

Feeding ecology of the highly threatened common bottlenose dolphin of the Gulf of Ambracia, Greece, through stable isotope analysis

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Abstract

The Gulf of Ambracia, in northwestern Greece, hosts a highly threatened community of about 150 common bottlenose dolphins (*Tursiops truncatus*). Until now, information on their feeding habits was derived exclusively from fish scale samples collected during surface-feeding events targeting small schooling epipelagic fish. The aim of this study was to determine the diet of bottlenose dolphins living in the Gulf of Ambracia through the application of Bayesian isotopic mixing models. Skin biopsy samples of 16 dolphins were analyzed and no difference related to sex or age-class was found in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Results suggested that the dolphin diet was mainly based on *Trachurus trachurus*, species belonging to the family Sparidae: *Diplodus annularis*, *Lithognathus mormyrus*, and *Sepia officinalis*, which represented together about $42\% \pm 15\%$ of the biomass ingested, followed by species belonging to the order Clupeiformes (*Engraulis encrasicolus*, *Sardinella aurita*, and *Sardina pilchardus*) and the genus *Gobius* ($37\% \pm 17\%$). A better understanding of the feeding habits of these dolphins sheds light on the feeding ecology

of this highly threatened population by, for instance, evidencing interactions with artisanal fisheries sharing the same target species, and is key for identifying adequate management measures consistent with an ecosystem-based approach.

KEYWORDS

diet, Mediterranean, mixing models, stable isotopes, *Tursiops truncatus*, $\delta^{13}\text{C}$ values, $\delta^{15}\text{N}$ values

1 | INTRODUCTION

The common bottlenose dolphin, *Tursiops truncatus* (hereafter referred to as bottlenose dolphin) is a cosmopolitan species that inhabits temperate and tropical marine waters and a variety of marine and estuarine habitats (Wells & Scott, 2018). Despite feeding habits of cetaceans being markedly difficult to observe, due to their high mobility and the high proportion of time spent underwater, bottlenose dolphins are well known for their foraging diversity, which can be population- or site-specific (Sargeant, Mann, Berggren, & Krützen, 2005). Bottlenose dolphins are frequently described as opportunistic feeders due to their capacity to feed mainly on the most available prey resources (Bearzi, Fortuna, & Reeves, 2009; Santos, Fernandez, López, Martínez, & Pierce, 2007).

This species is also able to exploit prey somehow facilitated by human activities. Bottlenose dolphins are frequently reported swimming behind fishing trawlers and feeding on discarded bycatch as well as around demersal trawlers (Gonzalvo, Valls, Cardona, & Aguilar, 2008; Scheinin, Kerem, Lojen, Liberzon, & Spanier, 2014), actively depredating set nets (Bearzi et al., 2009; Brotons, Grau, & Rendell, 2008; Díaz López, 2006; Gonzalvo, Giovos, & Moutopoulos, 2015), or foraging near fish farms (Bonizzoni et al., 2013; Díaz López, 2012; Piroddi, Bearzi, & Christensen, 2011), which may provide them a more energetically efficient food resource.

The analyses of bottlenose dolphins' stomach contents show that their diet mainly consists of demersal fish (Blanco, Salomón, & Raga, 2001; Santos et al., 2007), but crustaceans, cephalopods, and other invertebrates are also present in some dolphin populations (e.g., Santos, Clarke, & Pierce, 2001). In the Mediterranean and the Black sea, the main prey reported for the bottlenose dolphin include demersal resources such as *Merluccius merluccius*, *Conger conger*, *Ophidion* spp., and *Boops boops*, but also small pelagic resources such as *Engraulis encrasicolus*, *Sardina pilchardus*, *Trachurus* spp., *Sardinella aurita*, and *Mugil* spp., among others (Blanco et al., 2001; Gladilina & Gol'din, 2014; Milani et al., 2018; Scheinin et al., 2014).

While some studies indicate that the stomach contents from stranded dolphins are suitable for diet analyses (Dunsha et al., 2013), other studies highlight the limitations of this technique. The remains found in the gut may be biased due to differences in prey retention, digestion, and degradation rates of different organisms (Bowen & Iverson, 2013; Kaschner, Karpouzi, Watson, & Pauly, 2006; Santos et al., 2001). Moreover, these remains can only be associated with the feeding events taking place just before the dolphin's death, and thus provide information only about the food ingested on a short timescale (e.g., Giménez et al., 2017).

