



# Trophic position of dolphins tracks recent changes in the pelagic ecosystem of the Macaronesian region (NE Atlantic)

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**ABSTRACT:** Dolphins play a key role in marine food webs as predators of mid-trophic-level consumers. Because of their mobility and relatively long life span, they can be used as indicators of large-scale changes in the ecosystem. In this study, we calculated the trophic position (TP) of 5 dolphin species from the Canary, Madeira and Azores Islands using bulk and compound-specific stable isotope ratios from muscle tissue to assess trophic adaptations to recent changes in the availability of feeding resources. Dolphin TP values were then compared with those of 7 other species of cetaceans from this region. Analysis of stable nitrogen isotopes in amino acids of the common dolphin indicated non-significant effects of changes in the basal resources of the food web and thus supported the use of bulk samples for TP estimations. Dolphins occupied an intermediate TP (mean: 3.91 to 4.20) between fin (3.25) and sperm whales (4.95). Species-specific TP were equivalent among islands. However, TP increased for the common dolphin and decreased for the bottlenose dolphin (the latter also becoming more oceanic) between 2000 and 2018 in the Canary Islands. These results suggest different impacts of recent changes in the oceanography and in the pelagic food web of the Macaronesian region on the trophic ecology of dolphin species.

**KEY WORDS:** Cetaceans · Marine mammals · Canary Islands · Madeira · Azores · Stable isotopes · Amino acids · Temporal change

## 1. INTRODUCTION

Trophic level indicates the position of species in the flow of energy from autotrophs to top predators and

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<sup>§</sup>Corrections were made after online publication. For details see [www.int-res.com/abstracts/meps/v699/c\\_p167-180](http://www.int-res.com/abstracts/meps/v699/c_p167-180)  
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summarizes their role within food webs. This concept has been extensively used in ecological research as it enables the comparison of food webs containing different species across different systems (Yodzis 2001).

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Trophic levels are generally applied to a linear food chain where each level feeds exclusively on the preceding one. In this case, trophic levels can be designated by integer numbers, starting with primary producers (i.e. trophic level = 1). However, this approach is not appropriate for real food webs where predators typically feed on a range of prey species, each of which occupies a different position in the food web. Therefore, trophic positions (TPs) are more appropriate to account for the fraction of each different type of prey in the diet of consumers (Herendeen 2008). The estimation of TP is central to the analysis of food webs as it provides a measure of the use of available energy, stability or complexity in ecosystems (Vander Zanden & Fetzer 2007, Pavluk & bij de Vaate 2008). In marine ecosystems, the TP of large predators, such as cetaceans, is employed as a proxy of the length of the food chain, as they integrate changes in lower trophic levels (Ruiz-Cooley et al. 2017). Estimations of TP can be made from detailed analyses of the diet of each species, ecosystem models, or from stable isotope analysis (Pauly et al. 1998, Trites 2019). The latter technique is based on the progressive enrichment in heavy isotopes (mainly of nitrogen) from prey to consumers, reflecting the diet integrated over the time interval required for the turnover of the tissue analysed (Vander Zanden et al. 2015). However, TP estimations from stable isotopes require critical assumptions about the isotopic composition at the base of the food web (i.e. the isotopic baseline) and the degree of isotopic enrichment between prey and predator (i.e. the trophic enrichment factor, TEF).

Isotope-based estimations of TP are generally derived from the analysis of bulk tissues (Jennings & van der Molen 2015) but recent studies are increasingly using estimates based on the nitrogen stable isotopes in amino acids (Chikaraishi et al. 2009, Nielsen et al. 2015, Matthews et al. 2020, Troina et al. 2021). The latter approach has the advantage of using the isotopic values of certain amino acids with minimal variability across the food web (i.e. source amino acids) to estimate the isotopic baseline, thus avoiding the requirement of sampling basal species. The isotopic values of other amino acids that are enriched in heavy stable isotopes with each trophic step (i.e. trophic amino acids) provide the necessary information to calculate TP after accounting for the baseline and using an appropriate TEF value (Chikaraishi et al. 2009). However, in the case of cetaceans, current estimates of TP from stable isotopes are questioned because the universal TEF values applied to most food webs cannot reproduce the TP values expected from diet analysis (Matthews et al. 2020,

Ruiz-Cooley et al. 2021). One major limitation is the lack of appropriate TEF values for the different species and tissues, considering the large variability in diets. This is important because each tissue has a different turnover time, so it provides trophic information for a specific time window (Newsome et al. 2010, Caut et al. 2011, Browning et al. 2014, Vander Zanden et al. 2015, Teixeira et al. 2022). While some studies have assessed TEF in cetacean species, mostly these were based on skin and blood samples (Caut et al. 2011, Browning et al. 2014, Giménez et al. 2016), with only a few studies using muscle (e.g. Borrell et al. 2012). Taking these limitations into account, accurate determinations of cetacean TP are a prerequisite for further analysis of the variability in TP across species and ecosystems.

In this study, we estimated the TPs of dolphins and other cetacean species from a collection of samples taken from cetaceans found stranded in the Macaronesian islands, to determine whether they can be used as indicators of temporal changes in the food web at decadal scales. Macaronesia is a biogeographically complex oceanic region (Freitas et al. 2019), including diverse habitats (i.e. islands) under the influence of the oligotrophic North Atlantic Subtropical Gyre and the eutrophic NW African upwelling (Aristegui et al. 2009). Major changes in food webs in this region are expected as the result of variations in these major oceanic regimes in recent years. For instance, an intensification of the upwelling-favourable winds between 23° and 33° N (Benazzouz et al. 2015) was accompanied by decreases in primary production (Demarcq 2009) and in the stocks of planktivorous pelagic fish, such as sardines and anchovies (Sánchez-Garrido et al. 2019, 2021, Diogoul et al. 2021). Dolphins are among the top predators in this ecosystem and thus are good indicators of the impact of these changes across the food web. However, while there is a sound knowledge of their diversity and distribution (Martín et al. 2003, Freitas et al. 2012, Silva et al. 2014, Correia et al. 2020), there is much less information about the variability of their diets and feeding habits (Fernández et al. 2009, Quéroil et al. 2013) and there are no studies on the effects of the previously described environmental changes on their populations. Furthermore, there is no consistent study of the trophic hierarchy of cetaceans in the Macaronesian region, assessed with consistent and comparable estimations based on stable isotopes.

