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ORIGINAL RESEARCH

Spatial variation in the feeding strategies of Mediterranean fish: flatfish and mullet in the Gulf of Gaeta (Italy)

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Abstract Marine coastal areas are highly productive due to the presence of various inputs of organic matter, including terrestrial material, which fuels food webs. However, the ecological mechanisms underlying the productivity of benthic and demersal fish species in estuarine areas are poorly understood. By means of C and N stable isotope analysis and Bayesian mixing models, we investigated the trophic niches of three common fish species: *Citharus linguatula*, *Pegusa lascaris* (flatfish) and *Liza ramada* (mullet) in the Gulf of Gaeta (Italy). Fish were collected from the north-western area and the south-eastern area of the Gulf of Gaeta, the latter affected by organic inputs from the Garigliano River. The results highlighted the riverine terrestrial origin of the organic matter at the base of the food web in the south-eastern area and marine autochthonous input in the north-western area. All fish species increased their trophic specialisation in proximity to the river mouth. *L. ramada* specialised on seston of terrestrial origin, reducing its niche overlap with *C. linguatula* and *P. lascaris*. Away from the river mouth, all species were characterised by longer individuals, increased intraspecific diet variability and higher interspecific similarity in resource use. Organic input from the river represented a complementary trophic niche axis that enabled lower interspecific niche overlap in the south-eastern area, where fish populations were found at higher densities. In conclusion, this study provided information about the effects of the flow of material from the basal compartment up to abundant fish species in areas enriched by organic matter of varying origin.

Keywords Stable isotopes · Trophic niche · Estuarine and coastal waters · *Citharus linguatula* · *Pegusa lascaris* · *Liza Ramada*

Introduction

Marine coastal ecosystems are among the most productive ecosystems in the world, supporting great density and diversity of life. However, despite their value to humans, these ecosystems are becoming increasingly vulnerable, since they are exposed to strong pressures from anthropic activities including coastal urbanisation, tourism and fish farming (Halpern et al. 2008), as well as the consequent rise in organic and inorganic inputs (Careddu et al. 2015; Calizza et al. 2015; Jona-Lasinio et al. 2015; Rossi et al. 2018). In marine ecosystems, benthic predators such as decapods and fish, including important

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commercial species, are influenced by sediment and vegetation structure, faunal composition, energy fluxes and nutrient cycles (Fazi and Rossi 2000; Carrozzo et al. 2014; Careddu et al. 2017). In turn, fish are important consumers, influencing aquatic food web structure and functioning (Frederiksen et al. 2006; Mancinelli et al. 2007). Their foraging behaviour and feeding ecology are generally characterised by high trophic plasticity. Specifically, their dynamics seem to be closely related to the dynamics of organic matter pools (Darnaude et al. 2004; Buchheister and Latour 2011; Kostecki et al. 2012; di Lascio et al. 2013; Selleslagh et al. 2015; Bentivoglio et al. 2016), in many cases promoting exportation of organic matter via migration from highly productive to less productive areas (Le Loc'h et al. 2015).

In this context, the study of trophic niches represents a useful tool for describing the trophic pathways and foraging behaviour of species in coastal areas (Kostecki et al. 2012; Careddu et al. 2015). Stable Isotope Analysis (SIA) is increasingly being used as an efficient method for studying trophic niches and fish ecology (Buchheister and Latour 2011; Layman and Allgeier 2012; Le Loc'h et al. 2015; Parzanini et al. 2017; Adams et al. 2017; Costantini et al. 2018). Indeed, the isotopic signatures of carbon (δ^{13}C) and nitrogen (δ^{15}N) give information about the resources exploited by a consumer and its trophic position in the food web (Post 2002; Inger and Bearhop 2008; Kowskowsi and Trembaczowski 2015; Gallagher et al., 2017). Moreover, the δ^{13}C and δ^{15}N values of consumers within a given environment can be plotted on an isotopic biplot, allowing the quantification of isotopic niche metrics (Layman et al. 2007; Jackson et al. 2011; Calizza et al. 2017), which can provide useful insight concerning the trophic structure of a population and interspecific interactions. In addition, the recent improvement of multivariate Bayesian models and bootstrapped procedures has enabled the incorporation of isotopic signature uncertainty, providing more robust information about the diet and trophic niches of the studied species (Parnell et al. 2010; Jackson et al. 2011; Phillips 2012; Rossi et al. 2015).

As well as enabling reconstruction of trophic niche and diet, δ^{13}C also provides information on the various origins of consumer resources (Post 2002; Inger and Bearhop 2008; Kostecki et al. 2012; Faria et al. 2018), since it varies widely among primary producers (e.g. terrestrial C3 plants, marine producers) (Marshall et al. 2007; Rossi et al. 2010; Careddu et al. 2015). This makes it possible to determine the importance of terrestrial inputs to food webs in estuarine habitats (Kostecki et al. 2012; Careddu et al. 2015).

