



Trophic ecology of common bottlenose dolphins in a pelagic insular environment inferred by stable isotopes

Ester Dias · Morgane Dromby · Rita Ferreira · Ágatha Gil · Raquel Tejerina · L. Filipe C. Castro · Massimiliano Rosso · Isabel Sousa-Pinto · Joel C. Hoffman · Maria A. Teodósio · Ana Dinis · Filipe Alves

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Abstract The common bottlenose dolphin (*Tursiops truncatus*) is a top marine predator widely dispersed in coastal and pelagic habitats and with a generalist feeding behavior. Yet, information on the trophic ecology of animals inhabiting pelagic environments is still scarce. Using carbon ($\delta^{13}\text{C}$: $^{13}\text{C}/^{12}\text{C}$) and nitrogen ($\delta^{15}\text{N}$: $^{15}\text{N}/^{14}\text{N}$) stable isotope ratios, we identified and quantified the main groups of prey assimilated by bottlenose dolphins inhabiting an oceanic habitat (Madeira Island, East Atlantic). Bottlenose dolphins assimilated pelagic, schooling fish (such as

blue jack mackerel, *Trachurus picturatus*) and mesopelagic and demersal squids, which reinforces the pelagic dietary composition of insular/oceanic dolphins. Also, intra-seasonal differences were found in their stable isotope ratios, which suggest intraspecific variability in the feeding behavior among individuals living in the same area. Sex was not the main factor contributing to these differences, suggesting the lack of trophic niche segregation between adult males and females in this offshore environment. Nonetheless, further studies including different life stages and information on the ecophysiological requirements are necessary to disclose the factors responsible for the observed variability. This study showed that insular dolphins fed primarily on economically important pelagic prey, highlighting the need of developing management strategies that integrate conservation in fisheries plans.

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Ester Dias and Morgane Dromby contributed equally to this work.

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E. Dias · Á. Gil · L. F. C. Castro · I. Sousa-Pinto
CIIMAR - Interdisciplinary Centre of Marine
and Environmental Research, 4450-208 Matosinhos,
Portugal

M. Dromby · R. Ferreira · R. Tejerina · A. Dinis ·
F. Alves (✉)
OOM - Oceanic Observatory of Madeira,
9020-105 Funchal, Portugal
e-mail: filipe.alves@mare-centre.pt

M. Dromby · M. A. Teodósio
Faculty of Sciences and Technology, Universidade
do Algarve, 8005-139 Faro, Portugal

R. Ferreira · A. Dinis · F. Alves
MARE - Marine and Environmental Sciences Centre /
ARNET - Aquatic Research Network, Agência Regional
para o Desenvolvimento da Investigação Tecnologia e
Inovação (ARDITI), 9020-105 Funchal, Portugal

R. Ferreira
Faculty of Life Sciences, University of Madeira,
9020-105 Funchal, Portugal

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Introduction

Diet information on marine mammals is necessary to describe their role in marine ecosystems (Bowen, 1997; Gulka et al., 2017). Given their generally large sizes and ecological position (as high trophic level predators), they can have regulative effects on species at lower levels in marine food webs. Moreover, given their high degree of exposure to human-induced threats (Maxwell et al., 2013), this information is of paramount importance to design effective management and conservation strategies (Prato et al., 2013).

Characterizing variability of the feeding habits of marine mammals can increase the knowledge on how they explore their habitat, both temporally and spatially (horizontally and vertically in the water column). Such variability can be caused by seasonal fluctuations in the availability of food resources (e.g., O'Toole et al., 2015; Guerra et al., 2020), by interspecific competition for resources (e.g., Aurióles-Gamboa et al., 2013; Young et al., 2017),

or by intraspecific characteristics like sex or age class (e.g., Bolnick et al., 2011; Fernández et al., 2011; Quérouil et al., 2013). However, investigating the trophic interactions and diet of marine mammals, such as cetaceans, remains challenging given that some species are endangered or inhabit difficult-to-access environments (e.g., Hays et al., 2016).

Stable carbon and nitrogen isotope analysis has increasingly been used to investigate the trophic interactions within marine predator communities (including marine mammals), as well as spatial, ontogenetic, and sex variations of the diet of predators across a diversity of marine ecosystems (e.g., Hobson et al., 1996; Walker et al., 1999; Kiszka et al., 2014). Stable isotopes ratios provide a time-integrated signal of the food sources in the ecosystem that were incorporated into the consumers' structural components and energy reserves (Peterson & Fry, 1987). Moreover, stable isotope ratios in the consumers' tissues predictably reflect those of their prey, demonstrating an average trophic fractionation (i.e., the difference between the consumer and its diet) of approximately 0.4‰ $\delta^{13}\text{C}$ and 3.4‰ $\delta^{15}\text{N}$ per trophic level (Vander Zanden & Rasmussen, 2001; Caut et al., 2009). A major limitation of stable isotope analysis is that it does not provide a detailed description of the diet of consumers (i.e., taxonomy, size), which is typically obtained through the analysis of stomach (Spitz et al., 2011; Jansen et al., 2013) and fecal contents (Ford et al., 2016). However, mass-balanced isotopic models partially address this problem by estimating the dietary composition of a consumer based on the isotopic composition of candidate prey (Moore & Semmens, 2008; Parnell et al., 2013; Stock et al., 2018).

