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#### It is all about sharing and coexistence. Trophic assessment of three sympatric batoid species in the Southern Gulf of California

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individuals with different morphological or behavioral traits (e.g., maturity stages, sexes). 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 and stable isotope analyses. We found a clear differentiation between *H. dipterurus* and *N. entemedor*, where the latter had more benthic habits, supported by greater importance of infaunal prey and higher  $\delta^{13}$ C values. The degree of intra-specific differentiation was variable; however, we found a consistent differentiation among sexes and maturity stages, corresponding to changes in specialization (i.e., isotopic niche amplitude) or trophic spectrum (different prey importance and isotopic values per group). This work is a promising step towards understanding the biological and ecological mechanisms that allow the coexistence of these three species in a potentially important feeding area within the southern Gulf of California, Mexico.

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- It is all about sharing and coexistence.

  Trophic assessment of three sympatric batoid species in the Southern Gulf of California
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#### 28 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). 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 and stable isotope analyses. We found a clear differentiation between *H. dipterurus* and *N. entemedor*, where the latter had more benthic habits, supported by greater importance of infaunal prey and higher  $\delta^{13}C$  values. The degree of intra-specific differentiation was variable; however, we found a consistent differentiation among sexes and maturity stages, corresponding to changes in specialization (i.e., isotopic niche amplitude) or trophic spectrum (different prey importance and isotopic values per group). This work is a promising step towards understanding the biological and ecological mechanisms that allow the coexistence of these three species in a potentially important feeding area within the southern Gulf of California, Mexico.

#### 2 INTRODUCTION

- Species-specific trophic ecology studies are fundamental to comprehending their ecological role in the
- ecosystem (Coll et al., 2013; Ferreti et al., 2010). Batoids are a group of aquatic predators with a wide



range of shapes, sizes, and life-history strategies that have colonized marine and freshwater environments (Last et al., 2016). These organisms play a crucial role in transferring energy from lower to higher levels, having direct and indirect effects on 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, mainly composed of crustaceans, mollusks, polychaetes, and fishes (Barbini et al., 2018; Restrepo-Gómez et al., 2021; Serrano-Flores et al., 2021).

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

In Mexico, trophic ecology studies usually involve highly abundant and frequently captured species (Valenzuela-Quiñonez et al., 2018; Restrepo-Gómez et al., 2021; Serrano-Flores et al., 2021), but the mechanisms that allow their coexistence are less commonly analyzed (Navarro-González et al., 2012; Murillo-Cisneros et al., 2019). In the southern Gulf of California, the three most abundant and fished batoid species are (González-González et al., 2020): the diamond stingray [*Hypanus dipterurus* (Jordan & Gilbert, 1880)], the giant electric ray (\*Narnice entemedor\* Jordan & Starks, 1895)], and the golden cownose ray (*Rhinoptera steindachneri* Evermann & Jenkins, 1891). *Hypanus dipterurus* is a benthopelagic predator that feeds primarily on mollusks, bivalves, and stomatopods (Restrepo-Gómez et al., 2021). *Narcine entemedor*, on the other hand, feeds mainly on epibenthic prey, especially polychaetes and sipunculids (Valadez-González, 2007; Flores-Ortega et al., 2015; Cabrera-Melendez, 2017). Meanwhile, *R. steindachneri* feeds on bivalves (Simental-Anguiano, 2011), and other soft prey, such as echinoderms (Navarro-González et al., 2012) and, to a lesser extent, mysidaceans (Ehemann et al., 2019).

Most feeding ecology studies have been based on stomach content analyses (SCA), which allow a relatively precise taxonomic definition of the diet, requiring high sampling frequencies and sample sizes to obtain reliable, time-integrated overviews of dietary habits, among other inherent limitations (Hyslop, 1980; Cortés, 1997; Vinson and Budy, 2011). A widely used alternative that overcomes some of the limitations of the SCA is the stable isotope analysis (SIA), which traces the elemental flow 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 and Kaufman, 2007). These properties allow these ratios to closely identify the ecological niche (*sensu* Hutchinson 1978), where they 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 one year (Logan and Lutcavage, 2010; MacNeil et al., 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 et al., 2006; Vinson and Budy, 2011; Albo-Puigserver et al., 2015). Therefore, our study aims to analyze the coexistence of \*H.dipterurs\*, *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 about their trophic niche dynamics using SCA and SIA ( $\delta^{13}C$  and  $\delta^{15}N$ ).

