Accepted Manuscript

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

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Please cite this article as: Schaal, G., Nerot, C., Grall, J., Chouvelon, T., Lorrain, A., Mortillaro, J.-M., Savoye, N., Brind'Amour, A., Paulet, Y.-M., Le Bris, H., Stable isotope ratios in bentho-demersal biota along a depth gradient in the bay of biscay: A multitrophic study, *Estuarine, Coastal and Shelf Science* (2015), doi: 10.1016/j.ecss.2015.10.023.

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 :

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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 (δ^{13}C, δ^{15}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 δ^{13}C patterns along the gradient, the δ^{15}N patterns varied between SPOM,

33   scallops and carnivores. The δ^{15}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.

2

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, δ^{15}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 δ^{13}C values with decreasing terrestrial particles concentration, then decreasing

57   δ^{13}C values with increasing marine phytoplankton abundance. Concerning δ^{15}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 δ^{13}C patterns with depth, the δ^{15}N decrease along the depth gradient was stronger for

68   bivalves than for SPOM, resulting in bivalves displaying lower δ^{15}N than their supposed food source

3

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 asses 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 δ^{15}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 δ^{15}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 bentho-demersal fishes.

91

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

4

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

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125 international standards (Vienna Pee Dee Belemnite for $\delta^{13}C$ and N2 for $\delta^{15}N$) following the equation

126 $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard})-1] \times 10^3$ (in ‰) where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

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.

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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 ± standard deviations = -31.1±2.1‰,

136 respectively) and ^{15}N enriched values (13.8±3.0‰) (Figure 2). SPOM $\delta^{13}C$ decreased along the

137 gradient, from -19.9±0.3‰ at the most shallow station down to -23.9±0.4‰ at the deepest station

138 (155m depth) (figure 2). The $\delta^{15}N$ decreased from ca. 11.5 ‰ upstream the Vilaine estuary down to

139 6.4±0.4‰ at the deepest station. In contrast with SPOM, the $\delta^{13}C$ of SOM did not display any obvious

140 trend, and experienced high variability along the depth gradient (Table 1). In contrast, SOM $\delta^{15}N$

141 decreased with depth, from 7.7±0.6‰ for shallow stations down to 6.3±0.3‰ for the deepest sampling

142 station.

143   The $\delta^{13}C$ 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 $\delta^{13}C$ (ANCOVA, F=0.587, p=0.45). The $\delta^{15}N$ of *P. maximus* displayed a

147 marked ^{15}N depletion trend along the depth gradient, from 9.5±0.2‰ at 22m depth down to

148 4.0‰±0.3‰ 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

6

153 (*Arnoglossus spp., Callionymus lyra*). Both $\delta^{13}C$ and $\delta^{15}N$ 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 $\delta^{13}C$ gradient along the depth gradient (ANCOVA, F=1.168, p=0.28), their $\delta^{15}N$ 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 $\delta^{13}C$

158 (ANCOVAs, F= 30.7, p<0.001 and F=4.7, p=0.032 for fish and crustaceans, respectively) and $\delta^{15}N$

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 $\delta^{15}N$ and higher $\delta^{13}C$

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 $\delta^{13}C$ pattern.

164

165 DISCUSSION

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; Chouvelon 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

7

180   A marked $\delta^{13}C$ difference ($\approx 4‰$) 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ 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

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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 δ^{15}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 δ^{13}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 δ^{15}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 δ^{15}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

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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   <u>Depth-related isotopic patterns in carnivores</u>

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   bentho-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 $\delta^{15}N$, hence being potentially reliable to establish isotopic

249   baselines. However, the $\delta^{15}N$ 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 $\delta^{15}N$ 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 (Carlier

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 $\delta^{15}N$ 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

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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 δ^{15}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 δ^{15}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 δ^{15}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 bentho-demersal community. Although metabolic factors that can

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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

295 ACKNOWLEDGEMENTS

302

303   BIBLIOGRAPHY

304   Abello P., 1989. Feeding habits of *Macropipus tuberculatus* (Brachyura, Portunidae) off the catalan
305   coast (NW Mediterranean). Miscellania Zoologica 13, 45-50.

