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Feeding Habitats, Connectivity and Origin of Organic Matter Supporting Fish Populations in an Estuary with a Reduced Intertidal Area Assessed by Stable Isotope Analysis

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Abstract Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) were used to analyse the food web downstream of the largest estuary on the French coast: the Gironde. The different sources of organic matter supporting the most abundant and commercially important fish species were determined, as well as habitat connectivity for fish. Stable isotope analysis was performed in different producers (marine, freshwater and local sources), primary consumers (zooplankton and macrozoobenthos) and nine fish species (*Alosa alosa*, *Engraulis encrasicolus*, *Sprattus sprattus*, *Liza ramada*, *Pomatoschistus minutus*, *Platichthys flesus*, *Solea solea*, *Dicentrarchus punctatus* and *Argyrosomus regius*) in three habitats of the downstream area of the estuary in June–July 2012. All sources and invertebrates had significantly different isotopic signatures in different habitats. Only sole, *S. solea*, presented distinct dual isotopic signatures, indicating a higher feeding location fidelity, no other fish species showed significant differences in isotopic signatures. This overlap was interpreted as evidence that fish had not been feeding exclusively in the habitat where they were collected, instead ingesting food with different isotopic signatures, reflecting high habitat connectivity for these fish. As the base of the fish food web significantly differed among habitats, the present study indicated the suitability of stable isotopes in tracing fish movements and their fidelity/connectivity for habitats separated by less than 10 km, particularly estuarine habitats without salinity differences but located on opposite banks. The SIAR mixing model estimations of organic matter contribution to fish diets in the Gironde estuary were quite similar for the fish species investigated. The major organic source was marine-derived POM, with contributions >75 % for each species. Freshwater and local POM (generally indicated as the sources structuring estuarine food webs) contributed little to the overall fish food webs in the Gironde estuary. Only flounder, *P. flesus*, and shad, *A. alosa*, migratory amphihaline species, utilised freshwater POM in greater proportion than marine. The observed low freshwater POM-high marine POM contribution to the fish food web seems to be explained by the reduced intertidal surface of the system. This characterization of the trophic base and habitat connectivity for the most important Gironde estuary fish provides a novel insight for future management of the estuary, especially in the current context of global change.

Keywords Fish · Stable isotopes · Food web · Connectivity · Organic matter origin · Gironde estuary

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Introduction

Estuaries consist of a mosaic of different types of habitats (salt marshes, mudflats, seagrass meadows, bare sediments, etc.), often interconnected (Pihl et al. 2002). They are considered among the most productive aquatic areas (Costanza et al. 1997) and are associated with a diverse range of fish and crustaceans, including species of high recreational and commercial values, and with important ecological functions, such as, nurseries for marine juvenile fishes, feeding areas for resident and adult fishes, transitory environments for reproduction and growth and refuges from predation with high prey availability (Beck et al. 2001). Due to these ecological properties, estuaries are also associated with highly valuable goods and services for human activity (Costanza et al. 1997) and

consequently increasingly subjected to anthropogenic pressures such as construction of harbours, pollution, eutrophication and fishing (Post and Lundin 1996). These activities can cause extensive habitat loss and degradation (Chambers 1992), which could potentially affect fish ecology and hence fisheries since many fishery species spend part of their life in estuarine habitats (Pauly 1988; Lamberth and Turpie 2003). Knowledge of juvenile fish movements and spatial utilisation of estuarine habitats is thus crucial for our understanding of fish population ecology and constitutes a prerequisite for effective conservation and management (Hobson et al. 1999).

Methods to evaluate fish movements are multiple and often system-dependent. While tagging is usually employed for large individuals, methods to monitor the movement of small organisms (i.e. juvenile fishes <100 mm) are restricted (Durbec et al. 2010). Natural markers, in particular stable isotopes, are being increasingly used in this respect. Stable isotope analyses require only a small amount of material, making it possible to trace smaller individuals. Stable isotopes have been used to describe spatial patterns of fish movement at various scales but most studies deal with large spatial scales between different geographic areas, e.g., estuary and coastal areas (Kostecki et al. 2010; Vinagre et al. 2011a; Kopp et al. 2013), freshwater spawning sites and juvenile nurseries or between distant marshes within an estuary (Green et al. 2012). Relatively few studies have considered the fine spatial scale (i.e. a few kilometres; Durbec et al. 2010; França et al. 2011). The diversity of estuarine habitats makes an isotopic approach to trace the movement of fishes particularly appealing, by increasing the likelihood of finding habitat-specific isotopic signatures (Herzka 2005). Stable isotopes have recently been used to evaluate site fidelity and infer whether there is mixing among fish subpopulations, i.e. connectivity among fish habitats (e.g. Vinagre et al. 2011b; Green et al. 2012), as well as to quantify relative proportions of organic matter sources which support the fish food web (e.g. França et al. 2011).

However, in complex and constantly changing environments like estuaries, it is difficult to identify the source of organic matter at the base of food webs. Primary production sources have been studied, yet remain a major topic of debate (Litvin and Weinstein 2003) and a great challenge despite years of research (see França et al. 2011). Many studies in estuaries point out a predominant incorporation of allochthonous organic matter of continental origin into fish food webs, while in systems under low freshwater influence, in situ primary production can override other food sources and significantly contribute to fish growth (Wilson et al. 2009a, 2010). Such conclusions were based on $\delta^{13}C$ vs $\delta^{15}N$ biplot graphic interpretations (e.g. Darnaude et al. 2004) and very few, so far, have tried to quantify the relative contribution of different sources of organic matter to estuarine fish food webs using mixing models like SIAR (Wilson et al. 2009a, 2010; França et al. 2011; Kostecki et al. 2012; Le Pape et al. 2013). In addition, within European estuaries, studies generally focused on only one fish species (often the sole, *Solea solea*).

The Gironde estuary (SW France) has an important function for fish and fisheries in the Bay of Biscay, yet although it has been a well-studied ecosystem for several decades, there is still a severe lack of information, or confusing conclusions, on both fish connectivity and origin of organic matter supporting the juvenile fish food web. Pasquaud et al. (2008) suggested a marine predominance of food web organic matter whereas Lobry et al. (2008) suggested that the Gironde estuary is totally under river influence in terms of energy and feeding. Based on a stable isotope approach, the present study, conducted on the downstream part of the Gironde estuary, a large estuary with a low proportion of intertidal area, had two objectives. The first was to determine the level of habitat connectivity for the most abundant fish species and to test the efficiency of the stable isotope approach for studying fish connectivity/fidelity at a low spatial scale. We hypothesised that stable isotopes allow discrimination of estuarine habitats separated by less than 10 km. The second objective was to quantify the main origin of the organic matter supporting the fish food web. More specifically, we hypothesised that there is a low contribution of freshwater particulate organic matter (POM) in an estuary with reduced intertidal area. In the context of global change (climate change and anthropogenic freshwater use), the characterization of the trophic base and the connectivity of fish, including species with commercial value, are crucial for future conservation and management of estuarine habitats.

Materials and Methods

Study Area

The study area was located in the lower part of the Gironde estuary (SW France—45°26′N, 0°45′W; Fig. 1) which opens into the Atlantic Ocean. This is the largest estuary in Europe (Lobry et al. 2003), covering an area of 625 km^2 at high tide. It is 12 km wide at the mouth and 76 km long between the ocean and Ambès (the upstream salinity limit), where the Garonne and Dordogne rivers meet (see Fig. 1). The watershed covers 81,000 km^2 and the mean annual rate of freshwater flow is around 600–1000 $m^3\ s^{-1}$ (Sottolichio and Castaing 1999). The Gironde is a macrotidal estuary with a tidal range of 4.5 m at the mouth and over 5 m at Bordeaux, located 25 km upstream on the Garonne river. Nevertheless, it is characterized as a low-tidal estuary since the tidal surface, mainly composed of mudflats, represents only 10 % of the estuary (Selleslagh et al. 2012), small compared to numerous other similar systems (França et al. 2011; Le Pape et al. 2013). The hydrodynamic conditions are highly variable due to the interaction of marine

and fluvial flows, leading to strong temperature and salinity gradients. The Gironde is one of the most turbid estuaries in Europe (SPM >500 mg l^{-1}, Sautour and Castel 1995). Particulate matter is tidally re-suspended and concentrations may exceed 1 g l^{-1} at the upstream limit of salinity intrusion (Allen et al. 1974). This zone of maximum turbidity, which is due to an asymmetric tidal wave, migrates seasonally according to river flow and tidal cycles (Sottolichio and Castaing 1999). Although high turbidity limits primary production, there is a high zooplanktonic biomass (Castel 1993). Three habitats were investigated in the present study: two opposite intertidal areas, called *Right bank* (R) and *Left bank* (L), located at Chant Dorat and Phare Richard, respectively, and one in the main channel, called *Subtidal* (S) (Fig. 1). The two intertidal habitats are separated by about 11 km. They have a mud substratum and are 3 m deep at high tide. The sampled subtidal area is equidistant from both intertidal areas, is dominated by muddy-sand substratum and is 10 m deep.

