Journal of the Marine Biological Association of the United Kingdom

cambridge.org/mbi

Original Article

Cite this article: Mendes C, Ramos S, Elliott M, Bordalo AA (2020). Feeding strategies and body condition of juvenile European flounder *Platichthys flesus* in a nursery habitat. *Journal of the Marine Biological Association of the United Kingdom* **100**, 795–806. https://doi.org/10.1017/S0025315420000600

Received: 4 October 2019
Revised: 9 March 2020
Accepted: 18 June 2020
First published online: 28 July 2020

Key words:
Condition; feeding; habitat use; nursery habitat quality; nursery

Author for correspondence:
C. Mendes,
E-mail: claudiavrmendes@gmail.com

Feeding strategies and body condition of juvenile European flounder *Platichthys flesus* in a nursery habitat

C. Mendes[1,2,3,4], S. Ramos[2,4], M. Elliott[3,4] and A. A. Bordalo[1,2]

[1]Laboratory of Hydrobiology and Ecology, Institute of Biomedical Sciences, University of Porto (ICBAS-UP), Rua Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal; [2]Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal; [3]Department of Biological and Marine Sciences, University of Hull, Hull HU6 7RX, UK and [4]International Estuarine & Coastal Specialists Ltd, Leven HU17 5LQ, UK

Abstract

Estuarine habitats are major nurseries for the European flounder *Platichthys flesus*, with different year classes sharing food and space resources. Hence, an understanding of feeding strategies that optimize resource use and maintain carrying capacity is fundamental for sustainable and successful ecosystem management. The main feeding areas of juvenile European flounder (including 0-group and 1-group age classes) in the Lima estuary (northern Portugal) nursery ground were investigated by integrating stomach content analyses with stable isotopic values ($\delta^{13}C$ and $\delta^{15}N$) and fish condition indices (Fulton K and RNA:DNA ratio). The 0-group flounder that were associated with the upstream section of the estuary presented the lowest $\delta^{13}C$ value (-25.58 ± 1.86‰), while 1-group flounder exhibited a higher $\delta^{13}C$ value (-22.59 ± 2.51‰), indicating use of the more saline areas of the estuary (lower and middle sections). The two age groups did not differ in terms of $\delta^{15}N$ (0-group: 13.93 ± 0.29‰; 1-group: 13.50 ± 0.96‰), indicating similar trophic levels. The low salinity upper estuary was the main feeding area of 0-group flounder (74%), while 1-group flounder fed along the estuary both upstream (52%) and downstream (48%). Juvenile flounder showed high individual condition based on the Fulton K index (0-group: 1.05 ± 0.08; 1-group: 1.07 ± 0.05) and RNA:DNA (0-group: 1.70 ± 0.70; 1-group: 1.41 ± 0.47). These indices deal with fish health, and hence indicate nursery habitat quality. It is concluded that in this temperate nursery habitat, different feeding strategies sustained the condition of the European flounder juveniles, compared with other flounder populations.

Introduction

Estuaries are highly productive systems (McLusky & Elliott, 2004) that support nursery areas for many marine fish species (Kerstan, 1991; Beck *et al.*, 2001; Potter *et al.*, 2015). Early life stages of fishes find suitable conditions in nursery habitats that enhance body condition, growth and survival through high food availability and shelter from predation (Boesch & Turner, 1984; Gibson, 1994; Pihl *et al.*, 2007). Ultimately, nursery conditions promote recruitment, and therefore maintain the adult populations (Gibson, 1994; Rijnsdorp *et al.*, 1995; Beck *et al.*, 2001). The quantity and quality of habitat together with food availability are major determinants of nursery carrying capacity (Gibson, 1994; Nunn *et al.*, 2012; Le Pape & Bonhommeau, 2015), in terms of the maximum number of individuals or biomass that can ecologically be supported by an area (Elliott *et al.*, 2007). However, historically there has been a high loss of estuarine areas and habitats (Wolanski & Elliott, 2015; Amorim *et al.*, 2017), and there is a need for management measures promoting remediation of these habitats (Elliott *et al.*, 2016). Hence, it is important to know whether food and space resources have diminished for fishes, including economically valuable species, and whether this has in turn decreased the estuarine carrying capacity.

The dispersal of juveniles supports ecological interactions between the different units that comprise nursery habitats, ensuring connectivity (Sheaves, 2009; Palmer *et al.*, 2014). Losses in connectivity may decrease trophic interactions and lead to food web fragmentation, compromising the system resilience and nursery function (Vinagre *et al.*, 2011; Selleslagh *et al.*, 2015). Similarly, a loss of connectivity would increase competition for scarce resources within an area. Moreover, distribution patterns will reflect abiotic factors, such as temperature, salinity, depth, sediment type (Power *et al.*, 2000; Andersen *et al.*, 2005*a*; Ramos *et al.*, 2009; Vasconcelos *et al.*, 2010) and biotic factors such as prey availability, predation and competition (van der Veer *et al.*, 2000; Darnaude *et al.*, 2001; Amara *et al.*, 2009; Sheaves *et al.*, 2015). In particular, prey availability is likely to affect the condition and growth of juveniles (Cabral *et al.*, 2002; Amara *et al.*, 2009; De Raedemaecker *et al.*, 2012*b*), and hence nursery value. Therefore, migrations and recruitment to areas with high prey availability may either optimize resource use or prevent food limitation, thus minimizing competition (Tableau *et al.*, 2016). These migrations may shape habitat and resource partitioning between different life stages and species in nursery habitats (Darnaude *et al.*, 2001; Russo *et al.*, 2008). Apart from migration, other

resource use and resource partitioning strategies include feeding on highly abundant prey (Molinero & Flos, 1992; Vinagre *et al.*, 2005), ontogenetic shifts in the diet (Aarnio *et al.*, 1996; Kopp *et al.*, 2013) and temporal differences in habitat use (Cabral & Costa, 1999).

Stomach contents analysis offers a snapshot of the fish diet (Hyslop, 1980; Marshall & Elliott, 1997), which can be combined with stable isotope information to give broader temporal information (DeNiro & Epstein, 1978; Minagawa & Wada, 1984; Peterson & Fry, 1987) to gauge trophic relationships. Carbon ($\delta^{13}C$) stable isotope ratios help to explain the diet composition of consumers (DeNiro & Epstein, 1978; Fry & Sherr, 1989), while $\delta^{15}N$ can also indicate trophic position (DeNiro & Epstein, 1981; Post, 2002). Recently, stable isotope mixing models (Parnell *et al.*, 2013; Phillips *et al.*, 2014) have been used to identify the main prey and organic matter sources (Le Pape *et al.*, 2013; Hoffman *et al.*, 2015; Dias *et al.*, 2017), and to characterize trophic niche widths and niche overlap between species (Kostecki *et al.*, 2012; Vaslet *et al.*, 2015). This information may then be integrated to track movements over variable temporal and spatial scales, and identify key habitats regarding food use (Green *et al.*, 2012; Kopp *et al.*, 2013; Selleslagh *et al.*, 2015).

Indices of fish condition are valuable in assessing nursery habitat quality (Gilliers *et al.*, 2004; Fonseca *et al.*, 2006; De Raedemaecker *et al.*, 2012*a*), by measuring individual somatic nutritional status, and hence the potential for growth and reproduction. These indices, including morphometric and biochemical ones, respond to environmental conditions over various temporal scales (Suthers *et al.*, 1992; Fonseca *et al.*, 2006). The Fulton condition factor, K (Ricker, 1975) is a morphometric index widely used to measure fish somatic condition (Amara *et al.*, 2009; Vasconcelos *et al.*, 2009; De Raedemaecker *et al.*, 2012*a*), considering that for a given length, the heavier fish are in better condition. The Fulton K is sensitive to the nutritional status of the juveniles (Ferron & Leggett, 1994; Selleslagh & Amara, 2013) and integrates condition over the preceding weeks to months (e.g. Caldarone *et al.*, 2012; De Raedemaecker *et al.*, 2012*a*). Complementary to this, the biochemical RNA:DNA ratio indicates recent nutritional condition and growth (Clemmesen, 1994; Malloy & Targett, 1994; Chícharo & Chícharo, 2008). It assumes that the DNA content in a somatic cell remains constant, while the RNA concentration reflects changes in protein synthesis rates (Bulow, 1970; Buckley *et al.*, 1999), i.e. in a non-stressful situation, higher RNA:DNA ratio reflects higher somatic growth of the fish. Furthermore, the RNA:DNA responds to changes in food availability within days (e.g. Clemmesen, 1994; Selleslagh & Amara, 2013; Foley *et al.*, 2016).

The flatfish European flounder *Platichthys flesus* (L. 1758) uses estuarine habitats as nurseries (Henderson & Holmes, 1991; Kerstan, 1991; Martinho *et al.*, 2008), including the Lima estuary (northern Portugal) (Ramos *et al.*, 2010) where they are an important component of the estuarine fish community. The NW Portuguese coast represents the southern limit of the distribution of flounder (Nielsen, 1986), with a recent decline in abundance associated with increasing seawater temperatures (Cabral *et al.*, 2007). Moreover, food limitation has been linked to interspecific competition and higher metabolic demands in southern European nurseries, resulting in reduced growth rates of flounder compared with higher latitudes (Freitas *et al.*, 2012).

Flounder spawning typically occurs from winter to early spring (Summers, 1979; Muus & Nielsen, 1999; Primo *et al.*, 2013) although the exact location of the spawning grounds off the Portuguese Coast remains unknown (Amorim *et al.*, 2016). Larvae usually enter the Lima estuary between February and July (Ramos *et al.*, 2010; Amorim *et al.*, 2016). Direct settlement occurs inside the estuary (Ramos *et al.*, 2010; Amorim *et al.*, 2018), and most likely in the upper section (Ramos *et al.*, 2010), as indicated by the high larval (Ramos *et al.*, 2017) and newly settled flounder abundances found in this area (Ramos *et al.*, 2010; Mendes *et al.*, 2014; Amorim *et al.*, 2016). As in other estuaries (Elliott & Hemingway, 2002), the spatial distribution of flounder in the Lima estuary varies with age (Ramos *et al.*, 2009; Mendes *et al.*, 2014, Amorim *et al.*, 2018). Young-of-the-year flounder aggregate in the upper estuary, while older flounder juveniles are more abundant in the middle section (Ramos *et al.*, 2009; Mendes *et al.*, 2014; Amorim *et al.*, 2018). The juvenile flounder typically feed on common prey such as polychaetes (Summers, 1980; Piet *et al.*, 1998; Martinho *et al.*, 2008) and amphipods (Aarnio *et al.*, 1996; Vinagre *et al.*, 2005; Mendes *et al.*, 2014). These studies have led to the hypothesis that juvenile distribution and feeding in any nursery area will be structured to minimize competition through resource partitioning and ensure good growth and condition. In order to test this hypothesis, the present study aimed to: (1) determine the main feeding areas of 0-group (<1 year old) and 1-group (juveniles 1–2 year old) flounder, using dietary indices and stable isotope mixing models, and (2) assess flounder juveniles' condition through morphometric and RNA:DNA indices in the Lima estuary. This, in turn, is needed to indicate the feeding use of flounder in a nursery system near its southern geographic distribution limit, which is crucial information needed for better management decisions.

