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Revisiting the use of δ^{15}N in meso-scale studies of marine food webs by considering spatio-temporal variations in stable isotopic signatures – The case of an open ecosystem: The Bay of Biscay (North-East Atlantic)

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ABSTRACT

Most of the recent framework directives and environmental policies argue for the development and the use of indicators – notably trophodynamic indicators – that should be able to follow ecosystems' evolution in space and time, particularly under anthropogenic perturbations. In the last decades, the use of stable carbon and nitrogen isotopes ratios has increased exponentially, particularly in studies of marine ecosystems' trophic structure and functioning. This method is principally based on the assumption that the isotopic composition of a consumer directly reflects that of its food. Nevertheless, few studies have attempted to define the limits of this tool, before using it and drawing ecological conclusions from isotopic analysis. This study aimed to assess the importance of considering spatio-temporal variations in isotopic signatures of consumers when using δ^{13}C and especially δ^{15}N values in open ecosystems with complex food webs, using the Bay of Biscay (North-East Atlantic) as a case study. To this end, more than 140 species from this marine ecosystem were analysed for the isotopic signatures in their muscle tissue. They were sampled from coastal to oceanic and deep-sea areas and at different latitudes, to evaluate spatial variations of isotopic signatures. Selected species were also sampled over several years and in two seasons to account for inter-annual and seasonal variations. In the Bay of Biscay temperate ecosystem, which is subject to both coastal and oceanic influences – two main river inputs and upwelling areas – , δ^{13}C and δ^{15}N values significantly decreased from inshore to offshore species, and to a lesser extent from benthic to pelagic organisms. River discharges appeared to be the first factor influencing δ^{13}C and δ^{15}N values in consumers. From the important spatial variations detected in δ^{15}N values in particular, we suggest that in such contrasted ecosystem, nitrogen isotopic ratios may also be revisited as an indicator of the feeding area. Moreover, we demonstrate that several baselines should be used when calculating trophic levels from δ^{15}N values. From the temporal variations detected, we recommend concentrating on a short time scale for the sampling most organisms.

1. Introduction

Maintaining both a sustainable exploitation of natural marine resources and a good environmental status of marine ecosystems is a challenge for human societies, and a good knowledge of the ecosystems' structure and functioning is a pre-requisite for this. Such approaches guide the most recent framework directives and environmental policies for the management of marine ecosystems and fisheries (Garcia et al., 2003; OSPAR, 2010). These directives notably rely on the development of indicators that are easy to implement, and powerful enough to quickly detect changes in the environment. In this way, many authors have recently argued for the development of trophic indicators (Gascuel et al., 2005; Cury et al., 2005; Pauly and Watson, 2005), such as the trophic level of catches which previously led to the description of the famous "fishing down marine food webs" process (Pauly et al., 1998; Pauly and Palomares, 2005).

In the last decades, analysis of carbon and nitrogen stable isotopes ratios in consumers' tissues (δ^{13}C, δ^{15}N) has proved to be a powerful tool to describe the trophic ecology and trophic relationships between marine organisms at the ecosystem scale. Indeed, this method represents an alternative or complementary tool to the traditional methods of dietary studies (e.g., analysis of guts or stomach contents) (Hobson et al., 2002; Michener and Kaufman, 2007). The use of these ecological tracers is principally based on the fact that (1) primary producers of an ecosystem generally present different isotopic compositions, due to the different nutrients

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fixed and the biochemical cycle they use for photosynthesis (Peterson and Fry, 1987; France, 1995); (2) the enrichment in ^{13}C and ^{15}N between a source or a prey and its consumer (also called Trophic Enrichment Factor, TEF) is relatively predictable, being less important in ^{13}C ($\leqslant 1‰$) than in ^{15}N (3.4‰ on average) (De Niro and Epstein, 1978, 1981; Minagawa and Wada, 1984; Post, 2002; Michener and Kaufman, 2007). Hence, $\delta^{13}C$ values are generally used as a tracer of the habitat or the feeding zone of organisms (Hobson, 1999; France, 1995). $\delta^{15}N$ values are mainly used as an indicator of the trophic position of organisms and have been widely used to calculate the absolute trophic level of organisms in various ecosystems (Hobson and Welch, 1992; Lesage et al., 2001; Le Loc'h et al., 2008; Mèndez-Fernandez et al. 2012).

However, there is still a huge lack of experimental studies to support the many assumptions needed to fully interpret stable isotopic data from the field (Gannes et al., 1997; Martínez del Rio et al., 2009), as well as studies devoted to describe the mechanisms which are at the origin of the isotopic signature variations of consumers in the field. Recently, some studies have thus highlighted the importance of considering spatio-temporal variations from the base of food chains, linking these variations to environmental variables such as depth, temperature and salinity (Schell et al., 1998; Jennings and Warr, 2003; Hill et al., 2006; Barnes et al., 2009; Lefebvre et al., 2009a). If not considered these variations can effectively lead to misinterpretations or confusion in the assessment of the feeding zone, the food partitioning in a consumer's diet or the calculation of trophic levels from stable isotope analysis (Dubois et al., 2007; Guzzo et al., 2011). Nevertheless in general, these studies logically focused on lower trophic level consumers (e.g., zooplankton and/or suspension-feeders) that may be found all over the specific spatial scale considered. Very few have investigated such spatio-temporal variations of isotopic signatures in higher trophic level consumers (e.g., Revill et al., 2009; Kurle et al., 2011). Moreover, the problem of such isotopic variability may increase in intensity with ecosystem size and in open systems, notably because such ecosystems often support a high diversity of organisms and highly mobile species, and are under diverse influences (e.g., river plumes, oceanic streams, upwelling). However, the stable isotopic approach deserves to be developed in such cases as well because there is a clear need to develop indicators to follow ecosystems' evolution in space and time.

In this general context, the first objective of this study was therefore to determine the spatio-temporal variations of stable isotopic signatures in various representative taxa of an open marine ecosystem, using the Bay of Biscay (North-East Atlantic) as a case study. Particularly, this study focused on potential differences between neritic/coastal and oceanic/deep-sea organisms, and, between benthic and pelagic organisms, and then focused on potential inter-annual and seasonal differences. Considering the existence of such spatio-temporal variations, the second objective of this study was to assess implications and to state recommendations for the use of $\delta^{13}C$ and $\delta^{15}N$ as ecological tracers in the field of meso-scale studies of marine food webs, especially in contrasted and temperate open ecosystems.

2. Materials and methods

2.1. Study area and sampling

The Bay of Biscay is a very large bay opened on the North-East Atlantic Ocean, located from 1 to 10°W and from 43 to 48°N (Fig. 1). Along the French coast, the continental shelf covers over 220,000 km^2, the extends more than 200 km offshore in the north of the Bay and only 10 km in the south. Two main river plumes (i.e., the Loire and the Gironde) influence its hydrological structure (Planque et al., 2004; Puillat et al., 2004). The Bay of Biscay also presents a vast oceanic domain and a continental slope indented by numerous canyons (Koutsikopoulos and Le Cann, 1996). Overall, the Bay of Biscay supports a rich fauna including many protected species (e.g., marine mammals, seabirds, sharks) and is subjected to numerous anthropogenic activities such as important fisheries (Lorance et al., 2009; OSPAR, 2010).

In this study, more than 1820 individuals have been sampled, belonging to 142 species covering a wide range of representative taxa of the North East Atlantic food webs components, and

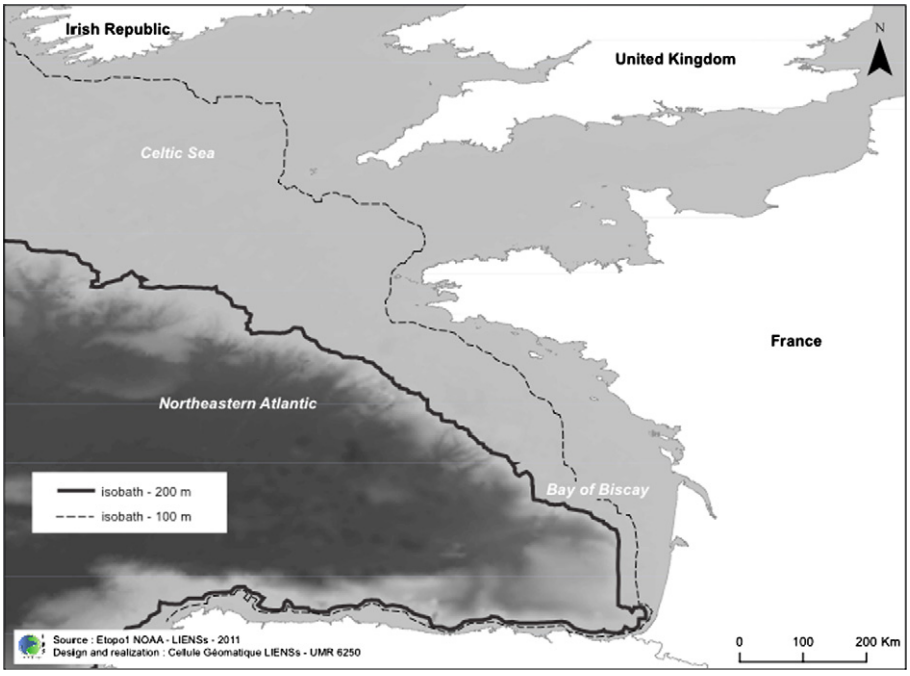

Fig. 1. Map of the study area.

Table 1
Characteristics of species – distribution, average trawling depth, number of individuals (*N*), size of individuals – and stable carbon and nitrogen isotope values (Mean ± Standard Deviation) in the muscle (except mesozooplankton, analysed as a whole) of the Bay of Biscay's food webs components. For all species, values correspond to autumn trawls (except marine mammals collected throughout the year; also, individuals of European pilchard and anchovy trawled in spring for seasonal variations analysis are not included here). Each row of the table corresponds to a dot Fig. 2. Species are classified by taxa, then by distribution on both horizontal and vertical axis, finally by increasing δ^{15}N values (see detailed grouping strategy in Material and Methods).

