

Trophic ecology of juvenile flatfish in a coastal nursery ground: contributions of intertidal primary production and freshwater particulate organic matter

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ABSTRACT: Coastal and estuarine nurseries are essential habitats for juvenile flatfish. These small but productive areas provide food supply and sustain adult fish populations. The Mont-Saint-Michel Bay (MSMB) supports an important flatfish nursery ground but differs from many other nursery habitats due to limited freshwater inputs. The objectives of the present study in the MSMB were to (1) use gut content analysis to identify prey of the 2 most abundant flatfish species (common sole *Solea solea* and plaice *Pleuronectes platessa*) for different juvenile age-classes (0-group and 1-group for sole, 0-group for plaice), (2) use C and N stable isotope analysis to model the production sources sustaining juvenile flatfish production, and (3) compare these results with previous knowledge of estuarine nursery grounds located in western Europe. Items found in gut contents differed between species and size classes, with juvenile plaice having a larger prey spectrum. Despite accounting for sensitivity to large uncertainties in source signatures and trophic enrichment factors, stable isotope mixing models led to robust outputs. In contrast to previous studies in estuarine nurseries, we found that microphytobenthos was the major carbon source contribution to juvenile flatfish. The contribution of freshwater particulate organic matter was nonetheless significant, in spite of the very limited river inputs to MSMB.

KEY WORDS: Microphytobenthos · Organic matter origin · Benthic food web · Coastal nursery ground · Juvenile flatfish

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INTRODUCTION

Coastal and estuarine ecosystems are the most productive environments in the world and represent half of oceanic secondary production (Costanza et al. 1997). They are essential fish habitats as they play the key role of nursery grounds for many marine species widely distributed on the continental shelf (Beck

et al. 2001, Peterson 2003), such as flatfish (van der Veer et al. 2000). The capacity and quality of these habitats have considerable influence on the renewal of marine populations (Rijnsdorp et al. 1992, Gibson 1994, Johnson et al. 1998, Peterson et al. 2000). Better understanding of the functioning of coastal and estuarine nursery grounds is crucial for the development of proper management policies (Beck et al. 2001).

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In many estuaries (Vilaine, Rhône, Thames, Gironde, Tagus, and Danube), the influence of river flow on nursery-ground carrying capacity has been established and incorporation of continental organic matter into juvenile-flatfish food webs has been documented (Le Pape et al. 2003, Darnaude et al. 2004, Leakey et al. 2008, Pasquaud et al. 2008, Vinagre et al. 2008, Banaru & Harmelin-Vivien 2009, Kostecki et al. 2010). However, the origin of food sources incorporated into juvenile-flatfish food webs is still not understood in the case of non-estuarine nursery areas.

The Mont-Saint-Michel Bay (MSMB) in France is located in the English Channel in western Europe (see Fig. 1). It is a vast, productive bay supporting nursery areas for several marine species (Kostecki et al. 2011), particularly the common sole *Solea solea* (L., 1758) and plaice *Pleuronectes platessa* (L., 1758). In this bay, freshwater influence is limited by low river discharge and high hydrodynamic circulation linked to the megatidal regime (Cugier et al. 2010). Primary production in the MSMB is largely based on salt marshes and benthic diatoms, and secondary production is enhanced by large mudflats (Lefeuvre et al. 2000, Arbach Leloup et al. 2008). We analyzed the origin of food sources incorporated into juvenile-flatfish food webs in this non-estuarine but productive nursery area.

First, we aimed to document resource-use overlap by juvenile flatfish species. Since gut examination has some limitations (e.g. under-estimation of prey diversity; Hyslop 1980), this method was coupled with C and N stable isotope analysis to determine energy sources for flatfish in the bay (Peterson & Fry 1987). Six different sources were traced into the flatfish food web using the R software package 'stable isotope analysis in R' (SIAR), a mixing model based on Bayesian methods (Parnell et al. 2010). A sensitivity analysis to different trophic enrichment factors (TEFs) was performed (Bond & Diamond 2011). Finally, we compared our results with those of common juvenile sole in estuarine nursery grounds elsewhere in western Europe.

MATERIALS AND METHODS

Mont-Saint-Michel Bay

The MSMB is located in northwest France, in the western part of the English Channel (48° 40' N, 1° 35' W; Fig. 1). The bay is a semi-diurnal macrotidal system with a high tidal range, reaching 15.50 m during the highest spring tides (Larsonneur 1994) and with limited freshwater inputs (average annual discharge: 7.1, 5.1, and 10.0 m³ s⁻¹ for the rivers Coues-

