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Trophic Dynamics of the Cape Stumpnose (*Rhabdosargus holubi*, Sparidae) Across Three Adjacent Aquatic Habitats

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Abstract Migratory fish species are major vectors of connectivity among aquatic habitats. In this study, conventional stomach contents and stable isotope methods (δ^{13}C and δ^{15}N) were combined to understand how fish of different sizes feed across contrasting aquatic habitats. The Cape stumpnose *Rhabdosargus holubi* (Sparidae, Perciformes) was selected as an abundant estuarine-dependent species in the permanently open Kowie system, South Africa. Three different habitats were sampled in the region, namely, river, estuary, and sea. Fish entered the estuary as post-larvae from the marine environment, resided in the estuary and lower part of the river as juveniles, and then returned to the sea as sub-adults. The diet varied among habitats, seasons, and fish sizes. "Stable Isotope Analysis with R" (SIAR) Bayesian mixing models mostly supported the results from the stomach content analyses, but also revealed the importance of some prey (e.g., insects) that were underestimated in the consumed diet. *Rhabdosargus holubi* δ^{13}C values indicated a clear spatial gradient in the origin of food sources assimilated across the habitats, with increasing δ^{13}C along the freshwater-marine continuum. The δ^{13}C ranges of sources and fish also overlapped within each habitat along this continuum, thus illustrating the fidelity of *R. holubi* to specific habitats at different life stages. By consuming prey in a particular habitat before migrating, either permanently or temporarily to another habitat, *R. holubi* participates in allochthonous fluxes among riverine, estuarine, and coastal marine environments, with approximately 7 tonnes of Cape stumpnose productivity being exported from the 142-ha Kowie Estuary to the sea each year.

Keywords Stomach contents · Stable isotopes · Connectivity · SIAR · Fish

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Introduction

Many marine fish species utilize estuaries and associated catchments as nurseries during their early life stages, with variable levels of dependency on the estuarine or freshwater environment (Secor and Hooker 2005). Furthermore, through their migrations to and from freshwater, estuarine, and coastal marine systems, fish contribute to a broad energy transfer across these systems, especially those species using one or several habitats as feeding grounds during their life cycle (Gillanders et al. 2003; Platell and Freewater 2009; Jardine et al. 2012). The latter taxa consume food, assimilate energy, and grow in one habitat before migrating, temporarily or permanently, to another. As such, fish diet, together with fish movements, constitutes the basis for potentially significant trophic connectivity among aquatic habitats. The understanding of how migrating fish species utilize food resources within

and among adjacent aquatic systems is therefore a crucial step toward designing appropriate measures for the integrative management of adjacent ecosystems (Deegan 1993; Peterson 2003; Ray 2005; Secor and Hooker 2005).

The Cape stumpnose (*Rhabdosargus holubi* Steindachner 1881) is a dominant fish in South African estuaries, including the Kowie system (Blaber 1973a; Whitfield et al. 1994). This species is estuarine-dependent during the juvenile life stages, but the adults are found almost exclusively in nearshore marine waters where spawning occurs mainly during spring and summer (Blaber 1974a; Whitfield 1998). Juveniles are characterized by a highly developed osmoregulatory capacity, thus allowing them to occupy areas with wide-ranging salinities (Blaber 1973b). In some estuaries, such as the Kowie, a proportion of *R. holubi* juveniles migrate into the freshwater environment above the ebb and flow region (Wasserman and Strydom 2011).

As with many fish species, larvae of *R. holubi* feed on zooplankton in the marine environment, while the juveniles consume mainly filamentous algae, aquatic macrophytes together with the associated epiphytes, and epifauna (Blaber 1974b; De Wet and Marais 1990). As they mature, *R. holubi* dentition changes from sharp tricuspid incisors in the outer row of both jaws into the molariform teeth characteristic of adults, a transformation that facilitates the consumption of echinoderms, molluscs, crustaceans, and polychaetes by adults in marine coastal waters (Buxton and Kok 1983). Because of its high abundance, complex life cycle, and strong ontogenetic variations in diet related to its utilization of freshwater, estuarine, and marine environments at different life stages, the Cape stumpnose represents an ideal species to investigate connectivity among adjacent habitats.

Most knowledge concerning the diet of *R. holubi* has relied on conventional methods such as stomach content analyses (e.g., Blaber 1974b; Whitfield 1984; De Wet and Marais 1990), with more recent studies using stable isotope analyses (Paterson and Whitfield 1997; Sheppard et al. 2012). While identifiable stomach contents provide short-term information about the diets of fish at different life stages and inhabiting particular regions (Hyslop 1980; Cortés 1997), dual stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) can reveal food resources assimilated by fish over longer time scales, ranging from weeks to months depending on fish tissues and life stages (e.g., Buchheister and Latour 2010).

Turnover metabolic rates vary depending on fish growth rates and species, so that specific time scales captured by specific isotopes should be estimated using experimental studies. Such studies, however, can rarely be conducted for all species of ecological interest, so that the difference in the temporal window revealed by stomach contents and stable isotopes is viewed on a relative scale, with isotopes always providing the longer time scale. Stable isotope ratios provide insights about the food items actually assimilated by a fish and the origin of consumed detrital material (e.g., terrestrial versus marine; Hobson 1999), both of which cannot be resolved with stomach contents. A dual approach combining both methodologies thus provides a more comprehensive picture of processes governing fish trophic variability through time and space (e.g., Rooker and Turner 2006; Allan et al. 2010; Bertrand et al. 2011; Buchheister and Latour 2011).

The objective of this study was to describe and analyze the seasonal, spatial, and size-related variability in the diet of *R. holubi* from the Kowie system using combined stomach contents and stable isotopes in three aquatic environments (river, estuary, and sea). We hypothesized that *R. holubi* used different food resources across these habitats during successive life stages, thereby contributing to the transfer of energy from one habitat to another during the species' life cycle.

Material and Methods

Study Area

The Kowie system is located in the Eastern Cape Province of South Africa (Fig. 1). The estuary provides a diversity of freshwater and estuarine habitats ideal for the study of fish dietary dynamics across contrasting aquatic ecosystems. The perennial Kowie River flows through the Waters Meeting Nature Reserve into the Kowie Estuary and enters the Indian Ocean at Port Alfred (Fig. 1). The distance between the estuary mouth and the tidal limit of the ebb and flow region is 21 km. Contrasting vegetation and sediment types are found in the estuarine and freshwater sections of the system, as described by Bergamino et al. (2014). The adjacent marine environment is characterized by rocky and sandy shores, submerged rocky reefs, and a high-wave-energy coastline.