#### METHODS

#### Study area and sample collection

This study did not require an "Ethical review and approval" because the specimens analyzed were caught by local artisanal fishers who worked under a commercial fishing permit (CONAPESCA-103053993316-

1) under Mexican laws and regulations. Hence, 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 Espíritu Santo Island, in "El Morrito" (24° 25' 17.55" N, 110° 18' 31.61" W), at Bahía de La Paz, southern Gulf of California, Mexico. The catching depths varied between 0 and 40 m using bottom gill nets (100 m long, 1.5 m wide, and 16 cm stretch mesh size). 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 et al. 2015).

#### Sample processing

#### Stomach contents

After sampling, the individuals were sexed, and their sexual maturity stages were determined following (Smith et al., 2007; Burgos-Vázquez et al., 2017, 2018). The stomachs were removed from the specimens, fixed, and preserved in 10% formaldehyde. Simultaneously,  $1 cm^3$  of muscle tissue was removed from the fresh specimens and kept frozen until SIA was performed to avoid possible alterations of the isotopic values (Stallings et al., 2015).

#### Stable isotope analysis

Upon collection, one gram of each sample was oven-dried at 60°C for 48 h, and lipids were removed using a chloroform:methanol (1:1) solution (Post et al., 2007; Carlisle et al., 2016) in a Microwave-Assisted Solvent Extraction System (1000 MARS-5, CEM Microwave Technology Ltd. Mathews, NC). Samples were then oven-dried at 60°C for 12 h to remove the remaining solvent (Kim and Koch, 2012; Li et al., 2016). 10 mL of deionized water were added to extract the urea accumulated in the tissue; the test tube was stirred for 15 minutes. Finally, the deionized water solution was discarded; this process was repeated twice. After extracting lipids and urea, the tissue was freeze-dried at -60°C for 36 hours and mechanically grounded in a porcelain mortar using a pestle until homogenization.

For stable isotope analyses, 400-1000 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., USA). Stable isotope ratios were measured in a Delta V Advantage isotope ratio mass spectrometer (IRMS, Thermo Fisher Scientific, Waltham, USA), interfaced in a continuous flow to a Thermo Scientific – Flash HT 2000 elemental analyzer (Thermo Fisher Scientific, Waltham, USA) and an Isodat Workspace version 3.0 (Thermo Scientific, Waltham, USA).

The stable isotope ratios are expressed in the  $\delta$  notation (DeNiro and 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 isotope (atmospheric  $N_2$  for nitrogen and Vienna PeeDee Belemnite for carbon, respectively) with a value of 0% (Brand et al., 2014).

#### Data Analyses

#### Stomach content analysis

Based on previous studies of SC (Cabrera-Melendez, 2017; Restrepo-Gómez et al., 2021), we constructed a single database to classify *H. dipterurus* and *N. entemedor* based on their diets; however, *R. steindachneri* was excluded from this analysis because the available stomach content data were insufficient to describe its diet adequately (Ehemann et al., 2019). Prey items were aggregated into ten broad taxonomic categories (amphipods, brachyuran and anomuran crabs, bivalves, echinoderms, other crustaceans, penaeid shrimps, polychaetes, sipunculids, stomatopods, and teleostean). 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 preys are ingested (Hyslop, 1980).

The trophic differences between the sexes, sexual maturity stages, and sampling seasons of *H. dipterurus* and *N. entemedor* 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 et al., 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 its 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 et al., 2018). These models were implemented in Python 3 (v. 3.8.6, Van Rossum and Drake 2009) using the Scikit-learn module (v. 1.0.1, Pedregosa et al. 2011).



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The model complexity was optimized using a grid search algorithm and 5-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 and 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 and 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: first, 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 et al. 2007. Further details and code are included in Supplemental Information 1.

#### 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., 0 divergences during the posterior sampling (No-U-Turn Sampler, NUTS, Betancourt 2017) and Gelman-Rubin statistics (Gelman et al., 2014) being less than 1.01 for every parameter. The corresponding Supplemental Information include 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 2000 (Martin, 2018). The posterior distributions were summarized in terms of their means and 95% Highest Density Intervals (*HDI*<sub>95%</sub>), 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 178 which the effects of the seasons (warm vs. cold), sexes, and maturity stages (adults vs. juveniles) on 179 both isotopic values are nested within each species. In consequence, the isotopic space of each species 180 is the result of 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 182 the parameters at the lower levels of the hierarchy and sequentially transfer it to the next; hence, they 183 effectively investigate cross-level hypotheses (Gelman et al., 2014). The model was implemented using 184 the PyMC3 library (v.3.11.4, Salvatier et al. 2016. Full details are provided in Supplemental Information 2. 185 Essential details are that 1) the hierarchical model allows a description at both the species and intra-species 186 187 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 188 the slopes, which results in an L1 regularization (i.e., a "Bayesian Lasso" regression, Park and Casella 189 2012; and 4) the heavy-tailed Student-\*t\* likelihood assigns a higher probability to extreme values; thus, 190 allowing to make robust estimations of the parameters (Kruschke, 2012). 191

