VIE ET MILIEU - LIFE AND ENVIRONMENT, 2016, 66 (3-4): 297-304

TROPHIC ECOLOGY OF COASTAL SOFT BOTTOMS: A DIVE INTO THE STEW OF MARINE SEDIMENT

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SOFT-BOTTOM SEDIMENTS
STABLE ISOTOPES
MEIOFAUNA
MACROFAUNA
CONTINENTAL SHELF
MEDITERRANEAN SEA

ABSTRACT. – Using stable isotope analyses, the present study looked at the fractionation of carbon and nitrogen isotopes between bulk sediment organic matter, particulates from the water column, and benthic consumers from the Northwestern Mediterranean continental shelf. Results showed that sedimentary organic matter in the area under study mainly consisted of phytoplankton detritus. In contrast to their available food source, consumers varied widely both in $\delta^{13}C$ and $\delta^{15}N$. Meiofauna fed selectively freshly settled organic particles and channeled energy and matter toward the next upper trophic level. Subsurface primary consumers that feed on less readily digestible sediment organic fractions showed enrichments in rare heavier isotopes as high as those of secondary consumers. Depth of feeding seemed to be a determining factor in their isotope fractionation. Within the sediment, infauna were relying on different food items with different isotope compositions, but metabolic pathways probably explained a great part of their ^{13}C and ^{15}N enrichment.

INTRODUCTION

"A man may fish with the worm that hath eat of a king, and cat of the fish that hath fed of that worm."

William Shakespeare, 1603. *Hamlet, Prince of Denmark, Act IV.*

Half a millennium ago, the Bard of Avon expressed brilliantly how the recycling of organic matter follows complicated pathways. Whether we take detrital organic matter to be at the base of a food chain or at its noble origin, clearly this material has a significant ecological role. In contemporary ecological work, organic matter transfer and transformation are central to characterizing nutrient and energy flows in ecosystems. However, determination of transfers and fluxes even along short food chains remains tricky, especially in marine soft-bottom environments. In these zones, many different organic matter sources, consumers, and sediment particles co-occur in complex mixtures (Darnaude *et al*. 2004). For example, sedimentary organic matter (SOM) is an undifferentiated mix of biogenic material undergoing various stages of decomposition. In addition to being extremely heterogeneous, organic matter from marine soft-bottoms also occurs in relatively low concentrations and has a low nutritional content (Lopez & Levinton 1987). Even so, benthic infauna are characterized by both high species diversity and secondary production (Gray 2002).

Benthic infauna are commonly split into micro-, meio- and macro-fauna depending on whether the largest dimension of the animals living within the sediment is less than 0.44 mm, between 0.44 and 1 mm or larger than 1 mm (Giere 2009). Due to high reproductive and metabolic rates, meiofauna make essential contribution to sediment bioturbation, nutrient cycling and secondary production (Kuipers *et al*. 1981, Coull 1999, Danovaro *et al*. 2007, Nascimento *et al*. 2012, Bonaglia *et al*. 2014). However, just how important is their role in benthic food webs remains an open question (Feller 2006, Nomaki *et al*. 2008). Many studies focused on the trophic position of meiofauna (Minagawa & Wada 1984, Riera *et al*. 1996, 1999, Moens *et al*. 2002, Riera & Hubas 2003, Moens *et al*. 2005, Van Oevelen *et al*. 2006, Nomaki *et al*. 2008, Rzeznik-Orignac *et al*. 2008, Lebreton *et al*. 2012), however the description of the interactions that connect them to other consumers of benthic food webs is still expected.

