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## 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

Using shallow marine coastal zones and estuaries as nursery areas is an important phase in the 36 life history of many marine organisms, including commercially valuable species (Amara, 37 2003; Vasconcelos et al., 2007). In temperate ecosystems, juvenile fish concentrate in nursery 38 grounds from late spring to early fall, which is the peak of macrobenthic prey biomass 39 (Amara and Paul, 2003; Pasquaud et al., 2010; Selleslagh et al., 2015). Refuge and feeding 40 areas for young fish in estuaries are considered important for fish survival and replenishing 41 42 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 43 44 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-45 associated fish, as well as for fish in general (Amara et al., 2001; Elliott et al., 2007; Elliott 46 47 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 48 understood (Selleslagh et al., 2015; Vinagre et al., 2008). In addition, there is still a lack of 49 information and confusing conclusions about the main origin of the organic matter that 50 sustains juvenile fish food webs in nursery grounds (Le Pape et al., 2013). For estuaries, some 51 studies (Darnaude et al., 2004; Leakey et al., 2008; Vinagre et al., 2008) indicated that 52 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, Vilaine). However, in small estuaries with little freshwater influence, in situ primary 56 production can override other food sources and contribute significantly to juvenile food webs 57 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 their spatial use of shallow nursery habitats are fundamental issues for effective conservation 60 and management of these essential fish habitats (Hobson et al., 1999). 61

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,

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 the fate of different sources of both freshwater and marine particulate organic matter (POM) 71 can be distinguished (Fry, 1999; Riera et al., 1999; Yokoyama et al., 2008). 72  $\delta$ 13C and  $\delta$ 15N are typically increased from prey to consumers by 3‰–4‰ for  $\delta$ 15N and 1‰ 73 for  $\delta 13C$  (De Niro and Epstein, 1981; Minagawa and Wada, 1984; Cabana and Rasmussen, 74 1994). Thus,  $\delta$ 15N can indicate the trophic position of an organism within the food web and 75 76  $\delta$ 13C 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 al. 2002) or fresh vs marine waters (Fry, 2006). Consequently,  $\delta 13C$  can be used as a tracer of 79 organic trophic sources for benthic consumers (Peterson, 1999). 80 81 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 86 estuary on the French coast of the Eastern English Channel (EEC) - the Canche estuary -87 based on stable carbon and nitrogen isotopes analysis. The Canche estuary is considered a 88 reference for small macrotidal estuaries because it is subject to low human disturbance and is 89 an important fish nursery ground (Selleslagh et al., 2009; Selleslagh and Amara, 2015) that 90 supports species with high commercial and recreational value during their juvenile stage. The 91 92 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 93 use both estuaries and shallow marine coastal waters, but the exact areas where they feed 94 95 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.

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101

### 102 2. Materials and Methods

#### 103 **2.1 Study area**

The Canche estuary (50°50'-50°56' N, 1°57'-1°67' E) is located in northern France along the 104 105 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 106 tidal range of ca. 1 m at neap tides and 6 m at spring tides, and is considered a macro/hyper-107 108 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. 13 109 110 m<sup>3</sup>.s<sup>-1</sup>. 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 111 112 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

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).

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

The POM was sampled at the surface at high tide using sterile pots and then was conserved in a cool box. In the laboratory, the water was filtered through pre-combusted Whatman GF/F filters (0.45 µm pore size, 47 mm diameter), with 3 replicates per site. The SOM was sampled at low tide by scraping the top first cm of sediment. Three replicates were performed at each site. The POM and SOM samples were conserved at -20 °C in the laboratory until transfer for 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 by scraping the surface of the sediment and were then extracted according to the protocol of 145 Riera et al. (1999). In the laboratory, we allowed MPB to migrate by leaving the sediment 146 containing benthic diatoms in flat trays to form a layer 1 cm thick. A nylon screen (60 µm 147 mesh) was placed on top of the sediment and covered with a layer of combusted sand powder 148 (60-200 µm) 5 mm thick. The trays were illuminated until the first dense brown mats 149 appeared on the surface. Meanwhile, the sand was kept moist by spraying filtered (GF/F) 150 151 seawater from the sampling site. The top 2 mm of sand were removed and sieved over a 60 µm mesh nylon screen to separate the diatoms from the remaining sand and nematodes or 152 copepods. The benthic diatoms were then collected on precombusted GF/F filters. Samples 153 were conserved at -20 °C until transfer for SIA. 154

155

### 156 2.5 Benthic organism sampling

157 In the surf zone (on both sides) and inside the estuary, macrobenthic fauna was sampled during low tide with a hand corer (0.025 m<sup>2</sup> area, 20 cm depth, 10 replicates for density and 158 159 biomass, and as many as needed for SIA). At the subtidal site, macrobenthic organisms were sampled using a Van Veen grab (sampling an area of 0.1 m<sup>2</sup>, 10 replicates for density and 160 biomass and as many as needed for SIA). Samples were washed, sieved through a 1 mm mesh 161 size and then washed again with milli-Q water to avoid contamination. In the laboratory, 162 benthic fauna was sorted and identified to the species level when is possible. Ash-free dry 163 164 weight (AFDW) of benthic invertebrates was determined using the method recommended by the Benthos Ecology Working Group of the ICES (Hamilton and Kingston, 1985). The fauna 165 166 was dried in an oven at  $60 \pm 1^{\circ}$ 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  $520 \pm 20^{\circ}$ 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**

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).

176

### 177 **2.7 Stable isotope analyses**

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

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 beforeencapsulation, while the other was encapsulated immediately after drying.

201  $\delta^{13}$ C and  $\delta^{15}$ 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  $\delta^{13}$ C and  $\delta^{15}$ N. Stable isotope ratios were expressed as parts per mil (‰) in 206 the  $\delta$  notation relative to the Pee Dee Belemnite standard for carbon and atmospheric N<sub>2</sub> for 207 nitrogen using the formula:

208 
$$\delta X(\%) = [(R \ sample/R \ standard) - 1] \times 1000$$

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

210

### 211 2.8 Data analysis

### 212 2.8.1 Statistical analysis

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

223

### 224 2.8.2 Layman metrics

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.

