each technique has its own strengths and weaknesses, the current trend in trophic ecology is to combine different methods (Jeffreys et al. 2009; Galloway et al. 2013; Gerringer et al. 2017) to cross-validate the results, as well as to improve the resolution of the investigation. In this thesis, three main techniques were applied to study a deep-sea food web sampled in the Northwest Atlantic: gut content, stable isotope and lipid analyses.

Gut content analysis

The analysis of gut contents is the most traditional approach in trophic studies (Gartner et al. 1997), and it has been developed to investigate diet in both marine and terrestrial organisms; however, it is often undervalued due to some limitations. The major criticism is that it merely provides snapshots of the diet, as it shows evidence only of the most recent feeding events or meals (Iverson et al. 2004; Würzberg et al. 2011; Couturier et al. 2013; Cresson et al. 2014). Furthermore, to obtain robust dietary information and to account for spatial, temporal, and biological variability, the approach ideally requires the collection of a large number of organisms from different locations and seasons, as well as specimens of different age and sex (Couturier et al. 2013; Hussey et al. 2014). However, extensive sampling is not always feasible, and depends on location, species type and behavior (Couturier et al. 2013), as well as on resource availability (e.g. funding, gear, and qualified personnel). In addition, the analysis requires taxonomic expertise for the identification of the prey/food items. Due to the differential digestion rates, soft-bodied prey may be digested faster than hard-bodied ones (Iverson et al.

2004; Würzberg et al. 2011; Hussey et al. 2014), thus making their identification even more challenging. Despite its limitations, gut content analysis represents an irreplaceable tool in trophic studies, as it clearly shows what animals have ingested (i.e. direct evidence of diet), and it provides a taxonomic resolution that no other method can offer.

Gut content analysis can be accomplished through different approaches, which span from a simple qualitative presence/absence survey to more complex quantitative analyses (e.g. prey-contribution by mass or volume; Hyslop 1980). In this research, for the goals of this project, the frequency of occurrence was chosen to characterize the diet of the organisms sampled. Specifically, this index represents the proportion of organisms containing a specific food item, out of the total number of individuals with food in their stomachs. Among all the indices, Baker et al. (2014) suggested the frequency of occurrence as the most reliable indicator of diet composition; not only does it involve lower effort and cost, but it is also subject to a lower probability of random error.

Bulk stable isotope analysis

To circumvent issues and limitations ascribed to gut content analysis, alternative indirect approaches have been developed and, among them, bulk stable isotope analysis (SIA) represents a primary tool in trophic ecology. SIA has successfully been applied to the study of ecosystem functioning and dynamics (McConnaughey and McRoy 1979); trophic position (Minagawa and Wada 1984; Post 2002; Gale et al. 2013); trophic relationships and food-web structure (Iken et al. 2001; Polunin et al. 2001; Iken et al. 2005; Reid et al. 2013); as well as energy sources and carbon flows (Fry and Sherr 1989; Budge et al. 2008; Trueman et al. 2014). In particular, stable nitrogen and carbon isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) are key indicators that provide time-integrated dietary data over longer intervals, from a few weeks to months. While the nitrogen stable isotope ratio

($^{15}N/^{14}N$ or $\delta^{15}N$) is particularly useful to study trophic positions due to its stepwise enrichment, from 2 to 4‰, between consumers and their food sources (Minagawa and Wada 1984; Iken et al. 2001; Post 2002); the carbon stable isotope ratio ($^{13}C/^{12}C$ or $\delta^{13}C$) is typically applied to assess the origin of the carbon source (McConnaughey and McRoy 1979). In fact, the fractionation of $\delta^{13}C$ between prey and predator is <1‰, and different carbon sources present specific $\delta^{13}C$ ratios (Fry and Sherr 1989; Budge et al. 2008). Although considered a routine method, the application of SIA and the interpretation of its results may be challenging. In fact, several assumptions and simplifications have to be made prior to analysis, including the choice of the appropriate trophic fractionation factor (Post 2002; Post et al. 2007), and food-web baseline (Cresson et al. 2014). Furthermore, depending on the organism/tissue being analyzed, the objective of the investigation, and the logistical availability, different protocols may be applied (Post et al. 2007). Lastly, biological and environmental variability may constitute a further source of misinterpretation.

Lipid and fatty acid analysis

Lipids represent a primary form of energy in aquatic environments (Parrish et al. 2000; Parrish 2009). Not only are they essential constituents of the vertical flux of organic matter (Parrish et al. 2005), thus providing high quality energy to heterotrophic organisms (Parrish 2009), but they are key components of cell membranes (Arts et al. 2001; Copeman and Parrish 2003), and are involved in numerous vital biological and physiological processes (Adams 1999; Phillips 2002; Bergé and Barnathan 2005; Parrish 2009). Among them, the phospholipid and sterol lipid classes, for example, play an important role by providing support, fluidity, and plasticity to cell membranes (Cossins and Lee 1985; Parrish 1999; Arts and Kohler 2009). Furthermore, triacylglycerols

constitute the main form of energy storage in the marine biosphere (Parrish 1999) and modulate survival and reproduction success in fish (Adams 1999) and other marine taxa (Fraser 1989). Due to their biological role, their ease of measurement (Parrish 2013), and their transferability through diet (Iverson 2009), lipids have been used as biomarkers to study health of organisms and ecosystems (Fraser 1989; Parrish et al. 2000; Parrish 2009); anthropogenic impact and carbon sources (Carreón-Palau et al. 2013); trophic interactions and food webs (Dalsgaard et al. 2003; Mercier et al. 2011; Kürten et al. 2013); as well as distribution patterns of marine species (Piatkowski and Hagen 1994; Smith et al. 1996).

As the major components of acyl lipids (e.g. triacylglycerols, wax esters, and phospholipids), fatty acids (FA) are defined as “building blocks” that play crucial biological functions (Iverson, 2008). FA typically consist of a straight chain of 14-24 carbons, with a carboxyl group (−COOH) at one end and a methyl group (-CH_3) at the other. Saturated FA have no double bonds while unsaturated FA have 1 to 6 or more double bonds. Several characteristics make FA excellent biomarkers. For example, they are not degraded during digestion and they are taken up by tissues with either no or limited (predictable) alteration (Iverson 2009). Furthermore, only a few organisms, such as primary producers, are able to synthesize fatty acids *de novo* (Iverson 2009). These organisms typically present unique FA signatures, allowing investigators to identify different organic carbon sources within an ecosystem (Iverson 2009; Jeffreys et al. 2009; Carreón-Palau et al. 2013). For all these reasons, FA represent a powerful tool in trophic ecology studies (Drazen et al. 2008; Stowasser et al. 2009; Kharlamenko et al. 2013). However, limitations of FA analysis include the strength of its signal, which usually decreases with increasing trophic level/position (Kürten et al. 2013). Moreover, in-depth interpretation of the results relies on secondary knowledge and can be quite complex,

especially since the same FA may represent the signature of more than one primary food source. For instance, palmitoleic acid 16:1ω7 is a biomarker for diatoms and bacteria (Parrish 2013).

Further analyses

The study of trophic relationships can be carried out using other techniques, such as video recording and feeding trials. However, the application of these approaches remains difficult when it comes to the deep sea, due to logistical and economic constraints. Nevertheless, alternative solutions can be used to indirectly infer trophic relationships and feeding habits. Examples include morphometric and elemental analysis (i.e. proportion of essential compounds within biological systems; %N, %C, C:N ratio), which were used in Chapters 2 and 4 respectively, in combination with the techniques described above.

The analysis of body shape and size of organisms can help infer their microhabitat use, lifestyle, feeding habits, as well as interactions (Scharf et al. 2000; Ward and Mehta 2010). Such analyses take into account that many organisms can grow several orders of magnitude during their life cycle (Scharf et al. 2000; Mindel et al. 2016a); and that they can also go through several developmental stages, thus experiencing changes in habitats and habits (Mauchline and Gordon 1985), as well as body shapes (Ward and Mehta 2010). In addition, organisms may display a whole suite of morphological adaptations (e.g. modified fins, special organs) which have been developed to cope with certain environmental conditions, and to contribute to their reproductive success, growth, and survival. Therefore, morphometric analysis has been used in the study of community and trophic ecology of marine species, to help

understand their functional role within communities (Scharf et al. 2000; Mindel et al. 2016a, b).

Elemental analysis is based on the idea that the main elemental compounds, such as C, N, and P, stand in a stoichiometric balance (e.g. Redfield Ratio, 106C:16N:1P) within organisms (Sterner and Elser 2002). However, variations can be observed that seem to be linked to both biological (e.g. size, diet, reproduction) and environmental factors (e.g. habitat, food availability; Elser et al. 1996; Ventura 2006; Connelly et al. 2012). Furthermore, elemental composition reflects that of major biological compounds, such as lipids, proteins, and nucleic acids, which have characteristic functional roles within organisms (Ventura 2006). Therefore, the study of elements and compounds has been applied to better understand energy flows and nutrient cycling, as well as to study the functional role of species in community energetics (Connelly et al. 2012).

Exploring functional diversity along a depth gradient

In the deep sea, light and, typically, temperature diminish while pressure increases with depth (Thistle 2003). In addition, both food quantity and quality decrease along the depth gradient (Campanyà-Llovet et al. 2017). To overcome physiological and biological issues related to such environmental variations, and to cope with these unique conditions, deep-sea organisms have evolved specific molecular, morphological and behavioral adaptations, and depth-related patterns have been detected (Sutton and Hopkins 1996; Simonato et al. 2006; Mindel et al. 2016a). For instance, Mindel et al. (2016b) observed that fractional size (i.e. a metric that accounts for ontogenetic changes in size) and body length of a demersal fish community increased along the depth gradient, with implications for community structure and composition. This trend was most likely driven

by changes in food availability across the depth range considered (Mindel et al. 2016b). In addition, temperature and pressure as functions of depth are known to influence cell membrane fluidity, hence its structure and function (DeLong and Yayanos 1985; Cossins and Macdonald 1989; Simonato et al. 2006). Deep-sea organisms respond to such variations through “homeoviscous adapation”, accomplished by the modification of the lipid composition of cell membranes (Macdonald and Cossins 1985).

Since the faunal assemblage analyzed in the present study was collected inside a tight temporal and latitudinal window (100 km radius, 7 days), but across a depth range of ~1000 m, morphometric together with biochemical (i.e. stable isotope, lipid, and elemental) analyses were used to investigate functional diversity along the depth gradient.

Study area

The study area is located in zone 3K of the NAFO Divisions, off insular Newfoundland, in the Northwest Atlantic (Fig. 1-2). Samples were opportunistically collected within 7 days, between November 30th and December 6th, 2013, inside a 100 km radius of the continental shelf/slope, and between 310 and 1413 m depth. The gear used (Campelen 1800 shrimp trawl; opened and closed at sampling depth), as described in Walsh and McCallum (1997) included a 16.9 m wide net with four panels of polyethylene twine. Mean bottom temperature at the sampling site ranged between 3.2 and 4.5°C, with a slight decrease with depth. This region of the Northwest Atlantic is characterized by high productivity levels, and affected by seasonal blooms of large-celled phytoplankton (Longhurst 2010) and strong lateral food inputs (Snelgrove and Haedrich 1985; Williams and Follows 1998; Afanasyev et al. 2001). Due to its well-known productivity, the area has been heavily exploited and fished. Furthermore, the study area is located in a

temperate-cold region, where Arctic-subarctic species are common (Parent et al. 2011). The area experiences the influence of the Labrador Current that transports cold waters from the Arctic (Lazier and Wright 1993; Parent et al. 2011).

Objectives and chapter structure

The general aim of this thesis was to draw a comprehensive picture of a deep-sea food web, sampled in the Northwest Atlantic, off the eastern coast of Canada. By combining different techniques, such as gut content, stable isotope, lipid, morphometric, and elemental analyses, I sought to meet the following objectives: (1) characterize the diet of different deep-sea species (Chapters 2 and 4); (2) assess the trophic relationships among the taxa collected (Chapters 2 and 4); (3) explore patterns of variations within and among taxa, in terms of diet and biochemical markers (Chapters 2, 3, 4, and 5); (4) detect organic carbon sources (Chapter 4); and (5) contribute to the understanding of broad geographic trends in the fatty acid and isotopic signatures of deep-sea organisms (Chapter 5). Such findings should make a significant contribution to current knowledge of deep-sea species and food webs, which remains limited; moreover, they will provide unique information on a geographic area that has not yet been explored in these terms. Lastly, this research will be useful in supporting management and conservation efforts in a heavily exploited marine region.

In Chapter 2, I studied the trophic ecology of a deep-sea fish assemblage collected above upper and mid-slope areas, within the study region. The investigation was carried out through the combined use of gut content, stable isotope, and morphometric analyses, and the main objectives were to assess the trophic relationships characterizing the fishes under study, as well as to detect any relationships among

feeding habits, habitats, and species function. A preliminary analysis on possible depth trends was also conducted.

In Chapter 3, I studied total lipid content, and lipid class and fatty acid composition in the entire deep-sea assemblage (138 species from 8 phyla). Samples comprised both vertebrate (e.g. fish) and invertebrate species (e.g. sponges, corals, and molluscs). The main goal of this extensive analysis was to provide baseline data on the lipid composition of deep-sea taxa. Furthermore, certain lipid groups, indicative of energy allocation strategies, physiological status, and nutritional value, were selected and used to study functional diversity across and within phyla. This chapter discusses both the environmental and biological factors that may drive the variability in the lipid and fatty acid composition of the taxa analyzed, and presents novel information for certain deep-sea species.

In Chapter 4, stable isotope, elemental, and fatty acid analyses were combined to explore the biochemical composition of deep-sea benthic species belonging to 7 focal phyla. Specifically, my goals were to characterize diet and trophic position of these species, as well as to study the fate of certain fatty acids, indicative of energy and nutrients, along the food web. As in Chapter 2, I ran an analysis of possible depth effects on the biochemical variables to help interpret the results. This study described the trophic relationships among the taxa analyzed; showing that not only energy, but also essential nutrients, were transferred throughout the food web; and presenting novel information such as the very long chain fatty acid composition of deep-sea sponges and corals.

Lastly, Chapter 5 used the findings presented in Chapters 2, 3 and 4, and compared them with those of previous studies conducted in other deep-water environments, aiming to detect large-scale (i.e. latitudinal and longitudinal) trends in the

isotopic and fatty acid composition of deep-sea species, as well as common patterns in deep-sea food webs.

Chapter 2 was published in October 2017 in the journal Marine Biology, whereas Chapter 4 is “Under revision” in Progress in Oceanography. A version of Chapter 3 was submitted to the journal PLoS One and was pending revision at the time of the publication. Lastly, Chapter 5 will be prepared for submission as a review paper. I also co-authored another lipid paper relevant to this thesis that is provided in Appendix 7-1.

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Tables

Table 1-1 List of deep-sea habitats, together with corresponding information of volume/area occupied (km^3/km^2), coverage (%), and proportion investigated (%). Modified from Ramirez-Llodra et al. (2010).

Habitat	Volume/Area occupied	Coverage	Proportion investigated
Deep-water pelagic system	$1.0*10^9$ km^3	73% of ocean water	< 0.0001%
Abyssal plains	$2.4*10^8$ km^2	75% of ocean floor	0.0001%
Continental slope (from 150 to 3500 m depth)	$4.0*10^7$ km^2	11% of ocean floor	< 1%
Mid-ocean ridge system	$3.0*10^7$ km^2	9.2% of ocean floor	10%
Seamounts	$8.5*10^6$ km^2	2.6% of ocean floor	0.25-0.28%
Hadal zone	~37 trenches (area not estimated)	1% of ocean floor	Minimal
Canyons	~448 canyons (area not estimated)	Unknown	Minimal
Oxygen minimum zone	$1.1*10^6$ km^2	0.35% of ocean floor	< 1%
Cold-water coral reefs	$2.8*10^5$ km^2	0.08% of ocean floor	Minimal
Hydrothermal vents	~2000 vents (area not estimated)	Unknown	10%
Cold seeps	$1.0*10^4$ km^2	0.003% of ocean floor	2%

Table 1-2 Examples of attributes describing food-web structure, together with their biological significance. Modified from Thompson et al. (2012).

Food-web attribute	Biological meaning
Taxa richness (S)	Number of taxa in the food web
Number of trophic links (L)	Number of directed feeding links between taxa
Linkage density (= L/S)	Number of links per taxon. A measure of mean dietary specialization across the food web
Connectance (= L / [S^2])	Proportion of potential trophic links that do occur
Generality	The mean number of prey per consumer
Vulnerability	Mean number of consumers per prey
Mean chain length	Average number of links found in a food chain across a food web
Maximum chain length	The maximum number of links found in any food chain in a food web
Number of basal taxa (b)	The number of taxa which do not consume any other taxa, by definition autotrophs
Number of intermediate taxa (i)	The number of taxa which are both consumed by, and consume, other taxa
Number of top taxa (t)	The number of taxa which are not consumed by any other taxa
Prey-predator (= {[b + i] / [t + i])	A measure of food-web 'shape'; high values are more triangular, low values are more 'square' in shape. When <1 the food web has an inverted structure that might indicate instability.
Robustness	The minimum level of secondary extinction that occurs in response to a particular perturbation (e.g. species removal)
Degree distribution	The frequency distribution of the number of interactions per taxa (termed its 'degree'). Can identify important interactors such as keystone species
Intervality	The degree to which the prey in a food web can be ordered so that the diets of all species are placed contiguously within a single dimension

Figures

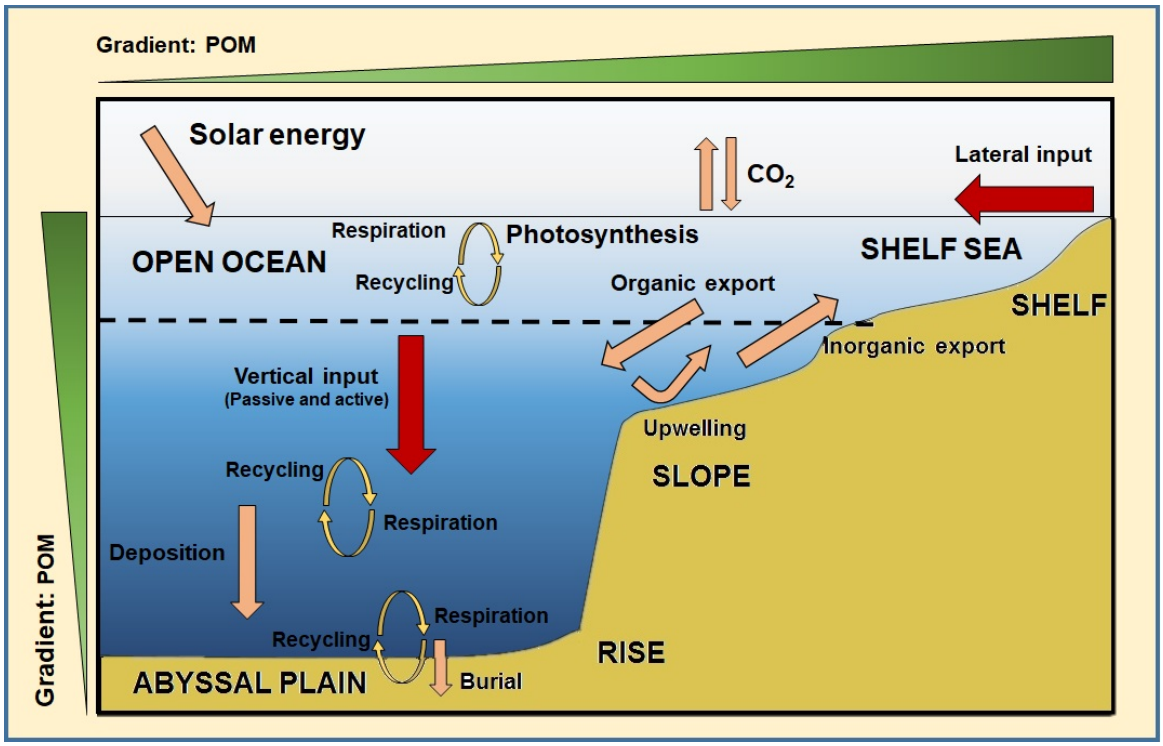

Fig. 1-1 Main biogeochemical and physical forces (pink arrows) involved in food transport (red arrows) at a continental margin. Gradient vectors (green wedges) represent the diminishing quality and quantity of particulate organic matter (POM) moving from the shelf to the open sea, and from shallow waters to the deep sea. Modified from Pfannkuche (2005).

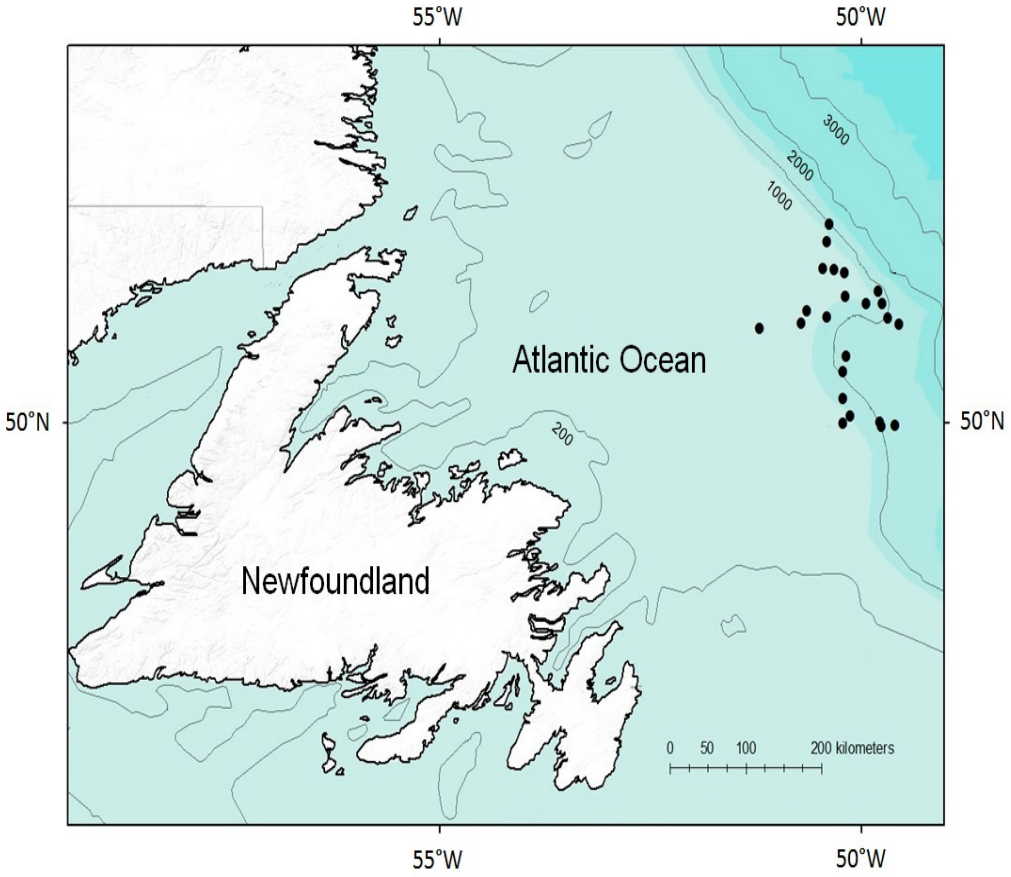

Fig. 1-2 Map of sampling sites off the northeastern coast of the Province of Newfoundland and Labrador (Northwest Atlantic). Dots (•) represent the locations of the sampling tows, and isobaths at 200,1000, 2000, and 3000 m are indicated by grey lines.

Co-authorship statement

This research was developed and carried out by Camilla Parzanini, together with the supervisory assistance of Annie Mercier and Chris Parrish, and the guidance of Jean-François Hamel. Jean-François Hamel was also responsible for animal collection during the expedition on board the CCGS *Teleost*, while Camilla Parzanini completed tissue collection, once animals were in the lab, and carried out all the sample processing and data analysis. The research papers prepared as an outcome of this thesis were written/assembled by Camilla Parzanini, and adjusted/edited with input from co-authors as follows:

Authorship for publication arising from Chapter 2, 3, 4, and 5 are C. Parzanini, C. C. Parrish, J-F. Hamel, A. Mercier.

The manuscripts in Chapters 2 and 4 have been prepared and formatted according to the style of the various scientific journals selected for publication.

Chapter 2 : **Trophic ecology of a deep-sea fish assemblage in the Northwest Atlantic**[1]

[1] A version of this manuscript was published in the journal Marine Biology in 2017 [Mar Biol 164(10):206].

Abstract Understanding the trophic ecology of deep-sea communities provides valuable insight into deep-water ecosystem functioning, and can help inform fisheries management and conservation initiatives. However, few deep-sea food webs have been studied so far in the Northwest Atlantic. Here, stable isotope, gut content, and morphometric analyses were combined to explore trophic relationships in a deep-water fish assemblage off eastern Canada. While there was a weak depth effect in the isotopic composition of the species analyzed, isotopic and dietary records revealed the existence of two main, strongly coupled trophic pathways. The pelagic pathway either comprised pelagic fishes (e.g. meso- and bathypelagic species), primarily feeding on zooplankton and fish, or benthopelagic predators that showed a more pelagic-oriented diet. Such fishes displayed the lowest stable N and C isotope ratios. In contrast, demersal fishes representing the benthic trophic pathway had significantly higher values of $\delta^{15}N$ and $\delta^{13}C$, and a taxonomically more benthic-oriented and diverse diet. Furthermore, smaller body sizes, larger mouths, and adaptations (e.g. bioluminescent structures and lures) prevailed in the pelagic species, consistent with living in a relatively food-poor environment. The largest average body sizes were found in demersal fishes suggesting enhanced food intake and growth investment for the species. Only juvenile individuals of threatened species, such as *Coryphaenoides rupestris* and *Rajella fyllae* were caught, suggesting vulnerability of such species to commercial fishing.

Introduction

The Atlantic region off Newfoundland in eastern Canada is characterized by high productivity (Snelgrove and Haedrich 1985) that has made the area a historical focus of commercial exploitation, centered particularly on groundfish fisheries until the late 1980s. However, since the commercial collapse of Atlantic cod, *Gadus morhua*, and the establishment of a moratorium in 1992, shellfish (e.g. northern shrimp, *Pandalus borealis*) has become the main target (Sherwood and Rose 2005). Furthermore, due to the severe ecological and socio-economic implications following the collapse of the cod population, several regulations have been introduced to manage fishing activities within eastern Canadian waters, including restricted fishing areas and quotas. However, very little effort has been put towards the protection of habitats and living resources in the deep sea (Baker et al. 2012). In fact, like shallow-water species, deep-water fishes have been affected by intense fishing activities within the region, resulting in drastic depletions of both target (e.g. *Coryphaenoides rupestris, Macrourus berglax*) and non-target stocks (*Malacoraja senta*, *Rajella fyllae*) (Devine et al. 2006; Baker et al. 2009). In addition, cold-water corals and sponge grounds, which are crucial habitats for fish and other biota (Baillon et al. 2012, 2014; Murillo et al. 2011) common in shelf and slope areas off Newfoundland (Sherwood et al. 2008; Murillo et al. 2011, 2012), are increasingly under threat. For instance, Murillo et al. (2011, 2012) showed that the biomass of sponges and corals was significantly lower in regularly exploited fishing areas, compared to untrawled or moderately trawled regions. The only regulation in place consists of a list of fish species “at risk” (COSEWIC 2016), and the designation of a few non-fishing areas by the Northwest Atlantic Fisheries Organization (NAFO) and Fisheries and Oceans Canada (DFO). In comparison, the deep-sea fish communities in the Northeast Atlantic are

among the most studied and well understood (Campbell et al. 2011; Godbold et al. 2013), and this information has allowed the establishment of a more solid management plan, which includes limitations on fishing efforts, total allowable catches, and fishing depth (Clarke et al. 2015). A better understanding of the trophic ecology of deep-water fish communities in the Northwest Atlantic could help devise more efficient conservation and protection programs.

In the last few decades, there has been growing attention devoted to food webs in marine conservation, as they have allowed a deeper understanding of communities and ecosystems (Iken et al. 2001; Reid et al. 2012), and informed management decisions (Rombouts et al. 2013). Current knowledge of food webs and trophic relationships has been gathered using different approaches and techniques, each with its own strengths and limitations. Analysis of gut contents is the most traditional approach (Gartner et al. 1997), and it has often been undervalued as providing mere snapshots of the diet (Hussey et al. 2014). Gathering robust dietary data requires extensive sampling, which is not always feasible in remote environments like the deep sea. Furthermore, the analysis involves considerable processing time, and taxonomic expertise. Nonetheless, this technique remains a cornerstone in trophic ecology, because it provides direct evidence, with a high taxonomic resolution, of what animals have recently eaten. Among alternative solutions, bulk stable isotope analysis has become a well-established method over the last decades (Altabet et al. 1999; Iken et al. 2001; Mintenbeck et al. 2007). In particular, stable carbon and nitrogen isotope ratios have successfully been used to evaluate ecosystem functioning and dynamics (McConnaughey and McRoy 1979), time-integrated diet records (Sherwood and Rose 2005), trophic position (Gale et al. 2013; Hussey et al. 2014), food-web structure (Iken et al. 2001; Polunin et al. 2001; Reid et al. 2012), as well as energy sources and carbon

flows (Trueman et al. 2014). Due to its stepwise enrichment between source and consumer (from 2 to 4‰; Minagawa and Wada 1984; Iken et al. 2001), the nitrogen stable isotope ratio ($^{15}N/^{14}N$ or $\delta^{15}N$) is an indicator of trophic position. Conversely, the carbon stable isotope ratio ($^{13}C/^{12}C$ or $\delta^{13}C$) undergoes smaller fractionation (i.e. increment of isotopic ratio from prey to predator; < 1‰) and, therefore, is mainly used to investigate the origin of the carbon source (Polunin et al. 2001). However, the interpretation of stable isotope data is complex due to several assumptions and implications (e.g. choice of food-web baseline, biological variability within and among taxa, and with the fractionation processes). A further approach to feeding ecology is through the analysis of body shape and traits (Scharf et al. 2000) to help understand the functional role of species within assemblages (Mindel et al. 2015, 2016). In the aquatic environment, body size and shape influence lifestyle, swimming mode, microhabitat use, and interactions (Ward and Metha 2010; Mindel et al. 2015, 2016). For example, body size of both predator and prey are closely linked to foraging success and prey escape abilities, respectively (Scharf et al. 2000). In addition, fishes go through several developmental stages during their life, experiencing changes in size, habitat, diet, and feeding behavior (Mauchline and Gordon 1985; Reid et al. 2013). In demersal fish species, the embryos, larvae and/or juveniles are often pelagic, whereas the adults are benthopelagic (Drazen and Sutton 2017). Moreover, the presence of special features (e.g. lures, photophores, and other bioluminescent structures) or modifications of the head, mouth, teeth and jaws, which have been described in deep-sea species (Ebeling and Cailliet 1974), may also provide insights into feeding interactions. In order to acquire reliable and high-resolution dietary data, as well as to overcome issues and limitations related to each method, the current trend in trophic ecology is to combine two or more techniques (Churchill et al. 2015). In the present study, stable isotopes were used in

combination with analysis of gut contents and morphological traits, to explore trophic relationships and gain new insights into a deep-sea fish assemblage sampled from upper and mid-slope areas in the Northwest Atlantic.

Variations in both isotopic ratios and composition of deep-water demersal communities have been shown to be depth-dependent (Polunin et al. 2001; Bergmann et al. 2009; Mindel et al. 2016). In fact, particulate organic matter (POM), which represents the main food source in deep-water systems, undergoes microbial degradation, hence isotopic fractionation, while sinking from surface water. Due to the preferential assimilation of the lighter stable N isotope (^{14}N) by microbes, POM is enriched in ^{15}N along a depth gradient (Altabet et al. 1999), and this trend can be reflected in the $\delta^{15}N$ composition of benthic consumers (Mintenbeck et al. 2007), with cascading effects along the food web (Iken et al. 2001; Trueman et al. 2014). Depth-related patterns have also been detected for morphological traits, such as body size and shape, which affect assemblage function and composition (Mindel et al. 2015, 2016). In addition, POM settling on the seafloor undergoes further degradation and, therefore, is more enriched in ^{15}N and ^{13}C than sinking POM. For this reason, organisms that primarily rely on settled POM display higher $\delta^{15}N$ and $\delta^{13}C$ than those feeding upon sinking POM, and two different trophic pathways, a pelagic and a benthic one, may be recognized within the same food web (Iken et al. 2001; Drazen et al. 2008).

The main objectives of the present investigation were to (1) assess the trophic structure of the fish assemblage sampled, which comprised both demersal and pelagic species; and (2) tease out any relationships among feeding habitat and species function. Depth effects on fish isotopic composition and size were explored as a preliminary analysis, followed by the examination of trophic structure and body traits as determinants of functional roles. As depth trends have been found in the isotopic composition of POM

and POM consumers, similar trends are expected for the fishes analyzed in this study when different functional groups are considered.

Methods

Sampling

Sampling centered on the by-catch of annual research surveys operated by DFO, Newfoundland Region, from November 30 to December 6, 2013. The sampling area, referred to as NAFO Division 3K, is located in the Northwest Atlantic Ocean, off the coast of insular Newfoundland, Canada (Fig. 2-1). Multiple species were collected through standard bottom trawl surveys conducted by the CCGS *Teleost* research vessel, following a stratified random design with a minimum of two sets per stratum, and tow durations of ~15 min (~4.8 km h^{-1} gear opened and closed at sampling depth). Further details on the gear used (Campelen 1800 shrimp trawl) can be found in McCallum and Walsh (1999). Samples were collected from a total number of 17 tows, between 310-1413 m depths (Table 2-1). Once on board, organisms were immediately vacuum packed and frozen at -20°C until analysis. Taxa were identified to species level directly on board or through photo-identification of frozen individuals. Total wet mass and total length (from the tip of the snout to the most posterior point of the caudal fin) were recorded for each fish as soon as they were removed from the freezer. Total length measurements were then compared to biological data from the literature (Froese and Pauly 2016), to assess whether individuals were juveniles or adults. A total of 43 species, belonging to 2 different classes, 15 orders, and 25 families were used in this study. The rare or sporadic occurrence of certain deep-sea fishes, together with the logistical constrains associated with deep-water sampling, resulted in low sample sizes

for some species (Table 2-2), which is not uncommon in studies of deep-water food webs (Iken et al. 2001; Polunin et al. 2001; Gerringer et al. 2017). In detail, about 25% of the species investigated are either considered rare or occur at low abundances according to previous studies; among them, *Lepidion eques* (Coad and Reist 2004; Baker et al. 2012) *Synaphobranchus kaupi* (Snelgrove and Haedrich 1985), *Alepocephalus bairdii* (Snelgrove and Haedrich 1985; Coad and Reist 2004), *Magnisudis atlantica*, *Trachyrincus murrayi*, *Amblyraja jenseni*, *Anoplogaster cornuta*, *Caristius* sp., *Cyclothone microdon*, *Glyptocephalus cynoglossus*, *Oneirodes* sp., and *Xenodermichthys copei* (Coad and Reist 2004). The combination of different methods was therefore fundamental to our approach; i.e. cross-referencing information retrieved from each technique increased the reliability of the results, especially for the few species in which a limited number of individuals were collected. Moreover, findings were compared to published literature on similar species for further validation.

Stable isotope analysis

White muscle was purposely selected because this tissue is characterized by low turnover rates and, therefore, it integrates diet records over a longer time (i.e. from weeks to months; Iken et al. 2001). Tissue was collected close to the anterior part of the dorsal fin, as recommended by previous investigators (Iken et al., 2001; Hoffman and Sutton 2010), dried in an oven at 70°C for 24 h, and ground into a fine powder using a mortar and pestle. Subsamples of 1 mg were analyzed for $\delta^{13}C$ and $\delta^{15}N$, as well as total C and total N, with a continuous-flow isotope-ratio mass spectrometer (Delta V Plus, Carlo Erba) at the Earth Resources Research and Analysis facility of Memorial University. Stable isotope ratios are expressed in the conventional (δ) notation as parts per thousand (‰), following the equation:

$$\delta^{13}C \text{ or } \delta^{15}N(‰) = [(R_{sample} / R_{standard}) - 1] \times 1000$$

where R_{sample} is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Results are reported relative to atmospheric N_2 for nitrogen stable isotopes, and Vienna PeeDee Belemnite (VPDB) for carbon stable isotopes. Internal and external reference material was used to calibrate the mass spectrometer data. EDTA and D-fructose were run for stable carbon isotope calibration, and IAEA-N-2 and EDTA for nitrogen isotopes. Additional standards were also used during the analysis. L-valine, USGS-24 graphite, IAEA-CH-6 sucrose, LSVEC, MUN-CO-1, and MUN-CO-2 were used for stable carbon isotopes. IAEA-N-1, USGS-25, USGS-26, and L-valine were used to assess accuracy and precision of stable nitrogen isotope data. B2155 protein was used as a standard for $\delta^{13}C$ and $\delta^{15}N$. The average standard deviation of selected replicates was ± 0.2‰ for $\delta^{13}C$ and ± 0.1‰ for $\delta^{15}N$.

C and N elemental proportions (%) were also analyzed in conjunction with stable isotopes, and were used to calculate C:N by mass and molar C:N ($C:N_{mol}$), to estimate the lipid content in fish muscle samples, and to lipid correct $\delta^{13}C$ data. For elemental calibration of N and C, sulfanilamide was used, whereas B2155 protein was run as reference material. The average standard deviation was ± 2.0 for %C and ± 0.6 for %N.

In line with previous results (Iken et al. 2001; Reid et al. 2013) and because of contradictory implications of defatting samples prior to analysis (Hoffman and Sutton 2010), lipids were not extracted in this study. However, the elemental C:N ratio of each individual was used to evaluate whether lipids might have affected their stable C isotope composition. In general, values of C:N by mass ≥ 3.5 indicate that lipid normalization should be applied (Post et al. 2007). In our dataset, the mean C:N was higher than this value whether or not elasmobranchs were included in the calculation (4.4 ± 2.8, comprising of elasmobranchs data; 5.0 ± 2.7, elasmobranchs excluded). Therefore, the following equation developed for multispecies data sets of deep-sea fishes was applied

(Hoffman and Sutton 2010):

$$\delta^{13}C_n = \delta^{13}C + [-6.39‰ * (3.76 - C{:}N_{mol})] / C{:}N_{mol}$$

where $\delta^{13}C_n$ and $\delta^{13}C$ are the lipid-normalized and the untreated carbon isotope ratios respectively, and $C{:}N_{mol}$ is the molar elemental ratio of the individual. $\delta^{13}C$ prior to normalization ranged from -25.0 to -16.7‰, whereas the $\delta^{13}C_n$ values ranged from -24.2 to -15.9‰. The lipid corrected values are size-independent.

A total of 5 species of elasmobranchs were sampled: the Squaliformes *Apristurus profundorum*, *Centroscyllium fabricii*, and the Rajiformes *Amblyraja jenseni*, *Malacoraja senta*, and *Rajella fyllae*. Elasmobranchs generally retain urea and trimethylamine oxide (TMAO) in their tissues as a buoyancy control mechanism. Since both urea and TMAO are ^{15}N-depleted, both the stable N isotope and C:N ratios of elasmobranchs may be affected when analyzing their isotopic compositions (Kim and Koch 2012; Hussey et al. 2012; Churchill et al. 2015). For the former issue, two approaches are suggested when studying the stable nitrogen isotope composition of elasmobranchs: the removal of both lipids and urea from muscle tissue prior to analysis or the use of arithmetic corrections. While these methods were explored, a decision was ultimately made not to use any treatment or correction because: 1) dissimilar results were obtained in previous studies attempting to compare bulk $\delta^{15}N$ *vs* treated $\delta^{15}N$ data from muscle tissues of various elasmobranch species; more (by 1.3‰; Kim and Koch 2012) or less (by 0.6 ± 0.6‰; Hussey et al. 2012) pronounced increases were reported, suggesting species-specific variability in urea and TMAO concentrations in shark muscle tissues (Hussey et al. 2012); 2) the quantity of urea and TMAO varies among elasmobranchs and species-specific discrimination factors need to be experimentally determined and used to assess trophic position (Kim and Koch 2012); 3) only a few elasmobranchs were present among the focal species, with relatively few individuals per species, providing no opportunity to

either assess variations in $\delta^{15}N$ data or discrimination factors; and 4) when attempting a mathematical correction using factors provided by Kim and Koch (2012) or Hussey et al. (2012), the differences between bulk and corrected values of trophic position were minimal (i.e. 0.3 and 0.2). The presence of urea and TMAO may also affect C:N, and create further obstacles when applying mathematical corrections for lipids based on those ratios. In fact, the correction we used in this study was developed by Hoffman and Sutton (2010) for a deep-sea fish dataset devoid of elasmobranchs. However, because of 1) the relative rarity of elasmobranchs in our samples, and 2) the small difference between the mean values of uncorrected *vs* corrected $\delta^{13}C$ measured (i.e. 0.5), lipid correction was applied to elasmobranch samples as well. Nonetheless, caution is needed when interpreting these data for elasmobranchs.

Species trophic position ($TP_{consumer}$) was based on the equation used by Gale et al. (2013), following Cabana and Rasmussen (1996):

$$TP_{consumer} = [(\delta^{15}N_{consumer} - \delta^{15}N_{base}) / \Delta^{15}N] + TP_{base}$$

where $\delta^{15}N_{consumer}$ is the mean stable N isotope ratio of each species; $\Delta^{15}N$ is the fractionation factor which, as applied to polar and deep-sea regions (Iken et al. 2005), corresponds to 3.8‰ in this study. $\delta^{15}N_{base}$ and TP_{base} respectively represent the nitrogen stable isotope composition and trophic position of the base of the food web. For the present investigation, crustacean zooplankton collected above shelf-edge areas off Newfoundland was used as the base of the food web. Values of $\delta^{15}N_{base}$ and TP_{base} relative to such base (i.e. 9.0‰ and 2.3, respectively) were obtained from Sherwood and Rose (2005). This choice was made to allow for comparisons with studies conducted by Sherwood and Rose (2005) and Gale et al. (2013) within the same geographic area.

Gut content analysis

Gut contents refer to all items present in the stomach and/or the intestine, in cases where the stomach was either empty or absent; to avoid biases due to post-capture ingestion, items found in the mouth or esophagus were not considered. Although different digestion rates exsist between stomach and intestine (Hynes 1950), potentially leading to the over-representation of certain food items, we chose to analyze the whole gastrointestinal tract to maximize the analysis of samples which are difficult to collect. The stomach and intestine were both removed from each individual, once completely thawed. To collect the entire content and prevent any invasive manipulation, each stomach was carefully cut open and its wall rinsed with filtered seawater (FSW), whereas FSW was directly pumped into the intestine. Effluents were then gathered in a petri dish and analyzed under a stereomicroscope (Nikon SMZ1500). In cases where stomach contents were not available (i.e. missing or damaged stomach), intestinal contents alone were analyzed. Food items, consisting of complete prey items and/or fragments, were recorded and identified to the lowest possible taxonomic level through direct observation and photo-identification using a digital camera (Nikon DXM1200F), coupled to the stereomicroscope and ACT-1 imaging software. The presence of sediment particles and undefined/highly digested material was also noted. Once analyzed, contents were immediately preserved in 75% ethanol. The frequency of occurrence of the main food-categories was subsequently calculated, based on the absence or presence of food items within the gut. Specifically, the frequency of occurrence refers to the percent number of fishes containing a specific food item, out of the total number of individuals with food in their gastrointestinal tract and, in this study, it was used to evaluate the diet composition of the fish assemblage, as well as of each trophic group. Although

apparently simple, the index has been considered a valuable and accurate descriptor of the diet composition (Baker et al. 2014). In fact, it has been shown that the occurrence technique provides similar results to those of more complex methods (Hynes 1950), even with relatively small sample sizes (Baker et al. 2014). Lastly, the various food-categories found in each species were summed and used as a proxy of diet diversity.

Biological data and morphological analysis

Fish species were divided according to their habitat into demersal or pelagic. Specifically, demersal species are those either living directly above (i.e. epibenthic) or near (i.e. benthopelagic) the ocean bottom, and mainly feeding upon epi- and hyperbenthic fauna (Gartner et al. 1997; Drazen and Sutton 2017). By contrast, pelagic species strictly live and feed within the water column; included within this category, for example, are zooplanktivores such as lantern fishes, as well as nektonectivores such as dragon fishes (Table 2-3). In this study, information on habitat was acquired from Drazen and Sutton (2017) and Coad and Reist (2004).

In addition, morphological characters that might influence the size and type of prey captured were assessed for each species. Body shape descriptors included: (1) laterally compressed, with maximum height greater than maximum width, as per Ribeiro et al. (2016); (2) flat, including laterally compressed or ventrally depressed species with eyes facing upward (e.g. halibut, skates); (3) spherical, with height similar to total length; and (4) elongate, with high ratios of standard length to height and greater number of vertebrae, as described by Reece and Metha (2013), including fusiform, rattail and anguilliform shapes. In addition, mouth size was scored as either small or large, depending on whether it extended beyond the middle of the eye, and the presence or absence of anatomic features (e.g. protruding jaws) favouring a wider oral opening was

noted. Finally, the presence of lures, photophores, bioluminescent organs or any other specialized feature was recorded. These morphological characters (presence or absence) were combined with the number of major food items retrieved in each species (i.e. diet diversity) into Table 2-3.

Statistical analysis

Spearman rank-order correlations were used to assess the existence of any depth effect on stable isotope ratios ($\delta^{15}N$, and lipid-normalized $\delta^{13}C_n$) across all samples, as well as in pelagic *vs* demersal species. Spearman rank-order was also performed to assess relationships between $\delta^{15}N$ and $\delta^{13}C_n$ in all individuals, and between stable isotope ratios ($\delta^{15}N$, $\delta^{13}C_n$) and fish size (i.e. total length and wet mass). Hierarchical cluster analysis (Ward's method of linkage, Euclidean distance) was performed on untransformed $\delta^{15}N$ and $\delta^{13}C_n$ data to identify functional groups or trophic niches among the species under study. According to Layman et al. (2007), the combination of the two ratios represents the trophic signature of a given organism, and species that display similar trophic signatures are assumed to belong to the same trophic niche, thus playing a similar functional role within an ecological system. After confirming data normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test), one-way analysis of variance (ANOVA) was used to study isotopic variability among the detected functional groups. Post-hoc Tukey tests were conducted to assess pairwise differences. In addition, multivariate statistics were run to further explore differences within the fish assemblage. In detail, PERMANOVA (permutational multivariate ANOVA) was performed to study the variability 1) in the isotopic composition among the functional groups; as well as 2) in the diet and 3) in the morphology (i.e. total length, wet mass, body shape, mouth size, and presence of special organs), by using both functional group and habitat (pelagic *vs*

demersal) as factors. Multivariate statistics (principal components analysis, PCA, and PERMANOVA) was also used was on a matrix of 31 species to investigate associations among normalized values of diet diversity, fish size (i.e. total length and wet mass), and isotopic ratios ($\delta^{15}N$, $\delta^{13}C_n$). In this analysis, only species represented by confirmed adults with gut contents were included; therefore, 12 species were removed from the dataset. Univariate statistics were conducted in SigmaPlot 11.0, whereas hierarchical cluster analysis was run using Minitab 17, and PCA and PERMANOVA were performed with PRIMER 6 + PERMANOVA software.

Results

Stable isotope ratios

Mean $\delta^{15}N$ values obtained from fish muscle samples ranged from 8.5 to 15.7‰, and data for the lipid-normalized stable carbon isotope ratios ($\delta^{13}C_n$) varied from -23.6 to -16.0‰ (Table 2-4). While there was no correlation between depth and $\delta^{15}N$ or $\delta^{13}C_n$ for pooled individuals, there was a weak but positive correlation between depth and isotopic ratios in pelagic species ($\delta^{15}N$, $r_s = 0.3$, $n = 48$, $P = 0.021$; $\delta^{13}C_n$, $r_s = 0.3$, $n = 52$, $P = 0.007$). A significant positive correlation was also found between $\delta^{15}N$ and $\delta^{13}C_n$ ($r_s = 0.7$, $n = 106$, $P < 0.001$). In addition, values of both $\delta^{15}N$ and $\delta^{13}C_n$ correlated with fish total length ($\delta^{15}N$, $r_s = 0.3$, $n = 102$, $P = 0.001$), and wet mass ($\delta^{15}N$, $r_s = 0.4$, $n = 102$, $P < 0.001$; $\delta^{13}C_n$, $r_s = 0.2$, $n = 102$, $P = 0.028$).

Hierarchical cluster analysis of isotopic ratios revealed the existence of four main functional groups of species (G1 - G4; Fig. 2-2), which were statistically different in terms of both $\delta^{15}N$ (ANOVA, $F_{3,39} = 105.2$, $P < 0.001$) and $\delta^{13}C_n$ (ANOVA, $F_{3,39} = 68.2$, $P < 0.001$). Species clustered within G1 (Fig. 2-3) were characterized by the lowest values of

both $\delta^{15}N$ and $\delta^{13}C_n$, with the former ranging from 8.5 to 10.9‰, and the latter from -20.5 to -19.1‰. Furthermore, analyses positioned the species in G1 at the lowest trophic positions (TP), with values between 2.2 and 2.8 (ANOVA, $F_{3,39} = 105.2$, $P < 0.001$; Table 2-4). Conversely, species in G4 exhibited the highest ratios for both $\delta^{15}N$ and $\delta^{13}C_n$, with mean values ± SD of 14.3 ± 0.2‰ and -17.3 ± 0.5‰, $n = 7$, respectively, as well as the highest TP (Fig. 2-3). Intermediate stable isotope ratios and, consequently, TP were exhibited by the species clustered in G2 and G3. Specifically, fishes in G2 showed significantly higher values of $\delta^{15}N$ (11.6 ± 0.1‰, $n = 14$; Tukey test, $q = 11.1$, $P < 0.001$) and TP scores (from 2.9 to 3.2; $q = 11.1$, $P < 0.001$) than those of the fishes in G1 (Table 2-4). However, there was no statistical difference in terms of $\delta^{13}C_n$. While stable isotope ratios and TP of the fishes clustered in G3 were significantly higher than those in G2 (for $\delta^{15}N$, 13.4 ± 0.2‰ and TP, 3.5 ± 0.1‰, $q = 9.2$, $P < 0.001$; for $\delta^{13}C_n$, -18.1 ± 0.6‰, $q = 6.6$, $P < 0.001$), the difference between species in G3 and G4 was less marked (for $\delta^{15}N$ and TP, $q = 3.9$, $P = 0.043$; for $\delta^{13}C_n$, $q = 10.4$, $P < 0.001$). PERMANOVA pairwise comparisons confirmed that the four groups of species were significantly different from each other in terms of isotopic composition ($P \leq 0.0005$).

Gut contents

Of the 106 fishes analyzed, 88 individuals belonging to 40 different species had contents in either their stomachs or intestines, whereas 28 were found with empty ($n = 8$), everted ($n = 14$), or missing ($n = 6$) stomachs. No contents were retrieved from *Glyptocephalus cynoglossus*, *Haplophryne mollis*, and *Notacanthus chemnitzii* (Table 2-2).

Once identified, food items were categorized using the main taxonomic groups Bivalvia, Cephalopoda, Chaetognatha, Crustacea, Gastropoda, Polychaeta, Pycnogonida, as well as fish, sediment, and other (Table 2-5). The last category included

all those taxa with a rare occurrence (< 3%), such as protozoans and sponges. Overall, crustaceans (56%), and fishes (23%) were the most frequent food items found in the gastrointestinal contents of the 88 fishes in which contents were found. Crustaceans were mostly found in the form of exoskeletons or broken appendages (full or partial); among them, Amphipoda, Copepoda, Lophogastrida and Misidacea species were the most common and, to a lesser extent, individuals of caridean shrimps were also retrieved. Fish remains represented by bones, spines, scales, eye lens, and muscle tissue could not be further characterized. Ingestion of polychaetes was confirmed by the presence of jaws, teeth, and soft body parts such as parapodia, with a frequency of occurrence of 16%. Cephalopod beaks, pens, and skin also occurred within gut contents (14%), while the remaining food categories occurred to a much lesser extent. Specifically, pycnogonids accounted for 6%, chaetognaths for 6%, shells of bivalves for 3%, sediment particles and skeletons of protozoans for 2%. Other food items detected on rare occasions included the columella pillar of a gastropod shell found in *Cottunculus microps*, and sponge spicules in *Gaidropsarus ensis* and *Trachyrincus murrayi* (Fig. 2-4).

Fig. 2-5 visually summarizes the gut contents of the species clustered in each isotopic group or trophic niche. Species clustered in G1 and G2 mainly fed on zooplankton (e.g. crustaceans, such as Copepoda, Mysidiacea, and Lophogastrida, and chaetognaths), and/or fish. Crustacean prey occurred with frequencies of 35 and 39%, and chaetognaths with frequencies of 6 and 9%, in the stomachs of the fish species in G1 and G2, respectively. Cephalopods were also found within the gut contents of the species in G2 (9%). Fishes grouped in G3 displayed greater diet diversity, represented by crustaceans (Mysidiacea, Spaelogriphacea, Amphipoda, and Lophogastrida; 64%), fish (36%), and cephalopods (23%), together with polychaetes (23%), chetognaths (5%), and pycnogonids (5%). Lastly, the diet of fishes in G4 was the most diverse: *C. microps*,

Macrourus berglax, and *G. ensis* had most food-categories represented in their gut contents (Table 2-3). As in the previous groups, crustaceans were the most frequent prey items (77%) in the stomachs of the fishes in G4, followed by polychaetes (35%), cephalopods (27%) and pycnogonids (19%). Furthermore, most of the food items found in the gut contents of G4 were clearly of benthic origin, including gastropods, sponges, and pycnogonids, as well as sediment. PERMANOVA showed that gut contents were significantly different in the four isotopic functional groups (PERMANOVA, pseudo-$F_{3,35}$ = 2.1, P = 0.042), and especially across the two habitats (PERMANOVA, pseudo-$F_{1,35}$ = 3.7, P = 0.016).

Biological data and morphological analysis

A total of 15 individuals belonging to five species (i.e. *Alepocephalus bairdii*, *n* = 2, *Coryphaenoides rupestris*, *n* = 2, *Macrourus berglax*, *n* = 4, *Rajella fyllae*, *n* = 4, and *Synaphobranchus kaupii, n* = 3) were determined to be juveniles based on maturity-length measurements reported in Fish Base (Froese and Pauly 2016). In particular, it is known that females of *A. bairdii* mature at a standard length (i.e. from the tip of the snout to the foremost end of the last vertebra) of 55 cm (Froese and Pauly 2016). Here, both individuals were juveniles with total lengths of 38.5 and 22.9 cm, respectively. Similarly, all the individuals of the Macrouridae *C. rupestris* and *M. berglax* were juveniles. While the mean lengths of *C. rupestris* and *M. berglax* analyzed here were 29.1 ± 4.2 and 31.2 ± 5.4 cm, respectively, their minimum standard length at maturity is reportedly 50.5 cm (Iwamoto 2015) and 54.3 cm (Froese and Pauly 2016). The skate *R. fyllae* reaches maturity at a mean total length of 49.5 cm in females and 45.5 cm in males (Kulka et al. 2009); all the individuals analyzed here were ~11 cm in length. Lastly, no information about length at first maturity was available in the literature for *S. kaupii*; however,

individuals may reach a maximum length of 100 cm (Reiner 1996), while the three analyzed here ranged from 45.4 to 54.5 cm and where thus considered juveniles.

Pelagic species had the highest frequency of individuals characterized by larger mouths (50%) and the presence of specialized organs (33%), such as the lures and photophores found in *Chauliodus sloani* and *Borostomias antarcticus*; whereas 11% of the demersal species had wide mouths, including the sculpins *Cottunculus microps* and *Cottunculus thompsonii*, and only *Centroscyllium fabricii* had special anatomical features (Table 2-3). With respect to body shape, demersal species either had elongate or flat bodies, whereas pelagic species exhibited more variable shapes, in addition to the elongated one; for example, the body of *Anoplogaster cornuta* and *Caristius macropus* is laterally compressed, and *Oneirodes macrosteus* and *Melanocetus johnsoni* are both spherical (Table 2-3). Such differences in morphology between demersal and pelagic species were further detected by PERMANOVA analysis (pseudo-$F_{1,35} = 4.3$, $P < 0.001$), whereas no significant differences were found between functional groups. On the other hand, in analyses of full-sized adults, both habitat and functional group explained significantly the variability of the fish assemblage sampled, with the first factor contributing the most (PERMANOVA, pseudo-$F_{1,25} = 6.6$, $P = 0.001$). Furthermore, PCA showed that demersal species were larger overall in size, had a more diverse diet, and higher values of stable N and C isotope ratios. By contrast, the pelagic species had the lowest scores in size, diet diversity, as well as $\delta^{15}N$ and $\delta^{13}C_n$ (Fig. 2-6).

Discussion

Depth effect

Depth represents a major abiotic factor in deep-water systems, and depth-related trends have been reported in the isotopic composition of deep-sea fish communities (Polunin et al. 2001; Mintenbeck et al. 2007; Bergmann et al. 2009), and their function (Mindel et al. 2015, 2016). While the present study did not detect any global relationship between depth and stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C_n$) in pooled samples, as expected, a separate analysis of demersal and pelagic species revealed a weak positive depth effect in the latter. This result is consistent with the effect of depth on the isotopic composition of sinking particulate organic matter (POM; Altabet et al. 1999). Since $\delta^{15}N$ and $\delta^{13}C_n$ of sinking POM both increase with increasing depth, due to degradation and fractionation processes (Altabet et al. 1999), the same depth trend is expected for benthic POM consumers, with cascading effects (Iken et al. 2001). However, trophic role and position may interfere (Trueman et al. 2014), whereby only consumers feeding upon suspension feeders have been found to exhibit strong positive depth effects on their isotopic ratios (Iken et al. 2001; Mintenbeck et al. 2007). Furthermore, values of $\delta^{15}N$ and $\delta^{13}C_n$ may vary with body size (Badalamenti et al. 2002; Galván et al. 2010; Trueman et al. 2014), which is known to increase with depth in deep-sea fish species (Mindel et al. 2015). In the present study, the interspecific variability in habitat (pelagic *vs* demersal) and trophic position, together with the absence of clear depth-size trends for deep demersal fishes off Newfoundland (Snelgrove and Haedrich 1985) and unequal sampling (not all species were represented at each depth) may explain the lack of any general effect of depth on isotopic composition. A similar lack of depth effect on the isotopic composition was reported in cold-water corals from slope areas off Newfoundland, which were determined

to feed on fresh, fast sinking resuspended POM (Sherwood et al. 2008). In fact, larger particles of POM sink faster than smaller particles, and they hence undergo minor stable N isotope fractionation in comparison to the slow-sinking ones (Altabet et al. 1999). Furthermore, water masses above the Northwest Atlantic slope areas are known to experience blooms of large phytoplankton species (Sherwood et al. 2008).

Trophic structure of the fish community

Two main trophic pathways were revealed within the fish community investigated. The pelagic trophic pathway comprised both pelagic and demersal predators belonging to isotopic groups G1 and G2, whose diet was dominated by zooplankton and nekton. Perhaps because they depend on fresher sinking POM depleted in ^{15}N and ^{13}C as the primary food source, such predators were characterized by low values of $\delta^{15}N$ and $\delta^{13}C_n$, and low TP. The lantern fishes *Lampanyctus* sp., *Myctophum* sp., and *Notoscopelus* sp. were the most representative examples of pelagic feeders in G1 (Haedrich 1996; Sherwood and Rose 2005). Lantern fishes feed on zooplankton and are important components of the diurnal vertical migration fauna that play a key role in transferring food from surface to deep demersal communities (Conley and Hopkins 2004; Trueman et al. 2014). Further examples of true pelagic feeders were *Arctozenus risso*, *Chauliodus sloani*, *Borostomias antarcticus*, *Bathylagus euryops*, *Cyclothone microdon*, *Magnisudis atlantica*, *Malacosteus niger*, *Xenodermichthys copei*, and *Serrivomer beanii*, which have a pelagic distribution and are well known zooplanktonivores and micronektonivores (Haedrich 1996; Coad and Reist 2004; Drazen and Sutton 2017). Still representing the pelagic trophic pathway according to isotopic composition and gut content records, the species clustered in G2 presented higher TP than those in G1. A diet mostly based on other nekton, rather than zooplankton, may explain differences in TP between G2 and

G1. In this regard, *Anoplogaster cornuta* and *Chiasmodon niger* are known predators of the mesopelagic and the bathypelagic zones (Mauchline and Gordon 1984a; Haedrich 1996), with the former being a pelagic generalist and the latter a micronektonivore (Drazen and Sutton 2017). Fish was the only prey type found in the stomachs of *A. cornuta* and *C. niger*, analyzed in this study. In addition to fish, zooplanktonic crustaceans made up the diet of *Bathytroctes macrolepis*, S*copeloberyx opisthopterus*, and *Oneirodes macrosteus*, thus supporting previous dietary records ascribing them a pelagic-based diet (Coad and Reist 2004). Although no dietary information was obtained from the analysis of gut contents of *Haplophryne mollis* and *Lampadena speculigera*, there are indications from the literature of pelagic-feeding habits for both species (Coad and Reist 2004, Drazen and Sutton 2017).

Apart from pelagic species, several demersal representatives were included in G1 and G2, based on their isotopic composition. These demersal species were shown, and confirmed by the existing literature (Coad and Reist 2004, Drazen and Sutton 2017), to either have pelagic or benthopelagic feeding habits, presumably explaining their low values of stable N and C isotope ratios. As for *Alepocephalus bairdii* and *Coryphaenoides rupestris*, their low $\delta^{15}N$ and $\delta^{13}C_n$ are likely an outcome of the smaller size and early age characterizing the individuals analyzed here (see below), although the values are consistent with those reported by Trueman et al. (2014) within a deep demersal fish community in the Northeast Atlantic. The Greenland halibut *Reinhardtius hippoglossoides* was also clustered together with pelagic species. Despite a flatfish morphology consistent with a benthic lifestyle and diet, the isotopic composition of *R. hippoglossoides,* in combination with records from the literature, ascribed it a pelagic occurrence and diet. Capelin (*Mallotus villosus*), for example, dominated the diet of *R. hippoglossoides* sampled in the Newfoundland shelf and slope areas (Bowering and Lilly

1992). On the other hand, among the benthopelagic representatives, *Sebastes mentella*, an important commercial species, *Antimora rostrata*, as well as deep-sea sharks and skates were included in G2. In line with existing literature, indicating benthopelagic dietary habits (Coad and Reist 2004; Drazen and Sutton 2017), crustaceans, chaetognaths, and polychaetes occurred within the stomach of *S. mentella* and *A. rostrata* analyzed in this study. Traces of crustaceans, fishes, and cephalopods were retrieved in addition to pycnogonids and polychaetes within the stomach of the deep-water elasmobranchs *Amblyraja jenseni, Apristurus profundorum,* and *Centroscyllium fabricii*. The presence of ^{15}N-depleted urea in their tissues (Hussey et al. 2012; Churchill et al. 2015) affects δ^{15}N and C:N ratios in elasmobranchs, which calls for caution when interpreting their trophic position and diet. For this reason, different solutions were explored in this study, and while we applied a mathematical correction for δ^{13}C data, we elected to interpret uncorrected bulk data for δ^{15}N.

