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Influence of seasonal variability on the trophic structure of pelagic communities

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Seasonal variations in hydrodynamic conditions play a critical role in prey availability at the base of the food web and thus have implications for trophic interactions at higher trophic levels. Here we use the combination of stomach content analysis (SCA) and stable isotope analysis (SIA) to investigate predator-prey interactions and trophic structure of the pelagic fish community of the Bay of Biscay (BoB) in spring and late summer of 2020 and 2021. We found that trophic guilds were more influenced by intra-specific affinities rather than seasonal variability. Main prey for the pelagic fish comprised a set of species belonging to different trophic positions (TPs), from low TP class Thaliacea (salps) to mid TP *Engraulis encrasicolus*. Vertically migrating meso- and macrozooplankton (mainly copepods and krill) also played an important role as food source for the pelagic fish community showing high trophic overlap among most of species. No differences were detected between the trophic structure in spring and late summer with fish, squids and chaetognaths at the top TPs, whereas krill, meso-zooplankton and salps had the lowest values. However, the TPs estimated using both SCA and SIA approaches were consistently lower in spring, likely due to higher densities and, thus, low TP prey availability in spring. When analyzing ontogenic changes in TPs with predator's size, significant and positive relationships were found when TP was estimated using SIA but not when using SCA. Overall, our results reveal new insights into the seasonal trophic dynamics of the pelagic fish community of the BoB.

KEYWORDS

trophic structure, pelagic fish, stomach contents, stable isotopes, Bay of Biscay, trophic position, seasonal variability

1 Introduction

Marine ecosystem functioning depends on its structure, diversity and integrity. Disturbance of one or several components of marine ecosystems can affect higher (e.g., bottom-up control) or lower (e.g., top-down control), and even the same trophic level (Cury et al., 2003; Checkley et al., 2009). For instance, drastic changes in one abundant prey

could have major consequences on potential predators and therefore on the functioning of the ecosystem. Forage fish are often abundant prey, playing a key role in marine pelagic ecosystems (Preciado et al., 2008; Corrales et al., 2022) jointly with phytoplankton and zooplankton, representing the main pathway by which energy and nutrients are transported from lower to upper trophic levels (Cury and Pauly, 2000). Therefore, studying seasonal variations not only in physical and oceanographic processes (e.g., Varela, 1996; Gil, 2008; Lassalle et al., 2011), but also in inter-specific trophic interactions (e.g., Bachiller and Irigoien, 2015) can contribute to better understand the overall functioning of marine ecosystems.

Trophic interactions in the pelagic environment have been traditionally studied through stomach content analysis (SCA). This approach provides qualitative and quantitative information on recent diet of a predator (Hyslop, 1980). However, this technique has several shortcomings, such as the disparate accuracy of prey taxonomic identification, given the uneven degree of prey digestion and short temporal resolution (Cresson et al., 2014). Complementing SCA, stable isotope analysis (SIA) has arisen as a powerful method in trophic ecology used to study the food assimilated by an organism over the previous weeks to months. The isotopic signature of a predator depends on the isotopic signature of its prey, corrected by an isotopic enrichment factor and as such, it can be used to understand trophic interactions within a community (Peterson and Fry, 1987). However, the taxonomic resolution of the SIA is much lower than of the SCA. Thus, the combination of both approaches, SCA and SIA, is highly recommended in trophic ecology to improve the estimate of predators' diet at an appropriate level of taxonomic resolution. Such data is key to discern complex interactions at a seasonal timeframe, and thus elucidate a trustworthy trophic structure for the pelagic environment (Post, 2002; Boyle et al., 2012).

In addition, δ^{13}C values provide information on the origin (e.g., terrestrial, benthic or pelagic) of the carbon source whereas δ^{15}N values can be used to estimate the trophic position of the organism. In fact, the relation between fish body size and trophic position (TP hereafter) has been widely studied worldwide in marine and freshwater ecosystems (Deudero et al., 2004; Jennings et al., 2007; Arim et al., 2010; Barnes et al., 2010; Ou et al., 2017; Moranta et al., 2020). Empirical studies have supported positive relationships between body size and TP, mainly in those species with ontogenic changes in their diet (piscivores and omnivores). However, a trophic position-body size relationship may not be apparent in some species because of their non-piscivorous diet, such as in planktivorous fish, because of their external morphological traits, because of the sex change effect, or because the predator size range sampled is too small (Galván et al., 2010; Ríos et al., 2019; Keppeler et al., 2020; Moranta et al., 2020).

In temperate pelagic ecosystems, as seasonal variations of hydrodynamic conditions trigger changes in nutrients, primary production and primary consumers, variations in predator-prey interactions and pelagic ecosystem functioning are expected. The Bay of Biscay (hereafter BoB) is one of such ecosystems where temporal variations can be expected due to variability in mesoscale oceanographic processes (e.g. upwelling) that influence on the distribution of nutrients, phytoplankton, zooplankton and ichthyoplankton (Gil, 2008), as well as

on the carbon and nitrogen isotopic signatures of pelagic fish (Bode et al., 2003; Bode et al., 2007; López-López et al., 2017). Important upwelling events occur in the Iberian and French shelves during summer (Botas et al., 1990; Jegou and Lazure, 1995; Gil, 2008), while the main rivers in the BoB are located in the French coast, providing approximately 80% of the freshwater discharge onto the French shelf mostly during winter and spring (Borja et al., 2018). The phytoplankton community in the shelf zone of the BoB is composed mainly of diatoms or dinoflagellates, which variation depends predominantly by river discharge variations, by water column stratification conditions, and by upwelling events (Morozov et al., 2013). In the off-shelf areas of the Cantabrian Sea there is a major phytoplankton spring bloom and a secondary one in autumn (Bode et al., 2011a). Zooplankton abundances and biomass in the BoB have two annual peaks, in spring and autumn, corresponding to, and just after, phytoplankton blooms (Valdés et al., 2007). Zooplankton communities are composed mainly of copepods (more than 70% of the total abundance), although other taxa such as Cladocera, Siphonophora, Thaliacea and Appendicularia also display high densities (Albaina and Irigoien, 2007). The pelagic fish community is mainly dominated by small species such as European anchovy *Engraulis encrasicolus*, sardine *Sardina pilchardus*, horse mackerel *Trachurus trachurus* and blue whiting *Micromesistius poutassou* all year round and Atlantic mackerel *Scomber scombrus* which seasonally enters the Bay during spring, as well as some migratory tuna species such as albacore which enters the BoB to feed on smaller pelagic fish (Goñi et al., 2011; Borja et al., 2018). Together with the latter, at the top of the pelagic food chain of the BoB are migratory and resident cetaceans and seabirds, including some endangered species (Pérez-Roda et al., 2017; Borja et al., 2018; Astarloa et al., 2019; García-Barón et al., 2019), although large demersal fish do also benefit from small pelagic forage fish regularly (Preciado et al., 2008; López-López et al., 2012).

The present study aimed to analyze seasonal changes in the trophic ecology of the pelagic community of the BoB using a multispecies and multitrophic approach. Based on information collected from integrated ecosystem-surveys, where information on oceanography, phytoplankton, zooplankton, small pelagic fish and marine megafauna is collected, we characterized the trophic ecology of the pelagic community using two complementary approaches (stomach contents and stable isotopes analysis). At the pelagic fish community level, different trophic metrics were estimated to assess prey contribution, diet overlap and trophic position. Specifically, trophic position was estimated from trophic information obtained from SCA and SIA to assess the influence of body condition and season. Our works provide novel insights on the trophic characterization of the pelagic fish community combining SCA and SIA. Our results might help elucidate the response of trophic variability in the Bay of Biscay that could contribute to the ecosystem monitoring of the pelagic domain.