The main objective of this study was to analyse whether Macaronesian dolphin species have adapted their TP to the changes in the availability of feeding resources in recent years. We hypothesized that the

reduction in pelagic fish availability would have induced a change in the TP of dolphins, as they would have to rely on alternative prey. For species consuming both pelagic and benthic prey, such as the bottlenose dolphin (e.g. Santos et al. 2007), the dominance of one type of prey would affect the isotopic baseline assimilated in the predator tissues. In addition, the variability in upwelling may have induced changes in the nitrogen inputs for primary producers, also affecting the isotopic baseline. Both effects were assessed from the stable isotope composition of dolphin muscle samples. For this purpose, we estimated the TP of dolphin species using the most recent stable isotope procedures, i.e. compound-specific stable isotope analysis of amino acids. Using a collection of samples from stranded cetaceans covering a period of 22 yr (1996–2018), we first determined the TPs of dolphin species and compared them to other cetaceans from the region. We subsequently assessed the relative importance of spatial variations (i.e. between archipelagos) and investigated inter-annual trends in dolphin TP and baseline nutrient sources in the pelagic food web.

## 2. MATERIALS AND METHODS

### 2.1. Sample collection

Muscle samples were obtained from local collections of stranded cetaceans in the Canary (n = 69), Madeira (n = 17) and Azores (n = 14) Islands between 1996 and 2018 (Fig. 1). As the study required samples covering several years, muscle samples were selected because they were available for a high number of species and years. Furthermore, muscle tissue provides trophic information from the last few months due to its medium turnover rates (Browning et al. 2014, Giménez et al. 2016, Teixeira et al. 2022). Six species of Family Delphinidae, including the common dolphin *Delphinus delphis* (n = 24), striped dolphin *Stenella coeruleoalba* (n = 23), Atlantic spotted dolphin *S. frontalis* (n = 21), bottlenose dolphin *Tursiops truncatus* (n = 18), Risso's dolphin *Grampus griseus* (n = 3), and short-finned pilot whale *Globicephala macrorhynchus* (n = 3), were studied. In addition, samples of sperm whale *Physeter macrocephalus* (n = 3), pygmy sperm whale *Kogia breviceps* (n = 1), Cuvier's beaked whale *Ziphius cavirostris* (n = 2), fin whale *Balaenoptera physalus* (n = 1), and Bryde's whale *B. edeni* (n = 1) were also analysed to characterize the trophic hierarchy of dolphins among other cetaceans from the Macaronesian region. Mus-

cle samples were stored frozen (−20°C) until analysis. Identification and sampling were carried out by experienced personnel following European Cetacean Society protocols (Kuiken & García Hartmann 1991), and the muscle samples used were from well-preserved and moderately decomposed carcasses (preservation codes ≤ 3, Kuiken & García Hartmann 1991), as recommended by Teixeira et al. (2022). The original data, along with the stable isotope results, are available in the PANGAEA repository (<https://doi.org/10.1594/PANGAEA.943603>).

### 2.2. Stable isotopes

#### 2.2.1. Bulk isotope analysis

Carbon and nitrogen stable isotopes were determined in bulk muscle tissues after freeze-drying and grinding of the original samples. Carbon isotopes were determined in 0.7 mg sample aliquots that were lipid-extracted with a 1:1 v/v mixture of trichloromethane:methanol (Bligh & Dyer 1959) prior to analysis, to obtain representative values of the muscle protein, as lipids are depleted in heavy carbon isotopes. In turn, nitrogen isotopes were analysed in untreated aliquots, as the removal of lipids may affect the results (Newsome et al. 2010, Teixeira et al. 2022). Samples were run into an isotope-ratio mass spectrometer (DeltaV Advantage, Thermo Scientific) coupled to an elemental analyser (FlashEA1112, ThermoFinnigan). Isotopic abundances of carbon and nitrogen were expressed as  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  relative to VPDB (Vienna PeeDee Belemnite carbonate) and atmospheric  $\text{N}_2$  isotope, respectively (Coplen 2011). International Atomic Energy Agency standards USGS40 and L-alanine, as well as internal acetanilide and sample standards, were analysed with each batch of samples to ensure offsets between certified and measured values were <0.1%. Mean precision (SE) of triplicate sample determinations were 0.20‰ and 0.18‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively. According to studies in bottlenose dolphin,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in muscle samples are comparable to those in skin (Arregui et al. 2017) and provide information on nutrients assimilated from the diet in the last 2–5 mo (Browning et al. 2014, Giménez et al. 2016).

#### 2.2.2. Amino acid compound-specific isotopic analyses

For 17 *D. delphis* samples, additional determinations of  $\delta^{15}\text{N}$  in amino acids were made to estimate

