Feeding ecology of Liza spp. in a tidal flat: Evidence of the importance of primary production (biofilm) and associated meiofauna

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Abstract

Grey mullets are unique among temperate-region fish species in their ability to feed on mudflat biofilm. In this study, we examined mullet feeding strategies on biofilm and associated meiofauna by using a diet study and stable isotope analysis to explore functional interactions between mullets and tidal flats. A stomach vacuity investigation showed that mullets did not import any materials from subtidal areas into the mudflat but exported mud, biofilm, and associated meiofauna. The results of mullet stomach content and fecal analyses, when compared to the availability of tidal flat resources, showed evidence of mullets' ability to ingest and assimilate biofilm and to concentrate major meiofauna grazers such as nematodes, copepods and, secondarily, foraminifers and ostracods. Isotopic ratios confirmed diet investigations, and as recently shown in salt marsh habitats, mullets exhibited an intermediate trophic position, supporting the hypothesis that they can assimilate both biofilm and major meiofauna grazers. The function of the tidal flat as a feeding habitat for grey mullets and the role of mullets as the main export pathway of biofilm from tidal flat ecosystems are discussed.

Keywords: Grey mullets; Feeding ecology; Mudflat; Microphytobenthos; Meiofauna

Introduction

Tidal flats represent a key system in the network of ecosystems at the continent–ocean interface. Biofilm, formed by microphytobenthos and bacteria, confers a high productivity to tidal flats (see Heip et al. (1995) for a review). The functioning of tidal flats is now well described, especially food web organization using carbon flow models (e.g., Degré et al., 2006; Leguerrier et al., 2003, 2004; Van Oevelen et al., 2006). In contrast, the role of biofilm production and associated primary consumers in the ecosystem remains less well known. To integrate the ecological functioning of tidal flat systems into a global flux scheme, it is indeed necessary to describe if and how primary production and associated primary consumers are integrated into the ecosystem and potentially exported to adjacent areas (e.g., subtidal waters) (Degré et al., 2006). If biofilm consumption by benthic suspension- and deposit-feeders (see Pascal et al., 2008a, 2008b, 2008c, 2009) has been greatly described, less is known about the role of vertebrates such as fishes or birds (see Bocher et al., this issue for birds).

The present study aimed to better understand how fish exploit biofilm in tidal flats and, conversely, what functions fish could have in such an ecosystem. Probably due to sampling difficulties, studies dealing with biofilm consumption by fish have focused on salt marshes (e.g., Laffaille et al., 2002; Lebreton et al., 2011) whereas tidal flats are rarely investigated (but see Almeida, 2003). We focused on mugilids, unique fish species in temperate regions able to consume biofilm. Mugilids are very abundant in coastal areas and occupy a large variety of habitats (e.g., Almeida, 2003; Bruslé, 1981a; Laffaille et al., 2000, 2002; Odum, 1970). This success may be due to unusual feeding behavior and diet (Almeida, 2003), combined with high salt tolerance in numerous species, including *Liza aurata* and *Liza ramada* (hereafter called grey mullets) (Cardona et al., 2008; Daverat et al., 2011). Because they have a highly adapted digestive tract (e.g., a protractile and thin mouth with developed lips, specialized gill rakers, and a gizzard (Thomson, 1966)), grey mullets are among the rare fish species able to graze biofilms on a variety of supports such as rocks and artificial items (pontoons, buoys, bridge pillars, reefs), but mainly tidal flats and salt marshes. Grazing by mullets could consequently represent an important role for primary production in intertidal mudflats (Almeida, 2003), and also a way of exchange between intertidal and subtidal areas (Laffaille et al., 1998). Because they appear very opportunistic and until recently most studies used only visual examination of stomach content, various terms have been used to describe grey mullets' diets. They can be based on the size of particles, such as "microbenthos and

