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Trophic behaviour of juvenile reef fishes inhabiting interlinked mangrove–seagrass habitats in offshore mangrove islets

A. Vaslet*†, D. L. Phillips‡, C. A. M. France§, I. C. Feller‖ and C. C. Baldwin¶

*Smithsonian Marine Station at Fort Pierce, 701 Seaway Dr., Fort Pierce, FL, 34949, U.S.A., ‡U.S. Environmental Protection Agency, National Health & Environmental Effects Research Laboratory, Western Ecology Division, 200 SW 35th St, Corvallis, OR, 97333, U.S.A., §Smithsonian Museum Conservation Institute, 4210 Silver Hill Rd, Suitland, MD, 20746, U.S.A., ‖Smithsonian Environmental Research Center, Edgewater, MD, 21037, U.S.A. and ¶National Museum of Natural History, Smithsonian Institution, P. O. Box 37012, Washington, DC, 20013-7012, U.S.A.

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Stable isotope (δ^{13}C and δ^{15}N) and gut content analyses were used to investigate size-related feeding habits of four reef fishes (the beaugregory *Stegastes leucostictus*, the french grunt *Haemulon flavolineatum*, the schoolmaster snapper *Lutjanus apodus* and the yellowtail snapper *Ocyurus chrysurus*) inhabiting an offshore (non-estuarine) mangrove islet off Belize, Central America. Comparisons of isotopic niche space and Schoener diet similarity index suggested a low to moderate degree of niche overlap between fish size groups. The δ^{13}C gradient between mangrove and seagrass prey as well as results of Bayesian mixing models revealed that sampled fishes relied mostly on seagrass prey items. Only small and large juveniles of the carnivorous species *L. apodus* derived a part of their diet from mangroves by targeting mangrove-associated Grapsidae crabs and fish prey, respectively. Isotopic niche shifts were particularly obvious for carnivorous fishes that ingested larger prey items (Xanthidae crabs and fishes) during their ontogeny. The utilization of mangrove food resources is less than expected and depends on the ecology and life history of the fish species considered. This research highlights that mangrove-derived carbon contributed relatively little to the diets of four fish taxa from an offshore mangrove islet.

Key words: fish foraging habitats; gut contents; marine ecosystems; SIAR; SIBER; stable isotopes.

INTRODUCTION

In tropical and subtropical environments, many juvenile reef fishes settle in shallow, back-reef ecosystems such as mangroves and seagrass beds that are often described as shelters and nurseries (Beck *et al.,* 2001; Adams *et al.,* 2006). These nursery grounds create favourable conditions enhancing the density, survival and growth rates of juveniles (Beck *et al.,* 2001). Nursery habitats are particularly attractive

†Author to whom correspondence should be addressed. Tel.: +00 590 690 248 461;
email: amandine.vaslet@gmail.com

to juveniles due to their structural complexity that limits predation and provides a diverse array of food sources and trophic niches (Laegdsgaard & Johnson, 2001). Along a mangrove–seagrass–reef continuum, reef fishes can be separated into several sub-populations with different size and biomass distributions. The presence of extensive and pristine mangroves in the vicinity of coral reefs enhances the biomass of adult reef fishes (Mumby *et al.,* 2004), thus revealing the influence of this nursery habitat on the structure of fish communities. Besides a change of habitats, juvenile reef fishes can experience a shift in resource use during their ontogeny, also known as ontogenetic diet shift (Cocheret de la Morinière *et al.,* 2003; Hammerschlag-Peyer *et al.,* 2011). These ontogenetic trophic shifts are not necessarily triggered by migrations towards adult habitats and can occur in back-reef habitats (Kimirei *et al.,* 2013). In this context, mangroves and seagrass meadows are expected to support feeding activities of some reef fish taxa during their juvenile stages (Cocheret de la Morinière *et al.,* 2003; Nagelkerken *et al.,* 2008).

In the Caribbean, mangroves and seagrass beds have been the subject of numerous studies investigating fish trophic behaviours and the relative importance of these back-reef habitats as feeding areas (Cocheret de la Morinière *et al.,* 2003; Kieckbusch *et al.,* 2004; Nagelkerken *et al.,* 2008; Vaslet *et al.,* 2012*a*). Most of these studies focused on nursery species, defined as reef fishes (*e.g.* Scaridae, Haemulidae and Lutjanidae) that spend their early life stages in nursery habitats (Cocheret de la Morinière *et al.,* 2002). Previous studies highlighted a limited contribution of mangrove food resources in fish diets, particularly for the nocturnally active carnivorous species such as the french grunt *Haemulon flavolineatum* (Desmaret 1823), the schoolmaster snapper *Lutjanus apodus* (Walbaum 1792) and the yellowtail snapper *Ocyurus chrysurus* (Bloch 1791) (Nagelkerken & van der Velde, 2004*a, b*). As they grow, carnivorous reef fishes, including grunts and snappers, consume larger prey items and increase their foraging range and trophic level (Cocheret de la Morinière *et al.,* 2003; Kimirei *et al.,* 2013). As these trophic shifts can occur at relatively small sizes (*i.e.* <5 cm, total length, L_T) (Kimirei *et al.,* 2013), it is important to assess size-related trophic behaviours of juvenile reef fishes in their back-reef habitats.

Previously considered as homogeneous habitats, mangroves differ in terms of physiochemical environment, landscape structure or physical configuration (*e.g.* fringing or riverine mangroves, dwarf mangroves and mangrove islets) (Gilmore & Snedaker, 1993). Mangrove configurations and hydrographic variables (such as tides) may influence fish feeding behaviours. In the Indian and Pacific Oceans, strong tidal currents dry or isolate mangrove areas, thus limiting consumers' access to and reliance on mangrove resources (Sheaves & Molony, 2000; Lugendo *et al.,* 2007). Even if Caribbean mangroves are flooded and accessible most of the time, differences in habitat configuration (*i.e.* fringing mangroves, ponds and islets) and hydrography affect the contribution of mangrove-derived organic matter in consumers' diets (Granek *et al.,* 2009; Vaslet *et al.,* 2012*a*). With these renewed investigations on the influence of habitat configurations on fish trophic habits, most Caribbean field research has focused on fringing mangroves, mangrove ponds, bays or lagoons, and has pooled relatively wide size ranges of juvenile fish specimens (small, medium and large individuals) (Cocheret de la Morinière *et al.,* 2003; Nagelkerken & van der Velde, 2004*b*; Vaslet *et al.,* 2012*a*). Less consideration has been given to offshore mangrove islets, which comprise fringing mangrove forests that are flooded by high tides and receive minimal terrestrial inputs (Gilmore & Snedaker, 1993).

