



Diet Composition and Isotopic Analysis of Nine Important Fisheries Resources in the Eastern Adriatic Sea (Mediterranean)

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We examined how the trophic ecology of nine economically important marine taxa varied across three distinct areas of the Adriatic Sea. These taxa included three species of demersal fishes (European hake *Merluccius merluccius*, red mullet *Mullus barbatus*, black-bellied angler *Lophius budegassa*) and two species of decapod crustaceans (Norway lobster *Nephrops norvegicus*, deep-water rose shrimp *Parapenaeus longirostris*) and four species of pelagic fishes (sardine *Sardina pilchardus*, anchovy *Engraulis encrasicolus*, Mediterranean horse mackerel *Trachurus mediterraneus*, Atlantic horse mackerel *Trachurus trachurus*). We used two complementary methods that differed in their temporal context to examine and compare diet. Stomach contents analysis was used to describe the short term diet while stable isotope analysis was used compare long-term assimilated diet. Results showed that although there were spatial differences in what each species consumed, and in their trophic and isotopic niches, each species fed at similar trophic position across locations, indicating similar ecological function. Comparisons of biomass-weighted trophic position ($\delta^{15}\text{N}$) and consumer body size (\log_2 mass) showed evidence for a common isotopic size spectrum across areas, indicating the existence of a size-structured food web. In turn this allowed us to provide a first estimate of the predator-prey body mass ratio (PPMR) for this area (655:1). Results obtained within this study, in future, could be used for ecological modeling and improved long-term management of the Adriatic Sea's marine resources.

Keywords: demersal fish, pelagic fish, food web, PPRM, trophic ecology, spatial variation, trophic position

INTRODUCTION

Recent decades have seen marked reductions in the biomass of several commercially important fish stocks across the Mediterranean region (FAO, 2018), including the Adriatic Sea. Although the Adriatic Sea only represents ca. 5% of the total surface area of the Mediterranean Sea, several stocks of pelagic and demersal fishes important to Mediterranean fisheries are located in this semi-enclosed sea, and are shared between bordering countries (UNEP-MAP-RAC/SPA, 2014).

Fisheries management relies on accurate information regarding the ecology and factors affecting the population dynamics of target stocks and how they vary over time and space.

In marine ecosystems, habitats and species are exposed to a wide range of natural and anthropogenic pressures. Over time, those can affect and drive shifts in marine ecosystems and taxa. The Adriatic Sea has experienced the combined effects of multiple stressors including climatic shift, pollution, and overexploitation in recent decades (Grbec et al., 2014; Ramirez et al., 2018). As such and reflecting patterns seen in other geographical areas (Burkhard, 2003; Nagelkerken et al., 2020) it is reasonable to expect that Adriatic food webs have also changed over the same period, with shifts in trophic structure, feeding patterns and nutritional relationships. Given the importance of commercial fisheries in the Adriatic Sea (mean annual yield (2000-2017) = 156 651 tonnes; FAO, 2020) there is a pressing need to provide an integrated characterization of the trophic ecology of key species as a first step toward future ecosystem-based management.

To date, the feeding habits of commercially exploited pelagic (sardine, anchovy, Atlantic horse mackerel, Mediterranean horse mackerel) and demersal (European hake, red mullet and black-bellied angler) fishes in the Adriatic have largely been studied at a species-specific level (Jardas et al., 2004; Šantić et al., 2004; Vrgoč et al., 2004; Tirelli et al., 2006; Borome et al., 2009; Zorica et al., 2017; Riccioni et al., 2018). However, a few comparative studies do exist (Zorica et al., 2016). Most existing information on trophic ecology is largely based on stomach content analysis (SCA), which provides information regarding the most frequent and abundant prey items consumed. Although this is a commonly used method in fish biology, it has its limitations. SCA provides information on prey that have been ingested most recently, while the contribution of digested prey items is neglected even though their assimilation has contributed to the energy budget of an individual (Hyslop, 1980; Wolf et al., 2009).

In order to provide a wider understanding of fish trophic ecology that counters some of the limitations of SCA, scientists have increasingly used alternative techniques, including stable isotope analysis (SIA) (Pethybridge et al., 2018). This method assumes that the ratio of heavy and light isotopes (e.g., $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$) in the tissues of an individual can indicate the relative contributions of prey items or prey characteristics (e.g., trophic position) assimilated over a longer time period than SCA. This time period depends on the tissues analyzed (e.g., liver, white muscle, blood), as their isotopic turnover rate can range from a few days to months (Hesslein et al., 1993; Bearhop et al., 2004).

Although SIA cannot provide the taxonomic resolution offered by SCA, it can reveal contributions of different energy sources, e.g., pelagic and benthic (Docmac et al., 2017) and it allows the estimation of consumer trophic position and food web length (Cabana and Rasmussen, 1996). Importantly, information can be gathered from individuals with empty or inverted stomachs, which can be a key limitation of SCA when examining the diet of demersal fishes. When interpreting the results of stable isotope analysis, one should be aware that consumer isotopic values may be the result of several combinations of possible food items. Both SCA and SIA have

their advantages and disadvantages (reviewed by Majdi et al., 2018; Nielsen et al., 2018; Pethybridge et al., 2018). However, their combined use can provide a robust characterization of species diet and of food web structure (Winemiller et al., 2007; Fanelli and Cartes, 2010; Young et al., 2018). Recently, analysis of stable nitrogen isotopes in the context of marine food webs has attracted considerable attention (Jennings et al., 2001; Bode et al., 2003; Funes et al., 2019). This has proved particularly useful as a mean of examining different theories regarding how ecosystems function. At a functional level, consumer body size can be considered as a proxy for trophic position, meaning that body size rather than the species identity can be considered as a trophic position identifier in marine ecosystems (Jennings et al., 2008). This has proved a useful tool to examine food webs and how they vary over space and time, and to rapidly estimate important parameters that characterize ecosystem function such predator:prey mass ratios (PPMR) and that are commonly required for ecosystem-based models (Jennings et al., 2008; McCormack et al., 2019). By analyzing the stomach content and stable isotopes of nine ecologically and economically most important fisheries resources in the Adriatic Sea, we aimed to provide some important parameters (TP, PPMR) that might be used in future ecological modeling. We also aimed to examine how spatial differences in habitat characteristics affect trophic ecology at the species and community level. To achieve these general aims, we characterized trophic ecology using SCA and SIA across three distinct geographic areas of the Adriatic Sea (Northern, Middle and Southern areas) of which differed in terms of depth, distance from the shore, and productivity. We also estimated the community isotopic size spectrum of each area and estimated PPMR.

More specifically, we examined: (i) how the diet of each species varied spatially, (ii) how species-level estimates of trophic position varied; and (iii) how community level measures of food web structure differed across three areas of the Adriatic Sea. Our data provide a first comparative survey of the feeding habits in commercially important species in the Adriatic, and how these vary spatially. Our estimates of trophic position for the different species and the community-level estimate of PPMR can be used to parameterize Ecopath with Ecosim models that can provide predictions of stock biomass in response to fishing pressure and various environmental factors as recommended by the General Fisheries Commission for the Mediterranean (GFCM, 2018).

MATERIALS AND METHODS

Study Area and Sampling Methods

The Adriatic Sea is the northernmost part of the Mediterranean Sea (Figure 1). It has a general cyclonic circulation driven by estuarine inputs, with pronounced seasonality induced by changing winds and thermal fluxes throughout the year (Cushman-Roisin et al., 2001). The water column is typically thermally stratified during summer, while substantial wind-mixing and winter surface cooling leads to stratification breaking down in the winter (Buljan and Zore-Armanda, 1974). The Adriatic Sea is characterized by varying oceanographic