meiobenthos feeder" (Hickling, 1970) or "benthic microphagous omnivore" (Blaber and Whitfield, 1977), or based on the location of exploited resources, such as "interface feeder" (Odum, 1970) or "limno-benthophagous" (Laffaille et al., 2002; Lebreton et al., 2011). For the same reasons, grey mullets have also been classified in a wide variety of trophic positions including herbivorous, omnivorous, plankton feeders, or micro-crustacean predators (Bruslé, 1981a). Indeed, it has been proven that many species of grey mullets ingest a great proportion of microphytobenthos (e.g., Laffaille et al., 1998), and also small benthic grazers such as copepods, foraminifers, nematodes, and polychaetes (Cardona, 2001; Ferrari and Chieregato, 1981; Laffaille et al., 1998). Nevertheless, to our knowledge, no studies occur on the prevalence of the whole diversity of biofilm components in mullet diets, especially for the main taxa of meiofauna. Except for younger pelagic zooplanktonophagous stages (Ferrari and Chieregato, 1981, Lebreton et al., 2011), it also remains unknown what exactly grey mullets assimilate from ingested prey. A recent investigation of stable isotopic ratios from salt marsh food webs placed grey mullets at a higher trophic position than previously described (Lebreton et al., 2011; Vinagre et al., 2012), with grey mullets being able to assimilate significant amounts of secondary consumers from the biofilm, in particular nematodes (Lebreton et al., 2011).

In light of these considerations and in the context of the tidal flat functioning in terms of the role of primary production, we investigated the feeding ecology and in particular the diet of adult mullets feeding in a natural tidal flat. We first explored, through the vacuity of their digestive tracts, the potential for material transport by mullet between subtidal and intertidal areas according to their tidal migrations. We then compared diet diversity in stomach contents and indices of what remains in feces with available food items in the biofilm. This instantaneous view was coupled with analyses of the natural isotopic ratios of the main components of the tidal flat food web, including mullets. From these investigations, we discuss a global perspective on the role of biofilm and associated meiofauna, and by extension tidal flats, for grey mullets and, conversely, the potential role of mullets in the global functioning of tidal flats.

Material and methods

1. Study site

Sampling was carried out in 2008 in a tidal flat called Brouage, the site of the VASIREMI ANR French program, in the eastern part of Marennes-Oléron Bay, located in the middle of the Atlantic coast of France. The surface of Marennes-Oléron Bay is 150 km^2 (Pouliquen, 1975), of which about 60% is exposed during spring low tide. This is a macrotidal bay in which well-mixed waters are often highly turbid because of Charente River inputs and the resuspension of sediment by currents and waves. A large mudflat about 3–4 km in width extends for more than 10 km from north to south, constituting a large silty–muddy flat of about 40 km^2 mainly bordered by a sand beach. Meteorological conditions exhibit a strong seasonality typical of a temperate climate. Details of numerous benthic organisms and processes are available in Leguerrier et al. (2003, 2004) and Degré et al. (2006).

2. Fish sampling

Fish were sampled in May, July, and September 2008 using 4 50-m gill nets (40-mm and 25-mm mesh size) to catch fish from 20 to 60 cm in length. One combination of 2 nets was set at ebb tide and a second set later from a boat at the high water slack. Nets were anchored in the mud to avoid being swept away by the flood tide. The first combination of nets was hauled in just before the high water slack to avoid capturing fish leaving the tidal flat. The second combination of nets was hauled in about 3 h after the high water slack in order to catch fish as they left the flat. Just after capture, feces, when available, were sampled by squeezing the belly of each individual and then conserved individually for further laboratory analyses in filtered seawater to prevent any contamination by other organisms. Fish were deep-frozen (-20°C) at the laboratory within 4 h after capture until analysis.

3. Laboratory

Stomach vacuity (percentage of individuals with an empty stomach) was compared between flood and ebb tide for all sampling months (n = 52 fish caught). Stomach content and feces analyses (meiofaunal taxa densities, viability of foraminifers after digestion process, chlorophyll *a* concentration, and %POM estimates, see below for details) were conducted over only 1 season because of strong variability in captures according to month and tide. Because of the small number of fish caught with a full stomach and feces in May (n = 4) and July (n = 11), we opted to focus on fish caught in September (n = 21 fish, with n = 21 for stomach contents and n = 10 feces). Feces and samples of stomach contents were analyzed under a stereomicroscope (×20) to check for occurrences and densities of common meiofaunal taxa, expressed in numbers of individuals per g of fresh stomach content or feces weight,