The present approach combines gut-content and stable-isotope analyses (SIA) in order to infer the origin of the organic matter consumed by fish species in offshore mangrove islets. Gut content analyses give a snapshot of a diet and are more often paired with SIA that provide temporally integrated information on prey assimilated by a consumer (Post, 2002; Cocheret de la Morinière *et al.*, 2003; Nagelkerken & van der Velde, 2004*a*). Significant differences of stable carbon-isotope ratios between mangrove and seagrass food resources can be used as proxies to reflect the habitat origin of carbon sources assimilated by consumers (Hemminga & Mateo, 1996; Bouillon *et al.,* 2008). In the ecological niche concept defined by Hutchinson (1978), SIA are also likely to provide information on bionomic axes (defined as species food resources) and scenopoetic axes (the species living habitats), with the δ-space representing species 'isotopic niche' (Newsome *et al.,* 2007). In this study, the foraging habitats of four reef fishes, *H. flavolineatum, L. apodus, O. chrysurus* and the beaugregory *Stegastes leucostictus* (Müller & Troschel 1848), were investigated from offshore mangrove islets in Belize. Fish specimens were separated into four size categories (adults, small, medium and large juveniles), gut content analysis and SIA were performed on fishes and their potential food sources (algae and animal prey items) to infer the diet composition and the foraging habitats of these reef fishes. Bayesian mixing models are used to assess inter and intraspecific variation in fish diet and to provide integrated metrics on species isotopic niche (Bearhop *et al.,* 2004; Jackson *et al.,* 2011). This research aimed (1) to investigate size-related and interspecific dietary shifts of juvenile reef fishes in an offshore mangrove islet and (2) to infer potential ontogenetic changes in resource and habitat use.

MATERIALS AND METHODS

STUDY SITE

The study was conducted at Twin Cays, a 92 ha archipelago of two mangrove islands located 2 km from the Belize barrier reef and 12 km off the coast of central Belize (16° 50′ N; 88° 06′ W; Fig. 1). These islets receive freshwater or terrigenous runoff from the mainland only during extreme climate events such as hurricanes (Koltes & Opishinski, 2009). Seasonality is thus not heavily marked in Twin Cays islets as the islets receive less terrestrial inputs compared with fringing mangroves from the mainland. An s-shaped channel separates West and East Islands, which are characterized by a complex network of channels and ponds (Fig. 1; Rützler *et al.,* 2004). The seaward vegetation of Twin Cays is dominated by red mangroves *Rhizophora mangle* (trees of 5–6 m high). The interior of the islands is dominated by dwarf *R. mangle* (1·0–1·5 m tall), with white mangroves *Laguncularia racemosa* and black mangroves *Avicennia germinans* in scattered locations (Woodroffe, 1995). The sediment bottom around Twin Cays is mainly covered by turtle grass *Thalassia testudinum* (Rützler *et al.,* 2004). Water salinity around the islands is oceanic (35–36) (Rützler *et al.,* 2004).

SAMPLE COLLECTION

From February to June 2010, four fish species, *S. leucostictus* (20 specimens), *H. flavolineatum* (43 specimens), *L. apodus* (51 specimens) and *O. chrysurus* (49 specimens), were sampled in the mangroves of Twin Cays using fishing lines, fish traps and hand-nets (Fig. 1). The examination of fish gonads and a literature review of length at maturity enabled separation of juveniles and adults (Table I; Cervigón, 1993; Froese & Pauly, 2013). Except for adult specimens of *S. leucostictus* (L_T: 9·0–9·6 cm) and *O. chrysurus* (L_T: 18·0–20·4 cm), all the fishes caught were juveniles and were sorted into size categories as small, medium and large juveniles (Table I

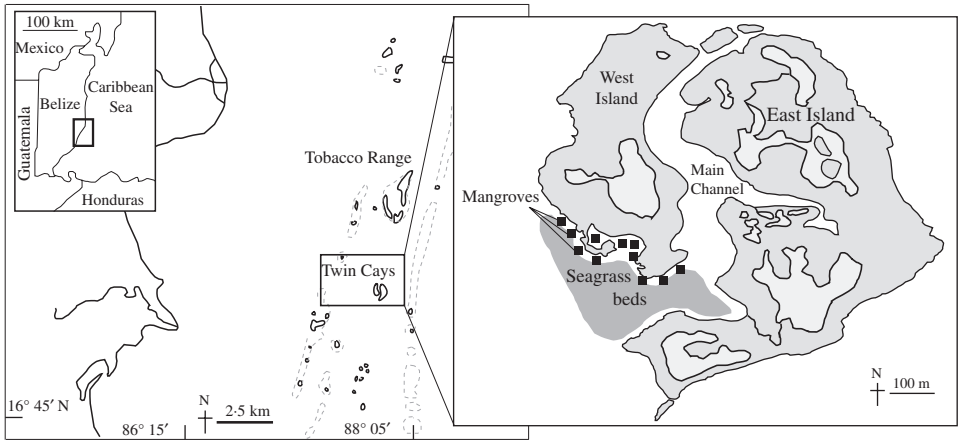

FIG. 1. Location of mangroves (■) and seagrass beds (■) sampled in the offshore islets of Twin Cays in Belize.

and Fig. S1, Supporting Information). Except for adults of *S. leucostictus* with nine specimens, each fish size category includes >10 specimens (Table I). For each fish specimen, gut content analyses were employed to identify ingested prey to the lowest possible taxonomic level. To estimate the relative contribution of each type of prey identified in the fish diet, the index of relative importance (I_{RI}) was calculated using the percentage of number (N_B), gravimetric values (M_D, dry mass) and frequency of occurrence (O) of the different food sources following the equation: $I_{RI} = (N_B + M_D)O$ (MacDonald & Green, 1983). The I_{RI} enables the separation of main prey ($I_{RI} > 50\%$), secondary prey ($25\% < I_{RI} < 50\%$) and rare prey ($I_{RI} < 25\%$) that were identified in the fish gut contents. These plant and animal prey items were then collected in mangroves (MAN) and nearby seagrass beds (SG) to assess fish foraging habitats (Fig. 1). Mangrove prop-root algae and filamentous algae from seagrass beds were sampled by hand. Microphytobenthos, composed of benthic motile microalgae, was collected by scraping the surface layer of the sediment that has been collected and spread in trays under light for at least 24 h (Riera & Richard, 1996). Zooplankton was sampled with a 0·1 mm mesh plankton net towed in the top 2 m of water. Benthic invertebrates (annelids and crustaceans) were collected with hand-nets and light traps. At least three samples were prepared for each food prey item, except for Mysidacea from mangroves and filamentous algae, zooplankton from seagrass beds for which two samples were collected and provided sufficient amount for SIA. Schoener's index (I_S) was used to assess dietary overlap between fish size classes (Schoener, 1968): $I_S = 1 - 0 \cdot 5 \sum |P_{iA} - p_{iB}|$, where p_{iA} and p_{iB} are the relative proportions of prey item$_i$ for fish size groups A and B. Index values $I_S > 0{\cdot}6$ indicate significant dietary overlap (Schoener, 1968).

