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1           **European flounder foraging movements in an estuarine nursery seascape**  
2                           **inferred from otolith microchemistry and stable isotopes**

3           Short Title: Fish foraging movements in nursery seascape

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## Abstract

Despite the importance of estuarine nurseries in the regulation of many fish stocks, temporal and spatial movements and habitat use patterns of juvenile fish remain poorly understood. Overall, combining several movement metrics allowed us to characterize dispersal patterns of juvenile flounder, *Platichthys flesus*, along an estuarine seascape. Specifically, we investigated otolith microchemistry signatures (Sr:Ca and Ba:Ca ratios) and stable isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) in muscles of these juveniles, during three consecutive years to assess inter-annual fluctuations in their home range and isotopic niches. The morphological condition and lipid content of individuals were lower in years of high as compared to low dispersal along the estuarine gradient. We discuss these results in relation to the ecosystem productivity and intra- and inter-specific competition level, which in turn affects movements and foraging behaviors of juvenile flounders.

**Keywords:** *Platichthys flesus*; Nursery; Otolith chemistry; Isotopes; Feeding strategy; Estuary; Dispersion; Home range

## 41 1. Introduction

42 Estuaries are complex ecosystems at the interface between marine and freshwater environments,  
43 associated with numerous ecological functions and ecosystem services (Barbier et al., 2011). In  
44 particular, estuaries provide diverse ecological niches to estuarine, freshwater, marine and diadromous  
45 fish (Potter et al., 2015), which benefit from the estuarine productivity during early stages of their life  
46 cycle (Sheaves et al., 2015). Evaluating the nursery value of estuarine habitats requires understanding  
47 resource dynamic and availability, connectivity patterns and ontogenetic migration of fish (Nagelkerken  
48 et al., 2015; Sheaves et al., 2015). Indeed, the quality of a nursery depends particularly on its ability to  
49 provide refuges against predators, and trophic resources (Beck et al., 2001). Changes in these conditions  
50 can therefore impact individual growth and morphological condition with ultimate consequences on  
51 survival and stock recruitment (Vasconcelos et al., 2009). Fluctuations of the estuarine productivity  
52 and/or competition pressures can thus lead to inter-annual variations of the nursery value, but also in  
53 habitat used and movement patterns of individuals (e.g. Mendes et al., 2014; Schloesser and Fabrizio,  
54 2019). When resources are limited and/or patchily distributed, individuals are expected to increase  
55 foraging movements to find their food, leading to additional metabolic costs (Bowler and Benton, 2005).  
56 Conversely, when resources are abundant and evenly distributed, juveniles are expected to limit costly  
57 movements and promote sedentary lifestyle while increasing their physiological condition.

58 Although nursery habitats are known to regulate many fish stocks (Beck et al., 2001), movement  
59 and dynamics of habitat use by early life stages of fish remain poorly understood in estuaries (Reis-  
60 Santos et al., 2015). Several tagging approaches are available for assessing connectivity patterns and  
61 home range of large fish (e.g. Le Pichon *et al.*, 2014). However, these methods are generally technically  
62 and/or ethically unsuitable for small vertebrates (Gillanders, 2009). Conversely, biological tracers of fish  
63 can deliver relevant information on connectivity patterns and home range of small-bodied fish,  
64 providing there is enough environmental heterogeneity (Elsdon et al., 2008; Secor et al., 1995).  
65 Biological tracers, such as the chemical composition of fish otoliths, or stable isotope ratios in soft

66 tissues and internal organs, have already demonstrated their usefulness to investigate fish movements  
67 among adjacent or segregated habitats within estuaries (e.g. Green *et al.*, 2012; Reis-Santos *et al.*, 2015;  
68 Mohan & Walther, 2018).

69 Carbon ( $^{13}\text{C}$ ) and nitrogen ( $^{15}\text{N}$ ) stable isotope ratios can be quantified in various fish tissues,  
70 including mucus, blood, fin, scale, liver or muscle, and these values reflect the spatial variation in food  
71 sources and position of the studied species in the food web (Hobson, 1999). During trophic transfer, the  
72 isotopic compositions of the consumer tissues compare to its prey change with variations in diet  
73 sources, tissue-specific turnover (i.e. time taken for a tissue to regenerate) and fractionation (i.e. how  
74 tissues fractionate the different isotopes and change the isotopic ratios) (Caut *et al.*, 2009). Therefore,  
75 the time required to reach a new isotopic equilibrium when the fish feed on a new food source is  
76 dependent on the tissue specific metabolic activity with, for example, higher turnover rates for the liver  
77 or blood than for muscle tissues (Buchheister and Latour, 2010). Accordingly, resident fish are expected  
78 to exhibit stable isotopic ratios aligned with local food webs, whereas immigrant fish may display mixed  
79 signatures or larger isotopic niches (Cunjak *et al.*, 2005; Fry *et al.*, 2003; Reis-Santos *et al.*, 2015). A  
80 gradual decrease in carbon isotope ratios ( $\delta^{13}\text{C}$ ) is expected along the salinity gradient from sea to  
81 freshwater through estuarine habitats (Herzka, 2005; Hobson, 1999; Peterson and Fry, 1987; Reis-  
82 Santos *et al.*, 2015).  $\delta^{13}\text{C}$  ratio primarily reflects variations in algal or detrital C sources at the bottom of  
83 the food chains (e.g. Kostecki *et al.*, 2012, 2010), while the nitrogen isotope ratio ( $\delta^{15}\text{N}$ ) increases with  
84 trophic levels from preys to predators through  $^{15}\text{N}$  accumulation (Peterson and Fry, 1987). Spatial  
85 changes in  $\delta^{15}\text{N}$  ratio are generally induced by anthropogenic activities (Herzka, 2005; Mohan and  
86 Walther, 2018). In estuarine nurseries, investigating shifts in stable C and N isotopic composition can be  
87 useful to estimate the proportion of migrant and resident fish, as well as the home range of their early  
88 life stages (Charles *et al.*, 2004; Green *et al.*, 2012; Herzka, 2005; Mohan and Walther, 2018; Reis-Santos  
89 *et al.*, 2015). However, stable C and N isotopic values of immigrant will dilute within few days or weeks  
90 depending on tissue investigated, meaning that evidences of migration will fade relatively quickly after  
91 settlement and feeding in their new habitat, the nursery (Cunjak *et al.*, 2005).

92 While stable isotope ratios provide an overview of organism movement and feeding behavior, the  
93 otolith chronological properties allow reconstructing patterns of habitat used and migration histories  
94 over long period (Secor et al., 1995). When a fish moves between habitats with distinct chemical  
95 signatures, some chemical elements are incorporated in the aragonitic matrix of its otoliths according  
96 to their different concentrations in the chemically distinct habitats (Daverat et al., 2005; Hüsey et al.,  
97 2020). Hence, the profile of chemical elements along an otolith transect records the fish movements  
98 between these habitats (Campana, 1999). Although a large panel of chemical elements can be tracked,  
99 not all provide information on the surrounding habitats, and some of them are more influenced by the  
100 fish physiology than by its environment (e.g. Mg, Limburg et al., 2018). Strontium:Calcium (Sr:Ca) and  
101 Barium:Calcium (Ba:Ca) elemental ratios are known to reflect changes in ambient water, while being  
102 little influenced by the fish physiology (Daverat et al., 2005; Hüsey et al., 2020). In estuarine waters, the  
103 Sr:Ca ratio commonly declines with the salinity gradient, while the Ba:Ca ratio increases in response to  
104 the growing freshwater input (Elsdon and Gillanders, 2006, 2005; Tabouret et al., 2010). These opposite  
105 dual patterns have thus been exploited to highlight fish migrations, and their movements at small-scale  
106 along the salinity gradient (Daverat et al., 2012; Laugier et al., 2015; Reis-Santos et al., 2015; Teichert et  
107 al., 2022; Williams et al., 2018). Using laser ablation-inductively coupled to a plasma mass spectrometer  
108 (LA-ICP-MS), the elementary signatures of otoliths can be quantified on very thin structures of three to  
109 ten  $\mu\text{m}$  wide, which respectively represents one day to a few weeks of a fish life (e.g. Selleslagh et al.,  
110 2016). Comparisons between the otolith and estuarine seascape elemental compositions provide a  
111 quantitative assessment of movement extent and home range during early life stages of a fish.

