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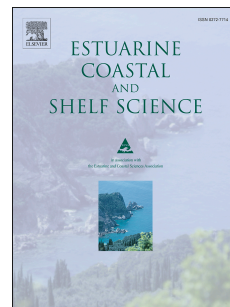
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1 STABLE ISOTOPE RATIOS IN BENTHO-DEMERSAL BIOTA ALONG A DEPTH GRADIENT
2 IN THE BAY OF BISCAY: A MULTITROPHIC STUDY

3

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24 Abstract :

25

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

41 INTRODUCTION

42
43 Coastal ecosystems are interface zones that receive high nutrient and particulate inputs of both
44 continental and marine origin. Consequently, these areas are characterized by strong environmental
45 gradients that can deeply impact the ecology of their associated organisms. In particular, the reliance
46 of coastal suspension-feeders on continental *versus* marine suspended particles has been investigated
47 using stable isotopes in several studies over the last twenty years (e.g. Riera and Richard 1996;
48 Darnaude et al. 2004; Nerot et al. 2012; Marchais et al. 2013). Because particulate material brought to
49 the ocean by rivers is typically ^{13}C -depleted (around -28‰, Peterson and Fry 1987) it contrasts with
50 ^{13}C -enriched coastal primary producers (i.e. microphytobenthos, kelps, seagrasses, around -14‰) and
51 marine phytoplankton (around -22‰). Besides, $\delta^{15}\text{N}$ values of particulate material at the vicinity of the
52 coastline may display ^{15}N -enriched values associated with the discharge of wastewaters from coastal
53 cities (McClelland et al. 1997; Riera et al. 2000; Costanzo et al. 2001). Because benthic suspension-
54 feeders directly rely on suspended particulate organic matter (SPOM) for food (Carlier et al. 2007; Le
55 Loc'h et al. 2008), they might be expected to reflect the same isotopic patterns along inshore-offshore
56 gradients: increasing $\delta^{13}\text{C}$ values with decreasing terrestrial particles concentration, then decreasing
57 $\delta^{13}\text{C}$ values with increasing marine phytoplankton abundance. Concerning $\delta^{15}\text{N}$, a decreasing pattern
58 revealing the dilution of anthropogenic inputs into coastal waters is expected (Chouvelon et al. 2012).
59 Benthic suspension-feeders, such as bivalves, have commonly been used to establish isotopic
60 baselines, because they integrate the short term spatial and temporal variability displayed by primary
61 producers (Post 2002; Rigolet et al. 2014). Under the assumption that the trophic structure of the
62 community is maintained across the continental shelf, the isotopic pattern is therefore expected to be
63 reflected in upper trophic levels, provided predators feed locally and to not exhibit significant
64 migration capacities.

65 In a recent article, such a pattern has been observed in SPOM and bivalves along a depth gradient
66 (from 20m to 220m) of Northeast Atlantic (Nerot et al. 2012). However, although bivalves and SPOM
67 displayed similar $\delta^{13}\text{C}$ patterns with depth, the $\delta^{15}\text{N}$ decrease along the depth gradient was stronger for
68 bivalves than for SPOM, resulting in bivalves displaying lower $\delta^{15}\text{N}$ than their supposed food source

69 at deepest sampling stations. This could result from the dilution of anthropogenic inputs, but the depth
70 (i.e. 190m) as well as the low freshwater input to the coastal ecosystem in this area (Mortillaro et al.
71 2014) makes this hypothesis rather unlikely. The alternative hypothesis proposed by Nerot et al.
72 (2012) consists in the influence of metabolic factors that would alter isotopic fractionation between
73 bivalves and their food source. Nerot et al. (2012) called for additional studies investigating this
74 pattern, that is of critical importance, since nitrogen isotopes, that displayed the most intriguing pattern
75 along this gradient, are commonly used to assess trophic level in a variety of marine organisms (e.g.
76 Page et al. 2013). Such confounding factors might result in isotopic approaches being invalid to
77 investigate the diet of benthic consumers at the deepest limit of their distribution range. A possible
78 way to address this issue is to investigate depth-related isotopic patterns in higher trophic level
79 organisms, i.e. predators. If low $\delta^{15}\text{N}$ observed in bivalves at the edge of the continental reflect
80 metabolic factors, one could expect that these factors would also affect predators' stable isotope
81 composition, which would therefore display a different depth-related isotopic pattern. In contrast,
82 similar isotopic patterns along the depth gradient between primary and secondary consumers would
83 suggest that $\delta^{15}\text{N}$ patterns reported in scallops were due to diet shifts.