SIA AND MIXING MODELS

Carbon and nitrogen isotope ratios were analysed to assess the origin of carbon food sources and fish trophic level, respectively. Isotope ratios were expressed in conventional delta notation (δ^{13}C and δ^{15}N) relative to standard references (Vienna pee-dee belemnite for δ^{13}C and atmospheric N_2 for δ^{15}N). SIA were performed on dorsal muscles of fishes, on soft tissue of crabs and shrimps after dissection from their exoskeleton and on the entire body of small prey (*i.e.* amphipods and annelids). For amphipods, sub-samples dedicated to δ^{13}C analyses were decalcified with HCl. Samples were washed, dried, homogenized and weighed into tin cups. SIA were performed at Smithsonian OUSS/MCI Stable Isotope Mass Spectrometry Laboratory (Suitland, MD) using a Thermo Scientific DeltaV isotope-ratio mass spectrometer (Thermo Scientific; www.thermoscientific.com) coupled to a C-N-S Costech Elemental Analyser (Costech Analytical Technologies; www.costechanalytical.com). Raw values were corrected to calibrated internal standards (acetanilide and urea). Repeated measurements of

TABLE I. Carbon and nitrogen stable-isotope values (mean ± S.D.) of fish species and their prey items sampled in Twin Cays mangrove islets. Fish codes consist of the first letter of the genus and the first three letters of the species name. Codes for size categories: S, small juveniles; M, medium juveniles; L, large juveniles; A, adults. *n*, number of samples; L_T, range of fish total length; L_M, length at maturity reported from the literature (Cervigón, 1993; Froese & Pauly, 2013)

Fish and prey items	Code	Size category	L_T (cm)	*n*	δ^{13}C (‰)	δ^{15}N (‰)
Omnivorous fishes						
Stegastes leucostictus	Sleu	S	4·0–4·6	11	−12·5 ± 0·3	7·1 ± 0·4
(L_M = 7·5 cm)	Sleu	A	9·0–9·6	9	−12·9 ± 0·3	7·2 ± 0·4
First-level carnivorous fishes (invertebrate feeder)						
Haemulon flavolineatum	Hfla	S	4·3–6·0	24	13·7 ± 0·4	6·2 ± 0·3
(L_M = 16·0 cm)	Hfla	M	11·0–12·4	19	−12·6 ± 0·4	7·0 ± 0·3
Second-level carnivorous fishes (invertebrate and fish feeder)						
Lutjanus apodus	Lapo	S	5·0–6·4	16	−14·6 ± 0·4	6·1 ± 0·4
(L_M = 26·0 cm)	Lapo	M	8·8–11·2	19	−13·5 ± 0·4	6·9 ± 0·5
	Lapo	L	15·2–19·1	16	−14·2 ± 0·5	7·7 ± 0·3
Ocyurus chrysurus	Ochr	S	8·1–10·2	19	−12·3 ± 0·2	5·8 ± 0·3
(L_M = 15·5 cm)	Ochr	M	11·5–12·7	14	−12·2 ± 0·3	6·2 ± 0·2
	Ochr	A	18·0–20·4	16	−12·6 ± 0·2	7·8 ± 0·3
Mangrove prey items						
Mangrove prop-root algae				5	−20·9 ± 0·5	1·3 ± 0·2
Filamentous algae	Filam. algae			4	−17·8 ± 0·1	−1·2 ± 0·4
Microphytobenthos	Microphyt.			3	−16·5 ± 0·1	0·6 ± 0·2
Crustacea larvae				3	−21·4 ± 0·6	6·4 ± 0·7
Mysidacea				2	−14·1 ± 0·6	5·8 ± 0·7
Zooplankton	Zoopl.			4	−17·7 ± 0·1	0·2 ± 0·1
Amphipods				4	−16·3 ± 0·7	3·5 ± 0·3
Annelids				3	−15·8 ± 0·3	3·7 ± 0·2
Xanthidae				3	−16·7 ± 0·9	5·1 ± 0·5
Grapsidae				3	−21·9 ± 0·1	3·7 ± 0·1
Penaidae				3	−17·6 ± 1·4	6·1 ± 0·5
Harengula humeralis	Hhum		12·7–13·0	3	−14·4 ± 0·9	8·1 ± 0·2
Haemulon aurolineatum	Haur		8·0–10·2	3	−13·2 ± 0·2	7·8 ± 0·3
Seagrass prey items						
Filamentous algae	Filam. algae			2	−13·1 ± 0·4	3·1 ± 0·1
Mysidacea				3	−13·9 ± 0·4	6·2 ± 0·9
Zooplankton	Zoopl.			2	−12·5 ± 0·1	2·9 ± 0·1
Amphipods				5	−12·4 ± 0·1	2·4 ± 0·1
Annelids				3	−12·6 ± 0·3	2·4 ± 0·2
Xanthidae				4	−14·6 ± 0·9	4·7 ± 0·9
Porcellanidae				4	−12·8 ± 0·2	3·7 ± 0·4
Hippolytidae	Hippol.	Small		3	−12·9 ± 0·1	4·1 ± 0·1
	Hippol.	Large		3	−13·9 ± 0·1	4·7 ± 0·1

internal standards showed a precision of ±0·2‰ for both δ^{13}C and δ^{15}N. Because the variation of lipid content in organism tissues affects δ^{13}C values and thus ecological interpretations, a mathematical normalization method was used to standardize lipid content in samples (Post *et al.,* 2007): $\delta^{13}C_{corrected} = \delta^{13}C_{untreated} - 3 \cdot 32 + 0 \cdot 99C:N$ (Fig. S1, Supporting Information). δ^{13}C values were corrected based on C:N ratios (*i.e.* C:N ratios >3·5 indicating lipid-rich tissue) and lipid contents in animal samples (Post *et al.,* 2007).

The mixing model Stable Isotope Analysis in R (SIAR; Parnell *et al.*, 2010) was used to estimate the relative contribution of mangrove *v.* seagrass prey items in the fish diet. SIAR model is based on a series of related linear equations that utilizes Bayesian inference to identify proportional contributions of food resources to consumer diet. The Bayesian approach incorporates numerous factors (multiple dietary sources, uncertainties or concentration dependence) in the model and generates true probability distributions (Parnell *et al.*, 2010; Phillips *et al.*, 2014). Therefore, SIAR accounts for uncertainty associated with specimen variability, numerous food resources, trophic enrichment and increases the ability to generate accurate estimates of source contributions (Parnell *et al.*, 2010).

For the omnivorous fish species, *S. leucostictus*, the model incorporates concentration dependence correction of food sources to consider difference in the C and N content of algal and animal prey (Phillips & Koch, 2002; Vaslet *et al.,* 2011). The assimilated carbon and nitrogen concentrations of each source were estimated using the source's digestibility reported in the literature and the elemental concentrations ([C] and [N]) measured in this study, following the methods outlined by Koch & Phillips (2002). *A posteriori* combinations of source proportions were determined as described by Phillips *et al.* (2005) to provide composite contributions of mangrove prey (D_{MAN}), seagrass prey (D_{SG}) and fish prey (D_F) to the fish diet. Prior to modelling, isotopic values of consumers (*i.e.* four reef fishes) were adjusted for trophic fractionation (Δ^{13}C and Δ^{15}N) following values from the literature reported for fishes (muscle tissue) feeding on either low (algae and plant material) or high (animal prey) protein content diets (McCutchan *et al.,* 2003; Mill *et al.,* 2007; Caut *et al.,* 2009): $\Delta^{13}C = 0{\cdot}8 \pm 0{\cdot}3$‰ and $\Delta^{15}N = 5{\cdot}4 \pm 0{\cdot}6$‰ for *S. leucostictus*; $\Delta^{13}C = 0{\cdot}7 \pm 0{\cdot}3$‰ and $\Delta^{15}N = 3{\cdot}3 \pm 0{\cdot}2$‰ for the three carnivorous fish species (Fig. S1, Supporting Information).

