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Stable isotope ratios in bentho-demersal biota along a depth gradient in the Bay of Biscay: A multitrophic study

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ABSTRACT

Although stable isotope ratios are increasingly used to investigate the trophic ecology of marine organisms, their spatial variations are still poorly understood in the coastal environment. In this study, we measured the stable isotope composition ($\delta^{13}C$, $\delta^{15}N$) of suspended particulate organic matter (SPOM) (primary producer), a suspension feeder, the great scallop *Pecten maximus* (primary consumer), megabenthic decapods and benthic fishes (secondary consumers) along a depth gradient (from 5 m to 155 m depth) across the continental shelf of the Bay of Biscay. Although the three trophic levels exhibited similar $\delta^{13}C$ patterns along the gradient, the $\delta^{15}N$ patterns varied between SPOM, scallops and carnivores. The $\delta^{15}N$ difference between SPOM and scallops decreased with increasing depth, suggesting that non trophic factors may affect the stable isotope composition of scallops at deepest sampling stations. An opposed trend was found between scallops and carnivores, suggesting that the trophic level of these carnivores increased at higher depth, possibly as an adaptation to lower prey abundances. Although our results suggest that primary consumers are suitable to establish isotopic baselines in coastal environments, we stress the need for further studies aiming at characterizing the variability of stable isotopes in coastal biota, and the respective effects of baseline, trophic and metabolic factors in their isotopic composition.

1. Introduction

Coastal ecosystems are interface zones that receive high nutrient and particulate inputs of both continental and marine origin. Consequently, these areas are characterized by strong environmental gradients that can deeply impact the ecology of their associated organisms. In particular, the reliance of coastal suspension-feeders on continental *versus* marine suspended particles has been investigated using stable isotopes in several studies over the last twenty years (e.g. Riera and Richard, 1996; Darnaude et al., 2004; Nerot et al., 2012; Marchais et al., 2013). Because particulate material brought to the ocean by rivers is typically ^{13}C-depleted (around −28‰, Peterson and Fry, 1987) it contrasts with ^{13}C-enriched coastal primary producers (i.e. microphytobenthos, kelps, seagrasses, around −14‰) and marine phytoplankton (around −22‰). Besides, $\delta^{15}N$ values of particulate material at the vicinity of the coastline may display ^{15}N-enriched values associated with the discharge of wastewaters from coastal cities (McClelland et al., 1997; Riera et al., 2000; Costanzo et al., 2001). Because benthic suspension-feeders directly rely on suspended particulate organic matter (SPOM) for food (Carlier et al., 2007; Le Loc'h et al., 2008), they might be expected to reflect the same isotopic patterns along inshore-offshore gradients: increasing $\delta^{13}C$ values with decreasing terrestrial particles concentration, then decreasing $\delta^{13}C$

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values with increasing marine phytoplankton abundance. Concerning $\delta^{15}N$, a decreasing pattern revealing the dilution of anthropogenic inputs into coastal waters is expected (Chouvelon et al., 2012). Benthic suspension-feeders, such as bivalves, have commonly been used to establish isotopic baselines, because they integrate the short term spatial and temporal variability displayed by primary producers (Post, 2002; Rigolet et al., 2014). Under the assumption that the trophic structure of the community is maintained across the continental shelf, the isotopic pattern is therefore expected to be reflected in upper trophic levels, provided predators feed locally and to not exhibit significant migration capacities.

In a recent article, such a pattern has been observed in SPOM and bivalves along a depth gradient (from 20 m to 220 m) of Northeast Atlantic (Nerot et al., 2012). However, although bivalves and SPOM displayed similar $\delta^{13}C$ patterns with depth, the $\delta^{15}N$ decrease along the depth gradient was stronger for bivalves than for SPOM, resulting in bivalves displaying lower $\delta^{15}N$ than their supposed food source at deepest sampling stations. This could result from the dilution of anthropogenic inputs, but the depth (i.e. 190 m) as well as the low freshwater input to the coastal ecosystem in this area (Mortillaro et al., 2014) makes this hypothesis rather unlikely. The alternative hypothesis proposed by Nerot et al. (2012) consists in the influence of metabolic factors that would alter isotopic fractionation between bivalves and their food source. Nerot et al. (2012) called for additional studies investigating this pattern, that is of critical importance, since nitrogen isotopes, that displayed the most intriguing pattern along this gradient, are commonly used to asses trophic level in a variety of marine organisms (e.g. Page et al., 2013). Such confounding factors might result in isotopic approaches being invalid to investigate the diet of benthic consumers at the deepest limit of their distribution range. A possible way to address this issue is to investigate depth-related isotopic patterns in higher trophic level organisms, i.e. predators. If low $\delta^{15}N$ observed in bivalves at the edge of the continental shelf reflect metabolic factors, one could expect that these factors would also affect predators' stable isotope composition, which would therefore display a different depth-related isotopic pattern. In contrast, similar isotopic patterns along the depth gradient between primary and secondary consumers would suggest that $\delta^{15}N$ patterns reported in scallops were due to diet shifts.

