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Ecological connectivity and niche differentiation between two closely related fish species in the mangrove–seagrass–coral reef continuum

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ABSTRACT: We aim to understand ontogenetic shifts in habitat use and feeding patterns by 2 fish species, *Lutjanus fulviflamma* and *L. ehrenbergii*, within a tropical seascape in East Africa. Stomach contents and stable isotope signatures of muscle tissues ($\delta^{13}C$ and $\delta^{15}N$) were compared between and within species. Fish of all life stages and potential food items were sampled from mangrove creeks, seagrass beds, and coral reefs around Mafia Island, Tanzania. Due to similarities in morphology between species, correct species identity was confirmed using genetic barcoding (mtDNA, partial sequence of cytochrome oxidase subunit I [COI]). Stable isotope analysis in R (based on mixing models) confirmed that $\delta^{13}C$ and $\delta^{15}N$ values in *L. fulviflamma* and *L. ehrenbergii* reflected those of prey items caught in different habitats. Diets and mean $\delta^{13}C$ and $\delta^{15}N$ values of muscle tissue differed between life stages of fish, indicating ontogenetic changes in habitat and diet. *L. fulviflamma* and *L. ehrenbergii* differed in diet and $\delta^{13}C$ and $\delta^{15}N$ values of muscle tissue, although they overlapped in habitat use, suggesting food resource partitioning between the 2 species. Furthermore, diet overlap indexes were low between subadult species in mangrove and seagrass or coral habitats. *L. fulviflamma* displayed a diet shift with decreasing importance of small crustaceans in juveniles and an increasing importance of prey fishes in subadults and adults. *L. ehrenbergii* showed the opposite pattern. The study verifies feeding interlinkage within the mangrove–seagrass–coral reef continuum in Mafia Island by providing strong evidence of ontogenetic migration. Understanding these connections will enhance our ability to manage tropical seascapes, and highlights the need to include multiple habitats in marine protected areas.

KEY WORDS: Stable isotopes · Stomach content · Ontogenetic shifts · Connectivity · Resource partitioning · Coral reef · Seagrass · Mangrove

INTRODUCTION

Seagrass beds and mangroves have been suggested to function as nurseries for a number of juvenile coral reef fish before undertaking ontogenetic migrations to coral reef habitats (Nagelkerken et al. 2001, Mumby et al. 2004, Lugendo et al. 2006, Nakamura et al. 2008). These habitats play important roles as sanctuaries from intense predation and sources of food that are thought to be in limited supply on coral reefs (Nagelkerken 2009). Most studies on ontogenetic migrations report higher densities of juvenile reef fish in mangroves and seagrass beds than on coral reefs, and generally lower total density of adult reef fish of the same species in mangroves and seagrass beds (e.g. Gillanders 1997, Appeldoorn et al. 2003, Nakamura & Sano 2004, Dorenbosch et al. 2006). Furthermore, studies have noted absence or low densities of adults from so-called 'nursery species' (species that use mangrove and seagrass beds as nursery habitat) on coral reefs where nursery habitats are very scarce or not present (e.g. Nagel-

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kerken et al. 2002, Mumby et al. 2004, Dorenbosch et al. 2005, 2007). Despite this indirect evidence, actual ontogenetic migration from nurseries to coral reefs has rarely been quantified (but see Tupper 2007, Verweij et al. 2007), possibly due to the difficulty of measuring movement of individuals (Beck et al. 2001). Seagrass beds and mangroves are also used as foraging grounds by many coral reef fish which transfer energy and nutrients from one habitat to another (Meyer et al. 1983). Diurnally active herbivores forage in seagrass beds during the day and migrate to the shelter of coral reefs at night (Maciá & Robinson 2005, Krumme 2009). Similarly, nocturnally active zoo-benthivores move from daytime resting areas on coral reefs or in mangroves to seagrass beds and sandflats to feed at night (Krumme 2009). Studies on diurnal and ontogenetic migrations are mostly descriptive and from the Caribbean. Only rarely they have been done in the western Indian Ocean (Berkström et al. (2012a).

Stable isotopes in animal tissue may be used to trace the origin or movement of fishes (Rubenstein & Hobson 2004, Herzka 2005). The isotopic signature in the tissue reflects those of local food webs and the aquatic habitat in which animals have grown (Hobson 1999). The ratio ^{13}C:^{12}C ($\delta^{13}C$) in its muscle tissue reflects the main source of carbon to a consumer (Fry 2006). Laboratory studies have confirmed that close isotopic similarity exists between animals and their diet (Peterson & Fry 1987). The various types of marine food sources often have different isotopic signatures that also differ between habitats, and hence stable carbon isotope analysis can be an effective tool for measuring connectivity (Fry & Ewel 2003, Rubenstein & Hobson 2004). Fish reside in isotopically distinguishable habitats, and the mangrove–seagrass–coral reef continuum can be viewed as an isoscape where each habitat displays different $\delta^{13}C$ signals (Hobson et al. 2010). This signal is then transferred through the diet of fish residing in a particular habitat. Stable isotopes can also be used to identify the trophic position of an individual organism. In this case, nitrogen is used. The ^{15}N:^{14}N ratio ($\delta^{15}N$) exhibits stepwise enrichment with trophic transfers and hence allows for estimation of trophic level (Minagawa & Wada 1984, Fry 2006). The $\delta^{15}N$ values can therefore be used when looking at ontogenetic diet changes within and between species.

We examine ecological connectivity through ontogenetic changes in habitat use and diet for 2 related species, *Lutjanus fulviflamma* and *L. ehrenbergii*, in an East African seascape using stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) and stomach content analysis. We also compare the 2 species, examining potential resource overlap. Juveniles and subadults of both *L. fulviflamma* and *L. ehrenbergii* have been reported from mangroves and seagrass beds (Gell & Whittington 2002, Dorenbosch et al. 2004, Mellin et al. 2007, McMahon et al. 2011), while adult individuals are found on coral reefs (Dorenbosch et al. 2005, Grandcourt et al. 2011, Kimirei et al. 2011). This suggests that both species display shifts in habitat use and thus contribute to ecological connectivity within the tropical seascape. Furthermore, *L. fulviflamma* and *L. ehrenbergii*, like other snappers are of commercial value, constituting large parts of local catches in many countries in the western Indian Ocean (WIO) region, including Tanzania (1984 to 1992 Tanzanian Annual Fisheries Statistics), Kenya (Ntiba et al. 1993), and the Emirate of Abu Dhabi (Hartmann et al. 2009). *L. fulviflamma* and *L. ehrenbergii* are very similar looking, especially as juveniles, and it can be problematic to distinguish between the 2 species based on morphological marks. Therefore we used DNA analysis to discriminate between the 2 species.

The overall aim of our paper was to understand ontogenetic shifts in habitat use and feeding patterns by 2 species of common macrocarnivores, *L. fulviflamma* and *L. ehrenbergii*, within a tropical seascape in East Africa. Furthermore, we aimed to understand resource partitioning between the 2 species. We hypothesize that (1) diet and habitat use changes through ontogeny in both species of fish and (2) diet composition (expressed as percent estimated volume of food items and stable isotope signatures of muscle tissues, $\delta^{13}C$ and $\delta^{15}N$) will be similar between species due to *L. fulviflamma* and *L. ehrenbergii* being found together in the same habitats.

