



# Trophic ecology of three sympatric batoid species (*Dasyatis pastinaca*, *Raja clavata*, and *Raja maderensis*) from the Azores, NE Atlantic

Paco Fontaine · João Pedro Barreiros · Sébastien Jaquemet

Received: 22 September 2022 / Accepted: 1 April 2023 / Published online: 20 April 2023  
© The Author(s), under exclusive licence to Springer Nature B.V. 2023

**Abstract** Describing the trophic structure and interactions of demersal elasmobranch assemblages is fundamental to understanding food web dynamics and developing ecosystem-based management approaches. Stomach content analysis (SCA) and stable isotope ratios (SIA) of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) from muscle were used to examine the dietary habits and intra- and interspecific trophic ecology of three sympatric batoid species (*Dasyatis pastinaca*, *Raja clavata*, and *Raja maderensis*) from the Azores, North-east Atlantic. Data were analyzed with respect to sex and maturity stages. SCA showed that *D.*

*pastinaca* feeds mostly on crustaceans, whereas *R. clavata* and *R. maderensis* prey almost exclusively on teleosts, but not on the same species. *Dasyatis pastinaca* displayed higher  $\delta^{13}\text{C}$  and lower  $\delta^{15}\text{N}$  values compared to *R. clavata* and *R. maderensis*. Trophic niche breadth was variable, *D. pastinaca* and *R. clavata* had the broadest and the narrowest trophic breadth, respectively. Relative trophic position categorized *D. pastinaca* as a mesopredator, while *R. clavata* and *R. maderensis* occupied higher trophic positions. With size, *R. clavata* and *R. maderensis* shifted from small prey such as crustaceans to larger prey such as teleosts, and they also exhibited significant increases in  $\delta^{15}\text{N}$  with size. Dietary and isotopic overlap was overall low among species, but it was higher between *R. clavata* and *R. maderensis*, suggesting more similarity in diet and habitat use between them than with *D. pastinaca*. This study depicts trophic interactions and functional roles of three co-existing batoid species in the Azorean food webs. In addition to presenting new information on the trophic ecology of *D. pastinaca* and *R. clavata*, the present study provides, to our knowledge, the first description of the diet composition and trophic level of the Macaronesian endemic batoid *R. maderensis*.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s10641-023-01412-2>.

P. Fontaine (✉) · S. Jaquemet  
UMR Entropie, Faculté Des Sciences Et Technologies,  
Université de La Réunion, 15, Avenue René Cassin - CS  
92003, 97744 Saint Denis Cédex 9, Réunion Island, France  
e-mail: 40007542@co.univ-reunion.fr

S. Jaquemet  
e-mail: sebastien.jaquemet@univ-reunion.fr

J. P. Barreiros  
cE3c- Centre for Ecology, Evolution and Environmental  
Changes, Azorean Biodiversity Group, CHANGE –  
Global Change and Sustainability Institute, Faculty  
of Agricultural Sciences and Environment, University  
of the Azores, Rua Capitão João d'Ávila, Pico da Urze,  
9700-042 Angra Do Heroísmo, Portugal  
e-mail: joao.ps.barreiros@uac.pt

**Keywords** Elasmobranch · Feeding ecology · Stomach contents · Trophic tracers · Macaronesia

## Introduction

Elasmobranchs often act as apex or mesopredators in marine food webs (Heupel et al. 2014). In coastal marine environments, batoids (superorder Batoidea comprising skates, stingrays, electric rays, and guitarfishes) are one of the most abundant and diverse groups of elasmobranchs, with ca. 633 species worldwide (Last et al. 2016; Flowers et al. 2021). They are important components of soft bottoms and vegetated marine systems, not only through predation (Grubbs et al. 2016; Barrios-O'Neill et al. 2017; Flowers et al. 2021) but also through bioturbation, a key process in biogeochemical cycles (Laverock et al. 2011; Takeuchi and Tamaki 2014; Flowers et al. 2021). While research on elasmobranchs has resulted in a comprehensive understanding of shark trophic ecology and their role in ecosystems (Heupel et al. 2014), such knowledge remains limited for batoids (Vaudo and Heithaus 2011). Additionally, although quantitative dietary information for batoids has progressively improved in recent years, there remains a lack of studies for many existing species in some areas, such as the Mediterranean and the Northeast Atlantic (Das and Afonso 2017; Coll-Calvo et al. 2020). The food preferences of batoids can vary regionally as a consequence of their opportunist/generalist foraging behavior in which prey availability and competition for trophic resources are important factors to determine the trophic niche of these elasmobranchs (Flowers et al. 2021). Additionally, batoids are characterized by a slow growth, late maturity and low fecundity, making their population extremely vulnerable to decline consequent to overexploitation and habitat destruction (Stevens et al. 2000; Dulvy et al. 2014).

Elasmobranch trophic ecology research has traditionally been based on stomach content analysis (SCA) (Hyslop 1980; Cortés 1999; Ebert and Bizzarro 2007). SCA provides invaluable information about the prey species consumed by the studied species (Hyslop 1980; Cortés 1999); however, the method is time consuming, typically lethal, and only captures short-term diet information (Hyslop 1980; Hussey et al. 2011). The analyses of stable isotopes (SIA) of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) have been used as complementary tools to investigate long-term dietary information (Vaudo and Heithaus 2011; Shiffman et al. 2012). The stable isotope of carbon ( $\delta^{13}\text{C}$ ) is used to track movements between isotopically

distinct habitats and to identify different sources of carbon, whereas the stable isotope of nitrogen ( $\delta^{15}\text{N}$ ) reveals relative trophic position within a food web (Hussey et al. 2011; Kinney et al. 2011). The combination of stomach contents and stable isotope analyses ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) provides a robust description of the trophic ecology of elasmobranchs within a food web (Shiffman et al. 2012).

The Azores islands, the north-westernmost archipelago of the Macaronesia region, is spread across 600 km in the Northeast Atlantic (Santos et al. 1997). The absence of an insular shelf limits fishing effort to the island's slopes and the surrounding seamounts within the area (Da Silva and Pinho 2007; Diogo et al. 2015). Despite the fact that the Azores archipelago hosts a rich marine fauna and is a hotspot of diversity of elasmobranchs (41 shark species and 16 batoid species) (Barreiros and Gadig 2011; Afonso et al. 2020; Santos et al. 2020), studies investigating the trophic habits of batoids remain scarce in this area (Morato et al. 2003; Ponte et al. 2016; Torres et al. 2016a; Santos et al. 2020). In the Azores, most of the literature on the trophic ecology of batoids focuses on the description of prey items in guts, while only one study is based on carbon and nitrogen stable isotope analysis (Torres et al. 2016a). In addition, previous studies usually describe the diet of one species, while studies on inter- and intraspecific interactions remain limited (Morato et al. 2003; Ponte et al. 2016). On the other hand, many batoids have declined in abundance across the Azores waters, and most of them are considered threatened or near-threatened according to the IUCN (Das and Afonso 2017). Most batoids captured in the Azores are not directly targeted but included in multispecies fisheries or are bycatch in the few traditional fisheries targeting commercially valuable teleost species (Torres et al. 2016b).

In the coastal zone of the Azores archipelago, skates and rays comprise a large proportion of the elasmobranch community (16 species) (Barreiros and Gadig 2011; Afonso et al. 2020; Santos et al. 2020). The common stingray *Dasyatis pastinaca* (Linnaeus 1758), the thornback ray *Raja clavata* (Linnaeus 1758), and the madeiran ray *Raja maderensis* (Lowe 1838) are three batoid species occurring in the region (Barreiros and Gadig 2011; Afonso et al. 2020; Santos et al. 2020). *Dasyatis pastinaca* occupies benthopelagic habitat down to 200 m deep but seems to be more common in shallow shelves (< 50 m)

(Ponte et al. 2016; Santos et al. 2020). This species is common in the Azores, and is also found from southern Norway to South Africa and the entire Mediterranean (Barreiros and Gadig 2011; Ponte et al. 2016). *Raja clavata* is a shallow-water, bottom-living elasmobranch whose distribution extends from Iceland to South Africa and throughout the Mediterranean (Morato et al. 2003; Barreiros and Gadig 2011). In the Azores, *R. clavata* is frequent in the shallow shelf slope/shelf break assemblage (<200 m) over sandy grounds and represents more than 90% of the biomass of the landed skates in this region (Barreiros and Gadig 2011; Santos et al. 2020, 2021). *Raja maderensis* is a poorly known endemic species, restricted to waters close to Madeira and the Azores (Barreiros and Gadig 2011). The species is rare in the Azores and conversely to *D. pastinaca* and *R. clavata*, it belongs to the upper slope fish assemblage (200–600 m) (Santos et al. 2020). *R. clavata* is classified as Least Concern (NT) worldwide (Ellis et al. 2016), while *D. pastinaca* and *R. maderensis* are classified as Vulnerable (VU) (Dulvy et al. 2015; Jabado et al. 2021).

Although elasmobranchs can be fundamental to the structure and functioning of marine food webs (Navia et al. 2017), especially batoids, few studies have described their diets in the Azores (Morato et al. 2003; Ponte et al. 2016; Torres et al. 2016a). The diet of *R. maderensis* particularly remains poorly documented throughout the distribution range of the species. Prior studies described *D. pastinaca* as a broad spectrum mesopredator exhibiting epibenthic feeding habits with a diet consisting of large proportions of decapod crustaceans (Ponte et al. 2016). In contrast, *R. clavata* in the Azores preys heavily upon fish at a higher trophic level (Morato et al. 2003; Torres et al. 2016a). Based on similarity with *R. clavata* (Serra-Pereira et al. 2011; Ball et al. 2016; Torres et al. 2016b), *R. maderensis* is presumed to mostly feed on teleosts. Despite their ecological importance and the worsening of their conservation status, there is a lack of studies on the trophic ecology of these species and its variability in relation with sex and age.

