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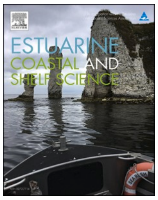

Hydro-morphological features and functional structure of fish assemblages mediate species isotopic niches in estuaries

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ABSTRACT

Despite the popularity of stable isotope analysis (Carbon and Nitrogen), the drivers of species isotopic niches and their consequences on food web functioning remained poorly described, especially in estuaries. Here, we hypothesised that species niche characteristics are influenced by ecosystem hydro-morphological features and the functional structure of fish assemblages. The trophic niches of four fish species (*Dicentrarchus labrax, Pomatoschistus minutus, Sprattus* and *Platichthys flesus*) and overlap between them were compared during two consecutive autumns in eight estuarine ecosystems of the northeast Atlantic Ocean. Our findings demonstrated a relative steadiness of trophic positions of fish in estuaries, despite a high level of interspecific variability. The seabass generally occupied the higher trophic position, followed by the sand goby, while the flounder and sprat were at the lowest position in the food web. Species isotopic niches and overlap were mediated by some estuarine features, including estuary size and intertidal extent, but also by biotic interactions at the intra- and inter-specific levels, as reflected by the influence of fish abundance and functional diversity metrics. Our results support the statement that the intertidal area is a pivotal factor in regulating trophic interactions, by promoting niche partitioning and diversification of trophic resources between species. Moreover, niche size and dietary divergence of species decreased with increasing regularity of trophic strategies within assemblages, suggesting that species occupied more restricted trophic niches when they were less subjected to competition for the same food resources. These findings emphasize the importance of local conditions and the functional composition of assemblages in modulating the trophic resources consumed by fish in estuarine environments.

1. Introduction

Estuaries and bays are complex ecosystems at the interface of marine and freshwater environments, associated with numerous ecological functions and ecosystem services (Barbier et al., 2011). Their environmental heterogeneity provides diverse ecological niches occupied by numerous fish species, including resident species but also many transient species, such as marine, freshwater, or diadromous fishes (Potter et al., 2015; Sheaves et al., 2015). As estuaries are productive ecosystems, juvenile fish, characterized by their sensitivity to environmental stress, generally find favorable growth conditions and shelters against large predators in shallow waters of estuarine nurseries (Beck et al., 2001). Juveniles fish concentrate in these areas from spring to early fall coinciding with seasonal peaks in benthic invertebrate preys (Saulnier et al., 2019). However, the spatio-temporal changes in resources supply, including food availability, can limit the secondary production and promote intra- and inter-specific competition for local resources (Le Pape and Bonhommeau, 2015; Saulnier et al., 2020). In

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particular, intertidal areas provide key feeding habitats for estuarine fish populations (Cattrijsse and Hampel, 2006; Nicolas et al., 2010a), as primary production and prey availability are higher, relative to other parts of the estuary (Hampel et al., 2003). Therefore, changes in habitat availability or productivity can affect the nursery value of estuaries, by altering competition level and diet strategy of species, with ultimate consequences on survival and stock recruitment (Vasconcelos et al., 2009).

Nitrogen (δ^{15}N) and carbon (δ^{13}C) stable isotope ratios are widely used to capture variations in the ecological structure and the organization of food webs within complex systems (Layman et al., 2012), as they provide complementarity insights on the species trophic niche (Bearhop et al., 2004). While the δ^{15}N mainly reflects the trophic position within the food chain (Peterson and Fry, 1987), the δ^{13}C informs on the origin of the carbon used by consumers, through variations in algal or detrital C sources at the bottom of the food chains (Kostecki et al., 2010, 2012). In estuaries, a gradual decrease in δ^{13}C is expected along the salinity gradient from sea to freshwater (Hobson, 1999; Reis-Santos et al., 2015; Teichert et al., 2022), whereas spatial changes in δ^{15}N ratio in coastal area are generally induced by anthropogenic activities (Herzka, 2005). Indeed, δ^{15}N in aquatic environment is strongly related to the intensity of N inputs related to agricultural land use (Anderson and Cabana, 2005). Accordingly, the niche metrics derived from the δ^{13}C–δ^{15}N space represent powerful indicators to describe intraspecific diet variability under different habitat conditions (Bearhop et al., 2004; Layman et al., 2007), by reflecting species trophic level but also their feeding habitat along the land-sea continuum. Basically, generalist exploiting variety of resources from different origins are expected to display a broad isotopic niche, while specialist, relying on a less diverse diet, should display a narrow niche (Van Valen, 1965; Bolnick et al., 2003; Svanbäck and Bolnick, 2005). In addition, overlap between species niches can provide insights on the consumers sharing similar trophic ecology, which provide an indirect assessment of the potential trophic competition for resources in the ecosystem (Bolnick, 2001; Bolnick et al., 2011). Such information appears especially relevant in estuarine nursery, where juveniles fish can be subjected to density-dependence processes and competition due to food limitation (Le Pape and Bonhommeau, 2015; Saulnier et al., 2020; Tableau et al., 2019). Despite the popularity of stable isotope analysis, the drivers of species isotopic niches and their consequences on food web functioning remained poorly described, especially in estuaries.

The optimal foraging theory predicts that the trophic niche size of a consumer increases with the level of competition and when preferred resources become scarce (MacArthur and Pianka, 1966; Svanbäck and Bolnick, 2007). Therefore, the isotopic niche size of consumers and overlap between them can vary with several environmental drivers, such as hydro-morphological estuarine features (Azevedo et al., 2022), seascape fragmentation (Layman et al., 2007b), changes in resource availability and diversity (Pool et al., 2017; Teichert et al., 2022), all contributing to the ecosystem productivity (Lesser et al., 2020). Beyond estuarine features, trait-based approaches can provide useful indications for assessing the level of biotic interactions and trait similarity in aquatic communities. In this context, species traits can be used to construct a multidimensional space to convert the species distributions and abundance into functional indices (Villéger et al., 2008). Then, the covariation between isotopic niche metrics and functional indices can be explored to determine the influence of the assemblage functional structure on species diet and niche segregation (Fitzgerald et al., 2017). In this context, increasing regularity and evenness in dietary strategies within assemblage is expected to promote niche partitioning and result in lower competitive interactions and narrower species isotopic niches. While previous studies investigated the relationship between functional diversity and isotopic diversity at the assemblage scale (e.g. Gajdzik et al., 2018; Włodarska-Kowalczuk et al., 2019), the influence of community traits on intraspecific isotopic variation remains largely unexplored.

Here, we investigated the influence of estuarine features and whole fish assemblage composition, including abundance and functional diversity, on the trophic niches of four fish species broadly distributed in estuaries of the northeast Atlantic Ocean. More particularly, species isotopic niches of seabass *Dicentrarchus labrax,* sand goby *Pomatoschistus minutus,* sprat *Sprattus* and flounder *Platichthys flesus* and overlap between them were compared during two consecutive autumns in eight estuarine ecosystems, which differed in term of environmental conditions and assemblage compositions. We hypothesised that species niche characteristics were influenced by hydro-morphological features and the functional structure of assemblages through variation in species traits and biotic interactions.