MATERIALS AND METHODS

Study area

The study was carried out around the southern part of Mafia Island (7° 40′ S, 40° 40′ E), off the east coast of Tanzania. A total of 21 sites comprising of mangrove, seagrass, and coral reef were surveyed (Fig. 1). Mafia Island is located 60 km south of Dar es Salaam and 21 km east of the Rufiji delta (Garpe & Öhman 2003). The area has 2 annual seasons (the northeast and southeast monsoon) and a large tidal range (McClanahan 1988). The weather is dry and sunny during the northeast monsoons (October to March), while the southeast monsoon (March to October) is windy, rainy and cloudy (McClanahan

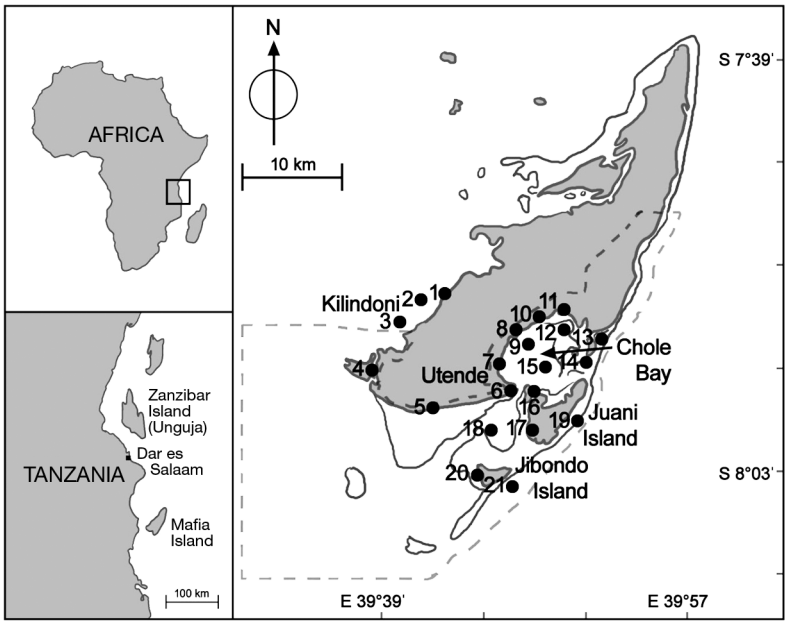

Fig. 1. Study sites around the southern part of Mafia Island, Tanzania. 1: Mfuruni Creek (m); 2: Mfuruni (s/c); 3: Kilindoni (s/c); 4: Mlongo Creek (m); 5: Changaramma (s/c); 6: Utende (s/c); 7: Utende Creek (m); 8: Adani Creek (m); 9: Adani (s); 10: Minaki Creek (m); 11: Mchangani Creek (m); 12: Mchangani (s); 13: Kinasi Pass 1 (c); 14: Kinasi Pass 2 (c); 15: Chole north (s/c); 16: Chole Island/Juani Island channel (m/s); 17: Juani Creek (m); 18: Maluzuku (s/c); 19: Juani Reef (c); 20: Jibondo west (s/c); 21: Jibondo Reef (c). m: mangrove; s: seagrass; c: coral. Dashed line: Mafia Island marine park (MIMP). Solid lines: reef areas

1988). The tides at Mafia Island are mixed semidiurnal and may reach average spring amplitudes of 3.3 m (Horrill et al. 1996, Garpe & Öhman 2003). Mafia Island is characterized by a high diversity of corals and fish (Garpe & Öhman 2007).

In 1995, Mafia Island Marine Park (MIMP), a multiuse national park, was established in the southern part of Mafia Island (Andersson & Ngazi 1995). The park is based on the concept of integrated coastal management with core zones of banned or restricted fishing (Kamukuru et al. 2004). It covers an area of 822 km^2 (Garpe & Öhman 2007). Most of the coastline within the marine park is fringed by mangroves, mainly *Xylocarpus granatum*, *Avicennia marina*, *Rhizophora mucronata*, *Brugueira gymnorrhiza*, and *Sonneratia alba*. Chole Bay is a shallow, sheltered bay with a maximum depth of 15 m. It is protected from intense wave action from the Indian Ocean by fringing coral reefs that run along the east coastline of Mafia Island. Strong tidal currents (up to 6 knots) provide water exchange with the open sea and outer reefs through 2 deep-water channels (Horrill et al. 1996). The interior of Chole Bay and shallow areas close to Juani and Jibondo Islands are comprised of a complex mosaic of seagrass beds and coral reefs. Intertidal flats are dominated by algae (mainly *Halimeda* spp.) and seagrasses (mainly *Thalassia hemprichii* and *Cymodocea* spp.), while the seagrasses *Enhalus acoroides* and *Thalassodendron ciliatum* form large monospecific or mixed-species beds in deeper water. The area between Utende (southern part of Chole Bay) towards Jibondo Island is covered by extensive seagrass beds with scattered patch reefs. Southwest of Jibondo Island, large and diverse coral reefs such as Mange and Kitutia are present.

Study species

The Dory snapper *Lutjanus fulviflamma* (Forsskål, 1775) and the blackspot snapper *L. ehrenbergii* (Peters, 1869), are widespread species, common in the Indian Ocean (Richmond 2002) and elsewhere (Randall et al. 1997). Both species reach a maximum total length (TL) of 35 cm and are found in various marine coastal habitats. In general, juveniles are found in mangrove habitats, and larger individuals on coral reefs, in large mixed-species aggregations (Lieske & Myers 2002). Both *L. fulviflamma* and *L. ehrenbergii* are described as fish-and-invertebrate feeders (de Troch et al. 1998, Baker & Sheaves 2005, Lugendo et al. 2006, Unsworth et al. 2009). They are commercially important (Lugendo et al. 2005, Shimose & Tachihara 2005, Grandcourt et al. 2006) and together with other snappers (Lutjanidae) and emperors (Lethrinidae) make up ~40% of the total fish catch in the area (1984 to 1992 Tanzanian Annual Fisheries Statistics).

Sample collection

Mangrove creeks, seagrass beds, and coral reefs around the southern half of Mafia Island were visited in order to gather general information on species occurrence and abundance in the region. Groundtruthing of major habitats gave a general overview of the Mafia Island seascape. A total of 388 samples of *Lutjanus fulviflamma* and *L. ehrenbergii* were collected in February–March 2010 and 2011 (see Table 1 for details). Juvenile fish were col-

Table 1. *Lutjanus fulviflamma* and *L. ehrenbergii*. Stomach content analysis for sites around Mafia Island, Tanzania, showing mean volumetric percentage (MVP) and percentage frequency of occurrence (PFO) of food items found in stomachs. **Bold**: values for the prey category constituting the largest part of the stomach contents. A: adult; S: subadult; J: juvenile. Code for site and habitat see Fig. 1 legend. n: number of full stomachs for each group of fish; numbers in ( ): empty stomachs; TL: total length. –: not observed