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 3). Isotopic niche overlap between species was assessed using the NicheROVER package (Swanson et al. 2015, Supplemental Information 4), 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

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#### Stomach Content Analyses

#### Inter-specific differentiation

The Random Forest (RF) model showed excellent performance, with a training AUC: 0.98, test AUC: 0.99), suggesting a clear boundary between the prey preferences of H. dipterurus (n = 205) and N. entemedor (n = 187). 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 (1. The optimized forest consisted of 200 trees, with a maximum depth of two and a maximum of three features (prey items) per tree.

#### Intra-specific differentiation

For *H. dipterurus*, the RFs for the seasons (n; cold = 96, warm = 109), sexes (138 females, 67 males), and maturity stages (44 adults, 161 juveniles) had moderate AUC values ( $\leq$  0.6; 2). The RF for the seasons showed higher weights of shrimps and crustaceans during the warm season. The model was optimized with 100 trees, a maximum depth of two and maximum one feature per tree. The main contributors to the classification of the sexes were bivalves, stomatopods, and shrimps. Higher weights of stomatopods and crabs were associated with females, while higher weights of shrimps and amphipods were associated with males. The final RF consisted of 50 trees with a maximum depth of 28 and one feature per tree, being the RF with the highest maximum depth, suggesting a fuzzy boundary between males and females. As for the maturity stages, the classes were balanced to 100 individuals, and stomatopods and bivalves were the most relevant prey groups to the classification (cumulative importance of  $\sim$  70%). Adults had higher weights of shrimps, polychaetes, echinoderms, and sipunculids, while juveniles haad higher weights of stomatopods, bivalves, crabs, and amphipods. The optimized forest consisted of 50 trees with a maximum depth of five and a maximum of four features per tree.

For *N. entemedor*, the AUCs were better than those of *H. dipterurus* (AUC > 0.8; 3), except for the seasons. Sipunculids had the highest impact on predicting the seasons (n; cold = 99, warm = 88) and sexes (n; females = 154, males = 33), while polychaetes were the most important for classifying maturity stages (n; adults = 173, juveniles = 14). Higher weights of sipunculids and shrimps were associated with the warm season; higher weights of sipunculids, polychaetes, and shrimps were associated with females; and higher weights of polychaetes and shrimps with juveniles. The optimized RF for the seasons consisted of 50 trees with a maximum depth of one and a maximum of nine features per tree. Regarding the sexes, the optimized RF consisted of 100 trees with a maximum depth and features of seven. As for the maturity stages, the RF consisted of 100 trees, with a maximum depth of four and a maximum of eight features per tree.

#### 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, with N. entemedor having the highest values  $(M, [HDI_{95\%}]: -12.63, [-12.63, -12.14])$ , followed by H. dipterurus (-14.0, [-14.39, -13.64]) and R. steindachneri (-16.12, [-16.34, -15.90]). For  $\delta^{15}N$ , R. steindachneri and H. dipterurus had similar values (16.18, [16.00, 16.34], and 16.16 [15.87, 16.46], respectively), which were lower than those of N. entemedor (18.05, [17.86, 18.25]). The comparisons of the posterior distributions showed high probabilities of differences in most cases (P > 99%, 4); 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  $\delta^{13}C$  (P(N.entemedor < R.steindachneri) = 100%; M = 3.7%; P(N) = 10.49; P(N) = 10

#### Intra-specific comparisons of isotopic values and niches

The results of analyzing the effect of the sex, seasonality, and maturity stage are summarized in 7. The effect of sex on isotopic values was relatively minor, with probabilities of differences lower than 75% in every case other than  $\delta^{13}C$  for *H. dipterurus* ( $\beta = -0.32\%$ ; p( $\beta < 0$ ) = 90%). The effect of the season was more evident in  $\delta^{15}N$ , with probabilities of differences between the warm and cold season exceeding 90% for most comparisons. The values of the three species were lower during the cold season compared to the warm season. For  $\delta^{13}C$ , only *H. dipterurus* showed higher values during the cold season as compared to the warm season ( $p(\beta < 0) \approx 98\%$ ;  $\beta = 0.76\%$ ;  $HDI_{95\%}$ : [0.028‰, 1.5‰]). Most comparisons between maturity stages showed that juveniles had lower values of both isotopes, with the exception of H. dipterurus in  $\delta^{13}C$ , where juveniles had higher values.