Stable isotope partitioning has already been investigated in soft-bottom communities but most investigations target specific sites or environments, such as the analysis of deep-sea communities by Iken *et al*. (2001) or for selected size ranges of consumers – either meiofauna (Pascal *et al*. 2008, Lebreton *et al*. 2012) or macrofauna (Darnaude *et al*. 2004, Carlier *et al*. 2007) – in isolation. Based upon distinct carbon isotopic signatures among different organic sources and little isotopic fractionation between sources and consumers, the $^{13}C/^{12}C$ ratio in animal tissues yields insights into feeding and carbon flow pathways (De Niro & Epstein 1978, Wada *et al*. 1991). Nitrogen fractionation ($^{15}N/^{14}N$) provides information about the trophic position of consumers as successive trophic levels become ^{15}N enriched along food chains (Minagawa & Wada 1984, Vander Zanden & Rasmus-

sen 1999, Iken *et al*. 2001, Vander Zanden & Rasmussen 2001, Post 2002).

With the aim of understanding the trophic ecology of soft-bottom sediments, this paper explored the fractionation of both carbon and nitrogen isotopes within the sedimentary organic matter (SOM), suspended particulate organic matter (SPOM), metazoan meiofauna, and larger-size deposit-feeders of the continental shelf of the Gulf of Lions (France). Our results were compared with earlier studies conducted along the northwestern Mediterranean to describe trophic pathways linking SOM, SPOM, meiofauna and benthic deposit-feeders in marine coastal soft-bottom environments.

MATERIALS AND METHODS

Study area and sampling: The study was conducted on the continental shelf of the western part of the Gulf of Lions (Mediterranean Sea) in June 2008. The sampling station (42°34.9'N, 3°12.4'E) was located 16 km from the shoreline at a mean depth of 80 m (Fig. 1). Sediment and meiofauna were collected using a "Midi" model multiple corer (inner core diameter, 59 mm) from OSIL® (Great-Britain). Benthic macrofauna was sampled with a Van Veen grab (0.1 m²). Sea water was also collected both at 3 m beneath the water surface (surface SPOM) and at 3 m above the sediment surface (bottom SPOM) using 10-L Niskin bottles for the characterization of suspended organic particles.

Suspended particulate organic matter (SPOM): Surface and bottom seawater samples were filtered through a 200 μm-mesh net immediately upon collection to remove large particles and zooplankton organisms. Back at the laboratory, chlorophyll pigments were determined spectrofluorimetrically. Three, 300-ml sub-samples were filtered through Whatman GF/F glass filters at low vacuum pressure (approximately 150 mm Hg). Photosynthetic pigments were extracted overnight in acetone 90 % at 5 °C in total darkness. The fluorescence of the supernatant was measured on an LS 55 spectrofluorimeter (Perkin Elmer Inc., USA) according to the method published in Neveux & Lantoine (1993) allowing the assay of 10 mixed pigments: chlorophylls *a*, *b*, and *c*, divinyl-chlorophylls *a* and *b*, and the five associated phaeopigments. The remaining of the water samples was filtered onto pre-combusted GF/F filters for the isotopic signature of surface SPOM and bottom SPOM. The material collected was acidified (HCl, 1 %), briefly rinsed with distilled water and freeze-dried, and then the filters were packed in tin capsules.

Sediment analyses: The top 3 cm of six sediment cores were analyzed. Each sample was gently homogenized and divided in four aliquots to determine grain size distribution, organic matter content, pigment contents and stable isotope ratios. The sediment granulometry was measured using a Malvern® Mastersizer 2000 laser microgranulometer. Grain size was expressed as d(0.5) which corresponds to the median size distribution based on the equivalent spherical volume diameters. Total organic matter content (expressed as relative %) was determined by measuring the weight loss of freeze-dried sediment samples, after combustion at 450 °C for 5 h.

Pigments were extracted from sediment slurries as described in Bourgeois *et al*. (2011). The fluorescence of the extracts was measured using the method developed by Neveux & Lantoine (1993). This method quantifies six chloropigments, namely chlorophylls *a*, *b*, and *c*, and their corresponding degradation products. The aliquots of sediment for stable isotope analyses of SOM were freeze-dried first and then the pellets were ground to fine powder. One aliquot of the dried powdered sediment was acidified with a 1 % HCl solution to remove carbonates (Pinnegar & Polunin 1999). Pre-weighed aliquots from the acidified and non-acidified sediment samples were added to separate tin capsules (Elemental Microanalysis Limited, UK) for the determination of C and N stable isotope ratios, respectively.