	L. fulviflamma										*L. ehrenbergii*					
Life stage:	A		S		S		J		J		S		S		J	
Habitat:	s/c		s/c		m		m		s/c		s/c		m		m	
Site:	2,3,6,9,15,19,20,21		2,6,9,15,16,18,20		4,8,11		4,7,8,10,11		5,9,15		16		8.11		1,4,7,8,11,17	
Size (TL, cm):	18.5–27.3		12.2–18.3		12.7–18.3		5–11.6		7–9		15–17.5		16.4–19.4		3.2–10	
n:	80 (1)		30 (2)		22 (10)		32 (4)		7		9 (1)		8 (3)		68 (13)	
Food items	MVP	PFO	MVP	PFO	MVP	PFO	MVP	PFO	MVP	PFO	MVP	PFO	MVP	PFO	MVP	PFO
Crabs	23.8	53.8	16.4	40.0	8.6	13.6	**46.5**	65.6	**28.3**	28.6	**58.7**	66.7	**62.5**	87.5	**24.3**	50.0
Shrimp/prawns	9.3	31.3	6.2	20.0	0.9	4.5	3.0	6.3	–	–	2.2	11.1	–	–	4.4	8.8
Stomatopods	5.3	13.8	3.0	10.0	–	–	0.3	3.1	–	–	–	–	–	–	–	
Appendages	9.3	46.3	**28.8**	46.7	16.8	13.6	16.4	31.3	7.6	14.3	5.0	11.1	8.8	12.5	16.2	33.8
Megalopae	0.4	5.0	–	–	–	–	–	–	0.3	14.3	–	–	2.5	25.0	0.5	11.8
Nauplii	0.4	1.3	–	–	–	–	–	–	0.3	14.3	–	–	–	–	1.0	8.8
Isopods	6.5	45.0	7.4	26.7	–	–	0.0	6.3	10.0	14.3	2.2	11.1	–	–	1.6	11.8
Amphipods	0.1	2.5	0.1	3.3	–	–	2.5	6.3	0.3	14.3	–	–	–	–	4.2	22.1
Copepods	–	–	–	–	–	–	0.6	3.1	–	–	–	–	–	–	–	
Ostracods	0.3	7.5	–	–	–	–	–	–	–	–	–	–	–	–	–	
Cirriped larvae	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.0	1.5
Fish	**30.4**	52.5	15.7	23.3	6.3	9.1	2.2	6.3	14.3	14.3	0.1	11.1	–	–	12.7	30.9
Cephalopods	1.0	1.3	–	–	–	–	–	–	–	–	–	–	–	–	–	
Bivalves	0.3	3.8	0.5	3.3	–	–	–	–	–	–	–	–	–	–	–	
Gastropods	–	–	–	–	–	–	0.3	3.1	–	–	0.2	11.1	–	–	1.2	1.5
Polychaetes	0.6	6.3	1.3	10.0	1.1	4.5	–	–	0.1	14.3	–	–	–	–	–	
Sipunculans	1.3	1.3	2.3	3.3	**17.0**	36.4	–	–	–	–	–	–	–	–	–	
Diatoms	–	–	–	–	–	–	–	–	0.1	14.3	–	–	–	–	–	
Porifera	0.5	5.0	–	–	0.0	4.5	–	–	–	–	–	–	–	–	–	
Sea squirts	0.4	1.3	–	–	–	–	–	–	–	–	–	–	–	–	–	
Eggs/eggmasses	0.1	1.3	–	–	–	–	–	–	–	–	–	–	–	–	0.1	4.4
Insects	–	–	–	–	–	–	–	–	–	–	1.1	11.1	–	–	1.3	1.5
Algae	0.4	11.3	–	–	15.9	22.7	3.1	3.1	–	–	–	–	–	–	1.8	4.4
Unidentified	9.9	47.5	18.3	36.7	33.3	54.5	25.0	53.1	38.7	57.1	30.4	55.6	26.3	50.0	30.7	57.4

lected at low tide in mangrove and seagrass habitats using a modified mosquito net (5 × 1.5 m, mesh size: 1 mm) or a small-scale gill net (6 × 1 m, mesh size: 15 to 20 mm). The mosquito net was slowly dragged along the bottom by 2 people, while a third person approached rapidly scaring fish into the net. In areas where fish congregated around roots or submerged dead tree branches, the gill net was laid out in a circle and slowly pulled together to shrink the net area and catch the fish inside. All adults and most subadults were purchased from local fishers. These were mainly caught in seagrass and coral reef habitats using traditional fishing methods, such as hook and line, small nets, and intertidal fence nets. Each fish was measured to the nearest millimeter to obtain total length, weighed to the nearest gram, photographed digitally, and had sex and gonad maturity recorded.

Fin clips from every caught specimen were stored in 95% alcohol for later DNA analysis. A piece of white muscle tissue (2 mm^2) was removed from each individual fish for isotope analysis, placed in a vial and frozen. Samples were later dried in an oven at 60 to 70°C for 48 to 72 h. The stomach and intestines, was removed and placed in 95% alcohol. Notes on the stomach (e.g. full, half full, or empty) were also recorded for each individual. Individuals <4 cm were placed whole in alcohol. Their digestive tract was later removed in the laboratory.

Potential food items (shrimps, crabs, and small fish) for use as reference specimens for the isotope study were collected in mangrove creeks, seagrass beds, and coral reefs within the Chole Bay area. These were frozen and later dried in an oven at 60 to 70°C for 48 to 72 h.

Genetic analysis

DNA extraction

DNA was extracted from the fish muscle samples using the DNeasy Blood & Tissue Kit (Qiagen). We followed the manufacturer's protocols including all optional additional steps. The final elution step was modified by eluting the samples in 50 µl heated elution buffer (70°C). Using a spectrophotometer Nd-1000 (Nano Drop), the amount of nucleic acids was quantified, and the samples were diluted to achieve approximately the same concentrations, i.e. 50 ng μl^{-1}.

mtDNA genotyping

The partial sequence of cytochrome oxidase subunit I (COI) region in the mitochondrial DNA was amplified using the primers Fish-F2 (5′ TCG ACT AAT CAT AAA GAT ATC GGC AC 3′) and FISH-R1 (5′ TAG ACT TCT GGG TGG CCA AAG AAT CA 3′) as outlined in Ward et al. (2005). PCR amplifications followed Ward et al. (2005), and the cycling conditions were as follows: 1 × 95°C (3 min); 35 × [30 s at 95°C, 30 s at 54.5°C, 1 min at 72°C]; and 1 × 72°C (10 min). The PCR products were diluted to 100 ng μl^{-1} and sent to Macrogen Korea for direct sequencing in both directions. A negative control was used for every PCR run, agarose gel analysis, and sequencing analysis to rule out contamination and genotyping errors. Furthermore, 5% of randomly chosen samples were re-amplified and re-sequenced on a separate date to ensure consistency of results.

Data analysis

All chromatograms were aligned by hand using MEGA 5.0 (Tamura et al. 2011) and trimmed to 689 bp. The different haplotypes were designated by DAMBE (Version 5.2.31; http://dambe.bio.uottawa.ca) identified using BLAST and aligned with reference sequences obtained from GenBank (NCBI) and The Barcode of Life Data Systems (BOLD; www.boldsystems.org).

Stomach content analysis

Each preserved digestive tract was opened and its contents placed in a Petri dish with a 1 cm^2 grid. All visible stomach contents were identified to the lowest practical taxonomic level. Estimated proportion volume (i.e. the volume of individuals of each prey type in all stomachs expressed as a proportion of the total volume of food items measured in all stomachs) was determined using methods described by Hyslop (1980) and Berkström et al. (2012b). A volumetric measure was chosen as it is a good estimation of biomass. Gravimetric methods can produce large errors in small volumes because of water content and blotting may damage samples in some cases (Cocheret de la Morinière et al. 2003a,b). In very small stomachs (such as those from juvenile fishes) individual prey items were difficult to weigh and hence a method (estimated proportion volume) that could be used in all size classes was chosen to avoid bias due to different methods.

