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Nursery habitat use and foraging ecology of the brown stingray *Dasyatis lata* determined from stomach contents, bulk and amino acid stable isotopes

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ABSTRACT: Identification of nursery habitats and knowledge of the trophic ecology and habitat use of juvenile fishes within these habitats are fundamental in developing sound management and conservation strategies. The brown stingray *Dasyatis lata* is a large benthic predator that inhabits the coastal waters of Hawai'i. Although abundant in these ecosystems, little is known about its basic ecology. Stomach content, bulk and amino acid stable isotope analyses were used to assess diet and habitat use of juvenile brown stingrays and to examine the possibility of competitive interactions with juvenile scalloped hammerhead sharks *Sphyrna lewini* that are sympatric with brown stingrays in Kāne'ohe Bay, Oahu. Based on stomach contents, brown stingrays fed almost exclusively on crustaceans. An ontogenetic shift in stingray diet and an increase in relative trophic position (TP) were apparent from stomach content and stable isotope analysis. Stingray bulk $\delta^{13}C$ and $\delta^{15}N$ values indicated long-term foraging fidelity to subregions of the bay. Use of Kāne'ohe Bay as a nursery habitat was supported by nitrogen isotopic analysis of individual amino acids from stingray muscle samples. Our results clearly demonstrated that stingrays foraged within the bay for the majority of their juvenile lives then shifted to offshore habitats with the onset of sexual maturity. Trophic enrichment factors used to estimate TPs from amino acid analysis in previous studies may underestimate TPs in elasmobranchs owing to urea retention for osmoregulation. Potential prey resources were partitioned between stingrays and juvenile scalloped hammerhead sharks, and TP estimates from each analytical method indicated that juvenile scalloped hammerhead sharks forage on higher TP prey than do juvenile brown stingrays. These results show that the study of foraging ecology and habitat use of marine animals can greatly benefit from integrating traditional stomach content and bulk stable isotopic analyses with nitrogen isotopic analyses of individual amino acids.

KEY WORDS: Elasmobranch · Amino acids · Trophic position · Ontogenetic shift · Resource partitioning

INTRODUCTION

Coastal ecosystems such as bays and estuaries are generally characterized by high productivity and a diverse range of habitats, and offer an abundant prey base for faunal constituents and refuge from predation (Shulman 1985, Beck et al. 2001). Because of these characteristics, coastal ecosystems commonly serve as nursery habitat for a wide range of fishes, including elasmobranchs (Beck et al. 2001, Heupel et al. 2007). These ecosystems are highly susceptible to degradation from anthropogenic influences (Lotze et al. 2006), which can have substantial effects on juvenile survivorship (e.g. Jennings et al. 2008). Because juvenile survivorship is often one of the most important factors regulating overall population size (Heppell et al. 1999,

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Cortés 2002), identification of nursery habitats and knowledge of the trophic ecology and habitat use of juvenile fishes within these habitats are fundamental in developing sound management and conservation strategies.

The trophic ecology of elasmobranchs has traditionally been studied through stomach content analysis (SCA) (Hyslop 1980). However, bulk tissue stable isotope analysis (SIA) has increasingly been used to compliment SCA (e.g. Graham et al. 2007). Stable isotope analysis is based on the observation that the ratio of carbon isotopes ($^{13}C/^{12}C$) in an animal's tissues primarily reflects the isotopic signatures of the primary producers at the base of its food chain and nitrogen isotope ratios ($^{15}N/^{14}N$) can indicate the trophic position (TP) of the animal (Post 2002). Thus, stable isotope analyses provide a complementary method to SCA for detecting ontogenetic shifts in diet and foraging habitat and calculation of TP (Post 2002, Fisk et al. 2002, Cocheret de la Morinière et al. 2003, MacNeil et al. 2005). Calculation of TP using bulk tissue SIA can require knowledge of the habitat-specific nitrogen isotopic composition of primary producers (Post 2002). These values may be difficult to obtain in habitats such as coral reefs, which have a diverse range of primary producers, or in deep water habitats that are not easily sampled. Recent studies have shown that analysis of the isotopic composition of individual amino acids (as opposed to bulk tissue) taken from a consumer can circumvent these challenges (Uhle et al. 1997, Fantle et al. 1999, McClelland & Montoya 2002, Chikaraishi et al. 2007, 2009, 2010, Popp et al. 2007, Hannides et al. 2009, Lorrain et al. 2009). This is because the $\delta^{15}N$ values of some amino acids, such as phenylalanine, retain the $\delta^{15}N$ values of primary producers. Other amino acids, such as glutamic acid, undergo large, consistent enrichments (~7.6‰) with each successive trophic position. The advantage of this approach is that the baseline $\delta^{15}N$ values of a consumer's foraging habitat as well as the consumer's TP can be determined by analyzing $\delta^{15}N$ values of individual amino acids in the consumer's tissues. Trophic positions have been estimated with this method for a variety of consumers across a range of TPs (Pakhomov et al. 2004, Schmidt et al. 2004, Popp et al. 2007, Chikaraishi et al. 2009, Hannides et al. 2009, Lorrain et al. 2009), yet TP has only been estimated for a single elasmobranch (Chikaraishi et al. 2010).

The brown stingray *Dasyatis lata* is a large (up to 133 cm disk width, 66 kg; J. Dale unpubl. data) benthic predator endemic to Hawai'i. Juveniles are abundant in shallow bays and estuaries, whereas adults are often found in deep offshore waters (Cartamil et al. 2003, Randall 2007, J. Dale unpubl. data). Despite their abundance in near-shore estuaries and potential for strong impacts on the trophic dynamics of these regions (VanBlaricom 1982, Thrush et al. 1994, Peterson et al. 2001), little is known about their general biology, foraging ecology and habitat use. The habitat use and activity patterns of brown stingrays actively tracked for short durations (days) overlapped significantly with that of juvenile scalloped hammerhead sharks *Sphyrna lewini* that are sympatric with brown stingrays in Kāne'ohe Bay, Oahu, Hawai'i (Holland et al. 1993, Lowe 2002, Cartamil et al. 2003). These results suggest that competition for prey resources between these 2 sympatric species may contribute to high rates of starvation found for neonate scalloped hammerhead sharks within their Kāne'ohe Bay nursery (Bush & Holland 2002, Lowe 2002, Duncan & Holland 2006).

The goals of this study were to quantify the foraging ecology and habitat use of brown stingrays in Kāne'ohe Bay and evaluate the extent of ecological interactions between brown stingrays and juvenile scalloped hammerhead sharks. Specifically, we used SCA and SIA to address the following questions. (1) What are the food habits of brown stingrays? (2) Do their food habits change through ontogeny? (3) Is Kāne'ohe Bay used as a nursery habitat by brown stingrays? (4) Do isotopic analyses of individual amino acids accurately reflect the TP of brown stingrays and juvenile scalloped hammerhead sharks? (5) Is there evidence of competition between brown stingrays and juvenile scalloped hammerhead sharks for prey resources? We found that the combined use of stomach content, bulk tissue and individual amino acid stable isotope analyses allowed for validation of results from each individual method and provided significant insight into the early life history and ecological relationships of brown stingrays. These results highlight the advantages of integrating multiple approaches in the study of foraging ecology and habitat use.

