Trophic assessment of three sympatric batoid species in the Southern Gulf of California

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ABSTRACT

The competitive exclusion principle establishes that the coexistence of closely related species requires a certain degree of resource partitioning. However, populations have individuals with different morphological or behavioral traits (*e.g.*, maturity stages, sexes, temporal or spatial segregation). This interaction often results in a multi-level differentiation in food preferences and habits. We explored such resource partitioning between and within three batoid species: *Hypanus dipterurus*, *Narcine entemedor*, and *Rhinoptera steindachneri* in the southern Gulf of California, Mexico, using a combination of stomach content (excluding *R. steindachneri*) and stable isotope analyses. We found a clear differentiation between *H. dipterurus* and *N. entemedor*, where the latter exhibited more benthic habitats, supported by a greater association to infaunal prey and higher $\delta^{13}C$ values. Though the degree and patterns of intra-specific segregation varied among species, there was a notable differentiation in both sex and stage of maturity, corresponding to changes in specialization (*i.e.*, isotopic niche breadth) or trophic spectrum (varying prey importance and isotopic values per group). This work is a promising step towards understanding the dietary niche dynamics of these species in a potentially important feeding area within the southern Gulf of California, as well as the biological and ecological mechanisms that facilitate their coexistence.

Subjects Ecology, Marine Biology, Zoology, Statistics
Keywords Resource partitioning, Stable isotopes, Bayesian inference, Machine learning

INTRODUCTION

Species-specific trophic ecology studies are fundamental to comprehending their ecological role in the ecosystem (*Coll, Navarro & Palomera, 2013*; *Ferreti et al., 2010*). Batoids are a group of aquatic predators with various shapes, sizes, and life-history strategies that occur in marine and freshwater environments (*Last et al., 2016*). As mesopredator organisms, they play a crucial role in transferring energy from lower to higher levels, directly and indirectly affecting all levels of the food web (*Heithaus et al., 2010*; *Barría et al., 2015*; *Navia et al., 2017*). Batoid species are generally considered carnivorous with a broad spectrum of prey, whose diet is mainly composed of crustaceans, mollusks, polychaetes, and fishes (*Barbini, Sabadin & Lucifora, 2018*; *Restrepo-Gómez et al., 2021*; *Serrano-Flores et al., 2021*).

Some studies have examined the competitive interactions of coastal batoids, finding that closely related and coexisting (*i.e.*, sympatric) coastal species display varying levels of interspecific dietary overlap and, therefore, resource partitioning (*e.g.*, *Mabragaña & Giberto, 2007*; *Navarro-González et al., 2012*; *Fontaine, Barreiros & Jaquemet, 2023*). This resource partitioning has been attributed to differential use of habitats and depth ranges in their ecosystem (*White, Platell & Potter, 2004*; *Marshall, Kyne & Bennett, 2008*; *Lim et al., 2018*) but also to differences in diet specialization (*Platell, Potter & Clarke, 1998*; *Espinoza et al., 2015*). Despite the similarity in trophic levels among batoid species, both prey type and size can vary considerably within and among them, including ontogenetic and sexual shifts (*e.g.*, *Moura et al., 2008*; *Schmitt et al., 2015*; *Restrepo-Gómez et al., 2021*), which allows them to play several trophic roles in the ecosystem in which they inhabit (*Navia et al., 2017*).

Thus far, studies on trophic ecology in Mexico have usually involved describing the diet of highly abundant and frequently captured species (*Valenzuela-Quiñonez et al., 2018*; *Restrepo-Gómez et al., 2021*; *Serrano-Flores et al., 2021*). However, comparative studies about dietary niche dynamics and the resource partitioning mechanisms enabling their coexistence (*sensu Hardin, 1960*; *Gause, 1934*) are less common (*Navarro-González et al., 2012*; *Murillo-Cisneros et al., 2019*). In the southern Gulf of California, the three most abundant and commercially fished batoid species are (*González-González et al., 2020*): diamond stingrays (*Hypanus dipterurus* (Jordan & Gilbert, 1880)), giant electric rays (*Narcine entemedor* Jordan & Starks, 1895), and golden cownose rays (*Rhinoptera steindachneri* Evermann & Jenkins, 1891). *Hypanus dipterurus* is a benthopelagic predator that feeds primarily on bivalves and stomatopods (*Restrepo-Gómez et al., 2021*). In contrast, *N. entemedor* mainly feeds on epibenthic prey, especially polychaetes and sipunculids (*Valadez-González, 2007*; *Flores-Ortega, Godínez-Domínguez & González-Sansón, 2015*; *Cabrera-Melendez, 2017*). Meanwhile, *R. steindachneri* primarily feeds on bivalves (*Simental-Anguiano et al., 2022*), echinoderms (*Navarro-González et al., 2012*) and, to a lesser extent, mysidaceans (*Ehemann et al., 2019*).

Most batoid feeding ecology studies have been based on stomach content analyses (SCA), which allow a relatively accurate taxonomic definition of the diet but require high sampling frequencies and sample sizes to obtain reliable, time-integrated overviews of

dietary habits. They also have other inherent limitations, such as often requiring lethal sampling (*Hyslop, 1980*; *Cortés, 1997*; *Vinson & Budy, 2010*). A widely used alternative that overcomes some of the limitations of the SCA is the stable isotope analysis (SIA), which traces the flow of elements throughout the food webs (*Ruiz-Cooley et al., 2006*). The more commonly used isotopic ratios are those of carbon ($^{13}C/^{12}C$ or $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$ or $\delta^{15}N$), where $\delta^{13}C$ values mainly reflect the carbon sources within an ecosystem (grazing, predation). While $\delta^{15}N$ reflects the consumers' trophic status, primarily in terms of position and amplitude (*Michener & Kaufman, 2007*). These properties allow these ratios to closely identify the ecological niche (*sensu Hutchinson, 1978*) and can be considered analogous to bionomic and scenopoetic axes, respectively (*Newsome et al., 2007*). Thus, the isotopic niche is considered a simplified approximation of the ecological niche.

Unlike SCA, SIA integrates information from the assimilated diet over a more extended period, which depends on the turnover rate of the tissue analyzed (*Sinisalo et al., 2008*). In muscle tissue of some elasmobranch species, this period has been estimated to be at least 1 year (*Logan & Lutcavage, 2010*; *MacNeil, Drouillard & Fisk, 2006*). The discrepancy in time integration has motivated the joint use of both techniques, allowing a more comprehensive description of trophic interactions in aquatic systems (*MacNeil, Drouillard & Fisk, 2006*; *Vinson & Budy, 2010*; *Albo-Puigserver et al., 2015*). Therefore, our study aims to analyze the coexistence of *H. dipterurus*, *N. entemedor*, and *R. steindachneri*, based on the feeding preferences of individuals captured by the artisanal fleet of La Paz Bay, southern Gulf of California, Mexico. We hypothesize that their coexistence is facilitated by the partitioning of trophic resources, which we test with integrative analyses of their trophic niche dynamics using SCA and SIA ($\delta^{13}C$ and $\delta^{15}N$). Our study also explores the intra-specific patterns of resource partitioning, thus providing the first integrated analysis of these batoids' feeding behaviors within the Gulf of California, both between and within species.

METHODS

Study area and sample collection

This study did not require an "Ethical review and approval" because the analyzed specimens were caught by local artisanal fishers who worked under a commercial fishing permit (CONAPESCA-103053993316-1) under Mexican laws and regulations. We did not participate in fishing operations, and at no point did we handle live animals.

The specimens were collected monthly from October 2013 to December 2015 off Espiritu Santo Island, in El Morrito (24 °25′17.55″N, 110 °18′31.61″W), at Bahia de La Paz, southern Gulf of California, Mexico (Fig. 1). The surface water temperature in this region changes seasonally from a warm season (30 °C, May to September) to a cold one (20 °C, October to April, *Guevara-Guillén, Shirasago-German & Pérez-Lezama, 2015*).

The organisms were caught at varying depths from 0 to 40 m using bottom gill nets (100 m long, 1.5 m wide, and 16 cm stretch mesh size). Fishers leave the nets overnight (approximately from 19:00–07:00 h) and immediately move the caught organisms to "El Morrito" fishing camp, where we collected the samples upon arrival.
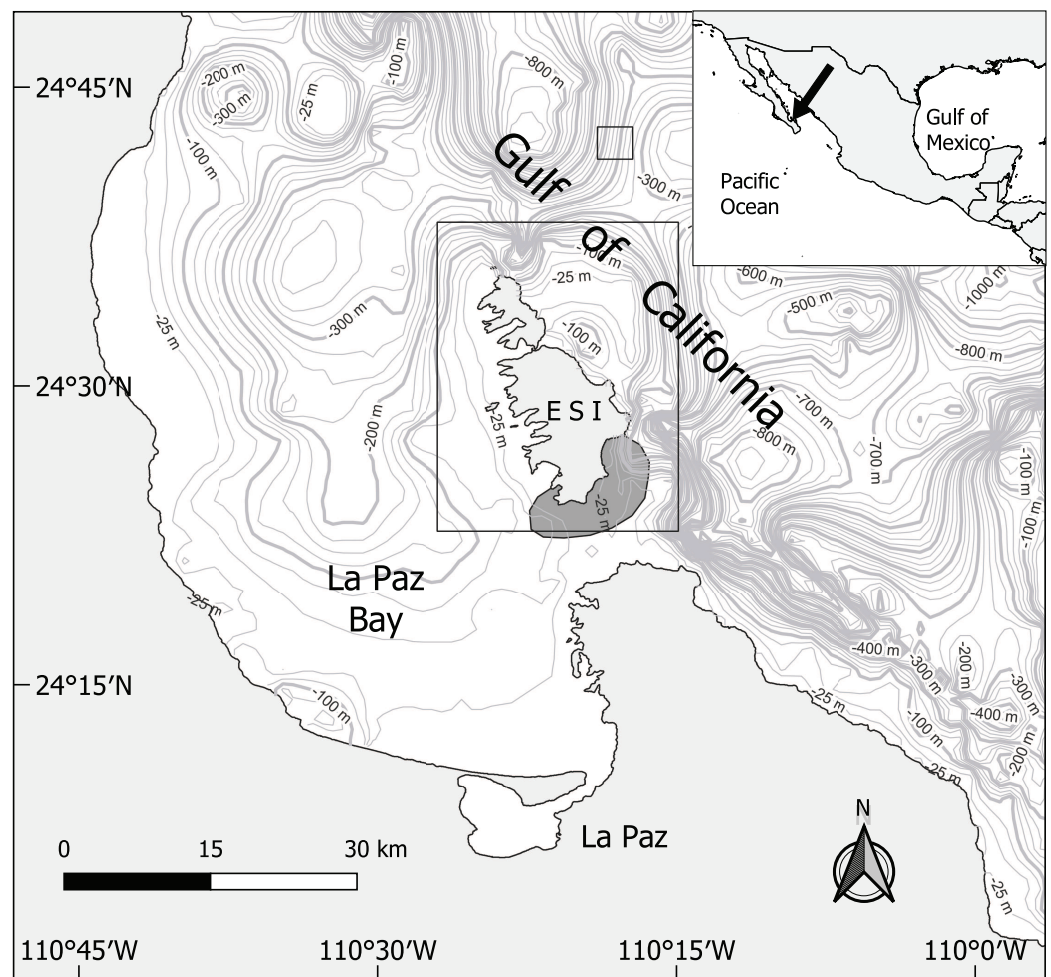
Figure 1 Map of the study area and sampling sites (rectangles). Contour lines represent the bathymetry (m). Courtesy of Gustavo De La Cruz-Agüero. ESI, Espiritu Santo Island.