Stable isotope analysis

Dried muscle samples were ground to a powder using mortar and pestle. Between samples all equipment was cleaned with distilled water and acetone to avoid contamination. Of each ground sample together with reference fish samples of Hoki *Macruronus novaezelandiae*, ~1 g were sent to the University of California Davis for stable isotope analysis. ^{13}C:^{12}C and ^{15}N:^{14}N ratios were measured using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (IRMS). Samples were combusted at 1000°C in a reactor packed with chromium oxide and silvered cobaltous/cobaltic oxide. Following combustion, oxides were removed in a reduction reactor (reduced copper at 650°C). The helium carrier then flowed through a water trap (magnesium perchlorate) and an optional CO_2 trap (for N-only analyses). N_2 and CO_2 were separated on a Carbosieve GC column (65°C, 65 mL min^{-1}) before entering the IRMS. The isotopic compositions of carbon and nitrogen were expressed in delta notation (δ). This refers to parts per thousand differences from an international standard V-PDB (Vienna PeeDee Belemnite) and air for carbon and nitrogen, respectively, according to the formula:

$$\delta X = [(R_{sample}:R_{standard}) - 1] \times 10^3$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio ^{13}C:^{12}C or ^{15}N:^{14}N.

Data analyses

In order to assess changes in diet and habitat use with ontogeny in *Lutjanus fulviflamma* and *L. eh-*

renbergii, individuals were sorted into 3 main life stages: juvenile (3 to 12 cm TL), subadult (12.1 to 18.5 cm TL), and adult (>18.5cm TL) following Nagelkerken & van der Velde (2002), where juveniles are <⅓, subadults ⅓ to ⅔ and adults >⅔ of the species' maximum length. However, the cut-off point between adults and subadults was slightly modified due to observations made while dissecting samples of *L. fulviflamma* in the current study. The smallest individual with ripe gonads was 18.5 cm in length and hence represented the new modified cut-off point between subadults and adults. Seagrass and coral were merged to 1 habitat category for the statistical analysis, resulting in 2 main habitats: mangroves and seagrass/coral.

Source contributions to diets

Stable isotope analysis in R (SIAR), a freeware package that runs in the R statistical computing environment, was used to examine the contribution of different food items to the isotopic signatures in the different species and life stages of fish. The program uses Bayesian inference to solve for the most likely set of dietary proportions given the isotopic ratios in a set of possible food sources and a set of consumers (Parnell et al. 2010). The model is similar in principle to IsoSource (Phillips & Gregg 2003), but allows all sources of uncertainty (such as in the sources or trophic fractionation values) to be propagated through the model to return a true probability distribution of estimated dietary proportions (Parnell et al. 2010). The trophic enrichment factors (TEFs; means ± SD) for nitrogen (3.2 ± 1.28‰) and carbon (1.74 ± 1.09‰) were extracted from Sweeting et al. (2007a,b). The SIAR mixing model was run for 500 000 iterations, discarding the first 50 000 samples.

Diet similarity between species

The diet similarity between *Lutjanus fulviflamma* and *L. ehrenbergii* was assessed using Schoener's diet overlap index (Schoener 1968):

$$D = 1 - 0.5\sum(pij - pik)$$

where D is the index value, and pij and pik are the relative proportion of each food item i for species j and k, respectively. On this scale, 1 represents complete overlap between the 2 species being compared and 0 represents no overlap. Significant dietary overlap is typically set to values >0.6 (Schoener 1968).

Statistical analyses

Stomach contents and mean $\delta^{13}C$ and $\delta^{15}N$ values were tested for differences between species (*Lutjanus fulviflamma* versus *L. ehrenbergii*), life stages (juvenile, subadult, and adult), and habitat (mangrove versus seagrass/coral) using a permutational multivariate ANOVA (PERMANOVA) in Primer 6 for stomach contents and a univariate PERMANOVA for $\delta^{13}C$ and $\delta^{15}N$ analysis, respectively. PERMANOVA is a multivariate variation of ANOVA that produces a pseudo *F*-statistic and significance (p) value by means of permutations methods (Anderson 2001). Stomach content data were forth-root transformed, and Bray-Curtis dissimilarity index was used. Food items were pooled into 7 categories (fish, crabs, crustacean species, crustacean appendages, sipunculans, algae, and other) to facilitate statistical analyses. Unidentified items were not included, as well-digested stomach contents may bias results. Unidentifiable material may contain remnants of 1 or more dietary categories and thus make it difficult to obtain reliable counts of certain prey items if they are included (Schafer et al. 2002). Furthermore, unidentified material was present in all categories, and the amounts were rather similar among all categories (25 to 39% of estimated volume) except for subadult (18% of estimated volume) and adult (10% of estimated volume) *L. fulviflamma* from seagrass/coral areas. The diet patterns would most likely remain similar whether or not unidentified items are included. Euclidian distances were used on the isotope data. Raw data were used for carbon isotopes, while nitrogen isotope data were forth-root transformed to meet assumption of homogeneity. One-way planned contrast PERMANOVA tests were carried out to compare differences in (1) stomach contents and (2) isotopic signatures between species (*L. fulviflamma* versus *L. ehrenbergii*). Two-way PERMANOVA tests were then used to test for differences in stomach content and isotopic signature between different life stages within species. *A posteriori* pairwise comparisons were performed to investigate significant terms (Anderson & Gorley, 2007).

Due to samples being collected in 2 different years (2010 and 2011), a planned contrast 1-way PERMANOVA test was performed on mean $\delta^{13}C$ values in order to account for possible differences due to year. Mainly juveniles of both species were collected dur-

ing 2011 in Chole Bay. There were no significant differences between groups that were possible to compare: juvenile *Lutjanus fulviflamma* in mangrove (F = 0.13663, p = 0.715), juvenile *L. fulviflamma* in seagrass/coral ($F = 1.8728 \times 10^2$, p = 0.8829), and juvenile *L. ehrenbergii* in mangrove (F = 3.036, p = 0.0882). Hence, we conclude that significant differences in our study are due to other factors than year.

RESULTS

Genetics

The genetic results allowed us to discriminate the 2 very similar-looking species *Lutjanus fulviflamma* and *L. ehrenbergii* (Fig. 2). The sequences revealed 17 *L. fulviflamma* haplotypes covering all life stages (Accession Numbers NCBI JQ639253 to JQ639269) and 10 juvenile and subadult *L. ehrenbergii* haplotypes (Accession Numbers NCBI JQ639270 to JQ639281); the 2 species could therefore be separated with certainty in the isotope analysis.

Stomach contents

A total of 290 fish stomachs were examined (256 with content and 34 empty) from 18 sites in the southern part of Mafia Island (Fig. 1, Table 1). Twenty-three categories of food items were identified in the examined stomachs of *Lutjanus fulviflamma* and *L. ehrenbergii*, almost half (n = 11) being crustaceans. The most common food items were crabs (Brachyura) and crustacean appendages, followed by fish, shrimp/prawns, stomatopods, isopods, and amphipods (Table 1, Fig. 3). Fish were more common in larger *L. fulviflamma*, comprising 34, 19, and 3% estimated volume of food items in adults, subadults, and juveniles, respectively (Fig. 3). However, juvenile *L. fulviflamma* caught in seagrass beds had a high percentage of fish in their diet (23% of the estimated volume).