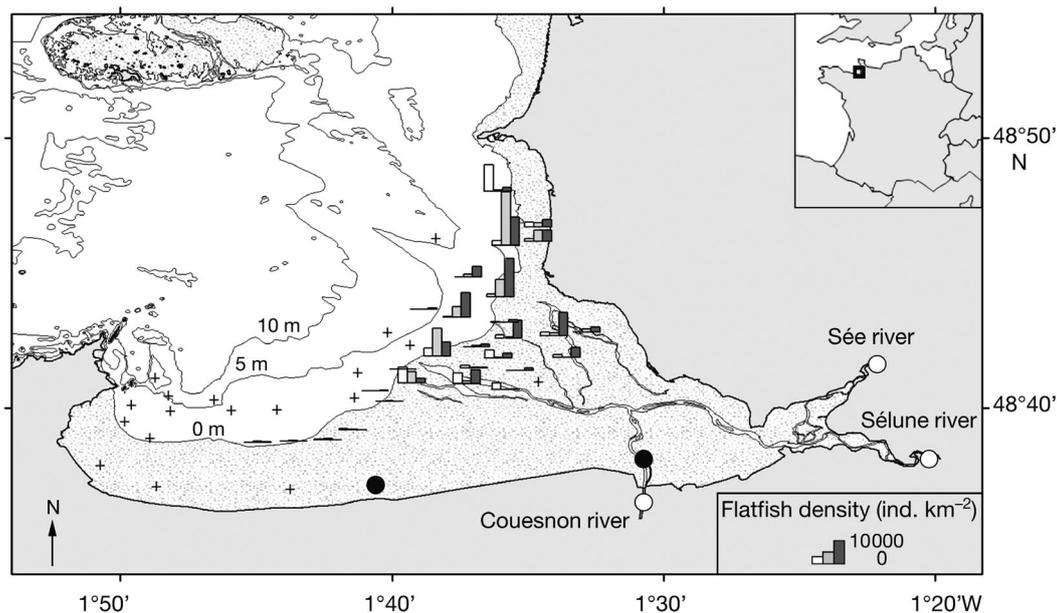


Fig. 1. Sampling stations in Mont-Saint-Michel Bay in August 2008: histograms show density of trawled age 0 (young-of-the-year) *Pleuronectes platessa* (0G-plaice) (white bar), age 0 (young-of-the-year) *Solea solea* (0G-sole) (light grey bar), and 1-group *S. solea* (1G-sole) (dark grey bar); +: no catch; circles: sampling sites for microphytobenthos (●) and freshwater particulate organic matter from rivers (○). Bathymetry lines are shown (0, 5, and 10 m). Inset (upper right) indicates general location of the study area

non, Sée, and Sélune, respectively). About half of this wide, shallow bay (500 km², depth <20 m) consists of mudflats (210 km²) and intertidal salt marshes (40 km²), which make the MSMB the largest salt marsh in Europe. The primary production of these large intertidal areas is central to ecosystem functioning (Lefeuvre et al. 2000), and estuarine influence is limited in the MSMB (Cugier et al. 2010). The MSMB is one of the most important nurseries in the English Channel for several fish species of commercial interest such as sea bass *Dicentrarchus labrax*, whiting *Merlangius merlangus*, elasmobranchs *Raja* spp., and flatfishes, mainly common sole and plaice, but also brill *Scophthalmus rhombus* (Legendre 1984, Laffaille et al. 1998, Kostecki et al. 2011).

Sample collection and preparation

Benthic macrofauna and flatfish were sampled during daytime with a beam trawl (2.9 m wide, 0.5 m high opening, and 10 mm stretched mesh net cod end) in August 2008. Forty-six hauls were carried out (Fig. 1) at 2.5 knots for 15 min, on average covering 3400 m² each haul. For each haul, benthic macrofauna and flatfish were sorted and identified to the lowest possible taxonomic level, generally to the species level. Due to proliferation of the invasive mollusk *Crepidula fornicata* in the western part of the MSMB, flatfish are restricted to the eastern part (Kostecki et al. 2011) (Fig. 1). Common sole and plaice were the 2 flatfish species for which catches were large enough to allow a quantitative study of both diet and stable isotope signatures. Fish were collected under the standardized conditions of mid- to high tide, sea bottom temperature ranging from 18.2 to 18.6°C, and bottom salinity ranging from 34.1 to 34.9. Individuals were measured and size-frequency histograms were used to discriminate cohorts. Two cohorts, age 0 (young-of-the-year) (0G-sole, total length <11 cm) and 1-group (1G-sole, total length <22 cm), were sampled for common sole, and only age 0 (young-of-the-year) (0G-plaice, total length <13 cm) were sampled for plaice (Table 1). Samples collected for flatfish gut content analysis were stored in 7% formaldehyde, and samples for stable isotope analysis (juvenile sole, plaice, and benthic invertebrates considered to be potential prey) were refrigerated on the boat and then stored at -20°C in the laboratory.

During the same survey, 3 samples of freshwater were collected in the Couesnon, Sée, and Sélune rivers (measured salinity: 0.0; Fig. 1). The water was first sieved (200 µm mesh) to extract detritus and

Table 1. Total length (TL) ranges and sample sizes (n) of flatfish analyzed for gut content and stable isotope analyses

Species	Gut content analysis		Stable isotope analysis	
	TL range (mm)	n	TL range (mm)	n
0G-plaice	42–125	120	47–114	68
0G-sole	53–106	178	56–100	49
1G-sole	118–212	51	116–206	47

large zooplankton. Particulate organic matter (POM) was obtained by filtering 1 l of this water over pre-combusted Whatman GF/F filters. Filters were acidified (10% HCl) to remove carbonates, rinsed with Milli-Q water, and oven-dried (50 to 60°C for 48 h).

Four samples (2 samples at each of 2 sites) of microphytobenthos (MPB; mainly benthic diatoms; Riera 2007) were collected at low tide on the intertidal section of the bay (Fig. 1) in August 2008. Benthic diatoms were extracted at the laboratory using the method suggested by Riera & Richard (1996) and described in Kostecki et al. (2010).