112 Among the fish species using estuaries as nursery areas, the flatfish European flounder,  
113 *Platichthys flesus* (Linnaeus, 1758), is widespread throughout the European coasts, from the White,  
114 Mediterranean to the Black seas. Flounders exhibit a facultative catadromous life cycle. Although  
115 spawning typically occurs in marine coastal waters, some individuals spawn in brackish or freshwater  
116 areas (Daverat et al., 2012). After hatching, larvae use selective tidal transport to reach estuarine  
117 nursery areas (Bos, 1999), where they generally settle in shallow upper reaches subjected to freshwater

118 influence (Bos and Thiel, 2006; Mendes et al., 2020). Although some individuals remain in freshwater  
119 for few years, older juveniles tend to move toward the middle or lower sections of the estuary (Amorim  
120 et al., 2018). Juvenile flounders are generalist feeders, but the young-of-the-year (i.e. 0+) tend to feed  
121 on fewer preys, essentially amphipods of the genus *Corophium* (e.g. Mendes et al., 2020). By contrast,  
122 the diet composition of larger individuals is more diversified, including small polychaetes and  
123 oligochaetes (Mendes et al., 2020, 2014; Summers, 1980). Large juveniles exhibit restricted home  
124 ranges and high site fidelity as demonstrated by mark–recapture surveys (Dando, 2011) and telemetry  
125 studies (Le Pichon et al., 2014; Wirjoatmodjo and Pitcher, 1984). However, the movement patterns of  
126 early settled flounders across estuarine seascape are far less known, mainly because of their small size  
127 (total length <90 mm). Given their specific diet, 0+ flounders are expected to strongly depend on prey  
128 availability and repartition, foraging on potentially large areas to find them.

129 In this study, we used biological tracers to investigate movement patterns of flounder juveniles in  
130 the Sélune estuary (Lower-Normandy, Western France) during three consecutive years. Specifically,  
131 both otolith microchemistry signatures (Sr:Ca and Ba:Ca ratios) and stable isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ )  
132 in muscle tissues were used to reconstruct home range and isotopic niches of juveniles. We  
133 hypothesized that inter-annual fluctuations in patterns of mobility and foraging behaviour will reflect in  
134 the morphological condition and lipid content of fish juveniles.

## 135 2. Materials and methods

### 136 2.1. Study area and fish sampling

#### 137 2.1.1. Study area

138 The Sélune River flows over 91 km from the source to the English Channel in the Mont Saint-Michel  
139 Bay, Lower-Normandy, France (Fig. 1). The catchment is 1 106 km<sup>2</sup> and median river discharge is 5.56  
140 m<sup>3</sup>.s<sup>-1</sup> at the river mouth. Since 1919, the upper part of the river network was disconnected (827 km<sup>2</sup>)  
141 by two large hydroelectric power dams (La-Roche-qui-Boit, H=16m, and Vezins, H=36 m). However,

142 environmental authorities and local operators decided to remove these two dams to restore the  
143 ecological continuity in compliance with the EU Water Framework Directive. Vezins dam (located 23 km  
144 upstream the estuary) was teared down in 2020, and removal of La-Roche-Qui-Boit dam (18 km  
145 upstream the estuary) will be achieved in 2023. Even if flounders are limited to the estuarine area and  
146 dams did not disturb their distribution on the river catchment, the input of nutriments and terrigenous  
147 organic matter into the estuary was likely affected during dismantling operations. The macro-tidal Mont  
148 Saint-Michel bay has the second highest tidal range in Europe (average: 10-11m and up to 16m) and its  
149 large intertidal zone covers 220 km<sup>2</sup> (Laffaille et al., 2001). During ebb tides in the Sélune estuary, the  
150 water supply is primarily ensured by river discharges, while the tidal influence during flood tides is  
151 perceptible up to the confluence with the Oir River (Fig.1). Accordingly, large salinity variations occur  
152 along the estuary, with highest fluctuations recorded in middle reaches, as observed in comparable  
153 macro-tidal estuaries (Robins et al., 2014). Salinity at the confluence between the Sée and Sélune rivers  
154 (Fig. 1) ranged from 2 to 34 ‰ during low and high tides respectively (Kostecki et al., 2012; unpublished  
155 data, Nils Teichert). In such estuaries, the influence of marine tidal flux dominates in the lower reaches  
156 leading to higher salinity range at high tide, but decreases toward upstream as inputs of freshwater  
157 increase. Therefore, the distance to the sea appeared to be a more relevant descriptor than punctual  
158 salinity measures to assess the marine influence along the estuary continuum. Here, we defined the  
159 distance to the sea ( $d_s$ , in km) as the distance between each estuarine position and the accepted limit  
160 of the transitional waters, following the main channel of the Sélune River (Fig. 1).

161 Daily records of river discharges were provided by the DREAL Basse-Normandie for the Ducey  
162 hydrological station (ref. I9241010), which is located on the Sélune River bank, just upstream of the tidal  
163 influence. Sampling was done during low hydrological conditions in late summer for three consecutive  
164 years; the mean ( $\pm$  sd) river discharge ( $\text{m}^3 \cdot \text{s}^{-1}$ ) during the month prior the samplings differed significantly  
165 between the three years ( $F = 45.54$ ,  $p < 0.001$ ), ranging from  $1.85 \text{ m}^3 \cdot \text{s}^{-1}$  ( $\pm 0.75$ ) in 2019 to  $2.45 \text{ m}^3 \cdot \text{s}^{-1}$   
166 ( $\pm 0.61$ ) in 2020 and  $3.31 \text{ m}^3 \cdot \text{s}^{-1}$  ( $\pm 0.44$ ) in 2021. However, these freshwater inputs remained far below



167 the median river discharge ( $5.56 \text{ m}^3 \cdot \text{s}^{-1}$ ), which suggests little estuarine salinity variation during the  
168 studied periods.

### 169 *2.1.2. Fish sampling*

170 In the first year (2019), juvenile flounders were collected in autumn at six sites along the Sélune  
171 estuarine continuum (Fig. 1, Table 1) to investigate isotopic and elemental signatures (see thereafter  
172 for method details). At site 1, 13 juvenile flounders were collected using a beam trawl (1.5 m large, 16  
173 mm mesh size) during high tide in September 2019. At site 2 and 3, 31 and 5 flounders were collected  
174 using a pushnet in September and November 2019 respectively, while 3, 8 and 4 flounders were  
175 respectively caught at sites 4, 5 and 6 by electrofishing in September 2019. Note that the upstream  
176 repartition of flounders was limited by two small weirs (approximately 1 m high) located upstream of  
177 sites 5 and 6 (Fig.1). Based on this first year of study, highest abundance of juvenile flounders was  
178 recorded at site 3, and this site was chosen to investigate movement patterns over in 2020 and 2021.  
179 Juveniles were collected using a pushnet ( $n = 31$  and  $34$  in September 2020 and 2021, respectively). All  
180 collected fish were anesthetized with a Benzocaine solution ( $15 \text{ mg} \cdot \text{L}^{-1}$ ) before being euthanized with a  
181 Benzocaine overdosed solution ( $200 \text{ mg} \cdot \text{L}^{-1}$ ), and then frozen ( $-20^\circ\text{C}$ ) until dissections at the laboratory.  
182 Juvenile flounders were measured (total length (TL) in mm) and weighed (total weight (TW) in g) to  
183 determine their morphological conditions using a length-independent relative condition index (Jakob et  
184 al., 1996). To this end, we fitted a linear regression model between the log-transformed body mass and  
185 standard length ( $\log(\text{TW}) = -12.15 + 3.14 \times \log(\text{TL})$ ,  $R^2 = 0.966$ ,  $p < 0.001$ ), where model residuals were  
186 then used as an index of body condition, with positive and negative residuals representing increased  
187 and decreased body condition respectively.