2. Material and methods

2.1. *Estuarine attributes*

Fish assemblages were investigated in eight estuaries distributed along the latitudinal gradient of the French coast (Fig. 1, three in the Bay of Biscay and five along the English Channel) which differed in terms of environmental attributes influencing estuarine fish community (Nicolas et al., 2010a; Teichert et al., 2018b). Three synthetic variables, the latitude, estuary area and intertidal extent, were collected to reflect habitat differences across estuaries (Table 1). The latitude (in degree), which was recorded at the mouth of the estuary, ranged from 45.9 to 50.2°N for the Charente and Somme estuaries respectively. It affects some regional biogeographic factors, such as productivity or temperature (Henriques et al., 2017a; Vasconcelos et al., 2015). The estuary area (in km^2), ranging from 1.88 for the Léguer estuary to 42.5 km^2 for the Mont St-Michel bay, is related to the diversity of habitat conditions available for aquatic organisms, and is commonly positively related to species richness and functional traits of estuarine assemblage (Henriques et al., 2017b; Nicolas et al., 2010b; Teichert et al., 2018b). Finally, the intertidal extent (in %) ranged from 26.2 to 95.7% for the Orne and Somme estuaries respectively. This variable is related to estuarine lateral connectivity and was defined as the proportion of the estuary occupied by the intertidal area, which is commonly used by juvenile and estuarine resident fish for foraging (Laffaille et al., 2000a; Teichert et al., 2018a).

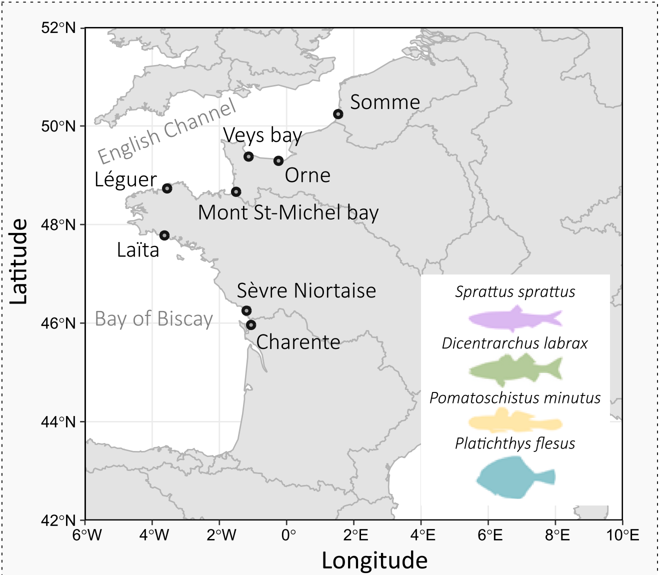

Fig. 1. Geographical distribution of the eight estuaries studied along the French coast (three in the Bay of Biscay: Laïta, Sèvre Niortaise and Charente, and five along the English Channel: Somme, Orne, Veys bay, Mont St-Michel bay and Léguer).

Table 1
Environmental attributes of the eight estuaries surveyed along the French coast. The number of beam trawl samples performed by survey (N samples) is provided for each year (N_{2020}/N_{2021}), as well as the number of individuals collected for the four target species (*Dicentrarchus labrax*, *Pomatoschistus minutus, Sprattus* and *Platichthys flesus*).

Estuary	Latitude	Estuary area (km^2)	Intertidal extent (%)	N samples	*P. minutus*	*D. labrax*	*P. flesus*	*S. sprattus*
Somme	50.2	40.8	95.7	24/24	9/-	31/32	-/32	28/32
Orne	49.3	5.58	26.2	14/14	35/-	29/31	14/32	10/-
Veys bay	49.4	31.5	39.5	13/13	33/32	34/30	9/-	-/-
Mont St-Michel bay	48.6	42.5	94.4	13/12	33/32	32/30	12/23	28/32
Léguer	48.7	1.88	73.4	12/12	33/-	20/10	-/-	-/-
Laïta	47.8	2.59	52.5	-/24	-/25	-/-	-/-	-/-
Sèvre Niortaise	46.3	47.8	79.8	17/18	32/31	32/17	12/-	-/32
Charente	45.9	23.0	55.0	-/25	-/32	-/-	-/12	-/9

2.2. Fish sampling

Fish data were collected through the monitoring program of the Water Framework Directive (WFD; 2000/60/EC) carried out in Autumn (2020); 2021, for respectively six and eight estuaries (Table 1). Fish samplings in the Laïta and Charente estuaries were not planned in 2020 by the French authorities in charge of the WFD, resulting in six surveys for this year. Fish abundances were estimated on the basis of beam trawl samples (1.5 m width, 0.5 m height and 16 mm stretched mesh size in codend) in application of the French standardized protocol (AFNOR, 2011; Delpech et al., 2010), which ensured the availability of homogeneous datasets. Nevertheless, the use of beam trawl can influence estimates of species proportion depending on their position in the water column, especially due to a greater efficiency in capturing benthic species. This can constitute a bias in abundance estimates within surveys, but it was assumed to be constant over surveys. For each survey (one estuary, one season), the whole upstream-downstream gradient of estuaries was sampled (range from 12 to 25 samples per survey, Table 1) to cover all salinity zones of the estuaries (oligohaline: ≤ 5, mesohaline: $5<$ and ≥ 18, and polyhaline: >18, when occurring tidal freshwater and oligohaline zones are merged as well as polyhaline and euhaline zones). For each sample, the fishing gear was towed between 10 and 15 min at a speed of 1.5–3 knots. The trawled area was estimated by multiplying the distance crossed by the width of beam trawl and used as proxy of fishing effort. Abundances of all fish species were then expressed in number of individuals per 100 m^2 trawled. Finally, the fish abundances at the survey scale were estimated by averaging abundances of samples.

During surveys, some specimens of seabass (*Dicentrarchus labrax*), sand goby (*Pomatoschistus minutus*), sprat (*Sprattus*) and flounder (*Platichthys flesus*) were collected for additional tissue sampling to describe their isotopic niche and overlap. These species were selected because they are broadly distributed across the studied estuaries, and they occupy different ecological niches and positions in the food web (Selleslagh and Amara, 2015). While *P. minutus* can be considered as an estuarine resident species mainly feeding on benthic invertebrates and meso-zooplankton, such as polychaetes, molluscs and amphipods (Leitão et al., 2006), the three others species use the estuary as a nursery area, and were therefore observed at their juvenile stage, i.e. young-of-the-year (Potter et al., 2015). At this stage, the seabass is a demersal species with an opportunistic feeding behaviour, including amphipods, mysids or polychaetes, and some little fish (Laffaille et al., 2001). Flounder is a benthic flatfish with a diversified diet, including small polychaetes and oligochaetes (Mendes et al., 2014), but the young-of-the-year tend to feed on planktonic crustacean like copepods (Thiel et al., 1996) but also preys such as amphipods of the genus *Corophium* (e.g. Mendes et al., 2020). Finally, sprat juveniles form pelagic schools in estuary and essentially feed on mesozooplankton, such as large copepodites and estuarine copepods (Maes and Ollevier, 2002).

