



Stable isotope analysis reveals trophic diversity and partitioning in territorial damselfishes on a low-latitude coral reef

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Abstract

Investigating the niche overlap of ecologically similar species can reveal the mechanisms that drive spatial partitioning in high-diversity systems. Understanding how food resources are used and whether the diets of neighboring species are different are particularly important when considering the coexistence and functional role of species. Territorial damselfish on coral reefs are considered to be herbivores that defend algal mats from other food competitors. However, this guild contains numerous small species whose functional role and dietary diversification is poorly understood. Here, the relationships between diet and spatial distribution of seven intermediate-sized territorial damselfishes at Kimbe Bay, Papua New Guinea (5°30'S, 150°05'E) were investigated. These species partition habitat across three reef zones with distinct patterns of fine-scale distribution. It was predicted that neighboring species partition food resources with minimal dietary overlap. Examination of isotope ratios of carbon and nitrogen delineated three distinct feeding strategies: pelagic, reef-based, and an intermediate group feeding on both prey types. None of the species appear to be strict herbivores. Adjacent species exhibited high-intermediate trophic niche partitioning when examining pelagic versus reef-based production sources, with two species previously described as benthic herbivores exhibiting pelagic feeding. The study demonstrates that diet reinforces the patterns of spatial partitioning and coexistence among ecologically similar damselfishes. These findings add to a growing view that interspecific differences among similar species are lost when categorizing species into broad functional classifications, and that previous studies suggesting that territorial damselfish are strictly reef-based feeders may not be applicable in all systems or for all species.

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Introduction

Coral reefs harbor exceptional diversity and two primary goals of reef fish ecology are to understand the mechanisms that allow similar species to coexist and the functional importance of each species. Niche partitioning has been viewed as one of the key factors in promoting the diversification of fishes (Cavender-Bares et al. 2009; Ndiribe et al. 2013; Gajdzik et al. 2016). Thus, investigating resource use of coral reef fishes (e.g., food and habitat) and describing species in the context of broad functional roles have received considerable attention (Pratchett et al. 2011; Hughes et al. 2017; Richardson et al. 2018). The identification of a species' trophic niche, defined as the role of an organism in the environment in relation to its food (Silvertown 2004), is one of the main ecological parameters that provides the basis of a species' functional group (outlined in Mouillot et al. 2013). However, interspecific differences among similar species are lost when categorizing species into broad functional classifications (Brandl and Bellwood 2014; Tebbett

et al. 2017). The species-specific differences are particularly important when considering the functional role and coexistence of a taxon that is abundant and important to the overall community.

Damselfishes (Pomacentridae) are a major component of coral reef fish assemblages (Choat 1991). Their diversity and abundance have resulted in more attention from researchers than any other family of coral reef fishes, and they were the focus of early theoretical advancements in reef fish ecology (Smith and Tyler, 1972, 1973; Sale 1976, 1977; Doherty 1983). The highly site-attached habits, relatively small bodies, and territorial behavior allow ecological parameters, niche properties, behavioral interactions and patterns of co-existence to be readily quantified (Robertson and Lassig 1980; Waldner and Robertson 1980). Within species, competitive effects on survival (Jones 1987a, b) and the drivers of abundance and distribution across coral reef habitats are well known (Meekan et al. 1995; Robertson 1996). Among species, habitat partitioning along the natural reef profile gradient—the reef flat, reef crest, and reef slope (Bay et al. 2001; Ceccarelli 2007; Chaves et al. 2012; Eurich et al. 2018a), and microhabitat partitioning (Medeiros et al. 2010, 2016) have been well documented. Recently, damselfish have again been used as a model species to test the mechanisms of coexistence due to the family's interspecific differences in habitat use and competitive interactions (Bonin et al. 2015; Eurich et al. 2018b). Ecological theory has since progressed from a focus on pairwise interactions among species to a multifactorial perspective of the process and mechanisms that govern competition within a community (Jones 1991; Hixon et al. 2002; Wiens et al. 2010; Pereira et al. 2015). Thus, when examining niche partitioning and coexistence within reef fish communities, it is important to document similarities and differences among species across important resource axes, such as depth (MacDonald et al. 2018). While studies of habitat partitioning have generated a substantial amount of evidence, the diet or trophic niche of pomacentrids has received much less attention at this scale (but see, Ceccarelli 2007; Frédérick et al. 2009; Gajdzik et al. 2016).

Territorial damselfish, commonly referred to as “farmers”, play an integral role in the benthic structure of coral reefs through their role as small-bodied consumers and aggressive holders of benthic space (Hixon and Brostoff 1983, 1996; Hata and Kato 2004; Ceccarelli et al. 2005). Early dietary research defined the guild as herbivorous, but studies were biased towards a few larger, more aggressive species that maintain conspicuous algal mats, such as extensive farming species from the genera *Stegastes*, *Dischistodus*, and *Hemiglyphidodon* (Meekan et al. 1995; Wilson and Bellwood 1997; Ceccarelli et al. 2001; Jones et al. 2006). In addition, early studies that examined the trophic niche of territorial damselfishes used gut content (stomach) analysis, which favors the observer to categorize matter as

algae or detritus instead of pelagic materials (Polunin and Klumpp 1989; Ceccarelli 2007; Feitosa et al. 2012). However, it is important to note there are ambiguities associated with the functional term “herbivorous” within the context of territorial damselfish and that different types of herbivory exist in the literature (reviewed by Horn 1989; Hata and Ceccarelli 2016; Clements et al. 2016). Commonly, herbivores are described as species that remove algal matter from the substratum for consumption, but species that incidentally or deliberately remove algae for other means, such as farming, were included in early studies (Lassuy 1980; Steneck 1988). For the purposes of this study, any species that removes algae from the substratum for direct consumption is described as herbivorous to differentiate between diet contributions, farming behavior, and ecological role (Clements et al. 2016). Successive studies have now provided contrary evidence indicating that not all farming damselfish are strictly herbivorous (reviewed by Hata and Ceccarelli 2016). Instead, territorial damselfish appear to act as benthic-associated omnivorous generalists, with highly opportunistic diets and feeding plasticity (Frédérick et al. 2016). Nonetheless, our empirical understanding of territorial damselfishes trophic niche has been limited by the absence of detailed information on the extent of diet and resource partitioning for the majority of smaller territorial species from the genera *Pomacentrus*, *Chrysiptera*, *Plectroglyphidodon*, and *Neoglyphidodon* (but see Ceccarelli 2007).