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

92 MATERIAL AND METHODS

93
94 This study was carried out in the Northern part of Bay of Biscay, from the Vilaine river estuary
95 down to the limit of the continental shelf (figure 1). The total area of the Vilaine river catchment is
96 10 500 km², and is characterized by the presence of urban areas (1 million inhabitants in the

97 catchment) and intensive farming (cereals, cattle and poultry), whose effluents can affect the nature of
98 inputs brought to the oceans by the river. Animal samples were collected in June 2010 using a beam
99 trawl (2.9m wide and 0.5m high opening), an otter trawl (average 11m wide and 2.5m high opening)
100 or scallops dredge (2m wide, 0,5 m opening). Although we tried as much as possible to collect species
101 representative of primary and secondary consumers that were present all along the depth gradient, this
102 objective was only achieved for the great scallop *Pecten maximus*. For crustaceans and fish, different
103 species with partial overlap in their depth distributions were sampled along the depth gradient.
104 Sediment was sampled using a Van Veen grab (only the upper 0.5 cm were analyzed), and bottom
105 water using a 8L Niskin bottle at 1m above the bottom.

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

114 Bottom water samples (three replicates per sampling station) were filtered on pre-combusted (4h,
115 450°C) GF/F filters that were briefly acidified, rinsed with distilled water, oven-dried (60°C, 48h),
116 folded and placed into tin capsules. Surface sediment was freeze-dried, sieved (500 µm mesh), the fine
117 fraction was ground into powder while larger particles were discarded. For C isotopic composition,
118 sediment powder was weighed in silver cups and decarbonated using HCl (1.2N). For N isotopic
119 composition, the bulk sample (i.e. not acidified) was analyzed.

120 Bivalves were analyzed at the Stable Isotopes in Nature Laboratory (New Brunswick, Canada) on
121 a Costech 4010 elemental analyzer coupled to either a Finnigan Delta Plus or a Finnigan Delta Plus
122 XP mass spectrometer. SPOM, SOM and predators (both crabs and fish) were analyzed at the LIENSs
123 laboratory (La Rochelle, France) using a Thermo Scientific Flash EA1112 elemental analyzer coupled
124 to a Delta V Advantage mass spectrometer. Results are expressed in standard δ notation based on

125 international standards (Vienna Pee Dee Belemnite for $\delta^{13}\text{C}$ and N2 for $\delta^{15}\text{N}$) following the equation
126 $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}})-1] \times 10^3$ (in ‰) where R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$.

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

130

131

132 RESULTS

133

134 Stable isotope ratios measured for bottom suspended particulate organic matter (SPOM) in the
135 Vilaine river displayed typically ^{13}C depleted (means \pm standard deviations = $-31.1 \pm 2.1\text{‰}$,
136 respectively) and ^{15}N enriched values ($13.8 \pm 3.0\text{‰}$) (Figure 2). SPOM $\delta^{13}\text{C}$ decreased along the
137 gradient, from $-19.9 \pm 0.3\text{‰}$ at the most shallow station down to $-23.9 \pm 0.4\text{‰}$ at the deepest station
138 (155m depth) (figure 2). The $\delta^{15}\text{N}$ decreased from ca. 11.5‰ upstream the Vilaine estuary down to
139 $6.4 \pm 0.4\text{‰}$ at the deepest station. In contrast with SPOM, the $\delta^{13}\text{C}$ of SOM did not display any obvious
140 trend, and experienced high variability along the depth gradient (Table 1). In contrast, SOM $\delta^{15}\text{N}$
141 decreased with depth, from $7.7 \pm 0.6\text{‰}$ for shallow stations down to $6.3 \pm 0.3\text{‰}$ for the deepest sampling
142 station.

143 The $\delta^{13}\text{C}$ of the suspension-feeder *Pecten maximus* decreased along the depth gradient, nearshore
144 stations being slightly ^{13}C enriched (between -17.3‰ and -15.5‰) compared to offshore stations
145 (between -18.3‰ and -17.1‰)(figure 2). No significant difference could be found between the slopes
146 of SPOM and *P. maximus* for $\delta^{13}\text{C}$ (ANCOVA, $F=0.587$, $p=0.45$). The $\delta^{15}\text{N}$ of *P. maximus* displayed a
147 marked ^{15}N depletion trend along the depth gradient, from $9.5 \pm 0.2\text{‰}$ at 22m depth down to
148 $4.0\text{‰} \pm 0.3\text{‰}$ for the deepest sampling station and differed from the trends exhibited by SPOM
149 (ANCOVA, $F=23.74$, $p<0.001$). Consequently, scallops sampled at more than 140m were ^{15}N depleted
150 compared SOM and SPOM values.

151 Secondary consumers were grouped in two taxo-functional groups for the analysis: predatory
152 decapods (*Macropipus tuberculatus*, *Munida rugosa.*, *Liocarcinus sp.*) and benthic fishes

153 (*Arnoglossus spp.*, *Callionymus lyra*). Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ decreased along the depth gradient for these
154 two taxo-functional groups (figure 3, table 2). However, although these two groups displayed the same
155 $\delta^{13}\text{C}$ gradient along the depth gradient (ANCOVA, $F=1.168$, $p=0.28$), their $\delta^{15}\text{N}$ varied differently, the
156 slope being steeper for decapods than for fishes (ANCOVA, $F=38.01$, $p<0.001$). Isotopic trends
157 displayed by both groups differed from the one displayed by *Pecten maximus*, for both $\delta^{13}\text{C}$
158 (ANCOVAs, $F=30.7$, $p<0.001$ and $F=4.7$, $p=0.032$ for fish and crustaceans, respectively) and $\delta^{15}\text{N}$
159 (ANCOVAs, $F=1286.6$, $p<0.001$ and $F=773.0$, $p<0.001$ for fish and crustaceans, respectively). Among
160 the different predator species sampled, *Munida rugosa* departed through lower $\delta^{15}\text{N}$ and higher $\delta^{13}\text{C}$
161 values, while the different depth ranges made it difficult to robustly identify consistent patterns at the
162 species level. Besides, the two *Arnoglossus* fish were consistently the most ^{15}N -enriched of co-
163 occurring carnivores, but did not display any specific $\delta^{13}\text{C}$ pattern.