accordingly. Both feces and stomach content were weighted before analyzing the content. Densities of meiofaunal taxa in the biofilm (n = 42 samples) from Brouage mudflat in July 2008 were also included in analyses. 70% alcohol fixed sediment cores (above 1st cm depth of a 15-cm diameter corer) were sieved through a 50-µm mesh and meiofauna taxa were then counted under a stereomicroscope (Orvain et al., unpublished data). Note that what is called "biofilm" for prey density comparison corresponded to the first cm of the upper layer of sediment and was in fact a mix of mud, biofilm, and associated fauna. Densities were compared according to origin (biofilm, stomach content, or feces) by a set of one-way analyses of variance (ANOVA). To check for the viability of foraminifers after the digestion process, shells found in feces (n = 10) were placed for 24 h in a seawater solution and regularly examined under a stereomicroscope (×20). If no pseudopodia emission occurred within 24 h, foraminifers were considered dead. To test for the ingestion and digestion of microphytobenthos, chlorophyll *a* concentration, a proxy of microphytobenthos, was estimated both in stomach contents (n = 21) and feces (n = 10) from fish caught in September and compared using a t-test. To explore the efficiency of mullets in concentrating particulate organic matter (POM), the percentage of POM (%POM) was also assessed in the stomach contents of mullets caught in September (n = 21) by comparing stomach content weight after cremation (SWC) (at 480°C for 48 h) and stomach content weight after desiccation (SWD) (at 50°C for 48 h): %POM = [1 − (SWC/SWD)] × 100.

It is well known that carbon and nitrogen turnover is much faster in the liver than in muscles (Guelinckx et al., 2007; MacAvoy et al., 2001). For example, in fish livers nitrogen and carbon isotopic compositions are characteristic of the food consumed within a few weeks before capture (Logan et al., 2006; MacNeil et al., 2006) while those of muscles indicate the average diets of adult fish within a few months before capture. Therefore, samples of liver and muscle tissues were collected (n = 52; all months) to unravel the recent individual feeding histories of mullets dwelling in the tidal mudflats. All samples were freeze-dried and ground using a ball mill. No delipidation was performed on samples since no significant effect on isotope values was found during preliminary tests (cyclohexane treatment according to De Niro and Epstein, 1977). The nitrogen and carbon isotopic compositions in liver and muscle tissues of mullet were determined using EA-IRMS (Isoprime, Micromass, UK). The carbon and nitrogen isotope ratios are expressed in the delta notation $\delta^{13}C$ and $\delta^{15}N$, where: $\delta X = [(R_{Sample}/R_{Reference}) - 1] \times 1000$, where $X = \delta^{13}C$ or $\delta^{15}N$ and R is the ratio $^{13}C:^{12}C$ or $^{15}N:^{14}N$ in the sample and in the reference material. Results are referred to Vienna Pee Dee Belemnite (VPDB) for C and to atmospheric nitrogen for N and expressed in units of ‰ ± standard

deviation (sd). Fish isotopic signatures were compared according to month and tissue (two-way ANOVAs) and also with signatures of food sources and main primary sources of C from freshly sampled materials. Most source data were reported by Bocher et al. (this issue, and unpublished data) and were sampled in 2008 in Marennes-Oléron Bay including seasonal variability (4 seasons, except for microphytobenthos that was not sampled in the autumn). It included microphytobenthos (n = 3), suspended organic matter (n = 20), particulate organic matter (n = 22), *Hydrobia ulvae* (n = 14), and nematodes (n = 17). Copepods (n = 5) and foraminifers (n = 2) isotopic values come from Pascal et al. (unpublished data) and were sampled in the spring and autumn of 2007. Note that foraminifers had not been acidified and included potential biased values in $\delta^{13}C$ (reaching potentially 100%, Pascal et al., 2008a).

Results

A total of 52 grey mullets were captured between May and September 2008 (Table 1). Their sizes ranged from 265 to 410 mm (mean and standard error of 340.17 ± 4.94 mm) and their sex ratio was constant (50/50) throughout the sampling period. In all sampling periods, the stomachs of fish caught during flood tide contained significantly less material (mud and biofilm, expressed as percentage of fish biomass without stomach content) than those leaving the mudflat during ebb tide (4.74 ± 0.88% and 11.03 ± 0.58% for fish caught during flood and ebb tide, respectively, $t = -4.63$, $p < 0.001$). The proportion of fish with a totally empty stomach was high (62%) during flood tide and 0% during ebb tide.

