

## Dynamics of an estuarine nursery ground: the spatio-temporal relationship between the river flow and the food web of the juvenile common sole (*Solea solea*, L.) as revealed by stable isotopes analysis

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### Abstract:

Estuaries are essential fish habitats because they provide nursery grounds for a number of marine species. Previous studies in the Bay of Vilaine (part of the Bay of Biscay, France) have underlined the estuarine dependence of juvenile common sole (*Solea solea*, L.) and shown that the extent of sole nursery grounds was positively influenced by the variability of the river flow. In the present study, stable carbon and nitrogen isotopes were used to describe the trophic network until the young-of-the-year sole and to compare interannual variations in the dominant trophic pathways in the sole nursery areas in this bay. Particulate organic matter (POM), sediment organic matter (SOM), microphytobenthos, benthic invertebrate sole prey and young-of-the-year common sole were collected during the summer over 4 years characterised by contrasting river discharges. POM isotopic signatures were used to identify the origins of nutrient and organic matter assimilated into the estuarine food web through benthic organisms to juvenile common sole. Interannual spatial variations were found in the POM carbon stable isotope signatures, with the importance of these variations depending on the interannual fluctuations of the river flow. Moreover, the spatio-temporal variability of this POM isotopic signature was propagated along the food webs up to juvenile sole, confirming the central role of river discharge and terrigenous subsidy input in the estuarine benthic food web in determining the size of the sole nursery habitat.

**Keywords:** Juvenile Common Sole; Stable Isotopes; Estuarine Nursery Grounds; Food Web; Eastern Atlantic

## 1. Introduction

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Coastal and estuarine systems are highly productive environments. They are essential fish habitats as they play the role of nursery grounds for many marine species that are widely distributed on the continental shelf (Beck *et al.*, 2001; Peterson, 2003), particularly flatfish (Van der Veer *et al.*, 2000). Hence, the available coastal and estuarine habitats and their quality have a considerable influence on recruitment levels (Rijnsdorp *et al.*, 1992; Gibson, 1994). Human pressure is especially high in these areas, and the natural communities may be threatened. Because juveniles are confined within these coastal and estuarine habitats, recruitment levels and population size can be affected by habitat loss and nutrient and contaminant loading (Johnson *et al.*, 1998; Peterson *et al.*, 2000; Le Pape *et al.*, 2007). It is thus crucial not only to protect these vulnerable habitats, but also to better understand their functioning and to determine optimal habitats in order to support decision-making concerning their management (Rubec *et al.*, 1999; Beck *et al.*, 2001).

Inshore waters of the Bay of Biscay (west coast of France) support nursery areas for several marine species, particularly the common sole *Solea solea* (L., 1758; Dorel *et al.*, 1991). In this site, young-of-the-year (YoY) sole are more abundant in shallow, soft-bottom areas of the estuarine and semi-enclosed sectors of the coast (Le Pape *et al.*, 2003b). In the Bay of Vilaine, an estuarine nursery of the Bay of Biscay, the extent of the nursery grounds and the related young sole abundance have also been shown to be influenced by the variability of the river flow in winter and spring, both before and during young sole settlement (Le Pape *et al.*, 2003c). A link between river flow and fish recruitment has also been observed for sole in the Rhone estuary, France (Salen-Picard *et al.*, 2002) and for other fish species in estuaries all over the world (Quiñones and Montes, 2001; Kimmerer, 2002; Staunton-Smith *et al.* 2004; Rowell *et al.*, 2005). It is assumed that the river flow facilitates the immigration of young fish stages by favouring the development of benthic invertebrates, which are suitable food for young fish (Gibson, 1994). However, the ecological processes implied in this link between river flow and recruitment are still largely unknown.

One way to improve our knowledge about the relationship between river flow, benthic invertebrates and juvenile common sole is to employ trophic tracers, such as stable isotopes. Trophic tracers constitute a powerful tool for ecological studies, allowing nutrient pathways and energy sources to be differentiated, as well as food web interactions and changes in trophic position to be better understood (Peterson and Fry, 1987). Stable isotopes of nitrogen ( $\delta^{15}\text{N}$ ) are often used in ecological studies to assess trophic levels, while stable isotopes of carbon ( $\delta^{13}\text{C}$ ) are used to distinguish production sources (DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Fry, 2006). Compared with marine primary producers, terrestrial producers are significantly  $^{13}\text{C}$ -depleted, and particulate organic matter (POM) from both origins can be differentiated by using stable isotopes of carbon (Vander Zanden and Rasmussen, 2001; Yokoyama and Ishihi, 2007). On the one hand, the transfer of terrestrial POM into benthic food webs (Peterson *et al.*, 1985; Riera *et al.*, 1999; Bouillon *et al.*, 2000; Salen-Picard and Arlhac, 2002; Choy *et al.*, 2008; Zeug and Winemiller, 2008) and the transfers from the lower trophic levels to flatfish juveniles have already been investigated (Gaston *et al.*, 2004; Winemiller *et al.* 2007). Using this methodological approach on benthic fish species in the Rhone, France (Darnaude *et al.* 2004; Darnaude, 2005), the Tagus, Portugal (Vinagre *et al.*, 2008) and the Thames, UK (Leakey *et al.*, 2008), benthic food webs have been shown to depend on freshwater nutrient and organic matter inputs. On the other hand, the extent of the river plume influences the size of the nursery habitat and the number of juveniles produced in the Bay of Vilaine (Le Pape *et al.* 2003c).

