BIODIVERSITY AND FOOD WEB PATTERNS IN THE DEEP SEA: WHY

FOOD QUALITY MATTERS

by

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Abstract

Despite multiple drivers of ocean change, few studies have addressed the role of food quality in structuring deep-sea benthic communities and food webs, and therefore its role in ecosystem processes (functioning). Based on a review of published data, I showed that food quality (i.e., nitrogen, pigment, lipid, carbohydrate, protein content) alters marine trophic guild composition, and thus food web structure, differently. In sampling heterogeneous submarine canyon and chemosynthetic ecosystems as model environments for food web and biodiversity studies, I found that patchy food quality distribution in Barkley submarine canyon influenced macroinfaunal community structure more strongly at smaller spatial scales (10's of m), whereas major stressors (i.e., oxygen/depth) acted over larger scales (100's of m). Increased food patchiness at a hydrate outcrop site (Barkley Hydrates), compared to sites located 20 and 600 m away, was related to increased macroinfaunal trophic diversity. Food quality explained substantial variation (~ 33 %) in macroinfaunal community structure, but H_2S toxicity likely explained much of the remaining variation. Increasing spatial resolution of analysis (i.e., ≤ 10 m) indicated a 9-15 m influence radius around the most methane-active site (i.e., more depleted $\delta^{13}C$, indicative of chemosynthesis). Macroinfaunal total abundance at Barkley Hydrates tracked temporal changes in chemosynthetic organic matter, however, some taxa (i.e., Ampharetidae) apparently matched recruitment to phytodetrital pulses, much like the background community. An *in situ* enrichment experiment demonstrated modest differences in infaunal community species and functional trait composition in food patches enriched with either *Chaetoceros calcitrans* (a diatom) or *Nannochloropsis*

1

oculata (a lipid rich eustygmatal alga). Megaepifaunal visits to the enrichment patches increased in *N. oculata* over the first two weeks of the experiment, perhaps responding to increased abundance of potential infaunal prey. This research demonstrates a structuring role for food quality both in benthic communities and in food webs, with different effects on organisms of different sizes (e.g., macrofauna and megafauna).

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Table of contents

List of Tables

List of Figures

15

List of appendices

List of common nomenclature and abbreviations

AIC – Akaike Information Criteria

ANOVA – Analysis of Variance

dbRDA – distance-based redundancy analysis

distLM – distance-based linear model

EFA – Essential fatty acid

MUN – Memorial University of Newfoundland

MGS – Mean grain size

nMDS – non-Metric Multidimensional Scaling

OE – Oceanic Explorer

OM – Organic matter

ONC – Ocean Networks Canada

PERMANOVA – Permutational multivariate analysis of variance

PERMDISP – homogeneity of dispersions

POM – Particulate organic mater

PCoA – Principal coordinates analysis

ROPOS – Remotely Operated Platform for Ocean Sciences

ROV – Remotely Operated Vehicle

SOM – Sediment Organic Mater

SIMPER – Similarity Percentage analysis

SPP – Surface Primary Productivity

TOC – Total Organic Carbon

VPDB – Vienna Pee Dee Belemnite

$\delta^{13}C$ – Stable carbon isotope ratio

δ^{15}N – Stable nitrogen isotope ratio

WP – Waypoint

Fatty acid nomenclature: A:BωC

A – Number of carbons of the fatty acid chain

B – Number of double bonds present

C – Position of the first double bond counting from the methyl end of the molecule

Co-authorship statement

The research detailed in this thesis was designed and conceptualized by Neus Campanyà i Llovet, with assistance from supervisor Dr. Paul Snelgrove, and committee members Drs. Christopher Parrish, Anna Metaxas, Kim Juniper, and Annie Mercier. All the data were collected and analyzed by Neus Campanyà i Llovet with assistance from Fabio de Leo Cabrera and Alice Bui. Dr. Paul Snelgrove helped formulate ideas, content, and structure of Chapters 2-6 and provided editorial guidance throughout. Chapter 2 was written in close collaboration with Dr. Christopher Parrish, and Chapter 3 with Dr. Fabio de Leo; both have been published in *Progress in Oceanography*. All manuscripts resulting from this research were written by Neus Campanyà i Llovet, with editorial guidance and creative direction from committee members and associated co-authors.

1. Introduction and overview

The remote locations of deep-sea ecosystems (> 200 m: Thistle 2003) limits current knowledge regarding ecological processes, however, evidence suggests these environments provide important ecosystem functions (defined here as processes such as bioturbation and nutrient cycling that take place within an ecosystem, considering the ecosystem as a whole) and services (provision of food, pH buffering, and carbon sequestration that provide direct benefits to humanity) that are vital for the ocean and biosphere (Thurber et al., 2014, Sweetman et al., 2017). Nonetheless, fisheries (destruction of habitat, loss of targeted and non-targeted species), global warming, deep-sea mining, and waste disposal threaten the delivery of these functions and associated services (Smith et al., 2008, Ramirez-Llodra et al., 2011).

Different benthic species and feeding guilds contribute to ecosystem functioning in multiple ways (Dauwe et al., 1998) and therefore, ecosystem function can vary according to community and food web structure. Different environmental and biological factors can influence deep-sea biodiversity and species composition. Local diversity in deep-sea bathyal depths usually exceeds diversity in most other deep-sea and coastal ecosystems (Sanders, 1968, Snelgrove and Smith, 2002, Rex and Etter, 2010). Early attempts to identify the main drivers of this unexpectedly high biodiversity pointed to biological interactions. For example, competition over long time periods (Sanders (1968) and his stability-time hypothesis) could facilitate speciation. Alternatively, predation cropping by megafauna (Dayton and Hessler, 1972) could limit population sizes, thus reducing or eliminating dominant species. Grassle and Sanders (1973) proposed the patch mosaic

theory, suggesting that small-scale periodic disturbances (sinking phytodetritus, dead falls, foraging activity, bioturbation, pits and mounds, and small-scale biogenic structures) enhance biodiversity by creating a mosaic of patches among which successional sequences are temporally out of phase. This theory became the principal paradigm for explaining local species coexistence (Snelgrove and Smith, 2002) but evidence to date has been mixed.

Food supply appears to be one of the primary environmental influences on deep-sea biodiversity, generally limiting deep-sea benthic fauna (abundance and biodiversity), which depend largely on organic material sinking from the euphotic zone (Ramirez-Llodra et al., 2010). Only a small percentage (0.5 to 2 %) of net primary production from the euphotic zone typically reaches deep-sea benthic communities below 2000 m (Buesseler et al., 2007). However, exceptions to strongly food-limited conditions include depocenters of organic matter at hadal depths, concentrations of organic material in submarine canyons, large food falls, and chemosynthetic environments (Danovaro et al., 2003, Vetter et al., 2010, Bernardino et al., 2010, 2012). Different factors influence the quantity and quality of food that reaches deep-sea benthic ecosystems from surface productive waters, including the biochemical composition of sinking material (Beaulieu, 2002), physical properties of the water column such as temperature, that influence the species composition of the plankton community (Beaugrand, 2009), degradation and transformation of slow sinking particles (Beaulieu, 2002), and transport through submarine canyons (Puig et al., 2014).

Some of the threats associated with human activity can also influence the quantity and quality of food that reaches the benthos. For example, global warming can modify the planktonic community (Beaugrand, 2009) with implications on the amounts and biochemical composition of food that reaches the deep seafloor (Smith et al., 2008). Rising temperatures, and therefore, ocean stratification, may favour small phytoplankton at the expense of diatoms, thereby altering biochemical composition and reducing export flux (Bopp et al., 2005). The gas hydrates that fuel chemosynthetic communities, though currently untapped as an energy resource, will eventually be targeted (Ramirez-Llodra et al., 2011), potentially reducing or eliminating energy sources that fuel microbes at the base of seep food webs (Ramirez-Llodra et al., 2011).

Given that food supply (quantity and quality) influences community structure (Gooday et al., 1990), changes in food supply can presumably alter the ecosystem functions carried out by these benthic communities. Billett et al. (1983) were among the first to report strong seasonality in phytodetrital deposition on the seafloor at temperate latitudes. Since then, numerous reports relate seasonal reproduction in deep-sea benthic species to surface-derived planktonic fluxes (Gooday, 2002, Hudson et al., 2003). Benthic communities respond to variation in particulate organic matter (POC) flux (Graf, 1989, Levin et al., 1999, Smith, 1999, Witte et al., 2003), however, different taxa respond in different ways. Bacteria and Foraminifera can take up fresh organic matter rapidly and reproduce in response to newly deposited phytodetritus; noting that these taxa account for most of the biomass within sediments, this activity plays a central role in Sediment Community Oxygen Consumption (SCOC) in the deep sea (Gooday et al., 1990, Witte et

al., 2003). Metazoan response can be as rapid or even exceed bacterial carbon uptake (Witte et al., 2003), however, substantive changes in macrofaunal and megafaunal population structure require more time. Seasonal macrofaunal (polychaetes, tanaidaceans, isopods) and meiofaunal (nematodes, harpacticoids, ostracods) density shifts have been reported approximately 9 months after phytodetritus deposition, coinciding with increased SCOC (Drazen et al., 1998). These examples illustrate how changes in amounts of food influence overall numbers, biomass, and/or SCOC. However, the quality of this food may play a different role. Dramatic shifts in megafaunal community structure were reported in the Porcupine Abyssal Plain, where an uncommon species (*Amperima rosea*) of holothuroid echinoderm became dominant within just months (Billett et al., 2010). The precise causes of population structure changes remain unclear, but likely link to changes in quality rather than quantity of phytodetritus (Billett et al., 2010). Furthermore, these food quality changes can be as subtle as changes in phytoplankton species, which result in changes in sedimentary pigment composition, which lead to selective feeding in some echinoderms (Wigham et al., 2003). Clearly, particulate organic carbon reaching the seafloor can significantly impact all sizes of benthic fauna, but more research is needed to understand the specific role of food quality and food quantity for different benthic communities and food webs.

This study aims to enhance knowledge on the role of food quality in structuring deep-sea benthic communities and food webs, which can, in turn, influence ecosystem function. To address the issue from different perspectives, I used multiple approaches including analysis of data from the literature to understand current knowledge of food quality,

correlations of species and feeding guild composition with food quantity and quality in space and time, and *in situ* experimentation.

The specific aims are:

- To investigate the importance of food quality in marine benthic communities and food webs by gathering and analysing data and information from the published literature (Chapter 2).

- To evaluate spatial variation in food quantity and quality in heterogeneous environments (i.e., submarine canyon and methane hydrates) through field sampling and identify its role as an environmental driver of infaunal communities and food webs (Chapter 3 and 4).

- To evaluate temporal variation in food quantity and quality in dynamic environments such as methane hydrates, and identify their role as an environmental driver of infaunal communities (Chapter 5).

- To identify the consequences of food pulses differing in quality, at the algal species level, on bathyal benthic ecosystems (i.e., macroinfaunal community structure, epifaunal disturbance, and food patch longevity) with an *in situ* experiment (Chapter 6).

1.1. References

Beaugrand, G. 2009. Decadal changes in climate and ecosystems in the North Atlantic Ocean and adjacent seas. Deep-Sea Research Part II: Topical Studies in Oceanography, 56, 656-673.

Beaulieu, S. 2002. Accumulation and fate of phytodetritus on the sea floor. Oceanography and Marine Biology: An Annual Review, 40, 171-232.

Bernardino, A.F., Levin, L.A., Thurber, A.R., Smith, C.R. 2012. Comparative composition, diversity and trophic ecology of sediment macrofauna at vents, seeps and organic falls. PLoS One, 7, e33515.

Bernardino, A.F., Smith, C.R., Baco, A., Altamira, I., Sumida, P.Y. 2010. Macrofaunal succession in sediments around kelp and wood falls in the deep NE Pacific and community overlap with other reducing habitats. Deep Sea Research Part I: Oceanographic Research Papers, 57, 708-723.

Billett, D.S.M., Bett, B.J., Reid, W.D.K., Boorman, B., Priede, I.G. 2010. Long-term change in the abyssal NE Atlantic: The *'Amperima* Event' revisited. Deep-Sea Research Part II: Topical Studies in Oceanography, 57, 1406-1417. doi: 10.1016/j.dsr2.2009.02.001.

Billett, D.S.M., Lampitt, R.S., Rice, A.L., Mantoura, R.F.C. 1983. Seasonal sedimentation of phytoplankton to the deep-sea benthos. Nature, 302, 520-522.

Bopp, L., Aumont, O., Cadule, P., Alvain, S., Gehlen, M. 2005. Response of diatoms distribution to global warming and potential implications: A global model study. Geophysical Research Letters, 32, L19606, doi:10.1029/2005GL023653.

Buesseler, K.O., Lamborg, C.H., Boyd, P.W., Lam, P.J., Trull, T.W., Bidigare, R.R., Bishop, J.K., Casciotti, K.L., Dehairs, F., Elskens, M. 2007. Revisiting carbon flux through the ocean's twilight zone. Science, 316, 567-570.

Danovaro, R., Della Croce, N., Dell'Anno, A., Pusceddu, A. 2003. A depocenter of organic matter at 7800m depth in the SE Pacific Ocean. Deep Sea Research Part I: Oceanographic Research Papers, 50, 1411-1420.

Dauwe, B., Herman, P.M., Heip, C. 1998. Community structure and bioturbation potential of macrofauna at four North Sea stations with contrasting food supply. Marine Ecology Progress Series, 173, 67-83.

Dayton, P., Hessler, R. 1972. Role of biological disturbance in maintaining diversity in the deep sea. Deep-Sea Research, 19, 199-208.

Drazen, J.C., Baldwin, R.J., Smith, K.L. 1998. Sediment community response to a temporally varying food supply at an abyssal station in the NE Pacific. Deep-Sea Research Part II: Topical Studies in Oceanography, 45, 893-913. doi: 10.1016/s0967-0645(98)00007-1.

Gooday, A.J. 2002. Biological responses to seasonally varying fluxes of organic matter to the ocean floor: A review. Journal of Oceanography, 58, 305-332. doi: 10.1023/a:1015865826379.

Gooday, A.J., Turley, C.M., Allen, J.A. 1990. Responses by benthic organisms to inputs of organic material to the ocean-floor - a review. Philosophical Transactions of the Royal Society a-Mathematical Physical and Engineering Sciences, 331, 119-138. doi: 10.1098/rsta.1990.0060.

Graf, G. 1989. Benthic pelagic coupling in a deep-sea benthic community. Nature, 341, 437-439. doi: 10.1038/341437a0.

Grassle, J.F., Sanders, H.L. 1973. Life histories and role of disturbance. Deep-Sea Research, 20, 643-659.

Hudson, I.R., Wigham, B.D., Billett, D.S.M., Tyler, P.A. 2003. Seasonality and selectivity in the feeding ecology and reproductive biology of deep-sea bathyal holothurians. Progress in Oceanography, 59, 381-407. doi: 10.1016/j.pocean.2003.11.002.

Levin, L.A., Blair, N.E., Martin, C.M., DeMaster, D.J., Plaia, G., Thomas, C.J. 1999. Macrofaunal processing of phytodetritus at two sites on the Carolina margin: *In situ* experiments using 13C-labeled diatoms. Marine Ecology Progress Series, 37-54.

Puig, P., Palanques, A., Martín, J. 2014. Contemporary sediment-transport processes in submarine canyons. Annual Review of Marine Science, 6, 53-77.

Ramirez-Llodra, E., Tyler, P.A., Baker, M.C., Bergstad, O.A., Clark, M.R., Escobar, E., Levin, L.A., Menot, L., Rowden, A.A., Smith, C.R. 2011. Man and the last great wilderness: human impact on the deep sea. PLoS One, 6, e22588.

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

Rex, M.A., Etter, R.J. 2010. Deep-sea biodiversity: pattern and scale. Harvard University Press.

Sanders, H.L. 1968. Marine benthic diversity: a comparative study. American Naturalist, 243-282.

Smith, C.R., De Leo, F.C., Bernardino, A.F., Sweetman, A.K., Arbizu, P.M. 2008. Abyssal food limitation, ecosystem structure and climate change. Trends in Ecology & Evolution, 23, 518-528.

Smith, K.L. 1999. Long-term discrepancy between food supply and demand in the deep Eastern North Pacific. Science, 284, 1174-1177. doi: 10.1126/science.284.5417.1174.

Snelgrove, P.V.R., Smith, C.R. 2002. A riot of species in an environmental calm: the paradox of the species-rich deep-sea floor. Oceanography and Marine Biology, 40, 311-342.

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

Thistle, D. 2003. The deep-sea floor: an overview. Ecosystems of the World, 5-38.

Thurber, A., Sweetman, A., Narayanaswamy, B., Jones, D., Ingels, J., Hansman, R. 2014. Ecosystem function and services provided by the deep sea. Biogeosciences, 11, 3941-3963.

Vetter, E.W., Smith, C.R., De Leo, F.C. 2010. Hawaiian hotspots: enhanced megafaunal abundance and diversity in submarine canyons on the oceanic islands of Hawaii. Marine Ecology, 31, 183-199.

Wigham, B.D., Hudson, I.R., Billett, D.S.M., Wolff, G.A. 2003. Is long-term change in the abyssal Northeast Atlantic driven by qualitative changes in export flux? Evidence from selective feeding in deep-sea holothurians. Progress in Oceanography, 59, 409-441. doi: 10.1016/j.pocean.2003.11.003.

Witte, U., Wenzhofer, F., Sommer, S., Boetius, A., Heinz, P., Aberle, N., Sand, M., Cremer, A., Abraham, W.R., Jorgensen, B.B., Pfannkuche, O. 2003. *In situ* experimental evidence of the fate of a phytodetritus pulse at the abyssal sea floor. Nature, 424, 763-766. doi: 10.1038/nature01799.

2. Rethinking the importance of food quality in marine benthic food webs [1]

2.1. Abstract

Current knowledge on the role of food for benthic communities and associated food webs focuses on quantity of available organic matter; however, the few studies that specifically address food quality show significant potential influences on food web and community structure. We examine current understanding of food quality, and consider its contribution to regulating benthic ecosystems. By assembling data from the literature we found that, whereas food quantity increases benthic stocks (i.e., abundances), various trophic groups respond differently to quality parameters, suggesting that food quality can alter benthic trophic structure substantially. Moreover, contrasting ecosystems respond differently to food quantity and quality inputs. Based on our literature review we find that, for many highly productive coastal marine ecosystems (coral reefs, seagrass meadows, kelp forests), the detrital compartment represents the most important primary food source by providing an indirect pathway of this high primary productivity, which is low in nutritional value (i.e., hard skeleton, lignin, deterrent substances, etc.), into the benthic food web. Strong seasonality in the flux of organic matter, such as in polar ecosystems, results in food webs based on relatively consistent but often poorer quality food sources (i.e., "food banks"). Benthic community structure may shift dramatically in food-poor deep-sea ecosystems where otherwise rare species become dominant in response to food pulses. These ecosystems appear to respond more strongly than other benthic ecosystems to quantity and quality of food input. In deep-sea chemosynthetic

environments, high food quantity and quality fuel benthic communities through resource partitioning of specialized chemosynthesis-based food sources. Lastly, we argue that food quality may have significant implications for benthic ecosystem functioning and services (e.g., bioturbation, nutrient fluxes, organic carbon preservation), particularly in the context of global warming. These implications point to several key gaps and opportunities that future food web studies should consider by applying knowledge gained in aquaculture to field studies, understanding the mechanisms for particle selection within the detrital compartment, and better understanding how rising temperatures and ocean acidification impact ecosystem functioning through changes in food quality.

Key words: benthos, biodiversity, food webs, food supply, food quality

2.2. Introduction

Many studies demonstrate the major role food quantity plays in structuring benthic communities and food webs (Pearson and Rosenberg, 1978, Rosenberg, 1995, Thiel, 1979, Wei et al., 2010). In contrast, far fewer benthic ecological studies address food quality which, limited evidence suggests, may be important in structuring benthic communities and food webs (Wigham et al., 2003a, Wieking and Kröncke, 2005, Billett et al., 2010). Food quality, defined here as the degree to which quantity and composition of accessible food fulfill consumer nutritional needs, cannot be fully disentangled from quantity because large amounts of low quality food may be as useful as small quantities of highly nutritious food (Müller-Navarra, 2008). Therefore, studies should examine both quality and quantity to understand the influence of food in structuring benthic communities and food webs.

Historically, marine ecologists have treated benthic and pelagic compartments somewhat separately, given contrasting species composition, community structure, processes, and even study methodologies. Most marine food web studies focus on pelagic rather than benthic ecosystems, creating a knowledge gap and a disconnection. Benthic food webs differ from their pelagic and terrestrial counterparts in supporting higher degrees of omnivory and connectivity or the number of links within the food web (Shurin et al., 2006). Sedimentary benthic ecosystems comprise the majority of benthic ecosystems (Snelgrove, 1999), and most examples in this review therefore focus on these habitats. Nonetheless, we include well-studied and productive coral reef and kelp systems to provide a comparison with highly productive shallow habitats.

In order to investigate the importance of food quality in marine benthic communities and food webs, we highlight major differences among seafloor ecosystems with contrasting pelagic and benthic primary productivity, thus spanning wide depths and latitudes. Different responses of benthic communities and food webs might be expected with plentiful food compared to food-poor environments with presumably greater competition for limited food resources. This review: 1) identifies different food quality components that influence benthic communities and food webs; 2) synthesizes evidence on how food quality, as opposed to quantity, structures benthic food webs; 3) contrasts the role of food quality between highly productive and food-limited ecosystems; 4) identifies the implications of differing food quality for contrasting marine benthic ecosystems; and 5) highlights areas of future research needs on these topics.

2.3. What is food quality?

In food science, properties contributing to total food quality include: 1) organoleptic and sensory attributes, 2) food safety, 3) nutritional value and 4) healthfulness (Giusti et al., 2008). In marine food webs these properties roughly translate to: 1) chemical and mechanical stimuli to encourage foraging, and palatability (Kleppel and Burkart, 1995), 2) toxicity, 3) nutritional value and digestibility (Perhar and Arhonditsis, 2009) and 4) beneficial health effects. Most of these properties are defined chemically and, in marine food webs, the units in the denominator are important. Thus analytes can be expressed as mass ratios (e.g. proportion of wet mass, dry mass, or organic mass, etc.) or concentrations (e.g. mass per litre, $g \cdot C^{-1}$, $g \cdot particle^{-1}$, or $g \cdot organism^{-1}$, etc.). The response of a consumer may differ depending on specific compound concentrations. When low concentrations of key compounds occur in excess particles, filter feeders, for example, cannot access them all, thus limiting food availability (Müller-Navarra, 2008). In contrast, when high concentrations occur in a few particles, resource intake depends on concentration per litre.

Important nutritional resources transferred to consumers include macronutrient proteins, lipids, and carbohydrates. Nitrogen, required for the formation of proteins, limits primary production in many marine ecosystems (Howarth, 1988), therefore carbon to nitrogen (C:N) ratios and nitrogen content provide insight into not only the degree of nitrogen-limitation in an ecosystem, but also into the nutritional value of a food source. Low C:N ratios in phytoplankton, and benthic microalgae (Hedges et al., 1988, Beaulieu, 2002), indicate higher nutritional value. The C:N ratio increases from Redfield ratio to values as

high as 10 with increasing organic matter decomposition (Henrichs, 2005). These easily digested food sources lack the refractory and deterrent compounds that characterize macroalgae, seagrasses, and terrestrial organic matter (Schell, 1983, Klumpp et al., 1989, Bolser and Hay, 1996).

Essential compounds (e.g. essential fatty acids, essential amino acids), by definition, cannot be synthesised de novo by benthic consumers but they play an important role in metabolism and in determining vital functions such as reproductive output. Carotenoids, in contrast, appear semi-essential for promotion of optimal survival and growth at low dietary inclusion levels (Wade et al., 2015). Most current knowledge on nutritional requirements comes from aquaculture experiments. This research demonstrates that finfish, crustaceans, and molluscs require ten indispensable amino acids (Li et al., 2009): arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. In aquatic animals, cysteine, glutamine, hydroxyproline, proline, and taurine are conditionally indispensable. In contrast, among the fatty acids, studies consistently identify the polyunsaturated fatty acids (PUFA) docosahexaenoic acid (DHA; 22:6ω3), eicosapentaenoic acid (EPA; 20:5ω3) and arachidonic acid (ARA; 20:4ω6) as indispensable to most cultured marine organisms (Glencross, 2009). Crustaceans and bivalves must obtain cholesterol, also an essential compound (Kanazawa, 2001, Müller-Navarra, 2008, Martin-Creuzburg and Elert, 2009).

Essential minerals also merit consideration. Iron fertilization substantially increases primary productivity in surface waters of high nitrate low chlorophyll (HNLC) environments such as the Southern Ocean, where low productivity occurs despite high

nutrient availability (Coale et al., 1996). Many trace metals play essential roles in the metabolism of invertebrates. For example, many enzymes require zinc, and the respiratory protein hemocyanine, found in some molluscs and malacostracan crustaceans, requires copper (Rainbow, 2002). Furthermore, tissue and skeleton formation, body-fluid chemistry, and photosynthesis all require magnesium (Harrison, 1990). Organisms require a specific quantity of each essential metal in order to meet essential metabolic needs; however, further accumulation of these essential trace metals in metabolically available form can potentially become toxic, requiring subsequent excretion and/or detoxification (Rainbow, 2002). Noting that because previous publications provide a detailed description of the chemical nature of nutrients, such detail is beyond the scope of this review (Appendix 2.1 summarizes the most relevant organic nutrients for benthic organisms).

Food may be available not only in solid but also in dissolved or colloidal form. Heterotrophic bacteria may utilize dissolved organic matter (DOM) as a food source, though DOM also adsorbs onto particle surfaces, transferring it back to the particulate fraction. DOM may be important as a food source for sediment-dwelling organisms. The aromaticity of DOM adds variable chemical reactivity (Weishaar et al., 2003), but aromatic compounds may represent an important energy source for microorganisms inhabiting deeper sediments in high depositional zones (Oni et al., 2015).

2.4. Methodology

Our analysis examined the general relationships between different food and food web structure parameters suggested by individual studies. It addresses food quantity by

examining total organic carbon (TOC) and quality by examining total nitrogen, C:N, pigments (chlorophyll *a*, chlorophyll *a*:phaeophorbides, chlorophyll *c*, fucoxanthin), and biopolymeric carbon (carbohydrates, proteins, lipids, proteins: carbohydrates). We consider total abundances as a measure of benthic standing stock and different feeding type abundances as a measure of trophic structure. For these analyses we separated taxa into different size groupings (i.e., meio-, macro-, and megafauna), and also considered their response in aggregate. We defined trophic groups broadly in order to accommodate all organisms from all studies: Surface deposit feeders (SD), sub-surface deposit feeders (SSD), suspension feeders (SF), and carnivores/scavengers (C/Sc). We omitted grazers because limited data availability precluded reasonable analysis.

Analyses based on trophic groups addressed all metazoan size groupings (i.e., meio-, macro-, and megafauna), although the vast majority of available data reported on macrofauna, with few megafaunal studies and even fewer meiofaunal trophic studies. Following a literature search using two search portals (ISI web of knowledge and SCOPUS) we assembled abundances of benthic trophic groups and organic matter data from different sedimentary ecosystems (deep-sea excluding chemosynthetic environments, polar, seagrass meadows, and other shallow-water). Initial inclusion of studies from highly disturbed areas (e.g. sewage, heavy metals, oxygen minimum zones, estuaries) in our analyses severely altered food-related patterns because of the strong influence of other (non-food related) environmental variables (e.g. contaminants, hypoxia, etc.), and we therefore excluded them. Particulate organic matter flux to the benthos can impact benthic communities but varies with depth (Wei et al., 2010) which

reflects time spent in the water column and, therefore, organic matter transformation. Because of this complexity, and the variable time lag between water column organic matter delivery and the response of benthic community structure, we only included sedimentary measures of food. We also included field observations only, because experiments in the laboratory may not necessarily reflect field responses in the absence of other environmental and biological parameters. The analysis presented here encompassed a total of 22 benthic studies (Appendix 2.2).

We completed all analyses using the R programming language (v3.3.0). Prior to running multiple regressions between benthic community and organic matter metrics we examined data for outliers, and log-transformed when necessary to achieve homogeneity of variance and normality in residual distributions. We tested normality with normal Q-Q plots, and homogeneity of variance by plotting residuals *vs* fitted values. High leverage data points (i.e. outliers) were tested with Cook's distance. The significance of each regression was re-tested, excluding data points with high leverage or outlier points from normality plots. If the model results differed, we report those without outliers but include the outlier data points on graphs (identified with brackets). A variance inflation factor (VIF<10) accounted for covariance between explanatory variables. We dropped the least biologically relevant variables or any variable with a higher VIF (total nitrogen, chlorophyll *c*, fucoxanthin, and biopolymeric carbon) to avoid inflating p-values and limit detection of significant effects.

2.5. Results and discussion

The studies included here could not account for the time lag between community and food dynamics because of the complexity of the response time of benthic communities to food variability in each ecosystem; benthic community structure could reflect organic matter input over the previous hours, days or even month(s). Furthermore, we based our comparisons on multiple regressions, acknowledging that fully resolving causation in food quantity and quality studies requires experimental manipulation. Even so, we believe our study identifies some intriguing patterns that point to potential research directions and hypotheses that our analyses cannot definitively address.

2.5.1. Food availability to benthic ecosystems: Quantity *vs* quality

Our results showed a statistically significant increase in meiofaunal, macrofaunal, and megabenthic densities with increasing TOC (Table 2.1, and Fig. 2.1, $p < 0.05$), a pattern widely accepted by ecologists (Table 2.2). Early studies correlated amounts of food with benthic stock in space, with patterns further supported by temporal observations (Table 2.2). Assessments of benthic stocks were subsequently related to decreasing organic carbon with depth, and researchers began to identify seafloor features such as submarine canyons as potential biodiversity and biomass "hot-spots" because they can aggregate organic matter compared to adjacent slopes (Table 2.2). More recent studies suggest varying relationships between total amounts of organic carbon and changes in biodiversity parameters (Table 2.2).

Table 2.1. Simple or multiple regressions considered for each combination of benthic fauna (density as ind/surface area) and food parameters. Significant models (p<0.05) are shown in bold with the variability explained by the model (R^2) and the coefficient estimate for each significant parameter in brackets. * Identifies statistically significant (p<0.05) individual variables.

Faunal Group	Food quantity	Nitrogen	Pigments	Labile carbon
Megafauna	**TOC* R^2=0.98 (0.44)**	C:N	Chl/TOC	----
Macrofauna	**TOC R^2=0.12 (1.61)**	C:N	Chl/TOC+Chl/Ph	CHO+LIPID
Meiofauna	**TOC* R^2=0.11 (3.47)**	**C:N* R^2=0.42 (-0.5)**	**Chl/TOC+Chl/Ph* R^2=0.57 (-3.84)**	**CHO+PROT*+LIPID*+PROT/CHO* R^2=0.99 (PROT: 0.45, LIPID: -1.31, PROT/CHO: 0.28)**
Surface deposit feeders	**TOC* R^2= 0.15 (1.79)**	**C:N* R^2= 0.75 (1.19)**	**Chl/Ph* R^2= 0.49 (9.15)**	----
Subsurface deposit feeders	TOC	C:N	**Chl/Ph* R^2= 0.37 (4.22)**	Density ~ CHO+LIPID
Suspension feeders	TOC	C:N	Chl/TOC+Chl/Ph	**CHO+LIPID* R^2= 0.99 (1391,39)**
Grazers	TOC	----	Chl/TOC+Chl/Ph	----
Carnivore/ scavenger	**TOC* R^2= 0.17 (-1.34)**	C:N	Chl/TOC+Chl/Ph	CHO+LIPID
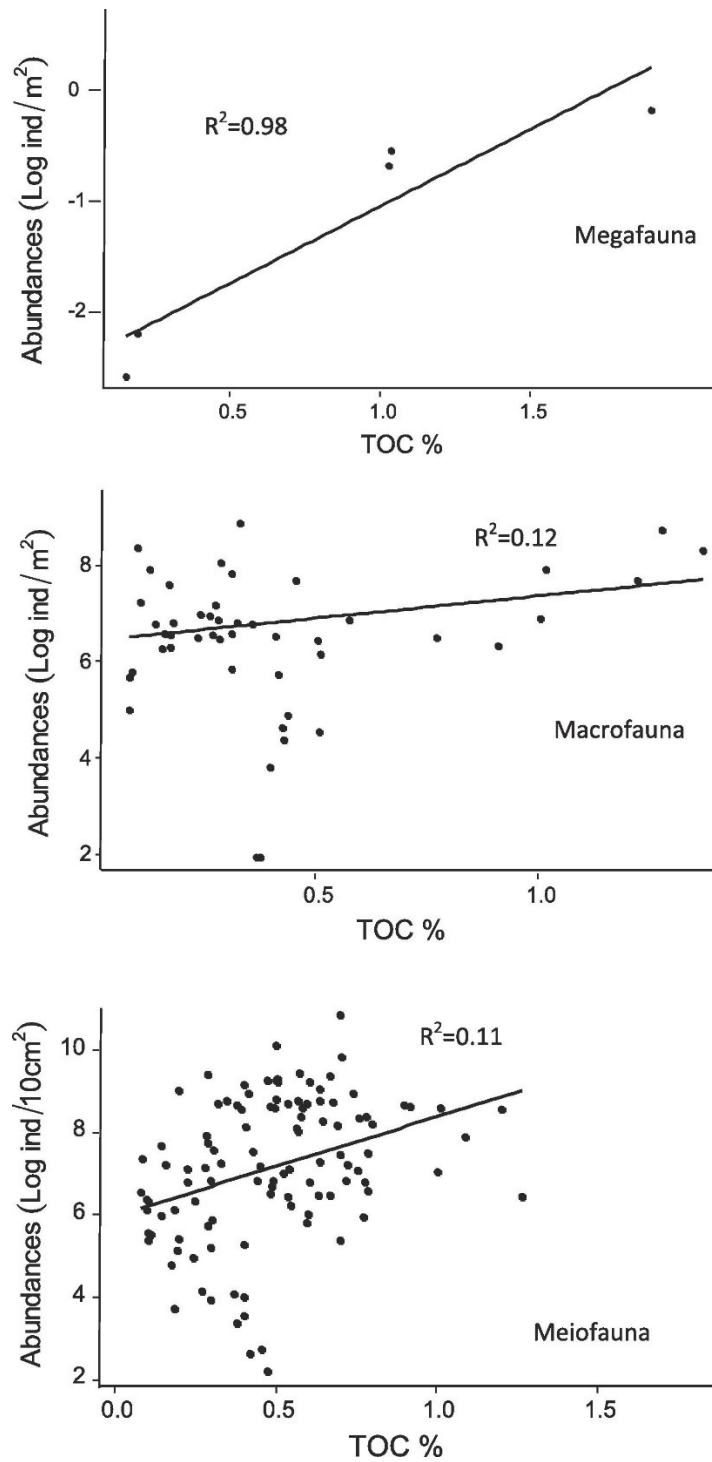
Figure 2.1. Response of benthic stocks (mega-, macro-, and meiofauna) to varying sediment total organic carbon (TOC) across all ecosystem types. All regression lines are statistically significant (p <0.05).

Table 2.2. Timeline describing major findings on the influence of food quantity on benthic communities.

Time	Finding	Reference
1970's	**Characterization of the influence of increased organic matter on benthic communities:**	
	Organic enrichment of benthic environments, whether from natural or artificial causes, results in higher abundances and lower number of species and biodiversity	Pearson and Rosenberg, 1978
	Lower meiofaunal abundances characterized the West African Angola Basin compared to the nearby Cape Basin were coastal upwelling occurs	Dinet 1973
	Lower meiofaunal abundances are found at the Faroe-Bank-Canal where strong currents in the limit settlement of particles, compared to the Norwegian Sea where currents are weak	Thiel 1979
1990's	**Temporal variation in benthic biomass related to organic flux variation:**	
	Temporal variation in organic matter supply to the abyssal NE Atlantic (BIOTRANS station) correlated with increased bacterial and protozoan biomass but not macro-mega-meio-benthos biomass	Pfannkuche, 1993
	Temporal variation in organic matter supply to the abyssal North Pacific correlated with increased epifaunal abundances (mostly holothurians and echinoids)	Smith et al., 1994
2010's	**Benthic stock with depth:**	
	Benthic biomass and abundances of all major taxa (meio-,macro-, and megafauna) generally decrease with depth (shallow to abyssal)	Rex and Etter, 2010
	Submarine canyons:	
	Submarine canyons can function as biomass hot spots	De Leo et al., 2010
	Currents within Barrow Canyon enhance carbon input to benthos where highest macrofaunal abundances occur in the Northeast Chukchi Sea	Schonberg et al., 2014
	Biodiversity:	
	Zonation patterns in the Gulf of Mexico correlate with POC and depth	Wei et al., 2010
	Inter-annual variation in density, species diversity and functional groups of abyssal polychaetes in the North Pacific correlated with depth, latitude and POC	Laguionie-Marchais et al., 2015

Irrespective of overall trends, each trophic group responded differently to different food parameters (Table 2.1). Whereas total megafaunal, macrofaunal, and meiofaunal abundances increased with TOC, individual trophic groups varied with respect to TOC (surface deposit feeders increased, carnivores decreased, and others showed no significant trend, Table 2.1 and Fig. 2.2). Contrary to expectation, surface deposit feeders responded positively to increases in C:N (Table 2.1), whereas other trophic groups were not significantly affected. The low number of available data points for C:N and trophic groups might explain these results, but it could also suggest a higher requirement for carbon or lower limitation by nitrogen, given the significant relationship between surface deposit feeders and TOC (Table 2.1). Surface and subsurface deposit feeders significantly increased with "freshness" of phytodetritus (i.e., high chlorophyll a/phaeophorbides; see Table 2.1 and Fig. 2.3). Our results show that suspension feeders significantly increased with lipid concentrations (Table 2.1), indicating a requirement for lipids, although admittedly the comparison was based on only a few data points, which might explain the unusually high R^2.
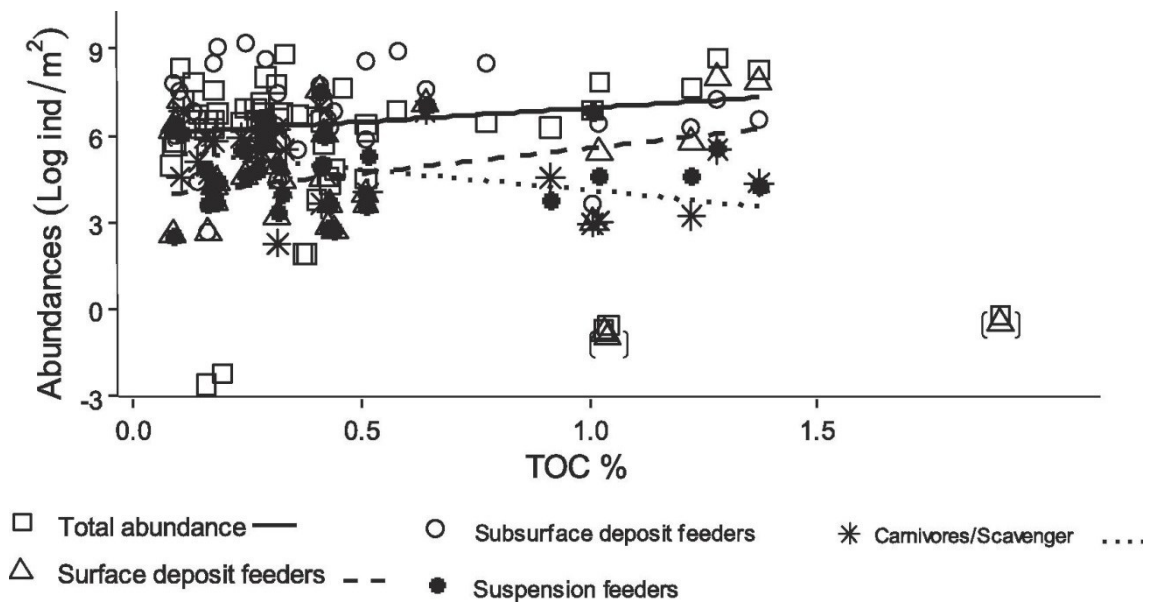
Figure 2.2 Total abundances and abundances per trophic group across gradients of TOC across all ecosystem types. Regression lines are only drawn for the statistically significant regressions (p <0.05). Because of the numerous comparisons summarized in the Figure, we report the R^2 values in Table 2.1.
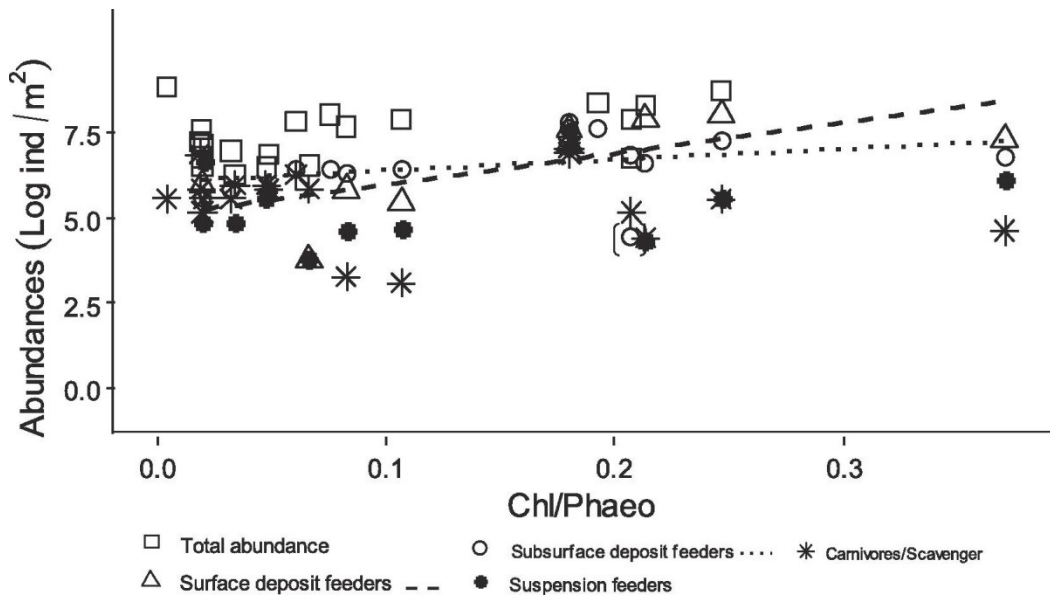
Figure 2.3. Total abundances and abundances per trophic group across gradients of Chl/Phaeo ratio across all ecosystem types. Regression lines are only drawn for the statistically significant regressions (p <0.05). Because of the numerous comparisons summarized in the Figure, we report the R^2 values in Table 2.1.

Interestingly, the lowest trophic levels responded most strongly to changes in food quality. Increases in food nutritional value increased abundances of deposit feeders and suspension feeders in different ways, depending on the food quality parameter and trophic group. However, we observed no response at the highest trophic levels (i.e., carnivores/scavengers). These results suggest a disconnection of the highest trophic levels from food quality, but a dependency (although negative) on total amounts of food (Table 2.1). These patterns suggest that varying composition of food should alter relative proportions of different trophic groups. Other studies that suggest that food quality may impact community and food web structure (Table 2.3) support our results.

Table 2.3. Timeline describing major findings on the influence of food quality on benthic communities.

Time	Finding	Reference
1980's – 1990's	**Patch mosaic theory and colonization experiments with different contrasting food sources:**	
	Patch mosaic theory: the unexpected high diversity found in deep-sea sedimentary habitats results from organic patches derived from differences in food supply (seasonal pulses of organic matter, organic remains of gelatinous zooplankton, wood falls, kelp, and seagrass)	Grassle and Sanders 1973
	Different species colonized azoic sediment trays and enriched with wood and macroalgae in the deep sea	Grassle et al 1987
	Different species colonized sediment trays enriched with different food qualities (microalgae, and macroalgae at different degradation stages) at 900 m depth	Snelgrove et al. 1992, 1994, 1996
	Evidence of temporal variation in food availability and quality supplied to benthos and tracers to benthic fauna:	
	Evidence of seasonal pulses of organic matter into the deep PAP (Porcupine Abyssal Plain)(1370-4100 m.), NE Atlantic	Billett et al. 1983
	Interannual variation in quality of flux of organic matter (i.e. salp faecal pellets *vs* plankton bloom) identified in the PAP, NE Atlantic	Pfannkuche 1993
	Tracing specific pigments found in the plankton community, in particular cyanobacteria, to benthic organisms, in particular, holothurians from the PAP	Pfannkuche et al. 1999

Table 2.3. Continued

Time	Finding	Ref
2000's	**Long-term changes in food web and community structure appear to be related to food quality changes:**	
	Dramatic shifts in the benthic community (epifauna and infauna) in the NE Atlantic (PAP) correlated with climate variation that can induce changes in food quality (i.e., increase in specific carotenoid concentrations)	Billett et al., 2001/2010
	Different pigment composition in gonads of different benthic holothurians suggested resource partitioning in the NE Atlantic (PAP)	Wigham et al., 2003b
	Dramatic shifts of benthic community (epifauna) in the NE Pacific correlated with climate variation, which the authors suggest could have altered food supply (quantity and quality: plankton composition)	Ruhl et al., 2004
	Food quality heterogeneity increased infaunal trophic diversity in the North Sea. Predators increased with presence of more degraded organic matter, whereas interface feeders and surface deposit feeders increased with "fresh" organic matter	Wieking et al., 2005
	Mineralization rates much faster in "fresh" organic matter enrichment than "altered" (more refractory) organic matter	Aspetsberger et al. 2007
	Climate can influence plankton community structure which can, in turn, alter biochemical and magnitude flux to the abyssal benthos	Smith et al. 2008
2010's	**Pulse-chase experiments with different food sources:**	
	Different bacterial and diatom organic matter utilized by nematode community in Nazaré Canyon (off Portugal)	Ingels et al. 2011
	Remineralisation of diatom organic matter much faster than for faecal pellet in the deep-sea of Northeast Atlantic	Mayor et al., 2012
	Different major taxa dominate the processing of organic matter depending on which phytodetritus or terrestrial detritus is present	Hunter et al., 2013

The "patch mosaic" theory and subsequent colonization experiments were the earliest studies on the topic, showing that different species colonized patches differing in food quality (Table 2.3). Subsequent studies looked at temporal variation in food quality reaching benthic communities, or at long-term changes in the benthic communities themselves (Table 2.3). More recent pulse-chase experiments studies show different fates (i.e., uptake by different taxa) of carbon depending on phytodetritus quality (Table 2.3). Detailed examples regarding the influence of food quality on food web structure follow below.

Environmental conditions, and ocean currents in particular, resulted in contrasting food quality (fresh *vs* degraded) in different areas of the North Sea (Wieking and Kröncke, 2005). As a consequence, macrofaunal trophic structure differed among sites, with interface feeders (i.e., feeding on suspended and surface sediment organic matter) dominating sites with intermediate quantities of high quality food (high chlorophyll *a*/C and low phaeophorbides/chlorophyll *a* ratios, indicative of phytodetritus "freshness"). Interface feeders also dominated sites with abundant food of intermediate quality, despite differences in species composition. The highest trophic diversity characterized sites with relatively lower food quantity but highly heterogeneous composition ("fresh" and degraded organic matter), where abundance of hyperbenthic predators exceeded other sites (Wieking and Kröncke, 2003, Wieking and Kröncke, 2005). Similarly, high trophic diversity of nematodes in the deep Mediterranean Sea correlated with high concentrations and high heterogeneity of potential food sources (Danovaro et al., 2004).

Ocean circulation patterns transport different water masses that influence benthic food webs, particularly in Arctic waters. Within the Arctic Chukchi Sea, for example, nutrient-rich and nutrient-poor water masses affected benthic food webs differently (Iken et al., 2010). Infaunal primary consumers (macrofauna) quantitatively dominated communities in nutrient-rich Anadyr water, capitalizing on abundant "fresh", labile material. In contrast, higher trophic levels exploited more refractory particle sources and dominated Arctic coastal water (i.e., nutrient-poor, with terrestrial influence: Iken et al., 2010). However, both areas exhibited similar relative biomass proportions within each trophic level for epifaunal communities (megafauna), with a larger proportion of biomass at higher trophic levels (Iken et al., 2010; see Appendix 2.3). This pattern suggests taxon-, community-, or even size-dependent effects of food quality. In fact, our results show different responses in total abundances of different size groupings with food quality parameters. The C:N ratio negatively influenced the total abundance of meiofauna ($p<0.05$) but not macro- or megafauna (Table 2.1). "Freshness" of the phytodetritus positively influenced meiofaunal abundances but not macro- or megafaunal abundances (Table 2.1). The labile fraction of organic carbon (proteins, lipids, carbohydrates) also played a role in structuring benthic communities. In particular, proteins, lipids, and the protein/carbohydrate ratio positively affected meiofauna (Table 2.1 and Fig. 2.4). These contrasting outcomes, particularly the stronger patterns in meiofauna, suggest differences in response to food quality with organism size, with greater sensitivity to gradients in food quality for smaller organisms.

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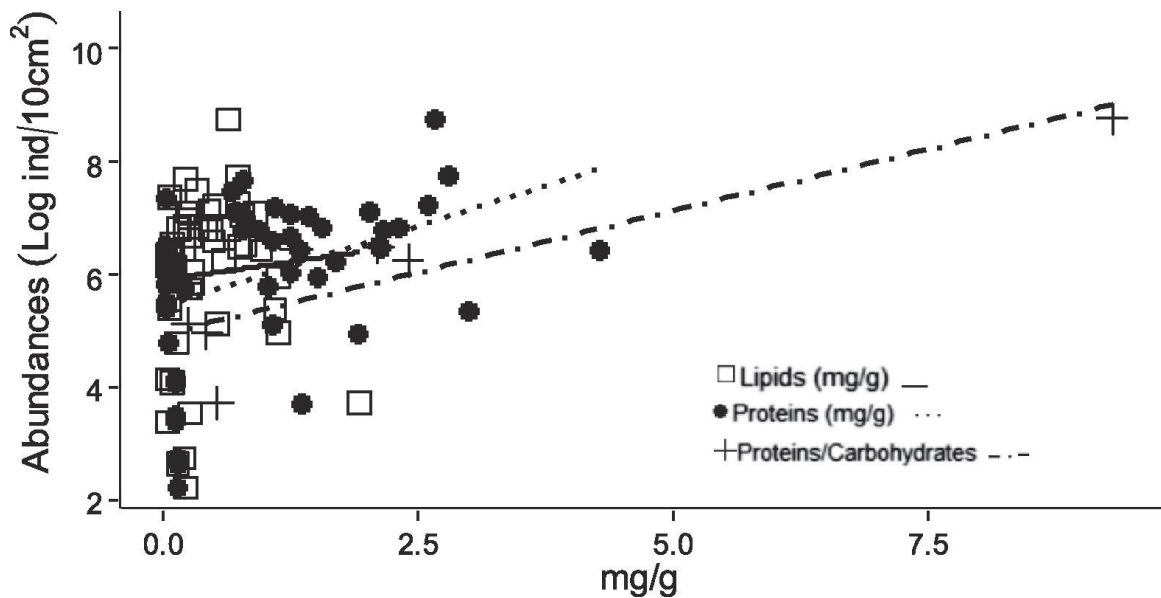
Figure 2.4. Response of meiofaunal abundances with varying lipids, proteins, and protein:carbohydates ratio across all ecosystem types. Regression lines are only drawn for the statistically significant regressions (p <0.05). Because of the numerous comparisons summarized in the Figure, we report the R^2 values in Table 2.1.

2.5.2. Importance of food quality in ecosystems with contrasting primary productivity

When considering ecosystem type, macrofauna and meiofauna increased with increasing TOC when combining all ecosystem types, but not in individual ecosystem types (Fig. 2.5); the trend is evident only when combining all ecosystems. These findings raise the question of the relative roles of food quality in highly productive ecosystems compared to food-limited ecosystems, which we explore below. Highly productive ecosystems might be expected to provide sufficient food resources to fulfill all the nutritional requirements of benthic fauna, in contrast to organisms in food limited, less productive ecosystems. In order to span a broad range of ecosystems, the following section considers non-sedimentary ecosystems (i.e., coral reefs, hydrothermal vents) and non-detrital food sources such as organic matter produced through symbiotic relationships (i.e., coral, tube worms from deep-sea chemosynthetic environments), as well as seagrasses and kelps.

52

The modest number of studies available for our empirical analysis and the added complexity of different environments did not allow us to formally test this hypothesis, so we address it from a more theoretical perspective.
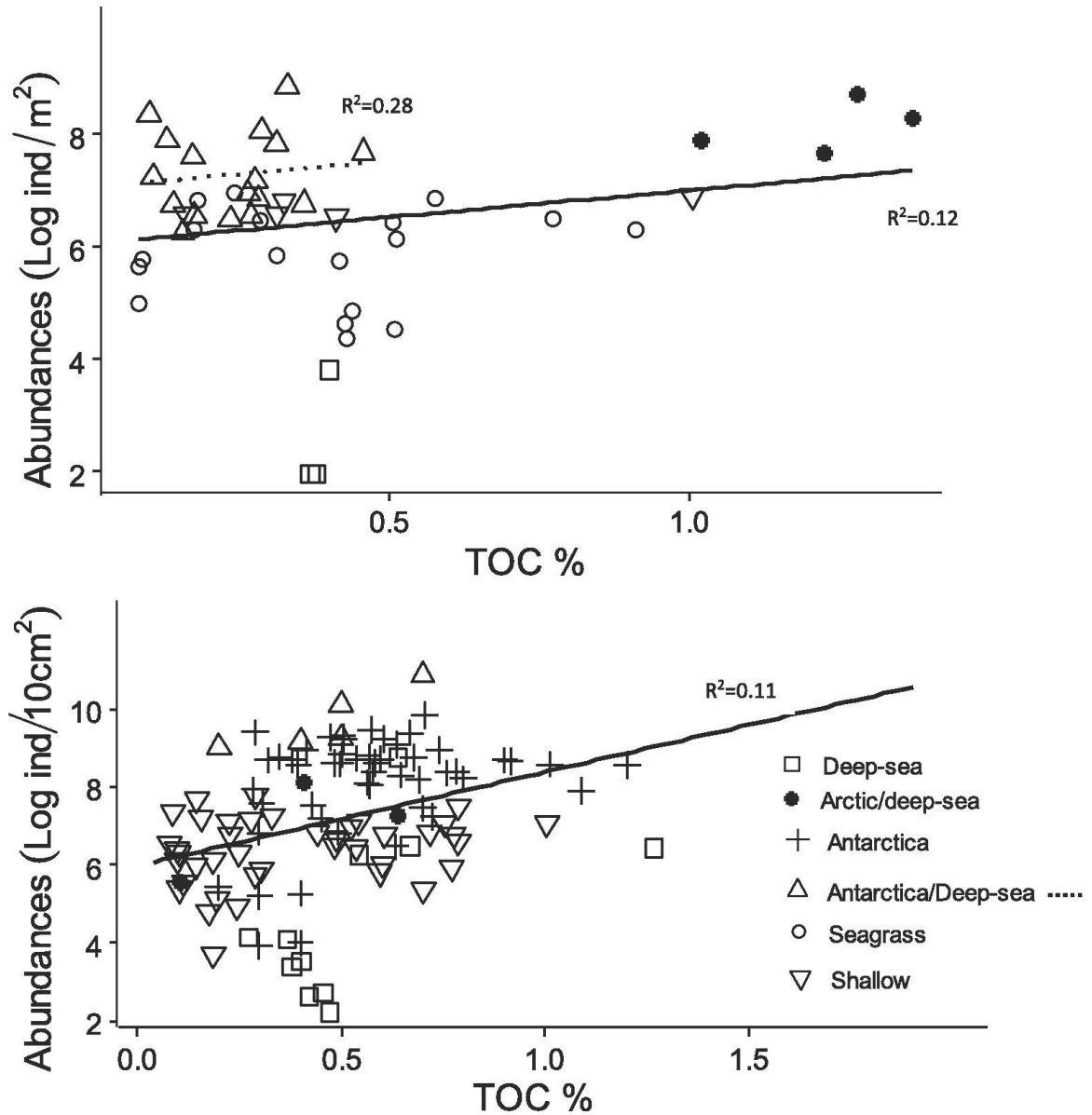
Figure 2.5. Response of macrofaunal (top) and meiofauna (bottom) abundances encompassing all feeding types in different ecosystem types along a gradient of TOC. Regression lines are only drawn for the statistically significant regressions (p <0.05). Solid regression line represents all ecosystems combined.

In terms of primary production, coral reefs (1500-5000 g C/m^2/yr), seagrass beds (500-1000 g C/m^2/yr), and kelp forests (800 g C/m^2/yr) comprise the most productive marine benthic ecosystems (Groombridge and Jenkins, 1996), in contrast to the strongly seasonal productivity of Antarctic and Arctic waters, which are highly productive for only short periods of time during the year. Overall, the deep sea encompasses the least productive marine benthic ecosystems, lacking autochthonous primary productivity, except for extraordinarily productive chemosynthetic ecosystems; the tube worm *Riftia pachyptila* alone, which dominates many hydrothermal vent systems on the East Pacific Rise, produces ~ 400 g C/m^2/year through symbiotic chemosynthetic bacteria (Shillito et al., 1999).

2.5.2.1. Coral reefs, seagrass meadows, and kelp forest: High food quantity but low food quality

Seagrasses, kelps, and corals provide important protection and habitat for other species, as well as a potential food source. Despite their high productivity, however, low palatability and nutritional content limit their value as a direct food source for many species (Klumpp et al., 1989, Sorokin, 1990, Fredriksen, 2003). Although their contribution as a direct food source can vary across food webs, large quantities of lignin and cellulose, and low nutrient content can result in poor nutritional value of seagrasses, whereas production of herbivore-deterrent compounds characteristic of kelps (Atkinson and Smith, 1983, Duggins and Eckman, 1997, Moncreiff and Sullivan, 2001, Norderhaug et al., 2003) and the hard skeleton in corals can reduce their appeal as food. Nonetheless, substantial productivity from these sources enters the food web through the detrital

pathway (Fig. 2.6 and Appendix 2.3), contributing significantly to food web inputs (Fredriksen, 2003, de Goeij et al., 2013).
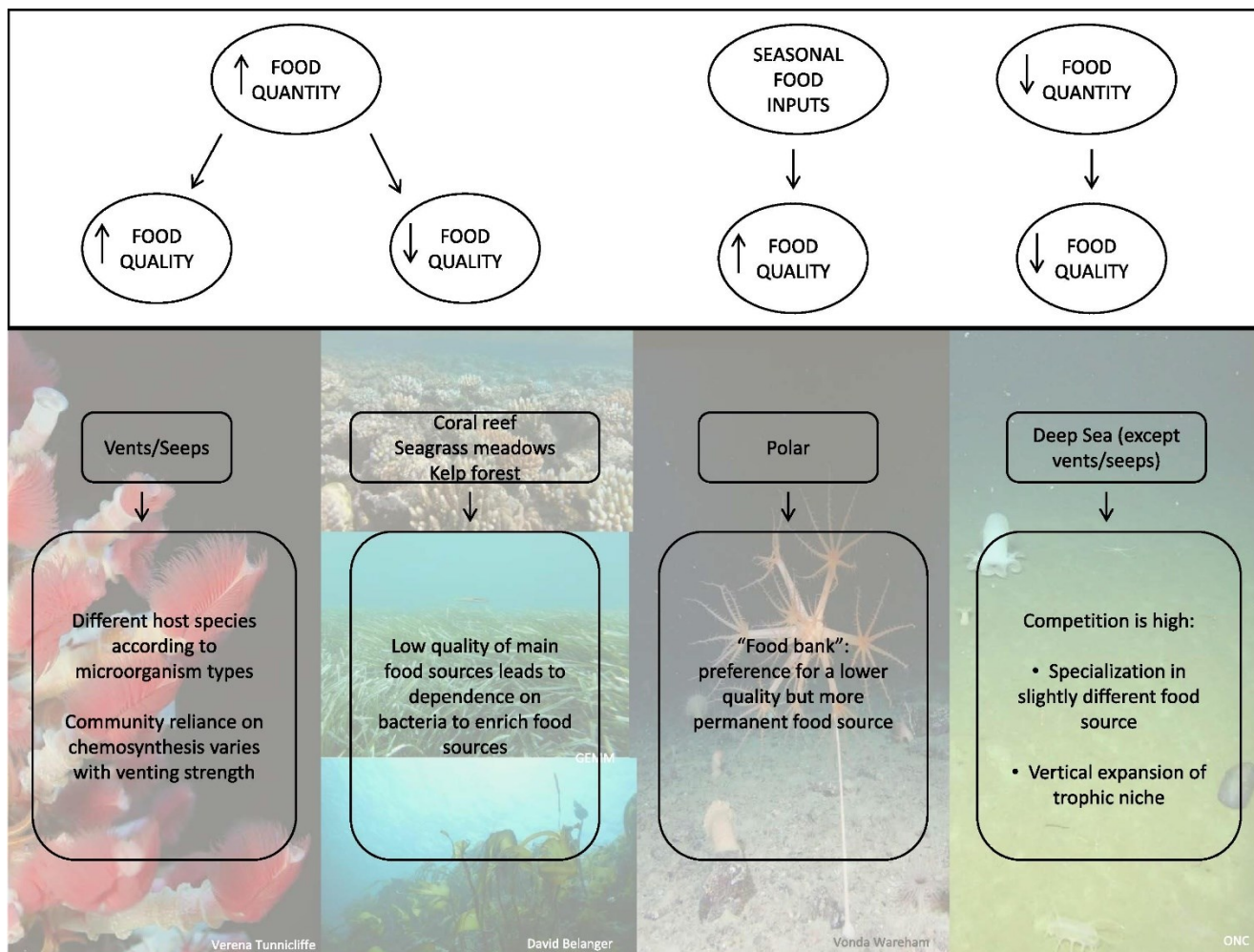
Figure 2.6. Schematic illustration of the main responses of benthic communities and species to different food quantities and qualities. Arrows next to food parameters indicate high/low.

Coral reef food webs are perhaps the best understood of highly productive marine benthic ecosystems, and we therefore consider them here in further detail. Despite high primary productivity and diverse food sources in coral reef ecosystems, the detritus pool and dissolved organic matter (DOM) provide the major energy pathway to higher trophic levels and thus form the foundation of reef ecosystem energy (Sorokin, 1990, de Goeij et al., 2013; see Appendix 2.3). In general, most production from coral-reef primary producers (e.g., macrophytes, seagrasses, calcareous algae) enters the reef food web through the detritus pool rather than through grazers feeding directly on primary producers (Sorokin, 1990). A recent breakthrough demonstrated that a significant portion of DOM enters higher trophic levels through the sponge loop (de Goeij et al., 2013). Significant uptake of DOM from surrounding waters (> 50 % of total) by sponges, driven by allocation to tissue turnover, results in massive shedding of tissue that becomes POM or detritus in the reef food web (de Goeij et al., 2013).

The quality of some types of benthic primary producers such as seagrasses and kelps varies with geographic location, which can influence benthic food web energy flows (Appendix 2.3). High leaf production and rapid turnover year round, lower fibre concentrations, and higher concentrations of carbohydrates result in higher nutritional value in tropical seagrasses than their temperate counterparts (Vonk et al., 2008). These attributes increase the range of grazers feeding directly on tropical seagrass leaves and therefore create a significant, more direct pathway from seagrasses to higher trophic levels (Vonk et al., 2008). Even so, the detrital pathway has greater relevance than direct grazing on leaves (Appendix 2.3). Production of higher concentrations of herbivore deterrents by seaweeds at low latitudes in response to grazing pressure reduces their palatability (Bolser and Hay, 1996; see Appendix 2.3). Subtle effects of changes in the food quality of macroalgae and seagrasses, in tandem with their high productivity, demonstrate that

abundant low quality food may serve a similar function as small amounts of highly nutritious food.