Many studies in the Mediterranean Sea have focused on the trophic ecology of pelagic fish species (Stergiou and Karpouzi 2002; Pinnegar et al. 2003; Zeug et al. 2017). However, less is known about benthic and demersal fish species in productive coastal areas. For these categories, our understanding of the influence of nutrient inputs and environmental factors on their diet and their role in the food web is relatively limited (Darnaude et al. 2004; Rumolo et al. 2016). Indeed, although estuarine areas are generally considered to be highly productive, the ecological mechanisms underlying such productivity in benthic and demersal fish communities have not been extensively investigated. Among others, flatfish are valuable as a product for human consumption, mullets are important low-cost sources of protein, and both are commercially exploited worldwide. Being bottom-feeders, they are able to use both autochthonous and allochthonous inputs, such as terrestrial-derived food inputs transported by river run-off in coastal areas, thereby linking grazing and detritus chains and thus playing a key ecological role in coastal and estuarine habitats (Darnaude et al. 2004; Buchheister and Latour 2011; Kostecki et al. 2012). Understanding the species' feeding behaviour and the main trophic sources supporting fish populations is fundamental to the management of areas crucial for fisheries. From this perspective, the aim of this study was to investigate, by stable isotope analysis, the trophic niches and overlap of flatfish and mullet in the Gulf of Gaeta. This is an important recruitment zone in the Mediterranean Sea (Colloca et al. 2015; Criscoli et al. 2017), crucial for fisheries and fish farming (Guerriero et al. 2017; Mazzola and Sarà 2001), characterised by a gradient of influence exerted by a large river mouth (Orlandi et al. 2014; Careddu et al. 2015, 2017; Rossi et al. 2018). We assessed the spatial variation of (i) trophic niches and (ii) the strength of interspecific interaction between the flatfish *Pegusa lascaris* (Risso 1810) and *Citharus linguatula* (Linnaeus 1758) and the mullet *Liza ramada* (Risso 1826), which are among the most common fish in the Gulf, in association with riverine input of terrestrial-derived organic matter.

Materials and methods

Study area and sampling activities

Sampling was carried out in October and November 2012 in the marine coastal area of the Gulf of Gaeta (Italy, central Tyrrhenian Sea), extending from the town of Gaeta (41°12′51″N, 13°34′16″E) to the Garigliano River mouth (41°13′23.36″N, 13°45′40.66″E; average discharge 120 m^3/s) (Fig. 1). Currents mostly flow from south-east to north-west following the cyclonic circulation of the Tyrrhenian Sea (La Rosa et al. 2002). However, there is another current flowing from offshore towards the coast near the village of Scauri (Aguzzi et al. 2012). These currents cause organic matter and nutrients to accumulate off the Garigliano River mouth in the south-eastern portion of the Gulf of Gaeta (Rossi et al. 2018). Two areas were selected for the sampling activities: the first in the north-western part of the Gulf (hereafter called NW) between the town of Gaeta and the promontory of Gianola-Monte Scauri, and the second between the promontory and the Garigliano River mouth to the south-east (hereafter called SE) (Fig. 1).

Within each area, benthic macroinvertebrates were collected in October and November 2012. In order to collect the diverse invertebrate taxa potentially included in the fish's diets, invertebrates were sampled by multiple methods at four sampling sites at depths ranging from 5 to 12 m in each area (Fig. 1). Three replicate samples per site were collected, using a Van Veen grab (0.035 m^2), leaf litter bags containing autochthonous leaf detritus (30 g dry weight) (Van Dokkum et al. 2002; Costantini et al. 2009; Calizza et al. 2013a) and fish traps for larger decapods and gastropods (Careddu et al. 2015). Given the different methods of collection, the abundance of invertebrates is indicated as the number of individuals per sampling site. Sediment collected with the grab was sieved through a 500-µm mesh to isolate the infauna specimens. Additional sediment replicates were collected in order to determine the isotopic composition of organic matter in the sediments (hereafter SOM) and to isolate samples of coarse particulate organic plant matter (hereafter detritus) from the sediment bulk (Calizza et al. 2013b). The organic matter component (dead and living) present in the water column (hereafter seston) was sampled by a 20-µm WP2 plankton net (Rolff 2000). The same procedure was used to obtain seston samples from the Garigliano River, 1 km upstream from the river mouth, which made it possible to measure the $\delta^{13}C$ and $\delta^{15}N$ values of organic matter transported by the river. Water samples were stored in 5 L refrigerated dark bottles and transported to the laboratory. Here, samples were prefiltered in order to collect particles within the range of 100–500 µm, which enabled us to exclude both small phytoplankton and coarse detritus particles.

In each area, adult specimens of *L. ramada*, *C. linguatula* and *P. lascaris* were captured with the help of professional fishermen at the end of November. In both areas, using an 8-m fishing boat, a 400-linear

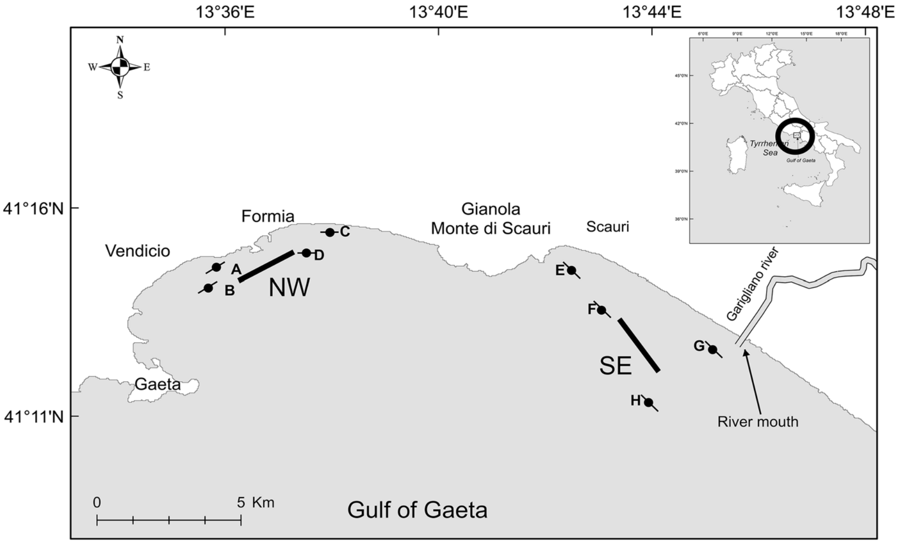

Fig. 1 Map of the Gulf of Gaeta showing the four sampling sites for fish resources in each of two study areas in the Gulf of Gaeta: A, B, C, D (north-west area, NW) and E, F, G, H (south-east area, SE). The two solid black lines indicate transects used for fish sampling using nets

metre gillnet (mesh size 10 mm) was lowered to a depth of 10 m for 12 h.