The main objective of this study was thus to trace the terrestrial inputs in the trophic network of juvenile sole in the Vilaine Bay and to analyse the spatio-temporal variations of the food sources assimilated by young flatfish in terms of the interannual variations of the river flow.

## 2. Materials and methods

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### 2.1. Vilaine estuary situation and sampling

The Vilaine estuary is located on a shallow coastal inlet of the northern Bay of Biscay (Fig. 1). In this estuary, the tide is blocked by a dam located 10 km upstream of the river mouth. The flow of the Vilaine River varies greatly depending on rainfall, with considerable seasonal and interannual fluctuations (Le Pape *et al.*, 2003d). The Vilaine estuary shelters important nursery grounds for the common sole within the Bay of Biscay (Koutsikopoulos *et al.*, 1989).

Four sampling surveys were undertaken in mid-July 2004, 2005, 2007 and 2008, just after the YoY sole finished colonizing the estuary (Marchand, 1991). Mid-July is a representative sampling period for assessing YoY sole distribution because during their first summer, juvenile sole have a very low mobility, and thus their distribution pattern remains relatively constant (Dorel *et al.*, 1991). These four annual surveys correspond to contrasting river discharge scenarios (Fig. 2). From February to June (*i.e.*, before and during the young sole settlement in the Vilaine estuary; Le Pape *et al.*, 2003cd), the river discharge averaged  $66 \text{ m}^3 \cdot \text{s}^{-1}$  and  $25 \text{ m}^3 \cdot \text{s}^{-1}$  in 2004 and 2005 respectively; thus these years can be considered as dry. Conversely, 2007 and 2008 were rainy years, and the river flow averaged  $115 \text{ m}^3 \cdot \text{s}^{-1}$  and  $123 \text{ m}^3 \cdot \text{s}^{-1}$ , respectively, during the same period.

These surveys were done in similar neap tide conditions. Sampling areas were designated in order to collect YoY sole in two different areas of the study site (Fig. 1). Area A is located at the mouth of the Vilaine river, in the inner estuary just after the Arzal dam, and is greatly influenced by freshwater inflows. Area B is in the external part of the estuary and benefits from the marine influence. These two locations were chosen based on previous knowledge of YoY sole distribution in this estuary (Dorel *et al.*, 1991; Nicolas *et al.*, 2007). They are always part of the nursery area, even during dry years when the extent of the nursery is limited (Le Pape *et al.*, 2003c).

### 2.2 Sample collection and preparation

Juvenile sole were sampled in 2004, 2005, 2007 and 2008 in areas A and B. They were caught by a beam trawl, with a 2.9 m wide and 0.5 m high opening and a 10 mm stretched mesh net cod end. Hauls were carried out at 2.5 knots for 15 min, covering approximately 3400 m<sup>2</sup> on average for each haul. Fourteen beam trawl hauls, distributed over the two study areas, were performed during the 4 surveys, allowing YoY sole to be caught (Table 1). For each haul, trawled sole were measured to estimate their age, and clearly identified YoY (*i.e.*, total fish length under 11 cm) were kept; at least 5 fish per sampling station (Table 1). All the samples were refrigerated on board during the survey, and once back in the laboratory stored at  $-20^\circ\text{C}$ .

At the same sampling station, benthic fauna was simultaneously sampled with a Van Veen grab (0.1 m<sup>2</sup>) (Table 1). Grab samples were sieved, and benthic fauna was sorted and extracted from sediment particles. Organisms were identified to the lowest possible taxonomic level, generally to the species level.

Sediment samples were also collected with the Van Veen grab (0.1 m<sup>2</sup>) at the same sampling stations (Table 1). Sediment Organic Matter (SOM) was obtained by removing carbonates from sediment through acid washing (1 M HCl). The SOM samples were rinsed several times with distilled water and then dried ( $60^\circ\text{C}$ ).

Water samples were collected just before the dam (Fig. 1), where there is no salinity intrusion (*i.e.*, in freshwater) and in area A (Table 1). Particulate Organic Matter (POM) was obtained by filtering 1 l of water over precombusted Whatman GF/F filters and then was stored at  $-20^\circ\text{C}$ . Subsequently, the filters were exposed to HCl vapor for 4 h in order to remove the carbonates, before being placed in tin cups.

Microphytobenthos (MPB) was sampled at low tide on the intertidal section of area A and extracted using the method suggested by Riera and Richard (1996). Two hours after sampling,

a 1 cm thick layer of the sediment was spread on flat trays in the laboratory. A nylon screen (63  $\mu\text{m}$  mesh) was placed on the sediment surface and covered with a 5 mm thick layer of combusted silica powder (60 to 210  $\mu\text{m}$ ). The trays were exposed to light for 12 hours, and the silica powder was kept moist with filtered seawater from the sampling site. Then the top 2 mm of the silica powder, into which the motile microalgae had migrated, were gently scraped and sieved through a 63  $\mu\text{m}$  mesh to separate the microphytobenthos from the remaining silica powder. Microphytobenthos was finally collected on previously combusted Whatman GF/F filters, washed with 1 M HCl, rinsed with Milli-Q water and freeze-dried. These filters were stored at  $-20^{\circ}\text{C}$ .