In contrast, the analysis of stable isotopes (SIA) ratios of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) of animal tissues is broadly used for exploring diet, feeding habitats, and trophic position of animals, including in marine mammals (Newsome, Clementz, & Koch, 2010). This is possible because the stable isotope composition of the consumer tissues reflects the one of the assimilated prey, providing indication of the averaged diet of the consumer, at least over the time needed to synthesize the tissue examined. Thus, the isotopic ratios of both consumer and possible prey can be interpreted through mathematical mixing models to determine the relative contribution of a variety of prey to the

consumer's diet (Hopkins & Ferguson, 2012; Phillips, 2012; Phillips & Gregg, 2003). Although the analysis of diet through SIA is somewhat limited when the potential prey species have similar isotopic values, since it cannot discriminate between them, this method plays an important role in the validation of independent diet estimates obtained through different methods (Bowen & Iverson, 2013).

In Greek waters, the bottlenose dolphin is found in coastal areas, including both rugged coastlines with no continental shelf (Bearzi et al., 2005; Bearzi, Agazzi, Bonizzoni, Costa, & Azzellino, 2008; Frantzis et al., 2003) and semi-enclosed gulfs of eutrophic waters, such as the Gulf of Ambracia (Bearzi et al., 2008; Gonzalvo et al., 2016).

The Gulf of Ambracia hosts one of the highest densities (0.37 animals/km²) of bottlenose dolphins observed in the Mediterranean Sea (Bearzi et al., 2008). Total population is estimated at about 150 ± 20 animals (Gonzalvo et al., 2016) and no other cetacean species has been reported in the area. While local density of dolphins is among the highest recorded anywhere in the Mediterranean Sea, bottlenose dolphins in the Gulf of Ambracia may be at risk due to their likely reproductive isolation, small population size, and small extent of occurrence. In addition, the Gulf is far from pristine and there is increasing acute anthropogenic impacts in this semiclosed shallow habitat (Gonzalvo et al., 2016), including locally intense small-scale fisheries, operating primarily with trammel nets (Gonzalvo et al., 2015). Such interactions may occasionally lead to incidental bycatch as well as to retaliation measures by fishermen that identify dolphins as direct competitors (Bearzi et al., 2008; Reeves, Read, & di Sciara, 2001).

This study aims to determine the diet of the bottlenose dolphin population inhabiting the Ambracia Gulf by means of the analysis of stable isotopes of carbon and nitrogen in skin samples of free-ranging individuals and the application of Bayesian isotopic mixing models. Understanding dietary preferences and feeding habits of bottlenose dolphin could shed light on the overlap with the fisheries operating in the area, providing useful information for management and conservation purposes.

2 | MATERIAL AND METHODS

2.1 | Study area

The Gulf of Ambracia, also referred to as Gulf of Amvrakikos (Figure 1), is a shallow, semiclosed embayment of 405 km² whose only communication with the open Ionian Sea is through the Preveza Channel, a narrow (minimum width of 370 m) and shallow (2–12 m deep) 3-km-long corridor. On average, the depth of the Gulf is approximately

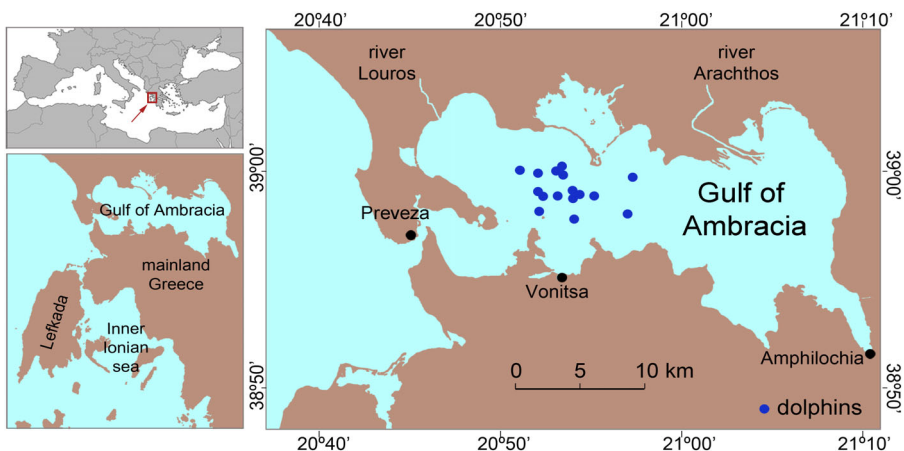


FIGURE 1 Map of the Gulf of Ambracia. Blue dots correspond to the dolphin sampling locations.

