
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

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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

35 1. Introduction

36 Using shallow marine coastal zones and estuaries as nursery areas is an important phase in the
37 life history of many marine organisms, including commercially valuable species (Amara,
38 2003; Vasconcelos et al., 2007). In temperate ecosystems, juvenile fish concentrate in nursery
39 grounds from late spring to early fall, which is the peak of macrobenthic prey biomass
40 (Amara and Paul, 2003; Pasquaud et al., 2010; Selleslagh et al., 2015). Refuge and feeding
41 areas for young fish in estuaries are considered important for fish survival and replenishing
42 coastal fish stocks (McLusky and Elliott, 2004). However, many authors have shown that
43 estuaries are only one component of broader nursery-use patterns and that shallow marine
44 coastal areas and estuaries may provide a mosaic of vital nursery habitat types for juvenile
45 marine fish (Beck et al., 2001). Although opportunism is widely reported for estuary-
46 associated fish, as well as for fish in general (Amara et al., 2001; Elliott et al., 2007; Elliott
47 and Hemingway, 2002), the importance of these different habitats (specific salinity zones
48 inside estuaries and adjacent marine subtidal and surf zones) for fish feeding is not well
49 understood (Selleslagh et al., 2015; Vinagre et al., 2008). In addition, there is still a lack of
50 information and confusing conclusions about the main origin of the organic matter that
51 sustains juvenile fish food webs in nursery grounds (Le Pape et al., 2013). For estuaries, some
52 studies (Darnaude et al., 2004; Leakey et al., 2008; Vinagre et al., 2008) indicated that
53 allochthonous organic matter of terrestrial origin predominated, while other studies suggested
54 that marine organic matter predominated (Pasquaud et al., 2008; Selleslagh et al., 2015). Most
55 of these studies were conducted in large estuaries (e.g. Tagus, Gironde, Thames, Rhone,
56 Vilaine). However, in small estuaries with little freshwater influence, *in situ* primary
57 production can override other food sources and contribute significantly to juvenile food webs
58 (Kostecki et al., 2012). Understanding the main ecological processes in ecosystems (e.g. food
59 sources, trophic transfer through the food web) and identifying juvenile fish feeding areas and
60 their spatial use of shallow nursery habitats are fundamental issues for effective conservation
61 and management of these essential fish habitats (Hobson et al., 1999).

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

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

73 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are typically increased from prey to consumers by 3‰–4‰ for $\delta^{15}\text{N}$ and 1‰
74 for $\delta^{13}\text{C}$ (De Niro and Epstein, 1981; Minagawa and Wada, 1984; Cabana and Rasmussen,
75 1994). Thus, $\delta^{15}\text{N}$ can indicate the trophic position of an organism within the food web and
76 $\delta^{13}\text{C}$ values of the food sources (primary producers as phytoplankton or microphytobenthos)
77 are also responsible for differences in the isotopic compositions of their consumers (Riera et
78 al. 1999), allowing distinctions to be made between pelagic and benthic food webs (Hobson et
79 al. 2002) or fresh vs marine waters (Fry, 2006). Consequently, $\delta^{13}\text{C}$ can be used as a tracer of
80 organic trophic sources for benthic consumers (Peterson, 1999).

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

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

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

100

101

102 2. Materials and Methods

103 2.1 Study area

104 The Canche estuary (50°50'-50°56' N, 1°57'-1°67' E) is located in northern France along the
105 coast of the Eastern English Channel (EEC). The Canche estuary is 12 km long and has a
106 maximum width of 1 km at its mouth. The estuary has a semi-diurnal tide, with an average
107 tidal range of ca. 1 m at neap tides and 6 m at spring tides, and is considered a macro/hyper-
108 tidal estuary according to the McLusky and Elliott (2004) classification. Water circulation
109 depends mainly on the tide and small freshwater inputs, with a mean annual rate of ca. 13
110 m³.s⁻¹. The Canche estuary is little impacted by human disturbances (Amara et al., 2007), it
111 belongs to the special conservation zone “Bay of Canche and corridor of the 3 estuaries” and
112 is classified as a “Natura 2000” site because it accounts as a major area of hosting juvenile
113 fish in the Eastern English Channel.