Gut content analysis

Because plaice and sole have a small stomach and a long alimentary tract (Beyst et al. 1999, Amara et al. 2001), the entire digestive tract (stomach and intestine) of each fish was removed from the body and stored in 70% alcohol. The number of empty digestive tracts was counted and the vacuity index (proportion of empty digestive tracts) was calculated. Prey items found in digestive tracts were sorted and counted under a binocular microscope. All prey items were identified to the lowest possible taxonomic level, generally the species. Indices of relative abundance (%N, the number of a particular prey item as a proportion of the total number of all prey items in the entire digestive tract) and percentage occurrence (%O, the percentage of gut in which a prey item occurred) of each prey were calculated for each group (species, age) of flatfish. To compare dietary overlap between 2 species or cohorts, the Schoener diet overlap index, α , was used (Schoener 1971):

$$\alpha = 1 - 0.5 \left(\sum_{i=1}^n |P_{xi} - P_{yi}| \right) \quad (1)$$

where α is the dietary overlap between species or cohorts x and y , P_{xi} and P_{yi} are proportions of food category i used by species or cohorts x and y respectively, and n is the total number of food categories. This index, varying between 0.1 (no overlap) and 1

(perfect overlap), is generally considered biologically significant when values exceed 0.6 (Banaru & Harmelin-Vivien 2009).

Stable isotope analysis

Sample preparation

The benthic invertebrate species chosen for isotopic analysis were (1) dominant in terms of abundance and biomass, (2) potential prey for sole and plaice juveniles, and (3) found in the fish gut contents, i.e. *Abra alba*, *Arenicola marina*, *Cerastoderma edule*, and *Nephtys* sp. (see 'Results—Gut content analysis' and Table 2). In order to minimize the effects of lipids on $\delta^{13}\text{C}$ (Bodin et al. 2007), muscle tissues (except for polychaetes) were used for stable isotope analysis. White muscle samples were taken from sole and plaice dorsal musculature (Table 1), as recommended for fish (Pinnegar & Polunin 1999, Sweeting et al. 2007), and from the siphon of bivalves. Viscera, setae, and the jaw were removed from large specimens of polychaetes by dissection, and stable isotope analysis was carried out on the remaining whole body (Le Loc'h & Hily 2005). After dissection, the tissue samples were washed with distilled water to prevent contamination by sediment carbonates (Kharlamenko et al. 2001, O'Reilly et al. 2002). Samples were individually frozen (-20°C) before freeze-drying. Dried samples were ground to obtain a homogeneous powder. All the samples (benthic invertebrates, juvenile flatfish, POM, and MPB) were weighed and encapsulated in tin foil.

Stable isotope analysis

Ratios of $^{13}\text{C}:^{12}\text{C}$ and $^{15}\text{N}:^{14}\text{N}$ were obtained from continuous-flow isotope-ratio mass spectrometry. Samples were analyzed using a Finnigan Delta Plus XP isotope-ratio mass spectrometer interfaced with a Carlo Erba NC2500 elemental analyzer. Isotope ratios were reported in conventional delta (δ) notation as parts per mil (‰) relative to the international standard for Peedee Belemnite Carbonate and atmospheric nitrogen. Repeat analyses of the International Atomic Energy Agency and laboratory standards (N1, N2, CH6, CH7, acetanilide, and peach leaf) showed that maximum standard deviations (SD) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were 0.14 and 0.18, respectively. SD for duplicate samples from the present data set averaged 0.13 for C and 0.22 for N. Single mea-

surements were carried out on all remaining samples. Because the C:N ratio used as a proxy for lipid content in samples was low and steady (<3.5), lipid normalization was not necessary (Post et al. 2007).

Additional stable isotope data

Stable isotope values from samples of marine POM and dead leaves of *Halimione portulacoides* and *Elymus athericus* collected in May 2002 in the MSMB (Riera 2007) were used to complete our organic matter sources. Since organic matter derived from marsh halophytes is known to contribute to the diet of all the tidal flat invertebrates (Lefeuvre et al. 2000), dead plant tissues were chosen instead of live plants, as their isotopic signatures are different (Riera 2007).

Only a fraction of the sedimentary pool can be assimilated by consumers (Zetsche et al. 2011). Accordingly, even if the flatfish are benthivorous, sediment samples were not collected for the present analysis.

Data analysis

Mean comparisons

ANOVAs coupled with Tukey's multiple comparison test were performed using the software program R to test whether $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of flatfish differed between species and age. A significance p-value level of 0.05 was used in all test procedures.

Stable isotope mixing model

Sources incorporated into food webs were identified using a mixing model that provides a combination of feasible solutions accounting for multiple possible sources that could explain a consumer's stable isotope values (Phillips & Greg 2003). In the present study, we used a Bayesian model developed by Parnell et al. (2010) and implemented this in the package SIAR with the software program R. This Bayesian model takes into account uncertainty and variability (SD) concerning sources and TEFs.

Freshwater POM data from the Sée and Sélune rivers were pooled in the mixing model since values were very close (Sée: $\delta^{13}\text{C}$, -25.51 and $\delta^{15}\text{N}$, 5.70 ; Sélune: $\delta^{13}\text{C}$, -24.80 and $\delta^{15}\text{N}$, 6.11 ; see Fig. 2). However, freshwater POM signatures from Couesnon River were different, i.e. ^{13}C -depleted ($\delta^{13}\text{C}$, -30.45)

and ^{15}N -enriched ($\delta^{15}\text{N}$, 8.55), so they were kept separate. Dead leaves of *Elymus athericus* were ^{13}C -depleted and ^{15}N -enriched compared to leaves of *Halimione portulacoides*, so these data were not pooled. Therefore, 6 sources were included in the model: 3 from the present survey (MPB, freshwater POM from Couesnon River, and freshwater POM from Sée and Sélune rivers) and 3 (marine POM, dead leaves of *E. athericus*, and dead leaves of *H. portulacoides*) from Riera (2007).