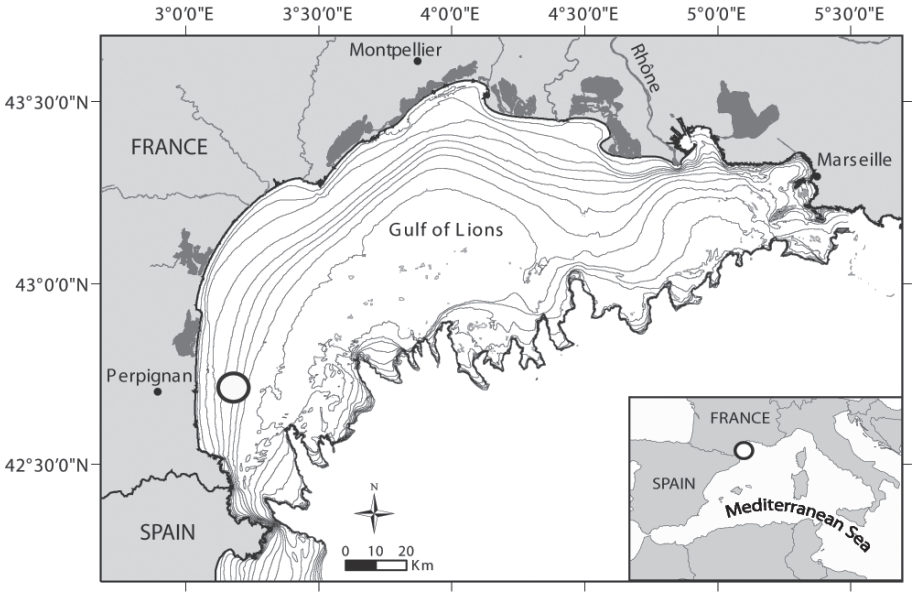

Fig. 1. – Sampled area in the eastern part of the Gulf of Lion (NW Mediterranean Sea).

Meiofauna: A preliminary sampling of meiofauna from the study site showed that nematodes and copepods were largely dominant in the assemblages, accounting respectively for 77 and 11 % of the total abundances. For the purpose of the study, in the laboratory, nematodes and copepods were then arbitrarily handpicked with a fine pipette under a stereomicroscope from the top 3 cm of three sediment cores. From each core, 150 copepods and 200 nematodes were isolated, starved for 12 h in GF/F filtered seawater, before to be briefly rinsed with Milli-Q water, frozen, freeze-dried and encapsulated in tin capsules. In addition, trophic types of the nematodes were identified according to the classification of Wieser (1960) on an extra set of one hundred individuals.

Macrofauna: As for meiofauna, macrobenthic invertebrates selected for isotopic analyses were species dominant in terms of abundance and biomass. A preliminary sampling led us to select four species that were found in most of the samples, contributed the most to the total biomass and differed by their feeding mode. We considered two crustaceans, the surface deposit-feeder *Alpheus glaber* (Olivi, 1792) and the epifaunal burrowing predator, *Goneplax rhomboides* (Linnaeus, 1758), and two sub-surface deposit feeders, *Sternaspis scutata* (Ranzani, 1817), an annelid, and the echinoderm *Oestergrenia digitata* (Montagu, 1815). The contents of 10 grabs were sieved on 1 mm mesh and organisms were immediately picked out and kept alive 24 h in filtered seawater to allow for gut evacuation. Except *S. scutata* from which we removed the horny plates of their ventro-caudal shield, animals were analyzed whole. Each specimen was freeze dried, ground into powder. One part of the dried powder was acidified with HCl (1 %) solution to remove carbonates (Pinnegar & Polunin 1999). Weighed masses from acidified and non acidified powder samples were encapsulated in tin capsules (Elemental Microanalysis Limited, UK) for the determination of C and N stable isotope ratios respectively.

