Journal of Sea Research
August 2013, Volume 81, Pages 40–48
http://dx.doi.org/10.1016/j.seares.2013.03.013

Spatial analysis of the trophic interactions between two juvenile fish species and their preys along a coastal–estuarine gradient

Dorothée Kopp[a,*], Hervé Le Bris[b], Lucille Grimaud[a], Caroline Nérot[c], Anik Brind'Amour[a]

[a] IFREMER, Unité Ecologie et Modèles pour l'Halieutique, Rue de l'île d'Yeu, B.P. 21105, 44311 Nantes Cedex 03, France
[b] Université Européenne de Bretagne, Agrocampus Ouest, UMR 985, 65 rue de St. Brieuc, CS 84215, 35042 Rennes Cedex, France
[c] LEMAR, UMR CNRS/UBO/IRD 6539, Institut Universitaire Européen de la Mer, Place Nicolas Copernic, 29280 Plouzané, France

*: Corresponding author : Dorothé Kopp, tel.: + 33 2 40 37 41 60 ; fax: + 33 2 40 37 40 75 ;
email address : koppdorothee@gmail.com

Abstract:

Coastal and estuarine systems provide nursery grounds for many marine fish species. Their productivity has been correlated with terrigeneous inputs entering the coastal–estuarine benthic food web, thereby favouring the establishment of fish juveniles. Studies in these ecosystems often describe the nursery as a single large habitat without verifying nor considering the presence of contiguous habitats. Our study aimed at identifying different habitats based on macrozoobenthic communities and morpho-sedimentary characteristics and assessing the trophic interactions between fish juveniles and their benthic preys within these habitats. It included 43 sampling sites covering 5 habitats in which we described taxonomically and quantitatively the invertebrates and fish communities with stable isotopes and gut contents. It suggested that the benthic common sole *Solea solea* displayed feeding plasticity at the population level, separating the juveniles (G0) from the older fish (G1) into different "feeding sub-populations". Size-based feeding plasticity was also observable in the spatial occupancy of that species in the studied bay. The demersal pouting, *Trisopterus luscus*, equally used the different habitats but displayed low feeding plasticity across and inside each habitat. Stable isotopes proved to be powerful tools to study the spatial distribution of trophic interactions in complex ecosystems like the bay of Vilaine and to define optimal habitats for fish that use the coastal–estuarine ecosystem as nursery grounds.

Highlights

►Identification of habitats based on benthic macrofauna and morpho-sedimentary data.►Assessment of trophic interactions between juveniles fish and their preys.►Juveniles of sole displayed size-based feeding plasticity according to habitats.►Juveniles of pouting equally used the different habitats.

Keywords: Stable isotopes ; Gut content ; Food web ; Nursery ground ; *Solea solea* ; *Trisopterus luscus*

1. **Introduction**

Located at the sea-continent interface, coastal ecosystems are known as productive areas (Costanza et al., 1997). They foster high primary and secondary production (Largier, 1993) and sometimes tertiary production as they are inhabited by marine species at various stages of their life cycle (Beck et al., 2001). Coastal zones are characterized by local production and inputs of organic matter originating from different sources (e.g. detritic, algal, planktonic) which vary greatly in time and space. These inputs may considerably boost up the coastal and marine production (Maslowski, 2003) and may significantly modify ecosystem functioning (Baird et al., 2004). For instance, seasonal river floods result in an increase in input of macronutrients leading to an increase in plankton production (Nielsen and Richardson, 1996). Benthic production also varies at various temporal scales via the cycling of nutrients between the sediments at the bottom and the overlying water column (Josefson and Conley, 1997). Nutrient enrichment and eventually eutrophication resulting from the pelagic-benthic coupling has a noticeable impact on food availability to the benthic fauna (Darnaude et al., 2004a). The impact of nutrient enrichment on the fluctuation of species abundance in coastal marine communities can be of prime importance in the functioning of these ecosystems (Salen-Picard et al., 2002). Thus terrestrial organic matter has been shown to play a significant role in the dynamics of coastal macrobenthic communities and on the productivity of commercial fish species and fisheries (Darnaude et al., 2004b).

Research on essential coastal habitats, such as nurseries, often describes them as a single large habitat without considering that it is composed of a mosaic of habitats (i.e. seascape) that provides resources for a diversity of species (Ray, 2005). Generally, when more than one habitat is studied, these are examined separately so that little is known about how they interact

69 and function together. The complexity of the spatial organisation of these habitats and the
70 multiplicity of potential organic matter sources that support secondary and tertiary consumers,
71 make fairly challenging the study of the nursery functioning (Deegan and Garritt, 1997).
72
73 Stable isotopes tracking is a powerful tool to apprehend the functional aspects of a nursery's
74 spatial organisation, yet allowing the definition of optimal habitats for fish species that use the
75 coastal-estuarine ecosystem as nursery grounds. Basic rationale of the stable isotopes
76 approach is that the isotopic composition of consumer tissues reflects this of their diet, which
77 in turn depends on the relative proportions of prey species assimilated over a specific time
78 period (De Niro and Epstein, 1978; Minagawa and Wada, 1984; Peterson and Fry, 1987).
79 Stable isotopes of carbon and nitrogen are commonly used to examine consumers' trophic
80 ecology providing a time-integrated measure of trophic position and energy sources. Nitrogen
81 stable isotope ratios in consumers are typically enriched in the heavier (^{15}N) isotope by from 2
82 to 4‰ per trophic level (Minagawa and Wada, 1984; Peterson and Fry, 1987), making $\delta^{15}N$
83 values useful in defining trophic positions of consumers (Post, 2002). The carbon isotope
84 ratios fractionate to a lesser extent (0 to 1‰) and are typically used to define diet
85 compositions or sources of energy (De Niro and Epstein, 1978).
86
87 In estuarine-coastal gradients, the natural variations of stable isotopes allow to distinguish
88 coastal from marine areas either on $\delta^{15}N$ or $\delta^{13}C$. Terrestrial and estuarine waters traditionally
89 present ^{13}C-depleted values compared to marine waters because carbon in materials originated
90 by photosynthesis (e.g. fixed carbon in terrestrial plants or phytoplankton) is depleted in ^{13}C
91 compared to atmospheric CO_2. Seawater $\delta^{13}C$ is supposedly at equilibrium with atmospheric
92 CO_2 (Oana and Deevey, 1960). In the same way, terrestrial waters have traditionally lower
93 $\delta^{15}N$ than marine waters (France, 1995), but nowadays, higher values in $\delta^{15}N$ are often

observed in coastal waters in comparison to marine waters, as coastal ecosystems receive ^{15}N-enriched sewage discharges (anthropisation, agriculture and industries) with river run-offs (Gartner et al., 2002; Schlacher et al., 2005). According to McClelland et al. (1997), wastewater with high NO_3^- derived from human and animal wastes is ^{15}N enriched (+10 to +20‰) and elevates the overall $\delta^{15}N$ signatures of water entering the trophic chain in coastal areas. These variations of stable isotope signatures along seaward gradient, make stable isotopes useful in the identification of the primary sources of organic carbon in the diet of organisms. In such conditions, the isotopic approach has been successfully used to trace the transfer of organic matter through estuarine and coastal food webs (Islam and Tanaka, 2006; Vinagre et al., 2008) and identify aspects of life history or movement patterns of species in nursery habitats (Fry, 2008). Stable isotope ratios ($\delta^{15}N$ or $\delta^{13}C$) were recently used in coastal areas to infer on the relative contribution of different resources use among juveniles of flatfish species (Vinagre et al., 2008; Kostecki et al., 2010; Le Pape et al., 2012) and to quantify the relative contribution of estuarine and coastal production in supporting juveniles fish (Leakey et al., 2008b). They have also proved to be powerful tools to assess ontogenetic size-based shift in fish diet and associated feeding plasticity (e.g. Leakey et al., 2008a for age 1+, 2+ and 3+ common sole; Ho et al., 2009).

The present study aimed to answer the following questions: Do juveniles of benthic and demersal fish species use the bay of Vilaine as one large habitat or as multiple habitats? If so, do these habitats display the same trophic interactions? In order to answer these questions, we defined and assessed the spatial organization of habitats along the estuarine-coastal gradient of the bay of Vilaine nursery ground. More precisely, we (i) identified different habitats based on macrozoobenthic communities and morpho-sedimentary characteristics, (ii) described and tested the biological and ecological differences of these biosedimentary habitats using stable

isotope analyses and fish stomach contents, and (iii) assessed the trophic interactions within these habitats. We hypothesized that benthic species would be more constrained in their use of habitats due to their close relationships with the substrate and associated fauna compared to demersal fish. In the same way we expected that age-0 fish (G0), due to a high site fidelity, would present lower feeding plasticity than older individuals (G1).