Full-size 🖼 DOI: 10.1016/j.jembe.2020.151359

Sample processing

After sampling, the individuals were sexed, and their sexual maturity stages were determined following *Smith, Cailliet & Melendez (2007)*; see *Burgos-Vázquez et al. (2017, 2018)*. The stomachs were removed from the specimens, fixed, and preserved in 10% formaldehyde. Simultaneously, using a scalpel, 1 cm^3 of ventral muscle tissue was removed from the fresh specimens. The tissue was then kept frozen at −20 °C until further processing for SIA to avoid possible alterations of the isotopic values (*Kaehler & Pakhomov, 2001*; *Stallings et al., 2015*).

Upon collection, one gram of each tissue sample was oven-dried at 60 °C for 48 h. Lipids are depleted in ^{13}C relative to protein and thus lead to lower $\delta^{13}C$ values (*Post et al., 2007*). To avoid such bias, we removed lipids using a chloroform:methanol (1:1) solution (*Post et al., 2007*) in a Microwave-Assisted Solvent Extraction System (1000 MARS-5; CEM Microwave Technology Ltd., Mathews, NC, USA). Samples were then oven-dried at 60 °C for 12 h to remove the remaining solvent. Moreover, the high levels of urea in

elasmobranchs' tissues due to osmotic balancing lead to a diminution in $\delta^{15}N$ values, caused by its enrichment in ^{14}N relative to pure protein (*Kim & Koch, 2012*). Hence, we removed the urea by adding 10 mL of deionized water, stirring the test tube for 15 min, and finally discarding the deionized water. This process was repeated three times (*Kim & Koch, 2012*; *Li et al., 2016*). After extracting lipids and urea, the tissue was freeze-dried at −60 °C for 36 h and mechanically grounded in a porcelain mortar using a pestle until homogenization.

For stable isotope analyses, 400–1,000 μg of dry-weight material were collected and weighed on a precision micro-balance (Mettler-Toledo Ltd., Singapore), encapsulated in tin capsules, and loaded into a Zero-Blank Autosampler (Costech Analytical Technologies Inc., Valencia, CA, USA). Stable isotope ratios were measured in a Delta V Advantage isotope ratio mass spectrometer (IRMS; Thermo Fisher Scientific, Waltham, MA, USA), interfaced in a continuous flow to a Thermo Scientific–Flash HT 2000 elemental analyzer (Thermo Fisher Scientific, Waltham, MA, USA) and an Isodat Workspace version 3.0 (Thermo Scientific, Waltham, MA, USA).

The stable isotope ratios are expressed in the conventional δ notation as parts per thousand (‰, *DeNiro & Epstein, 1976*), which represents the heavy-to-light isotope ratios of carbon or nitrogen ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) relative to international standards for each element (Vienna PeeDee Belemnite for carbon and atmospheric N_2 for nitrogen, *Brand et al. (2014)*). We placed several standards on each run, achieving an overall precision $\leq$ 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$ (n = 54).

Data analyses

Below is an overview of the data analyses performed. The complete details are available in at GitHub (arturobell.github.io/01072022).

Stomach content analysis

We constructed a single database to classify *H. dipterurus* and *N. entemedor* based on their diets, utilizing the SCA studies by *Cabrera-Melendez (2017)* for *N. entemedor* and *Restrepo-Gómez et al. (2021)* for *H. dipterurus*. We excluded *R. steindachneri* from this analysis because the SCA performed by *Ehemann et al. (2019)* did not to adequately describe its diet on the study area. The taxonomic determination of prey items was performed by trained personnel following standard identification guides (see the aforementioned references for details). Prey items were aggregated into ten broad taxonomic categories: amphipods, brachyuran and anomuran crabs (henceforth crabs), bivalves, echinoderms, other crustaceans, penaeid shrimps (shrimps), polychaetes, sipunculids, stomatopods, and teleosteans (fishes). Weight data from each prey taxon was used for subsequent analyses since they better represent the relative importance of each taxon, especially when different-sized prey items are ingested (*Hyslop, 1980*).

The trophic differences between species, sexes, sexual maturity stages, and sampling seasons were assessed using Random Forest (RF) classifiers. This method classifies objects (*i.e.*, individuals) by creating several uncorrelated decision trees and assigning each object

to its most frequently found class based on its feature values (*Carvajal, Maucec & Cullick, 2018*); hence, in this study, individuals are classified to either *H. dipterurus* or *N. entemedor* based on the weights of each prey item. This approach was preferred over traditional techniques (*e.g.*, PERMANOVA, ANOSIM, or SIMPER) because it is a non-linear model insensitive to biased distributions and extreme data points, it automatically includes the interaction between features (prey items) due to its hierarchical nature, and it does not require any data transformation (*Carvajal, Maucec & Cullick, 2018*). These models were implemented in Python 3 (v. 3.8.6, *Van Rossum & Drake, 2009*) using the Scikit-learn module (v. 1.0.1, *Pedregosa et al., 2011*).

The model complexity was optimized using a grid search algorithm and five-fold cross-validation during training to tune the maximum tree depth (*i.e.*, the maximum number of recursive partitions), the maximum number of prey species used per tree, and the number of trees in the ensemble. Model performance was evaluated using the area under the curve (AUC) of the Receiver Operating Characteristics (ROC) curve (*Meyer-Baese & Schmid, 2014*). A train-test (75–25%) data split was performed to assess whether the tuned models overfit. Prey (feature) importance was determined using the SHAP library (SHapely Additive exPlanations, v.0.39.0, *Lundberg & Lee, 2017*; *Lundberg et al., 2020*), which is a game-theoretic, model-agnostic approach that connects optimal prediction allocation with local explanations, based on Shapely values. Two preliminary steps were followed when classes were dramatically unbalanced: randomly under-sampling the overrepresented class and applying the Synthetic Minority Over-sampling Technique (SMOTE) using the imbalanced-learn library (v. 0.8.1, *Lemaitre, Nogueira & Aridas, 2007*).

Stable isotope analyses

We analyzed the stable isotope data using Bayesian Inference (BI). In general, BI reallocates the credibility of a parameter among a space of candidate possibilities, using Bayes' theorem for evaluation, given the data, the model, and prior knowledge about the parameter (*Bolstad, 2004*; *Kruschke, 2015*). Every posterior distribution was sampled with three Markov-Chains Monte Carlo (MCMC) algorithms that were run until convergence, *i.e.*, zero divergences during the posterior sampling (No-U-Turn Sampler, NUTS, *Betancourt, 2017*) and Gelman-Rubin statistics (*Gelman, Hwang & Vehtari, 2014*) being less than 1.01 for every parameter. The corresponding Supplemental Information include other graphical diagnostics such as posterior predictive checks and energy plots (*Betancourt, 2017*; *Gabry et al., 2017*). The size of the posterior sample for each model depended on the effective sample size for every parameter being over 2,000 (*Martin, 2018*). The posterior distributions were summarized in terms of their means and 95% Highest Density Intervals ($HDI_{95\%}$), which represent the areas of highest probability for the actual value of the parameter given the data and the model (*Bolstad, 2004*; *Kruschke, 2015*).

Inter and intra-specific analyses of isotopic values

The isotopic values of the three species were described using a custom hierarchical bivariate model, in which the effects of the seasons (cold *vs* warm), sexes, and maturity stages (adults *vs* juveniles) on both isotopic values are nested within each species.

Consequently, the isotopic space of each species results from two linear models (one per isotopic ratio), where the slopes represent the differences between both levels of each factor. Bayesian hierarchical models incorporate the uncertainty around the parameters at the lower levels of the hierarchy and sequentially transfer it to the next; hence, they effectively investigate cross-level hypotheses and minimize the effect of unbalanced data (*Gelman, Hwang & Vehtari, 2014*). The model was implemented using the PyMC3 library (v.3.11.4, *Salvatier, Wiecki & Fonnesbeck, 2016*). Essential details are that (1) the hierarchical model allows a description at both the species and intra-species levels; (2) the bivariate model accounts for the covariation between bulk isotopic values, which is relevant since these depend on the isotopic baseline and trophic discrimination; (3) a Laplacian prior was placed on the slopes, which results in an L1 regularization (*i.e.*, a "Bayesian Lasso" regression, *Park & Casella (2012)*; and (4) the heavy-tailed Student-*t* likelihood assigns a higher probability to extreme values; thus, allowing to make robust estimations of the parameters (*Kruschke, 2012*).

Isotopic niches and overlaps

The isotopic niche areas of each batoid species and their categories were estimated using Stable Isotope Bayesian Ellipse Areas (SEA_B), using the R package SIBER (Stable Isotope Bayesian Ellipses in R, v.2.1.0, *Jackson et al. (2011)*), and comparisons were based on their posterior distributions (Supplemental Information 1). Isotopic niche overlap between species was assessed using the NicheROVER package (*Swanson et al., 2015*), which uses BI to provide a directional estimate of the overlap, in the sense of the probability of finding one species' individual in the isotopic space of another.