114

115 2.2 Sampling strategy

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

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

127 The sampling sites consist of a variety of habitats. The middle part of the Canche estuary is a
128 muddy-sand shore that contains polychaetes and bivalves (EUNIS classification A2.24; Rolet
129 et al., 2015). The downstream part is characterized by a medium fine-sand benthic community
130 (EUNIS classification A2.223) and is dominated by amphipods and *Scolelepis* spp. (Rolet et
131 al, 2015) such as the surf zone (Sainte Cécile and Le Touquet beaches). The subtidal site is
132 muddy fine sand and contains polychaetes (*Magelona johnsoni*, *Nephtys* spp.) and bivalves
133 (*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 $-20\text{ }^{\circ}\text{C}$ 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 $-20\text{ }^{\circ}\text{C}$ 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 $60 \pm 1\text{ }^{\circ}\text{C}$ for at least 48 hours until a constant weight was obtained. It

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

170

171 **2.6 Fish, crab and shrimp sampling**

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

176

177 **2.7 Stable isotope analyses**

178 Species selected for SIA were dominant in both abundance and biomass to obtain a synthetic
179 image of the trophic structure within each community. As lipids are depleted in $\delta^{13}\text{C}$
180 compared to carbohydrates and proteins (DeNiro and Epstein, 1977; Griffiths, 1991), which
181 indicates that fatty tissues tend to be isotopically lighter than leaner ones, trophic
182 interpretations based on $\delta^{13}\text{C}$ composition may be confounded by lipid effects (Bodin et al.,
183 2007; Wada et al., 1993). To minimize these effects, mega- and macrofaunal (except for
184 polychaetes) low-lipid muscle tissue was used for SIA. Polychaetes were analysed after
185 removing their viscera by dissection. The valve muscle of bivalves, the abdomen muscle of
186 shrimp, the muscle in crab pincers and the white dorsal muscle of fish (even small ones) were
187 dissected and analysed for SIA. For other benthic organisms, the entire organism was
188 analysed after removing the digestive tract, jaws and cerci. The tissues were then washed with
189 milli-Q water to prevent contamination and freeze-dried before being encapsulated. For small
190 benthic organisms (*Bathyporeia pilosa*, *Eurydice pulchra*, *Gastrosaccus spinifer*, *Haustorius*
191 *arenarius*), each sample represented a combination of 2-4 individuals.

192 As fish size can influence isotope values, especially $\delta^{15}\text{N}$, due to ontogeny (Galván et al.,
193 2010; Wilson et al., 2009), we carefully selected individuals of similar size across species in
194 order to be sure that we select G0 juveniles. Before $\delta^{13}\text{C}$ analyses, POM and MPB filters were
195 divided into two subsamples: one was exposed to HCl vapour for 4 h to remove residual
196 carbonates (Cresson et al., 2012) before being placed in tin cups (Lorrain et al., 2003), while
197 the other was not treated and was used to measure $\delta^{15}\text{N}$. Sediment samples were dried at 60°C
198 for 24 h. They were divided into two subsamples: one was treated with HCl, to remove

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

201 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were measured using an elemental analyser Flash EA 2000 (Thermo
202 Scientific), connected to an Isotope Ratio Mass Spectrometer (Delta V+) with a ConFlo IV
203 interface (Thermo Scientific) at the Pôle Spectrométrie Océan in Plouzané, France. Replicate
204 analyses of international IAEA and laboratory USGS standards provided analytical errors
205 $<0.20\%$ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Stable isotope ratios were expressed as parts per mil (‰) in
206 the δ notation relative to the Pee Dee Belemnite standard for carbon and atmospheric N_2 for
207 nitrogen using the formula:

$$208 \quad \delta X(\text{‰}) = [(R \text{ sample}/R \text{ standard}) - 1] \times 1000$$

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

210

211 **2.8 Data analysis**

212 2.8.1 Statistical analysis

213 We first tested the hypothesis that potential sources and prey for fish had significantly
214 different isotopic compositions along the salinity gradient, and then that compositions at sites
215 inside vs. outside of the estuary differed significantly. For all samples, two-way ANOVAs
216 were performed separately for each ratio, factors are sites and seasons (after verifying that the
217 assumptions of parametric tests were met). ANOVA was used to test differences in $\delta^{13}\text{C}$ and
218 $\delta^{15}\text{N}$ in the POM from the water sources, considering site and season effects. Biplots of $\delta^{13}\text{C}$
219 vs. $\delta^{15}\text{N}$ were used to represent graphically means and standard deviations of isotopic
220 compositions of all compartments of the entire food web in each habitat. Besides, we
221 performed a Kruskal-Wallis test to test for fish species length homogeneity between sites and
222 seasons.

223

224 2.8.2 Layman metrics

225 We assessed isotopic niches of fish by calculating the three Layman metrics (Layman et al.,
226 2007): nitrogen range (NR), carbon range (CR) and total area (TA). We used a Bayesian
227 approach based on multivariate ellipse-based metrics (Jackson et al., 2011), in which the
228 location of the centroid represents the centre of the trophic niche in isotopic space. To
229 describe the spread of data points, we calculated the parameters developed by Layman et al.

