



# Dietary niche differentiation in a mesopredatory dasyatid assemblage

Chantel Elston<sup>1</sup> · Paul D. Cowley<sup>2</sup> · Rainer G. von Brandis<sup>3</sup> · Aaron Fisk<sup>4</sup>

Received: 25 October 2019 / Accepted: 17 April 2020 / Published online: 3 June 2020  
© Springer-Verlag GmbH Germany, part of Springer Nature 2020

## Abstract

Most batoids are mesopredators and are often hypothesised to play important ecological roles. However, a comprehensive understanding into these roles remains limited given the paucity of information of their trophic habits. St. Joseph Atoll (5.43° S, 53.35° E) is a remote ecosystem that hosts a resident assemblage of dasyatids (*Pastinachus ater*, *Urogymnus granulatus*, and *U. asperrimus*). Both stomach contents (SC) and stable isotope samples (SI) ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) were collected in 2015 and used to investigate the trophic niches of this dasyatid assemblage and whether these niches differed inter- and intra-specifically. Dasyatid muscle samples as well as baseline samples of potential prey species and primary producers were collected for SI analyses. SC data highlighted significant inter-specific differences in diet, *U. granulatus* juveniles mostly consumed decapod crustaceans (Callianassidae and Portunidae) and *P. ater* juveniles mostly consumed bivalve molluscs. The mean species trophic positions for juveniles of all three species ranged from 2.9 to 3.6 when calculated using  $\delta^{15}\text{N}$  and from 3.4 to 3.6 when calculated using stomach content data. Analysis of  $\delta^{13}\text{C}$  showed that all juveniles were reliant upon the same carbon resources (primarily derived from seagrass beds) at the base of the food web. Diet appeared to change with size as larger individuals displayed lower  $\delta^{13}\text{C}$  and higher  $\delta^{15}\text{N}$  compared to smaller juveniles. Additionally, isotope values of the smallest individuals were similar to the largest individuals, suggesting maternal meddling. The identified patterns of inter- and intra-specific trophic niche differentiation may be indicative of competitive effects and contributes to the understanding of mesopredators in community trophic dynamics.

## Introduction

Information regarding the trophic interactions and position of a species in a food web is required to understand its ecological role (Heithaus et al. 2013; Bornatowski et al. 2014). Understanding the trophic ecology of mesopredators is particularly relevant as they occupy an intermediate trophic

position, linking apex predators to lower levels in a food web (Ritchie and Johnson 2009).

In marine environments, small-bodied benthic batoids occupy mesopredatory positions as both predators and prey to larger sharks (Navia et al. 2017). Given both their trophic position and the potential that large sharks have to mediate communities through top-down control, batoids have been hypothesised to transmit effects of declining shark abundances down the food chain through a phenomenon called trophic cascades (Myers et al. 2007; Heithaus et al. 2008; Baum and Worm 2009). However, the roles of benthic batoids in these purported trophic cascades have recently been called into question (Grubbs et al. 2016). There are high levels of trophic complexity and redundancy (multiple species feeding on the same resources) in many marine food webs and batoids display conservative life history traits. These render ‘mesopredator release’ in response to declining shark populations unlikely in certain ecosystems (Navia et al. 2010, 2012, 2017). Nevertheless, benthic batoids have the potential to play important predatory roles (VanBlaricom 1982; Thrush et al. 1994; Peterson et al. 2001; Blanco-Parra et al. 2012) and could exert influence over prey

---

Communicated by J. Carlson.

---

Reviewed by J. Bizzarro and J. Logan.

---

✉ Chantel Elston  
chantel.elston@gmail.com

<sup>1</sup> Department of Ichthyology and Fisheries Science, Rhodes University, Grahamstown, South Africa

<sup>2</sup> South African Institute for Aquatic Biodiversity, Grahamstown, South Africa

<sup>3</sup> Save Our Seas Foundation - D’Arros Research Center, D’Arros Island, Seychelles

<sup>4</sup> School of the Environment, University of Windsor, Windsor, Canada

communities. Unfortunately, a comprehensive understanding into the trophic habits and thus ecological roles of benthic batoids is hindered by a lack of detailed dietary assessments for many species.

To fully assess the trophic roles of benthic batoids, not only is it necessary to quantify these vertical interactions in a food web (i.e. interactions between predator and prey), but also an understanding of inter-specific interactions is also required. Dasyatids often occur sympatrically and in abundance in tropical shallow sandy ecosystems (Vaudo and Heithaus 2009; O'Shea et al. 2013; Cerutti-Pereyra et al. 2014; Last et al. 2016) which could lead to high levels of competition if resources are limiting. The partitioning of resources along some ecological axis (trophic, spatial, and/or temporal) is a straightforward response to competitive effects (Hardin 1960; Scheffer and van Nes 2006) but species can also coexist if they are sufficiently similar (Scheffer and van Nes 2006; Vergnon et al. 2009, 2012). Competition can be a fundamental component of an environment that has the potential to influence community structure and the realised niche of individual organisms (Papastamatiou et al. 2006).

However, little is understood about the relationship between resource partitioning and competition in elasmobranch communities. A number of studies have investigated inter-specific patterns of dietary resource use, particularly in assemblages of urolophidae or rajidae, over small to large spatial scales (tens to hundreds of kilometres), and have identified inter-specific dietary differentiation (Platell et al. 1998; Yick et al. 2011; Marshall et al. 2008; Bornatowski et al. 2014; Yemişken et al. 2017). Only three studies have investigated inter-specific dietary differentiation between dasyatids, two of which found significant dietary overlap (Vaudo and Heithaus 2011; O'Shea et al. 2013) while the remaining study found inter-specific dietary differences (although sampling for the latter was conducted on a much larger scale of thousands of kilometres (Jacobsen and Bennett 2012)). Therefore, further investigations into these patterns of similar or dissimilar resource use in dasyatid communities are needed to provide further insights into the potential structuring effect of competition.

Studies have also investigated intra-specific dietary preferences and have found that diet composition often varies between batoids of different size classes or ontogenetic stages (Platell et al. 1998; White et al. 2004; Marshall et al. 2008; Valls et al. 2011; Yick et al. 2011; Jacobsen and Bennett 2012; O'Shea et al. 2013). These ontogenetic dietary changes may even cause the same species to occupy different trophic levels and perform different trophic roles throughout its life (Navia et al. 2017). It is important to consider these potential ontogenetic dietary changes when characterising the dietary habits of a species.

St. Joseph Atoll is a small and isolated atoll on the Amirantes Bank, Seychelles, which plays host to an

abundant sympatric dasyatid assemblage. Juveniles of three species, namely *Pastinachus ater*, *Urogymnus granulatus*, and *Urogymnus asperrimus*, are resident to this atoll and adults of the first two species are also encountered. This, in conjunction with the remoteness of the atoll, provides an opportunity to characterise the trophic niches of dasyatids in a pristine environment and to determine whether these niches differ intra- and inter-specifically.

The dietary composition of *U. asperrimus* juveniles in St. Joseph Atoll has been quantified (Elston et al. 2017) and individuals were found to specialise on infaunal prey, most notably the polychaete family Capitellidae. However, detailed dietary assessments of the other two species, *P. ater* and *U. granulatus*, have yet to be conducted, both within this ecosystem and across their distributions. Determining the dietary compositions of these species will not only provide insights into their roles as predators, but also information as to whether there is potential trophic niche differentiation in this ecosystem and what trophic level these species occupy. Further insights into food web dynamics and dietary differentiation within the atoll can be obtained through determining which basal carbon sources (i.e. the primary producers at the base of the food web) dasyatids were most reliant upon. Therefore, this study aimed to utilise both stomach content and stable isotope ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) analyses to assess the dietary habits of dasyatids that occur in St. Joseph Atoll. Specific objectives were to determine:

- The dietary compositions of *P. ater* and *U. granulatus* using stomach contents
- The basal carbon sources that each dasyatid species were most reliant upon using  $\delta^{13}\text{C}$  values
- Whether there was inter- and intra-specific trophic niche differentiation based on both stomach content and stable isotope data
- The trophic position of each dasyatid species based on stomach contents and  $\delta^{15}\text{N}$ .

## Materials and methods

### Study site

Fieldwork was conducted at the remote St. Joseph Atoll, Republic of Seychelles, South West Indian Ocean (5.43° S, 53.35° E). The atoll (area of ~ 22.5 km<sup>2</sup>) forms part of a small chain of outer islands known as the Amirantes Group, situated on the Amirantes Bank. It comprises 16 small uninhabited islands (1.4 km<sup>2</sup>) atop an uninterrupted sand flat (17.7 km<sup>2</sup>) that completely encloses a central shallow lagoon (4.8 km<sup>2</sup>) of 3–9 m depth (Stoddart et al. 1979). The flats are dominated by large areas of sand that are scattered with sea-grass beds (largely *Thalassia hemprichii* and *Thalassendron*

*ciliatum*). The lagoon is characterised by a sandy substrate with numerous large poritid and flaviid coral outcrops that rise to the surface.

### Dasyatid capture and handling procedure

Dasyatids were captured during daylight hours on the sand flats of St. Joseph Atoll using one of three methods. Larger *U. granulatus* and *P. ater* individuals (> 50 cm DW) were spotted from a motorised boat or on foot and were foul-hooked using a weighted treble-hook and line. Smaller individuals of these two species (< 50 cm DW) were caught using dip and cast nets in shallow (< 30 cm) water. Once an individual was captured, a person would carefully grab the tip of the tail and then place the other chainmail-gloved hand around the tail and spine to hold the latter flat against the former. This person would hold the spine against the tail for the duration of the dasyatid handling to ensure the safety of those working on the dasyatid. Given that *U. asperrimus* lack a spine on their tail, they were captured by hand. Individuals were approached from behind until close enough to lunge and place a gloved hand around the base of the tail.

Once individuals were caught and spines restrained (if necessary), they were placed ventral-side up on a water-filled working surface (a sit-on-top recreational kayak with plugged drainage holes was used) to ensure continued respiration and minimal stress throughout procedures. Individuals were sexed based on presence of claspers and disc-width (DW) was measured to the nearest cm using callipers. Maturity was assessed based on levels of clasper calcification in males and, if available, size-at-maturity estimates (Last and Stevens 2009). Captured individuals spanned a large size range as sample effort was directed across all of the size classes found in the atoll (27–140 cm DW), but the vast majority were considered juveniles (only ten individuals were identified as adults).

All captured individuals were released as close as possible to capture locations, and were monitored to determine the short-term post-release health of the individual. All individuals resumed normal behaviour upon release and no predation events were witnessed.

