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Trophic ecology of co-occurring fishes in the Sundays River Valley irrigation ponds, assessed using stable isotope and gut content analyses

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Abstract

The analysis of food web structures has increased the understanding of the dynamics of organisms belonging to different trophic levels. In this study, the diet of two native species, *Glossogobius callidus* and *Gilchristella aestuaria*, was assessed in the presence of two non-native species, *Oreochromis mossambicus* and *Gambusia affinis*, in irrigation ponds, Eastern Cape Province, South Africa. The proportion of dietary items consumed and assimilated by the four fish species were inferred from gut contents and carbon and nitrogen stable isotope analysis. Stable isotope analysis revealed that both *G. affinis* and *O. mossambicus* had a larger isotopic niche size than *G. callidus* and *G. aestuaria*. Although *G. callidus* fed on benthic resources and *G. aestuaria* fed on phytoplankton, gut content analysis showed that *G. callidus*, *O. mossambicus* and *G. affinis* fed predominantly on benthic resources, whereas *G. aestuaria* fed mainly on plankton resources. Considerable niche overlap corroborates the view that resource competition is a major factor shaping the composition of the four fish species. This study highlighted the low diversity of the food web within the Sundays River Valley irrigation ponds, where food items are shared by all the small-bodied fishes.

KEYWORDS
food web, invasion, niche, Sundays River, trophic position

* Deceased.

1 | INTRODUCTION

Freshwater ecosystems are the most imperilled as a result of the introduction of alien species, habitat destruction and biological invasions (Craig *et al*., 2017; Darwall *et al*., 2018; Strayer & Dudgeon, 2010). The introduction of fish through human actions can have negative effects on recipient systems (Nati *et al*., 2018; Vitousek *et al*., 1997). The negative effects of introduced fish include biodiversity loss (Ellender & Weyl, 2014), biotic homogenisation, introduction of pathogens, hybridisation and the destabilisation of native freshwater communities and food webs through biological interactions, such as consumptive and non-consumptive effects (Lombard *et al*., 2017; Mofu, South, *et al*., 2019a; Wasserman *et al*., 2016). The introduction of alien species in new environments is driven by many factors, such as inter-basin water transfer schemes, biological control, aquaculture and sport fishing (Cambray, 2003; Chapman *et al*., 2019; Pimentel *et al*., 2005).

The food web structure of small water bodies has been understudied, and their role remains largely unknown; therefore, understanding how species potentially compete for the same resources is of great practical and conceptual importance (Comte, Cucherousset, & Olden, 2016b; Sato *et al*., 2010). Quantifying interspecific interactions between native and alien species within a particular ecosystem is integral in understanding community biology by providing information on trophic dynamics related to competition, resource partitioning and predation (Copp *et al*., 2017; Hayden *et al*., 2014). Gut content analysis is considered standard practice for identifying food sources and can provide direct evidence of an organism's potential food resources (*i.e*., prey) (Foley *et al*., 2017; Hyslop, 1980). Stable isotope analysis is a comparatively cost-effective and integrative tool that provides information on the long-term assimilation of dietary food sources, as well as on the trophic ecology of predator–prey interactions (Comte, Cucherousset, Boulêtreau, & Olden, 2016a; Vander Zanden *et al*., 2015). In addition, stable isotope analysis can be used to provide insights into competition (Carbia *et al*., 2020) and resource availability (Park *et al*., 2017). Stable isotope analysis is an increasingly important tool in the study of ecological food webs and has been used in conjunction with stomach content analysis for investigating trophic dynamics in freshwater ecosystems (Eurich *et al*., 2019; Peterson & Fry, 1987).

In dietary studies, the composition of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) is the most commonly investigated (Post, 2002), especially in freshwater systems. The $\delta^{13}C$ signature provides information on the resource use of predators, the sources of carbon from the bottom of the food web and the overall diet preference of the predators (Davias *et al*., 2014), whereas the $\delta^{15}N$ is used to calculate trophic position and can be used to measure energy transfer (Vander Zanden *et al*., 1997). Often, both the $\delta^{13}C$ and $\delta^{15}N$ signatures differ between sources, thus providing information on a species diet (de Necker *et al*., 2020). Thus, using both isotopes together can also help better differentiate food sources and trophic relationships, allowing for the identification of main food chains, trophic niches, niche overlaps and interspecific diet variability (Abrantes & Sheaves, 2010; Post, 2003).

To better understand the effects of alien fish invasion on native fish communities, facilitated by anthropogenic water use, the Sundays River catchment in the Eastern Cape of South Africa was studied. The Sundays River Irrigation Scheme was completed in 1975 primarily to provide water from the Orange River to a network of small irrigation ponds, which resulted in the passive introduction of fishes, such as the native River goby *Glossogobius callidus* (Smith, 1937), Estuarine roundherring *Gilchristella aestuaria* (Gilchrist, 1914), alien Mozambique tilapia *Oreochromis mossambicus* (Peters, 1852) and Western mosquitofish *Gambusia affinis* (Baird and Girard, 1853) (Woodford *et al*., 2013). With increasing global biodiversity loss through introductions and translocations of species, new and integrative tools are needed to quantify the potential ecological effects of alien species on recipient ecosystems (Ehrenfeld, 2010; Vilizzi *et al*., 2019).

Successful invasion of new habitats can be aided by the invader having a broad trophic niche relative to native species, and high growth rates and early maturity are the main characteristics that are likely to increase invasion success (Rooke & Fox, 2018; Ruesink, 2005; Tayeh *et al*., 2015), thus, potentially counteracting biotic resistance under certain conditions, such as limited resources (Jackson & Britton, 2014; Shea & Chesson, 2002). In contrast, narrow trophic niches of native species can make them vulnerable to invaders, particularly if they overlap (enabling direct competition for resources) and are inferior competitors to the invader in such aspects as feeding efficiency (Alexander *et al*., 2014; Mofu, South, *et al*., 2019a). The current study was conducted to provide the first assessment of the diet and the trophic interrelationships between two native species, *G. callidus* and *G. aestuaria*, and two non-native species, *O. mossambicus* and *G. affinis*, from the Sundays River Valley irrigation ponds. The authors evaluated the hypothesis that *O. mossambicus* and *G. affinis* are utilising a vacant food niche, and all four species co-exist through minimal diet overlap.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

Collection of animals was carried out in compliance with the Eastern Cape Department of Economic Development and Environmental Affairs (DEDEA permit no. CRO 35/17CR and CRO 36/17CR), and ethical clearance was approved by the National Research Foundation – South African Institute for Aquatic Biodiversity (NRF–SAIAB reference no. 25/4/1/5_2017/03) and Rhodes University Ethical Standards Committee (reference no. 25/4/5).

2.2 | Site description

The Sundays River Valley irrigation ponds are located within a semi-arid region in Eastern Cape, South Africa. The climate in the Sundays River Valley is warm temperate with an average temperature of 15–45°C in summer and 5–18°C in winter (Kadye & Booth, 2012). The Sundays River Valley has an annual rainfall of 350–600 mm (Lombard *et al*., 2001). Each pond receives water up to an annual quota, which is *c*. 9000 m^3 per hectare irrigated. The vegetation near the ponds

consists of *Salix mucronata*, Asteraceae and *Ficus burkei*. The littoral zone of the ponds was characterised by *Atriplex semibaccata*, *Cynodon dactylon* and *Chloris* sp. (Mofu *et al*., 2021). Samples were collected from four irrigation ponds [*i.e*., Dungbeetle 1 (DB1; 33° 26' 33" S; 25° 40' 57" E), Dungbeetle 2 (DB2; 33° 26' 37" S; 25° 40' 45" E), ML Swart's (MLS; 33° 24' 33" S; 25° 29' 04" E) and River Bend (RBD; 33° 26′ 23" S; 25° 42' 25" E)]. DB1 is 150 m long and 95 m wide and covers an area of 14,250 m^2. DB2 is 270 m long and 105 m wide and covers an area of 28,350 m^2. MLS is 86 m long and 57 m wide and covers an area of 4902 m^2. RBD is 135 and 128 m wide and covers an area of 17,280 m^2. All four ponds are located in privately owned farms and are utilised for irrigation of citrus orchards (see Mofu *et al*., 2021; for ponds physico-chemical variables).

Samples for stable isotope and gut content analyses were collected in summer (December 2017). The sampling period was chosen as it coincides with peak species abundances. Phytoplankton and zooplankton were collected by pulling a 20 and 63 μm (40 cm diameter) plankton net through the water column from each pond ($n = 16$ samples per zoo or phytoplankton each: 4 ponds × 4 sites). Macroinvertebrates were collected using a 50 cm equilateral triangle-shaped net with a 1.5 m long handle and were identified to family level according to Gerber and Gabriel (2002) before being placed in individual Eppendorf tubes (*i.e*., 5–10 individuals per family depending on size; $n = 5$). Macroinvertebrates were assigned to one of the three functional feeding groups (FFGs), including scrapers (SCRA), collectors (COLL) and predators (PRED) (Cummins *et al*., 2005; Merritt & Cummins, 1996). Fish were collected using a 30-m-long × 2-m-deep seine net with 12 mm mesh wings and an 8 mm mesh-size cod end. Plants ($n = 4$ samples per pond) and detritus ($n = 4$ samples per pond) were collected by hand along the littoral zones of the ponds, with terrestrial plants also collected by hand from the nearby (*i.e*., "riparian") vegetation. All collected samples were placed in individual 1.5 ml Eppendorf tubes for further processing and analysis in the laboratory. On collection, all samples were stored separately based on site and pond in labelled Ziplock bags and were kept on ice in a cooler box, with sample processing being done within 24 h of collection.