306   Aberle N., Malzahn A.M., 2007. Interspecific and nutrient-dependant variations in stable isotope
307   fractionation: experimental studies simulating pelagic multitrophic systems. Oecologia 154,
308   291-303.

309   Bloomfield A.J., Elsdon T.S., Walther B.D., Gier E.J., Gillanders B.M., 2011. Temperature and diet
310   affect carbon and nitrogen isotopes of fish muscle: can amino acid nitrogen isotopes explain
311   effects? Journal of Experimental Marine Biology and Ecology 399, 48-59.

312   Carlier A., Riera P., Amouroux J., Bodiou J., Grémare A., 2007. Benthic trophic network in the Bay of
313   Banyuls sur Mer (northwest Maditerranean, France): an assessment based on stable carbon and
314   nitrogen isotopes analysis. Estuarine, Coastal and Shelf Science 72, 1-15.

315   Choi S.C., 1986. Natural diet and feeding habits of the crabs *Liocarcinus puber* and *L. holsatus*
316   (Decapoda, Brachyura, Portunidae). Marine Ecology Progress Series 31, 87-99.

317   Chouvelon T., Spitz J., Caurant F., Mèndez-Fernandez P., Chappuis A., Laugier F., Le Goff E.,
318   Bustamante P., 2012. Revisiting the use of δ^{15}N in meso-scale studies of marine food webs by
319   considering spatio-temporal variations in stable isotopic signatures – The case of an open
320   ecosystem: the Bay of Biscay (North-East Atlantic). Progress in Oceanography 101, 92-105.

321   Costanzo S.D., O'Donoghue M.J., Dennison W.C., Loneragan N.R., Thomas M., 2001. A new
322   approach for detecting and mapping sewage impacts. Marine Pollution Bulletin 42, 149-156.

323   Dang C., Sauriau P.-G., Savoye N., Caill-Milly N., Martinez P., Millaret C., Haure J., de Montaudouin
324   X., 2009. Determination of diet in Manila clams by spatial analysis of stable isotopes. Marine
325   Ecology Progress Series 387, 167-177.

326   Darnaude A.M., Salen-Picard C., Harmelin-Vivien M.L., 2004. Depth variation in terrestrial
327   particulate organic matter exploitation by marine coastal benthic communities off the Rhone
328   River delta (NW Mediterrean). Marine Ecology Progress Series 275, 47-57.

329   Deniel C., 1975. Régimes alimentaires d'*Arnoglossus thori* Kyle et d'*Arnoglossus imperialis*
330   Rafinesque (Teleosteens – Bothidae) en Baie de Douarnenez. Revue des Travaux de l'Institut
331   des Pêches Maritimes 38, 105-116.

332   Emmery A., Lefebvre S., Alunno-Bruscia M., Kooijman S.A.L.M., 2011. Understanding the dynamics
333   of δ^{13}C and δ^{15}N in soft tissues of the bivalve *Crassostrea gigas* facing environmental
334   fluctuations in the context of Dynamic Energy Budget (DEB). Journal of Sea Research 66, 361-
335   371.

336   Falkowski P.G., 1991. Species variability in the fractionation of ^{13}C and ^{12}C by marine phytoplankton.
337   Journal of Plankton Research 13(Suppl), 21-28.

338 Gannes L.Z., O'Brien D.M., Martínez del Rio C., 1998. Stable isotopes in animal ecology :
339   assumptions, caveats, and a call for more laboratory experiments. Ecology 78, 1271-1276.