### Sample collection and processing

#### Stomach contents

Stomach content samples were only collected from juveniles of *P. ater* and *U. granulatus* using non-lethal gastric lavage (adults were excluded given the small sample size). The procedure is detailed in Elston et al. (2015) but, briefly, a plastic hose that was connected to a bilge pump was eased into the stomach of the dasyatid via the mouth. Seawater was pumped into the stomach to facilitate the regurgitation of

stomach contents. The hose was removed once there was visual expansion of the abdomen. If individuals did not regurgitate stomach contents on the first lavage attempt, a second and sometimes third attempt was conducted. Flushed stomach contents were then collected by a mesh basket placed under the individual and transferred to a vial containing 70% ethanol. In the laboratory, prey items were identified to the lowest possible taxonomic level, blotted dry and items of a given taxon were weighed collectively (0.001 g). Stomach content that was too digested for identification was excluded from analyses.

#### Stable isotope samples

Muscle samples were collected from all three dasyatid species for stable isotope analyses (including juveniles and adults). A biopsy punch was used to remove a small (~ 1 cm<sup>3</sup>) section of muscle from the ventral surface of the pectoral fin of dasyatids. Primary producers and invertebrates were collected from the sand flats of St. Joseph Atoll to determine the dominant basal carbon sources dasyatids were reliant upon. Seagrasses, algae and filter-feeding bivalves were collected by hand and epiphytes were removed. All collected samples were then kept on ice until they could be frozen in the laboratory. Samples were subsequently thawed and rinsed with distilled water. For bivalves, muscle tissue was extracted for processing. Samples were then dried in an oven at 60°C for at least 48 h and homogenised using a mortar and pestle.

Lipid extraction is generally not required for batoids as their muscle tissue has a low lipid content (Carlisle et al. 2017). However, urea can potentially artificially lower  $\delta^{15}\text{N}$  values as it is thought to be depleted in <sup>15</sup>N, which is of particular concern for elasmobranchs as they produce urea as a waste product of metabolism (Fisk et al. 2002; Logan and Lutcavage 2010). The magnitude of this effect still remains unclear though; one study reported no change in  $\delta^{15}\text{N}$  values following urea extraction (Logan and Lutcavage 2010), two reported a significant increase in  $\delta^{15}\text{N}$  values (Kim et al. 2012; Burgess and Bennett 2017), while one reported variable outcomes for different species (Carlisle et al. 2017). To determine if lipid and/or urea extraction was required for this study, a sub-sample of muscle tissue from nine randomly chosen individuals across the size range were lipid extracted. While lipid extraction is not necessarily equivalent to urea extraction, several studies have noted an increase in  $\delta^{15}\text{N}$  following lipid extraction in a manner consistent with the removal of urea, suggesting lipid extraction removes urea as well as lipids (Hussey et al. 2010, 2012; Kim et al. 2012; Kim and Koch 2012; Li et al. 2016; Papiol et al. 2017). A bulk untreated sub-sample was used for comparison. Lipid extraction followed a modified Bligh and Dyer (1959) method: tissue was agitated twice in a 2:1

chloroform:methanol solution at 30°C for 24 h, the solvent decanted and the sample air dried. A Wilcoxon signed rank test revealed that  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  did not change significantly after lipid extraction ( $T = 28$ ,  $N = 9$ ,  $p = 0.57$  and  $T = 7$ ,  $N = 9$ ,  $p = 0.14$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, respectively). In addition,  $\delta^{15}\text{N}$  values decreased slightly, instead of the expected increase that urea removal would effect (mean  $\delta^{15}\text{N} = 10.45$  and 10.07 for bulk and lipid extracted samples, respectively). Finally, both bulk and treated samples had C:N ratios close to 3 (mean  $\pm$  SD =  $3.23 \pm 0.06$ ,  $N = 9$ ;  $3.13 \pm 0.08$ ,  $N = 9$ , respectively). These results indicated that neither lipid nor urea extraction was necessary in this study.

## Stable isotope analysis

Between 400 and 600  $\mu\text{g}$  of bivalve and dasyatid muscle tissue and between 1300 and 1500  $\mu\text{g}$  of flora were weighed into tin capsules using a microbalance. These samples were analysed for stable carbon and nitrogen isotope ratios and total percent carbon and nitrogen using a Delta V Advantage Mass spectrometer (Thermo) coupled to a Costech 4010 Elemental Combustion system and a ConFlo IV gas interface at the Great Lakes Institute for Environmental Research, University of Windsor. Analytical precision, based on the standard deviation of four standards (NIST1577c, internal lab standard (tilapia muscle), USGS40 and urea,  $N = 33$  for all) measured  $< 0.19\text{‰}$  for  $\delta^{15}\text{N}$  and  $< 0.18\text{‰}$  for  $\delta^{13}\text{C}$ .

Standard delta notation ( $\delta$ ) was used to express  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  as parts per thousand deviations from the international standards as follows:

$$\delta^{15}\text{N}/\delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}} - 1)] * 1000$$

where  $R = {}^{13}\text{C}/{}^{12}\text{C}$  or  ${}^{15}\text{N}/{}^{14}\text{N}$ . PeeDee Belemnite for carbon and atmospheric nitrogen were used as standard reference materials.

## Statistical analyses

### Dietary composition

Cumulative prey curves were constructed to determine whether a sufficient number of stomach contents had been sampled to accurately describe the diet of *P. ater* and *U. granulatus* juveniles (Ferry et al. 1997). A species accumulation curve was plotted using the R vegan package (Oksanen et al. 2008), and the order in which samples were analysed was randomised 100 times. If the curve reached an asymptote, it is assumed that a new prey type will only rarely be introduced into the diet and the sample number is considered sufficient. To quantitatively determine if the slopes reached an asymptote, a linear regression was conducted on the mean cumulative number of prey taxa in the

final four stomach content samples. The slope of the linear regression was statistically compared to a slope of 0 using a Student's *t* test ( $t = (b - 0)/S_b$  where  $b$  is the slope of the linear regression and  $S_b$  is the standard error of the slope) (Bizzarro et al. 2007).

Prey items identified from stomach contents were grouped to family level or higher. Although this likely artificially increases the dietary similarity identified in the assemblage, a finer taxonomic resolution would have reduced the accuracy of the identification. Three indices were calculated for each individual to describe dietary composition: percent frequency of occurrence (%F) i.e. the percentage of stomachs which contained a particular prey group, percent weight (%W) i.e., the weight of each prey group divided by the total weight of stomach contents (Hyslop 1980), and a percent Index of Importance (%IoI) defined as:

$$\%IoI_a = \frac{100 \times (\%F_a + \%W_a)}{\sum_{a=1}^n \%F + \%W}$$

The percent Index of Relative Importance (%IRI) was not calculated as prey items could not be accurately enumerated.

A feeding strategy plot was created by plotting %F against the prey-specific weight (%WP), calculated as the percentage weight of a prey item for all stomachs containing that prey item (i.e., this excludes samples not containing that prey item) (Amundsen et al. 1996). These plots demonstrate the importance of each prey item as a component of the diet, the feeding strategy (i.e. generalist or specialist feeder) and inter-individual and intra-individual niche width. If a prey item falls into the upper left of the diagram, there is specialisation by individual predators; if a prey item falls into the upper right, there is specialisation by the population; and if prey items fall into the lower quadrants, the population is thought to have a generalist strategy as prey items are eaten in low quantities (Amundsen et al. 1996).

### Basal carbon sources

Bayesian stable isotope mixing models (SIAR package, Parnell and Jackson 2013) were used to estimate the proportion of carbon derived from different primary producers in the muscle tissue of juvenile dasyatids. Individuals considered as adults were removed from this analysis given small sample size. Additionally, the smallest size class sampled was also excluded given the evidence for maternal meddling (see results and discussion). These mixing models allow for the incorporation of uncertainty associated with multiple prey sources of a consumer through the addition of error around consumer and prey stable isotope values, as well as error associated with trophic enrichment factors (TEFs) (Inger et al. 2010). The model produces a probability distribution for proportions of prey items consumed based on

their relationship with consumer tissue values. TEF values, i.e., the change in isotopic values between prey and consumer, are required for these models. To date, no accurate experimental TEF values have been calculated for dasyatids, however, Galván et al. (2016) estimated TEF values for the smallnose fanskate *Sympterygia bonapartii* based on a controlled feeding experiment, and Tilley and Strindberg (2013) suggested TEF values for dasyatids based on stomach content information available in the literature. Thus, the models were run using these two different TEF values.

Three potential carbon resource pools were assumed: benthic macroalgae, seagrass and phytoplankton in the atoll (the latter estimated from filter-feeding bivalves as a proxy, *Pinna muricata* and mactridae clams were chosen as these are known to be filter-feeding families (Chalermwat et al. 1991; Vázquez-Luis et al. 2016)). Each resource pool was assigned a trophic level (1 for primary producers and 2.1 for filter-feeders (Ebert and Bizzarro 2007)), and the mean trophic level for stingrays was calculated (see next section). TEF values of  $3.4 \pm 1\%$  for  $\delta^{15}\text{N}$  and  $0.4 \pm 1.3\%$  for  $\delta^{13}\text{C}$  were used for transfers between primary producers and primary consumers (Post 2002), TEF values of  $\delta^{15}\text{N} = 2.7 \pm 0.22\%$  and  $\delta^{13}\text{C} = 0.9 \pm 0.33\%$  (Tilley and Strindberg 2013), and  $\delta^{15}\text{N} = 1.5 \pm 0.2\%$  and  $\delta^{13}\text{C} = 1.3 \pm 0.25\%$  (Galván et al. 2016) were used for transfers between primary consumers and dasyatids (secondary consumers). These mean and SD TEF values were scaled to the number of trophic transfers between resource pools and dasyatids (i.e. mean and SD values were summed for each step up in TL).

### Inter- and intra-specific differences in trophic niche

#### Stomach contents

Multivariate analyses were conducted on the weight of prey items in juvenile stomach content samples to determine if dietary compositions differed significantly between species, sexes or with size. A permutational multivariate analysis of variance (PERMANOVA) was chosen as it is not as sensitive as a one-way analysis of similarities (ANOSIM) to the presence of zero values and it considers both categorical and continuous dependent variables (Anderson 2001). A Bray–Curtis dissimilarity matrix was developed, based on the weight of prey items for each individual, and the adonis function in the vegan package was used to perform the PERMANOVA (Anderson 2001). If the PERMANOVA highlighted significant differences, a similarity percentages analysis (SIMPER) was calculated to determine which prey categories contributed most to the dissimilarity.