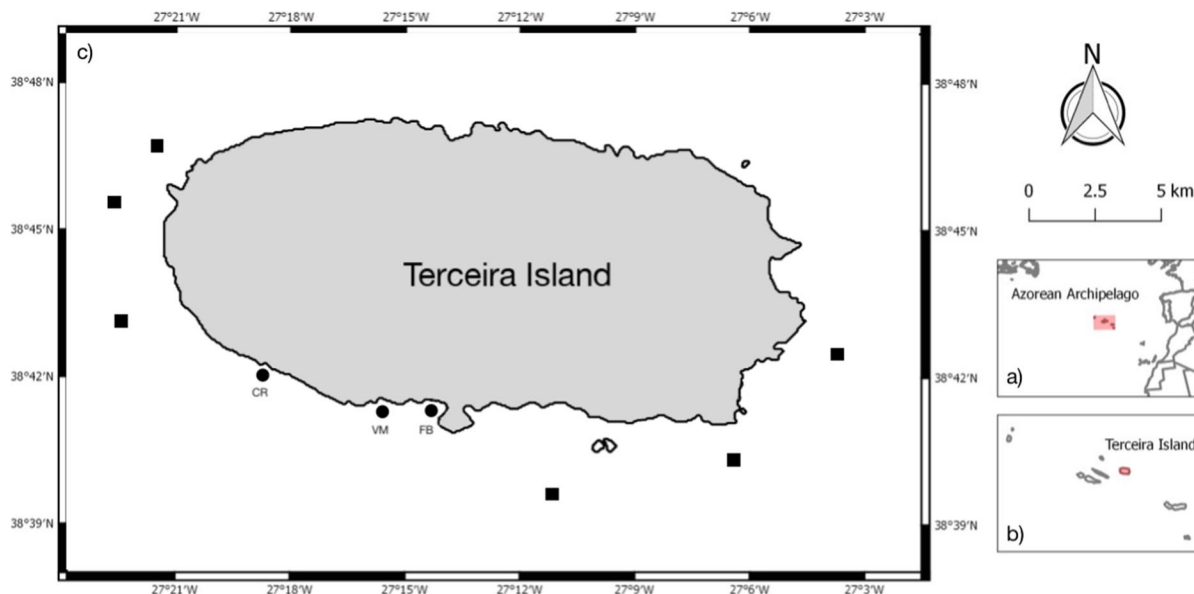
In this study, the trophic ecology of three sympatric batoids was assessed by combining stomach contents and stable isotope ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) approaches. Our specific objectives were to (1) investigate the diets of *D. pastinaca*, *R. clavata* and *R. maderensis*; (2) identify sex and age-related changes in diet; and

(3) assess any inter- and intraspecific dietary and trophic niche overlap between the three species in the Azores. The study provides new information about the ecological role of *D. pastinaca*, *R. clavata* and *R. maderensis* within the Azores region, offering new data on foraging habitats of these species.

## Materials and methods

### Study area and sample collection

This study was conducted between September 2021 and May 2022 in Terceira Island, Central Azores (Fig. 1). The convoluted coastline offers a variety of coastal habitats, including rocky reefs, boulder fields, cliffs, enclosed sandy bays, which are subject to various hydrodynamic conditions (Gomes and Pinto 2004). Specimens of *D. pastinaca* were captured at three sites along the coast of Terceira Island that are from west to east: Cinco Ribeiras (CR), Vila Maria Bay (VM), and Fanal Bay (FB) (Fig. 1). All individuals were caught using spearfishing techniques by a shot in the skull to limit animal suffering (Diggles et al. 2011). This method prevents regurgitation of stomach contents (Bowen 1996) and makes the selection of individuals easier (Frisch et al. 2012; Coll-Calvo et al. 2020). As commercial fisheries do not catch this species, this was the only possible way to obtain specimens. Two professional spearfishers conducted one to two dives a month during the study period by daylight (no night dives were undertaken) in water depths ranging from 0.5 to 20 m. Individuals of *R. clavata* and *R. maderensis* were obtained from bottom longlines targeting demersal species. This multi-species fishery uses mainly small vessels (<14 m) operating on the rocky slopes of the island and on seamounts down to 600 m (Da Silva and Pinho 2007; Carvalho et al. 2011). Line setting started one hour before sunrise and line retrieval started about 1.5 h after sunrise (Da Silva and Pinho 2007; Carvalho et al. 2011). After recording total length ( $T_L$ , to the nearest cm), sex and maturity were determined by macroscopic examination following Stehmann (2002). Life stages were classified as immature (immature, developing) or mature (spawning capable). All specimens were collected and processed in accordance with the University of Azores Ethics Committee approval (reference n°9/2021).



**Fig. 1** (a) Location of the Azorean Archipelago in the North-east Atlantic and (b) Terceira Island in the Azores. (c) Map of Terceira Island with the distribution of the sampling sites

(■ = samples collected during commercial fisheries; ● = samples collected during spearfishing). CR, Cinco Ribeiras; VM, Vila Maria Bay; FB, Fanal Bay

### Stomach content analysis (SCA)

The stomach was dissected from each individual and the contents were extracted and stored in individual sealable plastic bags. All stomach content samples were kept in a cool box following collection and thereafter frozen at  $-20\text{ }^{\circ}\text{C}$  in the laboratory until further analysis.

Stomach contents were emptied, rinsed with water, and prey were sorted and identified to the lowest taxonomic level possible under a dissecting microscope with the help of taxonomic guides (Cailliet et al. 1996; Hayward and Ryland 1990; Debelius 1997; Saldanha 2003). Prey items were individually counted and weighed (wet weight). As most food remains were highly digested, the identification of prey was limited, and as such all prey items were pooled into four prey categories: (1) Polychaeta, (2) Crustacea, (3) Mollusca, (4) Teleost. A vacuity index (%V) was calculated as the percentage of empty stomachs (Hyslop 1980). Cumulative prey curves were used to determine whether the number of stomachs with identifiable preys were adequate to accurately describe the diet of the species. The final four curve points

for each species, and for each subgroup (female, male, immature, mature) were statistically compared with the zero slope line using a Student's t-test (Bizzarro et al. 2007).

The importance of various prey species to the diet of *D. pastinaca*, *R. clavata*, and *R. maderensis* was assessed by calculating the percent frequency of occurrence (%FO), average percent number (%N), average percent weight (%W), prey-specific number (%PN), and prey-specific weight (%PW) (Hyslop 1980; Brown et al. 2012). The prey-specific index of relative importance (%PSIRI) was used to measure the relative importance of each prey group in the diet of *D. pastinaca*, *R. clavata*, and *R. maderensis* (Brown et al. 2012). These indices were calculated as follows:

Frequency of occurrence (FO):

$$\%FO_i = \frac{n_i}{n} \times 100,$$

Average percent abundance (%N<sub>i</sub>, %W<sub>i</sub>):

$$\%A_i = \frac{\sum_{j=1}^n \%A_{ij}}{n}$$

Prey-specific abundance (%PN<sub>*i*</sub>, %PW<sub>*i*</sub>):

$$\%PA_i = \frac{\sum_{j=1}^n \%A_{ij}}{n_i}$$

Prey-specific index of relative importance (%PSIRI<sub>*i*</sub>):

$$\%PSIRI_i = \frac{\%FO \times_i (\%PN_i + \%PW_i)}{2}$$

where %A<sub>*ij*</sub> is the per cent abundance (by number or weight) of prey category *i* in stomach sample *j*, *n<sub>i</sub>* is the number of stomachs containing prey *i*, and *n* is the total number of stomachs containing prey (Brown et al. 2012).

To measure the trophic niche breadth, the standardized Levin’s index (*Ba*) (Krebs 1999) was calculated:

$$Ba = \frac{\frac{1}{\sum P_{ij}^2} - 1}{n - 1}$$

where *P<sub>ij</sub>* is the proportion of the prey *j* in the diet of the species *i*, and *n* is the number of prey categories. This index ranges from 0 (minimum niche breadth and maximum specialization) to 1 (maximum niche breadth and minimum specialization).

The trophic position (TP<sub>*j*</sub>) was defined for species and subgroups according to Cortés (1999):

$$TP_j = 1 + \left( \sum_{j=1}^n DC_{ji} \times TP_i \right)$$

where TP<sub>*j*</sub> is the trophic level of the species (*j*), DC<sub>*ji*</sub> is the proportion of the prey category (*i*) in predator diet (*j*); *n* is the total number of prey categories, and TP<sub>*i*</sub> is the trophic level of the prey group (*i*). Trophic levels of prey categories were assigned based on the literature (Supplemental Table 1).

Mann–Whitney–Wilcoxon rank tests for non-normally distributed data were applied using the R function “pairwise.wilcox.test” (R Core Team 2022) to test for pairwise differences in TP between species. A second series of Mann–Whitney–Wilcoxon rank tests were used to test for sex and size-effect among species. Non-metric multidimensional scaling (NMDS) ordination was used to explore variation in the diet composition among species and between subgroups (males, females, immatures, matures). Bray–Curtis dissimilarity matrices were derived based on absolute volume (weight) data of the four prey categories

across all species and subgroups using the R function “vegdist” (Gardener 2014; R Core Team 2022). Given the low number of stomachs collected (see Table 1), gravimetric data pooling was designed to reduce the number of prey categories in the samples with zero values, thus increasing the efficiency of multivariate analysis (White et al. 2004). Prior to conduct permutational multivariate analysis of variance (PERMANOVA), a non-parametric permutational analysis of multivariate dispersions (PERMDISP) was run using the R function “permdisp” of the package vegan, to test the homogeneity of multivariate dispersions of data (Anderson 2006). PERMANOVA was used as implemented in the “adonis” function of the vegan R package (Oksanen et al. 2013) to test for possible statistical differences among species and subgroups’ diets. For pairwise differences between species diet compositions, PERMANOVA tests were run for all species pairs using the “adonis.pair” function of the EcoUtils R package (Salazar 2018; R Core Team 2022), and used Bonferroni correction to set significance levels for *p* values. In the case of significant differences in diet composition were observed, similarity percentage (SIMPER) analysis was conducted using the R function “simper” of the package vegan (Oksanen et al. 2013; R Core Team 2022) to detect which prey categories were the main contributors to the dissimilarity observed.