Stable isotopes are increasingly used in ecology to study diet and trophic niche analysis as they reflect tissue assimilation from prey and are not hindered by stomach content analysis biases [pomacentrids reviewed by Frédérick et al. (2016)]. While stomach content data are a snapshot that reflects the most recent meals and may not represent the overall diet, stable isotopes of a consumer are concurrently integrated from various prey tissues at a rate slower than digestion (Matley et al. 2016). Thus, stable isotope analysis provides a measure of the diet contributions over longer periods of time (Post 2002). Although stable isotope analysis does not provide high-resolution dietary information, it can be used to estimate contribution of production sources (Jackson et al. 2011; Parnell et al. 2012). The ratio of carbon ($\delta^{13}\text{C}$) is commonly used because it indicates food sources or habitat (i.e., limited enrichment between predator and prey; Michener and Schell 1994), while the ratio of nitrogen ($\delta^{15}\text{N}$) indicates trophic level (i.e., consistent enrichment between predator and prey; Minigawa and Wada 1984). Moreover, stable isotope analysis can be used to assess the trophic niche width and overlap among populations or species at similar spatial and temporal scales (Bearhop et al. 2004; Frédérick et al. 2009; Gajdzik et al. 2016). Few studies have segregated pelagic and reef-based diet contributions for a multi-species community of competing fishes to directly explore trophic niche partitioning within a guild.

Here, we explore the trophic niche diversification and partitioning in a guild of seven territorial damselfish. At our study location, Kimbe Bay, Papua New Guinea (PNG), these species partition the reef flat, reef crest, and upper reef slope habits on a fine scale (1–2 m) (Eurich et al. 2018a). The distinctive pattern of zonal distribution along this steep physical gradient of aspect and depth provides an experimentally tractable system for isolating niche partitioning. Eurich et al. (2018a) found that neighboring damselfish constrain their microhabitat use to facilitate the co-habitation of reef zones, but that microhabitat selectivity alone was insufficient in explaining the distinct zonation and limited distributional overlap. We hypothesize that in this highly partitioned ecological community, where there appears to be intense competition for resources, neighboring territorial damselfish may partition food resources. Studies have previously quantified diet contributions, but no isotopic analysis has been conducted for territorial damselfish to date to specifically target pelagic-based food sources. In the present study, we quantify trophic niches of the seven damselfish through the use of stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). Specifically, we asked: (1) To what degree does the generalization that territorial damselfish are herbivores apply to the seven PNG species?; (2) What is the variation in trophic position among species and to what extent does body size influence trophic diversification?; and (3) Are neighboring species more likely to partition food resources than non-neighboring species?

Materials and methods

Study site and species

Samples were collected from platform reefs in Kimbe Bay, PNG (5°30'S, 150°05'E) and prepared at Mahonia Na Dari Research and Conservation Centre. Kimbe Bay lies within the Coral Triangle of the Indo-Pacific, the region recognized for the greatest richness of marine species world-wide (Roberts et al. 2002). The two inshore reefs, Garbuna and Luba Luba, were selected as sample locations due to similarities in species composition and reef aspect. Both reefs are nearshore (< 1 km from land), and have a similar reef profile that can be clearly divided into typical coral reef zones, comprising a reef flat (exposed during low tides), reef crest, and reef slope.

At Kimbe Bay, seven species of territorial damselfish partition space along a gradient that extends from the reef flat to the reef crest and down the reef slope in the respective order; *Pomacentrus tripunctatus*, *Chrysiptera unimaculata*, *Pomacentrus bankanensis*, *Pomacentrus adelus*, *Plectroglyphidodon lacrymatus*, *Neoglyphidodon nigroris*, and *Pomacentrus burroughi*. Distribution patterns are characterized by a distinct zonation parallel to the reef crest with each species

restricted to subzones of just a few meters wide along the reef profile gradient (described in Eurich et al. 2018a). Further, the seven species are known to inhabit fixed territories (mean 1–1.5 m² territory sizes per individual) and are highly site attached (Ceccarelli et al. 2001). In Kimbe Bay, all species occupy rubble (dead coral fragments) and filamentous algae microhabitats, influence the algal assemblage within the territorial boundary by weeding or farming, and defend their territories against conspecifics, interspecific competitors, and other benthic feeding fishes (Hata and Kato 2004; Ceccarelli 2007; Eurich et al. 2018a, c).

All species have recently been categorized as predominantly (though not necessarily exclusive) benthic-feeding 'intermediate farmers', with the exception of *P. lacrymatus*, which was classified as an 'extensive farmer' [reviewed by Pratchett et al. (2016)]. Where, intermediate farmers maintain discrete, but significantly different to the surrounding environment, areas of algal turf (Emslie et al. 2012), and extensive farmers maintain small amounts of filamentous rhodophytes and a diverse assemblage of indigestible macroalgae (Hata and Ceccarelli 2016 and its citations). The act of farming makes it difficult for an observer to visually census the bite composition and targeted food resource of territorial damselfishes successfully. Thus, most recent studies have used gut content analysis to assess diet contribution, with only two employing isotopic analysis (Table 1). Prior isotopic research has included only two of the territorial damselfish species used in the present study (Frédérich et al. 2009; Gajdzik et al. 2016). There has been no common methodology to describe diet and information for most species is conflicting, limited, or non-existing.

Sample collection

All samples were collected in the morning (between 8 a.m. and 12 p.m.) within a 20-m-wide section of reef extending from the back of the reef flat to 25 m down the reef slope. To compare the seven territorial damselfish trophic niches, we aimed to capture dominant producers and consumers to represent the key functional groups common to all sites, rather than trying to sample all possible producers and consumers (Table 2). Four representative obligate fishes with known diets were selected as comparative species: mixed benthic feeder of algal material, cyanobacteria, detritus, and sediment *Siganus vulpinus* (Hoey et al. 2013), detritivore *Ctenochaetus striatus* (Tebbett et al. 2017), pelagic planktivore *Chromis xanthura* (Greenwood et al. 2010), and demersal planktivore *Pomacentrus nigromanus* (Pratchett et al. 2016). The focal territorial damselfish and obligate fishes were captured with clove oil or speared and brought to the surface immediately, euthanized in a 50% seawater–ice bath, and placed on ice for transport to the lab. The total length and details of capture of each fish were recorded. To

Table 1 Summary of previous studies on territorial damselfish diet contributions using different methods

Species	Method	Pelagic	Detritus	Algae	Invertebrates	Location	References
<i>P. tripunctatus</i>	GC		~70%	~30%		PNG	Ceccarelli (2007)
	GC		~55%	~45%		GBR	Ceccarelli (2007)
<i>C. unimaculata</i>	SI			Herbivore		M	Frédérich et al. (2009)
	GC		~65%	~35%		PNG	Ceccarelli (2007)
	GC	~3%	~7%	~90%		M	Frédérich et al. (2009)
	GC		~45%	~55%		PNG	Ceccarelli (2007)
<i>P. bankanensis</i>	VIS			Herbivore		GBR	Meekan et al. (1995)
	GC		~25%	~75%		PNG	Ceccarelli (2007)
<i>P. adelus</i>	GC		~5%	~85%	~10% ^a	GBR	Kramer et al. (2013)
	SI			Herbivore		FP	Gajdzik et al. (2016)
<i>P. lacrymatus</i>	SI		Omnivore ^b			M	Frédérich et al. (2009)
	GC		~25%	~75%		FP	Gajdzik et al. (2016)
	GC	~15%	~1%	~60% ^c		M	Frédérich et al. (2009)
	GC		~10%	~90%	~25%	PNG	Ceccarelli (2007)
	GC/VIS		NA	~95% ^d		PNG	Jones et al. (2006)
	VIS		NA	99.9%		GBR	Polunin and Klumpp (1989)
	VIS			Herbivore		GBR	Meekan et al. (1995)
<i>N. nigroris</i>	VIS		Omnivore			GBR	Allen (1975)
<i>P. burroughi</i>	GC		~40%	~60%		PNG	Ceccarelli (2007)