### 2.3 Stable isotopes

#### Sample preparation

The benthic species selected for isotopic analysis were those that were dominant in terms of abundance and biomass and those that were potential prey for sole juveniles. In order to minimize the lipid effects on  $\delta^{13}\text{C}$  (Bodin *et al.*, 2007), low-lipid muscle tissues were used for the mega- and macro-faunal samples (except polychaetes) used in the stable isotopes analysis. White muscle samples (Table 1) were taken from the dorsal sole musculature as recommended for fish (Pinnegar and Polunin 1999; Sweeting *et al.* 2007), from the abdomen of the Caridea and from the siphon of bivalves. Viscera, setae and the jaw were removed from large specimen of polychaetes by dissection, and the analyses were carried out on the remaining whole body (Le Loc'h and Hily, 2005). After dissection, the tissue samples from every taxon were washed with distilled water in order to prevent any contamination by sediment carbonates (Kharlmanenko *et al.*, 2001; O'Reilly *et al.*, 2002). All samples were frozen individually at  $-20^{\circ}\text{C}$  before freeze-drying. Each dried sample was then ground to obtain a homogeneous powder. Samples were weighed and encapsulated in a fold of tin before stable isotope analysis.

#### Isotopic analysis

The  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$  ratios were determined by continuous-flow isotope ratio mass spectrometry (CF-IRMS). Samples were analysed using a Europa Scientific ANCA-NT 20-20 Stable Isotope Analyser, together with an ANCA-NT Solid/ Liquid Preparation Module (2004 - 2005) and a Finnigan Mat Delta XP isotope ratio mass spectrometer interfaced with a Carlo Erba NC2500 elemental analyzer (2007 - 2008).

As the samples contained more than 10% nitrogen, the CF-IRMS was operated in dual isotope mode, allowing  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  to be measured in the same sample. Isotope ratios were reported in conventional delta ( $\delta$ ) notation as parts per mil (‰) relative to the international standard for Peedee Belemnite Carbonate (C) and atmospheric nitrogen (N). Repeat analyses of the IAEA and laboratory standards (N1, N2, CH6, CH7, acetaminide, cyclohexanone, nicotinamide and bovine liver) showed that maximum standard deviations for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were, respectively, 0.18 and 0.24‰. The standard deviations of the duplicate samples analysed averaged 0.18 and 0.48‰ for carbon and nitrogen. Single measurements were carried out; mean value and associated standard deviation were calculated on the replicated samples.

### 2.4. Data analysis

Dual  $\delta^{13}\text{C}$  -  $\delta^{15}\text{N}$  plots were used to present isotopic signatures of YoY sole *Solea solea* and potential C and N sources in the POM, SOM and MPB, and the benthic invertebrates (including *Hydrobia ulvae*, *Macoma balthica*, *Cerastoderma edule*, *Nephtys hombergii* and *Crangon crangon*) for the 2005 and 2007 surveys in area A. Associated standard deviation were plotted on this graph (points without error are related to single measure on sources). As the topic was

here to focus on nursery function, the entire trophic network in area B and for other years are not presented; general patterns were indicated in the text.

In addition to this graphic analysis, thorough analysis were led on YoY sole. In that aim, error plots and associated Tukey multiple means comparisons were performed after verifying the application conditions. The aim was to test whether or not the C stable isotopic values in YoY soles followed temporal and/or spatial variations. All data for the 2 areas from all 4 years were compared. The first test focused on spatial variability: annual area A and B values were compared (area A versus area B). A second comparison was made to see whether there was any spatial variation within each year (e.g., area  $A_{Year\ i}$  versus area  $B_{Year\ i}$ ,  $i$  is included in [2004,2005,2006,2008]). Finally, a third comparison was made to investigate the interannual variation for both areas in all survey years (e.g., area  $A_{Year\ i1}$  versus area  $A_{Year\ i2}$ ).

### 3. Results

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#### 3.1. YoY sole food web in the Vilaine estuary

In 2005, particulate organic matter (POM) from freshwater was  $^{13}\text{C}$ -depleted and  $^{15}\text{N}$ -enriched compared to POM from area A (Fig. 3 a). Sediment organic matter (SOM) was  $^{13}\text{C}$ -enriched compared with POM. Microphytobenthos (MPB) was the most  $^{13}\text{C}$ -enriched organic matter source compared to all other sources (SOM and POM) (Fig. 3a). Thus, the different freshwater and estuarine food sources (freshwater POM, estuarine POM and SOM, microphytobenthos only present on intertidal part of area A) had distinct  $\delta^{13}\text{C}$  values and can be traced into the trophic network.

Differences in isotopic signatures were also found among benthic invertebrates (Fig. 3a). The bivalves facultative deposit feeder *Macoma balthica* and suspension feeder *Cerastoderma edule* had similar  $\delta^{15}\text{N}$  values but differed in terms of their  $\delta^{13}\text{C}$  values, with *M. balthica* being more  $^{13}\text{C}$ -depleted than *C. edule* (Fig. 3a). The small gastropod *Hydrobia ulvae* was  $^{13}\text{C}$ - and  $^{15}\text{N}$ -enriched compared to SOM and MPB. Both carnivorous taxa *Nephtys hombergii* and *Crangon crangon* had higher  $\delta^{15}\text{N}$  signatures than the bivalves and gastropods. The C and N stable isotope signatures for the 0-group *Solea solea* were very close to *Crangon crangon*. As expected, we observed an increase in  $\delta^{15}\text{N}$  for species occupying higher trophic positions in the food web.