The common bottlenose dolphin *Tursiops truncatus* (Montagu, 1821) (hereafter, bottlenose dolphin) is undoubtedly among the most studied odontocete species because it is generally abundant in temperate and tropical marine waters around the world (reviewed in Wells & Scott, 2018). Its trophic ecology has been widely studied through several methods (e.g., stomach contents, fatty acids, stable isotopes; Barros & Wells, 1998; Samuel & Worthly, 2004; Mèndez-Fernandez et al., 2012; Kiszka et al., 2014; Bode et al., 2022), yet such studies are unbalanced in favor of coastal habitats where prey type and abundance can differ from those available in pelagic habitats.

Á. Gil

Department of Biology and Environment, Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro (UTAD), 5001-801 Vila Real, Portugal

Á. Gil

Instituto de Investigaciones Marinas (CSIC), Eduardo Cabello 6, 36208 Vigo, Spain

L. F. C. Castro · I. Sousa-Pinto

Department of Biology, Faculty of Sciences, University of Porto (FCUP), 4169-007 Porto, Portugal

M. Rosso

CIMA Research Foundation, 17100 Savona, Italy

J. C. Hoffman

Biology Department, University of Minnesota Duluth, Duluth, MN 55812, USA

M. A. Teodósio

CCMAR - Centre of Marine Sciences, Universidade do Algarve, 8005-139 Faro, Portugal

Madeira is among the most isolated archipelagos in the North Atlantic, offering a privileged location to study the ecology of pelagic predators in insular habitats. The island lies in a warm temperate latitude, is part of the Macaronesia biogeographical region (Spalding et al., 2007), and is characterized by a narrow continental shelf, steep submarine canyons, and deep waters (Geldmacher et al., 2000). This oceanic island benefits from complex geophysical wake flow such as fronts and eddies that can enhance primary productivity (known as island mass effect phenomena; Caldeira et al., 2002; Couvelard et al., 2012) attracting prey and consumers of all trophic levels (Kaufmann et al., 2015; Friedlander et al., 2017; Alves et al., 2018). Although surrounded by oligotrophic waters, the archipelago hosts rich marine biodiversity comprising several coastal and epipelagic fish species of economic and ecological interest (e.g., mackerel and tuna species; Wirtz et al., 2008; Hermida & Delgado, 2016; Tejerina et al., 2019), as well as a high number of species of megafauna (Ramos et al., 2016; Alves et al., 2018; Freitas et al., 2018; McIvor et al., 2022). The bottlenose dolphin is among Madeira's most frequently encountered cetacean species (Dinis et al., 2016a, b; Alves et al., 2018), where island-associated and transient animals are known to co-occur year-round (Dinis et al., 2016a, 2018; Fernandez et al., 2021). No genetic differentiation was found between animals sampled in Madeira and Azores, suggesting that bottlenose dolphins in Macaronesia belong to a large oceanic population (Quérouil et al., 2007). This is supported by a photographic-identification study showing individual movements between Madeira and the neighboring archipelagos of the Azores and the Canaries (Dinis et al., 2021).

Recently, a study based on nucleic acid-derived indices suggested that bottlenose dolphins occurring in Madeira waters are in good nutritional condition (Alves et al., 2020). Yet, that study provided only a general insight into feeding ecology without any inferences regarding diet. Further, there is no available information on stomach contents from stranded animals in Madeira and only one animal was analyzed in the neighboring Canary Islands (Fernández et al., 2009). Thus, the present study aimed to identify the main groups of prey assimilated by insular bottlenose dolphins from Madeira and investigate potential seasonal differences in their diet.

For that, carbon and nitrogen stable isotope ratios were combined with Bayesian mixing models to identify and quantify the groups of prey assimilated by bottlenose dolphins between 2017 and 2018. This will contribute to enlightening the trophic ecology of a worldwide distributed top predator in a poorly studied habitat.