Sampling Surveys

Considering the objectives of the present study, samples of water, sediment, fishes' main prey species (zooplankton, macrozoobenthos and shrimp) and fish were collected in the three investigated habitats in June–July 2012. Summer is indeed the best season to sample all the food web nodes and collect the number of individuals needed for analysis (Vinagre et al. 2012). This season was thus chosen for the present study due to higher species diversity and abundance, notably in the Gironde estuary (Selleslagh et al. 2012).

Fish and Shrimp Sampling

According to habitat type, fish and shrimp were collected using different methods. Nine fish and two shrimp species were collected from each habitat for stable isotope analysis: anchovy *Engraulis encrasicolus*, shad *Alosa fallax*, sprat *Sprattus sprattus*, mullet *Liza ramada*, sand goby *Pomatoschistus minutus*, flounder *Platichthys flesus*, common sole *S. solea*, spotted seabass *Dicentrarchus punctatus* and meagre *Argyrosomus regius* for fish, and brown shrimp *Crangon crangon* and white shrimp *Palaemon longirostris* for shrimp. As fish size may affect isotopic values, in particular δ^{15}N, due to ontogeny (Vinagre et al. 2008; Pasquaud et al. 2008; Wilson et al. 2009b; Galvan et al. 2010; Olin et al. 2012), we carefully selected individuals of similar size across species (Table 1). As two size classes were collected for *D. punctatus* and *E. encrasicolus*, individuals were divided into small and large sub-classes for further isotopic analyses (Table 1).

At the intertidal sites (*Right bank* R and *Left bank* L), sampling was performed during daylight hours using a 1.5 m beam trawl, towed by a zodiac against the current at 2 knots for 7 min. The fishing net was 5.5 m long, had a mesh

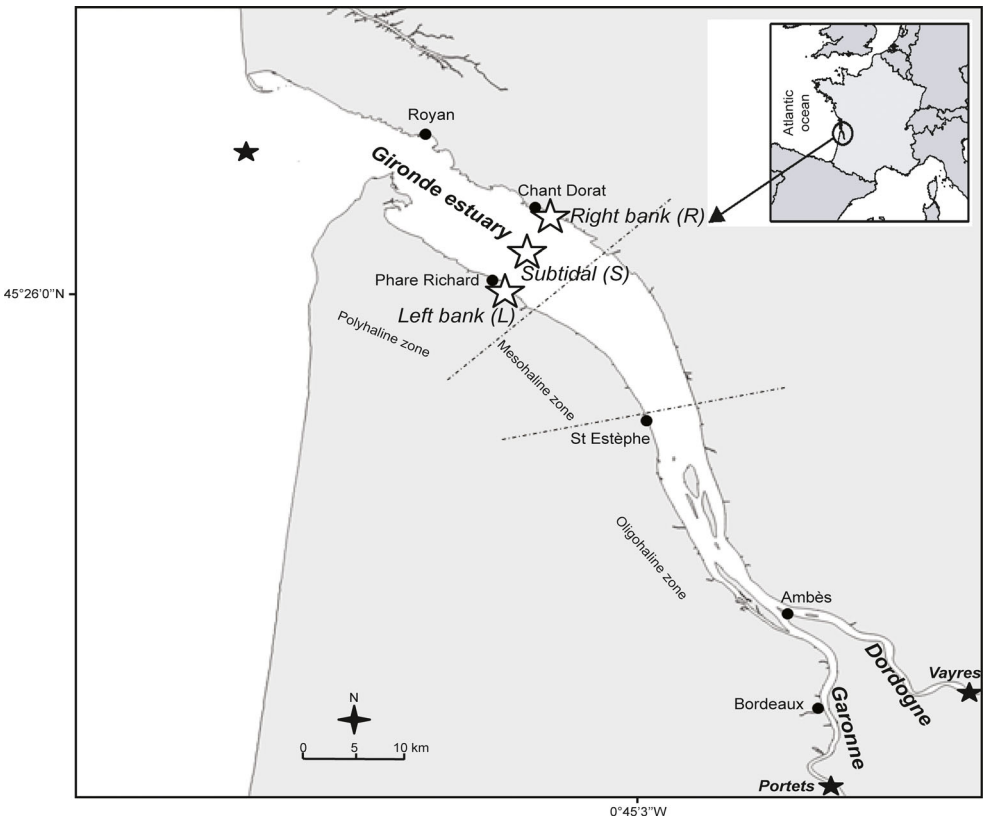

Fig. 1 Study area and location of the three sampling stations (*white stars*) in the Gironde estuary. *Black stars* indicated location of marine and freshwater sampling stations

Table 1 Total length (mm), weight (g) (both mean±standard error), trophic level (TL) and number of samples (N) of the nine fish species collected at each habitat in the Gironde estuary in July 2012 and used in the stable isotope analysis

Site	*E. encrasicolus* s	*E. encrasicolus* l	*D. punctatus* s	*D. punctatus* l	*P. flesus*	*P.minutus*	*A. regius*	*L. ramada*	*A. fallax*	*S.sprattus*	*S. solea*
Length											
R	–	–	37±7.0	148.7±15.9	65.2±13.0	46.0±6.4	–	–	35.0	38.5±5.8	117.2±76.5
L	34.0	84.6±11.6	39±5.3	–	60.8±14.2	43.2±4.8	–	–	39.0±1.8	45.0	84.0±11.9
S	–	85.6±10.8	–	122.6±20.9	49.5±3.5	44.6±4.9	330.0±84.8	205.8±85.7	38.0±6.0	49.5±2.1	119.6±53.0
Weight											
R	–		0.6±0.2	37.1±9.7	3.2±1.8	0.8±0.3	–	–	0.2	0.4±0.2	30.5±46.4
L	0.1	–	0.7±0.3	–	2.9±2.0	0.5±0.2	–	–	0.5±0.1	0.8	5.9±2.4
S	–	5.1±1.5	–	24.4±15.6	1.4±0.3	–	390.5±270.8	135.1±156.7	–	0.9±0.3	22.0±21.3
TL											
R	–	–	2.613	3.084	2.535	2.674	–	–	2.367	2.470	2.805
L	2.207	2.739	2.603	–	2.723	2.721	–	–	2.116	1.748	2.844
S	–	2.526	–	3.126	2.529	2.595	3.173	2.766	2.403	2.423	2.835
N											
R	0	0	3	3	5	5	0	0	1	4	5
L	1	3	4	0	5	5	0	0	4	1	5
S	0	5	0	6	2	5	2	5	3	2	5

size of 8×8 mm in the main body and 5×5 mm in the cod end, and was equipped with a tickler-chain in the ground rope. Between five and ten replicates were performed at each site to obtain a sufficient number of individuals per species. Although beam trawling is often considered a suitable method for sampling bentho-demersal species, it also catches a representative pelagic population in shallow waters (Selleslagh and Amara 2008), as in our case. As a consequence, both bentho-demersal and pelagic species were collected with beam trawl.

In the subtidal zone (*Subtidal* S), two surveys were performed simultaneously to collect organisms aboard the N-O "L'Esturial":

For the so-called 'Transect survey', simultaneous fishing samples were taken near the surface and near the bottom. Surface samples were collected using two 4.0×1.0 m rectangular frame nets, equipped with a flowmeter and fitted on both sides of the boat. The subconical nets had a stretched mesh size of 18 mm in the main section and 2.8 mm in the cod end. For the benthic samples, a dragnet with a 2.0×1.2 m frame was used. Runners kept the frame 0.2 m above the bed. The net meshes were identical to those used for surface samplings. Sampling lasted 5–7 min and was performed in daylight between mid-flood and high tide, with the gear being towed against the current. Triplicates were performed. The sampled fauna consisted mainly of small pelagic species, as well as shrimp.

Considering the second fish survey, the 'Trawling survey', sampling was carried out during daylight hours using a beam trawl (vertical opening 3 m and horizontal opening 0.5 m and with a mesh size of 5 mm in the cod end). Trawl tows lasted 7 min on average and were generally performed just after a 'Transect survey', at the beginning of the ebb tide. Two replicates were performed. The fish samples consisted mainly of bentho-demersal species.

For each survey, captured fish were immediately washed with milli-Q water, identified, counted, measured (total length with 0.5 mm precision) and frozen at −20 °C until transfer to the laboratory for isotopic analyses.

Macrozoobenthos and Sediment Sampling

Macrozoobenthos was also collected using different protocols according to habitat type. At intertidal sites, macrozoobenthic fauna was sampled during low tide with a hand corer (15 cm depth, 0.0066 m^2, 10 replicates) while at the subtidal site, macrozoobenthos was sampled using a van Veen grab (0.1 m^2, three to five replicates). Subtidal sampling took place during fish surveys. Samples were washed, sieved through a 0.5 mm mesh and then washed again with milli-Q water to avoid contamination. In the laboratory, macrozoobenthic fauna was sorted, identified to the species level (except oligochaetes) using a binocular microscope and frozen at −20 °C until analyses. Three additional sediment samples were taken in each habitat (as described above for macrozoobenthos) in order to determine sediment organic matter isotopic signatures. Sediment samples (top first centimetre) were handled within a few hours in the laboratory for isotopic analysis.