The Schoener and Pianka indices of overlap were also calculated using the spaa package to determine dietary overlap between *P. ater* and *U. granulatus*. Values range from 0 (no overlap)—1 (complete overlap). Schoener values of

> 0.6 are considered biologically relevant (Mabragana and Giberto 2007).

#### Stable isotopes

Given the lack of many life-history estimates for these species (e.g. size-at-birth and size-at-maturity), most individuals could not be placed into ontogenetic groups (i.e. young-of-year, juvenile, adult) a priori for statistical analyses, with the exception of mature males identified through clasper calcification. Therefore, intraspecific variation in isotope values was investigated using exploratory cluster analyses on size data as opposed to strict a priori hypothesis testing on ontogenetic groupings. Specifically, a hierarchical cluster analysis was performed to investigate differences in isotope values with the size of individuals. Species were still considered separately in this analysis given that sizes cannot be directly compared between species. Data were standardised and a cluster analysis was performed based on the Euclidean distance resemblance matrix of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values. The Ward's minimum variance method of linking of Euclidean distances was used to determine the clusters.

To further investigate potential isotopic niche differences, standard corrected ellipse areas (SEAc) (Jackson et al. 2011) were calculated for each identified cluster as a measure of trophic niche breadth. This metric was chosen because it uses multivariate ellipse-based Bayesian metrics and Bayesian inference techniques allow for robust statistical comparisons between samples of different sizes. The SEAc represents a measure of the total amount of isotopic niche exploited by a group and high values of SEAc represent a high trophic niche breadth. Each SEAc was calculated using the SIBER package (Jackson et al. 2011). The percentage overlap of each SEAc was also calculated to quantify the level of isotopic niche space overlap between clusters.

#### Trophic position

The trophic position (TP) for the juveniles of each species was calculated using stomach content ( $\text{TP}_{\text{SC}}$ ) and stable isotope ( $\text{TP}_{\text{SI}}$ ) data.  $\text{TP}_{\text{SC}}$  was determined as follows:

$$\text{TP}_{\text{sc}} = 1 + \left( \sum_{j=1}^n P_j \times \text{TP}_j \right),$$

where  $j$  represents the prey categories, TP is the trophic position and  $P$  is the proportion of each prey category (Cortés 1999). The TP of each prey category was taken from Ebert and Bizzarro (2007).

$\text{TP}_{\text{SI}}$  was calculated as follows:

$$\text{TP}_{\text{si}} = \frac{\lambda + (\delta^{15}\text{N}_{\text{secondary consumer}} - \delta^{15}\text{N}_{\text{base}})}{\Delta_n},$$

where  $\lambda$  is the trophic position of the base organism used to estimate  $\delta^{15}\text{N}_{\text{base}}$ ,  $\delta^{15}\text{N}_{\text{consumer}}$  is measured directly,  $\Delta$  is the TEF value per trophic level. The base species chosen was the Strombidae gastropod sampled from the sand flats of the atoll and its corresponding TP was 2.1 (Ebert and Bizzarro 2007). The two different TEF values used in the mixing models were also used to calculate two separate TPs (Tilley and Strindberg 2013; Galván et al. 2016).

## Results

A total of 70 *P. ater*, 50 *U. granulatus*, and 30 *U. asperrimus* individuals were caught for the collection of stomach content and/or muscle samples, details of which can be found in Table 1. Gastric lavage resulted in the successful collection of stomach contents from 71.4% of *P. ater* and 78% of *U. granulatus* individuals. The average lavage duration was 34 s and ranged from 5 s to 2 min 23 s. Short-term post-release survival was 100% with no observed shark predation/encounters and no obvious signs of distress. Three different primary producer species and two filter-feeding invertebrates were collected for stable isotope analyses at the same time that dasyatid muscle tissue was collected (Table 2). Samples were collected at various locations across the sand flats of the atoll.

## Dietary composition

Cumulative prey curves did not reach an asymptote for juveniles of both *P. ater* and *U. granulatus* as the slope of the regression line was statistically different from 0 ( $t = 30.93$  and  $18.75$ ;  $DF = 3$  and  $3$ ;  $p = 0.001$  and  $0.003$  for *U. granulatus* and *P. ater*, respectively). The number of stomach content samples was thus not yet sufficient to provide a complete insight into the dietary composition of these two species. Regardless, curves appeared to be nearing an asymptote and overall trends in the prey types consumed can still be evaluated (Fig. 1).

**Table 1** Details of dasyatids from which stomach contents and muscle samples (for stable isotope analyses) were collected.  $N$  = number of individuals

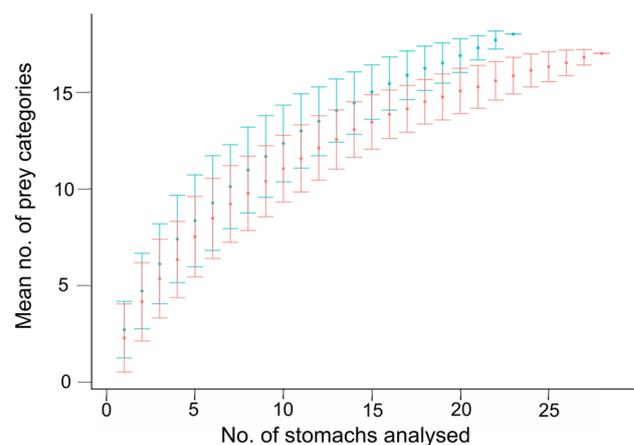
| Species                    | Disc width (cm) | $n$ (total, male, female) |
|----------------------------|-----------------|---------------------------|
| Stomach content collection |                 |                           |
| <i>P. ater</i>             | 28–140          | 50, 24, 26                |
| <i>U. granulatus</i>       | 28–100          | 39, 23, 16                |
| Muscle collection          |                 |                           |
| <i>P. ater</i>             | 28–140          | 39, 19, 20                |
| <i>U. granulatus</i>       | 27–100          | 34, 20, 14                |
| <i>U. asperrimus</i>       | 43–110          | 30, 16, 14                |

A variety of infaunal and epifaunal benthic organisms as well as small quantities of fish were identified in the stomach contents of both dasyatid species. Most prey items could only be identified to family level or higher and a total of 7 phyla and 13 families were identified. The majority of prey items were consumed whole and were not mechanically handled, apart from the molluscs and some brachyurans which had been crushed. Decapod crustaceans were cumulatively the most important prey component in the diet of *U. granulatus* juveniles (% $W = 70.27$  and % $\text{IoI} = 78.53$ ). Specifically, ghost shrimps Callinassidae and swimming crabs Portunidae were consumed by roughly half of the individuals sampled (albeit at low quantities) (Table 3 and Fig. 2). A small number of individuals consumed large proportions of annelids and fish (Actinopterygii), whilst remaining prey items were consumed infrequently and in low proportions (Fig. 2). Conversely for *P. ater*, molluscs were cumulatively the most important prey category (% $W = 55.17$  and % $\text{IoI} = 43.87$ ). Bivalves, specifically, was the only prey group to be

**Table 2** Details of the primary producers and invertebrates collected for stable isotope analyses

| Species                       | $n$ | Section sampled |
|-------------------------------|-----|-----------------|
| <b>Primary producers</b>      |     |                 |
| Chlorophyta                   | 25  | Whole           |
| <i>Thalassia hemprichii</i>   | 9   | Leaves          |
| <i>Thalassendron ciliatum</i> | 12  | Leaves          |
| <b>Filter-feeders</b>         |     |                 |
| Mactridae                     | 28  | Foot            |
| <i>Pinna muricata</i>         | 16  | Adductor muscle |

$N$  = number of individuals



**Fig. 1** Cumulative prey curves from collected stomach content samples of *P. ater* (blue) and *U. granulatus* (pink). Error bars denote standard deviation surrounding the mean number of cumulative prey categories for the number of stomach content samples analysed (after 100 randomisations)

**Table 3** Percentage weight (%W), percentage frequency (%F) and percentage index of importance (%IoI) of prey items collected from stomach contents of *U. granulatus* and *P. ater*

| Prey family              | <i>U. granulatus</i> |                           |      | <i>P. ater</i> |      |      |      |      |
|--------------------------|----------------------|---------------------------|------|----------------|------|------|------|------|
|                          | %W                   | %F                        | %IoI | %W             | %F   | %IoI |      |      |
| Crustacea                | 70.3                 |                           | 78.5 | 6.6            |      | 23.0 |      |      |
| Caridea                  |                      | Callianassidae            | 0.7  | 10.3           | 2.9  | 0.1  | 4.2  | 1.1  |
| Axiidea                  | 19.0                 |                           | 55.2 | 19.6           | 1.9  | 12.5 | 3.7  |      |
| Brachyura                |                      |                           |      |                |      |      |      |      |
|                          |                      | Calappidae                | 2.0  | 13.8           | 4.2  | –    | –    | –    |
|                          |                      | Ocypodidae                | 0.0  | 3.4            | 0.9  | 1.3  | 25.0 | 6.7  |
|                          |                      | Portunidae                | 22.6 | 41.4           | 16.9 | –    | –    | –    |
|                          |                      | Majidae                   | 1.6  | 10.3           | 3.1  | –    | –    | –    |
|                          |                      | Xanthidae                 | 0.3  | 3.4            | 1.0  | –    | –    | –    |
|                          |                      | Unidentifiable brachyura  | 6.1  | 27.6           | 8.9  | 1.2  | 8.3  | 2.4  |
| Dendrobranchiata         |                      | Penaeidae                 | 4.7  | 17.2           | 5.8  | 0.1  | 4.2  | 1.1  |
| Anomura                  |                      | Diogenidae                | –    | –              | –    | 0.0  | 4.2  | 1.1  |
|                          |                      | Hippidae                  | 1.3  | 3.4            | 1.3  | –    | –    | –    |
| Amphipoda                | –                    |                           | –    | –              | –    | 0.1  | 4.2  | 1.1  |
| Stomatopoda              | 2.5                  |                           | 10.3 | 3.4            | 0.0  | 4.2  | 1.1  |      |
| Unidentifiable Crustacea | 9.4                  |                           | 31.0 | 10.7           | 1.8  | 16.7 | 4.7  |      |
| Mollusca                 | 0.0                  |                           | 0.9  | 55.2           |      |      | 43.9 |      |
| Bivalvia Gastropoda      | –                    |                           | –    | –              | 52.7 | 75.0 | 32.6 |      |
|                          |                      | Nassariidae               | –    | –              | –    | 0.1  | 4.2  | 1.1  |
|                          |                      | Muricidae                 | –    | –              | –    | 0.4  | 4.2  | 1.2  |
|                          |                      | Unidentifiable gastropoda | 0.0  | 3.4            | 0.9  | 1.3  | 29.2 | 7.8  |
| Unidentifiable mollusca  | –                    |                           | –    | –              | –    | 0.7  | 4.2  | 1.2  |
| Annelida                 | 20.1                 |                           | 13.5 | 20.4           |      |      | 22.2 |      |
| Polychaeta               |                      |                           |      |                |      |      |      |      |
|                          |                      | Capitellidae              | 2.5  | 20.7           | 6.1  | 14.7 | 41.7 | 14.4 |
|                          |                      | Glyceridae                | 0.3  | 6.9            | 1.9  | 0.6  | 8.3  | 2.3  |
|                          |                      | Unidentifiable polychaeta | –    | –              | –    | 2.1  | 8.3  | 2.7  |
| Unidentifiable annelida  | 17.3                 |                           | 3.4  | 5.5            | 3.0  | 8.3  | 2.9  |      |
| Nemertea                 | 0.3                  |                           | 3.4  | 1.0            | 0.1  | 4.2  | 1.1  |      |
| Sipuncula                | –                    |                           | –    | –              | 17.6 | 12.5 | 7.7  |      |
| Chordata                 |                      |                           |      |                |      |      |      |      |
| Actinopterygii           | 9.3                  |                           | 10.3 | 5.2            | 0.2  | 8.3  | 2.2  |      |
| Echinodermata            |                      |                           |      |                |      |      |      |      |
| Echinoidea               | 0.1                  |                           | 3.4  | 0.9            | –    | –    | –    |      |