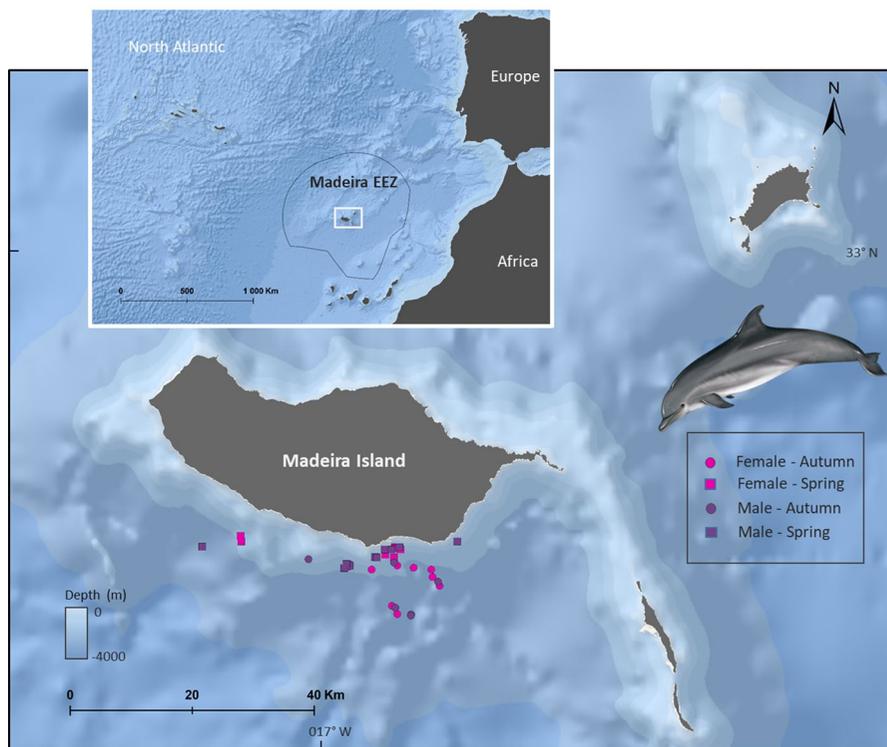
Materials and methods

Data collection

Samples were collected off the south coast of Madeira Island, Portugal (Fig. 1). To investigate seasonal variability in the feeding habits of bottlenose dolphins, tissue samples of wild animals were obtained during autumn (November) 2017 and spring (March and April) 2018 using a biopsy darting system (150-lb crossbow, with arrows and darts specially designed for small cetaceans by Finn Larsen, Ceta-Dart; Mathews et al., 1988). Biopsies were collected by experienced researchers carrying legal permits (see 'Ethics Approval') and targeted the flanks of large and robust animals with no signs of emaciation or carrying calves. Biopsy samples were stored in a liquid nitrogen container on board and kept at -80°C before being processed.

Bottlenose dolphins are generalist predators feeding on a large variety of pelagic fish and pelagic and demersal squid species (e.g., González et al., 1994; Barros et al., 2000). Therefore, several species (Table S1) of the most abundant demersal and pelagic squids and small schooling fish (Clarke & Lu, 1995; Clarke, 2006; Hermida & Delgado, 2016; Tejerina et al., 2019) were also sampled, because previous studies suggested they will likely prey on the locally available species (Barros & Odell, 1990; Hernandez-Milian et al., 2015). Additionally, two abundant tuna species (Hermida & Delgado, 2016, Table S1) were also considered as potential prey based on personal communication by local tuna fishers, which reported seeing dolphins preying on tunas in this area. Prey specimens were collected in the same general area as dolphins' biopsies (i.e., between the coastline and 20 km off the South coast of Madeira Island), except for tuna that were collected across the Madeira Exclusive Economic Zone (Fig. 1). Fish

Fig. 1 Location of Madeira Archipelago (with the contour of its EEZ—Economic Exclusive Zone in the inset picture) and of biopsied common bottlenose dolphins (*Tursiops truncatus*) during autumn 2017 and spring 2018 (basemap credits: Esri, Garmin, GEBCO, NOAA NGDC, and other contributors). Small pelagic fishes and squids used in this study were sampled in the same general area where biopsies were taken (i.e., between the coastline and 20 km off the south coast of Madeira Island), whereas tuna were sampled across the Madeira EEZ. Species illustration by E. Berninsson © ARDITI



were collected on board fishing boats between the summer of 2017 and spring of 2018, and squids were bought from fishermen or captured directly from the coastline during 2018 and 2019 (Table S1). All prey samples were kept at $-20\text{ }^{\circ}\text{C}$ before being processed.

Laboratory analyses

For stable isotope analyses (SIA) skin samples from bottlenose dolphins, and muscle (fish) and mantle (squids) samples of their potential prey, were dried in an oven at $60\text{ }^{\circ}\text{C}$ and ground to a fine powder with a mortar and pestle. Stable isotope ratios were measured using a Flash EA 1112 Series elemental analyser coupled online via Finnigan ConFlo III interface to a Thermo delta V S mass spectrometer (Marefoz, University of Coimbra, Portugal). Stable isotope ratios are reported in δ notation, $\delta X = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 10^3$, where X is the C (carbon) or N (nitrogen) stable isotope, and R is the ratio of heavy: light stable isotopes. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are expressed in units per mill (‰) relative to Vienna Pee Dee Belemnite and air, respectively. The analytical precision was better than 0.1‰ for $\delta^{13}\text{C}$ and 0.3‰ for $\delta^{15}\text{N}$. To control

for sample processing quality, samples with an SD between replicates (i.e., two sub-samples of the same sample) $> 0.2\text{‰}$ $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ were not included in subsequent data analyses.