Zooplankton and Water Sampling

Because of rapid changes in isotopic signatures in zooplankton (a few weeks) and turnover rates in fishes (within weeks for young fishes with fast growth; Herzka 2005), zooplankton samples were collected 1 month before other surveys, in June 2012. For that, a standard WP-2 net equipped with a 200 μm mesh was towed for 2 min at high tide in each investigated habitat. Three replicates were performed. The sampled zooplankton was filtered through 500 μm mesh to remove large debris and then purged in filtered estuarine water for 24 h at 15 °C until sorted in the laboratory. As copepods and mysids accounted for the majority of zooplanktonic abundance in the Gironde (>90 %; David et al. 2005), they were separated under a binocular microscope and frozen at −20 °C. Bottom water samples for POM analysis (2 L per replicate, three replicates) were also collected at high tide in the three habitats during fishing surveys. Additional water samplings were done at the mouth of the Gironde during high tide (in front of Royan, Fig. 1) and in both the Dordogne (at Vayres, Fig. 1) and Garonne (at Portets, Fig. 1) rivers at low tide for identification of marine and fluvial isotopic signatures of POM, respectively. Sampling was performed using a Niskin bottle and filtered until clogged through pre-combusted Whatman GF/F filters (0.7 μm) immediately after sampling. Filters were then frozen at −20 °C until their extraction. Microphytobenthos was not sampled in the present study since its biomass is relatively low in the investigated intertidal areas.

Stable Isotope Analyses

The standard preparation of samples for stable isotope analysis consisted of drying or freeze-drying samples and then grinding them to a fine and homogeneous powder with a mortar and a pestle. However, analyses of isotopic signatures require different pre-treatments, depending on the sample types. To limit lipid content-based variability on $\delta^{13}C$ (Bodin et al. 2007), dissection of low-lipid muscle tissue (e.g. dorsal white muscle for fish) was preferred (Pinnegar and Polunin 1999). To avoid carbonate contamination of the sample, which can bias isotopic analyses because carbonates present higher ^{13}C values than organic carbon, carbonates were removed by sample acidification prior to analysis (Ng et al. 2007; França et al. 2011). To test sample contamination, powder subsamples of all sample types were observed under a binocular microscope and acidified with 1 % HCl. If bubbling occurred, the sample was divided into two: one acidified with several

drops of 1 % HCl and used for $\delta^{13}C$ analysis, the other, for $\delta^{15}N$ analysis, was not acidified since acidification results in enrichment of $\delta^{15}N$ (Pinnegar and Polunin 1999).

Sediment samples were dried at 60 °C for 24 h and ground to a fine and homogeneous powder, before encapsulation (±2 and 15 mg for mud and sand sediment, respectively). Filters were freeze-dried and associated POM was recovered by scrubbing the filter. As contamination by carbonates was detected in sediment and filter samples, acidification was performed and two separate subsamples were used for $\delta^{13}C$ and $\delta^{15}N$ determination. Acidified subsamples were then rinsed several times with milli-Q water, re-dried at 60 °C for 24 h and ground to a fine powder before encapsulation. For zooplankton, each sample, consisting of a pool of several individuals, was freeze-dried and ground. The valve muscle for bivalves, the abdomen muscle for shrimp and the white dorsal muscle for fish (even small fish) were dissected and used for isotopic analysis, while for macrozoobenthos, the analysis was done on the whole organism, once digestive tracts, jaws and cerci were removed. The remaining tissues were then washed with milli-Q water to prevent contamination and freeze-dried before being encapsulated. For small macrozoobenthic organisms, each sample represented a pool of several individuals. None of the samples was contaminated, except for isopods where the acidification procedure described above was used. Approximately 0.4 mg of sample (depending on sample type) was accurately weighed and encapsulated into small tin cups for stable isotope analysis. Dissection tools, mortar and pestle and other materials used for stable isotope analysis sample preparation were washed with 10 % HCl, rinsed with milli-Q water and dried at 60 °C between each sample treatment.

$\delta^{13}C$ and $\delta^{15}N$ were determined by continuous-flow isotope ratio spectrometry (CF-IRMS) with a delta V advantage Isotope Ratio mass Spectrometer coupled with a Flash EA 1112 Elemental Analyser. As samples contained more than 10 % nitrogen, the CF-IRMS was operated in dual isotope mode, allowing $\delta^{13}C$ and $\delta^{15}N$ to be measured in the same sample. Replicate analyses of international IAEA and laboratory standards gave analytical errors of less than 0.1 and 0.2 % for carbon and nitrogen, respectively. Stable isotope ratios were expressed as parts per mil (‰) in the δ notation relative to the Pee Dee Belemnite standard for carbon and atmospheric N_2 for nitrogen using the formula:

$$\delta X(‰) = \left[\left(R_{\text{sample}}/R_{\text{standard}}\right)-1\right] \times 1000,$$

where X is ^{13}C or ^{15}N, R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$, and δ is the measure of heavy to light isotopes in the sample.

Data Analysis

The main goal of this paper was to investigate the connectivity of fish feeding habitats and define the source of organic matter at the base of the Gironde estuarine food web. Therefore, we first tested the hypothesis that potential sources and prey for fish displayed significantly different isotopic signatures among the three estuarine habitats. We then tested if fish showed different isotopic signatures among habitats. For all compartments of the food web, non-parametric Kruskal-Wallis (or Mann-Whitney when a compartment was collected in only two habitats) tests were performed separately for each isotope. Kruskal-Wallis was also used to test if collected fishes were of similar size and trophic level among the three habitats. When a significant difference was observed, a Dunn post hoc test was conducted. As significant differences in $\delta^{13}C$ and $\delta^{15}N$ values in a specific compartment between habitats does not necessarily imply a significant difference of the joint $\delta^{13}C$ and $\delta^{15}N$ isotopic signature, permutational-MANOVA (PERMANOVA) using a Euclidean distance similarity index was performed to better discriminate habitat signatures.

Dual $\delta^{13}C$-$\delta^{15}N$ plots were used to graphically represent isotopic signatures with associated standard deviations of all compartments of the entire food web of each habitat. This showed if one habitat's food web was more enriched or depleted based on isotopic signature differences between species and/or habitats. In addition, the percentage of individuals of each fish species within ("residents") and outside ("deviants") the central isotopic range, defined as the mean values for $\delta^{13}C$ and $\delta^{15}N$±1 % (Fry et al. 1999; Vinagre et al. 2011a), was calculated for the three habitats. This index allows the identification of individuals feeding in similar locations, and of deviants, i.e., individuals outside the central range.

Sources supporting fish populations of the Gironde estuary, and location of prey consumed by fish collected in each habitat, were identified using a mixing model. The Bayesian model, developed by Parnell et al. (2010) and implemented in SIAR package on R software, was used; this provided a combination of feasible solutions that could explain a consumer's isotopic signature (Phillips and Gregg 2003). Input parameters used in this mixing model are the signature of each source, with the associated standard error; the trophic enrichment factor (TEF) value, with its standard error; and consumer signatures, in our case fish signatures. Two different sets of TEF values, derived from Kostecki et al. (2012), were applied to our models: (i) 1±0.6 % and 3.4±1.5 % for $\delta^{13}C$ and $\delta^{15}N$, respectively, when models were used to identify prey consumed by fish in each habitat (in this case prey signatures of three habitats were considered) and (ii) 2±0.6 % and 5.6± 1.5 % for $\delta^{13}C$ and $\delta^{15}N$, respectively, when models were used to identify POM sources supporting fish populations (see Kostecki et al. 2012).

Based on stable isotope ratios and SIAR output parameters, fish trophic level (TL) was estimated as follows:

$$TL = \left[\left(\delta^{15}N_{fish} - \delta^{15}N_{prey}\right)/\Delta\delta^{15}N\right] + TL_{base},$$

where $\delta^{15}N_{fish}$ is the $\delta^{15}N$ signature of a given fish; $\Delta\delta^{15}N$ is the trophic fractionation of $\delta^{15}N$, estimated at 3.4 ‰ (Post 2002); TL_{base} is the trophic level of the baseline for fish (equal to 2, Pasquaud et al. 2010; Green et al. 2012) and $\delta^{15}N_{prey}$ is the $\delta^{15}N$ signature of the prey. In our study, $\delta^{15}N_{prey}$ is obtained through the following mixing equation:

$$\delta^{15}N_{prey} = X\delta^{15}N(P_1) + X\delta^{15}N(P_2) + \ldots + X\delta^{15}N(P_n),$$

where $\delta^{15}N_{prey}$ is the mixture of the proportions of different producers that contribute to fish diets; X is the relative contribution of each prey to the mixture, estimated from present SIAR models, and n is the number of prey contributing to the mixture.