Although the initial objective was to collect 35 specimens per species during each survey, density variations between estuaries and years were observed (Table 1). Accordingly, we selected species for which a minimum of 9 individuals were sampled per survey to limit the influence of sample size on the estimation of isotopic niches. From 9 to 35 individuals were thus randomly collected during 12 surveys for the seabass, 11 surveys for the sand goby, 8 surveys for the flounder and 7 surveys for the sprat (Table 1). Although the co-occurrence of target species was variable depending on the estuaries, it remained representative of the composition of local fish assemblages. All sampled fish were young-of-the-year (except *P. minutus*), of similar size within species (Supplementary material, Table S1), which limit potential bias induced by changes in isotopic signatures due to ontogenetic shifts in the diet (Pasquaud et al., 2008). Overall, the size classes of the four species were representative of sizes observed in estuaries during Autumn, while most juveniles of marine species grew for several months in the nursery. Although some larvae of the sand goby can still be found at this period, the mesh size of beam trawl (16 mm) was not suitable to sample them. Focusing on juveniles (and adults of goby) allowed to provide insights on isotopic niches of the four species for their most representative stages in estuaries during Autumn.

All collected fish were anesthetized (15 $mg.L^{-1}$) before being euthanized with an overdosed solution (200 $mg.L^{-1}$) of Benzocaine (AQUACEN Benzocaine), and preserved in absolute ethanol (VWR, AnalaR NORMAPUR® ACS $\geq$99.8 %). This research was conducted in accordance with all applicable international, national, and institutional guidelines of the French National Museum of Natural History for the care and use of animals.

2.3. Functional diversity indices

The diversity and structure of biotic interactions in the whole fish assemblages were assessed using functional diversity indices, incorporating information on species traits to determine their proximity in a multidimensional functional space (Villéger et al., 2008). Here, species functional positions were described from three complementary traits, position in the water column, diet and trophic level, influencing species habitat preference and their position in the food web. Fish attributes were retrieved from published studies (Teichert et al., 2018b, 2018c) and information available in FishBase (Froese and Pauly, 2000) for the most common life stage encountered in estuaries (Potter et al., 2015). Position in the water column influences species interactions and the set of available preys, as well as the bentho-pelagic energy flow (Vander Zanden et al., 1999). It was expressed in three categories: pelagic, demersal, and benthic. Diet drives the trophic interactions and affects the species repartition and habitat used depending on the availability of local resources (Seitz et al., 2014). Species were assigned in six categories according to the dominant food item in their diet (piscivorous, omnivorous, planktivorous, herbivorous, benthic invertebrate feeder and supra-benthic invertebrate feeder) (Teichert et al., 2018c). Finally, the trophic level reflects the position of species in the food web and was expressed as a continuous variable, as estimated in FishBase using the TROPH routine (Pauly et al., 2000).

The pairwise functional distances between species were computed using the Gower distance and used to build a multidimensional functional space by applying a Principal Co-ordinate analysis (PcoA, Villéger et al., 2008), using the R statistical software, v. 4.0.5 (R Core Team, 2018). For each survey, six functional diversity indices were calculated using the package 'mFD' (Magneville et al., 2022) by selecting the first three PCoA dimensions, which provided a satisfactory representation of species trait variability (Maire et al., 2015). While the functional richness (FRic) reflecting the volume of the functional space filled by species is supported by occurrence data, the other indices are abundance-based metrics that reflect evenness and distribution of species within the functional space: functional dispersion (FDis), functional divergence (FDiv), functional evenness (FEve), functional originality (FOri) and functional specialization (FSpe). For detailed explanations of diversity indices and their relevance see Magneville et al. (2022), Mouillot et al. (2013) and Villéger et al. (2008).

2.4. Stable isotope composition

For each collected fish, one muscle tissue sample was dissected to quantify nitrogen and carbon isotopic signatures and their elemental content (C and N). Isotopic signatures were expressed in the delta unit notation as deviation from international standards of PeeDee Belemnite for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$, following the formula: $\delta X = ((Rsample/Rstandard)-1) \times 1000)$, where X is ^{13}C or ^{15}N and R is the ratio (^{15}N:^{14}N or ^{13}C:^{12}C) in the sample and in the standard. For each muscle sample, nitrogen and carbon total quantities, and the isotopic ratios were measured by continuous flow isotope mass spectrometry (CF-IRMS) using a Thermo Scientific Delta V Advantage mass spectrometer coupled to a Thermo Scientific Flash 2000 elemental analyser. Analytical precision (standard deviation) was <0.15‰ of reference material. The $\delta^{13}C$ values were corrected for lipid content (C:N ratio) according to Post et al. (2007) because the ratio of carbon relative to nitrogen in some muscle samples exceeded the recommended threshold for correction (3.5).

2.5. Species isotopic niches

For each survey, the isotopic niches of species were described for lipid-corrected values based on eight metrics reflecting different aspects of trophic diversity (Layman et al., 2007a), using the packages 'SIBER' (Jackson et al., 2011) and 'rKIN' (Eckrich et al., 2020). The $\delta^{15}N$ range (NR) and the $\delta^{13}C$ range (NC) quantify respectively the trophic length and the diversity of basal resources of the population. The mean distance to centroid (CD) provides information on niche width but also on individual distribution within the $\delta^{13}C$ - $\delta^{15}N$ space. The mean nearest neighbour distance (MNND) measures the density and clustering of individuals within the isotopic space, with lower values indicating less divergence. The standard deviation of the nearest neighbour distance (SDNND) also gives information on the evenness of dietary divergence. Finally, the total area (TA), the corrected standard ellipse area (SEAc), and the kernel utilization density (KUD) provide estimate of dietary niche size. While TA can be sensitive to sample size because it was estimated by the convex hull encompassing the data points in the isotopic space, the SEAc corresponds to the 95% prediction ellipse interval and integrates a correction for sample size bias. The KUD is the 2D kernel density estimation of niche size at 95% confidence level, which is less sensitive to extreme values and provides a more realistic representation of niche when data are irregularly distributed (Eckrich et al., 2020). Although this metric does not include correction for sample size, it produced estimates of niche size like the SEAc ones (Pearson correlation, $n = 38$, $r = 0.97$, $P < 0.001$).

2.6. Trophic competition pressure indices

Isotopic niche proximity was used to assess the potential for competition between individuals or species, as competition per se, requiring information on limitation effect of food supply for fish assemblages could not be evaluated (Tableau et al., 2019).

The potential intraspecific competition was estimated for each survey using the Intraspecific Competition Pressure (ITP) index, proposed by Andrades et al. (2021). It was calculated by dividing the transformed (log+1) fish abundance (ind/100 m^2) of a species by its isotopic niche size (Fig. 2). Mechanically, highest ITP values are retrieved for abundant species with restricted dietary niche, whereas lowest ITP values are for non-abundant species with wide dietary niche (use of diverse resources). Although the SEAc was initially proposed as a measure of niche size for this index (Andrades et al., 2021), the KUD estimates was selected because it is a reliable assessment of niche sizes when data are not normally distributed. Moreover, calculated ITP values remained highly correlated whatever the niche size estimator, SEAc *versus* KUD (Pearson correlation, $n = 38$, $r = 0.99$, $P < 0.001$).