Taxa and Species	HAD[a]	VAD[a]	Depth (m)[b]		Size (cm or mm)[c]		δ^{13}C (‰)	δ^{15}N (‰)
			Mean	*N*	Mean ± SD	(min–max)	Mean ± SD	Mean ± SD
MARINE MAMMALS								
Phocoena phocoena	S/US	bp	NA	10	153 ± 20	(134–196)	−17.0 ± 0.4	13.0 ± 0.7
Globicephala melas	S/US	bp	NA	16	448 ± 73	(328–578)	−16.3 ± 0.8	13.2 ± 1.7
Tursiops truncatus	S/US	bp	NA	7	256 ± 38	(211–315)	−16.0 ± 0.7	14.5 ± 0.8
Stenella coeruleoalba	S/US	p	NA	11	185 ± 31	(130–219)	−17.5 ± 0.3	11.2 ± 0.9
Delphinus delphis	S/US	p	NA	26	200 ± 11	(183–230)	−17.4 ± 0.5	12.1 ± 0.6
Balaenoptera physalus	US/DS	p	NA	4	1785 ± 204	(1510–1950)	−18.2 ± 0.3	9.5 ± 1.3
Kogia breviceps	US/DS	p	NA	6	225 ± 47	(167–288)	−16.7 ± 0.3	11.1 ± 0.3
Physeter macrocephalus	US/DS	p	NA	3	1063 ± 27	1045–1095)	−16.5 ± 0.0	11.1 ± 1.0
Mesoplodon bidens	US/DS	p	NA	5	389 ± 51	(316–457)	−17.4 ± 0.8	12.2 ± 0.2
Ziphius cavirostris	US/DS	p	NA	11	521 ± 76	(387–600)	−16.2 ± 0.2	12.5 ± 0.5
Balaenoptera acutorostrata	US/DS	p	NA	7	576 ± 91	(455–690)	−18.3 ± 0.8	12.8 ± 0.8
FISH								
Chondrichthyans								
Leucoraja naevus	C/S	b	126	10	604 ± 28	(560–640)	−16.3 ± 0.3	12.3 ± 0.5
Raja clavata	C/S	b	128	11	735 ± 111	(570–940)	−16.1 ± 0.4	12.3 ± 0.4
Raja microocellata	C/S	b	21	5	694 ± 99	(590–810)	−14.6 ± 0.2	14.5 ± 0.3
Torpedo marmorata	C/S	b	33	3	383 ± 81	(310–470)	−16.4 ± 0.5	14.8 ± 0.5
Mustelus asterias	C/S	bp	112	11	874 ± 91	(740–1100)	−15.9 ± 0.5	13.0 ± 0.6
Mustelus mustelus	C/S	bp	108	4	935 ± 163	(790–1150)	−16.1 ± 0.4	13.0 ± 0.3
Scyliorhinus canicula	C/S	bp	126	10	579 ± 31	(530–630)	−16.7 ± 0.2	13.1 ± 0.3
Galeus melastomus	S/US	bp	289	12	606 ± 75	(500–720)	−17.2 ± 0.2	12.1 ± 0.6
Chimaera monstrosa	S/US	bp	669	17	592 ± 165	(340–810)	−16.5 ± 0.2	12.7 ± 0.7
Etmopterus spinax	S/US	bp	492	10	422 ± 25	(383–450)	−17.2 ± 0.1	12.8 ± 0.2
Deania profundorum	US/DS	bp	1033	4	445 ± 87	(320–520)	−18.1 ± 0.1	11.0 ± 0.1
Hydrolagus mirabilis	US/DS	bp	1116	5	420 ± 12	(410–440)	−16.9 ± 0.2	11.1 ± 0.3
Centroselachus crepidater	US/DS	bp	1147	5	678 ± 36	(650–740)	−17.5 ± 0.2	11.6 ± 0.3
Deania calcea	US/DS	bp	1033	10	934 ± 63	(840–1020)	−17.1 ± 0.3	12.2 ± 0.5
Large Actinopterygians								
Melanogrammus aeglefinus	C/S	bp	163	5	532 ± 44	(500–610)	−17.4 ± 0.2	12.6 ± 1.3
Chelidonichthys lucerna	C/S	bp	137	5	554 ± 63	(470–630)	−16.8 ± 0.6	13.2 ± 0.5
Labrus bergylta	C/S	bp	20	3	507 ± 25	(480–530)	−17.5 ± 0.0	13.5 ± 0.1
Zeus faber	C/S	bp	116	5	550 ± 19	(530–580)	−16.8 ± 0.1	14.0 ± 0.3
Dicentrarchus labrax (>400 mm TL)	C/S	bp	98	5	668 ± 24	(640–700)	−16.9 ± 0.5	14.1 ± 0.5
Merlangius merlangus (>350 mm TL)	C/S	bp	55	15	423 ± 36	(370–480)	−16.2 ± 0.3	14.8 ± 0.4
Conger conger	C/S	bp	67	5	1278 ± 88	(1150–1360)	−16.2 ± 0.6	15.3 ± 0.7
Lepidorhombus whiffiagonis	S/US	b	127	5	432 ± 24	(410–470)	−17.5 ± 0.2	12.2 ± 0.2
Malacocephalus laevis	S/US	bp	337	5	386 ± 21	(370–420)	−17.8 ± 0.3	12.0 ± 0.4
Molva macrophtalma	S/US	bp	492	5	646 ± 50	(600–730)	−17.6 ± 0.2	12.9 ± 0.2
Caelorhynchus caelorhynchus	S/US	bp	461	5	278 ± 19	(250–300)	−17.4 ± 0.3	13.0 ± 0.5
Helicolenus dactylopterus	S/US	bp	492	5	370 ± 22	(340–400)	−17.3 ± 0.1	13.2 ± 0.3
Trachyrincus scabrus	S/US	bp	536	5	408 ± 35	(360–450)	−17.5 ± 0.1	13.4 ± 0.1
Phycis blennoides	S/US	bp	259	5	510 ± 66	(440–580)	−17.0 ± 0.3	13.5 ± 0.1
Merluccius merluccius (350–550 mm TL)	S/US	bp	140	21	466 ± 56	(360–550)	−17.4 ± 0.3	13.6 ± 0.5
Scorpaena scrofa	S/US	bp	128	4	400 ± 45	(350–460)	−17.5 ± 0.2	13.6 ± 0.5
Lophius piscatorius (400–700 mm TL)	S/US	bp	193	18	570 ± 72	(450–690)	−16.9 ± 0.3	13.9 ± 0.3
Lophius piscatorius (>700 mm TL)	S/US	bp	313	12	831 ± 107	(720–1020)	−17.1 ± 0.6	14.0 ± 0.8
Lophius budegassa	S/US	bp	136	5	746 ± 88	(650–890)	−17.2 ± 0.1	14.0 ± 0.3
Merluccius merluccius (>550 mm TL)	S/US	bp	127	12	632 ± 59	(560–720)	−17.2 ± 0.3	14.0 ± 0.6
Molva molva	S/US	bp	203	4	812 ± 112	(680–910)	−17.5 ± 0.3	14.5 ± 0.3
Alepocephalus bairdii	US/DS	bp	1209	5	684 ± 65	(610–770)	−18.3 ± 0.1	10.4 ± 0.3
Beryx decadactylus	US/DS	bp	509	6	348 ± 58	(300–460)	−18.5 ± 0.2	11.0 ± 0.9
Coryphaenoides rupestris	US/DS	bp	1142	4	690 ± 60	(620–740)	−18.4 ± 0.7	11.6 ± 0.5
Alepocephalus rostratus	US/DS	bp	1109	11	522 ± 72	(370–600)	−18.2 ± 0.3	12.0 ± 0.6
Mora moro	US/DS	bp	1089	5	568 ± 32	(530–610)	−17.6 ± 0.3	12.4 ± 0.3
Lepidion eques	US/DS	bp	1177	5	362 ± 16	(340–380)	−17.8 ± 0.2	12.6 ± 0.1
Hoplostethus atlanticus	US/DS	bp	1153	5	514 ± 21	(490–540)	−17.7 ± 0.4	14.0 ± 0.5
Trachyscorpia cristulata	US/DS	bp	1125	10	401 ± 86	(280–510)	−17.3 ± 0.4	14.2 ± 0.7
Aphanopus carbo	US/DS	p	1033	5	996 ± 55	(920–1070)	−18.1 ± 0.1	12.5 ± 0.3
Small Actinopterygians								
Solea solea (<200 mm TL)	C/S	b	27	7	160 ± 33	(110–190)	−16.2 ± 0.4	12.0 ± 0.7

Table 1 (*continued*)