Laboratory procedures and stable isotope analysis

Samples of seston were concentrated by centrifugation (2000 rpm for 20 min). Water from the layer containing seston was then collected and passed through precombusted Whatman GF/F filters to obtain a sufficient amount of biomass, which was analysed as a whole for its isotopic signature (Kostecki et al. 2012; di Lascio et al. 2013). Samples of detritus and fresh seagrass were isolated and rinsed in distilled water. SOM was collected from the sediment surface (Mancinelli and Rossi 2002). All the collected macroinvertebrates were transferred to the laboratory in refrigerated plastic bags, sorted, identified using binocular microscopes and enumerated. Fish were measured for standard length (SL) to the nearest mm and weighed (W, wet weight) to the nearest g. For the stable isotope analysis, one sample of dorsal muscle was taken from each fish (Darnaude et al. 2004; Bentivoglio et al. 2016; Wolters et al. 2018). All the samples were stored at − 80 °C and then freeze-dried. Detritus samples were further observed under a binocular microscope to remove any inorganic residual impurities. Seagrass fragments were identified, gently scraped to remove epibionts and washed with distilled water. To remove carbonates, which could interfere with δ^{13}C signatures, subsamples of sediment and seston were acidified (HCl 1M) drop by drop and re-dried (60 °C, 72 h) before being analysed for δ^{13}C (Kennedy et al. 2005; Kostecki et al. 2012). In this case, the δ^{15}N signatures were obtained from unacidified subsamples. For analysis of macroinvertebrates, exoskeletal parts, valves and shells were removed under a dissection microscope, and only very small organisms were acidified drop by drop to remove inorganic carbon and then re-dried (60 °C, 72 h) before analysis. All samples were ground to a fine powder using a ball mill (Fritsch Mini-Mill Pulverisette 23). Subsamples of 0.20 ± 0.05 mg (animal tissues) and 3.0 ± 0.5 mg (basal resources) were placed in tin capsules (Post 2002; Rossi et al. 2007; Costantini et al. 2014). The samples were analysed using an Elementar Vario Micro-Cube elemental analyser (Elementar Analysensysteme GmbH, Germany) coupled with an IsoPrime100 isotope ratio mass spectrometer (Isoprime Ltd., Cheadle Hulme, UK). Stable carbon and nitrogen ratios were expressed in δ units in per mil (‰) as the difference with respect to international standards (Vienna Pee Dee Belemnite—PDB for carbon and atmospheric N_2 for nitrogen) in accordance with the equation: δX (δ^{13}C or δ^{15}N) = $[(R_{\text{sample}}/R_{\text{standard}})/R_{\text{standard}}] \times 10^3$ (Ponsard and Arditi 2000), where R is either the ^{13}C/^{12}C or the ^{15}N/^{14}N ratio. The internal laboratory standard was IAEA-600 Caffeine. Measurement error was found to be typically smaller than 0.05‰. In accordance with Post et al. (2007), for fish with C/N ratios > 3.5, the δ^{13}C signatures were corrected for lipid content using the equation: $\delta^{13}\text{C}_{\text{corrected}} = \delta^{13}\text{C}_{\text{measured}} - 3.32 + 0.99 \times \text{C/N}$ (Post et al. 2007). As in Abrantes et al. (2013), the δ^{13}C signatures of invertebrates were not corrected for lipid content because shifts in δ^{13}C associated with lipid removal can be highly variable, small and taxon-specific (Logan et al. 2008; Mateo et al. 2008).

Trophic niche analysis and mixing models

Isotopic niche metrics were applied to each species in accordance with Layman et al. (2007). The range between the lowest and highest δ^{15}N values (NR) indicates the maximum distance between the basal resource and the top consumer in the food web. The range between the lowest and highest δ^{13}C values (CR) gives information about the range of resources used by organisms. The mean nearest neighbour distance (MNND) is the mean distance between nearest values in the isotopic biplot space, providing information about the trophic redundancy of individuals (lower values indicate higher trophic redundancy), and its standard deviation (SDNND) gives information about the evenness of isotopic data distribution. The mean distance to centroid (CD) is the average Euclidean distance of each individual to the centroid, indicating the average trophic diversity (Layman et al. 2007). In addition to the Layman et al. (2007) metrics, standard ellipse areas were calculated (as SEAc; SIBER analysis, Jackson et al. 2011). The standard ellipse area is comparable to standard deviation for univariate cases applied to bivariate data. It provides robust information about the bidimensional isotopic niche area and allows comparisons for small population sizes (i.e. for $n \geq 5$, Perkins et al. 2018; Bašic et al. 2018) because it is largely insensitive to sample size (Jackson et al. 2011). Bayesian

Stable Isotope Mixing Models were run in order to evaluate the contributions of macroinvertebrates, SOM, detritus and seston to the fish diets in each area. Given (i) the relatively shallow sampling depth (10 m), and (ii) previous evidence demonstrating that seston contributes to the diet of flatfish in estuarine areas (Kostecki et al. 2012), seston was considered a potential trophic source for both the mullet and the two flatfish. The seagrass samples, mainly *Posidonia oceanica* (Delile 1813), were not included as input in the mixing models because they were only occasionally found in the northern area. In the northern area, the mullet *L. ramada* presented two separate isotopic δ^{13}C groups (see Results). Thus, specimens of these two groups were considered separately for the determination of their diet by means of Bayesian mixing models. The SIAR model requires the isotopic signatures of target consumers and potential resources (mean and standard deviation), as well as the Trophic Enrichment Factor (TEF), which is the difference between the isotopic signatures of consumers and their potential diets. We chose a TEF of 2.9 ± 0.3‰ for δ^{15}N and 1.3 ± 0.3‰ for δ^{13}C, as measured in muscle tissue, on the basis of literature data (McCutchan et al. 2003). The output of the model is a density function distribution of plausible proportion values, whose central tendency (mode, mean, median) and upper and lower limits of credibility intervals (CI: 50%, 75%, 95%) reveal the range of feasible contributions to fish diets of each resource (Parnell et al. 2010).