Comparisons of isotopic trophic niches between fish size groups were performed using Stable Isotope Bayesian Ellipses in R (SIBER; Jackson *et al.,* 2011). To determine core isotopic niche estimates, SIBER generates standard ellipse areas (S_{EA}) containing the central 40% of the data. S_{EA} were corrected to minimize bias caused by small sample sizes (S_{EAC}) and were used to calculate the degree of isotopic niche overlap. To use the same criteria as in the Schoener index (Schoener, 1968), isotopic niche overlaps were considered significant for values >60%. To obtain C.I. of isotopic niche areas, Bayesian estimates of S_{EA} were used, termed S_{EAB} in ‰^{2}, that incorporate greater uncertainty associated with small and unequal sample sizes (Jackson *et al.,* 2011; Syväranta *et al.,* 2013). The total convex hull area (T_A) method (convex polygon around the outermost isotope data) was used to consider the variability of stable-isotope values measured for fish species (Hammerschlag-Peyer *et al.,* 2011; Jackson *et al.,* 2011). Sequential steps performed on stable-isotope data and in SIAR and SIBER Bayesian models are summarized in the Fig. S1 (Supporting Information).

STATISTICAL ANALYSES

Principal component analysis (PCA) was performed on I_{RI} values of fish prey to investigate fish diet patterns and feeding habitats among fish size classes (de Crespin de Billy *et al.,* 2000). A hierarchical agglomerative cluster (Euclidean distance and Ward linkage) was performed on the co-ordinates produced by the factorial analysis and the three groups identified were reported on PCA biplot (Legendre & Legendre, 1998). Parametric *t*-tests were used when data met the assumptions of normality and homogeneity of variances (Shapiro–Wilks and Levene's tests). One-way ANOVAs were performed to test statistical differences in δ^{13}C and δ^{15}N values between fish size classes, except for δ^{15}N values of *S. leucostictus*. In this case, a Mann–Whitney *U*-test followed by *post hoc* Bonferroni correction was used because data did not meet assumptions of normality despite applying transformations (Legendre & Legendre, 1998). Statistical analyses (significance level of $P < 0{\cdot}05$), PCA, SIAR and SIBER packages were performed using R software (R project for Statistical Computing; www.r-project.org).

RESULTS

FISH DIETS

Histograms of I_{RI} values by size class within four fish species give the proportions of different prey taxa in the fish diets (Fig. 2). These histograms, along with I_S values, reveal the degree of dietary overlap between fish size categories (Fig. 2). A significant dietary overlap was observed between juvenile and adult specimens of *S. leucostictus* ($I_S = 0{\cdot}820$), which mainly foraged on filamentous algae ($I_{RI} > 60\%$) and secondly on crustacean larvae and benthic invertebrates (polychaetes and amphipods) [Fig. 2(a)]. With the two first axes explaining 88·1% of the variance, a PCA biplot provided a good assessment of the variation in diet composition between fish species and size classes (Fig. 3). Along the second axis of the PCA, *S. leucostictus* specimens were well separated from other carnivorous fish species due to their omnivorous diet consisting of both algal and invertebrate prey (Fig. 3). As confirmed by low Schoener index values ($I_S = 0{\cdot}080$; *i.e.* $I_S < 60\%$), no dietary overlap was observed between small and medium juveniles of *H. flavolineatum* [Fig. 2(b)]. Small juveniles of the carnivorous species *H. flavolineatum*, *L. apodus* and *O. chrysurus* consumed more small invertebrates (*i.e.* amphipods, annelids and zooplankton) compared to large juveniles which consumed Xanthidae crabs, Hippolytidae shrimps and fishes [Figs 2(b)–(d) and 3]. Among second-level carnivorous species, the higher values of Schoener's index ($I_S > 60\%$) observed between small and medium juvenile *L. apodus* ($I_S = 0{\cdot}639$) and *O. chrysurus* ($I_S = 0{\cdot}905$) revealed significant dietary overlap for these size categories [Figs 2(c), (d) and 3]. Large juveniles of *L. apodus* and adults of *O. chrysurus* consumed higher proportions of fish prey and had a more diverse diet than small and medium juveniles [Figs 2(c), (d) and 3]. This result was clearly observed along the first axis of the PCA that separated small carnivorous specimens feeding on small invertebrates on one side of the axis, and large and adult specimens consuming both invertebrates and fish prey on the other side of the axis (Fig. 3). Dietary analyses enabled separation of fishes into three trophic guilds (Figs 2 and 3): the omnivore *S. leucostictus* that feeds on both filamentous algae and small invertebrates; first-level carnivore (*H. flavolineatum*) foraging on invertebrates and second-level carnivores (*L. apodus* and *O. chrysurus*) consuming both invertebrates and fishes.

STABLE-ISOTOPE RATIOS OF CONSUMERS AND THEIR PREY

The δ^{13}C and δ^{15}N values (mean ± S.D.) were highly variable among fish food sources [Fig. 4(a)–(d) and Table I]. Mangrove prey items had ^{13}C-depleted carbon values ($\delta^{13}C_{mean} = -18{\cdot}0 \pm 0{\cdot}7$‰) compared with seagrass prey ($\delta^{13}C_{mean} = -13{\cdot}2 \pm 0{\cdot}3$‰) (Mann–Whitney *U*-test, d.f. = 54, $P < 0{\cdot}001$). A significant increase in δ^{15}N values was observed between primary producers and consumers (*t*-test, d.f. = 53, $P < 0{\cdot}001$): algae from mangroves and seagrass beds had the most ^{15}N-depleted values ($\delta^{15}N_{mean} = 0{\cdot}7 \pm 1{\cdot}0$‰), compared with invertebrates without decapods ($\delta^{15}N_{mean} = 2{\cdot}4 \pm 0{\cdot}5$‰), and decapods (crabs and shrimps, $\delta^{15}N_{mean} = 4{\cdot}7 \pm 0{\cdot}4$‰). Two fish species, the redear herring *Harengula humeralis* (Cuvier 1829) and the tomtate grunt *Haemulon aurolineatum* Cuvier 1830, were observed in the diets of the carnivorous fishes *O. chrysurus* and *L. apodus*, and had the most ^{15}N-enriched values among all fish food sources ($\delta^{15}N_{mean} = 8{\cdot}0 \pm 0{\cdot}2$‰) (*t*-tests, d.f. = 59, $P < 0{\cdot}001$) [Fig. 4(c), (d) and Table I].

