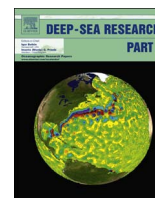




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Does upwelling intensity influence feeding habits and trophic position of planktivorous fish?



Lucia Lopez-Lopez^{a,*}, Izaskun Preciado^a, Isabel Muñoz^a, Moira Decima^b, Juan Carlos Molinero^c, Elena Tel^d

^a Spanish Institute of Oceanography. C.O., Santander, Spain

^b National Institute of Water and Atmospheric Research, Wellington, New Zealand

^c Helmholtz Centre for Ocean Research Kiel, Kiel, Germany

^d Spanish Institute of Oceanography. S.C., Madrid, Spain

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ABSTRACT

Food web configuration is shaped by many factors, including primary production patterns and oceanographic features, such as upwelling events. In this study, we investigate variability in the trophic position, food web interlinks and energy pathways of four planktivorous demersal fish in the Southern Bay of Biscay- NE Atlantic. The study area is exposed to upwelling events of varying intensity and shows a significant spatial gradient along the coast. The two sampling years were characterized by markedly different conditions, with weak summer upwelling in 2012 and an intense upwelling season in 2013. We used a complementary approach based on stomach content analysis (SCA) and stable isotope analysis (SIA) to test the effects of upwelling intensity and persistence on the food-web. In particular we investigated whether different intensities shift the main flow of energy between the pelagic and benthic energy pathways. We found conspicuous interannual changes in the isotopic POM, whose $\delta^{15}\text{N}$ was 2.5‰ lower during the productive 2013 season. Interannual changes in the isotopic signature of the fish species were also detected, although their values did not mirror variability at the basal level. The SCA results did not match the isotopic changes, which likely reflected dietary adjustments of the species during summer. The upwelling intensity gradient along the coast did not affect the nitrogen isotopic ratio of any group, however there was a significant effect of such gradient on the carbon isotopic ratio of the fish and euphausiid species. This effect was likely related to the higher primary production associated with intense upwelling conditions.

1. Introduction

Upwelling ecosystems contribute disproportionately to marine production, concentrating 20% of the world's marine fish catch in an area representing 5% of the global ocean (Chavez and Messié, 2009). While wind forcing on these systems is highly variable, the effect of temporal changes in upwelling intensity on trophic flows and local food webs remains largely unexplored (Chavez and Messié, 2009). Particular focus is currently centered on variability in the strength of benthic-pelagic coupling, linked to atmospheric forcing. For instance, demersal fish can shift their foraging from benthic to pelagic mode depending on upwelling intensity (as shown by *Acropoma japonicas*, Hamaoka et al., 2014), and primary production derived from strong upwelling events can be primarily assimilated by benthic carnivore decapods (Corbisier et al., 2014). This incipient body of literature suggests that variable upwelling intensity shapes trophic pathways and food web connection

nodes. This hypothesis, however, needs further support.

Food web studies have traditionally relied on stomach content analysis (SCA) to describe fish dietary habits and food web linkages, a straightforward method attempting to understand the trophic functioning of marine ecosystems. However, stable isotope analysis (SIA) has gained popularity over the last few decades, as a less time-consuming and complementary technique to identify these trophic links. SIA is based on the bioaccumulation of heavier isotopes along the food web, in a process called trophic fractionation (reviewed in Montoya (2007)). The use of nitrogen isotopes allows the estimation of consumer trophic level, as $\delta^{15}\text{N}$ usually increases by an average 3.4‰ from prey to predator (Post, 2002). Somewhat lower fractionation values are found at low trophic levels (Iken et al., 2005; Le Loc'h et al., 2008), however the 3.4‰ enrichment rate is routinely used in SIA studies. In contrast, the carbon isotope signature increases only 1–2‰ between diet and consumer (Wada et al., 1991), and therefore is

* Corresponding author. Present address: Centro Oceanográfico de Santander (IEO), Promontorio de San Martín s/n, 39004 Santander, Spain.
E-mail address: lucia.lopezlopez@gmail.com (L. Lopez-Lopez).

not useful to assess trophic ordering. However, it has proven useful to identify carbon sources of a consumer's diet. For instance, terrestrial primary production generally has a lower $\delta^{13}\text{C}$ values than its marine counterpart. This allows tracing the origin of carbon in the particulate organic matter pools of coastal environments (Riera and Richard, 1996; Bouillon et al., 2000). In addition, the composition of the phytoplankton community, which evolves along the primary production gradient, could also influence the $\delta^{13}\text{C}$ values due to the different fractionation of carbon isotopes among algal groups (Goericke et al., 1994). SCA gives an instantaneous and detailed description of diet, whereas SIA provides an estimation of the food source integrating diet over the medium term. This makes SCA quite comparable among different species and taxonomic groups, while SIA needs to consider tissue turnover rates to estimate the lag between dietary changes and response of the isotopic signature. Coupling of SCA and SIA provides a comprehensive picture of fish trophic relations by combining an instantaneous record of diet with the isotopic information, which reflects the food assimilated by an organism during an extended time preceding the sampling (Pasquaud et al., 2008; Preciado et al., (in Press)).

The northern edge of the Eastern North Atlantic upwelling system is subject to seasonal upwelling events of variable interannual intensity (Alvarez et al., 2011; Gonzalez-Nuevo et al., 2014), and a marked intensity gradient along the coast (Alvarez et al., 2011). Recent studies suggest that marine isoscapes experience long-term stability, reflecting their main hydrographic features (MacKenzie et al., 2014), but strong changes in hydrography may modify the isotopic baseline (Waite et al., 2007; Decima et al., 2013). The upwelling conditions in this area offers an excellent opportunity to test the flexibility of the food web under varying upwelling intensity. We address this question by investigating both spatial gradients (along the coast), and temporal variability, by comparing data from years characterized by contrasting upwelling intensities. To this aim, we target demersal species, which play a pivotal role by utilizing both benthic and pelagic resources potentially shifting the main energy channel in the ecosystem (Woodland and Secor, 2013).

The overall aim of this study is to test the effect of the intensity and persistence of upwelling conditions on a temperate shelf sea food-web, using demersal planktivorous fish, and to elucidate whether such effect stems from isotopic changes at the basal level, changes in the trophic structure, or both. In addition, using carbon isotopes we aim to determine whether different upwelling intensities shift the main flow of energy between the pelagic and benthic energy pathways.

2. Material and methods

2.1. Study area

The Northwest of the Iberian Peninsula (Fig. 1) in the southern edge Bay of Biscay, is a temperate shelf sea in the northern edge of Northeast Atlantic upwelling system. The small rivers discharging into the area have limited influence on the regional oceanography, excepting the Miño river, which enters the sea outside the southern edge of the study area. Nevertheless, numerous large coastal embayments exist in the southern part of the study area, whose outflow deposits the muddy sediments present in the inner and central shelf areas (Lopez-Jamar et al., 1992). During the summer, the regional oceanography is dominated by westerly winds causing upwelling with intensities generally decreasing from West to East (Gil, 2008; Alvarez et al., 2011). The upwelled water (Eastern North Atlantic Central Water) contributes new nutrients to the surface mixed layer (Alvarez-Salgado et al., 2002) boosting primary production (Tenore et al., 1995). During autumn, however, mesoscale features dominate the regional oceanography (Gil, 2008) configuring a highly heterogeneous pelagic environment (Lopez-Lopez et al., 2014). This continental shelf sea fosters a complex ecosystem with a well developed food web (Sánchez and Olaso, 2004).