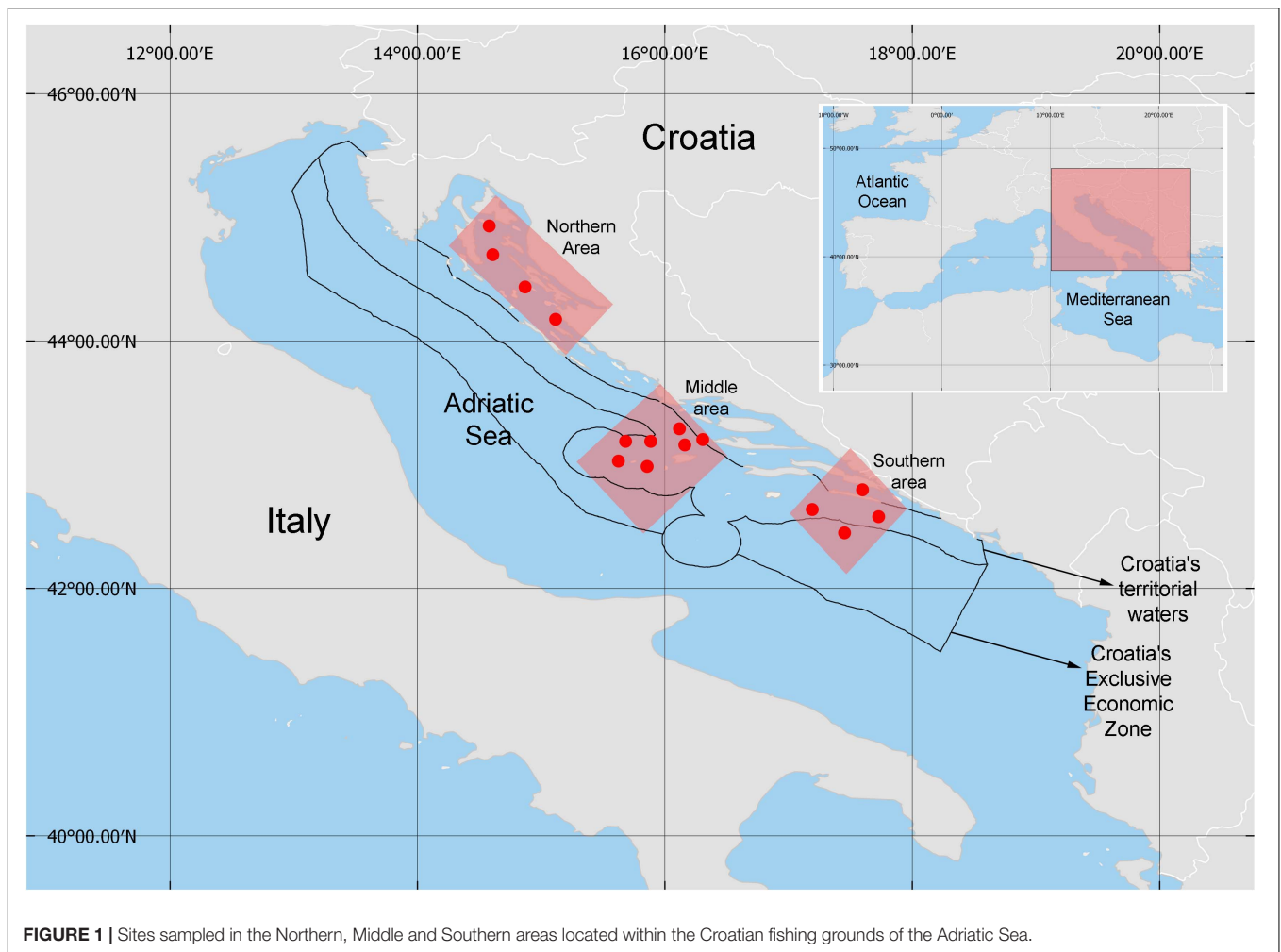


FIGURE 1 | Sites sampled in the Northern, Middle and Southern areas located within the Croatian fishing grounds of the Adriatic Sea.

conditions, especially regarding nutrient status (Zavatarelli et al., 2000). The shallow northern basin, particularly its north-western area, is influenced by materials from the discharge of the River Po, and is considered as productive and potentially eutrophic area. Conversely, the deeper central and southern basins are considered as oligotrophic, particularly along the eastern margin (Kovač et al., 2018). In general, the Adriatic Sea supports a diverse marine community (Coll et al., 2010; Dulčić et al., 2017). This study was conducted in three distinct areas within the Croatian part of the Adriatic Sea – Northern, Middle and Southern (**Figure 1**).

Here, we refer to the Northern area as the northern region of the eastern Adriatic Sea, where we sampled the channel area of Kvarnerić (four sampling stations; **Figure 1**). This area acts as a spawning and/or nursery ground of both pelagic and demersal species (Tičina et al., 2000; Sinovčić and Zorica, 2009; Piccinetti et al., 2012), associated with its productivity being enhanced by riverine inputs (Viličić et al., 2013).

The open Middle area (seven sampling stations; **Figure 1**) is characterized by a gradient of decreasing nutrient conditions from more productive coastal areas to oligotrophic open sea areas

(Ninčević Gladan et al., 2006; Šantić et al., 2014). Moreover, the open sea is influenced by discharge of the nutrient-enriched river Po along the western coast, and it is also an area of upwelling (Bergamasco and Gačić, 1996; Gačić et al., 1997; Mauri and Poulain, 2001). The Middle area acts as spawning and/or nursing habitat for several marine fishes (European hake, black-bellied angler, Mediterranean poor cod, Mediterranean scaldfish) and crustaceans (Norway lobster, deep-water rose shrimp) (Županović and Jardas, 1989; Piccinetti et al., 2012). A Fisheries Restricted Area (FRA) in the Jabuka/Pomo Pit was recently established in this area (GFCM, 2018) to protect spawning habitats of commercially important demersal taxa (Vrgoč et al., 2004).

The Southern area (four sampling stations; **Figure 1**) is characterized both by deeper water and by low relative productivity compared to the Northern and Middle areas, the latter reflecting water exchange between the Adriatic and the northern Ionian Sea across the Otranto Strait (Civitarese et al., 2010).

In order to study food web structure in each of the three areas of the Adriatic Sea we sampled demersal fishes (European

TABLE 1 | Frequency of occurrence (%F) and abundance (%N) of prey items found in the sardine, anchovy, Mediterranean horse mackerel, European hake [by size classes: small (S: TL < 20.0 cm), medium (M: 20.1 cm < TL < 30.0 cm) and large (L: 30.1 cm > TL)] and red mullet collected in the Northern area (Kvarnerić Bay) of eastern Adriatic Sea during MEDITS survey (June–July, 2018) along with number of analyzed specimens (N), its vacuity index (%V) and Levins' standardized index (B_i).

Prey item	Sardine (N = 10;%V = 0)		Anchovy (N = 10;%V = 0)		Mediterranean horse mackerel (N = 10;%V = 40.0)		European hake S (N = 10;%V = 0)		European hake M (N = 9;%V = 44.4)		European hake L (N = 2;%V = 0)		Red mullet (N = 10;%V = 0)	
	%F	%N	%F	%N	%F	%N	%F	%N	%F	%N	%F	%N	%F	%N
Copepoda	100	33.0	100	13.6	40	14.6	–	–	11	7.7	50	60.0	70	8.0
Amphipoda	~0	0.7	~0	0.2	10	4.9	10	9.1	–	–	–	–	100	67.2
Mysidacea	–	–	10	0.3	20	68.3	10	9.1	–	–	–	–	10	1.1
Euphausiacea	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cladocera	10	2.0	20	0.5	–	–	–	–	–	–	–	–	–	–
Decapoda larvae	20	5.1	40	1.0	30	12.2	50	54.5	33	23.1	–	–	40	2.9
Pisces ova	90	10.2	70	3.4	–	–	–	–	11	46.2	–	–	–	–
Pisces larvae	~0	7.5	–	–	–	–	10	9.1	33	23.1	100	40.0	–	–
Bivalvia larvae	80	35.9	100	80.0	–	–	–	–	–	–	–	–	30	4.0
Gastropoda larvae	–	–	10	0.3	–	–	–	–	–	–	–	–	–	–
Echinodermata larvae	~0	2.1	10	0.1	–	–	–	–	–	–	–	–	–	–
Ostracoda	–	–	20	0.3	–	–	–	–	–	–	–	–	–	–
Polychaeta larvae	~0	0.6	–	–	–	–	10	9.1	–	–	–	–	50	13.8
Diatomeae	10	1.1	10	0.2	–	–	–	–	–	–	–	–	–	–
Dinophyceae	~0	1.9	–	–	–	–	10	9.1	–	–	–	–	20	2.9
Levins' standardized niche index (B_i) =		0.05		0.08		0.33		0.39		0.69		–		0.18

hake *merluccius*, red mullet *Mullus barbatus*, black-bellied angler *Lophius budegassa* and decapod crustaceans (Norway lobster *Nephrops norvegicus*, deep-water rose shrimp *Parapenaeus longirostris*) as well as pelagic fishes (sardine *Sardina pilchardus*, anchovy *Engraulis encrasicolus*, Mediterranean horse mackerel *Trachurus mediterraneus*, Atlantic horse mackerel *Trachurus trachurus*). Samples were collected by bottom trawl (MEDITS trawl-net GOC73) during the scientific survey 'Mediterranean International Bottom Trawl-Survey' (MEDITS) carried out along the eastern Adriatic Sea (Croatian fishing ground, **Figure 1**) between 30 June and 21 July, 2018. The tows were carried out over the continental shelf between 40 and 800 m with duration of 30 to 60 minutes depending on the depth and a mean trawl speed of 3 knots.

Immediately after landing, the total length (TL \pm 0.1 cm) and wet mass (W \pm 0.01 g) of target specimens (up to 10 individuals from each area) were recorded onboard. Within these species, marked ontogenetic diet shifts have been reported for hake (Garrison and Link, 2000; Mahe et al., 2007). As such, hakes were sub-sampled by length classes: small (S: TL < 20.0 cm), medium (M: 20.1 cm < TL < 30.0 cm) and large (L: 30.1 cm > TL), while all other fishes were treated as a single category (**Supplementary Table 1**).

Stomach contents analysis were performed on 10 specimens of each fish species per area. Some European hake and black-bellied angler had inverted stomachs or had obviously regurgitated

some prey following capture. These individuals were excluded from SCA. Fish visceral cavities were carefully opened prior to preservation in 10% buffered formalin. Stomachs were removed and weighed (\pm 0.01 g) before and after (total mass of full W_{sf} , g; and empty stomach W_{se} , g) prey items were removed. Prey items were identified under a stereomicroscope (Zeiss Discovery. V12; magnification 40–80x) to class or family systematic level using relevant identification keys (Tréguiboff and Rose, 1957; Todd et al., 1996; Larink and Westheide, 2006) and counted.

