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Abstract :

Estuaries are highly important nursery habitats for a range of fish species because they provide refuge and food, especially for juveniles. However, the importance of specific salinity zones and adjacent shallow marine habitats (subtidal and surf zones) for fish feeding is not well understood, particularly in small macrotidal estuaries. Using the example of the Canche estuary, which is considered a reference for small macrotidal estuaries in France, we investigated the structure and seasonal variability in fish food webs based on stable carbon and nitrogen isotope analysis. Our results provide a new vision of the Canche estuary, which has been considered a major feeding ground for all marine fish that spend time there. Based on C results, our results revealed that organic matter of terrestrial origin has little influence on functioning of the Canche food web, except for flounders in the upstream area of the estuary. Conversely, microphytobenthos and marine particulate organic matter contribute most to the food web of fish in the estuary. Our study also revealed that some fish species visit the estuary for reasons other than feeding, such as to avoid predation or because they are carried by the tide. This work confirmed the suitability of using stable isotopes to trace fish fidelity to feeding grounds less than 10 km apart.

Keywords : Food web, macrotidal estuary, stable isotopes, seasonal variations, nursery

1. Introduction

Using shallow marine coastal zones and estuaries as nursery areas is an important phase in the life history of many marine organisms, including commercially valuable species (Amara, 2003; Vasconcelos et al., 2007). In temperate ecosystems, juvenile fish concentrate in nursery grounds from late spring to early fall, which is the peak of macrobenthic prey biomass (Amara and Paul, 2003; Pasquaud et al., 2010; Selleslagh et al., 2015). Refuge and feeding areas for young fish in estuaries are considered important for fish survival and replenishing coastal fish stocks (McLusky and Elliott, 2004). However, many authors have shown that estuaries are only one component of broader nursery-use patterns and that shallow marine coastal areas and estuaries may provide a mosaic of vital nursery habitat types for juvenile marine fish (Beck et al., 2001). Although opportunism is widely reported for estuary-associated fish, as well as for fish in general (Amara et al., 2001; Elliott et al., 2007; Elliott and Hemingway, 2002), the importance of these different habitats (specific salinity zones inside estuaries and adjacent marine subtidal and surf zones) for fish feeding is not well understood (Selleslagh et al., 2015; Vinagre et al., 2008). In addition, there is still a lack of information and confusing conclusions about the main origin of the organic matter that sustains juvenile fish food webs in nursery grounds (Le Pape et al., 2013). For estuaries, some studies (Darnaude et al., 2004; Leakey et al., 2008; Vinagre et al., 2008) indicated that allochthonous organic matter of terrestrial origin predominated, while other studies suggested that marine organic matter predominated (Pasquaud et al., 2008; Selleslagh et al., 2015). Most of these studies were conducted in large estuaries (e.g. Tagus, Gironde, Thames, Rhone, Vilaine). However, in small estuaries with little freshwater influence, *in situ* primary production can override other food sources and contribute significantly to juvenile food webs (Kostecki et al., 2012). Understanding the main ecological processes in ecosystems (e.g. food sources, trophic transfer through the food web) and identifying juvenile fish feeding areas and their spatial use of shallow nursery habitats are fundamental issues for effective conservation and management of these essential fish habitats (Hobson et al., 1999).

Reconstructing marine food webs is largely limited by methodological difficulties. A traditional approach to describe fish feeding ecology and determine their feeding niche has been stomach content analysis (Amara et al., 2001; Besyst et al., 1999). Although it may provide high taxonomic resolution, this method can be biased due to the difficulty in determining the origin of partially digested food items. Previous studies of estuarine fish food webs highlighted that stable isotope analysis (SIA), based on nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) signatures of various food-web compartments, can be a powerful tool. This technique,

which identifies trophic pathways and energy sources in a variety of ecosystems (Fry, 2006; Peterson and Fry, 1987), is particularly successful in coastal and estuarine systems, in which the fate of different sources of both freshwater and marine particulate organic matter (POM) can be distinguished (Fry, 1999; Riera et al., 1999; Yokoyama et al., 2008).

δ13C and δ15N are typically increased from prey to consumers by 3‰–4‰ for δ15N and 1‰ for δ13C (De Niro and Epstein, 1981; Minagawa and Wada, 1984; Cabana and Rasmussen, 1994). Thus, δ15N can indicate the trophic position of an organism within the food web and δ13C values of the food sources (primary producers as phytoplankton or microphytobenthos) are also responsible for differences in the isotopic compositions of their consumers (Riera et al. 1999), allowing distinctions to be made between pelagic and benthic food webs (Hobson et al. 2002) or fresh vs marine waters (Fry, 2006). Consequently, δ13C can be used as a tracer of organic trophic sources for benthic consumers (Peterson, 1999).

Given the diversity of juvenile nursery habitats, an isotopic approach to tracing fish movement is particularly appealing as it increases the likelihood of finding habitat-specific isotopic signatures (Herzka, 2005). Several authors have successfully used stable isotopes to study, for example, the connectivity of habitats (Fry et al., 2003; Selleslagh et al., 2015; Vinagre et al., 2008).

In the present study, we analysed for the first time the fish food web in a small macrotidal estuary on the French coast of the Eastern English Channel (EEC) – the Canche estuary – based on stable carbon and nitrogen isotopes analysis. The Canche estuary is considered a reference for small macrotidal estuaries because it is subject to low human disturbance and is an important fish nursery ground (Selleslagh et al., 2009; Selleslagh and Amara, 2015) that supports species with high commercial and recreational value during their juvenile stage. The role and importance of small macrotidal estuaries as feeding grounds for marine juvenile fish is not well understood. Along the French coast of the EEC, juvenile marine fish are known to use both estuaries and shallow marine coastal waters, but the exact areas where they feed remain unknown (Amara and Paul, 2003; Selleslagh and Amara, 2015).

The main objectives of the present study were to i) distinguish the origin of sources in the marine juvenile fish food web, ii) investigate seasonal variations in fish food-web structure and iii) assess the feeding-ground fidelity of marine juvenile fish that inhabit the Canche estuary and adjacent coastal nursery grounds.

2. Materials and Methods

2.1 Study area

The Canche estuary (50°50'-50°56' N, 1°57'-1°67' E) is located in northern France along the coast of the Eastern English Channel (EEC). The Canche estuary is 12 km long and has a maximum width of 1 km at its mouth. The estuary has a semi-diurnal tide, with an average tidal range of ca. 1 m at neap tides and 6 m at spring tides, and is considered a macro/hyper-tidal estuary according to the McLusky and Elliott (2004) classification. Water circulation depends mainly on the tide and small freshwater inputs, with a mean annual rate of ca. 13 $m^3.s^{-1}$. The Canche estuary is little impacted by human disturbances (Amara et al., 2007), it belongs to the special conservation zone "Bay of Canche and corridor of the 3 estuaries" and is classified as a "Natura 2000" site because it accounts as a major area of hosting juvenile fish in the Eastern English Channel.

2. 2 Sampling strategy

Samples of particular organic matter (POM), sediment organic matter (SOM), microphytobenthos (MPB) as well as fish and their main potential prey (i.e. benthic organisms, shrimp and crabs) were collected in the fall (October-November 2017) and spring (May-June 2018) at three sites inside the Canche estuary along a salinity gradient (upstream, middle and downstream).

To better understand the estuary's feeding role and connectivity with adjacent marine habitats, three additional sites were sampled outside the Canche estuary: two in the surf zone on both sides of the mouth of the estuary (Sainte Cécile beach and Le Touquet beach, respectively) and one in the subtidal zone in the plume of the estuary (Figure 1). For technical reasons, these sites were sampled only in spring (May-June 2018). We also described benthic communities and their biomass at all six sites only in spring (May-June 2018).

The sampling sites consist of a variety of habitats. The middle part of the Canche estuary is a muddy-sand shore that contains polychaetes and bivalves (EUNIS classification A2.24; Rolet et al., 2015). The downstream part is characterized by a medium fine-sand benthic community (EUNIS classification A2.223) and is dominated by amphipods and *Scolelepis spp*. (Rolet et al, 2015) such as the surf zone (Sainte Cécile and Le Touquet beaches). The subtidal site is muddy fine sand and contains polychaetes (*Magelona jonhsoni*, *Nephtys* spp.) and bivalves (*Donax vittatus*) (Desroy et al., 2003).