2.3 | Stable isotope analysis

Upon collection, fish samples were measured for total length (T_L) to the nearest 1.0 mm, and a muscle tissue sample was taken from each individual fish from the caudal peduncle, with all the scales and skin removed. For small-sized fish (<10 cm), the whole body was used after the head, intestines, scales and eyes were removed. Fish were grouped into the following broad functional groups: *G. callidus* (generalist invertivore), *G. aestuaria* (planktivorous), *O. mossambicus* (omnivorous) and *G. affinis* (omnivorous). In addition, fishes were grouped into two size classes ($n = 5$ per size class per species) based on length at maturity, and their diets were perceived to change with size (Supporting Information Table S1). Molluscs were separated from their carbonate-containing shells before drying.

Once sorted and identified, samples were oven-dried at 60°C for at least 48–72 h, then ground into a homogenous powder with a pestle and mortar and sent for isotopic analysis at the Stable Isotope Laboratory, Mammal Research Institute (MRI) at the University of Pretoria, South Africa. The stable isotope analysis was carried out using a Flash EA 112 Series coupled to a Delta V Plus stable light isotope ratio mass spectrometer *via* a ConFlo IV system (Thermo Fischer, Bremen, Germany). Standard delta notation (δ) was used to express stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). The isotope ratios in parts per thousand (‰) differences from a standard reference material were expressed as follows:

$$\delta^{13}C \text{ or } \delta^{15}N = \left[\left(\frac{Rsample}{Rstandard} - 1\right)\right] \times 1000$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$, respectively (Fry, 1991; Hobson & Clark, 1992). The C:N was in mass ratios. The standards used were referenced to atmospheric nitrogen for $\delta^{15}N$ (Ehleringer & Rundel, 1988), and $\delta^{13}C$ was referenced to Vienna Pee–Dee Belemnite (Craig, 1957). The average analytical precision was <0.15‰ for $\delta^{13}C$ and <0.1‰ for $\delta^{15}N$. All $\delta^{13}C$ values were corrected for lipid content (based on C:N ratios) using the equation described by Kiljunen *et al*. (2006).

For baseline $\delta^{15}N$ values, long-lived primary consumers are typically used (Post, 2002; Post *et al*., 2000; Vander Zanden *et al*., 1999). In this study, molluscs collected from the ponds were used to calculate an average $\delta^{15}N_{baseline}$ of 3.16‰ ± 0.87, $\delta^{13}C_{baseline}$ of −27.69‰ ± 2.57, $\delta^{13}C_{max}$ of −22.31‰, $\delta^{13}C_{min}$ of −30.69 ‰ and a carbon range $CR_{baseline}$ of 8.38‰.

All organisms in all ecosystems were then adjusted based on the following equations for $\delta^{15}N$ and $\delta^{13}C$:

$$Trophic\ position = \frac{\delta^{15}N_{organism} - \delta^{15}N_{baseline}}{\Delta^{15}N} + 2$$

where 2 represents the trophic position of the baseline organism, $\Delta^{15}N$ represents the fractionation factor calculated as 3.23‰, $\delta^{15}N_{organism}$ is the isotope ratio of the organism and $\delta^{15}N_{baseline}$ is the isotope ratio (3.16‰) of the primary consumers used for the baseline (Post, 2002).

$$\delta^{13}C_{corrected} = \frac{\delta^{13}C_{organism} - \delta^{13}C_{baseline}}{CR_{baseline}}$$

where $\delta^{13}C_{corrected}$ is the corrected carbon isotope ratio of the consumer, $\delta^{13}C_{organism}$ is the uncorrected isotope ratio of the organism, $\delta^{13}C_{baseline}$ is the mean primary consumer isotope ratio − 27.69 ‰ and $CR_{baseline}$ is the primary consumer carbon range ($\delta^{13}C_{max}$ – $\delta^{13}C_{min}$ = 8.38‰) (Jackson & Britton, 2014; Olsson *et al*., 2009). Individual isotopes for macroinvertebrates families were calculated prior to analyses.

To investigate the proportional contribution of each food to the diet of the four fish species across the four study ponds, Stable Isotope Mixing Models in R (SIMMR; Parnell & Inger, 2016) were applied using $\delta^{13}C$ and $\delta^{15}N$ values of fish muscle tissue and prey muscle and

tissue. The authors used (mean ± S.D.) discrimination factors of 1.3 ± 0.4‰ for $\delta^{13}C$ and 3.4 ± 1‰ for $\delta^{15}N$. The model was run with Markov chain Monte Carlo parameters of three chains of 300,000 iterations, a burn-in phase of 200,000 and a thinning of 100. Individuals as a random effect and both residual and process error were included in the model. The model generates posterior probability distributions that can be described by average estimates of the source contribution and their associated credible intervals. Convergence and diagnostic statistics were evaluated using the Gelman–Rubin test (all variables were ≤1.05). It should be noted that despite the incorporation of error terms and informative priors in Bayesian mixing models, which can include some variability in predictions (Parnell *et al*., 2010; Stock *et al*., 2018), there is still a mismatch in the time integration of food resources and consumers, with the latter being typically more time integrated (Phillips *et al*., 2014). Therefore, it is likely that the expected proportionate resource contributions to the irrigation ponds food webs will vary over time.

2.4 | Gut content sample collection and analysis

The gut contents of *G. callidus* ($n = 98$), *G. aestuaria* ($n = 99$), *O. mossambicus* ($n = 98$) and *G. affinis* ($n = 45$) were examined. The T_L of each fish was measured to the nearest 1.0 mm; then after, the guts were removed, opened and emptied into a 1 mm Petri dish, with graduated increments of 1 × 1 mm at the bottom, as described by Wasserman (2012). Gut content items were sorted according to the indirect volumetric method of gut content analysis (Hyslop, 1980). All gut content items were identified to the lowest taxonomic level possible according to Day *et al*. (2003), De Moor *et al*. (2003), Gerber and Gabriel (2002). To obtain the volume of each prey item, an indirect volumetric assessment was performed by flattening the gut content items under a microscope slide to a depth of *c*. 1 mm in thickness. The number (1 × 1 mm) of blocks occupied by non-faunal prey groups was estimated as the volume occupied. The volume was then calculated as the area an item covered (Wasserman, 2012). Gut contents were expressed as percentage volume (%V) of each prey category (Hyslop, 1980).

For analysis, fishes were grouped into the size classes as mentioned in stable isotope methods earlier. To identify the differences in diet between the fish, prey abundance (%N), frequency of occurrence (%F) and percentage volume (%V) were determined within each size class category per prey item as a percentage of all prey. As is common practice in gut content analyses (Hyslop, 1980), %N was calculated as the number of individuals of any prey item as a percentage of all prey items, %V was the volume occupied by any prey as a percentage of the total volume of all prey items and %F was the number of stomachs containing a given taxon as a proportion of all sampled stomachs. This allowed for the calculation of the Index of Relative Importance (IRI) for each prey category (Pinkas *et al*., 1971) as:

$$\mathrm{IRI} = (\%\mathrm{N} + \%\mathrm{V})\ x\ \%\mathrm{F}$$

recommended by Hyslop (1980). For each prey taxon, the IRI was then expressed as a percentage of the IRI values of all prey categories (%IRI).

2.5 | Statistical analysis

The normality of data and homogeneity of variance were checked using the Kolmogorov–Smirnov test and Levene's tests. Because the data were not normally distributed due to unequal population variance, they were transformed using the Box–Cox process to meet the assumptions of a parametric analysis. To test whether there were differences in carbon and nitrogen isotope values in the basal and different consumer groups, the authors used ANOVA. Specifically, a three-factor ANOVA with fish species, size class and ponds as fixed factors was used to compare differences in stable isotope values of fish ($\delta^{13}C$ and $\delta^{15}N$). A two-factor ANOVA with taxa and ponds as fixed factors was applied to compare the difference in stable isotope values of basal resources ($\delta^{13}C$ and $\delta^{15}N$). The authors tested for ontogenetic shifts in isotopic composition within each fish species using linear regression of isotopic values based on T_L. Comparison of resource use between species and across ponds was conducted by comparing 95% credibility limits of each prey source. The isotopic niche area of each species was calculated based on the standardised ellipse of $\delta^{13}C$ and $\delta^{15}N$ (Jackson *et al*., 2011). To overcome the disparity in sample sizes, the area of an ellipse corrected (SEAc) for small sample sizes, was calculated using SIBER (Jackson *et al*., 2011) to determine the isotopic area across ponds. Variation in niche width between species was calculated using the likelihood test in SIBER. Index of Relative Importance and Levin's' index were used to calculate the dietary niche width of each species (Levins, 1968).

To measure the trophic niche size and to test whether trophic niche overlap was not equivalently weighted among species, a probabilistic method was used (Swanson *et al*., 2015). This method measures a given 95% probability niche size and provides directional estimates of pair-wise niche overlap in multivariate space (Swanson *et al*., 2015). The proposed method defines the niche overlap of a particular species A onto species B as the fraction of the intersection area between niche of species A and niche of species B over the total niche area of species B and *vice versa* using the R package "NicheRover" (Lysy *et al*., 2021). To assess the relative proportion of each food source in the diet of the four fish species across all size classes per species, a Bayesian stable isotope mixing model that runs on the R platform (SIAR; Parnell *et al*., 2010; The R Development Core Team, 2017) was used.