Pooling stable isotope data for consumers and sources collected at different dates in the same analysis could be considered problematic because stable isotope signature of sources can change over space and time, leading to errors in estimates of source contributions. The interest of the SIAR model is to integrate such uncertainty. In the present study, the variation rates for marine POM data were especially high (see Table 3 & Fig. 2). We compared this variability with POM data collected over a 2 yr time period (2009 to 2010) from an adjacent sector of the western English Channel (Roscoff sampling point, data from SOMLIT, i.e. French national coastal observation system; <http://somlit.epoc.u-bordeaux1.fr>). The SD for marine POM data collected by Riera (2007) and used in the models (C: 1.01 and N: 1.17) was higher than the interannual variation in the time series from Roscoff (C: 0.94 and N: 1.09). Stable isotope signatures are less variable in dead leaves of marsh halophytes (Riera 2007), and lower SDs were associated with *Halimione portulacoides* and *Elymus athericus* (see Table 3). Overall, the uncertainty associated with sources from previous data was considered high enough to overcome, at least partly, the interannual variability in their stable isotope signatures.

The trophic level for common sole and plaice is 3.13 (SE: 0.32) and 3.26 (SE: 0.39), respectively (Froese & Pauly 2010), and closer to 3 for young post-settled juveniles (Darnaude et al. 2001). Thus, to trace organic matter in juvenile flatfish, 2 TEFs have to be considered: one from primary producer or organic matter to primary consumer (i.e. juvenile flatfish prey), and the second from primary consumer to secondary consumer (i.e. flatfish juveniles). Because TEFs have an impact on model outputs and estimates of source contributions (Bond & Diamond 2011), different TEFs were compared. From primary producer or organic matter to juvenile flatfish prey, one TEF value was used: 1.00 for C and 2.20 for N (DeNiro & Epstein 1978, 1981, Post 2002, Fry 2006). Then, from prey to flatfish juveniles, the following TEF values were used (see Table 4):

(a) a local TEF was calculated for each flatfish species or cohort as the difference between the mean C and N stable isotope signatures of the 4 benthic invertebrate species analyzed and each flatfish cohort or species (see Tables 3 & 4);

(b) a TEF from an experiment performed by F. Le Loc'h et al. (pers. comm.) concerning juvenile common sole reared with food sources of known $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in natural conditions (temperature and salinity) in the Thau lagoon (France);

(c) a TEF meta-analysis from the scientific literature (DeNiro & Epstein 1978, 1981, Post 2002, Fry 2006).

A larger review of TEFs in the scientific literature demonstrated that other values are possible (Minagawa & Wada 1984). Nevertheless, the 3 TEFs tested in the present analysis covered the range of values found in the scientific literature and allowed us to estimate the sensitivity of sources' contributions to TEFs. Without standardized information about the variability in estimates of these 3 TEFs, their associated standard error was maintained in SIAR as the default 0.5 value.

For each flatfish group, a Chi-squared (χ^2) test was performed to compare mean source contributions resulting from estimates obtained with the different TEFs used in the sensitivity analysis.

RESULTS

Gut content analysis

The highest vacuity index was observed in 0G-sole; 67% of stomachs and intestines were empty, compared with 20% and 21% for 1G-sole and 0G-plaice, respectively.

Juvenile flatfish prey differed among species and age groups in the MSMB (Table 2); plaice gut content analysis revealed the presence of 40 taxa, whereas diversity in 0G-sole (14 taxa) and 1G-sole (15 taxa) was lower. Cumacea (mainly *Pseudocuma longicorne* and *Cumopsis goodsir*) and Bivalvia (*Abra alba* and siphons of *Cerastoderma* sp.) were the most important items in 0G-plaice gut contents. Amphipoda (*Microprotopus maculatus*) and Cumacea (*P. longicorne*) were chiefly eaten by 0G-sole. In comparison, Polychaeta (*Arenicola marina* and *Nephtys* sp.) and Bivalvia (*Abra alba*) were the main components of 1G-sole diet. All flatfish groups ate Bivalvia, but siphons (sublethal cropping) were most frequently consumed by 0G fish.

Table 2. Relative abundance (%N) and percentage occurrence (%O) of taxa in juvenile flatfish diets from gut content analysis. n.i.: not identified