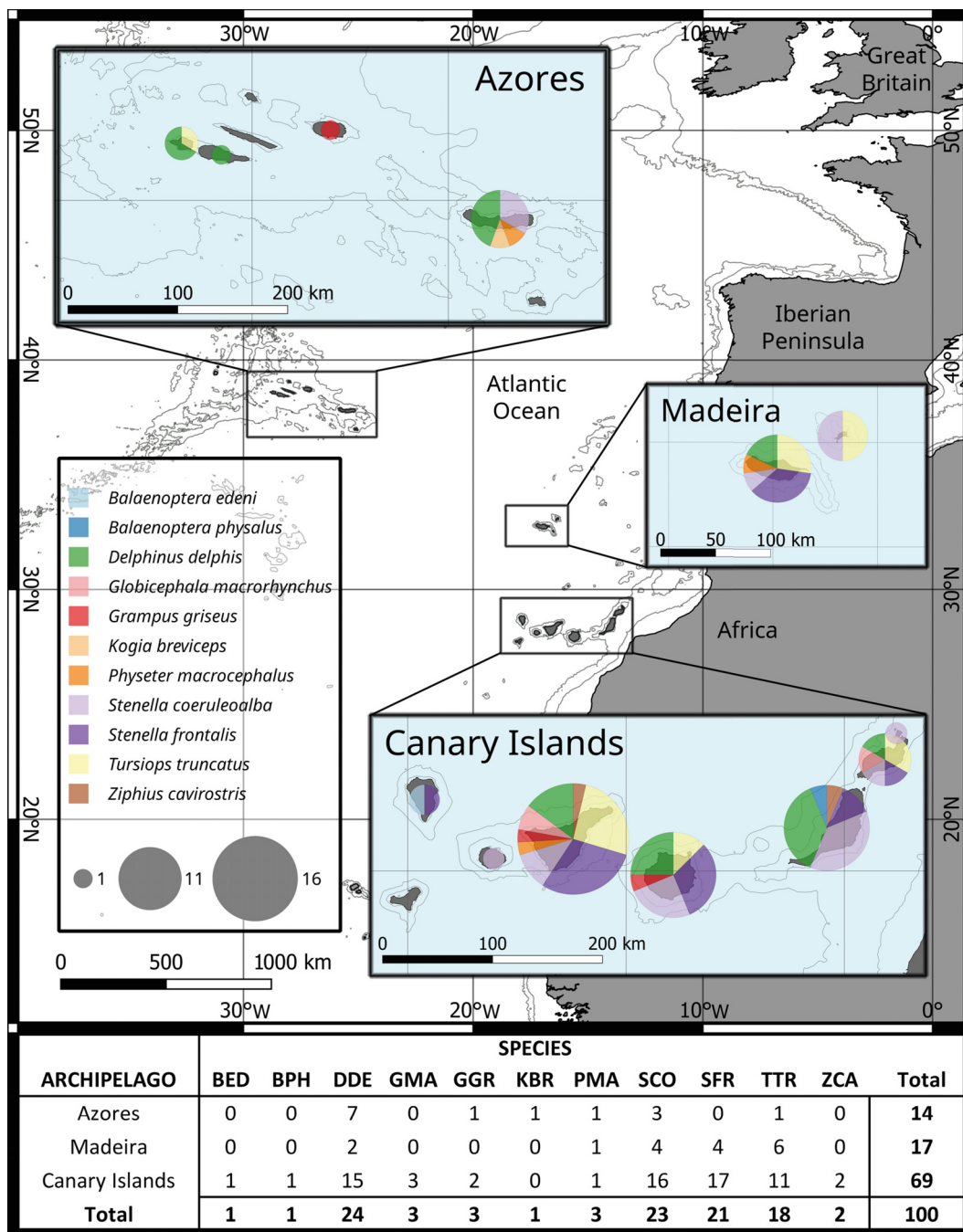


Fig. 1. Locations of the cetacean samples. BED: Bryde's whale *Balaenoptera edeni*, BPH: fin whale *B. physalus*, DDE: common dolphin *Delphinus delphis*, GMA: short-finned pilot whale *Globicephala macrorhynchus*, GGR: Risso's dolphin *Grampus griseus*, KBR: pygmy sperm whale *Kogia breviceps*, PMA: sperm whale *Physeter macrocephalus*, SCO: striped dolphin *Stenella coeruleoalba*, SFR: Atlantic spotted dolphin *S. frontalis*, TTR: bottlenose dolphin *Tursiops truncatus*, ZCA: Cuvier's beaked whale *Ziphius cavirostris*

the contributions of the nitrogen baseline and the TP to the bulk  $\delta^{15}\text{N}$ , using the information contained in the consumer tissues (McMahon & McCarthy 2016). These estimations do not require the use of additional samples from a baseline reference consumer

as is required for the TP estimations using  $\delta^{15}\text{N}$  in bulk tissues. The analytical procedure was described in detail in McCarthy et al. (2013) and Bode et al. (2021). Briefly, 10 mg sample aliquots were hydrolysed with 6 N HCl, and subsequently filtered

through 0.20 µm hydrophilic filters, evaporated to dryness under an N<sub>2</sub> stream, and then treated with 1:5 (v/v) acetyl chloride:2-propanol and 3:1 (v/v) dichloromethane:trifluoroacetic anhydride mixtures to obtain acylated forms of the individual amino acids. These derivatized products were separated using a gas chromatograph (Trace1310GC, Thermo Scientific) equipped with a TraceGOLD TG-5MS chromatographic column (60 m, 0.32 mm ID, and 1.0 µm film), before injection into a mass spectrometer (DeltaV Advantage, Thermo Scientific) using a continuous flow interface (Conflo IV, Thermo Scientific) and a combustion module (GCIsolink, Thermo Scientific). Sample individual amino acid δ<sup>15</sup>N values were calibrated with those obtained from isolated standards (Shoko Science) and analysed by combustion as described for bulk analysis. Additional corrections were made using an internal L-norleucine standard (SIGMA) added to each sample. The mean precision of triplicate samples (2 injections per sample) was 0.30‰ per individual amino acid. The amino acids analysed were categorized as trophic and source following previous studies (e.g. McMahon & McCarthy 2016). Source amino acids were lysine (Lys) and phenylalanine (Phe). Trophic amino acids included alanine (Ala), valine (Val), leucine (Leu), proline (Pro) and the mixtures of glutamine (Gln) and glutamic acid (Glu), and of aspartamine (Asn) and aspartic acid (Asp). The latter mixtures were caused by acid hydrolysis and were designated as Glx and Asx, respectively. All isotopic determinations were made at the Servicio de Análisis Instrumental of the Universidad da Coruña (Spain).

### 2.3. TP

Estimates of TP were computed for each species based on the δ<sup>15</sup>N values obtained for bulk muscle tissue using the canonical model (e.g. Post 2002):

$$TP_{\text{bulk}} = \frac{(\delta^{15}\text{N}_s - \delta^{15}\text{N}_p)}{\text{TEF}_{\text{bulk}}} + 2 \quad (1)$$

where δ<sup>15</sup>N<sub>s</sub> and δ<sup>15</sup>N<sub>p</sub> are, respectively, the values for the secondary (cetacean species) and primary consumers (reference baseline, TP = 2), and TEF<sub>bulk</sub> is the TEF (i.e. the average increase in δ<sup>15</sup>N) between adjacent trophic levels. In this study, we used mean ± SD values of 3.30 ± 0.26‰ for TEF (McCutchan et al. 2003), and 3.40 ± 1.24‰ (n = 19) for δ<sup>15</sup>N<sub>p</sub>, the latter obtained by averaging the values obtained in different surveys and seasons in the study region for calanoid copepods and mesozooplankton (Fernández

et al. 2014, Bode & Hernández-León 2018). The TEF<sub>bulk</sub> used in this study was selected after comparing the TP<sub>bulk</sub> values resulting from alternative models (Fig. S1 in the Supplement at [www.int-res.com/articles/suppl/m699p167\\_supp.pdf](http://www.int-res.com/articles/suppl/m699p167_supp.pdf)), including universal (Post 2002), cetacean-specific (Borrell et al. 2012), high-protein, diet-specific (McCutchan et al. 2003), or variable (Hussey et al. 2014) TEF<sub>bulk</sub> values with TP estimates from stomach-content analysis (Pauly et al. 1998). Error in TP estimations was calculated by propagating errors of the terms of Eq. (1) (Ku 1966). For comparative purposes, TP<sub>bulk</sub> values for 5 species (common, striped, spotted, bottlenose, and Risso's dolphin) in the Atlantic and Mediterranean were calculated using Eq. (1) from published studies reporting δ<sup>15</sup>N values (see Table S1), as a direct comparison of reported TP values is not feasible because of the different assumptions and models used in each study.