3 | RESULTS

3.1 | Stable isotope

A total of 349 samples were collected for stable isotope analysis, consisting of 43 basal resources (Table 1). The mean $\delta^{13}C$ values for phytoplankton was −25.7 ± 1.9‰ (Table 1 and Figure 1). The littoral zone aquatic and terrestrial plants had a mean $\delta^{13}C$ value of −16.1 ± 5.7‰ and − 25.6 ± 6.6‰, respectively. Detritus had a mean $\delta^{13}C$ value of −24.1 ± 5.2 ‰ (Table 1 and Figure 1). The mean $\delta^{15}N$ for phytoplankton was 6.5 ± 1.5‰. Aquatic and terrestrial plants had

TABLE 1 Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope values (mean ± S.D.) of basal resources, invertebrate groups and fish species sampled in the Sundays River Valley irrigation ponds

	DB1				DB2				MLS				RBD				
Group	***n***	**TP**	**δ13C**	**δ15N**	***n***	**TP**	**δ13C**	**δ15N**	***n***	**TP**	**δ13C**	**δ15N**	***n***	**TP**	**δ13C**	**δ15N**	**Functional Group**
Basal resources																	
Asteraceae	–		–	–	4		−16.4 ± 6.1	15.2 ± 2.9	–		–	–	4		−13.8 ± 0.7	9.7 ± 2.7	Aquatic plants
Atriplex semibaccata	3		−27.4 ± 0.9	9.5 ± 0.2	–		–	–	–		–	–	–		–	–	Aquatic plants
Chloris sp.									4		−12.4 ± 0.3	12.3 ± 1.6	–		–	–	Terrestrial plants
Cynodon dactylon	4		−12.6 ± 1.3	5.8 ± 1.5	4		−12.9 ± 0.7	10.9 ± 3.2	–		–	–	–		–	–	Aquatic plants
Ficus burkei	–		–	–	4		−29.2 ± 0.7	12.5 ± 1.8	4		−28.6 ± 0.9	8.6 ± 0.3	4		−29.0 ± 0.7	8.4 ± 0.7	Terrestrial plants
Salix mucronata	4		−27.8 ± 0.9	6.6 ± 1.2	–		–	–	–		–	–	–		–	–	Terrestrial plants
Detritus	4		−29.1 ± 0.7	6.6 ± 1.7	3		−18.1 ± 5.8	10.2 ± 4.5	4		−24.9 ± 1.7	6.1 ± 1.2	4		−22.8 ± 5.5	4.8 ± 1.6	Detritus
Phytoplankton	4		−26.9 ± 2.1	5.4 ± 0.6	4		−25.9 ± 1.3	8.1 ± 1.2	–		–	–	4		−24.5 ± 1.7	5.9 ± 1.2	Phytoplankton
Zooplankton	4	3.5	−25.8 ± 1.2	8.3 ± 0.8	4		−25.3 ± 0.4	8.3 ± 1.2	4		−25.7 ± 0.7	8.8 ± 1.2	4		−24.7 ± 1.0	6.4 ± 0.9	Zooplankton
Macroinvertebrates																	
Baetidae	–	2.3	–	–	–		–	–	4	3.5	−26.7 ± 1.8	8.2 ± 0.9	–		–	–	Scraper
Belostomatidae	4	3.8	−24.7 ± 1.6	9.4 ± 0.7	2	4.1	−25.3 ± 1.1	10.1 ± 2.3	4	3.9	−24.1 ± 0.4	9.8 ± 0.5	–		–	–	Predators
Chironomidae	2	3	−25.9 ± 0.6	6.6 ± 0.6	–		–	–	2	4.1	−29.8 ± 0.1	10.3 ± 0.4	–		–	–	Collectors
Corixidae	4	3.3	−26.7 ± 2.8	7.5 ± 1.7	4	3.3	−26.5 ± 0.5	7.6 ± 1.9	4	3.7	−21.9 ± 1.2	8.9 ± 0.1	4	3.1	−23.8 ± 0.9	6.9 ± 0.6	Predators
Hirudinea	3	4.3	−27.5 ± 0.8	10.9 ± 0.9	–		–	–	–		–	–	–		–	–	Predators
Notonectidae	4	4	−27.5 ± 0.4	9.9 ± 0.1	–		–	–	–		–	–	2	3.8	−23.9 ± 2.8	9.4 ± 0.9	Predators
Physidae	–		–	–	–		–	–	3		−23.5 ± 5.9	8.7 ± 1.7	–		–	–	Scraper
Teleost																	
GLC1	3	4.4	−26.4 ± 0.5	11.5 ± 0.5	5	5.1	−24.1 ± 0.3	13.8 ± 0.8	8	4.8	−25.3 ± 0.9	12.8 ± 0.7	11	4.7	−24.9 ± 0.3	12.3 ± 1.7	Generalist invertivore
GLC2	5	4.7	−25.5 ± 0.4	12.2 ± 0.9	11	5.5	−24.1 ± 0.6	15.1 ± 0.9	8	5.1	−24.7 ± 0.5	13.8 ± 0.5	12	5.3	−25.7 ± 0.5	14.5 ± 1.0	Generalist invertivore
GIA1	–		–	–	8	4.9	−26.8 ± 0.7	13.2 ± 0.4	7	4.9	−26.9 ± 0.6	13.1 ± 0.5	3	5.3	−26.4 ± 0.1	14.4 ± 0.6	Planktivorous
GIA2	–		–	–	7	5.6	−24.9 ± 0.2	15.5 ± 0.3	8	5.2	−24.6 ± 0.6	14.0 ± 0.4	22	5.2	−25.9 ± 0.5	14.1 ± 0.7	Planktivorous
ORM1	8	4.6	−26.1 ± 0.9	11.8 ± 1.4	–		–	–	7	3.8	−25.6 ± 1.6	9.6 ± 0.9	9	3.6	−25.4 ± 1.9	8.7 ± 2.1	Omnivorous
ORM2	13	4.2	−26.9 ± 0.9	10.6 ± 1.9	2	5.4	−25.4 ± 0.0	14.7 ± 0.0	7	4.5	−24.9 ± 0.7	11.5 ± 0.2	8	4.4	−26.1 ± 0.5	11.2 ± 0.2	Omnivorous
GAA1	8	4.8	−26.4 ± 0.6	12.6 ± 0.8	–		–	–	11	4.9	−24.7 ± 0.7	13.3 ± 0.9	10	4.1	−23.2 ± 1.5	10.3 ± 2.2	Omnivorous
GAA2	–		–	–	7	5.3	−24.0 ± 0.8	14.4 ± 0.6	–		–	–	3	5	−24.1 ± 0.5	13.4 ± 0.5	Omnivorous

Abbreviations: DB1, Dungbeetle 1; DB2, Dungbeetle 2; GAA1, *Gambusia affinis* (10–40 mm T_L); GAA2, *Gambusia affinis* (41–80 mm T_L); GIA1, *Gilchristella aestuaria* (20–40 mm T_L); GIA2, *Gilchristella aestuaria* (41–80 mm T_L); GLC1, *Glossogobius callidus* (40–80 mm T_L); GLC2, *Glossogobius callidus* (81–140 mm T_L); MLS, ML Swart's; *n*, number of samples; ORM1, *Oreochromis mossambicus* (10–40 mm T_L); ORM2, *Oreochromis mossambicus* (80–400 mm T_L); RBD, River Bend; TP, trophic position.

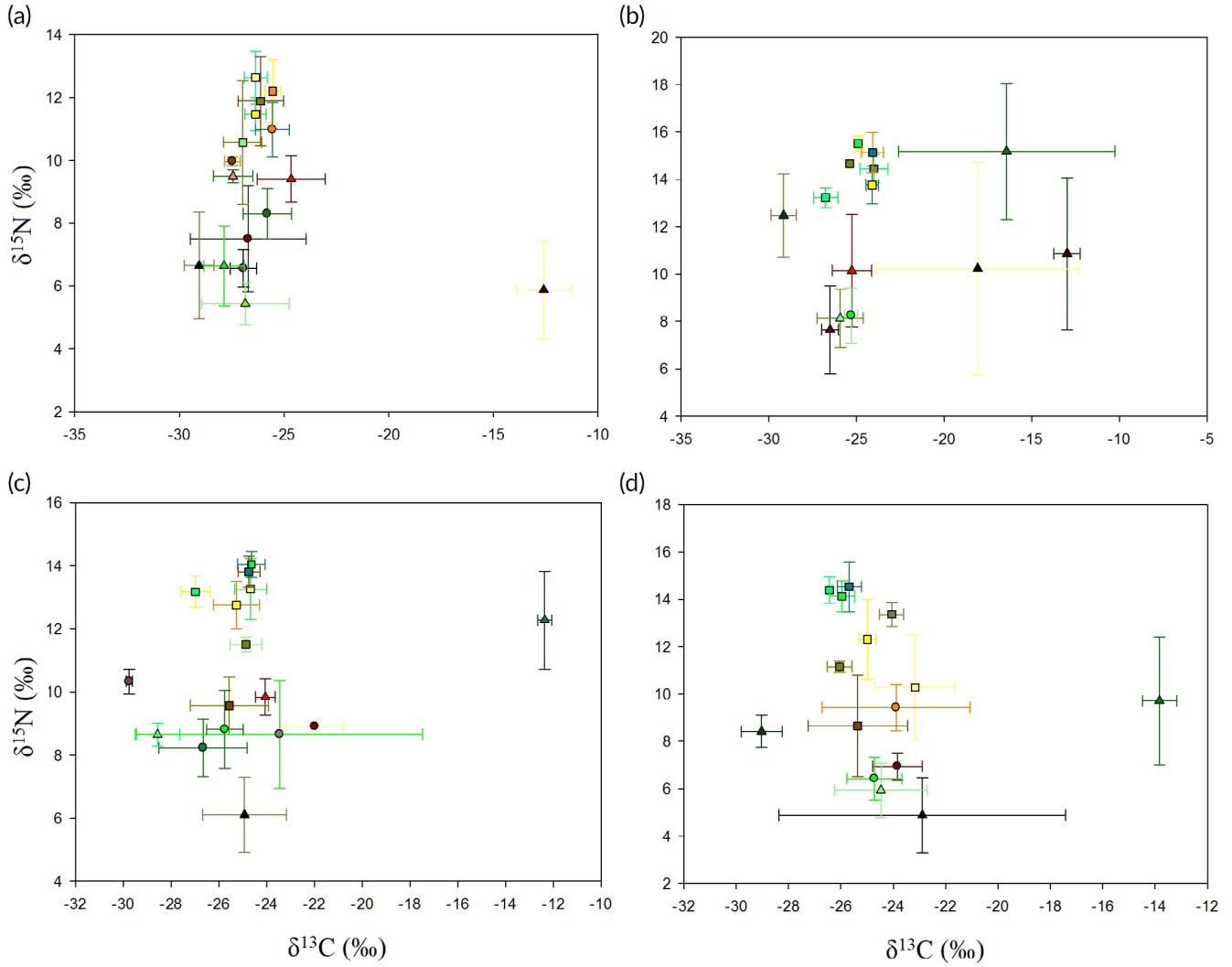

FIGURE 1 Stable $\delta^{13}C$–$\delta^{15}N$ isotopic bi-plots of fish and their food sources from (a) Dungbeetle 1 (DB1), (b) Dungbeetle 2 (DB2), (c) ML Swart's (MLS) and (d) River Bend (RBD) from the Sundays River Valley, Eastern Cape, South Africa. Data points represent the mean ± S.D. for each group. △: Asteraceae, ▲: *Atriplex semibaccata*, ▲: Chloris sp., ▲: *Cynodon dactylon*, ▲: *Ficus burkei*, ▲: *Salix mucronata*, △: Detritus, △: Phytoplankton, ●: Zooplankton, ●: Baetidae, ●: Belostomatidae, ●: Chironomidae, ●: Corixidae, ●: Hirudinea, ●: Notonectidae, ●: Physidae, ■: GLC1, ■: GLC2, ■: GIA1, ■: GIA2, ■: ORM1, ■: ORM2, ■: GAA1 and ■: GAA2