## 188 *2.2. Analysis of otolith microchemistry*

### 189 *2.2.1. Otolith preparation and elemental quantification*

190 Flounder sagittal otoliths were extracted and embedded in epoxy resin (Araldite 2020, Huntsman  
191 Corporation), then grounded along the transversal plane, and polished until the primordium was

192 reached. The otolith elemental composition (Sr and Ba) was quantified using femtosecond laser ablation  
193 (LA, IR 1030 nm; Alfamet-Novalase, France) coupled with an inductively plasma mass spectrometer (ICP-  
194 MS, DRCII; Perkin Elmer, Shelton). Ablations were performed with a raster scanning strategy along the  
195 longest growth axis of each otolith with a laser beam of 15  $\mu\text{m}$  of diameter, at a frequency of 20 Hz,  
196 moving forward at 5  $\mu\text{m}\cdot\text{s}^{-1}$ . Although each laser ablation was conducted from the primordium to the  
197 external edge of otolith, our analysis focused on the latest 150  $\mu\text{m}$  to study the last month of the juvenile  
198 life. The resulting elementary profile thus consisted of successive records taken every 5  $\mu\text{m}$ ,  
199 approximately representing one day of the juvenile flounder life (Amara et al., 2009), as also reported  
200 in the Sélune estuary (unpublished data, Nils Teichert). The external calibration was done by using the  
201 international reference materials NIST614, NIST612 and NIST610 (National Institute of Standards and  
202 Technology, USA). Calcium, an internal standard, is used to account for variations in the amount of  
203 ablation material and laser energy in ablation efficiency. Therefore, elementary compositions were  
204 standardized in elementary mass ratios, Sr:Ca and Ba:Ca. Analytical precision was measured using the  
205 otolith certified reference materials: fish NIES22 (National Institute for Environmental Studies, Japan;  
206 Yoshinaga, Nakama, Morita, & Edmonds, 2000) and FEBS-1 (National Research Council Canada, Canada).

### 207 *2.2.2. Spatio-temporal changes in marginal otolith signatures*

208 We investigated spatial changes in elemental composition along the estuarine gradient using the  
209 otolith marginal signatures of Sr:Ca and Ba:Ca ratios. This approach assumes that the signature at the  
210 otolith external edge, which corresponds to the last period of a fish life, indicates the chemical signature  
211 of their habitat just before being caught. The signatures of the two last elemental records from the  
212 otolith edge (i.e. 10  $\mu\text{m}$ , around 2 days) were compared between the six sampling sites using a  
213 permutation-based approach (permanova), with the Manhattan distance to account for dissimilarity  
214 between the scales of elemental ratios. For each elemental ratio, relationships between marginal  
215 signatures and the distance to the sea were then investigated using non-parametric Spearman  
216 correlation tests. Finally, the temporal consistency of otolith signatures was verified by comparing the  
217 marginal signatures of flounders caught at site 3 for the three sampling years, using a permanova.

218           2.2.3. *Inferring longitudinal positions from otolith signatures*

219           Longitudinal positions (i.e. distance to the sea) of flounders were estimated by fitting a Generalized  
220 Additive Model (GAM) adjusted with the otolith marginal signatures corresponding to the last two days  
221 of the fish before being caught (i.e. 10  $\mu\text{m}$ ). GAM model allows to estimate non-linear response curves  
222 (Wood, 2000). In the GAM model, the distance to the sea was used as response variable and the  
223 marginal elemental records as predictors, which were associated with cubic smoothing splines. The  
224 interaction between Sr:Ca and Ba:Ca ratios was considered in the model because preliminary  
225 examination indicated that its inclusion slightly improved the Akaike's information criterion (delta AIC :  
226 13.2 between models without and with interaction). All the marginal records were included in the model  
227 adjustment process, except for some flounders caught at site 3. At this site, a random sample of 30  
228 flounders was selected for fitting the model, whereas the other fish were used to evaluate the model  
229 performances on independent data. The significance and performance of the model were then tested  
230 using a deviance reduction test (F-test) and the adjusted r-squared ( $R_{\text{adj}}$ ) respectively. In addition, the  
231 positioning error (km) was calculated as the absolute distance separating the predicted position from  
232 the location of sampling site.

233           2.2.4. *Inter-annual movements inferred from otolith signatures*

234           Elemental records from the last 150  $\mu\text{m}$  of the otolith edge were used in the GAM model to predict  
235 successive positions of juvenile flounders during the month prior to the sampling. This distance  
236 threshold (i.e. 150  $\mu\text{m}$ ) corresponds roughly to a mean daily growth rate of 5  $\mu\text{m}\cdot\text{d}^{-1}$ , which is consistent  
237 with previous observations in other estuaries of similar characteristics in the English Channel (Amara et  
238 al., 2009). The predicted positions (i.e. distance to the sea) were used to calculate synthetic metrics  
239 reflecting how settled flounders occupied and moved along the estuarine seascape during the month  
240 preceding their capture (Table 2). While four metrics reflect how each individual used the longitudinal  
241 gradient (i.e. longitudinal position, individual niche extent, daily niche extent and daily niche used), the  
242 metric 'population niche extent' encompasses the estuarine gradient used by all caught fish of the year  
243 (i.e. annual cohort). Individual metrics were calculated for each fish caught at site 3 and inter-annual

244 differences (i.e. 2019, 2020 and 2021) were then investigated using an ANOVA, followed by pairwise  
245 comparisons with post-hoc Tukey HSD tests. For each sampling year, the population niche extent was  
246 estimated using bootstrap resampling with 1000 replicates. The niche extent for each year was  
247 considered statistically different from one another if the 95% confidence intervals did not overlap.

### 248 *2.3. Analysis of stable isotopes in fish muscles*

#### 249 *2.3.1. Quantification of stable isotopes*

250 For each collected fish, one muscle tissue sample was dissected to estimate its trophic position and  
251 origin of food sources based on its nitrogen and carbon isotopic signatures (Table 2, Hobson, 1999).  
252 Isotopic signatures were expressed in the delta unit notation as deviation from international standards  
253 of PeeDee Belemnite for  $\delta^{13}\text{C}$  and atmospheric  $\text{N}_2$  for  $\delta^{15}\text{N}$ , following the formula:  $\delta X =$   
254  $((R_{\text{sample}}/R_{\text{standard}})-1) \times 1000$ , where X is  $^{13}\text{C}$  or  $^{15}\text{N}$  and R is the ratio ( $^{15}\text{N}:^{14}\text{N}$  or  $^{13}\text{C}:^{12}\text{C}$ ) in the sample  
255 and in the standard. For each muscle sample, nitrogen and carbon total quantities, and the isotopic  
256 ratios were measured by continuous flow isotope mass spectrometry (CF-IRMS) using a Thermo  
257 Scientific Delta V Advantage mass spectrometer coupled to a Thermo Scientific Flash 2000 elemental  
258 analyzer. Analytical precision (standard deviation) was  $< 0.15\%$  of reference material. We considered  
259 that correction of  $\delta^{13}\text{C}$  values for lipid-rich tissues was not necessary because the ratio of carbon relative  
260 to nitrogen (C:N ratio) in muscle samples were almost all  $< 3.3$ , and exceptionally of 3.6 (Post et al.  
261 2007). Moreover, the C:N ratio was used as a proxy of lipid content stored in fish muscle  
262 (McConnaughey and McRoy, 1979; Mohan and Walther, 2018; Post et al., 2007). For each fish, the  
263 percentage of lipid content was estimated based on the C:N ratio following the linear approximation  
264 proposed by Post et al. (2007) for aquatic animals ( $\% \text{ lipid} = -20.54 + 7.24 \times \text{C:N}$ ).