MATERIALS AND METHODS

Study site and sample collection. Kāne'ohe Bay, located on the east coast of Oahu, covers an area of ~46 km^2 and is bounded on its seaward side by a barrier reef (Fig. 1). The inner bay, landward of the barrier reef, consists of deep lagoon (average depth, 13 m) interspersed with numerous patch reefs (Smith et al. 1981). Spatial differences in diet composition and habitat use were evaluated by dividing the bay into 3 zones: south, mid and north (Fig. 1). Zones were based on previous studies examining environmental and ecological processes within the bay (Smith et al. 1981, Duncan & Holland 2006). Brown stingrays were collected with standard demersal longlines in and outside

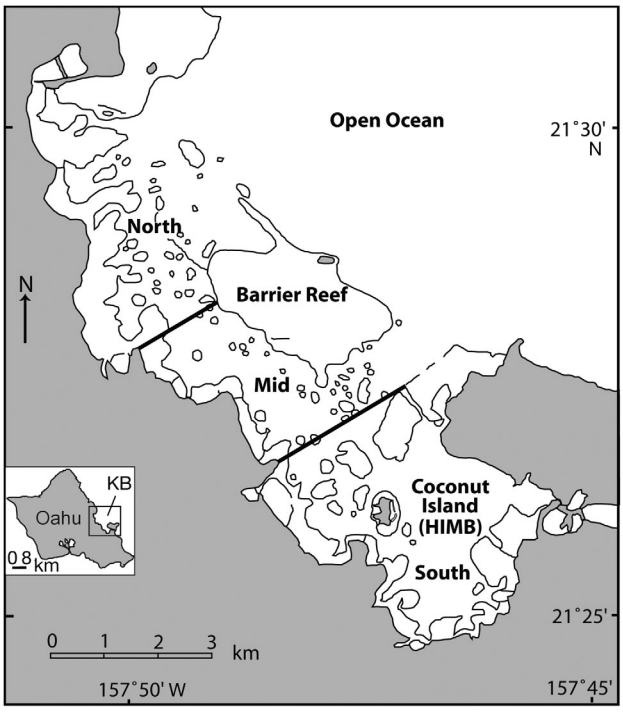

Fig. 1. Kāneʻohe Bay showing outline of patch reefs and bay zones (south, mid and north). HIMB: Hawaii Institute of Marine Biology. Inset: location of Kāneʻohe Bay (KB) on Oahu, Hawaiʻi

of Kāneʻohe Bay. Inshore longlines were set at least 5 m away from patch reefs to avoid entanglement on coral. Offshore longlines were set at depths of ~80 m based on catch records from a shark survey conducted in this area (D. Grubbs unpubl. data). All longlines were baited with tuna *Thunnus* spp., and/or squid *Loligo* spp. Lines were soaked for 3 h before being retrieved. Captured stingrays were landed, disk width (DW) measured to the nearest 1 mm, and euthanized. Maturity state (juvenile/adult) was assessed as part of a concurrent study on the age and growth of brown stingrays (J. Dale unpubl. data).

Stomach content analysis. Stomach contents were removed and preserved in 70% ethyl alcohol. Upon analysis, stomach contents were rinsed and individual prey items were identified to the lowest possible taxon. The total number and wet weight of each taxon was recorded and prey items were then dried to a constant weight at 60°C. The contribution of each prey taxon to the diet of brown stingrays was quantified with several metrics of dietary composition. Percent abundance ($\overline{\%N}$, [number of individuals in a prey category/total number of individuals among all prey categories] × 100) and percent weight ($\overline{\%W}$, [weight of individuals in a prey category/total weight of individuals among all prey categories] × 100) were calculated for each sample to provide mean and variability estimates (Bizzarro et al. 2007, Chipps & Garvey 2007). The percent frequency occurrence (%FO) was calculated as the number of stomachs containing a prey taxon/total number of stomach containing prey × 100 (Hyslop 1980). Individual metrics were also combined into a composite metric, the index of relative importance (IRI), for an additional description of diet composition (Pinkas et al. 1971), which is defined as:

$$\mathrm{IRI} = (\overline{\%N} + \overline{\%W}) \times \%\mathrm{FO} \quad (1)$$

The IRI values were expressed as a percentage ($\overline{\%IRI}$) to facilitate comparisons between prey taxon (Cortés 1997). Percent IRI values were calculated for each sample to provide mean and variability estimates for each prey taxon (Bizzarro et al. 2007).

There was no significant effect of sex or location on gravimetric diet data based on analysis of similarities (ANOSIM) procedures (see below); therefore, for ontogenetic comparisons data were pooled. By cluster analysis with the group average linkage method (PRIMER v. 6), 3 size classes were identified: small (35.0 to 54.9 cm DW), medium (55.0 to 69.9 cm DW) and large (70.0 to 94.9 cm DW). Brown stingrays were separated by DW into 5 cm bins, and the average $\overline{\%W}$ for each prey item was calculated. Bin averages were required because individual stomachs were too variable for meaningful interpretation. To compare dietary composition between identified size classes, non-metric multidimensional scaling (MDS) ordination plots were generated and ANOSIM was used to test for significant differences between size groups (PRIMER v. 6). The resultant global R-statistic ($-1 > R < 1$) describes the amount of similarity between each pair in the ANOSIM. A value of 0 indicates no difference while a value close to |1| indicates that the 2 groups are entirely separate. p-values generated from the R-statistic were considered statistically significant if they were <0.05. Multivariate dispersion (MVDISP) calculated the degree of dispersion between samples within size classes and similarity percentages (SIMPER) identified which dietary categories contributed most to the dissimilarities between size classes (PRIMER v. 6). For each size class, brown stingrays were randomly sorted into groups of 4 (i.e. dietary samples) and mean values for each prey taxon determined to overcome the problem of low prey diversity in the stomachs of individual stingrays (Platell & Potter 2001). Gravimetric diet data were square root transformed before multivariate analysis and, when appropriate, used to construct a Bray-Curtis similarity matrix. Only prey items representing >5% $\overline{W}$ were included to reduce effects of rare prey. Prey diversity was calculated for each size class with the Shannon-Wiener diversity index (H') (Krebs 1999). Trophic positions were calculated for each size class following Cortés

(1999). Trophic position estimates for prey categories were taken from the Sea Around Us Project database (www.seaaroundus.org) and Ebert & Bizzarro (2007).

To evaluate the possibility of competitive interactions, diet composition was compared between brown stingrays and juvenile scalloped hammerhead sharks. Raw data from Bush (2003) were reanalyzed to generate $\overline{\%W}$ values for each individual hammerhead shark sample. Dietary data were grouped into broad categories to eliminate biases in comparisons based on variable levels of taxonomic identification (Cortés 1997). Differences between hammerhead shark and stingray diets were analyzed following the procedures described for stingray size class comparisons (i.e. ANOSIM, MDS).

Bulk stable isotope analysis. A preliminary study conducted in September 2009 that examined the food web structure of Kāneʻohe Bay found red macroalgae to be the primary nitrogen source for benthic predators (mean $\delta^{15}N$ ± SD: 3.3 ± 0.7‰, n = 6, J. Dale unpubl. data). Macroalgae were collected by hand from sites throughout the bay, washed in distilled water and visually inspected for sources of contamination. Brown stingray and juvenile scalloped hammerhead shark epaxial white muscle tissue (~1 cm^3) was removed from each sample and frozen until further analysis. Samples were dried at 60°C and ground into a fine powder with a mortar and pestle. Lipid extraction was not performed owing to the low molar C:N ratios of stingray (mean ± SD: 2.97 ± 0.10) and hammerhead shark (3.00 ± 0.12) muscle samples, indicating low lipid concentrations in tissues and little variation between individuals (Post et al. 2007). The $\delta^{13}C$ and $\delta^{15}N$ values of white muscle tissue were determined with a carbon-nitrogen analyzer coupled with an isotope ratio mass spectrometer (Finnigan Conflo II/Delta-Plus). Isotope values are reported as δ-values (as ‰) relative to Vienna PeeDee Belemnite (VPDB) and atmospheric N_2 standards for carbon and nitrogen, respectively. Accuracy of isotopic measurements was determined through repeated analyses of a sample of glycine isotopically well characterized by long-term (>10 yr) analyses using multiple instruments in different laboratories and interspersed with samples. Average accuracy and precision of all stable isotopic analyses determined by replicate analysis of glycine and samples was <±0.1‰ (1 SD).

A general linear model (GLM) was used to test for differences in brown stingray bulk isotopic compositions between bay zones and sexes, with stingray DW as a covariate. If significant differences between zones or sex were found, a subsequent GLM was used to test for differences in isotopic values between size classes while controlling for variability due to significant factors, which were included as fixed effects. Trophic positions for each stingray size class and juvenile scalloped hammerhead sharks were calculated with the following equation:

$$TP_{bulk} = \frac{\delta^{15}N_{consumer} - 3.3}{2.7} + 1 \quad (2)$$

where 2.7‰ is the assumed trophic enrichment factor (TEF) (Vanderklift & Ponsard 2003) and 3.3‰ is the average nitrogen isotopic value for red macroalgae abundant in Kāneʻohe Bay (Stimson et al. 2001, J. Dale unpubl. data).