Once the primary food source mixes with the detritus it enters a very heterogeneous pool of organic matter. Although the detritus pool may appear homogenous as a food source, it often encompasses diverse components with different origins spanning different classes of dead phytoplankton, zooplankton moults, faecal pellets, terrestrial organic matter, and bacteria, among others (Beaulieu, 2002). Some studies suggest that plentiful food in highly productive shallow-water ecosystems can decrease competition for food resources, and therefore reduce niche separation (Hudson et al., 2003, Godbold et al., 2009), as shown in feeding experiments with echinoderms from 30-60 m in Gullmarfjord, Sweden (Godbold et al., 2009). Nonetheless, selective feeding on detritus also occurs (Jumars and Eckman, 1983). We refer readers to a previous review (Lopez and Levinton, 1987) that summarizes infaunal selection for organic compounds but we reiterate a few salient points. Lopez and Levinton (1987) inferred that completely nonselective deposit-feeding probably does not occur, in that organically enriched faecal pellets of some deposit feeders compared to surrounding sediments indicate some level of dietary selection. The limited mobility that characterizes most deposit-feeders constrains their ability to forage actively for patches. Taghon and Jumars (1984) tested an optimal foraging model that predicted covariance in ingestion rate and food value in order to maximise net rate of energy gain. Their experiments supported the model by comparing three polychaete species deposit-feeding on artificial sediments with varying protein content, confirming the assertion that deposit feeders can select among particle size, shape, surface texture, and specific gravity (Lopez and Levinton, 1987). Morphology of deposit feeders offers some insight into their selective abilities. For example, species with tentacles most likely feed selectively, in contrast to non-

selective species with eversible sac-like pharynxes (Lopez and Levinton, 1987). Selectivity starts at the ingestion stage but continues with digestion where digestive tract morphology promotes particle sorting by increasing residence time of preferred particles (Lopez and Levinton, 1987). Oligochaetes select particles of specific grain size and organic richness (Rodriguez et al., 2001), mussels select for specific particle sizes (Defossez and Hawkins, 1997), and some nematodes, benthic harpacticoids, and tubificids can select between diatoms and bacteria (Azovsky et al., 2005, Estifanos et al., 2013).

Examples of fine-level selectivity are few, but they exist. Oysters and harpacticoid copepods, for example, can selectively feed on different species of phytoplankton and even different species of diatoms (Loret et al., 2000, Cognie et al., 2001, Azovsky et al., 2005,). Heart urchins (*Echinocardium cordatum*) from the North Sea actively select for fatty acids and chlorophyll *a* (Boon and Duineveld, 2012), demonstrating selective deposit feeding on specific detrital pool compounds in shallow-water. Although this study demonstrates selective feeding in habitats with high organic matter inputs, much higher selection characterized the site with lower organic input. This topic requires further study; we found no field studies for our statistical analysis that reported on critical food quality parameters (e.g. essential fatty acids, essential amino acids, and semi-essential carotenoids), even though aquaculture research demonstrates the likely importance of these parameters in structuring benthic communities and food webs.

2.5.2.2. Temperate and polar environments: Intermittent availability of high quality food:
Benthic communities in polar and temperate regions often experience variable annual primary productivity with strong seasonal pulses of organic matter (Appendix 2.3). Linear inverse models from the Porcupine Abyssal Plain (PAP) show minimal contribution of labile detritus to the food web, representing less than 5 % of the total carbon requirements for all compartments (i.e., meio-

and macrobenthos: van Oevelen et al., 2012). Intra- and inter-annual variation in detrital input from the water column does not result in large variation in availability of the semi-labile fraction to the benthos (van Oevelen et al., 2012), indicating that benthic consumers base their diets on the low quality, but more permanent semi-labile fraction of detritus (Appendix 2.3, van Oevelen et al., 2012). We believe that the temperate location of PAP, which explains variability in food pulses, rather than depth (~ 4800 m), is responsible for the dependency on semi-labile detritus stated above.

Despite periods of high phytoplankton production in Antarctic ecosystems that represent a potential food source for benthic communities, most food enters benthic food webs through the detrital pathway via bacterial degradation (Glover et al., 2008, Berge et al., 2015), which persists year round as a permanent "food bank" (Fig. 2.6, Appendix 2.3). This "food bank" likely contributes to aseasonal reproductive patterns in most taxa near the Antarctic Peninsula, suggesting a greater dependence on the relatively permanent food source (Glover et al., 2008) rather than on seasonally variable chlorophyll *a*, enzymatically hydrolysable amino acids (EHAA), and bacterial biomass in near surface sediments (Mincks et al., 2005).

"Trophic buffering" has been suggested for temperate and northern benthic ecosystems where a large pool of sediment organic matter (SOM), may buffer benthic deposit feeders from year to year differences in spring bloom intensity (Levinton, 1972). The absence of seasonal denitrification and oxygen flux patterns in the northeast Chukchi Sea, despite seasonal fluxes of POM (Devol et al., 1997), support "trophic buffering", although in the Bering Sea, sediment oxygen demand varies with season and year, suggesting variable buffering (Lovvorn et al., 2005). The differences observed among Arctic areas may result from differences in food supply, such as the influence of more productive, richer quality waters of the Chuckchi Sea.

2.5.2.3. Deep-sea ecosystems: Low food quantity and quality

Deep-sea non-chemosynthetic ecosystems largely depend on external input of organic matter, primarily sinking from surface waters (Gage and Tyler, 1991), but also through lateral advection (Danovaro et al., 2003). In the open ocean, bacteria attach to and transform descending phytodetrital particles as they sink, degrading most labile organic compounds before they reach the deep-sea floor. Highly refractory organic matter persists to greater depths, as occurs with preferential delivery of carbohydrates over lipids and proteins in the Cretan Sea (Danovaro et al., 2000). Larger, faster-sinking aggregates arrive on the deep-sea floor first, typically representing 1-3 % of surface water primary production (Gage and Tyler, 1991). TOC and nitrogen content therefore typically decrease with depth, as do concentrations of proteins and individual lipids (Kiriakoulakis et al., 2001). At the same time, bacteria attached to settling particles add a potentially important food source for some deep-sea benthic ecosystems. In the Cretan Sea, bacterial carbon flux accounted for 5-6.5 % of the labile carbon flux, and for 22-41 % of the protein pool of settling particles (Danovaro et al., 2000). Several studies attributed increased particulate organic carbon (POC) and/or certain detritus fractions with depth to lateral advection of organic matter (Baldwin et al., 1998, Danovaro et al., 2000, Danovaro et al., 2003). This mechanism of food delivery may be more widespread than previously thought (Danovaro et al., 2014), which may explain the absence of consistent depth patterns in some aspects of organic matter, such as phytopigment concentration (Beaulieu, 2002).

Our results suggest greater sensitivity to changes in food quality in the deep sea, potentially reflecting the overall scarcity of food. Specifically, we found that benthic faunal responses varied substantially across ecosystems (i.e. shallow and deep) when considering certain food parameters (proteins, lipids, proteins:carbohydrates Fig. 2.7). In particular, deep-sea meiofauna increased in

abundance with increasing lipids and proteins, with no pattern observed in shallow-water meiofauna (Fig. 2.7). Dramatic shifts in benthic community structure were also reported in the deep sea in response to natural organic fluxes. *Amperima rosea*, a previously rare, opportunistic holothurian at the PAP, suddenly became the dominant megafaunal species, which the authors attributed to climate variation (correlated with NAO oscillations) that potentially changed the biochemistry of organic flux to the abyss (Billett et al., 2010). These specialised holothurians require semi-essential carotenoids and essential fatty acids to enhance their reproductive output, and effectively exploit the "freshest" organic matter that contains these essential compounds. Therefore, changes in the biochemistry of organic matter flux to the seafloor may support rapid increases in the abundance of *A. rosea* (Wigham et al., 2003b).
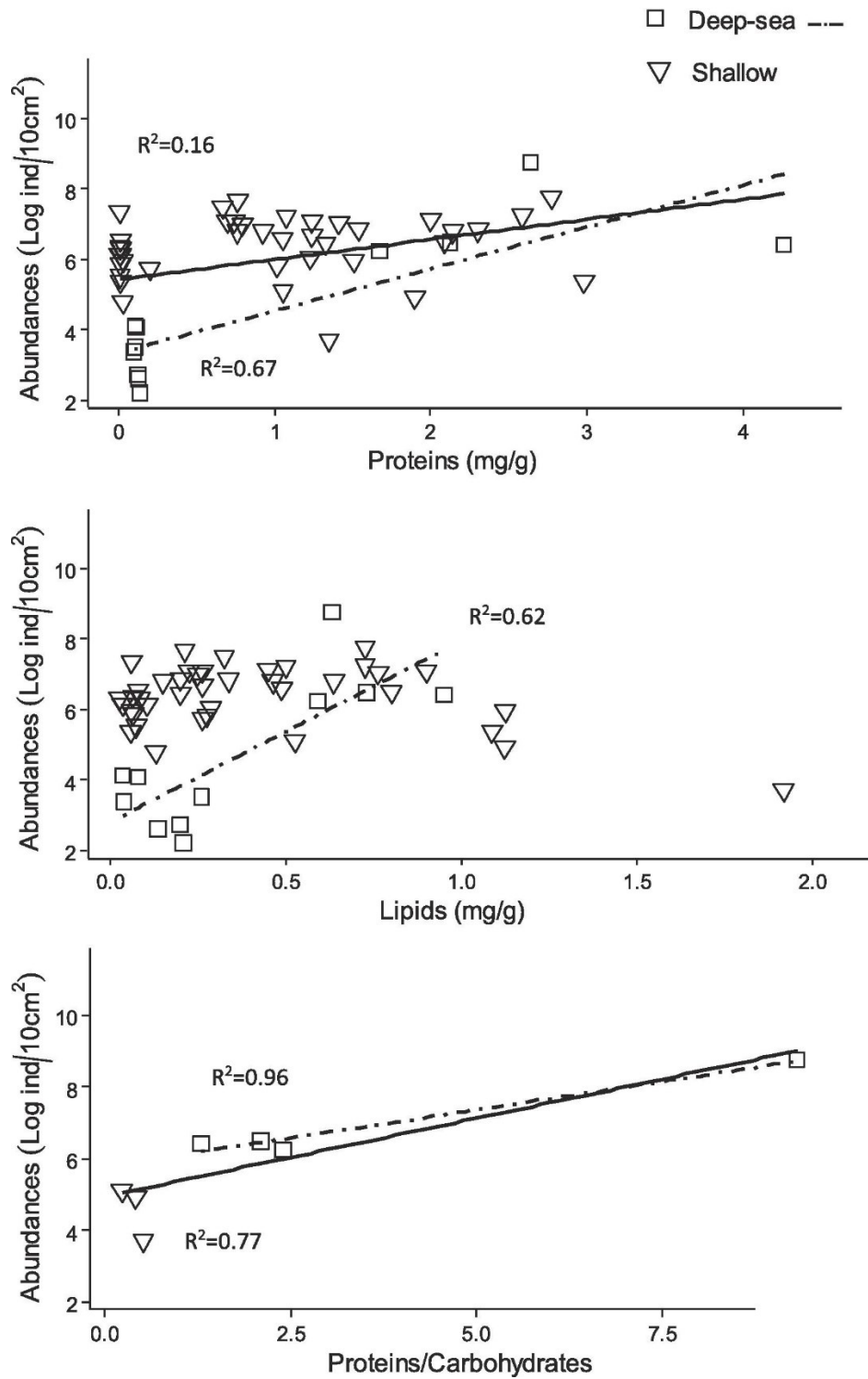
Figure 2.7. Response of total meiofauna from different ecosystems to increasing lipids, proteins and protein/carbohydrate ratio. In all cases, regression is significant (p <0.05). Solid regression line represents all ecosystems combined.

A similar result was reported for the northeast Pacific abyssal plain, where changes in epifaunal community structure correlated with El Niño/La Niña events, which can alter quantity and quality of food supply to the benthos (Ruhl and Smith Jr, 2004). The quantity and quality of food supply from surface water production, and changes in planktonic communities can influence the quality and export efficiency of sinking particles, and therefore ecosystem structure and function in the abyss (Smith et al., 2008). Therefore, changes in the quality of food delivered to deep-sea benthic ecosystems can dramatically shift species composition and numbers.

Some studies suggest significant changes in deep-sea benthic community structure with different food quality inputs. *In situ* experiments south of St. Croix, U.S. Virgin Islands (900 m depth) showed selectivity for food quality for macrofaunal colonizers, with higher abundance and lower diversity in sediments enriched with *Thalassiosira* sp. compared to *Sargassum* sp. (Snelgrove et al., 1992, Snelgrove et al., 1994). Poor quality and quantity of organic matter presumably promote competition. Competition for food may lead to two different evolutionary strategies, each of which involves niche separation along one of the two food web "axes" (horizontal and vertical) (Iken et al., 2001; see Fig. 2.6 and Appendix 2.3).

First, we examine food resource partitioning. Deposit feeders from food-limited environments with refractory material avoid competition by selecting for either "fresh" or refractory material (Iken et al., 2001, Wigham et al., 2003a). For example, the holothurians that often dominate megafaunal deposit feeders in abyssal plains may be classified as surface deposit feeders or as subsurface deposit feeders that feed on detritus of different refractiveness (Billett et al., 1988). The mechanism by which this partitioning occurs may relate to morphological differences in mouth parts, although this explanation requires further examination (Billett et al., 1988). Nematodes, a dominant abyssal meiofaunal component, can select between diatoms and bacteria

(Ingels et al., 2011). Mouth parts define nematode feeding types (selective deposit feeders, non-selective deposit feeders, epistratum feeders, predators+scavengers), but proportions of feeding type vary depending on available food sources (Ingels et al., 2011). Second, some species avoid competition by expanding their trophic niche vertically, defined as a broadening of the trophic level at which a given taxon feeds (Iken et al., 2001). Four to five trophic levels characterize abyssal plain benthic food webs in oligotrophic areas such as the tropics, as well as benthos in the food-poor Arctic Deep Canada Basin (Iken et al., 2005, Lin et al., 2014). In contrast, three trophic levels characterize abyssal plain benthic food webs beneath comparatively productive areas such as the PAP (Iken et al., 2001) and western Antarctic Peninsula (Mincks et al., 2008; see Appendix 2.3). Nonetheless, food web studies of the allochthonous and heterotrophic ecosystem of the PAP also suggest vertical expansion of trophic niches. Iken et al. (2001) identified numerous examples of trophic level separation between cnidarian species according to their lifestyles: a commensal species on a holothurian fed at the same lowest trophic level as its host (i.e., detritus), contrasted carnivory in a more mobile species (Iken et al., 2001). This trophic niche separation in food source and trophic level, supports the "Time Stability Hypothesis" described by Sanders (1969) which attributes high deep-sea diversity to a stable environment supportive of competitive interactions involving subdivision of available resources.

2.5.2.4. Chemosynthetic environments: High food quantity and quality

Hydrothermal vents and cold seeps, the most organic matter-rich deep-sea ecosystems, receive organic matter from surface waters just like other deep-sea ecosystems, but in most cases organic matter produced by chemosynthetic bacteria primarily drives seafloor production (Reid et al., 2013). Although other environmental factors such as toxicity structure benthic communities in chemosynthetic environments, this chemosynthesis supports distinct benthic species not found in

the ambient communities (Levin, 2005). Different chemosynthetic microorganisms support different megafaunal species. In New Zealand cold seeps, sulphide oxidation supports symbiont-bearing taxa such as solemyid and vesicomyid bivalves, in contrast to the methanotrophic symbionts in *Bathymodiolus* sp. (Thurber et al., 2010). Thurber et al. (2010) identified a broad range of isotopic signatures in different taxa from New Zealand cold seeps, which corresponded to multiple trophic pathways (methane oxidizing, sulphur oxidizing, and sulphate reducing bacteria, and phytodetritus).

The degree of dependency of these communities on the chemosynthetic food source depends on the strength of chemical fluxes (Fig. 2.6). A comparison of different venting areas in the Juan de Fuca Ridge (Limen et al., 2007), showed that lower trophic levels utilizing chemosynthetically-derived food dominate food webs in strong venting sites. California northern slope seeps function as ephemeral, small-scale disturbances that may not persist sufficiently long to support chemosynthetic-based, trophic specialization by most infauna and possibly epifauna. Even though chemosynthesis contributes substantially to overall seep production from the California northern slope (Levin and Michener, 2002), isotopic evidence identified chemosynthesis as a nutritional source for only one (a dorvilleid polychaete) of 14 macrofaunal taxa examined (Levin et al., 2000). Deeper cold-seep ecosystems depend more on chemosynthetic food sources than their shallower counterparts, probably reflecting the low quantity and quality of the sinking phytodetritus reaching deeper sites (Levin, 2005).

2.6. Implications

2.6.1. Ecosystem services and functions

An established infaunal community modifies its environment through bioturbation, which determines the fractionation, distribution, and availability of organic matter to the benthos

(Dauwe et al., 1998). Well-mixed sediment with organic matter and macrofauna penetrating into deeper layers (up to 20 cm) characterized a low food quality site in the North Sea, whereas subsurface deposit feeders, endobenthic (buried within the sediment) predators, and diffusive mixing (i.e., diffusion of particles, often enhanced by burrows and active pumping of animals) dominated sites with intermediate food quality (Dauwe et al., 1998). Minimal mixing characterized sites with high food quantity, quality, and surface deposition, where surface deposit-feeders, interface feeders, and suspension feeders dominated (Dauwe et al., 1998).

Bioturbation significantly influences nutrient fluxes, mineral dissolution rates, pollutant and trace metal cycling, and organic carbon preservation (Snelgrove et al., 2014). Therefore, food quality not only influences trophic structure of benthic food webs but may also strongly influence key ecosystem functions such as bioturbation and nutrient cycling.

2.6.2. Future environmental scenarios

Rising atmospheric greenhouse gas concentrations have increased global average temperatures with the world's oceans absorbing most of this added energy. As a result, the average temperature of upper ocean layers has increased by 0.6°C over the past 100 years (Hoegh-Guldberg and Bruno, 2010). Long-term studies of North Atlantic planktonic assemblages demonstrate changes in biogeographic distributions with changes in temperatures (Beaugrand, 2005); indeed, some studies propose plankton as a harbinger of climate change (Beaugrand, 2009). Changes in plankton composition can alter size and, therefore, transport efficiencies, and biochemical signatures of organic supply to the seafloor, with changes to important compounds that change with plankton classes, such as essential fatty acids, sterols, and carotenoids (Boyd and Newton, 1999, Smith et al., 2008).

Flux differences influence the quality and availability of food for benthic species (Smith et al., 2008). For example, the *A. rosea* (holothurians) bloom described earlier coincided with climatic forcing by the North Atlantic Oscillation that increased iron input to the upper ocean (Billett et al., 2010). Iron input to this iron-limited ocean region potentially increased cyanobacteria export, and *A. rosea* selectively ingested their pigments (Wigham et al., 2003b). Climate forcing in the Baltic Sea replaced phytoplankton-dominated assemblages with bacterial dominated plankton, and significantly reduced growth in a dominant benthic amphipod, *Monoporeia affinis* (Wiklund et al., 2009). These authors suggest that indirect impacts of climate change on benthic communities and their productivity will outweigh the direct effects of temperature.

Future environmental scenarios also predict ocean acidification resulting from increases in CO_2 concentrations (Doney et al., 2009). The oceans have absorbed approximately one third of the CO_2 injected into the atmosphere by human activities (Hoegh-Guldberg and Bruno, 2010). Anthropogenically-generated acidification will affect organismal physiology, food web dynamics, and biogeochemistry. For example, Rossoll et al. (2012) hypothesized that the projected increases in CO_2 partial pressures could indirectly affect zooplankton growth through alteration of phytoplankton biochemical composition. They analyzed direct and indirect effects of increasing CO_2 on a diatom-copepod system using fatty acids as measures of food quality because the diatom-copepod system commonly supports highly productive ecosystems. They confirmed that acidification decreased consumer growth and egg production by decreasing diet nutritional quality. They further suggested that increased saturated fatty acids in membranes reflected a response to lowered pH. Alteration of these food sources will likely produce significant change in a wide range of seafloor ecosystems, as predicted for deep-sea communities (Smith et al. 2008).

In summary total abundances responded to increases in food quantity (TOC), whereas different trophic groups responded differently to changes in food quantity and quality. In particular, lower trophic groups showed significant relationships with food quality, whereas carnivores/scavengers related only weakly to food quantity. Smaller size groupings (i.e., meiofauna) appear more sensitive to food quantity and quality than macro- and megafauna, and deep-sea ecosystems appear more sensitive to changes in quality of food supply than their shallow-water counterparts. Based on our literature review, high productivity systems are generally less efficient in processing low quality organic matter (i.e., seagrasses, kelp, corals) or are temporally variable (i.e., polar ecosystems); the detrital compartment plays a central role in carbon flow in these benthic food webs. Food webs limited by both quality and quantity (i.e., the deep sea) may promote greater competition, promoting food specialization and vertical expansion of the trophic niche. All these changes have implications for ecosystem functioning (e.g., bioturbation) and services (e.g., organic carbon preservation), and also suggest a threat from the different potential influences of climate change on food quantity and quality.

2.7. What's next?

1. Food quantity and quality should be considered as drivers of benthic trophic structure and energy flows.

2. The detrital compartment should be considered as a heterogeneous food source because of its varied origins and the ability of benthic deposit feeders to select within it.

3. Important food quality parameters such as biopolymeric carbon, essential fatty acids, essential amino acids, and semi-essential carotenoids, deserve further attention.

4. We encourage *in situ* experimentation to better understand the role of food quality in structuring benthic food webs.

5. The implications of climate change for ecosystems services point to the need for greater attention to food quality when studying benthic communities and food webs.

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2.9. References

Aspetsberger, F., Zabel, M., Ferdelman, T., Struck, U., Mackensen, A., Ahke, A., Witte, U. 2007. Instantaneous benthic response to different organic matter quality: *in situ* experiments in the Benguela Upwelling System. Marine Biology Research, 3, 342-356.

Atkinson, M., Smith, S. 1983. C: N: P ratios of benthic marine plants. Limnology and Oceanography, 28, 568-574.

Azovsky, A., Saburova, M., Chertoprood, E., Polikarpov, I. 2005. Selective feeding of littoral harpacticoids on diatom algae: hungry gourmands? Marine Biology, 148, 327-337.

Baldwin, R.J., Glatts, R.C., Smith, K.L. 1998. Particulate matter fluxes into the benthic boundary layer at a long time-series station in the abyssal NE Pacific: composition and fluxes. Deep-Sea Research Part II-Topical Studies in Oceanography, 45, 643-665. doi: 10.1016/s0967-0645(97)00097-0.

Beaugrand, G. 2005. Monitoring pelagic ecosystems using plankton indicators. ICES Journal of Marine Science: Journal du Conseil, 62, 333-338.

Beaugrand, G. 2009. Decadal changes in climate and ecosystems in the North Atlantic Ocean and adjacent seas. Deep Sea Research Part II: Topical Studies in Oceanography, 56, 656-673.

Beaulieu, S. 2002. Accumulation and fate of phytodetritus on the sea floor. Oceanography and Marine Biology: An Annual Review, 40, 171-232.

Berge, J., Daase, M., Renaud, P.E., Ambrose, W.G., Darnis, G., Last, K.S., Leu, E., Cohen, J.H., Johnsen, G., Moline, M.A. 2015. Unexpected levels of biological activity during the polar night offer new perspectives on a warming Arctic. Current Biology, 25, 2555-2561.

Billett, D., Llewellyn, C., Watson, J. 1988. Are deep-sea holothurians selective feeders? In: Burke et al. (Eds.), Echinoderm biology. Pub. Balkema Rotterdam, Victoria, B.C., pp. 421-429.

Billett, D.S.M., Bett, B.J., Reid, W.D.K., Boorman, B., Priede, I.G. 2010. Long-term change in the abyssal NE Atlantic: The 'Amperima Event' revisited. Deep-Sea Research Part II: Topical Studies in Oceanography, 57, 1406-1417. doi: 10.1016/j.dsr2.2009.02.001.

Billett, D.S.M., Bett, B.J., Rice, A.L., Thurston, M.H., Galeron, J., Sibuet, M., Wolff, G.A. 2001. Long-term change in the megabenthos of the Porcupine Abyssal Plain (NE Atlantic). Progress in Oceanography, 50, 325-348. doi: 10.1016/s0079-6611(01)00060-x.

Billett, D.S.M., Lampitt, R.S., Rice, A.L., Mantoura, R.F.C. 1983. Seasonal sedimentation of phytoplankton to the deep-sea benthos. Nature, 302, 520-522.

Bolser, R.C., Hay, M.E. 1996. Are tropical plants better defended? Palatability and defenses of temperate vs tropical seaweeds. Ecology, 77, 2269-2286.

Boon, A.R., Duineveld, G.C. 2012. Phytopigments and fatty acids in the gut of the deposit-feeding heart urchin Echinocardium cordatum in the southern North Sea: Selective feeding and its contribution to the benthic carbon budget. Journal of Sea Research, 67, 77-84.

Boyd, P.W., Newton, P.P. 1999. Does planktonic community structure determine downward particulate organic carbon flux in different oceanic provinces? Deep Sea Research Part I: Oceanographic Research Papers, 46, 63-91. doi: doi:10.1016/S0967-0637(98)00066-1.

Coale, K.H., Johnson, K.S., Fitzwater, S.E., Gordon, M.R., Tanner, S., Chavez, F.P., Ferioli, L., Sakamoto, C., Rogers, P., Millero, F., Millero, F., Steinberg, P., Nightingale, P., Cooper, D., Cochlan, W.P., Landry, M.R., Constantinou, J., Rollwagen, G., Trasvina, A., Kudela, R. 1996. A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. Nature, 383, 495-501.

Cognie, B., Barillé, L., Rincé, Y. 2001. Selective feeding of the oyster Crassostrea gigas fed on a natural microphytobenthos assemblage. Estuaries, 24, 126-134.

Danovaro, R., Della Croce, N., Dell'Anno, A., Pusceddu, A. 2003. A depocenter of organic matter at 7800m depth in the SE Pacific Ocean. Deep Sea Research Part I: Oceanographic Research Papers, 50, 1411-1420.

Danovaro, R., Dell'Anno, A., Pusceddu, A. 2004. Biodiversity response to climate change in a warm deep sea. Ecology Letters, 7, 821-828.

Danovaro, R., Snelgrove, P.V.R., Tyler, P. 2014. Challenging the paradigms of deep-sea ecology. Trends in Ecology & Evolution, 29, 465-475.

Danovaro, R., Della Croce, N., Dell'Anno, A., Fabiano, M., Marrale, D., Martorano, D. 2000. Seasonal changes and biochemical composition of the labile organic matter flux in the Cretan Sea. Progress in Oceanography, 46, 259-278.

Dauwe, B., Herman, P.M., Heip, C. 1998. Community structure and bioturbation potential of macrofauna at four North Sea stations with contrasting food supply. Marine Ecology Progress Series, 173, 67-83.

de Goeij, J.M., van Oevelen, D., Vermeij, M.J., Osinga, R., Middelburg, J.J., de Goeij, A.F., Admiraal, W. 2013. Surviving in a marine desert: the sponge loop retains resources within coral reefs. Science (New York, N.Y.), 342, 108-110. doi: 10.1126/science.1241981.

De Leo, F.C., Smith, C.R., Rowden, A.A., Bowden, D.A., Clark, M.R. 2010. Submarine canyons: hotspots of benthic biomass and productivity in the deep sea. Proceedings of the Royal Society B: Biological Sciences, 277, 2783-2792.

Defossez, J., Hawkins, A. 1997. Selective feeding in shellfish: size-dependent rejection of large particles within pseudofaeces from *Mytilus edulis*, *Ruditapes philippinarum* and *Tapes decussatus*. Marine Biology, 129, 139-147.

Devol, A.H., Codispoti, L.A., Christensen, J.P. 1997. Summer and winter denitrification rates in western Arctic shelf sediments. Continental Shelf Research, 17, 1029-1050.

Dinet, A. 1973. Distribution quantitative du méiobenthos profond dans la région de la dorsale de Walvis (Sud-Ouest Africain). Marine Biology, 20, 20-26.

Doney, S.C., Fabry, V.J., Feely, R.A., Kleypas, J.A. 2009. Ocean acidification: the other CO_2 problem. Marine Science, 1, 169-192.

Duggins, D., Eckman, J. 1997. Is kelp detritus a good food for suspension feeders? Effects of kelp species, age and secondary metabolites. Marine Biology, 128, 489-495.

Estifanos, T.K., Traunspurger, W., Peters, L. 2013. Selective feeding in nematodes: A stable isotope analysis of bacteria and algae as food sources for free-living nematodes. Nematology, 15, 1-13.

Fredriksen, S. 2003. Food web studies in a Norwegian kelp forest based on stable isotope (δ^{13}C and δ^{15}N) analysis. Marine Ecology Progress Series, 260, 71-81.

Gage, J.D., Tyler, P. 1991. Deep-sea biology: a natural history of organisms at the deep-sea floor. Cambridge University Press. New York, USA.

Giusti, A.M., Bignetti, E., Cannella, C. 2008. Exploring new frontiers in total food quality definition and assessment: From chemical to neurochemical properties. Food and Bioprocess Technology, 1, 130-142.

Glencross, B.D. 2009. Exploring the nutritional demand for essential fatty acids by aquaculture species. Reviews in Aquaculture, 1, 71-124.

Glover, A.G., Smith, C.R., Mincks, S.L., Sumida, P.Y., Thurber, A.R. 2008. Macrofaunal abundance and composition on the West Antarctic Peninsula continental shelf: Evidence for a sediment 'food bank'and similarities to deep-sea habitats. Deep Sea Research Part II: Topical Studies in Oceanography, 55, 2491-2501.

Grassle, J.F., Morseporteous, L.S. 1987. Macrofaunal colonization of disturbed deep-sea environments and the structure of deep-sea benthic communities. Deep-Sea Research Part A-Oceanographic Research Papers, 34, 1911-&. doi: 10.1016/0198-0149(87)90091-4.

Grassle, J.F., Sanders, H.L. 1973. Life histories and role of disturbance. Deep-Sea Research, 20, 643-659.

Groombridge, B., Jenkins, M. 1996. The diversity of the seas: a regional approach. World Conservation Press. Cambridge, UK.

Harrison, K.E. 1990. The role of nutrition in maturation, reproduction and embryonic development of decapod crustaceans: a review. Journal of Shellfish Research, 9, 1-28.

Hedges, J.I., Clark, W.A., Cowie, G.L. 1988. Fluxes and reactivities of organic matter in a coastal marine bay. Limnology and Oceanography, 33, 1137-1152.

Henrichs, S.M. 2005. Organic matter in coastal marine sediments. In: A.R. Robinson, K.H. Brink (Eds.), The Global Coastal Ocean: Multiscale Interdisciplinary Processes, The Sea, Vol. 13 Harvard University Press, Boston., pp. 129-162.

Hoegh-Guldberg, O., Bruno, J.F. 2010. The impact of climate change on the world's marine ecosystems. Science (New York, N.Y.), 328, 1523-1528. doi: 10.1126/science.1189930 [doi].

Howarth, R.W. 1988. Nutrient limitation of net primary production in marine ecosystems. Annual Review of Ecology and Systematics, 89-110.

Hudson, I.R., Wigham, B.D., Billett, D.S.M., Tyler, P.A. 2003. Seasonality and selectivity in the feeding ecology and reproductive biology of deep-sea bathyal holothurians. Progress in Oceanography, 59, 381-407. doi: 10.1016/j.pocean.2003.11.002.

Hunter, W.R., Jamieson, A., Huvenne, V., Witte, U. 2013. Sediment community responses to marine *vs* terrigenous organic matter in a submarine canyon. Biogeosciences, 10, 67-80.

Iken, K., Brey, T., Wand, U., Voigt, J., Junghans, P. 2001. Food web structure of the benthic community at the Porcupine Abyssal Plain (NE Atlantic): a stable isotope analysis. Progress in Oceanography, 50, 383-405.

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

Iken, K., Bluhm, B., Dunton, K. 2010. Benthic food-web structure under differing water mass properties in the southern Chukchi Sea. Deep Sea Research Part II: Topical Studies in Oceanography, 57, 71-85.

Ingels, J., Billett, D.S.M., Van Gaever, S., Vanreusel, A. 2011. An insight into the feeding ecology of deep-sea canyon nematodes - Results from field observations and the first in-situ C-13 feeding experiment in the Nazare Canyon. Journal of Experimental Marine Biology and Ecology, 396, 185-193. doi: 10.1016/j.jembe.2010.10.018.

Jumars, P.A., Eckman, J.E. 1983. 10. Spatial structure within deep-sea benthic communities. In: G. Rowe (Eds.), Deep-sea biology. Harvard University Press, USA, pp. 399-451.

Kanazawa, A. 2001. Sterols in marine invertebrates. Fisheries Science, 67, 997-1007.

Kiriakoulakis, K., Stutt, E., Rowland, S.J., Annick Vangriesheim, Lampitt, R.S., Wolff, G.A. 2001. Controls on the organic chemical composition of settling particles in the Northeast Atlantic Ocean. Progress in Oceanography, 50, 65-87. doi: doi: 10.1016/S0079-6611(01)00048-9.

Kleppel, G., Burkart, C. 1995. Egg production and the nutritional environment of *Acartia tonsa*: the role of food quality in copepod nutrition. ICES Journal of Marine Science: Journal du Conseil, 52, 297-304.

Klumpp, D., Howard, R., Pollard, P. 1989. Trophodynamics and nutritional ecology of seagrass communities. In: A. Larkum, A. McComb, S. Shepherd (Eds.), Biology of seagrasses. Elesevier, Amsterdam., pp. 394-457.

Laguionie-Marchais, C., Paterson, G.L., Bett, B.J., Smith, K.L., Ruhl, H.A. 2015. Inter-annual species-level variations in an abyssal polychaete assemblage (Sta. M, NE Pacific, 4000 m). Progress in Oceanography, 140, 43-53.

Levin, L.A. 2005. Ecology of cold seep sediments: interactions of fauna with flow, chemistry and microbes. Oceanography and Marine Biology: An Annual Review, 43, 1-46.

Levin, L.A., Michener, R.H. 2002. Isotopic evidence for chemosynthesis-based nutrition of macrobenthos: the lightness of being at Pacific methane seeps. Limnology and Oceanography, 47, 1336-1345.

Levin, L.A., James, D.W., Martin, C.M., Rathburn, A.E., Harris, L.H., Michener, R.H. 2000. Do methane seeps support distinct macrofaunal assemblages? Observations on community structure and nutrition from the northern California slope and shelf. Marine Ecology Progress Series, 208, 21-39.

Levinton, J. 1972. Stability and trophic structure in deposit-feeding and suspension-feeding communities. American Naturalist, 106, 472-486.

Li, P., Mai, K., Trushenski, J., Wu, G. 2009. New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. Amino acids, 37, 43-53.

Limen, H., Levesque, C., Juniper, S.K. 2007. POM in macro-/meiofaunal food webs associated with three flow regimes at deep-sea hydrothermal vents on Axial Volcano, Juan de Fuca Ridge. Marine Biology, 153, 129-139. doi: 10.1007/s00227-007-0790-1.

Lin, H., Lin, P., Chang, N., Shiao, J., Kao, S. 2014. Trophic structure of megabenthic food webs along depth gradients in the South China Sea and off northeastern Taiwan. Marine Ecology Progress Series, 501, 53-66.

Lopez, G.R., Levinton, J.S. 1987. Ecology of deposit-feeding animals in marine sediments. Quarterly Review of Biology, 62, 235-260.

Loret, P., Pastoureaud, A., Bacher, C., Delesalle, B. 2000. Phytoplankton composition and selective feeding of the pearl oyster *Pinctada margaritifera* in the Takapoto lagoon (Tuamotu Archipelago, French Polynesia): *in situ* study using optical microscopy and HPLC pigment analysis. Marine Ecology Progress Series, 199, 55-67.

Lovvorn, J.R., Cooper, L.W., Brooks, M.L., De Ruyck, C.C., Bump, J.K., Grebmeier, J.M. 2005. Organic matter pathways to zooplankton and benthos under pack ice in late winter and open water in late summer in the north-central Bering Sea. Marine Ecology Progress Series, 291, 135-150.

Martin-Creuzburg, D., Elert, E. 2009. Ecological significance of sterols in aquatic food webs. In: M. Kainz, M.T. Brett, M.T. Arts (Eds.), Lipids in Aquatic Ecosystems. Springer, New York., pp. 43-64; 3.

Mayor, D.J., Thornton, B., Hay, S., Zuur, A.F., Nicol, G.W., McWilliam, J.M., Witte, U.F. 2012. Resource quality affects carbon cycling in deep-sea sediments. The ISME Journal, 6, 1740-8. doi: 10.1038/ismej.2012.14.

Mincks, S.L., Smith, C.R., DeMaster, D.J. 2005. Persistence of labile organic matter and microbial biomass in Antarctic shelf sediments: Evidence of a sediment food bank. Marine Ecology Progress Series, 300, 3-19.

Mincks, S.L., Smith, C.R., Jeffreys, R.M., Sumida, P.Y. 2008. Trophic structure on the West Antarctic Peninsula shelf: detritivory and benthic inertia revealed by $\delta^{13}C$ and $\delta^{15}N$ analysis. Deep Sea Research Part II: Topical Studies in Oceanography, 55, 2502-2514.

Moncreiff, C.A., Sullivan, M.J. 2001. Trophic importance of epiphytic algae in subtropical seagrass beds: evidence from multiple stable isotope analyses. Marine Ecology Progress Series, 215, 93-106.

Müller-Navarra, D.C. 2008. Food web paradigms: the biochemical view on trophic interactions. International Review of Hydrobiology, 93, 489-505. doi: 10.1002/iroh.200711046.

Norderhaug, K., Fredriksen, S., Nygaard, K. 2003. Trophic importance of *Laminaria hyperborea* to kelp forest consumers and the importance of bacterial degradation to food quality. Marine Ecology Progress Series, 255, 135-144.

Oni, O.E., Schmidt, F., Miyatake, T., Kasten, S., Witt, M., Hinrichs, K., Friedrich, M.W. 2015. Microbial communities and organic matter composition in surface and subsurface sediments of the Helgoland Mud Area, North Sea. Frontiers in microbiology, 6, 1290. doi: 10.3389/fmicb.2015.01290.

Pearson, T., Rosenberg, R. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology Annual Review, 16, 229-311.

Perhar, G., Arhonditsis, G.B. 2009. The effects of seston food quality on planktonic food web patterns. Ecological Modelling, 220, 805-820.

Pfannkuche, O., Boetius, A., Lochte, K., Lundgreen, U., Thiel, H. 1999. Responses of deep-sea benthos to sedimentation patterns in the North-East Atlantic in 1992. Deep-Sea Research Part I-Oceanographic Research Papers, 46, 573-596. doi: 10.1016/s0967-0637(98)00081-8.

Pfannkuche, O. 1993. Benthic response to the sedimentation of particulate organic matter at the BIOTRANS station, 47 N, 20 W. Deep Sea Research Part II: Topical Studies in Oceanography, 40, 135-149.

Rainbow, P.S. 2002. Trace metal concentrations in aquatic invertebrates: why and so what? Environmental Pollution, 120, 497-507.

Reid, W.D., Sweeting, C.J., Wigham, B.D., Zwirglmaier, K., Hawkes, J.A., McGill, R.A., Linse, K., Polunin, N.V. 2013. Spatial differences in East Scotia ridge hydrothermal vent food webs: influences of chemistry, microbiology and predation on trophodynamics. PLoS One, 8, e65553.

Rex, M.A., Etter, R.J. 2010. Deep-sea biodiversity: pattern and scale. Harvard University Press.

Rodriguez, P., Martinez-Madrid, M., Arrate, J.A., Navarro, E. 2001. Selective feeding by the aquatic oligochaete *Tubifex tubifex* (Tubificidae, Clitellata). Hydrobiologia, 463, 133-140.

Rosenberg, R. 1995. Benthic marine fauna structured by hydrodynamic processes and food availability. Netherlands Journal of Sea Research, 34, 303-317.

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

Ruhl, H.A., Smith Jr, K.L. 2004. Shifts in deep-sea community structure linked to climate and food supply. Science, 305, 513-5. doi: 10.1126/science.1099759.

Sanders, H.L. 1969. Benthic marine diversity and the stability-time hypothesis. Brookhaven symposia in biology, 22, 71-81.

Schell, D.M. 1983. Carbon-13 and carbon-14 abundances in alaskan aquatic organisms: delayed production from peat in arctic food webs. Science (New York, N.Y.), 219, 1068-1071. doi: 219/4588/1068 [pii].

Schonberg, S.V., Clarke, J.T., Dunton, K.H. 2014. Distribution, abundance, biomass and diversity of benthic infauna in the Northeast Chukchi Sea, Alaska: Relation to environmental variables and marine mammals. Deep Sea Research Part II: Topical Studies in Oceanography, 102, 144-163.

Shillito, B., Ravaux, J., Gaill, F., Delachambre, J., Thiébaut, E., Childress, J. 1999. Preliminary data on carbon production of deep-sea vent tubeworms. Marine Ecology Progress Series, 183, 275-279.

Shurin, J.B., Gruner, D.S., Hillebrand, H. 2006. All wet or dried up? Real differences between aquatic and terrestrial food webs. Proceedings of the Royal Society B: Biological Sciences, 273, 1-9. doi: 10.1098/rspb.2005.3377.

Smith, K., Kaufmann, R., Baldwin, R. 1994. Coupling of near-bottom pelagic and benthic processes at abyssal depths in the eastern North Pacific Ocean. Limnology and Oceanography, 39, 1101-1118.

Smith, C.R., De Leo, F.C., Bernardino, A.F., Sweetman, A.K., Arbizu, P.M. 2008. Abyssal food limitation, ecosystem structure and climate change. Trends in Ecology & Evolution, 23, 518-528.

Snelgrove, P.V.R., Thrush, S.F., Wall, D.H., Norkko, A. 2014. Real world biodiversity–ecosystem functioning: a seafloor perspective. Trends in ecology & evolution, 29, 398-405.

Snelgrove, P.V.R., Grassle, J.F., Petrecca, R.F. 1992. The role of food patches in maintaining high deep-sea diversity - field experiments with hydrodynamically unbiased colonization trays. Limnology and Oceanography, 37, 1543-1550.

Snelgrove, P.V.R., Grassle, J.F., Petrecca, R.F. 1994. Macrofaunal response to artificial enrichments and depressions in a deep-sea habitat. Journal of Marine Research, 52, 345-369. doi: 10.1357/0022240943077082.

Snelgrove, P.V.R., Grassle, J.F., Petrecca, R.F. 1996. Experimental evidence for aging food patches as a factor contributing to high deep-sea macrofaunal diversity. Limnology and Oceanography, 41, 605-614.

Snelgrove, P.V. 1999. Getting to the bottom of marine biodiversity: Sedimentary habitats: Ocean bottoms are the most widespread habitat on earth and support high biodiversity and key ecosystem services. BioScience, 49, 129-138.

Sorokin, Y.I. 1990. Aspects of trophic relations, productivity and energy balance in coral-reef ecosystems. Ecosystems of the world, 25, 401-418.

Taghon, G.L., Jumars, P.A. 1984. Variable ingestion rate and its role in optimal foraging behavior of marine deposit feeders. Ecology, 65, 549-558.

Thiel, H. 1979. Structural aspects of the deep-sea benthos. Ambio Special Report, 6, 25-31.

Thurber, A.R., Kröger, K., Neira, C., Wiklund, H., Levin, L.A. 2010. Stable isotope signatures and methane use by New Zealand cold seep benthos. Marine Geology, 272, 260-269.

van Oevelen, D., Soetaert, K., Heip, C. 2012. Carbon flows in the benthic food web of the Porcupine Abyssal Plain: The (un) importance of labile detritus in supporting microbial and faunal carbon demands. Limnology and Oceanography, 57, 645-664.

Vonk, J.A., Christianen, M.J., Stapel, J. 2008. Redefining the trophic importance of seagrasses for fauna in tropical Indo-Pacific meadows. Estuarine, Coastal and Shelf Science, 79, 653-660.

Wade, N.M., Gabaudan, J., Glencross, B.D. 2015. A review of carotenoid utilisation and function in crustacean aquaculture. Reviews in Aquaculture, 0, 1-16.

Wei, C., Rowe, G.T., Hubbard, G.F., Scheltema, A.H., Wilson, G.D., Petrescu, I., Foster, J.M., Wicksten, M.K., Chen, M., Davenport, R. 2010. Bathymetric zonation of deep-sea macrofauna in relation to export of surface phytoplankton production. Marine Ecology Progress Series, 399, 1-14.

Weishaar, J.L., Aiken, G.R., Bergamaschi, B.A., Fram, M.S., Fujii, R., Mopper, K. 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environmental science & technology, 37, 4702-4708.

Wieking, G., Kröncke, I. 2003. Macrofauna communities of the Dogger Bank (central North Sea) in the late 1990's: spatial distribution, species composition and trophic structure. Helgoland Marine Research, 57, 34-46.

Wieking, G., Kröncke, I. 2005. Is benthic trophic structure affected by food quality? The Dogger Bank example. Marine Biology, 146, 387-400.

Wigham, B.D., Hudson, I.R., Billett, D.S.M., Wolff, G.A. 2003a. Is long-term change in the abyssal Northeast Atlantic driven by qualitative changes in export flux? Evidence from selective feeding in deep-sea holothurians. Progress in Oceanography, 59, 409-441. doi: 10.1016/j.pocean.2003.11.003.

Wigham, B.D., Tyler, P.A., Billett, D.S.M. 2003b. Reproductive biology of the abyssal holothurian *Amperima rosea*: an opportunistic response to variable flux of surface derived organic matter? Journal of the Marine Biological Association of the United Kingdom, 83, 175-188.

Wiklund, A.E., Dahlgren, K., Sundelin, B., Andersson, A. 2009. Effects of warming and shifts of pelagic food web structure on benthic productivity in a coastal marine system. Marine Ecology Progress Series, 396, 13-25.

2.10. Appendix

Appendix 2.1. Characterization of most relevant organic matter quality descriptors for benthic invertebrates

Organic matter	Description	Chemical structure	Relevance	Measurement	Reference
C:N:P	Basic elements of life	Part of basic organic molecules of life	Ratio reflects origin, degradation, and utilization and informs on limiting elements	C:N ratio	Meyers, 1994, Danovaro, 2010
Labile organic matter	Organic matter fraction easily degraded	Lipids, proteins and carbohydrates	Immediately digestible by benthic consumers	Biopolymeric fraction/carbon Chloropyhll *a*: phaeophorbide ratio	Fabiano et al., 1995, Danovaro, 2010
Refractory material	Organic matter fraction with low degradation rates	Humic and fulvic acids, structural carbohydrates, and "black" carbon	Most of the sedimentary organic matter	Chloropyhll *a*: phaeophorbide ratio Lignin Cellulose	Middelburg et al., 1999, Danovaro, 2010
Chloroplast pigments	Organic carbon of photosynthetic origin	Chlorophyll *a* Phaeophorbides	Key food source for the benthos: from small protozoa to meio- and macrofauna	Chlorophyll *a*/ TOC	Witte et al., 2003, Danovaro, 2010
Essential Fatty acids (EFA)	Fatty acids required for optimal health of organisms and which must be acquired through their diets	Some n-3 and n-6 polyunsaturated fatty acids (PUFAs)	Significant impact on physiology of consumers (e.g. growth) and their structural biochemistry (e.g. cell membrane)	E.g. DHA (22:6n-3), EPA (20:5n-3) and ARA (20:4n-6)	Parrish, 2009

Appendix 2.1. Continued

Organic matter	Description	Chemical structure	Relevance	Measurement	Reference
Semi-essential Carotenoids	Non-chloroplast pigments required for optimal health of organisms which must be acquired through their diets	Isoprenoid polyene pigments formally derived from lycopene	Significant impact on the reproduction of many different taxa, in particular echinoderms	E.g. Zeaxanthin, Astaxanthin, Canthaxanthin, β,β-carotenes, β-echineone, Lutein	Tsushlma et al., 1997, Plank et al., 2002, Hudson et al., 2003, Liaaen-Jensen, 2012, Wade et al., 2015
Essential amino acids (EAA)	Amino acids required for optimal health of organisms which must be acquired through their diets	Threonine (THR), valine (VAL), methionine (MET), isoleucine (ILEU), leucine (LEU), phenylalanine (PHE), histidine (HIS), tryptophan (TRP), lysine (LYS), and arginine (ARG)	Components of proteins, therefore, necessary in the production of enzymes and structural materials within animals. Free amino acids may function in osmoregulatory roles		Phillips, 1984
Sterols	Some are required for optimal health of some invertebrates (e.g. crustaceans, bivalves) and need to be acquired through their diets		Cholesterol is a precursor of a and fJ ecdysones (molting hormones)		Phillips, 1984, Martin-Creuzburg et al., 2009
Essential minerals	Elements required for optimal health of organisms which must be acquired through their diets	Zn, Fe, Mn, Cu	Important role in many metabolic processes		Phillips, 1984

Appendix 2.2. Studies used for the statistical analysis.

Reference	Taxon size	Latitude	Ecosystem	Number of food variables	Depth (m)
Vanhove 1995	Meiofauna	Polar	Antarctica/Deep-sea	6	211-2080
Pape 2013	Meiofauna	Temperate	Deep-sea	5	1063-3069
Wolff 2011	Megafauna	Polar	Antarctica/Deep-sea	7	4112-4174
Danovaro 2002	Meiofauna	Subtropical	Deep-sea	9	204-7800
Carroll 2012	Macrofauna	Polar	Arctic/deep-sea	4	221-319
Wlodarska-Kowalczuk 2016	Meiofauna	Polar	Arctic/deep-sea	4	80-305
Pasotti 2014	Meiofauna	Polar	Antarctica	4	15
Vanhove 1998	Meiofauna	Polar	Antarctica	3	10
Vanhove 2000	Meiofauna	Polar	Antarctica	7	10
Cummings 2010	Macrofauna	Polar	Antarctica/Deep-sea	4	100-500
Sumida 2014 & Mincks 2005	Megafauna	Polar	Antarctica/Deep-sea	4	585–600
leduc2011	Meiofauna	Temperate	Seagrass	4	intertidal
Baron1993	Macrofauna	Subtropical	Seagrass	2	intertidal
Zhou2009	Macrofauna	Subtropical	Seagrass	3	Inter-tidal
Olabarria2010	Macrofauna	Temperate	Seagrass	2	Intertidal
Harriague2014	Macrofauna	Temperate	Deep-sea	3	115-2646
Rosenberg 2003	Macrofauna	Temperate	Shallow	5	8-77
Danovaro 2002	Meiofauna	Temperate	Shallow	8	4
Danovaro 2004	Meiofauna	Temperate	Shallow	5	11
Pires-Vanin 2013	Macrofauna	Subtropical	Shallow	3	46-93
El-Serehy 2016	Meiofauna	Subtropical	Shallow	6	

Appendix 2.3. Food web structure and energy budget of different marine benthic ecosystems with contrasting food qualities and sources. POM = Particulate Organic Matter; DOM = Dissolved Organic Matter; DOC = Dissolved Organic Carbon; BMA = Benthic Macroalgae; BMI = Benthic Microalagae

Ecosystem type	Site	Food source	Food quality	Trophic structure	Energy budget
Coral reef	Curaçao, Kingdom of Netherlands Synthesis of coral reef systems	Detritus POM BMA Phytoplankton BMI Terrestrial organic matter Symbiotic zooxanthellae DOM Plankton and detritus of oceanic waters	BMA low C:N and presence of herbivore deterrent substances "junk-food" diet provided by symbiotic zooxantellae to corals	4 trophic levels High detritivore/herbivore rates Mixotrophy present in symbiont bearing corals (Diet: 65 % from symbiont zooxanthellae production – 15 % predatory feeding – 20 % sedimentary feeding on bacteria and DOM)	Microbial loop Sponge loop Detritus is main energy pathway Highly efficient mechanisms that recycle nutrients within the system ("oases in marine deserts")

* De Goeij et al. 2013; Sorokin 1990; Arias-gonzalez et al. 1997; Davies 1984

Appendix 2.3. Continued

Ecosystem type	Site	Food source	Food quality	Trophic structure	Energy budget
Kelp forests	Norway	Detritus	High C:N	Low number of grazers on kelp	Kelp detritus hypothesis
	NE Atlantic	POM	Herbivore deterrent substances		Kelp-derived organic matter
		BMA			
		Phytoplankton	Palatability of kelp vary with species		Epiphytes
		BMI			Phytoplankton?
		Kelp			Export to other ecosystems
		Kelp epiphytes			
		Terrestrial organic matter			

* Fredriksen 2003; Orr et al. 2013; Leclerc et al. 2013; Miller and Page 2012; Schaal et al. 2010

Appendix 2.3. Continued

Ecosystem type	Site	Food source	Food quality	Trophic structure	Energy budget
Seagrass meadows	Baltic Sea	Detritus	Epiphytes have a lower C:N ratio than seston and seagrass	Low number of grazers on seagrass	Seagrass' epiphytes
	Arcachon Bay, NE Atlantic	POM			Benthic microalgae
		BMA			Export to other ecosystems
	Mississippi sound (Gulf of Mexico)	Phytoplankton			
		BMI			
		Terrestrial organic matter			
		Seagrass			
		Seagrass epiphytes			

* Dubois et al. 2014; Jaschinski et al. 2008; Moncreiff et al. 2001

Appendix 2.3. Continued

Ecosystem type	Site	Food source	Food quality	Trophic structure	Energy budget
Tropical seagrass meadows	Sulawesi (Indonesia) Gazi Bay, Kenya	Detritus POM BMA Phytoplankton BMI Seagrass Seagrass epiphytes	C:N ratios seagrass and epiphytes similar Seagrass with low fibre content Seagrass high leaf production and fast turnover rate all year round Seagrass higher concentration of carbohydrates	Grazers compartment greater importance than in temperate seagrass meadows	Seagrass' epiphytes Seagrasses Benthic microalgae Export to other ecosystems

* Vonk et al. 2008; Marguillier et al. 1997

Appendix 2.3. Continued

Ecosystem type	Site	Food source	Food quality	Trophic structure	Energy budget
Eutrophic abyssal plain	Porcupine abyssal plain (PAP) West Antarctic Abyssal plain	Detritus POM Zooplankton DOM	Degraded organic matter	3 Trophic levels Dominance of lower trophic levels	Seasonal pulses of POM Detritus is main energy pathway
Oligotrophic abyssal plain	South China Sea abyssal plain (SCS) Arctic Abyssal plain	Detritus POM Zooplankton DOM	Degraded organic matter	4-5 Trophic levels Dominance of higher trophic levels	Detritus is main energy pathway

* Eutrophic abyssal plain: Iken et al. 2001; Billett et al. 1983; Mincks et al. 2005
* Oligotrophic abyssal plain: Lin et al. 2014; Iken et al. 2005

Appendix 2.3. Continued

Ecosystem type	Site	Food source	Food quality	Trophic structure	Energy budget
Chemosynthetic environments	Pacific hydrothermal vents and cold seeps MAR hydrothermal vents Antarctic hydrothermal vents	Free living bacteria Endosymbiotic bacteria Detritus DOM Terrestrial organic matter	Different carbon fixation pathways follow different bacteria (sulphur-oxidizers, sulphate reducers, methane oxidizers) Carbon pathways from vent (CBB-Calvin-Benson Cycle; rTCA-reductive tricarboxylic acid; both from sulphide oxidation)	Wide trophic base Reliance on lower trophic levels compared to adjacent ecosystems with low dependence on vent flows has been reported Macrofauna and meiofauna mostly heterotrophic Endosymbionts in certain megafaunal species Mixotrophy present in some symbiont-bearing species	Stronger dependence on chemosynthetically-derived organic matter with depth Importance of chemosynthetic trophic pathways vary regionally and among habitats

* Thurber et al. 2010; Rau 1981; Reid et al. 2013; Levin and Michener 2002; Levin 2005; De Busserolles et al. 2009; Riou et al. 2010; Limen 2007

Appendix 2.3. Continued

Ecosystem type	Site	Food source	Food quality	Trophic structure	Energy budget
Antarctica	West Antarctic Peninsula Eastern Antarctic (Vestfold Hills, Windmill Islands)	Sea Ice microalgae Detritus POM BMA Phytoplankton BMI DOM		Similar in all the areas Wide range of carbon sources	Seasonal pulses of POM "Food Bank" Low contribution of sea ice microalgae in benthic food webs
Nutrient-rich Arctic	Chuckchi Sea (Anadyr Water influence)	Sea Ice microalgae Detritus POM BMA Phytoplankton BMI DOM	Nutrient-rich water masses	Dominance of lower trophic levels	Detritus is main energy pathway Trophic buffering

* Antarctica: Gillies et al. 2013; Glover et al. 2008

*Nutrient-rich Arctic: Iken et al. 2010; Lovvorn et al. 2005; Levinton 1972; Berger et al. 2015

Appendix 2.3. Continued

Ecosystem type	Site	Food source	Food quality	Trophic structure	Energy budget
Nutrient-poor Arctic	Chuckchi Sea (Alaska coastal Water influence)	Sea Ice microalgae	Nutrient-poor water masses	Dominance of higher trophic levels	Detritus main energy pathway
		Detritus			
		POM	Refractory organic matter from terrestrial origin		Trophic buffering
		BMA			
		Phytoplankton			
		BMI			
		Terrestrial organic matter			
		DOM			

* Iken et al. 2010; Lovvorn et al. 2005

Appendix 2.3. Continued

Ecosystem type	Site	Food source	Food quality	Trophic structure	Energy budget
Estuaries	Yura estuary (Japan) Tagus estuary (Portugal) Kromme, Swartkops and Sundays estuaries (South Africa)	Phytoplankton Zooplankton Bacterioplankton Detritus Sediment bacteria Riverine POM Estuarine POM Marine POM Terrestrial vegetation BMA BMI DOC	Refractory organic matter from terrestrial origin	Spatial variation of trophic structure More even distribution of trophic structure at stations with high salinity Decrease of herbivores/detritivores towards coastal waters	Spatial variation of main food source High trophic efficiency at lower trophic levels with low riverine input

* Antonio et al. 2010; Gaudencio et al. 2007

Appendix 2.3. Continued

Ecosystem type	Site	Food source	Food quality	Trophic structure	Energy budget
Submarine Canyons	NW Mediterranean Sea	DOM	Heterotrophic	Gradation in different parts of the canyon (upper-middle-lower)	Feeding activity of macrofauna on terrigenous food source governed by strong nitrogen demand
	Nazare Canyon (off Portugal)	Terrestrial organic matter	Allochthonous		
		POM	ecosystems	Differ from adjacent slope at similar depths	
		Detritus	Degraded OM		
		Plant debris	Rapid transport of sediment can enhance om quality and quantity		Different fates for terrigenous and marine OM in submarine canyons
		Higher OM quantities than adjacent slopes at same depth			
			Accumulation of terrestrial organic matter		

* Van Oevelen et al. 2011; Ingels et al. 2009; Mamouridis et al. 2011; Garcia et al. 2007; Hunter et al. 2013; Sanchez-Vidal et al. 2013; Pasqual et al. 2013; Pedrosa-Pamies et al. 2013

2.11. Appendix references

Antonio, E.S., Kasai, A., Ueno, M., Won, N., Ishihi, Y., Yokoyama, H., Yamashita, Y. 2010. Spatial variation in organic matter utilization by benthic communities from Yura River–Estuary to offshore of Tango Sea, Japan. Estuarine, Coastal and Shelf Science, 86, 107-117. doi: 10.1016/j.ecss.2009.10.020.

Arias-Gonzalez, J., Delesalle, B., Salvat, B., Galzin, R. 1997. Trophic functioning of the Tiahura reef sector, Moorea Island, French Polynesia. Coral Reefs, 16, 231-246.

Berge, J., Daase, M., Renaud, P.E., Ambrose, W.G., Darnis, G., Last, K.S., Leu, E., Cohen, J.H., Johnsen, G., Moline, M.A. 2015. Unexpected levels of biological activity during the polar night offer new perspectives on a warming Arctic. Current Biology, 25, 2555-2561.

Billett, D.S.M., Lampitt, R.S., Rice, A.L., Mantoura, R.F.C. 1983. Seasonal sedimentation of phytoplankton to the deep-sea benthos. Nature, 302, 520-522.

Carroll, M.L., Ambrose Jr, W.G. 2012. Benthic infaunal community variability on the northern Svalbard shelf. Polar Biology, 35, 1259-1272.

Cummings, V., Thrush, S., Chiantore, M., Hewitt, J., Cattaneo-Vietti, R. 2010. Macrobenthic communities of the north-western Ross Sea shelf: links to depth, sediment characteristics and latitude. Antarctic Science, 22, 793-804.

Danovaro, R. 2010. Methods for the study of deep-sea sediments, their functioning and biodiversity. CRC Press. Boca Raton.

Danovaro, R., Gambi, C. 2002. Biodiversity and trophic structure of nematode assemblages in seagrass systems: evidence for a coupling with changes in food availability. Marine Biology, 141, 667-677.

Danovaro, R., Dell'Anno, A., Pusceddu, A. 2004. Biodiversity response to climate change in a warm deep sea. Ecology Letters, 7, 821-828.

Davies, P.S. 1984. The role of zooxanthellae in the nutritional energy requirements of *Pocillopora eydouxi*. Coral Reefs, 2, 181-186.

De Busserolles, F., Sarrazin, J., Gauthier, O., Gélinas, Y., Fabri, M., Sarradin, P., Desbruyeres, D. 2009. Are spatial variations in the diets of hydrothermal fauna linked to local environmental conditions? Deep Sea Research Part II: Topical Studies in Oceanography, 56, 1649-1664.

de Goeij, J.M., van Oevelen, D., Vermeij, M.J., Osinga, R., Middelburg, J.J., de Goeij, A.F., Admiraal, W. 2013. Surviving in a marine desert: the sponge loop retains resources within coral reefs. Science (New York, N.Y.), 342, 108-110. doi: 10.1126/science.1241981.

Dubois, S., Blanchet, H., Garcia, A., Massé, M., Galois, R., Grémare, A., Charlier, K., Guillou, G., Richard, P., Savoye, N. 2014. Trophic resource use by macrozoobenthic primary consumers within a semi-enclosed coastal ecosystem: stable isotope and fatty acid assessment. Journal of Sea Research, 88, 87-99.

El-Serehy, H.A., Al-Rasheid, K.A., Al-Misned, F.A., Al-Talasat, A.A.R., Gewik, M.M. 2016. Microbial–meiofaunal interrelationships in coastal sediments of the Red Sea. Saudi journal of biological sciences, 23, 327-334.

Fabiano, M., Danovaro, R., Fraschetti, S. 1995. A three-year time series of elemental and biochemical composition of organic matter in subtidal sandy sediments of the Ligurian Sea (northwestern Mediterranean). Continental Shelf Research, 15, 1453-1469.

Fredriksen, S. 2003. Food web studies in a Norwegian kelp forest based on stable isotope (δ^{13}C and δ^{15}N) analysis. Marine Ecology Progress Series, 260, 71-81.

Garcia, R., Koho, K., De Stigter, H., Epping, E., Koning, E., Thomsen, L. 2007. Distribution of meiobenthos in the Nazare canyon and adjacent slope (western Iberian Margin) in relation to sedimentary composition. Marine Ecology Progress Series, 340, 207-220.

Gaudêncio, M.J., Cabral, H. 2007. Trophic structure of macrobenthos in the Tagus estuary and adjacent coastal shelf. Hydrobiologia, 587, 241-251.

Gillies, C., Stark, J., Johnstone, G., Smith, S.D. 2013. Establishing a food web model for coastal Antarctic benthic communities: a case study from the Vestfold Hills. Marine Ecology Progress Series, 478, 27-41.

Giordani, P., Helder, W., Koning, E., Miserocchi, S., Danovaro, R., Malaguti, A. 2002. Gradients of benthic–pelagic coupling and carbon budgets in the Adriatic and Northern Ionian Sea. Journal of Marine Systems, 33, 365-387.

Glover, A.G., Smith, C.R., Mincks, S.L., Sumida, P.Y., Thurber, A.R. 2008. Macrofaunal abundance and composition on the West Antarctic Peninsula continental shelf: Evidence for a sediment 'food bank'and similarities to deep-sea habitats. Deep Sea Research Part II: Topical Studies in Oceanography, 55, 2491-2501.

Harriague, A.C., Bavestrello, G., Bo, M., Borghini, M., Castellano, M., Majorana, M., Massa, F., Montella, A., Povero, P., Misic, C. 2014. Linking environmental forcing and trophic supply to benthic communities in the Vercelli Seamount area (Tyrrhenian Sea). PLoS One, 9, e110880.

Hudson, I.R., Wigham, B.D., Billett, D.S.M., Tyler, P.A. 2003. Seasonality and selectivity in the feeding ecology and reproductive biology of deep-sea bathyal holothurians. Progress in Oceanography, 59, 381-407. doi: 10.1016/j.pocean.2003.11.002.

Hunter, W.R., Jamieson, A., Huvenne, V., Witte, U. 2013. Sediment community responses to marine *vs* terrigenous organic matter in a submarine canyon. Biogeosciences, 10, 67-80.

Iken, K., Brey, T., Wand, U., Voigt, J., Junghans, P. 2001. Food web structure of the benthic community at the Porcupine Abyssal Plain (NE Atlantic): a stable isotope analysis. Progress in Oceanography, 50, 383-405.