#### 265 *2.3.2. Inter-annual variations of isotopic niches*

266 Using fish collected at six sites, we first examined variations of isotopic ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) along  
267 the longitudinal estuarine gradient by investigating their linear relationships between the distance to  
268 the sea to sampling sites, and the longitudinal positions inferred from otolith signatures. We then

269 compared the annual isotopic signatures of fish caught at site 3 using an ANOVA, followed by post-hoc  
270 Tukey HSD tests, to investigate inter-annual changes in resource origin and trophic level (Table 2).  
271 Finally, we estimated the isotopic niche sizes for each year at site 3, from a 2D kernel density estimation  
272 at 95% confidence level using  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (Eckrich et al., 2020). We generated 1000 bootstrap  
273 replicates of isotopic niche size to determine significant differences between years, based on non-  
274 overlapping 95% confidence intervals.

275 All statistical analyses were performed in the R environment v. 4.0.5 (R Core Team, 2018) using the  
276 'stats' package for standard analysis, such as linear regressions, ANOVA and Tukey HSD post-hoc tests.  
277 The GAM models were adjusted using the 'mgcv' package (Wood, 2011). Kernel density estimation of  
278 isotopic niches were performed using the 'rKIN' package (Eckrich et al., 2020) and bootstrap replicates  
279 were generated using the 'boot' library (Canty and Ripley, 2017).

### 280 **3. Results**

281 Overall, juvenile flounders used to assess spatial variability in isotopic and elemental signatures  
282 (sites 1, 2, 4, 5 and 6) were significantly larger (TL between 32 and 205 mm, Table 1) than those caught  
283 at site 3 ( $F = 57.21$ ,  $p < 0.001$ ). In this latter nursery site, the total length (TL) of 0+ juveniles ranged from  
284 27 to 75 mm (mean = 41.5 mm) and differed significantly between the three sampling years, with larger  
285 sizes in 2021 ( $F = 14.18$ ,  $p < 0.001$ ) (Fig. 2a). The juvenile condition was significantly higher in 2020 than  
286 in other years for the morphological condition index ( $F = 6.51$ ,  $p = 0.002$ ; Fig. 2b) and the lipid content  
287 in muscles inferred from C:N ratio ( $F = 6.65$ ,  $p = 0.002$ ; Fig. 2c).

#### 288 ***3.1. Spatio-temporal changes in marginal otolith signatures***

289 Bivariate otolith marginal signatures varied significantly between sampling sites ( $R^2 = 0.54$ ,  $F = 60.7$ ,  
290  $p < 0.001$ ; Fig. 3). Following non-linear trends, the Ba:Ca elemental ratio increased ( $\rho = 0.56$ ,  $p <$   
291  $0.001$ ), while the Sr:Ca decreased significantly with the distance to the sea ( $\rho = -0.71$ ,  $p < 0.001$ ).

292 Signatures at site 3 remained similar between years ( $R^2 = 0.02$ ,  $F = 2.11$ ,  $p = 0.095$ ), which emphasized  
293 the temporal consistency of otolith signatures. By extension, we postulate that this is also the case  
294 across the estuarine gradient.

### 295 *3.2. Inferring movements from otolith microchemistry*

296 The GAM model adjusted from otolith marginal signatures explained 83.4% of deviance of the  
297 distance to the sea ( $R^2_{\text{adj}} = 0.81$ ,  $F = 27.51$ ,  $p < 0.001$ ). The predicted distance increased with increasing  
298 Ba:Ca ratio and decreasing Sr:Ca ratio, according to nonlinear relationships (Fig. 4). The mean ( $\pm$  SD)  
299 positioning error was  $2.46 (\pm 2.46)$  km for all records used for model adjustment, while it was  $2.12 \pm$   
300  $(1.93)$  km and  $1.87 (\pm 1.81)$  km respectively for the adjustment and validation of subsamples of fish  
301 caught at site 3.

302 Focusing on juvenile flounders caught at site 3, individual movement metrics revealed inter-annual  
303 differences during the last month of the fish life (Fig. 5). In 2021, juvenile flounders used upper estuarine  
304 habitats as revealed by the significant change in median longitudinal positions ( $F = 5.99$ ,  $p = 0.003$ ). In  
305 2019, the individual niche extent was two time larger than for other years ( $F = 27.7$ ,  $p < 0.001$ ), indicating  
306 that flounders used a larger range of estuarine habitats during lower hydrological conditions. Also, the  
307 daily niche extent decreased significantly from 2019 to 2021 ( $F = 20.9$ ,  $p < 0.001$ ), which suggests a  
308 lower propensity to disperse as freshwater flows increase. In comparison to the other years, juvenile  
309 flounders caught in 2020 traveled daily on a significantly larger part of their spatial niche ( $F = 20.9$ ,  $p <$   
310  $0.001$ ). At the population level, the niche extent displayed an opposite pattern where juvenile flounders  
311 caught in 2020 explored a narrower estuarine gradient than in other years, which stressed the low  
312 propensity of fish to disperse in 2020 (Fig. 6).

### 313 *3.3. Stable isotope signatures and trophic niches*

314  $\delta^{13}\text{C}$  values in fish muscles were significantly related to the distance to the sea of the sampling sites  
315 ( $R^2 = 0.506$ ,  $F = 129.08$ ,  $p < 0.001$ ). Interestingly, the relationship was even stronger using the  
316 longitudinal estuarine positions inferred from otolith signatures ( $R^2 = 0.725$ ,  $F = 340.17$ ,  $p < 0.001$ ),

317 suggesting that some individuals have recently immigrated in the sampling site (Fig. 7). By contrast,  $\delta^{15}\text{N}$   
318 values did not vary with longitudinal estuarine positions of juvenile flounders ( $R^2 = 0.00$ ,  $F = 0.024$ ,  $p =$   
319  $0.874$ ).

320 For juvenile flounders caught at site 3,  $\delta^{13}\text{C}$  signatures were slightly lower in 2021 in comparison to  
321 the other years ( $F = 17.70$ ,  $p = 0.002$ ), which appears congruent with upper positions previously  
322 highlighted by otolith signatures (Fig. 8). The trophic level, reflected by  $\delta^{15}\text{N}$  signatures, was higher in  
323 2019 ( $F = 11.47$ ,  $p < 0.001$ ), suggesting that preys consumed by juvenile flounders or their nitrogen  
324 signatures were different from the two other years. At the population scale, the isotopic niche size  
325 estimated for juvenile flounders caught in 2020 was very thin, indicating that individuals fed on a  
326 restricted range of prey items (Fig. 8). By contrast, isotopic niches of juvenile flounders caught in 2019  
327 and 2021 were much larger than in 2020, suggesting greater heterogeneity of food resources used by  
328 individuals.

## 329 **4. Discussion**

### 330 ***4.1. Biological tracers in estuarine environment***

331 Estuarine seascapes provide a suitable context to investigate animal movements based on  
332 environmental tracers because of the gradual changes in local conditions (Williams et al., 2018),  
333 including water chemistry (Walther and Nims, 2015). As reported in previous studies, Sr:Ca and Ba:Ca  
334 elemental ratios recorded in fish otoliths displayed opposite relationships with salinity, so that their  
335 simultaneous use provides a useful indication on fish position along the salinity gradient (e.g. Elsdon &  
336 Gillanders, 2005; Tabouret et al., 2010; Daverat et al., 2011, 2012; Reis-Santos et al., 2015; Williams et  
337 al., 2018; Nelson & Powers 2020). Here, we used the distance to the sea as a proxy of the estuarine  
338 continuum, mainly because sharp changes in local salinity induced by tides appeared poorly suitable to  
339 interpret otolith signatures. Despite the high laser beam resolution, the  $5\ \mu\text{m}$  elemental records yielded  
340 a buffered daily signature, which was insufficient to capture salinity variations over tidal cycle, but rather

341 reflected a spatial pattern shaped by the extent of marine intrusion along the estuary. This longitudinal  
342 discrimination was thus particularly relevant to reconstruct juvenile fish movements across the  
343 estuarine nursery (Mohan and Walther, 2018; Williams et al., 2018).