Stable nitrogen isotope analysis of individual amino acids. Before analysis, brown stingray and juvenile scalloped hammerhead shark muscle tissue was subjected to acid hydrolysis, esterification of the carboxyl terminus and trifluoroacetylation of the amine group (Macko et al. 1997, Popp et al. 2007). Muscle tissue (~5 mg) was hydrolyzed at 150°C for 70 min with sequanal grade 6 N hydrochloric acid (HCl) in a culture tube that was flushed with nitrogen gas (N_2) and fitted with a Teflon-lined cap. Glutamine is converted to glutamic acid during hydrolysis (Metges et al. 1996) so $\delta^{15}N$ values of glutamic acid include glutamine. The HCl was evaporated to dryness in a Thermo Savant Speed Vac concentrator coupled with a UVS400 at 55°C for 1.5 h. The residue was redissolved in 1 ml 0.01 N HCl and purified by filtration (0.45 µm hydrophilic filter), and the filter was washed with 1 ml 0.01 N HCl. Amino acids were further purified by cation exchange (~5 cm Dowex 50WX8-400 in a Pasteur pipette). The filtered hydrolysate was added to the ion exchange column in 0.01 N HCl and amino acids eluted with 4 ml ammonium hydroxide and evaporated to dryness under a stream of N_2 at 80°C. The samples were re-acidified by adding 0.5 ml of 0.2 N HCl; the vials were then flushed with N_2, heated to 110°C for 5 min and then dried in the Speed Vac concentrator for 1.5 h at 55°C. The hydrolyzed muscle samples were esterified with 2 to 3 ml of 1:4 acetyl chloride : isopropanol in N_2-flushed vials heated to 110°C for 60 min. Excess solvent was removed under a stream of N_2 at 60°C. Trifluoroacetylation of the amine group was accomplished by adding 3:1 methylene chloride : trifluoroacetic anhydride (TFAA) to each vial and heating to 100°C for 15 min. The samples were further purified by solvent extraction following Ueda et al. (1989) with 2 ml of P-buffer (KH_2PO_4 + Na_2HPO_4 in milli-Q water, pH 7). The acylated amino acids were partitioned into chloroform, the chloroform evaporated to dryness and the trifluoroacetylation step repeated to ensure full derivitization. Samples were stored at –20°C in 3:1 methylene chloride : TFAA for up to 1 mo until isotope analysis.

Just before isotope analysis, the 3:1 methylene chloride : TFAA mixture was evaporated under a stream

of N_2 at room temperature. Samples were redissolved in 100 µl of ethyl acetate. The stable N isotope composition of the amino acids were determined with either a Delta XP or Delta V Plus mass spectrometer interfaced with a Trace GC gas chromatograph through a GC-C III combustion furnace (980°C), reduction furnace (650°C) and liquid N cold trap. The samples (1 to 2 µl) were injected (split/splitless injector, 10:1 split ratio) onto a *forte* BPx5 capillary column (30 m × 0.32 mm × 1.0 µm film thickness) at an injector temperature of 180°C with a constant helium flow rate of 1.4 ml min^{-1}. The column was initially held at 50°C for 2 min and then increased to 190°C at a rate of 8°C min^{-1}. Once at 190°C, the temperature was increased at a rate of 10°C min^{-1} to 300°C where it was held for 7.5 min. Internal reference compounds, aminoadipic acid and norleucine of known nitrogen isotopic composition, were co-injected with samples and used to normalize the measured $\delta^{15}N$ values of unknown amino acids. All samples were analyzed at least in triplicate. Reproducibility associated with isotopic analysis of glutamic acid and phenylalanine averaged ±0.44‰ (1 SD) and ranged from ±0.06‰ to ±0.85‰. The accuracy of each measurement was determined by using the known $\delta^{15}N$ value for aminoadipic acid to determine the measured $\delta^{15}N$ value of norleucine as an unknown, and vice versa. We have found that the combustion reactor on the GC-C III is susceptible to rapid failure when used for nitrogen isotopic analyses of amino acids. Co-injection of aminoadipic acid and norleucine allows monitoring of combustion reactor degradation and provides an internal check on the accuracy of each sample injected. The accuracy averaged ±1.5‰ (1 SD) and ranged from ±0.36‰ to ±2.4‰.

Determination of trophic position using amino acid isotope analyses. The fractional trophic position of brown stingray and juvenile scalloped hammerhead shark samples was calculated with the measured $\delta^{15}N$ values of glutamic acid and phenylalanine as described by Chikaraishi et al. (2009) as follows:

$$TP_{Glu/Phe} = \frac{(\delta^{15}N_{Glu} - \delta^{15}N_{Phe}) - 3.4}{7.6} + 1 \quad (3)$$

where $TP_{Glu/Phe}$ is the trophic position determined with glutamic acid (Glu) and phenylalanine (Phe), 3.4 is the isotopic difference between glutamic acid and phenylalanine in the primary producers (β) and 7.6 is the TEF. A conservative error associated with the TP calculation was determined by propagation of error (e.g. Gelwicks & Hayes 1990) using the uncertainty in β and the TEF in Chikaraishi et al. (2009), as well as the measured reproducibility for glutamic acid and phenylalanine for each sample.

RESULTS

Stomach content analysis

A total of 156 juvenile brown stingrays were sampled from Kāne'ohe Bay (size range, 35.0 to 94.9 cm DW); 20 (12.8%) of these had empty stomachs. The number of empty stomachs varied significantly between size classes, with the greatest percentage occurring in small rays (22%), followed by large (17%) and medium (5%) rays ($\chi^2 = 6.4$, df = 2, p = 0.04). Prey from stomach contents of brown stingrays were separated into 16 prey categories (Table 1). Crustaceans, represented by alpheid shrimps, portunid crabs and stomatopods, were the most important prey group for all size classes ($\overline{IRI}$ ± SD: small, 93.25 ± 18.30; medium, 86.46 ± 25.52; large, 84.67 ± 23.32), with minor contributions from polychaete worms and teleost fish (Table 1). Gobies were the most important teleost component and only 1 other teleost family was identified (Table 1). With increasing stingray size, alpheid shrimps and polychaete worms declined in importance whereas the portunid crab, *Podophthalmus vigil*, and gobies increased in importance (Table 1). Another portunid crab, *Libystes villosus*, increased in importance from small to medium sized individuals, but then decreased from medium to large sized individuals (Table 1). There was a positive, linear relationship between stingray size (DW) and stingray mouth gape (r^2 = 0.86, p < 0.001) as well as stingray size and carapace width of *P. vigil* consumed ($r^2 = 0.51$, p < 0.001). Based on calculation of TP from prey in stingray stomachs, juvenile stingrays were classified as secondary consumers (TP < 4) and TP increased with increasing stingray size (range: 3.3 to 3.7, Table 1).

Gravimetric diet composition differed significantly overall between the 3 size classes (ANOSIM: Global R statistic = 0.428, p = 0.001) and also for each of the pairwise comparisons between size classes. The R-statistic values for pairwise comparisons were greatest between small and large size classes (0.856, p = 0.001) and comparably lower between small and medium (0.291, p = 0.004) and medium and large (0.298, p = 0.004) size classes. Ordination of the mean gravimetric dietary data showed a gradual dietary transition in small to large stingrays (Fig. 2). Sample dispersion was similar between the 3 size classes (MVDISP value range: 0.906 to 1.054). Although dietary samples overlapped between size classes, no samples from the small size class overlapped with samples from the large size class (Fig. 2). *Alpheus malabaricus* and *Podophthalmus vigil* were identified as the prey items most responsible for differences in dietary compositions between size classes by SIMPER analysis. Dietary breadth was lowest for small stingrays (H' = 0.563) and greatest for large stingrays (H' = 0.708) (Table 1).