RESULTS

The sample sizes per species and group for SCA and SIA are shown in Table 1.

Stomach content analyses

The tuning results of the Random Forest (RF) classifiers are presented in Table 2.

Inter-specific differentiation

A clear distinction between the prey preferences of *H. dipterurus* and *N. entemedor* was found (test AUC: 0.99). The main contributors (cumulative importance of approximately 70%) to their differences were sipunculids, followed by bivalves and polychaetes, where *N. entemedor* had higher weights of sipunculids and polychaetes and *H. dipterurus* had higher weights of bivalves (Fig. 2).

Intra-specific differentiation

The differences among the groups within *H. dipterurus* were not as clear (AUC $\leq$ 0.6; Table 2), but certain trends could still be identified with the SHAP explanations (Fig. 3). The diet of *H. dipterurus* during the warm season had a higher influence of shrimps and other crustaceans (AUC: 0.55). Females had higher weights of stomatopods and crabs, while males of shrimps and amphipods. As for the maturity stages (AUC: 0.6), the classes were balanced to 100 individuals, and stomatopods and bivalves were the most important

Table 1 Sample sizes for each species and their subgroups.

Group	SCA	SIA
H. dipterurus	205	81
Seasons (cold, warm)	96, 109	18, 63
Sexes (females, males)	138, 67	44, 37
Maturity stages (adults, juveniles)	44, 161	36, 45
N. entemedor	187	69
Seasons (cold, warm)	99, 88	15, 54
Sexes (females, males)	154, 33	55, 14
Maturity stages (adults, juveniles)	173, 14	55, 14
R. steindachneri		74
Seasons (cold, warm)		3, 71
Sexes (females, males)		31, 43
Maturity stages (adults, juveniles)		26, 48

Note:
SCA, stomach content analysis; SIA, stable isotope analysis.

Table 2 Tuning results of the random forest classifiers.

Classifier	Trees	Maximum depth	Maximum features	Test AUC
H. dipterurus vs N. entemedor	200	2	3	0.99
H. dipterurus				
Seasons	100	2	1	0.55
Sexes	50	28	1	0.6
Maturity stages	50	5	4	0.6
N. entemedor				
Seasons	50	1	9	0.6
Sexes	100	7	7	0.87
Maturity stages	100	4	8	0.83

Note:
AUC, area under the receiver operating characteristic (ROC) curve.

for the classification. Adults had higher weights of stomatopods, bivalves, crabs and amphipods.

In *N. entemedor*, the differences among groups were more definite than those of *H. dipterurus* (AUC > 0.8; Fig. 4), except among the seasons. The most important differences between both seasons (AUC: 0.6) and sexes (AUC: 0.87) were in sipunculids, while polychaetes were the most different between maturity stages. During the warm season, *N. entemedor* preyed more upon sipunculids and polychaetes. Females consumed more sipunculids, polychaetes, and shrimps, while juveniles fed more on polychaetes and shrimps. These results are strongly limited by the small sample sizes of the groups with fewer observations (juveniles and males, Table 1). Balancing the dataset with subsampling and SMOTE aids only for training and evaluating the RF; thus, the results should be taken cautiously.
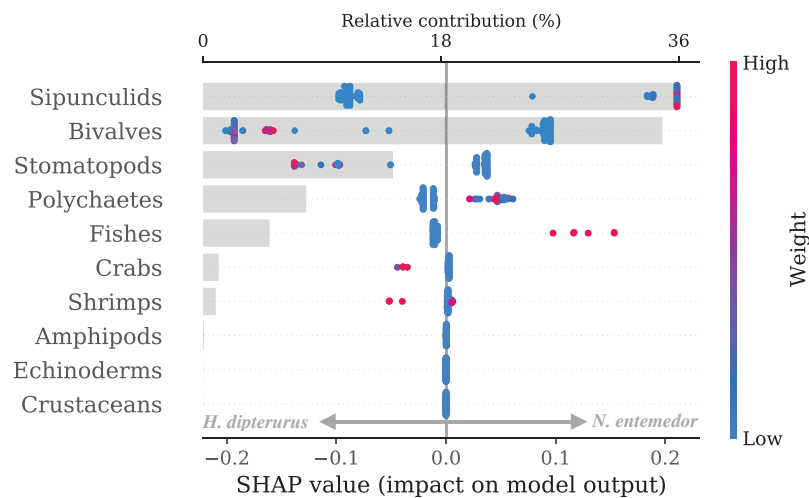
Figure 2 Bee swarm (points, bottom axis) and bar (top axis) plots of SHAP explanations for the random forest classifying *H. dipterurus* and *N. entemedor*. The bee swarm plot shows the SHAP values for every prey group per individual. A bluer color expresses lower prey weights, while a redder color indicates higher prey weights, and the position along the x-axis shows whether that prey weight contributed to *H. dipterurus* (left) or *N. entemedor* (right). The bar plot shows the net prey contribution to the prediction. Full-size ⬛ DOI: 10.7717/peerj-16117/fig-2

Stable isotope analyses

Inter-specific comparisons of isotopic values and niches

The MvST model showed a gradient in $\delta^{13}C$ values among the species (Table 3), with *N. entemedor* having the highest values, followed by *H. dipterurus* and *R. steindachneri*. For $\delta^{15}N$ (Table 3), *R. steindachneri* and *H. dipterurus* had similar values, lower than those of *N. entemedor*. The comparisons of the posterior distributions showed highly probable differences in most cases ($P > 99\%$; Fig. 5); the only exception being $\delta^{15}N$ values of *H. dipterurus* and *R. steindachneri*. The largest mean difference was found between *N. entemedor* and *R. steindachneri* in $\delta^{13}C$ ($M = 3.7‰$; $P(N.\ entemedor < R.\ steindachneri) = 100\%$). These results suggest inter-specific differences in both habitat and prey preferences.

Regarding the isotopic niche areas (Table 3 and Fig. 6A), *H. dipterurus* had the broadest niche, followed by *N. entemedor* and *R. steindachneri* with the narrowest. Every paired comparison showed highly probable differences ($P > 87\%$; Fig. 7). Additionally, the isotopic niche overlaps were higher in *N. entemedor* and *R. steindachneri* $\in$ *H. dipterurus* than vice-versa ($P > 70\%$, Table 4), explained by the dramatically broader isotopic niche of *H. dipterurus* and suggesting a high degree of plasticity when compared to the other species.