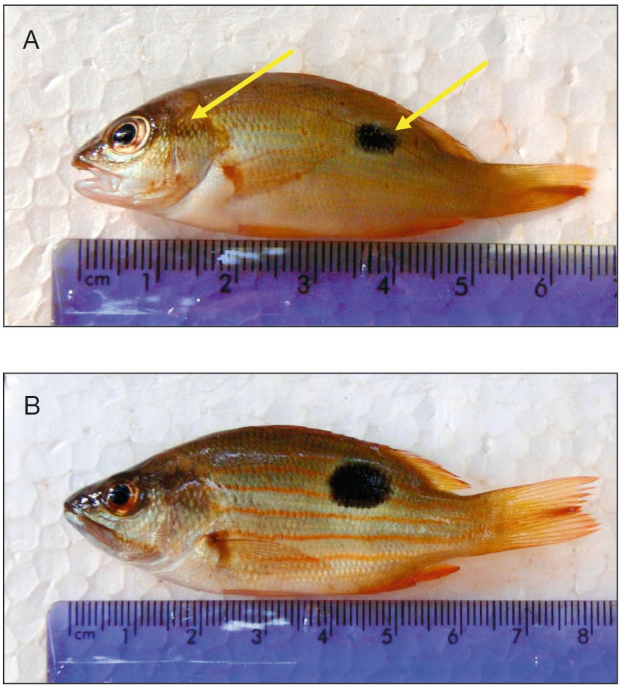

Fig. 2. *Lutjanus fulviflamma* and *L. ehrenbergii*. (A) Juvenile and subadult *L. fulviflamma* often display a small black line across the eye, less prominent horizontal lines, and a black dot on the peduncle area which is less pronounced and looks smudged at the edges compared to (B) *L. ehrenbergii*. These differences are less obvious in dead fish

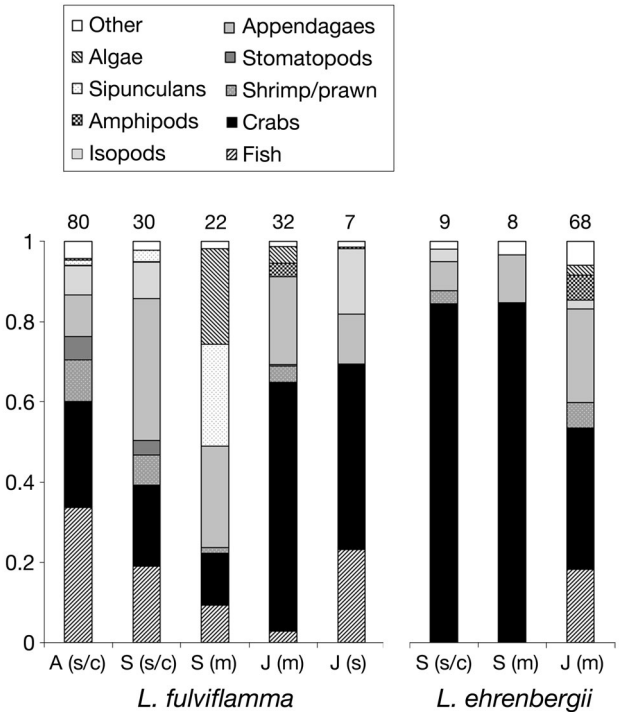

Fig. 3. *Lutjanus fulviflamma* and *L. ehrenbergii*. Estimated proportions of volume of major food categories present in stomachs from sites around Mafia Island, Tanzania. Number of analysed fish above the column. Less important categories (megalope- and naupli-stage crustaceans, copepods, ostracods, cirripedi, cephalopods, bivalves, gastropods, polychaetes, diatoms, poriferas, sea squirts, egg mass, and algae) have been lumped into the category 'other'. Unidentified items were removed from the graph. A: adult; S: subadult; J: juvenile; s: seagrass; c: coral; m: mangrove

The pattern was different in *Lutjanus ehrenbergii*. Fish were only found in juvenile *L. ehrenbergii* (18% of the estimated volume), while subadult stomachs contained no fish at all (Fig. 3). Subadult *L. ehrenbergii* contained >85% crabs. Worms such as polychaetes and sipunculids were only found in *L. fulviflamma*, mainly in subadults from mangrove areas. The diets of adult and subadult *L. fulviflamma* caught in seagrass/coral areas were similar in composition containing a variety of fish and crustaceans such as crabs, shrimp/prawns, stomatopods, and isopods (Fig. 3). Although isopods only comprised a small amount of the total stomach content, they were found in nearly half of all adult *L. fulviflamma* stomachs (Table 1). Subadults caught in mangroves differed however, with sipunculid worms and algae comprising 50% of the estimated volume of their diet (Fig. 3). Juvenile *L. fulviflamma* and juvenile *L. ehrenbergii* caught in mangrove areas had similar diets, mainly crabs and crustacean appendages.

Stomach contents differed significantly between the fish species *Lutjanus fulviflamma* and *L. ehrenbergii* in all comparable life stages and habitats (Table 2). Significant differences were found between juveniles of the 2 species in mangrove creeks, between subadults in mangrove creeks, and between subadults in seagrass/coral reef areas. Significant differences were also found within species. In *L. fulviflamma* there were significant differences between life stages, between habitats, and interactions between the 2 (Table 3). Post hoc tests showed that there were differences between all life stages and habitats, except for between juveniles in mangroves and juveniles in seagrass/coral and between juveniles in seagrass/coral and adults in seagrass/ coral. In *L. ehrenbergii* there were significant differences between life stages, but not habitat (Table 3).

Source contributions to diets

The contribution of different carbon sources (potential prey items including crabs, shrimp, and small fish from mangrove and seagrass habitats) to the diets of all fish examined, aligned well with dietary shifts documented in the stomach content analysis (Figs. 3 & 4). Small fish from seagrass areas made the dominant contribution in adult and subadult *Lutjanus fulviflamma* caught in seagrass/coral habitats, while crabs from seagrass beds made the dominant contribution in juvenile *L. fulviflamma* from seagrass/coral habitats (Table 1, Fig. 4). Furthermore, mangrove crabs made the dominant contribution to all examined life stages (juveniles and subadults) of *L. ehrenbergii* caught in both mangrove and seagrass/coral areas (Fig. 4).

Diet similarity between species

There were no significant diet overlaps between subadult *Lutjanus fulviflamma* and *L. ehrenbergii* in mangrove and seagrass/coral habitats. Schoeners' diet overlap index values were 0.47 and 0.50, respectively, consistent with low similarity in diets. There was however an overlap in juveniles caught in mangrove habitats with a Schoeners' diet overlap index of 0.78.