mean $\delta^{15}N$ values of 10.3 ± 3.8‰ and 9.8 ± 2.6‰, respectively, with detritus having a mean $\delta^{15}N$ value of 6.8 ± 2.8‰ (Table 1). A two-factor ANOVA found significant differences ($P < 0.05$) in $\delta^{13}C$ values across basal resources taxa and ponds (Supporting Information Table S2). There were significant differences in $\delta^{15}N$ values across basal resources taxa and ponds (Supporting Information Table S3).

For macroinvertebrate FFGs, scrapers, collectors and predators had mean $\delta^{13}C$ values of −24.8 ± 3.8‰, −28.4 ± 1.7‰ and − 25.1 ± 1.9‰, respectively (Table 1 and Figure 1). Scrapers, collectors and predators had mean $\delta^{15}N$ values of 9.3 ± 2.1‰, 8.4 ± 2.2‰ and 9.1 ± 1.8‰, respectively (Table 1). The mean $\delta^{13}C$ and $\delta^{15}N$ values for zooplankton were − 25.4 ± 0.9‰ and 7.9 ± 1.3‰, respectively (Table 1). For fish species, the most depleted $\delta^{13}C$ (−22.6 ± 0.9‰) and $\delta^{15}N$ values (9.9 ± 2.1‰) were for *O. mossambicus* (80–400 mm T_L) (ORM2) (Table 1). The most enriched $\delta^{13}C$ value was for *G. affinis* (41–80 mm T_L; GAA2) (−19.4 ± 0.6‰), whereas *G. aestuaria* (41–80 mm T_L; GIA2) had the most $\delta^{15}N$-enriched value (14.4 ± 0.7‰) (Table 1). A three-factor ANOVA revealed that $\delta^{13}C$ significantly ($P < 0.05$) differed across fish species, size classes and ponds (Supporting Information Table S2). There were significant differences ($P < 0.05$) in $\delta^{15}N$ values across fish species, size classes and ponds (Supporting Information Table S3).

Regressions of $\delta^{13}C$ and $\delta^{15}N$ against fish T_L revealed no significant ($P > 0.05$) relationships between $\delta^{13}C$ values and T_L of *G. callidus*, *O. mossambicus* and *G. affinis*; nonetheless, there was a negative significant ($P < 0.05$) relationship between $\delta^{13}C$ values and T_L of *G. aestuaria* ($R^2 = 0.43$, $P < 0.05$). *G. callidus* ($R^2 = 0.63$, $P < 0.05$), *G. aestuaria* ($R^2 = 0.23$, $P < 0.05$), *O. mossambicus* ($R^2 = 0.39$, $P < 0.05$) and *G. affinis* ($R^2 = 0.28$, $P < 0.05$) exhibited a positive significant relationship ($P < 0.05$) between $\delta^{15}N$ signatures and T_L.

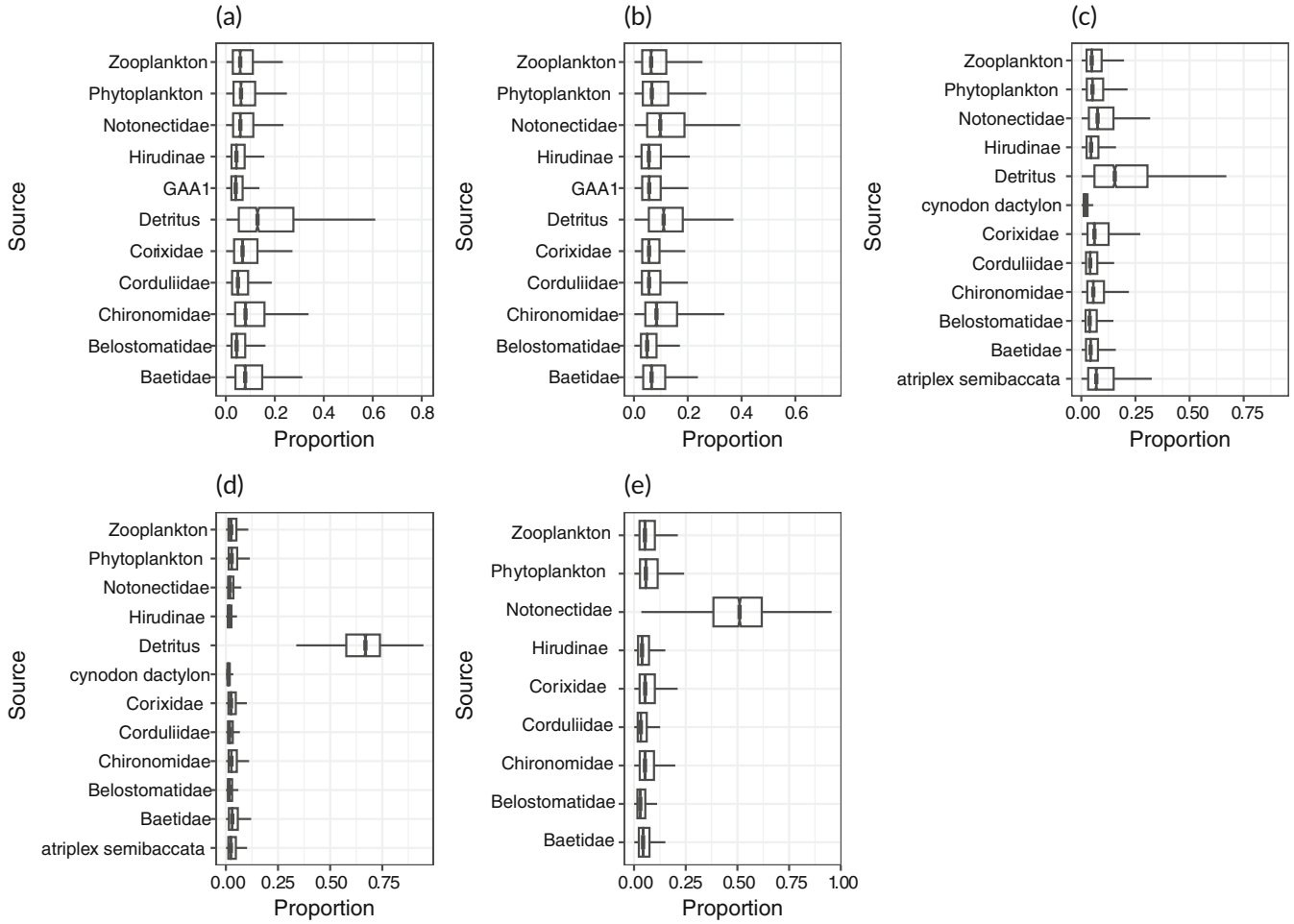

FIGURE 2 Proportional resource contributions to (a) GLC1 = *Glossogobius callidus* (40–80 mm T_L), (b) GLC2 = *Glossogobius callidus* (81–140 mm T_L), (c) ORM1 = *Oreochromis mossambicus* (10–40 mm T_L), (d) ORM2 = *Oreochromis mossambicus* (80–400 mm T_L) and (e) GAA1 = *Gambusia affinis* (10–40 mm T_L) as determined by Stable Isotope Mixing Models (SIMMR) for Dungbeetle 1 (DB1)

3.2 | Stable isotope mixing models

The SIMMR model output showed that detritus and Chironomidae had equal contribution to the diet of *G. callidus* (40–80 mm T_L) (GLC1) (Supporting Information Table S4; Figure 2a), whereas *G. callidus* (81–140 mm T_L) (GLC2) relied mostly on Chironomidae, Notonectidae and detritus (Supporting Information Table S4; Figure 2b). Detritus, Notonectidae and *A. semibaccata* contributed more to the diet of *O. mossambicus* (10–40 mm T_L) (ORM1), whereas detritus contributed more to the diet of *O. mossambicus* (80–400 mm T_L) (ORM2) (Supporting Information Table S4; Figure 2c,d). Notonectidae contributed more to the diet of *G. affinis* (10–40 mm T_L) (GAA1), whereas phytoplankton, zooplankton, Corixidae and Chironomidae contributed equally to the diet of *G. affinis* (10–40 mm T_L) (GAA1) (Supporting Information Table S4; Figure 2e).