Following Calizza et al. (2017), the potential limiting effect between species pairs (αij, i.e. the effect of species j on species (i) in each area was calculated from the ratio between the intra- and interspecific isotopic distance between specimens. Briefly, the mean isotopic distances of each specimen from (i) all its conspecifics (MND*intra*), and (ii) all specimens of each remaining species (MND*inter*) were calculated and compared. At the local scale, the ratio of MND*intra* to MND*inter* has been shown to be a robust measure of the strength of interspecific interaction between populations sharing trophic resources (Calizza et al. 2017; Britton et al. 2019). It is not affected by sample size and is directly dependent on the similarity in diet between specimens (Calizza et al. 2013b, 2017, 2018).

Data analysis

Data were tested for normality and transformed if necessary before performing statistical analyses. To test for potential differences between the isotopic distributions (δ^{13}C and δ^{15}N) of the three fish species, a Permanova test based on Euclidean distances was performed (Calizza et al. 2017).

The parameters a and b of the length–weight relationship were estimated by logarithmic transformation of the equation $W = a*L^b$, in which W is the body weight (g); L is the standard body length (mm); a is the intercept; and b is the slope indicating the condition of fish (Ricker 1973; Jones 2009). ANCOVA was performed at 0.05 significance in order to compare the condition of each fish species between areas, with significantly higher b values indicating better conditions (Jones 2009).

Results

Food sources

δ^{13}C and δ^{15}N values in seston collected in the Garigliano River were − 29.9 ± 1.0‰ and 7.2 ± 0.8‰ respectively. The δ^{13}C values of seston from marine water, detritus and SOM were lower in SE than in NW (*t* test: $t_{(\text{seston})} = 2.28$, $t_{(\text{detritus})} = 8.13$ and $t_{(\text{SOM})} = 4.02$, all $p < 0.05$), as were the δ^{15}N values of detritus and SOM (*t* test: $t_{(\text{detritus})} = 2.12$, $t_{(\text{SOM})} = 2.23$, both $p < 0.05$), whereas no differences were found in the δ^{15}N values of seston (Table 1). The abundance of macroinvertebrates did not differ significantly between the two areas: 693.26 ± 84.04 individuals/site were sampled in NW and 517.53 ± 72.59 individuals/site in SE (*t* test, $t = 1.58$, $p > 0.05$). In addition, average macroinvertebrate δ^{13}C and δ^{15}N values were similar (*t* test, δ^{13}C: $t = 1.1$, $p > 0.05$; δ^{15}N: $t = 0.04$, $p > 0.05$), and several taxa had overlapping isotopic values (Table 2). The most ^{13}C-enriched taxa were Cumacea and Tanaidacea in NW and SE respectively, while the most ^{13}C-depleted taxon was the sea urchin (Spatangoida) in both areas (Table 2).

Table 1 Mean $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) values (± standard error) of basal resources and macrobenthic fauna in two areas of the Gulf of Gaeta

Area	Resource type	$\delta^{13}C$	$\delta^{15}N$
North-west	SOM	− 23.60 ± 0.39	7.14 ± 0.65
	Detritus	− 14.81 ± 0.69	5.88 ± 0.42
	Macrobenthos	− 17.29 ± 0.14	7.97 ± 0.18
	Seagrass	− 13.68 ± 1.08	7.60 ± 1.83
	Seston	− 21.48 ± 0.47	8.38 ± 0.13
South-east	SOM	− 25.27 ± 0.15	5.61 ± 0.23
	Detritus	− 23.25 ± 0.76	4.12 ± 0.68
	Macrobenthos	− 17.93 ± 0.14	7.74 ± 0.18
	Seston	− 24.84 ± 1.52	8.04 ± 0.13

SOM Sediment organic matter. Detritus refers to coarse (i.e. > 2 mm) organic debris found in or above the sediment. Seston refers to organic matter sampled from the water column

Fish species

Overall, 70 specimens of *L. ramada* (8 in NW and 62 in SE), 18 specimens of *C. linguatula* (11 in NW and 7 in SE) and 45 specimens of *P. lascaris* (7 in NW and 38 in SE) were collected (Table 3). Individuals of *L. ramada, C. linguatula* and *P. lascaris* were longer in NW than in SE (Table 3; Student *t* test, $t_{(L.\ ramada)} = 4.20$, $t_{(C.\ linguatula)} = 3.97$ and $t_{(P.\ lascaris)} = 11.50$, p always < 0.001). The slopes (b) of the log-transformed length–weight relationships ($W = a*L^b$) of *L. ramada* ($b_{NW} = 2.30$, $b_{SE} = 3.12$; ANCOVA, $F = 5.5$, $p < 0.05$), *P. lascaris* ($b_{NW} = 1.92$, $b_{SE} = 2.67$; ANCOVA, $F = 4.7$, $p < 0.05$) and *C. linguatula* ($b_{NW} = 3.11$, $b_{SE} = 2.05$; ANCOVA, $F = 5.7$, $p < 0.05$) differed significantly between areas.