Additional TP estimations were made for common dolphin samples from δ<sup>15</sup>N in amino acids using the equation proposed by Chikaraishi et al. (2009):

$$TP_{\text{AA}} = \frac{(\delta^{15}\text{N}_{\text{Glx}} - \delta^{15}\text{N}_{\text{Phe}} - \beta)}{\text{TEF}_s} + 1 \quad (2)$$

where δ<sup>15</sup>N<sub>Glx</sub> and δ<sup>15</sup>N<sub>Phe</sub> are the values measured in Glx and Phe, the value of β (3.6 ± 0.5‰) is the value proposed for secondary and tertiary consumers (Bradley et al. 2015), and TEF<sub>s</sub> was calculated as in Ruiz-Cooley et al. (2021):

$$\text{TEF}_s = \frac{(\delta^{15}\text{N}_{\text{Glx}} - \delta^{15}\text{N}_{\text{Phe}} - \beta)}{\text{TP}_{\text{sc}} - 1} \quad (3)$$

from the expected value of 4.2 for TP<sub>sc</sub> based on stomach contents (Pauly et al. 1998).

### 2.4. Statistical analysis

Comparisons of TP among common, striped, spotted and bottlenose dolphins were made using ANOVA, while Student *t*-tests were employed for paired comparisons between TP estimations or archipelagos. For the latter, and after a preliminary exploration of the data, Madeira and Azores values were combined and compared with those from the Canary Islands to equilibrate the amount of data in each category. Normality and homoscedasticity of data were assessed using Shapiro-Wilks and Levene tests, respectively. The Welch correction was applied in case of unequal variances. Bonferroni post hoc tests were employed for paired comparisons. In addition, the Canary Islands samples were analysed to



test between-year variations in TP and isotopic values of these species using the Mann-Kendall trend test and linear regression (since exploratory analysis provided no clear indication of non-linear trends and the small dataset offers limited scope to characterize a non-linear relationship). A correction for the decadal decrease in atmospheric  $\delta^{13}\text{C}$  (Suess effect), equivalent to  $-0.022\text{‰ yr}^{-1}$  in the subtropical Atlantic (Quay et al. 2003), was applied prior to all statistical analyses. All these analyses were made using the statistical package PAST v. 4.09 (Hammer et al. 2001).

### 3. RESULTS

#### 3.1. Trophic hierarchy

The mean values of  $\text{TP}_{\text{bulk}}$  ( $\pm\text{SD}$ ) for the studied cetaceans ranged from  $3.25 \pm 0.48$  for fin whale and  $4.95 \pm 0.50$  for sperm whale (Fig. 2). Delphinids (Fig. 3) could be grouped as low TP (common and spotted dolphin) and high TP (pilot whale, Risso's, bottlenose, and striped dolphins) species according to significant differences between their  $\text{TP}_{\text{bulk}}$  values (ANOVA and Bonferroni post hoc tests,  $F_{3,83} = 6.055$ ,  $p < 0.05$ ).

Mean TP estimations for the common dolphin from  $\delta^{15}\text{N}$  in amino acids were not significantly different from those based on bulk  $\delta^{15}\text{N}$  (Student *t*-test,

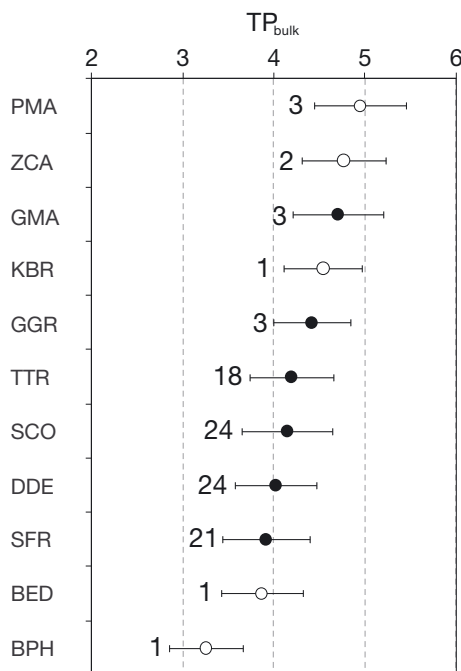


Fig. 2. Mean ( $\pm$  propagated SD) estimations of trophic position (TP) of the studied species from bulk measurements of  $\delta^{15}\text{N}$ . Species codes as in Fig. 1. Black dots: Delphinidae. The number of individuals sampled are indicated for each species

$p > 0.05$ ,  $n = 16$ , Fig. 4). Indeed, the values for trophic amino acids (but not those for source amino acids) were significantly correlated with bulk  $\delta^{15}\text{N}$  (Fig. S2). The mean ( $\pm\text{SD}$ ) value obtained for  $\text{TEF}_s$  was  $3.80 \pm 0.93\text{‰}$  ( $n = 16$ ).

#### 3.2. Spatial and temporal variations

There were no significant differences in  $\text{TP}_{\text{bulk}}$  or  $\delta^{15}\text{N}$  between archipelagos (Canary vs. Madeira + Azores) for the selected dolphin species (Table 1). However,  $\delta^{13}\text{C}$  for the striped dolphin was significantly higher in the Canary Islands than in Madeira + Azores (Table 1).

When considering only the samples from the Canary Islands, there were several significant trends in TP or isotopic values between 2000 and 2018 (Table S2, Fig. 5). In the case of the common dolphin, there was a significant increase in bulk  $\delta^{15}\text{N}$  (but not in  $\delta^{13}\text{C}$ ) that corresponded to a mean  $\pm$  SD increase of  $0.35 \pm 0.50$  TP per decade (Fig. 5A). Similar increases were also found for  $\delta^{15}\text{N}$  in trophic (but not in source) amino acids (Fig. 6). In contrast, the bottlenose dolphin showed significant linear decrease in bulk  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and particularly in  $\text{TP}_{\text{bulk}}$ , the latter changing at a rate of  $-0.50 \pm 0.19$  TP per decade (Fig. 5J). Striped and spotted dolphins did not show significant trends in either bulk TP or  $\delta^{15}\text{N}$ .