2 Material and methods

2.1 Study area and sample collection

Samples were collected in the Bay of Biscay during two ecosystem integrated surveys that cover the continental shelf and

oceanic areas in spring (BIOMAN survey) and late summer (JUVENA survey) in two consecutive years, 2020 and 2021 (Figure 1). The BIOMAN survey aims to evaluate the European anchovy and sardine populations in the BoB to advice on the regulation of these fisheries (Santos et al., 2018). The JUVENA survey is designed to monitor juvenile anchovy and other small pelagic fish over both Spanish and French continental shelf and slope waters (Boyra et al., 2013). Additionally, within these surveys, information on the oceanographic conditions, plankton communities, and marine megafauna species is collected. Sampling was conducted in 4 different oceanographic areas within the BoB, based on Santos et al. (2013) and Koutsikopoulos and Cann (1996): Central Cantabrian (CC), East Cantabrian (EC), Garonne area (GR) and South Britain area (SB) (Figure 1).

The pelagic community was characterized using pelagic trawls collecting fish, crab, krill and squid samples. A total of 90 pelagic hauls were carried out, at depths ranging from 1.2 to 310 m (Figure 1), based on registrations from acoustic sampling. Fish sampling included boarfish (*Capros aper*), anchovy, Mueller's pearlside (*Maurolicus muelleri*), hake (*Merluccius merluccius*), blue whiting, sardine, Atlantic chub mackerel (*S. colias*), Atlantic mackerel, sprat (*Sprattus sprattus*), Mediterranean horse mackerel (*T. mediterraneus*), horse

mackerel and poor cod (*Trisopterus minutus*). All trawls lasted around 50 minutes at 4 knots, keeping the initial fishing depth whenever possible. In each sampling station, 10 individuals of each species were set aside randomly. Crabs, krill, fish and squid samples were preserved frozen immediately after collection for further analyses of SIA (all samples) and SCA (fish). Zooplankton communities were sampled in each survey and each area at three different bathymetrical ranges: coast, continental shelf and upper slope. We used PairoVET (Pair of Vertical Egg Tow, Smith et al., 1985) nets with a mesh size of 150μm, sampling the water column with vertical tows in depths ranging from 15 to 100m (Figure 1). These samples were preserved in ethanol (96%) for later isotope analysis.

2.2 Stomach content analysis (SCA)

The stomach contents of 12 pelagic fish species were analyzed Table 1. In the laboratory, each fish was first sized (0.5cm) and weighted (0.1g) and then the stomach was dissected, weighted, and preserved in ethanol (96%) for later stomach content analysis.

A stereomicroscope was used for the identification of prey items. During processing, prey items were carefully separated,
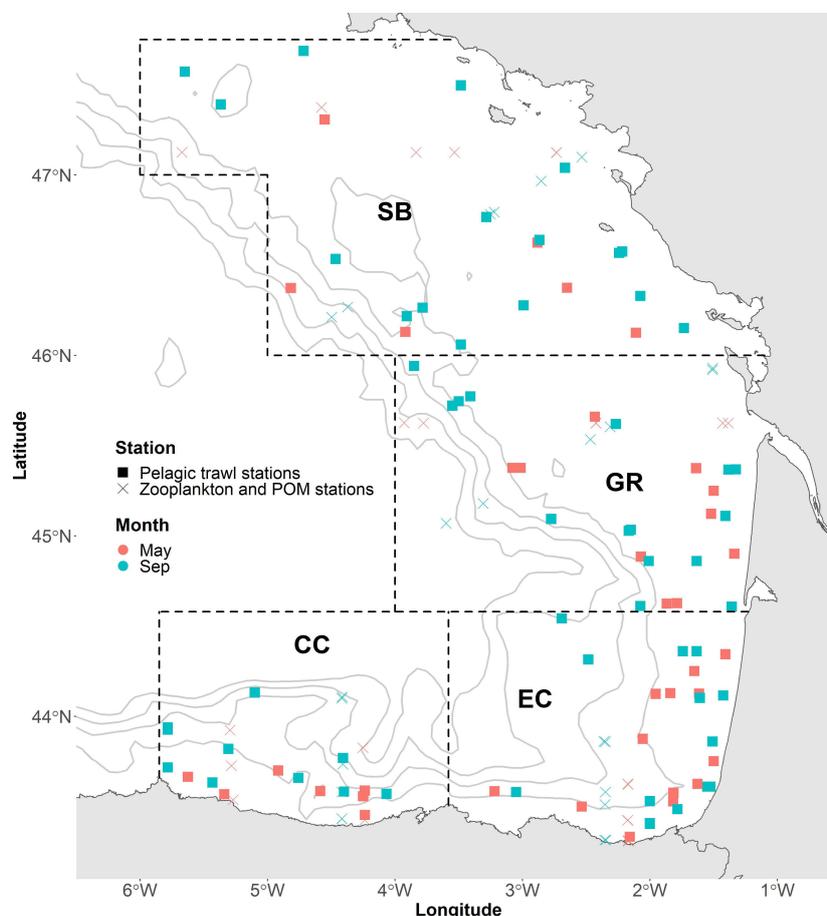

FIGURE 1
Pelagic trawl and zooplankton stations in each season (May and September) in the Bay of Biscay. Boundaries represent 4 different oceanographic areas, based on Santos et al. (2013) and Koutsikopoulos and Cann (1996): Central Cantabrian (CC), East Cantabrian (EC), Garonne area (GR) and south Britain area (SB).

counted and classified to the lowest identifiable taxonomic group, adding up to a total of 30 prey taxa identified. A subsample was taken and analyzed in those stomachs with large amounts of zooplankton. To make the illustrations clearer, the 30 types of prey were merged into 9 larger taxon groups. The unidentifiable items were considered as digested material and thus were not quantified. Then the percentage of each prey in the stomachs was estimated by the relative volume. Prey weight was calculated from the total stomach weight and the relative volume of the prey. In subsequent analyses, only those fish species with at least 10 stomachs in both seasons were used (8 species). The contribution of each food item to the diet of each species was expressed using common qualitative and quantitative metrics in the trophic ecology literature: the frequency of occurrence in percentage (%FO), the abundance by number (%N) and the abundance by weight (%W).

To study the trophic overlap among predators, the Schoener Index (S) was computed (Schoener, 1970). Prey weight was used for the calculations, since it better represents the relative importance of any prey item when it is partially digested or is difficult to count (Hyslop, 1980). The degree of overlap between the diet of two predators is considered biologically significant when values are above 0.6 (Wallace, 1981). Values between 0.3 and 0.6 are considered as medium overlap and values lower than 0.29 as low overlap (Langton, 1982). The R package *spaa* (Zhang, 2016) was used for the calculations.

To identify feeding patterns based on the diet composition, a quantitative matrix of predator-prey (prey weight) was calculated. Prey weight values were normalized by applying a logarithmic transformation. A hierarchical classification analysis was performed on a matrix containing 8 predators and 30 prey taxa based on the Bray-Curtis dissimilarity index and the distance matrix calculated with the UPGMA algorithm. Then, a SIMPER analysis was conducted to identify which prey contributed most to the dissimilarities between the predators' diet. The *vegan* package of R (Oksanen et al., 2022) was used for these analyses.

2.3 Stable isotope analysis (SIA)

Muscle tissue samples were extracted at the laboratory from fish, squids, crabs and krill for the stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analysis. The samples were placed in a drying oven for 48h at 60°C, then grounded until a fine powder was obtained, and finally a subsample of 1-1.5 mg of this powder was weighted and packed into tin capsules for the isotopic analysis. Zooplankton samples were first analyzed under a stereomicroscope. Individuals of the same taxonomic group were set aside and grouped and then they were placed in a drying oven for 48h at 60°C. Finally, they were packed (whole individuals and grouped) into tin capsules for the isotopic analysis. Some prey taxa present in the stomachs of fish could not be obtained from zooplankton samples in enough number (i.e., sufficient weight) to perform SIA.