Intra-specific comparisons of isotopic values and niches

The results of analyzing the effect of the sex, seasonality, and maturity stage are summarized in Fig. 8. The effect of sex on isotopic values was relatively minor, where the differences had probabilities lower than 75% in every case other than $\delta^{13}C$ for *H. dipterurus* ($\beta = -0.32‰$; $P(\beta < 0) \approx 90\%$). The effect of the season was more evident
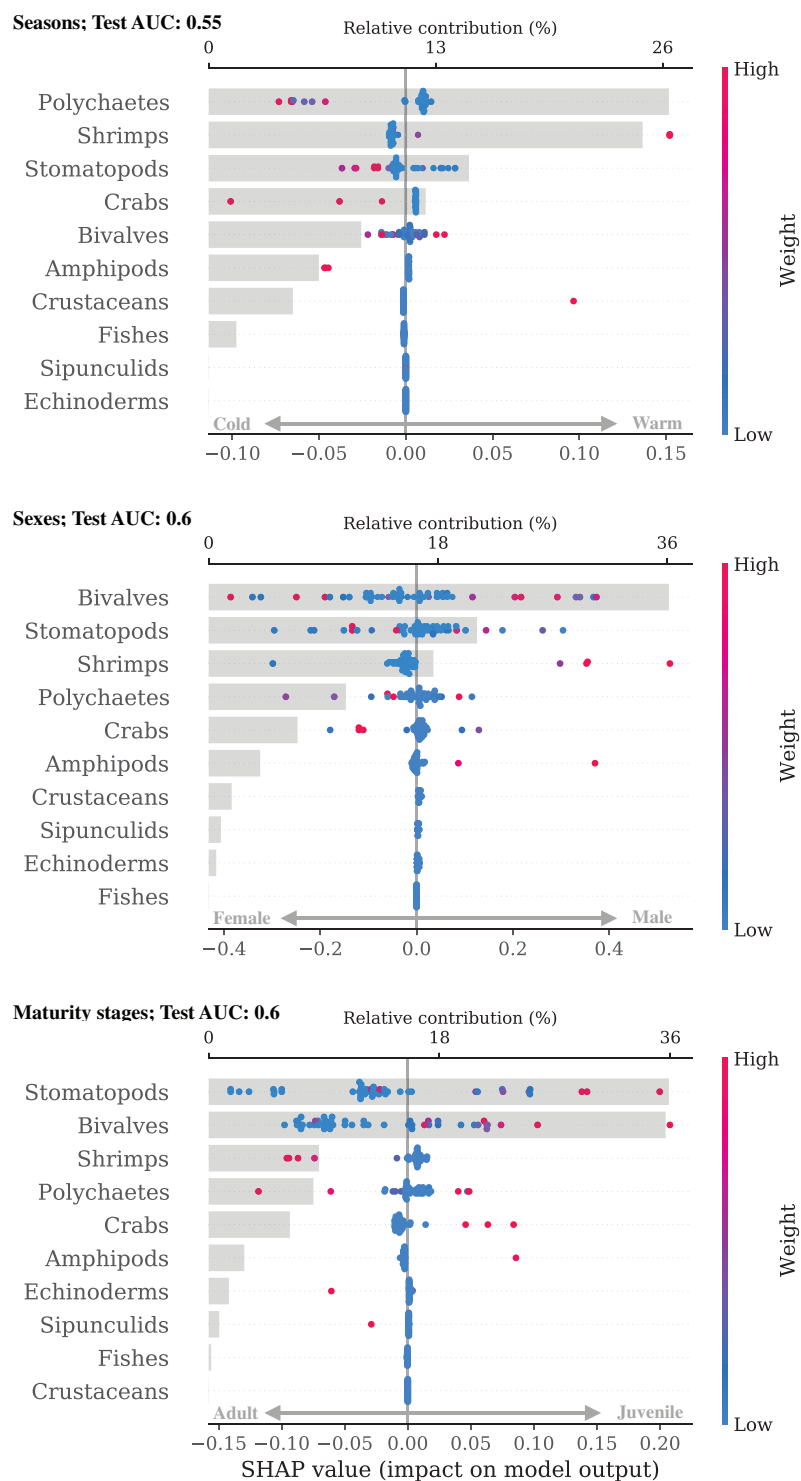
Figure 3 Bee swarm and bar plots of SHAP explanations for the intra-specific classifiers for *Hypanus dipterurus*.
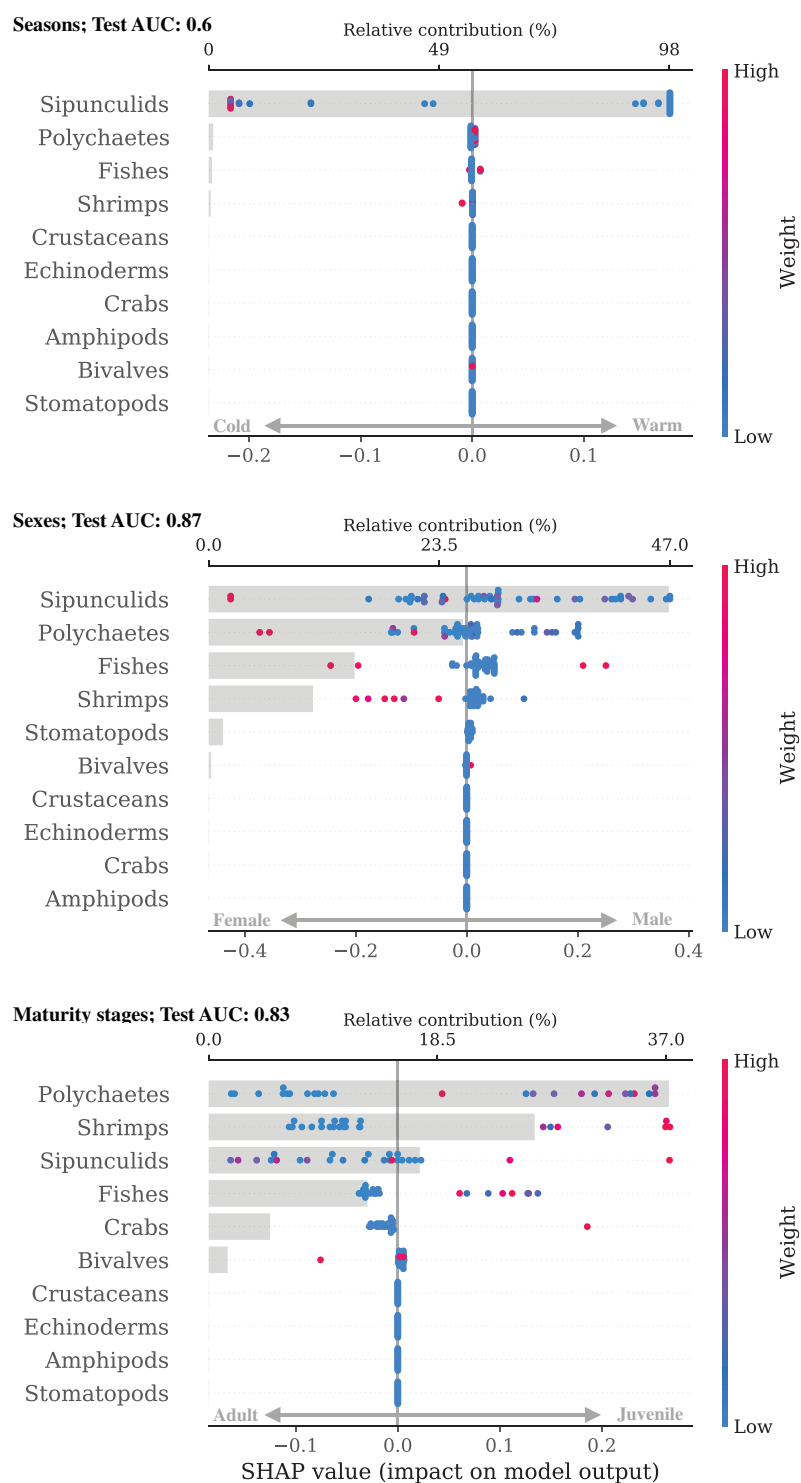Full-size ☐ DOI: 10.7717/peerj-16117/fig-3

Figure 4 Bee swarm and bar plots of SHAP explanations for the intra-specific classifiers for *Narcine entemedor*. Full-size ⊡ DOI: 10.7717/peerj-16117/fig-4

Table 3 Descriptive statistics of the posterior distributions for the mean $\delta^{13}C$ and $\delta^{15}N$ values (‰), and isotopic niche areas (‰^{2}) of each species, sorted in descending order.

Parameter	Mean	HDI$_{95\%}$
$\delta^{13}C$		
N. entemedor	−12.63	(−12.63, −12.14)
R. steindachneri	−14.0	(−14.39, −13.64)
H. dipterurus	−16.12	(−16.34, −15.90)
$\delta^{15}N$		
N. entemedor	18.05	(17.86, 18.25)
R. steindachneri	16.18	(16.00, 16.34)
H. dipterurus	16.16	(15.87, 16.46)
Isotopic niche area		
H. dipterurus	9.66	(7.5, 11.83)
N. entemedor	2.15	(1.65, 2.66)
R. steindachneri	1.68	(1.31, 2.08)

Note:
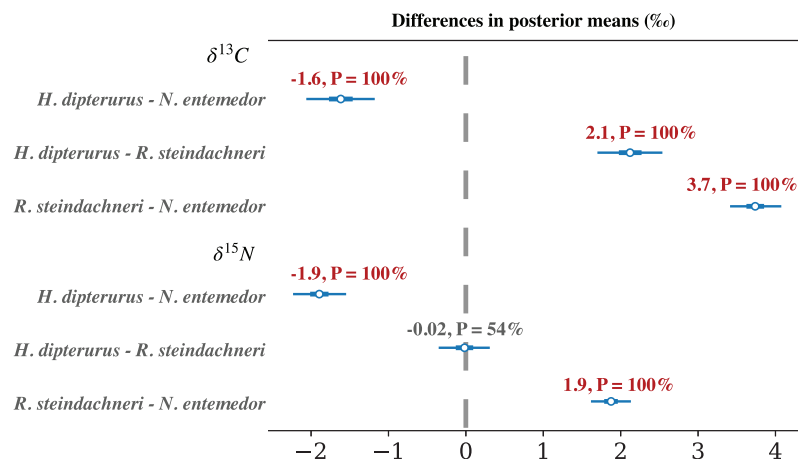 HDI$_{95\%}$, 95% highest density intervals.

Figure 5 Forest plot of the comparisons between the posterior means of each species' isotopic ratios. Shown as mean, probability of differences being greater or smaller than 0. Thin lines represent the 95% Highest Density Interval ($\delta^{13}C$), thick lines the $\delta^{15}N$, and points indicate the mean of the distribution.
Full-size 🖼 DOI: 10.7717/peerj-16117/fig-5

in $\delta^{15}N$, with the probabilities of the differences between the warm and cold seasons exceeding 90% for most comparisons. The values of the three species were lower during the cold season than during the warm season. For $\delta^{13}C$, only *H. dipterurus* showed higher values during the cold season as compared to the warm season [$\beta = 0.74‰$; $p\,(\beta > 0) \approx 98\%$]. Most comparisons between maturity stages showed that juveniles had lower values of both isotopes, except for *H. dipterurus* in $\delta^{13}C$, where juveniles had higher values than adults.

The degree of intra-specific differentiation in isotopic niche areas varied among species (Fig. 6B, and Fig. 7). For the seasons, *R. steindachneri* was the only species with highly
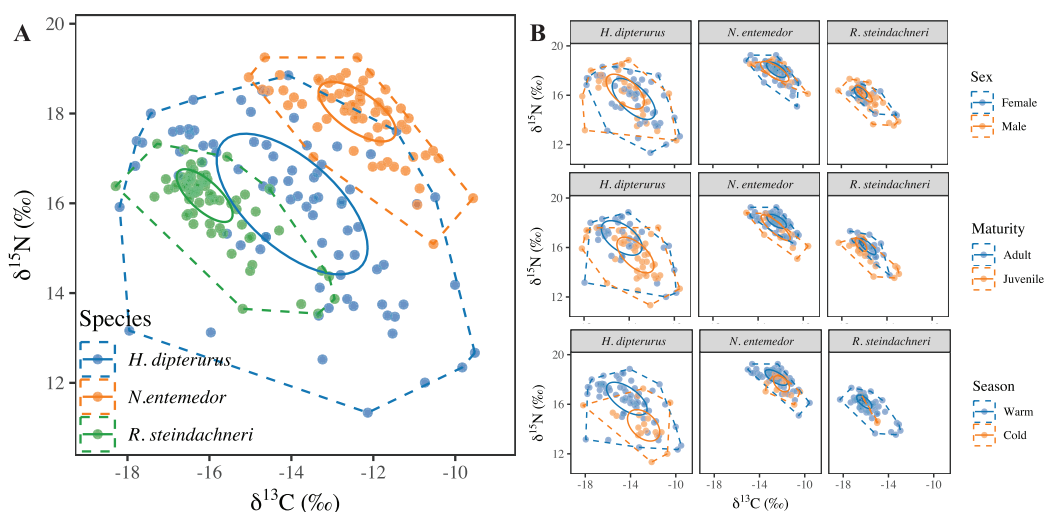
Figure 6 Isotopic niches of the three batoid species (A) and their categories (B), represented with standard ellipses and convex hulls. Full-size 🖼 DOI: 10.7717/peerj-16117/fig-6
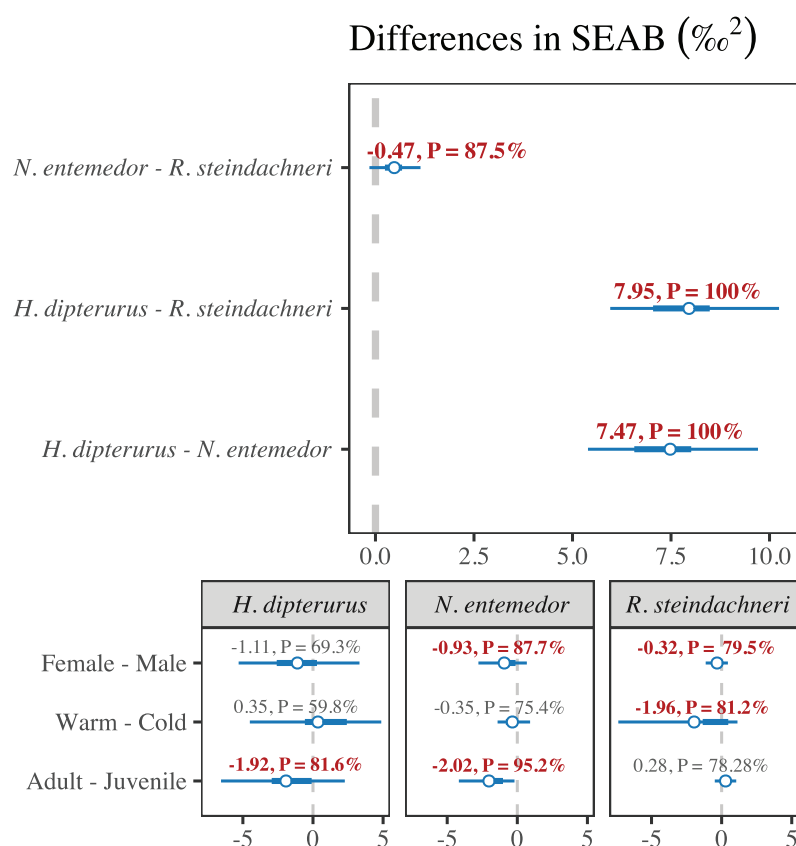