	0G-plaice		0G-sole		1G-sole	
	%N	%O	%N	%O	%N	%O
Arthropoda						
Crustacea n.i.	0.1	1.1	–	–	–	–
Amphipoda n.i.	0.3	3.2	4.7	5.2	–	–
<i>Abludomelita obtusata</i>	0.1	1.1	0	–	–	–
<i>Atylus falcatus</i>	0.5	5.3	–	–	–	–
<i>Corophium arenarium</i>	–	–	0.7	5.2	–	–
<i>Gammarus</i> sp.	–	–	4.9	10.3	–	–
<i>Microprotopus maculatus</i>	0.3	3.2	39.9	34.5	–	–
Cumacea n.i.	17.7	35.8	0.7	1.7	–	–
<i>Diastylis laevis</i>	0.3	3.2	–	–	–	–
<i>Diastylis</i> sp.	0.2	2.1	–	–	–	–
Bodotriidae n.i.	0.1	1.1	–	–	–	–
<i>Cumopsis goodsir</i>	25	18.9	1.1	6.9	–	–
<i>Eocuma dollfusi</i>	1	6.3	5.7	13.8	1	2.5
<i>Iphinoe</i> sp.	0.1	1.1	–	–	–	–
Pseudocumatidae n.i.	0.1	1.1	–	–	1	2.5
<i>Pseudocuma longicorne</i>	21.1	27.4	21.1	24.1	–	–
<i>Pseudocuma</i> sp.	1.4	8.4	–	–	–	–
Harpacticoida n.i.	2.7	4.2	2.9	10.3	–	–
Decapoda n.i.	0.1	1.1	–	–	–	–
<i>Crangon crangon</i>	0.2	2.1	–	–	1	2.5
<i>Diogenes pugilator</i>	2	2.1	–	–	–	–
Paguridae n.i.	0.2	2.1	0.1	1.7	1	2.5
<i>Pagurus bernhardus</i>	0.1	1.1	–	–	–	–
<i>Portumnus latipes</i>	1.6	7.4	–	–	–	–
Mysida n.i.	0.2	1.1	–	–	–	–
Ostracoda n.i.	0.2	2.1	–	–	–	–
Mollusca						
Bivalvia n.i.	0.7	9.5	–	–	3.9	10
Bivalvia n.i. (siphon)	0.2	1.1	14.6	17.2	2.9	2.5
<i>Abra alba</i>	10.8	41.1	2.1	12.1	31.4	25
Cardiidae n.i.	0.1	1.1	–	–	–	–
<i>Cerastoderma</i> sp. (siphon)	6.5	13.7	–	–	–	–
Mactridae n.i.	0.1	1.1	–	–	1	2.5
<i>Spisula elliptica</i>	4	9.5	–	–	–	–
<i>Nucula</i> sp.	–	–	–	–	1	2.5
Veneridae n.i.	0.1	1.1	–	–	–	–
Gastropoda n.i.	0.2	3.2	–	–	–	–
<i>Hydrobia ulvae</i>	0.2	1.1	–	–	–	–
Echinodermata						
<i>Amphiura</i> sp.	0.4	5.3	–	–	–	–
<i>Acrocynida brachiata</i>	0.1	1.1	–	–	–	–
Annelida						
Polychaeta n.i.	0.6	8.4	0.5	6.9	13.7	35
<i>Arenicola marina</i>	–	–	–	–	20.6	50
<i>Glycera convoluta</i>	0.1	1.1	–	–	4.9	7.5
<i>Nephtys</i> sp.	0.9	11.6	–	–	10.8	27.5
<i>Nephtys hombergii</i>	0.1	1.1	0.9	10.3	–	–
<i>Nereis</i> sp.	–	–	–	–	1	2.5
Cirratulidae n.i.	–	–	–	–	4.9	5
No. of taxa	40		14		15	

The Schoener diet overlap index between 0G-sole and 0G-plaice was 0.30, while it was 0.07 between 0G-sole and 1G-sole, and 0.14 between 0G-plaice and 1G-sole.

Food sources and flatfish stable isotope signatures

POM from the Couesnon River was ^{13}C -depleted and ^{15}N -enriched compared to POM from the Sée and Sélune rivers and marine POM (Fig. 2, Table 3). Dead leaves of *Elymus athericus* were ^{13}C -depleted and ^{15}N -enriched compared to *Halimione portulacoides*. MPB was the most ^{13}C -enriched organic matter source. Thus, the different freshwater, estuarine, and marine food sources (freshwater and marine POM, plants, and MPB) had distinct $\delta^{13}\text{C}$ values that could be traced into the trophic network.

Benthic invertebrate species exhibited differences in stable isotope signatures: bivalves (*Abra alba* and *Cerastoderma edule*) were ^{15}N -depleted compared to polychaetes (*Arenicola marina* and *Nephtys* sp.). Differences in $\delta^{13}\text{C}$ were also apparent, with *C. edule* being the most ^{13}C -depleted invertebrate (Fig. 2, Table 3).

The 0G-plaice, 0G-sole, and 1G-sole were not significantly different in $\delta^{13}\text{C}$ (ANOVA; $F_{2,160} = 0.93$,

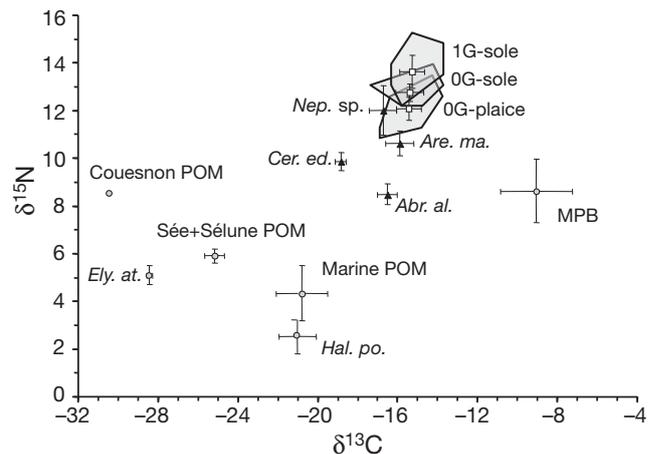


Fig. 2. Stable isotope signatures (mean \pm SD) of organic matter sources to flatfish diets, benthic invertebrates, and juvenile flatfish. Organic matter sources (\bullet): particulate organic matter (POM) from Couesnon River, POM from Sée and Sélune rivers, marine POM, microphytobenthos (MPB), and dead leaves of *Elymus athericus* (*Ely. at.*) and of *Halimione portulacoides* (*Hal. po.*). Benthic invertebrates (\blacktriangle): *Nephtys* sp. (*Nep. sp.*), *Arenicola marina* (*Are. ma.*), *Cerastoderma edule* (*Cer. ed.*), and *Abra alba* (*Abr. al.*). \square : Juvenile flatfish. The large grey shapes represent the smallest convex polygons containing all individuals of the same species and age-group

Table 3. Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and number of samples (n) of organic matter sources to flatfish diets, flatfish prey, and flatfish. POM: particulate organic matter