The isotopic analyses were carried out at the Servizos da Apoio á Investigación (SAI, Universidade da Coruña, Spain). The $\delta^{15}N$ and $\delta^{13}C$ determination was performed using a FlashEA1112

(ThermoFinnigan) analyzer coupled to a MAT253 ThermoFinnigan) mass spectrometer through a Conflo IV interface. $\delta^{15}N$ and $\delta^{13}C$ were expressed as parts per thousand (‰) with a standard deviation of ± 0.15‰. $\delta^{13}C$ values were corrected to avoid the potential effect of lipids on individuals with a C:N ratio higher than 3.5, following the formula proposed by Post et al. (2007):

$$\delta^{13}C_{(corrected)} = \delta^{13}C_{(bulk)} - 3.32 + 0.99 \times C:N$$

2.4 Trophic position based on SCA and SIA

The trophic position (TP) of each predator was calculated both based on the stable isotopes (TP_{SIA}) and stomach content analysis (TP_{SCA}), and both estimates were used to carry out a seasonal comparison of the variation in the TP.

The filter feeder class Thaliacea (salp) was used as a baseline for its consistent low values of $\delta^{15}N$ (Stowasser et al., 2012; Ménard et al., 2014). To calculate the TP_{SIA} the incremental approach suggested by Hussey et al. (2014) was applied:

$$TP_{SIA} = 2 + \frac{\log(\delta^{15}N_{lim} - \delta^{15}N_{base}) - \log(\delta^{15}N_{lim} - \delta^{15}N_{sample})}{k}$$

where $\delta^{15}N_{sample}$ and $\delta^{15}N_{base}$ are the N isotopic signatures of each sample and the reference baseline (salps), 2 is the trophic position of the reference baseline, $\delta^{15}N_{lim}$ is the saturating isotope limit as trophic position increases, and k the rate at which $\delta^{15}N_{sample}$ approaches $\delta^{15}N_{lim}$. Values for $\delta^{15}N_{lim}$ = 21.926 and k = 0.137 were provided by the meta-analysis of Hussey et al. (2014).

The TP_{SCA} was calculated applying the formula suggested by Pauly et al. (2000):

$$TP_{SCA_j} = 1 + \sum_{i=1}^{m} Wn_{ij} \times TP_i$$

where TP_i is the trophic position of prey i, Wn_{ij} is the weight fraction of prey i in the diet of predator j, and m the total number of preys. TP_i values were assigned based on our results of isotopic $\delta^{15}N$ signatures of the prey. For those prey taxa where TP_i was not available, data were obtained from the literature and TrophLab values (Fishbase, Froese and Pauly, 2000).

To analyze changes in the TP of pelagic fish along their ontogeny, we applied a Linear Mixed-effects Model (LMM) using the TP as dependent variable (both for TP_{SIA} and TP_{SCA}). We used a multispecies approach, including all fish species in the same model and establishing predator size (continuous) and season (categorical) as explanatory variables. To account for the potential effect of the predator identity, predator species was included as random variable. The *lmer4* package of R (Bates et al., 2015) was used for these analyses. To choose the final model, a selection of explanatory variables was carried out using a backwards/forwards stepwise selection process based on the Akaike's Information Criteria (AIC). The *MuMIn* package of R (Bartón, 2022) was used for this purpose. Those models with ΔAIC (the difference between the AIC value of the model and the model with the lowest AIC value) less than 2 were considered equivalent. AIC weights were used for the model's comparison.

3 Results

3.1 Diet composition, trophic overlap and trophic guilds

A total of 744 stomachs from 12 pelagic fish species were analyzed, 147 of which (19.76%) were empty or with unidentifiable food/prey items. A summary of the total number of stomachs and analyzed (full) stomachs, size range and depth range per season for each pelagic species is shown in Table 1. The size of the individuals varies in spring from 7.5 cm of *M. poutassou* to 45 cm of *M. merluccius* and in late summer from 3 cm of *M. muelleri* to 51cm of *M. merluccius*. Two species, *M. muelleri* and *T. minutus*, were only present during late summer. *C. aper*, *M. muelleri*, *T. minutus* and *T. mediterraneus* were not included in further analyses due to their low sample sizes.

Based on the prey weight (%W), copepods were the most common prey in both seasons for four species, European anchovy, sardine, sprat and horse mackerel, and for blue whiting

in spring with values ranging from 37.31%-99.07% (Table 1 and Figure 2). Blue whiting was the only predator mostly relying on krill, particularly in late summer (%W = 48.50%, Table 1 and Figure 2). Salps were also a common food resource in both seasons for Atlantic chub mackerel and in late summer for Atlantic mackerel, with %W ranging from 49.81%-69.79% (Table 1 and Figure 2). Anchovy was the main prey in both seasons for hake, as well as for Atlantic mackerel in spring (%W from 75.49%-86%, Table 1 and Figure 2). Other relevant prey (%W > 25%) found included fish eggs in the diet of sardines, gastropods in the diet of anchovies and hyperiids in the diet of blue whitings (Table S1 and Figure 2).

Differences in trophic overlap of most species were evident among seasons. In spring, small pelagic fishes (clupeid species, i.e., anchovy, sardine and sprat; horse mackerel; and blue whiting) showed medium or significant trophic overlap between them (S > 0.30; Table 2). On the other hand, hakes and Atlantic mackerel displayed significant overlap (S > 0.60) between them but no

TABLE 1 Summary of the collected pelagic fish in each season describing the size range, collected depth range, number of total analyzed stomachs, number of full stomachs, main prey and main prey weight (%W).

Predator	Month	Size range (cm)	Depth range (m)	Total stomachs	Full stomachs	Main prey	%W
Capros aper (Boarfish)	May	9-15	140-140	3	3	Copepod	95.58
	Sep	6-9.5	108-196	25	15	Copepod	50.02
Engraulis encrasicolus (European anchovy)	**May**	**9.5-15.5**	**5-80**	**107**	**106**	**Copepod**	**53.44**
	Sep	**3.5-15**	**1.2-118**	**80**	**60**	**Copepod**	**66.33**
Maurolicus muelleri (Mueller's pearlside)	May	–	–	–	–	–	–
	Sep	3-6	50-192	39	29	Copepod	91.77
Merluccius merluccius (European hake)	**May**	**19-45**	**10-127**	**25**	**8**	**Anchovy**	**83.12**
	Sep	**14-51**	**38-114**	**24**	**8**	**Anchovy**	**86**
Micromesistius poutassou (Blue whiting)	**May**	**7.5-31**	**17-215**	**35**	**30**	**Copepod**	**37.31**
	Sep	**12.5-22.5**	**116-205**	**18**	**8**	**Krill**	**48.50**
Sardina pilchardus (Sardine)	**May**	**10.5-20**	**6-80**	**50**	**50**	**Copepod**	**38.19**
	Sep	**10.5-19**	**9-50**	**56**	**56**	**Copepod**	**61.06**
Scomber colias (Atlantic chub mackerel)	**May**	**19.5-34.5**	**10-25**	**13**	**12**	**Salp**	**52.64**
	Sep	**10-27.5**	**8-50**	**22**	**22**	**Salp**	**49.81**
Scomber scombrus (Atlantic mackerel)	**May**	**19-37.5**	**5-75**	**39**	**35**	**Anchovy**	**75.49**
	Sep	**11-29.5**	**6-25**	**42**	**40**	**Salp**	**69.79**
Sprattus sprattus (European sprat)	**May**	**10.5-12.5**	**30-45**	**14**	**14**	**Copepod**	**99.07**
	Sep	**5.5-9**	**8-58**	**13**	**11**	**Copepod**	**72.20**
Trachurus mediterraneus (Mediterranean horse mackerel)	May	36-36	10-10	1	1	Hyperiid	100
	Sep	18-20	22-22	5	1	Cladoceran	100
Trachurus trachurus (Horse mackerel)	**May**	**8-31.5**	**5-22**	**59**	**42**	**Copepod**	**66.96**
	Sep	**5-31**	**3-50**	**68**	**43**	**Copepod**	**43.04**
Trisopterus minutus (Poor cod)	May	–	–	–	–	–	–
	Sep	16.5-19.5	108-108	10	4	Galatheoid	92.5