Materials and methods

Study area

The Lima estuary, NW Atlantic coast of Portugal, is a small open temperate estuary with a semidiurnal and mesotidal regime (3.7 m tidal range). Salt intrusion can extend up to 20 km upstream, with an average flushing rate of 0.4 m s^{-1}, and a residence time of 9 days (Ramos *et al.*, 2006). This study sampled at five stations covering the lower, middle and upper estuary (Figure 1). The lower estuary (station 1, average depth of 7.0 m), located within the first 2.5 km, is a narrow, 9-m deep navigational channel, industrialized, with walled banks, including a shipyard, commercial seaport and fishing harbour; the middle estuary (stations 2–3, average depth 5.1 m) comprises a broad shallow intertidal salt-marsh zone, mainly colonized by the common rush (*Juncus* spp.); the upper estuary (Stations 4–5, average depth 2.1 m) is a narrow shallow channel, less disturbed, with natural banks and small exposed sandbanks (Ramos *et al.*, 2010).

Data collection

Juvenile European flounder *Platichthys flesus*, macroinvertebrates, sediment and water samples were collected in August 2013. This sampling date ensured that 0-group flounder should have reached isotopic equilibrium to the estuarine habitats, considering that this process could take weeks to months (Herzka, 2005), given the typical late spring estuarine colonization (Martinho *et al.*, 2008; Ramos *et al.*, 2009). Vertical profiles of temperature and salinity were obtained at each site by means of a multi-parameter water quality probe YSI 6820. Bottom water samples for particulate organic matter (POM) analysis were collected at each sampling site with a Van Dorn bottle. Samples for sediment organic matter (SOM), and macroinvertebrate analysis were retrieved at each station with a Petite Ponar grab of area 0.023 m^2. Juvenile flounder, as well as the shore crab *Carcinus maenas* were collected during two-nightly ebb tides with a 2 m beam trawl, with a cod-end of 5 mm mesh, and a tickler chain, and sorted immediately. All samples were kept on ice until further processing in the laboratory. The geographic location of each sampling station was recorded with a Magellan 315 GPS, and vertical profiles of water

https://doi.org/10.1017/S0025315420000600 Published online by Cambridge University Press

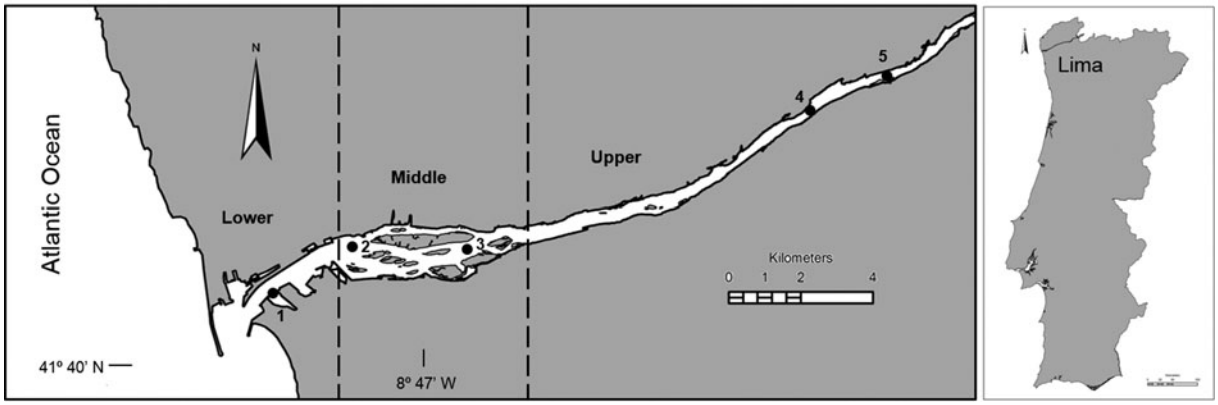

Fig. 1. The Lima estuary with the location of the sampling stations (1 – lower estuary; 2 and 3 – middle estuary; 4 and 5 – upper estuary).

temperature and salinity were obtained using a multi-parameter probe YSI 6820.

Stomach content analysis

Juvenile flounder were measured for total length (TL; ±1 mm), and wet weight (W; ±0.01 g) and sorted according to total length at first sexual maturity (assumed to be 200 mm; Dinis, 1986). The stomachs were excised, and the contents removed and preserved in 70% alcohol. The prey items were identified to the highest taxonomic separation possible, using a binocular microscope (Leica MZ12-5), counted and weighed (wet weight; 0.001 g) after blotting on a tissue paper (Mendes *et al.*, 2014).

Stable isotope analysis

POM samples were obtained by pre-filtering 1 litre of water sample through a 200 µm nylon mesh to remove zooplankton and debris, and then through pre-combusted GF/F glass fibre filters (Harmelin-Vivien *et al.*, 2008; Suzuki *et al.*, 2008). Filters were acidified with 10% HCl for carbonate removal (Vizzini *et al.*, 2002; Kennedy *et al.*, 2005), which is $\delta^{13}C$ enriched compared with organic carbon (DeNiro & Epstein, 1978), dried at 60°C for 48 h, and stored at −80°C. Sediment samples for SOM analysis were dried at 60°C, and ground to a fine powder. In addition, SOM samples for $\delta^{13}C$ analysis were acidified with 10% HCl to remove carbonates, and dried at 60°C. Samples were stored in a desiccator until further analysis. Bivalvia, *Carcinus maenas*, Chironomidae, *Corophium* spp., Gastropoda, Isopoda and Polychaeta were considered as main prey groups (Vinagre *et al.*, 2005; Martinho *et al.*, 2008; Mendes *et al.*, 2014) and sorted from the sediment samples. Samples from the upper estuary comprised Chironomidae, *Corophium* spp. and Polychaeta. The prey *C. maenas* and Polychaeta from the lower and middle sections were analysed, as well as Gastropoda from the lower estuary. Rare prey groups such as Bivalvia and Isopoda were not included due to insufficient material available for the stable isotope analysis. Whole individuals were used for prey stable isotope analysis, except for larger prey (*C. maenas*), for which muscle tissue was collected from the claws. Dorsal white muscle was removed from the flounder juveniles. Prey and fish samples were kept frozen (−80°C) until analysis. Prior to stable isotope analysis, animal tissue samples were dried and ground to a fine powder, using a mortar and pestle. *Corophium* spp. and gastropods were acidified (10% HCl) to remove carbonates (Ng *et al.*, 2007; Selleslagh *et al.*, 2015) from the samples for $\delta^{13}C$ analysis. Carbon and nitrogen stable isotope analysis were performed on individual samples of fish and crabs. For other prey groups, several individuals were pooled in order to have sufficient material for analysis. Ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ in each sample were determined by continuous flow isotope mass spectrometry (CF-IRMS) (Preston & Owens, 1983), using a Thermo Scientific Delta V Advantage IRMS via a Conflo IV interface. The delta (δ) notation was used to express the stable isotope ratios as ppt differences from a standard reference material:

$$\partial X\ (‰) = (R_{\text{sample}} \times R^{-1}_{\text{standard}} - 1) \times 10^3$$

where X is ^{13}C or ^{15}N and R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Isotope ratios were measured relative to the international standards of PeeDee Belemnite for carbon and atmospheric N_2 for nitrogen. Analytical precision (standard deviation) was ±0.2‰ of reference material for carbon and nitrogen.

Condition analysis

The individual somatic condition of the European flounder juveniles was assessed through the Fulton index and RNA:DNA ratio indices. The Fulton condition factor, K (Ricker, 1975) was determined following the formula:

$$K = W \times (\text{TL}^3)^{-1} \times 100,$$

where W is the wet weight (mg) and TL is the total length (mm). For the RNA:DNA, dorsal white muscle samples from European flounder juveniles were preserved in liquid nitrogen upon collection, and kept at −80°C until analysis. Prior to analysis, muscle samples were homogenized in 500 µl TEN-SDS buffer (0.05 M Tris, 0.01 M EDTA, 0.1 M NaCl, 0.01% SDS, pH 8). RNA:DNA was determined for three replicate samples of dorsal white muscle (10 mg) of each juvenile flounder, by the fluorometric method described in Caldarone *et al.* (2001), and modified by De Raedemaecker *et al.* (2012*b*). Fluorescence was measured on a FluoroSkan Ascent FL microplate reader with 535 nm excitation wavelength and 586 nm emission wavelength. RNA and DNA concentrations were determined based on standard curves prepared with baker's yeast RNA (Sigma) and pure calf-thymus DNA (Sigma). The RNA:DNA was determined as the ratio between average RNA and DNA concentrations of each sample. The ratio between the slopes of the RNA and DNA standard curve was 2.5, which can be used as a standardization factor for inter-calibration with other studies (Caldarone *et al.*, 2006).

Data analysis

Temperature and salinity parameters of the bottom water column (1 m above bottom depth) were averaged for each location.

https://doi.org/10.1017/S0025315420000600 Published online by Cambridge University Press

European flounder juveniles were sorted according to length that would broadly equate with <150 mm for 0-group flounder and <200 mm for 1-group flounder in Portuguese populations (Dinis, 1986; Martinho *et al.*, 2007; Primo *et al.*, 2013). These are literature classifications based on size-frequency distributions which may present an associated error due to inter-individual and inter-annual variability in growth rates. For each group, feeding activity was evaluated by the vacuity index (Iv), defined as the per cent of empty stomachs (Hyslop, 1980). Key prey were identified based on the numerical (NI) and weight (WI) percentages of each prey item in the diet (Hyslop, 1980). The average $\delta^{13}C$ of POM and SOM sources was determined for each estuarine sector. The high $\delta^{15}N$ variability between replicates for POM and SOM sources, possibly linked to the low nitrogen content of the samples, did not allow the use of this isotope to assess POM and SOM sources. The stable isotope signatures of POM and SOM, and samples from each prey category were sorted into estuarine classification, namely *upstream* (comprising the upper estuarine sector), and *downstream* (including pooled data from the lower and middle sections, see Supplementary material for details). The main feeding locations of juvenile flounder were also investigated. Therefore, the relative contribution of each prey to the diet of 0-group and 1-group flounder were determined with stable isotope mixing models, using the SIAR package in R software (Parnell *et al.*, 2010). The stable isotope mixing models estimate probability distributions of food source contributions based on Bayesian inference and accounting for different levels of uncertainty (sources, consumer and contributions of individual sources, Parnell *et al.*, 2010). One general set of trophic enrichment factors (TEF), 1% for $\delta^{13}C$ and 3.4% for $\delta^{15}N$ per trophic level, was used based on a meta-analysis from the scientific literature, and with an associated standard error of ±0.5 (Kostecki *et al.*, 2012). Differences of $\delta^{13}C$ and $\delta^{15}N$ between flounder groups were tested with a permutational multivariate analysis of variance (PERMANOVA). Multivariate dispersion was tested with the PERMDISP routine. The PERMANOVA and PERMDISP analyses were based on the Euclidean distance dissimilarity matrix, and performed with PRIMER v6.1 (Primer-E Ltd, UK) and PERMANOVA + 1.0.1 add-on software (Clarke & Gorley, 2006; Anderson *et al.*, 2008). Possible length effects were investigated through Pearson correlations between total length and (a) isotopic values of carbon and nitrogen, and (b) condition indices of the 0-group and 1-group flounder. The relationship between condition indices Fulton K and RNA:DNA was also investigated through Pearson correlations. Differences in condition indices between respective flounder age groups were investigated with a *t*-test. A significance level of $P < 0.05$ was used for all the statistical analyses that were performed using R software (R Development Core Team, 2007).