At the other end of the spectrum, the demersal species included in G3 and G4 represented the benthic trophic pathway, with higher values of δ^{15}N and $\delta^{13}C_n$ than those measured for fishes in G1 and G2. Their high isotopic ratios suggested a dependency on the more fractionated (i.e. ^{15}N-enriched) sedimentary organic matter as a primary food source. Furthermore, fishes in G3 and G4 displayed the greatest diversity in terms of prey-taxa composition, as exemplified by *Cottunculus microps*, *Gaidropsarus ensis*, and *Macrourus berglax*. Remains of bivalves, gastropods, pycnogonids, and sponges were in fact found in the stomach of these species, together with crustaceans and fishes. Similarly, the diets of *Lepidion eques* and *Trachyrincus murrayi* in G3, and *Nezumia bairdii* in G4, appeared highly diverse. Previous studies have ascribed a benthopelagic-based diet to these species (Mauchline and Gordon 1984b; Coad and Reist 2004), mainly involving small midwater fishes, as well as hyper-, and epibenthic invertebrates

(Drazen and Sutton 2017). Although many skates are considered micronektonivores (Drazen and Sutton 2017) only small benthic crustaceans were found in the stomach of *R. fyllae* (see below). The epifaunal browser *Polyacanthonotus rissoanus*, and the megafaunal cropper *Notacanthus chemnitzii* (Drazen and Sutton 2017), are further examples of trophic guilds characterizing the benthic trophic pathway. In general, the highly diverse diet and the relatively greater length and body mass of the fishes in G3 and G4 suggests trophic overlap among these demersal species.

The concept of dual trophic pathways has previously been proposed for deep-sea food webs studied in the central (Reid et al. 2012) and eastern North Atlantic (Iken et al. 2001; Trueman et al. 2014), western Mediterranean (Valls et al. 2014), Arctic Ocean (Iken et al. 2005), and Northeast Pacific (Drazen et al. 2008). Our findings, based on the combination of the three techniques, not only revealed the existence of two alternative energy pathways within the fish assemblage investigated; they also showed a clear interconnection. In fact, a strong positive correlation was found between $\delta^{15}N$ and $\delta^{13}C_n$, and such a result is indicative of a linear food web that relies on one main food source (Iken et al. 2001), namely large inputs of POM (Sherwood et al. 2008). In this regard, those demersal predators whose diet comprised a mix of pelagic and benthic prey play a key role in linking pelagic and benthic production.

Relationships among feeding habitat and species function

The study of body size and morphological traits has received greater attention over the last few decades, as it may provide further information on the role of individual species within systems, as well as on community function and composition (Sharf et al. 2000; Mindel et al. 2015). Moreover, it is known that morphology influences behavior, habitat use, and biological interactions (Scharf et al. 2000; Ward and Metha 2010; Mindel et al.

2015, 2016). In the current investigation, pelagic fish species were represented by comparatively smaller individuals that typically had larger mouths, and displayed long and sharp teeth, protruding jaws, and/or bioluminescent structures. Such adaptations are characteristic of mesopelagic and bathypelagic predators that live and feed within a dark and relatively food-poor environment (Ebeling and Cailliet 1974; Haedrich 1996). Smaller body sizes allow these pelagic predators to invest more energy into swimming, and to cover wider hunting areas, while larger mouths enable capture of a broader range of prey sizes (Ebeling and Cailliet 1974). Conversely, demersal predators in the present study were somewhat longer and heavier, and they did not present any special morphological features, except for the photophores of the deep-sea shark *C. fabricii*. Slope areas off Newfoundland are highly productive (Snelgrove and Haedrich 1985) and fueled by large inputs of POM (Sherwood et al. 2008). In addition, numerous cold-water coral and sponge grounds have been found within the study area (Sherwood et al. 2008; Murillo et al. 2011, 2012), which provide crucial shelter, nursery and feeding sites for fishes and many other taxa (Baillon et al. 2012, 2014). Therefore, the high prey availability above the deep-sea bottom may facilitate foraging and eliminate the need for long-distance movements, thus enhancing energy input towards growth.

A number of juveniles were found in the sampled community, i.e. 15 out of 106 individuals, belonging to the species *A. bairdii*, *C. rupestris*, *M. berglax*, *R. fyllae*, and *S. kaupii*. The dietary information obtained through the analysis of their stable isotope ratios and gut contents was not entirely consistent with that reported in previous studies of their adults. For example, isotopic ratios and gut contents of *A. bairdii*, *C. rupestris*, and *S. kaupii* were indicative of a pelagic-based diet. While these findings are in line with previous gut content records from *A. bairdii* sampled in the Canadian Arctic (Coad and Reist 2004), individuals previously collected in the Northeast Atlantic were ascribed

benthopelagic feeding habits (Mauchline and Gordon 1983). A similar discrepancy occurred for the roundnose grenadier *C. rupestris,* to which Mauchline and Gordon (1984b) and Coad and Reist (2004) respectively ascribed benthopelagic and benthic-based diets. Here, the N and C stable isotope ratios of *C. rupestris* was relatively low, and planktonic crustaceans and chaetognaths were found within its gut contents, consistent with a pelagic phase for earlier life stages of the species, which is known to experience ontogenetic migration along a depth gradient (Bailey et al. 2009). Juveniles may be more likely to access the diurnal vertical migration community than adults living at greater depths (Trueman et al. 2014). Lastly, *S. kaupii* collected from the Northeast Atlantic slope areas was determined to have a pelagic-based diet (Gordon and Mauchline 1996), while Coad and Reist (2004) included the species amongst benthic feeders and Bailey et al. (2007) recognized it as a scavenger. All species that were represented by juveniles in the present study have been previously demonstrated to change diets through ontogeny. Mauchline and Gordon (1984b), for example, noted a progressive variation in diet composition among different size-classes of *C. rupestris*, with smaller-sized individuals (< 12.5 cm) feeding mainly on small crustaceans and larger individuals (≥ 12.5 cm) incorporating fish into their diet. Similarly, evidence of ontogenetic shifts in diet were obtained for the populations of *A. bairdii* sampled in the Rockall Trough of the Northeast Atlantic (Mauchline and Gordon 1983) and *M. berglax* collected in Norwegian waters (Eliassen and Jobling 1985). Younger individuals of *M. berglax* had a more epibenthic-oriented diet, while older individuals fed on both pelagic and benthic prey (Eliassen and Jobling 1985). Moreover, while crustaceans represented the dominant food items in small individuals of *R. fyllae* collected above the Flemish Cap (Northwest Atlantic), medium-sized individuals mainly fed off polychaetes, and only larger-sized individuals included fish in their diet (Gonzáles et al. 2006). Lastly, Gordon

and Mauchline (1996) noted diet variations in *S. kaupii*, with larger individuals consuming larger prey. Here, we found an overall positive relationship between fish size (i.e. total length and wet mass) and stable isotopes. Therefore, we propose that both the small sizes and early ages of the individuals explain the inconsistencies between our findings and dietary information provided in previous studies. Not only may larger individuals display higher values of $\delta^{15}N$ (Badalamenti et al. 2002; Galván et al. 2010; Trueman et al. 2014), but ontogenetic shifts in diet, which are experienced by many deep-sea fishes (Mauchline and Gordon 1983, 1984a, b, 1985; Reid et al. 2013), may constitute an additional source of influence.

Interestingly, no adult-sized individuals of *A. bairdii*, *C. rupestris*, *M. berglax*, *R. fyllae*, and *S. kaupii* were collected. Due to population declines caused by fishing activities, most of these species have been added to the list of Canadian species at risk (COSEWIC 2016), as well as to the IUCN Red List of Threatened Species; *M. berglax* constitutes the only exception, since its status has not yet been evaluated. Specifically, *C. rupestris* represents one of the main deep-water fisheries in the North Atlantic; it is overexploited and listed as “Critically Endangered” (Baker et al. 2009; Iwamoto 2015). Furthermore, the species is characterized by slow growth, late age at maturity, and low reproductive rates (Clarke et al. 2003), which are features that most deep-sea fishes share, and that make them vulnerable to fishing activities (Drazen and Haedrich 2012). As bycatch of fisheries targeting *C. rupestris*, the populations of *A. bairdii* have been subjected to large declines over the past few decades; however, due to insufficient information, the species has been assigned to the “Data Deficient” category (Hulley 2015). Lastly, *R. fyllae* and *S. kaupii* have been categorized as “Least Concern”, despite reported declines in bycatch (Kulka et al. 2009; Smith et al. 2010). In particular, the skate *R. fyllae* is commonly captured by deep-water fishing gear such as trawls, longlines, and

gillnets. Although *M. berglax* has not been added to the Red List, Devine et al. (2006) and Baker et al. (2009) highlighted drastic declines (93.3% to 99.6%) in terms of abundance and mean population size, for both *M. berglax* and *C. rupestris* captured in Canadian waters over a period of 21 years. The fact that only small-sized individuals/juveniles of these species were collected in the present study suggests their vulnerability to commercial fishing.

This is the first study to investigate the trophic ecology of a deep-sea fish assemblage in the Northwest Atlantic, and to provide isotopic information on deep-water species from this region. Our findings reaffirm the importance of combining different techniques in an effort to overcome shortfalls and limitations of each method. Furthermore, this study emphasizes the importance of improving our understanding of deep-water ecosystems, in order to assist conservation efforts.

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Compliance with Ethical Standards

Conflict of interest All the authors, CP, CCP, J-FH, AM, have approved the manuscript submitted and declared they have no conflicts of interest in regard to this work.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Tables

Table 2-1 Temporal and spatial data associated with fish sampling. Start and end positions indicate the geographic locations (latitude and longitude) for each tow. Depths are reported as mean, minimum, and maximum values (m) during the tow.

Date	Tow	Start position		End position		Depth		
(dd/mm/yyyy)	#	Latitude (N)	Longitude (W)	Latitude (N)	Longitude (W)	Mean (m)	Min (m)	Max (m)
30/11/2013	7	49˚ 56' 24"	50˚ 12' 48"	50˚ 04' 00"	50˚ 12' 18"	488	483	492
	8	49˚ 58' 42"	49˚ 46' 12"	49˚ 58' 00"	49˚ 45' 24"	1090	1085	1097
	9	49˚ 59' 12"	49˚ 36' 00"	49˚ 58' 30"	49˚ 35' 36"	1282	1280	1284
01/12/2013	12	50˚ 04' 18"	50˚ 08' 06"	50˚ 03' 54"	50˚ 07' 00"	759	755	764
02/12/2013	19	50˚ 13' 42"	50˚ 13' 12"	50˚ 13' 18"	50˚ 12' 06"	889	888	890
	20	50˚ 28' 18"	50˚ 12' 12"	50˚ 29' 06"	50˚ 12' 24"	1094	1093	1096
	21	50˚ 30' 24"	49˚ 47' 00"	49˚ 31' 18"	49˚ 46' 54"	1321	1319	1322
	22	50˚ 37' 36"	50˚ 11' 30"	50˚ 37' 18"	50˚ 10' 24"	1122	1119	1127
03/12/2013	26	50˚ 52' 36"	51˚ 12' 00"	50˚ 53' 18"	51˚ 11' 24"	313	310	316
	29	50˚ 59' 12"	50˚ 23' 54"	50˚ 58' 24"	50˚ 24' 24"	868	866	871
04/12/2013	31	50˚ 55' 12"	49˚ 33' 12"	50˚ 55' 30"	49˚ 32' 12"	1365	1353	1369
	32	50˚ 57' 42"	49˚ 41' 00"	50˚ 58' 24"	49˚ 40' 24"	1084	1073	1089
	33	51˚ 07' 06"	49˚ 45' 00"	51˚ 06' 30"	49˚ 44' 30"	919	917	923
05/12/2013	35	51˚ 10' 06"	50˚ 11' 48"	51˚ 10' 00"	50˚ 10' 36"	707	695	714
	36	51˚ 13' 24"	49˚ 48' 18"	51˚ 13' 00"	49˚ 47' 12"	1027	1009	1053
06/12/2013	39	51˚ 26' 00"	49˚ 57' 30"	51˚ 25' 24"	50˚ 56' 42"	1324	1298	1351
	42	51˚ 50' 06"	50˚ 22' 48"	51˚ 50' 30"	50˚ 23' 12"	1407	1395	1413

Table 2-2 Taxonomic details (order and family) of species analyzed, together with relative number of individuals analyzed for gut content (GC) and stable isotope (SI) analysis. "ND" indicates that no data were retrieved for the species.

Order	Family	Species	Sample size	
			GC analysis	SI analysis
Rajiformes	Rajidae	*Amblyraja jenseni* (Bigelow & Schroeder, 1950)	1	1
		Malacoraja senta (Garman, 1885)	1	1
		Rajella fyllae Lütken, 1887	4	4
Squaliformes	Etmopteridae	*Centroscyllium fabricii* (Reinhardt, 1825)	2	2
	Galeomorphii	*Apristurus profundorum* (Goode & Bean, 1896)	3	3
Anguilliformes	Serrivomeridae	*Serrivomer beanii* Gill & Ryder, 1883	3	3
	Synaphobranchus	*Synaphobranchus kaupii* Johnson, 1862	2	3
Aulopiformes	Notosudidae	*Scopelosaurus lepidus* Krefft and Maul, 1955	1	1
	Paralepididae	*Arctozenus risso* (Bonaparte, 1840)	1	2
		Magnisudis atlantica (Krøyer, 1868)	2	2
Bercyformes	Anoplogastridae	*Anoplogaster cornuta* (Valenciennes, 1833)	3	4
Gadiformes	Lotidae	*Gaidropsarus ensis* (Reinhardt, 1837)	4	4
	Macrouridae	*Macrourus berglax* Lacepède, 1801	5	5
		Nezumia bairdii (Goode and Bean, 1877)	3	3
		Trachyrincus murrayi Günther, 1887	3	3
		Coryphaenoides rupestris Gunnerus, 1765	2	3
	Moridae	*Antimora rostrata* (Günther, 1878)	3	3
		Lepidion eques (Günther, 1887)	1	1
Lophiiformes	Linophrynidae	*Haplophryne mollis* (Brauer, 1902)	ND	1
	Oneirodidae	*Melanocetus johnsonii* Günther, 1864	1	1
		Oneirodes macrosteus Pietsch, 1974	1	1
Myctophiformes	Myctophidae	*Lampadena speculigera* Goode & Bean, 1896	1	1
		Lampanyctus sp.	3	4
		Myctophum sp.	1	1
		Notoscopelus sp.	2	2
Notacanthiformes	Notacanthidae	*Notacanthus chemnitzii* Bloch, 1788	ND	3
		Polyacanthonotus rissoanus (De Filippi & Vérany, 1857)	3	3
Osmeriformes	Alepocephalidae	*Alepocephalus bairdii* Goode & Bean, 1883	2	2
		Bathytroctes macrolepis Günther, 1887	2	2
		Xenodermichthys copei (Gill, 1884)	4	4
	Bathylagidae	*Bathylagus euryops* Goode & Bean, 1896	2	2
	Gonostomatidae	*Cyclothone microdon* (Gunter, 1878)	1	2
Perciformes	Cariistidae	*Caristius macropus* (Bellotti, 1903)	1	1
	Chiasmodontidae	*Chiasmodon niger* Johnson, 1864	2	3
Pleuronectiformes	Pleuronectidae	*Reinhardtius hippoglossoides* (Walbaum, 1792)	1	2
		Glyptocephalus cynoglossus (Linnaeus, 1758)	ND	3
Scorpaeniformes	Psychrolutidae	*Cottunculus microps* Collett, 1875	2	2
		Cottunculus thomsonii (Günther, 1882)	1	1
	Sebastidae	*Sebastes mentella* Travin, 1951	3	3
Stephanoberyciformes	Melamphaidae	*Scopeloberyx opisthopterus* (Parr, 1933)	2	2
Stomiiformes	Stomiidae	*Chauliodus sloani* Bloch & Schneider, 1801	3	6
		Borostomias antarcticus (Lönnberg, 1905)	4	4
		Malacosteus niger (Ayres, 1848)	2	2

Table 2-3 Descriptors of species under study, including size (i.e. total length and wet mass of frozen individuals), diet diversity (i.e. number of food-categories found in gut contents), habitat according to the list adapted from Drazen and Sutton (2017), and morphological traits (i.e. body shape, mouth size, and presence of special anatomical features).

Species	**Mean total length** (±SD)	**Mean wet mass** (±SD)	**Diet diversity**	**Habitat**	**Body shape**	**Mouth size**	**Special features**
A. bairdii	29.4 (±9.1)	161.2 (±140.0)	2	Demersal	Elongate	Small	
A. jenseni	49.5 (±0.0)	796.7 (±0.0)	2	Demersal	Flat	Small	
A. cornuta	16.7 (±2.0)	84 (±31.7)	1	Pelagic	Laterally compressed	Large	Long sharp teeth
A. profundorum	72.6 (±2.3)	1805.4 (±249.5)	4	Demersal	Elongate	Small	
A. risso	17.3 (±0.0)	4 (±0.0)	1	Pelagic	Elongate	Small	
A. rostrata	33.7 (±3.0)	263.2 (±92.3)	3	Demersal	Elongate	Small	
B. euryops	12.6 (±2.9)	7.5 (±2.4)	1	Pelagic	Elongate	Small	
B. macrolepis	20.5 (±2.8)	67.3 (±35.2)	2	Pelagic	Elongate	Small	
B. antarcticus	22.2 (±7.2)	44.9 (±46.7)	2	Pelagic	Elongate	Large	Chin barbel; photophores along the body; luminous post-orbital organ; sharp narrow teeth.
C. macropus	21.2 (±0.0)	176.3 (±0.0)	1	Pelagic	Laterally compressed	Small	
C. fabricii	58.3 (±3.5)	1177.7 (±72.8)	3	Demersal	Elongate	Small	Luminescent organs in its skin.
C. sloani	27.0 (±1.0)	45.8 (±7.4)	1	Pelagic	Elongate	Large	Photophores; long sharp teeth; lower jaw longer upper jaw.
C. niger	20.9 (±6.9)	80.3 (±64.2)	1	Pelagic	Elongate	Large	
C. rupestris	29.1 (±4.2)	64.5 (±21.8)	2	Demersal	Elongate	Small	

C. microps	20.0 (±5.1)	72.4 (±12.2)	8	Demersal	Elongate	Large	
C. thomsonii	48.5 (±0.0)	1379.3 (±0.0)	3	Demersal	Elongate	Large	
G. ensis	30.5 (±8.8)	174.6 (±139.1)	6	Demersal	Elongate	Small	
Lampanyctus sp.	14.9 (±0.7)	25.7 (±7.8)	3	Pelagic	Elongate	Large	
L. eques	29.4 (±0.0)	130.6 (±0)	1	Demersal	Elongate	Small	
M. berglax	31.2 (±5.4)	90.6 (±39.0)	8	Demersal	Elongate	Small	
M. atlantica	48.6 (±0.4)	342.5 (±28.8)	2	Pelagic	Elongate	Small	
M. senta	28.0 (±0.0)	81.7 (±0.0)	1	Demersal	Flat	Small	
M. niger	23.6 (±5.7)	38.75 (±0.9)	1	Pelagic	Elongate	Large	
M. johnsonii	12.5 (±0.0)	178.8 (±0.0)	2	Pelagic	Spherical	Large	Lure at the top of the head; long sharp teeth.
N. bairdii	30.5 (±3.0)	97.2 (±54.5)	5	Demersal	Elongate	Small	
Notoscopelus sp.	14.05 (±0.6)	21.7 (±6.8)	1	Pelagic	Elongate	Large	Photophores all along body.
O. macrosteus	13.0 (±0.0)	119.0 (±0.0)	1	Pelagic	Spherical	Large	Lure at the top of the head; long sharp teeth.
P. rissoanus	48.3 (±6.5)	94.2 (±34.6)	3	Demersal	Elongate	Small	
R. fyllae	10.5 (±0.5)	4.5 (±0.9)	1	Demersal	Flat	Small	
S. opisthopterus	7.8 (±0.4)	3.75 (±0.2)	1	Pelagic	Elongate	Small	
S. lepidus	34.2 (±0.0)	128.8 (±0)	1	Pelagic	Elongate	Small	
S. mentella	26.2 (±4.4)	200.0 (±108.1)	4	Demersal	Elongate	Small	
S. beanii	48.6 (±14.8)	49.5 (±20.9)	1	Pelagic	Elongate	Small	
S. kaupii	50.0 (±4.6)	100.0 (±14.7)	2	Demersal	Elongate	Small	
T. murrayi	32.83 (±1.6)	94.2 (±5.4)	4	Demersal	Elongate	Small	
X. copei	15.25 (±1.6)	18.6 (±4.8)	1	Pelagic	Elongate	Small	

Table 2-4 Isotopic group (G1, G2, G3, G4) recognized by cluster analysis, and mean values of $\delta^{15}N$, $\delta^{13}C_n$, and trophic position (TP), with standard deviation (SD), provided for each species. See Table 2-2 for species codes and sample sizes. Species are reported in order of trophic position.

Species	Isotopic group	$\delta^{15}N$	SD	$\delta^{13}C_n$	SD	TP	SD
Myctophum sp.	1	8.5		-19.6		2.2	
M. niger	1	9.3	0.1	-19.1	0.7	2.4	0.0
M. atlantica	1	9.4	0.5	-19.4	0.7	2.4	0.1
S. beanii	1	9.4	0.2	-20.2	0.3	2.4	0.1
X. copei	1	9.4	0.5	-19.7	0.2	2.4	0.1
A. risso	1	9.5	0.7	-19.8	0.2	2.4	0.2
Notoscopelus sp.	1	9.6	0.0	-19.2	0.5	2.5	0.0
B. euryops	1	9.6	0.9	-20.3	0.1	2.5	0.2
C. rupestris	1	9.7	0.6	-20.5	0.2	2.5	0.2
C. microdon	1	9.7	0.5	-19.6	0.3	2.5	0.1
C. sloani	1	9.9	0.5	-19.8	0.4	2.5	0.1
Lampanyctus sp.	1	10.2	0.2	-19.3	0.3	2.6	0.1
B. antarcticus	1	10.4	0.8	-19.4	0.3	2.7	0.2
A. bairdii	1	10.9	1.0	-19.4	0.8	2.8	0.3
S. lepidus	2	11.1		-19.8		2.9	
S. mentella	2	11.2	0.3	-19.8	0.2	2.9	0.1
A. rostrata	2	11.2	0.2	-19.9	0.6	2.9	0.1
C. niger	2	11.3	0.4	-19.3	0.6	2.9	0.1
A. profundorum	2	11.4	0.1	-19.5	0.2	2.9	0.0
L. speculigera	2	11.5		-20.0		3.0	
R. hippoglossoides	2	11.5	1.4	-19.5	1.0	3.0	0.4
A. cornuta	2	11.6	1.0	-18.6	0.2	3.0	0.3
C. fabricii	2	11.6	0.6	-19.7	0.2	3.0	0.1
A. jenseni	2	11.6		-19.4		3.0	
O. macrosteus	2	11.8		-19.8		3.0	
S. opisthopterus	2	12.0	2.0	-19.4	0.4	3.1	0.5
H. mollis	2	12.0		-20.0		3.1	
S. kaupii	3	12.2	0.6	-18.8	0.2	3.1	0.2
B. macrolepis	2	12.3	0.6	-19.8	0.2	3.2	0.2
M. senta	3	12.6		-19.3		3.3	
M. johnsonii	3	12.7		-18.6		3.3	
G. cynoglossus	4	12.9	0.1	-16.9	0.1	3.3	0.0
R. fyllae	4	13.2	0.2	-16.8	0.1	3.4	0.1
G. ensis	3	13.5	1.0	-18.7	0.5	3.5	0.3
C. macropus	3	13.6		-18.9		3.5	
C. microps	3	13.9	1.4	-18.3	0.4	3.6	0.4
L. eques	3	14.2		-19.0		3.7	
N. chemnitzii	4	14.5	0.5	-16.8	0.9	3.8	0.1
T. murrayi	3	14.6	0.1	-18.6	0.6	3.8	0.0
N. bairdiii	4	14.6	0.5	-18.1	0.3	3.8	0.1
P. rissoanus	4	14.7	0.5	-17.4	0.5	3.8	0.1
M. berglax	4	14.7	0.5	-17.9	0.4	3.8	0.1
C. thomsonii	4	15.7		-16.9		4.1	

Table 2-5 List of the main food items, showing number of fish individuals of each species that had that specific item in their gut contents. Frequencies of occurrence (%) of food items are reported in the last row.

Species code	Food items									
	Bivalvia	Cephalopoda	Chaetognatha	Crustacea	Fish	Gastropoda	Polychaeta	Pycnogonida	Sediment	Other
A. bairdii			1	1						
A. cornuta					2					
A. jenseni				1			1			
A. profundorum		2		3	3			1		
A. risso				1						
A. rostrata			1	1			1			
B. antarcticus				1	3					
B. euryops				2						
B. macrolepis				2	1					
C. fabricii		2		1	1					
C. macropus										1 (fish eggs?)
C. microdon										
C. microps	1	1		1		1	1	1	1	1 (Protozoa)
C. niger					1					
C. rupestris			1	1						
C. sloani			1		3					
C. thompsonii		1		1	1					
G. ensis		1	1	4			2	1		1 (Porifera)
L. eques				1						
L. speculigera										
Lampanyctus sp.				2	1					
M. antarctica		1		1						
M. berglax	2	2		5	2		2	1	1	1 (Protozoa)
M. johnsoni				1	1		1			
M. niger				1						
M. senta				1						

Myctophum sp.											
N. bairdii		1		3	1		2	1			
Notoscopelus sp.				2							
O. macrosteus					1						
P. rissoanus				2	1			1			
R. fyllae				1							
R. hippoglossoides											
S. beanii		1									
S. kaupii		1		1							
S. lepidus				1							
S. mentella				2	1		1				1 (Ostracoda)
S. opisthopterus				1							
T. murrayi		1		2			2				2 (Porifera)
X. copei				2							
Frequency of occurrence (%)	3	16	6	56	26	1	16	7	2	8	

Figures

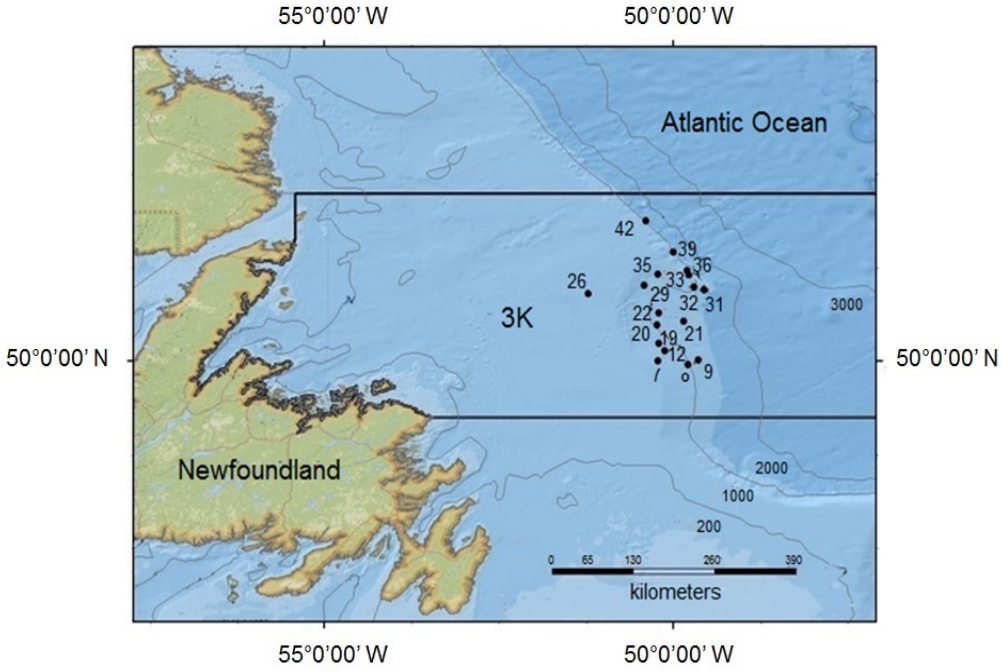

Fig. 2-1 Map of the sampling area (in NAFO subdivision 3K), off Newfoundland, Northwest Atlantic. Circles (●) correspond to each tow location (n = 17). Grey lines indicate the 200, 1000, 2000, and 3000 m isobaths. Geographical coordinates and sampling depths are reported in Table 2-1.

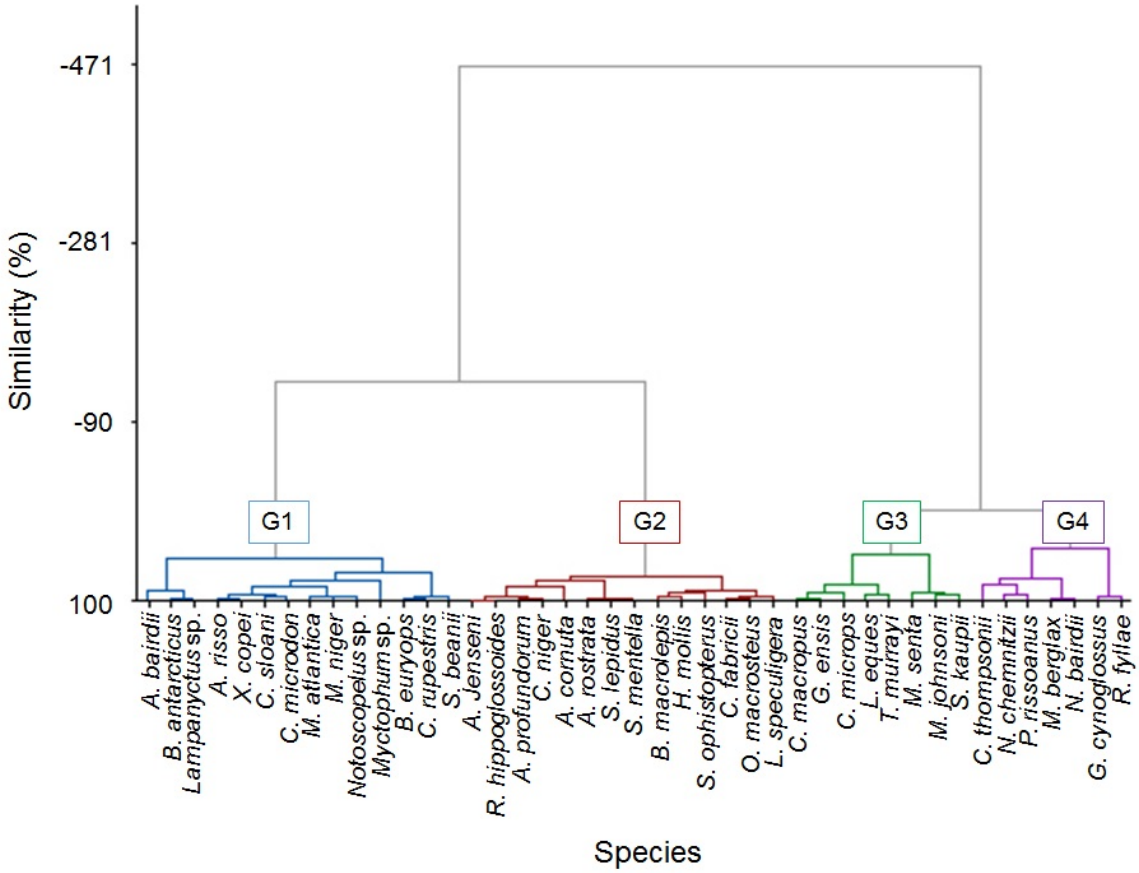

Fig. 2-2 The four isotopic groups of fishes (G1, G2, G3, and G4) determined through cluster analysis of mean values of $\delta^{15}N$ and $\delta^{13}C_n$ by Ward's method (Euclidean distance).

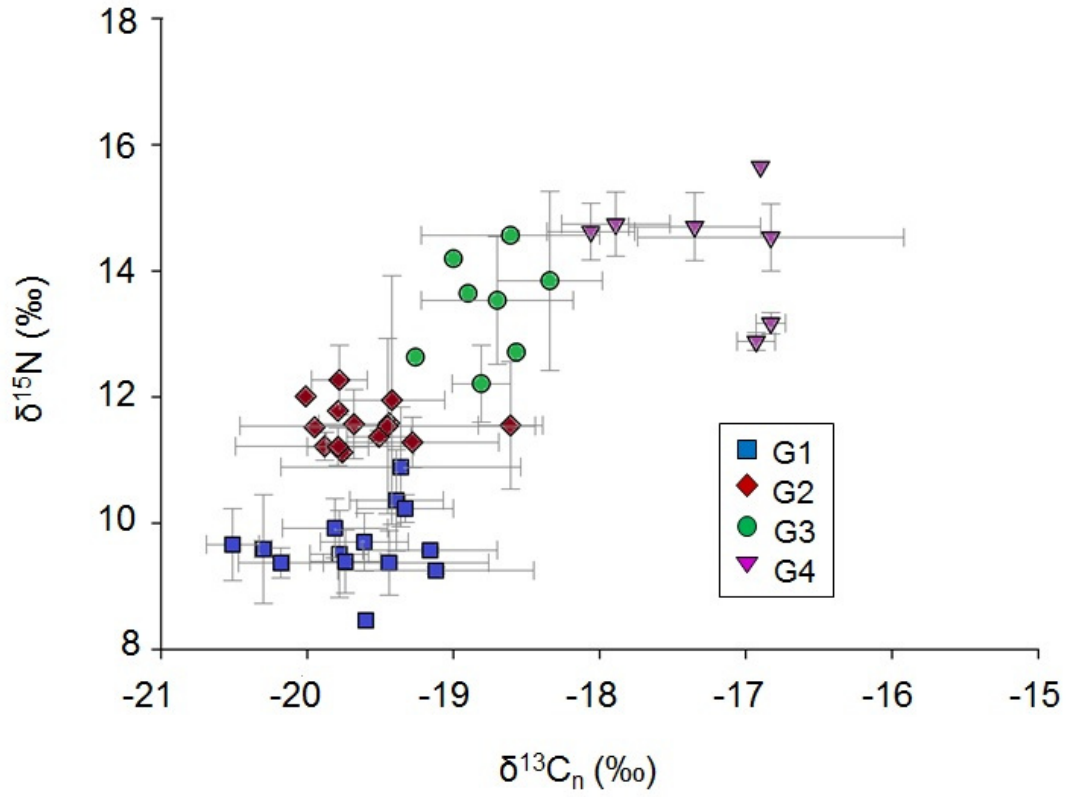

Fig. 2-3 Biplot of mean of stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C_n$) of the fish species under study. Four isotopic groups (G1, G2, G3, and G4) were revealed by cluster analysis (see Fig. 2-2). Error bars represent ± standard deviation (SD; *n* = 2-6).

Fig. 2-4 Examples of food items retrieved from the analysis of gut contents in the fish species under study. A) Fish scale in *A. cornuta*; B) fish spine in *B. antarcticus*; C) amphipod in *N. bairdii*; D) fish pectoral fin in *C. niger*; E) shrimp in *N. bairdii*; F) copepod in *P. rissoanus*; G) squid beak in *A. profundorum*; H) tubeworm in *G. ensis*; I) cephalic region of a polychaete in *A. jenseni;* J) cephalopod pen in *C. sloani*; K) columella pillar of gastropod in *C. microps*; and L) bivalve shell in *M. berglax*. Scale bars represent 1 mm.

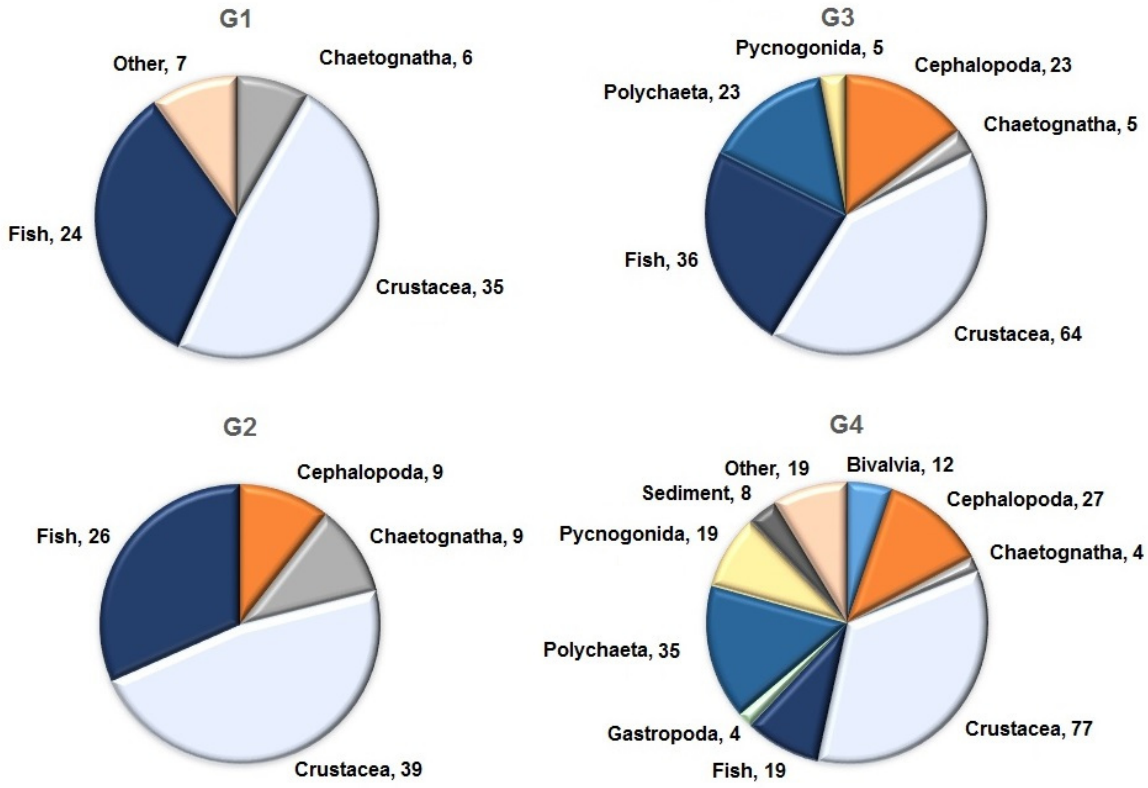

Fig. 2-5 Dietary distributions of fishes under study, distinguished by isotopic group (G1, G2, G3, and G4). Sample size for each group is *n* = 31, 25, 16, and 16, respectively. Labels indicate food-categories and their relative frequency of occurrence (%).

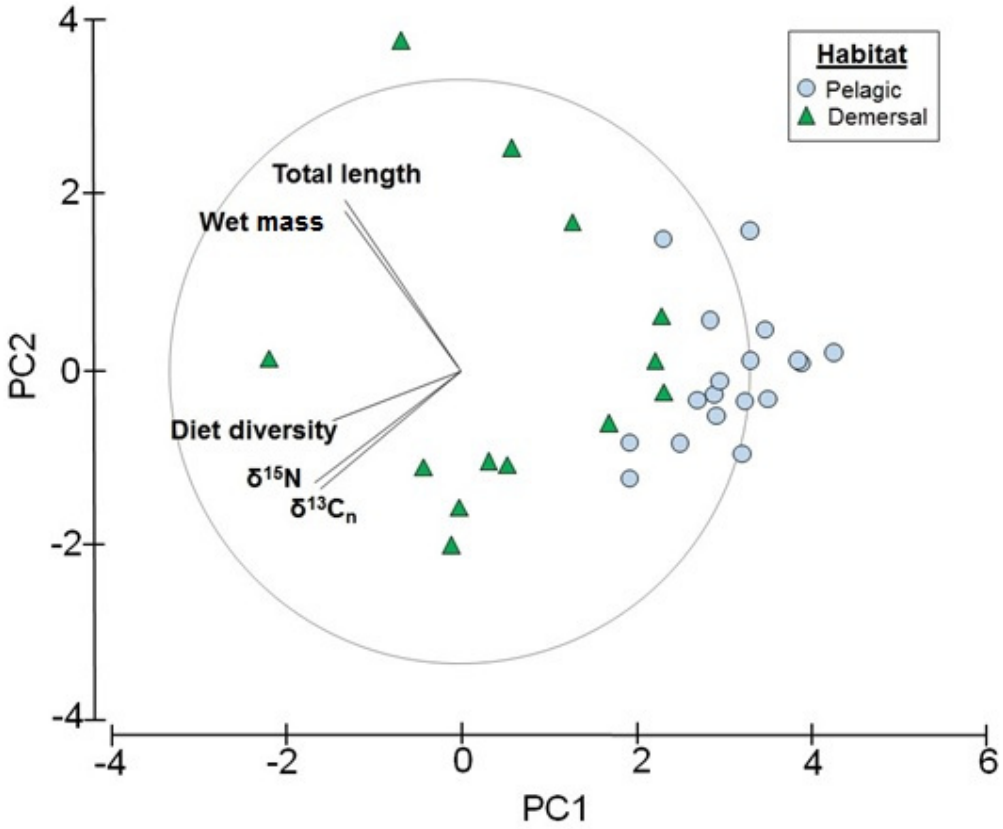

Fig. 2-6 Principal components analysis of fish size (mean total length and wet mass), diet diversity, and stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C_n$). Triangles (▲) refer to demersal species and circles (●) to pelagic species.

Chapter 3 : **Functional diversity and nutritional content in a deep-sea faunal assemblage through total lipid, lipid class, and fatty acid analyses**[2]

[2] A version of this manuscript was submitted to the journal PLoS ONE and was pending revision at the time of publication.

Abstract Lipids are key compounds in marine ecosystems being involved in organism growth, reproduction, and survival. Despite their biological significance and ease of measurement, the use of lipids in deep-sea studies is limited, as is our understanding of energy and nutrient flows in the deep ocean. Here, a comprehensive analysis of total lipid content, and lipid class and fatty acid (FA) composition, was used to explore functional diversity and nutritional content within a deep-sea faunal assemblage comprising 139 species from 8 phyla, including the Arthropoda, Chordata, and Mollusca. A wide range of total lipid content and lipid class composition suggested a diversified set of energy allocation strategies across taxa. Overall, phospholipid was the dominant lipid class. While triacylglycerol was present in most taxa as the main form of energy storage, a few crustaceans, fishes, jellyfishes, and corals had higher levels of wax esters instead. Type and amount of energy reserves may reflect dietary sources and environmental conditions for certain deep-sea taxa. Conversely, the FA composition was less diverse than was the lipid class composition, and large proportions of unsaturated FA were detected, consistent with the growing literature on cold and deep-water species. In addition, levels of unsaturation increased with depth, likely representing an adaptive strategy to maintain normal membrane structure and function in species found in deeper waters. Although proportions of ω3-FA were high across all phyla, later life stage representatives (juveniles/adults) of the phyla Chordata and Arthropoda were the main reservoirs of these essential nutrients.

Introduction

Lipids represent the densest form of energy in marine ecosystems since they provide about 1.5 and 2 times more energy per gram than proteins and carbohydrates, respectively (Glencross 2009; Parrish 2013). Moreover, they are key components of cell membranes (Parrish 2013), and are involved in numerous cellular and physiological processes crucial to the reproduction, growth, and general survival of organisms (Adams 1999; Bergé and Barnathan 2005; Glencross 2009). Lipids are, for example, deposited during oogenesis in marine crustaceans (Lee 1991; Hirche and Kattner 1993; Lee et al. 2006) and fishes (Sargent et al. 1999; Glencross 2009); they can be transferred as lipoprotein from mother to oocytes to provide energy to embryos (Lee 1991; Sargent et al. 1999). Overall, lipids are a highly diverse group of biomolecules (Iverson 2009), which not only vary in terms of structure, but also differ depending on their chemical and physical properties, origin, and function (Parrish 2009; Fahy et al. 2011). Nonetheless, by definition, they all share the characteristic of being insoluble in polar solvents, but soluble in non-polar organic solvents (Fahy et al. 2011) which makes them relatively easy to extract from biological tissues for analysis (Parrish 2009). For this reason, lipids have become a popular tool in investigations of the drivers of ecosystem health and functioning (Parrish et al. 2000), food-web structure and dynamics (Connelly et al. 2014), as well as carbon cycling (Connelly et al. 2012) and bioaccumulation (Signa et al. 2015) in the marine environment. However, while a vast body of literature exists for shallow-water species (Graeve et al. 1997; Budge and Parrish 1998; Copeman and Parrish 2003; Richoux et al. 2004a; Carreón-Palau et al. 2013), the study of lipids in deep-sea taxa lags behind, and is mostly limited to the analysis of fatty acids as trophic biomarkers (Howell et al. 2003; Drazen et al. 2008a; Drazen et al. 2008b) with a focus on certain

deep-water taxa or faunal groups, such as fishes, corals, and zooplankton (Lee et al. 1971; Cossins and Macdonald 1986; Økland et al. 2005; Hamoutene et al. 2007).

Lipid extracts of aquatic samples can be separated into different classes, including phospholipids (PL) and triacylglycerols (TAG), which are of primary interest in studies of marine ecosystems (Parrish 2013). Specifically, PL are the principal constituents of animal cell membranes (Parrish et al. 2000; Bergé and Barnathan 2005) and are hence found in all animal phyla; while TAG are the main form of energy storage in both terrestrial and marine animals (Lehninger 1975; Parrish et al. 2000). Other lipid classes, such as sterols (ST) and wax esters (WE), also play important roles in marine organisms. The former are key constituents of animal cell surface membranes (Crockett 1998) and are present in all eukaryotic taxa (Nes 1974; Morris and Culkin 1989); they are also precursors of steroid hormones (Lehninger 1975) and represent essential dietary nutrients for marine organisms (Napolitano et al. 1993; Paibulkichakul et al. 1998; Parrish 2013). A diet with limited cholesterol content (<1%), for instance, significantly decreased growth and survival rates of early stages in the crustacean *Panaeus monodon* (Paibulkichakul et al. 1998). Conversely, WE constitute the primary energy storage of certain shallow-water corals and sea anemones (Lee and Patton 1989), as well as deep-sea crustaceans and fishes (Benson and Lee 1972; Lee et al. 2006; Drazen et al. 2008b). Furthermore, WE also control buoyancy in myctophid fish (Phleger 1998) and diapausing zooplankton which overwinter in deep waters and re-enter the surface layers in spring to feed (Lee et al. 2006). Additional lipids may exert important physiological functions in a more taxon-specific manner. Among them, biogenic hydrocarbons and diacylglycerols have been detected in representatives of the phyla Arthropoda, Chordata, Cnidaria, Echinodermata, and Mollusca (Joseph 1989; Morris and Culkin 1989). Due to their low density, for example, certain hydrocarbons and

alkyldiacylglycerols are involved in buoyancy control, such as squalene in shallow-water sharks (Morris and Culkin 1989) and alkyldiacylglycerols in deep-sea sharks (Phleger 1998).

As major components of most lipids, fatty acids (FA) are commonly referred to as "building blocks" (Iverson 2009; Colombo et al. 2016). Two FA chains (or acyl chains) are for instance attached to the glycerol backbone of a PL molecule, whereas TAG is comprised of three FA chains. Dietary FA can be either oxidized to produce high-energy molecules (i.e. ATP), which in turn fuel metabolism, or they can be transferred into membrane PL, where they play a major role in membrane structure and function (Bergé and Barnathan 2005). Among them, certain FA are considered essential nutrients because they are required for optimal health and most organisms are unable to synthesize them *de novo* (Iverson 2009; Parrish 2009; Colombo et al. 2016). In marine ecosystems, three major essential FA can be identified, including docosahexaenoic (DHA; 22:6ω3) and eicosapentaenoic (EPA; 20:5ω3) acids from the ω3-series, and arachidonic acid (ARA; 20:4ω6) from the ω6-series. These specific polyunsaturated FA (PUFA) are precursors of docosanoids and eicosanoids, which regulate numerous cell processes (Parrish 2009). Through biochemical and biophysical processes, 22:6ω3, 20:5ω3, and 20:4ω6 are for example involved in neurological development and signaling (Simopoulos 2011), and support immunity (Calder 2015) and growth (Parrish 2009). However, the extent to which these three essential FA are required and occur within tissues may vary across taxa, or even intraspecifically with age, sex, season, and habitat (Luzia et al. 2003; Fernandes et al. 2014; Colombo et al. 2016). Typically, marine organisms present higher levels of ω3-PUFA than terrestrial counterparts, which instead have larger proportions of ω6-PUFA (Colombo et al. 2016). In addition, while a latitudinal trend has been found, whereby marine species from polar regions have higher levels of

PUFA than those from tropical areas (Colombo et al. 2016); a limited number of studies has compared shallow *vs* deep-water species. One study by Stowasser et al. (2009) observed that shallower (<4000 m) individuals of deep-sea macrourid and morid fish species, collected in the Northeast Atlantic, had higher proportions of PUFA in their liver than their deeper counterparts. Conversely, monounsaturated FA (MUFA) increased with depth, and no bathymetric trends were detected for either PUFA or MUFA when analyzing muscle tissue (Stowasser et al. 2009).

Environmental and biological variables may affect lipid content, as well as lipid and FA composition of marine organisms, including temperature and hydrostatic pressure (DeLong and Yayanos 1985; Simonato et al. 2006; Parrish 2013), and food availability (Drazen et al. 2008a; Drazen et al. 2008b). Factors such as type of tissue, age, sex, taxon, metabolic activity, and life style are also known modulators of the lipid signature (Henderson et al. 1984; Lockyer 1986; Fraser 1989; Hirche and Kattner 1993).

The Canadian Province of Newfoundland and Labrador is located in a cold-temperate region of the Northwest Atlantic, where species with subarctic/Arctic characteristics are common. While several studies have been carried out in coastal and other shallow-water ecosystems of the region (Jangaard and Ackman 1965; Ackman et al. 1969; Ackman and Hooper 1970; Budge and Parrish 1998; Parrish 1998; Copeman and Parrish 2003; Richoux et al. 2004a; Richoux et al. 2004b; Parrish et al. 2005), information on the lipid content and composition of deep-sea counterparts remains fragmentary. Hamoutene et al. (2007) determined total lipid contents and classes in corals collected at depths between 50 and 1500 m, while Salvo et al. (2018) focused on the FA composition of coral species sampled within 770 and 1370 m. Furthermore, Mercier et al. (2011) determined lipid contents, classes, and FA signatures in deep-sea gastropods and their epibiotic sea anemones collected between 191 and 627 m. In order

to provide novel information and baseline data for a broader range of deep dwelling taxa, the present investigation assessed total lipid content, and lipid classes and FA composition inside a deep-sea faunal assemblage sampled within a tight temporal and spatial window in the Northwest Atlantic. Such a choice in the sampling was made to limit data variation. The rich diversity analyzed here included 139 species across 8 major phyla, collected on the upper and mid-slope area off the east coast of Newfoundland. The opportunity was taken to conduct both a broad cross-taxa comparative analysis and an in-depth phylum-specific study of selected lipid and fatty acid groups indicative of energy-storage strategies, physiological processes, and dietary value for consumers, including humans. High levels of variability in lipid class and FA compositions are expected within and across taxa, given the broad taxonomic range represented. Moreover, high proportions of essential FA (ω3 and ω6) are anticipated, given their major role in marine ecosystems (Parrish 2013), as well as the cold and deep-water conditions. Lastly, both lipid class and FA composition are hypothesized to vary along the wide bathymetric gradient covered (~1000 m).

Materials and Methods

Sampling

Organisms belonging to various taxa were opportunistically collected within 7 days in November-December 2013, during one of the annual multispecies bottom-trawl surveys conducted by Fisheries and Oceans (DFO), Canada. Individuals were sampled onboard the CCGS *Teleost*, from a total of 23 tows inside a 100 km radius, and a depth range of 313 to 1407 m. The gear used to collect the samples (Campelen 1800 shrimp trawl) included a 16.9 m wide net with four panels of polyethylene twine. Further details are

found in Walsh and McCallum (1997). Mean bottom temperature at the sampling site was 4.0 ± 0.3°C, with a weak decrease with depth. The sampling area, referred to as NAFO Division 3K, is located off Newfoundland, eastern Canada, in the Northwest Atlantic (49° 31'- 51° 51'N, 49° 32'- 51° 13'W). Once on board, individuals were immediately vacuum packed and frozen at -20°C to minimize lipid oxidation and hydrolysis. Individuals were identified to the lowest possible taxonomic level, from direct observation and through photo-identification. A total of 283 deep-sea organisms, belonging to 139 species and 8 phyla, were processed for lipid analysis at the CREAIT-ARC Facility of Memorial University (Table 3-1). In this regard, tissues characterized by low turnover rates were purposely selected based on the literature, since they provide longer-term information; they were sampled from each individual one year after collection. This choice also allowed for comparisons across taxa to be drawn. The following were sampled: dorsal white muscle from fishes; body wall and tube feet from echinoderms; foot muscle from gastropods; mantle from cephalopods; non-gonad soft tissues or body walls from cnidarians; and dorsal abdominal muscle from crustaceans. When collection of target tissues was not feasible due to small body size, whole individuals, guts included, were processed. This was the case for 5 individuals of the phylum Annelida (i.e. *Alitta succinea*, Nereididae sp. 1, Polychaeta sp. 1, Polynoidae sp. 3, and Prionospio sp.), 10 of the Arthropoda (species of *Arcoscalpellum michelottianum* and *Nymphon* sp.), 2 of the Chordata (i.e. Ascidiacea sp. 3, and *Eudistoma vitreum*), and 3 of the Echinodermata (species of Gorgonocephalus sp., and *Ophioscolex glacialis*).

Lipid extraction

An aliquot of tissue (0.7 ± 0.2 g) was sampled from each still-frozen individual to limit lipid oxidation and hydrolysis. Prior to extraction, each sample was immersed in

chloroform (4 or 8 ml, depending on tissue amount), sealed under nitrogen gas, and stored in a freezer (-20ºC). Lipids were extracted and analyzed based on Parrish (1999). Briefly, samples were homogenized in a chloroform:methanol:water (2:1:1) mixture, sonicated, and centrifuged four times. Lipid extracts were pooled in a lipid-clean vial following each wash, and the total amount was concentrated down to volume under a gentle stream of nitrogen. Vials were sealed and stored at -20ºC until further analysis.

Total lipid content and lipid classes

Lipid extracts were analyzed using the Chromarod-Iatroscan TLC/FID system (Parrish 1987). In detail, the lipid extracts were spotted on silica-gel coated rods (Chromarods-SIII) and developed in three solutions of different polarity, to allow lipid class separation. Samples were first developed in a mixture of hexane:diethyl ether:formic acid (98.95:1:0.05), which allowed the separation of hydrocarbons, wax esters/steryl esters (WE/SE), ethyl esters, methyl esters, as well as ethyl and methyl ketones. Wax esters and steryl esters were considered together in this study as wax esters/steryl esters, since the method used does not allow the separation of the two lipid classes. The second development, consisting of hexane, diethyl ether, and formic acid 79.9:20:0.1 led to the separation of diacyl glyceryl ethers, triacyglycerols, free fatty acids (FFA), alcohols, sterols, and diacylglycerols. Lastly, acetone-mobile polar lipids and phospholipids, the most polar among the lipid classes, were separated by the third development of 100% acetone followed by chloroform:methanol:chloroform-extracted-water (5:4:1). After each development, lipid classes were scanned on the rods using an Iatroscan MK V, and quantified by combustion in a flame ionization detector. Lipid classes were identified and quantified through comparison with known standards, such as n-nonadecane for hydrocarbons, cholesteryl palmitate for SE, 3-hexadecanone for ketones, tripalmitin for

triacyglycerols, palmitic acid for FFA, 1-hexadecanol for alcohols, cholesterol for sterols, 1-monopalmitoyl-rac-glycerol for acetone-mobile polar lipids, and DL-α-phosphatidylcholine dipalmitoyl for phospholipids.The sum of the amount of all the lipid classes in each sample provided the total lipid content (mg g^{-1} wet mass), while each lipid class was measured as percent of total lipid. Proportions of lipid classes were then used to calculate the triacylglycerol to sterol ratio (TAG:ST), or condition index (Fraser 1989), and the phospholipid to sterol ratio (PL:ST) as a measure of membrane fluidity (Pernet et al. 2006; Parent et al. 2008).

FA analysis

FA were derivatized at 100ºC with H_2SO_4 in methanol, and quantified as methyl esters (FAME) by gas chromatography. Briefly, an aliquot of the lipid extract, calculated in relation to the total amount of lipids within each tissue sample, was transferred into a lipid clean vial and evaporated under N_2, to dryness. After adding 1.5 ml of dichloromethane and 3 ml of Hilditch reagent (i.e. H_2SO_4 dissolved in methanol), the vials were sonicated, sealed, and heated for 1 hour at 100ºC. On cooling, 0.5 ml of saturated sodium bicarbonate and 1.5 ml of hexane were added to the solution, thus creating two layers. The upper, organic layer was removed and transferred into a new lipid-clean vial. Finally, the solution was blown dry under N_2, and hexane (0.5 ml) was added to each vial. Samples were then sealed and loaded into a HP 6890 GC-FID equipped with a 7683 autosampler, for FA identification and quantification. FA peaks were identified by comparing them with those of known standards from Supelco, such as a 37 component FAME mix, a bacterial acid methyl ester mix, PUFA 1 and PUFA 3. In this study, FA were reported as sums. In detail, the sum of the saturated (∑Sat) was measured by summing the proportions of the following FA: 14:0, trimethyltridecanoic acid, 15:0,

pristanic acid, 16:0, phytanic acid, 17:0, 18:0, 19:0, 20:0, 21:0, 22:0, 23:0, and 24:0. The sum of the monounsaturated FA (∑MUFA) was obtained by summing 14:1, 15:1, 16:1ω11, 16:1ω9, 16:1ω7, 16:1ω5, 17:1, 18:1ω11, 18:1ω9, 18:1ω7, 18:1ω6, 18:1ω5, 20:1ω11, 20:1ω9, 20:1ω7, 22:1ω11(13), 22:1ω9, 22:1ω7, and 24:1; whereas the polyunsaturated 16:2ω4, 16:3ω3?, 16:4ω3?, 16:4ω1, 18:2a, 18:2b, 18:2ω6, 18:2ω4, 18:3ω6, 18:3ω4, 18:3ω3, 18:4ω3, 18:4ω1?, 18:5ω3, 20:2α?, 20:2β?, 20:2ω6, 20:3ω6, 20:4ω6, 20:3ω3, 20:4ω3, 20:5ω3, 21:5ω3?, 22:4ω6, 22:5ω6, 22:4ω3?, 22:5ω3, 22:6ω3, and the non-methylene-interrupted-dienoic 22:2 (i.e. 22:2NIMDa?, 22:2NIMDb?) were summed to calculate ∑PUFA. For the sum of the ω3- and ω6-FA, only those acids involved in the desaturation/elongation pathway were used, including 18:3ω3, 18:4ω3, 20:4ω3, 20:5ω3, 22:5ω3 and 22:6ω3 for ∑ω3, and 18:2ω6 18:3ω6, 20:3ω6, 20:4ω6, 22:4ω6 and 22:5ω6 for ∑ω6. Lastly, DHA+EPA represents the sum of the amounts of docosahexaenoic acid (22:6ω3) and eicosapentaenoic acid (20:5ω3) reported in g per 100-g of wet mass.

Statistical analysis

Two types of mean values were reported in Results and Tables: i) averages per phylum ± se and ii) averages per species ± sd. In addition, phyla are listed in decreasing order of mean lipid contents in the Results section and in the Tables. To study the relative magnitude of data variability among and within phyla, the coefficient of variation (CV) was calculated for selected metrics (i.e. wet mass, total lipid content, and proportions of PL, FFA, ST, TAG, WE/SE). Due to analytical artifacts related to blank correction and the consequent underestimation of the proportion of PL in individuals with low lipid content, n = 30 samples were removed from all the analyses involving lipid class composition (Table 3-1). After testing normality of data and heterogeneity of variance, Spearman

rank-order correlations were run to test for the presence of any relationship among depth of collection (mean value for each depth strata), total lipid content, lipid classes (PL, FFA, ST, TAG, and WE/SE), lipid ratios (TAG:ST and PL:ST), fatty acid indices (∑Sat, ∑MUFA, ∑PUFA, ∑ω3, ∑ω6), and wet mass of whole individuals. Furthermore, PERMANOVA (permutational multivariate ANOVA) and PCO (principal coordinate analysis) were performed on matrices of normalized values to explore differences in lipid and FA composition across taxa. Univariate analyses were run using the software Sigmaplot 11.0, and multivariate statistics was conducted in Primer 6 + PERMANOVA (Clarke and Gorley 2006).

Results

Lipid and fatty acid composition across phyla

Lipid analysis was performed on deep-sea organisms across a wide range of taxa, body sizes and depths (Table 3-1), and inside a tight temporal and geographical window. Representatives of the phyla Chordata and Arthropoda exhibited the highest mean concentrations of total lipids in their tissues, with marked variability (± se: Table 3-2). In particular, the Chordata displayed both the greatest lipid amounts (56.0 ± 12.1 mg g^{-1} wm, n = 105) and highest CV (221%), followed by the Arthropoda (24.8 ± 9.0 mg g^{-1} wm, n = 32; 206%). Conversely, the Porifera (5.9 ± 0.7 mg g^{-1} wm, n = 25) and the Sipuncula (5.1 ± 2.2 mg g^{-1} wm, n = 2) showed the lowest lipid quantities. Lipid contents of all remaining taxa along with CVs are listed in Table 3-2.

A total of 14 lipid classes were represented within the faunal assemblage. Overall, PL (35.3 ± 1.5%), FFA (19.4 ± 0.9%), ST (13.9 ± 0.6%), TAG (13.4 ± 1.3%), and WE/SE (4.3 ± 0.7%) were the most abundant lipid classes across all individuals analyzed

(n = 256). The remaining lipid classes (i.e. HC, EE, ME, EK, MK, GE, AL, DAG, and AMPL) occurred in smaller mean proportions (< 1.7%) and, for this reason, they were not further considered in the analysis; nonetheless, their proportions within each phylum is reported in Appendix 7-2. In particular, PL dominated the lipid class composition of all the phyla analyzed, with mean proportions ranging from 24.7 ± 2.1% in the Chordata to 66.4 ± 2.8% in the Mollusca (Table 3-2). FFA and ST were similarly detected in all the phyla, although to a generally lower extent than PL, ranging from 5.1 ± 2.8% in the Sipuncula to 25.1 ± 2.6% in the Arthropoda, for the former, and from 11.0 ± 0.9% in the Chordata to 35.9 ± 14.8% in the Sipuncula, for the latter (Table 3-2). While the Chordata had high levels of TAG in their tissues, i.e. 24.9 ± 2.7, this lipid class was less abundant in the other phyla (< 8%), and it was absent in the Sipuncula (Table 3-2). WE/SE were detected in the phyla Annelida, Arthropoda, Chordata, Cnidaria, Echinodermata, and Porifera, with the Arthropoda and Cnidaria having highest mean proportions (8.8 ± 3.1 and 12.7 ± 1.8%, respectively; Table 3-2). Overall, the lipid class composition varied significantly among phyla (PERMANOVA, Pseudo-$F_{7, 244}$ = 7.7, p = 0.0001) with PL and TAG contributing the most (90%) to the variability (Fig. 3-1). In addition, the mean CV measured for TAG and WE/SE were higher (> 150%) than that measured for PL, FFA, and ST (Table 2). Regarding the lipid ratios, the condition index TAG:ST ranged from values close to 0, as in the Mollusca, Porifera, and Annelida, to 7.7 ± 1.6 in the Chordata. Despite the low values of the index, the Mollusca also displayed the highest CV (Table 3-3). Conversely, results for the PL:ST were less variable across taxa overall, and values ranged from 1.8 ± 0.4 in the Annelida to 4.5 ± 0.5 in the Mollusca (Table 3-3).