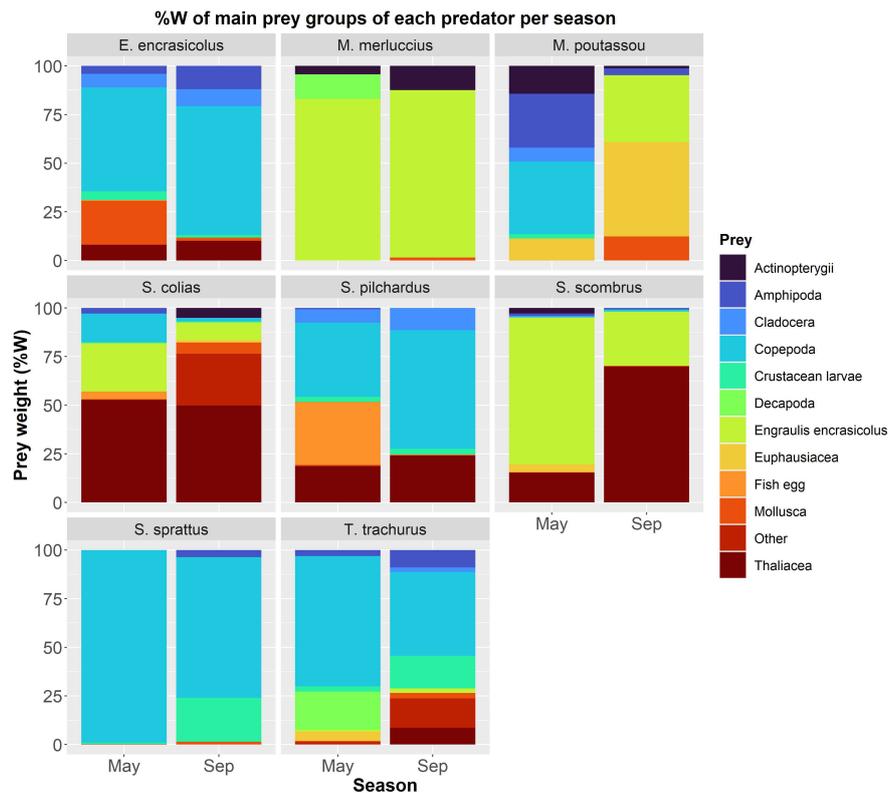Fish species with more than 10 stomachs in both seasons are highlighted in bold.

FIGURE 2
Prey weight (%W) for 12 prey groups of 8 fish species per season. Values can be found at Table S1.

overlap or low (S < 0.30) with the rest of species (Table 2). In late summer, also, a medium or significant dietary overlap was observed among small pelagic fishes, except for blue whiting, which only showed medium overlap with hakes (Table 2). The trophic overlap was also relevant between the two *Scombridae* species in both seasons (Table 2).

Considering similarities between predators' diets, the hierarchical classification analysis of prey affinities resulted in three groups of predators (Figure 3): i) planktivores with *E. encrasicolus*, *S. pilchardus* and *S. sprattus*, ii) piscivores which included *M. merluccius*, *S. scombrus* and *S. colias*, and iii) a mixed diet group composed of *T. trachurus* and *M. poutassou*, feeding on fish and plankton. SIMPER analysis revealed that the main taxa contributing to the average similarity within planktivores were copepods, salps and hyperiids (Table 3). Among piscivores, the European anchovy and salps were the main prey taxa. Finally, krill, European anchovies and hyperiids constituted the main prey in the diet composition of mixed-diet predators.

3.2 Stable isotope analysis

Nine fish species, one squid species, one krill species, one pelagic crab and five zooplankton taxonomic groups were analyzed (Table 4, C:N ratios in Table S2). The isotopic biplots (showing $\delta^{13}C$ and $\delta^{15}N$ values) displayed distinct isotopic

signatures between spring and late summer for the whole community (Figure 4). In May, $\delta^{13}C$ ranged between -24.8‰ and -17.9‰ whereas $\delta^{15}N$ ranged between 5.1‰ and 13.6‰. In September, values ranged between -22.5‰ and -17.5‰ for the $\delta^{13}C$ and between 4.9‰ and 14.5‰ for the $\delta^{15}N$. Salps displayed the greatest variability in $\delta^{13}C$ values between seasons. In both seasons, salps showed the lowest values of $\delta^{15}N$ and $\delta^{13}C$ (Table 4 and Figure 4, red circle); then there was a group with copepods, hyperiids, krill and pelagic crabs with higher $\delta^{15}N$ values (Table 4 and Figure 4, blue circle), and, finally, another group with the highest $\delta^{15}N$ values with fish, squids and chaetognaths (Table 4 and Figure 4, green circle). Boarfish showed lower values in September and was found in the middle group.

3.3 Trophic position based on SCA and SIA

Based on stable isotopes, salps showed the lowest trophic position in both seasons because it was considered as the baseline (TP_{May} and TP_{Sep} = 2, Table 4). *T. trachurus* displayed the highest TP_{SIA} in May and *M. merluccius* in September (TP_{May} = 3.36 and TP_{Sep} = 3.67, respectively, Table 4). Based on stomach contents (only fish species), *M. merluccius* had the highest TP in both seasons (TP_{May} = 3.93 and TP_{Sep} = 3.96, Table 4), whereas *E. encrasicolus* (TP_{May} = 3.1) and *S. colias* (TP_{Sep} = 3.19) showed the lowest TP (Table 4). Between-season comparisons showed lower TP in spring

TABLE 2 Trophic overlap (S: Schoener Index) between 8 fish species per season calculated using the estimated prey weight.

		E. encrasicolus	M. merluccius	M. poutassou	S. pilchardus	S. colias	S. scombrus	S. sprattus	T. trachurus
May	E. encrasicolus	x							
	M. merluccius	0	x						
	M. poutassou	0.46	0	x					
	S. pilchardus	0.56	0	0.47	x				
	S. colias	0.24	0.25	0.18	0.38	x			
	S. scombrus	0.1	0.75	0.06	0.17	0.42	x		
	S. sprattus	0.54	0	0.38	0.38	0.15	0.01	x	
	T. trachurus	0.59	0.01	0.42	0.39	0.16	0.06	0.68	x
		E. encrasicolus	M. merluccius	M. poutassou	S. pilchardus	S. colias	S. scombrus	S. sprattus	T. trachurus
Sep	E. encrasicolus	x							
	M. merluccius	0	x						
	M. poutassou	0.04	0.36	x					
	S. pilchardus	0.81	0	0	x				
	S. colias	0.12	0.11	0.1	0.26	x			
	S. scombrus	0.12	0.28	0.29	0.25	0.6	x		
	S. sprattus	0.73	0	0.04	0.64	0.02	0.02	x	
	T. trachurus	0.65	0.02	0.08	0.56	0.13	0.13	0.49	x