Results

Physical-chemical parameters

The bottom temperature ranged between 13.7 and 22.8°C, with an average of 17.1 ± 3.4°C. The temperature increased from the lower (14.6 ± 0.7°C) to the middle (14.8 ± 0.8°C) and upper (21.4 ± 1.1°C) sections. Salinity, as expected, decreased from the lower (29.9. ± 0.1) and middle (29.5 ± 0.3) sections to the upper estuary (7.2 ± 5.4).

Stomach contents analysis

A total of 42 European flounder juveniles were collected in the Lima estuary, which were allocated to two age groups based on total length (0-group flounder, N = 22; 1-group flounder, N = 20; Figure 2; Table 1). Most of the 0-group flounder were caught in the upper estuary (N = 20), but two 0-group flounder were retrieved from the middle estuary. The 1-group flounder were collected from the middle estuary. The 0-group flounder presented a lower vacuity index (8%), compared with the 70% vacuity of the 1-group flounder.

The diet of the 0-group flounder was dominated by the amphipod *Corophium* spp. (NI = 94%, WI = 88%), followed by the polychaete *Hediste diversicolor* that reached 12% of the WI (Figure 3A). Other prey such as *Crangon crangon*, Chironomidae, Mysidae and Oligochaeta were also identified as minor items (< 1%). The diet of the 1-group flounder comprised mainly polychaetes (NI = 48%; WI = 69%). Isopoda (NI = 20%, WI = 3%), and *C. crangon* (NI = 16%, WI = 17%) were also important prey items (Figure 3B). Other prey of 1-group flounder included Bivalvia, *Carcinus maenas* and *Corophium* spp. Both 0- and 1-groups also had plant debris and sand in their stomach contents.

Stable isotope analysis

Organic matter sources

The carbon signature of the upstream areas ranged from −32.66 to −25.55‰, and was significantly lower (see Supplementary material) than downstream areas with $\delta^{13}C$ varying from −24.57 to −19.45‰ (Table 2, Figure 4A).

Prey

The upstream prey (*Corophium* spp., Polychaeta and Chironomidae) presented lower $\delta^{13}C$ compared with other prey (Table 3). In upstream areas, *Corophium* spp. presented the lowest $\delta^{13}C$, while polychaetes presented enriched $\delta^{15}N$ compared with other prey. In the downstream areas, *C. maenas* and gastropods presented the most enriched $\delta^{13}C$, while polychaetes presented the lowest $\delta^{15}N$ values (Table 3, Figure 4B).

European flounder juveniles

European flounder juvenile carbon (0-group flounder: $R = 0.16$, $P = 0.46$; 1-group flounder: $R = -0.16$, $P = 0.50$) and nitrogen (0-group flounder: $R = 0.04$, $P = 0.86$; 1-group flounder: $R = 0.14$, $P = 0.56$) stable isotope ratios did not vary significantly with total length. Therefore, it was not necessary to correct the data for length effects. The stable isotopes varied significantly between flounder groups (PERMANOVA, Pseudo-F = 13.62, $P = 0.02$). The 1-group flounder had higher $\delta^{13}C$ and lower $\delta^{15}N$ values than the 0-group flounder (Table 1).

SIAR outputs

The diet of 0-group flounder depended primarily on *Corophium* spp. and other prey from the upper estuary (Figure 4B), with a total of 74% contribution according to SIAR (Figure 5A). However, some 0-group flounder had higher $\delta^{13}C$ values (Figure 4B), between −24 and −18‰, than expected if the diet depended only on the upper estuary resources. Indeed, polychaetes from the downstream areas constituted 26% of the diet. The dual isotope plots displayed one cluster of 1-group flounder (Figure 4B), with isotopic signatures similar to *Corophium* spp., and sources from upstream, while the remaining individuals presented more scattered values consistent with the sources and prey from downstream areas. Indeed, the SIAR model applied to 1-group flounder data showed that their diet relied equally on prey both from upstream (48%), and downstream areas (52%) (Figure 5B). Specifically, downstream polychaetes (46%), and upstream *Corophium* spp. (42%), represented the main prey of 1-group flounder.

https://doi.org/10.1017/S0025315420000600 Published online by Cambridge University Press

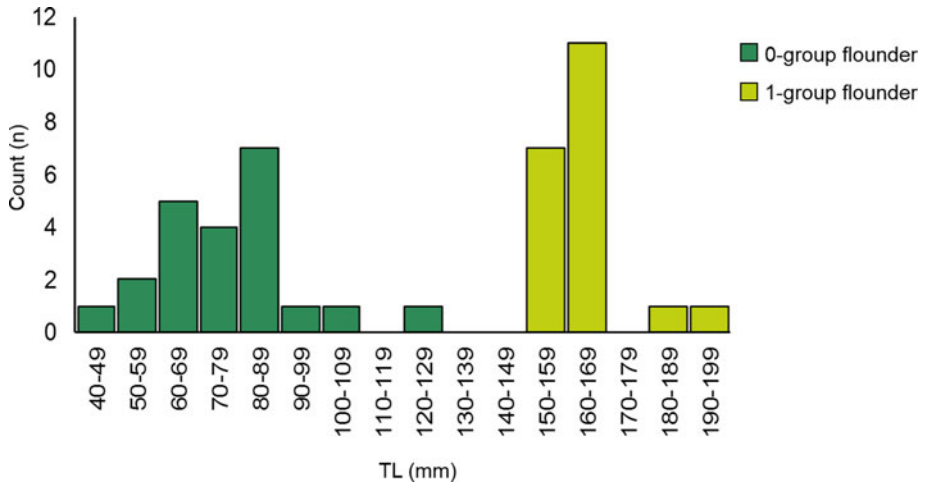

Fig. 2. Size frequency distribution of the sampled 0-group (N = 22) and 1-group (N = 20) European flounder in the Lima estuary.

Table 1. Number (*N*) of 0-group and 1-group European flounder sampled in the Lima estuary, mean total length (mm), total weight (g), Fulton K, RNA:DNA and muscle carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope signatures (‰)

Flounder	N	TL (mm)	WW (g)	*K*	RNA:DNA	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
0-group	22	75 ± 20	5.43	1.05 ± 0.08	1.70 ± 0.70	−25.58 ± 1.86	13.93 ± 0.29
1-group	20	163 ± 10	46.96	1.07 ± 0.05	1.41 ± 0.47	−22.59 ± 2.51	13.50 ± 0.96

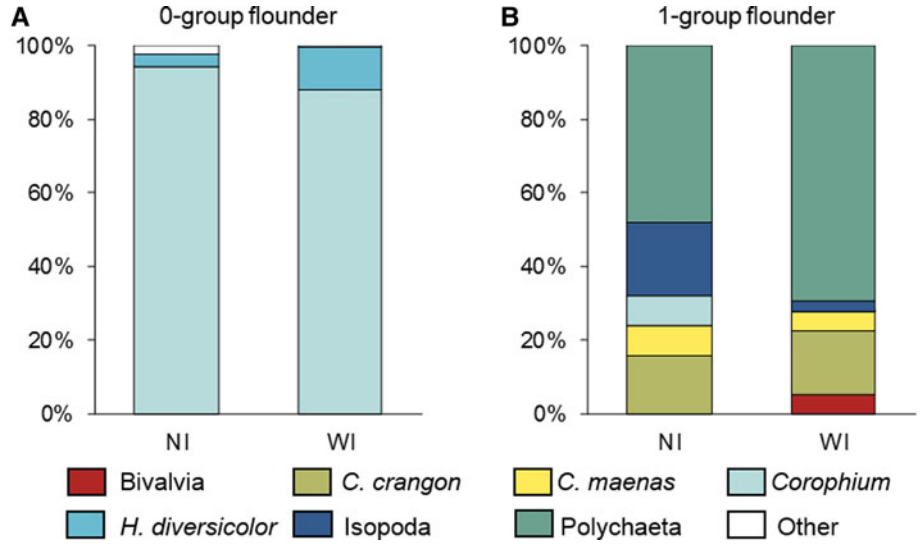

Fig. 3. Numerical (NI) and weight (WI) indices for stomach contents of (A) 0-group, and (B) 1-group European flounder in the Lima estuary.

Table 2. Mean carbon stable isotope $\delta^{13}C$ (‰) of particulate organic matter (POM) and sediment organic matter (SOM) sources in the upstream and downstream areas of the Lima estuary

	$\delta^{13}C$ (‰)	
Source	Downstream	Upstream
POM	−23.62 ± 0.32	−28.88 ± 3.34
SOM	−23.15 ± 2.47	−26.11 ± 0.39

Condition analysis

There was no correlation between flounder juvenile total length and Fulton K (0-group flounder: $R = 0.03$, $P = 0.87$; 1-group flounder: $R = -0.25$, $P = 0.28$), or RNA:DNA (0-group flounder: $R = 0.25$, $P = 0.24$; 1-group flounder: $R = 0.21$, $P = 0.40$). Therefore, no corrections for length effect were applied to the indices. Also, there were no significant differences in condition between 0-group flounder and 1-group flounder (Table 1), in terms of Fulton K (*t*-test, $t = -0.89$, df = 40, $P = 0.38$), or in terms of RNA:DNA (*t*-test, $t = 1.58$, df = 40, $P = 0.12$). The two condition indices Fulton K and RNA:DNA were also not correlated (0-group flounder: $R = 0.06$, $P = 0.78$, 1-group flounder: $R = -0.44$, $P = 0.06$).

Discussion

Integrating stomach contents and stable isotope analysis

Stomach content analysis provided a first indication of the recent diet of juvenile flounder, enabling identification of the main prey (polychaetes, *Corophium* spp. and *Carcinus maenas*) that could be included in the stable isotope analysis. A discrimination between sources and prey signatures was necessary to reconstruct the consumer diet based on stable isotope analysis (Vander

https://doi.org/10.1017/S0025315420000600 Published online by Cambridge University Press