The degree of intra-specific differentiation in isotopic niche areas varied among species (5, right, and 6). For the seasons, *R. steindachneri* was the only species with a high probability of differences  $(p(cold > warm) \approx 80\%; HDI_{95\%} : [-1.11^2, 7.78^2]; M = 2.05^2)$ . For the sexes, both *N. entemedor*  $(p(males > females) \approx 87\%; [-0.67^2, 2.78^2]; 0.93^2)$  and *R. steindachneri*  $(p(males > females) \approx 79\%; [-0.5^2, 1.1^2]; 2.05^2)$  showed differences. Regarding the maturity stages, both *H. dipterurus*  $(p(juveniles > adults) \approx 80\%; [-2.17^2, 4.19^2]; 2.02^2)$  and *R. steindachneri*  $(p(adults > juveniles) \approx 77\%; [-0.5^2, 1.06^2]; 0.27^2)$  showed differences in isotopic niche areas between juveniles and adults.

#### DISCUSSION

The competitive exclusion principle states that the coexistence of sympatric species requires a certain degree of resource partitioning (Gause, 1934; Hardin, 1960), hence heavily influencing these species' overall ecological niche (*sensu* Hutchinson 1978). Moreover, populations are not homogeneous but are formed by individuals with different morphological or behavioral traits (e.g., maturity stage, sexes) immersed in an ever-changing environment. This interaction results in a multi-dimensional differentiation in food preferences and resource exploitation, challenging the adequate management and conservation of populations (Coyle, 1998). We explored such differentiation in three coastal batoids by combining SCA and SIA, resulting in a comprehensive study of their foraging and coexistence strategies.

Differences in  $\delta^{13}C$  are usually associated with horizontal spatial segregations, namely an inshore-offshore gradient, but 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 and Epstein, 1971; Peterson and 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 and 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. At an inter-specific level, the results of SCA support the benthic-pelagic gradient due to the lower values of H. dipterurus relative to N. entemedor, and infaunal prey being more relevant to the latter. 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). Morphological differences and feeding strategies could explain this resource partitioning. The protractile and tubular ventral mouth, along with its electric discharge (Last et al., 2016), 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 et al., 2016; Last et al., 2016; González-González et al., 2020).

As for  $\delta^{15}N$ , the  $^{15}N$ -enriched values found are also coherent with those found in the Gulf of California, which is an area with particularly high  $\delta^{15}N$  values (Elorriaga-Verplancken et al., 2018), due to biochemical processes and the oceanic circulation. The 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 and Pauly, 2012). Testing whether this is the case is unfeasible



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with the available information. Bulk SIA can confound the effect of the diet and primary producers (Martínez del Río et al., 2009) due to the spatial and temporal variation of the isotopic composition at the base of the trophic webs (Whitehead et al., 2020). Nonetheless, these shortcomings could be solved 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

For H. dipterurus, the performances of the RF models for every factor were moderate, with AUCs around 0.6, which could be interpreted as a lack of marked differences between the different factors; 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 (7). 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. 5, 6); however, the effect of the maturity stage 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).

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 ontogenic changes in foraging habits, which agrees with previous studies on the species (Cabrera-Melendez, 2017). In this case, the ontogenic changes could also be related to an increased trophic spectrum due to an increase in body and mouth sizes during growth (Spath et al., 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. 6). 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 et al., 2019).

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 ontogenic changes in dietary habits, while differences in  $\delta^{15}N$  could indicate a change in the trophic spectrum of the species or could also be the consequence of the migratory behavior of the species (Schwartz, 1990). 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 while being affected by differential digestion rates of prey items, which, in turn, confounds the underlying ecological processes. This contrast also highlights the complementariness of both approaches. Nonetheless, an important consideration is that our SIA could reflect diet assimilation from over a year before sampling (Logan and Lutcavage, 2010; MacNeil et al., 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. Still, this study is a promising step towards understanding the biological and ecological mechanisms that allow the coexistence of the three species in the Southern Gulf of California, Mexico.