Except for foraminifers, there was a significant variability in the density of the main taxa of meiofauna between biofilm (n = 42), stomach contents (n = 21), and feces (n = 10) ($F_{2, 69}$ varied from 3.64 to 49.95, $p < 0.05$ for nematodes, copepods, ostracods, and platyhelminthes and $F_{2, 69} = 1.67$, NS for foraminifers) (Fig. 1). Density significantly increased from biofilm to stomach contents for nematodes, copepods, and ostracods (Tukey pairwise mean differences varied from 1.12 to 100.93, $p < 0.05$) but decreased for platyhelminthes (pairwise mean difference = -0.715, $p < 0.001$). Prey concentrations increased from biofilm to stomach content by 20% for nematodes, with mean densities of 218.4 ± 6.4 and 263.0 ± 69.6 $ind.g^{-1}$ (densities expressed in numbers of individuals per g of fresh biofilm or fresh total stomach content) in biofilm and stomach content, respectively; 4146% for copepods, with mean densities of 2.4 ± 0.1 and 101.9 ± 82.0 $ind.\cdot g^{-1}$ in biofilm and stomach content, respectively and 367% for ostracods, with mean densities of 0.3 ± 0.2 and 1.4 ± 1.0 $ind.\cdot g^{-1}$ in biofilm and stomach content, respectively. Conversely, there was a 60-fold decrease in platyhelminthes

concentration, with mean densities of 1.2 ± 0.1 and 0.02 ± 0.02 ind.$\cdot g^{-1}$ in biofilm and stomach content, respectively. A significant density decrease occurred between stomach content and feces only for nematodes (pairwise mean difference = 251.370, $p < 0.001$) (Fig. 1). Compared to stomach contents, all individuals found in feces were observed partly digested (e.g., nematode remains, copepod carapaces), nematode remains being more difficult to detect compared to indigestible foraminifer shells or copepod and ostracod carapaces. Even if the proportion of dead foraminifers in the sediment is not known, there was no doubt that mullet ingest living individuals since foraminifer's are well known to be active species in mudflat's biofilm (Pascal et al., 2008a). Nevertheless, as no activity was detected for foraminifers found in feces and examined under a stereomicroscope (×20) in a seawater solution, we concluded that even if their carapace remained intact, they died during the transit and their content was probably digested. Another interesting result was the absence in stomachs of *H. ulvae*, a common species homogeneously distributed (pers. obs.) on the mudflat of Brouage in large densities (17,191 ± 547 ind.$\cdot m^{-2}$, n = 168) in July 2008 (Orvain et al., unpublished data). Knowing the feeding behavior of grey mullet (grazing traces of 2 to 4 cm wide over variable length), it is obvious that they ingest some of these molluscs. This suggests that they were able to reject them before they reached the stomach. Chlorophyll *a* concentration was strongly different between feces and stomach content with a depletion of 74% (134,537 ± 16,181 µg.$\cdot g^{-1}$ in stomachs and 35,413 ± 8,252 µg.$\cdot g^{-1}$ in feces respectively, $t = -7.121$, $df = 20$, $p < 0.001$). This clearly confirms the ingestion and digestion of microphytobenthos by mullets. %POM was calculated for 21 stomach content samples from September and reached 6.79 ± 0.43%.

Although there was a significant temporal variability of $\delta^{13}C$ and $\delta^{15}N$ signatures, no clear seasonal pattern occurred (Fig. 2). Indeed, the $\delta^{13}C$ signature was constant among the months of study but not among tissues ($F_{1, 86} = 26.55$, $p < 0.001$ for tissue; $F_{2, 86} = 0.80$, NS for month; $F_{2, 86} = 0.68$, NS for intercept) (Fig. 2), while a significant variation was detected both among tissues and months for $\delta^{15}N$ ($F_{1, 86} = 68.31$, $p < 0.001$ for tissue; $F_{2, 86} = 4.58$, $p < 0.001$ for month; $F_{2, 86} = 8.55$, $p < 0.001$ for intercept). Interpretation of food web potential relationships are based on mean values of $\delta^{13}C$ of animals being typically enriched by 1.1‰ or less per trophic level within oceanic systems (e.g., France and Peters, 1997; Michener and Lajtha, 2007) and on the $\delta^{15}N$ of animals being enriched on average by 3‰ compared with their diet (Minagawa and Wada, 1984; Vander Zanden and Rasmussen, 2001; Post, 2002; MacCutchan et al., 2003; Caut et al., 2007). According to those values, the signatures from mullets appeared mainly linked to nematodes and potentially *H. ulvae*. Those taxa probably

exploit microphytobenthos as a primary source rather than SOM or POM, which exhibited too depleted $\delta^{13}C$ values to be significantly consumed ($\delta^{13}C$ was -15.4 ± 0.6‰, -19.4 ± 0.9‰, and -22.2 ± 1.8‰, for microphytobenthos, SOM, and POM, respectively). Since $\delta^{13}C$ signature of foraminifers could suffer bias due to the absence of acidification, it remained difficult to determine the importance of that prey for mullets. Nevertheless, as for copepods, foraminifers appeared in the same range of mullet $\delta^{15}N$ values, suggesting a secondary role as prey for mullets (differences between the $\delta^{15}N$ of mullet liver or muscle and the $\delta^{15}N$ of copepods or foraminifers ranged from -1.46 to 1.27‰).