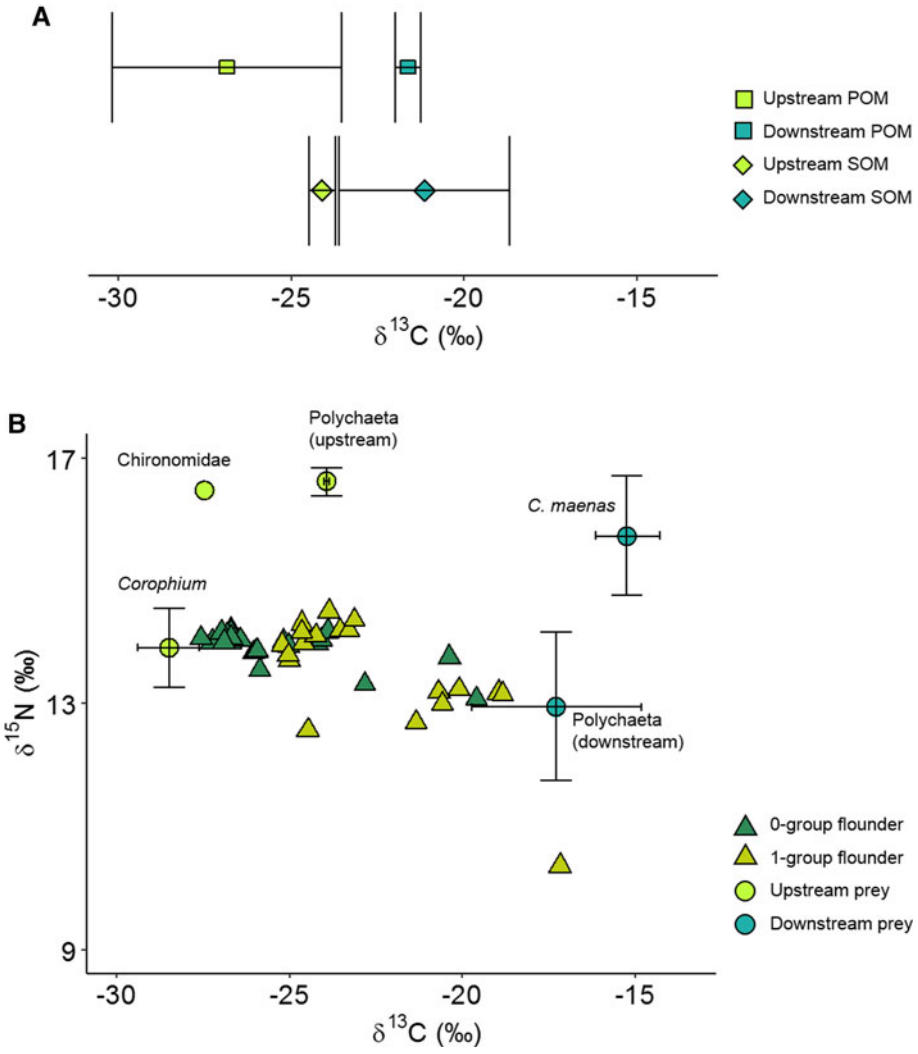

Fig. 4. Carbon ($\delta^{13}C$), and nitrogen ($\delta^{15}N$) stable isotopes (‰) of (A) sediment (SOM, upstream and downstream), and water particulate (POM, upstream and downstream) organic matter sources; (B) 0-group and 1-group European flounder, and respective upstream and downstream prey. Trophic enrichment factors were applied to sources ($\delta^{13}C$: ± 2‰, i.e. ±1‰ per trophic level), and to prey ($\delta^{13}C$: ± 1‰, $\delta^{15}N$: ± 3.4‰).

Table 3. Mean carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope signatures of the main prey groups of European flounder juveniles in the upstream and downstream areas of Lima estuary

Prey	Estuarine zone	$\delta^{13}C$	$\delta^{15}N$
Chironomidae	Upstream	−28.47 ± 0.05	13.05 ± 0.02
C. maenas	Downstream	−16.20 ± 0.93	12.32 ± 0.96
Corophium spp.	Upstream	−29.50 ± 0.90	10.50 ± 0.65
Gastropoda	Downstream	−15.58 ± 0.11	11.26 ± 1.38
Polychaeta	Upstream	−24.92 ± 0.08	13.20 ± 0.22
	Downstream	−18.26 ± 2.46	9.54 ± 1.21

Zanden *et al.*, 1997; Post, 2002). The $\delta^{13}C$ depletion usually increases along the horizontal salinity gradient from marine to estuarine and terrestrial sources (Fry, 2002). In the Lima estuary, the $\delta^{13}C$ similarity between lower and middle estuaries reflected the euhaline regime of both areas. The depleted $\delta^{13}C$ signatures in the upper estuary were related to the increasing terrestrial input in the upstream areas (Darnaude *et al.*, 2004; França *et al.*, 2011). The POM and SOM presented similar $\delta^{13}C$ values, providing good indicators of local $\delta^{13}C$ signatures.

In general, prey from upstream stretches consistently presented higher $\delta^{15}N$ values than the downstream prey, which may result from differences in the organic matter sources between estuarine sections. However, the high variability between replicates did not allow the determination of $\delta^{15}N$ of the organic matter sources to corroborate this hypothesis. The $\delta^{15}N$ variability between prey groups within each estuarine area may reflect differences in trophic position (Minagawa & Wada, 1984; Peterson & Fry, 1987; Post, 2002). For example, the epibenthic predator *C. maenas* (Raffaelli *et al.*, 1989) showed the highest $\delta^{15}N$ from the downstream prey, while the omnivorous polychaetes from upstream areas had higher $\delta^{15}N$ than Chironomidae and *Corophium*, which are opportunistic omnivores (Armitage *et al.*, 1995), and suspension and deposit feeders (Gerdol & Hughes, 1995), respectively. Prey from the upstream areas showed markedly depleted $\delta^{13}C$ compared with the more brackish downstream areas, thus reflecting the use of local OM sources. This discrimination between upstream and downstream prey enabled the use of stable isotope mixed models to identify the main feeding locations, and trace movements of European flounder juveniles in the Lima estuary.

The stomach content results complemented those provided by the stable isotope analysis. Discrepancies between these two methods may be related to the fact that stomach contents reflect recent feeding (Hyslop, 1980), while stable isotope analysis indicates and integrates long-term patterns (Vander Zanden *et al.*, 1997). Hence, easily digested prey, such as polychaetes, were probably underestimated in 0-group flounder dietary indices even though these were important prey, together with *Corophium*, according to SIAR results. Also, the near absence of the upper estuary prey *Corophium* spp. in 1-group flounder dietary indices contrary to SIAR, reflected the recent feeding on polychaetes in the middle estuary, where juveniles were caught.

https://doi.org/10.1017/S0025315420000600 Published online by Cambridge University Press

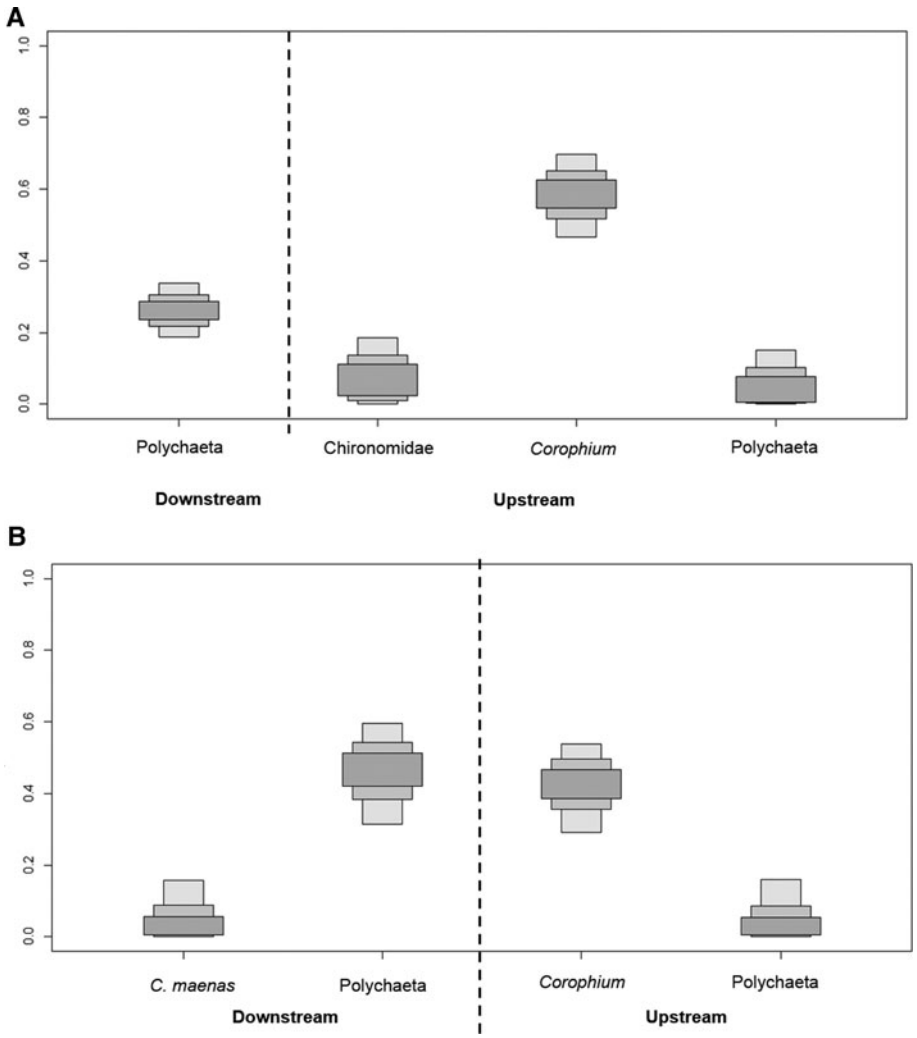

Fig. 5. Boxplots of the mixing models estimates of prey contribution to the diet of (A) 0-group and (B) 1-group European flounder. Prey groups divided according to upstream and downstream areas.

The diet of 0-group flounder was mostly composed by the amphipod *Corophium* as observed in other nursery areas, including the Douro (Vinagre *et al.*, 2005), Mondego (Martinho *et al.*, 2008), Schelde (Hampel *et al.*, 2005) and Ythan estuaries (Summers 1980). *Corophium* was highly abundant and mostly restricted to the Lima upper estuary where the 0-group flounder were also concentrated (Ramos *et al.*, 2009; Mendes *et al.*, 2014). *Corophium* is highly active and may become energetically more advantageous to the European flounder (Andersen *et al.*, 2005*b*; Grønkjær *et al.*, 2007) as a visual predator (De Groot, 1971), especially in the scarce vegetation conditions of the upper estuary (Mendes *et al.*, 2014). The polychaetes were the main prey of the 1-group flounder as observed in other estuaries (e.g. Summers *et al.*, 1980; Doornbos & Twisk, 1984; Pasquaud *et al.*, 2008). The 1-group flounder consumed less *Corophium*, reflecting their ability to handle larger prey such as polychaetes (Vinagre *et al.*, 2008), and the increased use of downstream areas (Ramos *et al.*, 2009; Mendes *et al.*, 2014) where amphipods were less abundant (Sousa *et al.*, 2006; Mendes *et al.*, 2014). Molluscs were more abundant in the lower estuary (Sousa *et al.*, 2006) and were only occasionally consumed by juvenile flounder that were distributed over other estuarine areas. Moreover, sedentary prey such as molluscs may become major prey in high turbidity systems such as the Severn estuary (Moore & Moore, 1976), where reduced visibility does not favour the predation on mobile prey. This is not the case in the Lima estuary, where low turbidity can be found in the summer months (Ramos *et al.*, 2006), associated with the presence of higher salinity coastal waters. Shrimps, crabs and isopods were only occasionally consumed as observed in other estuaries (Hampel *et al.*, 2005; Vinagre *et al.*, 2005).

European flounder juvenile movements and main feeding areas within the Lima estuary

The upper estuary was the main feeding area of 0-group flounder in line with the typical preference of early stage flounder for upstream areas (e.g. Kerstan, 1991; van der Veer *et al.*, 1991; Freitas *et al.*, 2009). Moreover, the lower $\delta^{13}C$ confirms the dependence on freshwater-derived sources observed from late larval (Dias *et al.*, 2017) to juvenile stages (Pasquaud *et al.*, 2008; Selleslagh *et al.*, 2015) of European flounder. Prey availability (Bos, 1999; Florin & Lavados, 2010; Vasconcelos *et al.*, 2010), and reduced competition for space and food (Beaumont & Mann, 1984; Złoch & Sapota, 2010; Souza *et al.*, 2013) may explain this preference for upstream areas, especially as prey availability and salinity have been previously correlated with the concentration of 0-group flounder in the Lima upper estuary (Ramos *et al.*, 2009; Mendes *et al.*, 2014). Despite this, 0-group flounder feeding was not restricted to the upper estuary, since polychaetes from downstream areas were also identified as prey items.