Fish Sampling

Seven sampling sites, distributed along the river-estuary-ocean salinity gradient, were visited in March/April (autumn), August (winter), November (spring) 2012, and February (summer) 2013 (Fig. 1; Table 1). Fish were collected at each site and season in the estuary using a purse seine net (50 m long × 2 m deep with a 3-cm stretch mesh in the wings and 1-cm stretch mesh in the bag) and in the river and estuary using a cast net (1.6 m diameter and 1 cm bar mesh). Spearfishing was employed to collect large juveniles and adults from the nearby marine environment. The exact location of marine fish capture varied slightly, depending mainly on weather conditions that influenced diving, but always occurred on subtidal reefs a few kilometers off Port Alfred harbor (Fig. 1). In addition, postlarvae immigrating into the Kowie Estuary from the sea were collected inside the mouth (ES4) using a dip net (1 m handle,
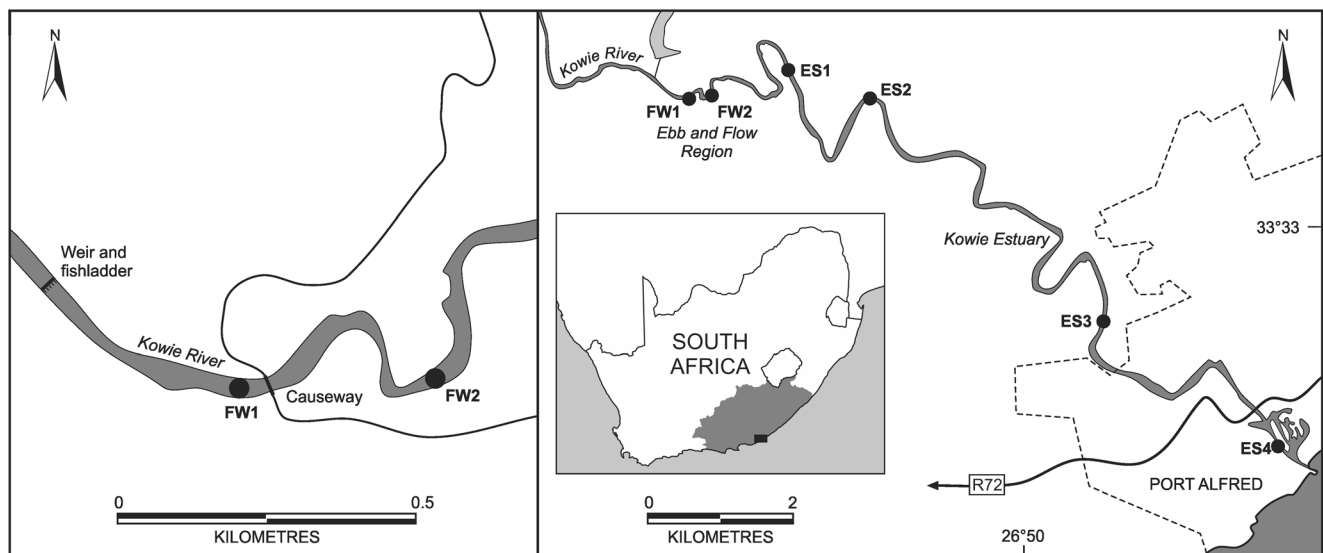
Fig. 1 Map of the lower Kowie system, South Africa, showing the sampling sites in the river and estuary. The marine site is not shown but was positioned on subtidal reefs a few kilometers south of the Kowie Estuary mouth

25 cm diameter ring net, and 2 mm mesh) during November 2013 (Fig. 1).

All fish collected were euthanized immediately after capture by immersion in a container filled with ice and then transported back to the Rhodes University laboratory for further analysis. This project received ethics clearance from Rhodes University (RU Ethics Clearance ZOOL-02-2012) and the South African Institute for Aquatic Biodiversity (SAIAB Ethics Clearance 2012/04). Water temperature (°C) and salinity were measured on each sampling occasion at every site using a portable meter (Eutech Instruments, CyberScan Series 600, model PC650).

Sampling of Food Sources

Sources of organic matter and major food items consumed by *R. holubi* were collected at similar locations and periods in the Kowie system, with the exception of the autumn (March/April 2012) and November 2013 periods (post-larval sampling). In addition, no sampling of food sources was conducted at the marine site. Sites for food source collection in the estuary were similar to those of the fish (i.e., ES1-3; Fig. 1), whereas in the river, fish were collected at two closely located sites (FW1 and FW2), but food sources were collected only at FW1 (Fig. 1). Due to weather-related and logistical constraints, sampling periods for food sources sometimes varied slightly when compared to those of the fish, but the different components were always collected within the same month.

Sampling methods for benthic algae, zooplankton, epiphyton, and macrophytes were described in Bergamino et al. (2014) and Bergamino and Richoux (2015), and corresponding data available from each site and season are given in Online Resource 1. Freshwater invertebrates were sampled in the river by repetitive kick sampling and sweeping using a South African Standard Scoring (SASS) net (mesh size 80 μm). Other benthic invertebrates from the estuary were obtained using a triangular dip net (mouth dimensions $0.3 \times 0.3 \times 0.3$ m, mesh size 1.0 mm) pushed perpendicular to the shore over a distance of 1–2 m (Froneman and Henninger 2009). Macrophytes were collected by hand, and epiphytes were brushed from the leaves of *Schoenoplectus brachyceras* and *Spartina maritima* in distilled water

Table 1 Number and size (standard length) of *Rhabdosargus holubi* specimens collected in the Kowie system

Site	Autumn	Winter	Spring	Summer	Total	Size [SL, cm] Mean (min–max)
FW1	10	0	6	12	28	6.4 (4.5–9.1)
FW2	10	0	10	10	30	6.6 (4.5–9.4)
ES1	29	0	10	17	56	7.4 (5.4–14.2)
ES2	9	0	10	10	29	7.9 (5.5–10.1)
ES3	11	21	11	11	54	7.7 (4.5–11.9)
ES4*	0	0	8	0	8	1.1 (1.0–1.2)
MAR	13	2	3	15	33	21.1 (13.1–29.7)

Sampling seasons and sites are described in the "Material and Methods" section and in Fig. 1. *ES4 is the site where post-larvae were collected in November 2013

(Bergamino et al. 2014; Bergamino and Richoux 2015). Surface sediment cores (10 cm deep) were collected in the estuary to obtain microphytobenthos signatures using the laboratory protocol described below. All samples were placed on ice in plastic containers and transported to the laboratory for further processing.