Methods: *SI* stable isotopes, *GC* gut content analysis (stomach content), and *VIS* visual observation (direct feeding). Location: *PNG* Papua New Guinea, *GBR* Great Barrier Reef, *M* Madagascar, Indian Ocean, and *FP* French Polynesia, Pacific Ocean. Species: *Pomacentrus tripunctatus*, *Chrysiptera unimaculata*, *Pomacentrus bankanensis*, *Pomacentrus adelus*, *Plectroglyphidodon lacrymatus*, *Neoglyphidodon nigroris*, *Pomacentrus burroughi*

^aKramer et al. (2013) recorded vagile invertebrates in the stomach of some individuals

^bFrédérich et al. (2009) stated that *P. lacrymatus* is an omnivore that feeds mainly on benthic algae

^cFrédérich et al. (2009) recorded ~25% vagile/sessile invertebrates in the stomach

^dJones et al. (2006) recorded ~40% epiphytic diatoms in the stomach, and ~5% bites on live coral substrate

represent planktonic production, we targeted filter feeding invertebrates: mussels *Septifer bilocularis* and barnacles Cirripedia (Post 2002; Baker et al. 2013). Tissue samples of epaxial and adductor muscles were dissected from the fishes and bivalves, respectively, and frozen (−20 °C) for isotopic analysis.

Different primary food sources (zooplankton, organic matter, algae, and benthic invertebrates) were taken from the same locations where the fishes were collected (Table 2). Zooplankton was sampled using a 250-µm plankton tow net by hand. The net was towed in a zig-zag pattern from the reef slope to the reef crest and reef flat at 1–2 m off the substrate avoiding prior sampled areas (Gajdzik et al. 2016). The tow was replicated three times within the fish-sampling period for two days (total six tows per site). Zooplankton samples were then pooled by day and site for analysis (4–5 mg per sample). Concurrently to strengthen the pelagic signature, particulate organic matter (POM) was obtained by collecting large quantities of settled particulates from thick algal turf mats (Frédérich et al. 2009). For the purposes of this study, POM is used in a broad sense, i.e., to describe amorphous material that

originated from the water column with no visible structure (Wilson and Bellwood 1997). The particulates were transferred from the substrate to seawater filled plastic bags by pipette dropper. Live materials such as microscopic algae (< 1 mm), crustaceans, and sand particles were later removed by sieve and microscope. While POM samples were collected from the substrate across the reef, this material was previously suspended in the water column and represents settled material that would have been available to pelagic feeders when suspended across the reef profile. Similar to the zooplankton, the POM samples were then pooled by day and site for analysis (4–5 mg per sample). For the benthic signature, four different types of algae were collected for dietary comparisons (following Ceccarelli 2007): red algae *Hypnea* spp., green algae, brown algae *Turbinaria ornata*, and mixed turf (incl. *Amphiroa* spp. and *Gelidiopsis* spp.; defined by Hay 1981). All alga samples were rinsed and cleaned of epiphytes, other alga species, crustaceans, and POM. Crustaceans (vagile invertebrates; portunid and xanthid crabs) were collected from the mixed turf samples under a microscope and pooled.

Table 2 Putative functional groups and sample metrics per site for focal territorial damselfish, representative obligates, diet sources, and primary producers that were collected at Kimbe Bay, Papua New Guinea

Taxa	Putative group	Abbreviation	Sampling method	No. of samples	Mean body size \pm SE (mm L_t)	Range of body sizes (mm L_t)
<i>Pomacentrus tripunctatus</i>	Focal	<i>P. trip</i>	Spearfishing	12	56 \pm 1.5	46–64
<i>Chrysiptera unimaculata</i>	Focal	<i>C. unim</i>	Spearfishing	12	61 \pm 0.8	56–66
<i>Pomacentrus bankanensis</i>	Focal	<i>P. bank</i>	Spearfishing	12	59 \pm 1.5	51–67
<i>Pomacentrus adelus</i>	Focal	<i>P. adel</i>	Spearfishing	12	65 \pm 0.9	58–69
<i>Plectroglyphidodon lacrymatus</i>	Focal	<i>P. lacr</i>	Spearfishing	12	83 \pm 1.5	75–91
<i>Neoglyphidodon nigroris</i>	Focal	<i>N. nigr</i>	Spearfishing	12	85 \pm 3.4	63–100
<i>Pomacentrus burroughi</i>	Focal	<i>P. burr</i>	Spearfishing	12	69 \pm 1.8	55–77
<i>Chromis xanthurus</i>	Pelagic planktivore	<i>C. xant</i>	Spearfishing	6	102 \pm 5.7	88–122
<i>Pomacentrus nigromanus</i>	Demersal planktivore	<i>P. nigr</i>	Spearfishing	6	72 \pm 2.5	62–80
<i>Siganus vulpinus</i>	Mixed benthic feeder ^b	<i>S. vulp</i>	Spearfishing	6	167 \pm 5.5	146–185
<i>Ctenochaetus striatus</i>	Detritivore	<i>C. stri</i>	Spearfishing	6	141 \pm 9.3	95–156
Mussel	Consumer	–	By hand	12	–	–
Barnacle	Consumer	–	By hand	4 ^c	–	–
Red algae (<i>Hypnea</i>)	Producer	–	By hand	12	–	–
Green algae	Producer	–	By hand	12	–	–
Brown algae (<i>Turbinaria</i>)	Producer	–	By hand	12	–	–
Mixed turf (incl. <i>Amphiroa</i> , <i>Gelidiopsis</i>) ^a	Producer	–	By hand	12	–	–
Vagile invertebrates (Crustacea)	Omnivore consumer	–	By hand	12	–	15–50
Zooplankton	Pelagic consumer	–	Tow net	4 ^c	–	0.25–0.50 ^d
Organic matter	Producer	POM	By hand	4 ^c	–	–

^aFor the purpose of this study, we used the definition of turf algae from Hay (1981) as masses of tightly packed upright branches that were dominated by filamentous species

^bAlgal material, cyanobacteria, detritus, and sediment (Hoey et al. 2013)

^cEach sample consisted of many pooled individuals or matter from one location and temporal period

^dPresumed range of body sizes based on mesh size of plankton net

Stable isotope analysis

Samples were rinsed and soaked in distilled water for > 30 min to remove salts, and dried for 48 h at 70 °C. After no residual water weight was found, samples were ground into homogeneous fine powder using a glass ball mill grinder (Biospec Mini Bead Beater Model 3110BX milling unit). Carbonate contamination of algae, zooplankton, barnacle, vagile invertebrate (ground whole) and POM samples were tested using three drops of 1 N HCl on a small subsample. If effervescence was visible, the presence of carbonates was removed from the subsample by overnight acidification, rinsing, and re-drying (Baker et al. 2013). The process was repeated until no inorganic carbon was found to ensure the carbon stable isotope ratios were unbiased (Frisch et al. 2014). For acid-treated samples (i.e., turf algae, zooplankton, vagile invertebrate, and POM), $\delta^{13}\text{C}$ was measured in the acid-treated subsample and $\delta^{15}\text{N}$ was measured in the untreated subsample (Pinnegar and Polunin 1999; Schlacher and Connolly 2014).