Iken, K., Bluhm, B.A., Gradinger, R. 2005. Food web structure in the high Arctic Canada Basin: evidence from $\delta C13$ and $\delta N15$ analysis. Polar Biology, 28, 238-249. doi: 10.1007/s00300-004-0669-2.

Iken, K., Bluhm, B., Dunton, K. 2010. Benthic food-web structure under differing water mass properties in the southern Chukchi Sea. Deep Sea Research Part II: Topical Studies in Oceanography, 57, 71-85.

Ingels, J., Kiriakoulakis, K., Wolff, G.A., Vanreusel, A. 2009. Nematode diversity and its relation to the quantity and quality of sedimentary organic matter in the deep Nazaré Canyon, Western Iberian Margin. Deep Sea Research Part I: Oceanographic Research Papers, 56, 1521-1539. doi: 10.1016/j.dsr.2009.04.010.

Jaschinski, S., Brepohl, D.C., Sommer, U. 2008. Carbon sources and trophic structure in an eelgrass (Zostera marina L.) bed based on stable isotope and fatty acid analyses. Marine Ecology Progress Series, 358, 103-114.

Leclerc, J., Riera, P., Leroux, C., Lévêque, L., Davoult, D. 2013. Temporal variation in organic matter supply in kelp forests: linking structure to trophic functioning. Marine Ecology Progress Series, 494, 87-105.

Levin, L.A. 2005. Ecology of cold seep sediments: interactions of fauna with flow, chemistry and microbes. Oceanography and Marine Biology: An Annual Review, 43, 1-46.

Levin, L.A., Michener, R.H. 2002. Isotopic evidence for chemosynthesis-based nutrition of macrobenthos: the lightness of being at Pacific methane seeps. Limnology and Oceanography, 47, 1336-1345.

Levinton, J. 1972. Stability and trophic structure in deposit-feeding and suspension-feeding communities. American Naturalist, 106, 472-486.

Liaaen-Jensen, S. 2012. Marine carotenoids. Marine natural products.Chemical and biological perspectives, 2, 1-73.

Limen, H., Levesque, C., Juniper, S.K. 2007. POM in macro-/meiofaunal food webs associated with three flow regimes at deep-sea hydrothermal vents on Axial Volcano, Juan de Fuca Ridge. Marine Biology, 153, 129-139. doi: 10.1007/s00227-007-0790-1.

Lin, H., Lin, P., Chang, N., Shiao, J., Kao, S. 2014. Trophic structure of megabenthic food webs along depth gradients in the South China Sea and off northeastern Taiwan. Marine Ecology Progress Series, 501, 53-66.

Lovvorn, J.R., Cooper, L.W., Brooks, M.L., De Ruyck, C.C., Bump, J.K., Grebmeier, J.M. 2005. Organic matter pathways to zooplankton and benthos under pack ice in late winter and open water in late summer in the north-central Bering Sea. Marine Ecology Progress Series, 291, 135-150.

Mamouridis, V., Cartes, J.E., Parra, S., Fanelli, E., Saiz Salinas, J. 2011. A temporal analysis on the dynamics of deep-sea macrofauna: influence of environmental variability off Catalonia coasts (western Mediterranean). Deep Sea Research Part I: Oceanographic Research Papers, 58, 323-337.

Marguillier, S., Van der Velde, G., De Hairs, F., Hemminga, M., Rajagopal, S. 1997. Trophic relationships in an interlinked mangrove-seagrass ecosystem as traced by delta C-13 and delta N-15. Marine Ecology Progress Series, 151.

Martin-Creuzburg, D., Elert, E. 2009. Ecological significance of sterols in aquatic food webs. In: M. Kainz, M.T. Brett, M.T. Arts (Eds.), Lipids in Aquatic Ecosystems. Springer, New York., pp. 43-64; 3.

Meyers, P.A. 1994. Preservation of elemental and isotopic source identification of sedimentary organic matter. Chemical Geology, 114, 289-302.

Middelburg, J.J., Nieuwenhuize, J., van Breugel, P. 1999. Black carbon in marine sediments. Marine Chemistry, 65, 245-252.

Miller, R.J., Page, H.M. 2012. Kelp as a trophic resource for marine suspension feeders: a review of isotope-based evidence. Marine Biology, 159, 1391-1402.

Mincks, S.L., Smith, C.R., DeMaster, D.J. 2005. Persistence of labile organic matter and microbial biomass in Antarctic shelf sediments: Evidence of a sediment food bank. Marine Ecology Progress Series, 300, 3-19.

Moncreiff, C.A., Sullivan, M.J. 2001. Trophic importance of epiphytic algae in subtropical seagrass beds: evidence from multiple stable isotope analyses. Marine ecology Progress Series, 215, 93-106.

Olabarria, C., Incera, M., Garrido, J., Rossi, F. 2010. The effect of wrack composition and diversity on macrofaunal assemblages in intertidal marine sediments. Journal of experimental marine biology and ecology, 396, 18-26.

Orr, K.K., Wilding, T.A., Horstmeyer, L., Weigl, S., Heymans, J.J. 2013. Detached macroalgae: Its importance to inshore sandy beach fauna. Estuarine, Coastal and Shelf Science.

Pape, E., Jones, D.O., Manini, E., Bezerra, T.N., Vanreusel, A. 2013. Benthic-pelagic coupling: effects on nematode communities along southern European continental margins. PLoS One, 8, e59954.

Parrish, C.C. 2009. Essential fatty acids in aquatic food webs. In: Arts et al. (Eds.), Lipids in Aquatic Ecosystems. Springer, New York., pp. 309-326.

Pasotti, F., Convey, P., Vanreusel, A. 2014. Potter Cove, west Antarctic Peninsula, shallow water meiofauna: a seasonal snapshot. Antarctic Science, 26, 554-562.

Pasqual, C., Goñi, M.A., Tesi, T., Sanchez-Vidal, A., Calafat, A., Canals, M. 2013. Composition and provenance of terrigenous organic matter transported along submarine canyons in the Gulf of Lion (NW Mediterranean Sea). Progress in Oceanography, 118, 81-94.

Pedrosa-Pàmies, R., Sanchez-Vidal, A., Calafat, A., Canals, M., Duran, R. 2013. Impact of storm-induced remobilization on grain size distribution and organic carbon content in sediments from the Blanes Canyon area, NW Mediterranean Sea. Progress in Oceanography, 118, 122-136.

Phillips, N.W. 1984. Role of different microbes and substrates as potential suppliers of specific, essential nutrients to marine detritivores. Bulletin of Marine Science, 35, 283-298.

Pires-Vanin, A.M.S., Arasaki, E., Muniz, P. 2013. Spatial pattern of benthic macrofauna in a sub-tropical shelf, São Sebastião Channel, southeastern Brazil/Aspectos ecológicos de la macrofauna bentónica del Canal de São Sebastião, sudeste de Brasil. Latin American Journal of Aquatic Research, 41, 42.

Plank, L.R., Lawrence, J.M., Lawrence, A.L., Olvera, R.M. 2002. The effect of dietary carotenoids on gonad production and carotenoid profiles in the sea urchin Lytechinus variegatus. Journal of the World Aquaculture Society, 33, 127-137.

Rau, G.H. 1981. Low $^{15}N/^{14}N$ in hydrothermal vent animals: ecological implications.

Reid, W.D., Sweeting, C.J., Wigham, B.D., Zwirglmaier, K., Hawkes, J.A., McGill, R.A., Linse, K., Polunin, N.V. 2013. Spatial differences in East Scotia ridge hydrothermal vent food webs: influences of chemistry, microbiology and predation on trophodynamics. PLoS One, 8, e65553.

Riou, V., Colaço, A., Bouillon, S., Khripounoff, A., Dando, P., Mangion, P., Chevalier, E., Korntheuer, M., Santos, R.S., Dehairs, F. 2010. Mixotrophy in the deep sea: a dual endosymbiotic hydrothermal mytilid assimilates dissolved and particulate organic matter. Marine Ecology Progres Serires, 405, 187-201.

Rosenberg, R., Grémare, A., Amouroux, J., Nilsson, H.C. 2003. Benthic habitats in the northwest Mediterranean characterised by sedimentary organics, benthic macrofauna and sediment profile images. Estuarine, Coastal and Shelf Science, 57, 297-311.

Sanchez-Vidal, A., Pasqual, C., Kerherve, P., Heussner, S., Calafat, A., Palanques, A., de Madron, X.D., Canals, M., Puig, P. 2009. Across margin export of organic matter by cascading events traced by stable isotopes, northwestern Mediterranean Sea. Limnology and Oceanography, 54, 1488-1500. doi: 10.4319/lo.2009.54.5.1488.

Schaal, G., Riera, P., Leroux, C. 2010. Trophic ecology in a Northern Brittany (Batz Island, France) kelp (Laminaria digitata) forest, as investigated through stable isotopes and chemical assays. Journal of Sea Research, 63, 24-35.

Sorokin, Y.I. 1990. Aspects of trophic relations, productivity and energy balance in coral-reef ecosystems. Ecosystems of the world, 25, 401-418.

Sumida, P., Smith, C., Bernardino, A., Polito, P., Vieira, D. 2014. Seasonal dynamics of megafauna on the deep West Antarctic Peninsula shelf in response to variable phytodetrital influx. Royal Society open science, 1, 140294.

Sumida, P.Y.G., Bernardino, A.F., Stedall, V.P., Glover, A.G., Smith, C.R. 2008. Temporal changes in benthic megafaunal abundance and composition across the West Antarctic Peninsula shelf: Results from video surveys. Deep Sea Research Part II: Topical Studies in Oceanography, 55, 2465-2477. doi: 10.1016/j.dsr2.2008.06.006.

Thurber, A.R., Kröger, K., Neira, C., Wiklund, H., Levin, L.A. 2010. Stable isotope signatures and methane use by New Zealand cold seep benthos. Marine Geology, 272, 260-269.

Tsushlma, M., Kawakami, T., Mine, M., Matsuno, T. 1997. The role of carotenoids in the development of the sea urchin *Pseudocentrotus depressus*. Invertebrate Reproduction & Development, 32, 149-153. doi: 10.1080/07924259.1997.9672616.

van Oevelen, D., Soetaert, K., Garcia, R., de Stigter, H.C., Cunha, M.R., Pusceddu, A., Danovaro, R. 2011. Canyon conditions impact carbon flows in food webs of three sections of the Nazare canyon. Deep Sea Research (Part II, Topical Studies in Oceanography), 58, 2461-2476.

Vanhove, S., Beghyn, M., Van Gansbeke, D., Bullough, L., Vincx, M. 2000. A seasonally varying biotope at Signy Island, Antarctic: implications for meiofaunal structure. Marine Ecology Progress Series, 202, 13-25.

Vanhove, S., Lee, H., Beghyn, M., Van Gansbeke, D., Brockington, S., Vincx, M. 1998. The metazoan meiofauna in its biogeochemical environment: the case of an Antarctic coastal sediment. Journal of the Marine Biological Association of the United Kingdom, 78, 411-434.

Vanhove, S., Wittoeck, J., Desmet, G., Van den Berghe, B., Herman, R., Bak, R., Nieuwland, G., Vosjan, J., Boldrin, A., Rabitti, S. 1995. Deep-sea meiofauna communities in Antarctica: structural analysis and relation with the environment. Marine Ecology Progress Series, 127, 65-76.

Vonk, J.A., Christianen, M.J., Stapel, J. 2008. Redefining the trophic importance of seagrasses for fauna in tropical Indo-Pacific meadows. Estuarine, Coastal and Shelf Science, 79, 653-660.

Wade, N.M., Gabaudan, J., Glencross, B.D. 2015. A review of carotenoid utilisation and function in crustacean aquaculture. Reviews in Aquaculture, 0, 1-16.

Witte, U., Wenzhofer, F., Sommer, S., Boetius, A., Heinz, P., Aberle, N., Sand, M., Cremer, A., Abraham, W.R., Jorgensen, B.B., Pfannkuche, O. 2003. *In situ* experimental evidence of the fate of a phytodetritus pulse at the abyssal sea floor. Nature, 424, 763-766. doi: 10.1038/nature01799.

Włodarska-Kowalczuk, M., Górska, B., Deja, K., Morata, N. 2016. Do benthic meiofaunal and macrofaunal communities respond to seasonality in pelagial processes in an Arctic fjord (Kongsfjorden, Spitsbergen)? Polar Biology, 1-15.

Wolff, G.A., Billett, D.S.M., Bett, B.J., Holtvoeth, J., FitzGeorge-Balfour, T., Fisher, E.H., Cross, I., Shannon, R., Salter, I., Boorman, B., King, N.J., Jamieson, A., Chaillan, F. 2011. The Effects of Natural Iron Fertilisation on Deep-Sea Ecology: The Crozet Plateau, Southern Indian Ocean. PLoS One, 6. doi: 10.1371/journal.pone.0020697.

3. Food quantity and quality in Barkley Canyon (NE Pacific) and its influence on macroinfaunal community structure [2]

3.1. Abstract

The highly heterogeneous nature of submarine canyon physical landscapes can influence organic matter spatial distribution and thus benthic community and food web structure. We therefore studied patterns in quantity and quality (i.e., nutritional value for benthic organisms) of sediment and bottom-water particulate organic matter and their influence on macroinfaunal community structure in Barkley Canyon (200-2000 m, NE Pacific), a shelf-incising and short submarine canyon, at multiple spatial scales (10's to 100's of meters). At large scales (100's of meters), we hypothesised that canyon heterogeneity would drive organic matter patterns: topographic features (slope, aspect, curvature, rugosity, and benthic positioning index) and other environmental variables (depth, dissolved oxygen concentration, surface primary productivity, and sediment type). Our multivariate distLM analysis identified mean grain size, surface primary productivity and benthic positioning index (concave/convex seafloor topography) as the primary drivers of organic distribution in sediments. Noting generally accepted different degradation rates among food variables, we inferred that the freshest organic matter reaches bottom waters of Barkley Canyon at 400 m, where ambient currents at the canyon head region likely concentrate primary productivity from surface waters. Evidence of deposition (increased amounts of fresh food) first appeared at 600 – 800 m, coincident with finer sediments, convex topography, and convergence of canyon branches; Degraded organic matter accumulated at 1500 and 2000 m where the comparatively fine sediments are known to adsorb a greater proportion of available organic material than at shallower depths but limited delivery of surface primary productivity reduces overall food quality. Despite clear differences in food quantity and quality among sites

(100's of m apart), dissolved oxygen primarily drove macrofaunal distribution, along with hydrocarbons, indicative of a chemosynthetic ecosystem. At smaller spatial scales (10's of meters) we found greater food patchiness associated with the topographically complex upper canyon (≤ 800 m). We also found distinct communities at smaller spatial scales (10's of m apart) at 200 m, where fatty acid biomarkers distinguished a food patch rich in zooplankton. Overall, canyon heterogeneity rather than depth primarily determines patterns of organic matter (quantity and quality) in Barkley Canyon, with greater food patchiness at sites ≤ 800 m depth. Organic matter distribution appears to influence macroinfaunal community structure more strongly at smaller spatial scales in contrast to major stressors (i.e., oxygen) that act over larger scales.

Key words: submarine canyons; organic matter; food quality; oxygen minimum zones; seafloor topography; benthic community structure; NE Pacific, Barkley Canyon.

3.2. Introduction

Organic matter (OM) produced by photosynthesis well above the deep-sea seafloor typically sinks through the water column and fuels deep-sea communities and food webs (Gage and Tyler, 1991). Therefore, we expect OM quantity and quality to decrease with depth in most deep-sea ecosystems. However, Smith (1987) found discrepancies between benthic community oxygen consumption and particle flux in the North Pacific, and Danovaro et al. (2003) found depocenters of OM at hadal depths, noting that lateral advection of organic material, including that produced by chemosynthesis, also provides important food sources for deep-sea benthic communities (Jahnke et al., 1990, Gage and Tyler, 1991). The heterogeneous nature of submarine canyons (e.g., steep and often sinuous topography, strong currents, sediment transport, and influence of surface primary productivity) could also modify OM distribution with depth. Barkley is a shelf-incising submarine canyon in the NE Pacific (200 -2000 m) (Allen et al., 2001) organic matter

input delivered from the shelf likely supports higher seafloor abundance, biomass, and biodiversity when compared to blind canyons, which do not reach the shelf edge (Harris and Whiteway, 2011). Strong hydrodynamics at the heads of canyons create periodic disturbances that can reduce organic matter deposition with related effects on macro- and megabenthic (including fish) communities (i.e., lower abundances and lower species richness: Vetter et al., 2010, De Leo et al., 2014). Submarine canyons transport sediments from continental shelves to the deep sea (Puig et al., 2014) along with the OM that supports benthic communities and associated food webs. Various processes control sediment transport through canyons, including internal tide/wave re-suspension and circulation, gravity flows such as turbidity currents induced by trawling activities, storms and/or inputs of fluvial sediments, or cascading of dense water formed by cooling, evaporation, or freezing of water over the continental shelf (Canals et al., 2006, Puig et al., 2014). Naturally occurring canyon-flank failures are also sediment transport mechanisms within submarine canyons contributing to focusing sediment deposition in specific canyon areas (Puig et al., 2014).

Previous studies report differences in within-canyon distribution of OM with depth not only among canyons (Pusceddu et al., 2010), but also within branches of the same canyon (Bianchelli et al., 2008). Temporal variability also contributes to canyon OM heterogeneity. Storm events and seasonal sea surface biological processes influence the composition, quantity, and deposition of particulate organic matter (POM) in canyon sediments (López-Fernández et al., 2013). Submarine canyon surroundings can also influence food quantity and composition within the canyon. For example, POM from Nazaré submarine canyon (Portugal) contains terrestrial organic matter inputs from the shelf and zooplankton-derived OM from Mediterranean Overflow Water (Kiriakoulakis et al., 2011). Benthic fauna can also modify OM that arrives on the

submarine canyon seafloor; for example, heterotrophic consumption/reworking by megafaunal organisms can affect the availability of food resources to other benthos (Amaro et al., 2010). These complexities in OM transport within canyons point to the complex distribution and heterogeneity of OM at depths far below the supply of sinking particulates from productive surface waters.

Multiple aspects of OM composition can provide insights regarding its quality for benthic consumers. We define food quality as the degree to which quantity and composition of accessible food fulfill consumer nutritional needs (Müller-Navarra, 2008). We describe different food variables in detail elsewhere (Campanyà-Llovet et al., 2017) and only provide a summary of the practical proxies that inform about OM quantity and quality here. Whereas total organic carbon (TOC) describes food quantity, Carbon:Nitrogen (C:N) ratios indicate food sources (i.e., terrestrial or marine), and its nitrogen limitations. Furthermore, although the OM $\delta^{13}C$ signature also provides insight into its source (i.e., terrestrial, marine, photosynthetic, chemosynthetic, etc.), the $\delta^{15}N$ signature describes its degradation state (Thornton and McManus, 1994) and origin (i.e., terrestrial, marine: Middelburg and Nieuwenhuize, 1998). Moreover, Chlorophyll a:Phaeophorbides (Chl *a*:Phaeo) pigment ratios describes the degree of OM freshness (Le Guitton et al., 2015) whereas the total OM lipids fraction comprise part of the highly labile (i.e. immediately digestible: Mayer 1995) carbon for benthic consumers, and thus its nutritional value (Parrish, 2013). In this study we consider acyl (i.e., triacylglycerols, glycolipid, phospholipids, and acetone-mobile polar lipids) and non-acyl lipids (i.e., hydrocarbons, esters, kethones, alcohols, and sterols) as part of the total lipids pool (Parrish, 1999). Non-acyl lipids provide valuable information, such as hydrocarbons as tracers of seepage (Parrish, 1999), methane hydrates in our study area. Furthermore, lipid classes and some fatty acids indicate OM origin

(e.g., wax esters are indicative of zooplankton, phospholipids of primary producers, 16:4ω1 of diatoms: Parrish, 2013) and certain fatty acids are considered essential (essential fatty acids or EFAs) because they are indispensable for survival, growth, and reproduction of a species (Parrish, 2009). Differences in degradation rates of molecules also allow exploration of organic matter quality at different time scales (Le Guitton et al., 2015). Algae and plant photosynthetic pigments degrade at faster rates compared to lipids, bulk OM, and amino acids, and can be used as a measure of freshness of OM (time-scales of days to weeks). Lower degradation rates in lipids compared to pigments but higher rates compared to bulk OM and amino acids indicate intermediate degradation time-scales of weeks to months. At the other end of the spectrum, longer degradation rates in bulk OM and amino acids compared to pigments and lipids reflect time scales of months to a year (Le Guitton et al., 2015). Therefore, we refer to short-term (pigments), intermediate-term (lipids), and long-term (TOC, C:N) food variables.

Food availability for benthic communities typically correlates positively with benthic abundances and/or biomass, however, many studies ignore food quality, which can also influence species composition and trophic structure (Wieking and Kröncke, 2005, Campanyà-Llovet et al., 2017). The "patch mosaic theory" proposed to explain unexpectedly high diversity in many deep-sea sedimentary habitats (Grassle and Sanders, 1973), posits that patches derived from differences in food supply (seasonal pulses of OM, organic remains of gelatinous zooplankton, wood falls, kelp, seagrass, etc.) provide the heterogeneity necessary to explain high biodiversity. Subsequent colonization experiments utilizing sediments enriched with different food sources: *Thalassiosira* sp. (diatom paste) and *Sargassum* sp. (brown algae) (Snelgrove et al., 1992, 1994, 1996) support this theory. Although multiple studies have identified submarine canyons as potential biomass and biodiversity "hot spots" because of their topographic effects in

channeling and accumulating OM in the seafloor (De Leo et al., 2010, Vetter et al., 2010, De Leo et al., 2014), fewer studies consider aspects of spatial heterogeneity in food quality and composition in submarine canyons (Pusceddu et al., 2010), and its structuring role for benthic communities. Because spatial heterogeneity in food quantity and quality occurs at multiple spatial scales, with depth (100's of meters: Johnson et al., 2007) or between patches (10's of meters: Snelgrove et al., 1992, 1994, 1996), our study encompasses both spatial scales, 10's and 100's of meters.

The inherent physical and topographic complexity that generates extreme habitat heterogeneity in productive and biodiverse submarine canyons, and the lack of in-depth studies in OM quality motivated us to study spatial and bathymetric patterns of food quantity and quality along Barkley Canyon in the NE Pacific, and its potential effects in structuring local benthic communities. Barkley Canyon has only recently received greater attention from the scientific community with respect to overall seafloor ecosystem functioning (Belley et al., 2016) and its role in enhancing local benthic productivity and biodiversity (Matabos et al., 2014, Doya et al., 2017, Thomsen et al., 2017, De Leo et al., 2018). In order to provide new insights into Barkley Canyon spatial heterogeneity and processes controlling OM deposition and availability for benthic communities, we established three working hypotheses: 1) Habitat/topographic heterogeneity rather than depth control organic matter (quantity and quality) in Barkley Canyon at large (100's of meters) spatial scales; 2) Organic matter (quantity and quality) is patchily distributed at small (10's of meters) spatial scales; and 3) food patches distinct in quantity and quality primarily drive benthic community structure at small and large spatial scales.

3.3. Materials and methods

3.3.1. Study area and sampling

Barkley Canyon is located in the NE Pacific, about 100 km offshore from Vancouver Island, British Columbia, Canada (Fig. 3.1). The canyon extends from the edge of the continental shelf at 200 m (Allen et al., 2001) through an oxygen minimum zone (OMZ) at intermediate depths (400-1000 m), to the Cascadia Basin at ~ 2000 m (Keeling et al., 2010, De Leo et al., 2018).
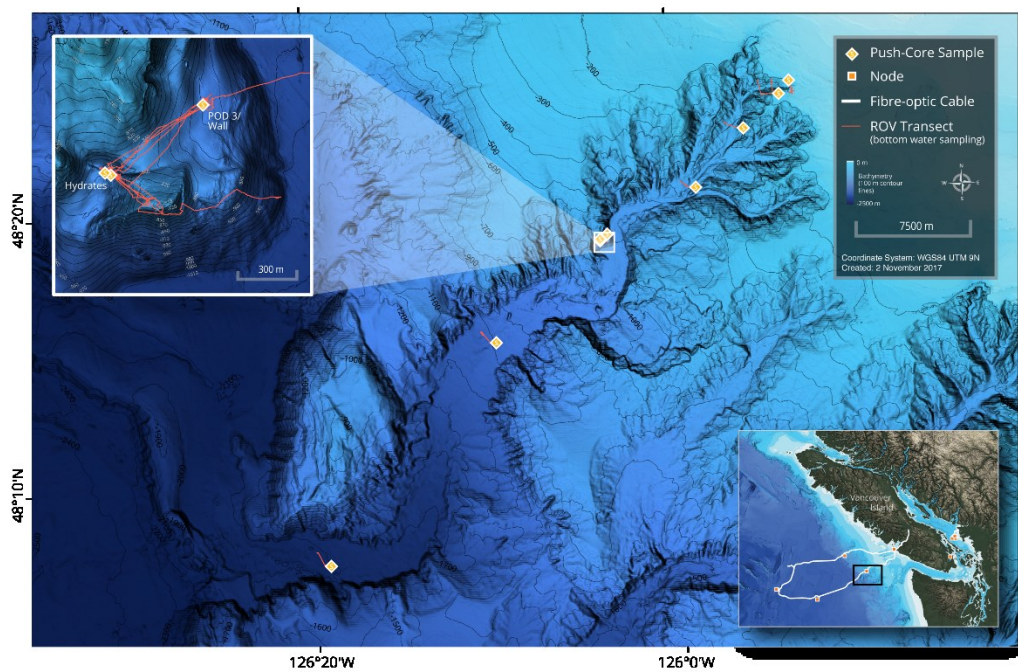
Figure 3.1. Location of Barkley Canyon (NE Pacific) and sampling sites.

We collected sediment samples for organic matter and faunal analyses during an expedition on board the Schmidt Ocean Institute's R/V *Falkor* in September 2013 (Table 3.1). Replicate push core sediment samples (i.d. = 6.7 cm, L = 35.6 cm) were collected using the Remotely Operated Vehicle (ROV) ROPOS (www.ropos.com) along Barkley Canyon axis (200 – 2000 m depth) and at the NE wall of the canyon (870-890 m depth; see Fig. 3.1, Table 3.1). The sampling areas near the NE canyon wall represent some of the long-term monitoring sites of Ocean Networks

Canada's (ONC) NEPTUNE cabled observatory (www.oceannetworkscanada.ca), including Barkley Hydrates at 870 m, and MidWest at 890 m (Table 3.1). A flat plateau characterizes Barkley Hydrates, with methane hydrate outcrops surrounded by patchily distributed cold-seep communities, including bacterial mats, live and dead vesycomid clams, and carbonate mounds (Doya et al., 2017). Specifically, we sampled Barkley Hydrates at the clam bed within the outcrop and at a location 20 m away. A mixture of mud and sand covers the seafloor at MidWest site, approximately 600 m to the NW of Barkley Hydrates (Juniper et al., 2013, Chauvet et al., 2018). We also subdivided the 200 m site into two sampling areas based on the presence or absence of a *Strongylocentrotus fragilis* (Echinoidea) aggregation ("urchin aggregation" and "no aggregation"). *S. fragilis*, a common megafaunal species, occurs at the shelf and slope depths in the NE Pacific where it tends to aggregate and thus significantly impacts megafaunal density patterns with depth (De Leo et al., 2017, Sato et al., 2017). We collected one push core for organic matter in each area and three for fauna in the "urchin aggregation" and four for fauna in the "no aggregation" area.

We also collected bottom-water particulate OM at the axis sites (B200, BC400, BC600, BC800, BC1500, and BC2000) with a McLane WTS-LV (Large Volume Water Transfer System) pump mounted on ROPOS, which sampled between 200-500 L of water during video/visual transects of variable length for another study assessing benthic megafaunal community structure and diversity (Fig. 3.1; Domke et al., 2017).

Table 3.1. Site, sample types and numbers, location, and depth of sites of Barkley Canyon in September 2013. SOM = Sediment Organic Matter, POM = Bottom-water Particulate Organic Matter.

Site	Sample type	Sample number	Setting	Lat	Long	Depth (m)
BC200	SOM/POM/fauna	2/2/7	Canyon axis	48.40401	-125.88811	200
BC300/400	SOM/POM	1/2	Canyon axis	48.39631/ 48.40448	-125.89787/ -125.9112	300/400
BC600	SOM/POM	2/2	Canyon axis	48.37629	-125.93118	600
BC800	SOM/POM	2/2	Canyon axis	48.3416	-125.97679	800
BC1500	SOM/POM	2/2	Canyon axis	48.2516	-126.16348	1500
BC2000	SOM/POM	2/2	Canyon axis	48.11991	-126.32055	2000
MidWest	SOM/fauna	3/7	Canyon wall	48.31496	-126.0588333	890
Barkley Hydrates- outcrop	SOM/fauna	4/6	Canyon wall	48.311945	-126.066022	870
Barkley Hydrates- 20m	SOM/fauna	4/6	Canyon wall	48.311855	-126.065722	870

3.3.2. Sample processing

Our SOM analyses focused on the 0-2 cm upper layer of each core to assess the sediment layer in immediate contact with canyon dynamics. Using a clean spatula, we collected subsamples for CHN and mean grain size analysis from each sample and transferred them to Whirl-pak® bags, whereas subsamples for pigment analyses were placed in 15 ml centrifuge tubes covered in aluminum tin foil to protect them from light. Both types of sample were stored at -20 °C until analyzed. Subsamples for lipid analyses were placed in aluminum tin foil, which was precombusted (450 °C for 8 h) to avoid contamination, and stored at -80 °C until analysis. POM samples collected with the McLane water pump system were filtered through two pre-weighed and pre-combusted (450 °C for 5 h) Whatman glass microfiber GF/F filters (420 mm); one was used for CHN and stable isotope analyses and stored at -20 °C, the other was stored at -80 °C and used for lipid analyses. Both sample types were wrapped in aluminum foil, which was also pre-combusted (450 °C for 8 h) to avoid contamination. The limited number of replicates available precluded pigment analysis on POM.

We extruded the upper 10 cm of each sediment core for biodiversity analyses. Sediment samples were processed through a 300 μm sieve prior to preservation in a 4 % seawater-formaldehyde solution on board, and later transferred into 70 % ethanol in the laboratory. Specimens were then sorted under a dissecting microscope and identified to family level, noting that many of the families at these sites contain few species (author's pers. obs). We identified a species of bivalves resembling the family thyasiridae because of the ferruginous patches on the anterior and posterior ends or elongated food and will refer to them as such from now on.

The multibeam sonar survey of Barkley Canyon was conducted by ONC from the R/V T.G. Thompson in 2012, and by ONC and Schmidt Ocean Institute on board the R/V *Falkor* 2013, in

both cases using a Kongsberg EM302 Echosounder 105. The surveys mapped different areas of the canyon head and mid-deep canyon respectively. The bathymetric data from the R/V *Falkor* cruise was evaluated visually to remove outliers and gridded at 25 m spatial resolution by ONC, but it has not been corrected for tides, which adds a minor error of several metres to reported depths.

3.3.3. Organic matter quantity and quality

3.3.3.1. Phytopigment content

We quantified sediment phytopigment content by estimating chlorophyll *a* (Chl *a*) and pheopigments (Pheo) fluorometrically, following a modified version of Riaux-Gobin & Klein (Riaux-Gobin and Klein, 1993). Pigments from 1-2 g of wet sediment were extracted in 90 % acetone (v/v) for 24 h at 4 ^{o}C and the supernatant analyzed using a Turner Designs 10-AU-005-CE fluorometer (Turner Designs, Sunnyvale, USA) with and without acidification. The remaining sediment was dried at 60 ^{o}C for 24 h and weighed to measure Chl *a* per g of dry weight.

3.3.3.2. Lipid content

Lipid samples were extracted with a combination of chloroform: methanol: water to 4:2:1.5 according to Parrish (1999), creating an upper inorganic and a lower organic layer. We removed the bottom organic layer, which contains the lipids, using a double pipetting technique, placing a 1 ml, lipid cleaned Pasteur pipette inside a 1 ml pipette to remove the organic layer without disturbing the top aqueous layer. Chloroform was then added back to the extraction test tube and the entire procedure was repeated three times. We then pooled all organic layers in a lipid-cleaned vial, concentrating samples under a flow of nitrogen gas to avoid lipid oxidation.

We determined lipid class composition with a three-step chromatographic development method (Parrish, 1987). The lipid extracts were applied to Chromarods and focused to a narrow sample band using 100 % acetone. The first development system was hexane/diethyl ether/formic acid (98.95:1.0:0.05, v/v/v). The rods were developed for 25 min, removed from the system for 5 min to dry, and replaced for 20 min. The second development ran for 40 min in hexane/diethyl ether/formic acid (79:20:1, v/v/v). The final development system entailed two 15 minute exposures to 100 % acetone, followed by two 10 min periods in chloroform/methanol/chloroform-extracted water (5:4:1, v/v/v). Before using each solvent system, we dried the rods in a constant humidity chamber. Samples were analysed using an Iatroscan Mark VI TLC-FID (thin-layer chromatography-flame ionization detector; Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan) after each development system (partial scanning), and collected the data using Peak Simple software (ver 3.67, SRI Inc). We calibrated the Chromarods with standards prior to and during their use on our samples.

A portion of each lipid extract was derivatized to obtain fatty acid methyl esters (FAME) with a mixture of BF_3/Ch3OH at 85 ^{o}C for 90 min under nitrogen. Purified water and hexane were then added. We transferred the upper, organic layer, where FAMEs occur, into a 2 ml vial, evaporated it under nitrogen, refilled the vial with hexane, and stored it at -20 ^{o}C under nitrogen. We removed hydrocarbons from FAMEs from the oversaturated samples collected at the Barkley Hydrates (outcrop and 20 m) sites in order to avoid damaging the GC column. We rinsed a Pasteur pipet packed with activated silica gel with 1 bed volume each of chloroform and hexane. FAMEs were placed at the head of the column and iso-octane (21 bed volume) was used to elute the contaminating hydrocarbons. Finally, we recovered FAMEs with 2 bed volume of 80:20 hexane:diethyl ether. All FAMEs were analyzed on a HP 6890 GC-FID (gas chromatography-

flame ionization detector) equipped with a 7683 autosampler. The GC column was a ZB wax + (Phenomenex, Torrance, CA, USA), 30 m in length with an internal diameter of 0.32 mm. Peaks were identified using retention times from standards purchased from Supelco (Bellefonte, PA, USA): 37 component FAME mix (Product number 47885-U), PUFA 3 (product number 47085-U) and PUFA 1 (product number 47033-U). Chromatograms were integrated using the Varian Galaxie Chromatography Data System, version 1.9.3.2 (Walnut Creek, CA, USA).

3.3.3.3. CHN and stable isotope analyses

We determined total organic carbon (TOC),total nitrogen (TN), and nitrogen and organic carbon stable isotope signatures by drying a sediment subsample of 1-5 g (wet weight) at 80 °C for 24 h, grinding it to a fine powder, and then weighing and acidifying (with pure HCl fumes) for 24 h to eliminate inorganic carbon. Samples were dried again at 80 °C for 24 h before starting CHN analysis. We then weighed an aliquot of dried decarbonated sediments (15 mg) in an aluminum capsule, and folded it tightly. TOC (%), TN (%), δ^{13}C (‰), δ^{15}N (‰) were determined in a Carlo Erba NA1500 Series II elemental analyser (EA). We present stable isotopic results based on the international standards of atmospheric nitrogen (AIR, N_2) for nitrogen and Vienna Pee Dee belemnite (V-PDB) for carbon. The average instrumental precision was ±0.1‰ for nitrogen and ±0.17‰ for carbon. The stable nitrogen and carbon isotope ratios are expressed as:

δ^{13}C or δ^{15}N = [(Rsample/Rstandard)-1] x 1000,

where R is ^{15}N/^{14}N for δ^{15}N and ^{13}C/^{12}C for δ^{13}C.

We used TOC as an indicator of food quantity, and the other variables (TN, C:N, δ^{13}C, δ^{15}N) as indicators of food quality.

3.3.4. Other environmental variables

3.3.4.1. Mean grain size

Granulometric properties (sediment mean grain size; MGS) were determined with a HORIBA Partica LA-950 laser diffraction particle size analyzer (Horiba Ltd, Kyoto, Japan). We deflocculated samples with calgon $(NaPO_3)_6$ prior to analysis to break down aggregates and ensure we were measuring actual sediment grain size.

3.3.4.2. Surface primary productivity

Data on surface water net primary productivity (SPP), a proxy for the available particulate organic matter, was derived from a Vertically Generalized Production Model (VGOPM) employing ocean colour and solar irradiance data from NADA MODIS and SeaWiFS satellites. The VGPM is a "chlorophyll based" model that estimates net primary production from chlorophyll using a temperature-dependent description of chlorophyll-specific photosynthetic efficiency to estimate net surface chlorophyll concentration (Behrenfeld and Falkowski, 1997). The VGPM model data products were extracted from the Ocean Productivity web portal, distributed by Oregon State University (www.science.oregonstate.edu/ocean.productivity/index.php). The original model accounted for 79 % of the observed variability in primary production (Behrenfeld and Falkowski, 1997). Net primary production data integrated over each month were extracted from the geographical locations (corresponding pixels in the model) where we collected our seafloor (sediment and bottom water) samples. We extracted data from two time periods, one coincident with our field sampling, and another one month prior, in order to account for two potential scenarios with two different time lags between primary productivity and deposition to the seafloor. The lack of knowledge of this time lag at each depth precludes more precise estimates of the influence of

primary productivity at each sampling site. VGPM model data was plotted and extracted in RStudio (v3.3.0).

3.3.4.3. Topographic features and dissolved oxygen concentration in bottom water

Seabed topographic features from Barkley Canyon were derived from the bathymetric raster using the Spatial Analyst tools in ArcGIS (version 10.4) for slope, aspect, and curvature, and the benthic terrain modeller (BTM) extension for fine and broad-scale BPI (Bathymetric Positioning Index), and rugosity – VRM (Vector Ruggedness Measure). BPI measures the bathymetric position of a specific pixel relative to surrounding pixels, and indicates whether any particular pixel forms part of a positive (e.g., crest) or negative (e.g., trough) feature of the surrounding terrain. Because the user specifies the radius used to compare bathymetric position, BPI can be calculated at different spatial scales: Broad-scale BPI (BBPI: scale factor of 1000 m in this study) and fine-scale BPI (FBPI: scale factor of 50 m in this study) (Weiss, 2001, Wilson et al., 2007). VRM measures terrain ruggedness as the variation in three-dimensional orientation of grid cells within a neighbourhood (Sappington et al., 2007). We chose derived variables according to their significance for sediment and OM transport and accumulation (i.e., slope, curvature, fine and broad-scale BPI, and VRM) and also current exposure (i.e., curvature, aspect, fine and broad-scale BPI). Sampling sites were plotted and derivatives extracted for each site using the "Extract Multi Values to Points" tool (Spatial Analyst). Dissolved oxygen (DO) concentrations near the seafloor were constantly measured by an optode (Seabird SBE-DO) installed on ROPOS.

3.3.5. Statistical analysis

3.3.5.1. Organic matter quantity and quality

In order to test the a priori hypothesis of patchy distribution of organic matter within Barkley Canyon as opposed to a gradual decrease with depth, we examined bathymetric patterns of food quantity (i.e., TOC), and food quality (i.e., C:N, Chl *a*:Pheo, total lipids, essential fatty acids (EFA), docosahexaenoic acid:eicosapentaenoic acid (DHA:EPA)) in Barkley Canyon with linear regressions. We checked normality with normal Q-Q plots, and homogeneity of variance by plotting residuals *vs* fitted values, transforming data when necessary prior to the analysis.

We examined the environmental drivers of spatial variation in OM in Barkley Canyon with the PERMANOVA+ add on to Primer 6 (Anderson et al., 2008). In this case, our data set resembled an environmental data set because of the absence of zeros, and we therefore based our resemblance matrix on Euclidean distances of standardized food variables (Anderson et al., 2008). We also performed a distance-based redundancy analysis (dbRDA) using a distance-based linear model (distLM) routine to identify potential environmental drivers (Anderson et al., 2008). In order to correct for data skewedness, we transformed the data (response and predictor variables) when needed, standardizing to make different variables inter-comparable (Clarke and Gorley, 2006). We checked multi-collinearity of the predictor variables ("Draftsman's plots" Primer 6) prior to analysis (Clarke and Gorley, 2006), and then eliminated temperature, salinity and SPP in September (sampling month). This process resulted in final predictor variables of mean grain size (MGS), % mud, depth, slope, aspect, curvature, BBPI, FBPI, VRM, surface water productivity in August (previous month), and bottom water DO. We then identified the model with the environmental variables that best explained variation in benthic fluxes using the "step-wise" selection procedure that employed 9999 permutations based on AICc selection

criterion. This criterion takes into account the number of variables added into the model and is well-suited for studies with low number of samples (Anderson et al., 2008).

In order to characterize within site heterogeneity or "patchiness" in OM variables (i.e., food quantity and quality), we calculated Euclidean distances (function "dist" in {stats} package) between samples from the same site and plotted them against depth. We could not attain homogeneity of variances, even after transformation, and therefore conducted a Kruskal-Wallis test in R with depth (levels: ≤ 800, >1500 m) as factor.

We evaluated the origin of OM within Barkley Canyon with a Principal Coordinates Analysis (PCoA: Anderson et al., 2008) from all the fatty acids using Primer 6. Our fatty acid data resembled a biological abundance data set because of numerous zeros, and we therefore based our resemblance matrix on Bray-Curtis similarity (Anderson et al., 2008).

3.3.5.2. Infaunal community

We investigated infaunal densities as well as diversity indices (species richness (S), Shannon-Wiener index, (H' log10), Simpson's diversity index (1-D), Pielou's evenness (J'), and expected number of species ($ES_{[10]}$)) per core, in order to describe community structure in space. We transformed density ($\sqrt{}$) to improve normality of the residuals and homogeneity of variance prior to a one-way analysis of variance (ANOVA) type (III) to account for an unbalanced design, with a site factor (levels: BC200, Barkley Hydrates, and MidWest). Barkley Hydrates site included the outcrop and 20 m sites. No transformation was required for the same analysis with S as response variable. For other biodiversity indices (i.e., H' log10, 1-D, J', $ES_{[10]}$), no transformation achieved assumptions of normality and homogeneity of variance, and we therefore analysed them with a (non-parametric) Kruskal-Wallis test. We performed a Tukey test

(parametric) or Dunn's test (non-parametric) whenever we found significant (P < 0.05) differences in one factor.

We used multivariate statistics to identify differences in benthic community structure with site (levels: BC200, Barkley Hydrates, MidWest). We log-transformed to account for zero-inflated data before plotting the results in nMDS (non-metric MultiDimensional Scaling) with PRIMER v6 (Clarke and Gorley, 2006). Because faunal data sets often include large number of zeros, Bray-Curtis similarity is an appropriate similarity measure (Clarke and Gorley, 2006). We tested for significant differences among sites with PERMANOVA performed with 9999 random permutations using the PERMANOVA+ add on to PRIMER v6 (Anderson et al., 2008). We verified homogeneity of multivariate dispersion using the PERMDISP routine (Anderson et al., 2008) and used a percent similarity procedure (SIMPER) analysis and nMDS vectors to identify the taxa that distinguished assemblages by site. In order to evaluate local differences in fauna, we performed the same PERMANOVA, PERMDISP, and SIMPER analysis at smaller spatial scales of 10's of meters instead of 100's of meters (Sites in BC200 levels: BC200-"urchin aggregation" and BC200-"no aggregation"). Because of the small number of unique permutations obtained (< 100) we report and based our interpretations on Monte Carlo p-value (Anderson et al., 2008).

We performed a step-wise distance-based linear model permutation test (DistLM: Anderson et al., 2008) to identify environmental variables that best predict multivariate variation in infaunal community structure. In order to correct for data skewedness, we first transformed the data (i.e., $\sqrt{}$ of each of hydrocarbon concentration, ARA, MGS, % mud, depth), and standardized the environmental matrix to make different variables inter-comparable (Clarke and Gorley, 2006). We then checked multi-collinearity of the predictor variables ("Draftsman's plots" Primer 6) prior to analysis (Clarke and Gorley, 2006), and removed strongly correlated ($r > 0.85$) variables.

We then identified the model with the environmental variables that best explained variation in benthic fluxes using the "step-wise" selection procedure that employed 9999 permutations based on AICc selection criterion. This criterion takes into account the number of variables added into the model and is well-suited for studies with low number of samples (Anderson et al., 2008). We obtained p-values using 9999 permutations of the raw data.

3.4. Results

3.4.1. Bathymetric patterns in organic matter (large spatial scale 100's of m)

Low amounts of TOC of low nutritional value characterized the axial sediments at the head of Barkley Canyon (BC200, BC300; see Fig. 3.2). Large amounts of TOC of high nutritional value, characterised the mid-canyon section (BC600, BC800), which was most evident at BC800 (Fig. 3.2). Finally, high amounts of TOC of lower nutritional value characterised the lower portion of the canyon (BC1500, BC2000; see Fig. 3.2). Therefore, total amounts of food (TOC) increased significantly with depth up to 800 m and then plateaued ($P < 0.05$, $R^2 = 0.88$; see Fig. 3.2). C:N, representing long-term food quality, decreased very quickly with depth until 600 m and then remained relatively constant until 2000 m ($P > 0.05$; see Fig. 3.2). Chl a:Phaeo ratios, representing short-term food quality, and total lipid concentrations, representing intermediate-term food quality, were highest at mid canyon depths, however, with large variability between sampling sites, and decreased again towards the deeper 1500 and 2000 m (Chl a:Phaeo: $P > 0.05$; total lipids: $P < 0.05$, $R^2 = 0.82$; see Fig. 3.2). Sediments at 200 m in Barkley Canyon differed markedly in OM quality between the "urchin aggregation" and "no aggregation" areas. We found lower percentages of carbon, but of higher nutritional value, at the "urchin aggregation" compared to "no aggregation" area (Fig. 3.2).
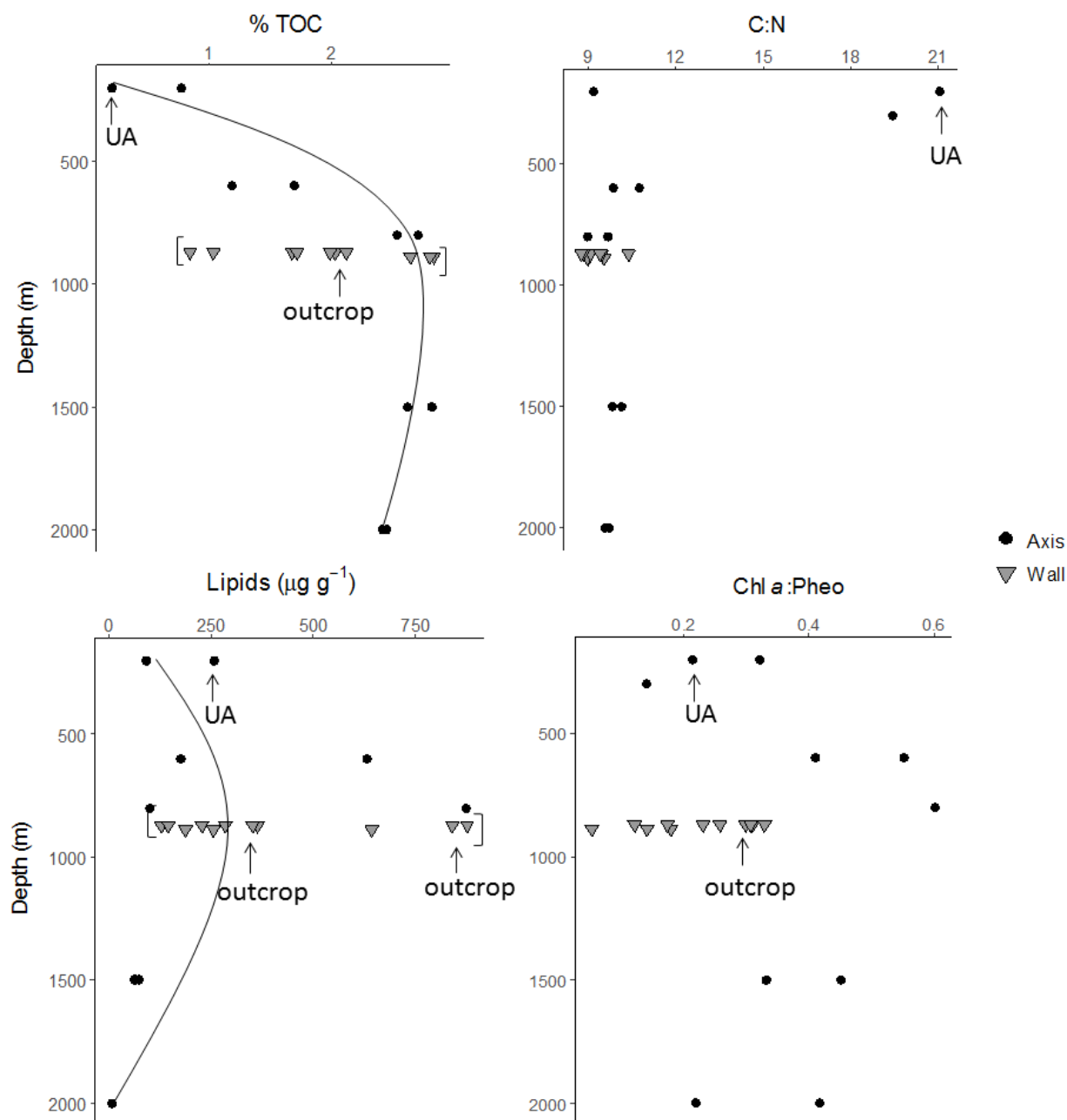
Figure 3.2. TOC, long-term food quality (C:N), intermediate-term food quality (total lipids), and short-term food quality (Chlorophyll *a*:Pheo) with depth, from Barkley Canyon sediment. Only statistically significant regression lines are shown: TOC: y = - 0.375 + 0.00451x - 1.57*10-6x2, R^2 = 0.88; Lipids: y = 4.59 + 0.00309x – 2.16*10-6x2; R^2 = 0.82. Data points excluded from the regression line (i.e., canyon wall samples) are plotted within brackets []. UA = "Urchin aggregation".

POM depth patterns within Barkley Canyon differed from that of SOM. TOC peaked at 800 and 1500 m (P > 0.05; see Fig. 3.3). The C:N ratio increased significantly with depth (P < 0.05, R^2=0.93), peaking at 1500 m (Fig. 3.3). Total lipids peaked at 400 m, because of increased phospholipids, and again at 800 m and 1500 m, because of increased wax esters/steryl esters (Fig. 3.3).
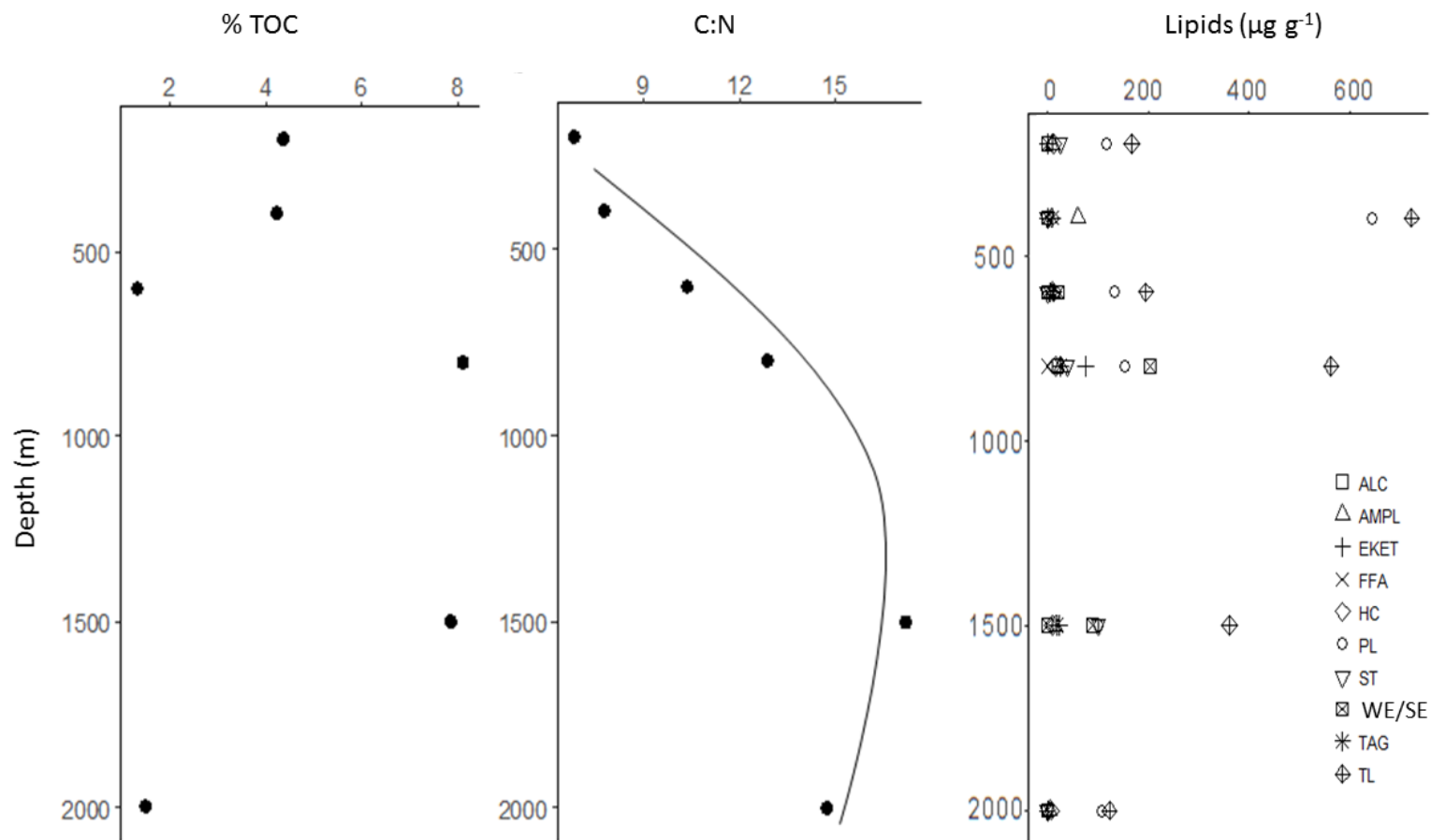
Figure 3.3. TOC, long-term food quality (C:N), and intermediate-term food quality (lipids), from bottom-water particulate organic matter of Barkley Canyon axis. Only statistically significant regression lines are shown: C:N: $y = 2.58 + 0\text{-}0173x - 5.49*10\text{-}6x2$, $R^2 = 0.96$. ALC= Alcohol, AMPL=Acetone mobile polar lipids, EKET=Ethyl Ketones, FFA= Free fatty acids, HC= Hydrocarbons, PL= Phospholipids, ST= Sterols, WE/SE = wax esters/steryl esters, TAG= Triacylglycerols, TL = Total lipids.

Different combinations of food quantity and food quality (POM and SOM) characterized different Barkley Canyon sites. The shallowest sites (200 m) exhibited the poorest food quantity and quality within the canyon, with low total organic carbon of low quality. In contrast, the deeper sites grouped together (1500-2000 m), with high food quantity but low quality. Intermediate depths (400-800 m sites) exhibited the freshest and highest concentrations of organic matter, and therefore, favourable conditions for consumers. Finally, a unique food source and high variability characterized the outcrop and 20 m sites (Fig. 3.2).

BC200 sites differentiated from the MidWest and deeper sites (i.e., BC1500, BC2000) because of lower amounts of TOC, higher $\delta^{13}C$, lower $\delta^{15}N$, and high C:N (Appendix 3.1). In general, the outcrop, 20 m site, and BC800 coincided with high TOC, depleted $\delta^{13}C$, enriched $\delta^{15}N$, and low C:N. The outcrop site differentiated from the other samples because of their high lipid content (Appendix 3.1).

3.4.2. Environmental drivers of organic matter quantity and quality (large spatial scale: 100's of m)

The PCoA reduced the environmental features that describe Barkley Canyon sites to 2 principal coordinates (PCO) axes (71.6 % of the total variance explained; see Appendix 3.2). BC200 was characterized by high SPP, high % mud, MGS, and therefore greater sediment heterogeneity, higher temperature, and lower depth and lower salinity (Appendix 3.2). In contrast, high salinity and low SPP, % mud, MGS, temperature characterized the deeper sites (i.e., BC1500, BC2000; see Appendix 3.2). MidWest, Barkley Hydrates (i.e., outcrop and 20 m) and 800 m sites differed from others because of their low DO (OMZ) and low FBPI (convex areas; see Appendix 3.2). A more irregular topography characterized the upper canyon ($\leq$ 800 m), with steeper slopes, changing aspect, BPI, and curvature and increased rugosity (Appendix 3.3).

The best distance-base linear model (distLM) explained 58.08 % of the sedimentary organic matter in Barkley Canyon (Fig. 3.4). SPP in the previous month (20.4 %), fine-scale BPI (12.6 %), % mud (13 %) and MGS (11.9 %) were the strongest environmental predictors in the model ($P < 0.05$; percent variability explained in brackets). The first axis of distLM accounted for 24.4 % of variation, whereas the second explained 27.8 %. The first axis separated BC200 and BC800 from each other and from other sites. Furthermore, the first axis separated two subsampled areas in BC200 (i.e., "urchin aggregation" and "no aggregation"). MGS, % mud, and fine-scale BPI were the primary contributors to the first axis. The second axis separated BC800 and BC200 from other sites, primarily driven by high SPP in the month prior to sampling (August).
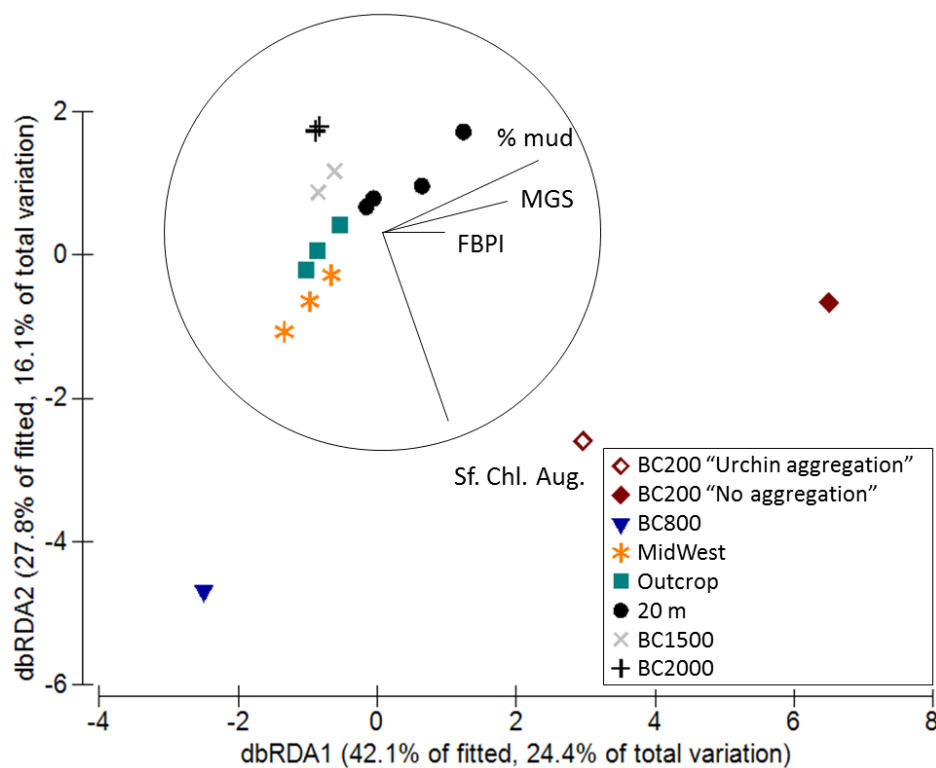
Figure 3.4. Distance-based redundancy analysis (dbRDA) based on the distance-based Linear Model (distLM) from Barkley Canyon sedimentary organic matter with predictor variables. Resemblance matrix based on euclidean distances. MGS= Mean Grain Size, FBPI = Fine-scale BPI, sf. Chl. Aug. = surface water chlorophyll in August 2013.

3.4.3. Organic matter patchiness (small spatial scales: 10's of m)

Euclidean distances between OM samples from a given site in Barkley Canyon demonstrate large within-site variability or patchiness. Shallow sites ($\leq$ 800 m) were more heterogeneous than deeper sites ($\leq$ 1500 m) (Kruskal-Wallis, $\chi^2(1) = 5.513$, P = 0.0189; see Fig. 3.5).
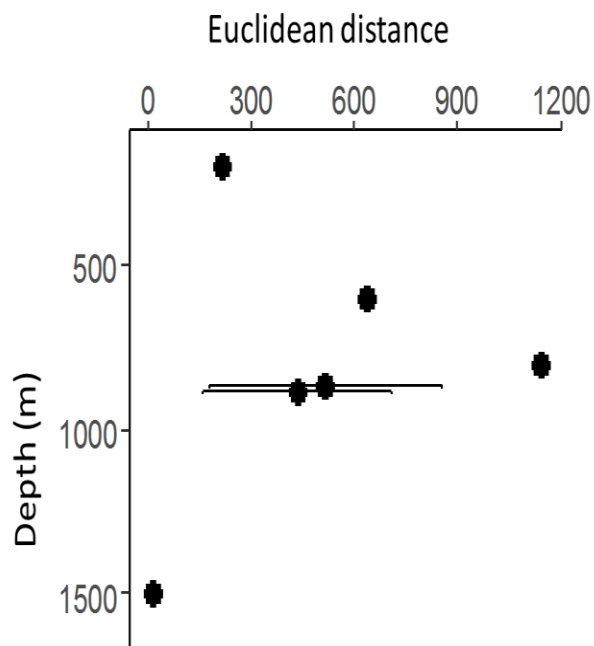
Figure 3.5. Within site Euclidean distances in organic matter samples of Barkley Canyon.