We collected zooplankton to compare food availability in the water column with prey consumed by fish. Zooplankton samples were collected on same occasion as fish samples using a standard WP2 plankton net of 200 μ m mesh size (mouth opening, 0.255 m²). The WP2 gear was hauled vertically from the near-bottom to the surface, performing double tows in on one sampling station in each of the three areas (Northern, Middle and Southern areas). Samples for SIA were frozen immediately after collection (-20° C), while samples for microscopic analysis of the zooplankton community were fixed with buffered 4% formaldehyde-seawater solution. Counting and species identification of zooplankton were performed using an inverted microscope (Olympus IX51), following standard laboratory practice. Subsamples for counting were obtained by the splitting method (1/32), while the entire sample was checked for rare species. Abundances were expressed as the number of individuals per cubic meter (ind. m⁻³). Taxonomic

TABLE 2 | Frequency of occurrence (%F) and abundance (%N) of prey items found in the sardine, anchovy, Atlantic horse mackerel, European hake [by size classes: small (S: TL < 20.0 cm), medium (M: 20.1 cm < TL < 30.0 cm) and large (L: 30.1 cm > TL)], red mullet and black-bellied angler collected in the Middle area of eastern Adriatic Sea during MEDITS survey (June–July, 2018) along with number of analyzed specimens (N), its vacuity index (%V) and Levins' standardized index (B_i).

Prey item	Sardine (N = 10; %V = 0)		Anchovy (N = 10; %V = 0)		Atlantic horse mackerel (N = 10; %V = 10.0)		European hake S (N = 15; %V = 86.6)		European hake M (N = 3; %V = 66.6)		European hake L (N = 4; %V = 75.0)		Red mullet (N = 10; %V = 30.0)		Black-bellied angler (N = 2; %V = 50.0)	
	%F	%N	%F	%N	%F	%N	%F	%N	%F	%N	%F	%N	%F	%N	%F	%N
Copepoda	100	2.2	80	1.5	70	94.3	–	–	–	–	–	–	20	23.1	–	–
Amphipoda	~0	1.0	10	0.3	10	2.9	–	–	–	–	–	–	30	23.1	–	–
Mysidacea	–	–	~0	0.5	–	–	–	–	–	–	–	–	10	3.8	–	–
Euphausiacea	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cladocera	20	2.9	10	0.8	–	–	–	–	–	–	–	–	–	–	–	–
Decapoda larvae	10	7.5	10	1.6	20	2.9	13	100.0	–	–	–	–	30	26.9	–	–
Pisces ova	10	15.7	90	4.5	–	–	–	–	33	99.2	–	–	–	–	–	–
Pisces larvae	10	10.8	–	–	–	–	–	–	33	0.8	25	100.0	–	–	50	100
Bivalvia larvae	40	51.6	60	89.2	–	–	–	–	–	–	–	–	–	–	–	–
Gastropoda larvae	–	–	~0	0.5	–	–	–	–	–	–	–	–	–	–	–	–
Echinodermata larvae	20	3.0	~0	0.3	–	–	–	–	–	–	–	–	–	–	–	–
Ostracoda	–	–	~0	0.6	–	–	–	–	–	–	–	–	–	–	–	–
Polychaeta larvae	10	0.8	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Diatomeae	~0	1.8	~0	0.3	–	–	–	–	–	–	–	–	–	–	–	–
Dinophyceae	30	2.7	–	–	–	–	–	–	–	–	–	–	20	23.8	–	–
Levins' standardized niche index (B_i) =		0.15		0.12		0.06		–		0.02		–		0.82		–

identification was generally performed to the species or genus level for holoplanktonic groups, while miscellaneous larvae were determined to lower taxonomic levels (class or phylum). Margalef's index was calculated to express the diversity of zooplankton community at each sampling station (PRIMER v6 software package; Clarke, 1993; Clarke and Warwick, 1994).

For stable isotope analysis, a total of 241 individuals from 9 species were sampled (Supplementary Table 2). All individuals were frozen (-20°C) immediately after sampling. In the laboratory, samples were defrosted, and in fish, a small piece of dorsal-anterior muscle was dissected, and skin and bones removed. Samples were dried in an oven at 60°C for 48 h and afterward ground using a mortar and pestle to homogenize the sample. To avoid possible inorganic carbon contamination due to residual bones in all small fish (e.g., sardine, anchovy), acid fumigation treatment was performed (following the procedure applied in Lorrain et al., 2003 for suspended particulate material). After acidification samples were dried at 60°C for 24 h. For decapod crustaceans, exoskeletons were removed and a sample of tail muscle tissue was taken for analysis, dried and homogenized in a similar way to fish. Plankton samples used as baseline were sieved through a $200\ \mu\text{m}$ sieve to remove smaller plankton size fraction and three subsamples were obtained from each sample. Each plankton subsample was dried in oven (60°C for 48 h), homogenized, exposed to acid fumigation treatment (Lorrain et al., 2003) and dried (at 60°C for 24 h). Due to potential acidification effects, $\delta^{15}\text{N}$ was analyzed separately on non-treated samples, both for small fish and plankton samples. Acidified

samples were packed into silver capsules to avoid corrosion and sample leakage.

Samples were analyzed at the Stable Isotope Facility at the University of California, Davis (United States). Stable isotope values of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$), and elemental %C and %N analyses were estimated using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, United Kingdom). The long-term standard deviation was $\pm 0.1\text{‰}$ for $\delta^{13}\text{C}$ and $\pm 0.3\text{‰}$ for $\delta^{15}\text{N}$. The final delta values are expressed relative to international VPDB (Vienna PeeDee Belemnite) standards and air for carbon and nitrogen, respectively. Fish $\delta^{13}\text{C}$ values were lipid-corrected following Kiljunen et al. (2006). Arithmetic lipid normalization was not applied to decapod crustaceans as Bodin et al. (2007) noted that it performed badly for these taxa. Although we not present lipid corrected $\delta^{13}\text{C}$ values for decapod crustaceans, their mean muscle C:N was < 3.5 , indicating a value close to that expected for protein, and as such there is unlikely a major lipid-effect on their $\delta^{13}\text{C}$ values.

Data Analyses

To assess the diet composition of each species the following indices were calculated:

The fullness index $\%J_r = (W_p/W) \times 100$, where W_p is the mass of prey items calculated as the difference between the mass of full and empty stomachs ($W_{sf} - W_{se}$); W refers to a total body mass.

TABLE 3 | Frequency of occurrence (%F) and abundance (%N) of prey items found in the sardine, anchovy, Atlantic horse mackerel, European hake [by size classes: small (S: TL < 20.0 cm), medium (M: 20.1 cm < TL < 30.0 cm) and large (L: 30.1 cm > TL)] and red mullet collected in the Southern area of eastern Adriatic Sea during MEDITS survey (June–July, 2018) along with number of analyzed specimens (N), its vacuity index (%V) and Levins' standardized index (B_i).

Prey item	Sardine (N = 10; %V = 0)		Anchovy (N = 9; %V = 33.3)		Atlantic horse mackerel (N = 10; %V = 0)		European hake S (N = 10; %V = 0)		European hake M (N = 6; %V = 50.0)		Red mullet (N = 10; %V = 10.0)		Black-bellied angler (N = 2; %V = 0)	
	%F	%N	%F	%N	%F	%N	%F	%N	%F	%N	%F	%N	%F	%N
Copepoda	100	57.1	57	58.8	30	2.6	50	3.4	17	20.0	60	17.5	–	–
Amphipoda	10	0.3	11	1.3	–	–	10	0.5	–	–	10	3.5	–	–
Mysidacea	–	–	11	1.3	–	–	–	–	–	–	10	1.8	–	–
Euphausiacea	–	–	–	–	90	95.1	–	–	–	–	–	–	–	–
Cladocera	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Decapoda larvae	10	0.6	11	1.3	–	–	60	6.0	50	73.3	40	6.1	–	–
Pisces ova	30	0.8	44	16.3	–	–	–	–	–	–	–	–	–	–
Pisces larvae	–	–	–	–	40	2.3	–	–	17	6.7	–	–	100	100
Bivalvia larvae	60	40.9	56	21.3	–	–	–	–	–	–	–	–	–	–
Gastropoda larvae	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Echinodermata larvae	10	0.3	–	–	–	–	–	–	–	–	–	–	–	–
Ostracoda	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Polychaeta larvae	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Diatomeae	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Dinophyceae	–	–	–	–	–	–	60	90.1	–	–	50	71.1	–	–
Levins' standardized niche index (B_i) =	0.20		0.37		0.05		0.07		0.33		0.21		–	

The vacuity index $\%V = E/N \times 100$, where E is the number of empty stomachs and N the total number of stomachs analyzed.

The frequency of occurrence $\%F = n/N \times 100$, where n is the number of stomachs containing a certain prey and N is the total number of analyzed stomachs containing any kind of prey.