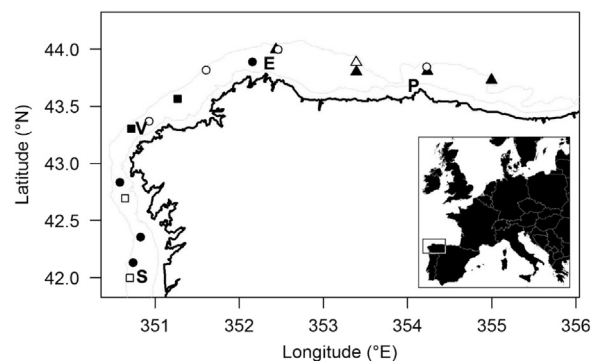


Fig. 1. Map of the study area showing sampling points in 2012 (solid symbol) and 2013 (open symbol). Local oceanographic conditions, i.e. upwelling conditions (square), stratification (circle) and anticyclonic eddies (triangle) are indicated. Buoys measuring upwelling intensity are marked by their first letter. Isobaths of 100 and 200 m are shown by grey lines.

2.2. Sampling design

Sampling was performed along a transect parallel to the coast in the central continental shelf, ~140–190 m depth (Fig. 1) between 20th September and 15th October of 2012 (10 sampling stations) and 2013 (7 sampling stations). We used as sampling platform the International Bottom Trawl Survey (IBTS) for the assessment of demersal and benthic ecosystem in the Galicia continental shelf and Cantabrian Sea (detailed survey information in Sánchez and Serrano (2003)). At each station a CTD (Seabird 25) was deployed to describe the water column characteristics. Fish and euphausiids were collected along the transect using a bottom trawl net with 20 mm mesh size in the cod-end towed during 30 min at 3 knots. Sediment samples were collected using a sediment collector attached to the ground rope of the trawl and a sample of subsurface water at 5 m. depth was obtained from the ship's water intake.

We targeted four species of fish, with demersal habitats and known planktivorous diet, for the experiment: silvery pout (*Gadiculus argenteus*), boar fish (*Capros aper*) and juveniles of European hake (*Merluccius merluccius*) and blue whiting (*Micromesistius poutassou*). We tried to keep consistency in the size of fish sampled in both years, however juvenile *M. poutassou* size was larger in 2013 than in 2012 (Table 1). Additionally, as euphausiids constituted an important trophic resource in the pelagic food web of the study area, we took samples of euphausiid muscle in the stations where it was present. *Meganyctiphanes norvegica* was directly sampled from the catch but *Nyctiphanes couchii* escapes the net due to its small size and needed to be biosampled, i.e. obtained from freshly ingested stomach contents of *M. poutassou*.

Table 1

Total length range of the four fish studied and number of samples used in stomach content analysis (SCA) and stable isotope analysis (SIA) in 2012 and 2013.

Species	Method SCA			Method SIA	
	Year	Size range (cm)	N Samples	Size range (cm)	N samples
<i>Micromesistius poutassou</i>	2012	12–18	70	12–14	21
	2013	18–21	27	18	15
<i>Merluccius merluccius</i>	2012	10–17	113	10–14	27
	2013	7–17	67	7–14	21
<i>Gadiculus argenteus</i>	2012	5–9	71	5–9	21
	2013	6–9	40	6–9	12
<i>Capros aper</i>	2012	5–8	78	5–8	24
	2013	6–9	60	6–9	21

2.3. Oceanographic parameters

We categorized the study stations into three types, based on water column characteristics inferred from CTD profiles, i.e. stratified, upwelling or anticyclonic eddy. These features are recurrent in the area during autumn (Gil, 2008) and were classified following the guidelines of Lopez-Lopez et al. (2014).

Upwelling data were obtained from wind shear speed at four buoys located in the study area: Silleiro, Vilano, Estaca de Bares y Cape Peñas (buoys belonged to Puertos del Estado, Spanish Government). The upwelling index (UI) was processed by the Spanish Oceanographic Institute (available at www.indicedeafloramiento.es) and expressed as flow by lineal kilometer of coast ($\text{m}^3 \text{s}^{-1} \text{km}^{-1}$). Previous studies have successfully used the UI to explain the dynamics of biological resources in the study area (Gonzalez- Nuevo et al., 2014).

2.4. Stable isotopes analyses

Carbon and nitrogen isotopic values were obtained by assaying at least three individuals per species, within a narrow size range (Table 1), at each station. Each specimen was measured and a tissue sample from the dorsal white muscle was dissected. Samples were frozen at -20°C prior to subsequent analyses.

To characterize the isotopic baseline, we sampled particulate organic matter in subsurface water (POM) and in the sediment (SOM). These samples were taken in triplicate, with the exception of POM in 2012, for which one only sample was taken per sampling station. POM samples were obtained from subsurface water (approx. 5 m depth), filtered through 200 μm mesh to eliminate large particles that could bias the measures, and then filtered through a pre-combusted Whatman GF/F until clogged. The filters were stored at -20°C for subsequent analyses. In the laboratory, the filters were thawed and carefully split into two subsamples to determine carbon and nitrogen isotopic ratios independently. The samples aimed for carbon analyses were placed in a vacuum desiccator with a beaker of concentrated HCl (37%) for 24 h and re-dried four times to remove excess of HCl, then placed in a drying oven for 48 h at 60°C . The samples aimed for nitrogen analysis were oven dried for 48 h at 60°C . Both sets of samples were packed into tin capsules for analysis.

Sediment samples were collected using a sediment collector attached to the ground rope of the trawl and freeze stored at -20°C on board. In the laboratory, they were dried for 48 h at 60°C and grounded and sieved (45 μm mesh size) after eliminating visible shell pieces and large pebbles. Three replicates of 20 mg for each sample station were encapsulated for nitrogen isotopic analyses. Three more replicates were acidified for removing traces of inorganic carbonates treating ca. 10 mg of sample with 1 mL of concentrated HCl (37%) for 24 h. Samples were subsequently centrifuged and rinsed with de-ionized water 3 times to remove excess of HCl, dried for 48 h at 60°C and encapsulated.

Isotopic analyses were performed at the Servicios Xerais de Apoio á Investigación (SXAIN, Universidade da Coruña, Spain) using a continuous flow isotope ratio mass spectrometry FlashEA1112 elemental analyzer (ThermoFinnigan, Italy) coupled to a Deltaplus Mass spectrometer (FinniganMat, Germany) through a ConFloII interface. The carbon and nitrogen stable isotopes ratio was expressed as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ parts per mille (‰) with a precision (standard deviation) of $\pm 0.15\text{‰}$ (1-sigma, $n=10$).

Isotopic data of each species were compared between years using a Student's *t*-test, or Kruskal-Wallis test for non-parametric distributions. The isotopic signature of POM was also related to the local oceanographic conditions in each sampling station using ANOVA. Trends in the isotopic signal along the sampling transect were explored fitting a linear regression to the data.

2.5. Stomach content analyses

Ten individuals of each target species were randomly chosen from each haul, measured and set aside for extended biological sampling. The stomach contents of juvenile hake and blue whiting diet were analyzed onboard. Boar fish and silvery pout were immediately frozen and analyzed in the laboratory, since their diet identification was thought to be more tedious with higher diet breadth and smaller prey (Macpherson, 1978; Lopes et al., 2006). The procedures to investigate fish prey items at sea and in the laboratory were identical. Quantitative diet estimates were obtained by measuring the stomach content volume using a trophometer (Olaso, 1990). Prey were separated and identified to the lowest possible taxonomic level, and the number of each prey category was recorded. Fresh prey or any items presumably consumed in the net were excluded from the analysis.