In each area, the isotopic distribution of $\delta^{13}C$ and $\delta^{15}N$ values differed between fish species (Fig. 2; NW: PERMANOVA, $F = 2.98$, $p < 0.05$; SE: PERMANOVA, $F = 113.5$, $p < 0.05$). In the NW area, *C. linguatula* had the highest $\delta^{15}N$ values (one-way ANOVA, $F = 14.89$, $p < 0.05$, Tukey test, $p < 0.05$). In contrast, the $\delta^{13}C$ values of the three species overlapped substantially, lying within the $\delta^{13}C$ range of marine resources. In SE, *L. ramada* had significantly lower carbon signatures (PERMANOVA, $F = 26.55$, $p < 0.05$), while the isotopic signatures of *C. linguatula* and *P. lascaris* were similar to what was observed in NW (PERMANOVA, F = 1.98 and $F = 2.37$ respectively, $p > 0.05$ for both species), and the three species overlapped on the $\delta^{15}N$ axis (Fig. 2). Overall, $\delta^{13}C$ values increased with body length in *C. linguatula* and *L. ramada* ($r = 0.78$, $p < 0.001$ and $r = 0.50$, $p < 0.001$ respectively), while $\delta^{15}N$ values increased with body length in *C. linguatula* only ($r = 0.50$, $p < 0.05$).

Isotopic and trophic niches

In both areas, the trophic niche area, calculated as the SEAc, was largest for *L. ramada* and smallest for *C.*

Table 2 Mean $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) values (± standard error) of each invertebrate taxon sampled in two areas of the Gulf of Gaeta

Taxon	North-west		South-east	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Amphipoda	− 16.29 ± 0.54	7.52 ± 0.54	− 17.67 ± 0.33	6.44 ± 0.58
Bivalvia	− 18.54 ± 0.42	6.75 ± 0.22	− 18.50 ± 0.60	6.02 ± 0.35
Cumacea	− 14.13 ± 1.20	5.15 ± 0.79	− 16.37 ± 0.31	5.52 ± 0.75
Decapoda	− 16.60 ± 0.54	8.31 ± 0.25	− 16.67 ± 0.82	7.92 ± 0.64
Gastropoda	− 16.72 ± 0.24	8.94 ± 0.29	− 17.60 ± 0.10	9.07 ± 0.17
Isopoda	− 15.00 ± 0.09	1.41 ± 0.10	− 16.88 ± 0.10	6.10 ± 0.10
Nemertea	− 18.34 ± 0.76	9.77 ± 0.42	− 17.29 ± 0.28	8.29 ± 0.61
Ophiurida	− 18.03 ± 0.24	8.56 ± 0.20	− 17.23 ± 1.14	8.04 ± 0.87
Polychaeta (Errantia)	− 17.22 ± 0.24	9.56 ± 0.25	− 18.22 ± 0.17	9.02 ± 0.27
Polychaeta (Sedentaria)	− 17.75 ± 0.27	8.11 ± 0.28	− 18.97 ± 0.17	7.67 ± 0.33
Scaphopoda	− 16.54 ± 0.28	8.87 ± 0.43	− 16.77 ± 1.61	7.64 ± 0.29
Spatangoida	− 19.24 ± 1.04	1.11 ± 0.45	− 21.13 ± 0.10	1.64 ± 0.90
Tanaidacea	− 15.43 ± 0.42	5.36 ± 1.11	− 15.27 ± 1.32	5.50 ± 1.53
Average	− 16.91 ± 0.41	6.88 ± 0.79	− 17.58 ± 0.40	6.84 ± 0.55

Average refers to mean $\delta^{13}C$ and $\delta^{15}N$ values across all taxa

Table 3 Number of individuals, mean body length (cm), $\delta^{13}C$ and $\delta^{15}N$ values (± standard error) and isotopic niche metrics (‰) of three fish species in two areas of the Gulf of Gaeta (NW: north-west and SE: south-east)

Area	Species	*n*	Length	$\delta^{13}C$	$\delta^{15}N$	CR	NR	CD	MNND	SDNND	SEAc
NW	*C. linguatula*	11	13.55 ± 2.24	− 17.58 ± 0.10	12.71 ± 0.22	1.08	2.33	0.71	0.3	0.17	0.80
	L. ramada	8	40.43 ± 3.62	− 19.15 ± 1.64	10.08 ± 0.57	9.71	5.08	4.11	1.23	1.22	20.12
	P. lascaris	7	24.43 ± 1.89	− 18.04 ± 0.52	11.33 ± 0.26	3.24	1.72	1.03	0.78	1.24	1.90
SE	*C. linguatula*	7	9.30 ± 1.15	− 18.40 ± 0.08	11.65 ± 0.08	0.47	0.52	0.25	0.12	0.08	0.15
	L. ramada	62	31.50 ± 5.06	− 26.54 ± 0.36	11.84 ± 0.28	14.23	9.79	3.05	0.59	0.63	18.69
	P. lascaris	38	15.08 ± 1.89	− 18.05 ± 0.14	12.12 ± 0.10	4.68	3.47	0.84	0.3	0.39	1.77

Isotopic niche metrics include CR: carbon range, NR: nitrogen range, CD: distance to centroid, MNND and SDNND: respectively mean and standard deviation of nearest neighbour distance, SEAc: Standard ellipse area, corrected for degrees of freedom (‰^{2}). For details of metrics, please refer to the materials and methods section