340 Grall J., Le Loc'h F., Guyonnet B., Riera P., 2006. Community structure and food web based on stable
341   isotopes (δ^{15}N and δ^{13}C) analysis of a North Eastern Atlantic maerl bed. Journal of Experimental
342   Marine Biology and Ecology 338, 1-15.

343 Hobson K.A., Clark R.G., 1992. Assessing avian diets using stable isotopes II: Factors influencing
344   diet-tissue fractionation. Condor 94, 189-197.

345 Hobson K.A., Ambrose W.G.Jr., Renaud P.E., 1995? Sources of primary production, benthic-pelagic
346   coupling, and trophic relationships within the Northeast Water Polynia: insights from δ^{13}C and
347   δ^{15}N analysis. Marine Ecology Progress Series 128, 1-10.

348 Kline T.C.Jr., 2009. Characterization of carbon and nitrogen stable isotope gradients in the northern
349   Gulf of Alaska using terminal feed stage copepodite-V *Neocalanus cristatus*. Deep Sea
350   Research II 56, 2537-2552.

351 Lagardère J-P., 1973. Distribution des décapodes dans le sud du Golfe de Gascogne. Revue des
352   Travaux de l'Institut des Pêches Maritimes 37, 77-95.

353 Le Loc'h F., Hily C., 2005. Stable carbon and nitrogen isotope analysis of *Nephrops norvegicus* /
354   *Merluccius merluccius* fishing grounds in the Bay of Biscay (Northeast Atlantic). Canadian
355   Journal of Fisheries and Aquatic Sciences 62, 123-132.

356 Le Loc'h F., Hily C., Grall J., 2008. Benthic community and food web structure on the continental
357   shelf of the Bay of Biscay (North Eastern Atlantic) revealed by stable isotope analysis. Journal
358   of Marine Systems 72, 17-34.

359 Leclerc J.-C., Riera P., Leroux C., Lévêque L., Laurans M., Schaal G., Davoult D., 2013. Trophic
360   significance of kelps in kelp communities in Brittany (France) inferred from isotopic
361   comparisons. Marine Biology 160, 3249-3258.

362 Lunven M., Guillaud J.-F., Youénou A., Crassous M.-P., Berric R., Le Gall E., Kérouel R., Labry C.,
363   Aminot A., 2005. Nutrient and phytoplankton distribution in the Loire River plume (Bay of
364   Biscay, France) resolved by a new Fine Scale Sampler. Estuarine, Coastal and Shelf Science 65,
365   94-108.

366 Marchais V., Schaal G., Grall J., Lorrain A., Nerot C., Richard P., Chauvaud L., 2013. Spatial
367   variability of stable isotope ratios in oysters (*Crassostrea gigas*) and primary producers along an
368   estuarine gradient (Bay of Brest, France). Estuaries and Coasts 36, 808-819.

369 McClelland J.W., Valiela I., Michener R.H., 19 97. Nitrogen-stable isotope signature in estuarine food
370   webs: a record of increasing urbanization in coastal watersheds. Limnology and Oceanography
371   42, 930-937.

372 Melville A.J., Connolly R.M., 2003. Spatial analysis of stable isotope data to determine primary
373   sources of nutrition for fish. Ocecologia 136, 499-507.

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

14

376 Moeri O., Sternberg L.D.L., Rodicio L.P., Walsh P.J., 2003. Direct effects of ambient ammonia on the
377   nitrogen isotope ratios of fish tissues. Journal of Experimental Marine Biology and Ecology 282,
378   61-66.

379 Montoya J.P., Wiebe P.H., McCarthy J.J., 1992. Natural abundance of ^{15}N in particulate nitrogen and
380   zooplankton in the Gulf Stream region and warm-core ring 86A. Deep Sea Research Part A –
381   Oceanographic Research 39, S363-S392.