164 DISCUSSION

165
166
167 Stable isotope analysis has become over the last twenty years a popular tool to investigate the diet
168 of marine consumers, including consumers sampled along depth gradients on continental shelves (e.g.
169 Kline 2009; Chauvelon et al. 2012; Nerot et al. 2012). This method is based on different assumptions,
170 among which the need to accurately know the fractionation occurring between a consumer and its prey
171 (Gannes et al. 1998). In marine soft-bottom ecosystems, most consumers rely on composite food
172 sources (e.g. SPOM, SOM), whose isolation of “pure” components is technically impossible, making
173 it challenging to characterize trophic fractionations, and then, trophic relationships. An alternative
174 option to characterize these relationships is the spatial analysis of both consumers and their potential
175 sources, under the assumption that co-varying sources and consumers are likely to be linked by a
176 trophic relationship (Melville and Connolly 2003; Vanderklift and Wernberg 2010, Leclerc et al.
177 2013).

178 179 Depth-related isotopic patterns in primary consumers

180 A marked $\delta^{13}\text{C}$ difference ($\approx 4\text{‰}$) was observed between SPOM and *P. maximus* all along the
181 depth gradient. Although this difference seems at odd with a direct trophic relationship between
182 scallops and SPOM, it has been repeatedly reported from various sublittoral ecosystems (e.g. Hobson
183 et al. 1995; Grall et al. 2006; Carlier et al. 2007; Le Loc'h et al. 2008). Different interpretations have
184 been proposed to explain this difference, including abnormal isotopic fractionation (including tissue-
185 specific isotopic fractionation), local variability in phytoplankton stable isotope composition, selective
186 feeding, or contribution of other sources (see Miller and Page 2012; Leclerc et al. 2013). In the present
187 study, the two food sources available to scallops, SPOM and SOM, are both ^{13}C depleted compared to
188 *P. maximus*, suggesting that the ^{13}C enriched isotopic ratios displayed by this bivalve arise from a
189 selective assimilation of a ^{13}C enriched fraction of SPOM (see Carlier et al. 2007 for instance) or from
190 an unexpectedly high carbon isotopic fractionation. For instance, a $\delta^{13}\text{C}$ fractionation of 3.5‰ has
191 been experimentally determined between phytoplankton and the clam *Ruditapes philippinarum* (Dang
192 et al. (2009). Another explanation for this $\delta^{13}\text{C}$ difference between POM, SOM and primary
193 consumers is the contribution of freshwater originating, ^{13}C -depleted particles, that would not be
194 assimilated by suspension-feeders, This explanation is however very unlikely for most sampling sites
195 (excepted the most inshore). The sampling period (early summer) corresponds to the smallest extent of
196 the Loire river plume, that is, anyway, restricted to the first meters of the water column (Lunven et al.
197 2005). It is therefore unlikely that isotopic patterns observed in the present study reflect the influence
198 of freshwater inputs. In any event, the consistency of this isotopic difference between SPOM and *P.*
199 *maximus* along the depth gradient would suggest that the diet of scallops does not drastically change
200 between shallow and deeper areas.

201 In a recent article, Nerot et al. (2012) observed that the $\delta^{15}\text{N}$ of suspension-feeders and SPOM did
202 not evolve similarly along a depth gradient, suggesting a diet shift along this gradient or a
203 modification in the trophic fractionation with increasing depth. An important finding of the present
204 study is a new observation in northern Bay of Biscay of the $\delta^{15}\text{N}$ decreasing pattern observed in
205 scallops, the slope of this trend being steeper than for their supposed food source. This new
206 observation suggests that this is common along the continental shelf, as reported for other areas of the
207 Bay of Biscay (Chouvelon et al. 2012) or in northern Pacific (Kline 2009). It has recently been