The potential interspecific competition was estimated by the niche overlap between species (e.g. Pelage et al., 2022). For each species, considered as receiver, the proportion of niche overlapped by others co-occurring species, considered as competitors, was calculated (Fig. 2). While the pairwise overlaps (Pov) reflect how much each competitor encroach on the niche of the receiver species, the multispecies overlap (Mov) quantify the competitive pressure induced by all co-occurring species. Here too, the 95% kernel estimate was selected because it leads to more accurate assessment of niche overlap (Eckrich et al., 2020). In complement, the distance to competitor centroids (CCD) was calculated. It was defined as the mean of Euclidean distances between centroid of receiver and those of their competitors. The CCD thus measures the dietary niche proximity of receiver with their potential competitors in the $\delta^{13}C$ - $\delta^{15}N$ space. Although all potential competitors of these estuarine assemblages can not be included in the analysis, the four species selected were assumed to represent an overview of the competition pressure, as they are representative of different ecological niches and their isotopic niches cover a large part of the dietary space of estuaries.

2.7. Drivers of isotopic niches and overlap

Linear mixed models (LMMs) were used to determine the drivers of species niches and trophic competition in estuaries. The four species were considered conjointly in the LMMs to increase the statistical power and to highlight common trends among species that use estuaries. Indeed, the number of replicas ranged from 7 to 12 depending on species, which prevented to carry out independent analyses for each species. LMMs were independently adjusted for the eight isotopic niche metrics (CR, NR, KUD, SEAc, TA, CD, MNND and SDNND) and the three competitions metrics (ITP, Mov and CCD) as response variables. The estuarine attributes (latitude, estuary area, and intertidal extent), the taxonomic descriptors (total fish abundance, receiver abundance, and fish species richness), and the functional diversity indices (FRic, FEve, FDiv, FDis, FSpe, and FOri) were used as explanatory variables. However, FDis was excluded form explanatory variables because of its strong positive correlation with estuary size ($r = 0.78$) and negative correlation with FDiv ($r = -0.73$). The correlation of others variables remained below the critical threshold of $|r| < 0.7$, which prevents collinearity problem in parameter estimations (Dormann et al., 2013). Models were adjusted using an identity link function and a Gaussian error distribution, using the "lme4" package (Bates et al., 2015). The estuary size and abundance variables were (log+1)-transformed to meet normality assumptions. Mean response and explanatory variables were centered to 0 and variance standardized to 1 to obtain standardized regression coefficients, which were interpreted as effect sizes (Ben-Shachar et al., 2020). Species and surveys were included as random effects in the LMMs to account for the influence of interspecific variability in niche parameters, as well as potential impact of basal sources variation among surveys. Accordingly, the effect sizes reveal consensual trends among species at the assemblage scale when other sources of variability are controlled by random intercepts. For each LLM, a backward elimination

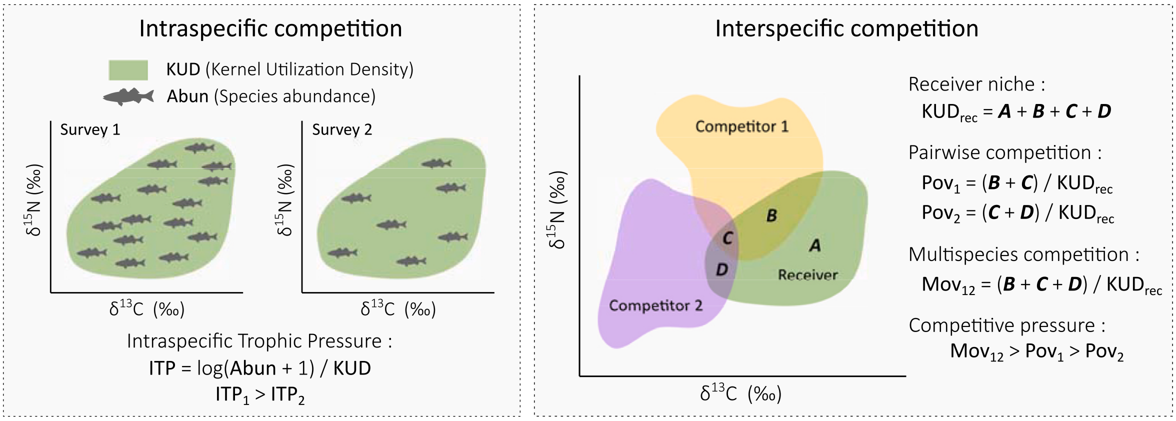

Fig. 2. Illustrative representations of the methodological approaches applied to assess the potential competition within and among species based on isotopic niche analysis. The intraspecific competition was assessed using the Intraspecific Competition Pressure (ITP) index, proposed by Andrades et al. (2021), whereas the interspecific competition was quantified by the proportion of a species niche (receiver) overlapped by others co-occurring species (competitors).

procedure based on deviance reduction tests (*F*-tests) was conducted to identify the explanatory variables that significantly affected the response variables (species niches and trophic competition metrics), using the package ‘lmerTest’ (Kuznetsova et al., 2017). Finally, the proportion of deviance explained by the LMMs was assessed using the marginal R^2m (variance explained by the fixed effects) and the conditional R^2c (variance explained by both the fixed and random effects; Nakagawa et al., 2017).

3. Results

3.1. Functional structure of fish assemblages

A total of 60 species were recorded from the 235 samples (93 in 2020 and 142 in 2021) collected during the 14 surveys (Table 1). Mean fish abundance in surveys was 10.6 ± 8.4 fish per 100 m^2, with on average 40.9 ± 27.5% of the captures within surveys being composed of the four target species. Among them, the sand goby was, on average, the most abundant in surveys (1.9 ± 2.7 fish.100 m^{-2}), followed by the seabass (0.9 ± 0.7 fish.100 m^{-2}), the sprat (0.2 ± 0.2 fish.100 m^{-2}), and the flounder (0.1 ± 0.2 fish.100 m^{-2}). The four species were representative of different ecological niches depending on their trophic and water column positions, as illustrated by their locations in the functional space defined by the PcoA (Fig. 3A). In surveys, the species richness ranged from 11 to 23 species, and was positively associated with FRic (Fig. 3B). Overall, the PCA conducted on functional diversity indices demonstrated their complementarity in providing information on the structure of fish assemblages, as well as on the high level of heterogeneity between surveys (Fig. 3B). For example, lowest FDis values were reported for the Charente and Laïta estuaries where FDiv was maximal, while highest FEve values were observed in the Veys bay and Léguer estuary (diversity values are detailed for each survey in Supplementary material, Table S2).