2. Material and methods

2.1. Study area and sampling protocol

The study was conducted in the bay of Vilaine located on the French Atlantic coast, south Brittany (Fig. 1). The bay covers a surface area of 230 km² and is characterized by an open shallow muddy estuarine area, under the influence of freshwater inflows. Surveys were carried out at the end of August 2008 using a stratified sampling design relying on a 5-stratum scheme (Fig. 1), in which each stratum was identified by depth range and sediment type. Depths ranged from 5 to 35 m and the sediment types varied from coarse-grained sand and gravel to fine sand and/or coarse silt. Sampling was conducted using a 2.9 m wide and 0.5 m high beam trawl, with a 20-mm-stretched mesh net in the cod-end. Each haul lasted 15 min and covered a mean surface of 4500–5000 m². A total of 43 hauls were performed (Fig. 1, see Table 1 for the number of hauls by habitat).

All fish were identified at the species level and weighted. This study focused however on two of the most abundant species in the bay of Vilaine: the benthic common sole and the demersal pouting. Individuals from these two species were aged according to their length-frequency distributions. The two size-classes corresponded respectively to the young-of-the-year group (G0) and age one group (G1): common sole (G0: 9.09 ± 0.76 cm, G1: 16.45 ± 1.25 cm) and pouting (G0: 7.88 ± 0.96 cm, G1: 11.49 ± 0.93 cm). These size classes were consistent with

144 our own database of otolith measures (unpublished data) and other studies (Merayo and
145 Villegas, 1994; Mérigot et al., 2007). As several studies have highlighted the nursery function
146 of the bay of Vilaine for these two species (Le Pape et al., 2003b), yet only the two size
147 classes associated with the juvenile phase were studied. Individuals of the two species were
148 frozen (-24°C) prior to isotopic and gut content analyses.
149
150 Concurrently, the benthic fauna was sampled at the same sites (43 sampling sites with 4
151 replicates per site) using a Van Veen grab (0.1 m^2). Sediments from the grab were sieved in a
152 cubic screen (1 mm mesh size). Retained fraction (sediments and macro-invertebrates) of 3 of
153 the 4 replicates was fixed and preserved in 10% seawater buffered formaldehyde. The
154 remaining grab replicate was frozen (-24°C) for isotopic analyses. In the laboratory,
155 invertebrates were sorted from the sediments and identified to the lowest taxonomic level,
156 before counting and weighing. Analyses of the benthic fauna were conducted on the summed
157 biomass of the 3 replicates by site. Benthic invertebrate macrofauna was categorized into
158 trophic guilds for the comparison between the habitats: carnivores, detritivores, deposit- and
159 suspension-feeders (Fauchald and Jumars, 1979; Rosenberg, 1993; Hily and Bouteille, 1999;
160 Appeltans et al., 2011). Bottom water was sampled using a Niskin bottle and filtered until
161 clogged through precombusted Whatman GF/F filters (0.5 μm) immediately after sampling.
162 Filters were kept frozen until their extraction to obtain particulate organic matter (POM).
163
164 *2.2. Stable isotope analyses (SIA)*
165 A sample of white dorsal muscle of the fish was dissected and used for SIA (Pinnegar and
166 Polunin, 1999). All samples were frozen individually at −80°C before freeze-drying. Each
167 dried sample was then ground into a homogeneous powder using a mixer mill. Approximately
168 0.2 mg of sample was accurately weighed into small tin cups, and stable isotope ratios of

carbon and nitrogen were analysed in a Carlo Erba NC2500 elemental analyser coupled to a Thermo Finnigan Mat Delta XP isotope ratio mass spectrometer. Isotope ratios were reported in delta notation as per international standards: Pee Dee belemnite carbonate for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. Data were corrected using working standards (bass muscle, bovine liver, nicotinamide; SD < 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$) that were previously calibrated against International Atomic Energy Agency (IAEA) standards.

Benthic invertebrates selected for SIA were those considered as potential preys for the bentho-demersal fish species and dominant in terms of abundance and biomass in the Vilaine coastal-estuarine ecosystem. Isotope analyses were conducted on the muscle of large benthic organisms (i.e. >1 cm), whereas analyses were done on the remaining tissues once the digestive tracts, jaws and cerci were removed for small organisms. Remaining tissues were then washed with distilled water in order to prevent any contamination by sediment carbonates. Samples of small invertebrates (e.g. bivalve juveniles, crustaceans, ophiurids) were divided into 2 subsamples: 1 subsample, for carbon isotope analysis, was acidified with 1% HCl solution to remove carbonates and rinsed with distilled water as carbonates present higher $\delta^{13}C$ values than organic carbon (De Niro and Epstein, 1978). The other subsample, for nitrogen isotope analysis, was not acidified since acidification results in enrichment of $\delta^{15}N$ (Pinnegar and Polunin, 1999). In many cases, several individuals of the same species at a given site had to be pooled to have sufficient biomass for SIA (0.2 mg of dried tissue). Preys were classified in the same trophic guilds as those used for the grab-sampled benthic macrofauna (Fauchald and Jumars, 1979; Rosenberg, 1993; Hily and Bouteille, 1999; Appeltans et al., 2011). Whatman GF/F filters containing particulate organic matter (POM) were oven-dried and subsequently exposed to HCl vapour for 4 hours in order to remove the carbonates (Lorrain et al., 2003). All stable isotope analyses were performed at the Stable

Isotopes in Nature Laboratory, University of New Brunswick, Canada. Number of samples analysed for SIA are given in Fig.2.

For invertebrate tissues, the observed C:N ratios were sometimes greater than 3.5, the value above which lipid normalization is recommended (Post et al., 2007). To account for the influence of lipid content on the $\delta^{13}C$, mathematical delipidation was performed after analysis using the following equation (Post et al., 2007):

$$\delta^{13}C_{normalized} = \delta^{13}C_{untreated} - 3.32 + 0.99 \times C{:}N$$

As $\delta^{15}N$ values provide indication of the trophic position (TP) of a consumer, TP for all prey and fish were calculated following Post equation (Post, 2002):

$$TP = (\delta^{15}N \text{ consumer} - \delta^{15}N\text{base}) / \Delta$$

where Δ is the assumed trophic-enrichment factor for $\delta^{15}N$ values, estimated at 3.4‰ (Minagawa and Wada, 1984). In each habitat, the $\delta^{15}N$ base referred to the lowest available $\delta^{15}N$ value, i.e. that of the particulate organic matter.

2.3. Gut content analyses

To assess the composition of fish diet, the gut contents of 89 common soles and 132 poutings were analysed. All prey items were identified to the highest possible taxonomic level, sorted and counted under a binocular microscope. The use of prey counts instead of prey biomass was preferred to avoid the bias (i.e. overestimation) potentially induced by preys that are partially ingested by juvenile fish (e.g. bivalve siphons). Prey items in gut contents were classified in the same trophic guilds as those used for the grab-sampled benthic macrofauna and SIA. Numbers of analysed gut contents are given in Fig.3.

2.4. Statistical analysis

A common approach to cope with biotic patterns is to select sampling sites along explicit gradients, such as depth and substrate gradients. That strategy is very much like using a stratified sampling in which the strata define habitat patches, and multiple samples within them are treated as approximate replicates, meaning, in practice, that samples within habitat patches are expected to be more similar than samples from different patches. This is the strategy that we have used to identify and characterize the spatial patterns of the bentho-demersal community in the bay of Vilaine. We identified habitat patches using Ward's hierarchical clustering analyses (Ward Jr, 1963) calculated on the Bray Curtis dissimilarity coefficient of the sites-species matrix. The latter was composed of macrobenthic biomass and morpho-sedimentary data (bathymetry, sediment type and organic matter). The Bray Curtis coefficient was chosen as it is acknowledged to be a good measure of ecological distance for species data (Faith et al., 1987). The number of clusters (i.e. habitat patches) was visually assessed and confirmed using the simple structure index SSI (Dolcinar et al., 1999).

Quantitative description of each habitat was done by estimating and comparing diverse indices (biomass, Simpson's diversity index, guild biomass) and isotopic signatures ($\delta^{13}C$, $\delta^{15}N$) of the POM, benthic preys, and fish. As the number of grabs slightly differed between habitats, we preferred the inverse Simpson's index over other measures of diversity: Simpson's index being less vulnerable to sampling effort (Buckland et al., 2005; Colwell, 2009). That index estimates the likelihood that two species selected randomly from the different habitats would be different species. We defined $p_{ij} = d_{ij} / \Sigma_i d_{ij}$ to be the proportion of individuals present in site $_j$ that belong to species $_i$. Simpson's index (D) for site $_j$ is then $_{Dj} = \Sigma_i p_{ij}^2$. For convenience, we used the transformed Simpson's index $-\ln D_j$ such that high values of the index indicate high values of diversity.