Dark green and bold: significant overlap, light green: medium overlap, red: no overlap.
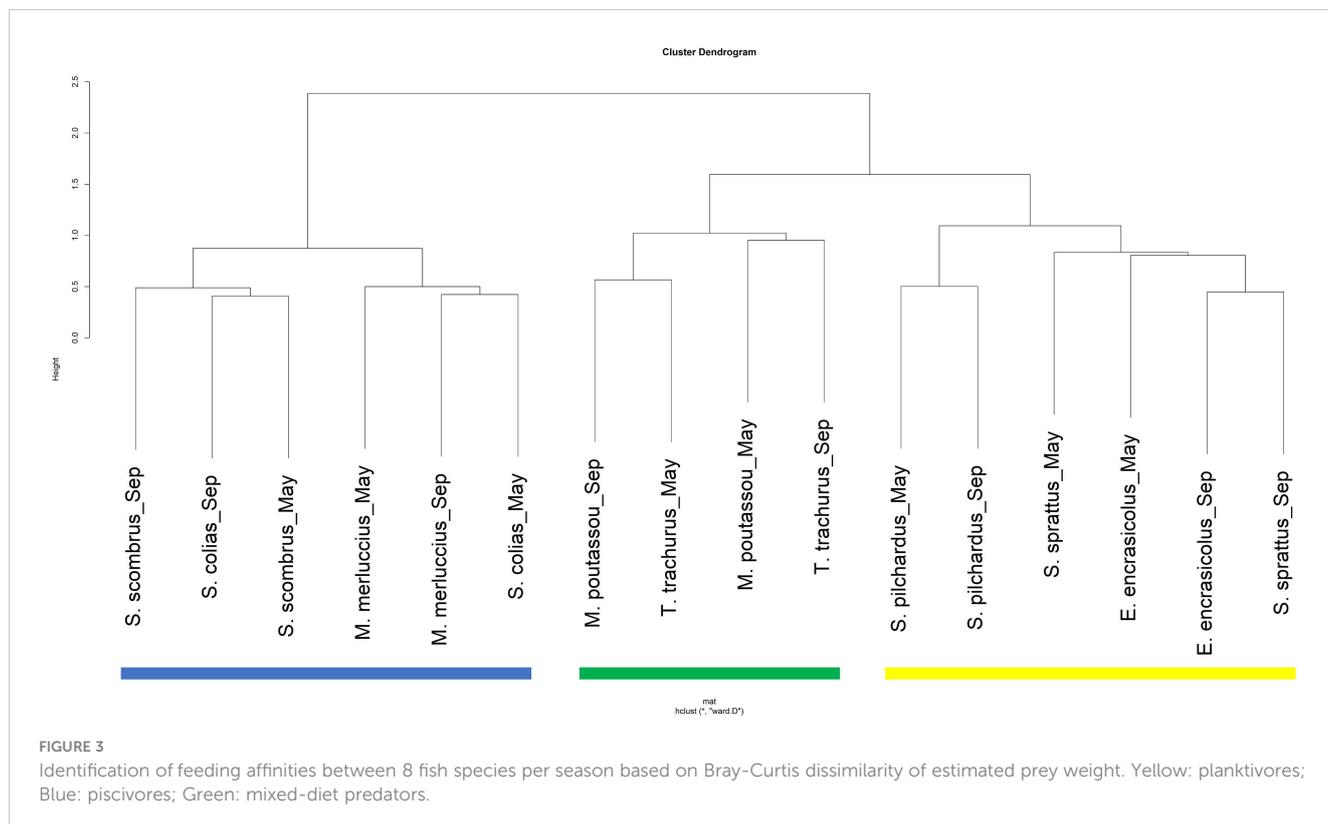

FIGURE 3

Identification of feeding affinities between 8 fish species per season based on Bray-Curtis dissimilarity of estimated prey weight. Yellow: planktivores; Blue: piscivores; Green: mixed-diet predators.

TABLE 3 Species contributing most to intra-group similarity resulting from the cluster analysis, with a 90% cut-off for low contribution.

Planktivores		Average similarity: 26.29			
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Copepod	0.02	15.20	1.39	57.82	57.82
Salp	0.03	5.34	0.54	20.31	78.13
Hyperiid	0.00	1.62	0.70	6.16	84.29
Decapod larvae	0.00	1.17	1.41	4.44	88.73
Euphausid larvae	0.00	0.90	1.12	3.41	92.14
Piscivores		Average similarity: 46.10			
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Anchovy	1.93	40	4.10	86.76	86.76
Salp	0.31	4.79	0.77	10.39	97.14
Mixed-diet predators		Average similarity: 15.26			
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Krill	0.57	8.67	0.55	56.81	56.81
Anchovy	0.19	4.40	0.59	28.81	85.62
Hyperiid	0.03	1.03	0.76	6.72	92.34

than in late summer using both approaches for all predator species, except for *S. colias* and *S. scombrus* in the TP_{SCA} value and *C. aper* in the TP_{SIA} value (Figure 5). Overall, TP_{SCA} was consistently higher than TP_{SIA}, with the exception of *T. trachurus* in spring and *S. colias* and *S. scombrus* in late summer (Table S3).

Based on the results of the best modelling of the TP_{SIA} with size (Table 5), the TP was influenced by the additive effect of the predator's size and the season, carrying 96% of the cumulative model weight. The TP_{SCA} however, it was influenced by the interaction of the season and the size (Table 5), with a cumulative model weight of 99%. TP increased in late summer using both approaches (see 'Estimate' in Table 6 and Figures S1, S2). The TP_{SIA} increased with predator size (see 'Estimate' in Table 6 and Figure S1). The TP_{SCA} increased with predator size in May, whereas in September it decreased slightly (see 'Estimate' in Table 6 and Figure S2).

Attending the random effect of predator species, the estimated variability in the intercept of the random effect was close to zero in both methods, indicating that predator species were not important in the model (see 'Variance' in Table S4). There was no general pattern of the TP variation between species.

4 Discussion

Our results confirm that seasonal variability has a significant impact on predator-prey interactions and trophic structure of the pelagic realm of the BoB. For the first time, we demonstrate this influence with a multispecies approach and using two complementary analyses (diet microscopy and stable isotopes) which give a more robust overview of the structure and functioning of the BoB pelagic ecosystem.