134

135 **2.3 Particulate organic matter and sediment organic matter sampling**

136 The POM was sampled at the surface at high tide using sterile pots and then was conserved in
137 a cool box. In the laboratory, the water was filtered through pre-combusted Whatman GF/F
138 filters (0.45 µm pore size, 47 mm diameter), with 3 replicates per site. The SOM was sampled
139 at low tide by scraping the top first cm of sediment. Three replicates were performed at each
140 site. The POM and SOM samples were conserved at -20 °C in the laboratory until transfer for
141 SIA.

142

143 **2.4 Microphytobenthos sampling**

144 Benthic diatoms were collected at the sites inside the estuary and in the surf zone at low tide
145 by scraping the surface of the sediment and were then extracted according to the protocol of
146 Riera et al. (1999). In the laboratory, we allowed MPB to migrate by leaving the sediment
147 containing benthic diatoms in flat trays to form a layer 1 cm thick. A nylon screen (60 µm
148 mesh) was placed on top of the sediment and covered with a layer of combusted sand powder
149 (60-200 µm) 5 mm thick. The trays were illuminated until the first dense brown mats
150 appeared on the surface. Meanwhile, the sand was kept moist by spraying filtered (GF/F)
151 seawater from the sampling site. The top 2 mm of sand were removed and sieved over a 60
152 µm mesh nylon screen to separate the diatoms from the remaining sand and nematodes or
153 copepods. The benthic diatoms were then collected on precombusted GF/F filters. Samples
154 were conserved at -20 °C until transfer for SIA.

155

156 **2.5 Benthic organism sampling**

157 In the surf zone (on both sides) and inside the estuary, macrobenthic fauna was sampled
158 during low tide with a hand corer (0.025 m^2 area, 20 cm depth, 10 replicates for density and
159 biomass, and as many as needed for SIA). At the subtidal site, macrobenthic organisms were
160 sampled using a Van Veen grab (sampling an area of 0.1 m^2, 10 replicates for density and
161 biomass and as many as needed for SIA). Samples were washed, sieved through a 1 mm mesh
162 size and then washed again with milli-Q water to avoid contamination. In the laboratory,
163 benthic fauna was sorted and identified to the species level when is possible. Ash-free dry
164 weight (AFDW) of benthic invertebrates was determined using the method recommended by
165 the Benthos Ecology Working Group of the ICES (Hamilton and Kingston, 1985). The fauna
166 was dried in an oven at 60 ± 1°C for at least 48 hours until a constant weight was obtained. It

was then weighed (with 10^{-1} mg precision) and placed in an oven at 520 ± 20°C for 6 h to calcinate the organic matter completely without altering the mineral matter, and the calcinated fauna was weighed again. The AFDW equalled the difference between the two weights.

2.6 Fish, crab and shrimp sampling

Fish, crabs and shrimps were sampled during daylight hours using a 1.5 m beam trawl, with one tickler chain and 5 mm mesh size in the cod end, towed by a semi-rigid boat against the current at 2 knots for 15 min. Fish, crabs and shrimp were identified to the species level, counted, and then measured (total length, with 1 mm precision).

2.7 Stable isotope analyses

Species selected for SIA were dominant in both abundance and biomass to obtain a synthetic image of the trophic structure within each community. As lipids are depleted in $\delta^{13}C$ compared to carbohydrates and proteins (DeNiro and Epstein, 1977; Griffiths, 1991), which indicates that fatty tissues tend to be isotopically lighter than leaner ones, trophic interpretations based on $\delta^{13}C$ composition may be confounded by lipid effects (Bodin et al., 2007; Wada et al., 1993). To minimize these effects, mega- and macrofaunal (except for polychaetes) low-lipid muscle tissue was used for SIA. Polychaetes were analysed after removing their viscera by dissection. The valve muscle of bivalves, the abdomen muscle of shrimp, the muscle in crab pincers and the white dorsal muscle of fish (even small ones) were dissected and analysed for SIA. For other benthic organisms, the entire organism was analysed after removing the digestive tract, jaws and cerci. The tissues were then washed with milli-Q water to prevent contamination and freeze-dried before being encapsulated. For small benthic organisms (*Bathyporeia pilosa*, *Eurydice pulchra*, *Gastrosaccus spinifer*, *Haustorius arenarius*), each sample represented a combination of 2-4 individuals.

As fish size can influence isotope values, especially $\delta^{15}N$, due to ontogeny (Galván et al., 2010; Wilson et al., 2009), we carefully selected individuals of similar size across species in order to be sure that we select G0 juveniles. Before $\delta^{13}C$ analyses, POM and MPB filters were divided into two subsamples: one was exposed to HCl vapour for 4 h to remove residual carbonates (Cresson et al., 2012) before being placed in tin cups (Lorrain et al., 2003), while the other was not treated and was used to measure $\delta^{15}N$. Sediment samples were dried at 60°C for 24 h. They were divided into two subsamples: one was treated with HCl, to remove

carbonate, then rinsed three times with distilled water, and freeze-dried for 48 hours before encapsulation, while the other was encapsulated immediately after drying.

$\delta^{13}C$ and $\delta^{15}N$ were measured using an elemental analyser Flash EA 2000 (Thermo Scientific), connected to an Isotope Ratio Mass Spectrometer (Delta V+) with a ConFlo IV interface (Thermo Scientific) at the Pôle Spectrométrie Océan in Plouzané, France. Replicate analyses of international IAEA and laboratory USGS standards provided analytical errors <0.20‰ for both $\delta^{13}C$ and $\delta^{15}N$. Stable isotope ratios were expressed as parts per mil (‰) in the δ notation relative to the Pee Dee Belemnite standard for carbon and atmospheric N_2 for nitrogen using the formula:

$$\delta X(‰) = [(R\ sample/R\ standard) - 1] \times 1000$$

where X is ^{13}C or ^{15}N, and R is the ratio of ^{13}C:^{12}C or ^{15}N:^{14}N.

2.8 Data analysis

2.8.1 Statistical analysis

We first tested the hypothesis that potential sources and prey for fish had significantly different isotopic compositions along the salinity gradient, and then that compositions at sites inside vs. outside of the estuary differed significantly. For all samples, two-way ANOVAs were performed separately for each ratio, factors are sites and seasons (after verifying that the assumptions of parametric tests were met). ANOVA was used to test differences in $\delta^{13}C$ and $\delta^{15}N$ in the POM from the water sources, considering site and season effects. Biplots of $\delta^{13}C$ vs. $\delta^{15}N$ were used to represent graphically means and standard deviations of isotopic compositions of all compartments of the entire food web in each habitat. Besides, we performed a Kruskal-Wallis test to test for fish species length homogeneity between sites and seasons.

2.8.2 Layman metrics

We assessed isotopic niches of fish by calculating the three Layman metrics (Layman et al., 2007): nitrogen range (NR), carbon range (CR) and total area (TA). We used a Bayesian approach based on multivariate ellipse-based metrics (Jackson et al., 2011), in which the location of the centroid represents the centre of the trophic niche in isotopic space. To describe the spread of data points, we calculated the parameters developed by Layman et al.

(2007). We constructed convex hulls to estimate the smallest TA that contained all individuals in the isotopic space. The TA can be interpreted as a measure of the total isotopic niche of a population (Layman et al., 2007). We estimated the niche width in each season using multivariate ellipse-based metrics (Jackson et al., 2011). The analysis generates standard ellipse areas (SEA), which are bivariate equivalents of standard deviations in univariate analyses.