Stable isotope analyses: Analyses of stable isotope ratios of C ($\delta^{13}C$) and N ($\delta^{15}N$) were carried out at the Institut des sciences de la mer (UQAR, Rimouski, Québec, Canada) using a COSTECH ECS 4010 Elemental Analyser coupled with a DeltaPlus XP Isotope Ratio Mass Spectrometer (IRMS, Thermo Electron Co). System control as well as acquisition and treatment of the data used the Isodat 2 software. Stable isotope ratios were expressed in δ notation as parts per thousand (‰) according to the equation:

$$\delta X = \left[\frac{(R_{sample})}{R_{standard}} - 1 \right] \times 1000 \qquad (1)$$

where X is ^{13}C or ^{15}N and R is the corresponding $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios. Standards used for the measurement of ^{13}C and ^{15}N were anhydrous caffeine (Sigma Chemical Co., St-Louis, USA), Mueller Hinton Broth (Becton Dickinson, USA) and *Nannochloropsis*. These homemade standards were cross-calibrated using standards from the National Institute of Standards and Technology (NIST, USA). Replicate analyses of standards gave analytical errors (SD) of ± 0.30 ‰ for C and ± 0.18 ‰ for N.

Statistical analyses: First, a hierarchical cluster analysis (Euclidean distance, average grouping method) was performed on $\delta^{13}C$ and $\delta^{15}N$ mean values to identify groups. Then one-way ANOVAs were performed to identify which isotopes were driving the cluster. If the ANOVA described significant differences, multiple mean comparisons (LSD tests) were done to distinguish significant differences between groups.

RESULTS

Sediment and water bulk characteristics

The average sediment grain size was in the range of 15-17 μm. Fine to very-fine muddy material (silt and clay, < 63 μm) was dominant and accounted for 86 to 90 % of total particle volume. Organic matter accounted for 0.6 % of sediment dry weight. Chloropigment concentrations are given in Table I. In surface waters, chlorophyll *a* was predominant, accounting for 70 % of the whole pigment consortium. The contribution of phaeophytins dramatically increased with depth comprising nearly all chloropigments in the bottom water and the sediment. Chlorophyll *c* concentrations were low or below detection limits as were chlorophyll *b* concentrations.

Groups distinguished by stable isotopes

Mean $\delta^{13}C$ and $\delta^{15}N$ values are given in Table II. $\delta^{13}C$ and $\delta^{15}N$ values ranged respectively from –23.5 to –16.1 ‰ and from 2.0 to 8.5 ‰. The hierarchical cluster analysis distinguished four groups. Group I accounted for organic matter sources, group II included nematodes and copepods, group III comprised *A. glaber*, *G. rhomboides* and *S. scutata,* group IV consisted of *O. digitata*. $\delta^{13}C$ and $\delta^{15}N$ values differed among these groups (one-way ANOVAs, F = 224.8 and F = 94.1, respectively; $p < 0.0001$ in both cases). Pair-wise comparisons concluded there are significant differences between any group pairs.

Table I. – Chloropigment composition in water and sediment samples. C: chlorophyll; P: phaeopigment; nd: not detectable. [*Pa/(Pa+Ca)*] accounts for the degree of degradation of the matter. nd: not detected.

	Ca	Pa	Cb	Pb	Cc	Pc	Pa/(Pa+Ca)
Surface water (mL L^{-1})	0.27(0.01)	0.01(0.00)	nd	0.06(0.01)	0.04(0.00)	nd	0.02
Bottom water (mL L^{-1})	nd	0.24(0.01)	nd	0.03(0.00)	nd	0.59(0.03)	1
Sediment (mg g^{-1})	1.16(0.21)	6.17(0.70)	nd	0.57(0.08)	0.07(0.01)	4.36(0.41)	0.84

Table II. – $\delta^{13}C$ and $\delta^{15}N$ compositions of sources and consumers (means ± SD), n : number of samples. $\Delta^{13}C$ and $\Delta^{15}N$ account for trophic fractionation between consumers and SOM. Trophic levels are: PC, primary consumer; SC, secondary consumer; O: omnivore. Feeding modes are: G, grazing; P, predation; SDF, surface deposit feeding; SSDF, sub-surface deposit feeding. a: de Juan *et al*. 2007, b: Fanelli *et al*. 2011.