30 m (maximum 60 m deep), and its bottom mostly consists of mud or sand. The northern side of the Gulf, a complex ecosystem, is composed of a double delta from the Arachthos and Louros Rivers and their associated marshes and lagoons, which are of particular importance for bird diversity (Ferentinos et al., 2010). The whole Gulf of Ambracia is a Natura 2000 site (GR2110001) and an Important Marine Mammal Area (IMMA; Hoyt, 2018), while the northern part is also a Special Protection Area (GR2110004) and a Ramsar site, and is protected by national, European, and international regulations. In 2008 it was designated as a National Park in accordance with the Greek national legislation (11989/08 KYA).

Commercial fisheries operating in the study area include only small-scale fisheries, working mainly with set nets, which comprise 280 boats fishing exclusively inside the Gulf. Their main target species are: *S. pilchardus*, *Mullus barbatus*, *Lithognathus mormyrus*, *Penaeus kerathurus*, *Sepia officinalis*, and species from the family Mugilidae and the genus *Solea* (European Commission, 2009; Piroddi, Moutopoulos, Gonzalvo, & Libralato, 2016).

2.2 | Sampling

Skin samples were obtained through remote biopsy sampling of 16 free-ranging bottlenose dolphins in the Gulf of Ambracia during summer 2013. All 16 biopsied dolphins were photo-identified during the sampling process, making it possible to classify them by age groups. Animals that were identified as newborns/calves in 2008/9 were classified as juveniles (i.e., younger than 5 years; $n = 6$), while individuals older than 5 years were classified as adults ($n = 10$; unknown $n = 1$). Sex of dolphins (6 females and 10 males) was determined by genetic analyses (Gonzalvo et al., 2016; Table 1). Figure 1 shows the area location where dolphins were sampled.

Because bottlenose dolphins exploit different food resources, depending on their availability in different geographical areas, potential prey species were collected directly from local fishermen at landing sites around the study area during September 2013. All species were captured inside of the Gulf of Ambracia, including one species of cephalopod (*S. officinalis*), and 11 species of fish (*E. encrasicolus*, *S. aurita*, *S. pilchardus*, *M. barbatus*, *Diplodus annularis*, *L. mormyrus*, *Mugil cephalus*, *Sparus aurata*, *Trachurus trachurus*, and *Solea solea*, *Gobius* spp.). As no previous studies on

TABLE 1 Biological data and results from N and C stable isotope analyses of the dolphins analyzed.

Sample #	Sampling date	Sex	Age class	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	%C	%N
1	July 2, 2013	M	juvenile	-17.46	14.92	46.45	16.29
2	July 2, 2013	F	juvenile	-19.66	13.38	45.88	15.16
3	July 3, 2013	M	adult	-19.85	13.16	46.02	15.36
4	July 3, 2013	M	adult	-19.31	13.71	29.62	9.90
5	July 3, 2013	F	juvenile	-18.76	14.47	46.16	15.99
6	July 4, 2013	M	adult	-17.34	14.85	46.23	16.61
7	July 5, 2013	M	juvenile	-20.05	13.92	47.72	15.69
8	July 5, 2013	F	juvenile	-20.29	13.22	45.94	14.99
9	July 6, 2013	M	adult	-18.81	12.83	47.13	16.16
10	July 6, 2013	M	adult	-17.96	14.51	45.12	16.09
11	July 7, 2013	F	adult	-18.53	13.92	46.77	16.20
12	July 7, 2013	M	unknown	-18.46	14.91	46.54	16.02
13	July 7, 2013	F	adult	-18.88	14.01	46.57	15.46
14	July 8, 2013	M	adult	-16.44	15.46	46.12	16.73
15	July 8, 2013	F	adult	-20.26	13.64	45.67	15.01
16	July 8, 2013	M	juvenile	-20.30	13.07	46.32	15.04

stomach content had been done in this population of dolphins, special care was taken in sampling the most frequent fish in the area and in securing the inclusion of species previously reported in the stomach contents of bottlenose dolphins in the Mediterranean and the adjacent seas. (e.g., Blanco et al., 2001; Dos Santos, Coniglione, & Louro, 2007; Giménez et al., 2017; Gladilina & Gol'din, 2014; Milani et al., 2018; Scheinin et al., 2014). For each prey species, five individuals were sampled by collecting muscle/mantle tissue. All samples were preserved frozen until analysis.

Fish were not sized before analysis, but special care was taken to select individuals of common length, which in most cases was shorter than 35 cm (Table 1). Bottlenose dolphins can feed on a wide range of prey sizes but generally target prey larger than 15 cm (MacLeod, Santos, Lopez & Pierce, 2006). Hernandez-Milian, Berrow, Santos, Reid and Rogan (2015) found that Irish bottlenose dolphins fed on fish 10–100 cm long, most of which were shorter than 30 cm, as were the majority of the fish analyzed in the current study. Similarly, Blanco et al. (2001) found that hake from the stomach contents of western Mediterranean bottlenose dolphins, ranged between 10 and 50 cm in length, with 80% of samples ranging between 15 and 30 cm.