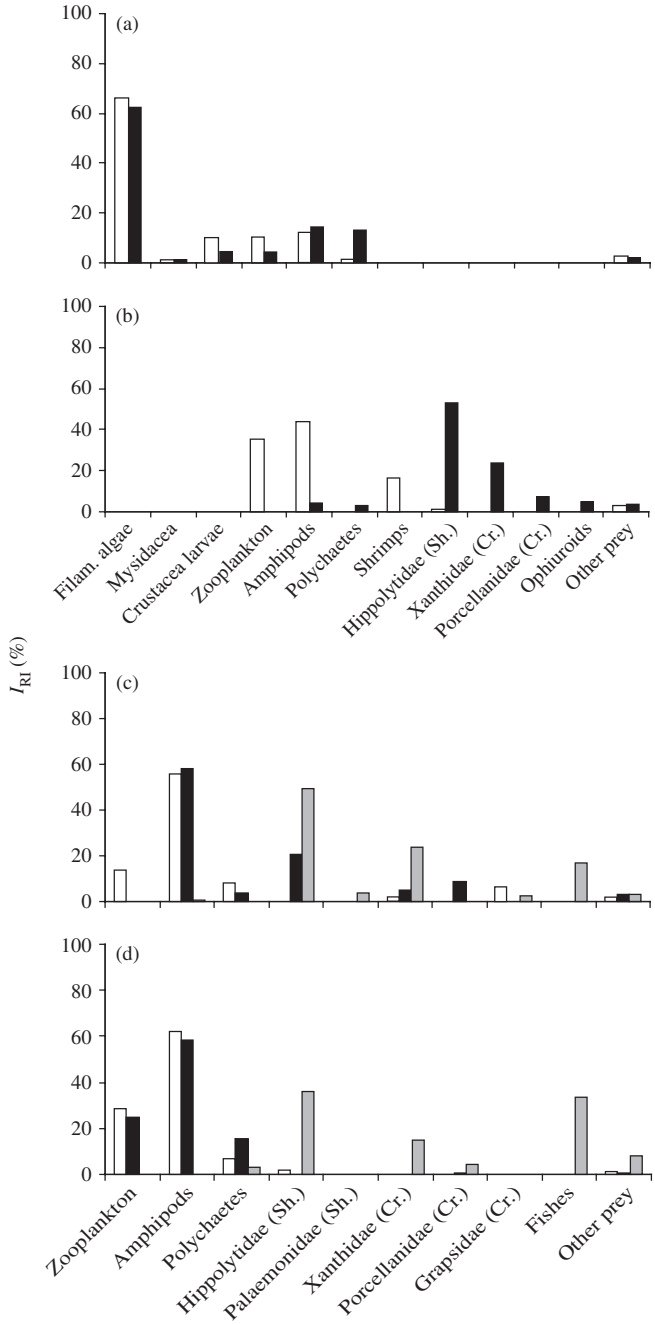

FIG. 2. Index of relative importance (I_{RI}) values of fish prey items identified in the gut contents of (a) *Stegastes leucostictus* (□, 11 small juveniles; ■, 13 adult specimens), (b) *Haemulon flavolineatum* (□, 22 small juveniles; ■, 18 adults), (c) *Lutjanus apodus* (□, 15 small specimens; ■, 17 medium juveniles; ▒, 15 adults) and (d) *Ocyurus chrysurus* (□, 15 small specimens; ■, 11 medium juveniles; ▒, 11 adults). Other prey, unidentified prey and prey with $I_{RI} < 2{\cdot}5\%$; Filam. algae, filamentous algae; Sh., shrimps; Cr., crabs.

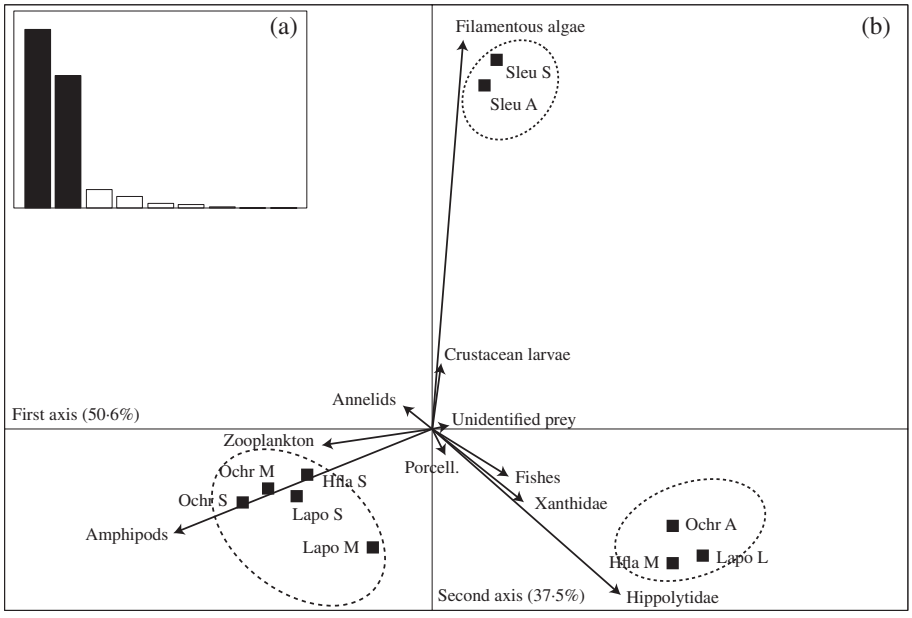

FIG. 3. Principal component analysis (PCA) biplot of fish size classes based on index of relative importance (I_{RI}) values of their prey items. (a) Histogram of eigenvalues (■ the two first values; □, remaining eigenvalues). (b) Distribution of fish diet composition (■) according to their prey (→). Groups from the hierarchical cluster are reported on the biplot. Prey with I_{RI} values < 9% are not plotted. Porcell., Porcellanidae crabs. Four-letter fish codes are given in Table I.

Fish species had wide ranges of carbon isotopic ratios, with values varying from −16·6‰ for small juveniles of *L. apodus* to −11·1 ‰ for medium juveniles of *H. flavolineatum* (Table I). For the omnivorous species *S. leucostictus*, no differences in carbon (one-way ANOVA, $F_{1,18} = 3{\cdot}63$, $P > 0{\cdot}05$) and nitrogen (Mann–Whitney *U*-test, d.f. = 18, $P > 0{\cdot}05$) isotopic values were observed between juvenile and adult specimens (Table II). For carnivorous fishes, significant differences in δ^{13}C and δ^{15}N values were observed between fish size classes (Table II). Small juveniles of *H. flavolineatum* had lower δ^{13}C and δ^{15}N ratios compared with medium juveniles (one-way ANOVA, $F_{1,41} = 17{\cdot}7$, $P < 0{\cdot}001$) (Table II). A similar pattern was observed for *L. apodus*, with small specimens characterized by lower δ^{13}C and δ^{15}N ratios compared with medium juveniles (one-way ANOVA, $F_{1,48} = 14{\cdot}0$, $P < 0{\cdot}05$; Table II). When comparing isotopic values among the three size classes of second-level carnivores, δ^{15}N values were significantly higher for large juveniles of *L. apodus* (one-way ANOVA, $F_{1,48} = 14{\cdot}75$, $P < 0{\cdot}05$) and for adults of *O. chrysurus* (one-way ANOVA, $F_{1,46} = 48{\cdot}56$, $P < 0{\cdot}001$) compared with small and medium juveniles of these two species (Table II). For *O. chrysurus* specimens, *post hoc* comparisons showed no difference in δ^{13}C values between the three size classes (Table II).