	Abbreviation	n	$\delta^{13}C$(‰)	$\delta^{15}N$(‰)	$\Delta^{13}C$(‰)	$\Delta^{15}N$ (‰)	Trophic level	Feeding
Sources								
surface SPOM	SPOMs	4	–23.5 + 0.4	2.0 + 0.3				
bottom SPOM	SPOMb	6	–23.4 + 0.7	3.1 + 1.5				
SOM	SOM	6	–23.4 + 0.3	2.7 + 0.5				
Meiofauna								
Copepods	Cop	6	–21.7 + 0.6	4.3 + 0.3	1.7	1.6	PC	G
Nematodes	Nem	6	–21.9 + 1.1	5.8 + 1.3	1.5	3.1	PC/SC	SDF/P
Macrofauna								
A. glaber	Ag	6	–18.2 + 0.9	7.0 + 0.7	5.2	4.3	O	SDF[a,b]
G. rhomboides	Gr	6	–18.5 + 0.4	7.8 + 0.2	4.9	5.1	SC	P[a]
S. scutata	Ss	6	–17.1 + 0.5	6.8 + 0.1	6.3	4.1	PC	SSDF[a,b]
O. digitata	Od	6	–16.1 + 0.4	8.5 + 0.2	7.3	5.8	PC	SSDF[a,b]

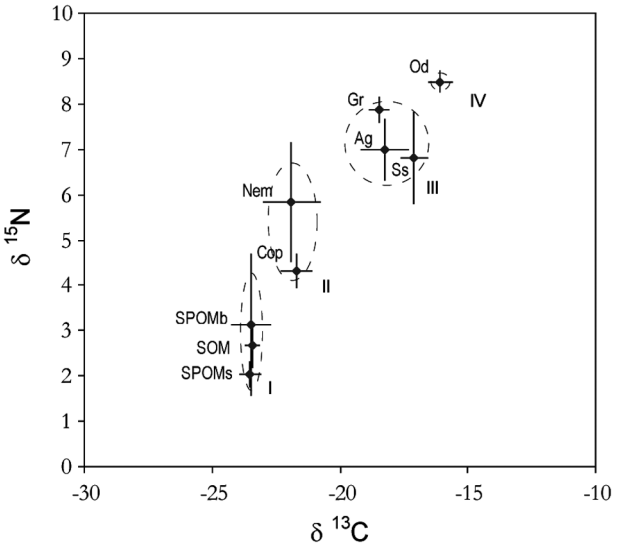

Fig. 2. – Scatterplot of mean $\delta^{13}C$ versus $\delta^{15}N$ of organic matter sources and consumers. Vertical and horizontal bars are standard deviation. Roman numbers and dotted lines accounts for groups defined by the hierarchical clustering analysis of $\delta^{13}C$ and $\delta^{15}N$ mean values.

Group I, the bulk SOM from the top 3 cm of the sediment layer and SPOM, was distinguishable from animal groups based on their relative depletion both in ^{13}C and ^{15}N (Fig. 2). $\delta^{13}C$ values of SOM varied between –23.8 and –23.4 ‰, whereas $\delta^{15}N$ values ranged from 2.4 to 3.5 ‰. SOM as well as SPOM collected in the bottom water were slightly more enriched in ^{15}N compared to SPOM from the surface water.

Within group II, $\delta^{13}C$ values for copepods were comprised between –22.2 and –20.9 ‰ and $\delta^{15}N$ ranged from 3.7 to 4.7 ‰. The range of $\delta^{15}N$ values was much wider for nematodes compared to that of copepods. The analysis of trophic types revealed that nematodes assemblages consisted of non-selective deposit feeders (57 %), predator/omnivores (22 %), selective deposit-feeders (11 %) and epigrowth-feeders (10 %).