Laboratory Processing

In the laboratory, the standard length (SL) of each fish was measured before dissection. Stomachs of 198 fish (i.e., all fish collected per site and season if less than 10 fish, or a minimum of 10 when more were available) were removed and preserved in 4 % formaldehyde for 72 h before being transferred to 70 % ethanol. The stomachs were later opened and the contents placed in a Petri dish. The contents were identified and separated into broad taxonomic groups, with the relative proportion of each prey item visually estimated (% of total content) according to the area it occupied under a dissecting microscope. Additionally, for the same fish, one sample of fresh dorsal muscle was excised and frozen at −80 °C before lyophilization using a VirTis BenchTop 2 K freeze-drier at −60 °C for a minimum of 30 h. The dried muscle samples were homogenized by grinding with an ethanol-cleaned mortar and pestle. Individual homogenized fish samples were weighed (±1 mg accuracy) on a Mettler Toledo XP205 analytical balance and placed in 8×5 mm tin capsules.

Laboratory processing methods for stable isotope analysis of benthic algae, estuarine invertebrates, and macrophytes are described in Bergamino et al. (2014) and Bergamino and Richoux (2015). To obtain microphytobenthos isotopic composition, surface sediment samples collected in the estuary were covered with a thin layer (5 mm thick) of pre-treated sand (washed with 1 N HCl and combusted at 500 °C for 5 h) and incubated for 15 h under artificial light to promote the movement of the microalgae toward the treated sand. Migrated microalgae were separated from the treated sand by pressure washing with distilled water and filtration through a 63-μm sieve and then concentrated onto pre-combusted (500 °C; 5 h) GF/F filters (protocol from Antonio et al. 2010, described in Bergamino et al. 2014).

Freshwater invertebrates were kept alive in plastic containers for approximately 6 h to allow for gut clearance and identified to the lowest possible taxon (mostly to family) prior to freezing and lyophilization. All samples were ground into a fine powder for stable isotope analysis. Insect samples intended for isotope analysis consisted of one to five whole individuals from each of the dominant taxa.

All $\delta^{13}C$ and $\delta^{15}N$ measurements were performed at IsoEnvironmental cc, Grahamstown, using a Europa Scientific 20–20 Isotope Radio Mass Spectrometer linked to an ANCA SL Prep Unit. Beet sugar, ammonium sulfate, and casein were the internal standards calibrated against the International Atomic Energy references (Vienna Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen). Precision of the analysis was ±0.1‰ for both elements. Isotopic carbon and nitrogen composition of all samples were expressed using the standard delta unit notation (δ) as follows:

$$\delta^{13}C \text{ or } \delta^{15}N = \left[(R_{sample}/R_{standard})-1 \right] \times 1000,$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$.

Data Analyses

The first step in data analysis consisted of summarizing habitat and ecosystem occupancy by *R. holubi* of different life stages. This procedure allowed us to confirm that the species migrated among adjacent aquatic habitats during its life cycle within the Kowie system, thereby contributing to biomass and energy transfers. The average sizes (cm SL) of fish collected in the different habitats and seasons (post-larvae excluded) were compared using one-way analyses of variance (ANOVA) and Student's *t* tests with factors as sites (*df*=5) or seasons (*df*=3). Similar statistics were used to test for differences in water temperature and salinity among sites and seasons.

Analyses of similarity (ANOSIM) were used to test for differences in stomach content composition among seasons, sites, and fish size classes. The latter factor was defined using five main size classes as follows: post-larvae (SL≤1.2 cm), very small (1.3 < SL≤5 cm), small (5.1 < SL≤7 cm), medium (7.1 < SL≤15 cm), or large (SL > 15 cm). ANOSIM analyses were based on Euclidean distance matrices. Stomach content data were first square-root-transformed to balance the weighting of dominant versus rarer prey in the data set (Zar 1999).

Analyses of contributions to the dissimilarity (SIMPER) in *R. holubi* diets were applied to the stomach content data, thereby identifying the prey items responsible for at least 10 % of the observed significant differences. The average proportions (%) and corresponding standard errors (SE) of the selected prey were then plotted to visualize patterns of variations in fish stomach content composition among seasons, sites, and size classes. Although often contributing more than 10 % of the stomach content SIMPER dissimilarities, unidentified detrital material was not analyzed further due to its unknown content and the difficulty in assigning it to a particular food source category. Results from stomach content analyses were used to select food sources for inclusion in the stable isotope models.

Averages and standard deviations (SD) of $\delta^{13}C$ and $\delta^{15}N$ measured in fish and their food sources were calculated for each site and season (see Online Resource 1 for food sources categories and availability). Variations in food source origin and fish trophic level as a function of fish ontogeny were tested using linear regressions between fish size class

(independent variable) and $\delta^{13}C$ and $\delta^{15}N$ (as dependent variables). Bayesian mixing models using the SIAR package (Parnell et al. 2010) were used to estimate the contributions of different food sources to the diet of *R. holubi* at various sites and seasons. Only food sources that occurred in fish stomach contents at sites and seasons similar to the fish were included in the models (Online Resource 1).

In order to include an appropriate number of possible sources into two-isotope-based models, sources were grouped into seven broad taxonomic categories corresponding to major food items reported from fish stomach content analyses (Fry 2013; Phillips et al. 2005, 2014). Furthermore, source categories that had mean $\delta^{15}N$ differences from *R. holubi* >4‰ were excluded, after subtracting the trophic enrichment ($\Delta\delta^{15}N$) of 3.4‰ as recommended by Miller et al. (2013). This procedure allowed the inclusion of only those sources having a strong probability of contributing to the fish diet, thus ensuring that each SIAR model had an ecologically relevant number of potential food source categories (i.e., three to five sources per model; Fry 2013; Phillips et al. 2005, 2014). The "siarsolo" command was used so that individual models included all fish collected at a particular site and season, thus allowing for a sufficient number of observations per model (i.e., $n \geq 5$, Parnell et al. 2010).

Trophic enrichment factors (TEF) were based on available literature from freshwater and estuarine systems (e.g., McCutchan et al. 2003; Cremona et al. 2010) and an examination of isotopic values from available food source data in the Kowie system (Bergamino et al. 2014; Bergamino and Richoux 2015; Moyo 2016). The sensitivity of SIAR models to TEF variability was tested by running each individual model with three different combinations of TEF values as follows: 0.4 ± 0.1 and 2.0 ± 0.3 for carbon and nitrogen, respectively (combination 1), following values for freshwater aquatic consumers in McCutchan et al. (2003); 1.3 ± 0.3 and 2.9 ± 0.3 for these two elements (combination 2) based on previous estuarine models from the same study sites (Bergamino et al. 2014; Bergamino and Richoux 2015); and 0.9 ± 1.3 and 3.4 ± 1.0 (combination 3) following Miller et al. (2013). These TEF for consumers are within confidence limits reviewed by Vanderklift and Ponsard (2003) and Caut et al. (2009). The SIAR models were set at 500,000 iterations, the initial 50,000 being discarded.