The present study aims at measuring the distribution of carbon and nitrogen stable isotopes in benthic sources (SPOM and sediment organic matter (SOM)) and secondary consumers along a depth gradient across the continental shelf of the Bay of Biscay. The aims were two fold: (1) to explore isotopic variation in SPOM and bivalves along a depth gradient (down to 155 m depth) in another part of the northern Bay of Biscay than that observed by Nérot et al. (2012) and (2) to investigate whether this pattern was transferred up to higher trophic level organisms, that is, megabenthic decapods and bentho-demersal fishes.

2. Material and methods

This study was carried out in the Northern part of Bay of Biscay, from the Vilaine river estuary down to the limit of the continental shelf (Fig. 1). The total area of the Vilaine river catchment is 10,500 km^2, and is characterized by the presence of urban areas (1 million inhabitants in the catchment) and intensive farming (cereals, cattle and poultry), whose effluents can affect the nature of inputs brought to the oceans by the river. Animal samples were collected in June 2010 using a beam trawl (2.9 m wide and 0.5 m high opening), an otter trawl (average 11 m wide and 2.5 m high opening) or scallops dredge (2 m wide, 0.5 m opening). Although we tried as much as possible to collect species representative of primary and secondary consumers that were present all along the depth gradient, this objective was only achieved for the great scallop *Pecten maximus*. For crustaceans and fish, different species with partial overlap in their depth distributions were sampled along the depth gradient. Sediment was sampled using a Van Veen grab (only the upper 0.5 cm were analyzed), and bottom water using a 8L Niskin bottle at 1 m above the bottom.

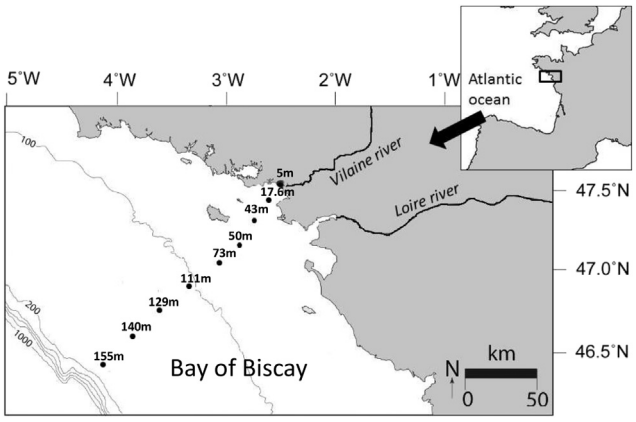

Fig. 1. Map of the sampling area in the Northern Bay of Bay. Black dots indicate sampling stations, and the depth is indicated for each station.

Animal samples were sorted onboard, and the great scallop *P. maximus*, the decapods *Liocarcinus holsatus*, *Liocarcinus marmoreus*, *Macropipus tuberculatus*, *Munida rugosa*, and the fish *Arnoglossus imperialis*, *Arnoglossus laterna* and *Callionymus lyra* were collected, measured and weighed (between 3 and ten replicates per station, according to their respective abundances in samples). Their muscles were then dissected and stored frozen (−25 °C) until further processing. In the laboratory, samples were freeze-dried and ground into a fine and homogeneous powder. Around 250 μg of powder was then weighed in tin capsules for isotopic analysis. Because only pure muscle tissues were analyzed, no acidification was performed on animal samples.

Bottom water samples (three replicates per sampling station) were filtered on pre-combusted (4 h, 450 °C) GF/F filters that were briefly acidified, rinsed with distilled water, oven-dried (60 °C, 48 h), folded and placed into tin capsules. Surface sediment was freeze-dried, sieved (500 μm mesh), the fine fraction was ground into powder while larger particles were discarded. For C isotopic composition, sediment powder was weighed in silver cups and decarbonated using HCl (1.2N). For N isotopic composition, the bulk sample (i.e. not acidified) was analyzed.

Bivalves were analyzed at the Stable Isotopes in Nature Laboratory (New Brunswick, Canada) on a Costech 4010 elemental analyzer coupled to either a Finnigan Delta Plus or a Finnigan Delta Plus XP mass spectrometer. SPOM, SOM and predators (both crabs and fish) were analyzed at the LIENSs laboratory (La Rochelle, France) using a Thermo Scientific Flash EA1112 elemental analyzer coupled to a Delta V Advantage mass spectrometer. Results are expressed in standard δ notation based on international standards (Vienna Pee Dee Belemnite for $\delta^{13}C$ and N2 for $\delta^{15}N$) following the equation $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard})-1] \times 10^3$ (in ‰) where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

The effects of depth on stable isotope ratios were assessed through linear regressions. In order to compare isotopic trends displayed by the different biota sampled, regression slopes were compared by means of ANCOVAs, using taxa as a categorical variable.