Group III included species with different feeding modes: surface selective deposit feeding, predation, and sub-surface deposit feeding. With $\delta^{13}C$ values ranging from –18.5 to –17.7 ‰, the carbon of animals from this group III was in all cases enriched in ^{13}C relative copepods and nematodes. Regarding nitrogen, $\delta^{15}N$ values for *A. glaber*, and *S. scutata* largely overlapped with nematodes.

Group IV consisted of *O. digitata*, a sub-surface deposit feeder. It differed from all other groups, as it showed the highest $\delta^{13}C$ and $\delta^{15}N$ values of the dataset, with $\delta^{15}N$ as high as 8.8 ‰.

DISCUSSION

This study provided an insight of carbon and nitrogen flows in the main consumers of sediment organic matter from the Mediterranean northwestern continental shelf. Within this single trophic level, the range of $\delta^{13}C$ and $\delta^{15}N$ was particularly wide. We thus took care to check the validity of our measurements by comparing them with literature values. We observed that for both ^{15}N and ^{13}C, enrichment factors of sub-surface deposit-feeders can be as high as those reported for secondary consumers, and that feeding depth is a determining factor for the isotope fractionation of deposit feeders. The different isotopic compositions of these consumers suggest the effect of

the heterogeneity of sediment organic particles and/or the diversity of the feeding preference.

Sediment organic matter

$\delta^{13}C$ and $\delta^{15}N$ of SOM (0-3 cm depth) from the study site were in good agreement with values available for the NW Mediterranean Sea. $\delta^{13}C$ ratios ranged from –22.5 to –24.2 ‰ and from 1.9 to 3.6 ‰ for $\delta^{15}N$ (Dauby 1989, Lepoint *et al*. 2000, Pinnegar & Polunin 2000, Darnaude *et al*. 2004, Carlier *et al*. 2007, Fanelli *et al*. 2011). Carbon isotope ratio in sediments was close to that of organic particles in suspension. The low $\delta^{13}C$ values and the absence of chlorophyll *b*, a marker of green algae but also terrestrial vascular plants (Jeffrey 1976), suggest that continental sources did not account for any significant part of SOM at the study site. The slight ^{13}C depletion of SOM and SPOM relative to pure marine-sourced organic matter (Meyers 1994) reflects that sediment and suspended matter not only consisted of fresh phytoplankton, but also contains an undifferentiated mixture of living (*e.g*. phytoplankton, bacteria, microfauna) and detrital particles (*e.g*. faecal pellets, phyto- or zooplankton detritus). ^{13}C depletion is attributed to the preferential degradation of ^{13}C enriched organic compounds (Lehmann *et al*. 2002). This degradation process was further illustrated by changes in the contribution of chlorophylls to total chloropigment contents (Table I). While chlorophyll pigments predominated compared to phaeopigments in the surface water, this ratio was reversed in the sediment. Concomitantly, sinking and sedimentary organic particles were ^{15}N enriched compared to suspended particles from the surface water. The slight ^{15}N enrichment of sediment organic nitrogen could result from the preferential remineralization of ^{14}N during early diagenesis (Mariotti *et al*. 1984). This suggests that at the study site SOM and bottom SPOM available for benthic primary consumers mainly consisted of marine phytoplankton detritus.