236

### 237 **3. Results**

### 238 **3.1 Benthic communities and biomass**

The upstream site was too muddy to sample benthic organisms. Among the five sites sampled 239 in spring, we identified 1288 individuals that belonged to 51 taxa (Table 1). Mean 240 macrobenthic species richness, abundance and biomass were higher outside than inside the 241 estuary (Wilcoxon test, p < 0.0001). Species richness was lowest in the middle of the estuary 242 (6 species) but higher at the downstream site (16), Sainte Cécile beach (13), Le Touquet beach 243 244 (11) and the subtidal site (32). The main species observed in the middle of the estuary were the polychaetes Hedistes diversicolor (326 ind.m<sup>-2</sup>; 2011 mg.m<sup>-2</sup>) and the bivalve 245 Scorbicularia plana (565 mg.m<sup>-2</sup>). The polychaete Scolelepis squamata (823 ind.m<sup>-2</sup>; 3 445 246 247 mg.m<sup>-2</sup>) and the bivalves Limecola balthica (1 282 mg.m<sup>-2</sup>) and Cerastoderma edule (640 mg.m<sup>-2</sup>) dominated the downstream site. The two surf zone sites (Le Touquet and Sainte 248 Cécile) were characterized by the polychaetes S. squamata (48-300 ind.m<sup>-2</sup>; 145-835 mg.m<sup>-2</sup>), 249 Nephtys cirrosa (124-160 ind.m<sup>-2</sup>; 559-831 mg.m<sup>-2</sup>) and Lanice conchylega (1 022 mg.m<sup>-2</sup> at 250 Sainte Cécile) and the bivalves M. balthica (153-1336 mg.m<sup>-2</sup>), C. edule (693 mg.m<sup>-2</sup> at 251 Sainte Cécile) and Fabulina fabula (706 mg.m<sup>-2</sup> at Le Touquet). More diverse taxa (N = 32) 252 and huge benthic biomass were observed at the subtidal site, which was dominated mainly by 253 the bivalves D. vittatus (516 ind.m<sup>-2</sup>; 108 035 mg.m<sup>-2</sup>), F. fabula (412 ind.m<sup>-2</sup>; 12 906 mg.m<sup>-2</sup>) 254 and Ensis leei (36 ind.m<sup>-2</sup>; 9 064 mg.m<sup>-2</sup>). 255



de the Canche estuary in spring.																																					
und outs																															r						
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f each bei	osition)	le the Canc	Le Touque	В							145.9	1336.6		3.3	831.72					9.5	3.4				257.4		59.6	1.8		706.4	0.4						
.m <sup>-2</sup> ) of	e comp	Outsic	e beach	D							300	4	4		124				4	28	40			16	∞ ;	24	28	52	16								
y (D) (ind	table isotop		Sainte Cécil	В							835.8	153.6	693.6		559.4		5		62.1	42.4	17.0			1022.8	447.9	295.6	57.7	28.0	7.1								
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$^{2}$ ) and	analyse	anche es	Downsi	В			85.5	1.4		15.1	3445.6	1282.7	640.5	246.5	146.2	29.1	4.5	11.6	8.5	4.1	0.5	0.4	0.3														
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(B)	nd to sp	Inside	Mid	В	2011.7	565.8	108.6	9.1	2.4	0.4																											
Table 1. Mean Biomass	(Abbreviations correspon		Species		Hediste diversicolor (H div)	Scrobicularia plana (S pla)	Carcinus maenas (C mae)	Oligochaeta	Gammarus zaddachi	Bathyporeia pilosa	Scolelepis squamata (S squ)	Limecola balthica (L bal)	Cerastoderma edule (C edu)	Eurydice pulchra (E pul)	Nephtys cirrosa (N cir)	Heteromastus filiformis	Bathyporeia sarsi	Eteone longa (E lon)	Glycera tridactyla	Haustorius arenarius (H are)	Urothoe poseidonis	Pygospio elegans	Mytilus edulis	Lanice conchilega (L con)	Portumnus latipes	Nepthys hombergi	Gastrosaccus spinifer (G spi)	Crangon crangon (C cra)	Spio martinensis	Fabulina fabula (F fab)	Cumopsis goodsiri	Donax vittatus ( $D$ vit)	Ensis leei (E lee)	Tritia reticulata	Ophiura albida	Abra alba (A alb)	Arenicola marina (A mar)

8 3 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	2 8 9 2	
$\begin{array}{c} 465.9\\ 443.1\\ 377.4\\ 218.7\\ 218.7\\ 1144.4\\ 118.8\\ 118.8\\ 8.4\\ 74.6\\ 74.6\\ 8.4\\ 8.4\\ 5.8\\ 5.8\\ 5.8\\ 2.4\\ 1.1\\ 1.1\\ 0.6\end{array}$	0.5	
	292	
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	648	
	223.0	
	101	
	922.4 1	
	348 2	
	2 698.1	
Liocarcinus vernalis Sigalion squamosus Anapagurus hyndmanni Nephtys assimilis Lagis koreni Phyllodoce mucosa (P muc) Phyllodoce mucosa (P muc) Phyllodoce mucosa (P muc) Phyllodoce mucosa (P muc) Panarian Phyllodoce mucosa Eteone picta Schistonysis kervillei Diastylis rugosa Capitella capitate (C cap) Leucothoe incisa Eumida sanguinea Nototropis swammerdamei Pariambus twirus	<b>TOTAL</b> <b>TOTAL</b>	

### 259 **3.2 Fish sampled for stable isotope analyses**

Nine fish species (eight in spring and six in fall) were collected for SIA: Buglossidium luteum, 260 261 Dicentrarchus labrax, Limanda limanda, Platichthys flesus, Pleuronectes platessa, Solea solea, Sprattus sprattus, Pomatoschistus microps and Pomatoschistus minutus (Table 2). 262 Most individuals were G0 juveniles, except P. microps, P. minutus, S. solea and P. platessa 263 (at the subtidal site in spring) and P. flesus (at Sainte Cécile beach in spring). S. solea, L. 264 265 *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. 266 *flesus* (p = 0.020,) and S. sprattus (p = 0.006), which were smaller in the middle of the estuary 267 than upstream or downstream. In spring, P. platessa were significantly longer at the subtidal 268 site (p < 0.0001). P. flesus were also significantly longer at Sainte Cécile than at the other 269 sites (p = 0.003). Inside the estuary, fish size varied between the two seasons for the four 270

- 271 species caught in both spring and fall (D. labrax, P. flesus, S. sprattus and P. platessa; p <
- 272 0.0001).



274 (fall and spring) analysed for carbon and nitrogen stable isotope composition.

Species Season Upstream									
opectes Season I and the	am Middle	Downst	ream	Sainte Céo	ile beach	Le Touquet	Beach	Subtidal	
Trangu	ı n Length n	Length	u	Length	u	Length	u	Length	u
Dicentrarchus. labrax (D lab) 5.9±0.9	0 10 5.2±0.9 1	0 6.3±0.5	11						
Platichtthys flesus (P fle) 6.0±3.0	0 11 3.8±0.2 1	0 6.9±1.3	6						
Pleuronectes platessa (P pla)		$6.6 \pm 1.3$	S						
Sprattus sprattus (S spr) 6.9±2.8	\$ 11 3.9±0.2 6	6.4±1.2	6						
Pomatochistus microps (P mic) 4.4±0.2	5 3.8±0.2 5	$4.6 \pm 0.7$	S						
Pomatochistus minutus (P min) 5.3±0.5	5 4.1±0.1 5	5.9±0.3	S						
Dicentrarchus labrax (D lab)	8.2±1.1 4	± 8.4±0.5	3						
Platichtthys flesus (P fle) 7.8±2.8	8 4 6.4±2.8 5	8.7±5.2	4	$13.0 \pm 1.2$	9	$10.6 \pm 3.1$	5	7.0± 5.4	10
Pleuronectes platessa (P pla)	3.9±4.8 5	i 4.6±1.0	4	5.5±7.4	5	$7.1 \pm 0.9$	6	14.5±5.2	4
Sprattus sprattus (S spr) 4.3±3.0	0 10 4.8±3.7 5	4.5±0.4	Э			$6.4{\pm}1.5$	5		
Pomatoschistus sp. (P sp) Spring	4.3±2.8 4	4.8±2.5	S						
Solea solea (S sol)				3.5±2.4	7	3.7±2.2	٢	13.1±2.1	$\infty$
Ruolossidium luteum (R lut)				7.6±3.8	S	4 1+7 3	v	$5.9 \pm 4.2$	Y
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						C-1-1-1-1	)		<u>،</u> ر
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### 275 **3.3 Results of stable isotope analyses**