344 In the same way,  $\delta^{13}\text{C}$  signature variations in flounder juvenile muscles clearly reflected the  
345 estuarine gradient, with highest values at the vicinity of the sea. Analogous results have been reported  
346 in other estuaries (e.g. Reis-Santos et al., 2015). These results allow to discriminate the origin of preys  
347 consumed by fish (Green et al., 2012; Mendes et al., 2020), as well as the relative contribution of marine  
348 and freshwater organic matters at the bottom of the food web (França et al., 2011; Kostecki et al., 2012,  
349 2010; Selleslagh et al., 2015). Accordingly, isotopic signatures can be used to infer foraging extents and  
350 fish movements, as geochemical gradients are reflected in tissues of locally foraging organisms (Winter  
351 et al., 2021). Additionally, fish assignments can depict their locations several days or weeks prior to the  
352 capture because of the isotopic turnover rate in fish muscles. Here, we assumed that the observed  
353 signatures reflected resources accumulated over one month before the fish sampling, which is in  
354 accordance with the turnover rates generally reported for fish juveniles (Herzka, 2005). This assumption  
355 was also supported by the congruence between  $\delta^{13}\text{C}$  signatures and longitudinal positions inferred from  
356 otolith signatures, which correctly relocated juvenile flounders that recently immigrated in the sampling  
357 sites.

358 Both otolith elemental and muscle isotopic signatures appeared as decisive tools for investigating  
359 fish spatial patterns in estuaries. However, exogenous factors such as river flow inputs, tidal cycles or  
360 wave energy can induce spatial variability in local environmental conditions, and limit or even impair  
361 the interpretation of movements inferred by natural tracers (e.g. Gillanders, 2005; Elsdon & Gillanders,  
362 2006). For a stationary estuarine location, the intensification of river flow is expected to decrease the  
363 Sr:Ca ratio, while increasing the Ba:Ca ratio, in response to the extra freshwater input (Walther and  
364 Nims, 2015; Williams et al., 2018). The inverse trend should be expected when the marine intrusion is  
365 enhanced, such as when the river flow is low, or during spring tides. In the present study, we



366 demonstrated that otolith marginal signatures of juvenile flounders caught in the median part of the  
367 estuary (site 3) remained stable over the three sampling years, while the river discharges were different.  
368 This result confirms the steadiness of microchemical signatures over the range of river flow considered  
369 in this study (during the low flow summer period). Unfortunately, the whole estuarine gradient was not  
370 sampled over the three years, so we had to assume stability of spatial patterns across the estuary.

#### 371 *4.2. Home range and movements across the seascape nursery*

372 We took advantage of the estuarine gradient to reconstruct small scale movements of juvenile  
373 flounders using a GAM model, which accounted for the non-linearity relation between otolith elemental  
374 ratios and salinity (Nelson and Powers, 2020). The simultaneous use of Sr:Ca and Ba:Ca substantially  
375 increased the model performance, leading to a mean positioning error of about 2 km, less than 7% of  
376 the estuarine length. Coherently with the stable C isotopes turnover rates in fish muscles, juvenile  
377 flounders' movements were reconstructed over the last month of their life, by focusing on the  
378 elemental records from the last 150  $\mu\text{m}$  of the otolith edge. We thus assumed a constant otolith growth  
379 rate between individual (i.e. 5  $\mu\text{m}\cdot\text{d}^{-1}$ ) because daily age estimates based on microstructures were  
380 difficult to obtain using otoliths prepared for elemental analysis.

381 Overall, movement metrics demonstrated the important site fidelity of early-settled juvenile  
382 flounders in the Sélune estuary. The individual niche extent over one month was restricted to about 6  
383 km during the drought hydrological year and about 3 km when the river flow was higher. This result is  
384 in accordance with studies highlighting that juvenile flounders forage on inundated mudflats at high  
385 tide, but remain in the main tidal channel supplied by the river flow during low tides (Summers, 1980;  
386 Wirjoatmodjo and Pitcher, 1984). Juvenile flounders are thus subjected to important physicochemical  
387 variations induced by tides, while juveniles of other marine species, such as sea bass for instance, reach  
388 the intertidal areas at high tide, and then move back to marine subtidal areas at low tide (Laffaille et al.,  
389 2001; Teichert et al., 2018). Even in the Sélune macro-tidal estuary, the resident behaviour of juvenile  
390 flounders resulted in small daily movements along the estuarine gradient (on average between 400 and

391 800 m), which emphasizes that they can find suitable foraging, sheltering and resting conditions in a  
392 reduced spatial extend. Similar results have been reported for late-stage juvenile flounders (2+, 3+) and  
393 mature flounders that moved respectively about 870 m (Le Pichon et al., 2014), and 270 m on average  
394 during one tidal cycle (Wirjoatmodjo and Pitcher, 1984).

395 Both isotopic and elemental signatures also highlighted inter-annual differences in the distribution  
396 of juvenile flounders, which tended to use upper estuarine habitats in 2021. It is probable that annual  
397 modifications in hydro-morphological conditions and in the spatial availability of prey contribute to the  
398 changes in their longitudinal positions. The prey availability has been reported as an essential driver of  
399 0+ flounder movements and their distribution in the nursery habitat (Bos, 1999; Florin and Lavados,  
400 2010; Mendes et al., 2020). Moreover, the Sélune estuary is part of the Mont-Saint Michel bay, and is  
401 therefore subjected to important sediment movements that recurrently modify the structure of tidal  
402 channels and the distribution of estuarine habitats (Levoy et al., 2017). Interestingly, the individual niche  
403 extent was larger in 2019, which suggests that individuals explored a longer range of the estuarine  
404 gradient maybe in response to lower resources availability during this year. In this case, the spatial  
405 distribution of food resources and competition for its accessibility must be the main drivers of individual  
406 mobility and home range of juveniles across the nursery seascape (Bolnick et al., 2003).

#### 407 ***4.3. Inter-annual changes in foraging movements***

408 Overall, our results revealed that the morphological condition and lipid storage of juvenile  
409 flounders, estimated from C:N ratio, were higher in 2020 than in 2019 and 2021. Our hypothesis to  
410 explain this is that inter-annual fluctuations lead to variations in ecosystem productivity and competition  
411 level, which in turn affects patterns of mobility and foraging behaviors of fish juveniles. Indeed, when  
412 resources are abundant and evenly distributed, individuals are expected to disperse regularly over small  
413 spatial niches because food accessibility is not a limiting factor (MacArthur and Pianka, 1966). In this  
414 case, the reduced energy cost of small-scale displacements and food abundance should increase  
415 individual condition, and their energetic reserves. Conversely, if resources are scarce or unevenly

416 distributed, individuals should display a more marked exploration behaviour to find their food and  
417 potentially face competition (Lesser et al., 2020; Svanbäck and Bolnick, 2007). In such a case, resources  
418 used by the population is expected to be more diversified and individual spatial niches larger and less  
419 depleted in terms of resources on a daily basis. Consequently, food limitation and energy expenditure  
420 caused by movements and competition are expected to decrease the individual reserves and  
421 morphological condition.