For sex determination, genomic DNA from bottlenose dolphins was extracted from the skin samples using a standard high-salt protocol as outlined in Sambrook et al. (1989). Single PCR reactions with only one set of primers (thus two PCR reactions per sample) were carried out to amplify both ZFX and SRY gene fragments (Bérube & Palsbøll, 1996) using Phusion Flash High-Fidelity PCR Master Mix (Thermo Scientific™) in $20\text{ }\mu\text{l}$ reactions. The amplification conditions used in this study are detailed in Alves et al. (2020). Several electrophoresis bands from different samples were sequenced to confirm whether the desired genes were amplified. The PCR products were cut from the gel, purified with the NZYGelpure (NZYTech), and sent to direct sequencing (Sanger sequencing) using the light run sequencing service of GATC Biotech. The DNA sequences were analyzed using the BioEdit Sequence Alignment

Editor version 7.0.4.1 (Hall, 1999) and aligned against reference sequences from GenBank.

Data analysis

The relative contribution of the most likely prey to the diet of bottlenose dolphins was quantified using the Bayesian stable isotope mixing model MixSIAR v3.1.12 (Stock & Semmens, 2016). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were adjusted for one trophic level using the trophic fractionation estimates ($\Delta\delta^{13}\text{C}=1.01\pm 0.37\text{‰}$, $\Delta\delta^{15}\text{N}=1.57\pm 0.52\text{‰}$) derived from the most extended feeding experiment available on bottlenose dolphins (350 days; following Giménez et al., 2016).

An exploratory analysis revealed that sex alone did not explain the observed intra-seasonal variability in bottlenose dolphins' stable isotope ratios because models including sex as a fixed factor showed multiplicative errors much greater than one ($\epsilon > 3$). This suggests that sources could be missing or that important consumer population structure was absent from the model (Stock et al., 2018). Thus, mixing polygon simulations were constructed to check the adequacy of the food sources and trophic fractionation values used for each season (Smith et al., 2013). If consumers fall within the 95% mixing region, that indicates that a mathematical solution can be found that satisfies the geometry of mixing models (Smith et al., 2013). Because no additional information was available on the consumers other than sex (e.g., age or size; although all the sampled dolphins were adults), we performed a hierarchical cluster analysis (linkage—Ward), which is a method of pattern mining (Han et al., 2011), to identify potential grouping in the bottlenose dolphins' stable isotopes by season. For that, the function *hclust* available in the package *stats* was used. The best number of clusters was determined using the function *NbClust* available in the package *NbClust* (Charrad et al., 2014).

Four models were fitted to the bottlenose dolphins' data: null (i.e., consider that all individuals in the population share the same diet), sex, group, and sex and group. The relative support for each model was evaluated with leave-one-out cross-validation (the best model should have $d\text{LOOic}=0$), and the corresponding Akaike weights were inspected (Vehtari et al., 2017). To run the models, the stable

isotope ratios of bottlenose dolphins and their most likely prey were input as raw data, using non-informative priors. Model convergence was assessed via Gelman–Rubin and Geweke diagnostics (Stock & Semmens, 2016). Posterior distributions obtained from the MixSIAR analyses are expressed as median and 95% credibility intervals.

When dealing with generalist predators that feed on multiple species, a reduced set of prey species or consolidating prey species is necessary due to overlapping $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Jansen et al., 2013). In this case, prey were grouped according to their taxonomic group (fish or squids) and habitat. Thus, the Atlantic chub mackerel (*Scomber colias*), European pilchard (*Sardina pilchardus*), sardines (*Sardinella* spp.), bogue (*Boops boops*), and slender snipefish (*Macroramphosus gracilis*) were grouped as 'small pelagic fish A'. The blue jack mackerel (*Trachurus picturatus*) formed the group 'small pelagic fish B'. This species is commonly observed in Madeira close to the surface (Tejerina et al., 2019), but ranges in depth to at least 370 m (Vasconcelos et al., 2006). Their ^{13}C - and ^{15}N -enriched composition, when compared to those from 'small pelagic fish A', suggest they may obtain their prey from deeper or coastal habitats, or that they feed on prey from higher trophic levels than those individuals comprising the 'small pelagic fish A' group. The skipjack tuna (*Katsuwonus pelamis*) and juvenile bigeye tuna (*Thunnus obesus*) were grouped as 'large pelagic fish'. The European squid (*Loligo vulgaris*) formed the group 'demersal squids', the webbed flying squid (*Ommastrephes caroli*) formed the group 'pelagic squids A', and the orangeback squid (*Sthenoteuthis pteropus*) formed the group 'pelagic squids B'. Although the last two squid species occupy similar habitats (Table S1), the difference in their mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of ca. 3‰ and 0.6‰, respectively, suggests that the orangeback squid occupies a higher trophic position than the webbed flying squid (e.g., Vander Zanden & Rasmussen, 2001). For that reason, they were separated into different prey groups.