276 3.3.1 Food sources

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



297 3.3.2 Isotopic compositions of benthic communities

Inside the estuary, the benthic invertebrate community followed the same isotopic trend in both seasons. The bivalve *Scrobicularia plana* and polychaetes *H. diversicolor* and *N. cirrosa* had the lowest  $\delta^{13}$ C values (-20‰ to -18‰), while the crustaceans *Carcinus maenas* and *Crangon crangon* had the highest  $\delta^{13}$ C values (-16‰ to -15‰) (Figures 2 and 3). The suspension feeder *L. balthica* (7.9-9.9‰) and the deposit feeders *S. squamata* (8.2-10.2‰) and *S. plana* (8.4-9.2‰) had the lowest  $\delta^{15}$ N values, while the crustaceans *C. crangon* and *C. 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).

In spring, the mean  $\delta^{13}$ C values for *C. crangon* and *C. maenas* were significantly lower inside

- 311 the estuary (C. crangon:  $-18.3\pm0.9\%$  at the upstream site to  $-15.5\pm0.7\%$  at Sainte Cécile
- beach; *C. maenas*:  $-17.4\pm0.5\%$  at the upstream site to  $-15.2\pm0.2\%$  at the intertidal site).
- 313

### 314 3.3.3 Isotopic composition of fish

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

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### 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.
- 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				Spr	ring		
		Inside			Inside			Outside	
Species	SEAc	CR	NR	SEAc	CR	NR	SEAc	CR	NR
Pomatoschistus minutus	17.58	9.01	2.63				2		
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				Y			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

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

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

377 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 378 379 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 380 381 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 382 383 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 producers (Fry, 2002). In the Canche estuary, POM, SOM and MPB  $\delta^{15}$ N were similar along 385 the salinity gradient during the same season, highlighting the relatively low nitrogen input 386 from the watershed (Guelinckx et al., 2006) due to the short length of the Canche river and 387 the low human modification of its catchment (Amara et al., 2007; Durou et al., 2007). 388

389

### **390 4.2 Structure of invertebrate communities**

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

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

One objective of the study was to assess the origin of the food source consumed by potential 407 prey throughout the estuary, from the intertidal zone upstream of the Canche estuary, with 408 low benthic species diversity and biomass, to the subtidal zone outside the estuary, with 409 higher species diversity and biomass. In the Canche estuary, suspension and deposit feeders 410 dominated the benthic invertebrate biomass. Their feeding activity is an important connection 411 412 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 413 414 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 415 different food sources and have plastic feeding behaviour depending on the environmental 416 conditions (Daggers et al., 2020; Herman et al., 2000). Nevertheless, benthic primary 417 consumers had higher d<sup>13</sup>C (around - 20 to -16‰) than fresh water POM (around -30‰), 418 revealing the latter's low contribution to the trophic functioning of the estuary. This finding, 419 even in the upstream of the estuary, may be due to the relatively weak flow of the Canche 420 River and consequently the small amount of organic matter that it carries (Selleslagh and 421 Amara, 2008). Thus, we can hypothesize that the marine POM, SOM and MPB which d<sup>13</sup>C 422 423 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 food; for example, suspension feeders can consume resuspended MPB, while deposit feeders 426 can consume sediment POM (Kang et al., 2015). 427

428

### 429 4.3 Fish structure and seasonal variations

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

439 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). 440 MPB production is high on the intertidal mud banks of the Canche estuary and provides food 441 for the potential prey of P. microps. Inside the estuary, the other fish species (e.g. D. labrax, 442 *P. minutus* or *S. sprattus*) had  $\delta^{13}$ C values from -19 to -17‰ upstream to – 16‰ downstream, 443 close to those of marine POM (around -20‰) and MPB (-16‰) revealing a food source 444 originating from both marine POM and MPB. 445 The seasonal comparison of fish SEA in the Canche estuary is informative, as the isotopic 446

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 invertebrates at the end of summer compared to the lower benthic biomass in spring (Rauch 449 and Denis, 2008). In the fall, SEA of the two Pomatoschistus sp. did not overlap, unlike 450 451 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 452 SEA of P. flesus and P. platessa overlapped in spring, perhaps due to the smaller amount of 453 available prey (Pape and Bonhommeau, 2015). 454

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.

468

### 469 **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 theCanche 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

Canche estuary and that fish species might visit the estuary for reasons other than feeding, 475 such as to avoid predation or because they are carried by the tide. We highlighted the need to 476 take into account the whole small macrotidal estuary and adjacent ecosystems to better 477 describe the flatfish nursery. This work demonstrated that potential prey and feeding sources 478 for fish had habitat-specific compositions, which confirms the suitability of SIA for tracing 479 fish movements, fidelity and connectivity inside and outside the Canche estuary for sites less 480 481 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 482 483 habitat (e.g. sediment type, foreshore position, salinity fluctuations) and to trophic 484 competition and predation.

485

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512 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 LeTouquet beaches) and subtidal site).
- 515







<b>8<sup>15</sup>N 8<sup>13</sup>C N</b>	<b>V δ<sup>15</sup>N</b> 3 9.2 ± 0.1 3 9.4 ± 0.5 10.7	$\begin{array}{c c} \mathbf{\delta}^{13}\mathbf{C} & 1\\ \hline -18.9 \pm 0.1 \\ -18.8 \pm 0.5 \\ -18.4 \end{array}$	N 8 <sup>15</sup> N			<b>:ile Beach</b>	Le To	iquet Beac	ľ	Subtio	lal
<b>8<sup>15</sup>N 6<sup>13</sup>C N</b> 3 3 3 3 3 3 4 9 1 3 4 0 9 1 3 4 0 9 1 8 3 4 0 9 1 1 9 1 9 1 9 1 9 1 9 1 9 1 9 1 9 1	<b>V 6<sup>15</sup>N</b> 3 9.2 ± 0.1 3 9.4 ± 0.5 10.7	$\frac{\delta^{13}C}{-18.9 \pm 0.1}$ -18.8 ± 0.5 -18.4	V 8 <sup>15</sup> N								
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11.3 ±0.9 -18.3 ±0.9 5									ŝ	$8.2 \pm 0.2$	$-16.6 \pm 0.2$
	$5 11.3 \pm 0.5$	$-17.4 \pm 0.5$	$11.5 \pm 0.2$	$-15.8 \pm 0.5$	5 13.6±1	9 $-15.5 \pm 0.7$	7 13.6 ±	1.0 -15.6	- 0.3		
$12.3 \pm 1.3$ $-17.4 \pm 0.5$ 5	$5 12.0 \pm 0.4$	-17.9 ± 1.1	$12.3 \pm 0.4$	$-15.3 \pm 1.2$	$11.6 \pm 0$	3 -15.8 ± 0.7	4 12.0 ±	0.3 -15.9	= 1.0 5	$11.9 \pm 0.5$	$-15.2 \pm 0.2$
60	$3 11.7 \pm 0.6$	$-16.8 \pm 0.4$	11.4	-17.9	2 11.8	-15.5	2 11.	-17	-	11.6	-15.9
			10.7	-15.9			1				
			$11.3 \pm 0.1$	$-21.9 \pm 0.6$	3 9.1 ± 0.	$-20.4 \pm 0.9$					
		7			$11.7 \pm 0$	$1 -18.5 \pm 1.3$					
									ŝ	$10.0 \pm 0.2$	$-17.0 \pm 0.5$
									5	$10.2 \pm 0.3$	$-18.2 \pm 1.5$
				3 11.3 ± 0.1	$3 11.3 \pm 0.1 - 21.9 \pm 0.6$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	3 11.3 $\pm$ 0.1 -21.9 $\pm$ 0.6 3 9.1 $\pm$ 0.9 -20.4 $\pm$ 0.9 -30.4 $\pm$ 1.7 $\pm$ 0.1 -18.5 $\pm$ 1.3	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	3 11.3 $\pm$ 0.1 -21.9 $\pm$ 0.6 3 9.1 $\pm$ 0.9 -20.4 $\pm$ 0.9 11.7 $\pm$ 0.1 -18.5 $\pm$ 1.3	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$