Samples were encapsulated in tin cups and weighed five times to the nearest 0.0001 g and averaged. Stable isotope ratios of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) were measured using an isotope ratio mass spectrometer (PDZ Europa 20-20, Sercon Ltd., Cheshire, UK) coupled in continuous-flow to an elemental analyzer (PDZ Europa ANCA-GSL, Sercon Ltd., Cheshire, UK) at the UC Davis Stable Isotope Facility, Davis, California, USA. Stable isotope ratio values are expressed in parts per thousand (‰) using the standard δ notation:

$$\delta X = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000, \quad (1)$$

where X is ^{15}N or ^{13}C , R_{sample} is the ratio ($^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$) in the sample, and R_{standard} is the ratio in the standard (Coplen 2011). The standard reference material was Pee Dee Belemnite (vPDB) carbonate and atmospheric N_2 for carbon and nitrogen samples, respectively. Experimental precision

(standard deviation of replicates of internal laboratory standard) was 0.1‰ for $\delta^{13}\text{C}$ and 0.2‰ for $\delta^{15}\text{N}$.

Data analyses

Isotopic biplots ($\delta^{13}\text{C}$ or $\delta^{15}\text{N}$) were created to visualize niche space by two main approaches. First, the mean \pm SE of all species sampled were plotted. The second approach utilized the SIBER package (Jackson et al. 2011) in R (R Core Team 2017), in which standard ellipses were fitted (40% confidence level) for each fish species. The size of ellipses was compared among damselfish after fitting Bayesian models (10^4 iterations) adjusted for small sample size (SEA_C) to the data as described by Jackson et al. (2011). Differences in SEA_C size were considered significant when $\geq 95\%$ of posterior draws for one species were smaller than the other. The area overlap between species/ellipses was also quantified as a percentage of shared isotopic space, to determine if neighboring species were more likely to partition food resources than non-neighboring species. Species with shared overlap $> 60\%$ were considered to have significant shared-niche space based on a criterion used by Schoener (1968). Standard ellipses were also plotted for the obligate consumers (*S. vulpinus*, *C. striatus*, *P. nigromanus*, and *C. xanthura*) as a reference considering their known feeding patterns. Data from Garbuna and Luba Luba were pooled for graphical presentation and final analysis where there were no differences among reefs.

The trophic positions (TP) of damselfish were calculated to reduce any inter-reef differences associated with baseline $\delta^{15}\text{N}$ values. Scaled TP were calculated following Hussey et al. (2014):

$$\text{TP}_{\text{scaled}} = \frac{\log(\delta^{15}\text{N}_{\text{lim}} - \delta^{15}\text{N}_{\text{baseline}}) - \log(\delta^{15}\text{N}_{\text{lim}} - \delta^{15}\text{N}_{\text{consumer}})}{k} + \text{TP}_{\text{baseline}}, \quad (2)$$

where $\delta^{15}\text{N}_{\text{lim}}$ represents the saturating isotope limit as TP increases, and occurs when rates of ^{15}N and ^{14}N uptake equal those of ^{15}N and ^{14}N elimination, as determined through meta-analysis for fish (21.93‰); and k is the rate at which $\delta^{15}\text{N}_{\text{consumer}}$ approaches $\delta^{15}\text{N}_{\text{lim}}$ per trophic transfer (0.14; Hussey et al. 2014). *Siganus vulpinus* ($\text{TP}_{\text{baseline}} = 2$) was selected as the TP baseline organism because it demonstrated consistent within-reef $\delta^{15}\text{N}$ values. Analysis of variance (ANOVA—type III error) tested if TP was influenced by damselfish species, reef, or habitat zone. The same ANOVA model was run with $\delta^{13}\text{C}$ as a response variable instead of TP to explore whether feeding habitat was affected

by the same explanatory variables. Tukey's HSD was applied as a post hoc test to determine within-factor differences if any of the above variables were significant ($P < 0.05$). Linear regressions were applied to each damselfish species to test for variation in TP or $\delta^{13}\text{C}$ and assess how body size influences trophic diversification.

To estimate the contributions of pelagic versus reef-based sources of primary production, we used Bayesian mixing models from the *simmr* package (Parnell 2016) in R (R Core Team 2017). A two-source model was used with all algal species combined to represent reef-based contribution, whereas zooplankton and POM were pooled for pelagic sources. POM was used as a pelagic source rather than a reef-based source due to the sampling method and the observed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (similar $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to planktivorous obligates and zooplankton). For comparative purposes, a separate model was produced using the following food items as independent source materials: zooplankton, POM, red algae, green algae, brown algae, mixed turf, and vagile invertebrates. Correction means (or diet-tissue discrimination factors) were set to 1.62‰ for $\delta^{13}\text{C}$ and 3.69‰ for $\delta^{15}\text{N}$. These values were chosen using stable isotope output from obligate consumers relative to food sources. Specifically, correction mean for $\delta^{13}\text{C}$ was calculated by subtracting the mean $\delta^{13}\text{C}$ values of zooplankton from mean $\delta^{13}\text{C}$ values of obligate planktivores (*P. nigromanus* and *C. xanthura*). Alternatively, $\delta^{15}\text{N}$ correction mean was derived by subtracting the mean $\delta^{15}\text{N}$ values of algae (pooled) from mean $\delta^{15}\text{N}$ values of the obligate reef-based feeder (*C. striatus*). This approach was deemed adequate because obligate consumers demonstrated consistent isotope values relative to the isotope axis ($\delta^{13}\text{C}$ or $\delta^{15}\text{N}$) pertaining to the animal's known diet regime (i.e., the reef-based feeder had little variation in $\delta^{15}\text{N}$ values; the planktivores had little variation in $\delta^{13}\text{C}$ values). Correction error was set at a conservative 0.5‰ for all diet items based on values calculated by Matley et al. (2016). Matrix plot correlations were used to evaluate if the model had difficulty separating prey sources due to proximity in isospace. No lipid extraction or normalization was performed on the isotope samples or data. White muscle of fish is typically low in lipid content (Pinnegar and Polunin 1999), so lipid removal was deemed unnecessary. Additionally, in the present study, consumers regularly had a C:N below 4, indicating that lipid content did not bias $\delta^{13}\text{C}$ values (Post et al. 2007). Dietary contributions (food-production source) are presented as the range between 25 and 75% credibility quantiles with error bars extending to the maximum and minimal values (97.5% and 2.5%, respectively).