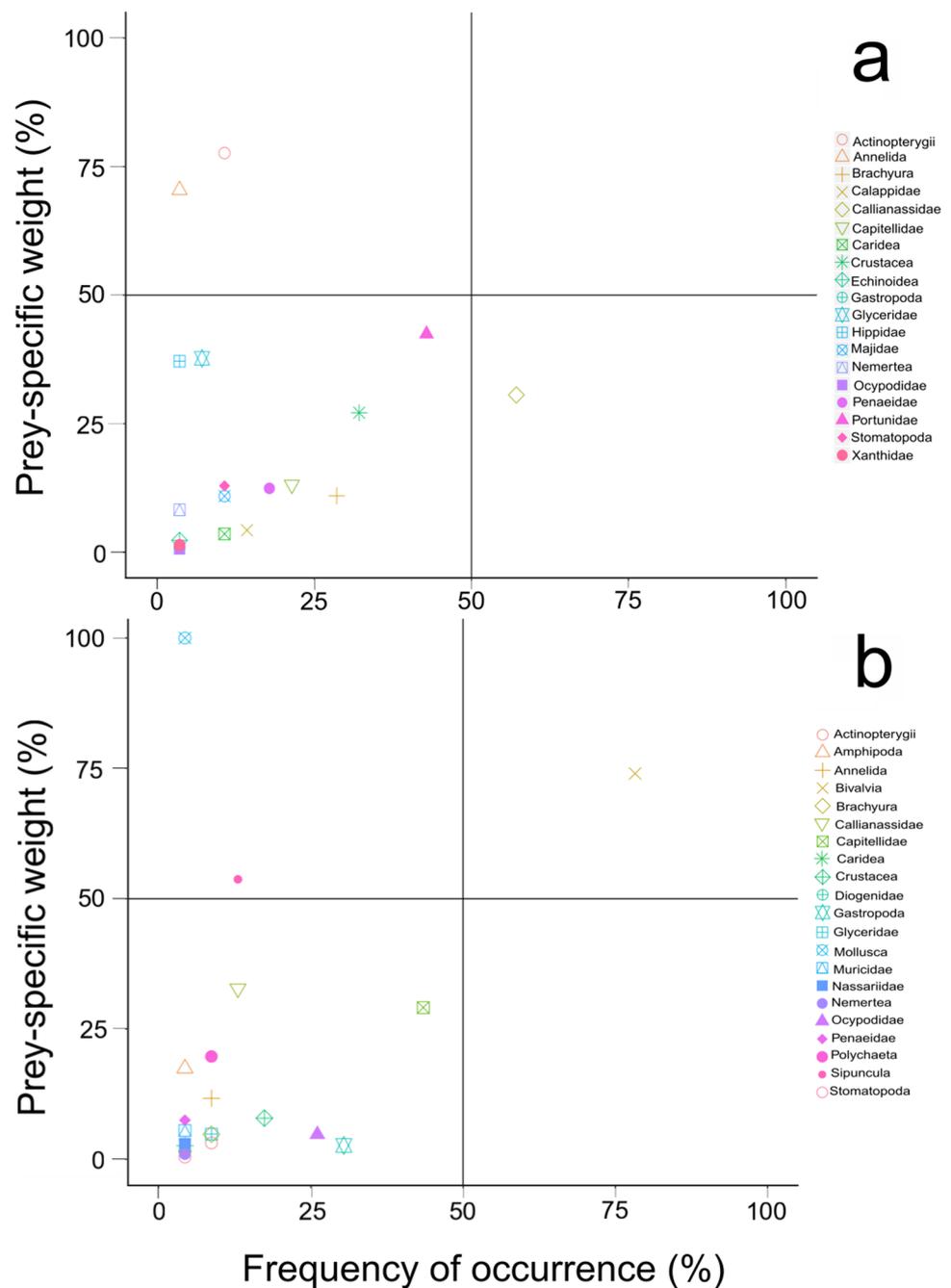
eaten in high quantities by a large proportion of the individuals sampled. Cumulatively, crustaceans and annelids were also important prey categories (%IoI = 22.96 and 22.23, respectively), but as with *U. granulatus*, individual families were consumed infrequently by a small number of individuals (Table 3 and Fig. 2).

### Basal carbon sources

Sampled primary producers had largely different  $\delta^{13}\text{C}$  values; seagrasses were the highest, benthic algae was the lowest and phytoplankton values estimated from filter feeders fell in between the two (mean  $\pm$  SD  $\delta^{13}\text{C}$  =  $-9.21 \pm 2.00$ ,

$N = 21$ ;  $-17.20 \pm 1.46$ ,  $N = 25$ ; and  $-12.00 \pm 1.37$ ,  $N = 44$ , respectively). Mixing models using the two different TEF values available for batoids produced slightly different results. Models using TEF values from Tilley and Strindberg (2013) revealed that both *P. ater* and *U. granulatus* juveniles were most reliant on carbon obtained from seagrass sources and there were lesser proportions of carbon obtained from phytoplankton sources. Conversely, in the models using TEF values from Galván et al. (2016), there were approximately equal contributions of carbon obtained from both seagrass and phytoplankton sources for all juvenile dasyatids. In all models, there were negligible contributions of carbon from chlorophyta algae (Fig. 3).

**Fig. 2** Feeding strategy plot for *U. granulatus* (a) and *P. ater* (b) based on stomach contents. Upper quadrants represent prey items that are eaten in large quantities, while those in the lower quadrants are eaten in small amounts. The right quadrants represent prey items that are eaten by a large portion of the population, while those in the left are eaten by few individuals in the population



### Inter- and intra-specific differences in trophic niche

#### Stomach contents

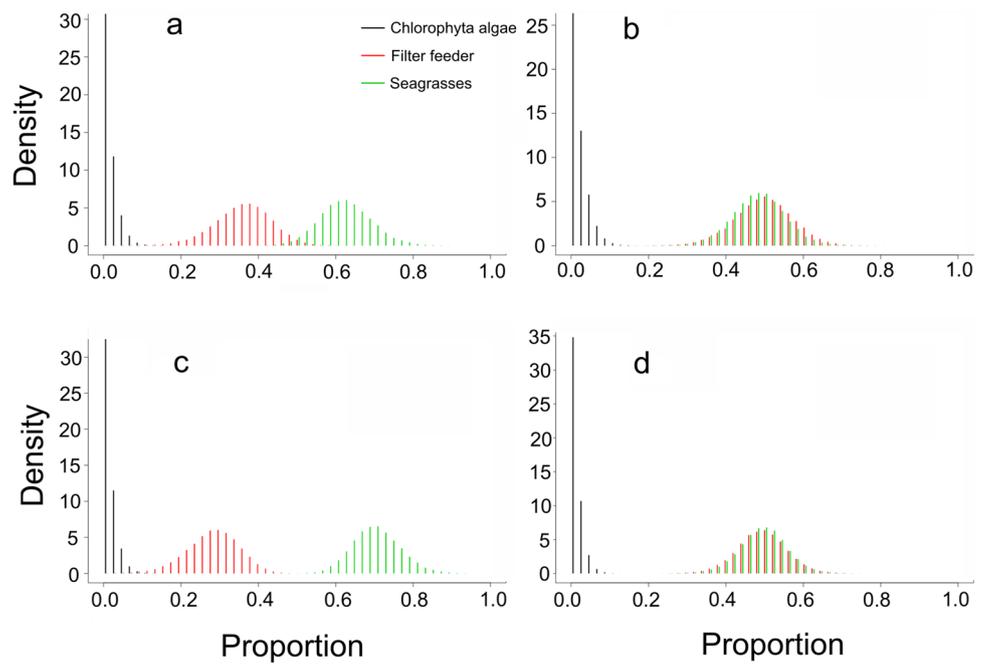
There were significant differences in the stomach content compositions between juveniles of *P. ater* and *U. granulatus* (Table 4) and three prey groups contributed most to this dissimilarity (Bivalvia contributed 40% to this dissimilarity, Portunidae 18% and Callianassidae 16%). *U. granulatus* consumed larger proportions of Callianassidae and Portunidae whereas *P. ater* consumed larger proportions of bivalves (Fig. 4). There was very little dietary

overlap between the two species (Schoener index = 0.16 and Pianka index = 0.09). Finally, there were significant dietary differences with size but not between sexes (Table 4).