Mean proportions (±se) of saturated FA (∑Sat) ranged from 14.9 ± 1.3% in the Echinodermata to 26.9 ± 2.1% in the Mollusca, and unsaturated FA (∑MUFA and ∑PUFA) were generally higher than saturated FA in all phyla, except Mollusca (Table 3-

4). In fact, this phylum was characterized by lower mean proportions of ∑MUFA than those of ∑Sat and ∑PUFA, as shown in Table 3-4. Regarding the essential FA, mean levels of ∑ω3 were higher overall (from 11.7 ± 2.0% in the Porifera to 42.4 ± 2.8% in the Mollusca) than those of ∑ω6 (from 2.1 ± 0.5% in the Porifera to 14.4 ± 1.8% in Echinodermata). Overall, the FA composition was significantly different across phyla (PERMANOVA, Pseudo-$F_{7, 271}$ = 11.7, p = 0.0001) (Fig. 3-2). Representatives of the phylum Chordata presented the highest mean concentrations of DHA+EPA in their tissues (0.5 ± 0.1 g per 100-g wm), followed by those belonging to the phyla Arthropoda, Mollusca, and Echinodermata (0.2 ± 0.0, 0.2 ± 0.0, 0.2 ± 0.1 g per 100-g wm, respectively; Table 3-4). Table 3-3 reports all the mean proportions of the various FA indices measured, with corresponding CV calculated by phylum. In general, the average CV measured for all the FA indices was <50%, with the only exceptions being those calculated for ∑ω6 and DHA+EPA, which were ≥85% (Table 3-4).

Analyses revealed that TAG, and ST were highly correlated with total lipid amounts (TAG, r_s = 0.6, n = 256, p = 0.000; ST, r_s = -0.6, n = 256, p = 0.000). Likewise, the TAG:ST ratio significantly correlated with total lipid content (r_s = 0.7, n = 250, p = 0.000). Although no significant relationship was detected between total lipid content and wet mass, ST correlated negatively with wet mass (r_s = -0.2, n = 256, p = 0.004). In addition, although weak, significant correlations were found between depth and various metrics, including FFA (r_s = -0.2, n = 256, p = 0.009); ST (r_s = -0.2, n = 256, p = 0.000); total lipid content (r_s = 0.2, n = 256, p = 0.001); wet mass (r_s = 0.2, n = 256, p = 0.001); PL:ST (r_s = 0.1, n = 238, p = 0.026); ∑MUFA (r_s = 0.2, n = 270, p = 0.002); and ∑ω6 (r_s = -0.2, n = 270, p = 0.003).

Lipid and fatty acid composition within phyla

Chordata

Several species of ray-finned fish (class Actinopterygii; n = 38 species), sharks (class Chondrichthyes; n = 5), and ascidians (class Ascidiacea; n = 6) comprised the phylum Chordata (Table 3-1). Overall, representatives of this phylum were characterized by the highest mean levels of fat in their tissues, as well as the greatest mean proportions of TAG. However, lipid data were highly variable across the taxa in the Chordata, with CV of mean values being ≥ 113% for both total lipid content and TAG (Table 3-2). Actinopterygii (ray-finned fish) showed higher amounts of lipid in their tissues than Chondrichthyes (sharks) and Ascidian (tunicates), with values ranging from 2.1 ± 1.1 mg g-1 wm in *Gaidropsarus ensis*, to 569.0 ± 417.0 mg g-1 wm in *Chiasmodon niger* (Table 3-1). Ray-finned fish also had a different lipid class composition, with high proportions of TAG, up to 82.9 ± 6.2% in *C. niger* (Appendix 7-3). In contrast, PL was the prevailing lipid class in the muscle tissue of sharks and ascidians, and with ST representing an important fraction in the body wall of the latter (≥ 23.7 ± 9.5%; Appendix 7-3). Although the phylum was characterized overall by low levels of WE/ST, the fishes *Arctozenus risso*, *Borostomias antarcticus*, *Caristius macropus*, *Lampadena speculigera*, and *Lampanyctus* spp. presented proportions of this lipid classes > 17% (Appendix 7-3). Conversely, variation in fatty acid data was smaller, and chordate species showed similar proportions of most FA indices, except for ∑ω6 where CVs reached 76% (Table 3-4). In detail, mean values of ∑ω6 ranged from 1.3% in Oneroides macrosteus to 12.8 ± 2.1% in Ascidiacea sp. 4 (Appendix 7-4). Ascidians were in general characterized by

higher mean levels of ω6-FA in their tissues, whereas sharks had larger proportions of PUFA and ω3, and fishes of ∑Sat (Appendix 7-3).

Arthropoda

This phylum was represented by 14 species across different classes (i.e. Hexanauplia, Malacostraca, and Pycnogonida), and characterized by a diverse set of lipid profiles (Tables 3-1 and 3-2). In particular, Malacostraca crustaceans had higher levels of lipids in their tissues than the Pycnogonida and Hexanauplia representatives (Table 3-1). Furthermore, most of the fat of these crustaceans was represented by WE/SE, as in *Acanthephyra pelagica*, Anonyx spp., and Gnathophausia zoea where this lipid class accounted for > 38% in (Appendix 7-3). Conversely, the lipid profile of both the Pycnogonida and Hexanauplia was mainly composed of PL and FFA, with the latter group also having high proportions of TAG (Appendix 7-3). In addition, WE/SE was either absent or present at trace levels within Pycnogonida and Hexanauplia (≤ 0.2 ± 0.3%), whereas TAG occurred in higher mean proportions (≥ 11.2 ± 3.0%). In the Arthropoda, CV levels for most of the FA indices were <45%, with the only exception was ∑ω6 whose CV was 106% (Table 3-4). In detail, the two species in the genus *Anonyx* presented the lowest proportions of ∑ω6 (0.8 and 0.7%) versus 10.1 ± 11.3% in *Steromastis sculpta* (Appendix 7-4). Appendix 7-4 shows the proportions of the different FA considered across the species of the Arthropoda; overall, decapods, such as *S. sculpta*, *Pandalus borealis*, and *Notostomus robustus*, displayed the lowest levels of MUFA within the phylum.

Echinodermata

This phylum was represented by 10 species of class Asteroidea (sea stars), 3 species of the class Echinoidea (sea urchins), and 4 of Ophiuroidea (brittle stars). Echinoderms had

relatively high amounts of lipids in their tissue (Table 3-2), dominated by PL (45.6 ± 3.4%). WE/SE were present only at trace levels in the sea star *Astropecten americanus*, the sea urchin Strongylocentrotus pallidus, and the brittle star *Ophiopholis aculeata*, whereas TAG was detected in most of the species, with particularly high mean proportions in the brittle stars *O. aculeata* and *Ophioscolex glacialis* (Appendix 7-3). While CVs of mean levels of ∑MUFA, ∑PUFA, and ∑ω3 was < 50%, greater variation was found for ∑Sat and ∑ω6 values across echinoderm species (Table 3-4). In fact, proportions of ∑Sat were spread in asteroids from 6.6% in *Mediaster bairdii* to 27.9% in *Brisaster fragilis*. The Echinoidea in general had higher levels of ∑Sat in their tissues than the Asteroidea and Ophiuroidea. On the other hand, the Asteroidea had greater percentages of ω6-FA in their tissues, peaking at 29.5% in *Myxaster sol* (Appendix 7-4).

Annelida

This phylum was represented by fewer species (n = 9; Table 3-1). The Annelida had intermediate amounts of lipids (10.1 ± 1.8 mg g-1 wm), which were mostly represented by PL, FFA, and ST (Table 3-2); nonetheless, both TAG (6.8 ± 2.8%) and WE/SE (3.5 ± 2.3%) were also detected. In particular, Polynoidae sp 3 and *Alitta succinea* respectively had the highest proportions of TAG and WE/SE within phylum. Proportions of saturated, unsaturated, ω3- and ω6-FA were similar overall across the Annelida. In addition, mean levels of MUFA and PUFA were higher than those of ∑Sat, and proportions of ∑ω3 were larger than those of ∑ω6 (Table 3-4).

Cnidaria

Phylum Cnidaria comprised 14 species belonging to class Anthozoa, which included representatives of the Actiniaria (sea anemones), Pennatulacea (sea pens), Alcyonacea (soft corals), and Scleractinia (stony corals). There were also 3 species belonging to

class Scyphozoa (jellyfishes). Cnidarians, in general, had low amounts of lipids in their tissues, although results were variable (CV = 98%; Table 3-2). The highest total lipid contents were found in pennatulaceans (sea pens) such as *Anthoptilum grandiflorum* and *Umbellula* sp. (35.4 and 31.1 mg g-1 wm, respectively), whereas lipid levels in jellyfishes were low at 2.0 ± 0.9 mg g-1 wm. Together with PL, FFA, and ST, WE/SE represented a significant fraction across cnidarians, with mean percentages of 12.7 ± 1.8% (Table 3-2), and the lipid class was particularly abundant in the corals *Paragorgia arborea* and *Umbellula* sp., as well as in the jellyfish *Periphyllia periphyllia* (Appendix 7-3). In addition, proportions of WE/SE were generally higher than those of TAG (Table 3-2). While proportions of ∑Sat, ∑MUFA, ∑PUFA and ∑ω3 were similar across species in Cnidaria, marked variation was noted for ∑ω6, especially within the class Anthozoa (Appendix 7-4). The sea anemone *Actinauge cristata* had the lowest levels of ω6-FA in its tissue (0.3 ± 0.1%), and the soft coral *Duva florida* had the largest proportions (40.4%).

Mollusca

A total of 9 species of class Cephalopoda and 4 of the Gastropoda represented the phylum Mollusca (Table 3-1). The low mean value of lipid content was largely consistent across species, with a CV of 44%. Likewise, the lipid class composition was similar among the species analyzed in this group: PL was the most abundant lipid class, occurring with percentages > 53%. Furthermore, no WE/SE were detected and TAG levels were low and measured only in the body wall of the cephalopods *Illex coindetii* and *Neorossia caroli*, and in the gastropod *Arrhoges occidentalis* (Appendix 7-3). Consistent with previous phyla, levels of ∑Sat, ∑MUFA, ∑PUFA, and ∑ω3 were similar across species, with CV < 40% and ∑ω6 showing the greatest variability (Table 3-4)

from 0.5% in the cephalopod *Rossia megaptera* to 16.7 ± 5.4% in gastropods of the genus *Colus* (Appendix 7-3).

Porifera

This phylum included 15 species of class Demospongia (demosponges) and 3 species of class Hexactinellida (glass sponges). Sponges were characterized overall by a low lipid content (5.9 ± 0.7 mg g-1 wm), with PL representing the largest fraction (45.6 ± 3.7%). Most of the variability among species was detected in TAG and WE/SE, with TAG presenting higher mean proportions in demosponges, and WE/SE in glass sponges (Appendix 7-3). Levels of PUFA, ω3-, and ω6-FA were highly variable across species (Table 3-4). In particular, the Hexactinellida had higher levels of PUFA than the Demospongiae; but the demosponge *Tentorium semisuberites* had the highest proportions of ω3- and ω6-FA in its tissue (30.7 and 6.8%, respectively).

Sipuncula

This phylum was represented by 2 species (Table 3-1). The Sipuncula had the lowest mean quantities of lipids among all the phyla analyzed (5.1±2.2 mg g-1 wm; Table 3-2), and most of these lipids were represented by PL, FFA, and ST; no TAG and WE/SE were detected in their tissues (Table 3-2). The 2 species of Sipuncula generally had higher mean levels of unsaturated FA, whereas those of ∑ω3 and ∑ω6 were similar (Table 3-4).

Discussion

As expected, there were marked differences in lipid content and composition both across the highest taxonomic groups (i.e. inter-phyla), as well as within phyla and within/among some of the lower taxonomic levels. Part of these differences may have been a reflection of phylogenetic diversity, as the PL composition of marine organisms is mostly driven by

phylogeny (Vaskovsky 1989) and PL represented the most abundant lipid fraction across the taxa analyzed. However, in the present study, most of the variability in lipid amounts appeared to be related to the lipid classes TAG and WE/SE (see paragraph below for assumptions and interpretations made for WE/SE), which also exhibited the largest coefficient of variation. In fact, a positive and significant correlation was detected between total lipid content and both these lipid classes. As TAG and WE are typical storage lipids in marine organisms (Fraser 1989; Lee et al. 2006), this variability was most likely reflective of the different energy allocation strategies (i.e. how energy is distributed towards growth, survival and reproduction) characterizing the taxa analyzed. Indeed, not all taxa accumulated energy reserves in either TAG or WE within the tissues analyzed and, among those that did accumulate lipid stores, different means (e.g. TAG *vs* WE) were used.

As previously shown by Lockyer (1986), Fraser (1989), and Lloret and Planes (2003), lipid content and composition of organisms may fluctuate on broad scales according to foraging and storage modes, metabolism, reproductive strategies, and food availability. Regarding the latter, studies suggest that high spatial and temporal variability in food supply selects for larger proportions of storage lipids (Childress et al. 1990). At the intraspecific level, age, size, and sex, as well as tissue type may also play a role (Henderson et al. 1984; Fraser 1989; Hirche and Kattner 1993; Pethybridge et al. 2010). Indeed, the size of organisms analyzed in the current investigation was highly variable within species, e.g. for the 3 individuals of *Zoroaster fulgens* (phylum Echinodermata) and the four individuals of *Borostomias antarcticus* (phylum Chordata), whereas age and sex were not determined. In addition, Pethybridge et al. (2010) showed that lipid class and FA composition of deep-water sharks substantially varied within each species, and across liver, kidney, muscle, pancreas, and stomach fluid tissues, with implications for

future studies. Indeed, depending on the main objective(s) of the investigation, the choice of the appropriate tissue type is crucial. In the current study, tissues characterized by low turnover rates were sampled from each taxon to reduce variability among tissue types and to optimize comparisons.

A positive correlation was also detected here between total lipid content and the condition index TAG:ST, thus that the fattier individuals were characterized by greater energy reserves than their conspecifics. This is mostly the case for the representatives of phylum Chordata, which had the highest variability in TAG:ST among and within species, as in *Notoscopelus* spp. and *Reinhardtius hippoglossoides*. In fact, as previously reported for shallow-water fishes (Herbinger and Friars 1991; Lloret and Planes 2003), corals (Glynn et al. 1985), crustaceans (Mourente et al. 1995), and bivalve larvae (Fraser 1989), the higher the lipid content and energy reserves within the representatives of these taxa, the higher their growth rate, reproductive success, or survival. The same idea may be applied to deep-sea organisms, taking into account that their metabolic rates and lipid stores may be lower than in their shallow-water counterparts, and the way the energy is partitioned among somatic growth, reproduction, and survival may hence be different (Childress et al. 1990).

Certain crustaceans, fishes, jellyfishes, and corals analyzed in this study used WE, rather than TAG, as the main form of energy storage. Although most terrestrial and aquatic organisms store energy in TAG (Lehninger 1975; Parrish 2013), these crustaceans, fishes, jellyfishes, and corals had greater levels of WE and/or SE, which could not be fully distinguished. Among them, the crustaceans *Acanthephyra pelagica*, *Anonyx* spp., and *Gnathophausia zoea*, the fishes *Lampanyctus* spp., *Caristius macropus*, and *Arctozenus risso*, the jellyfishes *Atolla wyvellei* and *Periphylla peryphylla,* and the corals *Paragorgia arborea* and *Umbellula* sp. showed proportions of WE/SE

>20% up to 60%. No indication was found in the literature about SE accumulation in these taxa. Whereas the technique applied in the current study did not allow for the separation of these two lipid classes; Kayama et al. (1974) showed that proportions of steryl esters were consistently smaller relative to those of wax esters in the roe of various shallow-water fish species, and Nevenzel (1970) indicated that small amounts of steryl esters are typically present in animal tissues. Therefore, the high proportions of WE/SE were assumed to mostly correspond to WE, which are known to play an important role as both energy storage (Benson and Lee 1972) and in buoyancy control (Phleger 1998; Lee et al. 2006).

Deep-water zooplankton and fish have previously been shown to accumulate large quantities of WE within their tissues (Lee et al. 1970; Lee and Hirota 1973; Phleger et al. 1997). In particular, herbivorous zooplankton (e.g. copepods) from the polar and sub-polar regions accumulated large quantities of WE over summer, and to use these lipids to store energy during long periods of starvation and to maintain neutral buoyancy at depths > 500 m (Visser and Jónasdóttir 1999; Lee et al. 2006). In fact, while TAG are used as a short-term deposit, WE provide a longer-term energy provision to such zooplankton overwintering at great depths (Hopkins et al. 1993; Lee et al. 2006). Furthermore, the use of WE for buoyancy control is beneficial for zooplankton living in cold deep waters, due to the thermal expansion and compressibility of such molecules (Visser and Jónasdóttir 1999). As for cold-water corals, the only study providing evidence of storage via WE is that conducted by Hamoutene et al. (2007), which was accomplished within the same region of the Northwest Atlantic during the same season. In particular, Hamoutene et al. (2007) proposed that corals stored their energy in WE, as well as in alkyldiacylglycerols. Here, the proportion of alkyldiacylglycerols (or glyceryl ethers) across all the Cnidaria species was minimal (0.7 ± 0.2%; Appendix 7-2).

Conversely, they showed higher levels of TAG (Appendix 7-2), suggesting these species may use both TAG and WE for energy storage as reported in shallow-water corals (Hamoutene et al. 2007). While herbivorous zooplankton are able to synthesize WE *de novo* (Lee et al. 2006), higher-level consumers may synthesize this lipid following the incorporation of dietary-derived fatty alcohols (Phleger et al. 1997). Therefore, it is likely that the crustaceans, jellyfishes, corals, and fishes presenting larger levels of WE in the current investigation preyed on WE-rich zooplankton.

Depth was an important driver of lipid content and composition of the species analyzed in this study, and the environmental conditions at sampling might also have contributed to the variability in their lipid levels. Although sampling was carried out within a tight geographical radius (100 km), organisms were indeed collected along a wide depth range of ~1000 m. Representatives of the phyla Mollusca and Echinodermata, for instance, which had the highest PL:ST ratios, were collected between 464 and 1407 m and between 313 and 1407 m, respectively. According to Cossins and Macdonald (1986), Hopkins et al. (1993), and Simonato et al. (2006), environmental variables such as temperature and pressure may modulate lipid content and composition, and both parameters vary along a bathymetric gradient (Thistle 2003). Positive trends were detected here between depth and the PL:ST ratio, an indicator of membrane lipid remodeling (Parrish 2013), as well as between depth and proportions of MUFA. However, depth negatively correlated with ST. These results suggest that both ST and unsaturated FA are involved in the bathymetric response and, specifically, that the species collected at deeper depths have higher overall levels of lipid unsaturation, mainly due to MUFA, and a lower ST content. Decreasing temperature and increasing pressure along the depth gradient have the ability to reduce membrane fluidity, thus compromising its general structure and function (Crockett 1998; Simonato et al. 2006; Parent et al.

2008). In response, organisms may adjust and remodel the lipid composition of their membranes, through a process known as homeoviscous adaptation, which involves changes in the cholesterol content, as well as changes in length and unsaturation levels of the membrane FA and in phospholipid headgroups and molecular species (Cossins and Macdonald 1989; Crockett 1998; Simonato et al. 2006). Specifically, cholesterol, the main form of ST in most animals (Drazen et al. 2008b), generally favours packing in the membranes, increasing their rigidity (Crockett 1998); in contrast, long-chain unsaturated FA are characterized by a higher molecular flexibility and lower melting points, thus providing more fluidity to membranes (DeLong and Yayanos 1985). Direct evidence of this type of lipid remodelling was documented in shallow-water bivalves (Pernet et al. 2006), as well as in deep-water microorganisms (Yano et al. 1998). It was also suspected to occur in fishes collected between 200 and 4000 m; specifically, deeper-water species displayed higher levels of unsaturation than shallow-water ones (Cossins and Macdonald 1986).

Interestingly, included in the present dataset were species known to undergo diel vertical migration, such as the myctophid fishes *Lampanyctus* spp. and *Myctophum* sp. (Watanabe et al. 1999) and the crustacean decapod *Acanthephyra pelagica* (Roe 1984). Since these species can travel vertically over a few hundred meters (Roe 1984), thus experiencing marked changes in temperature and pressure, it would be of particular interest to undertake a study to assess their ability to overcome such variations, in terms of membrane lipid composition. Pernet et al. (2007) found that while the level of unsaturation was adjusted in response to both long- and short-term acclimation to temperature fluctuations in the shallow-water oyster *Crassostrea virginica*, the modulation of the PL:ST ratio was only accomplished in response to long-term acclimation (7 days). As Hazel and Landrey (1988) noted that the modulation of

phospholipid molecular species and headgroups preceded (within 16-48 h) the adjustment of the unsaturation level (after 10-21 days) in the thermal acclimation of the rainbow trout *Salmo gairdneri*, it would be valuable to verify whether deep-sea species have the same time course for thermal acclimation.

In the present study, FA composition was more consistent across phyla than the lipid class composition and this, probably, was mostly driven by phylogeny, in accordance with Dalsgaard et al. (2003). Furthermore, higher proportions of unsaturated *vs* saturated FA were measured here, as well as higher levels of ∑ω3 *vs* ∑ω6 FA, which followed initial expectations. Certain PUFA (e.g. ω3-FA) are known key dietary components that are required by aquatic organisms for optimal health, both in shallow (Parrish 2009) and deeper waters (DeLong and Yayanos 1986). Such essential FA are for example involved in cell synthesis, neural development, somatic growth, membrane function and structure, reproduction, ionic regulation, and immune function in aquatic organisms (Simonato et al. 2006; Parrish 2009; Colombo et al. 2016). In particular, docosahexaenoic acid (DHA, 22:6ω3), eicosapentaenoic acid (EPA, 20:5ω3), and arachidonic acid (ARA, 20:4ω6) are all of primary importance for marine species (Parrish 2009), although the extent to which these essential FA occur within organisms may vary (Iverson et al. 2002; Bergé and Barnathan 2005). Typically, ARA occurs in lower proportions than EPA and DHA, according to the availability of these FA as dietary sources (Parrish pers. comm.). The present study was consistent with the literature, wherein proportions of ω6-FA were up to 9 times lower (e.g. in Arthropoda) than those of ω3-FA (values of individual FA are provided in Chapter 4).

Species of the phyla Chordata and Arthropoda represented the most important reservoir of essential nutrients within the faunal assemblage analyzed. Marine organisms, and fish in particular, are generally known to be a major source of PUFA,

such as ω3-FA (Arts et al. 2001; Huynh and Kitts 2009; Colombo et al. 2016). Marine species containing higher levels of ω3:ω6 FA, PUFA, and DHA+EPA are hence recommended for human consumption, due to their high nutritional value (Kris-Etherton et al. 2002; Huynh and Kitts 2009; Fernandes et al. 2014). Furthermore, as DHA, EPA, and to a lesser extent ARA, are likewise largely required by marine organisms and have to be gained through diet (Parrish 2009), feeding habits of marine organisms might be driven by their nutritional needs. In other words, PUFA and essential FA are required at every trophic level and are highly conserved in marine food webs (Arts et al. 2001). However, the transfer of these compounds throughout the food web is uneven, and depends on the biochemical and physiological requirements of each taxon (Arts et al. 2001). In the present investigation, taking into account that only certain tissues were analyzed for each taxon (see Material and Methods), the Mollusca, Arthropoda, and Chordata had the largest proportions of ω3-FA, while the Chordata, Arthropoda and Mollusca had the highest concentrations of DHA+EPA, and Mollusca had the highest levels of PUFA. Since neither eggs nor larvae were sampled here, these results suggest that later life stage representatives (juveniles/adults) of these phyla may all constitute important reservoirs of nutrients. However, the overall lipid content of the Mollusca was low and, therefore, the provision of PUFA and essential FA from these phyla may be limited. In contrast, the Chordata and Arthropoda presented the highest lipid levels in their tissues and, for the same mass, they therefore represent a greater reservoir of nutrients than the Mollusca. At the species level, the fishes *Coryphaenoides rupestris* and *Gaidropsarus ensis*, as well as the crustacean *Notostomus robustus*, presented the largest levels of essential FA in their tissues, and hence constitute key stores of nutrients among the species analyzed in the Northwest Atlantic. These species are widely distributed in the area (Van Guelpen et al. 2005), although the population of *C. rupestris*

underwent drastic declines over the last few decades (Baker et al. 2009). As a side note, the vertically migrating species *Lampanyctus* spp., *A. pelagica, P. borealis,* and *N. robustus*, included within the Chordata and Arthropoda, were also characterized by high levels of ∑Sat. Since ∑Sat are nutritionally important as a source of energy to consumers (Sargent et al. 1997), these migrating species may play a key role in enhancing the transfer of both essential nutrients and energy to deeper ecosystems.

Because of the small amount of samples required and the value of the information provided, lipid analysis has supported the investigation of still-poorly-known deep-sea fauna and ecosystems of different oceanic regions, such as the Northeast Pacific (Drazen et al. 2008a; Drazen et al. 2008b), Northeast Atlantic (Howell et al. 2003), and Antarctic (Würzberg et al. 2011). The present study extends this dataset to deep-sea taxa of the Northwest Atlantic and additionally highlights some important findings: i) the wide range of total lipid content and composition suggests a great diversity across deep-sea taxa in terms of energy allocation strategies, perhaps associated with diversified deep-sea adaptations (e.g. migratory behaviors, buoyancy and metabolic needs), and with a certain availability of food in the deep-sea; ii) the type and amount of energy storage are reflective of habitat (pelagic *vs* demersal), as well as of the type of preferred food sources for certain deep-sea taxa (e.g. WE-rich zooplankton); iii) by modulating ST and FA composition, some species are presumably able to counteract the effect of temperature and pressure along the depth gradient; and finally, iv) representatives of the phyla Chordata and Arthropoda constitute a major reservoir of essential nutrients, and the migrating species included in the two taxa may play a crucial role in transferring these nutrients to deeper food webs.

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Compliance with ethical standards

Conflict of interest All the authors have approved the manuscript submitted and declared they have no conflicts of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Tables

Table 3-1 Deep-sea macrofauna taxa sampled. Phylum, class, and species analyzed, together with sample size, mean depth of collection, and mean values of wet mass and total lipid content for each species are shown. Data are reported in decreasing order of lipid contents.

Phylum	Class	Species	n	Depth	Mean wet mass	Mean total lipids
				m	g ±sd	mg g^{-1} wm ±sd
Chordata						
	Actinopterygii					
		Alepocephalus bairdii	2	707-1321	161.2±140.0	41.0±31.2
		Anoplogaster cornuta	4	919-1365	80.2±26.9	148.0±30.6
		Antimora rostrata	3	1090	263.2±92.3	2.9±0.3
		Arctozenus risso	2	1090	15.1±15.6	67.3±75.2
		Bathylagus euryops	2	1090	7.5±2.4	19.7±11.7
		Bathytroctes macrolepis	2	1282	67.3±35.2	6.0±2.1
		Borostomias antarcticus	4	1090-1321	44.9±46.7	22.1±17.5
		Caristius macropus	1	1365	176.3	172.4
		Chauliodus sloani	6*	889-1365	36.7±12.3	25.9±15.9
		Chiasmodon niger	3	1365	78.9±45.4	568.9±417.4
		Coryphaenoides rupestris	3	759	54.3±23.4	4.8±1.9
		Cottunculus microps	2	889-919	72.4±12.2	7.7±8.3
		Cottunculus thomsonii	1	1090	1379.3	34.2
		Cyclothone microdon	2	1090	6.6±0.1	26.4±11.2
		Gaidropsarus ensis	4	919-1090	174.6±139.1	2.1±1.1
		Glyptocephalus cynoglossus	3	488	321.5	4.8±2.1
		Haplophryne mollis	1	1084	19.0	2.4
		Lampadena speculigera	1	1090	12.4	90.5
		Lampanyctus spp.	4	1090	24.9±6.5	52.2±32.2
		Lepidion eques	1	868	130.6	4.7
		Macrourus berglax	5*	759-1090	90.6±39.0	4.4±0.7
		Magnisudis atlantica	2	1122-1321	342.5±28.8	61.9±8.3
		Malacosteus niger	2	313-1094	38.8±0.9	68.0±2.9
		Melanocetus johnsonii	1	1407	178.8	7.4
		Myctophum sp.	1	1090	6.2	215.4
		Nezumia bairdii	3*	1090	97.2±54.5	4.7±2.8

		Notacanthus chemnitzii	3*	-	691.3±84.4	12.8±7.3
		Notoscopelus spp.	2	1090	21.7±6.8	270.1±9.2
		Oneirodes macrosteus	1	759	119.0	6.3
		Polyacanthonotus rissoanus	3*	1090-1321	94.2±34.6	33.0±17.6
		Reinhardtius hippoglossoides	2	759-1090	542.9	141.7±148.2
		Scopeloberyx opisthopterus	2	1090	3.8±0.2	22.8±2.8
		Scopelosaurus lepidus	1*	759	128.8	43.6
		Sebastes mentella	3	488	200.0±108.1	13.8±2.2
		Serrivomer beanii	3	-	49.5±20.8	11.7±2.8
		Synaphobranchus kaupii	3	1090	100.0±14.7	156.6±99
		Trachyrincus murrayi	3	868	94.2±5.4	2.8±0.4
		Xenodermichthys copei	4	759-889	18.6±4.8	28.2±11
	Ascidiacea					
		Ascidiacea sp 1	4**	759-1407	69.0±80.5	0.8±0.6
		Ascidiacea sp 2	1*	759	4.1	0.3
		Ascidiacea sp 3	1*	313	0.9	1.4
		Ascidiacea sp 4	2	759	7.4±0.6	3.9±0.9
		Didemnum sp.	1	759	0.9	1.9
		Eudistoma vitreum	1	1122	0.9	3.1
	Chondrichthyes					
		Amblyraja jenseni	1	919	796.7	8.1
		Apristurus profundorum	3	1324-1365	1805.4±249.5	9.0±3.1
		Centroscyllium fabricii	2	919	1177.7±72.8	6.8±0.5
		Malacoraja senta	1	759	81.7	11.1
		Rajella fyllae	4	919-1365	4.5±0.9	12.0±3.9
Arthropoda						
	Hexanauplia					
		Arcoscalpellum michelottianum	3	1094-1365	6.6±1.5	10.6±5.1
	Malacostraca					
		Acanthephyra pelagica	3*	1090	7.0±0.9	34.0±7.7
		Anonyx sp 1	1	1365	0.6	94.6
		Anonyx sp 2	1	1321	0.7	281.6
		Gnathophausia zoea	3*	1090-1282	1.5±0.6	20.5±2.3
		Munida tenuimana	1	868	1.1	1.3
		Munidopsis curvirostra	3	1084-1282	1.6±0.4	33.5±42.7
		Notostomus robustus	1	1365	11.9	5.2
		Pandalus borealis	3	488	5.6±0.2	15.8±7.5
		Pasiphaea tarda	3	1321	29.2±15.5	8.7±5.4
		Sabinea hystrix	3	1090-1094	7.0±3.2	11.6±3.1
		Stereomastis sculpta	3	1094-1321	4.6±2.1	4.4±2.3

		Themisto libellula	1	313	0.1	8.5
	Pycnogonida					
		Nymphon spp.	6*	347-868	0.3±0.2	8.7±6
Echinodermata						
	Asteroidea					
		Astropecten americanus	3	1122	14.3±3.02	18.2±13.0
		Brisingida spp.	2	1084-1365	52.6±41.9	24.1±16.8
		Cheiraster sp.	1	1365	3.5	0.4
		Ctenodiscus crispatus	3	313	3.1±1.0	2.6±0.4
		Freyella microspina	1	1407	70.2	103.8
		Leptychaster arcticus	3	353	2.4±0.3	5.9±1.3
		Mediaster bairdi bairdi	3	1090	14.8±3.5	4.2±0.2
		Myxaster sol	1	919	71.1	5.7
		Psilaster andromeda	2*	868-1365	19.6±19.1	31.5±42.7
		Zoroaster fulgens	3	759-1282	16.8±17.8	16.6±26.6
	Echinoidea					
		Brisaster fragilis	2*	759	3.7±2.5	1.9±2.6
		Phormosoma placenta	3	889	19.6±7.4	6.0±2.7
		Strongylocentrotus pallidus	2	353-379	20.5±22.1	2.6±0.9
	Ophiuroidea					
		Gorgonocephalus sp.	1	595	1.2	42.4
		Ophiopholis aculeata	2	353	0.9±0.5	17.3±13.3
		Ophioscolex glacialis	2	353	0.7±0.3	15.3±2.6
		Ophiura sarsii	3	1282	6.7±1.4	1.5±0.6
Annelida						
	Polychaeta					
		Alitta succinea	1	1027	0.3	16.8
		Laetmonice filicornis	1	595	3.1	7.4
		Nereididae sp 1	1*	868	0.0	8.5
		Nereididae sp 2	1	347	1.6	17.5
		Polynoidae sp 1	1	347	1.9	6.3
		Polynoidae sp 2	2	595	4±0.3	5.3±0.3
		Polynoidae sp 3	1	595	0.7	15.6
		Polychaeta sp 1	1	595	0.7	11.5
		Prionospio sp.	1	868	0.1	4.7
Cnidaria						
	Anthozoa					
		Acanella arbuscula	3*	759-1122	5.5±3.6	3.3±0.2
		Actinauge cristata	2*	759-889	101.9±44.5	0.8±0.4
		Actinoscyphia aurelia	3**	796-1027	33.9±21.1	0.4±0.2
		Actinostola callosa	3**	759	71.4±26.7	0.3±0.1

		Anthomastus agaricus	3	1027	12.2±7.1	4.1±1.9
		Anthomastus sp.	1	868	5.2	5.4
		Anthoptilum grandiflorum	1	759	4.8	35.4
		Duva florida	1	-	15.8	14.5
		Flabellum alabastrum	2	759	6.5±2.3	11.7±0.4
		Funiculina sp.	1	1084	2.1	13.1
		Paragorgia arborea	1	595	90.3	13.3
		Pennatula aculeata	3	1282	2.0±0.6	14.7±4.7
		Pennatula grandis	2	759-1282	4.2±2.2	18.7±8.2
		Umbellula sp.	1	1122	3.8	31.1
	Scyphozoa					
		Atolla wyvillei	3*	1090	25.5±24.5	0.7±0.7
		Periphylla periphylla	3*	759-1282	58.9±47.1	1.8±0.4
		Scyphozoa sp.	1*	1090	59.7	0.6
Mollusca						
	Cephalopoda					
		Bathypolypus arcticus	3	464-1321	19.2±14.1	7.4±1.0
		Bathypolypus bairdii	1	707	50.1	4.3
		Cephalopoda sp 1	1	1282	410.9	9.2
		Cephalopoda sp 2	1	1407	986.8±127.4	2.8±1.4
		Chiroteuthis veranii	1	1090	151.2	12.0
		Illex coindetii	3	1282	54.2±7.2	10.2±3.2
		Neorossia caroli	1	488	17.2	5.2
		Rossia megaptera	1	1407	36.7	4.5
		Stauroteuthis syrtensis	3	1090-1407	22.1	7.2
	Gastropoda					
		Arrhoges occidentalis	1	1282	6.2	4.4
		Buccinum sp.	3	759	5.8±2.6	6.9±1.3
		Colus spp.	3	759-889	22±30.3	4.8±1.0
		Neptunea despecta	1	889	7.1	4.8
Porifera						
	Demospongiae					
		Cliona sp.	1	1027	76.0	6.1
		Craniella cranium	3	464-595	13.1±6.1	6.9±1.0
		Geodia sp.	1	1027	577.9	5.1
		Haliclona sp.	2	1324	14.8±0.4	3.9±1.6
		Hamacantha (*Vomerula*) *carteri*	1	488	44.7	0.8
		Histodermella sp.	1	-	3.1	13.3
		Iophon piceum	1	353	157.2	7.8
		Mycale (Mycale) lingua	1	759	55.4	4.1
		Phakellia sp.	1	313	93.3	5.2

		Polymastia spp.	2	353	19.7±14.3	9.9±0.7
		Polymastia hemisphaerica	1	488	29.7	4.5
		Stelletta sp.	1	1122	26.1	4.3
		Stryphnus ponderosus	1	-	14.8	10.6
		Tentorium semisuberites	1	353	6.1	13.8
		Thenea muricata	4	353	16.2	2.6±1.2
	Hexactinellida					
		Euplectella sp.	2	1407-1094	87.7±107.4	4.3±3.7
		Hexactinellida sp 1	1	1027	228.6	4.8
		Hexactinellida sp 2	1*	1407	21.9	0.3
Sipuncula						
	Sipunculidea					
		Sipunculidea sp 1	1	1407	3.5	7.3
		Sipunculidea sp 2	1	1122	2.0	3.0

*, ** n = 1, 2 individual(s) removed from analysis of lipid composition

Table 3-2 Wet mass and lipids in deep-sea macrofauna phyla under study. Sample number (n), and mean values of wet mass, total lipids, and mean proportion of phospholipids (PL), free fatty acids (FFA), sterols (ST), triacylglycerols (TAG), wax esters or steryl esters (WE/SE). Coefficients of variation (CV; %) are also reported for each mean value, as well as grand means related to each variable.

Phylum	n	Wet mass		Total lipids		PL		FFA		ST		TAG		WE/SE	
		g ±se	CV	mg g^{-1} wm ±se	CV	% ±se	CV	% ±se	CV	% ±se	CV	% ±se	CV	% ±se	CV
Chordata	105	186.0±36.7	202	56.0±12.1	221	24.7±2.1	85	20.5±1.6	79	11.0±0.9	84	24.9±2.7	113	3.7±1.1	311
Arthropoda	32	6.2±1.6	146	24.8±9.0	206	31.7±3.8	68	25.1±2.6	59	15.2±1.6	58	7.3±2.3	180	8.8±3.1	199
Echinodermata	35	16.2±3.5	129	14.3±3.6	151	45.6±3.4	44	14.7±1.5	61	14.3±1.4	58	7.1±1.9	155	0.1±0.0	297
Annelida	9	1.8±0.5	85	10.1±1.8	53	38.2±6.3	49	21.6±4.7	65	21.5±4.9	68	6.8±2.8	123	3.5±2.3	193
Cnidaria	25	16.9±5.2	154	9.8±1.9	98	28.5±3.1	54	20.1±2.4	59	12.2±0.9	37	5.4±1.4	128	12.7±1.8	71
Mollusca	23	172.4±70.0	195	6.4±0.6	44	66.4±2.8	20	15.0±1.9	59	16.9±1.1	31	0.4±0.3	288	-	
Porifera	25	73.3±25.6	174	5.9±0.7	59	45.6±3.7	41	17.6±1.6	45	17.9±1.2	35	5.3±1.1	107	3.2±1.2	181
Sipuncula	2	2.8±0.8	39	5.1±2.2	59	52.8±16.4	44	5.1±2.8	79	35.9±14.8	58	-		-	
Mean CV			**141**		**111**		**51**		**63**		**54**		**156**		**209**

Table 3-3 Mean value of triacyglycerol to sterol (TAG:ST) ratio and phospholipid to sterol (PL:ST) ratio reported for each phylum, together with corresponding coefficients of variation (CV; %).

Phylum	TAG:ST		PL:ST	
	Mean±se	CV	Mean±se	CV
Chordata	7.7±1.6	203	3.2±0.5	147
Arthropoda	1.3±0.6	250	3.7±0.8	127
Echinodermata	0.9±0.3	173	4.0±0.5	72
Annelida	0.5±0.2	128	1.8±0.4	66
Cnidaria	0.6±0.2	141	2.5±0.3	53
Mollusca	0.0±0.0	325	4.5±0.5	49
Porifera	0.3±0.1	155	3.1±0.4	62
Sipuncula	-	-	2.0±1.3	91
Mean CV		**196**		**83**

Table 3-4 Fatty acid sums in deep-sea phyla under study. Sample number (n), mean value and related coefficient of variation (CV; %) of the sum of saturated (∑Sat), monounsaturated (∑MUFA), polyunsaturated (∑PUFA), ω3 and ω6 FA, as well as DHA+EPA are reported for each phylum.

Phylum	**n**	**∑Sat**		**∑MUFA**		**∑PUFA**		**∑ω3**		**∑ω6**		**DHA+EPA**	
		% ±se	CV	% ±se	CV	% ±se	CV	% ±se	CV	% ±se	CV	g per 100 g wm ±se	CV
Chordata	115	22.4±0.7	34	42.0±1.7	44	33.9±1.3	42	27.7±1.3	49	3.7±0.3	76	0.5±0.1	179
Arthropoda	35	16.5±1.2	44	43.8±1.6	22	37.3±1.3	21	30.4±1.7	32	3.5±0.6	106	0.2±0.0	81
Echinodermata	36	14.9±1.3	52	43.1±1.5	21	40.3±1.6	24	18.6±1.4	46	14.4±1.8	74	0.2±0.1	149
Annelida	9	20.4±1.3	20	38.8±2.1	16	39.5±2.6	20	27.6±2.7	29	4.6±0.6	39	0.1±0.0	59
Cnidaria	35	17.6±0.9	30	44.4±1.6	21	35.4±1.6	27	21.4±1.6	44	10.0±1.5	91	0.1±0.0	166
Mollusca	23	26.9±2.1	37	19.3±1.0	25	53.2±2.1	19	42.4±2.8	32	5.4±1.2	108	0.2±0.0	79
Porifera	24	20.8±2.2	51	50.3±3.2	31	20.8±3.7	86	11.7±2.0	85	2.1±0.5	115	0.04±0.0	118
Sipuncula	2	26.7±3.3	17	36.3±12.2	48	34.0±16.3	68	12.0±7.3	86	8.2±4.1	70	0.03±0.0	132
Mean CV			**36**		**29**		**38**		**50**		**85**		**120**

Figures

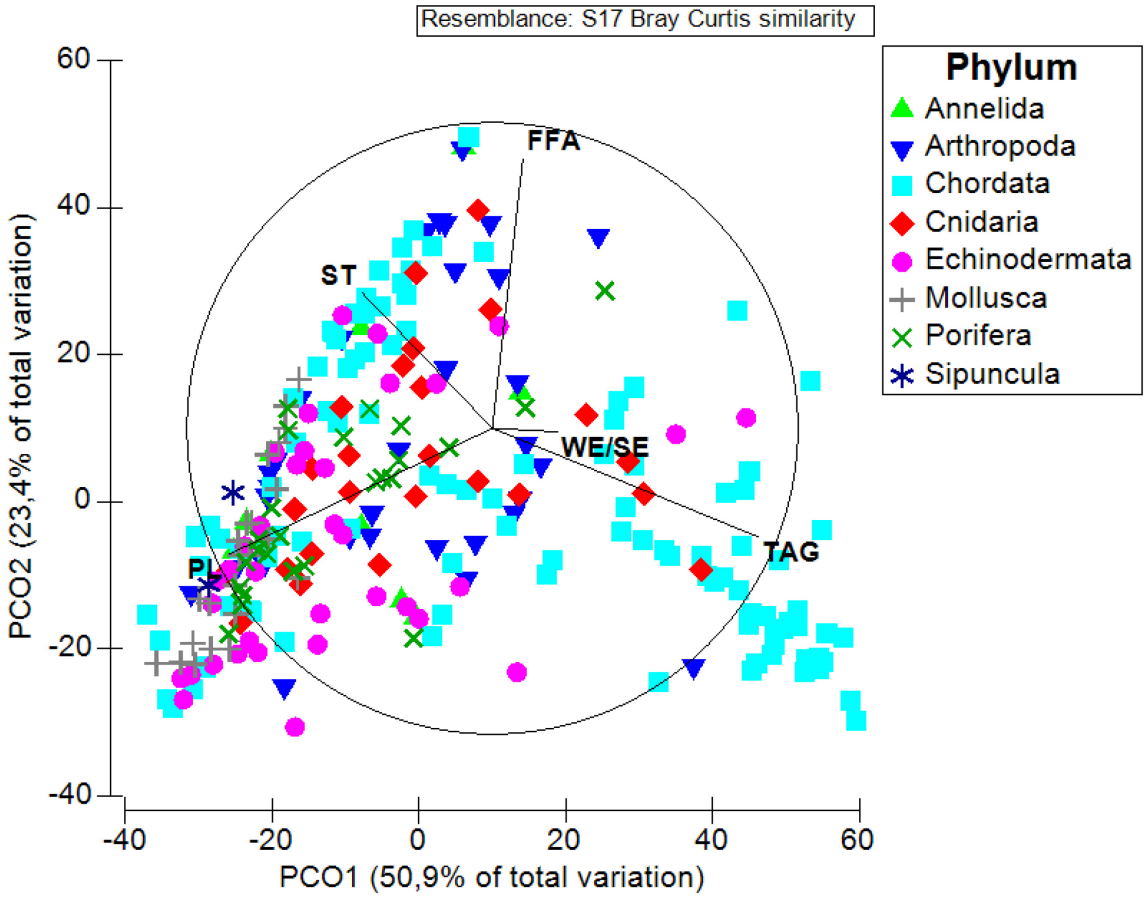

Fig. 3-1 Principal coordinate (PCO) analysis plot representing differences in terms of lipid class composition across phyla. The lipid classes reported occurred with proportions > 1.7%, including phospholipids (PL), free fatty acids (FFA), sterols (ST), triacylglycerols (TAG), and wax esters/steryl esters (WE/SE).

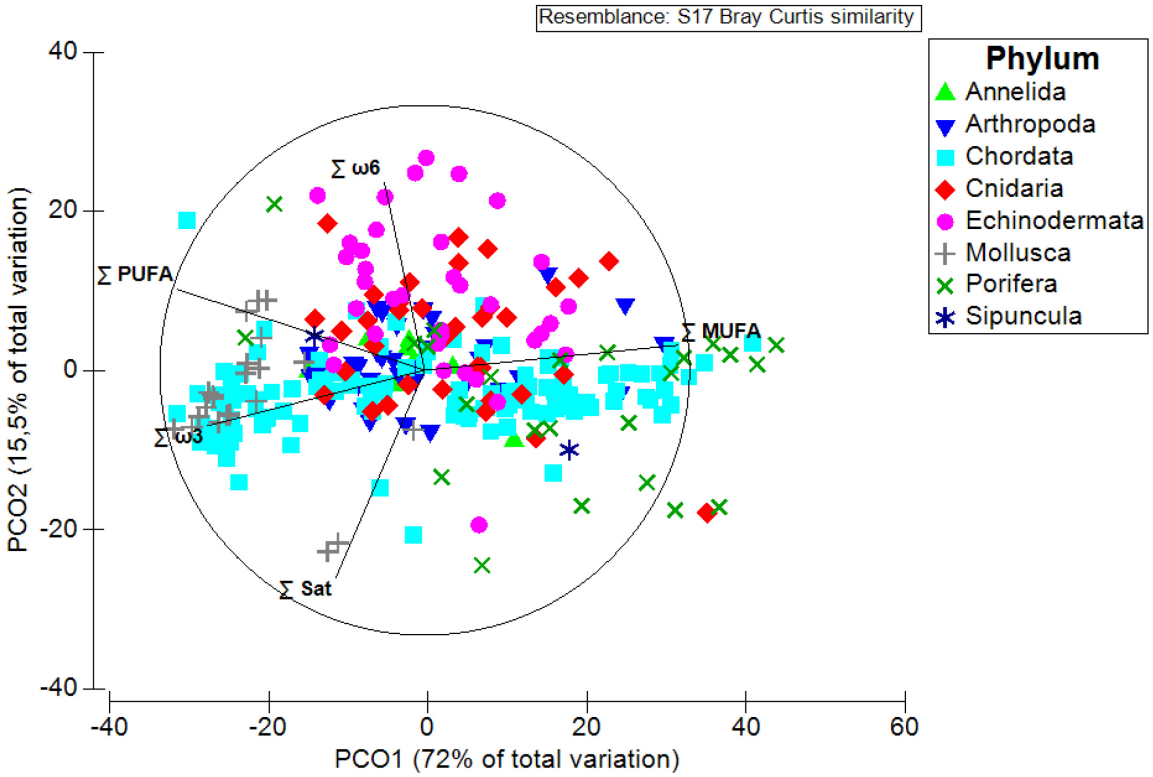

Fig. 3-2 Principal coordinate (PCO) analysis plot representing differences in terms of FA composition across phyla. The sums of saturated- (∑ Sat), monounsaturated- (∑ MUFA), and polyunsaturated FA (∑ PUFA), are reported together with those of ω3- and ω6 FA (∑ ω3 and ∑ ω6, respectively).

Chapter 4 : **Trophic relationships among deep-sea benthic organisms on a continental margin in the NW Atlantic inferred by stable isotope, elemental, and fatty acid composition**[3]

[3] A version of this manuscript was submitted to the journal Progress in Oceanography and was pending revision at the time of publication.

Abstract

As deep-sea benthic ecosystems of continental margins provide numerous functions and services to humans, a better understanding of these key habitats and their communities is needed to help predict climate-driven shifts and support conservation efforts. Here stable isotope ($\delta^{13}C$ and $\delta^{15}N$), elemental (%C, %N, and molar C:N), and fatty acid (FA) composition of 50 different deep-sea species, belonging to 7 major taxa, were analyzed in order to characterize their diet and trophic position, and to study the fate of energy and essential nutrients in the food web. In addition, relationships between depth and biochemical signatures ($\delta^{13}C$, $\delta^{15}N$, %C, %N, $C:N_{mol}$, and FA) were also investigated. In this regard, %C, oleic acid (18:1ω9), and arachidonic acid (ARA, 20:4ω6) increased with depth. While the increase of %C was likely due to the preferential assimilation of the more nutritious N along the gradient, that of 18:1ω9 was presumed to reflect the need for longer-term energy reserves in deeper organisms, and that of ARA to indicate a higher reliance on the benthic trophic pathway at greater depth. Analyses also revealed that the focal deep-sea taxa occupied a minimum of three trophic levels, whereas the weak correlation between $\delta^{13}C$ and $\delta^{15}N$ indicated that two or more trophic pathways were represented. Several feeding modes were also recognized within the assemblage. The lowest trophic positions were occupied by sponges most likely feeding on bacteria. Intermediate positions were mainly occupied by suspension feeders (e.g. sea anemones, corals), detritivores (e.g. the sea urchin *Phormosoma placenta*), and predators on small infaunal animals (e.g. the sea star *Leptychaser arcticus*). Conversely, predator/scavengers (e.g. various sea stars, gastropods, polychaete worms) occupied the highest trophic positions, together with sponges that were determined to be either carnivorous (e.g. *Iophon piceum*), or to feed on isotopically enriched organic matter.

Energetic compounds (i.e. 20:1ω11, 20:1ω9, and 22:1ω7) and essential nutrients (i.e. ARA) increased in proportion across trophic levels throughout this food web, emphasizing the importance of certain dietary FA for optimal organism health, and the key role of benthic communities in carbon cycling.

Introduction

Continental margins and associated benthic ecosystems (i.e. from 200 m down to 4000 m depth; Levin and Sibuet, 2012) are key features of the marine environment, as they provide numerous functions and services. Because they are characterized by a wide variety of highly heterogeneous ecosystems (Levin and Sibuet, 2012; Thurber et al., 2014), such as sponge grounds (Beazley et al., 2013), cold-water coral gardens (Bongiorni et al., 2010; Baillon et al., 2014), submarine canyons (Levin et al., 2010), cold seeps (Sibuet and Olu, 1998), and hydrothermal vents (Levin and Sibuet, 2012), these regions are often considered biodiversity hotspots. In addition, not only do continental margins play a major role in nutrient and biogeochemical cycling (Levin and Dayton, 2009; Levin and Sibuet, 2012), but they also sustain pelagic communities, through bentho-pelagic coupling and bottom-up forcing (Levin and Dayton, 2009). Continental margins also provide important resources, such as food, minerals and petroleum (Levin and Dayton, 2009), as well as natural products including antibiotics (Skropeta, 2008). The alteration of such ecosystems could therefore threaten both their functioning and the services they provide to humans.

Over the past decades, the study of food webs and trophic relationships has intensified, allowing a better understanding of various drivers of ecosystem health and stability (Thompson et al., 2012). Due to logistical and physical constraints related to deep-sea research, trophic ecologists have developed alternative methods to the classic study of gut contents to investigate feeding habits and interactions in deeper regions of the ocean. Among them, biomarkers such as stable isotopes and fatty acids have been successfully applied to study trophic positions (Post, 2002; Hussey et al., 2014), dietary habits (Gale et al., 2013), as well as carbon sources and energy flow (Parrish et al.,

2000; Budge et al., 2008; Trueman et al., 2014). The enrichment of stable nitrogen isotope ratios ($^{15}N/^{14}N$ or $\delta^{15}N$) between prey and predator ranges from 2 to 4‰ (Minagawa and Wada, 1984), making it a powerful tool to measure trophic positions. Conversely, the fractionation of stable carbon isotope ratios ($^{13}C/^{12}C$ or $\delta^{13}C$) between subsequent trophic positions is generally <1‰ (McConnaughey and McRoy, 1979), and because primary producers typically present distinct isotopic signatures, $\delta^{13}C$ is therefore used to study carbon sources (Budge et al., 2008).

Lipids represent an important fraction of the vertical flux of organic matter (Parrish et al., 2005), and they provide high quality energy to heterotrophic organisms on the sea floor (Parrish, 2009). As major constituents of most lipids, fatty acids (FA) are commonly used to assess diets (Howell et al., 2003; Drazen et al., 2008a, b), trophic dynamics and interactions (Stowasser et al., 2009; Kharlamenko et al., 2013; Kurten et al., 2013), and ecosystem health (Parrish et al., 2000; Parrish, 2009). Several characteristics make FA excellent biomarkers, including the fact that they are not degraded during digestion and they are usually taken up by tissues in the same way or with little alteration (Iverson, 2009). Furthermore, organisms can only synthesize a limited number of FA or modify them in a predictable way (Iverson, 2009). Because primary producers typically present unique FA compositions, their use facilitates the study of the source of organic carbon at the base of the food web (Parrish et al., 2000; Jeffreys et al., 2009; Kharlamenko et al., 2013). However, the strength of their signal typically decreases with increasing trophic level (Kurten et al., 2013).

Little information exists on the biochemical composition of deep-sea benthic species in the extensive region of the Northwest Atlantic, off eastern Canada. There are some data on lipid class composition in cold-water corals (Hamoutene et al., 2007), and studies of isotopic signatures in deep-sea corals (Sherwood et al., 2008) and deep-sea

demersal and pelagic fishes (Parzanini et al. 2017). Here, biomarkers (i.e. $\delta^{13}C$, $\delta^{15}N$, and FA) were combined with elemental analysis (%C, %N, and $C:N_{mol}$) to study trophic relationships and feeding habits inside a deep-sea benthic community sampled off Newfoundland. Main objectives were to characterize 1) the feeding habits and dietary sources of the organisms sampled, and 2) the fate of certain FA in the food web. Furthermore, 3) relationships between depth and samples' biochemical composition were investigated, as depth-related trends have been observed in the $\delta^{15}N$ profile of particulate organic matter (POM), in benthic consumers (Mintenbeck et al., 2007), in the C:N ratio of POM (Schneider et al., 2003), as well in the FA composition of marine organisms (Lewis, 1967; Cossins and Macdonald, 1986).

Materials and Methods

Sampling

Sampling occurred opportunistically and was conducted during the annual multispecies bottom-trawl surveys operated by Fisheries and Oceans (DFO), Canada, using the CCGS *Teleost* research vessel, between November 23 and December 6, 2013. Following a stratified-random design, multiple benthic taxa were collected with a Campelen 1800 shrimp trawl from a total of 23 tows, and at depths between 310 and 1413 m, within the NAFO Division 3K, off the island of Newfoundland (Northwest Atlantic; Table 4-1). Details of the type of gear and net can be found in Walsh and McCallum (1997). Once on board, individuals were immediately vacuum sealed and frozen at -20°C to prevent tissue degradation and lipid oxidation. Individuals were identified to the lowest possible taxonomic level through either direct observation or photo-identification. For the analyses, "long lived" tissue, i.e. characterized by low

turnover rates, hence able to provide long-term diet records (Iken et al., 2001), were collected in two separate aliquots. One aliquot was processed for stable isotope and elemental analysis and the second aliquot was used to assess individual FA signatures. The following tissues were sampled: tube feet and/or body wall from echinoderms when feasible, as in sea stars; foot muscle from gastropods; non-gonad soft tissues from soft corals and body walls from sea anemones and stony corals; body walls from sponges, annelid and sipunculid worms; and muscle from crustaceans. When collection of target tissues was not feasible due to small sizes of individuals (i.e. for the species *Arcoscalpellum michelottianum* in phyla Arthropoda and the following Annelida: Nereididae sp., Polychaeta sp. 1, 2 and 3, and Polynoidae sp.1), whole organisms, guts included, were split into equal portions, one for each set of analyses (see above). This choiced was merely made to obtain sufficient material for analysis.

Stable isotope and elemental analysis

Samples were oven-dried at 70°C for 24 hours, and then ground into a fine powder with mortar and pestle. HCl (1 M) was added dropwise to samples containing carbonates until bubbles stopped forming. Samples were then rinsed 3 times with distilled water, and oven dried again. This step was necessary to avoid carbonates affecting the stable C isotope ratio data, and to allow comparisons across taxa. Subsamples of 1 mg were packed into tin cups and simultaneously analyzed for stable isotope ratios (i.e. $\delta^{13}C$ and $\delta^{15}N$), and for elemental C and N, at The Earth Resources Research and Analysis (TERRA) of Memorial University's Core Research Equipment and Instrument Training Network (CREAIT), using a Delta V Plus (Carlo Erba) continuous-flow isotope-ratio mass

spectrometer. Isotope ratios are expressed in the conventional (δ) notation in parts per thousand (‰), following the equation:

$$\delta^{15}N \text{ or } \delta^{13}C = ((R_{sample} / R_{standard}) - 1) \times 1000$$

where R_{sample} is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Results are reported relative to international standards, such as atmospheric N_2 for stable nitrogen isotopes, and Vienna PeeDee Belemnite (VPDB) for stable carbon isotopes. Internal and external reference material was used to calibrate the mass spectrometer data. The average standard deviation of selected replicates was ±0.1‰ for $\delta^{15}N$ and ±0.1‰ for $\delta^{13}C$. Total elemental C and N were measured as proportions (%) of dry mass, and the average standard deviation was ±3.2 for %C and ±0.1 for %N. Elemental C and N were then used to calculate C:N ratio by mass (C:N) and moles ($C:N_{mol}$). Nine organisms out of the 89 analyzed had low proportions (< 10%) of both elemental C and N, including those of the species Polychaeta sp. 3 (n = 3), *Anthomastus agaricus* (n = 1), *Mediaster bairdii*, (n = 1), *Leptychaster arcticus* (n = 1), *Euplectella* spp. (n = 1), *Radiella hemisphaerica* (n = 1), and *Hamacantha* (*Vomerula*) *carteri* (n = 1), most likely due to analytical artifacts. As there was little difference between the $C:N_{mol}$ calculated from all the 89 individuals (5.1 ± 1.5) and that measured from a dataset excluding these 9 organisms (5.3 ± 1.6), it was chosen to include them into analysis. However, caution is needed when interpreting results for these organisms.

The lipid content measured from the individuals analyzed in this study was highly variable, spanning from low to large amounts (0.2-103.8 mg g^{-1} wet mass, n = 89). Because lipids have a more depleted ratio of ^{13}C to ^{12}C than proteins and carbohydrates, the interpretation of stable isotope data measured from lipid-rich tissues may be problematic (Post et al., 2007). Solutions recommended to improve the reliability of isotopic data include lipid extraction prior to analysis or the application of mathematical

corrections. Because lipid extraction may affect $\delta^{15}N$ data (Post et al., 2007), the second solution was preferred and applied in this study. Furthermore, due to the significant correlation between lipid content and C:N (r_s = 0.3, p = 0.007, n = 89), $\delta^{13}C$ data were lipid normalized applying the equation of Post et al. (2007), which utilizes C:N as a proxy for lipid content:

$$\delta^{13}C_n = \delta^{13}C - 3.32 + 0.99 \times C{:}N$$

in which $\delta^{13}C_n$ and $\delta^{13}C$ are the lipid normalized and the raw data of stable C isotopes respectively, and C:N is the ratio of carbon to nitrogen by mass. While values of uncorrected stable C isotope ratios ranged from -21.7 to -9.4‰, $\delta^{13}C_n$ spanned from -20.7 to -6.3‰.

Trophic positions (TP) were calculated following Cabana and Rasmussen (1996):

$$TP = [(\delta^{15}N - \delta^{15}N_{base}) / \Delta^{15}N] + TP_{base}$$

where $\delta^{15}N$ is the mean $\delta^{15}N$ for each consumer; $\Delta^{15}N$ is the fractionation factor which, in this study, corresponds to 3.8‰, as previously applied to deep-sea regions (Iken et al., 2005). As for the isotopic signature ($\delta^{15}N_{base}$) and trophic position (TP_{base}) of the base of the food web, values were taken from the individual with the lowest ratio (6.7‰) across our samples, the sponge *Craniella cranium*, which is a suspension feeder and hence a secondary consumer (TP = 2)..

Lipid content and FA analyses

Individuals were removed from the freezer, and processed as soon as thawed enough to allow dissection, to avoid lipid degradation. Sampled tissues were stored in chloroform, under nitrogen, at -20ºC, prior to extraction. Lipids were extracted in chloroform:methanol:water (2:1:1) following Folch et al. (1957) as modified by Parrish (1999). Samples were homogenized, sonicated, and centrifuged in the

chloroform:methanol:water mixture four times. The bottom, organic layers were removed following each of the four washes and pooled. The total lipid extract was then concentrated down to volume under a gentle stream of nitrogen, the tube was sealed with Teflon tape, and stored in a freezer (-20°C) until further analysis. Lipid content was quantified in a Chromarod-Iatroscan (Mark VI) TLC/FID system, using a three-step development method (Parrish, 1987), while FA were transesterified from the lipid extracts and analyzed as methyl esters (FAME) on a HP 6890 GC FID equipped with an 7683 autosampler. The GC column was 30 m x 0.32 mm (ZB-WAXplus, Phenomenex, U.S.A.). The column temperature was initially set at 65°C and held for 0.5 min. The temperature was subsequently raised to 195°C at a rate of 40°C min^{-1} and held for 15 min, before reaching the final temperature of 220°C at 2°C min^{-1}. This final temperature was held for 0.75 min. The flow rate of the hydrogen carrier gas was 2 ml min^{-1}. The initial injector temperature was 150°C and it reached the final temperature of 250°C at 12°C min^{-1}. The detector temperature stayed constant at 260°C. FA peaks were identified by comparing the retention times of samples with those of known standards using the software Varian Galaxie Chromatography Data System, version 1.9.3.2. Standards were purchased from Supelco, and included a 37 component FAME mix, a bacterial acid methyl ester mix, PUFA 1 and PUFA 3. FA were expressed according to the shorthand notation A:BωX, where A represents the number of carbons; B is the number of double bonds and X is the position of the first double bond relative to the terminal methyl group (CH_3). Further details on the various procedures can be found in Parrish (1999).