2.3 | Sample preparation and stable isotope analysis

Prior to stable isotope analysis, subsamples of each sample of dolphin skin, fish muscle, and cephalopod mantle were dried in an oven at 60°C for 48 hr and ground to a fine powder with mortar and pestle. Because lipids, compared with other biomolecules, are depleted in ^{13}C , and may affect the analytical results by decreasing the overall sample $\delta^{13}\text{C}$ value (De Niro & Epstein, 1977), all samples were treated with a 2:1 chloroform-methanol solution for lipid extraction. The C/N ratio for all samples varied between 2.75 and 3.22 (mean \pm SD: 3.08 ± 0.12). These values (<4) show that the lipid extraction process in the samples was effective. Lipid extraction may alter $\delta^{15}\text{N}$ values, producing discrepant effects in different tissues (e.g., Cloyed, DaCosta, Hodanbosi & Carmichael, 2020; Giménez et al., 2017; Ingram et al., 2007; Murry, Farrell, Teece, & Smyntek, 2006). However, as we used discrimination factors obtained from lipid-extracted tissues of prey and consumers, the variation in $\delta^{15}\text{N}$ values due to lipid extraction did not influence the result of diet composition analyses.

For most potential prey species analyzed, the percentage of water and lipids was calculated by weighing samples before and after drying and before and after lipid extraction (Table 2) (e.g., Evanson, Bornhold, Goldblatt, Harrison, & Lewis, 2000).

After these treatments and subsequent solvent removal, approximately 0.5 mg of each skin sample, 0.25–0.3 mg of each fish sample, and 0.3–0.35 mg of each cephalopod sample were weighed in tin capsules, automatically loaded, and combusted at 1,000°C to be analyzed in a continuous-flow isotope ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA Thermo Finnigan) coupled with an elemental analyzer.

Carbon isotope ratios were reported relative to Vienna Pee Dee Belemnite limestone (VPDB) and nitrogen relative to atmospheric nitrogen, though secondary calibrated standards. The accuracy for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements were 0.2‰ and 0.3‰, respectively.

Results were expressed following the delta (δ) notation:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} (\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 10^3$$

where R is the heavy-to-light isotope ratio ($^{15}\text{N}/^{14}\text{N}$, $^{13}\text{C}/^{12}\text{C}$) in the sample and in the reference standards, respectively. Analyses were performed at the Centres Científics i Tecnològics (CCiT), University of Barcelona.

2.4 | Data analysis

As $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ dolphin values followed a normal distribution (Shapiro–Wilk test) and the relative influence on them of sex, age-class, and their interaction was assessed through general linear modeling (GLM; SPSS 20). Statistical significance was set at $p = .05$.

TABLE 2 Means and standard deviations of N and C isotopic ratios of each prey analyzed and the group where they were classified, together with their water and lipid content (fresh weight) and the literature-derived lengths of the sampled species extracted from Froese and Pauly (2019) (fish) and from Reid, Jereb, & Roper (2005) (*Sepia officinalis*).

Species	Common length (cm)	Maximum length (cm)	H ₂ O (%)	Lipids (%)	$\delta^{13}\text{C}$ (‰) mean \pm SD	$\delta^{15}\text{N}$ (‰) mean \pm SD	Group number
<i>Engraulis encrasicolus</i>	13.5	20	74.20	3.36	-20.75 ± 0.30	11.64 ± 0.52	1
<i>Sardinella aurita</i>	25	36	77.20	0.48	-20.64 ± 0.65	11.03 ± 0.84	1
<i>Sardina pilchardus</i>	20	27.5	70.26	7.29	-20.93 ± 0.86	11.54 ± 0.50	1
<i>Gobius</i> spp.	<10	18	—	—	-20.31 ± 0.73	11.30 ± 0.78	1
<i>Diplodus annularis</i>	13	27.5	71.02	5.94	-19.13 ± 0.79	12.61 ± 0.62	2
<i>Lithognathus mormyrus</i>	30	55	76.04	0.69	-18.58 ± 0.75	12.45 ± 0.54	2
<i>Trachurus trachurus</i>	22	70	62.40	3.52	-18.89 ± 0.63	13.08 ± 1.06	2
<i>Sepia officinalis</i>		49	73.11	0.58	-18.26 ± 0.75	11.61 ± 0.37	2
<i>Solea solea</i>	35	70	52.17	2.36	-18.97 ± 0.56	10.97 ± 0.74	3
<i>Mullus barbatus</i>	20	33.2	75.36	3.23	-18.85 ± 0.84	10.54 ± 0.45	3
<i>Sparus aurata</i>	35	70	72.32	4.51	-19.14 ± 0.68	10.18 ± 1.30	3
<i>Mugil cephalus</i>	50	100	—	—	-16.65 ± 1.43	10.75 ± 1.23	4

Because mixing models cannot discriminate prey items with similar isotope ratios, mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for each prey species were used to identify statistically different clusters of prey through a Ward's hierarchical cluster analysis based on Euclidean distance (Philips, 2012).