### Stable isotope analysis (SIA)

Stable isotope analysis was performed on a subset of individuals for each species (see Table 1). Approximately 5 g of white muscle tissue was collected from the dorsal side of rays. Each muscle tissue was rinsed, frozen, dried at 50 °C for 48 h, and then ground into a homogeneous powder using a mortar and pestle. Ground samples were weighed (0.5–1 mg) into tin capsules. δ<sup>13</sup>C and δ<sup>15</sup>C were determined using a continuous flow mass spectrometer coupled to an elemental analyzer, at the Stable Isotope Laboratory of the University of Caen (icore.unicaen.fr). The isotopic composition is reported in the conventional delta (δ) per mil notation (‰), relative to Vienna Pee Dee Belemnite (δ<sup>13</sup>C) and atmospheric N<sub>2</sub> (δ<sup>15</sup>C). Lipids were not extracted from samples. Thus, correction equations were applied for δ<sup>13</sup>C (δ<sup>13</sup>C<sub>LE</sub> = 0.967 × δ<sup>13</sup>C<sub>bulk</sub> + 0.861) and δ<sup>15</sup>C (δ<sup>15</sup>N<sub>LE</sub> = 1.018 × δ<sup>15</sup>N<sub>bulk</sub> + 0.020), where δ<sup>13</sup>C<sub>LE</sub> and δ<sup>15</sup>N<sub>LE</sub> are corrected with

**Table 1** Size range ( $T_L$ , cm), number (% of empty) of stomach content (SCA) and stable isotope samples (SIA), standardized Levin's index (Ba), estimated trophic position based on the diet (TP), Bayesian isotopic ellipse area corrected (SEAc), and estimated trophic position by  $\delta^{15}\text{N}$  (TPSI) for *D. pastinaca*, *R.*

*clavata*, *R. maderensis*, and each of the subgroups. SE = standard error. Different letters indicate significant difference in TP and TPSI values between species (capital letters) and between sexes or maturity stages (lowercase) within each species

Species	Size range (cm)	SCA	SIA	$\delta^{13}\text{C} \pm \text{SE}$	$\delta^{15}\text{N} \pm \text{SE}$	Levin's index (Ba)	TP $\pm$ SE	SEAc	TPSI $\pm$ SE
<i>D. pastinaca</i>	32–59	15 (13%)	12	$-15.2 \pm 1.9^{\text{A}}$	$12.1 \pm 1.9^{\text{A}}$	0.51	$3.48 \pm 0.31^{\text{A}}$	0.68	$3.29 \pm 0.53^{\text{A}}$
Males	36–51	6	7	$-15.3 \pm 2.2^{\text{a}}$	$10.9 \pm 1.0^{\text{a}}$	0.44	$3.43 \pm 0.26^{\text{a}}$	0.71	$3.31 \pm 0.47^{\text{a}}$
Females	32–59	7	5	$-14.9 \pm 1.5^{\text{a}}$	$11.0 \pm 1.4^{\text{a}}$	0.54	$3.46 \pm 0.32^{\text{a}}$	0.66	$3.25 \pm 0.67^{\text{a}}$
Matures	45–59	7	6	$-15.8 \pm 1.8^{\text{a}}$	$11.5 \pm 0.9^{\text{a}}$	0.58	$3.52 \pm 0.38^{\text{a}}$	0.59	$3.52 \pm 0.53^{\text{a}}$
Immatures	32–43	6	6	$-14.5 \pm 1.9^{\text{a}}$	$10.4 \pm 1.2^{\text{a}}$	0.46	$3.41 \pm 0.36^{\text{a}}$	0.65	$3.27 \pm 0.49^{\text{a}}$
<i>R. clavata</i>	35–91	29 (41%)	11	$-17.3 \pm 1.7^{\text{B}}$	$12.1 \pm 0.5^{\text{B}}$	0.17	$4.19 \pm 0.39^{\text{B}}$	0.30	$3.57 \pm 0.32^{\text{A, B}}$
Males	35–85	7	5	$-18.3 \pm 1.1^{\text{a}}$	$12.1 \pm 0.5^{\text{a}}$	0.21	$4.13 \pm 0.42^{\text{a}}$	0.27	$3.53 \pm 0.51^{\text{a}}$
Females	39–91	10	6	$-16.4 \pm 1.7^{\text{a}}$	$12.2 \pm 0.6^{\text{a}}$	0.16	$4.21 \pm 0.44^{\text{a}}$	0.35	$3.58 \pm 0.44^{\text{a}}$
Matures	76–91	6	5	$-18.5 \pm 0.9^{\text{a}}$	$12.3 \pm 0.4^{\text{a}}$	0.12	$4.35 \pm 0.17^{\text{a}}$	0.23	$3.60 \pm 0.58^{\text{a}}$
Immatures	35–69	11	6	$-16.3 \pm 1.6^{\text{b}}$	$11.9 \pm 0.6^{\text{a}}$	0.23	$3.87 \pm 0.21^{\text{b}}$	0.34	$3.34 \pm 0.45^{\text{a}}$
<i>R. maderensis</i>	31–87	23 (39%)	12	$-18.3 \pm 1.4^{\text{B}}$	$12.7 \pm 1.1^{\text{B}}$	0.23	$4.14 \pm 0.33^{\text{B}}$	0.52	$3.76 \pm 0.42^{\text{B}}$
Males	31–79	5	7	$-17.9 \pm 1.6^{\text{a}}$	$13.2 \pm 1.0^{\text{a}}$	0.25	$4.12 \pm 0.38^{\text{a}}$	0.59	$3.69 \pm 0.52^{\text{a}}$
Females	40–87	9	5	$-18.6 \pm 1.5^{\text{b}}$	$12.6 \pm 1.1^{\text{a}}$	0.22	$4.23 \pm 0.41^{\text{a}}$	0.50	$3.73 \pm 0.57^{\text{a}}$
Matures	64–87	7	5	$-19.5 \pm 0.3^{\text{a}}$	$13.6 \pm 0.7^{\text{a}}$	0.16	$4.31 \pm 0.19^{\text{a}}$	0.43	$3.88 \pm 0.65^{\text{a}}$
Immatures	31–58	7	6	$-17.3 \pm 1.4^{\text{b}}$	$12.2 \pm 1.0^{\text{b}}$	0.27	$3.85 \pm 0.17^{\text{b}}$	0.58	$3.62 \pm 0.49^{\text{b}}$

lipid extraction (LE) (Logan et al. 2008). In addition, there was not prior urea extraction from the samples. To ensure a confident interpretation, the mathematical corrections developed for elasmobranchs by (Li et al. 2016) were applied to correct the  $\delta^{15}\text{N}$  ( $\delta^{15}\text{N}_{\text{LE} + \text{UE}} = 0.856 \times \delta^{15}\text{N}_{\text{LE}} + 2.813$ ), where  $\delta^{15}\text{N}_{\text{LE} + \text{UE}}$  is the value of  $\delta^{15}\text{N}$  corrected with LE and urea extraction (UE).

The normality and homoscedasticity of isotope data were tested using the Shapiro–Wilk and Levene's test, respectively. The nonparametric Wilcoxon signed-rank test was used to determine statistical differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  among batoid species and subgroups (sexes and maturity stages).

To evaluate the isotopic niche for each species and for each sex-maturation stage within each species, the smallest convex hulls that contain all individual  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values within a group were calculated to represent the total niche breadth of each species (Layman et al. 2007). As a measure of the trophic breadth, the Bayesian isotopic ellipse area corrected (SEAc) was calculated to reduce bias for small sample size (Jackson et al. 2011). The niche area is defined as the area occupied in bi-plot space in  $\%c^2$  and is considered to be a proxy for the extent of trophic diversity

(or trophic breadth) exploited by species or each sex-maturation stage within each species (high SEAc values indicate high trophic breadth). Due to the absence of isotopic signals of prey, the use of isotopic ellipses as a proxy of trophic diversity should be taken with caution because stable isotope analysis can have difficulty separating groups when the food web base has similar  $\delta^{13}\text{C}$  signatures (Shiple and Matich 2020). Inversely, when the food web base has different  $\delta^{13}\text{C}$  signatures, stable isotope analysis can indicate high trophic diversity while predators feed on few prey items (Shiple and Matich 2020). Bayesian standard ellipses corrected were also used to calculate isotopic niche overlap among groups and were measured as a percentage (%) of the shared isotopic space of each species (Jackson et al. 2011). Differences in SEAc size were considered significant if the 95% credibility intervals of posterior draws did not overlap. These analyses were done using the “SIBER” library in R statistical package v. R 4.0.0 (Jackson et al. 2011).

To estimate the trophic position for species and subgroups using nitrogen stable isotopes, a scaled  $\Delta^{15}\text{N}$  framework approach based on a dietary  $\delta^{15}\text{N}$  value-dependent model was used (Hussey et al.

2014). With knowledge of the  $\delta^{15}\text{N}$  value of a known baseline consumer ( $\delta^{15}\text{N}_{\text{base}}$ ), the  $\delta^{15}\text{N}$  value of the consumer ( $\delta^{15}\text{N}_{\text{TP}}$ ), the dietary  $\delta^{15}\text{N}$  value at which  $\delta^{15}\text{N}$  incorporation and  $\delta^{15}\text{N}$  elimination are equal ( $\delta^{15}\text{N}_{\text{lim}}$ ), and the rate at which the ratio between  $\delta^{15}\text{N}$  incorporation and  $\delta^{15}\text{N}$  elimination changes relative to dietary  $\delta^{15}\text{N}$  averaged across the food-web ( $k$ ), TPSI, is calculated as:

$$\text{TPSI} = \frac{\log(\delta^{15}\text{N}_{\text{lim}} - \delta^{15}\text{N}_{\text{base}}) - \log(\delta^{15}\text{N}_{\text{lim}} - \delta^{15}\text{N}_{\text{TP}})}{k} + \text{TP}_{\text{base}}$$

Given its abundance throughout the Central and North East Atlantic and since it has been reported to be the thornback ray's main prey (Morato et al. 2003), the boarfish *Capros aper* was used as the  $\delta^{15}\text{N}_{\text{base}}$  estimate.  $\delta^{15}\text{N}_{\text{base}}$  and  $\text{TP}_{\text{base}}$  were used according to data obtained from Torres et al. (2016a) for this species in the Azores. Values for  $\delta^{15}\text{N}_{\text{lim}}$  and  $k$  are estimated from meta-analysis (see Hussey et al. 2014). Mann–Whitney–Wilcoxon rank tests were applied to test for pairwise differences in TPSI between species. Differences in TPSI by species were assessed using Mann–Whitney–Wilcoxon rank (all sex-maturation stage from one species pooled together compared to all sex maturation stages of the other species). Differences in TPSI among species by subgroups were also analyzed using Mann–Whitney–Wilcoxon rank.