Sample	n	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Organic matter source			
Couesnon River POM	1	-30.45	8.54
Sée and Sélune rivers POM	2	-25.15 \pm 0.50	5.91 \pm 0.29
Marine POM ^a	4	-20.90 \pm 1.01	4.33 \pm 1.17
<i>Elymus athericus</i> ^a	3	-28.43 \pm 0.15	5.1 \pm 0.40
<i>Halimione portulacoides</i> ^a	3	-20.97 \pm 0.94	2.50 \pm 0.72
Microphytobenthos	4	-8.91 \pm 1.83	8.65 \pm 1.35
Prey			
<i>Abra alba</i>	10	-16.43 \pm 0.50	8.52 \pm 0.43
<i>Cerastoderma edule</i>	3	-18.79 \pm 0.26	9.90 \pm 0.37
<i>Arenicola marina</i>	20	-15.80 \pm 0.70	10.66 \pm 0.52
<i>Nephtys</i> sp.	13	-16.36 \pm 0.72	12.24 \pm 0.94
Flatfish			
0G-plaice	68	-15.35 \pm 0.62	12.13 \pm 0.49
0G-sole	49	-15.31 \pm 0.72	12.81 \pm 0.35
1G-sole	47	-15.18 \pm 0.61	13.70 \pm 0.70

^aAdditional data taken from Riera (2007)

$p = 0.40$; Fig. 2) but differed significantly in $\delta^{15}\text{N}$ (ANOVA; $F_{2,160} = 122.42$, $p < 0.001$). Tukey's multiple comparison test indicated that 0G-plaice were significantly ^{15}N -depleted compared with 0G- and 1G-sole (Fig. 2). The highest $\delta^{15}\text{N}$ values were observed in 1G-sole.

Source contributions

Mixing model estimations of each organic matter source contribution to fish diet were quite similar for the 2 juvenile sole cohorts and the 0-group plaice (Fig. 3). The major sources contributing to flatfish isotope signatures were MPB and POM from the Couesnon River. Conversely, contributions of Sée River and Sélune River POM, marine POM, dead leaves of *Elymus athericus*, and dead leaves of *Halimione portulacoides* were marginal.

Differences in TEFs (Table 4) led to significant differences in source contributions ($\chi^2_{0\text{G-plaice}} = 34.56$, $\chi^2_{0\text{G-sole}} = 45.45$, $\chi^2_{1\text{G-sole}} = 29.79$; $\text{df} = 8$, $p < 0.001$). Nevertheless, accounting for this variability, MPB consistently made a major contribution to the flatfish food web in all models (Fig. 3). Models differed in their estimated contributions of freshwater POM from the Sée and Sélune rivers and the Couesnon River, but differences in the total contribution of freshwater POM were minor.

Table 4. Trophic enrichment factors (TEFs) used for SIAR predictions with an SD of 0.5. The final column corresponds to the sum of TEFs from PP to C2: PP to C1 (taken from the literature^b) then C1 to C2 (based on the present study, an experiment^a, or taken from the literature^b). PP: primary producer or organic matter source, C1: primary consumer, C2: secondary consumer

Source	PP-C1	C1-C2	$\Sigma(\text{PP-C2})$
TEF for carbon			
Present study			
0G-plaice		1.53	2.53
0G-sole		1.57	2.57
1G-sole		1.69	2.69
Experiment ^a		2.43	3.43
Literature ^b	1.00	1.00	2.00
TEF for nitrogen			
Present study			
0G-plaice		1.32	3.52
0G-sole		2.00	4.20
1G-sole		2.88	5.08
Experiment ^a		2.27	4.47
Literature ^b	2.20	3.40	5.60

^aF. Le Loc'h et al. (pers. comm.)
^bDeNiro & Epstein (1978, 1981), Post (2002), Fry (2006)

DISCUSSION

Moderate trophic niche overlap between juvenile flatfish species in MSMB

Both plaice and sole juveniles ate benthic invertebrates, but taxonomic comparison showed dissimilarities among species present in gut contents, and no dietary overlap was observed among age groups or species in MSMB. Juvenile plaice had a larger prey spectrum than both 0G- and 1G-sole, while 0G-sole ate smaller prey items (mainly Amphipoda and Cumacea) compared to 1G-sole (mainly Bivalvia and Polychaeta). This latter difference reflects the larger gape width of the 1G-sole, allowing them to capture a wider range of prey, including invertebrates of higher trophic levels (Jennings et al. 2001). The $\delta^{15}\text{N}$ results support these observations based on diet. Juvenile plaice had significantly lower $\delta^{15}\text{N}$ values compared with 0G- and 1G-sole, indicating that they were feeding at a slightly lower trophic level. The changes in diet observed between 0G- and 1G-sole were similarly supported by small differences in $\delta^{15}\text{N}$.

A similar lack of dietary overlap for juvenile flatfish has been observed in other nursery grounds in Europe (Beyst et al. 1999, Amara et al. 2001). Juvenile plaice have a larger prey spectrum compared with juvenile common sole (Amara et al. 2001). The ontogenetic shift in diet between 0G- and 1G-sole observed