3.2. Isotopic niches and overlap

The four species displayed a wide range of carbon and nitrogen isotope composition depending on the estuary and sampling years, which suggests fluctuation in basal resources among surveys (Fig. 4). Nevertheless, the $\delta^{15}N$ mean values observed for the seabass (16.76 ± 1.78 ‰) and sand goby (15.69 ± 1.56 ‰) were overall higher than the ones for the flounder (14.56 ± 0.95 ‰) and sprat (13.81 ± 0.76 ‰; Kruskal-Wallis test, n = 38, $P < 0.001$). In contrast, the mean $\delta^{13}C$ values were highly variable and did not differ between the four species (Kruskal-Wallis test, n = 38, P = 0.414), reflecting the heterogeneity in carbon sources used by fish along the land-sea continuum. Overall, the four species displayed comparable niche features, as indicated by the lack of significant differences between species in the eight isotope niche

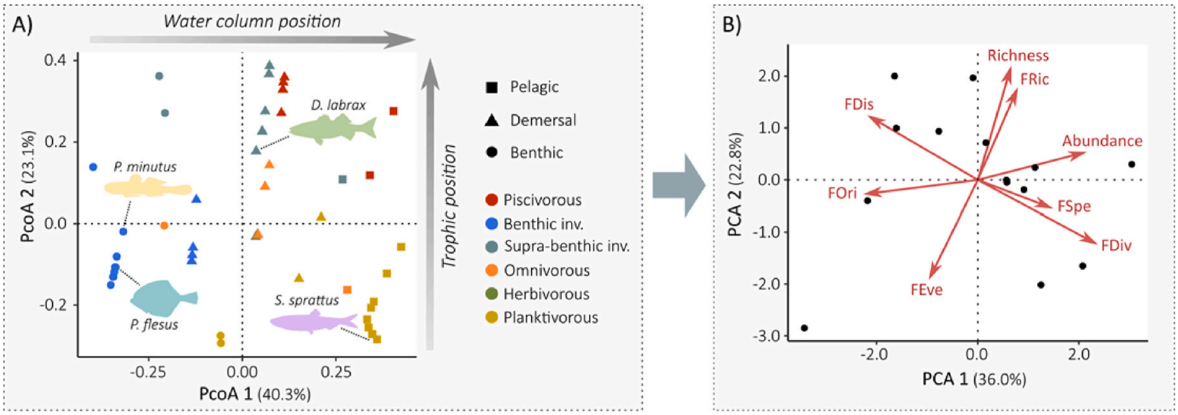

Fig. 3. Overview of the functional structure of fish assemblages in estuaries. A) Functional space defined by species traits (position in the water column, diet and trophic level) along the two first dimensions of the Principal Co-ordinate analysis (PcoA). The position of the four target species (*Dicentrarchus labrax*, *Pomatoschistus minutus, Sprattus* and *Platichthys flesus*) is provided as illustrative purpose. B) Principal component analysis illustrating the relationships between the functional diversity indices (red arrows) of the fish assemblages surveyed in estuaries (black dots). FDis: functional dispersion, FEve: functional evenness, FRic: functional richness, FDiv: functional divergence, FOri: functional originality, FSpe: functional specialization. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

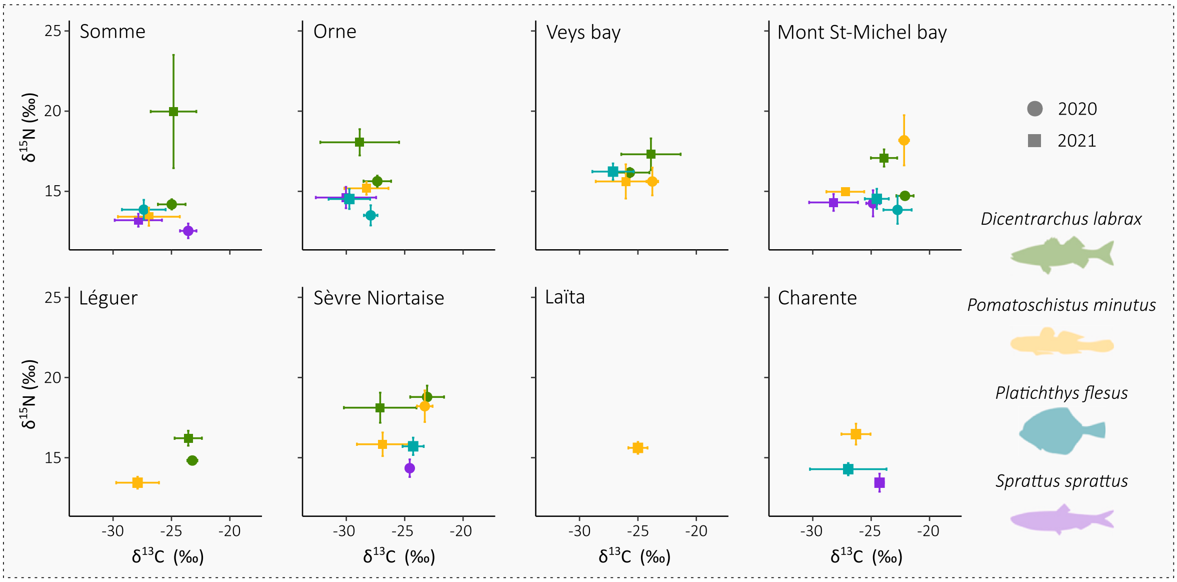

Fig. 4. Isotope signatures of the four target species (*Dicentrarchus labrax*, *Pomatoschistus minutus, Sprattus* and *Platichthys flesus*) within the δ^{13}C - δ^{15}N space (mean ± SD) for the eight estuaries sampled in Autumn (2020); 2021.

metrics (Table 2). While the δ^{15}N ranges (NR) remained lower than 3 ‰ on average, the δ^{13}C ranges (CR) extended from 4.66 to 6.63 ‰, which highlights its dominant effect on niche size variability. Although not significant, niche size (TA, KUD and SEAc) tended to be wider for the seabass and sand goby than for the flounder and sprat, but displayed high level of intraspecific variability as revealed by the high standard deviation values (Table 2). Metrics reflecting isotopic divergence (CD, NND and SDNND) remained almost comparable between species.

On average, the potential level of intraspecific competition was higher in the sand goby (ITP = 0.09 ± 0.1) and seabass (0.08 ± 0.12), than in the flounder (0.03 ± 0.05) and sprat (0.03 ± 0.03; Kruskal-Wallis test, n = 38, P = 0.047). Overall, the multispecies overlap values were lower for the seabass (Mov = 0.36 ± 0.36) than the sprat (0.53 ± 0.31), sand goby (0.62 ± 0.37) and flounder (0.65 ± 0.28; Kruskal-Wallis test, n = 38, P = 0.023), but remained highly variable between surveys (Fig. 5). Similarly, the distance to competitor centroids were longer for the seabass (CCD = 3.08 ± 1.41) and sprat (3.36 ± 0.76) than for the flounder (2.62 ± 0.62) and sand goby (2.52 ± 1.23), which underlined their proximity with potential competitors in the isotopic space. The pairwise overlaps between species isotopic niches revealed that the seabass was essentially in trophic competition with the sand goby, while the sprat niche was mainly overlapped by the flounder and sand goby ones, but to a lesser extent. In contrast, the flounder and sand goby appeared under the competitive pressure of the three other species (Fig. 5), because of their central position within the isotopic space (see Fig. 6).