230 (2007). We constructed convex hulls to estimate the smallest TA that contained all individuals
231 in the isotopic space. The TA can be interpreted as a measure of the total isotopic niche of a
232 population (Layman et al., 2007). We estimated the niche width in each season using
233 multivariate ellipse-based metrics (Jackson et al., 2011). The analysis generates standard
234 ellipse areas (SEA), which are bivariate equivalents of standard deviations in univariate
235 analyses.

236

237 3. Results

238 3.1 Benthic communities and biomass

239 The upstream site was too muddy to sample benthic organisms. Among the five sites sampled
240 in spring, we identified 1288 individuals that belonged to 51 taxa (Table 1). Mean
241 macrobenthic species richness, abundance and biomass were higher outside than inside the
242 estuary (Wilcoxon test, $p < 0.0001$). Species richness was lowest in the middle of the estuary
243 (6 species) but higher at the downstream site (16), Sainte Cécile beach (13), Le Touquet beach
244 (11) and the subtidal site (32). The main species observed in the middle of the estuary were
245 the polychaetes *Hedistes diversicolor* (326 ind.m⁻²; 2 011 mg.m⁻²) and the bivalve
246 *Scorbicularia plana* (565 mg.m⁻²). The polychaete *Scolecopsis squamata* (823 ind.m⁻²; 3 445
247 mg.m⁻²) and the bivalves *Limecola balthica* (1 282 mg.m⁻²) and *Cerastoderma edule* (640
248 mg.m⁻²) dominated the downstream site. The two surf zone sites (Le Touquet and Sainte
249 Cécile) were characterized by the polychaetes *S. squamata* (48-300 ind.m⁻²; 145-835 mg.m⁻²),
250 *Nephtys cirrosa* (124-160 ind.m⁻²; 559-831 mg.m⁻²) and *Lanice conchylega* (1 022 mg.m⁻² at
251 Sainte Cécile) and the bivalves *M. balthica* (153-1336 mg.m⁻²), *C. edule* (693 mg.m⁻² at
252 Sainte Cécile) and *Fabulina fabula* (706 mg.m⁻² at Le Touquet). More diverse taxa (N = 32)
253 and huge benthic biomass were observed at the subtidal site, which was dominated mainly by
254 the bivalves *D. vittatus* (516 ind.m⁻²; 108 035 mg.m⁻²), *F. fabula* (412 ind.m⁻²; 12 906 mg.m⁻²)
255 and *Ensis leei* (36 ind.m⁻²; 9 064 mg.m⁻²).

256

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

<i>Liocarcinus vermalis</i>					465.9	36
<i>Sigalion squamosus</i>					443.1	44
<i>Anapagurus hynchmanni</i>					377.4	12
<i>Nephtys assimilis</i>					244.3	4
<i>Lagis koreni</i>					218.7	36
<i>Phyllococe mucosa (P muc)</i>					144.4	244
Planarian					118.8	4
<i>Pinnotheres pisum</i>					74.6	12
Nemertians					42.2	32
<i>Eteone picta</i>					9.7	4
<i>Schistomysis kervillei</i>					8.4	4
<i>Diastylis rugosa</i>					5.8	4
<i>Capitella capitata (C cap)</i>					2.8	20
<i>Leucothoe incisa</i>					2.4	8
<i>Eumida sanguinea</i>					1.1	8
<i>Nototroptis swammerdami</i>					0.6	4
<i>Pariambus typicus</i>					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

259 3.2 Fish sampled for stable isotope analyses

260 Nine fish species (eight in spring and six in fall) were collected for SIA: *Buglossidium luteum*,
261 *Dicentrarchus labrax*, *Limanda limanda*, *Platichthys flesus*, *Pleuronectes platessa*, *Solea*
262 *solea*, *Sprattus sprattus*, *Pomatoschistus microps* and *Pomatoschistus minutus* (Table 2).
263 Most individuals were G0 juveniles, except *P. microps*, *P. minutus*, *S. solea* and *P. platessa*
264 (at the subtidal site in spring) and *P. flesus* (at Sainte Cécile beach in spring). *S. solea*, *L.*
265 *limanda* and *B. luteum* were collected only outside the Canche estuary.
266 In the fall, fish of the same species did not differ significantly in size among sites, except *P.*
267 *flesus* ($p = 0.020$,) and *S. sprattus* ($p = 0.006$), which were smaller in the middle of the estuary
268 than upstream or downstream. In spring, *P. platessa* were significantly longer at the subtidal
269 site ($p < 0.0001$). *P. flesus* were also significantly longer at Sainte Cécile than at the other
270 sites ($p = 0.003$). Inside the estuary, fish size varied between the two seasons for the four
271 species caught in both spring and fall (*D. labrax*, *P. flesus*, *S. sprattus* and *P. platessa*; $p <$
272 0.0001).