In 2007, POM from freshwater and POM from area A were not different (Fig. 3b); SOM was still  $^{13}\text{C}$ -enriched compared with POM (Fig. 3b). Nevertheless, on the whole, the benthic food web in the nursery depicted by stable isotope analyses did not show a major divergence between sampling years (Fig. 3). In area A, interannual variations were mostly observed for POM, which was  $^{13}\text{C}$ -depleted in 2007 compared with 2005 (Fig. 3). Similarly, benthic invertebrates and YoY sole were  $^{13}\text{C}$ -depleted in 2007 compared to 2005, except for *Cerastoderma edule*. Moreover, we observed an increase in the variance for *Macoma balthica*, *Crangon crangon* and *Solea solea* in 2007 compared to 2005. General pattern was similar for the outer estuary and other years, with the same pattern for different groups but a different  $\delta^{13}\text{C}$  scale.

#### 3.2. Spatial and interannual variations in the isotopic values of YoY soles

$\delta^{13}\text{C}$  values of YoY soles in area A were statistically more depleted than signatures observed in area B, all years included (Fig. 4 and Table 2). More precisely, we found that YoY sole in area A were significantly  $^{13}\text{C}$ -depleted compared to those in area B in 2007 and to a lesser extent in 2004, but not in 2005 nor in 2008.

We found no significant differences in the interannual variations of  $\delta^{13}\text{C}$  in area A between 2004 and 2005, but signatures were significantly higher during these two years than values obtained in 2007 and 2008 (Table 3a and Fig. 4). In area B, such interannual differences were mainly

observed for 2008, which was significantly more depleted than 2004, 2005 and 2007 (Table 3b and Fig.4).

To sum up, we observed a clear gradient between the two study areas, represented by a  $^{13}\text{C}$  depletion being more accentuated inside the estuary. Moreover, the  $\delta^{13}\text{C}$  signatures in juvenile sole in 2007 and 2008 (wet years, Fig. 2) in area A were significantly depleted compared with 2004 and 2005 (dry years). The same tendency was observed in area B, but only for 2008 (Table 3 and Fig. 4).

## 4. Discussion

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### 4.1. The trophic structure of the nursery ground in Vilaine estuary

In the present study, all samples were collected using a protocol comparable to previous studies of the trophic networks of juvenile common sole in estuarine nursery grounds (Darnaude *et al.*, 2004; Leakey *et al.*, 2008; Vinagre *et al.*, 2008). Since isotopic fractionation levels vary among species (Hesslein *et al.* 1993, MacAvoy *et al.* 2001, Guelinckx *et al.*, 2008), the present results cannot be used to provide a complete description of the trophic relationships in an estuarine nursery ground nor to determine accurate trophic levels for the different biological compartments of the food web. Nevertheless, our description of the structure of YoY sole food web in the Vilaine estuary allowed comparisons to be made with descriptions of other studied estuaries.

As in the Tagus estuary (Vinagre *et al.*, 2008), analyzing the food web through its isotopic composition highlighted a clear scheme of interactions between the system components. The nitrogen signatures of organic matter sources, benthic invertebrates and juvenile common sole were different, and this isotopic gradient confirmed the species' trophic levels. There is also a clear difference in origin of organic matter in benthic food webs. Carbon sources can be distinguished through their  $\delta^{13}\text{C}$  values in relation to their terrestrial origins (Peterson *et al.*, 1985; Riera and Richard, 1996; Riera *et al.*, 1999; Bouillon *et al.*, 2000; Yokoyama and Ishihi, 2007; Choy *et al.*, 2008; Zeug and Winemiller, 2008), since freshwater particulate organic matter had lower values than marine POM. Thus, the large range of isotopic signatures measured in the present study allowed the assimilation and transfer of food sources to be traced through the YoY sole nursery food web.

The isotopic signatures of the two bivalve species, *Cerastoderma edule* and *Macoma balthica*, were slightly different, which can be explained by their different diets: *C. edule* is a suspension feeder while *M. balthica* is a facultative deposit feeder (Riera *et al.*, 1999; Rossi *et al.*, 2004) so their diets are not based on the same proportions of POM, SOM and MPB. *Hydrobia ulvae*, a grazer gastropod that mainly feeds on seagrass and benthic diatoms, had an enriched carbon signature compared to the microphytobenthos. *Nephtys hombergii*, a carnivorous polychaete, had a higher isotopic nitrogen signature than the other benthic invertebrates. The other carnivorous invertebrate, the shrimp *Crangon crangon*, had C and N isotopic values very close to YoY *Solea solea*. These three carnivorous species occupy the highest trophic positions among the species sampled in the nursery ground. Based on these marked signatures, the main sources contributing to the energy flow towards the YoY sole originate in organic matter both from the water column and the sediment.

Finally, in the Vilaine estuary, the benthic food web structure (including YoY sole) depicted by stable isotopes is relatively similar to other case studies of the Tagus estuary (Portugal, Vinagre *et al.* (2008), the Gulf of Lions (France, Darnaude *et al.*, 2004) and the Thames estuary (UK, Leakey *et al.*, 2008). *Solea solea* occupied the highest trophic position among the species sampled in the nursery ground. Moreover, estuarine effect has been underlined by following terrestrial organic matter incorporation in benthic food web, from invertebrates to juvenile common sole.