Figure 7 Forest plot with the posterior differences in isotopic niche areas (SEA$_B$) and the probabilities of them being greater or smaller than 0. Full-size 🖼 DOI: 10.7717/peerj-16117/fig-7

Table 4 Mean overlaps (%) for a 95% Bayesian Ellipse, expressed as the probability of the species in the row being within the isotopic space of the one in the column $[P\,(row\,\in\,column)]$.

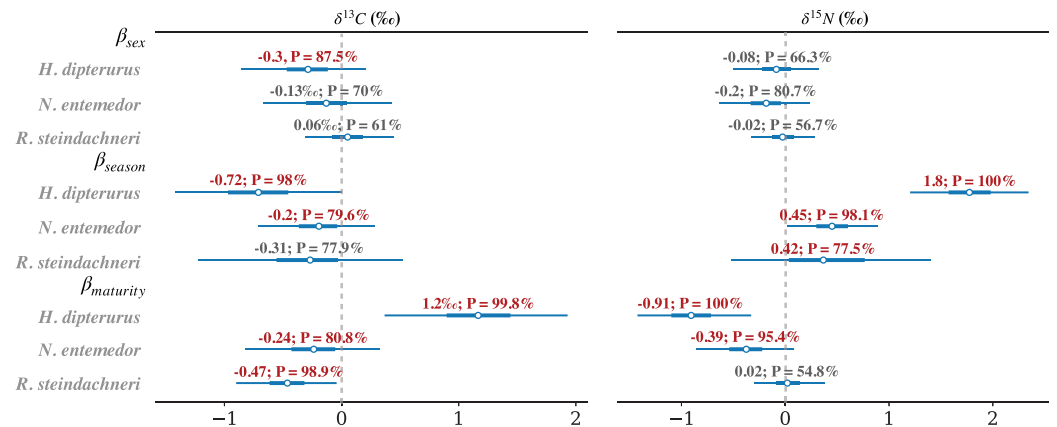|	*H. dipterurus*	*N. entemedor*	*R. steindachneri*
H. dipterurus		9	24
N. entemedor	73		0
R. steindachneri	100	0	

Figure 8 Forest plot of the posterior distributions of the slopes of every factor (β) for every species and isotopic ratio and the probabilities of them being greater or smaller than 0.

Full-size 🖾 DOI: 10.7717/peerj-16117/fig-8

probable differences ($p\,(cold > warm) \approx 80\%$). For the sexes, both *N. entemedor* and *R. steindachneri* showed differences, where males had broader areas than females ($p\,(males > females) \geq 79\%$). Regarding the maturity stages, both *H. dipterurus* ($p\,(juveniles > adults) \approx 80\%$) and *R. steindachneri* ($p\,(adults > juveniles) \approx 77\%$) showed differences in isotopic niche areas between juveniles and adults, albeit with inverse patterns.

As with SCA, some categories have limited sample sizes (Table 1). Although hierarchical Bayesian models aid in minimizing the effect of small sample sizes and unbalanced datasets, results should be carefully interpreted, and larger sample sizes are needed to validate them.

DISCUSSION

La Paz Bay and Espiritu Santo Island (ESI) are the most studied geographic zones within the Gulf of California, possibly due to their proximity to the national marine research institutes in La Paz (*Ehemann, García-Rodríguez & Cruz-Agüero, 2022*), where batoid studies have increased during the last decade. The result is a valuable scientific baseline that addresses topics such as diet (*Cabrera-Melendez, 2017*; *Ehemann et al., 2019*; *Restrepo-Gómez et al., 2021*), reproduction (*e.g.*, *Ehemann et al., 2017*; *Burgos-Vázquez et al., 2017, 2018*), artisanal fisheries (*González-González et al., 2020*), age and growth (*Mora-Zamacona et al., 2021*; *Pabón-Aldana et al., 2022*), and the role of ESI as a nursery habitat

(*e.g.*, pygmy devil rays, *Mobula munkiana*, *Palacios et al., 2021*). As a result, ESI has been pointed as a critical area for conserving batoid assemblages within the Gulf of California (*González-González et al., 2021*), providing batoid species with different niches, habitats, and resources. Nonetheless, this is the first study analyzing the dietary niche dynamics and resource partitioning patterns among and within the three most abundant and fished species in the area.

We found a gradient in $\delta^{13}C$ values, where *N. entemedor* had the highest, followed by *H. dipterurus* and *R. steindachneri*. Although differences in $\delta^{13}C$ are usually associated with horizontal spatial segregations, namely an inshore-offshore gradient, they could also indicate a benthic-pelagic gradient (*Newsome et al., 2007*). In this case, neither isotopic pattern can be discarded. Essentially, isotopic gradients follow the differences in isotopic fractionation, which, in turn, depend on primary productivity, carbon sources, and biochemical processes (*Smith & Epstein, 1971*; *Peterson & Fry, 1987*). In this regard, the Gulf of California is a highly productive environment due to seasonal upwellings and oceanic eddies (*Mercado-Santana et al., 2017*), thus favoring higher biomasses (both in size and abundance) of primary producers, which then have higher $\delta^{13}C$ signatures than the smaller and slower-growing phytoplankton found in oceanic environments (*Goericke & Fry, 1994*). Benthic habitats, on the other hand, yield higher $\delta^{13}C$ baselines than pelagic ones due to a couple of factors: a preferential photosynthetic uptake of ^{12}C in surface waters and the release of ^{12}C during the subsequent decomposition and sinking of organic matter, leading to an increase in $\delta^{13}C$ values in calcifiers (*Börner et al., 2017*).

Both inshore-offshore and benthic-pelagic segregations have biological explanations depending on the analyzed species and their behaviors. The inter-specific niche differentiation observed in this study from SCA and SIA approaches showed that *H. dipterurus* relies more heavily on benthic habitats and less on infaunal prey compared to *N. entemedor*. Moreover, their $\delta^{13}C$ values are similar to those reported for other coastal benthic predator species in the Gulf of California (*Aurioles-Gamboa et al., 2013*; *Valenzuela-Quiñonez et al., 2018*), which suggests that La Paz Bay is an important feeding area for these batoid species year-round. These findings are consistent with prior works on the feeding strategies of both species, where infaunal and epibenthic invertebrates are the most consumed by *H. dipterurus* (*Restrepo-Gómez et al., 2021*), while polychaetes have been reported as the primary prey of *N. entemedor* (*Valadez-González, 2007*; *Cabrera-Melendez, 2017*). Differences in oral myology (*Ramírez-Díaz et al., 2022*) and feeding strategies could explain this resource partitioning. The protractile and tubular ventral mouth, along with its electric discharge (*Last et al., 2016*; *Ramírez-Díaz et al., 2022*), make *N. entemedor* better suited to feed on benthic prey, while the different body shapes, teeth morphology, rostral fins, and locomotion systems of the other two species allow them to exploit the benthopelagic habitat (*Nelson, Grande & Wilson, 2016*; *Last et al., 2016*; *González-González et al., 2020*; *Ramírez-Díaz et al., 2022*).

The $\delta^{15}N$ values found in this study relate to the ^{15}N-enriched baseline found in the Gulf of California, due to biochemical processes and the oceanic circulation, leading to particularly high $\delta^{15}N$ values in consumers (*Elorriaga-Verplancken et al., 2018*). The Eastern Tropical Pacific (ETP) is characterized by low oxygen concentrations and a

shallow oxygen minimum zone, which result in deep denitrification processes that, in turn, mix with surface waters during upwellings (*Altabet et al., 1999*; *Takai et al., 2000*). The added denitrified water to the surface of the ETP generates a basal ^{15}N-enrichment due to the preferential removal of ^{14}N-enriched nitrate by bacteria, causing the emergence of ^{15}N-enriched residual nitrate during upwellings, which is then incorporated during primary production at the ocean surface (*Altabet et al., 1999*). Similar to $\delta^{13}C$, a gradient of $\delta^{15}N$ values was found, such that *N. entemedor* > *H. dipterurus* $\approx$ *R. steindachneri*. These differences could be associated with differences in trophic positions (TPs); however, the influence of the environment could be a confounding factor, given that previous reports suggest that these species share TPs around 3.5 (*Froese & Pauly, 2023*). Testing whether this is the case is unfeasible with the available information. Bulk SIA can confound the effect of the diet and primary producers (*Martínez del Río, Anderson-Sprecher & Gonzalez, 2009*) due to the spatial and temporal variation of the isotopic composition at the base of the trophic webs (*Whitehead et al., 2020*). Nonetheless, the isotopic differences can be explained by the differences in prey preference, where *N. entemedor* preyed more on sipunculids and polychaetes, while *H. dipterurus* preferred bivalves and stomatopods. Still, the confounding of these factors could be elucidated by measuring the isotope values of individual amino acids (Amino-Acid Compound-Specific SIA, AACSIA), given their unique and well-understood biochemical pathways, which would allow a direct assessment of the trophic web baseline (source AA) and the trophic status of the consumers (trophic AA, *Ruiz-Cooley et al., 2017*).