## Results

### Stomach content analysis

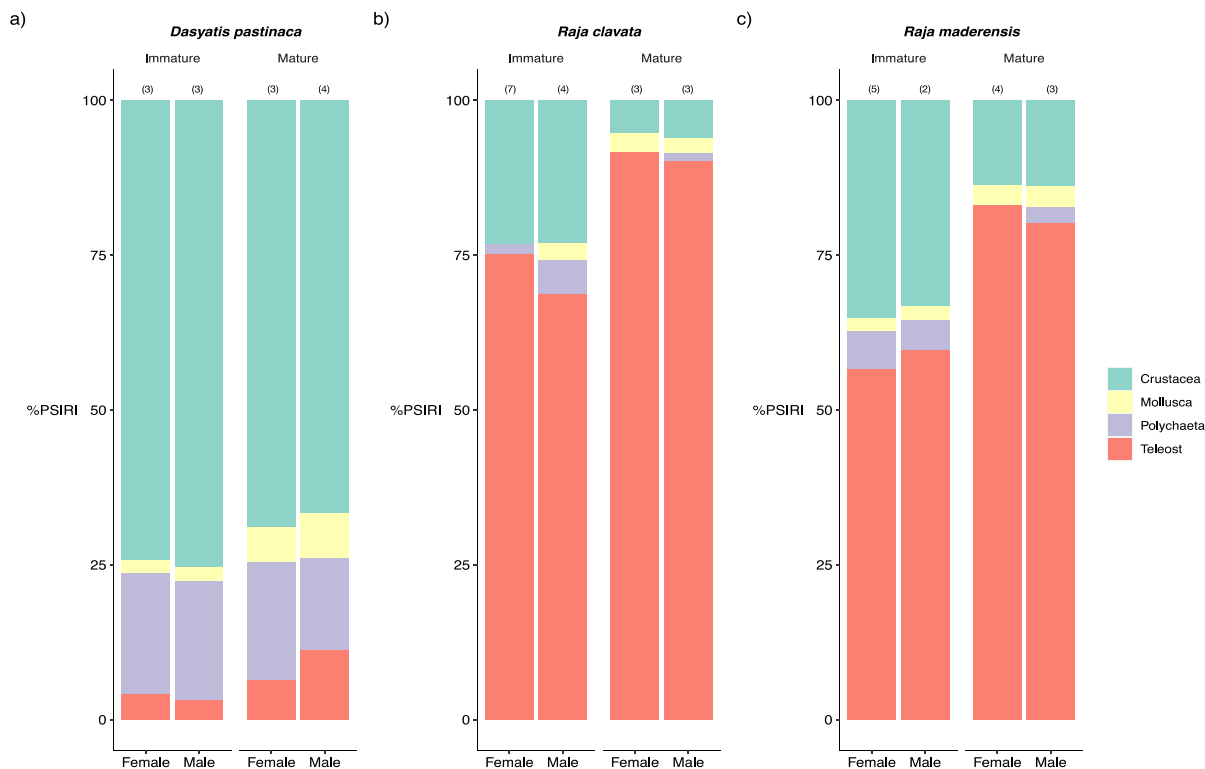
A total of 67 stomachs were analyzed, belonging to 15 *D. pastinaca* individuals measuring between 32 and 59 cm ( $T_L$ ), 29 *R. clavata* and 23 *R. maderensis* individuals measuring between 35–91 cm and 31–87 cm, respectively (Table 1). Cumulative curves of prey suggested that enough stomachs were examined to accurately describe the diet of the three studied species and of each subgroup as the cumulative prey curves always reached the asymptote (Supplemental Table 2, Supplemental Fig. 1). Of the analyzed stomachs, 2 *D. pastinaca* (13%V), 12 *R. clavata* (41%V), and 9 *R. maderensis* (39%V) stomachs were empty (Table 1). Overall, 634 individual prey items were found, belonging to four prey categories (Polychaeta, Crustacea, Mollusca, Teleost), from which five were identified to the species level (Supplemental

Table 3). These four prey categories were found in all studied species in different proportions (Fig. 2).

Stomach content analysis (SCA) revealed that *D. pastinaca*, *R. clavata*, and *R. maderensis* feed on a wide range of prey items, with crustaceans and teleosts as main categories of prey (Fig. 2, Supplemental Table 3). Crustaceans dominated the diet of *D. pastinaca* in frequency of occurrence, abundance and weight, while teleosts dominated the diet of *R. clavata* and *R. maderensis* (Fig. 2, Supplemental Table 3). Decapods were the most abundant and diversified of all prey species, and they were consumed by the three studied species. Even if teleosts were the main prey categories in *R. clavata* and *R. maderensis*, at lower taxonomic levels, differences in %FO, %PN, %PW, and %PSIRI were clear. *Raja clavata* preyed preferentially upon *Macroramphosus scolopax* (Linnaeus 1758) and *R. maderensis* on *Pagellus bogaraveo* (Brünnich 1768) but unidentified fish were also important for the two species (Fig. 2, Supplemental Table 3). *Capros aper* (Linnaeus 1758) was also an important fish prey of these two batoids.

Dietary breadths were similar in *R. clavata* ( $Ba=0.17$ ) and *R. maderensis* ( $Ba=0.23$ ) and smaller than in *D. pastinaca* ( $Ba=0.51$ ) (Table 1). According to the niche breadth size, immature individuals in *R. clavata* and *R. maderensis* were found to be generalist feeders, whereas adults exhibited a specialist feeding strategy (Table 1). The trophic position of *D. pastinaca*, estimated with the diet composition, was significantly lower (Table 1) than those of *R. clavata* and *R. maderensis*, which were not significantly different from each other (Table 1). There were no significant differences in TP between sexes within species. In all species, the trophic position increased with the maturity of the individuals, but this increase was significant for *R. clavata* and *R. maderensis* only (Table 1).

The NMDS revealed significant differences between the diet composition of *D. pastinaca* and both *R. clavata* and *R. maderensis*, but not between *R. clavata* and *R. maderensis* (Fig. 3a, Supplemental Table 4). Diet composition did not vary significantly between males and females in any of the studied species (Fig. 3b, d, Supplemental Table 4) and between maturity stages of *D. pastinaca* (Fig. 3e, Supplemental Table 4). PERMANOVA revealed significant differences between maturity stages in *R. clavata* and *R. maderensis* (Fig. 3f, g, Supplemental Table 4). The



**Fig. 2** Diet composition of three batoids from the coast of Terceira Island by sex and stage of maturity. Bar charts show the prey-specific index of relative importance (%PSIRI) of the four major prey categories (Crustacea, Mollusca, Polychaeta,

and Teleost) found in (A) *D. pastinaca*, (B) *R. clavata*, (C) *R. maderensis*. Numbers in parentheses above bars indicate stomach sample size for each combination

overall diet dissimilarity between *D. pastinaca* and *R. clavata* was 77.42%, with teleosts and crustaceans contributing the most to this dissimilarity (38.95% and 32.36% respectively, Supplemental Table 5). The second greatest dissimilarity occurred between the diets of *D. pastinaca* and *R. maderensis* (68.11%, Supplemental Table 5), with teleosts and crustaceans as the main drivers of this dissimilarity (33.71% and 29.19%, respectively, Supplemental Table 5). In *R. clavata*, the overall diet dissimilarity between immature and mature individuals was 46.36%, and this was significantly made up of crustaceans (23.03%), teleosts (21.56%), and mollusks (1.5%) (Supplemental Table 5). There was 51.45% dissimilarity between immature and mature individuals in *R. maderensis* diets, with teleosts and crustaceans being the prey categories that contributed the most to the dissimilarity (26.10% and 23.74%, respectively, Supplemental Table 5). Crustaceans dominated the diet of immature

*R. clavata* and *R. maderensis*, while teleosts were the most important prey in mature individuals.

#### Stable isotope analysis (SIA)

$\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were measured in 35 white muscle tissue samples, 12 for *D. pastinaca*, 11 for *R. clavata*, and 12 *R. maderensis* (Table 1). Results for stable isotope analysis for each species and subgroups are represented in Table 1. *D. pastinaca* exhibited  $\delta^{13}\text{C}$  values significantly enriched compared to those of *R. clavata* and *R. maderensis* (Table 1). *Raja clavata* and *R. maderensis* muscles were slightly  $\delta^{15}\text{N}$ -enriched compared to *D. pastinaca* muscle (Table 1). In all studied species, stable isotope values did not vary significantly between males and females; however, Mann–Whitney–Wilcoxon rank tests revealed significant differences between stages of maturity in *R. clavata* and *R. maderensis*. Immature and mature *R. clavata* exhibited a significant difference in  $\delta^{13}\text{C}$



values (Table 1). In *R. maderensis*, mature individuals had lower  $\delta^{13}\text{C}$  and higher  $\delta^{15}\text{N}$  than immature individuals (Table 1), as shown by their relative positions in isotopic niche space (Fig. 4g).

The niche breadths of the studied species were calculated from Bayesian isotope analysis as 0.68, 0.30, and 0.52 in *D. pastinaca*, *R. clavata*, and *R. maderensis*, respectively (Fig. 4a, Table 1). Among three pair-wise combinations between the studied species, isotopic overlap was only found between *R. clavata* and *R. maderensis* (Fig. 4a), with low SEAc overlap value (24%). No SEAc overlap between *D. pastinaca* and *R. clavata* or *R. maderensis* was found (Fig. 4a). There were no significant differences for *D. pastinaca*, *R. clavata*, and *R. maderensis* in isotopic niche breadths between sexes, with high SEAc overlap value (Fig. 4b, c, d, Table 1). The intraspecific analysis by maturity stages for *D. pastinaca* showed that SEAc values were close, with 29% of overlapping (Fig. 4e). Size-related SEAc differed in *R. clavata* and *R. maderensis*, with the immature stage having the higher SEAc values (Fig. 4f, g, Table 1), and the overlap between maturity stages was low in these two species (Fig. 4f, g).

*Dasyatis pastinaca* exhibited the lowest average trophic position based on  $\delta^{15}\text{N}$  values, with a value of  $3.29 \pm 0.53$ . *Raja clavata* and *R. maderensis* exhibited averaged values of  $3.57 \pm 0.32$  and  $3.76 \pm 0.42$ , respectively. *Raja maderensis* has significantly higher TPSI values than *D. pastinaca* (Table 1). *Raja clavata* TPSI did not significantly differ from both *D. pastinaca* and *R. maderensis* (Table 1). Neither significant interactions between sex and TPSI nor significant differences between sexes were detected in *D. pastinaca* and *R. clavata* or *R. maderensis* (Table 1). *Dasyatis pastinaca* and *R. clavata* had similar trophic positioning between immature and mature (Table 1). Mature *R. maderensis* showed higher trophic positions than immature (Table 1).