#### 4.2. Spatio-temporal variations in the trophic signature in relation to the river flow

To determine these spatio-temporal variations we focused on the dynamic of the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  inner and outer the Vilaine estuary, paying particular attention to interannual fluctuations in river discharge. Nursery ground trophic structures in July were very similar in wet and dry years. No difference in the relative isotopic gradients was detected in the food web (no difference in interspecies gradients between years), although the wet years were more  $\delta^{13}\text{C}$  depleted than the dry years. The POM carbon signature, which depends on the frequency and intensity of the rains and consecutive river inputs (Malet *et al.*, 2008), was quite variable over the years, no doubt because more terrestrial carbon comes into the estuary in wet years than in dry years. Statistical differences in the carbon signatures of areas A and B were also noticed between rainy (depleted) and dry (enriched) years. These spatio-temporal differences were verified for organic matter, some of the benthic invertebrates and YoY sole. For this reason, it appeared that varying river inputs over the years lead to differences in the POM carbon signatures that propagated throughout the entire benthic food web up to YoY sole in Vilaine estuary, with a downstream range depending on the intensity of the river flow.

This suggests a close relationship between benthic food webs and river input: during dry years (2004 and 2005), the terrestrial carbon signature was only found near the dam at the mouth of the Vilaine estuary, while this signature was also found in marine waters during rainy years (2007 and 2008). The Vilaine benthic food web shifted from a network with a mix of terrestrial and marine influences during dry years to an higher terrestrial influence when river flow is high. Yokoyama and Ishihi (2007) and Choy *et al.* (2008) have already used stable isotope to demonstrate a variation in the food sources of benthic macroinvertebrates along a land–sea transect. The present study confirms these results and provides two other pieces of information: the terrestrial influence differs in intensity and spatial extent from one year to another, with relation to river inputs, and it is propagated up to juvenile flatfish through their trophic interactions.

#### 4.3. Estuarine dependence and determinism in sole recruitment

Benthic invertebrates constitute the food supply for young fish in nursery grounds (Howell *et al.*, 1999; McConnaughey and Smith, 2000; Phelan *et al.*, 2001). For the Vilaine and Loire (an adjacent river) estuaries, Marchand and Masson (1989) and Marchand (1993) have shown the impact of salinity and turbidity on the settling of the benthic communities (dominated by young polychaetes and bivalves) preyed upon by euryhaline fishes, especially the common sole. The development of communities that tolerate low salinity levels provides suitable food for young sole (Costa and Bruxelles, 1989), thus also providing an energetic benefit of feeding in the estuary (Leakey *et al.*, 2008). Darnaude *et al.* (2004) concluded that high land-based run-off was favourable for the productivity of invertebrate prey, and thus for the juvenile common sole in the Mediterranean.

The present study confirms the estuarine opportunism of YoY sole and highlights the interannual variability of the river influence on estuarine nursery grounds, in terms of the river flow. The link between the amount of freshwater input and the spatial range of the nursery ground under estuarine influence has previously been demonstrated both for YoY density and spatial distribution (Le Pape *et al.*, 2003c). The present study adds a trophic dimension to this statistical relationship: the estuarine based trophic chain and its spatio-temporal dynamics could at least partially explain the positive influence of river flow on sole recruitment in the Vilaine estuary and the adjacent Bay (Le Pape *et al.*; 2003 bc), but also more generally in the Bay of Biscay at the level of population (Le Pape *et al.*; 2003 ac). Similarly, Dolbeth *et al.* (2008) have emphasized drought as a probable key reason for the decreased production of marine juvenile species in estuaries.

The ability of benthic fish to exploit terrestrial POM inputs depends on their ecology (Darnaude, 2005; Leakey *et al.*, 2008), and fish assemblages show a trend towards the enrichment of their carbon isotopic signatures from the upper towards the lower estuary (Pasquaud *et al.*, 2008).

Hence, these results on the common sole must be considered as specific, in terms of the life cycle of this species and its relative estuarine opportunism (Araujo *et al.*, 2000; Le Pape *et al.*, 2003ab). Nevertheless, these results about juvenile sole estuarine dependence can also be considered more generally, focusing on the important role played by estuaries as essential fish habitats. Estuaries play a determining role for marine fish population renewal (Beck *et al.*, 2001; Peterson, 2003), especially through the trophic link related to high productivity.

This dependence must be assessed to analyse the consequences of river discharge variations on marine fish populations and related fisheries (Darnaude, 2005). The consequences of lower river inputs linked to anthropogenic uptake of freshwater and climate changes could be harmful (Chicharo *et al.*, 2006; Dolbeth *et al.*, 2008). This could be particularly true if trophic enrichment of the marine fish food chain by terrestrial-derived subsidies is altered during the growth period of fish in estuarine nursery grounds.