The indices and the isotopic signatures of the POM and benthic preys were compared between (previously defined) habitat patches using non-parametric permutation-based one-way ANOVA using habitat as a fixed factor. Permutation procedures were used as the assumption of the normality of residuals was almost always violated. As isotopic signatures may vary according to fish size (Brischoux et al., 2010), comparison of the fish isotopic signatures was conducted using two-way permutation-based analyses of variance with habitat and fish size classes as fixed factors. The interaction of the habitat and fish size was also tested. Yet, the sole and pouting were classified in young-of-the-year (G0) and age-one group (G1). When significant differences were found, multiple comparison tests (i.e. Conover-Inman non-parametric multiple pairwise comparison test) were conducted (Conover, 1999).

Comparison of the fish gut contents between the different habitats was done using distance-based multivariate analyses of variance (MANOVA) with permutations (Anderson, 2001). A two-way design was used, testing the effect of habitat and fish size classes as well as the habitat*size interaction. Distance-based MANOVA is highly similar to its parametric univariate counterpart (ANOVA) in that it uses a multivariate analogue to the Fisher's F-ratio calculated directly from any distance or dissimilarity matrix. In practice, preys abundance from the gut contents of the sole and the pouting were grouped and summed in trophic guilds (i.e. invertebrate carnivores, detritivores, deposit- and suspension-feeders). Matrices of prey guilds were then transformed in distance matrices using Bray-Curtis pairwise distance (double-root transformation). The test was done using unrestricted permutation of raw data (Gonzalez and Manly, 1998) with 4999 random permutations on the full model. Data analyses and statistical tests were implemented using R software (2008) and a significant threshold of 0.05 was used.

3. Results

3.1. Habitats along the coastal-estuarine gradient

Cluster analyses computed on the biomass of the benthic macrofauna combined with morpho-sedimentary data detected five groups of sites along the coastal-estuarine gradient (Fig. 1), hereafter defined as Habitat 1 (H1) to Habitat 5 (H5). These habitats differed in terms of biotic (i.e. species assemblages, relative and total biomass, and species diversity; Table 1) and abiotic conditions (bathymetry, organic matter; Table 2).

3.1.1. Biotic characteristics

The habitat nearest to the mouth of the estuary, H1, was the habitat displaying the lowest mean biomass ($F_{(4,38)}$ = 6.13; p = 0.001). It was however the one displaying the highest Simpson's diversity index (although marginally significant) along with the *Haploops* habitat (H4). It was dominated by the suspension-feeder *Cerastoderma edule* (23% of the total biomass; Table 1). H2 displayed mean values of biomass in comparison to other habitats. Its macrobenthic assemblage was mainly dominated by the polychaete *Owenia fusiformis* and the deposit-feeders (69% of the total biomass). H3 was similar to H2 in terms of functional assemblage, i.e. dominated by a polychaete deposit-feeder, *Sternaspis scutata,* that accounted for almost 30% of the total biomass. The mean biomass in H3 was among the lowest with H1. The habitat H4 was quite different compared to the other four habitats of the bay. It was largely dominated by suspension-feeders and mainly the species *Haploops* sp. (Table 1), a tubiculous and gregarious amphipod considered as an autogenic engineer. It was also the habitat displaying the greatest average biomass (113.55 g/m^2). The fifth habitat (H5) was a marine-influenced habitat that displayed relatively high values of biomass (average: 95.47 g/m^2). It was dominated at 48% by the bivalve *Abra alba*, a facultative suspension- and deposit-feeder. The analyses of the benthic assemblages of the five habitats clearly showed

293 the dominance of species belonging to the deposit- and suspension-feeder trophic guilds: H1
294 and H4 being dominated by suspension-feeders whereas the other three habitats being mainly
295 characterized by deposit-feeders.
296 3.1.2. Abiotic conditions
297 The comparison of the abiotic environment of the habitats suggested the importance of
298 bathymetry (adjusted $R^2 = 0.734$, $p < 0.001$) and organic matter (OM; adjusted $R^2 = 0.319$, $p <$
299 0.001) as forcing variables (Table 2). The distribution of the five habitats was closely related
300 to the bathymetric gradient: H1 located near the mouth of the estuary was the shallower
301 habitat (mean bathymetry = 5.9 m) whereas H5, located far from the estuary was the deeper
302 habitat (mean bathymetry = 24 m). The *Haploops* (H4), located almost in the middle of the
303 bathymetric gradient displayed a significantly larger amount of organic matter (mean OM =
304 5.9% dry weight) in comparison to the other habitats (Table 2).
305
306 *3.2. Habitat distinctiveness using $\delta^{13}C$*
307 The $\delta^{13}C$ values for most of the studied compartments (POM, invertebrate guilds, fish)
308 increased gradually from the river mouth (H1) to the marine habitats (H5), yet confirming the
309 spatial influence of the terrigeneous enrichment in the bay of Vilaine (Fig. 2). That increasing
310 relationship was observable for the $\delta^{13}C$ POM signatures except for the *Haploops* habitat (H4)
311 which was ^{13}C-depleted. Deposit-feeders showed significant differences in their $\delta^{13}C$
312 signatures among habitats ($F_{(4,102)} = 18.26$; $p = 0.001$) and these differences were attributed to
313 H1 and H2 (habitats closest to the estuary) where deposit-feeders were ^{13}C-depleted compared
314 to the marine habitats H3, H4 and H5 (Fig. 2). Suspension-feeders also showed clear
315 distinction among habitats that somehow followed the bathymetric gradient ($F_{(4,64)} = 8.60$; $p =$
316 0.001). Suspension-feeders from H1, H2 and H3 were ^{13}C-depleted and significantly different
317 from those in H4 and H5 which displayed slightly enriched carbon signatures (Fig. 2).

318 Carnivores showed significant differences in their $\delta^{13}C$ signatures among habitats ($F_{(3,20)}$ =
319 4.27; p = 0.027) and these differences could be attributed to H1 where carnivores were
320 carbon-depleted compared to the marine habitat H5 (Fig. 2).
321
322 The fish signatures varied respectively with the species (sole *vs* pouting) and the size class
323 (G0 *vs* G1 sole). The range of variability of the sole $\delta^{13}C$ signatures was larger than for the
324 pouting (Fig. 2). Pouting $\delta^{13}C$ signatures showed a decreasing relationship with depth. The
325 $\delta^{13}C$ values ranged from –15.7 to –16.3 for G0 and from –15.61 to –15.90 for G1. We believe
326 that the decreasing trend is statistically significant probably due to an over power of the test
327 (sample size= 228 for G0 and 122 for G1). Ecologically speaking, the trend represents less
328 than 0.6‰ variation. Young sole were only captured in H1, H2 and H3 and differences were
329 found in their $\delta^{13}C$ signatures ($F_{(2,42)}$ = 4.95; p = 0.009). $\delta^{13}C$ in H1 was 1‰ on average lower
330 than in H2 (p =0.062, very close to the significant threshold of 0.05). That confirms that the
331 spatial distribution of that species age class is influenced by shallow depth. For G1
332 individuals of common sole, a significant difference among habitats was observed ($F_{(3,158)}$ =
333 7.85; p = 0.002): $\delta^{13}C$ signature increased with depth in the same way that fish prey signature
334 increased due to the enhanced influence of marine inputs in deeper waters. The only exception
335 to these sole trends was the signatures in H3 which displayed similar values to H1.
336
337 *3.3. Trophic interactions*
338 3.3.1. Nitrogen signatures and trophic levels
339 In general, $\delta^{15}N$ signatures of the benthic preys and fish were enriched at the mouth of the
340 river and depleted gradually moving towards the sea (Fig. 2). Interestingly, one compartment
341 did not follow that general pattern: G0 sole displayed similar stable $\delta^{15}N$ values (average =
342 14.6) notwithstanding the habitat in which they were captured.

Isotopic $\delta^{15}N$ signatures of POM displayed no significant difference between the five habitats, ($F_{(4,5)}$ = 8.23; p = 0.059). Deposit-feeders displayed significant differences in $\delta^{15}N$ among habitats ($F_{(4,102)}$ = 12.73; p = 0.001) and three groups of habitats could be distinguished. The habitat close to the estuary (H1) which displayed N-enriched signatures, the habitats far from the estuary (H3 and H5) which signatures were slightly lower than the ones in H1, and the habitats H2 and H4 which displayed intermediate values of $\delta^{15}N$. The suspension-feeders also showed differences among habitats ($F_{(4,64)}$ = 15.01; p = 0.001) as well as carnivores ($F_{(3,20)}$ = 9.74; p = 0.001): $\delta^{15}N$ significantly decreased with increasing distance to the estuary. Using the results of stable isotopes, we can well discriminate carnivores from suspension- or deposit-feeders. The $\delta^{15}N$ signatures of carnivores are one trophic level higher than the $\delta^{15}N$ signatures of the other two invertebrate trophic guilds (mean $\delta^{15}N$ of carnivores = 13.66; mean $\delta^{15}N$ of suspension-feeders = 10.42, mean $\delta^{15}N$ of deposit-feeders = 10.45). The stable isotope analyses did not allow to discriminate suspension- from deposit-feeders, as their food resources (e.g. suspended or sediment organic matter) probably display the same range of isotopic signatures and/or some species (e.g. *Abra alba*, *Owenia fusiformis*, …) from these two trophic guilds may display a great feeding plasticity between suspended and sediment organic matter.