Discussion

Functional interactions of grey mullets with tidal flat biofilm and associated meiofauna were examined using various approaches.

Quantitatively, 62% of captured fish had empty stomachs at flood tide and 100% had full stomachs during ebb tide. Moreover, nets were set at a middle distance from the tidal zone and fishes were caught within 1h after the beginning of the flood tide, so some of them had probably already begun to feed, explaining why some fish had stomach contents during flood tide. Laffaille et al. (1998, 2002) had previously checked for the vacuity of adult grey mullet stomachs (grey mullets >100 mm) in a salt marsh (Mont St Michel Bay in France). Unlike our results, they never found fish with an empty stomach as they entered the salt marshes. Nonetheless, a higher instantaneous ration (Ir = gut content weight/body weight) indicated that mullets came to feed actively on the salt marshes. This is likely due to the higher elevation of salt marshes that are flooded for 1 or 2 hours at the end of the flood tide and the beginning of the ebb tide, when mullets have started to feed before their capture. Similar results have been found by Lebreton et al. (2011) in Aiguillon Bay (France), where fishes were also caught in salt marshes lining the mudflat. Our results suggest that most grey mullets stay during the low tide without feeding in the subtidal area and wait for the next flood tide to reach the mudflats to forage. By extension, this proved that fish do not import anything from subtidal areas into tidal flats, which to our knowledge has never been shown before. Conversely, according to the mean biomass of stomach content (11.03 ± 0.58% of body weight), the export of materials from tidal flats to subtidal areas appeared concordant with those of Laffaille et al. (2002) (8% of body weight) but with a different %POM (31.3 ± 7.6% for Laffaille et al. (2002) compared to 6.79 ± 0.43% in our study). That difference in %POM most likely reflects different amounts of detritus (litter) mainly present in salt marshes compared to tidal flats where no macrophytes occur (e.g., Valéry et al., 2004). The absence of

salt marshes in Brouage Bay also permitted us to confirm the major role played by tidal flat biofilms for mullets. Indeed, studies dealing with mullets feeding on biofilm mainly concerned fish caught in salt marshes lining tidal flats (Laffaille et al., 1998, 2002; Lebreton et al., 2011; but see Almeida, 2003) where fish concentrate in canals, making them easier to sample. It was therefore impossible to distinguish where and when fish feed during their tidal migration.

Biofilm and associated meiofauna seem consequently to act as a unique resource for those species, at least during their presence on coastal mudflats, which is probably temporary. Indeed, during their life cycle, grey mullets exhibit a set of migration strategies, including amphidromous ones (Almeida, 1996, 2003). If most juveniles colonize estuarine or freshwaters (Trancart et al., 2011), adults exhibit various migration strategies notably between marine and freshwaters (Daverat et al., 2011). Therefore, more studies will be necessary to conclude about the relative contribution of biofilms to the overall diet throughout the entire life cycle of mullets.

Qualitatively, biofilm is a complex assemblage of primary producers associated with meiofauna and microfauna grazers. What exactly grey mullets ingest, select, and assimilate from biofilm and meiofauna grazers continues to be debated with sometimes contradictory results. Indeed, while Almeida (2003) suggested that mullets selectively reject particles <55 µm, a more recent study conversely observed retention of particles ranging between 20 and 55 µm (Pedro et al., 2008). It has also been proven that prey such as benthic diatoms or nematodes can be selectively ingested from sediment by the fish (Odum, 1970; Guinea and Fernandez, 1992; Pedro et al., 2008). This ability to select small-sized particles is in accordance with diatom sizes reported in Brouage Bay, where the assemblage structure of benthic diatoms is characterized by the dominance of a small species, *Navicula phyllepta* (around 20–30 µm in length), throughout the year with a seasonal succession of secondary species, including only 1 large diatom (*Gyrosigma peisonis*, around 50–60 µm in length) (Dupuy et al., this issue; Haubois et al., 2005). Concerning animal prey, our results appear consistent with recent evidence for the importance of meiofauna, especially nematodes, in grey mullet diets (organisms > 63 µm) (Lebreton et al., 2011), which mullets seem able to concentrate. Other preys are also more or less greatly concentrated by mullets, but appear more variable in diet as suggested by their relative isotopic signatures. It was nevertheless clear that, unlike foraminifers and platyhelminthes, mullets significantly concentrate copepods and ostracods. The observed variability of abundances of those preys in stomach contents could be due to their reduced availability (compared to nematodes) in sediments, relative