Fatty acid composition changed along Barkley Canyon in POM and SOM (See Appendix 3.4 for fatty acid names and biomarker nature). The first two axes of PCoA (Fig. 3.6) explained 83.77 % of variability of POM in Barkley Canyon. PCO1 explained 65.2 % of variability and separated BC2000 from other sites. PCO2 explained 18.6 % of variability and differentiated BC800. Bacterial (i17:0, 17:0, 18:1ω6) and other (18:3ω3, TMTD, 16:0, 18:0) markers characterized BC2000. Zooplankton (20:1ω4), diatom (18:4ω1, 16:4ω1, 16:3ω4? – tentatively identified), and other (14:0) markers separated BC800. EPA (20:5ω3), an essential fatty acid, separated the remaining sites (BC200, 600, 1500). The first two axes of the PCoA for the sediment samples

explained 86.8 % of variability (Fig. 3.6). PCO1 explained 78.7 % of the variability and separated the outcrop and 20 m sites from the other sites. PCO2 separated BC200-"urchin aggregation", and explained 8 % of total variability. The presence of 18:3ω6, 20:3ω6, 16:1ω5, 16:3ω4?, and 14:1 separated the outcrop and 20 m sites from the other sites. Zooplankton markers (22:1ω11(13), 20:1ω9) separated the "urchin aggregation". Bacterial markers (iso-, anteiso-, odd-chain fatty acids) separated the remaining sites. The first two PCoA axes for canyon axis sediment samples (i.e., excluding the canyon wall samples: MidWest, outcrop and 20 m sites; see Fig. 3.6) explained 59.2 % of between site variability and separated BC200-"Urchin aggregation" with zooplankton markers such as 22:1ω11(13), 20:1ω9, 22:1ω9, and others such as 18:1ω9, which can occur in crustaceans and other animals. EPA, DHA, and ARA, all of which are EFAs, separated BC800.
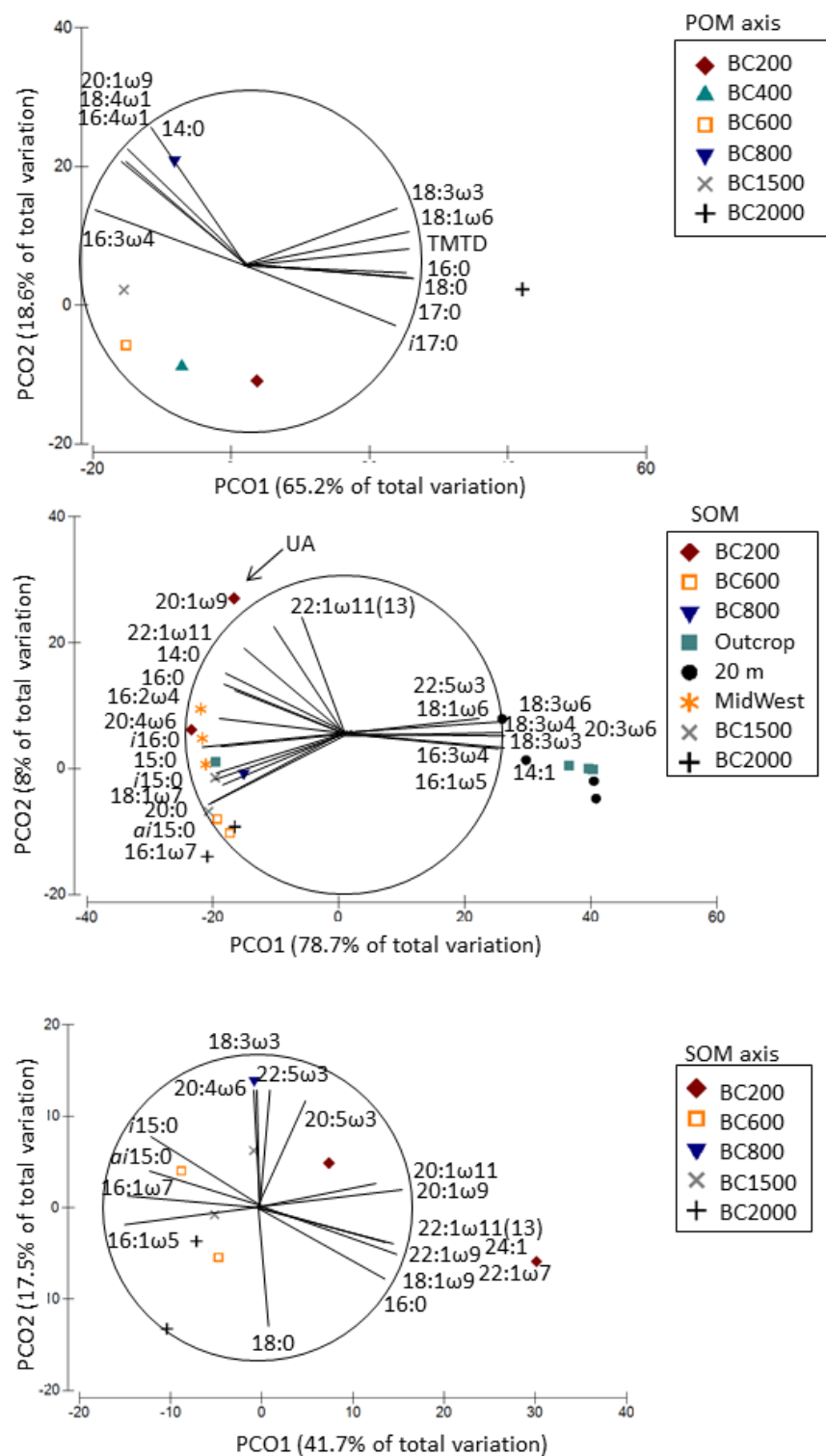
Figure 3.6. Principal coordinate analysis (PCoA) from Barkley Canyon fatty acids (%) within bottom water (POM), sediments (SOM), and sediments collected in the axis of the canyon only (SOM axis). Refer to appendix 3.4 for fatty acid names and biomarker nature.

Essential fatty acids (ARA, EPA and DHA) were more abundant in POM than SOM (Appendix 3.5). Within the canyon axis (i.e., excluding "urchin aggregation", outcrop, 20 m., and MidWest samples) all EFAs decreased significantly with depth (Appendix 3.5). A wide range of values occurred within the outcrop, 20 m, and MidWest samples for all EFAs and lower values characterized the "urchin aggregation" compared to "no aggregation" areas (Appendix 3.5). Although we found no significant patterns with depth for any EFA in POM samples, values generally decreased with depth (Appendix 3.5). Again we found no significant patterns with depth beyond an overall non-significant decreasing trend in DHA:EPA ratio (Appendix 3.6).

3.4.4. Infaunal community

In total we identified 750 individuals belonging to 59 different families from 26 cores collected at 3 different sites (BC200, Barkley Hydrates, and MidWest). We found the highest densities at BC200, which was more than twice the densities of infaunal communities at Barkley Hydrates (i.e., outcrop and 20 m sites), which were themselves twice the infaunal densities at the MidWest site (Table. 3.2). We also identified significant differences among sites in all biodiversity indices except J', with higher values of all diversity variables at BC200 compared to Barkley Hydrates and the MidWest samples (Table 3.3).

Table 3.2. Density and diversity variables from macrofaunal (> 300 μm) communities within Barkley Canyon sediments (± standard deviation). Species richness (S), Shannon-Wiener index, (H' log10), Simpson's diversity index (1-D), Pielou's evenness (J'), and expected number of species ($ES_{[10]}$).

Site	Density (ind./100 cm^2)	S	H'log10	1-D	J'	$ES_{[10]}$
BC200	154.78 ± 63.97	16.71 ± 3.73	1.07 ± 0.08	0.89 ± 0.02	0.38 ± 0.02	7.01 ± 0.39
Barkley Hydrates	67.13 ± 48.91	7.33 ± 3.08	0.66 ± 0.23	0.70 ± 0.17	0.35 ± 0.06	4.92 ± 1.79
MidWest	34.04 ± 17.33	5.57 ±1.40	0.68 ± 0.13	0.76 ± 0.08	0.40 ± 0.03	5.27 ± 1.49

Table 3.3. Statistical results of ANOVA type III (parametric) or Kruskal-Wallis (non-parametric), with subsequent post hoc tests, of density and diversity variables from macrofaunal (> 300 μm) communities within Barkley Canyon sediments (± standard deviation). One factor: Site [levels: BC200, Barkley Hydrates (BH), MidWest]. Species richness (S), Shannon-Wiener index, (H' log10), Simpson's diversity index (1-D), Pielou's evenness (J'), and expected number of species ($ES_{[10]}$)].

Variable	Test	df	F /χ2	P	Post-hoc test
N	ANOVA type III (Tukey HSD)	2,23	11.60	<0.001	P < 0.05, BC200 > BH, MidWest
S	ANOVA type III (Tukey HSD)	2,23	30.41	<0.001	P < 0.05, BC200 > BH, MidWest
H'log10	Kruskal – Wallis (Dunn)	2	14.36	0.001	P < 0.05, BC200 > BH, MidWest
1-D	Kruskal – Wallis (Dunn)	2	13.66	0.001	P < 0.05, BC200 > BH, MidWest
J'	Kruskal – Wallis (Dunn)	2	5.01	0.082	---
$ES_{[10]}$	Kruskal – Wallis (Dunn)	2	7.60	0.022	P < 0.05, BC200 > BH, MidWest

3.4.4.1 Broad spatial scale (100's of meters)

We found distinct infaunal communities based on families, at our three study sites (i.e., BC200, Barkley Hydrates, and MidWest), which separated on a two-dimensional nMDS (stress: 0.14; see Fig. 3.8). PERMANOVA showed significant differences in benthic community assemblages between sampling sites (Table 3.4). Pair-wise comparisons identified significant difference in benthic communities from all sites (Table 3.4). Within-site variability, however, also differed among sites (PERMDISP, P(perm) = 0.021), with greater dispersal in the Barkley Hydrates data cloud in the ordination space than BC200 or MidWest (P(perm) < 0.001). Therefore, the significant differences detected by PERMANOVA analysis between Barkley Hydrates and the other sites related not only to a difference in location in the ordination space but also to dispersion of the data points (Anderson et al., 2008; see Fig. 3.8). Annelida was the most diverse and abundant animal class at all sites. Vectors from nMDS distinguished BC200 from the other sites based on the presence of spionid, oweniid, syllid, cirratulid and capitellid polychaetes, and ingolfiellid amphipods which were mostly absent or rare at the other two sites. Barkley Hydrates was characterized by the presence of oligochaetes (F. Naididae), ampharetid polychaetes, and thyasirid bivalves. The high abundances of cirratulid polychaetes at BC200 compared to those at Barkley Hydrates further differentiated the two sites (SIMPER). Finally, paraonid and cossurid polychaetes characterized the MidWest site (SIMPER).
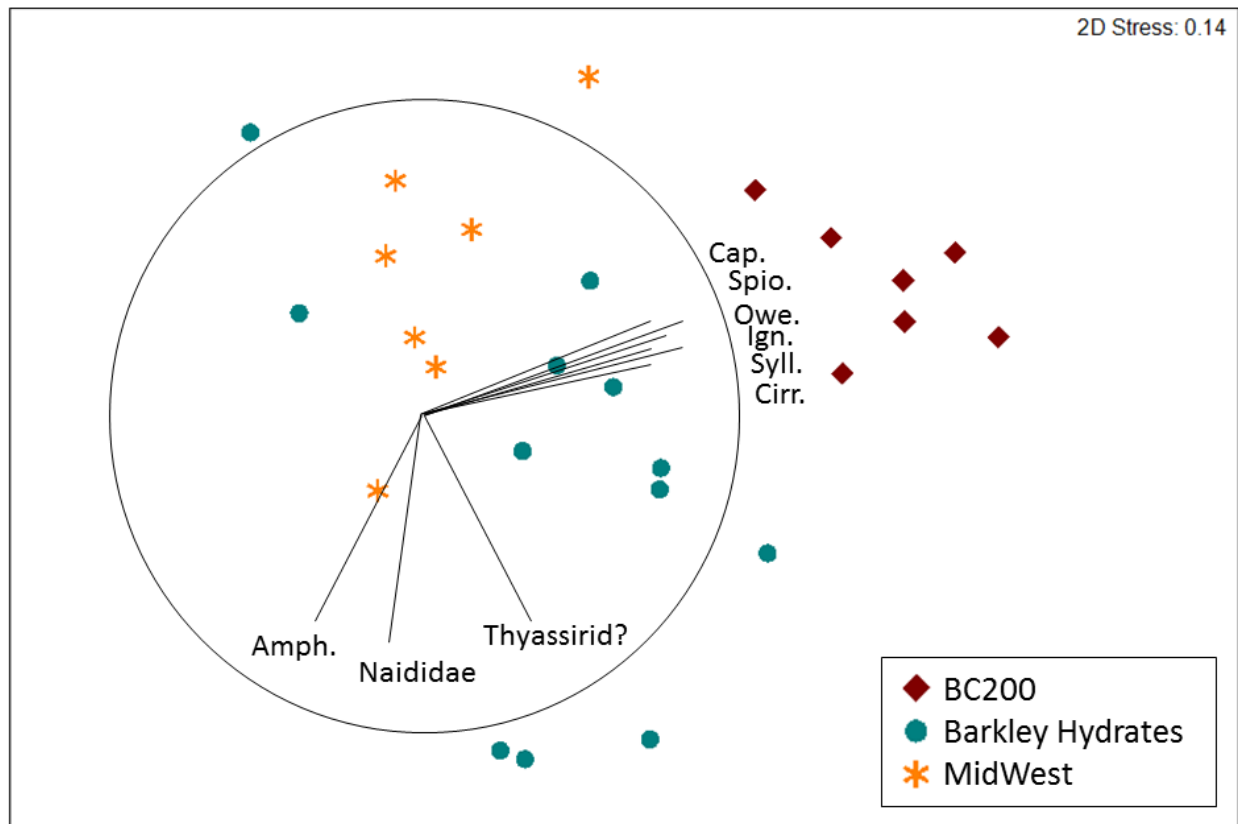
Figure 3.7. nMDS from Barkley Canyon infaunal communities. Resemblance matrix based on Bray-Curtis similarity from log(X+1) transformed data. Vectors shown at 0.5 Pearson correlation threshold. Amph. = Ampharetidae; Cap. = Capitellidae; Spio. = Spionidae; Owe. = Oweniidae; Ign. = Ingolfiellidea; Syll. = Syllidae; Cirr. = Cirratulidae.

Table 3.4. Statistical results of PERMANOVA main test, with consequent pairwise tests, of macrofaunal (> 300 μm) community structure within the sediments of Barkley Canyon.

Variable	df	SS	MS	Pseudo-F	P	Unique perms.	Pairwise test
Site	2	23843	11921	6.2987	< 0.001	9920	BC200 ǂ BH ǂ MidWest
Res	23	43531	1892.7				

DistLM analysis identified DO, hydrocarbon, and FA A (an undetermined fatty acid that persistently occurred in Barkley Hydrates samples) as significant predictor variables of Barkley Canyon infaunal communities. The best distance-base linear model (distLM) explained 50.86 % of family composition. DO, hydrocarbon, and FA A concentrations were the strongest

130

environmental predictors in the model (Table 3.5). The first axis of the dbRDA separated the BC200 samples from the other two sites, with DO as the major explanatory variables. The second axis separated Barkley Hydrates samples from the other site with hydrocarbon and FA A concentration as the primary explanatory variable (Fig. 3.9).

Table 3.5. Statistical results of DistLM method, of macrofaunal (> 300 μm) community structure within the sediments of Barkley Canyon with predictor variables.

Variable	AICc	SS (trace)	Pseudo-F	P	Prop. (%)	Cumul. (%)	Res. Df
+DO	200.97	17680	8.54	< 0.001	26.24	26.24	24
+HC	196.16	12278	7.55	< 0.001	18.22	44.46	23
+FA A	195.79	4314	2.87	0.004	6.40	50.87	22
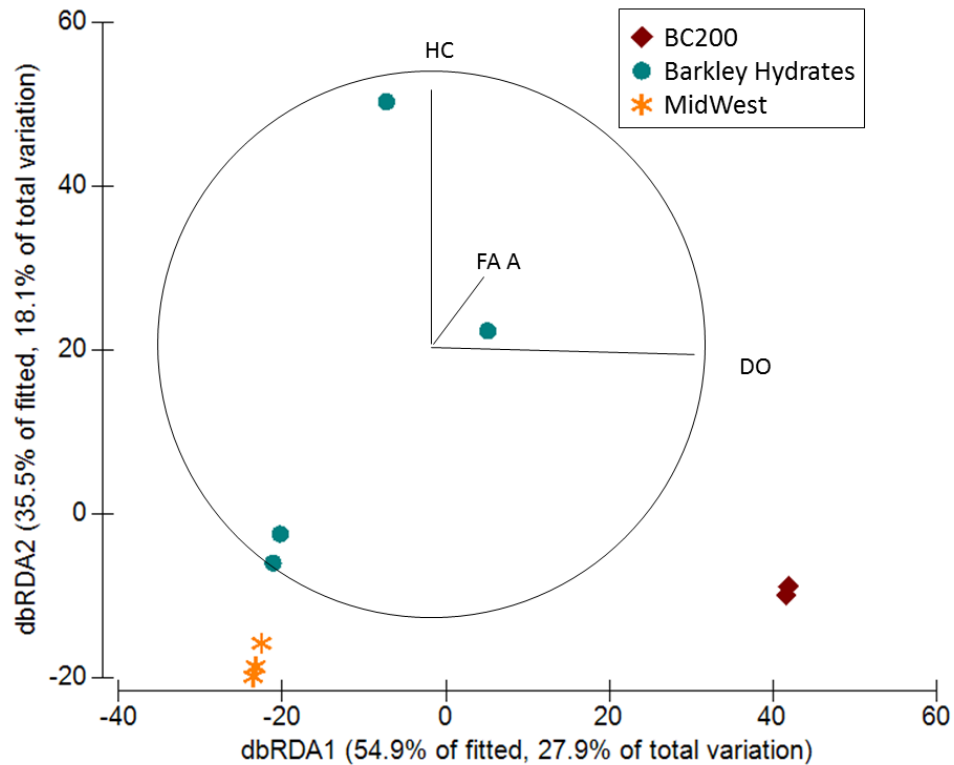
Figure 3.8. Distance-based redundancy analysis (dbRDA) based on the distance-based Linear Model (distLM) from Barkley Canyon macrofaunal communities (> 300 μm) with predictor variables. Resemblance matrix based on Bray-Curtis similarity of log (X+1) transformed data. HC= Hydrocarbon, DO = Dissolved oxygen concentrations, FA = Fatty acid.

3.4.4.2. Smaller spatial scale (10's of meters)

We found distinct infaunal communities, based on families, at the two BC200 sites (i.e., BC200-"urchin aggregation" and BC200-"no aggregation"), which separated in a 2D nMDS (stress: 0.03; see Fig. 3.10). PERMANOVA showed significant differences in benthic community assemblages between sampling sites (Table 3.6). Within-site variability did not differ among sites (PERMDISP, P(perm) = 0.458). Therefore, the significant differences detected by PERMANOVA analysis between the two sites reflected differences in location within the ordination space only (Fig. 3.10: Anderson et al., 2008). Annelida was the most diverse and abundant animal class at all sites. Cirratulid, oweniid, capitellid, and syllid polychaetes characterized the assemblages in BC200 "urchin aggregation", whereas cirratulid, syllid, maldanid, and oweniid polychaetes, and ingolfiellid amphipods characterized BC200 "no aggregation" (SIMPER). Vectors from nMDS distinguished BC200-"no aggregation" from BC200-"urchin aggregation" by the presence of sipunculid, trochochaetid, and fauvelopsid polychaetes, and SIMPER analysis separated the two sites based on the presence of maldanid, fauvelopsid, sipunculid, trochochaetid, and ingolfiellid amphipods in BC200- "no aggregation", which were absent at BC200-"urchin aggregation". Syllids and spionids were also more abundant in BC200- "no aggregation" whereas paraonids, capitellids and polychaetes recent recruits were more abundant in BC200-"urchin aggregation" (SIMPER).
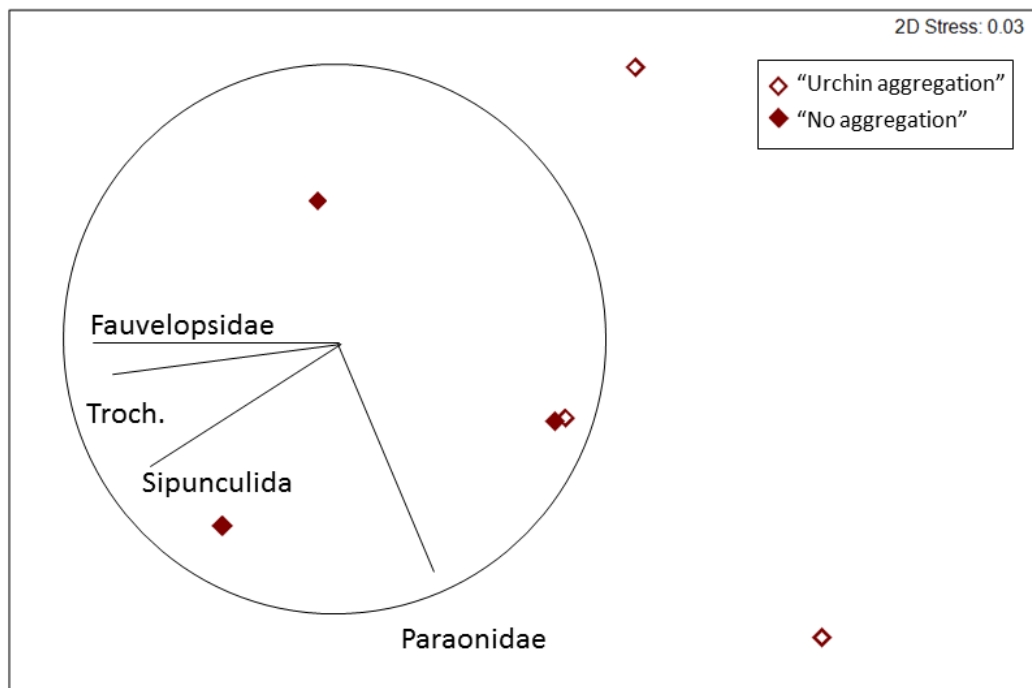
Figure 3.9. nMDS from Barkley Canyon infaunal communities. Resemblance matrix based on Bray-Curtis similarity from log(X+1) transformed data. Vectors shown at 0.8 Pearson correlation threshold. Troch. = Trochochaetidae.

Table 3.6. Statistical results of PERMANOVA main test of macrofauna (> 300 μm) community structure within the sediments of Barkley Canyon at 200 m depth. P(MC) = Monte Carlo P-value.

Variable	df	SS	MS	Pseudo-F	P (perm)	P(MC)	Unique perms.
Site	1	2581.6	2581.6	2.967	0.0289	0.0492	35
Res	5	4350.5	870.1				

3.5. Discussion

3.5.1. Food availability at large spatial scales (100's m): bathymetric patterns and sources

3.5.1.1. Bathymetric patterns along the canyon axis

Concentrations of various biochemical components within particulate (POM) and sediment organic matter (SOM) showed different bathymetric patterns in Barkley Canyon relating to sediment transport processes and water mass circulation. Degradation rates vary consistently among organic compounds (Cowie and Hedges, 1994, Veuger and van Oevelen, 2011, Veuger et al., 2012), which can explain the decoupling between food quantity and quality variables in Barkley Canyon, and sediments and bottom-water particles. Furthermore, these differences in degradation rates can help reconstruct a timeline of organic matter distribution within the canyon: biochemical compounds with higher degradation rates (e.g. chlorophyll *a*) indicate recent arrival whereas high amounts of TOC and the absence of fresh pigments or lipids indicate accumulation of organic material over time.

The bottom sediments at the shallowest site sampled in Barkley Canyon (i.e., 200 m) were the poorest in quantity and quality of organic matter, with markedly lower proportions of the diatom marker (EPA) compared to deeper sites. Previous studies demonstrated strong hydrodynamics at the head of Barkley Canyon (150-350 m) during up- and downwelling events, with measured bottom currents of up to 30 cm s^{-1} (Allen et al., 2001, Connolly and Hickey, 2014), which may result in entrained OM within the currents without deposition onto the sediments. Additionally, frequent storm- and along-shelf current-induced resuspension events over the shelf generate strong bottom and intermediate nepheloid layer-associated flows, laden with sediment and organic aggregates (Baker and Hickey, 1986, Carson et al., 1986, Hickey et al., 1986). Therefore, these two distinct processes combined may explain the absence of labile organic matter signals in

Barkley Canyon head sediments, but their presence in the particulate fraction of the near bottom water mass. Furthermore, the presence of coarse sediments at the shallower canyon head sites provides additional evidence for strong physical dynamism in the area (Baker and Hickey, 1986, Van Rijn, 1993). Some OM variables relate to sediment grain size (Longbottom, 1970), which is one of the primary environmental drivers of the OM distribution in our study (distLM results). Similar results were reported by Pedrosa-Pàmies et al. (2013), with lower TOC and coarser sediment grain size (up to 2500 μm) observed at the head of Blanes Canyon (NW Mediterranean) compared to depositional areas further down the canyon where much finer sediment (<4 μm) occurred.

Phospholipids made up much of the increased total lipids in bottom waters at 400 m depth. Phospholipids are indicative of living (bacteria, phytoplankton or zooplankton), or recently living, organisms given their essential structural role in cell membranes and labile nature (Parrish, 2009), and therefore, suggest fresh organic matter rather than re-suspension of sediments at that site. The slight increase in acetone-mobile polar lipids (AMPL) at 400 m POM, together with the presence of EPA (diatom biomarker), and the bright green colour of the lipid extraction compared to other sample extractions (authors' pers. obs.), suggest a phytoplankton origin. Because this site yielded the freshest organic matter of all our samples, we consider the bottom waters at 400 m as the canyon site where organic matter produced in surface waters first accumulates. We hypothesise that complex circulation at the canyon head triggered by upwelling and downwelling flows as well as by storm-induced re-suspension events concentrate fresh organic matter from productive surface waters down to 400 m bottom waters. Strong north-westerly, upwelling-favourable winds characterize non-"El Niño" summers off Vancouver Island (Allen et al., 2001). During upwelling, topographic steering of the along-shore upper flow by the

canyon rim (~ 150 m) triggers vertical vorticity, causing strong currents to flow upwards and towards the shore (Allen et al., 2001). Tidal movements may further concentrate the fresh POM at 400 m (Puig et al., 2014), which is likely phytoplanktonic in origin.

The fresh organic material in bottom waters at 400 m depth is not reflected in sediments at 300 m but does occur in sediments at 600-800 m, as indicated by high chlorophyll a:Pheo and total lipids (though variable). Increased phospholipids drove the peak in total lipids at 800 m, once again indicating the presence of living, or recently living, organisms. Biomarkers for protozoa and microeukaryotes, red algae, and kelp (20:4ω6), diatoms (20:5ω3), and dinoflagellates (22:6ω3) (Parrish, 2013) were also present at that site, likely indicating phytoplanktonic origin. Along with low POM total lipids, these results suggest deposition of fresh OM from 400 m POM in sediments at 600-800 m. Canyon head hydrodynamics likely limit OM deposition at 200 – 400 m depths, and result in deposition further down the canyon at 600-800 m. Additionally, the high C:N ratios at 800 m POM may have resulted from re-suspended material from shallower sites (BC200-BC300/400) where C:N ratios were highest. Other studies document sediment gravity flows within submarine canyons, which account for a portion of fresh OM deposited at Whittard Canyon (Amaro et al., 2015). This pattern suggests that a combination of processes such as internal tides, sinking OM from surface waters, and gravity flows may act in concern to transport OM down the canyon (De Stigter et al., 2007, Puig et al., 2014, Amaro et al., 2015). The topography of the canyon may contribute to the accumulation of fresh organic matter at 800 m. FBPI, one of the main drivers of OM distribution in Barkley Canyon, identified concave and convex seafloor areas and, therefore, areas of resuspension versus accumulation. Amaro et al. (2016) reported a general increase in TOC and TN in sediments towards the deepest parts of Whittard Canyon (~ 4000 m) west of the Bay of Biscay, where the branches merge to form

Whittard Channel. The increase in total sediment OM at 800 m in Barkley Canyon coincided with the point where the canyon branches merge, suggesting accumulation of OM transported through multiple canyon branches.

The high amounts (TOC) of low quality food within the sediments at 1500-2000 m in the canyon axis suggest an accumulation of OM and degradation or consumption of the more labile fraction (i.e., pigments and lipids). Previous studies point to surface primary production as one of the primary factors controlling biopolymeric carbon within canyons in the Atlantic and Eastern and Western Mediterranean Sea (Danovaro et al., 1999, Pusceddu et al., 2010). The decrease in the fresher fraction of SOM (i.e., Chlorophyll a:TOC) in Barkley Canyon at the deeper sites (i.e., 1500 and 2000 m) may reflect a combination of increased residence times in the water column and, therefore, more time for degradation, and decreased primary production towards the open ocean. The strong decrease in C:N and increase in TOC may relate to particle size. Deeper in the canyon, sediments become finer and the increased surface area per unit mass can adsorb more particles, thereby increasing TOC (Keil et al., 1994). Furthermore, increased nitrogen content associated with finer clay particles (Keil et al., 1994) commonly found in sediments from greater depth, explains the decrease in C:N.

3.5.1.2. Organic matter composition at the wall of the canyon

Some studies show greater accumulation of OM in canyon axes than walls (Garcia and Thomsen, 2008), which is true for some of our non-axis samples (i.e., outcrop, 20 m, and MidWest sites) for which concentrations of food-related variables in wall samples (i.e., ~ 800 m) were similar to or lower than in axis samples at the same depth. The canyon wall samples were highly heterogeneous because they encompassed chemosynthetic and non-chemosynthetic

environments (Campanyà-Llovet and Snelgrove, 2018a, Campanyà-Llovet and Snelgrove, 2018b).

3.5.1.3. Comparison of organic matter composition between sediments and bottom waters

Our results show a noticeable decoupling between amounts of food in SOM and POM and its overall quality. At the deepest parts of the canyon ($\geq$ 1500 m) food quantity remained high, whereas quality decreased. In POM, highest quantities of TOC appeared at 800-1500 m, in contrast to highest nutritional value at shallower depths (200-600 m), which also points to decoupling, in this case between SOM and POM. SOM reflects longer-term whereas POM shorter-term processes. For example, a substantial increase in total lipids coinciding with wax esters/steryl esters at 800-1500 m POM, suggests zooplankton input that was not evident in SOM. Wax esters in the combined wax ester/steryl ester Iatroscan peak reflect zooplankton energy stores (Lee et al., 1971). This result corroborates mass overwintering migrations of *Neocalanus* spp. (Copepoda Calanoidea) recently observed by means of seafloor video cameras from the NEPTUNE cabled observatory at the same location as our sampling (De Leo et al., 2018). The presence of overwintering zooplankton at 800 and 1500 m depth, suggested by fatty acid markers, and corroborated by data from NEPTUNE, coincides with decreased quality in POM that may result from heterotrophic reworking by zooplankton (Kiriakoulakis et al., 2011). Additionally, short-term variability in water mass circulation, such as upwelling and downwelling flows and re-suspension events (discussed above), may explain the higher variability within OM composition in the POM pool relative to the SOM pool. Discrepancies in several OM variables between POM and SOM also occur in shallow-water systems, with consistently older OM in sediments than in bottom waters (Le Guitton et al., 2015). Fatty acids corroborate the differences between POM and SOM. Higher proportions of polyunsaturated fatty

acids (PUFA) and lower monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA) characterized POM, whereas SOM exhibited the reverse trend. These results suggest an efficient utilization of PUFA within the water column by bacteria and zooplankton or within sediments by benthic invertebrates (Copeman and Parrish, 2003). Bacterial markers were higher in SOM than POM as reported in previous studies in Gilbert Bay, E Canada (~ 5 % POM, ~ 15 % SOM: Copeman and Parrish, 2003). However, some bacterial markers increased substantially in the outcrop and 20 m sites (25-30 % of total FA), presumably because of the presence of chemosynthetic bacteria. Essential ω3 PUFA were also substantially higher in POM (~ 20 %) than SOM (~ 5 %), with high values in the sediments of the MidWest samples (5-10 %).

3.5.2. Food availability at small spatial scales (10's of m): Patchiness and sources

Fatty acid biomarkers indicated different patches from varying food sources along the canyon. The presence of zooplankton and copepod biomarkers characterized the "urchin aggregation" at 200 m. The δ^{13}C and δ^{15}N signatures of SOM at those sites, together with C:N, corroborate a different origin for SOM at the two sites. The two samples from BC600 exhibited high variability in all of the OM compounds we measured. Fatty acid composition in one of the samples suggested bacterial markers (18:1ω7, ai15:0, and also 16:1ω7, although the latter is not exclusive to bacteria) that were not present in the other sample. Several chemosynthetic bacteria and algal markers clearly separated the outcrop and 20 m sites from the other sediment samples: 18:3ω6 (green macroalgae), 18:1ω6(?) (methanotrophic/sulphur oxidizing bacteria), 20:3ω6, 16:1ω5 (methanotrophic bacteria), 16:3ω4? (Diatoms), and 14:1 (proteobacteria) (Kelly and Scheibling, 2012, Parrish, 2013). The abundant hydrocarbons in sediment samples from the outcrop and 20 m sites (data not shown), confirm the presence of methane hydrates in the area. Finally, other bacterial markers (i.e., iso-, anteiso-, and odd chain fatty acids) characterized the

sediments from other Barkley Canyon sites (BC600, MidWest, BC1500, and BC2000). These patterns underscore the importance of heterogeneous OM (i.e., chemosynthetic, bacterial, zooplankton or phytodetritus), not only among Barkley Canyon sites but also within sites.

3.5.3. Implications for benthic communities at different spatial scales

Despite the overall importance of OM in driving benthic community structure, the effects of bottom water dissolved oxygen levels overrode the influence of food quantity and quality on taxonomic composition, abundance, and biodiversity patterns. Dissolved oxygen explained differences between BC200 infaunal communities shallower than the OMZ (1 ml O_2/l), and the deeper sites closer to the OMZ's core (0.17-0.18 ml O_2/l). Densities decreased considerably from BC200 to the Barkley Hydrates (i.e., outcrop and 20 m) and MidWest sites (56.63 % and 77.50 %, respectively), a finding consistent with previous reports on macrofaunal patterns in OMZs (Levin, 2002). Macrofaunal biodiversity frequently declines within OMZs, a generalization consistent with our results (S, H'log10, 1-D, $ES_{[10]}$), whereas dominance increases (Levin, 2002), a pattern inconsistent with our study (i.e., no significant difference in J' between sites). Despite considerably reduced overall number of families per core in OMZ sites, more than one annelid family nonetheless dominated the MidWest site (i.e., Cossuridae, Paraonidae, and Ampharetidae), and Barkley Hydrate sites (i.e., outcrop and 20 m sites) (i.e., Naididae, Dorvilleidae, and Ampharetidae). Naididae and Cossuridae commonly occur in low oxygen environments, whereas previous studies report Paraonidae and Ampharetidae within OMZs but also in well-oxygenated bathyal environments (Levin, 2002), suggesting tolerance of a wide spectrum of oxygen concentrations. The biology of the species we found within the OMZ suggest that, despite a strong correlation between depth and dissolved oxygen (DO) concentrations (> 0.9), DO itself plays an important role in determining species composition.

Of the environmental variables we measured, hydrocarbon concentrations contributed considerably to Barkley Canyon infaunal community structure. Hydrocarbons are indicative of seepage and clearly distinguished sediments from Barkley Hydrate sites, supporting the use of hydrocarbons as surrogates for specific habitats (i.e., methane hydrates) with food sources distinct from adjacent habitats. Habitat type, an important explanatory variable of benthic community structure at different spatial scales (Tews et al., 2004, Buhl-Mortensen et al., 2010, Wagner et al., 2013), has clear applicability to submarine canyons where habitat heterogeneity can be an important predictor of biodiversity (De Leo et al., 2014).

At spatial scales of hundreds of meters, DO and strong food and habitat changes such as chemosynthetically derived food in methane hydrates, were the strongest explanatory variables; but at smaller scales (10's of meters), and therefore, comparable DO and depth, sediment characteristics (i.e., food quantity, quality, and grain size) defined distinct habitats that help explain spatial distribution of species. At BC200 m remarkably different sediment characteristics (i.e., food quantity, quality, MGS) distinguished the "urchin aggregation" from surrounding habitats. Low amounts of TOC, EFAs, and Chl *a*:Pheo suggest poor nutritional value where urchins aggregate, whereas, low C:N, high total lipids, and higher proportion of Chl *a*:TOC ratios in the same area suggest better nutritional value. Furthermore, sediments rich in zooplankton fatty acid biomarkers in the "urchin aggregation", suggest food selectivity and some degree of selective feeding by sea urchins. Previous studies report *Strongylocentrotus fragilis* aggregations in Barkley Canyon (De Leo et al., 2017) and between 100 and 500 m offshore of California (Sato et al., 2017). These aggregations influence depth patterns in total megafaunal density (De Leo et al., 2017). *S. fragilis* aggregates on kelp falls on the California shelf and slope (Sato et al., 2017), much as we observed at ~ 400 m depth in Barkley Canyon (author's pers.

obs.). *S. fragilis* may aggregate to feed selectively on food differing in nutritional value. Previous studies report selective feeding in echinoderms (Wigham et al., 2003, FitzGeorge-Balfour et al., 2010, Boon and Duineveld, 2012). The two distinct sediment patches at the BC200 site coincide with two distinct infaunal communities, which may result from different food inputs, from *S. fragilis* feeding and reworking activities, or both. Megafauna can rework the sediments, changing their biochemistry. *Molpadia musculus* (Holothuroidea) produce such a change by digesting proteins more efficiently than carbohydrates or lipids (Amaro et al., 2010). Our results suggest that the drivers of infaunal community structure in Barkley Canyon vary with spatial scale, with dissolved oxygen concentrations and habitat type driving communities at 100's of meters but greater relevance for other variables, such as food quality and quantity and mean grain size, at spatial scales of 10's of meters.

3.6. Conclusions

The different degradation rates and processes that influence different OM measures and SOM and POM help in reconstructing OM time-lines of OM arrival and deposition within submarine canyons, and may contribute to variation in benthic community structure at different temporal and spatial scales. Within Barkley Canyon we observed food heterogeneity at broad scales (100's of m) resulting from differences in SPP, MGS, and % mud (which determine the adsorption of OM molecules to sediment grains), topographic features that influence where OM accumulates, and food patchiness at small scales (10's of m) related to the presence of distinct food sources (i.e., chemosynthetic, zooplankton rich) and possibly MGS. At broad scales (100's of meters), DO was the primary explanatory variable for infaunal communities, along with chemosynthetic food and habitat, whereas at smaller scales (10's of m) OM characteristics appeared to drive differences in community structure. Furthermore, although we could not document a statistical

difference, the striking contrast in OM characteristics from the "urchin aggregation" and 40 m away coincided with major differences in infaunal communities. Food patches can originate from differences in OM delivery related to environmental variables at broader and smaller spatial scales, or from re-working of SOM by benthic fauna at smaller spatial scales. Finally, the greater patchiness at shallower sites of canyons could contribute to higher diversity as well as diversity differences between shallow and deep areas of the canyons, consistent with a patch mosaic model of deep-sea diversity.

3.7. Acknowledgements

3.8. References

Allen, S., Vindeirinho, C., Thomson, R., Foreman, M.G., Mackas, D. 2001. Physical and biological processes over a submarine canyon during an upwelling event. Canadian Journal of Fisheries and Aquatic Sciences, 58, 671-684.

Amaro, T., Bianchelli, S., Billett, D., Cunha, M., Pusceddu, A., Danovaro, R. 2010. The trophic biology of the holothurian *Molpadia musculus*: implications for organic matter cycling and ecosystem functioning in a deep submarine canyon. Biogeosciences, 7, 2419.

Amaro, T., Huvenne, V., Allcock, A., Aslam, T., Davies, J., Danovaro, R., De Stigter, H., Duineveld, G., Gambi, C., Gooday, A. 2016. The Whittard Canyon – A case study of submarine canyon processes. Progress in Oceanography, 146, 38-57.

Amaro, T., de Stigter, H., Lavaleye, M., Duineveld, G. 2015. Organic matter enrichment in the Whittard Channel; its origin and possible effects on benthic megafauna. Deep Sea Research Part I: Oceanographic Research Papers, 102, 90-100.

Anderson, M., Gorley, R., Clarke, K. 2008. PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E. Plymouth, UK.

Baker, E.T., Hickey, B.M. 1986. Contemporary sedimentation processes in and around an active west coast submarine canyon. Marine Geology, 71, 15-34.

Behrenfeld, M.J., Falkowski, P.G. 1997. Photosynthetic rates derived from satellite-based chlorophyll concentration. Limnology and Oceanography, 42, 1-20.

Belley, R., Snelgrove, P.V.R., Archambault, P., Juniper, S.K. 2016. Environmental drivers of benthic flux variation and ecosystem functioning in Salish Sea and Northeast Pacific sediments. PLoS one, 11, e0151110.

Bianchelli, S., Gambi, C., Pusceddu, A., Danovaro, R. 2008. Trophic conditions and meiofaunal assemblages in the Bari Canyon and the adjacent open slope (Adriatic Sea). Chemistry and Ecology, 24, 101-109.

Boon, A.R., Duineveld, G.C. 2012. Phytopigments and fatty acids in the gut of the deposit-feeding heart urchin *Echinocardium cordatum* in the southern North Sea: selective feeding and its contribution to the benthic carbon budget. Journal of Sea Research, 67, 77-84.

Buhl-Mortensen, L., Vanreusel, A., Gooday, A.J., Levin, L.A., Priede, I.G., Buhl-• Mortensen, P., Gheerardyn, H., King, N.J., Raes, M. 2010. Biological structures as a source of habitat heterogeneity and biodiversity on the deep ocean margins. Marine Ecology, 31, 21-50.

Campanyà-Llovet, N., Snelgrove, P.V.R. 2018.a. Effects of temporal variation in food sources on infaunal community structure of chemosynthetic and non-chemosynthetic environments in

Barkley Hydrates, British Columbia, Canada. Deep-Sea Research Part I: Oceanographic Research Papers. In press.

Campanyà-Llovet, N., Snelgrove, P.V.R. 2018.b. Fine-scale infaunal community and food web patch mosaics from Barkley methane hydrates (British Columbia, Canada): the role of food quality. Deep-Sea Research Part I: Oceanographic Research Papers. In press.

Campanyà-Llovet, N., Snelgrove, P.V.R., Parrish, C. 2017. Rethinking the importance of food quality in marine benthic food webs. Progress in Oceanography, 156, 240-251.

Canals, M., Puig, P., de Madron, X.D., Heussner, S., Palanques, A., Fabres, J. 2006. Flushing submarine canyons. Nature, 444, 354-357.

Carson, B., Baker, E., Hickey, B., Nittrouer, C., DeMaster, D., Thorbjarnarson, K., Snyder, G. 1986. Modern sediment dispersal and accumulation in Quinault submarine canyon—a summary. Marine Geology, 71, 1-13.

Chauvet, P., Metaxas, A., Hey, A., Matabos, M. 2018. Annual and seasonal variations in the deep-sea megafaunal epibenthic communities in Barkley Canyon (British Columbia, Canada): a response to surface and deep-sea environment. Progress in Oceanography, in press.

Clarke, K., Gorley, R. 2006. PRIMER v6: User manual/tutorial. PRIMER-E. Plymouth, UK.

Connolly, T.P., Hickey, B.M. 2014. Regional impact of submarine canyons during seasonal upwelling. Journal of Geophysical Research: Oceans, 119, 953-975.

Copeman, L., Parrish, C. 2003. Marine lipids in a cold coastal ecosystem: Gilbert Bay, Labrador. Marine Biology, 143, 1213-1227.

Cowie, G.L., Hedges, J.I. 1994. Biochemical indicators of diagenetic alteration in natural organic matter mixtures. Nature, 369, 304 - 307.

Danovaro, R., Della Croce, N., Dell'Anno, A., Pusceddu, A. 2003. A depocenter of organic matter at 7800m depth in the SE Pacific Ocean. Deep Sea Research Part I: Oceanographic Research Papers, 50, 1411-1420.

Danovaro, R., Dinet, A., Duineveld, G., Tselepides, A. 1999. Benthic response to particulate fluxes in different trophic environments: a comparison between the Gulf of Lions – Catalan Sea (Western - Mediterranean) and the Cretan Sea (Eastern - Mediterranean). Progress in Oceanography, 44, 287-312.

De Leo, F.C., Vetter, E.W., Smith, C.R., Rowden, A.A., McGranaghan, M. 2014. Spatial scale-dependent habitat heterogeneity influences submarine canyon macrofaunal abundance and diversity off the Main and Northwest Hawaiian Islands. Deep Sea Research II: Topical studies in Oceanography, 104, 267–290.

De Leo, F.C., Smith, C.R., Rowden, A.A., Bowden, D.A., Clark, M.R. 2010. Submarine canyons: hotspots of benthic biomass and productivity in the deep sea. Proceedings of the Royal Society B: Biological Sciences, 277, 2783-2792.

De Leo, F.C., Gauthier, M., Nephin, J., Mihály, S., Juniper, S.K. 2017. Bottom trawling and oxygen minimum zone influences on continental slope benthic community structure off Vancouver Island (NE Pacific). Deep Sea Research Part II: Topical Studies in Oceanography, 137, 404-419.

De Leo, F.C., Ogata, B., Sastri, A., Heeseman, M., Mihály, S., Galbraith, M. 2018. High-frequency observations from a deep-sea cabled observatory reveal seasonal overwintering of *Neocalanus* spp. copepods in Barkley Canyon, NE Pacific: insights into unconstrained particulate organic carbon flux. Progress in Oceanography.In press.

De Stigter, H.C., Boer, W., de Jesus Mendes, Pedro A, Jesus, C.C., Thomsen, L., van den Bergh, Gert D, van Weering, T.C. 2007. Recent sediment transport and deposition in the Nazaré Canyon, Portuguese continental margin. Marine Geology, 246, 144-164.

Domke, L., Lacharite, M., Metaxas, A., Matabos, M. 2017. Influence of an oxygen minimum zone and macroalgal enrichment on benthic megafaunal community composition in a NE Pacific sumbarine canyon. Marine Ecology, 38(6). doi: 10.1111/maec.12481.

Doya, C., Chatzievangelou, D., Bahamon, N., Purser, A., De Leo, F.C., Juniper, S.K., Thomsen, L., Aguzzi, J. 2017. Seasonal monitoring of deep-sea megabenthos in Barkley Canyon cold seep by internet operated vehicle (IOV). PLoS One, 12, e0176917.

FitzGeorge-Balfour, T., Billett, D.S.M., Wolff, G.A., Thompson, A., Tyler, P.A. 2010. Phytopigments as biomarkers of selectivity in abyssal holothurians; interspecific differences in response to a changing food supply. Deep Sea Research Part II: Topical Studies in Oceanography, 57, 1418-1428. doi: 10.1016/j.dsr2.2010.01.013.

Gage, J.D., Tyler, P. 1991. Deep-sea biology: a natural history of organisms at the deep-sea floor. Cambridge University Press. New York, USA.

Garcia, R., Thomsen, L. 2008. Bioavailable organic matter in surface sediments of the Nazaré canyon and adjacent slope (Western Iberian Margin). Journal of Marine Systems, 74, 44-59.

Grassle, J.F., Sanders, H.L. 1973. Life histories and role of disturbance. Deep-Sea Research, 20, 643-659.

Harris, P.T., Whiteway, T. 2011. Global distribution of large submarine canyons: Geomorphic differences between active and passive continental margins. Marine Geology, 285, 69-86.

Hickey, B., Baker, E., Kachel, N. 1986. Suspended particle movement in and around Quinault submarine canyon. Marine Geology, 71, 35-83.

Jahnke, R.A., Reimers, C.E., Craven, D.B. 1990. Intensification of recycling of organic matter at the sea floor near ocean margins. Nature, 348, 50-54.

Johnson, N.A., Campbell, J.W., Moore, T.S., Rex, M.A., Etter, R.J., McClain, C.R., Dowell, M.D. 2007. The relationship between the standing stock of deep-sea macrobenthos and surface production in the western North Atlantic. Deep Sea Research Part I: Oceanographic Research Papers, 54, 1350-1360.

Juniper, S.K., Matabos, M., Mihály, S., Ajayamohan, R.S., Gervais, F., Bui, A.O.V. 2013. A year in Barkley Canyon: a time-series observatory study of mid-slope benthos and habitat dynamics using the NEPTUNE Canada network. Deep-Sea Research Part II: Topical Studies in Oceanography, 92, 114-123.

Keeling, R.F., Körtzinger, A., Gruber, N. 2010. Ocean deoxygenation in a warming world. Annual Review Marine Science, 2, 199–229.

Keil, R.G., Tsamakis, E., Fuh, C.B., Giddings, J.C., Hedges, J.I. 1994. Mineralogical and textural controls on the organic composition of coastal marine sediments: hydrodynamic separation using SPLITT-fractionation. Geochimica et Cosmochimica Acta, 58, 879-893.

Kelly, J.R., Scheibling, R.E. 2012. Fatty acids as dietary tracers in benthic food webs. Marine Ecology Progress Series, 446, 1-22. doi: 10.3354/meps09559.

Kiriakoulakis, K., Blackbird, S., Ingels, J., Vanreusel, A., Wolff, G.A. 2011. Organic geochemistry of submarine canyons: the Portuguese Margin. Deep Sea Research Part II: Topical Studies in Oceanography, 58, 2477-2488.

Le Guitton, M., Soetaert, K., Damsté, J.S., Middelburg, J. 2015. Biogeochemical consequences of vertical and lateral transport of particulate organic matter in the southern North Sea: A multiproxy approach. Estuarine, Coastal and Shelf Science, 165, 117-127.

Lee, R., Nevenzel, J., Paffenhöfer, G. 1971. Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. Marine Biology, 9, 99-108.

Levin, L.A. 2002. Oxygen minimum zone influence on the community structure of deep-sea benthos. Toxicology, and Water Quality, 121.

Longbottom, M. 1970. The distribution of *Arenicola marina* (L.) with particular reference to the effects of particle size and organic matter of the sediments. Journal of Experimental Marine Biology and Ecology, 5, 138-157.

López-Fernández, P., Bianchelli, S., Pusceddu, A., Calafat, A., Danovaro, R., Canals, M. 2013. Bioavailable compounds in sinking particulate organic matter, Blanes Canyon, NW Mediterranean Sea: effects of a large storm and sea surface biological processes. Progress in Oceanography, 118, 108-121.

Matabos, M., Bui, A.O.V., Mihály, S., Aguzzi, J., Juniper, S.K., Ajayamohan, R.S. 2014. High-frequency study of epibenthic megafaunal community dynamics in Barkley Canyon: A multi-disciplinary approach using the NEPTUNE Canada network. Journal of Marine Systems, 130, 56-68.

Mayer, L.M., Linda, L., Sawyer, T., Plante, C.J., Jumars, P.A., Sel, R.L. 1995. Bioavailable amino acids in sediments: a biomimetic, kinetics based approach. Limnology and Oceanography, 40, 511-520.

Middelburg, J.J., Nieuwenhuize, J. 1998. Carbon and nitrogen stable isotopes in suspended matter and sediments from the Schelde Estuary. Marine Chemistry, 60, 217-225.

Müller-Navarra, D.C. 2008. Food web paradigms: the biochemical view on trophic interactions. International Review of Hydrobiology, 93, 489-505. doi: 10.1002/iroh.200711046.

Parrish, C.C. 1987. Separation of aquatic lipid classes by chromarod thin-layer chromatography with measurement by latroscan flame ionization detection. Canadian Journal of Fisheries and Aquatic Sciences, 44, 722-731.

Parrish, C.C. 1999. Determination of total lipid, lipid classes, and fatty acids in aquatic samples. In: M.T. Arts, B.C. Wainman. Lipids in freshwater ecosystems. (pp. 4-20 (Chapter 1)). Springer. New York.

Parrish, C.C. 2009. Essential fatty acids in aquatic food webs. In: Arts et al. Lipids in Aquatic Ecosystems. (pp. 309-326). Springer. New York.

Parrish, C.C. 2013. Lipids in marine ecosystems. ISRN Oceanography, 2013, Article ID 604045, 16. doi: 10.5402/2013/604045.

Pedrosa-Pàmies, R., Sanchez-Vidal, A., Calafat, A., Canals, M., Duran, R. 2013. Impact of storm-induced remobilization on grain size distribution and organic carbon content in sediments from the Blanes Canyon area, NW Mediterranean Sea. Progress in Oceanography, 118, 122-136.

Puig, P., Palanques, A., Martín, J. 2014. Contemporary sediment-transport processes in submarine canyons. Annual Review of Marine Science, 6, 53-77.

Pusceddu, A., Bianchelli, S., Canals, M., Sanchez-Vidal, A., Madron, D.D., Heussner, S., Lykousis, V., de Stigter, H., Trincardi, F., Danovaro, R. 2010. Organic matter in sediments of canyons and open slopes of the Portuguese, Catalan, Southern Adriatic and Cretan Sea margins. Deep Sea Research Part I: Oceanographic Research Papers, 57, 441-457.

Riaux-Gobin, C., Klein, B. 1993. Microphytobenthic biomass measurement using HPLC and conventional pigment analysis. Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, 369-376.

Sappington, J.M., Longshore, K.M., Thompson, D.B. 2007. Quantifying landscape ruggedness for animal habitat analysis: a case study using bighorn sheep in the Mojave Desert. Journal of Wildlife Management, 71, 1419-1426.

Sato, K.N., Levin, L.A., Schiff, K. 2017. Habitat compression and expansion of sea urchins in response to changing climate conditions on the California continental shelf and slope (1994–2013). Deep Sea Research Part II: Topical Studies in Oceanography, 137, 377-389.

Smith, K. 1987. Food energy supply and demand: a discrepancy between particulate organic carbon flux and sediment community oxygen consumption in the deep ocean. Limnology and Oceanography, 201-220.

Snelgrove, P.V.R., Grassle, J.F., Petrecca, R.F. 1992. The role of food patches in maintaining high deep-sea diversity - field experiments with hydrodynamically unbiased colonization trays. Limnology and Oceanography, 37, 1543-1550.

Snelgrove, P.V.R., Grassle, J.F., Petrecca, R.F. 1994. Macrofaunal response to artificial enrichments and depressions in a deep-sea habitat. Journal of Marine Research, 52, 345-369. doi: 10.1357/0022240943077082.

Snelgrove, P.V.R., Grassle, J.F., Petrecca, R.F. 1996. Experimental evidence for aging food patches as a factor contributing to high deep-sea macrofaunal diversity. Limnology and Oceanography, 41, 605-614.

Tews, J., Brose, U., Grimm, V., Tielbörger, K., Wichmann, M., Schwager, M., Jeltsch, F. 2004. Animal species diversity driven by habitat heterogeneity/diversity: the importance of keystone structures. Journal of Biogeography, 31, 79-92.

Thomsen, L., Aguzzi, J., Costa, C., De Leo, F., Ogston, A., Purser, A. 2017. The Oceanic Biological Pump: Rapid carbon transfer to depth at Continental Margins during Winter. Scientific Reports, 7, 10763.

Thornton, S., McManus, J. 1994. Application of organic carbon and nitrogen stable isotope and C/N ratios as source indicators of organic matter provenance in estuarine systems: evidence from the Tay Estuary, Scotland. Estuarine. Coastal and Shelf Science, 38, 219-233.

Van Rijn, L.C. 1993. Principles of sediment transport in rivers, estuaries and coastal seas. Aqua publications Amsterdam. The Netherlands.

Vetter, E.W., Smith, C.R., De Leo, F.C. 2010. Hawaiian hotspots: enhanced megafaunal abundance and diversity in submarine canyons on the oceanic islands of Hawaii. Marine Ecology, 31, 183-199.

Veuger, B., van Oevelen, D. 2011. Long-term pigment dynamics and diatom survival in dark sediment. Limnology and Oceanography, 56, 1065-1074.

Veuger, B., van Oevelen, D., Middelburg, J.J. 2012. Fate of microbial nitrogen, carbon, hydrolysable amino acids, monosaccharides, and fatty acids in sediment. Geochimica et Cosmochimica Acta, 83, 217-233.

Wagner, J.K.S., McEntee, M.H., Brothers, L.L., German, C.R., Kaiser, C.L., Yoerger, D.R., Van Dover, C.L. 2013. Cold-seep habitat mapping: High-resolution spatial characterization of the Blake Ridge Diapir seep field. Deep-Sea Research Part II: Topical Studies in Oceanography, 92, 183-188.

Weiss, A. 2001. Topographic positions and landforms analysis (poster). ESRI International User Conference. San Diego, CA: ESRI.

Wieking, G., Kröncke, I. 2005. Is benthic trophic structure affected by food quality? The Dogger Bank example. Marine Biology, 146, 387-400.

Wigham, B.D., Hudson, I.R., Billett, D.S.M., Wolff, G.A. 2003. Is long-term change in the abyssal Northeast Atlantic driven by qualitative changes in export flux? Evidence from selective feeding in deep-sea holothurians. Progress in Oceanography, 59, 409-441. doi: 10.1016/j.pocean.2003.11.003.

Wilson, M.F.J., O'Connell, B., Brown, C., Guinan, J.C., Grehan, A.J. 2007. Multiscale terrain analysis of multibeam bathymetry data for habitat mapping on the continental slope. Marine Geodesy, 30, 3-35.

3.9. Appendix
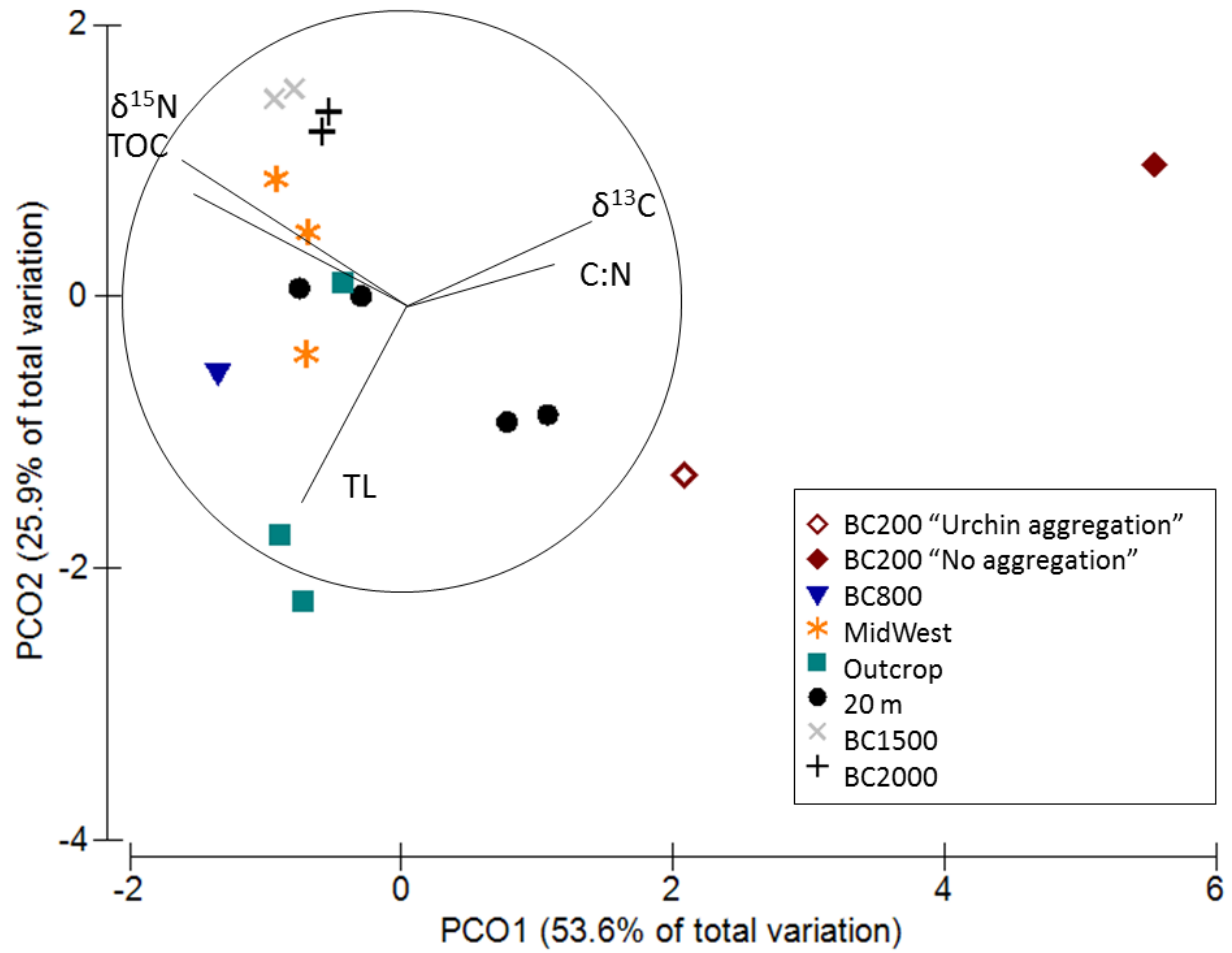
Appendix 3.1. Principal coordinate analysis (PCoA) from Barkley Canyon sedimentary organic matter (quantity and quality). Resemblance based on Euclidean distances. TOC = Total organic carbon, TL = Total lipids.
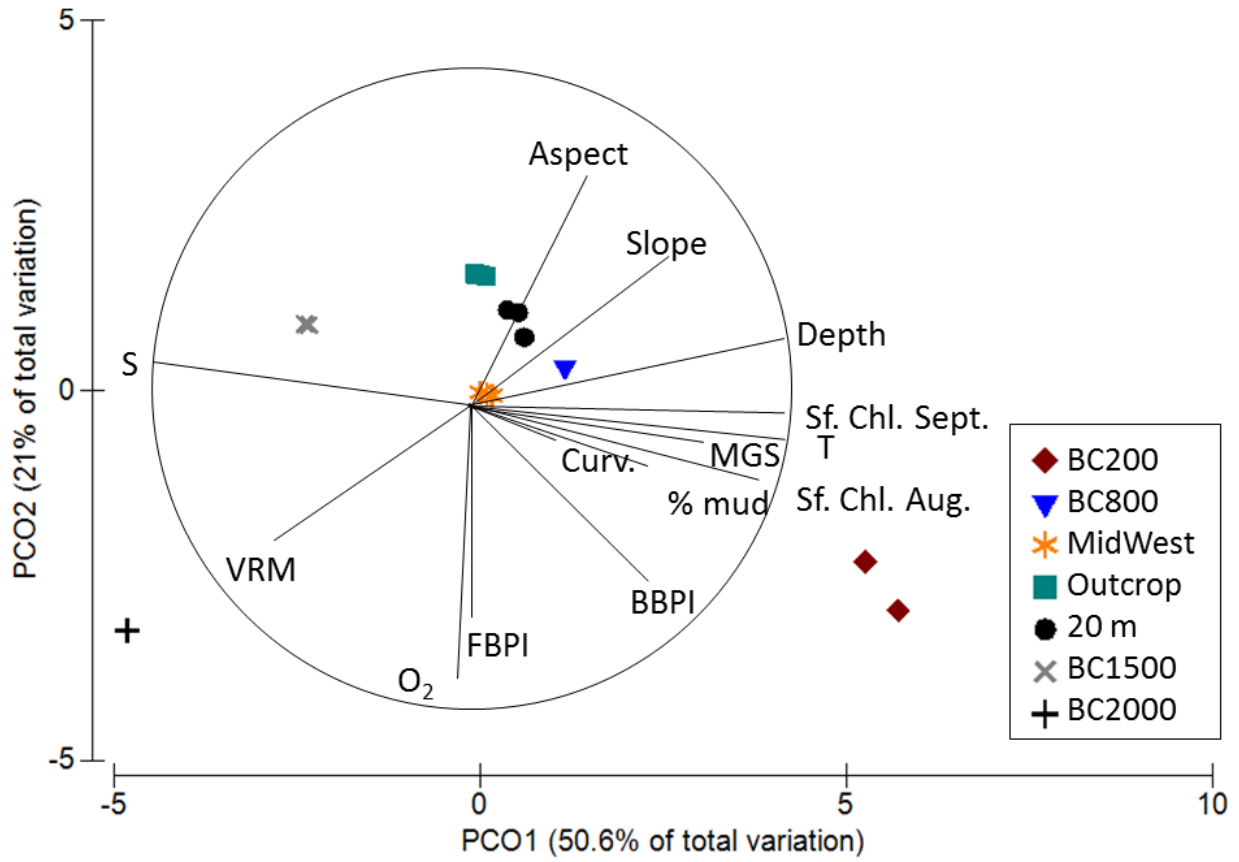
Appendix 3.2. Principal coordinates analysis (PCoA) from Barkley Canyon environmental variables. Resemblance matrix based on euclidean distances from normalized data. MGS= Mean Grain Size, FBPI = Fine-scale BPI, BBPI = Broad-scale BPI, VRM = Vector Ruggedness Measure, sf. Chl. Sept. = surface water chlorophyll in September 2013, sf. Chl. Aug. = surface water chlorophyll in August 2013, T= Temperature, S= Salinity, Curv. = Curvature.
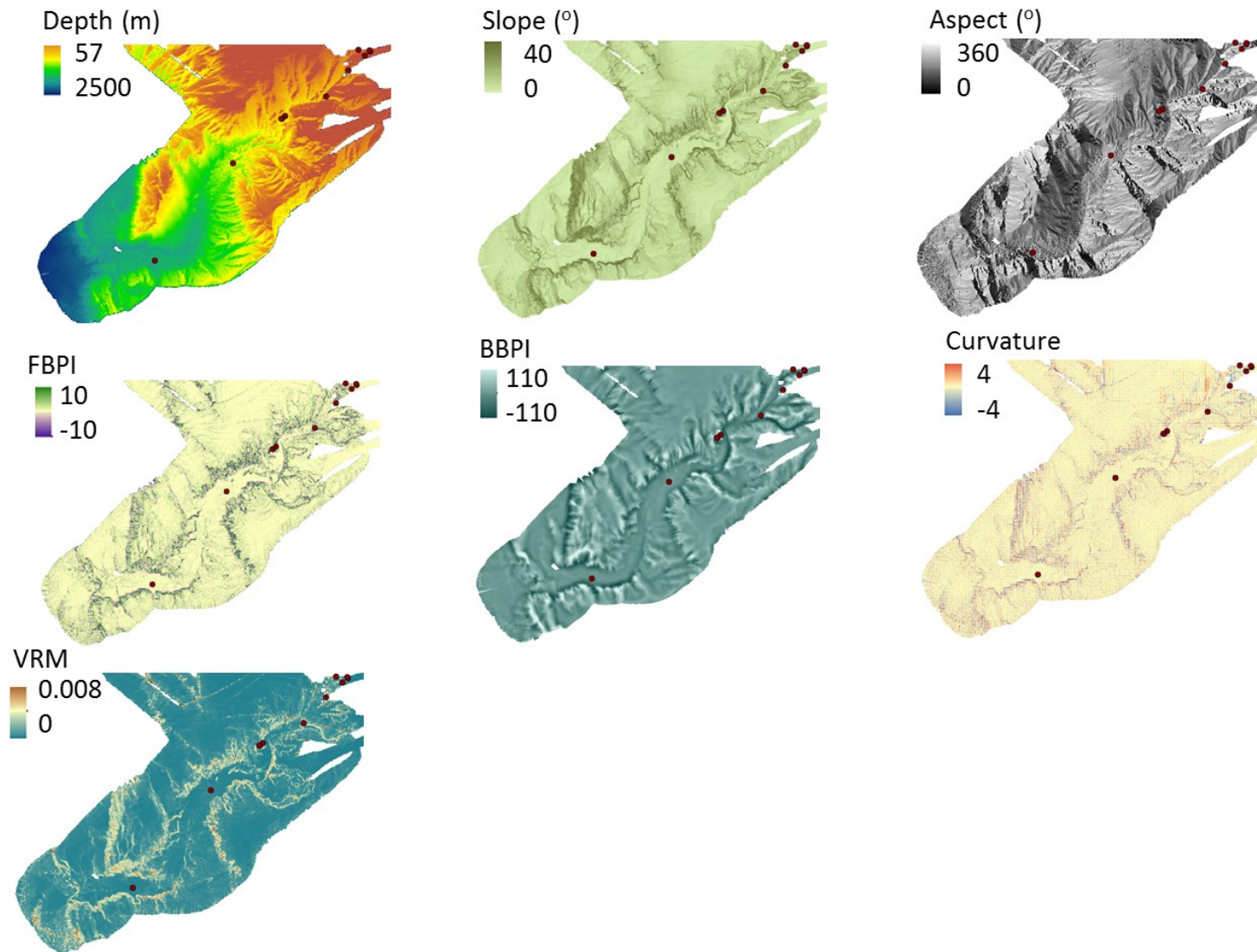
Appendix 3.3. Topographic variables from Barkley Canyon derived from ArcGis. Red marks represent sites sampled.