Results

Water Temperature and Salinity

Water temperature differed significantly among seasons ($F = 106.7$, $P < 0.0001$), but not among sampling sites ($F = 0.3$, $P > 0.05$). The lowest values were recorded in winter, the highest values in summer, and similar intermediate values

during autumn and spring (Fig. 2a). Conversely, salinity did not differ among seasons ($F = 0.6$, $P > 0.05$) but did vary among sites ($F = 7.0$, $P < 0.005$). Low mean salinity values occurred at the riverine sites (FW1, FW2), increasing slightly towards the middle reaches of the estuary (ES2), with the highest mean values recorded in the lower reaches (ES3) (Fig. 2b).

Fish Habitat and Ecosystem Use

A total of 238 *R. holubi* were collected over the study period, including 82, 23, 50, and 75 individuals in autumn, winter, spring and summer, respectively, with totals of 58, 147, and 33 individuals from the river, estuary, and sea, respectively (Table 1). Eight additional post-larvae were collected in the mouth region of the estuary (ES4) during November 2013. Because of their early ontogenetic stage, limited sample size, and corresponding small size range (10–12 mm SL), those particular specimens were not included in the average fish size comparisons.

The average sizes of *R. holubi* collected were similar among seasons ($F = 2.0$, $P > 0.05$), but varied significantly among sites ($F = 240.1$, $P < 0.0001$). The smallest juveniles were collected in the most upstream sites (FW1, FW2), with fish increasing in average size toward the sea (Table 1).

Stomach Contents

Among the 198 specimens dissected for their stomach contents, 21 (10 %) had empty stomachs. A total of 31 food types were recorded in the 178 remaining fish, with filamentous algae, amphipods, unidentified detrital material, and aquatic macrophytes the most frequent dietary items (>20 % of stomachs) in juvenile fish from the river and estuary, followed by isopods, unidentified crustaceans, and polychaetes (>10 % of stomachs; Table 2). Cladocerans, insects, arachnids, and diatoms were occasionally documented in the stomach contents (Table 2). Adult fish from the marine environment consumed mostly gastropods and cirripeds (>50 % of stomachs), followed by unidentified crustaceans, bivalves, cnidarians, and echinoderms (>10 % of stomachs). Surface-swimming post-larvae entering the estuary from the sea had all been feeding on planktonic copepods (Table 2).

The composition of *R. holubi* stomach contents varied significantly among seasons ($R = 0.057$, $P = 0.002$), sites ($R = 0.317$, $P = 0.001$), and fish size classes ($R = 0.192$, $P = 0.001$), with site being the strongest driver of variation, as indicated by the relatively high R statistic from the corresponding ANOSIM. Cirripeds were more often encountered as dietary items during winter, while aquatic macrophytes were more frequently consumed during spring (Fig. 3). The dominance of copepods during spring was due to the dietary dominance of this food item in immigrating post-larvae
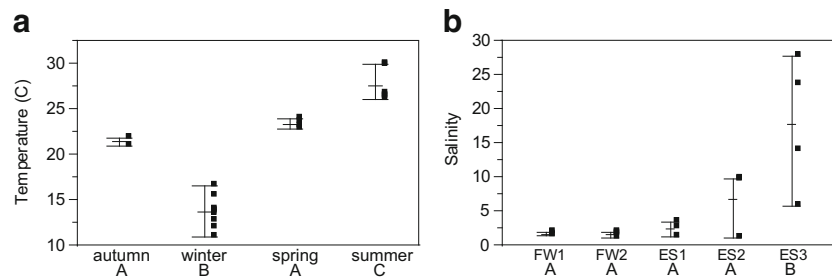
a

b

Fig. 2 Average and range (min–max) of **a** temperature (°C) during each season and **b** salinity at each site (shown in Fig. 1). *Capital letters* under the *season or site labels* indicate groupings obtained by ANOVA and pairwise Student's *t* test. *Dots* represent individual values and bars show the average, minimum, and maximum

collected in November 2013. Isopods were primarily consumed by very small juveniles from the river and upper reaches of the estuary (Fig. 3). The dominance of filamentous algae in the diet of *R. holubi* decreased from the river sites to the estuary mouth, concurrent with the abundance of this food source in the ebb and flow region and the increase in fish size along the same salinity gradient (Fig. 3). Amphipods dominated the diet of juvenile *R. holubi* in the upper reaches of the estuary, while aquatic macrophytes followed an inverse pattern and were more prevalent in the diets of fish from the lower estuary reaches. Gastropods and cirripeds were recorded exclusively in the stomachs of adult *R. holubi* from the marine environment (Fig. 3).

Stable Isotopes

The $\delta^{13}C$ values measured in fish muscle tissues increased along gradients of both fish size and salinity or habitat. The lowest values occurred in small juveniles from the river or upper reaches of the estuary, while the highest values were recorded in juveniles from the lower reaches of the estuary and adults from the marine environment (Table 3). The increase in $\delta^{13}C$ values with fish size was significant ($r^2 = 0.48$, $P < 0.0001$, $N = 134$), although the relationship between $\delta^{13}C$ and fish size was not linear due to a large variability in carbon values from juveniles in the estuary (Fig. 4a).

Fish larvae collected in November 2013 had relatively high $\delta^{13}C$ values, reflecting their previous dependence on zooplankton in the marine environment (Table 3). Most food sources collected from the river had lower carbon values than those collected from the estuary (Table 3), with the exception of aquatic macrophytes, for which carbon signatures were lowest in the upper and middle reaches of the estuary (Table 3). Fish always had relatively central positions within the range of carbon values represented by the sampled food sources, reflecting the omnivorous diet of *R. holubi* and its relative mobility within the study system. However, food sources consumed by *R. holubi* in particular habitats seemed to originate from these same habitats, as indicated by

overlapping $\delta^{13}C$ values of fish and food sources in specific habitats and seasons (Table 3).

Showing an opposite trend to $\delta^{13}C$, *R. holubi* $\delta^{15}N$ values consistently decreased along the gradients of fish size and salinity or habitat, with the highest values occurring in small juveniles from the river or upper estuary, and the lowest values occurring in larger juveniles from the lower reaches of the estuary (Table 3). Post-larvae collected in November 2013 also had relatively low $\delta^{15}N$ values which were similar to adults from the marine environment (Table 3). Furthermore, although statistically significant ($P < 0.0001$, $N = 134$), the relationship between fish size and $\delta^{15}N$ was nonlinear, with juveniles from the estuary displaying variable nitrogen composition (Fig. 4b) and thus contributing to a poor linear correlation ($r^2 = 0.30$).