As fish go through ontogenetic changes during their life and thus change their feeding habits, we tested the difference in $\delta^{15}N$ values between the habitats for the two fish species by looking at the Size*Habitat interaction (see Section 2.4 for details). The two-way ANOVAs on fish $\delta^{15}N$ signatures showed a significant interaction for the common sole, suggesting that the species likely fed in the different habitats on different food sources and that this relationship was influenced by the size of the fish ($F_{(2,176)}$ = 4.33; p = 0.015). Sole G0 displayed enriched $\delta^{15}N$ values in habitats H2 and H3 in comparison to G1 in the same

habitats (Fig. 2). G0 presented similar $\delta^{15}N$ values in all the sites where they were caught, and a signature equivalent to those observed for sole of G1 in H1 (Fig. 2). In opposition to the findings for the sole, no interaction was found for the pouting, meaning that the species fed on the same variety of preys in all the habitats whatever the size ($F_{(3,262)} = 1.28$; $p = 0.28$).

Calculation of the trophic position of the common sole and pouting confirmed the previous findings on the $\delta^{15}N$ values. G0 and G1 soles displayed a different trophic level in H1, the G0 belonged to 2nd consumers whereas G1 belonged to 3rd consumers. The two size classes had the same trophic position in H2 and H3 (the habitats in which the G0 are found). G0 and G1 individuals of pouting presented the same trophic position notwithstanding the habitats in which they were captured (Fig. 2).

3.3.2. Gut contents

Gut content analyses confirmed the trophic position results for the two studied species (Fig. 3). The common sole displayed a different diet in H1 compared to the other habitats. The diet of that species also differed accordingly with its size in H1 (habitat*size: $F_{(2,63)} = 2.44$; $p = 0.009$). Sole G0 preyed upon detritivores (e.g. *Gammarus* sp.) whereas sole G1 mainly preyed upon deposit-feeders. In the other habitats, common sole fed on carnivores (mainly *Philine aperta*) and suspension-feeders (e.g. *Ampelisca* sp. and small *Acanthocardia echinata*) notwithstanding its size. Pouting fed on similar preys notwithstanding the habitat in which they were sampled (Fig. 3), yet no difference was found in their diet in relation to habitats and size ($F_{(4,98)} = 1.47$; $p = 0.098$).

4. Discussion

393 This study showed that the bay of Vilaine functions as a mosaic of benthic habitats used by
394 the fish. Isotopic values ($\delta^{13}C$ and $\delta^{15}N$) of the organisms within each habitat contributed to
395 identify the spatial extent of the estuarine influence on the bay of Vilaine benthic food web,
396 thereby suggesting the presence of potential spatial food web subsidies in that ecosystem. The
397 large number of contiguous sampling sites allowed us to address the spatial relationships and
398 the connectivity along the coastal-estuarine gradient.
399 Isotopic values of organisms common to all the habitats of the estuary showed a strong
400 gradient from river to coastal habitats. The $\delta^{13}C$ signature of benthic organisms varied
401 accordingly to POM signature (except in H4): increasing gradually from the river mouth to
402 the marine habitats. The spatial heterogeneity was in part due to the terrigeneous-derived
403 organic matter which uptake was noticeable in several habitats, notably in the areas located at
404 the river mouth. Numerous studies from estuarine ecosystems have highlighted estuarine
405 signal in terms of organic matter supply in the benthic food web of coastal nursery grounds in
406 Europe (Darnaude et al., 2004a; Vinagre et al., 2008; Kostecki et al., 2010), America
407 (Simenstad and Wissmar, 1985; Deegan and Garritt, 1997) and Australia (Abrantes and
408 Sheaves, 2008). In these studies, the magnitude of such $\delta^{13}C$ depletion in the fish and
409 invertebrate tissues reached values of 0.5‰ and >1.5‰ in systems under low and high river
410 discharge influence respectively (Darnaude, 2005; Connolly et al., 2009). In our study, the
411 shift observed in $\delta^{13}C$ in the fish and invertebrate tissues captured close to the mouth of the
412 estuary and in the marine habitats were characteristic of large rivers (>4‰). The increasing
413 enrichment gradient in $\delta^{13}C$ from river to shelf was well reflected for species with low
414 mobility (invertebrate carnivores, deposit- and suspension- feeders) and to a certain extent for
415 benthic fish species strongly related to the substrate (e.g. common sole).
416 It is interesting to note that in H4, the habitat dominated by the ecosystem engineer *Haploops*
417 sp., POM $\delta^{13}C$ signatures did not follow the gradient observed in the other habitats and

418 harboured the lowest value. This might be explained by the geochemical process performed
419 by ecosystem engineers that affect the flux and fate of carbon in estuaries (D'Andrea and
420 DeWitt, 2009). Indeed, benthic communities displaying active tube- or burrow-dwelling
421 infauna are often associated with elevated rates of carbon and nutrient remineralization and
422 increased advection of dissolved inorganic carbon and nitrogen from sediments (Kristensen,
423 2008). Physical engineers oxygenate sediments and mix labile organic matter into sediments,
424 thereby stimulating the activity of microbial communities responsible for recycling of carbon
425 and nutrients (Kristensen, 2008; D'Andrea and DeWitt, 2009). As we did not measure
426 biochemical fluxes in the *Haploops* habitat, these are of course hypothetical mechanisms that
427 remain to be verified.
428
429 Mean values of $\delta^{15}N$ for the benthic macrofauna and the fish (other that the sole G0)
430 displayed a coastal-estuarine gradient where highest values were found close to the river
431 mouth and declined as we move towards the shelf. This gradient disagrees with what is
432 commonly found in the literature: a $\delta^{15}N$ depletion from estuarine to marine waters (Wada,
433 1993; France, 1994). However, according to France (1995), $\delta^{15}N$ can vary substantially as a
434 result of anthropogenic inputs and biogeochemical processes (Owens, 1985). Thus agriculture
435 and/or industries may all contribute to enrich terrestrial waters in $\delta^{15}N$. It has been
436 demonstrated that coastal ecosystems receiving sewage discharges display particularly high
437 $\delta^{15}N$ values (Gartner et al., 2002; Schlacher et al., 2005). Since the isotopic composition of
438 nitrogen can propagate throughout a local food web, organisms feeding in areas with high
439 anthropogenic nitrogen inputs can exhibit distinctive $\delta^{15}N$ values (Hansson et al., 1997). The
440 bay of Vilaine receives the waters of a 10 800 km² river watershed conveying inputs from
441 industry, urbanisation and agriculture. The annual flow of nutrients into the bay, evaluated to
442 16000 tonnes of nitrogen and 1030 of phosphorus (Le Bris and Glemarec, 1995), might

443 explain the high nitrogen values found in the POM and in the organisms close to the river
444 mouth.
445
446 Nitrogen rich effluents from the river might enhance the primary production, the productivity
447 of invertebrate preys and be favourable to fish that use the areas as nursery ground (Darnaude
448 et al., 2004a). Using stable isotopes in the bay of Vilaine, Kostecki et al. (2010) aimed to
449 determine the potential mechanisms of the "wet year nursery expansion" hypothesis stated
450 earlier by (Le Pape et al., 2003a). According to that hypothesis, benthic macrofauna would
451 benefit from the nitrogen-enriched terrestrial material sinking from plumes during years of
452 high river flow. By food web propagation, the benthic productivity would enhance fish
453 productivity and provide suitable food for young fish and notably the juveniles of common
454 sole. In that context, the larger the volume of river discharge (i.e. observed in wet years) the
455 further distance the terrestrial material will be carried out, thereby increasing the spatial extent
456 of productive areas (Connolly et al., 2009). Kostecki et al. (2010) showed that during the wet
457 years $\delta^{13}C$ signatures of the G0 sole were typical to those influenced by terrestrial inputs in
458 the habitat that spatially corresponded to H1 (our habitat near the mouth of the estuary). We
459 corroborated their result and hypothesized that the spatial effect of the Vilaine terrestrial
460 inputs can be observable far beyond Kostecki's outer estuary sampling sites, that is up to our
461 H3 (muddy habitat with depth $\leq$ 20m). That extent also corresponds to the limit at which no
462 young-of-the-year of the sole was captured and to recent habitat suitability models developed
463 for the juvenile soles (Trimoreau et al. submitted).
464
465 Studies on coastal food webs often ignore the size structure within species. However, fish
466 may undergo three to four ontogenetic changes during their life cycle and these size-specific
467 changes are frequently associated with habitat changes corresponding to a change in prey

availability (Werner and Gilliam, 1984). The combined use of stable isotopes, gut content analyses and calculation of the trophic position have provided a detailed picture of the spatialized productivity and trophic interactions that likely occur in the bay of Vilaine. This was notably observed in the habitat near the mouth of the estuary which displayed a supplementary trophic level in comparison to the other habitats. The isotopic signatures and gut content analyses in that habitat (H1) revealed that the common sole displayed size-based diet changes eating prey from different trophic guilds. Juveniles of that species preyed primarily upon detritivore species (mainly *Gammarus* sp.). In the Gironde, another French estuary, Pasquaud et al. (2008) found that polychaetes and *Gammarus* were the main food items of the common sole. These authors did not account however for the potential change of diet according to the size of the individuals. In our study, as the sole grew up, dietary changes occurred involving increased contributions of deposivore preys. Sole G1 displayed similar patterns to G0 but with less variability in $\delta^{13}C$ signatures (< 1.5‰ for G1 in comparison to > 2‰ for G0), thereby suggesting an increased in habitat connectivity (or habitat use) as the sole grew older.