Taxa and Species	HAD[a]	VAD[a]	Depth (m)[b]		Size (cm or mm)[c]		δ^{13}C (‰)	δ^{15}N (‰)
			Mean	*N*	Mean ± SD	(min–max)	Mean ± SD	Mean ± SD
Microchirus variegatus	C/S	b	47	5	162 ± 8	(150–170)	−17.3 ± 0.0	12.2 ± 0.1
Solea solea (>200 mm TL)	C/S	b	53	27	316 ± 59	(220–460)	−15.7 ± 0.6	13.2 ± 0.7
Dicologlossa cuneata	C/S	b	60	5	188 ± 16	(170–210)	−16.7 ± 0.3	13.3 ± 0.6
Boops boops	C/S	bp	99	5	262 ± 24	(230–290)	−18.0 ± 0.6	11.8 ± 1.1
Cepola macrophthalma	C/S	bp	109	5	554 ± 18	(530–570)	−18.2 ± 0.3	12.0 ± 0.3
Echiichthys vipera	C/S	bp	47	5	108 ± 8	(100–120)	−17.5 ± 0.2	12.3 ± 0.2
Spondyliosoma cantharus (<200 mm TL)	C/S	bp	30	5	142 ± 37	(100–190)	−16.6 ± 0.8	12.3 ± 0.3
Argentina sphyraena	C/S	bp	99	10	188 ± 13	(170–210)	−17.4 ± 0.2	12.3 ± 0.4
Callionymus lyra	C/S	bp	109	5	222 ± 16	(210–250)	−16.6 ± 0.3	12.5 ± 0.3
Pomatoschistus minutus	C/S	bp	60	5	56 ± 5	(50–60)	−17.5 ± 0.1	12.7 ± 0.3
Lesueurigobius friesii	C/S	bp	60	5	76 ± 5	(70–80)	−17.3 ± 0.3	12.8 ± 0.1
Trachinus draco	C/S	bp	40	10	237 ± 20	(200–270)	−16.7 ± 0.8	13.0 ± 1.3
Aspitrigla cuculus	C/S	bp	129	10	257 ± 12	(240–280)	−17.2 ± 0.3	13.1 ± 0.6
Trisopterus minutus	C/S	bp	114	65	181 ± 19	(145–235)	−17.1 ± 0.4	13.1 ± 0.6
Eutrigla gurnardus	C/S	bp	114	18	311 ± 62	(230–440)	−16.9 ± 0.3	13.1 ± 0.5
Dicentrarchus punctatus	C/S	bp	36	4	357 ± 15	(340–370)	−16.7 ± 0.0	13.9 ± 0.6
Trisopterus luscus	C/S	bp	63	14	180 ± 30	(145–235)	−16.6 ± 0.3	14.1 ± 0.2
Dicentrarchus labrax (⩽400 mm TL)	C/S	bp	29	6	373 ± 23	(340–400)	−15.8 ± 0.2	14.2 ± 0.5
Merlangius merlangus (⩽350 mm TL)	C/S	bp	36	32	211 ± 82	(80–350)	−16.8 ± 0.3	14.3 ± 0.6
Spondyliosoma cantharus (>200 mm TL)	C/S	bp	44	7	254 ± 34	(220–310)	−16.5 ± 0.6	15.1 ± 0.5
Engraulis encrasicolus	C/S	p	70	46	128 ± 15	(100–160)	−18.2 ± 0.7	10.7 ± 1.5
Scomber scombrus (>200 mm TL)	C/S	p	149	10	296 ± 12	(280–310)	−18.6 ± 0.3	11.2 ± 0.7
Sardina pilchardus (>150 mm TL)	C/S	p	110	78	205 ± 19	(167–241)	−18.0 ± 0.5	11.2 ± 0.7
Scomber japonicus	C/S	p	43	5	338 ± 19	(320–370)	−17.5 ± 0.3	11.7 ± 0.5
Sardina pilchardus (<150 mm TL)	C/S	p	76	25	115 ± 12	(100–140)	−18.2 ± 0.7	11.8 ± 0.8
Trachurus trachurus (<200 mm TL)	C/S	p	101	67	151 ± 40	(40–80)	−18.3 ± 0.9	11.8 ± 1.3
Scomber scombrus (<200 mm TL)	C/S	p	136	5	164 ± 5	(160–170)	−18.7 ± 0.4	11.8 ± 0.4
Sprattus sprattus	C/S	p	38	32	99 ± 21	(65–135)	−17.8 ± 0.3	12.2 ± 0.5
Ammodytes tobianus	C/S	p	58	5	290 ± 16	(270–310)	−17.1 ± 0.2	12.2 ± 0.2
Trachurus trachurus (>200 mm TL)	C/S	p	105	45	275 ± 62	(205–410)	−17.7 ± 0.3	12.4 ± 0.7
Hyperoplus lanceolatus	C/S	p	58	5	340 ± 14	(320–350)	−16.4 ± 0.3	14.3 ± 0.3
Atherina presbyter	C/S	p	25	5	110 ± 10	(100–120)	−16.5 ± 0.2	14.8 ± 0.4
Bathysolea profundicola	S/US	b	333	5	192 ± 13	(180–210)	−17.2 ± 0.2	12.7 ± 0.5
Argentina silus	S/US	bp	492	5	352 ± 27	(330–390)	−18.1 ± 0.2	10.5 ± 0.5
Micromesistius poutassou (<300 mm TL)	S/US	bp	224	78	182 ± 38	(116–255)	−18.2 ± 0.5	11.1 ± 0.7
Gadiculus argenteus	S/US	bp	47	5	110 ± 7	(100–120)	−18.4 ± 0.1	11.2 ± 0.2
Micromesistius poutassou (>300 mm TL)	S/US	bp	246	5	320 ± 7	(310–330)	−17.6 ± 0.4	11.9 ± 0.8
Merluccius merluccius (⩽350 mm TL)	S/US	bp	118	57	180 ± 80	(65–350)	−18.1 ± 0.4	12.2 ± 0.7
Polymetme thaeocoryla	US/DS	bp	506	5	134 ± 7	(125–145)	−18.9 ± 0.1	11.6 ± 0.2
Bathypterois dubius	US/DS	bp	1147	5	162 ± 4	(160–170)	−18.4 ± 0.2	13.2 ± 0.3
Nezumia aequalis	US/DS	bp	1033	5	286 ± 9	(280–300)	−17.2 ± 0.2	13.3 ± 0.2
Xenodermichthys copei	US/DS	p	1129	6	142 ± 13	(130–160)	−19.1 ± 0.2	9.2 ± 0.4
Benthosema glaciale	US/DS	p	800	5	39 ± 2	(35–40)	−18.7 ± 0.2	9.5 ± 0.6
Ceratoscopolus maderensis	US/DS	p	1316	5	67 ± 4	(60–70)	−19.2 ± 0.2	9.5 ± 0.1
Bathylagus greyae	US/DS	p	1980	5	125 ± 6	(120–135)	−19.5 ± 0.5	9.8 ± 0.2
Myctophum punctatum	US/DS	p	1316	5	71 ± 6	(65–80)	−19.5 ± 0.2	9.9 ± 0.4
Serrivomer beanii	US/DS	p	NA	5	724 ± 34	(670–760)	−18.8 ± 0.2	10.0 ± 0.5
Arctozenus risso	US/DS	p	1316	5	167 ± 11	(150–180)	−19.1 ± 0.1	10.0 ± 0.5
Argyropelecus olfersii	US/DS	p	1316	5	79 ± 4	(75–85)	−18.9 ± 0.1	10.1 ± 0.3
Lampanyctus crocodilus	US/DS	p	2250	5	115 ± 7	(105–125)	−18.5 ± 0.1	10.6 ± 0.5
Notoscopelus kroeyeri	US/DS	p	496	4	120 ± 9	(110–130)	−18.8 ± 0.1	10.7 ± 0.1
Stomias boa	US/DS	p	1033	5	278 ± 25	(260–320)	−18.3 ± 0.1	11.6 ± 0.4
Notacanthus bonaparte	US/DS	bp	1010	5	326 ± 73	(260–450)	−17.3 ± 0.6	12.1 ± 1.0
Normichthys operosa	US/DS	bp	2250	5	141 ± 9	(130–155)	−17.9 ± 0.3	13.1 ± 0.1
CEPHALOPOD MOLLUSCS								
Octopus vulgaris	C/S	b	39	5	129 ± 40	(78–180)	−16.9 ± 0.6	11.1 ± 0.4
Eledone cirrhosa	C/S	b	144	42	85 ± 27	(27–145)	−16.8 ± 0.6	11.6 ± 0.6
Sepia orbignyana	C/S	bp	122	10	79 ± 15	(46–100)	−17.5 ± 0.3	10.8 ± 0.7
Sepia elegans	C/S	bp	100	25	43 ± 16	(22–73)	−17.2 ± 0.3	11.7 ± 0.6
Sepietta neglecta	C/S	bp	99	17	25 ± 6	(14–36)	−17.1 ± 0.5	12.1 ± 0.5
Alloteuthis spp	C/S	bp	127	13	43 ± 12	(26–63)	−17.7 ± 0.2	12.3 ± 0.3
Sepia officinalis (<90 mm ML)	C/S	bp	28	11	68 ± 11	(48–83)	−16.5 ± 0.4	12.6 ± 0.8
Sepia officinalis (⩾90 mm ML)	C/S	bp	35	42	167 ± 52	(90–264)	−16.7 ± 0.5	12.7 ± 1.2
Loligo vulgaris (<100 mm ML)	C/S	bp	47	16	77 ± 11	(54–97)	−17.4 ± 0.5	12.8 ± 1.0

(*continued on next page*)

Table 1 (*continued*)