3. Results

3.1 Benthic communities and biomass

The upstream site was too muddy to sample benthic organisms. Among the five sites sampled in spring, we identified 1288 individuals that belonged to 51 taxa (Table 1). Mean macrobenthic species richness, abundance and biomass were higher outside than inside the estuary (Wilcoxon test, $p < 0.0001$). Species richness was lowest in the middle of the estuary (6 species) but higher at the downstream site (16), Sainte Cécile beach (13), Le Touquet beach (11) and the subtidal site (32). The main species observed in the middle of the estuary were the polychaetes *Hedistes diversicolor* (326 ind.m^{-2}; 2 011 mg.m^{-2}) and the bivalve *Scorbicularia plana* (565 mg.m^{-2}). The polychaete *Scolelepis squamata* (823 ind.m^{-2}; 3 445 mg.m^{-2}) and the bivalves *Limecola balthica* (1 282 mg.m^{-2}) and *Cerastoderma edule* (640 mg.m^{-2}) dominated the downstream site. The two surf zone sites (Le Touquet and Sainte Cécile) were characterized by the polychaetes *S. squamata* (48-300 ind.m^{-2}; 145-835 mg.m^{-2}), *Nephtys cirrosa* (124-160 ind.m^{-2}; 559-831 mg.m^{-2}) and *Lanice conchylega* (1 022 mg.m^{-2} at Sainte Cécile) and the bivalves *M. balthica* (153-1336 mg.m^{-2}), *C. edule* (693 mg.m^{-2} at Sainte Cécile) and *Fabulina fabula* (706 mg.m^{-2} at Le Touquet). More diverse taxa (N = 32) and huge benthic biomass were observed at the subtidal site, which was dominated mainly by the bivalves *D. vittatus* (516 ind.m^{-2}; 108 035 mg.m^{-2}), *F. fabula* (412 ind.m^{-2}; 12 906 mg.m^{-2}) and *Ensis leei* (36 ind.m^{-2}; 9 064 mg.m^{-2}).

257 Table 1. Mean Biomass (B) (mg.m^{-2}) and Density (D) (ind.m^{-2}) of each benthic species inside and outside the Canche estuary in spring.
258 (Abbreviations correspond to species analysed for stable isotope composition)

Species	Inside the Canche estuary				Outside the Canche estuary					
	Middle		Downstream		Sainte Cécile beach		Le Touquet beach		Subtidal	
	B	D	B	D	B	D	B	D	B	D
Hediste diversicolor (H div)	**2011.7**	**326**								
Scrobicularia plana (S pla)	**565.8**	**14**								
Carcinus maenas (C mae)	108.6	2	85.5	1						
Oligochaeta	9.1	2	1.4	5						
Gammarus zaddachi	2.4	2								
Bathyporeia pilosa	0.4	2	15.1	61						
Scolelepis squamata (S squ)			**3445.6**	**823**	**835.8**	**300**	145.9	48		
Limecola balthica (L bal)			**1282.7**	21	153.6	4	**1336.6**	12	**8990.1**	72
Cerastoderma edule (C edu)			**640.5**	1	**693.6**	4			**2709.8**	4
Eurydice pulchra (E pul)			246.5	3			3.3	16		
Nephtys cirrosa (N cir)			146.2	19	**559.4**	**124**	**831.72**	**160**		
Heteromastus filiformis			29.1	32						
Bathyporeia sarsi			4.5	36						
Eteone longa (E lon)			11.6	8						
Glycera tridactyla			8.5	3	62.1	4			7.8	4
Haustorius arenarius (H are)			4.1	4	42.4	28	9.5	4		
Urothoe poseidonis			0.5	4	17.0	40	3.4	8	2.7	4
Pygospio elegans			0.4	4						
Mytilus edulis			0.3	1						
Lanice conchilega (L con)					**1022.8**	16			**416.2**	**4196**
Portumnus latipes					447.9	8	257.4	8		
Nepthys hombergi					295.6	24			**981.6**	**124**
Gastrosaccus spinifer (G spi)					57.7	28	59.6	16		
Crangon crangon (C cra)					28.0	52	1.8	4	2.2	4
Spio martinensis					7.1	16				
Fabulina fabula (F fab)							**706.4**	12	**12906.6**	**412**
Cumopsis goodsiri							0.4	4		
Donax vittatus (D vit)									**108035.2**	**516**
Ensis leei (E lee)									**9604.8**	36
Tritia reticulata									**2630.4**	40
Ophiura albida									**898.6**	8
Abra alba (A alb)									**603.6**	68
Arenicola marina (A mar)									**586.0**	24

Liocarcinus vernalis					465.9	36
Sigalion squamosus					443.1	44
Anapagurus hyndmanni					377.4	12
Nephtys assimilis					244.3	4
Lagis koreni					218.7	36
Phyllodoce mucosa (P muc)					144.4	244
Planarian					118.8	4
Pinnotheres pisum					74.6	12
Nemertians					42.2	32
Eteone picta					9.7	4
Schistomysis kervillei					8.4	4
Diastylis rugosa					5.8	4
Capitella capitate (C cap)					2.8	20
Leucothoe incisa					2.4	8
Eumida sanguinea					1.1	8
Nototropis swammerdamei					0.6	4
Pariambus typicus					0.5	4
TOTAL	**2 698.1**	**348**	**5 922.4**	**1 404**	**4 223.0**	**648**	**3 356.0**	**292**	**150 536.3**	**5 992**

3.2 Fish sampled for stable isotope analyses

Nine fish species (eight in spring and six in fall) were collected for SIA: *Buglossidium luteum*, *Dicentrarchus labrax, Limanda limanda, Platichthys flesus, Pleuronectes platessa, Solea solea, Sprattus sprattus, Pomatoschistus microps* and *Pomatoschistus minutus* (Table 2). Most individuals were G0 juveniles, except *P. microps*, *P. minutus, S. solea* and *P. platessa* (at the subtidal site in spring) and *P. flesus* (at Sainte Cécile beach in spring). *S. solea, L. limanda* and *B. luteum* were collected only outside the Canche estuary.

In the fall, fish of the same species did not differ significantly in size among sites, except *P. flesus* ($p = 0.020$,) and *S. sprattus* ($p = 0.006$), which were smaller in the middle of the estuary than upstream or downstream. In spring, *P. platessa* were significantly longer at the subtidal site ($p < 0.0001$). *P. flesus* were also significantly longer at Sainte Cécile than at the other sites ($p = 0.003$). Inside the estuary, fish size varied between the two seasons for the four species caught in both spring and fall (*D. labrax*, *P. flesus*, *S. sprattus* and *P. platessa*; $p < 0.0001$).

273 Table 2. Total length (mean ± standard error, cm) and samples size (n) of the nine fish species collected in the Canche estuary in two seasons
274 (fall and spring) analysed for carbon and nitrogen stable isotope composition.

Species	Season	Upstream		Middle		Downstream		Sainte Cécile beach		Le Touquet Beach		Subtidal	
		Length	n	Length	n	Length	n	Length	n	Length	n	Length	n
Dicentrarchus. labrax (D lab)	Fall	5.9±0.9	10	5.2±0.9	10	6.3±0.5	11						
Platichthys flesus (P fle)		6.0±3.0	11	3.8±0.2	10	6.9±1.3	9						
Pleuronectes platessa (P pla)						6.6±1.3	5						
Sprattus sprattus (S spr)		6.9±2.8	11	3.9±0.2	6	6.4±1.2	9						
Pomatochistus microps (P mic)		4.4±0.2	5	3.8±0.2	5	4.6±0.7	5						
Pomatochistus minutus (P min)		5.3±0.5	5	4.1±0.1	5	5.9±0.3	5						
Dicentrarchus labrax (D lab)	Spring			8.2±1.1	4	8.4±0.5	3						
Platichtthys flesus (P fle)		7.8±2.8	4	6.4±2.8	5	8.7±5.2	4	13.0±1.2	6	10.6±3.1	5	7.0± 5.4	10
Pleuronectes platessa (P pla)				3.9±4.8	5	4.6±1.0	4	5.5±7.4	5	7.1±0.9	9	14.5±5.2	4
Sprattus sprattus (S spr)		4.3±3.0	10	4.8±3.7	5	4.5±0.4	3			6.4±1.5	5		
Pomatoschistus sp. (P sp)				4.3±2.8	4	4.8±2.5	5						
Solea solea (S sol)								3.5±2.4	7	3.7±2.2	7	13.1±2.1	8
Buglossidium luteum (B lut)								7.6±3.8	5	4.1±2.3	5	5.9 ± 4.2	5
Limanda Limanda (L lim)												13.4 ± 1.0	6