The absence of any spatial structure in $\delta^{15}N$ signatures of the G0 common sole confirms our precedent findings with the $\delta^{13}C$ and suggests strong feeding plasticity and opportunism for that age class. Opportunistic feeding was also supported by the gut content analyses which clearly show a large prey spectrum in the habitats they use (H1 *vs* H2 and H3). Studies of the feeding ecology of juvenile flatfishes highlighted opportunistic feeding by many species including the common sole (Lasiak and McLachlan, 1987; Beyst et al., 1999; Cabral, 2000). Feeding flexibility in estuaries and coastal zones allows to exploit peaks of available preys when fishes often need to share resources (Ley et al., 1994). Our conclusions regarding the feeding flexibility of the common sole is in agreement with Leakey (2008a, 2008b) who also found such feeding characteristic at the population level. According to these authors, G0 of

493 sole consumed both estuarine and coastal preys whereas older fish mainly consumed marine
494 food items.
495 The diet of the sole mimics the abundance distribution of the species across the estuarine-
496 coastal gradient: G0 remaining in coastal habitats during their first year and move towards
497 offshore habitats at the end of their first year (Costa et al., 2002). This marine intrusion may
498 indeed explain the carbon isotopic signatures of the G1 sole, reflecting the use of marine
499 sources. Gut content analyses revealed that in H2 and H3, common sole fed predominantly on
500 carnivorous preys. However, as the isotopic signatures of these preys were similar to those of
501 the soles, no such trophic interaction could be confirmed. This result might be explained by
502 the fact that the carnivore prey observed in sole gut contents were mainly composed of
503 *Philine aperta* (respectively 89% and 96% in H2 and H3) but unfortunately no isotope
504 samples of this species could be obtained in the present study. $\delta^{15}N$ signatures of *P. aperta*
505 were assessed in another study conducted in the bay of Vilaine (in habitats corresponding to
506 H2 and H3) and in another French nursery (the bay of Concarneau) by Houssin (2010).
507 Signatures of these species were close to 10‰, a value coherent to those expected here.
508 As opposed to common sole, pouting did not show any size-based feeding plasticity (i.e. low
509 variability in $\delta^{13}C$ signatures for two age classes). The G0 and G1 of that species used
510 indifferently the different habitats (as displayed by the $\delta^{13}C$ values) but showed low feeding
511 plasticity across and within each habitat (as displayed by the $\delta^{15}N$ values). These results
512 suggest that this species easily moves across the habitats, thereby integrating the various $\delta^{13}C$
513 signatures of low mobile preys belonging to these habitats. Similar findings were described in
514 the Thames estuary (Leakey et al., 2008a) where estuarine-caught whiting (another gadid
515 species) had intermediate $\delta^{13}C$ signatures ($\approx$ -18‰), suggesting that whiting moved and fed
516 on preys from estuarine and coastal waters. Similar results were also observed in Netherlands
517 where juveniles of pouting used both estuarine and coastal habitats as nursery grounds

(Hamerlynck and Hostens, 1993). According to Power et al. (2002), salinity would be the major forcing factor explaining the variability of pouting abundance and to a lesser extent food resources. For Gadidae in general, environmental factors such as temperature and salinity seem to have the strongest influence on the species distribution (Power et al., 2002). These comparisons of our results with those from other coastal areas (Gironde and Thames estuaries, South-west of Netherlands, bay of Concarneau) or other functionally similar benthic and demersal species (i.e. senegalese sole or whiting) make us confident that our findings might be generalized to other temperate coastal areas and species that present similar life strategies.

Our study was conducted during summer time, when the influence of the river flow is the least. The strength of the terrestrial signal in coastal food webs increases after periods of high river flow i.e. during autumn and winter for the present study (Bănaru et al., 2007). At this period, trophic subsidies of benthic compartments by river discharge are expected to be firmly installed. In that context, we believe that it is very unlikely that the season of our study influence the spatial patterns that we observed in here. Indeed, the seaward $\delta^{13}C$ gradient in the bay of Vilaine during winter is also apparent, only its amplitude differ (Mortillaro et al., unpublished data). In another ecosystem, França et al. (2011), observed similar results when studying two estuarine systems of the Portuguese coast. They concluded that seasonal differences were not pronounced enough to produce significant dissimilarities in the species isotopic signatures and the estuarine functioning between the two seasons.

Beside the *Haploops* habitat, the macrozoobenthic habitats defined in our study are generally common to all open shallow muddy estuarine area under the influence of freshwater inflows (e.g. Seine: Elkaim et al., 1982; Dauvin et al., 2006). We are confirming the general pattern

observed in these ecosystems, that terrestrial inputs are structuring coastal and estuarine food webs. Given that the bay of Vilaine offers different habitats for the G0 of several species, including the common sole (Le Pape et al., 2003b) and that strong environmental changes are actually occurring in these habitats (+1.5°C in temperature and +2 in salinity; Kopp et al., Submitted), our study raises important questions: Are all the habitats close to the river mouth equivalent in terms of G0 production? Will the environmental changes affect the species distribution? What is the relative importance of the trophic interactions versus the physical environment in all the described habitats? Do species inhabiting these habitats display similar growth rates? In the context of multiple coastal uses and coastal management, these are crucial questions that remain to be answered.

References

Abrantes, K., Sheaves, M., 2008. Incorporation of terrestrial wetland material into aquatic food webs in a tropical estuarine wetland. Estuar. Coast. Shelf. Sci. 80, 401-412.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32-46.

Appeltans, W., Bouchet, P., Boxshall, G.A., Fauchald, K., Gordon, D.P., Hoeksema, B.W., Poore, G.C.B., van Soest, R.W.M., Stöhr, S., Walter, T.C., Costello, M.J., 2011. World Register of Marine Species. Available at www.marinespecies.org [accessed on October 2012].

Bănaru, D., Harmelin-vivien, M., Gomoiu, M.-T., Onciu, T.-M., 2007. Influence of the Danube River inputs on C and N stable isotope ratios of the Romanian coastal waters and sediment (Black Sea). Mar. Poll. Bull. 54, 1385-1394.

Baird, D., Christian, R.R., Peterson, C.H., Johnson, G.A., 2004. Consequences of hypoxia on estuarine ecosystem function: Energy diversion from consumers to microbes. Ecol. Appl. 14, 805-822.

Beck, M.W., Heck, K.L., Able, K.W., Childers, D.L., Eggleston, D.B., Gillanders, B.M., Halpern, B., Hays, C.G., Hoshino, K., Minello, T.J., Orth, R.J., Sheridan, P.F., Weinstein, M.R., 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. Bioscience 51, 633-641.

Beyst, B., Cattrijsse, A., Mees, J., 1999. Feeding ecology of juvenile flatfishes of the surf zone of a sandy beach. J. Fish. Biol. 55, 1171-1186.

Brischoux, F., Bonnet, X., Cherel, Y., Shine, R., 2011. Isotopic signatures, foraging habitats and trophic relationships between fish and seasnakes on the coral reefs of New Caledonia. Coral Reefs 30, 155-165.

Buckland, S.T., Magurran, A.E., Green, R.E., Fewster, R.M., 2005. Monitoring change in biodiversity through composite indices. Philos. Trans. R. Soc. B-Biol. Sci. 360, 243-254.

Cabral, H.N., 2000. Comparative feeding ecology of sympatric *Solea solea* and *S. senegalensis*, within the nursery areas of the Tagus estuary, Portugal. J. Fish. Biol. 57, 1550-1562.

Colwell, R.K., 2009. Biodiversity: Concepts, Patterns, and Measurement, in: Levin, S.A. (Ed.), The Princeton Guide to Ecology. Princeton University Press, Princeton, NJ., pp. 257-263.