Taxa and Species	HAD[a]	VAD[a]	Depth (m)[b]		Size (cm or mm)[c]		$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
			Mean	*N*	Mean ± SD	(min–max)	Mean ± SD	Mean ± SD
Loligo vulgaris (>100 mm ML)	C/S	bp	31	47	183 ± 50	(102–302)	−16.6 ± 0.6	13.9 ± 1.3
Sepiola atlantica	C/S	bp	21	8	16 ± 2	(13–18)	−16.3 ± 0.4	15.1 ± 0.7
Bathypolypus sponsalis	S/US	b	514	16	54 ± 13	(35–78)	−17.7 ± 0.2	10.7 ± 0.5
Octopus salutii	S/US	b	227	8	75 ± 21	(33–105)	−17.5 ± 0.5	11.3 ± 0.5
Rossia macrosoma	S/US	bp	278	7	34 ± 13	(22–50)	−17.3 ± 0.3	10.6 ± 0.6
Illex coindetii	S/US	bp	256	32	158 ± 33	(107–225)	−18.2 ± 0.3	11.6 ± 0.6
Loligo forbesi (<170 mm ML)	S/US	bp	113	24	83 ± 34	(39–169)	−17.8 ± 0.4	12.2 ± 0.6
Todaropsis eblanae	S/US	bp	171	19	113 ± 39	(59–186)	−18.1 ± 0.4	12.3 ± 0.9
Loligo forbesi (>170 mm ML)	S/US	bp	195	38	290 ± 99	(172–490)	−17.5 ± 0.7	13.0 ± 1.0
Todarodes sagittatus	S/US	p	403	36	253 ± 39	(191–405)	−17.9 ± 0.4	11.9 ± 0.7
Opisthoteuthis agassizii	US/DS	b	1081	3	310 ± 73	(240–385)	−18.4 ± 0.4	11.1 ± 0.1
Teuthowenia megalops	US/DS	p	1939	4	134 ± 12	(118–147)	−18.6 ± 0.4	8.8 ± 0.4
Galiteuthis armata	US/DS	p	1844	3	252 ± 91	(147–308)	−18.5 ± 0.3	10.1 ± 0.8
Ancistrocheirus lesueurii (juveniles)	US/DS	p	1627	3	33 ± 15	(21–49)	−19.6 ± 0.2	11.6 ± 0.6
Histioteuthis bonnellii (juveniles)	US/DS	p	1525	6	38 ± 17	(27–73)	−19.2 ± 0.2	11.7 ± 0.2
Histioteuthis reversa	US/DS	p	2076	7	54 ± 22	(30–87)	−19.2 ± 0.2	12.2 ± 0.4
OTHER INVERTEBRATES								
Bivalve Molluscs								
Aequipecten opercularis	C/S	b (SF[d])	29	5	61 ± 1	(59–63)	−16.3 ± 0.2	9.0 ± 0.1
Pecten maximus	C/S	b (SF[d])	40	8	115 ± 9	(100–130)	−15.5 ± 0.2	9.4 ± 0.4
Pecten maximus	S/US	b (SF[d])	171	3	113 ± 6	(110–120)	−17.9 ± 0.5	5.4 ± 0.3
Gastropod Molluscs								
Buccinum undatum	C/S	b	29	5	76 ± 4	(71–80)	−14.8 ± 0.2	11.1 ± 0.3
Scaphander lignarius	C/S	b	63	5	39 ± 15	(25–56)	−14.9 ± 0.2	11.4 ± 0.5
Scaphander lignarius	S/US	b	150	8	42 ± 6	(36–55)	−17.3 ± 0.6	7.3 ± 0.8
Buccinum humphreysianum	S/US	b	511	5	35 ± 3	(33–40)	−17.4 ± 0.2	10.0 ± 0.4
Crustaceans								
Mesozooplankton (200–2000 μm)	C/S	p	36	4	NA	NA	−21.1 ± 0.6	6.6 ± 1.3
Mesozooplankton (200–2000 μm)	S/US	p	329	4	NA	NA	−21.5 ± 0.3	6.4 ± 0.5
Alpheus glaber	C/S	b	60	5	43 ± 1	(42–44)	−16.5 ± 0.5	9.7 ± 0.3
Munida intermedia	C/S	b	47	5	58 ± 12	(43–74)	−17.4 ± 0.3	9.7 ± 0.3
Plesionika heterocarpus	C/S	b	221	5	82 ± 1	(79–83)	−17.1 ± 0.1	10.0 ± 0.4
Nephrops norvegicus	C/S	b	60	5	147 ± 11	(135–164)	−15.9 ± 0.2	11.3 ± 0.2
Polybius holsatus	C/S	b	60	5	42 ± 3	(40–47)	−16.5 ± 0.4	11.3 ± 0.7
Goneplax rhomboides	C/S	b	60	5	34 ± 2	(32–38)	−16.4 ± 0.1	11.3 ± 0.3
Liocarcinus depurator	C/S	b	60	5	48 ± 2	(46–50)	−16.2 ± 0.3	11.7 ± 0.7
Cancer pagurus	C/S	b	155	11	197 ± 9	(180–210)	−15.8 ± 0.4	12.1 ± 0.6
Crangon crangon	C/S	b	40	5	53 ± 4	(50–59)	−15.6 ± 0.4	12.1 ± 0.3
Crangon allmanni	C/S	b	60	5	54 ± 5	(48–59)	−15.9 ± 0.2	12.2 ± 0.3
Systellaspis debilis	US/DS	p	1860	5	56 ± 2	(54–60)	−18.5 ± 0.2	8.0 ± 0.2
Meganyctiphanes norvegica	US/DS	p	1873	5x3[e]	8 ± 0	(7–8)	−19.8 ± 0.2	8.3 ± 0.2
Sergia robusta	US/DS	p	1316	5	75 ± 5	(67–79)	−18.9 ± 0.2	8.8 ± 0.2
Ephyrina hoskynii	US/DS	p	1860	5	98 ± 3	(94–100)	−17.7 ± 0.2	9.5 ± 0.3
Gnathophausia ingens	US/DS	p	2250	5	130 ± 12	(115–149)	−18.4 ± 0.3	11.9 ± 0.5

NA = Not available.

[a] HAD and VAD = Horizontal and Vertical Axis of the Distribution. See affiliation in Material and Methods; distribution is assumed to be the habitat and/or the feeding area of species. C/S = Coastal/Shelf; S/US = Shelf/Upper Slope; US/DS = Upper Slope/Deep sea; b = benthic; bp = benthopelagic; p = pelagic.

[b] Corresponds to the depth under the research vessel at the end of trawling.

[c] Sizes given in cm for marine mammals, in mm for all other taxa; Total Length (TL) for most fish, gastropod molluscs and "shrimp type" crustaceans; Dorsal Mantle Length (DML) for most cephalopod molluscs; Standard Width (SW) for bivalve molluscs and "crab type" crustaceans. Exceptions are described below: *Trachyrincus scabrus, Polymetme thaeocoryla, Bathypterois dubius, Nezumia aequalis, Xenodermichthys copei, Benthosema glaciale, Ceratoscopolus maderensis, Bathylagus greyae, Myctophum punctatum, Arctozenus risso, Argyropelecus olfersii, Lampanyctus crocodilus, Notoscopelus kroeyeri, Stomias boa, Notacanthus bonaparte, Normichthys operosa*: Standard Length (SL) instead of Total Length; *Chimaera monstrosa*, *Hydrolagus mirabilis* and *Coryphaenoides rupestris*: Pre-Anal Fin Length (PAFL) instead of Total Length; *Opisthoteuthis agassizii*: Total Length (TL) instead of Mantle Length; *Meganyctiphanes norvegica*: Cephalothorax Length (CL) instead of Total Length.

[d] SF = suspension feeder.

[e] Corresponds to five pools of three individuals (muscle tissue only).

including marine mammals, both cartilaginous and bony fish, molluscs, and crustaceans (Table 1). Almost all organisms were collected during the EVHOE groundfish surveys conducted by the Institut Français de Recherche pour l'Exploitation de la Mer

(IFREMER), from the continental shelf to the shelf-edge of the French part of the Bay of Biscay in the autumns of 2001–2010. During these surveys, bottom and pelagic trawls were also performed in the canyons indenting the continental slope to specifically collect oceanic and deep-sea organisms. Species selected for seasonal variations in isotopic signatures (i.e., European pilchard and anchovy, see below) were also collected during PELGAS cruises conducted by IFREMER, from the continental shelf to the shelf-edge of the Bay of Biscay in the springs of 2008–2010. Finally, mammal samples came from stranded animals along the French Atlantic coast and were recovered and examined by members of the French Stranding Network between 2000 and 2009.

As many species switch their diets with increasing size and ontogenesis (Hjelm et al., 2000; Karpouzi and Stergiou, 2003; Chouvelon et al., 2011), the different species have to be compared at equivalent stages of their life histories (Jennings et al., 2001). Thus, only adult individuals were sampled among most of the species analysed. When both juveniles and adults or several size classes were available, they were treated separately (see Table 1). Finally, for some rare species, only juveniles were available and thus sampled (these exceptions are indicated in Table 1).

Each individual was measured and a piece of muscle (except mesozooplankton, which was analysed as a whole) was taken for isotopic analysis. Indeed, muscle is the reference tissue in food web studies inferred from stable isotope analyses (Hobson and Welch, 1992; Pinnegar and Polunin, 1999). It allows comparisons of isotopic signatures between individuals and taxa, minimizing inter-tissue differences in terms of biochemical and physiological properties like protein turnover rate and metabolic routing (Cherel et al., 2009). After collection, samples were immediately placed in individual plastic bags, frozen at −20 °C and freeze-dried. Freeze-dried tissues were finally ground into a fine powder and stored in individual plastic vials until further analysis.

2.2. Samples preparation and isotopic analysis

As lipids are highly depleted in ^{13}C relative to other tissue components (De Niro and Epstein, 1977), they were extracted from muscle samples using cyclohexane, as described by Chouvelon et al. (2011). Then 0.40 ± 0.05 mg subsamples of lipid-free powder were weighed in tin cups for stable isotope analyses. Isotopic analyses were performed with a Thermo Scientific Delta V Advantage mass spectrometer coupled to a Thermo Scientific Flash EA1112 elemental analyser. The results are presented in the usual δ notation relative to the deviation from standards (Pee Dee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$), in parts per thousand (‰). Based on replicate measurements of internal laboratory standards, the experimental precision is ±0.15 and ±0.20‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

2.3. Data treatment

Major groups of species (see Table 1) were firstly defined following taxonomic criteria (e.g., Actinopterygian vs. Chondrichthyan fish, or Bivalve vs. Gastropod vs. Cephalopod molluscs). Such taxonomic groups limited variations due to physiological and metabolic differences that can considerably impact isotopic fractionation between different types of consumers (McCutchan et al., 2003; Vanderklift and Ponsard, 2003; Caut et al., 2009). Moreover, this grouping limited variations linked to excessive morphological differences that directly impact general feeding habits (Karpouzi and Stergiou, 2003). In this way, Actynopterygians that showed a wide range of individual lengths were subdivided into two groups, "large Actynopterygians" (56 ± 20 cm length on average), and "small Actynopterygians" (20 ± 9 cm length on average).

The spatial distribution (which we assume to correspond to the habitat and/or the feeding zone) of the studied species was defined following the published literature (Lorance et al., 2000; Quéro, 2003; Palomares and Pauly, 2010), and published diet data of the area (Spitz et al., 2006a, 2006b, 2011), or derived from shipboard and aerial surveys in the area (Trenkel et al., 2009; Centre de Recherche sur les Mammifères Marins, La Rochelle, France, unpublished data). This spatial distribution was determined both on the vertical axis (i.e., pelagic, benthopelagic or benthic) and on the horizontal axis (i.e., from the coastline to the oceanic area: coastal/shelf, shelf/upper slope, upper slope/deep-sea/oceanic) for each species. These classifications were in accordance with the depth and the area where organisms were actually trawled in the Bay of Biscay during surveys (Table 1). Furthermore, they enable spatial variations in stable isotopic signatures (taking all species into account) to be assessed on both the horizontal and the vertical axis of the distribution (Figs. 2 and 3).

To evaluate the inshore–offshore gradient (horizontal axis) in $\delta^{13}C$ and $\delta^{15}N$ values in particular, General Linear Models (GLMs) were used, modelling the relationship between $\delta^{13}C$ and $\delta^{15}N$ values separately and the average trawling depth of species (i.e., the average depth under the research vessel at the end of trawling), considering only individuals trawled in the autumn (Table 1).