From DB2, Belostomatidae contributed more to the diet of *G. callidus* (40–80 mm T_L) (GLC1), whereas zooplankton and phytoplankton contributed equally to the diet of *G. callidus* (40–80 mm T_L) (GLC1) (Supporting Information Table S4; Figure 3a,b). In addition, Belostomadiae contributed more, whereas phytoplankton and zooplankton contributed equally to the diet of *G. callidus* (81–140 mm T_L) (GLC2) (Supporting Information Table S4; Figure 3a,b). The estimated means of Belostomatidae were the main food sources for *G. aestuaria* (20–40 mm T_L) (GIA1). Phytoplankton and zooplankton contributed equally to the diet of *G. aestuaria* (20–40 mm T_L) (GIA1), whereas Corixidae and Belostomatidae contributed more to the diet of *G. aestuaria* (41–80 mm T_L) (GIA2) (Supporting Information Table S4; Figure 3c,d). Belostomatidae and Asteraceae were the main food sources for *O. mossambicus* (10–40 mm T_L) (ORM1) (Supporting Information Table S4; Figure 3e). Belostomatidae was the main food source for *G. affinis* (10–40 mm T_L) (GAA1) (Supporting Information Table S4; Figure 3f).

From MLS, Baetidae and Chironomidae contributed more to the diet of *G. callidus* (40–80 mm T_L) (GLC1) (Supporting Information Table S5; Figure 4a), whereas Chironomidae and zooplankton contributed more to the diet of *G. callidus* (81–140 mm T_L) (GLC2) (Supporting Information Table S5; Figure 4b). The estimated means of Chironomidae and Baetidae were the main food sources for *G. aestuaria* (20–40 mm T_L) (GIA1), whereas Chironomidae and Coenogronidae contributed more to the diet of *G. aestuaria* (41–80 mm T_L) (GIA2)

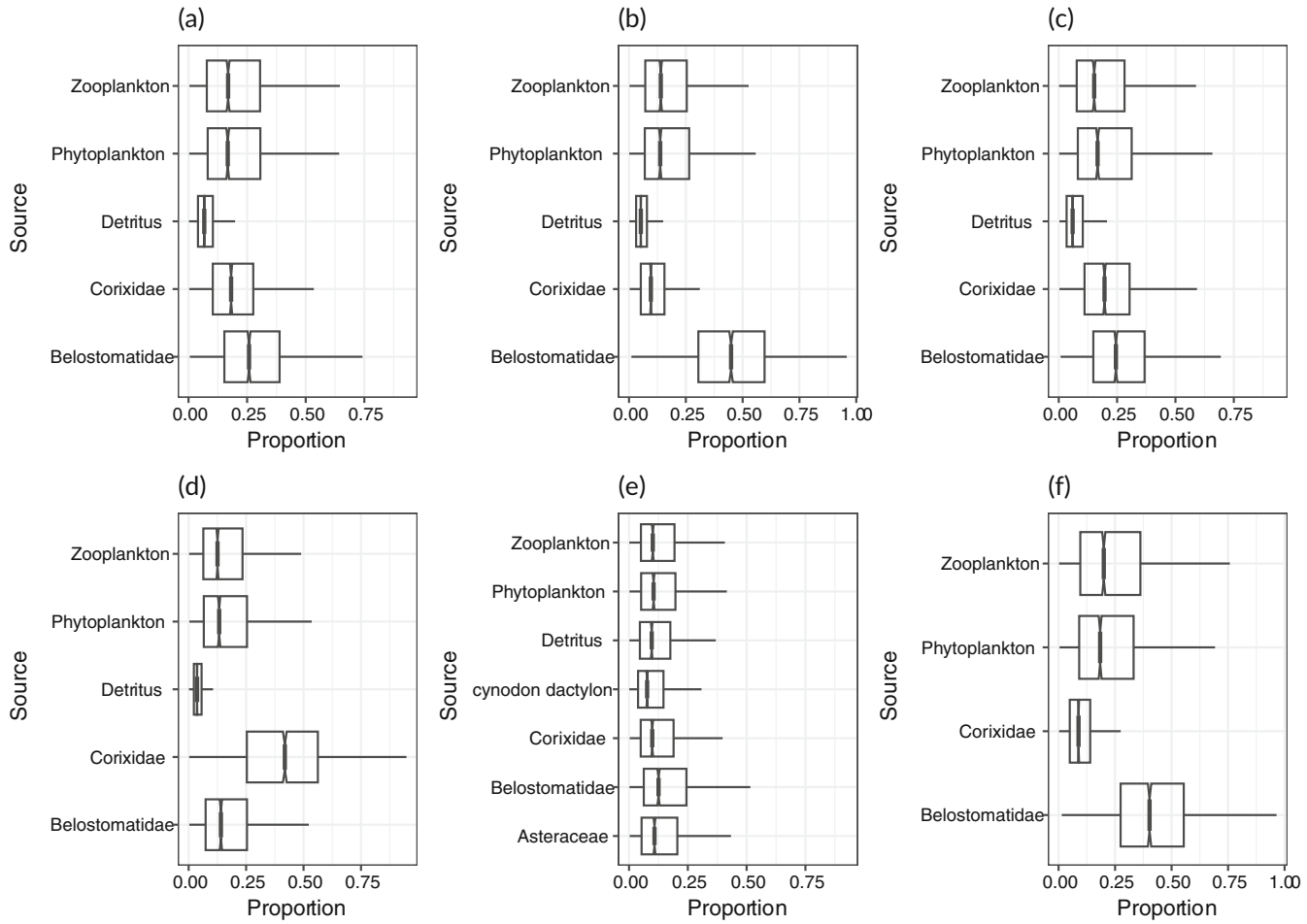

FIGURE 3 Proportional resource contributions to (a) GLC1 = *Glossogobius callidus* (40–80 mm T_L), (b) GLC2 = *Glossogobius callidus* (81–140 mm T_L), (c) GIA1 = *Gilchristella aestuaria* (20–40 mm T_L), (d) GIA2 = *Gilchristella aestuaria* (41–80 mm T_L), (e) ORM1 = *Oreochromis mossambicus* (10–40 mm T_L) and (f) GAA1 = *Gambusia affinis* (10–40 mm T_L) as determined by Stable Isotope Mixing Models (SIMMR) for Dungbeetle 2 (DB2)

(Supporting Information Table S5; Figure 4c,d). Baetidae and Chironomidae were the main food sources for *O. mossambicus* (10–40 mm T_L) (ORM1) and *O. mossambicus* (80–400 mm T_L) (ORM2), respectively (Supporting Information Table S5; Figure 4e,f). The main food source that contributed to the diet of *G. affinis* (10–40 mm T_L) (GAA1) and *G. affinis* (41–80 mm T_L) (GAA2) was Chironomidae (Supporting Information Table S5; Figure 4g,h). From RBD, *G. callidus* (40–80 mm T_L) (GLC1) relied mostly on detritus and Notonectidae, and *G. callidus* (81–140 mm T_L) (GLC2) relied mostly on Hydrophilidae and detritus (Supporting Information Table S5; Figure 5a,b), whereas *G. aestuaria* (20–40 mm T_L) (GIA1) relied mostly on Hydrophilidae and phytoplankton, and zooplankton and detritus contributed equally to the diet of *G. aestuaria* (20–40 mm T_L) (GIA1). *G. aestuaria* (41–80 mm T_L) (GIA2) relied mostly on Hydrophilidae and detritus (Supporting Information Table S5; Figure 5c,d). Corixidae and detritus contributed mostly to the diet of *O. mossambicus* (10–40 mm T_L) (ORM1), whereas phytoplankton and zooplankton had equal contributions to the diet of *O. mossambicus* (10–40 mm T_L) (ORM1), and *O. mossambicus* (80–400 mm T_L) (ORM2) relied mostly on Corixidae and detritus (Supporting Information Table S5; Figure 5e,f). Corixidae contributed more to the diet of *G. affinis* (10–40 mm T_L) (GAA1), whereas phytoplankton and zooplankton had equal contribution to the diet of *G. affinis* (10–40 mm T_L) (GAA1) (Supporting Information Table S5; Figure 5g). Notonectidae contributed more to the diet of *G. affinis* (41–80 mm T_L) (GAA2), whereas phytoplankton, zooplankton and detritus had equal contribution to the diet of *G. affinis* (41–80 mm T_L) (GAA2) (Supporting Information Table S5; Figure 5h).

The δ^{15}N‰ range and δ^{13}C range of the fish community in RBD were higher than that in DB1, DB2 and MLS, suggesting that the trophic length and range of basal resources used by the fish community in RBD was greater (Table 2). The average degree of trophic diversity mean distance to centroid (CD) was higher in RBD and similar for DB and MLS. Lower mean nearest neighbour distance and S.D. of nearest neighbour distance in DB1 indicate greater density and evenness of species packing in bi-plot space (Table 2). The resource use of each species, as derived from isotopic mixing models, varied between ponds. Both *O. mossambicus* (10–40 mm T_L) (ORM1) and *O. mossambicus* (80–400 mm T_L) (ORM2) exhibited a wide niche width, whereas *G.*