Appendix 3.4. The most relevant fatty acids in Barkley Canyon sediment and particulate organic matter: biomarkers, designations, and names. Some fatty acids can be biomarkers for more than one organism, whereas the utility of other fatty acids as biomarkers remains unclear. Information taken from Parrish 2013.

Biomarker	Fatty acid	Name
Protozoa and microeukaryotes	20:4w6	Arachidonic; ARA
Phytoplankton	15:0	Pentadecanoic
Diatoms	14:0	Myristic
	16:1w7	Palmitoleic
	16:4w1	Hexadecatetraenoic
	20:5w3	Eicosapentaenoic; EPA
Prymnesiophytes	14:0	Myristic
Mangrove	16:1w7	Palmitoleic
	18:1w9	Oleic
Macroalgae	20:5w3	Eicosapentaenoic; EPA
	18:1w9	Oleic
	18:3w6	γ-linolenic; GLA
red algae/Kelp	20:4w6	Arachidonic; ARA
Mangrove/Seagrass/Vascular plants	18:3w3	α-linolenic; ALA
Zooplankton	22:1w11(13)	Docosenoic
	22:1w9	Erucic
	24:1	Tetracosanoic
Copepod	20:1w9	Gondoic
	20:1w11	Gadoleic
Deep-sea fish/Crustaceans/Carnivory	18:1w9	Oleic
Proteobacteria	14:0	Myristic

Appendix 3.4. *Continued*

Biomarker	Fatty acid	Name
Bacteria	ai15:0	Methyltetradecanoic
	i15:0	Methyltetradecanoic
	16:1w7	Palmitoleic
	i16:0	Methylpentadecanoic
	16:0	Methylpentadecanoic
	16:1w5	Hexadecenoic
	i17:0	Methylhexadecanoic
	16:2w4	Hexadecadienoic
	17:0	Margaric
	18:1w6	Octadecenoic
	18:1w7	Vaccenic
No known markers	TMTD	Trimethyltridecanoic
	14:1	Tetradecenoic
	18:3w4	Octadecatrienoic
	18:4w1	Octadecatetraenoic
	20:0	Arachidic
	20:3w6	Dihomo-γ-linolenic
	16:3w4	Hexadecatrienoic
	18:0	Stearic
	22:1w7	Docosenoic
	22:5w3	Clupanodonic; DPA
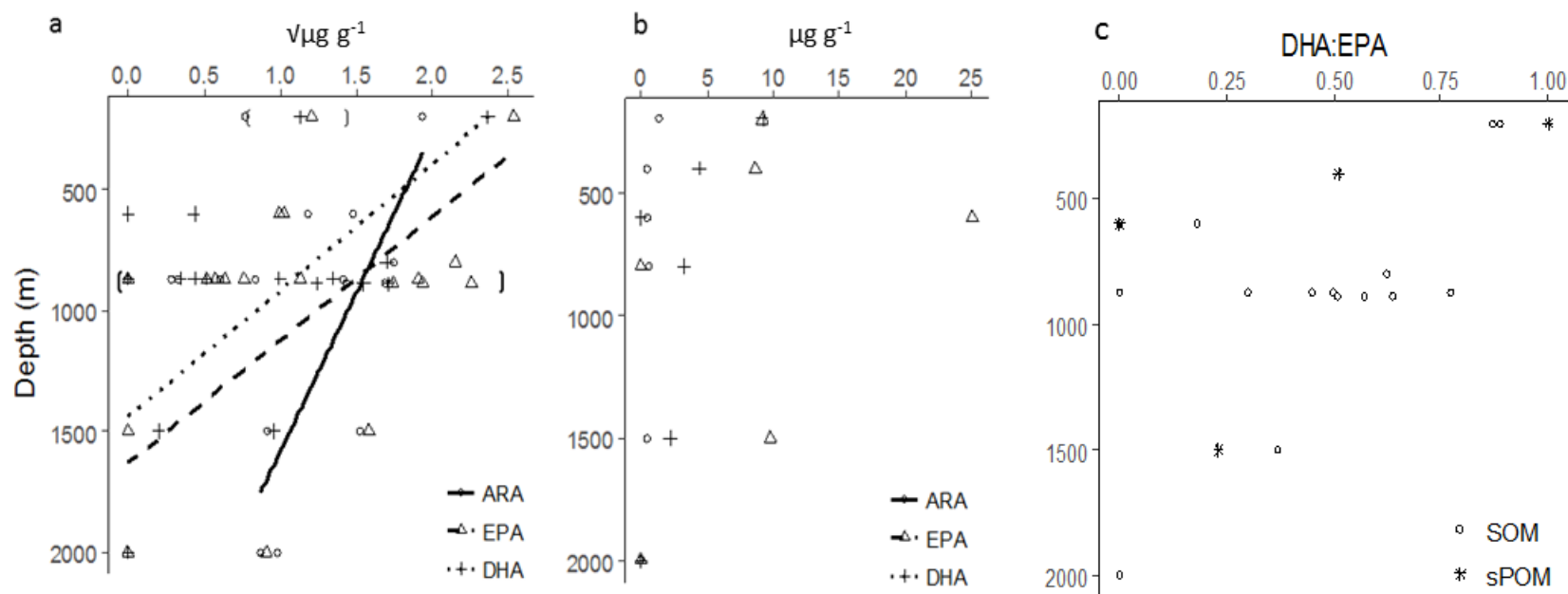
Appendix 3.5. Essential fatty acids (ARA: 20:4ω6 – arachidonic acid, EPA: 20:5ω3 – eicosapentaenoic acid, DHA: 22:6ω3 – docosahexaenoic acid) from Barkley Canyon within sediments (a), particulate organic matter (b), and DHA:EPA ratio from Barkley Canyon within sediments (SOM), and particulate organic matter (POM), with depth (c). We plotted only statistically significant regression lines with depth: Arachidonic acid: ARA = -0.0004Depth + 1.83, R^2=0.59; Eicosapentaenoic acid: EPA = -0.009Depth + 2.31, R^2=0.46; Docosahexaenoic acid: DHA = -0.0008 Depth + 1.76, R^2=0.40. Outliers in brackets were not considered in the regression.

4. Fine-scale infaunal community and food web patch mosaics from Barkley methane hydrates (British Columbia, Canada): the role of food quality [3]

4.1. Abstract

In order to assess the influence of organic matter patchiness on deep-sea biodiversity, we examined organic matter patchiness and macrofauna at small spatial scales (meters – 100's of meters) in a chemosynthetic environment (Barkley methane hydrates, British Columbia continental slope, ~ 900 m depth). Specifically, we assessed quantity, quality, and sources of organic matter and their influence on the associated infaunal community and food web structure (feeding guild composition) at the methane outcrop, as well as at sites 20 m and 600 m away. We found greater patchiness in food composition at the outcrop site (containing clam beds) than the more distant locations. Trophic diversity ($ES_{[10]}$) was significantly greater at the outcrop compared to that at the 20 m site, suggesting that the outcrop added rather than replaced trophic niche opportunities. Diversity (Expected Species rarefaction) was lower at the outcrop compared to the other two sites, although not significantly so. We re-analysed the community data at higher spatial resolution (5 to 10 m, and lower replication) to identify the distance of influence of the hydrates habitat and found a transition from outcrop to background at 9 and 15 m away from the most active site, with greater similarity in the communities at 28 m to those at 600 m than the outcrop itself. Although not major controlling factors, food quality (i.e., nutritional value for benthic organisms) and source played a significant role in determining food web structure, outweighing the influences of food quantity and sediment type. However, our distance-based linear model (distLM) using all variables, food quality, or topography explained only 33.56 %,

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33.54 %, and 33.18 % respectively of the total variation in feeding guild composition, suggesting important roles for other environmental variables. We conclude that the higher food patchiness in Barkley Hydrates compared to background sediments likely contributed to increased trophic niche opportunities. We emphasize the importance of sampling at adequate spatial scales to detect the limits of the influence of methane hydrates in infaunal communities and food webs.

Key words: macrofauna, diversity, cold seep, heterogeneity, sediment, food source

4.2. Introduction

Environmental heterogeneity can promote complex and diverse biological assemblages (Tews et al., 2004). The "patch mosaic theory" (Grassle, 1989) attributed high diversity in deep-sea sedimentary habitats to patchily distributed food resources of varying origin (e.g., seasonal pulses of organic matter, organic remains of gelatinous zooplankton, wood falls, kelp, and seagrass). Small-scale disturbances resulting from bioturbation processes that form pits and mounds contribute to this heterogeneity (Smith and Hessler, 1987). Subsequent colonization experiments (Snelgrove et al., 1992, 1994, 1996) supported this theory, in that different species colonized trays enriched with food sources (*Thalassiosira* sp., a diatom, and *Sargassum* sp., a brown macroalgae) differing in composition and age.

Despite widespread acceptance that increases in food supply generally result in increased abundances and/or biomass (Pearson and Rosenberg, 1978, Thiel, 1979, Rosenberg, 1995, Wei et al., 2010), limited evidence suggests a parallel role for food quality in structuring benthic communities and food webs (Wigham et al., 2003, Wieking and Kröncke, 2005, Campanyà-Llovet et al., 2017). Campanyà-Llovet et al. (2017) discussed different measures of food quantity and quality for benthic communities in detail, but we define quality as the degree to which

quantity and composition of accessible food fulfill consumer nutritional needs (Müller-Navarra, 2008).

Deep-sea chemosynthetic communities derive nutrition from two distinct sources: food produced by chemosynthetic microorganisms at the site and/or sinking organic matter produced from photosynthesis in surface waters. The availability of two food sources differing in quality at a given location raises questions regarding the relative contribution of each source to benthic communities (Levin, 2005, Zapata-Hernández et al., 2014). This situation suggests potential heterogeneity and therefore, patchiness in the sedimentary organic matter of deep-sea chemosynthetic ecosystems and their surroundings and, therefore, the capacity to influence infaunal communities and food webs. The scientific community has clearly recognized increased regional biodiversity (β-diversity) where chemosynthesis occurs in sedimentary habitats (Levin, 2005, Bernardino et al., 2012), attributing increases to different habitats created by chemosynthetic environments.

Gas hydrates originate from methane-supersaturated pore fluids under elevated pressure ($>6 \times 10^6$ Pa) and low temperature ($<4\ ^{\circ}$C), and occur at a few hundred meters depth on continental slopes (Chapman et al., 2004). Barkley Hydrates occur off Vancouver Island, British Columbia, Canada, at a depth of 870 m within Barkley Canyon. These hydrates differ from other sites nearby in the northern Cascadia Margin and Hydrate Ridge because of their thermogenic, as opposed to microbial, origin (Chapman et al., 2004). Chapman et al. (2004) speculate that these hydrates may indicate the presence of oil and gas deposits near Vancouver Island, because other thermogenic sites in the Gulf of Mexico and the Caspian Sea co-occur with petroleum reserves. From an ecological perspective, Barkley Hydrates form a patchy landscape with methane outcrops (i.e., ice-like methane hydrates protruding from the ground) resulting in 1-3 m high

mounds (Chapman et al. 2004). Such topographic features can promote heterogenous accumulation of organic matter, adding patchiness within the chemosynthetic environment. The mounds support bacterial mats and epifaunal communities that rely on chemosynthesis of organic matter and tolerate the associated toxicity (vesicomyid clams and bacterial mats: Chapman et al., 2004), however, Barkley Hydrates infauna remains unexplored.

Our study aims to understand patchiness in Barkley Hydrates habitats at different spatial scales (meters to 100's of meters), and to evaluate the role of food source and quality as drivers of macrofaunal patterns by: 1) investigating within-site heterogeneity (i.e., species, feeding guild, and food composition) with distance from the outcrop, 2) identifying the extent and location of any macrofaunal transition between outcrop and background communities, and 3) identifying the contribution of food quality (C:N, Chl *a*, Chl *a*: Pheo, total lipids, total hydrocarbons, and essential fatty acids) and source (δ^{13}C, δ^{15}N, Chl *a*:TOC, total hydrocarbons:TOC) to spatial changes in species and feeding guild composition of Barkley Hydrates infauna.

4.3. Materials and Methods

4.3.1. Study area

Barkley Hydrates are located at 870 m depth at the wall of Barkley submarine canyon, ~ 100 km offshore of British Columbia, Canada (Fig. 4.1). Barkley Hydrates form outcrops (mounds) 1-3 m in height containing gas and light oil of thermogenic origin (Chapman et al., 2004, Lu et al., 2007), which are surrounded by patchily distributed cold-seep communities, including bacterial mats at the top of the mound, live and dead *Calyptogena* spp. (vesycomid clams) at the base of the mound, and carbonate mounds (Doya et al., 2017). Within these ecosystems, environmental and biological characteristics, such as methane concentrations, bacterial and *Calyptogena* spp. distributions vary over short distances (< 1 m; Purser et al., 2013). Methane concentrations in

bottom waters of Barkley Hydrates range from 0.8 to 6.9 μM (Thomsen et al., 2012). The background (non-hydrate) community in this study was sampled 600 m to the NW of Barkley Hydrates, corresponding to the MidWest site of the ONC cabled observatory (890 m depth), we also sampled at the canyon wall. A mixture of mud and sand covers the seafloor at the MidWest site (Juniper et al., 2013, Chauvet et al., 2018). An oxygen minimum zone (OMZ) covers the region at intermediate depths (400 – 1000 m: Keeling et al., 2010), including our two study sites. Macrofaunal biodiversity frequently declines within OMZs, whereas dominance increases (Levin, 2003), a pattern we predict will characterize community structure at our study sites. Seasonal variation occurs in OMZs, with potential effects on infaunal communities (Levin, 2003).
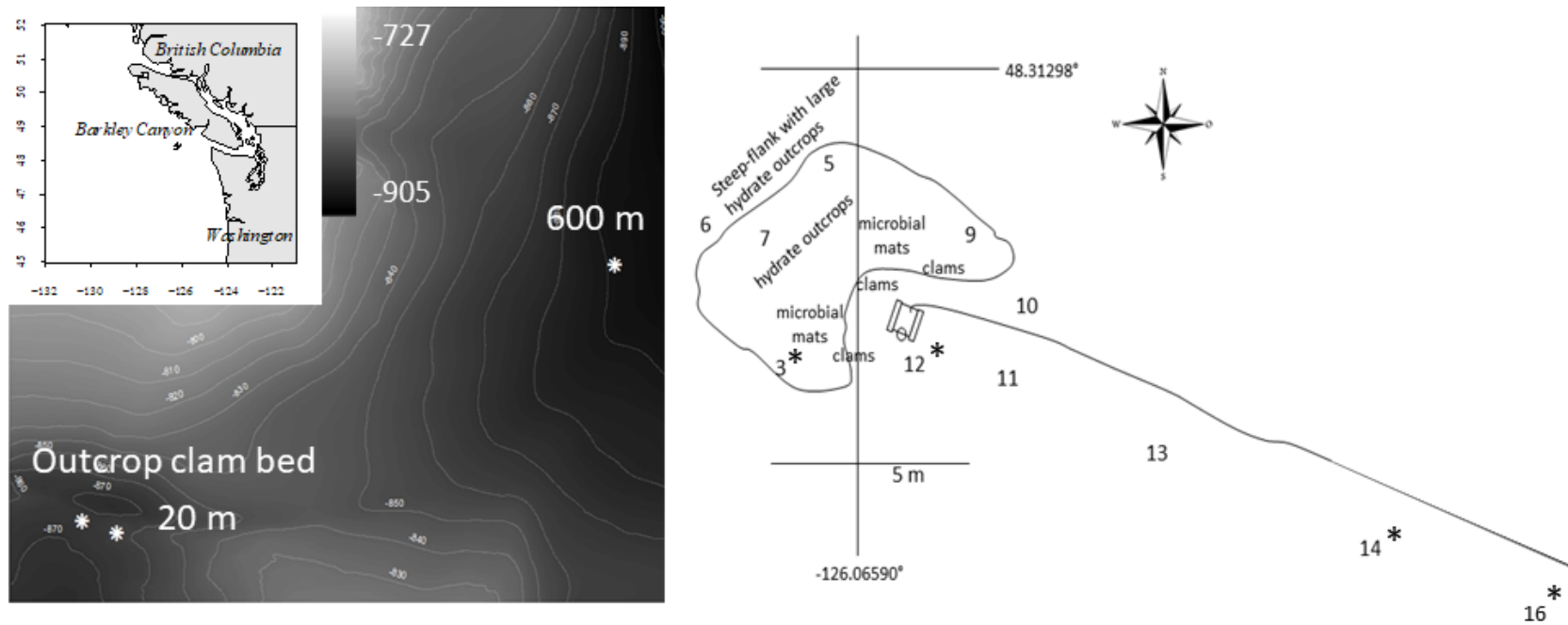
Figure 4.1. Map of sites sampled in Barkley Hydrates and surrounding sites, offshore from Vancouver Island, British Columbia, Canada (left). Map based on bathymetric map from MBARI (Monteray Bay Aquarium Research Institute). Map showing "Waypoints" arranged around a hydrate outcrop in Barkley Hydrates, with the Internet Operated Vehicle "Wally" and its floating tether to junction box of Ocean Networks Canada underwater observatory (right). Modified from Purser et al. (2013). Asterisks denote Waypoints sampled for this study.

4.3.2. Sampling

We sampled Barkley Hydrates sediments within the outcrop clam bed (hereafter referred to as the "outcrop"), where there was no overlap with the bacterial mats, and at two different distances from it (i.e., 20 and 600 m away; see Fig. 4.1) in September 2013 from the R/V *Falkor*. The 600-m site corresponds to site MidWest (890 m) of the Ocean Networks Canada cabled observatory. Push cores (i.d. = 6.7 cm, L = 35.6 cm) from the study sites were collected using the Remotely Operated Vehicle (ROV) ROPOS (www.ropos.com). A series of markers, referred to here as "Waypoints" (WP), provide a spatial reference for the crawler Wally, an Internet Operated Vehicle (IOV), and reference points for our sites; however, we did not use Wally in this study. At the outcrop, we collected five push cores at "Waypoint 3" (WP3) along with another five at "Waypoint 12" (WP12), 20 m away from the outcrop we collected five push cores at "Waypoint 14" (WP14) and five more at "Waypoint 16" (WP16), always avoiding Wally's tracks and any associated disturbance to the sediment. We collected additional sets of three, three, and four push cores, with each set at least 10 m away from the others at the third site, 600 m away from the outcrop. Each core was sectioned into 0-2, 2-5, and 5-10 cm layers. At the outcrop and 20-m sites, we retained four push cores for organic matter analysis and six for taxonomy at each site (2+3 in each WP), whereas at the 600-m site, we retained three cores for organic matter analysis and seven for taxonomy.

4.3.3. Macrofaunal identification and taxonomic diversity

Sediment samples were processed through a 300-µm sieve prior to preservation in a 4 % seawater-formaldehyde solution on board, and later transferred into 70 % ethanol in the laboratory. Specimens were sorted under a dissecting microscope and identified to the lowest taxonomic level possible. We identified a species of bivalves resembling the family Thyasiridae because of the ferruginous patches on the anterior and posterior ends or elongated food and will refer to them as such from now on. We defined feeding guilds following Jumars et al. (2015) and WoRMS Editorial Board (2017): surface deposit feeders (SDF), sub-surface deposit feeders (SSDF), suspension feeders (SF), omnivores (O), carnivores (C), scavengers (Sc), bacterivores (B), and unknown (U). We used fuzzy clustering, which allowed more than one feeding group for a given taxon, and scored them from 0 to 1 based on the extent to which they displayed each trait (Equihua, 1990). Except for comparisons between sediment layers, we combined the different vertical sections of the cores for faunal analyses.

4.3.4. Laboratory analysis of sediment organic matter

Food quantity and quality were analysed from the cores collected for organic matter. In this study, we used total organic carbon (TOC) as a measure of food quantity. We considered total nitrogen (TN), total organic carbon to total nitrogen ratio (C:N), chlorophyll a to pheopigments ratio (Chl a:Pheo), total lipids (TL), and essential fatty acids (EFAs), as measures of food quality, whereas $\delta^{13}C$, $\delta^{15}N$, chlorophyll a to total organic carbon ratio (Chl a:TOC), and total hydrocarbon to total organic carbon ratio (HC:TOC) characterized food source. From each sediment core, we removed separate subsamples for TOC, TN, and $\delta^{13}C$, for pigment analysis, and for lipids. We stored sediment samples for TOC, TN, $\delta^{13}C$, and $\delta^{15}N$ analysis in Whirl-pack bags at -20 °C, for pigment analysis in 15-ml centrifuge tubes covered in aluminium foil (to

protect them from light) at -20 °C, and for lipid analysis in pre-combusted (450 °C for 8 h) aluminium tin foil (to avoid contamination) at -80 °C.

4.3.4.1. TOC, TN, δ^{13}C, δ^{15}N

In order to determine TOC and TN in each sample, we dried a sediment subsample of 1-5 g (wet weight) at 80 °C for 24 h, ground it into a fine powder, and then weighed and acidified (under pure HCl fumes for 24 h) it to eliminate inorganic carbon. We then dried samples again at 80 °C for 24 h before CHN analysis. We weighed and folded an aliquot of dried decarbonated sediments (15 mg) in a tin capsule prior to analyzing TOC (%), TN (%), δ^{13}C (‰), and δ^{15}N (‰) in a Carlo Erba NA1500 Series II elemental analyser (EA). We present the stable isotopic results with respect to the international standards of atmospheric nitrogen (AIR, N_2) for nitrogen and Vienna Pee Dee belemnite (V-PDB) for carbon, noting average instrumental precision of ±0.1 ‰ for nitrogen and ±0.2 ‰ for carbon. The stable nitrogen and carbon isotope ratios are expressed as:

δ^{13}C = [(Rsample/Rstandard)-1]x1000,

δ^{15}N = [(Rsample/Rstandard)-1]x1000,

where R represents ^{13}C/^{12}C for δ^{13}C, and ^{15}N/^{14}N for δ^{15}N.

4.3.4.2. Phytopigment content

We measured chlorophyll *a* (Chl *a*) and pheopigments (Pheo) fluorometrically, following a modified version of Riaux-Gobin & Klein (1993). Pigments from 1-2 g of wet sediment were extracted in 90 % acetone (v/v) for 24 h at 4 °C and the supernatant analyzed using a Turner Designs 10-AU-005-CE fluorometer (Turner Designs, Sunnyvale, USA) with and without acidification. The remaining sediment was dried at 60 °C for 24 h and weighed.

4.3.4.3. Lipid content

Lipid samples were extracted with a combination of chloroform: methanol: water to 4:2:1.5 according to Parrish (1999), creating an upper inorganic and a lower organic layer. We removed the bottom organic layer, which contains the lipids, without disturbing the top aqueous layer by using a double pipetting technique, placing a long 1-ml, lipid cleaned Pasteur pipette inside a short 1-ml pipette. Chloroform was then added back to the extraction test tube and the entire procedure was repeated three times. We then pooled all organic layers in a lipid-cleaned vial, concentrating samples under a flow of nitrogen gas.

We determined lipid class composition with a three-step chromatographic development method (Parrish, 1987). The lipid extracts were applied to Chromarods and focused to a narrow band using 100 % acetone. The first development system was hexane/diethyl ether/formic acid (98.95:1.0:0.05, v/v/v). The rods were developed for 25 min, removed from the system for 5 min to dry, and replaced for 20 min. The second development ran for 40 min in hexane/diethyl ether/formic acid (79:20:1, v/v/v). The final development system entailed two 15-min exposures to 100 % acetone, followed by two 10-min periods in chloroform/methanol/chloroform-extracted water (5:4:1, v/v/v). Before using each solvent system we dried the rods in a constant humidity chamber. Samples were analysed using an Iatroscan Mark VI TLC-FID (thin-layer chromatography-flame ionization detector system; Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan) after each development using partial scanning until the last scan. We collected the data using Peak Simple software (ver 3.67, SRI Inc) and calibrated the Chromarods with standards prior to and during their use on our samples.

4.3.4.4. Fatty acids

The fatty acid composition is determined as the methyl esters of fatty acids by gas-liquid chromatography (GC) (Christie, 1982). We derivatized a portion of each lipid extract to obtain fatty acid methyl esters (FAME) with a mixture of BF3/CH3OH at 85 ^{o}C for 90 min under nitrogen and then added purified water and hexane. We transferred the upper, organic layer containing FAME, into a 2-ml vial, evaporated it under nitrogen, refilled the vial with hexane and stored it at -20 ^{o}C under nitrogen. All FAMEs were analyzed on a HP 6890 GC-FID (gas chromatography-flame ionization detector system) equipped with a 7683 autosampler. The GC column was a ZB wax + (Phenomenex, Torrance, CA, USA), 30 m in length with an internal diameter of 0.32 mm. Peaks were identified using retention times from standards purchased from Supelco (Bellefonte, PA, USA): 37 component FAME mix (Product number 47885-U), PUFA 3 (product number 47085-U) and PUFA 1 (product number 47033-U). Chromatograms were integrated using the Varian Galaxie Chromatography Data System, version 1.9.3.2 (Walnut Creek, CA, USA).

4.3.5. Mean grain size

Granulometric properties (sediment mean grain size; MGS) were determined with a HORIBA Partica LA-950 laser diffraction particle size analyzer (Horiba Ltd, Kyoto, Japan). We deflocculated samples with calgon $(NaPO_3)_6$ prior to analysis to break down aggregates and ensure we had measured actual sediment grain size.

4.3.6. Topography

We derived seabed topographic features from Barkley Hydrates from a high resolution bathymetric raster (1 m gird) obtained from Monterey Bay Aquarium Research Institute (MBARI). The data was originally acquired through an Autonomous Underwater Vehicle (AUV)

in 2009. We used the Spatial Analyst tools in ArcGIS (version 10.4) to derive slope, aspect, and curvature, and the benthic terrain modeller (BTM) extension for fine and broad-scale BPI, and rugosity – VRM (Vector Ruggedness Measure). Bathymetric Positioning Index (BPI) measures the bathymetric position of a specific pixel relative to surrounding pixels, and indicates whether any particular pixel forms part of a positive (e.g., crest) or negative (e.g., trough) feature of the surrounding terrain. Because the user specifies the radius used to compare bathymetric position, BPI can be calculated at different spatial scales: broad- scale BPI (BBPI) and fine-scale BPI (FBPI) (Weiss, 2001, Wilson et al., 2007). VRM measures terrain ruggedness as the variation in three-dimensional orientation of grid cells within a neighbourhood (Sappington et al., 2007). We chose derived variables based on their significance for benthic communities [i.e., slope, curvature, fine (< 10 m) and broad-scale (10's m) BPI and VRM] and because they accounted for topographic changes from the outcrop area (i.e., fine-scale BPI). Sampling sites were plotted and derivatives extracted for each site using the "Extract Multi Values to Points" tool (Spatial Analyst).

4.3.7. Statistical analysis

We investigated infaunal densities, as well as species and trophic diversity with Hulbert rarefaction curves, to describe spatial variation in community structure. We transformed density [log(x+0.1)] to achieve normality of residuals and homogeneity of variance prior to a one-way analysis of variance (ANOVA) type (III) to account for the unbalanced design, with site as a unique factor with levels: outcrop (n = 6), 20 m (n = 6), and 600 m (n = 7). We performed a post hoc comparison of significant effects ($P < 0.05$) using Tukey tests. We ran a one-way ANOVA on $ES_{[10]}$ with factor site [levels: outcrop (n = 6), 20 m (n = 6), and 600 m (n = 7)] based on species and on trophic guilds, separately.

In order to assess the effect of sediment depth on the communities at each site separately, we performed a non-parametric Kruskal-Wallis test with factor Sediment depth (levels: 0-2, 2-5, and 5-10 cm) at each site (i.e., outcrop, 20 m, and 600 m) separately. We performed a post hoc comparison of significant effects (P < 0.05) using Dunn's test.

We used multivariate statistics to identify differences in benthic community composition (species level) and food web structure (based on feeding guilds) in relation to site (outcrop, 20 m, and 600 m sites). Log-transformation accounted for zero-inflated data, and we zero-adjusted Bray-Curtis similarity applied to abundance matrices before plotting the results in nMDS (non-metric multidimensional scaling) with PRIMER v6 (Clarke and Gorley, 2006). Because fauna data sets often include large numbers of zeros, Bray-Curtis similarity was more suitable than Euclidean distance (Clarke and Gorley, 2006). We tested for significant differences among sites with PERMANOVA, performed with 9999 random permutations using the PERMANOVA+ add on in PRIMER v6 (Anderson et al., 2008). We ran the pair-wise comparisons as post hoc analysis whenever significant differences appeared. We used a percent similarity procedure (SIMPER) analysis and nMDS vectors to identify the taxa/feeding guilds that distinguished assemblages among sites.

As a measure of heterogeneity in community and food web structure, and food (quantity and quality) we ran a PERMDISP (Anderson et al., 2008) routine between sites (i.e., outcrop, 20 m, and 600 m) based on species composition, feeding-guild composition, and food composition (% TOC, % TN, C:N, δ^{13}C, δ^{15}N, Chl *a*, Chl *a*:TOC, Chl *a*: Pheo, total lipids, hydrocarbon, HC: TOC) separately.

In order to measure the spatial extent of the community that characterizes the outcrop we examined total abundance and biodiversity, by grouping the samples per WP [i.e., WP3 and

WP12 (outcrop); WP14 and WP16 (20 m site); 600 m remained the same] instead of actual sites (i.e., outcrop, 20 m, and 600 m). Because WP12 appeared to be most strongly influenced by the hydrates (i.e., most depleted δ^{13}C signature and higher concentrations of hydrocarbons) we assumed it was the most active and therefore considered it as the edge of the gradient in our study. We performed a one-way ANOVA with site (levels: WP3, WP12, WP14, WP16, and 600 m) as fixed factors, on total abundances and ES$_{[10]}$. Transformation (4√) of total abundances attained normality of residuals and homogeneity of variances. Whenever significant differences were found we conducted a post hoc Tukey test.

We used two principal coordinate analysis (PCoA) to describe, first, food quantity, quality, and source (based on Euclidean distance) and second, fatty acids (based on Bray-Curtis similarity) in space and sediment depth. Food quantity, quality, and source were standardized prior to analysis. We chose Bray-Curtis similarity for fatty acids rather than Euclidean distances commonly used for environmental variables (Clarke and Gorley, 2006) because it can handle zero-inflated data.

In order to identify the best environmental predictors of community composition and trophic structure, we performed distance-based linear models (DistLM) and visualised the results with ordination of distance-based redundancy analysis (dbRDA). We conducted separate analyses for different groupings of environmental variables: sediment type [mean grain size (MGS) and % mud], topography [depth, slope, aspect, curvature, planar curvature, profile curvature, FBPI, and VRM], food quantity (TOC), food quality (TN, C:N, Chl *a*:Phaeo, total lipids, and hydrocarbons), food source (δ^{13}C, δ^{15}N, Chl *a*:TOC, HC:TOC), and then all groupings together (i.e., sediment type, topography, food quantity, quality and source). Draftsman's plots of predictor variables were calculated to identify high correlations ($r \geq 0.95$) among predictors (i.e., we chose TOC over TN, and HC over HC:TOC) (Clarke and Gorley, 2006). We based the

constrained ordination on step-wise and AICc (small-sample-size corrected version of Akaike information criterion) procedures. We compared the AICc between different models for accuracy and examined R^2 to identify the best model and determine the proportion of the variation explained by the model.

4.4. Results

4.4.1. Benthic community and food web

4.4.1.1. Total abundances and diversity

In total we identified 360 individuals belonging to 85 different taxa from 19 cores representing 3 different sites (outcrop, 20 m, and 600 m). Total abundance per core decreased with distance from the outcrop, with outcrop > 20 m > 600 m (Fig. 4.2a). We found statistically significant differences among sites (one-way ANOVA, Site: $F_{2,16} = 4.55$, P = 0.027). Post hoc Tukey tests revealed significantly higher abundances at the outcrop than the 600 m site (P = 0.039) and borderline significance between the outcrop and 20 m site (P = 0.054). We also found significant differences in total abundances with sediment depth which differed at each site (Table 4.1 and Fig. 4.2b), with lower abundances in the 5-10 cm layer than in other sediment layers at the 20 and 600 m sites but no differences with sediment depth at the outcrop (Fig. 4.2). Even though diversity (rarefaction curves) was lower at the outcrop (Fig. 4.3), differences were not significant (one-way ANOVA, $ES_{[10]}$: $F_{2,16} = 0.42$, P = 0.667). Trophic diversity was significantly higher at the outcrop site than the other two sites (Fig. 4.3).
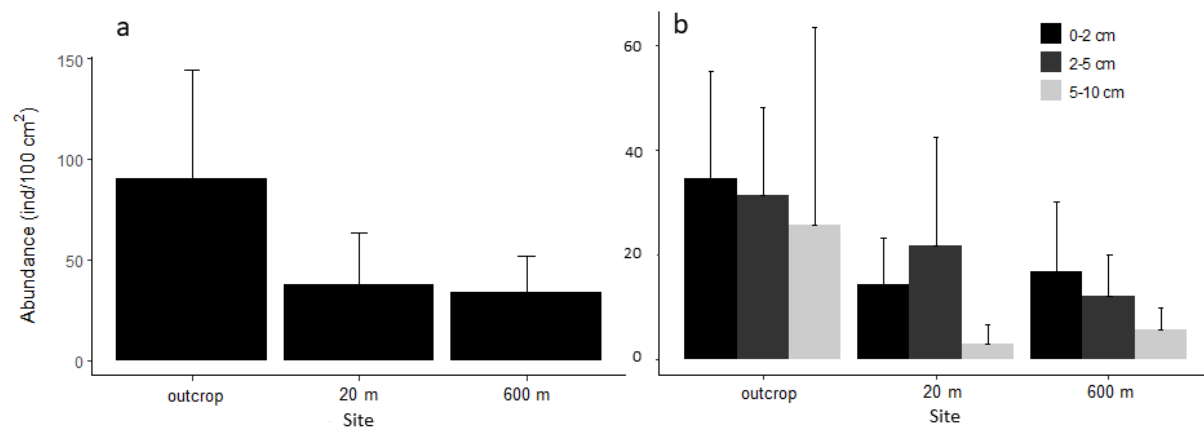
Figure 4.2. Mean infaunal abundances (ind/100 cm^2) (a) per core and (b) per sediment depth, at the outcrop clam bed (n = 6), 20 m (n = 6), and 600 m (n = 7) site sampled in September 2013 in Barkley Canyon (~ 900 m depth, NE Pacific). Error bars denote standard deviations.
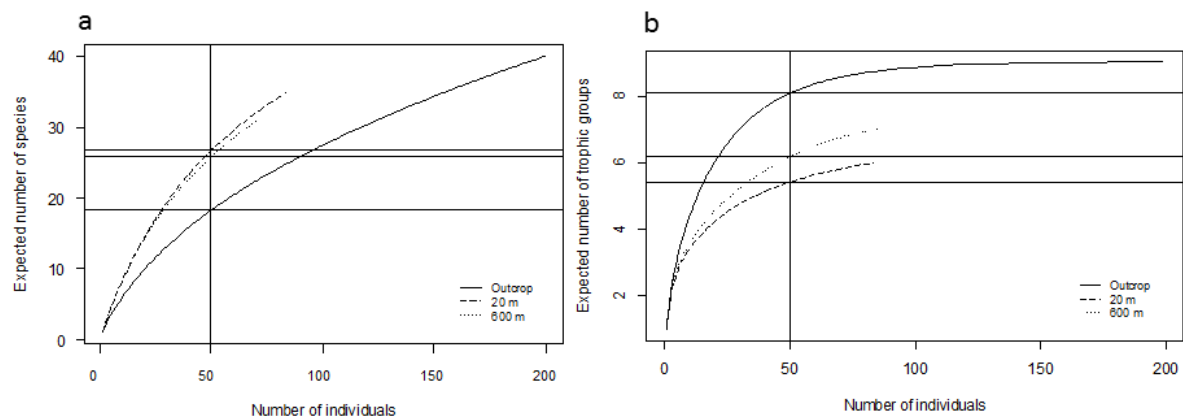
Figure 4.3. Rarefaction curves based on (a) species and (b) trophic guilds from each site sampled in Barkley Canyon (~ 900 m depth, NE Pacific). Expected number of species/trophic groups at each site, with straight horizontal lines indicating different Expected species for 50 individuals.

Table 4.1. Non-parametric Kruskal-Wallis and Dunn's test results of the effect of sediment depth (i.e., 0-2, 2-5, and 5-10 cm) on infaunal total densities at each Barkley Hydrates site (i.e., outcrop, 20 m, and 600 m). Significant p-values (< 0.05) in bold.

Site	df	χ2	P	Dunn's test
Outcrop	2	3.02	0.220	--
20 m	2	6.64	0.036	0-2 = 2-5 > 5-10
600 m	2	6.55	0.037	0-2 = 2-5 > 5-10

4.4.1.2. Species and feeding guilds composition

PERMANOVA showed significant differences in benthic community composition (analysis based on taxa) between sampling sites (pseudo-$F_{2,16}$ = 2.68, P < 0.001). Pairwise test revealed that the outcrop, 20 m, and 600 m communities all differed significantly from each other (P < 0.05), and also clearly separated in ordination space (Fig. 4.4a). Oligochaetes (Naididae) dominated the outcrop community (33 % contribution to group similarity: Table 4.2), together with thyasirid, dorvilleid polychaetes, *Anobothrus apaleatus* (Ampharetidae), and ampharetid recruites, which characterised the outcrop (Fig. 4.4a and Table 4.1). No single species dominated the other two sites, where representatives of paraonid, cossurid, ampharetid, and cirratulid polychaetes were common (Fig. 4.4a and Table 4.1). However, thyasirids, characteristic at the outcrop, were also present at the 20 m site (Table 4.1). A few taxa that occurred at the outcrop appeared at the 20 m site in very low abundances (i.e., Naididae, *Acharax* cf. *johnsoni*, Phoxocephalidae, and Dorvilleidae). SIMPER analysis identified oligochaetes (Naididae) as the main taxon differentiating the outcrop from the other two sites.
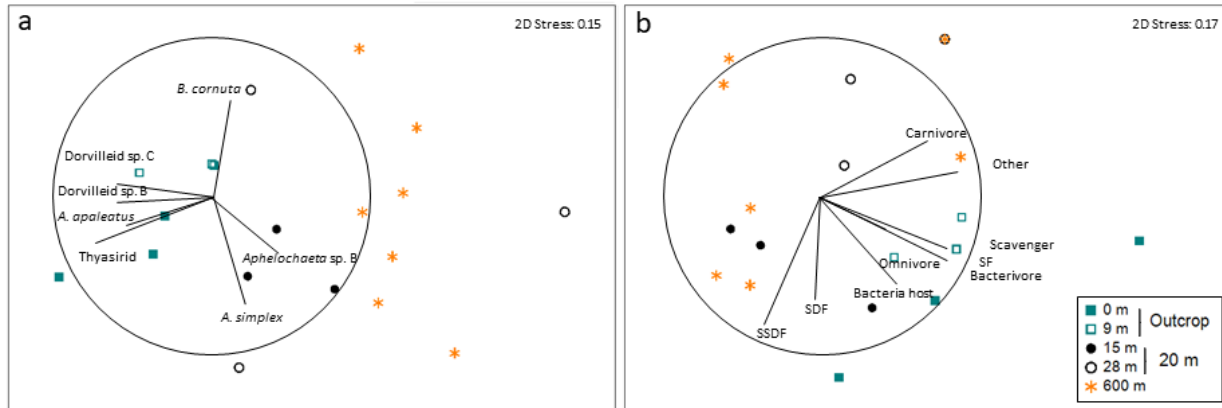
Figure 4.4. Nonmetric multidimensional scaling (nMDS) of the macrofaunal communities sampled in September 2013 in Barkley Canyon (~ 900 m depth, NE Pacific) based on (a) species and (b) trophic groups. Data has been log (X + 1) transformed and a dummy variable added to avoid double zero problems. (i.e., comparison between two samples that have no organisms). The associated matrix was based on Bray-Curtis similarity. *B. cornuta = Bipalponephtys cornuta*; *A. apaleatus = Anobothrus apaleatus*; *A. simplex = Aricidea (Acmira) simplex*; SSDF = Subsurface deposit feeder; SDF = Surface deposit feeder; SF = Suspension feeder.

Table 4.2. Output of SIMilarity PERcentage (SIMPER) analysis showing characterizing taxa contributing to similarity within sites at the outcrop and different distances from it. Note that Avg. Abund was calculated from the log-transformed data. Data analysed per core.

OUTCROP: Average similarity: 29.30

Species	Av.Abund	Av.Sim	Sim/SD	Contrib %	Cum. %
F. Naididae	1.93	9.74	1.06	33.25	33.25
Thyasirid	0.94	4.81	1.21	16.42	49.67
Dorvilleidae sp C	0.71	3.48	0.77	11.88	61.55
F. Ampharetidae Recruits	0.58	2.44	0.78	8.34	69.89
Dorvilleidae sp B	0.73	2.44	0.78	8.32	78.2

20 m: Average similarity: 14.75

Species	Av.Abund	Av.Sim	Sim/SD	Contrib %	Cum. %
Levinsenia occulata	0.81	2.37	0.47	16.04	16.04
Levinsenia gracilis	0.35	2.21	0.44	14.95	30.99
F. Ampharetidae recruits	0.41	1.61	0.47	10.92	41.92
Thyasirid	0.35	1.52	0.47	10.3	52.22
F. Cirratulidae sp. C	0.41	1.45	0.47	9.82	62.04
Cossura sp. A	0.35	1.38	0.48	9.32	71.36
Aricidea (Acmira) simplex	0.37	1.02	0.26	6.9	78.26

600 m: Average similarity: 19.53

Species	Av.Abund	Av.Sim	Sim/SD	Contrib %	Cum. %
Aphelochaeta sp. B	0.51	3.17	0.58	16.23	16.23
F. Ampharetidae recruits	0.64	2.99	0.61	15.3	31.53
Levinsenia occulata	0.5	2.75	0.61	14.08	45.61
Bipalponephtys cornuta	0.3	1.7	0.4	8.68	54.29
Levinsenia sp. recruits	0.41	1.5	0.4	7.66	61.95
Eclysippe trilobata	0.35	1.23	0.4	6.3	68.25
Cossura sp. A	0.4	1.19	0.39	6.1	74.35

PERMANOVA showed significant differences in feeding guilds among sampling sites (pseudo-$F_{2,16}$ = 3.81, P = 0.001). Pair-wise comparisons identified significantly (P < 0.05) different food

web structure at the outcrop from the 20 and 600 m sites. A larger variety of feeding guilds occurred at the outcrop site than the 20 and 600 m sited (Fig. 4.4b, and SIMPER). SIMPER analysis identified proportions of major feeding guilds (i.e., SDF and SSDF) as the major factor differentiating food webs at different sites.

4.4.2. Trophic characterization

The first two axes from PCoA based on sediment organic matter data (i.e., quantity, quality, and source) accounted for 88.8 % of the total variation (Fig. 4.5a). The first principal coordinate (PCO1) separated samples based on distance from the outcrop. Some of the outcrop site sites, corresponding to WP12, scored positively in PCO1, with higher amounts of total lipids, hydrocarbons, and proportion of hydrocarbons within the carbon pool, and a more depleted $\delta^{13}C$ signature. PCO1 did not resolve outcrop samples from WP3, showing high environmental heterogeneity within the outcrop site. The 600 m site scored negatively in PCO1 and was characterized by high amounts of chlorophyll *a* and a larger contribution of chloropigments to the organic carbon pool. Samples from the 20 m site appeared intermediate between the other two sites in trophic structure based on PCO1. The second principal coordinate (PCO2) separated the 20 m site from the other two sites; low amounts of food (TOC, TN) of high C:N ratio characterized the negatively scored 20 m site.
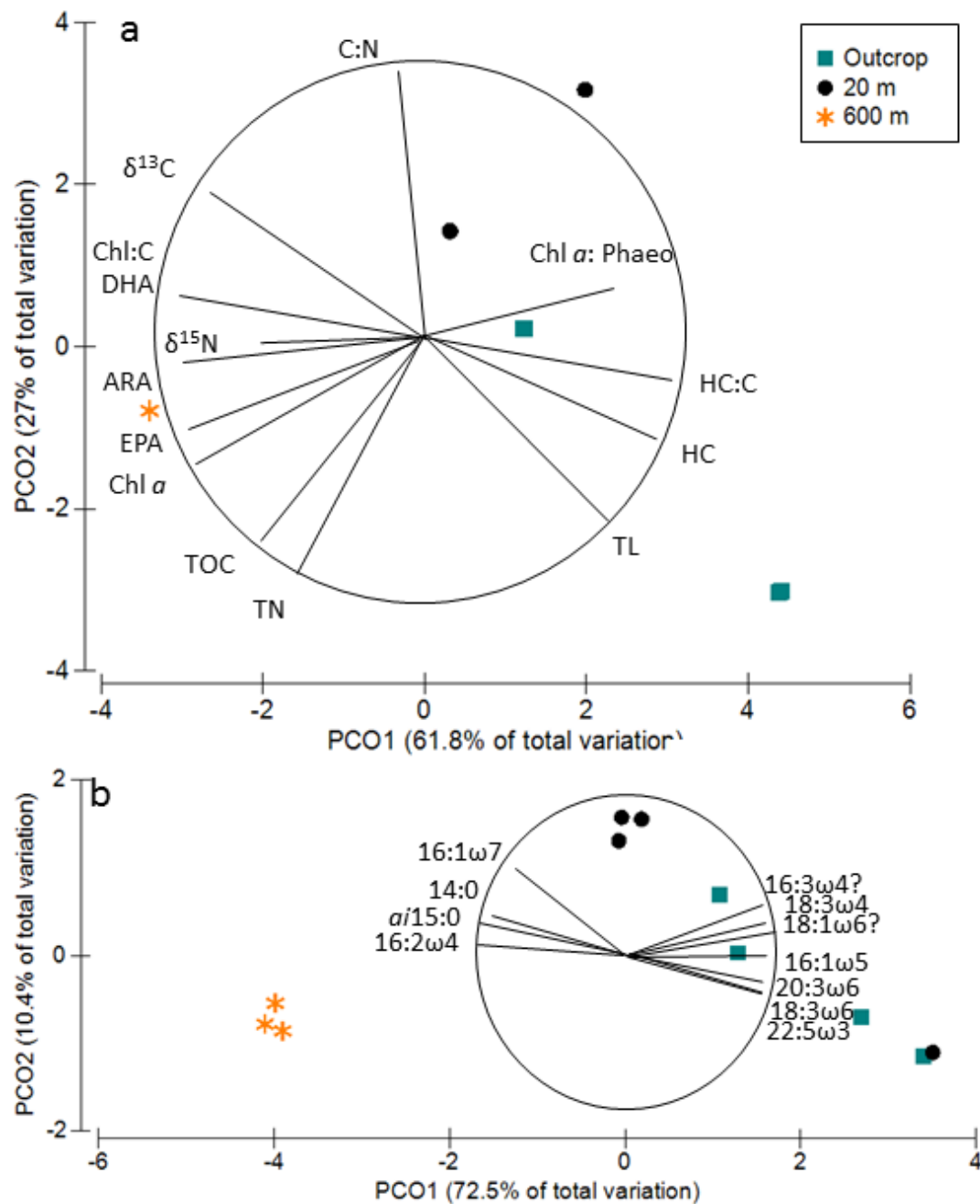
Figure 4.5. (a) Euclidean distance-based principal coordinates analysis (PCoA) performed on normalised sediment organic matter characteristics and (b) Principal coordinates analysis (PCoA) based on Bray-Curtis similarity on log(x+1) transformed data and performed on FA composition (%) sampled in Barkley Canyon, NE Pacific. Only vectors with >0.95 correlation shown. Essential fatty acids were forced into the dbRDA: ARA, EPA, DHA (0.8 correlation for all of them).

PCoA reduced the fatty acid (%) composition of the sediments to 2 principal coordinate axes (explaining 82.9 % of the total variation; see Fig. 4.5b). PCO1 separated sites with distance from the outcrop. Fatty acids 16:3ω4? (tentatively identified), 18:3ω4, 18:1ω6? (methanotrophic and sulfur-oxidizing bacteria), 16:1ω5 (bacteria), 20:3ω6, 18:3ω6 (macroalgae), and , 22:5ω3, characterised the outcrop and 20 m sites, whereas 16:1ω7 (mangrove, diatom, bacteria), 14:0 (proteobacteria, diatoms, prymnesiophytes), ai15:0 (bacteria), and 16:2ω4 (bacteria) characterised sediments at the 600 m. PCO2 separated the 20 m site from the outcrop sediments.

4.4.3. Patchiness

The different measures of heterogeneity used here imply a specific level of environmental patchiness. The PERMDISP analysis identified between-site differences in data-cloud dispersion of feeding guild and food composition but not of species composition (Table 4.3).

Table 4.3. Output of PERMDISP routing showing differences in data cloud dispersion, and therefore, heterogeneity/homogeneity, between sites (i.e., outcrop, 20 m, and 600 m). The analyses are based on species, feeding guild, and sediment food composition.

Source	df1	df2	F	P (perm)	Pairwise
Species composition	2	16	2.22	0.225	--
Feeding guild composition	2	16	0.06	0.948	--
Food composition	2	54	85.91	< 0.001	Outcrop > 20 m > 600 m

Analysis at greater spatial resolution but lower replication used individual WP as new sites, and were classified according to distance to the most chemosynthetically active site: WP12 (outcrop, most active site), WP 3 (outcrop, 9 m from the most active site), WP14 (20 m site): 15 m; WP16 (20 m site, 28 m form the most active site); and 600 m site (Fig. 4.1). The results of these

analyses indicated decreasing total abundance with distance from the most methane-active site sampled (two-way ANOVA, WP: $F_{4,14} = 6.74$, $P = 0.003$), with significant differences ($P < 0.05$) between WP12 and WP16, and between WP12 and the 600 m site (Fig. 4.6). Despite the obvious lower expected number of species in the most active site (WP12), we found no significant differences between Waypoints (ANOVA, $F_{4,14} = 2.68$, $P = 0.0751$).
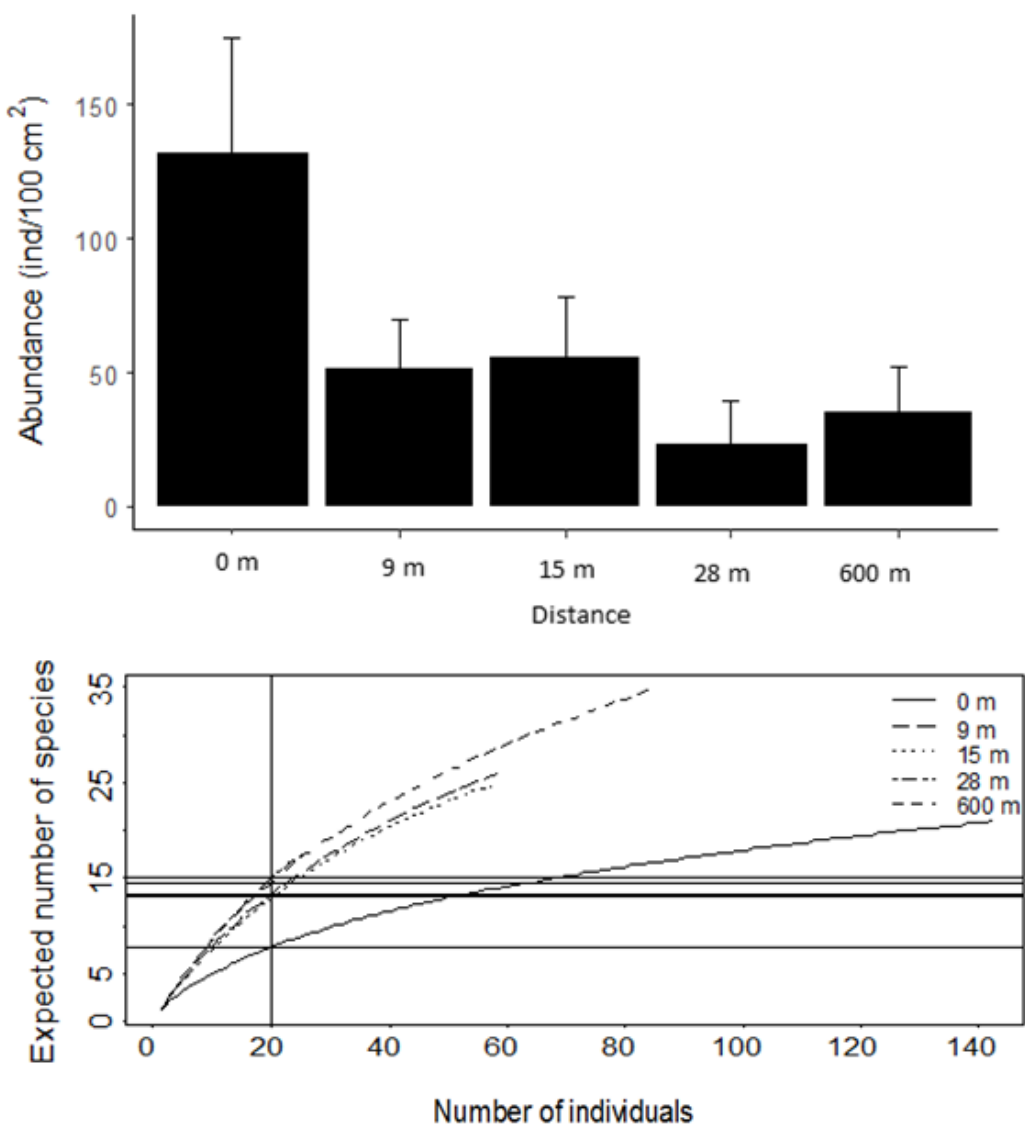
Figure 4.6. Total abundance and rarefaction curves from infaunal communities with distance from the most active site in Barkley Hydrates. Error bars denote standard deviations, with straight horizontal lines indicating different Expected species for 20 individuals.

4.4.4. Food and other environmental drivers

The distLM results (Table 4.4) better explained total variation with trophic guilds than with species composition. Topography, food quality, and the combination of all variables yielded the best models (higher % of total variability explained). The best distance-base linear model (distLM) explained 33.6 % of trophic structure. Hydrocarbons (21.9 %) and C:N ratio (11.6 %) were the strongest environmental predictors in the model (per cent of variability explained in brackets). The first axis of the dbRDA separated the samples from distance from the most active site in the outcrop (WP12), with hydrocarbons as the major explanatory variable. The second axis of the dbRDA separated WP16with C:N ratio as the major explanatory variable (Fig. 4.7).

Table 4.4. Distance-based linear models (distLM) results (AICc) for infaunal communities sampled in September 2013 in Barkley Canyon (~ 900 m depth, NE Pacific). Each model examines different faunal data sets (species or trophic group-based) and environmental predictors. MGS = Mean grain size, HC = Hydrocarbons, crv = curvature, Chl *a*/Phaeo = Chlorophyll *a*/Phaeopigments, TOC = total organic carbon. Best models per faunal-based analysis are highlighted in bold, overall best model highligted in bold and italicized.

Fauna	Environmental variables	Best model	AICc	R^2 %
Species	**Sediment type**	**~ MGS + % mud**	**159.2**	**19.0**
	Topography	**~ crv**	**156.9**	**16.7**
	Food quantity	~ TOC	158.5	9.1
	Food quality	**~ Chl *a*/Phaeo**	**157.0**	**16.0**
	Food source	~ δ^{13}C	157.0	15.9
	All variables	**~ Chl *a*/Phaeo**	**157.0**	**16.0**
Trophic guilds	Sediment type	~ MGS + % mud	129.1	27.3
	Topography	**~ slope + crv**	**127.5**	**33.2**
	Food quantity	~ TOC	Non-significant	Non-significant
	Food quality	*~ C:N + HC*	*127.4*	*33.6*
	Food source	~ δ^{13}C	127.7	21.9
	All variables	**~ δ^{13}C + C:N**	**127.4**	**33.5**
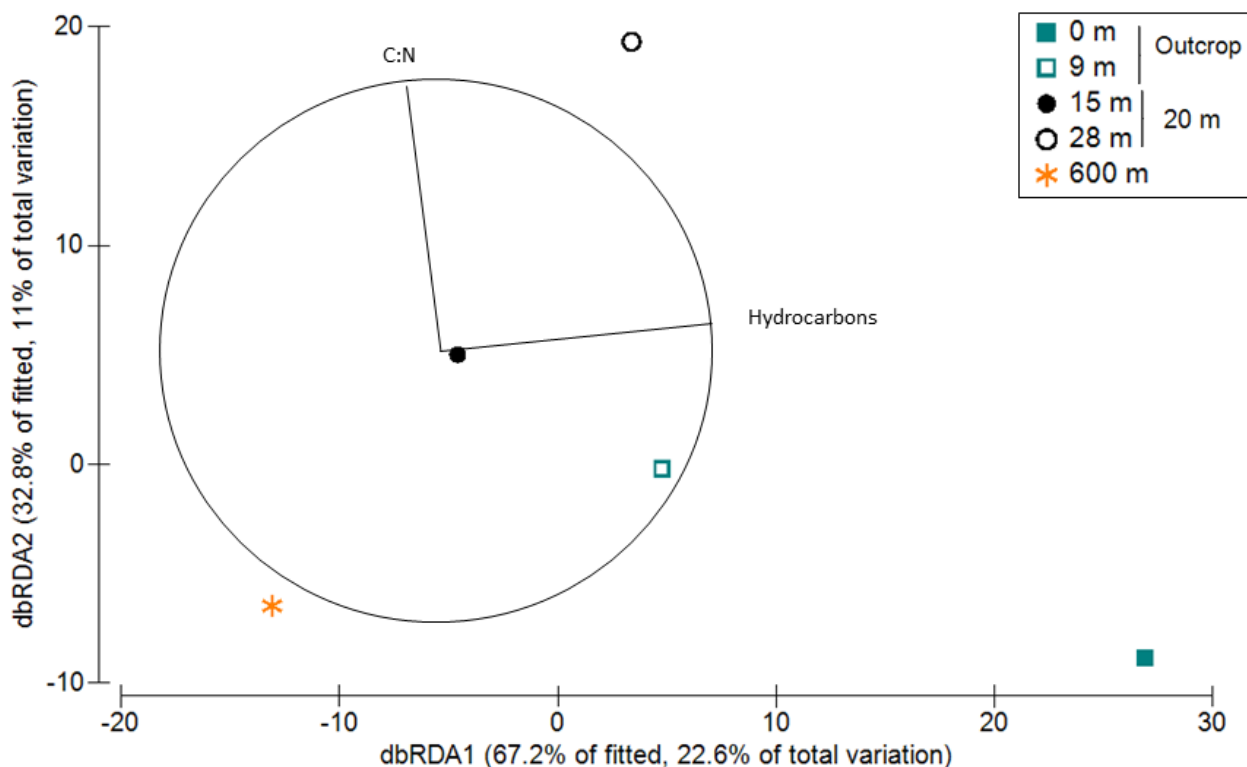
Figure 4.7. Redundancy analysis (dbRDA) from the best distance based linear model (distLM) of the trophic groups and all environmental variables sampled in Barkley Canyon (~ 900 m depth, NE Pacific).

4.5. Discussion

4.5.1. Food distribution in Barkley Hydrates and scale: patchiness

Food composition varied not only among sites but also within sites, with the most diverse and "patchy" trophic composition at the outcrop, followed by the 20 m and the 600 m sites. We attribute differences among within-site heterogeneity and therefore, patchiness, to the relative contributions of the two main food sources (i.e., phytodetritus and chemosynthetically-derived organic matter). The 600 m site only received phytodetrital food, as indicated by the high Chl a:TOC ratio, enriched $\delta^{13}C$ signature, and extremely low HC:TOC ratio. We found the opposite result for outcrop sediments, with the lowest Chl a:TOC ratio, depleted $\delta^{13}C$ signature, and an extremely high HC:TOC ratio. Despite the strong chemosynthetic signal of the sedimentary

organic matter at the outcrop, Chl *a* values, although low, nonetheless indicate the presence of phytodetrital food. The food composition of the 20 m site resembled that at the 600 m sites with some characteristics from the outcrop (e.g., presence of hydrocarbons, in particular at the deeper sediment layers). The high patchiness in food composition from the outcrop sediments suggests patchiness at small spatial scales (i.e., between WP from the same site and, therefore, less than 10 m).

Unexpectedly, the input of two distinct food sources at the outcrop did not result in higher total organic carbon (TOC) than the 600 m site where only one food source (from a photosynthetic origin) provided organic matter. At the same time, coarser sediments at the outcrop and 20 m sites compared to the 600 m site suggest stronger currents and therefore reduced deposition of finer sediments (Van Rijn, 1993) and thus, organic matter. Therefore, the two sites located away from the outcrop (i.e., 20 and 600 m) apparently differed in inputs of organic matter from the water column, which may explain an apparently higher TOC at 600 m compared to the outcrop. Despite the higher TOC at 600 m, total abundances were lower than at the outcrop and similar to those 20 m site suggest that variables other than TOC alone shape community structure parameters such as benthic stock. Another possible explanation for the high abundances and low TOC at the outcrop involves the reduction of TOC by infaunal consumption, as reported in some shallow-water environments (Canuel et al., 2007).

4.5.2. Barkley Hydrates trophic diversity and scale: Niche opportunity

The trophic groups separated in the nMDS parallel the patterns observed in community composition. We hypothesized that increased food heterogeneity or "food patchiness" within the outcrop could lead to more trophic niche opportunities, and in fact, a larger variety of feeding guilds occupied the surface layers of the outcrop (i.e., surface deposit feeders, suspension

feeders, carnivores, scavengers, bacterivores) compared to the other sites and sediment layers. Oligochaetes, the dominant taxon in the outcrop, are subsurface deposit feeders. Their rarity 600 m away from the methane source suggests some additional benefit from the hydrates, potentially related to food. The presence of bivalves in these deeper sediment layers, such as *Acharax* sp. which harbour sulfur oxidizers (pers. obs.) as symbionts, and thyasirids and dorvilleids, which can feed on free living bacteriasuggests use of chemosynthesised organic matter (Bernardino et al., 2012). The addition of scavengers, omnivores, and bacterivores to the abundant surface and subsurface deposit feeders are most responsible for this increase in trophic diversity at the outcrop compared to background sites. Surface deposit feeders and suspension feeders dominated the 20 and 600 m.