A significance p value of 0.05 was used in all test procedures. All statistical analyses and models were performed with R software (R Development Core Team 2005), while multivariate analyses were done with PRIMER 6 software.

Results

Source and Reservoir Isotopic Signatures

The main organic matter sources and reservoirs showed significant differences in $\delta^{13}C$ and $\delta^{15}N$ between habitats/sites (Table 2). The mean $\delta^{13}C$ of sources was significantly lower in the Dordogne river POM (−27.2 ‰±0.1) and higher in marine POM (−22.1 ‰±0.3), indicating a classical increase of POM from the river to the marine source. Regarding reservoirs, while $\delta^{13}C$ in water POM was significantly (p=0.01) lower in Right bank (−24.8 ‰±0.0) and higher in Subtidal (−23.3 ‰±0.0), $\delta^{13}C$ was significantly (p=0.005) lower in Subtidal (−25.5 ‰±0.4) and higher in Right bank (−24.6 ‰±0.1) in surface sediment. The mean $\delta^{15}N$ values also showed differences between habitats/sites. Regarding sources, the nitrogen signature of marine POM was significantly (p=0.02) higher than Dordogne (5.8 ‰±0.1) and Garonne river POM (6.3 ‰±0.1). The water POM signature was significantly (p=0.05) lower in Right bank (4.9 ‰±0.1) and higher in Left bank (5.8 ‰±0.5) (Table 2). Surface sediment showed a lower nitrogen value in Left bank (5.3 ‰±0.5) and a higher value in Right bank (6.1 ‰±0.5) (Table 2).

Table 2 Carbon and nitrogen stable isotope signatures (mean±standard error) of main sources and reservoirs of particulate organic matter (POM) in the three habitats of the downstream area of the Gironde estuary in June–July 2012

Source/reservoir	$\delta^{13}C$	$\delta^{15}N$
Main POM sources		
Dordogne River POM	−27.2±0.1	5.8±0.1
Garonne River POM	−26.7±0.1	6.3±0.1
Marine POM	−22.1±0.3	9.7±0.2
Main POM reservoirs		
Water POM		
R	−24.8±0.0	4.9±0.1
L	−24.4±0.1	5.8±0.5
S	−23.3±0.0	5.4±0.0
Surface sediment		
R	−24.6±0.1	6.1±0.5
L	−25.1±0.1	5.3±0.5
S	−25.5±0.4	6.1±0.3

Consumer Isotopic Signatures

Benthic Organisms

The $\delta^{13}C$ values of subtidal macrozoobenthic organisms were lower than in intertidal areas. Statistical analysis was not possible because of the scarcity of benthic organisms in Subtidal (only four measures, Table 3). Nevertheless, considering the base of the macrozoobenthic food web (organisms displaying the lowest $\delta^{15}N$ values: *Peringia ulvae*, Isopods, *H. diversicolor* and *Scrobicularia plana*), Subtidal organisms displayed much lower $\delta^{13}C$ values (−23.4 ‰, Isopod *Eurydice pulchra*, Table 3) than intertidal organisms with the lowest $\delta^{15}N$ values (−16.6, −14.3 and −13.4 ‰ for *S. plana*, *H. diversicolor* and *P. ulvae*, respectively, Table 3). In addition, intertidal oligochaetes displayed the lowest $\delta^{13}C$ value among intertidal macrobenthic organisms but this value was still higher (−21.0 ‰) than the highest value measured in Subtidal macrofauna (−22.6 ‰, *Heteromastus filiformis*).

Comparisons between the two intertidal sites were conducted by comparing the signatures of species that were retrieved in both sites, namely Isopod *Cyathura carinata*, *Nephtys* sp., and *S. plana*. PERMANOVA showed significant differences in isotope signatures between sites for the three species. There was however no consistent pattern of increased or decreased values between sites (Table 3; Fig. 2).

Shrimp

Both shrimp species (*C. crangon* and *P. longirostris*) displayed higher $\delta^{15}N$ values (12.8 and 11.8 to 13.2 ‰ respectively, according to habitats) than other macrobenthic

Table 3 $\delta^{13}C$ and $\delta^{15}N$ (mean±standard error) values of invertebrates and fish collected in the three habitats of the Gironde estuary in June–July 2012

Species (abbreviation)	Right bank		Left bank		Subtidal	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Copepods (Cop)	−24.2±0.1	10.3±0.1	−24.8	10.1	−25.7±0.2	10.9±0.0
Mysids (Mys)	−21.7±0.1	9.2±0.1	−22.4	8.4	−18.8±0.1	8.9±0.7
Peringia ulvae (*H ulv*)			−13.4±0.1	9.0±0.1		
Oligochaetes (Oli)	−21.0	10.2				
Isopods (Iso)	−16.0±0.1	11.5±0.1	−13.4±0.1	12.4±0.1	−23.4	8.6
Heteromastus filiformis (*H fil*)	−19.8	11.7			−22.6	11.9
Nephtys sp. (*Nep*)	−13.8±0.0	11.0±0.1	−15.3±0.1	11.2±0.1		
Hediste diversicolor (*N div*)	−14.3±0.1	8.7±0.1				
Scrobicularia plana (*S pla*)	−16.8±0.1	8.2±0.1	−16.6±0.0	9.7±0.1		
Macoma balthica (*M bal*)			−16.0±0.0	10.3±0.1		
Crangon crangon (*C cra*)	−16.4±0.0	12.9±0.1	−15.2±0.1	12.8±0.0	−19.5±0.1	12.8±0.0
Palaemon longirostris (*P lon*)	−19.7±0.5	13.2±0.1	−16.0±0.0	11.8±0.0	−20.8±0.1	12.7±0.0
Engraulis encrasicolus (*E enc*)			−17.5±0.2	12.4±0.3	−18.8±1.1	11.7±0.3
Alosa fallax (*A fal*)	−19.6	11.0	−22.0±0.6	10.2±0.3	−22.5±0.6	11.0±0.7
Sprattus sprattus (*S spr*)	−18.9±0.6	11.1±0.3	−20.8	11.1	−18.3±0.5	11.2±0.2
Liza ramada (*L ram*)					−19.1±4.0	11.2±1.5
Pomatoschistus minutus (*P min*)	−19.3±0.2	12.1±0.2	−19.2±0.8	12.1±0.4	−19.9±0.3	11.9±0.4
Platichthys flesus (*P fle*)	−24.2±0.5	12.1±0.6	−22.8±1.7	12.3±0.4	−23.9±0.8	12.4±0.3
Solea solea (*S sol*)	−15.8±0.8	12.6±0.3	−15.5±0.3	13.4±0.2	−17.8±1.2	13.0±1.4
Dicentrarchus punctatus (small) (*D puns*)	−20.6±0.8	12.5±0.9	−20.2±0.7	11.5±0.5		
Dicentrarchus punctatus (large) (*D punl*)	−15.8±0.6	14.6±0.4			−17.0±1.1	13.9±0.5
Argyrosomus regius (*A reg*)					−17.0±0.1	14.1±0.2

organisms, which all had values less than 12.4 % (Table 3). There were significant differences in isotope signatures among the three sites for both species (PERMANOVA and pairwise tests, $p<0.05$; Fig. 2).

Planktonic Organisms

Copepods displayed the most depleted $\delta^{13}C$ ratios among all sampled organisms in this study, with values in the range of −25.7 to −24.2 % whereas their $\delta^{15}N$ values were between 10.1 and 10.9 % (Table 3). The lowest $\delta^{13}C$ values for these organisms were obtained in Subtidal (Table 3).

Mysids displayed more depleted $\delta^{15}N$ ratios and higher $\delta^{13}C$ values than copepods (Table 3). Their isotopic signatures were different in Subtidal compared to intertidal stations (Right and Left banks) (PERMANOVA, $p<0.05$), with more depleted $\delta^{13}C$ ratios in intertidal areas (Table 3; Fig. 2).

Fish

While *P. flesus*, *P. minutus* and *S. solea* were caught in the three habitats, the other fish species were collected only in two habitats. Contrary to all sources and invertebrates, fish species/classes did not show any significant differences in isotope signatures among habitats (PERMANOVA, $p>0.05$), even if some species showed significant difference in either $\delta^{13}C$ (the majority of species) or $\delta^{15}N$ (only anchovy *E. encrasicolus*) ratios among habitats (Fig. 2). Dual isotopic signatures overlapped among sites for each fish species, except for the sole which showed a clear distinct dual isotopic signature for each habitat (Fig. 3). $\delta^{13}C$ and $\delta^{15}N$ ratios of sole were −15.8 %±0.8 and 12.6 %±0.3 at Right bank, −15.5 %± 0.3 and 13.4 %±0.2 at Left bank and −17.8 %±1.2 and 13.0 %±1.4 at Subtidal, respectively (Table 3), indicating a high fidelity to feeding locations. The other fish species, namely *Alosa alosa*, *S. sprattus*, *P. minutus*, *P. flesus* and *D. punctatus* displayed no significantly different dual isotopic signatures (PERMANOVA, $p>0.1$) and instead showed important isotopic overlap among areas (Fig. 3).