Stable isotopes

Stable isotope signatures were examined from tissue of a total of 183 fish and 30 potential prey samples from 18 sites in the southern part of Mafia Island (Fig. 1, Table 4). Potential prey items had mean $\delta^{13}C$

Table 2. *Lutjanus fulviflamma* and *L. ehrenbergii*. Results from planned contrast PERMANOVA tests (1-way) between species on gut contents and mean $\delta^{13}C$ and $\delta^{15}N$ values. df: degrees of freedom; SS: sums of squares; MS: mean square; p: significance level obtained under permutation; ns: non-significant. J: juvenile; S: subadult; Res: residual; m: mangrove; s: seagrass; c: coral

	df	SS	MS	Pseudo-*F*	p
Stomach contents (between species)					
J (m)	1	13278	13278	5.2915	0.0017
S (m)	1	21288	21288	6.9774	0.0002
S (s/c)	1	8013.6	8013.6	2.9449	0.0390
Res	144	3.8134×10^5	2648.2		
Total	149	4.4494×10^5			
Isotopes (between species)					
$\delta^{13}C$					
J (m)	1	17.625	17.625	4.3493	0.0444
S (m)	1	177.6	177.6	44.004	0.0001
S (s/c)	1	644.9	644.9	115.66	0.0001
Res	129	571.07	4.4269		
Total	134	1707			
$\delta^{15}N$					
J (m)	1	1.2883×10^4	1.2883×10^4	0.15019	0.7078 ns
S (m)	1	8.7423×10^4	8.7423×10^4	1.6085	0.2142 ns
S (s/c)	1	2.0834×10^3	2.0834×10^3	5.3769	0.0266
Res	129	8.7435×10^2	6.7779×10^4		
Total	134	0.20949			

Table 3. *Lutjanus fulviflamma* and *L. ehrenbergii*. Results from PERMANOVA tests (2-way) between life stages within species on gut contents and mean $\delta^{13}C$ and $\delta^{15}N$ values. df: degrees of freedom; SS: sums of squares; MS: mean square; p: significance level obtained under permutation; ns: non-significant

	df	SS	MS	Pseudo-*F*	p
Stomach contents (within species)					
L. fulviflamma					
Life stage	2	15946	7972.8	3.1871	0.001
Habitat	1	8643.7	8643.7	3.4553	0.01
Life stage × Habitat	1	9039.2	9039.2	3.6134	0.009
Residuals	156	3.9024×10^5	2501.6		
Total	160	4.5594×10^5			
L. ehrenbergii					
Life stage	1	11019	11019	4.6186	0.006
Habitat	1	1025	1025	0.42964	0.684 ns
Life stage × Habitat	0	0	–	No test	
Residuals	72	1.7178×10^5	2385.8		
Total	74	1.8665×10^5			
Isotopes (within species)					
$\delta^{13}C$, *L. fulviflamma*					
Life stage	2	124.14	62.07	12.765	0.001
Habitat	1	335.83	335.83	69.066	0.001
Life stage × Habitat	1	2.5788	2.5788	0.53035	0.472 ns
Residuals	113	549.46	4.8625		
Total	117	1090.8			
$\delta^{13}C$, *L. ehrenbergii*					
Life stage	1	43.016	43.016	14.539	0.001
Habitat	1	1.5333	1.5333	0.51825	0.467 ns
Life stage × Habitat	0	0	–	No test	
Residuals	57	168.64	2.9586		
Total	59	255.26			
$\delta^{15}N$, *L. fulviflamma*					
Life stage	2	0.15928	7.9639×10^2	104.33	0.001
Habitat	1	5.2029×10^3	5.2029×10^3	6.8163	0.004
Life stage × Habitat	1	5.1132×10^4	5.1132×10^4	0.66987	0.411 ns
Residuals	113	8.6253×10^2	7.6331×10^4		
Total	117	0.41246			
$\delta^{15}N$, *L. ehrenbergii*					
Life stage	1	1.7346×10^6	1.7346×10^6	24.546	0.001
Habitat	1	1.6986×10^4	1.6986×10^4	0.24037	0.64 ns
Life stage × Habitat	0	0	–	No test	
Residuals	57	4.028×10^2	7.0667×10^4		
Total	59	7.1474×10^2			

values ranging from −17.2 to −15.6 in small crabs, shrimps, and fish from mangrove creeks and from −18.8 to −8.1 in small crabs and fish from seagrass beds (Table 4).

Between species

There were significant differences in mean $\delta^{13}C$ and $\delta^{15}N$ values between the 2 fish species, *Lutjanus fulviflamma* and *L. ehrenbergii* (Table 2). *L. fulviflamma* and *L. ehrenbergii* differed significantly in mean $\delta^{13}C$ values between all life stages in all habitats (Table 2). Significant differences were found between juveniles of the 2 species in mangrove creeks, between subadults in mangrove creeks, and between subadults in seagrass/coral reef areas. Mean $\delta^{15}N$ value, on the other hand, only differed between *L. fulviflamma* and *L. ehrenbergii* among subadults in seagrass/coral areas (Table 2).

Within species

Significant differences were found in mean $\delta^{13}C$ and $\delta^{15}N$ values between life stages within each

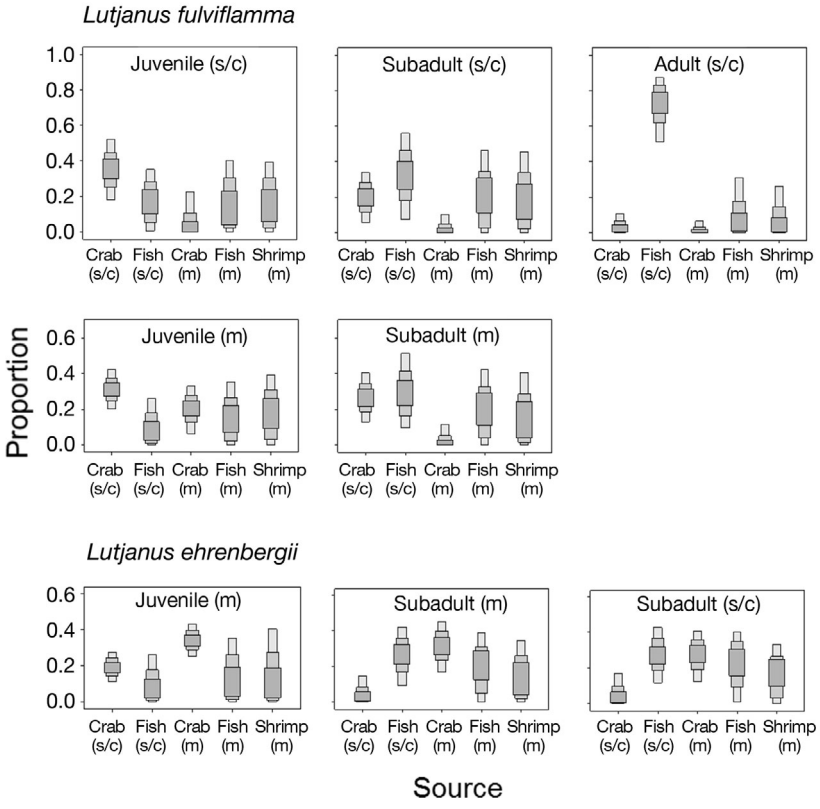

Fig. 4. *Lutjanus fulviflamma* and *L. ehrenbergii*. Boxplots derived from the stable isotope analysis in R (SIAR) showing the contribution of different potential food items to the diets using $\delta^{13}C$ and $\delta^{15}N$ isotopes. The proportions show 95, 75, and 50% credibility intervals. Potential food sources are labeled crab, fish, and shrimp from seagrass/coral areas (s/c) and mangrove areas (m)

species; juveniles, subadults, and adults of *Lutjanus fulviflamma* and juvenile and subadults of *L. ehrenbergii* (Table 3). The $\delta^{13}C$ values in *L. fulviflamma* overlapped to some extent, but post hoc tests showed significant differences between all life stages and all habitats, except for juveniles and subadults in seagrass/coral (Table 3, Fig. 5). The $\delta^{15}N$ values were also significantly different between all life stages and all habitats, with the exception of subadults in mangrove and subadults in seagrass/ coral, indicating differences in trophic level between all 3 life stages (Fig. 5). The mean $\delta^{15}N$ difference between juveniles and adults was >2.5‰ in *L. fulviflamma*, corresponding to a full tropic level (Vanderklift & Ponsard 2003; Fig. 5). Juvenile and subadult *L. ehrenbergii* also showed some overlap in $\delta^{13}C$ values, with significant differences between life stages, but not habitat (Table 3, Fig. 5). Significant differences between juvenile and subadult *L. ehrenbergii* were also found for $\delta^{15}N$ values (Table 3, Fig. 5).