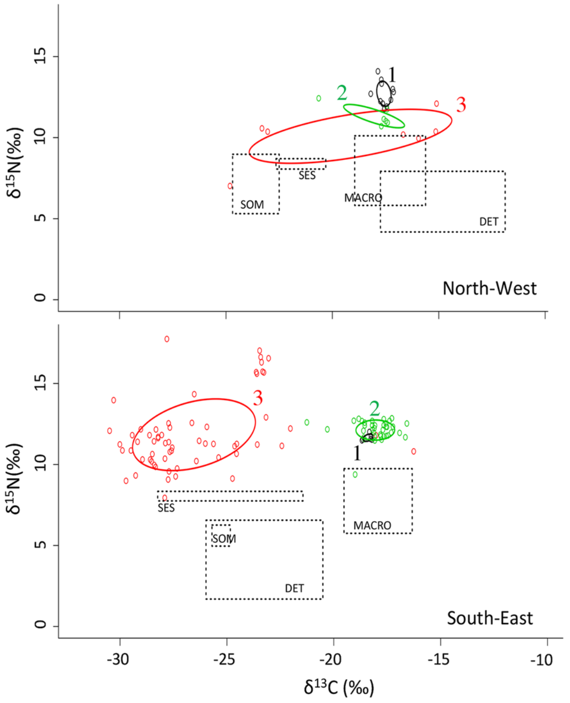

Fig. 2 Trophochemical graphs of $\delta^{13}C$ and $\delta^{15}N$ values with standard ellipses for the three fish species (1: *Citharus linguatula*, 2: *Pegusa lascaris* and 3: *Liza ramada*) sampled in two areas of the Gulf of Gaeta: north-west, south-east. Dashed polygons indicate the variation in isotopic signals (mean ± standard deviation) of the potential resources: seston (SES), sediment organic matter (SOM), Detritus (DET) and Macroinvertebrates (MACRO)

linguatula (Table 3 and Fig. 2). Despite the higher number of individuals of *L. ramada* and *P. lascaris* sampled, niche area was smaller and intraspecific similarity greater for all species in SE than in NW. *L. ramada* had the largest CR and NR. In NW, this species was characterised by two separate isotopic groups differing in their $\delta^{13}C$ values (− 23.72‰ ± 0.55 vs. − 15.72‰ ± 0.37, *t* test, $t = 12.03$, $p < 0.0001$) but with similar $\delta^{15}N$ values (9.32‰ ± 1.15 vs. 10.65‰ ± 0.48, *t* test, $t = 1.1$, $p > 0.05$) (Fig. 2). As a consequence, when considering all the specimens sampled in NW, *L. ramada* had the lowest trophic redundancy among individuals, as denoted by the highest CD, MNND and SDNND values (Table 3 and Fig. 2). In contrast, the flatfish *C. linguatula* had the lowest CD, MNND and SDNND values, indicating the highest degree of trophic similarity between individuals (Table 3 and Fig. 2). The SEAc ($R^2 = 0.17$), CD ($R^2 = 0.08$), MNND ($R^2 = 0.02$) and SDNND ($R^2 = 0.02$) values were all independent of the number of individuals sampled ($p > 0.05$ in all cases).

The trophic overlap (αij) between species was significantly higher in NW, and varied between species pairs (Fig. 3, Two-way ANOVA, effect of area: $F = 41.6$, $p < 0.0001$, effect of species pair: $F = 25.1$, $p < 0.0001$, interaction: $F = 10.9$, $p < 0.0001$). The only exception was the effect of *C. linguatula* on *P. lascaris*, which was higher in SE (Two-way ANOVA and post hoc comparisons, $p < 0.0001$). In NW, all species pair interactions were found to be asymmetric (i.e. $\alpha ij \neq \alpha ji$, one-way ANOVA and post hoc comparisons, $p < 0.05$), whereas in SE, asymmetry in interaction strength was observed only between *C. linguatula* and *P. lascaris* (Fig. 3). For each species, the mean αij measured at each location was not dependent on the number of individuals sampled ($R^2 = 0.05$, $p > 0.05$).

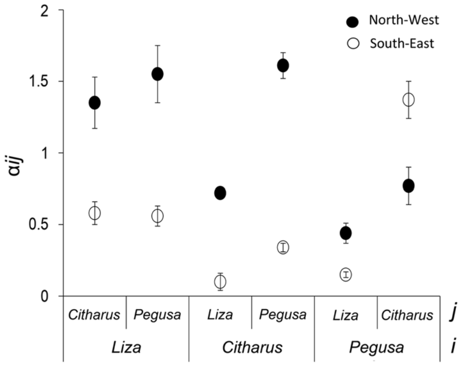

Fig. 3 Strength of the potential interspecific limiting effect between *Liza ramada*, *Citharus linguatula* and *Pegusa lascaris* in two areas of the Gulf of Gaeta (Italy) (black symbols: north-west; empty symbols: south-east). The interspecific limiting effect (*αij*) is measured with reference to intra- and interspecific isotopic similarity between specimens, and represents the potential effect of species *j* (upper row) on species *i* (lower row)

While the diet of the two flatfish species relied on both macroinvertebrates and seston in NW (Fig. 4a), they showed a marked preference for macroinvertebrates in SE (Fig. 4b). In contrast, the diet of *L. ramada* included all potential resources in NW while it specialised on seston in SE (Fig. 4). However, two groups of individuals were evident in NW: one characterised by more negative δ^{13}C values, consuming mainly SOM and seston, and one characterised by less negative δ^{13}C values, consuming mainly macroinvertebrates and detritus (Fig. 4a).

Discussion

Trophic niche of fish populations

This study investigated the trophic ecology of the flatfish *C. linguatula*, *P. lascaris* and the mullet *L. ramada* in a Mediterranean coastal sea, obtaining novel information on their feeding strategies and interspecific interactions in an important recruitment area influenced by run-off from a large river (the average discharge of the Garigliano is 120 m^3/s). For these and other more commercially important fish species, the Gulf of Gaeta is considered a crucial area in the central Mediterranean (Mazzola and Sarà 2001; Colloca et al. 2015; Criscoli et al. 2017; Guerriero et al. 2017). Here, fish species benefit from suitable conditions for growth, such as high food availability, favourable water temperature and potential refuge against predators (França et al. 2011).