Diet diversity for each fish species was calculated using the Shannon- Wiener diversity index. We assessed diet overlap quantitatively, because numeric data can be misleading without considering prey weight or volume, and is generally considered a poor descriptor of this metric (Wallace, 1981). Species diet between years was compared using Wilcoxon signed-rank test.

3. Results

3.1. Regional oceanography

The oceanographic conditions differed among sampling stations, and included stratified water column, upwelling, and anticyclonic eddy, both in 2012 and 2013 (Fig. 1).

Differences in upwelling intensity between 2012 and 2013 were outstanding, with significantly higher intensities during 2013 ($T=-21.524$, $p < 0.001$) (Fig. 2), which persisted throughout the entirety of the season.

Spatial patterns in upwelling were additionally investigated, by considering average values during summer. We found upwelling intensity decreased from Southwest to Northeast along the sampling transect, from $306 \text{ m}^3 \text{ s}^{-1} \text{ km}^{-1}$ (Silleiro) and $117 \text{ m}^3 \text{ s}^{-1} \text{ km}^{-1}$ (Vilano), to $-166 \text{ m}^3 \text{ s}^{-1} \text{ km}^{-1}$ (Estaca de Bares) and $31 \text{ m}^3 \text{ s}^{-1} \text{ km}^{-1}$ (Cape Peñas), during 2012. Patterns in 2013 were somewhat similar, with less marked decreases from Southwest to Northeast due to the higher upwelling intensity. The Southwest stations average values reached $589 \text{ m}^3 \text{ s}^{-1} \text{ km}^{-1}$ (Silleiro) and $414 \text{ m}^3 \text{ s}^{-1} \text{ km}^{-1}$ (Vilano), to $427 \text{ m}^3 \text{ s}^{-1} \text{ km}^{-1}$ (Estaca de Bares) and $136 \text{ m}^3 \text{ s}^{-1} \text{ km}^{-1}$ (Cape Peñas).

3.2. Feeding ecology using SIA

$\delta^{15}\text{N}$ values of the fish species clustered approximately 6‰ over the SOM basal level regardless of the year, but the distance to the POM basal level varied from 4.7‰ in 2012 to 7.1‰ in 2013 (Fig. 3a, b; Table 2). Blue whiting, hake and silvery pout values were statistically similar while boar fish was significantly higher than the others (Tukey HSD test, $p < 0.001$). In contrast, $\delta^{13}\text{C}$ values were not different among the four fish species (Fig. 3). The $\delta^{15}\text{N}$ values of euphausiids displayed an intermediate position between the basal level and the value of fish species (Fig. 3), 3.9‰ higher than SOM, and 2.6–5.1‰ above the POM isotopic baseline.

POM isotopic values were lower in 2013 for both carbon in nitrogen, with $\delta^{13}\text{C}$ values 0.9‰ ($T=2.41$, $df=12.93$, $p < 0.05$) and $\delta^{15}\text{N}$ 2.5‰ lower in 2013 ($T=4.19$, $df=13.18$, $p < 0.01$). The isotopic composition of the SOM basal level remained constant during both years, while no relationship between the oceanographic conditions at the sampling point and the isotopic signature of the POM were identified (Fig. 4).

We found interannual differences in fish isotopic content, yet the patterns were not consistent among species (Fig. 3; Table 2). Blue whiting $\delta^{15}\text{N}$ values were 0.5 units higher in 2013 than 2012 ($T=-3.01$,

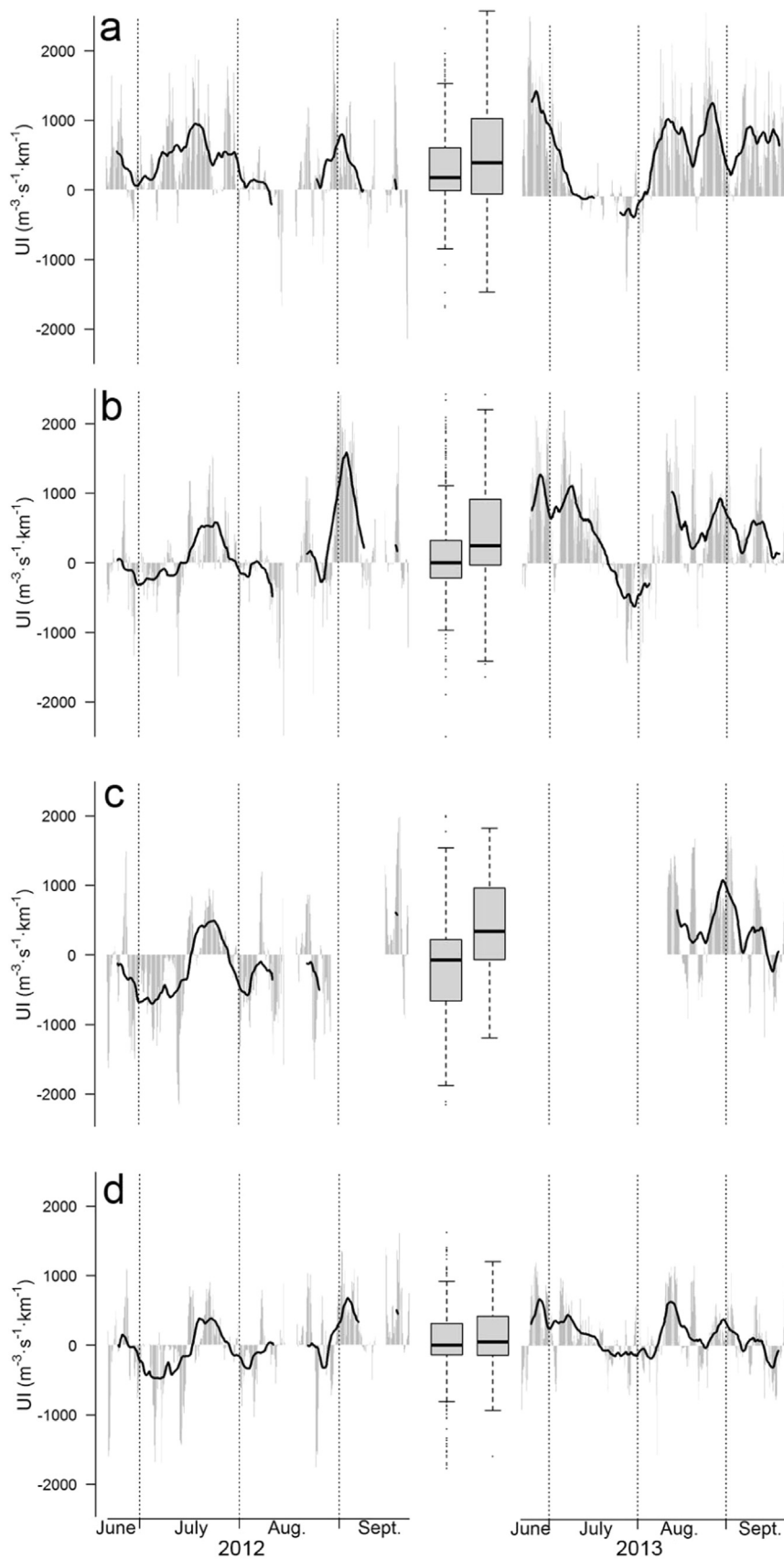


Fig. 2. Upwelling index during the summer months of 2012 and 2013 from buoy data at Silleiro (a), Vilano (b), Estaca (c) and Peñas (d). Each figure includes the daily upwelling during the summer season in 2012 and 2013, with the weekly mean superimposed as a solid line. Data from both years are separated by two boxplots which summarize the upwelling each summer. See Fig. 1 for their locations on the map.