Contrary to our expectations, diet of pelagic fish was determined more by intra-specific affinities than by seasonal variations. The three trophic guilds identified in the present study (planktivores, piscivores and mixed-diet predators) showed a high proportion and contribution of pelagic sources to the diet of pelagic fish. Copepods constituted the most important food source for half of the fish species (anchovy, sardine, sprat and horse mackerel) in both seasons, which is in accordance with other authors findings (Bachiller and Irigoien, 2015). The relative importance of copepods' consumption was higher in spring than in late summer for most of fish species, coinciding with the peak of abundance of copepods in the water column (Albaina and Irigoien, 2007); in late summer, larger prey such as krill were found in higher numbers in stomachs of blue whiting, and the same could be said regarding salps found in Atlantic mackerel, also being described by other authors in the study area (Olaso et al., 2005). Interestingly, the diet of anchovies and sardines showed the opposite trend, increasing the consumption of copepods in late summer, even though fewer copepods were presumably available (Albaina and Irigoien, 2007). This feeding behavior confirms that anchovies and sardines exhibit a clear prey preference for copepods, as it has already been demonstrated by other authors (Plounevez and Champalbert, 1999; Garrido et al., 2007). Copepods are indeed the dominant taxa in the offshore zooplankton communities of the Bay of Biscay during most of the year, with exception of early spring. However, larval stages of euphausiids and brachyuran, gelatinous organisms such as jellyfish, siphonophores and tunicates or fish eggs are also common within the size range of mesozooplankton within the BoB (Poulet et al., 1996), with meroplankton being more prevalent during the spring bloom (Bode et al., 2011b). In fact, an important
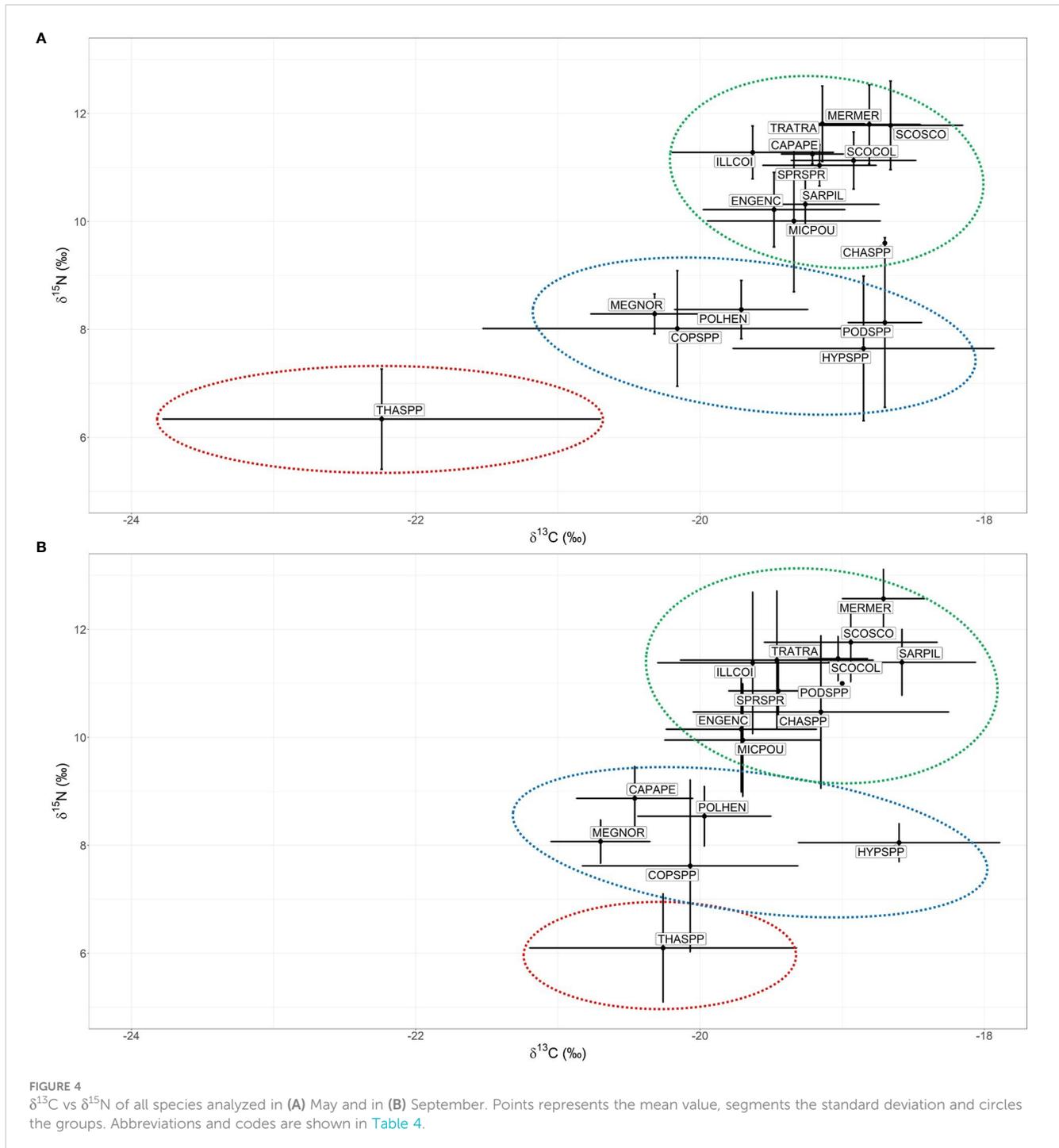
TABLE 4   Mean (δ¹⁵N and δ¹³C) and standard deviation (SD) of isotopic values, number of samples of the different groups analyzed for stable isotopes per season (N) and trophic position (TP) for each analysis method (SIA and SCA).

Group	Species	Code	Month	Size range (cm)	N	$\delta^{13}C$	$\delta^{15}N$	TP$_{SIA}$	TP$_{SCA}$
Fish	*Capros aper* (Boarfish)	CAPAPE	May	9-15	10	-19.21 ± 0.22	11.25 ± 0.18	3.13	–
			Sep	6-9.5	24	-20.46 ± 0.41	8.87 ± 0.59	2.69	–
	Engraulis encrasicolus (anchovy)	ENGENC	May	9.5-15.5	68	-19.48 ± 0.5	10.22 ± 0.69	2.82	3.10
			Sep	4-15	47	-19.71 ± 0.53	10.15 ± 1.16	2.96	3.38
	Merluccius merluccius (hake)	MERMER	May	19-45	16	-18.81 ± 0.36	11.8 ± 0.73	3.24	3.93
			Sep	14-51	11	-18.71 ± 0.29	12.57 ± 0.54	3.67	3.96
	Micromesistius poutassou (blue whiting)	MICPOU	May	7.5-31	24	-19.34 ± 0.61	10.01 ± 1.31	2.71	3.29
			Sep	12.5-17.5	6	-19.7 ± 0.55	9.95 ± 1.04	3.05	3.54
	Sardina pilchardus (sardine)	SARPIL	May	10.5-20	43	-19.26 ± 0.52	10.32 ± 0.49	2.86	3.37
			Sep	10.5-19	30	-18.58 ± 0.52	11.39 ± 0.61	3.28	3.37
	Scomber colias (Atlantic chub mackerel)	SCOCOL	May	19.5-34.5	13	-18.92 ± 0.44	11.13 ± 0.53	3.09	3.29
			Sep	10-27.5	20	-19.03 ± 0.21	11.46 ± 0.41	3.22	3.19
	Scomber scombrus (Atlantic mackerel)	SCOSCO	May	19-37.5	17	-18.66 ± 0.51	11.78 ± 0.82	3.26	3.62
			Sep	11-29.5	21	-18.94 ± 0.61	11.76 ± 0.73	3.45	3.25
	Sprattus sprattus (sprat)	SPRSPR	May	10.5-12.5	17	-19.16 ± 0.40	11.04 ± 0.38	3.03	3.13
			Sep	5.5-9	13	-19.45 ± 0.35	10.86 ± 0.57	3.22	3.33
	Trachurus trachurus (Atlantic horse mackerel)	TRATRA	May	8-31.5	38	-19.14 ± 0.30	11.81 ± 0.7	3.36	3.27
			Sep	5-31	35	-19.46 ± 0.68	11.43 ± 1.28	3.33	3.38
Squid	*Illex coindetii* (broadtail shortfin squid)	ILLCOI	May	6-18.3	35	-19.63 ± 0.57	11.28 ± 0.49	3.23	–
			Sep	3.1-15.6	65	-19.63 ± 0.67	11.38 ± 1.31	3.33	–
Krill	*Meganyctyphanes norvegica* (Northern krill)	MEGNOR	May	3.5-4.3	20	-20.32 ± 0.45	8.29 ± 0.37	2.35	–
			Sep	2.5-4.1	34	-20.7 ± 0.35	8.07 ± 0.4	2.37	–
Crab	*Polybius henslowii* (Henslow's swimming crab)	POLHEN	May	3.2-4.6	23	-19.71 ± 0.47	8.37 ± 0.54	2.36	–
			Sep	4-5.1	18	-19.97 ± 0.47	8.54 ± 0.55	2.56	–
Zooplankton	Chaetognatha sp (chaetognath)	CHASPP	May	–	1	-18.7	9.6	2.67	–
			Sep	–	4	-19.15 ± 0.9	10.47 ± 1.41	3.06	–
	Copepoda sp (copepod)	COPSPP	May	–	20	-20.16 ± 1.37	8.02 ± 1.07	2.29	–
			Sep	–	19	-20.07 ± 0.76	7.62 ± 1.59	2.39	–
	Hyperiidea sp (hyperiid)	HYPSPP	May	–	2	-18.85 ± 0.92	7.65 ± 1.34	2.21	–
			Sep	–	2	-18.60 ± 0.71	8.05 ± 0.35	2.63	–
	Podonidae sp (cladoceran)	PODSPP	May	–	3	-18.70 ± 0.26	8.13 ± 1.57	2.32	–
			Sep	–	1	-19.00	11	3.06	–
	Thaliacea sp (salp)	THASPP	May	–	5	-22.24 ± 1.54	6.34 ± 0.93	2	–
			Sep	–	8	-20.26 ± 0.94	6.1 ± 1	2	–