3.3 Results of stable isotope analyses

3.3.1 Food sources

The stable isotope composition of POM ranged from -29.70‰ to -20.15‰ for $\delta^{13}C$ and 5.20-7.30‰ for $\delta^{15}N$ (Table 3). POM $\delta^{13}C$ differed significantly among sites ($F = 9.536$; $p < 0.0001$, ANOVA)) and between seasons ($F = 9.448$; $p < 0.0001$, ANOVA). In spring, mean POM $\delta^{13}C$ was significantly lower inside the estuary ($F = 9.536$; $P < 0.0001$) and higher at the subtidal site (-20.89 ± 0.10‰) (Table 3). Mean $\delta^{15}N$ also differed significantly among sites ($F = 3.745$; $P < 0.0001$) and between seasons ($F = 3.762$; $P < 0.0001$). POM $\delta^{15}N$ was significantly higher at Le Touquet beach (7.30 ± 0.01‰ $P = 0.144$, Kruskal-Wallis) (Table 3). The stable isotope composition of SOM ranged from -24.18‰ to -19.62‰ for $\delta^{13}C$ and 4.67-7.19‰ for $\delta^{15}N$ (Table 3) and differed significantly among sites and between seasons for both $\delta^{13}C$ and $\delta^{15}N$. The ANOVA revealed a site effect on $\delta^{15}N$, as well as a season effect, with the lowest values in spring, and a significant effect of the site × season interaction ($F = 10.548$, $p < 0.0001$). SOM $\delta^{13}C$ showed significant enrichment from upstream to downstream along the estuary in both spring ($F = 9.352$, $p < 0.0001$) and fall ($F=4.531$, $p < 0.0001$) (Table 3). The MBP had the most depleted $\delta^{15}N$ ratios of the food sources sampled. The stable isotope composition of MPB ranged from -17.1‰ to -13.1‰ for $\delta^{13}C$ and 3.66-6.44‰ for $\delta^{15}N$ (Table 3). MBP $\delta^{13}C$ and $\delta^{15}N$ did not differ significantly among sites or between seasons.

Table 3. Carbon and nitrogen stable isotope compositions (mean ± standard error, ‰) of particulate organic matter (POM), sediment organic matter (SOM) and microphytobenthos (MPB) inside (Upstream, Middle, Downstream) and outside (Sainte Cécile and Le Touquet beaches, Subtidal) the Canche estuary in fall and spring. *only one replicate.

	season	Upstream		Middle		Downstream		Sainte Cécile beach		Le Touquet beach		Subtidal	
		$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
POM		6.61*	-29.70*	6.00 ± 0.05	-24.20 ± 0.14	6.96 ± 0.35	-27.43 ± 1.37						
SOM	Fall	6.00 ±0.96	-24.18 ±0.46	7.19 ±0.62	-23.04 ±0.36	6.07 ±0.57	-22.09 ±0.80						
MPB		6.44 ±1.90	-17.11 ±0.85	3.66 ±1.75	-16.18 ±0.44	5.94 ±0.75	-16.12 ±0.48						
POM		5.20 ± 0.66	-29.16 ± 0.21	6.57 ± 0.07	-20.89 ± 0.10	5.96 ± 1.24	-22.43 ± 1.24	6.08 ±0.72	-20.15 ± 0.23	7.30 ±0.01	-20.89 ± 0.10	6.50 ±0.40	-20.50 ±0.17
SOM	Spring	5.46 ±0.01	-24.18 ± 0.16	5.39 ±0.06	-19.62 ±0.05	4.67 ±0.94	-19.98 ±0.20	4.80 ±0.72	-19.95 ± 0.92				
MPB				6.40 ±0.59	-16.13 ±0.34	5.12 ±0.37	-15.71 ±1.41	6.42 ±0.16	-15.52 ± 0.65	4.88 ±0.49	-13.14 ±0.56		

297 3.3.2 Isotopic compositions of benthic communities

298 Inside the estuary, the benthic invertebrate community followed the same isotopic trend in
299 both seasons. The bivalve *Scrobicularia plana* and polychaetes *H. diversicolor* and *N. cirrosa*
300 had the lowest $\delta^{13}C$ values (-20‰ to -18‰), while the crustaceans *Carcinus maenas* and
301 *Crangon crangon* had the highest $\delta^{13}C$ values (-16‰ to -15‰) (Figures 2 and 3). The
302 suspension feeder *L. balthica* (7.9-9.9‰) and the deposit feeders *S. squamata* (8.2-10.2‰)
303 and *S. plana* (8.4-9.2‰) had the lowest $\delta^{15}N$ values, while the crustaceans *C. crangon* and *C.*
304 *maenas* (11.3-12.6‰) had the highest $\delta^{15}N$ values (Appendix 1, Appendix 2).
305 Outside the estuary, *S. squamata* (-18.8‰), *L. conchylega* (-18.6±0.4‰) and *E. pulchra*
306 (-20.4±0.9‰) had the lowest $\delta^{13}C$ values, while the predatory *C. maenas*, *C. crangon* and *N.*
307 *cirrosa* (-17.1‰ to -15.2‰) had the highest $\delta^{13}C$ values (Appendix 1). The suspension
308 feeders *D. vittatus*, *L. balthica* and *L. conchylega* had the lowest $\delta^{15}N$ values (7.1-9.6‰),
309 while the predatory shrimp *C. crangon* had the highest $\delta^{15}N$ values (13.6‰) (Appendix 1).
310 In spring, the mean $\delta^{13}C$ values for *C. crangon* and *C. maenas* were significantly lower inside
311 the estuary (*C. crangon*: -18.3±0.9‰ at the upstream site to -15.5±0.7‰ at Sainte Cécile
312 beach; *C. maenas*: -17.4±0.5‰ at the upstream site to -15.2±0.2‰ at the intertidal site).
313

314 3.3.3 Isotopic composition of fish
315 Inside the estuary in both seasons, *P. flesus* had the lowest $\delta^{13}C$ values (-24.2‰ to -16.8‰),
316 while in the fall, *P. microps* had the highest $\delta^{13}C$ values (-15.9‰ to -14.8‰). Inside the
317 estuary in the fall, all $\delta^{15}N$ values exceeded 13‰, except for *P. flesus* (12.5±0.6‰) and *P.*
318 *plastessa* (12.4±0.2‰), both downstream. Fish $\delta^{15}N$ was lower in spring (11.5-13.1‰),
319 except for *D. labrax* (ca. 14‰). Thus, inside the estuary we observed enrichment of
320 organisms from upstream to downstream (Figure 2). Outside the estuary in spring, carbon
321 ratios displayed the same patterns, with $\delta^{13}C$ values lowest for *P. flesus* (-20.7‰ to -17.2‰)
322 and highest for *P. microps* (-15.6±0.3‰). Fish had a wider range of $\delta^{15}N$ (10.2-13.8‰)
323 outside the estuary than inside.
324

325 3.3.4 Fish isotopic niches

326 Inside the estuary, isotopic niches overlapped strongly in fall, except for *P. platessa,* which
327 occupied a distinct isotopic space (Figure 4A). All niches overlapped in spring, except for two
328 pelagic fish (*S. sprattus* and *D. labrax*), which had distinct isotopic niches (Figure 4B). Inside

329 the estuary, total fish SEAc (corrected SEA) were larger in spring (21.79%) than in fall
330 (13.96%). In spring, fish isotopic niches were smaller outside the estuary than inside.

331 Table 5. The core isotopic niche area (SEAc, %), carbon range (CR) and nitrogen range (NR)
332 for fish species sampled inside and outside the Canche estuary in fall and spring.