In terms of the intra-specific differentiation, *H. dipterurus* did not have marked differences among the different factors (AUCs around 0.6); however, the MvST results for $\delta^{13}C$ values suggested differences among sexes, seasons and maturity stages. Likewise, for $\delta^{15}N$, the slopes suggested differences between seasons and maturity stages but not between sexes (Fig. 8). Two non-mutually exclusive hypotheses could bring the isotopic results together, including the broad isotopic niche. The first one is that adults of *H. dipterurus* may enter La Paz Bay during the warm season for reproductive activities, while juveniles enter during the cold season (*Burgos-Vázquez et al., 2018*). The second is that some individuals could have had isotopic values corresponding to a different isoscape (*i.e.*, an area with different baseline isotopic values) than those of the Gulf of California. Either way, a plausible explanation is the vagility of the species and its continuous distribution (*Last et al., 2016*), as has been reported with other migratory predator species in the Gulf of California (*Elorriaga-Verplancken et al., 2018*). These two hypotheses could also explain part of the much broader isotopic niche of the species relative to the other two (Figs. 6 and 7); however, the effect of the maturity stages on the isotopic niche cannot be discarded. Adults with a smaller isotopic niche than juveniles could indicate a change in prey items consumed or specialization, which is congruent with prior reports (*Restrepo-Gómez et al., 2021*). Such adaptive foraging behaviors of mesotrophic species are important to maintaining ecosystem function, where switching from generalists to specialists as they mature helps to maintain ecosystem stability and resilience by the exploitation of a broader range of resources and reducing possible competition with other individuals (*Schmitz et al., 2008*).

In contrast, both approaches yielded more consistent results for *N. entemedor* because they agreed upon differences within factors. The performances of the RF models for sexes and maturity stages were adequate (AUCs > 0.8), while MvST results suggested potential differences between maturity stages for $\delta^{13}C$ and between seasons and maturity stages for $\delta^{15}N$. These results indicate ontogenetic changes in foraging habits, agreeing with previous studies on the species (*Cabrera-Melendez, 2017*). In this case, the ontogenetic changes could also be related to an increased trophic spectrum due to an increase in body and mouth sizes during growth (*Spath, Barbini & Figueroa, 2013*), resulting in more efficient prey capture and handling mechanisms (*Valadez-González, 2007*). Consequently, adults have access to a broader array of potential prey, supported by their broader isotopic niche relative to juveniles (Fig. 7). Another interesting finding is the differences between sexes, where females had a smaller isotopic niche area than males, opposite to prior SCA results where males had the broader trophic niche (*Cabrera-Melendez, 2017*). This discrepancy could be explained not only by the different time resolutions represented by SCA and SIA but also by the body size dimorphism (larger females), allowing them to access a broader spectrum of prey, as has been mentioned for other marine species (*Phillips et al., 2011*; *Espinoza et al., 2015*; *Rosas-Hernández, Aurioles-Gamboa & Hernández-Camacho, 2019*). Moreover, whether these sex-related differences in batoid dietary habits could be related to reproduction has not been studied and is thus an ideal area for future research.

Although the SCA was not considered for *R. steindachneri*, the SIA results showed similar trends as in other species, with high probabilities of differences between maturity stages in $\delta^{13}C$ and between seasons in $\delta^{15}N$. As with *N. entemedor*, the differences in $\delta^{13}C$ agree with ontogenetic changes in dietary habits, while differences in $\delta^{15}N$ could indicate a change in trophic spectrum or could also be the consequence of the migratory behavior of the species (*Schwartz, 1990*; *Last et al., 2016*). These results are the first approach to shed light on the intra-specific variation in this species.

The contrasting results of SCA and SIA are likely due to differences in the periods reflected by both approaches. SCA reflects only a few hours before sampling and is affected by differential digestion rates of prey items, where soft-tissued organisms that are easily digested are not often present in stomachs (*Ehemann et al., 2019*). Consequently, this confounds the underlying ecological processes. This contrast also highlights the complementariness of both approaches. Nonetheless, two important considerations are that our SIA could reflect diet assimilation from over a year before sampling (*Logan & Lutcavage, 2010*; *MacNeil, Drouillard & Fisk, 2006*), meaning that data from the warm seasons could include information from the previous cold season and *vice-versa*. Thus, analyzing a more metabolically active tissue with a shorter integration period would be preferable to generate more comparable results. Isotopic turnover rates on elasmobranchs' tissues are mostly unknown, and most of the known tissues are slow (*Hussey et al., 2012*). Liver tissue and blood plasma, however, have the potential to be quicker and are probably better choices for future studies revolving around intra-annual variations or more directly comparing SIA with SCA (*Logan & Lutcavage, 2010*; *Matich, Heithaus & Layman, 2010*). This knowledge gap highlights the need to study isotopic turnover rates and trophic discrimination factors on different elasmobranch species and tissues. All things

considered, this study is a promising step towards understanding the dietary niche dynamics of these species, as well as the resource partitioning mechanisms that facilitate the coexistence of the three species in the Southern Gulf of California, Mexico.

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Arturo Bell Enríquez-García conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Víctor Hugo Cruz-Escalona conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- José D. Carriquiry conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the article, and approved the final draft.

- Nicolás R. Ehemann conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Paola A. Mejía-Falla conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Emigdio Marín-Enríquez conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Christina Treinen-Crespo conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- José R. Vélez-Tacuri conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Andrés F. Navia conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

The data is available at GitHub and Zenodo:

- https://arturobell.github.io/01072022/.

- Arturo Bell. (2023). ArturoBell/01072022: PeerJ Review 1 (PeerJ-rev1). Zenodo. https://doi.org/10.5281/zenodo.8024122

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REFERENCES

Albo-Puigserver M, Navarro J, Coll M, Aguzzi J, Cardona L, Sáez-Liante R. 2015. Feeding ecology and trophic position of three sympatric demersal chondrichthyans in the northwestern Mediterranean. *Marine Ecology Progress Series* **524**:255–268 DOI 10.3354/meps11188.

Altabet M, Pilskaln C, Thunell R, Pride C, Sigman D, Chavez F, Francois R. 1999. The nitrogen isotope biogeochemistry of sinking particles from the margin of the Eastern North Pacific. *Deep Sea Research Part I: Oceanographic Research Papers* **46(4)**:655–679 DOI 10.1016/S0967-0637(98)00084-3.

Aurioles-Gamboa D, Rodríguez-Pérez M, Sánchez-Velasco L, Lavín M. 2013. Habitat, trophic level, and residence of marine mammals in the Gulf of California assessed by stable isotope analysis. *Marine Ecology Progress Series* **488**:275–290 DOI 10.3354/meps10369.

Barbini SA, Sabadin DE, Lucifora LO. 2018. Comparative analysis of feeding habits and dietary niche breadth in skates: the importance of body size, snout length, and depth. *Reviews in Fish Biology and Fisheries* **28(3)**:625–636 DOI 10.1007/s11160-018-9522-5.

Barría C, Navarro J, Coll M, Fernandez-Arcaya U, Sáez-Liante R. 2015. Morphological parameters of abundant and threatened chondrichthyans of the northwestern Mediterranean Sea. *Journal of Applied Ichthyology* **31(1)**:114–119 DOI 10.1111/jai.12499.

Betancourt M. 2017. A conceptual introduction to Hamiltonian Monte Carlo. *ArXiv preprint* DOI 10.48550/arXiv.1701.02434.

Bolstad W. 2004. *Introduction to Bayesian statistics.* Hoboken: Wiley-Interscience.

Brand WA, Coplen TB, Vogl J, Rosner M, Prohaska T. 2014. Assessment of international reference materials for isotope-ratio analysis (IUPAC Technical Report). *Pure and Applied Chemistry* **86(3)**:425–467 DOI 10.1515/pac-2013-1023.

Burgos-Vázquez MI, Chávez-García VE, Cruz-Escalona VH, Navia AF, Mejía-Falla PA. 2018. Reproductive strategy of the Pacific cownose ray *Rhinoptera steindachneri* in the southern Gulf of California. *Marine and Freshwater Research* **70(1)**:93–106 DOI 10.1071/MF18096.

Burgos-Vázquez MI, Mejía-Falla P, Cruz-Escalona VH, Brown-Peterson NJ. 2017. Reproductive strategy of the giant electric ray in the southern Gulf of California. *Marine and Coastal Fisheries* **9(1)**:577–596 DOI 10.1080/19425120.2017.1370042.

Börner N, Baaere BD, Akita L, Francois R, Jochum K, Frenzel P, Zhu L, Schwalb A. 2017. Stable isotopes and trace elements in modern ostracod shells: implications for reconstructing past environments on the Tibetan Plateau, China. *Journal of Paleolimnology* **58**:191–211 DOI 10.1007/s10933-017-9971-1.

Cabrera-Melendez P. 2017. Hábitos alimentarios de *Narcine entemedor* en la Bahía de La Paz, México [Foraging habits of *Narcine entemedor* in Bahía de La Paz, México]. MSc thesis. Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas. La Paz, BCS: México. *Available at* https://www.repositoriodigital.ipn.mx/bitstream/123456789/25017/1/cabrerame1.pdf.

Carvajal G, Maucec M, Cullick S. 2018. *Components of artificial intelligence and data analytics.* Houston: Gulf Professional Publishing, 101–148.

Coll M, Navarro J, Palomera I. 2013. Ecological role, fishing impact, and management options for the recovery of a Mediterranean endemic skate by means of food web models. *Biological Conservation* **157**:108–120 DOI 10.1016/j.biocon.2012.06.029.

Cortés E. 1997. A critical review of methods of studying fish feeding based on analysis of stomach contents: application to elasmobranch fishes. *Canadian Journal of Fisheries and Aquatic Sciences* **54**:726–738 DOI 10.1139/cjfas-54-3-726.

DeNiro M, Epstein S. 1976. You are what you eat (plus a few per mil): the carbon isotope cycle in food chains. *Geological Society of America Abstracts with Programs* **8(6)**:834–835.

Ehemann NR, Abitia-Cárdenas LA, Navia AF, Mejía-Falla PA, Cruz-Escalona VH. 2019. Zeros as a result in diet studies, is this really bad? *Rhinoptera steindachneri* as a case study. *Journal of the Marine Biological Association of the United Kingdom* **99(7)**:1661–1666 DOI 10.1017/S0025315419000511.