SIAR AND SIBER MODELS

SIAR mixing models showed a high reliance of all size classes of fishes on food sources from seagrass beds. Mean contribution values (95% Bayesian credibility intervals, BCI) of seagrass prey ranged from 47·0% (34·9–59·2%) for small juveniles

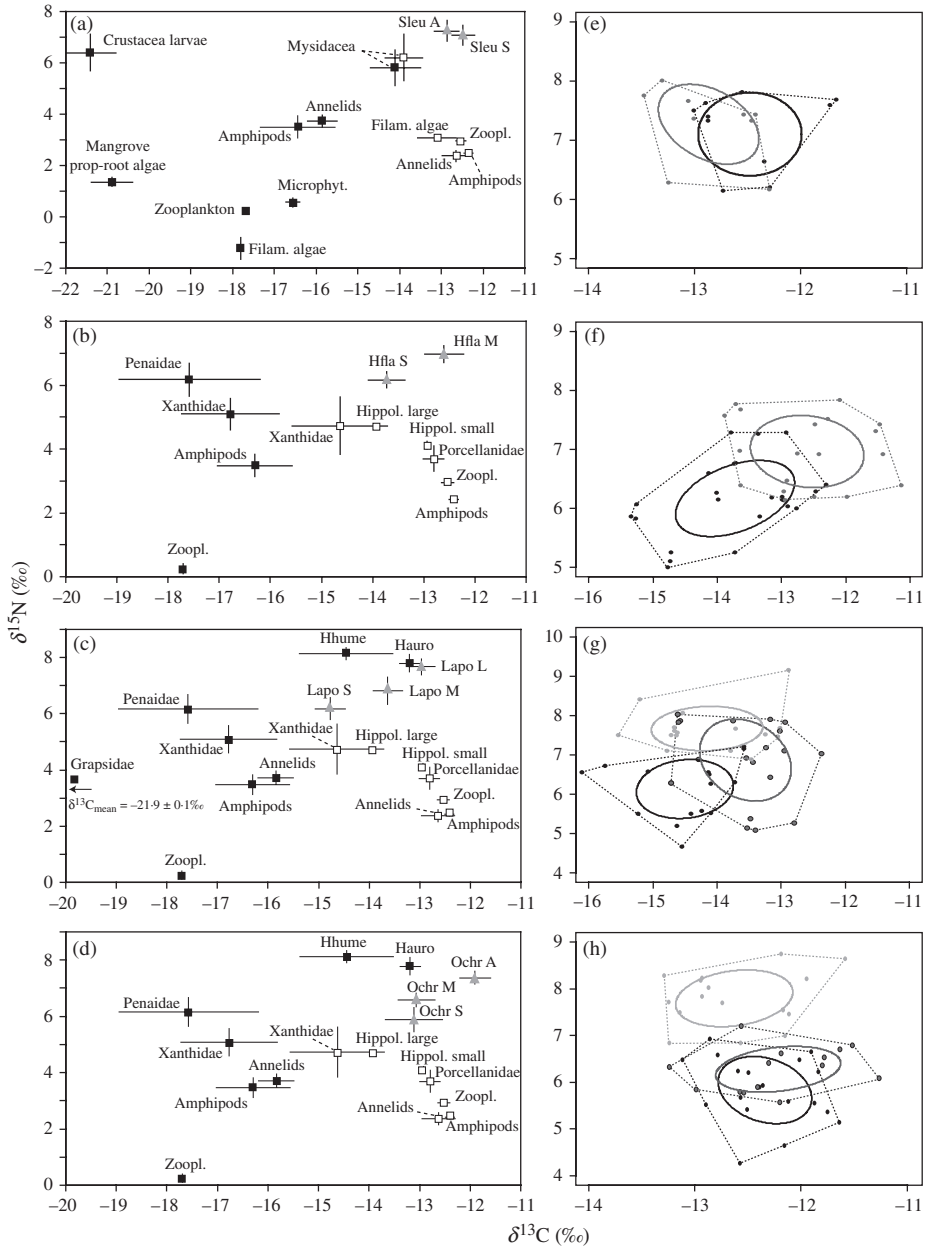

FIG. 4. Stable-isotope biplots of mean ± S.D. δ^{13}C and δ^{15}N values of the four fish species (▲) and prey items from mangroves (■) and seagrass beds (□): (a) *Stegastes leucostictus*, (b) *Haemulon flavolineatum*, (c) *Lutjanus apodus* and (d) *Ocyurus chrysurus*. δ^{13}C and δ^{15}N biplots of all fish specimens with convex hulls of total niche width (T_A, ...) and standard ellipse area corrected for small samples (S_{EAC}, ——): (e) *Stegastes leucostictus* (●, adults; ●, small juveniles), (f) *Haemulon flavolineatum* (●, small juveniles ; ●, medium juveniles) (g) *Lutjanus apodus* (●, small juveniles; ●, medium juveniles; ●, large juveniles), (h) *Ocyurus chrysurus* (●, small juveniles ; ●, medium juveniles; ●, adults). Four-letter fish codes, fish size groups, prey items, T_A and S_{EAC} values are given in Tables I and III. Note the different scales on *x*- and *y*-axes between biplots.

TABLE II. Results of ANOVAs and Mann–Whitney U-test for δ^{13}C, δ^{15}N and fish size classes. Four-letter fish codes are given in Table I

	δ^{13}C						
	One-way ANOVA			δ^{15}N	One-way ANOVA		
Fish species	F	P	Tukey HSD tests	Mann–Whitney U-test	F	P	Tukey HSD tests
Stegastes leucostictus							
Sleu S–Sleu A	3·63	>0·05		0·68			
Haemulon flavolineatum							
Hfla S–Hfla M	17·07	**<0·001**			17·76	<0·001	
Lutjanus apodus	7·09	<0·01**			14·75	<0·001	
Lapo S–Lapo M			0·001**				0·025*
Lapo S–Lapo L			0·408				0·013*
Lapo M–Lapo L			0·058				<0·001***
Ocyurus chrysurus	2·70	>0·05			48·56	<0·001***	
Ochr S–Ochr M							0·103
Ochr S–Ochr A							<0·001***
Ochr M–Ochr A							<0·001***

*$P < 0·05$; **$P < 0·01$; ***$P < 0·001$.

of *L. apodus* to 86·1% (79·5–92·2%) for small juveniles of *O. chrysurus* (Table III). Examination of 95% BCI and BCI overlap between mangrove and seagrass prey contributions revealed that small and large juveniles of *L. apodus* appeared to forage in both mangroves and seagrass areas (Table III). Seagrass prey items showed the highest contribution ranges and seagrass beds were therefore considered as important feeding areas for all the studied reef fishes except for the two size classes of *L. apodus* (Table III). Large juveniles of *L. apodus* and adult specimens of *O. chrysurus* showed a higher reliance on fish prey items (Table III).