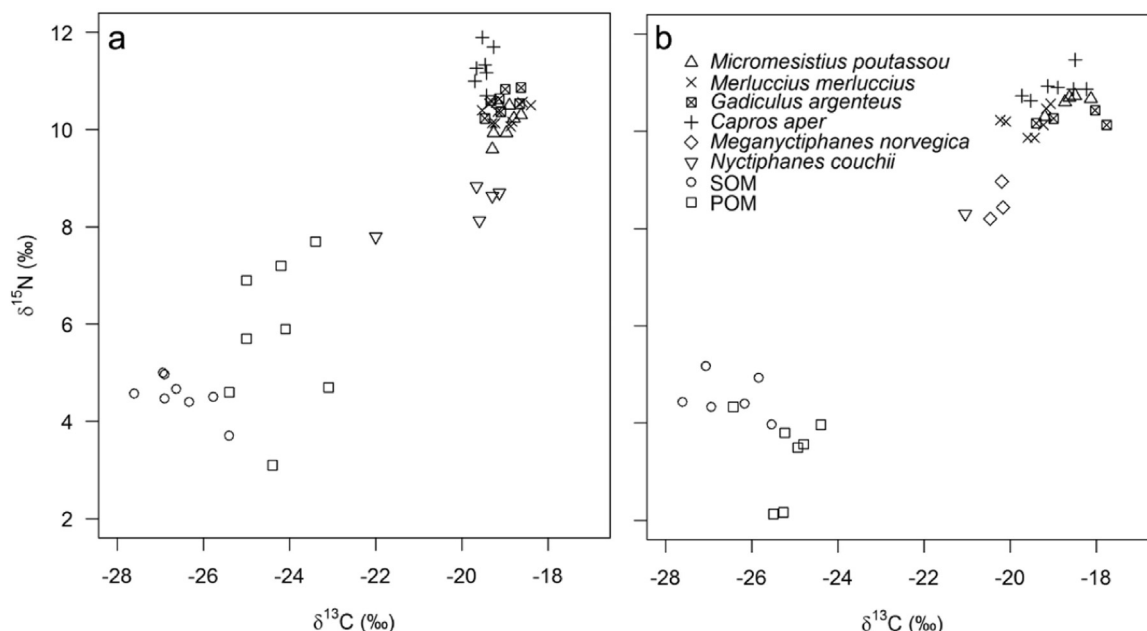


Fig. 3. Scatterplot showing the isotopic composition of the four studied species, two of its prey (euphausiid species) and the isotopic baseline in 2012 (a) and 2013 (b). Each species is identified with a different symbol, see legend in figure (b).

Table 2

Isotopic signature ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of the basal samples (POM and SOM) and all species analyzed.

	$\delta^{15}\text{N}$		$\delta^{13}\text{C}$	
	2012	2013	2012	2013
POM	5.83 ± 1.48	3.35 ± 0.87	-24.32 ± 0.80	-25.22 ± 0.64
SOM	4.53 ± 0.40	4.54 ± 0.44	-26.56 ± 0.71	-26.48 ± 0.74
<i>M. norvegica</i>	8.53 ± 0.39	8.53 ± 0.39	-20.28 ± 0.16	-20.28 ± 0.16
<i>N. couchii</i>	8.42 ± 0.44	8.3	-19.94 ± 1.17	-21.05
<i>C. aper</i>	11.22 ± 0.43	10.91 ± 0.27	-19.49 ± 0.14	-18.94 ± 0.56
<i>M. merluccius</i>	10.32 ± 0.20	10.19 ± 0.27	-19.03 ± 0.38	-19.55 ± 0.46
<i>M. poutassou</i>	10.15 ± 0.34	10.6 ± 0.18	-19.01 ± 0.25	-18.64 ± 0.39
<i>G. argenteus</i>	10.58 ± 0.23	10.25 ± 0.14	-19.05 ± 0.31	-18.55 ± 0.76

df=9.34, $p < 0.05$), in turn silvery pout was 0.3‰ lower in 2013 ($T=3.01$, $df=8.93$, $p < 0.05$), and $\delta^{13}\text{C}$ values showed no interannual variability in these two species. Hake and boar fish, however, displayed interannual differences only in their $\delta^{13}\text{C}$ values. The $\delta^{13}\text{C}$ of hake was

depleted by 0.5‰ in 2013 ($T=2.40$, $df=11.59$, $p < 0.05$), while the opposite pattern was observed for boarfish, with average $\delta^{13}\text{C}$ values ca. 0.5 units higher ($T=-2.55$, $df=6.66$, $p < 0.05$) in 2013 compared with 2012. The largest difference in carbon isotopic content we observed was at the basal level, with POM values differing notably, by up to 1‰ lower in 2013 (Fig. 3, Table 2).

The carbon isotope ratio along the sampling transect followed a similar pattern for all fish species, with an overall decrease following the Southwest-Northeast direction in 2013, which was also evident in 2012 for juvenile hake and blue whiting (Fig. 5). This pattern in $\delta^{13}\text{C}$ values along the transect was larger than 1‰ in all four fish species in 2013, while in 2012 it was moderate (Fig. 5). A similar decreasing pattern was observed when we combined the data available for euphausiids in 2012 and 2013. This trend, however, was not detected in the POM and SOM samples, which displayed no pattern in $\delta^{13}\text{C}$ values along the transect gradient. There was no general pattern in $\delta^{15}\text{N}$ values along the transect, although silvery pout displayed a significant decrease along the Southwest-Northeast sampling direction in 2012 (Fig. 6).

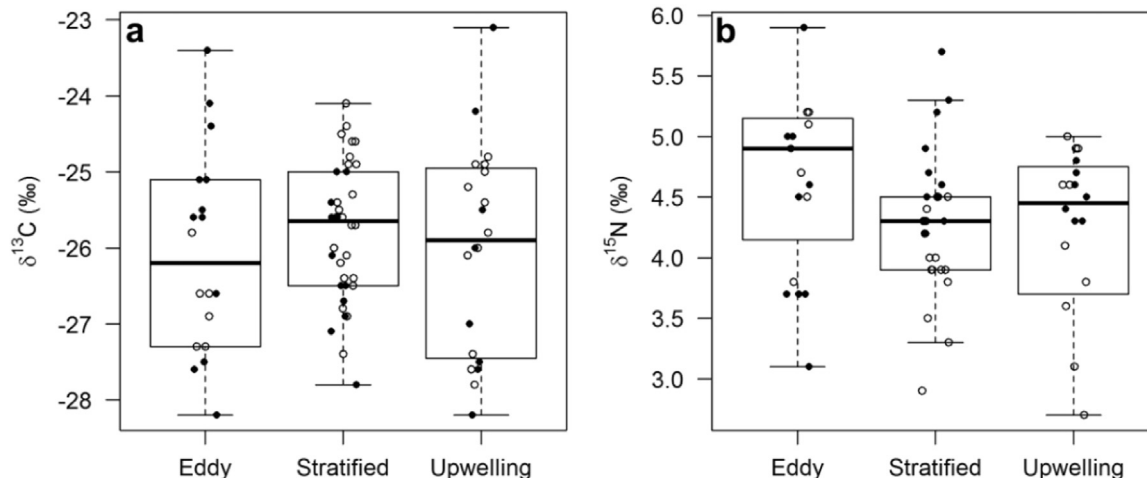


Fig. 4. Isotopic carbon (a) and nitrogen (b) ratios in the POM under the different oceanographic conditions encountered in the study area. Individual observations from 2012 (solid points) and 2013 (open points) are marked inside each box.