The dominance of oligochaetes at the outcrop reduced diversity but this site also featured the highest numbers of feeding guilds. Furthermore, greater patchiness in trophic composition at the outcrop coincided with greater patchiness in food web structure. These results suggest increased niche opportunities for different species and feeding guilds, likely arising from increased food patchiness. Patchiness may vary with spatial scale (e.g., region, habitat) and may influence benthic communities and food webs in different ways. For example, distinct habitats within chemosynthetic environments (e.g., bacterial mats, clam beds, background sediments) support distinct infaunal communities, thereby increasing overall diversity (Levin et al., 2010). In examining patchiness within sites, we found greater variability within outcrop community composition and in food web structure compared to the 20 and 600 m sites, suggesting that methane hydrates increase small-scale patchiness (i.e., meters to 100's of meters). A recent study of clam beds similar to those we sampled from within the outcrop reported similarly increased patchiness in infaunal community structure (Guillon et al., 2017). Other studies further suggest

patchiness within chemosynthetic environments (Cordes et al., 2010). Different habitats from cold seeps of the NE Atlantic varied in stable isotope signatures for infaunal food webs (Decker and Olu, 2012); the infauna from siboglinid fields varied most for both δ^{13}C and δ^{15}N signatures, suggesting additional trophic guilds and therefore niches. Dorvilleid polychaetes partition their trophic niches within the cold seeps from Hydrate Ridge and Eel River (NE Pacific) (Levin et al., 2013), suggesting some degree of food patchiness that increases diversity. Spatial and temporal variation in fluid supply also contributes to the patchy and ephemeral occurrence of chemosynthesis-dependent fauna (Sibuet and Olu, 1998). High variability in total abundances at the 5-10 cm sediment layer at our outcrop site support this idea, suggesting that variable methane supply contributes to greater patchiness at that site.

4.5.3. Barkley Hydrates infaunal community and spatial extent

Species diversity (ES) decreased slightly at the outcrop compared to the 20 m and 600 m sites, likely as a result of oligochaete dominance at the outcrop site, particularly in deeper sediment layers (i.e., 2-5 and 5-10 cm). Oligochaetes (Naididae) have been reported from other seeps along the NE Pacific at varying densities (Sahling et al., 2002, Levin et al., 2010). Sediments often become anoxic below the top few millimeters to centimeters, confining the vast majority of organisms to sediment surface layers (Snelgrove, 1999) as seen at the 20 and 600 m sites. Previous studies report oxygen penetration depth in Barkley Canyon, defined as the mean depth where oxygen concentration decreased below suboxic conditions (i.e., 5 μmol L^{-1}: Belley et al., 2016), and support this hypothesis. The suboxic conditions reached 7.3 mm in Barkley Hydrates (same outcrop than our study) and at the equivalent to our 600 m site (BMC in Belley et al., 2016). However, some oligochaetes tolerate extremely low oxygen concentrations, and may occur as deep as 20 cm in sediments (Giere and Pfannkuche, 1982). Those same species

reportedly tolerate hydrogen sulfide (H_2S) (Giere and Pfannkuche, 1982), which, although we did not explicitly measure it, is usually a major stressor for benthic fauna in chemosynthetic environments (Levin et al., 2013). These characteristics explain high abundances of oligochaetes in the deeper sediment layers at the outcrop, despite rarity at the 600 m site. Overall, total densities in the outcrop were lower than reported densities from cold seeps at similar depths but without the influence of an OMZ (Levin, 2003), suggesting a strong influence of bottom-water dissolved oxygen concentrations on total densities.

Dorvilleid and ampharetid polychaetes also characterized sediments from the outcrop with contributions from phoxocephalid amphipods. Dorvilleid polychaetes typically occur in disturbed sediments, including cold seeps (Bernardino et al., 2012), and can tolerate high levels hydrogen sulfide (H_2S) (Levin et al., 2013). Extremely dense ampharetid assemblages characterize other methane seeps (Thurber et al., 2013) and phoxocephalid amphipods have been reported from cold seeps at the Barbados accretionary prism (Bellan-Santini, 1997).

Similar contributions of paraonid, cossurid, and ampharetid polychaetes in sediments resulted in high diversity at the 600 m site. Paraonid polychaetes frequently dominate continental shelf and slope sediments (Blake et al., 1996a). Both cossurid and ampharetid polychaetes are often found in cold seeps and background sediments (Blake et al., 1996a, Blake et al., 1996b, Levin, 2005), however, in our study, only ampharetid polychaetes were common in both areas, although with different species represented. Cossurid polychaetes are widely distributed, and often play an important role in the community composition of deep-sea benthic assemblages (Blake et al., 1996a) and ampharetids occupy a wide range of environments, most likely because they adapt easily (in an evolutionary sense) to new environments, and they can also dominate some deep-sea ecosystems (Blake, et al., 1996b).

To understand the spatial extent of the hydrates influence on infaunal communities and food webs we examined our faunal samples at high spatial resolution (and thus decreased replication). These analyses showed intermediate communities with total abundances, diversities, and species composition in between the outcrop and the background levels at 9 (WP 3) and 15 m (WP 14) away from most active WP (WP 12) (lowest δ^{13}C organic matter signature), which decreased 28 m (WP 16) away. These results suggest an ecotone effect whereby taxa with different ecological requirements coexist at the transition between the two habitats (Risser, 1995). Temporal variability associated with methane seepage (Thomsen et al., 2012) can affect benthic epifauna (Purser et al., 2013) and infauna (Guillon et al., 2017, Campanyà-Llovet and Snelgrove,2018), thereby obscuring an otherwise distinct ecotone. Many ecosystem services associated with the interactions and transitions between chemosynthetic and background ecosystems point to a need to fully understand these transitional environments (Levin et al., 2016). In our study, we found methanotrophic and sulfur bacteria and archaea (Kelly and Scheibling, 2012) biomarkers 20 m from the main outcrop. FA biomarkers can be used as indicators or signatures of individual organisms or groupings of organisms, and have been broadly used as biomarkers in trophic transfer studies in aquatic food webs (Parrish, 2013). Through consumption of methane, these microorganisms act as biological filters that reduce concentrations of these climate-relevant molecules (Sommer et al., 2006). Therefore, in addition to the bacterial mats found in the outcrop, the ecotone, therefore, might play a significant role in the biological filter, although with less intensity, depending on its spatial extent.

4.5.4. Relative contribution of food to benthic community composition and food web structure

Environmental predictors chosen in this study better explained the variation observed in trophic than community structure. Food quality, source, and topographic features explained greater

variability in community and food web structure than sediment type, and food quantity alone. Sediment grain size alone could not explain a substantial portion of the variability in food web composition, although topography did. Many studies identify these variables as major environmental drivers (Harris and Baker, 2012). Admittedly our study addressed a relatively narrow range of sediments and topography and therefore focused on more subtle drivers of change. TOC usually predicts benthic stock of communities (Rex and Etter, 2010), however, in our study, highest TOC occurred at 600 m whereas total abundances were greatest at the outcrop. Because we did not measure biomass, we cannot rule out TOC as a driver of benthic stock, however, we also did not note any obvious differences in size structure in our samples. Alternatively, the high number of trophic niches at the outcrop could contribute to elevated abundances at that site. High amounts of hydrocarbons and lipids (i.e., outcrop) or high amounts of chlorophyll *a* (i.e., 600 m), characterize areas with high TOC (i.e., outcrop and 600 m site) (Appendix 4.1), demonstrating the distinct nature of the TOC increase.

Our best explanatory models included multiple food parameters (i.e., source and quality) and one parameter each for sediment type and topography. These results point to the relative importance of food in shaping infaunal food web structure, but also to the large number of contributing factors. Furthermore, our best models explained only ~ 33 % of faunal variation, pointing to contributions from other variables not measured in our study. Major environmental drivers of cold-seep benthic communities center on the toxic effects of H_2S as well as habitat heterogeneity (i.e., bacterial mats-clam beds-siboglinid beds, etc.: Levin et al., 2003, Levin et al., 2006, Bernardino et al., 2012, Levin et al., 2013), emphasising the important role they play in species' distributions. Hydrogen sulfide interferes with the aerobic metabolism of organisms, resulting in toxicity for most species, even at low concentrations (Bagarinao, 1992). Furthermore, other

studies suggest that the structure of food webs at cold seeps results from the complex interplay between environmental filtering, provision of energy, the engineering role of the large symbiont-bearing invertebrate as well as trophic and non-trophic interactions among species (Portail et al., 2016). Another explanation for the significant but limited explanatory power of our food web model is that smaller-sized fauna such as meiofauna may respond more strongly and directly to trophic composition than the larger-sized macrofauna that were the focus of our study (Campanyà-Llovet et al., 2017), and therefore, may better utilise organic matter resources. Furthermore, the high variability observed within sites suggests that samples for organic matter and infauna taken from the same core could reduce spatial differences associated with the use of separate push cores for each analysis. Our results showed that organic matter trophic composition explained a substantial percentage of variability in community composition but a greater percentage of food web structure, indicating that future studies should consider additional parameters (biological or environmental) in order to understand better macrofaunal food web structure at Barkley Hydrates.

4.6. Conclusions

The input of two different food sources at Barkley Hydrates contribute to greater food patchiness in the outcrop than at 20 or 600 m, and therefore, the "patch mosaic theory" may be particularly relevant for infaunal communities from deep-sea chemosynthetic environments. Insufficient spatial resolution in sampling may miss important gradients, which also depend on size ranges of individuals (e.g., meio-, macro-, and megafauna). Barkley hydrates support a transitional community at 9 to 15 m from the most active site at the outcrop, where macrofaunal α-diversity increased, albeit weakly. A strong mosaic in trophic patchiness may not only increase infaunal number of species but also structure infaunal food webs by increasing trophic niche

opportunities. Although environmental variables, including toxicity, play an important role in determining community composition and food web structure in deep-sea chemosynthetic environments, food quality and source also play significant roles in the spatial distribution of infaunal communities and food webs at Barkley Hydrates.

4.7. Acknowledgements

4.8. References

Anderson, M., Gorley, R., Clarke, K. 2008. PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E. Plymouth, UK.

Bagarinao, T. 1992. Sulfide as an environmental factor and toxicant: tolerance and adaptations in aquatic organisms. Aquatic Toxicology, 24, 21-62.

Bellan-Santini, D. 1997. Amphipods of the cold seep community on the South Barbados accretionary prism. Crustaceana, 70, 1-30.

Belley, R., Snelgrove, P.V.R., Archambault, P., Juniper, S.K. 2016. Environmental drivers of benthic flux variation and ecosystem functioning in Salish Sea and Northeast Pacific sediments. PLoS One, 11, e0151110.

Bernardino, A.F., Levin, L.A., Thurber, A.R., Smith, C.R. 2012. Comparative composition, diversity and trophic ecology of sediment macrofauna at vents, seeps and organic falls. PLoS One, 7, e33515.

Blake, J., Hilbig, B., Scott, P. 1996a. Taxonomic atlas of the benthic fauna of the Santa Maria Basin and western Santa Barbara Channel: vol. 6, The annelida part 3: Polychaeta: Orbiniidae to Cossuridae. Santa Barbara Museum of Natural History. Santa Barbara, California.

Blake, J., Hilbig, B., Scott, P.H. 1996b. Taxonomic atlas of the benthic fauna of the Santa Maria Basin and western Santa Barbara Channel: vol. 7, The annelida part 4: Polychaeta: Flabelligeridae to Ampharetidae. Santa Barbara Museum of Natural History. Santa Barbara, California.

Canuel, E.A., Spivak, A.C., Waterson, E.J., Duffy, J.E. 2007. Biodiversity and food web structure influence short-term accumulation of sediment organic matter in an experimental seagrass system. Limnology and Oceanography, 52(2): 590-602.

Campanyà-Llovet, N., Snelgrove, P.V.R. 2018. Effects of temporal variation in food sources on infaunal community structure of chemosynthetic and non-chemosynthetic environments in Barkley Hydrates, British Columbia, Canada. Deep-Sea Research Part I: Oceanographic Research Papers. In press.

Campanyà-Llovet, N., Snelgrove, P.V.R., Parrish, C. 2017. Rethinking the importance of food quality in marine benthic food webs. Progress in Oceanography, 156, 240-251.

Chapman, R., Pohlman, J., Coffin, R., Chanton, J., Lapham, L. 2004. Thermogenic gas hydrates in the northern Cascadia margin. Eos, Transactions, American Geophysical Union, 85, 361.

Christie, W.W. 1982. Lipid analysis. Pergamon press, Oxford, UK.

Clarke, K., Gorley, R. 2006. PRIMER v6: User manual/tutorial. PRIMER-E. Plymouth, UK.

Cordes, E.E., Cunha, M.R., Galeron, J., Mora, C., Olu-Le Roy, K., Sibuet, M., Van Gaever, S., Vanreusel, A., Levin, L.A. 2010. The influence of geological, geochemical, and biogenic habitat heterogeneity on seep biodiversity. Marine Ecology - an Evolutionary Perspective, 31, 51-65. doi: 10.1111/j.1439-0485.2009.00334.x.

Decker, C., Olu, K. 2012. Habitat heterogeneity influences cold-seep macrofaunal communities within and among seeps along the Norwegian margin–Part 2: contribution of chemosynthesis and nutritional patterns. Marine Ecology, 33, 231-245.

Equihua, M. 1990. Fuzzy clustering of ecological data. The Journal of Ecology, 519-534.

Giere, O., Pfannkuche, O. 1982. Biology and ecology of marine Oligochaeta, a review. Oceanography and Marine Biology, 20, 173-309.

Grassle, J.F. 1989. Species diversity in deep-sea communities. Trends in Ecology & Evolution, 4, 12-15.

Guillon, E., Menot, L., Decker, C., Krylova, E., Olu, K. 2017. The vesicomyid bivalve habitat at cold seeps supports heterogeneous and dynamic macrofaunal assemblages. Deep Sea Research Part I: Oceanographic Research Papers, 120, 1-13.

Harris, P.T., Baker, E.K. 2012. Seafloor gemorphology as benthic habitat: geohab atlas of seafloor geomorphic features and benthic habitats. Elsevier. London, UK.

Jumars, P.A., Dorgan, K.M., Lindsay, S.M. 2015. Diet of worms emended: an update of polychaete feeding guilds. Annual Review of Marine Science, 7, 497-520.

Kelly, J.R., Scheibling, R.E. 2012. Fatty acids as dietary tracers in benthic food webs. Marine Ecology Progress Series, 446, 1-22. doi: 10.3354/meps09559.

Levin, L.A. 2003. Oxygen minimum zone benthos: adaptation and community response to hypoxia. Oceanogr.Marine Biology Annual Review, 41, 1-45.

Levin, L.A., Baco, A.R., Bowden, D.A., Colaço, A., Cordes, E.E., Cunha, M.R., Demopoulos, A.W., Gobin, J., Grupe, B.M., Le, J. 2016. Hydrothermal vents and methane seeps: rethinking the sphere of influence. Frontiers in Marine Science, 3, 72.

Levin, L.A., Ziebis, W., Mendoza, G.F., Growney, V.A., Tryon, M.D., Brown, K.M., Mahn, C., Gieskes, J.M., Rathburn, A.E. 2003. Spatial heterogeneity of macrofauna at northern California methane seeps: influence of sulfide concentration and fluid flow. Marine Ecology Progress Series, 265, 123-139.

Levin, L.A., Ziebis, W., Mendoza, G.F., Growney-Cannon, V., Walther, S. 2006. Recruitment response of methane-seep macrofauna to sulfide-rich sediments: An *in situ* experiment. Journal of Experimental Marine Biology and Ecology, 330, 132-150.

Levin, L.A., Ziebis, W., Mendoza, G.F., Bertics, V.J., Washington, T., Gonzalez, J., T., A.R., Ebbe, B., Lee, R.W. 2013. Ecological release and niche partitioning under stress: Lessons from dorvilleid polychaetes in sulfidic sediments at methane seeps. Deep-Sea Research Part II: Topical Studies in Oceanography, 92, 214–233.

Levin, L.A. 2005. Ecology of cold seep sediments: interactions of fauna with flow, chemistry and microbes. Oceanography and Marine Biology: An Annual Review, 43, 1-46.

Levin, L.A., Mendoza, G.F., Gonzalez, J.P., Thurber, A.R., Cordes, E.E. 2010. Diversity of bathyal macrofauna on the northeastern Pacific margin: the influence of methane seeps and

oxygen minimum zones. Marine Ecology - an Evolutionary Perspective, 31, 94-110. doi: 10.1111/j.1439-0485.2009.00335.x.

Müller-Navarra, D.C. 2008. Food web paradigms: the biochemical view on trophic interactions. International Review of Hydrobiology, 93, 489-505. doi: 10.1002/iroh.200711046.

Parrish, C.C. 1987. Separation of aquatic lipid classes by chromarod thin-layer chromatography with measurement by latroscan flame ionization detection. Canadian Journal of Fisheries and Aquatic Sciences, 44, 722-731.

Parrish, C.C. 1999. Determination of total lipid, lipid classes, and fatty acids in aquatic samples. In: M.T. Arts, B.C. Wainman (Eds.), Lipids in freshwater ecosystems. Springer, New York., pp. 4-20 (Chapter 1).

Parrish, C.C. 2013. Lipids in marine ecosystems. ISRN Oceanography, 2013, Article ID 604045, 16. doi: 10.5402/2013/604045.

Pearson, T., Rosenberg, R. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology Annual Review, 16, 229-311.

Portail, M., Olu, K., Dubois, S.F., Escobar-Briones, E., Gelinas, Y., Menot, L., Sarrazin, J. 2016. Food-web complexity in Guaymas Basin hydrothermal vents and cold seeps. PLoS One, 11, e0162263.

Purser, A., Thomsen, L., Barnes, C., Best, M., Chapman, R., Hofbauer, M., Menzel, M., Wagner, H. 2013. Temporal and spatial benthic data collection via an internet operated Deep Sea Crawler. Methods in Oceanography, 5, 1-18.

Rex, M.A., Etter, R.J. 2010. Deep-sea biodiversity: pattern and scale. Harvard University Press.

Riaux-Gobin, C., Klein, B. 1993. Microphytobenthic biomass measurement using HPLC and conventional pigment analysis. Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, 369-376.

Risser, P.G. 1995. The status of the science examining ecotones. Bioscience, 45, 318-325.

Rosenberg, R. 1995. Benthic marine fauna structured by hydrodynamic processes and food availability. Netherlands Journal of Sea Research, 34, 303-317.

Sahling, H., Rickert, D., Lee, R.W., Linke, P., Suess, E. 2002. Macrofaunal community structure and sulfide flux at gas hydrate deposits from the Cascadia convergent margin, NE Pacific. Marine Ecology Progress Series, 231, 121-138.

Sappington, J.M., Longshore, K.M., Thompson, D.B. 2007. Quantifying landscape ruggedness for animal habitat analysis: a case study using bighorn sheep in the Mojave Desert. Journal of Wildlife Management, 71, 1419-1426.

Sibuet, M., Olu, K. 1998. Biogeography, biodiversity and fluid dependence of deep-sea cold-seep communities at active and passive margins. Deep-Sea Research Part II: Topical Studies in Oceanography, 45, 517-567. doi: 10.1016/s0967-0645(97)00074-x.

Smith, C.R., Hessler, R.R. 1987. Colonization and succession in deep-sea ecosystems. Trends in Ecology & Evolution, 2, 359-363.

Snelgrove, P.V.R. 1999. Getting to the bottom of marine biodiversity: Sedimentary habitats: Ocean bottoms are the most widespread habitat on earth and support high biodiversity and key ecosystem services. BioScience, 49, 129-138.

Snelgrove, P.V.R., Grassle, J.F., Petrecca, R.F. 1992. The role of food patches in maintaining high deep-sea diversity - field experiments with hydrodynamically unbiased colonization trays. Limnology and Oceanography, 37, 1543-1550.

Snelgrove, P.V.R., Grassle, J.F., Petrecca, R.F. 1996. Experimental evidence for aging food patches as a factor contributing to high deep-sea macrofaunal diversity. Limnology and Oceanography, 41, 605-614.

Snelgrove, P.V.R., Grassle, J., Petrecca, R. 1994. Macrofaunal response to artificial enrichments and depressions in a deep-sea habitat. Journal of Marine Research, 52, 345-369. doi: 10.1357/0022240943077082.

Sommer, S., Pfannkuche, O., Linke, P., Luff, R., Greinert, J., Drews, M., Gubsch, S., Pieper, M., Poser, M., Viergutz, T. 2006. Efficiency of the benthic filter: Biological control of the emission of dissolved methane from sediments containing shallow gas hydrates at Hydrate Ridge. Global Biogeochemical Cycles, 20. doi: 10.1029/2004gb002389.

Tews, J., Brose, U., Grimm, V., Tielbörger, K., Wichmann, M., Schwager, M., Jeltsch, F. 2004. Animal species diversity driven by habitat heterogeneity/diversity: the importance of keystone structures. Journal of Biogeography, 31, 79-92.

Thiel, H. 1979. Structural aspects of the deep-sea benthos. Ambio Special Report, 6, 25-31.

Thomsen, L., Barnes, C., Best, M., Chapman, R., Pirenne, B., Thomson, R., Vogt, J. 2012. Ocean circulation promotes methane release from gas hydrate outcrops at the NEPTUNE Canada Barkley Canyon node. Geophysical Research Letters, 39. doi: 10.1029/2012gl052462.

Thurber, A.R., Levin, L.A., Rowden, A.A., Sommer, S., Linke, P., Kröger, K. 2013. Microbes, macrofauna, and methane: A novel seep community fueled by aerobic methanotrophy. Limnology and Oceanography, 58(5), 1640-1656.

Van Rijn, L.C. 1993. Principles of sediment transport in rivers, estuaries and coastal seas. Aqua publications Amsterdam. The Netherlands.

Wei, C., Rowe, G.T., Hubbard, G.F., Scheltema, A.H., Wilson, G.D., Petrescu, I., Foster, J.M., Wicksten, M.K., Chen, M., Davenport, R. 2010. Bathymetric zonation of deep-sea macrofauna in relation to export of surface phytoplankton production. Marine Ecology Progress Series, 399, 1-14.

Weiss, A. 2001. Topographic positions and landforms analysis (poster). ESRI International User Conference. San Diego, CA: ESRI.

Wieking, G., Kröncke, I. 2005. Is benthic trophic structure affected by food quality? The Dogger Bank example. Marine Biology, 146, 387-400.

Wigham, B.D., Hudson, I.R., Billett, D.S.M., Wolff, G.A. 2003. Is long-term change in the abyssal Northeast Atlantic driven by qualitative changes in export flux? Evidence from selective feeding in deep-sea holothurians. Progress in Oceanography, 59, 409-441. doi: 10.1016/j.pocean.2003.11.003.

Wilson, M.F.J., O'Connell, B., Brown, C., Guinan, J.C., Grehan, A.J. 2007. Multiscale terrain analysis of multibeam bathymetry data for habitat mapping on the continental slope. Marine Geodesy, 30, 3-35.

WoRMS Editorial Board. 2017. World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2017-03-29. doi:10.14284/170.

Zapata-Hernández, G., Sellanes, J., Thurber, A.R., Levin, L.A., Chazalon, F., Linke, P. 2014. New insights on the trophic ecology of bathyal communities from the methane seep area off Concepción, Chile (~ 36° S). Marine Ecology, 35, 1-21.

4.9. Appendix
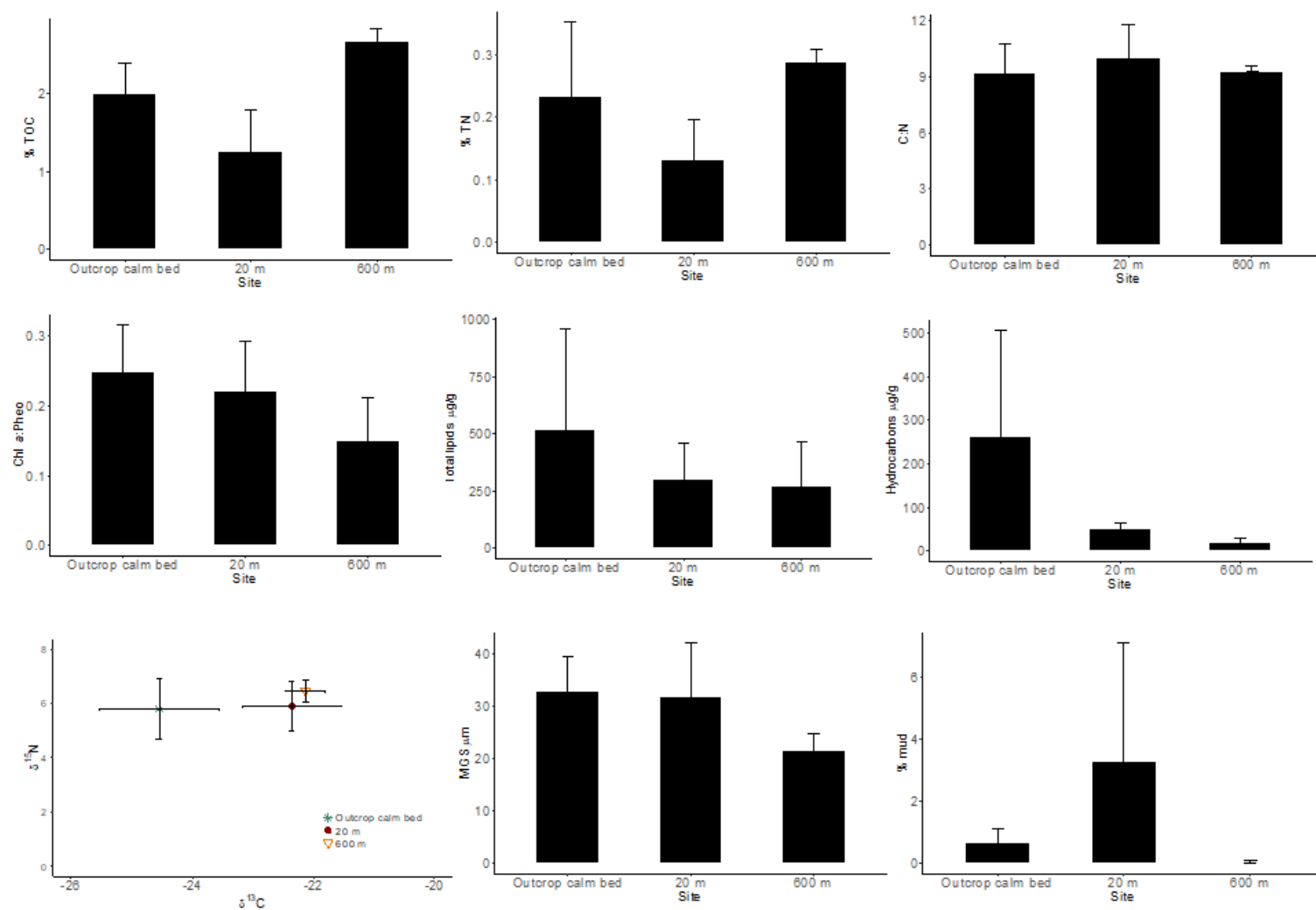
Appendix 4.1. Mean values and standard deviations (error bars) of sediment characteristics from Barkley Hydrates and background sediments.

Appendix 4.2. Trophic guild characterization of macroinfaunal communities from the outcrop of Barkley Hydrates and the 20 and 600 m sites. Abbreviations described in section 4.3.3.

Taxa	Feeding guild	Taxa	Feeding guild
F. Naididae	SSDF	*Levinsenia gracilis*	SDF/SSDF
Leitoscoloplos sp.	SSDF	*Levinsenia oculata*	SDF/SSDF
Leitoscoloplos sp. juv.	SSDF	*Levinsenia* sp.	SDF/SSDF
F. Cirratulidae sp. A	SDF (SF)	*Levinsenia* sp. juv.	SDF/SSDF
F. Cirratulidae sp. B	SDF (SF)	F. Paraonidae juv.	SDF/SSDF
F. Cirratulidae sp. C	SDF (SF)	F. Lumbrineridae sp. A	C
Cirratulus sp.	SDF (SF)	F. Lumbrineridae sp. A juv.	C
Aphelochaeta sp. A	SDF (SF)	*Lumbrinereis* sp.	C
Aphelochaeta sp. B	SDF (SF)	*Ancistrosyllis* cf. *groenlandica*	C
Aphelocaeta sp.	SDF (SF)	F. Dorvilleidae sp. A	O/B
Aphelocaeta sp juv.	SDF (SF)	F. Dorvilleidae sp. B	O/B
Cirratulidae juv.	SDF (SF)	F. Dorvilleidae sp. C	O/B
Prionospio (*Prionospio*) sp.	IF	F. Dorvilleidae sp. D	O/B
Prionospio (*Minuspio*) sp.	IF	F. Dorvilleidae sp. E	O/B
Prionospio sp.	IF	F. Dorvilleidae sp. F	O/B
Dipolydora sp.	IF	F. *Syllidae* sp. A	O
F. Ampharetidae	SDF	*Pionosyllis/Dioplosyllis* sp.	O
Anobothrus gracilis	SDF	*Bipalponephtys cornuta*	C(SDF)
Anobothrus cf. *apaleatus*	SDF	*Bipalponephtys cornuta* juv.	C(SDF)
Anobothrus apaleatus	SDF	F. Polynoidae	C(B/SDF)
Amage sp.	SDF	Polychaete unid.	U
Eclysippe trilobata	SDF	*Harmothoe* sp.?	U
F. Ampharetidae juv.	SDF	*Byblis* sp.	U
Cossura cf. *pygodactylata*	SSDF	*Harpinia* sp.	U
Cossura sp. juv.	SSDF	*Paraphoxus* sp.	U
Cossura sp. A	SSDF	F. Synopiidae	U
Cossura sp. B	SSDF	Amphipoda sp. A	U
Cossura modica	SSDF	Amphipoda sp. B	U
Cossura longocirrata	SSDF	Amphipoda juv.	U
Cossura longocirrata juv.	SSDF	Harpacticoida	U
Cossura cf. *rostrate*	SSDF	Crustacea	U
Cossura brunnea	SSDF	Cumacea	U
F. Maldanidae	SSDF(SF)	Isopoda	U
F. Paraonidae	SDF/SSDF	Asteroidea juv.	U
Aricidea (*Acmira*) *simplex*	SDF/SSDF	F. Thyasiridae	B
Aricidea sp. juv.	SDF/SSDF	Pectinoidea	U
Aricidea (*Strelzovia*) sp.	SDF/SSDF	*Acharax* cf. *johnsoni*	B
Aricidea (*Allia*) *ramosa*	SDF/SSDF	Gastropoda	U
Aricidea (*Strelzovia*) *monicae*	SDF/SSDF	Unid. sp A	U
Aricidea (*Strelzovia*) *mariannae*	SDF/SSDF	Unid. sp B	U
Aricidea (*Strelzovia*) *mariannae* juv.	SDF/SSDF	Unid. sp C	U
Aricidea sp.	SDF/SSDF		

5. Temporal variation in infaunal community structure of chemosynthetic and non-chemosynthetic environments in Barkley Hydrates, British Columbia, Canada [4]

5.1. Abstract

Increasing evidence points to greater temporal variation in deep-sea ecosystems than previously thought. In cold seeps, most available evidence focuses on successional stages of megafauna, with few studies on temporal variability in infaunal communities. We present a temporal study of infaunal communities and sedimentary organic matter characteristics from Barkley Hydrates outcrop, a chemosynthetic environment that receives organic matter from chemosynthetic and photosynthetic (phytodetritus) origins. In order to help interpret results from the outcrop, we contrast temporal variability in outcrop communities during two sampling periods with corresponding variation in an adjacent background sedimentary habitat (both habitats at ~ 900 m depth, offshore of British Columbia, Canada), that receives food input only from a photosynthetic source (phytodetritus). Comparisons with the background community help in differentiating chemosynthetic from photosynthetic food effects at the outcrop. Our results show contrasting temporal patterns for each community. First, lower (though not significantly) total density at the outcrop site in May 2014 corresponded to a significant change in species characteristic of that location in the previous sampling period (i.e., oligochaetes, dorvilleid polychaetes, and bivalves). Background communities did not change significantly in total density or species composition. Changes at the outcrop coincided with higher values of $\delta^{13}C$ in sediment layers at the outcrop site, indicating reduced contributions of chemosynthetic organic matter to the total organic carbon pool relative to September 2013, and low bottom water methane

concentrations. Second, we detected a recruitment event in September 2013 for multiple species (*Bipalponephtys cornuta*, *Cossura longocirrata*, ampharetid polychaetes, *Leitoscoloplos* sp., and *Levinsenia* sp.) at the background site, which could explain the higher (although not significant) total densities observed, but not at the outcrop site, following increased surface primary productivity in summer (June-August). The presence of recruits of ampharetid polychaetes at both sites in September 2013 suggests that, although most individuals from the hydrate site depend on chemosynthetic food sources and therefore track temporal variation of organic matter chemosynthesised from methane, some outcrop site species also track phytodetritus pulses, much as observed in the background community. Our results demonstrate the utility of temporal studies in identifying the primary food source(s) fueling seafloor communities, and the dynamic nature of deep-sea infaunal communities from contrasting sites in Barkley Hydrates.

Key words: macrofauna, diversity, cold seep, recruits, detritus, sediment

5.2. Introduction

Despite relatively constant physical variables (temperature, salinity), several lines of evidence such as seasonal pulses of organic matter (Billett et al., 1983, Smith et al., 1994) and bursts of animal activity (Smith et al., 1994, Billett et al., 2010) demonstrate dynamic aspects of deep-sea environments. Benthic community structure and/or biological aspects of some species (e.g., reproduction, population size-structure) can reflect fluctuation in quantity and/or composition of food inputs. For example, temporal changes in polychaete assemblages from the Porcupine Abyssal Plain (PAP, NE Atlantic, 4800 m depth) mirrored temporal variability in organic matter input (Soto et al., 2010). This variation occurred not only in total density, but also in density of specific families and trophic groups (Soto et al., 2010), corresponding to changes in species composition and food web structure. Population temporal dynamics revolve not only around

adult survival and migration but also recruitment and survival of those recruits. Seasonal reproduction in deep-sea invertebrates has long been known (Schoener, 1968) and, although not a ubiquitous phenomenon, deep-sea species can synchronize their reproductive cycles with organic matter pulses from the euphotic zone, thereby influencing the dynamics of deep-sea benthic communities (Ambrose and Renaud, 1997, Vanreusel et al., 2001, Gooday, 2002).

In the aphotic deep sea, most primary productivity derives from surface production, however, chemosynthetic microorganisms support some highly productive seafloor communities (Gage and Tyler, 1991). Macrofaunal sources of nutrition within chemosynthetic environments include organic matter derived chemosynthetically from endosymbiosis or ectosymbiosis, heterotrophic consumption of free-living chemolithotrophic microorganisms, or consumption of non-chemosynthetically derived material originating in the plankton, on land or coastal environments (Grassle, 1989, Levin, 2005). These sources can be grouped as organic matter from chemosynthetic or from photosynthetic origin. Macrofauna from shallow-water seeps depend mostly on organic matter from photosynthetic origin, probably because of its high density, whereas in deeper water, the reduced availability of organic matter contributes to greater variability in dependency on these contrasting food source from seep to seep (Levin, 2005). Different environmental variables govern chemosynthetic and photosynthetic food inputs, suggesting that temporal patterns in these environments may vary independently. At temperate latitudes, seasonal pulses of detritus sink to the deep-sea bed following plankton blooms from surface waters (Billett et al., 1983). Several studies reported temporal variability in methane discharge in the US Atlantic margin and also in Barkley Hydrates, NE Pacific (Thomsen et al., 2012, Skarke, 2015). Comparison of temporal changes in infaunal community structure at Barkley Hydrates with temporal changes in chemosynthetic and photosynthetic food sources can

clarify the degree of dependency of their respective infaunal communities on different food sources. Furthermore, comparison of temporal changes in infaunal community structure at Barkley Hydrates with temporal changes in the background community can help to differentiate the contributions of chemosynthetic and photosynthetic organic material.

Chemosynthetic communities typically transition into background communities over time with reduced flux and changes in fluid geochemistry, along with accompanying shifts in the dominant foundation (habitat creating) species (Bernardino et al., 2012). Previous studies show clear successional changes in methane seep communities associated with temporal changes in fluid flux over time scales of 10's to 100's of years (Levin et al., 2016). However, access to these environments has constrained studies of variation at shorter time scales (seasons and few years) within seep sites. Cabled observatories, such as Ocean Networks Canada (ONC), provide the tools and sampling opportunities to re-visit sites at regular intervals, facilitating temporal studies at shorter time scales that provide a more comprehensive understanding of deep-sea ecosystems. Such knowledge can help inform sustainable extraction of vast reservoirs of methane energy (Ramesh et al., 2014).

We evaluate the dependency of infaunal communities of Barkley methane hydrates (British Columbia, Canada, ~ 900 m) on chemosynthetic food sources by examining temporal variation in community structure and quality and quantity of food sources (i.e., from chemosynthetic and from photosynthetic origin). Food quality and quantity help regulate seafloor communities (Campanyà-Llovet et al., 2017), and we characterize food quantity based on bulk carbon (e.g. total organic carbon), and food quality, defined as the degree to which quantity and composition of accessible food fulfill consumer nutritional needs (Müller-Navarra, 2008). The C:N ratio increases from the standard Redfield ratio (~ 6.6) to values as high as 10 with ongoing marine

organic matter decomposition (Henrichs, 2005). Carbon stable isotopic signatures ($\delta^{13}C$) provide an indicator of the source of organic matter and can distinguish organic matter derived from photosynthetic versus chemosynthetic origin (Coleman and Fry, 2012). We investigate temporal changes in macrofaunal community structure (i.e., total density, species composition, and recruits density and composition) with respect to temporal changes in the two types of food source (i.e., surface primary productivity, and $\delta^{13}C$ signatures of chemosynthetically derived organic matter). Although seafloor communities may benefit from the two food sources simultaneously, temporal variation in macrofaunal community structure presumably matches temporal variation in its predominant food source. We examine temporal variation in community structure at the Barkley Hydrates outcrop and its dependency on photosynthetic and chemosynthetic food sources by: 1) comparing temporal variation in Barkley Hydrates communities with background communities that receive food only from a photosynthetic origin; and 2) examining temporal variation in the quantity and quality of organic matter available at Barkley Hydrates outcrop. In this manuscript, we examine only temporal variation in Barkley Hydrates macroinfaunal community; we do not include spatial comparisons between sites because we address this issue in further detail elsewhere (Campanyà-Llovet and Snelgrove, 2018).

5.3. Study area

Barkley Hydrates are located at 870 m depth at the wall of Barkley submarine canyon, ~ 100 km offshore of British Columbia (Canada). Barkley Hydrates form outcrops (mounds) 1-3 m in height containing gas and light oil of thermogenic origin (Chapman et al., 2004, Lu et al., 2007), which are surrounded by patchily distributed cold-seep communities, including bacterial mats, live and dead vesycomid clams, and carbonate mounds (Doya et al., 2017). The background

(non-hydrate) community in this study was sampled 600 m to the NW of Barkley Hydrates, corresponding to the MidWest site of the ONC cabled observatory (890 m depth), also at the canyon wall. A mixture of mud and sand covers the seafloor at the MidWest site (Juniper et al., 2013, Chauvet et al., 2018). An oxygen minimum zone (OMZ) covers the region at intermediate depths (400 – 1000 m; Keeling et al., 2010), including our two study sites. Macrofaunal biodiversity frequently declines within OMZs, whereas dominance increases (Levin, 2003), a pattern we predict will characterize community structure at our study sites. Seasonal variation occurs in OMZs, with potential effects on infaunal communities (Levin, 2003).

5.4. Materials and methods

5.4.1. Sampling

Barkley Hydrates (hereafter "outcrop") and the background communities were sampled from the R/V *Falkor* on September 2013 and the CCGS John P. *Tully* in May 2014. Sediment samples were collected at the clam bed area in the outcrop, and 600 m away from it (i.e., background). Push cores were collected from both sites using the Remotely Operated Vehicle (ROV) ROPOS (www.ropos.com) in 2013 and the ROV OE (http://itbsubsea.com/equipment/rovs/oceanic-explorer/) in 2014 (in both cases, i.d. = 6.7 cm, L = 35.6 cm). A series of markers, referred to here as "Waypoints", provide a spatial reference for the crawler Wally, an Internet Operated Vehicle (IOV) (Fig. 5.1). We collected a total of 10 push cores in the outcrop each sampling time (five push cores at Waypoint 3, and five at Waypoint 12, both located where the clam beds are at the outcrop mound), always avoiding Wally's tracks (a few meters away), as well as 10 cores from background sediments in September 2013 and another 7 in May 2014. For background sediments, we collected subsets of 3 or 4 cores at least 10 m apart. For September 2013, we used 4 push cores for organic matter analysis and 6 for taxonomy in the outcrop,

whereas for the background site, we used 3 for organic matter analysis and 7 for taxonomy. In May 2014, we dealt with limited sampling opportunity by removing 4 ml of sediment from each core layer to store at -20 °C for organic matter analysis, and preserving the remaining sediment for taxonomic identification.
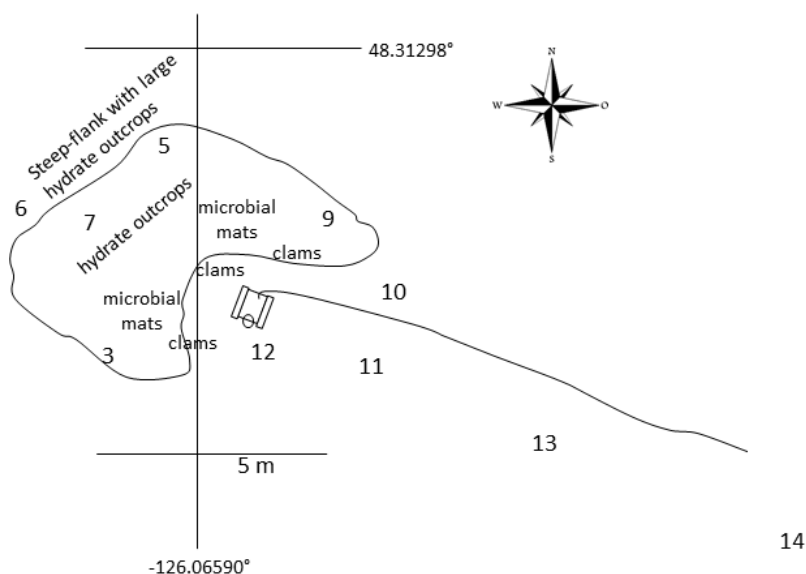
Figure 5.1. Map showing "Waypoints" arranged around a hydrate outcrop in Barkley Hydrates, with the Internet Operated Vehicle "Wally" and its floating tether to junction box of Ocean Networks Canada underwater observatory. Modified from Purser et al. (2013).

5.4.2. Macrofaunal identification and taxonomic diversity

Samples were processed over a 300 μm sieve prior to (September 2013) or after (May 2014) preservation in 4 % formaldehyde in seawater, and transferred to 70 % ethanol in the laboratory. Specimens were sorted under a dissecting microscope and identified to the lowest taxonomic level possible. We identified a species of bivalves resembling the family Thyasiridae based on ferruginous patches on the anterior and posterior ends or elongated foot, the latter of which is particularly useful on the smaller individuals; we will refer to them as such from now on. Abundances of organisms were standardized by sediment surface area prior to analysis to correct for differences in processing between sampling times.

To identify recruits, we measured the smallest individuals from each polychaete family and a subset of the larger organisms. We measured total length of complete organisms and a relevant thoracic parameter for non-complete organisms: thoracic length in cirratulids, ampharetids, cossurids, and paraonids, but prostomium length in lumbrinerids and nephtyids because their body is not clearly distinguished in regions. High correlation (Pearson > 0.6) between these parameters and total length confirmed thoracic length or prostomium length as size proxies in the appropriate families (Table 5.1). Based on these measurements we finally considered cirratulid, cossurid, and paraonid recruits if the individual had a thorax ≤ 1 mm, ampharetid recruits if thoracic length was equal or shorter than 2 mm, lumbrinerid recruits if prostomium length ≤ 0.4 mm, nephtyid recruits if prostomium length ≤ 0.08 mm, and orbiniid recruits if total length ≤ 2 mm.

Table 5.1. R^2 of the linear regression between anterior body part (thoracic length or prostomium length) and total length of a subset of polychaetes from families that included recruits individuals. No complete individuals were available for cossurid or lumbrinerid polychaetes. *denotes statistically significant relationships at $P < 0.05$, ** represent statistically significant relationships at $P < 0.1$.

Polychaete Family	Thoracic length (range in mm)	Prostomium length (range in mm)
Cirratulidae	0.49* (0.325 – 4.94)	
Ampharetidae	0.94* (0.59 – 9.65)	
Paraonidae	0.72** (0.43 – 9.21)	
Cossuridae	-- (0.48 – 4.54)	--
Nephtyidae		0.83** (0.05 – 0.18)
Lumbrineridae	--	-- (0.34 – 1.21)

5.4.3. Sediment organic matter

In order to determine total organic carbon (TOC) and nitrogen (TN), we dried a sediment subsample of 1-5 g (wet weight) at 80 °C for 24 h, ground it into a fine powder, and then weighed and acidified it (under pure HCl fume for 24 h) to eliminate inorganic carbon. We then dried samples again at 80 °C for 24 h before the CN analysis. An aliquot of dried decarbonated sediments (15 mg) was weighed in a tin capsule and folded, prior to analyzing TOC (%), TN (%), and $\delta^{13}C$ (‰) in a Carlo Erba NA1500 Series II elemental analyzer (EA). We present the stable isotopic results with respect to the international standards of Vienna Pee Dee belemnite (V-PDB) for carbon, noting average instrumental precision of ± 0.17 ‰. The stable carbon isotope ratios are expressed as:

$\delta^{13}C$ = [(Rsample/Rstandard)-1] x 1000,

where R represents $^{13}C/^{12}C$ for $\delta^{13}C$.

5.4.4. Other environmental conditions

The IOV (Internet Operated Vehicle) Wally measured methane concentrations 20 cm above the sediment in May 2014 at Waypoints 3 and 12. Unfortunately, these data were not available in September 2013 because of sensor issues, however we were able to use carbon isotopes in sediments (see above) as an indicator of the proportion of chemosynthesised organic carbon. The Wally mobile instrument platform (130 × 106 × 89 cm, LWH) can move over and monitor up to 2000 m^2 of sediment surface in real-time, controlled by any computer connected to the Internet through a 70-m long cable connected to a junction box at the ONC node. Background bottom water chlorophyll fluorescence and dissolved oxygen concentration were measured near the ONC MidWest site with a Wetlabs fluorometer and Sea-Bird SBE 63 dissolved oxygen sensor, respectively. The fluorometer measured backscattered fluorescence at 695 nm from a 470 nm

source at 1-min intervals. Fluorometry at the outcrop could not be measured and we therefore assume similar values as background fluorometry based on relative proximity (wall samples at similar bottom depth). The oxygen sensor measures dissolved oxygen concentration in seawater based on the ability of the sensing foil to act as a dynamic fluorescence quencher. Data from the three instruments were downloaded using the Oceans 2.0 software interface (dmas.uvic.ca). Net primary productivity (NPP) data for surface waters were downloaded from the Ocean Productivity group, Oregon State University (www.science.oregonstate.edu/ocean.productivity) and extracted and plotted in Rstudio (v3.3.0) using the {raster} package. Monthly NPP data were obtained with the VGPM (Vertically Generalized Production Model) based on MODIS.R2014 (Moderate-resolution Imaging Spectroradiometer) data, from June 2013 until May 2014. The spatial resolution of NPP was 18 km (cell size of 1/6 geographic degrees).

5.4.5. Statistical analyses

We initially compared temporal differences in community structure of the outcrop based on infaunal densities. We examined normality of the residuals (Q-Q plots) and homogeneity of variances (residuals *vs* fitted plot) prior to a one-way crossed ANOVA with Sampling time (levels: September 2013 and May 2014) as fixed factors. We ran the ANOVA with a type III sums of squares to account for the unbalanced design (n (2013) = 8, n (2014) = 10). We performed multivariate analysis using PRIMER v.6 (Clarke and Gorley, 2006) and used the add-on PERMANOVA+ module (Anderson et al., 2008) to identify significant differences in infaunal community structure between Sampling times (levels: September 2013 and May 2014), with a PERMANOVA type III (partial) sums of squares to account for the unbalanced design. We used a log-transformation to account for zero-inflated data, and Bray-Curtis similarity abundance data matrices. We used non-metric multidimensional scaling (nMDS) to visualize infaunal

communities by sampling times (levels: September 2013 and May 2014). Prior to these analyses we verified homogeneity of multivariate dispersion using the PERMDISP routine (Anderson et al., 2008). Lastly, we used a percent similarity procedure (SIMPER) analysis and plotted the vectors on nMDS to identify taxa that distinguished assemblages between sampling times. We repeated the same statistical analysis with samples from the background community. Because recruits can explain a significant portion of the temporal dynamics from a specific population (see Introduction), we repeated the above univariate analysis on total abundances and multivariate analyses on species composition considering recent recruits only.

We also examined temporal and sediment depth differences in food parameters (i.e., TOC, C:N, δ^{13}C) in the outcrop using one-way ANOVA with Sampling time as a fixed factor (September 2013 and May 2014). Prior to analysis, we examined whether the data met the assumptions of normality of residuals, homogeneity of variances, and looked for the presence of outliers. We also examined data for the presence of outliers, for example, the absence of δ^{13}C measurements in the deeper sediment layers of a core sample from the outcrop, which are the most depleted in 13-C, substantially modified the overall δ^{13}C signature of that sample. Noting the unbalanced design, we used Type III sums of squares (SS) ANOVAs. We repeated the same analysis for background sediments. Transformations of the C:N ratio in the outcrop sediments did not meet assumptions for ANOVA and we therefore ran a non-parametric Kruskal-Wallis test.

5.5. Results

5.5.1. Infaunal community structure

Total density at the outcrop decreased in May 2014 relative to September 2013 (Fig. 5.2), however, the difference was not significant (Table 5.2), likely because of high variability among samples. Polychaete dominated (79.0 %) the community at the outcrop, followed by Crustacea

(11.6 %), Bivalvia (6.4 %), other Molluska (2.7 %), and Echinodermata (0.2 %). At the outcrop site, we found significant differences in infaunal community structure over time (one-way PERMANOVA; Sampling time: Pseudo-$F_{1,14}$= 2.39, P (perm) = 0.003). The PERMIDSP routine did not identify significant differences in dispersion between Sampling times ($F_{1,14}$ = 1.82, P (perm) = 0.337). Oligochaetes (Naididae), thyasirid bivalves, ampharetid recruits and dorvilleid sp. C polychaetes characterized the sediments at the outcrop site in September 2013 (Table 5.3), whereas oligochaetes (Naididae), thyasirids recruits, *Aphelochaeta* sp. A (Cirratulidae) and *Paraphoxus* sp. (Gammaroidea) characterized sediments at the outcrop in 2014 (Table 5.3). Indeed, SIMPER analysis identified higher densities of oligochaetes, adult thyasirids, and dorvilleid sp. C in September 2013, together with higher densities of thyasirid recruits in May 2014 as the main contributors to dissimilarity between sampling times in outcrop sediments (Table 5.3). Vectors plotted over the nMDS coincide with higher densities of dorvilleid sp. C polychaetes in sediments at the outcrop site in September 2013 compared to May 2014 (Fig. 5.3).
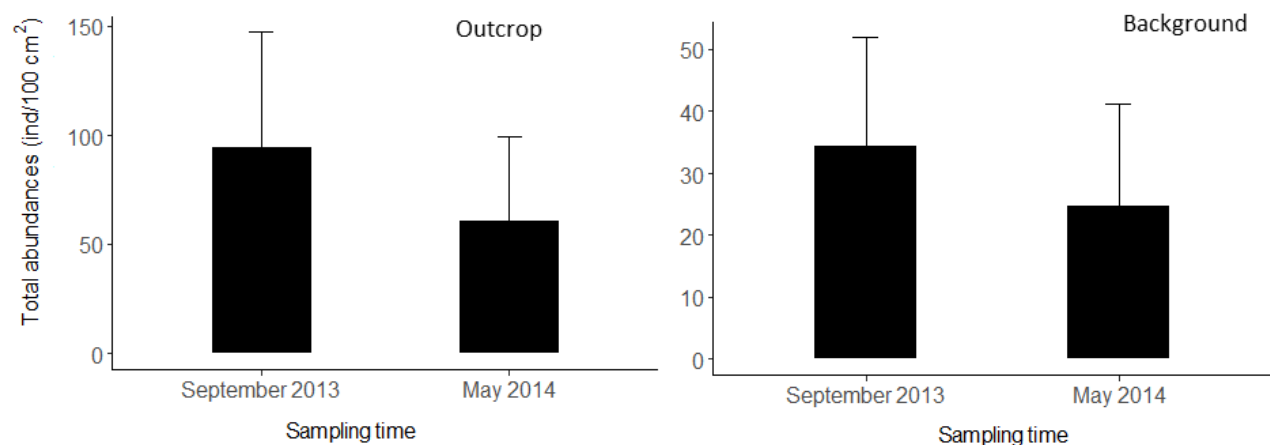
Figure 5.2. Total infaunal density (mean + sd) from Barkley Hydrates outcrop and background site sampled in September 2013 and May 2014.

Table 5.2. ANOVA (type III) results of the effect of year on infaunal total density at Barkley hydrates outcrop and the background site (600 m away from it).

OUTCROP

Source	Df	SS	MS	F value	P
Sampling time	1	4241	4241	2.16	0.163
Residuals	14	27460	1961.4		

BACKGROUND

Source	Df	SS	MS	F value	P
Sampling time	1	295.1	295.1	1.02	0.333
Residuals	11	3172.6	288.42		

Table 5.3. Results from SIMPER analysis per Sampling time on the infaunal community at Barkley Hydrates outcrop. Rec = recruit. Note that Avg. Abund was calculated from the log-transformed data. Data analysed per core.

Group September 2013; Average similarity: 31.22						Group May 2014; Average similarity: 23.22					
Species	Av.Abund	Av.Sim	Sim/SD	Contrib %	Cum. %	Species	Av.Abund	Av.Sim	Sim/SD	Contrib %	Cum. %
Naididae	2.9	9.36	1.14	29.97	29.97	Naididae	1.92	4.5	0.52	19.38	19.38
Thyasirid	1.65	5.17	1.24	16.56	46.53	Thyasirid rec.	1.36	3.09	0.61	13.31	32.69
Ampharetid rec.	1.22	3.98	1.3	12.73	59.27	*Aphelochaeta* sp. A	1.28	3.01	0.69	12.98	45.66
Dorvilleidae sp. C	1.46	3.23	0.78	10.34	69.61	*Paraphoxus* sp.	1.29	2.6	0.52	11.19	56.86

Groups September 2013 & May 2014; Average dissimilarity = 80.66

Species	Group Sept. 2013 Av.Abund	Group May 2014 Av.Abund	Av.Diss	Diss/SD	Contrib %	Cum. %
Naididae	2.9	1.92	5.74	1.07	7.12	7.12
Thyasirid	1.65	0	4.51	1.71	5.6	12.72
Dorvilleidae sp. C	1.46	0.87	4.3	1.01	5.33	18.05
Thyasirid rec.	0	1.36	3.46	0.96	4.29	22.34
Dorvilleidae sp. B	1.47	0.78	3.39	1.16	4.2	26.54
Harpacticoida	0.98	1.09	3.23	1.09	4	30.54
Ampharetid rec.	1.22	0	3.2	1.9	3.96	34.5
Anobothrus apaleatus	0.74	0.78	3.13	0.87	3.88	38.38
Paraphoxus sp.	0	1.29	3.07	0.95	3.8	42.18
Aphelochaeta sp. A	0.94	1.28	3.03	1.11	3.75	45.94
Harpinia sp.	1.21	0	2.86	0.9	3.54	49.48
Cirratulid rec.	0	1.1	2.63	0.92	3.25	52.73
Cossura sp. A	0.56	0.76	2.37	0.86	2.94	55.67
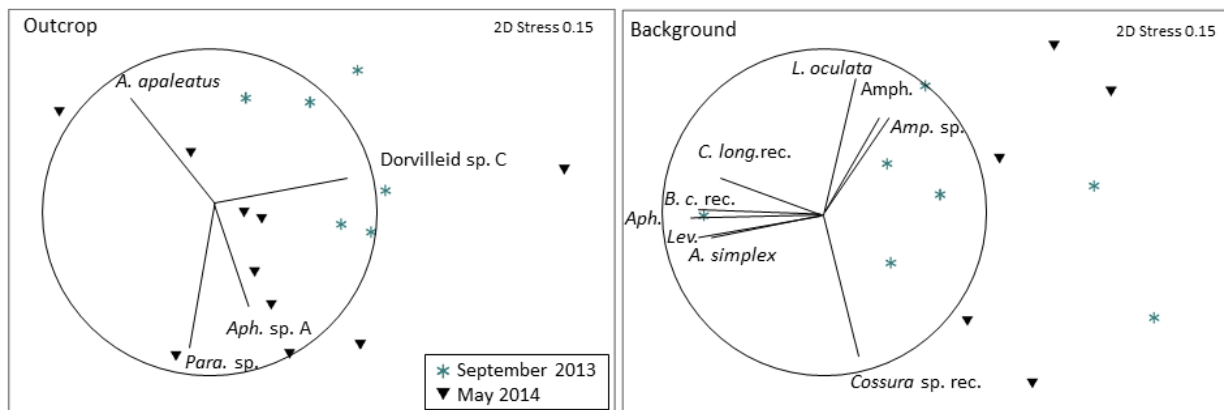
Figure 5.3. nMDS of outcrop and background community samples from Barkley Hydrates (890 m depth) per Sampling time. Assemblages are based on Bray-Curtis similarity with log (x+1) transformation. The large circle represents the correlation between species and nMDS axes. We only overlaid the most relevant vectors (Pearson's correlation threshold of 0.6). *A. apaleatus* = *Anobothrus apaleatus*; *Aph*. sp. A = *Aphelochaeta* sp. A; *Aph*. = *Aphelochaeta* sp. B; *Para*. = *Paraphoxus* sp.; *L. oculata*. = *Levinsenia oculata* Amph. = Ampharetidae; *Amp*. = *Ampelisca* sp.; *rec*. = recruit; *A. simplex*. = *Aricidea (Acmira) simplex*; *Lev*. = *Levinsenia* sp.; *B. c.* = *Bipalponephtys cornuta*; *C. long*.= *Cossura longocirrata; rec. = recruit*.

Total density in the background communities also decreased in May 2014 (Fig. 5.2), although not significantly (Table 5.2). Polychaetes also dominated the background community (91.9 %), followed by Crustacea (5.2 %), Bivalvia (1.5 %) and other Molluska (1.5 %). Background community species composition changed with time although not significantly (one-way PERMANOVA; Sampling time: Pseudo-$F_{1,10}$ = 1.29, P (perm) = 0.206; see Fig. 5.3). The PERMDISP analysis identified no differences in data dispersion between sampling times ($F_{1,10}$ = 0.59, P (perm) = 0.521). Ampharetid recruits, *Levinsenia oculata* (Paraonidae), and *Bipalponephtys cornuta* (Nephtyidae) characterized sediments of the background community in September 2013 (Table 5.4), whereas *L. gracilis*, *Cossura* sp. A, and *L. oculata* characterized sediments in May 2014 (Table 5.4). The presence of recruits (ampharetid and cossurid) and differences in densities of the most common species (i.e., *L. oculata, L. gracilis, B. cornuta*, and *Aricidea (Stretzovia) monicae*) played an important role in differentiating sediments at the background site between sampling times (Table 5.4, Fig. 5.3).

212

Table 5.4. Results from SIMPER analysis between years for the infaunal community at the background site. Rec = recruit. Note that Avg. Abund was calculated from the log-transformed data. Data analysed per core.

Group 2013; Average similarity: 17.30						Group 2014; Average similarity: 19.44					
Species	Av.Abund	Av.Sim	Sim/SD	Contrib %	Cum. %	Species	Av.Abund	Av.Sim	Sim/SD	Contrib %	Cum. %
Ampharetid juv.	1.49	2.99	0.6	17.28	17.28	*Levinsenia.gracilis*	1.81	4.26	0.62	21.91	21.91
Levinsenia occulata	1.45	2.94	0.6	17.01	34.29	*Cossura* sp. A	1.68	4.17	0.62	21.44	43.36
Bipalponephtys cornuta	1.1	1.57	0.4	9.1	43.39	*Levinsenia occulata*	1.71	3.83	0.62	19.7	63.06
Eclysippe trilobata	1.01	1.36	0.4	7.88	51.28						
Aphelochaeta sp. B	1.04	1.34	0.38	7.75	59.03						

Table 5.4. *Continued*

Groups 2013 & 2014; Average dissimilarity = 83.54

Species	Group Sept. 2013 Av.Abund	Group May 2014 Av.Abund	Av.Diss	Diss/SD	Contrib %	Cum. %
Levinsenia gracilis	1.01	1.81	4.03	1.16	4.83	4.83
Cossura sp. A	0.71	1.68	3.92	1.11	4.69	9.51
Levinsenia occulata	1.45	1.71	3.56	1.11	4.27	13.78
Ampharetid rec.	1.49	0.48	3.48	1.1	4.16	17.94
Cossura sp. rec.	0.69	1.2	3.35	0.93	4.01	21.95
Bipalponephtys cornuta	1.1	0.99	3.08	0.98	3.68	25.63
Aricidea (Stretzovia) monicae	1.04	0.93	3	1.02	3.6	29.23
Ampharetidae	0	1.2	2.78	0.79	3.33	32.55
Eclysippe trilobata	1.01	0.52	2.73	0.91	3.26	35.82
Aricidea (Allia) ramosa	0.28	1.04	2.7	0.88	3.24	39.05
Aphelochaeta sp. B	1.04	0	2.64	0.78	3.15	42.21
Cossura longocirrata rec.	0.85	0	2.38	0.62	2.84	45.05

5.5.2. Recruit community structure

Noting differences in the relative contribution of recruits of different species in September 2013, and recognizing those differences likely reflected recruitment events, we examined recruit species composition over time at each site (Fig. 5.4). Significant changes in outcrop recruit composition with time (one-way PERMANOVA, Pseudo-$F_{1,14}$ = 8.32, P (perm) < 0.001) resulted primarily from increased ampharetid densities in September 2013 and more cirratulids and bivalves in May 2014 (SIMPER) (Fig. 5.5). The PERMDISP routine did not identify significant differences in data dispersion between sampling times ($F_{1,14}$ = 1.79, P (perm) = 0.293). Recruit total abundances did not vary (Kruskal-Wallis, $\chi^2(1)$ = 0.37, P = 0.545) from September 2013 (4.72 ± 2.93 ind./100 cm^2) to May 2014 (8.96 ± 8.32 ind./100 cm^2), but were borderline significant in the background community (one-way ANOVA, $F_{1,11}$ = 4.55, P = 0.056), with 10.53 ± 8.46 ind./100 cm^2 in September 2013 and 3.98 ± 3.61 ind./100 cm^2 in May 2014. Temporal differences in background recruit composition (Fig. 5.5), although non-significant (one-way PERMANOVA Pseudo$F_{1,11}$ = 1.71, P (perm) = 0.115), resulted from more individuals of different species in September 2013 but only a few *Cossura* sp. recruits in May 2014 (SIMPER) (Fig. 5.5). The PERMDISP routine did not identify significant differences in data dispersion between sampling times ($F_{1,11}$ = 0.08, P (perm) = 0.804).
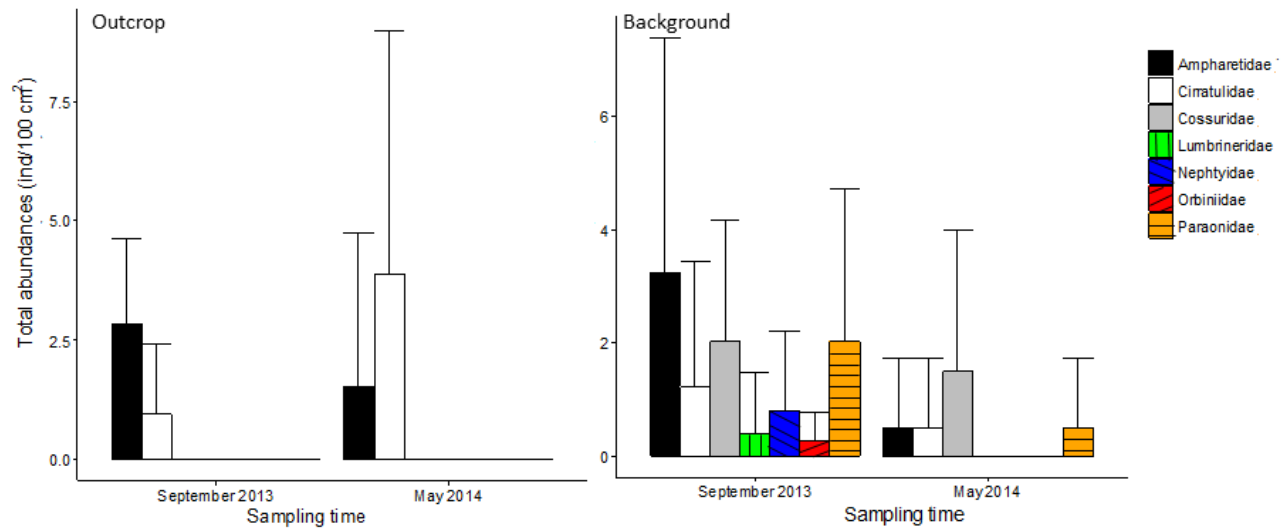
Figure 5.4. Polychaete recruit densities per family and sampling time (i.e., September 2013, May 2014) in Barkley Hydrates outcrop and background infaunal community. Error bars denote 1 standard deviation.
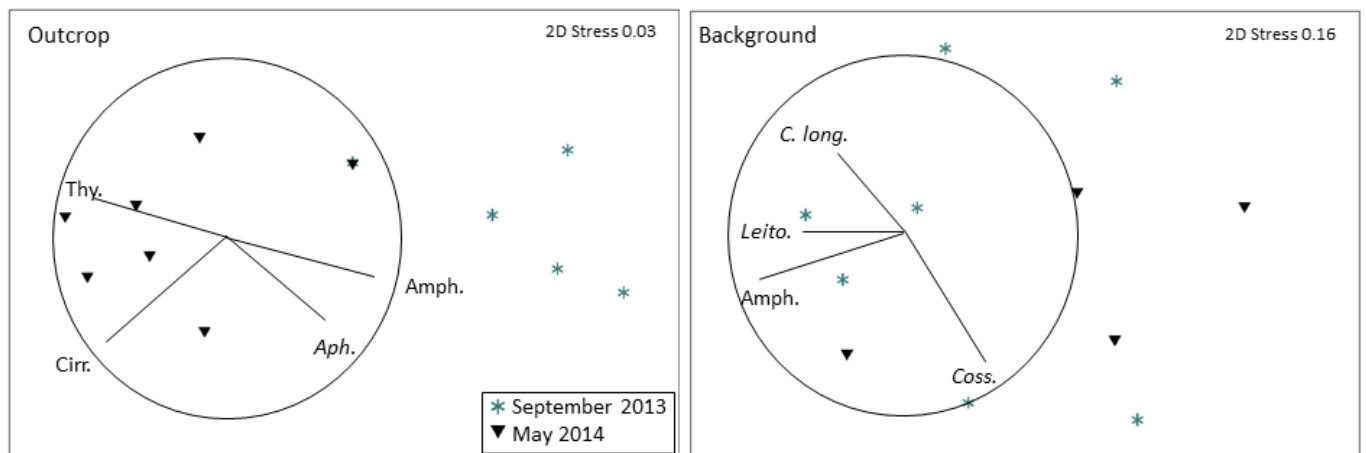
Figure 5.5. nMDS of the infaunal recruit composition from Barkley Hydrates outcrop , background site sampled in September 2013 and May 2014. Thy. = Thyasirid; Amph. = Ampharetidae; *Aph*. = *Ahphelochaeta* sp.; Cirr = Cirratulidae; *C. long.* = *Cossura longocirrata*; *Coss*. = *Cossura* sp.; *Leito*. = *Leitoscoloplos*.