Table 1. *Dasyatis lata*. Mean and SD of percent number ($\overline{\%N}$) and percent weight ($\overline{\%W}$), percent frequency occurrence (%FO) and mean and SD of percent index of relative importance ($\overline{\%IRI}$) of prey consumed by juvenile brown stingrays in Kāneʻohe Bay. Sample size, trophic level and Shannon-Wiener Index given at the bottom of each size class. DW: disk width

Prey	Size class (DW)																						
	35.0 to 54.9 cm							55.0 to 69.9 cm							70.0 to 94.9 cm								
	$\overline{\%N}$	SD	$\overline{\%W}$	SD	%FO	$\overline{\%IRI}$	SD	$\overline{\%N}$	SD	$\overline{\%W}$	SD	%FO	$\overline{\%IRI}$	SD	$\overline{\%N}$	SD	$\overline{\%W}$	SD	%FO	$\overline{\%IRI}$	SD
Crustacea	86.51	22.40	87.73	23.86	96.97	93.25	18.30	79.01	27.16	78.57	31.45	93.65	86.46	25.52	74.95	27.43	81.55	30.38	97.50	84.67	23.32
Alpheidae																					
Alpheus malabaricus	68.05	27.44	55.50	34.42	93.94	78.37	26.26	38.63	30.81	20.40	29.72	77.78	40.58	31.49	13.64	20.97	2.64	6.60	40.00	8.58	15.16
Unidentified alpheid	0.52	2.29	0.08	0.42	6.06	0.02	0.10	0.49	2.86	0.00	0.01	3.17	0.02	0.13	–	–	–	–	–	–	–
Portunidae																					
Podophthalmus vigil	3.76	7.56	9.50	22.32	24.24	2.78	6.72	15.38	26.92	20.83	35.16	38.10	16.63	29.44	36.36	36.54	47.11	43.36	70.00	50.70	39.58
Portunus granulatus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.50	15.81	2.50	15.81	2.50	2.50	15.81
Portunus longispinosus	0.18	1.02	0.86	4.94	3.03	0.02	0.14	0.29	2.29	0.14	1.10	1.59	0.02	0.16	1.25	7.91	1.87	11.82	2.50	0.22	1.38
Libystes villosus	10.95	21.45	17.39	28.95	42.42	11.03	22.24	16.25	21.49	27.48	34.21	50.79	23.21	27.85	8.76	16.14	12.49	25.44	30.00	8.96	16.78
Unidentified Portunidae	–	–	–	–	–	–	–	0.45	2.52	0.41	2.35	3.17	0.09	0.57	–	–	–	–	–	–	–
Unidentified crab	2.12	9.27	4.18	17.45	6.06	0.51	2.33	3.96	14.45	4.42	16.25	12.70	2.36	12.75	4.82	12.48	6.73	18.97	17.50	4.04	10.58
Stomatopoda																					
Oratosquilla oratoria	–	–	–	–	–	–	–	1.36	7.68	0.76	5.36	3.17	0.27	1.82	0.86	3.84	1.57	6.95	5.00	0.15	0.64
Gonodactylaceus falcatus	–	–	–	–	–	–	–	0.34	2.70	0.35	2.75	1.59	0.06	0.49	–	–	–	–	–	–	–
Pseudosquilla ciliata	–	–	–	–	–	–	–	1.33	6.38	2.54	12.46	4.76	0.62	2.82	3.01	15.07	3.80	17.54	5.00	1.77	9.41
Unidentified mantis	0.93	3.05	0.22	0.71	9.09	0.09	0.30	0.52	3.28	1.24	6.95	3.17	0.08	0.47	3.75	14.69	2.83	15.58	10.00	2.35	13.70
Polychaeta	9.33	20.84	6.69	18.24	36.36	5.50	17.70	7.06	18.65	6.01	19.49	26.98	4.47	17.72	1.80	6.97	1.44	7.70	7.50	0.28	1.31
Unidentified Polychaeta	9.33	20.84	6.69	18.24	36.36	5.83	17.76	7.06	18.65	6.01	19.49	26.98	5.27	17.96	1.80	6.97	1.44	7.70	7.50	0.43	2.01
Osteichthyes	2.34	7.25	1.25	4.11	15.15	0.34	1.05	9.50	17.51	8.82	20.56	39.68	6.15	15.65	20.76	24.23	13.88	24.24	55.00	14.26	22.04
Gobiidae	2.20	7.25	1.24	4.11	12.12	0.38	1.24	8.81	17.65	7.37	19.16	33.30	6.85	17.17	17.96	24.68	12.24	23.78	45.00	17.73	27.80
Ophichthidae	–	–	–	–	–	–	–	0.18	1.40	1.04	8.27	1.59	0.04	0.30	–	–	–	–	–	–	–
Unidentified teleost	0.14	0.79	0.01	0.05	3.03	0.00	0.02	0.52	2.42	0.41	3.15	4.76	0.05	0.28	2.80	8.16	1.64	7.92	12.50	1.08	3.54
Miscellaneous	1.89	5.43	4.46	16.87	15.15	0.95	3.98	4.42	14.06	6.60	18.14	22.22	2.92	12.92	2.49	6.38	3.13	9.71	15.00	0.78	2.33
Unidentified remains	1.89	5.43	4.46	16.87	15.15	1.00	4.07	4.42	14.06	6.60	18.14	22.22	3.86	13.96	2.49	6.38	3.13	9.71	15.00	1.49	4.26
Sample size (empty)				42 (9)							66 (3)							48 (8)			
Trophic level				3.3							3.5							3.7			
Shannon-Wiener index				0.563							0.691							0.708			

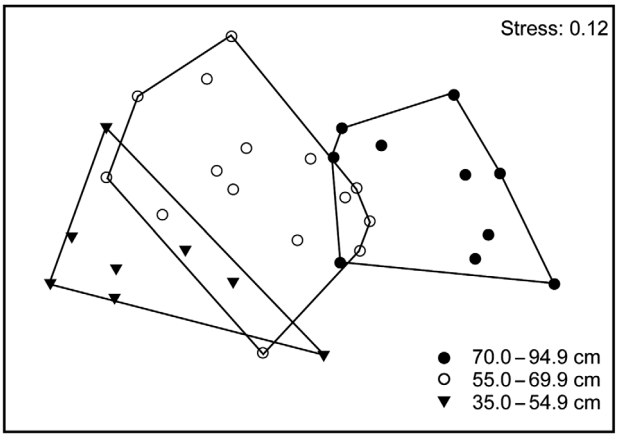

Fig. 2. *Dasyatis lata*. Non-metric multidimensional scaling of the mean gravimetric dietary data for the 3 size classes of juvenile brown stingrays

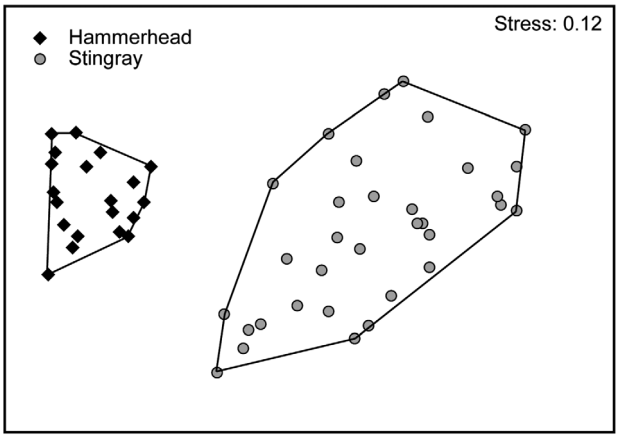

Fig. 3. *Dasyatis lata* and *Sphyrna lewini*. Non-metric multidimensional scaling of the mean gravimetric dietary data for juvenile brown stingrays and juvenile scalloped hammerhead sharks. Juvenile scalloped hammerhead dietary data were reanalyzed from Bush (2003)

Diet composition of juvenile scalloped hammerhead sharks was significantly different than that of brown stingrays in Kāneʻohe Bay (ANOSIM: Global R-statistic = 0.842, p = 0.001) and all pairwise comparisons between hammerhead sharks and stingray size classes were also significant (p = 0.001). Ordination of the dietary data revealed 2 distinct clusters with no overlap, one composed of juvenile scalloped hammerhead shark samples and the other composed of brown stingray samples (Fig. 3). SIMPER analysis identified teleosts and portunid crabs as contributing most to the dissimilarity between hammerhead sharks and all stingray size classes. Hammerhead sharks consumed larger amounts of teleosts and smaller amounts of portunids by weight compared with stingrays (Fig. 4). Alpheid shrimps also contributed strongly to the dissimilarity between juvenile scalloped hammerhead sharks and large brown stingrays, with hammerhead sharks consuming larger amounts of alpheids by weight compared with large stingrays (Fig. 4). Trophic position based on stomach contents for hammerhead sharks was 4.0, ~0.5 TPs higher than the average TP for all juvenile stingrays.