Due to a correlation between 'small pelagic fish A', 'demersal squids', and 'pelagic squids A' stable isotope values, the model could not fully discriminate between those groups. Therefore, we excluded the group 'small pelagic fish A' from the mixing models because previous studies based on stomach content

analyses estimated a small contribution of epipelagic fish to the diet of bottlenose dolphins from coastal (Santos et al., 2001, 2007; Hernandez-Milian et al., 2015; Giménez et al., 2017; Milani et al., 2018) and offshore areas (Mead & Potter, 1995).

The most likely prey assimilated by bottlenose dolphins in each season were identified using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ bi-plots, where bottlenose dolphins' $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (after adjusting for trophic fractionation) were compared to prey $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Prey specimens captured in summer and autumn were included in the diet analysis for the 'summer/autumn' season, while those caught in winter and spring were included in the 'winter/spring' season. This approach was used because cetacean skin has a half-life of 30–40 days (Giménez et al., 2016).

All the $\delta^{13}\text{C}$ values were corrected for lipid content because lipids are depleted in ^{13}C compared to protein and carbohydrates, which usually causes an inverse relationship between the C:N and $\delta^{13}\text{C}$ values in the muscle tissues of aquatic animals (DeNiro & Epstein, 1977). A correction factor of 1.5‰ was applied to the bottlenose dolphins' $\delta^{13}\text{C}$ values, following Wilson et al. (2014), because the mean (\pm SD) C:N was lower than 4.5 (4.2 ± 0.2). Prey tissue values were also corrected for lipid content following the mass balance correction for fish muscle tissue proposed by Hoffman and Sutton (2010, Eq. 6), which uses estimates of $\text{C:N}_{\text{protein}}$ and $\Delta\delta^{13}\text{C}_{\text{lipid}}$ similar to those from the muscle tissue found in other fish (e.g., Sweeting et al., 2006) and taxonomic groups (e.g., shrimp and zooplankton; Fry & Allen, 2003; Smyntek et al., 2007).

Standard deviation (\pm SD) was used as a measure of data dispersion when reporting mean values. All the analyses were performed using the open-source statistical language R software (R Core Team 2020).

Results

Thirty-four samples of bottlenose dolphins were analyzed in this study. It included 14 samples (six males and eight females) obtained during seven encounters (one to four samples per encounter) in autumn 2017 and 20 samples (11 males and nine females) during 12 encounters (one to five samples per encounter) in spring 2018 (Table 1).

Table 1 Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (‰) for common bottlenose dolphins per season and sex

Season	Sex	<i>n</i>	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	C:N
Autumn 2017	Female	8	-16.9 ± 0.3	9.6 ± 0.4	4.1 ± 0.2
	Male	6	-16.9 ± 0.4	10.1 ± 0.8	4.2 ± 0.2
	Mean		-16.9 ± 0.4	9.8 ± 0.6	4.2 ± 0.2
Spring 2018	Female	9	-17.3 ± 0.5	10.4 ± 0.6	4.4 ± 0.2
	Male	11	-17.1 ± 0.2	10.1 ± 0.6	4.2 ± 0.1
	Mean		-17.2 ± 0.4	10.3 ± 0.6	4.3 ± 0.2
Total	Mean	34	-17.0 ± 0.4	10.1 ± 0.7	4.2 ± 0.2

Hierarchical cluster analysis of bottlenose dolphins' data identified three distinct clusters in the season 'summer/autumn' (Fig. 2). The first branch separates ^{15}N -enriched individuals (group A) from ^{15}N -depleted (groups B and C) individuals: $10.3 \pm 0.3\text{‰}$ (group A), $9.5 \pm 0.2\text{‰}$ (group B), and $\delta^{15}\text{N}$: $9.4 \pm 0.2\text{‰}$ (group C). The mean $\delta^{13}\text{C}$ values of the individuals from groups A, B, and C were $-16.9 \pm 0.3\text{‰}$, $-17.2 \pm 0.2\text{‰}$, and $-16.6 \pm 0.3\text{‰}$, respectively.

In 'winter/spring', two clusters were identified, of which group D comprised six individuals and group E, 14 individuals (Fig. 2). The first branch separates ^{13}C - and ^{15}N -enriched individuals (group D; $\delta^{15}\text{N}$: $11.0 \pm 0.3\text{‰}$, $\delta^{13}\text{C}$: $-16.8 \pm 0.5\text{‰}$) from ^{13}C - and ^{15}N -depleted individuals (group E; $\delta^{15}\text{N}$: $9.9 \pm 0.4\text{‰}$, $\delta^{13}\text{C}$: $-17.3 \pm 0.3\text{‰}$).