Connolly, R.M., Schlacher, T.A., Gaston, T.F., 2009. Stable isotope evidence for trophic subsidy of coastal benthic fisheries by river discharge plumes off small estuaries. Mar. Biol. Res. 5, 164-171.

591 Conover, W.J., 1999. Practical non-parametric statistics, third ed. John Wiley and Sons, New
592 York.
593 Costa, M.J., Cabral, H.N., Drake, P., Economou, A.N., Fernandez-Delgado, C., Gordo, L.,
594 Marchand, J., Thiel, R., 2002. Recruitment and production of commercial species in
595 estuaries, in: Elliott M., Hemingway, K.L. (Eds), Fishes in Estuaries. Blackwell
596 Science LTD, Oxford, pp. 54-123.
597 Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K.,
598 Naeem, S., O'Neill, R.V., Paruela, J., Raskin, R.G., Sutton, P., van den Belt, M., 1997.
599 The value of the world's ecosystem services and natural capital. Nature 387, 253-260.
600 D'Andrea, A.F., DeWitt, T.H., 2009. Geochemical ecosystem engineering by the mud shrimp
601 *Upogebia pugettensis* (Crustacea: Thalassinidae) in Yaquina Bay, Oregon: Density-
602 dependent effects on organic matter remineralization and nutrient cycling. Limnol.
603 Oceanogr. 54, 1911-1932.
604 Darnaude, A.M., 2005. Fish ecology and terrestrial carbon use in coastal areas: implications
605 for marine fish production. J. Anim. Ecol. 74, 864-876.
606 Darnaude, A.M., Salen-Picard, C., Harmelin-Vivien, M.L., 2004a. Depth variation in
607 terrestrial particulate organic matter exploitation by marine coastal benthic
608 communities off the Rhone River delta (NW Mediterranean). Mar. Ecol. Progr. Ser.
609 275, 47-57.
610 Darnaude, A.M., Salen-Picard, C., Polunin, N.V.C., Harmelin-Vivien, M.L., 2004b.
611 Trophodynamic linkage between river runoff and coastal fishery yield elucidated by
612 stable isotope data in the Gulf of Lions (NW Mediterranean). Oecologia 138, 325-332.
613 Dauvin, J.C., Desroy, N., Janson, A.L., Vallet, C., Duhamel, S., 2006. Recent changes in
614 estuarine benthic and suprabenthic communities resulting from the development of
615 harbour infrastructure. Mar. Pol. Bull. 53, 80-90.

616 Deegan, L.A., Garritt, R.H., 1997. Evidence for spatial variability in estuarine food webs.
617 Mar. Ecol. Progr. Ser. 147, 31-47.
618 De Niro, M.J., Epstein, S., 1978. Influence of diet on distribution of carbon isotopes in
619 animals. Geochim. Cosmochim. Acta 42, 495-506.
620 Dolcinar, S., Grabler, K., Mazanec, J.A., 1999. Analyzing Destination Images: a Perceptual
621 Charting Approach,. Available at http://ro.uow.edu.au/commpapers/310 [accessed on
622 October 2011].
623 Elkaim, B., Ibanez, F., Proniewski, F., 1982. Le benthos subtidal de l'estuaire de la Seine:
624 analyse statistique des peuplements. J. Exp. Mar. Biol. Ecol. 64, 231-252.
625 Faith, D.P., Minchin, P.R., Belbin, L., 1987. Compositional dissimilarity as a robust measure
626 of ecological distance. Vegetatio 69, 57-68.
627 Fauchald, K., Jumars, P.A., 1979. The diet of worms: a study of polychaete feeding guilds.
628 Oceanogr. Mar. Biol. Ann. Rev. 17, 193-284.
629 França, S., Vasconcelos, R.P., Tanner, S., Máguas, C., Costa, M.J., Cabral, H.N., 2011.
630 Assessing food web dynamics and relative importance of organic matter sources for
631 fish species in two Portuguese estuaries: A stable isotope approach. Mar. Environ.
632 Res. 72, 204-215.
633 France, R., 1994. Nitrogen isotopic composition of marine and freshwater invertebrates. Mar.
634 Ecol. Prog. Ser. 115, 205-207.
635 France, R., 1995. Stable nitrogen isotopes in fish: Literature synthesis on the influence of
636 ecotonal coupling. Estuar. Coast. Shelf Sci. 41, 737-742.
637 Fry, B., 2008. Open bays as nurseries for Louisiana brown shrimp. Estuaries Coasts 31, 776-
638 789.

639 Gartner, A., Lavery, P., Smit, A.J., 2002. Use of delta N-15 signatures of different functional
640 forms of macroalgae and filter-feeders to reveal temporal and spatial patterns in
641 sewage dispersal. Mar. Ecol. Prog. Ser. 235, 63-73.
642 Gonzalez, L., Manly, B.F.J., 1998. Analysis of variance by randomization with small data
643 sets. Environmetrics 9, 53-65.
644 Hamerlynck, O., Hostens, K., 1993. Growth, feeding, production, and consumption in 0-
645 group bib (*Trisopterus luscus* L.) and whiting (*Merlangius merlangus* L.) in a shallow
646 coastal area of the South-west Netherlands. ICES J. Mar. Sci. 50, 81-91.
647 Hansson, S., Hobbie, J.E., Elmgren, R., Larsson, U., Fry, B., Johansson, S., 1997. The stable
648 nitrogen isotope ratio as a marker of food-web interactions and fish migration.
649 Ecology 78, 2249-2257.
650 Hily, C., Bouteille, M., 1999. Modifications of the specific diversity and feeding guilds in an
651 intertidal sediment colonized by an eelgrass meadow (*Zostera marina*) (Brittany,
652 France). C.R. Biol. 322, 1121-1131.
653 Ho, C.-T., Fu, Y.-C., Sun, C.-L., Kao, S.-J., Jan, R.-Q., 2009. Plasticity of feeding habits of
654 two *Plectroglyphidodon* damselfishes on coral reefs in Southern Taiwan: Evidence
655 from stomach content and stable isotope analyses. Zool. Stud. 48, 649-656.
656 Houssin, L., 2010. Diversité et structure trophique de la macrofaune chalutée associée aux
657 habitats à *Haploops* spp. de Bretagne. MSc thesis, Université de Bretagne Occidentale
658 - Institut Universitaire Européen de la Mer, Brest (France).
659 Islam, M.S., Tanaka, M., 2006. Spatial variability in nursery functions along a temperate
660 estuarine gradient: role of detrital versus algal trophic pathways. Can. J. Fish. Aquat.
661 Sci. 63, 1848-1864.
662 Josefson, A.B., Conley, D.J., 1997. Benthic response to a pelagic front. Mar. Ecol. Progr. Ser.
663 147, 49-62.

664 Kostecki, C., Le Loc'h, F., Roussel, J.M., Desroy, N., Huteau, D., Riera, P., Le Bris, H., Le
665 Pape, O., 2010. Dynamics of an estuarine nursery ground: the spatio-temporal
666 relationship between the river flow and the food web of the juvenile common sole
667 (*Solea solea*, L.) as revealed by stable isotopes analysis. J. Sea Res. 64, 54-60.
668 Kristensen, E., 2008. Mangrove crabs as ecosystem engineers with emphasis on sediment
669 processes. J. Sea Res. 59, 30-43.
670 Largier, J.L., 1993. Estuarine fronts – How important are they. Estuaries 16, 1-11.
671 Lasiak, T., McLachlan, A., 1987. Opportunistic utilization of mysid shoals by surf-zone
672 teleosts. Mar. Ecol. Progr. Ser. 37, 1-7.
673 Le Bris, H., Glémarec, M., 1995. Macrozoobenthic communities of an oxygen under-
674 saturated coastal ecosystem: The Bay of Vilaine (Southern Brittany). Oceanologica
675 Acta 18, 573-581.
676 Le Pape, O., Chauvet, F., Desaunay, Y., Guerault, D., 2003a. Relationship between
677 interannual variations of the river plume and the extent of nursery grounds for the
678 common sole (*Solea solea*, L.) in Vilaine Bay. Effects on recruitment variability. J.
679 Sea Res. 50, 177-185.
680 Le Pape, O., Chauvet, F., Mahevas, S., Lazure, P., Guerault, D., Desaunay, Y., 2003b.
681 Quantitative description of habitat suitability for the juvenile common sole (*Solea*
682 *solea*, L.) in the Bay of Biscay (France) and the contribution of different habitats to
683 the adult population. J. Sea Res. 50, 139-149.
684 Le Pape, O., Modéran, J., Beaunée, G., Riera, P., Nicolas, D., Savoye, N., Harmelin-Vivien,
685 M., Darnaude, A.M., Brind'Amour, A., Le Bris, H., Cabral, H., Vinagre, C., Pasquaud,
686 S., França, S., Kostecki, C., 2013. Sources of organic matter for flatfish juveniles in
687 coastal and estuarine nursery grounds: a meta-analysis for the common sole (*Solea*
688 *solea*) in contrasted systems of Western Europe. J. Sea Res. 75, 85-95.