The relative contribution of each cluster of prey to the diet of the bottlenose dolphin was determined through Bayesian stable isotope mixing models, using the MixSIAR package (Stock & Semmens, 2016a; Stock et al., 2018) in R v.3.6.3 (R Core Team, 2020). Models were run using a "Process * Residual" error structure, as suggested by Stock and Semmens (2016b), and run using a generalist type prior, three Markov chain Monte Carlo (MCMC) chains of 1,000,000 draws and a burn-in of 500,000 draws. Convergence was checked using the Gelman-Rubin and Geweke diagnostics.

The data used to run the model were: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ individual values of the bottlenose dolphin skin samples; $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ individual values of prey samples, grouped according to the results from the clusters analysis; and the discrimination factors between potential prey and the consumer tissue estimated by Browning, Dold, Jack and Worthy (2014) in captive bottlenose dolphins ($1.9\text{‰} \pm 0.08\text{‰}$ for $\delta^{15}\text{N}$ and $0.66\text{‰} \pm 0.07\text{‰}$ for $\delta^{13}\text{C}$). These discrimination factors were considered the most appropriate for our model (Smith, Mazumder, Suthers, & Taylor, 2013), as they were close to those obtained in similar studies with other cetacean species (Borrell, Abad Oliva, Gómez-Campos, Giménez, & Aguilar, 2012; Rossman et al., 2015a), and they were calculated using a diet with a lipid content of 5%, which was similar to the lipid content of our potential prey (ranging from 0.48% to 7.29%; Table 2). Since discrimination factors depend largely on the lipid content of the diet (Browning et al., 2014), this factor gave even more confidence in the choice.

The mixing polygon simulation approach was applied to exclude consumers occurring outside the mixing polygon delimited by the isotopic values of the prey clusters and validate the selected mixing model (Smith et al., 2013).

3 | RESULTS

The isotopic ratios (mean values \pm SD: $-18.9\text{‰} \pm 1.7\text{‰}$ for $\delta^{13}\text{C}$, and $14\text{‰} \pm 0.8\text{‰}$ for $\delta^{15}\text{N}$), as well as the biological characteristics of the dolphins, are shown in Table 1. Since we did not find a significant effect of sex, age class, or

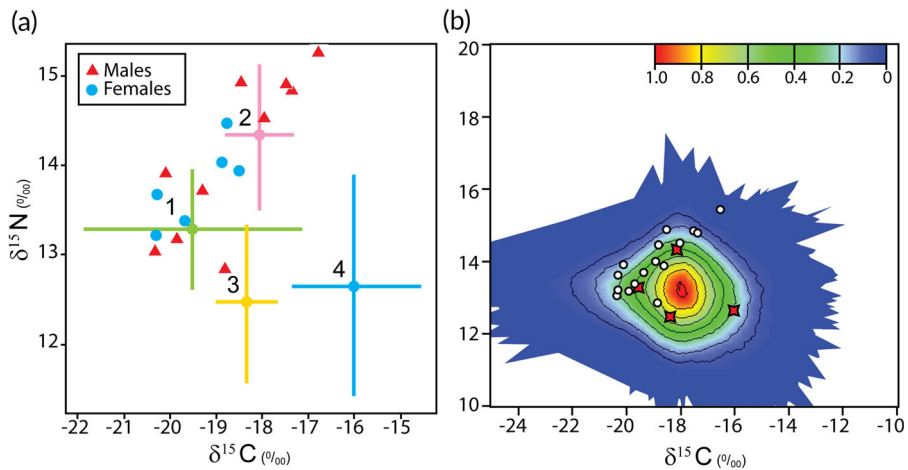


FIGURE 2 (a) Biplot of stable isotope ratios of bottlenose dolphins (split by sex) and potential prey groups, represented with the mean value of each group and the 95% confidence intervals, after correcting for the trophic discrimination factor. Group 1: *E. encrasicolus*, *S. aurita*, *S. pilchardus*, and *Gobius* spp.; Group 2: *D. annularis*, *L. mormyrus*, *T. trachurus*, and *S. officinalis*; Group 3: *S. solea*, *M. barbatus*, and *S. aurata*; Group 4: *M. cephalus*. (b) Mixing polygon for biplot a; bottlenose dolphins are represented with white dots and potential prey groups with red crosses. Probability contours are drawn every 10% level.

their interaction, on the isotope ratios of the dolphins (all $p > .05$), all samples were treated as a single group (see Figure 2a for sex and age class stable isotope distributions).