Small and medium juveniles of *H. flavolineatum* showed similar isotopic trophic niche metrics with mean S_{EAB} (95% BCI) varying between 1·88‰^{2} (1·18–2·67‰^{2}) and 1·87‰^{2} (1·10–2·73‰^{2}), respectively (Table III). The same trend was observed between juvenile and adult specimens of *S. leucostictus* and between the three size categories of *O. chrysurus* [Fig. 4(e), (h) and Table III]. Isotopic niche overlaps recorded between juvenile and adult specimens of *S. leucostictus* and between small and medium juveniles of *O. chrysurus* were relatively high (49·1 and 47·7%, respectively) but not significant [Fig. 4(e), (h) and Table III]. Isotopic niche widths varied significantly between the three size groups of *L. apodus* with medium juveniles characterized by higher T_A, S_{EAC} and S_{EAB} values (Table III). Among the situations where S_{EAC} overlapped, only the isotopic niche overlap between medium and large juveniles of *L. apodus* was deemed significant (threshold of 60%) [Fig. 4(g) and Table III].

DISCUSSION

FISH FEEDING HABITATS

Reef fishes sheltering in mangrove habitats during their juvenile stage appear to derive most of their carbon resources from prey items occurring in nearby seagrass beds

TABLE III. Mean contributions (95% Bayesian credibility intervals, BCI) of food sources in fish diets. Prey items were grouped as mangrove, seagrass and fish prey items. Main contributions are shown in bold. Fish size range in total length (L_T)

Fish species	SIAR, mean food source contributions (95% BCI)			SIBER, isotopic trophic niche metrics			
Fish code: L_T (cm)	Mangrove prey	Seagrass prey	Fish prey	T_A (‰^{2})	S_{EAC} (‰^{2})	S_{EAC} overlap (%)	S_{EAB} (‰^{2})
Stegastes leucostictus							
Small juvenile (S)	25·5 (13·6–38·2)	**74·4 (61·8–86·3)**		1·43	1·08	S–A = 49·1	1·51 (0·73–2·44)
Adult (A)	32·3 (20·3–44·9)	**67·6 (55·0–79·6)**		1·49	0·93		1·52 (0·66–2·57)
Haemulon flavolineatum							
Small juvenile (S)	35·7 (26·4–45·1)	**64·2 (54·8–73·5)**		4·11	1·71	M–L = 13·5	1·88 (1·18–2·67)
Medium juvenile (M)	14·8 (5·4–24·6)	**85·1 (75·3–94·6)**		3·7	1·69		1·87 (1·10–2·73)
Lutjanus apodus							
Small juvenile (S)	**48·0 (36·7–59·3)**	**47·0 (34·9–59·2)**	4·8 (0·2–10·3)	3·93	2·13	S–M = 26·5	2·29 (1·26–3·43)
Medium juvenile (M)	29·4 (19·2–39·8)	**61·9 (49·4–74·3)**	8·6 (0·9–16·6)	6·16	2·75	M–L = 66·5	2·81 (1·65–4·11)
Large juvenile (L)	**37·9 (27·0–48·8)**	**47·2 (35·2–59·5)**	14·8 (6·4–23·1)	4·78	1·85	S–L = 0	2·10 (1·16–3·18)
Ocyurus chrysurus							
Small juvenile (S)	10·8 (5·4–16·3)	**86·1 (79·5–92·2)**	3·1 (0·1–6·9)	2·50	0·97	S–M = 47·7	1·25 (0·74–1·84)
Medium juvenile (M)	12·7 (4·5–21·8)	**83·6 (73·3–92·6)**	3·7 (0·2–7·9)	2·06	0·88	M–L = 0	1·24 (0·66–1·91)
Adult (A)	12·8 (5·3–20·4)	**66·3 (55·1–77·5)**	21·0 (12·0–29·7)	2·34	1·03	S–L = 0	1·33 (0·74–1·98)

SIAR, stable-isotope analysis in R; SIBER, stable-isotope Bayesian ellipses in R. T_A, total convex hull area; S_{EAC}, standard ellipse area corrected for small samples; S_{EAB}, mean and 95% BCIs for standard ellipse area.

(Nagelkerken & van der Velde, 2004*a*; Vaslet *et al.,* 2012*a*; this study). This ecological trend is generally acknowledged for fringing mangroves where fishes have permanent access to adjacent habitats, such as seagrass meadows. But fish feeding habits can be site specific and depend on habitat configuration. Studies conducted in Indo-Pacific (Sheaves & Molony, 2000; Lugendo *et al.,* 2007) and Caribbean regions (Vaslet *et al.,* 2012*a*) have shown that fish species derive a substantial part of their diet from mangrove prey items when they remain in mangrove ponds, creeks or basin mangroves. Thus, mangrove contributions to fish diets vary depending on the habitat configuration and hydrologic features. This is particularly true for offshore mangrove islets, such as Twin Cays, where fish species from semi-enclosed mangrove ponds rely on mangrove prey, whereas fishes from fringing mangroves mostly feed in seagrass beds (Vaslet *et al.,* 2012*a, b*; this study). The limited contribution of mangrove prey to fish diets is more likely to be due to the chemical composition of mangrove organic carbon that has low nutritional value and high concentrations of refractory compounds, such as lignin-derived phenols or tannins (Kristensen *et al.,* 2008).

ONTOGENIC TROPHIC SHIFTS OF CARNIVOROUS FISHES

Carbon isotopic ratios of mangrove and seagrass prey items in Twin Cays were in the range of values previously reported, with a clear δ^{13}C gradient between mangrove prey (δ^{13}C on average < -15‰) and seagrass food sources (δ^{13}C > -15‰) (Hemminga & Mateo, 1996; Bouillon *et al.,* 2008). This significant segregation among fish food resources enables assessment of fish foraging habitats. The present research revealed several ontogenetic trophic shifts among the different size classes and the different species of the sampled reef fishes. The trophic study indicated that small and medium juveniles of *H. flavolineatum*, *L. apodus* and *O. chrysurus* fed mainly on small seagrass invertebrates (*i.e.* zooplankton and amphipods), whereas large juveniles and adult specimens fed predominantly on larger seagrass prey, such as decapods and prey fishes. This resource partitioning was particularly obvious along the δ^{15}N axis that showed an increase of δ^{15}N values with fish size due to the consumption of fish prey of higher trophic levels (Cocheret de la Morinière *et al.,* 2003; this study). These ontogenetic trophic shifts to larger prey are often observed for carnivorous species and can occur prior to ontogenetic habitat shifts (Kimirei *et al.,* 2013). Survival rates and predation risks affecting juvenile fishes control their nursery-to-reef migrations, even if coral reefs are more suitable in terms of food diversity and abundance (Kimirei *et al.,* 2013). Cocheret de la Morinière *et al.* (2003) hypothesized that this diet shift occurs when large fish specimens or sub-adults increase their foraging distance and find different prey assemblages. These authors also emphasized that these nursery fishes are more likely to find coral-reef habitats when they expand their foraging areas.