#### Stable isotopes

Hierarchical clustering performed on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values revealed two distinct groups, and individuals clustered together based on size rather than species. One identified group contained both the smallest and largest individuals for *P. ater* and *U. granulatus*, while the other identified group contained all the medium sized individuals for both species (Fig. 5 and

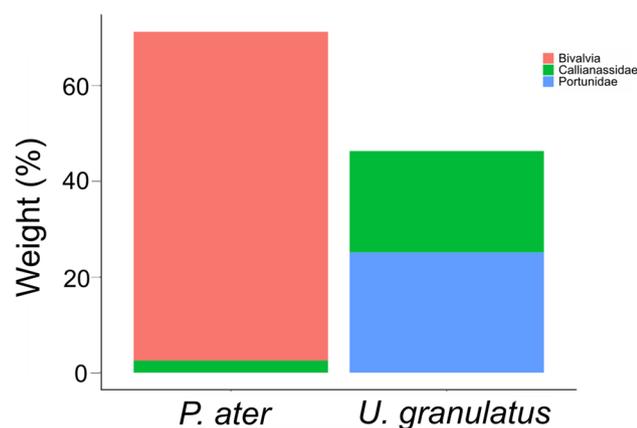
**Fig. 3** The probability distribution for proportional contributions of primary producers to the diet of *U. granulatus* (a, b) and *P. ater* (c, d) determined through Bayesian mixing models. Models were constructed using two available TEF values for batoids, (Tilley and Strindberg 2013) (a, c) and (Galván et al. 2016) (b, d) Primary producers included in models were chlorophyta algae, filter feeding *P. muricata* and Mactridae clams and Thalassendron and *Thalassia* seagrasses



**Table 4** Results of the PERMANOVA performed on stomach content data collected for *P. ater* and *U. granulatus* to determine if dietary composition varied between species, sexes and with size

|           | DF | F statistic | R2   | p value |
|-----------|----|-------------|------|---------|
| Species   | 1  | 9.16        | 0.15 | 0.001   |
| Size      | 1  | 3.19        | 0.05 | 0.002   |
| Sex       | 1  | 1.12        | 0.02 | 0.307   |
| Residuals |    |             | 0.78 |         |

DF degrees of freedom



**Fig. 4** Percentage weight contributions of prey items that contributed most to the dissimilarity of the stomach contents between *P. ater* and *U. granulatus* juveniles

Table 5). Conversely, *U. asperrimus* did not appear to cluster by size (Fig. 5).

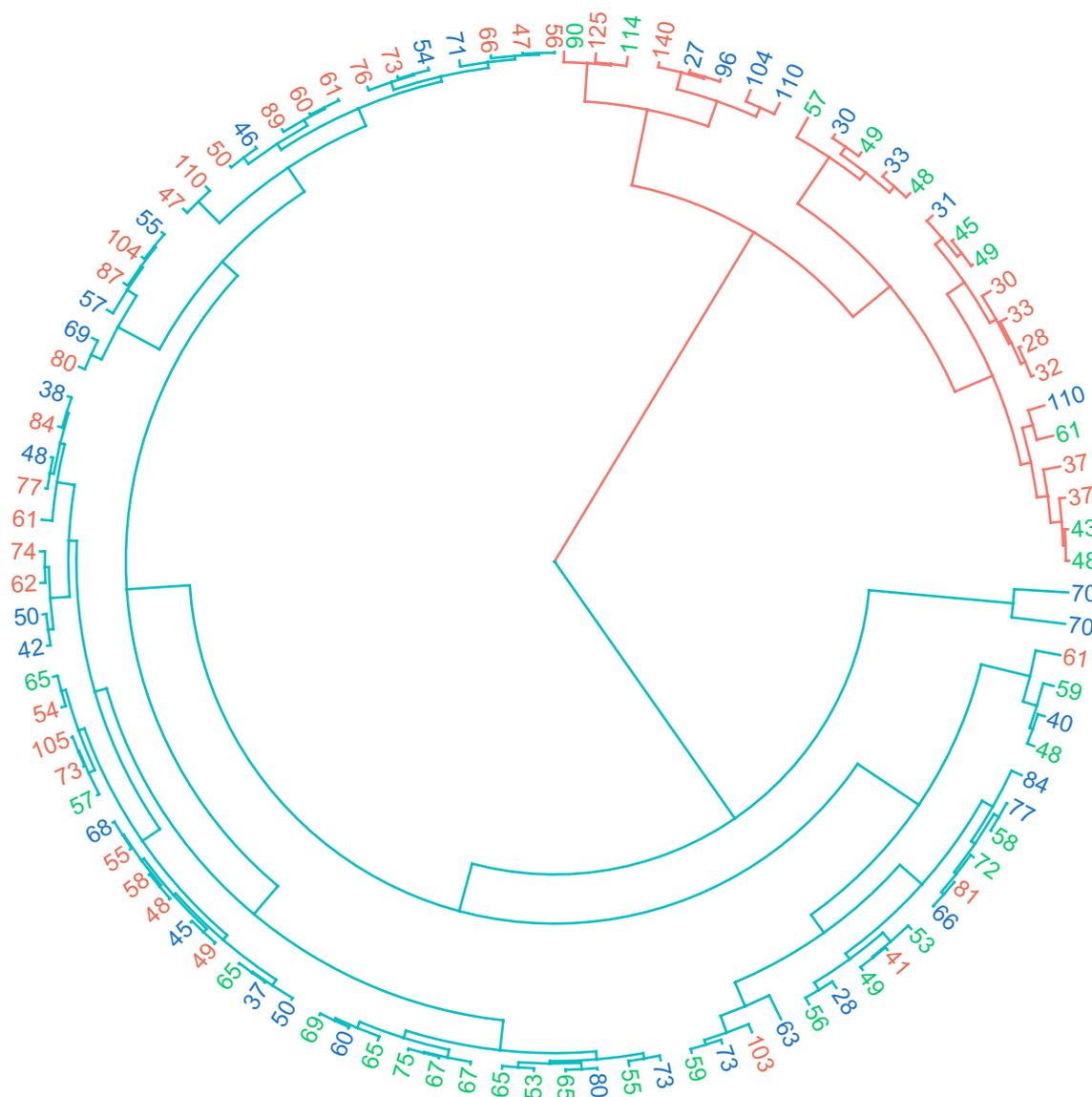
Two *U. granulatus* juveniles were outliers in the data, displaying both relatively lower  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. This could artificially enlarge SEAc values so these individuals were removed when calculating SEAc values. SEAc values could not be calculated for the large *P. ater* and *U. asperrimus* size classes due to small sample sizes (Table 5). SEAc estimates revealed the small *U. granulatus* and the medium *U. asperrimus* size classes to occupy the largest isotopic niche spaces and to display the largest levels of overlap, particularly with each other. Medium *U. granulatus* and *P. ater* size classes also had large degrees of overlap with each other, but lay completely separate to the somewhat overlapping isotopic niches of small *P. ater* and large *U. granulatus* size classes (Fig. 6).

### Trophic position

The trophic position (TP) estimates for juvenile dasyatids were similar when calculated with stable isotope and stomach content data. *P. ater* displayed the lowest estimates and *U. asperrimus* had the highest (Table 6).

### Discussion

The combination of stable isotope and stomach content data provided detailed insights into the trophic ecology of dasyatids in St. Joseph Atoll and their inter- and intra-specific



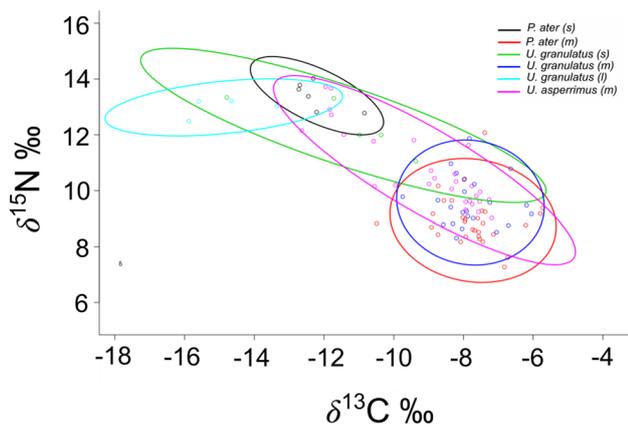
**Fig. 5** Output of hierarchical cluster analysis performed on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for *P. ater*, *U. granulatus* and *U. asperrimus*. Branch col-

ours represent the two major clusters identified. Numbers at the end of branches represent disc widths (cm) of *P. ater* (red), *U. granulatus* (blue), and *U. asperrimus* (green)

**Table 5** Details of size class groups defined for *P. ater*, *U. granulatus*, and *U. asperrimus* based on hierarchical cluster grouping of stable isotope data

| Species—size class           | <i>n</i> | DW(cm)  | $\delta^{15}\text{N}$ | $\delta^{13}\text{C}$ | C:N             |
|------------------------------|----------|---------|-----------------------|-----------------------|-----------------|
| <i>P. ater</i> —small        | 6        | 28–37   | $13.40 \pm 0.51$      | $-12.20 \pm 0.70$     | $3.18 \pm 0.03$ |
| <i>P. ater</i> —medium       | 30       | 41–110  | $8.94 \pm 0.89$       | $-7.73 \pm 0.96$      | $3.20 \pm 0.05$ |
| <i>P. ater</i> —large        | 2        | 125–140 | $13.58 \pm 0.74$      | $-14.14 \pm 0.68$     | $3.29 \pm 0.08$ |
| <i>U. granulatus</i> —small  | 5        | 27–33   | $12.34 \pm 0.98$      | $-11.44 \pm 2.06$     | $3.13 \pm 0.07$ |
| <i>U. granulatus</i> —medium | 25       | 37–84   | $9.33 \pm 1.29$       | $8.38 \pm 2.16$       | $3.22 \pm 0.10$ |
| <i>U. granulatus</i> —large  | 4        | 96–110  | $12.98 \pm 0.34$      | $14.88 \pm 1.14$      | $3.23 \pm 0.08$ |
| <i>U. asperrimus</i> —medium | 28       | 43–75   | $10.74 \pm 1.36$      | $9.12 \pm 1.74$       | $3.14 \pm 0.07$ |
| <i>U. asperrimus</i> —large  | 2        | 90–114  | $14.42 \pm 0.31$      | $14.06 \pm 0.45$      | $3.31 \pm 0.04$ |

Included are the size ranges of the groups (DW = disc-width), number of individuals in each group (*n*), and the mean  $\pm$  standard deviations of isotopic ratios for each group



**Fig. 6** Standard corrected ellipse areas (SEAc) calculated from  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for the different size classes of *P. ater*, *U. granulatus* and *U. asperrimus*. S = small, m = medium, l = large size classes

**Table 6** Mean  $\pm$  SD trophic position (TP) estimates calculated from stable isotope ( $\text{TP}_{\text{Si}}$ ) and stomach content ( $\text{TP}_{\text{Sc}}$ ) values for juvenile *P. ater*, *U. granulatus*, and *U. asperrimus*

| Species              | TP <sub>Si</sub> (Galvan et al. 2016) | TP <sub>Si</sub> (Tilley et al. 2013) | TP <sub>Sc</sub> |
|----------------------|---------------------------------------|---------------------------------------|------------------|
| <i>P. ater</i>       | 3.51 $\pm$ 0.6                        | 2.88 $\pm$ 0.3                        | 3.38             |
| <i>U. granulatus</i> | 3.77 $\pm$ 0.9                        | 3.03 $\pm$ 0.5                        | 3.56             |
| <i>U. asperrimus</i> | 4.71 $\pm$ 0.9                        | 3.55 $\pm$ 0.5                        | 3.58             |

The use of two different TEF values, from Tilley and Strindberg (2013) and Galván et al. (2016), resulted in two different  $\text{TP}_{\text{Si}}$  estimates

dietary differences. Similar to other dasytid species (Jacobsen and Bennett 2013), *P. ater* and *U. granulatus* juveniles were found to be continuous foragers given the presence of the same prey types in the stomachs at varying stages of digestion. The vast majority of prey consisted of infaunal and epifaunal organisms that reside on/in sand flats, suggesting these individuals forage on the surface and sub-surface layers of the sand flats when in the atoll. Although sample size was low and a full array of prey items potentially consumed were not identified, general trends in resource use were identified and can be discussed in the following ecologically relevant ways.