The isotopic ratios, water and lipid content, and the common and maximum length for each potential prey (Froese & Pauly, 2019; Reid, Jereb, & Roper, 2005) are shown in Table 2. The fish species that, according to their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively, shared a common food web base and similar trophic level, were combined by the cluster analysis in four groups: Group 1: *E. encrasicolus*, *S. aurita*, and *S. pilchardus* (belonging to the order Clupeiformes) and *Gobius* spp.; Group 2: *D. annularis* and *L. mormyrus* (belonging to the family Sparidae), *T. trachurus* and *S. officinalis*; Group 3: *S. solea*, *M. barbatus*, and *S. aurata*; and Group 4: *M. cephalus* (Table 2; see Figure 2a for the stable isotope values of each group of prey).

The mixing polygon simulation showed that the prey groups and the discrimination factors used for building the mixing model were valid, because all the consumer isotope ratios except one (which was excluded from the mixing model as an outlier) fell inside the 95% mixing region (Smith et al., 2013; Figure 2b).

The converged Bayesian mixing model, fitted with no variables, identified Group 2 and Group 1 as the main contributors to bottlenose dolphin diet, with mean contributions of $42\% \pm 15\%$ (mean \pm SD) and $37\% \pm 17\%$, respectively, followed by Group 3 ($14\% \pm 11\%$) and Group 4 ($7\% \pm 6\%$) (Figure 3).

4 | DISCUSSION

In recent decades, the analyses of carbon and nitrogen isotopes have been used effectively to reconstruct the diet of marine mammals (e.g., Fernández et al., 2011; Kiszka, Méndez-Fernandez, Heithaus, & Ridoux, 2014) and explore their trophic relationships (e.g., Gomez-Campos, Borrell, Cardona, Forcada, & Aguilar, 2011).

The isotope ratios of the bottlenose dolphins analyzed in this study showed large variation both in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Our results do not differ from those of other studies that report stable isotope ratios ranging from 2‰ to 5‰ in bottlenose dolphin individuals (e.g., Borrell et al., 2006; Browning et al., 2014; Kiszka et al., 2014; Lusseau & Wing, 2006). Large sample size in some dolphin populations highlighted small differences in stable isotope ratios

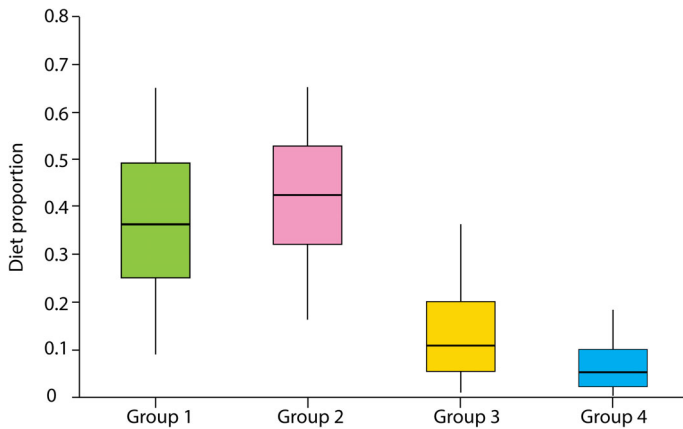


FIGURE 3 Dietary contribution of each potential prey group as estimated from the MixSIAR model outputs using stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values). Boxes and error bars represent 50% and 95% credible intervals, respectively. Group 1: *E. encrasicolus*, *S. aurita*, *S. pilchardus*, and *Gobius* spp.; Group 2: *D. annularis*, *L. mormyrus*, *T. trachurus*, and *S. officinalis*; Group 3: *S. solea*, *M. barbatus*, and *S. aurata*, Group 4: *M. cephalus*.

between age groups and sex (Fernandez et al., 2011; Rossman et al., 2015a), and some degree of specialization among individuals regardless of their biological characteristics (Rossman et al., 2015b). However, our analyses did not differentiate isotope ratios by age class or sex, probably due to the small number of samples, preventing the identification of the cause of such large variation among individuals.