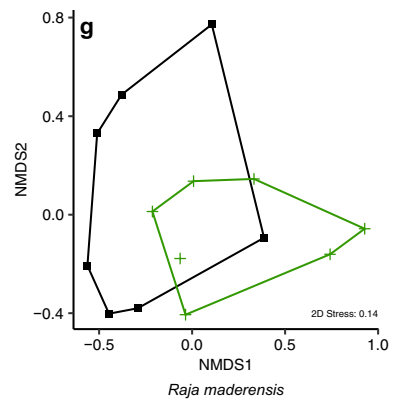
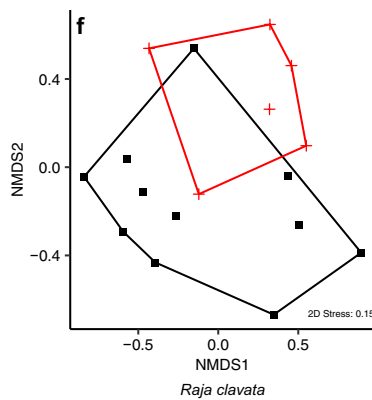
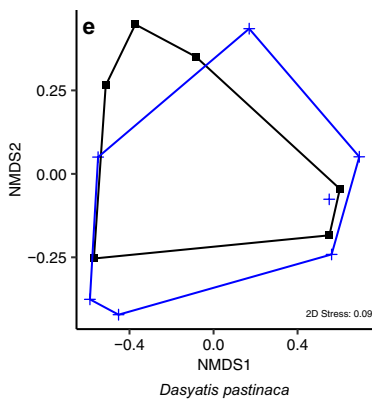
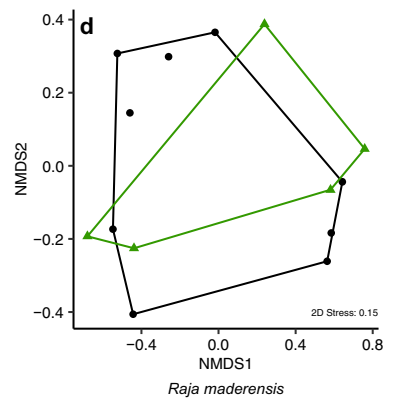
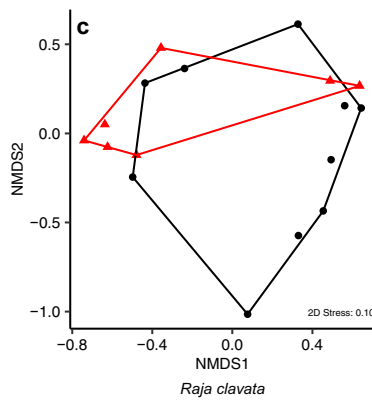
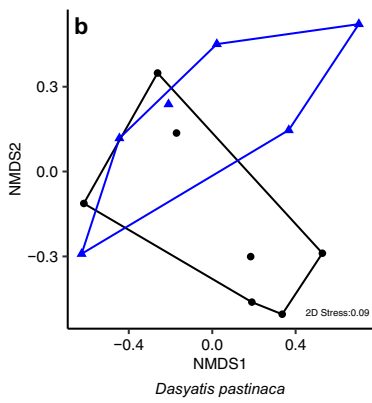
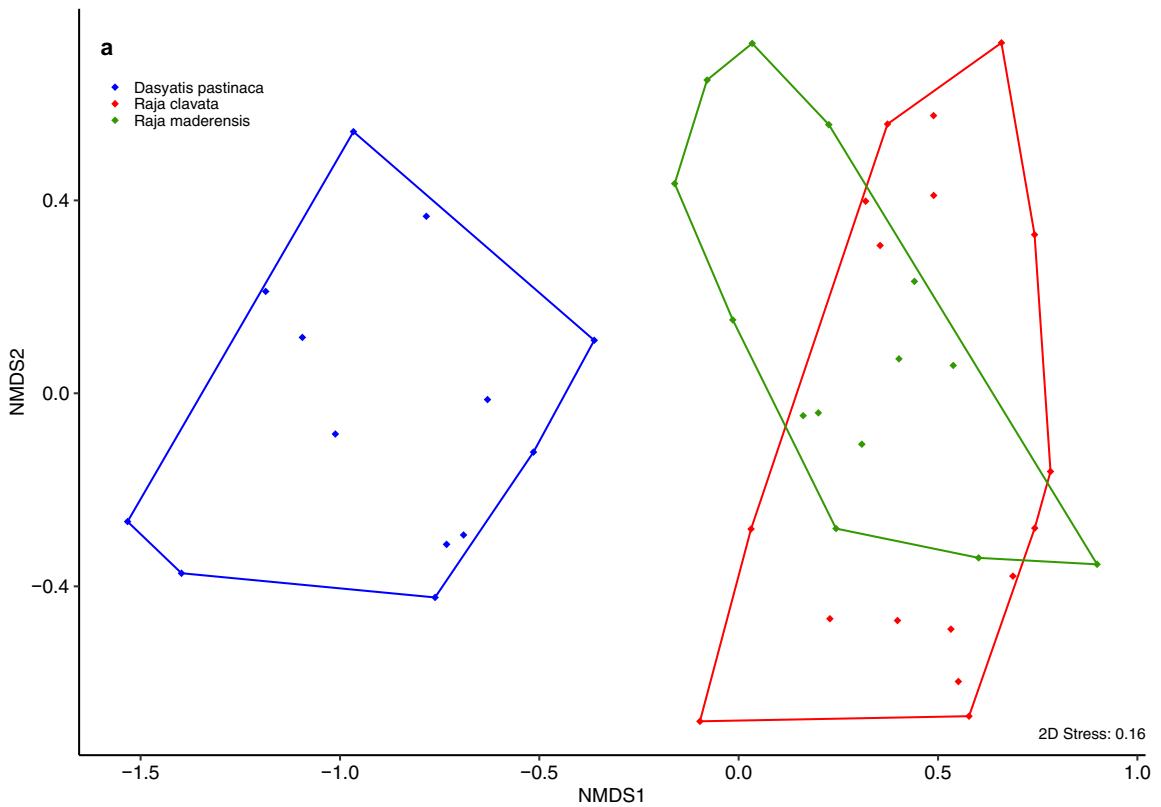
## Discussion

In the present study, we provide new information on the trophic ecology of three co-existing batoids in Terceira Island, in the Central Azores. Even if a limited number of studies were carried out in this area, they never attempt to understand inter- and intraspecific variability in the diet of *D. pastinaca*, *R. clavata*,

and *R. maderensis*. Both stomach content and stable isotope analysis showed differences in the trophic habits (diet composition, trophic niche, and trophic level) among the three studied species. In addition, *R. clavata* and *R. maderensis* showed a change in diet composition and foraging habitat with ontogeny. Although the results of the cumulative prey curves suggest a good sampling effort to describe the diet of the studied species, the low number of samples, especially in *D. pastinaca*, limits strong conclusions. The degree of prey identification and the absence of reconstituted biomass of prey are other weaknesses of the study. The information provided in this study remains useful to complement those dealing with trophic ecology of these species in the Azores and other locations for these three vulnerable species. Especially, this study provides new information on the trophic ecology of *R. maderensis*, a poorly studied endemic species of the Macaronesian region.

## Intraspecific assessment

There was some evidence for ontogenetic changes in the diets of *R. clavata* and *R. maderensis* although the results must be treated with caution because sample sizes may have not been sufficient to completely characterize the diet of juveniles. Both immature and mature individuals fed on fish, but this prey category was more important in mature individuals, whereas crustaceans were more abundant in the stomach contents of smaller individuals. The ontogenetic shift of *R. clavata* revealed in this study is consistent with previous studies, which reported that immatures preyed more on crustaceans than adults and individuals shift their diet toward teleosts as size increased (Šantić et al. 2012; Kadri et al. 2014; Daban et al. 2022; Gül and Demirel 2022). SIA also showed significant variations of carbon and nitrogen values with age in *R. clavata* and *R. maderensis*. Immatures of *R. clavata* and *R. maderensis* showed broader isotopic niches than adults. This suggests that these immatures may display movements across an isotopically heterogeneous environment and/or have more diverse food sources, which is in agreement with the diet results. *Raja maderensis* exhibited significant increases of  $\delta^{15}\text{N}$  values with age, indicating that as individuals grow, the contribution of prey