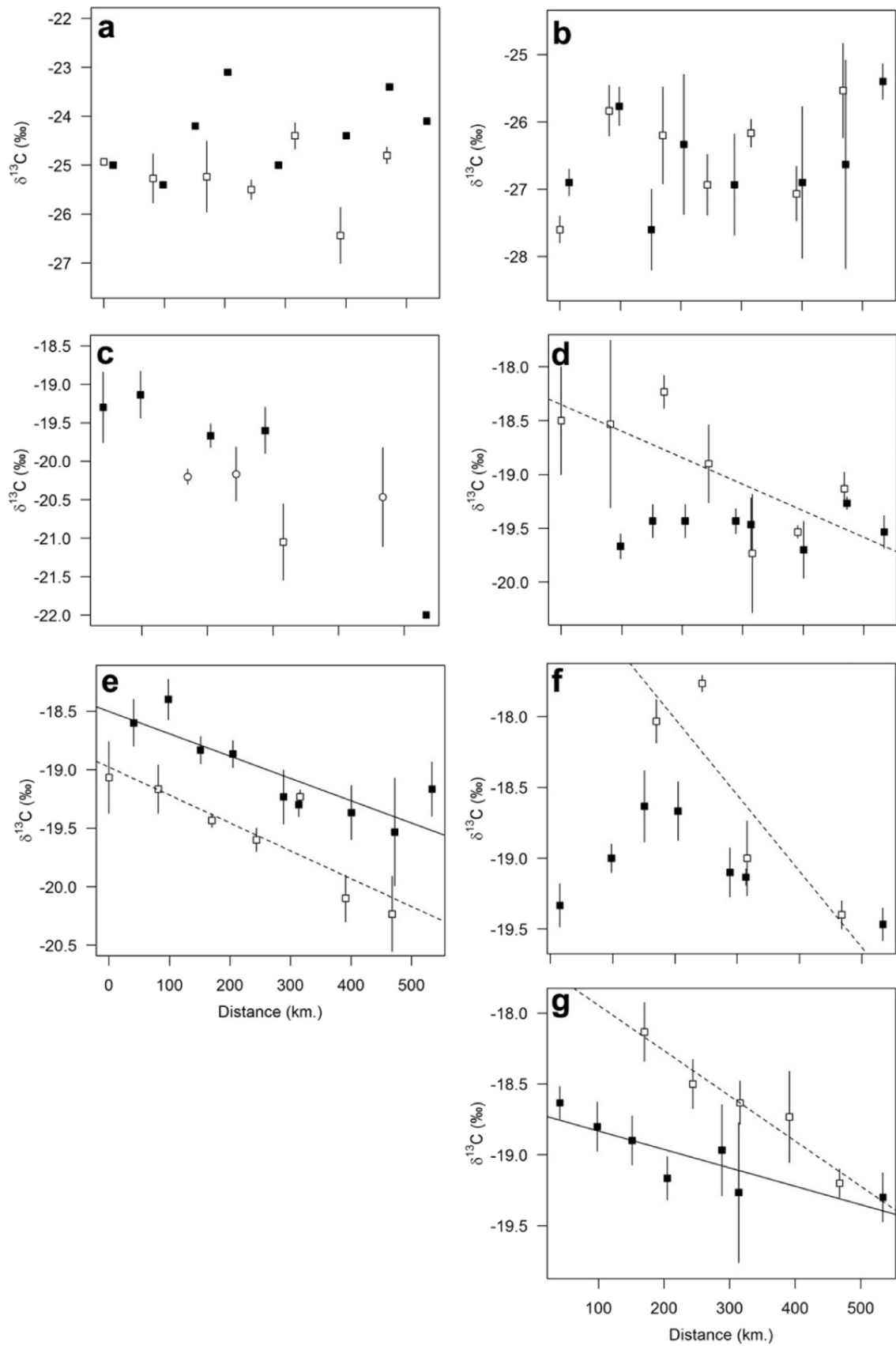


Fig. 5. Isotopic carbon ratio from SW to NE along the sampling transect in 2012 (solid symbol) and 2013 (open symbol) for (a) POM, (b) SOM, (c) euphausiids, (d) *Capros aper*, (e) juvenile *Merluccius merluccius*, (f) *Gadicus argenteus* and (g) juvenile *Micromesistius pouassou*. Within the euphausiids (c) *Nyctiphanes couchii* and *Meganyctiphanes norvegica* are indicated by square and circles respectively. Regression lines was added to the plot, as a solid line for 2012 data and/or a dashed line for 2013 data, if trends were statistically significant ($p < 0.05$) the. Note that the y-axis' scale differs among figures.