Mean and ranked prey contributions as estimated with SIAR models were generally consistent among TEF value combinations used, with the exception of winter samples at site ES3, which included the largest number of sources (Table 4). In spring, aquatic macrophytes made the largest contribution to fish diet at the freshwater sites, followed by insects and other invertebrates (Table 4). Invertebrates contributed greatly to *R. holubi* diet in the estuary, followed by epiphyton and microphytobenthos in the middle (ES2) and lower (ES3) estuarine reaches, respectively (Table 4). Copepods made minor contributions at sites ES1-ES2, and macrophytes at ES3 (Table 4). In summer, insects were the most important prey at the freshwater sites (Table 4). In the estuary, invertebrates dominated the summer diet, with copepods a major contributor at site ES3.

Discussion

Rhabdosargus holubi associated with the Kowie system relied on diverse food sources from adjacent ecosystems during its life cycle. Movements of fish across these ecosystems were confirmed by the presence of fish of successive size classes in the different habitats, with fish entering the estuary as post-larvae from the sea, moving between lower riverine and

Table 2 Frequency of occurrence of prey in the stomachs of *Rhabdosargus holubi*

Prey types	Occurrence (%)		
	Larvae (ES4)	Juveniles (FW1-ES3)	Adults (MAR)
Fish size (mean ± SD)	*1.1 ± 0.1*	*7.3 ± 1.5*	*21.1 ± 4.4*
Amphipods (crustaceans)	0	36.4	0
Ants (insects)	0	0.7	0
Aquatic breeding flies (insects)	0	2.0	0
Arachnidae (spiders)	0	0.7	0
Cirripeds (crustaceans)	0	1.3	55
Bivalves (molluscs)	0	9.3	15
Brachyuran decapods (crustaceans)	0	5.3	0
Chironomidae (insects)	0	9.9	0
Copepods (crustaceans)	100	0.7	0
Daphnia (cladocerans)	0	2.0	0
Diatoms	0	0.7	0
Dolichopodidae (insects)	0	1.3	0
Echinoidae (echinoderms)	0	0	10
Filamentous algae	0	77.5	0
Gastropods (molluscs)	0	4.6	75
Holothuroidea (echinoderms)	0	0	20
Hydrozoa (cnidarians)	0	0	15
Isopods (crustaceans)	0	14.6	0
Invertebrates	0	24.5	0
Macruran decapods (crustaceans)	0	2.6	5
Ophiureidae (echinoderms)	0	0	10
Unidentified detrital material	0	27.2	5
Plant seeds	0	0.7	0
Polychaetes (annelids)	0	10.6	5
Sand	0	1.3	0
Small damselflies (insects)	0	0.7	0
Stratiomyidae (insects)	0	1.3	0
Unidentified	0	1.3	0
Unidentified crustaceans	0	11.3	25
Unidentified decapods (crustaceans)	0	1.3	0
Unidentified molluscs	0	0	5

Frequency is expressed as a percentage of total number of fish dissected, excluding fish with empty stomachs, i.e. *n* = 7, 151, and 20 for larvae, juveniles, and adults, respectively. Fish size is given as standard length in cm

estuarine habitats as juveniles, and then migrating to the marine environment as adults. Stable isotope and stomach content data consistently confirmed the omnivorous diet of *R. holubi*, with ontogenetic variations in the nature of consumed prey following the salinity gradient, and an abrupt change between a juvenile population feeding on river and estuarine food sources and an adult population relying exclusively on marine resources. Macrophytes and insects dominated the diet of small juveniles in the river; larger juveniles consumed mostly invertebrates and a mixture of aquatic

macrophytes and associated microscopic algae (epiphyton, microphytobenthos) in the estuary, while adults depended upon benthic invertebrate prey at sea. Consumption of food resources originating from these three regions was also evidenced by a clear gradient of δ^{13}C in fish muscle tissues, from high values in adults and larvae feeding in or coming from the marine environment, respectively, to low values in juveniles collected from the upper reaches of the estuary or lower parts of the river. These results implied a degree of site fidelity by *R. holubi* at different stages during its life cycle within the Kowie system. By consuming prey in a particular habitat, and then migrating to another, *R. holubi* therefore contributed toward transferring energy among the river, estuary, and sea habitats during its life cycle.

Stomach content analysis showed that adult Cape stumpnose preyed upon invertebrates (mostly benthic gastropods and cirripeds) in the marine environment, whereas juveniles fed on filamentous algae, aquatic macrophytes, and invertebrates in the estuary. These results are consistent with other stomach content studies conducted in other South African estuaries (Blaber 1974b; Whitfield 1984; De Wet and Marais 1990; Sheppard et al. 2012) and coastal marine habitats (Buxton and Kok 1983). However, it was previously hypothesized that macrophytes, although dominant in the diet of *R. holubi* juveniles, were poorly assimilated as the species lack a cellulase (Blaber 1974b). More recent studies using stable isotope analyses supported the above view that only the epiphytic algae (especially diatoms) covering the leaves of aquatic plants are actually assimilated (Paterson and Whitfield 1997; Sheppard et al. 2012). In contrast, our data from the Kowie system suggest that some macrophytic material is digested by juveniles within the riverine habitat. Since our study is the first to investigate *R. holubi* diet from a riverine environment, this finding suggests that *R. holubi* can assimilate aquatic macrophytes at early stages of development when occupying low-salinity habitats, especially where this food source is readily available, while other potentially preferred dietary items (i.e., filamentous algae, estuarine invertebrates) are not.

The use of Bayesian mixing models for estimating prey proportions in a consumer diet relies on many assumptions, some of which may influence model output interpretations. The SIAR modeling method assumes no tissue-to-diet discrimination and no isotopic routing, assumptions that can lead to an over-estimation of the contributions of some prey to omnivorous endotherms (Martínez del Rio et al. 2009; Kelly and Martínez del Rio 2010). Uncertainties around TEF values used in particular SIAR models may also contribute to an increase in model uncertainty, since the TEF for a particular isotope varies depending on consumer and prey species, as well as on environmental conditions (Post 2002; McCutchan et al. 2003; Moore and Semmens 2008). The TEF