To assess spatial variations of isotopic signatures due to the potential influence of river inputs (Loire influence vs. Gironde influence, see Fig. 1), 10 species of fish and 7 species of cephalopods (inshore and offshore species) were selected, considering only individuals trawled in the autumn. They were trawled in both the northern part (continental shelf influenced by the Loire river

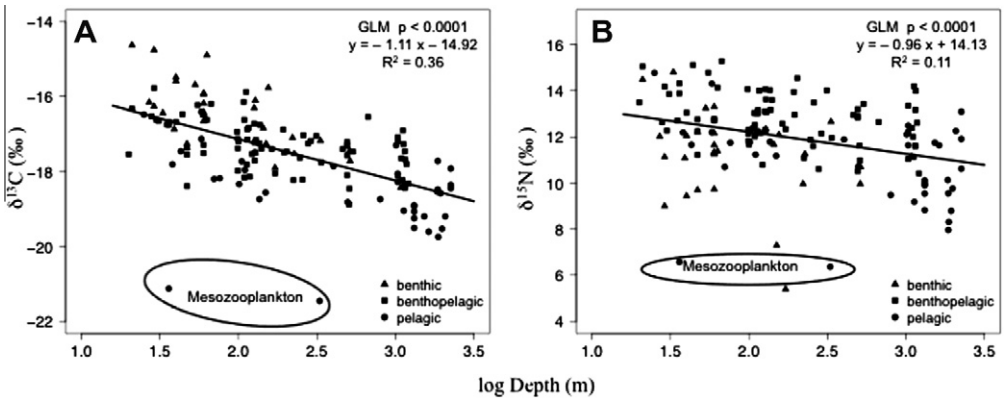

Fig. 2. Relationships (GLMs) between $\delta^{13}C$ (A) and $\delta^{15}N$ (B) values of species and depth of trawling (log-transformed values). Depth corresponds to the depth under the research vessel at the end of trawling. Marine mammals are not included, due to lack of depth associated with sampling (stranded animals).

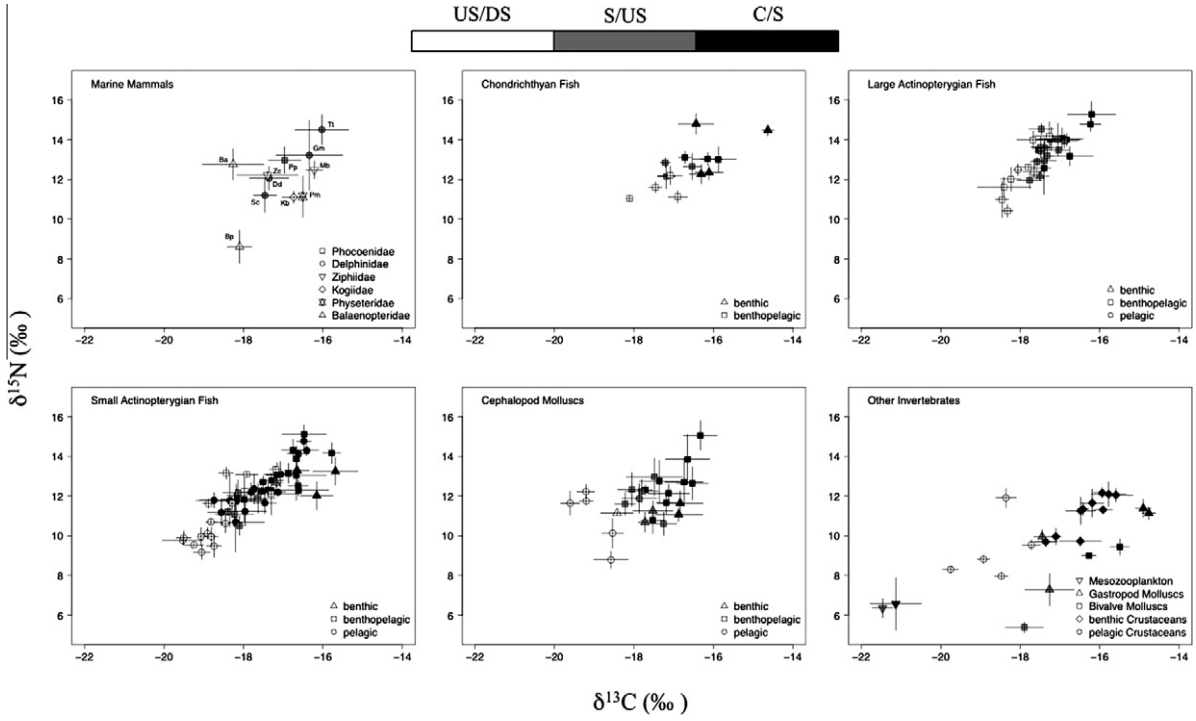

Fig. 3. δ^{13}C and δ^{15}N values (‰) for various taxa from the Bay of Biscay. Values are means ± Standard Deviation. Marine mammals: Bp = *Balaenoptera physalus*; Ba = *Balaenoptera acutorostrata*; Zc = *Ziphius cavirostris*; Mb = *Mesoplodon bidens*; Kb = *Kogia breviceps*; Pm = *Physeter macrocephalus*; Gm = *Globicephala melas*; Dd = *Delphinus delphis*; Sc = *Stenella coeruleoalba*; Tt = *Tursiops truncatus*; Pp = *Phocoena phocoena*. The same scale has been applied for all taxa, to facilitate the reading and comparisons between taxa. C/S = Coastal/Shelf; S/US = Shelf/Upper Slope; US/DS = Upper Slope/Deep sea (see details in Materials and methods).

inputs) and the southern part of the Bay of Biscay (continental shelf influenced by the Gironde river inputs) (Fig. 1). These selected species were analysed for their difference in δ^{13}C and δ^{15}N values as a function of the sampling zone (i.e., north vs. south), using a Student *t*-test or a Mann–Whitney–Wilcoxon test (depending whether the data satisfied the required conditions – normality and homogeneity of variances – for parametric statistics) (Table 2).

To assess inter-annual variations of isotopic signatures, four species of fish and four species of cephalopods were sampled each year in the autumns of 2005 or 2006–2010 (depending on species). Within each species, a narrow range of sizes was taken into account for comparison between years, to avoid potential distortion due to ontogenic effects (see comment above, and Tables 3 and 4). Also, when a difference between individuals trawled in the north of the Bay and those trawled in the south was previously revealed in some species, these zones were separated when they were both sampled in one year. As δ^{13}C and δ^{15}N values are generally correlated in marine ecosystems (Kelly, 2000), only δ^{15}N values (*a priori* proxy of trophic position) were tested for statistical difference between years, using a one-way analysis of variance (ANOVA) followed by a Tukey HSD multiple comparison test, or a Kruskal–Wallis (KW) followed by a multiple comparison test with the Holm adjustment method (depending whether the data satisfied the required conditions – normality and homogeneity of variances – for parametric statistics).

Finally, seasonal variations of isotopic signatures were tested in two species of small pelagic fish, the European pilchard *Sardina pilchardus* and the European anchovy *Engraulis encrasicolus*, sampled in the autumns and the springs of 2008–2010. As for inter-annual variations, a narrow and relatively same range of sizes was taken into account for comparison between seasons and years to avoid potential distortion due to ontogenic effects (*S. pilchardus*: 199 ± 16 mm Total Length; *E. encrasicolus*: 138 ± 24 mm Total Length). Again, only δ^{15}N values were tested for statistical difference between seasons within each year, using a Student *t*-test or a Mann–Whitney–Wilcoxon test (depending on whether the data satisfied the required conditions – normality and homogeneity of variances – for parametric statistics).

3. Results

3.1. Spatial isotopic variations

3.1.1. Inshore–offshore gradient

At the ecosystem scale (all species combined), isotopic signatures evidenced a gradient from coastal and neritic habitats to oceanic and deep-sea habitats: δ^{13}C and δ^{15}N values decreased from inshore to offshore organisms (Fig. 2). GLMs revealed a significant effect ($p < 0.0001$) of average trawling depth of species on average δ^{13}C and δ^{15}N values of species.

At a finer scale and when considering the major groups of species defined, the decrease in both δ^{13}C and δ^{15}N values from inshore to offshore organisms was consistent and accentuated, particularly for δ^{15}N values (Fig. 3). Moreover, the spread of signatures was the narrowest in higher trophic level consumers (i.e., large Actinopterygian fish, Chondrichthyan fish and marine mammals), intermediate in lower to medium trophic level consumers (i.e., small Actinopterygian fish and Cephalopod molluscs), and the widest in the lower trophic levels (i.e., other invertebrates) (Fig. 3).

Finally, at the species scale, very small coastal cephalopods or small coastal pelagic fish (lower to medium trophic level consumers) were particularly enriched in ^{15}N relative to some higher trophic level consumers from the same coastal/shelf habitat (e.g., *Sepiola atlantica*, *Hyperoplus lanceolatus*, *Atherina presbyter*, *Merlangius merlangus* < 35 cm) (Table 1). Individuals of *Pecten maximus* (Bivalve mollusc) and *Scaphander lignarius* (Gastropod mollusc)

Table 2
Muscle $\delta^{13}C$ and $\delta^{15}N$ values (‰) of species and individuals analysed for north–south difference in the Bay of Biscay, and results of the statistical tests performed. Within each species, a narrow range of sizes was taken into account for comparison (see table), to avoid potential distortion due to ontogenic effects (diet shift). Significant *p*-values are given in bold.