abundance being consistent with other studies in the same site (e.g., Rzeznik-Orignac et al. (2003) found that 95% of meiofauna taxa in numbers were nematodes while copepods and ostracods counted for only 2 and 1%, respectively). One can finally hypothesize that the extreme copepod density variability in stomach contents can be due to an aggregative distribution of that prey. Note that if foraminifer shells remained intact in feces, they were probably killed during the digestion process, even if their contribution to mullet diet appeared low or non-existent both in number and based on isotopic signatures. The hypothesis of potential dispersion of foraminifers by mullets seems consequently unlikely even if such observations were still revealed for molluscs surviving fish gut passage (see Brown, 2007). Finally, we also found that mullets are able to reject larger preys such as *H. ulvae*, probably by filtration through their gill rakers, since this species was absent from mullet diets, a result that would not be obvious based on isotopic signature methods.

It appears that the mullet diet in mudflats is a mix of meiofauna and primary sources. The balance between those different components of the diet remains relatively stable over seasons but could also explain inter-individual variability. It also depends on the ability of mullets to concentrate and, thus, filtrate what they ingest. It is likely that meiofauna contribute to the high trophic level found for mullets by Lebreton et al. (2011) and Vinagre et al. (2012). Note that Pasquaud et al. (2010) found that mullets in a temperate estuarine food web remained at the lowest trophic level among all fish species. This confirms the very special feeding niche that mullets occupy and probably their success in terms of occurrence and abundance, at least in temperate waters. Finally, those results also suggested that mullet diets in tidal flats remain consistent with those of salt marshes.

One can wonder why we found such a high mineral fraction in stomach contents (up to 90%), which tends to prove that the selection efficiency for biofilm by grey mullets remains low. The size of mud particles may be too low to be rejected or too similar to those of items mullets keep (microphytobenthos). Nevertheless, this could also be due to the reduced time mullets have for feeding (mudflats are immersed for only a few hours per day depending on the tide coefficient); mullets optimize the quantity of food ingested rather than the quality. This hypothesis is partially sustained by the observed frenzy of fish during the flood (obs. pers.), some individuals feeding with half of their body out of the water and most hurrying to reach immersed mudflat parts as quickly as possible. This behavior may also be linked to the microphytobenthos behavior at flood tide. Indeed, it has been observed that microphytobenthos migrate from 3 to 5 mm down to the sediment as immersion occurs

(Herlory et al., 2004) leading to a dilution of their concentration for mullet that feed by dragging the surface of the mud with their mouth.

Finally, if this study helped to clarify the diet and feeding behavior of grey mullets on tidal mudflats, the next question is then the role of the habitat and their resources for mullets and, conversely, the role of grey mullets in the global functioning of tidal flats. The great productivity of biofilm and the lack of competition, since grey mullets are the only fish species able to exploit it, could make temperate tidal mudflats an important habitat, especially for adult grey mullets. Indeed, sampling studies including mullets in salt marshes have found an absence of sub-adult G2 age fish (150 to 195 mm according to Lebreton et al., 2011) (e.g., Laffaille et al., 2000; Lebreton et al., 2011). The mesh sizes of the nets we have used were too large to catch fish smaller than 200 mm, but the smallest fish captured was 265 mm, in accordance with the results of the previous studies. One can hypothesize that tidal flats are transitional habitats for adult individuals waiting for gonad maturation before reaching the high sea to reproduce in autumn and winter at a minimum age of 3 years old (G3) (Bruslé, 1981b). Consequently, biofilm and associated meiofauna could be considered to be a main resource for grey mullets during gonad maturation. Nevertheless, it appears necessary to study grey mullets in other habitats where adults occur (e.g., harbors and coastal areas) to explore their strategy and life history when they use different feeding resources.

In conclusion, the comprehension of the functional interaction between mullets and tidal flats is improved notably by a better understanding of material exchange between tidal flats and adjacent subtidal ecosystems. As previously suggested by modeling (Degré et al., 2006), our results also confirmed the privileged export pathway for locally produced biofilm by grey mullets through ingestion during their tidal migration and their ability to select and concentrate some prey.