Food Webs

Comparing whole food webs among the three habitats, copepods had the most depleted $\delta^{13}C$ ratio (mean=−24.2 to −25.7 %; Table 3), followed by flounder *P. flesus* (mean= −22.8 to −24.2 %), while the polychaetes *H. diversicolor* (mean=−14.3 %) and *Nephtys* sp. (mean=−15.3 to −13.9 %), isopods (mean=−16.0 and −13.4 % in Right bank

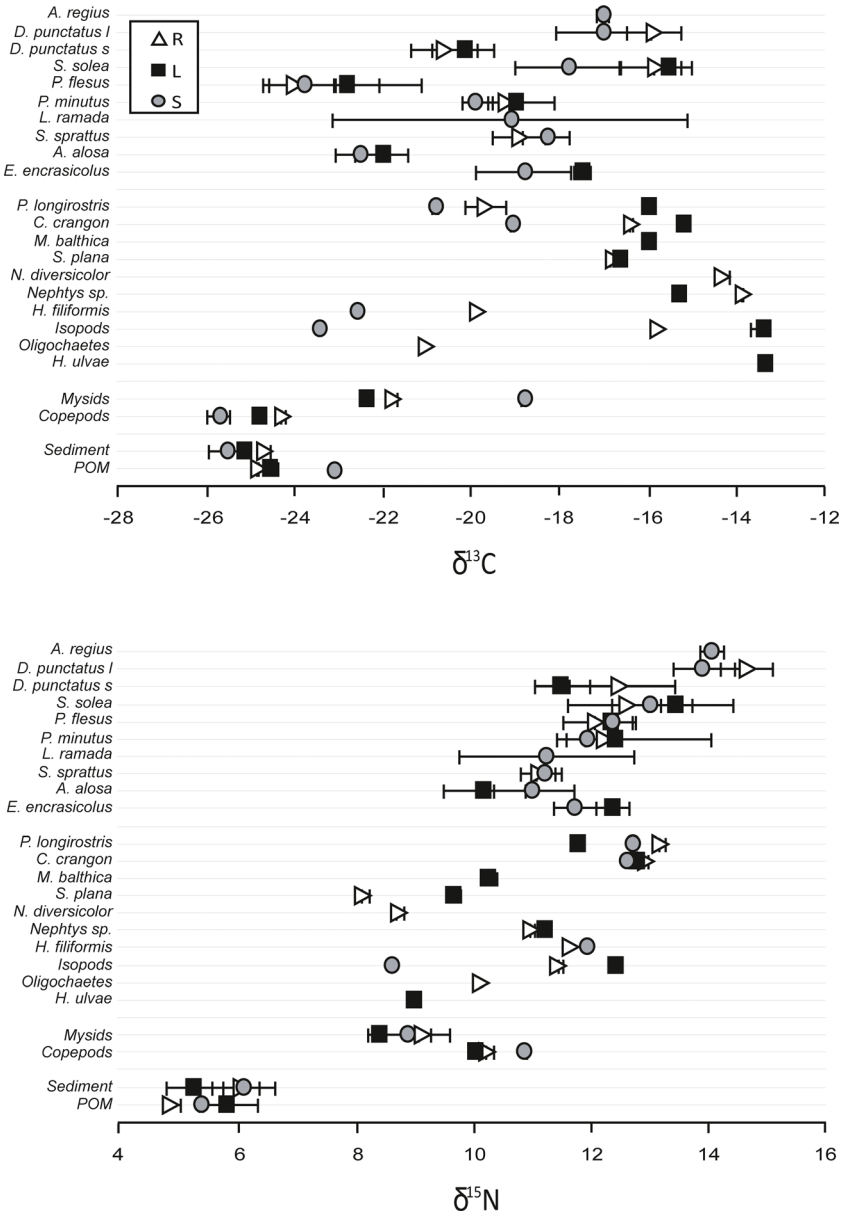

Fig. 2 δ^{13}C and δ^{15}N (mean± standard error) of the primary producers, invertebrates and fish collected in three habitats (*R* Right bank, *L* Left bank and *S* Subtidal) of the Gironde estuary in June–July 2012

and Left bank, respectively) and gastropod *P. ulvae* (mean= −13.4 %) had the most enriched δ^{13}C ratio (Table 3, Fig. 4). Mysids had the lowest δ^{15}N ratio (mean=8.4 to 9.2 %) while meagre *A. regius* (mean=14.1) and large spotted seabass *D. punctatus* (mean=13.9 to 14.6 %) had the highest δ^{15}N ratio (Table 3, Fig. 4), showing a classical increase in δ^{15}N ratio with increasing trophic level. The most enriched, or depleted, δ^{13}C and/or δ^{15}N ratios were not found at any particular site (Fig. 2), even if several organisms (e.g. isopods, *S. plana*, *C. crangon*, *P. longirostris*, *E. encrasicolus*, *A. alosa*, *P. minutus*, *S. solea* and small *D. punctatus*) showed more enriched δ^{13}C values in Left bank (Figs. 2 and 4). Thus, the food webs of Right bank, Left bank and Subtidal were relatively similar; they were composed of more or less the same organisms without any particular distinguishing pattern (Fig. 4). It is worth noting the high δ^{15}N of shrimp *C. crangon* and *P. longirostris* in all food webs.

Feeding Locations

According to isotope signatures, the highest percentage of residents was observed for *S. sprattus*, with 66.6 % of individuals within the central range of isotopic values (Table 4), followed by *P. minutus* and *S. solea* with 64.3 % and 53.3 % of residents, respectively (Table 4). *P. minutus* at Right bank, *S. sprattus* at Subtidal and *S. solea* at Left bank showed no isotopic deviants, i.e. 100 % residents. All other fish species showed a percentage of residents <50 %, suggesting that

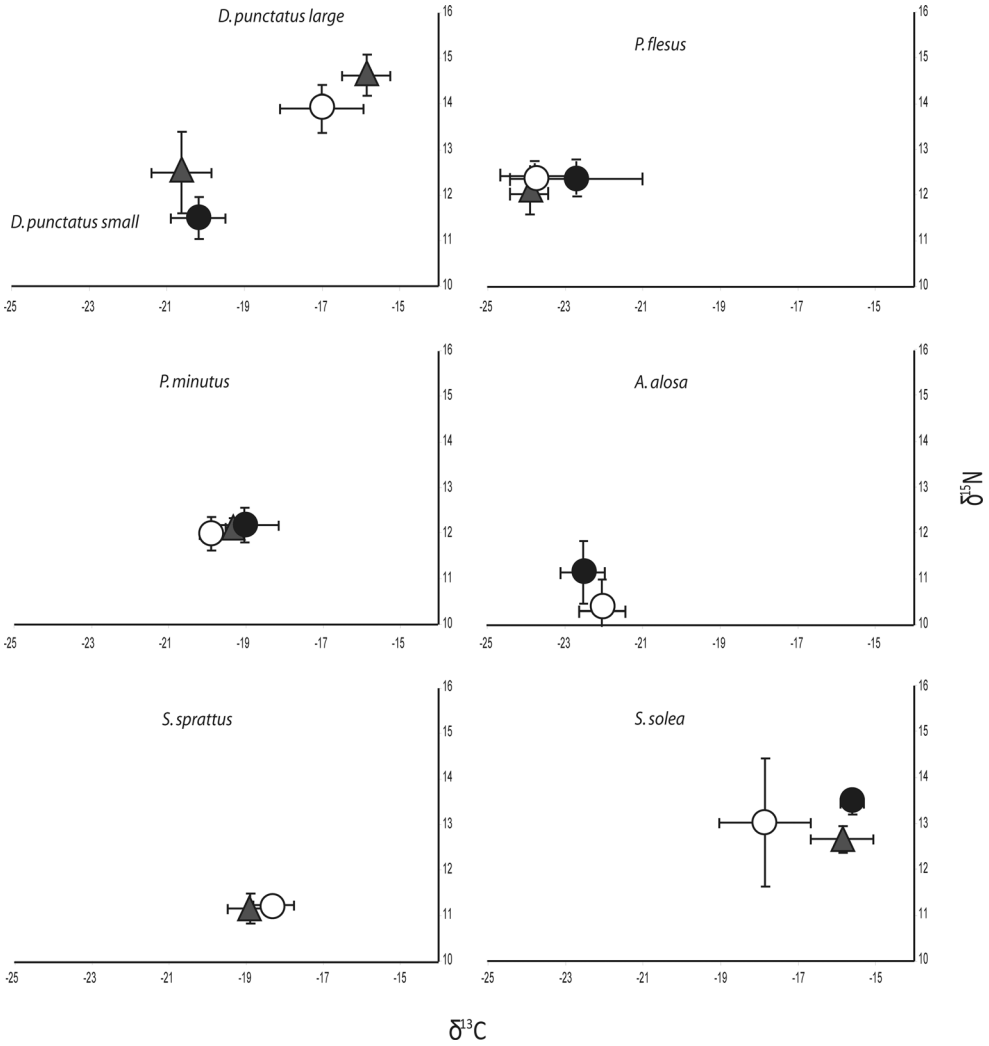

Fig. 3 Fish isotope signatures (mean±standard error) at each of the three habitats in the Gironde estuary in July 2012. *Triangles*, *black dots* and *white dots* refer to Right bank, Left bank and Subtidal, respectively

individuals caught in these habitats did not feed exclusively in these habitats but conversely fed in varied locations (Table 4). Although *S. sprattus* and *P. minutus* also showed a relatively high percentage of residents, i.e. a large number of individuals feeding in similar locations for each habitat suggesting high fidelity for a specific habitat, their dual isotopic signature did not differ among habitats. Only *S. solea* exhibited distinct dual isotopic signatures among the three habitats sampled (see above; Fig. 3), suggesting that this was the only fish species feeding in the habitat where it was collected.