	Fall			**Spring**					
	Inside			**Inside**			**Outside**		
Species	**SEAc**	**CR**	**NR**	**SEAc**	**CR**	**NR**	**SEAc**	**CR**	**NR**
Pomatoschistus minutus	17.58	9.01	2.63						
Platichthys flesus	16.41	7.67	3.59	23.49	8.50	4.74	12.79	4.77	3.95
Pomatoschistus microps	14.74	7.62	3.10						
Dicentrarchus labrax	13.28	7.19	2.68	17.60	7.30	4.17			
Pleuronectes platessa	5.45	6.99	2.86	25.04	8.86	4.67	14.65	6.50	3.59
Pomatoschistus sp				20.05	7.95	4.56			
Sprattus sprattus				18.92	8.43	3.89			
Buglossidium luteum							12.95	5.26	3.84
Limanda limanda							12.44	5.06	3.99
Solea solea							14.14	5.09	4.29
Total	**13.96**	**8.00**	**2.39**	**21.79**	**8.34**	**4.42**	**12.75**	**5.41**	**3.55**

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4. Discussion

4.1 Origin of and variability in organic matter in the Canche estuary

As estuaries are complex and changing ecosystems, it is challenging to distinguish the sources of organic matter at the base of food webs (Pasquaud et al., 2008; Selleslagh and Amara, 2015). Organic matter is a major component of suspended particles and fine sediment particles that determine many biogeochemical processes in marine environments (Bernasconi et al., 1997; Chen et al., 2012). Two natural sources of organic matter are generally considered in coastal ecosystems: allochthonous inputs and autochthonous production (Antonio and Richoux, 2014; Luo et al., 2016). Major sources of autochthonous organic matter include phytoplankton and aquatic macrophytes (Dalu and Froneman, 2016; Pearson et al., 2015). In estuarine ecosystems, organic matter of terrestrial origin is a major contributor to allochthonous organic matter (Duan et al., 2014; Lu et al., 2013). Analysis of the carbon and nitrogen stable isotopic compositions of estuarine organic matter can identify their contributions to the food web (Darnaude et al., 2004; Evans et al., 2019). Generally, POM in estuaries is composed of river POM (mixture of terrestrial POM and freshwater phytoplankton), estuarine-produced and marine phytoplankton, resuspended microphytobenthos and diverse detritus (e.g. faeces, macrophytes), which can make interpretation difficult (Kang et al., 2006).

In the Canche estuary, the POM stable isotopic signatures indicated a mixed organic matter composition that included freshwater/estuarine phytoplankton ($\delta^{13}C$ ranging from -29‰ to -20‰), with a clear increase in POM $\delta^{13}C$ values from fresh to marine waters, similar to previous estuarine studies in the Bay of Marennes-Oléron (Riera and Richard, 1996), the Vilaine estuary (Kostecki et al., 2010) and the Gironde estuary (Selleslagh et al., 2015). In the Canche estuary, salinity exhibits short-term changes, with a large amplitude from 0-35 due to the small size of the estuary, tide conditions, season and weather conditions (Amara et al., 2009; Selleslagh and Amara, 2008). MPB that live in intertidal flats in estuaries can contribute much of the total primary production in estuaries (Underwood and Kromkamp, 1999). MPB on intertidal flats is composed mainly of benthic diatoms (Méléder et al., 2007) and several studies have emphasised its key role in sustaining intertidal food webs (Christianen et al., 2017; Herman et al., 2000; Thrush et al., 2012). In the Canche estuary, the MPB is one of the main primary producers and could therefore be an important source of organic matter for benthic invertebrates. As in other European estuaries, the Canche MPB had the highest enriched carbon ratios (-17‰ to -15‰) among food sources, which allowed it to be traced in its consumers (Moens et al., 2002; Moncreiff and Sullivan, 2001; Riera and

Richard, 1996). SOM is a mixture of benthic and deposited pelagic microalgae, bacteria, aquatic and terrestrial plant debris and meiofauna. In the Canche estuary, SOM and POM $\delta^{13}C$ followed the same trend, with the lowest values upstream and highest values downstream due to the presence of freshwater phytoplankton, as measured in other nearby estuaries (Lambert et al., 2017; Middelburg and Nieuwenhuize, 1998).

In estuaries, variations in amounts and origins of nutrients such as nitrogen are common along salinity gradients, with a decrease in concentrations from fresh to marine waters due to mixing, which can be traced in food webs (Baeta et al., 2009; Connolly et al., 2013). The extent of nutrient mixing in estuaries varies spatially according to estuary size, and temporally at the seasonal and daily scales due to changing tides, wind, precipitation and temperature (Baeta et al., 2009; Hoeinghaus et al., 2011; Lautenschlager et al., 2014). $\delta^{15}N$ can be an accurate tracer for nitrogen inputs that originate from untreated domestic, industrial and/or agricultural activities that are incorporated in the food web through assimilation by primary producers (Fry, 2002). In the Canche estuary, POM, SOM and MPB $\delta^{15}N$ were similar along the salinity gradient during the same season, highlighting the relatively low nitrogen input from the watershed (Guelinckx et al., 2006) due to the short length of the Canche river and the low human modification of its catchment (Amara et al., 2007; Durou et al., 2007).

4.2 Structure of invertebrate communities

Two benthic communities were found in the Canche estuary: i) *S. squamata*/*E. pulchra*/*Bathyporeia* spp. (EUNIS A2.223), which corresponds to estuarine mid-shore medium-fine sand, and ii) *H. diversicolor*/*S. plana* on the upper shore mud banks (EUNIS A2.24; Rolet et al., 2015). Outside the estuary, Sainte Cécile and Le Touquet beaches were characterized by a low-shore fine sand *N. cirrosa*/*S. squamata* community (EUNIS A2.23), while the subtidal site had a muddy fine sand *Abra alba*/*D. vittatus*/*F. fabula* community (Desroy et al., 2003; Rolet et al., 2015). This distribution of benthic invertebrate communities is also present in the nearby estuaries of the Authie and Somme Rivers (Rolet et al., 2015). Biomasses inside the estuary and on adjacent beaches were lower (2.6-6.0 g AFDW.m^{-2}) than in the subtidal *A. alba* community (150 g AFDW.m^{-2}) dominated by the bivalve *D. vittatus*. In the EEC, benthic biomass within the *A. alba* community is heterogeneous, with a mean of 8.1 g AFDW.m^{-2} (Desroy et al., 2003), and a higher biomass ranging from 23.5-27.5 g AFDW.m^{-2} in the Seine Bay (Thiébaut et al., 1997) and from 45-3 000 g AFDW.m^{-2} in Gravelines (Desroy et al., 2003; Dewarumez et al., 1992; Ghertsos et al., 2000). This high subtidal

biomass of potential flatfish prey (mainly bivalves) outside the estuary created a major feeding ground for flatfish species.

One objective of the study was to assess the origin of the food source consumed by potential prey throughout the estuary, from the intertidal zone upstream of the Canche estuary, with low benthic species diversity and biomass, to the subtidal zone outside the estuary, with higher species diversity and biomass. In the Canche estuary, suspension and deposit feeders dominated the benthic invertebrate biomass. Their feeding activity is an important connection between suspended and sedimented organic matter originating from POM, SOM or MPB (Little, 2000; Mann and Wetzel, 2000). However, it is often difficult to distinguish food sources of macrozoobenthos in estuaries due to spatio-temporal variability in the isotopic compositions of food sources along the salinity gradient, and because macrofauna feed on different food sources and have plastic feeding behaviour depending on the environmental conditions (Daggers et al., 2020; Herman et al., 2000). Nevertheless, benthic primary consumers had higher $d^{13}C$ (around – 20 to -16‰) than fresh water POM (around -30‰), revealing the latter's low contribution to the trophic functioning of the estuary. This finding, even in the upstream of the estuary, may be dueto the relatively weak flow of the Canche River and consequently the small amount of organic matter that it carries (Selleslagh and Amara, 2008). Thus, we can hypothesize that the marine POM, SOM and MPB which $d^{13}C$ composition are around –22 to -15‰ are the main food sources for the benthic community inside the estuary. However, it may be difficult to distinguish suspension and deposit feeders isotopically as their feeding behaviour does not provide information about the origin of their food; for example, suspension feeders can consume resuspended MPB, while deposit feeders can consume sediment POM (Kang et al., 2015).