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Figure captions

Figure 1. Comparisons of mean densities of main meiofauna taxa (expressed in numbers of individuals per g of fresh biofilm (the first cm of the upper layer of sediment and in fact a mix of mud, biofilm, and associated fauna), stomach content, or feces weight, accordingly) between the Brouage tidal flat biofilm, grey mullet stomach contents (n = 21) and feces (n = 10). Densities were log-transformed to improve graphical representations of scarcer prey. *P < 0.05; **P < 0.01; ***P < 0.001; NS = not significant.

Figure 2. Mean ± sd $\delta^{15}N$ and $\delta^{13}C$ of grey mullet liver and muscle according to month. Prey and primary source signatures are also reported: particulate organic matter (POM), suspended organic matter (SOM), microphytobenthos (Mpb.), foraminifers (Foram.), copepods (Cop.), *Hydrobia ulvae* (*H. ulvae*), and nematodes (Nem.). Copepod and foraminifer isotopic values come from Pascal et al. (unpublished data); all sources and other preys come from Bocher et al., this issue and unpublished data.

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Tables

Table 1. Details of fish sampling in 2008 at Brouage mudflat according to months and tides, in numbers, mean sizes, and stomach contents (SC, expressed in standardized % of individual biomass) with standard errors (se).

	May			July			September			TOTAL	
	flood	ebb	total	flood	ebb	total	flood	ebb	total	flood	ebb
n	2	11	13	11	4	15	3	21	24	16	36
mean size (mm)	373.50	363.73	365.23	350.73	357.50	352.53	348.00	314.71	318.88	353.06	334.44
se	9.50	10.20	8.69	6.62	5.58	5.04	8.00	7.39	6.89	5.15	6.58
SC (%)	9.99	12.98	12.52	3.40	6.16	4.14	6.11	10.94	10.34	4.74	11.03
se	6.16	0.87	1.06	0.26	1.79	0.57	1.98	0.63	0.67	0.88	0.58

Fig. 1.