Ehemann N, García-Rodríguez F, Cruz-Agüero JDL. 2022. Morphological abnormalities in seven American round ray specimens: a review of America's batomorph anomalies. *Journal of Fish Diseases* **45(3)**:395–409 DOI 10.1111/jfd.13567.

Ehemann N, Pérez-Palafox X, Pabón-Aldana K, Mejía-Falla P, Navia A, Cruz-Escalona V. 2017. Biological notes on the reef stingray, *Urobatis concentricus*, an endemic species of Mexico. *Journal of Fish Biology* **91(4)**:1228–1235 DOI 10.1111/jfb.13398.

Elorriaga-Verplancken F, Sandoval-Sierra J, Paniagua-Mendoza A, Robles R. 2018. Seasonality and potential foraging grounds of migratory California sea lions from La Paz Bay, Southern Gulf of California. *Mexico Aquatic Mammals* **44(1)**:56–61 DOI 10.1578/AM.44.1.2018.56.

Espinoza M, Munroe S, Clarke T, Fisk A, Wehrtmann I. 2015. Feeding ecology of common demersal elasmobranch species in the Pacific coast of Costa Rica inferred from stable isotope and

stomach content analyses. *Journal of Experimental Marine Biology and Ecology* **470**:12–25 DOI 10.1016/j.jembe.2015.04.021.

Ferreti F, Worm B, Britten GL, Heithaus M, Lotze HK. 2010. Patterns and ecosystem consequences of shark declines in the ocean. *Ecology Letters* **13(8)**:1055–1071 DOI 10.1111/j.1461-0248.2010.01489.x.

Flores-Ortega JR, Godínez-Domínguez E, González-Sansón G. 2015. Ecología trófica de siete especies de batoideos (Batoidea) en el Pacífico Central Mexicano. *Revista de Biología Marina y Oceanografía* **50(3)**:521–533 DOI 10.4067/S0718-19572015000400010.

Fontaine P, Barreiros J, Jaquemet S. 2023. Trophic ecology of three sympatric batoid species (*Dasyatis pastinaca*, *Raja clavata*, and *Raja maderensis*) from the Azores, NE Atlantic. *Environmental Biology of Fishes* **106**:1477–1492 DOI 10.1007/s10641-023-01412-2.

Froese R, Pauly D. 2023. FishBase. *Available at www.fishbase.org* (accessed 20 February 2023).

Gabry J, Simpson D, Vehtari A, Betancourt M, Gelman A. 2017. Visualization in Bayesian workflow. *ArXiv preprint* DOI 10.1111/rssa.12378.

Gause G. 1934. *The struggle for existence*. Philadelphia: The Williams & Wilkins Company.

Gelman A, Hwang J, Vehtari A. 2014. Understanding predictive information criteria for Bayesian models. *Statistics and Computing* **24(6)**:997–1016 DOI 10.1007/s11222-013-9416-2.

Goericke R, Fry B. 1994. Variations in marine plankton δ^{13}C with latitude, temperature, and dissolved CO_2 in the world ocean. *Global Biochemical Cycles* **8(1)**:85–90 DOI 10.1029/93GB03272.

González-González LDV, Cruz-Escalona V, Ehemann N, Cruz-Agüero G, Abitia-Cárdenas L, Mejía-Falla P, Navia A. 2020. Riqueza y abundancia relativa de los batoideos de la pesquería artesanal en el archipiélago Espíritu Santo, BCS, México. *Hidrobiológica* **30(1)**:37–47 DOI 10.24275/uam/izt/dcbs/hidro/2020v30n1/Gonzalez.

González-González LDV, Mejía-Falla P, Navia A, de la Cruz-Agüero G, Ehemann N, Peterson M, Cruz-Escalona V. 2021. The Espiritu Santo Island as a critical area for conserving batoid assemblage species within the Gulf of California. *Environmental Biology of Fishes* **104(11)**:1359–1379 DOI 10.1007/s10641-021-01165-w.

Guevara-Guillén C, Shirasago-German B, Pérez-Lezama EL. 2015. The influence of large-scale phenomena on La Paz Bay hydrographic variability. *Open Journal of Marine Science* **5(1)**:146–157 DOI 10.4236/ojms.2015.51012.

Hardin G. 1960. The competitive exclusion principle. *Science* **131(3409)**:1292–1297 DOI 10.1126/science.131.3409.1292.

Heithaus MR, Frid A, Vaudo J, Worm B, Wirsing AJ. 2010. *Unraveling the ecological importance of elasmobranchs*. Boca Raton: CRC Press and Taylor & Francis Group.

Hussey N, MacNeil M, Olin J, McMeans B, Kinney M, Chapman D, Fisk A. 2012. Stable isotopes and elasmobranchs: tissue types, methods, applications and assumptions. *Journal of Fish Biology* **80(5)**:1449–1484 DOI 10.1111/j.1095-8649.2012.03251.x.

Hutchinson G. 1978. *An introduction to population ecology*. New Haven: Yale University Press.

Hyslop E. 1980. Stomach contents analysis-a review of methods and their application. *Journal of Fish Biology* **17(4)**:411–429 DOI 10.1111/j.1095-8649.1980.tb02775.x.

Jackson A, Inger R, Parnell A, Bearhop S. 2011. Comparing isotopic niche widths among and within communities: SIBER-stable isotope Bayesian ellipses in R. *Journal of Animal Ecology* **34(3)**:595–602 DOI 10.1111/j.1365-2656.2011.01806.x.

Kaehler S, Pakhomov E. 2001. Effects of storage and preservation on the δ^{13} C and δ^{15} N signatures of selected marine organisms. *Marine Ecology Progress Series* **219**:299–304 DOI 10.3354/meps219299.

Kim S, Koch P. 2012. Methods to collect, preserve, and prepare elasmobranch tissues for stable isotope analysis. *Environmental Biology of Fishes* **95(1)**:53–63 DOI 10.1007/s10641-011-9860-9.

Kruschke J. 2012. Bayesian estimation supersedes the t test. *Journal of Experimental Psychology: General* **142(2)**:573–603 DOI 10.1037/a0029146.

Kruschke J. 2015. *Doing Bayesian data analysis: a tutorial with R, JAGS, and Stan.* 2nd Edition. Cambridge: Academic Press.

Last P, Naylor G, Séret B, White W, de Carvalho M, Stehmann M. 2016. *Rays of the world.* Clayton: CSIRO Publishing.

Lemaitre G, Nogueira F, Aridas C. 2007. Imbalanced-learn: a Python toolbox to tackle the curse of imbalanced datasets in machine learning. *Journal of Machine Learning Research* **18**:1–5.

Li Y, Zhang Y, Hussey N, Dai X. 2016. Urea and lipid extraction treatment effects on δ^{15} N and δ^{13} C values in pelagic sharks. *Rapid Communications in Mass Spectrometry* **30(1)**:1–8 DOI 10.1002/rcm.7396.

Lim K, Chong V, Phaik-Eem L, Yurimoto T, Loh K. 2018. Feeding ecology of three sympatric species of stingrays on a tropical mudflat. *Journal of the Marine Biological Association of the United Kingdom* **99**:999–1007 DOI 10.1017/S0025315418000759.

Logan J, Lutcavage M. 2010. Stable isotope dynamics in elasmobranch fishes. *Hydrobiologia* **644**:231–244 DOI 10.1007/s10750-010-0120-3.

Lundberg SM, Erion G, Chen H, DeGrave A, Prutkin JM, Nair B, Katz R, Himmelfarb J, Bansal N, Lee S-I. 2020. From local explanations to global understanding with explainable AI for trees. *Nature Machine Intelligence* **2(1)**:56–67 DOI 10.1038/s42256-019-0138-9.

Lundberg S, Lee S-I. 2017. A unified approach to interpreting model predictions. *ArXiv preprint* DOI 10.48550/arXiv.1705.07874.

Mabragaña E, Giberto DA. 2007. Feeding ecology and abundance of two sympatric skates, the shortfin sand skate *Psammobatis normani* McEachran, and the Smallthorn sand skate *P. rudis* Gunther (Chondricthyes, Rajidae), in the southwest Atlantic. *ICES Journal of Marine Science* **64**:1017–1027 DOI 10.1093/icesjms/fsm078.

MacNeil M, Drouillard K, Fisk A. 2006. Variable uptake and elimination of stable nitrogen isotopes between tissues in fish. *Canadian Journal of Fisheries and Aquatic Sciences* **63**:345–353 DOI 10.1139/f05-219.

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. *Journal of Fish Biology* **72(4)**:883–898 DOI 10.1111/j.1095-8649.2007.01762.x.

Martin O. 2018. *Bayesian analysis with Python: introduction to statistical modeling and probabilistic programming using PyMC3 and ArviZ.* 2nd Edition. Birmingham: Pakt Publishing.

Martínez del Río CP, Anderson-Sprecher R, Gonzalez S. 2009. Dietary and isotopic specialization: the isotopic niche of three Cinclodes ovenbirds. *Oecologia* **161(1)**:149–159 DOI 10.1007/s00442-009-1357-2.

Matich P, Heithaus M, Layman C. 2010. Contrasting patterns of individual specialization and trophic coupling in two marine apex predators. *Journal of Animal Ecology* **80**:294–305 DOI 10.1111/j.1365-2656.2010.01753.x.

Mercado-Santana J, del Ángel ES, González-Silvera A, Sánchez-Velasco L, Gracia-Escobar M, Millán-Núñez R, Torres-Navarrete C. 2017. Productivity in the Gulf of California large marine ecosystem. *Environmental Development* **22**:18–29 DOI 10.1016/j.envdev.2017.01.003.

Meyer-Baese A, Schmid V. 2014. Chapter 7–foundations of neural networks. In: Meyer-Baese A, Schmid V, eds. *Pattern Recognition and Signal Analysis in Medical Imaging*. 2nd. Cambridge: Academic Press, 197–243.

Michener R, Kaufman L. 2007. *Stable isotope ratios as tracers in marine food webs: an update.* Hoboken: Blackwell Publishing, 238–282.