Fig. 3 Proportions of prey items, identified by SIMPER analysis, responsible for at least 10 % of the dissimilarity among seasons, sites, and fish size classes in *R. holubi* stomach content composition. *Vertical charts* are averages and *bars* are standard errors. *Au* autumn, *Wi* winter, *Sp* spring, *Su* summer, *Sp** fish post-larval sampling season (November 2013), *Lv* larvae, *Vs* very small, *Sm* small, *Me* medium, *Lg* large. Sampling periods and fish size ranges are given in the "Materials and Methods" section and Table 1, respectively
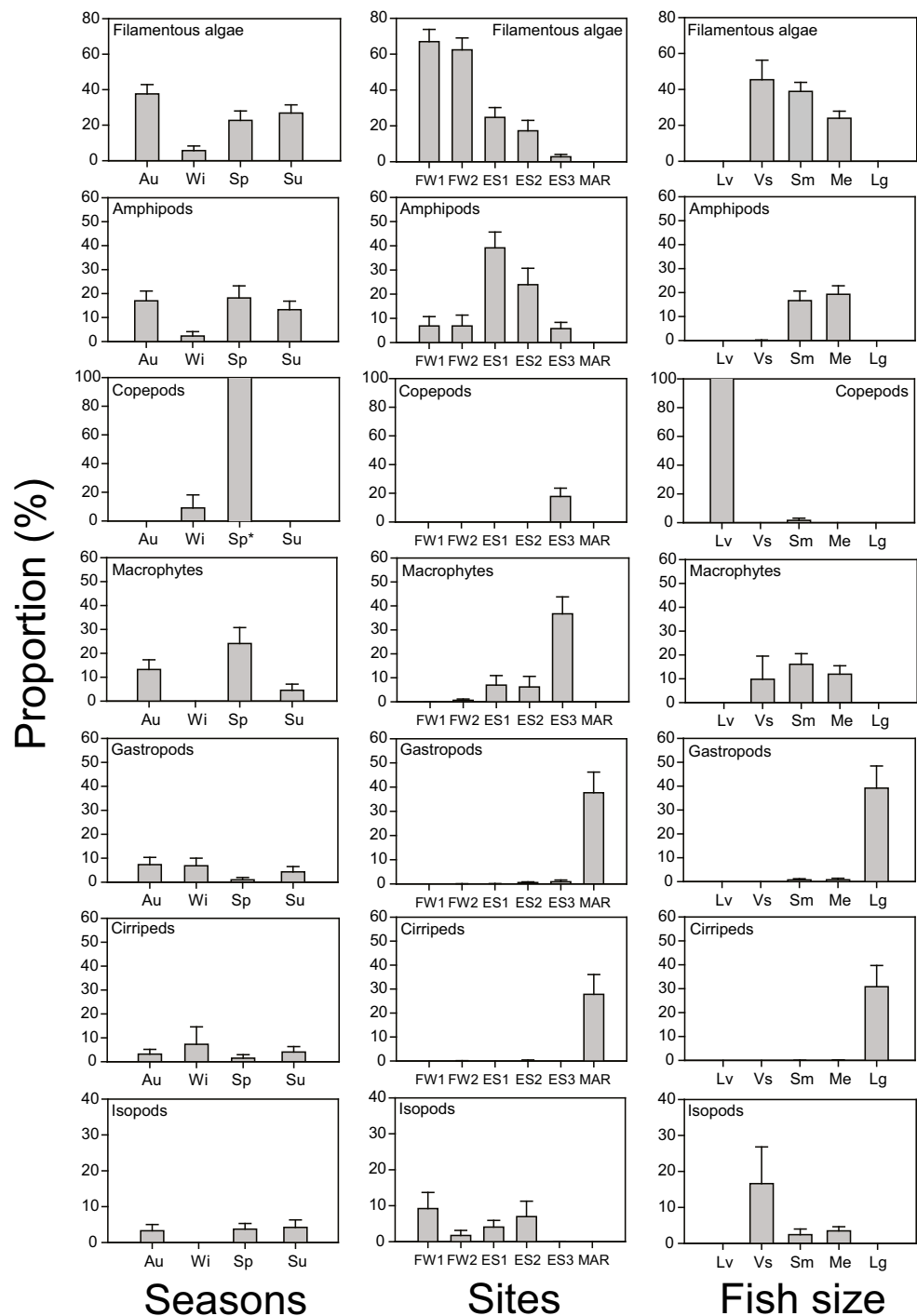
combinations used in our models were estimated based on isotopic values recorded from consumers and prey in the Kowie system during similar periods and from relevant literature in similar ecosystems (McCutchan et al. 2003; Cremona et al. 2010). Sensitivity tests also showed that variations in TEF values did not affect most model outputs in our study with the exception of the winter model, which was to be expected due to the inclusion of a larger number of possible food sources that resulted in higher uncertainties in prey

contribution estimations (Fry 2013; Semmens et al. 2013; Phillips et al. 2014). The procedure proposed by Miller et al. (2013) for the selection of appropriate prey categories to be included in SIAR models helped limit these uncertainties for our individual models. Results obtained by the Kowie SIAR models therefore closely matched those from the conventional stomach content analyses, thus emphasizing the validity of our prey selection approach and the resulting interpretations of the isotopic data.

Table 3 Average $\delta^{13}C$ and $\delta^{15}N$ values of *Rhabdosargus holubi* and potential food sources at each site and season in the Kowie system

| Isotope | Winter | | Spring | | | | | | | Summer | | | | | |
Source	ES3	MAR	FW1	FW2	ES1	ES2	ES3	ES4*	MAR	FW1	FW2	ES1	ES2	ES3	MAR
$\delta^{13}C$															
Benthic algae	−7.78	NA	NS		NS	NS	NS	NA	NA	NS		NS	NS	NS	NA
Macrophytes	−13.80	NA	−26.79		NS	NA	−14.16	NA	NA	−26.18		NS	−30.08	NS	NA
Epiphyton	−23.75	NA	NA		−27.59	−25.50	NS	NA	NA	NA		−26.24	NS	−20.22	NA
Microphytobenthos	−24.68	NA	NA		NS	NS	−24.27	NA	NA	NA		NS	NS	NS	NA
Copepods	NA	NA	NA		−29.87	−28.57	NA	NA	NA	NA		−27.11	−26.21	−24.90	NA
Invertebrates	−16.54	NA	−26.88		−24.25	−24.15	−18.73	NA	NA	−26.71		−25.21	−22.93	−20.53	NA
Insects	NA	NA	−28.48		NA	NA	NA	NA	NA	−26.98		NA	NA	NA	NA
Fish (*R. holubi*)	−20.66	−15.42	−24.25	−24.43	−23.69	−23.12	−20.01	−16.15	−14.62	−26.82	−26.65	−24.30	−22.64	−21.18	−15.90
$\delta^{15}N$															
Benthic algae	10.98	NA	NS		NS	NS	NS	NA	NA	NS		NS	NS	NS	NA
Macrophytes	12.40	NA	15.53		NS	NA	10.29	NA	NA	14.04		NS	10.08	NS	NA
Epiphyton	11.51	NA	NA		14.66	13.02	NS	NA	NA	NA		11.66	NS	10.45	NA
Microphytobenthos	12.22	NA	NA		NS	NS	13.67	NA	NA	NA		NS	NS	NS	NA
Copepods	NA	NA	NA		16.35	16.80	NA	NA	NA	NA		14.75	15.50	13.01	NA
Invertebrates	15.65	NA	15.19		17.32	17.19	14.63	NA	NA	17.12		16.76	15.07	13.58	NA
Insects	NA	NA	15.34		NA	NA	NA	NA	NA	17.48		NA	NA	NA	NA
Fish (*R. holubi*)	16.66	14.12	20.89	20.58	18.99	18.31	16.89	14.79	14.45	20.06	19.70	19.83	19.03	17.08	14.31