Bulk stable isotope analysis

White muscle tissue from 44 juvenile brown stingrays collected within Kāneʻohe Bay, ranging from 42.1 to 93.1 cm DW, were analyzed for bulk carbon and nitrogen isotopic compositions. These stingrays were a subset of the 156 individuals examined for SCA and represented the entire geographic distribution within Kāneʻohe Bay, full size range and sex of juvenile stingrays. There was no significant effect of sex on bulk $\delta^{13}C$ (p = 0.578) or $\delta^{15}N$ (p = 0.774) values (Table 2). Disk width and location explained 51 and 52% of the variance for $\delta^{13}C$ and $\delta^{15}N$ values, respectively. Of the total explained variance, 17 and 25% was explained by size and 33 and 26% was explained by location for $\delta^{13}C$ and $\delta^{15}N$ values, respectively (Table 2, Fig. 5). Stingrays captured in the south part of Kāneʻohe Bay were, on average, more depleted in ^{13}C (1.4‰, p < 0.001) and more enriched in ^{15}N (1.2‰, p < 0.002) compared with stingrays captured in the mid and north parts of the bay (Table 3, Fig. 5). There were significant differences between size classes for $\delta^{13}C$ values (GLM: $F_{2, 39}$ = 4.17, p = 0.023) and $\delta^{15}N$ values

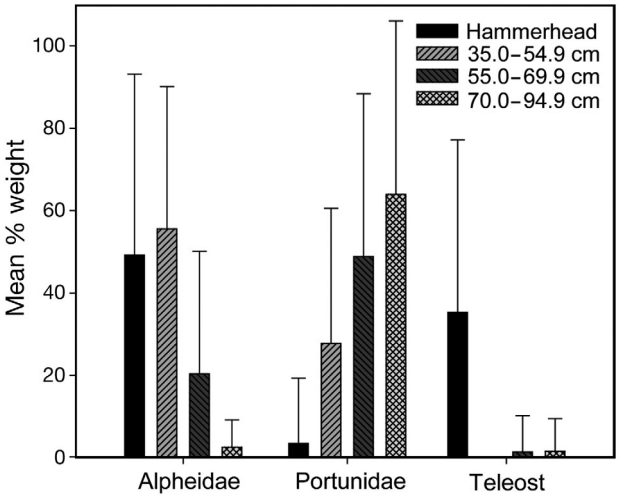

Fig. 4. *Dasyatis lata* and *Sphyrna lewini*. Gravimetric contribution (+1 SD) of the prey groups primarily responsible for differences in dietary composition between the 3 size classes of brown stingrays and juvenile scalloped hammerhead sharks. Juvenile scalloped hammerhead dietary data were reanalyzed from Bush (2003)

Table 2. *Dasyatis lata*. Results of GLM evaluating differences in juvenile brown stingray bulk $\delta^{13}C$ and $\delta^{15}N$ values between bay zones (location) and sex, with size (disk width) as a covariate

Source	df	$\delta^{13}C$				$\delta^{15}N$			
		SS	MS	*F*	p	SS	MS	*F*	p
Disk width	1	9.296	11.737	17.33	<0.001	14.469	5.612	8.04	0.007
Location	2	18.154	9.077	13.40	<0.001	15.053	7.526	10.78	<0.001
Sex	1	0.849	0.213	0.31	0.578	0.276	0.058	0.08	0.774
Error	39	26.420	0.677			27.229	0.698		
Total	43	54.719				57.026			

($F_{2, 39}$ = 4.74, p = 0.015). Small stingrays were more depleted in ^{13}C (p = 0.018) and ^{15}N (p = 0.013) compared with large stingrays (Table 3, Fig. 5). There were no significant differences between small and medium stingrays or between medium and large stingrays. For TP calculations, $\delta^{15}N$ values of stingrays captured in the south part of the bay were normalized by 1.2‰ to correct for spatial variation. Trophic positions for stingray size classes based on bulk nitrogen isotopic values were in close agreement to those based on SCA and increased with increasing stingray size (range: 3.3 to 3.6, Table 3).

Differences in dietary composition between juvenile scalloped hammerhead sharks and juvenile brown stingrays were also supported by bulk SIA. Juvenile hammerhead sharks were significantly more depleted in ^{13}C (mean $\delta^{13}C$ = –16.41 ± 0.34; ANOVA: $F_{3, 48}$ = 89.94, p < 0.001) and more enriched in ^{15}N (mean $\delta^{15}N$ = 11.83 ± 0.60; ANOVA: $F_{3, 48}$ = 15.61, p < 0.001) compared with all stingray size classes (stable isotopic values of brown stingrays and juvenile scalloped hammerhead sharks caught in the south bay normalized for spatial variation). The TP of juvenile hammerhead sharks based on bulk nitrogen isotopic values was 4.1, ~0.6 TPs higher than that of juvenile stingrays (south bay samples normalized for spatial variation).

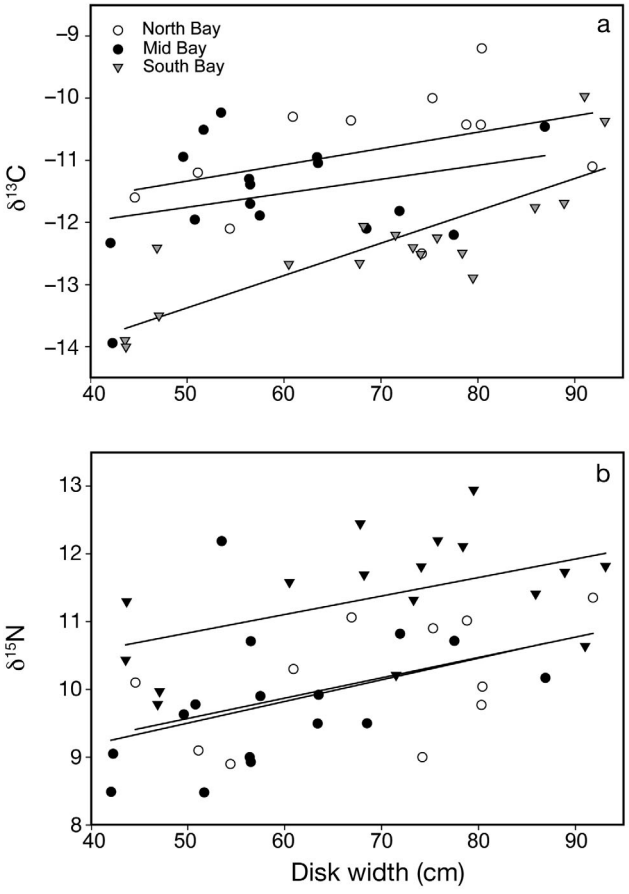

Fig. 5. *Dasyatis lata*. Effect of size and capture location of juvenile brown stingrays on bulk (a) $\delta^{13}C$ values (‰) and (b) $\delta^{15}N$ values (‰)

Stable nitrogen isotope analysis of individual amino acids

An additional 23 muscle samples were collected from very large (>95 cm DW) brown stingrays captured both within and outside Kāneʻohe Bay. Inclusion of these samples revealed a dramatic decrease in bulk tissue $\delta^{15}N$ values, and onset of this decrease varied by sex (Fig. 6). There are 2 competing hypotheses that could explain the observed decrease in $\delta^{15}N$ values: (1) stingrays of all sizes are feeding in habitats that are isotopically similar and the observed decrease in $\delta^{15}N$ values is due to very large stingrays changing to food that is on average 0.7 TPs lower than that of large (70 to 94.9 cm DW) juvenile stingrays, or (2) very large stingrays are feeding in a habitat with basal nitrogen isotopic values distinct (lower) from those within Kāneʻohe Bay. To test these hypotheses, we analyzed the $\delta^{15}N$ values of individual amino acids from 11 individuals representing the entire DW range that was used to determine the TP of brown stingrays. Based on this analysis, TP increased in very large stingrays compared with that in juvenile stingrays. This result is contrary to the trend in bulk tissue results and unambiguously supports the second hypothesis, namely that very large stingrays are feeding in a habitat isotopically different from Kāneʻohe Bay (Fig. 7).