			Upstrea	am		Midd	lle		Downst	ream
Trophic position	Species									
position		Ν	$\delta^{15}N$	δ <sup>13</sup> C	Ν	$\delta^{15}N$	$\delta^{13}C$	N	$\delta^{15}N$	δ <sup>13</sup> C
	Scrobicularia plana (S pla)				5	$9.1\pm0.2$	$-19.7 \pm 0.2$	3	$8.7 \pm 0.2$	$-16.6 \pm 0.1$
	Hediste diversicolor (H div)				10	$10.3\pm0.5$	$-18.7 \pm 0.6$			
primary consumers	Scolelepis squamata (S squ)							4	$10.2\pm0.7$	$\textbf{-16.7}\pm0.6$
•••••	Limecola balthica (L bal)							5	$9.9\pm0.3$	$\textbf{-16.1}\pm0.5$
	Cerastoderma edule (C edu)							7	$8.9\pm0.1$	$\textbf{-16.9}\pm0.3$
	Crangon crangon (C cra)	10	$12.6\pm0.7$	$\textbf{-16.7} \pm 1.0$	9	$12.5\pm0.5$	$-16.0 \pm 1.2$	8	$12.4\pm0.9$	$\textbf{-14.6}\pm0.4$
	Carcinus maenas (C mae)	5	$12.7\pm0.4$	$\textbf{-20.9} \pm 1.9$	6	$12.1 \pm 0.7$	-16.9 ± 2	7	$12.3\pm0.8$	$\textbf{-15.6} \pm 1.0$
secondary	Nephtys cirrosa (N cir)				5	$10.3\pm0.4$	$-18.8 \pm 0.5$	3	$12.3\pm1.1$	$\textbf{-14.5}\pm1.2$
	Eteone longa (E lon)							2	13.9	-15.9
	Eurydice pulchra (E pul)							4	$11.0\pm0.3$	$\textbf{-15.8}\pm0.2$

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

	Inside												Outside					
	Upstream				Middle				Downstream	-			Saint Cécile l	Beach	Le Touquet l	Beach	Subtidal	
Species (abbreviation)	fall		spring		fall		spring		fall		spring		spring		spring		spring	
	δ <sup>13</sup> C	8 <sup>15</sup> N	δ <sup>13</sup> C	δ <sup>15</sup> Ν	8 <sup>13</sup> C	8 <sup>15</sup> N	δ <sup>13</sup> C	8 <sup>15</sup> N	δ <sup>13</sup> C	δ <sup>15</sup> Ν	8 <sup>13</sup> C	8 <sup>15</sup> N	δ <sup>13</sup> C	δ <sup>15</sup> Ν	8 <sup>13</sup> C	δ <sup>15</sup> Ν	δ <sup>13</sup> C	ð <sup>15</sup> N
Pomatoschistus microps (P mic)	$-15.1 \pm 1.2$	$13.9 \pm 0.8$			-14.8 ± 2.4	$13.4\pm0.5$			$13.8\pm0.5$	$13.8\pm0.6$								
Sprattus sprattus (S spr)	$-17.1 \pm 0.7$	$13.5 \pm 0.5$	$-17.3 \pm 0.5$	$11.3\pm2.1$	$-16.6 \pm 0.8$	$13.7\pm0.6$	$-17.4 \pm 0.3$	$12.2 \pm 0.3$	$\textbf{-16.8}\pm0.8$	$13.5\pm0.8$	$-16.7 \pm 0.3$	$11.9\pm0.1$			$-17.4 \pm 0.6$	$11.7\pm0.7$		
Pomatoschistus minutus (P min)	$-17.6 \pm 1.3$	$14.0\pm0.6$		Ŕ	$-16.1 \pm 1.8$	$13.7\pm0.6$		_	$-15.9 \pm 0.2$	$13.6\pm0.1$								
Dicentrarchus labrax (D lab)	$-18.9 \pm 1.5$	$13.7\pm0.7$			$-17.7 \pm 1.0$	$13.9\pm0.3$	$-17.2 \pm 1.2$	$14.5 \pm 0.8$	$-16.4 \pm 1.2$	$13.6\pm0.3$	$-16.1\pm0.2$	$13.8\pm0.1$						
Platichthys flesus (P fle)	$-21.1 \pm 1.3$	$13.1\pm0.5$	-24.2 ± 4.5	$11.4\pm0.8$	$-17.6 \pm 2.8$	$13.2\pm1.0$	$-20.6 \pm 0.5$	$11.9 \pm 0.5$	$-16.8 \pm 2$	$12.5\pm0.6$	$-19.5 \pm 0.9$	$12.3\pm0.5$	$-18.5 \pm 3.1$	$13.8\pm1.6$	$-20.7 \pm 0.5$	$11.8\pm0.9$	$-17.2 \pm 0.5$	$13.3\pm0.8$
Buglossidium luteum (B lut)													$-16.2 \pm 0.3$	$11.8\pm0.9$	$-16.2 \pm 0.2$	$11.3\pm0.1$	$-17.1 \pm 0.4$	$11.9\pm0.2$
Pomatoschistus sp (P sp)			$-19.3 \pm 1.2$	$12.8\pm0.3$			$-17.27 \pm 0.9$	$12.9 \pm 0.3$			$-16.1\pm0.6$	$13.1\pm0.3$			$-15.6 \pm 0.3$	$12.7\pm0.3$		
Limanda Limanda (L lim)																	$-16.4 \pm 0.6$	$12.8 \pm 1$
Pleuronectes platessa (P pla)							$-21.3 \pm 0.5$	$11.5\pm0.5$	$-15.5 \pm 0.2$	$12.4\pm0.2$	$-17.2 \pm 1.0$	$11.4\pm1.0$	$-16.6 \pm 0.9$	$10.2\pm0.3$	$-16.0 \pm 0.6$	$10.8\pm0.3$	$-15.9 \pm 0.7$	$12.7\pm0.2$
Solea solea (S sol)							1						$-16.6 \pm 0.4$	$11.4\pm0.2$	$-16.3 \pm 0.5$	$11.3\pm0.2$	$-16.2 \pm 0.6$	$13.2\pm1.2$
538										N								
												7						
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**Appendix 3.** Fish carbon and mitrogen stable isotope compositions (mean  $\pm$  standard error. %) in fall and spring 537