382 Mortillaro J.-M., Schaal G., Grall J., Nerot C., Brind'Amour A., Marchais V., Perdriau M., Le Bris H.,
383   2014. Comparative study of isotopic trends in two coastal ecosystems of North Biscay : a
384   multitrophic spatial gradient approach. Estuarine, Coastal and Shelf Science, 136 : 149-156

385 Nerot C., Lorrain A., Grall J., Gillikin D.P., Munaron J.-M., Le Bris H., Paulet Y.-M., 2012. Stable
386   isotope variations in benthic filter feeders across a large depth gradient on the continental shelf.
387   Estuarine, Coastal and Shelf Science 96, 228-235.

388 Nerot C., Méziane T., Schaal G., Grall J., Lorrain A., Paulet Y.-M., Kraffe E., in revision. Spatial
389   changes in fatty acids signatures of the great scallop *Pecten maximus* across the Bay of Biscay
390   continental shelf. Submitted to Journal of Experimental Marine Biology and Ecology.

391 Page H.M., Brooks A.J., Kulbicki M., Galzin R., Miller R.J., Reed D.C., Schmitt R.J., Holbrook S.J.,
392   Koenig C., 2013. Stable isotopes reveal trophic relationships and diet of consumers in temperate
393   kelp forest and coral reef ecosystems. Oceanography 26, 180-189.

394 Perga M.E., Gerdeaux D., 2005. 'Are fish what they eat' all year round ? Oecologia 144, 598-606.

395 Peterson B., Fry B., 1987. Stable isotopes in ecological studies. Annual Review of Ecology and
396   Systematics 18, 293-320.

397 Post D.M., 2002. Using stable isotopes to estimate trophic positions: models, methods, and
398   assumptions. Ecology 83, 703-718.

399 Riera P., Richard P., 1996. Isotopic determination of food sources of *Crassostrea gigas* along a trophic
400   gradient in the estuarine bay of Marennes-Oléron. Estuarine, Coastal and Shelf Science 42, 347-
401   360.

402 Riera P., Stal L.J., Nieuwenhuize J., 2000. Heavy δ^{15}N in intertidal benthic algae and invertebrates in
403   the Scheldt estuary (The Netherlands): effects of river nitrogen inputs. Estuarine, Coastal and
404   Shelf Science 51, 365-372.

405 Rigolet C., Thiébaut E., Dubois S.F., 2014. Food web structure of subtidal benthic muddy habitats :
406   evidence of microphytobenthos contribution supported by an engineer species. Marine Ecology
407   Progress Series 500, 25-41.

408 Tenore K., 1983. What controls the availability to animals of detritus derived from vascular plants:
409   organic nitrogen or caloric availability? Marine Ecology Progress Series 10, 307-309.

410 Vander Zanden M.J., Rasmussen J.B., 2001. Variation in δ^{15}N and δ^{13}C trophic fractionation:
411   implications for aquatic food web studies. Limnology and Oceanography 46, 2061-2066.

412 Vanderklift M.A., Ponsard S., 2003. Sources of variation in consumer-diet δ^{15}N enrichment: a meta-
413   analysis. Oecologia 136, 169-182.

15

414 Vanderklift M.A., Wernberg T., 2010. Stable isotopes reveal a consistent consumer-diet relationship
415   across hundreds of kilometres. Marine Ecology Progress Series 403, 53-61.

416 Vuorio K., Meili M., Sarvala J., 2004. Taxon-specific variation in the stable isotopic signatures (δ^{13}C
417   and δ^{15}N) of lake phytoplankton; Freshwater Biology 51, 807-822.