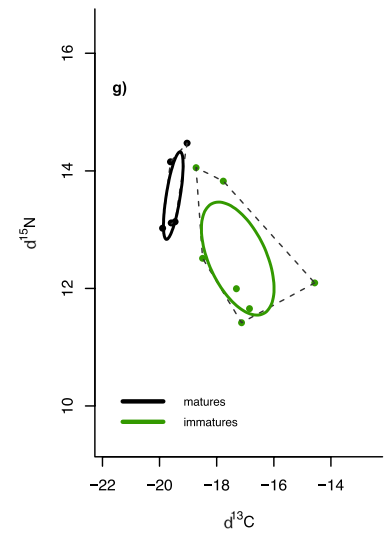
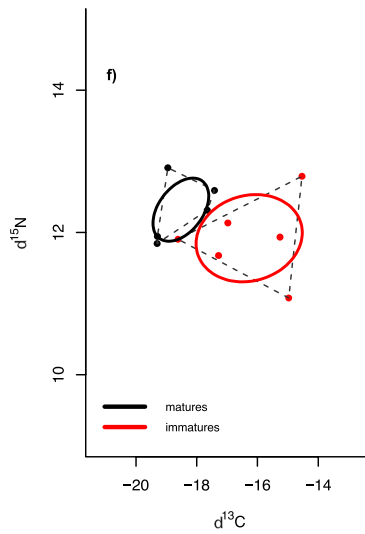
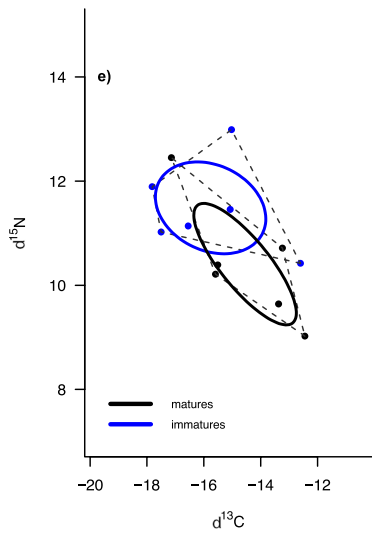
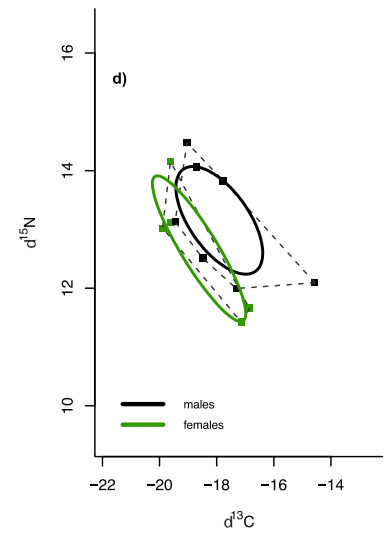
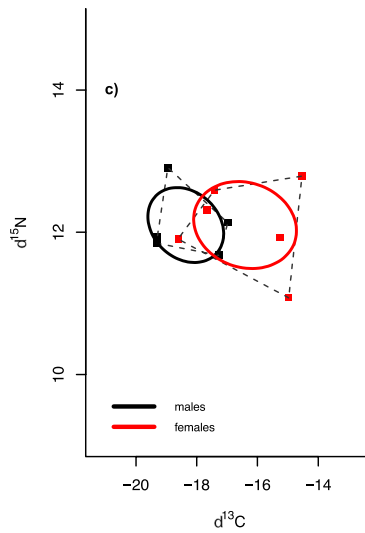
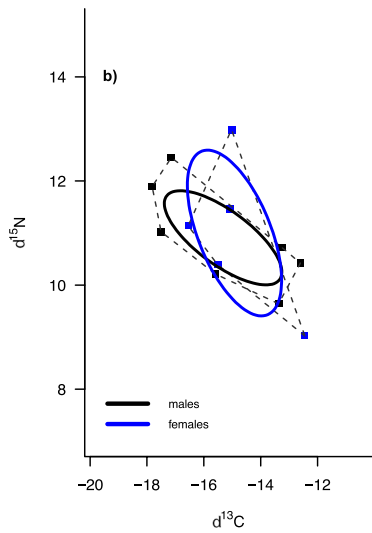
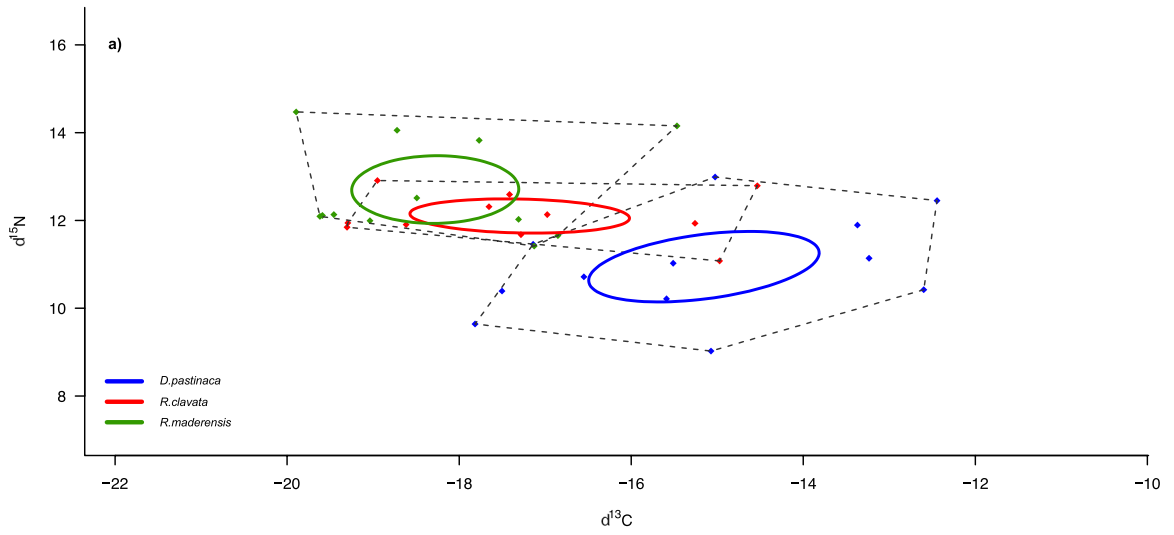
**Fig. 3** Non-parametric multidimensional scaling (nMDS) plot for (a) *D. pastinaca*, *R. clavata*, and *R. maderensis*; (b–d) for males (●) and females (▲) *D. pastinaca*, *R. clavata*, and *R. maderensis*; (e–g) for matures (+) and immatures (■) of *D. pastinaca*, *R. clavata*, and *R. maderensis*. Gravimetric data (% prey weight) of the four major prey categories (Polychaeta, Crustacea, Mollusca, and Teleost) were used to examine differences in diet composition

of higher trophic levels increases (Hussey et al. 2011). Although the sampling size is limited, the low dietary overlap between stages of maturity of *R. clavata* and *R. maderensis* may also indicate that these species change their diet as they grow, or experience ontogenetic habitat shifts. Ontogenetic changes have been reported for different species of batoids (Marshall et al. 2008; Treloar et al. 2009; Mulas et al. 2019). Several reasons explain this, including increased energy requirements as individuals mature (Lucifora et al. 2009), larger gap size and stronger bite as animals increase in size (Kolmann et al. 2015), and greater movements across foraging habitats as individuals get larger (Wetherbee et al. 2004). Morato et al. (2003) found in the Azores that specimens of *R. clavata* from different depths fed on different prey, with increasing consumption of penaeids, seabreams, and myctophids with depth. Interestingly, both mature groups of *R. clavata* and *R. maderensis* showed depleted  $\delta^{13}\text{C}$  values in comparison to immature groups. Because  $\delta^{13}\text{C}$  values decrease with distance from shore or depth (Hussey et al. 2011; Shipley et al. 2017), this finding could suggest that in *R. clavata* and *R. maderensis*, juveniles and adults segregate by depth or distance to the shore (Rigby et al. 2015). It may also reveal changes in habitat use or hunting strategies with age (Afonso and Hazin 2015). Whether this reflects foraging on different prey taxa, habitat segregation, or both remains to be elucidated. In contrast to the other two species, there was no significant change in diet composition and stable isotopic values with size for *D. Pastinaca*. Ismen (2003) observed a shift to bony fish consumption among adults in Iskenderun Bay in the eastern Mediterranean. Therefore, the lack of a relationship between age and diet composition or stable isotopic values could either be related to the sample size or be related to the prey availability in Terceira Island compared to Iskenderun Bay.

While sample sizes were limited, no evidence for sex-related dietary differences in the studied species was detected, in agreement with the results from several studies of batoids (Jacobsen and Bennett 2013; Mulas et al. 2019). Other studies have, however, noticed that some batoid species exhibit differential diet and habitat use patterns between sexes (Ajemian and Powers 2014; White et al. 2014; Jargowsky et al. 2020; Serrano-Flores et al. 2021).

#### Interspecific assessment

Overall, the results showed that the three species prey mostly upon crustaceans and fishes, but displayed different feeding habits. Polychaetes and mollusks were of minor importance. Crustaceans, especially decapods, were major prey for *D. pastinaca* in accordance with previous studies carried out in the Azores (Ponte et al. 2016), the Mediterranean Sea (Ismen 2003; Yeldan et al. 2009; Mulas et al. 2019; Tiralongo et al. 2020) and the Black Sea (Saglam et al. 2010). The diet of *R. clavata* was primarily composed of teleosts, confirming previous observations from the Azores (Morato et al. 2003; Torres et al. 2016a), the Black Sea (Demirhan et al. 2005), the Aegean Sea (Eronat and Özaydın 2015), and the Mediterranean Sea (Kadri et al. 2014), but our results indicate that some immature individuals feed on crustaceans. Several authors reported a dominance of crustaceans in the diet of *R. clavata* in the Mediterranean Basin (Kabasakal 2002; Saglam and Bascinar 2008; Šantić et al. 2012; Mulas et al. 2019; Daban et al. 2022). Although our results indicated that *R. clavata* has a specialist feeding strategy, previous studies have described the species as a generalist and opportunistic predator (Kadri et al. 2014; Mulas et al. 2019). The foraging plasticity of some batoids could allow them to exploit the most abundant prey, resulting in the unequal prey contribution (Serrano-Flores et al. 2021). In the Azores, the high degree of piscivory may result from the high availability of fish prey (Morato et al. 2003; Torres et al. 2016a). The diet composition of *R. maderensis* could not be compared with other studies, since none exist to our knowledge. Results agree with the proportion of crustaceans and teleosts consumed by several skates (Dolgov 2005; Ebert and Bizzarro 2007; Treloar et al. 2009), including the sympatric *R. clavata*, which has many ecological



◀**Fig. 4** Isotopic niche overlap plot of: (a) *D. pastinaca*, *R. clavata*, and *R. maderensis*; males and females of (b) *D. pastinaca*, (c) *R. clavata*, (d) *R. maderensis*; immature and mature individuals of (e) *D. pastinaca*, (f) *R. clavata*, (g) *R. maderensis*. Convex hulls are indicated by black dashed lines. Standard Bayesian ellipses corrected (SEAc) are indicated by solid-colored lines

and phylogenetic affinities (Dolgov 2005; Ebert and Bizzarro 2007; Treloar et al. 2009; Serrano-Flores et al. 2021; Ball et al. 2016; Torres et al. 2016b).

Although based on a small sample size, inter-specific differences in isotopic values were highlighted. As isotopic carbon ratios typically increase from offshore to near-shore ecosystems (Hussey et al. 2011), the significantly higher  $\delta^{13}\text{C}$  values in *D. pastinaca* supported that the species is predominantly a coastal feeder. Furthermore, the species occupied a limited spatial extent, in agreement with its neritic foraging habitat (Torres et al. 2016b; Das et al. 2022). The significantly lower  $\delta^{13}\text{C}$  values in *R. clavata* and *R. maderensis* suggested that these species derive their carbon from both benthopelagic and oceanic prey, as the organic matter resulting from pelagic sources is more depleted in  $\delta^{13}\text{C}$  when compared to the benthos (Hussey et al. 2011). *Dasyatis pastinaca* preyed mainly upon crustacean and other low trophic level preys, explaining the significantly lower  $\delta^{15}\text{N}$  compared to *R. clavata* and *R. maderensis* that rely mostly on fish. While *D. pastinaca* and *R. clavata* were described as generalist and specialist respectively, *R. maderensis* showed an intermediate feeding strategy based on SEAc. Important isotopic niche overlap was observed between *R. clavata* and *R. maderensis* in accordance with the diet results. This high overlap does not necessarily mean that species are feeding on the same prey (Hussey et al. 2011) because different prey species can have similar isotopic values. It has also been suggested that the metabolic turnover of white muscle may be too low (> 250 days) to reveal fine-scale insights, resulting in high overlap between potential interactive species (MacNeil et al. 2005). Therefore, future work should incorporate isotopic signal of prey and faster isotopic turnover rate tissues (e.g., plasma and whole blood) to provide even greater insight into the diets, foraging habitats, and home ranges of the studied species.

## Role of *D. pastinaca*, *R. clavata*, and *R. maderensis* in food webs

Our trophic positions estimated from stomach contents were generally close to the standardized values reported for skates (Ebert and Bizzarro 2007) and stingrays (Jacobsen and Bennett 2013), and consistent with values reported using stable isotope-based trophic positions (Torres et al. 2016a; Gül and Demirel 2020, 2022). Our estimation of trophic level based on stable isotopes was lower than based on stomach contents. Stable isotope and stomach content analyses can produce different results for trophic position due to the different temporal integration of the diet, and in our case, to the limited information available on baselines to estimate trophic position (Olin et al. 2013). Trophic position estimates ranged from  $3.25 \pm 0.67$  to  $4.35 \pm 0.17$  according to the method, the species, and the individuals. These estimates are coherent with a previous study conducted in the Azores (Torres et al. 2016a), confirming the mesopredator role of *D. pastinaca* and high trophic level of *R. clavata* and *R. maderensis*.