As in many diet studies of cetaceans, skin was the tissue of choice because it is sampled using minimally invasive techniques, such as biopsy darting, which allows the collection of samples from free-ranging, healthy individuals, representative of the wild populations (Aguilar & Borrell, 1994; Aguilar & Nadal, 1984; Noren & Mocklin, 2012). Moreover, in a recent study, Arregui, Josa, Aguilar and Borrell (2017) showed that there are no isotopic differences among skin samples taken from different positions of the dolphin body, excluding possible differences in isotopic discrimination across the dolphin skin.

For the analyses of prey, muscle and mantle were selected because they are easier to sample and process in the laboratory than the entire prey and they are the tissues that most closely reflect the isotopic ratios of the whole prey (Kelly, 2000). Moreover, experimental studies in captive bottlenose dolphins suggest that the diet-to-skin discrimination values do not differ when analyzing only fish muscle or the whole fish (Giménez, Ramírez, Almunia, Forero, & de Stephanis, 2016).

The results of the mixing model showed that the bottlenose dolphins in the Gulf of Ambracia have a varied diet, dominated by the prey group including *T. trachurus*, the family Sparidae and *S. officinalis* (mean values \pm SD: 42% \pm 15%), followed by the group including species belonging to the order Clupeiformes and *Gobius* spp. (38% \pm 17%), which together make up 80% of the bottlenose dolphin diet. In contrast, *M. cephalus* represented <7% of the diet (Figure 3).

No precise information is available on the stock status of any of the fish species in the Gulf of Ambracia (European Commission, 2009). Nevertheless, fish farms, agriculture, livestock, and discharges of domestic sewage from coastal towns and villages contribute to the nutrient enrichment of the Ambracian waters, which are highly eutrophic (Ferentinos et al., 2010; Gonzalvo et al., 2015, 2016). The western part of the Gulf is seasonally hypoxic, while the eastern part is seasonally anoxic (Kountoura & Zacharias, 2013). These conditions have likely led to a reduction of the fish diversity in the Gulf, affecting primarily demersal species, while favoring small pelagic/epipelagic fish and lagoon-dependent species. Most reliable information on the diversity of fish present in the Gulf comes from fisheries studies. According to interviews made to fishermen, the species composition of their catch consists primarily of small pelagic/epipelagic fish (Gonzalvo et al. 2015), with the most dominant target species being, in order of importance, *M. kerathurus*, *S. solea*, and *M. barbatus*. Another recent study based on interviews of fishermen (Ioannou, 2019) showed that 11 fish species contributed to the highest percentage of the fish captured (all of them sampled in our study), and discarded species included *S. aurita* and, to a lesser extent, *Raja* spp. (5%–20%), decapods (20%), *Trachinus* spp. (10%), *Sarda sarda* (10%), *Sarpa salpa* (10%), *Torpedo* spp. (60%), *E. encrasicolus* (10%–30%),

D. annularis (10%–30%), and *Pagellus bogaraveo* (40%). Based on such information, we believe we have included in our study the vast majority of species that may play a significant role in the dolphin's diet.

The low number of dolphins sampled ($n = 16$; representing 10% of the dolphin population based on the most recent abundance estimates) may account for the differences between our results, in which no differences were found between sex or age classes, and those of Blanco et al. (2001), who reported ontogenetic and sexual differences in diet composition of western Mediterranean bottlenose dolphins. According to their findings, females tended to eat more cephalopods than males, and juveniles smaller *M. merluccius* (the principal prey) than adults.

Similarly to dolphins in the western Mediterranean, stomachs of bottlenose dolphins from the Gulf of Cadiz contained principally *C. conger* and *M. merluccius*, even though SIA highlighted that the most assimilated prey items were Sparidae and a mixture of other species including *S. pilchardus* (Giménez et al., 2017). Also, bottlenose dolphins living in neighboring waters of the Inner Ionian Sea Archipelago around the island of Kalamos, were reported to feed predominantly on demersal prey (Bearzi et al., 2005).

Despite the proximity with the Ionian Sea, our results suggested instead that Ambracian bottlenose dolphins feed more on pelagic species (i.e., *T. trachurus* and Clupeiformes that aggregate in large schools near the surface and the shoreline (Food and Agriculture Organization, 2017) than on demersal ones. Stomach content analyses in dolphins from the North Aegean Sea (Greece) showed that Ophiidae (34%) and Sparidae (26%) occupied about 60% of their diet, while a contribution of 13% was also given by Clupeidae, and *M. merluccius* was rarely detected (Milani et al., 2018). Our findings are in accordance with these studies, showing that Sparidae and Clupeidae can also be an important part of the bottlenose dolphin's diet in the Mediterranean Sea.