4.3 Fish structure and seasonal variations

Two fish assemblages were observed, one inside the Canche estuary (*P. minutus*, *P. microps* and *D. Labrax* juveniles) and the other outside the estuary (*B. luteum*, *S. solea* and *L. limanda* juveniles). *P. flesus* and *P. platessa* juveniles occurred in both assemblages (Selleslagh and Amara, 2008). In both seasons in upstream Canche, *P. flesus* juveniles had the lowest $\delta^{13}C$ values, 5 to 8% away from that of the freshwater POM, revealing a slight contribution of organic matter from freshwater. This could be due to the diet of *P. flesus* juveniles, which is composed of meiofauna (nematodes, harpacticoides and ostracods (Aarnio et al., 1996; Selleslagh and Amara, 2015)) that may consume SOM. Conversely, *P. microps* had the highest $\delta^{13}C$ values, which were similar to those of MPB, revealing the contribution of MPB

to the feeding of *P. microps* prey. Previous studies indicated that *P. microps* feeds mainly on amphipods, polychaetes and meiofauna (Leclerc et al., 2014; Selleslagh and Amara, 2015). MPB production is high on the intertidal mud banks of the Canche estuary and provides food for the potential prey of *P. microps*. Inside the estuary, the other fish species (e.g. *D. labrax*, *P. minutus* or *S. sprattus*) had $\delta^{13}C$ values from -19 to -17‰ upstream to – 16‰ downstream, close to those of marine POM (around -20‰) and MPB (-16‰) revealing a food source originating from both marine POM and MPB.

The seasonal comparison of fish SEA in the Canche estuary is informative, as the isotopic space occupied is smaller in spring than in fall. Thus, a wider range of prey appears to be consumed in the fall. This could be due to the higher biomass and diversity of coastal benthic invertebrates at the end of summer compared to the lower benthic biomass in spring (Rauch and Denis, 2008). In the fall, SEA of the two *Pomatoschistus* sp. did not overlap, unlike *Platichthys flesus* and *Pleuronectes platessa* that inhabit the Canche estuary, which confirms that their diet may differ (Salgado et al., 2004; Selleslagh and Amara, 2015). Conversely, the SEA of *P. flesus* and *P. platessa* overlapped in spring, perhaps due to the smaller amount of available prey (Pape and Bonhommeau, 2015).

Outside the estuary, the $\delta^{13}C$ values of flatfish juveniles (*B. luteum*, *S. solea*, *L. limanda*) were ca. -16‰, revealing MPB and POM to be a major basic food source, except for *P. platessa*, which had much lower $\delta^{13}C$ values, similar to those found in the estuary. This suggests that *P. platessa* individuals caught outside the Canche estuary did not feed exclusively in the habitat in which they were collected, which indicates that this species has high mobility and habitat connectivity.

Flatfish SEA indicates a slight isotopic niche overlap of *S. solea*, *B. luteum* and *L. limanda*, which suggests trophic segregation of the three species. Conversely, the SEA of *P. flesus* completely overlapped those of juveniles of these flatfish species, which indicates that *P. flesus* consumes a wider range of prey and may have trophic competition with the three other flatfish species. Juvenile fish in estuaries usually follow an opportunistic feeding strategy, which is driven by intra- and inter-specific competition (Brown et al., 2019; Post et al., 1999) and prey availability.

5. Conclusion

We showed a significant difference in invertebrate biomass between subtidal and intertidal sites, which influences the quality of the feeding ground for juvenile fish. This is a classic

situation in European estuaries (Dubois et al., 2014). This result provides a new vision of the Canche estuary, which has been considered an important feeding ground for marine fish.

Our study revealed that ~~these~~ continental inputs have a minor role in the functioning of the Canche estuary and that fish species might visit the estuary for reasons other than feeding, such as to avoid predation or because they are carried by the tide. We highlighted the need to take into account the whole small macrotidal estuary and adjacent ecosystems to better describe the flatfish nursery. This work demonstrated that potential prey and feeding sources for fish had habitat-specific compositions, which confirms the suitability of SIA for tracing fish movements, fidelity and connectivity inside and outside the Canche estuary for sites less than 10 km apart. Estuarine nursery feeding grounds, even in small estuaries, appear to be complex due to the mosaic of benthic communities (potential prey), which are related to the habitat (e.g. sediment type, foreshore position, salinity fluctuations) and to trophic competition and predation.

Acknowledgements

The authors would like to acknowledge the University of Littoral Opal Coast, the University of Badji Mokhtar Annaba and the Parc Naturel Marin des Estuaires Picards et de la Mer d'Opale that funded this work.

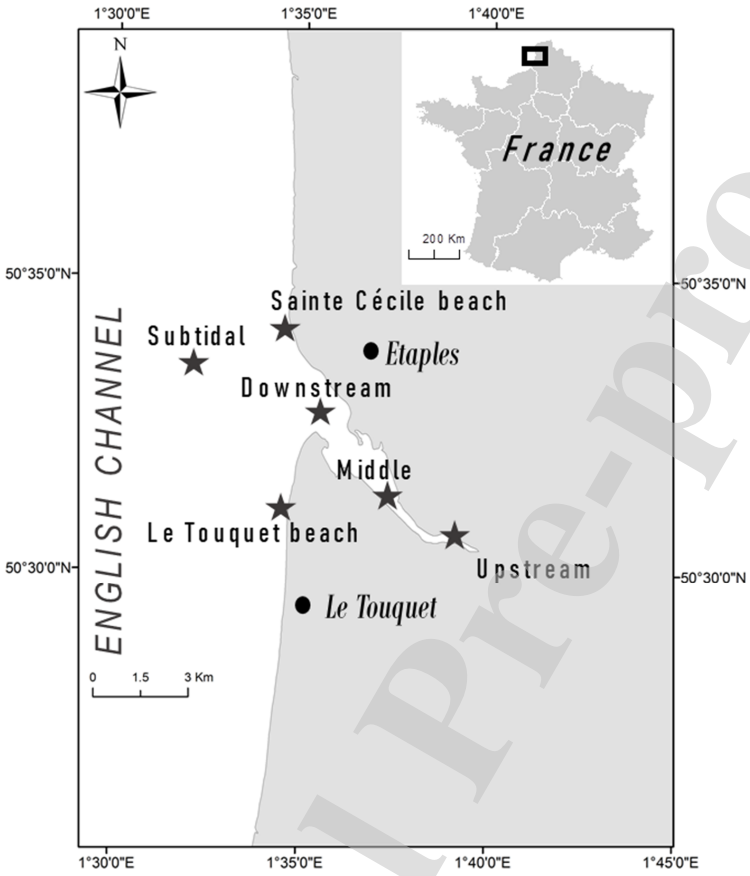


Figure 1. Map of the Canche estuary showing the sampling sites (stars) inside the estuary (upstream, middle, and downstream) and outside the estuary (surf zone (Sainte Cécile and Le Touquet beaches) and subtidal site).