208 suggested, based on fatty acid composition of *P. maximus* digestive gland, that the diet of scallops
209 differed between shallow and deeper sites, including a lower reliance on fresh phytoplankton and the
210 assimilation of degraded material as well as the different components of the microbial food web for
211 the deepest sampling stations (Nerot et al. in press). In our study, this hypothesis would involve that
212 SPOM at deepest sites is mostly composed of refractory ^{15}N enriched material, and that primary
213 consumers only assimilate a minor ^{15}N depleted fraction, resulting in the difference between the
214 isotopic composition of SPOM and bivalves food source. Such a ^{15}N depleted fraction could be
215 represented by degraded material, which is more readily available to consumers (Tenore et al. 1983).
216 However, degradation processes are known to cause an increase in the $\delta^{15}\text{N}$ of phytoplankton (e.g.
217 Montoya et al. 1992), while in the present study scallops seem to rely on a ^{15}N depleted source. Such
218 an heterogeneity in the isotopic composition of fractions that compose the pool of SPOM has already
219 been shown among microalgae in freshwater lakes (Vuorio et al. 2004) or among cultured marine
220 microalgae (Falkowski 1991). However, in the present study, similarities in the $\delta^{13}\text{C}$ patterns displayed
221 by SPOM, *Pecten maximus*, carnivorous decapods and benthic fishes along the depth gradient suggest
222 that no drastic diet shift occurs. Hence, our results suggest that discrepancies observed in the $\delta^{15}\text{N}$ of
223 biota sampled on the continental shelf of the Bay of Biscay arise, at least mainly, from variability in
224 nitrogen trophic fractionation between primary consumers and their food sources. Indeed, different
225 studies aiming to characterize factors affecting isotopic trophic-step fractionation found that
226 temperature, food availability or nutrient levels were likely to significantly affect the value of this
227 fractionation (e.g. Moeri et al. 2003; Aberle and Malzahn 2007; Bloomfield et al. 2011). For instance
228 the lower $\delta^{15}\text{N}$ fractionation observed in detritivores compared to herbivores (0.53‰ vs 2.98‰,
229 respectively, Vanderklift and Ponsard 2003) might contribute to the low values observed in deepest
230 scallops, that have been reported to rely on recycled material (Nerot et al. in press). The metabolism of
231 scallops is therefore probably affected by these different factors at deeper sampling stations, resulting
232 in a modified trophic-step fractionation. Although laboratory and modelling studies have shown in
233 past an effect of metabolic condition on isotopic fractionation (e.g. Hobson and Clark 1992; Emmery
234 et al. 2011), it had rarely been noticed from field studies. For stable isotope studies carried out over
235 environmental gradients, possibly encompassing optimal and sub-optimal ecological conditions, this

236 variability can strongly affect the outcomes of trophic inferences. The results from these studies must
237 therefore be analyzed with much care before conclusions could be drawn about the trophic ecology of
238 consumers.

239

240 Depth-related isotopic patterns in carnivores

241 Demersal fish, such as the dragonet *Callionymus lyra* or the scaldfish *Arnoglossus spp.*, as well as
242 megabenthic decapods sampled for this study, are known to feed mainly on small invertebrates or fish
243 juveniles (Deniel 1975; Choi 1986; Abello 1989). Stable isotope ratios measured in the different
244 benthic-demersal carnivores were in accordance with a diet mainly based on local primary consumers
245 for the most shallow sampling stations, being ^{15}N enriched of 2-4‰ compared to scallops (Vander
246 Zanden and Rasmussen 2001). Although the carnivores sampled in this study are unlikely to actually
247 feed on scallops because of their large size, this suggests that these suspension-feeders are good
248 estimators of average primary consumers $\delta^{15}\text{N}$, hence being potentially reliable to establish isotopic
249 baselines. However, the $\delta^{15}\text{N}$ difference between those carnivores and scallops increased with
250 increasing depth (Figure 4), in opposition to what was observed between SPOM and scallops. Lower
251 feeding levels for carnivores at higher depth could cause increased trophic enrichment factors that
252 could explain the differences in $\delta^{15}\text{N}$ trends observed for scallops and carnivores along the depth
253 gradient. It has for instance been reported that the feeding level could modify by more than 2‰ the
254 fractionation between a consumer and its food source (Emmery et al. 2011). The basal trophic resource
255 of shelf benthic communities is a mixture of resuspended sediment and sinking phytoplankton (Carrier
256 et al. 2007; Le Loc'h et al. 2008), whose abundance decreases with increasing depth. Hence, deep
257 benthic communities are sustained by a lower abundance of organic matter, and are therefore less
258 abundant (personal observation). If feeding levels were the main reason for the observed modification
259 of isotopic fractionation with depth, this should therefore be observed for both primary and secondary
260 consumers. The fact that isotopic fractionation displays an opposite pattern with depth for primary and
261 secondary consumers suggests that this is not the case in northern Bay of Biscay. An alternative
262 explanation for the higher $\delta^{15}\text{N}$ difference between scallops and carnivores at higher depth involves an
263 increase in the trophic level of those carnivores with increasing depth. All the carnivores sampled for

264 this study are known to feed on a wide array on vertebrate and invertebrate preys, including shrimps or
265 small fish, which are themselves carnivores (Deniel 1975; Choi 1986; Abello 1989). The low density
266 of potential prey on the deep continental shelf could lead them to rely on higher trophic level prey,
267 explaining higher $\delta^{15}\text{N}$ than those expected for strict secondary consumers.