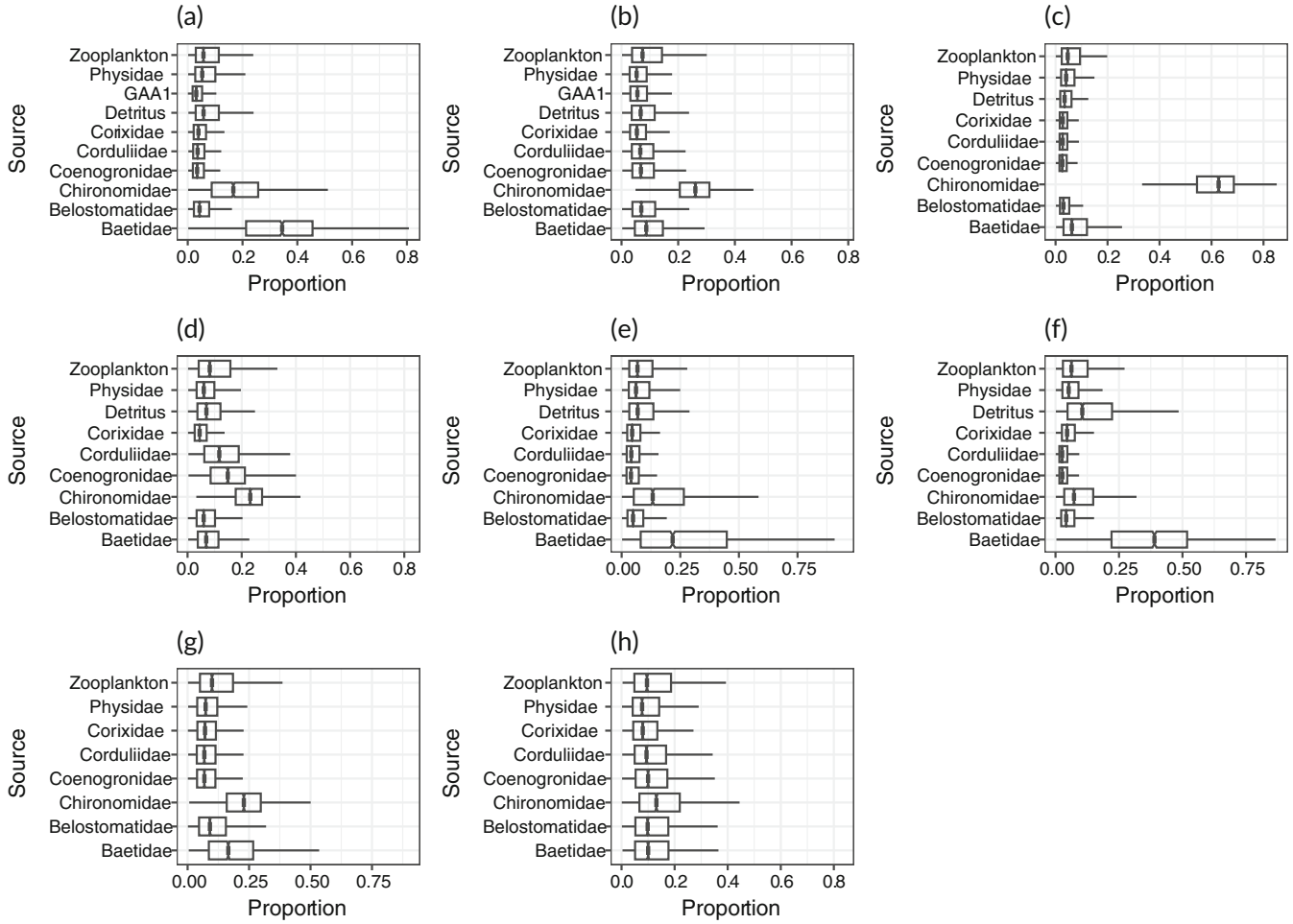

FIGURE 4 Proportional resource contributions to (a) GLC1 = *Glossogobius callidus* (40–80 mm T_L), (b) GLC2 = *Glossogobius callidus* (81–140 mm T_L), (c) GIA1 = *Gilchristella aestuaria* (20–40 mm T_L), (d) GIA2 = *Gilchristella aestuaria* (41–80 mm T_L), (e) ORM1 = *Oreochromis mossambicus* (10–40 mm T_L), (f) ORM2 = *Oreochromis mossambicus* (80–400 mm T_L), (g) GAA1 = *Gambusia affinis* (10–40 mm T_L) and (h) GAA2 = *Gambusia affinis* (41–80 mm T_L) as determined by Stable Isotope Mixing Models (SIMMR) for ML Swart's (MLS)

callidus (40–80 mm T_L) (GLC1) and *G. callidus* (81–140 mm T_L) (GLC2) had a narrow niche width (Supporting Information Figure S1). From DB2, all fish species exhibited a narrow niche width (Supporting Information Figure S1). From MLS, *O. mossambicus* (10–40 mm T_L) (ORM1) exhibited a wide niche width, followed by *G. affinis* (10–40 mm T_L) (GAA1) (Supporting Information Figure S1). Similar to RBD, *O. mossambicus* (80–400 mm T_L) (ORM2) had a wide niche width followed by *G. affinis* (10–40 mm T_L) (GAA1) (Supporting Information Figure S1).

3.3 | Probabilistic niche overlap

The application of the Bayesian posterior distribution of the probabilistic niche overlap metric showed niche overlaps among the different size classes (Supporting Information Table S6 and Figure S2). Overlap values showed low niche overlap between *G. callidus* (40–80 mm T_L) (GLC1) and *G. affinis* (10–40 mm T_L) (GAA1) (26.01%) and that *G. affinis* (10–40 mm T_L) (GAA1) had high niche overlap with *O. mossambicus* (10–40 mm T_L) (ORM1) and *O. mossambicus* (80–400 mm T_L) (ORM2) (94.79% and 92.37%), respectively (Supporting Information Table S6 and Figure S2). In addition, *G. callidus* (40–80 mm T_L) (GLC1) and *G. callidus* (81–140 mm T_L) (GLC2) had high niche overlap with *O. mossambicus* (10–40 mm T_L) (ORM1) (98.28%) and *O. mossambicus* (80–400 mm T_L) (ORM2) (84.44%), respectively (Supporting Information Table S6 and Figure S2). From DB2, the isotope niche size exhibited differences in niche overlap, where *G. affinis* (41–80 mm T_L) (GAA2) and *G. aestuaria* (20–40 mm T_L) (GIA1) had the lowest overlap (3.30%), and *G. affinis* (41–80 mm T_L) (GAA2) and *G. aestuaria* (41–80 mm T_L) (GIA2) also had low niche overlap (2.19%), whereas *G. affinis* (41–80 mm T_L) (GAA2) had a moderate niche overlap with *G. callidus* (40–80 mm T_L) (GLC1) (46.43%) and high niche overlap with *G. callidus* (81–140 mm T_L) (GLC2) (78.34%), respectively (Supporting Information Table S6). From MLS, the isotope niche size exhibited differences in niche overlap, where *G. affinis* (41–80 mm T_L) (GAA2) and *G. aestuaria* (20–40 mm T_L) (GIA1) had the lowest overlap (1.75%), and *G. affinis* (41–80 mm T_L) (GAA2) and *G. aestuaria* (41–80 mm T_L) (GIA2) also had a moderate niche overlap (38.88%), whereas *G. affinis*

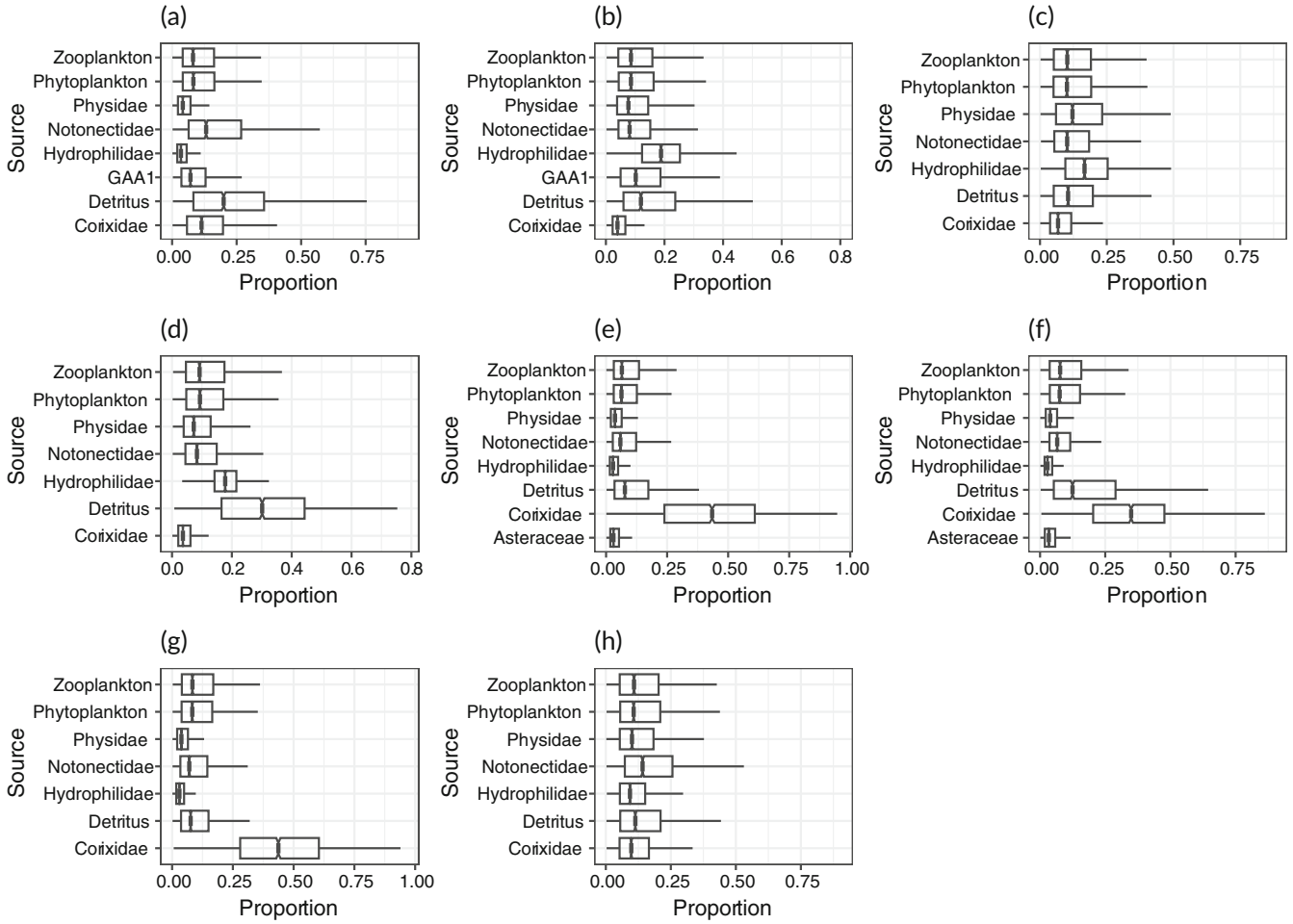

FIGURE 5 Proportional resource contributions to (a) GLC1 = *Glossogobius callidus* (40–80 mm T_L), (b) GLC2 = *Glossogobius callidus* (81–140 mm T_L), (c) GIA1 = *Gilchristella aestuaria* (20–40 mm T_L), (d) GIA2 = *Gilchristella aestuaria* (41–80 mm T_L), (e) ORM1 = *Oreochromis mossambicus* (10–40 mm T_L), (f) ORM2 = *Oreochromis mossambicus* (80–400 mm T_L), (g) GAA1 = *Gambusia affinis* (10–40 mm T_L) and (h) GAA2 = *Gambusia affinis* (41–80 mm T_L) as determined by Stable Isotope Mixing Models (SIMMR) for River Bend (RBD)