273 Table 2. Total length (mean \pm 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
<i>Dicentrarchus labrax</i> (D lab)		5.9 \pm 0.9	10	5.2 \pm 0.9	10	6.3 \pm 0.5	11						
<i>Platichthys flesus</i> (P fle)		6.0 \pm 3.0	11	3.8 \pm 0.2	10	6.9 \pm 1.3	9						
<i>Pleuronectes platessa</i> (P pla)	Fall	6.9 \pm 2.8	11	3.9 \pm 0.2	6	6.6 \pm 1.3	5						
<i>Sprattus sprattus</i> (S spr)		4.4 \pm 0.2	5	3.8 \pm 0.2	5	6.4 \pm 1.2	9						
<i>Pomatoschistus microps</i> (P mic)		5.3 \pm 0.5	5	4.1 \pm 0.1	5	4.6 \pm 0.7	5						
<i>Pomatoschistus minutus</i> (P min)						5.9 \pm 0.3	5						
<i>Dicentrarchus labrax</i> (D lab)				8.2 \pm 1.1	4	8.4 \pm 0.5	3						
<i>Platichthys flesus</i> (P fle)		7.8 \pm 2.8	4	6.4 \pm 2.8	5	8.7 \pm 5.2	4	13.0 \pm 1.2	6	10.6 \pm 3.1	5	7.0 \pm 5.4	10
<i>Pleuronectes platessa</i> (P pla)				3.9 \pm 4.8	5	4.6 \pm 1.0	4	5.5 \pm 7.4	5	7.1 \pm 0.9	9	14.5 \pm 5.2	4
<i>Sprattus sprattus</i> (S spr)		4.3 \pm 3.0	10	4.8 \pm 3.7	5	4.5 \pm 0.4	3			6.4 \pm 1.5	5		
<i>Pomatoschistus</i> sp. (P sp)	Spring			4.3 \pm 2.8	4	4.8 \pm 2.5	5						
<i>Solea solea</i> (S sol)								3.5 \pm 2.4	7	3.7 \pm 2.2	7	13.1 \pm 2.1	8
<i>Buglossidium luteum</i> (B lut)								7.6 \pm 3.8	5	4.1 \pm 2.3	5	5.9 \pm 4.2	5
<i>Limanda limanda</i> (L lim)												13.4 \pm 1.0	6

275 3.3 Results of stable isotope analyses

276 3.3.1 Food sources

277 The stable isotope composition of POM ranged from -29.70‰ to -20.15‰ for $\delta^{13}\text{C}$ and 5.20-
278 7.30‰ for $\delta^{15}\text{N}$ (Table 3). POM $\delta^{13}\text{C}$ differed significantly among sites ($F = 9.536$; $p <$
279 0.0001 , ANOVA) and between seasons ($F = 9.448$; $p < 0.0001$, ANOVA). In spring, mean
280 POM $\delta^{13}\text{C}$ was significantly lower inside the estuary ($F = 9.536$; $P < 0.0001$) and higher at the
281 subtidal site ($-20.89 \pm 0.10\text{‰}$) (Table 3). Mean $\delta^{15}\text{N}$ also differed significantly among sites (F
282 $= 3.745$; $P < 0.0001$) and between seasons ($F = 3.762$; $P < 0.0001$). POM $\delta^{15}\text{N}$ was
283 significantly higher at Le Touquet beach ($7.30 \pm 0.01\text{‰}$ $P = 0.144$, Kruskal-Wallis) (Table 3).
284 The stable isotope composition of SOM ranged from -24.18‰ to -19.62‰ for $\delta^{13}\text{C}$ and 4.67-
285 7.19‰ for $\delta^{15}\text{N}$ (Table 3) and differed significantly among sites and between seasons for both
286 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. The ANOVA revealed a site effect on $\delta^{15}\text{N}$, as well as a season effect, with the
287 lowest values in spring, and a significant effect of the site \times season interaction ($F = 10.548$, p
288 < 0.0001). SOM $\delta^{13}\text{C}$ showed significant enrichment from upstream to downstream along the
289 estuary in both spring ($F = 9.352$, $p < 0.0001$) and fall ($F=4.531$, $p < 0.0001$) (Table 3). The
290 MBP had the most depleted $\delta^{15}\text{N}$ ratios of the food sources sampled. The stable isotope
291 composition of MPB ranged from -17.1‰ to -13.1‰ for $\delta^{13}\text{C}$ and 3.66-6.44‰ for $\delta^{15}\text{N}$
292 (Table 3). MBP $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ did not differ significantly among sites or between seasons.