268 Although this pattern is shared by all the carnivores considered, it seems that the squat lobster
269 *Munida rugosa* feeds at a slightly lower trophic level than others, because its $\delta^{15}\text{N}$ is consistently
270 lower. Although *M. rugosa* is generally considered as a strict carnivore (Le Loc'h & Hily 2005), it is
271 possible that this species feed at a slightly lower trophic level than other decapods. In particular, this
272 species has been reported to be able to rely on sedimented planktonic particles, which would explain
273 the lower trophic level observed here (Lagardère 1973). In contrast, the two *Arnoglossus* species
274 display the highest $\delta^{15}\text{N}$ of co-occurring carnivores, which could result from a slightly higher trophic
275 level.

276

277 CONCLUSION

278

279 Although it was impossible, for logistical reasons, to repeat this sampling in time in order to
280 assess the seasonal variability of the patterns described here, we are quite confident that they may be
281 valid for larger time scales. Indeed, not only tissues (i.e. muscles) sampled in this study integrate
282 dietary information over long periods (several months for fish, see Perga and Gerdeaux 2005) but
283 offshore ecosystems are less temporally variable ecosystems than nearshore ones. No significant
284 influence of freshwater inputs on the benthos was observed in this study. Northward coastal currents in
285 this area of the Bay of Biscay result in these inputs being transferred to the open ocean along the
286 southern coast of Brittany. The continental shelf of the Northern Bay of Biscay is therefore under
287 limited freshwater influence. Identifying the spatial variability of basal trophic resources isotopic
288 composition is necessary in any isotopic study including a spatial perspective. In this study, the
289 differences in the depth-related isotopic patterns displayed by the different trophic levels suggest that
290 the use of primary consumers as proxies for ecological mechanisms occurring on the continental shelf
291 may not be valid for the entire benthic-demersal community. Although metabolic factors that can

292 contribute to this lag are difficult to untangle from baseline or trophic effects, it seems necessary to
293 seriously consider them for the interpretation of stable isotope results in future studies.

294

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- 418

ACCEPTED MANUSCRIPT

419 Table 1 : linear regressions between depth and stable isotope composition in *Pecten maximus*, benthic
420 fishes and predatory decapods along a depth gradient in the Northern Bay of Biscay

421

422			Slope	intercept	p-value	R ²
423	SPOM	$\delta^{13}\text{C}$	-0.028	-19.62	< 0.001	0.76
424		$\delta^{15}\text{N}$	-0.012	9.44	< 0.001	0.25
425	SOM	$\delta^{13}\text{C}$	0.013	24.54	0.30	0.08
426		$\delta^{15}\text{N}$	0.018	8.50	< 0.001	0.55
427	<i>Pecten maximus</i>	$\delta^{13}\text{C}$	-0.010	-16.00	< 0.001	0.46
428		$\delta^{15}\text{N}$	-0.042	10.72	< 0.001	0.95

429

430

431 Table 2: linear regressions between depth and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the different carnivores sampled in this
 432 study. Taxa: F=fish, C=crustacean.

433

434	Species		n	slope	intercept	R ²	p-value
435	Fish						
436	<i>Arnoglossus imperialis</i>	$\delta^{13}\text{C}$	46	-0.010	-16.52	0.63	<0.001
437		$\delta^{15}\text{N}$	46	-0.026	14.61	0.74	<0.001
438	<i>Arnoglossus laterna</i>	$\delta^{13}\text{C}$	14	-0.020	-15.32	0.76	<0.001
439		$\delta^{15}\text{N}$	14	-0.030	15.26	0.83	<0.001
440	<i>Callionymus lyra</i>	$\delta^{13}\text{C}$	32	-0.014	-16.04	0.51	<0.001
441		$\delta^{15}\text{N}$	32	-0.015	13.172	0.51	<0.001
442	Decapods						
443	<i>Liocarcinus holsatus</i>	$\delta^{13}\text{C}$	19	-0.008	-16.25	0.11	0.173
444		$\delta^{15}\text{N}$	19	-0.030	14.50	0.71	<0.001
445	<i>Liocarcinus marmoreus</i>	$\delta^{13}\text{C}$	21	-0.0123	-15.89	0.63	<0.001
446		$\delta^{15}\text{N}$	21	-0.016	12.93	0.52	<0.001
447	<i>Macropipus tuberculatus</i>	$\delta^{13}\text{C}$	19	-0.017	-15.59	0.39	0.004
448		$\delta^{15}\text{N}$	19	-0.043	16.12	0.33	0.010
449	<i>Munida rugosa</i>	$\delta^{13}\text{C}$	25	-0.014	-15.20	0.42	<0.001
450		$\delta^{15}\text{N}$	25	-0.026	13.01	0.86	<0.001

451

452 Figures captions

453 Figure 1:

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

456

457 Figure 2:

458 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of suspended particulate organic matter (open circles, large dashes), sediment (open
459 squares, little dashes) and *Pecten maximus* (black triangles, full line) across the continental shelf of the
460 Bay of Biscay. See table 1 for linear regressions parameters.

461

462 Figure 3:

463 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the different carnivores sampled across the continental shelf of the Bay of Biscay.