689 Leakey, C.D.B., Attrill, M.J., Jennings, S., Fitzsimons, M.F., 2008a. Retrospective
690 quantification of estuarine feeding activity by coastally caught marine fishes. J. Sea
691 Res. 60, 210-214.
692 Leakey, C.D.B., Attrill, M.J., Jennings, S., Fitzsimons, M.F., 2008b. Stable isotopes in
693 juvenile marine fishes and their invertebrate prey from the Thames Estuary, UK, and
694 adjacent coastal regions. Estuar. Coast. Shelf Sci. 77, 513-522.
695 Ley, J.A., Montague, C.L., McIvor, C.C., 1994. Food habits of mangrove fishes: a
696 comparison along estuarine gradients in Northeastern Florida Bay. Bull. Mar. Sci. 54,
697 881-899.
698 Lorrain, A., Savoye, N., Chauvaud, L., Paulet, Y.M., Naulet, N., 2003. Decarbonation and
699 preservation method for the analysis of organic C and N contents and stable isotope
700 ratios of low-carbonated suspended particulate material. Anal. Chim. Acta 491, 125-
701 133.
702 Maslowski, J., 2003. Effects of trophic conditions on benthic macrofauna in the vicinity of the
703 River Swina mouth (Pomeranian Bay; southern Baltic Sea). Oceanologia 45, 41-52.
704 McClelland, J.W., Valiela, I., Michener, R.H., 1997. Nitrogen-stable isotope signatures in
705 estuarine food webs: A record of increasing urbanization in coastal watersheds.
706 Limnol. Oceanogr. 42, 930-937.
707 Merayo, C.R., Villegas, M.L., 1994. Age and growth of *Trisopterus luscus* (Linnaeus, 1758)
708 (Pisces, Gadidae) off the coast of Asturias. Hydrobiologia 281, 115-122.
709 Mérigot, B., Letourneur, Y., Lecomte-Finiger, R., 2007. Characterization of local populations
710 of the common sole *Solea solea* (Pisces, Soleidae) in the NW Mediterranean through
711 otolith morphometrics and shape analysis. Mar. Biol. 151, 997-1008.

712 Minagawa, M., Wada, E., 1984. Stepwise enrichment of ^{15}N along food chains: further
713 evidence and the relation between ^{15}N and animal age. Geochim. Cosmochim. Acta
714 48, 1135-1140.
715 Nielsen, E., Richardson, K., 1996. Can changes in the fisheries yield in the Kattegat (1950-
716 1992) be linked to changes in primary production? ICES J. Mar. Sci. 53, 988-994.
717 Oana, S., Deevey, E.S., 1960. Carbon 13 in lake waters, and its possible bearing on
718 paleolimnology. Am. J. Sci. 256-A, 253-272.
719 Owens, N.J.P., 1985. Variations in the natural abundance of ^{15}N in estuarine suspended
720 particulate matter – a specific indicator of biological processing. Estuar. Coast. Shelf
721 Sci. 20, 505-510.
722 Pasquaud, S., Elie, P., Jeantet, C., Billy, I., Martinez, P., Girardin, M., 2008. A preliminary
723 investigation of the fish food web in the Gironde estuary, France, using dietary and
724 stable isotope analyses. Estuar. Coast. Shelf Sci. 78, 267-279.
725 Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. Ann. Rev. Ecol. Syst. 18,
726 293-320.
727 Pinnegar, J.K., Polunin, N.V.C., 1999. Differential fractionation of delta C-13 and delta N-15
728 among fish tissues: implications for the study of trophic interactions. Funct. Ecol. 13,
729 225-231.
730 Post, D.M., 2002. Using stable isotopes to estimate trophic position: Models, methods, and
731 assumptions. Ecology 83, 703-718.
732 Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., Montaña, C.G.,
733 2007. Getting to the fat of the matter: models, methods and assumptions for dealing
734 with lipids in stable isotope analysis. Oecologia 152, 179-189.

735 Power, M., Attrill, M.J., Thomas, R.M., 2002. Environmental influences on the long-term
736 fluctuations in the abundance of gadoid species during estuarine residence. J. Sea Res.
737 47, 185-194.
738 R Development Core Team, 2008. R: A language and environment for statistical computing R
739 Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0,
740 available at http://www.R-project.org.
741 Ray, G.C., 2005 Connectivities of estuarine fishes to the coastal realm. Estuar. Coast. Shelf
742 Sci. 64, 18-32.
743 Rosenberg, R., 1993. Suspension feeding in *Abra alba* (Mollusca). Sarsia 78, 119-121.
744 Salen-Picard, C., Darnaude, A.M., Arlhac, D., Harmelin-Vivien, M.L., 2002. Fluctuations of
745 macrobenthic populations: a link between climate-driven river run-off and sole fishery
746 yields in the Gulf of Lions. Oecologia 133, 380-388.
747 Schlacher, T.A., Liddell, B., Gaston, T.F., Schlacher-Hoenlinger, M., 2005. Fish track
748 wastewater pollution to estuaries. Oecologia 144, 570-584.
749 Simenstad, C.A., Wissmar, R.C., 1985. Delta C13 evidence of the origins and fates of organic
750 carbon estuarine and nearshore food webs. Mar. Ecol. Progr. Ser. 22, 141-152.
751 Vinagre, C., Salgado, J., Costa, M.J., Cabral, H.N., 2008. Nursery fidelity, food web
752 interactions and primary sources of nutrition of the juveniles of *Solea solea* and *S-*
753 *senegalensis* in the Tagus estuary (Portugal): A stable isotope approach. Estuar. Coast.
754 Shelf Sci. 76, 255-264.
755 Wada, E., Kabaya, Y., Kurihara, Y., 1993. Stable isotopic structure of aquatic ecosystems. J.
756 Biosci. 18, 483-499.
757 Ward Jr, J.H., 1963. Hierarchical grouping to optimize an objective function. J. Am. Stat.
758 Assoc. 58, 236-244.

759 Werner, E.E., Gilliam, J.F., 1984. The ontogenetic niche and species interactions in structured
760 populations. Ann. Rev. Ecol. Syst. 15, 393-425.
761

761 **Table 1.** Summary of the different biotic variables in each habitat. Mean values and standard

762 deviations (in brackets) are given.

	Habitat 1	Habitat 2	Habitat 3	Habitat 4	Habitat 5
Dominant species (in biomass)	*Cerastoderma edule*	*Owenia fusiformis*	*Sternaspis scutata*	*Haploops* sp.	*Abra alba*
Total biomass (g/m^2)	14.22 (20.79)	78.87 (53.89)	32.33 (14.63)	113.55 (72.45)	95.47 (39.17)
Inverse Simpson' index	2.63 (2.23)	1.74 (0.72)	1.63 (0.81)	3.30 (2.94)	1.83 (0.59)
Guild relative biomass (%)					
Carnivores	8.80	12.56	11.95	1.94	5.78
Detritivores	0.050	0.01	0.04	0.00	0.00
Deposit- feeders	40.44	69.43	50.21	7.38	79.66
Suspension-feeders	46.82	17.99	37.47	90.68	14.35
Scavengers	3.89	0.01	0.33	0.00	0.21

763

764

764 **Table 2.** Summary of the different abiotic variables in each habitat. Mean values and standard

765 deviations (in brackets) are given.

	Habitat 1	Habitat 2	Habitat 3	Habitat 4	Habitat 5
Bathymetry (m)	5.91 (0.61)	9.30 (1.79)	13.64 (3.80)	18.55 (1.95)	24.00 (6.46)
Organic matter (% dry weight)	5.81 (2.63)	5.80 (2.37)	7.92 (2.21)	10.46 (0.87)	6.60 (1.22)
Sediment type	Mud	Mud and sand	Mud	Consolidated mud	Mud and sand
Number of hauls	7	10	9	5	12

766

767

768

768 Figure captions

769

770 **Fig. 1.** Bathymetric map of the bay of Vilaine in the northern Bay of Biscay and geographic
771 position of the sampling sites. 1, 2, 3, 4 and 5 = habitats. Habitats were defined using a
772 hierarchical cluster analysis on a matrix of invertebrates biomass combined with morpho-
773 sedimentary data.