Table 2
Summary of the isotopic niche metrics of the four target species (*Dicentrarchus labrax*, *Pomatoschistus minutus, Sprattus* and *Platichthys flesus*) averaged (±SD) between surveys and estuaries. The significance of Kruskal-Wallis tests investigating differences between species is provided. NR: δ^{15}N range (‰), CR: δ^{13}C range (‰), TA: total area (‰^{2}), KUD: kernel utilization density (‰^{2}), SEAc: corrected standard ellipse area (‰^{2}), CD: mean distance to centroid, MNND: mean nearest neighbour distance, SDNND: standard deviation of the nearest neighbour distance.

Niche metric	*D. labrax*	*P. flesus*	*P. minutus*	*S. sprattus*	P-value
NR (‰)	2.81 (±2.60)	2.20 (±0.47)	2.99 (±1.56)	2.25 (±0.61)	0.778
CR (‰)	6.63 (±3.39)	5.72 (±2.59)	6.26 (±3.08)	4.66 (±3.38)	0.597
TA (‰^{2})	14.66 (±17.73)	7.31 (±3.76)	10.31 (±7.22)	5.50 (±4.08)	0.407
KUD (‰^{2})	22.84 (±22.93)	13.18 (±4.50)	16.09 (±13.80)	11.59 (±8.90)	0.827
SEAc (‰^{2})	4.56 (±5.56)	2.82 (±1.17)	3.03 (±2.38)	2.00 (±1.38)	0.595
CD	1.57 (±0.96)	1.32 (±0.58)	1.41 (±0.52)	1.20 (±0.70)	0.998
MNND	0.39 (±0.17)	0.55 (±0.22)	0.43 (±0.24)	0.41 (±0.25)	0.305
SDNND	0.33 (±0.11)	0.45 (±0.22)	0.43 (±0.19)	0.24 (±0.10)	0.111

3.3. *Drivers of isotopic niches and overlap*

The total proportion of deviance explained by the LMMs varied according to the isotopic niche metrics and trophic competition indices (R^2c = [0.12–0.49], R^2m = [0.00–0.35]). One to four explanatory variables were kept during the backward elimination procedure depending on the response variables, except for MNND for which all fixed effects remained non-significant (details on model adjustments and selection of variables are provided in Supplementary material, Table S3). Species isotopic niches were significantly influenced by some estuarine attributes, as well as by the taxonomic and functional structure of fish assemblages (Fig. 5). Niche size metrics (KUD, SEAc and TA) were negatively related to the proportion of intertidal areas and functional indices related to regularity (FEve) and importance of extreme traits (FDiv). Niche divergence metrics, such as CD, and MNND, were negatively associated with FDiv, but positively influenced by the total fish abundance in estuary. Similarly, CR reflecting the diversity of origin resources tended to increase with fish abundance. Latitude, estuary area, species richness, Fric and FOri did not influence species isotopic niches, while SEAc was positively associated with FSpe.

Overall, the competition metrics were more related to the estuarine attributes and fish density than to the functional diversity of assemblages (Fig. 5). The intensity of potential intraspecific competition (ITP) was only related to the abundance of conspecific species, while the multispecies overlap (Mov) increased with the total fish abundance and tended to decrease when the intraspecific density rose. The estuary area was positively associated with Mov and negatively with CCD, which indicates a greater dietary proximity of competitors in large estuaries. In contrast, CCD increased with the availability of intertidal areas.

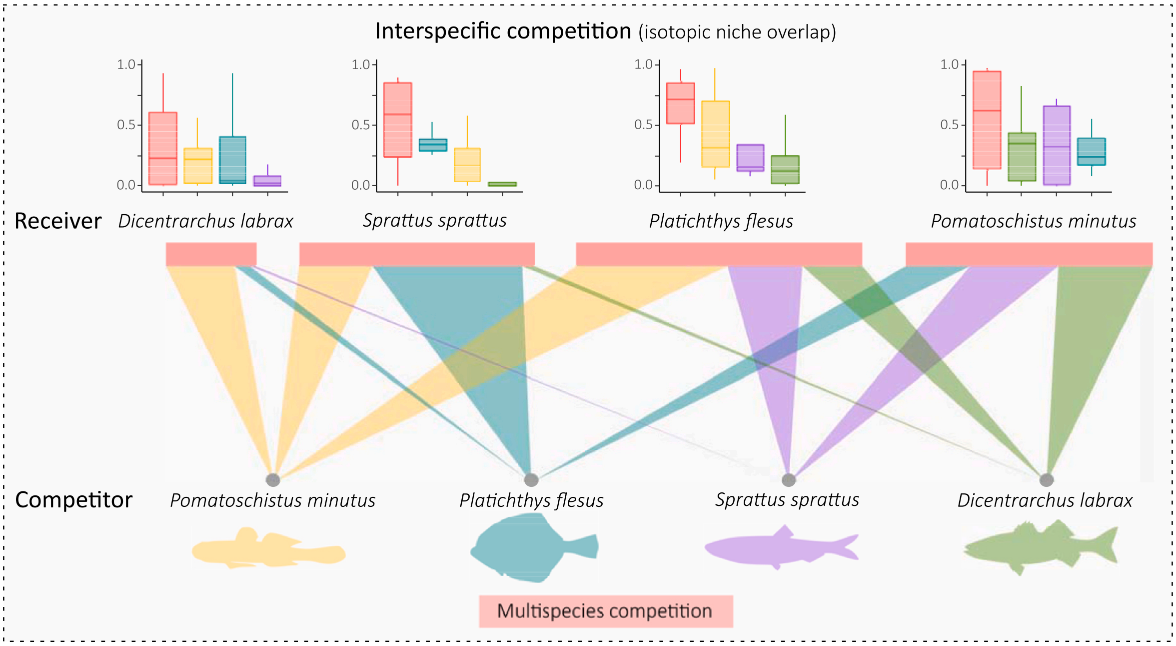

Fig. 5. Potential interspecific competition assessed by isotopic niche overlaps of the four target species (*Dicentrarchus labrax*, *Pomatoschistus minutus, Sprattus* and *Platichthys flesus*) within the surveys conducted in the eight estuaries. Bottom of the figure, the bipartite network illustrates the median proportion of the receiver niche overlapped by each competitor (pairwise overlaps, Pov), while the upper red box width is proportional to the median of multispecies overlap (Mov). Boxplots detail the variability of Pov and Mov in the surveys for each receiver. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

Despite the high variability in isotopic composition, the hierarchy in trophic positioning was overall consistent across years and estuarine systems. The seabass generally occupied the higher trophic position, followed by the sand goby, while the flounder and sprat were at a lower position in the food web. Such distribution within the isotopic space is in accordance with previous studies investigating the fish trophic structure in estuaries (e.g. Bouaziz et al., 2021; Pasquaud et al., 2010, 2008), and suggests a relative steadiness in species trophic positioning among estuaries. Fish in estuaries generally display dietary specialization, so that their diet do not merely reflect the prey diversity and availability of their environment (Selleslagh and Amara, 2015). This is also reported in marine fish assemblages of the English Channel (Cachera et al., 2017). Moreover, when species rely on the same food resources, spatial segregation can limit the potential interspecific competition, as observed between *P. flesus* and *P. microps* in the Canche estuary (Selleslagh and Amara, 2015). However, our results showed high variability in δ^{15}N and δ^{13}C values depending on sites and years. As dynamic ecosystems, estuaries are subjected to heavy environmental fluctuations, altering the abundance and availability of organic matter, which is the basal sources of food chains (Pasquaud et al., 2008; Selleslagh et al., 2015). Accordingly, hydro-morphological features such as marine connectivity, extent of intertidal mudflats, or changes in river flow can modify the relative contribution of marine, estuarine and terrestrial inputs (Bouaziz et al., 2021; Kostecki et al., 2010; Le Pape et al., 2013), with substantial repercussions on isotopic signatures along the food chain (Vinagre et al., 2011).