464 For clarity reasons, only regression lines are shown. For linear regression parameters, see table 2. 1-

465 *Arnoglossus imperialis*, 2- *Arnoglossus laterna*, 3- *Callionymus lyra*, 4- *Liocarcinus holsatus*,

466 5- *Liocarcinus marmoreus*, 6- *Macropipus tuberculatus*, 7- *Munida rugosa*.

467 Figure 4:

468 Relationship between depth and corrected $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{carnivore}} - \delta^{15}\text{N}_{\text{scallops}}$) of demersal fish (dashed line)

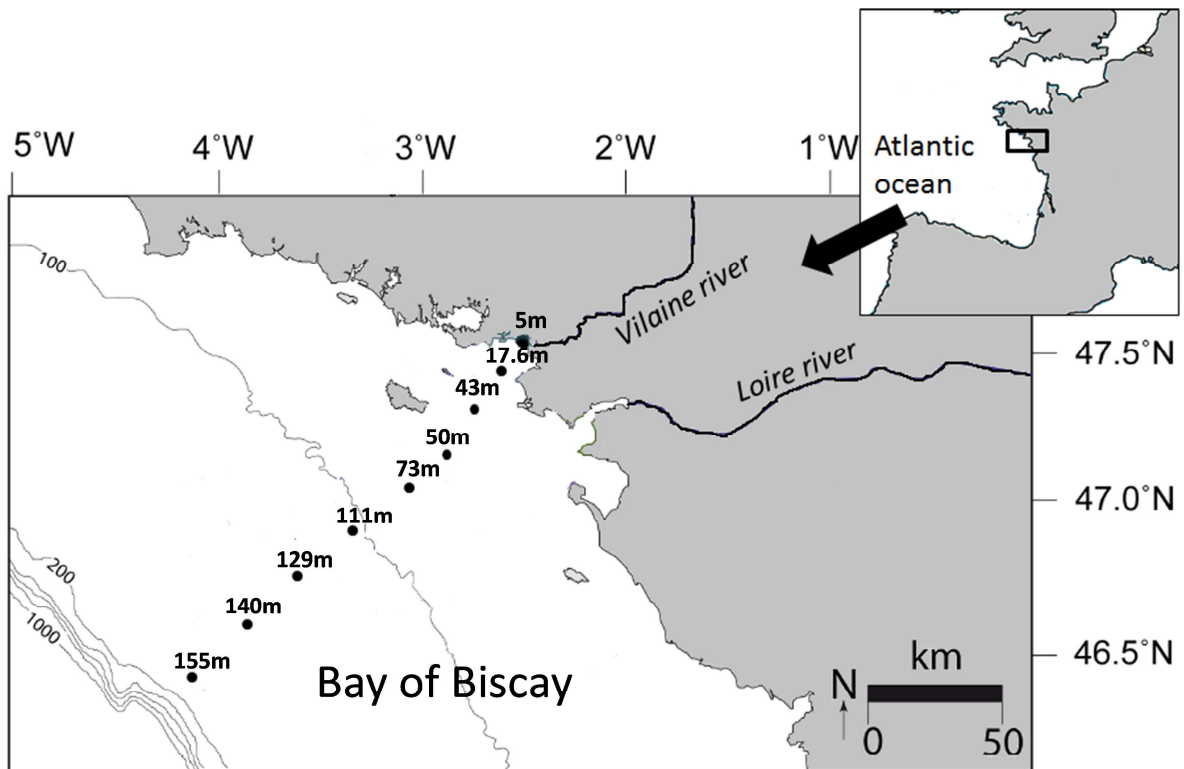
469 and megabenthic crustaceans (full line) across the continental shelf of the Bay of Biscay. Fish:

470 corrected $\delta^{15}\text{N} = 0.027 * \text{depth} + 2.58$; $R^2 = 0.74$; $p < 0.001$. Crustaceans: corrected $\delta^{15}\text{N} = 0.016 * \text{depth} + 2.81$;

471 $R^2 = 0.45$; $p < 0.001$.