TABLE 2 Stable isotope community metrics (mean with 95% C.I. in parentheses) comparing trophic structure of fish communities sampled from Dungbeetle 1 (DB1), Dungbeetle 2 (DB2), ML Swart's (MLS) and River Bend (RBD)

Species	DB1	DB2	MLS	RBD
NR	2.1 (1.2–3.5)	2.3 (1.8–3.2)	3.1 (2.4–4.0)	5.9 (5.2–7.6)
CR	1.4 (0.8–3.0)	2.8 (2.0–4.2)	1.1 (0.5–2.8)	3.3 (2.8–5.6)
CD	0.7 (0.8–1.5)	1.2 (1.1–2.6)	1.2 (0.8–1.4)	2.0 (1.8–2.4)
MNND	0.7 (0.5–1.4)	1.1 (1.0–1.4)	1.1 (0.8–1.4)	1.4 (1.0–2.2)
SDNND	0.2 (0.1–0.8)	0.9 (0.6–1.0)	1.2 (0.8–1.4)	0.9 (0.4–1.4)
SEA_c	0.9 (0.3–3.4)	3.2 (1.6–5.5)	1.5 (0.6–3.0)	11.6 (0.8–18.2)

Note: Species collected and their isotope metrics.
Abbreviations: CD, mean distance to centroid; CR, $\delta^{13}C$ range; MNND, mean nearest neighbour distance; NR, $\delta^{15}N‰$ range; SDNND, standard deviation of nearest neighbour distance; SEA_c, standard ellipse area.

(41–80 mm T_L) (GAA2) had a moderate niche overlap with *G. callidus* (40–80 mm T_L) (GLC1) (68.71%) and with *G. callidus* (81–140 mm T_L) (GLC2) (52.39%), respectively (Supporting Information Table S6). Niche overlap between *G. aestuaria* (41–80 mm T_L) (GIA2) and *G. callidus* (81–140 mm T_L) (GLC2) was very high (78.62%) (Supporting Information Table S6). Niche overlap between fish size class from RBD differed, and the highest recorded overlap was between *G. aestuaria* (41–80 mm T_L) (GIA2) and *G. callidus* (81–140 mm T_L) (GLC2) (86.95%) (Supporting Information Table S6). The niche overlap metric also showed moderate niche overlap between *G. callidus* (40–80 mm T_L) (GLC1) and *O. mossambicus* (10–40 mm T_L) (ORM1) (68.26%) (Supporting Information Table S6).

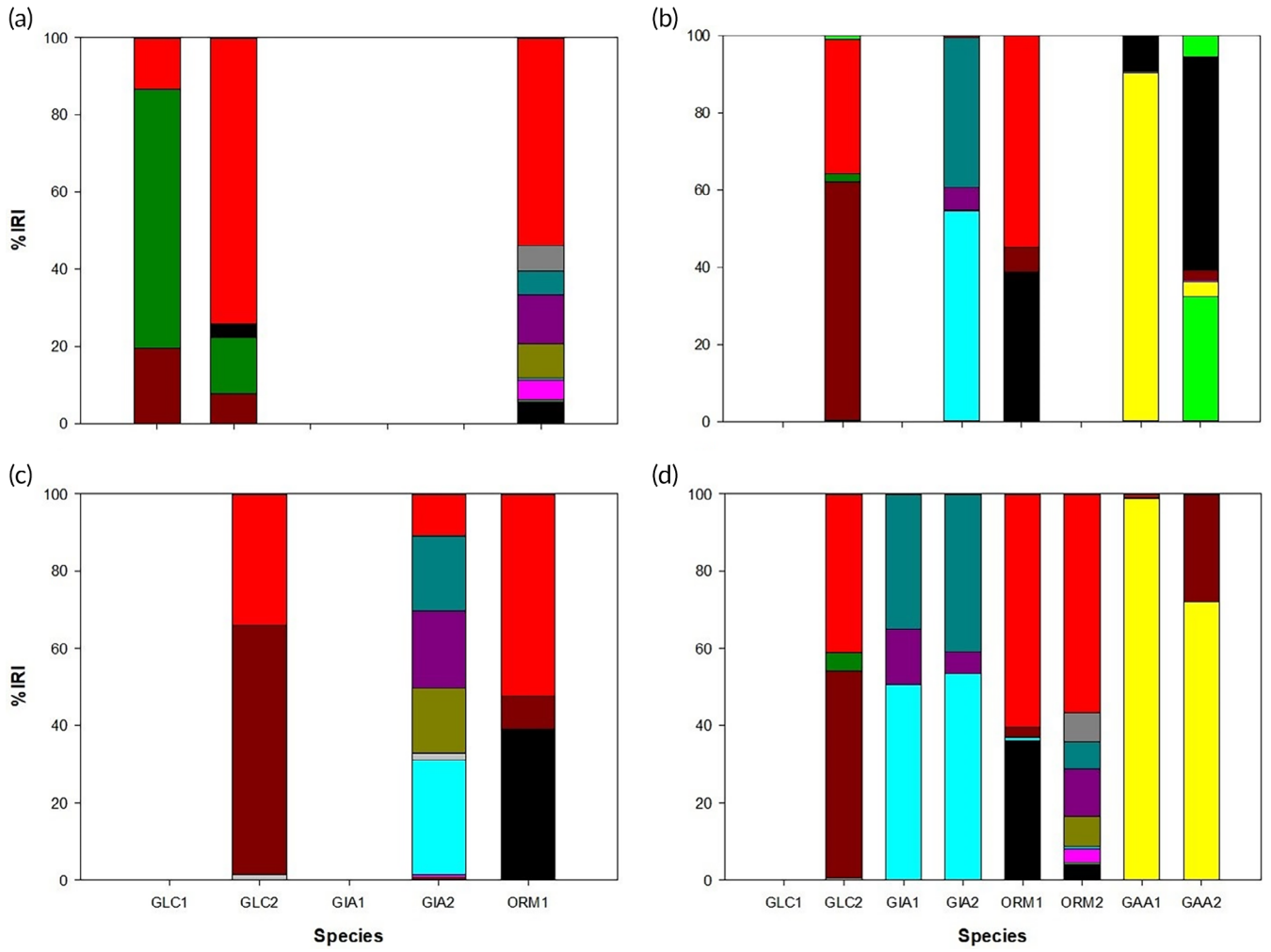

FIGURE 6 The percentage of relative importance (%IRI) of prey items from the gut contents of GLC1 = *Glossogobius callidus* (40–80 mm T_L), GLC2 = *Glossogobius callidus* (81–140 mm T_L), GIA1 = *Gilchristella aestuaria* (20–40 mm T_L), GIA2 = *Gilchristella aestuaria* (41–80 mm T_L), ORM1 = *Oreochromis mossambicus* (10–40 mm T_L), ORM2 = *Oreochromis mossambicus* (80–400 mm T_L), GAA1 = *Gambusia affinis* (10–40 mm T_L) and GAA2 = *Gambusia affinis* (41–80 mm T_L) from (a) Dungbeetle 1 (DB1), (b) Dungbeetle 2 (DB2), (c) ML Swart's (MLS) and (d) River Bend (RBD). ▬: Algae, ▬: Euglena, ▬: Melosira varians Agardh, ▬: Macrocyclops, ▬: Nauplii, ▬: Pannus, ▬: Xenococcus kemeri, ▬: Calopterygidae, ▬: Chironominae, ▬: Corixidae, ▬: Brachionus, ▬: Cephalodella gibba 1, ▬: Keratella tecta, ▬: Trichocerca similis, ▬: Polyarthra vulgaris, ▬: Gambusia, ▬: Ditritus and ▬: Inverts remains

3.4 | Gut contents

Of the 98 *G. callidus* [*i.e.*, 35 *G. callidus* (40–80 mm T_L) (GLC1) and 63 *G. callidus* (81–140 mm T_L) (GLC2)] gut contents examined, only 0.03% was empty. The gut contents of *G. callidus* (40–80 mm T_L) (GLC1) from DB1 contained mostly Corixidae (Insecta) (%IRI = 67.0%) and detritus (%IRI = 13.4%) (Supporting Information Table S7; Figure 6a). The diet composition of *G. callidus* (81–140 mm T_L) (GLC2) from DB1 was detritus (%IRI = 74.0%) and Corixidae (Insecta) (% IRI = 14.5%) (Supporting Information Table S7; Figure 6a). *Oreochromis mossambicus* (80–400 mm T_L) (ORM2) gut contents from DB1 contained detritus (%IRI = 53.8%) and *K. tecta* (rotifers) (% IRI = 12.6%). (Supporting Information Table S7; Figure 6a). The diet composition of *G. callidus* (81–140 mm T_L) (GLC2) from DB2 was Chironomidae (Insecta) (%IRI = 61.7%) and detritus (%IRI = 35.0%) (Supporting Information Table S7; Figure 6b). The *G. aestuaria* (20–40 mm T_L) (GIA1) gut contents from DB2 contained *Xenococcus kerneri* (cyanobacteria) (%IRI = 54.4%) and *Trichocerca similis* (rotifers) (%IRI = 32.4%) (Supporting Information Table S7; Figure 6b). *Oreochromis mossambicus* (10–40 mm T_L) (ORM1) gut contents from DB2 contained detritus (%IRI = 54.8%) and unidentified algae (algae) (% IRI = 38.6%) (Supporting Information Table S7; Figure 6b). The *G. affinis* (10–40 mm T_L) (GAA1) gut contents from DB2 contained mostly *Macrocyclops* sp. (Copepoda) (%IRI = 90.2%) and *G. affinis* (teleost) (% IRI = 9.2%), whereas *G. affinis* (41–80 mm T_L) (GAA2) guts contained *G. affinis* (teleost) (%IRI = 55.2%) and *M. varians* (algae) (%IRI = 32.3%) (Supporting Information Table S7; Figure 6b).