293

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

season	Upstream		Middle		Downstream		Sainte Cécile beach		Le Touquet beach		Subtidal	
	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
POM	6.61*	-29.70*	6.00 \pm 0.05	-24.20 \pm 0.14	6.96 \pm 0.35	-27.43 \pm 1.37						
SOM	6.00 \pm 0.96	-24.18 \pm 0.46	7.19 \pm 0.62	-23.04 \pm 0.36	6.07 \pm 0.57	-22.09 \pm 0.80						
MPB	6.44 \pm 1.90	-17.11 \pm 0.85	3.66 \pm 1.75	-16.18 \pm 0.44	5.94 \pm 0.75	-16.12 \pm 0.48						
POM	5.20 \pm 0.66	-29.16 \pm 0.21	6.57 \pm 0.07	-20.89 \pm 0.10	5.96 \pm 1.24	-22.43 \pm 1.24	6.08 \pm 0.72	-20.15 \pm 0.23	7.30 \pm 0.01	-20.89 \pm 0.10	6.50 \pm 0.40	-20.50 \pm 0.17
SOM	5.46 \pm 0.01	-24.18 \pm 0.16	5.39 \pm 0.06	-19.62 \pm 0.05	4.67 \pm 0.94	-19.98 \pm 0.20	4.80 \pm 0.72	-19.95 \pm 0.92				
MPB			6.40 \pm 0.59	-16.13 \pm 0.34	5.12 \pm 0.37	-15.71 \pm 1.41	6.42 \pm 0.16	-15.52 \pm 0.65	4.88 \pm 0.49	-13.14 \pm 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 $\delta^{13}\text{C}$ values (-20‰ to -18‰), while the crustaceans *Carcinus maenas* and
301 *Crangon crangon* had the highest $\delta^{13}\text{C}$ 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 $\delta^{15}\text{N}$ values, while the crustaceans *C. crangon* and *C.*
304 *maenas* (11.3-12.6‰) had the highest $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ values, while the predatory *C. maenas*, *C. crangon* and *N.*
307 *cirrosa* (-17.1‰ to -15.2‰) had the highest $\delta^{13}\text{C}$ values (Appendix 1). The suspension
308 feeders *D. vittatus*, *L. balthica* and *L. conchylega* had the lowest $\delta^{15}\text{N}$ values (7.1-9.6‰),
309 while the predatory shrimp *C. crangon* had the highest $\delta^{15}\text{N}$ values (13.6‰) (Appendix 1).

310 In spring, the mean $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values (-24.2‰ to -16.8‰),
316 while in the fall, *P. microps* had the highest $\delta^{13}\text{C}$ values (-15.9‰ to -14.8‰). Inside the
317 estuary in the fall, all $\delta^{15}\text{N}$ values exceeded 13‰, except for *P. flesus* (12.5±0.6‰) and *P.*
318 *platessa* (12.4±0.2‰), both downstream. Fish $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{15}\text{N}$ (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.

Species	Fall			Spring					
	Inside			Inside			Outside		
	SEAc	CR	NR	SEAc	CR	NR	SEAc	CR	NR
<i>Pomatoschistus minutus</i>	17.58	9.01	2.63						
<i>Platichthys flesus</i>	16.41	7.67	3.59	23.49	8.50	4.74	12.79	4.77	3.95
<i>Pomatoschistus microps</i>	14.74	7.62	3.10						
<i>Dicentrarchus labrax</i>	13.28	7.19	2.68	17.60	7.30	4.17			
<i>Pleuronectes platessa</i>	5.45	6.99	2.86	25.04	8.86	4.67	14.65	6.50	3.59
<i>Pomatoschistus</i> sp				20.05	7.95	4.56			
<i>Sprattus sprattus</i>				18.92	8.43	3.89			
<i>Buglossidium luteum</i>							12.95	5.26	3.84
<i>Limanda limanda</i>							12.44	5.06	3.99
<i>Solea solea</i>							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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338 4. Discussion

339 4.1 Origin of and variability in organic matter in the Canche estuary

340 As estuaries are complex and changing ecosystems, it is challenging to distinguish the sources
341 of organic matter at the base of food webs (Pasquaud et al., 2008; Selleslagh and Amara,
342 2015). Organic matter is a major component of suspended particles and fine sediment
343 particles that determine many biogeochemical processes in marine environments (Bernasconi
344 et al., 1997; Chen et al., 2012). Two natural sources of organic matter are generally
345 considered in coastal ecosystems: allochthonous inputs and autochthonous production
346 (Antonio and Richoux, 2014; Luo et al., 2016). Major sources of autochthonous organic
347 matter include phytoplankton and aquatic macrophytes (Dalu and Froneman, 2016; Pearson et
348 al., 2015). In estuarine ecosystems, organic matter of terrestrial origin is a major contributor
349 to allochthonous organic matter (Duan et al., 2014; Lu et al., 2013). Analysis of the carbon
350 and nitrogen stable isotopic compositions of estuarine organic matter can identify their
351 contributions to the food web (Darnaude et al., 2004; Evans et al., 2019). Generally, POM in
352 estuaries is composed of river POM (mixture of terrestrial POM and freshwater
353 phytoplankton), estuarine-produced and marine phytoplankton, resuspended
354 microphytobenthos and diverse detritus (e.g. faeces, macrophytes), which can make
355 interpretation difficult (Kang et al., 2006).