Results

Trophic diversification among species

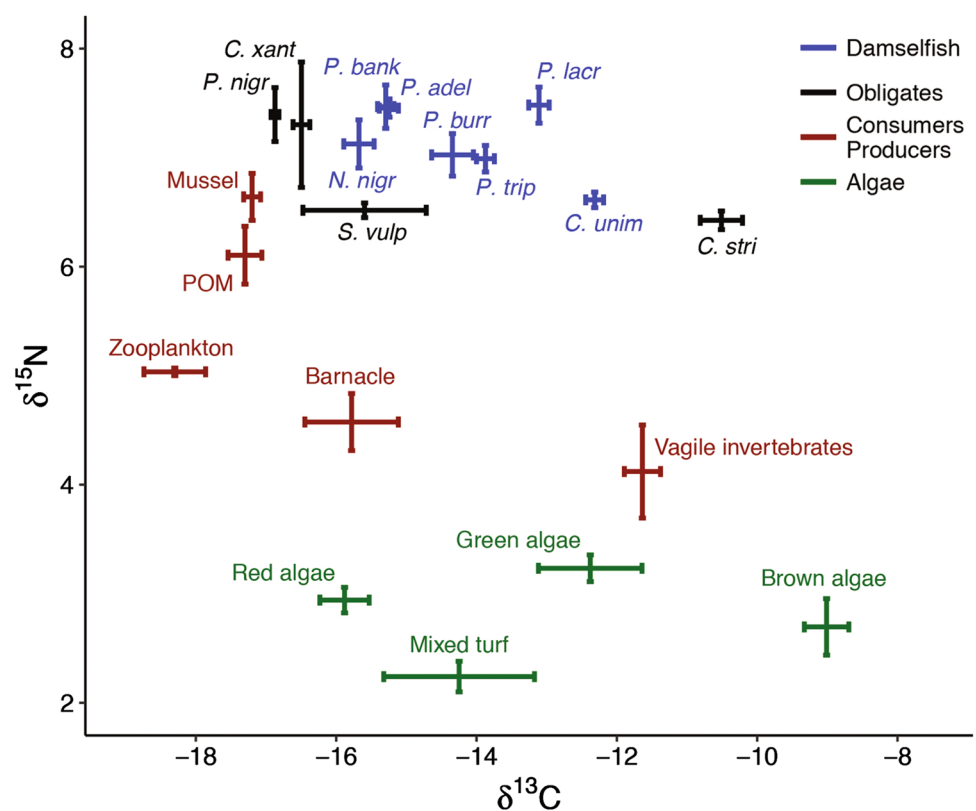
The stable isotope biplot of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for all organisms showed evident segregation between and within main trophic groups (e.g., algae, zooplankton/invertebrates, and fish; Fig. 1). Along the $\delta^{15}\text{N}$ axis (equivalent to TP), as expected, fish (damsel fish and obligate), had the highest values, followed by primary consumers/producers and algae, respectively. Within each trophic group, $\delta^{15}\text{N}$ values had limited variation (i.e., between 1 and 3‰). Greater variation existed along the $\delta^{13}\text{C}$ axis (i.e., 4–7‰) demonstrating a gradient between pelagic-based (e.g., POM and zooplankton; more negative $\delta^{13}\text{C}$ values) and reef-based (e.g., vagile invertebrates and *Ctenochaetus striatus*; less negative $\delta^{13}\text{C}$ values) input. Specifically for the fishes, the obligate consumers bounded the isotopic extent of the damselfish, again, with greater variation in $\delta^{13}\text{C}$ values compared to $\delta^{15}\text{N}$ values.

To assess the generalization that territorial damselfish are herbivores, a two-source mixing model was used to provide further insight into the proportion of pelagic input compared to that of reef-based contribution. Pelagic sources contributed at least 75% (based on 25–75% quantiles and max–min values) of prey for *Pomacentrus bankanensis* and

Neoglyphidodon nigroris, whereas reef-based sources contributed to the majority of prey for *Chrysiptera unimaculata* (Fig. 2a). Other species were more difficult to resolve within the two-source context because they likely feed on a variety of pelagic and reef-based sources, nevertheless, the mixing model showed *Pomacentrus adelus* consumed a greater proportion of pelagic prey, while *Pomacentrus tripunctatus*, *Plectroglyphidodon lacrymatus*, and *Pomacentrus burroughi* consumed more reef-based prey (Fig. 2a). Similar results were found when all prey were analyzed as independent sources; specifically, zooplankton was an important prey source for *P. bankanensis* and *N. nigroris*, and red algae contributed > 50% of prey (based on 25–75% quantiles and max–min values) to *P. tripunctatus*, *P. burroughi*, and *C. unimaculata* (Fig. A1). The mixing model for the obligate consumers verified the results of the present study with prior knowledge. The mixing model indicated high pelagic contribution in the muscle tissue of *Chromis xanthura* and *Pomacentrus nigromanus*, and high reef-based contribution in *Siganus vulpinus* and *C. striatus* (Fig. 2b).

The ANOVA testing whether species, reef, or habitat zone influenced TP or $\delta^{13}\text{C}$ showed that species (ANOVA, $F(6,82) = 3.89$, $P = 0.002$) and reef (ANOVA, $F(1,82) = 12.12$, $P < 0.001$; Garbuna and Luba Luba) influenced TP values, while only species (ANOVA, $F(6,82) = 36.41$, $P < 0.001$) influenced $\delta^{13}\text{C}$ values. Trophic

Fig. 1 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (expressed in ‰) signatures of producers and consumers at Kimbe Bay. Data points are group means with error bars representing \pm SE. Species abbreviations and sample sizes and are defined in Table 2



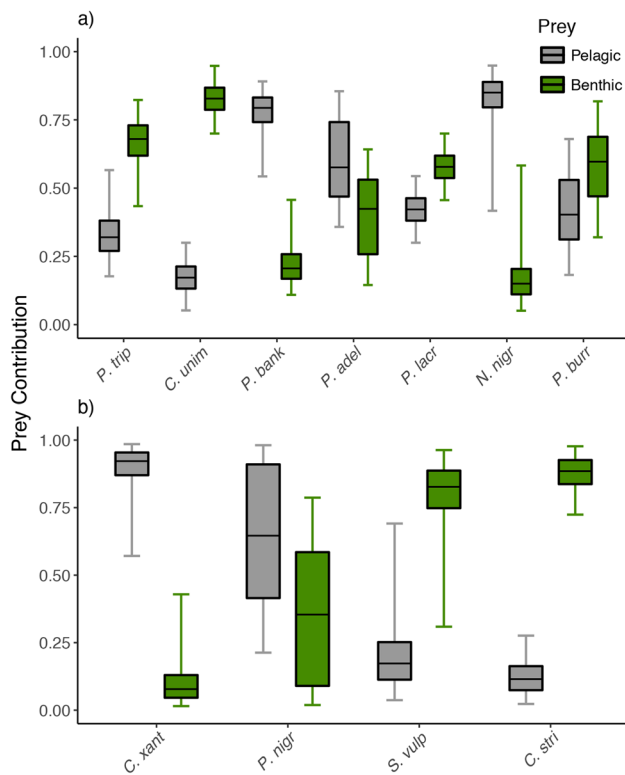


Fig. 2 Prey contribution of **a** territorial damselfish and **b** obligate consumers from the two-source pelagic (gray) versus reef-based (benthic; green) mixing model. Box and whisker plot displays the range between 25% and 75% credibility quantiles, with error bars extending to the maximum and minimal values (97.5% and 2.5%, respectively), and the median represented by the bold line. Species abbreviations and sample sizes are defined in Table 2