422 Here, isotopic niches and population niche extents displayed convergent annual patterns,  
423 highlighting that juvenile flounders caught in 2020 were in good condition and dispersed little, feeding  
424 on a restricted range of prey items. These fish efficiently used their individual niche extent as evaluated  
425 by the daily niche used ratio, which suggests that individuals can daily cross a large part of their home  
426 range, in search of suitable habitats and sufficient resources to grow and accumulate energy reserves.  
427 Contrastingly, the large spatial and isotopic niche sizes reported for the 2019 and 2021 populations  
428 could reflect an increase of intra-specific competition, where individuals had to adopt different foraging  
429 strategies and use distinct estuarine locations (Bolnick et al., 2003). Interestingly, processes involved in  
430 niche sizes delineation were likely different between years. In 2019, the large population niche extent  
431 was associated with an increase in the extent of individual niches, whereas they remained narrow in  
432 2021 but scattered along the estuarine gradient. This result confirms that changes in population niche  
433 size can be decoupled from changes in the individual niches within the population (Bolnick et al., 2010).  
434 During these periods, where juvenile flounders were in low condition, the daily use of individual spatial  
435 niches was less efficient than in 2020, suggesting that travel for daily activities was likely more expensive,  
436 either because of the involved distances and/or linked to hydrological constraints. Although our study  
437 does not clearly highlight the mechanism shaping the spatial and trophic niches of juvenile flounders,  
438 our results emphasize the strong inter-annual variability of their trophic movements. This variability is  
439 probably related to changes in resource availability and biotic interactions within the nursery. Future  
440 studies should thus focus on linking individual and population dispersal patterns with environmental

441 constraints, such as prey availability, conspecific abundance, competition, and/or the presence of  
442 predators.

## 443 **5. Credit authorship contribution statement**

444 **Nils Teichert:** Conceptualization, Project administration, Funding acquisition, Data curation,  
445 Methodology, Formal analysis, Writing - original draft. **Anne Lizé, H  l  ne Tabouret, Gilles Bareille,**  
446 **Anthony Acou, Thomas Trancart:** Methodology, Data curation, Writing - review & editing. **Jean-Marc**  
447 **Roussel:** Writing - review & editing. **Laure-sarah Virag:** Methodology, Data curation. **Alexandre**  
448 **Carpentier, Eric Feunteun:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing -  
449 review & editing.

## 450 **6. Declaration of Competing Interest**

451 The authors declare that they have no known competing financial interests or personal relationships  
452 that could have appeared to influence the work reported in this article.

## 453 **7. Data Availability**

454 Data that support the findings of this study are available from the corresponding author upon  
455 reasonable request.

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641

642 **Table 1:** Summary of the juvenile flounders, *Platichthys flesus*, collected in the six sampling sites of  
643 the estuarine nursery of the Sélune River. The sampling date, number and fish size range (total length,  
644 mm) are provided, as well as analyses in which they were involved. While all individuals were used to  
645 investigate spatial change in otolith microchemistry and isotopic signatures along the estuarine  
646 gradient, only fish from site 3 were used to describe the interannual variability of movement patterns  
647 and trophic niches.

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Site	Sampling date	Number	Size range (mm)	Analysis
1	28 Sept. 2019	13	35-91	spatial
2	12 Nov. 2019	5	32-53	spatial
3	16 Sept. 2019	31	27-65	spatial - temporal
3	17 Sept. 2020	31	30-50	spatial - temporal
3	17 Sept. 2021	34	33-75	spatial - temporal
4	9 Sept. 2019	3	43-113	spatial
5	9 Sept. 2019	8	48-187	spatial
6	23 Sept. 2019	4	142-205	spatial

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651 **Table 2:** List of movement and isotopic metrics derived from otolith elemental and muscle isotopic  
 652 signatures of juvenile flounder, *Platichthys flesus*, in the estuarine nursery of the Sélune River.  
 653 Movement metrics derived from elemental records from the last 150 µm of the otolith edge reflect how  
 654 flounder occupied and moved along the estuarine seascape the month before their capture. The  
 655 biological level of each metric is specified such as Ind: individual, Pop: fish population sampled the same  
 656 year.

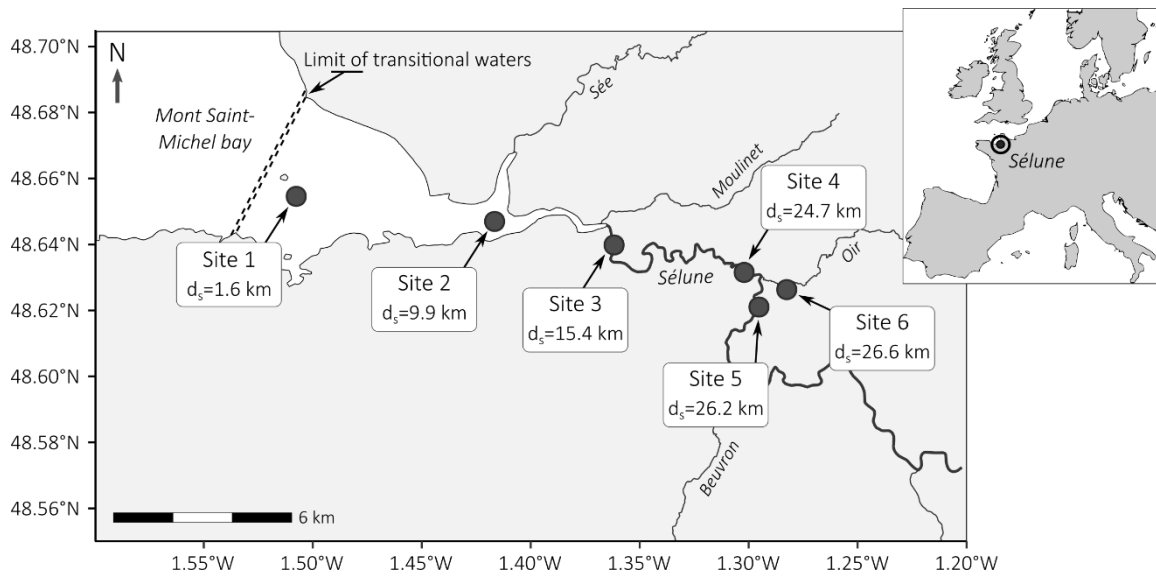
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Metrics	Scale	Unit	Description
<b><i>Otolith microchemistry</i></b>			
Longitudinal position	Ind.	km	Median of distances to the sea predicted during the last month of fish, reflecting the average longitudinal position occupied by fish on the estuarine gradient.
Individual niche extent	Ind.	km	Absolute difference between quantiles 5% and 95% of predicted positions during the last month of the fish, estimating the longitudinal extent occupied by the fish along the estuarine gradient (i.e. distance between extreme positions).
Daily niche extent	Ind.	km	Median of distances between successive predicted positions during the last month of the fish, representing the average longitudinal distance traveled in a day, assuming an otolith daily growth rate of 5 µm.d <sup>-1</sup> .
Daily niche used	Ind.	-	Ratio between the daily and individual niche extents, reflecting the amount of estuarine gradient traveled daily compared to the total extent used during the last month by the fish.
Population niche extent	Pop.	km	Absolute difference between upper and lower limits of the 95% kernel distribution density of longitudinal positions of fish population, representing the longitudinal extent occupied by the fish population along the estuarine gradient.
<b><i>Muscle isotopy</i></b>			
Resource origin	Ind.	‰	Carbon isotopic ratio ( $\delta^{13}\text{C}$ ), traducing variations in signatures of food sources consumed by the fish, which basically correspond to various contributions of terrigenous organic matter to the estuarine food web.
Trophic level	Ind.	‰	Nitrogen isotopic ratio ( $\delta^{15}\text{N}$ ), traducing the trophic level of fish through enrichment process from prey to predator or changes in $\delta^{15}\text{N}$ values at the basal level of the estuarine food web.
Isotopic niche size	Pop.	-	2D Kernel isotopic niche size estimated at the 95% confidence level, based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the fish population. It reflects the amount of heterogeneity in food source and origin consumed by the population.

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662 **Figure 1:** Location of the six sampling sites within the estuarine nursery of the Sélune River, Western  
663 Europe, France. The distance to the sea ( $d_s$ , km) is specified for each site, and corresponds to the  
664 distance from the limit of the transitional waters following the main river channel.