### Inter-specific dietary differences

Stomach content analysis revealed significant inter-specific differences in diet, even though species possess similar body forms, benthic lifestyles and size. Juveniles of *U. granulatus* and *P. ater* consumed significantly different proportions of infaunal prey (mostly decapod crustaceans and bivalves, respectively). Juveniles of *U. asperrimus* were previously found to feed largely on polychaetes (Elston 2016),

suggesting that each species exploits a different dietary niche in the atoll. Given these dietary differences, TP estimates were somewhat variable between species, with *P. ater* having the lowest TP and *U. granulatus* having the highest.

The only other studies that have investigated dietary differentiation between sympatric dasytids over a small scale have found high degrees of trophic overlap. These studies hypothesise that the lack of differential resource use was either due to competitive effects being absent due to an abundance of resources, or there was partitioning of other resources (e.g. space). Theoretical models show that differential resource use is one expected outcome when competitive effects are present (Scheffer and van Nes 2006). The evidence for differential resource use in this present study might possibly be indicative of competitive effects and that food may be limiting in St. Joseph Atoll. This is potentially due to the fact that this small and isolated atoll supports high abundances of juvenile dasytids (Elston unpubl data). However, experimental evidence would be needed to confirm if resources are indeed limiting and whether competitive effects are present.

Differential resource use may not necessarily only be a result of competition, but other factors too (Ross 1986). These species may have previously evolved morphological or behavioural differences which can facilitate dietary specialisation in the absence of competition. Factors that may facilitate this include mouth morphology and dentition, foraging behavior and depth, and temporal/spatial differences in foraging (Platell et al. 1998; Platell and Potter 2001; Marshall et al. 2008; Vaudo and Heithaus 2011). Although detailed dentition information for these species is absent, it has been noted that *P. ater* has unique hexagonal, high crowned rough teeth (Compagno et al. 1989) that may allow them to crush harder prey than most other dasytids. Indeed, molluscs consumed by *P. ater* in this study were mechanically crushed while *U. granulatus* and *U. asperrimus* largely consumed prey whole. Conversely, other studies have shown that *P. ater* consumes large proportions of annelids in Western Australia (Vaudo and Heithaus 2011; O'Shea et al. 2013), suggesting that this species may specialise on different prey items throughout its range. There appear to be subtle differences in foraging depth between dasytid species in St. Joseph Atoll. Previously conducted sediment sampling revealed that bivalves, which were consumed by *P. ater*, were located near the surface of the substratum, while polychaetes and decapods, which were consumed by *U. granulatus* and *U. asperrimus*, occurred deeper in the sediment (Elston pers. obs.). The distribution and composition of benthic fauna can vary across microhabitats (Darnell 1990), thus fine-scale habitat partitioning may be facilitating the differences in diet, but this still needs to be investigated.

Inter-specific dietary differences between juveniles of *P. ater* and *U. granulatus* were not reflected in the stable

isotope data. This might be due to the differences in time-scales between methods as stomach contents provide a ‘snapshot’ view of the diet whereas stable isotopes integrate dietary compositions over longer time periods (MacNeil et al. 2006). However, it is probably an artefact of similar isotopic values between the main prey items (Elston unpubl data).

### Intra-specific dietary differences

Stable isotope data suggested shifts in diet with size, with evidence to suggest that this corresponded with changes in ontogeny. Multiple studies have found that batoids change diet through ontogeny (Smale and Cowley 1992; Vaudo and Heithaus 2011; Yick et al. 2011; O’Shea et al. 2013; Tilley and Strindberg 2013) and these changes may lead to species occupying different trophic positions and thus performing different trophic roles throughout their life-history (Navia et al. 2017). Juveniles of all species had TP estimates consistent with mesopredators (Table 6) and may play important roles in structuring the benthic community of the St. Joseph Atoll. Large individuals (some identified as adults) had higher  $\delta^{15}\text{N}$ , suggesting they occupy higher trophic positions. The dietary preferences of these large individuals remain in question, but it may be that they shift to a diet based more on fish than on benthic organisms, which would affect the increase in  $\delta^{15}\text{N}$ . Indeed, studies have shown that larger rays have greater foraging capabilities and can ingest larger, more mobile species (Kyne and Bennett 2002; Collins et al. 2007; Marshall et al. 2008; Jacobsen and Bennett 2012). Alternatively, this increase in  $\delta^{15}\text{N}$  could be due to different baseline values in different ecosystems where these larger individuals may feed (see below).

Despite this apparent change in diet with size, few studies take size into account when estimating TP for dasyatids, a trend highlighted by a review of dietary studies that reported TP estimates for 25 dasyatid species (Jacobsen and Bennett 2013). TP estimates in this review ranged from 3.16 to 4.08 (mean = 3.62), which largely concur with the TP estimates of juveniles in this study (2.9–4.7, mean = 3.55). Specifically, the mean TP for *P. ater* juveniles in this study (mean TP = 3.26) was similar to the TP obtained in other studies (TP = 3.2 (Jacobsen and Bennett 2013) and 3.5 (Vaudo and Heithaus 2011)). The slightly higher value found by Vaudo and Heithaus 2011 might be due to the inclusion of adults, whereas the present study only included juveniles. López-García et al. (2012) was the only study in the review that took ontogenetic stage into account when calculating TP and found that adults of *Dasyatis longa* had a TP of 4.2. The variability in  $\delta^{15}\text{N}$  with size in the present study further highlights the importance of calculating TP for various size classes, especially in dasyatids, where shifts from a mesopredatory to a higher predatory lifestyle may be common.

Large dasyatids also had lower  $\delta^{13}\text{C}$  values compared to juveniles. Mixing models were used to determine the sources of carbon juvenile dasyatids were reliant upon and while there may be some inaccuracy introduced due to the assumptions required (e.g. accurate TEF values across multiple trophic positions even though the closest TEF values currently available in literature are for skates), overall relative trends can still be discussed. Most notably, seagrasses appeared to contribute large proportions of carbon to the diet of juveniles, providing further confirmation they feed in the atoll. A similar result was obtained by Vaudo and Heithaus (2011), who found that an elasmobranch mesopredatory assemblage was highly dependent on a seagrass-based food web. This suggests that even though mesopredatory elasmobranchs feed on prey from the sand flats, there are important nutritional flows from adjacent seagrass beds (potentially via detrital pathways) that sustain communities. This further emphasises the importance of seagrass beds, particularly to species that are not typically associated with seagrass (Vaudo and Heithaus 2011). Although seagrasses were found to be important sources of carbon, models also suggested that juveniles were reliant on carbon sources obtained from filter-feeding bivalves present on the sand flats, highlighting another important nutritional pathway in the atoll. Conversely, adult carbon isotope values were lower than those of juveniles. This may be characteristic of a more offshore system with phytoplanktonic primary producers (Michener and Kaufman 2007), suggesting that adults are likely feeding in a different food web, potentially outside of the atoll.

### Maternal meddling

It was also found that the smallest individuals and the largest individuals had similar isotopic values. However, rather than this being a function of similar dietary preferences, it is most likely a function of maternal meddling, a trend observed before in sharks (Olin et al. 2011). There is a transfer of maternal resources to individuals during gestation which ceases after birth, but elasmobranch muscle tissue has a long turn-over rate ( $\approx 1$  year, MacNeil et al. 2006). Thus, stable isotopes of individuals less than a year old are influenced by their mother’s diets and foraging location and do not reflect what the individuals themselves are consuming (Olin et al. 2011). This was also reflected in the large isotopic niche space occupied by the small *U. granulatus* size class. These individuals were not in isotopic steady state with their juvenile diet and stable isotope values become less influenced by maternal signatures as they grow, leading to greater variability in isotopic values between individuals. This also suggests that the smallest individuals caught can be classified as young-of-year individuals.

## Conclusions and ecological importance

This is the first study to identify both inter- and intra-specific dietary niche differentiation in a sympatric dasyatid assemblage, despite these species occupying a small isolated area and their similarity in body form and size. This differential resource use may be indicative of competitive effects however, evolutionary processes could have led to the stable coexistence of these similar species regardless of competition (Scheffer and van Nes 2006). Nevertheless, whether the partitioning of dietary resources is a function of competitive effects or evolutionary history, it likely facilitates the co-occurrence of these three similar dasyatid species over a small spatial scale (tens of kilometres).

Dasyatids have the potential to affect the structure and function of the food webs in which they occur because they participate in many of their trophic roles (Navia et al. 2017). Given that each species in St. Joseph Atoll specialises on a different prey group, they may have strong predator-prey interactions and may play influential roles in structuring these communities (Bascompte et al. 2005). In addition, given this specialisation, it suggests relatively low levels of trophic redundancy and the ecosystem might be sensitive to the removal of one of these species (Navia et al. 2012). However, species responses to change are difficult to predict and dasyatids may increase their dietary niche if one species is removed and competition is reduced, as has been seen in juvenile sharks (Matich et al. 2017). To investigate this, determining the diet of these species under variable levels of competition would be needed. In addition, it is important to remember that dasyatids are not the only mesopredators present in the atoll. Bonefish *Albula glossodonta*, mullet (Mugilidae), and juvenile blacktip *Carcharhinus melanopterus* and sickle-fin lemon *Negaprion acutidens* sharks all inhabit the atoll, some of which may feed on the same prey, thereby increasing the trophic redundancy and stability of the system. Finally, given that these species are trophic omnivores (with adults feeding at different trophic levels to juveniles (Novak 2013)), it increases the functional redundancy within trophic groups which is important in maintaining structure and robustness of food webs (Bascompte et al. 2005; Navia et al. 2017).