Food sources were grouped by categories for simplicity, but are detailed in Online Resource 1. *Data from fish larvae collected in November 2013 (see "Material and Methods" section for food source selection procedure)

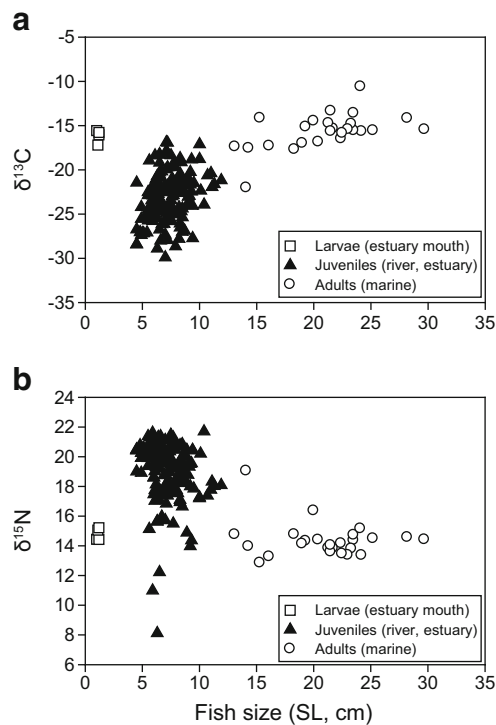*NA* not available, *NS* not selected

Fig. 4 **a** δ^{13}C and **b** δ^{15}N values measured in fish muscle tissue as a function of *R. holubi* size (SL, cm) in the river, estuary, and sea

Overall, stomach content and isotope data revealed similar patterns in terms of *R. holubi* trophic dynamics, with the exception of insects. Larval insects were recorded in the stomach contents of juvenile *R. holubi* collected from both the river and estuary, but with relatively low overall contributions. Conversely, SIAR models highlighted large dietary contributions by insects in spring and summer from the freshwater habitat. This result can be partly explained by the small size and associated quick digestion rate of insect larvae that dominate invertebrate assemblages from the freshwater habitat during this period (Hyslop 1980; Cortés 1997). In general, prey dominating the diet of *R. holubi* at different life stages reflected food source availability in the different habitats occupied by the fish at successive life stages. For example, larval insects were more abundant in the river in summer (Moyo 2016), and they contributed more to the fish diet during this season. Similarly, the higher contributions of epiphyton and microphytobenthos to the fish diet in spring illustrated the increased availability of microalgae in the estuary during this season (Dalu et al. 2016). The Cape stumpnose, along with the Cape moony *Monodactylus falciformis* and freshwater mullet *Myxus capensis*, are among the few marine fish species that make extensive use of estuarine headwaters in the Kowie system (Wasserman and Strydom 2011). Occupation of the lower reaches of the Kowie River by euryhaline marine species is facilitated by the elevated conductivity of the river, i.e., a relatively high geologically derived salt content in the water when compared to other riverine systems in South Africa. In addition, the abundance of submerged aquatic macrophytes and filamentous algae in the river-estuary interface (REI) zone may attract an omnivorous fish species such as *R. holubi* to this area.

Results from our isotope data analysis, in particular the overlapping δ^{13}C values of fish and their food sources within each habitat during each season, indicated a relatively low mobility by *R. holubi* following its life history-related movements from one aquatic environment to another. This finding implies that Cape stumpnose consumed prey in one particular habitat for extended periods before migrating to another, thereby growing and accumulating tissue in one habitat before moving to an adjacent habitat. These longitudinal movements in the estuary appear to occur gradually, as indicated by fish size data measured in the three aquatic habitats; i.e., post-larvae enter from the sea and then migrate up to the lower river or upper estuary where they feed as small juveniles (4.5 to 9.4 cm SL), followed by intra-estuarine movements as they migrate downstream towards the lower reaches (4.5 to 14.2 cm SL), and finally moving out the estuary into the marine coastal environment as sub-adults (≥13.1 cm SL). The final emigration of juveniles from the estuary to the sea also coincides with ontogenetic change in dentition reported for *R. holubi* (Blaber 1974b; Buxton and Kok 1983), thus supporting the hypothesis of a gradual move between these two environments. Researchers in aquatic systems around the world have documented similar longitudinal transfers by estuarine-associated marine or diadromous fish species (e.g., Deegan 1993; Gillanders et al. 2003; Herzka 2005; Ray 2005; Lugendo et al. 2006; Platell and Freewater 2009; Jardine et al. 2012). Isotopic studies such as ours provide additional evidence of such transfers, the importance of which for ecosystem functioning, including food web stabilization, is becoming increasingly apparent (McCann et al. 2005). For instance, Nelson et al. (2012) used stable isotopes to demonstrate the importance of fish migrations from temperate seagrass meadows to adjacent marine ecosystems for offshore fisheries in the northern Gulf of Mexico.

The estimated annual productivity of *R. holubi* in the Kowie Estuary (after mortalities) is conservatively estimated at 22 tonnes. This estimate is based on 29 % of annual fish production by Cape stumpnose in the Kowie Estuary (Whitfield et al. 1994) compared with 74 % of annual fish production in the nearby East Kleinemonde Estuary amounting to 41 g m^{-2} (Cowley and Whitfield 2002), and then extrapolating this figure to account for the total water surface area of the Kowie Estuary (142 ha). Fish mortality for *R. holubi* was estimated to account for about 3 % of annual production (Cowley and Whitfield 2002). Since sub-adult *R. holubi* only leave an estuary toward the end of their second year (Blaber 1974a), approximately 66 % of the annual fish production is likely to be retained within the estuary, with only about 33 % of production entering the

Table 4 Mean (%) and ranked contributions (between brackets, in increasing order) of different prey to the diet of *R. holubi* at different seasons and sites, estimated using SIAR models based on δ^{13}C and δ^{15}N measurements in fish and prey