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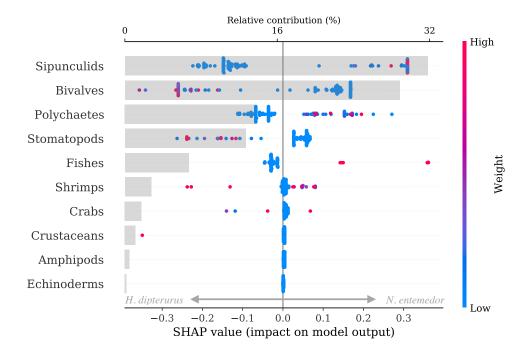
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#### DATA AVAILABILITY

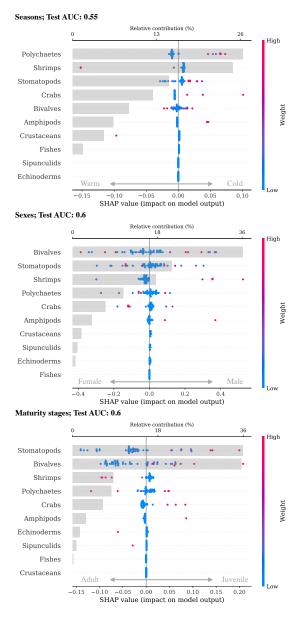
All data analyzed during this study are included in this published article and its Supplemental Informations, which are also stored in the GitHub repository ArturoBell/01072022.

#### 592 FIGURES AND TABLES



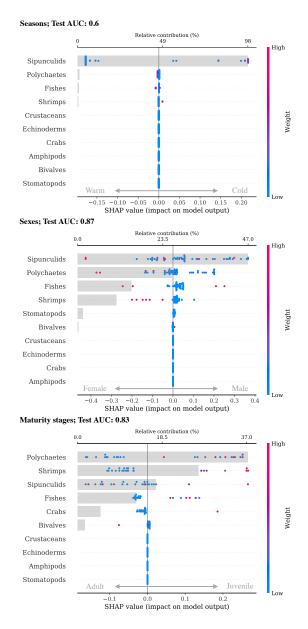
**Figure 1.** Bee swarm (points, bottom axis) and bar (top axis) plots of SHAP explanations for the random forest classifying *H. dipterurus* and *N. entemedor*. The bee swarm plot shows the SHAP values for every prey group per individual. A bluer color expresses lower prey weights, while a redder color 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.



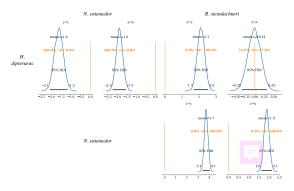


**Figure 2.** Bee swarm and bar plots of SHAP explanations for the intra-specific classifiers for *Hypanus dipterurus*.



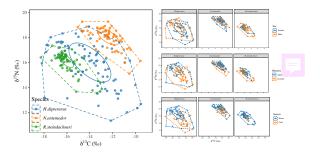


**Figure 3.** Bee swarm and bar plots of SHAP explanations for the intra-specific classifiers for *Narcine entemedor*.

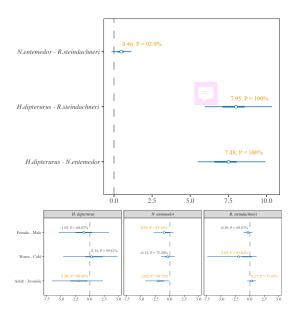


**Figure 4.** Comparisons between the posterior means of each species' isotopic ratios.

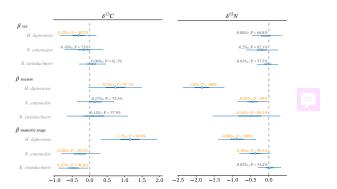




**Figure 5.** Isotopic niches of the three batoid species (left) and their categories (right), represented with standard ellipses and convex hulls.



**Figure 6.** Forest plot with the posterior differences in isotopic niche areas  $(SEA_B)$  and their probabilities of being greater or smaller than 0.



**Figure 7.** Forest plot of the posterior distributions of the slopes of every factor  $(\beta)$  for every species and isotopic ratio. Shown as mean; probability of  $\beta > 0 \lor \beta < 0$ . Thin lines represent the  $HDI_{95\%}$ , thick lines the  $HDI_{50\%}$ , and points indicate the mean of the distribution.