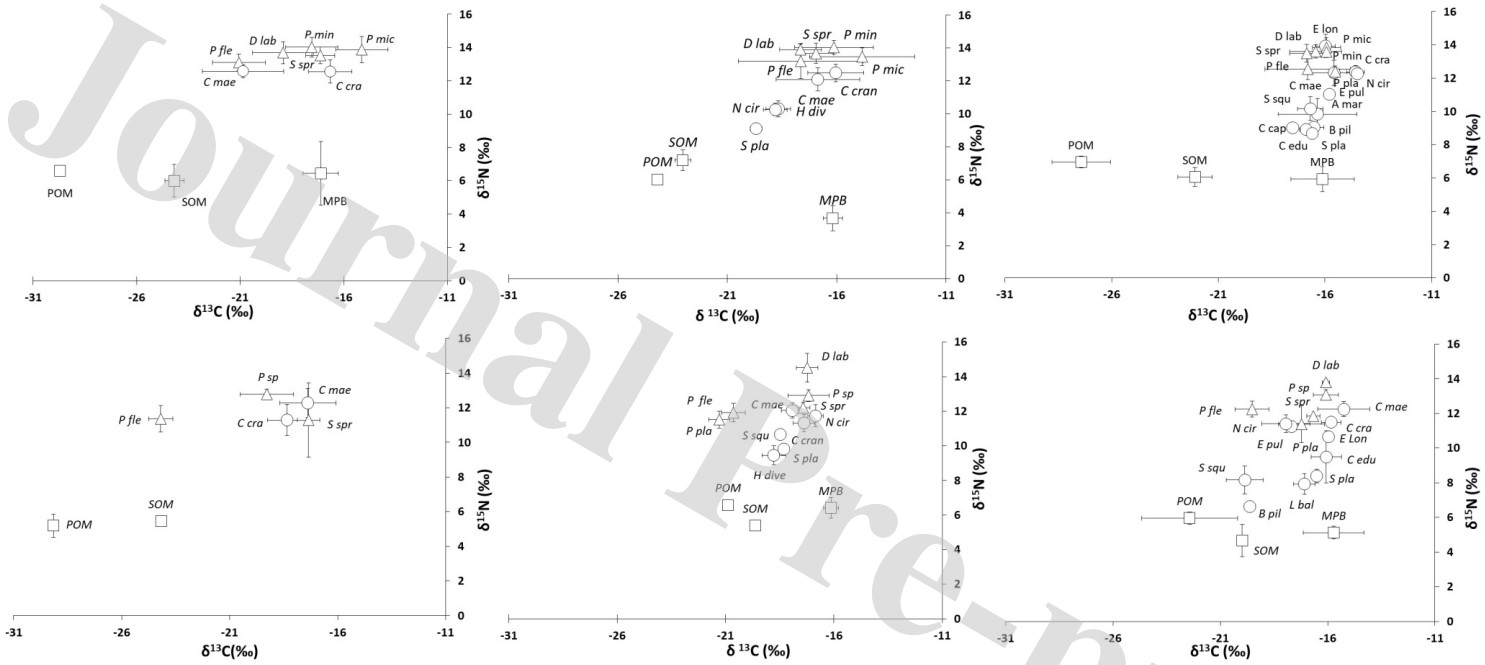
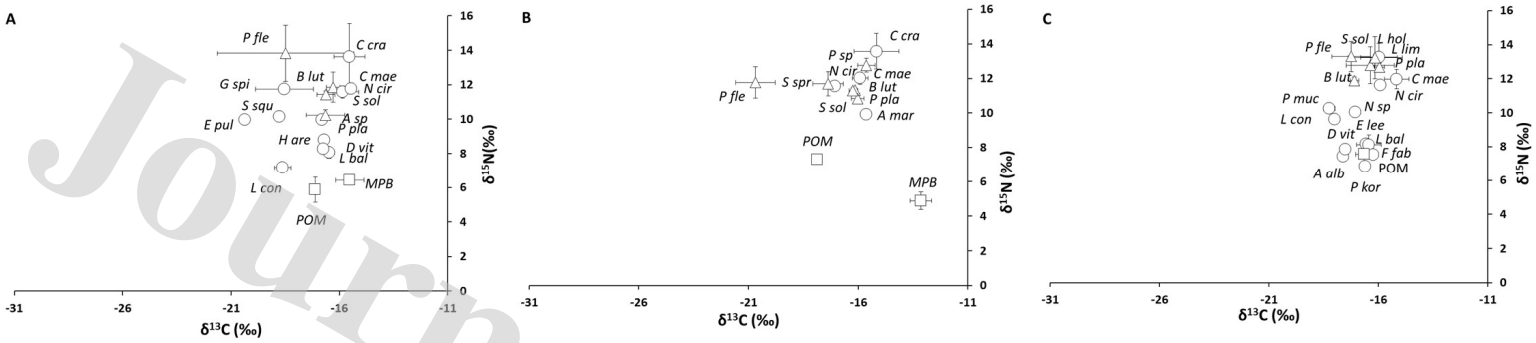


Figure 2. Mean (± standard deviation) $\delta^{13}C$ vs. $\delta^{15}N$ biplots of the food sources (□), invertebrates (○), and fish (Δ) inside the Canche estuary in the fall (A: upstream, B: middle, C: downstream) and spring (D: upstream, E: middle, F: downstream). See Tables 2 and 4 for abbreviations.



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522 Figure 3. Mean (± standard deviation) $\delta^{13}C$ vs. $\delta^{15}N$ biplots of the food sources (□), invertebrates (○), and fish (Δ) outside the Canche estuary in
523 the fall (A: Sainte Cecile beach, B: Le Touquet beach, C: subtidal site). See Tables 2 and 4 for abbreviations.

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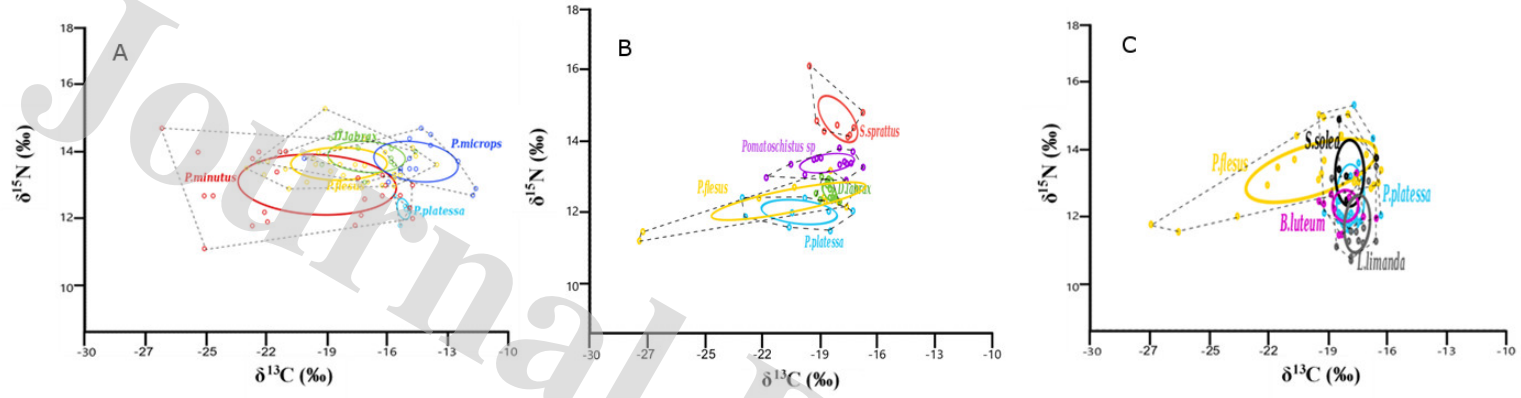

Figure 4. Convex hull polygons and corrected Standard Ellipse Areas (SEAc) of the fish community. Each convex hull is a measure of the total area, while each SEAc is a proxy of the mean isotopic niche of the core population, which is insensitive to sample size (A: inside the Canche estuary in the fall, B: inside the Canche estuary in spring, C: outside the Canche estuary in spring).

Appendix 1. Macrobenthic carbon and nitrogen stable isotope compositions (mean ± standard error, ‰), and number of individuals analysed (N) in spring