Calculations of TP from amino acid nitrogen isotopic analyses based on a TEF of 7.6‰ and a $\Delta\delta^{15}N_{Glu/Phe}$ of 3.4‰ consistently underestimated absolute TP for brown stingrays by ~1 (9 out of 11 samples had a TP <3) and juvenile scalloped hammerhead sharks by

Table 3. *Dasyatis lata*. Mean and SD of juvenile brown stingray muscle bulk $\delta^{13}C$ and $\delta^{15}N$ for the 3 size classes by bay zone. Trophic position estimates of each size class for all zones combined provided

Size class (Disk width) (cm)	Bay zone: South $\delta^{13}C$	SD	$\delta^{15}N$	SD	Mid $\delta^{13}C$	SD	$\delta^{15}N$	SD	North $\delta^{13}C$	SD	$\delta^{15}N$	SD	Trophic position[a]
35.0–54.9	−13.45	0.73	10.37	0.68	−11.65	1.39	9.6	1.38	−11.63	0.45	9.37	0.64	3.3
55.0–69.9	−12.66	0.34	11.91	0.47	−11.48	0.43	9.64	0.61	−10.33	0.04	10.68	0.54	3.5
70.0–94.9	−11.85	0.96	11.62	0.18	−11.49	0.91	10.57	0.35	−10.61	1.12	10.35	0.89	3.6

[a]Trophic position calculations based on corrected bulk $\delta^{15}N$ values for stingrays captured in the south bay (see 'Results')

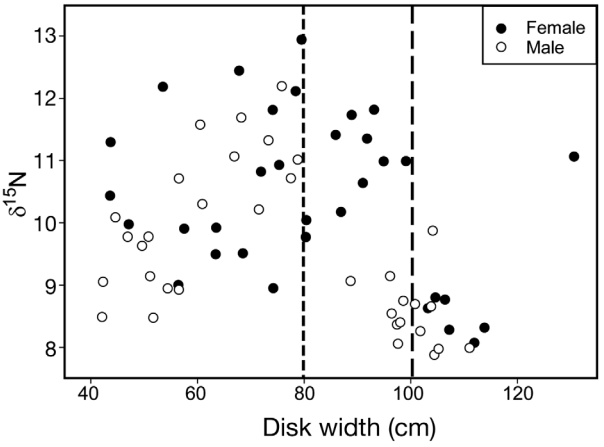

Fig. 6. *Dasyatis lata*. Effect of size on bulk $\delta^{15}N$ values (‰) for juvenile and adult brown stingrays captured both within and outside Kāneʻohe Bay. Vertical lines (males: short dash; females: long dash) represent approximate size of stingrays at sexual maturity (J. Dale unpubl. data)

~0.7 compared with independent TP estimates based on SCA and bulk SIA (Tables 1, 3 & 4). Based on the close agreement of TP estimates from SCA and bulk SIA, TP estimates from amino acid analysis were increased by 1 (stingrays) and 0.7 (hammerhead sharks) and a new TEF for each individual sample was calculated with a $\Delta\delta^{15}N_{Glu/Phe}$ of 3.4‰. New TEFs ranged from 4.1 to 5.9‰ with an overall mean of 5.0‰ (±0.6‰ SD, Table 4), which is 2.6‰ less than the 7.6‰ found by Chikaraishi et al. (2009). Trophic positions calculated with the new TEFs ranged from 3.2 for a 43.6 cm DW brown stingray to 4.2 for a 110.0 cm DW stingray and 3.9 to 4.1 for juvenile scalloped hammerhead sharks (Fig. 7, Table 4).

DISCUSSION

Foraging ecology

The diet of juvenile brown stingrays was dominated by crustaceans, particularly alpheid shrimps and portunid crabs. Brown stingrays primarily use the deep lagoonal portions of Kāneʻohe Bay, which are characterized by muddy substrate (Smith et al. 1981, Cartamil et al. 2003). Previous studies suggest that these mud habitats are of low productivity and faunal diversity and are dominated by alpheids, polychaetes, gobies and portunids (Harrison 1981, Smith et al. 1981, Smith & Kukert 1996, Bush 2003). Collectively, these results suggest that juvenile brown stingrays are opportunistic benthic foragers that use the most abundant prey resources of the Kāneʻohe Bay benthos.

These findings are consistent with dietary studies of dasyatid rays in other locations (Hess 1961, Struhsaker 1969, Gilliam & Sullivan 1993, Taniuchi & Shimizu 1993, Ismen 2003). Alpheids are a common prey item for dasyatids from Florida and the eastern Mediterranean Sea (Snelson & Williams 1981, Yeldan et al. 2009), whereas portunids were common in the diets of dasyatids from the central Bahamas and the western Atlantic Ocean (Struhsaker 1969, Gilliam & Sullivan 1993). Site-specific differences in prey abundances probably contribute to a lower importance of crustaceans for some dasyatid rays. For example, poly-

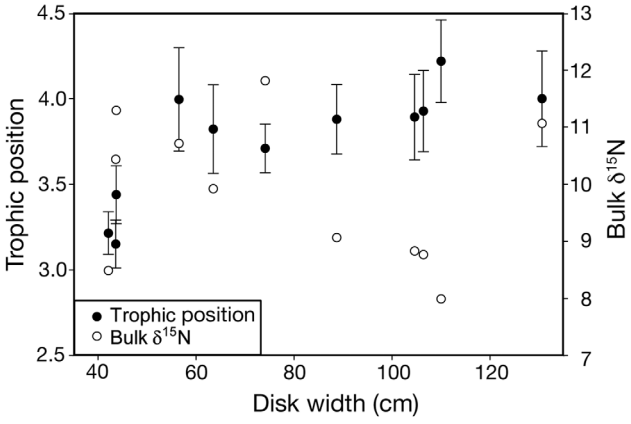

Fig. 7. *Dasyatis lata*. Relationship between size, amino acid-based trophic position (±1 SD of propagated error) and bulk $\delta^{15}N$ values (‰) for juvenile and adult brown stingrays captured both within and outside Kāneʻohe Bay and analyzed for the nitrogen isotopic composition of individual amino acids. Trophic position estimates are based on recalculated trophic enrichment factors for each individual (see 'Results')

Table 4. *Dasyatis lata* and *Sphyrna lewini*. $\delta^{15}N$ values of glutamic acid (Glu) and phenylalanine (Phe) for 11 stingrays (S1–S11) and 3 juvenile scalloped hammerhead sharks (JSH1–JSH3). Sizes are reported as disk width for stingrays and fork length for hammerhead sharks. TP1: trophic position estimated from $\delta^{15}N$ values Glu and Phe using the trophic enrichment factor (TEF) of 7.6‰ from Chikaraishi et al. (2009); see 'Materials and methods' for details. TP2: revised trophic position estimated by increasing TP1 by 1 (stingrays) and 0.7 (hammerhead sharks) trophic positions; see 'Results' for details. TEF2: recalculated TEF for each individual sample based on their corresponding TP2 value

Sample no.	Size (cm)	Bulk $\delta^{13}C$	Bulk $\delta^{15}N$	$\delta^{15}N$ Glu (±1 SD)	$\delta^{15}N$ Phe (±1 SD)	$TP1_{Glu/Phe}$ (±1 SD)	$TP2_{Glu/Phe}$	TEF2
S1	43.6	–13.9	10.4	18.1 ± 0.3	6.0 ± 0.3	2.2 ± 0.1	3.2	4.1
S2	43.7	–13.9	11.3	18.1 ± 0.3	3.8 ± 0.5	2.4 ± 0.2	3.4	4.5
S3	74.1	–12.5	11.8	22.1 ± 0.3	5.8 ± 0.1	2.7 ± 0.1	3.7	4.8
S4	104.6	–15.7	8.8	20.2 ± 0.7	2.4 ± 0.4	2.9 ± 0.2	3.9	5.0
S5	106.4	–16.7	8.8	20.6 ± 0.7	2.5 ± 0.1	2.9 ± 0.2	3.9	5.0
S6	42.1	–12.3	8.5	18.5 ± 0.1	5.8 ± 0.2	2.2 ± 0.1	3.2	4.2
S7	60.0	–11.7	10.7	21.6 ± 0.8	3.0 ± 0.5	3.0 ± 0.3	4.0	5.1
S8	110.0	–15.9	8.0	19.1 ± 0.3	–1.2 ± 0.6	3.2 ± 0.2	4.2	5.2
S9	130.6	–13.7	11.1	21.9 ± 0.6	3.3 ± 0.6	3.0 ± 0.3	4.0	5.1
S10	63.5	–11.0	9.9	19.1 ± 0.2	1.9 ± 0.8	2.8 ± 0.3	3.8	4.9
S11	88.7	–16.1	9.1	20.5 ± 0.5	2.8 ± 0.4	2.9 ± 0.2	3.9	5.0
JSH1	36.0	–15.9	13.6	26.1 ± 0.3	4.4 ± 0.8	3.4 ± 0.3	4.1	5.9
JSH2	37.5	–15.9	12.3	25.2 ± 0.4	5.2 ± 0.9	3.2 ± 0.3	3.9	5.8
JSH3	38.5	–16.7	13.0	25.1 ± 0.2	3.2 ± 0.4	3.4 ± 0.2	4.1	5.9

chaetes were important prey items for dasyatids from West and South Africa in habitats where polychaetes were abundant (Devadoss 1978, Ebert & Cowley 2003). Additionally, teleosts contributed significantly to the diets of dasyatids from the central Bahamas, West Africa and Japan (Devadoss 1978, Gilliam & Sullivan 1993, Taniuchi & Shimizu 1993).