418

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

		Slope	intercept	p-value	R²
SPOM	$\delta^{13}C$	-0.028	-19.62	< 0.001	0.76
	$\delta^{15}N$	-0.012	9.44	< 0.001	0.25
SOM	$\delta^{13}C$	0.013	24.54	0.30	0.08
	$\delta^{15}N$	0.018	8.50	< 0.001	0.55
Pecten maximus	$\delta^{13}C$	-0.010	-16.00	< 0.001	0.46
	$\delta^{15}N$	-0.042	10.72	< 0.001	0.95

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

Species		n	slope	intercept	R²	p-value
Fish						
Arnoglossus imperialis	$\delta^{13}C$	46	-0.010	-16.52	0.63	<0.001
	$\delta^{15}N$	46	-0.026	14.61	0.74	<0.001
Arnoglossus laterna	$\delta^{13}C$	14	-0.020	-15.32	0.76	<0.001
	$\delta^{15}N$	14	-0.030	15.26	0.83	<0.001
Callionymus lyra	$\delta^{13}C$	32	-0.014	-16.04	0.51	<0.001
	$\delta^{15}N$	32	-0.015	13.172	0.51	<0.001
Decapods						
Liocarcinus holsatus	$\delta^{13}C$	19	-0.008	-16.25	0.11	0.173
	$\delta^{15}N$	19	-0.030	14.50	0.71	<0.001
Liocarcinus marmoreus	$\delta^{13}C$	21	-0.0123	-15.89	0.63	<0.001
	$\delta^{15}N$	21	-0.016	12.93	0.52	<0.001
Macropipus tuberculatus	$\delta^{13}C$	19	-0.017	-15.59	0.39	0.004
	$\delta^{15}N$	19	-0.043	16.12	0.33	0.010
Munida rugosa	$\delta^{13}C$	25	-0.014	-15.20	0.42	<0.001
	$\delta^{15}N$	25	-0.026	13.01	0.86	<0.001

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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 δ^{13}C and δ^{15}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 δ^{13}C and δ^{15}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 δ^{15}N ($\delta^{15}N_{carnivore}$ - $\delta^{15}N_{scallops}$) of demersal fish (dashed line)
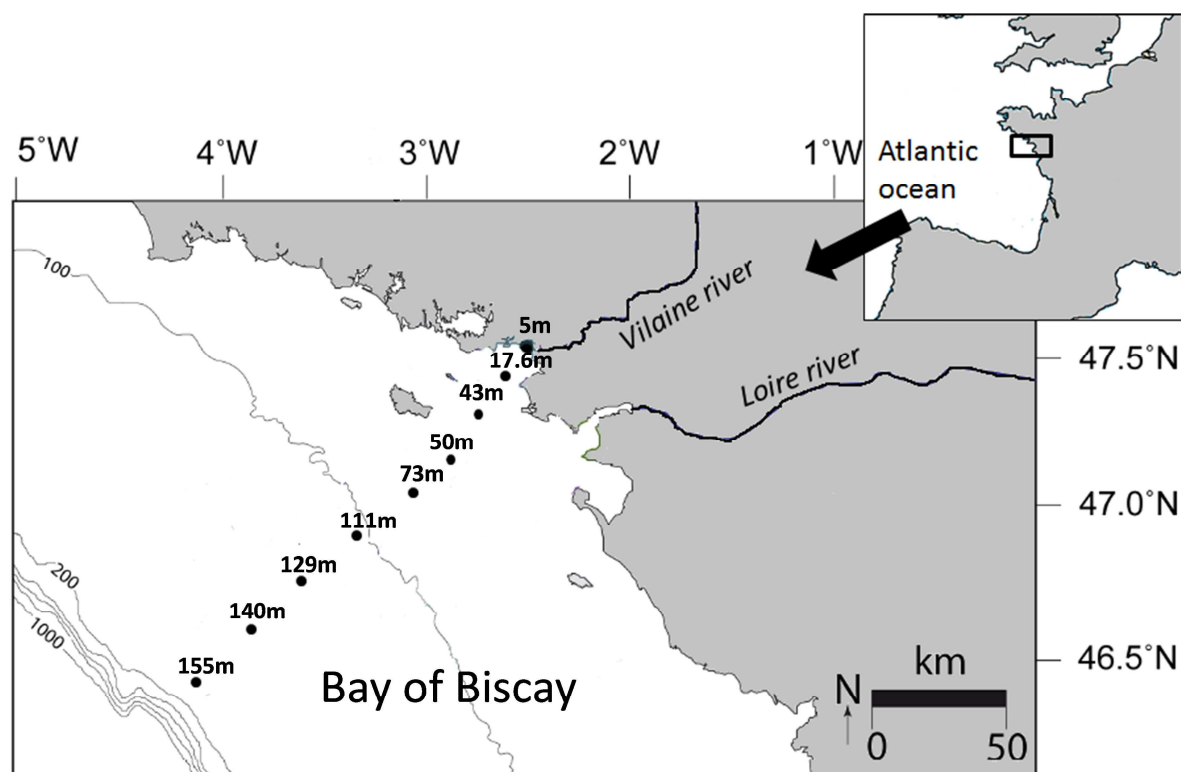
469 and megabenthic crustaceans (full line) across the continental shelf of the Bay of Biscay. Fish:

470 corrected δ^{15}N=0.027*depth+2.58; R²=0.74; p<0.001. Crustaceans: corrected δ^{15}N=0.016*depth+2.81;
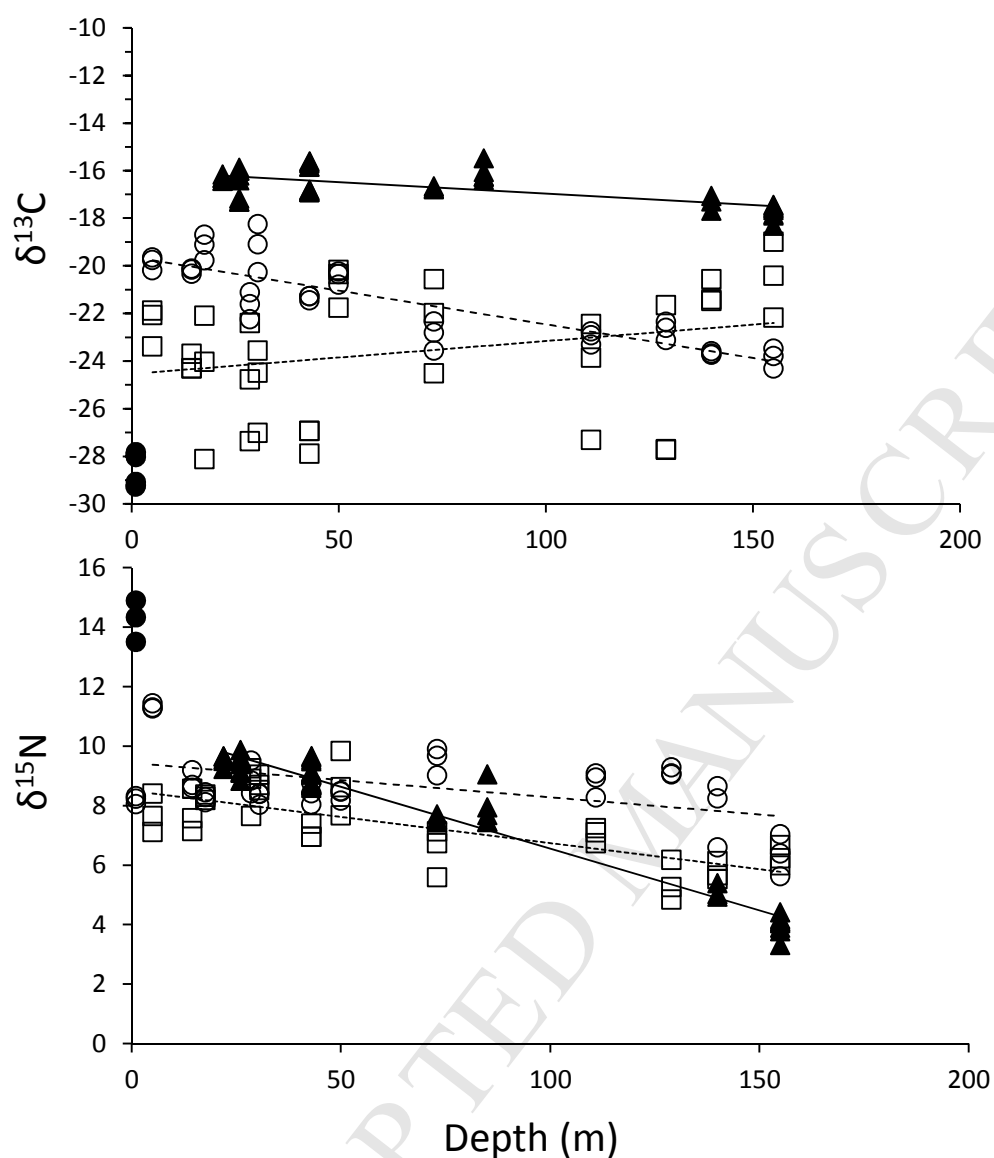
471 R²=0.45; p<0.001.