While a smaller niche was expected for specialist species (Van Valen, 1965; Bolnick et al., 2003; Svanbäck and Bolnick, 2005), such as for a zooplankton feeder like the sprat, our results revealed that variability within species were overall larger than between species, which remained non-significant. Niche size metrics (TA, KUD and SEAc) are function of both the origin of basal resources (CR) and trophic level variation (CN), but variability in δ^{13}C appeared to be determinant for the niche size variability. Similarly, the dietary divergence metrics (CD, MNND and SDNND) were similar between the four species but highly variable between surveys. This high intraspecific variability in niche parameters suggests an important contrast in mobility and habitat connectivity for fish between estuaries (Green et al., 2012; Selleslagh et al., 2015). Within an estuarine assemblage, interspecific differences in δ^{15}N values mainly reflect segregation of trophic position (Layman et al., 2007a), while changes in δ^{13}C values are related to spatial segregation across the land-sea continuum (Green et al., 2012; Teichert et al., 2022). Accordingly, isotopic niche overlap between species integrates both spatial and dietary components involved in trophic competitive interactions. Nevertheless, this measure may only reflect a propensity to trophic competition based on niche proximity, as it does not incorporate information on food limitation. Overall, seabass and sprat isotopic niches were more segregated than the ones of the sand goby and flounder, as indicated by the larger Mov and longer distance in CDD values. This result agrees with the specialist feeding strategy of these two species as demonstrated in stomach content analyses in the Gironde (Pasquaud et al., 2010) and Canche estuaries (Selleslagh and Amara, 2015). Among the four species investigated, the sand goby was the main potential trophic competitor of the seabass, while the sprat niche was principally overlapped by the flounder's one. In contrast, the trophic competitive pressure exerted on sand goby and flounder was more evenly distributed between competitors, resulting in higher Mov values. The central position of these species in the trophic chain probably makes them more sensitive to niche overlap with potential competitors. Although only four species were considered, these results suggest that species positioning in the food web strongly influences its vulnerability to trophic pressure, with species occupying marginal positions in the food web being less sensitive to the potential interspecific competition.

The high plasticity in the structure and size of fish isotopic niches suggests an important implication of the local context, especially in response to changes in availability and accessibility of trophic resources (MacArthur and Pianka, 1966). Even though the sample size of this study was not sufficient to describe independently the responses of each species, the outputs of the LMMs provided a consensual overview of the influence of estuarine parameters and biotic interactions on the isotopic

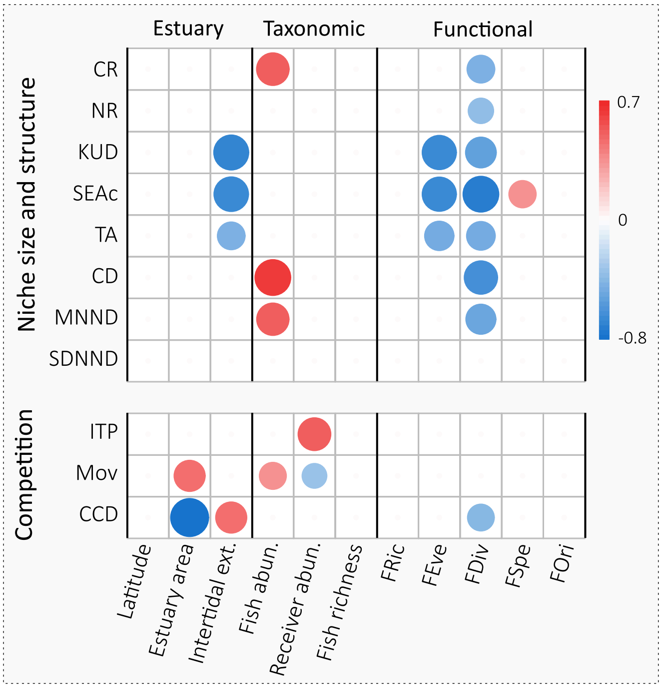

Fig. 6. Overview of the influence of estuarine attributes, taxonomic descriptors and functional diversity indices on the eight isotopic niche metrics and the three competition metrics derived from isotopic signatures of the four target species (*Dicentrarchus labrax, Pomatoschistus minutus, Sprattus* and *Platichthys flesus*). The color gradient reflects the standardized regression coefficients (effect sizes) estimated from the LMMs for the explanatory variables kept by the backward elimination procedure (details on variable selection are provided in Appendix C). NR: δ^{15}N range (‰), CR: δ^{13}C range (‰), TA: total area (‰^{2}), KUD: kernel utilization density (‰^{2}), SEAc: corrected standard ellipse area (‰^{2}), CD: mean distance to centroid, MNND: mean nearest neighbour distance, SDNND: standard deviation of the nearest neighbour distance, ITP: intraspecific competition pressure, Mov: multispecies overlap, CCD: distance to competitor centroids. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

niches occupied by fish in the estuarine ecosystem. Species niche parameters were not influenced by the latitude and total estuary area. Indeed, the geographical distribution of the estuaries sampled in this study (between 45.9° and 50.2° N), is likely too restricted to highlight a latitude effect, which is mostly perceptible at a larger scale (Henriques et al., 2017b). For example, Comte et al. (2016) demonstrated that niche segregation in freshwater fish assemblages depends on latitude, through productivity and temperature gradients. The lack of effect of the estuary size was more unsuspected because environmental heterogeneity, and therefore niche availability, commonly increases with estuary size and other correlated variables, such as annual discharge or entrance width (Henriques et al., 2017a). This environmental heterogeneity provides favorable conditions to a larger spectrum of species (Nicolas et al., 2010a; Vasconcelos et al., 2015), as well as the addition of functionally differentiated fish (Henriques et al., 2017b; Teichert et al., 2018b). Although one would have expected an increase in niche sizes with the diversity of prey available in larger estuaries (e.g. Van Valen, 1965), the steadiness of niche parameters suggests that fish species tended to select specific trophic resources, which thus promotes niche partitioning. Furthermore, the potential interspecific competition increased with the estuary size, as revealed by the positive relationship with multispecies overlap (Mov), as well as the reduction of isotopic distances to competitors (Dov) in large estuaries. In these large systems, the food web complexity and density could explain why species are more subjected to dietary overlap than in small estuaries, where species richness and fish density are generally limited. However, future investigations should confirm these trends because the description of four species, even regularly distributed in the functional space, is nonetheless insufficient to accurately reflect the levels of competition within assemblages.