539	References
540	Aarnio, K., Bonsdorff, E., Rosenback, N., 1996. Food and feeding habits of juvenile flounder
541	Platichthys flesus (L.), abd turbot Scophthalmus maximus L. in the aland archipelago,
542	northern Baltic Sea. Journal of Sea Research 36, 311–320. https://doi.org/10.1016/S1385-
543	1101(96)90798-4
544	Amara R 2003 Seasonal Ichthyodiversity and Growth Patterns of Juvenile Flatfish on a Nursery
5/5	Ground in the Southern Bight of the North Sea (France) Environmental Biology of
545	Eichos 67, 101, 201, https://doi.org/10.1027/A-1025646(22066)
540	Fishes 0', 191–201. https://doi.org/10.1025/4.1025040022000
547	Amara, R., Lallargue, P., Dewarumez, J.M., Marymak, C., Lagardere, F., Luzac, C., 2001. Feeding
548	ecology and growth of 0-group Hattish (sole, dab and plaice) on a nursery ground (Southern
549	Bight of the North Sea). Journal of Fish Biology 58, 788–803. https://doi.org/10.1111/j.1095-
550	8649.2001.tb00531.x
551	Amara, R., Meziane, T., Gilliers, C., Hermel, G., Laffargue, P., 2007. Growth and condition indices in
552	juvenile sole Solea solea measured to assess the quality of essential fish habitat. Marine
553	Ecology Progress Series 351, 201–208. https://doi.org/10.3354/meps07154
554	Amara, R., Paul, C., 2003. Seasonal patterns in the fish and epibenthic crustaceans community of an
555	intertidal zone with particular reference to the population dynamics of plaice and brown
556	shrimp, Estuarine, Coastal and Shelf Science 56, 807–818, https://doi.org/10.1016/S0272-
557	7714(02)00315-3
558	Amera R Selestark I Billon G Minier C 2009 Growth and condition of 0-group European
550	Hundr, R., Schestagh, J., Jinon, G., Hundr, C., 2007. Glow and condition of o-gloup European foundar, <i>Platishthys facus</i> as indicator of actuaring habitat quality. Hydroxiologia 627, 87
559	hounder, <i>Functionarys fiesus</i> as indicator of estuarme natival quarty. Hydrobiologia 627, 87.
560	https://doi.org/10.100//\$10/50-009-9/17-9
561	Antonio, E.S., Richoux, N.B., 2014. Irophodynamics of three decapod crustaceans in a temperate
562	estuary using stable isotope and fatty acid analyses. Marine Ecology Progress Series 504,
563	193–205. https://doi.org/10.3354/meps10761
564	Baeta, A., Pinto, R., Valiela, I., Richard, P., Niquil, N., Marques, J.C., 2009. $\delta^{13}N$ and $\delta^{13}C$ in the
565	Mondego estuary food web: Seasonal variation in producers and consumers. Marine
566	Environmental Research 67, 109–116. https://doi.org/10.1016/j.marenvres.2008.11.005
567	Beck, M.W., Heck, K.L., Able, K.W., Childers, D.L., Eggleston, D.B., Gillanders, B.M., Halpern, B.,
568	Hays, C.G., Hoshino, K., Minello, T.J., Orth, R.J., Sheridan, P.F., Weinstein, M.P., 2001. The
569	Identification, Conservation, and Management of Estuarine and Marine Nurseries for Fish and
570	Invertebrates: a better understanding of the babitats that serve as nurseries for marine species
571	and the factors that create site-specific variability in nursery quality will improve conservation
572	and management of these areas BioScience 51, 633, 641, https://doi.org/10.1641/0006
572	2569/200105116623-TICAMOUS Prosence 51, 055-041. https://doi.org/10.1041/0000-
575	Solo(2001)051[0055.11CAW0]2.0.CO,2
574	Bernasconi, S.M., Barbieri, A., Simona, M., 1997. Carbon and introgen isotope variations in
5/5	sedimenting organic matter in Lake Lugano. Limnology and Oceanography 42, 1755–1765.
576	https://doi.org/10.4319/10.1997.42.8.1755
577	Besyst, B., Cattrijsse, A., Mees, J., 1999. Feeding ecology of juvenile flatfishes of the surf zone of a
578	sandy beach. Journal of Fish Biology 55, 1171–1186. https://doi.org/10.1111/j.1095-
579	8649.1999.tb02068.x
580	Bodin, N., Le Loc'h, F., Hily, C., 2007. Effect of lipid removal on carbon and nitrogen stable isotope
581	ratios in crustacean tissues. Journal of Experimental Marine Biology and Ecology 341, 168-
582	175. https://doi.org/10.1016/j.jembe.2006.09.008
583	Brown, C.J., Broadley, A., Adame, M.F., Branch, T.A., Turschwell, M.P., Connolly, R.M., 2019, The
584	assessment of fishery status depends on fish habitats. Fish and Fisheries 20, 1–14
585	https://doi.org/10.1111/faf.123.18
586	Cabana G. Rasmussen I. B. 1994 Modelling food chain structure and contaminant bioaccumulation
500	using stable nitrogen isotones. Nature 372-255-257
J0/	using static introgen isotopes. Nature, 572, 255-257.
588	Chen, N., wu, J., Hong, H., 2012. Effect of storm events on riverine nitrogen dynamics in a
589	supropical watersned, southeastern China. Science of The Total Environment 431, 357–365.
590	nttps://doi.org/10.1016/j.scitotenv.2012.05.072
591	Christianen, M.J.A., Middelburg, J.J., Holthuijsen, S.J., Jouta, J., Compton, T.J., Heide, T. van der,
592	Piersma, L., Damsté, J.S.S., Veer, H.W. van der, Schouten, S., Olff, H., 2017. Benthic primary