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Figures

Figure 1

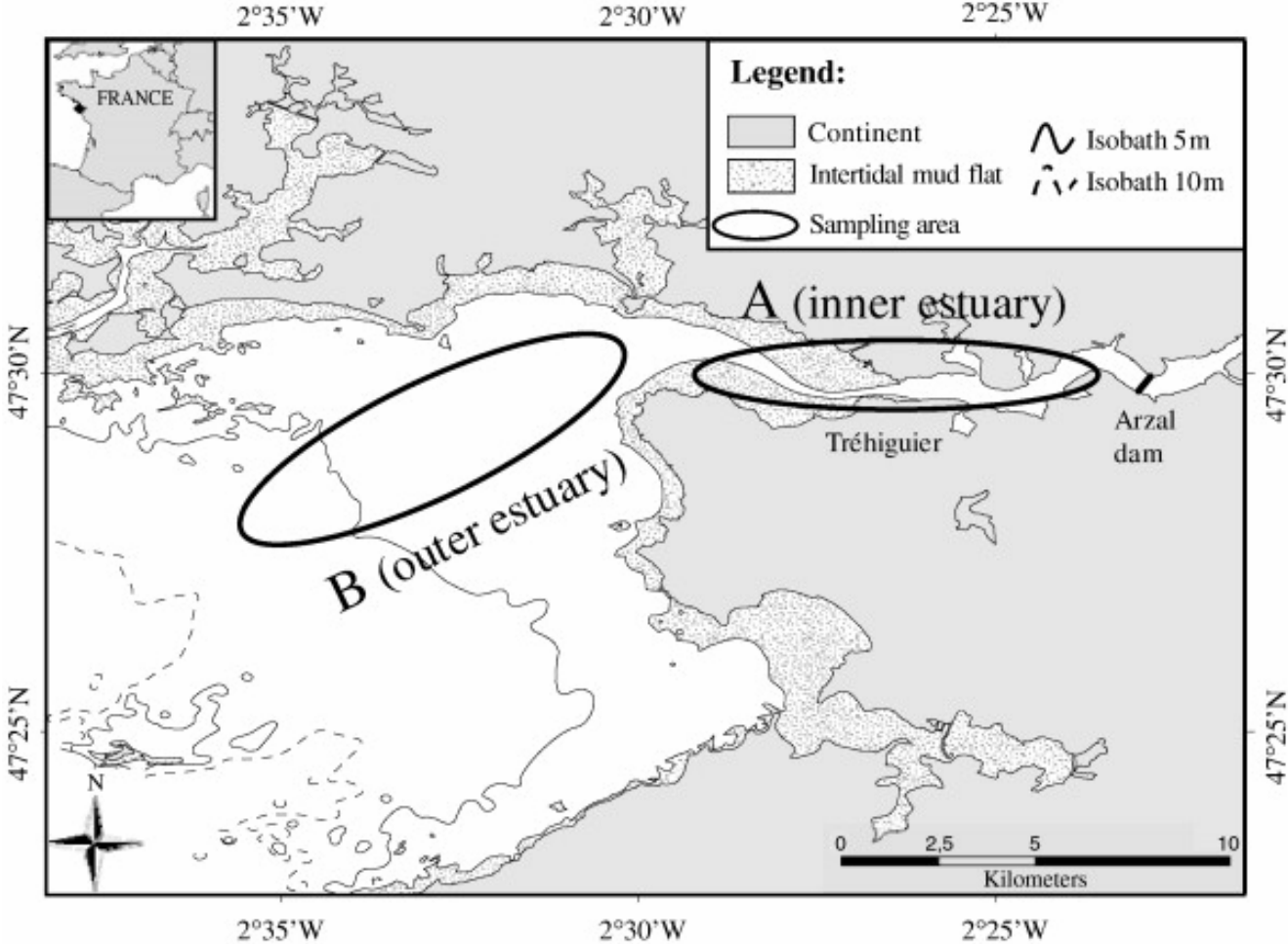


Fig. 1: Vilaine estuary and location of the sampling areas A and B. In the upper left corner of the figure: general location of the study area in France

Figure 2

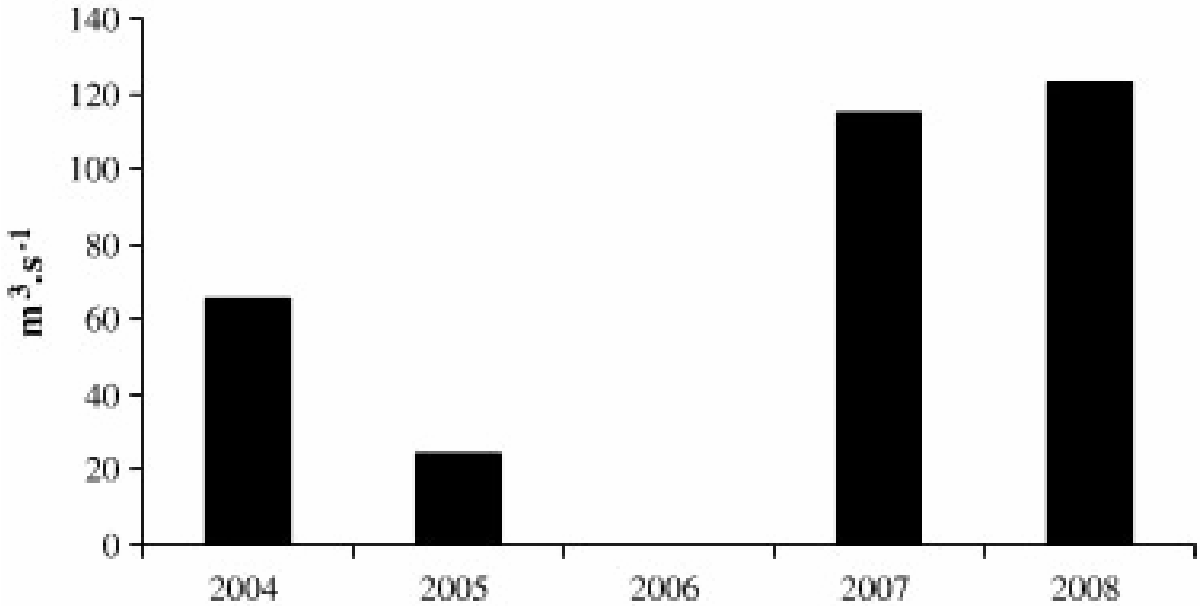


Fig. 2: Mean Vilaine river flow calculated from February to June at Arzal dam (in m³.s⁻¹)