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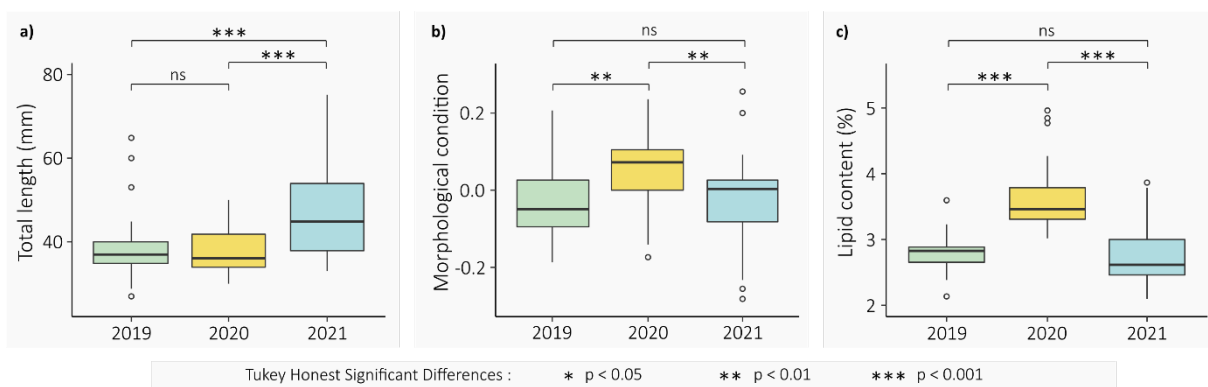
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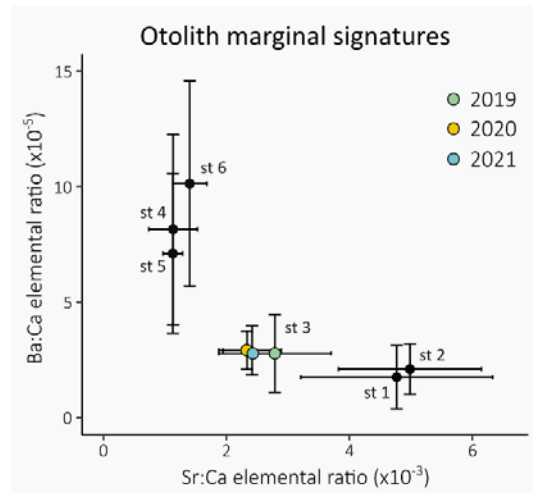


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672 **Figure 2:** Biological traits of juvenile flounders, *Platichthys flesus*, caught at site 3 of the Sélune estuarine  
673 nursery in September 2019 ( $n = 31$ ), 2020 ( $n = 31$ ) and 2021 ( $n = 34$ ). The boxplots show the a) total  
674 length (mm), b) morphological condition (no unit) and c) lipid content (%) whose estimation is based on  
675 the C:N ratio for each sampling year. Results of pairwise Tukey honest significant difference tests are  
676 provided.

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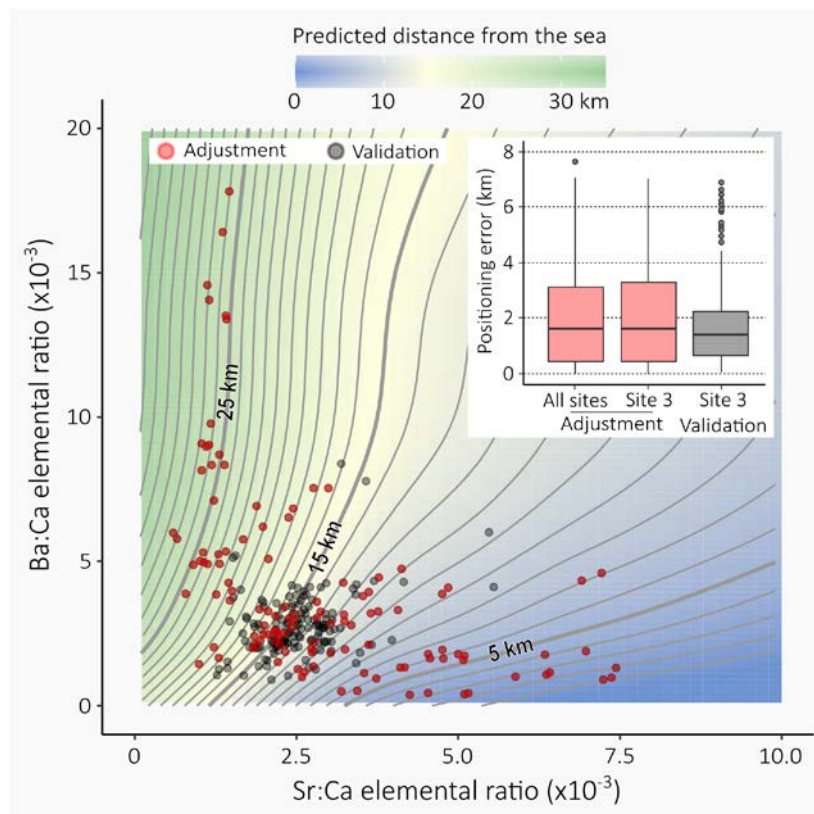


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680 **Figure 3:** Spatio-temporal changes in otolith marginal signatures of juvenile flounders, *Platichthys flesus*,  
 681 collected in the six sampling sites of the Sélune estuarine nursery. The bivariate plot displays the Sr:Ca  
 682 and Ba:Ca mean elemental ratios recorded from the otolith edge (10  $\mu\text{m}$ , around 2 days of life) according  
 683 to sampling sites (st 1-6) and years for the site 3. Vertical bars represent standard deviations.

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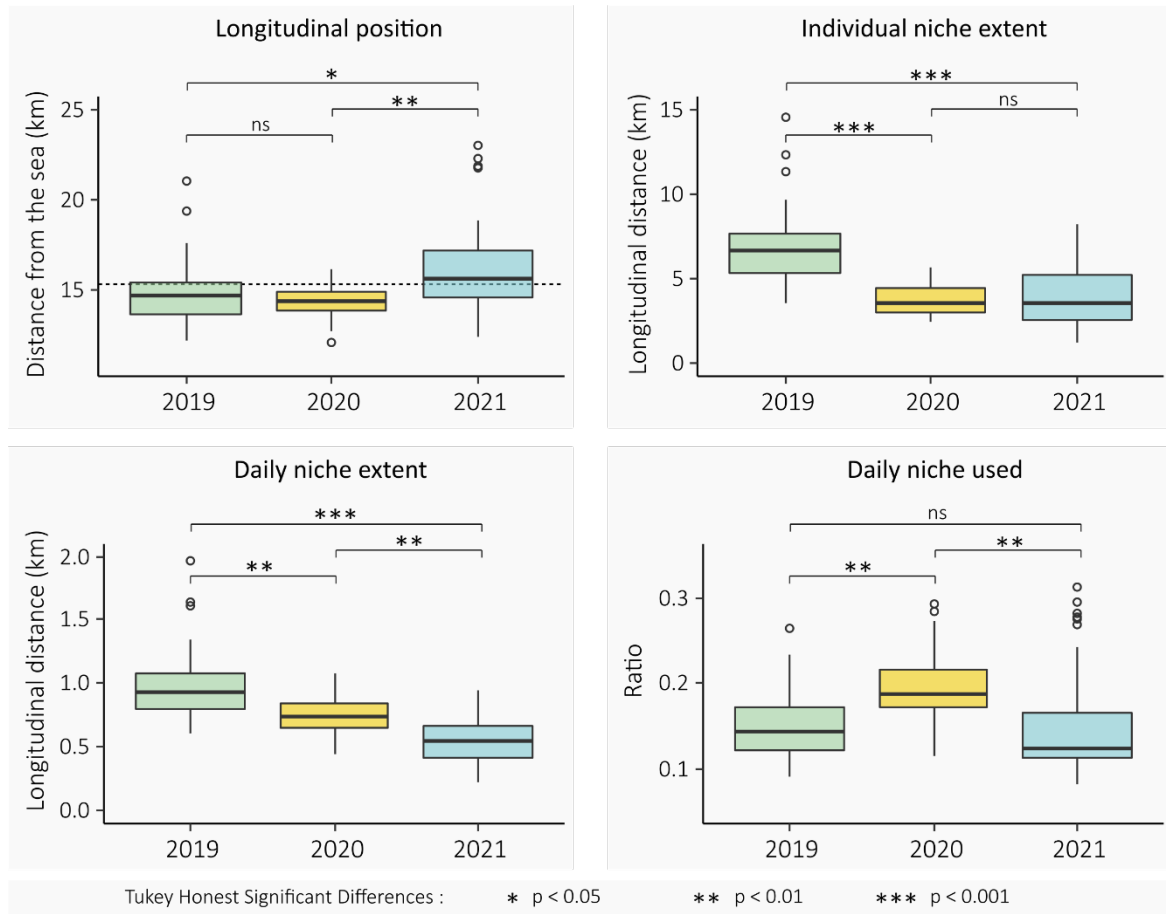