593 594 595 596 597	producers are key to sustain the Wadden Sea food web: stable carbon isotope analysis at landscape scale. Ecology 98, 1498–1512. https://doi.org/10.1002/ecy.1837 Connolly, R.M., Gorman, D., Hindell, J.S., Kildea, T.N., Schlacher, T.A., 2013. High congruence of isotope sewage signals in multiple marine taxa. Marine Pollution Bulletin 71, 152–158. https://doi.org/10.1016/j.marpolbul.2013.03.021
598 599 600 601	Cresson, P., Ruitton, S., Fontaine, MF., Harmelin-Vivien, M., 2012. Spatio-temporal variation of suspended and sedimentary organic matter quality in the Bay of Marseilles (NW Mediterranean) assessed by biochemical and isotopic analyses. Marine Pollution Bulletin 64, 1112, 1121, https://doi.org/10.1016/j.marnolhul.2012.04.003
602	Dagastra T. J. van Oavalan D. Harman D.M. Boashkar H.T. van der Wal D. 2020 Spetial
602 603	variability in macrofaunal diet composition and grazing pressure on microphytobenthos in
604	intertidal areas. Limnology and Oceanography. https://doi.org/10.1002/ino.11554
605 606	Dalu, T., Froneman, P.W., 2016. Diatom-based water quality monitoring in southern Africa:
000	channenges and luttice prospects. water SA 42, 531–539, https://doi.org/10.4514/wsa.v4214.05
607	Darnaude, A.M., Salen-Picard, C., Harmelin-Vivien, M.L., 2004. Depth variation in terrestrial
608	particulate organic matter exploration by marine coastal behavior communities on the know
609 610	River delta (NW Mediterranean). Marine Ecology Progress Series 2/5, 4/-5/.
611	Davies M L Enstein S 1077 Machanism of aerbon isotone fractionation associated with linid
612	synthesis. Science 197, 261–263. https://doi.org/10.1126/science.327543
613	Desroy, N., Warembourg, C., Dewarumez, J.M., Dauvin, J.C., 2003, Macrobenthic resources of the
614	shallow soft-bottom sediments in the eastern English Channel and southern North Sea. ICES
615	Journal of Marine Science 60, 120-131. https://doi.org/10.1006/jmsc.2002.1333
616	Dewarumez, JM., Belgrano, A., Craeymeersch, J.A., Duquesne, S., Heip, C., 1992. Influence de la
617	circulation des masses d'eaux dans la dynamique du peuplement à Abra alba de la baie Sud de
618	la Mer du Nord. Presented at the Le littoral, ses contraintes environnementales et ses conflits
619	d'utilisation, Nantes, 1-4 juillet 1991, pp. 73–73.
620	Duan, S., Amon, R.M.W., Brinkmeyer, R.L., 2014. Tracing sources of organic matter in adjacent
621 622	urban streams having different degrees of channel modification. Science of The Total Environment 485–486, 252–262. https://doi.org/10.1016/j.scitotenv.2014.03.066
623	Dubois, S., Blanchet, H., Garcia, A., Massé, M., Galois, R., Grémare, A., Charlier, K., Guillou, G.,
624	Richard, P., Savoye, N., 2014. Trophic resource use by macrozoobenthic primary consumers
625	within a semi-enclosed coastal ecosystem: Stable isotope and fatty acid assessment. Journal of
626	Sea Research 88, 87–99. https://doi.org/10.1016/j.seares.2014.01.004
627	Durou, C., Poirier, L., Amiard, JC., Budzinski, H., Gnassia-Barelli, M., Lemenach, K., Peluhet, L.,
628	Mouneyrac, C., Romeo, M., Amiard-Triquet, C., 2007. Biomonitoring in a clean and a multi-
629	Narais diversicolor Environmental Pollution 148 445.458
631	https://doi.org/10.1016/i.envpol.2006.12.022
632	Elliott M Hemingway K L 2002 Fishes in Estuaries Blackwell Science Oxford 636 nn
633	Elliott, M., Whitfield, A.K., Potter, I.C., Blaber, S.J.M., Cyrus, D.P., Nordlie, F.G., Harrison, T.D.,
634	2007. The guild approach to categorizing estuarine fish assemblages: a global review. Fish
635	and Fisheries 8, 241–268, https://doi.org/10.1111/j.1467-2679.2007.00253.x
636	Evans, T.M., Bellamy, A.R., Bauer, J.E., 2019. Radioisotope and stable isotope ratios ( $\Delta^{14}$ C, $\delta^{15}$ N)
637	suggest larval lamprey growth is dependent on both fresh and aged organic matter in streams.
638	Ecology of Freshwater Fish 28, 365-375. https://doi.org/10.1111/eff.12459
639	Fry, B., 2006. Isotope Additions, in: Stable Isotope Ecology. Springer, New York, NY, pp. 183-193.
640	https://doi.org/10.1007/0-387-33745-8_6
641	Fry, B., 2002. Conservative mixing of stable isotopes across estuarine salinity gradients: A conceptual
642	framework for monitoring watershed influences on downstream fisheries production. Estuaries
043 644	Ex. B 1900 Using stable isotopes to monitor watershed influences on aquatic tranhadynamics
645	Canadian Journal of Fisheries and Aquatic Sciences https://doi.org/10.1139/f90-152
646	Fry. B., Baltz, D.M., Benfield, M.C., Fleeger, J.W., Gace, A., Haas, H.L., Ouiñones-Rivera, Z.I.
647	2003. Stable isotope indicators of movement and residency for brown shrimp

648	(Farfantepenaeus aztecus) in coastal Louisiana marshscapes. Estuaries 26, 82–97.
649	https://doi.org/10.1007/BF02691696
650	Galvan, D.E., Sweeting, C.J., Reid, W.D.K., 2010. Power of stable isotope techniques to detect size-
651	based feeding in marine fishes. Marine Ecology Progress Series 40/, 2/1–2/8.
052	Chartese K. Luczek C. Dewerumez I.M. Dewin, I.C. 2000. Influence of anoticl cooles of
000	ohensos, K., Luczak, C., Dewarumez, JM., Dauvin, JC., 2000. Innuence of spanal scales of
655	from the English Channel and the southern North See, ICES Journal of Marine Science 57
656	1481 1487 https://doi.org/10.1006/imsc.2000.0016
657	Griffiths H 1991 Applications of Stable Isotone Technology in Physiological Ecology Functional
658	Ecology 5 254–269 https://doi.org/10 2307/2389263
659	Guelincky I Maes I De Brahandere I. Dehairs F Ollevier F 2006 Migration dynamics of
660	clupeoids in the Schelde estuary: A stable isotope approach. Estuarine, Coastal and Shelf
661	Science 66, 612–623. https://doi.org/10.1016/i.ecss.2005.11.007
662	Hamilton, S., Kingston, P. F. (1985). The effects of the preservatives alcohol, formalin and propylene
663	phenoxetol on the wet weights of some marine animals. Report to the ICES Benthos Working
664	Group, 2.
665	Herman, P.M.J., Middelburg, J.J., Widdows, J., Lucas, C.H., Heip, C.H.R., 2000. Stable isotopes as
666	trophic tracers: combining field sampling and manipulative labelling of food resources for
667	macrobenthos. Marine Ecology Progress Series 204, 79-92.
668	https://doi.org/10.3354/meps204079
669	Herzka, S.Z., 2005. Assessing connectivity of estuarine fishes based on stable isotope ratio analysis.
670	Estuarine, Coastal and Shelf Science 64, 58-69. https://doi.org/10.1016/j.ecss.2005.02.006
671	Hobson, K.A., Wassenaar, L.I., Taylor, O.R., 1999. Stable isotopes ( $\delta D$ and $\delta^{13}C$ ) are geographic
672	indicators of natal origins of monarch butterflies in eastern North America. Oecologia 120,
673	397–404. https://doi.org/10.1007/s004420050872
674	Hoeinghaus, D.J., Vieira, J.P., Costa, C.S., Bemvenuti, C.E., Winemiller, K.O., Garcia, A.M., 2011.
675	Estuary hydrogeomorphology affects carbon sources supporting aquatic consumers within and
676	among ecological guilds. Hydrobiologia 673, 79–92. https://doi.org/10.1007/s10750-011-
6//	
678	Jackson, A.L., Inger, K., Parnell, A.C., Bearnop, S., 2011. Comparing isolopic niche widths among
679	Easlogy 80, 505, 602
681	Kang C.K. Lee, V.W. Choy, F.L. Shin, L.K. Seo, L.S. Hong, L.S. 2006. Microphytohenthos
682	seasonality determines growth and reproduction in intertidal bivalves. Marine Ecology
683	Progress Series 315 113–127 https://doi.org/10.3354/mens315113
684	Kang, CK., Park, H.J., Choy, F.J., Choi, KS., Hwang, K., Kim, JB., 2015, Linking Intertidal and
685	Subtidal Food Webs: Consumer-Mediated Transport of Intertidal Benthic Microalgal Carbon.
686	PLOS ONE 10, e0139802, https://doi.org/10.1371/journal.pone.0139802
687	Kostecki, C., Le Loc'h, F., Roussel, J.M., Desroy, N., Huteau, D., Riera, P., Le Bris, H., Le Pape, O.,
688	2010. Dynamics of an estuarine nursery ground : the spatio-temporal relationship between the
689	river flow and the food web of the juvenile common sole (Solea solea, L.) as revealed by
690	stable isotopes analysis. Journal of Sea Research 64, 54-60.
691	https://doi.org/10.1016/j.seares.2009.07.006
692	Kostecki, C., Roussel, J.M., Desroy, N., Roussel, G., Lanshere, J., Le Bris, H., Le Pape, O., 2012.
693	Trophic ecology of juvenile flatfish in a coastal nursery ground: contributions of intertidal
694	primary production and freshwater particulate organic matter. Marine Ecology-Progress Series
695	449, 221–232. https://doi.org/10.3354/meps09563
696	Lambert, T., Bouillon, S., Darchambeau, F., Morana, C., Roland, F.A.E., Descy, JP., Borges, A.V.,
697	2017. Effects of human land use on the terrestrial and aquatic sources of fluvial organic matter
698	in a temperate river basin (The Meuse River, Belgium). Biogeochemistry 136, 191–211.
699 700	nups://doi.org/10.100//S10555-01/-058/-9
700	Lautenschlager, A.D., Mallnews, I.G., Quinn, G.P., 2014. Utilization of organic matter by
701	and Shelf Science 1/0, 232, 2/3, https://doi.org/10.1016/j.acs.201/.09.020
702	and onen berenet 177, 252 275. https://doi.org/10.1010/j.cc55.2014.00.020