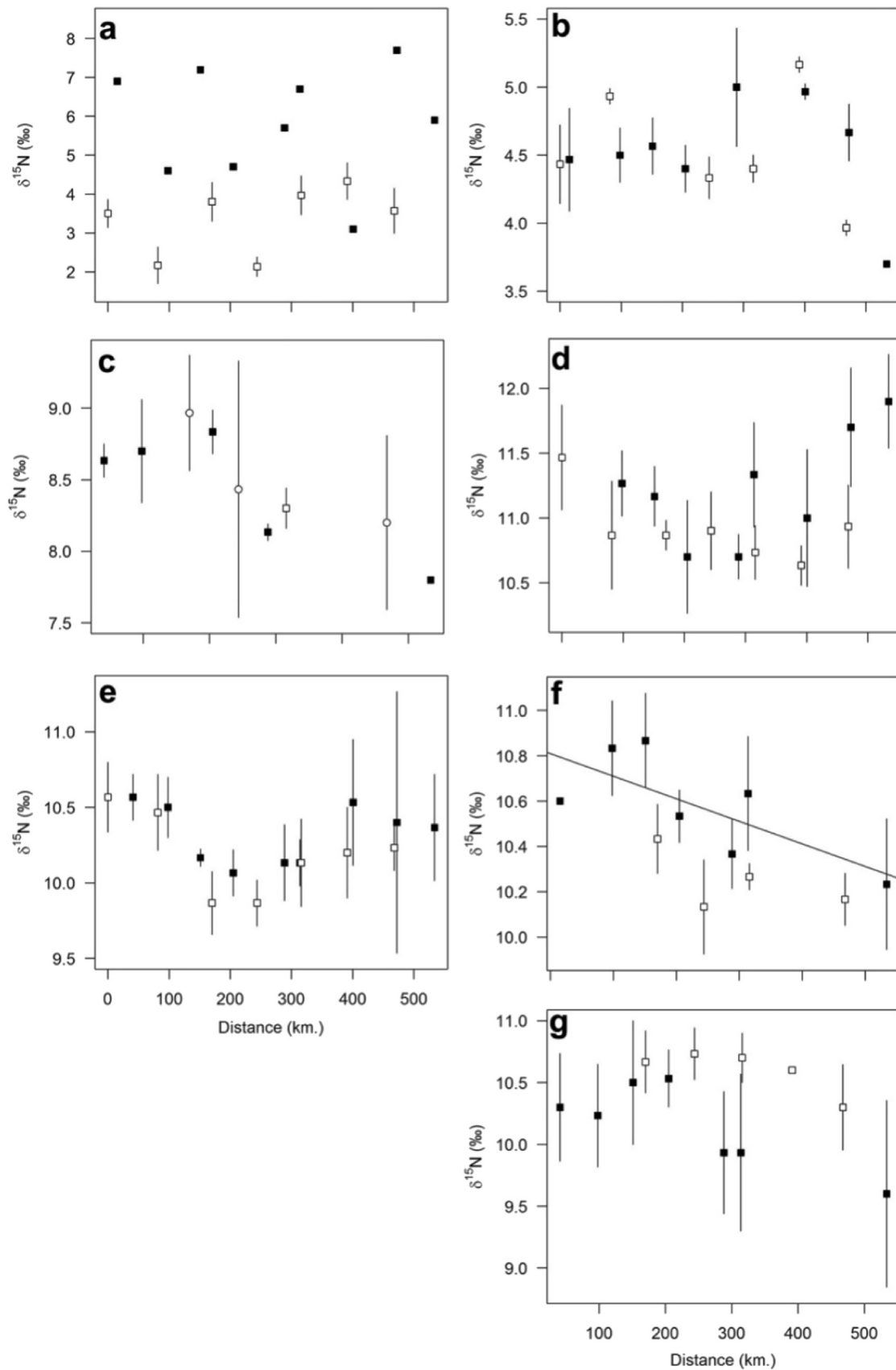


Fig. 6. Isotopic nitrogen ratio from SW to NE along the sampling in 2012 (solid symbol) and 2013 (open symbol) for (a) POM, (b) SOM, (c) euphausiids, (d) *Capros aper*, (e) juvenile *Merluccius merluccius*, (f) *Gadiculus argenteus* and (g) juvenile *Micromesistius poutassou*. Within the euphausiids (c) *Nyctiphanes couchii* and *Meganyctiphanes norvegica* are indicated by square and circles respectively. Regression line was added to the plot if trends were statistically significant ($p < 0.05$), as a solid line for 2012 data and/or a dashed line for 2013 data. Note that the y-axis' scale differs among figures.

Table 3

Prey identified in the diet of the four fish species in 2012 and 2013 quantified as the percentage of occurrence using number of prey. Prey identified to taxonomic level coarser than species or genus are added to the taxa pool without explicit acknowledgement, i.e. Mysiids include *Lophogaster typicus* and other unidentified mysiiids.

Prey group/species	<i>Capros aper</i>		<i>Gadiculus argenteus</i>		<i>Merluccius merluccius</i>		<i>Micromesistius poutassou</i>	
	2012	2013	2012	2013	2012	2013	2012	2013
Macrozooplankton	13.98	0.80	71.22	88.32	97.91	91.51	99.88	80.79
Euphausiids+Mysiids	10.31	0.80	70.73	88.32	97.08	90.57	97.42	46.36
Euphausiid	4.77	0.00	55.12	56.07	96.76	87.74	97.42	46.36
<i>Meganycitiphanes norvegica</i>	0.00	0.00	0.00	0.00	0.00	59.43	0.00	13.91
<i>Nyctiphanes couchii</i>	0.00	0.00	37.68	0.00	0.00	0.00	0.00	0.00
Euphausiid larvae (Calyptosis stage)	0.04	0.00	0.12	0.00	0.00	0.00	0.00	0.00
Euphausiid larvae (Furcilia stage)	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00
Mysidacea	2.01	0.00	0.85	0.00	0.31	2.83	0.00	0.00
<i>Lophogaster typicus</i>	0.00	0.00	0.00	0.00	0.31	2.83	0.00	0.00
Hyperiidae	3.35	0.00	0.49	0.00	0.84	0.94	2.46	34.44
Brachyuran larvae (Megalopa stage)	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Crustacean larvae (Zoea stage)	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mesozooplankton	61.45	48.97	27.07	9.81	0.00	0.00	0.00	0.00
Copepods	52.17	47.02	26.83	9.66	0.00	0.00	0.00	0.00
<i>Aechaetus</i> sp.	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Aetidius</i> sp.	1.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Calanus helgolandicus</i>	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Caligus</i> sp.	0.78	0.92	0.00	0.00	0.00	0.00	0.00	0.00
<i>Calocalanus</i> sp.	0.00	0.00	0.24	0.00	0.00	0.00	0.00	0.00
<i>Candancia armata</i>	2.86	0.11	3.29	0.16	0.00	0.00	0.00	0.00
<i>Ditrichius</i> sp.	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Macrosetella rosea</i>	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Metridia lucens</i>	0.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Paracalanus</i> sp.	0.00	0.00	0.24	0.00	0.00	0.00	0.00	0.00
<i>Paraeuchaeta</i> sp.	0.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Pleuromamma</i> sp.	0.11	0.00	0.37	0.00	0.00	0.00	0.00	0.00
<i>Pseudocalanus</i> sp.	0.00	0.00	0.49	0.00	0.00	0.00	0.00	0.00
<i>Temora</i> sp.	0.04	0.00	0.12	0.16	0.00	0.00	0.00	0.00
Copepod larvae (Nauplii stage)	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Harpacticoide	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Meroplankton	6.60	1.95	0.00	0.16	0.00	0.00	0.00	0.00
Cirripeda larvae	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00
Decapoda larvae	2.22	1.49	0.00	0.16	0.00	0.00	0.00	0.00
Polychaeta larvae	0.21	0.34	0.00	0.00	0.00	0.00	0.00	0.00
Gastropoda larvae	4.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cladocero	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ostracoda	2.58	0.00	0.24	0.00	0.00	0.00	0.00	0.00
Gelatinous zooplankton	2.26	0.69	0.00	0.00	0.00	0.00	0.00	0.00
Chaetognata	0.11	0.34	0.00	0.00	0.00	0.00	0.00	0.00
Appendicularia	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Jellyfish	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Salpidae	1.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Siphonophora	1.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Suprabenthos	11.65	22.82	0.61	0.00	0.63	0.00	0.00	0.00
Amphipoda	2.68	0.23	0.49	0.00	0.00	0.00	0.00	0.00
<i>Phronima sedentaria</i>	0.42	0.11	0.00	0.00	0.00	0.00	0.00	0.00
Caprellida	3.07	12.73	0.00	0.00	0.00	0.00	0.00	0.00
Gammaridea	0.88	7.91	0.12	0.00	0.00	0.00	0.00	0.00
Isopoda	0.18	1.83	0.00	0.00	0.00	0.00	0.00	0.00
Gnatidae	0.11	1.83	0.00	0.00	0.00	0.00	0.00	0.00
Tanaidacea	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Decapoda natantia	4.80	0.11	0.00	0.00	0.63	0.00	0.00	0.00
<i>Processa</i> sp.	0.00	0.00	0.00	0.00	0.31	0.00	0.00	0.00
<i>Solenocera membranacea</i>	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00
Epibenthos	0.14	0.69	0.00	0.00	0.00	0.00	0.00	0.00
Hydrozoa	0.14	0.23	0.00	0.00	0.00	0.00	0.00	0.00
Ophiuroidea	0.00	0.46	0.00	0.00	0.00	0.00	0.00	0.00
Teleost fish	0.00	0.00	0.12	0.31	1.36	7.55	0.12	17.22
<i>Arnoglossus</i> sp.	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00
<i>Gadiculus argenteus</i>	0.00	0.00	0.00	0.00	0.42	0.00	0.00	0.00
<i>Maurolicus muelleri</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.91
<i>Micromesistius poutassou</i>	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00
Unidentified Gobidae	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00
Other prey	10.52	26.03	0.98	1.56	0.10	0.94	0.00	1.99
Colonial diatoms	1.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Diatoms	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fish scales	1.02	5.05	0.00	0.00	0.00	0.00	0.00	0.00
Foraminifera	3.85	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Invertebrate eggs	3.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pycnogonida	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00