Mora-Zamacona P, Melo-Barrera F, Cruz-Escalona V, Navia A, Morales-Bojórquez E, Pérez-Palafox X, Mejía-Falla P. 2021. Growth modeling of the giant electric ray Narcine entemedor in the southern Gulf of California: analyzing the uncertainty of three data sets. *Animals* **21(1)**:19 DOI 10.3390/ani12010019.

Moura T, Figueiredo I, Farias I, Serra-Pereira B, Neves A. 2008. Ontogenetic dietary shift and feeding strategy of *Raja undulata* Lacepède, 1802 (Chondichtyes: Rajidae) on the Portuguese continental shelf. *Scientia Marina* **72(2)**:311–318 DOI 10.3989/scimar.2008.72n2311.

Murillo-Cisneros DA, O'Hara TM, Elorriaga-Verplancken FR, Curiel-Godoy P, Sánchez-González A, Marmolejo-Rodríguez AJ, Marín-Enríquez E, Galván-Magaña F. 2019. Trophic assessment and isotopic niche of three sympatric ray species of western Baja California Sur. *Mexico Environmental Biology of Fishes* **102(12)**:1519–1531 DOI 10.1007/s10641-019-00923-1.

Navarro-González J, Bohórquez-Herrera J, Navia A, Cruz-Escalona V. 2012. Composición trófica de batoideos en la plataforma continental frente a Nayarit y Sinaloa, México. *Ciencias Marinas* **38(2)**:347–362 DOI 10.7773/cm.v38i2.1999.

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? *Marine and Freshwater Research* **68(7)**:1342–1353 DOI 10.1071/MF16161.

Nelson JS, Grande TC, Wilson MV. 2016. *Fishes of the world.* Hoboken: John Wiley & Sons.

Newsome S, del Rio C, Bearhop S, Phillips D. 2007. A niche for isotopic ecology. *Frontiers in Ecology and the Environment* **(5)**:429–436 DOI 10.1890/060150.1.

Pabón-Aldana K, Melo-Barrera F, Pérez-Palafox X, Navia A, Cruz-Escalona V, Mejía-Falla P. 2022. Age and growth of Pacific cownose ray, *Rhinoptera steindachneri*: a species with intermediate growth and shorter lifespan than expected. *Marine and Freshwater Research* **73(8)**:1011–1024 DOI 10.1071/MF21103.

Palacios M, Hoyos-Padilla E, Trejo-Ramírez A, Croll D, Galván-Magaña F, Zilliacus K, O'Sullivan J, Ketchum J, González-Armas R. 2021. Description of first nursery area for a pygmy devil ray species (*Mobula munkiana*) in the gulf of California, Mexico. *Scientific Reports* **11**:132 DOI 10.1038/s41598-020-80506-8.

Park T, Casella G. 2012. The Bayesian Lasso. *Journal of the American Statistical Association* **103(482)**:681–686 DOI 10.1198/016214508000000337.

Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O, Blondel M, Prettenhofer P, Weiss R, Dubourg V, Vanderplas J, Passos A, Cournapeau D, Brucher M, Perrot M, Duchesnay E. 2011. Scikit-learn: machine learning in Python. *Journal of Machine Learning Research* **12**:2825–2830.

Peterson B, Fry B. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* **18**:293–320 DOI 10.1146/annurev.es.18.110187.001453.

Phillips R, McGill R, Dawson D, Bearhop S. 2011. Sexual segregation in distribution, diet and trophic level of seabirds: insights from stable isotope analysis. *Marine Biology* **158(10)**:2199–2208 DOI 10.1007/s00227-011-1725-4.

Platell M, Potter I, Clarke K. 1998. Resource partitioning by four species of elasmobranchs (Batoidea: Urolophidae) in coastal waters of temperate Australia. *Marine Biology* **131**:719–734 DOI 10.1007/s002270050363.

Post D, Layman C, Arrington D, Takimoto G, Quattrochi J, Montaña C. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia* **152**:179–189 DOI 10.1007/s00442-006-0630-x.

Ramírez-Díaz C, Peña R, Diogo R, Cruz-Escalona V. 2022. Comparative cranio-mandibular myology of three species of Batoidea from the Southern Gulf of California, Mexico. *Journal of Morphology* **284(1)**:e21547 DOI 10.1002/jmor.21547.

Restrepo-Gómez D, Cruz-Escalona V, Mejía-Falla P, Peterson M, Navia AF. 2021. Effects of age, maturity, sex, and seasonality on the feeding strategies of the diamond stingray, *Hypanus dipterurus*, in the southern Gulf of California. *Marine and Freshwater Research* **72**:469–480 DOI 10.1071/MF20165.

Rosas-Hernández M, Aurioles-Gamboa D, Hernández-Camacho C. 2019. Specialized foraging habits of adult female California sea lions *Zalophus californianus*. *Marine Mammal Science* **35(4)**:1463–1488 DOI 10.1111/mms.12603.

Ruiz-Cooley R, Gerrodette T, Fiedler P, Chivers S, Danil K, Ballance L. 2017. Temporal variation in pelagic food chain length in response to environmental change. *Science Advances* **3(e1701140)**:1–8 DOI 10.1126/sciadv.1701140.

Ruiz-Cooley R, Markaida U, Gendron D, Aguíñiga S. 2006. Stable isotopes in jumbo squid (*Dosidicus gigas*) beaks to estimate its trophic position: comparison between contents and stable isotopes. *Journal of the Marine Biological Association of the United Kingdom* **86**:437–445 DOI 10.1017/S0025315406013324.

Salvatier J, Wiecki TV, Fonnesbeck C. 2016. Probabilistic programming in Python using PyMC3. *PeerJ Computer Science* **2**:e55 DOI 10.7717/peerj-cs.55.

Schmitt J, Gedamke T, DuPaul W, Musick J. 2015. Ontogenetic and sex-specific shifts in the feeding habits of the barndoor skate. *Marine and Coastal Fisheries* **7(1)**:409–418 DOI 10.1080/19425120.2015.1063553.

Schmitz O, Grabowski J, Peckarsky B, Preisser E. 2008. From individuals to ecosystem function: toward an integration of evolutionary and ecosystem ecology. *Ecology* **89(9)**:2436–2445 DOI 10.1890/07-1030.1.

Schwartz F. 1990. Mass migratory congregations and movements of several species of cownose rays, Genus *Rhinoptera*: a world-wide review. *Journal of the Elisha Mitchell Scientific Society* **106(1)**:10–13.

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. *Marine Ecology* **42**:e12687 DOI 10.1111/maec.12687.

Simental-Anguiano M, Torres-Rojas Y, Galván-Magaña F, Tripp-Quezada A. 2022. Importance of shellfish in the diet of two ray species (*Rhinoptera steindachneri* and *Hypanus dipterurus* in the upper Gulf of California. *Journal of Shellfish Research* **41**:291–299 DOI 10.2983/035.041.0215.

Sinisalo T, Jones R, Helle E, Valtonen E. 2008. Changes in diets of individual Baltic ringed seals (*Phoca hispida botnica*) during their breeding season inferred from stable isotope analysis of multiple tissues. *Marine Mammal Science* **24(1)**:159–170 DOI 10.1111/j.1748-7692.2007.00170.x.

Smith W, Cailliet GM, Melendez EM. 2007. Maturity and growth characteristics of a commercially exploited stingray, *Dasyatis dipterura*. *Marine and Freshwater Research* **58(1)**:54–66 DOI 10.1071/MF06083.

Smith B, Epstein S. 1971. Two categories of $^{13}C/^{12}C$ ratios for higher plants. *Plant Physiology* **47**:380–384 DOI 10.1104/pp.47.3.380.

Spath C, Barbini S, Figueroa D. 2013. Feeding habits of the apron ray, *Discopyge tschudii* (Elasmobranchii: Narcinidae), from off Uruguay and northern Argentina. *Journal of the Marine Biological Association of the United Kingdom* **93(2)**:291–297 DOI 10.1017/S0025315412000665.

Stallings CD, Nelson JA, Rozar KL, Adams C, Wall KR, Switzer TS, Winner BL, Hollander DJ. 2015. Effects of preservation methods of muscle tissue from upper trophic level reef fishes on stable isotope values (δ^{13}C and δ^{15}N). *PeerJ* **3**:e874 DOI 10.7717/peerj.874.

Swanson HK, Lysy M, Power M, Stasko AD, Johnson JD, Reist JD. 2015. A new probabilistic method for quantifying n dimensional ecological niches and niche overlap. *Ecology* **96(2)**:318–324 DOI 10.1890/14-0235.1.

Takai N, Onaka S, Ikeda Y, Yatsu A, Kidokoro H, Sakamoto W. 2000. Geographical variations in carbon and nitrogen stable isotope ratios in squid. *Journal of the Marine Biological Association of the United Kingdom* **80**:675–684 DOI 10.1017/S0025315400002502.

Valadez-González C. 2007. Distribución, abundancia y alimentación de las rayas bentónicas de la costa de Jalisco y Colima, México [Distribution, abundance and feeding of the benthic rays of the coasts of Jalisco and Colima]. PhD thesis. Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas. La Paz, BCS: Mexico. *Available at https://tesis.ipn.mx/ bitstream/handle/123456789/4170/valadez_gonzalezCarmen.pdf*.

Valenzuela-Quiñonez F, Galván-Magaña F, Ebert DA, Aragón-Noriega EA. 2018. Feeding habits and trophic level of the shovelnose guitarfish (*Pseudobatos productus*) in the upper Gulf of California. *Journal of the Marine Biological Association of the United Kingdom* **98(7)**:1783–1792 DOI 10.1017/S0025315417000832.

Van Rossum G, Drake F. 2009. *Python 3 reference manual*. Scotts Valley, CA: CreateSpace.

Vinson MR, Budy P. 2010. Sources of variability and comparability between salmonid stomach contents and isotopic analyses: study design lessons and recommendations. *Canadian Journal of Fisheries and Aquatic Sciences* **68**:137–151 DOI 10.1139/F10-117.

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. *Marine Biology* **144**:439–448 DOI 10.1007/s00227-003-1218-1.

Whitehead D, Murillo-Cisneros D, Elorriaga-Verplancken F, Hacohen-Domené A, Parra RDL, González-Armas R, Galván-Magaña F. 2020. Stable isotope assessment of whale sharks across two ocean basins: Gulf of California and the Mexican Caribbean. *Journal of Experimental Marine Biology and Ecology* **527**:151359 DOI 10.1016/j.jembe.2020.151359.