Limited mobility, and consequent patchy segregation along upstream-downstream estuarine gradients, have been reported for 0-group flatfish (Raffaelli *et al.*, 1990; Le Pape & Cognez, 2016). However, these movements between feeding habitats (Summers, 1980; Wirjoatmodjo & Pitcher, 1984; Dando, 2011; Souza *et al.*, 2013) may be the result of the 0-group flounder's increased mobility given that the individuals sampled were

https://doi.org/10.1017/S0025315420000600 Published online by Cambridge University Press

approaching 1-group age class (≥150 mm). The risk from predation inherent in feeding in downstream areas was reduced as individuals attained size-based refuge from predation by the crustaceans *C. maenas* (>50 mm TL), and *C. crangon* (>30 mm TL) (van der Veer & Bergman, 1987; Burrows *et al.*, 2001). Laboratory experiments have showed that low salinity typically found in the upstream section may limit somatic condition and growth (Gutt, 1985; O'Neill *et al.*, 2011) of the juvenile flounder. The predator–prey trade-offs mentioned above may explain why the juveniles remained upstream despite these limitations. In parallel, the downstream areas may have promoted higher condition as the juveniles approached 1-group age size, and became less vulnerable to predation, while consuming larger and more diverse prey (Mendes *et al.*, 2014).

The 1-group fed throughout the estuary, in contrast to the 0-group flounder that fed mostly on upstream prey. These differences between feeding patterns of 0-group and 1-group flounder were clear and consistent both in stomach content and stable isotope data, despite the low number of sampled individuals (N = 42). The 1-group flounder relied equally on prey from downstream where they were more abundant (Ramos *et al.*, 2009), and upstream areas. It is of note that a limited home range and high site fidelity have been reported for juvenile flounder (Raffaelli *et al.*, 1990; Dando, 2011). However, diel and tidal migrations (Edwards & Steele, 1968; Edwards *et al.*, 1970; Gibson, 1973), to areas with high prey availability (Modin & Pihl, 1996), reflected movements between estuarine habitats. Moreover, a gradual use of the downstream areas throughout development (Kerstan, 1991; Primo *et al.*, 2013; Souza *et al.*, 2013), is typical of the flounder life cycle (Elliott & Hemingway, 2002). Thus, the wide range of 1-group flounder $\delta^{13}C$ may indicate recent migration from upstream to downstream areas, where most 1-group flounder were caught, given that muscle tissue takes many weeks to reach isotopic equilibrium (Vander Zanden *et al.*, 1997; Herzka, 2005). Overall, connectivity between upstream and downstream estuarine habitats has also been observed for 1-group flounder in other estuarine habitats (Vinagre *et al.*, 2011; Selleslagh *et al.*, 2015). Such a connectivity may also allow the use of alternative resources if one of the habitats is compromised (i.e. resource partitioning), hence increasing the ability of flounder to tolerate environmental change (Selleslagh *et al.*, 2015). Overall, these feeding strategies corroborated the life cycle described for the European flounder in the Lima estuary (Ramos *et al.*, 2010; Mendes *et al.*, 2014; Amorim *et al.*, 2018). The larvae settled in early summer in the upper estuary (Ramos *et al.*, 2010; Amorim *et al.*, 2016) that was also the main feeding habitat of the 0-group flounder. The juveniles increasingly used the middle estuary as they grew (Ramos *et al.*, 2009; Mendes *et al.*, 2014; Amorim *et al.*, 2018), with the 1-group flounder feeding between upstream and downstream areas. Adults representing 23% of the total population were mostly found in the lower and middle estuaries (Ramos *et al.*, 2010). These differences in habitat use and feeding strategies between life-stages may prevent intraspecific competition.

Feeding strategies promoting European flounder condition in the Lima estuary and management implications

The issue of food limitation in nursery habitats is still debated (Le Pape & Bonhommeau, 2015), as several authors suggest that feeding on abundant prey (van der Veer *et al.*, 2000; Amara *et al.*, 2009; Selleslagh & Amara, 2013), and resource partitioning strategies (Evans, 1983; Besyst *et al.*, 1999; Hampel *et al.*, 2005; Haynes *et al.*, 2011) may reduce the effects of niche overlap and prevent competition. Accordingly, the distinct isotopic signatures of 0-group and 1-group flounder and their feeding on highly abundant prey (e.g. *Corophium* and polychaetes) in the Lima estuary (Sousa *et al.*, 2006), did not support evidence for competition. Moreover, the morphometric condition (mean = 1.06 ± 0.07) of European flounder juveniles in the Lima estuary was within the same range observed for this species in estuaries such as Canche (mean = 0.89), Authie (mean = 1.0) (Amara *et al.*, 2009), Minho, Mondego and Douro (mean = 0.70, Vasconcelos *et al.*, 2009) suggesting good somatic condition. Thus, the differential distribution and feeding strategies of the 0-group and 1-group flounder may have minimized competition and sustained somatic condition over the summer months preceding collection of juveniles. Overall, these results did not suggest food limitation, i.e. the carrying capacity of the Lima estuary may not have been fully exploited, at least at the time scale integrated by the Fulton K and stable isotope analysis. However, it has been suggested that limitation of food resources may be underestimated by individual condition indices (Le Pape & Bonhommeau, 2015), as size-selective mortality favours survival of fast-growing individuals (Sogard, 1997). The growth of the surviving individuals would be close to maximal values even when there was food limitation (Le Pape & Bonhommeau, 2015). Thus, further studies are required to estimate the relative amounts of prey available to predators and carrying capacity.

The RNA:DNA of the European flounder in the Lima estuary (mean = 1.57 ± 0.62) was well above the critical starvation value (0.32), and close to optimal feeding values (1.71) defined for newly settled plaice *Pleuronectes platessa* L. 1758 (Selleslagh & Amara, 2013). However, it was lower than RNA:DNA of well-fed metamorphosing flounder (>2, O'Neill *et al.*, 2011), common sole *Solea solea* L., 1758 (>1.5, Richard *et al.*, 1991) and Japanese flounder *Paralichthys olivaceus* Temminck and Schlegel, 1846 (>2, Gwak & Tanaka, 2001). These comparisons must be taken with caution as RNA:DNA is species and size dependent (Ferron & Leggett, 1994; Buckley *et al.*, 2008; Tanaka *et al.*, 2008). Decreased growth rates of juvenile flounder have been linked to food limitation, which may be caused by changes in prey quality and availability (Teal *et al.*, 2008), and intraspecific (Edwards *et al.*, 1970; Laffargue *et al.*, 2007) and interspecific competition (Jager *et al.*, 1995; van der Veer *et al.*, 2010; Freitas *et al.*, 2012; Ciotti *et al.*, 2013*b*). It is of note that European flounder juveniles were sampled in the late summer when sub-optimal growth has been suggested for juveniles of this species (Jager *et al.*, 1995; Freitas *et al.*, 2012), and similar to other flatfish such as plaice (Freitas *et al.*, 2012; Ciotti *et al.*, 2013*a*; van der Veer *et al.*, 2016) and common sole (Fonseca *et al.*, 2006; Laffargue *et al.*, 2007; Teal *et al.*, 2008). However, Poiesz *et al.* (2019) found no trend in flounder growth over time in the Wadden Sea, and suggested that this species, as an epibenthic predator, was not affected by reduced infauna activity in late summer, and thus reduced prey availability, in contrast to plaice which is a benthic feeder (van der Veer *et al.*, 2016). The RNA:DNA ratio of European flounder in the Lima estuary was lower than in the nearby Minho and Douro (Vasconcelos *et al.*, 2009), while in the same range as Canche, Authie (Amara *et al.*, 2009) and Mondego (Vasconcelos *et al.*, 2009). Although this may suggest suboptimal food conditions, differences in methodology may limit comparisons of the RNA:DNA ratio between studies (Caldarone *et al.*, 2006), and temperature effects must also be considered. Indeed, temperature is a controller of enzymatic activity and metabolic reactions (Fry, 1971), and influences RNA synthesis and activity (Buckley *et al.*, 2008). Therefore, temperature affects RNA:DNA ratios and the relationship between RNA:DNA and growth (Ferron & Leggett, 1994; Buckley *et al.*, 1999). Lower temperatures were observed in Lima downstream areas (T = 14.7 ± 0.7) compared with upstream areas (T = 21.4 ± 1.1°C). Therefore, temperature differences between the Lima estuary (T = 17.1 ± 3.4°C) and other estuaries within the same geographic

https://doi.org/10.1017/S0025315420000600 Published online by Cambridge University Press

area (>20°C, Vasconcelos *et al.*, 2009) may have contributed to the lower RNA:DNA observed in this study, even though temperature in the upper estuary was within the optimal range (18–22°C) for flounder growth (Fonds *et al.*, 1992). The different lag response to environmental conditions of the Fulton K and the RNA:DNA ratio may explain the lack of a correlation between these indices (Suthers *et al.*, 1992; Ferron & Leggett, 1994; De Raedemaecker *et al.*, 2012*b*). Hence, the Lima estuary appeared to promote good condition of the juveniles during summer, while short-term changes in environmental conditions or prey availability and competition may justify the low RNA:DNA as this index is sensitive to fish recent feeding (Clemmesen, 1994; Malloy & Targett, 1994), and condition (Buckley, 1984; Gwak & Tanaka, 2001). These condition patterns observed over a brief summer period may not be reflected at the seasonal and interannual scales.

Historical habitat loss is a major environmental problem in estuaries (Wolanski & Elliott, 2015; Amorim *et al.*, 2017), and there are increasing measures to remedy that loss (Elliott *et al.*, 2016). Coordinated management initiatives (e.g. Lonsdale *et al.*, 2015) aim to balance the effects of the various estuarine users, and their demands on the systems. The Lima estuary is located at the southern limit of the distribution of flounder where increased temperatures, especially in the shallow upstream areas, may limit the food and habitat available with effects on juvenile flounder condition and growth (Freitas *et al.*, 2012). Hence, the present study is important in determining which species and stages use habitats within and adjacent to the estuary, thereby guiding the habitat management within the estuary. Further studies are required to assess how the juvenile flounder condition will respond to changes in food availability and temperature related to climate change.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0025315420000600.

Acknowledgements. The authors wish to thank two anonymous referees and the Editor for their valuable comments made on an earlier version of the paper.

Financial support. This research was partially supported by FCT – The Portuguese Foundation for Science and Technology through the PhD fellowship awarded to C. Mendes (SFRH/BD/86325/2012). This study was also partially funded through the project "Mytag – Integrating natural and artificial tags to reconstruct fish migrations and ontogenetic niche shifts" (PTDC/MAR-EST/2098/2014), under the Project 9471 – Reforçar a Investigação, o Desenvolvimento Tecnológico e a Inovação (Projeto 9471-RIDTI) and subsidized by the European Regional Development Fund (FEDER, POCI-01-0145-FEDER- 016787), and by the Strategic Funding UID/Multi/04423/2013 and UID/Multi/04423/2019 through national funds provided by FCT – Foundation for Science and Technology and European Regional Development Fund (ERDF), in the framework of PT2020.