356 In the Canche estuary, the POM stable isotopic signatures indicated a mixed organic matter
357 composition that included freshwater/estuarine phytoplankton ($\delta^{13}\text{C}$ ranging from -29‰
358 to -20‰), with a clear increase in POM $\delta^{13}\text{C}$ values from fresh to marine waters, similar to
359 previous estuarine studies in the Bay of Marennes-Oléron (Riera and Richard, 1996), the
360 Vilaine estuary (Kostecki et al., 2010) and the Gironde estuary (Selleslagh et al., 2015). In the
361 Canche estuary, salinity exhibits short-term changes, with a large amplitude from 0-35 due to
362 the small size of the estuary, tide conditions, season and weather conditions (Amara et al.,
363 2009; Selleslagh and Amara, 2008). MPB that live in intertidal flats in estuaries can
364 contribute much of the total primary production in estuaries (Underwood and Kromkamp,
365 1999). MPB on intertidal flats is composed mainly of benthic diatoms (Méléder et al., 2007)
366 and several studies have emphasised its key role in sustaining intertidal food webs
367 (Christianen et al., 2017; Herman et al., 2000; Thrush et al., 2012). In the Canche estuary, the
368 MPB is one of the main primary producers and could therefore be an important source of
369 organic matter for benthic invertebrates. As in other European estuaries, the Canche MPB had
370 the highest enriched carbon ratios (-17‰ to -15‰) among food sources, which allowed it to
371 be traced in its consumers (Moens et al., 2002; Moncreiff and Sullivan, 2001; Riera and

372 Richard, 1996). SOM is a mixture of benthic and deposited pelagic microalgae, bacteria,
373 aquatic and terrestrial plant debris and meiofauna. In the Canche estuary, SOM and POM
374 $\delta^{13}\text{C}$ followed the same trend, with the lowest values upstream and highest values
375 downstream due to the presence of freshwater phytoplankton, as measured in other nearby
376 estuaries (Lambert et al., 2017; Middelburg and Nieuwenhuize, 1998).

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

389

390 **4.2 Structure of invertebrate communities**

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

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

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

428

429 **4.3 Fish structure and seasonal variations**

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

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

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

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

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

468

469 5. Conclusion

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

472 situation in European estuaries (Dubois et al., 2014). This result provides a new vision of the
473 Canche estuary, which has been considered an important feeding ground for marine fish.
474 Our study revealed that ~~these~~ continental inputs have a minor role in the functioning of the
475 Canche estuary and that fish species might visit the estuary for reasons other than feeding,
476 such as to avoid predation or because they are carried by the tide. We highlighted the need to
477 take into account the whole small macrotidal estuary and adjacent ecosystems to better
478 describe the flatfish nursery. This work demonstrated that potential prey and feeding sources
479 for fish had habitat-specific compositions, which confirms the suitability of SIA for tracing
480 fish movements, fidelity and connectivity inside and outside the Canche estuary for sites less
481 than 10 km apart. Estuarine nursery feeding grounds, even in small estuaries, appear to be
482 complex due to the mosaic of benthic communities (potential prey), which are related to the
483 habitat (e.g. sediment type, foreshore position, salinity fluctuations) and to trophic
484 competition and predation.