FA typically contain from 14 to 24 atoms of carbon (Iverson, 2009). However, certain taxa, such as sponges and corals, possess FA > C_{24} (Bergé and Barnathan, 2005; Kornprobst and Barnathan, 2010; Monroig et al., 2013). Because they were first identified in sponges of the class Demospongiae, such very long chain FA (VLCFA) are

also called demospongic acids (Litchfield et al., 1976). A more detailed description of the demospongic acids can be found in Kornprobst and Barnathan (2010). In this study, VLCFA were detected following the same GC procedure as described above. The only differences concerned the column temperatures which, after holding at 65°C for 0.5 minutes, ramped to 160°C at a rate of 40°C/min, and finally to 250°C at a rate of 1°C /min, where it was held for 0.12 minutes; and the standard used to identify peaks, which was a mixture of the saturated FAs C_{26}, C_{27}, C_{28}, and C_{30}. Unsaturated FAs were tentatively identified by plotting C numbers against relative retention times along isolines, through literature review (Bergé and Barnathan, 2005; Rezanka and Sigler, 2009; Kornprobst and Barnathan, 2010; Imbs, 2013; Monroig et al., 2013), and by comparing the FA profiles across samples. In addition, hydrogenation was carried out to verify the correct number of C atoms for these VLCFA, confirming that most of the peaks identified were FA. However, the number of double bonds and position of first double bonds from the methyl-end remain tentative (Appendix 7-5).

Biomagnification of FA and essential nutrients

For those FA correlating with $\delta^{15}N$, a trophic multiplication factor was calculated. In detail, the trophic multiplication factor represents in numeric terms the extent to which certain compounds biomagnify along a given food web (Borgå et al., 2012; Connelly et al., 2014). Assuming that the proportion of the compound increases exponentially with increasing trophic position:

$$[\text{compound}] = e^{m \times TP}$$

which is equal to

$$\log_e [\text{compound}] = (m \times TP) + b$$

and, therefore,

$$TMF = e^{m}$$

where m and b respectively correspond to the slope and the intercept of the linear relationship between $\log_e$ [compound] and trophic position TP, and TMF is the trophic multiplication factor. Positive values of m and TMF indicate that the compound biomagnifies throughout the food web; whereas negative values suggest a depletion in proportion.

Statistical analysis

Statistical analyses were performed on a dataset of 89 individuals, classified into 50 species and 7 phyla; the results presented were reported as mean values both per species and per phylum ±SD. Pearson and Spearman rank-order correlations were run to test for the presence of any relationships between mean depth and stable isotopes ($\delta^{15}N$ or $\delta^{13}C_n$), elemental data (%N, %C, and $C:N_{mol}$), as well as FA, while permutational multivariate ANOVA (PERMANOVA) was performed on normalized data to study the FA composition variability among species. Only FA with mean proportions higher than 0.5% were included in the analysis (33 out of 71). Similarity percentages (SIMPER) analyses were run to further investigate differences/similarities of the FA composition among and within phyla. Results were plotted using multidimensional scaling (MDS), performed on Bray-Curtis similarity matrices. Discriminant analysis was used to assess whether FA were valid predictors of the taxa represented, and species represented by n = 1 individual were excluded from the test. Correlations were performed using the software

SigmaPlot 11.0, while Minitab 17 was used to run discriminant analysis and linear regressions, and PRIMER (+PERMANOVA) for PERMANOVA, SIMPER, and MDS.

Results

Bathymetric trends

Significant relationships were detected between mean depth and several biochemical markers measured within tissues of the organisms collected, including %C (r_s = 0.2, p = 0.022 , n = 89), 14:0 (r_s = -0.2, p = 0.032, n = 89), 18:1ω9 (r_s = 0.3, p = 0.001, n = 89), 20:4ω6 (r_s = 0.2, p = 0.031, n = 89), 22:1ω7 (r_s = -0.3, p = 0.005, n = 89), 22:2NIMDa (r_s = -0.3, p = 0.032, n = 89), and 24:1 (r_s = -0.3, p = 0.005, n = 89; Appendix 7-6). On the contrary, no significant trends between mean depth and either $\delta^{15}N$ or $\delta^{13}C_n$ were revealed.

Stable isotope and elemental analyses

Mean values of lipid-corrected stable C isotope ratios ($\delta^{13}C_n$) ranged from -20.1 ± 1.2‰ (n = 3) in *Acanthephyra pelagica* to -6.3‰ in *Zoroaster fulgens* (Table 4-2). Furthermore, in terms of $\delta^{13}C_n$, the Echinodermata and Cnidaria showed the greatest within phylum variability (Fig. 4-1). Mean values of stable N isotope ratios ($\delta^{15}N$) varied between 6.8‰ in Hexactinellida sp. and 16.9‰ in *Psilaster andromeda* (Table 4-2), with Porifera showing the greatest interspecific variation (Fig. 4-1).

Based on an enrichment factor of 3.8‰ per trophic level and given the difference of about 10.0‰ between the lowest and the highest $\delta^{15}N$ value measured within the assemblage investigated, analyses revealed that 3 trophic levels were represented within the assemblage analyzed. Two members of the phylum Porifera, i.e.

Hexactinellida sp. and *Craniella cranium*, occupied the lowest trophic positions within the assemblage analyzed, whereas representatives of the Echinodermata, such *Myxaster sol* and *Psilaster andromeda* were characterized by the highest values (Table 4-2). In addition, intra-phylum variability was high according to $\delta^{15}N$ data, and Porifera were characterized by the widest range of $\delta^{15}N$ values, hence trophic positions.

Polychaeta sp. 3 yielded the lowest values of both elemental N and C, accounting for 0.7 ± 0.2 and 4.1 ± 0.5% dry mass (n = 3), respectively. Conversely, Polychaeta sp. 1 had the highest concentration of elemental N (14.7%), while the sea pen *Umbellula* sp. presented the highest levels of %C (51.6%; Table 4-2). Regarding the C to N molar ratio ($C:N_{mol}$), mean values spanned from a minimum of 3.5 in the sea anemone *Actinoscyphia aurelia* to a maximum of 9.5 in the alcyonacean coral *Paragorgia arborea*, both extremes belonging to the phylum Cnidaria (Table 4-2). Overall, at the phylum level, the Echinodermata and Cnidaria had the highest mean values of $C:N_{mol}$, whereas the Sipuncula and Arthropoda had the lowest (Table 4-2).

When examining data from all individuals, significant correlations were found between $\delta^{13}C_n$ and all elemental biomarkers: $\delta^{15}N$ ($r_s = 0.3$, $p = 0.018$, n = 89), %C ($r_s = -0.5$, $P < 0.001$, n = 89), %N ($r_s = -0.6$, $p < 0.001$, n = 89), and $C:N_{mol}$ ($r_s = 0.6$, $p < 0.001$, n = 89).

Fatty acid analysis and trophic multiplication factor

Of the 71 FA retrieved from the samples, only 33 were included in the analysis as occurring with mean proportions > 0.5% (Table 4-3, 4-4, 4-5, 4-6). Among them, the most abundant across species were: eicosapentaenoic acid, 20:5ω3 (EPA; 11.7 ± 1.0%, n = 50); palmitic acid, 16:0 (8.5 ± 5.9%); vaccenic acid, 18:1ω7 (7.3 ± 1.0%); oleic acid, 18:1ω9 (6.9 ± 0.9%); palmitoleic acid, 16:1ω7 (6.4 ± 0.9%); eicosenoic acid, 20:1ω9 (5.9

± 0.7%); docosahexaenoic acid, 22:6ω3 (DHA; 5.8 ± 0.8%); and arachidonic acid, 20:4ω6 (ARA; 5.1 ± 1.1 %). SIMPER analysis revealed that species belonging to the same phylum were similar in terms of FA composition, with similarities ≥ 59% in all phyla except Porifera (43%) and Sipuncula (46%). Furthermore, discriminant analysis confirmed that FA were accurate predictors (97%) of a given species' phylum. However, the differences among phyla were significant (PERMANOVA, Pseudo-F = 6.1, *p* (perm) = 0.0001; Fig. 4-2). The FA profile of the Annelida was characterized by high mean levels of EPA (12.1 ± 3.5%), 16:0 (11.7 ± 1.5%), and DHA (10.4 ± 3.0%), which together contributed to 46% of the similarity within the phylum. Moreover, large proportions of the non-methylene-interrupted dienoic 22:2 (22:2NIMDa; 11.8%) were found in *Laetmonice filicornis*, whereas Polychaeta sp 1, 2 and 3, as well as Polynoidae sp 1 had high percentages (> 9.0%) of 18:1ω9 in their tissues. In addition, Polychaeta sp 3 had higher levels of 16:1ω7 than the other species of the Annelida. In the Arthropoda, 18:1ω9 (16.8 ± 4.2%), EPA (15.8 ± 5.4%), and DHA (14.8 ± 3.7%) were the most abundant across representatives, and together contributed to nearly 60% of the similarity within the phylum. All arthropods also had relatively high proportions of 16:0 in their muscle tissue, except *A. pelagica* (4.0%). Conversely, *A. pelagica* had large proportions of 22:1ω11(13) and 22:5ω3, which were much smaller across all the other Arthropoda (Table 4-3). The phylum Cnidaria was characterized by a universal presence and high levels of EPA and 20:1ω9 (Table 4-4). Furthermore, while DHA occurred at low mean proportions (2.8 ± 1.6%), levels of ARA were overall higher (4.9 ± 5.2%), especially in *Actinoscyphia aurelia*, *Anthomastus* spp, *Paragorgia arborea*, and *Pennatula aculeata* (Table 4-4). Gastropoda species (phylum Mollusca) were characterized by high proportions of all three main essential FA (i.e. ARA, EPA, DHA; Table 4-4) which, together with 18:0, were the most abundant FA within the phylum. Although the overall FA profile of the Porifera

was variable (57% dissimilarity), some similarities were detected across species. For instance, *Craniella cranium*, *Cliona* sp., *Haliclona* sp., *Geodia* sp. and Hexactinellida sp. were 80% similar, characterized by high levels of 16:1ω7 and 18:1ω7, and by the presence of *i*15:0, 16:1ω5, *i*17:0, *ai*17:0, and 18:1ω5 in their tissues. All the remaining species of poriferans (sponges) had much lower levels of these FA in their tissues, and they were instead characterized by larger proportions of DHA, as in *Iophon piceum* and *Polymastia* spp., or EPA, as in *Hamacantha* (*Vomerula*) *carteri*, *Tentorium semisuberites*, and *Euplectella* sp. (Table 4-5). Phylum Echinodermata had lipid compositions dominated by ARA, followed by EPA, and 20:1ω11 (Table 4-6), which together contributed to more than 50% of the similarity within the phylum. The sea stars *Mediaster bairdi*, *Myxaster sol*, and *Psilaster andromeda*, had particularly high proportions of ARA, >26%. On the other hand, similar to the Cnidaria, Echinodermata had negligible levels of DHA. The phylum Sipuncula was represented by only two species, which displayed different FA profiles: while DHA was present at trace levels in both species, Sipunculidea sp 1 presented large proportions of EPA and ARA in its tissue (11.4 and 11.3%, respectively), along with high levels of 16:0 and 20:2b; whereas Sipunculidea sp 2 was mostly characterized by the presence of 18:0 and 18:1ω7 (Table 4-6).

While 18:0, 20:1ω11?, 20:1ω9, ARA, and 22:1ω7 were biomagnified by a mean factor of 3.1% per level, 16:1ω7, 16:1ω5, *i*17:0, *ai*17:0, 16:3ω3, 18:1ω9, 18:1ω7, 18:1ω5, and 22:1ω9 exhibited a depletion of ~0.50% per TP in magnitude (Table 7). Table 7 also presents the significant relationships between either $\delta^{13}C_n$ or $C:N_{mol}$ and different FA. Among them, strong correlations were detected between $\delta^{13}C_n$ and 20:1ω11? ($r = 0.6$, $p < 0.001$, $n = 50$), ARA ($r = 0.6$, $p < 0.001$, $n = 50$), and DHA ($r = -0.5$, $p < 0.001$, $n = 50$); as well as between $C:N_{mol}$ and 22:1ω11(13) ($r = 0.6$, $p < 0.001$, n

= 50). In contrast to $\delta^{15}N$, $\delta^{13}C_n$ and $C:N_{mol}$ only correlated with even-chain C_{18}-C_{22} FA, and no correlations were found with bacterial biomarkers.

Very long chain fatty acids (VLCFA)

Since the focus of this study was on the most common FA (C14-C24), and because of the extensive time and effort required to investigate VLCFA, this additional, qualitative analysis was exclusively performed on taxa known to have such FA in their tissues (Bergé and Barnathan, 2005; Kornprobst and Barnathan, 2010; Monroig et al., 2013). As such, samples of Porifera (n = 4) and Cnidaria (n = 7), from a set of different species, were examined for VLCFA (i.e. FA > C24; Table 4-8). Overall, the analysis indicated the presence of 2 to 24 C23-C30 VLCFA in each sample. Corals (phylum Cnidaria) contained 24:1ω9, 23:5ω3, 24:5ω6, 24:5ω3, 24:6ω3, 25:5ω3, 26:4ω6, and 26:6ω3 (Table 4-8). Furthermore, 24:5ω6 was the most common FA across the coral samples, followed by 24:5ω3 and 24:6ω3; while the latter was present at the highest proportions (6.8 ± 4.0% of total VLCFA; n = 4). Sponges contained a more diverse array of VLCFA than corals, including 24:1ω11, 24:2ω6, 23:5ω3, 26:0, 24:6ω3, 26:1, 26:1ω9, 26:2, 26:2ω6, 25:5ω3, 27:0, 26:4ω6, 26:5ω6, 28:1ω9, 28:2, 28:2ω6, 27:5ω3, 28:5ω6, 28:6ω3, 30:1ω9 (Table 4-8). Among them, 24:1ω11, 23:5ω3, and 27:0 were the most common contributors, and the FA 28:2 occurred in the highest proportions (>25% of FA).

Discussion

Bathymetric trends

There were positive relationships between depth and proportions of carbon (%C), oleic acid (18:1ω9), and arachidonic acid (ARA, 20:4ω6); whereas the proportions of 14:0,

22:1ω7, 22:2NIMDa, and 24:1 decreased along the depth gradient of the area investigated (310 – 1413 m). Apart from specific aspects discussed below, these broad trends are generally consistent with biological and environmental processes occurring within the water column and along the bathymetric gradient. In particular, the increase of %C with depth is most likely a reflection of the preferential assimilation of N, an essential and limiting nutrient in aquatic ecosystems (Campanyà-Llovet et al., 2017). In fact, as described by Altabet et al. (1999) and Schneider et al. (2003), POM undergoes microbial degradation while sinking to deeper waters, thus leading to an overall decrease of POM %N, as well as increasing C:N with depth. As sinking POM is among the primary food sources for deep-sea communities (Gage, 2003; Pfannkuche, 2005), the isotopic and elemental composition of several benthic organisms feeding directly on POM may vary accordingly (Schneider et al., 2003; Mintenbeck et al., 2007; Lin et al., 2014).

There may be a few reasons why 18:1ω9 increased with depth, which are likely linked to the different roles ascribed to this FA within organisms. In particular, two explanations are related to the fact that 18:1ω9 is typically associate with wax esters (Nevenzel et al., 1965; Nevenzel et al., 1966; Lewis, 1967), and that this lipid class plays a major role in both energy storage and buoyancy in certain deep-water species (Phleger, 1998; Lee et al., 2006). In fact, because wax esters provide a longer term energy reserve than triacylglycerols (Lee et al., 2006), the typical storage lipids (Lehninger, 1975), they are thought to be advantageous for species living in environments characterized by long periods of food limitation, such as the deep sea (Lee et al., 2006; Drazen et al., 2008b). Furthermore, deep-dwelling crustaceans and fish are known to accumulate droplets of wax esters to maintain neutral buoyancy at great depths (Phleger, 1998; Lee et al., 2006). Lipid class data from a concurrent investigation (Chapter 3) indicate that species in the phyla Arthropoda (especially Malacostraca

crustaceans) and Cnidaria (including jellyfish, sea anemones, and corals) are rich in wax esters, with mean proportions per phylum ≥ 11.8%. In these same phyla investigated, levels of 18:1ω9 were high (~16.8 % in the Arthropoda and ~7.3% in the Cnidaria); therefore, it was assumed that 18:1ω9 was mostly linked to wax esters. Given the generally sporadic nature of the food supply coming from surface waters (Gage, 2003) and that this supply diminishes with increasing depth (Buesseler et al., 2007), relying on long-term energy reserves like wax esters may be more beneficial for deep-dwelling organisms, thus supporting the bathymetric patterns observed in this study. Moreover, consistent with our results, Lewis (1967) reported increasing levels of 18:1ω9 with depth in a study of 20 species collected from the coast down to 4400 m, off San Diego and Baja California (Pacific Ocean). Since most of the species studied by Lewis (1967) were fish and crustaceans with high levels of wax esters in their tissues, following findings of previous studies (Nevenzel et al., 1965; Nevenzel et al., 1966), the author suggested that the presence of 18:1ω9 might have helped these organisms maintain neutral buoyancy in deeper waters. In addition, Lee (1974) found that the FA composition of wax ester in zooplankton differed between deep and shallow-water species, and that the former were typically characterized by high levels of 18:1ω9, whereas the latter were richer in 20:1 and 22:1 fatty alcohols (i.e. long-chain alcohols deriving from fatty acids). In support of this last observation, a significant decrease in 22:1ω7 along the bathymetric gradient was detected in this study. Lastly, not only is 18:1ω9 involved in energy storage and buoyancy, but it has also been used as an indicator of carnivory/omnivory, especially when compared to 18:1ω7 (Graeve et al., 1997; Parrish, 2013). Therefore, increasing levels detected in deeper samples may reflect greater prevalence of this feeding strategy at greater depths, presumably to counteract the diminishing quantity of OM reaching the seafloor (Buesseler et al., 2007).

Like 18:1ω9, ARA increased along the bathymetric gradient in the focal assemblage. However, while 18:1ω9 is typically associated with wax esters, ARA is linked to membrane phospholipids (Olley and Duncan, 1965), thus suggesting different interpretations for the higher levels of the latter with depth. One possibility is a greater dependency of deeper-dwelling organisms on the benthic-detrital trophic pathway, rather than the pelagic pathway. In fact, the source of ARA has been ascribed to microorganisms (i.e. bacteria, protozoans, and microeukaryotes) from the sediment (Fullarton et al., 1995; Howell et al., 2003), and such organisms play a major role within the benthic-detrital trophic pathway by degrading the settling POM (Rowe and Deming, 1985). As the amount of POM decreases along a bathymetric gradient (Buesseler et al., 2007), benthic species living at greater depth most likely have to rely on heavily degraded detritus rather than on fresh organic material coming from surface waters. In addition, increasing proportions of ARA may also be related to the generally higher levels of polyunsaturated FA (PUFA) in membrane phospholipids at greater depth, following homeoviscous adapation theory (Macdonald and Cossins, 1985). Larger proportions of PUFA help maintain membrane fluidity at cold temperatures and high pressures, which are typical of the deep sea (Macdonald and Cossins, 1985). Conversely, according to this theory, the proportion of saturated FA is expected to diminish at deeper depths and, interestingly, a decrease of the saturated 14:0 was observed in the present study.

Similar to patterns determined by Parzanini et al. (2017) in deep-sea fishes from the same geographic area, no bathymetric trends were detected in the isotopic signatures of the benthic assemblage analyzed. These findings are also similar to those of Sherwood et al. (2008) who studied cold-water corals from slope areas off Newfoundland. However, they differ from the depth-related trends reported in the high-Antarctic Weddell Sea on shelf and slope areas (Mintenbeck et al., 2007), suggesting a

geographic/latitudinal influence. The lack of a variation with depth for deep-sea fish from the Northwest Atlantic was previously ascribed to several factors, such as differences in trophic roles/feeding guilds, trophic positions, as well as body sizes, which may have elicited variations in the $\delta^{15}N$ and $\delta^{13}C_n$ values and masked any depth effects (Parzanini et al., 2017). The same assumption might be extended to benthic organisms in the present study. Mintenbeck et al. (2007) observed that while the $\delta^{15}N$ of suspension feeders increased along a depth gradient (i.e. 50-1600 m), reflecting the signature of sinking POM, no bathymetric pattern was detected in deposit feeders. Species with different dietary habits were analyzed (see below) from different taxa and trophic guilds, decreasing the chance of detecting depth effects.

On the other hand, Sherwood et al. (2008) found a similar absence of bathymetric trends despite focusing their analyses on cold-water coral taxa, and they inferred that it was likely due to the fact that corals were feeding on large particles of fresh POM. Since larger particles sink faster than smaller ones, the former undergo less degradation and $\delta^{15}N$ fractionation, and their signal of any depth effect is minimal (Altabet et al., 1999). As suggested by Sherwood et al. (2008), it is likely that large particles of phytodetritus constituted a major food source for the benthic faunal assemblage analyzed.

Feeding habits and dietary sources

The benthic organisms analyzed in the current investigation were characterized by a diverse spectrum of $\delta^{15}N$ and $\delta^{13}C_n$ values, thus suggesting a wide variety of dietary habits and sources both across and within phyla. Furthermore, the weak positive relationship between $\delta^{15}N$ and $\delta^{13}C_n$ (Fig. 4-1) was indicative of a non-linear food web, characterized by a diverse set of food sources (Fanelli et al., 2013; Papiol et al., 2013),

and in which more than one trophic pathway was represented: typically, a pelagic and a benthic trophic pathway. While the former was characterized by ^{15}N- and $^{13}C_n$-depleted isotopic signatures, and isotopically depleted POM constituted the primary dietary source; the latter was characterized by higher isotopic ratios due to a greater dependence on more ^{15}N- and ^{13}C-enriched organic matter. Isotopic results were largely confirmed by FA analysis. To provide a clear overview and tease out the distinguishing elements of the various phyla, each of them is discussed separately below.

Annelida

Representatives of the phylum Annelida were polychaetes found to occupy various trophic positions; however, their $\delta^{13}C_n$ values were similar overall and, due to their relatively negative values, suggestive of a more pelagic trophic pathway. In this regard, FA analysis allowed further clarification of dietary sources. The species Polychaeta sp 3, sitting at the lowest trophic position for the phylum, was characterized by comparatively high proportions of 16:1ω7, which is of either algal or bacterial origin (Parrish, 2013), hence indicative of a diet based on sinking detritus. Polychaeta sp 1-2, Polynoidae sp 1-2, and *Laetmonice filicornis* were at much higher and similar trophic positions. While Polychaeta sp 1 and Polynoidae sp 2 were characterized by large proportions of EPA and 18:1ω7, Polychaeta sp 2 and Polynoidae sp 1 had high levels of 18:1ω9. Since EPA and 18:1ω7 are biomarkers for diatoms and bacteria, respectively (Parrish, 2013), and 18:1ω9 is an indicator of either carnivory or omnivory (Parrish, 2013), as discussed above, this result may be reflective of two different feeding modes for these species of Annellida: detritivory for Polychaeta sp 1 and Polynoidae sp 2 *vs* carnivory/omnivory for Polychaeta sp 2 and Polynoidae sp 1. Conversely, the FA signature of *L. filicornis* was not very informative because no specific biomarker was highlighted in the single

individual analyzed for the species. Nonetheless, records from the literature ascribe predatory behaviors for *L. filicornis*. In fact, Drazen et al. (2008b) found that this abyssal polychaete worm in the genus *Laetmonice* was most likely a carnivore feeding upon *Calanus* copepods based on high proportions of 20:1 FA measured in its tissues. Overall, the species of Annelida analyzed in the current investigation displayed a diverse range of trophic positions and feeding habits. It is acknowledged that some individuals had to be processed whole, inclusive of the digestive tract (i.e. Nereididae sp., Polychaeta sp 1, 2 and 3, and Polynoidae sp1); while subsamples of the body wall were collected in others (i.e. *L. filicornis* and Polynoidae sp 2). Nonetheless, these findings seem to be consistent with those of previous studies (Drazen et al., 2008b; Würzberg et al., 2011), which indicated that deep-sea polychaetes display a broad range of feeding strategies, from carnivory and omnivory to phytodetritivory.

Arthropoda

Similar to the Annelida, the species of Arthropoda analyzed in this study presented different trophic positions, but similar $\delta^{13}C_n$ ratios. However, the highly negative values of $\delta^{13}C_n$ were suggestive of a closer link to the pelagic food web for the Arthropoda than for the Annelida representatives. These isotopic results were partly confirmed by the FA analysis. Specifically, large proportions of the zooplankton biomarkers 20:1ω9 and 22:1ω11(13) were detected in the decapod crustacean *Acanthephyra pelagica,* likely because its feeds on copepods. Furthermore, the scalpellid barnacle *Arcoscalpellum michelottianum* and several mesopelagic decapods such as *Notostomus robustus*, *Pandalus borealis*, *Pasiphaea tarda*, *Stereomastis sculpta*, and *Sabinea hystrix* had high levels of EPA and DHA, which are known phytoplankton biomarkers (Parrish, 2013). While *A. michelottianum*, like most of the cirripeds, is a known filter-feeder (Buhl-

Mortensen and Høeg, 2006), the species *N. robustus*, *P. borealis*, *P. tarda*, *S. sculpta*, and *S. hystrix* analyzed in this study were thought to mainly consume phytodetrital material, consistent with previous records (Moore et al., 1993; Drazen et al., 2008b).

Cnidaria

All the species of Cnidaria were characterized by similar intermediate trophic positions. However, their $\delta^{13}C_n$ ratios were largely variable and suggestive of horizontal niche separation (i.e. same trophic position, but different diets) within the phylum. In fact, while the low values of $\delta^{13}C_n$ characterizing the sea anemones *Actinauge cristata* and *Actinostola callosa*, and the soft corals *Anthoptilum grandiflorum* and *Umbellula* sp. reflected a pelagic-based diet, those of the remaining species of Cnidaria were higher, suggestive of a more benthic-oriented diet. Such a variety of feeding modes for the representatives of the phylum Cnidaria was previously reported by Iken et al. (2001) and Sherwood et al. (2008) who used stable isotopes to analyze an abyssal food web in the Northeast Atlantic and a cold-water coral assemblage in the Northwest Atlantic, respectively. The isotopic data were further confirmed by FA signatures. In fact, both ARA and zooplankton biomarkers (e.g. 20:1ω9, 22:1ω11(13)) were detected within the species of Cnidaria analyzed. Our results were therefore consistent with those of previous studies, which ascribed several food sources to this group, including fresh phytodetritus, resuspended material, sediment, benthic animals, and pelagic prey, such as zooplankton (Iken et al., 2001; Sherwood et al., 2008).

Echinodermata

Representatives of this phylum occupied both intermediate, as well as the highest trophic positions within the assemblage analyzed. Furthermore, their relatively high $\delta^{13}C_n$ ratios evoked a more benthic-oriented diet. However, as highlighted for the Cnidaria, these

ratios were highly variable, thus suggesting niche separation across species. Indeed, intra-phylum variations in feeding modes and habits have been documented for deep-sea echinoderms using isotopic composition (Iken et al., 2001; Howell et al., 2003; Gale et al., 2013). In addition, although the echinoderms showed high overall levels of ARA, consistent with a benthic-based diet, intra-phylum variations were also reflected in their FA signatures. In particular, the sea urchin *Phormosoma placenta* and the branched ophiuoroid *Gorgonocephalus* sp., which displayed the most negative values of $\delta^{13}C_n$ within this phylum, contrasted with the sea stars occupying similar intermediate trophic positions (i.e. *Freyella microspina* and *Leptychaster arcticus*).

While *P. placenta* exhibited high levels of 16:1ω7 and 18:1ω7, suggesting a diet based on phytodetritus, the ophiuroid was characterized by high proportions of the copepod biomarker 20:1ω9, and ARA was present at only trace levels. A suspension-feeding strategy was hence ascribed to *P. placenta* and *Gorgonocephalus* sp. analyzed. Conversely, the sea stars *F. microspina* and *L. arcticus* had much higher values of $\delta^{13}C_n$. In support of this, *L. arcticus* was reported to feed infaunally upon bulk sediment and molluscs (Gale et al., 2013). Furthermore, although most of the sea stars of the order Brisingida, like *F. microspina*, are known suspension-feeders (Gale et al., 2014), they have also been reported to feed on foraminifers and small animals (Mortensen, 1927). High levels of the zooplankton biomarkers 20:1ω11 and 22:1ω11(13) were indeed detected within the tissues of *F. microspina* analyzed; however, their $\delta^{13}C_n$ data indicated a chiefly benthic-based diet, highlighting the opportunistic behavior of this species. In line with Lin et al. (2014), Iken et al. (2001), and Gale et al. (2013), species of sea stars occupied the highest positions in the benthic food web analyzed. *Zoroaster fulgens* is a generalist infaunal predator (Gale et al., 2013), and *Mediaster bairdi* and *Psilaster andromeda* are known predator/scavengers (Khanna, 2005; Gale et al., 2013).

Conversely, little information is available in the literature on the ecology of the sea star *Myxaster sol* (Khanna, 2005), and further research on the species is required.

Mollusca

Species of Gastropoda occupied intermediate to high trophic positions. While the $\delta^{13}C_n$ ratios for the representatives of this phylum were suggestive of a benthic-oriented diet, the analysis of their FA composition did not provide much additional information, since no specific biomarker were highlighted, except for EPA. However, a concurrent investigation revealed that body wall tissue of gastropods was characterized by high proportions of membrane lipids (i.e. phospholipids) and by comparatively lower levels of storage lipids (i.e. triacylglycerols; Chapter 3). Therefore, it is more likely the high levels of EPA detected were associated with the large presence of this FA in the species' membranes, rather than coming directly from the diet. Overall, our isotopic results were largely consistent with those of previous studies. Himmelman and Hamel (1993) have indicated that shallow-water species of the genus *Buccinum* are either carnivores or, less frequently, scavengers, feeding on other molluscs, polychaetes, and carrion. Furthermore, Kosyan (2007) ascribed predatory behaviors to buccinids of the Colinae subfamily, including *Colus islandicus*, by studying their external morphology and anatomy. In addition, sediment particles, foraminiferan shells, brittle stars, polychaetes, and amphipods were retrieved from the gut of the individuals analyzed by Kosyan (2007).

Porifera

The sponges analyzed in this study occupied very different trophic positions, broadly separated into two main groups. The first group included the species *Cliona* sp., *Craniella cranium*, *Geodia* sp., *Stelletta* sp., *Haliclona* spp., and Hexactinellida sp., and

was characterized by very low trophic positions. The second group was represented by *Euplectella* sp., *Iophon piceum*, *Phakellia* spp., *Hamacantha* (*Vomerula*) *carteri*, *Polymastia* spp., *Tentorium semisuberites*, and *Thenea muricata*, displaying the highest trophic positions within phylum. The two groups were also different in terms of FA signatures. Species in the first group showed high levels of algal/bacterial biomarkers, including 16:1ω7, 18:1ω5, 18:1ω7, *i*15:0, 16:1ω5, *i*17:0, and *ai*17:0. Reiswig (1975) showed that bacteria associated with POM constituted the main source of food for several species of marine sponges collected in temperate intertidal areas. Porifera is in fact the only phylum of benthic filter feeders able to efficiently retain small particles (i.e. 0.2-1.0 μm) of POM (Reiswig, 1975; Pile and Young, 2006). Given the low values of $\delta^{13}C_n$, it is likely that *Cliona* sp., *Craniella cranium*, *Geodia* sp., *Stelletta* sp., *Haliclona* spp., and Hexactinellida sp. were selectively feeding on the bacteria adhering to sinking POM. On the other hand, the sponges at the higher trophic positions were not characterized by the presence of algal/bacterial biomarkers, except for *T. muricata* which had large proportions of 16:1ω7 (> 10%). In this regard, the species exhibited a broad array of VLCFA, with both even and odd acyl chains; most of these FAs could be of bacterial origin, whether incorporated through diet or deriving from prokaryotes living within the sponge tissues. A few interpretations can be proposed based on the present results and records from the literature. The species *I. piceum*, in particular, had the highest trophic position within the phylum, consistent with the fact that sponges in the order Poecilosclerida are believed to have developed a unique carnivorous diet (Hestetun et al., 2016). Conversely, no records of carnivory were found for the other species, which were thus assumed to be filter-feeders like most sponges (Reiswig, 1975). It is possible that *Phakellia* sp., *H. carteri*, *Polymastia* spp., *T. semisuberites*, and *T. muricata* were feeding on resuspended material, which is typically more fractionated

(i.e. ^{15}N-enriched) than sinking POM, due to degradation processes occurring on the seafloor.

As a side note, while sponges may host microbial biomass within their body tissues, in this study, no step was taken to decontaminate them; therefore, it is likely that microbial material is included in the analysis. For this reason, it is also possible that the two groups of sponges had different microbial contributions, thus influencing their biochemical results. Indeed, high proportions of bacterial biomarkers were detected in the group of sponges at lower TP.

Sipuncula

The low sample size (n = 2) made inferences about the trophic habits of Sipuncula difficult; in addition, it generated marked variation in the mean biomarker values for the phylum. In fact, as the lipid turnover in the digestive tract is generally high, in order to obtain longer term dietary information, guts are usually removed from analyses. However, due to the small sizes of the 2 individuals, it was not possible to separate target tissues; but, importantly, it was still possible to gather some preliminary information and make comparisons with the literature. Isotopic results placed Sipunculidea sp. 1 and 2 at intermediate to high trophic positions. In addition, their values of $\delta^{13}C_n$ were suggestive of a more benthic-oriented diet. Such results were confirmed by FA analysis. In particular, Sipunculidea sp. 1 was characterized by high levels of ARA, a biomarker for microorganisms associated with sediment (Fullarton et al., 1995; Howell et al., 2003); and Sipunculidea sp. 2 showed high levels of 18:1ω7, which is a biomarker for either diatoms or bacteria (Parrish, 2013). Deep-sea sipunculids are known deposit-feeders (Jumars et al., 1990). Their relatively high trophic positions might

be explained by the fact that the two Sipuncula representatives were feeding on isotopically enriched settling POM.

Transfer of energy and essential nutrients

Several FA were correlated significantly with $\delta^{15}N$, an indicator of TP (Cabana and Rasmussen, 1996), suggesting either an increase or a depletion in the relative proportion of these FA along the food web analyzed. Specifically, 20:4ω6 (ARA) and some biomarkers of *Calanus* copepods, such as 20:1ω11, 20:1ω9, and 22:1ω7, where found in larger proportions in organisms ascribed to higher trophic levels, suggesting trophic accumulation of these FA, with major implications. Despite limited knowledge on the acquisition pathway, ARA is known to be of chief importance in marine ecosystems, together with EPA and DHA, as an essential nutrient and through its role in the reproduction, growth, and survival of marine organisms (Parrish, 2009). For example, ARA is responsible for sperm activation in the polychaete *Arenicola marina* (Bentley et al., 1990), and can enhance larval growth in fish (Koven et al., 2001). In this study, several species of echinoderms occupied the highest trophic positions and also had the highest levels of ARA. Echinoderms from shallow (Cook et al., 2000) to deeper-water ecosystems (Lewis, 1967; Howell et al., 2003; Drazen et al., 2008a) display large concentrations of ARA, making them a good source of this essential FA in marine communities. Conversely, long-chain monounsaturated are typically stored, such as C_{20} 20:1 and 22:1 in copepods, and hence represent major sources of energy for metabolic activities (Brockerhoff et al., 1963). Overall, the high trophic multiplication factor

measured for ARA, 20:1ω11, 20:1ω9, and 22:1ω7 (i.e. > 1.8) suggest these FA play a major role in the food web investigated in terms of energy and essential nutrient transfer.

The FA 16:1ω7, 16:1ω5, 18:1ω7, *i*17:0, *ai*17:0 are typical phytoplankton and bacterial biomarkers (Parrish, 2013). Their proportion was lower in organisms occupying higher trophic positions, probably due to the fact that higher trophic consumers are less likely to feed directly on POM or detritus. This result is in line with the heterotrophic nature of most deep-sea ecosystems, including the one analyzed in this study. They rely largely on the sinking OM as primary food source (Gage, 2003), which undergoes bacterial degradation along the depth gradient. Therefore, high proportions of bacterial FA can be found in organisms directly feeding either on sinking or settling POM. However, the strength of their signal may decrease along the food web, as secondary and tertiary consumers prey on primary consumers.

Not only does $\delta^{15}N$ indicate TP, but it also reflects the isotopic baseline. While variations in the $\delta^{15}N$ signature of the primary food source may occurr due to environmental and biological factors (Altabet et al. 1999; Mintenbeck et al. 2007), the food web analyzed in this study was complex and most likely charcterized by a diverse set of primary food sources, with potentially different stable N isotope signatures.

Conclusions

The current study, via the integrated use of stable isotope, elemental, and FA analyses, helped elucidate feeding habits and dietary sources of deep-water benthic taxa sampled in the Northwest Atlantic. Overall, the faunal assemblage relied upon a diverse set of sources, and representatives of several phyla (e.g. Annelida, Arthropoda, Cnidaria) exhibited niche separation, perhaps to limit food competition. In addition, this study detected general trends in the elemental and FA composition of deep-sea benthic

organisms collected along a bathymetric gradient, which most likely reflected both biological (e.g. microbial degradation and preferential assimilation of nutrients) and environmental (physical and chemical both) processes occurring within the water column and along the depth gradient; as well as flows of energy (20:1ω11, 20:1ω9, and 22:1ω7) and essential nutrients (e.g. ARA) throughout the food web analyzed. Lastly, novel data were provided, as for the VLCFA found within several sponges and corals, and the biochemical signature of poorly known deep-water species, such as the echinoderm *Myxaster sol*.

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Tables

Table 4-1 Details of sample collection. Date, bottom temperature (°C), coordinates (latitude and longitude) corresponding to start and end of gear deployment, and mean, minimum, and maximum depth (m) are reported for each tow.

Date	Tow	Bottom	Start position		End position		Depth		
(2013)		Temp	Latitude (N)	Longitude (W)	Latitude (N)	Longitude (W)	Mean	Min	Max
Nov, 30	7	4.5	49˚ 56' 24"	50˚ 12' 48"	50˚ 04' 00"	50˚ 12' 18"	488	483	492
	8	3.8	49˚ 58' 42"	49˚ 46' 12"	49˚ 58' 00"	49˚ 45' 24"	1090	1085	1097
	9	3.7	49˚ 59' 12"	49˚ 36' 00"	49˚ 58' 30"	49˚ 35' 36"	1282	1280	1284
Dec, 1	12	4.0	50˚ 04' 18"	50˚ 08' 06"	50˚ 03' 54"	50˚ 07' 00"	759	755	764
Dec, 2	19	4.1	50˚ 13' 42"	50˚ 13' 12"	50˚ 13' 18"	50˚ 12' 06"	889	888	890
	20	3.8	50˚ 28' 18"	50˚ 12' 12"	50˚ 29' 06"	50˚ 12' 24"	1094	1093	1096
	21	3.7	50˚ 30' 24"	49˚ 47' 00"	49˚ 31' 18"	49˚ 46' 54"	1321	1319	1322
	22	3.8	50˚ 37' 36"	50˚ 11' 30"	50˚ 37' 18"	50˚ 10' 24"	1122	1119	1127
Dec, 3	26	3.2	50˚ 52' 36"	51˚ 12' 00"	50˚ 53' 18"	51˚ 11' 24"	313	310	316
	27	3.7	50˚ 55' 18"	50˚ 42' 36"	50˚ 55' 54"	50˚ 42' 18"	353	351	356
	28	3.6	51˚ 02' 30"	50˚ 38' 24"	51˚ 01' 48"	50˚ 38' 48"	347	345	349
	29	4.3	50˚ 59' 12"	50˚ 23' 54"	50˚ 58' 24"	50˚ 24' 24"	868	866	871
Dec, 4	31	3.8	50˚ 55' 12"	49˚ 33' 12"	50˚ 55' 30"	49˚ 32' 12"	1365	1353	1369
	32	4.0	50˚ 57' 42"	49˚ 41' 00"	50˚ 58' 24"	49˚ 40' 24"	1084	1073	1089
	33	3.9	51˚ 07' 06"	49˚ 45' 00"	51˚ 06' 30"	49˚ 44' 30"	919	917	923
Dec, 5	34	4.2	51˚ 06' 12"	49˚ 55' 48"	51˚ 06' 24"	49˚ 56' 54"	796	791	803
	35	4.5	51˚ 10' 06"	50˚ 11' 48"	51˚ 10' 00"	50˚ 10' 36"	707	695	714
	36	4.1	51˚ 13' 24"	49˚ 48' 18"	51˚ 13' 00"	49˚ 47' 12"	1027	1009	1053
Dec, 6	38	4.5	51˚ 23' 42"	50˚ 12' 30"	51˚ 23' 36"	50˚ 11' 42"	464	458	471
	39	3.8	51˚ 26' 00"	49˚ 57' 30"	51˚ 25' 24"	50˚ 56' 42"	1324	1298	1351
	40	4.0	51˚ 25' 18"	50˚ 19' 18"	51˚ 25' 24"	50˚ 19' 00"	379	376	380
	41	4.4	51˚ 40' 30"	50˚ 23' 36"	51˚ 25' 24"	50˚ 23' 54"	595	592	599
	42	3.8	51˚ 50' 06"	50˚ 22' 48"	51˚ 50' 30"	50˚ 23' 12"	1407	1395	1413

Table 4-2 Sampling tow, sample size (n), and mean values and standard deviations (SD) of wet mass, stable isotopes ($\delta^{15}N$ and $\delta^{13}C_n$), elemental N (%N) and C (%C), carbon to nitrogen molar ratio ($C:N_{mol}$), and trophic position (TP) for each species. Phylum mean values and SD are also given. Wet mass is in g, stable isotope N and C ratios are in parts per million (‰), and elemental N and C are % dry mass.

Taxon	Tow	n	Wet mass	SD	$\delta^{15}N$	SD	$\delta^{13}C_n$	SD	%N	SD	%C	SD	$C:N_{mol}$	SD	TP	SD
Annelida																
Laetmonice filicornis	41	1	3.1		13.7		-16.0		5.4		19.7		4.2		3.8	
Nereididae sp.	28	1	1.6		13.9		-17.1		11.7		41.4		4.1		3.9	
Polychaeta sp 1	20	1	0.5		13.2		-15.7		14.7		48.3		3.8		3.7	
Polychaeta sp 2	22-31	2	1.5	0.1	11.1	0.2	-19.0	0.6	12.2	3.1	45.7	3.1	4.6	1.5	3.1	0.1
Polychaeta sp 3	27-28-42	3	2.4	0.5	7.9	0.6	-15.8	1.2	0.7	0.2	4.1	0.5	6.5	0.7	2.3	0.1
Polynoidae sp 1	28	1	1.9		12.6		-17.8		10.7		41.9		4.6		3.5	
Polynoidae sp 2	41	2	4.0	0.3	12.0	0.2	-17.5	0.6	12.3	3.1	39.7	3.1	3.8	1.5	3.4	0.2
Mean			2.1	1.1	11.2	2.3	-17.0	1.4	8.5	5.5	30.4	18.5	4.8	1.2	3.4	0.5
Arthropoda																
Acanthephyra pelagica	8	3	7.0	0.9	9.4	0.6	-20.1	1.2	12.5	0.2	44.2	0.5	4.1	0.7	2.7	0.1
Arcoscalpellum michelottianum	20-31	3	6.6	1.5	13.3	0.4	-18.9	0.3	10.0	0.6	38.5	4.1	4.5	0.3	3.7	0.1
Notostomus robustus	31	1	11.9	5.2	11.6		-19.8		12.2		40.8		3.9		3.3	
Pandalus borealis	7	3	5.6	0.2	11.1	0.6	-19.1	0.2	13.1	0.2	43.1	0.2	3.8	0.0	3.2	0.1
Pasiphaea tarda	21	2	29.2	15.5	11.4	0.2	-19.2	0.3	13.1	0.3	42.4	1.2	3.8	0.0	3.2	0.1
Sabinea hystrix	8-20	2	7.0	3.2	14.9	0.6	-16.4	0.1	12.5	1.0	41.2	2.0	3.9	0.1	4.2	0.2
Stereomastis sculpta	20-21	3	4.6	2.1	12.6	0.7	-19.2	0.1	11.8	0.6	39.5	1.7	3.9	0.0	3.5	0.2
Mean			10.3	8.7	12.1	1.8	-18.9	1.2	12.2	1.1	41.4	2.0	4.0	0.3	3.1	0.5

Cnidaria																
Actinauge cristata	12-19	2	101.9	44.5	11.1	0.1	-18.6	0.1	9.5	0.6	30.1	2.3	3.7	0.1	3.2	0.0
Actinoscyphia aurelia	34	3	33.9	21.1	11.7	2.0	-16.8	1.0	11.4	1.0	34.0	3.2	3.5	0.0	3.3	0.5
Actinostola callosa	12	3	71.4	26.7	10.8	0.1	-18.6	0.2	7.9	1.2	23.9	3.7	3.5	0.0	3.1	0.0
Anthomastus spp.	29-36	4	10.5	6.8	11.0	1.2	-13.8	4.3	5.6	4.2	24.4	15.8	6.5	2.7	3.2	0.3
Anthoptilum grandiflorum	12	1	4.8		11.6		-19.5		9.3		36.9		4.6		3.3	
Funiculina sp.	32	1	2.1		10.9		-14.8		4.8		26.4		6.5		3.1	
Paragorgia arborea	41	1	90.3		10.4		-11.5		2.7		22.0		9.5		3.0	
Pennatula aculeata	9	3	2.0	0.6	10.3	0.4	-8.6	1.8	2.8	0.5	20.5	1.6	8.7	0.9	2.9	0.1
Pennatula grandis	9-12	2	4.2	2.2	12.6	0.5	-17.0	0.9	12.1	0.3	44.0	0.2	4.2	0.1	3.0	0.1
Umbellula sp.	22	1	3.8		11.7		-19.3		9.0		51.6		6.7		3.3	
Mean			32.5	40.0	11.2	0.7	-15.8	3.6	7.5	3.4	31.4	10.2	5.7	2.2	3.1	0.1
Echinodermata																
Freyella microspina	42	1	70.2		12.3		-9.1		2.8		19.6		8.2		3.5	
Gorgonocephalus sp.	41	1	1.2		11.8		-14.5		4.5		29.5		7.7		3.3	
Leptychaster arcticus	27	3	2.4	0.3	12.4	0.6	-9.6	1.2	3.0	0.4	19.8	0.7	7.9	0.8	3.5	0.1
Mediaster bairdi	8	3	14.8	3.5	16.0	0.4	-8.2	1.7	3.2	0.9	19.0	3.1	7.2	1.2	4.4	0.1
Myxaster sol	33	1	71.1		16.8		-12.4		6.5		28.5		5.2		4.7	
Phormosoma placenta	19	3	19.6	7.4	12.3	0.3	-14.3	0.8	6.2	1.4	27.7	3.4	5.3	0.5	3.5	0.1
Psilaster andromeda	31	1	33.1		16.9		-13.0		6.3		29.8		5.5		4.7	
Zoroaster fulgens	9	1	37.3		14.1		-6.3		2.7		17.5		7.5		3.9	
Mean			31.2	27.5	14.1	2.2	-10.9	3.0	4.4	1.7	23.9	5.4	6.8	1.3	3.9	0.6
Mollusca																
Buccinum sp.	12	2	6.7	2.9	12.6	0.5	-17.0	0.9	12.1	0.3	44.0	0.2	4.2	0.1	3.6	0.1
Colus islandicus	19	1	56.9		14.2		-15.0		13.6		44.7		3.8		4.0	

Neptunea despecta	19	1	7.1		15.2		-15.4		9.2		38.6		4.9		4.2	
Mean			23.6	28.9	13.7	1.3	-16.1	1.2	11.8	1.8	42.8	2.8	4.3	0.4	3.9	0.3
Porifera																
Cliona sp.	36	1	76.0		7.7		-17.4		4.7		20.1		5.0		2.3	
Craniella cranium	38-41	3	13.1	6.1	7.5	0.7	-18.5	0.2	5.7	1.2	24.3	4.3	5.0	0.3	2.2	0.2
Geodia sp.	36	1	577.9		8.7		-17.1		4.0		17.0		5.0		3.7	
Iophon piceum	27	1	157.2		16.5		-18.0		4.0		18.0		5.3		3.6	
Stelletta sp.	22	1	26.1		8.0		-17.2		4.3		18.0		4.9		4.6	0
Phakellia sp.	26	1	93.3		14.9		-15.8		2.4		12.1		6.0		2	
Hamacantha (*Vomerula*) *carteri*	7	1	44.7		15.4		-14.6		0.9		5.7		7.0		4.1	
Haliclona spp.	39	2	14.8	0.4	7.9	0.1	-17.1	0.9	4.2	1.6	17.7	6.0	5.0	0.2	2.5	
Hexactinellida sp.	36	1	228.6		6.8		-17.3		4.0		16.5		4.9		4.3	
Euplectella sp.	20	1	11.7		12.9		-16.3		1.9		11.5		7.1		4.5	
Polymastia spp.	27	2	19.7	14.3	13.3		-16.7	0.3	3.8	2.8	17.6	12.5	5.4	0.1	2.3	
Tentorium semisuberites	27	1	6.1		15.5		-17.6		5.6		24.1		5.0		2.3	
Thenea muricata	27	4	16.2		14.2	0.3	-17.4	0.4	3.3	1.6	15.5	8.4	5.4	0.3	4.3	0.1
Mean			98.9	158.7	11.5	3.7	-17.0	1.0	3.8	1.4	16.8	5.0	5.4	0.8	3.3	1.0
Sipuncula																
Sipunculidea sp 1	42	1	7.3		11.4		-16.0		11.0		38.9		4.1		3.2	
Sipunculidea sp 2	22	1	2.0		15.0		-15.3		12.8		41.6		3.8		4.2	
Mean			4.7	3.7	13.2	2.5	-15.6	0.5	11.9	1.3	40.3	1.9	3.9	0.2	3.7	0.7

Table 4-3 FA composition of species of Annelida and Arthropoda reported as proportions (%).

	Annelida										Arthropoda											
FA	*L. filicornis*	Nereidae sp.	Polychaeta sp 1	Polychaeta sp 2	**SD**	Polychaeta sp 3	**SD**	Polynoidae sp 1	Polynoidae sp 2	**SD**	*A. pelagica*	**SD**	*A. michelottianum*	**SD**	*N. robustus*	*P. borealis*	**SD**	*P. tarda*	*S. hystrix*	**SD**	*S. sculpta*	**SD**
14:0	1.1	3.4	3.0	2.1	0.6	0.9	0.2	2.5	1.9	0.0	0.7	0.2	1.7	0.7	1.0	2.3	0.2	0.6	0.8	0.1	0.5	0.6
i15:0	0.0	0.1	0.2	0.4	0.5	0.1	0.0	0.1	0.0	0.1	0.1	0.0	0.2	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0
16:0	12.4	10.0	14.3	12.2	0.3	11.8	9.8	11.6	9.9	0.4	4.0	1.4	9.5	1.1	14.4	17.0	4.2	7.1	13.3	0.7	16.5	3.4
16:1ω9?*	0.5	0.2	0.2	0.6	0.7	3.6	5.6	0.1	0.2	0.2	0.1	0.0	0.3	0.3	0.3	0.1	0.1	0.0	0.2	0.0		
16:1ω7	5.0	5.3	5.5	4.8	1.0	8.4	7.2	4.7	4.0	0.0	3.0	0.5	5.6	1.8	1.8	5.5	1.0	4.2	9.1	2.3	6.2	1.4
16:1ω5	0.1	0.1	0.2	0.2	0.0	0.3	0.3	0.2	0.1	0.0	0.1	0.0	0.2	0.1	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.1
i17:0	0.6	0.3	0.8	0.2	0.1	0.8	0.1	0.2	0.3	0.0	0.2	0.1	0.3	0.1	0.1	0.1	0.1	0.1	0.5	0.0	0.2	0.2
ai17:0	0.2	0.2	0.9	0.1	0.0	0.5	0.3	0.1	0.1	0.0	0.1	0.0	0.2	0.1	0.0	0.1	0.1	0.2	0.4	0.0	0.0	0.0
17:1	1.6	0.2	0.2	0.5	0.2	0.2	0.2	0.4	1.5	0.6	0.3	0.0	0.4	0.2	0.2	0.3	0.4	0.4	0.6	0.0	0.3	0.1
16:3ω3?*	0.3		0.3	0.2	0.1	0.2	0.1	0.4	0.4	0.3	0.9	1.2	0.2	0.0	0.6	0.2	0.1	0.6	0.4	0.0	0.5	0.5
18:0	7.8	3.5	3.6	2.2	3.1	3.5	0.7	2.9	7.6	0.4	0.9	0.3	2.1	1.0	3.7	3.1	2.4	1.7	3.0	0.5	5.7	1.0
18:1ω9	4.1	5.4	2.3	17.6	1.5	9.1	8.3	9.4	3.7	0.2	16.0	4.2	13.8	2.3	17.7	15.3	6.5	25.5	12.8	0.6	16.7	1.5
18:1ω7	4.6	4.3	7.4	9.7	0.3	3.7	0.6	5.8	5.1	0.1	4.3	0.9	3.6	0.5	5.9	6.7	0.8	6.9	10.5	0.7	6.0	0.8
18:1ω5?*	0.4	0.4	0.6	0.9	0.1	0.5	0.0	0.4	0.3	0.0	0.5	0.0	0.5	0.1	0.5	0.4	0.3	0.9	0.8	0.1	0.6	0.2
18:2ω6	0.6	1.8	1.5	0.9	0.2	4.0	1.4	2.1	2.4	0.7	1.0	0.1	0.7	0.1	0.6	2.5	1.7	1.2	1.2	0.3	1.1	0.4
18:5ω3				0.0	0.1			0.1							0.6							
20:1ω11?*	7.7	12.2	8.2	1.4	1.4	0.4	0.4	5.3	7.4	1.1	0.8	0.7	3.2	2.1	3.0	0.2	0.2	0.9	0.7	0.1	1.1	0.6
20:1ω9	4.2	3.5	1.1	6.8	0.8	1.1	0.6	6.4	4.2	0.1	13.2	1.0	11.5	1.3	0.4	2.0	1.2	3.3	0.7	0.0	5.8	1.3
20:1ω7?*	0.5	1.8	1.5	1.8	0.6	3.2	1.9	1.2	0.8	0.4	1.0	0.3	0.9	0.2	0.0	1.3	1.4	0.1	0.8	0.2	0.1	0.1
20:2b	0.6	0.4	0.5					0.4			0.0	0.0	0.1	0.1					0.3	0.1		
20:2ω6	1.6	1.1	1.4	0.6	0.4	0.2	0.2	0.9	1.2	0.3	0.4	0.0	0.1	0.1	0.3	0.2	0.2	0.1	0.5	0.1	0.3	0.3
20:4ω6		1.5	1.0	0.8	0.9	0.5	0.5	2.6	0.6	0.2	0.5	0.1	1.1	0.4	1.5	0.8	0.7	1.4	2.0	0.4	8.2	10.2
20:5ω3	9.6	11.4	13.5	8.0	1.6	9.7	8.4	13.9	18.4	0.6	7.7	3.6	14.7	3.6	24.2	19.8	3.5	15.9	16.8	0.2	11.3	9.8
22:0		0.1				4.2	7.2				0.1	0.1	2.2	3.8							0.2	0.3
22:1ω11(13)	3.0	4.3		1.8	1.3	0.4	0.4	4.3	1.3	0.1	10.4	2.2	3.9	2.5		1.0	1.0	0.7	0.3	0.1	0.1	0.1
22:1ω9	0.7	0.7		1.3	0.5	0.2	0.2	0.7	1.6	2.0	1.7	0.3	1.1	0.6	1.1	1.2	1.6	0.7	0.4	0.3	0.2	0.2
22:1ω7	0.3	0.5		0.0	0.0	0.3	0.2	0.3			0.1	0.0	0.3	0.3	0.3				0.2	0.0		
22:2NIMDa?*	11.8	1.0		0.1	0.1	0.3	0.4	1.2	2.4	3.4					0.1							

23:0						0.0	0.1															
22:4ω6?*	1.3	0.5	2.0	0.5	0.2	0.1	0.1	0.9	1.9	0.1		0.0	0.0	0.0	0.0				0.5	0.1		
22:5ω3	2.3	2.1	8.3	10.1	8.7	0.8	0.7	2.9	5.2	0.3		16.6	3.8	1.1	0.2	5.6	0.5	0.5	0.3	2.0	0.2	0.9	1.5
22:6ω3	6.9	13.0	10.7	11.1	3.2	5.7	4.9	11.6	13.7	1.4		7.1	2.4	15.4	4.4	14.2	16.8	5.2	19.1	16.1	1.1	15.2	5.9
24:1	1.8	0.1	0.0	0.1	0.2	2.3	3.8	0.2	0.0	0.0		0.8	0.3	1.6	0.5	0.2	0.2	0.2	0.5	0.0	0.1	0.3	0.3

* '?' tentative identifications based on retention times and the literature (Ackman 1986)

Table 4-4 FA composition of species of Cnidaria and Mollusca reported as proportions (%).

	Cnidaria																Mollusca			
FA	*A. cristata*	**SD**	*A. aurelia*	**SD**	*A. callosa*	**SD**	*Anthomastus* spp.	**SD**	*A. grandiflorum*	*Funiculina* sp.	*P. arborea*	*P. aculeata*	**SD**	*P. grandis*	**SD**	*Umbellula* sp.	*Buccinum* sp.	**SD**	*C. islandicus*	*N. despecta*
14:0	1.3	0.5	0.7	0.9	0.4	0.4	1.0	0.7	1.4	1.0	2.5	0.8	0.7	1.8	0.5	0.5	1.8	0.2	1.1	0.9
i15:0	0.2	0.2	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.2	0.0	0.1	0.0	0.2	0.0	0.0	0.1	0.0	0.1	0.1
16:0	11.4	3.7	11.0	2.1	9.2	1.8	8.5	1.4	11.4	10.8	5.9	7.9	0.9	8.8	0.8	3.2	7.0	0.8	7.1	6.8
16:1ω9?*	0.2	0.3			0.2	0.3	0.1	0.1		0.2	0.1	0.1	0.1	0.0	0.0		0.1	0.0	0.1	0.1
16:1ω7	3.7	0.3	2.6	1.4	1.9	0.8	2.2	1.5	5.0	2.8	3.2	3.7	0.8	6.6	1.6	4.1	1.0	0.2	0.8	0.5
16:1ω5	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1
i17:0	1.3	0.5	1.2	0.7	0.7	0.3	0.7	0.4	0.5	0.5	0.1	0.6	0.0	0.8	0.2	0.4	0.3	0.0	0.1	0.2
ai17:0	0.9	0.7	0.4	0.2	0.3	0.3	0.4	0.3	0.3	0.2	0.1	0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.3	0.1
17:1	0.1	0.1	0.1	0.3	0.2	0.2	0.1	0.1		0.2	0.1	0.3	0.2	0.3	0.1	0.1	1.4	0.5	2.0	1.3
16:3ω3?*	0.8	1.2			0.4	0.4	0.3	0.6		1.5	3.0			1.6	2.3	10.2	0.2	0.0	0.2	0.4
18:0	7.6	3.9	9.7	4.0	5.0	1.1	5.3	2.0	2.5	2.5	1.8	2.4	0.3	1.6	0.1	0.5	12.0	0.0	11.7	10.6
18:1ω9	6.4	0.4	5.4	2.4	4.4	2.0	7.5	1.8	12.5	6.0	4.8	7.3	2.1	9.1	0.3	9.4	4.2	0.7	3.9	3.9
18:1ω7	2.8	0.5	2.9	1.0	0.5	0.7	3.2	0.6	4.3	2.1	1.8	6.0	1.1	4.5	0.1	4.4	4.4	0.4	2.7	2.1
18:1ω5?*	1.6	0.1	3.7	2.9	0.5	0.5	1.1	1.5	0.6	0.7	0.3	0.2	0.2	0.4	0.0	0.4	0.1		0.0	0.1
18:2ω6	0.1	0.2	0.3	0.5	0.4	0.2	0.6	0.6	1.1	0.4	0.8	0.4	0.5	1.0	0.4	1.0	1.2	0.1	1.5	1.4
18:5ω3																				
20:1ω11?*	0.8	0.5	0.4	0.5	0.2	0.4	0.6	0.4	0.5	0.6	0.5	0.7	0.6	0.6	0.0	0.6	5.5	0.8	5.5	5.3
20:1ω9	17.5	0.9	10.2	0.8	7.2	3.7	14.1	4.3	11.1	8.2	12.4	13.4	0.6	9.9	0.0	10.3	3.4	0.7	3.1	2.5
20:1ω7?*	2.9	1.7	2.4	1.0	1.1	1.1	3.5	1.2	2.2	8.4	1.9	3.2	0.2	2.6	0.4	1.9	0.8	0.3	1.3	0.9
20:2b					3.8	6.6											0.3	0.4		
20:2ω6			0.1	0.1	2.3	3.9	0.0	0.1		0.8				0.5	0.1	0.5	3.7	0.0	2.8	3.1
20:4ω6	0.2	0.3	8.4	14.5	0.3	0.2	13.4	6.2	2.8	2.6	7.8	12.7	3.4	1.3	1.9		3.5	0.9	13.1	13.2
20:5ω3	8.2	11.6	15.8	8.1	13.2	1.5	9.5	7.5	20.7	25.6	13.2	10.5	2.1	17.6	0.4	17.3	24.5	0.2	13.5	
22:0																	0.3	0.1		17.5
22:1ω11(13)	10.4	0.3	6.1	3.9	4.8	3.2	14.8	8.8	9.7	6.6	13.9	11.1	0.3	7.9	0.1	11.8	0.1	0.1	0.3	
22:1ω9	8.8	4.3	0.8	0.7	5.2	2.1	1.4	1.0	4.7	2.9	0.9	4.5	0.2	5.6	1.6	3.1	0.0	0.1		
22:1ω7										0.3		0.0	0.1	0.3	0.5					
22:2NIMDa?*										0.1							2.9	0.6	2.8	6.4

23:0																				
22:4ω6?*			1.6	2.8	3.3	2.5	1.2	2.3	0.2	0.3	0.9	7.4	1.7	0.5	0.2	0.1		0.7	0.0	1.7	1.9
22:5ω3			5.3	9.1	6.4	2.6	4.5	9.0	1.6	6.2	15.2	2.5	0.3	7.7	0.8	10.2		10.6	0.9	16.5	10.8
22:6ω3	5.1	7.2	1.0	1.7	5.2	4.2	1.8	1.8	3.3	4.4	1.8	1.7	0.6	2.1	0.4	1.4		4.0	0.8	2.9	2.4
24:1	0.2	0.2	0.0	0.0	16.7	28.8	0.3	0.4	0.9	0.9	0.5	0.3	0.3	0.7	0.0	0.9		0.0	0.0	0.0	1.7

* '?' tentative identifications based on retention times and the literature (Ackman 1986)

Table 4-5 FA composition of species of Porifera reported as proportions (%).