CARBON SOURCES SUPPORTING *L. APODUS* DIET DURING ONTOGENY

Most of the studied reef fishes foraged on seagrass prey items, but stomach content analyses and SIA indicated that small and large juveniles of *L. apodus* also derive a part of their diet from mangroves. The δ^{13}C values increased between small and medium juveniles of *L. apodus,* suggesting a gradual shift in carbon values and a diet shift between these two size classes. Dietary analyses performed in this study highlighted

that small juveniles consumed small mangrove tree crabs, *Aratus pisonii* (Family Grapsidae), which are particularly abundant on the *R. mangle* fringing habitats of Twin Cays (McKeon & Feller, 2004). *Aratus pisonii* live on mangrove trees and are the primary folivore of *R. mangle* leaves and can opportunistically feed on animal prey (Erickson *et al.,* 2008). Ovigerous females release their eggs in the seawater where planktonic larvae remain for several months before their recruitment to juvenile crabs (Warner, 1967). Fishes can thus prey on small Grapsidae during their aquatic life phases. Similar findings have been reported in Curaçao for juvenile *L. apodus* and in Florida for sub-adult of the grey snapper *Lutjanus griseus* (L. 1758), where these fish species expand their trophic niches to include *A. pisonii*, which have ^{13}C-depleted carbon values (Nagelkerken & van der Velde, 2004*b*; Yeager & Layman, 2011). As observed by these authors and in this study, mean carbon values of mangrove *A. pisonii* (*i.e.* $\delta^{13}C = -21{\cdot}9 \pm 0{\cdot}1‰$, this study) are much more depleted than the mean isotopic values of small juveniles of *L. apodus* (*i.e.* $\delta^{13}C_{LapoS} = -14{\cdot}6 \pm 0{\cdot}4‰$, this study), suggesting that these juveniles ingest invertebrates from both mangroves and seagrass beds. This is consistent with the study of Nagelkerken & van der Velde (2004*b*) revealing that the nocturnally active species *L. apodus* shelters and feeds in mangroves during the daytime and migrates at night in seagrass beds to complement their diet. Small juvenile fishes remain and feed in mangroves, as it would be too risky to leave the protection provided by the prop roots. A field experiment using artificial mangrove units (AMU) show that small juvenile fishes actively shelter and avoid leaving AMUs when a predator is introduced in the system (Laegdsgaard & Johnson, 2001). To increase their survival rates, juvenile reef fishes remain in back-reef habitats (mangroves and seagrass beds) and avoid more predation-prone habitats (coral reefs) even if they provide higher food abundance (Kimirei *et al.,* 2013). The risk of predation has a strong influence on the choice of living habitats for small juveniles and is more likely to determine juvenile feeding habits and habitats (Laegdsgaard & Johnson, 2001; Kimirei *et al.,* 2013).

In the mangroves of Twin Cays, larger juveniles of *L. apodus* not only fed on seagrass-associated invertebrates but also targeted small fishes that shelter and feed in mangroves, such as the transient species *H. humeralis* (Vaslet *et al.,* 2012*a*). This feeding behaviour explains the relative reliance of this second-level carnivore on both seagrass and mangrove prey. It has been shown that small piscivores are much more likely to access shallow waters, such as mangroves, to prey on small fishes (Baker & Sheaves, 2005). Shallow waters and physical constraints (*i.e.* mangrove prop roots) hinder the movement of large piscivores, but would favour small carnivorous and piscivorous fishes feeding at the interface between mangroves and seagrass beds (Nagelkerken & van der Velde, 2004*a*).

Further investigations need to encompass other fish size categories (such as adults of *H. flavolineatum* and *L. apodus*, or large juveniles of *O. chrysurus*) and to occur in similar offshore mangrove islets to assess foraging habits of reef fishes.

STEGASTES LEUCOSTICTUS FEEDING HABITS AND HABITATS

Although some dietary shifts have been observed along δ^{13}C and δ^{15}N axes for the three carnivorous fishes, this was not the case for juvenile and adult specimens of *S. leucostictus*. The present findings suggest that this species mainly foraged on seagrass prey items and that no major ontogenetic trophic shifts occurred between the two size groups. Previous studies occurring in Caribbean islands have shown similar

ranges of isotopic values for *S. leucostictus* and underlined that this omnivore feeds in seagrass beds even if specimens were sampled at <50 m from mangroves (Nagelkerken & van der Velde, 2004*a*; Nagelkerken *et al.,* 2006). These studies also showed that *S. leucostictus* has relatively high δ^{15}N values compared with herbivorous (parrotfish and surgeonfish) and other omnivorous [*i.e.* the western Atlantic seabream *Archosargus rhomboidalis* (L. 1758)] fishes. According to dietary studies, most of the diet of *S. leucostictus* consists of algal material, but they also consume animals (*i.e.* eggs, polychaetes and amphipods) as accessory prey items (Randall, 1967; Nagelkerken *et al.,* 2006; this study). In this study, *S. leucostictus* heavily foraged on filamentous algae from seagrass beds as mangrove prop-root algae had carbon values too depleted in ^{13}C to be considered as potential food resources for this species. Like most damselfishes, species of the genus *Stegastes* (Pomacentridae) are territorial species and are considered as 'farmers' that select and maintain algal communities inside their territories (Ceccarelli, 2007). These algal mats sustain abundant cryptic invertebrate assemblages that are consumed by damselfishes to complement nitrogen shortage in algae (Ferreira *et al.,* 1998; Dromard *et al.,* 2013). In Brazil, juveniles of the Brazilian damsel *Stegastes fuscus* (Cuvier, 1830) ($L_T < 8$ cm) target more motile invertebrates (amphipods and copepods) than adults, to increase the nutritional value of their diet during their fast-growing juvenile stage (Ferreira *et al.*, 1998). This feeding behaviour explains the relatively high δ^{15}N values of *Stegastes* species that are in the range of those reported for some carnivorous fishes (Nagelkerken *et al.,* 2006; this study). As few studies have focused specifically on *S. leucostictus*, complementary research is needed to understand their food assimilation, foraging habits and the effect of this species on algal communities.

This study showed the overall reliance of the four reef fish species on seagrass prey items. Dietary shifts were observed among fish size categories with a relatively low degree of niche overlap between fish size groups. Only small and large juveniles of some carnivorous fishes relied, for a part of their diet, on mangrove food resources, highlighting a low contribution of mangrove-derived carbon to the diets of reef fishes. Elucidating trophic functional linkages between mangroves and seagrass beds are important because these ecosystems constitute essential fish habitats for many ecologically or commercially important fishes.

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Supporting Information

Supporting Information may be found in the online version of this paper:

Fig. S1. Diagram for the methods used in this study. Datasets used for stable-isotope analyses and mixing models are specified. See Materials and Methods section for more details.

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