Season	Site	TEF	Benthic algae		Invertebrates		Macrophytes		Epiphyton		Microphytobenthos		Insects		Copepods	
Winter	ES3	1	0.1	(4–5)	38.7	(2)	0.1	(4–5)	60.3	(1)	0.8	(3)	–	–	–	–
		2	**0.1**	**(5)**	**62.9**	**(1)**	**0.2**	**(4)**	**1.2**	**(3)**	**35.6**	**(2)**	–	–	–	–
		3	2.4	(5)	27.6	(2)	2.7	(4)	17.2	(3)	50.1	(1)	–	–	–	–
Spring	FW1	**1**	–	–	**10.1**	**(2)**	**81.0**	**(1)**	–	–	–	–	**9.0**	**(3)**	–	–
		2	–	–	5.5	(2)	89.5	(1)	–	–	–	–	4.9	(3)	–	–
		3	–	–	16.6	(2)	71.5	(1)	–	–	–	–	11.9	(3)	–	–
	FW2	**1**	–	–	**11.1**	**(2)**	**79.6**	**(1)**	–	–	–	–	**9.4**	**(3)**	–	–
		2	–	–	5.0	(2)	90.5	(1)	–	–	–	–	4.5	(3)	–	–
		3	–	–	21.1	(2)	67.2	(1)	–	–	–	–	11.7	(3)	–	–
	ES1	1	–	–	99.4	(1)	–	–	0.3	(2–3)	–	–	–	–	0.3	(2–3)
		2	–	–	**99.5**	**(1)**	–	–	**0.3**	**(2)**	–	–	–	–	**0.2**	**(3)**
		3	–	–	97.4	(1)	–	–	1.5	(2)	–	–	–	–	1.1	(3)
	ES2	1	–	–	27.0	(2)	–	–	71.3	(1)	–	–	–	–	1.7	(3)
		2	–	–	**18.3**	**(2)**	–	–	**80.7**	**(1)**	–	–	–	–	**1.1**	**(3)**
		3	–	–	25.7	(2)	–	–	71.7	(1)	–	–	–	–	2.6	(3)
	ES3	1	–	–	98.6	(1)	0.3	(3)	–	–	1.0	(2)	–	–	–	–
		2	–	–	**98.7**	**(1)**	**0.4**	**(3)**	–	–	**0.9**	**(2)**	–	–	–	–
		3	–	–	96.9	(1)	1.0	(3)	–	–	2.1	(2)	–	–	–	–
Summer	FW1	**1**	–	–	**1.5**	**(3)**	**1.8**	**(2)**	–	–	–	–	**96.6**	**(1)**	–	–
		2	–	–	1.6	(2)	0.7	(3)	–	–	–	–	97.6	(1)	–	–
		3	–	–	32.9	(2)	14.8	(3)	–	–	–	–	52.2	(1)	–	–
	FW2	**1**	–	–	**0.8**	**(3)**	**1.4**	**(2)**	–	–	–	–	**97.8**	**(1)**	–	–
		2	–	–	0.9	(2)	0.7	(3)	–	–	–	–	98.4	(1)	–	–
		3	–	–	51.3	(1)	13.1	(3)	–	–	–	–	35.6	(2)	–	–
	ES1	1	–	–	92.9	(1)	–	–	2.4	(3)	–	–	–	–	4.7	(2)
		2	–	–	**97.7**	**(1)**	–	–	**1.0**	**(3)**	–	–	–	–	**1.4**	**(2)**
		3	–	–	80.7	(1)	–	–	6.6	(3)	–	–	–	–	12.7	(2)
	ES2	1	–	–	94.4	(1)	0.3	(3)	–	–	–	–	–	–	5.3	(2)
		2	–	–	**99.3**	**(1)**	**0.1**	**(3)**	–	–	–	–	–	–	**0.6**	**(2)**
		3	–	–	72.2	(1)	1.7	(3)	–	–	–	–	–	–	26.1	(2)
	ES3	1	–	–	38.5	(2)	–	–	2.1	(3)	–	–	–	–	59.4	(1)
		2	–	–	**12.0**	**(2)**	–	–	**2.0**	**(3)**	–	–	–	–	**86.1**	**(1)**
		3	–	–	58.0	(1)	–	–	4.6	(3)	–	–	–	–	37.4	(2)

See the "Materials and Methods" section for details on food source selection, SIAR implementation and criteria, and TEF value combinations (1, 2, and 3). Bolded rows highlight the most probable contributions (estimated considering TEF values from the environment in which fish and food sources were collected, i.e., freshwater TEFs = combination 1 for models from freshwater sites; estuarine TEFs = combination 2 for models from estuarine sites). The full list of food sources comprising each category is provided in Online Resource 1

marine environment each year, i.e., about 7 tonnes per annum.

Another potential sink for animal material transported from one habitat or ecosystem to another is through predation. *Rhabdosargus holubi* juveniles are consumed by piscivorous fish and some bird species in estuaries (Blaber 1973a; Whitfield and Blaber 1978) and by marine piscivores in the sea, particularly those predators associated with subtidal reefs where adult *R. holubi* congregate (Buxton and Kok 1983). Immigration of post-larvae from the marine environment into the Kowie Estuary and the REI zone, and later downstream migration of juveniles down the estuary towards the sea, makes these individuals available to predators in each of the respective habitats at different stages of their life cycle.

Through its consumption of insects in the river, *R. holubi* also contributed to lateral energy transfer across the boundary between riparian and aquatic ecosystems. Insects consumed by *R. holubi* in the freshwater habitat were indeed mostly larvae of aquatic breeding flies produced by adults occupying riparian terrestrial environments. Consumption of such prey

by *R. holubi* juveniles thus represented lateral energy transfers from the terrestrial habitat where the flies originated, to the river, then the estuary, and ultimately to the coastal marine environment. The feeding behavior displayed by *R. holubi* therefore highlights its important role in ecosystem function and resilience of the Kowie system, by contributing to the incorporation of terrestrial subsidies into the aquatic food web (e.g., Richardson et al. 2010; Marcarelli et al. 2011). Connectivity among habitats will, in turn, affect *R. holubi* feeding success and dynamics in the area. Besides the physical connectivity through water flow that allows for longitudinal fish movements, trophic exchanges that are facilitated by the lateral transfer of organic material and nutrients across the boundaries of adjacent habitats are very important (Polis et al. 1997; Baxter et al. 2005; Wasserman et al. 2011).

In conclusion, our findings from both stomach content and stable isotope analyses highlight the advantages of combining dietary approaches when addressing questions about fish trophic ecology. In this study, stable isotope data assisted in better estimating the importance of certain prey types that stomach content data underestimated (e.g., insects). Constraints related to dietary analyses of fish based on stomach contents alone necessitate the collection of a large number of specimens for dissection, primarily because of the high levels of individual variability characterizing fish stomach composition (Cortés 1997). Stable isotope analyses can be conducted with smaller numbers of samples, as the information provided encompasses food assimilation processes occurring over longer temporal scales (Post 2002). However, stomach content data do provide a useful guideline in designing a sampling strategy of the relevant food resources for inclusion in stable isotope models, an approach successfully used in this study. Overall, both methods should be combined to obtain the most accurate descriptions of fish trophic dynamics and therefore their role in aquatic ecosystem functioning.

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