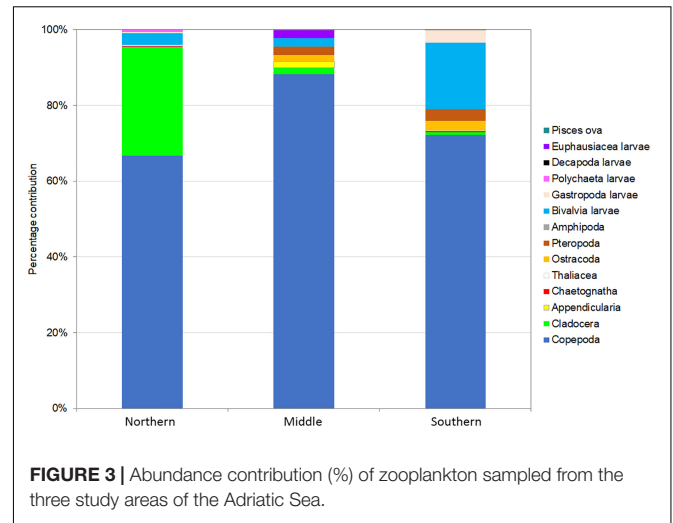
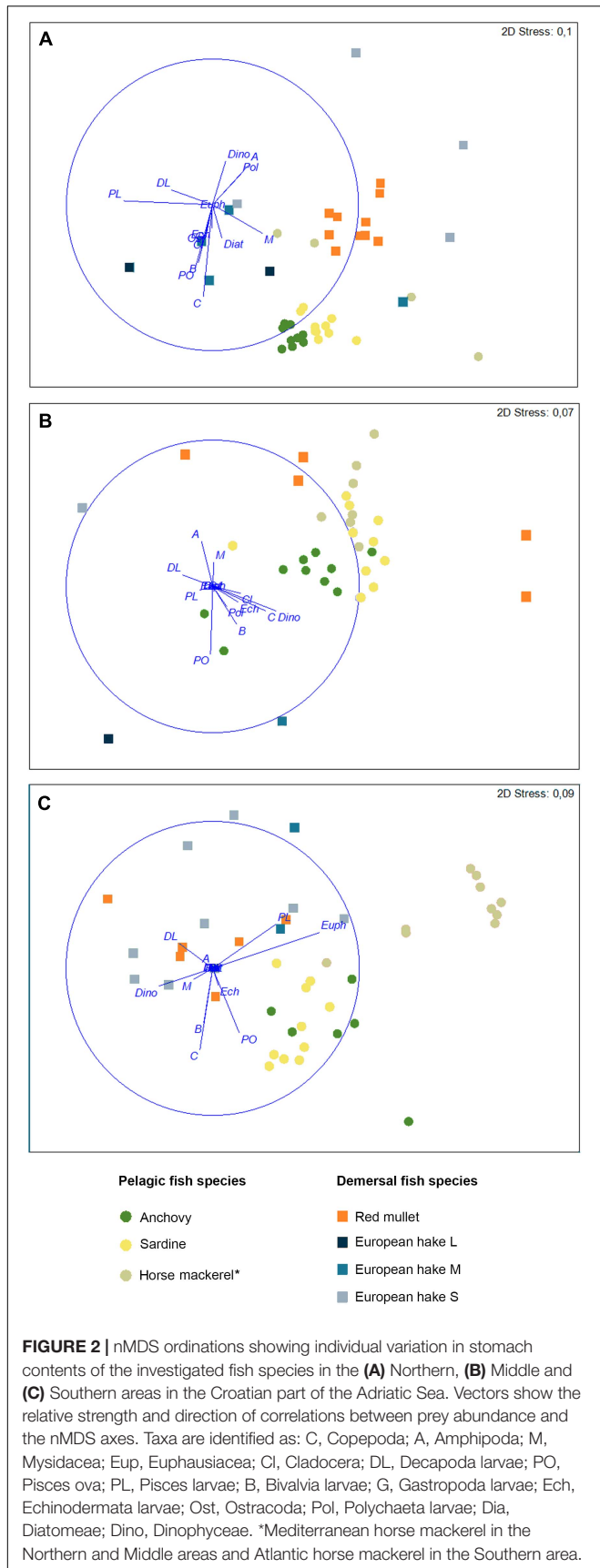
Numerical abundance $\%N = np/Np \times 100$, where np is the number of prey specimens in a specific group and Np is the number of all determined prey groups. The niche width of each species in each area of sampling was calculated by Levins' standardized index (Hurlbert, 1978; Krebs, 1989): $B_i = [1/(n-1)] / (1/(\sum_j p_{ij}^2) - 1)$ where B_i is Levins' standardized index for predator i; p_{ij} the proportion of diet of predator i that is made up of prey j; n the number of prey categories. The values of B_i ranges from 0 to 1; lower values indicate diets dominated by few prey items (specialist predators) while higher values indicate generalist diets (Krebs, 1989).

Stomach contents abundance data (for the species that had 3 or more individuals) from individual fish were $\log(x + 1)$ -transformed, and a Bray–Curtis similarity matrix was constructed using PRIMER v6 (Clarke, 1993; Clarke and Warwick, 1994). In order to compare whether the observed diet varied within each species (or size classes in the case of hake) we used two factor PERMANOVA (fixed factors were area and species) (PRIMER-E Ltd.; Clarke and Warwick, 2001). *Post hoc* pairwise PERMANOVA was used to identify species among which the

differences in diet were statistically significant. A one-way PERMANOVA test, followed by *post hoc* pairwise tests, was used to compare the diet of different European hake size classes. For species that had low number of permutations, Monte-Carlo P values were calculated. To visualize dietary variation, we used non-metric multidimensional scaling (nMDS) ordination based on a Bray–Curtis similarity matrix. Average dissimilarities between ambient mesozooplankton from the three study areas were determined using the SIMPER routine in PRIMER software package on a $\log(x + 1)$ -transformed abundance matrix (Clarke, 1993; Clarke and Warwick, 1994).

The size and overlap of isotopic niches between different species were estimated in the R package *SIBER* (Version 2.1.4, Jackson et al., 2011). Isotopic niches were estimated from each species (with $N > 4$ individuals) by calculating the Standard Ellipse Area corrected for small sample size (SEA_c) which is based on the core 40% of $\delta^{15}N$ and $\delta^{13}C$ values. Furthermore, overlap of isotopic niches between species was assessed by calculating the percentage of the overlap of SEA_c area.

We estimated and compared species (and for hake, size class) modal trophic position (TP) and 95% credibility interval (i.e., 95% of modeled estimates of TP) for each area using the R package *trophicPosition* (version 0.7.7; Quezada-Romegialli et al., 2018). We used mesozooplankton $\delta^{15}N$ values from the study area as the trophic baseline ($\lambda = 2$) and conducted



comparisons using the oneBaseline model option. We used a trophic discrimination factor (TDF) based on aquatic consumers from McCutchan et al. (2003) ($\Delta^{15}N = 2.9 \pm 0.3\text{‰}$).

We examined community isotopic size spectra and mean predator-prey body mass ratios (PPMR) for the three study areas to test the possible size-structuring of trophic relationships within each community. We followed Jennings et al. (2001) by using linear correlation to examine possible relations between biomass-weighted trophic position ($\delta^{15}N$) (Al-Habsi et al., 2008) and body size (\log_2 body mass) for each area of the Adriatic studied (Northern, Middle and Southern). Following equation was used:

$$\delta^{15}N_{mean} = (\delta^{15}N_1 \times Wt_1 + \delta^{15}N_2 \times Wt_2 + \delta^{15}N_{...i} \times Wt_{...i}) / \sum Wt_{1...i}$$

where $\delta^{15}N$ is the nitrogen isotopic values of individuals 1 to i and Wt is their respective mass. We compared the form of the relationships between each region using ANCOVA. We calculated mean PPMRs using the equation mass ratio = $2^{TDF/b}$, where TDF is the mean trophic discrimination factor and b is the slope of the relationship between biomass weighted $\delta^{15}N$ and \log_2 size class, and we used the mean TDF of 2.9 ‰ (McCutchan et al., 2003).

RESULTS

Diet Composition Obtained by Stomach Content Analysis

Overall, we examined 183 individuals belonging to seven species collected from the three areas of the Adriatic Sea (Supplementary Table 1). Of these, 141 individuals, (77%) had food remains in their stomachs. The greatest number of empty stomachs was observed in hake, especially in large (TL > 30.0 cm) individuals. In contrast, all sardine specimens had at least one prey item in their stomach.

The estimated abundance and frequency of occurrence of different prey categories in each species are given in Tables 1–3. In the Northern area, 14 different taxonomic

TABLE 4 | Standard Ellipse Area corrected for small sample (SEA_c) and SEA_c overlap percentage between the species sampled in the Northern area of the Adriatic Sea (values indicate SEA_c overlap of the species in the row with the species in the column).

Species	SEA_c (% 2)	Anchovy	Sardine	Mediterranean horse mackerel	European hake S	European hake M	Red mullet	Norway lobster
Anchovy	0.54	█	88	0	37	0	0	0
Sardine	2.14	22	█	34	30	0	0	0
Mediterranean horse mackerel	0.92	0	78	█	44	2	0	0
European hake S	1.13	18	56	36	█	14	0	0
European hake M	0.19	0	0	9	81	█	0	0
Red mullet	0.52	0	0	0	0	0	█	0
Norway lobster	0.24	0	0	0	0	0	0	█

groups were identified among prey items of five fish species, while in the Middle and Southern areas SCA was conducted on six fish species in each area and revealed the presence of 14 and 10 different prey items, respectively. Since both animal prey and phytoplankton items were identified in four fish species (anchovy, sardine, small-bodied European hake and red mullet), we classified these taxa as omnivores. Mediterranean and Atlantic horse mackerel, medium and large hake as well as black-bellied angler, were classified as being carnivorous, as they only had animal prey in their stomachs.