The common name and code of the analyzed species is provided.

ingestion of fish eggs was also observed in some species in spring, e.g., anchovy eggs ingested by sardines, also observed by (Bachiller et al., 2015), coinciding with the spawning season of some species, e.g., anchovies, sardines and Atlantic mackerel (Motos et al., 1996; Olaso et al., 2005; Bellier et al., 2007). Seasonal succession in the copepod community is also apparent; in the western edge of the BoB *Pseudocalanus elongatus* and *Acartica clausii* predominate in spring, while early stages of *Calanus helgolandicus* and *Oncaea media* are more abundant in late summer-autumn (Bode et al., 2011b). However, the taxonomic resolution of the present study

FIGURE 4
δ^{13}C vs δ^{15}N of all species analyzed in **(A)** May and in **(B)** September. Points represents the mean value, segments the standard deviation and circles the groups. Abbreviations and codes are shown in Table 4.
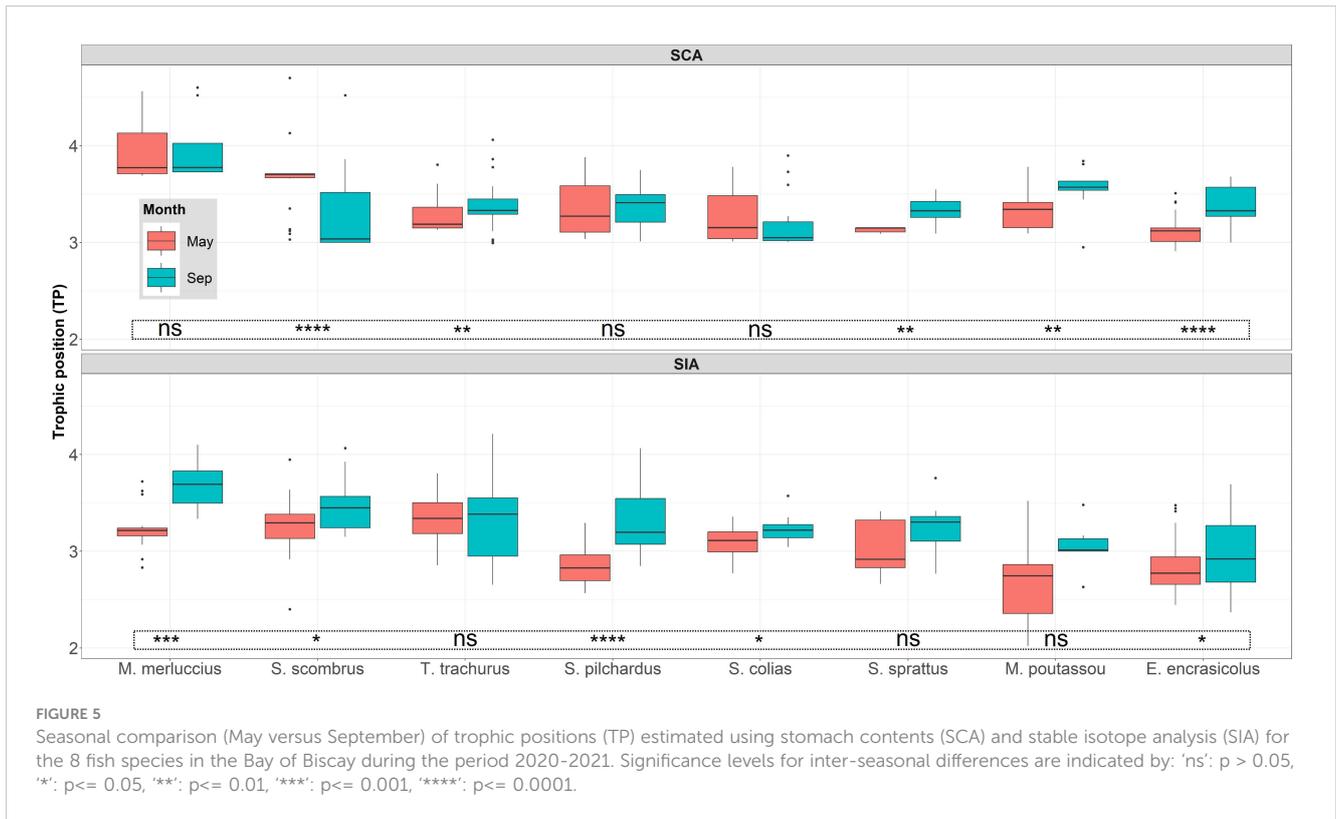
prevented the detection of shifts in plankton composition at the species level.

Among the mixed-diet species, blue whiting showed a significant seasonal change in the diet, feeding primarily on copepods in spring, while on *M. norvegica* (krill) in late summer. This seasonal change in diet, which shifts the main prey from meso- to macrozooplankton species, could be attributed to variations in prey abundances but also suggests a preference for this prey which could respond both to its larger size and/or shoaling behavior. Krill typically performs daily vertical migrations shoaling close to the bottom over the continental shelf during daytime and near the surface at dawn (Mauchline and

Fisher, 1967). Although the importance of krill in blue whiting's diet has also been observed in other regions (Cabral and Murta, 2002; Bachiller et al., 2016; López-López et al., 2017), seasonal variations in the diet are reported here for the first time. A high proportion of copepods was observed in horse mackerels' stomachs in both seasons, which is in line with previous observations (Bachiller and Irigoien, 2015), as well as the low proportion of anchovies in their diet (Olaso et al., 1999).

Our study confirms the piscivore behavior of European hake adults, which fed mainly on anchovies in both seasons. In other studies carried out in the BoB and surrounding regions however,

FIGURE 5
Seasonal comparison (May versus September) of trophic positions (TP) estimated using stomach contents (SCA) and stable isotope analysis (SIA) for the 8 fish species in the Bay of Biscay during the period 2020-2021. Significance levels for inter-seasonal differences are indicated by: 'ns': p > 0.05, '*': p<= 0.05, '**': p<= 0.01, '***': p<= 0.001, '****': p<= 0.0001.

blue whiting, horse mackerel and silvery pout were the most important prey species for hake (Velasco and Olaso, 1998; Cabral and Murta, 2002; Mahe et al., 2007). This difference in the diet could be attributed to the fishing depth at which hake were sampled (above 130m), where anchovies can be found more frequently than blue whiting (Velasco and Olaso, 1998) and to the spatial extent of the study area overlapping with the main recruitment areas for anchovy in the BoB (López-López et al., 2011). Cannibalism was also observed in hake's diet in concurrence with previous studies (Velasco and Olaso, 1998; Cabral and Murta, 2002; Mahe et al., 2007; Preciado et al., 2015). The size range sampled (with full

stomachs) in the present study (> 20cm) possibly prevented us to identify macrozooplankton as an essential part of hake diet, as this species shows an ontogenic change in diet (Velasco and Olaso, 1998), with only juveniles smaller than 19 cm being planktivores. Salps and anchovies were the main prey for Atlantic mackerel and Atlantic chub mackerel, in contrast to that observed in other studies where decapods, krill, copepods or blue whiting were the most important food source (Cabral and Murta, 2002; Olaso et al., 2005; Bachiller and Irigoien, 2015; Kvaavik et al., 2019). Olaso et al. (2005) however reported salps as important source of food for Atlantic mackerel in late summer-autumn in the BoB.

TABLE 5 Results of the GLMM models for the TP estimation for each method.