References

Aarnio K, Bonsdorff E and Rosenback N (1996) Food and feeding habits of juvenile flounder *Platichthys flesus* (L.), and turbot *Scophthalmus maximus* L. in the Åland archipelago, northern Baltic Sea. *Journal of Sea Research* **36**, 311–320.

Amara R, Selleslagh J, Billon G and Minier C (2009) Growth and condition of 0-group European flounder, *Platichthys flesus* as indicator of estuarine habitat quality. *Hydrobiologia* **627**, 87.

Amorim E, Ramos S, Elliott M and Bordalo AA (2016) Immigration and early life stages recruitment of the European flounder (*Platichthys flesus*) to an estuarine nursery: the influence of environmental factors. *Journal of Sea Research* **107**, 56–66.

Amorim E, Ramos S, Elliott M, Franco A and Bordalo AA (2017) Habitat loss and gain: influence on habitat attractiveness for estuarine fish communities. *Estuarine, Coastal and Shelf Science* **197**(Suppl. C), 244–257.

Amorim E, Ramos S, Elliott M and Bordalo AA (2018) Dynamic habitat use of an estuarine nursery seascape: ontogenetic shifts in habitat suitability of the European flounder (*Platichthys flesus*). *Journal of Experimental Marine Biology and Ecology* **506**, 49–60.

Andersen AK, Schou J, Sparrevohn CR, Nicolajsen H and Støttrup JG (2005*a*) The quality of release habitat for reared juvenile flounder, *Platichthys flesus*, with respect to salinity and depth. *Fisheries Management and Ecology* **12**, 211–219.

Andersen BS, Carl JD, Grønkjær P and Støttrup JG (2005*b*) Feeding ecology and growth of age 0 year *Platichthys flesus* (L.) in a vegetated and a bare sand habitat in a nutrient rich fjord. *Journal of Fish Biology* **66**, 531–552.

Anderson MJ, Gorley RN and Clarke KR (2008) *PERMANOVA + for PRIMER: Guide to Software and Statistical Methods*. Plymouth: Primer-E.

Armitage PD, Pinder LC and Cranston P (1995) *The Chironomidae: Biology and Ecology of Non-Biting Midges*. London: Chapman and Hall.

Beaumont WRC and Mann RHK (1984) The age, growth and diet of a freshwater population of the flounder, *Platichthys flesus* (L.), in southern England. *Journal of Fish Biology* **25**, 607–616.

Beck MW, Heck KL, Able KW, Childers DL, Eggleston DB, Gillanders BM, Halpern B, Hays CG, Hoshino K, Minello TJ, Orth RJ, Sheridan PF and Weinstein MP (2001) The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. *BioScience* **51**, 633–641.

Besyst B, Cattrijsse A and Mees J (1999) Feeding ecology of juvenile flatfishes of the surf zone of a sandy beach. *Journal of Fish Biology* **55**, 1171–1186.

Boesch DF and Turner RE (1984) Dependence of fishery species on salt marshes: the role of food and refuge. *Estuaries* **7**, 460–468.

Bos AR (1999) Tidal transport of flounder larvae (*Platichthys flesus*) in the Elbe River, Germany. *Archive of Fishery and Marine Research* **47**, 47–60.

Buckley LJ (1984) RNA-DNA ratio: an index of larval fish growth in the sea. *Marine Biology* **80**, 291–298.

Buckley L, Caldarone E and Ong T-L (1999) RNA–DNA ratio and other nucleic acid-based indicators for growth and condition of marine fishes. *Hydrobiologia* **401**, 265–277.

Buckley LJ, Caldarone EM and Clemmesen C (2008) Multi-species larval fish growth model based on temperature and fluorometrically derived RNA/DNA ratios: results from a meta-analysis. *Marine Ecology Progress Series* **371**, 221–232.

Bulow FJ (1970) RNA–DNA ratios as indicators of recent growth rates of a fish. *Journal of the Fisheries Research Board of Canada* **27**, 2343–2349.

Burrows MT, Gontarek SJ, Nash RDM and Gibson RN (2001) Shrimp predation on 0-group plaice: contrasts between field data and predictions of an individual-based model. *Journal of Sea Research* **45**, 243–254.

Cabral H and Costa MJ (1999) Differential use of nursery areas within the Tagus Estuary by sympatric soles, *Solea solea* and *Solea senegalensis*. *Environmental Biology of Fishes* **56**, 389–397.

Cabral HN, Lopes M and Loeper R (2002) Trophic niche overlap between flatfishes in a nursery area on the Portuguese coast. *Scientia Marina* **66**, 293–300.

Cabral HN, Vasconcelos R, Vinagre C, França S, Fonseca V, Maia A, Reis-Santos P, Lopes M, Ruano M, Campos J, Freitas V, Santos PT and Costa MJ (2007) Relative importance of estuarine flatfish nurseries along the Portuguese coast. *Journal of Sea Research* **57**, 209–217.

Caldarone EM, Wagner M, Onge-Burns JS and Buckley LJ (2001) *Protocol and Guide for Estimating Nucleic Acids in Larval Fish Using a Fluorescence Microplate Reader*. Northeast Fisheries Science Center (Reference Document) 01-11, 1–22.

Caldarone EM, Clemmesen CM, Berdalet E, Miller TJ, Folkvord A, Holt GJ, Olivar MP and Suthers IM (2006) Intercalibration of four spectrofluorometric protocols for measuring RNA/DNA ratios in larval and juvenile fish. *Limnology and Oceanography: Methods* **4**, 153–163.

Caldarone EM, MacLean SA and Sharack B (2012) Evaluation of bioelectrical impedance analysis and Fulton's condition factor as nonlethal techniques for estimating short-term responses in postsmolt Atlantic salmon (*Salmo salar*) to food availability. *Fishery Bulletin* **110**, 257–270.

Chícharo M and Chícharo L (2008) RNA:DNA ratio and other nucleic acid derived indices in marine ecology. *International Journal of Molecular Sciences* **9**, 1453–1471.

Ciotti BJ, Targett TE and Burrows MT (2013*a*) Decline in growth rate of juvenile European plaice (*Pleuronectes platessa*) during summer at nursery beaches along the west coast of Scotland. *Canadian Journal of Fisheries and Aquatic Sciences* **70**, 720–734.

Ciotti BJ, Targett TE, Nash RDM and Burrows MT (2013*b*) Small-scale spatial and temporal heterogeneity in growth and condition of juvenile fish on sandy beaches. *Journal of Experimental Marine Biology and Ecology* **448**, 346–359.

Clarke KR and Gorley RN (2006) *Primer V6: User Manual – Tutorial*. Plymouth: Plymouth Marine Laboratory.

Clemmesen C (1994) The effect of food availability, age or size on the RNA/DNA ratio of individually measured herring larvae: laboratory calibration. *Marine Biology* **118**, 377–382.

Dando PR (2011) Site fidelity, homing and spawning migrations of flounder *Platichthys flesus* in the Tamar estuary, South West England. *Marine Ecology Progress Series* **430**, 183–196.

Darnaude AM, Harmelin-Vivien ML and Salen-Picard C (2001) Food partitioning among flatfish (Pisces: Pleuronectiforms) juveniles in a Mediterranean coastal shallow sandy area. *Journal of the Marine Biological Association of the United Kingdom* **81**, 119–127.

Darnaude AM, Salen-Picard C and Harmelin-Vivien ML (2004) Depth variation in terrestrial particulate organic matter exploitation by marine coastal benthic communities off the Rhone River delta (NW Mediterranean). *Marine Ecology Progress Series* **275**, 47–57.

De Groot SJ (1971) On the interrelationships between morphology of the alimentary tract, food and feeding behaviour in flatfishes (Pisces: Pleuronectiformes). *Netherlands Journal of Sea Research* **5**, 121–196.

DeNiro MJ and Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* **42**, 495–506.

DeNiro MJ and Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* **45**, 341–351.

De Raedemaecker F, Brophy D, O'Connor I and Comerford S (2012*a*) Habitat characteristics promoting high density and condition of juvenile flatfish at nursery grounds on the west coast of Ireland. *Journal of Sea Research* **73**, 7–17.

De Raedemaecker F, Brophy D, O'Connor I and O'Neill B (2012*b*) Dependence of RNA:DNA ratios and Fulton's K condition indices on environmental characteristics of plaice and dab nursery grounds. *Estuarine, Coastal and Shelf Science* **98**(Suppl. C), 60–70.

Dias E, Morais P, Faria AM, Antunes C and Hoffman JC (2017) Benthic food webs support the production of sympatric flatfish larvae in estuarine nursery habitat. *Fisheries Oceanography* **26**, 507–512.

Dinis MT (1986) *Quatre Soleidae de l'estuaire du Tage. Reproduction et croissance. Essai d'elevage de Solea senegalensis Kaup* (Ph.D. dissertation). Université de Bretagne Occidentale, Brest, France.

Doornbos G and Twisk F (1984) Density, growth and annual food consumption of plaice (*Pleuronectes platessa* L.) and flounder (*Platichthys flesus* (L.)) in Lake Grevelingen, the Netherlands. *Netherlands Journal of Sea Research* **18**, 434–456.

Edwards R and Steele JH (1968) The ecology of 0-group plaice and common dabs at Loch Ewe I. Population and food. *Journal of Experimental Marine Biology and Ecology* **2**, 215–238.

Edwards RRC, Steele JH and Trevallion A (1970) The ecology of 0-group plaice and common dabs in Loch Ewe. III. Prey-predator experiments with plaice. *Journal of Experimental Marine Biology and Ecology* **4**, 156–173.

Elliott M and Hemingway KL (2002) *Fishes in Estuaries*. Oxford: Blackwell Science.

Elliott M, Burdon D, Hemingway KL and Apitz SE (2007) Estuarine, coastal and marine ecosystem restoration: confusing management and science – a revision of concepts. *Estuarine, Coastal and Shelf Science* **74**, 349–366.

Elliott M, Mander L, Mazik K, Simenstad C, Valesini F, Whitfield A and Wolanski E (2016) Ecoengineering with ecohydrology: successes and failures in estuarine restoration. *Estuarine, Coastal and Shelf Science* **176**, 12–35.

Evans S (1983) Production, predation and food niche segregation in a marine shallow soft-bottom community. *Marine Ecology Progress Series* **10**, 147–157.

Ferron A and Leggett WC (1994) An appraisal of condition measures for marine fish larvae. *Advances in Marine Biology* **30**, 217–303.

Florin A-B and Lavados G (2010) Feeding habits of juvenile flatfish in relation to habitat characteristics in the Baltic Sea. *Estuarine, Coastal and Shelf Science* **86**, 607–612.

Foley CJ, Bradley DL and Höök TO (2016) A review and assessment of the potential use of RNA:DNA ratios to assess the condition of entrained fish larvae. *Ecological Indicators* **60**, 346–357.

Fonds M, Cronie R, Vethaak AD and Van Der Puyl P (1992) Metabolism, food consumption and growth of plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*) in relation to fish size and temperature. *Netherlands Journal of Sea Research* **29**, 127–143.

Fonseca VF, Vinagre C and Cabral HN (2006) Growth variability of juvenile soles *Solea solea* and *Solea senegalensis*, and comparison with RNA:DNA ratios in the Tagus estuary, Portugal. *Journal of Fish Biology* **68**, 1551–1562.