The combination of the diet and isotope results indicates that *R. clavata* and *R. maderensis* occupy similar foraging habitats and exhibit close related dietary habits. This suggests that these species have very similar functions in the food web off Terceira Island. This functional redundancy is typical of high trophic level predators and it is associated with the wide sharing of food resources, and less trophic specialization (Heupel et al. 2014). High trophic level predators impact the prey community through diffuse predation, as several predators forage upon the same prey species (Heupel et al. 2014). Diffuse predation is important in maintaining structure and robustness of food webs (Bascompte et al. 2005; Navia et al. 2017). *Raja clavata* and *R. maderensis* exhibited ontogenetic changes in their diet. As they mature, their trophic levels change and, with that, the roles they play as predators change. Consequently, these changes can lead to a more important role of the species in structuring the prey community (Bascompte et al. 2005).

In conclusion, this study presents new information on the feeding ecology of *D. pastinaca*, *R. clavata*, and *R. maderensis* in the Azores, where limited information on the ecology of these species is available. The results revealed a clear feeding preference

for crustaceans for *D. pastinaca* and teleosts for *R. clavata* and *R. maderensis* off Terceira Island. The results are in overall agreement with previous studies on these species in other areas and with the general knowledge on batoids. They remain preliminary, as the number of samples was low, therefore limiting the robustness of the conclusions. Further studies should be conducted to better understand ontogenetic, sex-related, and seasonal changes in diet and habitat use. In addition, the variation of diet of these species in the Macaronesian region should be investigated, especially for the endemic *R. maderensis*, which is listed as vulnerable by the IUCN and is regularly by-caught in local fisheries.

**Author contributions** All authors contributed to the study conception and design. Material preparation, data collection, and analyses were per Paco Fontaine, João Pedro Barreiros, and Sébastien Jaquemet. The first draft of the manuscript was written by Paco Fontaine and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Data availability** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### Declarations

**Conflicts of interest** All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

#### References

- Afonso AS, Hazin FH (2015) Vertical movement patterns and ontogenetic niche expansion in the tiger shark, *Galeocerdo cuvier*. Plos One 10:e0116720
- Afonso P, Fontes J, Giacomello E, Magalhães MC, Martins HR, Morato T, Neves V, Prieto R, Santos RS, Silva MA (2020) The Azores: a mid-Atlantic hotspot for marine megafauna research and conservation. Front Mar Sci 6:826
- Ajemian MJ, Powers SP (2014) Towed-float satellite telemetry tracks large-scale movement and habitat connectivity of myliobatid stingrays. Environ Biol Fish 97:1067–1081
- Anderson MJ (2006) Distance-based tests for homogeneity of multivariate dispersions. Biometrics 62:245–253
- Ball RE, Serra-Pereira B, Ellis J, Genner MJ, Iglésias S, Johnson AF, Jones CS, Leslie R, Lewis J, Mariani S (2016) Resolving taxonomic uncertainty in vulnerable elasmobranchs: are the Madeira skate (*Raja maderensis*) and the thornback ray (*Raja clavata*) distinct species? Conserv Genet 17:565–576
- Barreiros JP, Gadig OB (2011) Catálogo ilustrado dos tubarões e raias dos Açores. Instituto Açoriano de Cultura, Angra do Heroísmo
- Barrios-O'Neill D, Bertolini C, Collins PC (2017) Trophic cascades and the transient keystone concept. Biol Conserv 212:191–195
- Bascompte J, Melian CJ, Sala E (2005) Interaction strength combinations and the overfishing of a marine food web. Proc Natl Acad Sci USA 102:5443–5447
- Bizzarro JJ, Robinson HJ, Rinewalt CS, Ebert DA (2007) Comparative feeding ecology of four sympatric skate species off central California. Environ Biol Fish 80:197–220
- Bowen SH (1996) Quantitative description of the diet. In: Murphy BR, Willis DW (eds) Fisheries techniques. American Fisheries Society, Bethesda, pp 513–522
- Brown SC, Bizzarro JJ, Cailliet GM, Ebert DA (2012) Breaking with tradition: redefining measures for diet description with a case study of the Aleutian skate *Bathyraja aleutica* (Gilbert 1896). Environ Biol Fish 95:3–20
- Cailliet GM, Love MS, Ebeling AW (1996) Fishes: a field and laboratory manual on their structure, identification and natural history. Waveland, Long Grove
- Carvalho N, Edwards-Jones G, Isidro E (2011) Defining scale in fisheries: Small versus large-scale fishing operations in the Azores. Fish Res 109:360–369
- Coll-Calvo E, Barría C, Recasens L, Navarro J (2020) Feeding ecology of a Mediterranean endemic mesopredator living in highly exploited ecosystems. Mar Environ Res 157:104932
- Cortés E (1999) Standardized diet compositions and trophic levels of sharks. ICES J Mar Sci 56:707–717
- Da Silva HM, Pinho MR (2007) Small-scale fishing on seamounts. In: Pitcher TJ, Morato T, Hart PJB, Clark MR, Haggan N, Santos RS (eds) Seamounts: ecology fisheries and conservation, fisheries and aquatic resource series, vol 12. Blackwell, Oxford, pp 333–360
- Daban IB, Cabbar K, Yiğın CÇ (2022) Feeding ecology of Thornback Ray, *Raja clavata* (Linnaeus 1758) in Gökçeada Island, Northern Aegean Sea, Turkey. Thalassas 38:197–211
- Das D, Afonso P (2017) Review of the diversity, ecology, and conservation of elasmobranchs in the Azores region, mid-north Atlantic. Front Mar Sci 4:354
- Das D, Gonzalez-Irusta JM, Morato T, Fauconnet L, Catarino D, Afonso P, Viegas C, Rodrigues L, Menezes G, Rosa A (2022) Distribution models of deep-sea elasmobranchs in the Azores, Mid-Atlantic Ridge, to inform spatial planning. Deep Sea Res I 182:103707
- Debelius H (1997) Mediterranean and Atlantic fish guide. IKAN-Unterwasserarchiv, Frankfurt
- Demirhan SA, Engin S, Seyhan K, Akama E (2005) Some biological aspects of thornback ray (*Raja clavata* L., 1758) in the southeastern Black Sea. Turk J Fish Aquat Sci 5:75–83
- Diggles B, Cooke S, Rose J, Sawynok W (2011) Ecology and welfare of aquatic animals in wild capture fisheries. Rev Fish Biol Fish 21:739–765
- Diogo H, Pereira JG, Higgins RM, Canha Â, Reis D (2015) History, effort distribution and landings in an artisanal bottom longline fishery: an empirical study from the North Atlantic Ocean. Mar Policy 51:75–85
- Dolgov A (2005) Feeding and food consumption by the Barents Sea skates. J Northwest Atl Fish Sci 35:495–503

- Dulvy NK, Fowler SL, Musick JA, Cavanagh RD, Kyne PM, Harrison LR, Carlson JK, Davidson LN, Fordham SV, Francis MP (2014) Extinction risk and conservation of the world's sharks and rays. *eLife* 3:e00590
- Dulvy NK, Walls R, Buscher E, Stehmann M (2015) *Raja maderensis*. In: IUCN 2023. IUCN red list of threatened species. Version 2023.2. [www.iucnredlist.org](http://www.iucnredlist.org). Accessed 17 Apr 2023
- Ebert DA, Bizzarro JJ (2007) Standardized diet composition and trophic levels of skates (Chondrichthyes: Rajiformes: Rajoidei). *Environ Biol Fish* 80:221–237
- Ellis J, Dulvy N, Serena F (2016) *Raja clavata*. In: IUCN 2023. IUCN red list of threatened species. Version 2022.2. [www.iucnredlist.org](http://www.iucnredlist.org). Accessed 17 Apr 2023
- Eronat EGT, Özyaydn O (2015) Diet composition of the thornback ray, *Raja clavata* Linnaeus, 1758 (Elasmobranchii: Rajidae) in the Turkish Aegean Sea. *Zool Middle East* 61:38–44
- Flowers KI, Heithaus MR, Papatamatiou YP (2021) Buried in the sand: uncovering the ecological roles and importance of rays. *Fish Fish* 22:105–127
- Frisch AJ, Cole AJ, Hobbs J-PA, Rizzari JR, Munkres KP (2012) Effects of spearfishing on reef fish populations in a multi-use conservation area. *PLoS One* 7:e51938
- Gardener M (2014) Community ecology: analytical methods using R and Excel. Pelagic Publishing, Exeter
- Gomes FV, Pinto FT (2004) Azores Islands (Portugal). EUROSION Case Study Instituto de Hidráulica e Recursos Hídricos, Lisboa
- Grubbs RD, Carlson JK, Romine JG, Curtis TH, McElroy WD, McCandless CT, Cotton CF, Musick JA (2016) Critical assessment and ramifications of a purported marine trophic cascade. *Sci Rep* 6:1–12
- Gül G, Demirel N (2020) Trophic interactions of uncommon batoid species in the sea of Marmara. *J Black Sea/Medit Environ* 26:294–309
- Gül G, Demirel N (2022) Ontogenetic shift in diet and trophic role of *Raja clavata* inferred by stable isotopes and stomach content analysis in the Sea of Marmara. *J Fish Biol* 101:560–572
- Hayward PJ, Ryland JS (1990b) The marine fauna of the British Isles and north-western Europe. Vol. 2. Molluscs to chordates. Clarendon Press, Oxford
- Heupel MR, Knip DM, Simpfendorfer CA, Dulvy NK (2014) Sizing up the ecological role of sharks as predators. *Mar Ecol Prog Ser* 495:291–298
- Hussey NE, Dudley SF, McCarthy ID, Cliff G, Fisk AT (2011) Stable isotope profiles of large marine predators: viable indicators of trophic position, diet, and movement in sharks? *Can J Fish Aquat Sci* 68:2029–2045
- Hussey NE, MacNeil MA, McMeans BC, Olin JA, Dudley SF, Cliff G, Wintner SP, Fennessy ST, Fisk AT (2014) Rescaling the trophic structure of marine food webs. *Ecol Lett* 17:239–250
- Hyslop E (1980) Stomach contents analysis—a review of methods and their application. *J Fish Biol* 17:411–429
- Ismen A (2003) Age, growth, reproduction and food of common stingray (*Dasyatis pastinaca* L., 1758) in Iskenderun Bay, the eastern Mediterranean. *Fish Res* 60:169–176
- Jabado R, Chartrain E, De Bruyne G, Derrick D, Dia M, Diop M, Doherty P, Leurs G, Metcalfe K, Pacoureaux N, Pires J, Ratão S, Seidu I, Serena F, Soares A, Tamo A, Vander Wright W, Williams A (2021) *Dasyatis pastinaca*. In: IUCN 2023. IUCN red list of threatened species. Version 2022.2. [www.iucnredlist.org](http://www.iucnredlist.org). Accessed 17 Apr 2023
- Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER—Stable Isotope Bayesian Ellipses in R. *J Anim Ecol* 80:595–602
- Jacobsen IP, Bennett MB (2013) A comparative analysis of feeding and trophic level ecology in stingrays (Rajiformes; Myliobatoidei) and electric rays (Rajiformes: Torpedinoidei). *PLoS One* 8:e71348
- Jargowsky MB, Cooper PT, Ajemian MJ, Colvin ME, Drymon JM (2020) Discerning the dietary habits of the smooth butterfly ray *Gymnura lessae* using two distinct methods, otolith identification and metagenetics. *J Fish Biol* 96:434–443
- Kabasakal H (2002) Cephalopods in the stomach contents of four Elasmobranch species from the northern Aegean Sea. *Acta Adriat* 43:17–24
- Kadri H, Marouani S, Bradai MN, Bouaïn A (2014) Diet and feeding strategy of thornback ray, *Raja clavata* (Chondrichthyes: Rajidae) from the Gulf of Gabes (Tunisia—Central Mediterranean Sea). *J Mar Biol Assoc UK* 94:1509–1516
- Kinney MJ, Hussey NE, Fisk AT, Tobin AJ, Simpfendorfer CA (2011) Communal or competitive? Stable isotope analysis provides evidence of resource partitioning within a communal shark nursery. *Mar Ecol Prog Ser* 439:263–276
- Kolmann MA, Huber DR, Motta PJ, Grubbs RD (2015) Feeding biomechanics of the cownose ray, *Rhinoptera bonasus*, over ontogeny. *Janat* 227:341–351
- Krebs CJ (1999) Ecological Methodology, 2nd edn. Benjamin Cummings, California
- Last PR, White WT, de Carvalho MR, Séret B, Stehmann MF, Naylor GJP (2016) Rays of the world. CSIRO Publishing, Clayton
- Laverock B, Gilbert JA, Tait K, Osborn AM, Widdicombe S (2011) Bioturbation: impact on the marine nitrogen cycle. *Biochem Soc Trans* 39:315–320
- Layman CA, Arrington DA, Montaña CG, Post DM (2007) Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology* 88:42–48
- Li Y, Zhang Y, Hussey NE, Dai X (2016) Urea and lipid extraction treatment effects on  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in pelagic sharks. *Rapid Commun Mass Spectrom* 30:1–8
- Logan JM, Jardine TD, Miller TJ, Bunn SE, Cunjak RA, Lutcavage ME (2008) Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. *J Anim Ecol* 77:838–846
- Lucifora LO, García VB, Menni RC, Escalante AH, Hozbor NM (2009) Effects of body size, age and maturity stage on diet in a large shark: ecological and applied implications. *Ecol Res* 24:109–118
- MacNeil MA, Skomal GB, Fisk AT (2005) Stable isotopes from multiple tissues reveal diet switching in sharks. *Mar Ecol Prog Ser* 302:199–206
- Marshall A, Kyne P, Bennett M (2008) Comparing the diet of two sympatric urolophid elasmobranchs (*Trygonoptera testacea* Müller & Henle and *Urolophus kapalensis* Yearsley & Last): evidence of ontogenetic shifts and possible resource partitioning. *J Fish Biol* 72:883–898
- Morato T, Solà E, Grós MP (2003) Diets of thornback ray (*Raja clavata*) and tope shark (*Galeorhinus galeus*) in