	Porifera																
FA	*Cliona* sp.	*C. cranium*	**SD**	*I. piceum*	*Geodia* sp.	*Stelletta* sp.	*Phakellia* sp.	*H. carteri*	*Haliclona* spp.	**SD**	*Polymastia* spp.	**SD**	*T. semisuberites*	*T. muricata*	**SD**	*Euplectella* sp	Hexactinellida sp.
14:0	2.0	3.2	0.1	1.6	1.7		1.7		1.4	0.2	3.1	1.9		0.8	1.6	1.6	2.4
i15:0	4.5	5.7	1.0	0.9	5.4	3.7	0.3		4.0	1.1	1.7	1.4	1.3	1.3	0.7	5.2	0.2
16:0	3.9	4.9	0.2	7.3	5.1	0.8	6.6	38.3	4.0	1.1	10.8	2.9	10.9	8.8	2.9	5.3	7.5
16:1ω9?*	0.7	0.8	0.2	0.3	0.8	23.0	0.1		0.5	0.1	0.4	0.1	0.4	0.8	0.6	0.7	0.2
16:1ω7	22.0	22.3	0.3	4.9	22.0	2.6	5.6	6.3	20.9	2.3	6.6	1.0	8.7	10.7	4.0	23.4	6.8
16:1ω5	2.7	3.3	0.4	0.4	2.5	5.0	0.1		2.9	0.1	0.4	0.2	0.7	0.6	0.6	3.6	0.2
i17:0	1.6	3.2	1.7	1.9	1.7	1.1	0.5		1.0	0.1	1.7	1.5	0.7	0.7	0.7	5.2	0.4
ai17:0	5.7	7.4	1.1	0.9	5.8		0.6		2.1	1.8	0.5	0.5	0.5	0.5	0.4	3.1	0.2
17:1	1.9	1.0	0.4	0.3	0.7				1.0	0.2	3.1	1.9	5.6	3.0	2.5	2.1	0.2
16:3ω3?*				0.5			0.2				0.2	0.1	0.1	0.0	0.1		0.1
18:0	2.9	2.6	0.1	3.6	3.1		4.1		2.4	0.4	1.6	1.5	1.5	2.2	1.0	3.4	2.5
18:1ω9				2.2		24.8	3.0	12.2			5.9	5.2	1.5	3.9	3.1		11.7
18:1ω7	26.3	25.0	2.7	8.4	26.7		4.1	1.4	25.7	4.0	4.8	0.6	9.6	7.6	4.2	30.3	4.0
18:1ω5?*	4.6	3.0	3.5		4.6		0.2		6.0	1.0	4.2	1.7	0.6	0.9	0.5	6.3	0.4
18:2ω6	0.1	0.2	0.0	0.8	0.1		0.5				1.1	0.1	1.8	1.0	0.6	0.2	0.9
18:5ω3																	
20:1ω11?*	0.2	0.0	0.1	1.1		0.4	0.5				0.6	0.9	0.8	0.4	0.3		1.4
20:1ω9	0.3	0.5	0.8	1.5			3.6	16.9	0.0	0.0	4.5	6.4	1.3	3.2	3.1	0.6	8.8
20:1ω7?*	0.1	0.1	0.1	1.1			1.1	2.6			0.5	0.8	8.0	3.6	3.6	0.3	0.7
20:2b																	
20:2ω6				0.8			0.2				0.3	0.0	0.6	0.2	0.3		0.4
20:4ω6	0.1	0.4	0.2								2.6	1.5	4.6	0.4	0.7		1.0
20:5ω3	5.6	0.4	0.3	7.7			5.0	12.0			6.7	0.2	12.0	6.4	4.1		10.2
22:0							0.2										
22:1ω11(13)							3.7		0.1	0.2	5.0	7.0		2.3	3.2	0.5	8.8
22:1ω9							0.4				0.5	0.7		0.2	0.5		1.4
22:1ω7		0.2	0.2	10.5			3.4				10.2	7.8	1.5	1.8	0.9		
22:2NIMDa?*														11.4	22.8		
23:0	0.2						42.1		10.3	11.6							11.8
22:4ω6?*				0.3			1.8		0.2	0.2							

22:5ω3	0.5	0.0	0.1	0.7		0.3			2.4	1.1	1.1	1.6	0.0	3.3	3.9		1.3
22:6ω3	1.8	0.6	0.3	19.2	5.9		1.5		0.0	0.1	10.3	6.9	5.3	7.0	3.5	0.2	7.9
24:1	0.4	1.9	0.5	9.7	3.7		5.2	10.4			2.9	0.6	3.4	4.4	1.6	3.3	1.2

* '?' tentative identifications based on retention times and the literature (Ackman 1986)

Table 4-6 FA composition of species of Echinodermata and Sipuncula reported as proportions (%).

	Echinodermata											Sipuncula	
FA	*F. microspina*	*Gorgonocephalus* sp.	*L. arcticus*	**SD**	*M. bairdi*	**SD**	*M. sol*	*P. placenta*	**SD**	*P. andromeda*	*Z. fulgens*	Sipunculidea sp 1	Sipunculidea sp 2
14:0	0.6	3.5	1.2	0.9	0.4	0.3	0.4	2.4	0.3	0.3	0.5	0.5	2.0
i15:0	0.2	0.1	0.2	0.1	0.4	0.1	0.2	0.5	0.2	0.2	0.1	0.4	
16:0	3.6	4.4	2.6	1.0	1.4	0.5	3.2	6.7	1.0	2.6	3.7	11.1	0.2
16:1ω9?*	0.1	0.1	0.2	0.2	1.0	0.6	0.4	0.5	0.2	0.2		0.7	7.0
16:1ω7	2.1	8.5	1.1	0.5	0.8	0.4	0.8	17.9	1.3	0.8	2.5	7.2	3.5
16:1ω5	0.1	0.2	0.1	0.0	0.2	0.1		0.4	0.0		0.1	0.5	0.9
i17:0	0.2	0.1	0.2	0.0	0.2	0.0	0.2	0.6	0.0	0.1	0.1	0.6	
ai17:0	0.2	0.1	0.0	0.0	0.7	0.3	0.3	0.1	0.1	0.3	0.1	0.4	1.3
17:1	0.5	0.4	1.1	0.6	3.1	1.6	4.2	0.5	0.1	0.5	0.2	0.9	1.3
16:3ω3?*	0.2	0.1	0.1	0.1	0.2	0.0	0.2	0.1	0.3	0.1		0.2	
18:0	4.5	2.7	8.2	0.5	3.5	1.8	7.3	3.8	0.4	7.7	4.2	4.2	10.6
18:1ω9	1.6	4.7	1.1	0.2	2.6	2.4	1.7	2.2	0.2	1.5	2.2	3.2	5.2
18:1ω7	4.0	4.5	9.7	2.3	2.6	2.4	3.4	9.5	0.4	3.5	4.3	6.1	17.4
18:1ω5?*	0.3	0.4	0.8	0.7	1.1	1.0	0.2	0.1	0.1	0.1	0.3	0.3	0.6
18:2ω6	0.1	0.8	0.2	0.2	0.1	0.1	0.1	0.3	0.0	0.1	0.0	0.5	0.4
18:5ω3	10.4						15.5						
20:1ω11?*	16.2	4.3	14.0	1.3	17.9	2.6	0.0	6.1	0.5	17.5	11.2	2.4	5.0
20:1ω9	0.8	12.6	6.3	3.5	4.0	1.3	11.5	2.7	0.8	10.4	17.8	0.4	1.1
20:1ω7?*	1.4	0.7	0.6	0.3	3.0	0.6	1.0	1.0	0.4	0.9	0.8	1.6	2.8
20:2b		0.2	0.5	0.1	0.6	0.3		1.1	0.2			17.3	4.6
20:2ω6	1.0	0.3	1.7	0.8	0.2	0.1	0.3	0.7	0.1	0.7	1.3	0.5	
20:4ω6	16.9	0.7	8.2	2.1	27.3	5.9	29.4	14.4	1.4	26.9	4.6	11.3	3.8
20:5ω3	13.2	18.5	24.8	2.8	6.4	1.4	12.3	11.1	1.6	17.7	17.1	11.4	1.6
22:0			0.2	0.3	0.1	0.1		1.4	0.2				
22:1ω11(13)	13.7	9.3	2.6	1.7	2.6	0.9	1.3	0.5	0.5	0.9	18.5		
22:1ω9	1.4	1.7	4.3	6.3	0.8	0.1	0.3	1.2	0.5		2.0		
22:1ω7	0.2	0.2	0.3	0.2	1.4	0.4					0.2		
22:2NIMDa?*			0.3	0.4	0.2	0.2							

23:0													5.7	
22:4ω6?*			0.1	0.1	0.9	0.3							0.5	0.0
22:5ω3		8.8	0.6	0.7	0.6	0.6	0.1	0.6	0.0				2.3	1.5
22:6ω3	2.1	2.0	1.4	0.6	0.8	0.1	0.2	1.8	0.4	2.4	3.3		1.7	0.3
24:1	2.1	1.1	1.2	0.7	2.8	0.5	1.2	0.3	0.1	1.4	1.4			

* '?' tentative identifications based on retention times and the literature (Ackman 1986)

Table 4-7 Trophic multiplication factor (TMF) measured for those FA that correlated significantly with $\delta^{15}N$. The strength of the Pearson correlation (r) is reported, together with values (± 95% confidence intervals) of slope (m), and the intercept (b) of the linear regression. Values of r are also reported for those FA that correlated significantly with $\delta^{13}C_n$ and $C:N_{mol}$.

$\delta^{15}N$				
FA	r	TMF	m	b
16:1ω7	-0.5	0.5	-0.7 (±0.2)	3.6 (±0.6)
16:1ω5	-0.4	0.4	-0.8 (±0.3)	0.9 (±0.9)
i17:0	-0.4	0.5	-0.6 (±0.2)	1.2 (±0.6)
ai17:0	-0.3	0.6	-0.6 (±0.3)	0.6 (±0.9)
16:3ω3?	-0.4	0.5	-0.7 (±0.3)	1.2 (±1.1)
18:0	0.3	1.4	0.3 (±0.1)	0.3 (±0.5)
18:1ω9	-0.5	0.5	-0.8 (±0.2)	4.2 (±0.6)
18:1ω7	-0.4	0.6	-0.4 (±0.2)	3.1 (±0.5)
18:1ω5	-0.5	0.4	-0.8 (±0.2)	2.1 (±0.7)
20:1ω11?	0.6	3.9	1.4 (±0.3)	-3.9 (±1.0)
20:1ω9	0.3	1.8	0.6 (±0.3)	0.6 (±0.9)
20:4ω6	0.6	3.9	1.4 (±0.3)	-3.4 (±1.1)
22:1ω9	-0.3	0.5	-0.8 (±0.4)	2.6 (±1.2)
22:1ω7	0.7	4.6	1.5 (±0.4)	-5.8 (±1.2)

$\delta^{13}C_n$	
FA	r
18:1ω9	-0.4
18:2ω6	-0.3
18:5ω3	0.3
20:1ω11?	0.6
20:4ω6	0.6
22:1ω11(13)	0.4
22:6ω3	-0.5

$C:N_{mol}$	
FA	r
18:0	-0.3
20:1ω9	0.4
22:1ω11(13)	0.6
22:6ω3	-0.4

Table 4-8 Presence ("x") of very long chain fatty acids (VLCFA) in various species of corals (phylum Cnidaria) and sponges (phylum Porifera). The VLCFA listed were tentatively identified by plotting C numbers against relative retention times (see Appendix 7-6), literature review (Bergé and Barnathan, 2005; Rezanka and Sigler, 2009; Kornprobst and Barnathan, 2010; Imbs, 2013; Monroig et al., 2013), and comparisons across samples.

VLCFA	Cnidaria							Porifera			
	A. cristata	*A. callosa*	*Anthomastus* spp.	*P. arborea*	*P. aculeata*	*P. grandis*	*Umbellula* sp.	*Cliona* sp.	*C. cranium*	*Haliclona* spp.	*T. muricata*
24:1ω11								x		x	x
24:1ω9							x				
24:2ω6								x			x
23:5ω3							x	x		x	x
24:5ω6			x	x	x	x	x				
24:5ω3	x			x		x	x				
26:0										x	
24:6ω3				x	x	x	x				x
26:1											x
26:1ω9											x
26:2								x			
26:2ω6											x
25:5ω3				x							x
27:0								x		x	x
26:4ω6	x			x			x				x
26:5ω6											x
26:6ω3				x		x	x				
28:1ω9								x			x
28:2											x
28:2ω6											x
27:5ω3								x			x
28:5ω6								x			x
30:1ω9											x

Figures

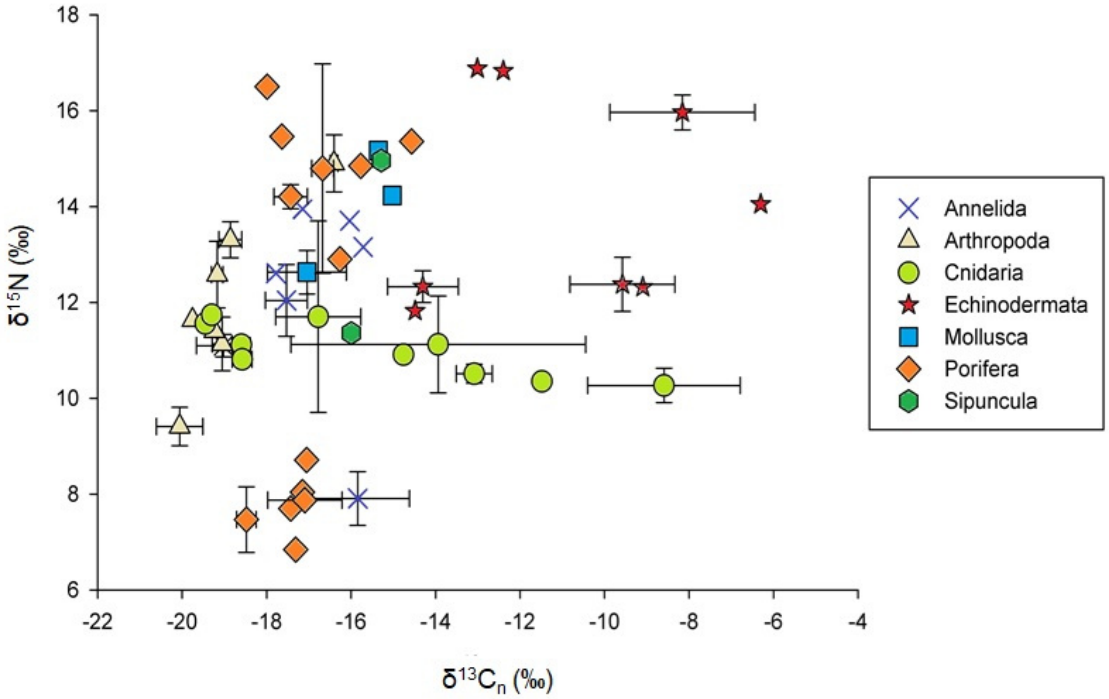

Fig. 4-1 Biplot of mean (± SD; *n* = 2-4) ratios of stable isotopes ($\delta^{15}N$ and $\delta^{13}C_n$) in benthic taxa analyzed.

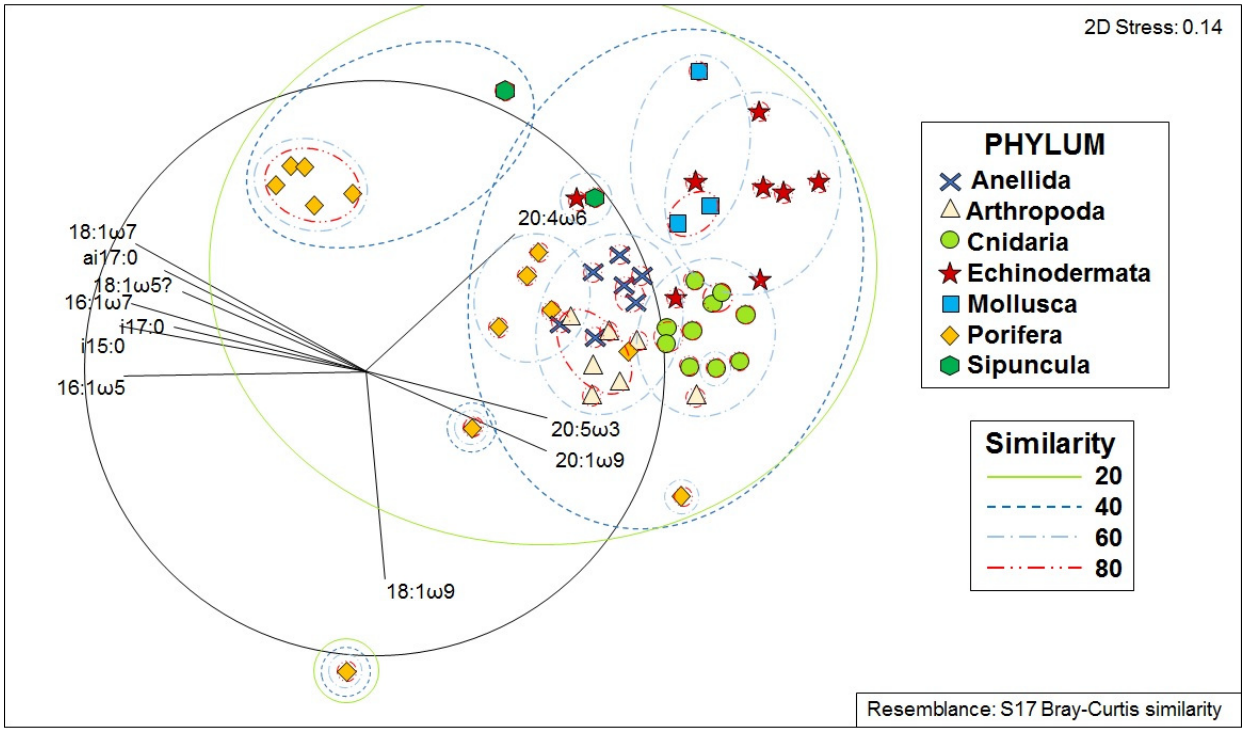

Fig. 4-2 MDS plot, based on Bray-Curtis similarity matrices, of FA composition of the deep-sea benthic taxa analyzed. The FA reported are those contributing to 60% of the variability among the species.

Chapter 5 : **Deep-sea food webs and trophic biomarkers: a review**

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, this review provided an opportunity for a preliminary analysis of global geographic (i.e. latitudinal and longitudinal) trends in the isotopic ($\delta^{15}N$, $\delta^{13}C$) and fatty acid composition of deep-sea benthic taxa collected in upper- and mid-slope areas (~200-2500 m depth). Results revealed significant relationships along the latitudinal gradient, with deep-sea benthic organisms sampled at temperate and polar latitudes displaying lower $\delta^{13}C$ ratios and greater proportions of essential ω3 long-chain polyunsaturated fatty acids (LC-PUFA). than tropical counterparts. Conversely, no latitudinal trends were found $\delta^{15}N$ nor in the levels of ω6 LC-PUFA. Since similar trends in the isotopic and fatty acid signatures were found in surface water phytoplankton, particulate organic matter, and organisms, these results highlight the link across latitudes between surface-water primary production and deep-water benthic communities. As the former represent the main food source for the latter, global climate change may have major impacts via alteration of dietary intake in deep-sea organisms. Importantly, methodological disparities were highlighted that prevented in-depth analyses, indicating that predictions derived from this early data set will need to be corroborated with further studies conducted using standardized methods.

Review

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 (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 the 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 $\delta^{13}C$) as trophic markers. In the same year, Polunin et al. (2001) published their study on the trophic relationships of a slope megafaunal assemblage collected off the Balearic Islands (western Mediterranean), also elucidated by $\delta^{15}N$ and $\delta^{13}C$. 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 trophodynamics. 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 (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 two decades ago, this review synthesizes current knowledge in this growing field of research. In addition, it provides a preliminary overview of large-scale geographic trends from the analysis of isotopic and FA data, along with guidance for future investigations. In particular, the present contribution i) briefly describes various trophic biomarkers and their respective advantages; ii) characterizes 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; iv) provides a preliminary quantitative analysis across studies by using subsets of data; and v) suggests future directions.

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 habits 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 is proposed to overcome many of the issues ascribed to gut content analysis (Michener and Kaufman 2007). In this regard, Table 5-1 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 5-1). 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 groups, 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 sophisticated instrumentation (e.g. gas chromatograph, mass spectrometer). Although each method necessitates a sufficient sample size, only gut content analysis provides a direct and clear evidence of the diet (Table 5-1). Therefore, as stated above, the latest trend in trophic ecology advocates a multifaceted approach, on the understanding that each technique may provide 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 isotopic tracers applied in food-web studies. 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 distiguish 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,b; Hamoutene et al. 2008). Their use is based on the principle that certain FA and amino acids are considered essential in organisms, being required for optimal fitness. However, most organisms cannot synthesize these essential compounds *de novo* and, therefore, they have to gain them through 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 acids show trophic enrichment (Bradley et al. 2015). Detailed information about FA analysis was outside the scope of this study, and is provided by Parrish (2009) and Iverson (2009); whereas the use of sterols as food-web tracers was outlined in Martin-Creuzburg and Von Elert (2009) and Parrish et al. (2000). McClelland and Montoya (2002) and Larsen et al. (2009), conversely, discuss the use of amino acids as trophic biomarkers.

Understanding deep-sea food webs through biochemical markers

As there is no photosynthetically-derived primary production in the deep sea, deep-water ecosystems are mostly heterotrophic (Gage 2003), and they largely rely on particulate organic matter (POM) that passively sinks from the surface waters as a primary source

of nutrients (Hudson et al. 2004). Nonetheless, food can also be actively transported down by those organisms that carry out vertical diel migrations through the water column (Trueman et al. 2014); it can also be provided by the occasional fall of large animal carcasses (Smith and Baco 2003); and/or by lateral inputs, from inland and shelf areas towards abyssal offshore regions (Pfannkuche 2005). Although most of the deep-water ecosystems are heterotrophic, a few, such as hydrothermal vents and cold seeps, are fuelled by chemical energy (e.g. methane CH_4 and hydrogen sulfide H_2S) and rely on chemosynthetic microorganisms for the production of organic matter. Each of these primary food sources has a specific isotopic composition and biochemical signature, resulting from a combination of chemical and physical processes reflective of its origin. By knowing the composition of the food source(s) that fuel(s) a given food web, it is possible to re-construct its trophic structure and dynamics. Conversely, by measuring the signatures of the food-web components, it is possible to assess upon which food source they rely. For instance, Iken et al. (2001) showed that phytodetritus was the primary energy input of the deep-sea benthic community at PAP, and also defined two different trophic pathways: a pelagic and isotopically lighter one in which sinking POM and small pelagic prey constituted the main food sources; and a benthic and more isotopically enriched trophic pathway, fuelled by degraded sedimented POM. In fact, once POM settles on the seafloor, it undergoes continuous degradation by microbes and is reworked through bioturbation and feeding activities, thus leading to a more enriched material relative to the sinking one (Iken et al. 2001). Depending on the primary food source they relied on, benthic organisms at PAP were thus characterized by either lower or higher values of $\delta^{15}N$. Similar scenarios of dual trophic pathways characterizing benthic systems were also found by Iken et al. (2005) in the Canadian Arctic; Drazen et al. (2008b) in the North Pacific; Reid et al. (2012) within the benthic community sampled

on the mid-Atlantic Ridge; Valls et al. (2014) in the western Mediterranean; and Parzanini et al. (2017) in the Northwest Atlantic. Moreover, Kharlamenko et al. (2013) used both stable isotopes and FA to study the dietary sources of benthic invertebrates collected along a slope area (500 – 1600 m depth) in the Sea of Japan. The authors recognized different trophic pathways (i.e. planktonic, benthic, microbial) and dietary sources by using biochemical tracers; and they proposed a strong link with the primary production of the surface waters, as the FA composition of the deep-sea invertebrates was similar to that of the shallow-water counterparts.

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

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

Deep-water systems are generally characterized by a limited food supply, as the quantity of material sinking from the surface water diminishes with increasing depth (Gage 2003). In addition, in temperate areas, food arrives as intermittent pulses, following the spring and late summer blooms of primary productivity. For this reason, deep-water benthic communities can only rely on fresh phytodetritus within short temporal windows following algal blooms; whereas reworked and resuspended POM fuels these communities for the rest of the year (Lampitt 1985). Deep-sea benthic organisms have hence developed adaptations and strategies to increase their feeding success and minimize competition for food, including trophic niche expansion and specialization. In this regard, certain benthic taxa (e.g. sea pens, hexactinellid sponges) and/or feeding groups (e.g. suspension and deposit feeders) at PAP showed vertical extension of their trophic niches (i.e. omnivory) which, according to Iken et al. (2001), was most likely driven by a strong competition for food. In other words, some species belonging to the same taxon or feeding group shared similar food sources (i.e. exhibiting similar $\delta^{13}C$ values), but they were located at different trophic levels (i.e. exhibiting a wide range of $\delta^{15}N$). Similarly, Jeffreys et al. (2009) reported trophic niche expansion among and within feeding groups sampled between 140 and 1400 m depth, at the Pakistan margin (Arabian Sea). Sea pens and other sestonivorous cnidarians, for

example, displayed the greatest niche expansion; they fed not only on POM, but also on small invertebrates (e.g. zooplankton). Moreover, ophiuroids, which are typically selective deposit feeders, were determined to switch 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. Holothuroids, 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 on FA proportions discriminated three different feeding groups among the asteroids analysed, including mud ingesters, predators/scavengers, and suspension feeders.

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 5-2).

Biological sources

Age, size, and sex, whether or not 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. On 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 fishes experience changes in diet with age, typically with younger individuals preying upon benthic organisms and adults feeding on larger and bentho-pelagic prey (Mauchline and Gordon 1984; Eliassen and Jobling 1985). Stowasser et al. (2009) combined 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). 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 depths, the authors were not able to distinguish whether these results were due to ontogenetic changes in diet or merely to an effect of increasing sizes, within the size-range sampled. Moreover, it has been shown that $\delta^{15}N$ and trophic position may increase with body size in adult shallow-water fish, as the predators' diet are determined by their sizes (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 on 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 which 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.

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 of 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; Mindel et al. 2016b). Likewise, deep-sea organisms may exhibit different lipid and FA compositions along a bathymetric gradient, reflecting physiological adaptations of changing temperature and pressure with depth (Chapter 3).

Season and geographic location (e.g. latitude, oceanic region), linked to level and type of surface primary production 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 the fish species varied temporally, and suggested 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 because the high trophic position of the species and the length of the food web analyzed obscured the effects of the seasonal POM inputs (Stowasser et al. 2009). In addition, Colombo et al. (2016) detected a latitudinal gradient in the FA composition of marine species, whith 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, while Fig. 5-1 shows where food-web studies accomplished via biochemical tracers have been carried out, highlighting important geographic

heterogeneity, especially the limited number of investigations in the southern hemisphere.

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 invertebrates, which are characterized by higher turnover rates of lipids than others (e.g. white muscle). For this reason, these tissues incorporate information only on the recent diet. To avoid biases caused by lipids, 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. (2011), 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 which are simultaneously run for $\delta^{15}N$ and $\delta^{13}C$, as the treatment may affect $\delta^{15}N$ data (Bunn et al. 1995). Whether feasible, depending on both financial possibilities 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), Fanelli et al. (2011), 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 concordance 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 anaysis are either stored at -80°C (recommended) or at -20°C prior to further processing in the lab. In the latter case, as -20°C might not completely prevent lipid degradation, especially if samples are analyzed after several years, vacuum packing and rapid processing time may compensate for such issues when freezing at -80°C is not logistically feasible (Parrish pers. comm). 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 of organisms (Arrington et al. 2002; Syväranta et al. 2011; Xu et al. 2011).

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 also allows us to make predictions and support conservation initiatives. While several studies already exists on large-scale distribution and biodiversity patterns of deep-sea species (Rex et al. 1993; Stuart et al. 2003; Ramirez-Llodra et al. 2010), a similar approach has yet to be applied to trophodynamics. This preliminary analysis attempted to detect global spatial trends (i.e. latitudinal and longitudinal) in the isotopic and FA composition of deep-water benthic organisms for the first time since the application of biochemical tracers to the study of trophic ecology in the deep sea.

Latitudinal gradients have been detected in $\delta^{13}C$ of plankton and POM collected from surface waters in both the southern and northern hemispheres, with decreasing values towards the polar regions (Sackett et al. 1965; Rau et al. 1982; Francois et al. 1993). Both environmental (e.g. temperature) and biological (e.g. plankton metabolism) factors have been proposed to explain such trends (Rau et al. 1982; Francois et al. 1993). In addition, latitudinal trends have also been detected in the FA composition of marine organisms, which seem to have higher levels of essential ω3 long-chain polyunsaturated fatty acids (LC-PUFA) in the polar and temperate regions in comparison to the tropical ones (Colombo et al. 2016). As POM is the main food source of most of deep-sea benthic food webs (Gage 2003; Hudson et al. 2004), we hypothesized that

similar gradients exist in the isotopic and essential PUFA composition of deep-water benthic organisms. Furthermore, as surface-water primary productivity varies regionally and seasonally, according to the physical condition of each area throughout the year (Ramirez-Llodra et al. 2010), we tested whether the biochemical composition of these organisms reflected any longitudinal pattern.

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 among deep-water macrofaunal and megafaunal organisms, as well as to study deep-sea benthic food webs. Studies on pelagic food webs and on chemosynthetic habitats (e.g. hydrothermal vents) were excluded *a priori*. For the former, this choice was based on the insufficient number of investigations available. As for chemosynthetic habitats, they are fuelled by a primary dietary source with completely different isotopic and FA compositions than POM (Rau and Hedges 1979; Saito and Osako 2007), and were thus excluded to avoid possible biases. Furthermore, following the selection criteria described below, data available for deep-sea benthic fish were minimal compared to those available for invertebrates; thus only the latter were considered. Table 5-3 outlines the full data set collated for the present analysis. The literature search was carried out through the Google Scholar portal using the following key words: stable isotopes, fatty acids, food webs, deep sea, trophic ecology, and trophic relationships. Only subsets of data that met strict criteria (outlined below) were used to analyze global latitudinal and longitudinal trends in $\delta^{15}N$, $\delta^{13}C$, and the essential arachidonic (ARA, 20:4ω6), eicosapentaenoic (EPA; 20:5ω3) and docosapentaenoic (DPA, 22:6ω3) acids. These LC-PUFA are the most important nutrients in aquatic ecosystems, highly required by organisms for optimal health (Parrish

2009), as well as excellent biomarkers. In fact, whereas EPA and DPA are typically used as a 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 present in all the organisms analyzed.

2.1.1 Latitudinal gradient in the isotopic and FA composition

To test the existence of a latitudinal gradient in the isotopic composition of deep-water benthic organisms (Sackett et al. 1965; Rau et al. 1982; Francois et al. 1993), following that of plankton and POM in the surface waters, food-web studies carried out at different latitudes (i.e. tropical, 0 - 30°; temperate, 30 - 60°; and polar, 60 - 90°) were compared. Specifically, both $\delta^{15}N$ and $\delta^{13}C$ were tested and, to limit other sources of variability outlined in previous sections, only those focusing on benthic invertebrates of upper and mid-slope areas (~200 -2500 m depth) were considered. Since both isotopic and FA composition might vary along a bathymetric gradient (Bergmann et al. 2009; Parzanini et al. 2017), food-web studies undertaken below that range (e.g. Drazen et al. 2008a, b) were not considered. To allow analyses with an adequate number of data points, variations across studies in i) tissue type, ii) acidification treatment and iii) the sampling season were not considered. In addition, tests were performed on lipid-corrected and uncorrected $\delta^{13}C$ data pooled simultaneously, after running analyses on either lipid-corrected or uncorrected values, or both simultaneously, and determining that results were not affected. For the tropical latitudes, data were obtained from Jeffreys et al. (2009). For temperate regions, data from Sherwood et al. (2008), Carlier et al. (2009), Boyle et al. (2012), Fanelli et al. (2013), Gale et al. (2013), Kharlamenko et al. (2013), Papiol et al. (2013), Preciado et al. (2017), and Parzanini et al. (Chapter 4) were used.

Finally, data for the polar regions were provided by Iken et al. (2005) and Bergmann et al. (2009; Appendix 7-7).

Colombo et al. (2016) found that marine organisms from polar and temperate latitudes had higher levels of ω3 LC-PUFA (i.e. linolenic and linoleic acids, EPA, and DHA) relative to those sampled from the tropical latitudes. To test whether the same trend exists in the FA composition of upper and middle slope dwelling benthic organisms, data for the essential ARA, EPA, and DHA were collected from Jeffreys et al. (2009) for the tropical latitudes; Howell et al. (2003), Hudson et al. (2004), Salvo et al. (2018), and Parzanini et al. (Chapter 4) for the temperate regions; and Würzberg et al. (2011a, b) for the polar ones. As a side note, while the studies for the tropical and temperate regions were representative of the northern hemisphere, those from Würzberg et al. (2011a) and Würzberg et al. (2011b) were carried out in the southern hemisphere (Appendix 7-8). This choice was led by the fact that no corresponding investigation was found in the literature for the northern hemisphere. As above, only those studies conducted above upper and mid-slope areas were used for comparisons.

2.1.2 Longitudinal gradient in the isotopic composition

To explore the existence of longitudinal trends in $\delta^{15}N$ and $\delta^{13}C$ among benthic food webs, several studies carried out within the same latitude (i.e. temperate region) were compared. The choice of using only studies within the temperate region was based on the fact there were no more than two studies representing either the polar or tropical latitudes. In detail, Kharlamenko et al. (2013) was chosen as representative for the Sea of Japan; Carlier et al. (2009) for the Ionian Sea (central Mediterranean); Fanelli et al. (2013) and Papiol et al. (2013) for the Balearic basin (western Mediterranean); Preciado et al. (2017) for the Northeast Atlantic; Sherwood et al. (2008), Gale et al. (2013), and

Parzanini et al. (Chapter 4) for the Northwest Atlantic; and Boyle et al. (2012) for the eastern North Pacific (Appendix 7-9). No analysis was run to test for longitudinal trends in the FA composition, as there were not enough data availabe.

Statistical analysis

After testing for a normal distribution of data and homogeneity of variance, comparisons among multiple groups of benthic organisms were run through 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) and oceanic regions (depending on the studies included), to detect any significant differences or trends. When the normality assumption was violated, Kruskal-Wallis one way ANOVA on ranks was performed instead. In addition, multivariate statistics, i.e. principal coordinate analysis (PCO) and permutational ANOVA (PERMANOVA) were used to study variations in the isotopic and FA composition across different latitudes and oceans. ANOVA and ANOVA on ranks were conducted using Sigmaplot 11.0, whereas PCO and PERMANOVA were run through Primer 6.0 with the add-on package PERMANOVA+ (Clarke and Gorley 2006).

Results

Analyses revealed latitudinal trends for $\delta^{13}C$ and FA composition. In particular, mean values of $\delta^{13}C$ (±SD) were significantly lower in deep-sea organisms sampled at polar latitudes, than in those collected from temperate and tropical areas (-20.3±1.8, -17.1±2.5, -16.9±1.3‰, respectively; ANOVA on Ranks, $H_2 = 107.2$, $p \leq 0.001$; Fig. 5-2). 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 latitudes, especially for the former FA (for EPA, 18.6±7.1, 13.0±7.2, and 8.3±5.1% respectively, ANOVA, $F_{134,2}$ = 14.1, $p \leq 0.001$; for DHA, 11.3±7.3, 5.1±5.3, and 9.7±10.7% respectively, ANOVA on Ranks, H_2 = 28.4, $p \leq 0.001$; Fig. 5-3). Similarly, PERMANOVA detected significant differences across latitudes in terms of both stable isotopes [Pseudo-F = 44.7, *p*(*perm*) = 0.0001; and essential FA Pseudo-F = 5.7, *p*(*perm*) = 0.0001]. The plots in Fig. 5-4 visually represent the results obtained with PERMANOVA analysis.

No longitudinal gradient was detected in the isotopic composition of deep-sea organisms studied across temperate regions, although there were significant differences among discrete oceanic regions in terms of stable N and C isotope signature [i.e. Northwest Atlantic, eastern North Pacific, central and western Mediterranean, Northeast Atlantic, and Sea of Japa; Pseudo-F = 40.6, *p*(*perm*) = 0.0001]. In particular, the largest differences in stable isotope ratios of deep-sea species were shown between the eastern North Pacific and the western and central Mediaterranean [t = 14.0, *p*(*perm*) = 0.0001 and t = 9.0, *p*(*perm*) = 0.0001, respectively], and Northeast Atlantic [t = 8.5, *p*(*perm*) = 0.0001]; whereas there was no difference between central Mediterranean and Northeast Atlantic. Data used for this analysis are available in Appendix 7-9.

Discussion

For the first time, this preliminary analysis suggests the existence of latitudinal trends in both stable isotope and essential FA composition of deep-sea benthic organisms, with decreasing $\delta^{13}C$ ratios and increasing ω3 LC-PUFA towards the poles. In addition, the current investigation highlights the link, across latitudes, between the primary production of the surface waters and the deep-water benthic consumers. The present findings align with reports of decreasing values of $\delta^{13}C$ in surface-waters plankton and POM towards

the polar regions, in both the southern and northern hemisphere (Sackett et al. 1965; Rau et al. 1982; Francois et al. 1993). Furthermore, Colombo et al. (2016) noticed that proportions of ω3 LC-PUFA were higher in marine organisms from polar and temperate regions in comparison to tropical regions. Water temperature, in combination with other abiotic and biological factors (e.g. depth, metabolism, and taxonomic group) seems to play a major role in this regard (Rau et al. 1982; Francois et al. 1993; Colombo et al. 2016). In particular, water temperature influences the stable carbon isotopes fractionation process during photosynthesis and, typically, higher fractionation is associated with lower temperatures (Sackett et al. 1965). Furthermore, water temperature affects membrane fluidity, and lower temperatures decrease the fluidity of cell membrane (Parrish 2013; Colombo et al. 2016). Thus, in order to maintain normal membrane function and condition, i.e. health, ectotherms may counteract variations in water temperature by readjusting their FA composition (Cossins and Lee 1985; Parrish 2013). For example, larger proportions of long chain unsaturated FA (e.g. EPA and DHA) within the lipid bilayer help increase membrane fluidity (Parrish 2013), as these molecules are characterized by a higher flexibility (DeLong and Yayanos 1985; Colombo et al. 2016). Colombo et al. (2016) did not take the factor depth into account when running their analysis, although data of deep-sea fish were included. Indeed depth, as a proxy for water temperature, may determine variations in the FA composition of marine organisms (Chapters 3 and 4). In the current investigation, we specifically targeted deep-sea organisms belonging to upper- and mid- slope areas to minimize the depth effect and provide new information.

Whereas surface water temperature varies with latitude, bottom-water temperature below 800 m depth is overall constant across latitudes, and stable at 2 – 4°C (Thistle 2003). Finding latitudinal trends in the biochemical composition of deep-

water organisms that mirror results from shallow depths provides further evidence of the link between the two habitats, in that deep-sea benthic communities rely on POM sinking from the surface water as a primary food source (Gage 2003; Hudson et al. 2004).

Close dependence of deep-sea food webs on near-surface processes raises important concerns. According to the latest climate predictions, while both air and water temperatures are rising, and continue to increase; seawater pH has already dropped by 0.1 units due to large CO_2 emissions, and is expected to decrease further (IPCC 2017). A recent study showed how oligotrophic marine waters are expanding due to increasing seawater temperatures and vertical stratification levels (Polovina et al. 2008). Furthermore, Hixson and Arts (2016) showed the FA composition of the six most common phytoplankton species, of both fresh- and salt waters, varied with temperature and, specifically, that their ω3 PUFA levels decreased along with increasing temperature. Not only do ω3 PUFA, such as EPA and DHA, play an important role in the response to temperature variations in water systems, but they are also essential nutrients and are highly required by aquatic organisms for optimal growth and health (Parrish 2009). In addition, in an experimental study, Rossol et al. (2012) showed 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. Our investigation, therefore, suggests that changes in amount and composition of surface-water production could result in changes in the biomarkers on deep-sea benthic organisms that feed on it, with cascading effects throughout deep-water food webs. Indeed, such variations may alter nutrient requirements of deep-sea benthic organisms, as well as trophodynamics; and they may also influence species' abilities to cope with deep cold waters.

Trends in the isotopic and FA composition of deep-sea benthic organisms were not only seen across latitudes, but also among oceanic regions, although without any consistent pattern. In this analysis, for feasibility reasons, only studies carried out within the northern temperate zone were considered. Water temperature may not be the only factor responsible for variability in the biochemical composition of organisms at large scales. Among the other potential drivers are the composition and distribution of the phytoplankton community characterizing the waters above the investigated areas. In fact, primary producers have distinct $\delta^{13}C$ and FA signatures which are reflected in the biochemical composition of consumers (McConnaughey and McRoy 1979; Parrish 2009). In turn, the phytoplankton composition and distribution may be regulated by the amount of nutrients available and their ability to utilize such resources (Litchman et al. 2007); and by the physical oceanographic conditions of a given area (Li 2002; Ramirez-Llodra et al. 2010). In addition, differences in the ability of primary producers to fix CO_2 (Rau et al. 1982) and produce FA *de novo* (Parrish 2013) may explain large scale variations. It seems, therefore, clear that a combination of both abiotic and biotic factors drives variability in the biochemical composition of organisms across oceanic regions. Furthermore, despite initial reports described it as an uniform and desolate place, the deep sea is actually diverse and dynamic, and biogeographic differences have been reported in deep-sea benthic and pelagic communities (Ramirez-Llodra et al. 2010). Such differences are also reflected in the biochemical composition of deep-sea benthic organisms according to our results.

The current investigation was successful in detecting global-scale patterns in the isotopic and essential FA composition of deep-sea benthic species. Importantly, a number of assumptions had to be made (i.e. no variability among tissue types, acidification treatments, and sampling seasons) to generate a sufficiently high number of

data points. On the other hand, the investigations considered in the present analysis were otherwise selected in order to minimize procedural differences across studies and detect large-scale spatial patterns. Although not entirely optimal, this represents the first attempt to draw global trends the stable isotopes and FA composition of deep-water species.

Outlook

This investigation is the first to summarize the information available on deep-sea food webs inferred by bulk stable isotope and FA analyses, providing some guidance for future studies, and to attempt preliminary detection of global-scale patterns in the biochemical composition of deep-water organisms. Food-web tracers represent a powerful tool which can help elucidate the structure and dynamics of food webs from shallow to deeper waters, and help support management initiatives. However, this tool becomes 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. Nonetheless, depending on the scale of the investigation, these differences are more or less susceptible to biases, suggesting that they have to be considered when attempting cross-comparisons but may be contextually acceptable. In this investigation, a preliminary analysis detected latitudinal trends in the isotopic and FA composition of deep-sea benthic species. Furthermore, in light of global climate change and the link between surface-water production and deep-sea benthic communities, changes in the amount and composition of surface-waters production may influence dietary intake, as well as the ability to cope with temperature variations of deep-water organisms. However, there is the need to corroborate the present results with further

investigations. In this regard, more studies are required to help detect global trends, especially in those areas that are still poorly or not yet investigated (e.g. in the southern hemisphere). In addition, it seems advisable to standardize analytical methods to limit and compensate for natural variability.

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Tables

Table 5-1 Comparison outlining the major strengths and drawbacks of gut content, stable isotope, and fatty acid analysis.

Gut content analysis	Stable isotope analysis	Fatty acid 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 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 possible 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 of sex in cases where there are dietary differences between sexes	Partly dependent of sex in cases where there are dietary differences between sexes	Partly dependent of sex in cases where there are dietary differences between sexes
May be sensitive to organism's size (e.g. onthogenetic dietary changes)	May be sensitive to organism's size	Dependent of organism's 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, and the evidence obtained cannot be misinterpreted, taxonomically speaking	Data interpretation is complex (post-analysis mathemathical corrections are often applied)	Data interpretation is complex
Long processing time	Relatively short processing time	Relatively short processing time
Low tech, low cost (unless high resolution scopes are used)	Med tech, med/high cost	Med tech, med/high cost

Table 5-2 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

Table 5-3 List of trophic ecology studies in deep-sea systems, that have been carried out using stable isotopes (bulk) and lipids, including fatty acids, as food-web tracers. Reference, method(s) applied, latitude, sampling depth, ocean region, and taxa analyzed are reported for each study. Polar latitudes include investigations between 60 - 90° N/S, whereas temperate and tropical latitudes represent studies carried out within 0 - 30° N and 30 - 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 organisms
Würzberg et al. 2011a	Lipids	Polar	600-5337	Weddell Sea (Antarctic)	Shelf and deep-sea peracarid crustaceans + foraminiferans
Würzberg et al. 2011b	Lipids	Polar	600-5337	Weddell Sea (Antarctic)	Shelf and deep-sea polychaetes
Würzberg et al. 2011c*	Lipids, Gut content	Polar	600-2150	Weddell Sea (Antarctic)	Demersal fish
Iken et al. 2005	Stable isotopes	Polar	800-2082	High Arctic Canadian Basin	Pelagic, benthic, and sympagic species
Petursdottir et al. 2008*	Stable isotopes, Lipids	Polar	1000-2000	Reykjanes Ridge (North Atlantic)	Mesopelagic shrimps and fish
Bergmann et al. 2009	Stable isotopes	Polar	1300-5600	HAUSGARTEN observatory, west Svalbard (Arctic)	Benthic organisms
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 coral species
Hamoutene et al. 2007*	Lipids	Temperate	50-1500	Cape Chidley, and southern Grand Bank (Northwest Atlantic)	Cold-water corals
Boyle et al. 2012	Stable isotopes, Gut content	Temperate	55-1280	eastern North Pacific	Benthic organisms
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 and pelagic species
Gale et al. 2013	Stable isotopes, Gut content	Temperate	258-1418	Northwest Atlantic	Deep-sea echinoderms
Carlier et al. 2009	Stable isotopes	Temperate	300-1100	Ionian Sea (central Mediterranean)	Species from a cold-water coral bank

Parzanini et al. 2017*	Stable isotopes, Gut content, Morphometrics	Temperate	310-1413	Northwest Atlantic	Pelagic and demersal fish
Parzanini et al. in prep	Lipids	Temperate	310-1413	Northwest Atlantic	Macro- and megafauna
Parzanini et al. under review	Stable isotopes, Lipids, Elemental	Temperate	310-1413	Northwest Atlantic	Benthic organisms
Madurell et al. 2008	Stable isotopes	Temperate	350-780	Balearic Basin (western Mediterranean)	Suprabenthic crustaceans
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 organisms
Trueman et al. 2014*	Stable isotopes	Temperate	500-1500	Porcupine Bank and western continental slope (Northeast Atlantic)	Demersal fish
Kharlamenko et al. 2013	Stable isotopes, Lipids	Temperate	500-1600	Sea of Japan	Megabenthos
Preciado et al. 2017	Stable isotopes, Gut content	Temperate	625-1800	Galicia Bank (Northeast Atlantic)	Benthopelagic species
Fanelli et al. 2009	Stable isotopes	Temperate	650-780	Algerian Basin (western Mediterranean)	Mesopelagic and suprabenthic species
Fanelli et al. 2011*	Stable isotopes, Gut content	Temperate	650-800	Balearic Basin (western Mediterranean)	Zooplankton and micronekton
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)	Holoturians
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 organisms
Reid et al. 2013*	Stable isotopes	Temperate	2404-2718	Mid-Atlantic Ridge (North Atlantic)	Deep-sea fish
Drazen et al. 2008a*	Lipids	Temperate	4100	eastern North Pacific	Ophiuroids and holoturoids
Drazen et al. 2008b*	Lipids	Temperate	4100	eastern North Pacific	Cnidarian, polychaete and crustacean species
Drazen et al. 2008c*	Stable isotopes, Gut content	Temperate	4100	eastern North Pacific	Macrourid fish

Drazen et al. 2009*	Lipids	Temperate	4100	eastern North Pacific	Macrourid fish
Iken et al. 2001*	Stable isotopes	Temperate	4840	Porcupine Abyssal Plain (Northeast Atlantic)	Meio-, macro-, and megbenthos
Lewis, 1967*	Lipids	Tropical	0-4000	Off San Diego and Baja California (eastern Pacific)	Macro- and megafauna
Jeffreys et al. 2009	Stable isotopes, Lipids	Tropical	140-1400	Arabian Sea	Macro- and megabenthos
Churchill et al. 2015*	Stable isotopes, Gut content	Tropical	250-1200	south-central Gulf of Mexico, off Florida to Louisiana (western Atlantic)	Deep water elasmobranchs

*The study was excluded from analyses because it did not meet the various criteria adopted in this investigation. See Section 2.1 Dataset

Figures

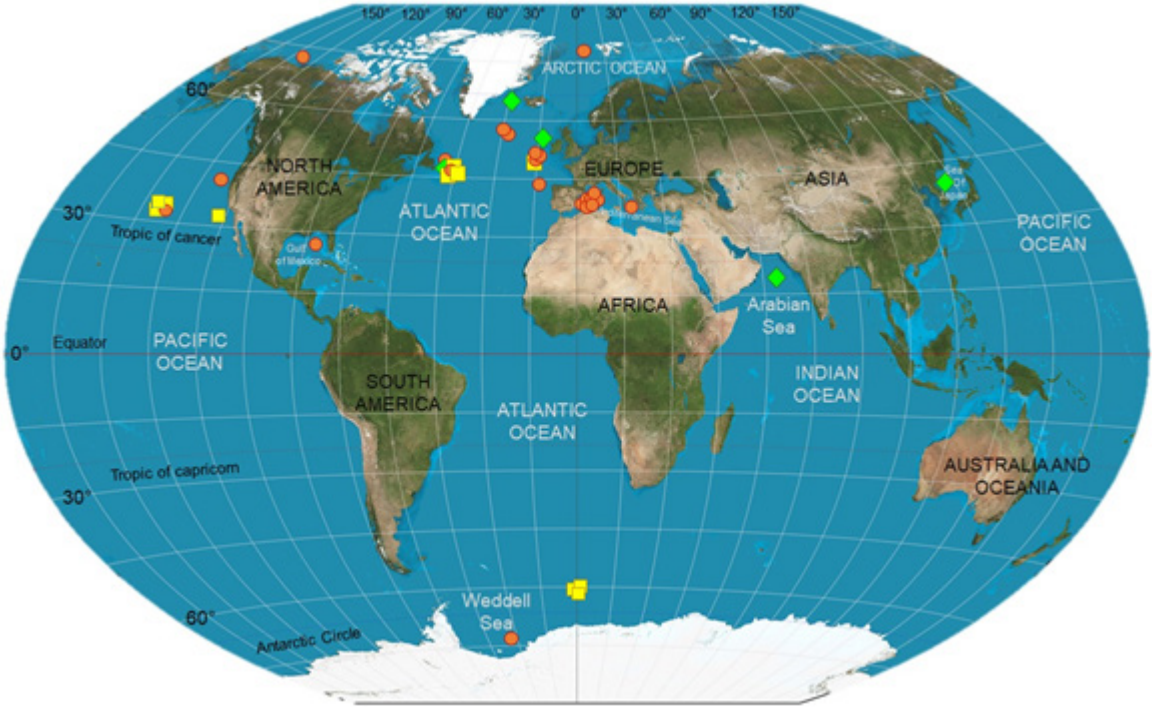

Fig. 5-1 Map of the world ocean. Symbols indicate where the studies listed in Table 5-3 have been carried out. Red circles represent those investigations that have used stable isotopes as food-web tracers; whereas yellow squares and green diamonds indicate those which used lipids and a combination of SIA and FA analysis, respectively.

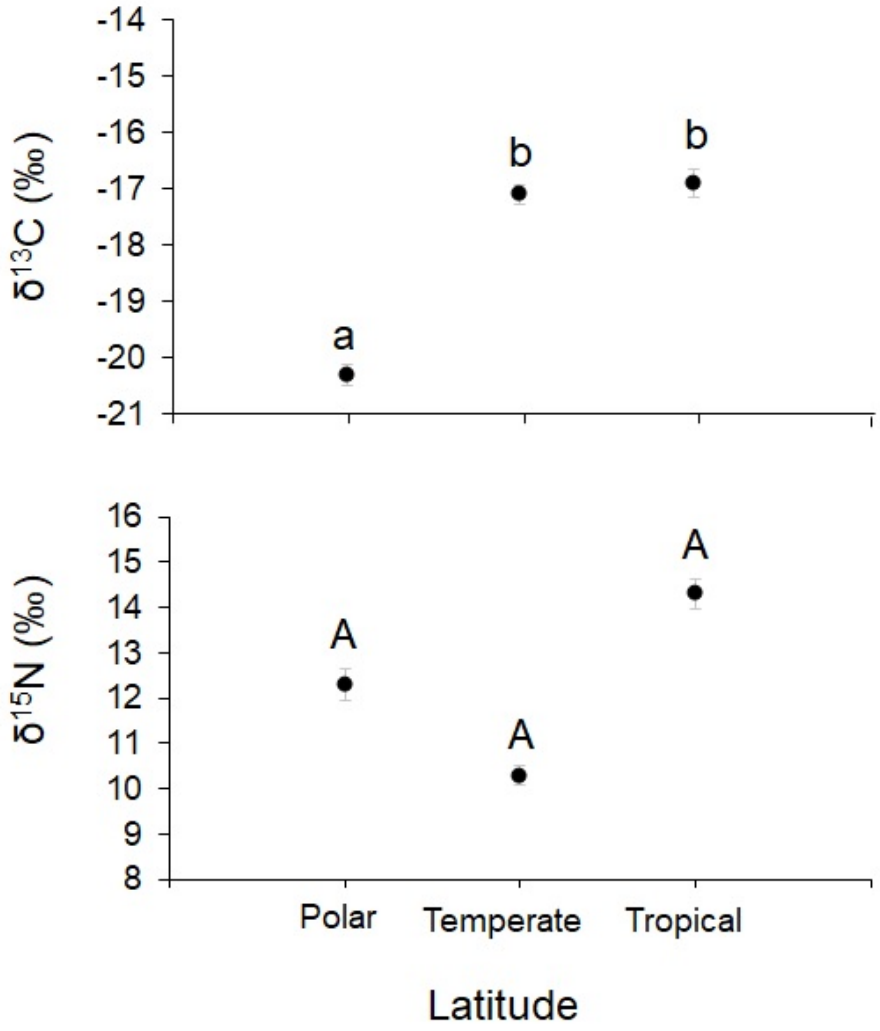

Fig. 5-2 Mean values of $\delta^{15}N$ and $\delta^{13}C$ (‰) across deep-sea benthic organisms collected within polar (90 - 60°N/S), temperate (60 - 30°N/S), and tropical latitudes (30°N - 30°S). Bars represent standard errors (n species = 26 – 199), and letters indicate significant differences ($p < 0.05$) across latitudes.

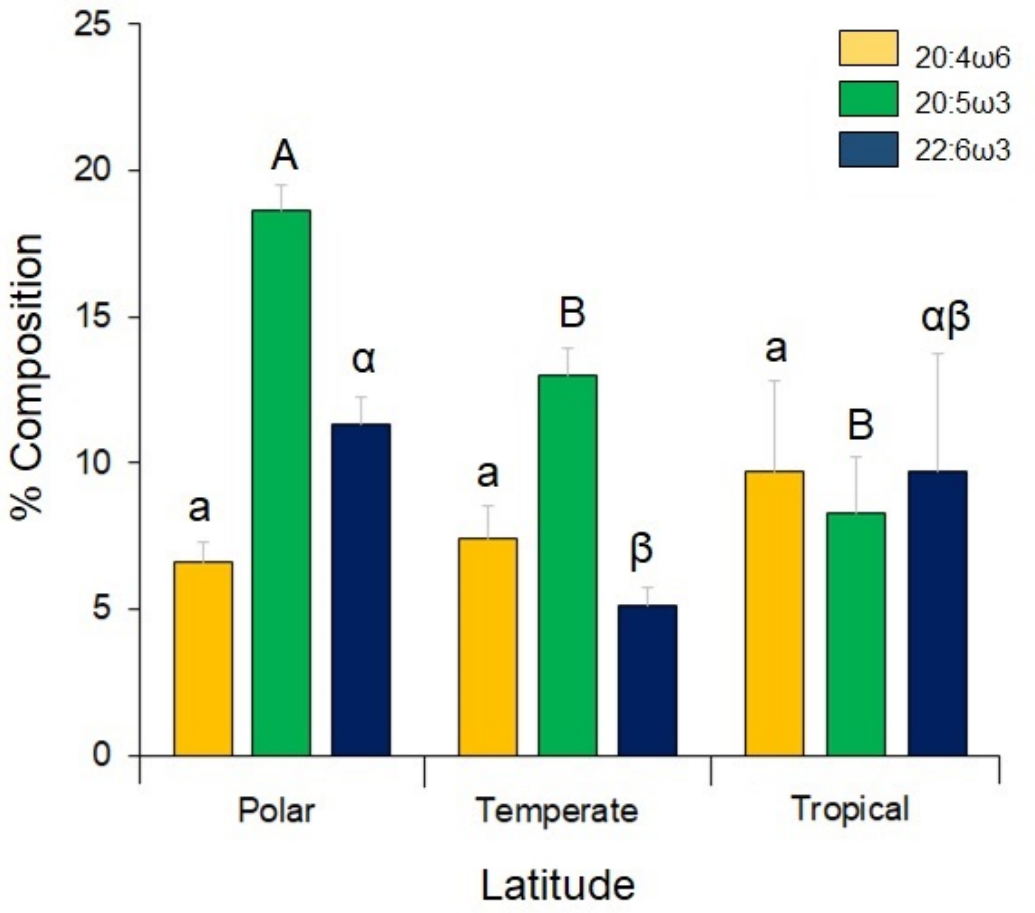

Fig. 5-3 Mean proportions of essential FA recovered from deep-sea benthic organisms collected at polar, temperate, and tropical latitudes. Bars represent standard error (n species = 7 – 67), and letters indicate significant differences ($p < 0.05$) across latitudes.

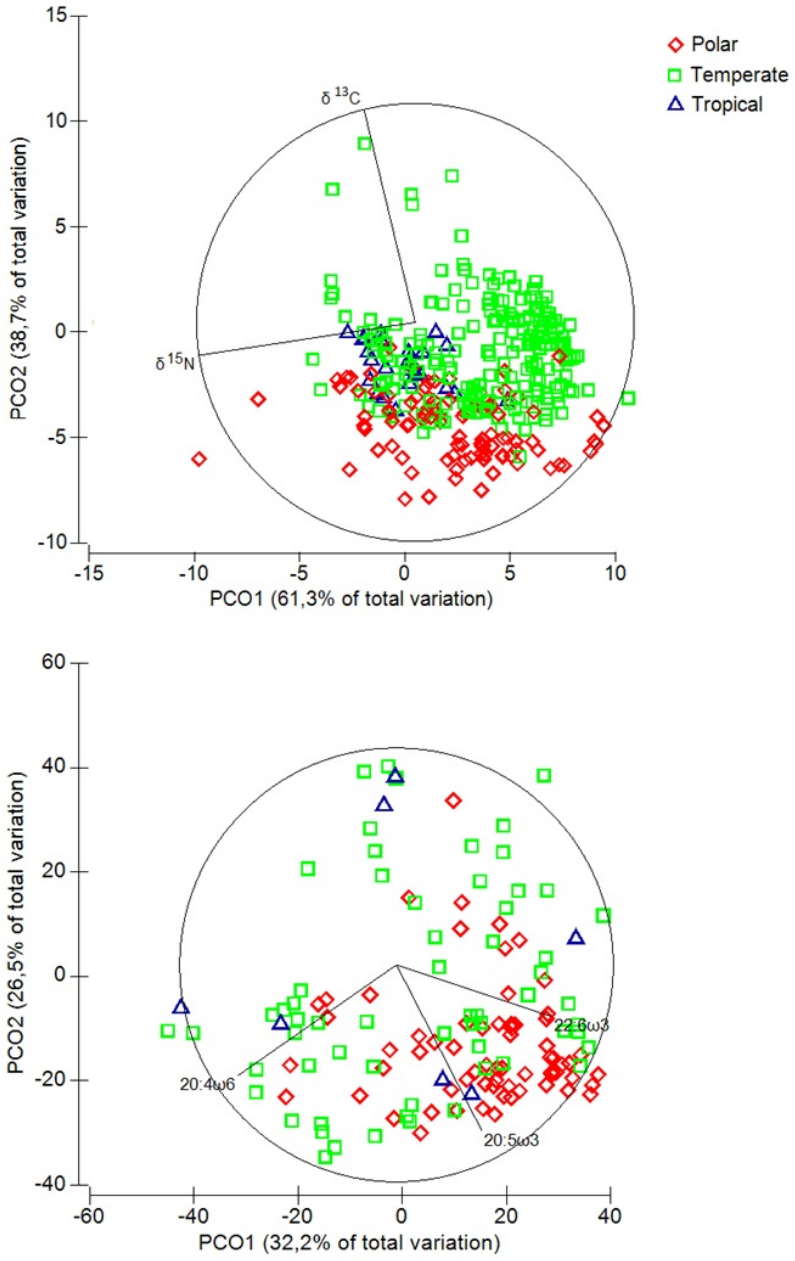

Fig. 5-4 Two dimensional configuration plots obtained from principal coordinate analysis (PCO) representing differences in terms of isotopic ($\delta^{15}N$, $\delta^{13}C$; top) and essential FA (20:4ω6, 20:5ω3, 22:6ω3; bottom) composition across species collected at different latitudes.

Chapter 6 : **General Conclusions**

Summary

The deep sea harbors vast and complex ecosystems that have yet to be fully explored and understood, but are presumably susceptible to a range of global threats such as anthropogenic climate change and fishing activities (Baker et al. 2009; Ramirez-Llodra et al. 2011). This dangerous combination underscores the need to improve knowledge of deep-water communities and processes to support conservation initiatives and ecosystem management plans. In this context, studies of the complex network of feeding interactions (i.e. food webs) represent a valuable tool (Sala and Sugihara 2005; Rombouts et al. 2013). The present thesis combined different methods, including gut content, bulk stable isotope, lipid, elemental, and morphometric analyses, to draw an overall picture of the food web sampled in an area of the Northwest Atlantic that had not yet been studied in these terms. In addition, with this thesis, I provided novel information about deep-water communities and energy and nutrients cycling; as well as suggested vulnerability of certain deep-sea fish species (*Coryphaenoides rupestris* and *Rajella fyllae*) to fishing activities. The key features characterizing this study are the high taxonomic-heterogeneity of the dataset, consisting of 143 deep-sea species from 8 phyla, as well as the narrow spatial and temporal frame (100 km radius, within 7 days in late fall 2013) in which organisms were collected. While most previous food web studies have favored a deeper investigation of few taxa/functional groups, the broad taxonomic approach of the present study aimed for a more comprehensive assessment of trophic interactions within the focal system. In addition, this sampling design was adopted to limit environmentally-driven variations in the biological parameters analyzed.

Overall, the faunal assemblage was composed of species strictly feeding within either the pelagic or benthic compartment, and by mobile predators able to prey within

both realms (Chapters 2 and 4, Appendix 7-1). The latter represented a major link between the two trophic pathways. Such duality was confirmed by gut content and biochemical (i.e. stable isotope ratios and FA biomarkers) analyses, as well as by morphometric data. In particular, species representing the pelagic pathway, such as the mesopelagic fishes *Lampanyctus* sp. and *Scopeloberyx opisthopterus*, were determined to feed directly on zooplankton (e.g. crustaceans and chaetognaths) and/or nekton; they had relatively smaller body sizes and larger mouths; and were characterized by special organs (e.g. lure in *Melanocetus johnsonii* and *Oneirodes macrosteus*; Chapter 2). In addition, deep-sea fish, but also crustaceans, anemones, and corals (e.g. *Acanthephyra pelagica*, Actinauge cristata, and *Anthoptilum grandiflorum*, respectively) had high levels of both algal (e.g. eicosapentaenoic acid EPA, and docosahexaenoic acid DHA) and zooplankton (e.g. 20:1ω9, 22:1ω11(13)) fatty acid (FA) biomarkers, indicative of a pelagic-based diet (Chapter 4, Appendix 7-1). Moreover, organisms representing the pelagic trophic pathway overall had the lowest values of $\delta^{15}N$ and $\delta^{13}C$ ratios, as well as the lowest trophic positions within the food web sampled, indicative of a diet based on ^{15}N-depleted sinking POM (Iken et al. 2001). Conversely, organisms with a benthic-based diet were characterized by higher isotopic ratios and trophic positions, and determined to prey on benthic invertebrates and/or detritus. Specifically, demersal fishes (e.g. *Antimora rostrata* and *Cottunculus thompsonii*) had somewhat larger bodies and smaller mouths than the pelagic counterparts, and preyed upon a wide set of benthic organisms (e.g. bivalves, gastropods, pycnogonids, and sponges; Chapter 2). In addition, benthic organisms of higher-trophic levels, such as the sea stars *Mediaster bairdi* and *Psilaster andromeda*, had large proportions of arachidonic acid, ARA (Chapter 4), a FA marker typical of microorganisms from the sediment (Howell et al. 2003).