França S, Vasconcelos RP, Tanner S, Máguas C, Costa MJ and Cabral HN (2011) Assessing food web dynamics and relative importance of organic matter sources for fish species in two Portuguese estuaries: a stable isotope approach. *Marine Environmental Research* **72**, 204–215.

Freitas V, Costa-Dias S, Campos J, Bio A, Santos P and Antunes C (2009) Patterns in abundance and distribution of juvenile flounder, *Platichthys flesus*, in Minho estuary (NW Iberian Peninsula). *Aquatic Ecology* **43**, 1143.

Freitas V, Kooijman S and van der Veer HW (2012) Latitudinal trends in habitat quality of shallow-water flatfish nurseries. *Marine Ecology Progress Series* **471**, 203–214.

Fry FEJ (1971) 1 – The effect of environmental factors on the physiology of fish. In Hoar WS and Randall DJ (eds), *Fish Physiology*, vol. 6. London: Academic Press, pp. 1–98.

Fry B (2002) Conservative mixing of stable isotopes across estuarine salinity gradients: a conceptual framework for monitoring watershed influences on downstream fisheries production. *Estuaries* **25**, 264–271.

Fry B and Sherr EB (1989) Δ13C measurements as indicators of carbon flow in marine and freshwater ecosystems. In Rundel PW, Ehleringer JR and Nagy KA (eds), *Stable Isotopes in Ecological Research*. New York, NY: Springer, pp. 196–229.

Gerdol V and Hughes RG (1995) Feeding behaviour and diet of *Corophium volutator* in an estuary in southeastern England. *Oceanographic Literature Review* **42**, 391.

Gibson RN (1973) Tidal and circadian activity rhythms in juvenile plaice, *Pleuronectes platessa*. *Marine Biology* **22**, 379–386.

Gibson RN (1994) Impact of habitat quality and quantity on the recruitment of juvenile flatfishes. *Netherlands Journal of Sea Research* **32**, 191–206.

Gilliers C, Amara R and Bergeron J-P (2004) Comparison of growth and condition indices of juvenile flatfish in different coastal nursery grounds. *Environmental Biology of Fishes* **71**, 189–198.

Green BC, Smith DJ, Grey J and Underwood GJC (2012) High site fidelity and low site connectivity in temperate salt marsh fish populations: a stable isotope approach. *Oecologia* **168**, 245–255.

Grønkjær P, Carl JD, Rasmussen TH and Hansen KW (2007) Effect of habitat shifts on feeding behaviour and growth of 0 year-group flounder *Platichthys flesus* (L.) transferred between macroalgae and bare sand habitats. *Journal of Fish Biology* **70**, 1587–1605.

Gutt J (1985) The growth of juvenile flounders (*Platichthys flesus* L.) at salinities of 0, 5, 15 and 35%%. *Journal of Applied Ichthyology* **1**, 17–26.

Gwak WS and Tanaka M (2001) Developmental change in RNA: DNA ratios of fed and starved laboratory-reared Japanese flounder larvae and juveniles, and its application to assessment of nutritional condition for wild fish. *Journal of Fish Biology* **59**, 902–915.

Hampel H, Cattrijsse A and Elliott M (2005) Feeding habits of young predatory fishes in marsh creeks situated along the salinity gradient of the Schelde estuary, Belgium and the Netherlands. *Helgoland Marine Research* **59**, 151–162.

Harmelin-Vivien M, Loizeau V, Mellon C, Beker B, Arlhac D, Bodiguel X, Ferraton F, Hermand R, Philippon X and Salen-Picard C (2008) Comparison of C and N stable isotope ratios between surface particulate organic matter and microphytoplankton in the Gulf of Lions (NW Mediterranean). *Continental Shelf Research* **28**, 1911–1919.

Haynes PS, Brophy D, De Raedemaecker F and McGrath D (2011) The feeding ecology of 0 year-group turbot *Scophthalmus maximus* and brill *Scophthalmus rhombus* on Irish west coast nursery grounds. *Journal of Fish Biology* **79**, 1866–1882.

Henderson PA and Holmes RHA (1991) On the population dynamics of dab, sole and flounder within Bridgwater Bay in the lower Severn Estuary, England. *Netherlands Journal of Sea Research* **27**, 337–344.

Herzka SZ (2005) Assessing connectivity of estuarine fishes based on stable isotope ratio analysis. *Estuarine, Coastal and Shelf Science* **64**, 58–69.

Hoffman JC, Kelly JR, Peterson GS and Cotter AM (2015) Landscape-scale food webs of fish nursery habitat along a river-coast mixing zone. *Estuaries and Coasts* **38**, 1335–1349.

Hyslop EJ (1980) Stomach contents analysis – a review of methods and their application. *Journal of Fish Biology* **17**, 411–429.

Jager Z, Kleef HL and Tydeman P (1995) Mortality and growth of 0-group flatfish in the brackish dollard (Ems Estuary, Wadden Sea). *Netherlands Journal of Sea Research* **34**, 119–129.

Kennedy P, Kennedy H and Papadimitriou S (2005) The effect of acidification on the determination of organic carbon, total nitrogen and their stable isotopic composition in algae and marine sediment. *Rapid Communications in Mass Spectrometry* **19**, 1063–1068.

Kerstan M (1991) The importance of rivers as nursery grounds for 0- and 1-group flounder (*Platichthys flesus* L.) in comparison to the Wadden Sea. *Netherlands Journal of Sea Research* **27**, 353–366.

Kopp D, Le Bris H, Grimaud L, Nérot C and Brind'Amour A (2013) Spatial analysis of the trophic interactions between two juvenile fish species and their preys along a coastal–estuarine gradient. *Journal of Sea Research* **81**, 40–48.

Kostecki C, Roussel JM, Desroy N, Roussel G, Lanshere J, Le Bris H and Le Pape O (2012) Trophic ecology of juvenile flatfish in a coastal nursery ground: contributions of intertidal primary production and freshwater particulate organic matter. *Marine Ecology Progress Series* **449**, 221–232.

Laffargue P, Lagardère F, Rijnsdorp AD, Fillon A and Amara R (2007) Growth performances of juvenile sole *Solea solea* under environmental constraints of embayed nursery areas. *Aquatic Living Resources* **20**, 213–221.

Le Pape O and Bonhommeau S (2015) The food limitation hypothesis for juvenile marine fish. *Fish and Fisheries* **16**, 373–398.

Le Pape O and Cognez N (2016) The range of juvenile movements of estuarine and coastal nursery dependent flatfishes: estimation from a meta-analytical approach. *Journal of Sea Research* **107**(Part 1), 43–55.

Le Pape O, Modéran J, Beaunée G, Riera P, Nicolas D, Savoye N, Harmelin-Vivien M, Darnaude AM, Brind'Amour A, Le Bris H, Cabral H, Vinagre C, Pasquaud S, França S and Kostecki C (2013) Sources of organic matter for flatfish juveniles in coastal and estuarine nursery grounds: a meta-analysis for the common sole (*Solea solea*) in contrasted systems of Western Europe. *Journal of Sea Research* **75**, 85–95.

Lonsdale J-A, Weston K, Barnard S, Boyes SJ and Elliott M (2015) Integrating management tools and concepts to develop an estuarine planning support system: a case study of the Humber Estuary, eastern England. *Marine Pollution Bulletin* **100**, 393–405.

Malloy KD and Targett TE (1994) The use of RNA:DNA ratios to predict growth limitation of juvenile summer flounder (*Paralichthys dentatus*) from Delaware and North Carolina estuaries. *Marine Biology* **118**, 367–375.

Marshall S and Elliott M (1997) A comparison of univariate and multivariate numerical and graphical techniques for determining inter- and intraspecific feeding relationships in estuarine fish. *Journal of Fish Biology* **51**, 526–545.

Martinho F, Leitão R, Neto JM, Cabral HN, Marques JC and Pardal MA (2007) The use of nursery areas by juvenile fish in a temperate estuary, Portugal. *Hydrobiologia* **587**, 281–290.

Martinho F, Leitão R, Neto JM, Cabral H, Lagardère F and Pardal MA (2008) Estuarine colonization, population structure and nursery functioning for 0-group sea bass (*Dicentrarchus labrax*), flounder (*Platichthys flesus*) and sole (*Solea solea*) in a mesotidal temperate estuary. *Journal of Applied Ichthyology* **24**, 229–237.

McLusky DS and Elliott M (2004) *The Estuarine Ecosystem: Ecology, Threats and Management*. Oxford: Oxford University Press.

Mendes C, Ramos S and Bordalo AA (2014) Feeding ecology of juvenile flounder *Platichthys flesus* in an estuarine nursery habitat: influence of prey–predator interactions. *Journal of Experimental Marine Biology and Ecology* **461**, 458–468.

Minagawa M and Wada E (1984) Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between δ15N and animal age. *Geochimica et Cosmochimica Acta* **48**, 1135–1140.

Modin J and Pihl L (1996) Small-scale distribution of juvenile plaice and flounder in relation to predatory shrimp in a shallow Swedish bay. *Journal of Fish Biology* **49**, 1070–1085.

Molinero A and Flos R (1992) Influence of season on the feeding habits of the common sole *Solea solea*. *Marine Biology* **113**, 499–507.

Moore JW and Moore IA (1976) The basis of food selection in flounders, *Platichthys flesus* (L.), in the Severn Estuary. *Journal of Fish Biology* **9**, 139–156.

Muus BJ and Nielsen JG (1999) *Sea Fish*. Hedehusene: Scandinavian Fishing Year Book.

Ng JSS, Wai T-C and Williams GA (2007) The effects of acidification on the stable isotope signatures of marine algae and molluscs. *Marine Chemistry* **103**, 97–102.

Nielsen JG (1986) Pleuronectidae. In Whitehead PJP, Bauchot ML, Hureau JC, Nielsen J and Tortonese E (eds), *Fishes of the North-Eastern Atlantic and Mediterranean*. Paris: UNESCO, pp. 1299–1307.

Nunn AD, Tewson LH and Cowx IG (2012) The foraging ecology of larval and juvenile fishes. *Reviews in Fish Biology and Fisheries* **22**, 377–408.

O'Neill B, De Raedemaecker F, McGrath D and Brophy D (2011) An experimental investigation of salinity effects on growth, development and condition in the European flounder (*Platichthys flesus* L.). *Journal of Experimental Marine Biology and Ecology* **410**, 39–44.

Palmer SCF, Coulon A and Travis JMJ (2014) Inter-individual variability in dispersal behaviours impacts connectivity estimates. *Oikos* **123**, 923–932.

Parnell AC, Inger R, Bearhop S and Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. *PLoS ONE* **5**, e9672.

Parnell AC, Phillips DL, Bearhop S, Semmens BX, Ward EJ, Moore JW, Jackson AL, Grey J, Kelly DJ and Inger R (2013) Bayesian stable isotope mixing models. *Environmetrics* **24**, 387–399.

Pasquaud S, Elie P, Jeantet C, Billy I, Martinez P and Girardin M (2008) A preliminary investigation of the fish food web in the Gironde estuary, France, using dietary and stable isotope analyses. *Estuarine, Coastal and Shelf Science* **78**, 267–279.

Peterson BJ and Fry B (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* **18**, 293–320.