The dietary composition of juvenile brown stingrays varied with stingray size. Although crustaceans were the dominant prey for all size classes, the relative contribution of different prey items varied. Small stingrays primarily fed on alpheids and to a lesser degree on the portunid crab, *Libystes villosus* and polychaetes. These are all small prey items, probably easy prey for small stingrays. As brown stingrays increase in size, the contribution of alpheids to their diet decreases substantially (<10% IRI for large stingrays), and a corresponding increase occurs in the contribution of portunids, particularly *Podophthalmus vigil* (>50% IRI for large stingrays). Larger stingrays also fed more evenly on potential prey items as indicated by an increase in dietary breadth with increasing size. The ontogenetic dietary shift indicated by stomach content analysis was supported by bulk and compound-specific stable isotope analyses. Both bulk tissue $\delta^{13}C$ and $\delta^{15}N$ values and the TP based on amino acid $\delta^{15}N$ values increased with increasing size, which is consistent with a dietary shift from smaller, lower trophic level prey (alpheids and polychaetes) to larger, higher trophic level prey (*P. vigil* and gobies). Ontogenetic diet shifts are common for elasmobranchs and these shifts have been linked to morphological, behavioral and physiological characteristics (Scharf et al. 2000, Wetherbee & Cortés 2004, Lucifora et al. 2006). In this study, a positive correlation between the size of *P. vigil* consumed and brown stingray morphology suggests that the abundance of small prey items in the diet of small stingrays is largely due to gape limitations. Ingestion of less abundant but larger, more energetically valuable prey may decrease the amount of time and energy larger stingrays spend foraging (Scharf et al. 2000) and would limit competition with smaller conspecifics. Lucifora et al. (2006) suggested that small sharks have higher consumption rates, and therefore a lower proportion of empty stomachs, owing to higher mass-dependent metabolic rates. However, small brown stingrays had the largest percentage of empty stomachs in the present study, potentially owing to slow development of foraging skills as has been suggested for newborn scalloped hammerhead sharks in Kāneʻohe Bay (Lowe 2002, Bush 2003).

Trophic position calculations based on stomach content and bulk SIA were in close agreement, classifying juvenile brown stingrays as secondary consumers. To calculate stingray TP from bulk $\delta^{15}N$ values, we assumed a TEF of 2.7‰. However, several studies have indicated significant variation in TEFs owing to variables such as species, tissue type, diet, environment, method of excretion and biochemical composition (Pinnegar & Polunin 1999, Vander Zanden & Rasmussen 2001, McCutchan et al. 2003, Vanderklift & Ponsard 2003, MacNeil et al. 2006, Barnes et al. 2007, Caut et al. 2009). Although TEFs have not yet been experimentally determined for stingrays, Hussey et al. (2010a) determined a TEF of 2.3 ± 0.2‰ for 3 adult sand tiger sharks *Carcharias taurus* and 1 subadult

lemon shark *Negaparion brevirostris* in a long-term (12 mo) controlled feeding experiment. However, the concordance of TP estimates between stomach content and stable isotope methods in our study supports the assumed ecosystem-level TEF of 2.7‰. Stomach content and bulk tissue SIA both indicated an increase in TP with increasing size for juvenile brown stingrays, although these increases were generally small (0.3 to 0.4 TPs). This small increase is predominantly driven by stingrays shifting to larger, higher trophic level prey while retaining crustaceans as their primary prey resource. There is little information on TPs for other stingray species. A TP of 3.7 was estimated for marbled stingrays *Dasyatis marmorata* from the Mediterranean Sea and skates primarily feeding on decapods had TPs ranging from 3.5 to 3.9, values similar to those observed in this study (Stergiou & Karpouzi 2002, Ebert & Bizzarro 2007).

Habitat use

Bulk SIA indicated a significant effect of capture location on stingray $\delta^{13}C$ and $\delta^{15}N$ values. Brown stingrays captured in the southern part of Kāneʻohe Bay were consistently depleted in ^{13}C and enriched in ^{15}N compared with stingrays caught in the mid and north bay. A lack of spatial variation in juvenile brown stingray diet composition suggests stable isotopic differences are due to variability in baseline values between bay zones. Variation in baseline values is supported by the independence of stingray bulk tissue $\delta^{15}N$ values and TPs calculated from amino acid isotopic analysis, which indicates stingray bulk tissue $\delta^{15}N$ values are controlled primarily by variations in baseline $\delta^{15}N$ values within the bay. The south part of Kāneʻohe Bay is characterized by relatively long water residence times (13 d versus 8 d for the remainder of the bay), receives 30% of the stream runoff entering the bay and is bordered by relatively high levels of human population (Smith et al. 1981). In addition, sediments may still be heavily affected by treated sewage dumped into the south bay from 1951 to 1978. Recent analyses showed that high rates of dissolved inorganic nitrogen efflux continue to support macroalgae growth within the south bay (Smith et al. 1981, Stimson et al. 2001). Cumulatively, these factors probably contribute substantially to the low $\delta^{13}C$ and high $\delta^{15}N$ values found in brown stingrays captured in the south part of the bay because freshwater and anthropogenic inputs can decrease $\delta^{13}C$ and increase $\delta^{15}N$ values, respectively, in coastal ecosystems (McClelland & Valiela 1998, Vizzini et al. 2005). These results highlight the importance of understanding spatial variability of basal stable isotopic values for foraging ecology studies, even in relatively small ecosystems. Our results show that incorporation of stable isotopic analysis of individual amino acids can provide the information necessary for gaining insight into the underlying causes of variations in bulk tissue $\delta^{15}N$ values.

Stable isotope analysis has been used to determine residence time and detect small-scale migration within and between habitats for a variety of fishes (e.g. Cocheret de la Morinière et al. 2003). Despite relatively long nitrogen isotopic turnover times for muscle in elasmobranchs (>1 yr, MacNeil et al. 2006, Logan & Lutcavage 2010), we were able to detect both site fidelity within and recent migrations between bay zones. Capture location for the majority of stingrays sampled during this study could be identified by their bulk stable isotopic values, indicating long-term foraging in that area. In a previous study, brown stingrays actively tracked in the south part of Kāneʻohe Bay remained in the area for the duration of the study (>3 d, Cartamil et al. 2003). However, some stingrays displayed stable isotopic values distinctly different from their capture location suggesting recent migration between bay zones. Alternative methods such as passive acoustic tracking and/or SIA that use tissues with faster turnover times (e.g. blood, liver) might be required to more accurately describe the movement patterns of brown stingrays throughout the bay.

Analysis of individual amino acids in stingray muscle samples allowed us to evaluate competing hypotheses generated from bulk SIA and calculate TPs for brown stingrays (including very large stingrays from offshore habitats) and juvenile scalloped hammerhead sharks without prior knowledge of the $\delta^{15}N$ values of primary producers. Sampling of all potential primary producers in offshore habitats would have been challenging owing to the depths at which very large stingrays typically occur (>40 m). Trophic positions based on recalculated TEFs (stingrays range: 3.2 to 4.2; hammerhead sharks range: 3.9 to 4.1) were consistent with those based on stomach content and bulk SIA. The increase in stingray TP based on amino acid analysis was independent of bulk $\delta^{15}N$ values (especially evident for very large stingrays) and confirmed a foraging habitat shift from inside Kāneʻohe Bay to offshore waters. The onset of this shift coincides closely with the onset of sexual maturity. Males mature and migrate to offshore habitats at a smaller size than do females (Fig. 6, J. Dale unpubl. data).

Heupel et al. (2007) proposed 3 criteria for defining an area as a nursery. (1) Juveniles are more commonly encountered in the nursery than other areas. In our case, juvenile brown stingrays were rarely captured during ongoing longline fishing surveys outside Kāneʻohe Bay (J. Dale unpubl. data). (2) Juveniles have a tendency to remain or return for extended periods.