Overall, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of bottlenose dolphins analyzed for the seasons 'summer/autumn' and 'winter/spring'—after adjusting for trophic fractionation—were intermediate between several potential prey groups, indicating reliance on multiple sources (Fig. 3). The low $\delta^{15}\text{N}$ values of individuals analyzed for 'summer/autumn' suggest the assimilation of ^{15}N -depleted prey such as demersal squids, pelagic fish, and pelagic squids. The range of $\delta^{13}\text{C}$ values of bottlenose dolphins also suggests the assimilation of pelagic fish belonging to the group 'small pelagic fish B' (Fig. 3). On the other hand, the individuals analyzed for the season 'winter/spring' showed high variability in their $\delta^{15}\text{N}$ values, suggesting the assimilation of ^{15}N -enriched prey such as 'large pelagic fish' (Fig. 3).

After correcting for trophic fractionation, bottlenose dolphins' $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values fell within

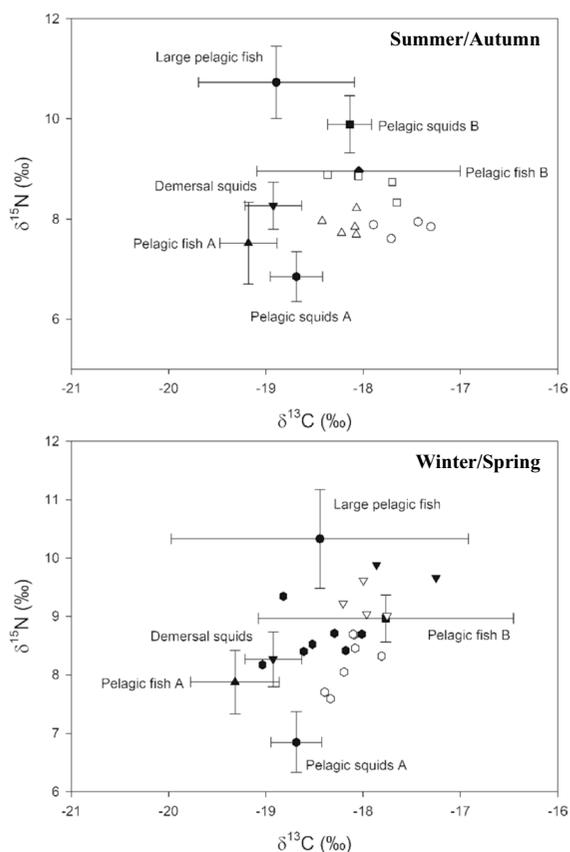


Fig. 3 Mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (‰) of each group of bottlenose dolphins formed by the hierarchical clustering (following Fig. 2), adjusted for one trophic level fractionation ($\delta^{13}\text{C}=1.01 \pm 0.37\text{‰}$, $\delta^{15}\text{N}=1.57 \pm 0.52\text{‰}$, Giménez et al. 2016), and their potential prey in 'summer/autumn' (top) and 'winter/spring' (bottom). Bottlenose dolphins' groups (following Fig. 2) in 'summer/autumn' include A (open squares), B (open triangles), and C (open circles), and in 'winter/spring' include D (closed inverted triangles—females, open inverted triangles—males) and E (closed diamonds—females, open diamonds—males). The 'small pelagic fish A' comprises the Atlantic chub mackerel, European pilchard, sardines, bogue, and slender snipefish, whereas the 'small pelagic fish B' is formed by the blue jack mackerel. The 'large pelagic fish' comprises the skipjack and bigeye tuna. The 'demersal squids' is formed by the European squid, the 'pelagic squids A' by the webbed flying squid, and the 'pelagic squids B' by the orange-back squid (see 'Materials and Methods' for details)

dolphins (e.g., Connor et al., 2001; Lusseau & Newman, 2004; Möller et al., 2006), resource partitioning may correspond to population social structure as highly associated individuals occupy the same habitat and encounter the same resources