Regardless of sampling area, copepods and bivalve larvae were the most frequent and abundant prey items in two small pelagic fish species (sardine and anchovy). Copepods, euphausiids and decapod larvae were the most frequent and abundant prey items in both mackerel species. Of the three demersal fish species, only the black-bellied angler was an entirely piscivorous species, while copepods and amphipods were the most frequent and abundant prey in red mullet. The most frequent and abundant prey items all size classes of European hake specimens were decapod larvae and fish eggs, respectively.

There was no obvious relationship between individual fish size and stomach fullness (%Jr) in the different fishes examined, with only European hake showing a statistically significant negative regression between %Jr and TL (%Jr = $-0.22TL + 4.44$; $r^2 = 0.33$; $p < 0.05$), with large-size class individuals having a greater vacuity index than medium- or small-sized specimens. Diet content analysis between three size categories of European hake revealed a statistically significant difference (PERMANOVA, Pseudo-F = 3.04, df = 2, 30, $p = 0.03$) between size classes when locations were pooled. The most marked differences (PERMANOVA Pairwise test, $p(MC) = 0.001$) were observed between the diet of S- and L-sized classes of European hake. Smaller specimens consumed decapod larvae and dinoflagellates, which were their most frequent (35% and 21%, respectively) and abundant (8% and 88%, respectively) prey items, while large specimens consumed large amounts of fish larvae (%F = 43 and %N = 50, respectively). There was no evidence for a linear relationship between %Jr and TL in other species ($r < 0.25$; $p > 0.05$).

PERMANOVA results of SCA data showed significant differences both between areas and among species (Supplementary Table 3). Furthermore, interaction between

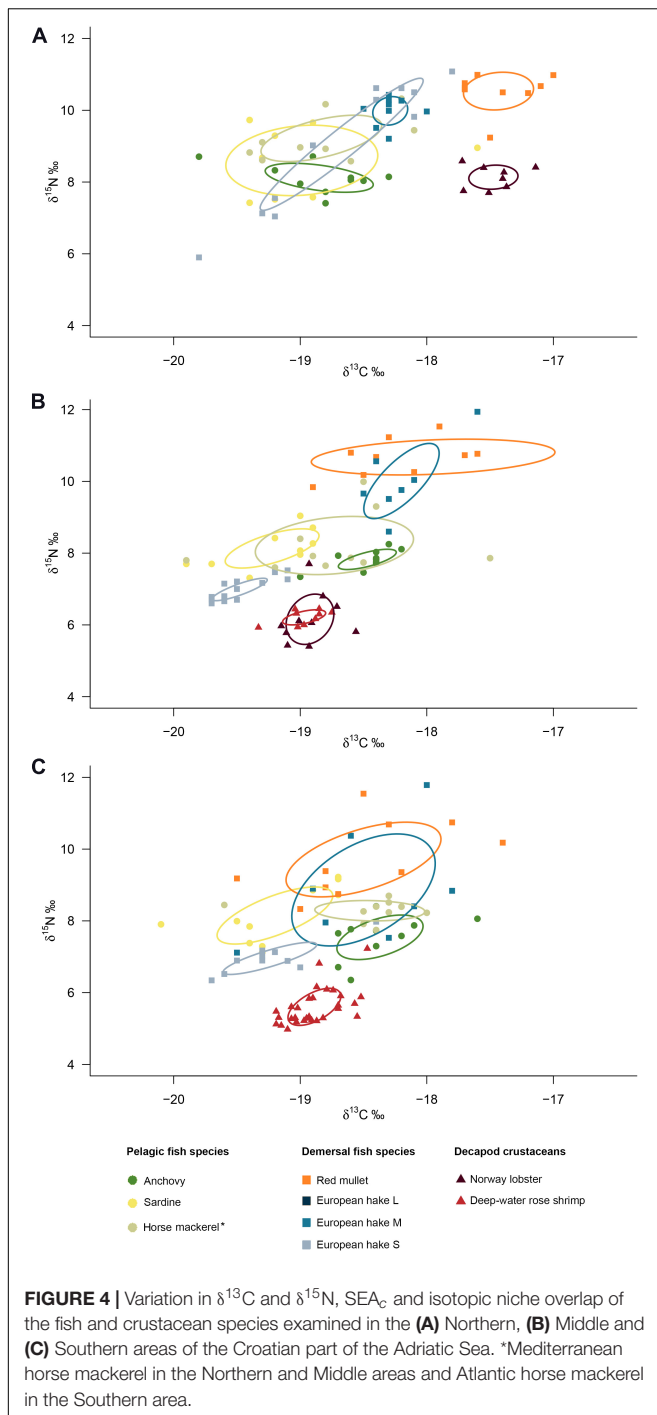
those two factors was also significant. In general, only medium-sized European hake did not show significant differences between the three study areas. For all other species, some levels of diet dissimilarity were apparent between the three areas.

Pairwise PERMANOVA (Supplementary Table 3) and nMDS (Figure 2) showed some evidence for diet overlap between species within each area. In general, diet composition differed between pelagic and demersal fishes, as well as among pelagic species in all study areas. An exception was seen in mackerel, a putatively pelagic fish, but whose diet overlapped with that of demersal species. The diet of demersal fishes was similar across the three areas, with the exception of red mullet, where individuals from the Northern area differed significantly from other demersal species.

Levins' standardized index (B_i) values indicated that sardine and anchovy had smaller trophic niche widths in the Northern area and larger niche widths in the Southern area. Conversely, other species showed the largest niche widths in the Northern area (Tables 1–3). Only red mullet had a larger trophic niche width in the Middle area.

Prey-Availability: Composition and Abundance of Zooplankton in the Water Column

The highest total zooplankton abundance was recorded in the Northern area (919 ind. m^{-3}), while in the Middle and Southern areas values were 3- and 2.5-fold lower, respectively. Overall, we determined a high dominance of copepods in all samples, accounting for > 65% of the total community (Figure 3). Structure-wise, there was a distinct prevalence of small and medium-sized copepods, mostly belonging to families Oithonidae, Oncaeiidae, Clausocalanidae and Paracalanidae (> 64%). In the Middle and Southern areas there was a higher contribution of Euchaetidae (20% and 7% respectively), while Acartiidae were better represented (6%) in the Northern areas. Ostracods and amphipods were not found in the Northern area, but were present, albeit in low abundances, in the Middle and Southern areas. Appendicularians and chaetognaths both occurred in very low abundances (< 5 ind. m^{-3}), mostly represented by juvenile individuals and only contributed < 2% to the total community. Cladocerans were abundant in the Northern area (27%), but were very scarce in both the Middle and



Southern areas (< 2%). Miscellaneous larvae were dominated mainly by Bivalvia larvae and showed a somewhat increased contribution in the Southern area (21%), mostly due to the abundance of molluscan veligers (Bivalvia and Gastropoda). Additionally, euphausiid furciliae were found in the Middle area, decapod larvae in the Southern and Northern areas, while Polychaeta larvae were apparent in the Northern area. Fish eggs were scarce (< 1%) in zooplankton samples, with anchovy eggs found in the Northern and Middle areas and sardine eggs in the

Southern area. A SIMPER analysis showed that the Southern and Middle areas both showed higher average dissimilarity with the Northern area (55.1 and 67.9%, respectively) than between each other (44.2%), with *Penilia avirostris* (Cladocera) highlighted as the top discriminant species. Margalef's index of species richness was lower in the Northern area (4.13), in comparison with the Middle (7.3) and Southern (5.7) areas.

Isotopic Niche Overlap and Trophic Position of Investigated Species

The SEAc values of the fish species from the Northern Adriatic Sea ranged from 0.2 ‰² for the M-sized European hake to 2.1 ‰² for the sardine (Table 4). Relatively high isotopic overlap was recorded between sardine and other pelagic species (Table 4 and Figure 4A). Furthermore, among the demersal fish species only European hake overlapped with pelagic fish species. The isotopic niches of small European hake overlapped with that of sardine and Mediterranean horse mackerel by 56% and 36%, respectively. Between demersal species, a high degree of niche overlap was also recorded between medium- and small-sized European hake (81%) while isotopic niche overlap was not recorded between other demersal species. Red mullet did not show isotopic overlap with any other species. The only crustacean species examined in this region was Norway lobster, and this species did not show isotopic niche overlap with any of the fish species.

In the Middle area, fish SEAc's ranged from 0.1 ‰² for small-sized European hake and deep-water rose shrimp to 1.8 ‰² for Atlantic horse mackerel (Table 5). Among pelagic fish species, sardine and anchovy isotopic niches did not overlap, but both species showed a high degree of overlap with Atlantic horse mackerel. Demersal and pelagic fish species did not overlap (Table 5 and Figure 4B). The medium-sized European hake did not overlap with small-sized European hake. Furthermore, medium-sized European hake exhibited high overlap (42%) with red mullet. Among the crustacean species, deep-water rose shrimp had a smaller SEAc value (0.1 ‰²) in comparison with Norway lobster (0.5 ‰²), but they showed almost complete overlap (96%). However, their isotopic niches did not overlap with any of the fish species.