Results from SIA suggest that juvenile brown stingrays forage within Kāneʻohe Bay for the majority of their juvenile lives. (3) The habitat is repeatedly used across years. Juvenile stingrays were captured across multiple years during this study indicating repeated use of the bay habitat. Thus, based on the criteria proposed by Heupel et al. (2007), Kāneʻohe Bay is an important nursery habitat for brown stingrays.

The relative ontogenetic and interspecific variation in TPs calculated from amino acid analysis, regardless of the TEF used, was consistently represented for brown stingrays and juvenile scalloped hammerhead sharks in the present study and further demonstrates the value of this method for understanding the foraging ecology of elasmobranchs. All elasmobranchs are carnivores, requiring a minimum TP of 3.0 (Wetherbee & Cortés 2004). However, in the present study, initial calculations of absolute TPs systematically underestimated TP by ~1 TP for stingrays (range: 2.2 to 3.2) and ~0.7 TP for juvenile scalloped hammerhead sharks (range: 3.2 to 3.4). Assuming the $\Delta\delta^{15}N_{Glu/Phe}$ of 3.4‰ is correct (Chikaraishi et al. 2009), we estimated an average TEF of 5.0 ± 0.6‰ for brown stingray and scalloped hammerhead shark muscle. A previous study that used stable isotopic compositions of amino acids found a TEF of 3.6‰ more accurately represented penguin TPs (Lorrain et al. 2009). That difference was attributed to the use of blood samples for isotopic analysis, whereas earlier studies had used whole animal or muscle tissue (McClelland & Montoya 2002, Chikaraishi et al. 2007, 2009, Popp et al. 2007, Hannides et al. 2009).

The use of muscle in the present study precludes tissue biochemical effects as a viable explanation for the lower observed TEF from amino acid analysis. Alternatively, lower observed TEFs in brown stingray and juvenile scalloped hammerhead shark muscle may be due to lower reaction rates involving glutamic acid as a consequence of urea retention for osmotic regulation (Evans et al. 2004). This has been suggested from elasmobranch bulk tissue isotopic analysis (e.g. Fisk et al. 2002, Hussey et al. 2010b) and is consistent with retention of ^{15}N-depleted waste products. Trophic enrichment of glutamic acid in elasmobranchs is more difficult to explain but probably results from its frequent use in transamination and deamination reactions (Gannes et al. 1998, Chikaraishi et al. 2007). Glutamate can be converted to α-ketoglutarate by glutamate dehydrogenase (GDH) for use in the citric acid cycle (Ballantyne 1997) or converted to glutamine by glutamine synthetase to provide nitrogen to the ornithine–glutamine–urea pathway (Anderson 1991). Activity levels of GDH provide a measure of importance of amino acids as an oxidative substrate and a strong inverse relationship was found between hepatic GDH and urea levels in elasmobranch muscle (Speers-Roesch et al. 2006). Increased importance of the glutamate–glutamine–urea pathway could result in lower glutamate catabolism (Speers-Roesch et al. 2006) and reduced ^{15}N enrichment in glutamic acid in muscle tissue. Elasmobranchs use a unique carbamoyl phosphate synthetase (CPSase III) that utilizes glutamine in the pool of free amino acids in liver mitochondrial cells as the nitrogen-donating substrate rather than ammonia for urea formation (Julsrud et al. 1998). We speculate that reduced hepatic glutamate catabolism resulted in lower ^{15}N enrichment of glutamic acid in muscle tissue of brown stingrays and juvenile scalloped hammerhead sharks. Nitrogen isotope fractionation associated with cleavage of the amide functional group of glutamine should be smaller than that associated with cleavage of the single amine group of glutamate because glutamine has 2 nitrogen atoms and only 1 nitrogen bond is broken. Therefore we suggest that ^{15}N enrichment is lower in glutamic acid exiting the liver to form muscle protein in elasmobranchs with high muscle urea concentrations and perhaps in other animals that excrete urea (or uric acid) as a waste product.

These results suggest that urea retention in elasmobranchs can have important impacts on metabolic pathways involving glutamic acid that could influence TPs determined with amino acid compound-specific isotopic analyses. Consequently, TP estimates based on a TEF of 7.6‰ estimated from non-ureosmotic species would result in an underestimation of absolute stingray and hammerhead shark TPs. However, in the only other study to use nitrogen isotopic analysis of individual amino acids to estimate TP for an elasmobranch, Chikaraishi et al. (2010) estimated a TP of 4.8 for a *Squalus* sp. using a TEF of 7.6‰. This is 0.6 to 0.9 TPs higher than TPs estimated from stomach content studies for this genus (3.9 to 4.2, Cortés 1999). It is known that amino acids rather than lipids can be important oxidative substrates in elasmobranch muscle tissue (Ballantyne 1997), thus in addition to ureagenesis, TEF could vary with relative animal activity. For example, the higher TEF we found for juvenile scalloped hammerhead sharks could be due to lower urea production in the liver or higher muscle activity in hammerhead sharks relative to brown stingrays. These contradictory results emphasize the need to further investigate the relationship between urea content in elasmobranchs, hepatic GDH activity, animal activity and the extent of ^{15}N enrichment in glutamic acid for white muscle tissue. In order to adapt to hypotonic environments, freshwater elasmobranchs retain low levels of urea (Thorson et al. 1967, Tam et al. 2003) and express higher levels of hepatic GDH activity than do marine elasmobranchs (Speers-Roesch et al. 2006).

Therefore, future experimental studies should also focus on both marine and freshwater species to evaluate the potential effects of urea on amino acid TEFs.

Ecological interactions

Significant differences exist in the foraging ecology of brown stingrays and juvenile scalloped hammerhead sharks, which suggests available prey resources are partitioned between these 2 species in Kāneʻohe Bay. Differences between diets were primarily due to a larger contribution of teleosts to the diet of juvenile hammerhead sharks and a larger contribution of portunids to the diet of juvenile stingrays. A larger contribution of teleosts to the hammerhead shark diet is also supported by bulk tissue and amino acid stable isotope analysis. Hammerhead shark bulk muscle tissue was enriched in ^{15}N by ~1.5‰ compared with juvenile stingray muscle, and the amino acid based TP was higher by ~0.5, indicating that the hammerhead sharks are feeding higher in the food web. These dietary differences can potentially be explained by differences in mouth morphology and prey capture behavior between species. Brown stingrays have larger mouths than juvenile scalloped hammerhead sharks, which would allow them to forage on larger prey such as portunids. The prey capture behavior of brown stingrays is similar to that described for the blue stingray *Dasyatis chrysonota*, Ebert & Cowley 2003). Once a prey item is located, the stingray quickly settles upon the substrate, trapping prey under its body, which is followed by suction of prey into the mouth (J. Dale pers. obs.). Although this foraging mechanism is well suited for benthic prey, it may limit the stingrayʼs ability to catch faster, more mobile prey such as teleost fish. In contrast, the use of ram feeding by the hammerhead sharks would facilitate capture and ingestion of teleosts. Similar mechanisms were suggested to explain low dietary overlap between a rhinobatid ray and sharks in an Australian bay (White et al. 2004).

CONCLUSIONS

Brown stingrays use Kāneʻohe Bay as a nursery ground and, while present, tend to show restricted movements within subsections of the bay. Stingrays move out of the bay when they become sexually mature although some adults are occasionally found within the bay. Juvenile brown stingrays are generalist benthic predators whose diets reflect the low diversity of potential prey items in Kāneʻohe Bay. Ontogenetic shifts in dietary composition are correlated with mouth size, with larger individuals able to forage on larger, more energetically valuable prey. Future studies focusing on the growth rates and energetics of brown stingrays would provide further insight into their role in ecosystem energy flow and their effect on prey populations (e.g. Lowe 2002). This study demonstrated that analysis of the nitrogen isotopic composition of individual amino acids is a viable method for detecting ontogenetic foraging habitat shifts and determining relative trophic positions for elasmobranchs. However, our results suggest that the TEF used to calculate absolute TP in previous studies requires adjustment for elasmobranchs, potentially owing to retention of urea as an osmolyte and its effect on biochemical reactions involving glutamic acid. Controlled experiments are required to better understand the effect of urea on the metabolic pathways of amino acids, particularly glutamic acid. Prey resource partitioning between brown stingrays and juvenile scalloped hammerhead sharks in Kāneʻohe Bay indicate competition for prey resources is not a factor contributing to high mortality rates of hammerhead shark pups.

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