Phillips DL, Inger R, Bearhop S, Jackson AL, Moore JW, Parnell AC, Semmens BX and Ward EJ (2014) Best practices for use of stable isotope mixing models in food-web studies. *Canadian Journal of Zoology* **92**, 823–835.

Piet GJ, Pfisterer AB and Rijnsdorp AD (1998) On factors structuring the flatfish assemblage in the southern North Sea. *Journal of Sea Research* **40**, 143–152.

Pihl L, Cattrijsse A, Codling I, Mathieson S, McLusky DS and Roberts C (2007) Habitat use by fishes in estuaries and other brackish areas. *Fishes in Estuaries*. Oxford: Blackwell Science, pp. 10–53.

Poiesz SSH, de Vries A, Cardoso JFMF, Witte JIJ, van der Veer HW and Freitas V (2019) A comparison of growth in two juvenile flatfish species in the Dutch Wadden Sea: searching for a mechanism for summer growth reduction in flatfish nurseries. *Journal of Sea Research* **144**, 39–48.

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* **83**, 703–718.

Potter IC, Tweedley JR, Elliott M and Whitfield AK (2015) The ways in which fish use estuaries: a refinement and expansion of the guild approach. *Fish and Fisheries* **16**, 230–239.

Power M, Attrill MJ and Thomas RM (2000) Environmental factors and interactions affecting the temporal abundance of juvenile flatfish in the Thames Estuary. *Journal of Sea Research* **43**, 135–149.

Preston T and Owens NJP (1983) Interfacing an automatic elemental analyser with an isotope ratio mass spectrometer: the potential for fully automated total nitrogen and nitrogen-15 analysis. *The Analyst* **108**, 971–977.

Primo AL, Azeiteiro UM, Marques SC, Martinho F, Baptista J and Pardal MA (2013) Colonization and nursery habitat use patterns of larval and juvenile flatfish species in a small temperate estuary. *Journal of Sea Research* **76**(Suppl. C), 126–134.

Raffaelli D, Conacher A, McLachlan H and Emes C (1989) The role of epibenthic crustacean predators in an estuarine food web. *Estuarine, Coastal and Shelf Science* **28**, 149–160.

Raffaelli D, Richner H, Summers R and Northcott S (1990) Tidal migrations in the flounder (*Platichthys flesus*). *Marine Behaviour and Physiology* **16**, 249–260.

Ramos S, Cowen RK, Ré P and Bordalo AA (2006) Temporal and spatial distributions of larval fish assemblages in the Lima estuary (Portugal). *Estuarine, Coastal and Shelf Science* **66**, 303–314.

Ramos S, Ré P and Bordalo AA (2009) Environmental control on early life stages of flatfishes in the Lima Estuary (NW Portugal). *Estuarine. Coastal and Shelf Science* **83**, 252–264.

Ramos S, Ré P and Bordalo AA (2010) Recruitment of flatfish species to an estuarine nursery habitat (Lima Estuary, NW Iberian Peninsula). *Journal of Sea Research* **64**, 473–486.

Ramos S, Paris CB and Angélico MM (2017) Larval fish dispersal along an estuarine–ocean gradient. *Canadian Journal of Fisheries and Aquatic Sciences* **74**, 1462–1473.

https://doi.org/10.1017/S0025315420000600 Published online by Cambridge University Press

R Development Core Team (2007) *R: A Language and Environment for Statistical Computing*. Vienna: R foundation for Statistical Computing.

Richard P, Bergeron J-P, Boulhic M, Galois R and Person-Le Ruyet J (1991) Effect of starvation on RNA, DNA and protein content of laboratory-reared larvae and juveniles of *Solea solea*. *Marine Ecology Progress Series* **72**, 69–77.

Ricker WE (1975) *Computation and Interpretation of Biological Statistics of Fish Populations*. Ottawa: Bulletin of the Fisheries Research Board of Canada.

Rijnsdorp AD, Berghahn R, Miller JM and Van Der Veer HW (1995) Recruitment mechanisms in flatfish: what did we learn and where do we go? *Netherlands Journal of Sea Research* **34**, 237–242.

Russo T, Pulcini D, O'Leary Á, Cataudella S and Mariani S (2008) Relationship between body shape and trophic niche segregation in two closely related sympatric fishes. *Journal of Fish Biology* **73**, 809–828.

Selleslagh J and Amara R (2013) Effect of starvation on condition and growth of juvenile plaice *Pleuronectes platessa*: nursery habitat quality assessment during the settlement period. *Journal of the Marine Biological Association of the United Kingdom* **93**, 479–488.

Selleslagh J, Blanchet H, Bachelet G and Lobry J (2015) Feeding habitats, connectivity and origin of organic matter supporting fish populations in an estuary with a reduced intertidal area assessed by stable isotope analysis. *Estuaries and Coasts* **38**, 1431–1447.

Sheaves M (2009) Consequences of ecological connectivity: the coastal ecosystem mosaic. *Marine Ecology Progress Series* **391**, 107–115.

Sheaves M, Baker R, Nagelkerken I and Connolly RM (2015) True value of estuarine and coastal nurseries for fish: incorporating complexity and dynamics. *Estuaries and Coasts* **38**, 401–414.

Sogard SM (1997) Size-selective mortality in the juvenile stage of teleost fishes: a review. *Bulletin of Marine Science* **60**, 1129–1157.

Sousa R, Dias S and Antunes JC (2006) Spatial subtidal macrobenthic distribution in relation to abiotic conditions in the Lima Estuary, NW of Portugal. *Hydrobiologia* **559**, 135–148.

Souza AT, Dias E, Nogueira A, Campos J, Marques JC and Martins I (2013) Population ecology and habitat preferences of juvenile flounder *Platichthys flesus* (Actinopterygii: Pleuronectidae) in a temperate estuary. *Journal of Sea Research* **79**(Suppl. C), 60–69.

Summers RW (1979) Life cycle and population ecology of the flounder *Platichthys flesus* (L.) in the Ythan estuary, Scotland. *Journal of Natural History* **13**, 703–723.

Summers RW (1980) The diet and feeding behaviour of the flounder *Platichthys flesus* (L.) in the Ythan estuary, Aberdeenshire, Scotland. *Estuarine and Coastal Marine Science* **11**, 217–232.

Suthers IM, Fraser A and Frank KT (1992) Comparison of lipid, otolith and morphometric condition indices of pelagic juvenile cod *Gadus morhua* from the Canadian Atlantic. *Marine Ecology Progress Series* **84**, 31–40.

Suzuki KW, Kasai A, Isoda T, Nakayama K and Tanaka M (2008) Distinctive stable isotope ratios in important zooplankton species in relation to estuarine salinity gradients: potential tracer of fish migration. *Estuarine, Coastal and Shelf Science* **78**, 541–550.

Tableau A, Brind'Amour A, Woillez M and Le Bris H (2016) Influence of food availability on the spatial distribution of juvenile fish within soft sediment nursery habitats. *Journal of Sea Research* **111**, 76–87.

Tanaka Y, Satoh K, Yamada H, Takebe T, Nikaido H and Shiozawa S (2008) Assessment of the nutritional status of field-caught larval Pacific bluefin tuna by RNA/DNA ratio based on a starvation experiment of hatchery-reared fish. *Journal of Experimental Marine Biology and Ecology* **354**, 56–64.

Teal LR, de Leeuw JJ, van der Veer HW and Rijnsdorp AD (2008) Effects of climate change on growth of 0-group sole and plaice. *Marine Ecology Progress Series* **358**, 219–230.

van der Veer HW and Bergman MJN (1987) Predation by crustaceans on a newly settled 0-group plaice *Pleuronectes platessa* population in the western Wadden Sea. *Marine Ecology Progress Series* **35**, 203–215.

van der Veer HW, Bergman MJN, Dapper R and Witte JIJ (1991) Population dynamics of an intertidal 0-group flounder *Platichthys flesus* population in the western Dutch Wadden Sea. *Marine Ecology Progress Series* **73**, 141–148.

van der Veer HW, Berghahn R, Miller JM and Rijnsdorp AD (2000) Recruitment in flatfish, with special emphasis on North Atlantic species: progress made by the Flatfish Symposia. *ICES Journal of Marine Science* **57**, 202–215.

van der Veer HW, Freitas V, Koot J, Witte JIJ and Zuur AF (2010) Food limitation in epibenthic species in temperate intertidal systems in summer: analysis of 0-group plaice *Pleuronectes platessa*. *Marine Ecology Progress Series* **416**, 215–227.

van der Veer HW, Jung AS, Freitas V, Philippart CJM and Witte JIJ (2016) Possible causes for growth variability and summer growth reduction in juvenile plaice *Pleuronectes platessa* L. in the western Dutch Wadden Sea. *Journal of Sea Research* **111**, 97–106.

Vander Zanden MJ, Cabana G and Rasmussen JB (1997) Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios (δ^{15}N) and literature dietary data. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 1142–1158.

Vasconcelos RP, Reis-Santos P, Fonseca V, Ruano M, Tanner S, Costa MJ and Cabral HN (2009) Juvenile fish condition in estuarine nurseries along the Portuguese coast. *Estuarine, Coastal and Shelf Science* **82**, 128–138.

Vasconcelos RP, Reis-Santos P, Maia A, Fonseca V, França S, Wouters N, Costa MJ and Cabral HN (2010) Nursery use patterns of commercially important marine fish species in estuarine systems along the Portuguese coast. *Estuarine, Coastal and Shelf Science* **86**, 613–624.

Vaslet A, Phillips DL, France CAM, Feller IC and Baldwin CC (2015) Trophic behaviour of juvenile reef fishes inhabiting interlinked mangrove–seagrass habitats in offshore mangrove islets. *Journal of Fish Biology* **87**, 256–273.

Vinagre C, França S, Costa MJ and Cabral HN (2005) Niche overlap between juvenile flatfishes, *Platichthys flesus* and *Solea solea*, in a southern European estuary and adjacent coastal waters. *Journal of Applied Ichthyology* **21**, 114–120.

Vinagre C, Cabral H and Costa MJ (2008) Prey selection by flounder, *Platichthys flesus*, in the Douro estuary, Portugal. *Journal of Applied Ichthyology* **24**, 238–243.

Vinagre C, Salgado J, Cabral HN and Costa MJ (2011) Food web structure and habitat connectivity in fish estuarine nurseries – impact of river flow. *Estuaries and Coasts* **34**, 663–674.

Vizzini S, Sarà G, Michener RH and Mazzola A (2002) The role and contribution of the seagrass *Posidonia oceanica* (L.) Delile organic matter for secondary consumers as revealed by carbon and nitrogen stable isotope analysis. *Acta Oecologica* **23**, 277–285.

Wirjoatmodjo S and Pitcher TJ (1984) Flounders follow the tides to feed: evidence from ultrasonic tracking in an estuary. *Estuarine, Coastal and Shelf Science* **19**, 231–241.

Wolanski E and Elliott M (2015) *Estuarine Ecohydrology: An Introduction*. Boston, MA: Elsevier.

Złoch I and Sapota M (2010) Trophic interactions between preadult and adult *Pomatoschistus minutus* and *Pomatoschistus microps* and young *Platichthys flesus* occurring in inshore waters of the Gulf of Gdańsk (Southern Baltic). *Oceanological and Hydrobiological Studies* **39**, 37–53.

https://doi.org/10.1017/S0025315420000600 Published online by Cambridge University Press