position of territorial damselfish at Garbuna was typically higher than at Luba Luba, and TP values of *C. unimaculata* were lower than *P. lacrymatus*, *P. adelus*, and *P. bankanensis* (Fig. 3a). For $\delta^{13}\text{C}$, Tukey's test showed that *C. unimaculata* and *P. lacrymatus* had higher (less negative) values than all other species. Also, *P. tripunctatus* had higher $\delta^{13}\text{C}$ values than *P. adelus*, *N. nigroris*, and *P. bankanensis*. Finally, $\delta^{13}\text{C}$ values of *P. burroughi* were higher than *N. nigroris* (Fig. 3b).

The range in body size and stable isotope values for each damselfish species sampled were small (Table 2). Additionally, the influence of body size on both TP and $\delta^{13}\text{C}$ values was not uniform among species (Fig. A2). Consequently, linear regressions were calculated separately to determine whether body size affected isotope values at a species level. There was a significant positive relationship between body size and TP values for *P. tripunctatus*, *P. bankanensis*, and *N. nigroris*. Carbon isotope values did not show any significant relationship with body size for any species (Fig. A2).

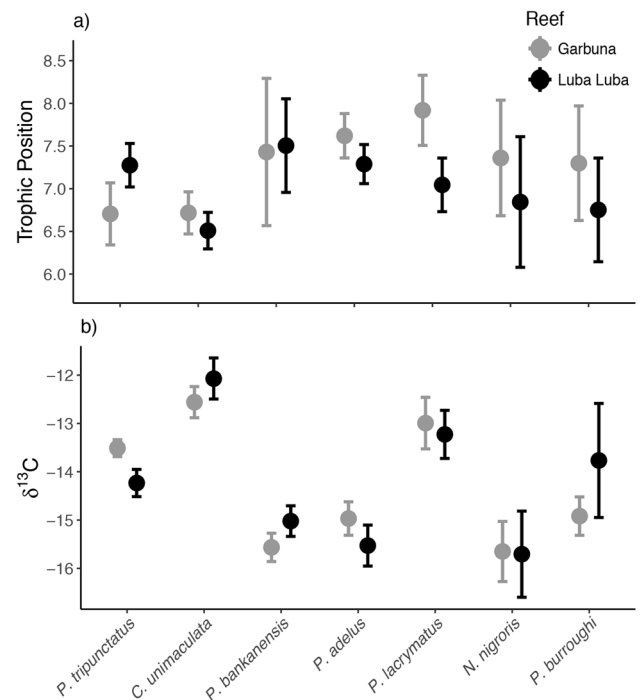


Fig. 3 Territorial damselfish **a** trophic position and **b** $\delta^{13}\text{C}$ (expressed in ‰) signatures between Garbuna (gray) and Luba Luba (black). Data points are group means with error bars representing \pm SE. Species abbreviations and sample sizes are defined in Table 2. Vertical axes are not comparable

Trophic partitioning between neighbors

The isotopic biplots of territorial damselfish differed markedly within each of the three reef zones (Fig. 4a). There were significant differences in isotopic space, with minimal–no overlap among neighboring species for all but one co-occurring pair (Table 3). There was no significant shared isotopic overlap among *P. tripunctatus* and *C. unimaculata* (0%), the species co-occurring on the back and middle reef flat, respectively. Additionally, on the other distribution boundary of *C. unimaculata*, there was no observed isotopic overlap between the three neighboring reef crest species: *C. unimaculata* and *P. adelus* (0%), *C. unimaculata* and *P. bankanensis* (0%), and *C. unimaculata* and *P. lacrymatus* (0%). On the reef crest, no significant isotopic overlap was observed between *P. adelus* and *P. lacrymatus* (0%) and *P. bankanensis* and *P. lacrymatus* (0%), although *P. adelus* and *P. bankanensis* (62%) demonstrated significant isotopic overlap. The ellipses of both *N. nigroris* and *P. burroughi*, the co-occurring reef slope species, were larger than all other damselfish (i.e., $\geq 95\%$ posterior draws of these species were larger than others), but had limited overlap in isotopic space (13%).

The obligate consumers showed clear trends in isospace reflecting their feeding modes (Fig. 4b; Fig. A1 for

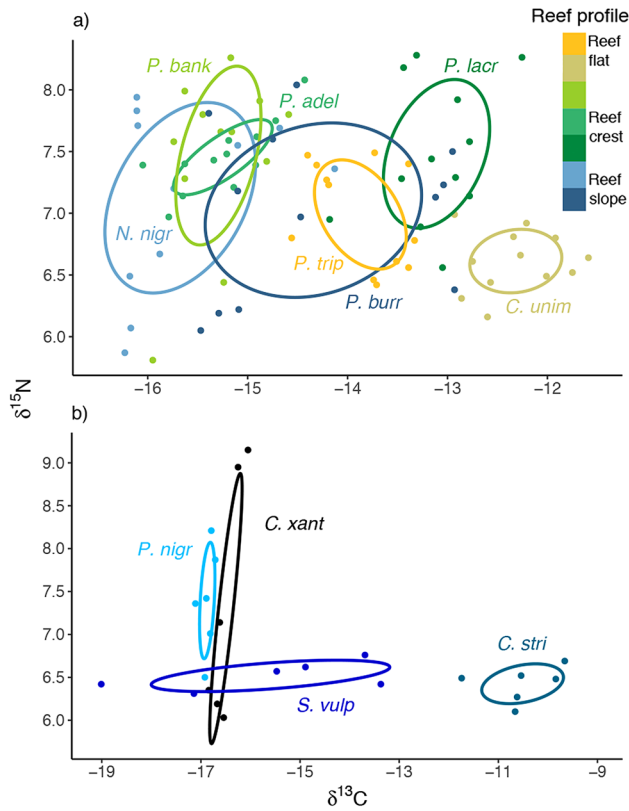


Fig. 4 Isotopic niche overlap for **a** territorial damselfish and **b** obligate consumers as a reference considering their known feeding patterns. Standard ellipse area (SEA_C) is depicted by a solid line with $\delta^{15}N$ and $\delta^{13}C$ values expressed in ‰. Territorial damselfish **a** are colored to match the distribution patterns along the reef profile gradient—reef flat (orange/yellow), reef crest (green), and reef slope (blue). Obligate consumers **b** are not colored to the reef profile gradient. Species abbreviations and sample sizes are defined in Table 2. Horizontal and vertical axes are not comparable

independent prey sources). Specifically, the planktivorous damselfish, *P. nigromanus* and *C. xanthura*, showed little variation in $\delta^{13}C$ values but a large range along the $\delta^{15}N$ -axis with values typically larger than the herbivorous *S. vulpinus*. In contrast, *S. vulpinus* exhibited a large range along the $\delta^{13}C$ -axis, while $\delta^{15}N$ values remained stable across individuals. *C. striatus* was centered at low $\delta^{15}N$ and high $\delta^{13}C$ within the biplot distinct from the other obligates.