703	Layman, C.A., Arrington, D.A., Montaña, C.G., Post, D.M., 2007. Can Stable Isotope Ratios Provide
704	for Community-Wide Measures of Trophic Structure? Ecology 88, 42–48.
705	https://doi.org/10.1890/0012-9658(2007)88[42:CSIRPF]2.0.CO;2
706	Le Pape, O., Modéran, J., Beaunée, G., Riera, P., Nicolas, D., Savoye, N., Harmelin-Vivien, M.,
707	Darnaude, A.M., Brind'Amour, A., Le Bris, H., Cabral, H., Vinagre, C., Pasquaud, S., França,
708	S., Kostecki, C., 2013. Sources of organic matter for flatfish juveniles in coastal and estuarine
709	nursery grounds: A meta-analysis for the common sole (Solea solea) in contrasted systems of
710	Western Europe. Journal of Sea Research 75, 85–95.
711	https://doi.org/10.1016/j.seares.2012.05.003
712	Leakey, C.D.B., Attrill, M.J., Jennings, S., Fitzsimons, M.F., 2008. Retrospective quantification of
713	estuarine feeding activity by coastally caught marine fishes. Journal of Sea Research 60, 210–
714	214. https://doi.org/10.1016/j.seares.2008.05.002
715	Leclerc, JC., Riera, P., Noël, L.ML.J., Leroux, C., Andersen, A.C., 2014. Trophic ecology of
716	<i>Pomatoschistus microps</i> within an intertidal bay (Roscoff, France), investigated through gut
717	content and stable isotope analyses. Marine Ecology 35, 261–270.
718	https://doi.org/10.1111/maec.12071
719	Little, C., 2000. The Biology of Soft Shores and Estuaries. Oxford University Press, Oxford, 262 pp.
720	Lorrain, A., Savoye, N., Chauvaud, L., Paulet, YM., Naulet, N., 2003. Decarbonation and
721	preservation method for the analysis of organic C and N contents and stable isotope ratios of
722	low-carbonated suspended particulate material. Analytica Chimica Acta 491, 125–133.
723	https://doi.org/10.1016/S0003-2670(03)00815-8
724	Lu, F., Liu, Z., Ji, H., 2013. Carbon and nitrogen isotopes analysis and sources of organic matter in the
725	upper reaches of the Chaobai River near Beijing, China. Science China Earth Sciences 56,
726	217–227. https://doi.org/10.1007/s11430-012-4525-x
727	Luo, Z., Ma, JM., Zheng, SL., Nan, CZ., Nie, LM., 2016. Different hydrodynamic conditions on
728	the deposition of organic carbon in sediment of two reservoirs. Hydrobiologia 765, 15–26.
729	https://doi.org/ 10.1007/s10750-015-2410-2
730	Mann, C.J., Wetzel, R.G., 2000. Hydrology of an impounded lotic wetland-wetland sediment
/31	characteristics. Wetlands 20, 23–32. https://doi.org/10.16/2/02//-
732	5212(2000)020[0023:HOATLW]2.0.CO;2
/33	McLusky, D.S., Elliott, M., 2004. The Estuarine Ecosystem: Ecology, Threats and Management. 3 <sup>rd</sup>
734	edition. Oxford: OUP; 2004. 216 pp.
/35	Meleder, V., Kince, Y., Barlie, L., Gaudin, P., Kosa, P., 2007. Spatiotemporal changes in
730	Discrete 42 1177 1100 kerset (10 1111/1 1520 8817 2007 00422 rt
/3/	Phycology 45, 1177–1190. https://doi.org/10.1117/j.1529-8817.2007.00425.x
738	Middelourg, J.J., Niedweinnutze, J., 1996. Carbon and mitogen stable isotopes in suspended matter and
739	seminents from the Scheroe Estuary. Marine Chemistry 00, 217–223.
740	$\frac{1}{10000000000000000000000000000000000$
741	Minagawa, M., wada, E., 1964. Stepwise enterment of 151x along tood chains: further evidence and the relation between 151x and a comparison of Comparison of the state 49, 1125
742	1140
744	Moens T Luvten C Middelburg II Herman PMI Vincx M 2002 Tracing organic matter
745	sources of estuarine tidal flat nematodes with stable carbon isotones. Marine Ecology Progress
746	Series 234, 127–137, https://doi.org/10.3354/mens234127
747	Moncreiff, C.A., Sullivan, M.I. 2001. Trophic importance of epiphytic algae in subtropical seagrass.
748	beds: evidence from multiple stable isotore analyses. Marine Ecology Progress Series 215.
749	93–106. https://doi.org/10.3354/meps215093
750	Pape, O.L., Bonhommeau, S., 2015. The food limitation hypothesis for iuvenile marine fish. Fish and
751	Fisheries 16, 373–398. https://doi.org/10.1111/faf.12063
752	Pasquaud, S., David, V., Lobry, J., Girardin, M., Sautour, B., Elie, P., 2010. Exploitation of trophic
753	resources by fish under stressful estuarine conditions. Marine Ecology Progress Series 400,
754	207-219. https://doi.org/10.3354/meps08387
755	Pasquaud, S., Elie, P., Jeantet, C., Billy, I., Martinez, P., Girardin, M., 2008. A preliminary
756	investigation of the fish food web in the Gironde estuary, France, using dietary and stable