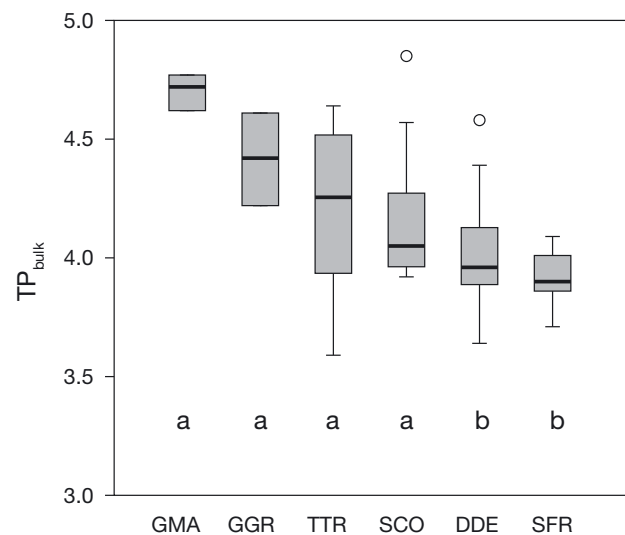


Fig. 3. Box plot of trophic position estimations ( $\text{TP}_{\text{bulk}}$ ) of dolphin species from  $\delta^{15}\text{N}$  in bulk muscle and herbivorous zooplankton as the baseline. Different lowercase letters: significant differences (ANOVA, Bonferroni,  $p < 0.05$ ); box: 25 and 75% quartiles; whiskers: 1.5 $\times$  the interquartile range; horizontal line: median; circles: outliers ( $>1.5\times$  the interquartile range). Species codes as in Fig. 1

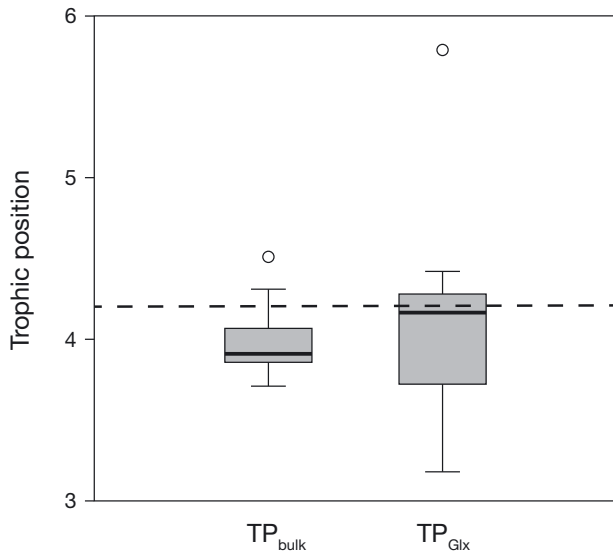


Fig. 4. Trophic position (TP) estimations for the common dolphin from  $\delta^{15}\text{N}$  in bulk muscle ( $\text{TP}_{\text{bulk}}$ ) or amino acids ( $\text{TP}_{\text{Glx}}$ ). Dashed line: TP value expected from stomach content analysis (Pauly et al. 1998). Boxplot parameters as in Fig. 3

## 4. DISCUSSION

### 4.1. Trophic role of dolphins in the region

Our estimations confirm that oceanic dolphins (common, striped, spotted, bottlenose, and Risso's dolphins) are secondary predators in the Macaronesian food web, with mean  $\text{TP}_{\text{bulk}}$  values falling between those of baleen whales (fin and Bryde's whales), which are mainly plankton consumers, and those of pilot, beaked and sperm whales, which are tertiary predators. These estimations are all in the

range of values previously estimated for marine mammals from diet composition. For instance, Trites (2019) report a mean TP of 4.21 for 32 species of Delphinidae, almost coincident with the mean value determined in this study for the 6 species considered (mean TP: 4.23, ranging from 3.91 for the spotted dolphin to 4.70 for the short-finned pilot whale). The estimated values for the Macaronesian dolphins are also within the range of values for the same species computed using published  $\delta^{15}\text{N}$  values in other areas of the Atlantic and Mediterranean (Table S1). They represent the first TP estimates for these species for the Macaronesian region obtained by using the same model and assumptions on TEF and baselines. It should be noted that only a few studies directly reported TP values for these dolphin species, as most of the studies use the stable isotope values for other purposes (e.g. correlations with pollutant content).

Taking into account the estimated errors, the TP values computed in our study agree reasonably well with those originally reported in the literature for other areas, in some cases despite substantial differences in the assumed baseline  $\delta^{15}\text{N}$  and TEF values employed in each particular study. Values of TEF derived from experimental studies in captive cetaceans are only representative of the isotopic fractionation of the trophic step from the diet to the consumers (Caut et al. 2011, Browning et al. 2014, Giménez et al. 2016). The same applies to those derived from the stomach contents of recently captured animals (Borrell et al. 2012). Therefore, it is not surprising that such values are  $<3\%$ , as they reflect the assimilation of high-quality, protein-rich diets, while higher values are generally associated with

Table 1. Student *t*-test comparisons of mean ( $\pm$ SD) values for trophic position estimations ( $\text{TP}_{\text{bulk}}$ ), bulk  $\delta^{15}\text{N}$  and lipid-free  $\delta^{13}\text{C}$  of selected dolphin species by zones (Canary vs. Madeira + Azores). Values of  $\delta^{13}\text{C}$  corrected for Suess effect (see Section 2.4). Species codes as in Fig. 1. **Bold**: significant ( $p < 0.05$ ). The number of data values for each species indicated in parentheses

		DDE	SCO	SFR	TTR
$\text{TP}_{\text{bulk}}$	Canary Islands	$3.97 \pm 0.11$ (15)	$4.13 \pm 0.23$ (16)	$3.91 \pm 0.12$ (17)	$4.13 \pm 0.38$ (11)
	Azores + Madeira	$4.09 \pm 0.31$ (9)	$4.19 \pm 0.26$ (7)	$3.92 \pm 0.12$ (4)	$4.28 \pm 0.26$ (7)
	Student <i>t</i>	1.186	0.515	0.207	0.958
	p	0.265	0.618	0.845	0.353
$\delta^{15}\text{N}$	Canary Islands	$9.90 \pm 0.38$ (15)	$10.42 \pm 0.75$ (16)	$9.69 \pm 0.39$ (17)	$10.44 \pm 1.24$ (11)
	Azores + Madeira	$10.32 \pm 1.03$ (9)	$10.62 \pm 0.86$ (7)	$9.75 \pm 0.40$ (4)	$10.92 \pm 0.87$ (7)
	Student <i>t</i>	1.178	0.520	0.233	0.952
	p	0.268	0.615	0.826	0.355
$\delta^{13}\text{C}$	Canary Islands	$-17.75 \pm 1.16$ (15)	$-17.33 \pm 0.57$ (16)	$-17.40 \pm 0.49$ (17)	$-17.80 \pm 0.65$ (11)
	Azores + Madeira	$-17.86 \pm 0.56$ (9)	$-17.16 \pm 0.43$ (7)	$-17.24 \pm 0.85$ (4)	$-17.53 \pm 0.34$ (7)
	Student <i>t</i>	0.315	<b>2.946</b>	0.367	1.141
	p	0.756	<b>0.010</b>	0.735	0.271