Vertically migrating organisms, such as the lantern fish *Lampanyctus* sp. and the decapod *A. pelagica*, together with active demersal predators feeding within both the pelagic and the benthic realms (e.g. *Sebastes mentella* and *Antimora rostrata*) played a key role in connecting the two major trophic pathways, by providing energy (e.g. saturated FA), and organic matter to the benthic organisms (Chapter 2 and 3). In conclusion, by combining different techniques to depict the food web sampled, I was able to i) verify that dietary, biochemical, and morphological variations of species were reflective of feeding habits and habitat (Chapters 2, 3, and 4, and Appendix 7-1); ii) recognize the presence of two major trophic pathways within the demersal faunal assemblage analyzed (Chapters 2 and 4), which was in line with results from other geographic regions (Iken et al. 2001; Trueman et al. 2014); iii) observe that greater food inputs within the benthic realm favoured a higher growth investment for the demersal fish species, whereas pelagic fishes were most likely adapted to live in a food-limited environment, such as the meso- and bathypelagic zones (Chapter 1); and, lastly, iv) provide evidence of the bioaccumulation of energy (e.g. 20:1ω11, 20:1ω9, and 22:1ω7) and essential nutrients (i.e. ARA) within deep-sea benthic organisms (Chapter 4; Appendix 7-1).

Since organisms were collected along a depth gradient (310 – 1413 m), I carried out a preliminary analysis to test the effect of depth on biochemical ($\delta^{15}N$, $\delta^{13}C$, lipid content, lipid classes and FA composition, as well as elemental N and C) and morphological parameters (total length and wet mass; Chapters 2,3, and 4). Such an analysis was necessary to avoid biases in data interpretation, as depth-related trends of these metrics have been detected in previous studies (Lewis 1967; Polunin et al. 2001; Bergmann et al. 2009; Mindel et al. 2016). Not only did the findings enable a better understanding of trophic structure and dynamics within the faunal assemblage sampled

(Chapters 2 and 4), but they also highlighted presumed adaptive strategies and behaviors with depth (Chapters 3 and 4). Among the most interesting results, bathymetric trends were observed in the isotopic composition of deep-sea fishes only when pelagic and demersal fishes were studied separately (Chapter 2), thus emphasizing the importance of considering feeding habits and trophic positions when interpreting data. Moreover, variations along the same depth gradient were also encountered in the lipid content and composition of the deep-sea macrofaunal assemblage analyzed in Chapter 3, inclusive of vertebrate and invertebrate taxa. In particular, depth significantly correlated with the lipid class, sterols; the phospholipid to sterol ratio, an indicator of membrane fluidity (Pernet et al. 2006; Parent et al. 2008); and the sum of monounsaturated FA, most likely representing an adaptive strategy of organisms to maintain normal cell membrane activities in deeper waters (Chapter 3). Lastly, proportions of ARA and oleic acid, 18:1ω9, from the FA composition of benthic organisms were determined to increase, whereas 22:1ω7 decreased along the bathymetric gradient (Chapter 4). These findings suggest a higher reliance on the benthic trophic pathway, together with increasing carnivory/omnivory behaviors of species at greater depths, presumably as a reflection of the decreasing amounts of OM and energy reaching the seafloor (Buesseler et al. 2007).

Apart from bathymetric gradients, large-scale latitudinal trends were found in the isotopic and FA composition of deep-sea benthic taxa (Chapter 5). In fact, deep-sea benthic organisms from upper- and mid-slope areas displayed lower $\delta^{13}C$ ratios and larger proportions of essential nutrients (i.e. EPA, DHA, and ARA) at higher latitudes, relative to tropical latitudes. Furthermore, Chapter 5 attempted to detect such large-scale trends and draw preliminary global conclusions by exploring published studies on deep-sea food webs carried out with bulk stable isotope and FA analyses, thus putting the

current investigation in a more comprehensive context. Indeed, this review chapter provided further evidence of the relationship between surface-water production and deep-water benthic communities; and it pointed out the potential repercussions of global warming and ocean acidification on quantity and quality of dietary intake, and abilities of deep water organisms to cope with temperature shifts. Thus, the study of food webs and trophic relationships is crucial to make large-scale predictions and support management plans involving the deep sea.

Future directions

The various techniques applied in this project have enabled me to obtain an overall picture of diet and feeding habits of the focal organisms. However, due to both logistical and methodological limitations, it was not always possible to obtain clear and species-specific dietary data. For example, highly digested prey items were often retrieved from fish gut contents (Chapter 2), thus preventing their identification to the species-level. Moreover, as already amply discussed in Chapters 2 and 4, the lack of specificity of biomarkers means that they only provided a broad indirect idea of the diet. In this regard, DNA and compound isotope specific (on either fatty acids or amino acids) analyses may allow for a more rigorous identification of the prey items, hence depiction of the diet, as well as a more accurate assessment of trophic position, thus leading to a finer understanding of trophic interactions. Furthermore, combining the current data with *in-situ* observation by Remote Operated Vehicles (ROVs) and laboratory feeding trials, would provide considerable support in assessing the trophic ecology of these species. In fact, not only would both approaches furnish further evidence of what species feed on, but they would also allow the direct observation of their feeding behaviors. The application of models (e.g. Bayesian mixing models, Ellipsed based-metrics) would also

represent a next step for future studies, as they would allow the study of the relative importance of the various dietary components and of trophic niches in a quantitative manner; as well as prediction of future scenarios.

The organisms in this study were collected over a period of 7 days in late fall, thus representing a snapshot of that specific time of the year. As seasonal variations may occur in the food supply from the surface water, as well as in other abiotic and biotic processes (e.g. stratification regimes, inter-seasonal changes in biological communities and diet, migrations), it would be compelling to perform a longer-term study (e.g. over several years) to either confirm the results of the current investigation or corroborate them with further data. Indeed, given the opportunistic nature of this study, together with the rare occurrence of certain deep-water organisms (e.g. Chapter 2), several species analyzed were poorly represented (i.e. < 3 replicates). While the combination of several analytical techniques, in addition to literature research, increased the reliability of our results, a multi-season/year approach would help consolidate them.

Chapter 3 presented an overall analysis of lipid content, lipid class, and FA composition of the deep-water organisms collected on shelf and slope areas off Newfoundland. While this study provided an extensive dataset and baseline information on organisms which have been poorly or never studied before, its magnitude, together with the broad taxonomic range represented, prevented a more in depth-analysis of lipid content and composition of these species. For this reason, future research on specific taxa and on several tissue types should be carried out to clarify their biochemical composition. In addition, taxa could be subdivided into functional groups before running correlations with depth.

Finally, since the organisms analyzed were representative of a demersal food web, it would be valuable to study and incorporate data from the pelagic counterpart to

gain a more comprehensive overview of the trophic connections existing within the area, and especially of the link between the benthic and pelagic compartments. This information would be particularly useful in the design and application of ecosystem-based management strategies. In addition, it would help enable prediction of potential effects of increasing water temperature and decreasing pH due to global climate change.

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Chapter 7 : **Appendices**

Appendix 7-1

Neutral and polar lipid fatty acids in five families of demersal and pelagic fish from the deep Northwest Atlantic

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Abstract

Neutral and polar lipid fatty acids were used to investigate trophic connections in species from five families of demersal (*Rajella fyllae*, *Malacoraja senta*, *Alepocephalus bairdii*, *Borostomias antarcticus*) and pelagic fish (*Bathytroctes macrolepis*, *Lampanyctus* sp., *Chaulidos sloani*, *Serrivomer beanii*) sampled in the deep Atlantic Ocean off Newfoundland, Canada. Lipid extracts were fractionated into neutral, acetone-mobile polar, and phospholipids to separate fatty acids in storage from those in membranes. Multivariate analysis of fatty acids showed that there were greater differences among the three lipid fractions than there were among the species when all fatty acid fractions were considered together. Neutral lipid fatty acids were characterized by monoenes, acetone-mobile polar lipids by C_{18} polyenes, and

phospholipids by 16:0 and docosahexaenoic acid (DHA, 22:6ω3). Multivariate analysis of fatty acids in phospholipids showed a strong grouping by taxonomic family (>80% similarity), while the neutral lipid fatty acids showed a weaker grouping by family (72.5% similarity) but groupings that also related to habitat and vertical migration. The neutral lipid data support the use of 20:1ω9 as a biomarker of calanoid copepods and of 16:1ω7 as a marker of diatoms to determine food web connections in deep-sea fish, but not some other common markers (e.g. DHA/EPA and 18:1ω9/18:1ω7 ratios). In addition, correlations with $\delta^{15}N$ showed that series of ω6 and ω7 fatty acids are trophically transferred though neutral lipids, especially the essential fatty acid, arachidonic acid (ARA, 20:4ω6).

Introduction

The deep sea is the largest ecosystem on Earth, covering about 65% of its surface, but it is still poorly understood (Herring 2002; Thistle 2003; Danovaro et al. 2014). Deep-sea ecosystems include waters and sediments beyond the shelf break, generally from 130-200 m down to about 11,000 m (Snelgrove 1999; Duarte, 2006; Watling et al. 2013). Only 5% of the deep sea has been explored and less than 0.01% of the deep-sea floor has been scientifically investigated (Ramirez-Llodra et al. 2011). Nevertheless, it is recognized that the deep sea supports one of the highest levels of biodiversity on Earth (Etter and Mullineaux 2001, Grassle and Macioleck, 1992; Snelgrove and Smith, 2002; Stuart et al., 2003), as well as important biological and mineral resources (Baker and German 2009; UNEP 2007).

Fatty acids in consumer tissues can provide diet information (Sargent and Whittle 1981; Sargent et al. 1987; Graeve et al. 1994b; Stübing and Wilhelm 2003) and certain ones have been successfully used as trophic markers to track energy transfer, as well as to study predator-prey relationships (Falk-Peterson et al. 1990, 2002, 2004; Dalsgaard et al. 2003; Budge et al. 2006; Litzow et al. 2006). Fatty acids are distributed among different lipid classes representing an essential and integral part of these compounds. Lipid classes can be broadly divided into neutral and polar lipids. In turn, the latter can be divided into phospholipids and acetone-mobile polar lipids (AMPL). Neutral lipids predominantly consist of storage triacylglycerols and wax esters while polar lipids mainly comprise membrane glycolipids and phospholipids. Neutral and polar lipids have different functions and structures in different species (Roessler 1990). One major role of polar lipids is to provide the basic matrix of the cellular membranes into which cholesterol, proteins, and other membrane constituents are embedded (Spector and Yorek 1985; Stubbs and Smith 1990; Cook 1996; Vance 1996). Furthermore, the dual structural and functional role of polar lipids limits the type of fatty acids that are incorporated (Vaskovsky 1989). These particular

fatty acids provide special conformational properties to the biomembranes and assist tissue specific cells in reacting to external stimuli such as changing environmental conditions (Sargent et al. 1993; Cook 1996). In contrast, the neutral lipid content and the constituent fatty acids are related to the physiological status of the organism. An organism experiencing a dietary surplus of energy may accumulate lipids directly, in which case the fatty acid composition is similar to the diet (Sargent and Whittle 1981).

This study describes and compares fatty acid compositions in the neutral, acetone-mobile polar, and phospholipid fractions of muscle of some demersal and pelagic fish species in five families from the north-west Atlantic Ocean. The aim was to investigate trophic interactions through fatty acid analyses of lipid fractions of different polarities in fish tissues. This is the first study to examine the trophic transfer of fatty acids in all three of these fractions in marine organisms.

Materials and methods

Sampling area and samples

The sampling area was located within the NAFO Division 3K, in the Northwest Atlantic Ocean off Newfoundland, Canada. Samples were collected by the CCGS *Teleost* research vessel during routine multi-species surveys conducted by the Canadian Department of Fisheries and Oceans. All specimens were sampled in a 100 km radius between 750-1370 m, over two days in December 2013. Four demersal and four pelagic fish species were analyzed. The demersal species were *Rajella fyllae* (n=3) and *Malacoraja senta* (n=1) of the family Rajidae, *Alepocephalus bairdii* (n=1) of the Alopecephalidae, *Borostomias antarcticus* (n=3) of the Stomiidae, and the pelagic ones were *Lampanyctus* sp. (n=2) of the Myctophidae, *Bathytroctes macrolepis* (n=2) of the Alpoecephalidae, *Chaulidos sloani* (n=4) of the Stomiidae, and *Serrivomer beanii* (n=3) of the Serrivomeridae. The identification of species as either demersal or pelagic was based on information provided by FishBase (Froese and Pauly 2017). White muscle tissue (0.5-1.0 g) was taken from close to the dorsal

fin in each fish. Then each muscle sample was placed in lipid clean vials, where 4 ml of chloroform was added to preserve tissue samples. Each muscle sample was then sealed under N_2, and stored in a freezer (-20°C) until lipid extraction.

Lipid extraction

Lipids were extracted in chloroform:methanol:water (1:2:1) following Folch et al. (1957), as modified by Parrish (1999). Tissues were homogenized in chloroform:methanol (2:1) and chloroform extracted water (1:2). Briefly, samples were ground, sonicated (4 min) and centrifuged (3000 rpm) in the chloroform:methanol:water mixture three times. Lipid layers were removed using a double pipetting technique and pooled following each of the three chloroform washes. Total lipid extracts were then concentrated down to volume under a gentle stream of nitrogen (N_2), sealed with Teflon tape and stored in the freezer (-20°C) until further analyses.

Column chromatography

Neutral lipid, AMPL and phospholipid fractions were separated by passing each extract through 2 ml glass pipettes containing 0.8 g silica gel which had been heated in a muffle furnace overnight (450°C). The silicic acid was poured onto glass wool at the bottom of each pipette. The pipettes were heated at 100°C for 1 h to activate the silicic acid. The pipettes were cooled at least 30 min before column chromatography was started.

Neutral lipids were eluted with 8 ml chloroform:methanol:formic acid (98:1:0.5 v/v). AMPL with 6 ml (2 x 3 ml) acetone (100%), and phospholipids with 2 bed volumes (6 ml) of methanol followed by 9 ml chloroform:methanol:chloroform extracted water (5:4:1 v/v) into 15 ml vials(Parrish et al., 1996). The eluents were concentrated under N_2.

Derivatization and analysis of fatty acids

Fatty acid methyl esters (FAME) were prepared by transesterification with 5% HCl in methanol (modified from Sato and Murata, 1988). 800 µL of sample was transferred to sample vials. 2.5 ml HCl-MeOH was added and the mixture heated for 2.5 h at 85°C. Then 1.5 ml hexane was added and the upper phase removed. The samples were dried under N_2. The products were then extracted into hexane and stored at -20°C for FAME analyses.

The FAME were analysed on a HP 6890 GC FID equipped with a 7683 autosampler. The GC column was a ZB wax+ (Phenomenex, USA). The column length was 30 m with an internal diameter of 0.32 mm. The column temperature began at 65°C where it was held for 0.5 min. The temperature ramped to 195°C at a rate of 40°C/min, where it was held for 15 min and then it was ramped to a final temperature of 220°C at a rate of 2°C/min. This final temperature was held for 0.75 min. The carrier gas was hydrogen flowing at 2 ml/min. The injector temperature started at 150°C and ramped to a final temperature of 250°C at a rate of 120°C/min. The detector temperature then stayed constant at 260°C. Peaks were identified using retention times from standards purchased from Supelco: 37 component FAME mix (Product number 47885-U), PUFA 1 (product number 47033) and PUFA 3 (product number 47085-U). Fatty acid peaks were integrated using Galaxie chromatography software (version 1.9.3.20).

Determination of lipid classes

Lipid classes were determined using thin-layer chromatography with flame ionization detection (TLC-FID) with a MARK V Iatroscan (Parrish, 1987). Extracted samples were spotted on silica gel-coated Chromarods and a three-stage development system was used to separate lipid classes into neutral lipid, acetone-mobile polar lipid and phospholipid classes. The first system consisted of two developments in hexane:diethyl ether:formic acid (98.95:1:0.05 by vol.). The first development was for 25 min followed by a second of 20 min, and the rods were then scanned in the

Iatroscan to behind the ketone peak. The second system consisted of a 40 min development in hexane:diethyl ether:formic acid (79.9:20:0.1 by vol.), and the rods were scanned to behind the diacylglycerol peak. The last system consisted of two 15 min developments in 100% acetone followed by two 10 min ones in chloroform: methanol: water (5:4:1 by vol.). The rods were then scanned in the Iatroscan to quantify the polar lipids. Scan data were collected using Peak Simple software (ver 3.67, SRI Inc). Peak areas were quantified using calibration curves obtained from lipid standards (Sigma Chemical Inc.). Total lipid concentration was determined as mg/g wet weight (WW).

Statistical analysis

Differences among data obtained from lipid classes and fatty acids in neutral, acetone-mobile polar, and phospholipids were tested using the PERMANOVA add-on to PRIMER v6. Principal coordinates analysis (PCO) was conducted to visualize the data set and permutational MANOVA was used to examine average similarity between/within groups and to test for significant differences among groups.

Results

Lipid classes

Lipid classes were identified in each of the column chromatography fractions in 19 fish specimens belonging to 4 demersal species (*R. fyllae, M. senta*, *A. bairdii*, *B. antarcticus*) and 4 pelagic fish species (*B. macrolepis*, *Lampanyctus* sp., *C. sloani*, *S. beanii*) across 5 families. Tables A1 and A2 show total amounts recovered in the neutral, acetone-mobile polar, and phospholipid fractions (NL, AMPL, PL). The major lipid classes in the three fractions of the demersal and pelagic fish species were steryl ester/wax ester, triacylglycerol, acetone-mobile polar lipid, and phospholipid. While Iatroscan-determined neutral lipids were the major contributors in the NL fraction and phospholipids in the PL fraction, there were significant levels of both neutral and phospholipids in the AMPL fraction. This is because acetone-mobile polar

lipids represented only 2-5% of the total lipids in the unseparated fish sample extracts. Nonetheless, in all species except *B. antarcticus*, comparatively more sample AMPL appeared in this minor fraction than did sample neutral acyl lipids or phospholipids. Despite the presence of the other sources of fatty acids, there were significant differences in the fatty acid profiles in the AMPL fraction compared to the other fractions (see below).

Fatty acids

Up to 67 fatty acids were identified in each sample. Appendix Tables 3 and 4 show the fatty acid composition of the demersal and pelagic fish species, in their neutral, acetone-mobile polar, and phospholipid fractions. The fatty acid composition of all deep-sea demersal and pelagic fish species analysed comprised polyunsaturated fatty acids (PUFA), mainly eicosapentaenoic acid (EPA), DHA, monounsaturated fatty acids (MUFA), mainly 20:1ω9, 22:1ω11(13), and 18:1ω9 and the saturated fatty acid 16:0.

In this investigation, it was determined that both the demersal and pelagic fishes tended to have high levels of PUFA (on average 42% across all lipid fractions) and a lower content of MUFA (on average 34%). Saturates averaged 23%. The PUFA 18:2ω6 and 18:4ω3 were always highest in AMPL, while DHA proportions were always highest in polar lipids, and the monoene 20:1ω9 was always highest in the neutral lipids of all taxa (Appendix Tables 3 and 4).

All fatty acids were used in the multivariate analyses of the 111 samples. In PERMANOVA analyses of the entire fatty acid data set factored by species, lipid class (NL, PL, AMPL), or habitat (demersal or pelagic: FishBase), lipid class gave the highest Pseudo-F value (20.7) and the lowest P(perm) value (0.001). Pair-wise tests revealed that the fatty acid distributions in all lipid classes were significantly different from each other: P(perm) = 0.001

Figure 1 shows a two-dimensional configuration plot of a PCO analysis of total fatty acid data. Phospholipids were characterized by 22:6ω3 (DHA) and 16:0, neutral lipids by monoenes, and AMPL by a variety of fatty acids (Fig. 1). Because of the distinct separation of fatty acid profiles when samples were factored by lipid classes, subsequent PCO analyses of species (*R. fyllae*, *B. antarcticus*, *B. macrolepis*, *C. sloani*, *S. beanii*, *Lampanyctus* sp., *M. senta*, *A. bairdii*) were performed on neutral lipid and phospholipid fatty acids separately.

Figure 2 shows a two-dimensional configuration plot of a PCO analysis of fatty acids in neutral lipids. *R. flyllae* from the Rajidae family and *S. beanii* from the Serrivomeridae family were located together in the same area with 72.5% similarity. The two *Lampanyctus* sp. samples from the Myctophidae family are close. Also, *B. antarcticus* and *C. sloani* from the Stomidae family are in the same area with 72.5% similarity. Figure 2 shows that different species from the same family group together: *Rajella fyllae* and *Malacoraja senta* of the family Rajidae, *Alepocephalus bairdii* and *Bathytroctes macrolepis* of the Alepocephalidae, and *Borostomias antarcticus* and *Chaulidos sloani* of the Stomiidae. Families that group together, however, do not all come from the same habitat, although the group to the right consists mainly of demersal species and that to the left consists mainly of pelagic species.

Figure 3 shows a two-dimensional configuration plot of a PCO analysis of a resemblance matrix of fatty acids in phospholipids. As in the neutral lipids, some members of the same family group together but with an even higher level of similarity. *C. sloani* and *B. antarcticus* from the Stomiidae family group in the same area with 87.4% similarity. Likewise, the skates *R. flyllae* and *M. senta* from the Rajidae family group closely together with 91.5% similarity. *A. bairdii* and *B. macrolepis* of the Alepoecephalidae do not group together at this level but do so at 81.26% similarity. Again, families that group together, generally do not come from the same habitat, although the group to the left consists solely of pelagic species.

After removing fatty acids representing <1% of total, the six fatty acids showing the greatest difference across all samples were 16:0, 18:1ω9, 20:1ω9, 22:1ω11(13), 22:5ω3 and DHA. Figure 4 shows the top five fatty acids with the greatest differences between the highest and lowest values among groups, along with another essential fatty acid, 20:5ω3 (EPA). Species were grouped together in Figures 4 and 5 and in Appendix Tables 3 and 4 on the basis of the PCO analyses (Fig. 3 and especially Fig. 2). Where there were single representatives of a fish species they were grouped together with other samples of the same family on the basis of their close proximity in Figure 2. Then the groups were separated so that the group to left comprises the Myctophidae and Stomiidae and that to the right the Alepocephalidae, the Rajidae and the Serrivomeridae. The group to the left comprises mainly pelagic species and mainly vertical migrators and the group to the right comprises mainly demersal species and mainly non-migrators. The group to the left has more significant differences among the lipid fractions and is characterized by higher proportions of 20:1ω9 and 22:1ω11(13) in the neutral lipids. The group to the right has higher proportions of 16:0, EPA and DHA in the neutral lipids, and slightly more 20:1ω9 in the phospholipids.

Out of eight summary data of fatty acid ratios and groupings (Tables A3 and A4), ∑SAT, ∑MUFA, ∑PUFA, ∑ω3, ∑zooplankton fatty acids and the 18:1ω9/18:1ω7 ratio showed the greatest difference between highest and lowest values. On the other hand, 16:1ω7/16:0 and DHA/EPA showed less variability. Figure 5 illustrates the four with the greatest difference between the highest and lowest values as well along with the 18:1ω9/18:1ω7 and 16:1ω7/16:0 ratios. The group on the left comprising the families Myctophidae and Stomiidae has many more significant differences than the slope community to the right and was characterized by higher levels of MUFA generally and zooplankton fatty acids specifically. They had neutral lipids with the highest proportions of MUFA across all lipid fractions in all taxa (Fig. 5), especially

those with 20 or 22 carbons (Fig. 5) such as 20:1ω9 and 22:1ω11(13) (Fig. 4). In addition the ratio 16:1ω7/16:0 is higher in neutral and acetone-mobile polar lipids on the left than on the right. The slope community to the right is characterized by higher levels of PUFA in neutral lipids generally and ω3 PUFA specifically.

Individual neutral lipid fatty acids and summary data (Tables A3 and A4) were also correlated with $\delta^{15}N$ measured in the same white muscle samples in a companion study (Parzanini et al. 2017). The monoenes 18:1ω7, 20:1ω7, 22:1ω7 had Pearson correlations ranging from 0.767 to 0.831 with P values ranging between 0.011 and 0.026, and with 18:1ω7 showing the most significant correlation. The polyenes 18:2ω6, 20:4ω6, 22:4ω6 and 22:5ω6 had Pearson correlations ranging from 0.736 to 0.868 with P values ranging between 0.005 and 0.037, and with 20:4ω6 showing the most significant correlation. None of the summary data of fatty acid ratios and groupings correlated significantly with $\delta^{15}N$.

Discussion

Fatty acids in demersal and pelagic fish of the deep Northwest Atlantic

In this study, fatty acid proportions were shown to change among the taxa and among the different lipid fractions. However, the fatty acids identified were generally the same. Only a limited number of fatty acids were not represented in certain fractions and/or fish species. For example, 19:0 was not present in any lipid fraction of *B. macrolepis*, whereas 21:0 was not detected in the phospholipid fraction of *B. macrolepis*, *Lampanyctus* sp., and *S. beanii*, and 23:0 was not present in the neutral lipid fraction *B. antarcticus*. However, when present amounts differed among species and fractions. The presence/absence of these particular saturated fatty acids is notable because they are often used as internal standards in fatty acid analyses (e.g. Parrish et al. 2015).

The lantern fishes in family Myctophidae and the ray-finned fishes in family Stomiidae were characterized by having neutral lipids with the highest proportions of

monoenoic 20:1ω9 and 22:1ω11(13) which are markers for copepods of the genus *Calanus* (Dalsgaard et al. 2003). Myctophids are opportunistic predators on primary consumers like copepods providing an ecological link to top predators (Catul et al. 2011). Thus the stomiids may have acquired the copepod markers from feeding on vertically migrating myctophids as well as on zooplankton (Sutton and Hopkins 1996).

The distribution of fatty acids among lipid fractions

Fish neutral lipid fatty acids were most strongly associated with monoenoic fatty acids, especially those generally associated with zooplankton. There are very few phospholipid molecular species containing long-chain monoenes in fish (Boselli et al. 2012) probably because their length hinders their accommodation in membranes (Sargent 1995). The herbivorous calanoid marker 20:1ω9 had the highest Pearson correlation at 0.963 in the PCO ordination (Fig. 1) and is strongly associated with the neutral lipid fatty acids. This monoene was always highest in the neutral lipids of all taxa, suggesting it is an excellent biomarker. Phospholipids showed the tightest grouping in Figure 1 and they were characterized by DHA and 16:0. This combination of fatty acids occurs in all phospholipid molecular species of fish and shellfish, and usually in abundance (Boselli et al. 2012).

Proportions of DHA were always highest in polar lipids, usually phospholipids, and EPA was usually highest in phospholipids too. This suggests some caution is required in using the DHA/EPA ratio as a trophic biomarker in these fish taxa, especially in unfractionated lipids. Previously, DHA/EPA correlated strongly with $\delta^{15}N$ trophic position in copepods when 4 species were pooled (El-Sabaawi et al. 2009). In addition, the DHA/EPA ratio reflects the relative proportions of dinoflagellates to diatoms in the diets of herbivorous and omnivorous copepods, because dinoflagellates are rich in DHA, whereas diatoms are rich in EPA (Viso and Marty 1993). Here the DHA/EPA ratio in the neutral lipids did not correlate significantly with $\delta^{15}N$ measured in the same samples during a companion study (Parzanini et al.

2017), but interestingly series of ω6 and ω7 fatty acids did. The polyenes 18:2ω6, 20:4ω6, 22:4ω6 and 22:5ω6 and the monoenes 18:1ω7, 20:1ω7, 22:1ω7 all had significant ($P<0.04$) positive Pearson correlations ($r>0.73$). Increases in proportions of these fatty acids with trophic position not only suggests their importance in this assemblage but also conversions among fatty acids of the same family. Importantly, the highest correlation with $\delta^{15}N$ was with the essential fatty acid 20:4ω6. The essential fatty acids EPA and DHA are often quantitatively dominant, but the ω6 long-chain PUFA 20:4ω6, which is commonly found in lipid extracts from aquatic food webs, is also nutritionally important for fish (Bell et al. 2003).

The fact that DHA was sometimes highest in AMPL is interesting and validates the isolation of a separate AMPL fraction. However, this together with the fact that EPA was sometimes highest in the neutral lipid fraction suggests some caution is also required in examining the DHA/EPA ratio in unfractionated lipids in terms of membrane biochemistry. EPA and DHA differentially influence membrane lipid dynamics and structural organization (Mason et al., 2016).

The PUFA 18:2ω6 and the dinoflagellate marker 18:4ω3 (Mansour et al. 1999) were always highest in AMPL fractions in the fish taxa, again validating the isolation of a separate AMPL fraction. The term “acetone-mobile polar lipids” was coined for a heterogeneous class of compounds that could be separated by Chromarod thin-layer chromatography (Parrish 1987). The use of an acetone development permits quantification of glycolipids and pigments and reduces contamination of phospholipids. The same principle was applied here in order to reduce the presence of non-phospholipid fatty acids in the phospholipid fraction which in the case of deep-sea fish were C_{18} PUFA.

The ratios 18:1ω9/18:1ω7 and 16:1ω7/16:0 are commonly used trophic markers (Dalsgaard et al., 2003) but the denominator was not consistently highest in the neutral lipid fraction in these fish from the deep north-west Atlantic. The monoene

18:1ω9 was always highest in neutral lipids but 18:1ω7 was not, half the time the latter was highest in phospholipids. As with the DHA/EPA ratio, 18:1ω9/18:1ω7 correlated strongly with $\delta^{15}N$ trophic position in copepods when 4 species were pooled (El-Sabaawi et al., 2009). Here again there was no significant correlation between 18:1ω9/18:1ω7 in the neutral lipids and $\delta^{15}N$ in fish from the deep north-west Atlantic

The microalgae marker 16:1ω7 was also always highest in NL but 16:0 was not, instead it was usually highest in PL. Nonetheless, in all but one fish species, 16:1ω7/16:0 was highest in the neutral lipid fraction and lowest in the phospholipid one. 16:1ω7/16:0 has been used to infer a dominant diatom versus dinoflagellate food web base (Auel et al., 2002). While 16:1ω7 is found in cyanobacteria, dinoflagellates, and a specific isomer, the *trans* one, is found in bacteria, 16:1ω7 is most prevalently associated with diatoms (Sargent et al., 1987).

Neutral lipid fatty acids in different species

Triacylglycerol was one of the major components of the neutral lipid classes in all species under study, indicating it was the major form of energy storage in these taxa. The monoenoic fatty acids were most strongly associated with the neutral lipids, contributing on average 50%, with the highest amount (67%) occurring in the neutral lipids of *C. sloani*. The monene 16:1ω7 and its chain elongation product 20:1ω7 were always highest in the neutral lipids.

PCO analysis of fatty acids in neutral lipids (Fig. 2) showed that different fish species from the same family grouped together, but that families that grouped together did not always come from the same habitat; although the group to the left consists mainly of pelagic representatives. They did, however, tend to group according to whether their migration behaviours. Thus one group was composed mainly of known diel vertical migrators while most of the slope fish community members to the right are not known to vertically migrate. Lantern fish, including

Lampanyctus sp., are known to be vertical migrators (Conley and Hopkins, 2004; Catul et al., 2011); however, *Lampancytus regalis* is a non-migrator (Catul et al., 2011). Both *B. antarcticus* and *C. sloanii* belong to the family Stomiidae which contains many vertical migrators (Sutton and Hopkins, 1996). *C. sloani* was reported to migrate vertically in the Arabian Sea (Butler et al. 2001), although there was no evidence of diel vertical migration in the mid-North Atlantic Ocean (Cook et al. 2013; Sutton et al. 2013).

PCO analysis showed the skate, *R. fyllae* and the sawtooth eel, *S. beanii* group together with 72.5% similarity in an area where the vectors indicate the importance of SFA presumably for energy, and essential fatty acids. Both are micronektonivores but the latter is pelagic and the former demersal and *S. beanii* may migrate vertically (Cook et al. 2013). The importance of the essential DHA and EPA in the ordination of the neutral lipids of the Alepocephalidae, the Rajidae and the Serrivomeridae (Fig. 2) and their significantly higher levels than in the neutral lipids of other taxa (Fig. 4) suggests enhanced intake of good quality food. This is because the enzymes esterifying FA into neutral lipids are not very selective (Koussoroplis et al. 2011) compared to those esterifying PUFA into membranes (Stillwell and Wassall 2003, Petursdottir et al., 2008), suggesting a dietary excess in these taxa.

The two *Lampanyctus* sp. grouped closely together in the neutral lipid PCO and much closer than in polar lipid fatty acids. Here the location was in an area where the vectors indicate the importance of zooplankton, consistent with the Lampanyctinae being known to prey on copepods (Conley and Hopkins, 2004). *B. antarcticus* and *C. sloani* of the family Stomiidae group together with 72.5% similarity, and it should be noted that remains which were probably of myctophid fishes (e.g. *Lampanyctus* sp.) were found in both *C. sloani* and *B. antarcticus* (Parzanini et al. 2017) providing an indirect path to copepod lipids.

The FishBase database was used to designate fish as pelagic or demersal. However, in the case of *B. antarcticus* some researchers (e.g. Heino, 2004; Klimpel et al., 2006; Sutton et al. 2008) have identified it as a pelagic fish. The co-location of *B. antarcticus* with *Lampanyctus* sp. and *C. sloani* (Fig. 2) supports its classification as pelagic, suggesting the possibility of an intermediate status. However, for the purpose of this study we have elected to remain with the FishBase designation as demersal.

Phospholipid lipid fatty acids in different species

In the PCO analysis of the phospholipid fatty acids, 16:0 and DHA have Pearson correlations greater than 0.85 and the central group between these vectors consists almost exclusively of specimens of the Stomiidae, whose representatives are not found to the left or right suggesting a strong phylogenetic signature for this group. In addition to 16:0 and DHA, 22:5ω6 characterized this group. Phospholipid molecular species containing both the ω6 and ω3 isomer of this PUFA are common in fish and shellfish (Boselli et al. 2012), and 22:5ω6 has been hypothesized to be an essential fatty acid in fish and shellfish and one that may have originally been synthesized through the polyketide synthesis pathway (Parrish et al. 2007).

Hierarchical cluster analysis revealed *C. sloani* and *B. antarcticus* from the Stomidae family to group with >82% similarity. Likewise, *R. fyllae* and *M. senta* grouped closely together with >82% similarity and they belong to the same family, the Rajidae. In addition, *B. macrolepis* and *A. bairdii* group together but with a slightly lower similarity of 81%. The vectors with high correlations in the location of the Rajidae indicate the importance of ω6 long-chain PUFA and bacterial fatty acids which is consistent with a more benthic diet (Sargent et al. 1987). The demersal species *M. senta* and *R. fyllae* had the highest uncorrected bulk data for $\delta^{15}N$ in this group (Parzanini et al. 2017). Their high isotopic fractionation suggested a benthic trophic pathway originating in sedimentary organic matter as a primary food source.

As in the PCO of the neutral lipids, some members of the same family group together in the phospholipid PCO but families that group together generally do not come from the same habitat. Again, the groupings are similar to Figure 2 in that the group to the left comprises known diel vertical migrators while those slope fish community members to the right are not known to vertically migrate. However, the group in the middle consists of both. Nonetheless the slope fish community member in the middle, *A. bairdii* does group with *R. fyllae, M. senta* and *B. macrolepis* at 81% similarity.

The monene 16:1ω7 was always lowest in the phospholipid fraction with the exception of one fish group in which it was the second lowest. Examination of the phospholipid molecular species composition of twelve species of fish and shellfish presented by Boselli et al. (2012) reveals little incorporation of 16:1ω7 into their phospholipids. Molecular species containing 16:1ω7 were absent from most phospholipids with the exception of phosphatidyl choline where a 16:1ω7 containing molecular species reached a maximum of 16% of the species in a mussel (Boselli et al. 2012). The lack of inclusion of 16:1ω7 in phospholipids and the fact it was always highest in the neutral lipids suggests it is an excellent biomarker.

Conclusions

Increases in proportions of ω6 and ω7 unsaturated fatty acids in neutral lipids with trophic position not only suggests their significance in this food web but also conversions among fatty acids of the same fish family. The importance of the essential DHA and EPA in the neutral lipids of the Alepocephalidae, Rajidae and Serrivomeridae suggests enhanced intake of good quality food.

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Figures

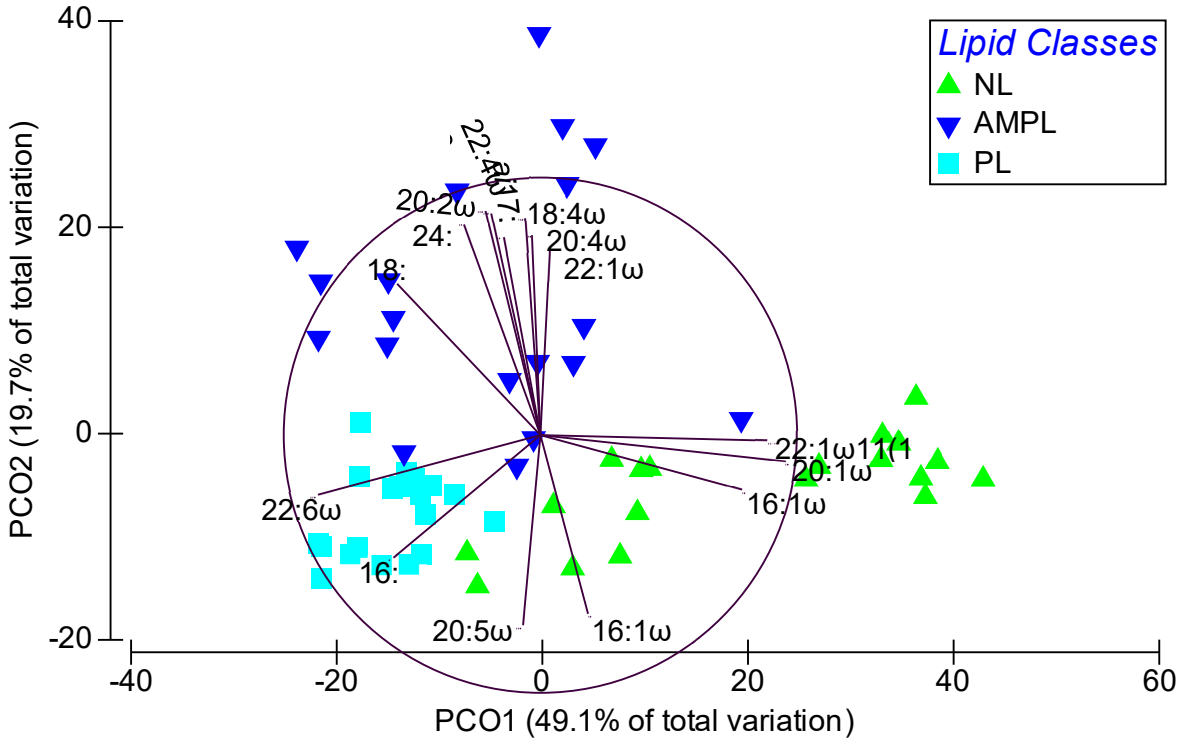

Figure 1. Total fatty acids. Principal coordinates analysis (PCO). The lower triangular matrix was created using Bray-Curtis similarity coefficients. Pearson correlation > 0.7.

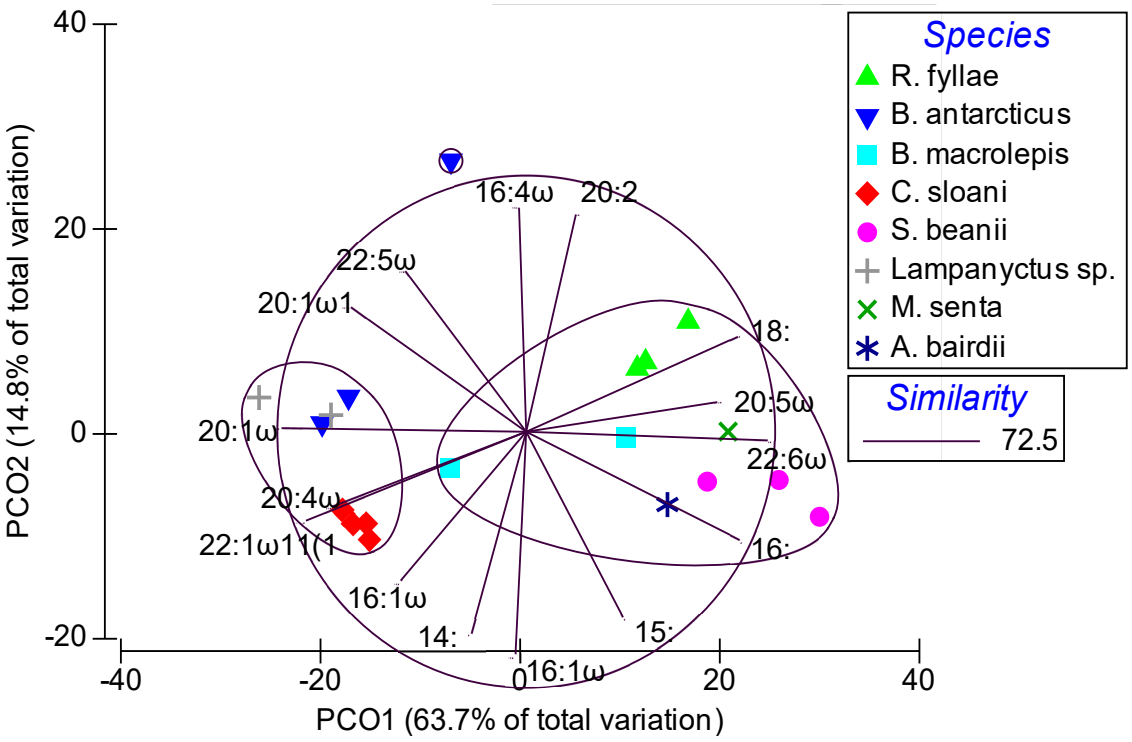

Figure 2. Neutral fatty acids. PCO with a Bray-Curtis similarity resemblance measure;

Pearson Correlation > 0.75

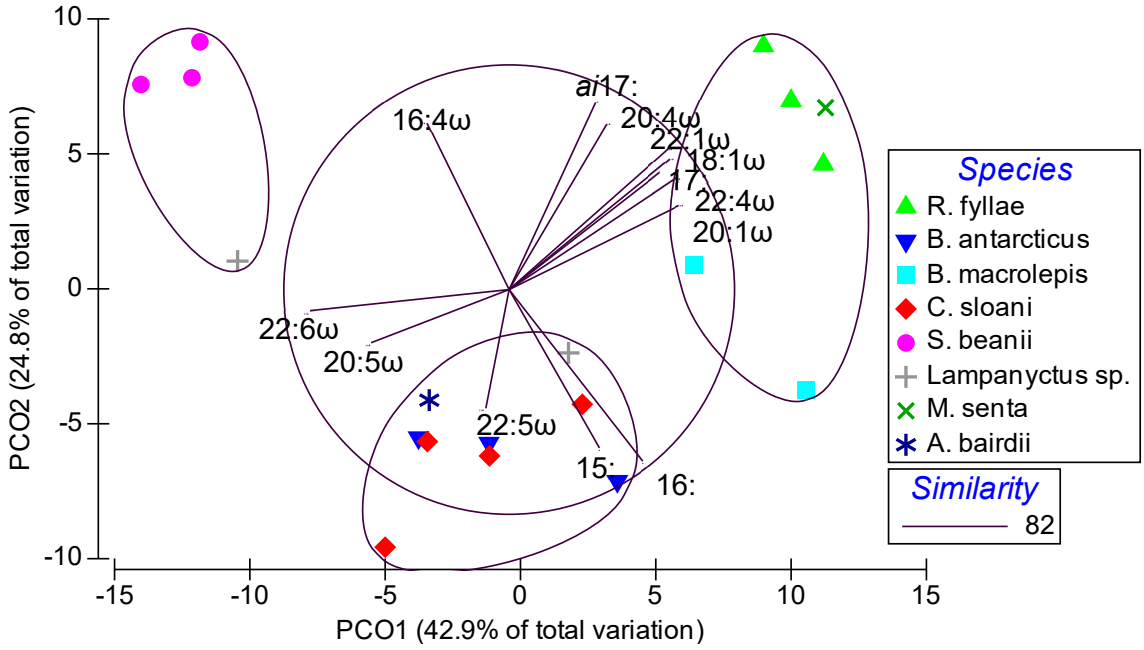

Figure 3. Phospholipid fatty acids; PCO with a Bray-Curtis similarity resemblance measure; Pearson Correlation > 0.81 except EPA and 22:5ω6 which have Pearson correlations of 0.657 and 0.539.

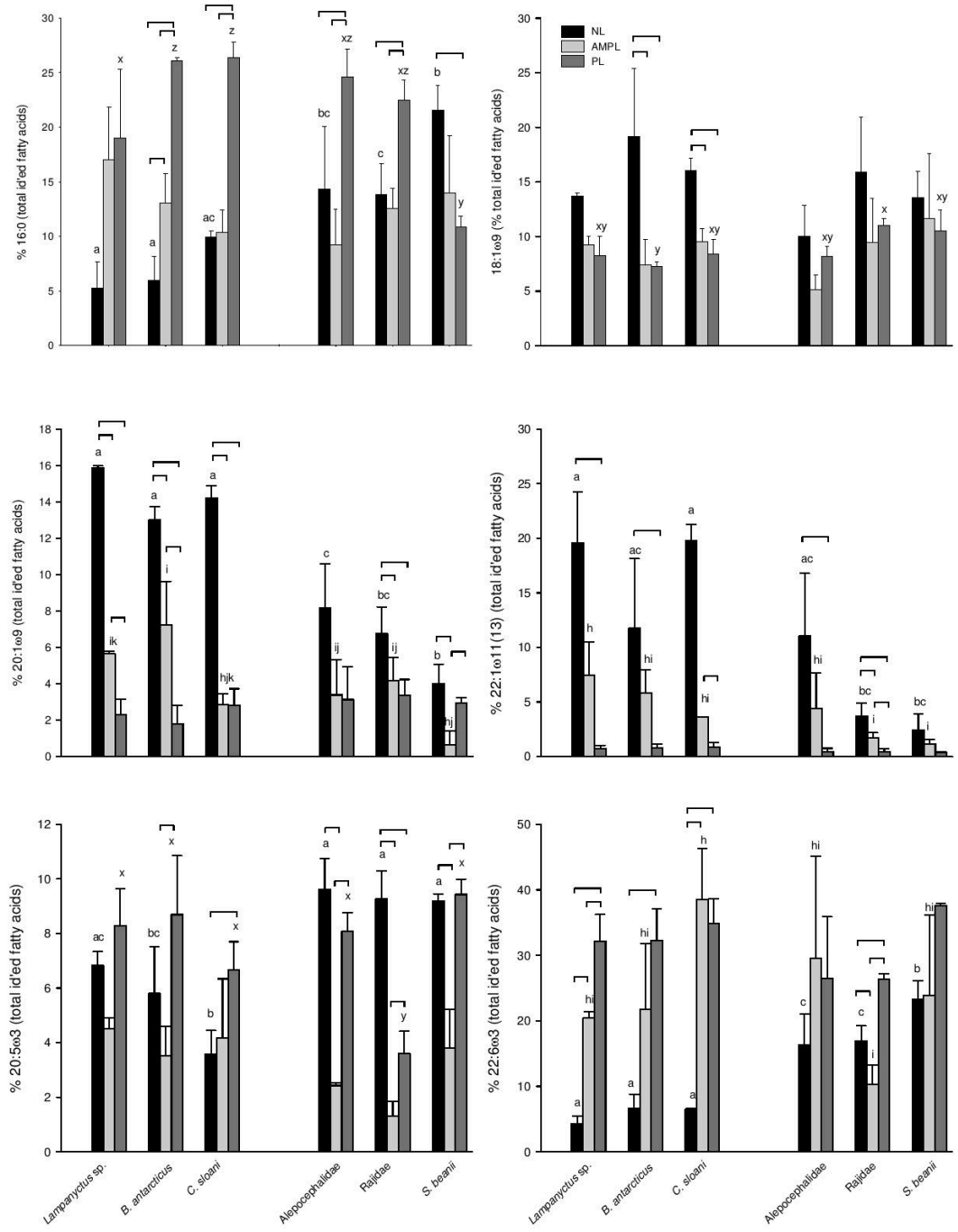

Figure 4. Fatty acids showing the greatest difference between the highest and lowest values in the demersal and pelagic fish species. After removing fatty acids <1.00% of total identified fatty acids (Tables A4 and A5), the six five fatty acids with the largest differences across all samples were plotted as well as EPA.

Brackets indicate significant differences among the lipid fractions for individual taxa. The letters are the result of separate one way ANOVAs.

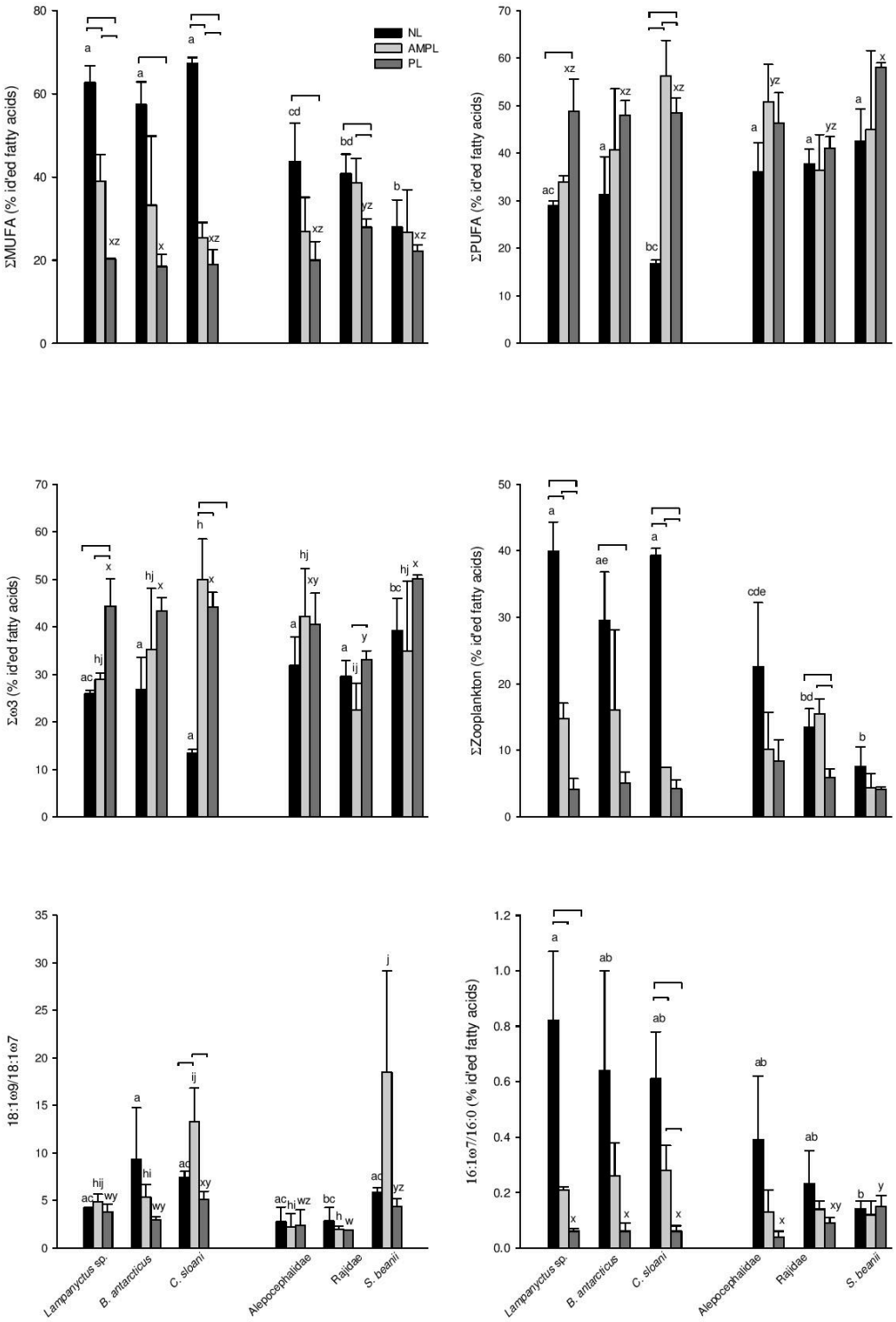

Figure 5. Fatty acid sums and proportions showing the greatest difference between the highest and lowest values in the demersal and pelagic fish species. Out of 8 summary data (Tables A4 and A5) all the fatty acid sums and the 18:1ω9/18:1ω7 ratio showed the largest differences across all samples. The top four most variable of the summary data are plotted together with the 18:1ω9/18:1ω7 and 16:1ω7/16:0 ratios. Brackets indicate significant differences among the lipid fractions for individual taxa. The letters are the result of separate one way ANOVAs among the taxa for individual fractions.

Supplementary material

Appendix Table 1. Concentrations of lipid fractions (mg/g wet wt ± 1 s.d.) in Alepocephalidae, Rajidae and Serrivomeridae from the Northwest Atlantic in December 2013

Classes	Alepocephalidae			Rajidae			***S. beanii***		
(µg/g WW)	**NL**	**AMPL**	**PL**	**NL**	**AMPL**	**PL**	**NL**	**AMPL**	**PL**
Total Lipid	0.18±0.03	0.08±0.04	0.26±0.19	0.12±0.02	0.10±0.04	0.21 ±0.10	0.15±0.07	0.13±0.05	0.18±0.05
∑NL	0.11±0.04	0.01±0.01	0.15±0.09	0.08±0.03	0.02±0.01	0.03±0.02	0.09±0.03	0.04±0.01	0.03±0.01
∑PL	0.04±0.02	0.04±0.01	0.10±0.05	0.04±0.01	0.04±0.01	0.15±0.07	0.04±0.01	0.05±0.01	0.12±0.03
∑AMPL	0.04±0.02	0.02±0.01	0.01±0.01	0.01±0.01	0.03±0.02	0.03±0.02	0.03±0.01	0.04±0.01	0.02±0.01
*Total Lipid		0.52±0.09			0.43±0.06			0.47±0.03	

*Total lipid is the sum of the NL, AMPL and PL fractions

Appendix Table 2. Concentrations of lipid fractions (mg/g wet wt ± 1 s.d.) in Myctophidae and Stomiidae from the Northwest Atlantic, in December 2013

Classes	***Lampanyctus* sp.**				***B. antarcticus***			***C. sloani***	
(µg/g WW)	**NL**	**NL**	**AMPL**	**PL**	**AMPL**	**PL**	**NL**	**AMPL**	**PL**
Total Lipid	1.56±0.73	1.12±0.45	0.10±0.08	0.14±0.07	0.12±0.01	0.12±0.01	0.26±0.07	0.0	0.12±0.03
∑NL	1.14±0.64	0.70±0.39	0.04±0.03		0.03±0.02	0.03±0.02	0.20±0.06	6±0.01	0.01±0.003
∑PL	0.23±0.05	0.	0.04	0.01±0.01	0.04±0.01	0.04±0.01	0.04±0.03		0.1±0.02
∑AMPL	0.19±0.03	24±0.05	±0.01	0.1±0.04	0.04±0.01	0.04±0.01	0.02±0.01	0.01±0.004	0.01±0.001
		0.18±0.09	0.02±0.01						
				0.04±0.01				0.04±0.001	
								0.01±0.01	
*Total Lipid			1.37±0.58			1.80±0.83			0.43±0.13

*Total lipid is the sum of the of NL, AMPL, and PL fractions

Appendix Table 3. Fatty acid composition (% total fatty acids ± 1 s.d.) in Alepocephalidae, Rajidae and Serrivomeridae from the Northwest Atlantic, in December 2013

	A. agassizii			***R. fyllae***			***B. antarcticus***		
FAs	**NL**	**AMPL**	**PL**	**NL**	**AMPL**	**PL**	**NL**	**AMPL**	**PL**
14:0	2.19±0.51	0.54±0.06	0.70±0.38	0.98±0.26	0.97±0.06	0.78±0.14	1.94±1.41	2.38±1.32	1.30±0.34
TMTD	0.01±0.01	0.05±0.03	0.01±0.00	0.03±0.03	0.02±0.01	0.02±0.00	0.01±0.00	0.05±0.03	0.02±0.00
14:1	0.06±0.04	0.01±0.01	0.02±0.01	0.04±0.02	0.02±0.01	0.02±0.01	0.06±0.02	0.05±0.01	0.03±0.01
i15:0	0.09±0.02	0.07±0.01	0.03±0.01	0.16±0.03	0.20±0.04	0.30±0.09	0.09±0.04	0.22±0.12	0.05±0.02
ai15:0	0.02±0.01	0.17±0.25	0.04±0.01	0.04±0.02	0.15±0.15	0.04±0.01	0.02±0.01	0.04±0.01	0.04±0.01
15:0	0.30±0.04	0.22±0.09	0.29±0.10	0.19±0.02	0.22±0.03	0.26±0.02	0.18±0.07	0.25±0.12	0.34±0.05
15:1	0.01±0.00	0.05±0.02	0.04±0.01	0.03±0.02	0.02±0.01	0.04±0.01	0.01±0.00	0.05±0.01	0.10±0.12
i16:0	0.04±0.01	0.09±0.08	0.04±0.02	0.20±0.05	0.08±0.04	0.14±0.03	0.31±0.16	0.22±0.10	0.05±0.01
ai16:0	0.03±0.01	0.58±0.03	0.48±0.18	0.16±0.09	0.36±0.15	0.32±0.19	0.04±0.02	0.28±0.15	0.31±0.10
16:0	14.33±5.7	9.26±3.25	24.63±2.52	13.85±2.79	12.55±1.88	22.47±1.87	5.98±2.21	13.04±2.70	26.11±0.2
16:1ω11	3	0.03±0.03	0.02±0.02	0.08±0.01	0.05±0.01	0.18±0.08	0.04±0.02	0.03±0.03	6
16:1ω9	0.02±0.00	0.28±0.09	0.37±0.17	0.29±0.03	0.27±0.07	0.37±0.13	0.22±0.11	0.37±0.18	0.04±0.02
16:1ω7	0.69±0.14	1.08±0.46	1.04±0.47	3.26±0.73	1.74±0.66	1.99±0.43	4.09±2.72	3.26±2.17	0.28±0.04
16:1ω5	4.97±1.29	0.06±0.04	0.15±0.05	0.15±0.05	0.07±0.03	0.16±0.01	0.16±0.10	0.10±0.04	1.59±0.78
i17:0	0.29±0.03	0.09±0.10	0.25±0.03	0.32±0.08	0.28±0.14	0.38±0.07	0.23±0.16	0.30±0.14	0.31±0.12
ai17:0	0.25±0.04	0.69±0.34	0.14±0.03	0.41±0.20	0.60±0.27	0.26±0.04	0.12±0.02	0.31±0.10	0.36±0.06
16:2ω4	0.06±0.01	0.42±0.22	0.33±0.04	0.26±0.16	0.41±0.25	0.14±0.06	0.38±0.18	0.47±0.12	0.07±0.01
phytanic	0.47±0.06	0.02±0.00	0.02±0.02	0.01±0.00	0.01±0.01	0.03±0.03	0.02±0.01	0.02±0.01	0.45±0.10
17:0	0.01±0.00	0.19±0.04	0.28±0.03	0.37±0.10	0.38±0.15	0.51±0.14	0.07±0.01	0.16±0.10	0.04±0.01
16:3ω4	0.12±0.03	0.11±0.03	0.19±0.05	0.29±0.05	0.22±0.08	0.24±0.13	0.42±0.08	0.30±0.15	0.23±0.03
17:1	0.26±0.10	0.06±0.04	0.02±0.01	0.05±0.02	0.10±0.07	0.05±0.01	0.02±0.01	0.13±0.06	0.27±0.06
16:3ω3	0.07±0.03	0.15±0.07	0.36 ±0.10	0.61±0.31	0.92±0.36	0.97±0.54	1.62±0.47	0.30±0.30	0.05±0.04
16:4ω3	0.22±0.02	1.25±0.64	0.09±0.01	0.07±0.02	0.47±0.34	0.15±0.09	0.06±0.03	0.86±0.42	0.29±0.03
16:4ω1	0.03±0.03	0.08±0.02	0.34±0.14	0.39±0.09	1.42±0.51	1.05±0.64	0.47±0.23	0.12±0.08	0.08±0.02
18:0	0.12±0.05	9.85±1.80	6.51±1.02	4.28±0.61	7.81±1.16	5.28±0.08	1.94±1.20	8.94±3.40	0.13±0.05
18:1ω11	2.58±0.53	0.20±0.12	0.34±0.08	0.90±0.66	0.12±0.15	0.39±0.08	0.96±0.32	1.29±0.54	4.55±0.45
18:1ω9	0.52±0.14	5.14±1.38	8.16±0.92	15.89±5.02	9.44±4.03	11.04±0.61	19.15±6.2	7.42±2.32	0.91±0.38
18:1ω7	10.06±2.7	2.28±0.45	3.44±0.56	5.49±1.14	4.81±1.76	5.98±0.46	2	1.35±0.34	7.26±0.43
18:1ω6	9	0.03±0.03	0.03±0.01	0.02±0.01	0.01±0.00	0.03±0.01	2.29±0.72	0.03±0.02	2.47±0.14
18:1ω5	3.61±0.64	0.08±0.01	0.21±0.06	0.47±0.06	0.25±0.13	0.43±0.10	0.03±0.01	0.16±0.06	0.05±0.01