The gut contents of *G. callidus* (81–140 mm T_L) (GLC2) from MLS contained mostly Chironomidae (Insecta) (%IRI = 64.4%) and detritus (%IRI = 33.9%) (Supporting Information Table S7; Figure 6c). The diet

composition of *G. aestuaria* (41–80 mm T_L) (GIA2) from MLS was *X. kerneri* (cyanobacteria) (%IRI = 29.6%) and *Keratella tecta* (rotifers) (%IRI = 20.0%) (Supporting Information Table S7; Figure 6c). *O. mossambicus* (10–40 mm T_L) (ORM1) gut contents from MLS contained detritus (%IRI = 52.5%) and unidentified algae (algae) (%IRI = 39.1%) (Supporting Information Table S7; Figure 6c). The diet composition of *G. callidus* (81–140 mm T_L) (GLC2) from RBD was Chironomidae (Insecta) (%IRI = 53.7%) and detritus (%IRI = 41.0%) (Supporting Information Table S7; Figure 6d). The *G. aestuaria* (20–40 mm T_L) (GIA1) gut contents from RBD contained *X. kerneri* (cyanobacteria) (%IRI = 53.3%) and *T. similis* (rotifers) (%IRI = 40.7%) (Supporting Information Table S7; Figure 6d). *O. mossambicus* (10–40 mm T_L) (ORM1) gut contents from DB2 contained detritus (%IRI = 60.4%) and unidentified algae (algae) (%IRI = 35.8%) (Supporting Information Table S7; Figure 6d). The guts of *O. mossambicus* (80–400 mm T_L) (ORM2) from RBD contained mostly detritus (%IRI = 56.6%) and *K. tecta* (rotifers) (%IRI = 12.0%). The *G. affinis* (10–40 mm T_L) (GAA1) gut contents from DB2 contained mostly *Macrocyclops* sp. (Copepoda) (%IRI = 98.8%) and detritus (%IRI = 0.8%), whereas *G. affinis* (41–80 mm T_L) (GAA2) guts contained *Macrocyclops* sp. (Copepoda) (%IRI = 72.1%) and Chironomidae (algae) (%IRI = 27.9%) (Supporting Information Table S7; Figure 6d). Niche breadth varied with size class and across ponds. *G. callidus* (40–80 mm T_L) (GLC1) had the broadest niche as compared to *G. callidus* (81–140 mm T_L) (GLC2) from DB1 (Supporting Information Figure S3). From DB2, *G. affinis* (10–40 mm T_L) GAA1 had the broadest niche. In addition, from MLS, *O. mossambicus* (10–40 mm T_L) (ORM1) had the broadest niche, whereas from RBD, *G. aestuaria* (20–40 mm T_L) (GIA1) had the broadest niche (Supporting Information Figure S3).

4 | DISCUSSION

Overall, stable isotope and gut content analyses showed that all four fish species can consume a wide range of resources. With regard to the hypothesis of this study, the authors found no evidence that *O. mossambicus* and *G. affinis* utilised a vacant niche, but they found considerable niche overlap among the four fish species. This study showed that co-existence in the Sundays River Valley irrigation ponds is achieved through differences in prey preferences and feeding behaviour. Regression analysis showed a significant increase in $\delta^{15}N$ with increasing fish size suggesting an ontogenetic shift in the diet of the four fish species. Ontogenetic niche shift is important particularly in fishes, as it helps maximise their fitness by reducing competition with conspecifics (Werner & Gilliam, 1984), reduces predation risk through changes in habitat (Werner *et al*., 1983) and increases growth rates through changes in diet (Aggus & Elliot, 1975). In addition, stable isotope and gut content analyses supported previous results that *G. callidus* is a generalist invertivore and feeds predominantly on benthic resources (Mofu, *et al*., 2019b). This study was in support of the studies by Wasserman (2012) and Mofu, *et al*. (2019b). Mofu, *et al*. (2019b) found that the *G. callidus* demonstrated an ontogenetic shift in their diet, and this was in agreement with Wasserman (2012), where ontogenetic shift was observed across seasons and size classes.

Based on the SIMMR result, the proportion of food resources increased with increasing fish size and this was in concordance with gut content analyses. The SIMMR results confirmed the results of regression analysis, where the model explicitly showed differences in food source contribution between the size classes of *G. callidus*. The SIMMR results demonstrated that *G. callidus* became more oriented towards piscivorous feeding behaviour with increasing body size. Both stable isotope and gut content analyses showed an ontogenetic shift towards a preference in larger-sized prey, including juvenile *G. affinis*. The estimates from stable isotope mixing models suggest that both size classes of *G. callidus* would probably assimilate detritus through their benthic feeding behaviour, whereas the gut content analysis found that detritus formed a bulk component of *G. callidus* diet. This suggests that high contributions of detritus may be ingested, during benthic foraging for invertebrates (Bowen, 1983). This is also observed in other species, such as the common carp, *Cyprinus carpio* and common goldfish, *Carassius auratus* (Busst & Britton, 2017; Kelleyway *et al*., 2010). This is in concordance with research in estuaries, where Boulle (1990) showed that non-epi-benthic and terrestrial invertebrates dominated the diet of the *G. callidus*. The authors hypothesize that low contribution of certain food sources, such as Coenogronidae and Corduliidae, could be driven by the living behaviour of these sources or the feeding depth in the sediment of these food sources.

Overall, the results of stable isotope and gut content analyses of this study demonstrated that *G. aestuaria* is a generalist feeder and is capable of exploiting resources ranging from phytoplankton to collectors and scrapers. This further suggests patterns of resource partitioning, which could be achieved through selective feeding by *G. aestuaria*. To date, only limited literature exists on resource use by *G. aestuaria*, and current literature is based on estuaries where *G. aestuaria* feeds predominantly on zooplankton, including copepod eggs, and Ostracoda. The stable isotope component of this study corroborates the findings by Coetzee (1981) who showed that when phytoplankton and zooplankton densities are low, *G. aestuaria* is often seen feeding on larger prey items, such as chironomid larvae.

Ontogenetic shifts in the diet of *G. aestuaria* have been described in the St. Lucia estuary, where size-based shifts in foraging resulted in increased predation of larger-sized prey by larger *G. aestuaria* (Blaber, 1979). Changes in the diet of *G. aestuaria* with increasing length are caused by a decrease in the filtering efficiency for copepods, and an increased ability to feed on macroinvertebrates (Blaber, 1979; Coetzee, 1981). This is supported by findings from the current study and from the study by Costalago *et al*. (2015), who demonstrated that in turbid water, *G. aestuaria* adapted their feeding strategy to a non-filter feeding strategy. Results from stable isotope and gut content analyses suggest that *O. mossambicus* is an omnivorous feeder, which undergoes an ontogenetic shift in diet. The trophic position occupied by the different size classes is in accordance with previous findings, that *O. mossambicus* can consume different kinds of prey, such as algae, phytoplankton, zooplankton, insects and fish (Dyer *et al*., 2013; Zengeya

et al., 2011). This study agrees with the finding of Tomojiri *et al*. (2019), who studied the food habits of three alien cichlid fish, namely, Mayan cichlid, *Mayaheros urophthalmus*, *O. mossambicus* and Nile tilapia, *O. niloticus* in the Chao Phraya River basin in Thailand, that detritus was the main food resource, whereas algae and small aquatic animals were secondary prey items. The results obtained from stable isotope and gut content analyses agree with those from previous studies that suggested that *G. affinis* has a broad dietary niche (Garcia, 1999; Ruehl & DeWitt, 2005). Differences in diet across size classes of *G. affinis* suggest that large-sized *G. affinis* had greater efficiency in predation and had greater propensity to move up and down the water column to exploit different biotopes when feeding. Such behaviour could allow *G. affinis* to avoid direct competition with *G. callidus* and *O. mossambicus* through active prey switching across different habitats, as resources within the ponds fluctuate over time.

The isotopic niche of the small size class of these species showed a variable niche overlap between species. Nonetheless, despite the overall high degree of overlap observed from the SIBER results, subtle differences were observed for large-size classes. According to Sakai *et al*. (2001), invasive species are expected to have broader trophic niches, and the isotope metrics showed that both *G. affinis* and *O. mossambicus* utilised a wide range of resources and trophic levels, and in addition, both species (*G. affinis* and *O. mossambicus*) had larger isotopic niche widths (SEA_c) than *G. callidus* and *G. aestuaria*.

Dietary interactions between introduced species and native species can strongly influence predator–prey interactions and resource competition (Alexander *et al*., 2014; Busst & Britton, 2017; Guo *et al*., 2017). This study suggests that there is potential interspecific competition between *G. callidus* and *G. affinis*, supporting prior evidence of negative spatial interactions between the two species (Howell *et al*., 2013), which may indicate avoidance behaviour to limit competitive interactions. The findings of the present study have wider implications beyond the Sundays River irrigation ponds, indicating the resource use of *G. affinis* and *O. mossambicus* is likely to alter the resource use of *G. callidus* and *G. aestuaria*.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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