Table 4. Stable isotope data for samples of fish and potential prey from sites around Mafia Island, Tanzania. $\delta^{13}C$ and $\delta^{15}N$ values (means ± SE) for each group of organisms are displayed. A: adult; S: subadult; J: juvenile; m: mangrove; s: seagrass; c: coral

Organism	Life stage	Habitat	Site	Size TL (cm)	Isotope samples (n)	$\delta^{13}C$ ± SE	$\delta^{15}N$ ± SE
Fish species							
Lutjanus fulviflamma	J	m	1,4,7,8,10,11	5–12	34	−13.8 ± 0.4	7.3 ± 0.1
	J	s/c	5,9,16	7–9.9	12	−9.2 ± 0.2	7.7 ± 0.1
	S	m	4.8	12.8–18.3	17	−11.6 ± 0.5	8.4 ± 0.1
	S	s/c	6,16,18,20	12.2–17.8	24	−7.3 ± 0.4	8.7 ± 0.1
	A	s/c	3,6,19,20	21–27.5	31	−10.6 ± 0.4	10.0 ± 0.1
Lutjanus ehrenbergii	J	m	1,7,8,11,17	3.2–10	39	−14.8 ± 0.3	7.3 ± 0.1
	S	m	8.11	16.4–19.4	11	−17.0 ± 0.5	8.1 ± 0.1
	S	s/c	16	15–17.5	10	−17.5 ± 0.7	8.3 ± 0.1
Epinephelus fasciatus	A	c	12	15–22	6	−15.5 ± 0.2	10.8 ± 0.1
Potential prey items							
Crabs		m	8.11	<2	6	−17.2 ± 0.4	3.8 ± 0.2
Shrimp		m	8.11	<2	6	−15.6 ± 0.6	6.1 ± 0.2
Small fish	J	m	8.11	<2	6	−16.4 ± 0.3	6.7 ± 0.1
Crabs		s	9.12	<2	6	−8.1 ± 0.2	2.9 ± 0.2
Small fish	J	s	9.12	<2	6	−18.8 ± 0.1	8.2 ± 0.1

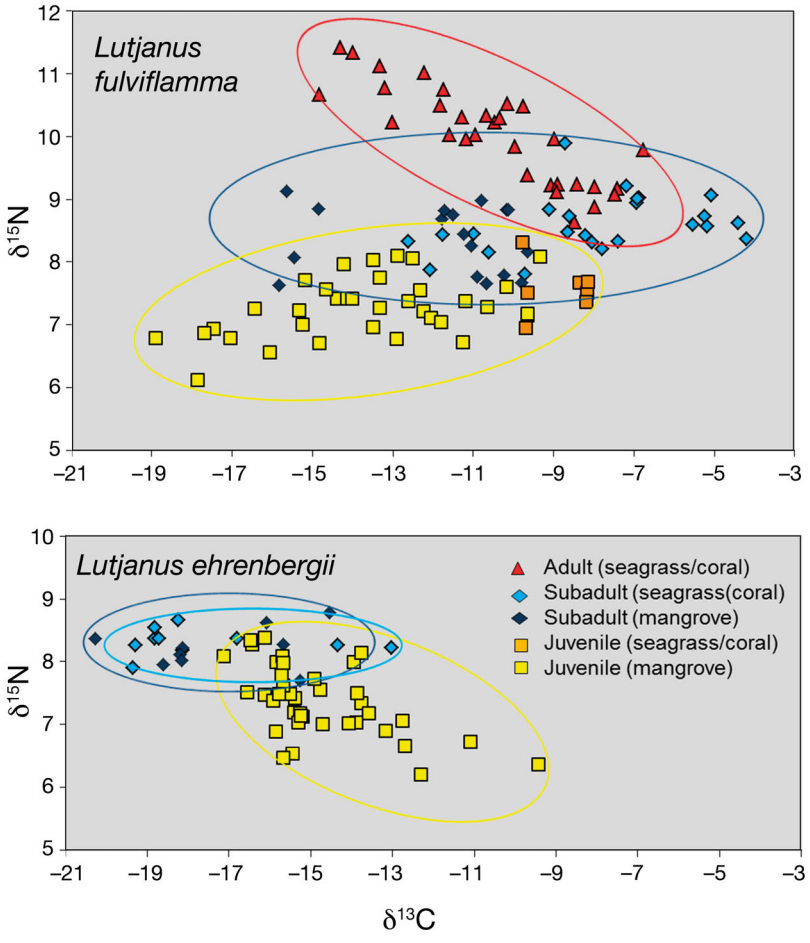

Fig. 5. *Lutjanus fulviflamma* and *L. ehrenbergii*. Biplots of $\delta^{13}C$ and $\delta^{15}N$ values for different life stages from sites around Mafia Island. Adult values are found within the red ellipse; subadult values within the blue (light and dark) ellipses; and juvenile values within the yellow ellipses

DISCUSSION

According to predictions, $\delta^{13}C$ and $\delta^{15}N$ values in *Lutjanus fulviflamma* and *L. ehrenbergii* reflected those of prey items caught in different habitats. Furthermore, mean $\delta^{13}C$ and $\delta^{15}N$ values differed between different life stages of fish, indicating ontogenetic changes in habitat and diet. However, contrary to predictions, *L. fulviflamma* and *L. ehrenbergii* differed in $\delta^{13}C$ and $\delta^{15}N$ values, although they overlapped in habitat use, suggesting food resource partitioning between the 2 species. Furthermore, diet overlap indexes were low between subadult *L. fulviflamma* and *L. ehrenbergii* in mangrove habitats and in seagrass/coral habitats. Resource partitioning in diet among snappers has also been documented in a tropical Brazilian estuary where nursery habitats overlapped (Pimentel & Joyeux 2010). Food overlap was low between the ecologically similar species and a combination of inter-specific differences in size, spatial distribution, microhabitat preferences, and seasonal patterns of abundance of prey choice were suggested as main factors explaining the differences in diet. We did not examine microhabitat preferences, seasonality, or day and night differences in stomach contents between species; therefore, resource partitioning cannot be proven. Nevertheless, as stable isotopes reflect food intake over a longer period of time, the differences between species in our study indicate that diets are consistently different; hence, resource partitioning may be a plausible reason.

Ontogenetic diet and habitat shifts

Lutjanus fulviflamma and *L. ehrenbergii*, showed evidence of ontogenetic shifts in habitat and diet. *L. fulviflamma* displayed a diet shift with a decreasing importance of small crustaceans in juveniles and an increasing importance of prey fishes in subadults and adults. This pattern corresponds well to what Kamukuru & Mgaya (2004) and Lugendo et al. (2006) previously found in *L. fulviflamma* and to what has been found among other snappers in the Caribbean (Rooker 1995, Cocheret de la Morinière et al. 2003a,b). The increase in larger prey such as fish in *L. fulviflamma* corresponded with higher $\delta^{15}N$ values, indicating an increase in trophic position with age. *L. ehrenbergii*, on the other hand, did not seem to include more fish in their diet with age. The lack of adult *L. ehrenbergii* specimens in our study may however distort the results, and a similar trend in this species cannot be rejected. There was, however, a difference in stomach contents in juvenile and subadult *L. ehrenbergii* (regardless of habitat), with an opposite pattern to that of *L. fulviflamma*. Subadult *L. ehrenbergii* in both mangrove and seagrass/coral habitats had higher amounts of crabs in their diet than juveniles. Usmar (2012) found a similar trend in a snapper *Pagrus auratus* from New Zealand, where juveniles mainly consumed benthic copepods, mysids,

and shrimp, while subadults and adults shifted to feed on larger crabs and bivalves.