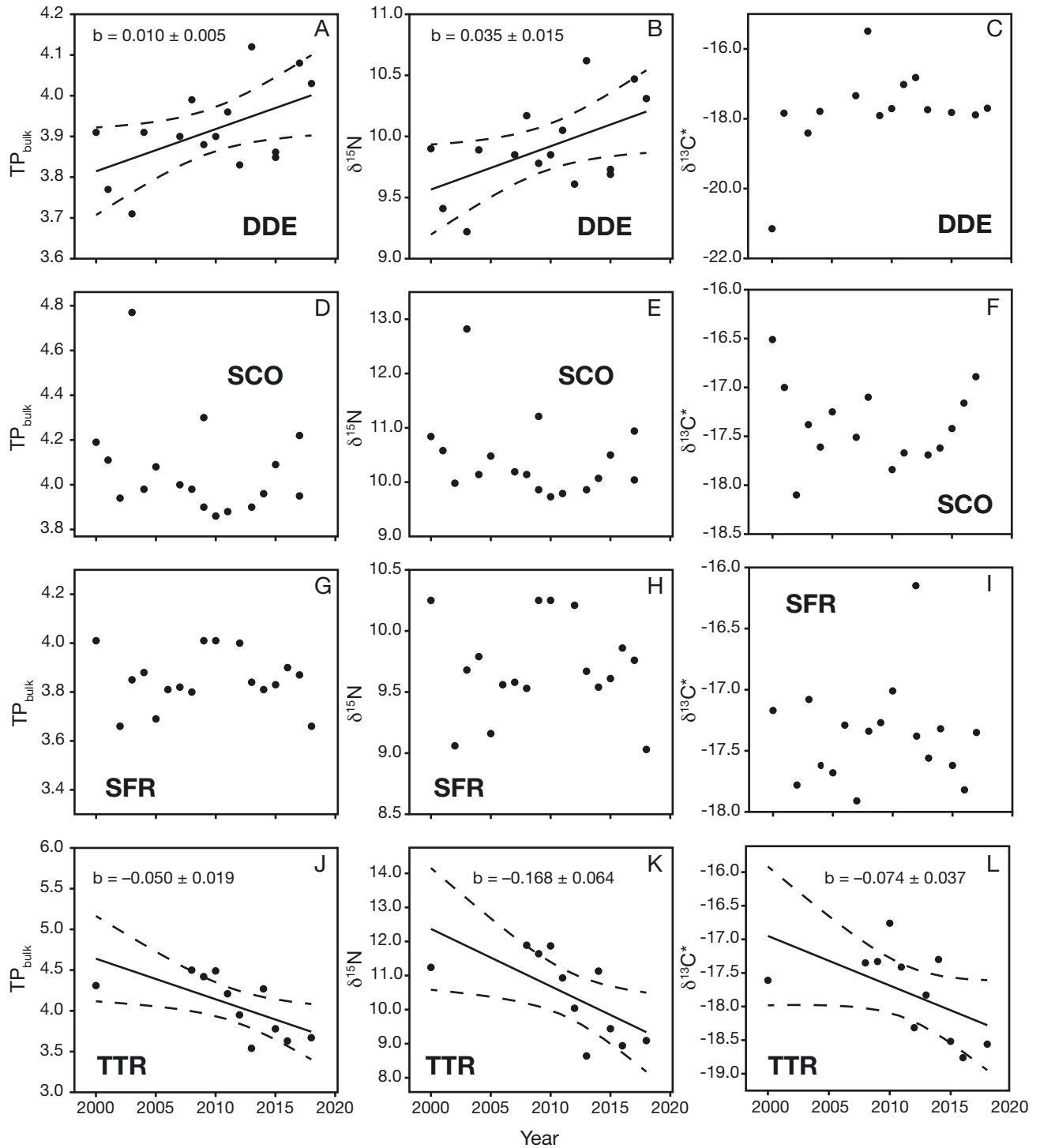


Fig. 5. For (A–C) common dolphin, (D–F) striped dolphin, (G–I) Atlantic spotted dolphin and (J–L) bottlenose dolphin from the Canary Islands: variation of (A,D,G,J) trophic position estimations ( $TP_{bulk}$ ), (B,E,H,K) bulk  $\delta^{15}N$  or (C,F,I,L) lipid-free  $\delta^{13}C^*$  adjusted for the Suess effect ( $\delta^{13}C^*$ ) by year of sampling. Continuous line: significant ( $p < 0.05$ ) regression line; dashed lines: 95% confidence intervals; b: regression slope ( $\pm SE$ ). Species codes as in Fig. 1

low-protein diets (McMahon & McCarthy 2016). In contrast, the TEF values employed in TP determinations, as in the present study, aimed to represent the

whole food web, from lower trophic levels feeding on low-quality diets to higher trophic levels relying on high-quality food. Our TP estimations using a  $TEF_{bulk}$