216

5.5.3. Organic matter

Quantity and quality of sedimentary organic matter varied little over time. Analysis of temporal variation showed no significant differences in outcrop TOC between sampling times (one-way ANOVA; $F_{1,10}$ = 1.23, P = 0.294), although TOC decreased in May 2014 (Fig. 5.6). We found no significant differences in C:N ratios between sampling times (Kruskal-Wallis, $\chi^2(1)$ = 0.69, P = 0.405; see Appendix 5.1), and significant differences in the δ^{13}C signature between sampling times (one-way ANOVA; Sampling time: $F_{1,8}$ = 7.81, P = 0.023), given the higher values in May 2014 (Fig. 5.6). We found no significant differences either in the background sediment TOC between sampling times, (one-way ANOVA; Sampling time: $F_{1,7}$ = 0.00, P = 0.975) (Fig. 5.6), or in C:N ratio between sampling times (Kruskal-Wallis, $\chi^2(1)$ = 1.8, P = 0.180; see Appendix 5.1), although the C:N ratio increased in May 2014 (Appendix 5.1). We also found no differences in δ^{13}C between sampling times (Kruskal-Wallis, $\chi^2(1)$ = 1.07, P = 0.302) in background sediments (Fig. 5.6). Sea surface primary productivity from the 3-month period prior to bottom sampling was substantially higher in 2013 (i.e., June-August prior to September sampling) than 2014 (February-April prior to May sampling; see Fig. 5.7), with surface primary productivity in spring 2014 approximately half of that in the fall and winter months of 2013, and a ~ 60 % higher value in the April 2014 spring bloom.
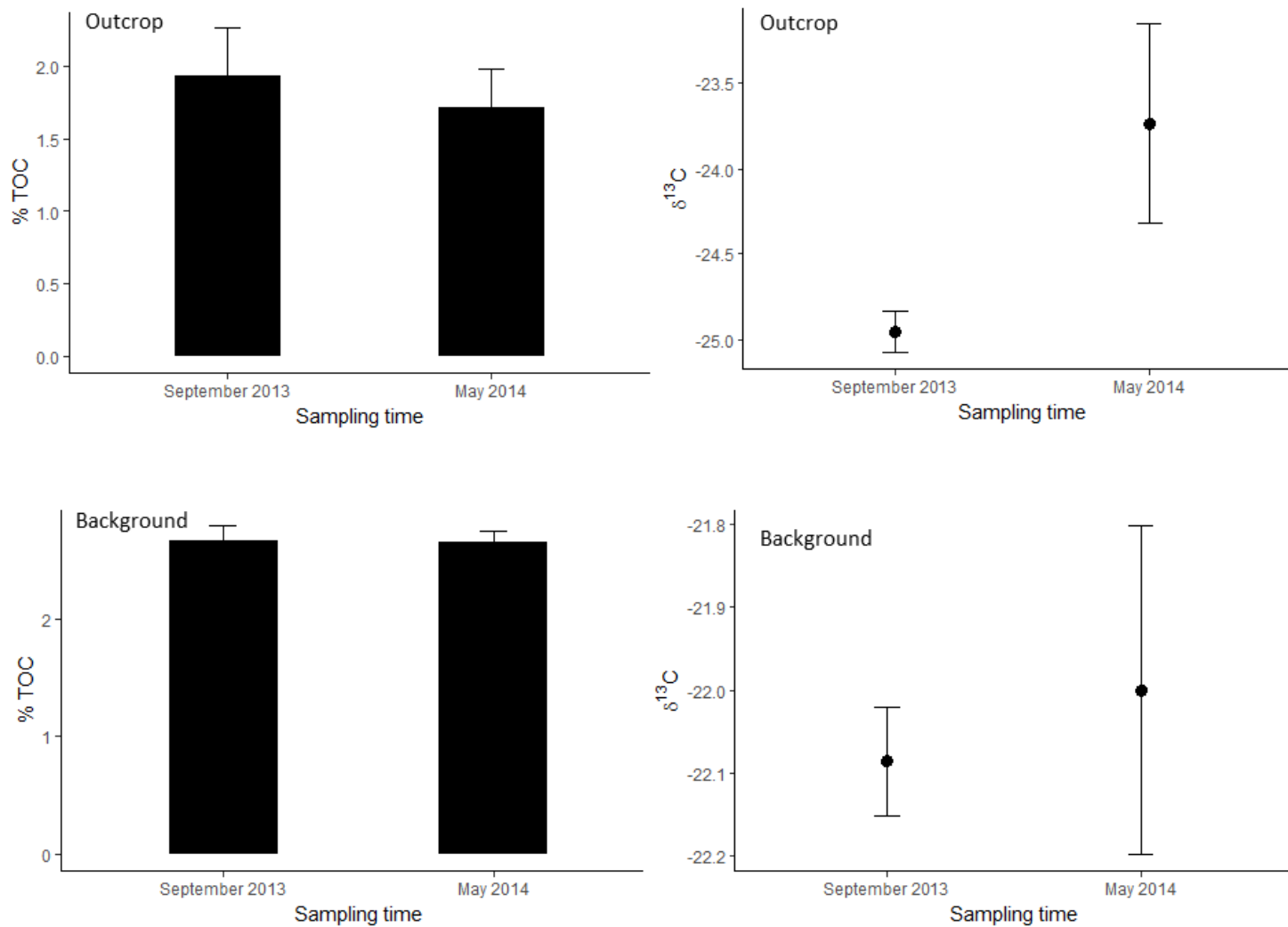
Figure 5.6. % TOC and δ^{13}C (mean ± sd) between years from Barkley Hydrates outcrop and the background sites.
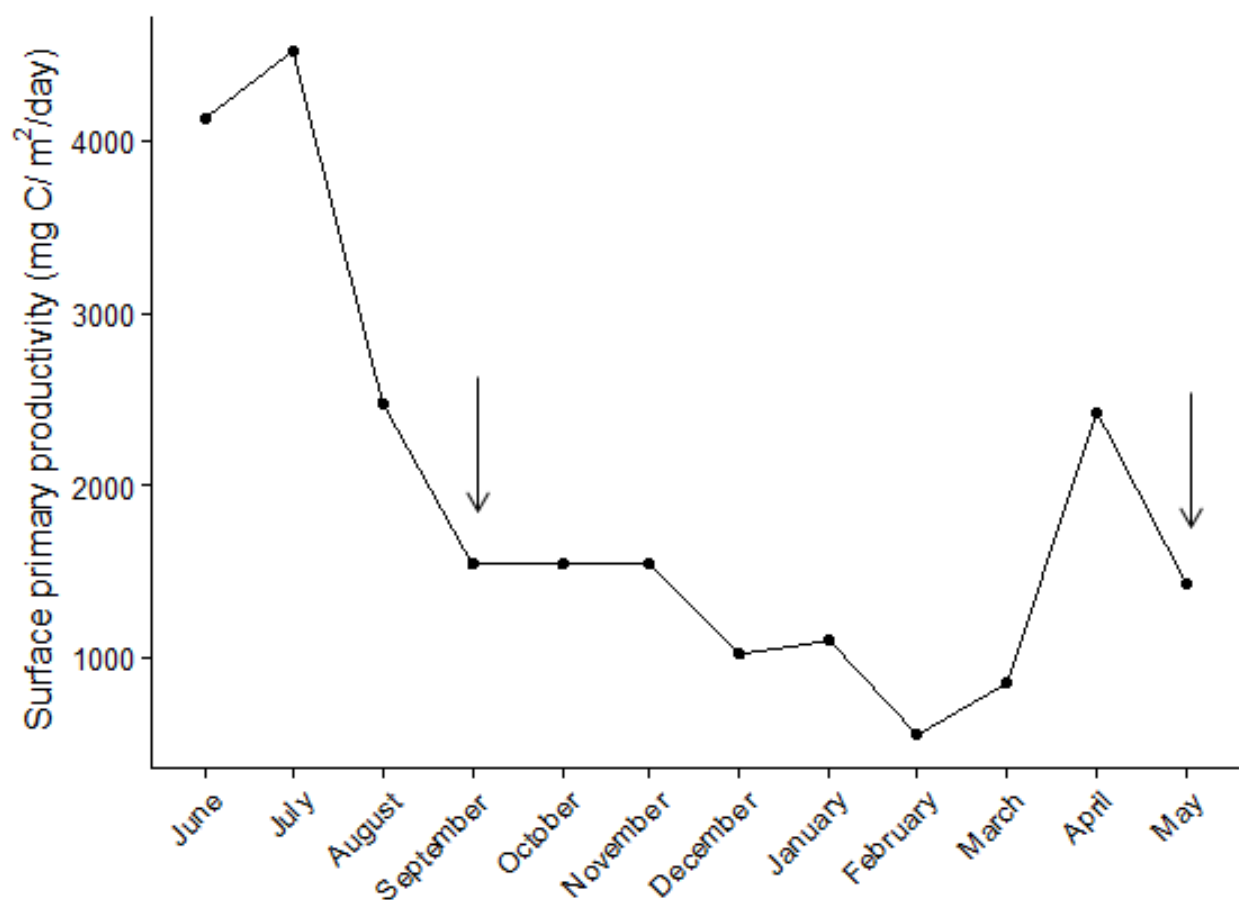
Figure 5.7. Monthly net surface primary productivity overlaying Barkley Hydrates outcrop and background site from June 2013 to May 2014. Arrows indicate sedimentary community sampling months. Data obtained with the VGPM (Vertically Generalized Production Model) based on MODIS.R2014 (Moderate-resolution Imaging Spectroradiometer) data, from June 2013 until May 2014. The spatial resolution was 18 km (cell size of 1/6 geographic degrees).

5.6. Discussion

5.6.1. Temporal variation in community structure from Barkley Hydrates outcrop

Infaunal community structure from the outcrop varied between the two sampling times of our study together with variation in organic matter composition, in contrast to more modest differences in the background community. We attribute these differences in response to temporal variation in quality and quantity of available food, although we acknowledge that fine-scale spatial patchiness could have also contributed to these differences. Oligochaetes, dorvilleid sp. C, and thyasirid were the greatest contributors to temporal changes at the outcrop as a result of reduced densities in May 2014 relative to September 2013. The differences observed in total densities and species composition between the two sampling times were mostly driven by samples collected in Waypoint 12, which was the more methane active location (i.e., higher lipid concentrations and more depleted $\delta^{13}C$ signatures: Campanyà-Llovet and Snelgrove, 2018). In September 2013 surface primary productivity (SPP) was higher and $\delta^{13}C$ more depleted than in May 2014. We discuss the influence of SPP in the sampled communities in section 5.2. Depleted signatures of $\delta^{13}C$ indicate organic carbon of chemosynthetic origin (Coleman and Fry, 2012), and the bottom water methane concentrations from the outcrop in May 2014 corroborated the $\delta^{13}C$ results.

Previous research in Barkley Hydrates attributed changes in bottom water methane concentrations to ocean circulation patterns, specifically diurnal shelf waves, internal semidiurnal tides, and wind-generated near inertial motions that affect methane release from hydrates (Thomsen et al., 2012). Furthermore, changes in the spatial extent of

220

bacterial mats and distribution of vesycomid bivalves at Barkley Hydrates (Purser et al., 2013) suggest changing availability of chemical flux for chemosynthesis over time. We found reduced methane concentrations (0.2 to 0.3 μmol/l in Waypoint samples) compared to previous temporal studies in the same area (0.8-6.9 μmol/l: Thomsen et al., 2012), suggesting low methane availability for chemosynthesis in May 2014. Unfortunately no methane concentrations were available for September 2013, which precludes comparison between sampling times.

Our results do not imply causality, however, decreasing densities of species exclusive to or characteristic of the outcrop (e.g., dorvilleid, thyasirid, oligochaetes: Campanyà-Llovet and Snelgrove, 2018), may have resulted from a decline in the proportion of organic matter from a chemosynthetic origin, suggesting a dependency on chemosynthetic food sources. Some species of dorvilleid are known to feed on chemosynthetic bacterial mats (Wiklund et al., 2009, Levin et al., 2013, Salvo et al., 2014) and some species of thyasirid bivalves harbor chemosynthetic symbionts (Dufour and Felbeck, 2003). Despite the presence of chemosynthetic symbionts (Dubilier et al., 2006) in so-called gutless oligochaetes, our oligochaete species does not fall into this category. Oligochaetes are highly opportunistic species that can tolerate hypoxic conditions and therefore typically occur deeper in sediments than most macrofauna (Giere and Pfannkuche, 1982); their absence in the background community suggests a preference for a chemosynthetic environment and a potential reliance on organic matter of chemosynthetic origin. The increased surface primary productivity in September 2013 can explain the presence of

ampharetid recruits which coincides with a presumed recruitment event in the background community in that sampling time (see below).

Previous studies report ampharetids from both chemosynthetic and non-chemosynthetic environments. Methane-derived organic carbon can fuel ampharetid polychaetes, such as the ampharetid-based assemblage from New Zealand methane seep sites at 662 – 1172 m (Thurber et al., 2013). Intriguingly, they also inhabit non-chemosynthetic environments where they time their life cycles to coincide with seasonal phytoplankton blooms (Glover et al., 2008). Even within seeps, species from this family can switch food sources. Mixotrophy has been reported in seep ampharetids as a means to cope with variation in supply of hydrocarbon-rich fluids, supporting survival during decreased seep activity through increased use of photosynthetically-derived carbon (Levin et al., 2016). Other recruits contributed to the differences in community structure between sampling times as well (i.e., cirratulid polychaete and thyasirid bivalve). However, in contrast to ampharetid recruits, cirratulid and thyasirid recruits increased in density in May 2014 with respect to the previous sampling time. Their absence in September 2013 suggests intolerance to chemosynthetic environments, and reduced methane in May 2014 allowed recruits of these other taxa to colonize. Another possible explanation could simply be a different reproductive cycle in these taxa than for ampharetid polychaetes. The presence of thyasirid adults in September 2013 but recruits in May 2014 suggest a full reproductive cycle and/or ontogenetic shifts in environmental conditions in that species.

5.6.2. Temporal variation in background community structure

Carbon stable isotopic signature from sediment organic matter from the background community ($\delta^{13}C > -23$ ‰) confirms a photosynthetic origin with no traces of a chemosynthetic source (Coleman and Fry, 2012). The low total background community density compared to other studies from similar depth and region without the influence of an OMZ (Levin, 2003), suggests that low dissolved oxygen concentrations in bottom waters, impacted these communities. Indeed, macroinfaunal total densities from Barkley Hydrates were also low compared to cold seeps from the NE Pacific not influenced by low dissolved oxygen concentrations (Levin, 2003). Seasonal changes in dissolved oxygen concentrations (DO) can modify benthic community structure (Levin, 2003), however, this is not the case for the changes observed in the communities from our study sites given the negligible variation in DO between sampling times (i.e, 0.27 ± 0.004 ml O_2/l in September 2013 and 0.27 ± 0.01 ml O_2/l in May 2014) shown by mid-slope oxygen sensors from Ocean Networks Canada.

An increase in the presence of recruits in the background community suggests a recruitment event occurred around September 2013. Despite the absence of *in situ* photosynthetic primary productivity in the deep sea, many deep-sea species couple their reproductive cycles with seasonal surface phytoplankton blooms (Gage and Tyler, 1991). Sea surface primary productivity seasonality was high prior to sampling in September 2013, particularly in June and July but also August 2013, when surface primary productivity doubled relative to the fall and winter months, and was ~ 60 % greater than the spring bloom in April 2014 that preceded our May 2014 sampling. We observed a

temporal peak in chlorophyll in bottom waters similar to that observed in surface imagery, suggesting a temporal pattern in bottom water consistent with surface primary production, but because of calibration concerns with the flourometer (see also Juniper et al. (2013)) we do not present that data here. This relative increase in bottom water chloropigment concentrations shortly before (~ 2 and 4 weeks) sampling in September 2013 but not May 2014 suggests higher phytodetritus input following a surface bloom. Billett et al. (1983) suggest a 2-3 week lag for transport of organic matter from surface waters to 2000 m in the NE Atlantic. Our comparatively shallower sites (870 and 890 m) and enhanced dynamism and horizontal transport of sediments and organic matter expected in submarine canyons, suggest a shorter time lag between surface production and organic matter deposition to the benthos. The strong phytoplankton signature in surface waters (June-July) may reach the sediments in late June-July-early August, where organic matter not used by the benthos moves further down the canyon (e.g. by September 2013, our sampling month). This export may explain the absence of temporal differences in TOC at our sites. Furthermore, we measured a subset of food parameters, but not lipids, proteins, chlorophyll *a*, essential fatty acids, and other aspects that can play an important role in structuring benthic communities and food webs (Campanyà-Llovet et al., 2017). The composition of organic matter may or may not have changed with time even though total amounts of food (TOC) remained somewhat constant. Alternatively, the signature of some of our food quality parameters may have changed with time but during periods when we could not sample.

Polychaete reproductive cycles vary among families and species (Giangrande, 1997), and polychaete planktonic larval duration may vary from a few hours to months before metamorphosis and settlement (Scheltema, 1986). Distributions of some infaunal species span from bathyal depths to shallow waters, where strong seasonal reproductive patterns occur (Blake et al., 1996a, Blake et al., 1996b), thus straddling our sampling depths (870 and 890 m). Furthermore, many deep-sea species display seasonal reproductive cycles (Gage and Tyler, 1991). The taxa that characterized the recruitment event in September 2013 (i.e., ampharetid, Levinsenia sp. (F. Paraonidae), *Cossura longocirrata* (F. Cossuridae), *Leitoscoloplos* sp. (F. Orbiniidae)) show some seasonality in their reproductive cycles elsewhere. In general, ampharetid polychaetes spawn lecithotrophic larvae seasonally, presumably with a limited pelagic phase (Blake et al., 1996b). Some paraonid species reproduce seasonally with direct development (Giangrande, 1997). C. longocirrata breeds continuously, with fluctuating gamete production that apparently increases in summer, with spring and summer recruitment (Blake et al., 1996a). Planktotrophy is absent from some families such as Orbiniidae (Giangrande, 1997), a family in which direct development occurs in most species, though seasonally (Giangrande, 1997). A species of *Leitoscoloplos* (i.e., L. pugettensis) exhibits direct development, with recruits crawling free into the sediment about a month after egg deposition in spring (Blake et al., 1996a). Despite limited knowledge on the reproductive cycles of most species that recruited as recruits in September 2013 in our study, available information suggests seasonal reproduction for these taxa, apparently recruiting during the summer months, consistent with our observation of late summer recruitment. The lag-time (months) between expected recruitment and the recruits reported here may relate to

225

location, to year to year variation, or indicate that these recruits recruited and metamorphosed less recently than we assumed.

5.7. Conclusion

By comparing temporal variation in food and community structure we infer some degree of dependency on different food sources for the benthic community in its entirety or for specific taxa. Previous studies near the Antarctic Peninsula used a similar approach in examining temporal variation (or its absence) in the dependency of infaunal communities on the seasonal phytoplankton bloom (or to a more permanent but partly degraded detrital food source, the "food bank": Mincks et al., 2005, Glover et al., 2008). Our results suggest that the outcrop associated community (870 m), as a whole, appears more dependent on organic carbon produced by chemosynthesis because total density decreased when the $\delta^{13}C$ signature was higher and presumably supported less chemosynthesis. This decline in total density at the outcrop was admittedly non-significant, likely because of our weak statistical power, but we nonetheless observed clear changes in species composition that were not apparent in background communities. The appearance of ampharetid recruits in the outcrop in September 2013 suggests dependency on phytodetritus in this species similar to that seen in the background community. Background communities at Barkley Canyon depend strongly on phytodetritus from seasonal surface blooms, and some species match their reproductive cycles to these inputs. Therefore, even though the Barkley Hydrates outcrop community appears more dependent on chemosynthetic food sources, some taxa nonetheless utilize photosynthetically derived food sources.

5.8. Acknowledgements

5.9. References

Ambrose, W.G., Renaud, P.E. 1997. Does a pulsed food supply to the benthos affect polychaete recruitment patterns in the Northeast Water Polynya? Journal of Marine Systems, 10, 483-495. doi: http://dx.doi.org/10.1016/S0924-7963(96)00053-X.

Anderson, M., Gorley, R., Clarke, K. 2008. PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E. Plymouth, UK.

Bernardino, A.F., Levin, L.A., Thurber, A.R., Smith, C.R. 2012. Comparative composition, diversity and trophic ecology of sediment macrofauna at vents, seeps and organic falls. PLoS One, 7, e33515.

Billett, D.S.M., Bett, B.J., Reid, W.D.K., Boorman, B., Priede, I.G. 2010. Long-term change in the abyssal NE Atlantic: The '*Amperima* Event' revisited. Deep-Sea Research Part II: Topical Studies in Oceanography, 57, 1406-1417. doi: 10.1016/j.dsr2.2009.02.001.

Billett, D.S.M., Lampitt, R.S., Rice, A.L., Mantoura, R.F.C. 1983. Seasonal sedimentation of phytoplankton to the deep-sea benthos. Nature, 302, 520-522.

Blake, J., Hilbig, B., Scott, P. 1996a. Taxonomic atlas of the benthic fauna of the Santa Maria Basin and western Santa Barbara Channel: vol. 6, The annelida part 3: Polychaeta: Orbiniidae to Cossuridae. Santa Barbara Museum of Natural History. Santa Barbara, California.

Blake, J., Hilbig, B., Scott, P.H. 1996b. Taxonomic atlas of the benthic fauna of the Santa Maria Basin and western Santa Barbara Channel: vol. 7, The annelida part 4: Polychaeta: Flabelligeridae to Ampharetidae. Santa Barbara Museum of Natural History. Santa Barbara, California.

Campanyà-Llovet, N., Snelgrove, P.V.R. 2018. Fine-scale infaunal community and food web patch mosaics from Barkley methane hydrates (British Columbia, Canada): the role of food quality. Deep-Sea Research Part I: Oceanographic Research Papers. In press.

Campanyà-Llovet, N., Snelgrove, P.V.R., Parrish, C. 2017. Rethinking the importance of food quality in marine benthic food webs. Progress in Oceanography, 156, 240-251.

Chapman, R., Pohlman, J., Coffin, R., Chanton, J., Lapham, L. 2004. Thermogenic gas hydrates in the northern Cascadia margin. Eos, Transactions, American Geophysical Union, 85, 361.

Chauvet, P., Metaxas, A., Hey, A., Matabos, M. 2018. Annual and seasonal variations in the deep-sea megafaunal epibenthic communities in Barkley Canyon (British Columbia, Canada): a response to surface and deep-sea environment. Progress in Oceanography, in press.

Clarke, K., Gorley, R. 2006. PRIMER v6: User manual/tutorial. PRIMER-E. Plymouth, UK.

Coleman, D.C., Fry, B. 2012. Carbon isotope techniques. Academic Press. San Diego, USA.

Doya, C., Chatzievangelou, D., Bahamon, N., Purser, A., De Leo, F.C., Juniper, S.K., Thomsen, L., Aguzzi, J. 2017. Seasonal monitoring of deep-sea megabenthos in Barkley Canyon cold seep by internet operated vehicle (IOV). PLoS One, 12, e0176917.

Dubilier, N., Blazejak, A., Rühland, C. 2006. Symbioses between bacteria and gutless marine oligochaetes. In: J. Overmann. Molecular Basis of Symbiosis. (pp. 251-275). Springer. Germany.

Dufour, S.C., Felbeck, H. 2003. Sulphide mining by the superextensile foot of symbiotic thyasirid bivalves. Nature, 426, 65.

Gage, J.D., Tyler, P. 1991. Deep-sea biology: a natural history of organisms at the deep-sea floor. Cambridge University Press. New York, USA.

Giangrande, A. 1997. Polychaete reproductive patterns, life cycles and life histories: an overview. Oceanography and marine Biology, 35, 323-386.

Giere, O., Pfannkuche, O. 1982. Biology and ecology of marine Oligochaeta, a review. Oceanography and Marine Biology, 20, 173-309.

Glover, A.G., Smith, C.R., Mincks, S.L., Sumida, P.Y., Thurber, A.R. 2008. Macrofaunal abundance and composition on the West Antarctic Peninsula continental shelf: Evidence for a sediment 'food bank'and similarities to deep-sea habitats. Deep Sea Research Part II: Topical Studies in Oceanography, 55, 2491-2501.

Gooday, A.J. 2002. Biological responses to seasonally varying fluxes of organic matter to the ocean floor: A review. Journal of Oceanography, 58, 305-332. doi: 10.1023/a:1015865826379.

Grassle, J.F. 1989. Species diversity in deep-sea communities. Trends in Ecology & Evolution, 4, 12-15.

Henrichs, S.M. 2005. Organic matter in coastal marine sediments. In: A.R. Robinson, K.H. Brink. The Global Coastal Ocean: Multiscale Interdisciplinary Processes, The Sea, Vol. 13. (pp. 129-162). Harvard University Press. Boston.

Juniper, S.K., Matabos, M., Mihály, S., Ajayamohan, R.S., Gervais, F., Bui, A.O.V. 2013. A year in Barkley Canyon: a time-series observatory study of mid-slope benthos and habitat dynamics using the NEPTUNE Canada network. Deep-Sea Research Part II: Topical Studies in Oceanography, 92, 114-123.

Keeling, R.F., Körtzinger, A., Gruber, N. 2010. Ocean deoxygenation in a warming world. Annual Review Marine Science, 2, 199–229.

Levin, L.A. 2003. Oxygen minimum zone benthos: adaptation and community response to hypoxia. Oceanogr.Marine Biol.Annu.Rev, 41, 1-45.

Levin, L.A. 2005. Ecology of cold seep sediments: interactions of fauna with flow, chemistry and microbes. Oceanography and Marine Biology: An Annual Review, 43, 1-46.

Levin, L.A., Baco, A.R., Bowden, D.A., Colaço, A., Cordes, E.E., Cunha, M.R., Demopoulos, A.W., Gobin, J., Grupe, B.M., Le, J. 2016. Hydrothermal vents and methane seeps: rethinking the sphere of influence. Frontiers in Marine Science, 3, 72.

Levin, L.A., Ziebis, W., Mendoza, G.F., Bertics, V.J., Washington, T., Gonzalez, J., T., A.R., Ebbe, B., Lee, R.W. 2013. Ecological release and niche partitioning under stress: lessons from dorvilleid polychaetes in sulfidic sediments at methane seeps. Deep-Sea Research Part II: Topical Studies in Oceanography, 92, 214–233.

Lu, H., Seo, Y., Lee, J., Moudrakovski, I., Ripmeester, J.A., Chapman, N.R., Coffin, R.B., Gardner, G., Pohlman, J. 2007. Complex gas hydrate from the Cascadia margin. Nature, 445, 303-306.

Mincks, S.L., Smith, C.R., DeMaster, D.J. 2005. Persistence of labile organic matter and microbial biomass in Antarctic shelf sediments: evidence of a sediment food bank. Marine Ecology Progress Series, 300, 3-19.

Müller-Navarra, D.C. 2008. Food web paradigms: the biochemical view on trophic interactions. International Review of Hydrobiology, 93, 489-505. doi: 10.1002/iroh.200711046.

Purser, A., Thomsen, L., Barnes, C., Best, M., Chapman, R., Hofbauer, M., Menzel, M., Wagner, H. 2013. Temporal and spatial benthic data collection via an internet operated Deep Sea Crawler. Methods in Oceanography, 5, 1-18.

Ramesh, S., Vedachalam, N., Ramesh, R., Prasad, N.T., Ramadass, G., Atmanand, M. 2014. An approach for methane hydrates reservoir dissociation in a marine setting, Krishna Godhavari Basin, east coast India. Marine and Petroleum Geology, 58, 540-550.

Salvo, F., Wiklund, H., Dufour, S.C., Hamoutene, D., Pohle, G., Worsaae, K. 2014. A new annelid species from whalebones in Greenland and aquaculture sites in Newfoundland: *Ophryotrocha cyclops*, sp. nov. (Eunicida: Dorvilleidae). Zootaxa, 3887, 555-568.

Scheltema, R.S. 1986. On dispersal and planktonic larvae of benthic invertebrates: an eclectic overview and summary of problems. Bulletin of Marine Science, 39, 290-322.

Schoener, A. 1968. Evidence for reproductive periodicity in the deep sea. Ecology, 49, 81-87.

Skarke, A. 2015. New Insights into the temporal variability of seafloor methane discharge on the northern US Atlantic margin. American Geophysical Union, Fall Meeting.

Smith, K., Kaufmann, R., Baldwin, R. 1994. Coupling of near-bottom pelagic and benthic processes at abyssal depths in the eastern North Pacific Ocean. Limnology and Oceanography, 39, 1101-1118.

Soto, E.H., Paterson, G.L.J., Billett, D.S.M., Hawkins, L.E., Galeron, J., Sibuet, M. 2010. Temporal variability in polychaete assemblages of the abyssal NE Atlantic Ocean. Deep-Sea Research Part II: Topical Studies in Oceanography, 57, 1396-1405. doi: 10.1016/j.dsr2.2009.02.003.

Thomsen, L., Barnes, C., Best, M., Chapman, R., Pirenne, B., Thomson, R., Vogt, J. 2012. Ocean circulation promotes methane release from gas hydrate outcrops at the NEPTUNE Canada Barkley Canyon node. Geophysical Research Letters, 39. doi: 10.1029/2012gl052462.

Thurber, A.R., Levin, L.A., Rowden, A.A., Sommer, S., Linke, P., Kröger, K. 2013. Microbes, macrofauna, and methane: A novel seep community fueled by aerobic methanotrophy. Limnology and Oceanography, 58(5), 1640-1656.

Vanreusel, A., Cosson-Sarradin, N., Gooday, A.J., Paterson, G.L.J., Galéron, J., Sibuet, M., Vincx, M. 2001. Evidence for episodic recruitment in a small opheliid polychaete species from the abyssal NE Atlantic. Progress in Oceanography, 50, 285-301.

Wiklund, H., Glover, A.G., Dahlgren, T.G. 2009. Three new species of Ophryotrocha (Annelida: Dorvilleidae) from a whale-fall in the North-East Atlantic. Zootaxa, 2228, 43-56.

5.10. Appendix

Appendix 5.1. Mean (± standard deviation) C:N ratio from sedimentary organic matter from the outcrop and background sites sampled in September 2013 and May 2014.

C:N	September 2013	May 2014
Outrcrop	9.2 ± 0.82	11.2 ± 3.87
Background	9.3 ± 0.05	9.3 ± 0.33

6. The role of phytodetrital quality in macroinfaunal community structure and epifaunal response [5]

6.1. Abstract

In order to evaluate how food quality influences deep-sea macroinfaunal and mega-epifaunal communities, we deployed experimental food pulses at 890 m depth in Barkley Canyon, British Columbia, Canada. Pulses of two different microalgal species (i.e., *Chaetoceros calcitrans* and *Nannochloropsis oculata*) differed in nitrogen, carotenoid, and lipid content (i.e., quality). After 8 months we sampled enriched patches and controls with push cores to assess the effect of food quality. Analysis of deeper sediment layers indicated significant differences in community structure among treatments, driven by larger differences between the two algal treatments. We found no significant ($P > 0.05$) differences among total densities of macroinfauna in the surface sediment layers between the two enrichment patches and the control. Even so, the community from surface sediments enriched with *N. oculata* separated in ordination space (non-metric multidimensional scaling), driven primarily by the presence of recruits of *Aricidea* sp. (Paraonidae, Polychaeta) in enrichment treatments. These results suggest that the duration of the experiment exceeded the appropriate timeframe to detect initial colonizers, limiting the observed effect of food enrichment to the deeper sediment later. Analysis of functional traits indicated no significant differences among treatments and controls when comparing adults and recruits (i.e., horizontal migration and colonization), sessile, discretely motile, and motile organisms (i.e., foraging capacity), or the abundance of taxa

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with tentacles, palps or pharynxes (i.e., capacity for food selectivity). Analysis of functional traits of both recruits and adults nonetheless separated controls from both algal treatments in ordination space, and organisms with tentacles separated some samples enriched with *C. calcitrans* on the ordination space. Based on fixed camera images of each treatment over the course of the experiment, we observed more epifaunal Brachyura visits to a patch enriched with *N. oculata* than to a patch enriched with *C. calcitrans* or the procedural control patch during the first two weeks of the experiment. The visible area of one enrichment patch per algal treatment disappeared (> 90 %) ~ 1.5 months after deployment. Our study points to influences of food quality, at the algal class level, on macroinfaunal community structure and function, epifaunal disturbance, and sediment reworking.

6.2. Introduction

Although several lines of evidence emphasize the importance of differences in quantity (e.g. Grassle and Morse-Porteous 1987) and quality (e.g. Snelgrove et al. 1992; 1994; 1996) of food supply to structuring deep-sea benthic communities, supporting evidence remains scant. This gap limits our understanding of how food supply influences biodiversity patterns and biogeography of deep-sea ecosystems, noting that food supply can also influence ecosystem functioning and services (e.g., carbon recycling) (Levin et al., 2001, Snelgrove and Smith, 2002). Expected changes in food supply from surface waters to the deep-sea benthos associated with climate change (Smith et al., 2008, Sweetman et al., 2017), add urgency in understanding how food quality influences one of the largest species pools on Earth (Snelgrove and Smith, 2002).

The quality of a particular food source can be defined as the degree to which quantity and composition of that food source fulfill consumer nutritional needs (Müller-Navarra, 2008), and food quality can play a role in structuring communities and food web structure (Campanyà-Llovet et al., 2017). Most evidence on the role of food quality for marine organisms comes from the field of aquaculture, which optimizes diets to enhance growth, survival, and reproductive outputs of the reared species (Glencross, 2009). Nitrogen content (Howarth, 1988), degradation state (Beaulieu, 2002), biopolymeric carbon (i.e., carbohydrates, lipids, and proteins: Pusceddu et al., 2011), fatty acids and sterols (Parrish, 2013), and algal pigment composition (Roy et al., 2011), all represent potentially important sources of variation in food quality for benthic organisms.

Several studies on megafauna and macrofauna suggest specific benefits for organisms from food sources differing in quality. For example, some carotenoids improve reproductive output of echinoderms (Tsushlma et al., 1997), which can feed selectively on food patches containing specific carotenoids or fatty acids (Wigham et al., 2003, Boon and Duineveld, 2012), resulting in dramatic shifts in benthic communities (Billett et al., 2010). Furthermore, structural changes in benthic communities and food webs can influence ecosystem processes such as bioturbation (Dauwe et al., 1998). Polychaetes, the dominant macroinfaunal group in our Barkley Canyon (Campanyà-Llovet et al., in review) study site, can feed selectively on particles differing in size and/or biochemistry (Galéron et al., 2001, Jumars et al., 2015). Oligochaetes select particles of specific grain size and organic richness (Rodriguez et al., 2001), and mussels select for specific particle sizes (Defossez and Hawkins, 1997). Some nematodes and benthic harpacticoids exhibit selective feeding at a finer level (finer than bulk organic matter or biopolymeric carbon such as proteins, carbohydrates, and lipids), differentiating between diatoms and bacteria (Azovsky et al., 2005, Estifanos et al., 2013). In shallow water, oysters and harpacticoids can feed selectively on different classes of phytoplankton and even different species of diatoms (Loret et al., 2000, Cognie et al., 2001). Heart urchins (*Echinocardium cordatum*) from the North Sea actively selected for fatty acids and chlorophyll *a* (Boon and Duineveld, 2012). Changes in food quality may favour some selective benthic feeders, broadly affecting community structure, however, the mechanisms for selective feeding are not fully understood.

The "patch mosaic" theory posits that food patchiness and disturbance enhance overall numbers of species, explaining in part the unexpectedly high diversity in the deep sea (Grassle and Sanders, 1973). Colonization experiments consisting of enriched trays with contrasting food sources at different degradation stages, showed food selectivity at the colonization stage (Snelgrove et al., 1992, 1994, 1996). More recent studies trace food uptake by isotopically labelling the food added to the sediments, in "pulse-chase" experiments (Witte et al., 2003, Mäkelä et al., 2017). This type of experiment delineates trophic pathways and identifies the taxa that dominate and/or initiate food uptake from differing food sources (Hunter et al., 2013). Our study focuses on changes in macrofaunal community structure with varying qualities of food (i.e., phytoplankton classes differing not only in nitrogen content but also lipid and pigment composition). Most deep-sea experiments to date altered sediments to enrich them or confined the enrichment to a closed patch; in contrast, adding food to otherwise undisturbed sediments without barriers to migration, adds realism to the experiment (Smith and Brumsickle, 1989). Changes in macroinfaunal community structure have been reported after pulses of high-quality phytodetritus in shallow waters (Quijón et al., 2008), among naturally occurring patches of more or less degraded seagrass (Gallmetzer et al., 2005), and following the addition of wood or brown macroalgae to the deep sea floor (Grassle and Morse-Porteous, 1987).

Enrichment experiments are time-sensitive. For example, numbers of individuals and species in mudflat sediments enriched with green macroalgae decreased within the first two months of the experiment, then increased after about four months, and finally declined to background abundances five months later after detrital resources were

depleted (Kelaher and Levinton, 2003). Quijón et al. (2008) observed changes in macroinfaunal community structure one week after but not five months after the addition of an algal pulse. Furthermore, algae added in the fall produced a greater impact on the benthic community than when added during the summer. "Pulse-chase" experiments demonstrate macroinfaunal uptake of organic matter within a few days of addition (Witte et al., 2003, Hunter et al., 2013, Mäkelä et al., 2017), but colonization of food patch and changes in community structure typically become evident after weeks or months (Snelgrove et al., 1996, Galéron et al., 2001). Specific environmental variables and community structure determine the time response to food enrichments in each ecosystem.

In order to test the effects of food quality on Barkley Canyon benthos, we selected two biochemically distinct algal species for our *in situ* experiment. The two species differed in nitrogen content but also represent different algal classes, which implicitly affects their lipid and pigment composition. We chose the diatom *Chaetoceros calcitrans* to mimic the spring phytoplankton bloom, and the euglenophyte *Nannochloropsis oculata* for its high lipid content (Liu et al., 2017). *N. oculata* has been widely used in aquaculture because of its high polyunsaturated fatty acids (PUFA) and sterol content (Cohen, 1999). We aimed to quantify the consequences of contrasting food pulses on deep-sea benthic ecosystems by: 1) identifying shifts in macroinfaunal community structure between patches differing in food quality; 2) monitoring the number of visits of different epifaunal species to food patches differing in quality with time; and 3) comparing food patch disappearance and therefore, longevity, between algal treatments.

6.3. Materials and Methods

6.3.1. Experimental deployment and recovery

We deployed a food enrichment experiment at Barkley submarine canyon (890 m; see Fig. 6.1) in September 2013 from the vessel RV *Falkor* using the Remotely Operated Vehicle (ROV) ROPOS (www.ropos.com). The location of the experiment corresponded to the MidWest platform of the Ocean Networks Canada (ONC, www.oceannetworkscanada.ca) observatory. Food quality and quantity in Barkley Canyon vary with depth (Campanyà-Llovet et al., 2018), where sediments at the MidWest platform are characterized by high amounts (total organic carbon – TOC) of relatively high quality (high lipid concentrations and low C:N ratios), though relatively degraded (low Chl *a*: Phaeo ratio) organic matter (Campanyà-Llovet et al., 2018). The presence of an oxygen minimum zone (OMZ) (400 - 1000 m depth) within the canyon (Keeling et al., 2010, Domke et al., 2017) likely limits macroinfaunal densities compared to those reported elsewhere (Levin et al., 2003).
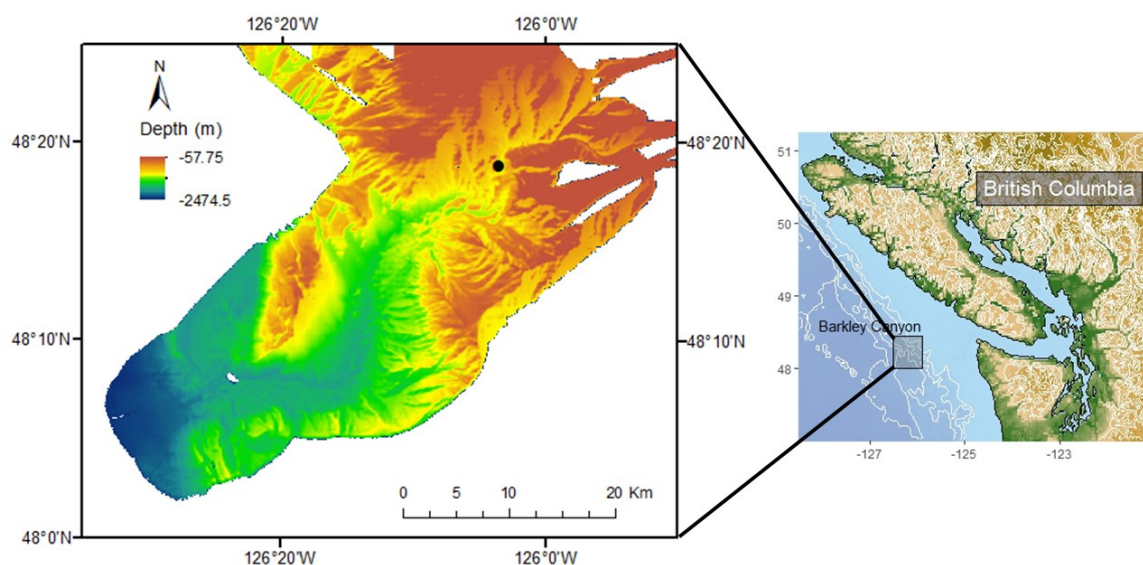
Figure 6.1. Enrichment experiment location within Barkley Canyon (NE Pacific). Bathymetric map generated from two original rasters sampled from T.G. Thompson 2002 and R/V *Falkor* 2013 by Ocean Networks Canada

We spread the same amount (88.2 mg) of organic carbon onto the sediment generating an organic matter "patch", in the form of either the North Pacific diatom (*Chaetoceros calcitrans*) or the Eustigmatophycea (*Nannochloropsis oculata*); this organic input corresponds to the maximum flux of organic matter arriving at 500 m depth in Barkley Canyon (60 mg C/m^2/d: Wu et al., 1999) integrated over a one-month period. In addition to the implicit differences in lipid and pigment composition of algae from two different classes that differ in food quality, we measured total organic carbon and nitrogen (Section 6.3.2), to evaluate differences in food quality, at least at a bulk level.

The algae were spread onto the seafloor using an Oceanlab spreader (Dr. Ursula Witte, Oceanlab, University of Aberdeen, Scotland, UK). Each spreader consists of a polycarbonate tube (diameter 25 cm, length 30 cm) with a plunger mechanism that

releases the treatment upon triggering. The resulting patches could potentially cover an area of 490 cm^2; however, actual distributions were non-homogenous and covered approximately 25 % of the area (122.5 cm^2) heavily. The spreaders were left in place for 4-6.5 h to allow the treatment to settle, noting a minimum reported settling time of 30 min to 2 hr (Fornes et al. 1999, Hunter et al. 2012). To expedite settlement of the algae we mixed it with inert kaolin clay (40 g / patch: Fornes et al., 1999, Hunter et al., 2012) which adds a clear white color to the enrichment patch that distinguishes it from surrounding sediments. Given that one of the main concerns with this study was that bottom currents (< 20 cm s^{-1} in Barkley Canyon: Thomsen et al., 2012) or megafauna would disperse the organic matter, we added 0.5 g of inert fluorescent tracer to provide visible evidence of whether the patches were still present when we re-sampled at the end of the experiment. In the end, the kaolin clay clearly stood out from the background sediment and proved a better marker. Preliminary experiments within a flume tank simulating currents as strong as 20 cm s^{-1} showed no substantial removal of kaolin clay over a period of 2-3 days. To minimize disturbance by the abundant sablefish, we placed cage frames (50 cm x 50 cm x 40 cm, no mesh) over each treatment patch. One video record shows a sablefish clearly diverted by the cages from swimming on top of the patch (pers. obs.). Because the experiment was deployed in front of ONC's AXIS P1347 colour camera with two ROS MV-LED Lights and a pan/tilt unit that allowed complete coverage of all three treatments (±90° Tilt and ±180° Pan), we could monitor patch disappearance and megafaunal visits to the enrichment patches over time. Because of the large spatial extent of the experiment and the availability of only one camera, we could only record one patch per treatment (*N. oculata*, *C. calcitrans*, and procedural control), precluding

any replication. The camera lights were turned on every 2 hours for 5 min, during which time the camera was directed at each treatment and control patch for 80 seconds. All videos and still imagery are archived and available through ONC's Oceans 2.0.

Each patch was placed a minimum of 3 m away from adjacent patches. We deployed 6 replicates of each algal treatment and 6 cages with no enrichment patch as a control for cage frames (Fig. 6.2). Limitations in bottom time precluded the deployment of a procedural control with kaolin and fluorescent dye onto sediments protected with cage frames and without algal material. Instead our procedural control only included cage frames. Background sediment samples provided a natural control. After 8 months (May 2014), we were finally able to return to the site to recover the experiment from the C.C.G. *Tully* using the ROV OE (http://itbsubsea.com/equipment/rovs/oceanic-explorer/). We collected 24 push cores (6 from the background community, 6 from the procedural controls, 6 from *C. calcitrans* treatments, and 6 from *N. oculata* treatments) to evaluate macroinfaunal community structure and any remaining traces of the algal enrichment. We vertically sectioned each core into 0-5 and 5-10 cm layers, removed four ml sediment samples from each core layer with a syringe, and froze them at -20 ºC in Whirl-pack bags for later organic matter analysis. The remainder of the core was preserved in a 4 % seawater-formaldehyde solution for taxonomic analysis.

Figure 6.2. Food enrichment experiment layout in MidWest Ocean Networks Canada (ONC) platform at Barkley Canyon. Procedural control controls for the cage frame effects on the benthic communities.

6.3.2. Sediment samples

Organic matter samples were analyzed for total organic carbon as a measure of food quantity and TN and C:N as measures of food quality (Campanyà-Llovet et al., 2017). We quantified TOC and TN by drying a sediment subsample of 1-5 g (wet weight) at 80 °C for 24 h, grinding the subsample to a fine powder, and weighing and acidifying it (under pure HCl fumes for 24 h) to eliminate inorganic carbon. Samples were dried again at 80 °C for 24 h before the C:N analysis, for which we weighed and folded an aliquot of dried decarbonated sediments (15 mg) into a tin capsule to analyse for TOC and TN in a Carlo Erba NA1500 Series II elemental analyser (EA).

We transferred samples for taxonomy into 70 % ethanol and processed them through a 300-μm sieve in the laboratory for identification. Specimens were sorted under a dissecting microscope and identified to the lowest taxonomic level possible using a variety of published sources (Hartman, 1968, Hartman, 1969, Bousfield, 1973, Barnard and Karaman, 1991, Blake et al., 1996a, Blake et al., 1996b, Blake et al., 1996c, Reuscher et al., 2009).

6.3.3. Video analysis

In order to determine rates of patch disappearance, we extracted still images from the videos available in Sea Tube Pro (Oceans 2.0, www.dmas.uvic.ca/SeaTube) from Ocean Networks Canada every day from the beginning to the end of the experiment (13th of September 2013 until 25th of April 2014). We measured the surface area of each patch with "image J" software (https://imagej.nih.gov), using the width of the cage frame to calculate scale in each picture.

In order to account for any diel behaviour, we estimated the number of epifaunal visits at each food patch and control four sampling times each day (00:00, 06:00, 12:00, and 18:00) focusing on the the first two weeks of the experiment (13th – 28th of September 2013) to assess the initial epifaunal response to food pulses of differing quality. We counted and identified the organisms found on the sediment within the area delimited by each cage frame at a broad taxonomic level following Juniper et al. (2013), constrained by the lack of physical specimens to confirm species identities.

We further investigated the impact of the cage frames by identifying the taxa and counting the number of individuals from each taxon that interacted with different areas of

the cage frame: on top, underneath but in contact with the cage frame, on the legs, at the base of the legs, and on the sediment surface but under the cage frame.

6.3.4. Statistical analysis

6.3.4.1. Infauna

Because we expected a different response from organism in the surface sediment layers, and therefore, in direct contact with the algal treatment compared to the deeper sediment layers (5-10 cm), and therefore, away from the influence of the food enrichment, we ran separate statistical analyses for the two sediment depths (0-5 cm and 5-10 cm). We compared macroinfaunal densities and biodiversity (i.e., $ES_{[10]}$ and rarefaction curves) in each experimental treatment and procedural control. We examined normality of the residuals prior to a one-way ANOVA (density – log transformed - and biodiversity - no transformation) with one fixed factor: Treatment (levels: procedural control, *C. calcitrans*, *N. oculata*). We could not achieve normality of the residuals in total abundances or biodiversity ($ES_{[10]}$) from the deeper sediment layers and therefore ran a non-parametric test (Kruskal-Wallis). We then used the multivariate PERMANOVA routine from the add-on PERMANOVA+ module in PRIMER v.6 (Clarke and Gorley, 2006, Anderson et al., 2008) to identify differences in macroinfaunal community structure with the same factors and levels as above. Abundance data were log-transformed into Bray-Curtis similarity matrices and zero-adjusted in the deeper sediment layers (5-10 cm) where organisms were sometimes absent. This correction forces 100 % similarity between two samples with no fauna, as is appropriate for such data. We assume lack of oxygen at those sediment depths affected all samples similarly, and we used the zero-adjusted Bray-

Curtis similarity method. We used non-metric multidimensional scaling (nMDS) to visualize macroinfaunal community variation with treatment and sediment depth within the ordination space, and superimposed correlation vectors to show the most important taxa in explaining the observed pattern. We complemented these results with the SIMilarity PERcentage (SIMPER) routine, which tests for significant differences in discriminating taxa. We used a PERMANOVA with one fixed factor (Treatment levels: Procedural control, *C. calcitrans*, *N. oculata*) to assess differences in community structure among treatments in each sediment depth separately. Prior to these analyses we verified homogeneity of multivariate dispersion using the PERMDISP routine (Anderson et al., 2008). We repeated the same multivariate analyses based on functional traits of the different taxa (Jumars et al., 2015): life stage (i.e., recruit and adults based on size), which differentiate between colonization from larvae that develop into recruits and horizontal migration of adults; mobility (i.e., sessile – organisms that can feed without moving - , discretely motile – organisms that stay in place indefinitely and can feed without moving but remain capable of moving, and motile – organisms that move in order to eat), which represents the degree of foraging capacity of the organism; and feeding structure (i.e., palps, tentacles, and pharynx), which indicate capacity to selectively feed on different food particles, with palps and tentacles as the most selective structures and pharynxes as the least. In order to assess the effect of cage frames on macroinfaunal community structure, we also repeated the uni- and multivariate analyses based on number of species, modifying the "Treatment" factor (levels: procedural control and background). We used a Kruskal-Wallis non-parametric test when comparing total abundances among procedural

control and background for the surface and deeper sediment layers because we could not attain normality and homogeneity of variances, even after transformation.

6.3.4.2. Epifauna

We used multivariate analysis to visually identify differences in epifaunal composition with Treatment (levels: procedural control, *C. calcitrans*, *N. oculata*). We could not use statistical tests because of lack of replication (only one patch per treatment). Because of the relatively high proportions of "unknown" taxa (organisms that could not be identified because of limited camera resolution), we focused on proportion of dominant taxa within the identified organisms, Brachyura (Crustacea, Decapoda) and Caridea (Crustacea, Decapoda). We used those proportions to classify the "unknown" organisms into Brachyura, Caridea, or others (18.4 % of counts). All multivariate and ordination analyses were performed in PRIMER v.6 (Clarke and Gorley, 2006) and the add-on PERMANOVA+ module (Anderson et al., 2008). Count data were log-transformed and zero-adjusted. Bray-Curtis similarity matrices were used for resemblance-based methods. We used non-metric multidimensional scaling (nMDS) to visualize epifaunal visitors per treatment and overlaid species vectors in order to assess the taxa contributing to the nMDS distribution. We used the SIMPER routine and nMDS vectors to assess the primary contributors to differences observed among treatments.

6.3.4.3. Sediment organic matter

We first investigated food quality (i.e., TN, and C:N) of the algae used to enrich the sediments with a one-way ANOVA with one fixed factor (Algae: *C. calcitrans*, *N. oculata*). We then investigated food composition from the sediments at the end of the

experiments (i.e., TOC, TN, and C:N) with a two-way ANOVA with fixed and crossed factors (Treatment: procedural control, *C. calcitrans*, and *N. oculata*; Sediment depth: 0-5 and 5-10 cm) on TN and C:N, which was possible because of the inferred independence of samples between sediment depths for each food variable (one-way ANOVA, P > 0.05). We also ran a Kruskal-Wallis test for each factor above on TOC because we could not attain normality of the residuals and homogeneity of variances, even after transformation.

6.4. Results

6.4.1. Infauna

In total, we identified 279 individuals representing 77 taxa from 57 cores that sampled four different treatments (background, procedural control, *C. calcitrans*, *N. oculata*). Our multivariate analysis based on species composition, showed significant differences between treatments only in the deeper sediment samples (5-10 cm) (PERMANOVA, Pseudo-$F_{2,15}$ = 1.34, P(perm) = 0.0362). Pairwise comparisons indicated largest differences among the two algal treatments, however, differences were not significant (P(perm) > 0.05), indicating differences in the effect sizes between each pair. The PERMDISP routine did not identify differences in the data cloud dispersion among treatments (PERMDISP, $F_{2,15}$ = 1.58, P(perm) = 0.360). *Aricidea (Acmira) simplex*, thyasirid, and *Aphelochaeta* sp. A were the main contributors in the separation of the sediments enriched with *N. oculata* in the deeper layers (5-10 cm), according to the superimposed vectors in the nMDS (Fig. 6.3), and SIMPER analyses confirmed these results (Appendix 6.1). The absence of organisms in some cores and low number of

species resulted in zero similarities within the procedural control and the *C. calcitrans* treatment.

Figure 6.3. Non-metric multidimensional scaling (3D nMDS) based on infaunal response to a food enrichment in Barkley Canyon per treatment and per sediment depth. Assemblages are based on Bray-Curtis similarity log (x+1) transformation. The large circle represents the correlation between species and nMDS axes. We only overlaid the most relevant vectors (Pearson's correlation threshold of 0.75). N (Control) = 6, N (*C. calcitrans*) = 6, N (*N. oculata*) = 6. *A. monicae = Aricidea (Stretzovia) monicae*; *B. c. = Bipalponephtys cornuta*; Amph. juv. = Ampharetid juvenile, *Aph.* sp. A = *Aphelochaeta* sp. A.

Multivariate analysis on surface sediment samples (0-5 cm) indicated no significant differences (PERMANOVA, Pseudo-$F_{2,15}$ = 0.80, P = 0.786; PERMDISP, $F_{2,15}$ = 0.12, P = 0.898) in species composition of *N. oculata* sediment samples from *C. calcitrans* and procedural control samples, which, despite some overlap, separated in ordination space (Fig. 6.3). Superimposed vectors on the nMDS plot based on Pearson correlation > 0.75, identified recruits belonging to the genera *Aricidea* sp. and *Cossura* sp. as the main contributor to sediments enriched with the *N. oculata* treatment, whereas ampharetid recruits and the paraonid *Aricidea (Stretzovia) monicae* were more prevalent in the control and *C. calcitrans* treatments. SIMPER analysis confirmed *Aricidea* sp. recruits as the main driver of differences between sediments enriched with *N. oculata* and the other two treatments (procedural control and *C. calcitrans*; see Appendix 6.2). The paraonids *Levinsenia gracilis*, *L. oculata*, and the dorvilleid *Pettiboneia* sp. (Appendix 6.2) characterized surface sediments in the procedural control. *Aricidea (Stretzovia) monicae* (Paraonidae), *Pettiboneia* sp. (Dorvilleidae), and *Eclysippe* sp. (Ampharetidae) characterized the surface sediments enriched with the *C. calcitrans* whereas *Aricidea* sp. recruits, *Cossura* cf. *rostrata*, and *Bipalponephtys cornuta* recruits characterized surface sediments enriched with *N. oculata* (Appendix 6.2).

Despite non-significant differences among treatments in functional traits (P > 0.05), recruits in surficial sediments separated from both algal treatments in nMDS ordination space (Fig. 6.4). For adults, some surficial sediment samples enriched with *C. calcitrans* separated from sediments enriched with *N. oculata* and procedural controls, and both

algal treatments separated from the control when considering only organisms with tentacles as feeding structure (Fig. 6.4).
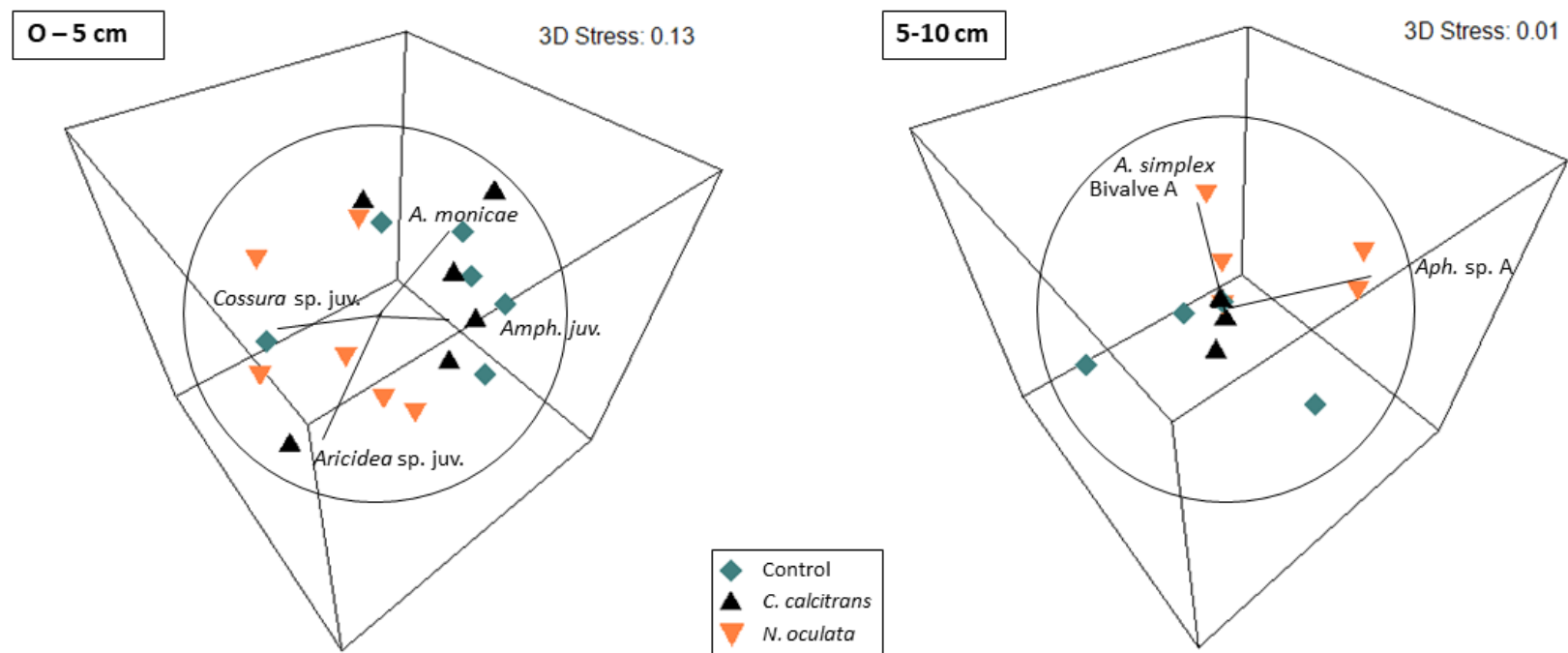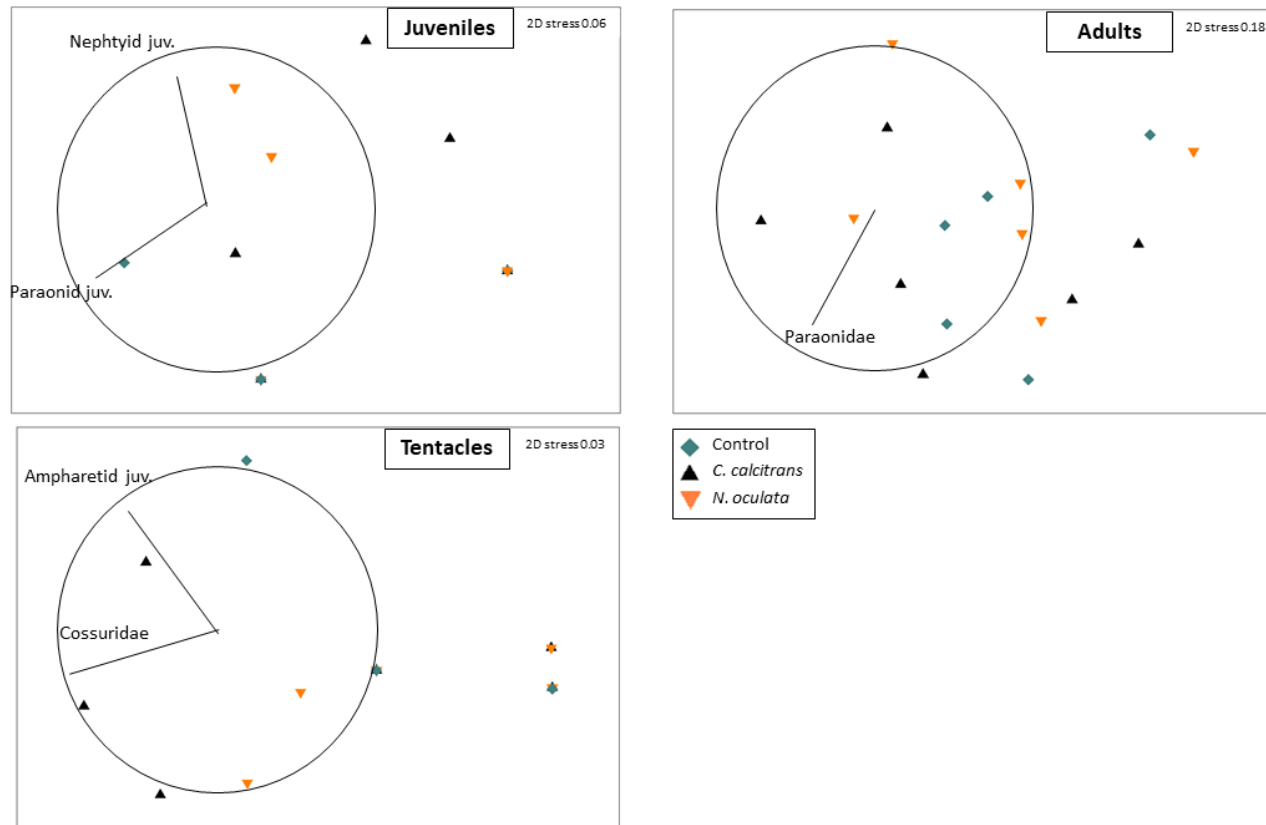
Figure 6.4. Non-metric multidimensional scaling (2D nMDS) based on infaunal response to a food enrichment in Barkley Canyon per treatment and per sediment depth based on recruits, adults, and organisms with tentacles. Assemblages are based on Bray-Curtis similarity log (x+1) transformation. The large circle represents the correlation between species and nMDS axes. We only overlaid the most relevant vectors (Pearson's correlation threshold of 0.75). N (Control) = 6, N (*C. calcitrans*) = 6, N (*N. oculata*) = 6.