Figure 3

Fig. 3: Mean ( $\pm$  Standard Deviation)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in area A in 2005 (a) and 2007 (b) for carbon and nitrogen sources [particulate organic matter from freshwater (POM fw) and from area A (POM); sediment organic matter (SOM) and microphytobenthos (MPB)], benthic invertebrates [*Macoma balthica* (Mac), *Cerastoderma edule* (Cer), *Hydrobia ulvae* (Hyd), *Nephtys hombergii* (Nep), *Crangon crangon* (Cra)] and young of the year common sole (Sole)

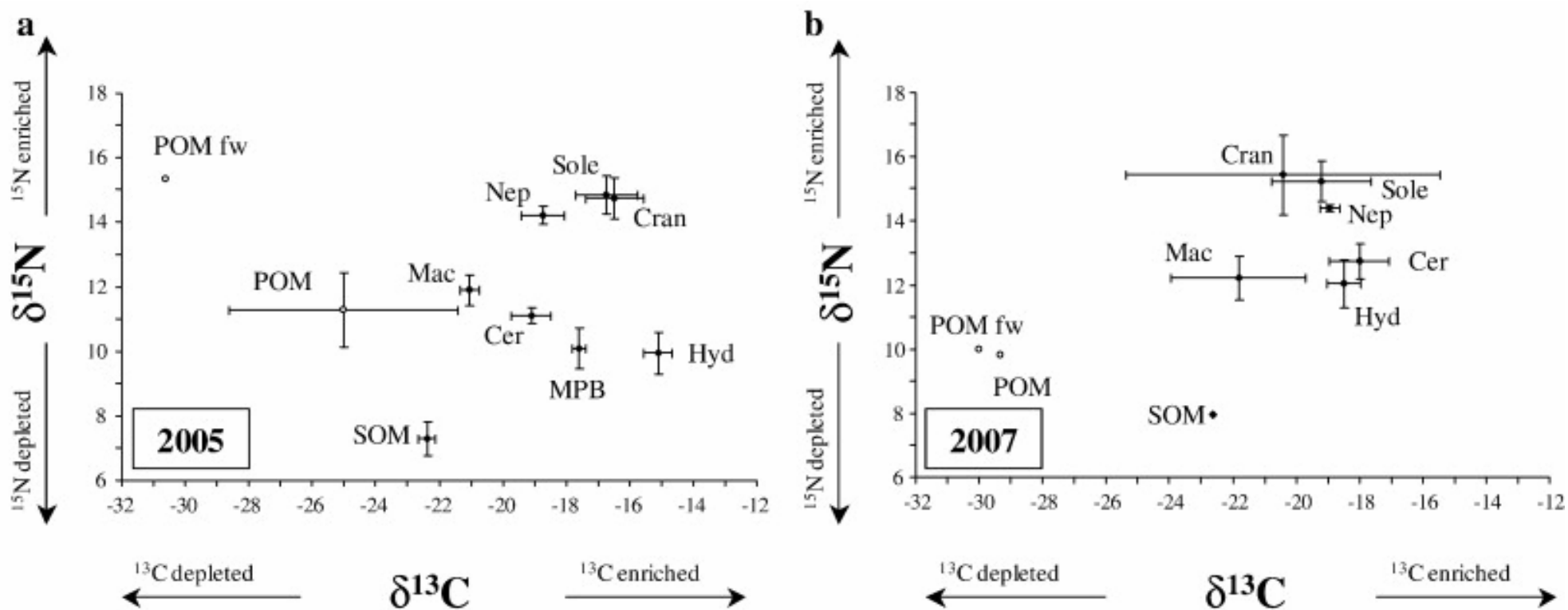




Figure 4

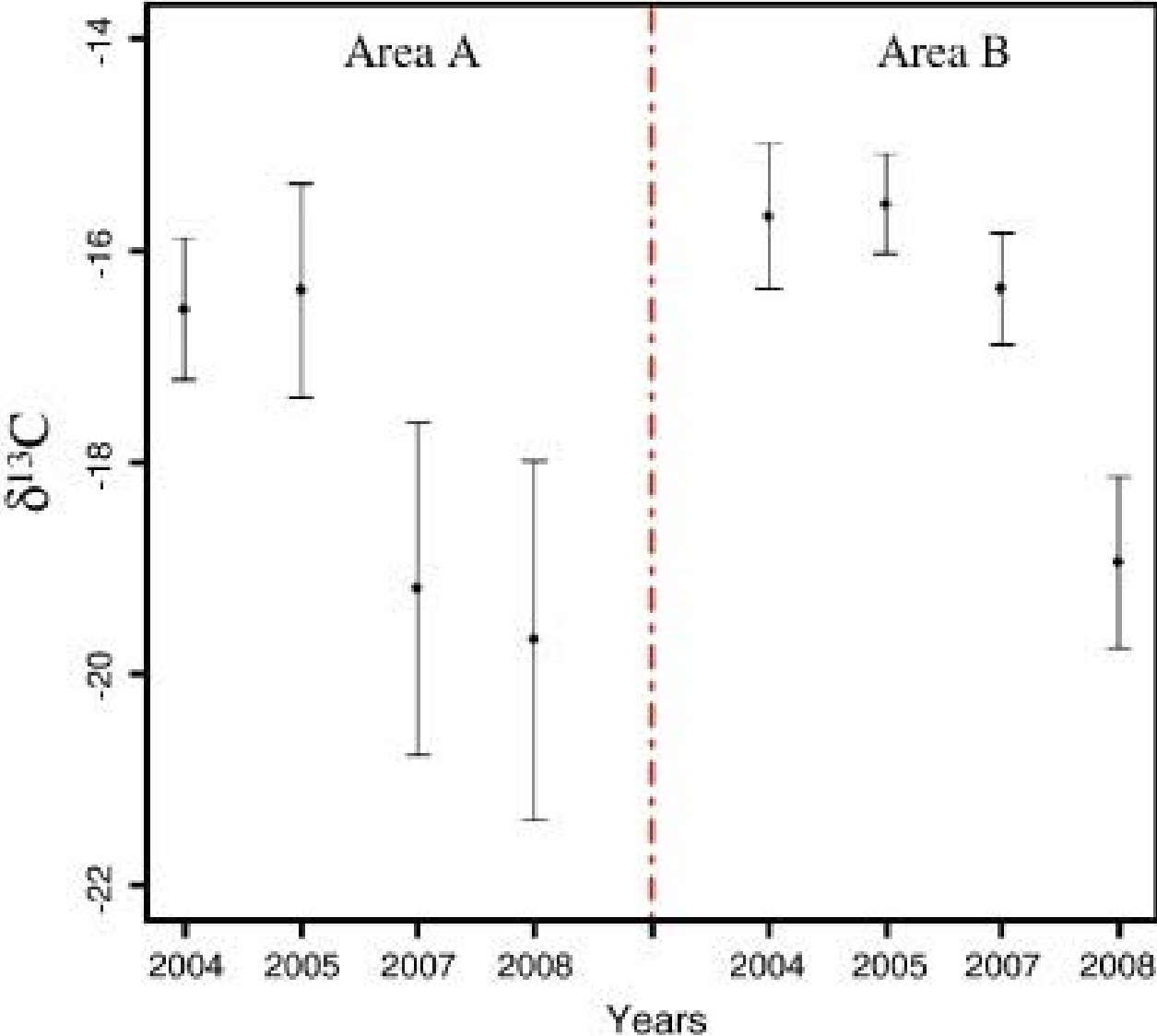


Fig. 4: Mean ( $\pm$  Standard Deviation) stable carbon isotope values for young of the year sole in each area (A and B) for all years (2004, 2005, 2007 and 2008)

## Tables

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Table 1: Number of samples (*Solea solea*, invertebrates and organic matter: POM (Particulate Organic Matter), SOM (Sediment Organic Matter) and MPB (Microphytobenthos)) used in this study for each area and each year

	<b>AREA A</b>	<b>AREA B</b>
<b>2004</b>	<i>Solea solea</i> : 9 individuals Invertebrates: 23 individuals Organic matter: 0 sample	<i>Solea solea</i> : 15 individuals Invertebrates: 15 individuals Organic matter: 0 sample