To verify this hypothesis, and show if fish fed exclusively on prey where they were caught or also on prey from other sampled habitats, mixing model contributions of each invertebrate prey from each habitat for each fish species in each habitat were calculated by habitat to estimate the contribution of all prey from each habitat to the diet of each species in each habitat. In general, models showed that fish species collected in a given habitat did not feed exclusively on prey from that habitat (Fig. 5). Conversely, prey from the other two habitats contributed to the diet of fish species caught in each habitat. For example, the diet of large *D. punctatus* collected in Right bank consisted of 42.5 % local prey, 44.0 % prey from Left bank and 13.5 % Subtidal prey (Fig. 5a). Only *S. solea* in Right bank and Left bank and *P. flesus* in Subtidal showed a diet of primarily local prey, with a total contribution nearly 50 % or more (48.5 % for *S. solea* and 60.0 % for *P. flesus*) (Fig. 5a, b, c).

Thus, combining isotope analysis methodologies showed that fish in this study fed in different locations, except *S. solea*, the only species displaying a relevant habitat fidelity.

Source Contributions

The SIAR mixing model estimations of organic matter source contributions to diets of fish in the Gironde estuary were quite similar for eight of the 10 fish species/classes investigated. The major source contributing to these fish species (*E. encrasicolus*, large and small *D. punctatus*, *P. minutus*, *A. regius*, *L. ramada*, *S. solea* and *S. sprattus*) was marine POM with a contribution >75 % for each species (Fig. 6).

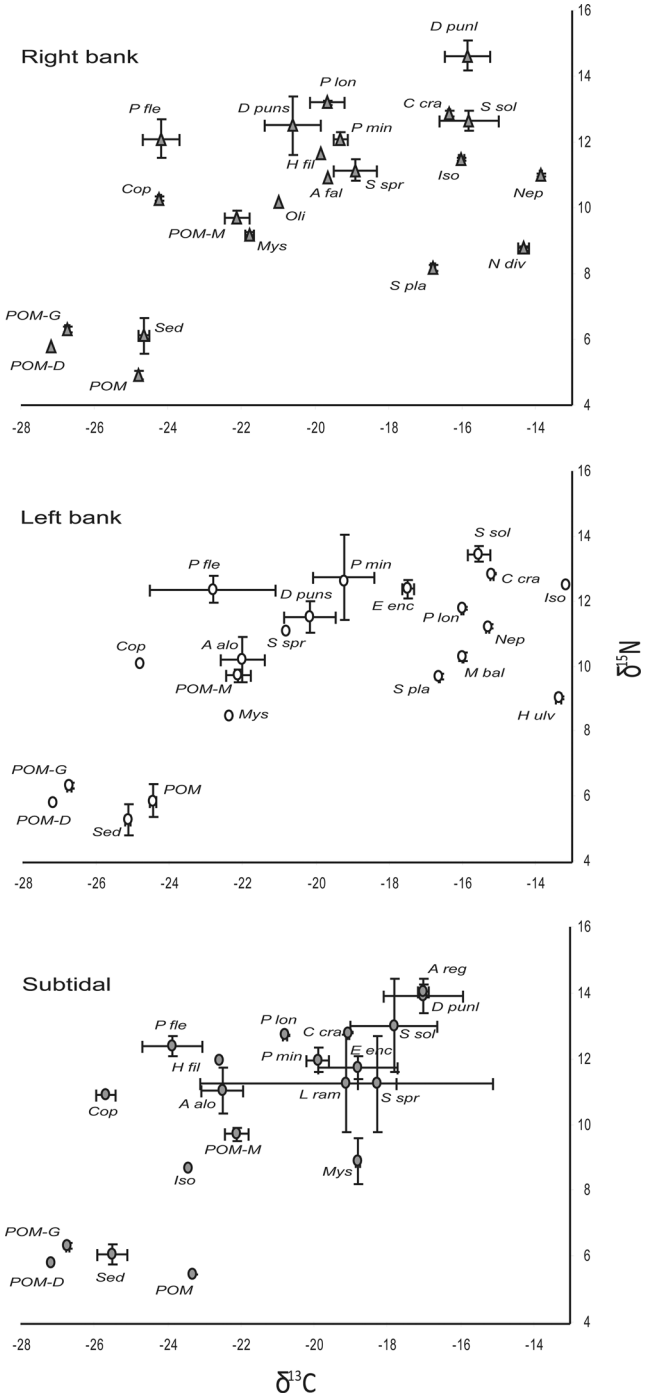

Fig. 4 $\delta^{13}C$ vs $\delta^{15}N$ biplots of food web at each of the three habitats (Right bank, Left bank and Subtidal) in the Gironde estuary in June–July 2012. See Table 3 for species abbreviations

Contributions of sediment, local POM, Garonne POM and Dordogne POM were marginal for these eight species (Fig. 6). Conversely, all sources contributed in more or less similar proportions for *A. alosa* and *P. flesus*, with a noticeable contribution of Garonne POM and Dordogne POM for these two diadromous species, in particular for *P. flesus* where contributions were 20.0 and 19.3 %, respectively (Fig. 6).

Discussion

Food Web Functioning

It was beyond the scope of this study to determine the source of organic matter for the macrozoobenthic community, however, our results suggest strong differences between subtidal and intertidal areas considered in this study. Particularly, the intertidal macrozoobenthic community displayed a range of isotope signatures clearly shifted toward less depleted $\delta^{13}C$ values compared to Subtidal organisms. This indicates that the main Subtidal organic matter sources should be characterized by $\delta^{13}C$ values between −23 and −21 %, considering the isotope signature of Isopod *E. pulchra* (Table 3). Such a value would, according to our results, correspond to marine POM. In contrast, the main source of organic matter for macrozoobenthic primary consumers, such as the suspension/deposit-feeding species *S. plana* and *Macoma balthica* or the grazing/deposit-feeding *P. ulvae*, correspond to $\delta^{13}C$ values around −18 to −16 % which did not match any of the organic matter values for sources or reservoirs sampled in this study. These results strongly suggest that most macrozoobenthic species at this downstream position in the estuary do not use organic matter of terrestrial origin. Sources displaying such a relatively high $\delta^{13}C$ value usually correspond to epipelic microphytobenthic cells as documented by Riera and Richard (1996) or Lebreton et al. (2011). Despite this evidence, we cannot definitively state the importance of this source for macrozoobenthos in the absence of further measurements. In contrast to fish, macrozoobenthic organisms displayed significant, station-specific isotope signatures in direct relation to their very low mobility as adults. These differences between intertidal areas were clearly secondary in terms of magnitude compared to differences between subtidal and intertidal situations and to differences related to the feeding behaviour of species (e.g. Dubois et al. 2014) and probably reflected minor local differences in the availability of organic matter sources.