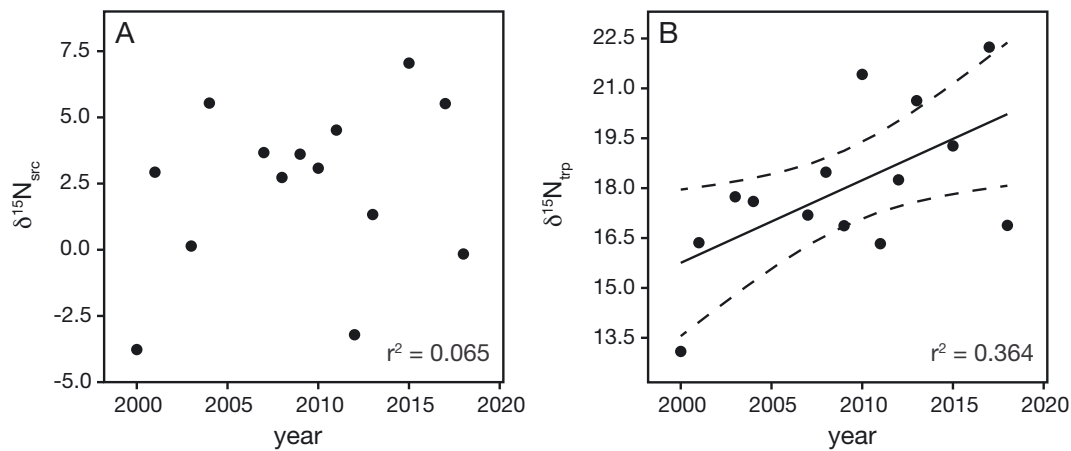


Fig. 6. Variation of  $\delta^{15}\text{N}$  averaged by (A) source ( $\delta^{15}\text{N}_{\text{src}}$ ) or (B) trophic ( $\delta^{15}\text{N}_{\text{tp}}$ ) amino acids by sampling year in the common dolphin from the Canary Islands. Solid line: significant regression line ( $p < 0.05$ ); dashed lines: 95% confidence limits;  $r^2$ : determination coefficient

value of 3.3‰ (McCutchan et al. 2003) are close to the average value estimated from the diet (Pauly et al. 1998, Trites 2019), while those using a cetacean muscle TEF value (but obtained for a planktivorous species, Borrell et al. 2012) were higher than diet-based TP (Fig. S1). However, the application of TEF values scaled to the  $\delta^{15}\text{N}$  values in primary consumers, as proposed for predatory fish (Hussey et al. 2014), produced unrealistically low TP values for dolphin species.

We also demonstrated that TP estimations from  $\delta^{15}\text{N}$  in bulk muscle tissue of the common dolphin were equivalent to those derived from  $\delta^{15}\text{N}$  in amino acids. The latter required an estimation of  $\text{TEF}_s$  which resulted in a value of 3.8‰, similar to that of 3.1‰ reported in a previous study by Ruiz-Cooley et al. (2021) of the same species in the Gulf of Mexico. These new values proposed for cetaceans feeding on fish and cephalopods were much lower than the universal  $\text{TEF}_s$  values (Chikaraishi et al. 2009, Nielsen et al. 2015) which were shown to underestimate TP values for cetacean species (Matthews et al. 2020, Ruiz-Cooley et al. 2021). Our results further support the recent evidence of reduced  $\text{TEF}_s$  for top predators to account for the length expected for marine food webs (McMahon & McCarthy 2016, Ruiz-Cooley et al. 2021). Therefore, using appropriate estimations of  $\text{TEF}_s$  values for cetacean species, as obtained for the common dolphin in this study, would result in realistic and more constrained TP values, thus avoiding the underestimation of TP caused by universal  $\text{TEF}_s$ . Indeed, the correspondence between bulk and amino acid TP estimates, at least in this species and for muscle samples, supports the continued use of the former in trophic studies of cetaceans because of the

lower costs and analytical efforts required. However, the information provided by the  $\delta^{15}\text{N}$  amino acid profiles allows for a more detailed analysis of the food web, as illustrated in this study by the common dolphin samples. The correlations between amino acid and bulk  $\delta^{15}\text{N}$  indicate that, at least in this species, bulk values were more affected by the changes in TP than by changes in the nitrogen baseline. Also, the lack of correlation between  $\delta^{15}\text{N}$  of source amino acids and  $\delta^{13}\text{C}$  suggests a common carbon baseline for this species. These results support the subsequent interpretation on temporal and spatial variability of TPs, at least for the common dolphin, as discussed in the following sections.

Nevertheless, the compilation of TP values (Table S1) highlights major regional differences for some species but not for others. Mean TP values for common and striped dolphins showed a general decreasing pattern from northern to southern regions, with the highest values in Ireland and the English Channel, intermediate values in the Macaronesian region, and the lowest values in southern Brazil (for the common dolphin). In contrast, mean TP values for adult bottlenose dolphins varied between 4.21 and 5.39 with no apparent geographic pattern. In addition, the observed variability in TP among the species analysed was higher than the variability estimated for these species from global average values of diet data, as reported in Pauly et al. (1998). A comparison of mean TP values showed that diet estimates were restricted to a much narrower range than our TP estimates, particularly for Delphinidae (Fig. S3). The differential preservation of the various types of prey in the stomachs of cetaceans undoubtedly affects the reconstruction of diets from such

data. For instance, cephalopod beaks could remain in the stomachs for longer periods than parts of less robust prey, such as crustacean exoskeletons, thus causing an overestimation of the importance of cephalopods as prey items (Pierce & Boyle 1991, Fernández et al. 2009). Notwithstanding their utility in the absence of other information on TP, our results indicate that global TP estimates from diet data are less sensitive to changes in trophic behaviour than estimates from stable isotopes, at least for cetacean species with a large feeding plasticity. Using regional data (both for diets and stable isotopes) would improve the comparison of TP estimates and the assessment of resource competition between the different species.

The TP values estimated in this study agree, in general, with the expected local diets considering the limited information available for the Macaronesian region. However, while the diet reported for delphinid species in Macaronesia was dominated by cephalopod remains, our results point out to a large variety of TP values depending on the species. For instance, cephalopod remains were the only diet component identified in 3 common dolphin stomachs analysed in the Canary Islands (Fernández et al. 2009) but reached 7% of the mean diet weight in NW Spain and Portugal, where this species consumed mostly fish (Santos et al. 2013, Marçalo et al. 2018). This discrepancy could be an artefact of natural dietary variability and the much lower number of specimens analysed in the Canary Islands compared to the other regions. It is also possible that bias could arise due to the low digestibility of cephalopod beaks in the acidic conditions in the stomach compared to remains of fish (see Pierce & Boyle 1991), coupled with a possibly longer average delay between stranding and discovery of carcasses in Macaronesia than on mainland coasts. Studies in oceanic waters in other regions of the NE Atlantic report relatively high proportions of mesopelagic fish in the diet of this species (Pauly et al. 1998, Pusineri et al. 2007). The reported fish consumption of one bottlenose dolphin in the Canary Islands amounted to >10% of mean diet weight (Fernández et al. 2009), which is consistent with mean TP values higher than those for the common dolphin. Even though both diet and isotope estimations were derived from a relatively low number of individuals in the present study, these differences suggest trophic adaptations of some species to the available resources in each region. If the dominance of cephalopod prey in dolphins from the Canary Islands can be confirmed by studies of more individuals, it could be due to the lower local availability of

fish caused by fishery overexploitation, thus mimicking the switch observed in fisheries catches in the Saharan Bank after the decline in catches of Sparidae (Balguerías et al. 2000). In addition, the open ocean is likely to contain more deep-water cephalopods than areas with large continental shelves (Clarke 1996). Furthermore, there is evidence of an increase in the abundance of cephalopods at a global scale, particularly in temperate regions, because of fishery effects and environmental changes (Doubleday et al. 2016), thus favouring their consumption by cetacean predators. In any case, the different dolphin species seem well adapted to withstand changes in the availability of prey, as they show a high foraging plasticity (Pusineri et al. 2007, Santos et al. 2007, 2013, Fernández et al. 2009, Giménez et al. 2017, 2018, Marçalo et al. 2018).