Discussion

This study provides evidence for high levels of trophic diversification and niche partitioning in a guild of territorial damselfish on a high-diversity coral reef. The isotopic biplots of all seven territorial damselfish differed in $\delta^{15}N$ and $\delta^{13}C$ values. The isotopic distributions were linked to distribution patterns along the reef profile gradient—reef flat, reef crest, and reef slope. Where the distribution of species overlapped, these species were characterized by differences in source contributions. Adjacent species exhibited high–intermediate trophic niche partitioning when examining pelagic versus reef-based (benthic) production sources. We argue diet plays a supplementary role to habitat partitioning and competition in reinforcing the patterns of spatial partitioning and coexistence among ecologically similar species. In addition, our research also demonstrates the importance of considering interspecific differences when categorizing a guild of species to a single functional classification. We support prior evidence that territorial damselfish act as omnivorous generalists, with potentially opportunistic diets and feeding plasticity.

Table 3 Results of pairwise percent niche overlap (gray, top right) and isotopic area overlap (blue, bottom left) of territorial damselfish. Data are the mean of a species (row) relative to another species (column). One cell represents the overlap between the two species. Species are in order of distribution from the back of the reef flat to the reef crest and down the reef slope from left to right and top to bottom. Neighboring species are shaded darker (dark gray and dark blue) to highlight ecologically important pairs. Species abbreviations and sample sizes and are defined in Table 2

		Percent niche overlap						
		<i>P. trip</i>	<i>C. unim</i>	<i>P. bank</i>	<i>P. adel</i>	<i>P. lacr</i>	<i>N. nigr</i>	<i>P. burr</i>
Overlap area (‰ ²)	<i>P. trip</i>		0%	0%	0%	4%	0%	61%
	<i>C. unim</i>	0		0%	0%	0%	0%	0%
	<i>P. bank</i>	0	0		62%	0%	59%	15%
	<i>P. adel</i>	0	0	28		0%	50%	7%
	<i>P. lacr</i>	3	0	0	0		0%	14%
	<i>N. nigr</i>	0	0	68	26	0		13%
	<i>P. burr</i>	57	0	19	4	19	25	

Damselfishes, including known planktivores, corallivores, and territorial herbivorous species, have been previously assigned to three trophic groups based on feeding strategies—pelagic feeders: zooplanktivores, reef-based feeders: corallivores, algivores or herbivores, and an intermediate group: omnivores (Frédérich et al. 2009, 2016; Gajdzik et al. 2016). While comparative studies have not focused solely on territorial damselfish, a similar trophic diversity was observed within the guild of territorial damselfish in the present study. The trophic diversity within this group was much higher than expected based on the literature. Territorial damselfish are commonly classified as herbivorous or omnivorous (Ceccarelli et al. 2001; Frédéricich et al. 2016). However, we found evidence of planktivory within the guild. While this is not unique for pomacentrids, as damselfish (e.g., Chrominae) have served as a model for this trophic strategy (Frédérich et al. 2013), territorial damselfish have not previously been described as pelagic feeders. Through the integrated perspective of trophic role provided by stable isotopes, the present study documents *Pomacentrus bankanensis* and *Neoglyphidodon nigroris* as pelagic feeders (supporting prior J. G. Eurich pers. obs.) under the suggested criterion of a species to consume $\geq 70\%$ zooplanktonic prey or filamentous algae to not be considered an intermediate omnivore (Frédérich et al. 2016). This designation is supported based on similar trends in isospace as the obligate planktivores *Pomacentrus nigromanus* and *Chromis xanthurus*. Both *P. bankanensis* and *N. nigroris* were previously described as herbivores and benthic feeders under the intermediate trophic grouping (see Table 1; Hata and Ceccarelli 2016). The present study represents the first isotopic values for these species and highlights the need for species-specific evidence when assigning a species to broad taxa-related functional classifications.

Based on the isotope values and source-based mixing models, only one of the seven territorial damselfish species, *Chrysiptera unimaculata*, can be defined strictly as a reef-based (benthic) feeder and none were specialized herbivores. Our evidence supports a comparable isotopic study from Madagascar (Frédérich et al. 2009) and gut content analysis from the same region as the present study, Kimbe Bay (Ceccarelli 2007). However, while we define the species as a reef-based feeder, we explicitly do not provide evidence for this species to be classified functionally as an algivore (as in Frédéricich et al. 2009) due to the mixed benthic feeding obligate, *Siganus vulpinus*, having a more enriched $\delta^{13}\text{C}$ signature. While algal material comprises $\sim 30\%$ of the stomach content for *S. vulpinus*, Hoey et al. (2013) found cyanobacteria contributed $\sim 60\%$, which is known to enrich $\delta^{13}\text{C}$ signatures (Shahraki et al. 2014). Instead of classifying *C. unimaculata* as an algivore, based on prior literature and the isotopic values, we argue the species primary source of nutrients is detritus. In addition to detritus comprising

$\sim 65\%$ of the stomach content for *C. unimaculata* in a prior study (Ceccarelli 2007), the isotopic values in the present study are consistent with *Ctenochaetus striatus*, a known detritivore (Tebbett et al. 2017). Consequently, the broad trophic groups traditionally used to classify reef fishes need to be refined and subdivided to reflect differences in trophic partitioning as new methodology is established (e.g., Brandl and Bellwood 2014; Pratchett et al. 2016; Clements et al. 2016; Tebbett et al. 2018).

The intermediate group, also considered generalists or omnivorous, are composed of species that consume planktonic and benthic copepods, detritus, small vagile invertebrates, and filamentous algae (Kramer et al. 2013; Frédéricich et al. 2016; Hata and Ceccarelli 2016). Our research confirms the paradigm from recent literature (see Table 1) that the majority of territorial damselfish conform to this grouping. In the present study, four species belong to the intermediate feeding strategy: *Pomacentrus tripunctatus*, *Pomacentrus adelus*, *Pomacentrus burroughi*, and *Plectroglyphidodon lacrymatus*. While the resolution of stable isotopes does not provide dietary information at the prey species level (i.e., to corroborate vagile invertebrates; Ceccarelli 2007; Kramer et al. 2013), the four species exhibited distinct isotope ratios for C and N. We argue the four species are omnivorous with a diet comprising zooplankton, detritus and local, benthic prey. Ceccarelli (2007) found *P. tripunctatus*, *P. adelus*, and *P. burroughi* were generalists feeding on detritus, corticated algae, and filamentous algae in (almost) equal proportions, with *P. lacrymatus* acting as a specialist consuming microalgae. While Ceccarelli (2007) found no evidence for pelagic-derived materials, this was likely a limitation of gut content analysis. Furthermore, only the diet of *P. lacrymatus* has been previously examined through stable isotope analysis (Frédérich et al. 2009; Gajdzik et al. 2016). The discrepancies in diet at the species level between studies within the intermediate group can also be linked to opportunistic feeding. Frédéricich et al. (2009) documented planktivorous species shifting to demersal fish eggs to opportunistically exploit nutrient-rich food. Similarly, species with reef-based diets, including *P. lacrymatus* (Kuo and Shao 1991), occasionally switch exclusively to coral propagules in the water column during mass coral spawning events (Pratchett et al. 2001; McCormick 2003). While damselfish are known to feed opportunistically, the explicit partitioning of resources is a key factor in promoting the coexistence of closely related and otherwise ecologically equivalent species (Robertson and Lassig 1980; Waldner and Robertson 1980).