Total abundances did not differ among treatments (i.e., procedural control, *C. calcitrans*, *N. oculata*) in surface sediment layers (one-way ANOVA, $F_{2,15}$ =0.98, P = 0. 397; see Fig. 6.5a), nor in the deeper sediment layers (Kruskal-Wallis, χ^2 = 1.64, df = 2, P = 0.439). Similarly, $ES_{[10]}$ did not vary significantly among treatments in surface sediment layers (one-way ANOVA, $F_{2,15}$ = 0.92, P = 0.419), nor deeper sediment layers (Kruskal-Wallis, χ^2 = 1.55, df = 2, P = 0.460; see Fig. 6.5b).
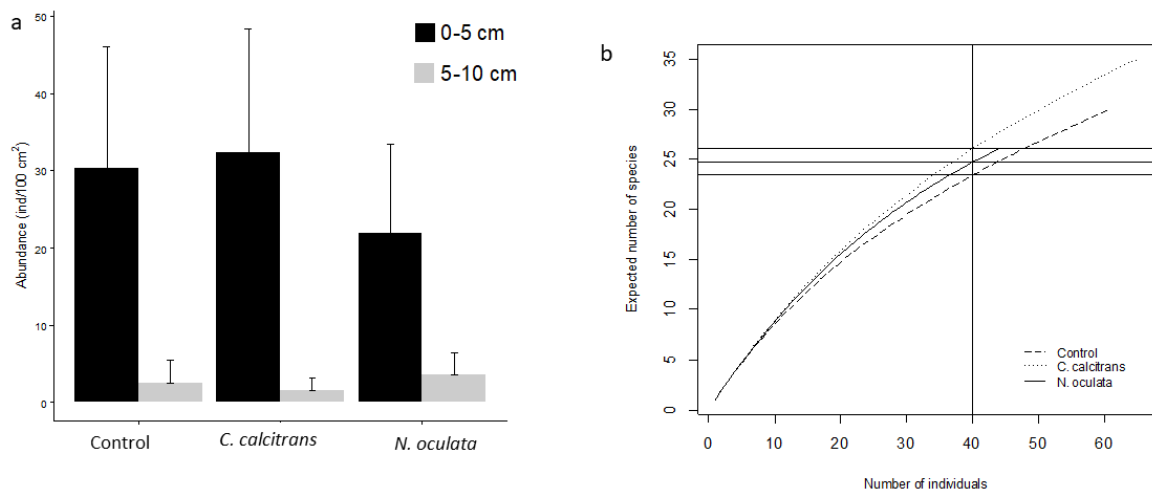
Figure 6.5. Effects of food enrichment with different algal classes on macroinfaunal communities from Barkley Canyon: (a) Total infaunal abundances per treatment and sediment, error bars denote standard deviation, and (b) expected number of species per treatment in surface sediment layer (0-5 cm), lines denote number of species at 50 individuals. *C. calcitrans* = *Chaetoceros calcitrans*, *N. oculata* = *Nannochloropsis oculata*. N (Control) = 6, N (*C. calcitrans*) = 6, N (*N. oculata*) = 6, per sediment layer.

We compared the background community with the procedural control assemblages to identify any potential effects of the cage frames (treatment factor levels: procedural control and background) on macroinfaunal community structure. Total abundances did not significantly differ among treatments (i.e., background and procedural control) in the

surface sediments (Kruskal-Wallis χ^2 (1)= 1.28, P = 0.257) nor in the deeper sediments (Kruskal-Wallis χ^2 (1)= 0.08 , P = 0.768; see Fig. 6a). Similarly, $ES_{[10]}$ did not vary significantly among treatments (one-way ANOVA, Treatment: $F_{1,9}$ = 0.12, P = 0.733) but decreased with sediment depth (one-way ANOVA, $F_{1,21}$ = 17.81, P < 0.001; see Fig. 6.6b). PERMANOVA analysis identified no significant differences between background and procedural control samples (Pseudo-$F_{1,9}$ = 0.76, P = 0.779), and statistically significant differences only between sediment depths (Pseudo-$F_{1,21}$ = 3.94, P = 0.002)(Fig. 6.7). We found no significant differences in the data cloud dispersion of the procedural control and background samples based on treatment (PERMDISP, $F_{1,9}$=0.73, P(perm) = 0.477) but significant differences based on sediment depth (PERMDISP, $F_{1,21}$ = 72.29, P(perm) < 0.001).
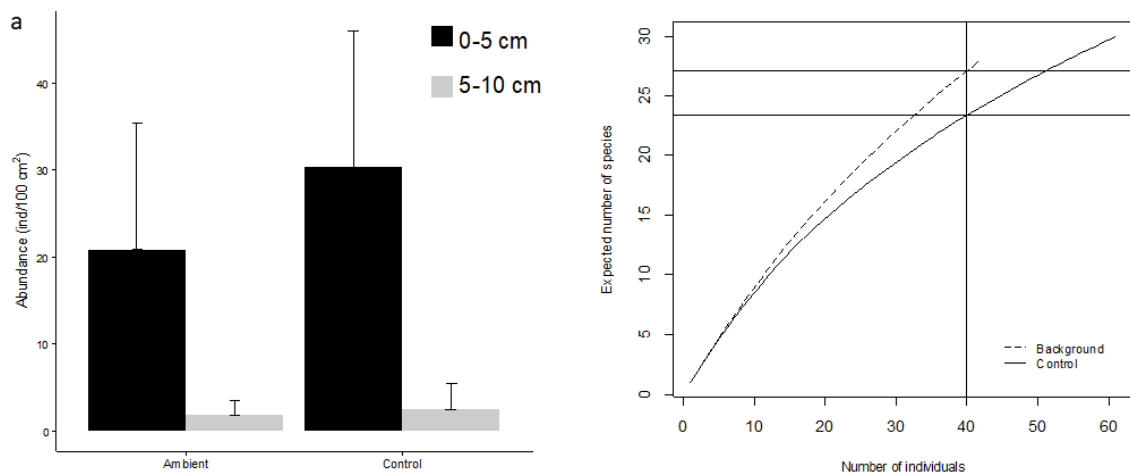
Figure 6.6. Effects of cage frames on macroinfaunal communities from Barkley Canyon: (a) Total infaunal abundances per treatment and sediment, error bars denote standard deviation, and (b) expected number of species per treatment, lines denote number of species at 40 individuals. N (Background) = 6, N (Control) = 6, per sediment layer.
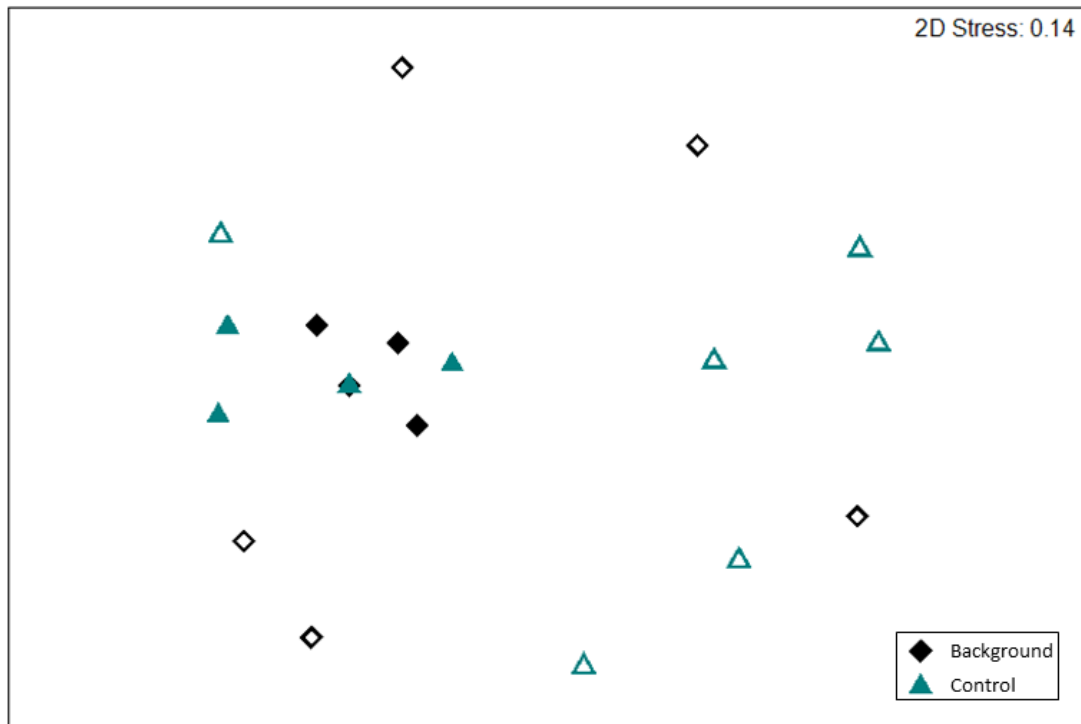
Figure 6.7. Non-metric multidimensional scaling (nMDS) based on infaunal response to cage frames used in a food enrichment in Barkley Canyon. Assemblages are based on Bray-Curtis similarity with dummy variable and log (x+1) transformation. Blank symbols represent 0-5 cm sediment depth layer and filled symbols the 5-10 cm layer. N (Background) = 6, N (Control) = 6, per sediment layer.

We measured a significantly higher (one-way ANOVA, $F_{1,5}$ = 7306, P < 0.001) C:N ratio in *N. oculata* (11.29 ± 0.15) compared to *C. calcitrans* (5.49 ± 0.04) algae before deploying the experiment. Sediment organic matter at the end of the experiment did not differ significantly among treatments (P > 0.05) or sediment depth for any of the food variables (i.e., TOC, TN, C:N; see Table 6.1), although the C:N ratio was substantially reduced in the *N. oculata* treatment (Fig. 6.8).

Table 6.1. Results from ANOVA analysis on total nitrogen (TN) and C:N ratios from sedimentary organic matter from Barkley Canyon (NE Pacific, 890 m depth) and Kruskal-Wallis from total organic matter (TOC) between treatments (i.e., control, *Chaetoceros calcitrans*, and *Nannochloropsis oculata*) and sediment depth (i.e., 0-5 and 5-10 cm).

Variable	Source	df	SS	MS	F value/ χ^2	Pr (>F) / P - value
TOC	Treatment	2	--	--	1.76	0.415
	Sediment depth	1	--	--	2.18	0.14
TN	Treatment	2	0.00	0.00	0.29	0.749
	Sediment depth	1	0.00	0.00	2.39	0.144
	Treatment: sediment depth	2	0.00	0.00	0.81	0.465
	Residuals	14	0.00	0.00	--	--
C:N	Treatment	2	0.63	0.32	2.35	0.132
	Sediment depth	1	0.05	0.05	0.39	0.541
	Treatment: sediment depth	2	0.06	0.03	0.25	0.785
	Residuals	14	1.90	0.14	--	--
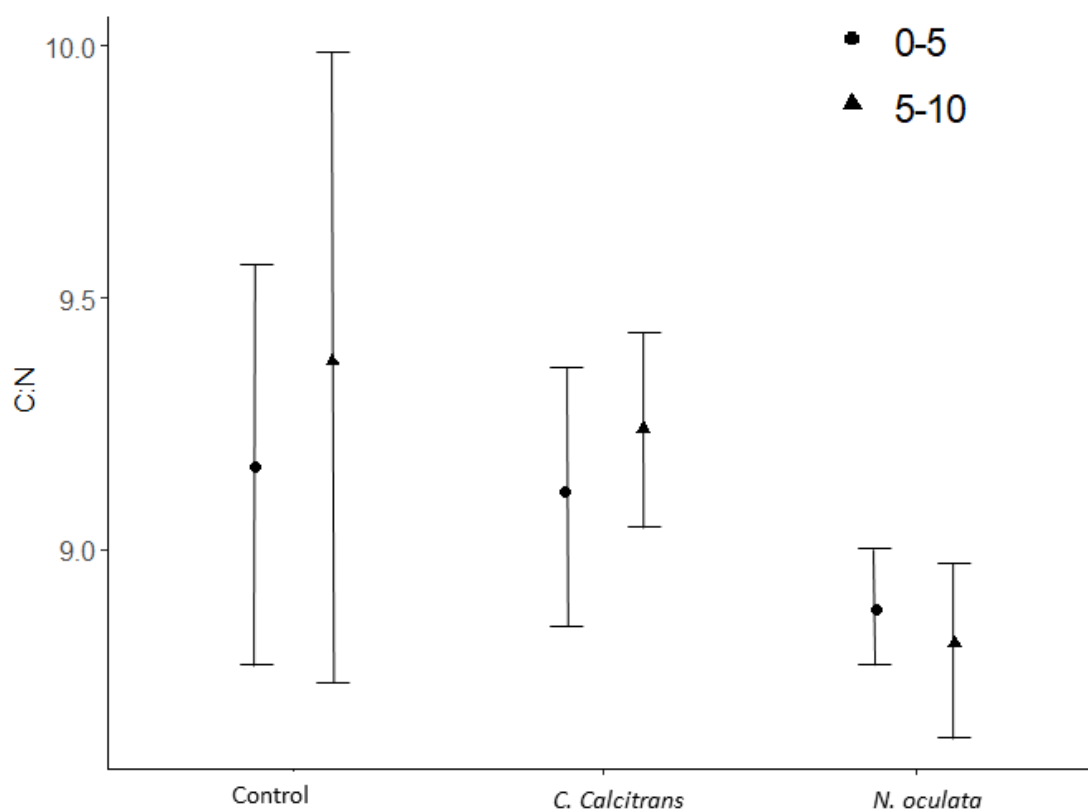
Figure 6.8. Mean C:N ratio of sediment organic matter at three different treatments and two sediment depths (i.e., 0-5 and 5-10 cm) from an enrichment experiment in Barkley Canyon. *C. calcitrans = Chaetoceros calcitrans, N. oculata = Nannochloropsis oculata.* Error bars represent standard deviations and N=3 for each treatment and sediment depth. N (Control) = 3, N (*C. calcitrans*) = 3, N (*N. oculata*) = 3, per sediment layer.

6.4.2. Epifauna

Nannochloropsis oculata enrichment attracted more visitors (total of 178) in the first 15 days after experimental deployment than the other two treatments (procedural control: 99; *C. calcitrans*: 114). Taxonomic composition differed among treatments (Fig. 6.9). Brachyura dominated *N. oculata* and *C. calcitrans*, with lower proportions of Caridea; however, Brachyura strongly dominated *N. oculata* treatments (SIMPER). The nMDS vectors also indicated Brachyura dominance in both algal treatments (Fig. 6.9). Brachyura

and Caridea visited the procedural control treatment in equal proportions. Finally, we observed that the cage frames played a sheltering and support role for epifauna. We observed Brachyura positioned underneath the cage frame and close to the leg bases, Caridean on top of the cage frame, and Munidospis sp. on the legs of the cage frame.
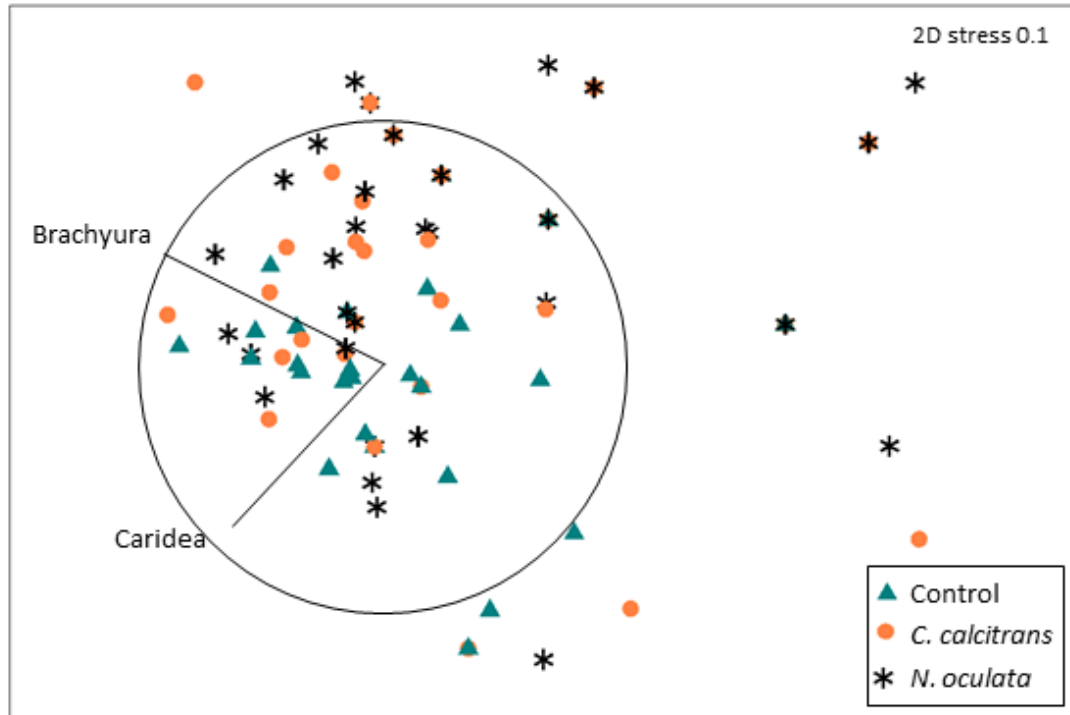
Figure 6.9. Non-metric multidimensional scaling (nMDS) based on the epifaunal counts from a food enrichment experiment in Barkley Canyon (890 m depth). Each dot represent a sampling time over the 15 day study. Assemblages are based on Bray-Curtis similarity. *C. calcitrans = Chaetoceros calcitrans*, *N. oculata = Nannochloropsis oculata*. The large circle represents the correlation between taxa and nMDS axes. We only overlaid the most relevant vectors nMDS (Pearson's correlation threshold of 0.8).

6.4.3. Food patch surface area

Longevity of the two different food patches (*C. calcitrans* and *N. oculata*) differed by four months (Fig. 6.10). *C. calcitrans* disappeared in April 2014 (7 months after deployment) whereas *N. oculata* disappeared in December 2013 (3.5 months after deployment). However, the majority of the food patch (>90 %) vanished in both treatments by the end of October 2013 (i.e., 1.5 months after deployment). The two treatments differed in disturbance frequency. The patch size of *C. calcitrans* decreased abruptly twice (arrows in Fig. 6.10), whereas *N. oculata* relative surface area decreased more gradually until mid-October 2013 (arrow in Fig. 6.10), when surface area briefly increased, then continued to decline.
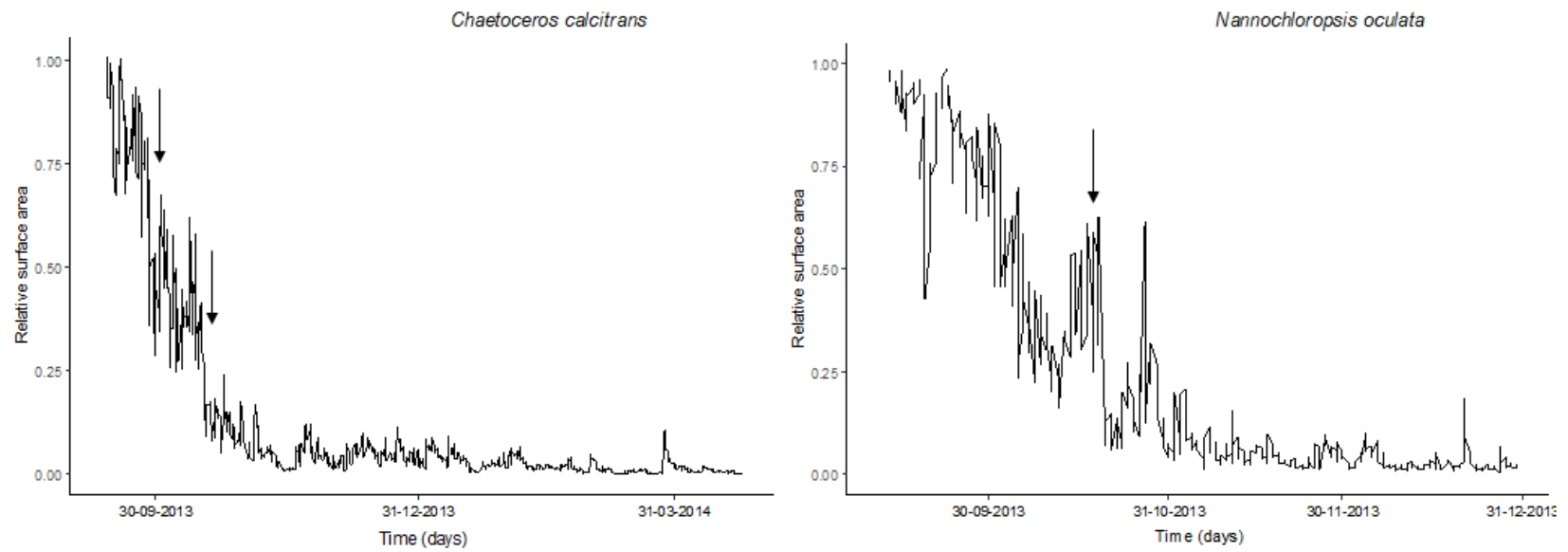
Figure 6.10. Relative surface area of food patches (i.e., *Chaetoceros calcitrans* and *Nannochloropsis oculata*) at Barkley Canyon (890 m depth) with time. Arrows denote episodic events with sudden increases or decreases in relative surface area covered.

6.5. Discussion

6.5.1. Infaunal community structure

Food enrichment experiments often compare very distinct food qualities (e.g., terrestrial *vs* marine organic matter, macroalgae *vs* microalgae) to ensure a strong signal in the response variable of interest. Few studies base their *in situ* food experiments on different phytoplankton species (Ingels et al., 2011, Mäkelä et al., 2017). A clear signal in these kinds of experiments requires strong selective feeding (Jumars, 1993, Wigham et al., 2003). For example, nematodes from polar regions ingest bacterial carbon more rapidly than diatom carbon, thus indicating food selectivity (Ingels et al., 2010). Our experiment showed changes in benthic communities enriched with similar food sources and therefore, tested more subtle food selectivity (i.e., between algal classes) than most experiments.

Deeper layers of sediments are usually disconnected from on-going processes at the sediment-water interface. Sediments often become anoxic below the top few millimeters to centimeters, confining the vast majority of organisms to sediment surface layers (Snelgrove, 1999). Even so, several processes can connect surface and subsurface sediments, for example, through bioturbation. Biological activity can mix the surface layer of deep-sea sediments to depths of ~ 10 cm (Guinasso and Schink, 1975), potentially transporting added food particles from surface to deep sediments, and thus influencing community structure at those sediment depths.

We observed differences, albeit weak, in overall species distributions among treatments in surface sediment layers, which differed slightly in C:N ratios at the end of the experiment. Our analyses identified *Aricidea* sp. recruits as distinctive within *N. oculata*

enrichment patches, suggesting a degree of selectivity in these recruits. Feeding modes of paraonid polychaetes vary greatly but selectivity has been observed in some species (Jumars et al., 2015). Even though we could not prove the inert effect of kaolin clay and fluorescent dye on benthic macroinfauna, we nonetheless demonstrated that observed differences in community response were not simply an artefact of the cage frames we deployed to protect the enrichment patches from sablefish disturbance.

Pulses of organic matter to the sea floor can influence both ecosystem functioning (e.g., carbon mineralization, biological traits) and/or community structure (Gallmetzer et al., 2005, Quijón et al., 2008, Mayor et al., 2012). We only found weak differences in response of functional groups to food pulses differing in quality (ie., different algal classes). Recruits of some species contributed to separation of algal treatments from the procedural control in ordination space, pointing to selection of organic patches by some organisms. Motility and feeding mode are linked (Jumars et al., 2015): the degree of motility of an organism depends on its distance from, and longevity of resource availability (Grünbaum, 2002). For example, there are no completely sessile macrophagous feeders (Jumars et al., 2015) because they must search for large food items (e.g., macroalgae or prey). Even so, we found no treatment separation based on organism motility. Among the different feeding structures present (i.e., palps, tentacles, and pharynx), tentacles can be associated with selective feeding, rather than an eversible pharynx (Jumars et al., 2015); species with tentacles as feeding structures helped to separate sediments enriched with *C. calcitrans* from control sediments and those enriched with *N. oculata*.

Food quality can influence benthic community and food web structure (Campanyà-Llovet et al., 2017). Carbon to nitrogen ratios and TN provide insight into the nutritional value of a food source because protein and most membrane lipid formation require nitrogen, which limits primary production in many marine ecosystems (Howarth, 1988, Parrish, 2013). Preferential consumption of organic nitrogen by certain benthic fauna (Evrard et al., 2010), which is controlled by organismal C:N budgets and thus their demands (Hunter et al., 2012), demonstrates the relevance of C:N as a metric of food quality for benthic organisms and/or the capability of benthic organisms to modify this ratio. Low carbon uptake of terrestrial organic matter by benthic macrofauna from Whittard Canyon, NE Atlantic, most likely related to high C:N, and therefore, nitrogen limitation of the food source (Hunter et al., 2013). The differences observed in C:N ratios from algal treatments used in our experiment, together with the differences in lipid and fatty acid composition could explain the differences observed in the respective community structures. However, the C:N ratios at the end of the experiment result from the initial differences between treatments and the consumption of the organic matter over the entire length of the experiment.

Polyunsaturated fatty acids, essential fatty acids, and some sterols, which differ between algal species (Banerjee et al., 2011), are indispensable to most cultured marine organisms (Müller-Navarra, 2008, Glencross, 2009). Essential fatty acids cannot be synthesised de novo by benthic consumers but they play an important role in metabolic function and in determining vital functions such as reproductive output (Parrish, 2009) [e.g., 20:5ω3 (EPA – eicosapentaenoic acid), 20:4ω6 (ARA – arachidonic acid), and 22:6ω3 (DHA –

docosapentaenoic acid)]. *N. oculata*, like all Eustigmatophiceae have high proportions of EPA and ARA, in contrast to a high proportion of EPA and the presence of ARA and DHA in diatoms (Hodgson et al., 1991, Brown et al., 1997). *Nannochloropsis* spp. differ also from other microalgae because they build up high concentrations of a range of pigments such as astaxanthin, zeaxanthin, and canthaxanthin (Lubián et al., 2000). These differences in food quality may explain structural changes observed in the infaunal community in our experiment although the exact mechanisms are not fully understood.

Differences in species composition of the infaunal community among food treatments can only be explained by selective feeding. Some polychaete species selectively feed on apparently homogeneous detrital material (Self and Jumars, 1988). Tentaculate deposit feeders (such as spionids, and ampharetid polychaetes observed in our study) can select for specific (generally small) particle sizes (Jumars et al., 1982). Our results showed non-significant differences among tentaculate polychaetes, which may select for a specific algal treatment. Deposit feeder particle selectivity may be largely passive and mechanical: the surface texture of natural grains increases probability of selection by tentacles, and bacteria-coated grains appear to be picked up selectively because of adhesion to the bacterial coating (Guieb et al., 2004). Tentaculate feeders can select for particles using muscles, cilia, and mucus with different adhesive properties that enhance the proportion of protein in ingested food (Lopez and Levinton, 1987). Selective feeding also occurs in suspension- and deposit-feeding bivalves, with species-specific processes based on physical and chemical characteristics of the particles (Ward and Shumway, 2004).

Previous enrichment experiments in the field reported responses by colonizers after the addition of organic matter into the sediments. Total abundance and biodiversity changed among trays enriched with different food sources (i.e., diatom *vs* brown macroalgae) and degradation state. (Snelgrove et al., 1992, 1996). Although our food patches occupied ~ 120 cm^2, they fall within the areal range (50 – 1750 cm^2) in which different colonization modes generally balance (i.e., settlement from water column *vs* reproduction by species with benthic development *vs* immigration of rectruits from the surrounding sediments: Smith and Brumsickle, 1989). Studies examining trophic pathways through food enrichments have shown differential uptake of pulses of organic matter by different taxa (Witte et al., 2003, Hunter et al., 2013), and even among polychaete families (Sweetman and Witte, 2008). These differences suggest taxon-dependent efficiency in carbon uptake, and could explain increases in abundances of some taxa, leading to community structure changes. Whereas experiments with enriched trays evaluate recruitment, "pulse-chase" experiments focus on which food resource particular organisms select and ingest more efficiently; a community experiment such as ours considers both, and determines changes in community structure.

We expected a rapid increase in numbers of individuals in algal patches shortly after the experiment deployments (first few days or weeks). However, because our experiment ran for 8 months, any initial attraction to a food pulse had presumably attenuated by the time we recovered the experiment. Based on the longevity of our patches (see below 4.3.), ideally we would have resampled the experiment within a month of deployment to detect maximum numbers of organisms migrating to selected food patches and/or rectruit

colonizers, and associated reworking and uptake of the introduced organic carbon. The high organic matter content at that site compared to other canyon locations (Campanyà-Llovet et al., 2018) may dampen the need for niche speciation and therefore, selective feeding and macrofaunal response. Furthermore, the influence of an environmental stressor such as an OMZ may obscure the effects of food quality on benthic communities (Campanyà-Llovet et al., 2017).

6.5.2. Epifaunal number of visits to patch

Highly mobile and visible crustaceans dominated the visitors in the area: Brachyura, Caridea, *Munidopsis* sp., and Paguroidea were all reported previously in the same season (fall) in Barkley Canyon (Juniper et al., 2013). Grooved tanner crab (*Chionoecetis tanneri*), a common brachyurid, commonly occurs at that site (Juniper et al., 2013); although we lack data on feeding preferences, its congener, *C. opilio*, is a carnivore and scavenger that feeds on bivalves, polychaetes, amphipods, and other crustaceans (Divine et al., 2017). We suggest that the increased number of Brachyura in the algal treatments in the first two weeks following experiment deployment resulted from increased prey abundances within the sediment, rather than attraction of Brachyura to the actual food treatment. We also observed brachyuran feeding on larger food falls (i.e., gelatinous material) outside the cage frames, adding scavenging to a predatory diet (pers. obs.). The irregular terrain surrounding the *N. oculata* enrichment patch compared to the *C. calcitrans* or procedural control patches may have played a role. Again, the absence of imagery from other experimental replicates precludes de finitive conclusions on differences in patch visitors.

The higher counts of mega-epifauna during the first two weeks after the deployment of the experiment suggest an increased predatory pressure at the sediments enriched with *N. oculata*. The deeper sediment layers occasionally provide refuge from predators to infaunal species. Total abundances in sediment patches enriched with *N. oculata* were slightly lower than in the procedural control and *C. calcitrans* surface sediment layers, but slightly higher in the deeper sediment layer. These results, although not statistically significant, support the idea of a predatory refuge in deeper sediments and the need to acknowledge interaction between different size-class organisms when interpreting the results of a food enrichment experiment.

6.5.3. Patch disappearance

The organic matter we added to Barkley Canyon (~ 890 m depth) was likely remineralized and/or buried. Most organic matter produced in the photic zone and deposited onto the sediment surface is recycled and remineralized, with burial of a small proportion (Rullkötter, 2006). Deep-sea benthic organisms begin to ingest organic matter only a few days after its arrival (Witte et al., 2003). The organic matter consumed by benthic organisms enters each size compartment (i.e., bacteria, meiofauna, macrofauna, megafauna) in different proportions, which varies with ecosystem (Smith et al., 2008). Given the negligible effect of bottom currents on patch movement at our site (based on our preliminary experiments), our results suggest that benthic organisms (i.e., bacteria, meiofauna, macrofauna, and megafauna) in Barkley Canyon at 890 m depth, reworked ~ 90 % of 88.2 mg of carbon in ~ 1.5 months. Even so, we found differences in the removal of the visible food patch with time between the two algal treatments. First, the organic

matter remaining after ~ 1.5 months persisted longer in *C. calcitrans* (an additional 5.5 months) than in *N. oculata* (an additional 2 months). Second, episodic events varied among treatments. Two episodic events substantially reduced *C. calcitrans* patch size, suggesting a sudden uptake or disturbance by a large organism. At the same time, the *N. oculata* patch actually increased in surface area, likely as a result of sediment movement and reworking that flattened the food patch, thereby increasing its surface area. As noted earlier, although these results offer valuable information on patch longevity at bathyal depths, lack of replication limits our conclusions.

6.6. Conclusions

Food supply can depend on environmental variation in the form of increases/decreases in particulate organic carbon flux, and therefore, amounts of food, and/or in the form of changes in organic matter biochemistry and, therefore, food quality (Smith et al., 2008). Changes in planktonic species composition are expected to result in shifts from large to smaller-sized plankton with climate change, which have different export efficiencies and biochemistry, altering in this way the carbon flux (Smith et al., 2008). In this study, we show that differences in food quality, at the algal class level, can modify benthic infaunal community structure and attract different epifaunal species, although, we recommend a shorter time-frame (e.g. one month) for this type of experiment to achieve more conclusive results. These kinds of integrative studies are useful in understanding interactions among size compartments (e.g., macroinfauna and mega-epifauna), in that the *N. oculata* food patch received more epifaunal visitors. However, more robust conclusions require shorter experiments and higher numbers of replicates.

These findings have ramifications for predicting climate change impacts on benthos. The sensitivity of planktonic species to temperature change can lead to phenological alterations and biogeographic shifts at the species level, resulting in trophic mismatch within the food web, or abrupt shifts in planktonic community structure (Beaugrand, 2009). These changes in species composition can result in changes in the biochemistry of this flux (i.e., algal composition: Smith et al., 2008).

6.7. Acknowledgements

6.8. References

Anderson, M., Gorley, R., Clarke, K. 2008. PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E. Plymouth, UK.

Azovsky, A., Saburova, M., Chertoprood, E., Polikarpov, I. 2005. Selective feeding of littoral harpacticoids on diatom algae: hungry gourmands? Marine Biology, 148, 327-337.

Banerjee, S., Hew, W.E., Khatoon, H., Shariff, M., Yusoff, F.M. 2011. Growth and proximate composition of tropical marine *Chaetoceros calcitrans* and *Nannochloropsis oculata* cultured outdoors and under laboratory conditions. African Journal of Biotechnology, 10, 1375-1383.

Barnard, J.L., Karaman, G.S. 1991. The families and genera of marine gammaridean Amphipoda (except marine gammaroids). Records of the australian museum, Suppl. 13, 866.

Beaugrand, G. 2009. Decadal changes in climate and ecosystems in the North Atlantic Ocean and adjacent seas. Deep-Sea Research Part II: Topical Studies in Oceanography, 56, 656-673.

Beaulieu, S. 2002. Accumulation and fate of phytodetritus on the sea floor. Oceanography and Marine Biology: An Annual Review, 40, 171-232.

Billett, D.S.M., Bett, B.J., Reid, W.D.K., Boorman, B., Priede, I.G. 2010. Long-term change in the abyssal NE Atlantic: The '*Amperima* Event' revisited. Deep-Sea Research Part II: Topical Studies in Oceanography, 57, 1406-1417. doi: 0.1016/j.dsr2.2009.02.001.

Blake, J., Hilbig, B., Scott, P. 1996a. Taxonomic atlas of the benthic fauna of the Santa Maria Basin and western Santa Barbara Channel: vol. 6, The annelida part 3: Polychaeta: Orbiniidae to Cossuridae. Santa Barbara Museum of Natural History. Santa Barbara, California.

Blake, J., Hilbig, B., Scott, P.H. 1996b. Taxonomic atlas of the benthic fauna of the Santa Maria Basin and western Santa Barbara Channel: vol. 4, The annelida part 1: Oligochaeta and Polychaeta: Phyllodocida (Phyllodocidae to Paralacydoniidae). Santa Barbara Museum of Natural History. Santa Barbara, California.

Blake, J., Hilbig, B., Scott, P.H. 1996c. Taxonomic atlas of the benthic fauna of the Santa Maria Basin and western Santa Barbara Channel: vol. 7, The annelida part 4: Polychaeta: Flabelligeridae to Ampharetidae. Santa Barbara Museum of Natural History. Santa Barbara, California.

Boon, A.R., Duineveld, G.C. 2012. Phytopigments and fatty acids in the gut of the deposit-feeding heart urchin *Echinocardium cordatum* in the southern North Sea: selective feeding and its contribution to the benthic carbon budget. Journal of Sea Research, 67, 77-84.

Bousfield, E.L. 1973. Shallow-water gammaridean amphipoda of New England. Cornell University Press. UK.

Brown, M., Jeffrey, S., Volkman, J., Dunstan, G. 1997. Nutritional properties of microalgae for mariculture. Aquaculture, 151, 315-331.

Campanyà-Llovet, N., Snelgrove, P.V.R. 2018. Fine-scale infaunal community and food web patch mosaics from Barkley methane hydrates (British Columbia, Canada): the role of food quality. Deep-Sea Research Part I: Oceanographic Research Papers. In press.

Campanyà-Llovet, N., Snelgrove, P.V.R., de Leo, F. 2018. Food quantity and quality in Barkley Canyon (NE Pacific) and it influence on macroinfaunal community structure. Progress in Oceanography, in press.

Campanyà-Llovet, N., Snelgrove, P.V.R., Parrish, C. 2017. Rethinking the importance of food quality in marine benthic food webs. Progress in Oceanography, 156, 240-251.

Clarke, K., Gorley, R. 2006. PRIMER v6: User manual/tutorial. PRIMER-E. Plymouth, UK.

Cognie, B., Barillé, L., Rincé, Y. 2001. Selective feeding of the oyster *Crassostrea gigas* fed on a natural microphytobenthos assemblage. Estuaries, 24, 126-134.

Cohen, Z. 1999. Chemicals from microalgae. CRC Press, UK.

Dauwe, B., Herman, P.M., Heip, C. 1998. Community structure and bioturbation potential of macrofauna at four North Sea stations with contrasting food supply. Marine Ecology Progress Series, 173, 67-83.

Defossez, J., Hawkins, A. 1997. Selective feeding in shellfish: size-dependent rejection of large particles within pseudofaeces from *Mytilus edulis*, *Ruditapes philippinarum* and *Tapes decussatus*. Marine Biology, 129, 139-147.

Divine, L.M., Bluhm, B.A., Mueter, F.J., Iken, K. 2017. Diet analysis of Alaska Arctic snow crabs (*Chionoecetes opilio*) using stomach contents and δ^{13}C and δ^{15}N stable isotopes. Deep Sea Research Part II: Topical Studies in Oceanography, 135, 124-136. doi: https://doi.org/10.1016/j.dsr2.2015.11.009.

Domke, L., Lacharite, M., Metaxas, A., Matabos, M. 2017. Influence of an oxygen minimum zone and macroalgal enrichment on benthic megafaunal community composition in a NE Pacific sumbarine canyon. Marine Ecology, 38(6). doi: 10.1111/maec.12481.

Estifanos, T.K., Traunspurger, W., Peters, L. 2013. Selective feeding in nematodes: A stable isotope analysis of bacteria and algae as food sources for free-living nematodes. Nematology, 15, 1-13.

Evrard, V., Soetaert, K., Heip, C.H., Huettel, M., Xenopoulos, M.A., Middelburg, J.J. 2010. Carbon and nitrogen flows through the benthic food web of a photic subtidal sandy sediment. Marine Ecology Progress Series, 416, 1-16.

Fornes, W.L., DeMaster, D.J., Levin, L.A., Blair, N.E. 1999. Bioturbation and particle transport in Carolina slope sediments: A radiochemical approach. Journal of Marine Research, 57, 335-355. doi: 10.1357/002224099321618245.

Galéron, J., Sibuet, M., Vanreusel, A., Mackenzie, K., Gooday, A., Dinet, A., Wolff, G. 2001. Temporal patterns among meiofauna and macrofauna taxa related to changes in sediment geochemistry at an abyssal NE Atlantic site. Progress in Oceanography, 50, 303-324.

Gallmetzer, I., Pflugfelder, B., Zekely, J., Ott, J. 2005. Macrofauna diversity in *Posidonia oceanica* detritus: distribution and diversity of mobile macrofauna in shallow sublittoral accumulations of *Posidonia oceanica* detritus. Marine Biology, 147, 517-523.

Glencross, B.D. 2009. Exploring the nutritional demand for essential fatty acids by aquaculture species. Reviews in Aquaculture, 1, 71-124.

Grassle, J.F., Morse-Porteous, L.S. 1987. Macrofaunal colonization of disturbed deep-sea environments and the structure of deep-sea benthic communities. Deep-Sea Research Part I: Oceanographic Research Papers, 34, 1911-&. doi: 10.1016/0198-0149(87)90091-4.

Grassle, J.F., Sanders, H.L. 1973. Life histories and role of disturbance. Deep-Sea Research, 20, 643-659.

Grünbaum, D. 2002. Predicting availability to consumers of spatially and temporally variable resources. Hydrobiologia, 480, 175-191.

Guieb, R.A., Jumars, P.A., Self, R.F. 2004. Adhesive-based selection by a tentacle-feeding polychaete for particle size, shape and bacterial coating in silt and sand. Journal of Marine Research, 62, 260-281.

Guinasso, N., Schink, D. 1975. Quantitative estimates of biological mixing rates in abyssal sediments. Journal of Geophysical Research, 80, 3032-3043.

Hartman, O. 1968. Atlas of the errantiate polychaetous annelids from California. Allan Hancock Foundation. Los Angeles, California.

Hartman, O. 1969. Atlas of the sedentariate polychaetous annelids from California. Allan Hancock Foundation. Los Angeles, California.

Hodgson, P.A., Henderson, R.J., Sargent, J.R., Leftley, J.W. 1991. Patterns of variation in the lipid class and fatty acid composition of *Nannochloropsis oculata* (Eustigmatophyceae) during batch culture. Journal of Applied Phycology, 3, 169-181.

Howarth, R.W. 1988. Nutrient limitation of net primary production in marine ecosystems. Annual Review of Ecology and Systematics, 89-110.

Hunter, W.R., Levin, L.A., Kitazato, H., Witte, U. 2012. Macrobenthic assemblage structure and organismal stoichiometry control faunal processing of particulate organic carbon and nitrogen in oxygen minimum zone sediments. Biogeosciences, 9, 993-1006. doi: 10.5194/bg-9-993-2012.

Hunter, W.R., Jamieson, A., Huvenne, V., Witte, U. 2013. Sediment community responses to marine *vs* terrigenous organic matter in a submarine canyon. Biogeosciences, 10, 67-80.

Ingels, J., Van den Driessche, P., De Mesel, I., Vanhove, S., Moens, T., Vanreusel, A. 2010. Preferred use of bacteria over phytoplankton by deep-sea nematodes in polar regions. Marine Ecology Progress Series, 406, 121-133.

Ingels, J., Billett, D.S.M., Van Gaever, S., Vanreusel, A. 2011. An insight into the feeding ecology of deep-sea canyon nematodes - results from field observations and the first in-situ 13C feeding experiment in the Nazare Canyon. Journal of Experimental Marine Biology and Ecology, 396, 185-193. doi: 10.1016/j.jembe.2010.10.018.

Jumars, P.A., Dorgan, K.M., Lindsay, S.M. 2015. Diet of worms emended: an update of polychaete feeding guilds. Annual Review of Marine Science, 7, 497-520.

Jumars, P.A., Self, R.F., Nowell, A.R. 1982. Mechanics of particle selection by tentaculate deposit-feeders. Journal of Experimental Marine Biology and Ecology, 64, 47-70.

Jumars, P.A. 1993. Gourmands of mud: diet selection in marine deposit feeders. In: R.N. Hughes. Mechanisms of Diet Choice. (pp. 124-156). Blackwell Scientific Publishers. Oxford.

Juniper, S.K., Matabos, M., Mihály, S., Ajayamohan, R.S., Gervais, F., Bui, A.O.V. 2013. A year in Barkley Canyon: a time-series observatory study of mid-slope benthos and habitat dynamics using the NEPTUNE Canada network. Deep-Sea Research Part II: Topical Studies in Oceanography, 92, 114-123.

Keeling, R.F., Körtzinger, A., Gruber, N. 2010. Ocean deoxygenation in a warming world. Annual Review Marine Science, 2, 199–229.

Kelaher, B.P., Levinton, J.S. 2003. Variation in detrital enrichment causes spatio-temporal variation in soft-sediment assemblages. Marine Ecology Progres Serires, 261, 85-97.

Levin, L.A., Ziebis, W., Mendoza, G.F., Growney, V.A., Tryon, M.D., Brown, K.M., Mahn, C., Gieskes, J.M., Rathburn, A.E. 2003. Spatial heterogeneity of macrofauna at northern California methane seeps: influence of sulfide concentration and fluid flow. Marine Ecology Progress Series, 265, 123-139.

Levin, L.A., Etter, R.J., Rex, M.A., Gooday, A.J., Smith, C.R., Pineda, J., Stuart, C.T., Hessler, R.R., Pawson, D. 2001. Environmental influences on regional deep-sea species diversity. Annual Review of Ecology and Systematics, 51-93.

Liu, J., Song, Y., Qiu, W. 2017. Oleaginous microalgae *Nannochloropsis* as a new model for biofuel production: Review & analysis. Renewable and Sustainable Energy Reviews, 72, 154-162.

Loret, P., Pastoureaud, A., Bacher, C., Delesalle, B. 2000. Phytoplankton composition and selective feeding of the pearl oyster *Pinctada margaritifera* in the Takapoto lagoon (Tuamotu Archipelago, French Polynesia): *in situ* study using optical microscopy and HPLC pigment analysis. Marine Ecology Progress Series, 199, 55-67.

Lubián, L.M., Montero, O., Moreno-Garrido, I., Huertas, I.E., Sobrino, C., González-del Valle, M., Parés, G. 2000. *Nannochloropsis* (Eustigmatophyceae) as source of commercially valuable pigments. Journal of Applied Phycology, 12, 249-255.

Mäkelä, A., Witte, U., Archambault, P. 2017. Ice algae *vs* phytoplankton: resource utilization by Arctic deep sea macroinfauna revealed through isotope labelling experiments. Marine Ecology Progress Series, 572, 1-18.

Mayor, D.J., Thornton, B., Hay, S., Zuur, A.F., Nicol, G.W., McWilliam, J.M., Witte, U.F. 2012. Resource quality affects carbon cycling in deep-sea sediments. The ISME Journal, 6, 1740-8. doi: 10.1038/ismej.2012.14.

Müller-Navarra, D.C. 2008. Food web paradigms: the biochemical view on trophic interactions. International Review of Hydrobiology, 93, 489-505. doi:10.1002/iroh.200711046.

Parrish, C.C. 2009. Essential fatty acids in aquatic food webs. In: Arts et al. Lipids in Aquatic Ecosystems. (pp. 309-326). Springer. New York.

Parrish, C.C. 2013. Lipids in marine ecosystems. ISRN Oceanography, 2013, Article ID 604045, 16. doi: 10.5402/2013/604045.

Pusceddu, A., Bianchelli, S., Gambi, C., Danovaro, R. 2011. Assessment of benthic trophic status of marine coastal ecosystems: Significance of meiofaunal rare taxa. Estuarine, Coastal and Shelf Science, 93, 420-430.

Quijón, P.A., Kelly, M.C., Snelgrove, P.V.R. 2008. The role of sinking phytodetritus in structuring shallow-water benthic communities. Journal of Experimental Marine Biology and Ecology, 366, 134-145. doi: 10.1016/j.jembe.2008.07.017.

Reuscher, M., Fiege, D., Wehe, T. 2009. Four new species of Ampharetidae (Annelida: Polychaeta) from Pacific hot vents and cold seeps, with a key and synoptic table of characters for all genera. Zootaxa, 2191, 1-40.

Rodriguez, P., Martinez-Madrid, M., Arrate, J.A., Navarro, E. 2001. Selective feeding by the aquatic oligochaete *Tubifex tubifex* (Tubificidae, Clitellata). Hydrobiologia, 463, 133-140.

Roy, S., Llewellyn, C.A., Egeland, E.S., Johnsen, G. 2011. Phytoplankton pigments: characterization, chemotaxonomy and applications in oceanography. Cambridge University Press. New york (USA).

Rullkötter, J. 2006. Organic Matter: the driving force for early diagenesis. In H.D. Schulz, M. Zabel. Marine Geochemistry.Springer. Berlin, Heidelberg.

Self, R.F., Jumars, P.A. 1988. Cross-phyletic patterns of particle selection by deposit feeders. Journal of Marine Research, 46, 119-143.

Smith, C.R., Brumsickle, S.J. 1989. The effects of patch size and substrate isolation on colonization modes and rates in an intertidal sediment. Limnology and Oceanography, 34, 1263-1277.

Smith, C.R., De Leo, F.C., Bernardino, A.F., Sweetman, A.K., Arbizu, P.M. 2008. Abyssal food limitation, ecosystem structure and climate change. Trends in Ecology & Evolution, 23, 518-528.

Snelgrove, P.V.R., Butman, C.A., Grassle, J.F. 1995. Potential flow artifacts associated with benthic experimental gear - deep-sea mudbox examples. Journal of Marine Research, 53, 821-845. doi: 10.1357/0022240953213016.

Snelgrove, P.V.R., Butman, C.A., Grassle, J.P. 1993. Hydrodynamic enhancement of larval settlement in the bivalve *Mulinia lateralis* (Say) and the polychaete *Capitella* sp. I in microdepositional environments. Journal of Experimental Marine Biology and Ecology, 168, 71-109. doi: 10.1016/0022-0981(93)90117-7.

Snelgrove, P.V.R., Grassle, J.F., Petrecca, R.F. 1992. The role of food patches in maintaining high deep-sea diversity - field experiments with hydrodynamically unbiased colonization trays. Limnology and Oceanography, 37, 1543-1550.

Snelgrove, P.V.R., Grassle, J.F., Petrecca, R.F. 1994. Macrofaunal response to artificial enrichments and depressions in a deep-sea habitat. Journal of Marine Research, 52, 345-369. doi: 10.1357/0022240943077082.

Snelgrove, P.V.R., Grassle, J.F., Petrecca, R.F. 1996. Experimental evidence for aging food patches as a factor contributing to high deep-sea macrofaunal diversity. Limnology and Oceanography, 41, 605-614.

Snelgrove, P.V.R. 1999. Getting to the bottom of marine biodiversity: Sedimentary habitats: Ocean bottoms are the most widespread habitat on earth and support high biodiversity and key ecosystem services. BioScience, 49, 129-138.

Snelgrove, P.V.R., Smith, C.R. 2002. A riot of species in an environmental calm: The paradox of the species-rich deep-sea floor. Oceanography and Marine Biology, 40, 311-342.

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

Sweetman, A.K., Witte, U. 2008. Macrofaunal response to phytodetritus in a bathyal Norwegian fjord. Deep-Sea Research Part I: Oceanographic Research Papers, 55, 1503-1514. doi: 10.1016/j.dsr.2008.06.004.

Thomsen, L., Barnes, C., Best, M., Chapman, R., Pirenne, B., Thomson, R., Vogt, J. 2012. Ocean circulation promotes methane release from gas hydrate outcrops at the NEPTUNE Canada Barkley Canyon node. Geophysical Research Letters, 39. doi: 10.1029/2012gl052462.

Tsushlma, M., Kawakami, T., Mine, M., Matsuno, T. 1997. The role of carotenoids in the development of the sea urchin *Pseudocentrotus depressus*. Invertebrate Reproduction & Development, 32, 149-153. doi: 10.1080/07924259.1997.9672616.

Ward, J.E., Shumway, S.E. 2004. Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. Journal of Experimental Marine Biology and Ecology, 300, 83-130. doi: 10.1016/j.jembe.2004.03.002.

Wigham, B.D., Hudson, I.R., Billett, D.S.M., Wolff, G.A. 2003. Is long-term change in the abyssal Northeast Atlantic driven by qualitative changes in export flux? Evidence

from selective feeding in deep-sea holothurians. Progress in Oceanography, 59, 409-441. doi: 10.1016/j.pocean.2003.11.003.

Witte, U., Wenzhofer, F., Sommer, S., Boetius, A., Heinz, P., Aberle, N., Sand, M., Cremer, A., Abraham, W.R., Jorgensen, B.B., Pfannkuche, O. 2003. *In situ* experimental evidence of the fate of a phytodetritus pulse at the abyssal sea floor. Nature, 424, 763-766. doi: 10.1038/nature01799.

Wu, J.P., Calvert, S.E., Wong, C.S. 1999. Carbon and nitrogen isotope ratios in sedimenting particulate organic matter at an upwelling site off Vancouver Island. Estuarine Coastal and Shelf Science, 48, 193-203. doi: 10.1006/ecss.1998.0409.

7. Conclusions and future directions

7.1. Consequences of changes in food quality for deep-sea benthic ecosystems

This research demonstrates a significant relationship between food quality and different community and food web variables. Some food quality parameters were better predictors of several feeding guilds, relationships that, not surprisingly, were stronger for lower trophic levels (i.e., deposit feeders and suspension feeders), the feeding guilds in direct contact with the detritus (Chapter 2). Knowing that each feeding guild contributes to specific functions (e.g., bioturbation) in different ways (Dauwe et al., 1998), these results imply an indirect influence of food quality on ecosystem functions.

Food heterogeneity in space and time coincided with differences in infaunal community and food web structure (Chapters 3-6). Therefore, patches with distinct food qualities can harbour distinct infaunal communities and areas with high food heterogeneity, such as the clam bed at Barkley Hydrates or the head of Barkley Canyon (Chapter 3 and 4), can support higher species and/or feeding guild diversity (Chapter 4). These results also confirm some degree of macroinfaunal specialization in the apparently homogeneous but, in fact, heterogeneous detrital pool (Beaulieu, 2002). Selective feeding by benthic fauna

on detritus has been reported for macroinfauna and mega-epifauna (Wigham et al., 2003, Guieb et al., 2004). Organism morphology offers insights into selection mechanisms. Tentaculate polychaetes can select actively for particle size, shape, and/or coating whereas polychaetes with an eversible pharynx cannot feed selectively on detritus (Guieb et al., 2004). Although stomach contents of some echinoderms clearly demonstrate selective feeding (Wigham et al., 2003, Boon and Duineveld, 2012) the mechanism of selectivity is not fully understood and cannot be directly inferred from morphology (Wigham et al., 2003).

Finally, these results indicate that quantity of organic material also merits consideration in tandem with quality. The amount of food, which usually relates to benthic stocks (i.e., total abundance and biomass: Johnson et al., 2007), sometimes modifies species composition (Wei et al., 2010), and although my study emphasizes the role of food quality in structuring benthic communities and food webs, quantity cannot be overlooked. In fact, food quantity and quality cannot be fully disentangled (Müller-Navarra, 2008) when studying environmental drivers of benthic communities.

7.2. Responses to food quality from different taxonomic size groupings

My analyses showed different responses to changes in food quantity and quality in different size classes of benthic fauna (i.e., bacteria, meiofauna, macrofauna, and megafauna). Organic matter biochemistry might be more relevant to smaller organisms living within the sediment than to larger organisms. For example, analysis of data compiled from the literature showed that food quantity was a stronger predictor of meiofaunal biomass than for macrofauna or megafauna (Chapter 2). The interactions

between different size classes of benthic organisms also merit consideration. In Chapter 3, distinct infaunal communities may result from differing food inputs (i.e., more zooplankton detritus in one patch), and reworking of organic matter by the pink sea urchin aggregation. Megafauna can modify sediment biochemistry (Amaro et al., 2010), which may affect infaunal community structure. At the same time, structural changes in infaunal community (e.g., abundance and biomass) can attract dominant carnivorous megafauna (e.g., Brachyura response to the *Nannochloropsis oculata* food patch described in Chapter 6).

7.3. Food quality as an environmental driver

The variation in food quality necessary to alter benthic community and food web structure span from disparate (e.g., chemosynthetic and photosynthetic; see Chapters 4 and 5) to relatively similar (e.g., phytoplankton class levels) food sources (Chapter 6). Even so, environmental stressors (e.g., low dissolved oxygen concentrations, toxicity, depth, and pressure) and pollutants can override the effects of food quality on benthic communities and food webs. Most of the chapters in my thesis support this assertion. For example, studies that included polluted environments in the initial data compilation appeared repeatedly as outliers from the general trend (Chapter 2); dissolved oxygen concentration was a key driver of infaunal community differences in Barkley Canyon over large scales (100's of meters); and food quality explained an important fraction of the variability in the community structure of Barkley Hydrates in space (~ 33 %), and the chemical toxicity characteristic of chemosynthetic environments likely contributed a significant proportion of the unexplained variability toxicity present (Levin, 2005). Few

species can survive under such environmental conditions, which therefore drastically modify species composition.

7.4. Methodology

Differences in sample storage requirements add a significant challenge in studying community and food web structure simultaneously (i.e., frozen for food web analysis but fixed in 10 % formalin and posterior transfer to 70 % ethanol for community analysis). Samples fixed in formalin and/or ethanol cannot be used for stable isotope or lipid analyses, whereas frozen samples typically lose key morphological characteristics over time, thus complicating taxonomic identification. Several approaches could help overcome this obstacle. First, where time and expertise permit, organisms should be identified on board prior to freezing. Second, some studies report no significant differences in the nitrogen isotopic signature between frozen and fixed samples, and no differences in the carbon isotopic signature between frozen samples and those stored in formalin and ethanol for less than six months (Fanelli et al., 2010). Even so, these findings appear taxon-dependent, time sensitive, and not applicable to lipid analysis. Third, if the isotope signature between food sources differs substantially (e.g., 10 ‰) as is the case in comparing methanotrophs to phytoplankton, a few per mil artifact from fixation with formalin and preservation in ethanol will not obscure the source of organic carbon or nitrogen. Fourth, review papers on feeding guilds (e.g., Jumars et al., 2015) can help in identifying functional groups and feeding modes where sufficient information exists. Fourth, temporal studies help understand how communities utilize a specific food source (Chapter 4). By matching temporal patterns in food sources with life cycles of

individual species and with temporal patterns in community structure, I inferred that some species require a specific food source and identified the major food sources driving temporal patterns in the broader infaunal community.

In considering the role of temporal changes in community and food web analysis, the different degradation states of organic matter along Barkley Canyon axis helped reconstruct the flow of organic matter within the canyon. Sites with indicators of relatively fresh organic matter within the sediment suggested recent deposition of organic matter, whereas sites with primarily degraded bulk organic matter suggested accumulation over time, rather than recent deposition (Chapter 3). The food enrichment experiment (Chapter 6) added another time-related aspect to the thesis, but the opportunistic recovery on the earliest cruise opportunity, eight months after deployment, limited its utility. This kind of experiment requires sufficient time for recruitment of species but also mobilization of organisms (immigration and emigration). However, because ~ 90 % of the food patches disappeared after 1.5 months of deployment, an experimental time frame of 1-2 months would be ideal for a food enrichment experiment of this kind in Barkley submarine canyon.

7.5. Future directions
The aims of this thesis were primarily to consider whether food quality can be as relevant as quantity when considering species distribution and community and food web structure with space and time, to evaluate food quality as an environmental driver in ecosystems with high heterogeneity in food (i.e., a submarine canyon and a chemosynthetic environment), and finally, to simulate the effects of changing phytoplankton composition

as a phytodetrital food source for deep-sea benthic ecosystems. Through this thesis I have also identified areas of future research:

- Chapter 2 demonstrated that different food quality variables can predict abundances of different feeding guilds, but the mechanisms by which each food quality variable influences organisms remain obscure. Aquaculture offers some useful information, although most aquaculture efforts focus on specific fish, shellfish, and crustaceans rather than the polychaetes and nematodes that typically dominate deep-sea ecosystems.

- Chapter 2 also showed how food quality predicts some feeding guilds better than others. No food quality variable predicted carnivores effectively, in contrast to the multiple predictors of surface deposit feeders. Furthermore, most taxa lack the rich literature on selective feeding that has amassed for echinoderms. Some taxa/feeding guilds appear more sensitive to food quality than others and further research should clarify such differences.

- In chapter 6, I also identified potentially different roles for different phytoplankton classes in structuring benthic communities in Barkley Canyon. Future research should focus on which phytoplankton classes impact benthic communities most and the scenarios in which those impacts occur.

- Future research should determine the mechanisms involved in selective feeding within the seemingly homogeneous detrital compartment. Organisms could select for

physical and chemical properties of food particles, but we must improve understanding of

the mechanisms by which these particles are selected.

7.6. References

Amaro, T., Bianchelli, S., Billett, D., Cunha, M., Pusceddu, A., Danovaro, R. 2010. The trophic biology of the holothurian *Molpadia musculus*: implications for organic matter cycling and ecosystem functioning in a deep submarine canyon. Biogeosciences, 7, 2419.

Beaulieu, S. 2002. Accumulation and fate of phytodetritus on the sea floor. Oceanography and Marine Biology: An Annual Review, 40, 171-232.

Boon, A.R., Duineveld, G.C. 2012. Phytopigments and fatty acids in the gut of the deposit-feeding heart urchin *Echinocardium cordatum* in the southern North Sea: selective feeding and its contribution to the benthic carbon budget. Journal of Sea Research, 67, 77-84.

Dauwe, B., Herman, P.M., Heip, C. 1998. Community structure and bioturbation potential of macrofauna at four North Sea stations with contrasting food supply. Marine Ecology Progress Series, 173, 67-83.

Fanelli, E., Cartes, J., Papiol, V., Rumolo, P., Sprovieri, M. 2010. Effects of preservation on the δ^{13}C and δ^{15}N values of deep-sea macrofauna. Journal of Experimental Marine Biology and Ecology, 395, 93-97.

Guieb, R.A., Jumars, P.A., Self, R.F. 2004. Adhesive-based selection by a tentacle-feeding polychaete for particle size, shape and bacterial coating in silt and sand. Journal of Marine Research, 62, 260-281.

Johnson, N.A., Campbell, J.W., Moore, T.S., Rex, M.A., Etter, R.J., McClain, C.R., Dowell, M.D. 2007. The relationship between the standing stock of deep-sea macrobenthos and surface production in the western North Atlantic. Deep Sea Research Part I: Oceanographic Research Papers, 54, 1350-1360.

Jumars, P.A., Dorgan, K.M., Lindsay, S.M. 2015. Diet of worms emended: an update of polychaete feeding guilds. Annual Review of Marine Science, 7, 497-520.

Levin, L.A. 2005. Ecology of cold seep sediments: interactions of fauna with flow, chemistry and microbes. Oceanography and Marine Biology: An Annual Review, 43, 1-46.

Müller-Navarra, D.C. 2008. Food web paradigms: the biochemical view on trophic interactions. International Review of Hydrobiology, 93, 489-505. doi: 10.1002/iroh.200711046.

Wei, C., Rowe, G.T., Hubbard, G.F., Scheltema, A.H., Wilson, G.D., Petrescu, I., Foster, J.M., Wicksten, M.K., Chen, M., Davenport, R. 2010. Bathymetric zonation of deep-sea macrofauna in relation to export of surface phytoplankton production. Marine Ecology Progress Series, 399, 1-14.

Wigham, B.D., Hudson, I.R., Billett, D.S.M., Wolff, G.A. 2003. Is long-term change in the abyssal Northeast Atlantic driven by qualitative changes in export flux? Evidence from selective feeding in deep-sea holothurians. Progress in Oceanography, 59, 409-441. doi: 10.1016/j.pocean.2003.11.003.