	Species	Upstream			Middle			Downstream			Saint Cécile Beach			Le Touquet Beach			Subtidal		
		N	$\delta^{15}N$	$\delta^{13}C$	N	$\delta^{15}N$	$\delta^{13}C$	N	$\delta^{15}N$	$\delta^{13}C$	N	$\delta^{15}N$	$\delta^{13}C$	N	$\delta^{15}N$	$\delta^{13}C$	N	$\delta^{15}N$	$\delta^{13}C$
	Scorbicularia plana (S pla)				3	9.2 ± 0.1	-18.9 ± 0.1	3	8.4 ± 0.4	-16.5 ± 0.1									
	Hediste diversicolor (H div)				3	9.4 ± 0.5	-18.8 ± 0.5												
	Scolelepis squamata (S squ)				2	10.7	-18.4	4	8.2 ± 0.8	-19.9 ± 0.8	2	10.2	-18.8						
	Limecola balthica (L bal)							4	7.9 ± 0.6	-17.1 ± 0.5	2	8.2	-16.7				3	8.1 ± 0.6	-16.4 ± 0.3
	Cerastoderma edule (Cedu)							5	9.5 ± 1.5	-16.1 ± 0.7									
primary	*Lanice conchilega (L con)*										4	7.1 ± 0.1	-18.6 ± 0.4				2	9.6	-18.0
consumers	*Donax vittatus (D vit)*										5	8.1 ± 1.3	-16.5 ± 0.5				3	7.8 ± 0.2	-17.5 ± 0.3
	Haustorius arenarius(H are)										2	8.8	-16.7						
	Arenicola marina (A mar)										2	9.9	-16.8	1	9.9	-15.6			
	Abra alba (A alb)																2	7.4	-17.6
	Fabulina fabula (F fab)																3	7.5 ± 0.1	-16.2 ± 0.2
	Ensis leei (E lee)																5	8.2 ± 0.2	-16.6 ± 0.2
	Crangon crangon (C cra)	6	11.3 ±0.9	-18.3 ±0.9	5	11.3 ± 0.5	-17.4 ± 0.5	5	11.5 ± 0.2	-15.8 ± 0.5	6	13.6 ± 1.9	-15.5 ± 0.7	7	13.6 ± 1.0	-15.6 ± 0.3			
	Carcinus maenas (C mae)	6	12.3 ±1.3	-17.4 ± 0.5	5	12.0 ± 0.4	-17.9 ± 1.1	5	12.3 ± 0.4	-15.3 ± 1.2	4	11.6 ± 0.3	-15.8 ± 0.7	4	12.0 ± 0.3	-15.9 ± 1.0	5	11.9 ± 0.5	-15.2 ± 0.2
	Nephtys cirrosa (N cir)				3	11.7 ± 0.6	-16.8 ± 0.4	2	11.4	-17.9	2	11.8	-15.5	2	11.6	-17.1	1	11.6	-15.9
secondary	*Eteone longa (E lon)*							1	10.7	-15.9									
consumers	*Eurydice pulchra (E pul)*							3	11.3 ± 0.1	-21.9 ± 0.6	3	9.1 ± 0.9	-20.4 ± 0.9						
	Gastrosaccus spinifer (G spi)											11.7 ± 0.1	-18.5 ± 1.3						
	Nemertes sp																3	10.0 ± 0.2	-17.0 ± 0.5
	Phyllodoce mucosa (P muc)																5	10.2 ± 0.3	-18.2 ± 1.5

534 **Appendix 2.** Macrobenthic carbon and nitrogen stable isotope compositions (mean ± standard
535 error, ‰), and the number of individuals analysed (N) in the fall

Trophic position	Species	Upstream			Middle			Downstream		
		N	$\delta^{15}N$	$\delta^{13}C$	N	$\delta^{15}N$	$\delta^{13}C$	N	$\delta^{15}N$	$\delta^{13}C$
primary consumers	*Scrobicularia plana (S pla)*				5	9.1 ± 0.2	-19.7 ± 0.2	3	8.7 ± 0.2	-16.6 ± 0.1
	Hediste diversicolor (H div)				10	10.3 ± 0.5	-18.7 ± 0.6			
	Scolelepis squamata (S squ)							4	10.2 ± 0.7	-16.7 ± 0.6
	Limecola balthica (L bal)							5	9.9 ± 0.3	-16.1 ± 0.5
	Cerastoderma edule (C edu)							7	8.9 ± 0.1	-16.9 ± 0.3
secondary consumers	*Crangon crangon (C cra)*	10	12.6 ± 0.7	-16.7 ± 1.0	9	12.5 ± 0.5	-16.0 ± 1.2	8	12.4 ± 0.9	-14.6 ± 0.4
	Carcinus maenas (C mae)	5	12.7 ± 0.4	-20.9 ± 1.9	6	12.1 ± 0.7	-16.9 ± 2	7	12.3 ± 0.8	-15.6 ± 1.0
	Nephtys cirrosa (N cir)				5	10.3 ± 0.4	-18.8 ± 0.5	3	12.3 ± 1.1	-14.5 ± 1.2
	Eteone longa (E lon)							2	13.9	-15.9
	Eurydice pulchra (E pul)							4	11.0 ± 0.3	-15.8 ± 0.2

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537 **Appendix 3.** Fish carbon and nitrogen stable isotope compositions (mean ± standard error, ‰) in fall and spring

Species (abbreviation)	Inside												Outside					
	Upstream				Middle				Downstream				Saint Cécile Beach		Le Touquet Beach		Subtidal	
	fall		spring		fall		spring		fall		spring		spring		spring		spring	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Pomatoschistus microps (P mic)	-15.1 ± 1.2	13.9 ± 0.8			-14.8 ± 2.4	13.4 ± 0.5			13.8 ± 0.5	13.8 ± 0.6								
Sprattus sprattus (S spr)	-17.1 ± 0.7	13.5 ± 0.5	-17.3 ± 0.5	11.3 ± 2.1	-16.6 ± 0.8	13.7 ± 0.6	-17.4 ± 0.3	12.2 ± 0.3	-16.8 ± 0.8	13.5 ± 0.8	-16.7 ± 0.3	11.9 ± 0.1			-17.4 ± 0.6	11.7 ± 0.7		
Pomatoschistus minutus (P min)	-17.6 ± 1.3	14.0 ± 0.6			-16.1 ± 1.8	13.7 ± 0.6			-15.9 ± 0.2	13.6 ± 0.1								
Dicentrarchus labrax (D lab)	-18.9 ± 1.5	13.7 ± 0.7			-17.7 ± 1.0	13.9 ± 0.3	-17.2 ± 1.2	14.5 ± 0.8	-16.4 ± 1.2	13.6 ± 0.3	-16.1 ± 0.2	13.8 ± 0.1						
Platichthys flesus (P fle)	-21.1 ± 1.3	13.1 ± 0.5	-24.2 ± 4.5	11.4 ± 0.8	-17.6 ± 2.8	13.2 ± 1.0	-20.6 ± 0.5	11.9 ± 0.5	-16.8 ± 2	12.5 ± 0.6	-19.5 ± 0.9	12.3 ± 0.5	-18.5 ± 3.1	13.8 ± 1.6	-20.7 ± 0.5	11.8 ± 0.9	-17.2 ± 0.5	13.3 ± 0.8
Buglossidium luteum (B lut)													-16.2 ± 0.3	11.8 ± 0.9	-16.2 ± 0.2	11.3 ± 0.1	-17.1 ± 0.4	11.9 ± 0.2
Pomatoschistus sp (P sp)			-19.3 ± 1.2	12.8 ± 0.3			-17.27 ± 0.9	12.9 ± 0.3			-16.1 ± 0.6	13.1 ± 0.3			-15.6 ± 0.3	12.7 ± 0.3		
Limanda Limanda (L lim)																	-16.4 ± 0.6	12.8 ± 1
Pleuronectes platessa (P pla)							-21.3 ± 0.5	11.5 ± 0.5	-15.5 ± 0.2	12.4 ± 0.2	-17.2 ± 1.0	11.4 ± 1.0	-16.6 ± 0.9	10.2 ± 0.3	-16.0 ± 0.6	10.8 ± 0.3	-15.9 ± 0.7	12.7 ± 0.2
Solea solea (S sol)													-16.6 ± 0.4	11.4 ± 0.2	-16.3 ± 0.5	11.3 ± 0.2	-16.2 ± 0.6	13.2 ± 1.2

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AUTHORSHIP STATEMENT

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All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in **Regional Studies in Marine Science**

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This statement is signed by all the authors (*a photocopy of this form may be used if there are more than 10 authors***):**

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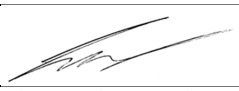
Rym Bouaziz		December 31, 2020

François Le Loc'h		December 31, 2020
Céline Rolet	The coauthor gave her agreement but the signature is missing due to year-end holidays. We will obtain it the first week of January.	December 31, 2020
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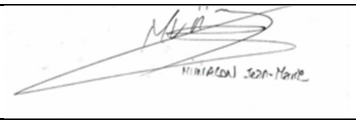
Jean Marie Munaron		December 31, 2020
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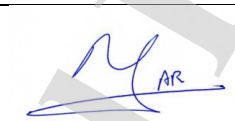
Rachid Amara		December 31, 2020
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Conflict of interest

Structure and seasonal variability in fish food webs in a small macrotidal estuary (Canche estuary, Eastern English Channel) based on stable carbon and nitrogen isotope analysis

The authors declare they don't have any conflict of interest.