Ecological partitioning of distinct non-overlapping spatial distributions of damselfish along the reef gradient has been well documented globally (Bay et al. 2001; Ceccarelli 2007; Medeiros et al. 2010; Chaves et al. 2012). At Kimbe Bay, the seven species of territorial damselfish partition space

along the reef profile gradient with each species restricted to subzones of just a few meters wide. Eurich et al. (2018a) found that the species distributions were linked to levels of microhabitat selectivity and aggression between species. Further, through the use of a large-scale removal experiment, it was demonstrated that both direct and indirect competition among neighboring species helps to maintain the population boundaries (Eurich et al. 2018b). The present study provides additional evidence of trophic niche partitioning between adjacent species reinforcing the patterns of coexistence. The diets of co-occurring territorial damselfish within each of the three reef zones differed markedly with the (1) reef flat comprising a reef-based omnivore *P. tripunctatus* and detritivore *C. unimaculata*, (2) reef crest comprising a planktivore *P. bankanensis*, pelagic-based omnivore *P. adelus*, and a reef-based omnivore *P. lacrymatus*, and (3) reef slope comprising a planktivore *N. nigroris*, and a reef-based omnivore *P. burroughi*. This level of partitioning may reflect the high species diversity of territorial damselfish within the region. Previous work has clearly shown that pomacentrids can partition food types along various functional axes of their niches that relate to biological, ecological, and environmental factors (Frédérich et al. 2009, 2014; Gajdzik et al. 2016), but did not demonstrate differences in the trophic ecology among territorial damselfish. Kimbe Bay's high richness likely drives the need for ecological diversification within the guild of territorial damselfish (Gajdzik et al. 2018).

The dietary diversification observed is likely refined by differences in ecological versatility between adjacent species. While opportunistic feeding has been previously documented (as discussed above), neighboring territorial damselfish individuals exploited different feeding strategies over long-term diet trends during mutually inclusive time periods. Bearhop et al. (2004) discriminated between different types of generalists within a population, with species either all taking a wide range of food types (type A) or specializing in a different but narrow range of food types (type B). The large variation of $\delta^{13}\text{C}$ values with little difference in trophic position in the present study could be related to perpetual intra-population variability in prey choice (i.e., type B), thus facilitating coexistence (Frédérich et al. 2016). For example, the pelagic feeding species, *P. bankanensis* and *N. nigroris*, are within each respective neighboring species habitat space (i.e., reef zone and microhabitat use; Eurich et al. 2018a), but by foraging in the water column on pelagic resources competition is reduced. The generalist and flexible feeding strategies observed in this guild likely support the coexistence of multiple territorial damselfish species within a reef zone.

While this study focuses on adults due to the strong territorial interactions and spatial partitioning of adult territorial damselfish species (Bay et al. 2001; Ceccarelli 2007; Eurich et al. 2018a), it is notable that ontogenetic

shifts in diet have been observed for pomacentrids and other fishes. Damsel-fishes mainly consume pelagic copepods during the larval phase and switch to benthic invertebrates and algae upon settlement (Sampey et al. 2007). Further, some species shift between pelagic and reef-based foraging strategies from the juvenile to adult stage. Thus, for the purposes of determining dietary overlap and niche widths among species with known small-scale distribution differences, only adult individuals were used in the present study. While we observed a significant positive relationship between body size and TP values for *P. tripunctatus*, *P. bankanensis*, and *N. nigroris*, no significant relationship was found between carbon isotope values and body size for any species. Ontogenetic niche shifts can also lead to intraspecific variation between co-occurring individuals when sampling at different life-history stages (Araújo et al. 2011). In Kimbe Bay, territorial damselfish juveniles and adults occupied similar distributions within the reef profile but more sampling is required to empirically corroborate the findings of Araújo et al. (2011) and assess if ontogenetic size-related shifts or intraspecific variation is occurring (Pereira et al. 2015; Frédéricich et al. 2016). Explicit spatial and temporal sampling (e.g., multiple tissues and sampling periods) is required to further explore how feeding regimens and diet assimilation change within a species.

Here, we used stable isotope analysis to obtain estimates of dietary diversification. Similar protocols have commonly been used to quantify food-production source values for reef fishes (Bearhop et al. 2004; Frédéricich et al. 2009; Gajdzik et al. 2016). While this methodology is sufficient in assessing pelagic versus reef-based signatures, output can be influenced by several factors (Robbins et al. 2010). Due to variable diet-tissue discrimination factors (DTDFs) among species and prey types (e.g. Post 2002), different effects of prey quality (Robbins et al. 2010), and potentially variable effects of lipid content (Post et al. 2007), we caution against over-interpreting the specific details of the data in the present study, as these issues can complicate or bias interpretations. Due to the variety of potential dietary items for each species, we were unable to directly estimate DTDFs in situ and instead derived estimates from the obligate consumers. We acknowledge that DTDFs can be influenced by the above factors; however, this approach was used as more intensive prey sampling was outside the scope of this project. The overall findings of the present study are suggestive of trophic niche partitioning of food-production source values, which is sufficient in explaining the distribution patterns observed in this assemblage of closely related fishes.

Conclusions

We demonstrate that trophic niche partitioning contributes to fine-scale partitioning of space within reef zones and the limited distributional overlap of species on a high-diversity coral reef. Evidence presented here suggests that neighboring species utilize different feeding strategies that may facilitate coexistence. Dietary diversification, with microhabitat selectivity, likely minimizes direct and indirect competition for space, potentially a limiting factor on coral reefs. Despite territorial damselfish typically being described functionally as intermediate omnivores or herbivores, we found novel evidence of pelagic-based feeding within the guild through stable isotope analysis. These findings add to a growing view that interspecific differences among similar species are lost when categorizing species into broad functional classifications, and that previous studies suggesting that territorial damselfish are strictly reef-based feeders may not be applicable in all systems or for all species.

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Author contributions JGE, RB, MIM, and GPJ conceptualized and designed the study. JGE conducted the field sampling, data collection, and sample preparation. JKM performed statistical analysis. JGE and JKM interpreted the data and prepared the figures. JGE wrote the manuscript text. All authors reviewed the manuscript and gave final approval of the submitted version.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval All work carried out herein was in accordance with the James Cook University Animal Ethics Guidelines (JCU Animal Ethics approval A2106).

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