Habitat	Species	Depth (m)[a]	*N*		Size (mm)[b] Mean ± SD		$\delta^{13}C$ (‰) Mean ± SD			$\delta^{15}N$ (‰) Mean ± SD		
		Mean (Range)	North	South	North	South	North	South	*p-value*	North	South	*p-value*
CEPHALOPODS												
Benthic to benthopelagic species												
Coastal	*Sepia officinalis*	35 (21–134)	28	16	158 ± 48	171 ± 66	−16.5 ± 0.5	−16.9 ± 0.4	**0.002**	13.4 ± 0.7	11.6 ± 0.9	**<0.001**
↓	*Sepia elegans*	100 (40–152)	8	17	61 ± 11	34 ± 10	−17.1 ± 0.2	−17.2 ± 0.3	0.360	12.1 ± 0.5	11.5 ± 0.5	**0.012**
	Eledone cirrhosa	146 (43–650)	18	18	89 ± 25	94 ± 17	−16.7 ± 0.5	−16.7 ± 0.6	0.982	11.8 ± 0.7	11.6 ± 0.3	0.263
Oceanic	*Bathypolypus sponsalis*	514 (459–650)	8	8	52 ± 12	55 ± 14	−17.7 ± 0.1	−17.8 ± 0.2	0.467	10.5 ± 0.5	10.8 ± 0.4	0.282
Benthopelagic to pelagic species												
Coastal	*Loligo vulgaris*	33 (24–58)	32	23	159 ± 63	182 ± 51	−16.4 ± 0.5	−17.2 ± 0.4	**<0.001**	14.6 ± 0.7	12.5 ± 0.8	**<0.001**
↓	*Alloteuthis sp*	126 (122–130)	8	8	35 ± 13	43 ± 14	−17.6 ± 0.1	−17.8 ± 0.1	**0.038**	12.2 ± 0.1	12.4 ± 0.3	0.180
Oceanic	*Todarodes sagittatus*	394 (92–536)	8	23	245 ± 30	250 ± 20	−17.8 ± 0.2	−17.9 ± 0.5	0.619	11.9 ± 0.3	12.0 ± 0.9	0.672
FISH												
Benthic to benthopelagic species												
Coastal	*Trachinus draco*	40 (33–47)	5	5	238 ± 29	236 ± 9	−16.1 ± 0.2	−17.2 ± 0.7	**0.025**	14.2 ± 0.4	11.9 ± 0.5	**<0.001**
	Trisopterus minutus	114 (31–148)	34	25	175 ± 15	179 ± 11	−17.2 ± 0.3	−16.9 ± 0.5	0.054	12.8 ± 0.7	13.3 ± 0.4	**0.003**
↓	*Eutrigla gurnardus*	114 (101–122)	11	7	313 ± 55	309 ± 76	−16.8 ± 0.4	−16.9 ± 0.3	0.751	13.4 ± 0.4	12.8 ± 0.5	0.025
	Scyliorhinus canicula	126 (122–130)	5	5	588 ± 34	570 ± 29	−16.7 ± 0.2	−16.7 ± 0.1	0.600	12.9 ± 0.3	13.3 ± 0.2	0.124
Oceanic	*Lophius piscatorius*	193 (44–485)	10	8	568 ± 61	572 ± 88	−16.9 ± 0.4	−16.8 ± 0.2	0.626	13.9 ± 0.4	13.9 ± 0.2	0.969
Benthopelagic to pelagic species												
Coastal	*Trachurus trachurus*	99 (39–147)	39	25	197 ± 29	191 ± 18	−17.7 ± 0.4	−18.0 ± 0.3	**0.004**	12.8 ± 0.6	11.8 ± 0.9	**<0.001**
	Argentina sphyraena	99 (47–150)	5	5	198 ± 8	178 ± 8	−17.3 ± 0.1	−17.5 ± 0.2	0.148	12.3 ± 0.4	12.3 ± 0.3	0.923
↓	*Sardina pilchardus*	111 (33–166)	40	30	206 ± 18	212 ± 14	−18.0 ± 0.5	−18.1 ± 0.5	0.411	11.2 ± 0.6	11.0 ± 0.7	**0.037**
	Scomber scombrus	149 (147–150)	5	5	302 ± 8	290 ± 12	−18.7 ± 0.4	−18.4 ± 0.1	0.202	11.1 ± 0.9	11.3 ± 0.4	0.309
Oceanic	*Micromesistius poutassou*	221 (107–650)	57	20	184 ± 36	169 ± 34	−18.2 ± 0.5	−18.2 ± 0.4	0.599	11.0 ± 0.6	11.3 ± 0.7	0.150

[a] Corresponds to the depth under the research vessel at the end of trawling.
[b] Mantle Length (ML) for cephalopods, Total Length (TL) for fish.

trawled in the coastal/shelf habitat were also enriched in both ^{13}C (2.4‰ in both species) and ^{15}N (4‰ difference in both species) relative to individuals of the same species trawled in the shelf/upper slope habitat (Table 1).

3.1.2. North–south difference

There was a significant difference in both $\delta^{13}C$ and $\delta^{15}N$ values between individuals trawled in the north of the Bay and those trawled in the south among the four most coastal species only (i.e. *Sepia officinalis* and *Loligo vulgaris* for cephalopods, *Trachinus draco* and *Trachurus trachurus* for fish; Mann–Whitney–Wilcoxon or Student *t*-tests, $p < 0.05$) (Table 2). These significant differences were almost always in favour of enriched $\delta^{13}C$ (0.5‰ on average) and $\delta^{15}N$ values (1.2‰ on average) in individuals trawled in the north (Table 2). The only exception was *Trisopterus minutus*, whose individuals trawled in the north were 0.5‰ depleted in ^{15}N on average relative to individuals trawled in the south; however, standard deviations associated with average $\delta^{15}N$ values were relatively high (i.e., 12.8 ± 0.7‰ and 13.3 ± 0.4‰ in the north and south, respectively). Furthermore, $\delta^{15}N$ values were more frequently significantly different than $\delta^{13}C$ values between individuals trawled in the south of the Bay and those trawled in the north (8 species vs. 5 species out of 17 species analysed) (Table 2).

3.1.3. Pelagic–benthic difference

Excluding very small coastal ^{15}N-enriched species mentioned above, small pelagic fish (e.g., *E. encrasicolus*, *S. pilchardus*, *Scomber scombrus* or *T. trachurus* from neritic waters; *Xenodermichthys copei*, *Myctophum punctatum* or *Serrivomer beanii* from oceanic waters) generally displayed lower $\delta^{13}C$ and $\delta^{15}N$ values than small benthic or benthopelagic fish from the same areas (e.g., *Dicologlossa cuneata*, *Callionymus lyra*, *Lesueurigobius friesii* or *T. minutus* from neritic waters; *Polymetme thaeocoryla*, *Bathypterois dubius* or *Nezumia aequalis* from oceanic waters) (Table 1).

Moreover, in lower trophic level invertebrates, the benthic surface deposit feeder *S. lignarius* was enriched in ^{13}C and ^{15}N relative to the suspension feeder *P. maximus* and to relative the pelagic mesozooplankton in both coastal/shelf and shelf/upper slope habitats (Table 1). For instance, there was a 2‰ difference between $\delta^{15}N$ values of *S. lignarius* and *P. maximus* in both environments (Table 1).

3.2. Temporal isotopic variations

3.2.1. Inter-annual variations

There were some significant differences in $\delta^{15}N$ values from 1 year to another in both cephalopods and fish analysed in this respect, but these differences between some years did not follow any clear or consistent pattern among all species (Tables 3 and 4); that is, all species did not display $\delta^{15}N$ values that increased or decreased with time, or a consistent cycle of variations of these $\delta^{15}N$ values (Tables 3 and 4). When averaging all years sampled for each species, the coastal squid *L. vulgaris* displayed the highest $\delta^{15}N$ values, while the more oceanic squids *Illex coindetii* and *Todarodes sagittatus* presented lower $\delta^{15}N$ values (Table 3). In fish, the coastal and benthopelagic *T. minutus* displayed the highest $\delta^{15}N$

Table 3
Muscle δ^{15}N values (‰) of cephalopod species and individuals analysed for inter-annual variations of isotopic signatures in the Bay of Biscay, and results of the statistical tests performed. Within each species, a narrow range of sizes was taken into account for comparison (see table), to avoid potential distortion due to ontogenic effects (diet shift). Groups (same letter) indicate that years are not significantly different (post hoc Tukey test in the case of ANOVA, multiple comparison tests with Holm adjustment method in the case of Kruskal Wallis). Average δ^{15}N values over years, per species and/or per location, are given in bold.

Species	Zone	Year	*N*	δ^{15}N (‰) Mean ± SD (min–max)	Test and characteristics	Groups (post-hoc tests) a	b	c
Loligo vulgaris	North BB[a]	2008	11	15.0 ± 0.3 (14.6–15.4)	*t*-test; $t = 3.4$; $df = 3.9$	|		
		2009	4	14.2 ± 0.4 (13.7–14.6)	$p =$ **0.023**		|	
				14.6				
	South BB	2006	5	12.2 ± 0.2 (12.0–12.5)	KW	|		
		2008	3	12.8 ± 0.2 (12.6–13.1)	$\chi^2 = 2.7$; $df = 3$	|		
		2009	5	12.7 ± 0.8 (11.8–13.5)	$p = 0.448$	|		
		2010	6	12.7 ± 1.0 (11.4–14.2)		|		
				12.6				
Eledone cirrhosa	Whole BB	2006	5	11.0 ± 0.5 (10.2–11.4)	1-way ANOVA	|		
		2008	22	11.8 ± 0.3 (11.1–12.3)	$F = 17.2$; $df = 3$		|	
		2009	3	12.8 ± 0.5 (12.3–13.1)	$p <$ **0.001**			|
		2010	5	11.5 ± 0.4 (11.2–12.1)		|	|	
				11.8				
Illex coindetii	Whole BB	2005	5	12.2 ± 0.8 (11.1–13.1)	1-way ANOVA	|		
		2008	9	11.5 ± 0.2 (11.2–12.0)	$F = 5.6$; $df = 3$	|	|	
		2009	7	11.5 ± 0.4 (10.8–12.0)	$p =$ **0.005**		|	
		2010	5	11.1 ± 0.3 (10.8–11.4)			|	
				11.6				
Todarodes sagittatus	Whole BB	2006	6	12.4 ± 0.2 (12.2–12.7)	KW	|		
		2007	7	12.6 ± 0.9 (10.7–13.4)	$\chi^2 = 19.0$; $df = 4$	|		
		2008	6	11.5 ± 0.2 (11.2–11.8)	$p <$ **0.001**		|	|
		2009	7	11.9 ± 0.4 (11.3–12.4)		|	|	
		2010	4	10.7 ± 0.4 (10.3–11.1)				|
				11.8				

[a] BB = Bay of Biscay.

values, in comparison to the pelagic species *T. trachurus* and *S. pilchardus*, which presented lower δ^{15}N values, while the more oceanic *Micromesistius poutassou* finally presented the lowest values (Table 4).

3.2.2. Seasonal variations

In the European pilchard *S. pilchardus*, there was no significant difference in δ^{15}N values between individuals trawled in spring and those trawled in autumn, for the 3 years analysed in this respect (Student *t*-tests, $p > 0.05$, Fig. 4A). In the European anchovy *E. encrasicolus*, there was only a significant difference in δ^{15}N values between individuals trawled in spring and those trawled in autumn for the year 2008 (Student *t*-test, $p < 0.001$, Fig. 4B). However, the variability in δ^{15}N values was generally higher in individuals trawled in autumn compared to those trawled in spring, particularly in anchovy (Fig. 4).