Our results highlighted differences in the trophic behaviour of three species in the Gulf in response to changing environmental conditions. Specifically, mullets were found to vary their diet significantly across and within areas whereas flatfish exhibited a more consistent and homogeneous feeding pattern. Indeed, flatfish relied for most of their diet on macroinvertebrates, displaying very narrow trophic niches with limited variation among individuals. Interindividual diet variability increased in all three species in NW, far from the river mouth, where populations were characterised by greater average length of individuals and terrestrial-derived organic inputs were limited. Of the three species, only *L. ramada* shifted its food preferences towards terrestrial-derived food near the river mouth, which reduced its niche overlap and strength of interaction with the other two species. The independence of SEAc, CD, MNND, SDNND and *αij* from population densities makes it possible to state confidently that niche breath, intraspecific trophic similarity and the strength of interspecific interactions were not biased by the lower number of specimens of *L. ramada* and *P. lascaris* sampled in NW (Calizza et al. 2017).

The slopes between the length and weight of the three species differed between areas. When comparing conspecific populations or subpopulations, these slopes can be used as a proxy of fish condition, with a higher slope generally being considered indicative of a better condition (Jones 2009). Specifically, for *L. ramada* and *P. lascaris*, the slope was significantly higher in SE than in NW. This suggests that additional resource inputs from riverine transport, coupled with lower interspecific limitation associated with reduced niche overlap, may translate into better conditions for fish specimens observed in the estuarine area than in the NW sector of the Gulf. On the other hand, the greater incorporation of marine-derived organic matter through the benthic food chain can explain the correlation between δ^{13}C values and length in individuals sampled in the northern area of the Gulf. Indeed, such intraspecific changes in isotopic carbon signatures with body length suggest migration of larger specimens from the river mouth-influenced area to the north-western one. The lower δ^{13}C values measured in marine seston sampled in SE strongly resembled those measured 1 km upstream from the

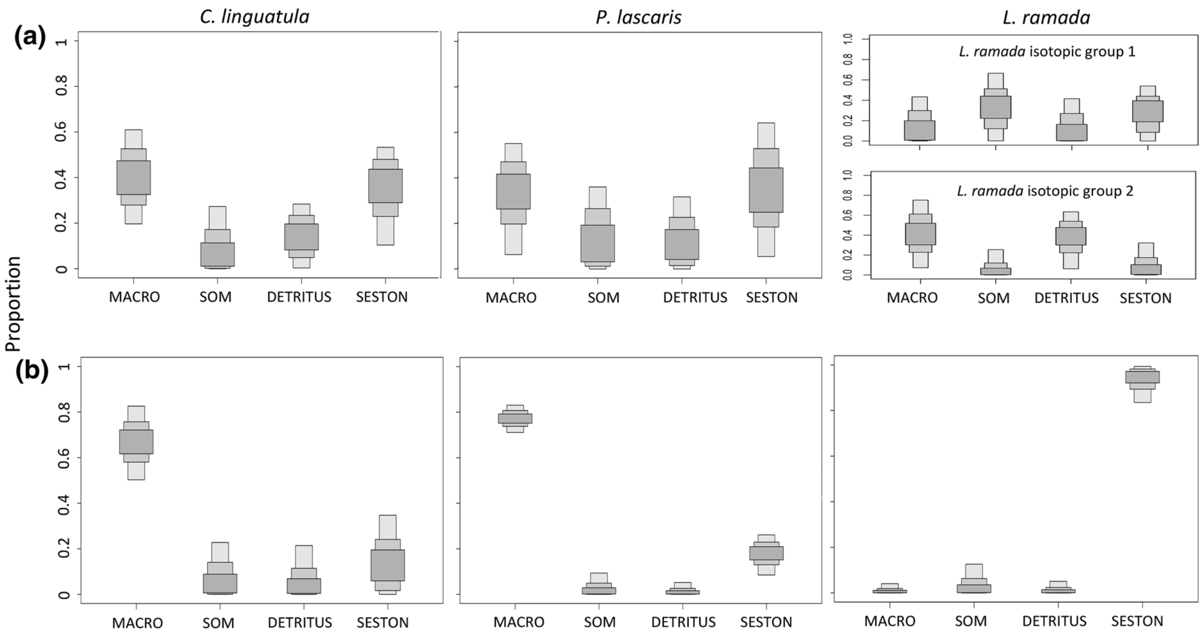

Fig. 4 Proportional contribution of food sources (Proportion) to the diet of *Citharus linguatula*, *Pegusa lascaris* and *Liza ramada* in the north-west (a) and south-east (b) areas of the Gulf of Gaeta (Italy). In the north-west, the diet of *L. ramada* is presented separately for two subsets of specimens, which showed significantly different $\delta^{13}C$ values. Macro: macroinvertebrates; SOM: sediment organic matter; Detritus: coarse organic detritus; Seston: suspended particles in the water column, including plankton and dead organic matter. Grey shades, from dark to light, represent 50%, 75% and 95% credible intervals around the modal contribution of each food source. Data obtained through isotopic Bayesian mixing models (SIAR package, R software)

river mouth, further confirming that more negative $\delta^{13}C$ values observed in *L. ramada* in SE were dependent on the assimilation of river-transported organic matter (Careddu et al. 2015).