Significance of Marine POM in the Gironde Fish Food Web

Organic matter origin in the estuarine fish food web was made possible by noting increasing isotopic signatures of primary producers along the salinity gradient (e.g. Darnaude et al. 2004; Pasquaud et al. 2008; Kostecki et al. 2010; Vinagre et al. 2011a; Kopp et al. 2013) and a low increase in $\delta^{13}C$ from prey to predator of 0–1 % (Paterson and Whitfield 1997). In the present study, food sources followed this well-documented pattern in estuarine systems, with increasing $\delta^{13}C$ values of POM from fresh (−27.2±0.1 % and −26.7±0.01 % for the Dordogne and Garonne rivers, respectively) to marine waters (−22.1±0.3 %) (this study; Savoye et al. 2012). Values were sufficiently different to accurately identify the main origin of

Table 4 Isotopic deviants percentage for each fish species at each habitat and total %residents and deviants for each species collected in the Gironde estuary in July 2012

Species	Isotopic deviants			Total % residents	Total % deviants
	R	L	S		
D. punctatus s	66.6 %	50.0 %	/	42.8 %	57.2 %
D. punctatus l	66.6 %	/	83.4 %	22.2 %	77.8 %
P. flesus	75.0 %	100.0 %	100.0 %	9.1 %	90.9 %
P. minutus	0.0 %	75.0 %	40.0 %	64.3 %	35.7 %
A. alosa	/	75.0 %	66.6 %	28.5 %	71.5 %
S. sprattus	50.0 %	/	0.0 %	66.6 %	33.4 %
S. solea	60.0 %	0.0 %	75.0 %	53.3 %	46.7 %

organic matter. In a prior modelling approach, Lobry et al. (2008) suggested that the Gironde estuary food web was totally under river influence in terms of energy and feeding while Pasquaud et al. (2008) concluded that a mixture of estuarine-enriched sources could better explain high $\delta^{13}C$ values observed in fish. With mean values between −24.2 and −15.5 %, the $\delta^{13}C$ values of fishes recorded in that study are closer to marine signals than terrestrial signals, hence toward a marine predominance of the main organic matter source. The observed difference in OM source previously cited is not surprising considering the spatio-temporal scales and number of fluxes investigated in those studies.

In complex and constantly changing ecosystems like estuaries, the identification of sources of organic matter at the base of fish estuarine food webs appeared difficult (Pasquaud et al. 2008), contradictory and difficult to quantify yet according recent literature. While França et al. (2011) reported that ultimate nutrition sources for fish such as *Solea senegalensis*, *Dicentrarchus labrax* and *Pomatoschistus microps* in the Tagus and Mira estuaries were predominantly saltmarsh-derived, Riera et al. (1999) in the Aiguillon cove reported that not, despite the wide availability of saltmarsh plants. Numerous authors showed that in different nursery grounds of Western Europe 0-group fish (often soles) mainly relied on freshwater organic matter (Darnaude et al. 2004, the Rhone river; Kostecki et al. 2010, the Vilaine; Vinagre et al. 2008, the Tagus; Leakey et al. 2008, the Thames and Green et al. 2012, the Blackwater-Colne and Tour-Orwell estuary complexes), even in low flow conditions (Vinagre et al. 2011b). In all cases, a mixture of sources, contributing to different degrees to juvenile fish diets, is probably incorporated into the food web (Riera et al. 1999; Vinagre et al. 2008; França et al. 2011).

Yet, it seems difficult to use the analysis of the carbon isotope ratio to precisely identify and quantify which proportions of organic matter sources are assimilated by fish, especially as the majority of studies are based on $\delta^{13}C$ vs $\delta^{15}N$ biplots (see e.g. Darnaude et al. 2004). Although stable isotope mixing models can be sensitive to variations in Trophic Enrichment Factors (Bond and Diamond 2011), Kostecki et al. (2010) and Le Pape et al. (2013), leading sensibility analyses, recently argued for the combined use of quantitative approaches like SIAR mixing models for accurate estimation of source contributions from stable isotope data. Using this modelling method, organic matter sources for juvenile fishes in the downstream area of the Gironde estuary, formerly identified graphically based on $\delta^{13}C$ vs $\delta^{15}N$ biplots (Pasquaud et al. 2008), could be quantified for the first time. The stable isotope signatures of most fish and invertebrates sampled shows that freshwater and local POM contribute little. Only the diadromous fish flounder *P. flesus* and shad *A. alosa* would use freshwater-derived POM. Given the ecology of these species, they may have spent a part of summer upstream from the zone studied, and hence, assimilated organic matter from the Dordogne or Garonne rivers. We found that marine POM was the main carbon source contribution to juvenile of most fish species in the low Gironde estuary. This confirms the meta-analysis made by Le Pape et al. (2013) which showed the general decrease in freshwater POM exploitation by 0-group soles in their estuarine nurseries and disproved the widespread hypothesis of a larger exploitation of freshwater inputs by juveniles in large estuaries. More interesting, these authors suggested that the contribution of benthic primary production (microphytobenthos+macrophytes) to 0-group growth could be very low in non-tidal nursery habitats and that freshwater-derived POM contribution is proportional to intertidal surface. The observed low freshwater POM-high marine POM contribution in fish food webs reinforces this hypothesis and seems to be explained by the low (10 %) available intertidal surface of the Gironde. Vinagre et al. (2011b) indicated that the Tagus estuary is an area of sediment deposition with ca. 40 % intertidal area, composed mainly of mudflats; thus, much of the sediments and POM carried by river floods get deposited here and can be transferred to fish thorough the food web, also supporting our hypothesis.

In Europe, the dependence of marine fish production on river inputs has been well demonstrated (e.g. Darnaude et al. 2004; Kostecki et al. 2010), including the large contribution of terrestrial organic matter to estuarine fish food webs (Darnaude et al. 2004; Kostecki et al. 2010; Vinagre et al.

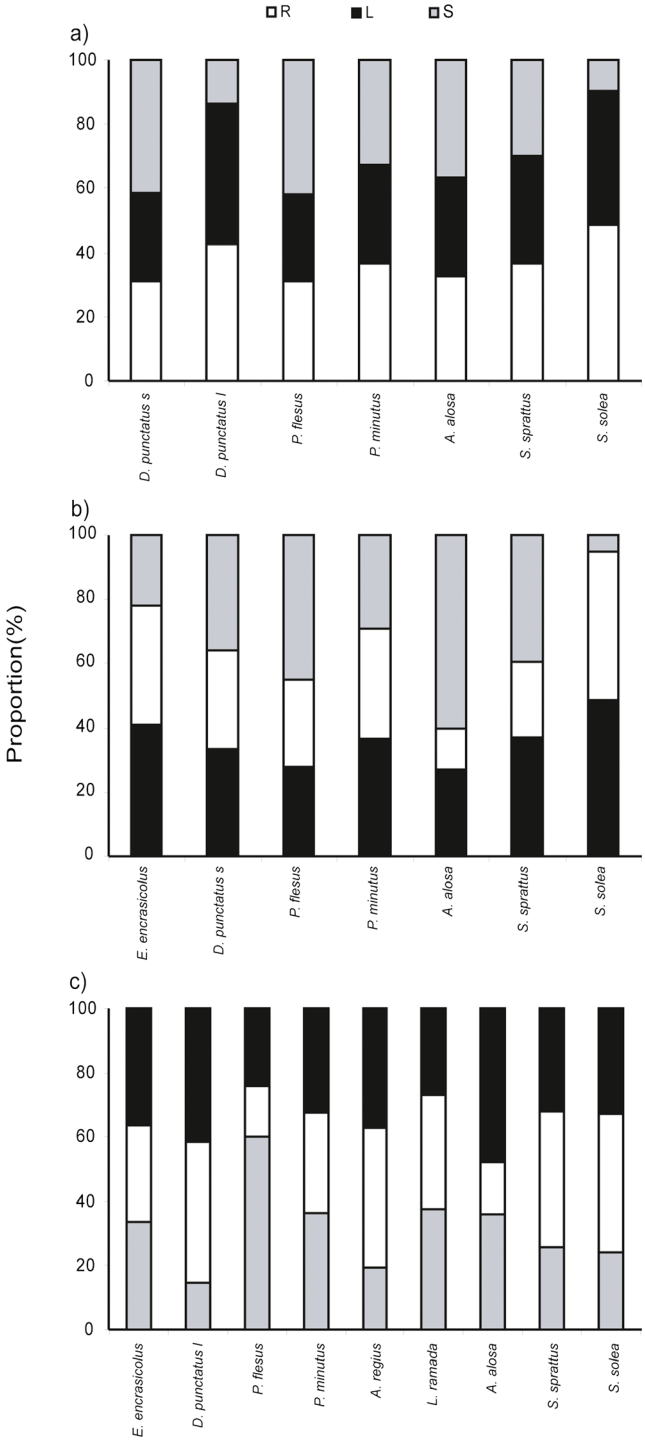

Fig. 5 Mean percentage contribution of habitat food for fish species collected at each of the three habitats (Right bank (**a**); Left bank (**b**); and Subtidal (**c**)) in the Gironde estuary in July 2012

2011b). Hence, drought events have been emphasized as a probable key reason for the decreased production of marine juveniles in estuaries, and hence recruitment (Dolbeth et al. 2008). In the context of global change and increasing anthropogenic freshwater use, more frequent droughts or river input modifications should lower the connectivity of estuarine fish nursery food webs, leading to their fragmentation into subwebs with consequent losses in complexity and resilience and severe consequences on the nursery function of estuarine and coastal ecosystems (Dolbeth et al. 2008). It seems this will not be true for the Gironde estuary, considered an important nursery area for several commercially important fish species in the Bay of Biscay (Lobry et al. 2003), since we show here that, in spite of the high river flow, marine POM was the major carbon contribution to fish food webs.