18:2ω6	0.04±0.02	2.45±1.16	1.60±0.92	1.72±0.16	5.41±4.65	1.62±0.37	0.34±0.16	2.48±0.57	0.33±0.11
18:2ω4	0.37±0.13	0.02±0.00	0.03±0.01	0.06±0.01	0.05±0.02	0.04±0.01	1.17±0.16	0.02±0.01	1.31±0.09
18:3ω6	1.07±0.06	0.09±0.02	0.14±0.03	0.09±0.02	0.07±0.02	0.07±0.01	0.06±0.02	0.06±0.02	0.04±0.02
19:0	0.04±0.01	-	-	0.02±0.01	-	-	0.08±0.03	0.01±0.00	0.04±0.00
18:3ω4	0.08±0.01	0.13±0.06	0.03±0.00	0.02±0.01	0.07±0.02	0.09±0.03	0.05±0.02	0.07±0.02	-
18:3ω3	-	0.16±0.06	0.76±1.04	0.15±0.07	0.35±0.39	0.07±0.03	0.14±0.08	0.23±0.13	0.07±0.03
18:4ω3	0.03±0.02	2.37±1.49	0.38±0.19	0.33±0.13	2.95±1.84	0.18±0.08	0.36±0.15	1.74±0.24	0.21±0.07
18:4ω1	0.33±0.13	0.07±0.03	0.22±0.00	0.03±0.01	0.22±0.09	0.16±0.11	0.56±0.20	0.08±0.03	0.16±0.05
20:0	0.48±0.21	0.21±0.10	0.12±0.06	0.09±0.03	0.37±0.20	0.07±0.02	0.25±0.15	0.09±0.01	0.02±0.01
18:5ω3	0.05±0.02	0.03±0.03	0.01±0.00	0.07±0.09	0.03±0.01	0.01±0.00	0.14±0.04	0.11±0.04	0.04±0.02
20:1ω11	0.10±0.02	0.27±0.19	0.35±0.11	1.14±0.48	1.28±0.72	0.60±0.19	-	0.98±0.34	0.01±0.00
20:1ω9	0.03±0.03	3.38±1.95	3.13±1.80	6.74±1.49	4.16±1.27	3.35±0.88	2.59±0.52	7.22±2.41	0.71±0.78
20:1ω7	0.64±0.28	0.32±0.25	0.47±0.35	0.90±0.23	0.83±0.56	0.45±0.07	12.99±0.7	0.17±0.10	1.79±1.01
20:2a	8.18±2.40	0.24±0.23	0.04±0.03	0.27±0.17	0.08±0.02	0.03±0.01	5	0.12±0.07	0.06±0.02
20:2b	0.90±0.39	0.29±0.33	0.05±0.04	0.06±0.02	0.02±0.03	0.01±0.00	0.71±0.08	0.05±0.03	0.02±0.01
20:2ω6	0.07±0.04	2.14±0.77	0.71±0.26	0.59±0.08	3.30±2.73	0.45±0.08	0.22±0.11	1.94±1.51	0.06±0.02
20:3ω6	0.08±0.08	0.06±0.01	0.07±0.03	0.10±0.04	0.06±0.05	0.05±0.02	0.33±0.15	0.03±0.01	0.24±0.08
21:0	0.19±0.02	0.02±0.01	0.00±0.00	0.02±0.01	0.04±0.04	0.01±0.00	0.34±0.14	0.04±0.02	0.11±0.03
20:4ω6	0.05±0.01	1.70±0.52	1.32±0.49	3.57±0.35	2.09±0.69	3.17±0.64	0.04±0.01	1.08±0.67	0.01±0.00
20:3ω3	0.02±0.01	0.12±0.07	0.11±0.04	0.07±0.06	0.19±0.20	0.02±0.01	0.03±0.01	0.88±0.16	1.24±0.45
20:4ω3	1.13±0.58	1.55±1.23	0.28±0.15	0.12±0.08	2.97±2.93	0.13±0.10	0.33±0.19	1.77±0.40	0.03±0.01
20:5ω3	0.05±0.01	2.43±0.94	8.08±0.69	9.26±1.03	1.32±0.52	3.66±0.82	0.07±0.02	3.51±1.08	0.50±0.11
22:0	0.25±0.19	0.08±0.03	0.03±0.00	0.15±0.14	0.41±0.11	0.10±0.04	0.59±0.26	0.06±0.02	8.69±2.16
22:1ω11(13)	9.61±1.14	4.42±3.21	0.45±0.30	3.69±1.20	1.71±0.46	0.42±0.32	5.80±1.72	5.81±2.10	0.03±0.02
22:1ω9	0.03±0.01	1.40±1.00	0.44±0.22	0.78±0.11	5.91±1.66	0.92±0.08	0.04±0.02	0.70±0.33	0.77±0.37
22:1ω7	11.03±5.7	0.41±0.11	0.09±0.04	0.17±0.05	1.59±0.61	0.14±0.05	11.74±6.3	0.15±0.09	0.07±0.01
22:2NIMDa	6	0.05±0.02	-	0.06±0.02	0.07±0.02	0.01±0.00	9	0.05±0.02	0.03±0.01
22:2NIMDb	1.67±0.96	0.04±0.03	-	0.02±0.02	0.02±0.00	0.01±0.00	1.46±0.44	0.01±0.00	0.01±0.00
21:5ω3	0.15±0.02	0.18±0.09	0.13±0.06	0.20±0.05	0.06±0.04	0.04±0.01	0.12±0.09	0.13±0.08	0.03±0.02
23:0	0.04±0.01	0.06±0.05	0.01±0.00	0.04±0.01	0.28±0.41	0.02±0.01	0.02±0.01	0.05±0.02	0.06±0.03
22:4ω6	0.03±0.01	0.22±0.14	0.17±0.11	0.39±0.23	0.43±0.36	0.39±0.12	0.01±0.00	0.10±0.03	0.01±0.00
22:5ω6	0.23±0.09	0.53±0.03	0.63±0.06	0.31±0.17	0.15±0.07	0.40±0.04	0.19±0.06	0.21±0.19	0.02±0.00
22:4ω3	0.02±0.01	1.54±0.77	0.26±0.10	0.12±0.06	2.24±2.14	0.10±0.03	-	0.95±0.37	0.53±0.23
22:5ω3	0.18±0.10	2.95±2.16	3.51±2.45	1.60±0.06	0.65±0.27	1.41±0.26	0.06±0.02	1.28±1.08	0.08±0.03
24:0	0.27±0.03	0.07±0.03	0.01±0.01	0.08±0.02	0.09±0.03	-	0.14±0.08	0.17±0.22	1.03±0.07
22:6w3	0.05±0.01	29.52±15.61	26.52±9.35	16.96±2.30	10.28±2.94	26.37±0.82	0.07±0.04	21.76±10.06	0.01±0.01

24:1	4.29±3.13	7.36±1.33	1.23±0.10	0.69±0.20	6.27±1.76	1.35±0.32	10.84±3.13	3.43±1.83	32.25±4.88
	0.01±0.00						0.03±0.01		
∑SFA	16.30±4.74	20.56±5.20	32.63±1.87	20.11±2.85	23.17±1.93	29.55±1.95	24.89±7.21	1.64±0.33	
∑MUFA	0.50±0.15	26.87±8.23	20.00±4.49	40.78±4.76	38.64±5.81	27.92±2.01	6.69±2.13	33.22±16.63	
∑PUFA		50.89±7.79	46.40±6.33	37.82±3.04	36.52±7.43	41.08±2.40	0.46±0.33	40.67±12.88	32.68±0.15
∑ω3		42.25±10.11	40.51±6.58	29.56±3.41	22.44±5.66	33.12±1.87		35.17±12.89	
DHA/EPA	19.71±6.25	12.77±7.58	3.34±1.30	1.83±0.12	8.05±1.33	7.54±2.02	10.44±2.51	5.03±2.74	18.49±2.98
Zooplankton		10.21±5.53	8.37±3.22	13.43±2.82	15.47±2.26	5.88±1.36		16.13±12.05	
(∑20:1+∑22:1)	43.78±9.16	0.13±0.08	0.04±0.02	0.23±0.12	0.14±0.03	0.09±0.02	57.44±5.46	0.26±0.12	47.96±3.15
16:1ω7/16:0		2.25±1.37	2.38±1.64	2.86±1.40	1.94±0.36	1.85±0.06		5.32±1.36	
18:1ω9/18:1ω7	36.03±6.19						31.31±7.99		43.39±2.79
	31.87±5.95						26.85±6.75		3.94±1.41
	1.68±0.33						1.24±0.88		5.05±1.69
	22.57±9.68						29.61±7.21		0.06±0.03
	0.39±0.23						0.64±0.36		2.95±0.33
	2.76±1.55						9.30±5.47		

Appendix Table 4. Fatty Acid Composition (% total fatty acids ± 1 s.d.) of Pelagic Fish from Northeast of Newfoundland in December 2013

	Lampanyctus sp.			*S. beanii*			*C. sloani*		
FAs	**NL**	**AMPL**	**PL**	**NL**	**AMPL**	**PL**	**NL**	**AMPL**	**PL**
14:0	0.97±0.27	3.18±1.21	0.57±0.11	2.32±0.26	1.68±0.60	0.86±0.09	3.07±0.45	2.17±0.13	1.24±0.24
TMTD	0.03±0.01	0.07±0.03	0.02±0.01	0.01±0.00	0.01±0.01	0.02±0.01	0.01±0.01	0.03±0.01	0.02±0.01
14:1	0.04±0.02	0.03±0.00	0.02±0.01	0.05±0.01	0.10±0.01	0.03±0.01	0.09±0.02	0.03±0.01	0.01±0.01
i15:0	0.06±0.02	0.17±0.08	0.07±0.02	0.06±0.02	0.11±0.06	0.06±0.02	0.17±0.01	0.18±0.07	0.05±0.01
ai15:0	0.02±0.01	0.04±0.00	0.05±0.01	0.02±0.00	0.13±0.05	0.03±0.01	0.05±0.01	0.23±0.10	0.03±0.01
15:0	0.11±0.05	0.35±0.10	0.20±0.05	0.39±0.06	0.22±0.06	0.20±0.03	0.32±0.02	0.27±0.02	0.35±0.03
15:1	0.02±0.00	0.02±0.00	0.02±0.01	0.01±0.01	0.03±0.01	0.06±0.04	0.02±0.01	0.07±0.00	0.02±0.01
i16:0	0.55±0.12	0.12±0.01	0.06±0.01	0.03±0.02	0.11±0.03	0.06±0.03	0.10±0.01	0.13±0.01	0.03±0.01
ai16:0	0.02±0.00	0.20±0.09	0.24±0.02	0.05±0.01	0.61±0.30	1.37±0.42	0.09±0.03	0.59±0.29	0.10±0.04
16:0	5.27±2.43	17.02±4.8	19.02±6.32	21.59±2.22	13.99±5.22	10.86±0.9	9.96±0.54	10.40±2.01	26.40±1.39
16:1ω11	0.03±0.01	3	0.03±0.02	0.05±0.02	0.07±0.02	9	0.04±0.01	0.03±0.00	0.03±0.01
16:1ω9	0.18±0.07	0.03±0.01	0.22±0.05	0.38±0.28	0.34±0.09	0.18±0.07	0.52±0.63	0.43±0.20	0.27±0.05
16:1ω7	4.01±0.65	0.53±0.18	1.10±0.14	2.94±0.70	1.74±1.08	0.25±0.03	6.08±1.65	2.52±0.03	1.63±0.39
16:1ω5	0.17±0.02	3.51±0.93	0.14±0.05	0.24±0.03	0.03±0.02	1.60±0.28	0.29±0.02	0.07±0.04	0.33±0.06
i17:0	0.27±0.01	0.10±0.03	0.23±0.07	0.23±0.13	0.20±0.09	0.14±0.03	0.35±0.03	0.21±0.07	0.29±0.05
ai17:0	0.07±0.00	0.09±0.03	0.16±0.01	0.04±0.01	0.35±0.16	0.31±0.08	0.14±0.04	0.69±0.27	0.03±0.01
16:2ω4	0.43±0.04	0.36±0.09	0.24±0.00	0.28±0.13	0.68±0.13	0.17±0.05	0.45±0.06	0.45±0.03	0.42±0.06
phytanic	0.01±0.00	0.67±0.09	0.01±0.00	0.03±0.01	0.05±0.02	0.47±0.17	0.02±0.01	0.01±0.00	0.02±0.01
17:0	0.03±0.02	0.00±0.00	0.23±0.04	0.20±0.03	0.14±0.08	0.05±0.03	0.08±0.04	0.08±0.02	0.20±0.03
16:3ω4	0.32±0.01	0.11±0.02	0.19±0.06	0.26±0.09	0.28±0.02	0.21±0.06	0.60±0.05	0.19±0.09	0.30±0.04
17:1	0.04±0.02	0.36±0.03	0.02±0.00	0.01±0.01	0.16±0.06	0.23±0.06	0.04±0.01	0.02±0.00	0.02±0.01
16:3ω3	0.12±0.02	0.03±0.01	0.31±0.10	0.23±0.06	0.31±0.17	0.04±0.02	0.26±0.03	0.09±0.01	0.22±0.03
16:4ω3	2.34±0.79	0.21±0.07	0.24±0.10	0.04±0.01	0.73±0.30	0.27±0.09	0.01±0.00	1.11±0.20	0.05±0.03
16:4ω1	0.30±0.12	0.46±0.03	0.27±0.18	0.06±0.03	0.76±0.21	0.26±0.05	0.09±0.04	0.02±0.01	0.18±0.05
18:0	0.59±0.17	0.12±0.05	9.56±0.49	3.38±0.48	10.16±2.56	2.60±0.59	1.39±0.11	3.19±1.09	3.65±0.47
18:1ω11	0.25±0.27	4.70±1.25	0.26±0.05	0.42±0.14	0.35±0.12	5.37±0.82	1.33±0.36	0.28±0.14	0.83±0.31
18:1ω9	13.68±0.29	0.53±0.11	8.24±1.76	13.57±2.41	11.68±5.93	1.20±0.42	16.07±1.1	9.56±1.17	8.40±1.35
18:1ω7	3.29±0.15	9.24±0.81	2.16±0.01	2.34±0.54	1.03±0.08	10.49±1.9	0	0.76±0.05	1.64±0.21
18:1ω6	0.04±0.02	1.90±0.14	0.04±0.01	0.16±0.12	0.08±0.03	4	2.16±0.12	0.01±0.01	0.03±0.00
18:1ω5	0.43±0.07	0.01±0.00	0.29±0.05	0.11±0.13	0.12±0.05	2.40±0.05	0.06±0.01	0.05±0.02	0.31±0.03
18:2ω6	0.89±0.11	0.24±0.05	0.70±1.00	0.77±0.51	2.05±0.82	0.01±0.00	0.49±0.04	2.59±1.02	1.27±0.43
18:2ω4	0.08±0.02	1.58±0.09	0.04±0.01	0.03±0.01	0.02±0.01	0.18±0.04	1.33±0.07	0.01±0.01	0.03±0.01

18:3ω6	0.05±0.02	0.05±0.02	0.09±0.02	0.12±0.03	0.13±0.05	0.79±0.09	0.07±0.02	0.05±0.01	0.05±0.01
19:0	0.07±0.04	0.10±0.01	-	0.99±1.70	0.01±0.00	0.03±0.02	0.10±0.01	0.01±0.00	-
18:3ω4	0.13±0.08	0.01±0.00	0.09±0.07	0.11±0.16	0.10±0.05	0.20±0.05	-	0.05±0.03	0.04±0.06
18:3ω3	0.31±0.01	0.03±0.01	0.20±0.01	3.72±6.20	0.15±0.10	-	0.04±0.01	0.31±0.02	0.28±0.06
18:4ω3	0.50±0.16	0.26±0.07	0.66±0.25	0.83±0.89	2.20±1.03	0.06±0.01	0.68±0.03	1.76±0.54	0.18±0.09
18:4ω1	0.09±0.03	1.31±0.15	0.05±0.01	0.06±0.06	0.26±0.12	0.13±0.01	0.98±0.14	0.04±0.00	0.03±0.01
20:0	0.15±0.03	0.04±0.01	0.12±0.01	0.11±0.13	0.21±0.13	0.24±0.11	0.06±0.04	0.07±0.01	0.02±0.01
18:5ω3	-	0.14±0.05	0.01±0.00	0.19±0.29	0.06±0.03	0.95±0.74	0.09±0.01	0.04±0.06	-
20:1ω11	1.77±0.33	0.01±0.00	0.66±0.21	0.65±0.27	2.25±1.61	0.04±0.01	-	0.58±0.44	0.38±0.07
20:1ω9	15.87±0.11	0.72±0.15	2.28±0.86	4.01±1.06	0.62±0.77	-	1.64±0.23	2.86±0.59	2.82±0.90
20:1ω7	0.80±0.13	3	0.12±0.01	0.09±0.09	0.08±0.02	0.61±0.11	14.21±0.6	0.09±0.04	0.07±0.04
20:2a	0.02±0.01	5.64±0.13	0.02±0.01	0.02±0.01	0.20±0.06	2.92±0.32	8	0.04±0.02	0.01±0.00
20:2b	0.02±0.01	0.15±0.05	0.01±0.00	0.04±0.03	0.28±0.12	0.07±0.02	0.35±0.09	0.09±0.04	0.01±0.01
20:2ω6	0.32±0.01	0.04±0.02	0.64±0.14	0.30±0.07	2.26±1.03	0.03±0.01	0.04±0.01	1.82±0.47	0.13±0.02
20:3ω6	0.09±0.01	0.00±0.00	0.06±0.00	0.05±0.01	0.11±0.05	0.04±0.02	0.01±0.01	0.07±0.03	0.09±0.03
21:0	0.01±0.00	0.78±0.05	0.00±0.00	0.03±0.02	0.04±0.01	0.48±0.06	0.23±0.07	0.02±0.01	0.01±0.00
20:4ω6	0.23±0.01	0.05±0.00	1.13±0.03	0.92±0.47	2.46±1.24	0.01±0.00	0.03±0.01	0.42±0.35	1.05±0.26
20:3ω3	0.09±0.04	0.01±0.00	0.08±0.02	0.11±0.11	0.06±0.02	0.00±0.00	0.01±0.00	0.65±0.04	0.04±0.02
20:4ω3	1.00±0.18	0.41±0.03	0.62±0.15	0.32±0.24	1.41±0.15	1.84±0.22	0.22±0.10	1.20±0.38	0.63±0.22
20:5ω3	6.83±0.51	0.05±0.03	8.29±1.36	9.19±0.26	3.80±1.41	0.18±0.03	0.07±0.04	4.17±2.17	6.67±1.03
22:0	0.05±0.02	0.21±0.09	0.18±0.08	0.01±0.00	0.06±0.02	0.13±0.04	0.67±0.19	0.03±0.01	0.01±0.00
22:1ω11(13)	19.56±4.71	4.51±0.40	0.73±0.29	2.44±1.45	1.16±0.42	9.43±0.56	3.58±0.87	3.59±0.04	0.87±0.40
22:1ω9	1.87±0.21	0.16±0.09	0.16±0.10	0.32±0.17	0.23±0.09	0.02±0.01	0.02±0.01	0.09±0.04	0.05±0.03
22:1ω7	0.12±0.02	7.43±3.07	0.20±0.09	0.04±0.02	0.05±0.02	0.35±0.09	19.77±1.4	0.18±0.08	0.01±0.01
22:2NIMDa	0.02±0.00	0.58±0.00	0.00±0.00	0.02±0.01	0.08±0.05	0.17±0.01	9	0.02±0.00	0.01±0.00
22:2NIMDb	0.01±0.02	0.25±0.19	0.00±0.00	0.02±0.02	0.12±0.05	0.02±0.01	3.23±0.09	0.03±0.02	0.01±0.00
21:5ω3	0.18±0.01	0.11±0.03	0.23±0.04	0.16±0.11	0.25±0.09	0.01±0.00	0.09±0.03	0.35±0.18	0.17±0.02
23:0	0.00±0.00	0.07±0.03	0.11±0.05	0.02±0.01	0.04±0.02	-	0.01±0.00	0.02±0.00	0.01±0.01
22:4ω6	0.07±0.04	0.46±0.02	0.05±0.03	0.01±0.01	0.09±0.03	0.09±0.04	0.01±0.01	0.13±0.10	0.03±0.01
22:5ω6	0.07±0.01	0.18±0.17	0.84±0.16	0.22±0.08	0.16±0.02	0.01±0.00	0.20±0.06	0.31±0.19	0.61±0.18
22:4ω3	0.06±0.01	0.23±0.29	0.44±0.12	0.10±0.09	1.32±1.01	0.02±0.01	0.01±0.00	1.06±0.28	0.07±0.03
22:5ω3	10.13±0.17	0.42±0.09	1.16±0.13	1.02±0.42	0.76±0.31	0.29±0.08	0.06±0.04	0.68±0.24	1.06±0.05
24:0	0.01±0.00	0.50±0.03	0.13±0.03	0.04±0.04	0.16±0.03	0.16±0.06	0.06±0.04	0.06±0.03	0.01±0.01
22:6w3	4.31±1.17	0.53±0.43	32.17±4.06	23.30±2.80	23.91±12.2	1.68±0.36	0.04±0.01	38.53±7.78	34.87±3.73
24:1	0.55±0.10	0.05±0.03	3.52±0.70	0.13±0.08	1	0.01±0.00	0.46±0.05	4.09±3.52	1.27±0.14
		20.42±1.0			6.65±2.55	37.57±0.3	0.01±0.00		
∑SFA	7.28±3.20	1	30.14±6.85	29.12±1.33		7	6.45±0.23	16.37±2.91	31.95±1.16
∑MUFA	62.70±3.99	8.10±4.74	20.21±0.28	27.96±6.55	26.80±6.80	1.44±0.29	0.85±0.17	25.33±3.77	18.99±3.49

∑PUFA	29.02±0.89		48.84±6.74	42.50±6.81	26.74±10.2			56.28±7.50	48.52±3.07
∑ω3	25.89±0.67	26.00±5.1	44.41±5.63	39.20±6.80	2	17.66±0.7	14.99±0.8	49.95±8.53	44.25±3.01
DHA/EPA ratio	0.63±0.13	7	3.89±0.15	2.53±0.24	44.96±16.6	7	9	10.01±3.20	5.38±1.35
Zooplankton	39.98±4.38	39.02±6.3	4.15±1.61	7.55±2.97	1	22.17±1.4	67.33±1.4	7.39±0.09	4.20±1.38
(∑20:1+∑22:1)	0.82±0.25	5	0.06±0.01	0.14±0.03	34.95±14.6	5	2	0.28±0.09	0.06±0.02
16:1ω7/16:0	4.16±0.10	33.99±1.2	3.82±0.80	5.85±0.52	0	58.17±0.8	16.78±0.8	13.33±3.51	5.14±0.81
18:1ω9/18:1ω7		1			6.79±2.07	8	0		
		28.93±1.3			4.39±2.06	50.13±0.7	13.40±0.7		
		9			0.12±0.05	6	7		
		4.56±0.63			18.49±10.6	4.00±0.28	1.83±0.38		
		14.76±2.3			8	4.13±0.39	39.29±1.0		
		3				0.15±0.04	7		
		0.21±0.01				4.37±0.85	0.61±0.17		
		4.90±0.78					7.45±0.62		

Appendix 7-2 Mean proportion % ±se of hydrocarbons (HC), ethyl ethers (EE), methyl esters (ME), ethyl ketones (EK), methyl ketones (MK), glyceryl ethers (GE), alcohols (ALC), diacylglycerols (DAG), and acetone-mobile polar lipids (AMPL) are reported from the phylum containing the highest amounts of lipids to the phylum characterized by the lowest contents.

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Phylum	HC	EE	ME	EK	MK	GE	ALC	DAG	AMPL
Chordata	1.4±0.1	0.5±0.3	0.1±0.1	1.0±0.3	1.9±0.4	2.3±1.0	4.1±0.6	0.3±0.1	3.5±0.3
Arthropoda	2.3±0.3	0.3±0.1	0.8±0.4	1.3±0.5	1.8±1.0	-	3.1±1.3	0.0±0.0	2.2±0.6
Echinodermata	1.9±0.4	3.0±0.7	0.2±0.1	1.5±0.6	5.7±1.4	1.3±0.9	1.6±0.7	1.0±0.4	2.1±0.5
Annelida	0.7±0.3	0.6±0.3	0.7±0.4	1.1±0.7	-	0.4±0.4	2.3±2.3	-	2.5±1.5
Cnidaria	2.9±0.5	4.0±1.1	1.7±0.9	1.4±0.6	2.0±1.1	0.8±0.3	2.6±0.5	0.1±0.1	5.7±1.5
Mollusca	0.8±0.2	-	0.0±0.0	0.3±0.2	0.0±0.0	-	-	-	0.1±0.1
Porifera	1.7±0.4	4.8±1.5	1.5±0.5	0.4±0.2	0.1±0.1	-	0.3±0.1	0.3±0.2	1.3±0.3
Sipuncula	2.6±1.6	-	3.6±2.8	-	-	-	-	-	-

Appendix 7-3 Mean proportion of phospholipids (PL), free fatty acids (FFA), sterols (ST), triacylglycerols (TAG), wax esters/steryl esters (WE/SE), as well as the triacyglycerol to sterol (TAG:ST) and phospholipid to sterol (PL:ST) ratios measured for each species analyzed in this study.

Phylum	Taxon	PL	FFA	ST	TAG	WE/SE	TAG:ST	PL:ST
		% ±sd	% ±sd	% ±sd	% ±sd	% ±sd	Mean±sd	Mean±sd
Chordata								
	Actinopterygii							
	A. bairdii	23.8±29.9	24.1±5.2	7.2±7.2	29.1±41.1	-	26.8	2.4±1.7
	A. cornuta	10.4±5.7	13.8±4	5.3±2	51.4±8.3	-	11.7±7.6	1.9±0.4
	A. rostrata	22.1±18	50.3±17.2	11.1±13.7	-	-	-	7.5±7.4
	A. risso	17.4±7.3	10.2±9.2	6.2±1.2	7.52±0.0	23.1±32.7	1.2±0.2	3.0±1.7
	B. euryops	19.2±8.7	20.6±7.1	5.4±2.9	40.0±27.8	-	10.4±10.8	3.7±0.4
	B. macrolepis	6.5±0.2	37.8±17.9	10.6±15.1	29.9±1.4	-	1.4	0.3
	B. antarcticus	23.1±22.3	12.5±2.6	10.6±10.0	20.3±24.1	17.2±21.0	9.1±1.3	2.1±2.0
	C. macropus	2.0	7.2	5.6	28.3	39	5.1	0.4
	C. sloani	11.1±13.1	17.7±12.3	7.7±5.8	50.8±20.6	0.1±0.3	12.0±9.8	1.8±2.0
	C. niger	3.9±0.8	7.4±2.3	1.7±1.3	82.9±6.2	-	69.5	3.0±1.3
	C. rupestris	38.1±7.1	31.4±8.6	12.0±3.7	9.70±10.5	0.09±0.2	0.9±1.1	3.3±1.1
	C. microps	36.6±16.6	26.2±29.0	5.70±8.1	1.21±1.7	-	0.2	4.2
	C. thomsonii	3.1	12.6	6.8	68.7	-	10.1	0.5
	C. microdon	12.5±6.8	14.8±4.4	9.1±0.3	52.6±16.3	0.2±0.3	5.8±1.6	1.4±0.8
	G. ensis	18.3±6.6	54.1±5.2	17.5±2.9	1.5±3	0.2±0.4	0.3	1.1±0.5
	G. cynoglossus	28.4±3.6	46.3±14.1	4.9±8.6	-	-	-	2.0
	H. mollis	64.6	13.9	19.5	-	-	-	3.3
	L. speculigera	28.8	3.5	0.8	8.4	49.9	11	37.8
	Lampanyctus spp.	12.6±6	12.3±2.4	3.8±2.4	11.5±3.7	40.8±1.7	4.0±2.3	3.6±0.5
	L. eques	37.7	39.7	15.3	-	-	-	2.5
	M. berglax	24.8±6.9	45.8±65.7	20.7±4.0	-	-	-	1.3±0.6
	M. atlantica	13.1±1.2	16.0±0.9	5.7±2	48.2±3.1	-	9.1±3.8	2.5±1.1
	M. niger	2.7±2.3	6.7±9.4	1.2±1.7	41.4±54.9	-	33.8	0.4
	M. johnsonii	10.4	23.3	18	28.4	-	1.6	0.6
	Myctophum sp.	7.3	12.2	4.5	65.1	-	14.5	1.6
	N. bairdii	16.2±7.6	32.0±18.2	15.1±0.6	12.9±15.5	-	0.9±1.1	1.1±0.5
	N. chemnitzii	4.4±5.9	22.7±1.0	10.0±1.8	48.7±5.3	0.4±0.6	5.0±1.4	0.5±0.7
	Notoscopelus spp.	9±4.8	9.9±1.1	2.4±1.4	71.5±5.9	-	36.3±23.3	3.8±0.2
	O. macrosteus	44.2	3.6	24.2	1.0	-	0.0	1.8
	P. rissoanus	11.0±1.6	12.4±3.5	5.8±1.8	60.3±17.4	0.9±0.1	11.5±6.6	2.0±0.3
	R. hippoglossoides	24.8	9.3	1.2	56.6	-	49.2	21.5
	S. opisthopterus	14.9±0.9	28.7±7.3	10.1±2.2	31.8±4.9	-	3.2±0.2	1.5±0.4

S. mentella	33.1±2.6	24.4±0.6	13.7±1.7	12.0±7.2	-	0.9±0.6	2.5±0.4
S. beanii	39.6±18.5	17.8±7.3	13.1±3.6	-	-	-	3.5±2.6
S. kaupii	7.7±3.0	10.3±1.9	4.3±0.6	68.1±5	-	16.0±3.1	1.7±0.4
T. murrayi	37.1±6.90	42.2±5.1	17.2±0.9	-	-	-	2.2±0.5
X. copei	3.8±3.9	14.8±2.6	7.7±3	64.1±7.3	-	9.6±4.6	0.6±0.8
Ascidiacea							
Ascidiacea sp 1	60.9±22.8	1.8±2.5	31.4±13.3	-	-	-	2.3±1.7
Ascidiacea sp 4	64.7±4.8	7.4±1.5	23.7±9.5	2.3±3.2	-	0.3	3.0±1.4
Didemnum sp.	20.6	27.8	35.6	-	-	-	0.6
E. vitreum	15.6	19.8	54.4	-	-	-	0.3
Chondrichthyes							
A. jenseni	80.5	1.4	15.9	-	-	-	5.1
A. profundorum	68.3±20.5	8±3.9	8.5±1.2	7.5±13	0.0±0.1	2.4	8.3±3.2
C. fabricii	80.3±0.9	1.8±0.7	7.4±1.1	0.1±0.1	0.1±0.1	0.0	11.0±1.8
M. senta	50.3	3.5	14.3	3.1	-	0.2	3.5
R. fyllae	38.8±5.6	3.5±3.4	17±8.6	3.4±2.8	5.2±5.9	0.4±0.3	3.0±2.3
Arthropoda							
Hexanauplia							
A. michelottianum	40.5±9.4	18.2±5.7	13.1±4.7	17.5±7.1	0.2±0.3	1.5±1.1	3.4±1.4
Malacostraca							
A. pelagica	7.8±4.2	16.6±12.3	13.9±9.1	0.3±0.4	40.9±15.3	0.1	0.8±0.8
Anonyx sp 1	22.5	4.3	1.8	5.0	56.8	2.8	12.7
Anonyx sp 2	22.1	16.5	0.9	8.7	41.8	9.4	23.8
G. zoea	20.2±14.7	12.8±3.1	8.0±0.1	-	38.4±13.3	-	2.5±1.8
M. tenuimana	25.8	26.8	14.4	30.8	0.0	2.1	1.8
M. curvirostra	43.5±22.7	13.4±4.8	16.9±12.5	21.5±37.2	-	15.9	3.4±1.6
N. robustus	31.3	33.0	26.6	-	6.2	-	1.2
P. borealis	7.2±5.0	39.5±13.2	23.7±6.6	-	-	-	0.3±0.3
P. tarda	22.1±18.6	41.4±12.7	17.1±14.2	0.2±0.4	-	0.0	2.9±2.9
S. hystrix	59.5±5.5	20.9±3.4	13.5±1.6	0.5±0.8	-	0.1±0.1	4.5±0.9
S. sculpta	44.5±27.9	22.5±16.5	24.4±11.1	-	-	-	2.3±1.7
T. libellula	19.1	29.7	12.4	12.6	18.6	-	-
Pycnogonida							
Nymphon spp.	37.0±27.0	33.5±21.4	12.3±4.3	11.2±3.0	-	1.0±0.2	4.2±4.8
Echinodermata							
Asteroidea							
A. americanus	73.6±9.8	2.2±1.3	9±1.9	1.1±1.0	0.1±0.2	0.2±0.0	8.3±1.1
Brisingida spp.	33.1±37.4	12.3±13.0	6.8±3.4	11.1±12.1	-	2.4±3.0	4.0±3.5
Cheiraster sp.	5.8	10.5	24.0	-	-	-	0.2
C. crispatus	40.5±5.7	17.7±5.9	20.2±0.9	-	-	-	2.0±0.3
F. microspina	53.3	10.9	6.1	7.4	-	1.2	8.7

	L. arcticus	54.9±7.3	12.3±1.1	16.6±3.4	8.1±12.2	-		3.3±0.3
	M. bairdi	39±21.3	25.7±15.6	22.2±3.6	0.5±0.8	-	0.1	1.9±1.3
	M. sol	67.0	9.1	7.6	1.1	-	0.2	8.9
	P. andromeda	63.6	9.0	8.2	3.2	-	0.4	7.8
	Z. fulgens	32.0±14.6	13.4±1.1	24.5±15.2	3.9±6.8	-	1.5	2.5±2.9
	Echinoidea							
	B. fragilis	53.0	11.9	-	1.6	-	-	-
	P. placenta	36.8±17.7	22.5±12.2	15.5±4.0	13.5±5.4		0.9±0.5	2.6±1.6
	S. pallidus	43±7.0	17.9±2	18±3.2	5.4±4.4	0.4±0.6	0.3±0.2	2.4±0.0
	Ophiuroidea							
	Gorgonocephalus sp.	45.8	11.7	5.5	5.2	-	0.9	8.3
	O. aculeata	38.6±6	13.9±8.7	5.8±0.9	27.9±5.8	0.5±0.1	4.8±0.3	6.8±2.0
	O. glacialis	32.1±43.6	18.8±15.3	5.7±8.0	29.5±26.6	-	4.3	0.1
	O. sarsii	61.5±9.6	14.6±9.5	17.7±1.5	-	-	-	3.5±0.7
Annelida								
	Polychaeta							
	A. succinea	17.6	26.2	12.4	11.3	20.7	0.9	1.4
	L. filicornis	43.5	15.7	18.3	10.0	6.1	0.5	2.4
	Nereididae sp 2	46.9	13.1	13.2	18.2	4.5	1.4	3.5
	Polychaeta sp 1	55.7	8.3	33.3	1.4	0.6	0.0	1.7
	Polynoidae sp 1	30.8	42.7	-	-	-	-	-
	Polynoidae sp 2	54.7±7.1	18.4±4.7	25.5±1.9	-	-	-	2.2±0.4
	Polynoidae sp 3	38.5	6.6	14.2	20.2	-	1.4	2.7
	Prionospio sp.	1.7	45.3	51.3	-	-	0.0	0.0
Cnidaria								
	Anthozoa							
	A. arbuscula	9.5±0.6	35.5±20.2	13.6±3.7	6.9±9.7	12.9±1.6	0.6±0.9	0.7±0.2
	A. cristata	29.5	21.4	16.7	-	-	-	1.8
	A. aurelia	18.3	34.8	26.9	-	13.8	-	0.7
	A. callosa	60.1	8.6	18.4	-	-	-	3.3
	A. agaricus	30.8±18.5	14.6±5.1	12.3±2.4	7.4±12.8	15.2±7.7	2.1	2.4±1.4
	Anthomastus sp.	46.4	12.1	14.9	4.2	8.5	0.3	3.1
	A. grandiflorum	17.9	42.5	7.6	5.6	7.6	0.7	2.4
	D. florida	34.4	5.3	8.4	2.3	1.8	0.3	4.1
	F. alabastrum	26.7±2.4	31.9±6.2	11.3±0.6	4.0±0.3	8.5±1.7	0.4±0.0	2.4±0.3
	Funiculina sp.	38.8	24.5	11.5	3.1	7.8	0.3	3.4
	P. arborea	29.7	7.7	6.3	2.2	24.4	0.4	4.7
	P. aculeata	27.7±1.5	24.3±12.3	12.9±2.4	6.6±2.7	10.4±3.2	0.5±0.1	2.2±0.4
	P. grandis	15.1±9.9	17.1±0.5	8±1.5	12.0±3.8	9.9±1.7	1.6±0.8	1.8±0.9
	Umbellula sp.	15.4	13.1	6.8	4.9	32.7	0.7	2.3
	Scyphozoa							

	A. wyvillei	44.7±24.0	8.6±0.6	12.9±3.0	-	15.0±5.7	-	3.8±2.7
	P. periphylla	27.8±29.8	14.6±5.3	10.3±4.1	12.4±17.5	25.2±18.0	1.7±2.4	2.3±2.0
Mollusca								
	Cephalopoda							
	B. arcticus	82.7±1.7	4.7±1.1	11.9±1.3	-	-	-	7.0±0.9
	B. bairdii	73.0	6.7	19.5	-	-	-	3.7
	Cephalopoda sp 1	67.6	16.7	13.7	-	-	-	4.9
	Cephalopoda sp 2	80.9	7.6	11	-	-	-	7.3
	C. veranii	85.9	0.5	13.5	-	-	-	6.4
	I. coindetii	69.5±9.8	15.4±4.3	11.5±2.9	2.5±3.0	-	0.3±0.2	6.5±2.8
	N. caroli	53.7	19.7	22.3	1.8	-	0.1	2.4
	R. megaptera	73.7	8.0	17.4	-	-	-	4.2
	S. syrtensis	63.4±14.8	16.5±6	17.5±5.9	-	-	-	4.2±2.4
	Gastropoda							
	A. occidentalis	48.2	27.7	22.7	0.9	-	0.0	2.1
	Buccinum sp.	57.2±13.7	22.5±8.6	19.9±5.3	-	-	-	3.1±1.6
	Colus spp.	55.7±12.4	22.0±11.3	21.0±3.9	-	-	-	2.7±1.0
	N. despecta	59.7	15.1	23.6	-	-	-	2.5
Porifera								
	Demospongiae							
	Cliona sp.	70.6	12.9	12.8	0.5	-	0.0	5.5
	C. cranium	60.5±4.5	15.3±0.6	16.0±2.9	1.3±1.8	-	0.1±0.1	3.9±0.8
	Geodia sp.	74.8	10.2	10.2	-	-	-	7.3
	Haliclona sp.	56.6±14.4	27.3±8.9	13.6±4.7	-	-	-	4.6±2.7
	H. carteri	24.3	7.4	32.9	8.3	-	0.3	0.7
	Histodermella sp.	39.3	24.0	20.3	4.5	7.5	0.2	1.9
	I. piceum	65.6	7.5	18.3	3.9	-	0.2	3.6
	M. lingua	47.4	14	22.2	0.8	-	0	2.1
	Phakellia sp.	49.9	9.7	20	2.7	-	0.1	2.5
	Polymastia spp.	18.2±20.8	29.8±6.9	21.7±1.71	11.5±9.8	11.7±3.08	0.5±0.4	0.9±1.0
	P. hemisphaerica	36.8	18.0	17.9	7.4	13.1	0.4	2.1
	Stelletta sp.	48	31.9	13.3	-	-	-	3.6
	S. ponderosus	28.4	19.1	25.7	7.2	8.6	0.3	1.1
	T. semisuberites	37.4	24.9	15.8	11.2	6.8	0.7	2.4
	T. muricata	37.7±20.5	16.0±3.3	21.8±6.84	10.8±5.8	-	0.5±0.1	2.1±1.6
	Hexactinellida							
	Euplectella sp.	32.7±3.7	11.1±6.1	9.9±5.72	8.5±8.1	10.5±14.8	1.3±1.6	4.1±2.7
	Hexactinellida sp 1	69.5	13.1	13.3	-	-	-	5.2
Sipuncula								
	Sipunculidea							
	Sipunculidea sp 1	69.2	7.9	21.1	-	-	-	3.3
	Sipunculidea sp 2	36.4	2.3	50.7	-	-	-	0.7

Appendix 7-4 Mean value of the sum of saturated FA (∑Sat), monounsaturated FA (∑MUFA), polyunsaturated FA (∑PUFA), ω3-FA (∑ω3), ω6-FA (∑ω6), and the sum of docosahexaenoic acid and eicosapentaenoic acids (DHA+EPA) retrieved from the species studied. Materials and Methods of Chapter 3 gives the fatty acids considered in these sums.

Phylum	Taxon	n	∑Sat	∑MUFA	∑PUFA	∑ω3	∑ω6	DHA+EPA
			% ±sd	% ±sd	% ±sd	% ±sd	% ±sd	g per 100 g wm ±sd
Chordata								
	Actinopterygii							
	A. bairdii	2	29.3±16.7	43.6±20.1	26.5±3.7	22.9±5.4	1.8±0.3	0.5±0.3
	A. cornuta	3	14.6±3.8	74.1±6.2	10.7±2.2	7.4±3.1	1.4±0.3	0.7±0.2
	A. rostrata	3	29.1±4.8	17.1±1.8	53.6±3.1	49.4±3.6	2.9±1.0	0.1±0.0
	A. risso	2	17.8±6.8	37.5±10.5	40.4±5.4	30.4±8.9	2.4±0.9	0.6±0.4
	B. euryops	2	20.2±1.4	51.5±4.3	27.6±5.9	23.6±6.1	2.2±0.6	0.3±0.1
	B. macrolepis	2	19.0±3.3	40.7±7.2	39.9±3.9	35.7±4.4	3.0±0.1	0.1±0.0
	B. antarcticus	4	17.1±4.7	48.9±17.5	33.2±13.5	27.2±11.2	2.6±0.9	0.2±0.1
	C. macropus	1	1.1	7.2	51.8	19.1	2.5	0.1
	C. sloani	6	18.2±3	60.0±8.5	20.9±6.8	17.3±7	2.2±0.3	0.1±0.1
	C. niger	3	18.4±1.7	65.6±5.3	15.3±3.7	11.6±3.7	1.6±0.1	4.1±2.4
	C. rupestris	3	23.5±2.7	28.0±8.8	47.1±6.1	43.5±5.7	1.8±0.6	0.1±0.0
	C. microps	2	21.3±2.1	28.0±6.2	50.3±3.9	38.8±2.8	10.9±7.2	0.2±0.2
	C. thomsonii	1	11.7	73.1	14.4	10.0	2.7	0.1
	C. microdon	2	18.4±0.8	59.5±1.1	21.3±1.9	17.3±1.4	2.1±0.2	0.3±0.1
	G. ensis	4	24.1±1.8	20.6±2.6	54.9±4	51.1±2.8	3.2±1.1	0.1±0.0
	G. cynoglossus	3	32.6±12.0	17.8±2.3	48.8±10.1	36.3±11.7	9.2±0.9	0.1±0.0
	H. mollis	1	29.9	22.4	47.5	43.8	3.2	0.1
	L. speculigera	1	15.0	44.3	38.6	28.9	2.2	0.8
	Lampanyctus spp.	4	13.9±4.4	55.9±14.3	28.7±9.4	24.1±10.1	1.5±0.8	0.4±0.2
	L. eques	1	33.3	13.8	52.6	49	3.3	0.2
	M. berglax	5	35.0±8.1	13±1.5	51.5±7.8	44.7±6.5	6.3±1.8	0.1±0.0
	M. atlantica	2	24.8±1.0	47.5±1.3	26.7±2.1	22.8±1.7	2.2±0.2	0.8±0.1
	M. niger	2	20.5±1.1	58.6±3.5	19.9±2.4	15.8±2.5	2.1±0.1	0.7±0.1
	M. johnsoni	1	16.4	60.9	22.3	18.5	2.7	0.1
	Myctophum sp.	1	23.8	46.1	29.0	24.4	2.0	3.2
	N. bairdii	3	21.7±3.3	20.4±6.0	40.4±16.0	36.7±15.1	2.5±0.9	0.1±0.1
	N. chemnitzii	3	21.7±3.3	46.6±12.5	30±10.9	22.6±11.9	4.3±2.1	0.1±0.0
	Notoscopelus spp.	2	16.2±2.7	56.8±14.9	26.5±12.2	14.1±1.1	1.8±1.0	2.7±0.4
	O. macrosteus	1	17.1	65.1	17.3	13.2	1.3	0.0
	P. rissoanus	3	20.1±0.5	55.4±3.1	23.3±2.9	19.2±3.2	2.7±0.1	0.4±0.2
	R. hippoglossoides	2	19.1±0.7	66.2±3.2	14.0±3.9	10.3±3.3	1.5±0.1	0.6±0.5

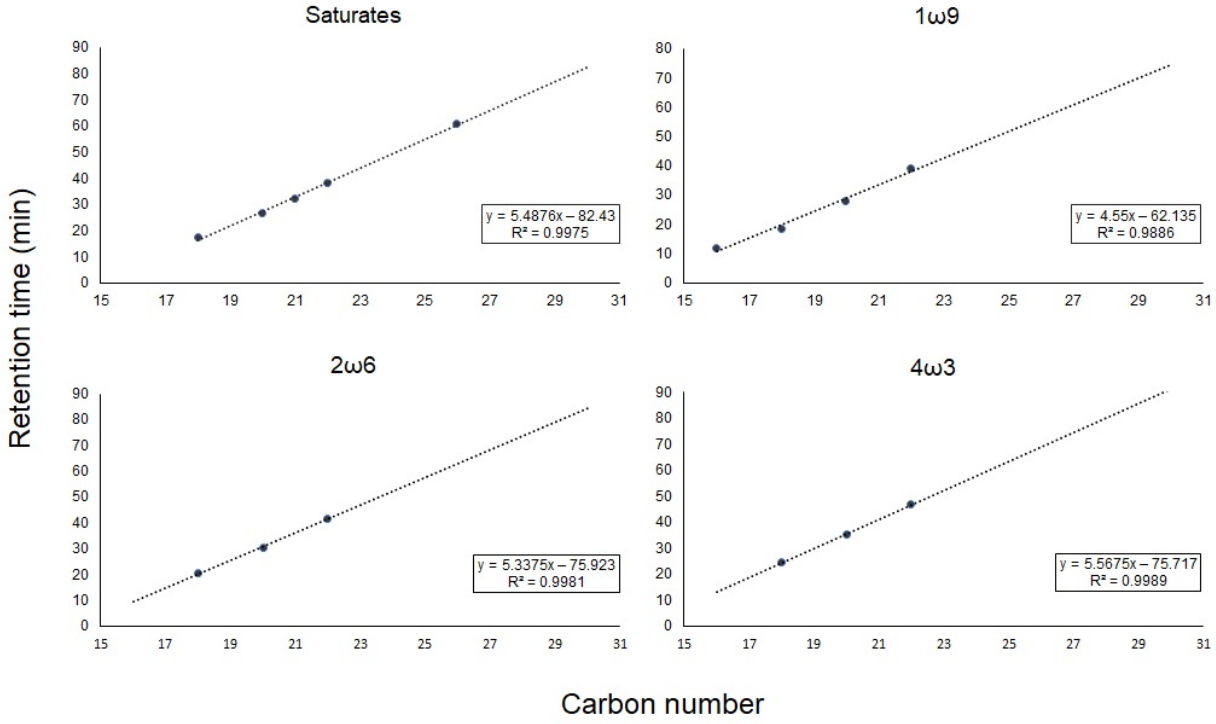
	S. opisthopterus	2	22.0±2.8	51.6±0.3	25.7±2.3	20.0±1	2.5±0.3	1.6±0.1
	S. lepidus	1	18.8	61.1	18.8	15.5	1.5	0.5
	S. mentella	3	24.8±6	32.4±12.2	42.5±6.2	38.07±6.2	2.7±0.5	0.3±0.0
	S. beanii	3	25.8±3.5	28.4±3.5	45.4±1.9	41.6±1.6	2.7±0.3	0.2±0.1
	S. kaupii	3	13.9±0.8	72.6±2.5	12.7±1.9	9.4±1.9	1.6±0.1	0.9±0.6
	T. murrayi	3	30.2±7	18.9±2.9	50.5±4.4	44.1±3.6	5.5±0.5	0.1±0.0
	X. copei	4	22.4±1.1	49.0±2.0	27.4±1.2	21.8±1.3	2.5±0.4	0.4±0.1
	Ascidiacea							
	Ascidiacea sp 1	4	24.2±7.7	46.0±2.9	24.4±7.1	11.1±4.1	8.2±4.4	0.0
	Ascidiacea sp 2	1	24.5	53.8	19.4	9.7	7.6	0.0±0.0
	Ascidiacea sp 3	1	21.8	26	40.9	27.7	6.6	0.0
	Ascidiacea sp 4	2	17.0±0.4	35.5±3.6	41.4±2.8	27.5±1.1	12.8±2.1	0.0
	Didemnum sp.	1	18.9	36.7	39.5	30.2	8.8	0.0±0.0
	E. vitreum	1	21.2	47.2	23.7	16	4.8	0.0
	Chondrichthyes							
	A. jenseni	1	26.9	23.9	48.6	40.5	6.7	0.2
	A. profundorum	3	23.9±4.5	27.1±6.8	48.2±9	41.8±8.3	4.6±0.9	0.2±0.0
	C. fabricii	2	23.3±0.3	30.7±0.1	45.5±0.5	38.2±1.3	5.1±1.3	0.1±0.0
	M. senta	1	8.0	56.0	31.9	27.0	1.5	0.1
	R. fyllae	4	25.7±1.8	40.6±7.1	32.7±5.4	25.9±5.1	5.1±0.5	1.9±1.2
Arthropoda								
	Hexanauplia							
	A. michelottianum	3	16.2±2.2	47.1±8.1	35.8±7.8	32.1±7.7	2.1±0.2	0.2±0.1
	Malacostraca							
	A. pelagica	3	8.9±2.4	52.6±5	35.6±5.8	32.5±4.8	1.6±0.2	0.2±0.1
	Anonyx sp 1	1	2.0	57.6	38.4	11.1	0.8	0.2
	Anonyx sp 2	1	5.3	55.5	26.1	7.0	0.7	0.8
	G. zoea	3	9.3±1.2	55.5±10.6	31.1±13.3	27.0±12.6	1.5±0.2	0.2±0.1
	M. tenuimana	1	20.0	38.1	41.5	36.4	2.2	0.0
	M. curvirostra	3	17.5±0.4	54.3±11.5	27.8±11.2	25.1±11.8	2.0±0.7	0.4±0.4
	N. robustus	1	19.7	31.5	48.5	44.6	2.1	0.1
	P. borealis	3	23.0±6.4	34.3±3.5	42.1±8.8	37.8±9.3	3.4±1	0.2±0.0
	P. tarda	3	12.6±8.5	41.9±4	41.1±2.7	36.7±2.1	2.7±0.2	0.2±0.1
	S. hystrix	3	18.1±1.1	38.7±2.7	41.5±1.8	34.8±1.4	4.3±0.5	0.2±0.1
	S. sculpta	3	23.8±3.9	37.4±5	38.7±8.6	27.7±14	10.1±11.3	0.1±0.0
	T. libellula	1	12.5	38.0	39.2	17.9	3.1	0.1
	Pycnogonida							
	Nymphon spp.	6	22.0±7.2	37.9±5.9	38.0±5.0	31.2±5.3	4.8±1.1	0.1±0.1
Echinodermata								
	Asteroidea							
	A. americanus	3	13.0±1.9	42.8±10.3	49.8±1.8	23.9±1.8	22.7±1.6	0.3±0.2

	Brisingida spp.	2	7.9±3.6	42.2±10.3	47.2±10.8	27.1±5.1	0.9±0.9	0.4±0.3
	Cheiraster sp.	1	17.5	37.3	28.1	21.6	9.9	0.0
	C. crispatus	3	23.3±20.6	39.5±11.7	38.7±16.5	7.1±0.7	24.4±18.9	0.0±0.0
	F. microspina	1	10.0	39.3	44.4	7.4	17.3	1.0
	L. arcticus	3	13.2±1.4	45.2±1.6	42.2±3.6	23.5±7.2	9.3±2.5	0.1±0.0
	M. bairdi	3	6.6±1.6	43.2±3.3	44.6±4.8	13.9±10.5	28.8±5.8	0.0±0.0
	M. sol	1	12.2	48.6	60.5	8.2	29.5	0.0
	P. andromeda	2	12.1±1.0	31.9±8.3	39.5±14.7	16.4±5.2	28.2±1.1	0.4±0.6
	Z. fulgens	3	10.1±4.5	52±13.1	42.8±12.0	23.4±8.0	9.3±4.2	0.2±0.3
	Echinoidea							
	B. fragilis	1	27.9	40.9	45.3	33.2	11.3	0.0
	P. placenta	3	16.5±1.6	38.6±10.7	36.6±2.7	15.6±3.9	16.4±1.4	0.1±0.0
	S. pallidus	2	20.1±0.3	36.7±9.4	45.1±4.3	21.8±10.8	9.9±3.0	0.0±0.0
	Ophiuroidea							
	Gorgonocephalus sp.	1	11.3	34.1	38.1	23.5	1.6	0.5
	O. aculeata	2	19±0.7	46.9±3.0	34.1±2.2	28.7±5.2	3.5±1.4	0.2±0.2
	O. glacialis	2	17.7±6.6	47.3±0.2	26.2±0.2	20.4±2.7	2.6±1.0	0.2±0.0
	O. sarsii	3	18.8±8.3	54.8±10.9	28.4±0.7	14.8±1.9	9±0.6	0.0±0.0
Annelida								
	Polychaeta							
	A. succinea	1	15.5	37.5	44.5	33.2	3.9	0.2
	L. filicornis	1	23.9	34.9	40.2	19.8	2.6	0.1
	Nereididae sp 1	1	19.5	39.5	40.2	22.4	8.4	0.1
	Nereididae sp 2	1	18.0	40.5	37.5	27.2	4.1	0.3
	Polynoidae sp 1	1	17.8	41.9	39.6	28.9	6.0	0.1
	Polynoidae sp 2	2	21.1±0.7	30.3±0.7	48.1±0.2	38±1.6	4.9±0.3	0.1±0.0
	Polynoidae sp 3	1	29.0	46.5	22.9	14.7	2.5	0.1
	Prionospio sp.	1	18.0	47.6	34.2	26.2	4.4	0.1
Cnidaria								
	Anthozoa							
	A. arbuscula	3	16.2±1.4	44.0±2.4	35.3±4	20.0±4.4	12.3±2.6	0.0±0.0
	A. cristata	2	21.2±9.2	54.0±9.6	17.6±20.6	7.2±10.2	0.3±0.1	0
	A. aurelia	3	21.8±5.1	40.1±9.9	40.1±3.3	18.9±8.3	10.3±13.8	0.0±0.0
	A. callosa	3	16.4±3.2	41.7±17.4	39.2±14	26.1±9.4	6.5±4.3	0.0±0.0
	A. agaricus	3	15.6±3.8	51.3±11.3	27.0±3.0	15.8±15.3	17.4±0.3	0.0±0.0
	Anthomastus sp.	1	15.8	48.6	49.8	12.3	8.8	0.1
	A. grandiflorum	1	16.1	31.9	31.0	38.4	4.2	0.6
	D. florida	1	18.0	51.6	49.1	25.7	40.4	0.0
	F. alabastrum	2	17.9±2.3	31.5±9.9	41±0.2	13.6±8.4	19±1.2	0.1±0.0
	Funiculina sp.	1	15.2	38.3	43.4	22.0	3.3	0.2
	P. arborea	1	11.3	40.1	45.9	36.9	9.5	0.1

	P. aculeata	3	11.9±1.3	49.0±8.1	35.7±6.6	19.9±10.7	20.6±4.8	0.1±0.1
	P. grandis	2	13±0.6	47.6±1.1	36.4±1.3	22.5±8.6	6.3±2.8	0.2±0.1
	Umbellula sp.	1	5.1	49.0	46.6	26.8	3.2	0.4
	Scyphozoa							
	A. wyvillei	3	24.4±2.3	42.1±5.5	36.7±4.8	27.7±4.1	4.9±0.9	0.0±0.0
	P. periphylla	4	23.6±2.7	46.3±6.3	28.7±7.0	19.6±4.3	2.7±0.7	0.0±0.0
	Scyphozoa sp.	1	18.1	49.0	25.1	21.2	2.2	0.0
Mollusca								
	Cephalopoda							
	B. arcticus	3	26.6±0.7	24.4±20.9	58.9±3.6	37.8±30.1	2.5±0.4	0.2±0.1
	B. bairdii	1	30.3	18.6	31.9	48.9	3.6	0.1
	Cephalopoda sp 1	1	25.8	16.0	55.8	24.5	0.8	0.3
	Cephalopoda sp 2	1	25.1	18.3	55.4	53.5	1.6	0.3
	C. veranii	1	25.4	37.5	54.9	24.6	2.3	0.4
	I. coindetii	3	21.7±0.8	18.4±0.9	60.3±0.8	55.3±3.7	1.8±0.7	0.4±0.1
	N. caroli	1	24.4	17.9	60.0	55.0	1.3	0.2
	R. megaptera	1	55.3	15.4	28.3	55.5	0.5	0.1
	S. syrtensis	3	34.7±18.3	19.3±3.5	45.9±16.3	50.5±3.8	2.8±0.3	0.1±0.0
	Gastropoda							
	A. occidentalis	1	15.5	16.7	59.8	22.7	10.0	0.1
	Buccinum sp.	3	21.8±1.0	21.8±1.8	55.9±1.0	38.9±1.6	5.7±0.8	0.1±0.0
	Colus spp	3	21.6±0.5	20.7±1.0	56.9±1.7	36.1±4.6	16.7±5.4	0.0±0.0
	N. despecta	1	36.9	20.4	43.8	25.0	16.6	0.0
Porifera								
	Demospongiae							
	Cliona sp.	1	10.5	18.5	9.4	13.2	0.3	0.0
	C. cranium	3	13.3±1.6	59.0±4.0	5±1.8	4.0±4.0	0.6±0.3	0.0±0.0
	Geodia sp.	1	27.6	68.5	24.1	2.3	0.1	0.0
	Haliclona sp.	2	23.7±4.7	28.6±24.8	36±39.8	4.4±3.4	0.2±0.2	0.0
	H. carteri	1	13.9	53.4	10.9	4.1	0.0	0.0
	Histodermella sp.	1	38.3	63.6	12.0	12.0	5.2	0.1
	I. piceum	1	11.1	49.7	1.2	12.3	6.0	0.1
	Phakellia sp.	1	29.8	73.2	4.4	29.6	2.3	0.0
	Polymastia spp.	2	16.8±0.5	59.2±8.4	29.2±12.2	4.9±2.5	2.6±3.6	0.1±0.1
	R. hemisphaerica	1	55.2	40.5	11.8	26.8	2.7	0.0
	Stelletta sp.	1	26.4	31.2	3.3	13.8	0.0	-
	S. ponderosus	1	13.6	70.3	33.7	0.5	4.8	0.1
	T. semisuberites	1	21.6	42.4	18.0	30.7	6.8	0.2
	T. muricata	4	20.2±9.6	52.4±14.6	25.0±10.1	16.1±5.1	3.0±2.6	0.0±0.0
	Hexactinellida							
	Euplectella sp.	1	22.6	39.1	28.9	27.6	1.8	0.1

	Hexactinellida sp 1	1	9.0	43.2	68.1	20.9	0.2	0.0
	Hexactinellida sp 2	1	18.4	20.5	28.7	1.0	0.2	0.0
Sipuncula								
	Sipunculidea							
	Sipunculidea sp 1	1	23.4	65.2	50.4	4.1	12.3	0.1
	Sipunculidea sp 2	1	29.9	24.1	17.7	19.3	4.2	0.0

Appendix 7-5 Plots for saturated, :1ω9, :2ω6, and :4ω3 unsaturated fatty acids used to identify very long chain FA in corals and sponges. The X axis represents the FA carbon number, whereas the Y axis gives relative retention times (min). Each plot also gives the regression equation and coefficient of determination (R^2).



Appendix 7-6 Summary results of the Spearman correlation test between depth and various biological parameters conducted on a dataset of 89 observations. Spearman correlation coefficient (r_s) and *p-value* (*p*) are reported, and significant correlations are marked in bold.

Variable	r_s	*p*
$\delta^{15}N$	-0.12	0.248
$\delta^{13}C_n$	-0.04	0.725
%N	0.20	0.055
%C	**0.24**	**0.022**
C:N	-0.07	0.492
$C:N_{mol}$	-0.07	0.492
Lipid content	0.16	0.142
Trophic position	-0.12	0.248
14:0	**-0.23**	**0.032**
i15:0	-0.10	0.346
16:0	-0.04	0.733
16:1ω9?	-0.08	0.445
16:1ω7	-0.06	0.557
16:1ω5	-0.12	0.256
i17:0	-0.15	0.165
ai17:0	-0.06	0.584
17:1	-0.06	0.599
16:3ω3?	0.11	0.326
18:0	-0.09	0.424
18:1ω9	**0.34**	**0.001**
18:1ω7	-0.04	0.743
18:1ω5?	0.05	0.672
18:2ω6	-0.18	0.093
18:5ω3	0.13	0.235
20:1ω11?	0.15	0.149
20:1ω9	0.13	0.239
20:1ω7?	-0.04	0.685
20:2b?	-0.04	0.718
20:2ω6	-0.11	0.310
20:4ω6	**0.23**	**0.031**
20:5ω3	-0.01	0.926
22:0	-0.11	0.289
22:1ω11(13)	0.10	0.337
22:1ω9	0.13	0.223
22:1ω7	**-0.30**	**0.005**
22:2NIMDa?	**-0.23**	**0.032**
23:0	0.09	0.422
22:4ω6?	0.06	0.594
22:5ω6	0.01	0.894
22:6ω3	0.03	0.763
24:1	**-0.29**	**0.005**

Appendix 7-7 Dataset applied to test for latitudinal gradients in the isotopic composition of deep-sea benthic organisms. Values of $\delta^{15}N$ and $\delta^{13}C$ are expressed in parts per thousand (‰). For the polar latitudes, data were provided by Iken et al. (2005) and Bergmann et al. (2009). For temperate regions, data from Sherwood et al. (2008), Carlier et al. (2009), Boyle et al. (2012), Fanelli et al. (2013), Gale et al. (2013), Kharlamenko et al. (2013), Papiol et al. (2013), Preciado et al. (2017), and Parzanini et al. (Chapter 4) were used. Finally, data for tropical latitudes were obtained from Jeffreys et al. (2009).