Our results highlighted the significant impact of specific habitat availability in shaping species isotopic niches. Niche sizes (KUD, SEAc and TA) were negatively affected by the extent of intertidal area, reflecting that individuals tended to forage on a narrower range of prey items when these essential areas were available. The intertidal areas of estuaries, including mudflats or saltmarshes, play an essential role as feeding area for many fish species, which find abundant food resources and refuges (Cattrijsse and Hampel, 2006; Nicolas et al., 2010a; Teichert et al., 2018a). Several studies pointed out the importance of these areas for sustaining estuarine food web (e.g. Bouaziz et al., 2021; França et al., 2011; Kostecki et al., 2012), because of their importance in organic matter production by microphytobenthos or saltmarsh macrophytes (Le Pape et al., 2013) and abundance in preys highly specific to these areas (Mantzouki et al., 2012; Saint-Béat et al., 2014). In estuaries with large intertidal areas, some species can favor feeding on these highly productive areas, and therefore depend closely on the carbon inputs produced by the mudflats or saltmarshes (Laffaille et al., 2001; Day et al., 2021; Lafage et al., 2021). Conversely, when intertidal areas are reduced, fishes must explore other habitats to find their preys (Selleslagh et al., 2015). Accordingly, the availability of intertidal areas likely promotes species trophic specialization, but also niche partitioning between species and life stage that use different trophic sources (Laffaille et al., 2000b), as indicated by the increase of isotopic distances between competitors (Dov). In this sense, our results highlight the importance of lateral habitats, as a foraging area, but also as a factor regulating trophic interactions in estuaries.

The total abundance in fish assemblages significantly impacted niche divergence metrics (CR, CD, MNND), but not species niche sizes. In estuaries where fish densities are high, the isotopic signatures of individuals within species tended to be more diversified, suggesting that individuals used a wider range of habitats to find their food resources, probably as a response to the competition (Svanbäck and Bolnick, 2007). Indeed, the positive relationship of CR with fish density indicates a diversification of carbon origin in fish isotopic signatures probably relying to a broader range of foraging habitats along the land-sea continuum. This niche dispersal is associated with an increase in multispecies niche overlap (Mov), which can reflect an intensification of interspecific competition with fish densities. These observations support the classical assumption of the foraging theory stating that competition for resources can drive niche expansion (MacArthur and Pianka, 1966). When food is diverse, abundant and freely available, individuals are expected to specialize on a small set of high-value resources, but when resources become scarce (for example due to resource competition), individuals should display a more marked exploration behaviour to find their food (Lesser et al., 2020; Svanbäck and Bolnick, 2007). In estuarine nurseries, competition for limited resources can negatively impact individual growth and survival, through density-dependent processes (Le Pape and Bonhommeau, 2015). For example, food supply in the Seine estuary may have decreased juvenile fish production when settlement is high and/or prey availability is low (Saulnier et al., 2020). Although this issue can not be addressed with our data, our results suggest that large fish concentration can induce changes in trophic behaviour, through the diversification of prey items and their origin along the estuarine gradient. Interestingly, the abundance of conspecific did not significantly influence the size and structure of isotopic niches, which suggests that niche expansion was more related to interspecific interactions than to variations in intraspecific abundance. Nevertheless, increasing abundances within species appeared to be the only factor affecting the level of intraspecific competition (ITP), which is not surprising since abundance is an integral part of the index proposed by Andrades et al. (2021). In contrast, the multispecies overlap (Mov) was negatively related to the abundance of receiver species, suggesting that species subjected to niche overlap, and thus potential interspecific competition, display lower abundance in estuarine assemblages.

Our results also demonstrated the significance of assemblage functional structure in shaping species isotopic niches. By selecting traits related to space occupancy and species diet, the functional indices calculated in this study reflect the diversity and structure of biotic relationships of co-occurring fish in assemblages (Villéger et al., 2008). Accordingly, the proximity of species in the functional space indicates convergence in trophic regime and position in the water column, while species with divergent traits are expected to be less subjected to interspecific competition. This concept is clearly illustrated by the location of the four target species within the trait-based functional space, which is consistent with species isotopic positioning and overlapping previously reported. Our results demonstrated that the size and structure of species isotopic niches were related to the regularity (FEve) and divergence (Fdiv) of the trophic traits within assemblages, while the functional richness had no influence (in the line of species richness). Niche size and dietary divergence of species decreased with increasing regularity of trophic strategies within assemblages, suggesting that species occupied more restricted trophic niches when they were less subjected to competition for the same food resources. These results agree with the niche partitioning hypothesis stating that limiting similarity favors the coexistence of functionally dissimilar species by promoting the exploitation of different resources (MacArthur and Levins, 1967; Mason et al., 2008). The diversity of prey available for fish in estuaries does not lead to generalist diets, the species are rather specialized on a few taxa (Pasquaud et al., 2010; Selleslagh and Amara, 2015), which contributes to limit the interspecific trophic competition (Cabral, 2000). Similarly, isotopic niche contractions were reported when assemblages were dominated by species with extreme traits (FDiv), even if the isotopic proximity of competitors was high (Dov). As previously exposed, using marginal trophic niches should contribute to favor niche resource partitioning and limit the interspecific competition pressure, as species feed singular trophic resource.

Globally, our findings demonstrated a relative steadiness of trophic position, despite a high level of interspecific variability. Nevertheless, our findings were only based on two years of surveys conducted in Autumn. Future studies should confirm our results with larger sample size and investigate the influence of seasonal variations in fish food webs. Indeed, the fluctuation of species abundance related to seasonal recruitment of marine fish, associated with diet shift during ontogeny, can affect the structure of trophic relationships in estuarine fish assemblages (Bouaziz et al., 2021). In the present study, the species isotopic niches were mediated by topographic features but also by biotic interactions at the intra- and inter-specific levels, as reflected by the influence of fish abundance and functional diversity metrics. Our results supported the statement that intertidal areas are pivotal factor in regulating trophic interactions, by promoting niche partitioning and diversification of trophic resources between species. The erosion of intertidal areas thus appears as a critical concern for management of estuarine ecosystems to limit species competition and favor their co-existence. Similarly, functional changes in fish assemblages due to human stressors can affect food web functioning through an alteration of biotic interactions, which contribute to shape niche size and dietary divergence of species. The preservation of environmental heterogeneity is probably a key issue to favor co-existence of functionally dissimilar species, limiting competition and maximizing diversity in estuaries.

CRediT authorship contribution statement

Nils Teichert: Writing – original draft, Project administration, Funding acquisition, Formal analysis, Data curation. **Anne Lizé:** Writing – review & editing, Methodology. **Mario Lepage:** Writing – review & editing, Methodology. **Henrique Cabral:** Writing – review & editing, Methodology. **Thomas Trancart:** Writing – review & editing, Methodology. **Anthony Acou:** Writing – review & editing, Methodology. **Marie Larregieu:** Data curation. **Eric Feunteun:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Alexandre Carpentier:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Nils Teichert reports financial support was provided by French Biodiversity Office.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2024.108686.

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