It is noteworthy that in the Gulf of Ambracia, *M. merluccius* is not a potential prey, as it has never been observed during monitoring of fish landings (Katselis, Moutopoulos, Dimitriou, & Koutsikopoulos, 2013; Koutsikopoulos et al., 2008). This is in contrast with the western Mediterranean: in the Balearic Archipelago, for instance, bottlenose dolphins and the local trawling fleet reportedly behave as what may be interpreted as two sympatric species (Gonzalvo et al., 2008), where dolphins play a parasitic role in a fishing activity that has *M. merluccius* as one of its main target species (Massutí & Reñones, 2005). Trawling and purse seining are prohibited in the Gulf of Ambracia year-round since 1953 by the Royal Fishing Law 23.3/8–4-53 (European Commission, 2009; Koutsikopoulos et al., 2008), and small-scale fisheries, mainly using set nets (i.e., trammel and gill nets), are the only ones allowed inside the Gulf.

Diet and foraging behavior of bottlenose dolphins appear to vary widely depending on the area, season or trophic niche occupied. Even geographically contiguous or overlapping groups may show dramatically different foraging behaviors (Genov, Centrih, Kotnjek, & Hace, 2019) and prey preferences (Bearzi et al., 2009). Given the highly adaptable and opportunistic nature of bottlenose dolphins, a possible explanation for the difference in their diet in the Gulf of Ambracia, with respect to their conspecifics in adjacent Ionian waters, is the increase of the pelagic fish populations of sardines and anchovies, in detriment of the demersal species, during the last decades, as a result of the extensive water blooms occurring throughout the Gulf and the degradation of the demersal compartments of the food web (Piroddi et al., 2016).

It is noteworthy that in the Gulf of Ambracia bottlenose dolphins have frequently been seen engaging in surface-feeding targeting small schooling fish (Bearzi et al., 2008). Drifting fish scale samples collected during these foraging events belonged mainly (99.8%) to two species of Clupeidae, *S. pilchardus* and *S. aurita*, which likely represent the main prey of the bottlenose dolphins during surface feeding bouts (Bräger, Gonzalvo, Agazzi, & Bearzi, 2016). However, scales fall out of the scale pockets (Szymczyk, 1978) more easily in clupeids than in other species such as *T. trachurus*, which might have biased the information derived from Bräger et al. (2016) by exaggerating the percentage of ingested clupeids.

We also found that the Ambracian bottlenose dolphin diet included demersal species of the Sparidae family, which represents a regular catch of local small-scale fisheries operating with trammel nets (European Commission, 2009). Dolphin depredation appears to be recurrently perceived by Mediterranean fishermen to be causing economic hardship, particularly as far as small-scale fisheries are concerned (Bearzi, 2002), and the Gulf of

Ambracia is no exception. Fishermen working in the Gulf regularly report damage to their nets by bottlenose dolphins (Gonzalvo et al., 2015). This reportedly strong dolphin-fisheries interaction may be one of the possible pathways through which Sparidae species become part of the dolphin diet.

Gaining a better understanding of the dolphins' feeding habits provides important information about this species, including how the individuals interact and what strategies they adopt to cope with the stress derived from living in increasingly degraded and fragile coastal ecosystems. Such understanding will be key to better informing the ecosystem models required to evaluate the consequences of fishery management measures (Marçalo et al., 2018). Moreover, it will also help to assess the extent and nature of dolphin-human interactions, such as the dolphin predation on fishing gear and on wild fish aggregations around open-cage farms. Solutions to protect endangered marine fauna are already provided for by national and European Union (EU) legislation, and fisheries management measures within Natura 2000 sites are mandated by the EU Common Fisheries Policy. However, dietary information may be crucial for defining effective management measures to mitigate such frequently conflictual interactions, while ensuring continued ecosystem services and biodiversity conservation.

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AUTHOR CONTRIBUTIONS

Asunción Borrell: Conceptualization; funding acquisition; investigation; project administration; resources; supervision; validation; visualization; writing-original draft; writing-review and editing. **Morgana Vighi:** Conceptualization; formal analysis; methodology; software; supervision; validation; writing-review and editing. **Tilen Genov:** Methodology; resources; writing-review and editing. **Ioannis Giovos:** Methodology; resources; writing-review and editing. **Joan Gonzalvo:** Conceptualization; data curation; funding acquisition; methodology; project administration; resources; supervision; validation; writing-review and editing.

ETHICAL APPROVAL

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Biopsies were collected in accordance with the best practices of the Society for Marine Mammalogy and with permit number 147434/2810 from the Greek Ministry of Environment, Energy and Climate Change.

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