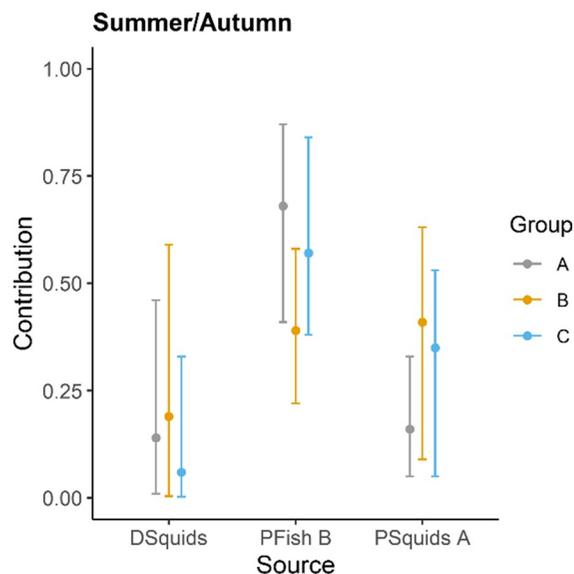
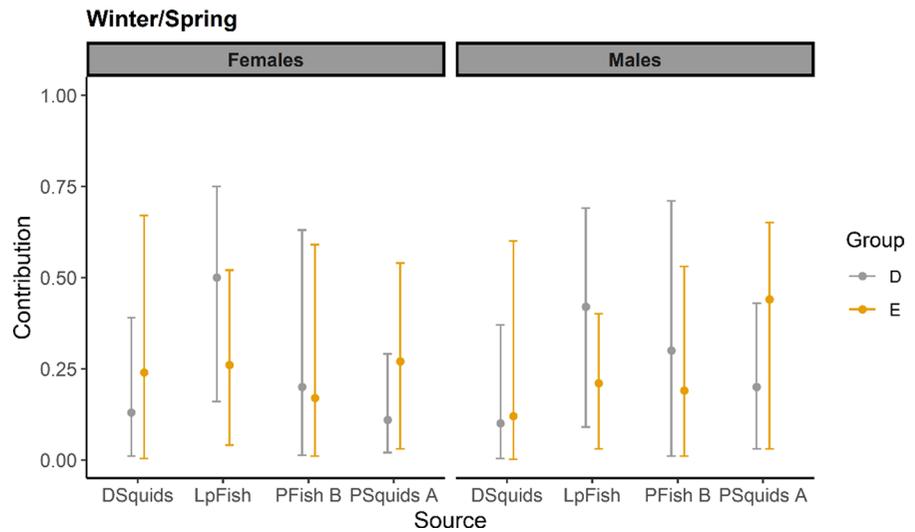


Fig. 4 Relative contribution of each type of prey to bottlenose dolphins' groups (A, B, and C, following Fig. 2) during 'summer/autumn' based on the stable isotope mixing models. The types of prey included in the model were 'demersal squids' (DSquids), 'small pelagic fish B' (PFish B), and 'pelagic squids A' (PSquids A). Closed circles indicate the median value and lines indicate the 95% Bayesian credibility intervals

(Semmens et al., 2009; Nicholson et al., 2021). The present study and others (e.g., Barros & Odell, 1990) suggest that sex does not fully explain intrapopulation differences in diet, while others identified differences between the diet of males and females. For instance, on the Israeli coast, it was found that males ate a more diverse diet than females (Sheinin et al., 2014), whereas, in the western Mediterranean, males ate larger but fewer fish than females (Blanco et al., 2001). Off the west coast of Florida (USA), females showed broader foraging habits than males due to habitat specialization (Rossman et al., 2015). Moreover, compared to inshore ecosystems, pelagic environments such as the waters surrounding Madeira lack multi-habitat specificities, and thus other factors are likely contributing to the isotopic differences observed between individuals.

Recently, it was found that individual foraging variation can drive social organization in bottlenose dolphins (Methion & Díaz López, 2019). Based on social network analysis, it was concluded that bottlenose dolphins prefer to affiliate with

Fig. 5 Relative contribution of each type of prey to bottlenose dolphins' groups (D and E, following Fig. 2) during 'winter/spring' based on the stable isotope mixing models. The types of prey included in the model were 'demersal squids' (DSquids), 'large pelagic fish' (LpFish), 'small pelagic fish B' (PFish B), and 'pelagic squids A' (PSquids A). Closed circles indicate the median value and lines indicate the 95% Bayesian credibility intervals



individuals displaying similar foraging strategies, which likely promotes segregation of the population into behaviourally distinct groups (Methion & Díaz López, 2019). For instance, individuals facing a patchy and irregular prey distribution may benefit from increased cooperation and reduced intragroup competition leading to strong intragroup associations (Methion & Díaz López, 2019). Our sample strategy consisted of sampling only large and robust animals with no signs of emaciation nor carrying calves. However, we lacked associated information about the sampled individuals (e.g., age, residency patterns, feeding tactics) which impairs further conclusions about the role of behavior, or other factors such as metabolic and physiological requirements or habitat use, on their feeding ecology. Notwithstanding it, differences were found between the diet of bottlenose dolphins living in coastal and in offshore/oceanic habitats, with the latter ecotype consuming higher relative proportions of pelagic squids (Mead & Potter, 1995; Hernandez-Milian et al., 2015). Thus, given the observed intra-seasonal variability in the stable isotope values, it is possible that individuals of different residency patterns were analyzed in this study (Dinis et al., 2016a, 2018). Yet, the lack of photographic-identification of the biopsied animals does not allow discussing it, nor the possibility of duplicates influencing the results. To test this hypothesis, further studies should complement photographic-identification with the stable isotope values of the baselines in both coastal and offshore

habitats to investigate the origin of the prey assimilated by the bottlenose individuals in Madeira. These findings highlight the need to investigate this topic further, given that the data analysis was consistent with distinct feeding groups within the assemblage sampled and that bottlenose dolphins' feeding strategies may expose them differently to local human threats (e.g., fisheries, pollution).