Latitude	Ocean/Ocean region	Class	Species	$\delta^{15}N$	$\delta^{13}C$
Polar	Svalbard	Hexactinellida	*Caulophacus arcticus*	12.2	-21.2
Polar	Svalbard	Demospongiae	Axinellidae sp.	7.8	-23.0
Polar	Svalbard	Demospongiae	*Cladorhiza gelida*	9.1	-19.5
Polar	Svalbard	Demospongiae	*Cladorhiza gelida*	9.6	-18.2
Polar	Svalbard	Demospongiae	*Cladorhiza gelida*	11.2	-21.2
Polar	Svalbard	Demospongiae	*Esperiopsis* sp.	11.3	-21.3
Polar	Svalbard	Demospongiae	*Esperiopsis* sp.	16.6	-19.5
Polar	Svalbard	Demospongiae	*Tentorium semisuberites*	13.0	-21.8
Polar	Svalbard	Hydrozoa	*Thecate hydroid*	7.6	-23.1
Polar	Svalbard	Hydrozoa	*Turbulariidae* cf. *Bouillonia cornucopia*	6.3	-22.7
Polar	Svalbard	Anthozoa	*Gersemia rubiformis*	8.9	-21.7
Polar	Svalbard	Anthozoa	*Gersemia rubiformis*	11.2	-21.9
Polar	Svalbard	Anthozoa	Acontiaria gen. et sp. nov.	10.8	-21.6
Polar	Svalbard	Anthozoa	*Hormathiidae* cf. *Amphianthus sp.*	12.2	-22.0
Polar	Svalbard	Anthozoa	*Kadosactis rosea*	15.3	-19.1
Polar	Svalbard	Anthozoa	*Amphianthus* sp 1	11.4	-21.8
Polar	Svalbard	Anthozoa	*Amphianthus* sp 2	11.3	-22.1
Polar	Svalbard	Anthozoa	*Amphianthus* sp 4, Isophelliidae gen.1	9.8	-22.6
Polar	Svalbard	Anthozoa	*Bathyphellia margaritacea*	11.2	-21.4
Polar	Svalbard	Anthozoa	*Bathyphellia margaritacea*	11.9	-21.9
Polar	Svalbard	Anthozoa	*Bathyphellia margaritacea*	16.6	-19.6
Polar	Svalbard	Nemertea	Nemertea	12.4	-19.0
Polar	Svalbard	Priapulidae	*Priapulus caudatus*	13.8	-17.2
Polar	Svalbard	Gastropoda	*Cryptonatica affinis*	9.7	-19.1
Polar	Svalbard	Gastropoda	*Mohnia mohni*, *Tacita danielsseni*	13.4	-18.2
Polar	Svalbard	Gastropoda	*Mohnia mohni*, *Tacita danielsseni*	10.9	-19.0
Polar	Svalbard	Gastropoda	*Mohnia mohni*, *Tacita danielsseni*	13.2	-18.0
Polar	Svalbard	Gastropoda	*Mohnia mohni*, *Tacita danielsseni*	10.6	-19.7
Polar	Svalbard	Bivalvia	*Katadesmia kolthoffi*	10.5	-19.7
Polar	Svalbard	Polychaeta	*Bylgides* cf. *groenlandica*	11.8	-19.8

Polar	Svalbard	Polychaeta	*Nereis* cf. *gracilis*	15.8	-18.0
Polar	Svalbard	Polychaeta	*Praxillura longissima*	15.1	-21.3
Polar	Svalbard	Echiurida	*Hamingia arctica*	12.9	-18.0
Polar	Svalbard	Echiurida	*Hamingia arctica*	14.7	-16.0
Polar	Svalbard	Pantopoda	*Ascorhynchus abyssi*	9.7	-21.6
Polar	Svalbard	Pantopoda	*Colossendeis proboscidea*	12.2	-18.0
Polar	Svalbard	Pantopoda	*Colossendeis proboscidea*	9.5	-19.7
Polar	Svalbard	Hexanauplia	*Verum striolatum*	11.3	-21.5
Polar	Svalbard	Hexanauplia	*Verum striolatum*	10.0	-22.2
Polar	Svalbard	Malacostraca	*Eurythenes gryllus*	14.8	-22.1
Polar	Svalbard	Malacostraca	*Eurythenes gryllus*	12.8	-22.8
Polar	Svalbard	Malacostraca	*Eurythenes gryllus*	12.7	-21.7
Polar	Svalbard	Malacostraca	*Halirages quadridentatus*	10.0	-21.3
Polar	Svalbard	Malacostraca	*Haploops* sp.	8.7	-22.1
Polar	Svalbard	Malacostraca	*Liljeborgia fissicornis*	10.9	-19.8
Polar	Svalbard	Malacostraca	*Lysianassidae* cf. *tryphosa*	14.2	-23.4
Polar	Svalbard	Malacostraca	*Unciola sp.*	5.6	-21.1
Polar	Svalbard	Malacostraca	*Diastylis* spp.	5.4	-21.6
Polar	Svalbard	Malacostraca	*Caecognathia stygia*	9.7	-21.9
Polar	Svalbard	Malacostraca	*Caecognathia stygia*	10.4	-22.1
Polar	Svalbard	Malacostraca	*Mesidothea megalura*	11.3	-19.6
Polar	Svalbard	Malacostraca	*Mesidothea megalura*	10.5	-19.6
Polar	Svalbard	Malacostraca	*Bythocaris* spp.	15.2	-18.3
Polar	Svalbard	Malacostraca	*Bythocaris* spp.	13.0	-19.6
Polar	Svalbard	Malacostraca	*Bythocaris* spp.	13.2	-19.4
Polar	Svalbard	Malacostraca	*Bythocaris* spp.	13.8	-19.7
Polar	Svalbard	Crinoidea	*Bathycrinus* cf. *carpenteri*	8.3	-23.1
Polar	Svalbard	Crinoidea	*Bathycrinus* cf. *carpenteri*	10.4	-22.2
Polar	Svalbard	Crinoidea	*Bathycrinus* cf. *carpenteri*	11.0	-22.8
Polar	Svalbard	Asteroidea	*Bathybiaster vexillifer*	15.8	-17.1
Polar	Svalbard	Asteroidea	*Bathybiaster vexillifer*	16.8	-17.1
Polar	Svalbard	Asteroidea	*Bathybiaster vexillifer*	17.0	-17.1
Polar	Svalbard	Asteroidea	*Hymenaster pellucidus*	13.1	-19.5
Polar	Svalbard	Asteroidea	*Hymenaster pellucidus*	11.9	-19.9
Polar	Svalbard	Asteroidea	*Hymenaster pellucidus*	24.6	-19.5
Polar	Svalbard	Asteroidea	*Poraniomorpha tumida*	21.3	-17.3
Polar	Svalbard	Holothuroidea	*Elpidia heckeri*	6.0	-22.2
Polar	Svalbard	Ascidiacea	Ascidiacea	13.1	-18.9
Polar	Canadian Arctic	Bivalvia	*Bathyarca* sp.	12.7	-22.3
Polar	Canadian Arctic	Bivalvia	Thyasiridae	12.4	-21.2
Polar	Canadian Arctic	Bivalvia	*Dacrydium* cf. *viteum*	10.2	-20.1
Polar	Canadian Arctic	Bivalvia	Yoldiidae	14.2	-18.8
Polar	Canadian Arctic	Gastropoda	*Limacina helicina*	5.9	-22.4
Polar	Canadian Arctic	Scaphopoda	*Siphonodentalium lobatum*	10.7	-21.0
Polar	Canadian Arctic	Polychaeta	Polychaeta sp 1	15.3	-19.0
Polar	Canadian Arctic	Polychaeta	Polychaeta sp 2	15.5	-20.7
Polar	Canadian Arctic	Polychaeta	Polychaeta sp 3	10.2	-21.3
Polar	Canadian Arctic	Polychaeta	Polychaeta sp 4	13.7	-19.5

Polar	Canadian Arctic	Polychaeta	Polychaeta sp 5	13.4	-19.7
Polar	Canadian Arctic	Polychaeta	Polychaeta sp 6	8.6	-20.3
Polar	Canadian Arctic	Polychaeta	*Ophelina cylindricaudata*	15.3	-19.5
Polar	Canadian Arctic	Polychaeta	*Dorvillea* cf. *rudolphi*	16.2	-20.7
Polar	Canadian Arctic	Polychaeta	*Prionospio* sp.	14.5	-19.8
Polar	Canadian Arctic	Polychaeta	*Chaetozone setosa*	15.3	-23.3
Polar	Canadian Arctic	Polychaeta	*Glycinde wireni*	17.7	-21.4
Polar	Canadian Arctic	Polychaeta	*Nephtys* cf. *malmgreni*	17.4	-17.1
Polar	Canadian Arctic	Polychaeta	*Terebellides stroemi*	16.5	-19.0
Polar	Canadian Arctic	Polychaeta	Nereidae	17.4	-17.4
Polar	Canadian Arctic	Echiura	Echiura sp 1	16.6	-17.8
Polar	Canadian Arctic	Ophiuroidea	Ophiuroidea sp 1	11.7	-23.5
Polar	Canadian Arctic	Ophiuroidea	Ophiuroidea sp 2	12.2	-20.8
Polar	Canadian Arctic	Crinoidea	Crinoidea sp 1	6.9	-18.0
Temperate	Northwest Atlantic	Polychaeta	*Laetmonice filicornis*	13.7	-16.0
Temperate	Northwest Atlantic	Polychaeta	Nereididae sp.	13.9	-17.1
Temperate	Northwest Atlantic	Polychaeta	Polychaeta sp 1	13.2	-15.7
Temperate	Northwest Atlantic	Polychaeta	Polychaeta sp 2	11.1	-19.0
Temperate	Northwest Atlantic	Polychaeta	Polychaeta sp 3	7.9	-15.8
Temperate	Northwest Atlantic	Polychaeta	Polynoidae sp 1	12.6	-17.8
Temperate	Northwest Atlantic	Polychaeta	Polynoidae sp 2	12.0	-17.5
Temperate	Northwest Atlantic	Malacostraca	*Acanthephyra pelagica*	9.4	-20.1
Temperate	Northwest Atlantic	Malacostraca	*Notostomus robustus*	11.6	-19.8
Temperate	Northwest Atlantic	Malacostraca	*Pandalus borealis*	11.1	-19.1
Temperate	Northwest Atlantic	Malacostraca	*Pasiphaea tarda*	11.4	-19.2
Temperate	Northwest Atlantic	Malacostraca	*Sabinea hystrix*	14.9	-16.4
Temperate	Northwest Atlantic	Malacostraca	*Stereomastis sculpta*	12.6	-19.2
Temperate	Northwest Atlantic	Hexanauplia	*Arcoscalpellum michelottianum*	13.3	-18.9
Temperate	Northwest Atlantic	Anthozoa	*Actinauge cristata*	11.1	-18.6
Temperate	Northwest Atlantic	Anthozoa	*Actinoscyphia aurelia*	11.7	-16.8
Temperate	Northwest Atlantic	Anthozoa	*Actinostola callosa*	10.8	-18.6
Temperate	Northwest Atlantic	Anthozoa	*Anthomastus* spp.	11.1	-13.9
Temperate	Northwest Atlantic	Anthozoa	*Anthoptilum grandiflorum*	11.6	-19.5
Temperate	Northwest Atlantic	Anthozoa	*Paragorgia arborea*	10.4	-11.5
Temperate	Northwest Atlantic	Anthozoa	*Funiculina* sp.	10.9	-14.8
Temperate	Northwest Atlantic	Anthozoa	*Pennatula aculeata*	10.3	-8.6
Temperate	Northwest Atlantic	Anthozoa	*Pennatula grandis*	10.5	-13.1
Temperate	Northwest Atlantic	Anthozoa	*Umbellula* sp.	11.7	-19.3
Temperate	Northwest Atlantic	Anthozoa	*Freyella microspina*	12.3	-9.1
Temperate	Northwest Atlantic	Asteroidea	*Leptychaster arcticus*	12.4	-9.6
Temperate	Northwest Atlantic	Asteroidea	*Mediaster bairdi*	16.0	-8.2
Temperate	Northwest Atlantic	Asteroidea	*Myxaster sol*	16.8	-12.4
Temperate	Northwest Atlantic	Asteroidea	*Psilaster andromeda*	16.9	-13.0
Temperate	Northwest Atlantic	Asteroidea	*Zoroaster fulgens*	14.1	-6.3
Temperate	Northwest Atlantic	Echinoidea	*Phormosoma placenta*	12.3	-14.3
Temperate	Northwest Atlantic	Ophiuroidea	*Gorgonocephalus* sp.	11.8	-14.5
Temperate	Northwest Atlantic	Gastropoda	*Buccinum* sp.	12.6	-17.0
Temperate	Northwest Atlantic	Gastropoda	*Colus islandicus*	14.2	-15.0

Temperate	Northwest Atlantic	Gastropoda	*Neptunea despecta*	15.2	-15.4
Temperate	Northwest Atlantic	Demospongiae	*Cliona* sp.	7.7	-17.4
Temperate	Northwest Atlantic	Demospongiae	*Craniella cranium*	7.5	-18.5
Temperate	Northwest Atlantic	Demospongiae	*Iophon piceum*	16.5	-18.0
Temperate	Northwest Atlantic	Demospongiae	*Geodia* sp.	8.7	-17.1
Temperate	Northwest Atlantic	Demospongiae	*Stelletta* spp.	8.0	-17.2
Temperate	Northwest Atlantic	Demospongiae	*Phakellia* spp.	14.9	-15.8
Temperate	Northwest Atlantic	Demospongiae	*Hamacantha (Vomerula) carteri*	15.4	-14.6
Temperate	Northwest Atlantic	Demospongiae	*Haliclona* sp.	7.9	-17.1
Temperate	Northwest Atlantic	Demospongiae	*Polymastia* spp.	14.8	-16.7
Temperate	Northwest Atlantic	Demospongiae	*Tentorium semisuberites*	15.5	-17.6
Temperate	Northwest Atlantic	Demospongiae	*Thenea muricata*	14.2	-17.4
Temperate	Northwest Atlantic	Hexactinellida	Hexactinellida sp.	6.8	-17.3
Temperate	Northwest Atlantic	Hexactinellida	*Euplectella* spp.	12.9	-16.3
Temperate	Northwest Atlantic	Sipunculidea	Sipunculidea sp 1	11.4	-16.0
Temperate	Northwest Atlantic	Sipunculidea	Sipunculidea sp 2	15.0	-15.3
Temperate	eastern North Pacific	Anthozoa	Hormathiidae	14.0	-18.1
Temperate	eastern North Pacific	Anthozoa	Hormathiidae	15.3	-15.8
Temperate	eastern North Pacific	Holothuroidea	*Parastichopus leukothele*	13.5	-16.6
Temperate	eastern North Pacific	Holothuroidea	*Pseudostichopus mollis*	14.6	-17.6
Temperate	eastern North Pacific	Holothuroidea	*Pseudostichopus mollis*	15.5	-15.5
Temperate	eastern North Pacific	Holothuroidea	*Scotoplanes* sp.	13.2	-16.0
Temperate	eastern North Pacific	Holothuroidea	*Scotoplanes* sp.	14.2	-16.8
Temperate	eastern North Pacific	Holothuroidea	*Scotoplanes* sp.	15.2	-17.6
Temperate	eastern North Pacific	Gastropoda	*Neptunea* sp.	15.5	-17.7
Temperate	eastern North Pacific	Asteroidea	*Crossaster borealis*	18.4	-15.9
Temperate	eastern North Pacific	Asteroidea	*Luidia* spp.	18.3	-17.4
Temperate	eastern North Pacific	Malacostraca	*Chionoecetes tanneri*	13.8	-19.5
Temperate	eastern North Pacific	Malacostraca	*Chionoecetes tanneri*	13.2	-19.7
Temperate	eastern North Pacific	Malacostraca	*Euphausia pacifica*	13.9	-20.3
Temperate	eastern North Pacific	Malacostraca	*Euphausia pacifica*	10.2	-20.8
Temperate	eastern North Pacific	Polychaeta	Onuphidae	16.2	-18.6
Temperate	eastern North Pacific	Malacostraca	*Eualus biunguis*	14.9	-18.0
Temperate	eastern North Pacific	Malacostraca	*Eualus biunguis*	15.2	-19.3
Temperate	eastern North Pacific	Malacostraca	*Eualus macrophthalmus*	15.8	-18.9
Temperate	eastern North Pacific	Malacostraca	*Eualus macrophthalmus*	14.5	-18.9
Temperate	central Mediterranean	Demospongiae	*Pachastrella monilifera*	7.4	-17.3
Temperate	central Mediterranean	Demospongiae	*Poecillastra compressa*	11.1	-18.6
Temperate	central Mediterranean	Anthozoa	*Madrepora oculata*	7.3	-20.7
Temperate	central Mediterranean	Anthozoa	*Madrepora oculata*	8.6	-20.6
Temperate	central Mediterranean	Anthozoa	*Lophelia pertusa*	8.0	-20.6
Temperate	central Mediterranean	Anthozoa	*Lophelia pertusa*	10.1	-19.1
Temperate	central Mediterranean	Anthozoa	*Desmophyllum dianthus*	8.8	-19.1
Temperate	central Mediterranean	Anthozoa	*Leiopathes glaberrima*	7.3	-19.4
Temperate	central Mediterranean	Anthozoa	*Leiopathes glaberrima*	8.2	-19.6
Temperate	central Mediterranean	Anthozoa	*Leiopathes glaberrima*	7.3	-20.2
Temperate	central Mediterranean	Anthozoa	*Paramuricea* cf. *macrospina*	9.7	-18.5
Temperate	central Mediterranean	Bivalvia	*Asperarca nodulosa*	6.7	-17.4

Temperate	central Mediterranean	Bivalvia	*Delectopecten vitreus*	8.0	-18.1
Temperate	central Mediterranean	Polychaeta	*Serpula* cf. *vermicularis*	6.5	-17.4
Temperate	central Mediterranean	Polychaeta	*Eunice norvegica*	10.2	-17.1
Temperate	central Mediterranean	Polychaeta	*Eunice norvegica*	10.1	-17.3
Temperate	central Mediterranean	Polychaeta	*Eunice norvegica*	10.7	-19.9
Temperate	central Mediterranean	Malacostraca	*Platyscelidae* sp.	5.8	-19.8
Temperate	central Mediterranean	Malacostraca	*Phrosinidae* sp.	6.6	-19.8
Temperate	central Mediterranean	Malacostraca	*Euphausiidae* sp.	7.1	-19.3
Temperate	central Mediterranean	Malacostraca	*Rochinia rissoana*	9.5	-17.8
Temperate	central Mediterranean	Echinoidea	*Cidaris cidaris*	9.3	-14.0
Temperate	central Mediterranean	Asteroidea	*Ceramaster grenadensis*	10.6	-15.8
Temperate	western Mediterranean	Malacostraca	*Aristeus antennatus*	9.7	-15.3
Temperate	western Mediterranean	Malacostraca	*Aristeus antennatus*	8.8	-15.5
Temperate	western Mediterranean	Malacostraca	*Acanthephyra eximia*	8.4	-16.0
Temperate	western Mediterranean	Malacostraca	*Acanthephyra eximia*	7.5	-16.2
Temperate	western Mediterranean	Malacostraca	*Acanthephyra pelagica*	6.9	-17.4
Temperate	western Mediterranean	Malacostraca	*Munida tenuimana*	7.4	-15.6
Temperate	western Mediterranean	Malacostraca	*Munida tenuimana*	6.9	-15.1
Temperate	western Mediterranean	Malacostraca	*Nematocarcinus Nexi exilis*	7.2	-15.3
Temperate	western Mediterranean	Malacostraca	*Nephrops norvegicus*	7.0	-16.1
Temperate	western Mediterranean	Malacostraca	*Plesionika acanthonotus*	8.1	-15.0
Temperate	western Mediterranean	Malacostraca	*Plesionika martia*	6.7	-16.2
Temperate	western Mediterranean	Malacostraca	*Pasiphaea multidentata*	7.4	-16.4
Temperate	western Mediterranean	Malacostraca	*Pasiphaea multidentata*	8.3	-16.4
Temperate	western Mediterranean	Malacostraca	*Pasiphaea multidentata*	8.1	-16.9
Temperate	western Mediterranean	Malacostraca	*Pontophilus norvegicus*	9.4	-13.5
Temperate	western Mediterranean	Malacostraca	*Pontophilus norvegicus*	9.0	-13.8
Temperate	western Mediterranean	Malacostraca	*Polycheles typhlops*	9.0	-15.9
Temperate	western Mediterranean	Malacostraca	*Sergia robusta*	7.3	-19.8
Temperate	western Mediterranean	Malacostraca	*Sergia robusta*	6.7	-19.6

Temperate	western Mediterranean	Malacostraca	*Sergia robusta*	6.2	-18.2
Temperate	western Mediterranean	Malacostraca	*Stereomastis sculpta*	9.6	-14.6
Temperate	western Mediterranean	Cephalopoda	*Bathypolipus sponsalis*	7.2	-16.2
Temperate	western Mediterranean	Cephalopoda	*Heteroteuthis dispar*	9.0	-17.4
Temperate	western Mediterranean	Cephalopoda	*Histioteuthis reversa*	8.2	-18.0
Temperate	western Mediterranean	Cephalopoda	*Opisthoteuthis calypso*	6.6	-17.1
Temperate	western Mediterranean	Malacostraca	*Aristeus antennatus*	8.7	-15.9
Temperate	western Mediterranean	Malacostraca	*Aristeus antennatus*	8.3	-14.3
Temperate	western Mediterranean	Malacostraca	*Aristeus antennatus*	8.5	-13.8
Temperate	western Mediterranean	Malacostraca	*Acanthephyra eximia*	8.1	-16.0
Temperate	western Mediterranean	Malacostraca	*Acanthephyra eximia*	6.9	-16.3
Temperate	western Mediterranean	Malacostraca	*Acanthephyra pelagica*	6.7	-16.7
Temperate	western Mediterranean	Malacostraca	*Geryon longipes*	9.5	-16.6
Temperate	western Mediterranean	Malacostraca	*Munida tenuimana*	7.3	-14.3
Temperate	western Mediterranean	Malacostraca	*Munida tenuimana*	7.5	-14.6
Temperate	western Mediterranean	Malacostraca	*Nematocarcinus Nexi exilis*	7.7	-14.9
Temperate	western Mediterranean	Malacostraca	*Nephrops norvegicus*	6.1	-16.6
Temperate	western Mediterranean	Malacostraca	*Plesionika acanthonotus*	7.1	-15.8
Temperate	western Mediterranean	Malacostraca	*Plesionika martia*	7.0	-15.9
Temperate	western Mediterranean	Malacostraca	*Pasiphaea multidentata*	7.5	-16.6
Temperate	western Mediterranean	Malacostraca	*Pontophilus norvegicus*	10.3	-13.8
Temperate	western Mediterranean	Malacostraca	*Pontophilus norvegicus*	10.5	-12.8
Temperate	western Mediterranean	Malacostraca	*Polycheles typhlops*	9.6	-15.7
Temperate	western Mediterranean	Malacostraca	*Sergia robusta*	6.6	-18.2

Temperate	western Mediterranean	Malacostraca	*Sergia robusta*	7.0	-17.0
Temperate	western Mediterranean	Malacostraca	*Stereomastis sculpta*	9.2	-14.8
Temperate	western Mediterranean	Cephalopoda	*Heteroteuthis dispar*	8.0	-16.7
Temperate	western Mediterranean	Cephalopoda	*Histioteuthis reversa*	8.5	-18.3
Temperate	western Mediterranean	Cephalopoda	*Opisthoteuthis calypso*	8.8	-16.4
Temperate	western Mediterranean	Malacostraca	*Aristeus antennatus*	9.5	-15.7
Temperate	western Mediterranean	Malacostraca	*Pontophylus norvegicus*	9.0	-14.9
Temperate	western Mediterranean	Malacostraca	*Geryon longipes*	9.1	-15.6
Temperate	western Mediterranean	Malacostraca	*Paromola cuvieri*	9.7	-14.9
Temperate	western Mediterranean	Malacostraca	*Nephrops norvegicus*	7.4	-16.9
Temperate	western Mediterranean	Malacostraca	*Polycheles typhlops*	8.7	-15.8
Temperate	western Mediterranean	Malacostraca	*Monodaeus couchii*	7.6	-15.6
Temperate	western Mediterranean	Malacostraca	*Pasiphaea multidentata*	7.0	-18.4
Temperate	western Mediterranean	Malacostraca	*Sergestes arcticus*	6.7	-18.9
Temperate	western Mediterranean	Malacostraca	*Sergia robusta*	6.4	-18.2
Temperate	western Mediterranean	Malacostraca	*Munida tenuimana*	7.4	-15.0
Temperate	western Mediterranean	Malacostraca	*Acantephyra eximia*	8.2	-17.1
Temperate	western Mediterranean	Malacostraca	*Plesionika acantonothus*	7.0	-16.6
Temperate	western Mediterranean	Malacostraca	*Plesionika martia*	7.7	-16.9
Temperate	Northeast Atlantic	Malacostraca	*Acanthephyra pelagica*	8.5	-19.9
Temperate	Northeast Atlantic	Malacostraca	*Chaceon affinis*	10.3	-18.2
Temperate	Northeast Atlantic	Malacostraca	*Gennadas elegans*	7.8	-19.6
Temperate	Northeast Atlantic	Malacostraca	*Gnatophausia zoea*	8.1	-18.8
Temperate	Northeast Atlantic	Malacostraca	*Meganyctiphanes norvegica*	8.1	-20.8
Temperate	Northeast Atlantic	Malacostraca	*Pagurus alatus*	9.3	-20.5
Temperate	Northeast Atlantic	Malacostraca	*Pasiphaea multidentata*	9.6	-19.7
Temperate	Northeast Atlantic	Malacostraca	*Pasiphaea sivado*	9.3	-19.5
Temperate	Northeast Atlantic	Malacostraca	*Phronima sedentaria*	4.0	-20.5
Temperate	Northeast Atlantic	Malacostraca	*Sergestes arcticus*	8.2	-19.4
Temperate	Northeast Atlantic	Malacostraca	*Sergia robusta*	8.8	-19.8

Temperate	Northeast Atlantic	Malacostraca	*Systellaspis debilis*	7.8	-19.8
Temperate	Northwest Atlantic	Anthozoa	*Anthomastus grandiflorus*	11.2	-18.7
Temperate	Northwest Atlantic	Anthozoa	*Duva florida*	11.5	-18.6
Temperate	Northwest Atlantic	Anthozoa	*Acanthogorgia armata*	11.3	-18.9
Temperate	Northwest Atlantic	Anthozoa	*Acanella arbuscula*	10.5	-19.4
Temperate	Northwest Atlantic	Anthozoa	*Paragorgia arborea*	11.3	-19.9
Temperate	Northwest Atlantic	Anthozoa	*Paramuricea* sp.	11.7	-18.9
Temperate	Northwest Atlantic	Anthozoa	*Primnoa resedaeformis*	10.3	-20.3
Temperate	Northwest Atlantic	Anthozoa	*Pennatula* sp.	10.9	-19.0
Temperate	Northwest Atlantic	Anthozoa	*Bathypathes* sp.	10.8	-19.8
Temperate	Northwest Atlantic	Anthozoa	*Flabellum alabastrum*	12.8	-17.2
Temperate	Sea of Japan	Bivalvia	*Robaia robai*	8.2	-18.2
Temperate	Sea of Japan	Bivalvia	*Robaia robai*	8.0	-18.9
Temperate	Sea of Japan	Bivalvia	*Megayoldia* sp.	6.0	-18.1
Temperate	Sea of Japan	Bivalvia	*Megayoldia* sp.	6.6	-17.6
Temperate	Sea of Japan	Bivalvia	*Megayoldia* sp.	6.5	-18.0
Temperate	Sea of Japan	Bivalvia	*Cardiomya beringensis*	12.8	-20.0
Temperate	Sea of Japan	Bivalvia	*Cardiomya beringensis*	12.9	-19.8
Temperate	Sea of Japan	Scaphopoda	*Fustiaria nipponica*	9.3	-18.5
Temperate	Sea of Japan	Malacostraca	*Chionoecetes japonicus*	13.5	-19.9
Temperate	Sea of Japan	Malacostraca	*Eualus biungus*	11.5	-19.7
Temperate	Sea of Japan	Crinoidea	*Heliometra glacialis*	9.6	-22.3
Temperate	Sea of Japan	Ophiuroidea	*Ophiura leptoctenia*	9.1	-21.1
Temperate	Sea of Japan	Asteroidea	*Ctenodiscus crispatus*	12.6	-16.7
Temperate	Sea of Japan	Asteroidea	*Ctenodiscus crispatus*	13.6	-17.4
Temperate	Sea of Japan	Asteroidea	*Ctenodiscus crispatus*	15.5	-16.1
Temperate	Northwest Atlantic	Asteroidea	*Novodinia americana*	12.2	-18.0
Temperate	Northwest Atlantic	Asteroidea	*Zoroaster fulgens*	11.6	-12.9
Temperate	Northwest Atlantic	Asteroidea	*Leptychaster arcticus*	12.1	-17.2
Temperate	Northwest Atlantic	Asteroidea	*Ctenodiscus crispatus*	12.4	-14.3
Temperate	Northwest Atlantic	Asteroidea	*Ceramaster granularis*	17.0	-13.2
Temperate	Northwest Atlantic	Asteroidea	*Hippasteria phrygiana*	15.8	-15.1
Temperate	Northwest Atlantic	Asteroidea	*Mediaster bairdi*	16.5	-14.2
Tropical	Arabian Sea	Asteroidea	*Astropecten* sp.	13.9	-16.8
Tropical	Arabian Sea	Asteroidea	*Astropecten* sp.	12.0	-16.5
Tropical	Arabian Sea	Malacostraca	*Pontocaris* sp.	15.0	-15.3
Tropical	Arabian Sea	Malacostraca	*Solenocera* sp.	14.0	-17.0
Tropical	Arabian Sea	Malacostraca	*Solenocera* sp.	13.2	-16.6
Tropical	Arabian Sea	Malacostraca	*Solenocera* sp.	13.8	-17.1
Tropical	Arabian Sea	Ophiuroidea	*Amphiura* sp.	13.9	-16.4
Tropical	Arabian Sea	Ophiuroidea	*Amphiura* sp.	12.4	-15.8
Tropical	Arabian Sea	Ophiuroidea	*Ophiura euryplax*	12.1	-18.8
Tropical	Arabian Sea	Ophiuroidea	*Ophiura euryplax*	9.7	-19.6
Tropical	Arabian Sea	Echinoidea	*Echinoptilum* sp.	15.0	-19.1
Tropical	Arabian Sea	Echinoidea	*Echinoptilum* sp.	15.4	-18.3
Tropical	Arabian Sea	Malacostraca	*Munidopsis* aff *scobina*	15.1	-17.0
Tropical	Arabian Sea	Malacostraca	*Munidopsis* aff *scobina*	15.7	-18.1
Tropical	Arabian Sea	Anthozoa	*Pennatula* aff*. grandis*	15.7	-16.5

Tropical	Arabian Sea	Anthozoa	*Pennatula* aff. *grandis*	15.9	-17.4
Tropical	Arabian Sea	Anthozoa	*Actinoscyphia* sp.	15.9	-15.4
Tropical	Arabian Sea	Anthozoa	*Actinoscyphia* sp.	15.8	-16.1
Tropical	Arabian Sea	Anthozoa	*Actinauge* sp.	15.9	-15.4
Tropical	Arabian Sea	Anthozoa	*Actinauge* sp.	16.5	-15.0
Tropical	Arabian Sea	Anthozoa	*Actinoscyphia* sp.	15.0	-15.7
Tropical	Arabian Sea	Anthozoa	*Actinoscyphia* sp.	15.8	-15.4
Tropical	Arabian Sea	Polychaeta	*Hyalinoecia* sp.	14.1	-17.9
Tropical	Arabian Sea	Polychaeta	*Hyalinoecia* sp.	13.8	-17.3
Tropical	Arabian Sea	Echinoidea	*Phormosoma placenta*	12.4	-18.5
Tropical	Arabian Sea	Echinoidea	*Phormosoma placenta*	13.5	-17.6

Appendix 7-8 Dataset applied to test for latitudinal gradients in the FA composition of deep-sea benthic organisms. The FA 20:4ω6, 20:5ω3, and 22:6ω3 are expressed as proportions (%). Data were collected from Würzberg et al. (2011a, b) for the polar latitudes; Howell et al. (2003), Hudson et al. (2004), Salvo et al. (2018), and Parzanini et al. (Chapter 4) for the temperate regions; and from Jeffreys et al. (2009) for the tropical latitudes.

Latitude	Ocean/Ocean region	Class	Species	20:4ω6	20:5ω3	22:6ω3
Polar	Weddell Sea	Polychaeta	Acrocirridae sp.	4	29	4
Polar	Weddell Sea	Polychaeta	Capitellidae sp.	5	33	2
Polar	Weddell Sea	Polychaeta	Euphrosinidae sp.	20	17	5
Polar	Weddell Sea	Polychaeta	Fauveliopsidae sp.	5	15	11
Polar	Weddell Sea	Polychaeta	Flabelligeridae sp.	1	26	17
Polar	Weddell Sea	Polychaeta	Flabelligeridae sp.	2	3	5
Polar	Weddell Sea	Polychaeta	Bathyglycinde sp.	1	13	7
Polar	Weddell Sea	Polychaeta	Maldanidae sp.	8	12	13
Polar	Weddell Sea	Polychaeta	Nephtyidae sp.	25	12	3
Polar	Weddell Sea	Polychaeta	Nephtyidae sp.	24	14	1
Polar	Weddell Sea	Polychaeta	Aglaophamus sp.	4	20	20
Polar	Weddell Sea	Polychaeta	*Ammotrypanella* cf. *arctica*	4	29	12
Polar	Weddell Sea	Polychaeta	*Kesun abyssorum*	3	9	3
Polar	Weddell Sea	Polychaeta	*Kesun abyssorum*	11	15	5
Polar	Weddell Sea	Polychaeta	*Ophelina breviata*	4	26	6
Polar	Weddell Sea	Polychaeta	*Ophelina breviata*	11	10	6
Polar	Weddell Sea	Polychaeta	cf. *Ophelina* sp.	4	30	5
Polar	Weddell Sea	Polychaeta	*Travisia kerguelensis*	12	11	3
Polar	Weddell Sea	Polychaeta	*Travisia kerguelensis*	11	10	3
Polar	Weddell Sea	Polychaeta	*Travisia kerguelensis*	3	20	7
Polar	Weddell Sea	Polychaeta	Orbiniidae sp.	3	23	19
Polar	Weddell Sea	Polychaeta	Phyllodocidae sp.	2	27	20
Polar	Weddell Sea	Polychaeta	Polynoidae sp.	4	17	23
Polar	Weddell Sea	Polychaeta	Polynoidae sp.	3	19	22
Polar	Weddell Sea	Polychaeta	Polyodontidae sp.	4	16	20
Polar	Weddell Sea	Polychaeta	Polyodontidae sp.	3	14	17
Polar	Weddell Sea	Polychaeta	*Axiokebuita millsii*	5	17	1
Polar	Weddell Sea	Polychaeta	Scalibregmatidae sp.	6	16	3
Polar	Weddell Sea	Polychaeta	Sigalionidae sp.	2	19	21
Polar	Weddell Sea	Polychaeta	Spionidae sp.	2	26	11
Polar	Weddell Sea	Polychaeta	Spionidae sp.	3	21	13
Polar	Weddell Sea	Polychaeta	Spionidae sp.	9	39	3
Polar	Weddell Sea	Polychaeta	*Pionosyllis epipharynx*	7	25	13

Polar	Weddell Sea	Polychaeta	*Eusyllis kerguelensis*	9	28	13
Polar	Weddell Sea	Polychaeta	*Eusyllis kerguelensis*	10	23	14
Polar	Weddell Sea	Polychaeta	*Trypanosyllis gigantea*	4	21	17
Polar	Weddell Sea	Polychaeta	*Trypanosyllis gigantea*	9	22	1
Polar	Weddell Sea	Polychaeta	*Brania rhopalophora*	5	14	14
Polar	Weddell Sea	Polychaeta	*Eupistella grubei*	1	14	11
Polar	Weddell Sea	Malacostraca	*Eurythenes gryllus*	3	14	17
Polar	Weddell Sea	Malacostraca	*Abyssorchomene* spp.	2	11	11
Polar	Weddell Sea	Malacostraca	*Abyssorchomene* sp.	1	11	7
Polar	Weddell Sea	Malacostraca	*Lysianassidae* sp.	9	14	24
Polar	Weddell Sea	Malacostraca	*Eurycope* sp.	10	17	15
Polar	Weddell Sea	Malacostraca	*Betamorpha* sp.	19	17	16
Polar	Weddell Sea	Malacostraca	*Syneurycope* sp 1	6	19	16
Polar	Weddell Sea	Malacostraca	*Syneurycope* sp 2	8	18	20
Polar	Weddell Sea	Malacostraca	*Ilyarachna* sp.	12	18	13
Polar	Weddell Sea	Malacostraca	*Chaulioniscus* sp.	4	13	11
Polar	Weddell Sea	Malacostraca	*Mastigoniscus* sp.	5	15	13
Polar	Weddell Sea	Malacostraca	*Haploniscus* sp.	3	24	40
Polar	Weddell Sea	Malacostraca	*Macrostylis* sp 1	10	14	13
Polar	Weddell Sea	Malacostraca	*Macrostylis* sp 2	4	10	7
Polar	Weddell Sea	Malacostraca	*Chaetarcturus* cf. *bovinus*	6	26	12
Polar	Weddell Sea	Malacostraca	*Ischnomesus* sp.	5	18	25
Polar	Weddell Sea	Malacostraca	*Notoxenus* cf. *spinifer*	8	23	14
Polar	Weddell Sea	Malacostraca	*Stenetrium weddellensis*	4	17	10
Polar	Weddell Sea	Malacostraca	Leuconidae sp 1	3	30	16
Polar	Weddell Sea	Malacostraca	Leuconidae sp 2	6	21	10
Polar	Weddell Sea	Malacostraca	*Eudorella* sp.	3	28	11
Polar	Weddell Sea	Malacostraca	Bodotriidae spp.	6	29	7
Polar	Weddell Sea	Malacostraca	Nannastacidae sp.	2	19	3
Polar	Weddell Sea	Malacostraca	Diastylidae sp.	4	24	4
Polar	Weddell Sea	Malacostraca	*Apseudomorpha* sp.	4	8	7
Polar	Weddell Sea	Malacostraca	*Neotanais* sp.	16	15	7
Polar	Weddell Sea	Malacostraca	*Apseudes* sp.	9	15	9
Polar	Weddell Sea	Malacostraca	*Paranarthrura* sp.	19	5	8
Temperate	Northwest Atlantic	Anthozoa	*Stauropathes arctica*	1	11	2
Temperate	Northwest Atlantic	Anthozoa	Average value gorgonians (*Acanthogorgia armata*, *Keratoisis grayi, Acanella arbuscula*, *Radicipes gracilis, Paramuricea* spp.)	16	8	4

Temperate	Northwest Atlantic	Anthozoa	Average value sea pens (*Pennatula* sp., *Funiculina quadrangularis*, *Pennatula grandis*, *Anthoptilum grandiflorum, Distichoptilum gracile, Halipteris finmarchica*)	5	17	3
Temperate	Northwest Atlantic	Anthozoa	Average value soft corals (*Nephtheidae* sp., *Anthomastus* sp., *Duva florida*)	17	9	3
Temperate	Northwest Atlantic	Anthozoa	*Flabellum alabastrum*	10	9	1
Temperate	Northwest Atlantic	Polychaeta	Nereididae sp.	1	11	13
Temperate	Northwest Atlantic	Polychaeta	Polychaeta sp 1	1	13	11
Temperate	Northwest Atlantic	Polychaeta	Polychaeta sp 2	1	8	11
Temperate	Northwest Atlantic	Polychaeta	Polychaeta sp 3	1	10	6
Temperate	Northwest Atlantic	Polychaeta	Polynoidae sp 1	3	14	12
Temperate	Northwest Atlantic	Polychaeta	Polynoidae sp 2	1	18	14
Temperate	Northwest Atlantic	Malacostraca	*Acanthephyra pelagica*	0	8	7
Temperate	Northwest Atlantic	Malacostraca	*Notostomus robustus*	1	24	14
Temperate	Northwest Atlantic	Malacostraca	*Pandalus borealis*	1	20	17
Temperate	Northwest Atlantic	Malacostraca	*Pasiphaea tarda*	1	16	19
Temperate	Northwest Atlantic	Malacostraca	*Sabinea hystrix*	2	17	16
Temperate	Northwest Atlantic	Malacostraca	*Stereomastis sculpta*	8	11	15
Temperate	Northwest Atlantic	Hexanauplia	*Arcoscalpellum michelottianum*	1	15	15
Temperate	Northwest Atlantic	Anthozoa	*Actinauge cristata*	0	8	5
Temperate	Northwest Atlantic	Anthozoa	*Actinoscyphia aurelia*	8	16	1
Temperate	Northwest Atlantic	Anthozoa	*Actinostola callosa*	0	13	5
Temperate	Northwest Atlantic	Anthozoa	*Anthomastus spp*	13	9	2
Temperate	Northwest Atlantic	Anthozoa	*Anthoptilum grandiflorum*	3	21	3

Temperate	Northwest Atlantic	Anthozoa	*Paragorgia arborea*	8	13	2
Temperate	Northwest Atlantic	Anthozoa	*Funiculina* sp.	3	26	4
Temperate	Northwest Atlantic	Anthozoa	*Pennatula aculeata*	13	11	2
Temperate	Northwest Atlantic	Anthozoa	*Pennatula grandis*	1	18	2
Temperate	Northwest Atlantic	Anthozoa	*Umbellula* sp.	0	17	1
Temperate	Northwest Atlantic	Asteroidea	*Freyella microspina*	17	13	2
Temperate	Northwest Atlantic	Asteroidea	*Leptychaster arcticus*	8	25	1
Temperate	Northwest Atlantic	Asteroidea	*Mediaster bairdi*	27	6	1
Temperate	Northwest Atlantic	Asteroidea	*Myxaster sol*	29	12	0
Temperate	Northwest Atlantic	Asteroidea	*Psilaster andromeda*	27	18	2
Temperate	Northwest Atlantic	Asteroidea	*Zoroaster fulgens*	5	17	3
Temperate	Northwest Atlantic	Echinoidea	*Phormosoma placenta*	14	11	2
Temperate	Northwest Atlantic	Ophiuroidea	*Gorgonocephalus* sp.	1	19	2
Temperate	Northwest Atlantic	Gastropoda	*Buccinum* sp.	4	25	4
Temperate	Northwest Atlantic	Gastropoda	*Colus islandicus*	13	14	3
Temperate	Northwest Atlantic	Gastropoda	*Neptunea despecta*	13	0	2
Temperate	Northwest Atlantic	Demospongiae	*Cliona* sp.	0	6	2
Temperate	Northwest Atlantic	Demospongiae	*Craniella cranium*	0	0	1
Temperate	Northwest Atlantic	Demospongiae	*Iophon piceum*	0	8	19
Temperate	Northwest Atlantic	Demospongiae	*Geodia* sp.	0	0	6
Temperate	Northwest Atlantic	Demospongiae	*Stelletta* spp.	0	0	0
Temperate	Northwest Atlantic	Demospongiae	*Phakellia* spp.	0	5	1
Temperate	Northwest Atlantic	Demospongiae	*Hamacantha* (*Vomerula*) *carteri*	0	12	0
Temperate	Northwest Atlantic	Demospongiae	*Haliclona* sp.	0	0	0

Temperate	Northwest Atlantic	Demospongiae	*Polymastia* spp.	3	7	10
Temperate	Northwest Atlantic	Demospongiae	*Tentorium semisuberites*	5	12	5
Temperate	Northwest Atlantic	Demospongiae	*Thenea muricata*	0	6	7
Temperate	Northwest Atlantic	Hexactinellida	Hexactinellida sp.	0	0	0
Temperate	Northwest Atlantic	Hexactinellida	*Euplectella* spp.	1	10	8
Temperate	Northwest Atlantic	Sipunculidea	Sipunculidea sp 1	11	11	2
Temperate	Northwest Atlantic	Sipunculidea	Sipunculidea sp 2	4	2	0
Temperate	Northeast Atlantic	Asteroidea	*Hyphalaster inermis*	23	15	0
Temperate	Northeast Atlantic	Asteroidea	*Styracaster chuni*	24	11	1
Temperate	Northeast Atlantic	Asteroidea	*Dytaster grandis grandis*	23	22	1
Temperate	Northeast Atlantic	Asteroidea	*Bathybiaster vexillifer*	24	19	2
Temperate	Northeast Atlantic	Asteroidea	*Hymenaster membranaceus elegans*	20	22	1
Temperate	Northeast Atlantic	Asteroidea	*Freyella elegans*	14	24	6
Temperate	Northeast Atlantic	Asteroidea	*Brisingella coronata*	11	27	9
Temperate	Northeast Atlantic	Asteroidea	*Brisinga endecacnemos*	12	23	5
Temperate	Northeast Atlantic	Asteroidea	*Zoroaster longicauda*	15	25	3
Tropical	Arabian Sea	Asteroidea	*Astropecten* sp.	17	4	1
Tropical	Arabian Sea	Malacostraca	*Pontocaris* sp./*Solenocera* sp.	4	2	17
Tropical	Arabian Sea	Malacostraca	*Solenocera* sp.	12	15	29
Tropical	Arabian Sea	Anthozoa	*Echinoptilum* sp.	21	9	4
Tropical	Arabian Sea	Anthozoa	*Actinoscyphia* sp.	13	15	14
Tropical	Arabian Sea	Anthozoa	*Actinoscyphia* sp.	0	6	2
Tropical	Arabian Sea	Polychaeta	*Hyalinoecia* sp.	1	7	1

Appendix 7-9 Dataset applied to test for longitudinal gradient in the isotopic composition of deep-sea benthic organisms. Values of $\delta^{15}N$ and $\delta^{13}C$ are expressed in parts per thousand (‰). Data were collected from Sherwood et al. (2008), Gale et al. (2013), and Parzanini et al. (Chapter 4) for the Northwest Atlantic; Boyle et al. (2012) for the eastern North Pacific; Fanelli et al. (2013) and Papiol et al. (2013) for the Balearic basin (western Mediterranean); Preciado et al. (2017) for the Northeast Atlantic; Kharlamenko et al. (2013) for the Sea of Japan; and Carlier et al. (2009) for the Ionian Sea (central Mediterranean).

Ocean/Oceanic region	Class	Species	$\delta^{15}N$	$\delta^{13}C$
Northwest Atlantic	Polychaeta	*Laetmonice filicornis*	13.7	-16.0
Northwest Atlantic	Polychaeta	Nereididae sp 2	13.9	-17.1
Northwest Atlantic	Polychaeta	Polychaeta sp 1	13.2	-15.7
Northwest Atlantic	Polychaeta	Polychaeta sp 2	11.1	-19.0
Northwest Atlantic	Polychaeta	Polychaeta sp 3	7.9	-15.8
Northwest Atlantic	Polychaeta	Polynoidae sp 1	12.6	-17.8
Northwest Atlantic	Polychaeta	Polynoidae sp 2	12.0	-17.5
Northwest Atlantic	Malacostraca	*Acanthephyra pelagica*	9.4	-20.1
Northwest Atlantic	Malacostraca	*Notostomus robustus*	11.6	-19.8
Northwest Atlantic	Malacostraca	*Pandalus borealis*	11.1	-19.1
Northwest Atlantic	Malacostraca	*Pasiphaea tarda*	11.4	-19.2
Northwest Atlantic	Malacostraca	*Sabinea hystrix*	14.9	-16.4
Northwest Atlantic	Malacostraca	*Stereomastis sculpta*	12.6	-19.2
Northwest Atlantic	Hexanauplia	*Arcoscalpellum michelottianum*	13.3	-18.9
Northwest Atlantic	Anthozoa	*Actinauge cristata*	11.1	-18.6
Northwest Atlantic	Anthozoa	*Actinoscyphia aurelia*	11.7	-16.8
Northwest Atlantic	Anthozoa	*Actinostola callosa*	10.8	-18.6
Northwest Atlantic	Anthozoa	*Anthomastus* spp.	11.1	-13.9
Northwest Atlantic	Anthozoa	*Anthoptilum grandiflorum*	11.6	-19.5
Northwest Atlantic	Anthozoa	*Paragorgia arborea*	10.4	-11.5
Northwest Atlantic	Anthozoa	*Funiculina* sp.	10.9	-14.8
Northwest Atlantic	Anthozoa	*Pennatula aculeata*	10.3	-8.6
Northwest Atlantic	Anthozoa	*Pennatula grandis*	10.5	-13.1
Northwest Atlantic	Anthozoa	*Umbellula* sp.	11.7	-19.3
Northwest Atlantic	Anthozoa	*Freyella microspina*	12.3	-9.1
Northwest Atlantic	Asteroidea	*Leptychaster arcticus*	12.4	-9.6
Northwest Atlantic	Asteroidea	*Mediaster bairdi*	16.0	-8.2
Northwest Atlantic	Asteroidea	*Myxaster sol*	16.8	-12.4
Northwest Atlantic	Asteroidea	*Psilaster andromeda*	16.9	-13.0
Northwest Atlantic	Asteroidea	*Zoroaster fulgens*	14.1	-6.3
Northwest Atlantic	Echinoidea	*Phormosoma placenta*	12.3	-14.3
Northwest Atlantic	Ophiuroidea	*Gorgonocephalus* sp.	11.8	-14.5
Northwest Atlantic	Gastropoda	*Buccinum* sp.	12.6	-17.0
Northwest Atlantic	Gastropoda	*Colus islandicus*	14.2	-15.0

Northwest Atlantic	Gastropoda	*Neptunea despecta*	15.2	-15.4
Northwest Atlantic	Demospongiae	*Cliona* sp.	7.7	-17.4
Northwest Atlantic	Demospongiae	*Craniella cranium*	7.5	-18.5
Northwest Atlantic	Demospongiae	*Iophon piceum*	16.5	-18.0
Northwest Atlantic	Demospongiae	*Geodia* sp.	8.7	-17.1
Northwest Atlantic	Demospongiae	*Stelletta* spp.	8.0	-17.2
Northwest Atlantic	Demospongiae	*Phakellia* spp.	14.9	-15.8
Northwest Atlantic	Demospongiae	*Hamacantha (Vomerula) carteri*	15.4	-14.6
Northwest Atlantic	Demospongiae	*Haliclona* sp.	7.9	-17.1
Northwest Atlantic	Demospongiae	*Polymastia* spp.	14.8	-16.7
Northwest Atlantic	Demospongiae	*Tentorium semisuberites*	15.5	-17.6
Northwest Atlantic	Demospongiae	*Thenea muricata*	14.2	-17.4
Northwest Atlantic	Hexactinellida	Hexactinellida sp 1	6.8	-17.3
Northwest Atlantic	Hexactinellida	*Euplectella spp.*	12.9	-16.3
Northwest Atlantic	Sipunculidea	Sipunculidea sp 1	11.4	-16.0
Northwest Atlantic	Sipunculidea	Sipunculidea sp 2	15.0	-15.3
Northwest Atlantic	Anthozoa	*Duva florida*	11.5	-18.6
Northwest Atlantic	Anthozoa	*Acanthogorgia armata*	11.3	-18.9
Northwest Atlantic	Anthozoa	*Acanella arbuscula*	10.5	-19.4
Northwest Atlantic	Anthozoa	*Keratoisis ornate*		-19.7
Northwest Atlantic	Anthozoa	*Paragorgia arborea*	11.3	-19.9
Northwest Atlantic	Anthozoa	*Paramuricea* sp.	11.7	-18.9
Northwest Atlantic	Anthozoa	*Primnoa resedaeformis*	10.3	-20.3
Northwest Atlantic	Anthozoa	*Pennatula* sp.	10.9	-19.0
Northwest Atlantic	Anthozoa	*Bathypathes* sp.	10.8	-19.8
Northwest Atlantic	Anthozoa	*Flabellum alabastrum*	12.8	-17.2
Northwest Atlantic	Asteroidea	*Novodinia americana*	12.2	-18.0
Northwest Atlantic	Asteroidea	*Zoroaster fulgens*	11.6	-12.9
Northwest Atlantic	Asteroidea	*Leptychaster arcticus*	12.1	-17.2
Northwest Atlantic	Asteroidea	*Ctenodiscus crispatus*	12.4	-14.3
Northwest Atlantic	Asteroidea	*Ceramaster granularis*	17.0	-13.2
Northwest Atlantic	Asteroidea	*Hippasteria phrygiana*	15.8	-15.1
Northwest Atlantic	Asteroidea	*Mediaster bairdi*	16.5	-14.2
Northwest Atlantic	Anthozoa	*Duva florida*	11.5	-18.6
Northwest Atlantic	Anthozoa	*Acanthogorgia armata*	11.3	-18.9
Northwest Atlantic	Anthozoa	*Acanella arbuscula*	10.5	-19.4
Northwest Atlantic	Anthozoa	*Keratoisis ornate*		-19.7
Northwest Atlantic	Anthozoa	*Paragorgia arborea*	11.3	-19.9
eastern North Pacific	Anthozoa	Hormathiidae	14.0	-18.1
eastern North Pacific	Anthozoa	Hormathiidae	15.3	-15.8
eastern North Pacific	Holothuroidea	*Parastichopus leukothele*	13.5	-16.6
eastern North Pacific	Holothuroidea	*Pseudostichopus mollis*	14.6	-17.6
eastern North Pacific	Holothuroidea	*Pseudostichopus mollis*	15.5	-15.5
eastern North Pacific	Holothuroidea	*Scotoplanes sp.*	13.2	-16.0
eastern North Pacific	Holothuroidea	*Scotoplanes* sp.	14.2	-16.8

eastern North Pacific	Holothuroidea	*Scotoplanes* sp.	15.2	-17.6
eastern North Pacific	Gastropoda	*Neptunea* sp.	15.5	-17.7
eastern North Pacific	Asteroidea	*Crossaster borealis*	18.4	-15.9
eastern North Pacific	Asteroidea	*Luidia* spp.	18.3	-17.4
eastern North Pacific	Malacostraca	*Chionoecetes tanneri*	13.8	-19.5
eastern North Pacific	Malacostraca	*Chionoecetes tanneri*	13.2	-19.7
eastern North Pacific	Malacostraca	*Euphausia pacifica*	13.9	-20.3
eastern North Pacific	Malacostraca	*Euphausia pacifica*	10.2	-20.8
eastern North Pacific	Polychaeta	Onuphidae	16.2	-18.6
eastern North Pacific	Malacostraca	*Eualus biunguis*	14.9	-18.0
eastern North Pacific	Malacostraca	*Eualus biunguis*	15.2	-19.3
eastern North Pacific	Malacostraca	*Eualus macrophthalmus*	15.8	-18.9
eastern North Pacific	Malacostraca	*Eualus macrophthalmus*	14.5	-18.9
central Mediterranean	Demospongiae	*Pachastrella monilifera*	7.4	-17.3
central Mediterranean	Demospongiae	*Poecillastra compressa*	11.1	-18.6
central Mediterranean	Anthozoa	*Madrepora oculata*	7.3	-20.7
central Mediterranean	Anthozoa	*Madrepora oculata*	8.6	-20.6
central Mediterranean	Anthozoa	*Lophelia pertusa*	8.0	-20.6
central Mediterranean	Anthozoa	*Lophelia pertusa*	10.1	-19.1
central Mediterranean	Anthozoa	*Desmophyllum dianthus*	8.8	-19.1
central Mediterranean	Anthozoa	*Leiopathes glaberrima*	7.3	-19.4
central Mediterranean	Anthozoa	*Leiopathes glaberrima*	8.2	-19.6
central Mediterranean	Anthozoa	*Leiopathes glaberrima*	7.3	-20.2
central Mediterranean	Anthozoa	*Paramuricea* cf. *macrospina*	9.7	-18.5
central Mediterranean	Bivalvia	*Asperarca nodulosa*	6.7	-17.4
central Mediterranean	Bivalvia	*Delectopecten vitreus*	8.0	-18.1
central Mediterranean	Polychaeta	*Serpula cf. vermicularis*	6.5	-17.4
central Mediterranean	Polychaeta	*Eunice norvegica*	10.2	-17.1
central Mediterranean	Polychaeta	*Eunice norvegica*	10.1	-17.3
central Mediterranean	Polychaeta	*Eunice norvegica*	10.7	-19.9
central Mediterranean	Malacostraca	*Platyscelidae* sp.	5.8	-19.8
central Mediterranean	Malacostraca	*Phrosinidae* sp.	6.6	-19.8
central Mediterranean	Malacostraca	*Euphausiidae* sp.	7.1	-19.3
central Mediterranean	Malacostraca	*Rochinia rissoana*	9.5	-17.8
central Mediterranean	Echinoidea	*Cidaris cidaris*	9.3	-14.0
central Mediterranean	Asteroidea	*Ceramaster grenadensis*	10.6	-15.8
western Mediterranean	Malacostraca	*Aristeus antennatus*	9.7	-15.3
western Mediterranean	Malacostraca	*Aristeus antennatus*	8.8	-15.5
western Mediterranean	Malacostraca	*Acanthephyra eximia*	8.4	-16.0
western Mediterranean	Malacostraca	*Acanthephyra eximia*	7.5	-16.2
western Mediterranean	Malacostraca	*Acanthephyra pelagica*	6.9	-17.4

western Mediterranean	Malacostraca	*Munida tenuimana*	7.4	-15.6
western Mediterranean	Malacostraca	*Munida tenuimana*	6.9	-15.1
western Mediterranean	Malacostraca	*Nematocarcinus Nexi exilis*	7.2	-15.3
western Mediterranean	Malacostraca	*Nephrops norvegicus*	7.0	-16.1
western Mediterranean	Malacostraca	*Plesionika acanthonotus*	8.1	-15.0
western Mediterranean	Malacostraca	*Plesionika martia*	6.7	-16.2
western Mediterranean	Malacostraca	*Pasiphaea multidentata*	7.4	-16.4
western Mediterranean	Malacostraca	*Pasiphaea multidentata*	8.3	-16.4
western Mediterranean	Malacostraca	*Pasiphaea multidentata*	8.1	-16.9
western Mediterranean	Malacostraca	*Pontophilus norvegicus*	9.4	-13.5
western Mediterranean	Malacostraca	*Pontophilus norvegicus*	9.0	-13.8
western Mediterranean	Malacostraca	*Polycheles typhlops*	9.0	-15.9
western Mediterranean	Malacostraca	*Sergia robusta*	7.3	-19.8
western Mediterranean	Malacostraca	*Sergia robusta*	6.7	-19.6
western Mediterranean	Malacostraca	*Sergia robusta*	6.2	-18.2
western Mediterranean	Malacostraca	*Stereomastis sculpta*	9.6	-14.6
western Mediterranean	Cephalopoda	*Bathypolipus sponsalis*	7.2	-16.2
western Mediterranean	Cephalopoda	*Heteroteuthis dispar*	9.0	-17.4
western Mediterranean	Cephalopoda	*Histioteuthis reversa*	8.2	-18.0
western Mediterranean	Cephalopoda	*Opisthoteuthis calypso*	6.6	-17.1
western Mediterranean	Malacostraca	*Aristeus antennatus*	8.7	-15.9
western Mediterranean	Malacostraca	*Aristeus antennatus*	8.3	-14.3
western Mediterranean	Malacostraca	*Aristeus antennatus*	8.5	-13.8
western Mediterranean	Malacostraca	*Acanthephyra eximia*	8.1	-16.0

western Mediterranean	Malacostraca	*Acanthephyra eximia*	6.9	-16.3
western Mediterranean	Malacostraca	*Acanthephyra pelagica*	6.7	-16.7
western Mediterranean	Malacostraca	*Geryon longipes*	9.5	-16.6
western Mediterranean	Malacostraca	*Munida tenuimana*	7.3	-14.3
western Mediterranean	Malacostraca	*Munida tenuimana*	7.5	-14.6
western Mediterranean	Malacostraca	*Nematocarcinus Nexi exilis*	7.7	-14.9
western Mediterranean	Malacostraca	*Nephrops norvegicus*	6.1	-16.6
western Mediterranean	Malacostraca	*Plesionika acanthonotus*	7.1	-15.8
western Mediterranean	Malacostraca	*Plesionika martia*	7.0	-15.9
western Mediterranean	Malacostraca	*Pasiphaea multidentata*	7.5	-16.6
western Mediterranean	Malacostraca	*Pontophilus norvegicus*	10.3	-13.8
western Mediterranean	Malacostraca	*Pontophilus norvegicus*	10.5	-12.8
western Mediterranean	Malacostraca	*Polycheles typhlops*	9.6	-15.7
western Mediterranean	Malacostraca	*Sergia robusta*	6.6	-18.2
western Mediterranean	Malacostraca	*Sergia robusta*	7.0	-17.0
western Mediterranean	Malacostraca	*Stereomastis sculpta*	9.2	-14.8
western Mediterranean	Cephalopoda	*Heteroteuthis dispar*	8.0	-16.7
western Mediterranean	Cephalopoda	*Histioteuthis reversa*	8.5	-18.3
western Mediterranean	Cephalopoda	*Opisthoteuthis calypso*	8.8	-16.4
western Mediterranean	Malacostraca	*Aristeus antennatus*	9.5	-15.7
western Mediterranean	Malacostraca	*Pontophylus norvegicus*	9.0	-14.9
western Mediterranean	Malacostraca	*Geryon longipes*	9.1	-15.6
western Mediterranean	Malacostraca	*Paromola cuvieri*	9.7	-14.9
western Mediterranean	Malacostraca	*Nephrops norvegicus*	7.4	-16.9

western Mediterranean	Malacostraca	*Polycheles typhlops*	8.7	-15.8
western Mediterranean	Malacostraca	*Monodaeus couchii*	7.6	-15.6
western Mediterranean	Malacostraca	*Pasiphaea multidentata*	7.0	-18.4
western Mediterranean	Malacostraca	*Sergestes arcticus*	6.7	-18.9
western Mediterranean	Malacostraca	*Sergia robusta*	6.4	-18.2
western Mediterranean	Malacostraca	*Munida tenuimana*	7.4	-15.0
western Mediterranean	Malacostraca	*Acantephyra eximia*	8.2	-17.1
western Mediterranean	Malacostraca	*Plesionika acantonothus*	7.0	-16.6
western Mediterranean	Malacostraca	*Plesionika martia*	7.7	-16.9
Northeast Atlantic	Malacostraca	*Acanthephyra pelagica*	8.5	-19.9
Northeast Atlantic	Malacostraca	*Chaceon affinis*	10.3	-18.2
Northeast Atlantic	Malacostraca	*Gennadas elegans*	7.8	-19.6
Northeast Atlantic	Malacostraca	*Gnatophausia zoea*	8.1	-18.8
Northeast Atlantic	Malacostraca	*Meganyctiphanes norvegica*	8.1	-20.8
Northeast Atlantic	Malacostraca	*Pagurus alatus*	9.3	-20.5
Northeast Atlantic	Malacostraca	*Pasiphaea multidentata*	9.6	-19.7
Northeast Atlantic	Malacostraca	*Pasiphaea sivado*	9.3	-19.5
Northeast Atlantic	Malacostraca	*Phronima sedentaria*	4.0	-20.5
Northeast Atlantic	Malacostraca	*Sergestes arcticus*	8.2	-19.4
Northeast Atlantic	Malacostraca	*Sergia robusta*	8.8	-19.8
Northeast Atlantic	Malacostraca	*Systellaspis debilis*	7.8	-19.8
Northwest Atlantic	Anthozoa	*Anthomastus grandiflorus*	11.2	-18.7
Sea of Japan	Bivalvia	*Robaia robai*	8.2	-18.2
Sea of Japan	Bivalvia	*Robaia robai*	8.0	-18.9
Sea of Japan	Bivalvia	*Megayoldia* sp.	6.0	-18.1
Sea of Japan	Bivalvia	*Megayoldia* sp.	6.6	-17.6
Sea of Japan	Bivalvia	*Megayoldia* sp.	6.5	-18.0
Sea of Japan	Bivalvia	*Cardiomya beringensis*	12.8	-20.0
Sea of Japan	Bivalvia	*Cardiomya beringensis*	12.9	-19.8
Sea of Japan	Scaphopoda	*Fustiaria nipponica*	9.3	-18.5
Sea of Japan	Malacostraca	*Chionoecetes japonicus*	13.5	-19.9
Sea of Japan	Malacostraca	*Eualus biungus*	11.5	-19.7
Sea of Japan	Crinoidea	*Heliometra glacialis*	9.6	-22.3
Sea of Japan	Ophiuroidea	*Ophiura leptoctenia*	9.1	-21.1
Sea of Japan	Asteroidea	*Ctenodiscus crispatus*	12.6	-16.7
Sea of Japan	Asteroidea	*Ctenodiscus crispatus*	13.6	-17.4
Sea of Japan	Asteroidea	*Ctenodiscus crispatus*	15.5	-16.1