<b>2005</b>	<i>Solea solea</i> : 18 individuals Invertebrates: 35 individuals Organic matter: 12 samples (6 POM + 3 SOM + 3 MPB)	<i>Solea solea</i> : 6 individuals Invertebrates: 4 individuals Organic matter: 3 samples (3 POM + 0 SOM)
<b>2007</b>	<i>Solea solea</i> : 5 individuals Invertebrates: 39 individuals Organic matter: 4 samples (2 POM + 2 SOM)	<i>Solea solea</i> : 12 individuals Invertebrates: 32 individuals Organic matter: 3 samples (2 POM + 1 SOM)
<b>2008</b>	<i>Solea solea</i> : 24 individuals Invertebrates: 39 individuals Organic matter: 10 samples (8 POM + 2 SOM)	<i>Solea solea</i> : 5 individuals Invertebrates: 9 individuals Organic matter: 4 samples (3 POM + 1 SOM)

Table 2: Tukey test on the stable carbon isotope values for YoY sole between areas

<b>4.1. AREA A / AREA B</b>	
<b>2004</b>	0.008 **
<b>2005</b>	0.105
<b>2007</b>	$<10^{-4}$ ***
<b>2008</b>	0.358
<b>All years</b>	$<10^{-5}$ ***

\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$

Table 3: Tukey test on the stable carbon isotope values for YoY sole between years

5. a) 6. 6.1. AREA A					b) 6.2. 6.3. AREA B				
7.	8.	2005	2007	2008		2005	2007	2008	
	2004	0.992	0.01 *	<10 <sup>-5</sup> ***		2004	0.986	0.044 *	<10 <sup>-7</sup> ***
	2005		0.002 **	<10 <sup>-7</sup> ***		2005		0.103	<10 <sup>-7</sup> ***
	2007			0.895		2007			<10 <sup>-6</sup> ***

\* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001