#### 4.2. Decadal changes in TP and origin of resources (Canary Islands)

The trends observed in the isotopic composition and TP for Canary Island common and bottlenose dolphins between 2000 and 2018 point to major decadal changes in the ecosystem and/or a gradual feeding adaptation in these species. Because of the limited number of samples available for the studied period, these patterns need to be interpreted with caution, but at the same time they seem to confirm our hypothesis of species-specific changes in the diet or in the isotopic baseline. Linear trends were assumed, since visualization of the data did not suggest non-linear trends. However, there is wide variation around the fitted lines and the dataset is small, so it is difficult to rule out non-linear changes. The increase in TP for the common dolphin was not accompanied by a change in the baseline, thus implying a change in feeding habits related to prey availability rather than a change in the nutrients provided by the upwelling. This species would have to progressively increase the ratio between high- vs. low-TP prey to reach the TP values found in recent years. One possible explanation could be increased feeding on large cephalopods or fish with high TP (Murphy et al. 2020), and decreased feeding on smaller cephalopods and planktivorous fish with low TP, such as sardines and anchovies (Bode et al. 2018). As discussed above, it is likely that the diet of this species in the Canary Islands would include not only cephalopods, whose beaks are generally well preserved in the stomachs of the stranded dolphins (Fernández et al. 2009), but also variable proportions of

fish, as reported in other regions (Santos et al. 2013, Giménez et al. 2018).

For the bottlenose dolphin, a decadal decrease in both TP and baseline (i.e. carbon isotopes) implies both a change in the type of prey and in their origin. In this case the decreasing trend was evident even without taking into account the only sample available prior to 2008 (Fig. 5L). For instance, such changes would be achieved by an increase in the consumption of planktivorous fish (i.e. low TP) that would be captured progressively further offshore, as  $\delta^{13}\text{C}$  values of plankton and pelagic consumers become more negative (i.e. depleted in  $^{13}\text{C}$  vs.  $^{12}\text{C}$ ) in oceanic, less productive and deeper waters (Fry & Wainright 1991, Perry et al. 1999). Feeding on fish by this species was reported for the Canary Islands (Fernández et al. 2009) and other Atlantic regions (Santos et al. 2007, Giménez et al. 2017). Along the Atlantic coast of Spain there is evidence that coastal and offshore populations of bottlenose dolphins have different diets, with offshore dolphins characterized by lower values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Fernández et al. 2011). It not clear whether there is more than one ecotype present in Macaronesia (Tobeña et al. 2014), and this may require further exploration. In contrast, no trends in TP or in the baseline were apparent for striped and spotted dolphins in this study, further supporting a differential impact of environmental changes on each species.

The trends described are consistent with recent oceanographic changes in the region. One major factor was likely the significant increase in the upwelling-driving winds reported for recent decades in the 26° N–33° N area (Benazzouz et al. 2015). With more intense winds, the mean cross-shore Ekman transport was estimated to increase about 32% per decade between 1981 and 2011, thus supporting the hypothesis of Bakun (1990), predicting an intensification of upwelling because of the higher thermal gradient between the land and water masses caused by global warming. While moderate upwelling favours high local primary production supporting high biomasses of consumers (e.g. plankton and planktivorous fish), an excess of the Ekman transport hinders the efficient use of resources in the upwelling zone. Intense upwelling mixing prevents large phytoplankton blooms (Largier et al. 2006) and dilutes key trophic prey (e.g. zooplankton) in the oligotrophic ocean (Bakun & Weeks 2006). The increased upwelling may explain the decreasing trend reported for primary production in this region between 1998 and 2007 (Aristegui et al. 2009, Demarcq 2009). Correspondingly, there was also a decrease in the other-

wise abundant sardine and anchovy populations after 2000, as evidenced both by annual fisheries catches and model results (Sánchez-Garrido et al. 2019, 2021). A similar decrease in both plankton and planktivorous fish biomass since the late 1990s in the region north of Cape Blanc (21° N) was reported based on ecological models and acoustic surveys, respectively (Diogoul et al. 2021). All these trends are consistent with the increase in TP of common dolphin and the decrease in TP and possible change in habitat for bottlenose dolphin. The changes in the common dolphin suggest an adaptation to prey availability, as reported for waters near the Iberian Peninsula where this species changed its diet following decreases in sardine stocks (Santos et al. 2013, but see Marçalo et al. 2018). Our conclusions align with those found in other upwelling ecosystems, challenging the paradigm of the stability of biogeochemical cycles and food web structure in these systems (Ruiz-Cooley et al. 2014, 2017).

## 5. CONCLUSIONS

Based on  $\delta^{15}\text{N}$  in their muscle, oceanic dolphins ranked as secondary predators in the Macaronesian food web. Our TP estimates from bulk samples were comparable to those expected from the published diet for the studied species in this region. In the case of the common dolphin, these estimates agreed well also with those obtained from nitrogen isotopes in amino acids. These results support the continued use of isotopic determinations in bulk tissues for TP estimations, but also the more informative estimations based on  $\delta^{15}\text{N}$  in amino acids.

Despite the limited number of samples available, the relatively large trophic variability displayed by the studied dolphins supports their use as indicators of major changes in the regional ecosystem at spatial and decadal timescales. For instance, trends in stable isotope data for some dolphin species in the Canary Islands were consistent with a decrease in primary production, plankton and planktivorous fish since the late 1990s. However, these changes varied among the different dolphin species. While there was a progressive increase in TP of common dolphin, a typically oceanic species, there was a decrease in TP for bottlenose dolphin that apparently moved towards more oceanic habitats, as indicated by  $\delta^{13}\text{C}$  values (although other possible explanations need to be explored in this case).

While these conclusions need to be confirmed with more data, including direct observations on the dol-

phins' diet and by analysing changes in the species distributions related to environmental variables, this study adds further evidence to the value of ecological indicators derived from stable isotopes to understand large-scale changes in oceanic food webs and biogeochemical cycles.

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