The species with the smallest isotopic niche in the Southern area (Table 6) was deep-water rose shrimp (SEAc: 0.3 ‰²), while the largest niche was recorded for medium-sized European hake (2.9 ‰²). Relatively small isotopic niche overlap was recorded between pelagic fish species (Table 6 and Figure 4C), with Atlantic horse mackerel and anchovy showing the largest overlap (13%). Demersal and pelagic fish species overlapped partially, and the highest overlaps were those of Atlantic horse mackerel with medium-sized European hake (93%) while lower overlap was between anchovy (38%) and sardine (35%) and medium-sized hake. Among demersal fish species, only the niches of red mullet and European hake medium size showed higher degrees of isotopic overlap (> 50%). The deep-water rose shrimp niche did not overlap with any fish species.

We estimated that mean TP ranged between 2.5 and 4.4 in the species examined here (Supplementary Table 2), which indicates that the taxa sampled here consumed prey over approximately

three different trophic levels. Trophic position increased from decapod crustaceans, followed by pelagic fishes, with demersal fish species at the highest trophic position (Figure 5). Generally, each species belonged to the same trophic position, regardless of the sampling area. However, in small-sized European hake and Norway lobsters, individuals from the Northern area fed at a higher trophic level compared to conspecifics from the Southern Adriatic and the Middle, respectively. This possibly reflects differences in consumer size between the different study areas as analyzed specimens of small-sized European hake and Norway lobsters in average were larger in the Southern and the Middle Adriatic, respectively.

Predator–Prey Body Mass Ratio Estimates

Community isotopic size spectra are shown in Figure 6. All three linear regressions indicated that the component of the marine food web studied here were strongly size-based. According to ANCOVA results there was no significant difference in estimates for either the slope (area \times Log₂ Class: $F_{2,27} = 1.01$, $p = 0.377$) or the intercept (area: $F_{2,29} = 2.05$, $p = 0.146$) (Figure 6) between areas. As such, data were pooled and a single regression was conducted for the overall study area, which indicated a strong relationship between trophic position and consumer size (biomass-weighted $\delta^{15}\text{N} = 6.59(\text{SE} \pm 0.49) + 0.31(\pm \text{SE } 0.06)$ Log₂ Mass Class; $r^2 = 0.49$; $p < 0.0001$). Using a mean TDF of 2.9‰ and the value of the slope ($b = 0.31$) from this relationship, a mean predator-prey body-mass ratio for the Eastern part of the Adriatic Sea was estimated as 654.8:1.

DISCUSSION

Diet Composition by the Stomach Content Analysis

The exploration and understanding of intra-/inter-species relationships are challenging within any marine ecosystem. Here, we examined spatial and taxonomic variation in the diet of seven commercially exploited species of fish across three different areas in the Eastern Adriatic Sea. Our SCA data were in line with the few available previous studies performed in the Adriatic Sea, while some slight differences were observed with studies from wider geographical areas (Mediterranean Sea, Atlantic Ocean). Our data from pelagic fishes (sardine, anchovy, Atlantic horse mackerel and Mediterranean horse mackerel) were in accordance with previously published data for the same area (sardine: Zorica et al., 2016, 2017; anchovy: Borme et al., 2009; Zorica et al., 2016; Atlantic horse mackerel: Jardas et al., 2004; Mediterranean horse mackerel: Šantić et al., 2004). Those data indicated that small pelagic fish species (sardine, anchovy) preferred to feed on animal food items, specifically on copepods, Bivalvia larvae and fish ova/larvae, although both fish species also had phytoplankton cells within their stomachs. Medium-sized pelagic fishes (Atlantic and Mediterranean horse mackerel) consumed larger animal prey including adult Euphausiacea and Mysidacea or Decapoda larvae (Tables 1–3).

Qualitatively, the diet of the Adriatic pelagic fish species examined in this study was quite similar to those reported for the same species distributed in the Mediterranean area (small pelagics: Costalago et al., 2015; Mazlum et al., 2017; medium-sized pelagics: Rumolo et al., 2016; Karachle, 2017; Georgieva et al., 2019; Saglam and Yıldız, 2019). The slight differences in their relative proportions reported between studies likely reflect spatial and temporal variation in availability. In general, our SCA data indicated that pelagic fish had narrow trophic niche widths (Levins' index: $B_i < 0.37$) and consumed a limited range of prey species, potentially indicating trophic specialism. Alternatively, these species may have opportunistically consumed the most abundant food source (Figure 3) available (Castro Hernández and Ortega, 2000; Ganas, 2014).

Demersal trophic niche width values (i.e., based on SCA) were high (Tables 1–3) relative to those of pelagic species. This suggests that demersal fishes can be considered not only as generalist, but also as opportunistic feeders (Esposito et al., 2014; Mellon-Duval et al., 2017; Riccioni et al., 2018). Among the demersal fish species examined, only the black-bellied angler proved to be strictly piscivorous. This supports earlier studies from the Adriatic (Jardas, 1987), while in the area of Mediterranean sea some studies reported have noted that black-bellied anglers also consume other taxa, including crustaceans, cephalopods and molluscs (Negzaoui-Garali et al., 2008; Stagioni et al., 2013; López et al., 2016). The lack of these other prey in our dataset may reflect the limited number of individuals, especially larger size fishes, sampled here. This is most probably related to the fact that majority of the Adriatic fisheries occurs on the continental shelf and/or near its edge (sea depth $< 200 - 250$ m) where the abundance of those fishes is not high as their abundance is related to the bathymetry - larger fish inhabit greater depths according to Drazen and Haedrich (2012). Furthermore, the number of empty stomachs, which is also size related (Vinson and Angradi, 2011), and the fact that most black-bellied angler specimens, similar to the European hake specimens, had inverted stomachs affected our results. Reflecting this problem, our use of SIA provided a useful means to complement the study of the trophic ecology of European hake and black-bellied angler.

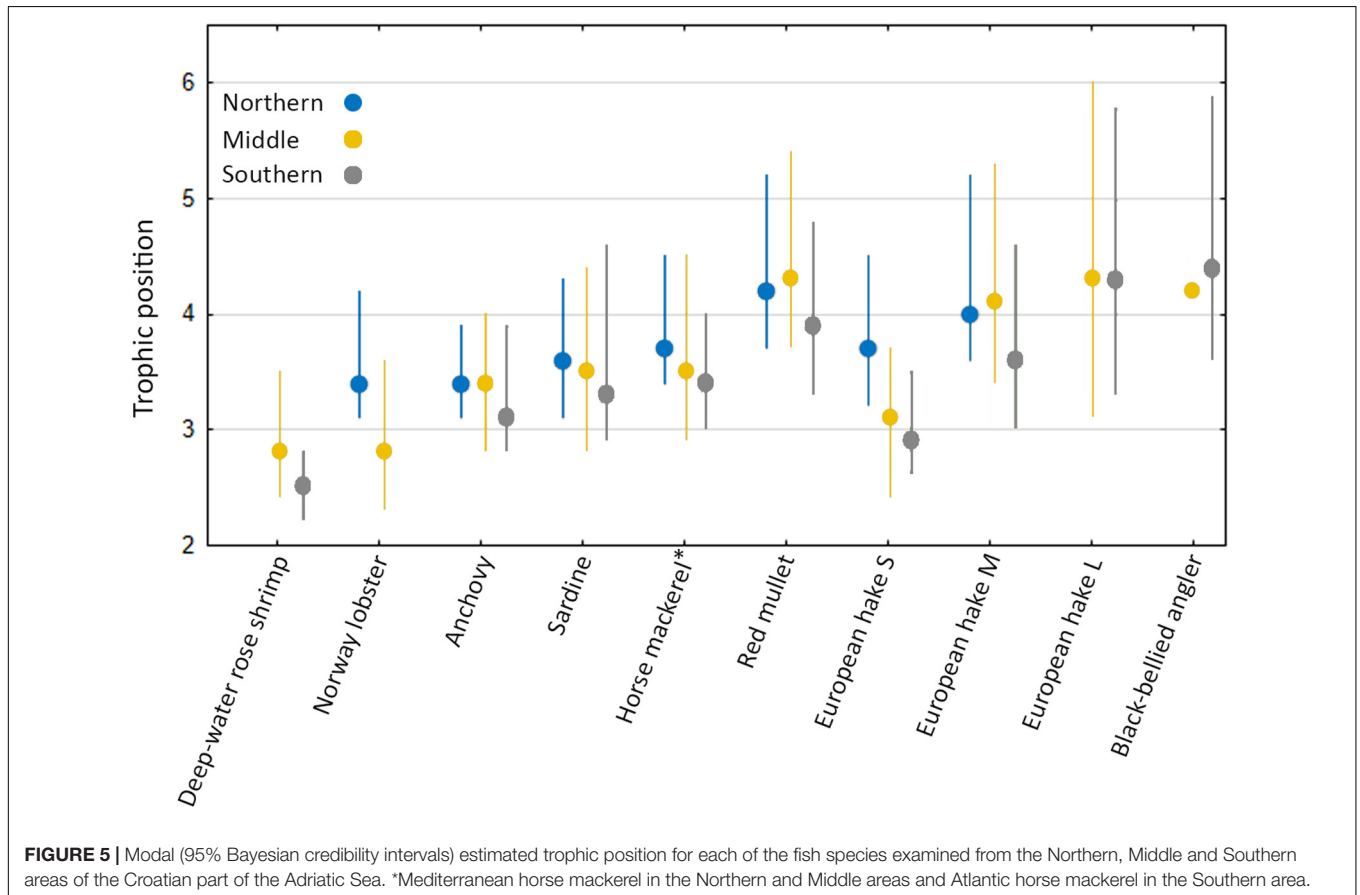
Red mullet largely preyed on copepods, amphipods, decapods and polychaete larvae. This result is in accordance with data previously reported from the Adriatic (Vrgoč et al., 2004) and Mediterranean populations of this species (Vassilopoulou and Papaconstantinou, 1993; Bautista-Vega et al., 2008; Chérif et al., 2011; Esposito et al., 2014; Boudraa et al., 2018; Onay and Dalgic, 2019). Our data on the diet of European hake were qualitatively similar to that reported in previous studies from both the Adriatic (Vrgoč et al., 2004; Riccioni et al., 2018) and Mediterranean seas (Bozzano et al., 2005; Carpentieri et al., 2005; Philips, 2012; Modica et al., 2015; Carrozzi et al., 2019). These studies have typically characterized European hake as an opportunistic feeder, exhibiting dietary shifts as the individuals grow (Vrgoč et al., 2004; Modica et al., 2015; Carrozzi et al., 2019). We obtained similar results, with hake showing a shift in diet from smaller zooplankton toward fishes as their total body length increased.