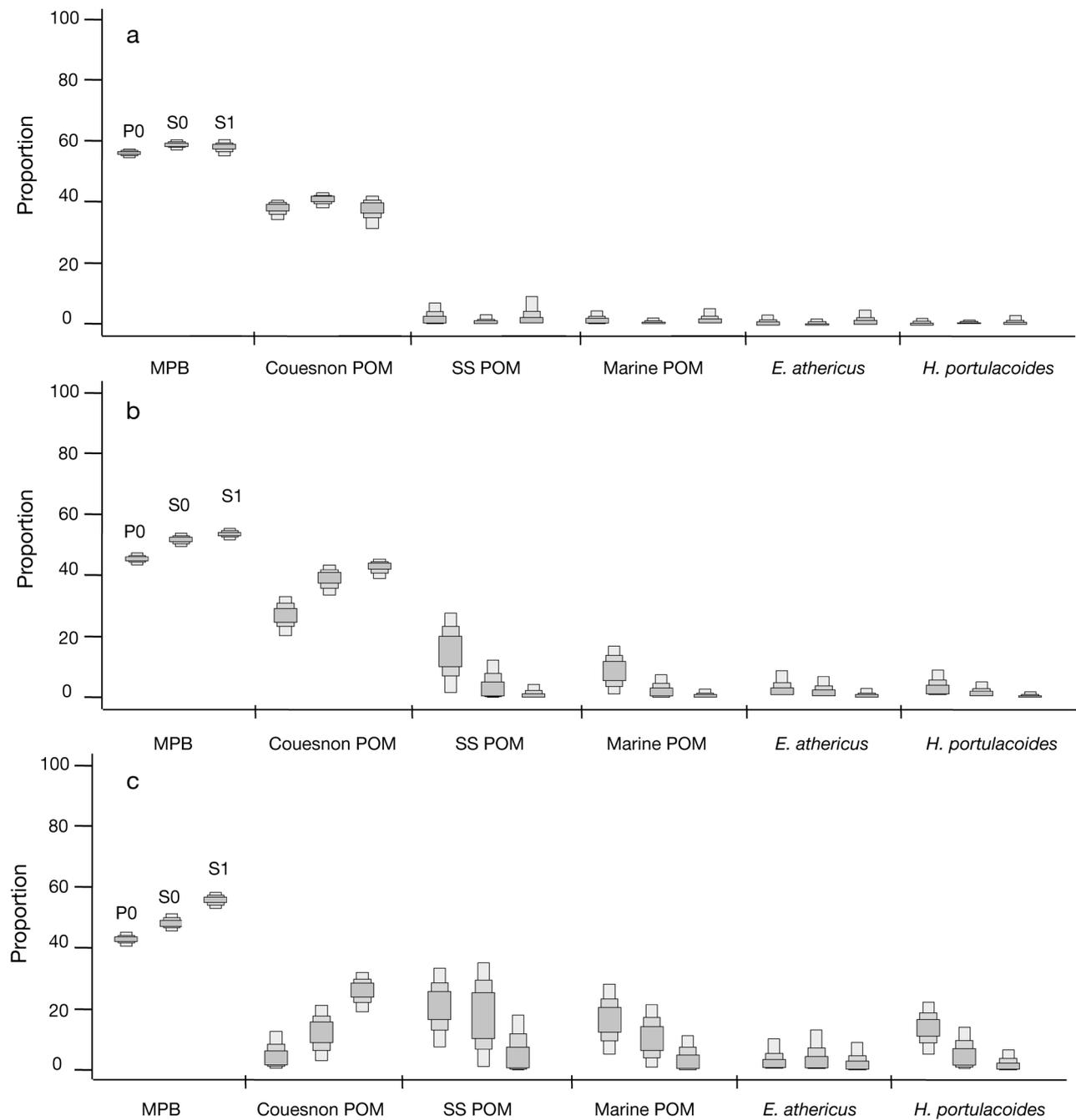


Fig. 3. Boxplots of 3 mixing-model estimates of contributions to flatfish diets from 6 organic matter sources: microphytobenthos (MPB), Couesnon River particulate organic matter (POM), POM from Sée and Sélune rivers (SS POM), marine POM, *Elymus athericus*, and *Halimione portulacoides*, using trophic enrichment factors: (a) calculated using data from the present study, (b) based on *in situ* experiments (F. Le Loc'h pers. comm.), and (c) taken from the literature (see Table 4). Bars represent 25th, 75th, and 95th Bayesian credibility intervals. P0: 0G-plaice, S0: 0G-sole, S1: 1G-sole

in the MSMB is similar to results obtained by Lagardère (1987), Molinero & Flos (1991), and Cabral (2000). While bivalve siphons were an important prey item for both 0G flatfish, only 0G-plaice were found to eat *Cerastoderma* sp. siphons in the present study

and in the Seine estuary (Amara et al. 2001), another estuarine nursery system of the English Channel. By contrast, in the Rance estuary (France), close to the MSMB, competition for *Cerastoderma* sp. occurs between young sole and plaice (Le Mao 1986).

Both sole and plaice have a small mouth size, small esophagus and stomach, and a complicated intestinal loop, which are features appropriate for consumption of small-sized benthic prey (De Groot 1971, Braber & De Groot 1973). The dissimilarities observed in gut contents can be explained by differences in feeding strategy: plaice are visual feeders, specializing on slow-moving bottom-dwellers such as active crustaceans, while sole are night feeders, preying on sessile or barely mobile organisms (Batty & Hoyt 1995, Harvey 1996, Beyst et al. 1999). Benthic invertebrates eaten by young plaice were mainly deposit- and suspension-feeders, while common sole's prey were deposit-feeders and carnivorous (with a higher trophic level).

Because of the differences in time of feeding, the stomachs of daytime-feeding plaice were more full compared to nighttime-feeding sole because sampling was undertaken during the daytime (De Groot 1971). While our daytime sampling was probably not optimal to encompass the variability in resource consumption by juvenile flatfish because we were obtaining single snapshots of a consumer's diet that may not correspond to relevant ecological time-scales (Hyslop 1980), the differences in diet we observed were consistent with the stable isotope results. Stable isotope analysis of a consumer's tissues provide for time- and space-integrated representations of the trophic ecology of organisms and energy assimilated by the consumer (Peterson & Fry 1987), giving us confidence that the differences in feeding strategies among flatfish juveniles in the MSMB we observed were real.