Method	Model	df	logLik	AIC	ΔAIC	Weight
SIA	**Size+Season**	**5**	**-86.34531**	**182.6906**	**0**	**0.9640046**
	Size*Season	6	-88.63301	189.2660	6.575411	0.03599537
	Season	4	-105.53661	219.0732	36.382619	1.21E-8
	Size	4	-118.81265	245.6253	62.934692	2.08E-14
	Null	3	-124.01235	254.0247	71.334086	3.12E-16
SCA	**Size*Season**	**6**	**-31.60663**	**75.36939**	**0**	**0.9998902**
	Season	4	-43.64337	95.36082	19.99144	4.56E-5
	Null	3	-44.67769	95.39974	20.03036	4.47E-5
	Size+Season	5	-43.53427	97.17985	21.81046	1.84E-5
	Size	4	-47.37337	102.82081	27.45142	1.09E-6

Selected models are in bold.
* means interaction.

TABLE 6 Summary statistics of the selected models in Table 5 for explaining TP based on each method.

Method	Effect	Estimate	Std. Error	t value
SIA	Size	0.01922	0.00266	7.225
	SeasonMay	2.69402	0.07698	34.996
	SeasonSep	2.95170	0.07291	40.485
SCA	Size	0.014963	0.002528	5.918
	SeasonMay	3.091455	0.088240	35.035
	SeasonSep	3.466281	0.087683	39.532
	Size : SeasonSep	-.018928	0.003211	-5.898

Attending to the dietary overlap among species, high potential competition for food source was evident between planktivore species especially in late summer, since all of them fed mainly on copepods. As shown in previous studies (Raab et al., 2011; Bachiller and Irigoien, 2015; Le Bourg et al., 2015), the three clupeid species had a large diet overlap, and also with larger fish like Atlantic horse mackerel. The trophic overlap among piscivore species (European hake and Atlantic mackerel) was also significant since both species fed mainly on anchovies. Diet overlap between the two species of scombrids was mainly due to the high presence of salps in their diet. However, as gelatinous prey experience a rapid digestion, their contribution to the diet using microscopy analyses is likely underestimated (Arai, 2005). The seasonal variation in the diet of blue whiting (no anchovies in spring, but in late summer) led to a medium overlap with hake in September indicating generally a high resource partitioning and low potential competition between these two species.

The seasonal changes detected in species diets did not respond to a change in the community structure, which displayed a similar structure between seasons with salps at the base of the food web (TP around 2), a second group primarily composed of meso and macrozooplankton and a third group of fish and squids with the highest TPs. Nevertheless, the whole community showed consistently lower TPs in spring, regardless of the trophic levels, which could be explained by the shortened trophic chains that dominate the pelagic pathway under high primary productivity pulses, such as those experienced during this season (Calbet, 2001; Jennings and Mackinson, 2003). Seasonal variations in trophic positions are likely caused by changes in prey availability and secondarily by the changes in feeding behaviour of each prey species which ultimately are largely influenced by changes in primary production. An enhanced input of nutrients (e.g., river discharge, upwelling) may trigger a bottom-up cascade effect by increasing primary production and consequently phytoplankton and zooplankton biomasses and/or changes in their associated diversity that will affect the upper trophic positions (Checkley et al., 2009; García-Comas et al., 2016).

Attending to Søreide et al. (2006) classification, all fish and squid species were found to have a carnivorous diet ($TP_{SIA} \geq 2.8$) showing the highest TP_{SIA} in both seasons. The TP_{SIA} values of blue whiting were relatively low in comparison with other studies (Pétursdóttir et al., 2012; Mir-Arguimbau et al., 2020), possibly

due to the high consumption of copepods in the present study. Squids also showed high TP_{SIA} in both seasons in agreement with previous studies, which characterize these species as meso- and top predators (Lassalle et al., 2014; Albo-Puigserver et al., 2016). Secondary consumers with mid TP species such as *M. norvegica* and the pelagic crab *P. henslowii* displayed significant lower values in spring, once again pointing to a more direct trophic transfer along the food web during this season (Calbet, 2001; Jennings and Mackinson, 2003). The TP_{SIA} showed by *M. norvegica* ($TP_{SIA} < 2.4$) suggests a herbivorous diet, probably composed of the dominant species of diatoms and dinoflagellates, whereas other studies showed an omnivorous feeding strategy (Pétursdóttir et al., 2008; Agersted et al., 2014; López-López et al., 2017). Similar discrepancies occurred with the pelagic crab *P. henslowii* with TP_{SIA} (< 2.6) much lower than those expected for a carnivore species (Signa et al., 2008). European hake showed the highest TP_{SCA} in both seasons likely due to their piscivorous diet based on anchovies. On the contrary, anchovies and the two species of scombrids displayed the lowest TP_{SCA} most probably due to the consumption of gastropods and salps in spring and late summer, respectively. For those species which had a TP_{SIA} and a TP_{SCA} estimate available, the stable isotopes estimate was consistently lower than that based on the microscopy of the diet. This discrepancy could be attributed to many different factors, including an overestimation of the TP of prey items (using either SIA or published estimates) when calculating TP_{SCA}, to the availability of prey with lower TPs in the weeks-months previous to the sample, or to inaccurate estimation of the SIA baseline and/or enrichment factors. It should be noted, however, that TP_{SIA} and TP_{SCA} represent different temporal resolution, giving thus a complementary perspective (assimilated food vs. snapshot respectively) of the feeding behaviour of the species.

Differences between TP_{SIA} and TP_{SCA} persisted when analyzing how these estimates varied across fish size. According to the results of the linear mixed model, the TP_{SIA} was influenced by the additive effect of the predator's size and season, whereas the interaction between season and size was affecting the TP_{SCA}. TP_{SCA} in May and TP_{SIA} showed a positive relationship with body-size suggesting a dietary change with ontogeny, which could be attributed to either an increase in the ingestion of prey species with higher TPs, or to larger prey within the same species, not

requiring in this latter case a taxonomic change in the diet (Deudero et al., 2004; Jennings et al., 2007; Ou et al., 2017). Although the general model stated an increase of TP_{SIA} with size and, also, for TP_{SCA} in May, such positive relationship was not evident for all species. European hake is well known to display ontogenic changes in diet which represent an increase in trophic position with size (Badalamenti et al., 2002; Le Loc'h and Hily, 2005). In contrast, a negative or null increase of the trophic position with size was found in planktivore species, such as sardine (Bode et al., 2007), which suggests the absence of ontogenic changes in the diet, which is corroborated by our diet results. Overall, these outcomes should be interpreted with caution since the size ranges of some species could be undersampled in the present study, and this has been suggested a crucial aspect to interpret changes in trophic position along species ontogeny (Galván et al., 2010).

Therefore, the resulting trophic groups in the pelagic fish community of the BoB were determined more by intra-specific feeding affinities than by seasonal differences, as the trophic structure was similar in both seasons. The trophic position of the analyzed species was consistently lower in spring than in late summer. To better understand the seasonal food web dynamics in the pelagic ecosystem of the BoB, longer time series are needed. Our study reflects the importance of analyzing the seasonal changes in diet using different techniques, since each approach provides different information. Taking advantage of the multidisciplinary oceanographic surveys that are conducted in the BoB throughout the year, the monitoring of systematic sample collection of pelagic components would allow the study of temporal (seasonal and annual) and spatial differences in pelagic trophic interactions. It would also allow the exploration of potential changes in the ecosystem under different stressors (e.g., changes in fishing pressure or climate change), as changes in this trophic guild of small pelagics have a major impact on other species in the ecosystem, thus moving towards an ecosystem-based management.

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 study on animals in accordance with the local legislation and institutional requirements

Author contributions

BI, IP, ML, MS conceived and designed the study. BI did the lab work and performed the analyses. BI, IP and ML wrote the first draft of manuscript. EB, LL-L, MS, GB, EA and UC provided feedback on the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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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.2023.1185376/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Model visualization for the TP_{SIA}. The effect of the predator's size can be observed in the left and the effect of the season in the right.

SUPPLEMENTARY FIGURE 2
Model visualization for the TP_{SCA}. The effect of the interaction between predator's size and season can be observed.

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