485

486 **Acknowledgements**

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

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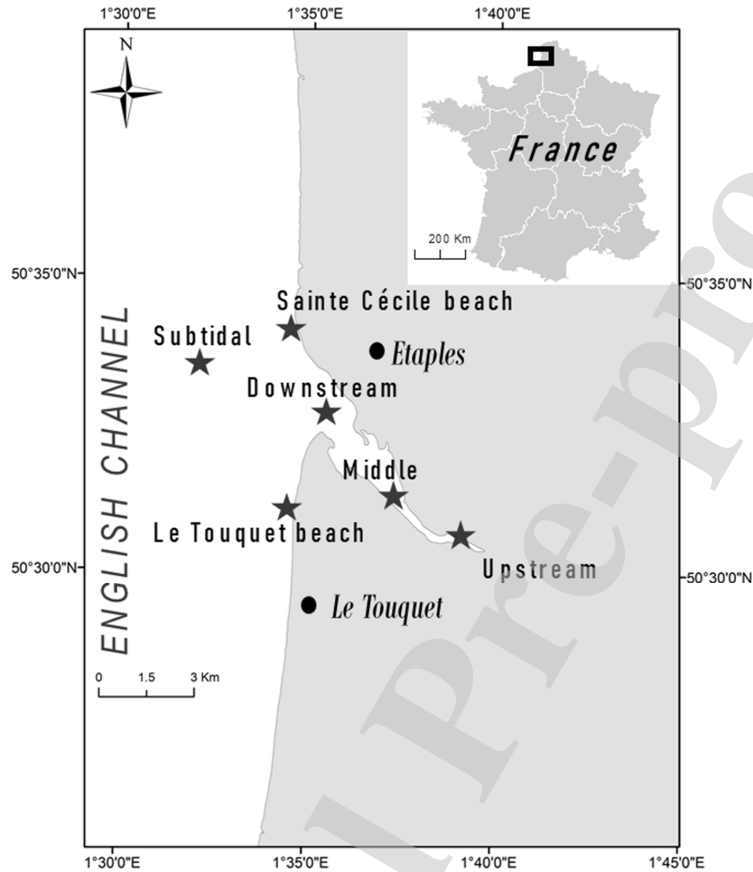
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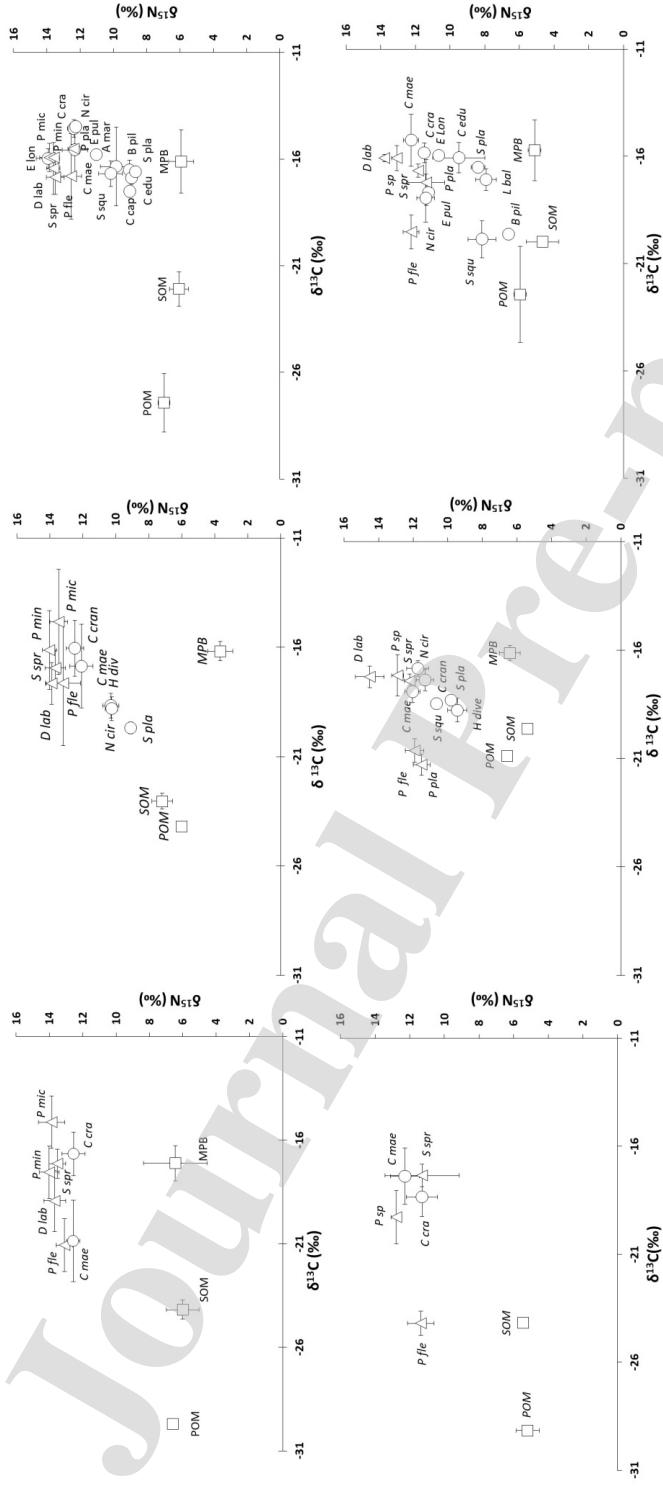
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512 Figure 1. Map of the Canche estuary showing the sampling sites (stars) inside the estuary
 513 (upstream, middle, and downstream) and outside the estuary (surf zone (Sainte Cécile and Le
 514 Touquet beaches) and subtidal site).