Moderate sensitivity to uncertainty in production source data and TEFs

Trophic sources were well discriminated from their signatures in spite of large SDs. The $\delta^{13}\text{C}$ values for freshwater POM were lower than other organic matter sources, following a well-documented pattern of trophic sources in coastal and estuarine systems (Darnaude et al. 2004, Kostecki et al. 2010). An increasing $\delta^{13}\text{C}$ of POM from fresh to marine waters was observed, which is consistent with previous studies (Yokoyama & Ishihi 2007). Detrital salt-marsh species had distinct C and N stable isotope values, allowing discrimination, and MPB displayed the highest $\delta^{13}\text{C}$ signature of all sources in MSMB. These patterns fit with previous stable isotope analyses in this bay (Lefeuvre et al. 2000, Riera 2007) and elsewhere (Yokoyama & Ishihi 2007, Choy et al. 2008).

Stable isotope mixing models can be sensitive to variation in TEFs (Wilson et al. 2009, Bond & Diamond 2011). However, the present sensitivity analysis (Fig. 3) demonstrated that SIAR model outputs appeared relatively insensitive to changes in TEF values and found consistency in the most important source contributions, despite accounting for moderate differences in TEFs. Thus by accounting for variability in sources' data and TEFs, we were able to quantify food sources in the juvenile flatfish trophic chain from SIAR model outputs.

Significance of MPB for juvenile-flatfish food webs in MSMB

The origin of organic matter in food webs of flatfish was similar for juvenile plaice (0-group) and sole (0- and 1-group) in the MSMB, despite differences in prey preferences and, to a lesser extent, in trophic levels. The most important organic matter sources were MPB (i.e. mainly benthic diatoms; Riera 2007) and, to a lesser proportion, freshwater POM subsidies. The origin of organic matter for juvenile flatfishes contrasts with the diet of shellfish (cultivated mussels and oysters, and invasive slipper limpet), the dominant secondary producers in the MSMB, representing 40% of all secondary production in the bay (Arbach Leloup et al. 2008, Cugier et al. 2010). The diet of these filter-feeding mollusks was primarily based on marine plankton, with no significant contribution from benthic diatoms (Riera 2007). An investigation of organic matter and nutrient fluxes between salt marshes and marine waters in the MSMB (Lefeuvre et al. 2000) using stable isotopes and fatty acids demonstrated the importance of organic matter produced by salt marshes in the diet of tidal-flat invertebrates and for transient fish species that colonize salt marshes to forage or graze. Conversely, the present study found that salt marshes, represented by 2 C_3 plants, did not seem to be an important source of organic carbon to flatfish nurseries. This could be related to their limited surface (40 km^2), 5 times lower than the intertidal mudflats (210 km^2), where MPB production is important (Arbach Leloup et al. 2008).

Our results in the MSMB system tend to underline the large influence of local primary production on coastal food chains when freshwater inputs of organic matter are limited. Many studies have shown the importance of benthic algae primary production in estuaries and lagoons. Quan et al. (2010) characterized the major pathways for energy flow in an arti-

ficial lagoon using 4 primary producers; >50% of organic carbon in the food chain was derived from epibenthic microalgae, suggesting that the lagoon food web was mostly based on this production source. Melville & Connolly (2003) used stable isotopes to determine the autotrophic sources supporting production of fish in a tropical estuary. They demonstrated the importance of seagrass, epiphytic algae, and local POM (including phytoplankton), in addition to organic matter from adjacent mangroves, for resident fish species. In the MSMB, different sources of primary production appear to be significant to the different components of the MSMB benthic food web; e.g. phytoplankton for cultivated and invasive shellfish (Riera 2007, Arbach Leloup et al. 2008) and MPB for juvenile flatfish in their nursery ground.

MSMB nursery ground: a contrasting function compared with estuarine nursery grounds?

In MSMB, production of MPB and continental organic matter present in freshwater POM entered the flatfish-juvenile food web. This combination of local primary production and freshwater organic matter has already been demonstrated to sustain juvenile flatfish in nursery sectors, especially in estuaries (Darnaude et al. 2004, Leakey et al. 2008, Pasquaud et al. 2008, Vinagre et al. 2008, Kostecki et al. 2010). The present study provides new insight about the importance of MPB, which is usually considered a subsidiary for juvenile flatfish in estuaries.

Continental subsidies can vary in space (Pasquaud et al. 2008, Kostecki et al. 2010) and time (dry years vs. wet years; Kostecki et al. 2010), but are frequently considered predominant in estuaries. In non-estuarine areas, and especially in bays, which are important nursery grounds (Riou et al. 2001, Le Pape et al. 2003), the importance of intertidal primary production, and especially MPB, may be predominant and sustain nursery function.

Nevertheless, the contribution of freshwater POM in the juvenile-flatfish food web is quite important (i.e. about one-third) in the MSMB. In this bay, juvenile flatfish are concentrated in a restricted area (Kostecki et al. 2011) in front of the 3 river mouths (Fig. 1), where the estuarine influence is moderated but higher than elsewhere in the bay (Cugier et al. 2010). Even if the common sole faced a reduction of its habitat in this bay, as a consequence of the proliferation of the invasive slipper limpet, the heart of its distribution has been located in the same place for 3

decades (Kostecki et al. 2011). This situation allows us to understand the significant contribution of freshwater POM in spite of the very limited river discharge at the MSMB scale. This has implications for the maintenance of freshwater flow to this coastal system: freshwater loadings provide both inorganic nutrients enhancing intertidal primary production from MPB (Underwood & Provot 2000) and organic matter, thus it is an important input to sustain the nursery function in the bay.

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