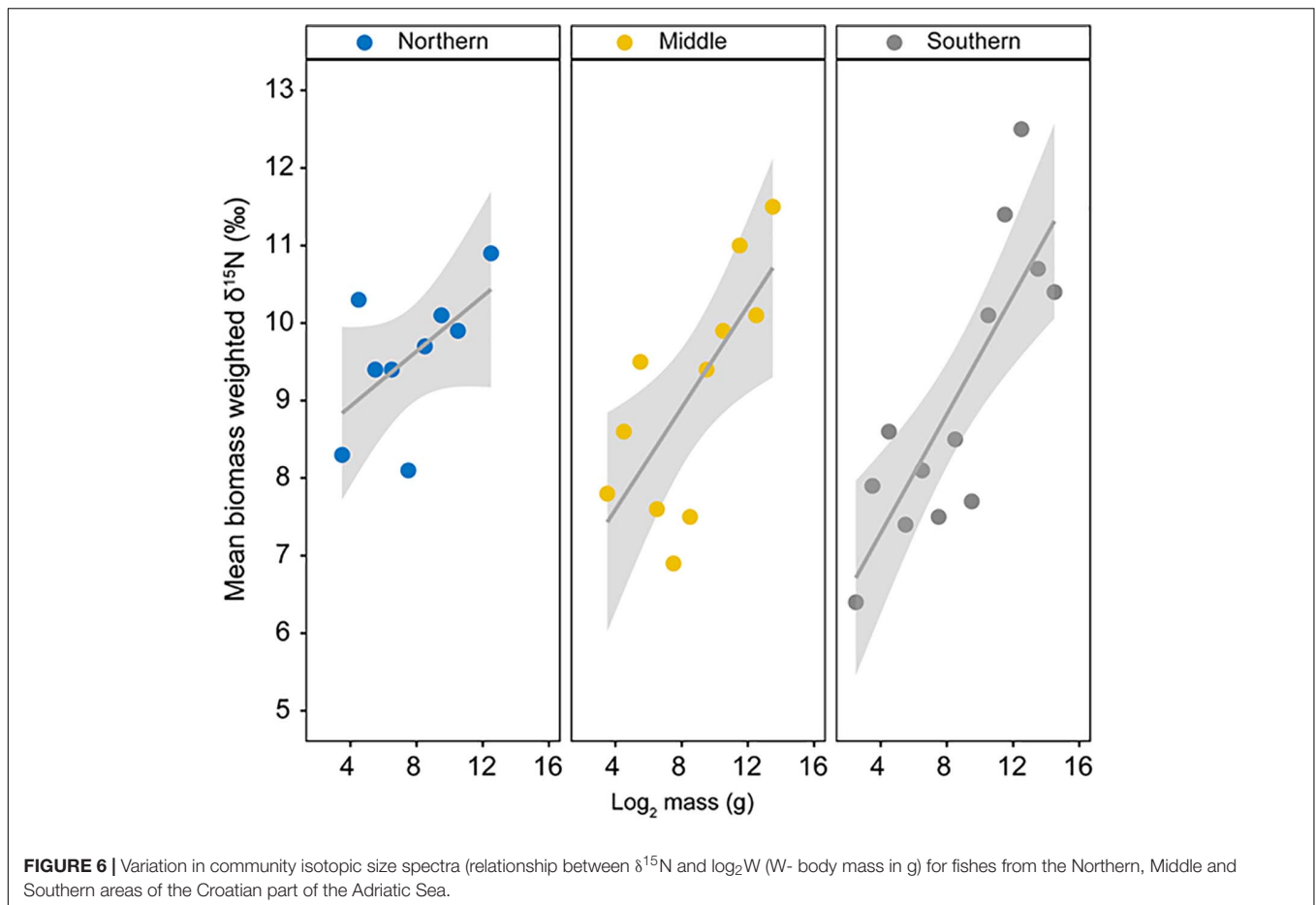
TABLE 5 | Standard Ellipse Area corrected for small sample (SEA_c) and SEA_c overlap percentage between sampled species in the Middle area of the Adriatic Sea (values indicate SEA_c overlap of the species in the row with the species in the column).

Species	SEA_c (% 2)	Anchovy	Sardine	Atlantic horse mackerel	European hake S	European hake M	Red mullet	Norway lobster	Deep-sea rose shrimp
Anchovy	0.16		0	100	0	0	0	0	0
Sardine	0.55	0		72	0	0	0	0	0
Atlantic horse mackerel	1.75	9	23		0	2	0	0	0
European hake S	0.12	0	0	0		0	0	0	0
European hake M	0.80	0	0	3	0		42	0	0
Red mullet	1.63	0	0	0	0	20		0	0
Norway lobster	0.46	0	0	0	0	0	0		22
Deep-water rose shrimp	0.11	0	0	0	0	0	0	96	

TABLE 6 | Standard Ellipse Area corrected for small sample (SEA_c) and SEA_c overlap percentage between sampled species in the Southern Adriatic Sea (values indicate SEA_c overlap of the species in the row with the species in the column).

Species	SEA_c (% 2)	Anchovy	Sardine	Atlantic horse mackerel	European hake S	European hake M	Red mullet	Deep-water rose shrimp
Anchovy	0.66		0	8	0	38	0	0
Sardine	0.96	0		3	0	35	13	0
Atlantic horse mackerel	0.42	13	6		0	93	0	0
European hake S	0.32	0	0	0		6	0	0
European hake M	2.86	9	12	14	1		46	0
Red mullet	1.89	0	6	0	0	70		0
Deep-water rose shrimp	0.29	0	0	0	0	0	0	





The trophic niche of small-bodied European hake was smaller than that of their larger conspecifics, a pattern similar to that noted by Modica et al. (2013).

Our SCA data showed spatial differences in diet within all species apart from European hake and highlighted how individuals captured from the shallow Northern area (Kvarnerić Bay) differed in their diet from conspecifics from the Middle and Southern areas. This observation reflects earlier information from this and other eastern Adriatic channels (Vrgoč et al., 2004), which led to them being designated as key habitats for planktivorous pelagic fishes (Vučetić, 1963; Sinovčić and Zorica, 2009). Channels located along the eastern coast of the Northern Adriatic support higher zooplankton densities than the open waters of the Middle and Southern Adriatic, with productivity driven by the nutrient enrichment from freshwater sources (submarine springs, smaller rivers), high precipitation over the Kvarnerić Bay and strong vertical mixing due to katabatic 'bora' winds (Viličić, 2014; Vilibić et al., 2018). Variation in trophic niche width also reflected differences between functional groups and study area. Within the productive channel areas of the Northern area, pelagic fish trophic niche widths became increasingly smaller, indicating specialist feeding behavior. Conversely, demersal fish niches increased, indicating a more generalist strategy.

Potential Zooplankton Prey in the Water Column

We showed a distinct decrease in total zooplankton abundance through the water column toward the most southerly station, in line with the general longitudinal gradient observed in the Adriatic Sea (Fonda Umani, 1996; Hure and Kršinić, 1998). Overall, zooplankton abundance was very low, especially in the Middle and Southern area ($> 350 \text{ ind. m}^{-3}$). This may have reflected selectivity of the $200 \mu\text{m}$ mesh size, which does not effectively retain smaller-bodied mesozooplankton (e.g., copepodite stages, juvenile appendicularians and early larvae of benthic taxa). Alternatively, it may have reflected a temporal effect, given that the annual zooplankton peak is usually reported in spring (Hure et al., 1980), and we sampled in summer. Nevertheless, the presence and relative proportions of prey categories determined in fish guts (especially in sardine and anchovy) corresponded well with the abundance of zooplankton in the water column. Indeed, the most abundant prey items – copepods, cladocerans and bivalve larvae combined – represented $> 90\%$ of ambient mesozooplankton at all stations sampled. The overall mesozooplankton composition across the three different areas of the Adriatic was relatively uniform, with minor discrepancies reflecting the regular distribution patterns

of individual mesozooplankton groups, e.g., an increased contribution of cladocerans and an absence of euphausiids in the Northern area (Lipej et al., 1997; Gangai et al., 2018). The least-represented groups of ambient zooplankton (e.g., chaetognaths, appendicularians, pteropods and thaliaceans) were absent from the fish guts. Conversely, planktivorous fish species are likely more efficient at capturing hyperbenthic mysids than the standard net vertical hauls, which generally do not sample the first few meters above the sea bottom. The observed concordance between prey categories and zooplankton composition/abundance in the water column points at opportunistic feeding behavior in the planktivorous fishes examined here, where individuals consume the most abundant prey, resulting in indiscriminate feeding on mesozooplankton. However, it is possible that further and subtler differences in prey composition per area might be revealed at zooplankton species, rather than group level.