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687 **Figure 4:** Isolines of distances to the sea predicted from the GAM model using Sr:Ca and Ba:Ca elemental  
 688 ratios of juvenile flounder otoliths, caught at the six sampling sites of the Sélune estuarine nursery. Dots  
 689 represent the marginal elemental records (10  $\mu\text{m}$ , around 2 days of life) used to fit the model (red) and  
 690 those used for validation purpose for site 3 (grey). The boxplot in the upper box details the positioning  
 691 error (km) for the adjustment and validation subsamples.

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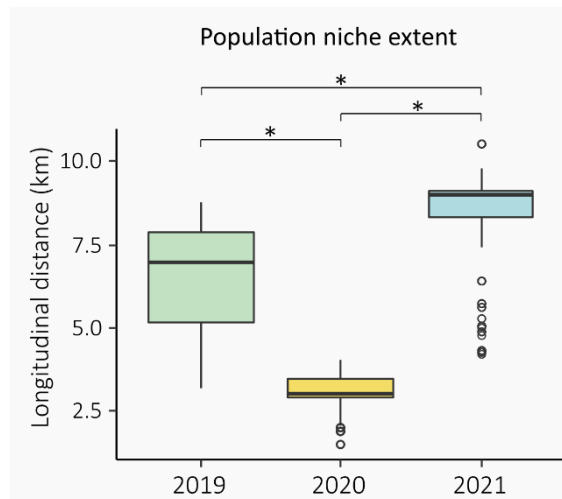
695 **Figure 5:** Inter-annual variations in longitudinal position, individual niche extent, daily niche extent and  
696 daily niche used by juvenile flounders, *Platichthys flesus*, caught at site 3 of Sélune estuarine nursery in  
697 2019 (n = 31), 2020 (n = 31) and 2021 (n = 34). See Table 1 for a detailed description of each metric. The  
698 dashed line represents the longitudinal position of site 3. Results of pairwise Tukey honest significant  
699 differences tests are provided.

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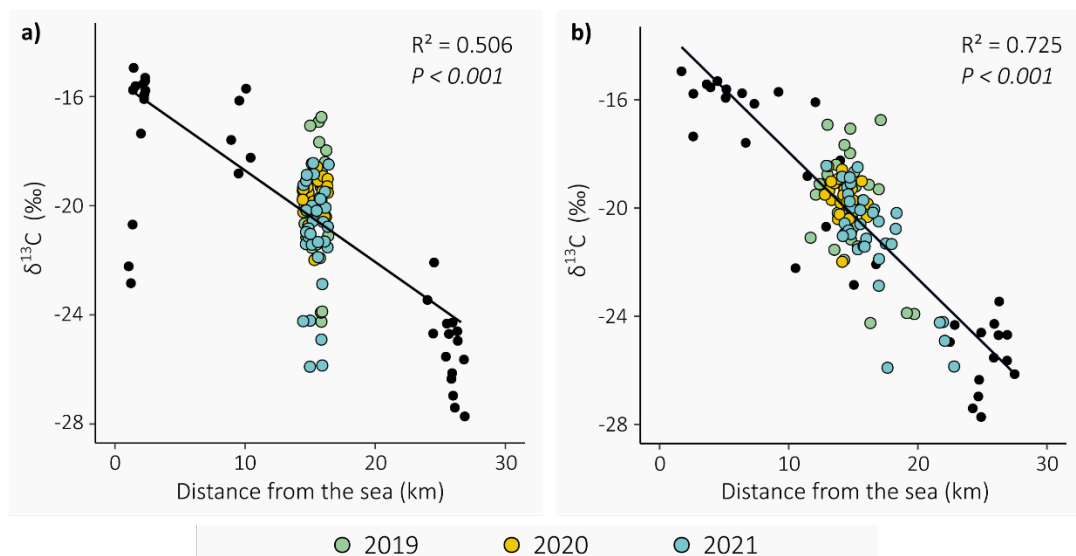
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704 **Figure 6:** Population niche extent (km) of juvenile flounders caught at site 3 of the Sélune estuarine  
 705 nursery in 2019 (n = 31), 2020 (n = 31) and 2021 (n = 34). See Table 1 for a detailed description of this  
 706 metric. Boxplots show the niche extent generated from 1000 bootstrap replicates. Niche extends for  
 707 each year were assumed to be statistically different from each other (\*) if the 95% confidence intervals  
 708 did not overlap.

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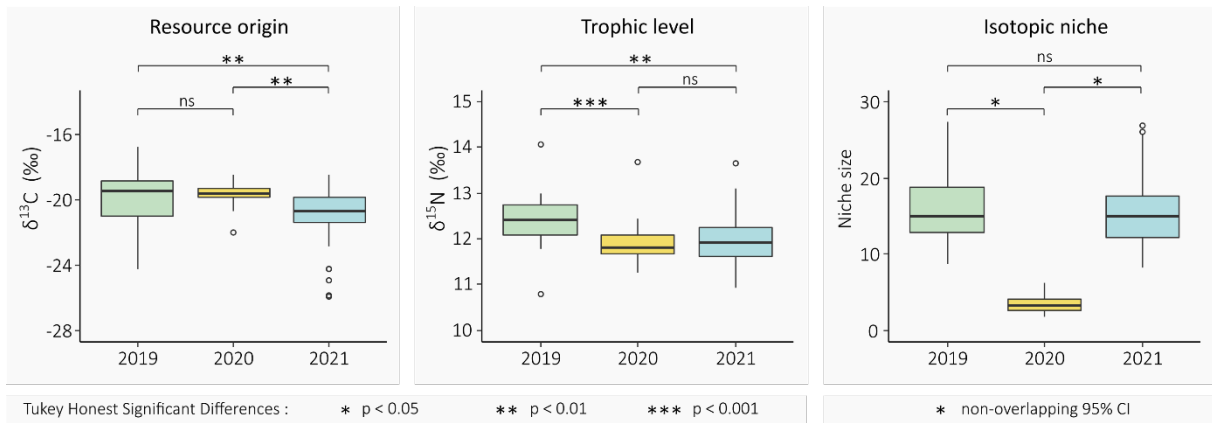
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713 **Figure 7:** Linear relationship between the  $\delta^{13}\text{C}$  signatures (‰) in muscle of juvenile flounders caught at  
 714 the six sampling sites of the Sélune estuary and distance to the sea a) from its catching location and b)  
 715 from its predicted location based on elemental composition of otoliths. Coloured dots represent the  
 716 fish caught at site 3 for the three sampling years, while black ones gather fish caught at the other sites.

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721 **Figure 8:** Inter-annual variations in resource origin ( $\delta^{13}\text{C}$ , ‰), trophic level ( $\delta^{15}\text{N}$ , ‰) and population  
 722 isotopic niche size of juvenile flounders caught at site 3 of the Sélune estuarine nursery in 2019 (n = 31),  
 723 2020 (n = 31) and 2021 (n = 34). See Table 1 for a detailed description of each metric. Results of pairwise  
 724 Tukey honest significant difference tests are provided for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures. Isotopic niche sizes,  
 725 which were generated from 1000 bootstrap replicates, and years were assumed to be statistically  
 726 different from each other if the 95% confidence intervals did not overlap.

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