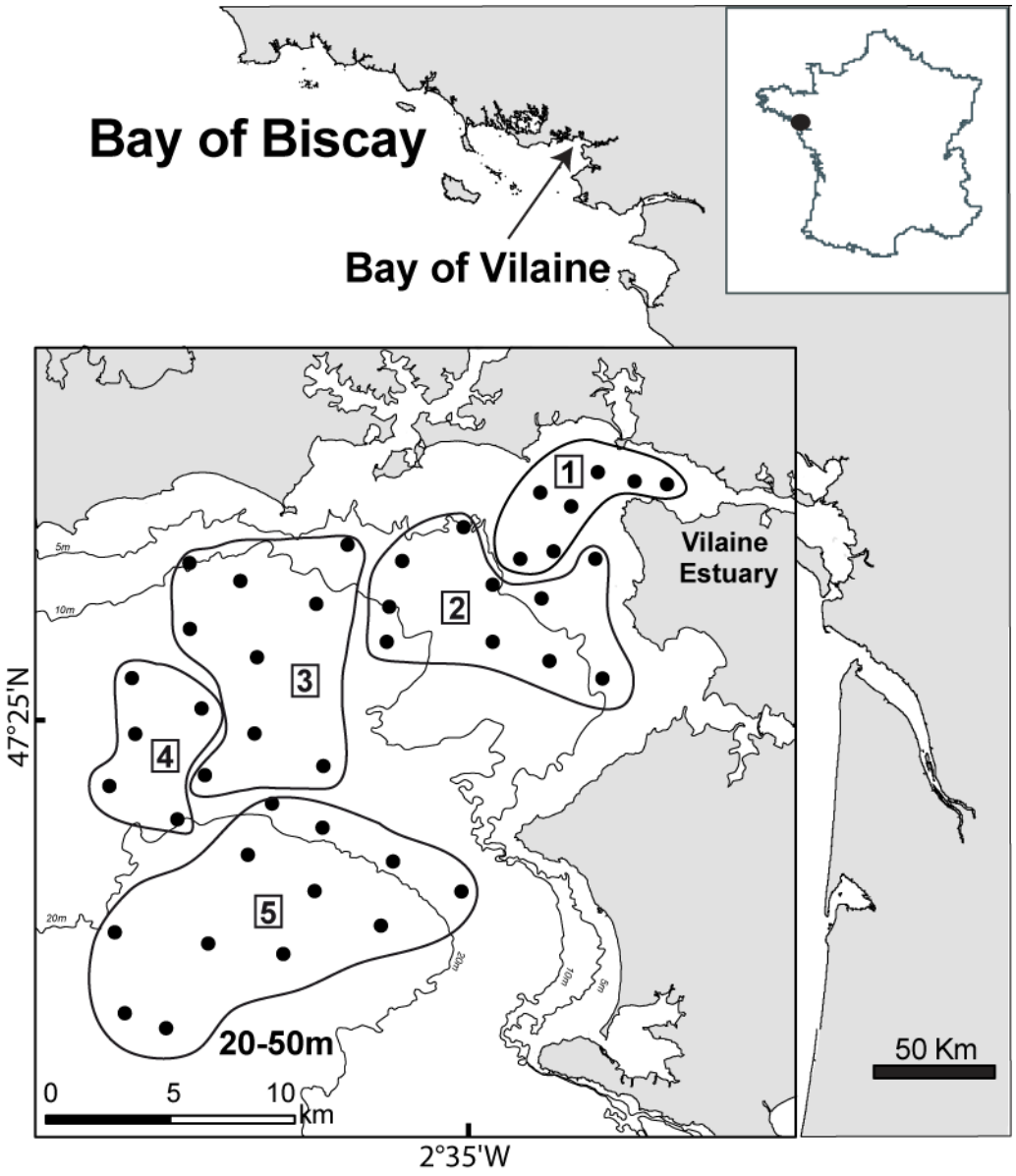
774

775 **Fig. 2.** $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SD) for POM, carnivores, deposit- and suspension-
776 feeders, and fish in the five described habitats. The fish were separated in two age classes (G0
777 for the young-of-the-year and G1 for age 1 individuals; see Material and Methods for details).
778 Prey were classified in trophic guilds. Number of individuals of fish and preys in the different
779 habitats are indicated (N).

780

781 **Fig. 3.** Relative abundance (%) of the different trophic guilds in the gut contents of common
782 sole and pouting in the 5 studied habitats (0: G0, 1: G1). No sole was captured in the
783 *Haploops* habitat (H4), nor any sole G0 was observed in the deeper habitat (H5). Prey items
784 in gut contents were classified in trophic guilds, only scavengers are absent from gut contents.
785 Numbers of gut contents analysed are given above the barplot.

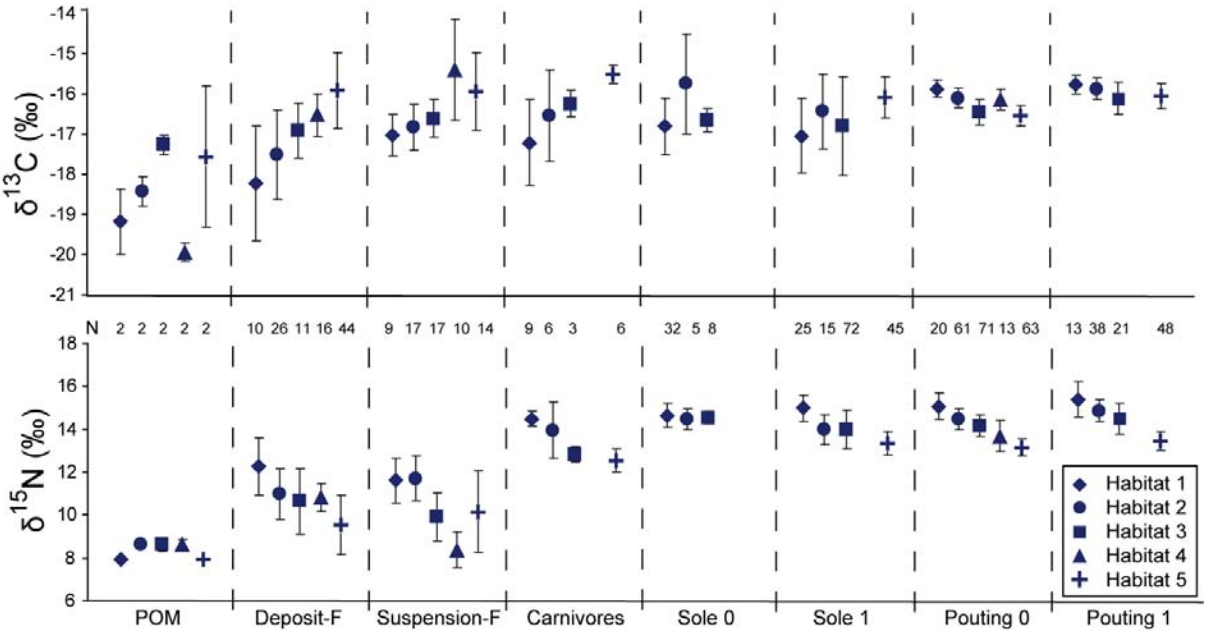
786



786

787 Figure 1

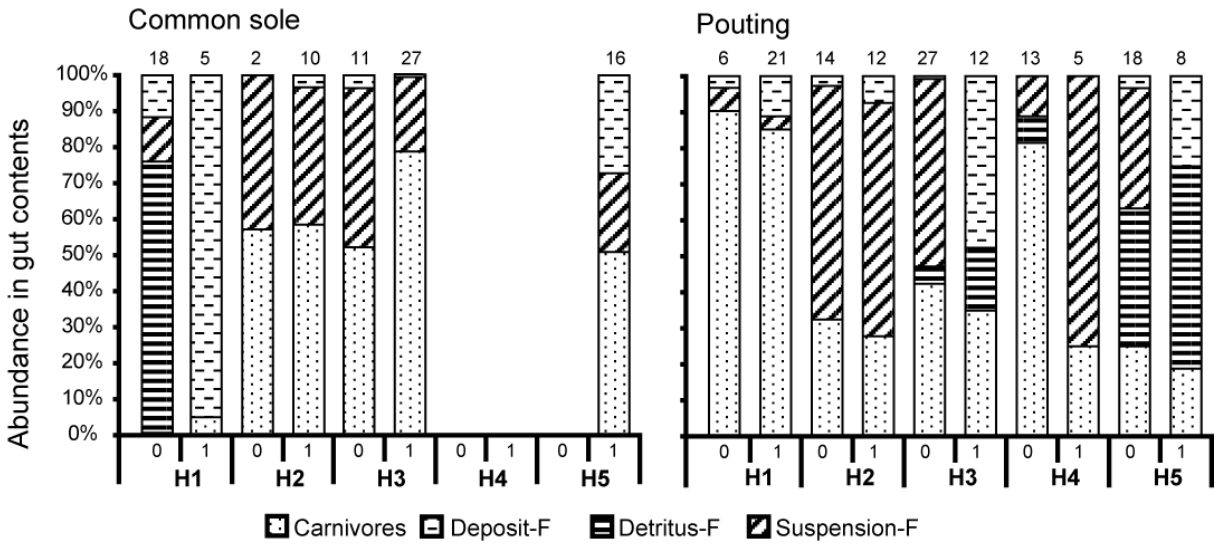
788



788

789 Figure 2

790



790

791 Figure 3