Isotopic Niche Overlap and Trophic Position of Investigated Species

In most cases, SIA confirmed the results obtained through SCA in terms of niche overlap. However, some discrepancies were observed, which likely reflect the strengths and weaknesses of the two approaches (Majdi et al., 2018; Nielsen et al., 2018; Pethybridge et al., 2018). SCA provides information on recently consumed items, while bulk SIA of muscle tissue provides data about source utilization over a period of several months. In general, the isotopic niches of pelagic and demersal species did not show high degree of overlap. Among pelagic fish species, anchovy had the smallest isotopic niche, which rarely overlapped with the other pelagic species. Such a narrow isotopic niche for anchovy suggests a specialist diet. Furthermore, it might be related to the fact that the sampling time corresponded to the spawning time of this species (April – July; Zorica et al., 2020), when its individuals tend to feed on larger and therefore higher-energy-content prey (e.g., decapod larvae) according to Zorica et al. (2016), as we also confirmed through SCA (Tables 1–3). Apart from in the most productive Northern area, the isotopic niches of anchovy and sardine did not overlap. Anchovy frequently shifts from filter feeding to particulate feeding (Rumolo et al., 2016), meaning that they are probably capable of foraging in deeper layers and utilizing larger and more nutritious prey items. Although SCA data indicated that anchovy diet was distinct from demersal fishes, there was some overlap with small- and medium-sized European hake in the Northern and Southern areas, respectively. This indicates that over the longer temporal window afforded by SIA, anchovy and certain life stages of European hake may partially share a similar trophic niche. The isotopic niche of sardine overlapped with other pelagic fish species, particularly with the Atlantic horse mackerel. In areas of high (Northern area) and low (Southern area) prey availability, sardine isotopic niche overlapped with the niche of demersal fish (small and medium size European hake). Both mackerel species displayed overlap in their isotopic niche with sardine, except in the Southern area where its niche overlapped with medium-sized European hake. This likely

reflects limitations of prey availability in this area, where mackerel species had the smallest isotopic niche that showed elevated overlap with medium-sized European hake. The isotopic niche of red mullet did not overlap with any pelagic fish species but showed certain degree of overlap with demersal European hake in the Middle and Southern areas. This was supported by stomach contents data, where PERMANOVA indicated similar prey composition in the stomachs of these species. Bautista-Vega et al. (2008) noted that red mullet with a TL > 11.0 cm are surface/sub-surface deposit feeders; hence, the slight overlap with other fish species can be explained by their different feeding mechanisms. Small- and medium-sized European hake showed isotopic overlap with some pelagic fishes (sardine and horse mackerel). This is in line with the fact that smaller hakes tend to move throughout the water column in order to capture prey (Bozzano et al., 2005; Carpentieri et al., 2005; Riccioni et al., 2018). The niche-overlap of medium European hake with red mullet was high in the Middle and Southern Adriatic, where isotopic niche overlap between demersal fish species was the most pronounced.

Comparisons showed that the isotopic niches of the two commercially important decapod crustaceans did not overlap with any of the fish examined: they did however show almost complete mutual isotopic overlap (Table 5). Both species were only encountered in sympatry in the Middle area. Here, the relatively small isotopic niche of the deep-water rose shrimp overlapped completely with the much wider niche of the Norway lobster. Although there are no comparative studies concerning the diets of those two species, our data are in accordance with studies dealing with each species separately, which revealed that their feeding habits are relatively similar. Norway lobster is a scavenger but also a predator capable of active feeding (Oakley, 1979; Parslow-Williams et al., 2002) and filter feeding (Loo et al., 1993). According to dietary analysis performed by Parslow-Williams et al. (2002), Norway lobster largely consumes molluscs, crustaceans, polychaetes and echinoderms. Furthermore, a recent isotopic study highlighted the importance of suspended particulate organic matter to Norway lobster during certain seasons, and noted that its contribution was likely underestimated in other studies (Santana et al., 2020). The deep-water rose shrimp has a similar prey range to the Norway lobster, and exhibits two feeding phases: hunting and digging (Tursi et al., 1999). Hunting is the more active phase, during which it preys on small fish, cephalopods and crustaceans, while during the digging phase it forages in the mud, consuming mainly polychaetes, bivalves, echinoderms and benthic foraminifera (Tursi et al., 1999). The larger isotopic niche of Norway lobster compared to the rose shrimp could be due to its ability to utilize various food sources including decomposing food, plankton and live prey. Analysis of the gut contents of decapod crustaceans would probably detect different prey taxonomic groups, but unfortunately those analyses were not included within this study. Future studies should consider such analyses in these taxa as a priority. Isotopic niche differentiation between the two demersal crustaceans and most of the fishes studied here likely reflects the consumption of benthic prey, which were not observed from fish stomachs.

Although the target species showed dietary differences across the three study areas of the Eastern Adriatic Sea, they had similar trophic position across locations, indicating equivalent ecological function. This suggests that although their diet may differ spatially, they feed at similar trophic levels regardless of location. However, a common community-level relationship was shown between $\delta^{15}\text{N}$ and body weight across the three basins. Furthermore, it was obvious that smaller size classes of European hake feed at lower trophic levels than the larger size-class, as was confirmed by its diet analysis within this study. European hake, as they grow, change their feeding habits toward higher-energy-content prey such as fishes. In general, our estimates of trophic position for the species examined here were in accordance with those obtained by Coll et al. (2006) for the wider Mediterranean Sea and by Albo-Puigserver et al. (2016) for the North-western Mediterranean. The slight differences we observed are most probably due to differences in the environments sampled, but in general the trophic position of the study species did not show pronounced differences across the different areas. Mesozooplankton $\delta^{13}\text{C}$ values shown in this study (mean $\delta^{13}\text{C} -23.0\text{‰}$) were slightly ^{13}C depleted in comparison with those reported from other marine areas (e.g., the global mean \pm SD for zooplankton $\delta^{13}\text{C}$ included in Magozzi et al. (2017) is $-21.0 \pm 2.1\text{‰}$), and compared to mesoplankton collected off Crete and Cyprus (e.g., Koppelman et al. (2009) mean = $-19.7 \pm 0.8\text{‰}$), further south in the Mediterranean. The reason for the apparent ^{13}C depletion in mesozooplankton $\delta^{13}\text{C}$ reported here possibly reflects terrestrial inputs (e.g., from the Po River), effects of local oceanographic conditions or even variation in mesozooplankton lipid contents. Our values do however coincide with the modeled isoscape $\delta^{13}\text{C}$ values presented for the Adriatic Sea in McMahon et al. (2013). A more detailed study is required to resolve the effects of such influences on spatial variation in stable isotope values in different functional groups across our study region.

Since $\delta^{15}\text{N}$ values reflect the trophic position at which species are feeding (Peterson and Fry, 1987), it is apparent that the species in question feed at different trophic levels; more precisely, that the food webs in each sampling area and overall were size-structured. This is also consistent with the findings of Jennings et al. (2001, 2008), who reported that in marine food webs based on phytoplankton the mentioned relationships are strong and might reflect the TP of each body mass class. Additionally, these authors estimated similar slope values for the North Sea community ($b = 0.34$) as we estimated for the Adriatic Sea ($b = 0.31$). In some other areas where community size structuring was studied, like the Western Arabian Sea (Al-Habsi et al., 2008) and the Galician upwelling area (Bode et al., 2003), slope values were somewhat lower ($b = 0.26$ and $b = 0.28$, respectively), most probably due to weak cross-species relationship or variation in food web length. The PPMR value (655:1) estimated for the Eastern Adriatic Sea was different from the PPMR reported for Western Arabian Sea (7762:1; Al-Habsi et al., 2008), Galician upwelling area (4500:1; Bode et al., 2003) and North Sea (1136:1; Jennings et al., 2001). Observed differences were expected since they used different TDF values

(e.g., 2.5‰ and 3.4‰) and their studies covered wider and/or narrower range of trophic levels.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because performed research involved sampling of marine animals including vertebrates (fishes) and invertebrates (crustaceans). Animals were collected through scientific surveys and caught with methodology normally used in the scientific sampling or in fishery practices and were not exposed to any unnecessary pain, injuries or suffering in this study. Furthermore, special attention was given to the avoidance of accidental catches of sensitive or endangered species (sea turtles, marine mammals, etc.).

AUTHOR CONTRIBUTIONS

BZ did the conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, resources, supervision, validation, visualization, and writing. DE-B did the conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, resources, supervision, validation, visualization, and writing. OV did the zooplankton analysis, visualization, and writing. VV did the fieldwork. MŠ and VČK did the stomach content analysis. II did the resources and fieldwork. NV did the funding acquisition and investigation. CH did the conceptualization, visualization, and writing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2021.609432/full#supplementary-material>

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