**Acknowledgements** The authors gratefully acknowledge the Save Our Seas Foundation which provided the funds to carry out this study, as well as the National Research Foundation and South African Institute of Aquatic Biodiversity that provided additional funds. We also thank the staff and volunteers of the Save Our Seas Foundation - D'Arros Research Center (SOSF-DRC) for their assistance in the field and use of facilities and equipment, in particular, R. and C. Daly, K. Bullock, C. Boyes, R. Bennett and E. Moxham. Finally, we are grateful to the staff of the Great Lakes Institute for Environmental Research, in particular A. Hussey, for their expertise and assistance with the stable isotope analyses. We would also like to thank the anonymous reviewers who improved the quality of this manuscript. These methods were approved

by the South African Institute for Aquatic Biodiversity Animal Ethics Committee (reference number 2014/12) and by the Ministry of Environment, Energy, and Climate Change, Seychelles.

## Compliance with ethical standards

**Compliance with ethical standards** This research was supported by a grant obtained from the Save Our Seas Foundation Keystone Grant. The primary author also received a scholarship from the South African National Research Foundation. The authors declare that they have no conflict of interest. All applicable international, national, and institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the South African Institute for Aquatic Biodiversity Animal Ethics Committee (Reference No. 2014/12) and by the Ministry of Environment, Energy, and Climate Change, Seychelles.

**Availability of data and material** The datasets created during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

## References

- Amundsen P, Gabler H, Staldivik FJ (1996) A new approach to graphical analysis of feeding strategy from stomach contents data—modification of the Costello (1990) method. *J Fish Biol* 48(4):607–614
- Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. *Aust Ecol* 26(1):32–46. <https://doi.org/10.1046/j.1442-9993.2001.01070.x>
- Bascompte J, Melian CJ, Sala E (2005) Interaction strength combinations and the overfishing of a marine food web. *Proc Natl Acad Sci USA* 102(15):5443–5447
- Baum JK, Worm B (2009) Cascading top-down effects of changing oceanic predator abundances. *J Anim Ecol* 78(4):699–714. <https://doi.org/10.1111/j.1365-2656.2009.01531.x>
- Bizzarro JJ, Robinson HJ, Rinewalt CS, Ebert DA (2007) Comparative feeding ecology of four sympatric skate species off central California, USA. *Environ Biol Fishes* 80(2–3):197–220. <https://doi.org/10.1007/s10641-007-9241-6>
- Blanco-Parra MDP, Galván-Magaña F, Márquez-Farías JF, Niño-Torres CA (2012) Feeding ecology and trophic level of the banded guitarfish, *Zapteryx exasperata*, inferred from stable isotopes and stomach contents analysis. *Environ Biol Fishes* 95(1):65–77. <https://doi.org/10.1007/s10641-011-9862-7>
- Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 37(8):911–917
- Bornatowski H, Wosnick N, Carmo WPD, Corrêa MFM, Abilhoa V (2014) Feeding comparisons of four batoids (Elasmobranchii) in coastal waters of southern Brazil. *J. Mar Biol Assoc UK* 94(7):1491–1499
- Burgess KB, Bennett MB (2017) Effects of ethanol storage and lipid and urea extraction on  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  isotope ratios in a benthic elasmobranch, the bluespotted maskray *Neotrygon kuhlii*. *J Fish Biol* 90(1):417–423. <https://doi.org/10.1111/jfb.13164>
- Carlisle AB, Litvin SY, Madigan DJ, Lyons K, Bigman JS, Ibarra M, Bizzarro JJ (2017) Interactive effects of urea and lipid content confound stable isotope analysis in elasmobranch fishes. *Can J Fish Aquat Sci* 74(3):419–428
- Cerutti-Pereyra F, Thums M, Austin CM, Bradshaw CJA, Stevens JD, Babcock RC, Pillans RD, Meekan MG (2014) Restricted movements of juvenile rays in the lagoon of Ningaloo Reef, Western

- Australia—evidence for the existence of a nursery. *Environ Biol Fishes* 97(4):371–383. <https://doi.org/10.1007/s10641-013-0158-y>
- Chalermwat K, Jacobsen TR, Lutz RA (1991) Assimilation of bacteria by the dwarf surf clam *mulinia lateralis* (bivalvia: Mactridae). *Mar Ecol Prog Ser* 71:27–35
- Collins AB, Heupel MR, Motta PJ (2007) Residence and movement patterns of cownose rays *Rhinoptera bonasus* within a south-west Florida estuary. *J Fish Biol* 71(4):1159–1178. <https://doi.org/10.1111/j.1095-8649.2007.01590.x>
- Compagno LJ, Ebert DA, Smale MJ (1989) Guide to the sharks and rays of southern Africa. New Holland, London
- Cortés E (1999) Standardized diet compositions and trophic levels of sharks. *ICES J Mar Sci* 56:707–717. <https://doi.org/10.1006/jmsc.1999.0489>
- Darnell RM (1990) Mapping of the biological resources of the continental shelf. *Am Zool* 30:15–21
- Ebert DA, Bizzarro JJ (2007) Standardized diet compositions and trophic levels of skates (Chondrichthyes: Rajiformes: Rajoidei). *Dev Environ Biol Fishes* 80(2–3):221–237. <https://doi.org/10.1007/s10641-007-9227-4>
- Elston C (2016) The trophic and spatial ecology of juvenile porcupine rays *Urogymnus asperrimus* at the remote St. Joseph Atoll. In: Masters thesis. Rhodes University, Grahamstown
- Elston C, von Brandis RG, Cowley PD (2015) Gastric lavage as a non-lethal method for stingray (Myliobatiformes) diet sampling. *Afr J Mar Sci* 37(3):415–519. <https://doi.org/10.2989/1814232X.2015.1076519>
- Elston C, Von Brandis RGV, Cowley PD (2017) Dietary composition and prey selectivity of juvenile porcupine rays *Urogymnus asperrimus*. *J Fish Biol* 91(2):429–442
- Ferry LA, Clark SL, Cailliet GM (1997) Food habits of spotted sand bass (*Paralabrax maculatofasciatus*, Serranidae) from Bahia de Los Angeles, Baja California. *Bull South Calif Acad Sci* 96(1):1–21
- Fisk AT, Tittlemier SA, Pranschke JL, Norstrom RJ (2002) Using anthropogenic contaminants and stable isotopes to assess the feeding ecology of greenland sharks. *Ecology* 83(8):2162–2172
- Galván DE, Jañez J, Irigoyen AJ (2016) Estimating tissue-specific discrimination factors and turnover rates of stable isotopes of nitrogen and carbon in the smallnose fanskate *Sympterygia bonapartii* (Rajidae). *J Fish Biol* 89:1258–1270. <https://doi.org/10.1111/jfb.13024>
- Grubbs RD, Carlson JK, Romine JG, Curtis TH, McElroy WD, McCandless CT, Cotton CF, Musick JA (2016) Critical assessment and ramifications of a purported marine trophic cascade. *Sci Rep* 6:20970. <https://doi.org/10.1038/srep20970>
- Hardin G (1960) The ecompetitive exclusion principle. *Science* 131:1292–1297
- Heithaus MR, Frid A, Wirsing AJ, Worm B (2008) Predicting ecological consequences of marine top predator declines. *Trends Ecol Evol* 23(4):202–210. <https://doi.org/10.1016/j.tree.2008.01.003>
- Heithaus MR, Vaudo JJ, Kreicker S, Layman CA, Krützen M, Burkholder DA, Gastrich K, Bessey C, Sarabia R, Cameron K, Wirsing A, Thomson JA, Dunphy-daly MM (2013) Apparent resource partitioning and trophic structure of large-bodied marine predators in a relatively pristine seagrass ecosystem. *Mar Ecol Prog Ser* 481:225–237. <https://doi.org/10.3354/meps10235>
- Hussey NE, Brush J, Mccarthy ID, Fisk A (2010)  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  diet—tissue discrimination factors for large sharks under semi-controlled conditions. *Comp Biochem Physiol Part A* 155(4):445–453. <https://doi.org/10.1016/j.cbpa.2009.09.023>
- Hussey NE, MacNeil MA, Olin JA, McMeans BC, Kinney MJ, Chapman DD, Fisk AT (2012) Stable isotopes and elasmobranchs: Tissue types, methods, applications and assumptions. *J Fish Biol* 80(5):1449–1484. <https://doi.org/10.1111/j.1095-8649.2012.03251.x>
- Hyslop EJ (1980) Stomach contents analysis—a review of methods and their application. *J Fish Biol* 17:411–429
- Inger R, Jackson A, Parnell A, Bearhop S (2010) SIAR V4 (Stable Isotope Analysis in R). In: *An Ecologist's Guide*. Dublin, Ireland
- Jackson AL, Parnell AC, Inger R, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER—Stable Isotope Bayesian Ellipses in R. *J Anim Ecol* 80:595–602
- Jacobsen IP, Bennett MB (2013) A comparative analysis of feeding and trophic level ecology in stingrays (Rajiformes; Myliobatoidei) and electric rays (Rajiformes; Torpedinoidei). *PLoS One*.1, <https://doi.org/10.1371/journal.pone.0071348>
- Jacobsen IP, Bennett MB (2012) Feeding ecology and dietary comparisons among three sympatric Neotrygon (Myliobatoidei: Dasyatidae) species. *J Fish Biol* 80(5):1580–1594. <https://doi.org/10.1111/j.1095-8649.2011.03169.x>
- Kim SL, Koch PL (2012) Methods to collect, preserve, and prepare elasmobranch tissues for stable isotope analysis. *Environ Biol Fishes* 95:53–63. <https://doi.org/10.1007/s10641-011-9860-9>
- Kim SL, Casper DR, Galván-Magaña F, Ochoa-Díaz R, Hernández-Aguilar SB, Koch PL (2012) Carbon and nitrogen discrimination factors for elasmobranch soft tissues based on a long-term controlled feeding study. *Environ Biol Fishes* 95(1):37–52. <https://doi.org/10.1007/s10641-011-9919-7>
- Kyne PM, Bennett MB (2002) Diet of the eastern shovelnose ray, *Aptychotrema rostrata* (Shaw & Nodder, 1794), from Moreton Bay, Queensland, Australia. *Mar Freshw Res* 53(3):679–686. <https://doi.org/10.1071/MF01040>
- Last PR, Stevens JD (2009) *Sharks and Rays of Australia*. CSIRO publishing
- Last P, Naylor G, Séret B, White W, de Carvalho M, Stehmann M (eds) (2016) *Rays of the World*. CSIRO publishing
- Li Y, Hussey NE, Zhang Y (2016) Quantifying ontogenetic stable isotope variation between dermis and muscle tissue of two pelagic sharks. *Aquat Biol* 25:53–60. <https://doi.org/10.3354/ab00657>
- Logan JM, Lutcavage ME (2010) Stable isotope dynamics in elasmobranch fishes. *Hydrobiologia* 644(1):231–244. <https://doi.org/10.1007/s10750-010-0120-3>
- López-García J, Navia AF, Mejía-Falla PA, Rubio EA (2012) Feeding habits and trophic ecology of *Dasyatis longa* (Elasmobranchii: Myliobatiformes): Sexual, temporal and ontogenetic effects. *J Fish Biol* 80(5):1563–1579. <https://doi.org/10.1111/j.1095-8649.2012.03239.x>
- Mabragana E, Giberto DA (2007) Feeding ecology and abundance of two sympatric skates, the shortfin sand skate *Psammodontus normani* McEachran and the smallthorn sand skate *P. rudis* Gunther (Chondrichthyes, Rajidae), in the southwest Atlantic. *ICES J Mar Sci* 64(5):1017–1027
- MacNeil MA, Drouillard KG, Fisk AT (2006) Variable uptake and elimination of stable nitrogen isotopes between tissues in fish. *Can J Fish Aquat Sci* 63(2):345–353. <https://doi.org/10.1139/f05-219>
- Marshall AD, Kyne PM, Bennett MB (2008) Comparing the diet of two sympatric urolophid elasmobranchs (*Trygonoptera testacea* Muller and Henle and *Urolophus kapalensis* Yearsley and Last): evidence of ontogenetic shifts and possible resource partitioning. *J Fish Biol* 72(4):883–898. <https://doi.org/10.1111/j.1095-8649.2007.01762.x>
- Matich P, Ault JS, Boucek RE, Bryan DR, Gastrich KR, Harvey CL, Heithaus MR, Kiszka JJ, Paz V, Rehage JS, Rosenblatt AE (2017) Ecological niche partitioning within a large predator guild in a nutrient-limited estuary. *Limnol Oceanogr* 62(3):934–953. <https://doi.org/10.1002/lno.10477>
- Michener RH, Kaufman L (2007) Stable isotope ratios as tracers in marine food webs: an update. *Stable Isot Ecol Environ Sci* 2:238–282
- Myers RA, Baum JK, Shepherd TD, Powers SP, Peterson CH (2007) Cascading effects of the loss of apex predatory sharks from a