Of the two flatfish, *P. lascaris* was more generalist than *C. linguatula*. However, like *C. linguatula,* its diet was based on macroinvertebrates, in accordance with Marinaro and Bouabid (1983) and what is generally reported for flatfish (Link et al. 2002; Darnaude et al. 2004). Specifically, *P. lascaris* juveniles are known to feed preferentially on macroinvertebrates, such as Cumacea, Bivalvia, Decapoda and Amphipoda (Cabral et al. 2002). In addition, Le Pape and Cognez (2016) observed that flatfish species used estuaries mainly as nursery areas or temporary habitats as an alternative to coastal areas, and that the range of movement increased with body length (Kramer and Chapman 1999; Le Pape and Cognez 2016). These observations support our hypothesis that bigger flatfish specimens migrate from SE to NW, which may thus explain the lower body size and higher abundance of specimens sampled in the area directly affected by river run-off.

The mullet *L. ramada* was the most generalist and omnivorous of the three species and showed wide trophic plasticity and intraspecific variability, particularly evident in the NW area. Here, mullets relied on all the basal resource compartments, although the two distinct $\delta^{13}C$ isotopic groups reflected different contributions to the diets, showing that the marked trophic generalism observed at the population level was mainly dependent on intraspecific diet differentiation between individuals. In contrast, seston dominated the diet of mullets near the mouth of the Garigliano River. This result is consistent with Rumolo et al. (2016), who dealt with pelagic fish, and Careddu et al. (2015, 2017), who demonstrated the uptake of terrestrial organic matter supplied by run-off from the Garigliano River in benthic food webs in the Gulf of Gaeta, thereby indicating the presence of distinct ecological conditions. A clear spatial isotopic carbon gradient from riverine terrigenous to marine input has been already observed in estuarine areas by other authors (Vizzini et al. 2005; Kopp et al. 2013; Selleslagh et al. 2015). Various studies also show that land-derived C3 carbon reaches higher food web trophic levels via consumption by predators of detritivores and filter feeders, which rely in turn on the terrestrial detrital pathway (Darnaude et al. 2004; Calizza et al. 2012; Costantini et al. 2012; Careddu et al. 2015; Leal et al. 2017). Here, the shift from higher to lower terrestrial input was accompanied by falling fish abundance and body condition for *L. ramada* and *P. lascaris*, the two most abundant species, coupled with stronger interspecific limitation due to stronger overlap of food sources between species pairs.

Mullets are highly abundant in coastal areas (Carpentier et al. 2014) and this success may be due to opportunistic feeding behaviour, combined with their high tolerance to variation in salinity, which enables them to enter rivers. Indeed, they are able to feed on both live and dead organic matter from primary producers (Almeida 2003), thanks to a highly adapted digestive tract (e.g. a protractile and thin mouth with developed lips, specialised gill rakers and a gizzard) (Carpentier et al. 2014). In terms of trophic role, they have therefore been variously described as herbivorous, omnivorous, plankton feeders, or invertebrate predators (Carpentier et al. 2014). Phytoplankton have been found to be a food item for *L. ramada* especially in the upper reaches of estuaries (Cardona and Castelló 1994; Almeida 2003), and this particular trophic behaviour has encouraged many studies of their foraging strategy (Lebreton et al. 2011; Carpentier et al. 2014; Le Loc'h et al. 2015). In our study, the δ^{13}C signatures of mullets near the river mouth were congruent with the depleted δ^{13}C values typical of inland carbon sources (the average δ^{13}C values of C-3 plants is about − 27‰; Marshall et al. 2007), which was also consistent with the δ^{13}C values measured in seston in this area and upstream from the river mouth. Lastly, the observed shift from depleted to enriched δ^{13}C signatures typical of marine carbon sources in larger mullet specimens in NW indicates migration from the river mouth to the northern area of the Gulf during specimen growth. This is acknowledged as a common feeding strategy in this opportunistic species (Lebreton et al. 2011) and was also observed for the two flatfish species. Larger specimens are expected to enlarge their home range due to higher resource requirements and the lower energetic costs of habitat exploration in comparison to smaller ones (Kramer and Chapman 1999). Migration may also reduce their overlap in resource use with smaller conspecifics and thus can limit intraspecific competition and promote differences in habitat use between size classes (Svanbäck and Bolnick 2006; Chapman et al. 2012).

In conclusion, this study helps to clarify the diet and feeding behaviour of *C. linguatula*, *P. lascaris* and *L. ramada* in an important breeding and fishing area of the central Mediterranean Sea. The study showed that the presence of the Garigliano River mouth affects the trophic habits of these fish species in different ways, as previously described for invertebrate consumers and predators in the area (Careddu et al. 2015, 2017). Far from the river, all species populations were characterised by longer individuals and increased interindividual variability in diet. However, while the flatfish species did not substantially change their trophism near the river mouth, the river provided basal organic inputs of terrestrial origin, which made up a large part of the diet of the mullets. Thus, our results suggest that food input from river run-off represents a key factor promoting stable coexistence between the three studied species in the Gulf at the high densities observed in the south-eastern area. Indeed, representing an abundant supplementary food source, seston from the river allows specimens of *L. ramada* to specialise on it, reducing niche overlap with other fish species, thereby lowering potential interspecific limitations on population density (Calizza et al. 2017; Duarte et al. 2017).

Finally, this study confirms stable isotope analysis as a useful tool for tracing organic matter flow, supplying information about resources and foraging areas exploited by fish species during successive life stages. This technique can thus help to evaluate ad hoc management strategies for ecosystems that are fundamental foraging habitats for marine species and are characterised by dynamic environmental conditions, such as coastal and estuarine Mediterranean ecosystems. In our specific study case focusing on the Gulf of Gaeta, the results suggest that both fish populations and catches will benefit from fishing in the river mouth area where, in spite of higher fish density, interspecific competition seems to be less limiting for the fish populations due to high resource availability. Fishing costs will increase away from the mouth of the river, since fishing efforts will concentrate on less dense fish assemblages with larger specimens, which are expected to contribute more than the smaller ones to population recruitment, due to their higher egg production, with consequent damage for fish populations.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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