4. Discussion

In open marine systems, the complexity of prey/predator fluxes increases, making it possible to define clear food web and ecosystem boundaries (Polis and Strong, 1996; Vander Zanden and Fetzer, 2007). Moreover, temperate systems such as the Bay of Biscay are often characterised by a mosaic of ecosystems supporting a high biodiversity able to feed on different sources. All of this may lead to higher difficulties in using ecological tracers such as stable isotopic ratios to study a global ecosystem's structure and functioning at meso-scale, in comparison to "simpler" ecosystems (e.g., polar ecosystems), that is, ecosystems subject to less variability in terms of sources, diet preferences of predators, or anthropogenic influences.

The knowledge of spatio-temporal variations driving stable isotopic signatures of organisms is thus essential to increase the robustness of the isotopic tool for studying such contrasted ecosystems. Using a wide range of taxa and species, our study principally highlights the potential of this tool in distinguishing the feeding zones of organisms from δ^{13}C values, as well as from δ^{15}N values (i.e., consistent inshore–offshore gradient) at the ecosystem scale. Moreover, the ^{15}N-enrichment of very small coastal species in particular implies a careful use of δ^{15}N values in the calculation of trophic levels' (see below). As for temporal variations, they did not appear to influence inter-specific comparisons of isotopic signatures at the ecosystem scale, despite evident intra-specific variations. These temporal variations were thus minor in comparison to spatial variations and their consequences for the use of stable isotope ratios in meso-scale and marine open ecosystem studies.

4.1. Spatial meso-scale drivers of isotopic signatures

At the ecosystem scale and on the horizontal axis of the distribution, δ^{13}C and δ^{15}N values of species varied greatly, decreasing considerably from inshore to offshore organisms (Figs. 2 and 3). At the species scale, δ^{13}C and δ^{15}N values of coastal species also varied with latitude, with individuals trawled in the north displaying significantly higher δ^{13}C and δ^{15}N values than individuals trawled in the south (Table 2). Furthermore, differences in δ^{15}N values between the different habitats (inshore vs. offshore, north vs. south) were likely to be higher than differences in δ^{13}C values. If this information is slightly distorted when considering all organisms analysed together (i.e., all trophic levels within a habitat or depth range, see Fig. 2), due to the fact that the TEF between sources and a consumer is higher in nitrogen (3.4‰ on average; Post, 2002) than in carbon ($\leqslant 1$‰), large variations in δ^{15}N values between habitats are highlighted when considering *a priori* similar trophic level species or single species (Fig. 3, Table 2).

Rather than being linked to variations in trophic structure and feeding habits between the different environments, such spatial differences in δ^{15}N values in particular may be more linked to

Table 4
Muscle δ^{15}N values (‰) of fish species and individuals analysed for inter-annual variations of isotopic signatures in the Bay of Biscay, and results of the statistical tests performed. Within each species, a narrow range of sizes was taken into account for comparison, to avoid potential distortion due to ontogenic effects (diet shift). Groups (same letter) indicate that years are not significantly different (post hoc Tukey test in the case of ANOVA, multiple comparison test with Holm adjustment method in the case of Kruskal Wallis). Average δ^{15}N values over years, per species and/or per location, are given in bold.

Species	Zone	Year	*N*	δ^{15}N (‰) Mean ± SD (min–max)	Test and characteristics	Groups (post hoc tests)	
						a	b
Trisopterus minutus	North BB[a]	2006	10	12.6 ± 0.5 (11.9–13.4)	KW	|	
		2007	5	12.4 ± 0.3 (11.9–12.9)	χ^2 = 8.6; *df* = 4	|	
		2008	5	12.5 ± 0.3 (12.2–12.9)	*p* = 0.072	|	
		2009	4	13.9 ± 0.2 (13.6–14.0)			|
		2010	10	13.0 ± 0.9 (11.9–14.2)		|	|
				12.9			
	South BB	2006	10	13.1 ± 0.3 (12.5–13.6)	1-way ANOVA	|	
		2007	5	13.9 ± 0.2 (13.8–14.3)	*F* = 10.1; *df* = 3		|
		2008	5	13.3 ± 0.2 (13.1–13.7)	*p* < **0.001**	|	
		2010	5	13.0 ± 0.4 (12.5–13.5)		|	
				13.3			
Trachurus trachurus	North BB	2006	10	12.4 ± 0.5 (11.8–13.3)	1-way ANOVA	|	
		2007	9	13.2 ± 0.4 (12.7–13.9)	*F* = 5.8; *df* = 4		|
		2008	6	13.3 ± 0.4 (12.8–13.8)	*p* = **0.001**		|
		2009	9	12.7 ± 0.6 (11.7–13.5)		|	|
		2010	5	12.5 ± 0.5 (11.8–13.2)		|	|
				12.8			
	South BB	2006	10	11.4 ± 0.7 (9.4–11.8)	KW	|	
		2007	5	13.1 ± 0.5 (12.4–13.6)	χ^2 = 11.3; *df* = 2		|
		2010	10	11.7 ± 0.5 (11.0–12.6)	*p* = **0.004**	|	
				12.1			
Sardina pilchardus	North BB	2006	3	11.5 ± 0.4 (11.1–11.7)	1-way ANOVA	|	
		2007	14	11.2 ± 0.7 (9.8–12.0)	*F* = 0.6; *df* = 4	|	
		2008	5	11.2 ± 0.7 (10.4–12.2)	*p* = 0.678	|	
		2009	11	11.4 ± 0.4 (10.9–12.2)		|	
		2010	7	11.1 ± 0.5 (10.5–11.9)		|	
				11.3			
	South BB	2006	20	11.1 ± 0.9 (9.9–13.1)	KW	|	
		2008	5	10.9 ± 0.3 (10.6–11.4)	χ^2 = 0.3; *df* = 2	|	
		2009	5	11.0 ± 0.3 (10.7–11.3)	*p* = 0.856	|	
				11.0			
Micromesistius poutassou	Whole BB	2006	29	10.9 ± 0.6 (9.3–12.0)	KW	|	
		2007	15	11.4 ± 0.8 (9.5–12.5)	χ^2 = 15.4; *df* = 2	|	
		2008	15	11.4 ± 0.5 (10.0–11.9)	*p* = **0.004**	|	
		2009	5	10.6 ± 0.7 (9.3–11.2)		|	
		2010	13	11.0 ± 0.5 (10.2–12.2)		|	
				11.1			

[a] BB = Bay of Biscay.

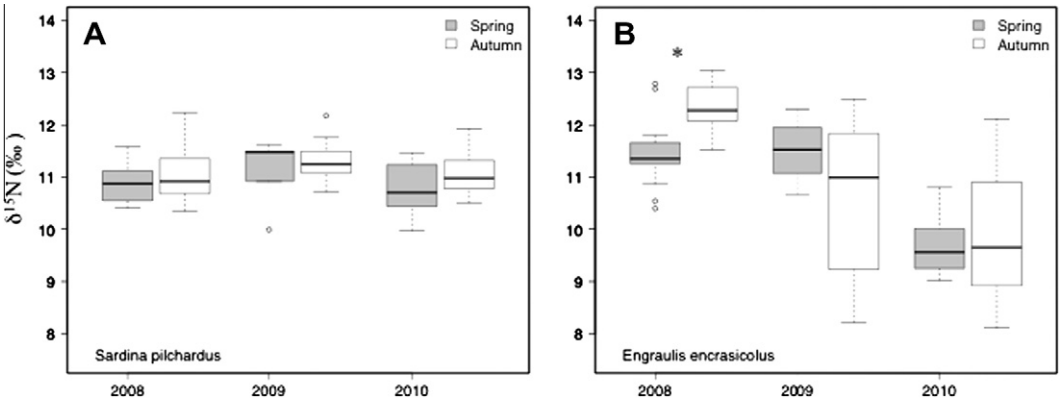

Fig. 4. Boxplots of muscle δ^{15}N values (‰) as a function of the season and year of sampling in fish species analysed for seasonal variations in isotopic signatures in the Bay of Biscay. (A) European pilchard *Sardina pilchardus*; (B) European anchovy *Engraulis encrasicolus*. Between 5 and 21 individuals have been analysed for each season within each year. The same scale has been applied for both species, to facilitate the reading and comparisons between them. * Indicates the only one significant difference between spring and autumn individuals (Student *t* test, $p < 0.05$).

processes occurring at the dissolved inorganic nitrogen (DIN) level, as described by Sherwood and Rose (2005) or Montoya (2007). Also, many processes can enrich δ^{15}N values of the available DIN pool in particular (see reviews by Sherwood and Rose, 2005; Montoya, 2007; and references therein), and the following general conclusions can be drawn: (1) when DIN demand is higher than the

supply of nutrients, primary producers may be faced with a δ^{15}N-enriched nitrogen source (e.g., "recycled" or-ammonium-enriched, especially if it comes from higher trophic levels), which is then reflected in the local food chain. Alternatively, during upwelling events in areas subject to this, the physical supply of "new" nutrients overwhelms the biological uptake rate, favouring δ^{15}N-depleted nitrogen sources for producers of this environment. Moreover, high primary production (blooms) during spring on the continental shelf reduces nutrient quantities, thus favouring δ^{15}N-enrichment of the available DIN. Even short in time, this effect may be lasting for benthic consumers in particular, due to the sinking of particles to the bottom; (2) rivers may be a vector of δ^{15}N-enriched organic matter into coastal waters as well, linked to δ^{15}N-enriched anthropogenic inputs derived from human waste for example (Fry, 1988; Hansson et al., 1997; McClelland et al., 1997; Vizzini and Mazzola, 2006).

In the particular case of the Bay of Biscay, it may be difficult to assess whether one mechanism or the other is more important. Both processes can be involved and the prevalence of one or the other can change temporally. Indeed, this ecosystem is characterised by contrasted hydrological landscapes, with regions under upwelling influence, regions largely under river plume influence, and intermediate areas (Koustikopoulos and Le Cann, 1996). Nevertheless, these landscapes vary greatly in their spatial extent seasonally and from 1 year to another. This is primarily due to the amount of river runoff and river plumes (i.e., the Loire and the Gironde), which also vary considerably over time according to the river regime (Planque et al., 2004; Puillat et al., 2004, 2006). Nutrient availability for primary production is thus highly dependent on these temporal variations as well (Herbland et al., 1998; Lunven et al., 2005), while they can strongly affect δ^{15}N values of primary producers, as commented above.