The main seasonal difference between the resources assimilated by bottlenose dolphins was the relative contribution of pelagic fish, which was higher during 'winter/spring' than during 'summer/autumn'. This increase was driven by the apparent contribution of small tunas to their diet during 'winter/spring', which coincides with the seasonal increase in their availability in Madeira, especially bigeye tuna (Gouveia et al., 2017). To the best of our knowledge, no study reported the consumption of tuna by bottlenose dolphins, suggesting caution when interpreting this result. However, previous studies have identified similar-sized fishes from the family Scombridae (e.g., bonito *Sarda* spp., little tunny *Euthynnus* spp., king mackerel *Scomberomorus cavalla*) in their diet in other regions of the Atlantic (reviewed in Mead & Potter, 1990). This possibility was also considered based on in situ observations made by local fishermen, which reported dolphins preying on skipjack tuna and juveniles from bigeye tuna (fish of 4–5 kg). There is also the possibility that access to this prey may result not from direct attacks but from opportunistic associations with other species

that feed on tuna. Nonetheless, we cannot exclude the possibility that individuals sampled during spring could have recently migrated from coastal or other geographic areas to the area where sampling took place or that other ^{15}N -enriched prey are missing in our analyses.

One critical assumption of investigating the trophic ecology of bottlenose dolphins in Madeira was that the seasonal variability in their stable isotope ratios resulted from seasonal differences in the prey assimilated. A previous study conducted with captive bottlenose dolphins estimated half-life turnover rates in skin tissue of 24.16 ± 8.19 days for $\delta^{13}\text{C}$ and 47.63 ± 19 days for $\delta^{15}\text{N}$ (Giménez et al., 2016). Such rates enabled us to properly investigate seasonal variations in the dietary habits of this consumer, which has been rarely approached in the trophic ecology of cetaceans (but see Guerra et al., 2020). Also, for modeling purposes, the stable isotope ratios from bogue, European squid, and webbed flying squid collected during 'summer/autumn' were used in the analysis conducted for 'winter/spring'. While inter-seasonal variability is likely to occur in the stable isotopes of these prey, it was expected to be low and similar to that observed in other prey species collected during this study. For example, the mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the small pelagic European pilchard, which is zooplanktivore (e.g., Costalago & Palomera, 2014; Hure & Mustac, 2020), were similar throughout the studied period (Table S1). The mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the individuals collected between summer and autumn were $-19.3 \pm 0.3\text{‰}$ and $7.6 \pm 0.4\text{‰}$, respectively, while the mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of those collected between winter and spring were $-18.9 \pm 0.4\text{‰}$ and $7.6 \pm 0.4\text{‰}$, respectively. These prey species are expected to reflect changes occurring at the base of the food web as a result of their feeding behavior. Thus, the minor differences observed in their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values suggest that there were no major isotopic changes at the base of the pelagic food web throughout the studied period or that they were not prolonged enough in time to translate into major changes in the stable isotope ratios of the European pilchard. Small changes in the mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between seasons were also observed for epipelagic (i.e., blue jack mackerel, and Atlantic chub mackerel) and other pelagic species (i.e., *Sardinella* spp.) (Table S1), thus suggesting small temporal

changes in the isotopic composition at the base of the food webs they rely upon.

As an overview, this study was the first attempt to analyze bottlenose dolphins' trophic ecology in a truly pelagic environment. We suggest that bottlenose dolphins living off the coast of Madeira assimilated prey of intermediate (i.e., mackerels) and high (i.e., tunas) trophic levels and that their relative contribution varied between seasons. Also, intra-seasonal differences were found in their stable isotope ratios, which suggest intraspecific variability in the feeding behavior among individuals living in the same area, although the origin for that variability remains unknown. Future studies should include coastal and other demersal prey species and additional information on individuals (e.g., age, habitat use, physiological condition) and groups of individuals (social organization) to identify the main factors responsible for the diet's intrapopulation variability observed in Madeira. Moreover, DNA analysis on stomach contents from stranded bottlenose dolphins or biologgers equipped with cameras to observe their feeding behavior in situ, would allow obtaining detailed information about their prey, especially to disclose if they prey on tunas. Finally, this study suggests that bottlenose dolphins assimilated small pelagic fish that are among the most commercially important captured species in Madeira (Hermida & Delgado, 2016; Tejerina et al., 2019). This outcome highlights the need for developing monitoring programs to evaluate their interaction with fisheries and the potential to be caught due to their feeding activities.

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Author contributions FA, AD, MAT, and ED conceived the study, FA, AD, MAT, and IS-P obtained funding for data collection and laboratory analyses, MD, RF, ÁG, RT, MR, AD, and FA collected the data, MD, JCH, and ED analyzed

the data, LFCC performed genetic sexing, MD, ED, and FA drafted the manuscript, and all authors critically reviewed the manuscript and approved the version for publication.

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Data availability Available as Electronic Supplementary Material S3.

Declarations

Conflict of interest We have no competing interests.

Ethical approval Biopsies were obtained following the relevant guidelines and regulations imposed by Instituto de Flores-tas e Conservação da Natureza IP-RAM under sampling permits 308 1.856/2017, 508/2018, and 10661/2018.

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