Isotope fractionation

Stable isotope analysis has proved to be a very effective method for tracking energy and nutrient flows in ecosystems (Fry & Sherr 1984, Post 2002). The common assumption of this approach is the stepwise enrichment in heavy isotopes along food chains. According to seminal empirical datasets, carbon isotopic composition of animals should reflect their diet within about 1 ‰ (De Niro & Epstein 1978, Peterson & Fry 1987) and trophic nitrogen enrichment should be near 3.4 ‰ (Minagawa & Wada 1984). Even if $\delta^{13}C$ and $\delta^{15}N$ fractionation can deviate from these commonly assumed fractionation factors (Pinnegar & Polunin 1999), the variability in fractionation has rarely been considered (Macko *et al*. 1982, Vander Zanden & Rasmussen 2001). In our survey, $\delta^{13}C$ of animals were enriched over that of their diet (*i.e*. SOM) by 1.5 to 7.3 ‰, while $\Delta^{15}N$ ranged from 1.6 to 5.8 ‰ (Table II). Highest fractionation values for both isotopes concerned *O. digitata* a sub-surface deposit-feeder that burrows deep in the sediment. Variability in nitrogen fractionation has already been pointed out in primary consumers (Vander Zanden & Rasmussen 1999, 2001). Compared to prey, plants are nitrogen-depleted diets and some of their components are not readily digestible (*e.g*. molecules produced for defensive functions and molecules with structural roles). As a consequence, nitrogen fractionation in herbivores results not only from metabolic but also from assimilative processes. This explains why trophic nitrogen enrichment factors in primary consumers stand out from what is usually reported for secondary consumers (Vander Zanden & Rasmussen 2001). With respect to the ^{13}C isotope, several data sets showed that carbon fractionation in primary consumers and especially deposit feeders (up to 5.9 ‰ in Darnaude *et al*. 2004, 7.1 ‰ in Carlier *et al*. 2007; 6.8 ‰ in Fanelli *et al*. 2011) can be different from the fractionation factor usually expected between a prey and its predator.

Trophic pathways

Most consumers considered here were supposed to rely on sediment organic particles to meet their energy requirements (Table II). However, distinct mean values of $\delta^{13}C$ and $\delta^{15}N$ characterized three main groups. Within these, copepods and nematodes were distinguished by the smallest isotope enrichments relative to SOM; *O. digita* which digs galleries deep in the sediment showed the highest fractionation factors; while intermediary fractionation values accounted for the two epifaunal burrowing *A. glaber* and *G. rhomboides* and the shallow burrowing infaunal deposit feeder *S. scutata*.

Copepods were slightly ^{13}C and ^{15}N enriched in comparison with SOM suggesting that they did feed on this source and might channel energy towards secondary consumers. In contrast, $\delta^{15}N$ values in nematodes spanned a relatively large range. Such a wide variation was expected since the nematodes sampled at the studied site displayed different feeding guilds. Trophic structure of the nematode community could account for the observed difference in $\delta^{15}N$ enrichment; the predators that feed on protozoans, selective deposit feeders and other nematodes that feed on bacteria represented up to 40 % of relative abundance. The $\delta^{13}C$ of copepods and part of nematodes were closer to $\delta^{13}C$ of organic particles collected right above the sediment than to that of sediment. Copepods and some nematodes might thus have preferentially selected freshly settled organic particles as food source. Because of a shorter life span and so quicker elemental turnover in their tissues, copepods and nematodes were also expected to follow more closely sediment isotopic changes compared with larger organisms.

With respect to the meiofauna, isotope fractionation factors of the benthic macrofauna were high. Data aggregation led to pool species from different trophic guilds living at various depths in the sediment (Fig. 2; Table II). However pooling spatial scales and trophic pathways obscured the ability to correlate process and trends. Fractionation values of consumers were consistent with those of the same or related species from other studies (Darnaude *et al*. 2004, Carlier *et al*. 2007, Fanelli *et al*. 2011). The isotope fractionation of *G. rhomboides* with respect to that of meiofauna coincided with the expected position of a predator. The isotopic signature of *A. glaber* with a mean value close to that of *G. rhomboides* was much more variable. This variability was consistent with the feeding behavior of a species described as an epifaunal selective deposit feeder relying on omnivory for its nutrition. Nitrogen fractionation in sub-surface deposit-feeders (*S. scutata* and *O. digitata*) was as high as that of the epifaunal shallow burrowing predator *G. rhomboides* (de Juan *et al*. 2007). Such enrichments in sub-surface deposit feeders have already been reported for ^{15}N (Iken *et al*. 2001, Darnaude *et al*. 2004, Carlier *et al*. 2007, Fanelli *et al*. 2011) and obviously cannot be used for dietary analysis and for determination of the trophic level of this guild.