The method used to estimate relative volumetric quantities in fish diet through stomach content analysis may be considered rather rough and subject to a fair amount of bias (Hyslop 1980). Furthermore, the technique assesses diet over very small temporal scales (hours). However, by combining stomach content analysis with stable isotope analysis many of these weaknesses can be circumvented and both short- and long-term dietary changes can be studied. Due to stable isotope ratios in animal tissue being based on actual food assimilation, they reflect, on average, the diet over the previous weeks to months (Hobson 1999). In our study, stomach content data corroborate the isotope pattern of a shift in resource use between juvenile, subadult, and adult fish. Stable isotope analysis was consistent with stomach content findings, suggesting that both methods have given a representative picture of *Lutjanus fulviflamma* and *L. ehrenbergii* diets, despite some limitations in stomach sample sizes.

A number of explanations have been suggested as to why diet shifts occur. According to optimal foraging theory, larger predators tend to consume larger prey to maximize the energetic gain relative to capture effort (Schoener 1971). In our study, prey size increased with fish life stage and size (authors' pers. obs.), consistent with the theory. However, ontogenetic changes in morphology, such as jaw size and strength, have also been suggested as reasons for ontogenetic diet changes (Usmar 2012). An alternative explanation may be an ontogenetic shift in habitat. Results from the SIAR analysis show that isotope signals in *Lutjanus fulviflamma* and *L. ehrenbergii* correspond well to those of food items collected in the same habitats. For example, the contribution of prey fish from seagrass/coral areas was high in adult *L. fulviflamma* caught in similar habitats. Similarly, the contribution of prey crabs from mangrove areas was high in juvenile *L. ehrenbergii* caught in mangrove creeks. Furthermore, the $\delta^{13}C$ values of juveniles from both species differed significantly from the $\delta^{13}C$ values found in subadult and adult specimens caught in seagrass beds or coral reefs, implying ontogenetic changes in habitat. One can argue that $\delta^{13}C$ signatures will change as fish grow and give a false habitat signal. However, Vinagre et al. (2011) concluded that muscle $\delta^{13}C$ and $\delta^{15}N$ did not vary with body size or mass in 7 bony fishes from Portugal, suggesting that $\delta^{13}C$ values may be accurate base signatures and representative of the different habitats in our study. We do however acknowledge limitations in our results due to some overlap in isotope values between potential food items from mangrove and seagrass/coral areas. Furthermore, movement during different tidal regimes, as described by Dorenbosch et al. (2004), by juvenile snappers may also occur at Mafia Island, confounding our results. Further tagging studies and visual surveys are needed to clarify ontogenetic changes in habitat and diet.

In resemblance of our study, Cocheret de la Morinière et al. (2003a) and Verweij et al. (2008) found that $\delta^{13}C$ and $\delta^{15}N$ ratios in fish tissue from the juvenile snappers *Lutjanus apodus*, *L. griseus*, and *Ocyurus chrysurus* were similar to those of seagrass habitats and differed from those of adults on Caribbean coral reefs. Furthermore, Verweij et al. (2007) quantified movement of *L. apodus* between seagrass nursery areas and adult coral reef habitat by following artificially tagged juveniles and subadults. Recent studies, analyzing $\delta^{13}C$ values in otolith amino acids in *L. ehrenbergii*, also found that juveniles and adults utilize different habitats (McMahon et al. 2011, 2012). Migration corridors between inshore seagrass nurseries and offshore coral reefs were identified. Interestingly, some adults on oceanic reefs were found to have settled directly into reef habitats, although the majority of individuals on coastal reefs had used seagrass nurseries as juveniles (McMahon et al. 2012). The reason why mangroves and seagrass beds are used as nurseries are many, but a high abundance of food and shelter are the most commonly cited (Nagelkerken 2009). Experimental studies indicate that habitat complexity or food availability in mangroves and seagrass beds attract juvenile fishes (Cocheret de la Morinière et al. 2004, Verweij et al. 2006a). Recently Igulu et al. (2011) studied microhabitat selection in the settlement of *L. fulviflamma* larvae at Kunduchi, Tanzania. They found that *L. fulviflamma* larvae prefer seagrass and coral to mangrove roots and prefer to settle where conspecifics were present. The distribution pattern by juvenile *L. fulviflamma*, being more common in mangrove creeks than seagrass beds around Mafia Island (authors' pers. obs.), may hence reflect a refuge in mangroves compared to the generally higher predation pressure in seagrass beds and coral reefs, although these habitats are preferred.

Feeding migrations

Adult *Lutjanus fulviflamma* isotope values differed from those of *Epinephelus fasciatus*, a grouper with similar feeding habits as adult *L. fulviflamma* resid-

ing on coral reefs (Froese & Pauly 2009). *E. fasciatus* is known to live and feed on coral reefs. *L. fulviflamma* were observed on coral reefs during the day, and were caught in seagrass beds adjacent to coral reefs at night. According to local fishers, *L. fulviflamma* disperse from their schools on coral reefs and scatter to feed in seagrass beds at night. Stomach content analysis by Kamukuru & Mgaya (2004) on adult *L. fulviflamma* at Mafia Island revealed full stomachs around dusk and dawn, indicating that adults feed almost exclusively at night. Surprisingly, no adult *L. ehrenbergii* were observed or caught in seagrass beds at night, suggesting that they may feed differently from adult *L. fulviflamma* and not perform diel migrations to seagrass beds. A tagging study by Kaunda-Arara & Rose (2004) showed that adult *L. fulviflamma* swam distances up to 2 km, confirming that this species is capable of migrating between coral reefs and seagrass beds. Feeding migrations from coral reefs to adjacent seagrass beds have been documented for other snappers in the Florida Keys, USA (Luo et al. 2009), and in the US Virgin Islands (Hitt et al. 2011). The lack of potential food items from Mafia coral reefs in our study, however, limit our results, and further studies, tagging and following large individuals on coral reefs, are needed to confirm feeding migrations between coral reefs and seagrass beds by *L. fulviflamma*.

In summary, *Lutjanus fulviflamma* and *L. ehrenbergii* overlap in habitat use but differ significantly in diet and isotope values, indicating resource partitioning between the 2 species in Tanzanian waters. Juveniles and adults of *L. fulviflamma* seem to be ecologically separated for a considerable period of time and feed on more fish at increasingly higher trophic levels as they migrate from nursery habitat to coral reef. Inter- and intraspecific differences in diet, combined with size-related changes in dietary compositions and the occupation of different habitats by juvenile and adult *L. fulviflamma* and *L. ehrenbergii* may reduce the potential for competition for food resources among and within species. Increased knowledge of movement and feeding habits in commercial species such as *L. fulviflamma* and *L. ehrenbergii* is needed for proper management. Our results suggest that isolated management of the adult stocks would be insufficient to maintain their productivity, since different life stages occupy different habitats within the mangrove–seagrass–coral reef continuum. Furthermore, their role in food-web interactions across boundaries is of importance to understand the ecological connectivity within the tropical seascape.

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