757	isotope analyses. Estuarine, Coastal and Shelf Science 78, 267-279.
758	https://doi.org/10.1016/j.ecss.2007.12.014
759	Pearson, J.A., Haddow, S.D., Hillson, S.W., Knüsel, C.J., Larsen, C.S., Sadvari, J.W., 2015. Stable
760	carbon and nitrogen isotope analysis and dietary reconstruction through the life course at
761	Neolithic Çatalhöyük, Turkey. Journal of Social Archaeology 15, 210–232.
762	https://doi.org/10.1177/1469605315582983
763	Peterson, J., 1999. Stable isotopes as tracers of organic matter input and transfer in benthic food webs :
764	A review. Acta Oecologica, 20(4), 479-487.
765	Peterson, B.J., Fry, B., 1987. Stable Isotopes in Ecosystem Studies. Annual Review of Ecology and
766	Systematics 18, 293–320. https://doi.org/10.1146/annurev.es.18.110187.001453
767	Post, J.R., Parkinson, E.A., Johnston, N.T., 1999. Density-Dependent Processes in Structured Fish
768	Populations: Interaction Strengths in Whole-Lake Experiments. Ecological Monographs 69,
769	155-175. https://doi.org/10.1890/0012-9615(1999)069[0155:DDPISF]2.0.CO;2
770	Rauch, M., Denis, L., 2008. Spatio-temporal variability in benthic mineralization processes in the
771	eastern English Channel. Biogeochemistry 89, 163–180. https://doi.org/10.1007/s10533-008-
772	9191-x
773	Riera, P., Richard, P., 1996. Isotopic Determination of Food Sources of Crassostrea gigas Along a
774	Trophic Gradient in the Estuarine Bay of Marennes-Oléron. Estuarine, Coastal and Shelf
775	Science 42, 347–360. https://doi.org/10.1006/ecss.1996.0023
776	Riera, P., Stal, L.J., Nieuwenhuize, J., Richard, P., Blanchard, G., Gentil, F., 1999. Determination of
777	food sources for benthic invertebrates in a salt marsh (Aiguillon Bay, France) by carbon and
778	nitrogen stable isotopes: importance of locally produced sources. Marine Ecology Progress
779	Series 18/, 301–30/.
780	Rolet, C., Spilmont, N., Dewarumez, J. M., Luczak, C. 2015. Linking macrobenthic communities
781	structure and zonation patterns on sandy shores: Mapping tool toward management and
782	conservation perspectives in Northern France. Continental Shelf Research, 99, 12-25.
783	Salgado, J.P., Cabral, H.N., Costa, M.J., 2004. Feeding ecology of the gobies <i>Pomatoschistus minutes</i>
784	(Pallas, 1770) and Pomatoschistus microps (Krøyer, 1838) in the upper Tagus estuary,
785	Portugal. Scientia Marina 68, 425–434. https://doi.org/10.3989/scimar.2004.68n3425
786	Selleslagh, J., Amara, R., 2015. Are Estuarine Fish Opportunistic Feeders? The Case of a Low
787	Anthropized Nursery Ground (the Canche Estuary, France). Estuaries and Coasts 38, 252-
788	267. https://doi.org/10.1007/s12237-014-9787-4
789	Selleslagh, J., Amara, R., 2008. Environmental factors structuring fish composition and assemblages
790	in a small macrotidal estuary (eastern English Channel). Estuarine, Coastal and Shelf Science
791	79, 507–517. https://doi.org/10.1016/j.ecss.2008.05.006
792	Selleslagh, J., Amara, R., Laffargue, P., Lesourd, S., Lepage, M., Girardin, M., 2009. Fish
793	composition and assemblage structure in three Eastern English Channel macrotidal estuaries:
794	A comparison with other French estuaries. Estuarine, Coastal and Shelf Science 81, 149–159.
795	https://doi.org/10.1016/j.ccss.2008.10.008
796	Selleslagh, J., Blanchet, H., Bachelet, G., Lobry, J., 2015. Feeding Habitats, Connectivity and Origin
797	of Organic Matter Supporting Fish Populations in an Estuary with a Reduced Intertidal Area
798	Assessed by Stable Isotope Analysis. Estuaries and Coasts 38, 1431–1447.
/99	https://doi.org/10.100//s1223/-014-9911-5
800	Integrati, E., Cabicon, L., Dauvin, J.C., Reitere, C., Genti, F., 1997. Spatio-temporal persistence of
801	the <i>Abra abou-recimaria koreni</i> muday-tine sand community of the eastern Bay of Sene.
802 802	Thrush S.E. Howitt J.E. Lobrer, A.M. 2012. Interaction notworks in coastal coff adimente
005	midsh, S.F., flewin, J.E., Loniet, A.M., 2012. Interaction networks in coastar soft-seminents
804 805	1223 https://doi.org/10.1800/11-1103.1
805	Underwood G. Kromkamp, I. 1990 Primary Production by Phytonlankton and Microphytohenthos
807	in Estuaries Advances in Ecological# 12 in G/WResearch Elsevier
808	Vasconcelos R P Reis-Santos P Fonseca V Maia A Ruano M Franca S Vinagre C Costa
809	M.J., Cabral, H., 2007. Assessing anthropogenic pressures on estuarine fish nurseries along
810	the Portuguese coast: A multi-metric index and concentual approach. Science of The Total
811	Environment 374, 199–215. https://doi.org/10.1016/j.scitotenv.2006.12.048

- Vinagre, C., Salgado, J., Costa, M.J., Cabral, H.N., 2008. Nursery fidelity, food web interactions and
  primary sources of nutrition of the juveniles of *Solea solea* and *S. senegalensis* in the Tagus
  estuary (Portugal): A stable isotope approach. Estuarine, Coastal and Shelf Science 76, 255–
  264. https://doi.org/10.1016/j.ecss.2007.07.006
- Wada, E., Kabaya, Y., Kurihara, Y., 1993. Stable isotopic structure of aquatic ecosystems. Journal of
   Biosciences 18, 483–499.
- Wilson, R.M., Chanton, J., Lewis, G., Nowacek, D., 2009. Combining organic matter source and relative trophic position determinations to explore trophic structure. Estuaries and Coasts 32, 999–1010. https://doi.org/10.1007/s12237-009-9183-7
- Yokoyama, H., Ishihi, Y., Yamamoto, S., 2008. Diet-tissue isotopic fractionation of the Pacific oyster
   *Crassostrea gigas*. Marine Ecology Progress Series 358, 173–179.
   https://doi.org/10.3354/meps07306
- 824
- 825

### 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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**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.