Deep-burrowing deposit-feeders were also more enriched in heavy isotopes than sub-surface deposit feeders, a feature observed in the other datasets (Darnaude *et al*. 2004, Carlier *et al*. 2007, Fanelli *et al*. 2011). Within the sediment, sub-surface deposit feeders must rely on different food items or trophic pathways (Iken *et al*. 2001). Benthic infauna rely on a food source that is limited, sediment organic content here was 0.6 %, of which only a minor part is readily available to consumers. Furthermore, this digestible part generally decreases during degradation processes and thus with sediment depth. The increase in ^{15}N of SOM resulting from denitrification under anoxic conditions (Delwiche & Steyn 1970, Mariotti *et al*. 1984) may explain to some extent the relative ^{15}N enrichment of *O. digitata* which feed deep in the sediment. However, the sediment enrichment of $\delta^{13}C$ and $\delta^{15}N$ due to diagenetic processes is less than 1 ‰ (Freudenthal *et al*. 2001). This reduces very little the enrichment factors in ^{15}N and ^{13}C of sub-surface deposit feeders relative to the isotopic composition of the SOM.

Trophic enrichment factors in consumers result from the metabolic fractionation during synthesis of the tissues (Macko *et al*. 1986, Vander Zanden & Rasmussen 2001). For deposit feeders, it probably results from the uptake of ^{13}C and ^{15}N enriched compounds or their preferential assimilation during digestion. Michener and Kaufmann (2007) in their review pinpointed quality rather than quantity as a key factor of isotope fractionation. During biogeochemical processing, C/N increases due to a preferential loss of nitrogen (Prahl *et al*. 1994); polysaccharides are preferentially removed over molecules with structural roles, cellulose and lignin (Benner *et al*. 1987); polyunsaturated fatty acids over high-weight molecular and complex lipid classes (Canuel & Martens 1996). Considering that lipids are isotopically lighter than other biochemical fractions including amino acids (De Niro & Epstein 1977, Hedges *et al*. 1997, Pinnegar & Polunin 1999), a low lipid diet should produce tissues with low lipid content and then higher $\delta^{13}C$. Low nitrogen diet increases the ^{15}N enrichment as consumers might lose more ^{15}N depleted nitrogen as excretion products (Stephenson *et al*. 1986). This would explain why *O. digitata* and sub-surface deposit feeders which are specialized in the use of a low quality and diluted food resource exhibited high values of $\delta^{15}N$ and $\delta^{13}C$. The high trophic enrichment factors of subsurface deposit feeders reported here and in other works (Darnaude *et al*. 2004, Carlier *et al*. 2007, Fanelli *et al*. 2011, Iken *et al*. 2011) are countering some of the assumptions formed from the initial trophic ecology papers. Understanding the trophic ecology of marine soft bottoms requires studying specifically stable isotope fractionation along food chains based on the use of detrital organic matter.

CONCLUSION

In a benthic food web mainly based on phytodetritus, the range of $\delta^{13}C$ and $\delta^{15}N$ within primary consumers of the sediment organic matter was particularly wide. Selective and surface deposit feeders relied on freshly settled organic particles as a food resource and channelled that matter and energy towards higher trophic levels. In contrast with surface deposit feeders, $\delta^{13}C$ and $\delta^{15}N$ enrichment factors of sub-surface deposit feeders were as high as those observed for secondary consumers. Within the sediment, deposit feeders were unlikely to feed a single source from a single depth with the most similar isotopic composition, but rather on several sources at different depths with distinct isotopic signatures. The depth of feeding is not the only factor explaining the isotope fractionation pattern of deposit feeders, selective assimilation and distinct metabolic pathways must also be involved.

ACKNOWLEDGEMENTS – This study was supported by grants from the EC2CO – CNRS ECOPOP project and from the Natural Sciences and Engineering Research Council of Canada to CN. We thank the crew of the R/V Nereis II (CNRS-INSU), JC Roca and B Hesse for their excellent work and assistance during the SESAME cruise. Thanks to V Chamberland and C Dubé for their sampling and analytical support.

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Received on May 2, 2016
Accepted on September 2, 2016
Associate editor: N Coineau