Our results are consistent with a prevalence of the influence of river discharges on δ^{13}C and especially δ^{15}N values (McClelland et al., 1997; Vizzini and Mazzola, 2006), followed by the potential influence of slope currents or upwellings, because: (1) very small coastal cephalopods or small coastal pelagic fish species are particularly enriched in ^{13}C and ^{15}N relative to their known predators in the area (e.g., small cetaceans; Spitz et al., 2006a, 2006b; Meynier et al., 2008); (2) only the more coastal species are affected by a significant difference in both δ^{13}C and δ^{15}N values when comparing north and south; (3) the pattern is consistent in almost all those coastal species (i.e., there is an enrichment of individuals trawled in the north of the Bay, under the Loire's influence, compared with individuals trawled in the south, under the Gironde's influence).

Finally, in open ecosystems such as the Bay of Biscay, the pelagic–benthic difference appears as the third factor influencing δ^{13}C and δ^{15}N values in consumers. Indeed, on the vertical axis of the distribution in a given area, there was some clear evidence of enrichment in ^{13}C and ^{15}N of species depending more on the benthic environment, in comparison to those depending almost exclusively on the pelagic environment (Table 1). In marine coastal environments, benthic algae are effectively enriched by 5‰ on average relative to phytoplankton (France, 1995). This is due to the differential carbon fixation and greater diffusion resistance by benthic algae, which present larger boundary layers in thickness and occur in lower a turbulence lentic system, finally resulting in more positive δ^{13}C values in these algae (see France, 1995, and associated references). The potential sinking of δ^{15}N-enriched particles to the bottom (see explanation above, and review by Sherwood and Rose, 2005) can also explain the higher δ^{15}N values displayed by benthic organisms, as well as their potential scavenger behaviour (e.g., necrophagous crustaceans).

However, this benthic–pelagic difference was only detectable in lower trophic level species, that is, benthic invertebrate' feeder fish vs. zooplankton feeder fish, or sub-surface deposit feeder/grazing gastropod molluscs and benthic crustaceans vs. suspension feeder bivalve molluscs and pelagic mesozooplankton (Table 2, Fig. 3). This may be due to the greater difficulty of correctly defining the trophic environment of higher trophic level consumers. Indeed, they are probably more mobile on the vertical axis of the distribution when foraging, and represent a greater mixture of sources than lower trophic level species.

4.2. Temporal meso-scale drivers of isotopic signatures

As river plumes (i.e., the Loire and the Gironde in the French part of the Bay of Biscay) seem to largely influence isotopic signatures from a spatial standpoint, one potential source of temporal variations in consumers' isotopic signatures should be the temporal variations of river plumes in the Bay of Biscay (Puillat et al., 2004, 2006). Indeed, the hydrological meso-scale variability (often associated with river discharges) has biological consequences, notably in terms of fish spawning areas survival of or eggs and larvae (Mion et al., 1998; Bellier et al., 2007).

However, species collected over several years did not follow a consistent pattern in the variations of δ^{15}N values over years. Such a consistent pattern would have suggested a possible change in the baseline over years. Also, if coastal fish species tended to be more affected (e.g., *T. minutus*, *T. trachurus*) than the less coastal species (e.g., *S. pilchardus*, *M. poutassou*) by inter-annual variations in isotopic signatures (Table 4), this was not the case in cephalopods (Table 3). In fact, there was no clear trend in cephalopods, or, on the contrary, only a slight decreasing trend 2005/2006 and 2010 in the more oceanic species *I. coindetii* and *Todarodes sagittatus*. Furthermore, if the absolute trophic position of a species could change over years, the average and relative trophic position of the species in the whole food web, and its affiliation to one or another habitat on both horizontal and vertical axes of the distribution was not impacted. Indeed, annual variations did not affect the discrimination of species' isotopic niche (as defined by Newsome et al., 2007) when all years of sampling were averaged within each species (Tables 3 and 4).

Thus, rather than being linked to an isotopic change in the baseline, inter-annual variations of the species' isotopic signatures may be more linked to an adjustment of the species facing variations in the food supply, to avoid competition with other species (e.g., Lefebvre et al., 2009b). Seasonal and inter-annual variations in pilchard and anchovy, both zooplankton feeders, also favour this theory. Indeed, if no significant difference between seasons was revealed in general, the spread of signatures in individuals sampled in autumn was often larger than that in individuals sampled in spring, particularly in anchovy (Fig. 4 B). When a type of food is very abundant (e.g., some mesozooplankton species following phytoplankton blooms in spring), individuals and/or species may tend to feed on and share the same overabundant prey, minimizing variations around the average isotopic signature within a species and/or isotopic differences between species.

Another hypothesis regarding such inter-individual and temporal variations at the species scale is the high mobility of these fish and cephalopod species (Nøttestad et al., 1999; Semmens et al., 2007): thus, we cannot exclude the feeding of some individuals and/or part of the population in different areas presenting different baseline signatures in δ^{15}N in the Bay of Biscay (see comment above, i.e., neritic vs. oceanic domain), particularly in autumn when food supply is less abundant in neritic waters (no blooms). For like-sized individuals, such an inter-individual difference in δ^{15}N values is effectively intriguing (more than 4‰ difference between individuals from autumns 2009 and 2010) (Fig. 4 B). Factors explaining this phenomenon in detail, at the individual and species scales, remain to be explored in the Bay of Biscay (e.g., different life

history traits, prey preferences, prey distribution and spatio-temporal variations of this distribution, etc.).

4.3. Implications and recommendations for further studies

This meso-scale study of spatio-temporal variations of isotopic signatures from various representative taxa of a complex open marine ecosystem revealed that spatial variations (principally due to river discharges influence) are greater than temporal variations (inter-annual and seasonal, at the species scale) in terms of implications for further studies on the structure and functioning of this type of marine system, even if confounding effects (spatio-temporal patterns combined) may obviously occur.

First, δ^{13}C and δ^{15}N values proved to be powerful indicators of the feeding zone on the horizontal axis of the distribution (i.e., evident inshore–offshore discrimination). This finding is of course to nuance for the more mobile species, such as marine mammal species. Indeed, for instance, some mammal species (e.g., *Globicephala melas*, *Kogia breviceps*, *Physeter macrocephalus*, *Ziphius cavirostris*) presented relatively high δ^{13}Cand δ^{15}N values, which we did not expect (Table 1, Fig. 3). Those species are however known to be deep diving foraging species which mostly feed on oceanic/deep-sea cephalopods (Spitz et al., 2011). Thus, isotopic signatures could suggest some incursions on the continental shelf by some of those species (Mèndez-Fernandez et al., 2012), foraging occasionally on more coastal and/or demersal species, as also demonstrated by the analysis of their stomach contents in the Bay of Biscay (Spitz et al., 2011). Secondly, to a lesser extent because the observation is only evidenced in lower trophic level species, both δ^{13}C and δ^{15}N values also distinguished between pelagic and benthic trophic environments.

All these results highlight the difficulty of assessing the feeding zone and diet of higher trophic level consumers (as well as highly mobile species) through stable isotopic signatures only and the necessity of combining them with other approaches and/or published data on species. At temperate latitudes, higher trophic level consumers effectively represent a greater mixture of sources (Chassot et al., 2008), and integrate all variations that may already affect lower trophic level consumers. This is well illustrated by the fact that the spread of both δ^{13}C and δ^{15}N values becomes very narrow in consumers with increasing trophic level (Fig. 3A–C vs. E–F).

Finally, the principal implication of the spatial variations revealed by this study is that δ^{15}N values may be revisited as an indicator of the feeding area as previously suggested by Hansson et al. (1997), Sherwood and Rose (2005), or Ménard et al. (2007) for other areas. This is especially important when considering the horizontal axis of the distribution. Up to now, δ^{15}N values are almost always only used as an indicator of the trophic position and as a basis for the calculation of absolute trophic levels' (Hobson and Welch, 1992; Lesage et al., 2001; Le Loc'h et al., 2008; Mèndez-Fernandez et al., 2012). Much more important is precisely what is implied when δ^{15}N values are used to calculate absolute trophic levels from a single baseline for a whole ecosystem. For instance, if the trophic levels of the sperm whale *P. macrocephalus* (δ^{13}C: -16.5 ± 0.0‰; δ^{15}N: 11.1 ± 1.0‰) and of the Atlantic bobtail squid *S. atlantica* (δ^{13}C: -16.3 ± 0.4‰; δ^{15}N: 15.1 ± 0.7‰) were calculated from the same δ^{15}N baseline, with a TEF of 3.4‰ per trophic level, we should conclude that the Atlantic bobtail squid is more than one trophic level higher relative to the sperm whale in the Bay of Biscay, which is total nonsense. This demonstrates that in such open and contrasted marine ecosystems, it is crucial to consider several baselines and to use an appropriate baseline for the different environments defined (on the horizontal axis of the distribution in particular in this Bay of Biscay case study) (Jennings and Warr, 2003; Barnes et al., 2009). Several authors have also argued for the use of primary consumers instead of primary producers and/or Particulate Organic Matter as baselines (Cabana and Rasmussen, 1996; Vander Zanden and Rasmussen, 1999; Post, 2002). Indeed, primary consumers – and especially sessile species – appear more appropriate to reflect spatial variations in the relatively long-term, contrary to primary producers, which are temporally highly variable (due to fluctuations in nutrient availability in particular) (Lefebvre et al., 2009a). The knowledge and the consideration of such spatial variations in δ^{15}N values on the horizontal axis of the distribution in particular also has important consequences for using stable isotopic ratios and/or derived trophic levels to correctly study, for instance, the transfer of contaminants in foods webs (Hobson et al., 2002; Dehn et al., 2006).

5. Conclusions

From the Bay of Biscay case study, spatial variations of isotopic signatures highlighted that δ^{15}N values vary with and clearly reflect the feeding area of organisms, which is usually expected from δ^{13}C values only. Thus, the calculation of trophic levels through δ^{15}N values in such a contrasted ecosystem should absolutely respect the following conditions: (1) the different environments of the ecosystem must be separated on the horizontal axis of the distribution in particular (i.e., coastal/shelf vs. shelf/upper slope vs. upper slope/deep sea/oceanic); (2) different baselines – representative of each environment – must be taken into account. However, in higher trophic level and highly mobile consumers, information derived from stable analysis should be combined with information derived from other approaches, to fully elucidate the trophic ecology of those organisms. Temporal variations suggested that when studying such an ecosystem using the isotopic tool, the sampling of species should be performed over a short time scale (e.g., at one season of one year). Nonetheless, for rare species, it may be possible to use individuals sampled over several years to obtain an average value for those species.

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