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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

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478   Figure 2:

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480     $\delta^{13}C$ and $\delta^{15}N$ 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)

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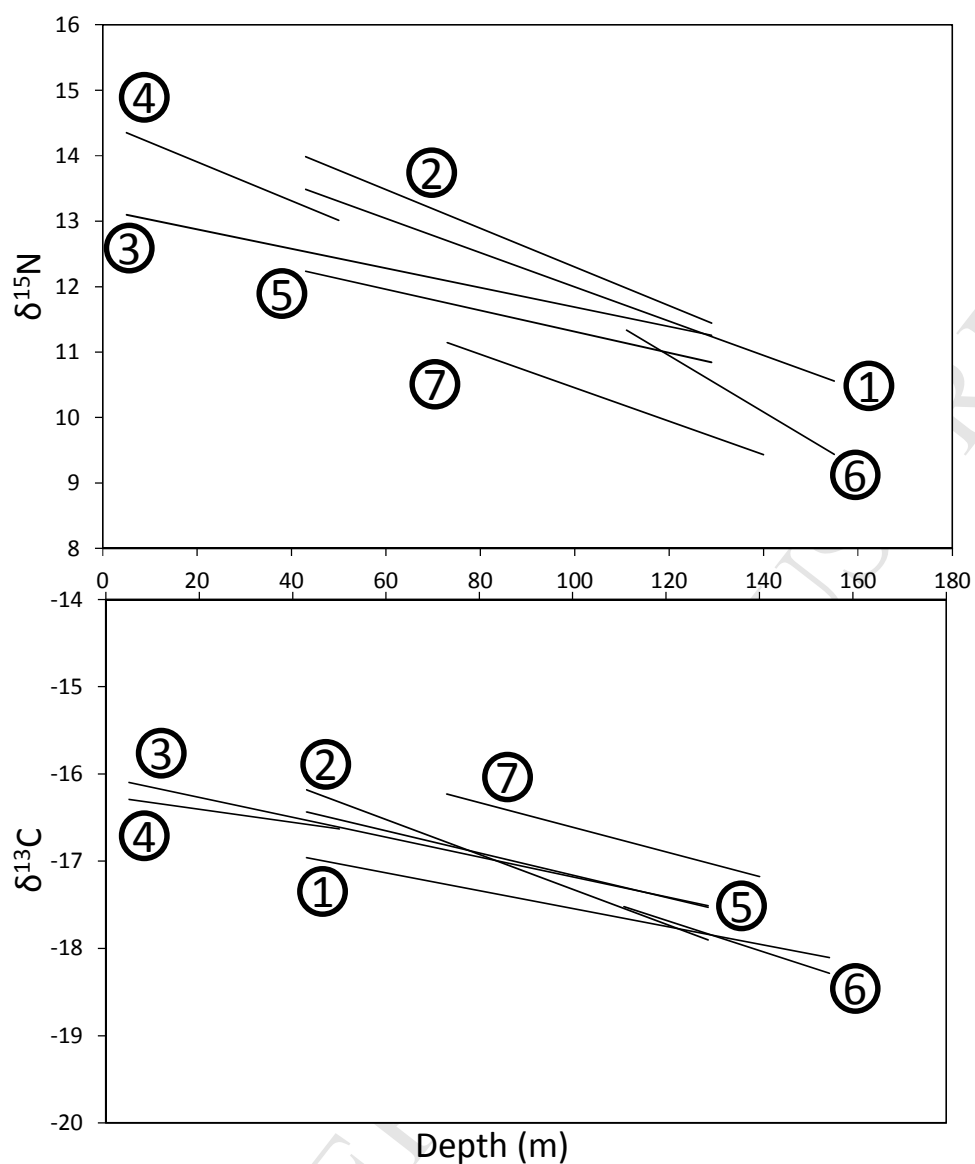
485   Figure 3:

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487   δ¹³C and δ¹⁵N of the different carnivores sampled across the continental shelf of the Bay of Biscay.
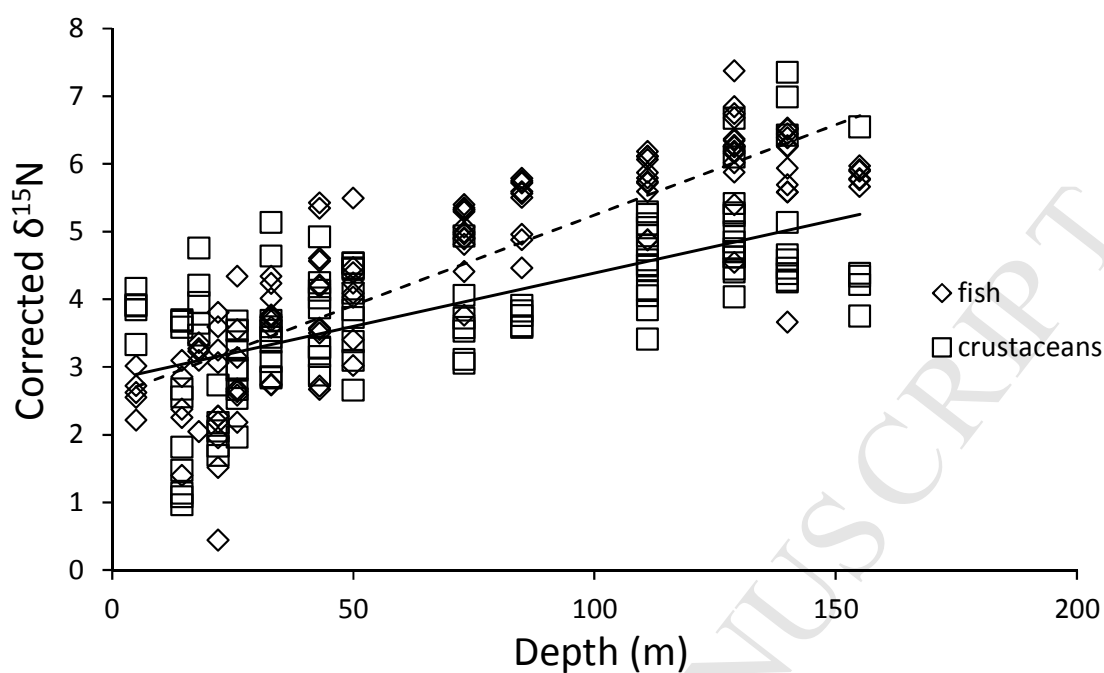
488   For clarity reasons, only regression lines are shown. For linear regression parameters, see table 2.

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492   Figure 4:

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

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

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

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