Since our study was conducted in summer, a winter study, when the river flow of the Gironde estuary is maximum and strength of the terrestrial signal increased (see França et al. 2011; Le Pape et al. 2013), could lend further support to the hypothesis that freshwater POM contribution is influenced by the proportion of intertidal area, rather than river flow. The characterization of the trophic bases, as well as habitat connectivity, for some of the most commercially important fish occurring in the Gironde provides a novel insight for integrated management and conservation of estuarine habitats.

Habitat Connectivity

Isotopic analyses were proposed to study habitat connectivity in estuarine fish (Herzka 2005), however, to do so several requirements must be met. First, differences in the isotopic signatures of local sources and prey among habitats must be established and shown to be reasonably consistent within the time frame and spatial scale of the study (Herzka 2005). Our study demonstrated that potential prey and feeding sources for fish had habitat-specific signatures, confirming the suitability of stable isotopes in tracing fish movements, fidelity and connectivity among estuarine habitats separated by less than 10 km (Durbec et al. 2010), including among habitats with no salinity difference but located on opposite banks, as demonstrated in the present work.

Connectivity is defined as the rate of exchange of individuals of the same species among spatial units (Polis et al. 1997); this can be transposed to the number of "deviants". Here, the number of "deviants" was generally high (±59.0 %). In addition, isotopic signatures generally showed important overlap among habitats for fish species. These considerations suggest that individuals caught in the lower part of the Gironde estuary did not feed exclusively in the habitat in which they were collected, indicating high mobility and habitat connectivity for fish. In comparison, Vinagre et al. (2011a), using this methodology, indicated that 50–87 % of juvenile fish in a bay adjacent to the Tagus estuary were "residents". This divergence with our study could be explained by the different fish species studied, the more varied habitats and/or the much larger spatial scale investigated (Weinstein et al. 2000). In two estuary complexes in the UK, Blackwater-Colne and Stour-Orwell, Green et al. (2012) showed distinctive isotopic

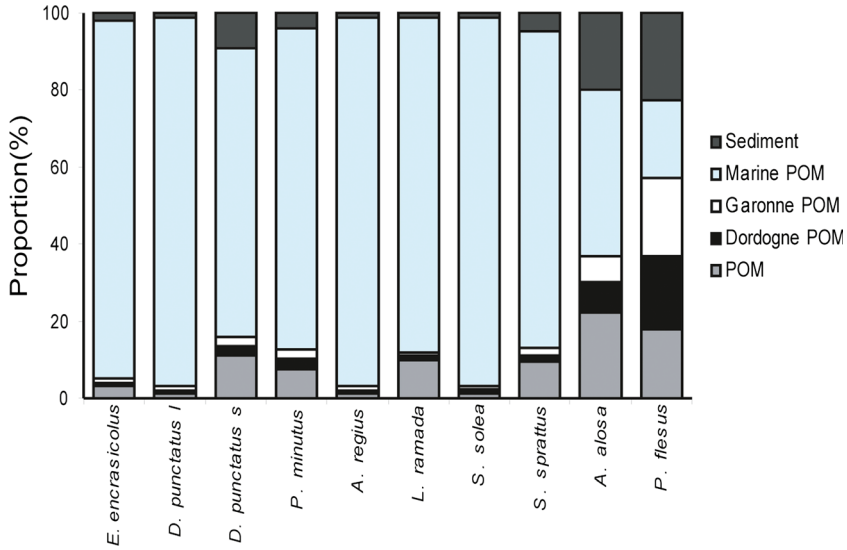

Fig. 6 Mean percentage contribution of organic matter sources supporting fish species in the Gironde estuary in June–July 2012

signatures for several fish species and hence little connectivity among closely located salt marshes. In North American marshes, limited movement of *Fundulus heteroclitus*, ecologically equivalent to the common goby *P. minutus*, were shown, with an estimated home area range of 15 ha (387× 387 m) (Cattrijsse and Hampel 2006). For *D. labrax*, Holden and Williams (1974) reported a short range of movement of the species (16 km). Variations in $\delta^{13}C$ and $\delta^{15}N$ ratios in estuarine fish collected in at least two of the studied habitats here were too small to be significant, although isotopic signatures of sources and invertebrates clearly differed. There was no clear relationship between the isotopic values of fish sampled in a particular habitat and the isotopic signature characteristic of that habitat, except for the sole *S. solea*. According to SIAR models, *S. solea* was the only fish species that predominantly fed on local prey, indicating a narrow range of movement and feeding area for this species which explains the better isotopic distinction among habitats (see Camusso et al. 1999). Conversely, all other species, namely *D. punctatus*, *P. flesus*, *P. minutus*, *A. alosa* and *S. sprattus*, fed from different habitats, suggesting high habitat connectivity/low fidelity for these species. This trend was confirmed by fish trophic levels, which were similar among habitats and could be related to the fact that fish move within the estuary and consume prey from different habitats (França et al. 2011).

Results concerning *S. solea* are in agreement with previous studies showing that this species has a strong relation to the estuarine area it occupies during its first months (e.g. Vinagre et al. 2008). The distinct isotope ratios of juvenile sole identified in this study indicate they would have spent at least 1 month feeding within the locality of any particular habitat, rather than widely dispersing at each tide. Although isotopic signatures differed among habitats, it must however be kept in mind that isotopic turnover rate was not determined for the species measured. Nevertheless, Herzka (2005) reported that young fishes with faster growth rates will equilibrate within days or weeks. Limited movement had already been exhibited by the sole *S. solea* in tagging experiments (Coggan and Dando 1988) or isotope analyses (Vinagre et al. 2008). Vinagre et al. (2008) observed distinct isotopic signatures between nursery areas for 0-group soles *S. solea* and *S. senegalensis* in the Tagus estuary, concluding that there is a low connectivity between the two studied sites due to high site fidelity exhibited by these fish. On the other hand, they reported that although 1-group sole presented different isotopic signatures among nursery areas, they exhibited lower site fidelity with 35.5 % of migrant individuals identified and consequently a larger connectivity. The authors indicated this is probably due to an increase in locomotor capacity and energetic demand with increasing size which leads to broadening of feeding areas. Kopp et al. (2013) also indicated an increase in habitat connectivity, or habitat use, as sole aged. In the present study, the mean length of *S. solea* was 10.7 cm, neighboring 1-group size, which could explain why the isotopic distinction was not as clear as in other studies. It should be interesting in the future to conduct the same work on smaller individuals (4–5 cm) to verify this hypothesis.

Isotopic investigations are very scarce on other fish species studied here (*D. punctatus*, *P. minutus*, *A. alosa*, *P. flesus* and *S. sprattus*). A recent work on ecologically equivalent species (*D. labrax*, *P. microps* and *Clupea harengus*) indicated a clear distinction of isotopic signatures and hence high fidelity among closely located salt marshes (Green et al. 2012). While Green et al. (2012) investigated connectivity among five salt marshes located in two estuaries separated by 9.7 to 59.5 km, the maximum distance between habitats in this study was 11 km, which could explain these different results. Furthermore, salt marshes, because of their associated vegetation, are known to be more isolated habitats with specific

and constant fish assemblages (e.g. Nagelkerken and van der Velde 2004; França et al. 2009), compared to bare mudflats or subtidal areas investigated here. As mentioned above for *S. solea*, it is also known that there is a change in site fidelity as these fish species mature, leading to changes in isotopic values with increasing size. Vinagre et al. (2011b) showed that the smallest individuals of *D. labrax* show low connectivity in the Tagus estuary. They first reported a clear isotopic distinction between 0-groups of *D. labrax* from adjacent nursery areas (21 km separation) and a low level of connectivity between them. In the present study, even small *D. punctatus* showed overlap in isotopic signatures, indicative of high habitat connectivity.

The fact that primary consumers presented isotopic distinction between habitats, while fish did not (except *S. solea*), means that parallel food webs may exist, but with high levels of interaction among them (Vinagre et al. 2008). This seems to give a certain "strength" to the Gironde system. Having a uniquely large food web instead of various relatively discrete sub-webs increases the likelihood that there will be species able to cope in different ways with environmental change (Levin 1999; McCann 2000). Also, it is more likely that in a larger food web, some species are able to replace the function of others (Levin 1999). Yet, the loss of interactions among habitats will lead to a loss in the number of links and thus lowered complexity and connectivity, which can result in increased fragility (McCann 2000). Lower connectivity of estuarine fish nursery food webs leads to their fragmentation into sub-webs with consequent loss in complexity and resilience (Vinagre et al. 2011b). At a time when biological communities will be adapting to alterations induced by climate change, a decrease in resilience may have important consequences for the viability of this ecosystem and its ability to play a functional role for fish (Vinagre et al. 2011b). The importance that an individual estuarine habitat can have in supporting its associated fish community and its interactions with others should be taken into consideration when planning future habitat/estuary conservation measures.

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