- coastal ocean. *Science* 315(5820):1846–1850. <https://doi.org/10.1126/science.1138657>
- Navia AF, Cortés E, Jordán F, Cruz-Escalona VH, Mejía-Falla PA (2012) Changes to Marine Trophic Networks Caused by Fishing. In: *Ecosystem Book*, vol 1. pp 417–452
- Navia AF, Cortés E, Mejía-falla PA (2010) Topological analysis of the ecological importance of elasmobranch fishes: a food web study on the Gulf of Tortugas, Colombia. *Ecol model* 221:2918–2926. <https://doi.org/10.1016/j.ecolmodel.2010.09.006>
- Navia AF, Mejía-Falla PA, López-García J, Giraldo A, Cruz-Escalona VH (2017) How many trophic roles can elasmobranchs play in a marine tropical network? *Mar Freshw Res* 68:1–12. <https://doi.org/10.1071/MF16161>
- Novak M (2013) Trophic omnivory across a productivity gradient: intraguild predation theory and the structure and strength of species interactions. *Proc R Soc B Biol Sci* 280(1766):20131415
- Oksanen J, Blanchet FG, Kindt R, Legendre P, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2008) *Vegan: community ecology package R package version 1.17-4*. <http://CRAN.R-project.org/package=vegan>. Accessed Jan 2018
- Olin JA, Hussey NE, Fritts M, Heupel MR, Simpfendorfer CA, Poulakis GR, Fisk AT (2011) Maternal meddling in neonatal sharks: implications for interpreting stable isotopes in young animals. *Rapid Commun Mass Spectrom* 25:1008–1016. <https://doi.org/10.1002/rcm.4946>
- O'Shea OR, Thums M, van Keulen M, Kempster RM, Meekan MG (2013) Dietary partitioning by five sympatric species of stingray (*Dasyatidae*) on coral reefs. *J Fish Biol* 82(6):1805–1820. <https://doi.org/10.1111/jfb.12104>
- Papastamatiou YP, Wetherbee BM, Lowe CG, Crow GL (2006) Distribution and diet of four species of carcharhinid shark in the Hawaiian Islands: evidence for resource partitioning and competitive exclusion. *Mar Ecol Prog Ser* 320:239–251
- Papiol V, Fanelli E, Cartes JE, Rumolo P, López-pérez C (2017) A multi-tissue approach to assess the effects of lipid extraction on the isotopic composition of deep-sea fauna. *J Exp Mar Biol Ecol* 497:230–242. <https://doi.org/10.1016/j.jembe.2017.10.001>
- Parnell A, Jackson A (2013) *siar: stable isotope analysis in R URL* <https://cran.r-project.org/package=siar>. Accessed Jan 2019
- Peterson CH, Fodrie FJ, Summerson HC, Powers SP (2001) Site-specific and density-dependent extinction of prey by schooling rays: generation of a population sink in top-quality habitat for bay scallops. *Oecologia* 129(3):349–356. <https://doi.org/10.1007/s004420100742>
- Platell ME, Potter IC (2001) Partitioning of food resources amongst 18 abundant benthic carnivorous fish species in marine waters on the lower west coast of Australia. *J Exp Mar Biol Ecol* 261:31–54
- Platell ME, Potter IC, Clarke KR (1998) Resource partitioning by four species of elasmobranchs (*Batoidea: Urolophidae*) in coastal waters of temperate Australia. *Mar Biol* 131(4):719–734. <https://doi.org/10.1007/s002270050363>
- Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83(3):703–718. [10.2307/3071875](https://doi.org/10.2307/3071875), [http://www.esajournals.org/doi/abs/10.1890/0012-9658\(2002\)083\[0703:USITET\]2.0.CO;2](http://www.esajournals.org/doi/abs/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2)
- Ritchie EG, Johnson CN (2009) Predator interactions, mesopredator release and biodiversity conservation. *Ecol Lett* 12(9):982–998. <https://doi.org/10.1111/j.1461-0248.2009.01347.x>
- Ross ST (1986) Resource partitioning in fish assemblages: a review of field studies. *Copeia* 1986(2):352–388
- Scheffer M, van Nes EH (2006) Self-organized similarity, the evolutionary emergence of groups of similar species. *Proc Natl Acad Sci USA* 103(16):6230–6235
- Smale MJ, Cowley PD (1992) The feeding ecology of skates (*Batoidea: Rajidae*) off the Cape south coast, South Africa. *South Afr J Mar Sci* 12(1):823–834. <https://doi.org/10.2989/02577619209504744>
- Stoddart DR, Coe MJ, Fosberg FR (1979) D'Arros and St. Joseph, Amirante Islands. *Atoll Res Bull* 223(223):1–48. <https://doi.org/10.5479/si.00775630.223.1>
- Thrush S, Pridmore R, Hewitt J (1994) Impacts on soft-sediment macrofauna: the effects of spatial variation on temporal trends. *Ecol Appl* 4(1):31–41
- Tilley A, Strindberg S (2013) Population density estimation of southern stingrays *Dasyatis americana* on a Caribbean atoll using distance sampling. *Aquat Conserv Mar Freshw Ecosyst* 23(2):202–209. <https://doi.org/10.1002/aqc.2317>
- Valls M, Quetglas A, Ordines F, Moranta J (2011) Feeding ecology of demersal elasmobranchs from the shelf and slope off the Balearic Sea (western Mediterranean). *Sci Mar* 75(4):633–639. <https://doi.org/10.3989/scimar.2011.75n4633>
- VanBlaricom GR (1982) Experimental analyses of structural regulation in a Marine sand community exposed to Oceanic Swell. *Ecol Monogr* 52(3):283–305
- Vaudo JJ, Heithaus MR (2009) Spatiotemporal variability in a sand-flat elasmobranch fauna in Shark Bay, Australia. *Mar Biol* 156(12):2579–2590. <https://doi.org/10.1007/s00227-009-1282-2>
- Vaudo JJ, Heithaus MR (2011) Dietary niche overlap in a nearshore elasmobranch mesopredator community. *Mar Ecol Prog Ser* 425:247–260. <https://doi.org/10.3354/meps08988>
- Vázquez-Luis M, Morató M, Campillo JA, Guitart C, Deudero S (2016) High metal contents in the fan mussel *pinna nobilis* in the balearic archipelago (western mediterranean sea) and a review of concentrations in marine bivalves (pinnidae). *Sci Mar* 18(1):1–12
- Vergnon R, Dulvy NK, Freckleton RP (2009) Niches versus neutrality: uncovering the drivers of diversity in a species-rich community. *Ecol Lett* 12:1079–1090. <https://doi.org/10.1111/lj.1461-0248.2009.01364.x>
- Vergnon R, van Nes EH, Scheffer M (2012) Emergent neutrality leads to multimodal species abundance distributions. *Nat Commun* 3:663–666. <https://doi.org/10.1038/ncomms1663>
- White WT, Platell ME, Potter IC (2004) Comparisons between the diets of four abundant species of elasmobranchs in a subtropical embayment: implications for resource partitioning. *Mar Biol* 144(3):439–448. <https://doi.org/10.1007/s00227-003-1218-1>
- Yemişken E Esken, Forero MG, Megalofonou P, Eryilmaz L, Navarro J (2017) Feeding habits of three Batoids in the Levantine Sea (north-eastern Mediterranean Sea) based on stomach content and isotopic data. *J Mar Biol Assoc UK*. <https://doi.org/10.1017/S002531541700073X>
- Yick JL, Tracey SR, White RWG (2011) Niche overlap and trophic resource partitioning of two sympatric batoids co-inhabiting an estuarine system in southeast Australia. *J Appl Ichthyol* 27(5):1272–1277. <https://doi.org/10.1111/lj.1439-0426.2011.01819.x>