(continued on next page)

Table 3 (continued)

Prey group/species	<i>Capros aper</i>		<i>Gadiculus argenteus</i>		<i>Merluccius merluccius</i>		<i>Micromesistius poutassou</i>	
	2012	2013	2012	2013	2012	2013	2012	2013
Sand	0.28	12.61	0.00	0.00	0.00	0.00	0.00	0.00
Unidentified cephalopoda	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00
Unidentified crustacea	0.21	0.00	0.73	0.47	0.00	0.94	0.00	1.99
Unidentified prey	0.49	8.37	0.24	1.09	0.00	0.00	0.00	0.00

3.3. Feeding ecology using SCA

The feeding ecology of each species during autumn was described by their stomach content analysis. From the 526 individuals analyzed (Table 1), 404 (77%) contained prey and were thus considered in the analysis. Emptiness differed between the four species ranging from 41% in hake juveniles, 34% in blue whiting juveniles, 14% in silvery pout and 7% in boar fish.

The four fish species displayed three main feeding modes, with differing diet diversity and reliance of benthic and pelagic prey. Juvenile hake and blue whiting fed almost exclusively on macrozooplankton, mainly euphausiids (Table 3), yielding Shannon-Wiener diversity values of 0.97 and 1.08, respectively. Silvery pout had a mixed diet of macro- and mesozooplankton, with a Shannon-Wiener index of 1.59, although euphausiids also constituted the main part of its diet. Contrastingly, boar fish had the most diverse diet (Shannon-Wiener index 2.34) combining macro- and mesozooplankton with suprabenthos and other prey (Table 3). Although we cannot assert diets were statistically different between 2012 and 2013 (Wilcoxon signed-rank test resulted in p-values > 0.1 in all cases), small differences are noteworthy. For instance, in 2013 juveniles of hake and blue whiting increased piscivory, but in a small percentage, whereas silvery pout increased its consumption of macrozooplankton at the expense of mesozooplankton (Table 3). Boarfish consumption of planktonic prey (macro- and mesozooplankton and gelatinous zooplankton) was higher in 2012, while in 2013 suprabenthos and sand/sediment increased in its diet (Table 3).

4. Discussion

4.1. Effect of upwelling on the isotopic baseline

During the summer months, upwelling dominates the regional oceanography despite the interannual variability in intensity. The upwelling intensities of 2012 and 2013 were amongst the lowest and highest ranges of the available time series data (see data at www.indicedeafloramiento.ieo.es), respectively. In addition, the upwelling gradient from South-West to North-East depicted by buoy data is a recurrent pattern in the area. This gradient exists not only in the total flow of upwelled water, but also in the persistence of upwelling conditions, arising from both the number of days per upwelling season and the number of consecutive days with upwelling conditions (Alvarez et al., 2011). Upwelling of deep water boosts primary production by delivering nutrient rich waters to the euphotic zone, enhancing phytoplankton uptake and growth (Montoya, 2007). Indeed, as a consequence of the summer upwelling, Chl a and production values in autumn are similar or even larger than those observed during the spring bloom (Bode et al., 1996, 2009).

The nutrient dynamics during the highly productive phases of the ecosystem leave their footprint on the isotopic composition of the phytoplankton, which is subsequently transferred through the food web. We found POM $\delta^{15}\text{N}$ was significantly higher during the weak 2012 upwelling season, contrasting with the 2013 intense upwelling conditions. Our results are consistent with the mechanism operating

during blooming conditions. Phytoplankton preferential uptake of ^{14}N leads to a residual nitrate pool available that becomes progressively enriched in ^{15}N , resulting in higher $\delta^{15}\text{N}$ POM values over the duration of the productive season (reviewed by Montoya (2007)). Alternatively, during upwelling events the supplies of deep water nutrients overwhelm the biological uptake rate, thereby favoring ^{15}N -depleted nitrogen sources for producers (Chouvelon et al., 2012). We did not identify effects of the local oceanographic conditions (sampled at each station) on the isotopic signature of POM, suggesting longer-lasting regional scale processes determine the nutrient uptake dynamics and resulting isotopic signatures in the study area.

We found no interannual variation in the sediment basal isotopic signature, which was surprising as a certain degree of variability in $\delta^{13}\text{C}$ was expected *a priori* along the sampling transect. This expectation stems from the predominance of fine sediments flowing out from the rías in the first hundreds of kms of the sampling transect, likely enriched in terrigenous organic matter. The isotopic signature of riverine and terrestrial sediments is usually depleted in $\delta^{13}\text{C}$ compared to purely marine sediments (Riera and Richard, 1996; Bouillon et al., 2000; Chouvelon et al., 2015 and references therein). However, grain size *per se* has been shown to be a poor predictor for sediment isotopic composition (Alt-Epping et al., 2007). The lack of variability in $\delta^{13}\text{C}$ values in SOM along the sampling transect suggests these sediments are not heavily influenced by river outflows. Nitrogen isotopic patterns in areas near this study are also influenced by riverine discharge. In the adjacent Portuguese Shelf, the outflow of important rivers (as the Douro and Tagus) leave a conspicuous print on the sediment, increasing the proportion of organic matter from terrestrial origin up to 30–40%, and yielding $\delta^{15}\text{N}$ values over 5‰ (Alt-Epping et al., 2007; Vinagre et al., 2011). Outside the influence of these rivers, the upwelling effect evidenced by light $\delta^{15}\text{N}$ values (below 5‰) can be detected in the sediment. Indeed, this increase in $\delta^{15}\text{N}$ near river-mouths seems to be widespread (Chouvelon et al., 2015 and references therein). The $\delta^{15}\text{N}$ values of our SOM samples are below this 5‰ threshold, regardless of the year considered, suggesting that terrigenous inputs have little influence on the sediment organic matter.

The carbon and isotopic patterns in POM and SOM in our study indicate rapid sedimentation of primary production and reduced water-column processing by mesozooplankton. The remarkable similarity between $\delta^{13}\text{C}$ SOM and POM values rules out substantial particle residence-time in the water column, as this would lead to decreases in the carbon isotopic ratio, owing to the selective degradation of ^{13}C -rich compounds (Nakatsuka et al., 1997), which we did not observe. In fact, exhaustion of nutrients in the Cantabrian Sea are known to be quick, resulting in blooms of short duration (Bode et al., 1996). This hinders the effective channelization of energy through the plankton, and results in settling phytoplankton directly fuelling the benthic energy channel, supporting organisms that preferentially rely on the vertical flux of pelagic OM (Romero-Romero et al., 2016).