472

473 Figure 1:

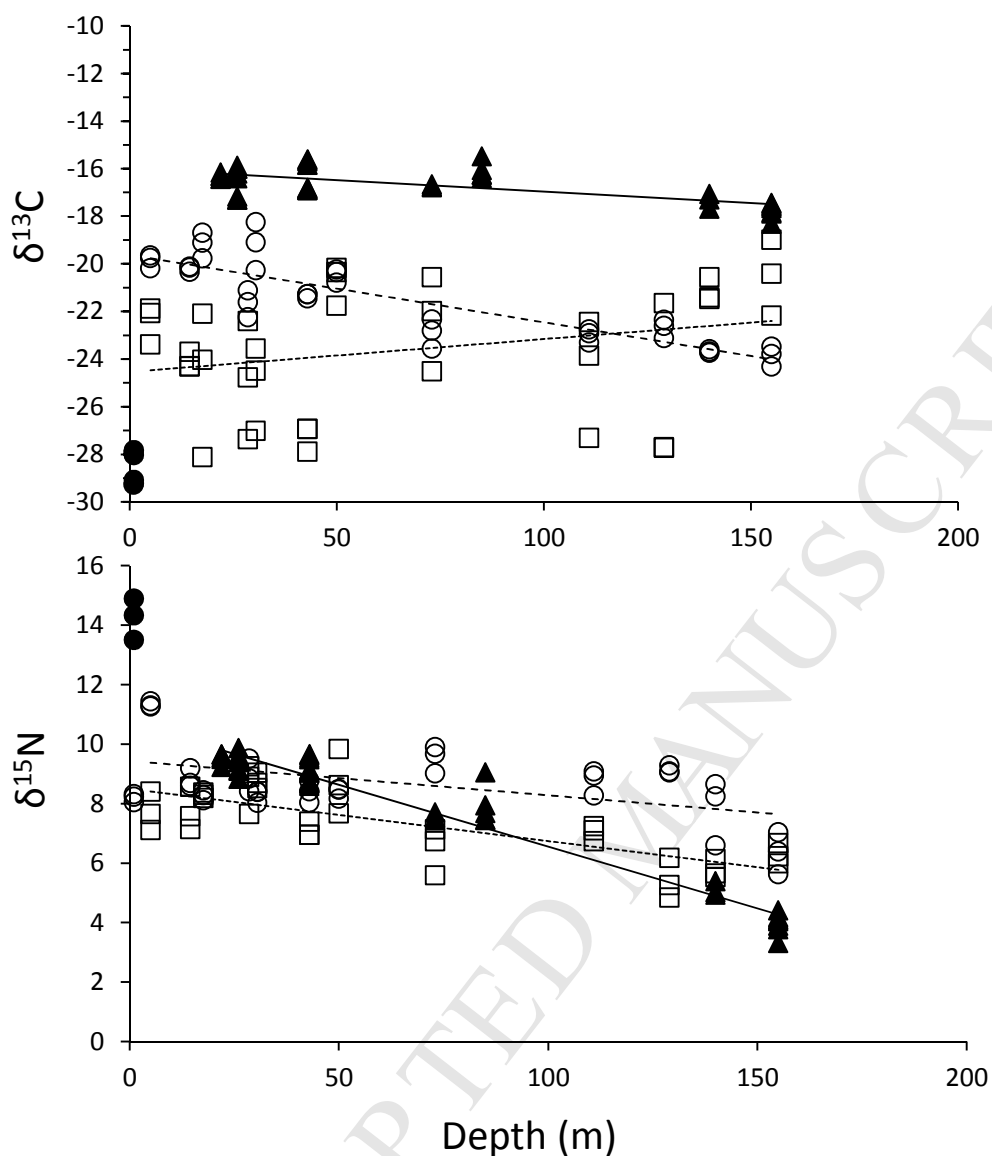


474

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

477

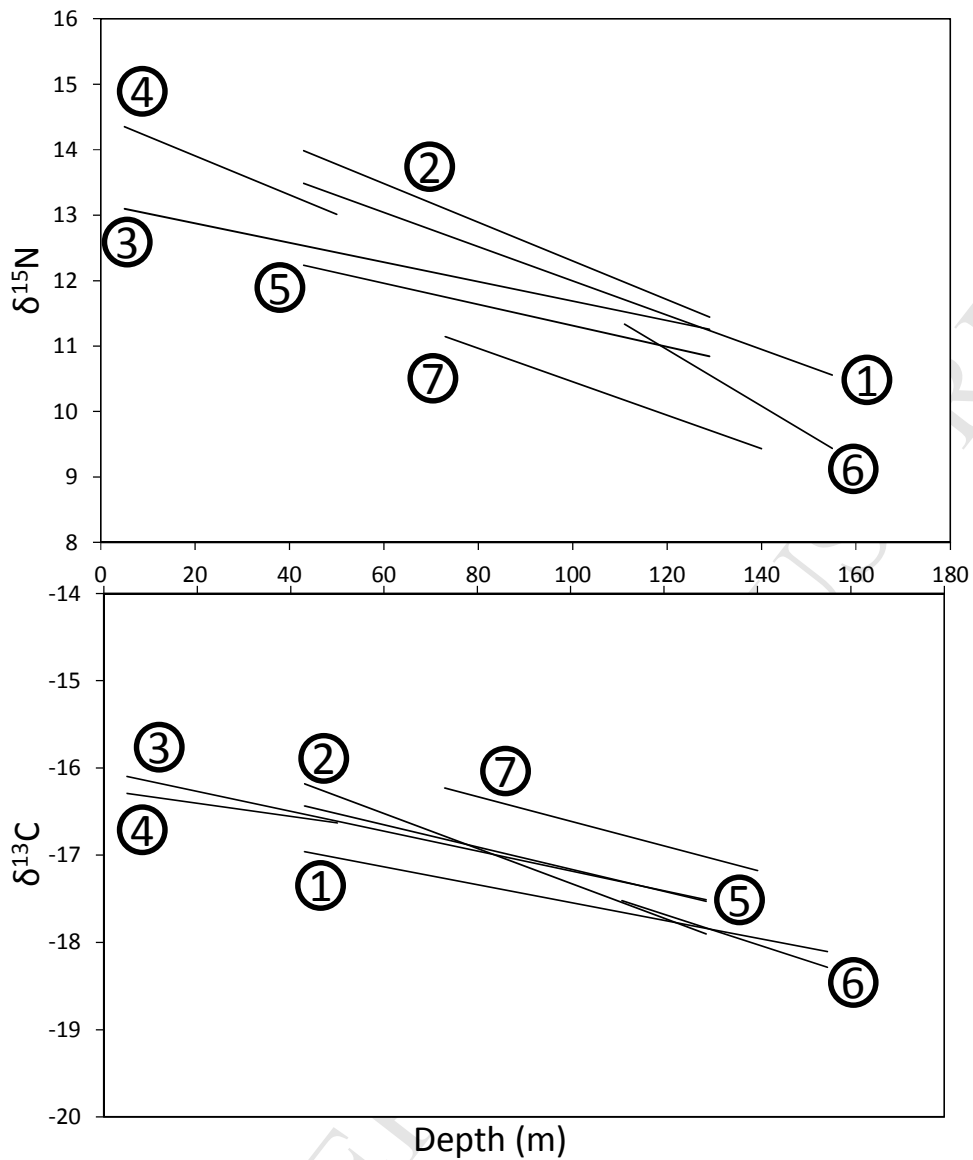
478 Figure 2:



479
 480 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of suspended particulate organic matter (open circles, large dashes), sediment (open
 481 squares, little dashes) and *Pecten maximus* (black triangles, full line) across the continental shelf of the
 482 Bay of Biscay. See table 1 for linear regressions parameters. Filled symbols indicates samples from
 483 the upper reach of the estuary (salinity=0)

484

485 Figure 3:



486

487 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the different carnivores sampled across the continental shelf of the Bay of Biscay.

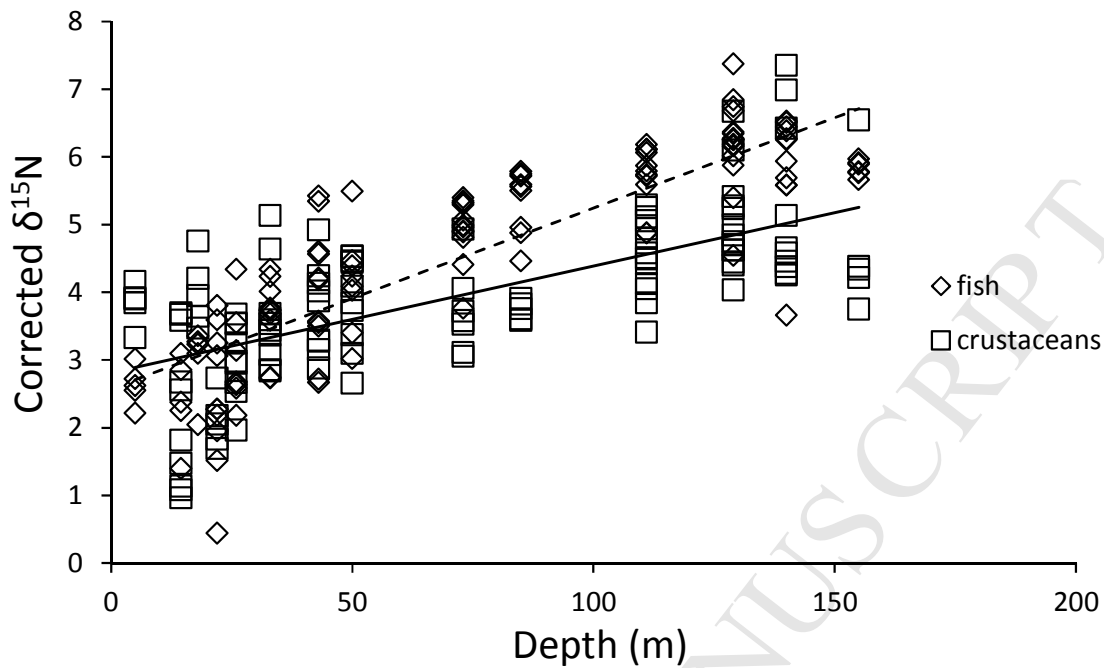
488 For clarity reasons, only regression lines are shown. For linear regression parameters, see table 2.

489

490

491

492 Figure 4:



493

494 Relationship between depth and corrected $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{carnivore}} - \delta^{15}\text{N}_{\text{scallops}}$) of demersal fish (dashed line)
 495 and megabenthic crustaceans (full line) across the continental shelf of the Bay of Biscay. Fish:
 496 corrected $\delta^{15}\text{N} = 0.027 * \text{depth} + 2.58$; $R^2 = 0.74$; $p < 0.001$. Crustaceans: corrected $\delta^{15}\text{N} = 0.016 * \text{depth} + 2.81$;
 497 $R^2 = 0.45$; $p < 0.001$.