- the bottom longline fishery of the Azores, Northeastern Atlantic. *Fish Bull* 101:590–602
- Mulas A, Bellodi A, Cannas R, Carbonara P, Cau A, Marongiu MF, Pesci P, Porcu C, Follesa MC (2019) Resource partitioning among sympatric elasmobranchs in the central-western Mediterranean continental shelf. *Mar Biol* 166:1–16
- Navia AF, Mejía-Falla PA, López-García J, Giraldo A, Cruz-Escalona VH (2017) How many trophic roles can elasmobranchs play in a marine tropical network? *Mar Freshw Res* 68:1342–1353
- Oksanen J, Blanchet F, Kindt R, Legendre P, Minchin P, O'hara R, Simpson G, Solymos P, Stevens M, Wagner H (2013) Package 'vegan'. Community ecology pack- age, version, 2. Retrieved from <https://cran.ism.ac.jp/web/packages/vegan/vegan.pdf>
- Olin JA, Hussey NE, Grgicak-Mannion A, Fritts MW, Wintner SP, Fisk AT (2013) Variable  $\delta^{15}\text{N}$  diet-tissue discrimination factors among sharks: implications for trophic position, diet and food web models. *PLoS One* 8:e77567
- Ponte DDS, Barcelos LMD, Santos CS, Medeiros J, Barreiros JP (2016) Diet of *Dasyatis pastinaca* and *Myliobatis aquila* (Myliobatiformes) from the Azores, NE Atlantic. *Cybiu* 40:209–214
- R Core Team (2022) R: A language and environment for statistical computing. Austria, Vienna
- Rigby CL, Daley RK, Simpfendorfer CA (2015) Comparison of life histories of two deep-water sharks from eastern Australia: the piked spurdog and the Philippine spurdog. *Mar Freshw Res* 67:1546–1561
- Saglam H, Bascinar NS (2008) Feeding ecology of thornback ray (*Raja clavata* Linnaeus, 1758) on the Turkish coast of the south-eastern Black Sea. *Mar Biol Res* 4:451–457
- Saglam H, Kutlu S, Aydin I (2010) Diet and feeding strategy of the common stingray *Dasyatis pastinaca* (Linnaeus, 1758) on the Turkish coast of southeastern Black Sea. *Cah Biol Mar* 51:37–44
- Salazar G (2015) EcolUtils: utilities for community ecology analysis. R package version 0.1. <https://github.com/Guill emSalazar/EcolUtils>
- Saldanha L (2003) Fauna submarina Atlântica: Portugal continental, Açores. Publicações Europa-América, Madeira
- Šantić M, Rađa B, Pallaoro A (2012) Diet and feeding strategy of thornback ray *Raja clavata*. *J Fish Biol* 81:1070–1084
- Santos R, Medeiros-Leal W, Novoa-Pabon A, Crespo O, Pinho M (2021) Biological knowledge of thornback ray (*Raja clavata*) from the Azores: improving scientific information for the effectiveness of species-specific management measures. *Biology* 10:676
- Santos R, Novoa-Pabon A, Silva H, Pinho M (2020) Elasmobranch species richness, fisheries, abundance and size composition in the Azores archipelago (NE Atlantic). *Mar Biol Res* 16:103–116
- Santos RS, Porteiro FM, Barreiros JP (1997) Marine fishes of the Azores. Annotated checklist and bibliography. A catalogue of the Azorean marine ichthyodiversity. *Arquipel Suppl* 1:1–242
- Serra-Pereira B, Moura T, Griffiths AM, Serrano Gordo L, Figueiredo I (2011) Molecular barcoding of skates (Chondrichthyes: Rajidae) from the southern Northeast Atlantic. *Zool Scr* 40:76–84
- Serrano-Flores F, Torres-Rojas YE, Ajemian MJ, Mendoza-Carranza M, Pérez-Jiménez JC (2021) Advances in the study of the trophic niche of batoids with distribution in Mexican waters. *Mar Ecol* 42:e12687
- Shiffman D, Gallagher A, Boyle M, Hammerschlag-Peyer C, Hammerschlag N (2012) Stable isotope analysis as a tool for elasmobranch conservation research: a primer for non-specialists. *Mar Freshw Res* 63:635–643
- Shipley ON, Matich P (2020) Studying animal niches using bulk stable isotope ratios: an updated synthesis. *Oecologia* 193:27–51
- Shipley ON, Polunin NV, Newman SP, Sweeting CJ, Barker S, Witt MJ, Brooks EJ (2017) Stable isotopes reveal food web dynamics of a data-poor deep-sea island slope community. *Food Webs* 10:22–25
- Stehmann M (2002) Proposal of a maturity stages scale for oviparous and viviparous cartilaginous fishes (Pisces, Chondrichthyes). *Arch Fish Mar Res* 50:23–40
- Stevens J, Bonfil R, Dulvy NK, Walker P (2000) The effects of fishing on sharks, rays, and chimaeras (chondrichthyan), and the implications for marine ecosystems. *ICES J Mar Sci* 57:476–494
- Takeuchi S, Tamaki A (2014) Assessment of benthic disturbance associated with stingray foraging for ghost shrimp by aerial survey over an intertidal sandflat. *Cont Shelf Res* 84:139–157
- Tiralongo F, Messina G, Lombardo BM (2020) Biological Aspects of Juveniles of the Common Stingray, *Dasyatis pastinaca* (Linnaeus, 1758) (Elasmobranchii, Dasyatidae), from the Central Mediterranean Sea. *J Mar Sci Eng* 8:269
- Torres P, da Cunha RT, Micaelo C, dos Santos RA (2016a) Bioaccumulation of metals and PCBs in *Raja clavata*. *Sci Total Environ* 573:1021–1030
- Torres P, da Cunha RT, dos Santos RA (2016b) The elasmobranch fisheries of the Azores. *Mar Policy* 73:108–118
- Treloar MA, Laurenson LJ, Stevens JD (2009) Dietary comparisons of six skate species (Rajidae) in south-eastern Australian waters. *Environ Biol Fish* 80:181–196
- Vaudo JJ, Heithaus MR (2011) Dietary niche overlap in a nearshore elasmobranch mesopredator community. *Mar Ecol Prog Ser* 425:247–260
- Wetherbee BM, Cortés E, Bizzarro JJ (2004) Food consumption and feeding habits. In: Carrier JC, Musick JA, Heithaus MR (eds) *Biology of sharks and their relatives*. CRC Press, Boca Raton, pp 225–246
- White J, Simpfendorfer C, Tobin A, Heupel M (2014) Spatial ecology of shark-like batoids in a large coastal embayment. *Environ Biol Fish* 97:773–786
- White W, Platell M, Potter I (2004) Comparisons between the diets of four abundant species of elasmobranchs in a subtropical embayment: implications for resource partitioning. *Mar Biol* 144:439–448
- Yeldan H, Avsar D, Manaslı M (2009) Age, growth and feeding of the common stingray (*Dasyatis pastinaca*, L., 1758) in the Cilician coastal basin, northeastern Mediterranean Sea. *J Appl Ichthyol* 25:98–102

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.