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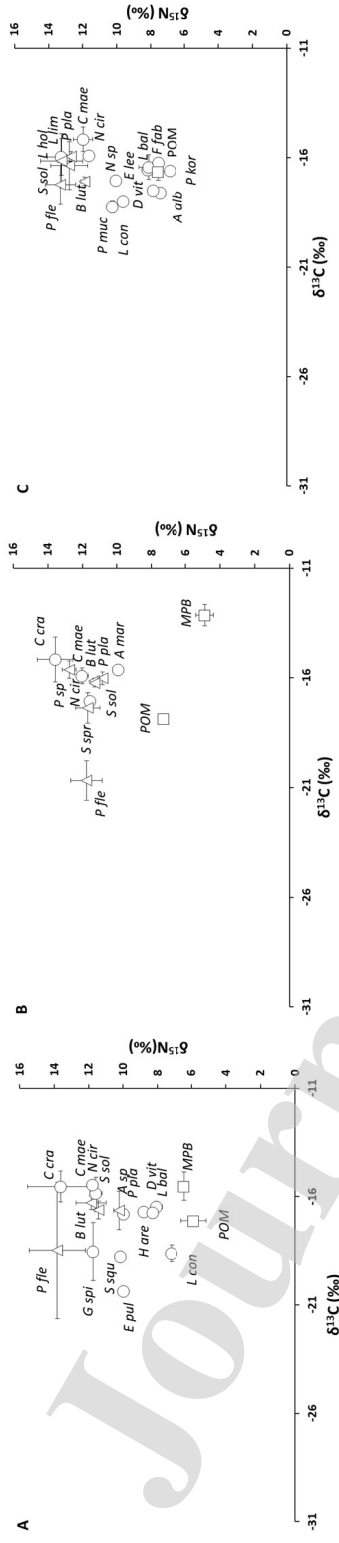
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518 Figure 2. Mean (\pm standard deviation) $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$ biplots of the food sources (\circ), invertebrates (\square), and fish (Δ) inside the Canche estuary in
 519 the fall (A: upstream, B: middle, C: downstream)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

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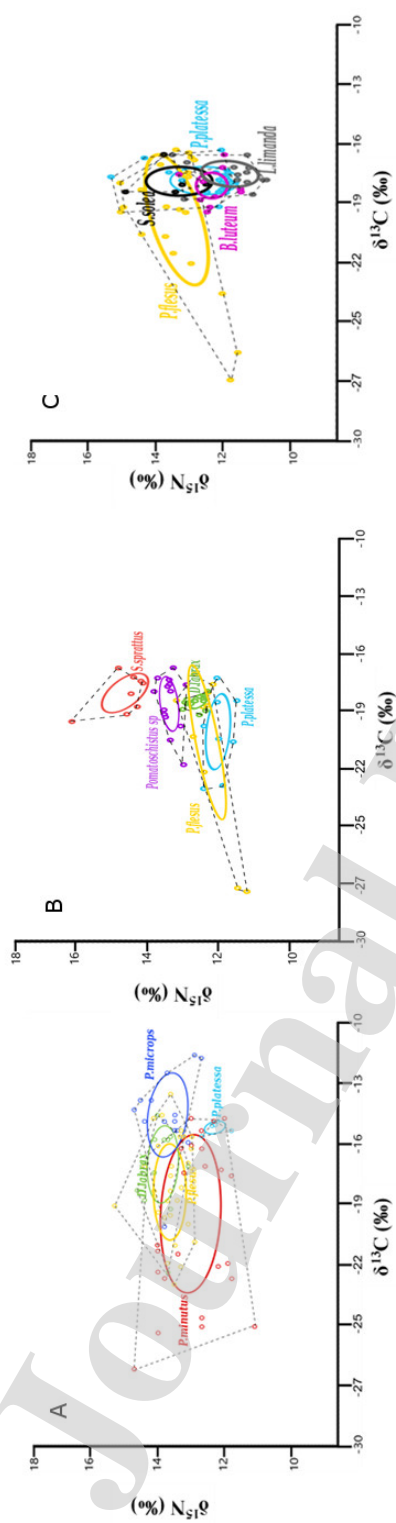


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522 Figure 3. Mean (\pm standard deviation) $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$ biplots of the food sources (\square), invertebrates (\circ), and fish (Δ) outside the Canche estuary in

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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526
527 Figure 4. Convex hull polygons and corrected Standard Ellipse Areas (SEAc) of the fish community. Each convex hull is a measure of the total
528 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
529 estuary in the fall, B: inside the Canche estuary in spring, C: outside the Canche estuary in spring).

530

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

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

533

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

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

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824
825

AUTHORSHIP STATEMENT

Manuscript title: 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.

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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Acknowledgements

All persons who have made substantial contributions to the work reported in the manuscript (e.g., technical help, writing and editing assistance, general support), but who do not meet the criteria for authorship, are named in the Acknowledgements and have given us their written permission to be named. If we have not included an Acknowledgements, then that indicates that we have not received substantial contributions from non-authors.

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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.