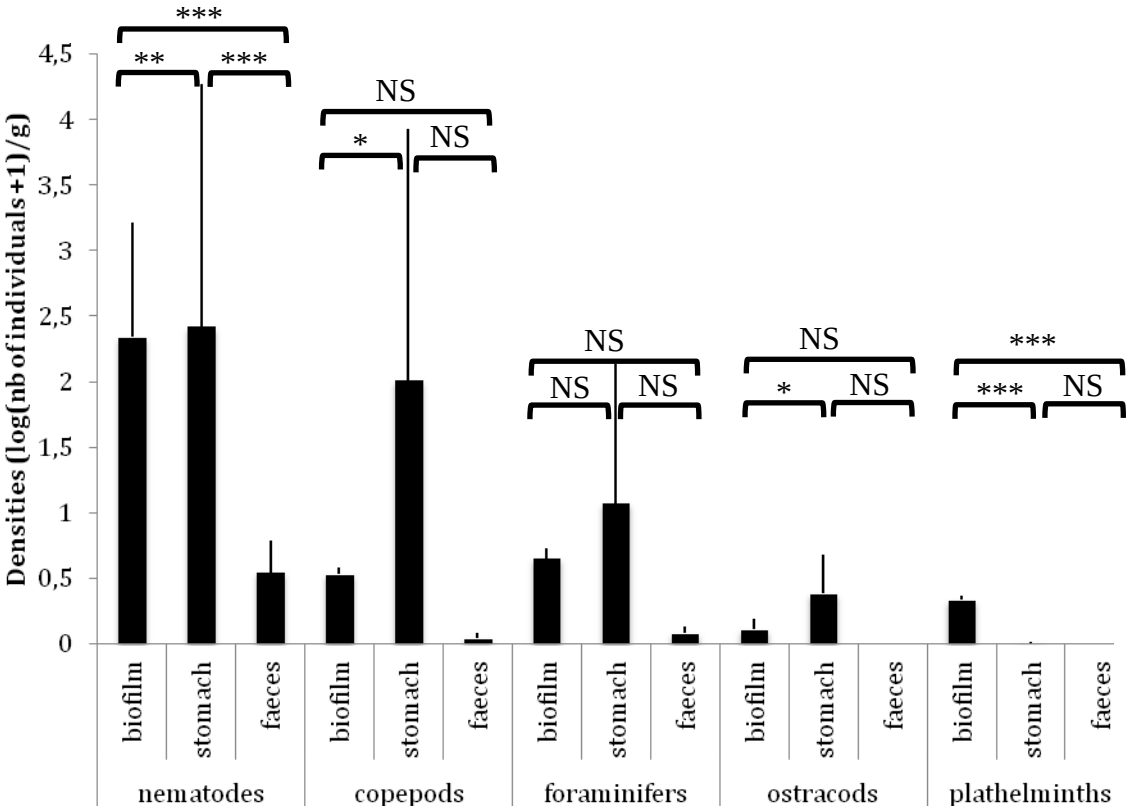
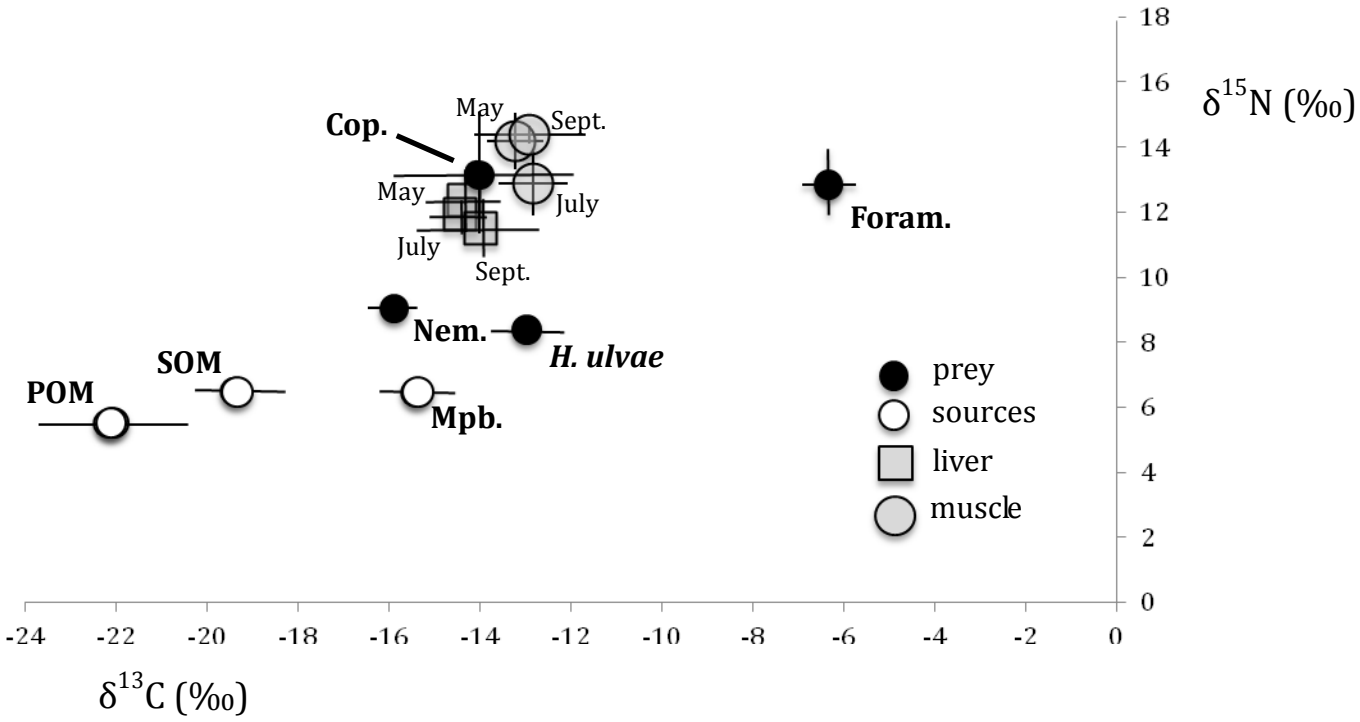

Densities (log(nb of individuals+1)/g)
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
***
**
***
NS
*
NS
NS
NS
NS
NS
*
NS
***
***
NS
biofilm
stomach
faeces
nematodes
copepods
foraminifers
ostracods
plathelminths

Fig. 2


Cop.
May
Sept.
May
July
July
Sept.
Foram.
Nem.
H. ulvae
SOM
POM
Mpb.
prey
sources
liver
muscle
δ15N (‰)
δ13C (‰)
18
16
14
12
10
8
6
4
2
0
-24
-22
-20
-18
-16
-14
-12
-10
-8
-6
-4
-2
0