4.2. Effect of upwelling on further trophic levels

Upwelling affects not only the isotopic content of POM, which transfers to higher trophic levels, but also influences the structure of

the food web by altering the relative abundances of prey and predators (Van der Lingen et al., 2006). The euphausiids *Meganyctiphanes norvegica* and *Nyctiphanes couchii* had an invariant isotopic signature in 2012 and 2013 both in nitrogen and carbon isotopes, but the isotopic distance from this group to the basal isotopic signal differed greatly among years. The small enrichment in $\delta^{15}\text{N}$ of the euphausiids in 2012 (2.6‰), taking POM isotopic signature as the baseline, points towards the reliance on benthic production and/or an increase in herbivory during this first year. In 2013, however, the isotopic distance to POM along the $\delta^{15}\text{N}$ axis is higher (5.1‰), while the distance to the SOM basal level remains constant (3.9‰) suggesting that euphausiids have an omnivorous diet. This interpretation of the isotopic distances needs to be considered cautiously, as the isotopic integration time ranges from a few weeks for euphausiids to the order of days for POM. Although the proportion of phytoplankton and zooplankton in *M. norvegica* diet is variable, studies at high latitudes have found an increased consumption of algae during spring and summer (Kaartvedt et al., 2002). Diatoms co-dominate the phytoplankton in Northwestern Iberia during upwelling events (Espinoza-González et al., 2012), and may constitute a main feeding resource for *M. norvegica*, whose mandibles allow efficient grinding of diatoms (reviewed in Schmidt (2010)). *M. norvegica* is known to feed both in the water column and at the benthic boundary layer (Schmidt, 2010), but the similar values we found for $\delta^{13}\text{C}$ in SOM and POM prevent identifying the main energy channel used by the species.

The pelagic food web in NW Iberia displays two main configurations that are concomitant with the governing oceanographic settings. During the upwelling period the nutrient-rich upwelled water supports high plankton abundances and a "metazoan food web". In contrast, a poleward current in autumn-winter combined with summer stratification, leading to a lower nutrient load, might favor a microbial based food web (reviewed by Bode et al. (2007)). Indeed, regarding the phytoplankton succession, the dominance of diatoms during upwelling events is followed by large numbers of microflagellates during the upwelling relaxation and summer stratification (Casas et al., 1999). The latter phytoplankton group can regenerate a large portion of the production of the upwelling phase through the microbial loop (Bode and Varela, 1994). Despite the seasonality of microflagellates, they may represent up to 70% of the phytoplankton during all periods (Casas et al., 1999). Hence, the food web can generally be considered multi-voorous all year around with the microbial loop existing as a permanent feature (Espinoza-González et al., 2012). While isotopic enrichment by microbial and protozoan links is virtually absent (Gutierrez-Rodriguez et al., 2014), phytoplankton growing on remineralized nutrients are expected to have lower nitrogen isotopic ratios than plankton grown from new production (Wada et al., 1991; Montoya, 2007). The isotopic turnover rates in euphausiids are in the order of weeks (Frazer et al., 1997) and in planktivorous fish round 2–3 months, mainly due to growth (Macavoy et al., 2001; Miller, 2006; Bode et al., 2007). Therefore, our sampling provides insights of the target species' diets during the upwelling season.

All four planktivorous fish examined were secondary consumers, as shown either by their diet or isotopic signature. Our results are consistent with previous studies, although Chouvelon et al. (2012) reported slightly higher $\delta^{15}\text{N}$ values for hake, blue whiting and silvery pout. This was probably due to the smaller organisms sampled in our study (the mean fish size in their work was larger than ours in all three cases) as isotopic enrichment increases with ontogeny (Chouvelon et al., 2012, 2014). The prevalence of euphausiids in the diet of silvery pout, juvenile hake and blue whiting further support their role as secondary consumers. In turn, the slightly higher $\delta^{15}\text{N}$ signature of boar fish possibly responds to a higher consumption of carnivorous zooplankton. Conspicuous genera include the copepods *Paraeuchaeta* sp., *Pleuromamma* sp., *Metridia lucens* and *Candancia armata* (Bode and Alvarez-Osorio, 2004), carnivore gelatinous zooplankton, and possibly predatory caprellidae. While the caprellidae species were not

identified, the lack of plumose setae in the antenna and the relative large size of the gnathopods (personal observation) point toward a predatory/carnivore feeding mode (Caine, 1977). Results of the feeding analysis of the four fish species match diet descriptions found in the literature (Macpherson, 1978; Velasco and Olaso, 1998; Cabral and Murta, 2002; Mahe et al., 2007; Lopes et al., 2006; Velasco, 2007). Differences in the fish diet between 2012 and 2013 were not statistically significant, however we observed a tendency to consume larger prey in 2013, which was not reflected in the isotopic signature of the species. While our results suggest that upwelling variability had limited effect on the feeding ecology of these species, further work is needed using coupled time series of upwelling, isotopic and diet analysis. Indeed, Chouvelon et al. (2012) allot the interannual variability they found in isotopic signatures to dietary adjustments following variations in food supply rather than processes occurring at the baseline level.

Along the sampling transect (SW to NE), the nitrogen isotopic ratio did not show any generalized trend. However, all fish species decreased their $\delta^{13}\text{C}$ values along the transect, either in 2013 or in both sampling years. The same trend was also evident in the euphausiid data when considering both years together (Fig. 5c). This decreasing trend in the carbon isotopic ratio is not consistent with the presence of terrestrial or riverine organic matter. These processes would induce the opposite pattern with $\delta^{13}\text{C}$ -depleted samples during the first half of the sampling transect, under the influence of the rías. The carbon isotopic ratio is known to increase related to primary production and Chl *a* although the mechanism, likely related to differences in carbon fractionation among phytoplankton groups, is not fully understood (Miller et al., 2008).

Since the upwelling of nutrient-rich deep water fuels primary production, the trends we observed in the consumers' $\delta^{13}\text{C}$ are consistent with the upwelling intensity gradient. Such gradient shapes the base of the food web and could be transferred to the production of higher trophic levels, as the transfer efficiency depends on the number of trophic links and the energy loss associated to each of them (Sommer et al., 2002). Our results provide empirical support to the traceability of upwelling gradients by the isotopic signature of planktivorous fish. We stress, however, that depicting the influence of interannual changes in upwelling intensity in the food web is not a simple task, as variability in baseline processes can be confounded by species adjusting their dietary intake following changes in relative prey abundances.

4.3. Conclusions

The high $\delta^{15}\text{N}$ values observed in subsurface POM after the weak upwelling season (2012) is consistent with the biological enrichment of the nitrogen pool, via preferential uptake of ^{14}N . The lower $\delta^{15}\text{N}$ values of POM in 2013 (intense upwelling) are likely derived from the input of nitrate-rich, deep waters. Mesoscale local oceanographic conditions, however, seemed to have no effect on POM isotopic content. The similar isotopic ratios found in the POM and SOM suggest fast sedimentation processes. The relatively ^{13}C -depleted SOM further indicates that any influence from the rías is overwhelmed by the sedimentation of pelagic production. All four fish species displayed small differences in carbon and/or nitrogen isotopic ratios between 2012 and 2013, but such differences did not mirror variability at the basal level nor variations in diet. We hypothesize that dietary adjustments during the summer season may cause this interannual variability in isotopic signatures. No clear, generalized trend in $\delta^{15}\text{N}$ values could be related to the upwelling intensity gradient. However, the carbon isotopic ratio of the fish and euphausiids displayed a clear and linear trend along the sampling transect, likely related to the higher primary production associated with intense upwelling conditions.

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